Tissue- and Stage-Specific Roles of the Ubiquitously Expressed Transcription Factor Sp1 in Haematopoietic Development

Leigh O'Connor

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College of Medical and Dental Sciences

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Abstract

Sp1 is a ubiquitously expressed transcription factor and regulates a range of genes including housekeeping and tissue-specific genes. Studies using a DNA binding domain (DBD)-deficient Sp1 have shown that Sp1 is required for haematopoietic specification.

Here, we generated a Sp1-DBD deficient ESC line to recapitulate the previous model, as well as a novel Sp1 null ESC line. Sp1 knockout cells demonstrated a complete absence of haematopoietic differentiation, indicating a crucial role for Sp1 at the early stages of blood cell specification. In contrast, Sp1 DBD-deficient cells were able to differentiate to haematopoietic progenitors, but failed to terminally differentiate, suggesting a different mechanism of Sp1-mediated transcriptional regulation in early and later stages.

Gene expression analysis in Sp1 knockout cells indicated a novel role for Sp1 in ESC differentiation potential and mesoderm formation, while chromatin accessibility profiling revealed changes in chromatin structure in the absence of Sp1. We found Sp3, a close family member of Sp1, is able to compensate for loss of Sp1 at most sites, but not at some important genes encoding developmental regulators. This work provides novel insights into the interplay between Sp1 and Sp3 and furthers our understanding of the function of one of the earliest discovered TFs.

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Abbreviations

AGM – aorta-gonad-mesonephros

ATAC – assay for transposase accessible chromatin

ATP – adenosine triphosphate

bp – base pair

BSA – bovine serum albumin

ChIP – chromatin immunoprecipitation

cDNA – complementary DNA

CRISPR – clustered regularly interspaced short palindromic repeats

DAM-ID – DNA-adenine methyltransferase identification

DBD – DNA binding domain

DMEM – Dulbecco's Modified Eagle's Medium

DNMT – DNA methyltransferase

DSG – disuccinimidyl glutarate

E – embryonic day

EB – embryonic body

EHT – endothelial-haematopoietic transition

ESC – embryonic stem cell

FACS – fluorescence activated cell sorting

FCS – foetal calf serum

FPKM – Fragments per kilobase of transcript per million mapped reads

GSEA – gene set enrichment analysis

GTF – general transcription factor

HAT – histone acetyltransferase

HDAC – histone deacetylase

HE – haemogenic endothelium

HSC – haematopoietic stem cell

ID – inhibitory domain

IMDM – Iscove Modified Dulbecco Medium

ITR – inverted terminal repeat sequences

Kb – kilobase

LIF – leukaemia inhibitory factor

MACS – magnetic activated cell sorting

M-CSF - macrophage colony stimulating factor

MEF - mouse embryonic fibroblast

MEP – megakaryocyte-erythroid progenitor

NDR – nucleosome-depleted region

NHEJ – non-homologous end joining

nt – nucleotides

PB – PiggyBac

PBS - phosphate buffered saline

PCR – polymerase chain reaction

PIC – protease inhibitor cocktail

PTM – post-translational modification

qPCR – quantitative PCR

RIPA – radioimmunoprecipitation assay

RNAPII – RNA polymerase II

Seq – sequencing

Sp1 – specificity protein 1

TAD – transactivation domain

TALEN – transcription activator-like effector nuclease

TF – transcription factor

TSS – transcription start site

WT – wildtype

YS – yolk sac

ZFN – zinc-finger nuclease

1. INTRODUCTION

1.1 Chromatin

Every human cell contains its genetic information encoded in ~2 metres of DNA. The DNA is compacted into chromatin, enabling it to fit inside the nucleus of the cell. This function of chromatin was first discovered in the 1970's, but a torrent of follow-up studies found chromatin also acts as a regulatory mechanism, controlling access of proteins, such as transcription factors (TFs), to the DNA (Weintraub & Groudine, 1976; Wu, 1980; Li et al., 2007).

Chromatin is formed by repeating units of nucleosomes (Kornberg & Thonmas, 1974). The nucleosome is formed of ~146 base pairs (bp) of DNA wrapped around a histone core, which is made up of two molecules each of the four histone proteins – H2A, H2B, H3 and H4. The histone octamer forms a globular structure, except for unstructured N-terminal tails that extend out of the complex (Luger et al., 1997) (Figure 1.1). The N-terminal tails are subject to an array of post-translational modifications, which can influence both the stability of the nucleosome structure and the binding of regulatory proteins and enzymatic complexes (Strahl & Allis, 2000).

Nucleosomes then assemble into a higher order structure (a 30 nm fibre) with the help of linker proteins, such as histone H1, which binds on the edge of the nucleosome, constraining an extra ~20 bp DNA, as well as determining the trajectory of the DNA entering and exiting the nucleosome to aid in formation of the higher order structure (Routh et al., 2008). The structure of the 30 nm fibre is still debated. The zigzag model suggests the nucleosomes stack on top of each other, forming a double helix while the

linker DNA zigzags between them (Schalch et al., 2005). In contrast, the solenoid model states nucleosomes from adjacent coils are interdigitated (Robinson et al., 2006).

Chromatin can be further ordered in 3D space in the nucleus. Imaging studies have shown areas of condensed, inactive 'heterochromatin' and more accessible 'euchromatin', containing active genes (Heitz, 1928; Gilbert et al., 2004; Cockerill, 2011). Histone modifications can aid in the establishment and preservation of these chromatin domains. For example, euchromatin is associated with acetylation, H3 lysine 4 trimethylation (H3K4me3), H3K79me3 and H3K36me3, whereas heterochromatin is associated with repressive marks, such as H3K9me3 and H3K27me3 (Yan & Boyd, 2006; Kouzarides, 2007). Indeed, more recently, the genome-wide mapping of histone modifications, as well as chromatin-binding proteins, in *Drosophila* led to the identification of several subcategories of euchromatin and heterochromatin (Filion et al., 2010).

Chromatin can also form large megabase-sized domains called topologically associated domains (TADs), defined as intrachromasomal regions that display a high frequency of physical interaction (Dixon et al., 2012). They are stable across cell division/differentiation and are often conserved between species. TADs can function to cluster similarly-regulated genes, such as lineage-specific genes, and promote/direct contacts between genes and their regulatory elements (Neems et al., 2016).

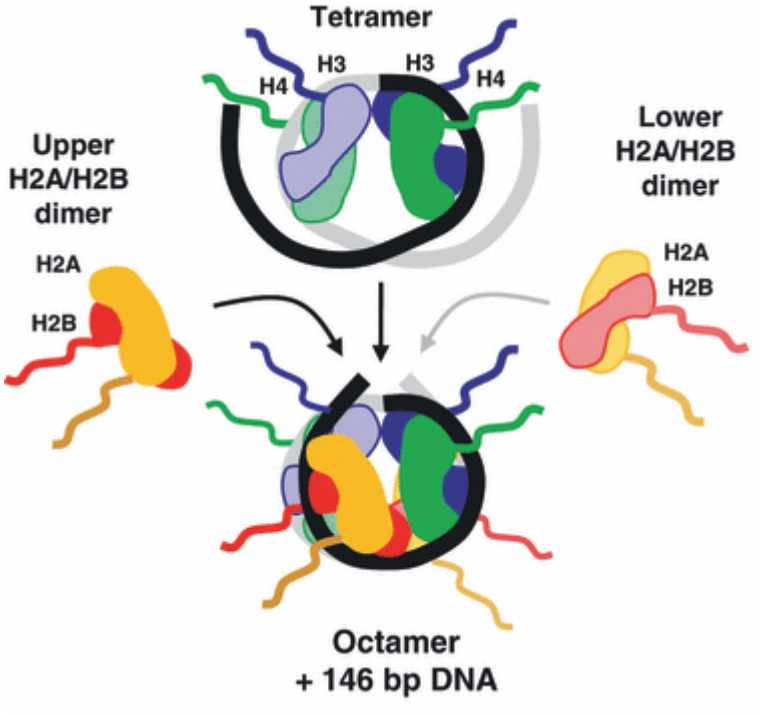

Figure 1.1 – Nucleosome structure
The nucleosome is made up of ~146 bp DNA wrapped around a core histone octamer. Two molecules of each H3 and H4 first form a tetramer and bind ~60 bp DNA, before two H2A/H2B dimers bind the flanking DNA and assemble above and below the H3/H4 tetramer. The unstructured N-terminal tails extend from the core complex into solution. Adapted from (Cockerill, 2011). (Permission to reproduce this figure has been granted by John Wiley and Sons.)

1.2 Transcriptional Regulation

The genetic information in DNA is transcribed to RNA by RNA polymerases – RNA polymerase II (RNAPII) in the case of protein-coding genes. Each cell in the body contains an identical genome, but each cell type has a different phenotype and performs distinct functions. This diversity comes about through the control of gene expression, with different cell types having a unique pattern of gene activation. Thus, transcription is highly regulated, often at the level of individual genes, at all stages of gene expression, from transcription initiation and elongation to mRNA processing, transport and translation. General transcription factors (GTFs) are sufficient to initiate a basal level of transcription

at any gene, but transcription frequency is modulated by specific activators and cofactors, as well as the chromatin state.

1.2.1 Transcription factors

TFs control the expression of genes involved in all cellular processes, including cell cycle progression, differentiation/development and homeostasis. Indeed, TFs are overrepresented among oncogenes (Furney et al., 2006) and a third of developmental disorders are thought to be caused by dysfunctional TFs (Boyadjiev & Jabs, 2000), highlighting their importance.

TFs function to modulate the rate of transcription of their target genes. They generally have modular structures, split into distinct domains, including the DNA-binding domain (DBD), transactivation domain (TAD) (through which its effector functions are mediated) and dimerization/interaction domain (Brent & Ptashne, 1985). The DBD gives TFs the ability to recognise and bind specific sequences in the DNA – typically a stretch of 6-12 bp degenerate sequences called motifs. TFs can be characterised into families, often distinguished by the type of DBD, such as zinc fingers and helix-loop-helix (Luscombe et al., 2000). These motifs denote fairly low sequence specificity in the genome, meaning additional levels of regulation are required to ensure specific TF binding, including accessibility of the site in the chromatin and combinatorial TF binding (Biggin, 2011). Indeed, TFs regularly interact together to affect each other's binding and, thus, activity. For instance, TFs can cooperatively bind, thereby increasing their affinities for the DNA sequence, or they can bind and synergistically activate transcription, upregulating gene expression more than by any individually (Spitz & Furlong, 2012). Given the relatively few types of TFs compared to the number of genes, each requiring specific regulation, the

ability of TFs and cofactors to act together adds complexity and diversity to gene regulation and creates dynamic transcription networks.

TFs activate transcription by promoting recruitment of the basal transcriptional machinery. Some TFs can interact directly with the GTFs; others can interact with non-DNA binding cofactors, which can, in turn, recruit the GTFs or increase accessibility of the DNA for the transcription machinery (Kadonaga, 2004).

1.2.2 Promoters

Transcription is regulated through the integrated action of many regulatory elements in the DNA, both those near to the 5' end of the gene and others more distal. The core promoter contains the transcription start site (TSS), typically spanning ~40 bp to either side, and is where the transcription machinery assembles (Zhang, 1998). The proximal promoter stretches up to a few hundred bp upstream from the core promoter and contains a number of binding sites for sequence-specific TFs (activators) to fine-tune transcription initiated at the core promoter (Maston et al., 2006).

The core promoter is enriched for a variety of conserved motifs (Smale & Kadonaga, 2003; Kadonaga, 2012). The TATA box is the most widely used in nature, and so was the first found and the most studied. It is positioned ~30 bp upstream of the TSS (Hu & Manley, 1981). Inr is the most common core promoter element, which spans the TSS (Smale & Baltimore, 1989). Other motifs include BRE, DPE and MTE (Figure 1.2). The motifs can bind components of the general transcription machinery – for example, TBP (TFIID) can bind the TATA box and TFIIB can bind BRE (Kim et al., 1993; Lagrange et al., 1998). The motif composition can affect gene regulation as different sets of factors

mediate basal transcription from different types of core promoter (Lewis et al., 2005; Wright et al., 2006).

However, not all promoters contain these core motifs. Indeed, only 10-20% of promoters are thought to have the canonical TATA box (Gershenzon & Ioshikhes, 2005; Cooper et al., 2006). Some promoters are associated with an enrichment of unmethylated CG dinucleotides called a CpG island, which tend to lack the conventional core promoter motifs (Blake et al., 1990). While promoters containing the conserved motifs have a very defined TSS ("sharp"), defined by sequences such as Inr, CG-rich promoters have several TSSs throughout the promoter ("broad") (Carninci et al., 2006). This formed the basis for the different classes of promoter (Lenhard et al., 2012). Type I promoters normally contain a TATA box, have low CG content and typically regulate expression of inducible genes and tissue-specific genes (Schug et al., 2005; Yamashita et al., 2005; Carninci et al., 2006). Type II promoters are generally associated with constitutively-expressed housekeeping genes. They tend to have a short CpG island over the TSS and are TATA-depleted, but can have DREs and other weaker, less-characterised motifs (FitzGerald et al., 2006). In contrast, type III promoters often have several large CpG islands that can extend into the gene body and, while still classed as "broad", have a sharper TSS than type II (Akalin et al., 2009). They are mostly associated with developmentally-regulated genes, which are often highly-regulated by enhancers and can be repressed by Polycomb proteins (Ernst et al., 2011). The promoter classes are also associated with specific epigenetic marks. For example, while all active promoters have a nucleosome-depleted region (NDR), broad CG-rich promoters have precisely positioned -1,+1 nucleosomes either side of the promoter, whereas nucleosomes flanking sharp TATA-rich promoters have less defined positions (Cairns, 2009; Rach et al., 2011).

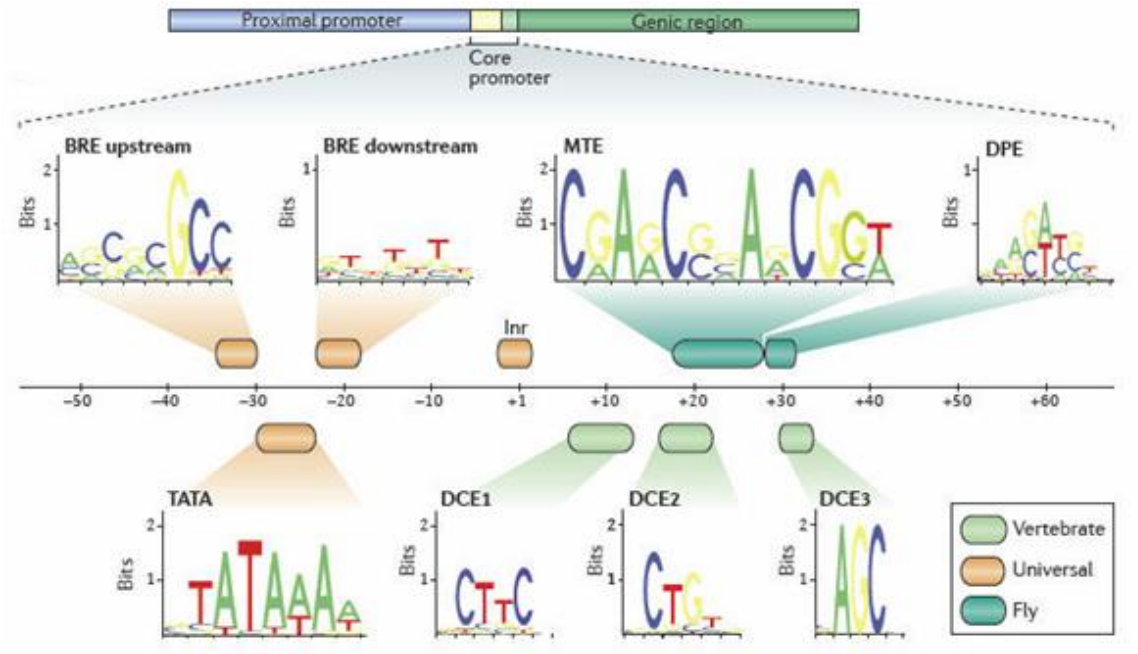

Figure 1.2 – Conserved sequences in core promoters
The consensus sequence and common location in the core promoter relative to the TSS of known motifs is illustrated. Most promoters contain only one or a selection of the elements. The frequency and combinations of the sequence elements in the promoter can vary between species (e.g. both vertebrate and fly sequences are shown, as many of the studies into promoter structure/function have been performed in Drosophila). Adapted from (Lenhard et al., 2012). (Permission to reproduce this figure has been granted by Springer Nature.)

1.2.3 CpG Islands

DNA methylation is the addition of a methyl group at the 5' carbon of the pyrimidine ring of cytosine and in vertebrates occurs predominantly at CG dinucleotides. Most CG dinucleotides in the genome are thought to have been depleted over the course of evolution due the high rate of mutation of methyl-cytosine to thymine by spontaneous deamination (Bird, 1980). However, there are regions where CG dinucleotides are overrepresented compared to the rest of the genome, called CpG islands (Gardiner-Garden & Frommer, 1987), which are generally unmethylated (Larsen et al., 1992). CpG islands are commonly found at promoters: indeed, ~70% of promoters have a CpG island

and it is thought to be one of the most reliable indicators of a promoter element (Saxonov et al., 2006). Generally, CpG island promoters are associated with widely-expressed housekeeping genes (Larsen et al., 1992; Yamashita et al., 2005).

CpG islands are inherently transcriptionally permissive. They are usually associated with a NDR. In vitro nucleosome formation studies have suggested that the DNA sequence itself makes the assembly of stable nucleosomes unfavourable, thus enabling some TFs to bind their target sites within chromatin, which would otherwise require the help of nucleosome remodelling factors (Ramirez-Carrozzi et al., 2009). Furthermore, in ESCs, RNAPII was found bound at the CpG island promoters of many inactive genes, suggesting transcription can readily initiate at these elements (Guenther et al., 2007).

CpG islands are commonly marked by the histone modification H3K4me3, which is linked to actively transcribed genes (Figure 1.3). H3K4me3 can act as a binding site for activating factors, such as the NuRF remodelling complex (Wysocka et al., 2006) and histone acetyltransferases (HATs) (Saksouk et al., 2009), which help to create a permissive chromatin structure. It can also recruit the transcriptional machinery, for example via TFIID (van Ingen et al., 2008). H3K4me3 at CpG islands is thought to be established by CXXC-domain containing proteins (which preferentially bind unmethylated CpG sequences), such as Setd1, a H3K4 methyltransferase (Thomson et al., 2010). The transcriptional machinery can also recruit Setd1; hence it is likely that active transcription from CpG island promoters contributes to the levels of H3K4me3. However, H3K4me3 can often persist even when the gene is inactive (Guenther et al., 2007) and CpG density in the CpG island correlates with levels of H3K4me3, suggesting the underlying DNA sequence is important (Illingworth et al., 2010). Interestingly, H3K4me3 can repress the binding of DNA methyltransferases (DNMTs), thus helping to maintain an active, unmethylated promoter (Ooi et al., 2007). Another theory is that DNA methylation is

actively removed at CpG islands promoters, as the demethylating enzyme Tet1 contains a CXXC domain and preferentially binds to CpG islands (Wu et al., 2011).

CpG island promoters are often targets of Polycomb-mediated silencing, through the deposition of the negative histone modification H3K27me3 (Mikkelsen et al., 2007). In embryonic stem cells (ESCs), CpG islands are associated with bivalent promoters, marked with both active H3K4me3 and repressive H3K27me3, thought to silence developmentally-regulated genes in ESCs, but keep them poised for activation upon differentiation (Bernstein et al., 2006; Ku et al., 2008). While the DNA sequence has a role in Polycomb recruitment, as it preferentially binds at CpG islands, the presence of TF motifs/binding can also play a role in preventing Polycomb binding (Ku et al., 2008).

CpG island promoters do not tend to contain core promoter motifs, such as TATA box (although there are exceptions, such as α-globin and erythropoietin) (Deaton & Bird, 2011). Thus, CpG island promoters often rely on the binding of TFs to recruit the transcriptional machinery, for example Sp1 can recruit TBP/TFIID to TATA-less promoters (Pugh & Tjian, 1991). Many TF motifs are CG-rich and contain CpG dinucleotides, such as Sp1, ETS and E-box, and these binding sites are enriched at CpG island promoters (Rozenberg et al., 2008). Therefore, CpG islands can promote TF binding.

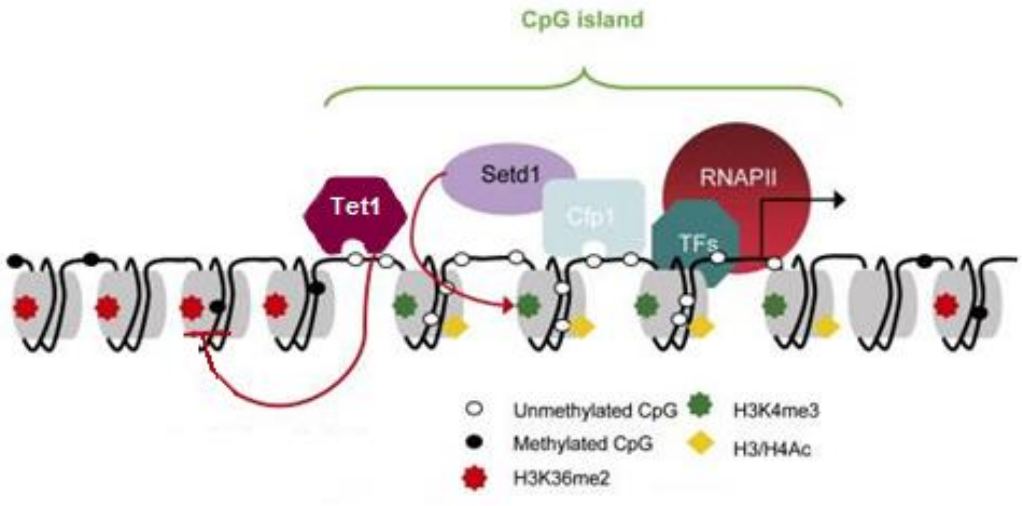

Figure 1.3 – Active chromatin state at CpG islands
CpG islands are normally unmethylated and transcriptionally permissive. They are marked by active histone modifications, such as acetylation and H3K4me3 (which is mediated through the recruitment of Setd1 via its CXXC-domain Cfp1). They are also associated with nucleosome deficiency and TF binding, which can recruit RNAPII. DNA demethylation may be mediated by Tet1, also recruited by a CXXC domain. Adapted from (Deaton & Bird, 2011) (available under a Creative Commons license).

1.2.4 Enhancers

Enhancers are regulatory elements that modulate expression of its target gene through the recruitment of TFs and cofactors (Banerji et al., 1981). They often regulate tissue/developmental stage-specific or inducible genes, giving rise to specific patterns of temporal and spatial activity (Spitz & Furlong, 2012). Enhancers can be positioned upstream, downstream or within an intron of its target gene, as well as at large distances from its promoter, as far as megabases away (Sanyal et al., 2012). They are also associated with a NDR and H3K4me1 and H3K27ac marks (Rada-Iglesias et al., 2011).

Gene expression levels correlate with the number of associated enhancers (Chepelev et al., 2012). One promoter can interact with several enhancers, either at the same time to

give high expression levels, or different enhancers can be active in different cell types to alter the transcriptional programme of the cell (Ernst et al., 2011) (Figure 1.4). Enhancers commonly contain multiple TF motifs, with combinatorial TF binding resulting in diverse and precise patterns of transcription, dependent on levels of each TF and response to signalling pathways in the cell.

Enhancers act by physically interacting with its target promoter and thereby recruiting the transcriptional machinery (often via the mediator complex (Allen & Taatjes, 2015)) and histone modifying enzymes to generate a permissive chromatin structure. The DNA between the enhancer and promoter is looped out, so bringing the enhancer and promoter into close proximity in the nucleus, as demonstrated by chromosome conformation assays (Marsman & Horsfield, 2012). This enhancer-promoter interaction is cell-type specific, occurring when the enhancer is active (Figure 1.4). For example, in brain cells, the β-globin locus is linear, while in erythrocytes, the enhancer region is in close proximity to the β-globin genes, coinciding with their expression, while intervening inactive globin genes are looped out (Tolhuis et al., 2002). Looping is mediated by proteins such as CTCF and cohesin (Splinter et al., 2006; Ebmeier & Taatjes, 2010), as well by interactions between specific TFs bound at the enhancer and promoter (Song et al., 2007; Nolis et al., 2009), which also help ensure specific enhancer-promoter activity. Moreover, some studies have suggested that enhancers have preferences for different core promoter motifs or classes of promoter (Engström et al., 2007; Zabidi et al., 2015), while the formation of TADs can promote specific enhancer-promoter contacts and minimise erroneous enhancer interactions (Zabidi & Stark, 2016). Insulators, another type of regulatory sequence, can prevent genes being affected by the activities of neighbouring elements, for example by blocking enhancer-promoter communications and stopping the spread of heterochromatin.

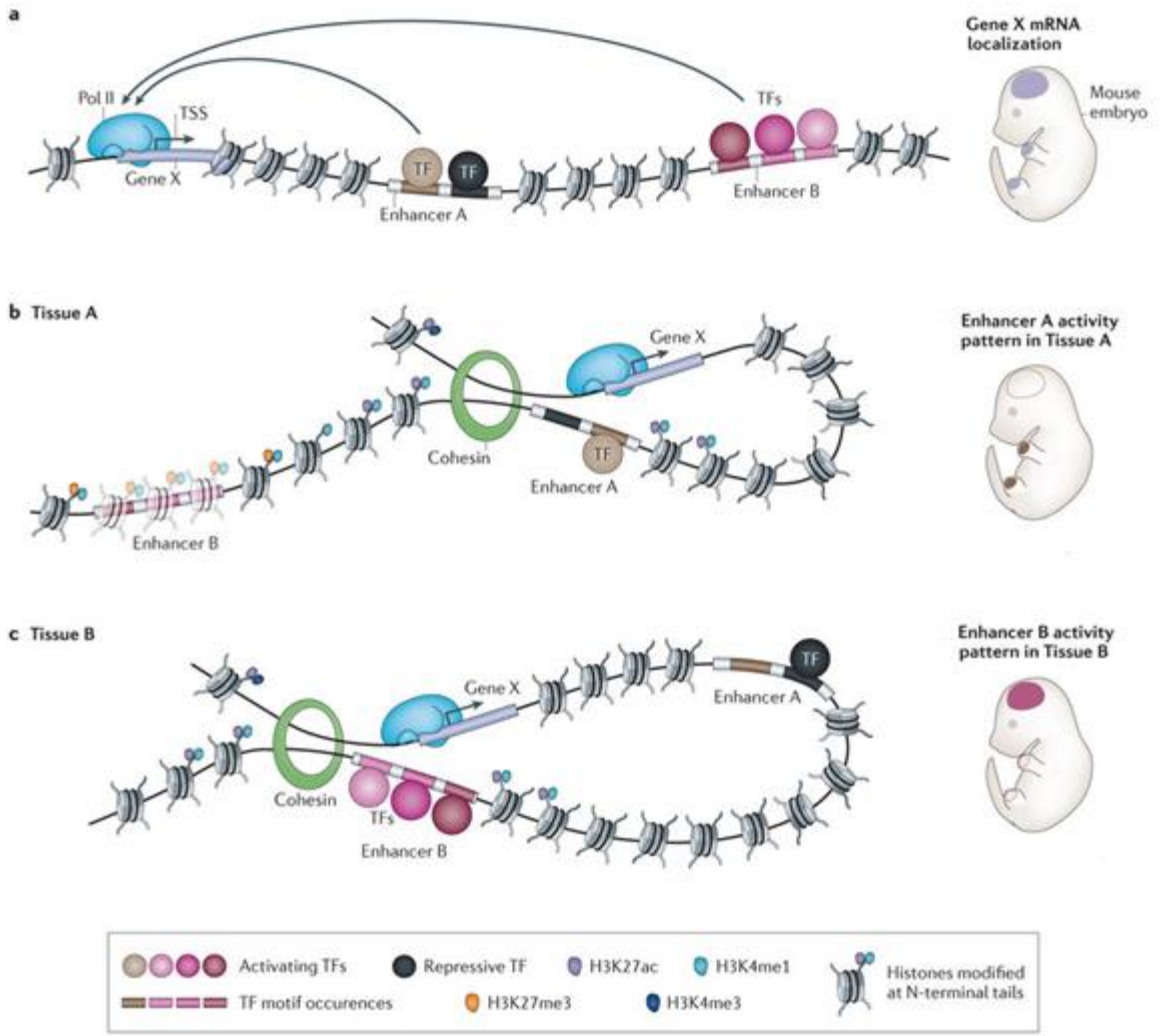

Figure 1.4 – Enhancers can mediate tissue-specific patterns of gene expression

(A) Enhancers are distal regulatory elements that can bind specific TFs and upregulate the expression of its associated gene. (B,C) Enhancers may only be active in certain cell types to elicit a tissue-specific pattern of transcription. Active enhancers are bound by TFs and brought into close spatial proximity with their target promoters through looping of the intervening DNA. Looping is mediated by cohesin and other proteins, such as CTCF. Active enhancers are commonly marked by H3K4me1 and H3K27ac. Inactive enhancers can be silenced by H3K27me3, be kept away from the promoter in space (e.g. by CTCF) or be bound by repressor proteins. Adapted from (Shlyueva et al., 2014). (Permission to reproduce this figure has been granted by Springer Nature.)

1.2.5 Chromatin and epigenetic transcription regulation

The chromatin environment at a gene's regulatory elements can impact on transcription regulation. Many TFs cannot bind to its target site if within a nucleosome (Li et al., 1994; Gutiérrez et al., 2000) and the transcription machinery cannot assemble on the promoter if the TSS is covered by a nucleosome (Workman & Roeder, 1987; Beato & Eisfeld, 1997). Chromatin remodelling complexes, recruited by specific TFs, use ATP to change the positioning of the nucleosome by sliding the nucleosome (such as ISWI) or displacing the nucleosome (e.g. SWI/SNF) (Clapier et al., 2017). Remodellers can also mediate exchange of the core histones for histone variants. H3.3 and H2A.Z are commonly found at promoters as they form less stable nucleosomes (Zhang et al., 2005a; Jin et al., 2009). Pioneer TFs can also bind within compacted chromatin, disrupting the nucleosome structure to allow the binding of other TFs, for example FoxO1, which has a similar structure to H1 to enable it to compete for chromatin binding (Cirillo et al., 2002; Hatta & Cirillo, 2007; Zaret & Mango, 2016).

Histone modifications can also affect transcriptional activation (Kouzarides, 2007; Zhang et al., 2015). They can act as binding sites to recruit TFs and cofactors, for example H3K4me3 at promoters can recruit histone acetyltransferases and TFIID to promote transcription (van Ingen et al., 2008). Histone modifications can also impact on chromatin stability. Lysine acetylation neutralises its basic charge, weakening DNA-histone interactions and making histones easier to displace (Ito et al., 2000; Reinke & Hörz, 2003). Acetylation can also interfere with higher order chromatin structure, as H4K16 acetylation inhibits formation of 30 nm fibres (Shogren-Knaak et al., 2006). Correspondingly, levels of acetylation at promoters correlate with gene expression (Pokholok et al., 2005). Histone modifications are also associated with transcription progress, for example, H3K4me3 is present at the 5' end of genes and H3K36me3 is

present in the body of the gene to inhibit cryptic transcription initiation (Barth & Imhof, 2010).

Furthermore, DNA methylation can inhibit transcription by preventing binding of some TFs (Domcke et al., 2015) or by recruiting other repressors such as histone deacetylases (HDACs) (Jones et al., 1998). However, some TFs can still bind despite DNA methylation, such as CEBPβ (Mann et al., 2013) and Sp1 (Höller et al., 1988), while others can prevent DNA methylation, e.g. REST, which occupies enhancers during ESC differentiation to neural progenitors and prevents their methylation (Stadler et al., 2011). Sp1 has also been shown to prevent DNA methylation at the *Aprt* locus (Macleod et al., 1994) and may have a role in preventing DNA methylation at CpG island promoters in general by its constitutive binding (Höller et al., 1988). DNA methylation is also targeted to gene bodies during transcription to inhibit cryptic initiation (Neri et al., 2017).

1.3 Transcription

1.3.1 Initiation

For transcription initiation, GTFs assemble on the core promoter in a step-wise manner (Thomas & Chiang, 2006; Luse, 2014) (Figure 1.5). TFIID binds to the core promoter motifs. TFIID consists of TBP, which recognises TATA box, as well as 13-14 TBP-associated factors (TAFs), which can assist in promoter binding, for example TAF1/2 can bind Inr and TAF6 can bind DPE. TFIIA then binds, which in turn recruits TFIIB. TFIIB recruits the pre-formed TFIIF-RNAPII complex and together, TFIIB and TFIIF orient the DNA template and select the TSS. Finally, TFIIE and TFIIH are recruited, the latter of

which has helicase activity to melt the DNA and it phosphorylates the C-terminal domain of RNAPII (Ser5 of the repeat) (Tirode et al., 1999).

1.3.2 Elongation

Once the initiation complex has been established, RNAPII moves into the gene body, while TFIID, TFIIA, TFIIE and TFIIH remain at the promoter as a scaffold to promote re-initiation (Yudkovsky et al., 2000). RNAPII pauses ~20-60 nucleotides downstream of the TSS and is stabilised in this state by NELF and DSIF (Lee et al., 2008a). This pausing acts as a regulatory step, ensuring that the mRNA is capped at the 5' end. P-TEFb is recruited, often by TFs or cofactors such as mediator (Takahashi et al., 2011), to trigger release of the paused RNAPII by phosphorylating both NELF and DSIF, as well as RNAPII on Ser2 of the C-terminal repeat, which can in turn recruit necessary elongation factors (Lis et al., 2000; Jonkers & Lis, 2015). RNAPII can then proceed to productive elongation. The rate of elongation can vary between genes, depending on histone modifications and features of the gene itself, e.g. the number of exons, which link to co-transcriptional processes such as splicing (de la Mata et al., 2003).

1.3.3 Termination

Transcription termination is closely linked to 3' end processing of the nascent mRNA (Porrua & Libri, 2015). Cleavage and polyadenylation factors are recruited to the RNAPII complex and recognise the polyadenylation signal in the mRNA, triggering cleavage ~18-30 nt downstream. This enables XRN2, an RNA exonuclease, to degrade the RNA, travel to the paused RNAPII and displace it (West et al., 2004). However, another model suggests that conformational changes in RNAPII and loss of elongation factors following transcription of the polyadenylation signals cause decreased processivity and finally

termination (Zhang et al., 2005b). A combination of the two models is likely (Lemay & Bachand, 2015).

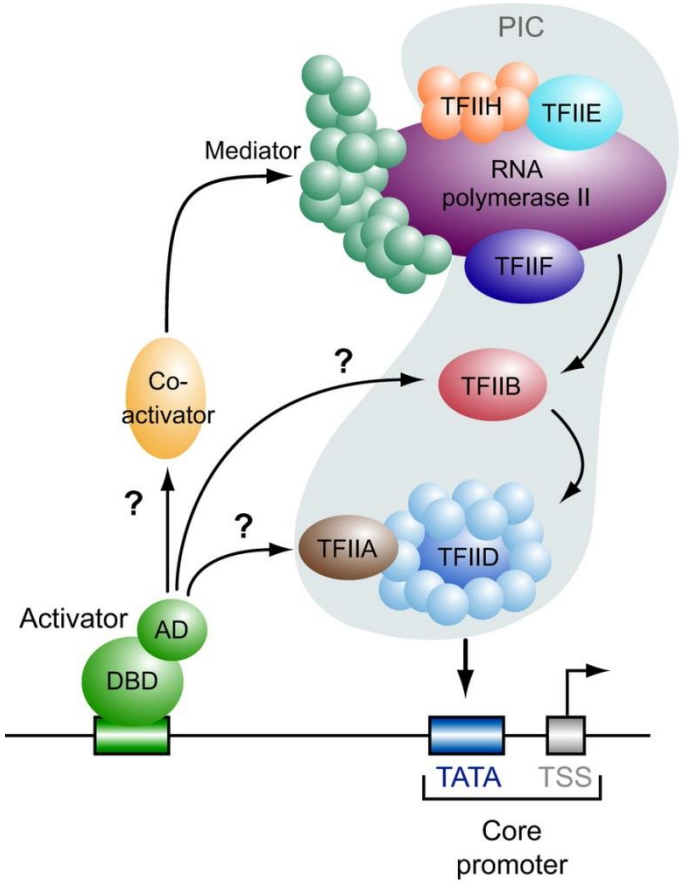

Figure 1.5 – Transcription initiation
GTFs assemble on the promoter in a stepwise manner, along with RNAPII, to form a preinitiation complex (PIC), which position RNAPII on the TSS. TFIID recognises many of the core promoter sequence elements to aid binding to the promoter. The transcriptional machinery can also be recruited by sequence-specific TFs (activators), either directly or via co-factors such as mediator. (Maston et al., 2006). (Available to reproduce from Annual Reviews).

1.4 Haematopoiesis

Haematopoiesis is the process of blood cell development, which in adult mammals occurs in the bone marrow. Haematopoietic stem cells (HSCs), formed during embryogenesis, represent a life-long pool to generate the relatively short-lived mature blood cell types found in adult blood and peripheral tissues such as lymph nodes, thymus and spleen. Each blood cell type performs individual, crucial functions from oxygen supply to the body to immune defence. HSCs differentiate through a series of intermediate progenitors, each gradually more restricted to a specific lineage, to the final mature blood cell types (Figure 1.6). This process is controlled by the expression of stage-specific TFs, which in turn establish a cell-type specific pattern of gene expression. It is worth noting that this traditional stepwise hierarchical model of haematopoiesis is now being questioned, with recent advances in single-cell technology suggesting a continuous differentiation process along one trajectory from the HSC, with the intermediate progenitors (e.g. MPPs) representing transitory states, rather than discrete cell types (Naik et al., 2013; Velten et al., 2017).

HSCs are defined as multipotent (able to generate all blood cell types), have the capacity to self-renew and provide long-term engraftment and reconstitution of the entire haematopoietic system upon transplant into irradiated adult recipients. The process of HSC formation in the embryo has been extensively studied and still represents a much-used system in which to investigate principles of development and the roles of TFs in cell-fate decisions. Our current knowledge of embryonic haematopoiesis will be discussed.

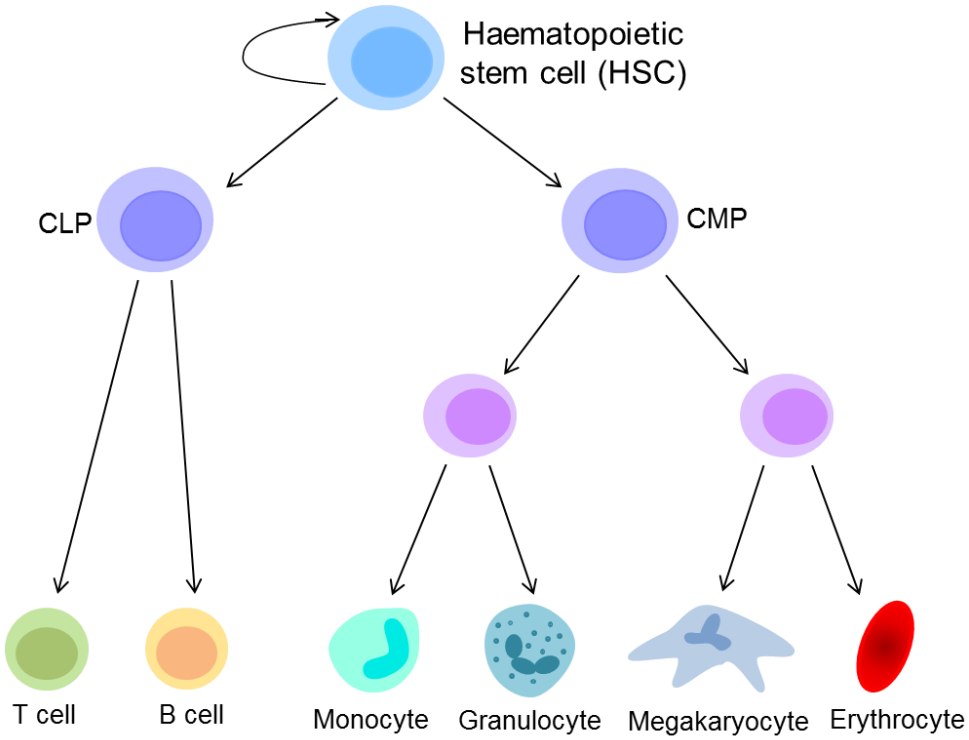

Figure 1.6 – Classical hierarchy model of haematopoietic cell differentiation
Adult bone marrow contains a self-renewing population of HSCs, which can differentiate through various multipotent progenitors to generate the mature blood cells that make up the haematopoietic system. However, recent models suggest that, rather than differentiating through discrete progenitors, the HSCs gradually and continuously acquire the transcriptome of a single lineage.

1.4.1 Anatomical sites of embryonic haematopoiesis

During embryonic development, gastrulation gives rise to the three germ layers – ectoderm, mesoderm and endoderm – which form the basis of the body plan. The extra- and intra-embryonic territories become defined during this process, constituting the yolk sac (YS) and embryo, respectively.

The first haematopoietic cells were identified in the YS in the 1920's (Sabin, 1920) and it was long thought to be the first source of HSCs, before they migrate to the embryo proper (Moore & Metcalf, 1970). However, this theory was questioned in the 1970's when no long-term repopulating HSCs could be found in the YS before the circulation was formed. An elegant chimera study involved engraftment of a quail embryo to a chick YS and found all cells in the haematopoietic organs (the spleen and thymus) were of the quail, suggesting an intra-embryonic source of HSCs (Dieterlen-Lievre, 1975). Indeed, a number of later studies confirmed an intra-embryonic site of HSC generation. Transplantation of cells derived from different embryonic tissues found that only those from the aorta-gonad-mesonephros (AGM) region (derived from the mesodermal germ layer) were capable of long-term reconstitution of the haematopoietic system in lethally irradiated mice (Muller et al., 1994). Furthermore, to remove the compounding factor of cellular exchange between the YS and AGM region in the embryo, the YS, AGM and liver were cultured individually in vitro. Only the AGM could both maintain HSCs and increase the number of HSCs in culture, indicating it is the main source of HSCs in the embryo (Medvinsky & Dzierzak, 1996). More specifically, HSC development is localised to the dorsal aorta (as well as the vitelline artery and umbilical vessels) of the AGM (de Bruijn et al., 2000). However, there is some controversy in whether the intra-embryonic environment is the defining factor in HSC formation, as a study has found culturing YS derived cells with AGM stromal cells is sufficient to generate HSCs (Matsuoka et al., 2001).

These studies led to the model that the haematopoietic system is established in successive waves during embryogenesis (Figure 1.7) (Lacaud & Kouskoff, 2017). The first wave occurs in mesoderm-derived blood islands in the YS shortly after gastrulation at around embryonic day 7.25 (E7.25) in the mouse (Palis et al., 1995; Palis et al., 1999), giving rise to primitive progenitors. These cells have reduced differentiation potential

compared to HSCs, being primed to form only a subset of haematopoietic lineages (Cumano et al., 1996). Firstly, primitive erythroid cells are formed, found only during early embryogenesis (containing foetal haemoglobin) and function to deliver oxygen rapidly to the growing embryo (Palis et al., 1999). Macrophage and megakaryocyte precursors are also formed during this first stage (Palis et al., 1999; Tober et al., 2007). The second, partially overlapping, wave of haematopoiesis begins at ~E8.25 with the emergence of erythromyeloid progenitors (EMPs) in the YS (Palis et al., 1999; Bertrand et al., 2005; McGrath et al., 2015). The EMPs colonise the foetal liver and give rise to the first definitive (or adult-like) erythrocytes and myeloid cells (Palis et al., 1999; McGrath et al., 2011). The first lymphoid progenitors emerge at ~E9.5 (Yoshimoto et al., 2011; Yoshimoto et al., 2012). These transient populations are essential to maintain the embryo before HSC formation and expansion (McGrath et al., 2015). Recent studies have also found that tissue-resident macrophages that remain throughout life are generated in the first wave of haematopoiesis (Schulz et al., 2012; Gomez Perdiguero et al., 2015).

The third wave occurs at E10.5 at the ventral part of the dorsal aorta in the AGM region of the embryo. It is here that the definitive HSCs are generated (Medvinsky & Dzierzak, 1996; de Bruijn et al., 2000). They migrate to through the newly developed circulation to the foetal liver where they are expanded, before moving to the bone marrow at E16.5, which remains the haematopoietic niche throughout adult life. This dynamic production of distinct haematopoietic cells throughout embryogenesis provides the embryo with the cells it requires at each stage as it develops.

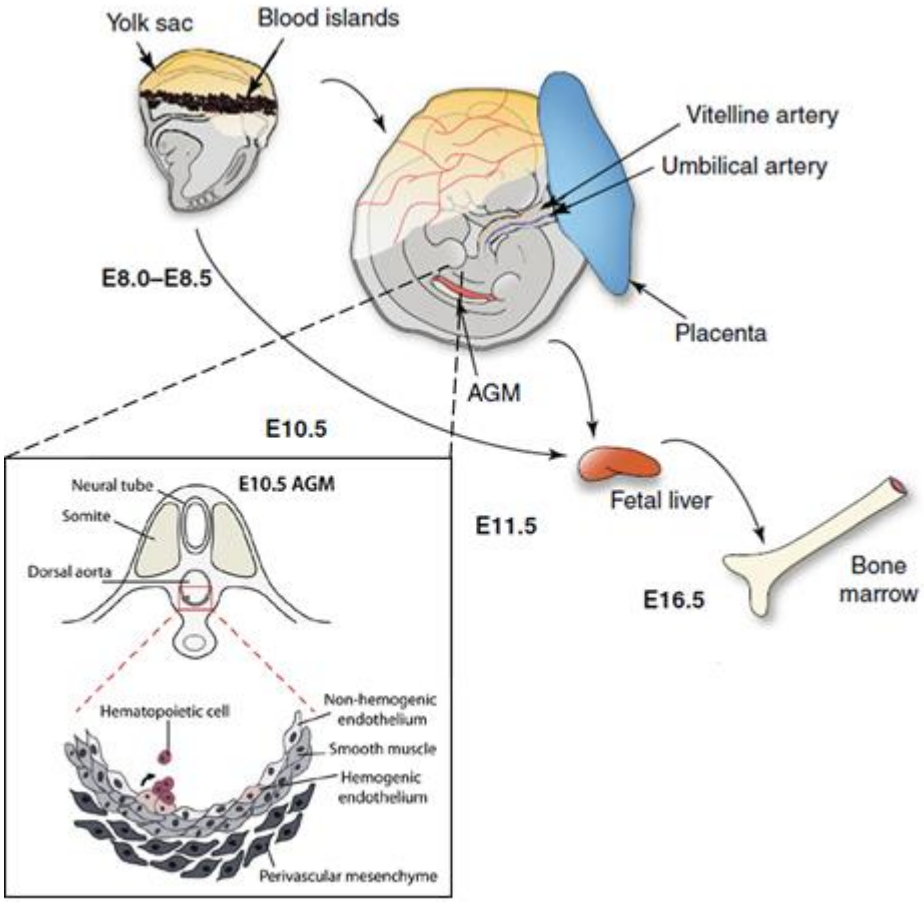

Figure 1.7 – Embryonic haematopoiesis occurs in distinct anatomical sites at different times

In murine embryos, the first haematopoietic progenitors are generated in blood islands in the YS at ~E8. At ~E10.5, specialised cells called the HE emerge in the dorsal aorta of the AGM region, as well as in the placenta and vitelline and umbilical arteries, which give rise to the HSCs. Once circulation is established, the HSCs and primitive cells from the YS colonise the foetal liver, where they are expanded. Finally, the HSCs move to the bone marrow before birth, which remains the main haematopoietic niche during adult life. Adapted from (Costa et al., 2012; Swiers et al., 2013). (Permission granted by Elsevier.)

1.4.2 Cellular origin of embryonic haematopoiesis

The blood islands in the YS are formed from an aggregation of mesoderm cells. As they develop, the central cells differentiate to embryonic haematopoietic cells, while the peripheral cells differentiate to endothelium to form vasculature. This close association in

the development of haematopoietic and endothelial cells led to the hypothesis that they arise from a common mesodermal precursor, named the haemangioblast (Sabin, 1920; Murray, 1932).

The first evidence for the haemangioblast came from in vitro studies involving differentiation of mouse ESCs to the haematopoietic lineage. ESCs can be differentiated to form 3D colonies called embryoid bodies (EBs), before culturing to form blast colonies containing both haematopoietic and endothelial precursors. Mixing studies (using two ESC lines with different resistance genes) showed both the haematopoietic and endothelial cells within the blast colony were derived from the same cell, suggesting a common precursor (Choi et al., 1998). Similar results were found using cells derived from the embryos of mice containing different markers, again supporting the existence of the haemangioblast (Huber et al., 2004). It was found to be a rare and transient population, present in a narrow and defined time window of development (Huber et al., 2004). The haemangioblast was confirmed as having a mesodermal origin and is identified by the expression of mesodermal marker Brachyury and Flk1 (VEGF receptor) (Fehling et al., 2003). Indeed, the origin of all blood cells in the mouse can be traced to a Flk1+ mesoderm progenitor (Lugus et al., 2009). However, there is still no in vivo evidence (e.g. based on lineage tracing) for the existence of the haemangioblast.

An endothelial origin of HSCs was proposed when clusters of haematopoietic precursor cells were found on the endothelial lining of the dorsal aorta (Jaffredo et al., 1998; Bertrand et al., 2005). Lineage tracing confirmed an endothelial precursor of HSCs (Jaffredo et al., 1998; Zovein et al., 2008), while the use of transgenic markers in the mouse embryo showed the first HSCs localised to the endothelial cell layer of the dorsal aorta wall (de Bruijn et al., 2002). This precursor was termed the haemogenic endothelium (HE).

Our understanding of the transition from endothelium to haematopoietic precursors has been enhanced by live imaging studies. Continuous long-term imaging at the single cell level was used to monitor differentiation of an in vitro ESC-derived mesodermal cell population to endothelial cells. Using morphological and molecular markers, the detachment of round haematopoietic precursors from tightly packed endothelial cells was visualised, with a concomitant gradual loss of endothelial markers and adhesion (Eilken et al., 2009).

Furthermore, high resolution imaging of live zebrafish embryos showed the emergence of HSCs directly from the endothelial floor of the dorsal aorta into the sub-aortic space to form clusters, before detaching into the blood stream. Specific cells in the endothelium (the HE) upregulate haematopoietic markers and transform into round haematopoietic cells before detaching. This endothelial-haematopoietic transition (EHT) occurs not by asymmetric cell division, but rather a bending and rounding of the cell before losing its cell contacts in the endothelial layer (Bertrand et al., 2005; Kissa & Herbomel, 2010). Imaging of the AGM region of dissected mouse embryos supported the process of an EHT and confirmed the haematopoietic cells in the intra-aortic clusters expressed markers consistent with long-term repopulating HSCs (Boisset et al., 2010).

In vitro ESC differentiation studies proved the existence of an HE intermediate in HSC development. Flk1-expressing haemangioblast cells were cultured to differentiate to blast colonies containing clusters of endothelial cells with haemogenic potential. The HE upregulated haematopoietic markers (e.g. CD41) and concomitantly downregulated endothelial markers (such as Tie2) as they proceeded through the EHT and committed to the haematopoietic lineage. This identified the HE as an intermediate in haematopoiesis:

the haemangioblast (Flk1+, Brachyury+) differentiates via the HE (Tie2+, Kit+) to generate haematopoietic progenitors (CD41+, Kit+, Tie2-) (Lancrin et al., 2009) (Figure 1.8).

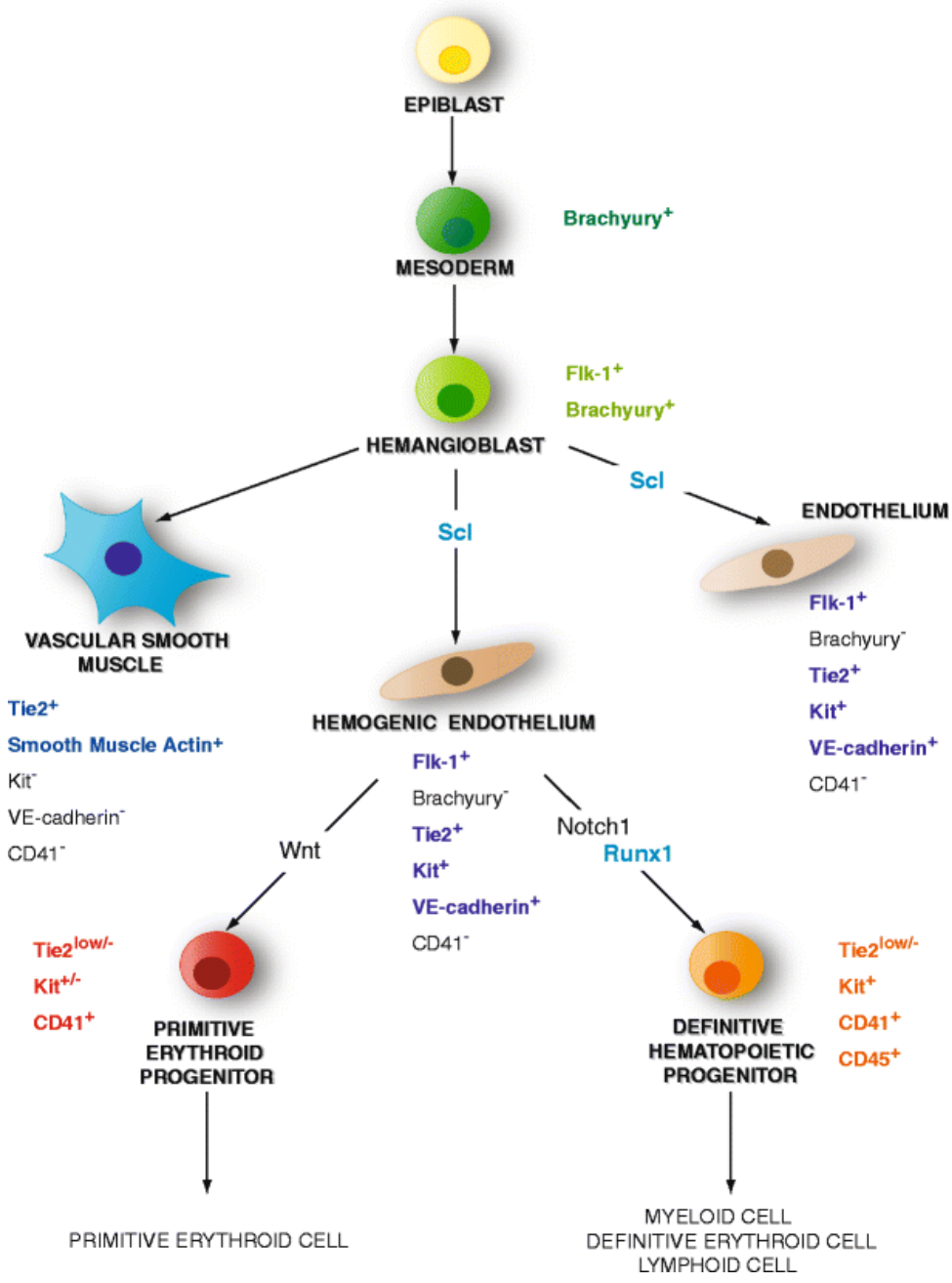

Figure 1.8 – Blood cell formation in embryogenesis
During embryo development, the haematopoietic system is formed by the differentiation of mesoderm through various transient cell types to generate HSCs and finally, the mature blood cells. Some of the key markers at each cell stage are indicated, as well as some of the significant factors (TFs and signalling molecules) that help determine the differentiation path. (Lancrin et al., 2010). (Permission to reproduce this figure has been granted by Springer Nature.)

1.4.3 In vitro ESC differentiation as a model of embryonic haematopoiesis

The first few divisions in embryo development give rise to identical daughter cells. During blastocyst formation, the cells begin to differentiate, with some cells forming the trophoectoderm and others forming the inner cell mass. It is the inner cell mass which will develop into the foetus, and so the cells of the inner cell mass are pluripotent – i.e. able to differentiate into all tissues of the adult body. These cells can be isolated from the embryo and cultured in vitro as ESCs (Evans & Kaufman, 1981). ESCs can self-renew when cultured in appropriate conditions (commonly grown on mouse embryonic fibroblasts [MEFs] in the presence of leukaemia inhibitory factor [LIF]) and have the capacity to differentiate into the three germ layers. The differentiation of ESCs in vitro can be driven to a variety of cell types using specific culture conditions, growth factors and cytokines. The differentiation conditions to generate haematopoietic cells have been well-established and this system has been extensively studied (Keller, 2005; Sroczynska et al., 2009; Garcia-Alegria et al., 2016).

Briefly, ESCs are differentiated to form EBs, before sorting the cells to isolate Flk1-expressing cells, containing haemangioblast cells. This cell population can be further differentiated in a blast culture containing BMP4, Activin A and VEGF to smooth muscle, endothelial and HE cells (Pearson et al., 2015). The HE undergo an EHT, with a gradual loss of the endothelial programme and concurrent upregulation of the haematopoietic programme, to generate round, floating haematopoietic progenitor cells (Lancrin et al., 2009). ESCs can also be plated into methylcellulose, in the presence of appropriate cytokines, to form colonies that differentiate to the various blood cell lineages, which can be quantified and identified (Kennedy et al., 1997).

Generally, the in vitro ESC differentiation system is thought to be an excellent model of embryonic haematopoiesis (Ottersbach et al., 2010). The generation of haematopoietic cells occurs through the same intermediate cell types (Fehling et al., 2003; Keller, 2005; Lancrin et al., 2009) and the temporal emergence of each of the blood lineages closely resembles the sequential emergence seen in vivo (Irion et al., 2010). Furthermore, the same network of TFs appear to drive each stage of haematopoietic specification, resulting in a similar gene expression profile in ESC-derived cells compared to those found in vivo (Keller et al., 1993; Moignard et al., 2013). However, it is important to note that HSCs are not generated in the ESC differentiation system; therefore, it can be considered an excellent model of YS embryonic haematopoiesis, but not of AGM haematopoiesis.

ESC haematopoietic differentiation therefore offers a quick and inexpensive model to study haematopoiesis. Previously, studies have been hampered by the sparsity of cells in the embryo. However, the ability to generate haematopoietic lineages in cultures provides access to early precursors, as well as to rare and transient cell populations (such as the haemangioblast, (Choi et al., 1998)) to analyse the molecular mechanisms controlling development. Advancing technology has also made genetic manipulation of ESCs relatively easy, enabling the manipulation of genes encoding specific TFs to elucidate their role in haematopoiesis (discussed further below) - particularly useful for those factors that are essential for development and can lead to embryonic lethality at an early stage.

1.4.4 Role of TFs in haematopoietic specification

Cell fate is determined by TFs and their interaction with epigenetic machinery to modulate the chromatin state, through the establishment of cell-type specific programmes of gene expression. During haematopoiesis, developmental stage-specific TFs become activated

in a temporal manner to regulate transcription of stage-specific genes. Together with the genes they regulate, TFs form a dynamic network, with successive activation of distinct TFs to drive differentiation along a particular lineage (Goode et al., 2016).

Many of the TFs that are essential for the specification of distinct stages of embryonic haematopoiesis have been identified, often using an in vitro model. For example, ETV2 is expressed in early mesoderm in a subset of Flk1+ cells that have endothelial/haematopoietic potential. Knockout of the gene in mouse embryos is lethal, as blood islands in the YS fail to develop, resulting in no primitive erythropoiesis or vasculogenesis (Lee et al., 2008b). However, conditional knockout at a later stage had little effect, suggesting ETV2 is no longer needed after Flk1+ cells have developed and therefore, there is a precise time-window in which ETV2 is required for haemangioblast formation (Kataoka et al., 2011; Wareing et al., 2012).

ETV2 activates the expression of a number of TFs required for the next stage of differentiation, such as SCL/TAL1, FLI1 and GATA2 (Wareing et al., 2012). Deletion of the gene encoding SCL/TAL1 in mouse embryos caused vascular defects and a failure to generate any haematopoietic cells (Robb et al., 1995). In vitro studies found that the haemangioblast was formed, but it was unable to generate endothelial and haematopoietic cells, instead forming clusters of smooth muscle cells (D'Souza et al., 2005). SCL/TAL1 was later confirmed to be required for HE development (Lancrin et al., 2009). Intriguingly, DNA-binding by SCL/TAL1 was found to be dispensable for establishment of haematopoietic and vascular programmes in ESCs and a zebrafish model (Porcher et al., 1999). Although SCL/TAL1 DNA-binding was required for the production of normal numbers of definitive haematopoietic cells and for maturation of erythrocyte and megakaryocyte precursors, these studies suggested that DBD-deficient SCL/TAL1 was highly active at early stages of haematopoiesis (Porcher et al., 1999;

Kassouf et al., 2010). It is possible that it is tethered to DNA in a transcriptional regulatory complex through its partner proteins (such as LMO2). These studies also indicate alternative mechanisms of gene expression regulation by transcription factors.

SCL/TAL1 is involved in regulating the expression of the TF RUNX1 in the HE. Knockout of *Runx1* in mouse embryos causes lethality at ~E12.5 with no haematopoietic clusters formed in the dorsal aorta (North et al., 1999). Knockout in ESCs revealed that RUNX1 is essential for the EHT (Lancrin et al., 2009) and in vivo studies showed RUNX1 is required for the formation of HSCs (Okuda et al., 1996; Chen et al., 2009; Lancrin et al., 2009). RUNX1 establishes a haematopoietic gene expression programme through the activation of haematopoietic genes and TFs, but it also orchestrates the down-regulation of the endothelial programme, for example by the activation of transcriptional repressors GFI1 and GFI1B (Lancrin et al., 2012). GFI1 and GFI1B act by binding to the regulatory regions of a large set of genes involved in cardiovascular and blood vessel development. They then recruit the histone demethylase LSD1, which is part of the coREST complex with histone deacetylases HDAC1 and HDAC2, thus epigenetically silencing the endothelial programme in the HE (Thambyrajah et al., 2016). This is an example of how TFs interact with epigenetic regulatory machinery to establish specific patterns of gene expression.

TFs can also play a role in priming the genes required later on in development. The inducible expression of RUNX1 in a *Runx1* knockout background revealed that SCL/TAL1, FLI1 and CEBPβ bind regulatory elements of haematopoietic genes, priming them for expression at later stages (Lichtinger et al., 2012). Upon RUNX1 activation in the HE, there is a re-organisation of TF complexes (including changes in the binding of SCL and FLI1), along with local changes in histone acetylation. Thus, RUNX1 establishes a

haematopoietic-specific programme of TF binding and gene expression (Lichtinger et al., 2012; Goode et al., 2016).

The importance of priming is highlighted in the differentiation to mature blood cells, which requires the expression of additional factors, such as PU.1 (coded for by *Spi1*) and CSF1R for myeloid specification. SCL/TAL1, FLI1 and CEBPβ bind and prime a *Spi1* enhancer, with mutation of these binding sites resulting in delayed expression of *Spi1* (Lichtinger et al., 2012). *Csf1r* expression, however, requires the activity of RUNX1, PU.1 and other PU.1-induced factors, meaning it is expressed later (Hoogenkamp et al., 2009).

A number of studies have examined cell-fate decisions at specific stages of embryonic haematopoiesis or during the differentiation of HSCs to mature blood cells. However, a recent study analysed the cell transitions across six consecutive developmental stages of haematopoiesis, from ESCs to macrophages (Goode et al., 2016). Goode et al. integrated global gene expression, chromatin accessibility, histone modification and TF binding data at each differentiation stage to investigate how the stage-specific gene expression programme is established. A dynamic regulatory network, involving the interplay of stage-specific TFs and the chromatin landscape, was proposed to describe haematopoietic specification. These data also provide a resource for future studies. For example, the analyses indicated the time course of TF binding and which factors were most important in controlling lineage specification. This data was then used to identify factors important in cellular reprogramming, while analysis of TF motifs at specific stages identified a novel role for a known TF in haematopoietic specification (Goode et al., 2016). The data can be applied in the study of other factors and their role in the haematopoietic pathway.

Ubiquitously-expressed TFs can also play a role in establishing tissue-specific programmes of gene expression. For example, TEAD4, a TF involved in Hippo-signalling, can bind to the DNA with tissue-specific TFs, such as SCL, and is essential for the generation of haematopoietic cells in vitro (Goode et al., 2016; Obier et al., 2016). Moreover, the ubiquitous TF Sp1 has a role in embryonic haematopoiesis. Deletion in ESCs causes a failure to terminally differentiate to mature blood cells, while conditional deletion at later stages had little effect, suggesting a role at early stages of haematopoietic specification (Marin et al., 1997; Gilmour et al., 2014).

This data highlights the interplay between TFs to ensure correct temporal expression of stage-specific genes to regulate gene expression at specific developmental stages. Highly dynamic transcription networks are established, involving tissue-specific and ubiquitous TFs, as well as their impact on the chromatin structure, to regulate developmental transitions and cell fate decisions.

1.5 Transcriptional regulation by Sp1 and the Sp family of TFs

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REVIEW

YJBM

The Role of the Ubiquitously Expressed Transcription Factor Sp1 in Tissue-specific Transcriptional Regulation and in Disease

Leigh O'Connor*, Jane Gilmour, Constanze Bonifer*

Institute of Cancer and Genomic Sciences, Institute of Biomedical Research, College of Medical and Dental Sciences, University of Birmingham, UK

Sp1 belongs to the 26 member strong Sp/KLF family of transcription factors. It is a paradigm for a ubiquitously expressed transcription factor and is involved in regulating the expression of genes associated with a wide range of cellular processes in mammalian cells. Sp1 can interact with a range of proteins, including other transcription factors, members of the transcription initiation complex and epigenetic regulators, enabling tight regulation of its target genes. In this review, we discuss the mechanisms involved in Sp1-mediated transcriptional regulation, as well as how a ubiquitous transcription factor can be involved in establishing a tissue-specific pattern of gene expression and mechanisms by which its activity may be regulated. We also consider the role of Sp1 in human diseases, such as cancer.

INTRODUCTION

Gene expression needs to be tightly regulated as the specific pattern of gene activation or repression is decisive for establishing fates. The gene expression program of a cell is controlled by the activities and the interactions of the epigenetic regulatory machinery and sequence-specific transcription factors. The epigenetic machinery consists of enzymes that post-translationally modify histone proteins, such as histone acetyltransferases (HATs†), histone deacetylases (HDACs), histone kinases and methyltransferases, as well as ATP-dependent chromatin remodeling complexes [1]. These factors regulate gene expression by altering the conformation of DNA and allowing access to key regulatory elements of transcription. Transcription factors bind to specific regulatory sequences in the DNA and regulate transcription of the associated gene by promoting recruitment of the transcription initiation machinery. Additionally, transcription factors are capable of directing histone modifying enzymes and chromatin remodeling complexes to specific sites, such as gene promoters, thus preparing the gene for transcription or, in the case of repressors, blocking it.

Transcription factors interact in a combinatorial fashion to uniquely regulate genes and, in response to different stimuli, regulate tissue-specific and developmental stage-specific gene expression. Many transcription factors are expressed in a tissue-specific manner and regulate the specialized functions of a particular cell; therefore elimination of these factors can result in a block in development/differentiation. For example, SCL/TAL1 is a crucial transcription factor in the hematopoietic system and the deletion of its gene in mice results in a failure to generate hematopoietic precursors and embryonic death [2]. Other transcription factors are ubiquitously expressed and are generally involved in the expression of ubiquitously expressed "housekeeping" genes in all cell types. However, they can also interact with tissue-specific proteins or be post-translationally modified in a tissue-specific manner to elicit a particular pattern of gene expression. Nuclear Factor I (NFI) family members are ubiquitously expressed and are involved in the regulation of constitutive genes and those that are controlled by hor-

*To whom all correspondence should be addressed: Leigh O'Connor, Tel: +44 121 4158683, email: lxo314@bham.ac.uk; Constanze Bonifer, Tel: +44 121 4148881, email: c.bonifer@bham.ac.uk.

†Abbreviations: AML, acute myeloid leukemia; BTD, Buttonhead domain; DNMT, DNA methyltransferase; EMSA, electrophoretic mobility shift assay; EMT, epithelial-mesenchymal transition; ER, estrogen receptor; ESC, embryonic stem cell; HAT, histone acetyltransferase; HCC, Hepatocellular carcinoma; HDAC, histone deacetylase; Htt, Huntingtin; KLF, Krüppel-like factor; MS, multiple sclerosis; NFI, Nuclear Factor I; Sp, Specificity protein; TAF, TBP-associated factors; TBP, TATA-binding protein; TK, Thymidine Kinase.

Keywords: Sp1 transcription factor, gene regulation, tissue specificity, human disease, epigenetics

monal, nutritional, and developmental signaling [3]. However, the knockout of individual NFI members results in tissue-specific defects. For example, NFI-A knockout mice have a defect in brain development (formation of the corpus callosum) but few other anatomical problems [4]. This suggests that the four NFI family members have overlapping roles and can compensate for the lack of NFI-A activity, but there are distinct functions in specific tissues, revealed by the defects, indicating ubiquitous transcription factors have important roles in development and tissue-specific gene expression. Conversely, knockout of the gene encoding OCT1, another ubiquitously expressed transcription factor, leads to developmental arrest at a very early embryonic stage [5], suggesting widespread roles. Although OCT1 is important in the expression of housekeeping genes, such as *H2B* [6], it also activates tissue-specific genes, often via interactions with cell-specific proteins [7-9]. The *IL3* locus is an example of such a target. T Cell Receptor signaling leads to activation of an inducible T cell-specific enhancer upstream of the *IL3* gene, containing NFAT and OCT1 binding sites. Studies have shown the two transcription factors interact at the enhancer to synergistically activate T cell-specific *IL3* expression [8]. Importantly, this system demonstrates how cooperation with a cell-specific protein can alter the binding or the activity of a ubiquitous transcription factor to bring about tissue-specific gene expression.

Sp1 is a transcription factor that has been found to be present in all mammalian cell types [10]. Thus, it was long thought to be solely a regulator of housekeeping genes and indeed, knockout of Sp1 in mice causes embryonic lethality at an early stage of development (around day 10.5 of gestation) with a broad range of phenotypic abnormalities, suggesting a general function in many cell types [11]. However, Sp1 is now also known to be involved in the regulation of tissue-specific, cell cycle, and signaling pathway response genes [12], with chromosome mapping studies estimating there are at least 12,000 Sp1 binding sites in the human genome, associated with genes involved in most cellular processes [13]. Furthermore, its expression levels were seen to vary in different cell types and through different stages of mouse development [14], and it is required for the transcriptional activation of Hsp70.1, one of the first genes expressed after fertilization in mouse embryos [15], highlighting Sp1's importance in development. It has also been shown to play a role in numerous human diseases, including cancer. Therefore, it is important to fully understand Sp1's mode of action and contribution to gene regulation.

THE SP/KLF FAMILY

Sp1 was the first mammalian transcription factor to be cloned and characterized, named originally according to the purification procedure used (Sephacryl and phosphocellulose columns), but now more commonly named Specificity protein 1 [16,17]. When whole cell extracts were prepared from HeLa cells to study the factors required for transcription initiation *in vitro*, Dynan and Tjian identified that one of these factors, Sp1, showed sequence specificity and was able to bind to the SV40 early promoter and activate transcription of the gene [18].

Sp1 is the founding member of the Specificity protein/Krüppel-like factor (Sp/KLF) family of transcription factors, which currently has a total of 26 members [19]. The family is characterized by the highly conserved DNA binding domain (sequence identity > 65 percent) near to the C-terminus of all members, which recognize GC (consensus sequence: GGGGCGGGG), as well as GT/CACC (GGTGTGGGG) boxes [16,20-22]. The DNA binding domain is made up of three adjacent Cys2His2-type zinc fingers consisting of exactly 81 amino acids in every protein [17]. Not only are the amino acids within the individual zinc finger structures conserved, but there are also constraints on the residues in the interfinger regions, with the conserved linker sequence T/S-G-Q-R/K-P, suggesting the zinc fingers act as a single unit [23]. The residues that are directly in contact with the DNA, and therefore providing the specific base recognition, are the most conserved parts of the protein. In Sp1, these residues were identified as KHA in the first zinc finger, RER in the second and RHK in the third, although there are slight changes in some of the other family members that correspond with differing preference to GT boxes rather than GC boxes, or differing binding affinities [16,24].

The Sp/KLF family is split into two groups based on the structure at the N-terminus: Sp-like transcription factors (Sp1-9) (Figure 1) and the KLF-like transcription factors, named from the Cys2His2 zinc finger Krüppel protein in *Drosophila*. In general, the Sp-like family recognize GC boxes in preference to GT boxes, while the reverse is found for the KLF-like family, which comprises both transcriptional activator and repressor proteins. In terms of structure, the nine Sp-like members are distinguished from the KLF-like proteins by the presence of a conserved Buttonhead domain (BTD, first identified in the *Drosophila* Sp1 homologue Buttonhead) N-terminal to the DNA binding domain [23,25]. Its function is debated, but studies suggest it is involved in the transactivation or synergistic activities of the Sp proteins [26,27]. Another feature in most Sp-like proteins is a conserved stretch of amino acids at the N-terminus of the protein with the sequence SPLALLAATCSR/KI, termed the Sp box [23]. Again, the precise function of this motif is unknown, but as it contains an endoproteolytic cleavage site and is located close to the region in Sp1 that targets proteasome-dependent cleavage [28], one theory is that it may have a function in regulation of protein degradation.

The Sp-like protein family can be further subdivided into Sp1-4 and Sp5-9, with Sp1-4 being distinguishable by the presence of N-terminal glutamine-rich transcriptional activation domains. Overall, Sp1-4 have a very sim-

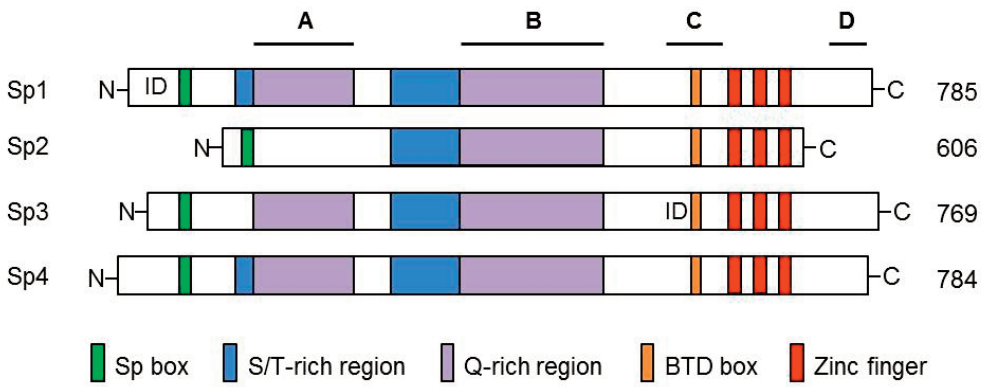

Figure 1. Primary structure of the Sp-like transcription factors.
Conserved domains of the Sp-like transcription factors are illustrated. Two glutamine (Q)-rich domains (A and B) form the transactivation domains, while the inhibitory domains (ID) present in Sp1 and Sp3 are also indicated. Three zinc fingers at the C-terminus comprise the DNA binding domain and domain C contains a highly charged region adjacent to the DNA binding domain. Domain D in Sp1 enables synergistic binding with other Sp1 proteins. The conserved sequence motifs, BTD and Sp boxes, are also shown and the length of the protein in amino acids is indicated on the right.

ilar modular domain structure with Sp1, Sp3 and Sp4 being more closely related in structure and activity than Sp2. The former proteins have a highly conserved DNA binding domain sequence and bind to GC boxes (and to a lesser extent, GT boxes) with similar affinities. Sp2, however, preferentially binds GT boxes due to changes from the consensus zinc finger DNA-binding residues, with a leucine substituted for the conserved histidine residue in the first zinc finger [21,29]. Sp1, Sp3, and Sp4 each contain two glutamine-rich transactivation domains, termed A and B, located near to a serine/threonine-rich sequence, which is the target of many posttranslational modifications. Sp2, on the other hand, only contains one glutamine-rich domain, but they share a highly charged region adjacent to the DNA binding domain (domain C) [21].

Both Sp1 and Sp3 are ubiquitously expressed in mammalian cells. They have highly similar structures, with their DNA binding domains sharing over 90 percent DNA sequence homology, meaning that the two transcription factors recognize the same DNA sequence element and bind with similar affinity [21,30]. However, while knockout of Sp1 in mice causes death at around day 10.5 of gestation [11], Sp3 knockout mice die postnatal, apparently of respiratory failure [31,32]. In addition, mice heterozygous for either transcription factor appear normal, albeit slightly smaller, but being heterozygous for both proteins leads to embryonic lethality with a diverse range of phenotypes [32]. This suggests that, despite having very similar DNA specificity and affinity, the two transcription factors perform distinct functions in the cell. In support, high resolution fluorescent microscopy studying immunolocalization of Sp1 and Sp3 in the MCF-7 cell line revealed that both proteins were concentrated in discrete regions of the nucleus and are part of different promoter complexes [33]. However, the expression of many Sp target genes in *Sp1*$^{-/-}$ mice was found to be unaffected, suggesting that Sp3 may be able to compensate, in part, for loss of Sp1-mediated transcription and thus there is potential redundancy between Sp1 and Sp3 functions [11].

The biggest differences between the structures of Sp1 and Sp3 are a) the presence of a domain D at the C-terminal end of Sp1 only (important for synergy and multimerization, see below) [34] and b) the position of the inhibitory domain, which suppresses the transcription activation potential. In Sp1, the inhibitory domain is located at the N-terminus and acts by interacting with co-repressor molecules [35], while in Sp3, it is positioned just N-terminal to the three zinc fingers [36]. Transfection of Sp3 into *Drosophila* SL2 cells showed that it could only activate a portion of Sp target promoters and could not activate reporter gene constructs [37]. Mutation analysis identified the inhibitory domain, which worked to silence the two transactivation domains, and highlighted the importance of a highly charged amino acid motif 'KEE' for inhibitory behavior. Mutation of the KEE motif to alanine residues converted Sp3 into a strong transcriptional activator, identifying a means by which Sp3 activity could be regulated [36]. It is theorized that these structural differences are responsible for the functional differences of Sp1 and Sp3 [29,31].

The functions of other Sp proteins have also been studied. Expression of Sp2 is detectable in embryonic stem cells (ESCs) and in all tissues during embryogenesis (except the heart). It was initially found to be unable to stimulate transcription from promoters that are activated by other Sp members [38], but knockout of Sp2 in mice led to embryonic death at day 9.5, showing it is an essential transcription factor in mouse development [39]. Sp4 knockout mice show no obvious abnormalities, but two thirds die within their first month and the surviving mice

are smaller in size with abnormal reproductive organs [40,41]. Overall, Sp4 knockout studies suggest that the transcription factor is required for specification of the cardiac conduction system [42] and normal brain development [43]. Knockout of members of the KLF family have also been found to be embryonic lethal (KLF1, 2, 5 and 6), with the others displaying abnormalities in a range of tissues [44]. These deletion studies show the importance of the Sp/KLF transcription factors, while the variety of phenotypes shows that despite their structural similarity, they have distinct functions.

SP1-MEDIATED TRANSCRIPTION REGULATION

The Sp-like family of transcription factors generally function to activate transcription, whereas the KLF-like subgroup contains both activators and repressors of gene expression. Sp1 functions by recruiting the basal transcription machinery and, specifically, interacting with members of the TFiiD complex. TFiiD is composed of the TATA-binding protein (TBP) and multiple TBP-associated factors (TAFs). It is the first component of the transcription machinery to bind to the promoter, which then triggers formation of the pre-initiation complex. These interactions are known to be regulated by various transcription activators [45]. An *in vitro* transcription system first revealed that TFiiD was necessary and sufficient for Sp1 mediated transcription [46]. Characterization of the components of the TFiiD complex showed that the *Drosophila* protein dTAFii110 (the human homologue is hTAFii130) contained both glutamine- and serine/threonine-rich domains, similar to that of Sp1, leading to the hypothesis that the two proteins were functionally linked. Indeed, a yeast two hybrid assay demonstrated interactions between the transactivation domains of Sp1 and the N-terminus of dTAFii110, and transcription activation by Sp1 was increased with the addition of the dTAFii110 N-terminal fragment [46]. Deletion mutants revealed that each Sp1 transactivation domain interacts with distinct regions of hTAFii130. Of the four glutamine-rich domains (Q1 to Q4) within hTAFii130, the interaction with transactivation domain B of Sp1 was only disrupted upon deletion of Q1, whereas transactivation domain A made multiple contacts to hTAFii130 at Q2, Q3 and Q4 [47]. Interestingly, it was found that it was the hydrophobic residues within the transactivation domains that were important for the binding to dTAFii110 and subsequent transcription activation, whereas mutation of the glutamine residues had no effect [48]. These studies were then followed by the discovery that Sp1 also binds to TBP with the interaction occurring via the Sp1 glutamine-rich transactivation domains and the conserved C-terminus of TBP. There was a correlation between the extent of binding and the level of transcription *in vitro*, but TBP alone was not sufficient to activate Sp1-mediated transcription [45]. Therefore, Sp1 regulates transcription by communication with the RNA polymerase II transcription machinery.

Sp1 also regulates gene expression by affecting the chromatin state. It has been found to interact with histone modifying enzymes, including the HAT p300 [1,49]. The interaction between the DNA binding domain of Sp1 and the acetyltransferase catalytic domain of p300 leads to increased binding of Sp1 to DNA. Despite the DNA binding domain of Sp1 being acetylated by p300 during the interaction, there was little effect on DNA binding under varying acetylation conditions, suggesting that the increase in DNA binding was due to the direct interaction of the two proteins [49]. Thus, gene expression can be promoted both by changing the chromatin modifications at the promoter towards a more permissive structure and by the binding of a transcription activator, in this case Sp1. DNA binding of Sp1 then causes a release of p300, allowing it to regulate expression at further genes [49]. An example of cooperation between Sp1 and p300 is found during neuronal differentiation (in response to nerve growth factor signaling) when they activate the *p21* promoter to bring about withdrawal of the progenitor neural cells from the cell cycle [50].

Sp1 can also interact with negative epigenetic modifiers to cause down-regulation of gene expression [12,33]. Trichostatin A, an HDAC inhibitor molecule, was shown to lead to activation of the Thymidine Kinase (*TK*) gene. This gene is a target of Sp1 and indeed, co-immunoprecipitation experiments showed HDAC1 and Sp1 to be part of the same complex with interactions occurring via the C-terminal DNA binding domain of Sp1. The presence of Sp1 was also required for HDAC1-mediated *TK* repression, showing that Sp1 is involved in HDAC-mediated transcription inhibition [51]. HDAC1 was similarly found to be associated with Sp1 at the *p21* promoter in proliferating cells, so regulating cell cycle progression [52]. In addition, the DNA methyltransferase DNMT1 was found to bind to Sp1 and elicit repression of some Sp1 target genes, such as *MAZ* [53]. DNMT1 bound to seven consensus amino acids in the N-terminus of Sp1 and, at the Survivin promoter, were found to act together to inhibit gene expression (in response to p53 signaling and cell stress). In addition, Sp1 gradually recruited other transcriptional repressors, such as HDAC1 [54], to control gene expression in response to changing cell conditions. In contrast, Sp1 is involved in the maintenance of a methylation free state at the CpG islands in target gene promoters, for example at the *APRT* gene [55,56]. The methylation free CpG islands on *APRT* corresponded to three GC boxes, which footprinting revealed were bound by Sp1. The promoter region became methylated upon deletion or mutagenesis of the Sp1 binding sites, suggesting that Sp1 sites are required for the maintenance of CpG islands and the activation of gene expression [56]. Similar interactions with epigenetic regulators have also been reported for Sp3 [57-59].

Sp1 is capable of synergistically activating transcription [60]. Early studies involving co-transfection of an Sp1 expression vector and reporter constructs in *Drosophila* SL2 cells showed that while one Sp binding site gave modest activation of the reporter gene, two sites produced 78-fold greater transcriptional activation [34]. Similar experiments also demonstrated the ability of Sp1 to activate transcription from both proximal and distal sites (using reporter constructs containing sites near the transcription start site or 1.7 kb away), with the presence of both sites eliciting efficient and strong activation of transcription [61]. Electron microscopy revealed that this synergistic activation between proximal and distal sites was achieved through looping of the intervening DNA to allow Sp1 protein interactions [62]. The ability of Sp1 to regulate looping of DNA between enhancers and promoters was more recently confirmed using chromosome conformation capture assays [63]. Furthermore, crosslinking showed that there were interactions between individual Sp1 proteins, with dimers, trimers and tetramers forming both in solution and bound to DNA [34,61]. Additional electron microscopy studies imaged Sp1 as initially forming a tetramer at the promoter site. Upon DNA looping it assembled into multiple tetramers with those at the distal element at the DNA loop junction [64]. This suggests that transcriptional synergy occurs through interaction of Sp1 monomers to form multimer complexes at regulatory elements. Using EMSA and titration of Sp1 protein, it was demonstrated that Sp1 bound initially as a monomer until most of the free DNA template was occupied, followed by a second Sp1 molecule with increasing protein concentration [34]. This shows that the increase in transcription activation is not due to cooperative binding between Sp1 molecules, but rather to synergism, i.e. they do not affect each other's DNA binding affinity, but together can activate transcription to a greater extent than the sum of each alone.

Deletion analysis of the distinct domains of Sp1 revealed that, in addition to the transactivation domains A and B, the C-terminal domain D is required for multimer formation and synergistic transactivation [34]. The domain D deficient Sp1 mutant was able to activate transcription with equal efficiency to wildtype at promoters with a single GC site, but there was a decreased transactivation at promoters with multiple binding sites [61]. For example, the *p21* promoter contains 6 GC boxes and a deletion of domain D in Sp1 gave just a 12-fold increase in transcription of *p21*, in contrast to a 47-fold increase with wildtype Sp1 [65], indicating domain D is required for synergy. Further analysis of the Sp1 domain structure reported that a form of Sp1 unable to bind DNA (missing the zinc finger domain) had no transcriptional activity when expressed alone in SL2 cells, but could interact with a DNA-bound wild type Sp1 protein and significantly enhance transcription, showing superactivation of Sp1 mediated transcription [34,61].

As most members of the Sp/KLF family have similar DNA sequence specificity, yet varying transcriptional stimulation activities, the relative levels of expression of each member in the cell can influence the gene expression pattern [66]. The ratio of Sp1 and Sp3 levels in the cell is particularly important due to their highly similar, indeed almost identical, DNA binding specificity and affinity [21,37]. The relevance of the ratio of the two proteins was highlighted in primary keratinocytes, in which Sp3 levels exceed those of Sp1. However, upon differentiation of the cells *in vitro*, Sp1 levels increase and the Sp3/Sp1 ratio is inverted, suggesting that Sp1 and Sp3 are differentially involved in the regulation of transcription of some cell type-specific genes [29,67]. Sp1 and Sp3 can both cooperate to synergistically activate transcription, such as at the tumor suppressor gene *RASSF1A* [68] and transactivate genes independently, e.g. the gene encoding prostate-specific antigen [68]. However, this is still a contentious issue, as the theory that Sp1 and Sp3 cooperate to regulate transcription is contradictory to findings that Sp1 and Sp3 are present in distinct transcription complexes [33].

Early studies into Sp3 activity reported that Sp3 was not able to initiate expression of several genes with different Sp site-containing promoter elements in *Drosophila* SL2 cells [37]. In fact, Sp3 could repress Sp1 mediated transcription in this system. The repression was dependent on the DNA binding domain, suggesting Sp3 functioned through competition with Sp1 at Sp recognition sites [37]. In light of such studies, it was first thought that Sp3 functioned as a transcriptional repressor molecule. However, co-transfection of Sp1 and Sp3 expression vectors with a number of different Sp1 target genes revealed that only the promoters consisting of multiple GC/GT boxes were subject to Sp3 mediated repression of Sp1 transcription [70]. The ability of Sp3 to repress transcription was found to be due to both the C terminal inhibitory domain (as discussed above) [36] and that Sp3 cannot transactivate synergistically at two or more Sp binding sites [30,71]. Despite Sp3 binding to DNA as a monomer, it can form highly stable complexes with those proteins at nearby Sp recognition sites, which are slower to dissociate than either monomeric Sp3-DNA or multimeric Sp1-DNA complexes. This means Sp3 can outcompete Sp1 for binding at promoters consisting of multiple Sp sites. Moreover, Sp3's inhibitory domain and lack of a domain D like Sp1 means that Sp3 cannot synergistically activate transcription. Thus, when Sp3 displaces the stronger transactivator Sp1 at a regulatory element, there is a net repression of Sp1-mediated transcription [30,60]. This is exemplified by co-transfection studies in *Drosophila*: Sp1 can give about 100 fold increase in *BCAT2* expression, whose promoter has multiple GC boxes, whereas there is only a slight increase with Sp3 [30]. Therefore, the differential expression of different transcription factors and their interplay is important for determining the specific gene expression pattern of a cell.

SP1 REGULATION

While Sp1 is active in all cell types and conditions, it is also tightly regulated enabling Sp1 activity to alter in response to signaling pathways and changing cellular conditions, giving differential expression of inducible and cell cycle/growth genes (including many tumor suppressor genes and oncogenes). One such mechanism is through interactions with other proteins. Transcription factors interact to generate unique patterns of gene expression, meaning the cell can function with a relatively small number of transcription factor proteins. Sp1 has a variety of binding partners dependent on the cell conditions and extracellular signals, which regulate Sp1-dependent transcription [60,72]. Some proteins can bind and enhance Sp1 activity. Oct1, for example, was found to interact with domain B and the adjacent serine/threonine-rich region of Sp1 and increase its DNA binding affinity by cooperatively binding to the distal regulatory element of the U2 snRNA gene to increase transcription [73]. Other proteins can bind to Sp1 and activate transcription synergistically, such as estrogen receptor (ER) proteins. ER binding to Sp1 increases Sp1-DNA binding to estrogen responsive elements independently of estrogen, but the transactivation of the gene is only enhanced in the presence of estrogen [74], illustrating how Sp1-mediated transcription can be altered to respond to signaling pathways. Alternatively, transcription factors can superactivate Sp1-dependent transcription by interacting with DNA-bound Sp1, but not binding to DNA directly, for example AP2, first shown using GAL4 transactivation assays [75].

There are also examples of protein-protein interactions mediating negative effects on Sp1 activity. The cell cycle regulator p53 can bind to Sp1 and interfere with its binding to the *hTERT* promoter (encoding the human telomerase reverse transcriptase gene), thus preventing expression and contributing to tumor suppression [76]. Alternatively, p53 can inhibit expression of the cyclin B1 gene without interfering with Sp1 binding: the inhibition of cyclin B1 was dependent on the Sp1 binding sites and an Sp1/p53 complex was identified at the promoter. However, p53 did not bind the DNA directly, suggesting the inhibition was not through competition/preventing DNA binding, but possibly by disruption of the recruitment of transcription machinery [77]. Conversely, p53 can interact with Sp1 to positively regulate transcription at the *p21* promoter. In proliferating cells, the *p21* promoter is inhibited by HDAC1 binding to Sp1 at the promoter, but upon cell stress and p53 induction, p53 displaces HDAC1 from Sp1 to activate *p21* transcription and halt the cell cycle [52,78].

Sp1 is also highly post-translationally modified, altering Sp1 activity and enabling specific responses to a range of signals. One of the most well studied post-translational modifications of Sp1 is phosphorylation. There are thought to be 23 putative phosphorylation sites in Sp1 and various kinases have been identified, resulting in diverse functional effects [79,80]. Most kinases affect the DNA binding of Sp1, for example, Cyclin B1-Cdk phosphorylates Sp1 at T739 at the C-terminus during mitosis, causing reduced Sp1 DNA binding and facilitating chromatin condensation [81]. In contrast, phosphorylation of S59 at the Sp1 N-terminus by cyclin A-Cdk leads to increased DNA binding and transcription [82]. It can also impact on Sp1 protein stability. During mitosis, JNK phosphorylates T278 and T739, however, with kinase inhibitors to target JNK, Sp1 becomes ubiquitinated and proteasomally degraded [83]. The phosphorylation at T739 was shown to prevent binding to the E3 ubiquitin ligase, thus shielding Sp1 from degradation during mitosis and maintaining levels for cell cycle progression [84].

In addition, Sp1 can be acetylated at K703 in the DNA binding domain, which is linked to its interactions with HAT and HDAC epigenetic regulators. As described above, p300 increases Sp1 binding to DNA, albeit independently of acetylation of Sp1 [49], but acetylation at K703 by p300 reduces their interaction and so decreases the Sp1 transcriptional activity [85]. Sp1 can also be deacetylated by HDAC1, which increases its binding to promoters important for cell cycle and cell death, such as *p21* and *Bak* [86]. Furthermore, Sp1 undergoes various other post-translational modifications, including glycosylation [87,88], poly(ADP-ribosyl)ation [89], methylation [90] and sumoylation [91], which, along with phosphorylation and acetylation modifications, are extensively reviewed in Chu, 2012 [92] and Chang and Hung, 2012 [79].

More recently, several miRNAs have been identified which can post-transcriptionally modulate Sp1 expression, thus providing an additional level of regulation. Examples of such miRNAs are discussed below and reviewed in Safe, 2015 [93]. Together, these different aspects of regulation allow a ubiquitous factor, such as Sp1, to carry out diverse functions in a wide range of cell types.

TISSUE-SPECIFIC ROLES OF SP1

A common theme in gene regulation is the cooperation of ubiquitous transcription factors (including Sp1) with tissue/development stage-specific transcription factors. Much of the research into this mechanism has been performed in the hematopoietic system, one of the most widely studied differentiation systems. Indeed, levels of Sp1 were shown to be high in hematopoietic cells in the mouse embryo [14] and Sp binding sites were identified at many hematopoietic genes [94]. To trigger activation of specific gene programs at certain developmental stages or tissues, Sp1 could either be modified to increase binding/transactivation at a specific site, or Sp1 could bind at the site in only that cell type [95]. These two mechanisms are evident in myeloid differentiation. Firstly, Sp1 undergoes post-translational modification during myeloid spec-

ification. The phosphorylated form of Sp1 increases in myeloid progenitors. This causes increased Sp1 binding to its target site in the promoter for the CD14 cell surface protein, thus giving monocyte-specific promoter activity [96]. Secondly, epigenetic changes could alter the availability of the binding site. The myeloid transcription factor Pu.1 binds close to the Sp1 site at the integrin *CD11b* promoter. Pu.1 binding exposes the binding site for Sp1 in the chromatin, allowing it to bind and regulate transcription in a tissue-specific manner [97].

In addition, Sp1 can interact with tissue-specific transcription factors to generate tissue-specific gene expression programs. In erythroid cells, Sp1 can cooperate synergistically with the transcription factor GATA1 at erythroid-specific promoters. Sp1 and GATA1 binding sites can be seen in close proximity at many promoters and enhancers of erythroid-specific genes. The two proteins physically interact at the DNA binding domains to synergistically activate transcription, for example at the *EpoR* promoter [98], the *Tal1* promoter [99] and the *ALAS2* gene (required for heme synthesis), where they also recruit the activator p300 [100]. Sp1/GATA1 complexes have also been identified at promoters without GATA sites, suggesting Sp1 can recruit tissue-specific transcription factors to particular regulatory elements [98]. Furthermore, Sp1 and GATA1 could interact from a distance in reporter constructs modelling the architecture of globin locus control regions, suggesting the two proteins can interact to stabilize loops between regulatory regions and synergistically activate the globin gene [98]. SCL/TAL1 is an important regulator of hematopoietic specification. It forms a complex with many other proteins (e.g. LMO2, Ldb1, E2A), which has been reported to enhance *Kit* expression, encoding a receptor needed in hematopoiesis. The complex is tethered to the promoter by Sp1, with the interaction between the cell type-specific factors and a ubiquitous transcription factor determining the gene expression profile and cell fate [94]. These studies also demonstrate that Sp1 can recruit SCL and GATA1, plus other restricted transcription factors, to specific promoters, but not to all, indicating that the promoter architecture is also important in regulation of tissue-specific genes, likely to position the proteins to enable functional interactions [94].

Despite Sp1 knockout causing embryonic lethality in mice, Sp1-deficient (with a knockout of the DNA binding domain) ESCs could grow normally in culture [11]. During *in vitro* differentiation to mimic embryonic hematopoiesis in culture, Sp1-deficient embryonic stem cells could proceed through most stages of blood cell development, but Sp1 was required for terminal differentiation. Gene expression analysis of purified cells representing successive stages of hematopoietic specification revealed a progressive deregulation of gene expression: most Sp1 target genes were unaffected, but Cdx and some Hox genes were downregulated at an early stage, and the number of affected genes increased through later stages as a result, causing a failure in terminal hematopoietic differentiation. Interestingly, the deletion of Sp1 at later developmental stages, in this case at the myeloid progenitor stage had no effect, indicating that the defects in the Sp1 knockout mice were cumulative [101].

Additional tissue- and developmental-specific roles have been discovered for Sp1 and other Sp factors, including in the nervous system. The *NR1* gene encodes an essential component of the *N*-methyl-D-aspartate receptor, which is important for neuronal differentiation. Sp factors bind to and activate an NFκB site in the promoter to upregulate *NR1* expression: specifically Sp3 during neuronal differentiation and Sp1 in differentiated neuronal cells [102]. Sp1, Sp3 and Sp4 interact to activate neuronal-specific transcription of cyclin-dependent kinase 5/p35, which is critical for brain function [103]. However, Sp3 and Sp4 together repress expression of superoxide dismutase 2 in neurons, but the substitution of Sp4 for Sp1 in astroglia causes upregulation of transcription [104]. Moreover, Sp1 and Sp3 mediate expression of cyclooxygenase-2 in response to oxidative stress in neurons to aid in neuronal survival [105]. This highlights the importance of the balance between the levels and activities of related Sp transcription factors in the function of the nervous system, particularly the tissue-specific Sp4 and ubiquitous Sp1 and Sp3.

These examples demonstrate the ability of ubiquitously expressed factors to contribute to lineage specific regulatory programs and highlights important general principles in developmentally controlled gene regulation.

SP1 IN DISEASE

Given the role of Sp1 in a multitude of cellular pathways and processes, it is unsurprising that it is associated with the pathogenesis of a number of diseases, with perhaps the best studied being cancer. Sp1 overexpression is seen in a host of cancer cell types, where levels of Sp1 also correlate with tumor stage and a poor prognosis [12]. Knockdown of Sp1 in cancer cell lines (including breast, kidney, pancreatic, lung, and colon cancers) led to decreased survival and the inhibition of cell growth and migration. Similarly, tumor formation and metastasis was reduced in mouse xenograft models with Sp1 knockdown. Furthermore, the changes in gene expression following knockdown correlated with the observed phenotypic changes of the cells [106]. Indeed, several anticancer agents in clinical use act by inhibiting Sp1 action [107]. Mithramycin A (and its analogues) can alter the binding of Sp1 to DNA and downregulate Sp1-mediated transcription [108,109]. Tolfenomic acid increases the ubiquitination and degradation of Sp1 [110], while anthracyclines, one of the most effective anticancer treatments, bind DNA at GC-rich sequences, preventing Sp1 binding [111,112], though this may not be its sole mechanism of action. Other

drugs can act on Sp1 indirectly, such as curcumin, which increases reactive oxygen species in the cell, causing activation of ZBTB4/10 proteins that displace Sp1 from GC-rich sites and decreased Sp1 expression [113].

The role of Sp1 in cancer stems from its regulation of genes that are involved in all of the hallmarks of cancer: growth factor-independent proliferation, immortality, evasion of apoptosis, angiogenesis, tissue invasion and metastasis [72,114]. Sp1 is involved in the regulation of genes required for the progression of the cell cycle and entry into S-phase, such as cyclins and *MYC*, as well as in growth factor signaling pathways e.g. *IGF1R* has up to eight Sp sites at its promoter and IGF signaling is commonly used by cancer cells to maintain proliferation [115,116]. However, it also regulates the transcription of cell cycle inhibitor genes, for example, synergizing with p53 under conditions of cell stress to activate transcription of *p21* [65]. Sp1 regulates the expression of telomerase subunits involved in the maintenance of telomeres and cell immortality. It can bind to five Sp sites present at the *hTERT* promoter to activate gene expression [117], or conversely, interact with HDACs to repress *hTERT* expression [57]. Sp1 is involved in the control of both pro- and anti-apoptotic factors, which have a direct role in cancer development. Survivin is a protein that promotes cell survival by inhibiting apoptosis and is essential in many tumors: its overexpression is directly associated with an increase in levels of Sp1 [118]. The pro-angiogenic factor VEGF has Sp1 binding sites at its promoter: estrogen signaling in breast cancer can result in interaction of Sp1 with ERα and subsequent upregulation of the *VEGF* gene [119]. Sp1 is also involved in maintaining genome stability via regulation of DNA damage factors and inflammatory signaling to drive oncogenesis [72].

While the deregulation of signaling pathways and transcription factor networks has been well studied, the impact of aberrantly expressed miRNAs in cancer is a newly developing field. Specific miRNAs have been found to be downregulated in certain cancers, such as miRNA223 in gastric cancer [120]. In this example, Sp1 protein levels were also found to increase, but with no change in mRNA levels, suggesting post-transcriptional regulation. miRNA223 was found to bind to the 3' untranslated region of Sp1 mRNA and inhibit its translation. The increase in Sp1 led to enhanced epithelial-mesenchymal transition (EMT), involved in promoting cell migration and invasion in tumorigenesis, whereas overexpression of miRNA-223 in a gastric cancer model caused decreased EMT and proliferation, and induced apoptosis [120]. A similar action was discovered for miRNA-324-5p in Hepatocellular carcinoma (HCC) [121] and miRNA-23b in multiple myeloma [122]. Additionally, Sp1 has been found to regulate the expression of miRNAs. miRNA-195 promotes cell apoptosis and suppresses cancer cell proliferation/metastasis; its expression is frequently reduced in various cancers. Characterization of its promoter region found an Sp1 site required for *miRNA-195* expression, but in HCC cells, Sp1 interacted with HDAC3 at the promoter to repress transcription [123]. The miRNA-23a-27a-24-2 cluster is deregulated in many cancers. The promoter, containing 2 Sp1 sites, was found to be demethylated in Hep2 cells, compared to control HEK293 cells, leading to upregulation of the cluster and promotion of proliferation and cell survival of cancer cells [124]. Furthermore, Sp1 was found to be involved in a regulatory network with another transcription factor (NFκB), an epigenetic regulator (HDAC) and a miRNA (miRNA-29b) to modulate *KIT* expression in a subset of acute myeloid leukemia (AML) [125]. miRNA-29b acts to post-transcriptionally inhibit Sp1. Conversely, Sp1, along with NFκB, binds to the *miRNA-29b* enhancer and interacts with HDAC1/3 to form a repressive complex and inhibit *miRNA-29b* expression. Aberrant activation of *KIT* in AML cells leads to upregulation of *MYC*, which in turn results in downregulation of *miRNA-29b* and an increase in Sp1 expression. Sp1, along with NFκB, activates *KIT* transcription, thus completing the regulatory loop and contributing to the disease state [125].

Sp1 can be linked to the changes in DNA methylation often observed in cancer cells. Sp1 can be involved in the protection of regulatory regions of genes (especially housekeeping genes) from methylation [55,56] and when methylation spreads to Sp1 sites, binding is inhibited, contributing to gene silencing [126]. Sp1 mediates transcription of the tumor suppressor *RASSF1A*, whose promoter has four Sp sites. In cancer, a change in histone modifications (H3 deacetylation and K9 trimethylation) causes a reduction in Sp1 binding, followed by methylation of the promoter and gene silencing [127]. This suggests that the increase in Sp1 levels in cancer cells is not sufficient to overcome the silencing of its target genes through DNA methylation. However, Sp1 can also interact with DNMT1 to promote methylation at specific sites [53,54], suggesting a role for Sp1 in establishing the epigenetic state of both normal and cancer cells. More studies are needed to completely understand its mechanism in transcription activation and epigenetics. The ability of Sp1 to regulate oncogenes and tumor suppressors, pro-survival and pro-apoptotic genes, highlights the need to fully understand Sp1's activity at different promoters and in different cell conditions to develop a therapy that can specifically target Sp1 in cancer.

Sp1 has been implicated in Huntington's disease, a dominantly inherited neurodegenerative disorder caused by expansion of a polyglutamine tract in the Huntingtin (Htt) protein. Htt was found to bind to Sp1 and TAFII130 and inhibit DNA binding, while overexpression of both factors in striatal cells from a mouse model of Huntington's led to an improvement of symptoms and reversed inhibition of the dopamine D2 receptor gene, known to be a marker of the disease [128]. Further studies suggested the protective role of Sp1 overexpression involved acti-

vation of cystathione γ-lyase gene expression, the biosynthetic enzyme for cysteine, which is depleted in disease tissues [129]. However, this issue is still disputed, as other studies have found Sp1 contributes to the pathology in Huntington's disease. Sp1 was found to be overexpressed in the brains of mouse models and in model cell lines: inhibition or knockout of Sp1 led to amelioration of toxicity caused by mutant Htt and the mice survived longer, possibly due to Sp1 negatively regulating the Dopamine D2 gene. This suggests Sp1 is a potential therapeutic target in Huntington's disease [130].

A positive role of Sp1 has been found in Alzheimer's disease, where inhibition of Sp1 with mithramycin A in transgenic mouse models led to further memory impairment and an increase in the levels of Amyloidβ peptides (a major hallmark of the disease) [131]. A polymorphism in an Sp1 binding site of the *COL1A1* gene, encoding collagenα1, a major protein in bone, is associated with a predisposition to osteoporosis by altering the ratio of collagen α1 to α2 chains, causing reduced biomechanic strength in the bones [132]. In contrast, there was a negative correlation between the same polymorphism and hip osteoarthritis, suggesting there is a reduced risk of the disease [133]. Furthermore, Sp1 has been implicated in the development of multiple sclerosis (MS). Polymorphisms in the *IRF5* and *CD24* genes, factors involved in MS, can lead to increased Sp1 binding at these genes and an increased risk of MS [134,135]. Gene expression analysis in MS patients suggested the involvement of Sp1 in gender-specific gene signatures and inhibition of Sp1 transcription reduced the incidence and severity of experimental autoimmune encephalomyelitis in mice (the model of MS), highlighting Sp1 as a potential therapeutic target in MS [136].

CONCLUSION

Sp1 was the first characterized and still is one of the best studied mammalian transcription factors. It functions as a transcriptional activator of a variety of genes including house-keeping genes, cell cycle regulators and tissue-restricted genes. It is ubiquitously expressed, but its activity can be modified to respond to external stimuli, different stages of the cell cycle and different cell functions through post-translational modification and interaction with other transcriptional regulators. It can also regulate tissue- and developmental stage-specific gene expression, but there is still little known about the protein interactions and/or post-translational modifications that occur to elicit the specific patterns of Sp1-mediated gene expression. Therefore, more work is needed to both further understand the role of ubiquitous transcription factors in tissue-specific gene regulation and the dynamic transcription network controlling cell specification. This review highlights the importance of models of development to elucidate the mechanism of transcription activation, as well as the need to further our understanding of Sp1-mediated transcription in development and disease.

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1.6 Aims and objectives

Our group have previously reported a novel role for Sp1 in haematopoietic specification. Using in vitro differentiation of Sp1 DBD-deficient ESCs, Gilmour et al. found Sp1 was dispensable for the formation of haematopoietic progenitors, but it was essential for terminal haematopoietic differentiation. The loss of functional Sp1 was accompanied by gene expression changes, including several important developmental regulators. However, many questions still need to be addressed to better understand the mechanism behind the mutant phenotype. In this study, we aim to:

1) *Generate a complete Sp1 knockout ESC line and recapitulate the Sp1 DBD-deficient model in a different ESC background*

We will first delete the Sp1 DBD coding region in a different ESC line (A17 2lox), which could allow for the generation of an inducible expression system in a mutant background. The results from these cells will also be compared to the previous Sp1 DBD-deficient model to confirm the role of Sp1 in haematopoiesis. However, no studies have yet investigated the effect of deletion of the entire *Sp1* gene; therefore, we aim to knockout the complete Sp1 protein in ESCs and compare the phenotype to those lacking only the DBD.

2) *Characterise the gene expression changes associated with loss of Sp1*

Gilmour et al. analysed changes in gene expression through in vitro haematopoietic differentiation using microarrays. We aim to validate their findings, plus examine the changes in the transcriptome in more detail using RNA-seq in our Sp1 mutant cells.

3) Analyse epigenetic changes in Sp1 mutant cells

The binding of Sp1 is widespread across the genome and it is involved in the regulation of genes in a range of cellular processes. It also interacts with several epigenetic factors, but the effect of Sp1 deficiency on the chromatin profile of the cell has never before been investigated. We will therefore show how the chromatin structure changes with loss of Sp1 function using ATAC-seq.

4) Investigate the interplay between Sp1 and Sp3

Sp3 is a ubiquitously expressed transcription factor with a highly similar structure and binding specificity to Sp1. We plan to examine how Sp3 functions to regulate gene expression in the absence of Sp1 by mapping the binding sites of Sp1 and Sp3 using ChIP-seq in Sp1 mutant cells. We will also show the binding of Sp1 and Sp3 relative to each other in WT cells and how this changes upon ESC differentiation to gain insight into the relationship between Sp1 and Sp3.

2. MATERIALS AND METHODS

2.1 ESC culture

ESCs were plated onto mitomycin-C–treated murine embryonic fibroblasts (MEFs) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma) supplemented with 1 mM L-glutamine, 15% (v/v) foetal calf serum (FCS, Stem Cell Technologies), 1 mM sodium pyruvate, 0.15 mM MTG, 25 mM HEPES buffer, 1x non-essential amino acids, 100 units/ml penicillin and 100 µg/ml streptomycin, and 10^3 units/ml Leukaemia Inhibitory Factor (LIF, ESGRO, Millipore), at 37°C. For passaging, the cells were washed in phosphate buffered saline (PBS) and incubated at 37°C with TrypLE dissociation reagent (Life Technologies). Medium was then added and the cells re-plated at the appropriate density.

2.2 ESC differentiation

The differentiation procedure was performed as described in Gilmour et al., 2014. Briefly, ESCs were cultured on gelatinised plates for one day in DMEM ESC and for a second day in Iscove's Modified Dulbecco Medium (IMDM, Sigma) ESC medium. The cells were transferred to low adherence plates (Sterilin) in IVD media (IMDM supplemented with 15% (v/v) FCS (Gibco), 100 units/ml penicillin and 100 µg/ml streptomycin, 1 mM glutamine, 0.45 mM MTG, 0.18 mg/ml human transferrin (Roche) and 50 µg/ml ascorbic acid) to form embryoid bodies (EBs). After 3.75 days, the EBs were harvested and dispersed to a single cell suspension using TrypLE reagent (Life Technologies) and passing through a cell strainer. Flk1+ cells were isolated by magnetic activated cell sorting (MACS). The cells were incubated with a biotin-conjugated Flk1 antibody (eBioscience 13-5821) at 5

µl/10^7 cells for 15 min on ice. The cells were washed, incubated with MACS anti-biotin beads at 20 µl/10^7 cells and passed through MACS LS columns (Miltenyi Biotec). The Flk1+ cells were cultured for 2-3 days in blast medium (IMDM supplemented with 10% FCS (v/v), 100 units/ml penicillin and 100 µg/ml streptomycin, 1 mM glutamine, 0.45 mM MTG, 0.18 mg/ml human transferrin (Roche), 25 µg/ml ascorbic acid, 20% D4T conditioned media (v/v), 5 µg/L VEGF [Peprotech] and 10 µg/L IL-6 [Peprotech]) to form haematopoietic progenitors.

2.3 Macrophage release assay

ES cells were seeded at an appropriate density in methylcellulose media: IMDM (Sigma) with 1.1% (v/v) methylcellulose (Methocult, Stem Cell Technologies), 1x Pen/Strep, 1 mM glutamine, 10% (v/v) FCS (Gibco), 10 µg/ml insulin, 0.45 mM MTG, 10% (v/v) M-CSF conditioned media (obtained from the culture of L cells), 5% (v/v) IL-3 conditioned media (harvested from a culture of X63 Ag8-653 myeloma cells carrying an IL-3 expression cassette), 100 units/ml IL-1, supplemented with 25 ng/ml recombinant macrophage colony stimulating factor (M-CSF) and 5 ng/ml IL-3 cytokines (Peprotech) to promote differentiation to macrophages. The cells were incubated at 37°C for approximately 2 weeks, at which time macrophage-releasing EBs were counted.

2.4 Flow cytometry and fluorescence activated cell sorting (FACS)

Approximately 1x10^5 cells were harvested, washed in MACS buffer (PBS, 0.5% bovine serum albumin [BSA] and 2 mM EDTA) and resuspended in the remaining supernatant (approx. 50 µl). The antibody was added (0.5 µl) (Table 2.1) and the cell suspension was incubated on ice, in the dark, for 15-30 min. The cell suspension was then washed with MACS buffer and the cell composition was analysed using a CyAn ADP machine

(Beckman Coulter) and Summit 4.3 (Beckman Coulter) software, initially gating for live cells and single cells.

Sorted haematopoietic populations were prepared at day 2 of blast culture. Cells were harvested, washed with PBS and resuspended in IMDM/20% FCS at $5x10^6$ cells/ml. The cells were stained with KIT (1:50 dilution), CD41 (1:100) and Tie2 (1:200) antibodies (Table 2.1) for 30 min at 4°C on a rotating wheel. After two washes with MACS buffer, the cells were resuspended at $20x10^6$ cells/ml in IMDM/20% FCS and filtered through a cell strainer. The cells were separated on a BD FACSAria Fusion (Special Order System) (BD Biosciences) into HE1 (KIT+, CD41-, Tie2+), HE2 (KIT+, CD41+, Tie2+) and progenitor (KIT+, CD41+, Tie2-) cell populations according to surface marker expression. An unstained sample was used as a control and a triple isotype control was used to set the gates. The cells were initially gated for live and single cells.

Table 2.1 – Antibodies used in flow cytometry experiments

Antibody	**Manufacturer**	**Serial Number**
TIE2-PE	eBioscience	12-5987-81
CD41-PE Cy7	eBioscience	25-0411-82
KIT-APC	BD Pharmingen	553356
Flk1-PE	eBioscience	12-5821-83
IgG Isotype Control PE Cy7	eBioscience	25-4727-81
IgG Isotype Control PE	eBioscience	12-4321-82
IgG Isotype Control APC	eBioscience	17-4031-82

2.5 Western blotting

Cells were harvested, washed with PBS and centrifuged (300 x g, 5 min). The pellet was resuspended in radioimmunoprecipitation assay (RIPA) buffer (150 mM NaCl, 10 mM Tris [pH 7.2], 0.1% [w/v] SDS, 1% [v/v] Triton X-100, 1% [w/v] Sodium Deoxycholate, 5 mM EDTA) supplemented with protease inhibitor cocktail (PIC) (Sigma P8340, at 1:100 dilution) at a ratio of 100 µl per $1x10^6$ cells and incubated at 4°C for 30 min on a rotating wheel. The samples were centrifuged (13.5 x g, 10 min, 4°C) and the supernatant was collected. Protein concentrations were measured using the Pierce BCA protein assay (Thermo Scientific), according to manufacturer's instructions. Appropriate amounts of the sample were mixed with 5x Laemmli buffer (0.3125M Tris [pH6.8], 10% [w/v] SDS, 25% [v/v] glycerol, 10% [v/v] β-2 Mercaptoethanol, 0.05% [v/v] Bromophenol blue) and incubated for 5 min at 95°C to denature the protein.

The samples were loaded onto Mini-PROTEAN TGX precast gradient polyacrylamide gels (Bio-Rad) in running buffer (0.025 M Tris, 0.2 M Glycine, 3.5 mM SDS) and the proteins separated by SDS-PAGE. The protein was transferred to a nitrocellulose membrane (Bio-Rad) using the Trans-Blot Turbo transfer system (Bio-Rad) and blocked with 5% non-fat dry milk (Marvel) in TBST (0.2% [v/v] Tween™ 20, 0.075 M sodium chloride [NaCl] and 0.01 M Tris [pH 7.5]) for at least 30 min. The membrane was incubated with primary antibody at the appropriate dilution (see Table 2.2) in 2.5% milk on a rotary shaker for a minimum of 3 hours. The blot was washed with TBST, and then peroxidase-linked secondary antibody (anti-mouse and anti-goat by Jackson ImmunoResearch; anti-rabbit by Cell Signaling) (diluted in 2.5% milk) was added for 1-2 hours. The blot was washed for 3x10 min with TBST on a rotary shaker, before the addition of ECL detection reagent (GE Healthcare) and development using Bio-Rad Universal Hood II.

Table 2.2 – Antibodies used for Western blotting

Antibody	Dilution	Species	Supplier/code
GAPDH	1:10000	Mouse	Abcam 8245
Sp1 (TADs)	1:10000	Rabbit	Millipore 07-645
Sp1 (DBD)	1:7000	Goat	Abcam ab157123
Sp3	1:10000	Goat	Santa Cruz sc-644x

2.6 RNA extraction, cDNA synthesis and qPCR gene expression analysis

For RNA extraction, cell pellets were resuspended in Trizol (Invitrogen) and purified according to manufacturer's instructions. For cDNA synthesis, up to 1.5 µg RNA was treated with 1 µl RQ1 DNase enzyme (Promega) for 30 min at 37^{o}C, before the reaction was ended with the addition of DNase stop solution. The RNA was denatured by heating at 65^{o}C for 10 min and an oligo-dT primer annealed at 70^{o}C for 5 min. First-strand cDNA synthesis was performed by incubation with M-MLV reverse transcriptase (Promega) at 37^{o}C for 60 min, followed by 5 min at 95^{o}C, according to manufacturer's instructions. The cDNA was appropriately diluted and 2.5 µl used in each 10 µl qPCR reaction, along with 0.25 µM primers (Table 2.3) and SYBR green PCR master mix (Applied Biosystems). The qPCR was performed in duplicate on a StepOnePlus (Applied Biosystems) or 7900HT (Applied Biosystems) machine. Standard curves were generated for each primer pair with various dilutions of cDNA to account for primer efficiencies and the results were normalised to levels of *Gapdh.*

Table 2.3 – Primers used for gene expression analysis

Gene	Forward (5'-3')	Reverse (5'-3')	Reference
Sp1 exon3-4	TAGCAAACACCCCAGGTGATC	TCCTTCTCCACCTGCTGTCTC	(Gilmour et al., 2014)
Sp1 exon5-6	TCATATTGTGGGAAGCGCTTT	CAGGGCAGGCAAATTTCTTCT	(Gilmour et al., 2014)
hSP1 exon3-4	GCGAGAGGCCATTTATGTGT	GGCCTCCCTTCTTATTCTGG	-
hSP1 exon5-6	TAGCAAATGCCCCAGGTGATC	TTCCTCTCCACCTGCTGTGTC	(Gilmour et al., 2014)
Nanog	TCTTCCTGGTCCCCACAGTTT	GCAAGAATAGTTCTCGGGATGAA	Primerbank 31338864a1
Bmp4	AGCCCGCTTCTGCAGGA	AAAGGCTCAGAGAAGCTGCG	(Le Bouffant et al., 2011)
Gp5	CATCGCTGAGCTAGGTCTGC	GCTGAAGGACTGTCATGCC	Primerbank 31982322a1
Brachyury	GCTTCAAGGAGCTAACTAACGAG	CCAGCAAGAAAGAGTACATGGC	Primerbank 6678203a1
Etv2	CTGGGAGCGGAATTTGGTTTC	GTAAAGCGGGGTTCCAGTCC	(Liu et al., 2015)

2.7 RNA-sequencing library preparation

For RNA extraction, cells were pelleted by centrifugation (300 x g, 5 min), resuspended in Trizol (Invitrogen) and purified according to the manufacturer's instructions. Glycogen was added during the procedure to increase RNA yield from small cell numbers. The RNA was incubated with 0.1 units/µl DNase1 and 1x DNase1 Buffer (Ambion, Invitrogen) at 37°C for 30 min and further purified using Minelute RNeasy columns (Qiagen), according to manufacturer's protocol.

RNA-seq libraries were prepared using the TruSeq Stranded Total RNA Library Prep Kit (Illumina), according to manufacturer's instructions. Briefly, 10 µl of the RNA sample was incubated with 5 µl rRNA binding buffer and 5 µl rRNA removal mix at 68°C for 5 min, then rRNA removal beads added to remove rRNA by magnetic separation. The RNA was further purified using RNAClean XP magnetic beads and fragmented at 94°C for 8 min.

For first strand cDNA synthesis, 8 µl of a master mix consisting of one part SuperScript II Reverse Transcriptase to nine parts First Strand Act D Mix was added and incubated at 25°C for 10 min, 42°C for 15 min and 70°C for 15 min. To synthesise the second cDNA strand, 20 µl Second Strand Marking Mix was added, along with 5 µl resuspension buffer, and incubated for 1 hour at 16°C. The cDNA was purified using 1.8 x AMPure XP beads (Beckman Coulter) and eluted in 15 µl resuspension buffer.

The cDNA ends were adenylated by adding 2.5 µl resuspension buffer and 12.5 µl A-tailing Mix, before incubating at 37°C for 30 min, then 70°C for 5 min. Next, indexing adapters (Illumina) were ligated to the ends of the double-stranded cDNA to enable hybridisation onto a flow cell. Appropriate adapters were diluted 1 in 4 and 2.5 µl added to the sample, along with 2.5 µl resuspension buffer and 2.5 µl ligation mix, before incubating at 30°C for 10 min. The reaction was stopped with 5 µl Stop Ligation Buffer and the cDNA purified by two successive 1 x AMPure XP magnetic clean ups, resulting in 20 µl sample in resuspension buffer. To amplify the cDNA, 5 µl PCR Primer Cocktail and 25 µl PCR Master Mix was added and a PCR performed (98°C for 30 sec, followed by 15 cycles of 98°C for 10 sec, 60°C for 30 sec and 72°C for 30, then hold at 72°C for 30 sec). The cDNA then underwent a final clean up using 1 x AMPure XP magnetic beads and eluting in 30 µl resuspension buffer.

The library was validated by running on Bioanalyzer 2100 (Agilent) and quantified using KAPA KK4835 Library Quantification Kit (Kapa Biosystems) according manufacturer's instructions. Samples were sequenced on a Hiseq 2500 (Illumina) using rapid run chemistry with 100 bp paired end reads.

2.8 Generation of *SP1*-expressing ESCs to rescue the Sp1-deficient phenotype

A DNA fragment coding for the PGK promoter and *SP1* gene was first generated, from two separate vectors, using fusion PCR. The human *SP1* gene was isolated by PCR from a cDNA clone, courtesy of Sjaak Phillipsen, using primers to give the addition of a 3' HindIII restriction site (5'- CAGTAAAAGCTTTCAGAAGCCATTGCCACTG -3'), and a 5' Kozak sequence, preceded by a 20 bp sequence overlapping that found at the 3' end of the PGK promoter (5'- CATCTCCGGGCCTTTCGACCCCGCCATGGATGAAATGACAGC TGTGG -3'). The PGK promoter was isolated from the p2lox plasmid (courtesy of Michael Kyba), using primers with a 5' SalI site overhang (5'- ATTGTGTCGACCGACCTCGAAATT CTACCGG -3') and a 3' sequence overlapping with the 5' region of *SP1* (5'- CTGTCATTTCATCCATGGCGGGGTCGAAAGGCCCGGAGATG -3').

The PCR products were then annealed and amplified as one DNA fragment by incubating equimolar amounts of *SP1* and PGK products in a PCR reaction for 30 sec at 90°C, then thermocycling for 5 cycles of 98°C for 10 sec, 65°C for 30 sec and 72°C for 30 sec, followed by 25 cycles of 98°C for 10 sec and 72°C for 3 min, and finally holding at 72°C for 10 min. The reactions were purified using a Qiagen PCR Purification Minelute kit, according to manufacturer's instructions.

The PCR product was digested with SalI and HindIII enzymes (NEB), along with the PB-TRE-*Gata1*-2A-mCherry plasmid, courtesy of George Lacaud, thus removing the TRE-*Gata1* section of the plasmid. The reactions were separated by gel electrophoresis and the desired fragments cut out and purified using the Qiagen Gel Extraction Minelute kit, according to manufacturer's instructions. The fragments were ligated together using T4 DNA ligase (NEB) at a ratio of 3x insert (totalling 30-50 ng) to 1x plasmid for 2-3 hours at room temperature to generate a PB-PGK-*SP1*-2A-mCherry plasmid.

The vector was transformed into DH5α *E. coli* by heat shock and the bacteria were plated onto ampicillin LB plates to select for successfully transformed cells. Single colonies were picked and cultured in ampicillin-containing LB broth, then the plasmid was purified using Macherey-Nagel NucleoBond Xtra Midi EF kit according to manufacturer's instructions. The resulting DNA was sequenced to ensure correct incorporation of the PGK promoter and *SP1* gene into the plasmid using the PCR primers above, plus: 5'-AGCAGGATG GTTCTGGTCAA -3', 5'- GAGCAAAACCAGCAGACACA -3', 5'- AAGACAGTGAAGGAAG GGGC -3', 5'- AGTCAGAAACAACTTTGGCACA -3' and 5'- TCCTGGACCTGGATTGCT TT -3'.

$Sp1^{del/del}$ and $Sp1^{-/-}$ ES cells were co-transfected with the PB-PGK-*SP1*-2A-mCherry vector and a PiggyBac (PB) transposase expression vector, PL623, at a two to one ratio (as described in (Wang et al., 2008)) using the P3 4D-nucleofector kit (Lonza V4XP-3024), according to manufacturer's guidelines, and a 4D-Nucleofector™ X Unit. The PB-PGK-*SP1*-2A-mCherry plasmid contains a PB transposon element, made up of inverted terminal repeat sequences (ITRs), either side of the transgene. The PB transposase recognises the PB ITRs and cuts at these sites, triggering a transposition reaction to insert the intervening transgene into the genome randomly at TTAA sites.

Cells were sorted after 48 hours for mCherry expression, replated onto MEFs for approximately 5 days, before individual clones were picked and expanded. *SP1* expression was then assayed by qPCR analysis and Western blotting.

For the expression of the Sp1 DBD in $Sp1^{del/del}$ cells, the protocol as above was repeated, but primers to isolate the DBD fragment from the *SP1* cDNA were based on those used in Koutsodontis et al. 2005: 5'- GACCCTTAAGCAATTGCCGCCATGGGGGATCCTGGCAA AAAGAAAC -3' and 5'- CAGTAAAAGCTTGAAGCCATTGCCACTGATATTAATG -3' (Koutsodontis et al., 2005).

2.9 CRISPR-Cas9 in vitro system

The efficiency of guide sequences was tested in vitro using the SureGuide Cas9 Nuclease Kit (Agilent), according to manufacturer's instructions. The target region (between the beginning of exon 5 of *Sp1* and the end of the gene) was isolated from mESC genomic DNA using PCR (F: 5'-GGTCAGCCTTGTCTACTTAGTAA-3'; R: 5'- CAGTGACATTGGG TGCCA-3') and purified using Qiagen PCR MinElute Purification kit, according to manufacturer's instructions. Guide RNAs, along with a tracrRNA, were obtained from Dharmacon. Guides 1 and 3 were designed to target the start of exon 5, while guides 14 and 18 were designed to cut at the end of the gene (Table 2.4).

In a 20 µl reaction, 50 ng DNA (1425 bp) was mixed with 1 µl Cas9, 50 nM gRNA, 50 nM tracrRNA and 1x Cas9 Digestion Buffer, made up to 20 µl with water. The sample was incubated at 37°C for 30 min, followed by 65°C for 15 min, before analysing the digestion pattern by gel electrophoresis on a 1% agarose gel.

Table 2.4 – Sequences of guide RNAs used in the in vitro Cas9 assay.

Guide	RNA oligo sequence (5'-3')
1	CAUAUACUUUGCCGCAUCCU
3	AAUAUUAUGAUUUAUCACUC
14	CCUUGCAUCCCGGGCUUAGU
18	AUCCCGGGCUUAGUGGGUAU

Note, the sequence GUUUUAGAGCUAUGCUGUUUUG was added at the 3' end to act as a scaffold and promote Cas9 activity.

2.10 CRISPR-Cas9 targeting of Sp1 in ESCs

Four guide sequences, two to target the start of *Sp1* exon 5 (1 and 3) and two at the end of the gene (14 and 18) (thus flanking the DBD-coding region), were designed using The CRISPR Design Tool by the Zhang lab (a programme based on the specificity analysis performed Hsu et al. (Hsu et al., 2013)). The guides were then shortened from 20 nucleotides (nt) to 18 nt (taken from the opposite end to the PAM sequence), which was found to reduce off-target effects (Fu et al., 2014) (Table 2.5).

Table 2.5 – Sequences of CRISPR guides used to target *Sp1* DBD-coding region.

Guide	Sense Sequence (5'-3')
1	TATACTTTGCCGCATCCT
3	TATTATGATTTATCACTC
14	TTGCATCCCGGGCTTAGT
18	CCCGGGCTTAGTGGGTAT

The guide sequences were cloned into the PX458 plasmid (Addgene), encoding Cas9-2A-GFP, based on the protocols described in Ran et al., 2014 and Bauer et al., 2015. Briefly, the sequences were ordered as oligos (Sigma) in both the sense and antisense direction to allow them to anneal as double-stranded DNA. Flanking sequences were incorporated

at each end of the oligo to generate sticky ends for direct cloning into the PX458 plasmid. Thus, the sense oligo sequence was: 5'-CACCGNNN…-3', while the antisense oligo had the sequence: 5'-AAACNN…NNC-3'. The two complementary sense and antisense oligos were annealed and phosphorylated by incubating 10 µM of each oligo with 1 µl T4 10x ligation buffer (NEB) and 1 µl T4 Polynucleotide Kinase (NEB) in a 10 µl reaction for 37°C for 30 min, then 95°C for 5 min, before cooling to 25°C at a rate of 5°C/min. The annealed oligos were diluted 1:10 and added to a reaction to both digest PX458 with BbsI (NEB), and ligate the guide oligos into the plasmid. 1 µl diluted oligo duplex was mixed with 100 ng PX458, 5 µl RE buffer 2.1 (NEB), 2 µl BbsI, 1 mM ATP, 0.5 µl BSA and 1.875 µl T4 ligase (NEB), making up to 50 µl with water. The sample was incubated at 37°C for 5 min, then 20°C for 5 min, repeated for 20 cycles, followed by 80°C for 20 min.

The vector was transformed into DH5α *E. coli* by heat shock and the bacteria were plated onto ampicillin LB plates to select for successfully transformed cells. Single colonies were picked and cultured in ampicillin-containing LB broth, then the plasmid purified using Macherey-Nagel NucleoBond Xtra Midi EF kit according to manufacturer's instructions. The resulting DNA was sequenced by Sanger sequencing to ensure correct incorporation of the guide sequence using a primer in the U6 promoter (5'-GAGGGCCTATTTCCCATG ATTCC-3').

A17 2lox murine ESCs (courtesy of Michael Kyba) were transfected with two collaborating plasmids (each expressing a guide RNA targeting either side of the *Sp1* DBD coding region) using the P3 4D-nucleofector kit (Lonza V4XP-3024), according to manufacturer's guidelines, and a 4D-Nucleofector™ X Unit. The cells were plated onto gelatine for 24 hours, before isolating GFP-expressing cells by FACS and seeding a proportion as single cells in a 96-well plate on MEFs.

The GFP-positive bulk population was harvested for DNA extraction using the Macheray-Nagel Nucleospin Tissue kit, according to manufacturer's protocol. Disruption of the *Sp1* gene was assayed using PCR with primers F: 5'-TGGCACACATACCTTTAATCCT-3' and R: 5'-ACCTGGGATGAGATAAATGCTG-3'. The product obtained from WT DNA was 1564 bp, whereas successful deletion of the target region resulted in a product of 496 bp.

When a successful targeting event was detected in the bulk population, the single cell cultures were expanded, splitting into two 96-well plates – one to freeze on MEFs and one on gelatine for DNA extraction. The cells were washed with PBS, and then incubated at 60°C for 2-3 hours with 50 µl lysis buffer (10 mM Tris-HCl pH7.5, 10 mM EDTA, 10 mM NaCl, 0.5% Sarcosyl [Sigma], 1 mg/ml proteinase K [Roche]). 150 µl precipitation buffer (100 mM NaCl, 100% EtOH) was added for 30 min at room temperature, followed by centrifugation (5 min, 1750 x g). The supernatant was discarded and the DNA pellet washed with 70% EtOH. The DNA was resuspended in 0.1x TE. PCR was performed with 0.8 µl DNA from each well and the primer as above, and the products analysed on a gel to detect clones with disruption at the target site.

2.11 Chromatin Immunoprecipitation (ChIP)

Cells were harvested and washed with PBS, before resuspending in PBS at $3.3x10^6$ cells/ml and double crosslinking. Disuccinimidyl glutarate (DSG) (Sigma) was added at 830 µg/ml and incubated at room temperature for 45 mins on a roller. The cells were washed four times with PBS and subsequently resuspended in IMDM/20% FBS with 1% (v/v) formaldehyde (Thermo Scientific Pierce) at 1 ml/10^6 cells. The cells were incubated on a roller for 10 min and the reaction quenched with the addition of a tenth of the volume 2 M glycine. The cells were then washed twice with ice-cold PBS.

To isolate the chromatin, the cell pellet was resuspended in ice-cold buffer A (10 mM HEPES pH 8.0, 10 mM EDTA, 0.5 mM EGTA, 0.25% Triton X-100, 1:1000 PIC [Roche UK] and 0.1 mM PMSF) at $1x10^7$ cells/ml and rotated at 4°C for 10 min. The sample was centrifuged (500 x g, 5 min, 4°C) and pelleted nuclei were resuspended in buffer B (10 mM HEPES pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.01% Triton X-100, 1:1000 PIC and 0.1 mM PMSF) at $1x10^7$ cells/ml, followed by rotation for 10 min at 4°C. The nuclei were pelleted again (at 500 x g, 5 min, 4°C) and resuspended in ice-cold IP buffer I (25 mM Tris-HCl pH 8.0, 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.25% SDS, 1:1000 PIC and 0.1 mM PMSF) at $2x10^7$ cells/ml.

To fragment the chromatin, the sample was sonicated using Diagenode Bioruptor for the appropriate number of cycles (high 240 W, 30 sec on, 30 sec off, 4°C) for the cell type. The sample was centrifuged (16,000 x g, 10 min, 4°C) and the supernatant diluted by added 2 volumes of ice-cold IP buffer II (25 mM Tris-HCl pH 8.0,150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 7.5% glycerol, 1:1000 PIC and 0.1 mM PMSF).

For immunoprecipitation (IP), 15 µl Dynabeads-Protein G (Invitrogen) was washed with 0.1 M citrate-phosphate buffer pH5 (CP buffer) by magnetic separation. The beads were resuspended in 15 µl CP buffer, plus 0.5% BSA (v/w) and 2 µg primary antibody (Table 2.6), then rotated at 4°C for 1-2 hours to allow the antibody to bind the Dynabeads-Protein G. The mixture was washed with CP buffer to remove non-bound antibodies and the beads resuspended in 15 µl CP buffer/BSA. The antibody-Dynabeads were added to the chromatin ($1\text{-}2x10^6$ cells per IP) and rotated at 4°C overnight (10% of the chromatin material was taken as an input control).

The sample was then washed by magnetic separation with: wash buffer 1 (150 mM NaCl, 1% Triton X-100, 20 mM Tris-HCl, 0.1% SDS and 2mM EDTA), twice with wash buffer 2

(500 mM NaCl,1% Triton X-100, 20 mM Tris-HCl, 0.1% SDS and 2mM EDTA), LiCl buffer (250 mM LiCl , 0.5% NP-40, 10 mM Tris-HCl pH 8.0, 0.5% Na-deoxycholate and 1 mM EDTA) and twice with TE/NaCl buffer (50 mM NaCl,10 mM Tris-HCl pH 8.0 and 1 mM EDTA). The DNA was eluted from the beads with 100 µl elution buffer (1% SDS, 100mM NaHCO3) for 30 min with shaking. Reverse crosslinking was performed by adding 0.2 M NaCl and 25 µg Proteinase K, and incubating overnight at 65°C. The input control was incubated with 10 µg RNaseA for 30 min at 37°C and made up to 100 µl with 1% SDS (w/v), 50 µg Proteinase K and water, before also reverse crosslinking at 65°C. The DNA was then purified using Agencourt AMPure beads (Beckman Coulter), according to manufacturer's protocol, and eluted in 100 µl 0.1 x TE.

The resulting DNA was assessed by qPCR. The results were normalised to the values obtained with the input control and at a gene-poor control region (Chr2). The enrichment was analysed using primers at known binding sites (positive control regions), compared to regions that are not bound by the protein of interest (negative controls) (Table 2.7). qPCR validations of Sp1 and Sp3 ChIPs are shown in Figure 2.1 and Figure 2.2, respectively.

Table 2.6 – Antibodies used in ChIP

Antibody	**Company**	**Product number**
Sp1	Millipore	17-601
Sp3	Santa Cruz	sc-644x

Table 2.7 – Primers used in Sp1 and Sp3 ChIP qPCR validations

Primer region	**Forward (5'-3')**	**Reverse (5'-3')**
Chr2	AGGGATGCCCATGCAGTCT	CCTGTCATCAGTCCATTCTCCAT
Sp1 promoter	CGAGAGAGCGAGTCCTACCA	TAATCCCCGCCCCTTATCTA
Flad1 promoter	GCCAGGCCCCTATTTTTACT	ATCGCGGAGCTAGAGGAAAT
Caprin2 promoter	CGGACTGTGGTGGTCTAGC	GCCTATTTCCGGTCCTCTG
Sp1 gene body	GTGCATTGGGTACTTCAGGGA	AGGATGTCTTACCTGGGGTGT
Itga2b gene body	CAGCGACACGTTGAGGCTTA	CCCAGCACAGAGACAGATAGC

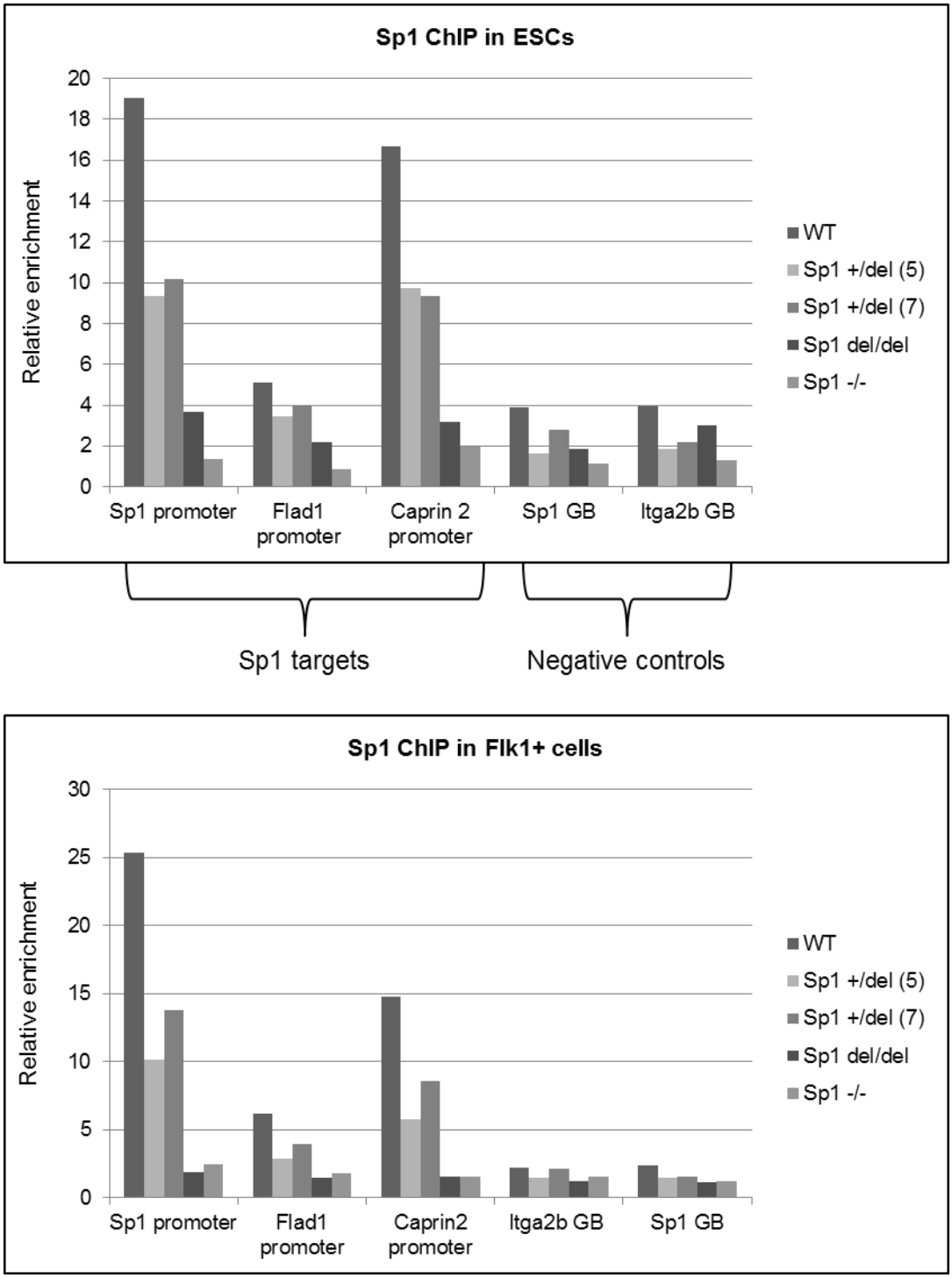


Figure 2.1 – Sp1 ChIP-qPCR shows enrichment at known Sp1 binding regions

Sp1 ChIP was performed in ESC and Flk1+ cells of each of the Sp1 mutant cell clones, followed by qPCR at known Sp1-binding regions and negative control regions. The enrichment is shown as normalised to input and Chr2 (a gene-poor control region). Libraries were made from this ChIP and sequenced to determine Sp1 binding sites across the genome.

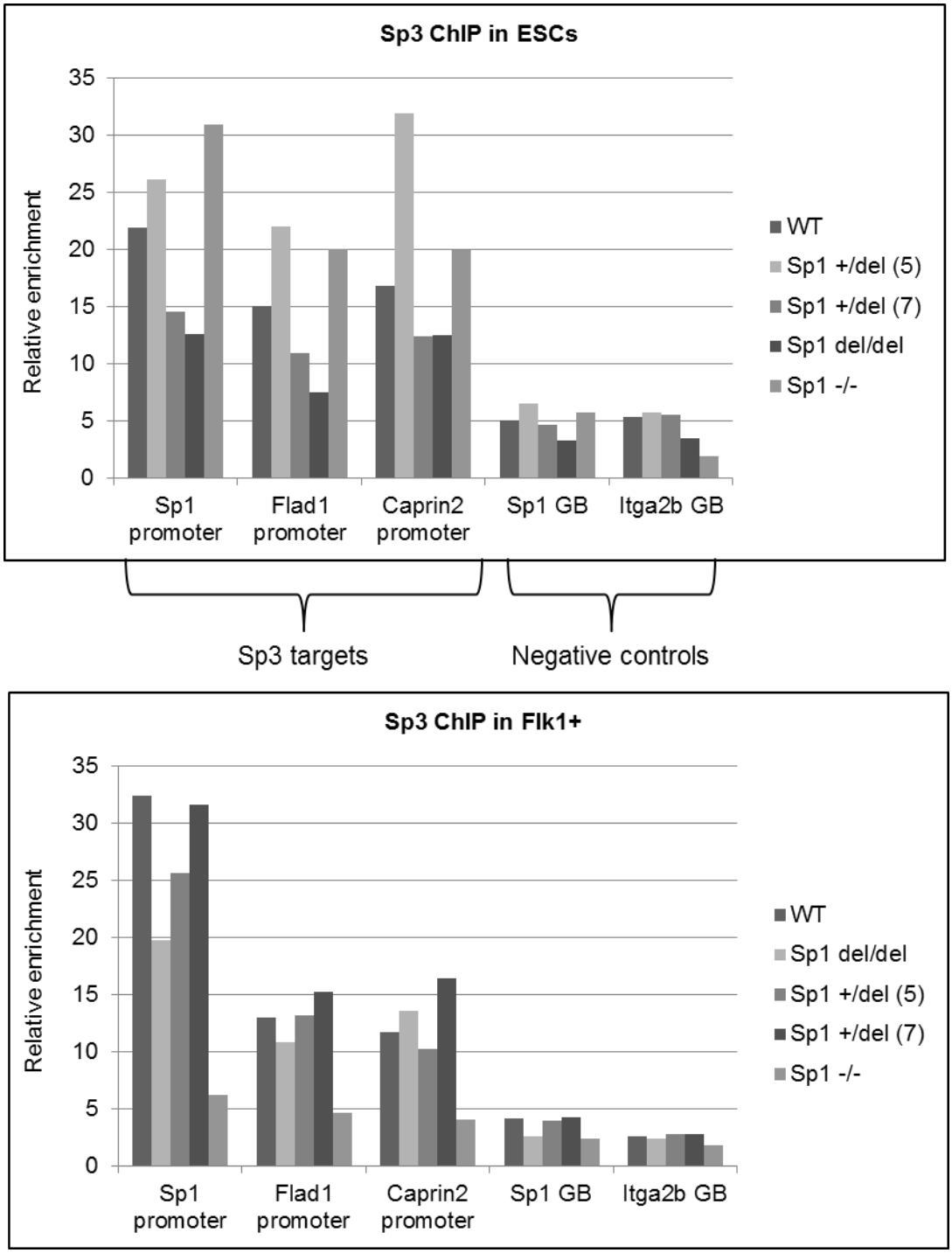


Figure 2.2 – qPCR validation of Sp3 ChIP

Sp3 ChIP was performed in ESC and Flk1+ cells of each of the Sp1 mutant cell clones. qPCR was performed using primers at known Sp1-binding regions and negative control regions, and the results normalised to input and Chr2 (a gene-poor control region). ChIP-seq libraries were then made.

2.12 ChIP-sequencing (ChIP-seq) library preparation

ChIP-seq libraries were prepared using the KAPA Hyper Prep Kit (Illumina Platforms), following the manufacturer's recommended protocol. Briefly, 50 µl of the ChIP-obtained DNA (re-purified using Agencourt AMPure beads to elute in the correct volume, if required) was incubated with 7 µl End Repair and A-tailing buffer and 3 µl of the corresponding enzyme at 20°C for 30 min and 65°C for 30 min. To ligate the sequencing adapter, 30 µl ligation buffer, 5 µl DNA ligase, 5 µl adapter (diluted 1:6) and 5 µl water were added and incubated at 20°C for 15 min. The DNA was purified using 1.2x Agencourt AMPure XP beads (Beckman Coulter), according to manufacturer's instructions, and eluted in 20 µl 10 mM Tris-HCl pH8. Half of the library was then amplified by adding 12.5 µl 2x KAPA HiFi HotStart ReadyMix and 5 µl 10x primer mix and incubated at 98 for 45 sec, an appropriate number of cycles (often 16 cycles) of 98°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec, and then finally 72°C for 1 min. The remaining half was reserved if a suboptimal number of cycles was used. The reaction was then purified using AMPure XP reagent. Size selection was performed by running the sample on a 2% agarose gel, separating the DNA fragments by electrophoresis and isolating the ~250-450bp fragments by cutting them out of the gel and purifying using the Qiagen MinElute Gel Extraction kit. Finally, libraries were quantified by Kapa library quantification kit (Kapa Biosystems) and run in a pool of indexed libraries on a HiSeq 2500 (Illumina).

2.13 ATAC-seq

Assay for transposable accessible chromatin, followed by sequencing (ATAC-seq), was performed based on Buenrostro et al., 2015 (Buenrostro et al., 2015a). Briefly, $5x10^4$ cells were pelleted by centrifugation (500 x g, 5 min, 4°C) and resuspended in a transposition reaction mix consisting of 0.27 µl 1 x digitonin (Promega G9441), 25 µl 2 x Tagment DNA

Buffer (Illumina #FC-121-1030), 2.5 µl Tn5 Transposase (Illumina #FC-121-1030) and 22.23 µl water. The sample was incubated at 37°C for 30 min with shaking at 300 rpm. The Qiagen MinElute Reaction Clean up Kit was used to purify the DNA and elute in 11 µl EB buffer. To amplify the transposed DNA fragments, the following were combined: 10 µl transposed DNA, 10 µl water, 25 µl NEBNext High-Fidelity 2x PCR Master Mix (New England Biolabs) and 2.5 µl each of 25 µM customised Nextera PCR primer 1 and 2 (sequences as described in Buenrostro et al., 2013) (Buenrostro et al., 2013). The following PCR conditions were used: 72°C for 5 min, 98 °C for 30 sec, and thermocycling at 98°C for 10 sec, 63°C for 30 sec and 72°C for 1 min. In order to reduce GC and size bias in the PCR, the reaction was monitored to stop amplification before saturation. The PCR was initially performed for 5 cycles and an aliquot of 5 µl was taken (with the remaining kept on ice) and analysed by qPCR by adding 5 µl NEBNext High-Fidelity 2x PCR Master Mix, 4.44 µl water, 0.06 µl 100x Sybr Green and 0.25 µl each of 25 µM customised Nextera PCR primer 1 and 2. The qPCR was performed for 30 cycles and the number of cycles that corresponded to a third of the maximum fluorescence intensity calculated (accounting for fluorescence from water control reaction). The remaining 45 µl of PCR sample was then ran for the appropriate number of cycles in the reaction: 98°C for 30 sec, then thermocycling at 98°C for 10 sec, 63°C for 30 sec and 72 °C for 1 min. The reaction was purified with the Qiagen PCR purification kit, eluting in 15 µl EB, and subsequently with 1.2x AMPure XP beads (Beckman Coulter), according to manufacturer's recommendations, eluting in 20 µl 0.1x TE. The libraries were validated by running on Bioanalyzer 2100 (Agilent) and sequenced on an Illumina HiSeq 2500. An illustration of how ATAC-seq works to elucidate regions of open chromatin is shown in Figure 2.3.

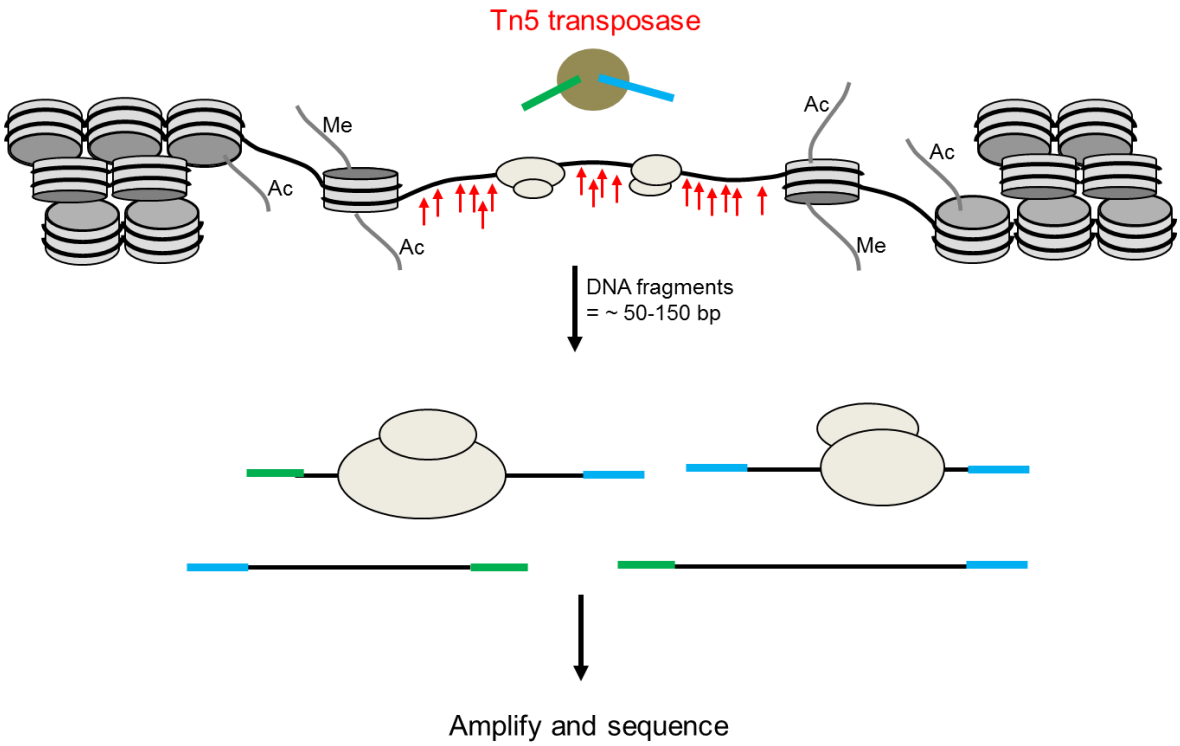


Figure 2.3 – Schematic of the ATAC-seq technique
The enzyme Tn5 transposase simultaneously cuts the DNA to fragment the chromatin and tags the open DNA ends with adapters for high throughput sequencing. The enzyme preferentially targets accessible chromatin and thus amplifiable DNA fragments are preferentially located in regulatory regions.

2.14 Immunofluorescence Assays

Approximately 10,000 ESCs were resuspended in 100 µl PBS and adhered to a microscope slide using Cytospin 4 (300 rpm, 3 min). The slides were washed in PBS and the cells fixed with 4% (v/v) formaldehyde for 10 min at room temperature. The cells were washed twice with PBS and the cells permeablised with 0.3% (v/v) Triton-x-100 in PBS for 5 min. The cells were washed again with PBS and blocked with 3% BSA/10% FCS in PBS for 1 hour. The antibody was then added, diluted to the appropriate dilution in blocking solution, and the cells incubated at 4°C, overnight, in a humidified atmosphere. The cells were washed three times in 0.1% PBS-Tween 20 for 5 mins, before the

secondary antibody was added, diluted in blocking solution, and incubated for 1 hour at room temperature. The slides were washed three times again, dipped in water and left to dry, before adding a drop of ProLong Diamond Antifade Mountant with DAPI (ThermoFisher Scientific) and topping with a coverslip. A secondary antibody only slide was also made. The cells were imaged using a Zeiss LSM 510 Meta Confocal microscope.

2.15 Bioinformatics Data Analysis

These analyses were performed by Chris Middleton and Salam Assi

2.15.1 ATAC-seq and ChIP-seq data analysis

Sequencing quality was verified using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adaptor sequences were removed using Cutadapt (Martin, 2011) for ATAC-seq reads and with Trimmomatic v0.35 (Bolger et al., 2014) for ChIP-seq. Sequencing reads were aligned to mm10 mouse genome with Bowtie2 v2.3.1 (Langmead & Salzberg, 2012) using default settings. Any identical duplicate reads in the ChIP-seq were removed using Picard (broadinstitute.github.io/picard) to reduce effects of PCR bias.

Peak calling for ATAC-seq and ChIP-seq was performed with MACS v1.4.2 (Zhang et al., 2008) with default settings. ATAC-seq peaks were further filtered against previously generated DNaseI-sequencing datasets performed at each cell stage through ESC in vitro haematopoietic differentiation (Goode et al., 2016) in order to obtain a set of high-quality peaks which can be replicated across multiple assays. The DNaseI hypersensitive site

peaks were merged across all cell types to identify any possible regions of open chromatin. Overlaps between ATAC-seq and DNaseI-seq were accepted if the summit of the ATAC-seq peak was positioned between the start and end coordinates of a peak in the merged DNaseI-seq dataset. ChIP-seq peaks were filtered against the ATAC-seq peaks using BEDTools and Homer (Heinz et al., 2010) to generate a set of high confidence ChIP-seq peaks representing a binding site in an open chromatin region. Peaks were annotated to gene promoters if within 2 kb of a TSS and as distal, otherwise. Peaks were annotated to the closest gene using Homer AnnotatePeaks.pl (Heinz et al., 2010).

To perform clustering analysis, the ATAC-seq datasets were first merged to identify any potential regions of open chromatin. The peaks were defined as being the same open region between datasets if the summits were within 200 bp of each other using BEDTools v2.25.0 intersect function. The peaks were normalised by total read depth within each sample. Pearson correlation coefficients were calculated between samples using the log2 of the normalised read counts of each peak. Hierarchical clustering of ATAC-seq peaks was performed with average linkage clustering of the Euclidean distances using R. Heatmaps were generated using Mev (Saeed et al., 2006) or with R. ChIP data was clustered in the same way.

The read count across the summit over a 2 kb window was measured using Homer AnnotatePeaks.pl function with the wig file generated by MACS. Average profiles of the average read counts across the 2 kb window of the peak were plotted using R. Density plots were created by first calculating the fold-difference of the read count within peaks between each dataset being compared. The fold-difference was calculated by counting the number of mapped reads in a 400 bp window centred on the peak summit, and then normalised by total tag count across all peaks. Peaks were then ranked according to this

fold-difference, and the read density was calculated in a 2kb window surrounding the peak summits. The density plot was then plotted using Java TreeView (Saldanha, 2004).

De novo motif analysis was performed with the Homer FindMotifsGenome.pl script. Motifs were filtered based on similarity to the consensus motif and presence in at least 2% of sites with a significant p value ($p \leq 0.01$). Motif enrichment plots were made by plotting the position of the motif across the 2 kb window in the union of peaks used in the density plot.

2.15.2 RNA-seq data analysis

Sequencing quality was verified using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adaptor sequences and low-quality bases were removed from the raw sequencing data using Trimmomatic v0.35 (Bolger et al., 2014). RNA-Seq paired-end reads were aligned to the mm10 mouse genome build using STAR (Dobin & Gingeras, 2002) with the default parameters. Separate density tracks for the positive and negative strand were generated for RNA-Seq data using bedtools (http://bedtools.readthedocs.io/en/latest/) and were uploaded to the UCSC genome browser. Fragments per kilobase of transcript per million mapped reads (FPKM) values for each gene were calculated using Cufflinks (http://cole-trapnell-lab.github.io/cufflinks/) and differentially expressed genes were determined using Cuffdiff function (using individual replicates). All genes with FPKM ≥ 1 in at least one condition were considered. The replicates were merged using Cuffdiff and the average FPKM value across replicates was used as a summary metric in the clustering analyses and subsequent plotting of heatmaps to show how the expression between cell types clustered (simplifying the heatmaps due to the large number of samples/conditions in the study). Note the high correlation between replicates enabled them to be merged (see Results chapter 3.2.1 for replicate correlations).

RNA-seq data between samples was normalised using the entire distribution of FPKM values for all genes, rather than just those of housekeeping genes to avoid introducing false variation between samples. The RNA-seq data was validated by checking the expression of developmentally-regulated genes were expressed as expected across the course of differentiation (e.g. *Fli1, Runx1, Oct4*) and checking if differentially expressed genes (found by RNAseq) are also differentially expressed by manual qPCR (both approaches discussed in more detail in results section 3.2.1).

The correlation between any two samples was obtained with Pearson correlation coefficient of log2 FPKM values over all genes. A correlation matrix was generated for all the samples and hierarchically clustered using Mev (Saeed et al., 2006).

For comparisons in gene expression between samples within the same cell type, a two-fold cut off was used to classify deregulated genes. For changes in expression between samples of genes that are differentially expressed between cell stages of differentiation, deregulated genes were identified by a two-fold change between clone and WT if the gene is differentially expressed by a two-fold change between cell stages in both the clone or WT cells, while only 1.5-fold change used if the change was at least two-fold in either WT or clone between stages. This was identified as the most significant threshold to consider.

Clustering of gene expression was carried out on log2 FPKM for all expressed genes and on fold-changes for genes associated with fold changes described above. Hierarchical clustering was used with Euclidean distance and average linkage clustering. Heatmaps were generated using Mev (Saeed et al., 2006). Gene expression fold changes were grouped according to patterns of expression throughout differentiation. Twelve clusters of expression patterns were identified for each differentiated stage – this covers all the ways

the gene expression between cell stages could change compared with WT (e.g. gene is upregulated in both WT and Sp1 mutant, more upregulated in Sp1 mutant, less downregulated in Sp1 mutant etc.).

Gene ontology analysis was performed using DAVID v6.8 (Huang et al., 2008). The GSEA software (Subramanian et al., 2005) was used to perform gene set enrichment analysis on groups of genes.

3. RESULTS

3.1 Knockout of Sp1 in ESCs disrupts haematopoietic specification

3.1.1 Targeting of Sp1 in ESCs by the CRISPR-Cas9 system

Deletion of the DBD-coding region of the *Sp1* gene in mouse ESCs has been previously shown, by use of the in vitro differentiation system, to lead to a failure in terminal differentiation of the haematopoietic lineage (Gilmour et al., 2014). However, the precise role of Sp1 in haematopoietic specification and the mechanism behind the impaired haematopoiesis in Sp1 DBD-deficient ESCs is still not well understood. Additionally, there is no complete Sp1 knockout model available, which could help to elucidate the function of Sp1 in haematopoiesis. Therefore, to further investigate these questions, we aimed to recapitulate the previous model by deleting the DBD-coding region of *Sp1* in A17 2lox murine ESCs, as well as develop a complete Sp1 knockout ESC model.

A number of genome editing techniques have been developed over recent years, including zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) that involve generating unique protein DNA-binding domains tethered to a nuclease domain. However, the CRISPR-Cas9 system is becoming more widely used due to its high efficiency, specificity and ease compared to ZFNs and TALENs. The CRISPR-Cas9 technique (reviewed by (Zhang et al., 2014)) allows targeted knockout/mutation with the use of a vector expressing the endonuclease Cas9 and an RNA molecule complementary to the target site. The ‘guide’ RNA forms a hybrid structure with a transactivating crRNA (tracrRNA), which is recognised by Cas9. Subsequently, Cas9 is directed to the DNA target sequence, where, providing there is a 5’ protospacer adjacent motif (PAM) present, Cas9 cleaves the DNA to cause a double-strand break.

Thus, the combination of two guide RNA molecules can give deletion of the intervening DNA. The DNA double-strand break can also be repaired by error-prone non-homologous end-joining (NHEJ), which can introduce random mutations (Figure 3.1A).

Guide RNAs were initially designed to target the DBD-coding region of *Sp1* – at the start of exon 5 and at the end of the coding region (after exon 6). PCR primers were designed to isolate the target region and enable screening of the treated cells (Figure 3.1B). Two guide sequences were initially identified at each site (guides 1 and 3 at the start of the DBD-coding region; guides 14 and 18 at the end) and the efficiency to target Cas9 was assessed using an in vitro CRISPR-Cas9 system (Agilent). The target region was amplified from genomic DNA by PCR, before incubating with the RNA guides in two alternative combinations and the Cas9 protein. DNA cutting efficiency at the target sites was analysed from the resulting digestion pattern. The results showed that a combination of guides 1 and 18, as well as guides 3 and 14, were predominantly targeting to only one site. However, the major product of the alternate guide pairing was the desired fragment generated by cutting at both target sites (Figure 3.1C) and thus, this guide combination was used in in vivo experiments.

The guide sequences were cloned into PX458, a Cas9-GFP expressing plasmid, and were expressed under the control of the U6 promoter. Upon transfection of the pairs of plasmids, the A17 2lox ESCs were sorted for GFP expression and the bulk populations were analysed by PCR. A successful gene targeting event was detected in the 1+14 population (Figure 3.1D). The cells were further sorted as single cells and the expanded clones screened for *Sp1* mutations by PCR.

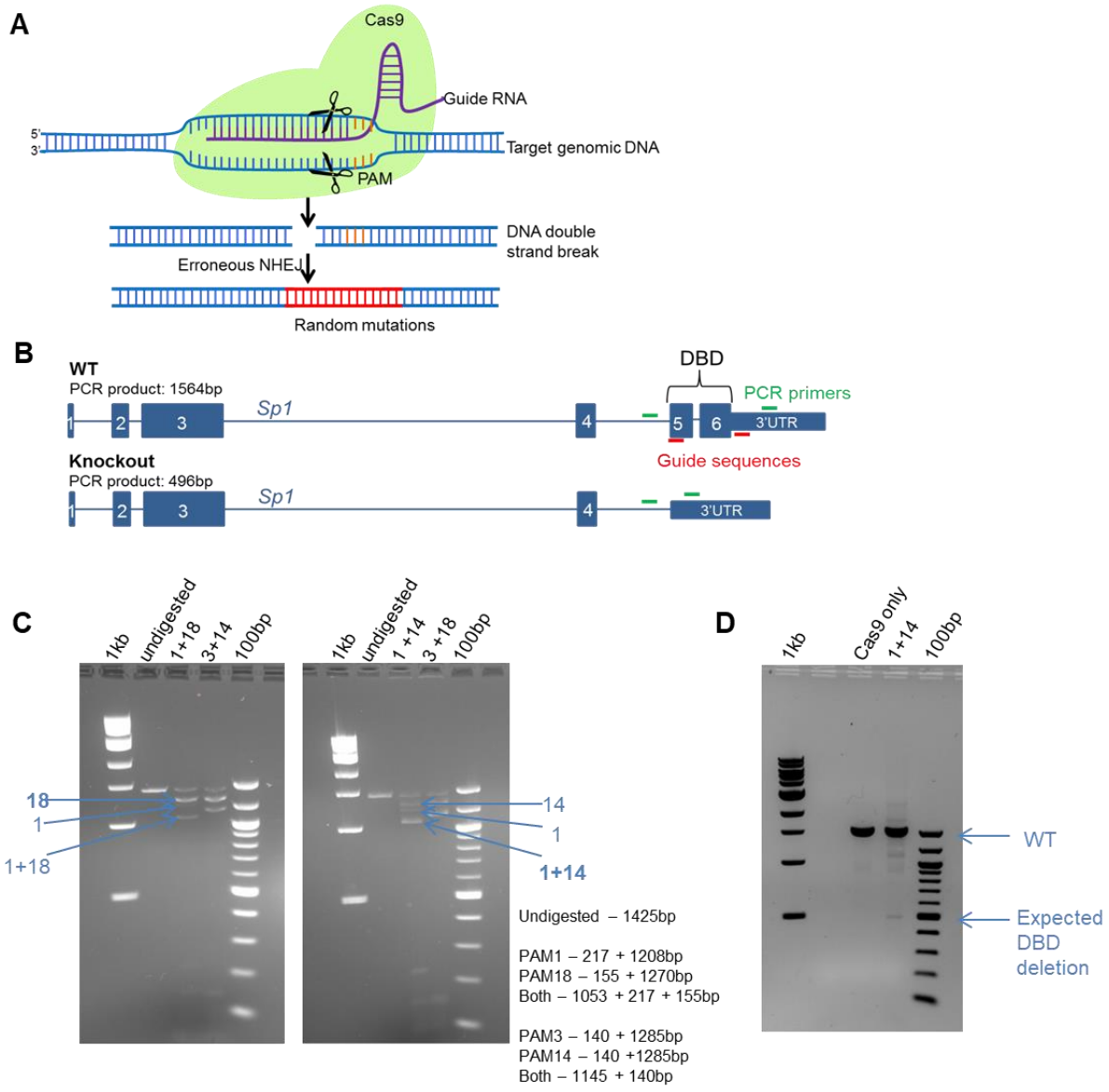


Figure 3.1 – CRISPR-Cas9 targeting strategy to knockout the Sp1 DNA binding domain

(A) Schematic representation of the CRISPR-Cas9 strategy to introduce random mutations at a target site via non-homologous end joining (NHEJ). (B) Illustration of the *Sp1* gene, showing the position of the targeted sites flanking the DBD-coding region (red) and the position of the primers used to isolate the target region and screen the CRISPR-Cas9 targeted cells (green). The expected product of the CRISPR-Cas9 targeting is depicted, showing deletion of the DBD-coding exons. (C) The efficiency of the guide sequences to target Cas9 was tested using an in vitro CRISPR kit (Agilent). The target region was amplified by PCR using genomic DNA as the template. The PCR product was incubated with the RNA guides (1 and 3 designed to target the start of the DBD-coding region; 14 and 18 to target the end) in two alternative combinations, plus Cas9 recombinant protein, before the digestion pattern was analysed by gel electrophoresis. The expected fragment sizes from cuts at the corresponding sites are indicated. (D) A17 2lox cells were transfected with PX458 plasmids encoding guides 1 and 14. The cells were sorted for GFP expression, the genomic DNA was isolated and a PCR performed with primers flanking the target region of *Sp1*. Expected WT product size is 1564 bp, while the DBD knockout would be 496 bp.

3.1.2 Generation of ESC lines targeted deletions in the Sp1 locus

ESC clones that had been successfully targeted to disrupt the *Sp1* gene were expanded and deletions were verified by PCR analysis (Figure 3.2A). The clones were further characterised by qPCR to analyse *Sp1* gene expression using primers in the target region (exons 5-6) and upstream in the gene, which should be unaffected by CRISPR-Cas9 activity (exons 3-4) (Figure 3.2B). The Sp1 protein expression was also analysed by Western blotting (Figure 3.2C). Although PCR analysis of clone 12 detected the presence of a mutant allele (in addition to a wildtype allele), there was no change in the Sp1 protein; therefore clone 12, along with clone 2 which appeared similar to WT in all analyses, was excluded from further investigations. The PCR products derived from DNA of each of the remaining clones were integrated into the pBlueScript plasmid to allow sequencing of the individual alleles, with the resulting mutations illustrated in Figure 3.2D and the full sequence information in Supplementary Data 5.1.

Clone 4 was the only clone that carried the desired DBD knockout (and so termed $Sp1^{del/del}$): the DNA sequence corresponded to accurate cutting at the targeted sites and high fidelity repair, and Western blotting showed it expressed only a truncated Sp1 protein (of a size corresponding to a DBD deletion). Interestingly, there was a much lower level of Sp1 protein compared to in WT cells, suggesting the truncated protein is less stable. A similar phenomenon was seen with the previous Sp1 DBD-deficient model (Gilmour et al., 2014), which gave us confidence that this was indeed the correct protein. Furthermore, while no *Sp1* gene expression was detected in exons 5-6, as expected, there was unusually high expression of exons 3-4 compared to WT cells, suggesting the cell may be trying to compensate for the lack of a functional Sp1 protein.

PCR analysis of clone 14 showed a slightly smaller product than that of WT cells, lower RNA expression levels by qPCR and, surprisingly, no Sp1 protein. DNA sequencing revealed a small out-of-frame deletion around the first cut site, causing a nonsense mutation and potentially RNA degradation due to nonsense mediated decay. Thus, a complete Sp1 knockout ESC line was generated (termed $Sp1^{-/-}$). This also meant that although we originally planned to use the CRISPR-Cas9 system to delete the whole *Sp1* gene, it was no longer necessary.

Clone 5 and 7 were heterozygous clones, with only one allele targeted and mutated, thus generating cells expressing both WT and truncated Sp1 protein (so termed $Sp1^{+/del}$), with RNA expression of a comparable levels to that of WT. Clone 11 was also heterozygous, but both alleles were targeted – one allele had a large deletion in exon 5, whereas the second allele had just a small disruption in the 5' UTR, resulting in the expression of both a truncated Sp1 and a protein of approximately equal size to wildtype Sp1.

In summary, we generated another Sp1 DBD-deficient ESC line, in order to recapitulate the previous model. In addition, we created ESC lines that are heterozygous for this mutation, as well as a complete Sp1 knockout ESC clone, which together will be used in in our studies aimed at elucidating the role of Sp1 in development.

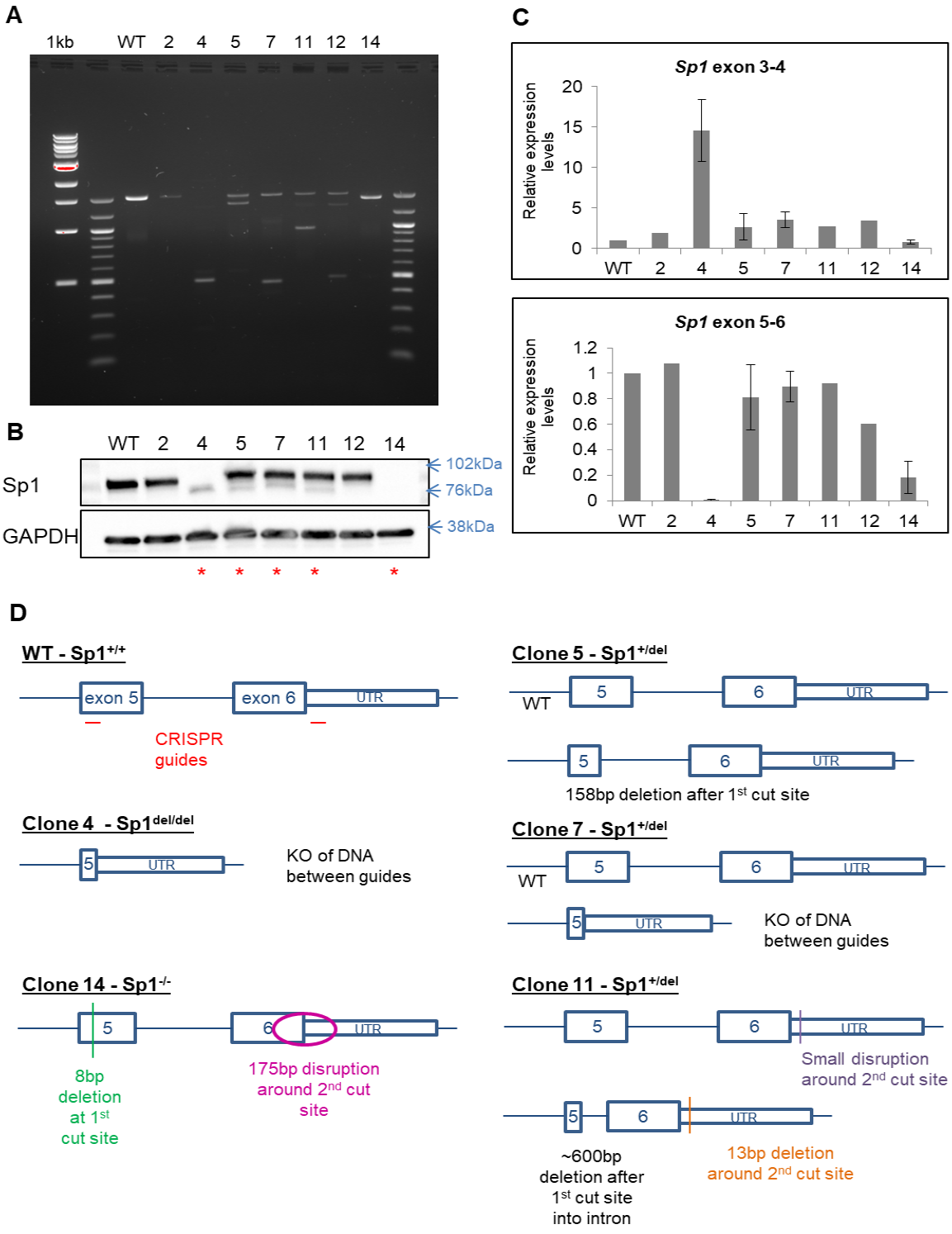


Figure 3.2 – Generation of ESC lines lacking the Sp1 DBD, heterozygous cells expressing WT Sp1 and DBD-deficient Sp1, and a complete Sp1 null line.

(A) ESCs were transfected with Cas9-GFP plasmids, also expressing guides sequences 1 and 14 to target the Sp1 DBD. Successfully targeted clones were analysed for genomic DNA mutations by PCR. (B) Sp1 protein levels in the clones were analysed by Western blotting (asterisk indicates a mutant phenotype) (n=3). (C) RNA expression levels of *Sp1* were analysed by qPCR using primers spanning exons 5 and 6 (the region targeted for deletion), and exons 3 and 4 (which should be unaffected). Expression levels were normalised to those of WT cells. Error bars represent standard deviation (n=3). Clone 4

showed significantly different expression levels ($p \leq 0.01$) to WT cells at both exons 3-4 and exons 5-6, while clone 14 was significantly different to WT at exons 5-6 only (as calculated by an unpaired t-test). (D) Illustration of the mutations present in the successfully-targeted clones within each individual allele.

3.1.3 Sp1 mutation impairs differentiation of ESCs to the haematopoietic lineage

To examine the differentiation potential of the targeted Sp1 ESC clones, the cells were differentiated towards the haematopoietic lineage using the previously described in vitro differentiation system (Gilmour et al., 2014; Obier et al., 2016). Briefly, the cells are cultured to form colonies called embryoid bodies (EBs), before being sorted for Flk1-expressing cells. The Flk1+ cell population contains cells that are equivalent to the haemangioblast stage of haematopoietic specification and which possess smooth muscle, endothelial and haematopoietic potential. The cells are then cultured to generate blast cell colonies containing the haemogenic endothelium (HE), which progress through two stages as they acquire haematopoietic markers and lose endothelial features (HE1 Kit+ Tie2+ CD41-; HE2 Kit+ Tie2+ CD41+) and subsequently differentiate into non-adherent multipotent haematopoietic progenitors (Kit+ Tie2- CD41+) (fully committed to the blood lineage) (Figure 3.3).

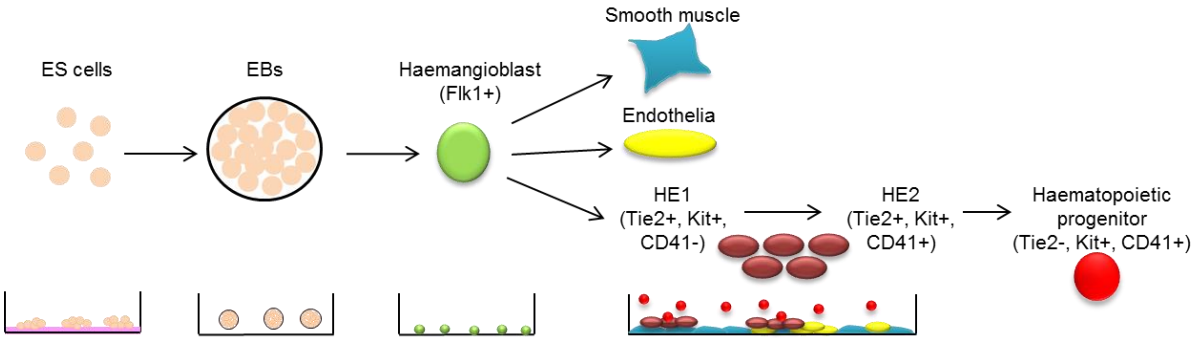


Figure 3.3 – In vitro model of embryonic haematopoiesis
Schematic of in vitro ESC differentiation to mimic embryonic haematopoiesis. ESCs are maintained on embryonic fibroblasts in LIF-containing medium before beginning culture as embryoid bodies (EBs) for 3.75 days. Flk1+ cells, which contain haemangioblast cells, are sorted from dissociated EBs and, during a blast culture of 2-3 days, give rise to smooth muscle and endothelial lineages, as well as haematopoietic progenitors via a haemogenic endothelium (HE) intermediate stage.

The cells were analysed at the Flk1+ stage for the proportion of Flk1+ cells in the EBs by cell staining and flow cytometry. The blast culture was analysed by staining for Kit, CD41 and Tie2, followed by flow cytometry (Figure 3.4). The results show that the differentiation of the heterozygous clones was largely unaffected: $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7) cells differentiated to give a good proportion of Flk1+ cells in the EB culture and further differentiated to form both HE and progenitors at levels comparable to that of wildtype cells. However, the differentiation of $Sp1^{del/del}$ cells was impaired, generating consistently lower levels of Flk1+ cells than WT cells, but generally forming a similar proportion of HE and progenitors in the blast culture. A complete loss of Sp1 caused a severe impairment in haematopoietic specification. $Sp1^{-/-}$ cells failed to differentiate to the Flk1+ stage, forming a very low number of Flk1+ cells, suggesting Sp1 is essential for this stage of haematopoiesis. When the few Flk1+ cells were taken to the next stage of differentiation, while the blast culture looked poor and the proportion of haematopoietic cells was lower

than for WT, $Sp1^{-/-}$ cells were capable of forming HE and progenitors. These results show the importance of Sp1 in the process of haematopoietic specification. However, as $Sp1^{-/-}$ cells are unable to generate Flk1+ cells, it may be that Sp1 is essential at an early stage of the process, particularly in mesoderm formation (as not all Flk1+ are haemangioblasts). These results also suggest the Sp1 DBD-deficient cells (and indeed, the previously used model) likely still possess some Sp1 function – i.e. they are hypomorphs.

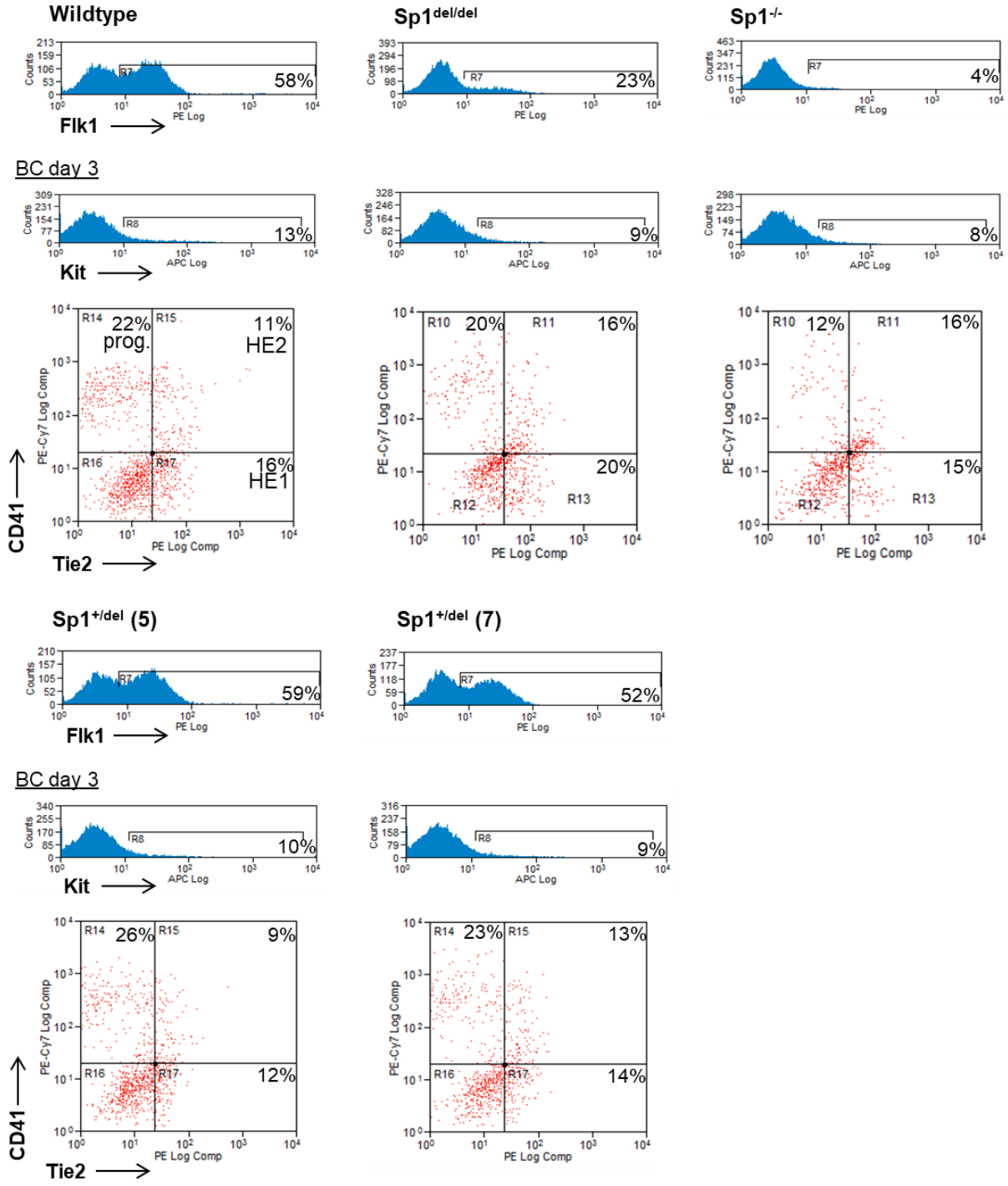


Figure 3.4 – Differentiation of Sp1 mutant cells is impaired

In vitro differentiation was performed with wildtype ESCs and with each of the CRISPR-generated Sp1 mutant clones. At the EB stage, the proportion of cells expressing Flk1 (a marker of the haemangioblast) was analysed by staining and flow cytometry (top plot). After three days blast culture, the cell population was analysed by staining for Kit, CD41 and Tie2. The cells were first gated for Kit expression (indicating cells with haematopoietic potential), before analysing the relative levels of CD41 and Tie2, which showed the proportion of HE and progenitors in the blast culture. n=4, except for $Sp1^{-/-}$ blast culture, which was only performed once due to low numbers of Flk1+ cells.

Previously, it was found that Sp1 DBD-deficient ESCs were impaired in terminal haematopoietic differentiation (Gilmour et al., 2014). To confirm this finding with the $Sp1^{del/del}$ ESC clone, and to investigate the terminal differentiation potential of the $Sp1^{-/-}$ cells, macrophage release assays were performed with each of the Sp1 clones (Figure 3.5). Briefly, ESCs were cultured in methylcellulose medium, supplemented with cytokines to promote macrophage differentiation. Consistent with the results from the in vitro differentiation experiments, the heterozygous clones efficiently formed macrophages, at similar levels to that of WT cells. $Sp1^{del/del}$ cells were significantly impaired in macrophage differentiation, whereas $Sp1^{-/-}$ cells were unable to form macrophages. These findings support those of previous study (Gilmour et al., 2014), which showed $Sp1^{del/del}$ cells can form haematopoietic progenitors, but fail to terminally differentiate to macrophages. However, the stages of haematopoiesis cannot be distinguished in the macrophage release assay. Therefore, it may be that the reduction in macrophage formation in the $Sp1^{del/del}$ and $Sp1^{-/-}$ cells stems from the defect in these cells to form Flk1+ cells.. Again, $Sp1^{del/del}$ cells show a less severe phenotype than $Sp1^{-/-}$ cells, suggesting they retain some Sp1 function.

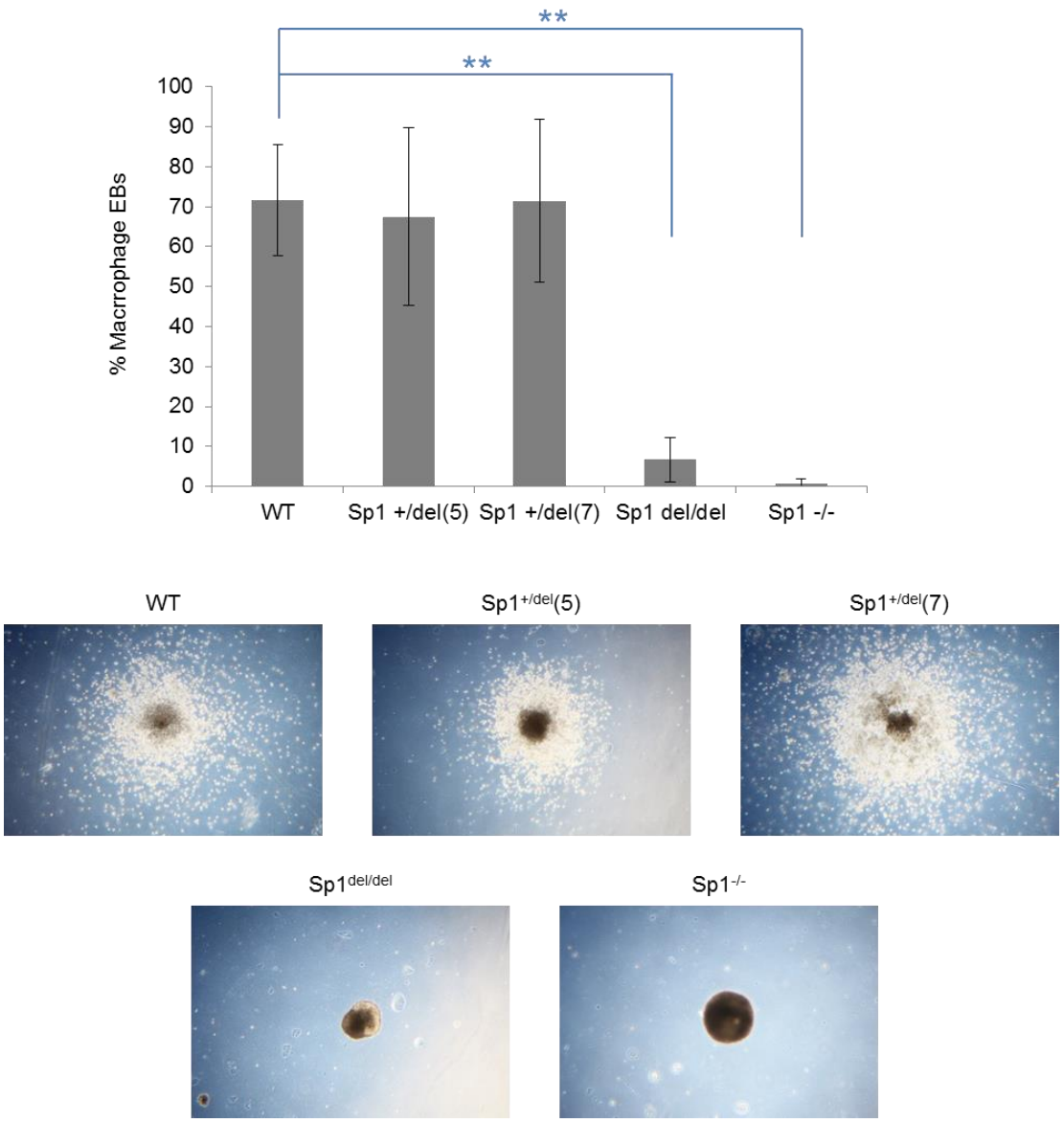


Figure 3.5 – Absence of Sp1 abolishes the ability to terminally differentiate into mature haematopoietic cells

Each of the Sp1 mutant ESC clones were placed in a macrophage release assay. EBs were allowed to form in methylcellulose under macrophage-promoting conditions. $Sp1^{-/-}$ and $Sp1^{del/del}$ cells showed a reduced capacity to form macrophage-releasing Ebs, while heterozygous cells that still contain a WT *Sp1* allele are unaffected. Error bars represent s.d. (n=3). ** indicates a $p \leq 0.01$, as calculated by an unpaired t-test with Bonferronni correction.

3.1.4 Haematopoiesis in the Sp1 mutant clones can be rescued with expression of WT Sp1

To verify the observed phenotypic effects were due to the relating Sp1 mutations and not off-target effects of CRISPR-Cas9 editing, $Sp1^{del/del}$ and $Sp1^{-/-}$ ESCs were transfected with a PiggyBac vector containing the WT human *SP1* gene with a mCherry reporter gene (section 2.8). The *SP1*-2A-mCherry gene (under the control of the constitutive PGK promoter) was randomly integrated into the genome and the cells sorted for mCherry expression, before individual clones were isolated. *SP1* expression was identified in two clones with a $Sp1^{del/del}$ background and one clone of $Sp1^{-/-}$. The gene expression was verified by qPCR (Figure 3.6A) and the protein expression was analysed by Western blotting, showing expression of a WT Sp1 protein in each PiggyBac-SP1 clone, albeit at lower levels than normally found in WT cells (Figure 3.6B).

The differentiation potential of the PiggyBac-transfected *SP1*-expressing clones was investigated using the in vitro differentiation system. All of the clones generated consistently higher levels of Flk1+ cells than the corresponding Sp1 mutant cells (Figure 3.6C) (see Results 1.1.3 for differentiation profiles of the original clones and Supplementary Figure 5.1 for Flk1+ levels of WT cells compared with Sp1 mutant clones). They could also differentiate further to HE and progenitors in the blast culture (Figure 3.6D). Thus, expression of a WT Sp1 protein was able to rescue the impairment in haematopoietic specification in $Sp1^{del/del}$ and $Sp1^{-/-}$ cells.

This result confirms that the defects in haematopoietic differentiation in $Sp1^{del/del}$ and $Sp1^{-/-}$ cells are caused by their corresponding *Sp1* mutations, rather than any off-target effects of the CRISPR-Cas9 targeting system.

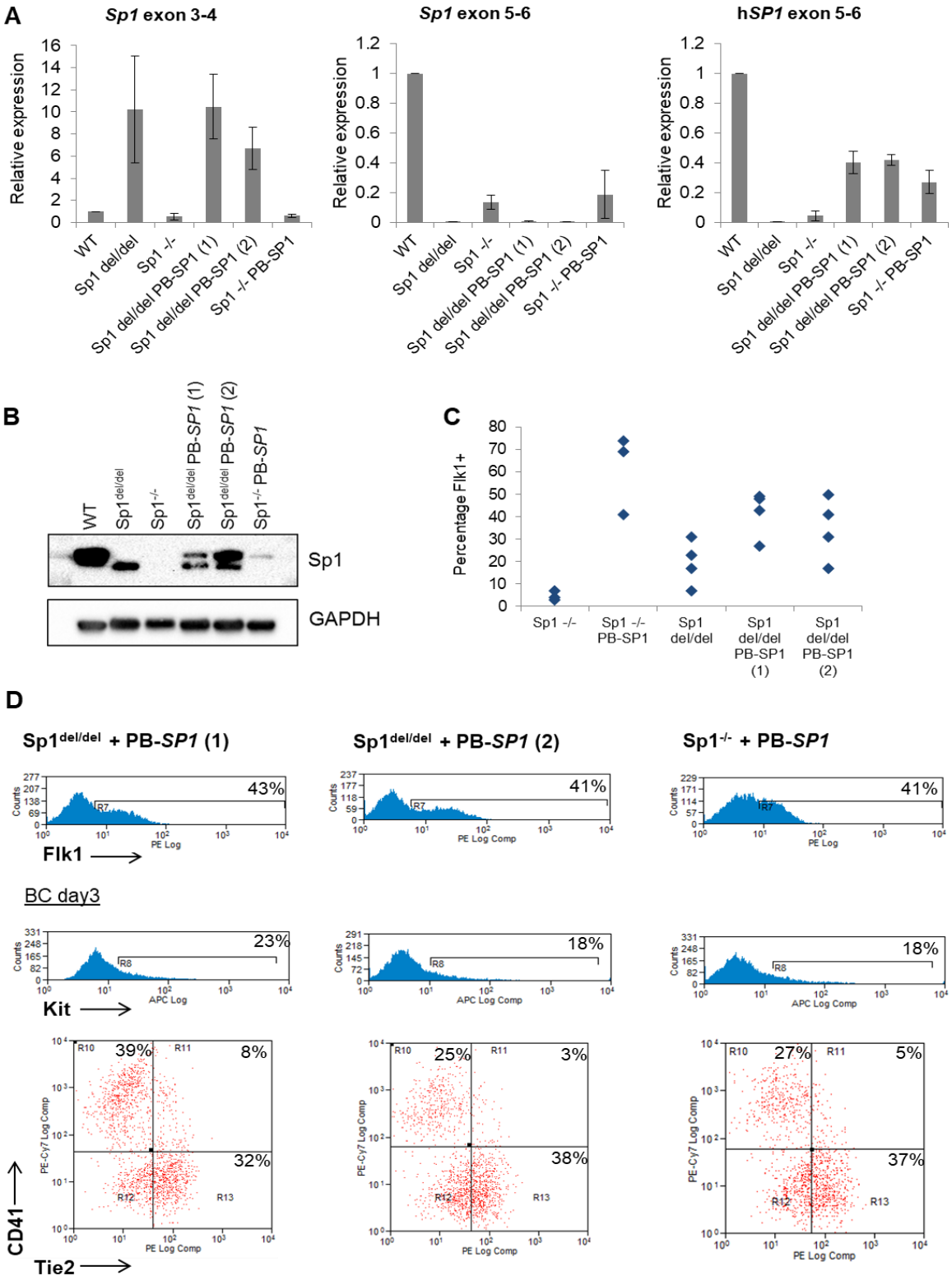

A
Sp1 exon 3-4
Sp1 exon 5-6
hSP1 exon 5-6
Relative expression
WT
Sp1 del/del
Sp1 -/-
Sp1 del/del PB-SP1 (1)
Sp1 del/del PB-SP1 (2)
Sp1 -/- PB-SP1
B
Sp1
GAPDH
C
Percentage Flk1+
Sp1 -/-
Sp1 -/- PB-SP1
Sp1 del/del
Sp1 del/del PB-SP1 (1)
Sp1 del/del PB-SP1 (2)
D
Sp1del/del + PB-SP1 (1)
Sp1del/del + PB-SP1 (2)
Sp1-/- + PB-SP1
43%
41%
41%
Flk1
BC day3
23%
18%
18%
Kit
39%
8%
32%
25%
3%
38%
27%
5%
37%
CD41
Tie2

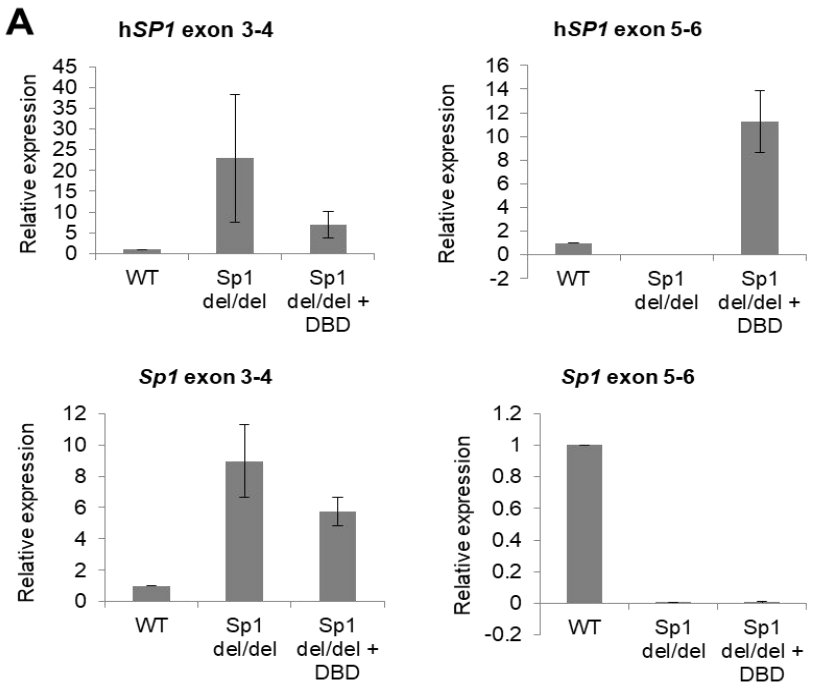
Figure 3.6 – The Sp1 mutant phenotype can be rescued by the constitutive expression of WT Sp1

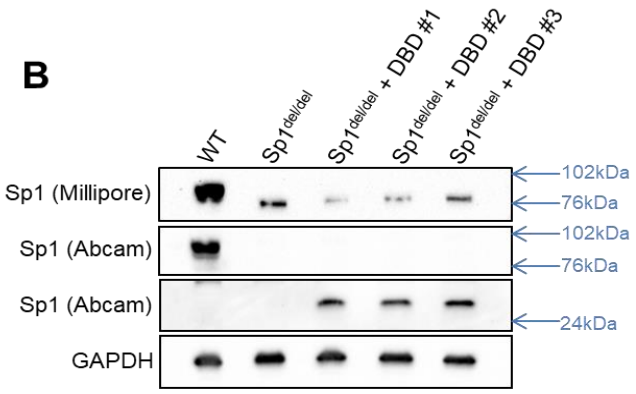
(A,B) The WT human *SP1* gene was integrated into $Sp1^{-/-}$ and $Sp1^{del/del}$ ESCs using the PiggyBac system and individual clones selected. Expression of the *SP1* gene was analysed by qPCR (A) and protein levels were measured by Western blotting (B) (n=3). Each of the PiggyBac-*SP1* treated clones expressed significantly ($p \leq 0.01$) higher levels of h*SP1* at exons 5-6 than its corresponding Sp1 mutant cell clone, as calculated by unpaired t-test. (C,D) *SP1*-expressing ESCs were differentiated in vitro and levels of Flk1 at the EB stage, then levels of Kit, CD41 and Tie2 at day 3 of blast culture, were measured by staining and flow cytometry. The percentage of Flk1+ cells over 3 differentiation experiments generated by *SP1*-expressing cells, compared to $Sp1^{-/-}$ and $Sp1^{del/del}$ cells, were plotted (C). Flow cytometry plots from a representative differentiation culture are shown (D). n=4 for $Sp1^{del/del}$ and its corresponding rescue clones; n=3 for $Sp1^{-/-}$ and its corresponding rescue clones.

3.1.5 Expression of the Sp1 DBD in $Sp1^{del/del}$ may be sufficient to recover haematopoietic potential

The Sp1 protein is made up of distinct domains, such as two TADs and a DBD. Would the expression of individual Sp1 protein domains be able rescue the mutant phenotype to varying extents? $Sp1^{del/del}$ cells express all of the Sp1 protein except the C-terminal DBD. Therefore, we asked whether the expression of the complementary DBD region in $Sp1^{del/del}$ cells would be sufficient to rescue the impaired haematopoietic phenotype. The PiggyBac system was used to express the human Sp1 DBD in $Sp1^{del/del}$ ESCs and the expression in a single clone was verified by qPCR (Figure 3.7A) and Western blotting (Figure 3.7B). In vitro differentiation of the DBD-expressing $Sp1^{del/del}$ clone showed a recovery of haematopoietic potential, forming higher levels of Flk1+ cells than $Sp1^{del/del}$ cells and forming a good percentage of haematopoietic progenitors in the blast culture (Figure 3.7C). These results suggest expression of the complementary regions of the Sp1 protein in $Sp1^{del/del}$ hypomorphic cells is sufficient to rescue the impaired haematopoietic differentiation. It is possible that the DBD is able to associate with the N-terminal portion of the Sp1 protein to elicit gene expression changes. However, this is only a preliminary

result; only one clone was found to express the DBD efficiently (likely due to the instability of expressing an individual domain away from the context of the complete protein) and so the influence of ESC clonal differences on the results cannot be excluded.





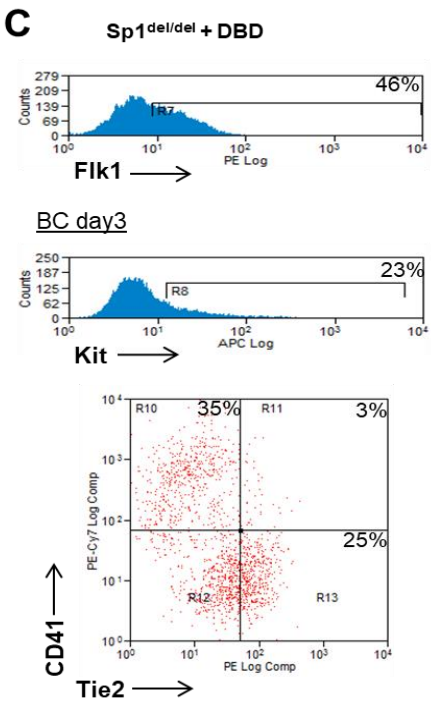


Figure 3.7 – Expression of the DBD of the Sp1 protein in Sp1$^{del/del}$ ESCs can rescue haematopoietic specification

The PiggyBac system was used to express the human Sp1 DBD in Sp1$^{del/del}$ cells. (A) Expression of the *SP1* DBD in a single clone was verified by qPCR using primers specific to both mouse and human Sp1 regions (n=3, error bars represent standard deviation). The expression of the DBD, as shown by h*SP1* exon 5-6 primers, was significantly ($p \leq 0.01$) higher in Sp1$^{del/del}$+DBD cells than WT or Sp1$^{del/del}$ cells, as calculated by unpaired t-test. (B) Expression of the Sp1 DBD protein (over 3 replicates) was measured by Western blot using a Millipore Sp1 antibody recognising the full-length protein, except DBD, and an Abcam antibody that recognises only the C-terminus. (C) In vitro differentiation of the Sp1$^{del/del}$ + DBD ESCs. The proportion of Flk1+ cells in the EBs was measured and the levels of haematopoietic populations in the blast culture at day 3 was analysed by flow cytometry after staining with antibodies against Kit, CD41 and Tie2 surface molecules. The experiment was performed three times and representative plots are shown.

3.2 Disruption of Sp1 is associated with gene expression changes related to defects in haematopoietic differentiation

3.2.1 Global gene expression in WT and mutant cells at each stage of differentiation

In order to investigate whether the defects in Sp1 result in changes in gene expression, and whether these changes could explain the observed defects in haematopoietic specification in the Sp1 mutant clones, we performed RNA-seq throughout differentiation. Purified cell populations were obtained at each stage of differentiation: ESCs, Flk1+ cells (obtained by MACS), and HE1, HE2 and progenitors (obtained by harvesting the blast culture and immunostaining for Tie2, Kit and CD41, followed by FACS) (Supplementary Figure 5.2). The purity of the resulting populations was verified by cell staining for the associated markers and flow cytometry (Figure 3.8). RNA was purified from the cells and next generation sequencing performed to analyse gene expression levels genome-wide. RNA-seq was performed in duplicate, with the duplicate correlations shown in Supplementary Figure 5.1, and combined for further analyses. Unsurprisingly, the RNA-

seq browser tracks show an absence of reads over exons 5 and 6 of *Sp1* in $Sp1^{del/del}$ cells (Figure 3.9A). Furthermore, developmental stage-specific genes can be seen to change expression across differentiation stages as expected, such as *Fli1* (Figure 3.9B), thus validating the RNA-seq experiments.

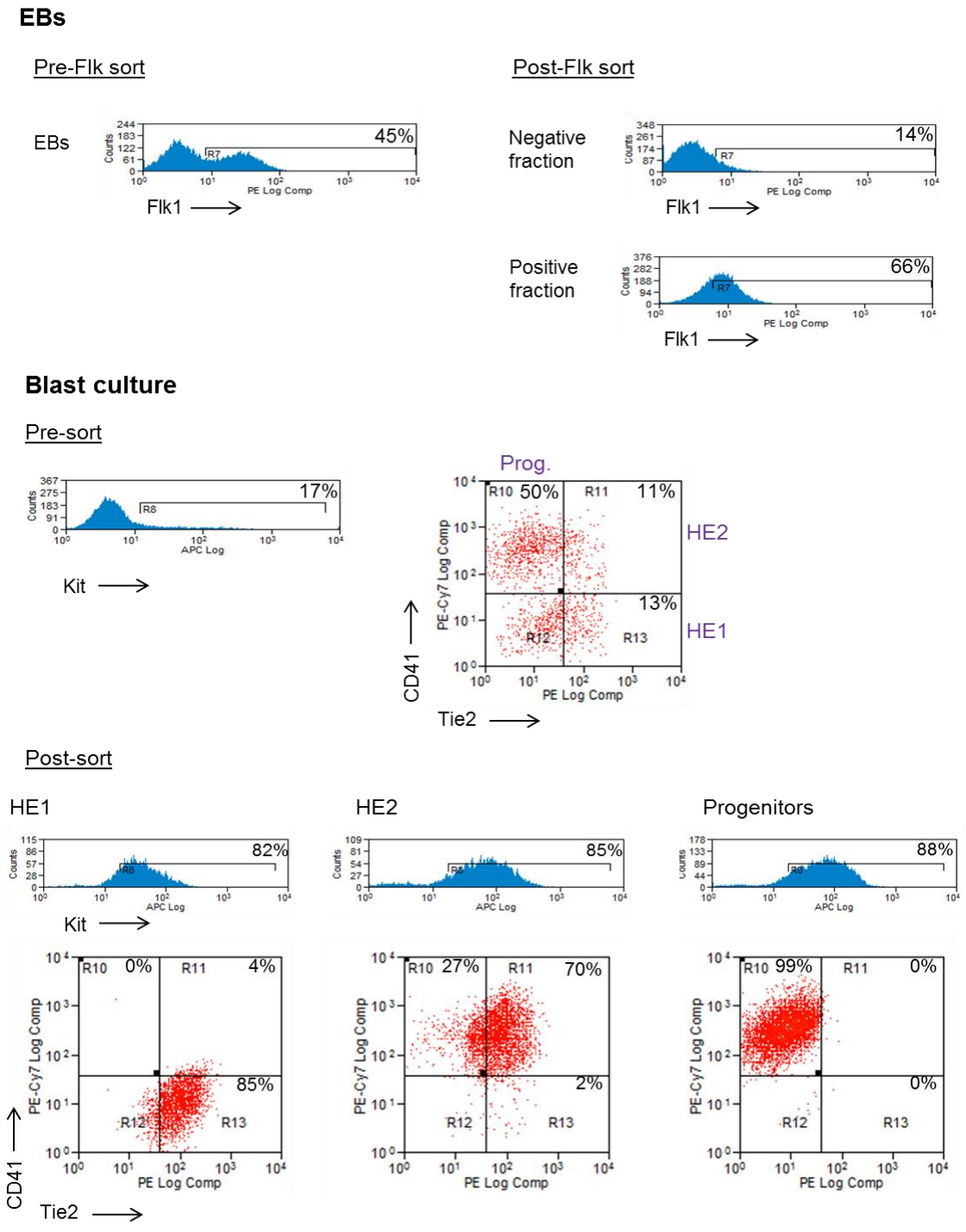


Figure 3.8 – Specific cell populations through differentiation were isolated for RNA-seq

Flk1+ cells were isolated from EBs and the Flk1 levels in both the negative and positive fractions verified by cell staining and flow cytometry, before taking the Flk1+ cells for RNA. Flk1+ cells were also plated for blast culture and harvested the cells at day 2. The cells were sorted into HE1, HE2 and progenitor populations by levels of Kit, CD41 and Tie2 and the resulting population verified by flow cytometry, before using the cells for RNA extraction. Performed twice for each cell clone (except $Sp1^{-/-}$) to obtain biological RNA-seq duplicates.

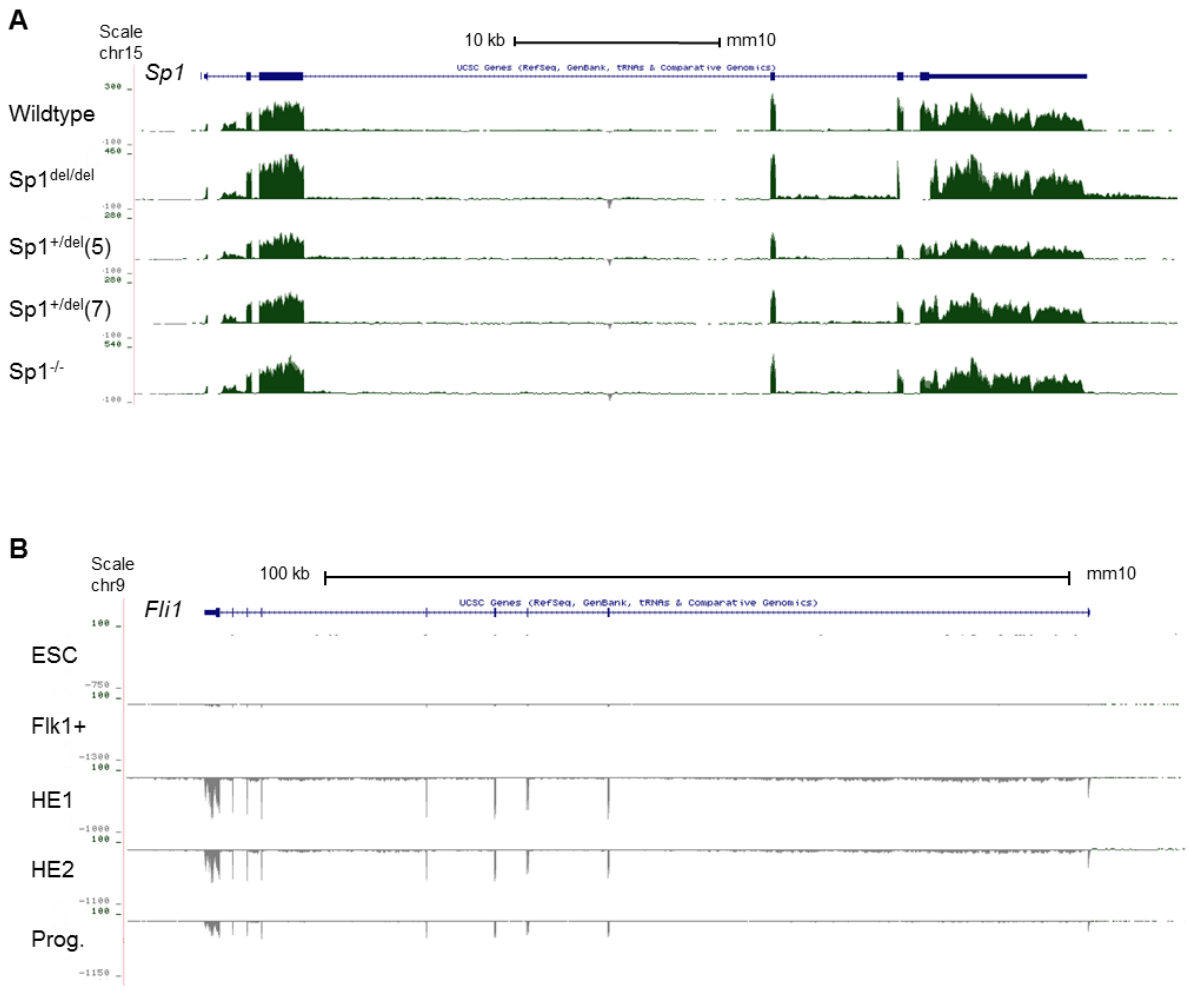


Figure 3.9 – RNA-seq data confirm the *Sp1* mutation and show differential expression of key developmental regulator genes

(A) RNA-seq analysis was performed for each of the CRISPR-generated Sp1 mutant clones. The browser track at ESC stage at the *Sp1* gene shows the deletion of exons 5 and 6 in $Sp1^{del/del}$ cells, with a concomitant increase in gene expression. (B) RNA-seq was carried out at each stage of haematopoietic specification. *Fli1*, an example of a stage-specific transcriptional regulator gene, is shown with expression levels changing through differentiation in WT cells.

3.2.2 Global gene expression patterns are largely unaffected by the Sp1 mutation

We then asked if gene expression profiles were changed as a result of *Sp1* mutation. Hierarchical clustering analysis of the similarity between the RNA-sequences of each Sp1 mutant cell clone at each stage of differentiation showed that gene expression patterns clustered based on cell type (or differentiation stage), rather than by *Sp1* mutation. A high correlation was also seen within each cell type between the different Sp1 mutant clones and WT cells (Figure 3.10). Moreover, hierarchical clustering of the expression of all expressed genes at each individual stage of differentiation showed a generally similar pattern of gene expression in each clone compared to the WT (Figure 3.11). However, while the overall patterns of highly and lowly expressed genes were similar, there were some differences in the actual expression levels; this was particularly apparent in the progenitor cells.

Overall, these results suggest that the gene expression profiles are largely unaffected with *Sp1* mutation and the global expression pattern is very similar in the Sp1 mutant clones and WT cells. Indeed, there were only a few hundred genes significantly deregulated (by at least two-fold) at each stage in the Sp1 mutant clones compared to WT cells (Figure 3.12). However, the number of deregulated genes was higher in $Sp1^{del/del}$ and $Sp1^{-/-}$ cells than in the heterozygous clones, while these clones also generally clustered away from WT and heterozygous cells when examining expression levels of every expressed gene (Figure 3.11), suggesting gene expression changes upon Sp1 mutation could be linked to impaired haematopoietic differentiation. Intriguingly, the number of deregulated genes was larger in hypomorph ESCs than complete Sp1 knockout cells, despite the less severe phenotype, which may indicate heterogeneity in the population and reflect the diverse phenotypes seen in the Sp1 DBD-deficient mouse model (Marin et al., 1997). Interestingly, many histone genes were consistently downregulated in $Sp1^{-/-}$ and $Sp1^{del/del}$

cells across the differentiation stages, likely reflecting their slower growth observed in culture. Slower growth can be expected, as Sp1 regulates a number of genes involved in the cell cycle and growth (Li & Davie, 2010).

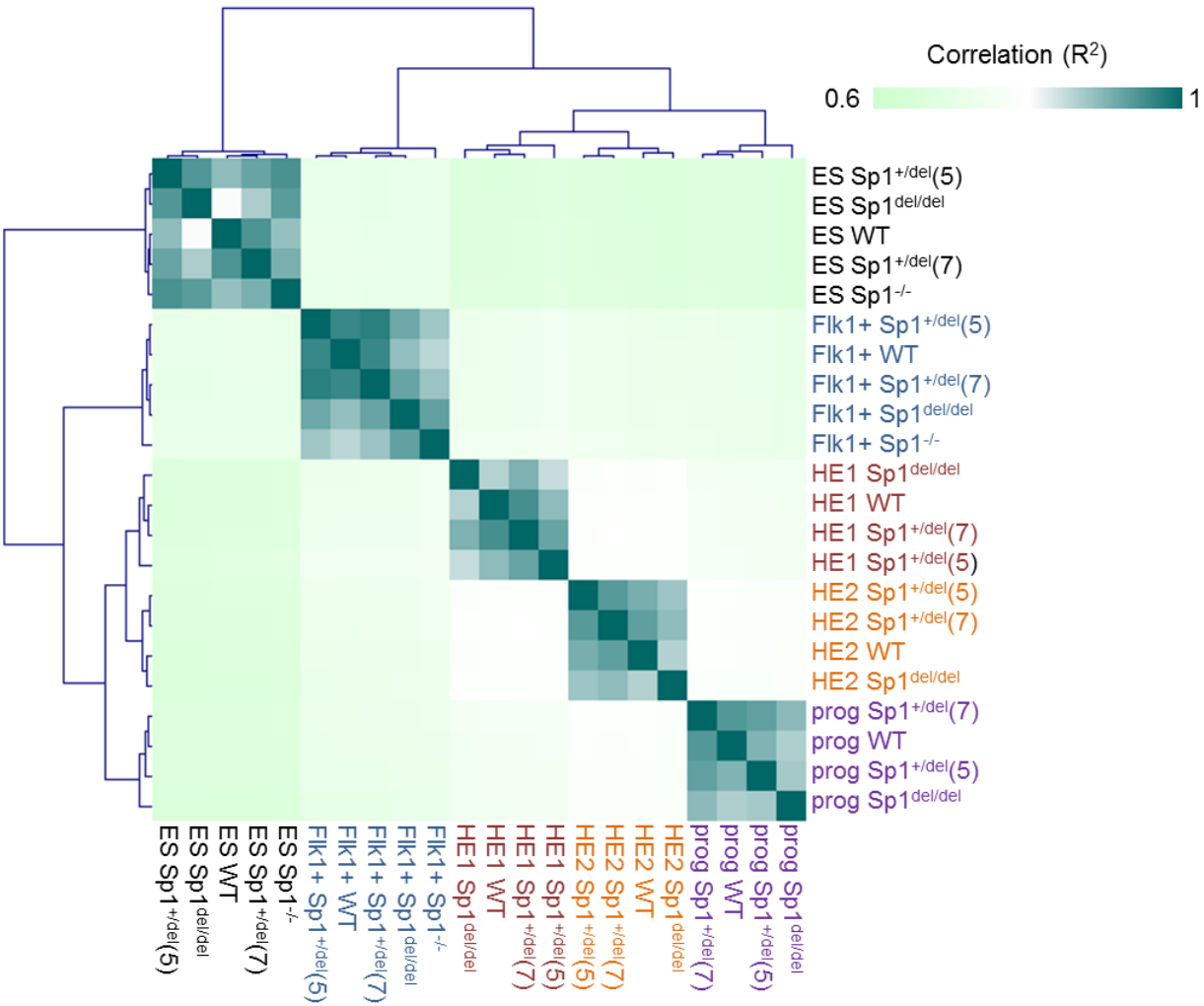


Figure 3.10 – Gene expression of WT and Sp1 mutant clones cluster based on differentiation cell type

RNA-seq was performed in WT cells and Sp1 mutant clones at each stage of in vitro haematopoietic differentiation. The Pearson correlation of the global gene expression levels between all clones (pairwise), at each cell stage, was calculated. Hierarchical clustering of the Pearson correlations was performed and the results shown as a heatmap.

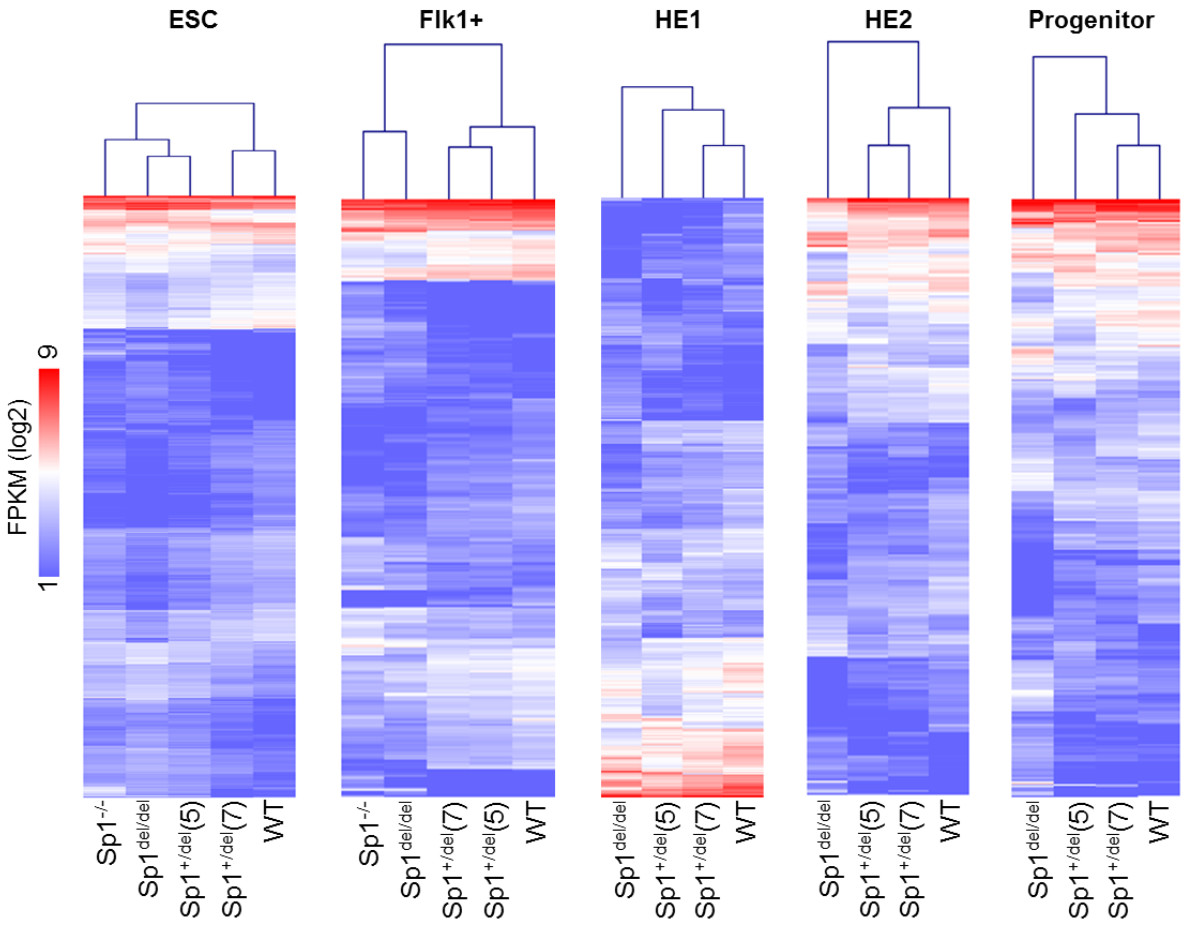


Figure 3.11 - Global gene expression is similar at each stage of differentiation between the different Sp1 mutant clones

RNA-seq was performed in duplicate in each Sp1 clone at each stage of differentiation and duplicates were combined. Hierarchical clustering of all expressed genes (based on level of expression FPKM) was performed at each differentiation stage in all clones compared to WT.

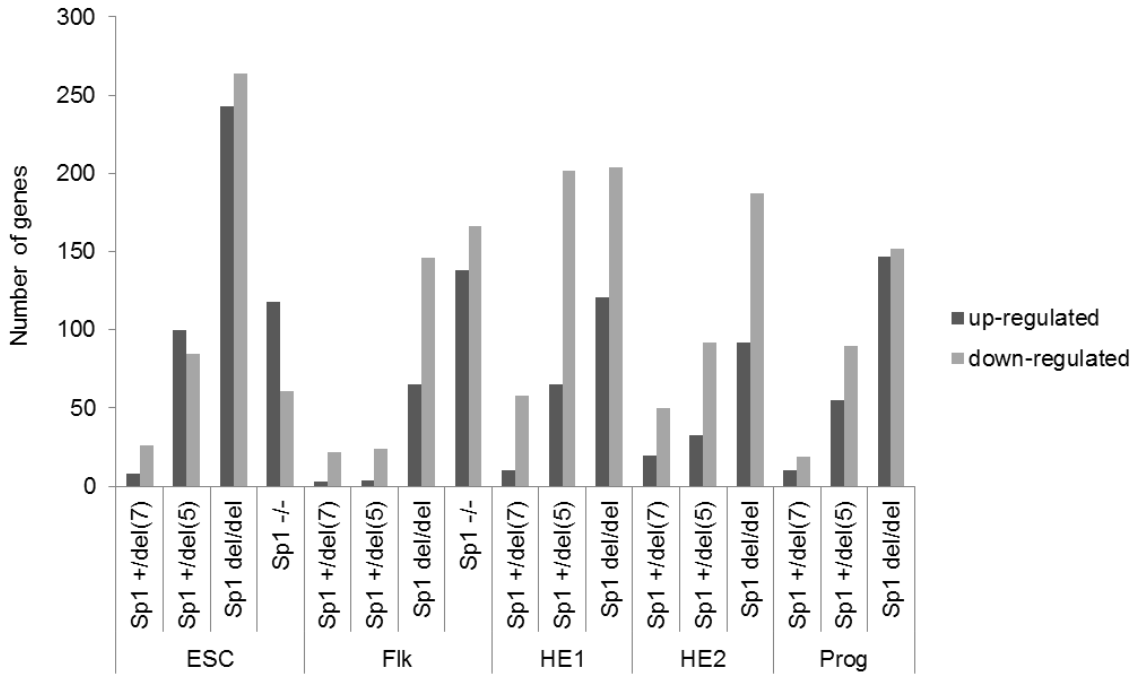


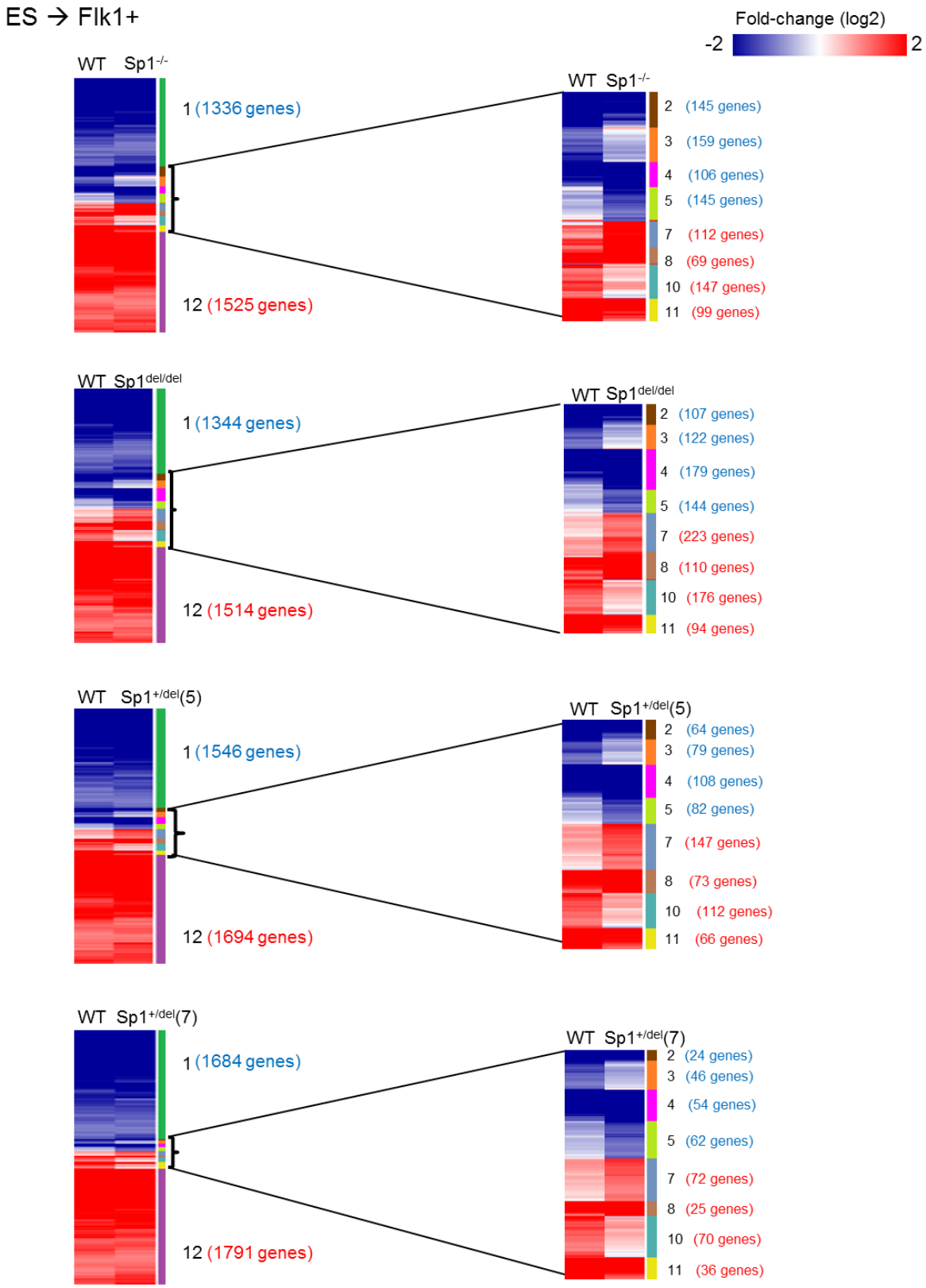
Figure 3.12 – A limited numbers of genes are deregulated by Sp1 deficiency
RNA-seq was performed in each Sp1 mutant clone at each stage of in vitro haematopoietic differentiation. The graph shows the number of genes that are deregulated by at least two-fold compared to WT at each cell stage.

3.2.3 The trajectory of the Sp1 mutant cells through differentiation is altered

In spite of the ubiquitous expression of Sp1, the majority of genes were similarly expressed between WT and mutant clones at each distinct stage of differentiation. However, we questioned whether there was a change in the expression of developmentally-regulated genes, whose levels can vary between stages of differentiation. As key developmental regulator genes fall into this category, it may explain a link to the observed haematopoietic defects.

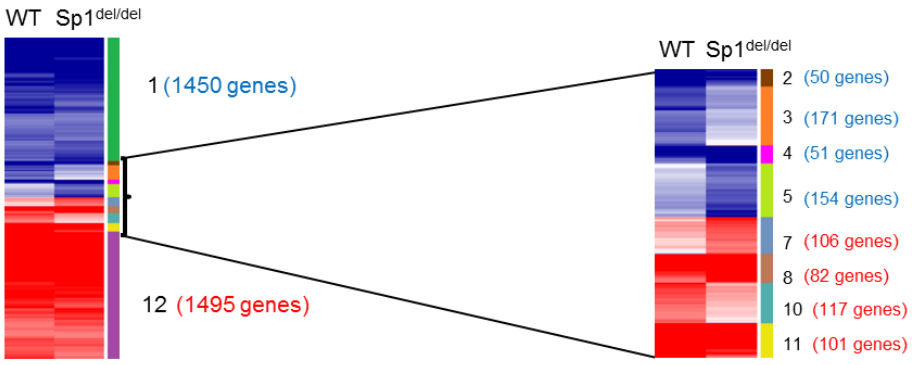
Therefore, the genes that are normally differentially expressed between stages of differentiation were identified (such as between ESC and Flk1+) and the expression of

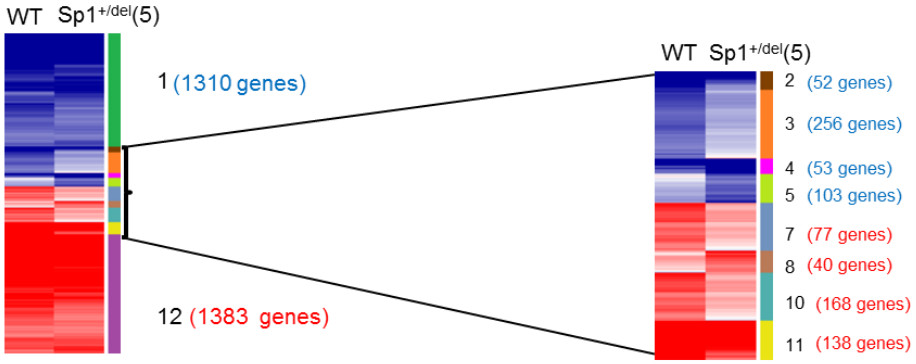
these genes were examined in the Sp1 mutant clones. The gene expression was then grouped for each clone, in each transition stage, based on how the gene expression levels were changing between differentiation stages compared to the WT. This generated 12 clusters for each condition, showing for example: less down-regulated compared to WT, up-regulated but unchanged in WT (Figure 3.13, Supplementary Tables 5.1-5.5). This analysis showed many more genes were deregulated, in contrast to looking at changes within discrete cell types, suggesting the trajectory of the cells through differentiation was altered. The number of deregulated genes was also higher at the ESC-Flk1+ transition than at later stages. This could indicate that the first stage is when Sp1 is crucially required, which corresponds with the failure of $Sp1^{-/-}$ cells to form Flk1+ cells. Alternatively, it could indicate a threshold effect – only those $Sp1^{del/del}$ cells (and indeed $Sp1^{+/del}$ cells) with sufficient Sp1 activity could proceed to the next differentiation stage and so fewer genes are deregulated at later stages.

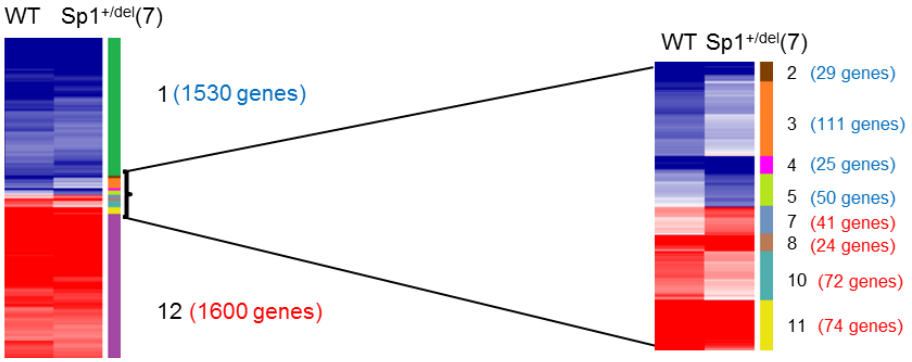

ES → Flk1+
Fold-change (log2)
-2
2
WT Sp1-/-
1 (1336 genes)
12 (1525 genes)
WT Sp1-/-
2 (145 genes)
3 (159 genes)
4 (106 genes)
5 (145 genes)
7 (112 genes)
8 (69 genes)
10 (147 genes)
11 (99 genes)
WT Sp1del/del
1 (1344 genes)
12 (1514 genes)
WT Sp1del/del
2 (107 genes)
3 (122 genes)
4 (179 genes)
5 (144 genes)
7 (223 genes)
8 (110 genes)
10 (176 genes)
11 (94 genes)
WT Sp1+/del(5)
1 (1546 genes)
12 (1694 genes)
WT Sp1+/del(5)
2 (64 genes)
3 (79 genes)
4 (108 genes)
5 (82 genes)
7 (147 genes)
8 (73 genes)
10 (112 genes)
11 (66 genes)
WT Sp1+/del(7)
1 (1684 genes)
12 (1791 genes)
WT Sp1+/del(7)
2 (24 genes)
3 (46 genes)
4 (54 genes)
5 (62 genes)
7 (72 genes)
8 (25 genes)
10 (70 genes)
11 (36 genes)

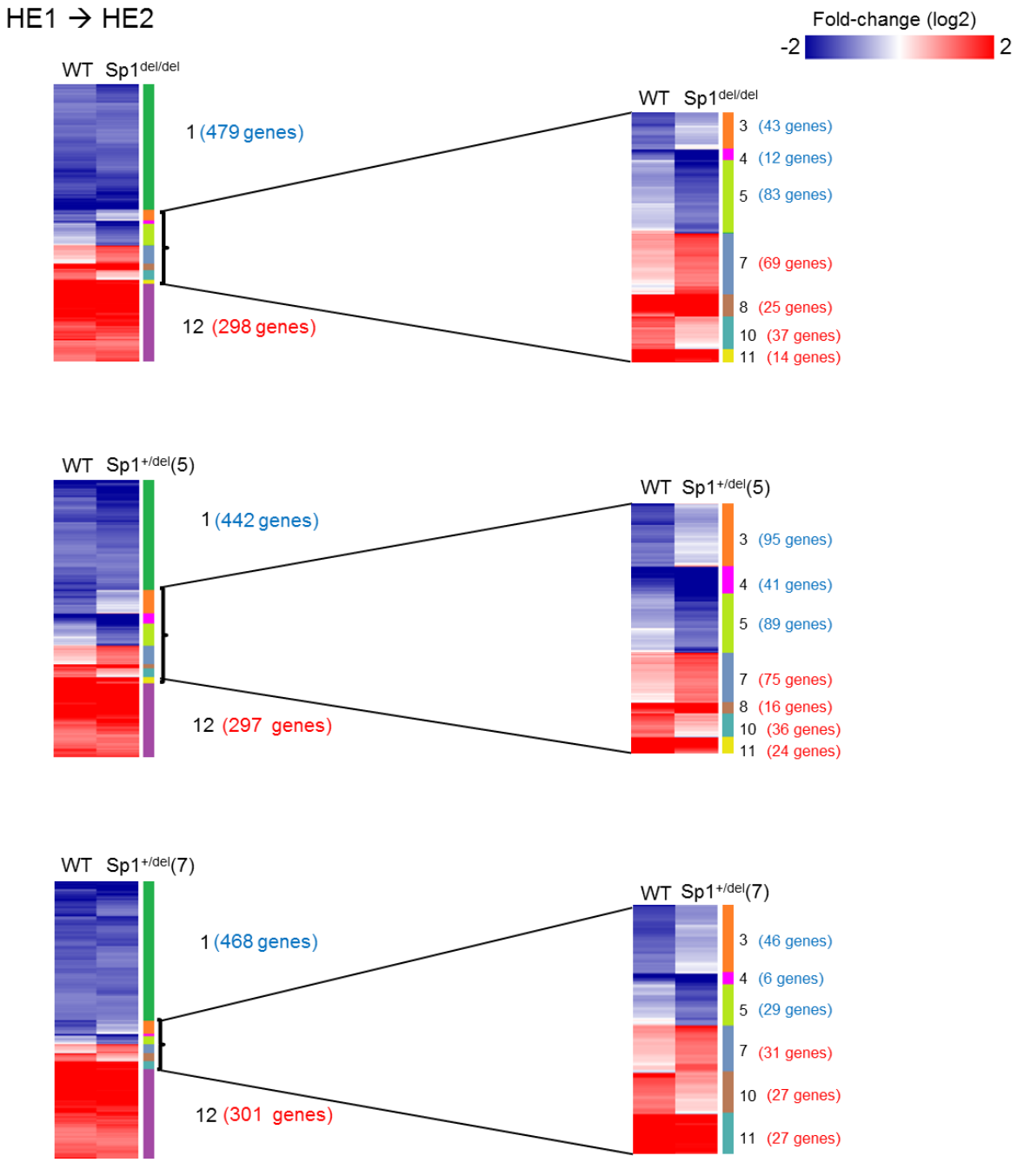
Flk1+ → HE1


Fold-change (log2)
-2
2


WT Sp1del/del
1 (1450 genes)
12 (1495 genes)
WT Sp1del/del
2 (50 genes)
3 (171 genes)
4 (51 genes)
5 (154 genes)
7 (106 genes)
8 (82 genes)
10 (117 genes)
11 (101 genes)


WT Sp1+/del(5)
1 (1310 genes)
12 (1383 genes)
WT Sp1+/del(5)
2 (52 genes)
3 (256 genes)
4 (53 genes)
5 (103 genes)
7 (77 genes)
8 (40 genes)
10 (168 genes)
11 (138 genes)


WT Sp1+/del(7)
1 (1530 genes)
12 (1600 genes)
WT Sp1+/del(7)
2 (29 genes)
3 (111 genes)
4 (25 genes)
5 (50 genes)
7 (41 genes)
8 (24 genes)
10 (72 genes)
11 (74 genes)


HE1 → HE2
Fold-change (log2)
-2
2
WT Sp1del/del
1 (479 genes)
12 (298 genes)
WT Sp1del/del
3 (43 genes)
4 (12 genes)
5 (83 genes)
7 (69 genes)
8 (25 genes)
10 (37 genes)
11 (14 genes)
WT Sp1+/del(5)
1 (442 genes)
12 (297 genes)
WT Sp1+/del(5)
3 (95 genes)
4 (41 genes)
5 (89 genes)
7 (75 genes)
8 (16 genes)
10 (36 genes)
11 (24 genes)
WT Sp1+/del(7)
1 (468 genes)
12 (301 genes)
WT Sp1+/del(7)
3 (46 genes)
4 (6 genes)
5 (29 genes)
7 (31 genes)
10 (27 genes)
11 (27 genes)

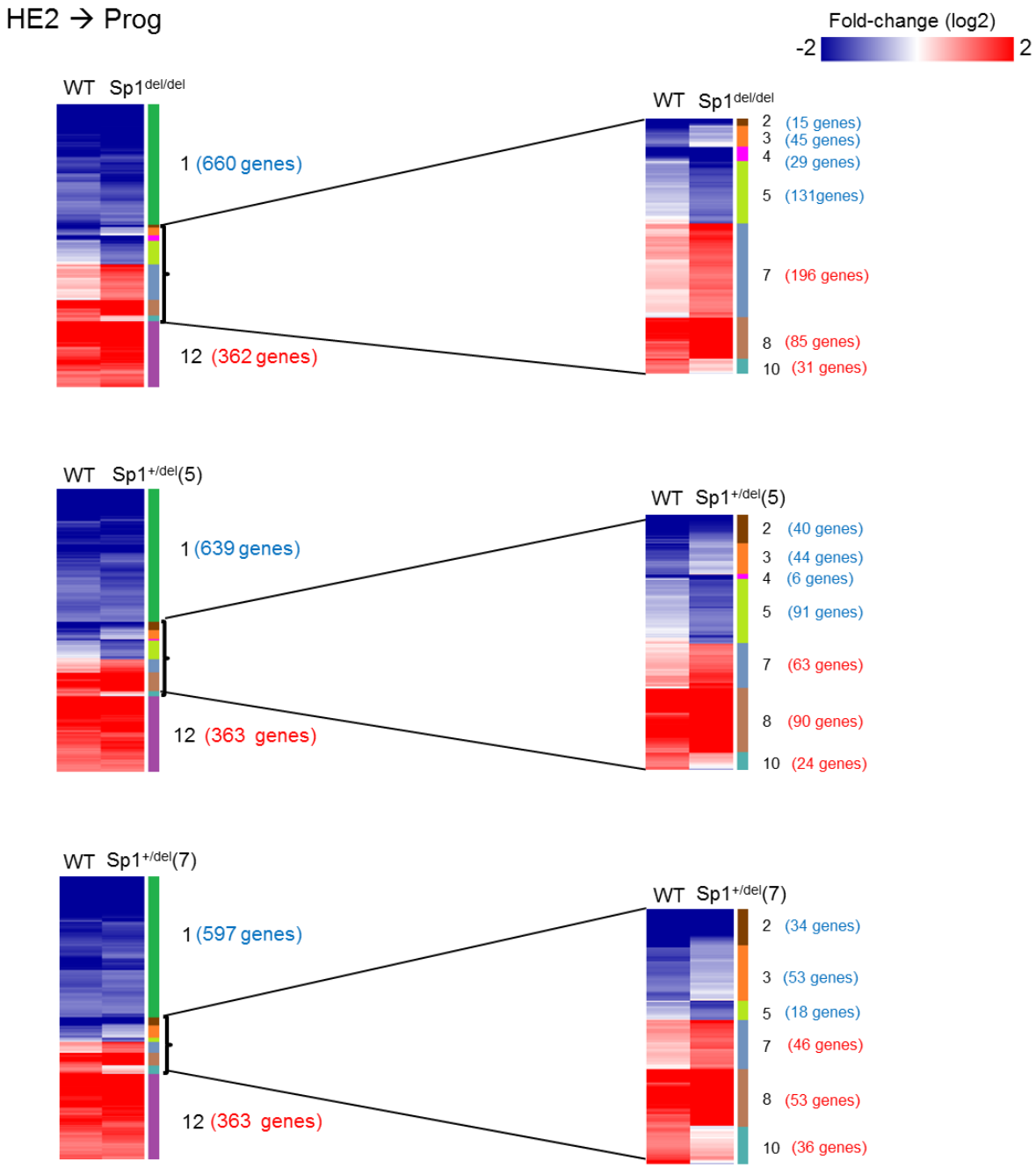


Figure 3.13 - The differential expression of genes as the cells transition between differentiation stages is altered with Sp1 mutation

Genes that were differentially expressed (at least two-fold) between consecutive differentiation stages in WT cells were first identified. The same list was generated in each of the Sp1 mutant clones and the genes that were differently differentially regulated between differentiation stages in the Sp1 clone, compared to the WT, were identified. The expression fold change (log2 value), compared to WT, of the differential genes is shown and grouped into how the gene expression is altered upon Sp1 mutation, for each of the four differentiation stage transitions.

Clusters: 1 – still downregulated in mutant, 2 – less downregulated in mutant, 3 – not downregulated in mutant, 4 – more downregulated in mutant, 5 – downregulated in mutant/no change in WT, 6 – downregulated in mutant/up-regulated in WT, 7 – upregulated in mutant/ no change in WT, 8 – more upregulated in mutant, 9 – upregulated in mutant/downregulated in WT, 10 – not upregulated in mutant, 11 – less upregulated in mutant, 12 – still upregulated in mutant.

When we examined these deregulated genes more closely, we identified several key developmental genes, which could be involved in causing the defects in haematopoietic differentiation. The $Sp1^{-/-}$ ESC to Flk1+ transition showed a deregulation of genes associated with ESC differentiation potential, BMP/Wnt signalling and mesoderm markers (Table 3.1). BMP and Wnt signalling is known to be important in determining cell fate during gastrulation and beyond, such as regulating the differentiation from mesoderm to haemangioblast or cardiac mesoderm (Murry & Keller, 2008). Correspondingly, there was a downregulation of a number of important mesoderm regulators/markers, such as *Brachyury* (*T*) and *Bmp4* (Kispert & Herrmann, 1994; Winnier et al., 1995). The first stage of in vitro differentiation is the specification to mesoderm, before differentiating to Brachyury+ Flk1+ haemangioblast cells. Thus, the deregulation of key mesoderm-defining factors and signalling pathways may underlie the inability of $Sp1^{-/-}$ cells to reach the first Flk1+ stage. Correspondingly, there was also a downregulation of *Etv2*, which is important for haemangioblast specification (Wareing et al., 2012). The deregulation of *Brachyury*, *Bmp4* and *Etv2* was confirmed by qPCR analysis (Figure 3.14). This data suggests Sp1 is important for mesoderm/haemangioblast specification.

Sp1$^{-/-}$ ES → Flk1+

	Name	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk FC	Sp1$^{-/-}$ ES/Flk FC	WT FC/Sp1$^{-/-}$ FC	
	Sfrp1	7.7	5.7	2.3	6.6	-1.7	0.2	1.9	BMP/Wnt signalling
	Bmp2	0.3	0.2	28.8	36.9	6.4	7.4	0.9	
	Frzb	0.2	0.2	15.8	25.0	6.5	7.4	0.8	
	Bmp4	46.8	30.5	129.8	60.1	1.5	1.0	-0.5	
	Bambi	3.2	3.7	21.3	13.7	2.8	1.9	-0.9	
	Bmper	0.3	0.4	34.9	21.0	6.9	5.9	-1.0	
	Wnt5a	0.6	1.3	38.0	29.8	5.9	4.5	-1.4	
*	*Dkk1*	0.1	0.2	29.6	15.7	7.8	6.1	-1.7	
*	*Smad6*	1.4	6.0	25.7	14.9	4.3	1.3	-2.9	
*	*Spp1*	124.0	258.7	0.7	8.7	-7.5	-4.9	2.6	ESC/differentiation
	Esam	10.2	8.7	2.5	9.6	-2.1	0.2	2.2	
	Wt1	4.6	1.3	0.3	0.3	-3.7	-2.0	1.8	
	Dppa4	97.2	99.1	1.5	4.7	-6.0	-4.4	1.6	
	Flt1	0.5	0.5	5.8	14.9	3.6	5.1	1.5	
*	*Pecam1*	8.5	21.0	1.2	7.4	-2.9	-1.5	1.4	
*	*Amd1*	0.1	0.1	6.5	12.7	5.7	7.0	1.3	
	Txnip	71.9	72.9	80.5	164.9	0.2	1.2	1.0	
	Lefty1	84.3	164.6	2.3	7.8	-5.2	-4.4	0.8	
	Lmo2	0.5	0.8	49.1	34.5	6.5	5.4	-1.2	
	Etv2	0.9	1.1	24.7	13.9	4.8	3.7	-1.2	
*	*Lefty2*	58.4	157.9	33.4	38.2	-0.8	-2.1	-1.2	
	Fgfr2	9.9	13.7	9.3	5.0	-0.1	-1.5	-1.4	
*	*Msgn1*	0.1	0.1	17.1	5.5	7.4	5.8	-1.6	Mesoderm
	Foxf1	0.1	0.2	17.6	7.6	6.9	5.5	-1.5	
	Msx2	0.6	0.9	53.3	33.8	6.6	5.3	-1.3	
	Tbx6	0.2	0.2	6.0	2.3	4.9	3.6	-1.3	
	Tbx2	0.1	0.2	9.1	5.0	6.1	4.9	-1.2	
	Hand2	0.1	0.1	22.0	14.5	7.8	6.9	-0.9	
	Hand1	0.2	0.2	65.6	27.6	8.2	7.3	-0.9	
	Evx1	0.4	0.4	27.7	18.7	6.3	5.5	-0.8	
	Tbx20	2.1	1.8	56.2	29.5	4.7	4.1	-0.7	
	Kdr	0.3	0.4	211.7	166.8	9.3	8.7	-0.6	
	Bmp4	46.8	30.5	129.8	60.1	1.5	1.0	-0.5	
	Mesp1	0.2	0.1	70.1	35.3	8.6	8.2	-0.4	
	Mixl1	0.3	0.3	91.7	66.2	8.3	8.1	-0.2	
	Msx1	0.6	0.4	64.2	42.1	6.8	6.7	-0.1	
	Fgf4	40.9	23.7	0.9	0.7	-5.5	-5.1	0.4	
	T	2.8	1.4	64.6	44.7	4.5	5.0	0.5	

Table 3.1 – Deregulation of developmental genes between ESC and Flk1+ with Sp1 knockout.

Genes that were significantly differently expressed between ESC and Flk1+ cell stages in Sp1$^{-/-}$ cells compared to WT were identified. The list was filtered for genes known to be important in ESC differentiation or Flk1+ cell formation. The FPKM values in each cell type are shown, as well as the respective fold change (FC, log2 value) between ESC and Flk1+ stages, and the fold change between these values in WT and Sp1$^{-/-}$ cells. Yellow – Sp1 target, based upon Sp1 ChIP-seq (chapter 3.4.1). Green – Sp3-specific target, based on Sp3 ChIP-seq (note, many genes are bound by both Sp1 and Sp3). Asterisk – also downregulated in at least one heterozygous Sp1 clone.

The $Sp1^{del/del}$ ESC to Flk1+ transition (Table 3.2) indicated a deregulation of genes involved in ESC maintenance and differentiation potential, as well as some genes involved in the first stages of haematopoietic differentiation, such as *Etv2* and the mesoderm marker *Gsc* (Tada et al., 2005; Wareing et al., 2012). Most noticeably, there was a group of genes involved in ESC pluripotency that were commonly deregulated. Those involved in promoting pluripotency were generally up-regulated, such as *Nanog* (which is validated by qPCR analysis in Figure 3.14) and *Esrrb* (Silva et al., 2009; Festuccia et al., 2012), while those involved in promoting differentiation were downregulated, such as *Eif2s3y* and *Satb1* (Savarese et al., 2009; Li et al., 2016). These effects could explain the impaired differentiation of $Sp1^{del/del}$ cells, thus forming lower numbers of Flk1+ cells. However, many of the ESC pluripotency-associated genes were also deregulated in one or both of the heterozygous clones, which differentiated normally. Both the $Sp1^{del/del}$ and $Sp1^{+/del}$ cells express a truncated form of Sp1, although the heterozygous cells also express WT Sp1. Therefore, it may be that the truncated Sp1 protein interferes with transcriptional regulation, for example by inhibiting complex formation with partner proteins. Thus, the gene deregulation in all three mutant clones may come about through the same mechanism, but with WT Sp1 present in $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7), the effects are less severe. Alternatively, the truncated Sp1 could be associating with DNA (e.g. via its binding partners), but is only able to form transient complexes, decreasing transcription initiation efficiency. The lower concentration of Sp1 found in $Sp1^{+/del}$ cells relative to WT may also elicit inefficient transcription initiation, but to a lesser extent than in $Sp1^{del/del}$ cells. Indeed, this could again suggest a threshold level of Sp1 activity for the cells to progress to the next stage of differentiation, meaning differentiation is less efficient in $Sp1^{del/del}$ cells.

$Sp1^{del/del}$ ES → Flk1+

	Name	ES WT FPKM	ES $Sp1^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ $Sp1^{del/del}$ FPKM	WT ES/Flk FC	$Sp1^{del/del}$ ES/Flk FC	WT FC/ $Sp1^{del/del}$ FC	
*	*Satb1*	5.0	1.3	6.8	8.2	0.4	2.6	2.2	ESC/pluripotency
*	*Tex19.1*	23.6	86.7	0.4	3.9	-5.9	-4.5	1.4	
	Eif2s3y	22.8	2.2	18.8	5.1	-0.3	1.2	1.5	
	Prdm5	17.0	17.1	9.0	5.9	-0.9	-1.5	-0.6	
*	*Esrrb*	44.5	63.9	0.6	0.4	-6.1	-7.2	-1.1	
*	*Hck*	41.7	73.4	0.2	0.2	-7.5	-8.6	-1.1	
*	*Nr5a2*	16.4	31.4	0.3	0.2	-6.1	-7.3	-1.2	
	Nanog	90.7	180.1	10.6	8.9	-3.1	-4.3	-1.2	
*	*Prdm14*	3.0	9.2	1.8	1.4	-0.8	-2.7	-1.9	
*	*Dppa3*	11.6	72.2	0.2	0.3	-5.9	-7.8	-1.9	
*	*Dppa5a*	1558.9	686.8	31.9	3.1	-5.6	-7.8	-2.2	
*	*Lefty2*	58.4	149.9	33.4	17.1	-0.8	-3.1	-2.3	
*	*Amd1*	0.1	13.7	6.5	0.1	5.7	-6.9	-12.6	
	Hoxb1	0.1	0.1	4.0	12.3	4.9	6.7	1.8	Differentiation potential/haematopoiesis
	Hmgn5	4.5	1.3	4.7	3.7	0.1	1.5	1.5	
	Cdx2	2.0	1.4	2.4	4.4	0.3	1.6	1.4	
	Vegfc	7.7	2.7	12.5	10.5	0.7	1.9	1.3	
*	*Gli3*	8.0	4.4	11.6	12.9	0.5	1.6	1.0	
	Fgf4	40.9	24.0	0.9	1.1	-5.5	-4.5	1.0	
*	*Bmp1*	9.2	5.1	14.7	16.0	0.7	1.7	1.0	
	Efnb2	11.3	4.9	20.1	16.2	0.8	1.7	0.9	
	Eomes	1.6	2.4	122.5	90.1	6.3	5.2	-1.0	
	Lmo2	0.5	1.0	49.1	43.1	6.5	5.5	-1.0	
	Igf2	8.7	22.2	225.1	277.7	4.7	3.7	-1.0	
	Gsc	0.1	0.2	9.2	5.9	6.1	5.0	-1.1	
	Hck	41.7	73.4	0.2	0.2	-7.5	-8.6	-1.1	
*	*Snai1*	0.6	1.2	17.3	14.6	4.8	3.7	-1.1	
	Kdr	0.3	0.7	211.7	188.0	9.3	8.0	-1.3	
	Tbx6	0.2	0.3	6.0	3.2	4.9	3.6	-1.3	
*	*Dkk1*	0.1	0.2	29.6	19.5	7.8	6.4	-1.4	
	Etv2	0.9	1.9	24.7	17.6	4.8	3.2	-1.6	
	Mesp1	0.2	0.4	70.1	41.3	8.6	6.8	-1.9	
	Otx2	6.5	23.6	23.0	17.2	1.8	-0.5	-2.3	
*	*Smad6*	1.4	7.8	25.7	23.4	4.3	1.6	-2.7	

Table 3.2 – Deregulation of key genes in differentiation of ESC to Flk1+ cells in Sp1 hypomorph cells

Differentially expressed genes between ESC and Flk1+ cell stages that were significantly deregulated in $Sp1^{del/del}$ cells compared to WT were identified. The table shows those selected for involvement in ESC differentiation. The FPKM values in each cell type are shown, as well as the respective fold change (FC, log2 value) between ESC and Flk1+ stages, and the fold change between these values in WT and $Sp1^{del/del}$ cells. Yellow – Sp1 target, based upon Sp1 ChIP-seq. Green – Sp3-specific target, based on Sp3 ChIP-seq (note, many genes are bound by both Sp1 and Sp3) (see chapter 3.4.1). Asterisk – also downregulated in at least one heterozygous Sp1 clone.

The gene expression during the $Sp1^{del/del}$ Flk1+ to HE1 transition (Table 3.3) showed the deregulation of a number of genes associated with angiogenesis (as blood vessels can also be formed at this stage) and in HE/haematopoietic specification. Again, most of these genes were also deregulated in the $Sp1^{+/del}$ cells, suggesting haematopoietic specification is less efficient in $Sp1^{del/del}$ cells.

The latter differentiation stages of $Sp1^{del/del}$ cells displayed fewer gene expression changes. The HE1 to HE2 transition showed only small changes in gene expression and no significant changes in known regulators were seen. In contrast, the HE2 to progenitor transition (Table 3.3) showed an upregulation of megakaryocyte and platelet-associated genes (not seen in $Sp1^{+/del}$ clones), suggesting a bias for this haematopoietic lineage and explaining their impaired differentiation to macrophages. In support, *Gp5*, which encodes a platelet cell surface protein that is important in haemostasis (Calverley et al., 1995), is highly upregulated in $Sp1^{del/del}$ progenitors by qPCR analysis (Figure 3.14).

Sp1$^{del/del}$ Flk1+ → HE1

	Name	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	HE1 WT FPKM	HE1 Sp1$^{del/del}$ FPKM	WT Flk/HE1 FC	Sp1$^{del/del}$ Flk/HE1 FC	WT FC/ Sp1$^{del/del}$ FC	
	Angpt2	0.2	0.2	1.5	3.0	3.1	4.2	1.2	Angiogenesis
	Epcam	10.2	5.1	0.5	0.5	-4.4	-3.3	1.1	
	Angpt1	0.2	0.2	3.6	5.6	3.9	5.0	1.1	
*	*Acvrl1*	0.8	1.1	37.8	26.1	5.6	4.6	-1.0	
*	*Col18a1*	19.1	25.6	354.6	243.3	4.2	3.3	-1.0	
*	*Rasip1*	2.0	2.9	78.7	56.8	5.3	4.3	-1.0	
*	*Flt4*	4.7	4.4	30.1	14.0	2.7	1.7	-1.0	
*	*Ldb2*	1.8	3.2	18.6	14.6	3.4	2.2	-1.2	
*	*Plxnd1*	8.9	4.4	174.5	34.1	4.3	3.0	-1.3	
*	*Vash1*	1.1	1.5	16.5	9.0	3.9	2.6	-1.3	
*	*Tie1*	1.2	2.7	180.6	161.8	7.3	5.9	-1.4	
	Itgb3	0.6	0.9	7.4	3.8	3.6	2.1	-1.5	
*	*Pecam1*	1.2	3.1	211.0	183.4	7.5	5.9	-1.6	
*	*Gja5*	0.1	0.1	8.5	1.1	5.9	3.0	-2.9	
	T	64.6	52.0	0.2	0.4	-8.7	-6.9	1.8	HE / haematopoiesis
	Cav1	0.4	0.3	18.4	34.9	5.5	7.1	1.6	
*	*Pdgfra*	45.5	35.8	9.8	18.4	-2.2	-1.0	1.3	
	Sox17	1.0	0.8	11.9	20.6	3.6	4.8	1.2	
	Tek	2.0	3.4	150.4	131.5	6.3	5.3	-1.0	
*	*Icam2*	1.2	2.3	94.7	79.1	6.3	5.1	-1.2	
	Etv2	24.7	17.6	3.3	1.0	-2.9	-4.1	-1.2	
*	*Aplnr*	92.3	90.6	81.0	33.3	-0.2	-1.5	-1.3	
*	*Sox18*	0.6	0.8	27.7	14.6	5.5	4.2	-1.3	
	Itga2b	1.5	2.0	36.7	18.6	4.7	3.3	-1.4	
	Esam	2.5	3.9	138.3	77.5	5.8	4.3	-1.5	
*	*Pecam1*	1.2	3.1	211.0	183.4	7.5	5.9	-1.6	
*	*Cd34*	0.5	0.6	177.9	61.3	8.6	6.7	-1.9	
*	*Dll4*	0.2	0.3	8.5	1.6	5.7	2.5	-3.3	
	Hba-x	0.2	0.6	11.5	5.6	5.7	3.2	-2.5	Blood
	Hbb-y	0.2	0.4	10.2	4.2	5.5	3.5	-2.0	
	Hbb-bh1	3.2	4.2	119.1	42.7	5.2	3.4	-1.9	

Sp1$^{del/del}$ HE2 → Progenitor

	Name	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	HE1 WT FPKM	HE1 Sp1$^{del/del}$ FPKM	WT Flk/HE1 FC	Sp1$^{del/del}$ Flk/HE1 FC	WT FC/ Sp1$^{del/del}$ FC	
	Gp5	1.0	1.1	2.8	15.4	1.5	3.8	2.3	Megakaryocyte/Platelets
	Gp9	0.8	0.6	3.5	10.4	2.0	4.2	2.2	
*	*Tubb1*	4.0	2.8	18.4	53.4	2.2	4.3	2.1	
	Vwf	8.5	19.2	1.9	14.2	-2.2	-0.4	1.8	
	Clec1b	12.9	12.8	11.1	36.1	-0.2	1.5	1.7	
	Fermt3	52.3	20.5	76.2	91.1	0.5	2.1	1.6	
	F2rl2	5.4	5.6	6.9	20.7	0.4	1.9	1.5	
	G6b	0.3	0.6	1.3	7.1	2.1	3.5	1.4	
	Klf1	1.0	0.7	6.2	11.4	2.7	4.0	1.4	
	Prkcq	4.0	3.7	7.3	16.9	0.9	2.2	1.3	
*	*Gata1*	4.6	3.2	31.7	48.7	2.8	3.9	1.1	
	Gp1ba	4.1	4.6	6.0	14.0	0.6	1.6	1.1	
	Syk	13.0	9.0	26.5	38.1	1.0	2.1	1.1	
	Nfe2	54.2	37.3	140.6	173.8	1.4	2.2	0.8	
	Mpl	14.1	9.5	24.9	29.7	0.8	1.6	0.8	
	Vps33b	11.3	9.8	14.7	22.5	0.4	1.2	0.8	
	Itgb3	133.0	96.8	182.4	229.8	0.5	1.2	0.8	
*	*F10*	4.0	4.0	6.6	10.0	0.7	1.3	0.6	

Table 3.3 – Gene expression changes through differentiation of Sp1 hypomorph cells in blast culture

Genes that are differentially expressed in WT cells between differentiation stages were identified and the expression levels compared to that in Sp1$^{del/del}$ cells. Important regulators and markers that were deregulated between Flk1+ and HE1, and HE2 to progenitor are shown. Few genes were identified during HE1 to HE2 stages. The FPKM values in each cell type are shown, as well as the respective fold change (FC, log2 value) between ESC and Flk1+ stages, and the fold change between these values in WT and Sp1$^{del/del}$ cells. Asterisk represents those that were also deregulated in at least one Sp1 heterozygous clone.

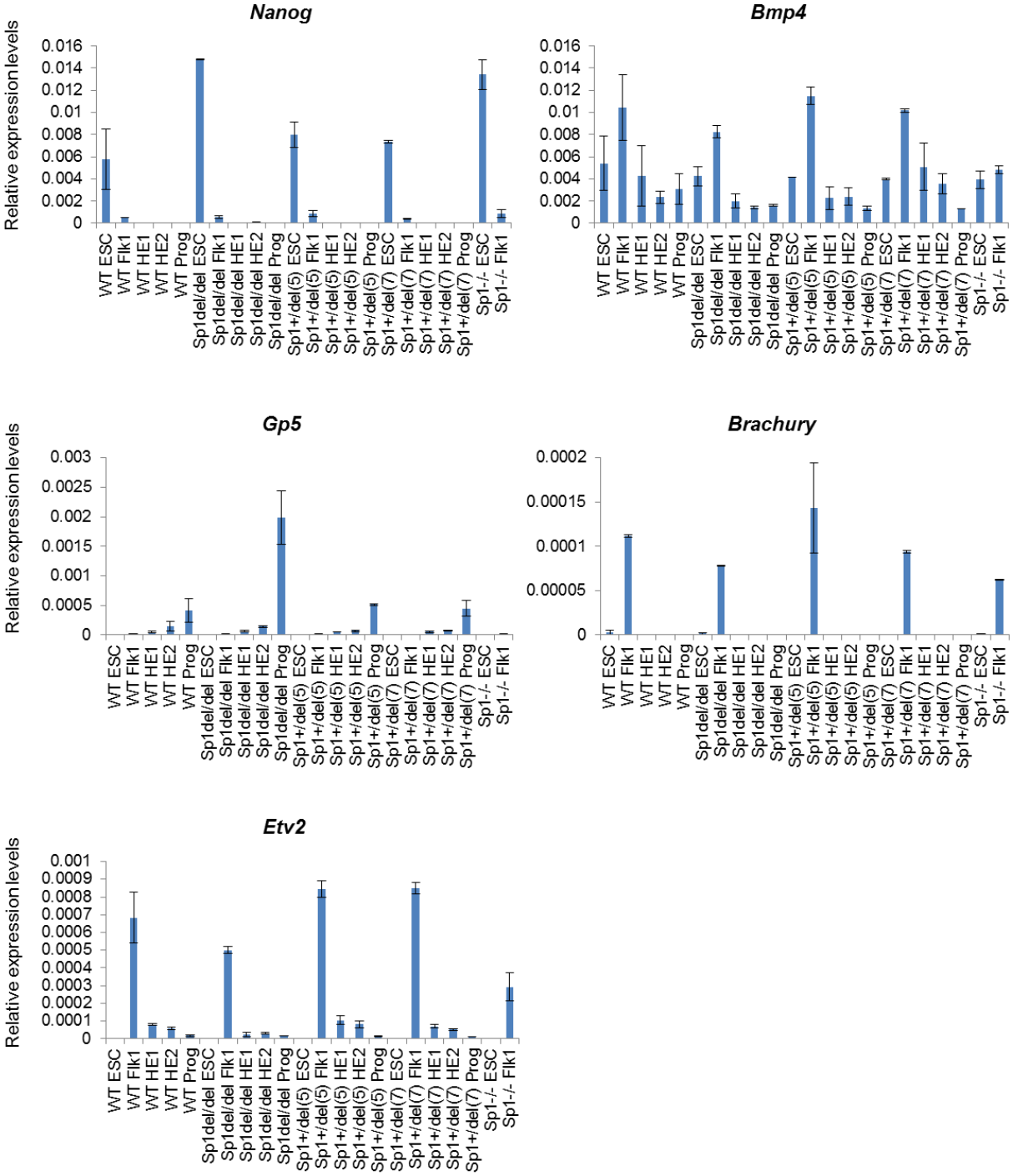


Figure 3.14 – qPCR analysis supports the RNA-seq results

RNA was obtained from WT and four Sp1 mutant clones at each stage of in vitro haematopoietic differentiation. This was performed in duplicate and RNA-seq carried out. The RNA-seq duplicates were merged for further analysis and the genes of several key developmental regulators found to be deregulated in $Sp1^{-/-}$ and/or $Sp1^{del/del}$ cells. The deregulation of five of these candidate genes was validated by reverse transcription, followed by qPCR analysis, of the RNA used for the RNA-seq libraries. The graphs show the average of the relative gene expression calculated from the two biological replicates and the error bars show the variation of the two independent replicates from the mean.

Gilmour et al. previously analysed the gene expression in Sp1 DBD-deficient cells through in vitro haematopoietic differentiation using microarrays. To compare the gene expression changes in the two $Sp1^{del/del}$ cell lines, GSEA plots were made by analysing the enrichment of genes that were either up- or downregulated in $Sp1^{del/del}$ cells vs. WT cells in Gilmour et al., as compared to the differentially expressed genes in $Sp1^{del/del}$ vs. WT cells of the present study (Figure 3.15). The plots show that generally the same genes were up- or downregulated in the DBD-deficient cells, which is consistent with the highly similar phenotype between the $Sp1^{del/del}$ cell clones. However, there were some differences. Indeed, some of the key deregulated genes identified (e.g. several Hox and Cdx genes) are not shared between the two studies.

In summary, while the global gene expression programme is generally maintained in Sp1 mutant clones, there is a deregulation of genes across differentiation stages, suggesting that the trajectory of the cells has changed. Groups of deregulated genes encoding important cell fate regulators have been identified. These analyses showed $Sp1^{-/-}$ cells likely fail to differentiate to Flk1+ cells due to a deregulation of mesoderm regulators. $Sp1^{del/del}$ cells, however, seem to be able to differentiate to the haematopoietic lineage, but they do so less efficiently than WT cells. This feature is associated with the deregulation of genes for pluripotency and differentiation potential at early stages, haematopoietic specification genes at HE, and finally an upregulation of megakaryocyte/platelet genes, suggesting a bias in their differentiation.

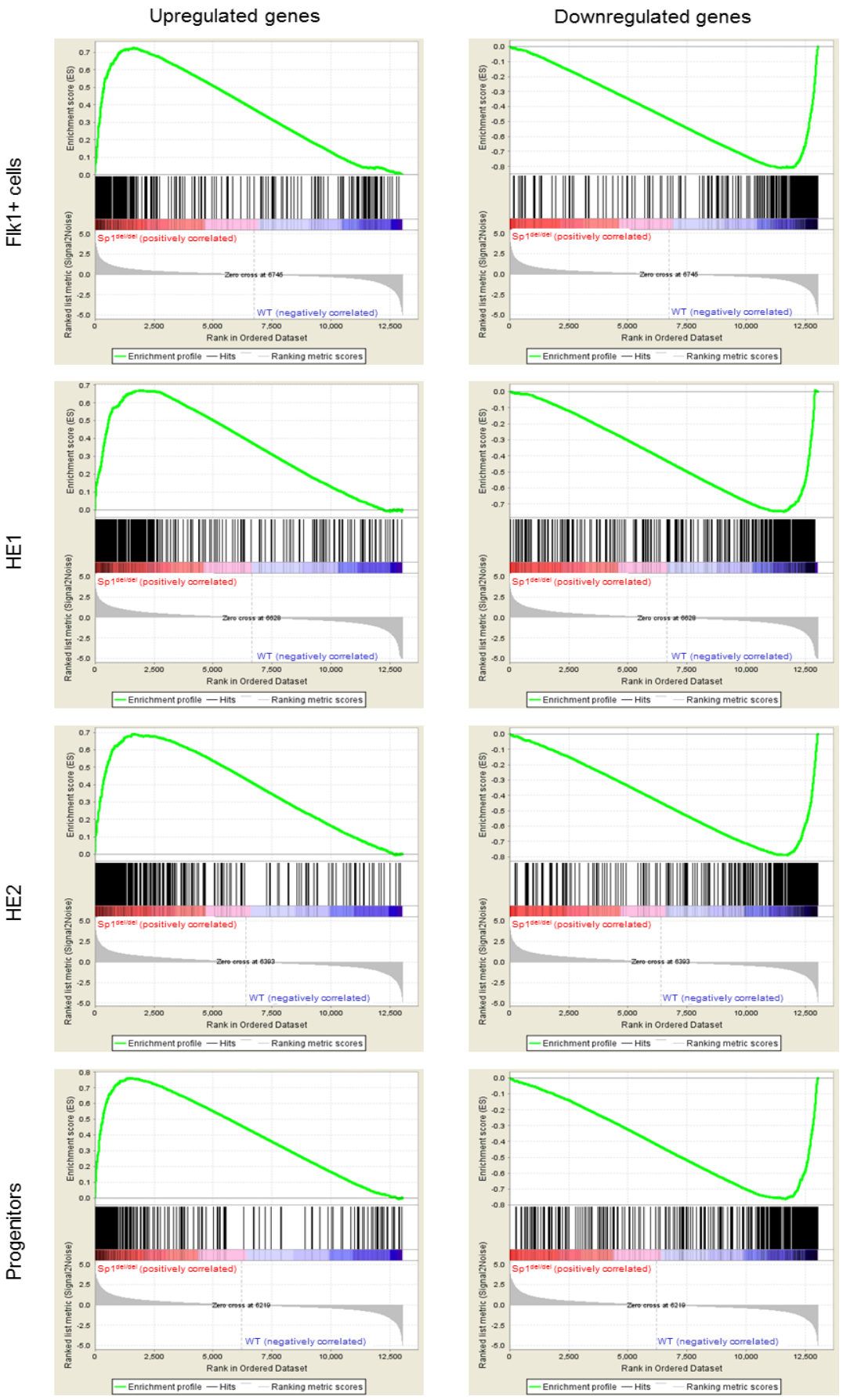

Upregulated genes
Downregulated genes
Flk1+ cells
HE1
HE2
Progenitors
Enrichment score (ES)
Ranked list metric (Signal2Noise)
Sp1del/del (positively correlated)
WT (negatively correlated)
Zero cross at 6745
Zero cross at 6628
Zero cross at 6393
Zero cross at 6219
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure 3.15 – Gene expression profiles of two different Sp1 DBD-deficient cell clones are similar

Gene set enrichment analysis was performed using GSEA software. The differential gene expression between $Sp1^{del/del}$ and WT cells (used in the current study) was calculated and the genes ranked based on fold change. The genes that were identified as differentially expressed between Sp1 DBD-deficient and WT cells in Gilmour et al. were grouped into upregulated and downregulated (at least two-fold different) genes and the genes plotted based on the prior ranking. An enrichment score was calculated based on how many genes were found in both at the same place in the ranking. For example, if the same genes were upregulated in both cell lines, there would be a high enrichment. The analysis was performed for Flk1+, HE1, HE2 and progenitor cells. Generally, the two datasets show high similarity.

3.3 Gene expression changes upon disruption of *Sp1* correlate with changes in chromatin structure

3.3.1 Chromatin accessibility profiles are altered in Sp1 null Flk1+ cells

The regulation of chromatin accessibility by transcription factors is important in establishing a cell type-specific pattern of gene regulation and indeed, the chromatin structure changes through differentiation of ESCs to haematopoietic cells (Goode et al., 2016). To examine how the chromatin profile changed upon *Sp1* manipulation and how this correlated with changes in gene expression, we performed ATAC-seq. ATAC-seq enables the identification of open chromatin regions genome-wide (Buenrostro et al., 2015b). It was performed at ESC and Flk1+ cell stages in biological replicates, which seem to be the most crucial stages for Sp1 function and where there is most gene deregulation.

Comparison of the open chromatin regions across the Sp1 clones in ESC and Flk1+ cells showed that the two cell types formed distinct clusters with a high level of correlation

between WT and Sp1 mutant clones, suggesting the chromatin structure is generally similar (Figure 3.16). However, Sp1$^{-/-}$ Flk1+ cells, while still clustering with the other Flk1+ cells, showed a weaker correlation with WT, suggesting Sp1$^{-/-}$ Flk1+ cells have an altered chromatin structure. As only very small numbers of Flk1+ cells were obtained during differentiation and the RNA-seq results suggested a block of Sp1$^{-/-}$ in mesoderm formation, it may be that these are a different cell type (rather than conventional Flk1+ haemangioblast cells).

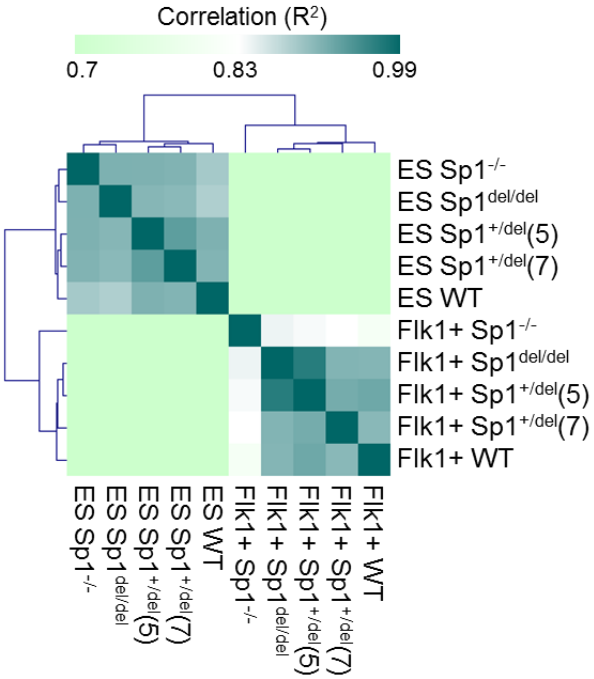


Figure 3.16 – ATAC-seq profiles cluster by cell type
ATAC-seq was performed in WT and Sp1 mutant cells at ESC and Flk1+ stages (biological replicates) to identify regions of open chromatin. The read counts (log2 value) of each peak in the ATAC-seq datasets (merged duplicates) were obtained and a Pearson correlation calculated between each sample. The Pearson correlation values were then clustered using unsupervised hierarchical clustering and plotted as a heatmap to compare ESC and Flk1+ stages, as well as the different clones.

3.3.2 Changes in chromatin accessibility correlate with changes in gene expression

To look in more detail at the changes in chromatin accessibility within cells carrying the different Sp1 mutations, we compared each clone to WT in a pairwise comparison in ESCs (Figure 3.17) and in Flk1+ cells (Figure 3.18). As before, the density plots showed a generally similar pattern of chromatin accessibility in WT and Sp1 mutant clones. However, while there were few uniquely open regions, there were a considerable number of regions that were at least two-fold different (more or less open based on number of reads) in cells with the *Sp1* mutation compared to WT cells. The extent of these differences compared to WT was similar in all four clones in ESCs. In Flk1+ cells, again the numbers of clone- or WT-specific sites were comparable in $Sp1^{del/del}$, $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7), whereas $Sp1^{-/-}$ Flk1+ cells showed a very different chromatin profile, again suggesting they may be a different cell type.

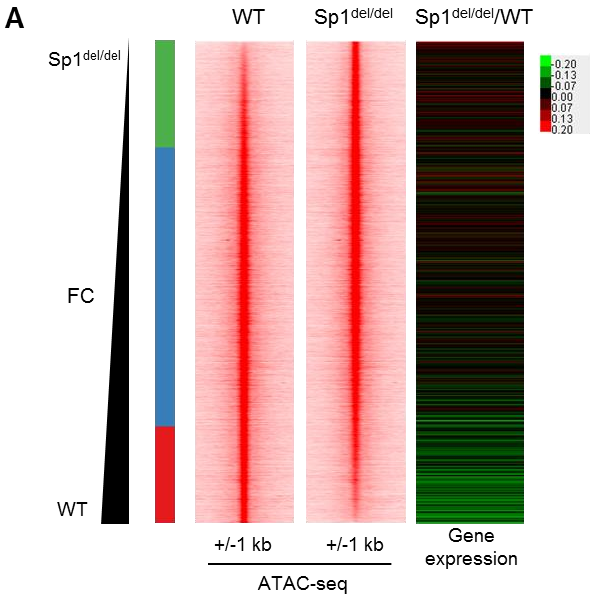
We then analysed whether the changes in chromatin structure were having an impact on gene expression. The open chromatin regions identified by ATAC-seq peaks were assigned to the nearest gene and the differential gene expression in Sp1 mutant clone compared to WT was plotted (Figure 3.17, Figure 3.18). It must be noted that only small changes in gene expression were identified, but there was a correlation between gene expression changes and changes in chromatin accessibility. For example, in Figure 3.17A, the WT-specific open chromatin regions were associated with a downregulation of gene expression in the $Sp1^{del/del}$. This trend was most prominent in $Sp1^{del/del}$ and $Sp1^{-/-}$ cells, rather than the heterozygous clones, which showed less gene deregulation. These results suggest the changes in chromatin accessibility are associated with changes in gene regulation and that Sp1 has a role in establishing or maintaining chromatin structure.

We further investigated the common, Sp1 mutant- and WT-specific regions of open chromatin by performing de novo motif discovery (Figure 3.17, Figure 3.18) using the HOMER software (Heinz et al., 2010). This method identifies consensus TF-binding motifs that are enriched in each group of sites in an unbiased way, thus suggesting which transcription factor family may be bound there. The common sites in each pairwise comparison were enriched in promoter-associated motifs, such as those bound by the transcription factor NFY. The differential sites were more enriched with enhancer-associated motifs and showed motifs for cell type-specific factors, e.g. Pou5f1 (OCT4) which were found in ESCs, whereas GATA motifs were identified in Flk1+ cells. This is consistent with the fact that the majority of promoters are commonly associated with open chromatin regions, while the accessibility at enhancers is often induced by TFs, chromatin remodellers and histone modifiers to ensure cell type- or stage-specific activation. Furthermore, in Flk1+ cells, motifs linked with differentiation progression, such as GATA and TEAD, were associated with WT-specific sites, which may underlie a defect in haematopoietic differentiation.

Importantly, the Sp1 motif – the GC-box – was enriched in WT-specific and common sites of open chromatin, but not in Sp1 mutant-specific sites. Overall, these results suggest the chromatin structure in the Sp1 mutant clones may be altering to allow the cells to adjust for the lack (or decrease) of functional Sp1. Thus, it may be that sites are opening that do not bind Sp1, but which bind factors that can compensate for the loss of Sp1, to some extent, and therefore, resulting in minimal changes in gene regulation.

ESC

A



Sp1$^{del/del}$ specific (6741 sites)

Motif	Name	p value	% sites
	CTCF	1e-1439	12.24
	NFY	1e-524	9.92
	ETS	1e-220	21.67
	Pou5f1	1e-85	5.00

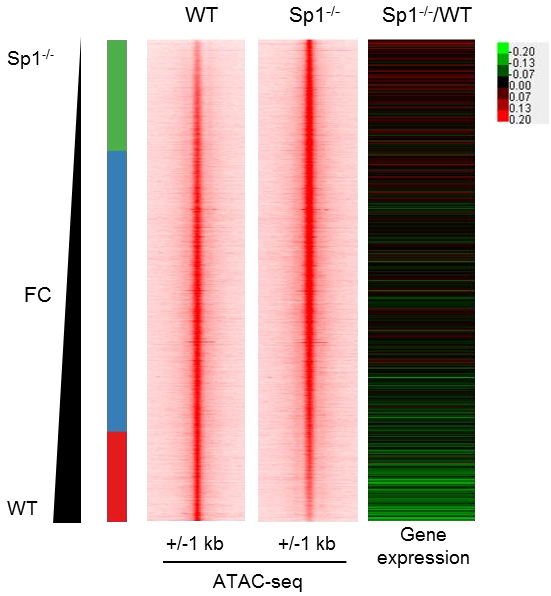
Common (17451 sites)

Motif	Name	p value	% sites
	NFY	1e-744	15.75
	GC-box	1e-688	29.12
	CTCF	1e-630	8.26
	ETS	1e-281	15.74

WT specific (6066 sites)

Motif	Name	p value	% sites
	CTCF	1e-573	13.3
	GC-box	1e-341	31.77
	Pou5f1	1e-103	4.48

B



Sp1$^{-/-}$ specific (6982 peaks)

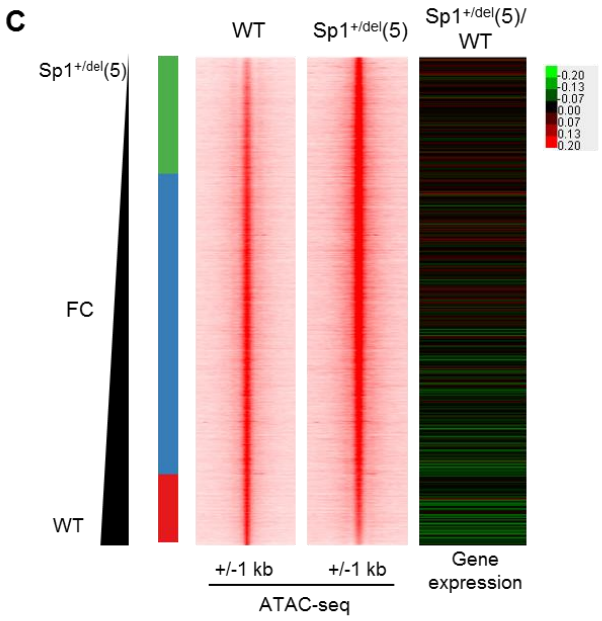
Motif	Name	p value	% sites
	CTCF	1e-973	19.98
	Pou5f1	1e-108	3.85
	ETS	1e-53	16.74

Common (17830 peaks)

Motif	Name	p value	% sites
	CTCF	1e-701	9.26
	GC-box	1e-691	28.81
	NFY	1e-594	12.61
	ETS	1e-282	4.14

WT specific (5705 peaks)

Motif	Name	p value	% sites
	CTCF	1e-507	14.27
	GC-box	1e-300	26.33
	Pou5f1	1e-90	3.33
	NFY	1e-48	3.98



Sp1$^{+/del}$(5) specific (7521 sites)

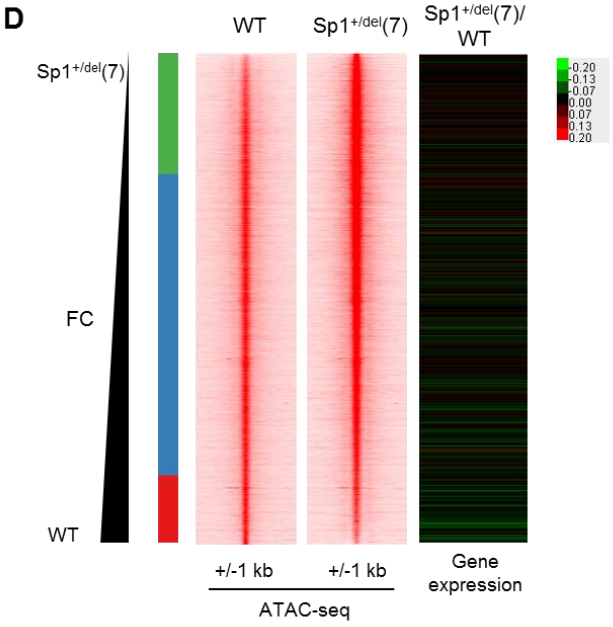
Motif	Name	p value	% sites
	CTCF	1e-1111	22.76
	Pou5f1	1e-373	2.90

Common (19087 sites)

Motif	Name	p value	% sites
	GC-box	1e-823	29.39
	CTCF	1e-659	8.35
	NFY	1e-586	11.84
	ETS	1e-239	16.29

WT specific (4314 sites)

Motif	Name	p value	% sites
	CTCF	1e-499	19.12
	GC-box	1e-156	21.70
	Pou5f1	1e-71	5.91



Sp1$^{+/del}$(7) specific (7752 sites)

Motif	Name	p value	% sites
	CTCF	1e-915	18.33
	Pou5f1	1e-190	5.53

Common (19212 sites)

Motif	Name	p value	% sites
	GC-box	1e-831	32.54
	CTCF	1e-642	7.65
	NFY	1e-628	12.00
	ETS	1e-263	9.62

WT specific (4237 sites)

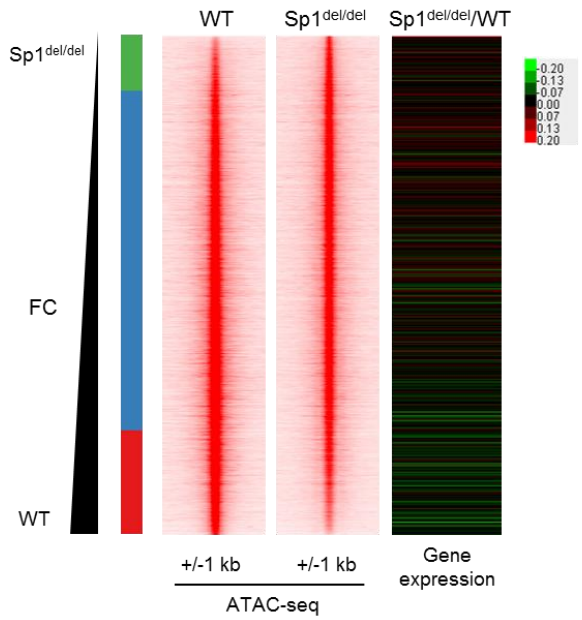
Motif	Name	p value	% sites
	CTCF	1e-542	16.10
	GC-box	1e-109	20.13
	Pou5f1	1e-56	4.81
	NFY	1e-35	4.01

Figure 3.17 – Changes in chromatin accessibility in Sp1 mutant ESCs correlate with gene expression

Density plots showing fold change (FC) of read count (log2 value) in ATAC-seq peaks in CRISPR-generated Sp1-mutant ESCs compared to WT ESCs. The peaks that are at least 2-fold different are indicated by the green and red sections of the coloured bar (showing regions of open chromatin in each Sp1 mutant clone that are gained and lost, respectively). A de novo motif search was performed using Homer to identify any motifs that were enriched in the common, WT-specific or Sp1 mutant-specific peaks, as shown in the tables. Each ATAC-seq peak was assigned to the nearest gene and the corresponding gene expression fold change (log2 value) in the Sp1 clone vs WT plotted alongside. Sp1$^{del/del}$ (A), Sp1$^{-/-}$ (B), Sp1$^{+/del}$(5) (C) and Sp1$^{+/del}$(7) (D) profiles are shown.

Flk1+ cells

A



Sp1$^{del/del}$ specific (3476 sites)

Motif	Name	p value	% sites
	CTCF	1e-673	26.62
	RFX	1e-26	21.53
	ETS	1e-17	7.51

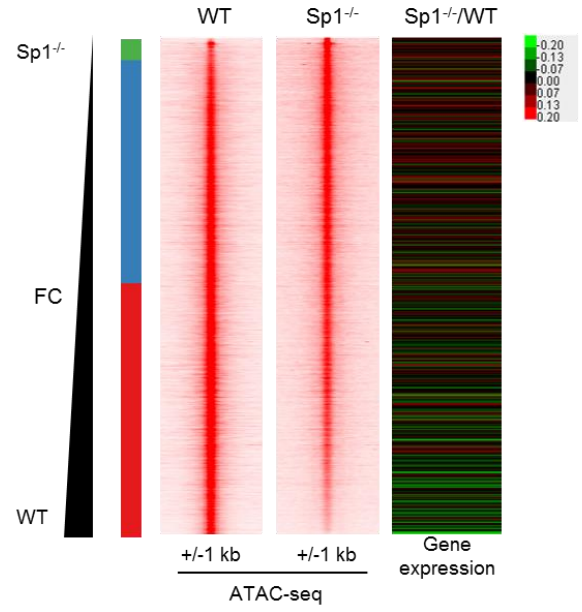
Common (20854 sites)

Motif	Name	p value	% sites
	CTCF	1e-793	7.02
	GC-box	1e-626	27.13
	NFY	1e-443	10.56
	ETS	1e-290	10.93

WT specific (6377 sites)

Motif	Name	p value	% sites
	CTCF	1e-668	13.61
	GATA	1e-296	13.02
	GC-box	1e-109	17.27
	TEAD	1e-164	10.84

B



Sp1$^{-/-}$ specific (1213 sites)

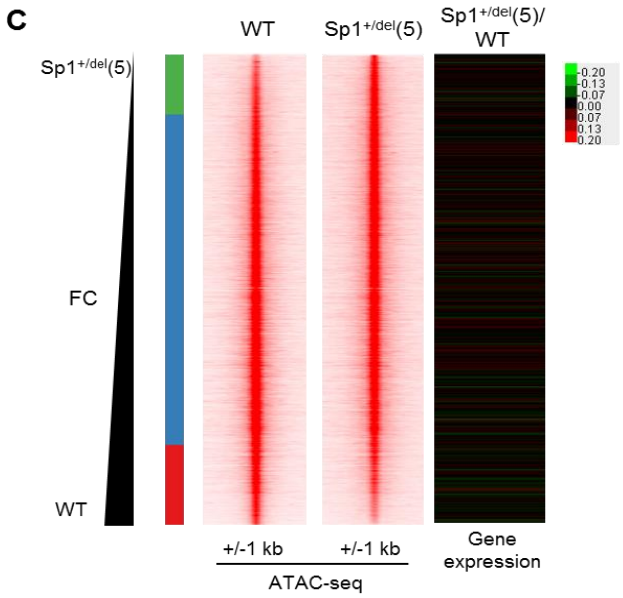
Motif	Name	p value	% sites
	CTCF	1e-54	9.89

Common (12733 sites)

Motif	Name	p value	% sites
	GC-box	1e-657	37.32
	NFY	1e-640	18.02
	ETS	1e-293	12.42
	CTCF	1e-241	5.44

WT specific (14483 sites)

Motif	Name	p value	% sites
	CTCF	1e-1223	10.51
	GATA	1e-601	14.98
	GC-box	1e-276	28.90
	TEAD	1e-200	8.34



Sp1$^{+/del}$(5) specific (3974 sites)

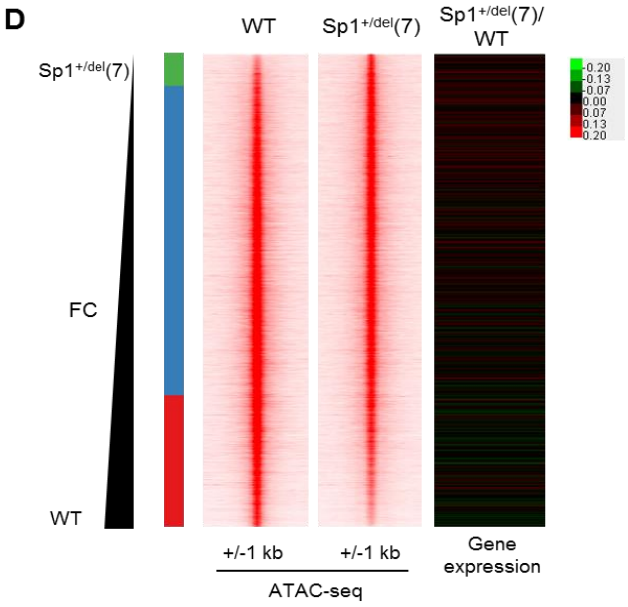
Motif	Name	P-value	% sites
	CTCF	1e-673	26.62
	RFX	1e-24	4.86

Common (21800 sites)

Motif	Name	p value	% sites
	CTCF	1e-859	7.27
	GC-box	1e-590	14.98
	NFY	1e-404	10.94
	GATA	1e-382	15.40
	ETS	1e-307	15.47

WT specific (5240 sites)

Motif	Name	p value	% sites
	CTCF	1e-497	12.67
	GATA	1e-225	13.80
	TEAD	1e-140	12.73
	GC-box	1e-97	21.24



Sp1$^{+/del}$(7) specific (2046 sites)

Motif	Name	p value	% sites
	CTCF	1e-378	25.12
	GATA	1e-15	7.09
	Myb	1e-13	8.88

Common (19125 sites)

Motif	Name	p value	% sites
	GC-box	1e-634	34.49
	CTCF	1e-591	8.47
	NFY	1e-483	11.92
	ETS	1e-280	5.19

WT specific (8090 sites)

Motif	Name	p value	% sites
	CTCF	1e-795	12.74
	GATA	1e-327	13.07
	GC-box	1e-120	23.04
	TEAD	1e-154	13.88

Figure 3.18 – Changes in chromatin profile in Sp1 mutant Flk1+ cells compared to WT correlate with gene expression changes

The fold change (FC) of read count (log2 value) in ATAC-seq peaks (indicating accessible chromatin) in Sp1 mutant Flk1+ cells compared to WT cells are shown as density plots. The green and red sections of the bar indicate the peaks that were significantly different (two-fold) between cell types. The motifs (identified using Homer de novo motif discovery) that were enriched in the common, WT-specific or Sp1 clone-specific peaks are shown in the tables. The gene expression change in the Sp1 mutant clones vs WT of the nearest gene to the ATAC-seq peak (fold change, log2 value) is plotted on the same axis. Sp1$^{del/del}$ (A), Sp1$^{-/-}$ (B), Sp1$^{+/del}$(5) (C) and Sp1$^{+/del}$(7) (D) profiles are shown.

The motif analysis suggests that chromatin structure at promoters is generally unchanged, while the sites that change with *Sp1* mutation are associated with enhancers. To test this idea, we divided the ATAC-seq peaks into promoter and distal open chromatin regions and compared them separately by ranking read counts (Section 2.15.1). Figure 3.19 shows the results for $Sp1^{del/del}$ at ESC (A) and Flk1+ cells (B). As predicted, the read count comparison of promoter regions was similar in WT and $Sp1^{del/del}$ cells, but there was more change at distal sites. Similar results were found for the other Sp1 clones (Supplementary Figure 5.2). This suggests that the chromatin of promoter-regulated constitutively expressed genes is unaffected, while the accessibility of tissue-specific regulatory regions is changing. This finding is somewhat surprising, as Gilmour et al., showed that Sp1 binding occurs to a large extent in promoters, rather than distal regions (Gilmour et al., 2014). However, whether change in chromatin accessibility is a direct or indirect effect of the lack of (or a reduction in) functional Sp1 needs to be further investigated.

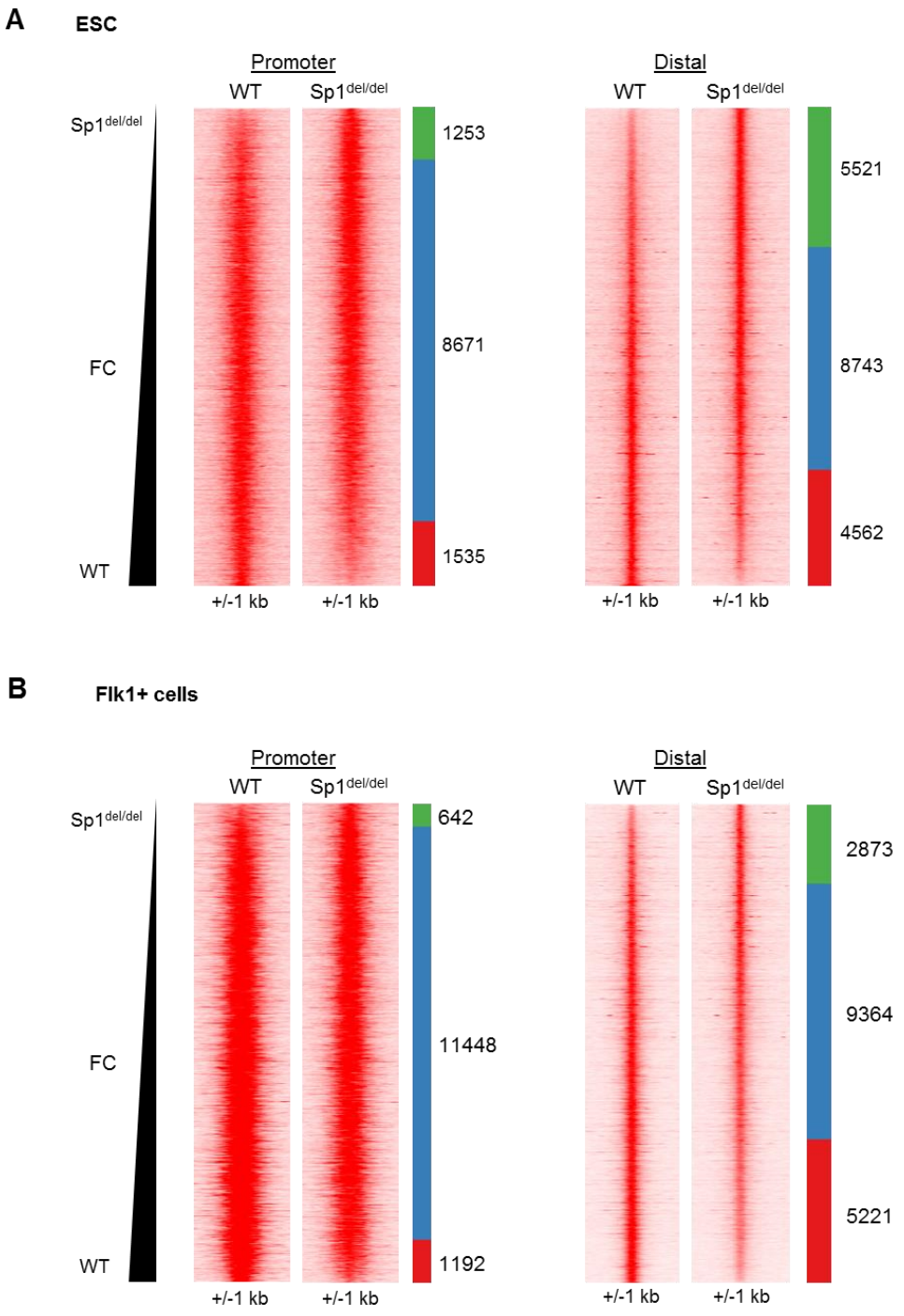


Figure 3.19 – Most of the changes in chromatin accessibility following Sp1 manipulation occur at distal sites

ATAC-seq was performed in WT and Sp1$^{del/del}$ cells to identify regions of open chromatin. ATAC-seq peaks in both WT and Sp1$^{del/del}$ cells were divided into their position relative to a TSS – either at promoters (if within 2 kb of a TSS) or at distal sites (further than 2 kb from TSS). Density plots show the fold change (FC) of read counts at promoter and distal ATAC-seq peaks in Sp1$^{del/del}$ relative to WT in ESC (A) and Flk1+ cells (B). The plot is divided into common peaks (blue) and specific peaks, which are at least two-fold different (red is WT-specific and green is Sp1$^{del/del}$-specific).

Density plots showed changes in chromatin accessibility are associated with gene expression changes. For example, *Hand1*, a mesoderm marker found to be downregulated in Sp1$^{-/-}$ Flk1+ cells, showed a decrease in chromatin accessibility at the promoter in Sp1$^{-/-}$ cells (Figure 3.20). In contrast, no change in chromatin accessibility was seen at a housekeeping gene (*Tbp*), whose expression is not altered, thus confirming the effect is not due to a difference in ATAC-seq data quality (Figure 3.21). Therefore, changes in chromatin structure could be a contributing factor to the impaired haematopoietic phenotype.

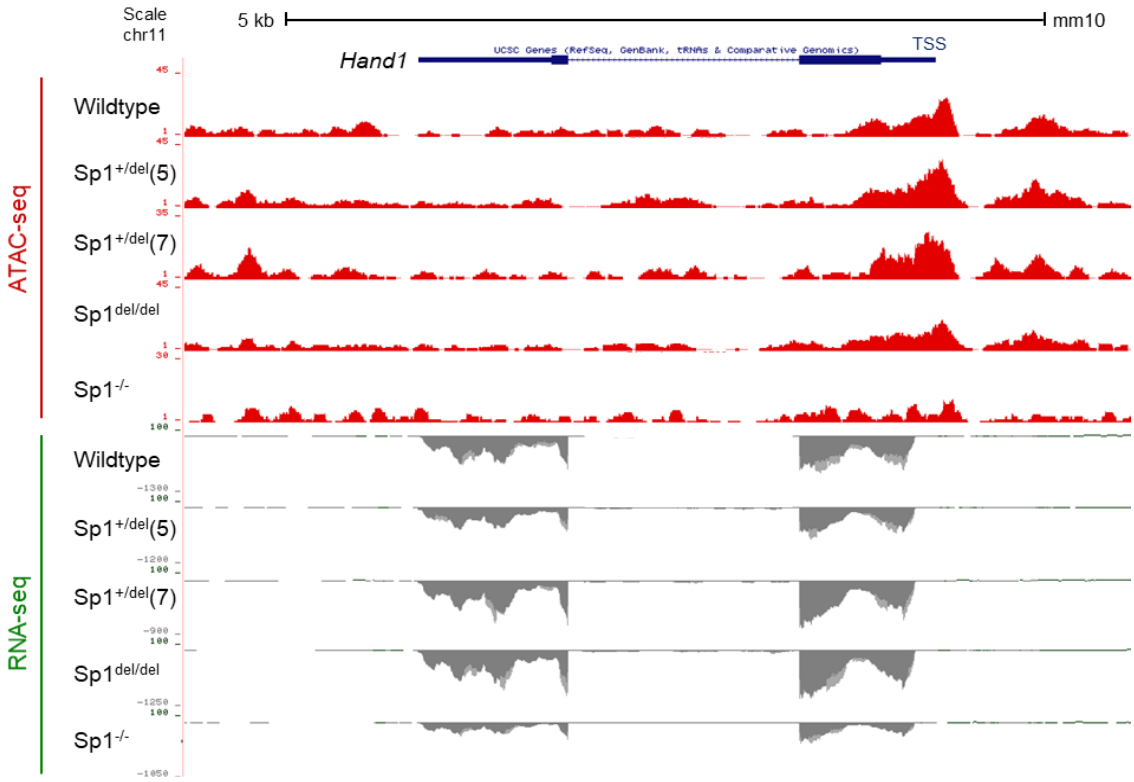


Figure 3.20 – Differential open chromatin regions are detected by ATAC-seq and correlate with changes in gene expression

ATAC-seq was performed with the five indicated clones (mutant, heterozygote and WT). A screenshot from the ATAC-seq browser track in Flk1+ cells at the *Hand1* gene is shown (top). *Hand1* is significantly downregulated in Sp1$^{-/-}$ Flk1+ cells compared to WT (shown by RNA-seq data, bottom). The ATAC-seq peak present at the start of the gene is lost in Sp1$^{-/-}$ cells, compared to the other clones, showing a reduction in chromatin accessibility at the promoter in Sp1$^{-/-}$ cells.

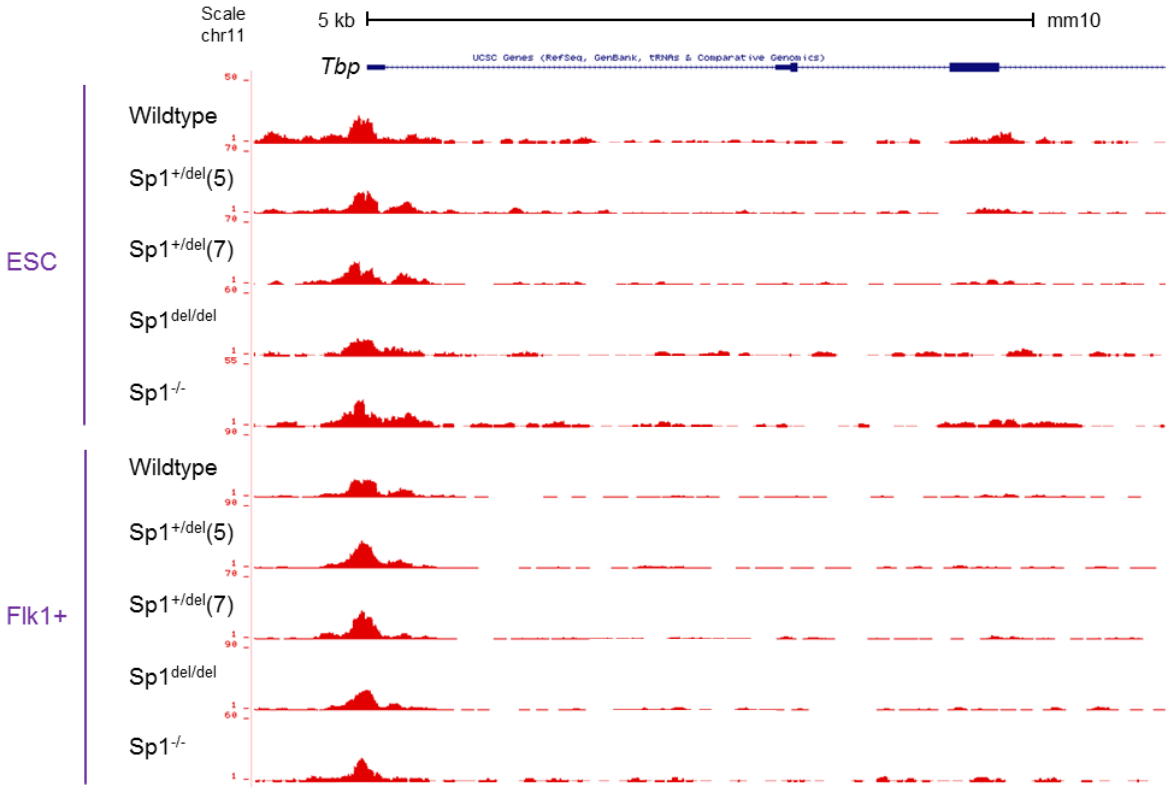


Figure 3.21 – Quality of ATAC-seq data is consistent between different cell clones
ATAC-seq was performed in WT and Sp1 mutant cell clones at ESC and Flk1+ cell stages. UCSC browser screenshot at the housekeeping gene *Tbp* shows an ATAC-seq peak at the start of the gene in all samples.

3.4 Differences in $Sp1^{del/del}$ and $Sp1^{-/-}$ cannot be explained by residual Sp1 binding to DNA

3.4.1 ChIP-seq reveals genome-wide binding sites of Sp1

The in vitro differentiation experiments (described in chapter 3.1) showed that haematopoietic specification is severely impaired with $Sp1^{-/-}$ cells, failing to form Flk1+ cells. In contrast, $Sp1^{del/del}$ cells are more mildly impaired – they are able to differentiate to Flk1+ cells and haematopoietic progenitors, albeit with reduced efficiency, but they fail to terminally differentiate. We therefore asked the question of the underlying cause of this difference in differentiation potential. $Sp1^{-/-}$ cells express no Sp1 protein; $Sp1^{del/del}$ cells express a truncated Sp1 protein that lacks the DBD. As the truncated Sp1 cannot directly bind DNA, we would expect it to be non-functional. However, the differentiation results suggest that it is hypomorphic. Consequently, we asked whether DBD-deficient Sp1 could be binding indirectly to the DNA, for example through protein-protein interactions with its DNA-binding partner proteins, and impacting on gene regulation through the action of its intact TADs. To investigate this, ChIP-seq was performed (with double crosslinking) in ESC and Flk1+ cells, in each of the Sp1 clones, to identify sites of Sp1 binding to DNA, genome-wide. (Note: ChIP-seq was not possible in $Sp1^{-/-}$ Flk1+ cells due to low cell numbers.) Furthermore, while Sp1 ChIP-seq was performed in WT cells by Gilmour et al., residual Sp1 binding was not explored in Sp1 DBD-deficient cells, thus these experiments provide novel datasets (Gilmour et al., 2014). High confidence binding site data was generated by filtering Sp1 ChIP-seq peaks with ATAC-seq peaks (merged of all clones), thereby identifying binding sites that were present in regions of open chromatin (Figure 3.22). We found that a high proportion of Sp1 binding sites were located at open CpG islands in WT ESC and Flk1+ cells (Figure 3.23). Conversely, ~91% of accessible CpG islands were bound by Sp1 in both cell types, showing that nearly all CpG islands contain Sp1 binding sites. In addition, the majority of key deregulated genes identified by RNA-

seq analyses were direct targets of Sp1, as highlighted in Tables 3.1 and 3.2, suggesting a direct effect of the Sp1 mutation on their deregulation.

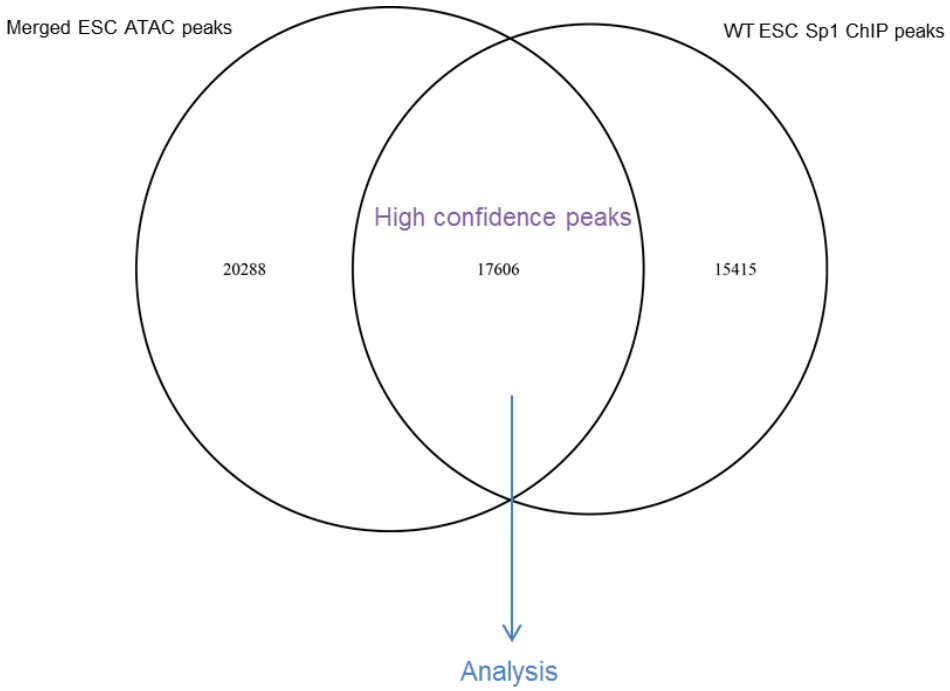


Figure 3.22 – High confidence protein-DNA binding sites were generated by filtering for open chromatin regions.
ChIP-seq was performed in each Sp1 mutant clone and WT cells to identify Sp1 binding sites. The ChIP-seq peaks were overlapped with ATAC-seq peaks from a merged dataset of all clones at each individual cell stage. An example using Sp1 ChIP-seq in WT ESCs and merged ESC ATAC-seq peaks is shown.

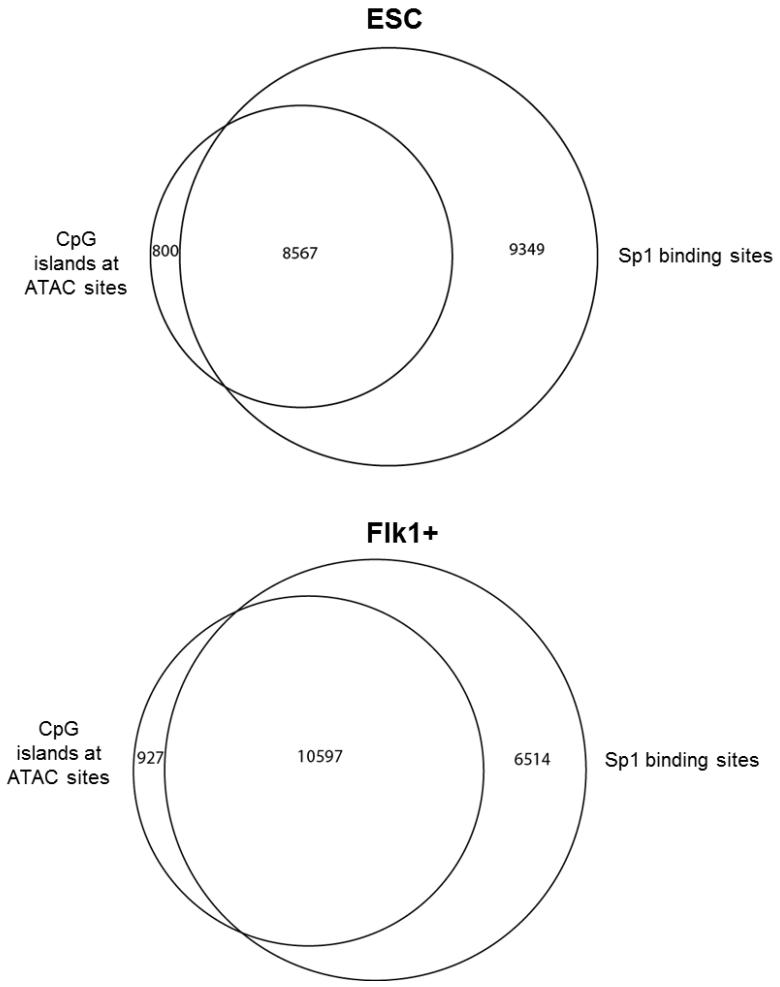


Figure 3.23 – Most accessible CpG islands are bound by Sp1
Sequences containing annotated CpG islands were filtered for those in open chromatin by selecting those within ATAC-seq peaks. The accessible CpG islands were then overlapped with Sp1 ChIP-seq peaks in WT ESC and Flk1+ cells to identify the proportion of CpG islands that are bound by Sp1.

3.4.2 The truncated protein expressed in Sp1$^{del/del}$ cells does not bind to DNA despite its nuclear localisation

We asked whether the different phenotypes between Sp1 hypomorph and knockout cells could be explained by residual Sp1 binding in Sp1$^{del/del}$ cells. To explore this idea, the average number of ChIP-seq reads at each high confidence binding site in each of the

Sp1 mutant clones and the wild type was plotted (Figure 3.24). No Sp1 binding was found in either $Sp1^{-/-}$ or $Sp1^{del/del}$ cells, suggesting the differences in haematopoietic potential cannot be explained by indirect binding of the Sp1 truncated protein to DNA in $Sp1^{del/del}$ cells. Reduced binding was also seen in $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7) cells compared to WT, possibly reflecting lower levels of WT Sp1 in the cell (as only one allele is WT). Alternatively, the truncated form of Sp1 protein in the cells could be interfering with the activity of the WT protein, for example interacting with its binding partners, thus resulting in reduced average levels of binding. These results were confirmed by the Sp1 ChIP-seq browser tracks (Figure 3.25).

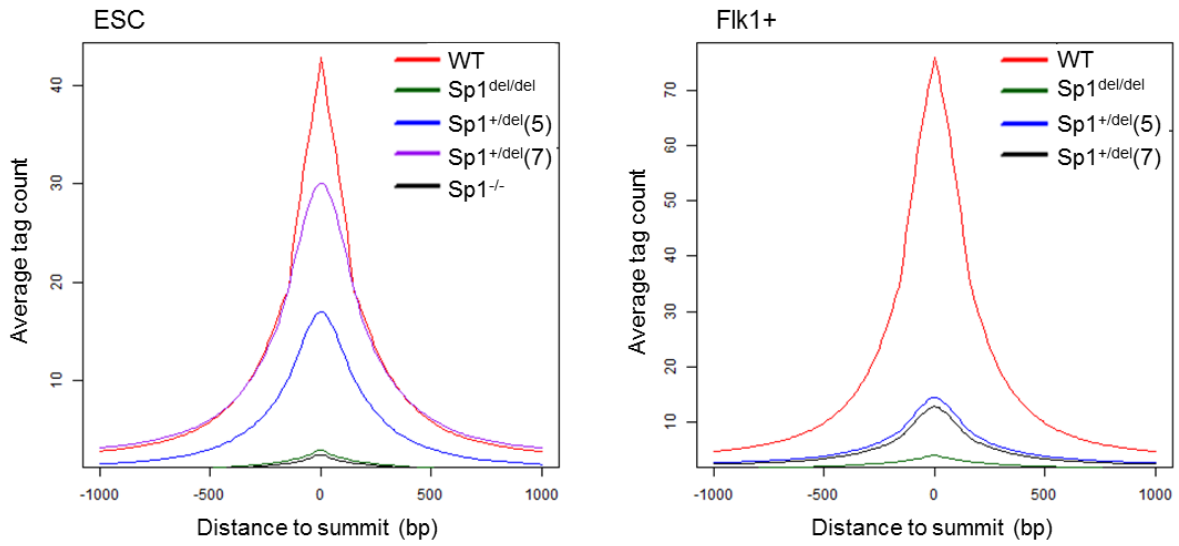


Figure 3.24 – There is no Sp1 binding in Sp1 hypomorph cells and reduced Sp1 binding in heterozygous cells

Sp1 ChIP-seq was performed in each Sp1 clone at ESC and Flk1+ cell stages. High confidence Sp1 binding sites were identified (by calling the ChIP peaks that also had an ATAC-seq peak) and the average number of reads at each site plotted relative to the summit of the peak.

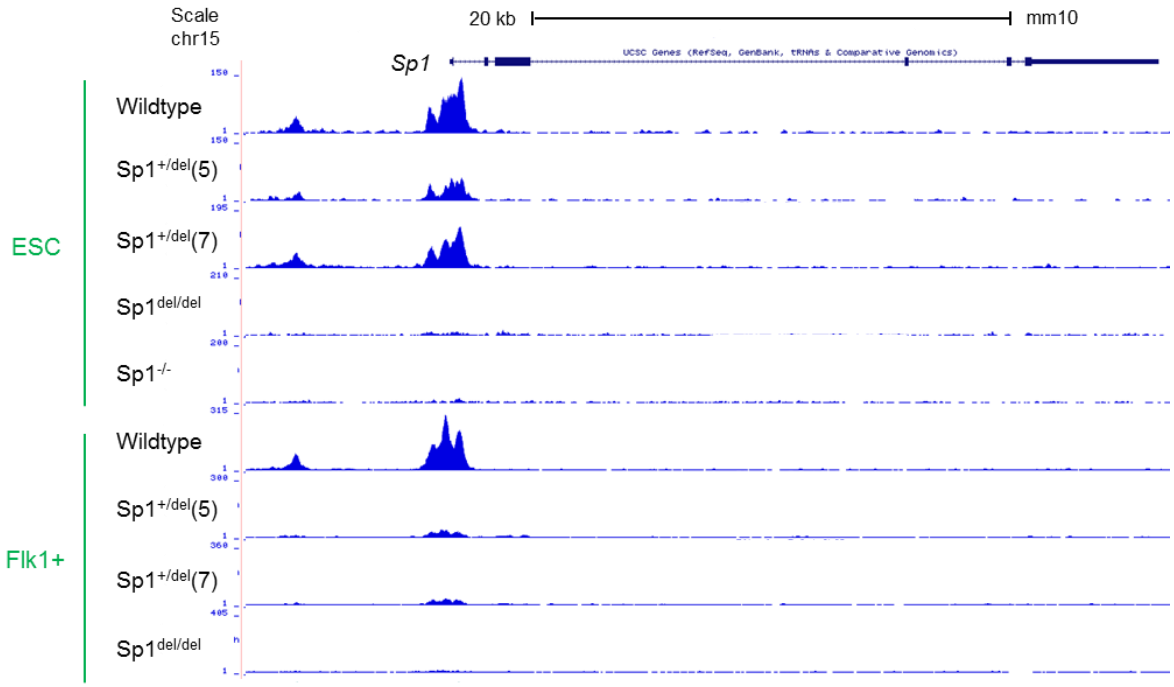


Figure 3.25 – Sp1 ChIP-seq showed no binding in Sp1$^{del/del}$ cells
Sp1 ChIP-seq was carried out in all Sp1 clones at ESC and Flk1+ cell stages. UCSC browser screenshot at the *Sp1* locus shows differential Sp1 binding (a peak) at the promoter.

The nuclear localisation signal of Sp1 is thought to reside in the C-terminal DBD (Ito et al., 2009). Thus, to assess whether the DBD-deficient Sp1 protein in the Sp1$^{del/del}$ clone is able to enter the nucleus, we performed immunofluorescence staining assays, using an antibody to target Sp1 and β-actin, to view Sp1 localisation in WT and Sp1$^{del/del}$ ESCs (Figure 3.26). The results confirmed the truncated Sp1 protein was present in the nucleus of Sp1$^{del/del}$ cells, similar to levels seen for WT Sp1 in WT cells.

Altogether, Sp1 ChIP-seq experiments have shown there is no Sp1 binding to DNA in Sp1$^{del/del}$ cells, despite its presence in the nucleus. Therefore, the mechanisms behind the differences in knockout and hypomorph phenotypes need to be further investigated.

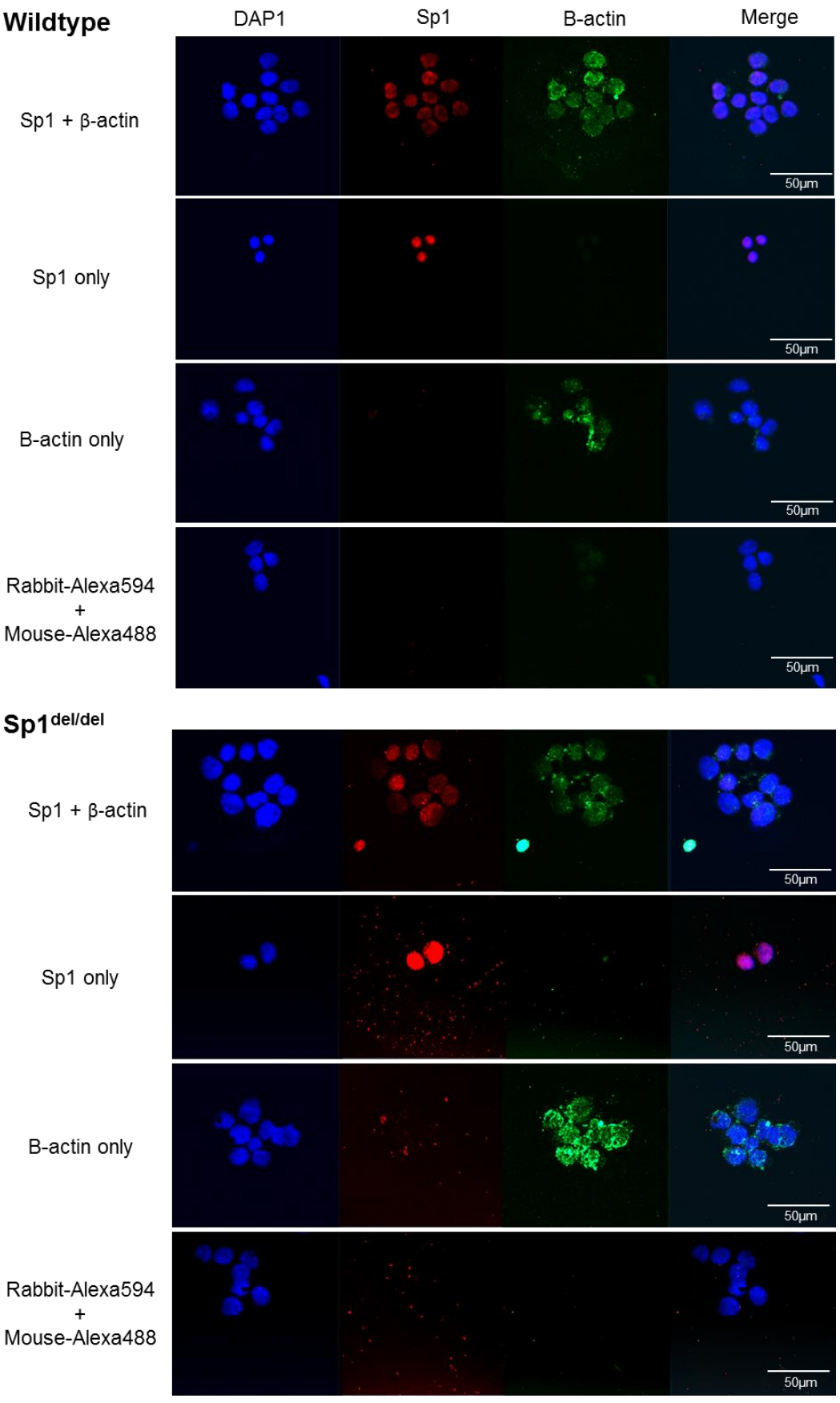

Wildtype
DAP1
Sp1
B-actin
Merge
Sp1 + β-actin
Sp1 only
B-actin only
Rabbit-Alexa594
+
Mouse-Alexa488
50μm
Sp1del/del
Sp1 + β-actin
Sp1 only
B-actin only
Rabbit-Alexa594
+
Mouse-Alexa488
50μm

Figure 3.26 – DBD-deficient Sp1 protein is localised to the nucleus
Sp1 localisation in WT and $Sp1^{del/del}$ ESCs was measured by immunofluorescence and confocal microscopy. Antibodies specific to Sp1 and β-actin were used with fluorescently tagged secondary antibodies, DAPI was used to stain the nucleus and secondary antibody only samples used as a control (n=3).

3.5 Sp3 can partially compensate for the loss of Sp1

Sp3 is a close family member of Sp1. It has a very similar structure, including a highly conserved DBD, and so can often recognise the same sites in the DNA. Like Sp1, it is also ubiquitously expressed. Consequently, we speculated whether Sp3 could be compensating for the lack of a functional Sp1 in $Sp1^{del/del}$ and/or $Sp1^{-/-}$ cells. This may explain why the ESCs are relatively unaffected and why there are only limited changes in the global gene expression programme.

Due to the high similarity of the *Sp1* and *Sp3* DNA sequence, we first verified that levels of Sp3 protein were unaffected by the CRISPR-Cas9 system in the Sp1 mutant cell clones by Western blotting (Figure 3.27A). The blot showed the levels of Sp3 were comparable in all of the CRISPR-generated Sp1 mutant clones and WT cells. However, interestingly, there appeared to be shift in the relative levels of the four isoforms of Sp3 with loss of Sp1 activity. Quantification of the Sp3 protein levels in the Western blot showed an increase in the ratio of long/short isoforms in $Sp1^{-/-}$ and $Sp1^{del/del}$ cells (Figure 3.27B).

We next performed Sp3 ChIP-seq in each of the Sp1 mutant cells as ESCs and Flk1+ cells. High confidence sites in open chromatin regions were identified as with the Sp1 ChIP-seq.

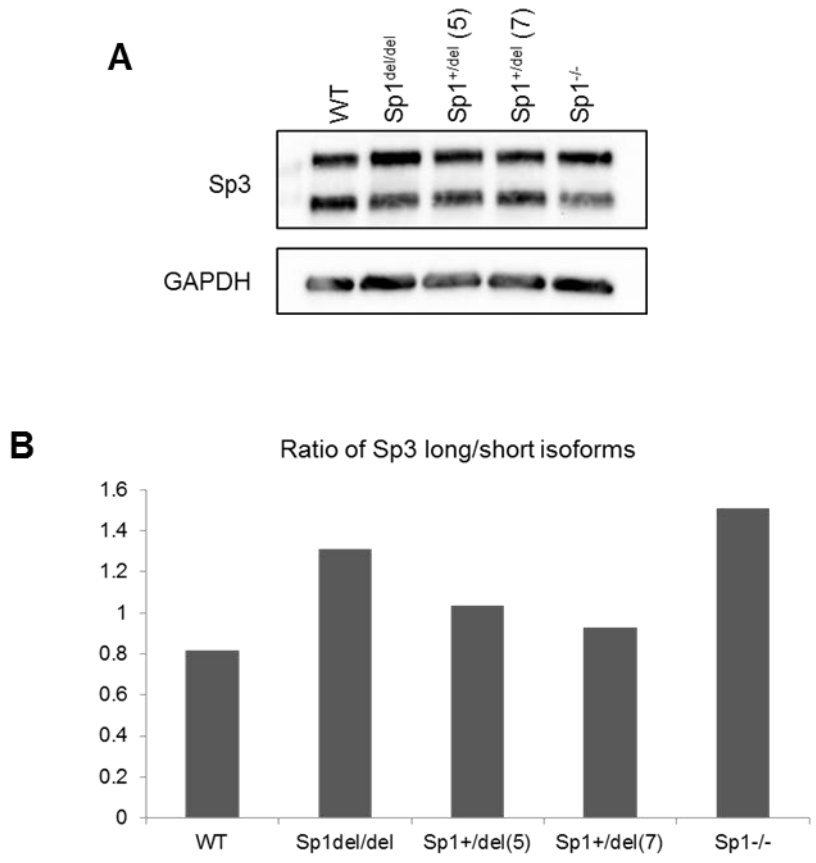


Figure 3.27 – Sp3 protein expression is unchanged with CRISPR-Cas9 targeting of *Sp1*

Protein was extracted from ESCs of each of the CRISPR-generated Sp1 mutant cell clones and a Western blot performed to measure levels of Sp3 expression (A) (n=2). The protein was resolved into two bands – one representing the two long isoforms and the second showing the two short isoforms. The levels were quantified by normalising to levels of GAPDH and the ratio between long and short isoforms calculated (B).

3.5.1 The binding of both Sp1 and Sp3 shifts in the ESC-Flk1+ transition

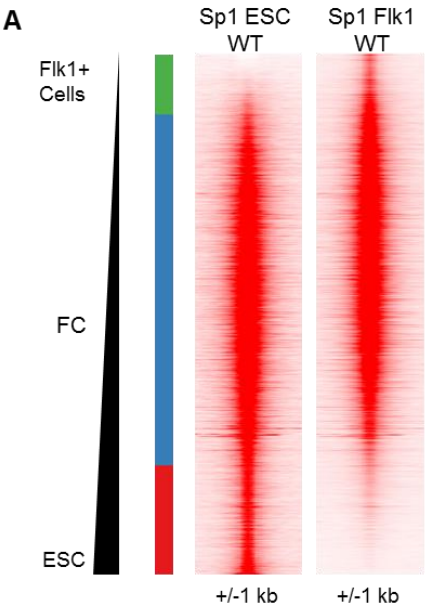
Both Sp1 and Sp3 are ubiquitously expressed proteins and involved in the regulation of many housekeeping genes. Earlier results (chapter 3.1), in addition to a previous study (Gilmour et al., 2014), have suggested that Sp1 can have a tissue-specific role in

haematopoietic specification. Therefore, we investigated whether the binding patterns of Sp1 and Sp3 change as the ESCs differentiate to Flk1+ cells.

A comparison between the Sp1 binding profile in ESCs and Flk1+ cells (Figure 3.28A) showed a large peak overlap. These shared sites were enriched for promoter-associated transcription factor binding motifs, such as GC-box and NFY, suggesting they may be associated with commonly-expressed genes, such as housekeeping genes. However, there was also a cell type-specific Sp1 binding pattern. As expected, the Flk1+ cell-specific sites are enriched for the binding motifs of factors associated with the early stages of haematopoietic specification, such as GATA and TEAD (in addition to the GC-box). Indeed, both GATA2 and TEAD4 transcription factors are known to be important, binding along with SCL/TAL1 and FLI1, in establishing the gene expression programme required for early haematopoietic specification (Pimanda et al., 2007; Goode et al., 2016). This suggests Sp1 may associate with these factors and is possibly involved in regulating cell fate. However, while ESC-associated motifs were detected in the ESC-specific binding sites, such as Pou5f1, they were only present at a small percentage of sites. This suggests Sp1 rarely interacts with ESC-associated factors, such as pluripotency factors.

To confirm that most of the changes in Sp1 binding occurred at enhancer elements, rather than promoters, we separated the binding sites into promoter and distal genomic positions (chapter 2.15.1) (Figure 3.28B,C). Consistent with the motif analysis from the genome-wide binding, we observed that binding at promoters was highly conserved between ESC and Flk1+ cells, and again show enrichment of conventional promoter-associated motifs. In contrast, there was a large change in the distribution of Sp1 binding at distal sites between the two differentiation stages, suggesting that most changes in Sp1 binding occur at enhancer elements. Again, we see enrichment of cell type-specific factors, which commonly drive tissue-specific patterns of gene expression via enhancer binding.

A



Flk1+ specific (2725 sites)

Motif	Name	p value	% sites
	GATA	1e-186	19.56
	GC-box	1e-61	52.84
	TEAD	1e-60	13.21
	PBX	1e-32	8.90

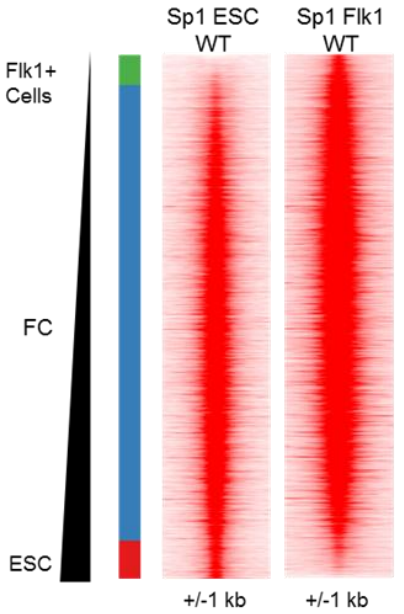
Common (15970 sites)

Motif	Name	p value	% sites
	GC-box	1e-1980	49.74
	NFY	1e-452	15.42
	ETS	1e-278	16.78
	CREB	1e-264	16.71
	GFY	1e-254	3.02
	CTCF	1e-108	3.49

ESC specific (4980 sites)

Motif	Name	p value	% sites
	GC-box	1e-713	42.27
	Pou5f1	1e-162	5.92
	NFY	1e-70	2.95

B



Flk1+ specific (745 sites)

Motif	Name	p value	% sites
	CREB	1e-24	10.74
	NFY	1e-22	28.46
	GATA	1e-14	9.13
	ETS	1e-13	19.33
	GC-box	1e-12	21.61

Common (11446 sites)

Motif	Name	p value	% sites
	GC-box	1e-1659	54.03
	NFY	1e-473	17.96
	ETS	1e-303	9.79
	NRF1	1e-251	12.05

ESC specific (969 sites)

Motif	Match	p value	% sites
	GC-box	1e-123	50.57
	NFY	1e-15	3.41
	HIF	1e-14	17.03

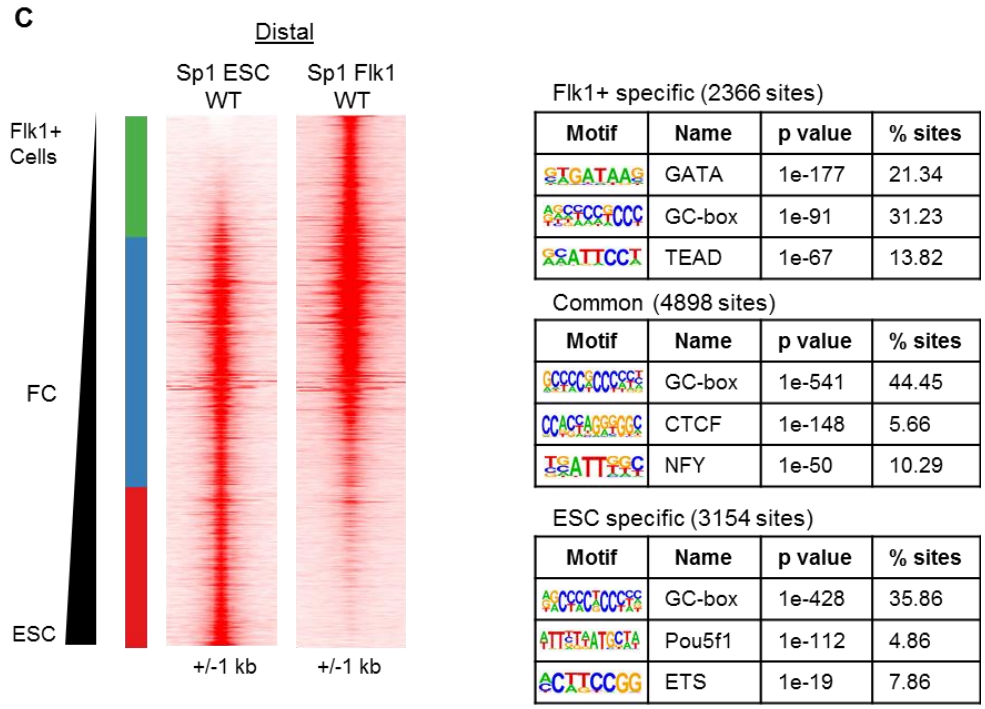
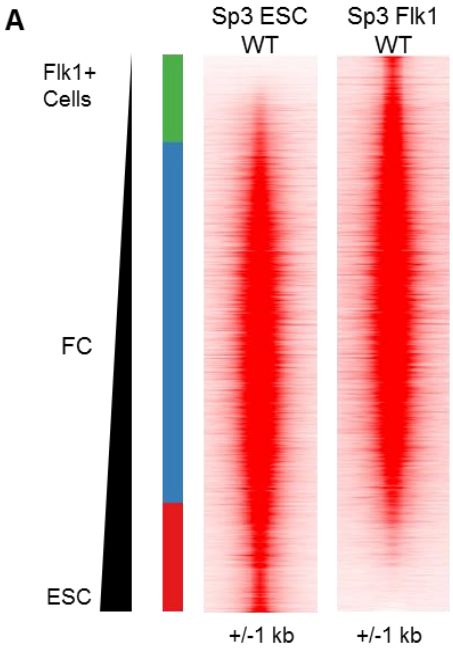


Figure 3.28 – Sp1 binding changes between ESC and Flk1+ cells
Sp1 ChIP-seq was performed in WT ESC and Flk1+ cells and high confidence Sp1 binding sites identified (with an overlapping ATAC-seq peak). Density plots show the fold change (FC) between ESC and Flk1+ Sp1 binding genome-wide (A) and specifically at promoters (B) and distal elements (C) (Promoters were classified if within 2 kb of a TSS and as distal otherwise). Green and red sections of the bar indicate ChIP-seq peaks that are at least two-fold different. Motifs (identified using Homer de novo motif discovery) that are significantly enriched at common, ESC- and Flk1-specific sites are shown.

Sp3 binding in ESCs and Flk1+ cells shows a remarkably similar pattern, with common binding predominantly at promoters and cell type-specific binding associated with distal regulatory elements and tissue-specific transcription factor binding motifs (Figure 3.29). However, surprisingly, there was an enrichment of CTCF binding sites, particularly at distal sites in Flk1+ cells. CTCF is a common motif in distal regions, with CTCF involved in DNA looping between promoters and enhancers, as well as having a role as an insulator of chromatin domains (Zlatanova & Caiafa, 2009). Therefore, ChIP-seq experiments show a potential role of Sp1 and Sp3 in early haematopoiesis by binding

tissue-specific enhancer elements and possibly interacting with cell type-specific transcription factors.



Flk1+ specific (4718 sites)

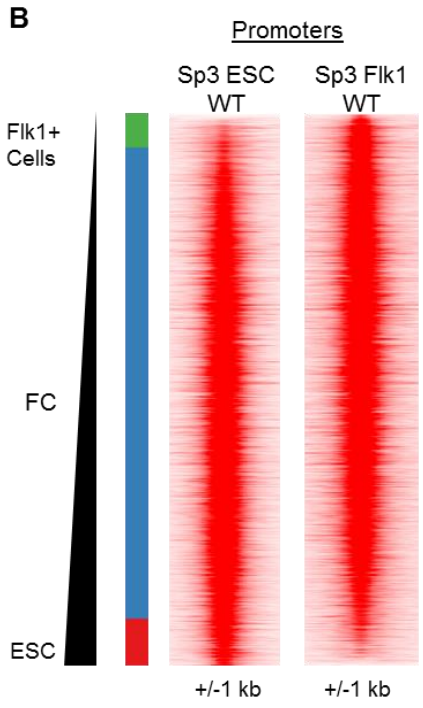
Motif	Name	p value	% sites
	CTCF	1e-453	15.41
	GATA	1e-186	17.44
	GC-box	1e-51	35.91
	NFY	1e-47	2.33
	ETS	1e-29	6.66

Common (19405 sites)

Motif	Name	p value	% sites
	GC-box	1e-1491	36.71
	CTCF	1e-1332	13.40
	NFY	1e-360	11.44
	CREB	1e-208	12.71

ESC specific (5885 sites)

Motif	Name	p value	% sites
	GC-box	1e-495	24.23
	Pou5f1	1e-132	5.28



Flk1+ specific (858 sites)

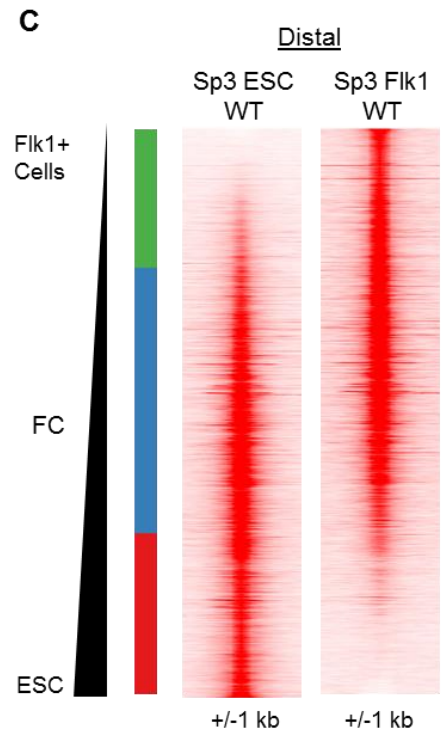
Motif	Name	p value	% sites
	NFY	1e-75	20.40
	GC-box	1e-32	58.39
	ETS	1e-31	29.14

Common (11746 sites)

Motif	Name	p value	% sites
	GC-box	1e-1136	38.90
	NFY	1e-337	15.24
	ETS	1e-291	15.00
	ZBTB33	1e-229	10.71
	NRF	1e-178	10.86

ESC specific (1164 sites)

Motif	Name	p value	% sites
	GC-box	1e-67	41.29



Flk1+ specific (3986 sites)

Motif	Name	p value	% sites
	CTCF	1e-578	20.57
	GATA	1e-172	16.16

Common (7602 sites)

Motif	Name	p value	% sites
	CTCF	1e-1644	26.81
	GC-box	1e-368	28.77
	NFY	1e-27	7.60

ESC specific (4605 sites)

Motif	Name	p value	% sites
	GC-box	1e-386	42.71
	SOX	1e-274	23.79
	Pou5f1	1e-149	4.21

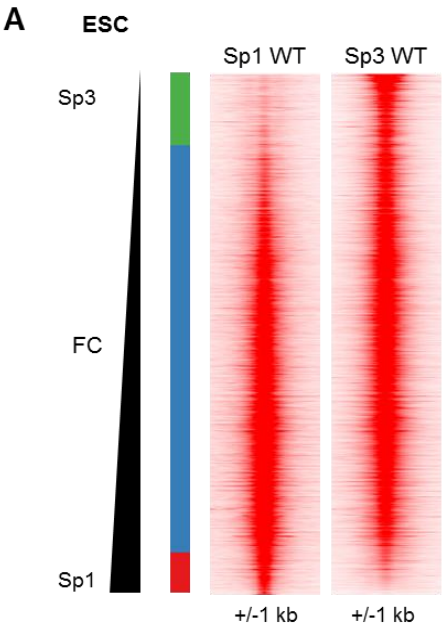
Figure 3.29 – ESC and Flk1+ cells show different Sp3 binding patterns
Sp3 ChIP-seq was performed in WT ESC and Flk1+ cells. Density plots of the fold change (FC) of Sp3 binding (based on high confidence ChIP-seq peaks) between ESC and Flk1+ cells genome-wide (A) and specifically at promoters (B) and at distal elements (C). Green and red sections of the coloured bar indicate sites with differential Sp3 binding (at least two-fold difference). Motifs (identified using Homer de novo motif discovery) that are significantly enriched at common, ESC- and Flk1-specific sites are shown.

3.5.2 A high level of overlap between Sp1 and Sp3 binding

Sp1 and Sp3 have been shown to bind to the same DNA sites in in vitro studies and at individual loci. We investigated whether the same was true on a genome-wide level by comparing the Sp1 and Sp3 binding sites identified by ChIP-seq in WT ESC and Flk1+ cells (Figure 3.30). Indeed, we found a large overlap in Sp1 and Sp3 binding in both ESC and Flk1+ cells, with the vast majority of sites bound by both transcription factors. However, at some sites we observed specific binding by Sp1 or Sp3. The Sp1-specific

sites show a very similar pattern of motif enrichment to the shared sites – common promoter-associated transcription factor motifs such as GC-box, NFY and ETS. This is consistent with the important role of Sp1 in regulating housekeeping genes and recruiting the transcriptional machinery to TATA-less promoters (Zhou & Chiang, 2002).

Surprisingly, the Sp3-specific sites are not enriched for the canonical GC-box. This suggests Sp3 may be recruited to chromatin via a binding partner protein. Interestingly, the CTCF motif is enriched in the Sp3-specific sites (consistent with the results of Figure 3.29). In support, ZBTB33 (Kaiso) motif is also enriched, which is known to interact with CTCF (Defossez et al., 2005).



Sp3 specific (3722 sites)

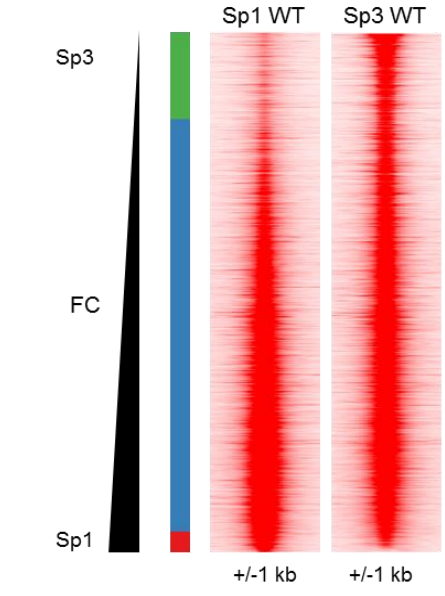
Motif	Name	p value	% sites
	CTCF	1e-630	33.61
	ZBTB33	1e-306	8.95
	STAT	1e-77	15.13
	ETS	1e-68	16.55

Common (20892 sites)

Motif	Name	p value	% sites
	GC-box	1e-1999	46.06
	NFY	1e-308	10.32
	ETS	1e-249	15.78
	ATF	1e-185	11.52

Sp1 specific (2075 sites)

Motif	Name	p value	% sites
	GC-box	1e-199	33.22
	NFY	1e-53	9.40
	ETS	1e-24	2.51



Sp3 specific (3859 sites)

Motif	Name	p value	% sites
	CTCF	1e-1461	37.42
	ETS	1e-43	13.19

Common (18398 sites)

Motif	Name	p value	% sites
	GC-box	1e-1789	42.15
	NFY	1e-517	13.74
	ETS	1e-345	11.88
	CTCF	1e-333	5.69

Sp1 specific (948 sites)

Motif	Name	p value	% sites
	GC-box	1e-198	61.25
	NFY	1e-24	11.71

Figure 3.30 – Sp1 and Sp3 show overlapping, yet distinct, binding patterns

Sp1 and Sp3 ChIP-seq was performed in WT ESCs (A) and Flk1+ cells (B). Density plots show the fold change (FC) in Sp1/Sp3 binding (based on high confidence ChIP-seq peaks). The Sp3- and Sp1-specific binding sites are indicated by the green and red bars, respectively. Motif discovery was carried out at the specific and common peaks and the significantly enriched motifs shown.

To further investigate a possible association between Sp3 and CTCF, we plotted the binding of CTCF (obtained from a publicly available ChIP-seq dataset in ESCs) against the Sp1/Sp3 binding comparison (Figure 3.31). CTCF binding was indeed found to be associated with Sp3-specific binding (with no or little Sp1 present). Furthermore, a plot of the localisation of Sp1 (GC-box) and CTCF motifs found that they negatively correlate. This suggests that Sp3, and not Sp1, is associated with CTCF binding. These Sp3-specific, CTCF-enriched sites, also show low enrichment of the GC-box motif, suggesting Sp3 may be associating with other factors (CTCF?) in order to bind to DNA. Sp3 may also be able to bind to certain CTCF motifs, which display some sequence similarity to GC-boxes.

Overall, these results show that Sp1 and Sp3 have very similar binding patterns, but there are differences, indicating they perform different functions in the cell.

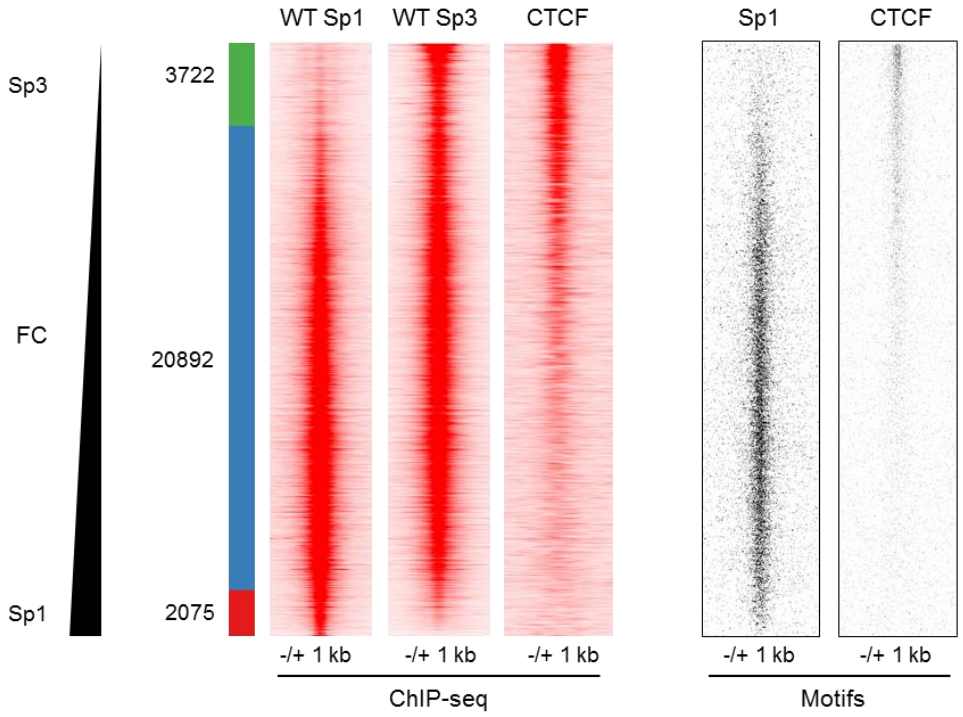


Figure 3.31 – Sp3 binding is correlated with CTCF binding
ChIP-seq was performed in WT ESCs to identify binding sites of Sp1 and Sp3. Density plots show the fold change (FC) in Sp1/Sp3 binding. The Sp3- and Sp1-specific binding sites are indicated by the green and red bars, respectively. A publicly available ESC CTCF ChIP-seq dataset (accession number: GSM2418860) was used to identify CTCF binding sites and plotted along the Sp1/Sp3 axis. The peaks were search for the presence of Sp1 and CTCF consensus motifs and the position of the motif relative to the peak summit plotted.

3.5.3 Sp3 binding distribution is mostly unchanged in Sp1 mutant cells

$Sp1^{del/del}$ and $Sp1^{-/-}$ ESCs grew fairly normally (although with a slightly lower rate of proliferation), which is surprising considering the important role of Sp1 in regulating genes in a wide variety of cellular processes, including metabolism and cell cycle. Also in the mouse, knockout of Sp1 was embryonic lethal, but the expression of many target genes was unaffected, suggesting Sp3 may be able to compensate, in part, for loss of Sp1 (Marin et al., 1997). Indeed, Sp1 and Sp3 show very similar binding profiles in WT cells,

however, there are also unique sites. Could the binding distribution of Sp3 be altered upon Sp1 knockout to now bind at Sp1-specific sites to rescue gene regulation? To investigate this, we compared the binding of Sp1 in WT (and $Sp1^{+/del}$ clones) to Sp3 in the Sp1 mutant clones using ChIP-seq.

Generally, the binding of Sp3 in each of the Sp1 wild type and mutant cell clones appeared very similar to each other and to the binding of Sp1 in WT cells (Figure 3.32). Moreover, we found a strong correlation overall between Sp1 and Sp3 binding (Figure 3.33), but there were also differences, consistent with the results in Figure 3.30. The binding correlations clustered based on the cell type (ESC and Flk1+) and on Sp1 or Sp3 specificity, rather than by any differences between the individual clones, suggesting that the overall binding of Sp3 pattern is similar with or without *Sp1* mutations. However, due to the relatively small number of Sp1- and Sp3-specific sites compared to shared sites, changes may not be detected in this heatmap.

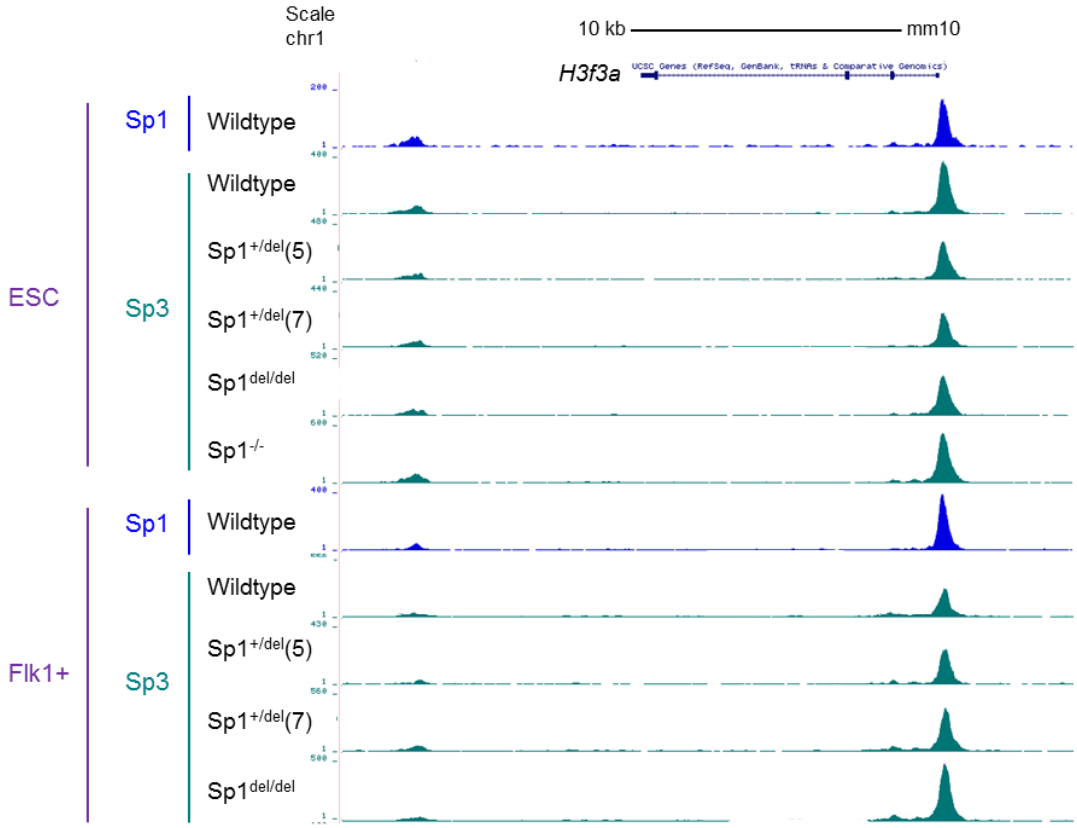


Figure 3.32 – Sp1 and Sp3 generally show similar binding patterns

Sp1 and Sp3 ChIP-seq was performed in WT and Sp1 mutant clones. A UCSC browser screenshot at the *H3f3a* locus shows Sp3 binding in all Sp1 mutant clones in ESC and Flk1+ cells, as compared to Sp1 binding in WT cells.

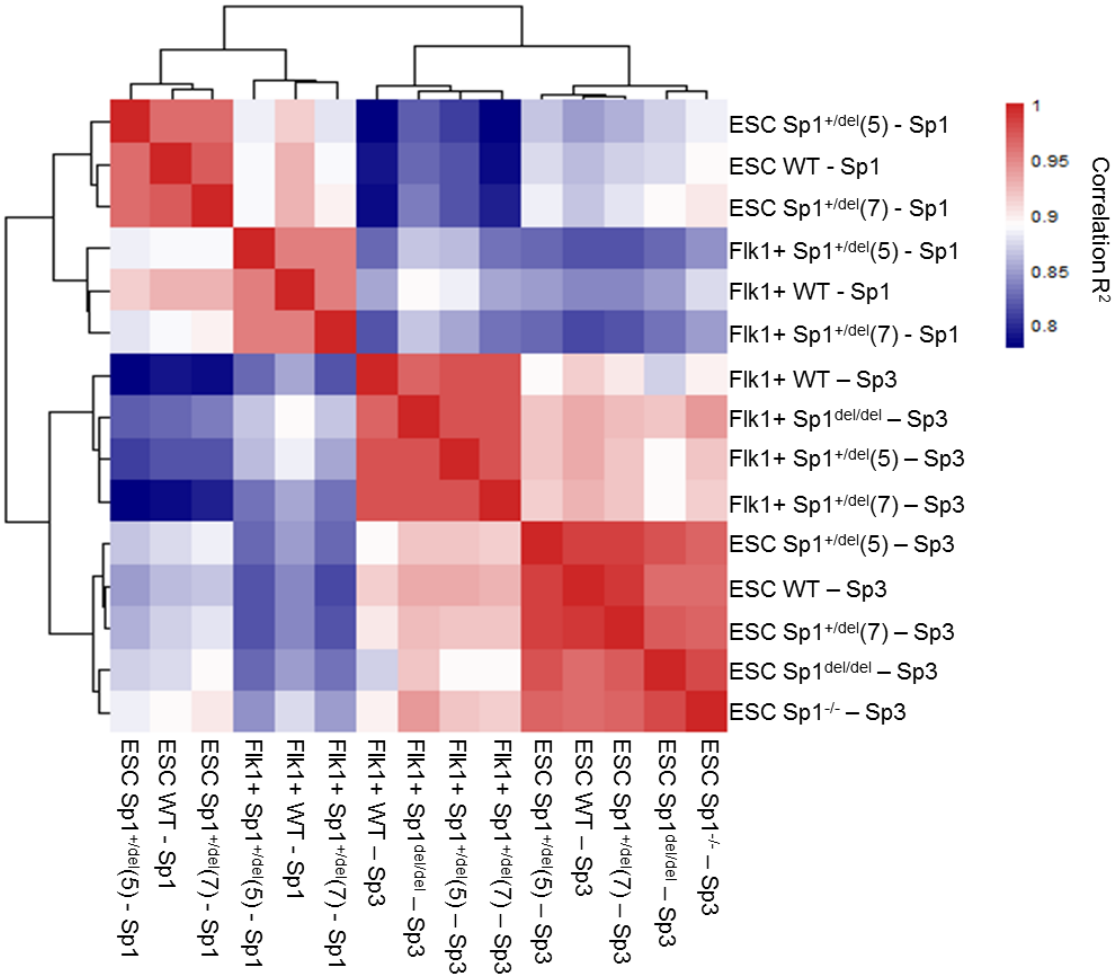


Figure 3.33 – Sp1 and Sp3 binding patterns are well correlated
ChIP-seq was performed in WT and Sp1 mutant clones in ESC and Flk1+ cells to identify genome-wide Sp1 and Sp3 binding. High confidence Sp1 and Sp3 ChIP-seq peaks were correlated using log2 read counts of the peaks. Hierarchical clustering was performed on the Pearson correlations (R^2 values) and a heatmap plotted to show the comparisons of Sp1 and Sp3 binding in each Sp1 clone in each cell type.

We further examined whether the binding of Sp3 was altered in cells with Sp1 deficiency by analysing the genome-wide distribution in WT, $Sp1^{-/-}$ and $Sp1^{del/del}$ cells (Figure 3.34). As expected, Sp1 was predominantly bound in promoter regions in both ESC and Flk1+ cells. The same was true for Sp3, but this factor occupied a higher proportion bound of intergenic and intragenic regions. We did not find a change in the binding distribution of Sp3 in $Sp1^{del/del}$ or $Sp1^{-/-}$ cells.

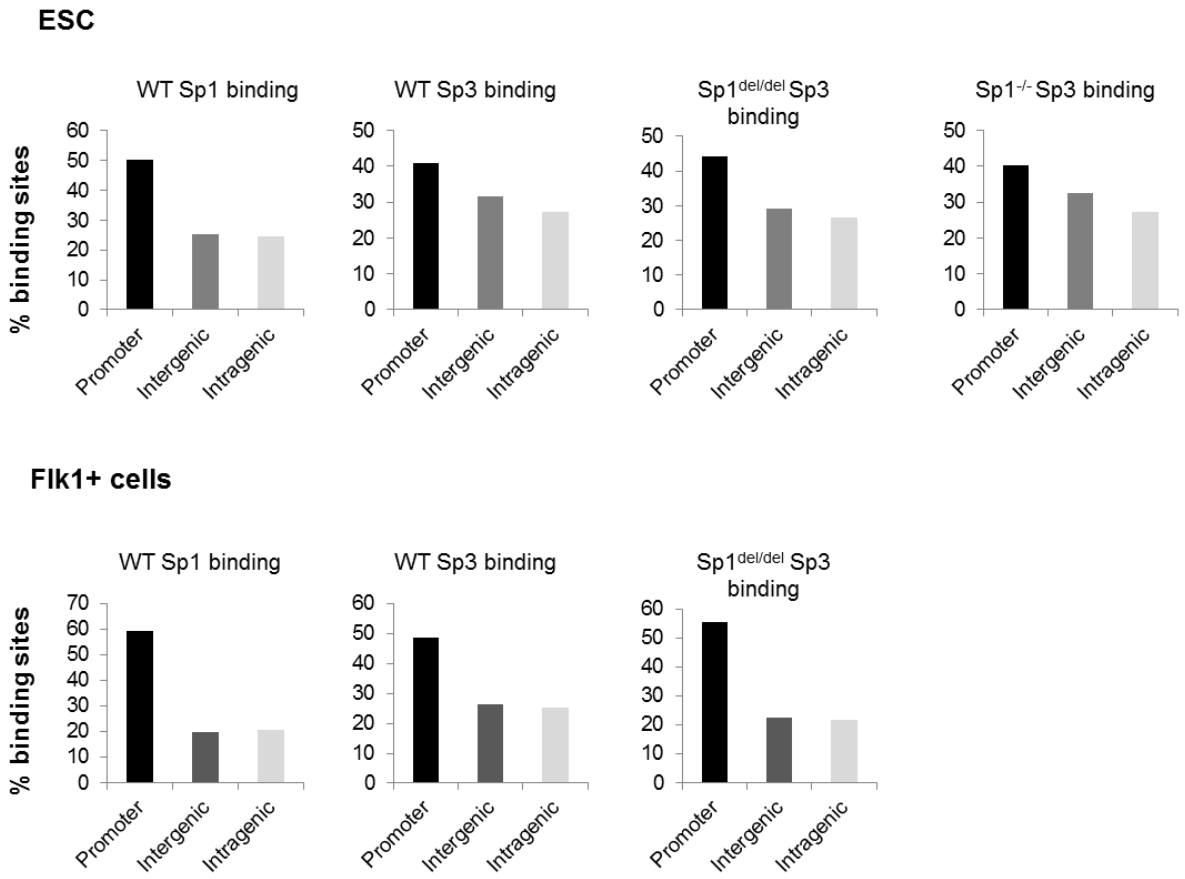


Figure 3.34 –The genomic distribution of Sp3 binding does not change in cells with Sp1 knockout

Sp1 and Sp3 ChIP-seq was performed in WT, $Sp1^{del/del}$ and $Sp1^{-/-}$ ESCs and in WT and $Sp1^{del/del}$ Flk1+ cells. High confidence peaks were identified that also have an ATAC-seq peak and then divided by their genomic position – promoter, intergenic or intragenic.

To confirm the absence of significant changes in the sites of Sp3 binding in Sp1 mutant clones, a density plot was made showing the fold change between Sp1 and Sp3 binding in WT cells, highlighting the Sp1- and Sp3-specific regions. Sp1 and/or Sp3 binding sites in the Sp1 clones were plotted along the same axis, in order to show the binding relative to that in WT ESCs (Figure 3.35A) and Flk1+ cells (Figure 3.35B). Sp1 binding density plots in $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7) cells showed a reduction in Sp1 binding, consistent with the results from the average profiles (Figure 3.24). Interestingly, most of the loss of Sp1 seemed to be at the sites with lower Sp1 binding in WT cells, suggesting Sp1 binding in

$Sp1^{+/del}$ cells is first lost at the sites with lowest binding affinity. Despite this alteration in Sp1 binding, possibly due to interference by the truncated Sp1 molecule in WT Sp1 complex formation, we observed no change in the binding of Sp3 in $Sp1^{+/del}$ ESC or Flk1+ cells. No change was also seen in $Sp1^{-/-}$ and $Sp1^{del/del}$ cells, suggesting that even with the loss of Sp1 DNA binding, Sp3 does not bind at the Sp1-specific sites. Therefore, Sp3 may be able to compensate for Sp1 knockout (or loss of function) at shared genes, but not at all Sp1-target genes.

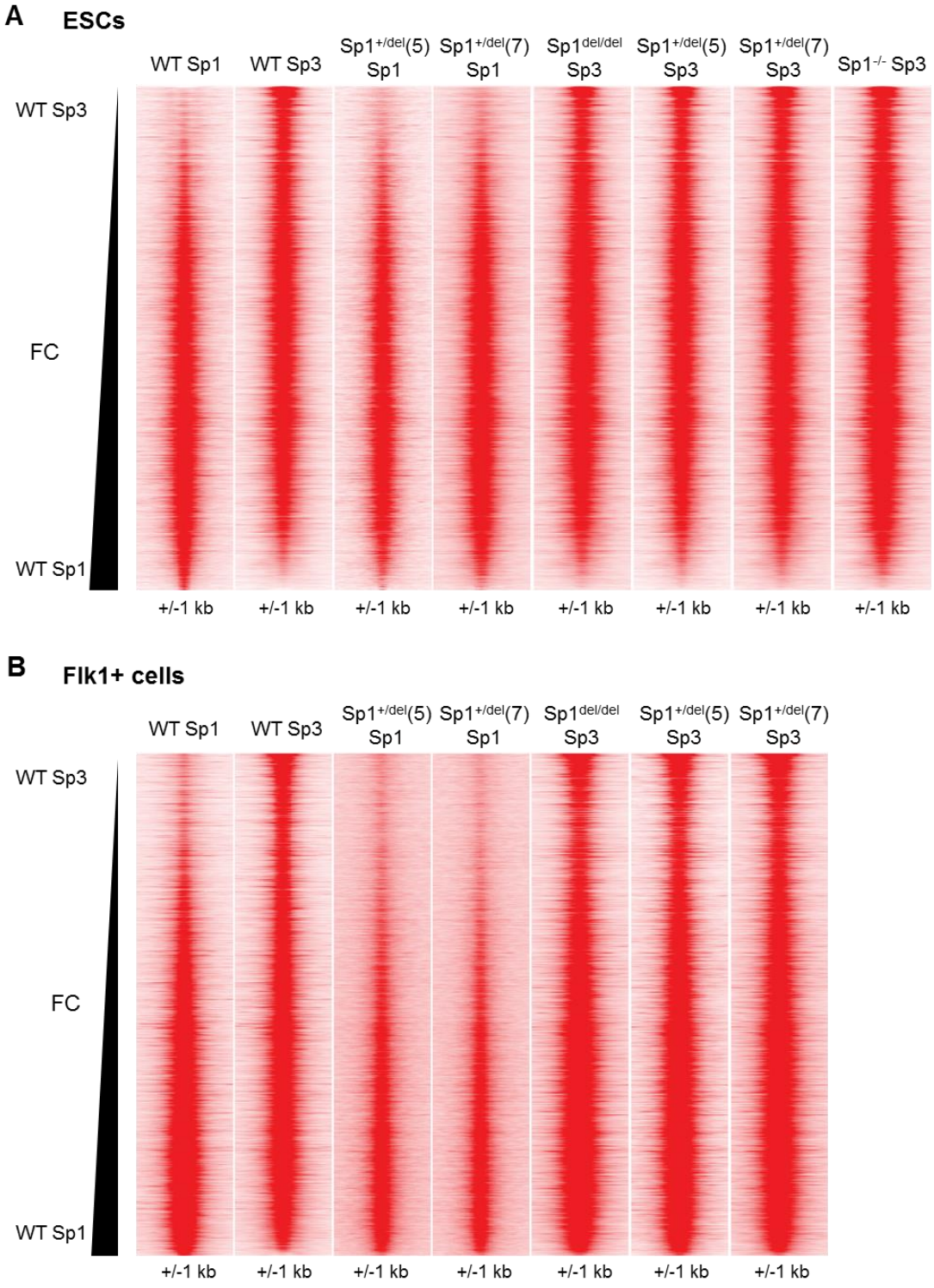


Figure 3.35 – The binding pattern of Sp3 does not change in the absence of Sp1

High confidence Sp3 and Sp1 binding sites were identified using ChIP-seq in each Sp1 clone in ESCs (A) and Flk1+ cells (B). The fold change between Sp1 and Sp3 binding in WT cells (based on number of reads in the peak) is plotted. The binding in the Sp1 mutant clones is plotted along the same axis in order to compare the regions of Sp1 and Sp3 binding in the mutant clones compared to that in WT.

To obtain quantitative data, we determined the mean levels of Sp3 binding in WT and Sp1 mutant cells by averaging the number of reads at each high confidence ChIP-seq peak (Figure 3.36). This analysis showed an increase in average Sp3 binding in $Sp1^{-/-}$ ESCs compared to WT cells. This suggests that although we saw no change in where Sp3 is bound, there was a global increase in the amount of Sp3 bound at its normal sites. This finding may be explained by a loss of competition from Sp1 for the same sites. Sp3 therefore appears to contribute to the maintenance of gene regulation in ESCs by compensating for the lack of Sp1.

In contrast, Sp3 binding was only slightly reduced overall in $Sp1^{del/del}$, $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7) ESCs. The fact that it occurred in all three clones suggests that the presence of the truncated Sp1 protein was causing this effect, possibly by interfering with normal complex formation. However, we saw no change in levels of Sp3 binding in Flk1+ cells. Thus, it may be that only those cells that can bind Sp3 at a sufficient level, and are able to compensate for loss of Sp1 function in the case of $Sp1^{del/del}$ cells, can successfully differentiate to Flk1+ cells.

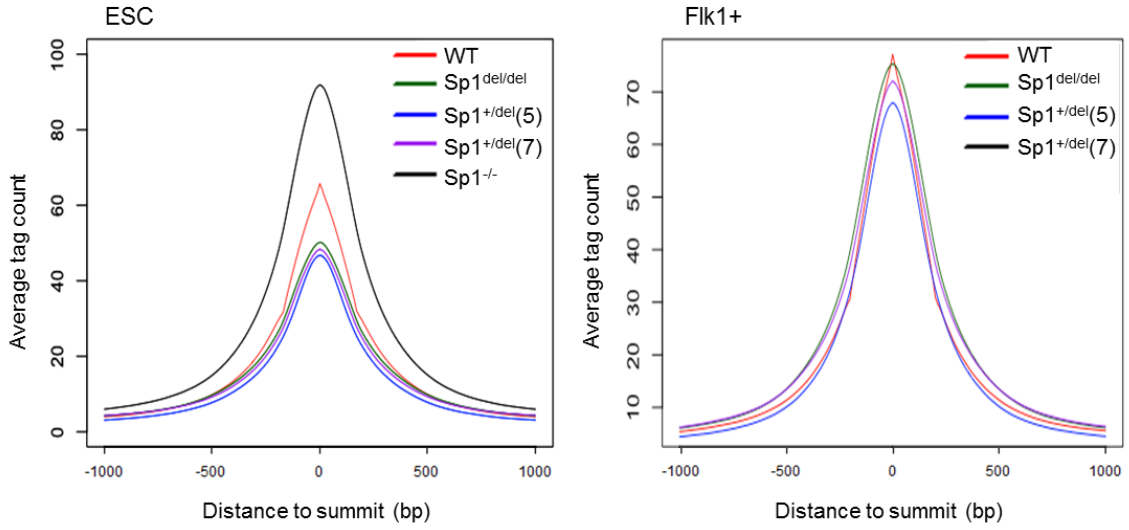


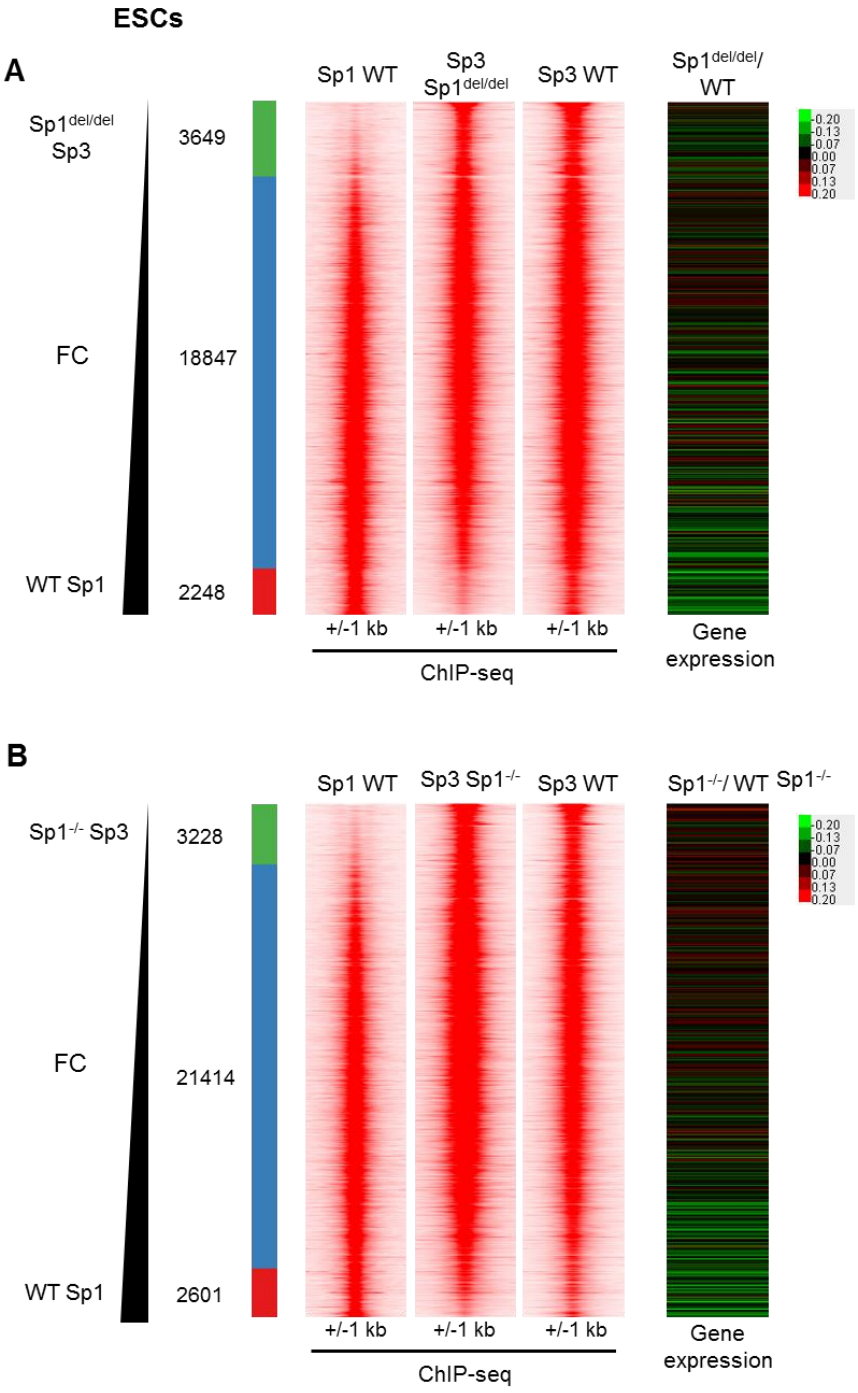
Figure 3.36 – Sp3 binding is increased in ESCs lacking Sp1
Sp3 ChIP-seq was performed in each Sp1 clone at ESC and Flk1+ cell stages. High confidence Sp3 binding sites were identified (by calling the ChIP peaks that also had a ATAC-seq peak) and the average reads at each site plotted relative to the summit of the peak.

3.5.4 The difference in Sp1 binding in WT cells and Sp3 binding in Sp1$^{-/-}$ and Sp1$^{del/del}$ cells is associated with changes in gene expression

To further investigate the role of Sp3 in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells, we performed pairwise comparisons of Sp1 binding in WT cells and Sp3 binding in Sp1 mutant cells at ESC (Figure 3.37) and Flk1+ cell stages (Figure 3.38). This enabled us to view the differences in Sp1 and Sp3 binding with each type of *Sp1* mutation. Similar to what we found in the WT Sp1/Sp3 density plots, there was a large overlap between Sp1 binding in WT cells and Sp3 binding in Sp1 mutant cell clones, but we also observed specific sites of Sp1 binding in WT cells and Sp3-specific binding sites in Sp1 mutant cells. This pattern was seen in all mutant clones in both ESCs and Flk1+ cells. The Sp3 binding in WT cells was

ranked by the fold change between Sp1 binding in WT cells and Sp3 binding in mutant cells and showed Sp3 binding was overall very similar between WT and the Sp1 clones.

To examine how the interplay of Sp1 and Sp3 regulated gene expression, we assigned the binding sites to the nearest gene, as the most likely gene for which it was regulating, and analysed the expression fold change in the Sp1 mutant cell clones vs. WT cells. In $Sp1^{-/-}$ and $Sp1^{del/del}$ cells, we found an association between WT cell Sp1-specific sites and genes displaying a minor downregulation of expression. This result suggests that Sp3 is unable to compensate at these genes. However, despite the presence of WT cell Sp1-specific binding sites in $Sp1^{+/del}$(5) or $Sp1^{+/del}$(7) cells, they were not associated with gene expression changes. This finding can be explained by the presence of WT Sp1 protein that can still bind and regulate expression of Sp1-specific genes.


ESCs
A
Sp1 WT
Sp3 Sp1del/del
Sp3 WT
Sp1del/del/ WT
Sp1del/del Sp3
3649
FC
18847
WT Sp1
2248
-0.20
-0.13
-0.07
0.00
0.07
0.13
0.20
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
Gene expression
B
Sp1 WT
Sp3 Sp1-/-
Sp3 WT
Sp1-/-/ WT
Sp1-/-
Sp1-/- Sp3
3228
FC
21414
WT Sp1
2601
-0.20
-0.13
-0.07
0.00
0.07
0.13
0.20
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
Gene expression

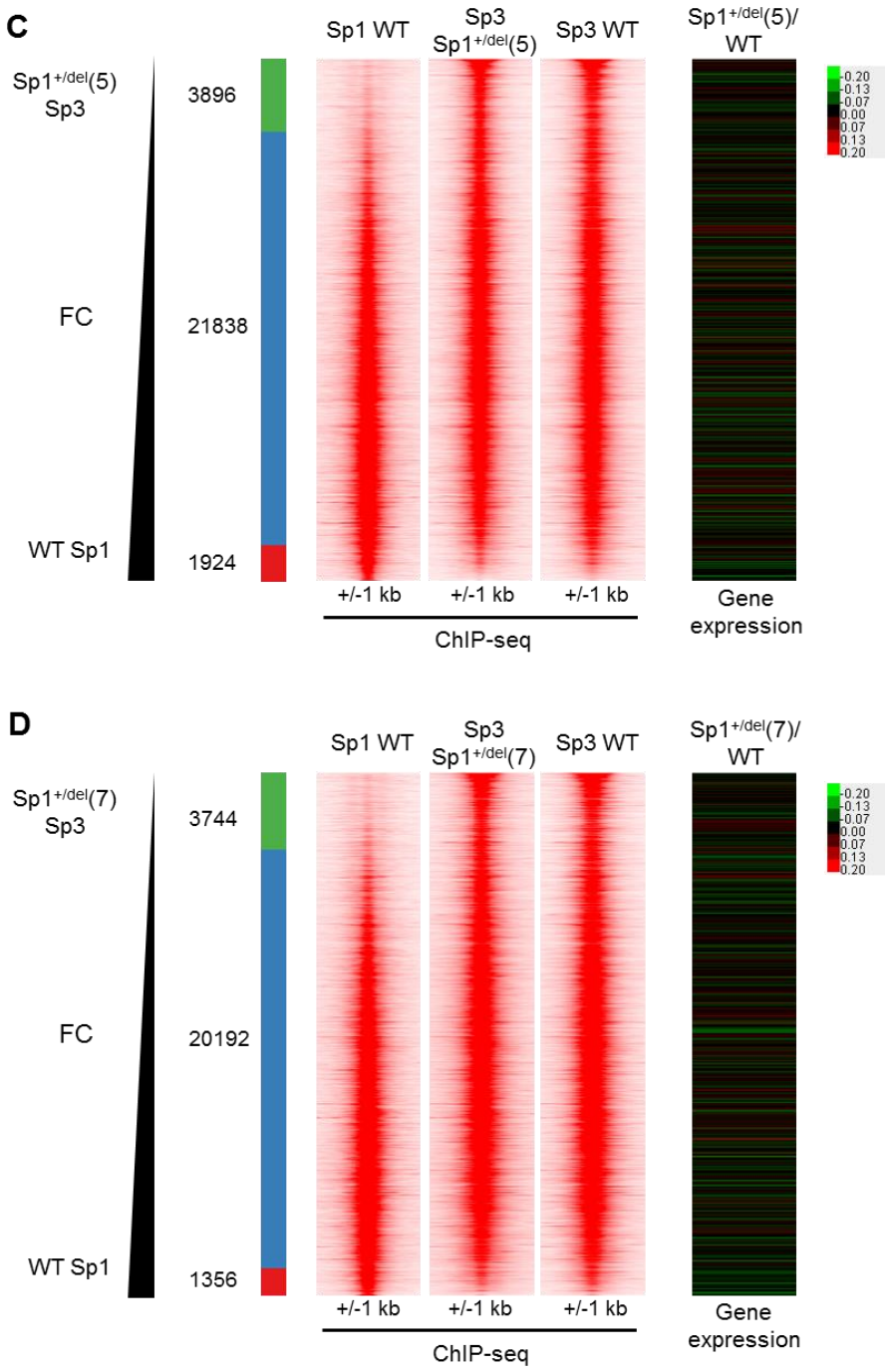
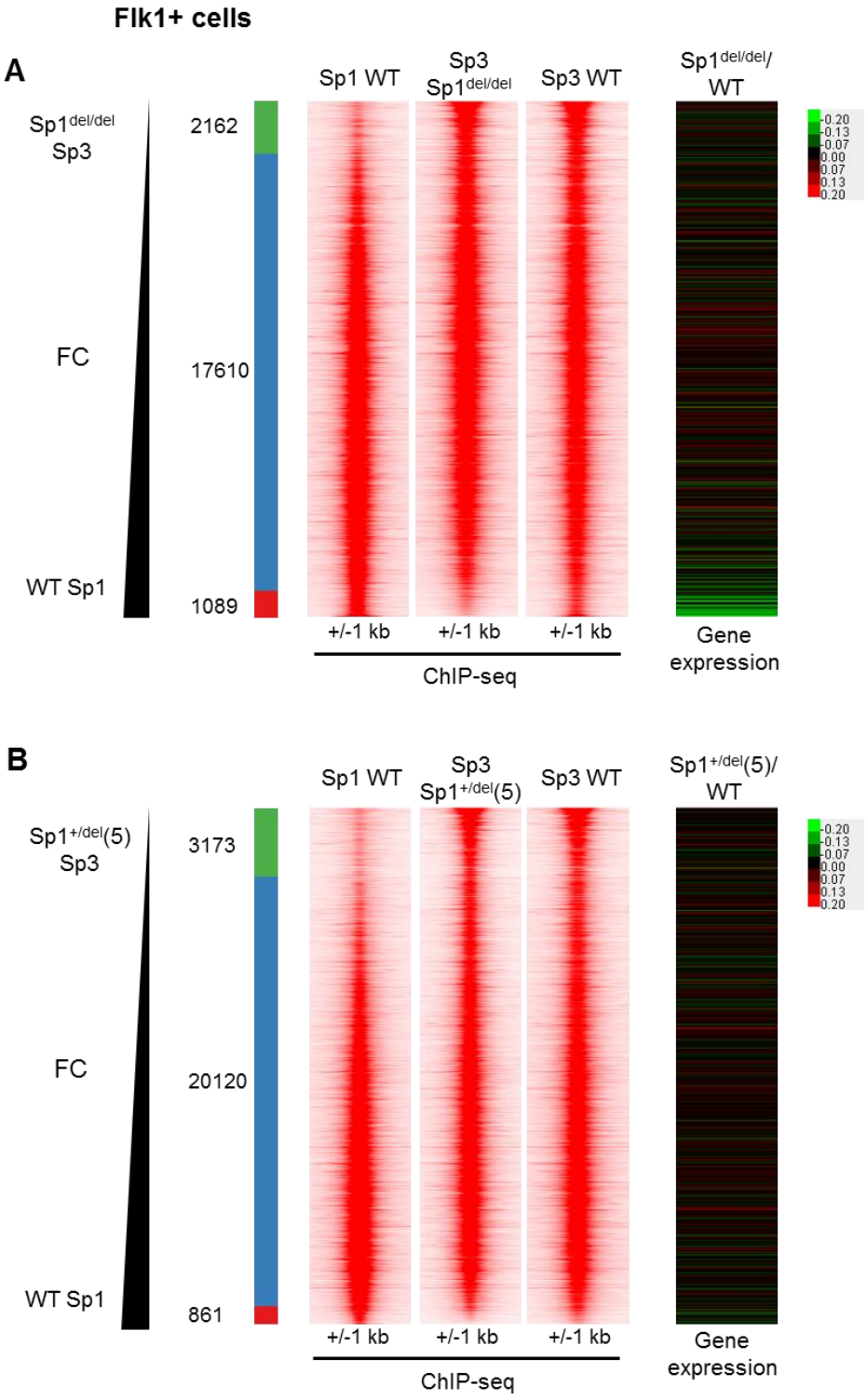


Figure 3.37 – Sp3 binding in Sp1$^{del/del}$ and Sp1$^{-/-}$ ESCs relative to Sp1 binding in WT ESCs correlates with subtle changes in gene expression, but not in Sp1$^{+/del}$ clones

Density plots showing fold change (FC) of Sp3 ChIP-seq peaks in CRISPR-generated Sp1-mutant ESCs compared to Sp1 ChIP-seq peaks in WT ESCs. The differential binding sites (peaks with at least 2-fold different number of reads) are indicated by the green and red sections of the coloured bar (showing clone Sp3-specific and WT Sp1-specific sites, respectively). Sp3 binding in WT ESCs was plotted along the same axis to show any changes in Sp3 binding with Sp1 manipulation and the position of such changes. Each ChIP-seq peak was assigned to the nearest gene and the corresponding fold change (log2 value) in gene expression in the Sp1 clone vs WT cells was plotted alongside. Sp1$^{del/del}$ (A), Sp1$^{-/-}$ (B), Sp1$^{+/del}$(5) (C) and Sp1$^{+/del}$(7) (D) profiles are shown.


Flk1+ cells
A
Sp1 WT
Sp3 Sp1del/del
Sp3 WT
Sp1del/del/ WT
Sp1del/del Sp3
FC
WT Sp1
2162
17610
1089
-0.20
-0.13
-0.07
0.00
0.07
0.13
0.20
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
Gene expression
B
Sp1 WT
Sp3 Sp1+/del(5)
Sp3 WT
Sp1+/del(5)/ WT
Sp1+/del(5) Sp3
FC
WT Sp1
3173
20120
861
-0.20
-0.13
-0.07
0.00
0.07
0.13
0.20
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
Gene expression

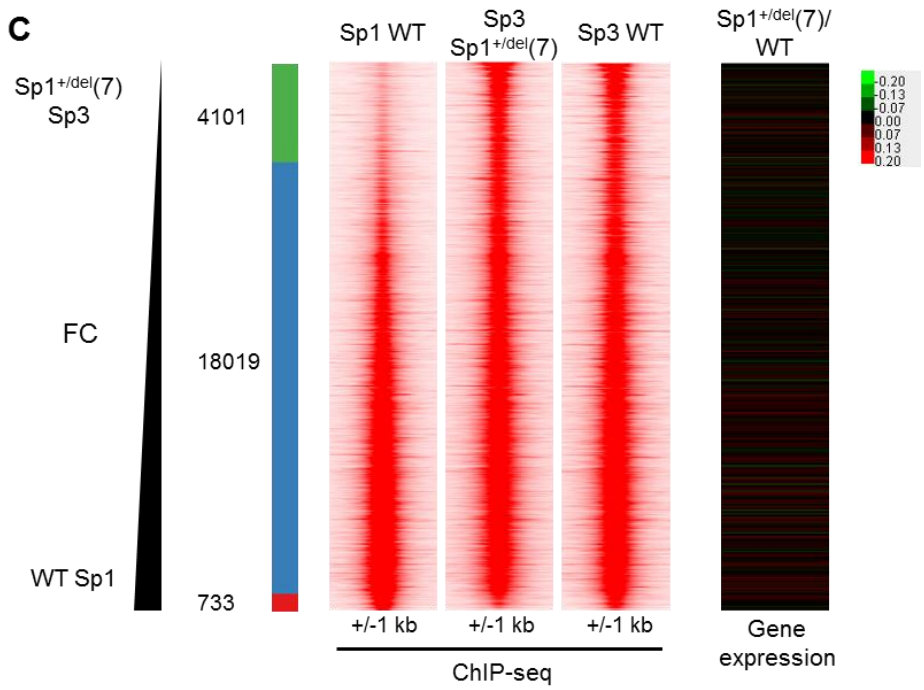
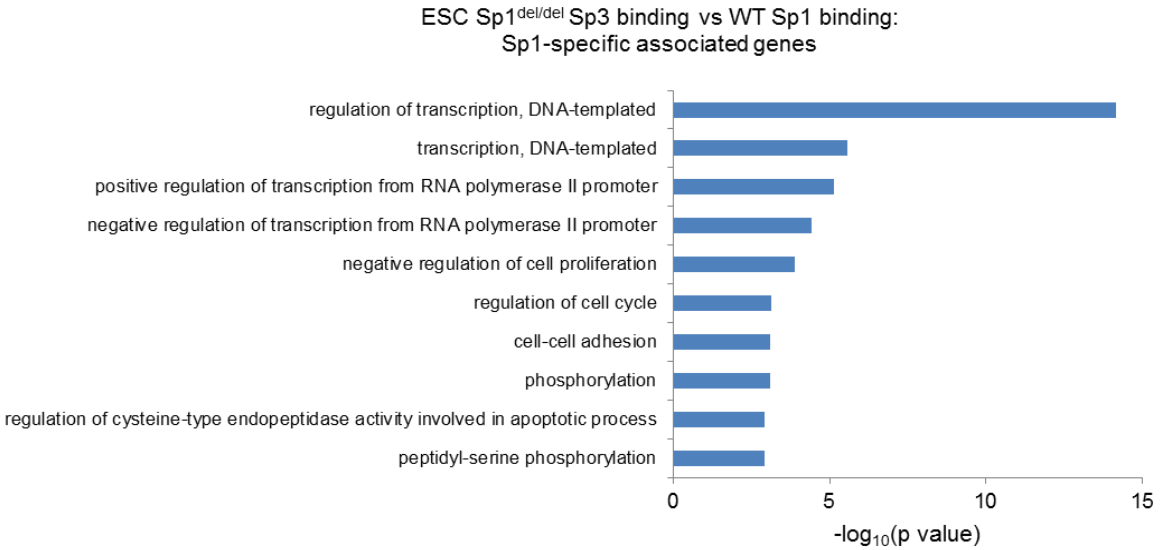


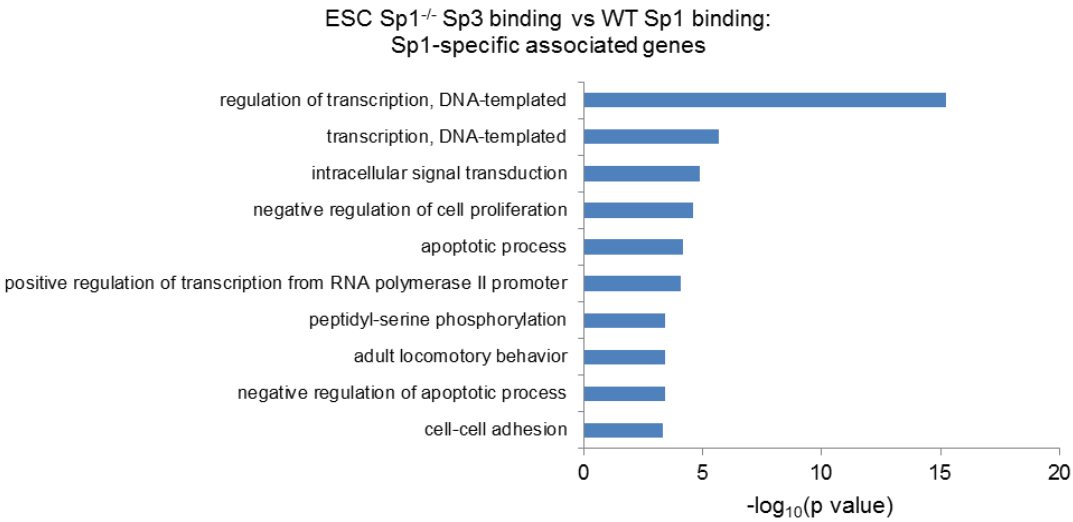
Figure 3.38 – Genes that normally bind Sp1 and do not bind Sp3 in Sp1$^{del/del}$ Flk1+ cells were slightly downregulated

Density plots showing fold change (FC) of Sp1 ChIP-seq peaks in WT Flk1+ cells compared to Sp3 ChIP-seq peaks in Sp1 mutant Flk1+ cell clones. Differential binding sites (at least 2-fold difference in number of reads) are indicated by the green and red sections of the coloured bar (showing clone Sp3-specific and WT Sp1-specific sites, respectively). Sp3 binding in WT Flk1+ cells was plotted along the same axis to show any changes in Sp3 binding with Sp1 mutation. Each ChIP-seq peak was assigned to the nearest gene and the corresponding fold change (log2 value) in gene expression in the Sp1 clone vs WT cells plotted alongside. Sp1$^{del/del}$ (A), Sp1$^{+/del}$(5) (B) and Sp1$^{+/del}$(7) (C) profiles are shown.

Absence of Sp3 was associated with genes that were slightly downregulated in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells. To examine whether downregulation of important regulator genes could be involved in the impairment of haematopoietic differentiation, the gene ontology terms of the WT cell Sp1-specific genes (relative to Sp1$^{del/del}$ and Sp1$^{-/-}$ Sp3 binding individually) were determined (Figure 3.39). In ESC, gene ontology analysis identified groups of genes associated with regulation of transcription, signalling and cell proliferation in both Sp1$^{del/del}$ and Sp1$^{-/-}$ clones. These groups included genes encoding TFs (such as numerous zinc-finger proteins), epigenetic modifiers (such as the DNA methyltransferase *Dnmt3a* (Okano

et al., 1999) and chromatin remodeller *Smarca2* (Wilson & Roberts, 2011)) and signalling molecules (e.g. *Notch1* and growth factor *Gdf11* (Andersson et al., 2006; Gerhardt et al., 2014)). Genes associated with development, cell adhesion and angiogenesis were enriched in $Sp1^{del/del}$ Flk1+ cells, such as *Mesp1*, a regulator of mesoderm cell fate, e.g. to haemangioblasts or cardiac cells (Chan et al., 2013), and *Sox17*, encoding a TF important in the development of HE (Clarke et al., 2013). Collectively, the identified gene ontology terms represent important cellular pathways and their deregulation due to a loss of Sp1 (and inability of Sp3 to bind) may be impacting on the cells' ability to differentiate.


ESC Sp1del/del Sp3 binding vs WT Sp1 binding:
Sp1-specific associated genes
regulation of transcription, DNA-templated
transcription, DNA-templated
positive regulation of transcription from RNA polymerase II promoter
negative regulation of transcription from RNA polymerase II promoter
negative regulation of cell proliferation
regulation of cell cycle
cell-cell adhesion
phosphorylation
regulation of cysteine-type endopeptidase activity involved in apoptotic process
peptidyl-serine phosphorylation
0
5
10
15
-log10(p value)


ESC Sp1-/- Sp3 binding vs WT Sp1 binding:
Sp1-specific associated genes
regulation of transcription, DNA-templated
transcription, DNA-templated
intracellular signal transduction
negative regulation of cell proliferation
apoptotic process
positive regulation of transcription from RNA polymerase II promoter
peptidyl-serine phosphorylation
adult locomotory behavior
negative regulation of apoptotic process
cell-cell adhesion
0
5
10
15
20
-log10(p value)

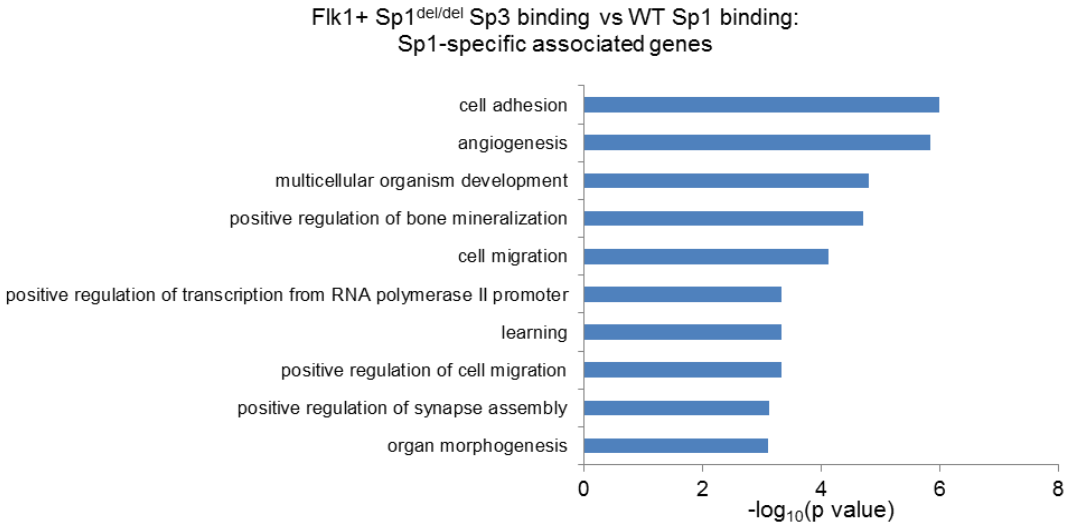

Flk1+ Sp1del/del Sp3 binding vs WT Sp1 binding:
Sp1-specific associated genes
cell adhesion
angiogenesis
multicellular organism development
positive regulation of bone mineralization
cell migration
positive regulation of transcription from RNA polymerase II promoter
learning
positive regulation of cell migration
positive regulation of synapse assembly
organ morphogenesis
0
2
4
6
8
-log10(p value)

Figure 3.39 – WT Sp1 sites, which Sp3 is not able to bind in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells, are associated with downregulation of genes involved in important cellular processes

Pairwise comparison of Sp1 binding in WT cells and Sp3 binding in Sp1 mutant cells showed a group of sites that are specifically bound by Sp1 in WT cells. When the sites are assigned to the corresponding nearest gene, the Sp1-specific sites were associated with a general downregulation of gene expression in the Sp1 clone compared to WT. These genes were used in a gene ontology analysis and the resulting significant terms shown.

An example of the impact of differential Sp3 binding on gene expression is the *Eif2s3y* gene, which is involved in suppressing the pluripotency of ESCs (Li et al., 2016). *Eif2s3y* is significantly downregulated by approx. ten-fold in Sp1$^{del/del}$ ESCs and showed reduced Sp3 binding at the promoter in Sp1$^{del/del}$ ESCs, while the binding and gene expression in other clones was unaffected (Figure 3.40). This suggests the ability of Sp3 to compensate for Sp1 or not can impact on gene regulation and influence the cell phenotype.

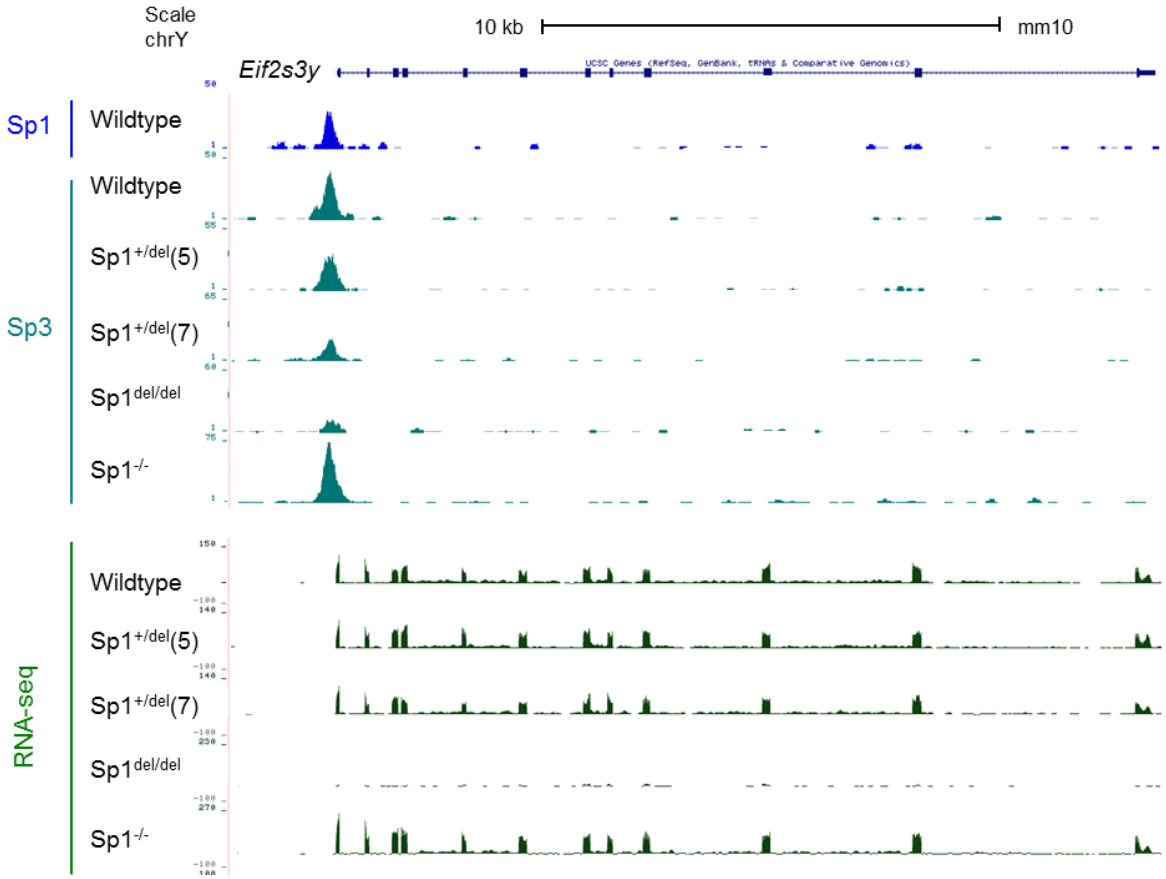


Figure 3.40 – Differential Sp3 binding correlates with change in gene expression
UCSC browser tracks at the *Eif2s3y* locus showing Sp1 and Sp3 ChIP-seq, as well as RNA-seq, in ESC Sp1 clones. *Eif2s3y* is significantly downregulated in Sp1$^{del/del}$ hypomorph cells and correlates with a decrease in Sp3 binding compared to WT cells.

However, while there was an association between absence of Sp3 where Sp1 is normally bound and the downregulation of gene expression in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells, the changes in gene expression are small overall (Figure 3.37, Figure 3.38). Indeed, many of the target genes identified as deregulated by the RNA-seq analysis, which are likely important in causing impaired of haematopoiesis, are not found in the WT Sp1-specific list. However, Sp3 does have different transcriptional activities and is often considered a weaker activator than Sp1. Therefore, it is possible that, even though Sp3 can bind to

Sp1-target genes, it may not be able to fully compensate and establish normal levels of expression.

In conclusion, it is likely that a combination of mechanisms is involved in establishing the genome-wide gene regulation programme in the Sp1 mutant cell clones. We have shown that the inability of Sp3 to bind at all Sp1 target genes causes a downregulation of gene expression (albeit to a small extent), which is associated with important cellular processes such as transcription and development. We have also shown that differential Sp3 binding at an individual locus can significantly deregulate gene expression when there is no Sp1 binding. Finally, we have shown that many significantly deregulated genes are bound by Sp3 (at a level comparable to WT cells), but Sp3 may not be able to fully restore gene expression because it is a weaker activator. However, the majority of Sp1-target genes are not deregulated upon loss of Sp1 binding, suggesting Sp3 can compensate to some degree and likely maintain ESC function.

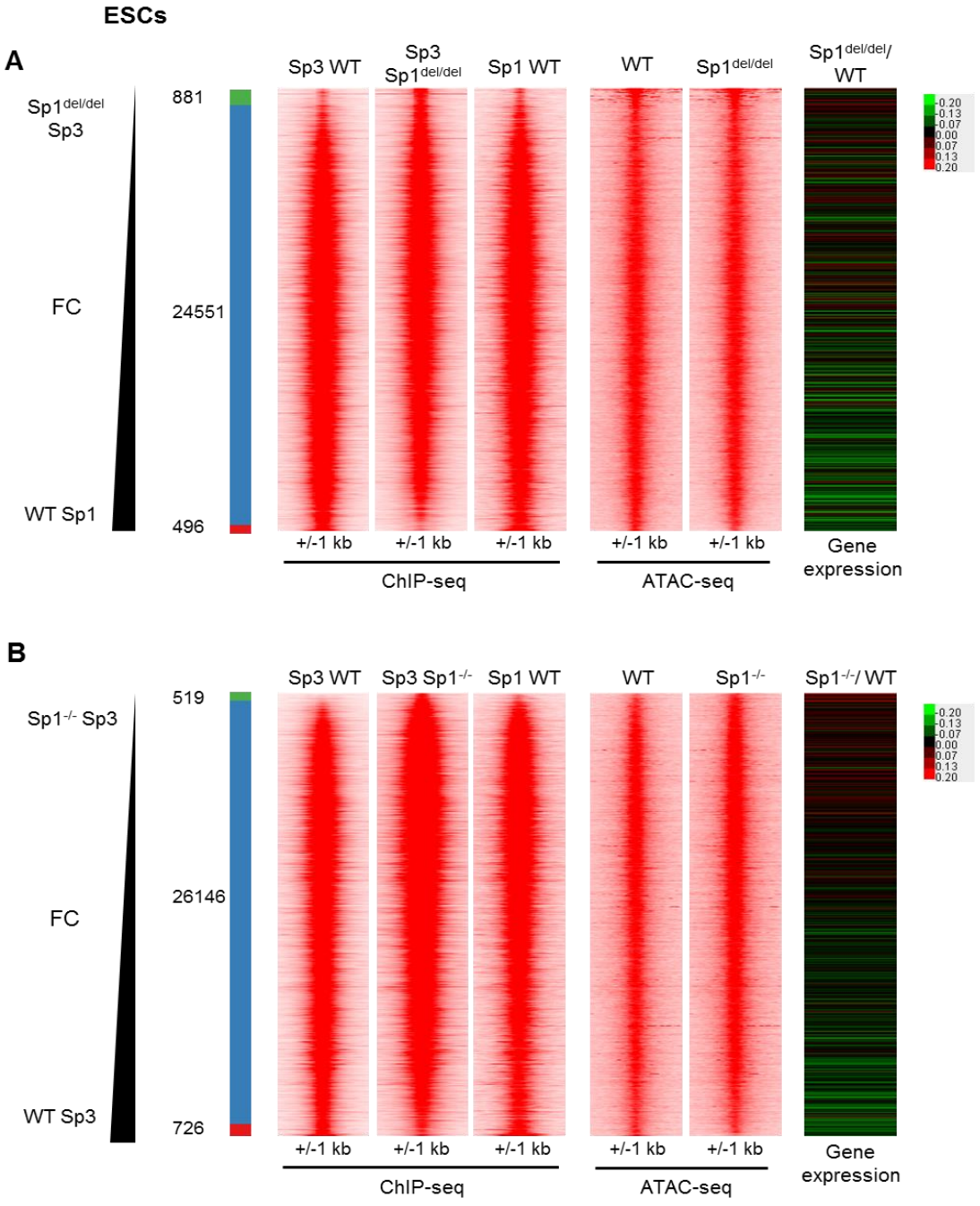
3.5.5 Changes in Sp3 binding in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells correlate with changes in gene expression

While the distribution of Sp3 relative to normal Sp1 binding did not change substantially in cells with Sp1 deficiency, we next questioned whether the overall binding of Sp3 genome-wide was affected. To examine this, we compared WT Sp3 binding to Sp3 binding in each Sp1 mutant clone using pairwise density plots in ESCs (Figure 3.41) and Flk1+ cells (Figure 3.42).

Once again, we saw a very similar Sp3 binding pattern in WT and Sp1 mutant cell clones in ESCs (Figure 3.41), suggesting there was little change in global Sp3 binding with loss of Sp1. We also found a large overlap between Sp1 binding sites, both in WT cells and in the corresponding $Sp1^{+/del}$ cell clones, consistent with previous results. Despite, the little change in Sp3 binding between WT and $Sp1^{-/-}$ or $Sp1^{del/del}$ cells, the fold change in Sp3 binding did correlate with gene expression changes between the Sp1 mutant cell clones and WT cells. For example, the expression of genes where Sp3 binding was lower in $Sp1^{-/-}$ cells compared to WT cells was generally downregulated, albeit to a small extent (Figure 3.41B). However, no association was found between fold change in Sp3 binding and gene expression changes between WT cells and $Sp1^{+/del}$(5) and $Sp1^{+/del}$(7) cells. The reason for this finding may be that $Sp1^{-/-}$ and $Sp1^{del/del}$ are more dependent on Sp3 for transcriptional regulation, due to a lack of Sp1 (or Sp1 binding to DNA), than $Sp1^{+/del}$ cells, which also express WT Sp1 protein. Thus, the gene expression profile changes as a result of the intrinsic differences in the ability of Sp3 to activate transcription compared to Sp1. However, these gene expression profiles only show a trend towards gene deregulation and overall, the changes in gene expression were small, suggesting that Sp3 is largely able to compensate for Sp1 in maintaining gene expression.

Similar results were found in Flk1+ cells: Sp3 binding in Sp1 mutant clones was very similar to that of WT cells with a high overlap of Sp3 binding and Sp1 in WT cells, and Sp3 binding in WT/$Sp1^{del/del}$ correlating with gene expression changes (but not in $Sp1^{+/del}$ cells). However, at this cell stage, we found slightly more alterations in Sp3 binding in the Sp1 mutant cell clones, for example 1710 sites lose Sp3 binding by at least two-fold in $Sp1^{del/del}$ Flk1+ cells, while this number is 1615 in $Sp1^{+/del}$(5) cells and 864 in $Sp1^{+/del}$(7) cells. We also observed a gain of Sp3 binding at some sites. To investigate whether changes in the chromatin accessibility of such regions could explain the differences, we plotted the ATAC-seq profile along the same axis. The ATAC-seq shows similar results in WT and

Sp1 mutant cell clones at these regions (although perhaps some slight reduction in accessibility is seen at the regions that lose Sp3 binding) (Figure 3.42). However, gene regulation in differentiated cells (Flk1+ cells) is generally considered more dependent on chromatin state than in ESCs, in which chromatin is largely permissive to enable the cells to activate/repress a wide range of genes required for different cell lineages (Chen & Dent, 2014). Thus, the role of chromatin in gene regulation and the changes in chromatin accessibility that occur with Sp1 mutation (Section 3.3), may be the basis of the changes seen in Sp3 binding in Flk1+ cells, but not (or to a lesser extent) in ESCs.


ESCs
A
Sp3 WT
Sp3 Sp1del/del
Sp1 WT
WT
Sp1del/del
Sp1del/del/ WT
Sp1del/del Sp3
FC
WT Sp1
881
24551
496
+/-1 kb
+/-1 kb
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
ATAC-seq
Gene expression
0.20
0.13
0.07
0.00
0.07
0.13
0.20
B
Sp3 WT
Sp3 Sp1-/-
Sp1 WT
WT
Sp1-/-
Sp1-/-/ WT
Sp1-/- Sp3
FC
WT Sp3
519
26146
726
+/-1 kb
+/-1 kb
+/-1 kb
+/-1 kb
+/-1 kb
ChIP-seq
ATAC-seq
Gene expression
0.20
0.13
0.07
0.00
0.07
0.13
0.20

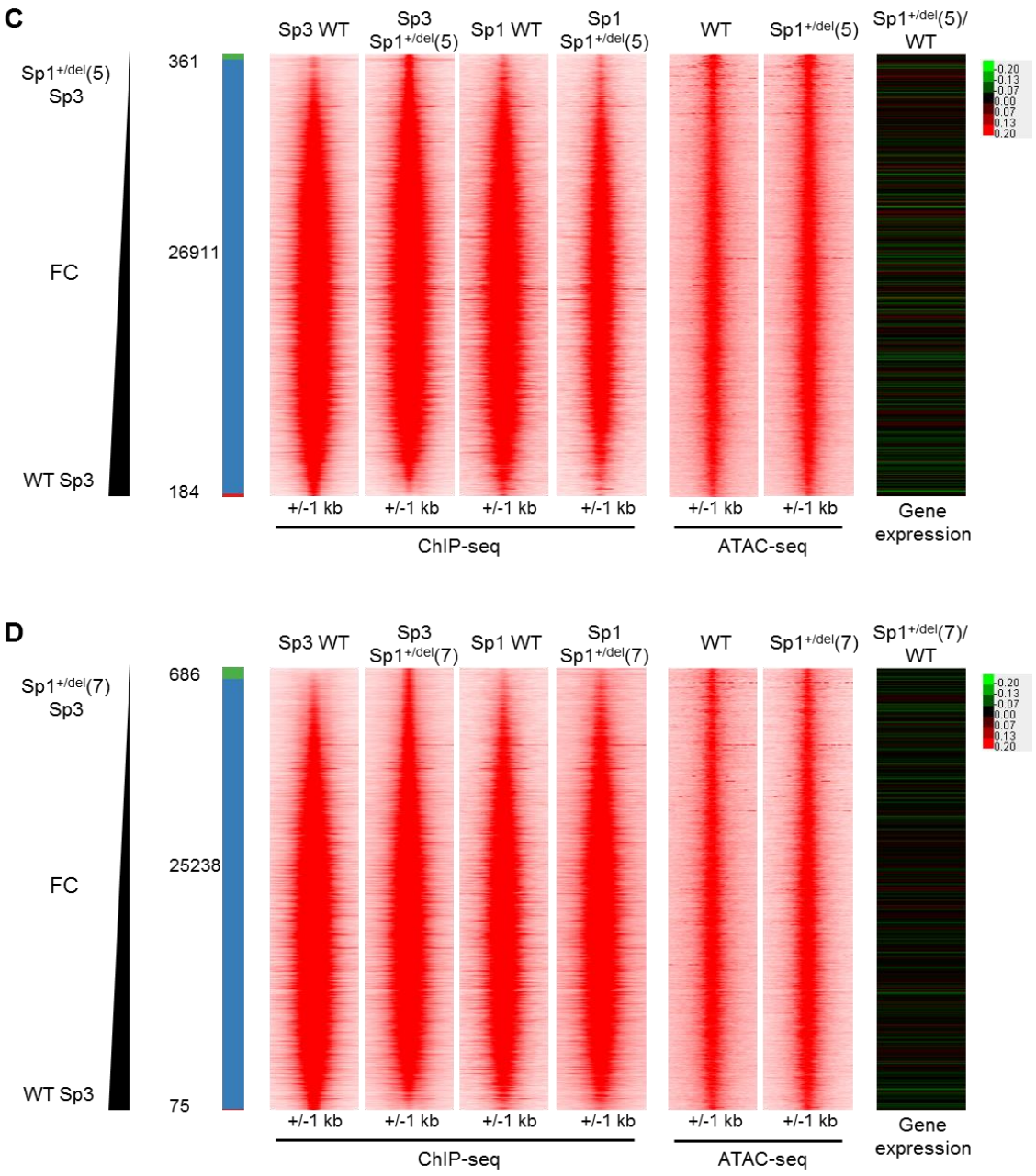
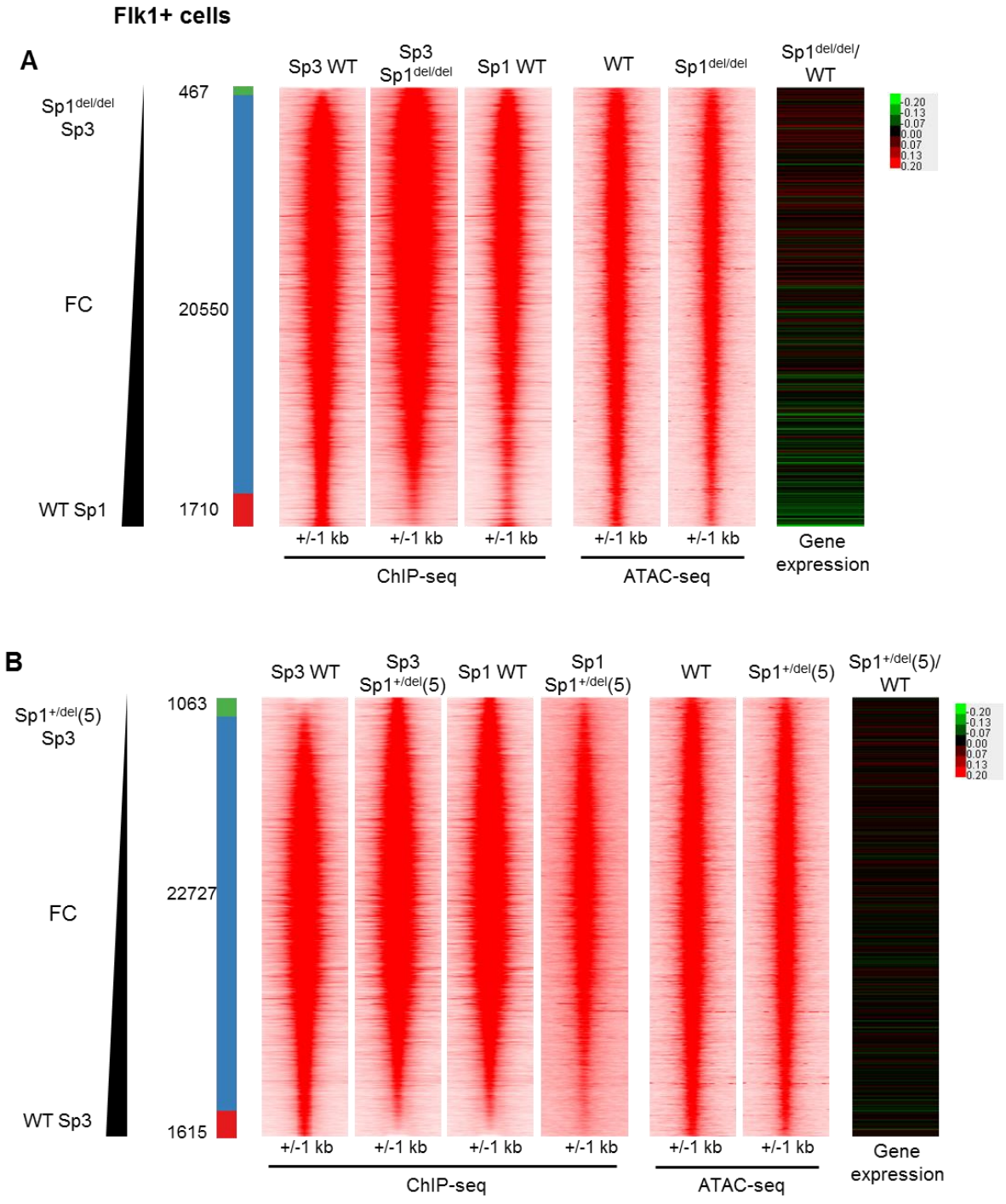


Figure 3.41 – Sp3 binding and gene expression in Sp1-deficient ESCs are unaffected

ChIP-seq was performed to identify Sp3 binding sites throughout the genome in WT and Sp1 mutant ESCs. Density plots show the fold change (FC) between Sp3 ChIP-seq peaks (based on read count of each peak) in WT and Sp1 mutant cells, in order to view any changes in Sp3 binding upon loss of Sp1. The differential binding sites (at least two-fold different) are indicated by the red and green sections of the bar. Sp1 binding (based on ChIP-seq peaks) in WT cells (and $Sp1^{+/del}$ where appropriate) was plotted along the same axis to compare Sp1 and Sp3 binding. ATAC-seq peaks, showing regions of open chromatin, at each binding site in WT and the corresponding clone were plotted along the same axis. Each ChIP-seq peak was assigned to the nearest gene and the corresponding fold change (log2 value) in gene expression in the Sp1 clone vs WT cells plotted alongside. $Sp1^{del/del}$ (A), $Sp1^{-/-}$ (B), $Sp1^{+/del}$(5) (C) and $Sp1^{+/del}$(7) (D) profiles are shown.


Flk1+ cells
A
Sp3 WT
Sp3 Sp1del/del
Sp1 WT
WT
Sp1del/del
Sp1del/del/ WT
Sp1del/del Sp3
FC
WT Sp1
467
20550
1710
-0.20
-0.13
-0.07
0.00
0.07
0.13
0.20
+/-1 kb
ChIP-seq
ATAC-seq
Gene expression
B
Sp3 WT
Sp3 Sp1+/del(5)
Sp1 WT
Sp1 Sp1+/del(5)
WT
Sp1+/del(5)
Sp1+/del(5)/ WT
Sp1+/del(5) Sp3
FC
WT Sp3
1063
22727
1615

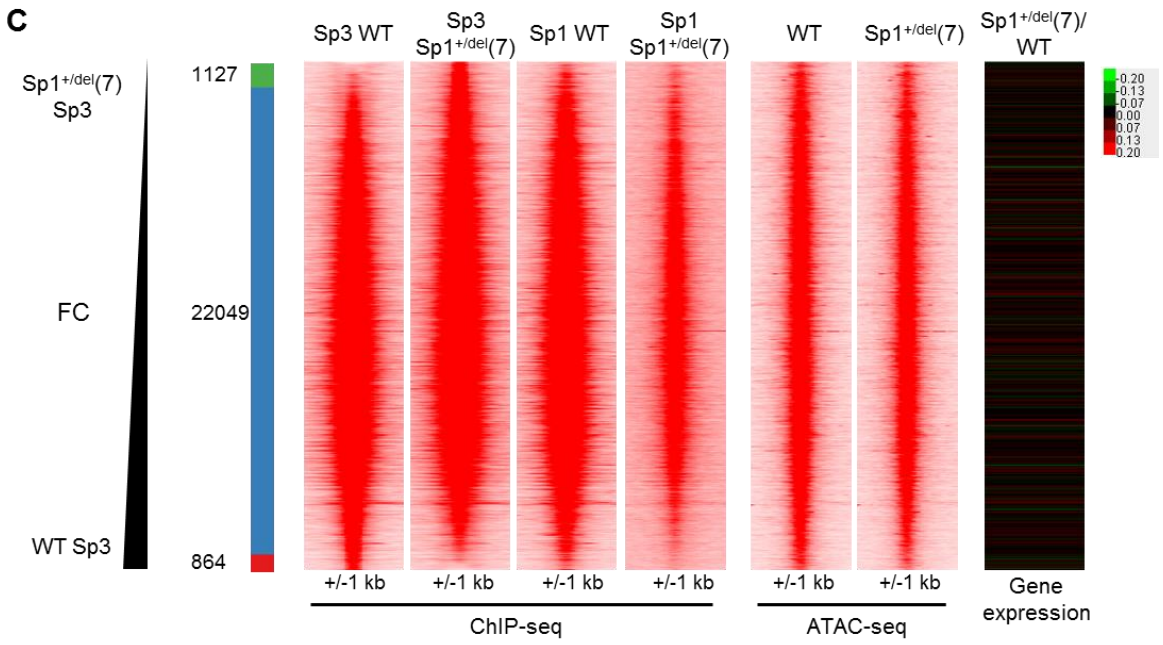


Figure 3.42 – Changes in Sp3 binding in Sp1$^{del/del}$ Flk1+ cells correlate with minor changes in gene expression

Density plots showing fold change (FC) of Sp3 ChIP-seq peaks in WT compared to Sp1 mutant Flk1+ cells. Differential binding sites (at least two-fold difference in number of reads) are indicated by the green and red sections of the coloured bar (showing clone-specific and WT-specific sites, respectively) showing any changes in Sp3 binding with *Sp1* mutation. Sp1 binding in WT cells (and in Sp1$^{+/del}$ cells where appropriate) was plotted along the same axis to compare Sp1 and Sp3 binding. ATAC-seq peaks, showing regions of open chromatin, at each binding site in WT and the corresponding mutant clone were plotted along the same axis. Each ChIP-seq peak was assigned to the nearest gene and the corresponding fold change (log2 value) in gene expression in the Sp1 clone vs WT cells plotted alongside. Sp1$^{del/del}$ (A), Sp1$^{+/del}$(5) (B) and Sp1$^{+/del}$(7) (C) profiles are shown.

In summary, our study of genome-wide Sp1 and Sp3 binding using ChIP-seq has indicated that Sp1 and Sp3 share the majority of binding sites, but show some differences in binding, which may come about through the association with different proteins (such as Sp3 with CTCF). The fold change between Sp1 binding in WT cells and Sp3 binding in Sp1$^{del/del}$ and Sp1$^{-/-}$ cells correlates with gene expression changes, suggesting Sp3 cannot compensate fully, possibly due to differences in its transactivation potential. However, the changes in gene expression overall are small and the number of significantly deregulated genes is relatively few, suggesting Sp3 is able to compensate for the lack of Sp1 at most sites. Therefore, Sp1 and Sp3 have overlapping, but not completely redundant functions.

4. DISCUSSION AND FUTURE EXPERIMENTS

In this study, we find novel insights into the function of the ubiquitously expressed TF Sp1 in haematopoietic development and, more generally, further our understanding of the role of a ubiquitous TF in tissue-specific gene regulation. We manipulated Sp1 to generate ESC lines that express (i) no Sp1, (ii) DBD-deficient Sp1 (recapitulating the previous model (Gilmour et al., 2014)) and (iii) both WT and DBD-deficient Sp1. Our work highlighted the importance of Sp1 in haematopoietic specification, investigated the effects of Sp1 disruption on chromatin structure and gene expression and uncovered novel insights into how the interplay of Sp1 and its close relative Sp3 establish and maintain gene expression programmes.

4.1 Generation of Sp1 mutant cells

Gilmour et al. discovered that Sp1 is crucial for haematopoietic specification during embryogenesis, but the precise stage that Sp1 is required in the process was still unknown (Gilmour et al., 2014). To answer this question, we aimed to delete the DBD-coding region of the *Sp1* gene in murine ESCs to recapitulate the previous Sp1 null ESC model as well as generate a complete Sp1 knockout ESC line, which has never before been studied. Thus, we targeted the *Sp1* gene in A17 2lox ESCs, which contain an expression cassette in the genome, with the reverse tetracycline transactivator stably expressed at the ROSA26 locus and a targeting site at the HPRT locus to enable site-specific integration of an expression construct (Kyba et al., 2002). This integration places the target gene under the control of a tetracycline-inducible promoter. We hoped to inactivate Sp1 in these cells, before inducing Sp1 expression at different time points through in vitro differentiation to elucidate at which stage Sp1 is crucially required. However, our A17 2lox cell clones appeared to be sensitive to doxycycline alone; addition

of doxycycline caused impaired differentiation even on cells not containing the transgene (data not shown). Therefore, this strategy was abandoned, but the generation of complete Sp1 null and DBD-deficient Sp1 cells in the same background meant these cells could still provide new insights into the role of Sp1 in haematopoiesis.

The CRISPR-Cas9 system was used to target Sp1, as it has shown to be effective and relatively simple experimentally, requiring only the design of the guide RNA sequences (Jinek et al., 2012; Wang et al., 2013). However, several studies have found that the CRISPR-Cas9 system could have unwanted off-target effects. Indeed, while Cas9 is targeted by the 20 nt guide sequence (in addition to requiring the presence of an adjacent PAM sequence) it could tolerate three to five bp mismatches to the genomic DNA sequence, particularly in the region of the guide sequence distal to the PAM (Fu et al., 2013; Hsu et al., 2013). A number of methods have been reported to minimise off-target effects, for example a mutant nickase Cas9, which cleaves only one strand of DNA and thus, requires two guide sequences to generate a DNA double-strand break. While this increases the specificity, it may also decrease on-target cleavage efficiency and limit the targeting region (Hsu et al., 2013; Ran et al., 2013; Cho et al., 2014). Alternatively, shortening the 20 nt guide sequence to 18 nt was found to improve specificity and decrease off-target cleavage (Fu et al., 2014). Therefore, 18 nt guide RNAs were designed in this study. The CRISPR/Cas9-targeted heterozygous $Sp1^{+/del}$ cell clones grew and differentiated well, and the expression of the majority of genes in the $Sp1^{-/-}$ and $Sp1^{+/del}$ ESCs was unchanged, suggesting minimal unwanted effects. In addition, the differentiation of $Sp1^{-/-}$ and $Sp1^{del/del}$ cells could be rescued by expression of the WT *SP1* gene (constitutively expressed to avoid doxycycline-related effects), indicating that the phenotypes were caused by *Sp1* mutation.

We successfully generated an ESC line expressing a truncated Sp1, lacking the DBD. The differentiation of these cells to the haematopoietic lineage was impaired, forming Flk1+ mesoderm cells, which include haemangioblast cells, with decreased efficiency compared to WT cells and being unable to terminally differentiate to macrophages. The size of the Sp1 protein and levels of expression in this ESC clone were very similar to that seen with the previous Sp1 DBD-deficient ESC model used by Gilmour et al. Moreover, the phenotypes of the two cell lines were highly comparable, showing that we successfully recapitulated the previous model, providing a system to validate, as well as build on, past findings.

Interestingly, the expression level of the DBD-deficient Sp1 protein was much lower than that of WT Sp1, suggesting it may be less stable. Sp1 is highly post-translationally modified, including by phosphorylation, glycosylation, sumoylation and acetylation, which occur on various residues along the length of the protein and that can regulate Sp1's activity e.g. by modulating DNA binding or protein-protein interactions (Tan & Khachigian, 2009; Chang & Hung, 2012). Post-translational modification of Sp1 can also affect protein stability. For example, phosphorylation of T739, which is absent in the truncated Sp1 protein, prevents Sp1 degradation, particularly during mitosis (Chuang et al., 2008; Wang et al., 2011). Therefore, an alteration in the post-translational modifications of DBD-deficient Sp1, as compared to the WT protein, may result in changes in its stability (as well as its transcriptional activity). Despite the decrease in protein level, there was an increase in mRNA expression levels, possibly due to the cell trying to compensate for the lack of functional Sp1 protein. Similarly high expression levels were seen in the $Sp1^{del/del}$ cells that were rescued by expression of WT Sp1. It is possible that we do not see a reduction in the mRNA expression levels in these cells because this upregulation has been selected for and is epigenetically stable.

Fortuitously, an ESC clone that expressed no Sp1 protein was also generated. Random mutations caused by the repair of the Cas9-induced DNA double strand break at exon 5 of the *Sp1* gene resulted in a premature stop codon. Nonsense-mediated decay is a process in the cell that acts as a surveillance pathway, triggering the degradation of mRNA containing premature stop codons to prevent the expression of truncated proteins, which may have adverse effects for the cell (Hug et al., 2016). While the mutation occurred late into the gene sequence, the presence of another intron and exon following the stop codon would signal for its degradation (Zhang et al., 1998). In support of this theory, the mRNA expression is significantly lower in $Sp1^{-/-}$ ESCs compared with WT ESCs. However, this effect is not seen in the RNA-seq browser tracks (Figure 3.9A). The RNA-seq results are normalised by total number of reads and by expression of housekeeping genes (e.g. *Gapdh*) to enable comparison between samples. It may be that loss of Sp1 causes a global reduction in transcription and the decrease in Sp1 transcription in $Sp1^{-/-}$ cells is minimised by the normalisation.

Only one clone of each the $Sp1^{del/del}$ and $Sp1^{-/-}$ mutations were generated, which may limit the reliability of the results due to possible clonal differences, as well as potential unwanted gene targeting effects. However, the similarity of the $Sp1^{del/del}$ phenotype compared with the previous DBD-deficient ESC line (Gilmour et al., 2014), as well as the ability of both clones to be rescued with WT Sp1 expression suggests the mutant phenotypes of the cells are caused by the manipulation of *Sp1*. Future studies could generate additional Sp1 mutant clones to further support our results.

A complete Sp1 knockout model has never before been studied. Surprisingly, the ESCs grew well, albeit slightly slower, likely due to the role of Sp1 in regulating growth and cell cycle associated genes. However, in vitro differentiation of the $Sp1^{-/-}$ cells showed they failed to reach the Flk1+ cell stage, a much more severe phenotype than $Sp1^{del/del}$ cells,

suggesting that DBD-deficient cells are actually hypomorphic. This is a novel finding, which has repercussions on a number of other studies using this model, including the Sp1 knockout mouse model. Marin et al. deleted the Sp1 DBD coding region in mice (termed a Sp1 knockout model) and found that although the mutation was embryonic lethal, the embryos did not die until ~E11. Many normal embryonic structures and tissues had also formed in some of the best developed Sp1 knockout embryos. Therefore, they concluded that Sp1 is dispensable for growth and differentiation of primitive cells, but Sp1 is important for the maintenance of differentiated cells (Marin et al., 1997). However, our results suggest Sp1 is important for differentiation and a mouse with complete loss of Sp1 may have a more severe phenotype.

Sp1 is made up of distinct protein domains. Preliminary experiments suggested that expression of the Sp1 DBD in the DBD-deficient $Sp1^{del/del}$ cells was sufficient to rescue the mutant phenotype. The DBD may therefore be able to associate with the remainder of the protein to complement protein function. Conversely, another study used expression of the DBD alone as a dominant negative protein – i.e. it inhibited the activity of Sp1 and transcription of Sp1-regulated reporter genes was diminished (Al-Sarraj et al., 2005). The DBD was expressed in the presence of the full-length Sp1 protein; a different effect may be seen with only a truncated Sp1 with limited function. However, our experiments were only performed with one ESC clone (likely due to the difficulty of expressing a single protein domain stably), so we cannot exclude clonal effects. Therefore, to confirm the results, we plan to generate additional DBD-expressing $Sp1^{del/del}$ ESC clones and test their differentiation potential. ChIP experiments could also be performed to show whether binding of the N-terminal TAD-containing region of Sp1 to DNA has increased with expression of the DBD. Furthermore, additional domains or portions of Sp1 could be expressed in $Sp1^{del/del}$ cells as well as $Sp1^{-/-}$ cells to determine if any other Sp1 domains can partially rescue the phenotypes. For example, is expression of one or both of the

TADs sufficient to rescue $Sp1^{-/-}$ cells? This will also provide additional information on the functions of the individual Sp1 domains in the cell.

4.2 Sp1 regulates key developmental regulators

We next aimed to investigate the changes in gene expression with Sp1 mutation during haematopoietic specification. Gilmour et al. used microarray experiments to examine the gene expression profiles of WT E14 ESCs compared to the Sp1 DBD-deficient ESCs. In this study, we used RNA-seq to a) confirm the findings of the previous study (Gilmour et al., 2014) and b) examine the changes in the transcriptome in more detail. RNA-seq is more sensitive, enabling the detection of low abundance transcripts; it is unbiased (unlike microarray which relies on transcript probes), thereby enabling detection of splicing variants, sequence variants and novel transcripts, and it can identify TSSs and promoter usage (Zhao et al., 2014). Thus, these experiments have provided a data resource for future studies to further investigate how Sp1 mutation impacts on the transcriptome.

Gene expression analysis at each stage of differentiation in the current study showed that only a limited number of genes were substantially deregulated and overall, the gene expression pattern was similar between WT cells and Sp1 mutant cells. This result was surprising considering the drastic phenotype and the role of Sp1 in a wide variety of cellular processes and its expression in every cell type (Li & Davie, 2010). In addition, only small changes in gene expression were observed when correlated against changes in chromatin accessibility (shown by ATAC-seq). While compensation by other TFs, such as Sp3, is likely to play an important part in the maintenance of gene regulation in the absence of functional Sp1, population effects may also play a role. Indeed, the RNA-seq, ATAC-seq and ChIP-seq experiments in this study were performed using a bulk population of cells. Therefore, any effects seen in just a subset of cells may be lessened

when averaged in the population. Heterogeneity in the population could be a particularly important factor when analysing dynamic gene expression programmes. Population heterogeneity has been particularly well studied in ESCs. Many TFs have been found to be heterogeneously expressed in an ESC population, including pluripotency factors such as NANOG that acts as the gatekeeper between self-renewal and differentiation (Chambers et al., 2003; Silva et al., 2009; Navarro et al., 2012; Torres-Padilla & Chambers, 2014). *Nanog* can switch between expressed and non-expressed states, with the non-expressing cells more prone to differentiation (Chambers et al., 2007). Transcription of lineage-associated TFs can also increase in cells that have lost NANOG (but can be silenced if *Nanog* is re-expressed), thus creating a fluctuating population of lineage-biased, but not committed, cell types, enabling the population of ESCs to be able to both self-renew and commit to different cell fates (Canham et al., 2010; MacArthur et al., 2012). These studies highlight the potential heterogeneity within a cell population.

Furthermore, the inactivation of Sp1 in the mouse (by deletion of the DBD-coding region) generated a variety of phenotypes. Sp1 inactivation was embryonic lethal at ~E11, with phenotypes ranging from developmental arrest as an undefined cell mass to retarded embryos with some recognisable tissues and structures (Marin et al., 1997). Therefore, $Sp1^{del/del}$ cells may represent a heterogeneous population, with varying residual Sp1 functionality and ability to differentiate. This diversity may also explain the higher number of deregulated genes found in $Sp1^{del/del}$ cells as compared to $Sp1^{-/-}$ cells, despite the milder phenotype. To study this effect further, single cell experiments could be performed. Recent advances in single cell technology now mean gene expression, chromatin accessibility and TF binding can be analysed on a genome-wide scale in a single cell (Trott & Martinez Arias, 2013; Buenrostro et al., 2015b; Rotem et al., 2015; Ye et al., 2017). These studies could show the effects of Sp1 knockout or inactivation in each cell

and draw attention to more dynamic gene expression changes or transient protein-DNA interactions.

Analysis of the expression of genes that alter between stages of differentiation rather than at discrete cell stages identified many more genes that are deregulated in the Sp1 mutant cells, indicating that the dynamics of differentiation were altered. We observed both an upregulation and downregulation of gene expression, showing the dual role of Sp1 as both a transcriptional activator and a repressor. In addition, genes that are differentially expressed through differentiation often encode developmental regulators, such as lineage-specific TFs. Therefore, the deregulation of these genes may underlie the defect in haematopoietic differentiation and suggest that the trajectory of the Sp1 mutant cells through differentiation is altered.

Deregulation of gene expression in Sp1$^{-/-}$ cells: In spite of the small number of deregulated genes, a number of key regulators in the early stages of haematopoietic differentiation were deregulated between Sp1$^{-/-}$ ESC and Flk1+ cells. Flk1+ haemangioblast cells originate from a mesodermal precursor (Fehling et al., 2003). Among the genes that were deregulated in Sp1$^{-/-}$ cells were several encoding factors important in mesoderm formation. Brachyury (*T*), as mentioned in the introduction, is involved in mesoderm specification and is often used as a marker (along with Flk1) of haemangioblast cells. Knockout of *T* in mouse embryos causes a reduction in the number of mesodermal cells, with an increase in ectoderm, and abnormalities in mesoderm derivative structures, such as the primitive streak and allantois (Yanagisawa et al., 1981; Yanagisawa, 1990), showing its importance in development. MIXL1 is co-expressed with Brachyury during embryogenesis and is required for mesoderm and endoderm patterning (Pereira et al., 2011). Both *T* and *Mixl1* were downregulated with loss of Sp1. Other genes acted downstream of these factors. Brachyury activates expression of the

transcription factor TBX6, which in turn activates *Msgn1* that is required for presomitic mesoderm maturation (Wittler et al., 2007). MESP1 acts to determine mesoderm patterning to haemangioblasts (by activating *Tal1* and *Etv2* expression) or cardiac precursors, in a context-dependent manner (Chan et al., 2013). Indeed, *Msgn1*, *Mesp1*, *Tbx6*, *Kdr* (Flk1) and *Etv2* are downregulated in $Sp1^{-/-}$ cells.

Additionally, the expression factors that are part of signalling pathways involved in mesoderm development were altered. FGF signalling via FGF4, whose gene was downregulated in $Sp1^{-/-}$ cells, promotes mesoderm formation through activation of T box factors (such as *T* and *Tbx6*) (Ciruna & Rossant, 2001). Wnt and BMP signalling also play an important role in mesoderm specification. Wnt signalling is involved in mesoderm formation early in gastrulation, while both Wnt (including the protein Wnt5a) and BMP signalling, particularly BMP4, drive mesoderm differentiation to the haemangioblast (Murry & Keller, 2008; Nostro et al., 2008). Altogether, these results show there is downregulation of TFs and signalling molecules involved in mesoderm and haemangioblast specification with the loss of Sp1, which may explain the defect in differentiation. Future studies could further explore the consequence of the deregulation of these factors. For example, knockdown studies using siRNA could be used to downregulate identified genes and analyse the effect on differentiation in our in vitro system. If a similar phenotype is seen, it would indicate the deregulation of these genes causes the differentiation failure. Moreover, while mesoderm differentiation appears to be blocked, it may be the cells are instead moving to a different lineage. No significant upregulation of endoderm or ectoderm markers was detected; however, the cells were selected for Flk1 expression. Therefore, future studies could analyse the population of cells in the EBs to see if there is a higher proportion of endoderm or ectoderm cells compared to WT.

Deregulation of gene expression in Sp1$^{del/del}$ cells: Gene expression analysis of Sp1$^{del/del}$ cells showed an upregulation of pluripotency-associated genes in the ESC to Flk1+ cell transition indicating that the timing of exit of pluripotency was altered. For example, *Nanog* was highly expressed in Sp1$^{del/del}$ ESCs. NANOG maintains pluripotency in ESCs is often considered the gatekeeper of differentiation (Chambers et al., 2007). Similarly, *Essrb*, *Dppa5a* (*Esg1*) and *Amd1*, which were also upregulated, are all involved in ESC self-renewal and maintaining pluripotency (Tanaka et al., 2002; Festuccia et al., 2012; Zhang et al., 2012). Conversely, factors which promote differentiation of ESCs such as *Eif2s3y*, which is involved in suppressing pluripotency (Li et al., 2016), and *Satb1*, knockdown of which causes an upregulation of *Nanog* and impaired differentiation, were downregulated (Savarese et al., 2009). The result of the deregulation of these genes may be decreased differentiation potential. Correspondingly, we found that Sp1$^{del/del}$ ESCs formed smaller, tighter colonies that were less prone to spontaneous differentiation. We also identified the deregulation of several genes important for regulating differentiation and cell fate, including *Kdr* (Flk1) and *Etv2* in haemangioblast specification, discussed above, and *Gsc* and *Hoxb1*, which are involved in organising the body plan in vertebrate embryos (McGinnis & Krumlauf, 1992; Ulmer et al., 2017).

At the Flk1+ cell to HE1 transition, we saw a deregulation (generally downregulation) of genes associated with specification of the HE, including the markers *Tek* (Tie2) and *Itga2b* (CD41). Several genes involved in angiogenesis and vascular development were also identified, such as *Angpt1/2*, which are growth factors to promote angiogenesis (Maisonpierre et al., 1997), *Plxnd1*, which is expressed in endothelial cells of the developing vasculature (Gitler et al., 2004) and *Pecam1*, encoding an endothelial cell-cell adhesion molecule (DeLisser et al., 1997). Again, this deregulation may result in impaired haematopoietic specification.

No genes were significantly deregulated at the HE1 to HE2 transition, but there was an upregulation of genes associated with megakaryocyte/platelet cells between HE2 and progenitor cells. For example, VWF is a plasma glycoprotein, which binds a receptor on the surface of platelets (Wise et al., 1991). GP5 forms part of this receptor and is cleaved during coagulation to mediate platelet adhesion to blood vessel walls (Calverley et al., 1995). G6B is present on the surface of platelets and acts to inhibit aggregation (Newland et al., 2007), while CLEC1B is involved in signalling pathways to activate platelets during haemostasis (Suzuki-Inoue et al., 2011). The expression of each of these genes was upregulated in $Sp1^{del/del}$ cells. These findings suggest $Sp1^{del/del}$ cells are biased towards one haematopoietic lineage and may explain their impaired ability to form macrophages.

While the deregulation of these genes may explain the impaired differentiation potential of $Sp1^{del/del}$ cells, it was interesting to find that many of the same genes (until the progenitor stage) were deregulated in $Sp1^{+/del}$ cells, suggesting that the genes were being deregulated by the same mechanism. It is possible that the truncated Sp1, while still retaining some function, interferes with interactions between TFs. Indeed, Sp1 has been reported to interact with some of its binding partners via the TADs (Gill et al., 1994; Koutsodontis et al., 2005). The decreased levels of Sp1 in the cell may also impact on the levels of transcription initiation. However, $Sp1^{+/del}$ cells can differentiate efficiently despite the same genes being deregulated, suggesting that $Sp1^{del/del}$ cells differentiate less efficiently and in a biased way, rather than being incapable of differentiation (as $Sp1^{-/-}$ cells are). Only at the HE2 to progenitor transition do $Sp1^{del/del}$ cells show a different set of deregulated genes to $Sp1^{+/del}$ cells, which reflects the inability of $Sp1^{del/del}$ as compared to $Sp1^{+/del}$ cells to terminally differentiate. This idea could be further investigated by analysing the rate of $Sp1^{del/del}$ differentiation compared to WT cells. The formation of Flk1+ cells, while normally taking ~3.75 days, may be delayed in $Sp1^{del/del}$, which could be examined with time course experiments. Finally, it was interesting to find that most of the

deregulated genes, while the majority were direct targets of Sp1, were different between $Sp1^{-/-}$ and $Sp1^{del/del}$ cells, suggesting a different mechanism at work and shows that DBD-deficient Sp1 still has some function. Given the slower growth of $Sp1^{del/del}$ and $Sp1^{-/-}$ cells, it would also be interesting to investigate the number of haematopoietic cells generated in the blast culture per Flk1+ input cell in these cells compared with WT and $Sp1^{+/del}$ cells.

A question still to be answered following the study by Gilmour et al. was at which stage Sp1 is required in haematopoiesis? The failure of $Sp1^{-/-}$ cells to form Flk1+ cells in our in vitro differentiation system, along with the deregulation of important genes in mesoderm specification suggests a role of Sp1 very early on in embryonic haematopoiesis. While there has been no reported role of Sp1 specifically in mesoderm or haemangioblast formation, many genes associated with these developmental stages were deregulated upon loss of Sp1 (the majority of which are direct targets of Sp1) suggesting Sp1 is important in this process.

Furthermore, the highest number of deregulated genes in $Sp1^{del/del}$ cells was found at the ESC to Flk1+ cell transition, supporting the theory that Sp1 is most important at this early stage. However, $Sp1^{del/del}$ cells are able to continue to make progenitor cells, albeit with decreased efficiency than WT cells and with a megakaryocyte bias. It is possible that Sp1 functions via a threshold effect: only the cells that are less affected are able to differentiate further, so we see fewer deregulated genes at later stages. Interestingly, studies of DBD-deficient SCL/TAL1 in mice have shown that DNA-binding activity of SCL/TAL1 is not required at early stages of haematopoietic specification, but it is required for terminal differentiation to erythroid cells (Kassouf et al., 2008), suggesting a TF's mechanism of action can change in different contexts or developmental stages. Therefore, Sp1 DNA-binding may not be essential for early stages of haematopoiesis, although the presence of

the TADs is required, but DNA-binding may be needed for terminal differentiation. This hypothesis could be investigated in future studies by expressing WT Sp1 in $Sp1^{del/del}$ cells at a later stage, such as in progenitors, and testing their ability to make macrophages.

Conditional Sp1 knockout mouse studies support the requirement of Sp1 at early stages. When the Sp1 DBD-coding region was deleted at the myeloid progenitor stage in mice, the cells were still able to efficiently generate macrophages in vitro (Gilmour et al., 2014). In contrast, deletion of the DBD in ESCs prevented differentiation to macrophages. To conclusively show at which stage Sp1 is crucially required in haematopoiesis, Sp1 could be induced at specific time points during differentiation in a Sp1-null background. While the A17 2lox cells used in this study are not amenable to this strategy, *Sp1* could be targeted again in a doxycycline-insensitive ESC line. Alternatively, a conditional Sp1 knockout ESC line could be generated.

4.3 $Sp1^{del/del}$ cells are comparable to previous models

Another study by our lab first showed a role for Sp1 in haematopoiesis using ESCs expressing DBD-deficient Sp1 (Gilmour et al., 2014). The $Sp1^{del/del}$ cells produced in the present study with a different ESC line background show a remarkably similar phenotype – the cells differentiate to haematopoietic progenitors, albeit with reduced efficiency, but fail to terminally differentiate to macrophages. Indeed, the gene expression data was very comparable from both cell lines that lack the Sp1 DBD, with GSEA plots showing that generally the same genes were up- or downregulated in the DBD-deficient cells, although there were some differences.

However, in spite of the broad similarities, most of the deregulated genes that were identified as likely to be contributing to the phenotype are different between the two.

Gilmour et al. found a downregulation of *Cdx1* and *Cdx2*, which are important in early haematopoiesis, and the consequent deregulation of several Hox genes, in $Sp1^{del/del}$ Flk1+ cells. However, only the upregulation of *Hoxb1* was consistent with our results. Several genes involved in the BMP and Wnt signalling pathways were deregulated in the previous study. While we identified a number of deregulated genes encoding BMP and Wnt signalling proteins in $Sp1^{-/-}$ cells, the same was not found in our $Sp1^{del/del}$ cells. Interestingly, Gilmour et al. found an upregulation of erythroid-associated genes at the progenitor cell stage, such as *Klf1* and *Gata1*, which are essential TF regulators of the erythroid lineage (Weiss et al., 1994; Tallack et al., 2010). Instead, we observed the upregulation of numerous genes associated with the megakaryocyte/platelet lineage. We also detected an upregulation of *Gata1*, which in addition to its well-known role in erythroid development, is essential for megakaryocyte growth and platelet development (Shivdasani et al., 1997; Noh et al., 2015). Moreover, erythroid and megakaryocytes originate from the same precursor, the megakaryocyte-erythroid progenitor (MEP), suggesting these cells may be predisposed to differentiate to the same pathway.

Gilmour et al. also identified a progressive gene deregulation throughout differentiation (Gilmour et al., 2014). They found a smaller number of deregulated genes in Flk1+ cells, but a larger number were direct Sp1 targets, suggesting that a deregulation of key developmental regulators at an early stage led to increasing gene deregulation as the cells progressed, until they failed to terminally differentiate. However, while still suggesting a crucial role for Sp1 at early stages, our results show no increasing numbers of deregulated genes through differentiation and the highest number actually in ESCs.

These differences may be the outcome of various changes in experimental method. As discussed above, gene expression analysis was performed using RNA-seq in the present study, but with microarrays in Gilmour et al. which are less sensitive and rely on specific

probes to identify transcripts. Furthermore, cell culture conditions were different, for example with different types of serum, which could introduce altered differentiation biases. Gene expression was also not analysed in ESCs in Gilmour et al. Thus, it would be interesting in future work to perform RNA-seq with the original Sp1 DBD-deficient cells, including in ESCs, and compare the results to our findings and the previous microarray results. As the phenotypes are very similar, it is likely the commonly deregulated genes are most important in causing impaired haematopoietic specification.

Furthermore, the $Sp1^{del/del}$ cells in Gilmour et al. and the present study were generated in a different ESC line, thus a different genetic background. We have also shown that loss of Sp1 activity results in changes in the chromatin profile of the cell. Therefore, there may be epigenetic drift in ESCs in the absence of functional Sp1 that could explain the differences between the two studies and possibly making the cells predisposed to differentiate in different directions. Such effects could be avoided in future studies by using a conditional Sp1 knockout model.

4.4 Sp1 deficiency impacts on chromatin structure

Our study as well as previous reports (Gilmour et al., 2014), have demonstrated a deregulation of the gene expression programme upon Sp1 loss or inactivation and a consequent defect in haematopoietic specification. However, no studies have investigated the impact of Sp1 deficiency on chromatin structure during haematopoiesis. Therefore, we aimed to analyse chromatin accessibility in the Sp1 mutant cells using ATAC-seq and link any changes to gene expression.

Our results showed the chromatin state in $Sp1^{del/del}$ and $Sp1^{-/-}$, as well as $Sp1^{+/del}$ cells, was altered. Although there were few unique sites of open chromatin in WT or Sp1 mutant cells, there were several thousand sites at which the accessibility was significantly different (based on a two-fold difference in the ATAC-seq reads). While the number of differential sites was comparable in $Sp1^{del/del}$ and $Sp1^{+/del}$ ESC and Flk1+ cells, the chromatin structure appeared to be vastly different in $Sp1^{-/-}$ Flk1+ cells compared to WT cells, suggesting mesoderm or haemangioblast formation is severely impaired and the cells are altered from conventional Flk1+ cells. This is consistent with the deregulation of mesoderm/haemangioblast associated genes in $Sp1^{-/-}$ cells. Most of the changes in each Sp1 mutant cell occurred at distal regions, indicating a role for Sp1 at enhancers and distal regulatory regions, as well as at promoters. Furthermore, the ATAC-seq dataset was initially filtered using high-read depth DNAse1 hypersensitive site data generated from WT cells throughout haematopoiesis (Methods 2.15.1). This approach allowed the generation of a set of high-confidence peaks, but it is possible that some open sites unique to Sp1 mutant cells were missed in the analysis. This could be investigated in further analyses.

Each Sp1 mutant cell clone contained sites at which accessibility was gained and sites that were lost. Sp1 has a number of reported roles in regulating chromatin state. Sp1 interacts with histone modifying enzymes – both positive, such as p300 (Suzuki et al., 2000), and negative, such as HDAC1 (Doetzlhofer et al., 1999) (discussed further in the introduction). Thus the disruption of Sp1 binding may affect the histone modifications at its binding sites and lead to a change in accessibility. Sp1 is also reported to have a more direct role in regulating chromatin accessibility as it can recruit the remodelling complex SWI/SNF, which likely acts to maintain a nucleosome depleted region at its target promoters and enhancers (Kadam & Emerson, 2003; Iwafuchi-Doi et al., 2016). Therefore, a loss of Sp1 DNA binding may result in more closed chromatin structures at

these regions. As open chromatin is required for TF binding at regulatory regions (with the exception of pioneer factors) and for transcription initiation, a change in accessibility would likely result in altered gene expression. Indeed, the change in chromatin accessibility in the Sp1 mutant cell clones compared to WT cells did correlate with a difference in gene expression. However, the gene expression changes were generally very small, suggesting minimal effects on the cell.

Some sites became more accessible following manipulation of Sp1, which may be a sign of the cell trying to compensate for the loss of Sp1 activity. Other factors may increase their DNA-binding and transcription activity to maintain the gene expression programme, thus resulting in generally small gene expression changes in the Sp1 mutant cells. To investigate this, the Sp1 mutant-specific open regions could be analysed to find which TFs are binding. De novo motif analysis in ESC Sp1 mutant-specific sites showed an enrichment of common promoter and enhancer associated motifs, such as NFY and ETS, as well as an enrichment of Pou5f1 motifs, which may indicate the pluripotency factor Pou5f1 (OCT4) was acting to maintain the ESC gene expression programme. In contrast, CTCF was most commonly found in the Sp1 mutant-specific open regions in Flk1+ cells. Our binding data could be integrated with the data obtained by Goode et al., who performed ChIP-seq on several TFs that are important throughout haematopoiesis (Goode et al., 2016), to analyse in more detail what factors may be bound. Furthermore, it would be interesting to examine in future studies whether the same sites are opening/closing in each Sp1 mutant cell clone. These comparisons may reveal whether there is a global modification of the chromatin structure due to Sp1 deficiency, or whether specific Sp1 binding sites are affected. The ATAC-seq experiments could also be performed in the later stages of haematopoiesis, i.e. HE1, HE2 and progenitors cells, to investigate whether the impact of Sp1 mutation is different at the various stages.

Sp1 has also been found to have a role in higher chromatin structure. In vitro studies have shown Sp1 can mediate looping between gene regulatory elements to activate gene transcription. Looping can occur through interactions with other Sp1 molecules, forming Sp1 tetramers at the DNA loop junction (Mastrangelo et al., 1991; Nolis et al., 2009; Deshane et al., 2010), as well as with other TFs, including tissue-specific TFs to establish cell type-specific patterns of gene expression. An example is the synergistic interaction of Sp1 and GATA1 in erythroid cells between the locus control region and the promoter at the β-globin locus (Merika & Orkin, 1995). The impact of Sp1 on higher order chromatin and the establishment of promoter-enhancer contacts could be explored in future studies by chromatin conformation assays, such as Hi-C (Belton et al., 2012), in WT and Sp1 mutant cells.

4.5 $Sp1^{del/del}$ vs. $Sp1^{-/-}$ cells

In this study, we generated a novel Sp1 null ESC line. All previous Sp1 loss of function studies used a DBD-deficient Sp1 model, including Gilmour et al. 2014. While Sp1 DBD-deficient ESC lines reproducibly showed impaired differentiation to haematopoietic progenitors, Sp1 null ESCs were unable to differentiate to haemangioblasts, showing for the first time that cells lacking the Sp1 DBD were hypomorphs and Sp1 likely retained some activity.

Similar properties were found for the TF SCL/TAL1. Deletion of *Tal1* in mouse embryos resulted in embryonic lethality due to a complete lack of haematopoiesis (Robb et al., 1995; D'Souza et al., 2005). However, HSCs were detected in embryos lacking just the DBD of SCL/TAL1, but the maturation of erythrocytes was severely impaired, suggesting DNA-binding activity of SCL/TAL1 is not required for haematopoietic specification, but it is

crucial for erythroid development (Porcher et al., 1999; Kassouf et al., 2008). A similar phenotype was found for Sp1 DBD-deficient cells: they could differentiate in vitro to haematopoietic progenitors, but failed to form macrophages. Thus, Sp1 DNA-binding may not be essential for haematopoietic specification, but it is for terminal differentiation. It was proposed that SCL/TAL1 binds to DNA indirectly via binding partners, and indeed it was found to bind to ~20% of its targets in primary foetal liver erythrocytes using ChIP-seq (Kassouf et al., 2010). However, this was at a late stage when DNA-binding was required, rather than at earlier in haematopoietic specification.

Therefore, we hypothesised Sp1 may still be binding to DNA indirectly as part of a complex with other TFs. Sp1 retains its two TADs, meaning it may still be able to activate transcription. However, Sp1 ChIP-seq with double crosslinking in $Sp1^{del/del}$ cells showed no Sp1 binding. This result is consistent with electrophoretic mobility shift assays (EMSA) performed with the original Sp1 DBD-deficient ESCs, which showed no binding of the truncated Sp1 protein to DNA (Marin et al., 1997).

Despite this result, the difference in phenotype of $Sp1^{-/-}$ and $Sp1^{del/del}$ cells still points to some residual function of the DBD-deficient Sp1. Although the antibody was able to bind the truncated Sp1 in Western blotting, it may be that it cannot recognise the native structure of the protein in cells. The ability of the antibody to bind DBD-deficient Sp1 could be confirmed by immunoprecipitation assays to verify the Sp1 protein can be isolated in the $Sp1^{del/del}$ cells. The double-crosslinking used in the ChIP method may also not have been sufficient to capture indirect TF binding to DNA. The use of double-crosslinking in ChIP to detect indirect binding could be validated with a control ChIP experiment of known transcription cofactors that bind indirectly to DNA.

Alternatively, it may be the ChIP-seq protocol used was not sensitive enough to detect the potentially very transient or unstable interactions between truncated Sp1 and its DNA-bound partner proteins. Recently, ChIP-seq has been modified to increase sensitivity, for example ChIP-exo (which uses an exonuclease to remove flanking DNA) has been shown to identify low-occupancy binding sites at a higher resolution (Rhee & Pugh, 2011). DNA-adenine methyltransferase identification (DAM-ID) may also be used. The technique takes advantage of the near absence of adenine methylation in eukaryotes, while it is abundant in prokaryotes and catalysed by the DAM enzyme. DAM is fused to the desired TF, resulting in adenine methylation at its binding sites. The DNA is then extracted, fragmented using the methylation-sensitive DpnI and the methylated fragments amplified and sequenced (Steensel & Henikoff, 2000). This technique allows identification of sites with even transient binding and with low levels of protein. For example, it was used to map RUNX1 binding in the HE, which is itself a transient population (hence present in low cell numbers) and with very low RUNX1 expression (Lie-A-Ling et al., 2014). Therefore, future investigations could use these techniques to discover any DBD-deficient Sp1 binding in $Sp1^{del/del}$ cells and analyse which proteins it may be bound with.

4.6 Interplay between Sp1 and Sp3

Sp1 and its homologue Sp3 have very similar structures, including a highly conserved DBD, meaning they recognise the same DNA motif. Therefore, we aimed to investigate how the interplay between Sp1 and Sp3 regulates transcription in ESC and Flk1+ cells, and how this relationship is affected with the loss of Sp1 activity. To this end, we identified the genome wide binding sites of Sp1 and Sp3 in WT and Sp1 mutant cells by ChIP-seq.

A large overlap was found between Sp1 and Sp3 binding sites, as we could have expected. However, there is still some debate over whether Sp1 and Sp3 occupy the same sites simultaneously, or whether they compete for the same sites. For example, Sp1 and Sp3 were found to physically interact at the promoter for the HGF receptor gene (*c-met*) and synergistically activate transcription (Zhang et al., 2003). However, other studies have shown Sp1 and Sp3 do not associate with each other: Sun et al. was only able to immunoprecipitate either Sp1 or Sp3, never both, while He et al. used immunofluorescence to show they localised to distinct parts of the nucleus (Sun et al., 2002; He et al., 2005). Reports at several different loci have shown Sp1 and Sp3 compete for binding and can elicit different transcriptional effects (Yu et al., 2003; Lee et al., 2004; Liu et al., 2004; de León et al., 2005). The relationship between Sp1 and Sp3 binding at their shared sites could be further examined using re-ChIP experiments. Re-ChIP involves consecutive immunoprecipitations in order to show simultaneous co-occupancy of proteins at genomic sites or protein-protein interactions, for example if a TF binds as part of a complex (Chaya et al., 2001; Kinkley et al., 2016).

The significant overlap between Sp1 and Sp3 binding, as well the minor changes in gene expression when correlated to the Sp1 binding in WT cells vs Sp3 binding in Sp1 mutant cells, suggest Sp3 is largely able to compensate for the absence of Sp1. However, the mutant phenotype suggests that Sp3 cannot functionally compensate completely, particularly at genes important in developmental progression. This result is supported by knockout mice studies. Deletion of the Sp1 DBD in mice is embryonic lethal at ~E11 with a range of phenotypes, although only a small number of genes are deregulated, suggesting that other factors (likely Sp3) were compensating for loss of Sp1 (Marin et al., 1997). On the other hand, mice lacking Sp3 die postnatally, apparently from respiratory defects (Bouwman et al., 2000), indicating that while there is some redundancy between their functions, Sp1 and Sp3 have specific roles in individual tissues.

While the majority of binding sites are shared by Sp1 and Sp3, we did identify some Sp1- and Sp3-specific sites in WT cells. The Sp3 binding distribution was unchanged in the Sp1 mutant cells, suggesting that Sp3 cannot bind at Sp1-specific sites, even without Sp1 bound. However, it is interesting to note that total Sp3 binding was higher in $Sp1^{-/-}$ ESCs, which may be responsible for the lack of phenotype in ESCs. Therefore, Sp3 cannot compensate at sites normally bound solely by Sp1. These sites were associated with a deregulation of genes associated with important cellular processes, such as transcription and development, thus there may be detrimental effect on the cell – possibly indirectly impacting on differentiation. However, many of the genes that were identified as being significantly deregulated with Sp1 deficiency and likely to be important in haematopoiesis were not found to be regulated by Sp1 specifically. Therefore, their deregulation may be an effect of Sp3's different transactivation abilities. There are numerous examples of where both Sp1 and Sp3 can activate transcription, such as *p21* (Gartel et al., 2000), *COL1A2* (Ihn & Trojanowska, 1997) and *POLD1* (Zhao & Chang, 1997). In contrast, Sp3 can elicit different effects to Sp1 at some genes. Indeed, Sp3 is often thought of as a weaker activator or even a repressor of Sp1-mediated transcription. For example, Sp1 activates gene expression, whereas Sp3 represses transcription at the topoisomerase IIα promoter (Williams et al., 2007) and c-myc promoter (Majello et al., 1995). Secretin expression is regulated by the ratio of Sp1/Sp3: in expressing cells, NeuroD, E2A, Sp1 and Sp3 bind the promoter, but there are high levels of Sp3 bound in non-expressing cells, which repress transcription (Lee et al., 2004).

The basis of Sp3 repression of Sp1-mediated transcription is thought to be its inability to multimerise. At sites with multiple GC-boxes, Sp1 can form a complex and synergistically activate transcription. Sp3, which lacks the interaction domain D (Hagen et al., 1994), cannot synergise, but can bind more stably to DNA at some of these sites, thus out-

competing Sp1 and eliciting weaker activation of transcription than Sp1 (Yu et al., 2003). In future studies, it would be interesting to analyse if there was a difference in the composition of sites at which Sp3 cannot compensate in the Sp1 mutant cell – for example, do they commonly contain multiple GC boxes?

Sp3 also contains a unique inhibitory domain (ID) adjacent to the zinc fingers (Dennig et al., 1996). Sumoylation in the ID is thought to recruit negative histone modifiers and chromatin remodellers, such as HP1 and SETDB1 (Stielow et al., 2008). Mutation of a KEE sequence in the ID can transform Sp3 into a strong activator of Sp1 target genes (Dennig et al., 1996), showing the presence of the ID in Sp3, as opposed to Sp1, can explain some of the differences in activity. Further studies could try to rescue $Sp1^{-/-}$ and $Sp1^{del/del}$ cells with the expression of Sp3 with a mutant ID, as the expression of Sp3 that is able to activate expression from Sp1 target genes may be sufficient to partially rescue differentiation.

There are four isoforms of Sp3, which originate from alternative translational start sites. The two long form (781 and 769 amino acids) can behave as activators or repressors, depending on the promoter, while the two short isoforms (496 and 479 amino acids), which only contain one TAD, are always inactive (Sapetschnig et al., 2004). Again, this shows Sp3 is not simply a functional equivalent of Sp1. Furthermore, a significant shift towards the long isoforms is seen in Sp1 deficient ESCs, showing the expression of the individual isoforms is regulated and it may be a mechanism to compensate for loss of Sp1 (Sapetschnig et al., 2004). Quantification of the Sp3 protein levels showed a shift in $Sp1^{del/del}$ and $Sp1^{-/-}$ ESCs towards the longer isoforms, suggesting a similar effect is occurring. The impact of the ratio of Sp3 isoforms on Sp3's ability to compensate for lack of Sp1 could be investigated in future studies by allowing the separation of all four isoforms and comparing their relative expressions in WT compared to Sp1 mutant cells.

Overall, we conclude that a combination of differential Sp1 and Sp3 binding, as well as the different transactivation properties, means Sp3 cannot completely compensate for the loss of Sp1 activity. As well as controlling the expression of ubiquitous genes, Sp1 can regulate tissue-specific gene expression e.g. by interacting with tissue-specific TFs (such as GATA1) and altering PTMs depending on cell context (see section 1.5). Thus, the defect in haematopoietic specification likely stems from the inability of Sp3 to fully compensate at important cell type specific genes that regulate haematopoiesis. We propose that Sp3 can effectively activate Sp1 target housekeeping genes (hence minimal overall gene deregulation and ESCs that grow well), but Sp3 cannot completely compensate at tissue-specifically expressed genes, for example as it cannot interact with some tissue-specific TF binding partners of Sp1 (Figure 4.1). The PTMs of Sp1 and Sp3 are also different which may have an effect on tissue-specific activity (Sapetschnig et al., 2004). The ability of Sp1 to multimerise and mediate DNA looping between regulatory elements would also be important in tissue-specific gene regulation, which often utilises enhancers, thus Sp3 may not be able to activate transcription to the same level. Therefore, there is a deregulation of developmental/haematopoietic regulator genes, such as those involved in mesoderm formation. However, a milder phenotype is observed in $Sp1^{del/del}$ cells compared to $Sp1^{-/-}$ cells. We suggest this is due to residual activity of the truncated Sp1 protein, which may still be able to interact with its DNA-binding partners and activate transcription via its TADs (Figure 4.1). Overall, the disparity between Sp1 and Sp3 function could mean that the differentiation dynamics of the Sp1 mutant cells is affected; hence differentiation is slower and less effective.

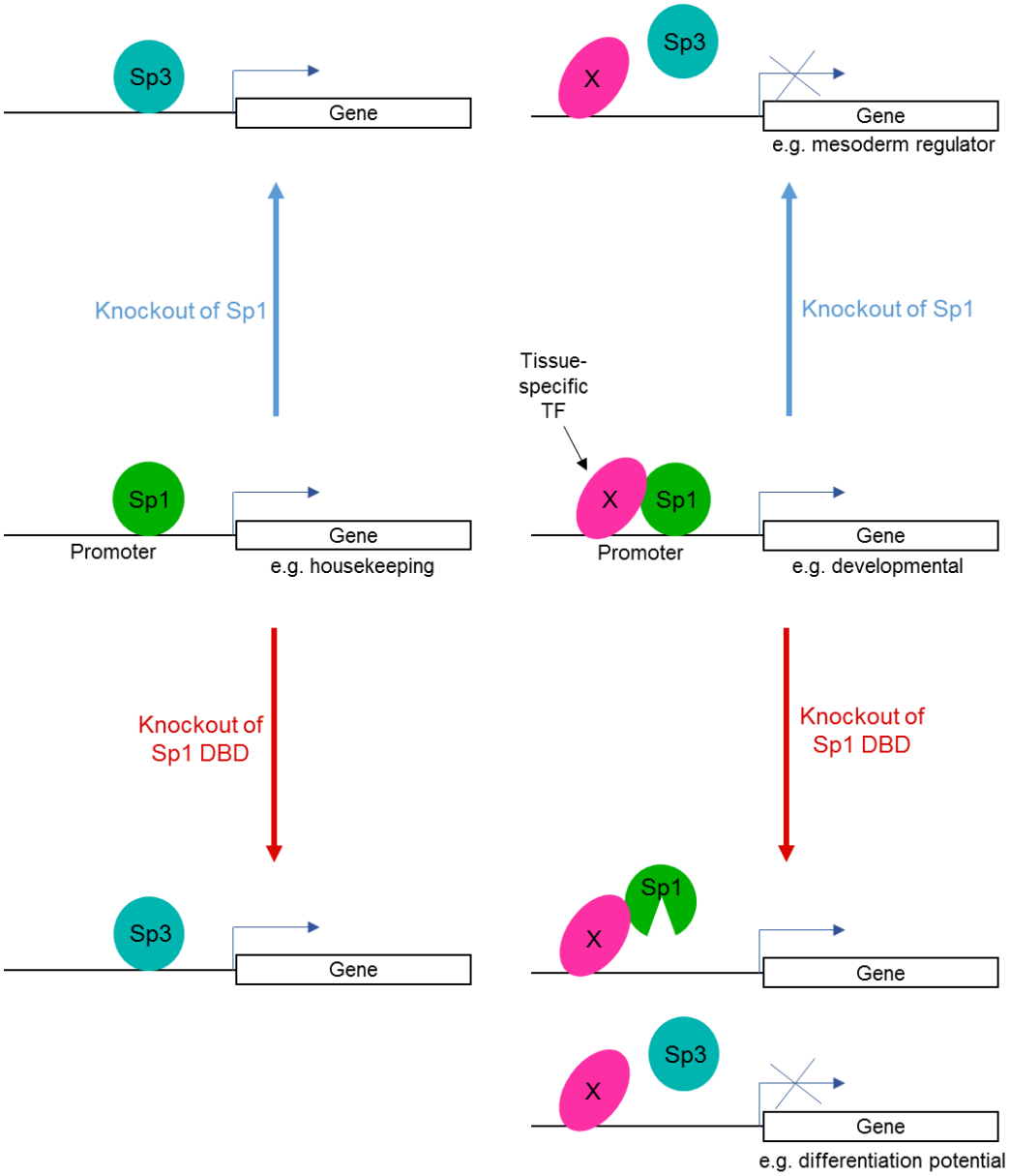


Figure 4.1 – Potential mechanism of Sp3 compensation in Sp1 deficient cells

Sp1 binds at promoter (and enhancer) elements of both housekeeping and tissue-specific genes to regulate gene expression. Sp1 can bind alone (or as Sp1 multimers) or interact with other TFs, such as tissue-specific TFs, to fine-tune its specificity or activity at its target genes. Upon complete knockout of Sp1, or deletion of its DBD, Sp3 can compensate at the majority of genes and maintain their expression (left). This includes at housekeeping genes, meaning the Sp1-deficient ESCs grow relatively normally. However, Sp3 cannot compensate at all genes, such as genes important in developmental progression, thus there is a defect in the differentiation of $Sp1^{-/-}$ and $Sp1^{del/del}$ cells to the haematopoietic lineage (right). This may occur as Sp3 cannot interact with Sp1 partner proteins, thus causing gene deregulation, such as of mesoderm regulators in $Sp1^{-/-}$ cells. The phenotype is less severe with knockout of just the Sp1 DBD, suggesting the truncated Sp1 protein may still be recruited to some gene regulatory elements via its partner proteins to activate transcription with its TADs. DBD-deficient Sp1 may also play a role in binding partner TFs and preventing them interacting with Sp3.

4.7 Tissue-specific gene regulation by Sp1

ChIP-seq experiments in this study have provided a wealth of information on the binding of Sp1 relative to Sp3 and how the binding changes between stages of differentiation. Motif analysis was performed at the Sp1 and Sp3 sites in an aim to identify potential TF interaction partners. We found some differences in the TF motifs that were enriched at the Sp1 and Sp3 sites, including the CTCF motif which was enriched in Sp3-specific sites, but not at Sp1-bound sites. Publicly available CTCF ChIP-seq data in ESCs confirmed this pattern, with CTCF binding strongly negatively correlated with the fold change of Sp1/Sp3 binding. CTCF is a ubiquitously expressed eleven-zinc finger protein. It has various functions, including insulating regions of chromatin to prevent spreading of active/repressive states and regulating contacts between promoters and enhancers (Zlatanova & Caiafa, 2009). We were surprised to find a potential link between CTCF and Sp3, however CTCF's binding is widespread across the genome, particularly at distal regions (Kim et al., 2007; Chen et al., 2012). The ZBTB33 motif was also identified in Sp3-specific regions, which is a known binding partner of CTCF, thus supporting the possibility of an association between Sp3 and CTCF (Defossez et al., 2005).

Recently, CTCF has been found to interact with TFs in gene regulation. For example, the tissue-specific TF LDB1 binds at the enhancers of some erythroid-specific genes and contacts CTCF bound at the promoter, via DNA looping, to activate transcription (Lee et al., 2017). Thus, CTCF is involved in mediating long range interactions between regulatory regions to establish a cell type-specific pattern of gene expression. Sp3 may be interacting with CTCF in a similar way. Sp3 lacks the domain D that enables Sp1 to form multimers and mediate DNA looping (Hagen et al., 1994; Yu et al., 2003), therefore it may interact with CTCF to enable it to still regulate gene expression from distal elements. However, the inability of Sp3 to form multimers and establish DNA loops, in contrast to

Sp1, has previously been proposed to be a reason for Sp3's weaker transactivation properties, or even its repressive activity at some loci, for example by displacing Sp1 and preventing looping between regulatory elements (Williams et al., 2007). Alternatively, one study has demonstrated the presence of an overlapping CTCF and Sp1/Sp3 binding site at the promoter of the *ALF* gene, with the GC-box beginning within the CTCF motif. CTCF and Sp3 repress transcription, while Sp1 can activate it, suggesting the ratio between TFs determines the expression of the gene at specific developmental stages or specific tissues (e.g. CTCF levels decrease in germ cells where *ALF* is expressed) (Kim et al., 2006). Therefore, the Sp3 binding sites could be further examined for a novel longer motif combining the two binding sites. However, as this is the first time a potential relationship between Sp3 and CTCF has been observed, the findings could be verified by repeating the ChIP-seq with different antibodies, to ensure there was no cross-reactivity.

The Sp1 and Sp3 ChIP-seq data provide a resource for future studies to potentially identify new binding partners. A large proportion of Sp1 binding sites were located in CpG islands, while nearly all open CpG islands were bound by Sp1, supporting a role for Sp1 in maintaining a DNA methylation-free state and the promoters active (Brandeis et al., 1994; Macleod et al., 1994). Correspondingly, a number of TF motifs commonly associated with promoters were enriched at both Sp1 and Sp3 sites. For example, NFY binds to a CCAAT box, which is one of the most common elements in promoters, regulating a range of tissue-specific, inducible and housekeeping genes (Bucher, 1990). NFY interacts with Sp1 at several promoters to cooperatively bind or synergistically activate transcription (Wright et al., 1995; Yamada et al., 2000; Iwano et al., 2001; Schardt et al., 2015). The ETS motif was also enriched, which can be bound by a number of TFs, such as FLI1 which is important in embryonic development and haematopoiesis and can interact with Sp1 (Shirasaki et al., 1999).

Sp1 also has roles in tissue-specific gene regulation, often with the interaction with tissue-specific TFs. However, it is still unknown what interactions occur to give Sp1 its tissue-specificity during embryonic haematopoiesis. While there was a low enrichment of ESC-associated motifs, such as Pou5f1, at Sp1 sites in ESCs, there was an enrichment of GATA and TEAD motifs at Sp1 sites in Flk1+ cells. GATA2 is required for haematopoiesis and is expressed at the haemangioblast stage (Tsai et al., 1994; Pimanda et al., 2007). GATA sites, commonly with ETS sites, are crucial for the activation of early haematopoietic genes, such as *Fli1*, *Tal1* and *Runx1* (Pimanda et al., 2007; Wilson & Roberts, 2011). Furthermore, GATA2, FLI1 and SCL/TAL1 interact to establish the gene expression programme at early stages of haematopoiesis (Pimanda et al., 2008). Sp1 has previously been found to interact with these factors, for example Sp1 interacts with the "SCL complex" (containing SCL/TAL1, E2A, GATA2, LMO2 and LDB1) and tethers it to the DNA at the *Kit* promoter (Lecuyer et al., 2002). Moreover, the interaction between Sp1 and GATA1 in erythroid cells has been extensively studied. Sp1 and GATA1 interact via the DBD and synergistically activate transcription of erythrocyte-specific genes, such as β-globin (Merika & Orkin, 1995). Indeed, Sp1 can recruit GATA1 to a promoter in the absence of GATA binding sites and vice versa. Thus, due to similarity in GATA family members, a similar interaction may occur with GATA2 at earlier stages of haematopoiesis. Recently, TEAD4 was identified as required in early stages of embryonic haematopoiesis, prior to the EHT. The motif was particularly enriched at the haemangioblast stage, consistent with our data, and TEAD4 bound several important genes in haematopoietic development, including *Runx1* and *Kit* (Goode et al., 2016). As the TEAD motif was enriched at Sp1 binding sites, there may be an interaction between the proteins, leading to tissue-specific gene regulation by Sp1.

The interactions of Sp1 with developmental stage-specific TFs could be further investigated by integrating our data with the ChIP-seq results obtained in Goode et al.

which mapped the binding of various TFs across the different stages of haematopoietic differentiation (Goode et al., 2016). This analysis could show factors that co-localise with Sp1 and may interact to regulate transcription, thus revealing novel binding partners of Sp1. Our study has shown Sp1 is crucial for haematopoietic specification, thus it would be interesting to examine what determines the tissue-specific expression of some Sp1 target genes (e.g. via the interaction of cell type-specific TFs). Sp3 is unable to compensate for the loss of Sp1 at some of these genes encoding haematopoietic regulators (hence a defect in differentiation of Sp1-deficient ESC). Therefore, the Sp3 ChIP-seq data may show differences in the ability of Sp1 and Sp3 to bind with other factors. For example, Sp3 may not be able to interact with Sp1's partner proteins. Future studies could also investigate the role of PTMs in generating cell type-specific gene regulation by Sp1, e.g. phosphorylation in myeloid progenitors can promote binding to monocyte-specific promoters (Zhang et al., 1994).

In conclusion, we have developed a novel complete Sp1 null ESC model, which has demonstrated a crucial role for Sp1 at the early stages of haematopoiesis. The contrasting ability of Sp1 DBD-deficient cells to form haematopoietic progenitors, but not macrophages, suggests different mechanisms of action at different stages of development. We found that Sp1's family member, Sp3, is able to compensate to some extent for the loss of Sp1, but not at some important genes in developmental progression, possibly due to differences in protein-protein interactions with tissue-specific TFs. This study therefore gathered novel biological insights into the function of one of the first discovered TFs and has generated a resource for future work to further investigate the tissue-specific roles of a ubiquitously expressed TF.

5. Supplementary Data

5.1 Sequence data of CRISPR-generated Sp1 mutant clones

WT

DNA sequence
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Introns are shown in lower case, exons in upper case
Guide 1 (PAM)
Guide 14 (PAM)
Stop codon
PCR primers

Amino acid sequence
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A L I T T N M V A M E A I C P E G I A R L A N S G I N V M Q V T E L Q S I N I S G N G
F

Sp1$^{del/del}$

DNA sequence
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CAATGTCACTG

Amino acid sequence
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V V G R I G R W Y K K K E M G L W H P M S L
(changes to sequence indicated in blue)

Sp1$^{-/-}$

8bp deletion

DNA sequence
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DNA sequence changes
between the highlighted bases

Amino acid sequence
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Stop

Sp1$^{+/del}$ (5)

Allele 1:

158bp deletion

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Amino acid sequence
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Sp1$^{+/del}$(7)

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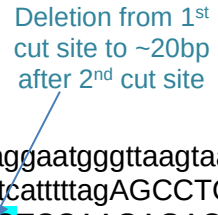

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Amino acid sequence
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Allele 2:
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Sp1$^{+/del}$(11)

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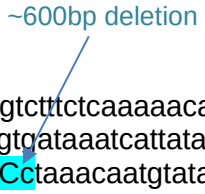

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13bp deletion

Amino acid sequence
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C P E G I A R L A N S G I N V M Q V T E L Q S I N I S G N G F Stop

Allele 2:

DNA sequence
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Change and addition of bases

Amino acid sequence:
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S D H L S K H I K T H Q N K K G G P G V A L S V G T L P L D S G A G S E G T A T P S
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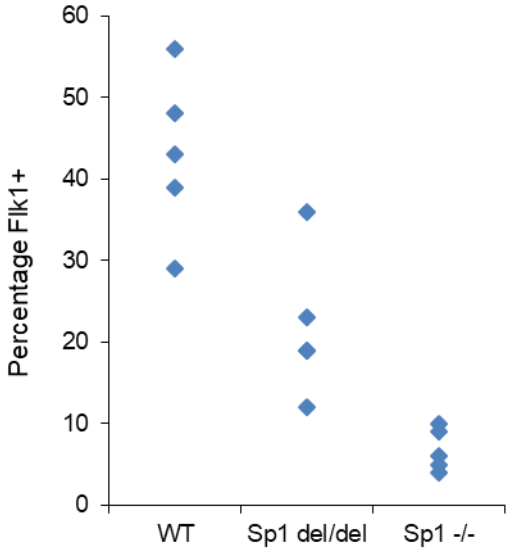
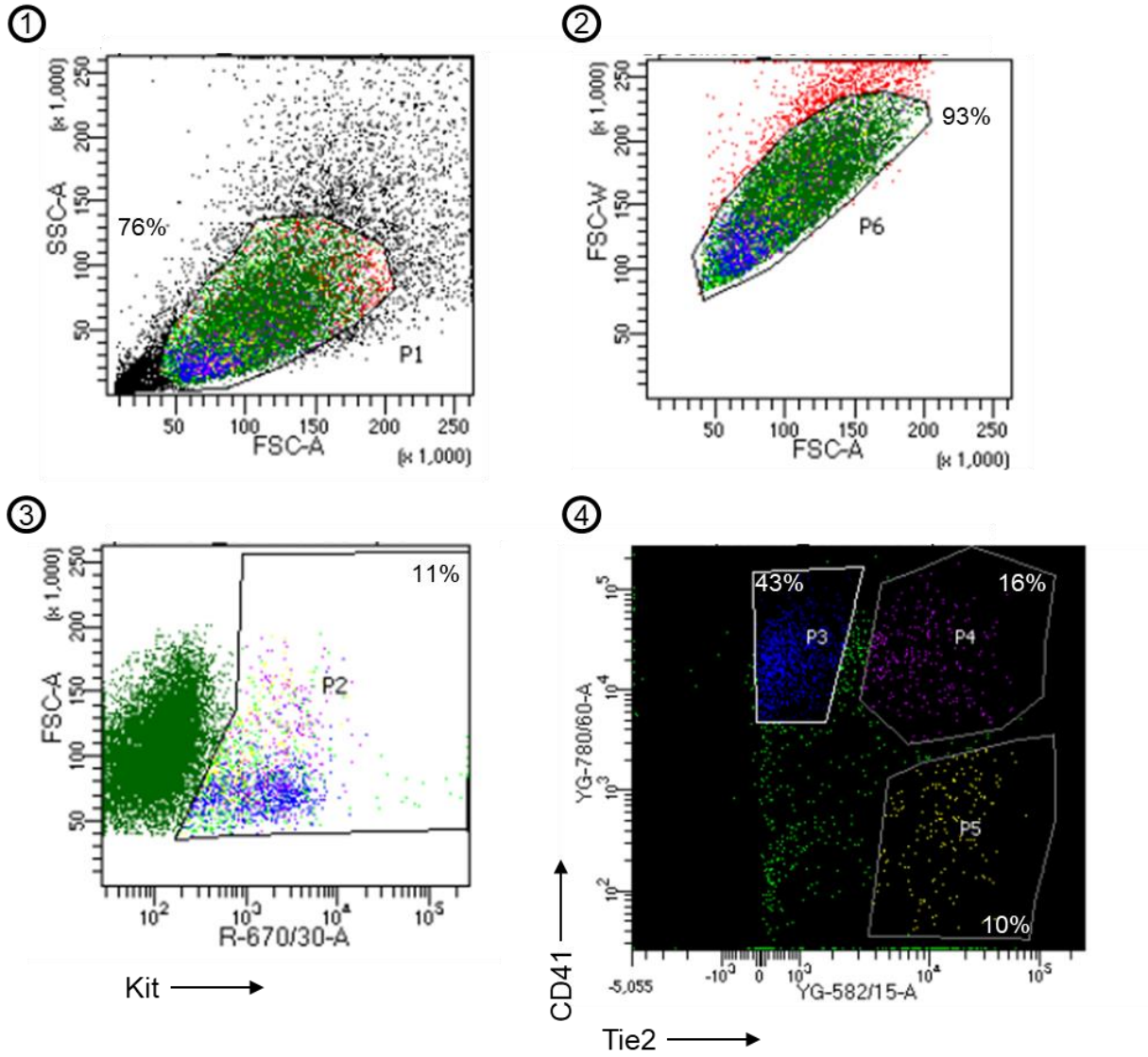
F Stop



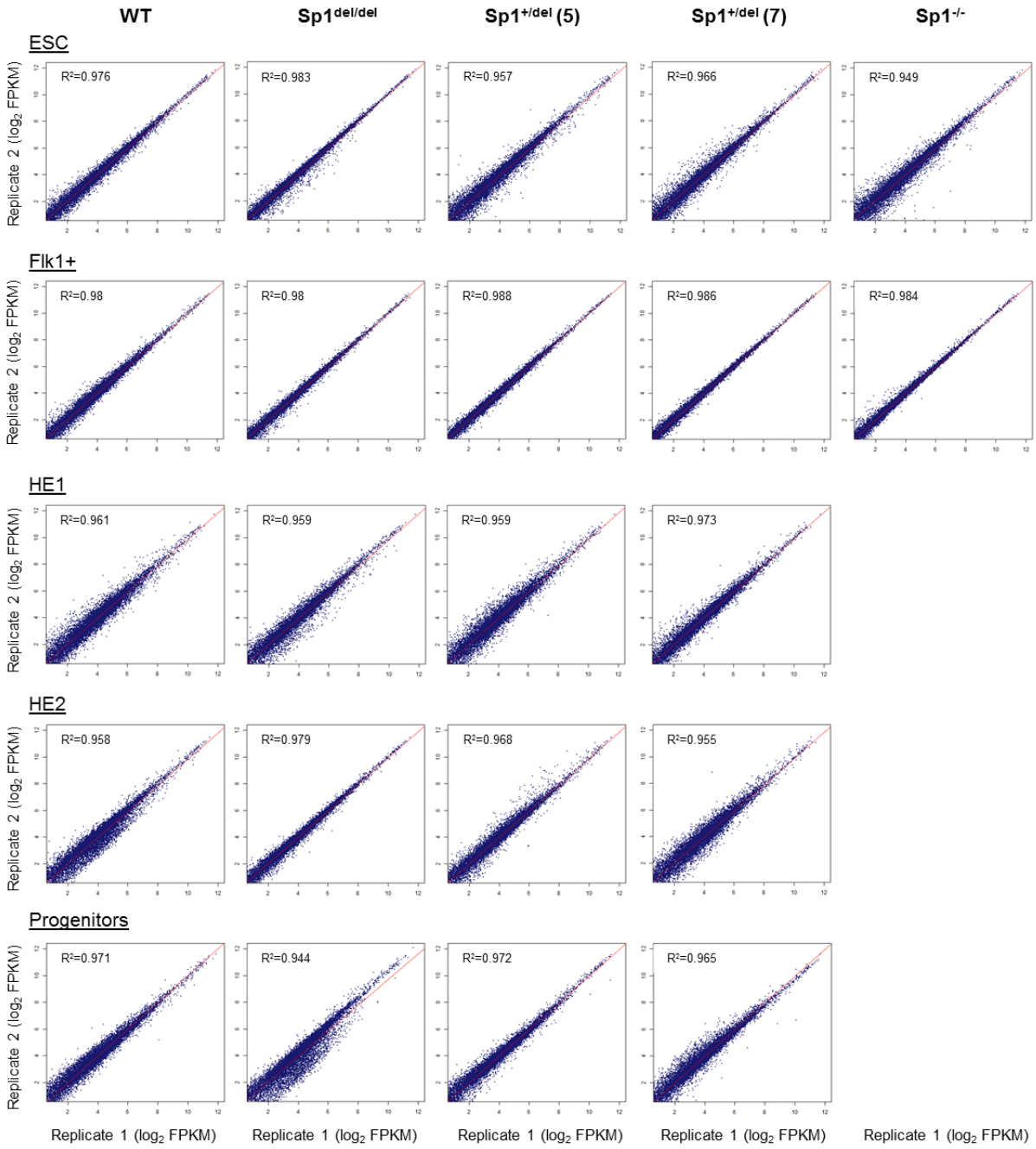
Supplementary Figure 5.1 – Variation in Flk1+ cell generation in WT, $Sp1^{del/del}$ and $Sp1^{-/-}$ cells

WT, $Sp1^{del/del}$ and $Sp1^{-/-}$ ESCs were differentiated to Flk1+ cells in vitro at the same time. The percentage of Flk1+ cells over 5 experiments is plotted.



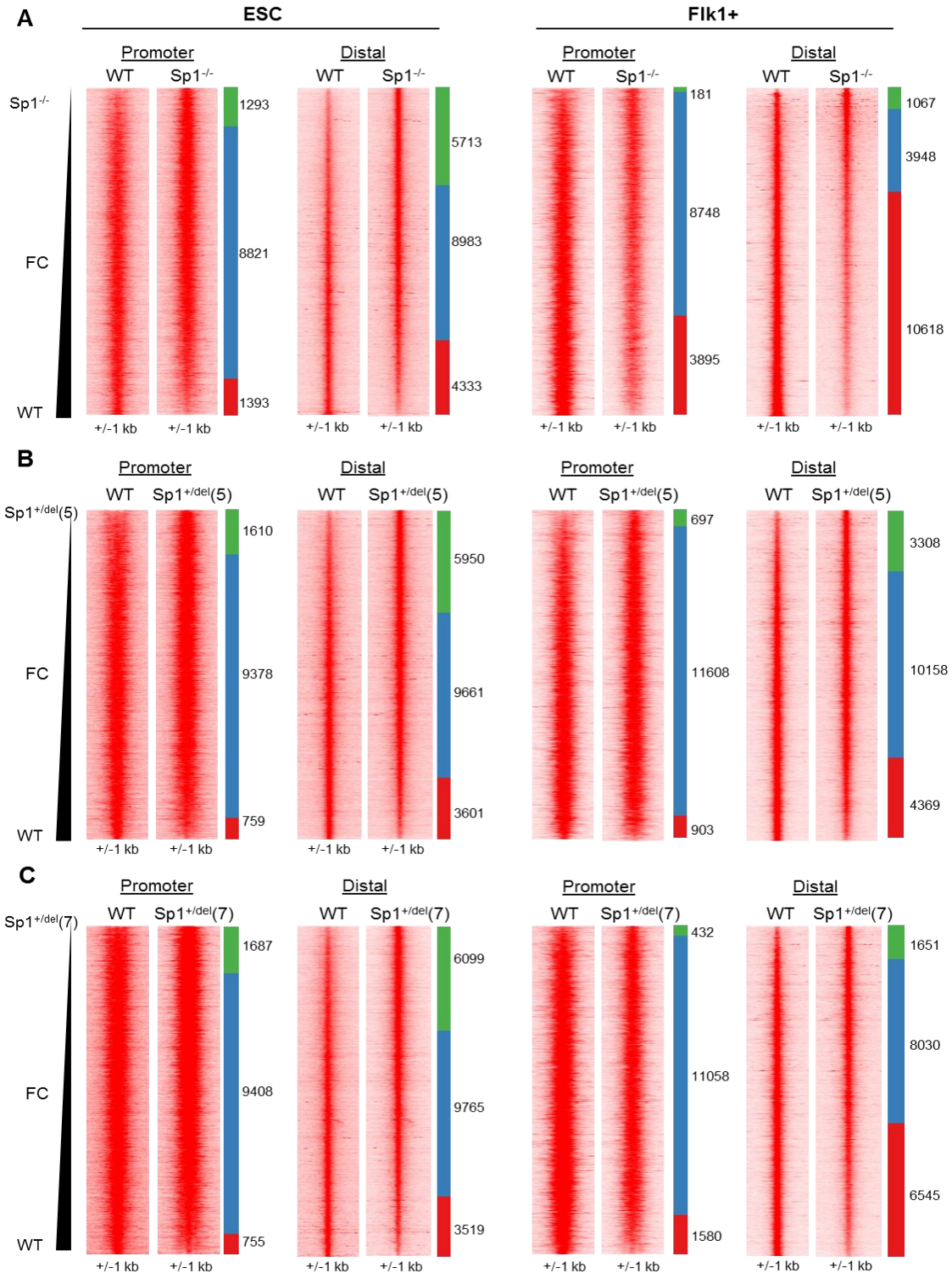
Supplementary Figure 5.2 – Specific cell populations from a clast culture were isolated by FACS

Flk1+ cells were cultured to form blast cell colonies. The blast culture was harvested at day 2 and the cells processed by FACS to isolate the distinct haematopoietic populations. The cells were first gated by forward and side scatter (area parameter) (FSC-A and SSC-A) to isolate living cells, before gating for forward scatter (area and width parameters) (FSC-A and FSC-W) to isolate single cells (removing doublets). The cells were sorted for expression of Kit to identify haematopoietic cells, and finally for levels of Tie2 and CD41 expression to isolate HE1, HE2 and progenitor populations. An unstained sample was used as a control and a triple IgG isotype control sample (PE Cy7, PE and APC) was used to set the gates. A representative WT sort is shown and the resulting populations verified in Figure 3.8.



Supplementary Figure 5.3 – RNA-seq replicates are well correlated

RNA-seq was performed in duplicate at each stage of differentiation in each Sp1 clone. Log2 (FPKM) values were plotted for each RNA-seq biological replicate and the R^2 value calculated to indicate the correlation between replicates.



Supplementary Figure 5.4 – Changes in chromatin accessibility in Sp1 mutant clones occurs at distal elements

ATAC-seq was used to measure chromatin accessibility in $Sp1^{-/-}$ (A), $Sp1^{+/del}(5)$ (B) and $Sp1^{+/del}(7)$ cells at ES and Flk1+ stages. The ATAC-seq peaks were divided into promoter and distal classes based on their position relative to a TSS of the nearest gene. The fold change (FC) of the ATAC-seq peaks in the Sp1 mutant clone vs WT is plotted and the regions that are at least two-fold different are indicated by the green and red sections.

Supplementary Table 5.1

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Trap1a	56.59	65.89	0.7	18.09	-6.34	-1.86	4.5
Rhox6	14.14	22.48	0.23	6.4	-5.93	-1.81	4.1
Rhox9	9.5	13.3	0.15	3.58	-5.94	-1.89	4.1
Laptm5	16.93	10.48	0.57	3.58	-4.88	-1.55	3.3
Tex11	3.18	5.09	0.15	1.49	-4.43	-1.77	2.7
Mfng	4.74	2.15	0.24	0.68	-4.3	-1.66	2.6
Hap1	15.21	10.37	0.42	1.79	-5.18	-2.54	2.6
Pde2a	9.61	5.57	0.65	2.24	-3.88	-1.32	2.6
Spp1	124.01	258.72	0.71	8.67	-7.46	-4.9	2.6
4933402E13Rik	8.72	6.5	0.13	0.57	-6.04	-3.51	2.5
Anxa1	28.43	41.98	0.43	3.53	-6.06	-3.57	2.5
Pla2g7	8.2	4.3	0.72	2.02	-3.52	-1.09	2.4
Scrn1	7.34	5.06	0.2	0.68	-5.2	-2.89	2.3
Rnf17	11.87	11.52	1.15	5.33	-3.37	-1.11	2.3
Ddx4	10.94	13.63	0.54	3.17	-4.34	-2.1	2.2
Tex19.1	23.63	66.68	0.39	5.26	-5.9	-3.66	2.2
Crmp1	23.15	13.32	1.99	5.03	-3.54	-1.4	2.1
Fam19a4	3.92	1.36	0.22	0.33	-4.17	-2.05	2.1
Mup6	13.57	18.06	0.51	2.83	-4.74	-2.67	2.1
Hmga1-rs1	4.73	1.13	0.1	0.1	-5.52	-3.5	2.0
Psma8	10.86	11.38	0.19	0.8	-5.85	-3.83	2.0
Hopx	13.07	4.9	0.38	0.56	-5.11	-3.13	2.0
Mael	7.73	6.19	0.13	0.4	-5.92	-3.94	2.0
Lrp2	14.85	28.7	0.63	4.64	-4.56	-2.63	1.9
Calca	16.36	8.41	0.65	1.22	-4.66	-2.78	1.9
Samd12	1.91	1.35	0.17	0.44	-3.49	-1.64	1.9
Adgrv1	4.75	4.08	0.17	0.54	-4.78	-2.93	1.9
Stk31	4.77	5.9	0.18	0.81	-4.7	-2.87	1.8
Gprc5a	3.67	2.46	0.29	0.68	-3.67	-1.85	1.8
Hsf2bp	20.29	21.2	0.52	1.93	-5.28	-3.46	1.8
Fam25c	22.63	27.19	0.1	0.43	-7.82	-6	1.8
Itga3	43.63	28.81	1.35	3.11	-5.01	-3.21	1.8
Tacstd2	2.99	2.54	0.38	1.1	-3	-1.21	1.8
Pde9a	3.5	1.23	0.49	0.58	-2.83	-1.08	1.8
Wt1	4.57	1.32	0.34	0.33	-3.74	-1.99	1.8
Enpp3	37.8	44.91	0.64	2.48	-5.89	-4.18	1.7
Sncg	3.21	1.81	0.14	0.25	-4.57	-2.88	1.7
Camk2b	2.22	1.76	0.31	0.8	-2.82	-1.14	1.7
Aire	4.49	2.3	0.1	0.16	-5.49	-3.81	1.7
Vat1l	3.69	2.5	0.32	0.68	-3.53	-1.88	1.7
Dppa4	97.16	99.05	1.5	4.72	-6.02	-4.39	1.6
Ddx58	6.04	5.14	0.88	2.29	-2.79	-1.17	1.6
Abca1	10.14	10.26	0.24	0.73	-5.42	-3.82	1.6
Wipf3	1.73	1.09	0.3	0.57	-2.54	-0.95	1.6
Trf	1.52	1.31	0.17	0.43	-3.18	-1.6	1.6
Vsig2	4.24	1.56	0.55	0.6	-2.93	-1.39	1.5
Gstp2	9.14	5.35	0.31	0.53	-4.88	-3.34	1.5
Ntn1	14.21	9.32	2.13	4	-2.74	-1.22	1.5
Cd74	3.22	1.85	0.53	0.86	-2.61	-1.1	1.5
Pdzd2	9.09	5.4	0.25	0.42	-5.17	-3.67	1.5
Pipox	20.43	22.86	0.25	0.78	-6.38	-4.88	1.5
Pnldc1	11.8	13.2	1.94	6.03	-2.6	-1.13	1.5
Triml2	37.76	33.45	0.34	0.82	-6.82	-5.35	1.5
Mmp25	3.59	2.87	0.34	0.75	-3.39	-1.93	1.5
Akr1b8	18.61	12.04	0.87	1.55	-4.42	-2.96	1.5
Smagp	23.68	13.33	0.99	1.52	-4.58	-3.13	1.5
Slco4a1	3.13	1.82	0.16	0.26	-4.25	-2.82	1.4
AU018091	42.27	52.24	0.3	1	-7.13	-5.7	1.4

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Ptk2b	17.26	14.25	0.78	1.73	-4.46	-3.04	1.4
Alox5ap	6.95	2.3	0.47	0.41	-3.9	-2.49	1.4
Plaur	35.04	25.26	1.47	2.82	-4.57	-3.16	1.4
Sox2	90.05	108.55	0.86	2.75	-6.71	-5.3	1.4
Rps6kl1	4.27	2.34	0.57	0.83	-2.9	-1.5	1.4
E2f2	9.24	4.72	1.16	1.55	-2.99	-1.6	1.4
Nkx6-3	3.98	1.56	0.1	0.1	-5.32	-3.93	1.4
Pecam1	8.45	21.01	1.16	7.43	-2.87	-1.5	1.4
Cldn7	11.56	7.57	1	1.7	-3.53	-2.16	1.4
Zbtb32	8.3	6.01	0.37	0.7	-4.48	-3.11	1.4
Cryab	8.2	11.3	0.85	2.98	-3.27	-1.92	1.4
Bmp8b	2.37	1.66	0.16	0.28	-3.92	-2.57	1.4
Rnf125	15.69	16.97	0.49	1.34	-5.01	-3.66	1.4
Urah	32.86	22.88	0.27	0.49	-6.9	-5.55	1.4
Spta1	16.2	12.3	0.13	0.26	-6.91	-5.56	1.4
Arc	10.54	5.49	0.41	0.54	-4.67	-3.35	1.3
Crct1	3.38	1.35	0.1	0.1	-5.08	-3.76	1.3
Gdf3	62.35	70.76	0.4	1.12	-7.29	-5.98	1.3
Slc44a4	4.41	2.19	0.29	0.35	-3.94	-2.66	1.3
Rbmxl2	23.55	23.13	0.27	0.64	-6.46	-5.18	1.3
Itgb4	3.84	1.52	0.22	0.2	-4.16	-2.89	1.3
Slc52a3	2.85	1.06	0.12	0.1	-4.63	-3.36	1.3
Ppl	5.5	2.87	0.21	0.26	-4.72	-3.45	1.3
Dnmt3l	260.79	233.53	0.55	1.18	-8.89	-7.62	1.3
Cd37	4.6	2.14	0.16	0.17	-4.89	-3.63	1.3
Tmprss11d	3.17	1.33	0.1	0.1	-4.99	-3.73	1.3
Tec	2.48	1.31	0.31	0.39	-2.99	-1.75	1.2
Arhgap30	4.36	1.51	0.32	0.26	-3.77	-2.53	1.2
Cdh1	175.73	146.84	7.73	15.18	-4.51	-3.27	1.2
Gpx2	5.33	1.92	0.12	0.11	-5.42	-4.19	1.2
Zcwpw1	25.12	36.65	5.13	17.51	-2.29	-1.07	1.2
Kank4	2.1	1.13	0.33	0.4	-2.69	-1.49	1.2
Dtx4	2.84	2.05	0.62	1.01	-2.2	-1.02	1.2
Stk38l	10.11	10.78	1.88	4.51	-2.43	-1.26	1.2
Hist3h2ba	48.96	69.51	5.74	18.35	-3.09	-1.92	1.2
Ocln	6.22	3.99	0.61	0.88	-3.35	-2.18	1.2
Prrg4	38.82	33.51	1.58	3.04	-4.62	-3.46	1.2
Atp1b1	29.13	22.45	0.93	1.59	-4.97	-3.82	1.2
Cdkn1c	108.1	40.99	17.23	14.43	-2.65	-1.51	1.1
Mtus1	8.74	8.47	1.77	3.77	-2.3	-1.17	1.1
Col18a1	137.7	150.6	19.11	45.67	-2.85	-1.72	1.1
Ppp1r1a	14.24	16.27	1.83	4.58	-2.96	-1.83	1.1
Mov10l1	2.6	2.58	0.17	0.36	-3.97	-2.84	1.1
Dtna	6.4	6.78	0.25	0.58	-4.66	-3.53	1.1
Cd109	3.79	3.74	0.82	1.77	-2.2	-1.08	1.1
Syk	8.33	6	1.45	2.24	-2.53	-1.42	1.1
Zbbx	1.84	1.21	0.1	0.14	-4.21	-3.1	1.1
Tex14	2.14	1.88	0.3	0.55	-2.85	-1.76	1.1
Mcam	18.1	20.71	2.18	5.29	-3.06	-1.97	1.1
Syt13	2.58	1.76	0.3	0.44	-3.1	-2.01	1.1
Palm3	17.15	17.48	0.61	1.32	-4.82	-3.73	1.1
Slain1	13.03	11.27	1.47	2.68	-3.15	-2.07	1.1
Rasgrp2	15.62	13.49	1.15	2.11	-3.76	-2.68	1.1
Tdh	295.87	257.45	1.54	2.84	-7.58	-6.5	1.1
Kcnj3	5.55	5.64	1.31	2.8	-2.08	-1.01	1.1
Bcl3	13.37	11.46	0.39	0.69	-5.11	-4.04	1.1
Slc5a11	5.23	2.92	0.14	0.17	-5.18	-4.11	1.1
Arhgef3	10.98	8.63	1.65	2.69	-2.73	-1.68	1.1
Syce1	10.22	8.71	0.21	0.37	-5.61	-4.56	1.1
Bmp8a	3.02	1.62	0.23	0.25	-3.72	-2.68	1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Mfap5	1.59	1.99	0.12	0.3	-3.76	-2.72	1.0
Hist1h2aa	13.62	12.65	0.1	0.19	-7.09	-6.05	1.0
Tcte2	2.88	2.11	0.59	0.88	-2.29	-1.26	1.0
Pla2g5	3.11	2.73	0.17	0.31	-4.18	-3.15	1.0
Tdrd12	13.51	33.42	0.39	1.98	-5.11	-4.08	1.0
Cbx7	48.7	59.14	7.87	19.4	-2.63	-1.61	1.0
Ccdc125	3.63	3.59	0.48	0.96	-2.92	-1.9	1.0
Tbc1d2	3.6	2.53	0.31	0.43	-3.55	-2.54	1.0
Robo4	4.76	4.24	0.35	0.62	-3.78	-2.77	1.0
Eppk1	3.41	2.34	0.21	0.29	-4.01	-3	1.0
Fam178b	5.84	6.44	0.27	0.59	-4.45	-3.44	1.0
Hook1	41.05	31.04	5.28	7.96	-2.96	-1.96	1.0
Coro1a	7.61	6.38	0.9	1.5	-3.09	-2.09	1.0
Bdh2	6.98	6.7	0.65	1.26	-3.42	-2.42	1.0
Larp1b	12.47	13.2	2.53	5.29	-2.3	-1.32	1.0
Syt11	11.72	15.83	1.94	5.2	-2.59	-1.61	1.0
Mcf2	5.1	3.17	0.17	0.21	-4.92	-3.94	1.0
Afap1l2	1.51	1.4	0.35	0.64	-2.1	-1.13	1.0
Nbeal2	3.29	2.89	0.71	1.22	-2.21	-1.24	1.0
Lgals9	5.61	4.97	0.49	0.86	-3.51	-2.54	1.0
Fam129a	20.7	8.75	0.33	0.27	-5.98	-5.01	1.0
Plk2	38.17	39.34	5.52	11.03	-2.79	-1.83	1.0
Syngr1	23.57	22.49	1.76	3.26	-3.75	-2.79	1.0
Dppa3	11.58	32.79	0.19	1.05	-5.92	-4.96	1.0
Ccdc88c	16.57	14.43	1.72	2.89	-3.27	-2.32	1.0
Camk1d	8.56	4.36	0.4	0.4	-4.41	-3.46	1.0
Lamc2	11.57	13.85	0.5	1.15	-4.54	-3.59	1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
3830417A13Rik	3.07	1.86	0.19	1.57	-4.03	-0.25	3.8
Cd34	3.04	1.14	0.46	1.97	-2.73	0.79	3.5
Rhox5	50.29	149.14	8.51	183	-2.56	0.3	2.9
Krt7	3.32	3.07	0.5	3	-2.73	-0.03	2.7
Thsd1	2.06	1.21	0.7	2.12	-1.55	0.8	2.4
Btg3	5.43	3.07	1.51	4.32	-1.84	0.49	2.3
Esam	10.23	8.65	2.46	9.64	-2.06	0.16	2.2
Aim2	11.99	13.47	1.8	9.19	-2.73	-0.55	2.2
Plvap	2.33	2.06	0.77	3.07	-1.59	0.57	2.2
Cntfr	7.52	2.88	1.29	2.2	-2.54	-0.39	2.2
Rec8	4.02	2.13	0.69	1.61	-2.54	-0.4	2.1
Dysf	5.74	1.99	1.96	2.96	-1.55	0.58	2.1
Pim2	24.37	16.63	4.33	12.03	-2.49	-0.47	2.0
Edn1	1.89	1.7	0.26	0.94	-2.88	-0.86	2.0
Fmr1nb	5.1	8.85	1.14	7.73	-2.16	-0.2	2.0
Sfrp1	7.65	5.74	2.33	6.64	-1.71	0.21	1.9
Sh3tc1	2.23	1.01	0.54	0.92	-2.05	-0.13	1.9
Nuak2	1.95	1.74	0.52	1.67	-1.91	-0.06	1.9
Sepp1	19.75	20.56	9.88	34.14	-1	0.73	1.7
Map4k2	3.94	2.84	1.91	4.55	-1.05	0.68	1.7
Stmn3	5.14	2.04	0.83	1.06	-2.64	-0.94	1.7
Bst2	19.9	13.89	6.49	13.92	-1.62	0	1.6
Gatsl2	2.34	1.18	0.64	0.97	-1.88	-0.28	1.6
Plek2	3.97	2.25	0.84	1.42	-2.25	-0.66	1.6
Ltb	4.37	2.6	0.85	1.5	-2.36	-0.79	1.6
Gap43	4.26	4.65	1.15	3.67	-1.89	-0.34	1.6

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Tek	7	7.05	1.97	5.6	-1.83	-0.33	1.5
Specc1	6.14	4.58	2.41	4.97	-1.35	0.12	1.5
Dnah8	7.62	1.87	1.69	1.15	-2.17	-0.71	1.5
Trh	153.88	176.14	39.6	119.66	-1.96	-0.56	1.4
Card10	2.62	1.26	0.99	1.23	-1.4	-0.03	1.4
Plcg2	8.36	4.66	2.22	3.2	-1.91	-0.54	1.4
4930447C04Rik	10.24	14.35	2.16	7.87	-2.24	-0.87	1.4
Sptb	2.91	1.15	0.91	0.92	-1.68	-0.33	1.4
Slc6a1	3.38	1.11	0.8	0.67	-2.08	-0.73	1.4
Tnfsfm13	3.84	1.35	0.96	0.85	-2	-0.66	1.3
Lyn	3.47	2.2	1.67	2.64	-1.06	0.26	1.3
Adgrg1	1.27	1.3	0.39	0.97	-1.73	-0.42	1.3
Pik3r3	3.35	2.92	0.93	2.02	-1.84	-0.53	1.3
Tram2	4.44	3.01	2.05	3.35	-1.12	0.15	1.3
Trp53i11	15.4	12.31	5.06	9.65	-1.61	-0.35	1.3
Epha1	13.13	7.89	5.5	7.75	-1.26	-0.03	1.2
Dab1	19.55	10.89	6.32	8.24	-1.63	-0.4	1.2
Npnt	1.54	1.04	0.35	0.56	-2.14	-0.91	1.2
Prkcb	1.58	1.16	0.69	1.18	-1.18	0.02	1.2
C130074G19Rik	1.01	1	0.29	0.66	-1.78	-0.6	1.2
Rsg1	1.92	1.06	0.84	1.03	-1.19	-0.03	1.2
Lfng	8.53	7.22	3.48	6.41	-1.29	-0.17	1.1
Arhgef28	2.65	4.24	0.79	2.7	-1.75	-0.65	1.1
Fam198b	4.04	6.53	1.08	3.76	-1.9	-0.8	1.1
Ica1	5.4	4.98	2.29	4.49	-1.24	-0.15	1.1
Qprt	7.79	3.48	3.32	3.14	-1.23	-0.15	1.1
Enc1	51.4	39.93	15.23	24.97	-1.76	-0.68	1.1
Lrp11	15.59	13.9	7	13.12	-1.15	-0.08	1.1
Dclk2	5.32	4.74	2.58	4.76	-1.05	0.01	1.1
Wfdc2	15.4	11.36	4.69	7.21	-1.72	-0.66	1.1
Als2cl	4.34	2.63	1.37	1.72	-1.66	-0.61	1.1
Nudt12	6.24	2.95	2.73	2.64	-1.19	-0.16	1.0
Npr1	6.55	5.49	2.67	4.54	-1.29	-0.27	1.0
Rasl11a	6.7	4.96	3.19	4.76	-1.07	-0.06	1.0
5730507C01Rik	3.1	2.69	1.19	2.09	-1.37	-0.36	1.0
Pkp2	21.92	17.96	5.92	9.76	-1.89	-0.88	1.0
Ptrf	15.52	12.33	6.23	9.72	-1.32	-0.34	1.0
Mgat4a	4.9	4.85	1.83	3.55	-1.42	-0.45	1.0
Slc25a23	2.35	1.78	0.86	1.28	-1.45	-0.48	1.0
Ptrh1	5.51	4.1	1.7	2.5	-1.69	-0.72	1.0
Unc13a	1.38	1.36	0.42	0.82	-1.7	-0.74	1.0
Slitrk5	4.48	3.75	1.34	2.17	-1.75	-0.79	1.0
Vill	2.96	1.41	1.31	1.2	-1.18	-0.23	1.0
Fam111a	23.82	13.39	11.34	12.13	-1.07	-0.14	0.9
Abca3	2.52	2.52	1.08	2.06	-1.22	-0.29	0.9
Nudt18	1.2	1.1	0.45	0.79	-1.4	-0.48	0.9
Cnp	9.35	5.3	4.8	5.13	-0.96	-0.05	0.9
Rab3d	2.51	1.69	0.81	1.02	-1.63	-0.72	0.9
Sntb1	2.68	1.96	0.86	1.18	-1.63	-0.72	0.9
Pde4b	1	1.2	0.51	1.14	-0.97	-0.07	0.9
Dock4	4.48	4.37	2.09	3.79	-1.1	-0.2	0.9
Rasip1	4.74	5.87	2.01	4.66	-1.23	-0.33	0.9
Bcl6b	4.96	3.94	1.75	2.58	-1.51	-0.61	0.9
Kif5c	11.26	12.54	5.11	10.52	-1.14	-0.25	0.9
Sdr39u1	1.45	1.65	0.59	1.25	-1.29	-0.4	0.9
Rasef	3.49	2.9	1.39	2.16	-1.32	-0.43	0.9
Mtm1	3.01	2.89	1.14	2.03	-1.4	-0.51	0.9
Zfhx2	2.93	2	0.9	1.13	-1.71	-0.82	0.9
Nes	22.25	16.72	8.98	12.44	-1.31	-0.43	0.9
Celsr2	2.19	2.09	0.65	1.14	-1.75	-0.87	0.9

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Rassf3	18.29	12.92	5.76	7.38	-1.67	-0.81	0.9
Lmbr1	3.78	3.09	1.91	2.81	-0.99	-0.14	0.9
Prmt8	1.46	1.39	0.73	1.23	-1.01	-0.17	0.8
Lekr1	1.21	1.15	0.44	0.73	-1.47	-0.66	0.8
9930012K11Rik	10.59	7.49	3.22	3.98	-1.72	-0.91	0.8
Cadps2	2.06	1.25	1.06	1.12	-0.96	-0.16	0.8
4930432K21Rik	2.1	2.29	0.8	1.51	-1.4	-0.6	0.8
Moap1	4.88	3.87	2.42	3.3	-1.01	-0.23	0.8
Rbm12b1	3.97	3.23	1.43	1.99	-1.48	-0.7	0.8
Hspa4l	3.76	4.17	1.32	2.51	-1.51	-0.73	0.8
Samd9l	1.42	2.36	0.49	1.38	-1.55	-0.77	0.8
Dnajb5	8.17	7.44	2.61	4.09	-1.64	-0.86	0.8
Ankrd33b	2.52	1.14	1.11	0.85	-1.18	-0.42	0.8
Ddx3y	5.52	1.08	2.12	0.7	-1.38	-0.62	0.8
Rundc3b	1.94	2.21	0.67	1.29	-1.52	-0.77	0.8
9030624G23Rik	3.91	3.06	1.87	2.43	-1.07	-0.33	0.7
Nmrk1	3.55	4.5	1.49	3.14	-1.26	-0.52	0.7
Rfng	8.86	6.16	3.53	4.09	-1.33	-0.59	0.7
Dhrs11	3.66	3.68	1.28	2.15	-1.51	-0.77	0.7
Myo5b	1.5	1.14	0.52	0.66	-1.52	-0.78	0.7
Rltpr	1.77	1.2	0.58	0.66	-1.6	-0.86	0.7
Hvcn1	9.11	6.69	4.19	5.01	-1.12	-0.42	0.7
Sat1	42.34	28.19	20.59	22.18	-1.04	-0.35	0.7
Slc6a8	24.2	15.38	11.54	11.86	-1.07	-0.38	0.7
Uchl4	1.99	1.12	0.71	0.64	-1.5	-0.81	0.7
Nrgn	1.72	2.1	0.57	1.13	-1.59	-0.9	0.7
Gabrb3	3.09	2.11	1.59	1.72	-0.96	-0.29	0.7
Gabarapl2	61.54	45.11	30.77	35.77	-1	-0.33	0.7
Rdh10	5.77	4.89	2.79	3.69	-1.05	-0.4	0.7
Stmn2	33.61	38.07	13.4	23.82	-1.33	-0.68	0.7
Jmjd1c	69.51	58.86	32.08	42.24	-1.12	-0.48	0.6
Synrg	5.74	5.04	2.54	3.48	-1.17	-0.53	0.6
F11r	43.31	39.99	14.62	21.03	-1.57	-0.93	0.6
Spry2	97.93	70.59	43.56	48.39	-1.17	-0.54	0.6
Tbc1d9	7.17	6.65	3.61	5.13	-0.99	-0.37	0.6
3110002H16Rik	14.41	13.18	5.68	7.99	-1.34	-0.72	0.6
Fam129b	40.4	32.66	15.56	19.34	-1.38	-0.76	0.6
Syne1	2.36	2.01	1.17	1.51	-1.02	-0.41	0.6
Tmem19	12.8	13.69	5.3	8.65	-1.27	-0.66	0.6
Bik	1.73	1.72	0.61	0.92	-1.5	-0.89	0.6
Akr1b10	11.85	9.49	5.22	6.36	-1.18	-0.58	0.6
Procr	2.87	3.18	1.01	1.69	-1.51	-0.91	0.6
Hdac6	23.2	32.85	10.82	23.08	-1.1	-0.51	0.6
Kbtbd7	14.57	13.15	6.01	8.15	-1.28	-0.69	0.6
Synj2	3.98	3.87	1.88	2.73	-1.08	-0.5	0.6
Sorl1	7.44	6.67	3.45	4.62	-1.11	-0.53	0.6
Rtn4ip1	6.46	5.56	2.86	3.69	-1.17	-0.59	0.6
Nfib	3.62	1.74	1.55	1.11	-1.22	-0.64	0.6
Isyna1	73.82	53.3	31.08	33.51	-1.25	-0.67	0.6
G3bp2	162.39	118.91	66.73	73.35	-1.28	-0.7	0.6
Amt	41.32	34.01	18.75	22.89	-1.14	-0.57	0.6
Ehmt2	83.96	57.75	36.69	37.67	-1.19	-0.62	0.6
Arhgap44	3.01	2.42	1.3	1.55	-1.21	-0.64	0.6
Rab15	9.17	5.01	3.42	2.77	-1.42	-0.85	0.6
Abhd3	1.66	1.05	0.61	0.57	-1.45	-0.89	0.6
Gbe1	9.18	5.38	4.25	3.65	-1.11	-0.56	0.6
Clip4	4.09	3.98	1.62	2.3	-1.34	-0.79	0.6
Igsf9	13.87	11.95	5.47	6.93	-1.34	-0.79	0.6
Fam222a	1.25	1.05	0.48	0.59	-1.37	-0.82	0.6
Cib1	25.83	22.96	9.82	12.76	-1.4	-0.85	0.6

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Frem2	9.7	10.16	4.78	7.3	-1.02	-0.48	0.5
B4galt4	4.12	2.09	1.85	1.37	-1.15	-0.61	0.5
Pitpnc1	24.31	16.92	9.68	9.79	-1.33	-0.79	0.5
AI464131	2.35	2.92	0.91	1.65	-1.36	-0.82	0.5
Sec24a	10.34	9.4	5.32	6.96	-0.96	-0.43	0.5
Aktip	16.69	18.52	8.39	13.44	-0.99	-0.46	0.5
Znfx1	7.51	10.62	3.48	7.09	-1.11	-0.58	0.5
Gsap	2.42	1.39	1	0.83	-1.27	-0.74	0.5
Dtx3l	10.13	9.93	3.98	5.58	-1.35	-0.83	0.5
Celsr3	2.01	2.18	0.73	1.14	-1.46	-0.94	0.5
Slc31a2	8.48	8.02	3.63	4.86	-1.23	-0.72	0.5
Lig4	6.43	5.23	2.68	3.08	-1.27	-0.76	0.5
Cfap74	3.55	2.97	1.41	1.67	-1.34	-0.83	0.5

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Igfbp7	2.32	9.86	0.22	0.15	-3.42	-6.03	-2.6
Inhba	1.27	6.51	0.13	0.11	-3.32	-5.89	-2.6
Lox	1.56	13.45	0.13	0.2	-3.64	-6.09	-2.5
Trim47	1.52	6.94	0.38	0.32	-2.01	-4.42	-2.4
Cav1	2.71	13.74	0.41	0.41	-2.74	-5.08	-2.3
Ptgs2	1.82	9.01	0.29	0.28	-2.67	-4.99	-2.3
Sigirr	13.12	8.96	1.38	0.21	-3.25	-5.39	-2.1
Timp1	29.16	45.4	6.62	2.61	-2.14	-4.12	-2.0
Bcl2l14	1.09	1	0.46	0.12	-1.24	-3.1	-1.9
Grem1	1.37	5	0.24	0.24	-2.54	-4.4	-1.9
Pga5	1	2.84	0.31	0.24	-1.69	-3.54	-1.9
Tnk1	3.35	3.29	0.84	0.23	-1.99	-3.83	-1.8
Celf4	5.21	5.57	1.23	0.36	-2.09	-3.93	-1.8
Dppa5a	1558.91	1102.11	31.85	6.4	-5.61	-7.43	-1.8
Rsph1	1.37	2.51	0.65	0.34	-1.08	-2.89	-1.8
Neurog3	1.66	4.55	0.13	0.1	-3.71	-5.49	-1.8
Gjb1	1.69	5.92	0.1	0.1	-4.08	-5.86	-1.8
Naaa	2.85	7.04	1.04	0.76	-1.45	-3.21	-1.8
Tcfl5	25.17	14.54	4.97	0.85	-2.34	-4.1	-1.8
Chmp4c	10.49	10.68	2.98	0.91	-1.81	-3.55	-1.7
Raet1a	13.48	14.22	0.95	0.31	-3.82	-5.52	-1.7
Pla2g4a	1.75	3.23	0.3	0.18	-2.54	-4.18	-1.6
Anxa8	2.55	4.98	0.18	0.11	-3.82	-5.44	-1.6
Bnipl	11.39	8.89	1.74	0.45	-2.71	-4.3	-1.6
Slco4c1	4.23	10.79	0.26	0.22	-4.05	-5.62	-1.6
Rbp7	4.81	4.01	1.36	0.39	-1.82	-3.38	-1.6
Calcoco2	4.51	9.4	0.16	0.11	-4.82	-6.38	-1.6
Rab27a	6.66	14.13	2.39	1.73	-1.48	-3.03	-1.6
Apoc1	28.06	24.72	5.68	1.72	-2.31	-3.85	-1.5
Nkx2-9	2.23	5.44	0.14	0.11	-4.03	-5.57	-1.5
S100a4	8.57	58.94	0.16	0.38	-5.75	-7.29	-1.5
Rln3	1.08	2	0.21	0.14	-2.35	-3.87	-1.5
Mia	3.25	3.55	0.63	0.24	-2.38	-3.88	-1.5
Col9a2	3.33	5.06	0.4	0.22	-3.06	-4.54	-1.5
Tekt1	1.1	1.55	0.25	0.13	-2.14	-3.61	-1.5
4930519F16Rik	2.49	3.42	0.21	0.1	-3.59	-5.05	-1.5
Tmem40	4.22	10.3	0.41	0.37	-3.36	-4.81	-1.5
Tex21	2.79	4.05	0.33	0.18	-3.07	-4.51	-1.4
Cpne5	3.95	10.14	1.08	1.04	-1.87	-3.29	-1.4
Cbs	6.16	3.69	1.66	0.37	-1.89	-3.31	-1.4

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Steap3	5.46	9.05	0.42	0.26	-3.71	-5.13	-1.4
Upk1a	1.54	2.13	0.66	0.34	-1.22	-2.63	-1.4
Glod5	5.06	11.56	0.14	0.12	-5.18	-6.59	-1.4
Phf19	3.14	4.95	0.9	0.54	-1.81	-3.21	-1.4
Ceacam1	8.97	6.01	1.97	0.52	-2.19	-3.54	-1.4
Dnajc6	5.43	11.35	0.19	0.16	-4.82	-6.17	-1.4
D630023F18Rik	4.19	9.31	0.34	0.3	-3.62	-4.94	-1.3
Zfp750	1.39	3.09	0.16	0.14	-3.11	-4.42	-1.3
Gsn	8.49	13.7	3.66	2.41	-1.21	-2.51	-1.3
2610305D13Rik	114.23	100.3	9.38	3.33	-3.61	-4.91	-1.3
Smox	3.47	8.21	1.35	1.31	-1.37	-2.65	-1.3
Notum	17.56	19.95	2.35	1.1	-2.9	-4.18	-1.3
Stac2	1.76	2.97	0.82	0.57	-1.11	-2.37	-1.3
Plcxd1	3.71	5.36	0.87	0.52	-2.1	-3.36	-1.3
Ccno	3.19	3.11	0.71	0.29	-2.17	-3.43	-1.3
Fcgrt	2.47	3.56	0.24	0.14	-3.37	-4.63	-1.3
Hecw2	2.14	6.04	0.6	0.72	-1.84	-3.06	-1.2
Tmem45a	2.2	2.02	0.57	0.22	-1.96	-3.18	-1.2
Tlr2	5.51	6.45	1.8	0.92	-1.62	-2.81	-1.2
Anxa11	21.88	22.2	5.62	2.5	-1.96	-3.15	-1.2
Glrx	6.92	8.36	1.32	0.7	-2.39	-3.58	-1.2
Plekha4	1.51	2.57	0.38	0.29	-1.98	-3.16	-1.2
A830018L16Rik	1.65	2.75	0.35	0.26	-2.25	-3.43	-1.2
Myo5c	1.62	1.95	0.37	0.2	-2.13	-3.3	-1.2
Ddit4l	3.92	3.23	0.88	0.32	-2.16	-3.33	-1.2
Plau	1.24	2.82	0.4	0.41	-1.63	-2.78	-1.2
Calcr	1.79	2.22	0.49	0.27	-1.87	-3.02	-1.2
Fbxo2	6.97	10.24	0.76	0.5	-3.19	-4.34	-1.2
Tert	5.46	4.17	2.16	0.76	-1.33	-2.46	-1.1
Mok	5.06	8.52	1.91	1.47	-1.41	-2.54	-1.1
Fzd9	1.44	1.44	0.39	0.18	-1.88	-3.01	-1.1
Gjb3	24.41	37.73	2.62	1.84	-3.22	-4.35	-1.1
Tmem102	2.01	2.05	0.71	0.33	-1.51	-2.63	-1.1
Fam83g	2.8	1.51	1.08	0.27	-1.38	-2.49	-1.1
Sdc4	22.96	50.47	7.08	7.19	-1.7	-2.81	-1.1
Ass1	19.68	24.02	4.67	2.65	-2.07	-3.18	-1.1
Ggnbp1	2.5	4.7	0.79	0.71	-1.65	-2.73	-1.1
Cpsf4l	13.36	34.98	0.43	0.53	-4.96	-6.04	-1.1
Sptbn2	8.14	16.85	0.88	0.87	-3.21	-4.28	-1.1
Celf5	1.94	1.79	0.6	0.27	-1.69	-2.75	-1.1
C030039L03Rik	2.2	4.34	1.08	1.03	-1.02	-2.07	-1.1
Arl5c	1.25	1.66	0.33	0.21	-1.92	-2.97	-1.1
Rims1	2.08	4.24	0.32	0.31	-2.71	-3.76	-1.1
Htatip2	1.32	1.73	0.18	0.12	-2.85	-3.89	-1.0
Sgol2b	1.27	2.66	0.1	0.1	-3.66	-4.7	-1.0
Papolb	1.44	2.13	0.58	0.42	-1.32	-2.35	-1.0
Serping1	4.74	7.28	0.98	0.74	-2.27	-3.3	-1.0
Ckb	106.41	205.42	17.4	16.63	-2.61	-3.63	-1.0
Tmem51	6.92	5.31	2.65	1.01	-1.38	-2.39	-1.0
Pdk4	1.95	3.5	0.55	0.49	-1.84	-2.84	-1.0
Mpzl2	11.9	11.4	1.46	0.7	-3.03	-4.03	-1.0
Sox15	9.73	11.14	0.7	0.4	-3.79	-4.79	-1.0
Gtsf1l	38.07	31.46	0.37	0.15	-6.7	-7.7	-1.0
Prr19	4.23	2.87	0.59	0.2	-2.83	-3.82	-1.0
Hist1h2ba	8.13	8.85	0.37	0.2	-4.47	-5.46	-1.0
2200002D01Rik	18.63	13.45	4.06	1.48	-2.2	-3.18	-1.0
Ap1m2	29.57	29.12	4.04	2.02	-2.87	-3.85	-1.0
Aifm2	5.97	4.67	0.81	0.32	-2.88	-3.86	-1.0
Ephx1	2.96	4.15	0.29	0.2	-3.36	-4.34	-1.0
Msc	5.9	10.49	0.11	0.1	-5.72	-6.7	-1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Alcam	1.18	1.9	0.38	0.32	-1.62	-2.59	-1.0
Prss23	4.31	14.06	0.69	1.16	-2.63	-3.6	-1.0
Ckmt1	5.71	7.29	0.67	0.44	-3.08	-4.05	-1.0
Tspan17	2.23	1.88	0.79	0.34	-1.5	-2.46	-1.0
AI467606	6.13	4.79	1.03	0.41	-2.58	-3.54	-1.0
Abcb1b	20.26	20.22	2.13	1.1	-3.25	-4.2	-1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
E030030I06Rik	1.16	4.92	1.17	0.45	0.01	-3.45	-3.5
Aspg	2.86	1.7	3.68	0.26	0.36	-2.72	-3.1
Tmem159	4.53	3.3	5.66	0.6	0.32	-2.46	-2.8
Hist1h4j	5.56	8.09	4.99	1.3	-0.16	-2.64	-2.5
Bgn	3.46	15.2	3.4	2.96	-0.02	-2.36	-2.3
Sycp3	12.44	9.19	10.33	1.58	-0.27	-2.54	-2.3
Smim20	15.29	10.56	14.69	2.34	-0.06	-2.17	-2.1
Mgmt	13.53	5.75	7.85	0.78	-0.79	-2.88	-2.1
Prdm14	3.01	7.21	1.75	0.99	-0.78	-2.86	-2.1
Tcaf2	2.48	1.25	2.39	0.29	-0.05	-2.12	-2.1
Neurl1a	3.71	4.42	4.71	1.35	0.34	-1.71	-2.1
Fam184b	1.33	1.1	1.03	0.21	-0.36	-2.4	-2.0
Ctsz	34.51	28.2	59.5	12.13	0.79	-1.22	-2.0
Efemp1	1.36	2.19	2.08	0.86	0.61	-1.35	-2.0
H60b	2.64	6.23	3.17	1.93	0.26	-1.69	-2.0
Klk8	12.48	9.22	10.02	1.92	-0.32	-2.27	-2.0
Selm	9.02	10.28	9.17	2.83	0.02	-1.86	-1.9
Csf1	1.89	8.25	2	2.42	0.08	-1.77	-1.9
Gm20594	2.92	5.29	2.95	1.51	0.02	-1.81	-1.8
Galnt12	3.76	3.31	4.85	1.26	0.37	-1.39	-1.8
Trim67	1.39	2.41	1.03	0.53	-0.42	-2.18	-1.8
Insl6	1.24	1.02	0.98	0.24	-0.34	-2.08	-1.7
Hddc2	26.86	28.78	42.82	14.36	0.67	-1	-1.7
Smim22	1.3	1.45	1.19	0.42	-0.12	-1.79	-1.7
Wdr86	4.36	3.19	3.05	0.72	-0.52	-2.15	-1.6
Aldoc	5.01	3.27	6.44	1.38	0.36	-1.24	-1.6
Nrk	2.65	4.41	2.78	1.53	0.07	-1.53	-1.6
Prelid2	9.49	2.62	10.23	0.94	0.11	-1.48	-1.6
Hist2h3b	31.25	32.63	38.87	13.83	0.31	-1.24	-1.6
Cenpm	13.45	22.09	10.51	5.92	-0.36	-1.9	-1.5
Raet1e	2.34	2.32	2.61	0.89	0.15	-1.38	-1.5
Tex15	3.88	3.32	2.69	0.8	-0.53	-2.06	-1.5
B230217C12Rik	1.87	2.91	2.27	1.29	0.28	-1.18	-1.5
Psors1c2	2.46	2.8	1.35	0.59	-0.86	-2.24	-1.4
Rbfa	25.37	16.9	17.2	4.44	-0.56	-1.93	-1.4
Mkx	5.84	7.32	3.73	1.8	-0.65	-2.02	-1.4
Fgfr2	9.88	13.66	9.29	5.01	-0.09	-1.45	-1.4
Ghdc	4.32	2.69	3.1	0.76	-0.48	-1.83	-1.4
Alpl	23.96	28.25	23.64	11.01	-0.02	-1.36	-1.3
Gsto1	29.73	34.09	29.03	13.45	-0.03	-1.34	-1.3
Radil	2.57	3.45	2.09	1.15	-0.3	-1.59	-1.3
Gng10	20.81	14.32	16.83	4.76	-0.31	-1.59	-1.3
Pkd2	13.21	11.24	12.25	4.36	-0.11	-1.37	-1.3
Gstt3	6.76	5.73	4.39	1.57	-0.62	-1.87	-1.3
Lefty2	58.4	157.91	33.38	38.24	-0.81	-2.05	-1.2
Qpct	1.61	1.52	1.06	0.43	-0.6	-1.83	-1.2
Myl9	14.47	27.24	11.13	8.99	-0.38	-1.6	-1.2

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Dnaaf3	1.24	2.13	1	0.74	-0.31	-1.52	-1.2
Gulo	1.98	2.81	1.34	0.82	-0.57	-1.78	-1.2
Fam58b	9.45	9.09	11.17	4.69	0.24	-0.95	-1.2
F3	7.69	8.43	5.77	2.8	-0.41	-1.59	-1.2
Gprc5b	5.04	8.86	3.51	2.75	-0.52	-1.69	-1.2
Serpinb6a	50.87	60.87	34.56	18.36	-0.56	-1.73	-1.2
Top1mt	5.03	3.45	5.39	1.7	0.1	-1.02	-1.1
Lmo7	10.44	11.22	8.38	4.12	-0.32	-1.44	-1.1
Zbtb7c	2.47	2.51	1.59	0.75	-0.63	-1.75	-1.1
Nbl1	1.42	1.86	0.81	0.49	-0.81	-1.92	-1.1
Mtmr11	1.61	1.22	1.17	0.41	-0.47	-1.56	-1.1
Tdrd5	2.74	3.12	1.85	0.99	-0.57	-1.66	-1.1
Lipt1	6.38	6.39	4.42	2.11	-0.53	-1.6	-1.1
Dok1	5.82	7.06	4.24	2.47	-0.46	-1.52	-1.1
Axl	2.76	6.44	1.81	2.04	-0.61	-1.66	-1.1
Agpat2	18.77	12.56	16.01	5.21	-0.23	-1.27	-1.0
Acbd4	5.19	4.87	3.32	1.51	-0.65	-1.69	-1.0
Myof	5.34	10.18	2.81	2.61	-0.93	-1.96	-1.0
Crtap	22.03	15.59	11.85	4.16	-0.89	-1.9	-1.0
Dcxr	9.68	9.09	8.76	4.11	-0.14	-1.14	-1.0
Cr1l	15.78	16.7	14.23	7.5	-0.15	-1.15	-1.0
Echdc2	22.94	24.79	23.18	12.86	0.01	-0.95	-1.0
Fkbp9	66.57	54.63	60.63	25.74	-0.13	-1.09	-1.0
Mta3	43.67	50.5	30.16	18.13	-0.53	-1.48	-1.0
Ccdc102a	6.78	6.64	4.65	2.36	-0.54	-1.49	-1.0
Cpne8	3.23	2.22	2.04	0.73	-0.66	-1.61	-1.0
Bola1	14.8	14.91	14.55	7.64	-0.02	-0.96	-0.9
Mpp1	13.3	16.36	10.79	6.94	-0.3	-1.24	-0.9
Cyp2s1	4.72	2.5	4.3	1.2	-0.14	-1.07	-0.9
Hyal1	2.56	2.57	1.85	0.98	-0.47	-1.39	-0.9
Hist1h2af	30.49	27.74	19.51	9.38	-0.64	-1.56	-0.9
Sp1	54.8	42.84	43.29	18.22	-0.34	-1.23	-0.9
Stx3	24.33	23.87	15.79	8.43	-0.62	-1.5	-0.9
Bbc3	7.06	9.48	5.11	3.74	-0.47	-1.34	-0.9
Adcy1	3.64	4.76	2.57	1.85	-0.5	-1.36	-0.9
Tefm	17.66	17.29	14	7.58	-0.34	-1.19	-0.9
Nhej1	10.69	12.34	7.24	4.65	-0.56	-1.41	-0.9
C030006K11Rik	3.91	3.53	2.28	1.15	-0.77	-1.62	-0.9
Urgcp	4.01	6.06	3.54	3	-0.18	-1.02	-0.8
Gnat2	1.4	1.4	1	0.56	-0.48	-1.32	-0.8
Syce2	63.65	48.7	40.4	17.42	-0.66	-1.48	-0.8
Fancf	2.45	2.69	1.89	1.2	-0.37	-1.17	-0.8
Ntpcr	8.75	6.92	6.65	3.03	-0.4	-1.19	-0.8
Spock2	1.46	3.23	1.04	1.33	-0.5	-1.28	-0.8
Rnh1	42.46	35.73	28.97	14.26	-0.55	-1.33	-0.8
Tnip2	3.34	4.11	1.89	1.36	-0.82	-1.6	-0.8
Dusp28	1.97	2.23	1.33	0.89	-0.57	-1.33	-0.8
Slc1a3	9.11	10.09	5.64	3.72	-0.69	-1.44	-0.8
Lpar1	4.17	6.11	2.64	2.35	-0.66	-1.38	-0.7
Ficd	1.54	2	1.11	0.89	-0.47	-1.17	-0.7
Ecm1	1.67	3.3	0.97	1.19	-0.78	-1.47	-0.7
2010107G23Rik	2.94	5.08	1.56	1.67	-0.91	-1.6	-0.7
Dtd2	6.17	6.15	4.95	3.07	-0.32	-1	-0.7
Npepl1	9.58	9.53	7.53	4.67	-0.35	-1.03	-0.7
Pon2	10.06	11.18	7.13	4.93	-0.5	-1.18	-0.7
Tarsl2	5.95	5.9	3.18	1.97	-0.9	-1.58	-0.7
B4galnt1	4.87	3.75	3.23	1.57	-0.59	-1.26	-0.7
Lrpap1	31.75	43.82	18.56	16.31	-0.77	-1.43	-0.7
Atp5sl	26.49	28.46	20.12	13.77	-0.4	-1.05	-0.7
Fzd6	3.83	4	2.75	1.83	-0.48	-1.13	-0.7

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Ggact	3.3	5.14	2.33	2.32	-0.5	-1.15	-0.7
Phgdh	53.47	67.39	42.85	34.57	-0.32	-0.96	-0.6
Tmem160	17.16	16.5	13.68	8.4	-0.33	-0.97	-0.6
Zfp710	27.23	42.69	20.06	20.26	-0.44	-1.08	-0.6
Cradd	8.68	10.91	5.44	4.4	-0.67	-1.31	-0.6
Mtg2	13.01	14.87	7.69	5.64	-0.76	-1.4	-0.6
Mgst1	1.91	1.34	1.06	0.48	-0.85	-1.49	-0.6
Rbm41	12.71	17.85	9.09	8.27	-0.48	-1.11	-0.6
Hsd17b11	14.72	11.75	8.52	4.46	-0.79	-1.4	-0.6
Fbxl15	1.96	1.86	1.24	0.77	-0.67	-1.27	-0.6
Ptrhd1	2.88	4.28	1.65	1.62	-0.8	-1.4	-0.6
Dsg2	11.43	6.91	6.95	2.78	-0.72	-1.31	-0.6
Aprt	195.76	214.96	146.07	107.67	-0.42	-1	-0.6
Nudt19	20.27	22.23	14.22	10.43	-0.51	-1.09	-0.6
Prph	2.31	1.86	1.47	0.78	-0.66	-1.24	-0.6
Rps6ka4	3.18	1.53	1.74	0.56	-0.87	-1.45	-0.6
Prdm5	16.96	17.56	9.01	6.25	-0.91	-1.49	-0.6
Gstm1	10.52	14.09	8.09	7.29	-0.38	-0.95	-0.6
Tmem106a	1	1.07	0.62	0.45	-0.68	-1.25	-0.6
Bex4	63	74.2	34.89	27.82	-0.85	-1.42	-0.6
Tmem191c	1.02	1.31	0.53	0.46	-0.94	-1.51	-0.6
Trim46	1.85	1.76	1	0.64	-0.89	-1.45	-0.6
Ino80b	7.91	8.31	5.81	4.14	-0.45	-1	-0.6
Srebf1	14.41	16.75	9.5	7.54	-0.6	-1.15	-0.6
Chic1	10.91	10.84	6.48	4.42	-0.75	-1.3	-0.6
Aim1l	1.67	1.52	0.96	0.6	-0.81	-1.36	-0.6
Tmco4	1.29	1.8	0.84	0.8	-0.62	-1.16	-0.5
Pced1b	10.96	17.09	6.62	7.11	-0.73	-1.27	-0.5
Snx10	16.59	27.86	9.57	11.08	-0.79	-1.33	-0.5
Plekhf1	7.15	8.35	4.11	3.3	-0.8	-1.34	-0.5
BC022687	2.27	3.39	1.5	1.55	-0.6	-1.13	-0.5
Cryl1	5.98	10.04	3.67	4.27	-0.7	-1.23	-0.5
Zfp182	4.57	4.64	3.36	2.38	-0.44	-0.96	-0.5
4930452B06Rik	6.81	9.58	5	4.89	-0.45	-0.97	-0.5
Nr1d2	14.11	20.06	8.24	8.16	-0.78	-1.3	-0.5
Otulin	33.05	45.61	18	17.3	-0.88	-1.4	-0.5
Sp6	1.87	1.7	1.17	0.75	-0.67	-1.18	-0.5
Amd2	55.19	51.35	32.49	21.29	-0.76	-1.27	-0.5

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 6: downregulated in mutant/upregulated in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Col1a1	1.48	10.02	7.59	4.21	2.36	-1.25	-3.6
Col1a2	1.38	11.5	3.59	4.96	1.38	-1.21	-2.6
Fbln2	2.24	18.97	19.3	9.58	3.11	-0.98	-4.1
Loxl3	0.64	2.48	1.98	0.91	1.63	-1.45	-3.08
Mgll	0.51	1.39	1.23	0.66	1.28	-1.09	-2.37
Ppp1r14a	1.38	5.16	3.38	1.83	1.29	-1.5	-2.8

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Atp2a3	3.48	0.48	1.87	1.29	-0.9	1.41	2.3

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Mecom	1.01	0.59	1.25	3.44	0.31	2.55	2.2
Coro2b	1.96	0.62	1.16	1.72	-0.75	1.47	2.2
Ecscr	6.32	3.77	4.33	11.92	-0.55	1.66	2.2
Nfkbie	1.94	0.96	1.62	3.23	-0.26	1.75	2.0
Uty	4.06	0.58	3.26	1.8	-0.31	1.63	1.9
Gdpd5	1	0.54	1.75	3.56	0.8	2.72	1.9
Col8a2	1.06	0.46	1.68	2.75	0.67	2.58	1.9
Plet1	5.58	3.16	4.38	7.72	-0.35	1.29	1.6
Tal2	2.05	1.43	2.29	4.79	0.16	1.75	1.6
Satb1	5.04	2.16	6.78	8.58	0.43	1.99	1.6
Pcdh17	1.1	0.69	1.61	2.95	0.55	2.1	1.6
Sema7a	1.55	0.78	2.41	3.39	0.64	2.12	1.5
Xylt1	1.86	1.01	1.58	2.34	-0.24	1.22	1.5
Cnr1	0.84	0.85	1.29	3.55	0.62	2.06	1.4
Psmb9	2.1	0.88	2.29	2.47	0.12	1.49	1.4
Arnt2	1.73	0.92	2.03	2.73	0.23	1.56	1.3
Cib2	2.18	1.95	2.94	6.5	0.43	1.74	1.3
Bmf	2.49	1.59	2.34	3.65	-0.09	1.2	1.3
Slc9a7	1.34	0.51	2.09	1.93	0.65	1.93	1.3
Tnfsf9	1.64	0.73	1.54	1.66	-0.09	1.18	1.3
Adamts15	0.84	0.5	1.1	1.53	0.38	1.63	1.3
Morn1	1.74	0.75	2.45	2.39	0.49	1.66	1.2
Atf3	2.7	1.63	4.46	6.11	0.73	1.9	1.2
Bcl11a	1.71	0.54	3.14	2.22	0.88	2.05	1.2
Mapk12	5.09	3.85	6.91	11.7	0.44	1.6	1.2
Rassf2	1.64	1.06	2.23	3.23	0.45	1.61	1.2
Sema6a	3.57	2.54	5.42	8.5	0.6	1.74	1.1
Gpm6b	1.03	0.69	1.73	2.54	0.75	1.87	1.1
Tmem151b	1.26	0.91	1.22	1.91	-0.04	1.07	1.1
Ppp1r13b	3.64	3.44	3.65	7.32	0	1.09	1.1
Dapk1	17.55	13.29	31.49	49.83	0.84	1.91	1.1
Syne2	3.27	2.47	5.86	9.24	0.84	1.91	1.1
Trim34a	0.83	0.51	1.06	1.35	0.35	1.41	1.1
Ccdc112	2.47	1.98	3	4.96	0.28	1.33	1.1
Zkscan7	1.04	0.86	1.51	2.56	0.54	1.58	1.0
Txnip	71.88	72.93	80.45	164.94	0.16	1.18	1.0
Rnd2	11.48	5.76	17.35	17.52	0.6	1.61	1.0
Capn2	38.54	22.01	43.17	48.82	0.16	1.15	1.0
Akap5	1.09	1.19	1.93	4.18	0.83	1.82	1.0
Ssbp2	1.74	1.07	3.28	4.03	0.92	1.91	1.0
Ogfrl1	3.81	2.05	3.79	3.99	-0.01	0.96	1.0
Trib1	6.19	4.2	11.15	14.83	0.85	1.82	1.0
Itpkb	1.37	0.89	2.42	3.03	0.82	1.78	1.0
Asap2	4.82	3.12	8.75	11.04	0.86	1.82	1.0
Dusp18	1.48	1.21	1.87	2.93	0.33	1.27	0.9
Tm6sf1	0.95	0.82	1.61	2.62	0.75	1.68	0.9
Fhod3	1.43	0.56	2.72	2.04	0.93	1.86	0.9
Stat6	7.63	3.61	10.47	9.42	0.46	1.38	0.9
Col11a1	1.23	1.24	1.27	2.4	0.05	0.96	0.9
Has3	1.21	0.86	2.04	2.72	0.75	1.66	0.9
Celsr1	2.55	2.05	4.45	6.64	0.8	1.7	0.9
Aldh1a2	1.78	1.44	3.18	4.8	0.84	1.74	0.9
Car4	7.97	8.01	11.08	20.47	0.47	1.35	0.9
Rerg	0.73	0.43	1.14	1.25	0.65	1.52	0.9
Gbp2	10.11	7.24	15.09	19.7	0.58	1.44	0.9
Ttc39c	0.8	0.47	1.52	1.62	0.92	1.78	0.9
Cmklr1	1.56	1.24	2.19	3.13	0.49	1.34	0.9
Ppp2r2c	2.73	2.76	4.58	8.3	0.75	1.59	0.8
Tagln2	80.28	62.79	117.28	161.8	0.55	1.37	0.8
D830031N03Rik	6.45	5.25	9.67	13.74	0.58	1.39	0.8

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Ypel1	2.83	2.82	4.74	8.33	0.75	1.56	0.8
Farp2	0.96	0.53	1.79	1.74	0.9	1.71	0.8
Coq10a	1.65	1.28	2.05	2.76	0.31	1.11	0.8
Eepd1	1.43	1.01	2.2	2.7	0.63	1.42	0.8
Fam89a	1.87	1.07	2.33	2.29	0.32	1.1	0.8
Sort1	9.39	7.65	16.31	22.46	0.8	1.55	0.8
Bmp1	9.16	6.95	14.74	18.45	0.69	1.41	0.7
Zbtb42	0.62	0.45	1.09	1.3	0.82	1.54	0.7
Nrbp2	3.44	2.95	6.03	8.47	0.81	1.52	0.7
Ptpdc1	2.39	1.81	3.04	3.73	0.35	1.04	0.7
Tmod2	3.65	3.19	5.18	7.21	0.5	1.18	0.7
Panx1	5.11	3.4	9.24	9.84	0.85	1.53	0.7
Psmb10	28.78	20.05	37.67	41.86	0.39	1.06	0.7
Dpysl4	1.4	1.25	2.04	2.91	0.55	1.22	0.7
Fzd4	1.85	1.08	2.9	2.7	0.65	1.32	0.7
Slc35c1	2.51	1.34	3.53	2.99	0.5	1.16	0.7
Myb	2.09	1.57	3.74	4.45	0.84	1.5	0.7
Clip3	3.99	3.75	4.97	7.3	0.31	0.96	0.7
Ak4	9.62	6.04	14.53	14.28	0.59	1.24	0.7
Tmtc2	0.53	0.39	1.01	1.15	0.92	1.57	0.7
3110052M02Rik	0.88	0.53	1.49	1.39	0.75	1.39	0.6
Nckap5l	3.67	2.43	6.93	7.13	0.92	1.55	0.6
Armcx6	1.17	0.7	1.87	1.71	0.68	1.3	0.6
Murc	4.61	3.03	7.78	7.83	0.75	1.37	0.6
Oasl2	0.68	0.76	1.15	1.98	0.77	1.39	0.6
H2-D1	7.64	7.39	10.02	14.81	0.39	1	0.6
Kctd21	1.14	1.2	1.48	2.38	0.38	0.98	0.6
Zfp3	1.13	0.88	2.15	2.53	0.93	1.53	0.6
Rasgef1b	1.24	1.01	1.65	2.02	0.41	1	0.6
Tubb6	66.53	47.46	99.15	106.73	0.58	1.17	0.6
Mttp	2.39	1.59	3.62	3.61	0.6	1.18	0.6
Map3k1	10.87	7.42	17.93	18.3	0.72	1.3	0.6
Vat1	17.2	12.26	22.46	23.64	0.38	0.95	0.6
Tet3	3.78	2.94	5.61	6.49	0.57	1.14	0.6
Hdgfrp3	3.43	2.89	5.79	7.2	0.75	1.32	0.6
Src	13.52	10.12	17.85	19.69	0.4	0.96	0.6
Trib2	5.34	3.53	8.8	8.49	0.72	1.27	0.6
Dennd1a	3.94	2.98	6.72	7.39	0.77	1.31	0.5
Fam89b	11.21	8.84	15.15	17.23	0.43	0.96	0.5
Zfp69	2.32	2.12	3.18	4.18	0.45	0.98	0.5
Homez	1.51	1.18	2.27	2.58	0.59	1.12	0.5
Glcci1	2.31	2.44	3.53	5.37	0.61	1.14	0.5
Slc4a7	23.21	16.68	31.18	32.29	0.43	0.95	0.5
Myo6	2.26	2.43	3.29	5.07	0.54	1.06	0.5
Gngt2	10.69	8.39	14.68	16.41	0.46	0.97	0.5
Umad1	1.68	1.13	2.31	2.21	0.46	0.97	0.5
Pih1d2	1.19	0.69	1.67	1.39	0.5	1.01	0.5
Notch1	7.27	6.21	12.72	15.55	0.81	1.32	0.5
Sh3rf1	5.03	3.29	9.69	9.01	0.94	1.45	0.5
Inpp5f	8.92	7.69	12.23	14.97	0.46	0.96	0.5
Purg	5.81	5.38	8.81	11.52	0.6	1.1	0.5

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Ctla2b	0.27	0.21	1.67	7.91	2.64	5.26	2.6
Slc18a2	0.13	0.1	1.39	6.81	3.45	6.06	2.6

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Icam2	0.13	0.1	1.23	5.43	3.22	5.72	2.5
Slc38a5	0.59	0.13	1.33	1.36	1.16	3.38	2.2
Cdh5	0.47	0.38	4.12	14.41	3.14	5.26	2.1
Erg	0.16	0.14	1.14	3.94	2.8	4.82	2.0
Mef2c	0.41	0.24	1.05	2.43	1.35	3.34	2.0
Samd3	0.1	0.1	1.09	4.14	3.45	5.37	1.9
Vav3	1.01	0.43	3.45	4.9	1.77	3.52	1.8
Sox17	0.19	0.13	1.01	2.29	2.42	4.16	1.7
Tie1	0.4	0.65	1.17	5.96	1.56	3.21	1.7
Spns2	0.46	0.32	1.17	2.41	1.35	2.94	1.6
Ptprb	0.16	0.16	1.37	4.28	3.12	4.71	1.6
Cer1	0.1	0.11	2.5	7.95	4.64	6.22	1.6
Gm15284	0.38	0.1	1.58	1.21	2.07	3.6	1.5
Egr2	0.44	0.26	1.03	1.75	1.22	2.73	1.5
Fignl2	2.67	0.9	10.55	10	1.98	3.47	1.5
Samsn1	0.1	0.1	1.14	3.3	3.51	5	1.5
Itga8	3.97	1.8	8.66	11	1.13	2.61	1.5
Flt1	0.48	0.45	5.81	14.93	3.59	5.07	1.5
Ubash3b	0.83	0.42	5.34	7.25	2.68	4.11	1.4
Jakmip1	0.77	0.42	1.94	2.82	1.34	2.74	1.4
Slc32a1	0.14	0.1	1.13	2.25	3.04	4.44	1.4
Sall3	2.27	0.82	10.84	10.26	2.25	3.64	1.4
Dusp2	1.09	0.63	12.85	19.43	3.56	4.94	1.4
Optn	2.41	0.85	8.72	7.76	1.85	3.19	1.3
Hbb-bh1	0.1	0.1	3.18	7.92	4.99	6.31	1.3
Gfi1b	0.18	0.13	1.24	2.16	2.77	4.07	1.3
Dll3	2.28	1.6	12.38	20.94	2.44	3.71	1.3
Amd1	0.12	0.1	6.49	12.72	5.7	6.95	1.3
Dpysl5	3.96	2.21	28.7	37.76	2.86	4.09	1.2
Hhex	2	1.03	6.59	7.85	1.72	2.94	1.2
Arhgef26	2.12	1.27	4.72	6.56	1.16	2.37	1.2
Myl4	5.76	2.46	13.21	13.07	1.2	2.41	1.2
Parm1	0.97	0.65	2.55	3.95	1.4	2.61	1.2
Myl7	3.52	1.77	65	74.95	4.21	5.4	1.2
Ctsc	11.92	7.26	34.3	47.57	1.53	2.71	1.2
Gfra2	0.59	0.31	13.26	16.04	4.5	5.68	1.2
Nol4l	0.86	0.39	5.4	5.57	2.65	3.82	1.2
Pkdcc	1.87	1.54	9.79	17.72	2.39	3.53	1.1
Robo3	0.21	0.16	1.77	2.95	3.04	4.18	1.1
Cdh8	0.31	0.16	2.37	2.68	2.94	4.07	1.1
Gad2	0.12	0.13	1.58	3.85	3.73	4.85	1.1
Hivep2	2.36	1.32	4.64	5.57	0.97	2.08	1.1
Grik5	2.38	1	5.46	4.97	1.2	2.31	1.1
Rgs3	0.54	0.39	2.64	4.08	2.28	3.39	1.1
Ndufa4l2	2.57	0.31	13.3	3.49	2.37	3.48	1.1
Sp5	1.05	0.66	9.28	12.65	3.15	4.25	1.1
Nxph4	0.29	0.11	3.6	2.86	3.65	4.74	1.1
Itga2b	0.25	0.24	1.45	3	2.55	3.63	1.1
Dbndd1	0.85	0.54	1.9	2.57	1.17	2.24	1.1
Skap1	0.13	0.1	2.03	3.21	3.92	4.99	1.1
Cntnap2	0.59	0.47	2.6	4.21	2.13	3.16	1.0
Tmem164	6.32	3.68	31.48	37.6	2.32	3.35	1.0
Gbp9	0.35	0.23	3.01	3.94	3.09	4.1	1.0
Calcrl	0.35	0.41	3.48	8.16	3.3	4.31	1.0
Man1c1	1.58	0.69	15.97	14.21	3.34	4.35	1.0
Gata4	1.35	0.61	77.28	69.82	5.84	6.84	1.0
Optc	0.1	0.11	4.99	10.44	5.64	6.63	1.0
Epm2a	0.27	0.26	2.06	3.86	2.93	3.91	1.0
Zic2	0.98	0.5	10.98	11.02	3.48	4.46	1.0
Ptprj	4.43	1.9	13.6	11.39	1.62	2.59	1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Srgap1	0.63	0.55	2.08	3.56	1.73	2.7	1.0
Mlycd	1.31	0.6	4.43	3.95	1.75	2.72	1.0
Bcorl1	1.47	0.91	10.13	12.3	2.79	3.76	1.0
Podxl2	0.33	0.3	1.09	1.93	1.71	2.67	1.0
Pcp4	0.4	0.1	3	1.47	2.92	3.88	1.0
Cdc14a	2.48	1.43	5.2	5.83	1.07	2.02	1.0
Tspan18	0.22	0.14	4.6	5.79	4.4	5.35	1.0

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 9: upregulated in mutant/downregulated in WT

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Afap1l1	3.29	2.07	1.71	4.59	-0.95	1.15	2.1
Ebf1	1.9	0.82	0.83	1.76	-1.19	1.11	2.3
Nos3	2.67	0.81	1.14	1.79	-1.22	1.14	2.4
Ulbp1	3.29	3.49	1.49	8.01	-1.14	1.2	2.3

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
3830417A13Rik	3.07	1.86	0.19	1.57	-4.03	-0.25	3.8
Cd34	3.04	1.14	0.46	1.97	-2.73	0.79	3.5
Rhox5	50.29	149.14	8.51	183	-2.56	0.3	2.9
Krt7	3.32	3.07	0.5	3	-2.73	-0.03	2.7
Thsd1	2.06	1.21	0.7	2.12	-1.55	0.8	2.4
Btg3	5.43	3.07	1.51	4.32	-1.84	0.49	2.3
Esam	10.23	8.65	2.46	9.64	-2.06	0.16	2.2
Aim2	11.99	13.47	1.8	9.19	-2.73	-0.55	2.2
Plvap	2.33	2.06	0.77	3.07	-1.59	0.57	2.2
Cntfr	7.52	2.88	1.29	2.2	-2.54	-0.39	2.2
Rec8	4.02	2.13	0.69	1.61	-2.54	-0.4	2.1
Dysf	5.74	1.99	1.96	2.96	-1.55	0.58	2.1
Pim2	24.37	16.63	4.33	12.03	-2.49	-0.47	2.0
Edn1	1.89	1.7	0.26	0.94	-2.88	-0.86	2.0
Fmr1nb	5.1	8.85	1.14	7.73	-2.16	-0.2	2.0
Sfrp1	7.65	5.74	2.33	6.64	-1.71	0.21	1.9
Sh3tc1	2.23	1.01	0.54	0.92	-2.05	-0.13	1.9
Nuak2	1.95	1.74	0.52	1.67	-1.91	-0.06	1.9
Sepp1	19.75	20.56	9.88	34.14	-1	0.73	1.7
Map4k2	3.94	2.84	1.91	4.55	-1.05	0.68	1.7
Stmn3	5.14	2.04	0.83	1.06	-2.64	-0.94	1.7
Bst2	19.9	13.89	6.49	13.92	-1.62	0	1.6
Gatsl2	2.34	1.18	0.64	0.97	-1.88	-0.28	1.6
Plek2	3.97	2.25	0.84	1.42	-2.25	-0.66	1.6
Ltb	4.37	2.6	0.85	1.5	-2.36	-0.79	1.6
Gap43	4.26	4.65	1.15	3.67	-1.89	-0.34	1.6
Tek	7	7.05	1.97	5.6	-1.83	-0.33	1.5
Specc1	6.14	4.58	2.41	4.97	-1.35	0.12	1.5
Dnah8	7.62	1.87	1.69	1.15	-2.17	-0.71	1.5
Trh	153.88	176.14	39.6	119.66	-1.96	-0.56	1.4
Card10	2.62	1.26	0.99	1.23	-1.4	-0.03	1.4
Plcg2	8.36	4.66	2.22	3.2	-1.91	-0.54	1.4
4930447C04Rik	10.24	14.35	2.16	7.87	-2.24	-0.87	1.4
Sptb	2.91	1.15	0.91	0.92	-1.68	-0.33	1.4
Slc6a1	3.38	1.11	0.8	0.67	-2.08	-0.73	1.4

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Tnfsfm13	3.84	1.35	0.96	0.85	-2	-0.66	1.3
Lyn	3.47	2.2	1.67	2.64	-1.06	0.26	1.3
Adgrg1	1.27	1.3	0.39	0.97	-1.73	-0.42	1.3
Pik3r3	3.35	2.92	0.93	2.02	-1.84	-0.53	1.3
Tram2	4.44	3.01	2.05	3.35	-1.12	0.15	1.3
Trp53i11	15.4	12.31	5.06	9.65	-1.61	-0.35	1.3
Epha1	13.13	7.89	5.5	7.75	-1.26	-0.03	1.2
Dab1	19.55	10.89	6.32	8.24	-1.63	-0.4	1.2
Npnt	1.54	1.04	0.35	0.56	-2.14	-0.91	1.2
Prkcb	1.58	1.16	0.69	1.18	-1.18	0.02	1.2
C130074G19Rik	1.01	1	0.29	0.66	-1.78	-0.6	1.2
Rsg1	1.92	1.06	0.84	1.03	-1.19	-0.03	1.2
Lfng	8.53	7.22	3.48	6.41	-1.29	-0.17	1.1
Arhgef28	2.65	4.24	0.79	2.7	-1.75	-0.65	1.1
Fam198b	4.04	6.53	1.08	3.76	-1.9	-0.8	1.1
Ica1	5.4	4.98	2.29	4.49	-1.24	-0.15	1.1
Qprt	7.79	3.48	3.32	3.14	-1.23	-0.15	1.1
Enc1	51.4	39.93	15.23	24.97	-1.76	-0.68	1.1
Lrp11	15.59	13.9	7	13.12	-1.15	-0.08	1.1
Dclk2	5.32	4.74	2.58	4.76	-1.05	0.01	1.1
Wfdc2	15.4	11.36	4.69	7.21	-1.72	-0.66	1.1
Als2cl	4.34	2.63	1.37	1.72	-1.66	-0.61	1.1
Nudt12	6.24	2.95	2.73	2.64	-1.19	-0.16	1.0
Npr1	6.55	5.49	2.67	4.54	-1.29	-0.27	1.0
Rasl11a	6.7	4.96	3.19	4.76	-1.07	-0.06	1.0
5730507C01Rik	3.1	2.69	1.19	2.09	-1.37	-0.36	1.0
Pkp2	21.92	17.96	5.92	9.76	-1.89	-0.88	1.0
Ptrf	15.52	12.33	6.23	9.72	-1.32	-0.34	1.0
Mgat4a	4.9	4.85	1.83	3.55	-1.42	-0.45	1.0
Slc25a23	2.35	1.78	0.86	1.28	-1.45	-0.48	1.0
Ptrh1	5.51	4.1	1.7	2.5	-1.69	-0.72	1.0
Unc13a	1.38	1.36	0.42	0.82	-1.7	-0.74	1.0
Slitrk5	4.48	3.75	1.34	2.17	-1.75	-0.79	1.0
Vill	2.96	1.41	1.31	1.2	-1.18	-0.23	1.0
Fam111a	23.82	13.39	11.34	12.13	-1.07	-0.14	0.9
Abca3	2.52	2.52	1.08	2.06	-1.22	-0.29	0.9
Nudt18	1.2	1.1	0.45	0.79	-1.4	-0.48	0.9
Cnp	9.35	5.3	4.8	5.13	-0.96	-0.05	0.9
Rab3d	2.51	1.69	0.81	1.02	-1.63	-0.72	0.9
Sntb1	2.68	1.96	0.86	1.18	-1.63	-0.72	0.9
Pde4b	1	1.2	0.51	1.14	-0.97	-0.07	0.9
Dock4	4.48	4.37	2.09	3.79	-1.1	-0.2	0.9
Rasip1	4.74	5.87	2.01	4.66	-1.23	-0.33	0.9
Bcl6b	4.96	3.94	1.75	2.58	-1.51	-0.61	0.9
Kif5c	11.26	12.54	5.11	10.52	-1.14	-0.25	0.9
Sdr39u1	1.45	1.65	0.59	1.25	-1.29	-0.4	0.9
Rasef	3.49	2.9	1.39	2.16	-1.32	-0.43	0.9
Mtm1	3.01	2.89	1.14	2.03	-1.4	-0.51	0.9
Zfhx2	2.93	2	0.9	1.13	-1.71	-0.82	0.9
Nes	22.25	16.72	8.98	12.44	-1.31	-0.43	0.9
Celsr2	2.19	2.09	0.65	1.14	-1.75	-0.87	0.9
Rassf3	18.29	12.92	5.76	7.38	-1.67	-0.81	0.9
Lmbr1	3.78	3.09	1.91	2.81	-0.99	-0.14	0.9
Prmt8	1.46	1.39	0.73	1.23	-1.01	-0.17	0.8
Lekr1	1.21	1.15	0.44	0.73	-1.47	-0.66	0.8
9930012K11Rik	10.59	7.49	3.22	3.98	-1.72	-0.91	0.8
Cadps2	2.06	1.25	1.06	1.12	-0.96	-0.16	0.8
4930432K21Rik	2.1	2.29	0.8	1.51	-1.4	-0.6	0.8
Moap1	4.88	3.87	2.42	3.3	-1.01	-0.23	0.8
Rbm12b1	3.97	3.23	1.43	1.99	-1.48	-0.7	0.8

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Hspa4l	3.76	4.17	1.32	2.51	-1.51	-0.73	0.8
Samd9l	1.42	2.36	0.49	1.38	-1.55	-0.77	0.8
Dnajb5	8.17	7.44	2.61	4.09	-1.64	-0.86	0.8
Ankrd33b	2.52	1.14	1.11	0.85	-1.18	-0.42	0.8
Ddx3y	5.52	1.08	2.12	0.7	-1.38	-0.62	0.8
Rundc3b	1.94	2.21	0.67	1.29	-1.52	-0.77	0.8
9030624G23Rik	3.91	3.06	1.87	2.43	-1.07	-0.33	0.7
Nmrk1	3.55	4.5	1.49	3.14	-1.26	-0.52	0.7
Rfng	8.86	6.16	3.53	4.09	-1.33	-0.59	0.7
Dhrs11	3.66	3.68	1.28	2.15	-1.51	-0.77	0.7
Myo5b	1.5	1.14	0.52	0.66	-1.52	-0.78	0.7
Rltpr	1.77	1.2	0.58	0.66	-1.6	-0.86	0.7
Hvcn1	9.11	6.69	4.19	5.01	-1.12	-0.42	0.7
Sat1	42.34	28.19	20.59	22.18	-1.04	-0.35	0.7
Slc6a8	24.2	15.38	11.54	11.86	-1.07	-0.38	0.7
Uchl4	1.99	1.12	0.71	0.64	-1.5	-0.81	0.7
Nrgn	1.72	2.1	0.57	1.13	-1.59	-0.9	0.7
Gabrb3	3.09	2.11	1.59	1.72	-0.96	-0.29	0.7
Gabarapl2	61.54	45.11	30.77	35.77	-1	-0.33	0.7
Rdh10	5.77	4.89	2.79	3.69	-1.05	-0.4	0.7
Stmn2	33.61	38.07	13.4	23.82	-1.33	-0.68	0.7
Jmjd1c	69.51	58.86	32.08	42.24	-1.12	-0.48	0.6
Synrg	5.74	5.04	2.54	3.48	-1.17	-0.53	0.6
F11r	43.31	39.99	14.62	21.03	-1.57	-0.93	0.6
Spry2	97.93	70.59	43.56	48.39	-1.17	-0.54	0.6
Tbc1d9	7.17	6.65	3.61	5.13	-0.99	-0.37	0.6
3110002H16Rik	14.41	13.18	5.68	7.99	-1.34	-0.72	0.6
Fam129b	40.4	32.66	15.56	19.34	-1.38	-0.76	0.6
Syne1	2.36	2.01	1.17	1.51	-1.02	-0.41	0.6
Tmem19	12.8	13.69	5.3	8.65	-1.27	-0.66	0.6
Bik	1.73	1.72	0.61	0.92	-1.5	-0.89	0.6
Akr1b10	11.85	9.49	5.22	6.36	-1.18	-0.58	0.6
Procr	2.87	3.18	1.01	1.69	-1.51	-0.91	0.6
Hdac6	23.2	32.85	10.82	23.08	-1.1	-0.51	0.6
Kbtbd7	14.57	13.15	6.01	8.15	-1.28	-0.69	0.6
Synj2	3.98	3.87	1.88	2.73	-1.08	-0.5	0.6
Sorl1	7.44	6.67	3.45	4.62	-1.11	-0.53	0.6
Rtn4ip1	6.46	5.56	2.86	3.69	-1.17	-0.59	0.6
Nfib	3.62	1.74	1.55	1.11	-1.22	-0.64	0.6
Isyna1	73.82	53.3	31.08	33.51	-1.25	-0.67	0.6
G3bp2	162.39	118.91	66.73	73.35	-1.28	-0.7	0.6
Amt	41.32	34.01	18.75	22.89	-1.14	-0.57	0.6
Ehmt2	83.96	57.75	36.69	37.67	-1.19	-0.62	0.6
Arhgap44	3.01	2.42	1.3	1.55	-1.21	-0.64	0.6
Rab15	9.17	5.01	3.42	2.77	-1.42	-0.85	0.6
Abhd3	1.66	1.05	0.61	0.57	-1.45	-0.89	0.6
Gbe1	9.18	5.38	4.25	3.65	-1.11	-0.56	0.6
Clip4	4.09	3.98	1.62	2.3	-1.34	-0.79	0.6
Igsf9	13.87	11.95	5.47	6.93	-1.34	-0.79	0.6
Fam222a	1.25	1.05	0.48	0.59	-1.37	-0.82	0.6
Cib1	25.83	22.96	9.82	12.76	-1.4	-0.85	0.6
Frem2	9.7	10.16	4.78	7.3	-1.02	-0.48	0.5
B4galt4	4.12	2.09	1.85	1.37	-1.15	-0.61	0.5
Pitpnc1	24.31	16.92	9.68	9.79	-1.33	-0.79	0.5
AI464131	2.35	2.92	0.91	1.65	-1.36	-0.82	0.5
Sec24a	10.34	9.4	5.32	6.96	-0.96	-0.43	0.5
Aktip	16.69	18.52	8.39	13.44	-0.99	-0.46	0.5
Znfx1	7.51	10.62	3.48	7.09	-1.11	-0.58	0.5
Gsap	2.42	1.39	1	0.83	-1.27	-0.74	0.5
Dtx3l	10.13	9.93	3.98	5.58	-1.35	-0.83	0.5

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Celsr3	2.01	2.18	0.73	1.14	-1.46	-0.94	0.5
Slc31a2	8.48	8.02	3.63	4.86	-1.23	-0.72	0.5
Lig4	6.43	5.23	2.68	3.08	-1.27	-0.76	0.5
Cfap74	3.55	2.97	1.41	1.67	-1.34	-0.83	0.5

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Cdh11	0.65	3.9	111.21	78.93	7.41	4.34	-3.1
Smad6	1.35	5.96	25.65	14.92	4.25	1.33	-2.9
Grrp1	0.13	0.44	24.21	13.78	7.55	4.96	-2.6
Pdlim4	0.8	2.3	28.44	15.29	5.16	2.73	-2.4
Ifi27l2a	0.1	0.28	6.56	3.75	6.04	3.72	-2.3
Tmem176a	0.1	0.32	13.77	9.95	7.11	4.94	-2.2
Angptl2	0.44	1.56	6.13	4.93	3.81	1.66	-2.2
Col4a5	0.36	1.23	3.11	2.46	3.12	1	-2.1
Ifi27	0.14	0.94	3.54	5.81	4.63	2.63	-2.0
Stard8	3.11	9.45	32.9	25.41	3.4	1.43	-2.0
Ism2	0.27	0.68	2.53	1.73	3.26	1.35	-1.9
Cd59a	0.7	1.23	8.58	4.07	3.61	1.73	-1.9
Lbh	0.38	1.78	3.08	4.13	3.04	1.22	-1.8
Nrp1	0.76	2.13	77.77	63.75	6.68	4.9	-1.8
Rgs4	0.11	0.53	3.66	5.42	5.08	3.36	-1.7
Hoxb2	0.1	0.23	5.47	3.88	5.77	4.05	-1.7
Dkk1	0.13	0.23	29.64	15.67	7.81	6.1	-1.7
Fabp7	0.27	0.57	2.94	1.95	3.46	1.77	-1.7
Sema3a	0.24	0.85	12.54	13.8	5.69	4.03	-1.7
Msgn1	0.1	0.1	17.05	5.49	7.41	5.78	-1.6
Efnb3	0.9	2.14	15.83	12.16	4.13	2.51	-1.6
Alx3	0.1	0.18	2.62	1.5	4.71	3.09	-1.6
Pcdh18	0.39	0.95	31.26	25.18	6.34	4.72	-1.6
Pcdh7	0.41	0.99	73.99	60.15	7.51	5.92	-1.6
Pygl	0.35	0.29	3.92	1.09	3.47	1.92	-1.6
Efna1	1.29	2	12.2	6.5	3.24	1.7	-1.5
Isg15	0.32	0.53	1.86	1.07	2.52	1.01	-1.5
Dapk2	0.66	1.46	7.35	5.75	3.47	1.98	-1.5
St3gal6	0.52	0.76	13.26	6.9	4.67	3.18	-1.5
Foxf1	0.14	0.17	17.58	7.61	6.94	5.45	-1.5
Ackr3	2.79	4.5	26.01	15.04	3.22	1.74	-1.5
Lhfp	0.78	2.83	11.74	15.44	3.91	2.45	-1.5
Jdp2	0.6	0.95	3.73	2.16	2.63	1.19	-1.4
Slc39a8	1.35	3.11	14.26	12.07	3.4	1.96	-1.4
Zfp703	0.22	0.29	10.6	5.09	5.57	4.16	-1.4
Six2	0.12	0.18	7.07	4.09	5.89	4.48	-1.4
Wnt5a	0.63	1.31	37.98	29.84	5.9	4.51	-1.4
Atp2b4	0.54	2.09	3.3	4.89	2.61	1.23	-1.4
Amhr2	1.71	1.91	13.46	5.82	2.97	1.61	-1.4
Gjb2	0.22	0.25	5.22	2.32	4.59	3.24	-1.4
Zfp516	3.04	6.11	32.47	25.79	3.42	2.08	-1.3
Daam2	0.1	0.15	4.69	2.7	5.55	4.22	-1.3
Msx2	0.56	0.88	53.33	33.75	6.58	5.26	-1.3
Greb1l	3.25	8.69	23.1	25.17	2.83	1.53	-1.3
Ifngr2	1.81	2.79	17.73	11.08	3.29	1.99	-1.3
Sat2	0.37	0.84	2.3	2.15	2.64	1.35	-1.3
Tbx6	0.2	0.19	5.96	2.33	4.89	3.62	-1.3
Letmd1	1.55	3.47	7.44	6.93	2.26	1	-1.3
Cyp26a1	0.8	1.65	215.35	185.47	8.06	6.81	-1.3

Sp1$^{-/-}$ ESC – Flk1+ cells
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{-/-}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{-/-}$ FPKM	WT ES/Flk log2 FC	Sp1$^{-/-}$ ES/Flk log2 FC	WT FC/Sp1$^{-/-}$ FC
Lgals3bp	0.16	0.55	3.94	5.77	4.62	3.38	-1.2
Itpr2	0.25	0.93	2.93	4.69	3.56	2.34	-1.2
Tnfaip2	0.3	0.55	5.62	4.38	4.21	2.99	-1.2
Tmem176b	0.17	0.39	9.42	9.35	5.81	4.59	-1.2
Hs3st3b1	1.76	3.31	23.55	19.15	3.74	2.53	-1.2
Tbx2	0.13	0.16	9.06	4.98	6.14	4.93	-1.2
Myzap	1.07	2.16	6.9	6.08	2.69	1.49	-1.2
Lats2	1.41	3.44	6.33	6.75	2.16	0.97	-1.2
Alas2	0.14	0.11	3.05	1.11	4.49	3.3	-1.2
Amer3	0.11	0.13	26.33	14.66	7.96	6.77	-1.2
Ano1	0.28	0.52	1.28	1.03	2.17	1	-1.2
Fam149a	0.28	0.45	1.58	1.11	2.49	1.32	-1.2
Amot	7.14	14.85	244.2	225.67	5.1	3.93	-1.2
Fst	1.53	3.32	25.95	25.14	4.08	2.92	-1.2
Etv2	0.87	1.1	24.66	13.86	4.82	3.66	-1.2
Lmo2	0.53	0.84	49.09	34.48	6.52	5.37	-1.2
Pth1r	0.97	2.33	12.13	13.29	3.64	2.51	-1.1
Mfsd12	2.02	4.02	10.41	9.57	2.37	1.25	-1.1
Gata3	0.43	0.41	6.52	2.85	3.92	2.81	-1.1
Col9a1	0.16	0.15	3.52	1.58	4.5	3.39	-1.1
Bace2	0.15	0.12	4.48	1.6	4.86	3.75	-1.1
Sct	0.9	0.79	8.83	3.63	3.3	2.2	-1.1
Zfpm1	0.68	1.32	27.75	24.8	5.34	4.24	-1.1
Cxcr4	0.42	0.73	91.02	73.58	7.75	6.65	-1.1
Zc3hav1	3.95	6.8	41.18	33.32	3.38	2.29	-1.1
Hey1	2.17	4.01	18.26	15.99	3.07	1.99	-1.1
Tnnt3	0.29	0.37	5.24	3.14	4.17	3.09	-1.1
Frem1	0.4	0.51	2.84	1.73	2.84	1.78	-1.1
Zbtb4	0.3	0.51	2.25	1.83	2.89	1.84	-1.1
Prickle3	0.47	0.58	1.9	1.13	2	0.96	-1.0
Unc5c	0.17	0.26	22.44	16.46	7.03	6	-1.0
Rsph3a	0.34	0.44	1.84	1.18	2.44	1.42	-1.0
Smim1	0.19	0.21	2.68	1.45	3.8	2.78	-1.0
Rhoj	0.22	1.04	3.8	8.82	4.1	3.08	-1.0
Rbms3	0.14	0.26	2.58	2.37	4.21	3.19	-1.0
Tmem119	0.29	0.26	16.41	7.31	5.84	4.82	-1.0
Gm5617	1.24	1.9	4.82	3.67	1.96	0.95	-1.0
Cd99l2	1.53	3.17	9.77	10.12	2.68	1.67	-1.0
Tgfb1i1	0.73	1.28	18.18	15.83	4.64	3.63	-1.01
Pdlim3	2.98	3.54	81.87	48.41	4.78	3.77	-1.0
Fat4	0.15	0.29	5.25	4.92	5.11	4.1	-1.0
Rai1	4.83	7.79	21.22	17.22	2.14	1.14	-1.0
Stbd1	1.21	0.99	7.89	3.23	2.71	1.71	-1.0
Slit3	0.85	2.11	10.7	13.35	3.65	2.66	-1.0
Ms4a4d	0.61	0.95	8.48	6.65	3.79	2.8	-1.0
Pik3ip1	2.05	4.66	15.83	18.26	2.95	1.97	-1.0
Bmper	0.3	0.36	34.92	21	6.85	5.87	-1.0
Tle3	12.05	19.88	51.92	44.27	2.11	1.15	-0.96
Alox15	0.46	0.28	4.52	1.43	3.31	2.35	-1.0
Ccnd2	4.93	9.15	42.02	40.4	3.09	2.14	-1.0

Supplementary Table 5.1 – Deregulated genes between ESC and Flk1+ cells in Sp1$^{-/-}$ cells compared to WT cells

Genes that were differentially expressed (at least two-fold) between ESC and Flk1+ cell differentiation stages in WT cells and Sp1$^{-/-}$ cells were first identified. The genes that were differently differentially regulated between differentiation stages in the Sp1$^{-/-}$ cells, compared to the WT, were identified. The expression (FPKM) in each cell type, the fold

change (log2 value) between the stages in each cell clone, and the FC between these values in $Sp1^{del/del}$ compared to WT is shown. The genes were grouped into clusters based on how the gene expression is altered upon loss of Sp1.

Supplementary Table 5.2

$Sp1^{del/del}$ ESC – Flk1+ cells
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES $Sp1^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ $Sp1^{del/del}$ FPKM	WT ES/Flk log2 FC	$Sp1^{del/del}$ ES/Flk log2 FC	WT FC/$Sp1^{del/del}$ FC
Trap1a	56.59	72.82	0.7	16.75	-6.34	-2.12	4.2
Anxa1	28.43	19.74	0.43	2.66	-6.06	-2.89	3.2
Laptm5	16.93	10.11	0.57	1.95	-4.88	-2.38	2.5
Pde2a	9.61	2.78	0.65	0.91	-3.88	-1.61	2.3
Rnf17	11.87	11.62	1.15	5.32	-3.37	-1.13	2.2
Hopx	13.07	2.32	0.38	0.31	-5.11	-2.89	2.2
Tex11	3.18	6.07	0.15	1.26	-4.43	-2.26	2.2
Mroh6	8.84	1.71	0.2	0.16	-5.47	-3.39	2.1
Crmp1	23.15	8.72	1.99	2.96	-3.54	-1.56	2.0
Sned1	8.12	1.82	0.39	0.34	-4.37	-2.44	1.9
Serpini1	8.08	7.02	0.32	1.01	-4.64	-2.8	1.8
Soga3	10.82	2.39	1.23	0.97	-3.14	-1.3	1.8
Nkx6-3	3.98	1.47	0.1	0.13	-5.32	-3.49	1.8
Akr1b8	18.61	12.6	0.87	2.09	-4.42	-2.59	1.8
Pla2g7	8.2	3.49	0.72	1.03	-3.52	-1.77	1.8
Scrn1	7.34	3.27	0.2	0.3	-5.2	-3.46	1.7
Zfp936	50.5	25.6	0.42	0.7	-6.9	-5.19	1.7
3830417A13Rik	3.07	1.44	0.19	0.28	-4.03	-2.35	1.7
Pde9a	3.5	1.22	0.49	0.54	-2.83	-1.17	1.7
E2f2	9.24	4.55	1.16	1.8	-2.99	-1.34	1.7
Ddx4	10.94	15.71	0.54	2.41	-4.34	-2.7	1.6
Gm13242	34.06	11.41	0.31	0.31	-6.8	-5.18	1.6
Syt13	2.58	1.04	0.3	0.37	-3.1	-1.49	1.6
Mfng	4.74	1.77	0.24	0.27	-4.3	-2.7	1.6
Hap1	15.21	9.67	0.42	0.8	-5.18	-3.6	1.6
Vat1l	3.69	1.69	0.32	0.43	-3.53	-1.97	1.6
EU599041	34.36	30.91	1.03	2.67	-5.06	-3.53	1.5
Samd12	1.91	1.83	0.17	0.47	-3.49	-1.96	1.5
Stmn3	5.14	1.6	0.83	0.74	-2.64	-1.12	1.5
Slco4a1	3.13	1.46	0.16	0.22	-4.25	-2.75	1.5
Zfp709	3.62	2.1	0.66	1.08	-2.45	-0.95	1.5
Dock3	2.9	1.51	0.42	0.6	-2.8	-1.32	1.5
Rhox6	14.14	29.28	0.23	1.33	-5.93	-4.47	1.5
Coro1a	7.61	5.61	0.9	1.81	-3.09	-1.63	1.5
Cd37	4.6	2.32	0.16	0.21	-4.89	-3.46	1.4
Tex19.1	23.63	86.72	0.39	3.87	-5.9	-4.48	1.4
Adgrv1	4.75	3.05	0.17	0.29	-4.78	-3.37	1.4
Ppl	5.5	1.68	0.21	0.17	-4.72	-3.32	1.4
Fam129a	20.7	4.83	0.33	0.2	-5.98	-4.63	1.4
Palm3	17.15	18.03	0.61	1.63	-4.82	-3.47	1.4
Stk31	4.77	7.17	0.18	0.7	-4.7	-3.35	1.4
Epas1	28.76	13.86	0.97	1.18	-4.9	-3.56	1.3
Rex2	13.64	4.06	0.22	0.17	-5.94	-4.61	1.3
Pdzd2	9.09	3.67	0.25	0.25	-5.17	-3.86	1.3
Zfp534	41.88	17.89	1.72	1.81	-4.61	-3.31	1.3
Fam19a4	3.92	1.34	0.22	0.18	-4.17	-2.89	1.3
Cdkn2a	2.2	2	0.32	0.71	-2.78	-1.5	1.3
Tcte2	2.88	1.72	0.59	0.85	-2.29	-1.02	1.3
Jam2	26.77	12.12	0.46	0.5	-5.87	-4.61	1.3

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Mapt	2.24	1.42	0.18	0.27	-3.63	-2.38	1.3
Urah	32.86	19.68	0.27	0.39	-6.9	-5.67	1.2
Gbx2	26.89	16.6	0.77	1.11	-5.13	-3.9	1.2
Cysltr1	3.5	1.45	0.24	0.24	-3.84	-2.61	1.2
Rtn1	4.54	2.11	0.88	0.97	-2.36	-1.13	1.2
Tmtc1	2.4	1.08	0.48	0.5	-2.32	-1.1	1.2
Calca	16.36	8.27	0.65	0.76	-4.66	-3.45	1.2
Syt11	11.72	15.54	1.94	5.95	-2.59	-1.38	1.2
Spta1	16.2	7.86	0.13	0.15	-6.91	-5.71	1.2
Cisd3	40.83	14.96	6.13	5.15	-2.74	-1.54	1.2
Naa11	6.6	2.64	0.12	0.11	-5.77	-4.58	1.2
Hsf2bp	20.29	26.02	0.52	1.53	-5.28	-4.09	1.2
Pnldc1	11.8	14.46	1.94	5.45	-2.6	-1.41	1.2
Ramp3	7.42	4.17	0.38	0.48	-4.29	-3.11	1.2
Apobec1	6.31	4.9	0.12	0.21	-5.71	-4.54	1.2
Camk2b	2.22	1.39	0.31	0.44	-2.82	-1.66	1.2
Itgb4	3.84	1.12	0.22	0.14	-4.16	-3.01	1.2
Mfap3l	7.02	3.44	0.42	0.45	-4.07	-2.92	1.2
Plch1	4.37	2.46	0.58	0.72	-2.91	-1.76	1.2
Mylpf	81.29	49.02	1.47	1.96	-5.79	-4.65	1.1
Dtx4	2.84	1.7	0.62	0.82	-2.2	-1.06	1.1
Zfp600	17.81	4.92	0.24	0.14	-6.24	-5.11	1.1
Gm7325	12.96	9.78	0.21	0.35	-5.92	-4.79	1.1
Mgat4c	2.73	1.2	0.11	0.1	-4.67	-3.54	1.1
Jakmip2	3.38	4.25	0.22	0.59	-3.97	-2.84	1.1
Kbtbd11	2.65	1.31	0.27	0.29	-3.28	-2.16	1.1
Sfmbt2	85.27	40.24	11.84	12.13	-2.85	-1.73	1.1
Gm1564	4.63	2.89	0.65	0.88	-2.84	-1.72	1.1
Plek2	3.97	2.2	0.84	1	-2.25	-1.14	1.1
Zbtb32	8.3	7.36	0.37	0.71	-4.48	-3.38	1.1
2410137M14Rik	1.44	1.21	0.1	0.18	-3.85	-2.75	1.1
Slc52a3	2.85	1.43	0.12	0.12	-4.63	-3.54	1.1
Zp3	5.16	3.12	0.1	0.13	-5.69	-4.62	1.1
Dok2	28.3	13	2.86	2.76	-3.3	-2.24	1.1
Tnfsf12	4.97	2.67	0.5	0.56	-3.3	-2.25	1.1
Nckap1l	2.92	1.9	0.15	0.2	-4.28	-3.25	1.0
Rhox5	50.29	502.59	8.51	173.6	-2.56	-1.53	1.0
Gm13247	56.21	17.61	0.75	0.48	-6.22	-5.2	1.0
Cltb	53.41	40.96	1.47	2.3	-5.18	-4.16	1.0
Prr19	4.23	1.99	0.59	0.57	-2.83	-1.81	1.0
Ell3	5.22	3.2	1.05	1.3	-2.31	-1.29	1.0
4930519F16Rik	2.49	1.16	0.21	0.19	-3.59	-2.58	1.0
Fgf4	40.93	23.96	0.89	1.05	-5.52	-4.52	1.0
Sap25	6.1	5.29	0.43	0.75	-3.81	-2.81	1.0
Rps6kl1	4.27	2.77	0.57	0.74	-2.9	-1.9	1.0
Itpk1	52.82	23.18	10.28	9.04	-2.36	-1.36	1.0
Rbmxl2	23.55	25.2	0.27	0.57	-6.46	-5.47	1.0
Myrf	15.17	9.06	1.88	2.24	-3.01	-2.02	1.0
Cacng7	9.13	9.01	1.62	3.15	-2.49	-1.51	1.0
Gm13251	10.44	3.87	2.44	1.78	-2.1	-1.12	1.0
Rapgef4	2.31	1.23	0.11	0.11	-4.42	-3.45	1.0
Mfap5	1.59	1.68	0.12	0.24	-3.76	-2.79	1.0
Galnt3	4.16	2.13	0.52	0.52	-3.01	-2.04	1.0
Inhbb	9.17	4.55	0.12	0.12	-6.23	-5.27	1.0
4930447C04Rik	10.24	14.74	2.16	6.05	-2.24	-1.28	1.0
Bmp8a	3.02	1.2	0.23	0.18	-3.72	-2.77	1.0
Prex2	6.51	5.36	0.65	1.04	-3.32	-2.37	1.0
Lrch4	14.29	8.85	3.23	3.88	-2.14	-1.19	1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cdkn1c	108.1	21.45	17.23	22.03	-2.65	0.04	2.7
Cntfr	7.52	1.81	1.29	1.73	-2.54	-0.06	2.5
Dysf	5.74	1.14	1.96	1.8	-1.55	0.66	2.2
Slc29a4	3.36	1.63	0.94	1.64	-1.84	0	1.8
Aim2	11.99	13.33	1.8	7.02	-2.73	-0.92	1.8
Fmr1nb	5.1	9.09	1.14	6.72	-2.16	-0.44	1.7
Tnfsfm13	3.84	1.41	0.96	1.14	-2	-0.31	1.7
Rec8	4.02	2.15	0.69	1.16	-2.54	-0.89	1.7
Sdr39u1	1.45	1.1	0.59	1.4	-1.29	0.34	1.6
Acap1	3.2	1	1.57	1.46	-1.03	0.55	1.6
Slitrk5	4.48	2.64	1.34	2.34	-1.75	-0.18	1.6
Dnah8	7.62	1.28	1.69	0.83	-2.17	-0.63	1.5
Ulbp1	3.29	4.89	1.49	6.43	-1.14	0.4	1.5
Lfng	8.53	5.59	3.48	6.49	-1.29	0.21	1.5
Btg3	5.43	4.16	1.51	3.26	-1.84	-0.35	1.5
Gbe1	9.18	3.17	4.25	4.07	-1.11	0.36	1.5
Dab1	19.55	6.88	6.32	5.97	-1.63	-0.2	1.4
Fmn2	2.94	1.28	0.72	0.83	-2.02	-0.62	1.4
Nfib	3.62	1.15	1.55	1.3	-1.22	0.18	1.4
Uchl4	1.99	1.02	0.71	0.94	-1.5	-0.11	1.4
Map4k2	3.94	2.75	1.91	3.25	-1.05	0.24	1.3
Pik3r3	3.35	2.56	0.93	1.71	-1.84	-0.58	1.3
Inpp1	8.06	3.04	2.18	1.95	-1.88	-0.64	1.2
Rasl11a	6.7	4.19	3.19	4.69	-1.07	0.16	1.2
Nefm	1.18	1.05	0.29	0.61	-2.01	-0.79	1.2
Nudt12	6.24	2.89	2.73	2.95	-1.19	0.03	1.2
Prkcb	1.58	1.17	0.69	1.19	-1.18	0.02	1.2
Necab3	1.98	1.57	0.5	0.9	-1.98	-0.81	1.2
5730507C01Rik	3.1	2.37	1.19	2	-1.37	-0.24	1.1
Zfp423	28.8	19.07	9.6	13.69	-1.59	-0.48	1.1
Kif5c	11.26	11.02	5.11	10.64	-1.14	-0.05	1.1
Sepp1	19.75	20.37	9.88	21.57	-1	0.08	1.1
Pkp2	21.92	12.69	5.92	7.19	-1.89	-0.82	1.1
Fbxo17	3.46	1.82	1.41	1.56	-1.29	-0.22	1.1
Epha1	13.13	6.74	5.5	5.92	-1.26	-0.19	1.1
Ptrf	15.52	7.5	6.23	6.26	-1.32	-0.26	1.1
Isyna1	73.82	40.4	31.08	35.52	-1.25	-0.19	1.1
Cacna2d1	3.93	1.58	1.49	1.24	-1.4	-0.35	1.1
Fam111a	23.82	10.69	11.34	10.53	-1.07	-0.02	1.1
Lyn	3.47	1.63	1.67	1.61	-1.06	-0.01	1.1
Ankrd33b	2.52	1.14	1.11	1.03	-1.18	-0.14	1.0
Fam198b	4.04	5.75	1.08	3.15	-1.9	-0.87	1.0
Nes	22.25	10.84	8.98	8.91	-1.31	-0.28	1.0
E130309D14Rik	4.11	1.07	1.71	0.91	-1.26	-0.23	1.0
Afap1l1	3.29	2.27	1.71	2.35	-0.95	0.05	1.0
Gabrb3	3.09	1.97	1.59	2.01	-0.96	0.03	1.0
Slc6a8	24.2	14.47	11.54	13.28	-1.07	-0.12	1.0
Pias3	7.45	5.51	3.43	4.87	-1.12	-0.18	0.9
Rab3d	2.51	1.65	0.81	1.02	-1.63	-0.7	0.9
Lekr1	1.21	1.18	0.44	0.8	-1.47	-0.55	0.9
Cnp	9.35	5.38	4.8	5.25	-0.96	-0.04	0.9
Neto2	5.33	3.72	1.51	1.95	-1.82	-0.93	0.9
Pitpnc1	24.31	13.81	9.68	10.22	-1.33	-0.44	0.9
Map1a	5.95	4.49	2.83	3.96	-1.07	-0.18	0.9
Rfng	8.86	6.83	3.53	4.95	-1.33	-0.46	0.9
Tram2	4.44	3.21	2.05	2.71	-1.12	-0.25	0.9
2810474O19Rik	83.07	53.62	25.94	30.43	-1.68	-0.82	0.9
Capn1	11.52	5.9	5.38	5	-1.1	-0.24	0.9
Lmbr1	3.78	3.45	1.91	3.16	-0.99	-0.13	0.9
Bik	1.73	1.08	0.61	0.68	-1.5	-0.66	0.8

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ehmt2	83.96	51.57	36.69	40.46	-1.19	-0.35	0.8
Specc1	6.14	3.56	2.41	2.48	-1.35	-0.52	0.8
Rasef	3.49	2.46	1.39	1.76	-1.32	-0.49	0.8
Spry2	97.93	57.6	43.56	45.46	-1.17	-0.34	0.8
Tbc1d9	7.17	5.34	3.61	4.76	-0.99	-0.17	0.8
Kcnc3	1.76	1.91	0.63	1.19	-1.49	-0.68	0.8
Mtss1	27	18.23	10.11	11.94	-1.42	-0.61	0.8
Wfdc2	15.4	10.34	4.69	5.46	-1.72	-0.92	0.8
Jmjd1c	69.51	42	32.08	33.55	-1.12	-0.32	0.8
Moap1	4.88	4.53	2.42	3.9	-1.01	-0.22	0.8
Rassf3	18.29	9.43	5.76	5.08	-1.67	-0.89	0.8
Trp53i11	15.4	11.33	5.06	6.37	-1.61	-0.83	0.8
Hes6	22.81	16.09	8.85	10.7	-1.37	-0.59	0.8
Asphd2	3.16	2.45	1.4	1.85	-1.18	-0.41	0.8
Plce1	2.46	1.25	1.16	1	-1.08	-0.32	0.8
9030624G23Rik	3.91	2.77	1.87	2.22	-1.07	-0.32	0.8
Als2cl	4.34	2.03	1.37	1.07	-1.66	-0.92	0.7
Mgat4a	4.9	5.2	1.83	3.26	-1.42	-0.68	0.7
Ank	6.37	3.22	2.85	2.41	-1.16	-0.42	0.7
Hspa4l	3.76	3.25	1.32	1.89	-1.51	-0.78	0.7
Vash1	2.66	2.22	1.1	1.52	-1.28	-0.55	0.7
Bst2	19.9	12.42	6.49	6.6	-1.62	-0.91	0.7
Dock4	4.48	4.04	2.09	3.07	-1.1	-0.39	0.7
Rims3	3.75	1.55	1.67	1.11	-1.17	-0.47	0.7
Plvap	2.33	2.83	0.77	1.51	-1.59	-0.9	0.7
D16Ertd472e	11.08	7.85	3.87	4.41	-1.52	-0.83	0.7
Dact2	3.85	1.12	1.56	0.73	-1.3	-0.61	0.7
Zfp654	15.35	8.35	7.95	6.87	-0.95	-0.28	0.7
Ripk1	9.43	1.86	4.4	1.37	-1.1	-0.44	0.7
Sat1	42.34	24.61	20.59	18.96	-1.04	-0.38	0.7
Zfp493	6.04	3.89	2.41	2.43	-1.33	-0.68	0.7
Qprt	7.79	3.69	3.32	2.47	-1.23	-0.58	0.7
Ap1g2	5.68	1.76	2.74	1.34	-1.05	-0.4	0.7
G3bp2	162.39	107.96	66.73	69.3	-1.28	-0.64	0.6
Slc25a23	2.35	1.59	0.86	0.9	-1.45	-0.82	0.6
Pnma2	16.12	5.48	6.23	3.27	-1.37	-0.75	0.6
Dhrs11	3.66	3.14	1.28	1.67	-1.51	-0.91	0.6
Baiap2	13.83	10.49	4.85	5.53	-1.51	-0.92	0.6
Adam22	3.07	4.05	1.35	2.66	-1.19	-0.6	0.6
Adam23	44.19	35.18	19.71	23.77	-1.16	-0.57	0.6
Dclk2	5.32	4.88	2.58	3.55	-1.05	-0.46	0.6
Npr1	6.55	5.69	2.67	3.49	-1.29	-0.71	0.6
3110009E18Rik	9.29	8.5	3.82	5.22	-1.28	-0.7	0.6
Slc2a1	294.61	189.03	135.48	130.03	-1.12	-0.54	0.6
Uso1	27.54	20.54	14.27	15.89	-0.95	-0.37	0.6
Eid3	7.9	5.18	3.7	3.62	-1.09	-0.52	0.6
P2rx7	1.39	1.05	0.5	0.56	-1.48	-0.92	0.6
Tmem263	25.99	17.56	11.33	11.23	-1.2	-0.64	0.6
Pdhb	103.8	74.77	45.46	48.32	-1.19	-0.63	0.6
Dpf1	8.24	7.54	3.71	5	-1.15	-0.59	0.6
Abca3	2.52	2.29	1.08	1.44	-1.22	-0.67	0.6
Rdh10	5.77	5.22	2.79	3.7	-1.05	-0.5	0.6
Whrn	3.29	2.97	1.63	2.14	-1.01	-0.47	0.5
Chrna7	4.02	5.64	1.63	3.31	-1.3	-0.77	0.5
Gli2	8.34	8.46	3.4	4.99	-1.29	-0.76	0.5
Lrp11	15.59	15.06	7	9.81	-1.15	-0.62	0.5
1110008P14Rik	9.23	9.23	3.89	5.55	-1.25	-0.73	0.5
Sec24a	10.34	8.75	5.32	6.46	-0.96	-0.44	0.5
Akr1b10	11.85	8.56	5.22	5.38	-1.18	-0.67	0.5
Haus4	29.67	19.09	13.65	12.5	-1.12	-0.61	0.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Prune	28.82	22.47	13.07	14.43	-1.14	-0.64	0.5
Gale	17.01	13.58	8.66	9.8	-0.97	-0.47	0.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Trim47	1.52	9.11	0.38	0.21	-2.01	-5.42	-3.4
1700019A02Rik	2.6	5.14	0.37	0.1	-2.81	-5.68	-2.9
Apoc1	28.06	21.32	5.68	0.64	-2.31	-5.05	-2.7
Rln3	1.08	3	0.21	0.1	-2.35	-4.91	-2.6
Npw	1.1	1.77	0.51	0.14	-1.1	-3.66	-2.6
Pga5	1	3.47	0.31	0.19	-1.69	-4.2	-2.5
Sigirr	13.12	7.61	1.38	0.15	-3.25	-5.7	-2.5
Rab27a	6.66	18.5	2.39	1.29	-1.48	-3.84	-2.4
Gm6792	8.38	15.69	0.25	0.1	-5.05	-7.29	-2.2
Aldh1b1	1.38	7.97	0.41	0.5	-1.76	-3.99	-2.2
Smox	3.47	10.09	1.35	0.84	-1.37	-3.59	-2.2
Dppa5a	1558.91	686.82	31.85	3.1	-5.61	-7.79	-2.2
Nkx2-9	2.23	7.03	0.14	0.1	-4.03	-6.13	-2.1
A830018L16Rik	1.65	3.25	0.35	0.16	-2.25	-4.32	-2.1
Bcl2l14	1.09	1.7	0.46	0.17	-1.24	-3.31	-2.1
Igfbp7	2.32	4.27	0.22	0.1	-3.42	-5.42	-2.0
Sptbn2	8.14	20.46	0.88	0.56	-3.21	-5.2	-2.0
Rbm47	15.2	31.1	0.95	0.51	-4	-5.93	-1.9
Calcoco2	4.51	17.91	0.16	0.17	-4.82	-6.74	-1.9
Dppa3	11.58	72.22	0.19	0.32	-5.92	-7.83	-1.9
D630023F18Rik	4.19	13.56	0.34	0.29	-3.62	-5.53	-1.9
Snai3	2.24	4.56	0.19	0.11	-3.54	-5.43	-1.9
Esrp1	19.89	31.42	2.45	1.05	-3.02	-4.9	-1.9
Il17b	1.27	4.59	0.1	0.1	-3.67	-5.52	-1.9
Erbb3	10.1	19.52	1.26	0.69	-3	-4.83	-1.8
Grem1	1.37	2.79	0.24	0.14	-2.54	-4.37	-1.8
Cd1d1	1.6	4.27	0.33	0.25	-2.26	-4.08	-1.8
Raet1a	13.48	12.54	0.95	0.26	-3.82	-5.6	-1.8
Col9a2	3.33	5.56	0.4	0.19	-3.06	-4.84	-1.8
Rnd1	2.48	5.56	0.41	0.27	-2.6	-4.36	-1.8
Prdm1	4.39	12.39	1.2	1	-1.87	-3.63	-1.8
Cpsf4l	13.36	41.04	0.43	0.39	-4.96	-6.71	-1.8
Fabp3	24	43.04	8.17	4.4	-1.56	-3.29	-1.7
Sdc4	22.96	64.05	7.08	6.02	-1.7	-3.41	-1.7
Celf5	1.94	2.68	0.6	0.26	-1.69	-3.36	-1.7
Sohlh2	2.31	5.42	0.17	0.13	-3.74	-5.4	-1.7
Anxa8	2.55	4.57	0.18	0.1	-3.82	-5.46	-1.6
Lypd2	2.17	4.1	0.16	0.1	-3.76	-5.36	-1.6
Ap1m2	29.57	37.29	4.04	1.68	-2.87	-4.47	-1.6
Phldb3	1.17	1.22	0.32	0.11	-1.86	-3.46	-1.6
Tfap2c	13.35	29.59	2.12	1.57	-2.65	-4.23	-1.6
Fcgrt	2.47	4.77	0.24	0.16	-3.37	-4.93	-1.6
Mt1	30.28	22.36	11.38	2.85	-1.41	-2.97	-1.6
Plcxd1	3.71	6.38	0.87	0.51	-2.1	-3.65	-1.6
Klk10	1.51	3.71	0.12	0.1	-3.67	-5.21	-1.5
Gjb1	1.69	4.86	0.1	0.1	-4.08	-5.6	-1.5
Cav1	2.71	4.8	0.41	0.25	-2.74	-4.26	-1.5
Tnk1	3.35	2.87	0.84	0.26	-1.99	-3.48	-1.5
Tmem45a	2.2	1.45	0.57	0.13	-1.96	-3.45	-1.5
1700019D03Rik	28.03	28.7	1.6	0.59	-4.13	-5.61	-1.5
Zfp750	1.39	3.8	0.16	0.16	-3.11	-4.59	-1.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Timp1	29.16	27.56	6.62	2.24	-2.14	-3.62	-1.5
Pdk4	1.95	4.59	0.55	0.46	-1.84	-3.32	-1.5
Sgk1	90.84	196.32	31.03	24.21	-1.55	-3.02	-1.5
Fbxo15	51.7	112.9	0.96	0.76	-5.75	-7.21	-1.5
Lox	1.56	5.47	0.13	0.16	-3.64	-5.1	-1.5
Meox1	1.81	6.29	0.28	0.35	-2.69	-4.15	-1.5
Cdh3	12.78	32.6	1.78	1.68	-2.84	-4.28	-1.4
Cox7a1	19.02	15.88	9.37	2.89	-1.02	-2.46	-1.4
Zfp296	20.9	24.6	1.38	0.6	-3.92	-5.35	-1.4
Calml4	22.1	46.15	0.16	0.13	-7.07	-8.49	-1.4
Gtsf1l	38.07	33.88	0.37	0.12	-6.7	-8.12	-1.4
Tmem30b	2.86	6.3	0.23	0.19	-3.64	-5.06	-1.4
Ceacam1	8.97	8.86	1.97	0.73	-2.19	-3.61	-1.4
Phf19	3.14	5.17	0.9	0.55	-1.81	-3.23	-1.4
Ifitm3	123.18	148.81	63.2	28.73	-0.96	-2.37	-1.4
Spata22	1.26	3.32	0.1	0.1	-3.66	-5.06	-1.4
Gadd45b	7.05	18.38	3.23	3.19	-1.13	-2.53	-1.4
Map7	15.51	22.72	0.89	0.49	-4.13	-5.52	-1.4
Ly6a	1.86	2.48	0.53	0.27	-1.8	-3.19	-1.4
Tjp3	2.45	4.67	0.45	0.32	-2.46	-3.84	-1.4
Sfn	65.84	44.77	3.09	0.81	-4.41	-5.78	-1.4
Cldn4	27.53	40.01	2.07	1.17	-3.73	-5.1	-1.4
Dnajc6	5.43	14.42	0.19	0.2	-4.82	-6.18	-1.4
Mab21l3	1.28	2.08	0.65	0.41	-0.97	-2.33	-1.4
Kctd12b	1.35	5.25	0.31	0.47	-2.14	-3.49	-1.4
Naaa	2.85	4.7	1.04	0.67	-1.45	-2.8	-1.4
Slco4c1	4.23	11.84	0.26	0.28	-4.05	-5.39	-1.3
Cnksr1	2	2.12	0.41	0.17	-2.3	-3.63	-1.3
Ano9	19.43	20.36	0.6	0.25	-5.02	-6.34	-1.3
Atp6v0a4	1.79	3.41	0.14	0.11	-3.7	-5.02	-1.3
Plekha4	1.51	3.84	0.38	0.39	-1.98	-3.3	-1.3
Ephx2	10.01	3.43	4.11	0.57	-1.28	-2.59	-1.3
Klhl13	104.93	223.76	13.17	11.46	-2.99	-4.29	-1.3
Gjb3	24.41	39.06	2.62	1.71	-3.22	-4.51	-1.3
Neurog3	1.66	3.18	0.13	0.1	-3.71	-4.99	-1.3
Prss8	10.79	9.8	3.28	1.23	-1.72	-3	-1.3
Fam110b	5.42	9.1	2	1.38	-1.44	-2.72	-1.3
Gls2	8.07	11.92	3.56	2.18	-1.18	-2.45	-1.3
Cd55	5.91	7.32	3.07	1.57	-0.95	-2.22	-1.3
Tmem102	2.01	2.21	0.71	0.32	-1.51	-2.77	-1.3
Ttyh1	2.24	2.99	1.02	0.57	-1.13	-2.39	-1.3
Tex21	2.79	5.06	0.33	0.25	-3.07	-4.32	-1.3
Nanos3	3.66	9.31	0.47	0.5	-2.97	-4.22	-1.3
Ptgs2	1.82	3.83	0.29	0.25	-2.67	-3.92	-1.3
Nr5a2	16.4	31.38	0.25	0.2	-6.06	-7.3	-1.2
Dpys	2.95	7.09	0.13	0.13	-4.51	-5.75	-1.2
Sult6b1	3.24	6.97	0.3	0.27	-3.43	-4.67	-1.2
Nanog	90.69	180.1	10.59	8.87	-3.1	-4.34	-1.2
Ckb	106.41	237.53	17.4	16.46	-2.61	-3.85	-1.2
Tekt1	1.1	3.2	0.25	0.31	-2.14	-3.38	-1.2
Mok	5.06	11.56	1.91	1.85	-1.41	-2.65	-1.2
1700007K13Rik	13.2	16.64	1.08	0.58	-3.61	-4.84	-1.2
Trp73	1	1.87	0.13	0.1	-2.97	-4.2	-1.2
Pnp2	1.93	2.88	0.45	0.28	-2.11	-3.34	-1.2
Celf4	5.21	6.99	1.23	0.7	-2.09	-3.32	-1.2
Fgfbp1	28.63	82.26	0.4	0.5	-6.15	-7.37	-1.2
Khdc3	5.44	12.75	0.1	0.1	-5.77	-6.99	-1.2
Kcnk5	17.27	23.24	1.75	1.01	-3.3	-4.52	-1.2
Pde1b	8.85	18.64	1.19	1.07	-2.9	-4.12	-1.2
Serping1	4.74	8.13	0.98	0.72	-2.27	-3.49	-1.2

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ccno	3.19	3.25	0.71	0.31	-2.17	-3.39	-1.2
Manba	20.72	31.32	1.1	0.72	-4.24	-5.44	-1.2
Sgol2b	1.27	2.99	0.1	0.1	-3.66	-4.86	-1.2
Stat4	5.25	6.66	0.44	0.25	-3.57	-4.75	-1.2
AI467606	6.13	6.01	1.03	0.44	-2.58	-3.76	-1.2
Tert	5.46	4.3	2.16	0.76	-1.33	-2.51	-1.2
Glod5	5.06	14.11	0.14	0.17	-5.18	-6.35	-1.2
Foxj1	2.34	3.01	0.21	0.12	-3.5	-4.67	-1.2
Fbxo2	6.97	13.94	0.76	0.68	-3.19	-4.36	-1.2
Six1	5.66	11.84	0.67	0.62	-3.07	-4.24	-1.2
Cdkn2b	2.76	2.88	0.49	0.23	-2.49	-3.66	-1.2
Mt2	29.77	16.26	6.69	1.63	-2.15	-3.32	-1.2
Cpne5	3.95	11.4	1.08	1.39	-1.87	-3.04	-1.2
Aes	36.55	58.69	10.23	7.31	-1.84	-3.01	-1.2
Arntl2	2.44	3.79	0.88	0.61	-1.47	-2.64	-1.2
Ildr1	3.94	7.49	0.19	0.16	-4.35	-5.51	-1.2
Zdhhc23	2.1	2.14	0.36	0.17	-2.54	-3.7	-1.2
2610305D13Rik	114.23	71.44	9.38	2.65	-3.61	-4.75	-1.1
Slc9b1	12.34	11.81	1.18	0.51	-3.39	-4.53	-1.1
Grb7	21.25	21.38	2.96	1.35	-2.84	-3.98	-1.1
Hck	41.71	73.39	0.23	0.18	-7.51	-8.64	-1.1
Acyp2	2.99	8.28	1.21	1.53	-1.3	-2.43	-1.1
Pik3cd	4.09	9.23	0.78	0.81	-2.4	-3.52	-1.1
Glrx	6.92	9.66	1.32	0.85	-2.39	-3.51	-1.1
Steap3	5.46	8.43	0.42	0.3	-3.71	-4.82	-1.1
Fbxw17	5.38	7.65	2.36	1.56	-1.19	-2.3	-1.1
Cd9	100.9	103.93	13.98	6.73	-2.85	-3.95	-1.1
Tmem37	3.06	4.97	1.47	1.11	-1.06	-2.16	-1.1
Acbd7	4.86	4.95	0.43	0.21	-3.5	-4.59	-1.1
Anxa11	21.88	23.4	5.62	2.82	-1.96	-3.05	-1.1
C030039L03Rik	2.2	6.4	1.08	1.48	-1.02	-2.11	-1.1
Neurod1	2.45	5.3	0.1	0.1	-4.62	-5.7	-1.1
Angptl4	8.58	8.46	0.88	0.41	-3.28	-4.36	-1.1
Chmp4c	10.49	12.1	2.98	1.64	-1.81	-2.89	-1.1
Fam167a	1.83	4.84	0.57	0.71	-1.69	-2.77	-1.1
Procr	2.87	4.41	1.01	0.73	-1.51	-2.59	-1.1
Fkbp6	1.07	3.82	0.12	0.21	-3.15	-4.22	-1.1
Mvp	23.81	19.83	4.62	1.82	-2.37	-3.44	-1.1
Esrrb	44.46	63.93	0.63	0.43	-6.14	-7.2	-1.1
Hecw2	2.14	6.53	0.6	0.87	-1.84	-2.9	-1.1
Ggnbp1	2.5	5.53	0.79	0.84	-1.65	-2.71	-1.1
Msrb2	7.47	13.95	2.87	2.57	-1.38	-2.44	-1.1
Nrn1l	1.1	1.1	0.48	0.23	-1.19	-2.25	-1.1
Hspb1	341.54	222.16	12.35	3.94	-4.79	-5.82	-1.0
Slc25a12	23.95	47.15	2.52	2.42	-3.25	-4.28	-1.0
Slamf9	1.08	1.55	0.25	0.18	-2.11	-3.14	-1.0
2200002D01Rik	18.63	8.47	4.06	0.91	-2.2	-3.22	-1.0
Capg	29.8	25.51	7.1	3	-2.07	-3.09	-1.0
Tmem51	6.92	6.09	2.65	1.16	-1.38	-2.4	-1.0
Rsph1	1.37	2.61	0.65	0.61	-1.08	-2.1	-1.0
Msc	5.9	12.62	0.11	0.12	-5.72	-6.73	-1.0
Cd38	3.81	5.87	1.92	1.47	-0.99	-2	-1.0
Tdh	295.87	286.84	1.54	0.75	-7.58	-8.58	-1.0
Esrp2	6.86	7.77	0.4	0.22	-4.11	-5.11	-1.0
Abcb1b	20.26	19.42	2.13	1.02	-3.25	-4.25	-1.0
Kcnk1	21	25.18	5.97	3.59	-1.81	-2.81	-1.0
Raet1b	6.11	8.39	2.7	1.86	-1.18	-2.18	-1.0
Stac2	1.76	2.77	0.82	0.64	-1.11	-2.11	-1.0
Sec1	1.04	3.74	0.25	0.45	-2.07	-3.06	-1.0
Coch	18.29	18.73	7.26	3.76	-1.33	-2.32	-1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Msln	5.94	7.43	0.16	0.1	-5.24	-6.22	-1.0
Tmem125	3.72	6.64	0.16	0.14	-4.58	-5.56	-1.0
Vwa2	2.36	3.21	0.23	0.16	-3.37	-4.35	-1.0
Arhgap8	18.36	18.58	1.94	0.99	-3.25	-4.23	-1.0
Pqlc1	10.92	23.73	3.21	3.53	-1.77	-2.75	-1.0
Gsn	8.49	7.28	3.66	1.59	-1.21	-2.19	-1.0
Lgals9	5.61	6.09	0.49	0.28	-3.51	-4.47	-1.0
Noxred1	1.98	3.99	0.34	0.36	-2.53	-3.48	-1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
E030030I06Rik	1.16	7.26	1.17	0.7	0.01	-3.38	-3.4
Prelid2	9.49	1.62	10.23	0.32	0.11	-2.35	-2.5
Lefty2	58.4	149.87	33.38	17.14	-0.81	-3.13	-2.3
Aspg	2.86	1.03	3.68	0.27	0.36	-1.96	-2.3
Gm20594	2.92	6.44	2.95	1.52	0.02	-2.09	-2.1
Mgmt	13.53	1.86	7.85	0.28	-0.79	-2.73	-1.9
Prdm14	3.01	9.18	1.75	1.44	-0.78	-2.67	-1.9
H60b	2.64	5.03	3.17	1.71	0.26	-1.56	-1.8
Ifitm1	97.4	284.19	161.52	141.3	0.73	-1.01	-1.7
Prrc1	5.61	16.82	8.56	7.78	0.61	-1.11	-1.7
Rhpn2	12.32	31.98	16.32	13.24	0.41	-1.27	-1.7
As3mt	5.67	5.4	6.34	1.93	0.16	-1.49	-1.7
Tmem106a	1	1.51	0.62	0.3	-0.68	-2.31	-1.6
Acta1	5.14	18.77	3.4	4.03	-0.6	-2.22	-1.6
Wdr86	4.36	2.85	3.05	0.66	-0.52	-2.11	-1.6
Cenpm	13.45	26.95	10.51	7	-0.36	-1.95	-1.6
Klk8	12.48	8.81	10.02	2.35	-0.32	-1.91	-1.6
Apol8	1.02	2.09	0.68	0.48	-0.58	-2.13	-1.6
Tmem159	4.53	1.78	5.66	0.77	0.32	-1.21	-1.5
Gulo	1.98	3.12	1.34	0.74	-0.57	-2.08	-1.5
Dnaaf3	1.24	2.58	1	0.73	-0.31	-1.82	-1.5
Nrk	2.65	4.78	2.78	1.85	0.07	-1.37	-1.4
Nudt6	1.16	1.2	1.45	0.57	0.32	-1.08	-1.4
Myof	5.34	10.01	2.81	2.02	-0.93	-2.31	-1.4
Nkx3-1	1.43	2.81	0.99	0.74	-0.54	-1.92	-1.4
Sycp3	12.44	5.94	10.33	1.89	-0.27	-1.65	-1.4
B230217C12Rik	1.87	5.08	2.27	2.37	0.28	-1.1	-1.4
Psors1c2	2.46	5.02	1.35	1.09	-0.86	-2.21	-1.4
2010107G23Rik	2.94	6.39	1.56	1.37	-0.91	-2.22	-1.3
Gimap9	1.76	4.31	1.55	1.53	-0.18	-1.49	-1.3
Fam58b	9.45	8.57	11.17	4.34	0.24	-0.98	-1.2
Gprc5b	5.04	9.13	3.51	2.77	-0.52	-1.72	-1.2
Selm	9.02	8.42	9.17	3.74	0.02	-1.17	-1.2
Lrpap1	31.75	52.17	18.56	13.52	-0.77	-1.95	-1.2
Alpl	23.96	29.19	23.64	12.72	-0.02	-1.2	-1.2
Birc3	2.59	3.85	2.28	1.52	-0.19	-1.34	-1.2
Smim20	15.29	5.44	14.69	2.37	-0.06	-1.2	-1.1
Tnip2	3.34	4.31	1.89	1.12	-0.82	-1.95	-1.1
Id4	1.75	3.54	1.11	1.03	-0.66	-1.78	-1.1
Prkch	1.69	3.73	1.22	1.25	-0.47	-1.58	-1.1
Bgn	3.46	6.55	3.4	2.99	-0.02	-1.13	-1.1
Hyal1	2.56	2.41	1.85	0.86	-0.47	-1.49	-1.0
Rbm43	3.12	7.66	2.77	3.35	-0.17	-1.19	-1.0
Urgcp	4.01	7.34	3.54	3.22	-0.18	-1.19	-1.0
Rundc3a	1.28	3.69	0.8	1.16	-0.67	-1.67	-1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Plin5	1.26	1.61	0.69	0.45	-0.87	-1.85	-1.0
Trmt2b	3.67	7.23	3.06	3.05	-0.26	-1.24	-1.0
Prss36	1.94	5.66	1.78	2.64	-0.12	-1.1	-1.0
Rnh1	42.46	34.8	28.97	12.18	-0.55	-1.52	-1.0
Pced1b	10.96	21.04	6.62	6.52	-0.73	-1.69	-1.0
Lpar1	4.17	7.73	2.64	2.52	-0.66	-1.62	-1.0
Mrm1	6.54	12.49	4.67	4.58	-0.49	-1.45	-1.0
Crtap	22.03	13.11	11.85	3.66	-0.89	-1.84	-1.0
Stx3	24.33	23.53	15.79	7.98	-0.62	-1.56	-0.9
Cryl1	5.98	12.99	3.67	4.23	-0.7	-1.62	-0.9
Serpinb6a	50.87	48.04	34.56	17.24	-0.56	-1.48	-0.9
Fbxo44	1.54	2.97	1.44	1.46	-0.1	-1.02	-0.9
Mkx	5.84	6.27	3.73	2.15	-0.65	-1.54	-0.9
Acbd4	5.19	5.65	3.32	1.96	-0.65	-1.53	-0.9
Tmco4	1.29	1.77	0.84	0.63	-0.62	-1.5	-0.9
Fam184b	1.33	1.02	1.03	0.43	-0.36	-1.23	-0.9
Ptpn6	9.43	8.79	8.5	4.33	-0.15	-1.02	-0.9
Tmem63a	4.78	5.97	2.61	1.8	-0.87	-1.73	-0.9
Snx10	16.59	31.5	9.57	10.06	-0.79	-1.65	-0.9
Ghdc	4.32	1.61	3.1	0.63	-0.48	-1.34	-0.9
Hspb2	1.39	2.13	1.02	0.86	-0.45	-1.31	-0.9
Rbfa	25.37	11.15	17.2	4.21	-0.56	-1.41	-0.9
Zfp710	27.23	51.92	20.06	21.29	-0.44	-1.29	-0.9
Cr1l	15.78	15.82	14.23	7.89	-0.15	-1	-0.9
Plekhf1	7.15	12.19	4.11	3.9	-0.8	-1.64	-0.8
Ifi30	26.36	24.18	17.54	8.97	-0.59	-1.43	-0.8
Gbp3	1.8	4.13	1.21	1.56	-0.57	-1.41	-0.8
Bex4	63	75.37	34.89	23.46	-0.85	-1.68	-0.8
Parp9	7.54	11.13	4.35	3.64	-0.79	-1.61	-0.8
Ficd	1.54	2.37	1.11	0.97	-0.47	-1.29	-0.8
Dok1	5.82	5.94	4.24	2.45	-0.46	-1.28	-0.8
Fzd8	1.02	1.72	0.76	0.73	-0.43	-1.24	-0.8
Phgdh	53.47	72.05	42.85	33.03	-0.32	-1.13	-0.8
Bbc3	7.06	10.14	5.11	4.21	-0.47	-1.27	-0.8
Lpar4	10.22	12.38	8.68	6.08	-0.24	-1.03	-0.8
Tagln	72.05	99.05	49.97	40.07	-0.53	-1.31	-0.8
Trim67	1.39	1.22	1.03	0.53	-0.42	-1.2	-0.8
Gstm1	10.52	14.2	8.09	6.36	-0.38	-1.16	-0.8
Spock2	1.46	3.37	1.04	1.4	-0.5	-1.27	-0.8
Abhd6	3.98	5.01	3	2.21	-0.41	-1.18	-0.8
Gpc4	9.1	13.44	5.33	4.64	-0.77	-1.53	-0.8
Tdrd5	2.74	3.11	1.85	1.25	-0.57	-1.32	-0.8
Npepl1	9.58	10.21	7.53	4.78	-0.35	-1.1	-0.8
Zbtb38	6.28	9.4	5.09	4.55	-0.3	-1.05	-0.8
Cradd	8.68	11.58	5.44	4.35	-0.67	-1.41	-0.7
Tnnt1	2.13	3.71	1.25	1.32	-0.77	-1.49	-0.7
Mta3	43.67	50.64	30.16	21.26	-0.53	-1.25	-0.7
Tmem160	17.16	16.35	13.68	7.9	-0.33	-1.05	-0.7
Dcaf4	27.17	35.9	19.22	15.52	-0.5	-1.21	-0.7
Mpp1	13.3	16.25	10.79	8.1	-0.3	-1.01	-0.7
Ggact	3.3	5.39	2.33	2.36	-0.5	-1.19	-0.7
Chac2	5.24	5.86	3.12	2.18	-0.75	-1.43	-0.7
Nabp1	23	38.7	12.87	13.56	-0.84	-1.51	-0.7
Slc9a3r1	36.23	45.57	24.29	19.15	-0.58	-1.25	-0.7
Sms	30.41	45.94	17.86	17	-0.77	-1.43	-0.7
Pon2	10.06	9.68	7.13	4.35	-0.5	-1.16	-0.7
Nid1	24.07	36.35	19.74	18.86	-0.29	-0.95	-0.7
Nbl1	1.42	1.6	0.81	0.58	-0.81	-1.45	-0.6
Mtg2	13.01	17.18	7.69	6.51	-0.76	-1.4	-0.6
Ntpcr	8.75	6.04	6.65	2.94	-0.4	-1.04	-0.6

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Lmo7	10.44	9.2	8.38	4.74	-0.32	-0.96	-0.6
Prdm5	16.96	17.14	9.01	5.89	-0.91	-1.54	-0.6
Tsacc	8.3	13.41	5.13	5.37	-0.69	-1.32	-0.6
Aim1l	1.67	1.56	0.96	0.58	-0.81	-1.43	-0.6
Syce2	63.65	50.02	40.4	20.54	-0.66	-1.28	-0.6
Zfp583	3.44	3.81	2.25	1.62	-0.61	-1.23	-0.6
Qpct	1.61	1.33	1.06	0.57	-0.6	-1.22	-0.6
Parp12	5.09	7.12	3.86	3.5	-0.4	-1.02	-0.6
Lace1	4.49	5.9	3.47	2.98	-0.37	-0.99	-0.6
Zbtb24	9.56	13.64	7.08	6.69	-0.43	-1.03	-0.6
Plec	21.45	23.61	12.27	8.95	-0.81	-1.4	-0.6
Tpk1	6.34	11.56	3.67	4.45	-0.79	-1.38	-0.6
Nr1d2	14.11	22.27	8.24	8.63	-0.78	-1.37	-0.6
L2hgdh	24.48	36.5	15.57	15.41	-0.65	-1.24	-0.6
Tarsl2	5.95	6.58	3.18	2.36	-0.9	-1.48	-0.6
Mrpl36	36.31	49.58	22.43	20.55	-0.7	-1.27	-0.6
Rpusd1	7.19	10.53	4.47	4.41	-0.69	-1.26	-0.6
Nudt14	15.33	19.28	11.29	9.58	-0.44	-1.01	-0.6
Arxes2	13.16	13.13	9.95	6.72	-0.4	-0.97	-0.6
Slc10a3	3.67	5.19	2.29	2.19	-0.68	-1.24	-0.6
Prr5l	3.37	5.03	1.78	1.82	-0.92	-1.47	-0.6
Zfp185	4.25	5.22	2.64	2.21	-0.69	-1.24	-0.6
Engase	2.59	4.32	1.87	2.14	-0.47	-1.02	-0.6
Aprt	195.76	225.67	146.07	115.24	-0.42	-0.97	-0.6
Rbl2	3.11	4.61	1.73	1.75	-0.85	-1.39	-0.5
Hist1h2af	30.49	28.6	19.51	12.59	-0.64	-1.18	-0.5
Ormdl2	20.44	21.34	13.64	9.83	-0.58	-1.12	-0.5
Enoph1	34.81	50.66	23.97	23.93	-0.54	-1.08	-0.5
Top3b	33.37	51.4	20.38	21.69	-0.71	-1.24	-0.5
Slc1a3	9.11	11.51	5.64	4.93	-0.69	-1.22	-0.5
Jund	7.95	10.96	5.19	4.95	-0.62	-1.15	-0.5
4930452B06Rik	6.81	10.62	5	5.4	-0.45	-0.98	-0.5
Angptl6	1.55	3.62	0.83	1.35	-0.9	-1.42	-0.5
Pak4	24.47	29.46	15.73	13.22	-0.64	-1.16	-0.5
Zbed5	11.82	14.64	7.21	6.3	-0.71	-1.22	-0.5
Rnf19b	15.76	20.73	11.65	10.74	-0.44	-0.95	-0.5
Tmem2	29.18	53.33	21.46	27.65	-0.44	-0.95	-0.5
Cpt1c	14.62	16.04	7.76	6.05	-0.91	-1.41	-0.5
Fgd1	20.15	27.88	12.42	12.17	-0.7	-1.2	-0.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Coro2b	1.96	0.52	1.16	1.9	-0.75	1.86	2.6
Ogfrl1	3.81	0.85	3.79	4.26	-0.01	2.33	2.3
Satb1	5.04	1.31	6.78	8.16	0.43	2.64	2.2
Zfp438	2.16	0.42	2.32	2.09	0.1	2.3	2.2
Osbpl10	3.14	0.9	3.64	4.06	0.21	2.17	2.0
Aldh1a2	1.78	0.91	3.18	5.71	0.84	2.65	1.8
Mecom	1.01	0.48	1.25	2	0.31	2.05	1.7
Cib2	2.18	1.19	2.94	5.34	0.43	2.16	1.7
Emc9	1.46	0.58	1.12	1.42	-0.38	1.3	1.7
Xylt1	1.86	0.88	1.58	2.36	-0.24	1.42	1.7
Capn2	38.54	13.02	43.17	45.4	0.16	1.8	1.6
Slco3a1	2.28	0.56	3.49	2.55	0.61	2.18	1.6
Eif2s3y	22.76	2.2	18.84	5.12	-0.27	1.22	1.5
Hmgn5	4.53	1.28	4.74	3.69	0.06	1.53	1.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Fzd4	1.85	0.95	2.9	4.05	0.65	2.09	1.4
Arnt2	1.73	0.88	2.03	2.79	0.23	1.66	1.4
Rasal1	1.2	0.49	1.04	1.14	-0.21	1.21	1.4
Bcl11a	1.71	0.45	3.14	2.2	0.88	2.29	1.4
Smarca1	15.08	6.65	21.45	24.8	0.51	1.9	1.4
Ttc39c	0.8	0.33	1.52	1.62	0.92	2.29	1.4
Fhod3	1.43	0.43	2.72	2.07	0.93	2.28	1.4
Cdx2	2.02	1.43	2.41	4.36	0.26	1.61	1.4
Acadl	13.65	3.01	16.88	9.38	0.31	1.64	1.3
Basp1	46.7	20.76	46.33	51.52	-0.01	1.31	1.3
Zbtb42	0.62	0.33	1.09	1.42	0.82	2.1	1.3
Pacsin3	5.95	2.5	5.14	5.21	-0.21	1.06	1.3
Vegfc	7.74	2.74	12.49	10.5	0.69	1.94	1.3
Ncs1	5.81	2.98	10.79	13	0.89	2.13	1.2
Fam89a	1.87	0.69	2.33	2.03	0.32	1.56	1.2
Tnfsf9	1.64	0.61	1.54	1.34	-0.09	1.13	1.2
Mafk	7.46	3.31	6.73	6.92	-0.15	1.07	1.2
Mapk12	5.09	3.54	6.91	10.83	0.44	1.61	1.2
Ckap4	43.04	18.6	40.49	39.05	-0.09	1.07	1.2
Gdpd5	1	0.59	1.75	2.27	0.8	1.95	1.2
Ak4	9.62	4.95	14.53	16.29	0.59	1.72	1.1
Rap1gds1	21.39	9.56	35.75	34.68	0.74	1.86	1.1
Gngt2	10.69	6.24	14.68	18.61	0.46	1.58	1.1
Zfp948	11.06	3.64	19.37	13.74	0.81	1.92	1.1
Ptprn	3.71	1.74	4.79	4.84	0.37	1.47	1.1
Ssbp2	1.74	1.06	3.28	4.27	0.92	2.01	1.1
Pgbd1	1.57	0.86	2.15	2.51	0.45	1.54	1.1
Cnr1	0.84	0.55	1.29	1.78	0.62	1.7	1.1
Slc35c1	2.51	1.07	3.53	3.17	0.5	1.57	1.1
Casp3	39.06	14.93	47.39	37.75	0.28	1.34	1.1
Nrbp2	3.44	2.44	6.03	8.87	0.81	1.86	1.1
Tet3	3.78	2.18	5.61	6.74	0.57	1.62	1.1
Bend4	16.25	6.72	18.35	15.72	0.18	1.23	1.1
Col8a2	1.06	0.7	1.68	2.3	0.67	1.71	1.0
Rnd2	11.48	5.73	17.35	17.84	0.6	1.64	1.0
Gli3	8.04	4.35	11.58	12.89	0.53	1.57	1.0
Ldlrap1	8.38	3.36	8.54	7.01	0.03	1.06	1.0
Akap9	13.78	6.7	17.43	17.25	0.34	1.36	1.0
Zdbf2	25.92	15.51	28.14	34.09	0.12	1.14	1.0
Ankrd12	2.72	1.38	4.12	4.2	0.6	1.61	1.0
Farp2	0.96	0.54	1.79	2.02	0.9	1.9	1.0
Selo	2.7	1.1	4.83	3.94	0.84	1.84	1.0
Gpr161	2.07	0.79	3.64	2.78	0.82	1.82	1.0
Sema7a	1.55	0.83	2.41	2.58	0.64	1.64	1.0
Inpp5f	8.92	5.11	12.23	14.07	0.46	1.46	1.0
Ccdc112	2.47	1.89	3	4.6	0.28	1.28	1.0
Kctd12	5.32	2.33	6.16	5.4	0.21	1.21	1.0
Kif1a	4.49	1.42	4.5	2.86	0	1	1.0
Asap2	4.82	2.92	8.75	10.49	0.86	1.85	1.0
Slc30a4	3.8	1.61	6.77	5.65	0.83	1.81	1.0
Kcnt2	0.85	0.37	1.4	1.21	0.72	1.7	1.0
Irgq	3.7	2.06	3.81	4.18	0.04	1.02	1.0
Atf3	2.7	1.07	4.46	3.49	0.73	1.7	1.0
Cxcl12	0.86	0.68	1.04	1.62	0.29	1.26	1.0
Sort1	9.39	6.14	16.31	20.77	0.8	1.76	1.0
Bmp1	9.16	5.07	14.74	15.96	0.69	1.65	1.0
Tmem158	0.71	0.39	1.11	1.21	0.65	1.61	1.0
Arl4c	8.97	5.06	9.64	10.52	0.1	1.06	1.0
D1Ertd622e	4.6	2.43	5.44	5.55	0.24	1.19	1.0
6720489N17Rik	6.47	3.42	10.88	11.03	0.75	1.69	0.9

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Gpr173	0.66	0.36	1.01	1.06	0.62	1.56	0.9
Parvb	9.36	4.3	11.29	9.97	0.27	1.21	0.9
Tmem150a	4.21	2.4	5.04	5.52	0.26	1.2	0.9
Psen2	2.82	1.64	3.09	3.44	0.13	1.07	0.9
Gulp1	3.54	1.9	6.41	6.53	0.86	1.78	0.9
Pfkfb3	4.25	2.06	6.58	6	0.63	1.55	0.9
Celsr1	2.55	2.05	4.45	6.71	0.8	1.71	0.9
Tpi1	275.17	114.43	455.3	356.56	0.73	1.64	0.9
Tagln2	80.28	41.55	117.28	114.02	0.55	1.46	0.9
Kdelr3	5.61	2.9	6.34	6.16	0.18	1.09	0.9
Tal2	2.05	1.57	2.29	3.3	0.16	1.07	0.9
Efnb2	11.33	4.9	20.1	16.18	0.83	1.72	0.9
4932438A13Rik	15.71	9.27	19.7	21.32	0.33	1.2	0.9
Garnl3	3.18	2.67	3.57	5.45	0.16	1.03	0.9
Fam107b	9.57	6.72	10.56	13.53	0.14	1.01	0.9
Tubb6	66.53	37	99.15	100.6	0.58	1.44	0.9
Rassf2	1.64	1.09	2.23	2.7	0.45	1.31	0.9
Kdm2b	23.4	15.32	24.92	29.53	0.09	0.95	0.9
Hmgn3	3.74	2.13	4.65	4.79	0.31	1.16	0.9
Armcx6	1.17	0.61	1.87	1.75	0.68	1.52	0.8
Zfp800	5.7	2.38	6.92	5.19	0.28	1.12	0.8
Gpr153	1.38	0.63	1.62	1.34	0.24	1.08	0.8
Phip	30.59	17.45	39.51	40.15	0.37	1.2	0.8
Hps6	3.32	1.57	4.02	3.37	0.28	1.11	0.8
Lrrc8c	4.85	3.37	7.87	9.67	0.7	1.52	0.8
Mttp	2.39	1.35	3.62	3.61	0.6	1.42	0.8
Gcnt1	11.73	5.22	15.55	12.24	0.41	1.23	0.8
Nckap5l	3.67	2.34	6.93	7.79	0.92	1.73	0.8
Limd1	12.27	7.79	20.44	22.85	0.74	1.55	0.8
Jph1	4.31	1.36	6.53	3.61	0.6	1.41	0.8
Mark1	2.89	1.81	4.31	4.75	0.58	1.39	0.8
Tpr	90.37	55.33	101.17	108.27	0.16	0.97	0.8
B4galt6	21.02	14.38	23.38	27.93	0.15	0.96	0.8
Fgf13	2.89	1.45	3.36	2.94	0.22	1.02	0.8
Pcdh17	1.1	0.74	1.61	1.86	0.55	1.34	0.8
Ktn1	71.3	39.8	87.42	84.4	0.29	1.08	0.8
Gpm6b	1.03	0.41	1.73	1.19	0.75	1.53	0.8
Magee1	0.87	0.62	1.28	1.54	0.55	1.32	0.8
Pfkl	94.96	47.44	136.86	116.89	0.53	1.3	0.8
Man1a	3.17	1.93	5	5.17	0.66	1.42	0.8
Mthfsl	3.81	0.84	4.77	1.76	0.32	1.08	0.8
Aldh9a1	27.92	11.56	34.67	24.35	0.31	1.07	0.8
Chml	7.43	6.14	8.99	12.49	0.27	1.03	0.8
Mapk8ip2	1.19	1.21	2.26	3.91	0.93	1.68	0.8
Bnip3	33.91	17.55	60.15	52.54	0.83	1.58	0.8
Ppap2c	11.1	4.37	17.29	11.48	0.64	1.39	0.8
Tmod2	3.65	3.15	5.18	7.5	0.5	1.25	0.8
Zfp943	4.31	2.71	5.32	5.63	0.3	1.05	0.8
Hdx	5.72	3.08	9.46	8.52	0.73	1.47	0.7
Clip3	3.99	3.92	4.97	8.1	0.31	1.05	0.7
Rps6ka2	7.65	4.52	9.41	9.26	0.3	1.04	0.7
Trib1	6.19	3.64	11.15	10.9	0.85	1.58	0.7
Zc3h7b	22.26	12.52	35.73	33.35	0.68	1.41	0.7
Primpol	3.91	2.53	6.07	6.56	0.64	1.37	0.7
Src	13.52	8.3	17.85	18.18	0.4	1.13	0.7
Invs	1.49	1.13	2.79	3.47	0.9	1.62	0.7
Rps16	356.56	163.92	591.44	447.77	0.73	1.45	0.7
Aff3	6.06	4.34	9.46	11.14	0.64	1.36	0.7
Phf10	46.78	26.7	69.33	65.51	0.57	1.29	0.7
Prdm11	1	0.82	1.37	1.84	0.45	1.17	0.7

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Murc	4.61	2.56	7.78	7.04	0.75	1.46	0.7
Slc4a7	23.21	12.73	31.18	28.15	0.43	1.14	0.7
Rin3	1.66	1.34	2.15	2.85	0.38	1.09	0.7
Lcorl	5.54	2.95	6.95	6.09	0.33	1.04	0.7
Dennd2a	12.24	8.49	23.15	26.19	0.92	1.62	0.7
Lrrfip1	15.19	8.76	23.72	22.22	0.64	1.34	0.7
1700017B05Rik	9.74	4.76	14.08	11.18	0.53	1.23	0.7
Slc9a7	1.34	0.67	2.09	1.7	0.65	1.34	0.7
Amn1	5.53	4.58	8.29	11.04	0.58	1.27	0.7
Zfp319	1.9	1.6	3.44	4.67	0.86	1.54	0.7
D830031N03Rik	6.45	4.98	9.67	11.89	0.58	1.26	0.7
Nagk	6.07	3.85	9.08	9.21	0.58	1.26	0.7
Fam189b	1.83	1.24	2.56	2.78	0.48	1.16	0.7
Ctsf	5.4	2.93	7.3	6.32	0.43	1.11	0.7
Plekha2	14.82	9.14	17.82	17.61	0.27	0.95	0.7
Maff	5.17	3.07	9.22	8.7	0.83	1.5	0.7
Hmgcll1	1.78	1.17	2.71	2.82	0.6	1.27	0.7
Plxna3	4.56	3.63	6.32	8.01	0.47	1.14	0.7
Nipbl	27.29	16.07	36.79	34.46	0.43	1.1	0.7
Gpx7	6.61	2.38	8.66	4.96	0.39	1.06	0.7
Impad1	27.5	14.05	50.19	40.53	0.87	1.53	0.7
Panx1	5.11	3.28	9.24	9.32	0.85	1.51	0.7
Ppp2r2c	2.73	1.83	4.58	4.82	0.75	1.4	0.7
2810417H13Rik	53.58	36.81	71.39	76.99	0.41	1.06	0.7
Dusp16	9	5.36	11.14	10.41	0.31	0.96	0.7
Prkaca	23.26	19.81	28.8	38.44	0.31	0.96	0.7
Tmem86b	1.39	0.88	2.3	2.25	0.73	1.36	0.6
Trim44	24.83	12.2	38.82	29.5	0.64	1.27	0.6
Homez	1.51	1.23	2.27	2.87	0.59	1.22	0.6
Simc1	7.58	3.33	11.17	7.61	0.56	1.19	0.6
Slc30a9	24.28	16.57	35.12	36.97	0.53	1.16	0.6
Pde4dip	7.89	6.04	12.86	15.12	0.7	1.32	0.6
Elovl1	13.45	8.16	19.67	18.34	0.55	1.17	0.6
Exoc6b	15.26	10.07	20.53	20.82	0.43	1.05	0.6
Arhgef11	8.84	6.13	11.26	12.02	0.35	0.97	0.6
Zdhhc8	4.06	3	5.51	6.21	0.44	1.05	0.6
Ptpdc1	2.39	1.98	3.04	3.85	0.35	0.96	0.6
Dusp22	5.96	3.6	10.97	10.03	0.88	1.48	0.6
Myo9a	12.14	9.46	16.75	19.78	0.46	1.06	0.6
Dync2h1	5.28	3.53	9.78	9.87	0.89	1.48	0.6
Repin1	4.76	3.42	8.82	9.52	0.89	1.48	0.6
Slc43a3	1.99	1.14	3.28	2.85	0.72	1.31	0.6
Cenpf	33.42	19.66	53.02	46.97	0.67	1.26	0.6
Zkscan7	1.04	0.76	1.51	1.67	0.54	1.13	0.6
Nbeal1	10.47	7.1	13.8	14.07	0.4	0.99	0.6
Adamts15	0.84	0.52	1.1	1.02	0.38	0.97	0.6
Scd2	70.34	64.5	133.51	182.17	0.92	1.5	0.6
Ptk7	55.7	43.82	101.35	118.63	0.86	1.44	0.6
Sptbn1	61.35	43.61	111.35	118.21	0.86	1.44	0.6
Cnot6l	5.67	3.67	10.1	9.73	0.83	1.41	0.6
Dvl3	5.2	3.72	7.94	8.48	0.61	1.19	0.6
Car4	7.97	7.49	11.08	15.51	0.47	1.05	0.6
Ap1m1	18.81	14.99	25.25	29.97	0.42	1	0.6
Fgfr3	1.45	1.53	2.74	4.3	0.92	1.49	0.6
Nlgn3	1.02	1.45	1.64	3.44	0.68	1.25	0.6
Wrn	6.17	4.12	9.78	9.76	0.67	1.24	0.6
Slc4a3	1.12	0.93	1.54	1.91	0.46	1.03	0.6
Vat1	17.2	9.93	22.46	19.19	0.38	0.95	0.6
Myb	2.09	1.52	3.74	4	0.84	1.4	0.6
Syne2	3.27	2.76	5.86	7.28	0.84	1.4	0.6

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Asap1	8.88	6.41	15.06	15.99	0.76	1.32	0.6
Stat6	7.63	3.44	10.47	6.97	0.46	1.02	0.6
Stag2	42.56	28.41	81.81	79.82	0.94	1.49	0.6
Mcoln3	1.07	0.67	2.02	1.84	0.91	1.46	0.6
Dapk1	17.55	13.03	31.49	34.2	0.84	1.39	0.6
Rufy3	8.5	7.02	14.57	17.7	0.78	1.33	0.6
Edem1	14.95	11.74	23.01	26.37	0.62	1.17	0.6
Pcm1	34.44	24.74	52.74	55.09	0.61	1.16	0.6
Zfp40	2.83	2.14	3.83	4.24	0.44	0.98	0.5
Atp2b1	21.12	15.58	35.57	37.73	0.75	1.28	0.5
Akt1	21.9	14.69	36.38	35.06	0.73	1.26	0.5
Ptprs	30.81	27.42	47.73	61.45	0.63	1.16	0.5
Lrp12	2.85	2.29	4.36	5.05	0.61	1.14	0.5
Cenpe	31.47	17.07	45.96	35.97	0.55	1.08	0.5
Igsf10	2.6	1.69	3.6	3.37	0.47	1	0.5
Ralgapa1	11.28	9.45	17.38	20.76	0.62	1.14	0.5
Galnt7	12.97	8.78	19.78	19.25	0.61	1.13	0.5
Tulp3	10.88	7.61	16.58	16.68	0.61	1.13	0.5
Zfp62	20.05	13.96	29.39	29.3	0.55	1.07	0.5
Nphp1	4.68	3.76	6.8	7.83	0.54	1.06	0.5
Cep97	9.37	6.22	13.56	12.86	0.53	1.05	0.5
Celf2	1.88	1.56	2.66	3.16	0.5	1.02	0.5
Hdgfrp3	3.43	2.94	5.79	7.03	0.75	1.26	0.5
Ago2	31.84	23.65	48.61	51.4	0.61	1.12	0.5
Fosb	2.35	0.75	3.39	1.54	0.53	1.04	0.5
Notch1	7.27	5.39	12.72	13.4	0.81	1.31	0.5
Ypel1	2.83	3.69	4.74	8.78	0.75	1.25	0.5
Kif1c	9.98	7.37	14.48	15.19	0.54	1.04	0.5
Diap3	38.42	29.91	55.28	60.51	0.52	1.02	0.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ndufa4l2	2.57	0.1	13.3	5.57	2.37	5.8	3.4
Nphp3	3.61	1	7.58	10.16	1.07	3.35	2.3
Slc16a3	46.83	7.91	132.11	97.53	1.5	3.62	2.1
Sall3	2.27	0.67	10.84	13.44	2.25	4.32	2.1
Lrp8	1.91	0.49	4.12	4	1.11	3.03	1.9
Lrrc3	0.94	0.35	2.13	2.94	1.18	3.06	1.9
Tmem164	6.32	1.99	31.48	35.25	2.32	4.15	1.8
Trim21	2.93	0.37	5.77	2.57	0.98	2.78	1.8
Hoxb1	0.14	0.12	4.03	12.32	4.88	6.67	1.8
Efna5	2.32	0.78	9.05	10.13	1.96	3.7	1.7
Tmsb15a	0.58	0.1	1.94	1.07	1.74	3.42	1.7
Reep1	1.83	0.49	4.3	3.66	1.24	2.91	1.7
Fignl2	2.67	0.7	10.55	8.79	1.98	3.64	1.7
Des	1.67	0.36	6.64	4.52	1.99	3.64	1.7
Man1c1	1.58	0.61	15.97	18.13	3.34	4.9	1.6
Micall1	2.15	0.66	6.51	5.9	1.6	3.16	1.6
Tbc1d4	5.38	1.39	15.07	11.52	1.49	3.05	1.6
Reln	0.59	0.22	7.3	8.03	3.63	5.18	1.6
Mef2c	0.41	0.21	1.05	1.53	1.35	2.89	1.5
Tnnc1	1.35	0.6	17.89	22.35	3.72	5.21	1.5
Vstm2b	0.44	0.12	48.56	37.33	6.79	8.27	1.5
Dpysl5	3.96	1.67	28.7	33.86	2.86	4.34	1.5
Nfe2	0.29	0.17	2.12	3.52	2.86	4.34	1.5
Ntm	0.45	0.14	2.51	2.13	2.48	3.95	1.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Pde5a	2.53	0.71	8.12	6.25	1.68	3.13	1.5
Trim62	0.53	0.31	1.33	2.13	1.33	2.78	1.5
Soga1	3.22	1.37	11.82	13.68	1.88	3.32	1.4
Cdh8	0.31	0.11	2.37	2.13	2.94	4.34	1.4
Itga8	3.97	1.22	8.66	7	1.13	2.52	1.4
Slc30a3	0.54	0.19	4.53	4.2	3.06	4.44	1.4
Zfp629	2.18	0.75	11.43	10.14	2.39	3.75	1.4
Gas1	0.71	0.2	24.72	17.56	5.12	6.47	1.4
Slc32a1	0.14	0.11	1.13	2.23	3.04	4.39	1.4
Rsph3b	0.99	0.23	2.22	1.32	1.17	2.52	1.4
Il3ra	1.3	0.48	2.83	2.66	1.13	2.48	1.4
Mapre3	0.53	0.26	1.21	1.51	1.2	2.51	1.3
Zmiz1	5.04	2.36	17.95	20.48	1.83	3.12	1.3
Dusp10	2.63	1.07	10.09	9.94	1.94	3.22	1.3
E330009J07Rik	2.87	1.05	9.49	8.44	1.73	3.01	1.3
Grb10	47.73	20.41	158.29	163.92	1.73	3.01	1.3
Ubash3b	0.83	0.41	5.34	6.24	2.68	3.94	1.3
Nol4l	0.86	0.42	5.4	6.32	2.65	3.91	1.3
Prrg1	0.79	0.29	2.97	2.63	1.91	3.17	1.3
Emilin2	0.83	0.47	4.69	6.4	2.51	3.76	1.3
Klhl29	0.25	0.14	1.05	1.41	2.07	3.32	1.3
Dact1	2.11	1.11	6.26	7.78	1.57	2.81	1.2
Prkd1	1.16	0.53	22.11	23.91	4.25	5.48	1.2
Ager	0.46	0.26	1.95	2.6	2.09	3.32	1.2
Ccdc109b	0.24	0.18	2.07	3.66	3.12	4.33	1.2
Ccdc136	7.11	3.58	14.92	17.33	1.07	2.27	1.2
Gfra2	0.59	0.3	13.26	15.57	4.5	5.69	1.2
Has2	4.58	1.76	85.58	74.42	4.22	5.4	1.2
Col26a1	0.32	0.17	3.51	4.15	3.47	4.65	1.2
Stc1	0.62	0.29	2.59	2.76	2.06	3.24	1.2
Ptprj	4.43	1.59	13.6	11.08	1.62	2.8	1.2
Axin2	4.39	2.05	26.2	27.43	2.58	3.75	1.2
Slc38a5	0.59	0.3	1.33	1.53	1.16	2.33	1.2
Bmp6	0.32	0.18	1.96	2.5	2.61	3.76	1.2
Ptprd	0.61	0.32	1.89	2.2	1.64	2.79	1.2
Ano10	2.03	0.31	15.22	5.19	2.91	4.05	1.1
Kcnmb4	0.47	0.24	7.92	8.84	4.07	5.2	1.1
Plac1	0.14	0.1	2.28	3.58	4.03	5.16	1.1
Asxl3	0.93	0.43	5.25	5.3	2.49	3.61	1.1
Nfxl1	9.61	6.74	34.74	52.82	1.85	2.97	1.1
Optn	2.41	0.8	8.72	6.31	1.85	2.97	1.1
Apln	0.57	0.23	2.03	1.78	1.84	2.96	1.1
Usp51	1.58	0.58	7.24	5.75	2.19	3.3	1.1
Siah2	1.48	0.73	5.9	6.34	2	3.11	1.1
Zbtb16	0.17	0.1	1.24	1.59	2.83	3.93	1.1
Farp1	3.55	1.95	11.71	13.8	1.72	2.82	1.1
Sacs	3.14	1.43	6.07	5.95	0.95	2.05	1.1
Cep112	0.39	0.23	2.11	2.65	2.43	3.51	1.1
Zyx	15.47	8.67	31.73	37.63	1.04	2.12	1.1
Erg	0.16	0.11	1.14	1.66	2.8	3.87	1.1
Adra2a	0.25	0.14	1.62	1.98	2.71	3.78	1.1
Ccnjl	2.47	1.16	10.86	10.68	2.13	3.2	1.1
Ppic	18.1	7.32	171.04	144.04	3.24	4.3	1.1
Arhgap10	4.78	2.12	10.12	9.35	1.08	2.14	1.1
Zcchc24	4.11	2.42	8.44	10.35	1.04	2.1	1.1
Ptgds	0.75	0.1	9.23	2.53	3.62	4.66	1.0
Slc18a2	0.13	0.11	1.39	2.42	3.45	4.49	1.0
Sema4c	1.36	0.59	10.24	9.18	2.92	3.96	1.0
Clip1	4.28	2.41	14.24	16.43	1.74	2.77	1.0
Fras1	0.83	0.49	2.38	2.83	1.52	2.55	1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rasgrp4	0.54	0.3	2.01	2.33	1.91	2.93	1.0
Vav3	1.01	0.53	3.45	3.68	1.77	2.79	1.0
Cgnl1	9.8	5.53	24.97	28.67	1.35	2.37	1.0
Olfm2	0.88	0.33	1.97	1.48	1.17	2.19	1.0
Ptprt	0.38	0.22	2.16	2.56	2.5	3.51	1.0
Pcca	2.12	0.82	5.82	4.52	1.46	2.47	1.0
Slc19a3	0.68	0.31	1.45	1.33	1.09	2.1	1.0
Mex3b	1.97	1.01	9	9.17	2.19	3.19	1.0
Sh2b3	3.26	1.87	10.07	11.6	1.63	2.63	1.0
Lef1	13.55	8.05	41.75	49.49	1.62	2.62	1.0
Amfr	16.66	7.31	32.98	28.96	0.99	1.99	1.0
Prkar1b	0.65	0.36	2.63	2.84	2.01	3	1.0
Snx32	0.49	0.39	1.34	2.12	1.45	2.44	1.0
Arhgef26	2.12	1.09	4.72	4.84	1.16	2.15	1.0
Nab1	4.8	2.32	10.44	10.01	1.12	2.11	1.0
Cobll1	1.99	0.86	5.46	4.63	1.45	2.43	1.0
Atp11c	8.09	4.85	18.38	21.7	1.18	2.16	1.0
Samsn1	0.1	0.1	1.14	2.23	3.51	4.48	1.0
H2-Ab1	0.34	0.13	2.8	2.06	3.03	4	1.0
Kdm6b	2.83	1.71	17.81	21.11	2.65	3.62	1.0
Sccpdh	7.52	4.12	25.11	27.02	1.74	2.71	1.0
Jak2	3.17	1.61	12.56	12.49	1.99	2.95	1.0
Lrrc8b	2.29	1.46	5.89	7.32	1.36	2.32	1.0
Pam	5.08	2.39	14.21	13	1.49	2.44	1.0
St3gal4	3.07	1.5	8.34	7.86	1.44	2.39	1.0
Igdcc3	2.82	1.78	6.71	8.19	1.25	2.2	1.0

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 9: upregulated in mutant/downregulated in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Amd1	0.12	13.71	6.49	0.12	5.7	-6.9	-12.6
Penk	0.67	3.61	1.77	1.37	1.4	-1.4	-2.8
Ppp1r14a	1.38	5.97	3.38	2.18	1.29	-1.45	-2.7
Car12	0.51	1.5	1.16	0.64	1.19	-1.22	-2.4

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Clmp	0.69	3.72	5.39	4.26	2.97	0.2	-2.8
Raet1d	0.72	2.9	3.04	1.91	2.07	-0.6	-2.7
Amhr2	1.71	17.2	13.46	23.25	2.97	0.43	-2.5
Lax1	0.27	1.31	1.68	1.4	2.62	0.09	-2.5
Fbln2	2.24	6.99	19.3	10.5	3.11	0.59	-2.5
Otx2	6.48	23.57	22.98	17.23	1.83	-0.45	-2.3
Pakap	0.45	2.35	2.32	2.58	2.37	0.13	-2.2
Cdkl1	0.37	2.08	1.66	2.04	2.18	-0.03	-2.2
Atp2b4	0.54	2.94	3.3	4	2.61	0.44	-2.2
Loxl3	0.64	1.8	1.98	1.32	1.63	-0.45	-2.1
Myzap	1.07	2.33	6.9	3.59	2.69	0.62	-2.1
Oas1a	0.55	1.39	1.48	1.04	1.43	-0.41	-1.8
Jdp2	0.6	1.86	3.73	3.26	2.63	0.81	-1.8
Sat2	0.37	0.83	2.3	1.55	2.64	0.91	-1.7
Col1a2	1.38	4.29	3.59	3.37	1.38	-0.35	-1.7

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rbp1	2.99	7.37	7.83	5.93	1.39	-0.31	-1.7
Mfsd12	2.02	6.71	10.41	11	2.37	0.71	-1.7
Hipk2	7.13	20.16	17.01	16.38	1.25	-0.3	-1.6
Col1a1	1.48	3.3	7.59	5.83	2.36	0.82	-1.5
Ifitm2	145.73	210.34	298.01	149.87	1.03	-0.49	-1.5
Gad1	0.46	2.68	1.28	2.65	1.47	-0.02	-1.5
Atp8a1	3.24	7.04	7.37	5.72	1.18	-0.3	-1.5
Fkbp7	1.04	1.36	4.56	2.12	2.12	0.65	-1.5
Samd5	1.26	2.28	4.7	3.11	1.91	0.45	-1.5
Adck3	1.19	2.95	2.3	2.1	0.95	-0.49	-1.4
Gnrh1	0.57	1.09	1.58	1.12	1.46	0.03	-1.4
Emp3	4.41	1.98	14.51	2.44	1.72	0.3	-1.4
Fah	6.79	3.18	14.62	2.58	1.11	-0.3	-1.4
AA414768	0.63	0.95	2.28	1.31	1.86	0.47	-1.4
Fgf5	0.85	2.2	3.97	3.96	2.22	0.85	-1.4
Tnik	0.55	1.26	1.74	1.53	1.65	0.28	-1.4
Ldhb	26.66	51.03	59.87	44.58	1.17	-0.19	-1.4
Gm12942	0.99	1.13	3.29	1.49	1.73	0.39	-1.3
Abat	0.61	1	1.91	1.23	1.64	0.3	-1.3
Car11	0.7	0.98	1.86	1.03	1.41	0.07	-1.3
Kif26a	2.53	3.74	4.94	2.88	0.96	-0.38	-1.3
Pycr1	0.52	1.5	2.13	2.45	2.04	0.71	-1.3
Scand1	0.87	1.06	2.35	1.14	1.43	0.1	-1.3
St8sia1	0.7	1.48	2.56	2.22	1.88	0.59	-1.3
Gng11	0.81	2.73	2.3	3.15	1.5	0.21	-1.3
Gstm7	0.75	1.43	2.74	2.16	1.87	0.59	-1.3
Ccpg1os	3.31	7.37	11.3	10.4	1.77	0.5	-1.3
Ccdc173	0.89	1.42	1.94	1.31	1.13	-0.11	-1.2
Gstk1	2.72	2.73	5.43	2.33	1	-0.23	-1.2
Rrad	0.83	2.43	1.72	2.16	1.05	-0.17	-1.2
Rab3il1	2.68	5.18	10.95	9.15	2.03	0.82	-1.2
Gm5617	1.24	2.38	4.82	4.11	1.96	0.79	-1.2
Cpne2	2.04	2.12	4.22	1.96	1.05	-0.12	-1.2
Prickle3	0.47	0.61	1.9	1.1	2	0.84	-1.2
Eci2	14.95	8.94	31.9	8.54	1.09	-0.07	-1.2
Lysmd4	1.42	3.17	4.88	4.93	1.78	0.64	-1.1
Tspo	8.78	7.09	25.64	9.43	1.55	0.41	-1.1
Naalad2	0.78	1.07	1.95	1.21	1.32	0.18	-1.1
Wbscr27	3.49	7.22	9.32	8.81	1.42	0.29	-1.1
Hsd11b2	0.82	2.41	2.24	3.03	1.45	0.33	-1.1
Dqx1	0.69	0.8	1.86	1	1.44	0.33	-1.1
Rwdd3	1.61	4.22	5.16	6.32	1.68	0.58	-1.1
Msrb3	1.22	2.45	2.65	2.51	1.12	0.04	-1.1
Psme2b	1	1.71	3.56	2.89	1.83	0.76	-1.1
Lix1	0.35	1.06	1.02	1.46	1.54	0.47	-1.1
Fam213b	5.72	7.47	13.05	8.13	1.19	0.12	-1.1
Txndc16	1.32	4.11	4.69	6.99	1.83	0.77	-1.1
1700109H08Rik	0.65	0.99	2.03	1.49	1.65	0.6	-1.1
Inafm1	0.7	2.22	2.24	3.47	1.68	0.64	-1.0
Camk1	3.12	4.74	9.94	7.46	1.67	0.65	-1.0
Gstm5	5.57	5.89	16.7	8.72	1.58	0.57	-1.0
Cyba	11.67	8.55	29.47	10.81	1.34	0.34	-1.0
Tsku	4.66	10.36	16.18	18.16	1.8	0.81	-1.0
She	0.88	1.5	2.66	2.3	1.59	0.62	-1.0
Npff	0.73	1.17	1.72	1.41	1.24	0.27	-1.0
Bcor	7.34	13.16	22.21	20.6	1.6	0.65	-1.0
Tsga10	0.67	4.88	1.98	7.48	1.57	0.62	-1.0
Eogt	3.79	6.48	9.61	8.47	1.34	0.39	-1.0
Lclat1	2.03	4.27	6.89	7.56	1.76	0.82	-0.9
Lppr2	0.69	1.58	2.49	2.97	1.85	0.92	-0.9

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Tmem80	2.19	5.29	5.72	7.24	1.38	0.45	-0.9
Dnajb4	1.88	3.62	4.81	4.9	1.36	0.44	-0.9
Nme4	35.35	42.19	72.93	45.74	1.04	0.12	-0.9
Prob1	0.6	0.94	1.23	1.01	1.03	0.11	-0.9
Dynlt3	4.44	11.03	13.99	18.66	1.66	0.76	-0.9
Spata1	0.43	1.75	1.36	2.93	1.64	0.74	-0.9
B4gat1	4.48	7.42	9.24	8.19	1.04	0.14	-0.9
Zfp672	1.84	5.79	3.68	6.19	1	0.1	-0.9
Csf2ra	0.95	0.9	2.65	1.35	1.48	0.59	-0.9
B9d2	1.15	1.76	3.06	2.52	1.41	0.52	-0.9
Ehd2	1.83	2.92	3.78	3.29	1.05	0.17	-0.9
Mpst	5.48	12.31	10.73	13.09	0.97	0.09	-0.9
Serpinf1	0.98	2.17	2.51	3.02	1.35	0.48	-0.9
Ssh3	0.87	0.78	2.17	1.06	1.32	0.45	-0.9
Hist1h2ak	91.34	109.31	191.49	125.8	1.07	0.2	-0.9
Trpv2	0.74	1.16	1.45	1.25	0.97	0.11	-0.9
Aldh1l1	0.82	0.72	2.63	1.28	1.68	0.84	-0.8
Zfp712	0.92	2.05	2.37	2.96	1.37	0.53	-0.8
Kitl	1.85	3.16	4.07	3.88	1.14	0.3	-0.8
Sox4	6.39	11.15	20.19	20.09	1.66	0.85	-0.8
Stox2	1.06	2.84	2.98	4.56	1.49	0.68	-0.8
Stradb	2.24	4.46	5.4	6.18	1.27	0.47	-0.8
Rab39	0.55	1.14	1.27	1.53	1.22	0.42	-0.8
Dnajb14	2.33	2.95	7.43	5.42	1.67	0.88	-0.8
Stard13	1.35	1.56	3.58	2.4	1.41	0.62	-0.8
Mrpl23	21.57	36.77	47.6	47.03	1.14	0.35	-0.8
Cep131	3.25	6.29	6.84	7.65	1.07	0.28	-0.8
Gjc1	16.34	33.18	32.27	37.77	0.98	0.19	-0.8
Rtkn	4.17	7.29	10.05	10.4	1.27	0.51	-0.8
Map3k5	3.34	4.76	6.49	5.5	0.96	0.21	-0.8
Rasl11b	1.61	3.29	3.7	4.54	1.2	0.47	-0.7
Ift57	5.36	9.56	12.13	13.03	1.18	0.45	-0.7
Fam78a	0.89	1.75	1.98	2.34	1.15	0.42	-0.7
Cyth1	1.4	2.33	3.06	3.08	1.13	0.4	-0.7
Tapbpl	1.18	1.34	2.58	1.75	1.12	0.39	-0.7
Tpm2	11.5	6.77	22.34	7.96	0.96	0.23	-0.7
Lenep	1.2	2.1	3.37	3.6	1.5	0.78	-0.7
Hsd17b7	4.34	6.45	10.58	9.55	1.29	0.57	-0.7
Zbed3	1.95	2.19	4.64	3.16	1.25	0.53	-0.7
Yif1a	10.33	13.47	22.81	18.07	1.14	0.42	-0.7
Slc27a3	6.55	8.25	13.44	10.28	1.04	0.32	-0.7
Pcdhga4	0.97	1.47	2.77	2.57	1.51	0.8	-0.7
Ubxn11	0.7	0.85	1.5	1.12	1.11	0.4	-0.7
Hist1h4h	237.53	260.79	489.67	328.52	1.04	0.33	-0.7
Hspb6	0.69	0.9	1.68	1.34	1.28	0.58	-0.7
Plat	0.78	1.45	1.81	2.08	1.22	0.52	-0.7
Efna4	9.04	13.73	18.77	17.5	1.05	0.35	-0.7
Slc25a47	0.57	0.91	1.6	1.6	1.49	0.8	-0.7
Sft2d3	0.87	1.29	2.22	2.05	1.35	0.67	-0.7
Ccdc107	4.2	6.12	9.7	8.86	1.21	0.53	-0.7
Cdc42ep4	15.68	25.19	34.06	34.29	1.12	0.44	-0.7
Lyrm2	7.44	15.23	19.66	25.19	1.4	0.73	-0.7
Pcdhgb8	0.9	1.34	1.86	1.72	1.04	0.37	-0.7
Ggta1	9.96	9.63	19.85	11.98	0.99	0.32	-0.7
Srd5a3	4.29	5.73	11.31	9.58	1.4	0.74	-0.7
Sh3pxd2a	8.31	12.68	17.89	17.35	1.11	0.45	-0.7
Cers4	5.15	1.38	10.22	1.73	0.99	0.33	-0.7
Mdfic	0.48	0.71	1.36	1.28	1.5	0.85	-0.7
Ppapdc2	0.93	0.94	2.01	1.3	1.11	0.46	-0.7
Tmem218	1.84	4.38	3.97	6.03	1.11	0.46	-0.7

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Tmsb15b2	3.59	6.62	7.19	8.47	1	0.35	-0.7
Mob3c	1.07	0.81	2.66	1.29	1.31	0.67	-0.6
Lrig3	6.43	8.43	15.51	13.1	1.27	0.64	-0.6
Fam214a	1.53	1.73	3.16	2.31	1.05	0.42	-0.6
Dpm3	10.04	11.07	19.46	13.89	0.96	0.33	-0.6
Afmid	0.64	0.92	1.47	1.38	1.2	0.58	-0.6
Ngfrap1	130.62	191.49	266.06	256.47	1.03	0.42	-0.6
Gas6	4.14	3.88	8.23	5.04	0.99	0.38	-0.6
Hist1h3f	85.93	89.54	169.95	115.43	0.98	0.37	-0.6
Mmd	2.92	6.03	8.43	11.49	1.53	0.93	-0.6
Bahd1	1.92	3.29	5.18	5.83	1.43	0.83	-0.6
Pcdhga3	1.23	1.76	3.32	3.14	1.43	0.83	-0.6
Tceanc	2.28	5.26	5.27	8.11	1.21	0.63	-0.6
Ninj1	4.64	2.99	9.64	4.16	1.06	0.48	-0.6
Nkiras2	9	15.14	25.43	28.87	1.5	0.93	-0.6
Per3	1.81	3.99	4.89	7.29	1.44	0.87	-0.6
Snrnp27	36.38	50.95	78.17	73.44	1.1	0.53	-0.6
Cox6b2	16.65	16.97	35.22	24.15	1.08	0.51	-0.6
B3glct	2.91	5.94	7.28	10.09	1.32	0.76	-0.6
Hscb	7.12	9.09	16.21	14.06	1.19	0.63	-0.6
Chpt1	5	5.87	10.87	8.72	1.12	0.57	-0.6
Copg2	13.88	36.56	26.83	48.08	0.95	0.4	-0.6
Spa17	0.97	1.81	2.67	3.43	1.46	0.92	-0.5
Sfxn3	1.06	1.7	2.68	2.94	1.33	0.79	-0.5
Cuedc1	1.41	2.71	2.86	3.79	1.02	0.48	-0.5
Dram2	4.55	6.73	11.41	11.66	1.32	0.79	-0.5
Map4k4	36.33	53.11	90.84	91.56	1.32	0.79	-0.5
E130311K13Rik	0.92	1.31	2.24	2.2	1.28	0.75	-0.5
Eme2	2.36	3.36	5.63	5.58	1.26	0.73	-0.5
3010026O09Rik	7.95	12.08	16.68	17.51	1.07	0.54	-0.5
Cpxm1	7.02	6.41	14.73	9.31	1.07	0.54	-0.5
2810006K23Rik	6.59	9.38	13.49	13.25	1.03	0.5	-0.5
Pard6b	11.68	17.23	31.22	32.17	1.42	0.9	-0.5
C330013J21Rik	0.68	1.1	1.64	1.86	1.27	0.75	-0.5
Gna12	18.05	26.22	43.4	44.16	1.27	0.75	-0.5
Ring1	2.56	4.91	5.37	7.19	1.07	0.55	-0.5
Cst3	47.24	61.54	105.07	95.68	1.15	0.64	-0.5
Rnf215	2.37	3.39	4.88	4.89	1.04	0.53	-0.5
Bmi1	3.35	5.36	7.64	8.66	1.19	0.69	-0.5
Dixdc1	1.45	2.55	3.12	3.88	1.11	0.61	-0.5
Acat1	6.82	8.98	14.06	13.01	1.04	0.54	-0.5

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ifi27l2a	0.1	0.46	6.56	3.33	6.04	2.85	-3.2
Grrp1	0.13	0.76	24.21	16.34	7.55	4.43	-3.1
Smad6	1.35	7.82	25.65	23.41	4.25	1.58	-2.7
Cyp26a1	0.8	3.29	215.35	147.18	8.06	5.48	-2.6
Cd59a	0.7	1.25	8.58	2.57	3.61	1.04	-2.6
Pdlim4	0.8	3.41	28.44	23.58	5.16	2.79	-2.4
Tmem176a	0.1	0.4	13.77	11.95	7.11	4.89	-2.2
Hs3st3b1	1.76	5.7	23.55	18.19	3.74	1.68	-2.1
Slc39a8	1.35	5.49	14.26	14.21	3.4	1.37	-2.0
Efnb3	0.9	3.33	15.83	14.65	4.13	2.14	-2.0
Tmem119	0.29	0.52	16.41	7.7	5.84	3.88	-2.0
Cxcr4	0.42	1.19	91.02	68.98	7.75	5.85	-1.9

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Lgals3bp	0.16	0.65	3.94	4.33	4.62	2.74	-1.9
Mesp1	0.18	0.38	70.14	41.32	8.64	6.77	-1.9
Itpr2	0.25	1.39	2.93	4.74	3.56	1.77	-1.8
Hey1	2.17	5.58	18.26	13.68	3.07	1.29	-1.8
Cfc1	1.62	4.44	65.04	54.28	5.33	3.61	-1.7
Stard8	3.11	12.04	32.9	38.9	3.4	1.69	-1.7
Efna3	3.37	8.52	38.32	30.47	3.51	1.84	-1.7
Etv2	0.87	1.92	24.66	17.59	4.82	3.19	-1.6
Sct	0.9	1.75	8.83	5.64	3.3	1.69	-1.6
Cbln1	0.39	0.74	5.72	3.69	3.89	2.31	-1.6
Col6a1	0.27	0.76	1.73	1.63	2.68	1.11	-1.6
H2-T22	0.51	1.71	4.51	5.36	3.16	1.65	-1.5
Usp18	0.12	0.14	19.36	8.01	7.31	5.82	-1.5
Heph	0.12	0.69	3.06	6.51	4.71	3.23	-1.5
Dapk2	0.66	1.26	7.35	5.19	3.47	2.04	-1.4
Rgs4	0.11	0.33	3.66	4.17	5.08	3.66	-1.4
Dkk1	0.13	0.23	29.64	19.47	7.81	6.4	-1.4
Slit3	0.85	2.28	10.7	10.79	3.65	2.24	-1.4
Pik3ip1	2.05	5.44	15.83	15.79	2.95	1.54	-1.4
Amot	7.14	16.73	244.2	217.14	5.1	3.7	-1.4
Rab37	0.18	0.63	1.46	1.91	2.99	1.59	-1.4
Syne3	0.31	0.57	1.67	1.15	2.41	1.01	-1.4
Cd44	1.36	2.52	7.63	5.41	2.49	1.1	-1.4
Sfxn4	0.26	0.76	1.55	1.7	2.54	1.16	-1.4
Ppp1r3b	0.25	0.32	2.54	1.3	3.35	2.01	-1.3
Tmem53	0.35	0.85	1.78	1.72	2.36	1.02	-1.3
Greb1l	3.25	9.16	23.1	25.91	2.83	1.5	-1.3
Ms4a4d	0.61	1.49	8.48	8.43	3.79	2.5	-1.3
Ppil6	0.14	0.42	1.32	1.64	3.24	1.95	-1.3
Fam132a	1.65	1.47	12.89	4.71	2.96	1.68	-1.3
Nme5	0.19	0.62	1.12	1.5	2.55	1.27	-1.3
Cdh11	0.65	1.47	111.21	103.66	7.41	6.14	-1.3
Ablim1	0.78	1.81	28.64	27.31	5.19	3.92	-1.3
Tbx6	0.2	0.26	5.96	3.21	4.89	3.62	-1.3
Kdr	0.34	0.71	211.65	188.02	9.29	8.04	-1.3
Letmd1	1.55	4	7.44	8.07	2.26	1.01	-1.3
Serpinb6b	0.22	0.46	3.39	3.01	3.94	2.72	-1.2
Rbm24	0.11	0.25	12.21	12.14	6.81	5.6	-1.2
Pdlim3	2.98	4.38	81.87	52.31	4.78	3.58	-1.2
Pth1r	0.97	2.84	12.13	15.41	3.64	2.44	-1.2
St6galnac4	1.91	3.98	20.32	18.48	3.41	2.22	-1.2
Zfpm1	0.68	1.54	27.75	27.44	5.34	4.16	-1.2
Bmp7	0.54	1.22	16.03	15.93	4.88	3.71	-1.2
Tmem176b	0.17	0.46	9.42	11.56	5.81	4.65	-1.2
Fam212a	0.64	1.03	20.14	14.46	4.97	3.81	-1.2
Ackr3	2.79	4.3	26.01	17.98	3.22	2.06	-1.2
Ctla2a	0.13	0.5	5.76	9.99	5.47	4.32	-1.2
Pdzk1ip1	0.3	0.31	2.46	1.16	3.05	1.91	-1.1
1110032A03Rik	1.17	2.15	5.4	4.51	2.21	1.07	-1.1
Spata24	2.69	10.59	11.81	21	2.13	0.99	-1.1
Snai1	0.63	1.15	17.28	14.61	4.79	3.66	-1.1
Tuba8	0.12	0.15	1.95	1.1	3.97	2.84	-1.1
Naglu	0.56	0.86	3.99	2.81	2.83	1.7	-1.1
S100a1	0.9	1.48	15.52	11.79	4.11	2.99	-1.1
Gsc	0.13	0.18	9.19	5.93	6.13	5.02	-1.1
Gpc3	9.46	21.88	482.12	515.68	5.67	4.56	-1.1
Gbp4	0.41	1.11	2.45	3.07	2.58	1.47	-1.1
Evx1	0.36	0.72	27.65	26.15	6.28	5.19	-1.1
Ifi27	0.14	0.3	3.54	3.51	4.63	3.55	-1.1
Dgkg	0.15	0.25	2.21	1.77	3.9	2.82	-1.1

Sp1$^{del/del}$ ESC – Flk1+ cells
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Slc35g2	0.38	0.71	4.48	3.96	3.55	2.47	-1.1
Fam114a1	1.11	0.87	6.15	2.29	2.47	1.4	-1.1
Lbh	0.38	0.85	3.08	3.36	3.04	1.98	-1.1
Cd302	0.58	1.4	3.3	3.8	2.5	1.44	-1.1
Pcdhb22	0.18	0.23	1.85	1.15	3.37	2.32	-1.1
Igf2	8.71	22.16	225.07	277.7	4.69	3.65	-1.0
Lmo2	0.53	0.96	49.09	43.06	6.52	5.49	-1.0
Msx2	0.56	1.26	53.33	59.56	6.58	5.56	-1.0
Scube2	0.22	0.32	4.65	3.45	4.43	3.41	-1.0
Smim1	0.19	0.25	2.68	1.71	3.8	2.78	-1.0
Eomes	1.61	2.39	122.5	90.13	6.25	5.24	-1.0
Limch1	0.62	1.24	8.17	8.14	3.72	2.72	-1.0
Zfp516	3.04	6.63	32.47	35.49	3.42	2.42	-1.0
Zc3hav1	3.95	6.52	41.18	34.02	3.38	2.38	-1.0
Igtp	0.37	0.41	3.14	1.76	3.1	2.11	-1.0
Mycl	2.49	3.98	14.72	11.87	2.57	1.58	-1.0
Meis2	0.41	0.83	10.99	11.17	4.73	3.75	-1.0
Tnni1	0.1	0.28	5.61	7.96	5.81	4.84	-1.0
Cox4i2	0.64	1.3	4.34	4.5	2.77	1.8	-1.0
Rilpl2	2.85	3.95	10.88	7.75	1.93	0.97	-1.0
Tcstv3	0.19	0.15	14.23	5.74	6.23	5.28	-1.0
C8g	0.64	1.32	2.72	2.89	2.08	1.13	-1.0

Supplementary Table 5.2 – Deregulated genes between ESC and Flk1+ cells in Sp1$^{del/del}$ cells compared to WT cells

Genes that were differentially expressed (at least two-fold) between ESC and Flk1+ cell differentiation stages were identified. Tables show the genes that were differently differentially expressed between the cell stages in Sp1$^{del/del}$ cells compared to WT cells. The expression fold change (log2 value), compared to WT, of the differential genes is shown and grouped into clusters based on how the gene expression is altered upon loss of Sp1. Note no genes were found in cluster 6 (upregulated in WT/downregulated in mutant). Clusters 1 and 12 represent clusters that are not significantly different between WT and Sp1$^{del/del}$ and are not shown.

Supplementary Table 5.3

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Amhr2	13.46	23.25	0.22	11.48	-5.94	-1.02	4.9
Ifi27l2a	6.56	3.33	0.1	0.31	-6.04	-3.43	2.6
Usp18	19.36	8.01	1	2.21	-4.28	-1.86	2.4
Ap1m2	4.04	1.68	0.15	0.32	-4.75	-2.39	2.4

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Krtcap3	3.78	1.78	0.24	0.58	-3.98	-1.62	2.4
4930447C04Rik	2.16	6.05	0.14	1.8	-3.95	-1.75	2.2
Ptgds	9.23	2.53	0.1	0.1	-6.53	-4.66	1.9
Cyp2s1	4.3	2.51	0.33	0.7	-3.7	-1.84	1.9
T	64.59	52.02	0.16	0.44	-8.66	-6.89	1.8
Stk26	8.3	3.79	0.97	1.4	-3.1	-1.44	1.7
Ctsc	34.3	34.18	1.93	5.63	-4.15	-2.6	1.6
Ntm	2.51	2.13	0.13	0.31	-4.27	-2.78	1.5
Kcnk1	5.97	3.59	0.81	1.34	-2.88	-1.42	1.5
L1td1	7.34	4.27	0.77	1.2	-3.25	-1.83	1.4
Gjb5	2.75	1.01	0.32	0.31	-3.1	-1.7	1.4
Pdzk1ip1	2.46	1.16	0.29	0.36	-3.08	-1.69	1.4
Podn	2.15	1.34	0.4	0.65	-2.43	-1.04	1.4
Tcstv3	14.23	5.74	0.1	0.1	-7.15	-5.84	1.3
Pgr	1.93	1.43	0.15	0.27	-3.69	-2.4	1.3
Rxrg	2.24	1.97	0.25	0.54	-3.16	-1.87	1.3
Ndufa4l2	13.3	5.57	0.1	0.1	-7.06	-5.8	1.3
Penk	1.77	1.37	0.18	0.33	-3.3	-2.05	1.3
Pdgfra	45.47	35.75	9.82	18.4	-2.21	-0.96	1.3
Jakmip1	1.94	1.28	0.2	0.31	-3.28	-2.05	1.2
Ptpn3	2.81	1.81	0.47	0.71	-2.58	-1.35	1.2
Mt2	6.69	1.63	0.69	0.38	-3.28	-2.1	1.2
Tbx6	5.96	3.21	0.44	0.53	-3.76	-2.6	1.2
Ptprj	13.6	11.08	2.1	3.81	-2.7	-1.54	1.2
Arhgap22	3.62	1.98	0.25	0.3	-3.86	-2.72	1.1
Ripk4	9.56	5.94	0.94	1.28	-3.35	-2.21	1.1
Sp8	2.5	1.99	0.36	0.63	-2.8	-1.66	1.1
Epcam	10.24	5.1	0.48	0.52	-4.42	-3.29	1.1
Dnajc22	2.24	2.38	0.4	0.91	-2.49	-1.39	1.1
Mesp2	1.7	1.43	0.13	0.23	-3.71	-2.64	1.1
Tsacc	5.13	5.37	1.12	2.42	-2.2	-1.15	1.1
Egflam	3.94	2.75	0.6	0.86	-2.72	-1.68	1.0
Aldoc	6.44	1.68	0.47	0.25	-3.78	-2.75	1.0
Tmem243	11.9	11.63	2.24	4.47	-2.41	-1.38	1.0
Alox15	4.52	2.59	0.13	0.15	-5.12	-4.11	1.0
Dkk1	29.64	19.47	1.58	2.09	-4.23	-3.22	1.0
Bmp7	16.03	15.93	0.2	0.4	-6.32	-5.32	1.0
Ppp4r4	3.88	2.24	0.32	0.37	-3.6	-2.6	1.0
Stmn2	13.4	18.04	3.04	8.17	-2.14	-1.14	1.0
Sulf1	40.64	47.49	10.43	24.48	-1.96	-0.96	1.0
Tnfrsf19	30.65	32.61	1.14	2.41	-4.75	-3.76	1.0
Bnip3	60.15	52.54	6.63	11.52	-3.18	-2.19	1.0
Chic1	6.48	5.16	1.56	2.48	-2.05	-1.06	1.0
Fam49a	8.13	6.27	2.08	3.16	-1.97	-0.99	1.0
Esrp1	2.45	1.05	0.18	0.15	-3.77	-2.81	1.0
Nptx2	3.74	4.25	0.77	1.7	-2.28	-1.32	1.0

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Acta1	3.4	4.03	1.54	7.04	-1.14	0.8	1.9
Gbp2b	5.54	3.49	2.31	5.42	-1.26	0.64	1.9
Ifi30	17.54	8.97	5.18	9.82	-1.76	0.13	1.9
Cox7a1	9.37	2.89	2.56	2.81	-1.87	-0.04	1.8
Cbln1	5.72	3.69	1.34	2.6	-2.09	-0.51	1.6
Gja6	1.53	1.21	0.41	0.9	-1.9	-0.43	1.5
Mt1	11.38	2.85	4.37	2.95	-1.38	0.05	1.4

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Slc6a15	6.43	5.09	1.62	3.31	-1.99	-0.62	1.4
Cdh1	7.73	6.05	2.24	4.34	-1.79	-0.48	1.3
2610305D13Rik	9.38	2.65	4.16	2.88	-1.17	0.12	1.3
Lgals3	4.87	1.81	1.4	1.23	-1.8	-0.56	1.2
H2-DMa	1.42	1.1	0.46	0.83	-1.63	-0.41	1.2
Gbp2	15.09	9.01	5.93	8.24	-1.35	-0.13	1.2
Zfp65	9.83	8.89	4.09	8.58	-1.27	-0.05	1.2
Cd68	6.18	3.29	2.28	2.8	-1.44	-0.23	1.2
Mybl1	6.4	6.04	2.22	4.68	-1.53	-0.37	1.2
Gria4	3.12	3.25	1.43	3.32	-1.13	0.03	1.2
Cth	11.93	6.86	3.92	4.97	-1.61	-0.46	1.2
Gm13154	3.83	2.36	1.1	1.49	-1.8	-0.66	1.1
Spint2	11.16	5.27	2.73	2.81	-2.03	-0.91	1.1
Acy1	19.38	20.13	6.21	13.59	-1.64	-0.57	1.1
Loxl4	1.16	1.08	0.55	1.07	-1.08	-0.01	1.1
Sp4	4.8	11.57	1.45	7.27	-1.73	-0.67	1.1
St14	2.14	1.05	1.02	1.04	-1.07	-0.01	1.1
Shisa2	1.53	1.77	0.4	0.95	-1.94	-0.9	1.0
Gbp8	1.36	1.16	0.41	0.71	-1.73	-0.71	1.0
Stbd1	7.89	3.94	2.12	2.12	-1.9	-0.89	1.0
Zfp760	13.48	14.16	4.63	9.73	-1.54	-0.54	1.0
Adcy2	2.6	1.58	1.02	1.24	-1.35	-0.35	1.0
Esf1	36.71	28.58	11.93	18.48	-1.62	-0.63	1.0
Pdgfa	11.84	10.37	4.38	7.67	-1.43	-0.44	1.0
Dusp9	45.57	33.59	17.22	25.28	-1.4	-0.41	1.0
Gm14124	1.54	1.31	0.61	1.03	-1.34	-0.35	1.0
Zfp53	5.14	4.32	2.37	3.96	-1.12	-0.13	1.0
Vps13a	6.85	3.65	3.23	3.42	-1.08	-0.09	1.0
Zfp729b	11.01	9.08	3.83	6.23	-1.52	-0.54	1.0
Slc16a1	109.73	90.48	41.63	67.8	-1.4	-0.42	1.0
Ube2v2	4.92	3.86	1.86	2.86	-1.4	-0.43	1.0
Rgs4	3.66	4.17	1.89	4.24	-0.95	0.02	1.0
Zfp85	3.73	3.63	1.8	3.38	-1.05	-0.1	1.0
Tmem53	1.78	1.72	0.71	1.3	-1.33	-0.4	0.9
AU041133	5.92	4.99	2.36	3.7	-1.33	-0.43	0.9
Rnase4	7.12	4.22	2.38	2.61	-1.58	-0.69	0.9
Thoc2	32.38	28.41	13.56	21.98	-1.26	-0.37	0.9
Stxbp6	2	1.62	0.72	1.07	-1.47	-0.6	0.9
Clstn2	1.33	1.63	0.56	1.25	-1.25	-0.38	0.9
Sfmbt2	11.84	12.13	4.57	8.5	-1.37	-0.51	0.9
Pdgfrl	2.92	2.69	1.33	2.23	-1.13	-0.27	0.9
Wdr35	10.71	9.54	4.97	8.01	-1.11	-0.25	0.9
Arhgdig	8.05	8.92	2.48	4.95	-1.7	-0.85	0.9
Lrif1	15.6	11.99	6.62	9.13	-1.24	-0.39	0.9
Zfp758	5.66	4.72	1.68	2.5	-1.75	-0.92	0.8
Cep290	6.86	6.82	2.25	3.96	-1.61	-0.78	0.8
Zfp40	3.83	4.24	1.36	2.69	-1.49	-0.66	0.8
Rab38	2.96	1.68	1.49	1.5	-0.99	-0.16	0.8
Frs3	1.82	1.61	0.67	1.05	-1.44	-0.62	0.8
1700109H08Rik	2.03	1.49	1.05	1.36	-0.95	-0.13	0.8
Zfp942	15.03	13.2	4.78	7.37	-1.65	-0.84	0.8
Etohi1	13.31	11.59	4.45	6.74	-1.58	-0.78	0.8
Oasl2	1.15	1.06	0.51	0.82	-1.17	-0.37	0.8
Nbeal1	13.8	14.07	6.21	11.07	-1.15	-0.35	0.8
Bmp4	129.83	95.92	40.04	51.19	-1.7	-0.91	0.8
Zfp617	5.88	5.58	2.94	4.82	-1	-0.21	0.8
Wnk3	6.96	7.23	2.2	3.92	-1.66	-0.88	0.8
Ankrd12	4.12	4.2	2.12	3.7	-0.96	-0.18	0.8
Dock11	48.32	45.3	16.25	25.93	-1.57	-0.8	0.8
Fam126b	5.35	4.46	1.86	2.65	-1.52	-0.75	0.8

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rassf7	10.05	10.79	3.54	6.45	-1.51	-0.74	0.8
Dscam	1.03	1.31	0.53	1.15	-0.96	-0.19	0.8
Gm14325	5.55	5.08	2.2	3.42	-1.33	-0.57	0.8
Atp11c	18.38	21.7	6.43	12.69	-1.52	-0.77	0.8
Nol8	20.44	17.26	8.2	11.63	-1.32	-0.57	0.8
Mpp5	25.49	23.62	12.43	19.36	-1.04	-0.29	0.8
Dzip3	25.19	20.93	9.3	12.89	-1.44	-0.7	0.7
Zfp120	5.91	4.98	2.54	3.56	-1.22	-0.48	0.7
Ston2	2.41	2.3	1.16	1.86	-1.05	-0.31	0.7
Dsp	49.24	39.51	15.96	21.22	-1.63	-0.9	0.7
Psmg4	26.58	11.67	10.64	7.73	-1.32	-0.59	0.7
Zfp280d	23.29	26.52	10.62	20.14	-1.13	-0.4	0.7
Zfp944	6.96	6.83	2.77	4.49	-1.33	-0.61	0.7
Atrx	44.82	46.4	18.21	30.95	-1.3	-0.58	0.7
Cenpe	45.96	35.97	21.98	28.39	-1.06	-0.34	0.7
Zfp729a	6.11	5.38	2.93	4.26	-1.06	-0.34	0.7
Slc4a7	31.18	28.15	11	16.33	-1.5	-0.79	0.7
Nrcam	2.63	2.82	1.05	1.85	-1.32	-0.61	0.7
Trnt1	44.07	36.75	21.97	30.08	-1	-0.29	0.7
Rev3l	15.49	14.97	7.86	12.44	-0.98	-0.27	0.7
Cited1	8.99	6.13	2.98	3.3	-1.59	-0.89	0.7
Fam117a	7.98	6.34	3.39	4.35	-1.24	-0.54	0.7
Atf2	36.46	34.41	16.34	25.07	-1.16	-0.46	0.7
Garem	3.78	4.7	1.95	3.95	-0.95	-0.25	0.7
Zfp738	7.66	6.75	2.8	3.99	-1.45	-0.76	0.7
Zfp292	36.43	34.27	13.64	20.66	-1.42	-0.73	0.7
Slit3	10.7	10.79	5.09	8.29	-1.07	-0.38	0.7
Ccdc55	11.65	9.3	5.61	7.26	-1.05	-0.36	0.7
Fsd1l	3.21	3.16	1.44	2.26	-1.16	-0.48	0.7
Nin	34.85	20.36	15.66	14.72	-1.15	-0.47	0.7
Hnrnpa1	328.52	285.69	153.88	215.74	-1.09	-0.41	0.7
Mrm1	4.67	4.58	2.19	3.45	-1.09	-0.41	0.7
Tceal1	7	6.28	2.31	3.3	-1.6	-0.93	0.7
Car11	1.86	1.03	0.71	0.62	-1.39	-0.73	0.7
Fgfrl1	14.64	15.76	5.25	8.87	-1.48	-0.83	0.7
Fam199x	14.29	13.6	6	8.95	-1.25	-0.6	0.7
B4galnt1	3.23	2.35	1.39	1.58	-1.22	-0.57	0.7
Zfp947	1.86	1.48	0.93	1.16	-1	-0.35	0.7
Zfp322a	21.45	19.57	7.52	10.71	-1.51	-0.87	0.6
Susd4	1.89	1.72	0.78	1.1	-1.28	-0.64	0.6
Timm8a1	11	9.85	4.75	6.63	-1.21	-0.57	0.6
Appl1	24.14	27.83	11.13	19.96	-1.12	-0.48	0.6
Taf4b	7.06	5.76	3.38	4.3	-1.06	-0.42	0.6
Pdlim3	81.87	52.31	39.65	39.47	-1.05	-0.41	0.6
AI987944	5.58	5.84	2.43	3.94	-1.2	-0.57	0.6
Kif20b	37.88	32.88	12.82	17.16	-1.56	-0.94	0.6
Zfp51	9.96	8.21	3.64	4.62	-1.45	-0.83	0.6
Klf8	6.04	5.39	3.07	4.21	-0.98	-0.36	0.6
Fah	14.62	2.58	5.02	1.35	-1.54	-0.93	0.6
Zfp953	4.46	3.23	1.66	1.83	-1.43	-0.82	0.6
Zfp820	5.1	4.37	2.22	2.9	-1.2	-0.59	0.6
Bod1l	25.83	24.31	12.07	17.2	-1.1	-0.5	0.6
Fgf13	3.36	2.94	1.19	1.56	-1.5	-0.91	0.6
Raet1c	1.39	1.07	0.51	0.59	-1.45	-0.86	0.6
Sowahc	5.99	6.37	2.35	3.75	-1.35	-0.76	0.6
Smyd3	6.61	5.98	3.17	4.32	-1.06	-0.47	0.6
Phip	39.51	40.15	14.37	21.78	-1.46	-0.88	0.6
Amd2	32.49	37.61	13.14	22.66	-1.31	-0.73	0.6
Zfp329	7.44	7.39	3.18	4.72	-1.23	-0.65	0.6
Bdp1	22.29	21.04	10.62	14.98	-1.07	-0.49	0.6

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Zfp943	5.32	5.63	2	3.14	-1.41	-0.84	0.6
Smc3	69.92	65.79	27.61	38.61	-1.34	-0.77	0.6
Zfp182	3.36	2.7	1.66	1.98	-1.02	-0.45	0.6
C330027C09Rik	34.54	28.94	17.43	21.66	-0.99	-0.42	0.6
Mbtd1	35.33	32.32	13.09	17.65	-1.43	-0.87	0.6
Acrbp	3.3	2.66	1.26	1.5	-1.39	-0.83	0.6
Pcmtd1	18.45	16	7.03	9	-1.39	-0.83	0.6
Smc6	44.73	44.89	18.16	26.8	-1.3	-0.74	0.6
Zfp874b	8.5	7.07	3.7	4.53	-1.2	-0.64	0.6
Upf2	17.09	18.33	7.52	11.95	-1.18	-0.62	0.6
Capn6	45.42	43.38	20.3	28.6	-1.16	-0.6	0.6
Twist1	11.24	7.26	5.23	5.01	-1.1	-0.54	0.6
Ncl	174.21	140.72	83.07	99.15	-1.07	-0.51	0.6
Sorl1	3.45	3.76	1.28	2.04	-1.43	-0.88	0.6
St8sia2	2.11	2.27	0.98	1.54	-1.11	-0.56	0.6
Snapc1	4.66	4.92	2.33	3.61	-1	-0.45	0.6
Prrg1	2.97	2.63	1.52	1.97	-0.97	-0.42	0.6
Carf	4.53	4.16	1.96	2.61	-1.21	-0.67	0.5
Chpf	7.12	6.86	2.67	3.69	-1.42	-0.89	0.5
Senp8	6.21	9.03	2.41	5.06	-1.37	-0.84	0.5
Aasdhppt	22.95	19.7	9.18	11.43	-1.32	-0.79	0.5
Atxn3	17.4	20.63	7.09	12.06	-1.3	-0.77	0.5
Hspbap1	5.56	4.94	2.29	2.94	-1.28	-0.75	0.5
Mrps36	22.64	18.56	10.07	11.95	-1.17	-0.64	0.5
2300009A05Rik	2.33	2.1	1.1	1.43	-1.08	-0.55	0.5
Tbc1d16	12.97	12.47	6.3	8.77	-1.04	-0.51	0.5
Imp3	55.56	38.71	27.87	27.93	-1	-0.47	0.5
Glmn	20.66	25.86	10.61	19.23	-0.96	-0.43	0.5
Dusp28	1.33	1.55	0.49	0.82	-1.44	-0.92	0.5
Mettl5	19.08	17.69	7.32	9.73	-1.38	-0.86	0.5
Dixdc1	3.12	3.88	1.24	2.21	-1.33	-0.81	0.5
Tmem88	71.73	36.56	28.48	20.85	-1.33	-0.81	0.5
Zfp518a	16.43	18.64	6.68	10.87	-1.3	-0.78	0.5
Gm20939	2.97	2.65	1.32	1.69	-1.17	-0.65	0.5
Nova1	1.8	1.74	0.69	0.95	-1.38	-0.87	0.5
Dctd	26.4	19.61	10.3	10.85	-1.36	-0.85	0.5
Trim12c	9.13	8.99	3.77	5.27	-1.28	-0.77	0.5
Ythdc2	7.04	8.04	3.11	5.04	-1.18	-0.67	0.5
Rsf1	18.65	18.39	9.34	13.12	-1	-0.49	0.5
Nars2	8.17	7.67	4.12	5.5	-0.99	-0.48	0.5
Helb	5.55	4.96	2.19	2.78	-1.34	-0.84	0.5
Zfp62	29.39	29.3	12.54	17.64	-1.23	-0.73	0.5
Akap7	8.35	7.63	3.62	4.65	-1.21	-0.71	0.5
2210404O09Rik	4.05	2.91	1.84	1.87	-1.14	-0.64	0.5

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ccdc106	2.63	5.69	0.39	0.24	-2.75	-4.57	-1.8
Rbp4	2.06	3.37	0.51	0.25	-2.01	-3.75	-1.7
Il18	1.36	2.46	0.46	0.25	-1.56	-3.3	-1.7
Rims3	1.67	1.11	0.84	0.17	-0.99	-2.71	-1.7
Scara5	1.05	2.11	0.26	0.16	-2.01	-3.72	-1.7
Asb4	51.6	75.26	13.47	6.04	-1.94	-3.64	-1.7
Nlgn3	1.64	3.44	0.33	0.22	-2.31	-3.97	-1.7
Slc32a1	1.13	2.23	0.22	0.14	-2.36	-3.99	-1.6

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Hoxb1	4.03	12.32	0.1	0.1	-5.33	-6.94	-1.6
Raet1e	2.61	2.16	0.43	0.12	-2.6	-4.17	-1.6
Slc22a21	2.2	1.45	1.09	0.26	-1.01	-2.48	-1.5
Asic4	2.46	3.5	0.36	0.19	-2.77	-4.2	-1.4
Grik4	1.15	1.33	0.39	0.17	-1.56	-2.97	-1.4
Zcwpw1	5.13	14.91	1.43	1.59	-1.84	-3.23	-1.4
H2-Bl	1.22	1.19	0.26	0.1	-2.23	-3.57	-1.3
Kcnj5	3.58	3.6	0.5	0.2	-2.84	-4.17	-1.3
Tcf7	21.31	19.51	4.88	1.81	-2.13	-3.43	-1.3
Myb	3.74	4	1.14	0.5	-1.71	-3	-1.3
Gfra2	13.26	15.57	2.53	1.23	-2.39	-3.66	-1.3
Hoga1	1.49	1.35	0.56	0.21	-1.41	-2.68	-1.3
EU599041	1.03	2.67	0.13	0.14	-2.99	-4.25	-1.3
Lrrc3	2.13	2.94	0.96	0.56	-1.15	-2.39	-1.2
Tnni3	1.02	1.39	0.17	0.1	-2.58	-3.8	-1.2
Lrrc16b	2.24	3.48	0.6	0.4	-1.9	-3.12	-1.2
Slc8a2	2.27	3.1	0.27	0.16	-3.07	-4.28	-1.2
Neurl1a	4.71	2.52	2.42	0.56	-0.96	-2.17	-1.2
Etv2	24.66	17.59	3.34	1.04	-2.88	-4.08	-1.2
Lrfn1	3.13	3.01	1.12	0.47	-1.48	-2.68	-1.2
Grin2c	2.84	1.98	0.68	0.21	-2.06	-3.24	-1.2
Rangrf	22.65	16.66	9.97	3.25	-1.18	-2.36	-1.2
Slc12a7	16.78	15.64	6.94	2.9	-1.27	-2.43	-1.2
BC051142	1.02	2.01	0.27	0.24	-1.92	-3.07	-1.2
Syndig1	7.5	9.12	0.23	0.13	-5.03	-6.13	-1.1
Glt1d1	11.54	10.01	1.06	0.43	-3.44	-4.54	-1.1
Megf6	1.45	2.22	0.74	0.53	-0.97	-2.07	-1.1
Ccdc109b	2.07	3.66	0.29	0.24	-2.84	-3.93	-1.1
Nyap1	2.96	2.49	0.8	0.32	-1.89	-2.96	-1.1
Mpp2	3.45	4.23	1.78	1.06	-0.95	-2	-1.1
Celsr1	4.45	6.71	0.64	0.47	-2.8	-3.84	-1.0
Ubxn11	1.5	1.12	0.49	0.18	-1.61	-2.64	-1.0
Il15ra	2.02	2.31	0.92	0.52	-1.13	-2.15	-1.0
Pygo1	2.88	3.2	1.33	0.73	-1.11	-2.13	-1.0
Lgi2	1.67	3.65	0.23	0.25	-2.86	-3.87	-1.0
Col23a1	9.72	9.37	1.77	0.85	-2.46	-3.46	-1.0
Fgf8	24.7	20.56	0.24	0.1	-6.69	-7.68	-1.0
Ak8	1.27	2.02	0.55	0.44	-1.21	-2.2	-1.0
Efr3b	1.39	1.74	0.68	0.43	-1.03	-2.02	-1.0
Pcdhac2	1.73	2.42	0.17	0.12	-3.35	-4.33	-1.0
Padi3	5.18	6.5	0.61	0.39	-3.09	-4.06	-1.0
Cmklr1	2.19	2.93	0.89	0.61	-1.3	-2.26	-1.0
Dnase2a	3.27	2.32	1.63	0.6	-1	-1.95	-1.0

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Gstk1	5.43	2.33	6.95	0.65	0.36	-1.84	-2.2
Ifitm5	1.84	1.15	1.19	0.2	-0.63	-2.52	-1.9
Rnaset2a	2.49	2.81	1.97	0.7	-0.34	-2.01	-1.7
Hoxd1	1.53	2.53	1.06	0.58	-0.53	-2.13	-1.6
Efna1	12.2	11.76	13.11	4.45	0.1	-1.4	-1.5
Epor	3.84	3.69	2.63	0.9	-0.55	-2.04	-1.5
Map1a	2.83	3.96	2.32	1.15	-0.29	-1.78	-1.5
Rfx2	1.02	1.97	1.03	0.71	0.01	-1.47	-1.5
Ccdc8	16.92	13.88	11.99	3.64	-0.5	-1.93	-1.4
Sapcd1	7.13	4.29	7.45	1.77	0.06	-1.28	-1.3

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Nmb	10.14	12.56	5.9	2.93	-0.78	-2.1	-1.3
Gm20604	5.46	5.66	3.47	1.47	-0.65	-1.94	-1.3
Gfi1b	1.24	1.69	0.86	0.48	-0.53	-1.82	-1.3
Mylpf	1.47	1.96	1.41	0.77	-0.06	-1.35	-1.3
Aplnr	92.27	90.6	80.97	33.27	-0.19	-1.45	-1.3
Thbs3	6.65	12.39	3.86	3.03	-0.78	-2.03	-1.3
Fam221a	1.67	2.05	1.08	0.56	-0.63	-1.87	-1.2
Rad9b	1.99	3.6	1.4	1.08	-0.51	-1.74	-1.2
Tmem121	1.19	1.11	1.11	0.44	-0.1	-1.33	-1.2
Slc46a3	5.98	7.44	4.92	2.7	-0.28	-1.46	-1.2
1700010I14Rik	1.18	1.02	1.02	0.39	-0.21	-1.39	-1.2
Impa2	15.23	13.81	8.15	3.34	-0.9	-2.05	-1.2
Nme5	1.12	1.5	0.68	0.41	-0.72	-1.87	-1.2
Lad1	5	7.51	5.29	3.59	0.08	-1.06	-1.1
Trim62	1.33	2.13	0.89	0.67	-0.58	-1.67	-1.1
Nsmf	12.5	11.8	10.47	4.74	-0.26	-1.32	-1.1
Fbxo36	5.15	4.61	4.01	1.74	-0.36	-1.41	-1.1
Fsbp	2.36	3.62	1.25	0.93	-0.92	-1.96	-1.0
Sema3f	9.87	10.59	7.85	4.1	-0.33	-1.37	-1.0
Myo7a	1.78	1.68	1.15	0.53	-0.63	-1.66	-1.0
Clcf1	1.42	2	0.83	0.58	-0.77	-1.79	-1.0
Tnks1bp1	16.91	17.31	14.55	7.32	-0.22	-1.24	-1.0
Fancf	1.89	2.11	1.38	0.77	-0.45	-1.45	-1.0
Fam98c	5.32	8.9	3.54	2.98	-0.59	-1.58	-1.0
Acaa2	34.73	30.95	27.64	12.39	-0.33	-1.32	-1.0
Slc30a3	4.53	4.2	3.4	1.61	-0.41	-1.38	-1.0
Trim46	1	1.21	0.95	0.59	-0.07	-1.04	-1.0
Sh3bp2	4.44	4.99	2.35	1.36	-0.92	-1.88	-1.0
Smim20	14.69	2.37	11.52	0.96	-0.35	-1.3	-1.0
Kif24	5.12	6.04	2.89	1.78	-0.83	-1.76	-0.9
Mblac1	1.8	1.84	1.5	0.81	-0.26	-1.18	-0.9
Zfp41	12.29	15.04	8.21	5.37	-0.58	-1.49	-0.9
Dnmt3a	19.82	20.1	14.18	7.65	-0.48	-1.39	-0.9
Ccdc24	3.43	5.81	2.6	2.38	-0.4	-1.29	-0.9
Pif1	3.72	3.84	3.38	1.88	-0.14	-1.03	-0.9
Shf	6.82	9.13	4.86	3.54	-0.49	-1.37	-0.9
Fam129c	1.27	1.61	1.06	0.74	-0.26	-1.12	-0.9
Cetn4	1.08	1.03	0.97	0.51	-0.15	-1.01	-0.9
Mks1	2.88	5.6	1.78	1.93	-0.69	-1.54	-0.9
Nipsnap1	23.07	22.02	15.01	7.95	-0.62	-1.47	-0.9
4931428F04Rik	5.98	7.21	4.7	3.13	-0.35	-1.2	-0.9
Cenpv	7.22	4.41	4.09	1.4	-0.82	-1.66	-0.8
H1f0	63	49.85	34.94	15.61	-0.85	-1.68	-0.8
Tmem218	3.97	6.03	2.6	2.23	-0.61	-1.44	-0.8
Hist1h1b	510.96	507.15	343.33	192.46	-0.57	-1.4	-0.8
Mypop	1.13	1.81	0.62	0.56	-0.87	-1.69	-0.8
Zfp446	2.13	2.6	1.39	0.96	-0.62	-1.44	-0.8
Nat2	2.83	1.88	2.1	0.79	-0.43	-1.25	-0.8
Ndrg2	10.61	4.1	5.83	1.29	-0.86	-1.67	-0.8
Creb3l4	1.76	1.03	0.99	0.33	-0.83	-1.64	-0.8
Dtx3	27.58	34.64	18.15	13.04	-0.6	-1.41	-0.8
Loxl3	1.98	1.32	1.55	0.59	-0.35	-1.16	-0.8
Ldhal6b	1.04	1.19	0.83	0.54	-0.33	-1.14	-0.8
Pstpip2	1.4	1.44	0.76	0.45	-0.88	-1.68	-0.8
Stag3	5.81	28.7	3.31	9.4	-0.81	-1.61	-0.8
Lfng	3.48	6.49	2.52	2.7	-0.47	-1.27	-0.8
Eid2b	1.33	1.59	1.07	0.74	-0.31	-1.1	-0.8
Hoxb2	5.47	8.32	2.95	2.64	-0.89	-1.66	-0.8
Hacl1	4.58	3.72	3.29	1.56	-0.48	-1.25	-0.8
Nup210	6.53	6.79	3.46	2.13	-0.92	-1.67	-0.8

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cttnbp2	2.51	3.25	1.68	1.29	-0.58	-1.33	-0.8
Dcp1b	2.29	2.79	1.97	1.42	-0.22	-0.97	-0.8
Wdr76	12.34	13.25	8.1	5.21	-0.61	-1.35	-0.7
Rnaseh2c	29.35	32.16	22.22	14.64	-0.4	-1.14	-0.7
Slc15a2	7.67	8.08	4.04	2.58	-0.92	-1.65	-0.7
Ptms	121.25	148.53	92.92	68.87	-0.38	-1.11	-0.7
Tep1	3.28	1.49	2.77	0.76	-0.24	-0.97	-0.7
Nphp1	6.8	7.83	4.37	3.05	-0.64	-1.36	-0.7
Pck2	11.36	5.73	6.87	2.11	-0.73	-1.44	-0.7
Slc46a1	1.76	1.83	1.31	0.83	-0.43	-1.14	-0.7
Xndc1	12.18	14.49	6.64	4.84	-0.88	-1.58	-0.7
Mdc1	27.46	41.05	18.25	16.82	-0.59	-1.29	-0.7
Hjurp	13.02	17.16	9.43	7.62	-0.47	-1.17	-0.7
Cdh24	2.11	1.59	1.66	0.77	-0.35	-1.05	-0.7
Timeless	30.58	32.74	17.44	11.6	-0.81	-1.5	-0.7
B930041F14Rik	1.5	1.32	0.99	0.54	-0.6	-1.29	-0.7
Ppox	6.04	7.38	4	3.04	-0.59	-1.28	-0.7
Sfi1	11.44	31.84	6.11	10.62	-0.9	-1.58	-0.7
Dpysl4	2.04	3	1.23	1.13	-0.73	-1.41	-0.7
A730008H23Rik	29.6	36.46	17.25	13.32	-0.78	-1.45	-0.7
Pbk	65.32	112.9	39.85	43.51	-0.71	-1.38	-0.7
Rbl1	9.44	15.79	5.94	6.23	-0.67	-1.34	-0.7
Zc3h8	5.01	6.62	3.84	3.19	-0.38	-1.05	-0.7
Hlcs	4.6	5.32	3.6	2.62	-0.35	-1.02	-0.7
Pomt1	7.72	7.88	6.2	3.98	-0.32	-0.99	-0.7
Pdk2	2.37	2.51	1.32	0.89	-0.84	-1.5	-0.7
Prom1	7.14	8.5	4.48	3.39	-0.67	-1.33	-0.7
Mdm1	13.82	18.9	8.79	7.64	-0.65	-1.31	-0.7
Nadsyn1	4.9	5.82	3.32	2.5	-0.56	-1.22	-0.7
Mcm3	119.66	125.66	82.31	54.8	-0.54	-1.2	-0.7
Stxbp2	23.7	14.9	18.04	7.21	-0.39	-1.05	-0.7
Zfp692	10.11	12.79	6.85	5.54	-0.56	-1.21	-0.7
Traf3ip1	3.28	3.84	2.63	1.96	-0.32	-0.97	-0.7
Fanca	5.93	9.25	4.73	4.72	-0.33	-0.97	-0.6
Mcm5	94.81	93.63	57.54	36.8	-0.72	-1.35	-0.6
Ppp1r3f	1.26	1.69	0.86	0.75	-0.55	-1.17	-0.6
Zkscan4	1.43	1.51	0.94	0.65	-0.61	-1.22	-0.6
Camk1	9.94	7.46	5.28	2.62	-0.91	-1.51	-0.6
Lig1	70.39	66.68	38.67	24.28	-0.86	-1.46	-0.6
Mok	1.91	1.85	1.27	0.81	-0.59	-1.19	-0.6
Klf11	10.87	11.91	7.34	5.28	-0.57	-1.17	-0.6
Swsap1	4.74	5.7	3.48	2.75	-0.45	-1.05	-0.6
Bard1	9.76	13.86	7.25	6.77	-0.43	-1.03	-0.6
Ift27	12.5	14.36	9.48	7.2	-0.4	-1	-0.6
Nodal	4.08	4.03	2.65	1.74	-0.62	-1.21	-0.6
Espl1	21.16	23.98	15.41	11.55	-0.46	-1.05	-0.6
Nuggc	1.19	1.88	0.65	0.69	-0.87	-1.45	-0.6
Rgs10	4.61	3.77	2.73	1.49	-0.76	-1.34	-0.6
Mamdc4	1.4	1.62	0.81	0.63	-0.79	-1.36	-0.6
Pole	20.66	26.23	11.94	10.27	-0.79	-1.35	-0.6
Mllt6	8.33	9.33	4.89	3.7	-0.77	-1.33	-0.6
Mterf1b	1.57	1.8	0.99	0.77	-0.67	-1.23	-0.6
Primpol	6.07	6.56	3.82	2.8	-0.67	-1.23	-0.6
Nup85	66.42	72.54	43.75	32.57	-0.6	-1.16	-0.6
Gpt	2.13	2.79	1.18	1.06	-0.85	-1.4	-0.6
Acacb	1.44	1.68	0.87	0.69	-0.73	-1.28	-0.6
Phf10	69.33	65.51	43.63	28.17	-0.67	-1.22	-0.6
Pttg1	68.34	40.31	44.58	17.89	-0.62	-1.17	-0.6
Sh3bp1	17.9	18.61	12.14	8.62	-0.56	-1.11	-0.6
Thra	15.01	19.9	10.16	9.25	-0.56	-1.11	-0.6

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Nacc2	1.05	1	0.72	0.47	-0.54	-1.09	-0.6
Cdc45	33.3	31.96	19.1	12.62	-0.8	-1.34	-0.5
Clspn	18.86	21.89	11.31	8.99	-0.74	-1.28	-0.5
Ppp1r16a	13.08	14.4	7.81	5.93	-0.74	-1.28	-0.5
Rad9a	16.35	19.54	10.96	9.01	-0.58	-1.12	-0.5
Rnf145	36.55	43.43	26.82	21.89	-0.45	-0.99	-0.5
Lenep	3.37	3.6	1.82	1.35	-0.89	-1.42	-0.5
Cdt1	35.68	33.33	20.27	13.11	-0.82	-1.35	-0.5
Uhrf1	127.12	151.58	76.08	62.67	-0.74	-1.27	-0.5
Chaf1b	36.4	49.97	23.59	22.34	-0.63	-1.16	-0.5
Helq	6.51	7.25	4.67	3.61	-0.48	-1.01	-0.5
Ftsj2	5.74	5.49	4.29	2.85	-0.42	-0.95	-0.5
Psmc3ip	19.66	19.08	10.39	7.03	-0.92	-1.44	-0.5
Rnf113a1	3.47	4.4	1.95	1.73	-0.83	-1.35	-0.5
Fan1	4.93	5.56	2.92	2.29	-0.76	-1.28	-0.5
Tipin	85	86.8	50.75	36.21	-0.74	-1.26	-0.5
Setdb2	5.94	5.78	3.69	2.49	-0.69	-1.21	-0.5
Ptpn6	8.5	4.33	4.61	1.65	-0.88	-1.39	-0.5
Sv2a	2.88	3.23	1.64	1.29	-0.81	-1.32	-0.5
Arhgef25	33.42	31.9	22.31	15.01	-0.58	-1.09	-0.5
Nek3	6.12	7.07	4.35	3.53	-0.49	-1	-0.5
Ticrr	13.09	14.88	7.15	5.74	-0.87	-1.37	-0.5
Rtel1	18.16	18.9	11.64	8.57	-0.64	-1.14	-0.5
Cchcr1	5.12	6.83	3.45	3.25	-0.57	-1.07	-0.5

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Gm20594	2.95	1.52	1.57	5.78	-0.91	1.93	2.8
Naalad2	1.95	1.21	2.16	4.67	0.15	1.95	1.8
Ube2ql1	2.7	1.89	1.88	4.23	-0.52	1.16	1.7
Nbl1	0.81	0.58	1.08	2.4	0.42	2.05	1.6
Nudt6	1.45	0.57	1.25	1.53	-0.21	1.42	1.6
Nr1d1	1.86	1.4	1.52	3.36	-0.29	1.26	1.6
Lpar4	8.68	6.08	7.93	15.33	-0.13	1.33	1.5
Acyp2	1.21	1.53	1.53	5.14	0.34	1.75	1.4
Krt19	20.21	8.17	34.93	37.28	0.79	2.19	1.4
Fstl3	0.92	0.7	1	1.87	0.12	1.42	1.3
Efemp1	2.08	1.07	2.13	2.64	0.03	1.3	1.3
Arap2	3.01	2.17	4.65	7.91	0.63	1.87	1.2
Abracl	63.7	33.56	67.86	81.29	0.09	1.28	1.2
Zfp52	1.52	0.81	2.09	2.5	0.46	1.63	1.2
Arhgap42	5.09	3.52	4.83	7.5	-0.08	1.09	1.2
Pltp	4.82	2.69	6.39	7.97	0.41	1.57	1.2
Flrt3	10.97	10.47	17.33	36.4	0.66	1.8	1.1
Apln	2.03	1.78	3.4	6.52	0.74	1.87	1.1
Lmo7	8.38	4.74	10.24	12.69	0.29	1.42	1.1
Plekhh2	0.92	1.03	1.36	3.28	0.56	1.67	1.1
Parp3	1.92	1.56	3.38	5.74	0.82	1.88	1.1
Lpar1	2.64	2.52	2.83	5.55	0.1	1.14	1.0
Gsto1	29.03	15.4	53.47	57.75	0.88	1.91	1.0
Fzd6	2.75	1.61	2.65	3.17	-0.05	0.98	1.0
Tmed5	9.85	9.25	10.98	21.03	0.16	1.18	1.0
Rnf128	11.25	8.02	11.6	16.57	0.04	1.05	1.0
Gadd45b	3.23	3.19	3.5	6.93	0.12	1.12	1.0
Fam13c	0.54	0.55	1	2.02	0.89	1.88	1.0
F8	1.42	1.65	1.94	4.39	0.45	1.41	1.0

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Adora1	1.74	1.51	1.96	3.17	0.17	1.07	0.9
Gm7694	0.98	0.83	1.23	1.93	0.33	1.22	0.9
Creld1	3.09	1.87	5.49	6.13	0.83	1.71	0.9
Kif1a	4.5	2.86	5.33	6.23	0.24	1.12	0.9
Ghr	8.19	6.71	12.08	18.1	0.56	1.43	0.9
Cnn1	6.24	6.36	6.9	12.77	0.15	1.01	0.9
Plxdc2	3.94	3.84	5.06	8.88	0.36	1.21	0.9
Fam84b	3.5	2.06	5.07	5.3	0.53	1.36	0.8
Dact2	1.56	0.73	2.04	1.7	0.39	1.22	0.8
Cd24a	162.66	161.52	200.54	354.59	0.3	1.13	0.8
Slc30a2	0.79	0.79	1.37	2.41	0.79	1.61	0.8
Nhs	2.99	2.88	5.32	8.97	0.83	1.64	0.8
Mreg	1.57	1.47	2.65	4.37	0.76	1.57	0.8
Elf1	13.53	8.81	24.64	27.88	0.86	1.66	0.8
Fam134b	2.14	1.57	3.67	4.69	0.78	1.58	0.8
Rhod	8.68	5.39	9.99	10.77	0.2	1	0.8
Cd63	84.87	72.05	152.14	223.76	0.84	1.63	0.8
Anxa4	13.52	9.94	17.13	21.71	0.34	1.13	0.8
Serpine2	12.73	11.98	14.66	23.83	0.2	0.99	0.8
Wbp5	223.76	186.84	306.08	437.59	0.45	1.23	0.8
Tnnc1	17.89	22.35	28.53	60.68	0.67	1.44	0.8
Llgl2	4.72	3.51	7.17	9.06	0.6	1.37	0.8
Samd5	4.7	3.11	5.87	6.64	0.32	1.09	0.8
Rbks	0.94	0.62	1.73	1.92	0.88	1.63	0.8
Paqr8	1.66	1.46	2.88	4.18	0.79	1.52	0.7
Cdh13	3.51	3.2	5.56	8.41	0.66	1.39	0.7
Rprm	1.24	1.31	1.86	3.23	0.58	1.3	0.7
Dmd	0.69	0.58	1	1.39	0.54	1.26	0.7
Pdlim5	31.65	24.25	48.2	60.22	0.61	1.31	0.7
Erc2	1.01	1.32	1.52	3.23	0.59	1.29	0.7
Ptprd	1.89	2.2	2.44	4.63	0.37	1.07	0.7
Pfkfb3	6.58	6	8.26	12.27	0.33	1.03	0.7
Sec14l2	2.12	1.55	3.25	3.84	0.62	1.31	0.7
Bin1	27.34	16.71	49.99	48.79	0.87	1.55	0.7
Serpinb6a	34.56	17.24	59.56	47.8	0.79	1.47	0.7
Dcxr	8.76	6.81	13.53	16.9	0.63	1.31	0.7
Ghdc	3.1	0.63	4.59	1.5	0.57	1.25	0.7
Smim10l1	12.21	7.43	17.74	17.34	0.54	1.22	0.7
Plcb4	3.98	3.81	5.22	8.02	0.39	1.07	0.7
Car4	11.08	15.51	17.46	39.12	0.66	1.33	0.7
Tradd	4.41	2.62	6.13	5.82	0.48	1.15	0.7
Ncoa7	4	3.73	7.7	11.33	0.94	1.6	0.7
Dgkk	5.43	4.04	8.18	9.6	0.59	1.25	0.7
Pkhd1l1	1.37	1.6	1.69	3.09	0.3	0.95	0.7
Nabp1	12.87	13.56	24.6	40.29	0.93	1.57	0.6
Tom1l1	6.39	4.04	10.69	10.49	0.74	1.38	0.6
Plcd3	2.01	1.36	2.89	3.04	0.52	1.16	0.6
C1galt1c1	7.4	5.91	10.03	12.39	0.44	1.07	0.6
Rb1	7.34	8.55	9.78	17.54	0.41	1.04	0.6
Bex4	34.89	23.46	59.87	61.75	0.78	1.4	0.6
Gbp7	0.6	0.6	1.02	1.57	0.77	1.39	0.6
Cacna2d1	1.49	1.24	2.57	3.27	0.79	1.4	0.6
Celf2	2.66	3.16	3.92	7.05	0.56	1.16	0.6
Lypla1	23.76	22.69	37.13	53.21	0.64	1.23	0.6
Mob1b	9.95	9.54	12.89	18.6	0.37	0.96	0.6
Tpm2	22.34	7.96	38.57	20.52	0.79	1.37	0.6
Npl	7.68	9.45	13.22	24.28	0.78	1.36	0.6
Zrsr1	1.98	1.58	2.77	3.3	0.48	1.06	0.6
Fam20c	1.05	0.74	1.96	2.05	0.9	1.47	0.6
Polk	9.65	8.69	15.1	20.26	0.65	1.22	0.6

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Bmpr2	20.15	18.49	27.79	37.2	0.46	1.01	0.6
Cgref1	3.77	4.46	7.23	12.42	0.94	1.48	0.5
Ezr	18.96	14.42	32.28	35.87	0.77	1.31	0.5
Lamp2	48.87	44.48	83.48	110.24	0.77	1.31	0.5
Cers5	35.21	22.06	55.94	50.87	0.67	1.21	0.5
Snx24	7.4	6.81	11.33	15.13	0.61	1.15	0.5
Radil	2.09	1.56	3.13	3.39	0.58	1.12	0.5
Fam214a	3.16	2.31	5.64	5.96	0.84	1.37	0.5
Usp2	0.99	0.86	1.6	2	0.69	1.22	0.5
Cast	12.94	12.65	21.4	30.18	0.73	1.25	0.5
Phldb2	27.17	23.38	46.4	56.97	0.77	1.28	0.5
Ucp2	19.32	5.23	30.65	11.87	0.67	1.18	0.5
Ttll3	1.18	1.1	1.86	2.47	0.66	1.17	0.5
Aif1l	11.38	9.92	17.42	21.61	0.61	1.12	0.5
Ikzf2	2.08	2.04	3	4.2	0.53	1.04	0.5
Atp13a3	31.95	33.27	45.94	68	0.52	1.03	0.5
Slc30a4	6.77	5.65	12.65	14.9	0.9	1.4	0.5

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Nppb	0.28	0.13	2.14	5.7	2.93	5.45	2.5
Ccl17	0.67	0.21	17.28	29.34	4.69	7.13	2.4
1700007K13Rik	1.08	0.58	2.53	6.48	1.23	3.48	2.3
Plscr2	0.11	0.1	1.16	4.49	3.4	5.49	2.1
Foxq1	0.57	0.3	2.93	5.8	2.36	4.27	1.9
Myh7	0.15	0.17	2.03	8.54	3.76	5.65	1.9
Gsn	3.66	1.59	23.4	37.62	2.68	4.56	1.9
Tnfaip6	0.19	0.1	3.71	7.06	4.29	6.14	1.9
Fabp3	8.17	4.4	18.24	34.44	1.16	2.97	1.8
Ly96	0.64	0.35	2.98	5.48	2.22	3.97	1.8
Ptgs2	0.29	0.25	4.84	13.84	4.06	5.79	1.7
Akr1cl	0.1	0.1	1.93	6.29	4.27	5.97	1.7
Perp	4.83	1.82	17.16	20.93	1.83	3.52	1.7
Hspb1	12.35	3.94	36.83	37.74	1.58	3.26	1.7
Gm5082	0.17	0.12	1.12	2.46	2.72	4.36	1.6
Cav1	0.41	0.25	18.38	34.86	5.49	7.12	1.6
Esrp2	0.4	0.22	2.46	3.97	2.62	4.17	1.6
Nts	0.34	0.39	5.1	17.07	3.91	5.45	1.5
F3	5.77	2.14	28.76	29.79	2.32	3.8	1.5
Masp1	1.46	1.05	2.88	5.65	0.98	2.43	1.5
S100a11	33.4	15.53	112.74	141.58	1.76	3.19	1.4
Mfap3l	0.42	0.45	3.02	8.58	2.85	4.25	1.4
Dpp4	0.18	0.15	1.33	2.91	2.89	4.28	1.4
Scel	0.16	0.15	4.73	11.53	4.89	6.26	1.4
Ankrd1	2.18	2.1	81.81	197.71	5.23	6.56	1.3
Mocos	0.14	0.1	1.2	2.13	3.1	4.41	1.3
Qpct	1.06	0.57	2.48	3.31	1.23	2.54	1.3
S100a6	18.83	4.1	157.63	85	3.07	4.37	1.3
Npnt	0.35	0.22	7.43	11.32	4.41	5.69	1.3
Camk4	0.43	0.3	1.71	2.89	1.99	3.27	1.3
Met	1.43	1.05	3.01	5.28	1.07	2.33	1.3
Egfr	1.74	0.96	4.08	5.35	1.23	2.48	1.3
Pcdh9	0.6	0.56	1.34	2.97	1.16	2.41	1.3
Cxcl10	0.29	0.18	1.26	1.83	2.12	3.35	1.2
Tinagl1	0.74	0.47	13.76	20.2	4.22	5.43	1.2
Slc25a2	1.01	0.79	2.47	4.48	1.29	2.5	1.2

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Abcb1b	2.13	1.02	4.32	4.78	1.02	2.23	1.2
Tmem37	1.47	1.11	2.9	5.06	0.98	2.19	1.2
Sox17	1.01	0.76	11.94	20.61	3.56	4.76	1.2
Gcnt2	0.97	0.8	2.44	4.61	1.33	2.53	1.2
Cdkn2b	0.49	0.23	18.87	20.25	5.27	6.46	1.2
Gpr39	0.22	0.18	1.3	2.43	2.56	3.75	1.2
Pqlc3	2.4	1.09	5.57	5.77	1.21	2.4	1.2
Tshr	0.15	0.13	1.07	2.08	2.83	4	1.2
Bdnf	0.3	0.18	1.23	1.67	2.04	3.21	1.2
2200002D01Rik	4.06	0.91	9.77	4.94	1.27	2.44	1.2
Mme	3.18	3.17	9.01	20.07	1.5	2.66	1.2
Angpt2	0.18	0.16	1.5	2.97	3.06	4.21	1.2
Slitrk4	0.31	0.33	1.27	3	2.03	3.18	1.2
Prnd	0.38	0.22	49.77	63.33	7.03	8.17	1.1
Sgcd	0.24	0.19	2.56	4.48	3.42	4.56	1.1
Ier3	2.51	0.83	8.73	6.35	1.8	2.94	1.1
Mbnl3	1.9	1.95	4.38	9.89	1.2	2.34	1.1
Nexn	1.21	1.46	3.96	10.47	1.71	2.84	1.1
Tgfbi	1.4	0.72	10.63	11.82	2.92	4.04	1.1
Upk1b	0.1	0.12	3.05	7.87	4.93	6.04	1.1
Prkg1	1.29	0.84	3.61	5.05	1.48	2.59	1.1
Tagln	49.97	40.07	420.42	718.61	3.07	4.16	1.1
Apol8	0.68	0.48	4.03	6.08	2.57	3.66	1.1
Gadd45a	2.86	2.23	8.98	14.93	1.65	2.74	1.1
Birc7	0.1	0.1	1.04	2.2	3.38	4.46	1.1
Nrg1	1.9	1.13	10.1	12.72	2.41	3.49	1.1
Galnt14	1.07	0.79	2.99	4.66	1.48	2.56	1.1
Angpt1	0.24	0.18	3.59	5.61	3.9	4.96	1.1
Stard13	3.58	2.4	11.7	16.39	1.71	2.77	1.1
Cd1d1	0.33	0.25	1.99	3.12	2.59	3.64	1.1
Lif	0.36	0.32	1.23	2.25	1.77	2.81	1.0
Parm1	2.55	1.95	8.41	13.18	1.72	2.76	1.0
Lypd6	1.75	1.2	3.37	4.78	0.95	1.99	1.0
Ccl7	0.1	0.1	2.25	4.6	4.49	5.52	1.0
Cpz	0.29	0.13	1.33	1.21	2.2	3.22	1.0
Glipr1	0.12	0.14	13.03	30.62	6.76	7.77	1.0
Itga1	1	0.65	23.64	30.79	4.56	5.57	1.0
Tnfaip8l3	0.59	0.27	1.93	1.78	1.71	2.72	1.0
Cpne2	4.22	1.96	11.15	10.4	1.4	2.41	1.0
Pitx1	0.21	0.13	1.19	1.46	2.5	3.49	1.0
Sema3b	0.67	0.45	1.98	2.63	1.56	2.55	1.0
Fosb	3.39	1.54	16.21	14.6	2.26	3.24	1.0
Aldh4a1	2.93	1.97	10.58	14.03	1.85	2.83	1.0
Gab3	0.56	0.41	1.24	1.8	1.15	2.13	1.0
Pdyn	0.38	0.45	3.82	8.76	3.33	4.28	1.0
Dkk2	0.16	0.13	1.11	1.74	2.79	3.74	1.0

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 9: upregulated in mutant/downregulated in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Isg15	1.86	0.8	0.96	2.15	-0.95	1.43	2.4

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES $Sp1^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ $Sp1^{del/del}$ FPKM	WT ES/Flk log2 FC	$Sp1^{del/del}$ ES/Flk log2 FC	WT FC/$Sp1^{del/del}$ FC
Slfn2	0.29	1.5	2.12	1.42	2.87	-0.08	-3.0
Hoxd9	0.36	0.84	2.47	1.57	2.78	0.9	-1.9
Plcd1	2.07	2.07	5.43	1.52	1.39	-0.45	-1.8
Lat	0.85	1.07	4.83	1.88	2.51	0.81	-1.7
Kcnk5	1.75	1.01	5.77	1.14	1.72	0.17	-1.6
Crip1	29.51	12.4	164.61	23.84	2.48	0.94	-1.5
Ptp4a3	2.57	2.86	5.15	2	1	-0.52	-1.5
Peg12	1.08	3.07	3.79	4.14	1.81	0.43	-1.4
E2f2	1.16	1.8	2.66	1.65	1.2	-0.13	-1.3
Tnfrsf22	1.55	2.15	3.02	1.66	0.96	-0.37	-1.3
Npm2	0.74	1.61	2.02	1.77	1.45	0.14	-1.3
Atp2b4	3.3	4	14.93	7.46	2.18	0.9	-1.3
Tnfsf9	1.54	1.34	3.94	1.47	1.36	0.13	-1.2
Scd1	11.89	17.29	40.36	25.19	1.76	0.54	-1.2
Ebf1	0.83	1.16	2.14	1.38	1.37	0.25	-1.1
Inafm2	1.29	2.45	3.52	3.1	1.45	0.34	-1.1
Insig1	29.59	41.11	64.56	42.56	1.13	0.05	-1.1
Hap1	0.42	0.8	1.05	1	1.32	0.32	-1.0
Palm3	0.61	1.63	1.28	1.73	1.07	0.09	-1.0
Fads1	35.36	36.66	84.03	44.48	1.25	0.28	-1.0
Btg3	1.51	3.26	5.12	5.7	1.76	0.81	-1.0
Rhox5	8.51	173.6	22.21	234.23	1.38	0.43	-1.0
Tmem176b	9.42	11.56	27.93	18.07	1.57	0.64	-0.9
Gimap9	1.55	1.53	5.38	2.84	1.8	0.89	-0.9
Relb	3.72	5.17	11.96	8.84	1.68	0.77	-0.9
Slc2a12	1.06	0.92	2.87	1.33	1.44	0.53	-0.9
Fdps	92.55	114.93	214.96	143.56	1.22	0.32	-0.9
Tm7sf2	3.21	5.98	9.21	9.27	1.52	0.63	-0.9
Slc43a2	0.91	1.27	2.57	1.95	1.5	0.62	-0.9
Paqr4	1.31	1.97	3.17	2.58	1.27	0.39	-0.9
Nr4a1	8.69	9.95	27.29	17.21	1.65	0.79	-0.9
Hyal1	1.85	0.86	5.6	1.45	1.6	0.75	-0.9
Sepp1	9.88	21.57	33.95	41.52	1.78	0.94	-0.8
Armcx6	1.87	1.75	5.49	2.87	1.55	0.71	-0.8
Fads2	22.51	28.36	65.89	46.42	1.55	0.71	-0.8
Clic6	4.85	4.13	12.76	6.07	1.4	0.56	-0.8
Noxo1	0.46	0.97	1.48	1.78	1.69	0.88	-0.8
Ccdc60	0.92	1.27	2.3	1.81	1.32	0.51	-0.8
Aacs	13.66	13.31	41.96	23.49	1.62	0.82	-0.8
Zhx3	1.57	2.91	3.47	3.69	1.14	0.34	-0.8
St6galnac3	5.2	6.57	15.32	11.21	1.56	0.77	-0.8
Nmrk1	1.49	3.72	2.89	4.22	0.96	0.18	-0.8
Tns2	7.43	9.06	19.61	14.02	1.4	0.63	-0.8
Myl9	11.13	10.29	21.7	11.73	0.96	0.19	-0.8
Rin2	3.65	4.66	9.9	7.49	1.44	0.68	-0.8
Mapk12	6.91	10.83	17.67	16.32	1.35	0.59	-0.8
Znfx1	3.48	7.04	7.65	9.14	1.14	0.38	-0.8
Mmp2	19.31	12.5	41.79	15.98	1.11	0.35	-0.8
Nrgn	0.57	0.85	1.73	1.53	1.6	0.85	-0.8
Ncald	0.4	0.78	1.03	1.19	1.36	0.61	-0.8
Dock6	10.74	11.06	32.36	19.88	1.59	0.85	-0.7
Pkn3	6.32	7.53	18.78	13.34	1.57	0.83	-0.7
Maged2	59.71	57.93	175.73	102.48	1.56	0.82	-0.7
Tmem86a	0.83	0.99	2.42	1.75	1.54	0.82	-0.7
Gpr4	0.7	1.09	1.94	1.83	1.47	0.75	-0.7
Gypc	2.77	2.97	8.29	5.43	1.58	0.87	-0.7
Ccdc85b	1.2	1.51	3.01	2.32	1.33	0.62	-0.7
Arrdc1	3.68	2.33	9.01	3.49	1.29	0.58	-0.7
Srebf1	9.5	8.46	21.29	11.56	1.16	0.45	-0.7
Tcp11l2	0.96	1.37	2.04	1.79	1.09	0.39	-0.7
Lcat	0.52	0.84	1.59	1.6	1.61	0.93	-0.7
Dpysl2	106.08	160.06	270.03	255.41	1.35	0.67	-0.7

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Gpsm3	3.85	5.87	9.26	8.81	1.27	0.59	-0.7
Mxd1	1.72	2.1	3.55	2.71	1.05	0.37	-0.7
Tnfrsf1a	12.36	14.31	27.76	20.26	1.17	0.5	-0.7
Sall2	8.87	11.92	19.13	16.13	1.11	0.44	-0.7
Hmgcr	72.75	108.38	156.19	146.4	1.1	0.43	-0.7
Fbxo9	7.33	8.18	14.81	10.34	1.01	0.34	-0.7
Tiam1	14.85	17.1	30.76	22.36	1.05	0.39	-0.7
Slc35e1	8.31	9.6	19.35	14.24	1.22	0.57	-0.7
Dchs1	4.81	5.91	10.52	8.25	1.13	0.48	-0.7
Trap1a	0.7	16.75	1.36	20.76	0.96	0.31	-0.7
Celsr2	0.65	0.94	1.76	1.64	1.44	0.8	-0.6
Serpinb6b	3.39	3.01	8.92	5.09	1.4	0.76	-0.6
Ripply3	1.26	1.06	3.14	1.7	1.32	0.68	-0.6
Spred3	2.25	3.18	4.78	4.34	1.09	0.45	-0.6
B4galt1	21.53	25.85	44.82	34.53	1.06	0.42	-0.6
Il17ra	2.68	2.75	5.35	3.52	1	0.36	-0.6
Acly	86.72	93.24	168.85	116.19	0.96	0.32	-0.6
Slc48a1	2.61	3.46	6.56	5.62	1.33	0.7	-0.6
Rab11fip3	11.49	15.97	32.02	29.03	1.48	0.86	-0.6
Trabd2b	0.72	1.17	1.82	1.93	1.34	0.72	-0.6
2310047M10Rik	2.43	2.8	4.82	3.62	0.99	0.37	-0.6
Por	18.64	24.36	36.72	31.25	0.98	0.36	-0.6
Sirpa	8.57	6.99	17.57	9.42	1.04	0.43	-0.6
Rnf141	10.22	9.2	19.96	11.82	0.97	0.36	-0.6
Nr4a2	1.01	0.94	2.46	1.51	1.28	0.68	-0.6
Rsc1a1	7.92	10.82	17.48	15.73	1.14	0.54	-0.6
Asb12	0.83	1.91	2.33	3.57	1.49	0.9	-0.6
Inpp5a	7.78	10.55	19.62	17.67	1.33	0.74	-0.6
Ppp1r9b	11.51	12.09	24.84	17.3	1.11	0.52	-0.6
Eif2ak3	5.17	5.67	11.09	8.12	1.1	0.52	-0.6
Sorbs1	6.79	9.57	18.84	17.81	1.47	0.9	-0.6
Cnnm2	3.08	3.21	7.68	5.39	1.32	0.75	-0.6
3110062M04Rik	4.26	4.79	9.65	7.3	1.18	0.61	-0.6
Npr2	1.91	2.43	4.32	3.72	1.18	0.61	-0.6
Pafah2	3.06	4	6.67	5.87	1.12	0.55	-0.6
Zfp319	3.44	4.67	7.05	6.48	1.04	0.47	-0.6
Plekhb2	2.96	3.53	7.98	6.43	1.43	0.87	-0.6
Lrmp	1.06	2.26	2.49	3.6	1.23	0.67	-0.6
Habp4	3.33	5.44	7.33	8.11	1.14	0.58	-0.6
Glrx	1.32	0.85	3.36	1.48	1.35	0.8	-0.6
Msmo1	46.6	53.97	92.63	73.17	0.99	0.44	-0.6
Tbc1d10b	9.41	12.73	19.43	18.08	1.05	0.51	-0.5
Pcdhga11	2.91	3.71	7.62	6.75	1.39	0.86	-0.5
Uaca	13.59	17.06	31.98	27.63	1.23	0.7	-0.5
Tpcn1	6.2	8.15	14.34	13.06	1.21	0.68	-0.5
Sqle	35.71	48.84	68.87	65.51	0.95	0.42	-0.5
Stk25	21.34	25.81	49.63	42	1.22	0.7	-0.5
Cnrip1	2.68	3.41	5.4	4.78	1.01	0.49	-0.5
Fgd5	21.56	20.84	42.16	28.51	0.97	0.45	-0.5
Fam149a	1.58	1.18	3.07	1.6	0.96	0.44	-0.5
Exoc3l	9.61	8.13	18.55	10.94	0.95	0.43	-0.5
Fdft1	49.8	63.7	100.3	90.13	1.01	0.5	-0.5
Ubc	8.62	11.26	23.35	21.6	1.44	0.94	-0.5
Xkr8	1.12	1.13	2.2	1.57	0.97	0.47	-0.5
Pkd1	8.01	10.77	15.63	14.78	0.96	0.46	-0.5

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Dll4	0.16	0.3	8.53	1.64	5.74	2.45	-3.3
Gja5	0.14	0.13	8.47	1.07	5.92	3.04	-2.9
Pi16	0.13	0.28	12.8	4.97	6.62	4.15	-2.5
Hba-x	0.23	0.62	11.53	5.62	5.65	3.18	-2.5
Anxa1	0.43	2.66	143.14	163.56	8.38	5.94	-2.4
C1qtnf6	1.04	0.98	11.29	2.16	3.44	1.14	-2.3
Gimap6	0.11	0.36	41.24	29.69	8.55	6.37	-2.2
Tspan32	0.36	0.41	6.52	1.66	4.18	2.02	-2.2
Hoxd8	0.45	1.23	9.09	6.16	4.34	2.32	-2.0
Hbb-y	0.23	0.37	10.2	4.15	5.47	3.49	-2.0
Gimap4	0.62	0.52	50.44	11.09	6.35	4.41	-1.9
Gpr182	0.1	0.12	4.82	1.51	5.59	3.65	-1.9
Cd34	0.46	0.58	177.92	61.27	8.6	6.72	-1.9
Hbb-bh1	3.18	4.19	119.13	42.69	5.23	3.35	-1.9
Fermt3	0.73	0.89	11.72	4.24	4	2.25	-1.8
Fmo1	0.11	0.41	33.64	37.83	8.26	6.53	-1.7
Tmem204	0.13	0.27	31.92	20.67	7.94	6.26	-1.7
Clec1b	0.1	0.18	3.49	2.03	5.13	3.5	-1.6
Syt13	0.3	0.37	8.52	3.41	4.83	3.2	-1.6
Art4	0.31	0.9	3.8	3.57	3.62	1.99	-1.6
Pecam1	1.16	3.1	210.99	183.35	7.51	5.89	-1.6
Sema3g	0.18	0.22	4.18	1.77	4.54	3.01	-1.5
Esam	2.46	3.9	138.26	77.51	5.81	4.31	-1.5
Rcsd1	0.55	0.86	17.08	9.56	4.96	3.47	-1.5
Cldn5	0.18	0.29	8.76	5.14	5.6	4.15	-1.5
Itgb3	0.63	0.88	7.39	3.78	3.55	2.1	-1.5
Tmem173	0.34	0.65	3.43	2.44	3.33	1.91	-1.4
Itga2b	1.45	1.95	36.69	18.57	4.66	3.25	-1.4
8430408G22Rik	0.12	0.2	1.86	1.17	3.95	2.55	-1.4
Tie1	1.17	2.73	180.56	161.8	7.27	5.89	-1.4
Hoxd4	0.3	0.44	2.07	1.18	2.79	1.42	-1.4
Nova2	0.2	0.46	5.99	5.35	4.9	3.54	-1.4
Adgrl4	0.2	0.56	49.69	54.99	7.96	6.62	-1.3
Plxnd1	8.89	4.37	174.52	34.09	4.3	2.96	-1.3
Vash1	1.1	1.52	16.5	9.04	3.91	2.57	-1.3
Car8	0.23	0.23	3	1.19	3.71	2.37	-1.3
Sorcs2	0.71	0.89	3.64	1.81	2.36	1.02	-1.3
Sost	0.12	0.31	11.12	11.4	6.53	5.2	-1.3
Ccm2l	0.13	0.25	8.31	6.39	6	4.68	-1.3
Ednrb	1.1	1.25	65.75	29.91	5.9	4.58	-1.3
Sox18	0.61	0.79	27.72	14.59	5.51	4.21	-1.3
Entpd1	0.32	0.35	2.54	1.13	2.99	1.69	-1.3
Galnt18	0.64	0.67	3.56	1.52	2.48	1.18	-1.3
Adgrf5	0.52	0.9	24.09	17.04	5.53	4.24	-1.3
Csf2rb	0.15	0.28	4.76	3.65	4.99	3.7	-1.3
Dhrs3	0.44	0.85	7.35	5.81	4.06	2.77	-1.3
Akr1b8	0.87	2.09	10.21	10.01	3.55	2.26	-1.3
Myct1	0.22	0.5	18.65	17.58	6.41	5.14	-1.3
Slc43a3	3.28	2.85	16.05	5.91	2.29	1.05	-1.2
Plvap	0.77	1.51	89.13	74.58	6.85	5.63	-1.2
Ushbp1	0.19	0.38	11.6	9.99	5.93	4.72	-1.2
Nr2f2	0.17	0.43	5	5.47	4.88	3.67	-1.2
C130074G19Rik	0.29	0.44	4.4	2.88	3.92	2.71	-1.2
Megf9	0.94	3.8	7.28	12.65	2.95	1.74	-1.2
Grap	0.59	1.09	25.19	20.38	5.42	4.22	-1.2
Map4k2	1.91	3.25	12.22	9.09	2.68	1.48	-1.2
Akr1c13	0.1	0.13	6.15	3.51	5.94	4.75	-1.2
Slc25a45	0.16	0.19	4.16	2.16	4.7	3.51	-1.2
Ldb2	1.78	3.19	18.56	14.57	3.38	2.19	-1.2
Icam2	1.23	2.31	94.7	79.05	6.27	5.1	-1.2

Sp1$^{del/del}$ Flk1+ - HE1
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ppp1r13b	3.65	6.15	55.6	41.66	3.93	2.76	-1.2
S100a16	0.47	0.32	6.9	2.09	3.88	2.71	-1.2
Gja4	2.01	2.36	56.63	29.8	4.82	3.66	-1.2
Adgrg1	0.39	0.54	1.84	1.14	2.24	1.08	-1.2
Fhod1	5.24	9.27	24.77	19.77	2.24	1.09	-1.2
Fam198b	1.08	3.15	20.47	26.92	4.24	3.1	-1.1
Cd93	0.81	1.42	109.95	88.39	7.08	5.96	-1.1
Hcls1	0.1	0.18	2.3	1.91	4.52	3.41	-1.1
Arhgef3	1.65	2.62	13.72	10.1	3.06	1.95	-1.1
Cnr2	0.13	0.22	7.35	5.85	5.82	4.73	-1.1
Arhgef15	0.72	1.04	22.46	15.21	4.96	3.87	-1.1
Thsd1	0.7	1.15	31.1	24.21	5.47	4.4	-1.1
Ehd2	3.78	3.29	47.07	19.52	3.64	2.57	-1.1
Ackr1	0.84	1.42	5.86	4.74	2.8	1.74	-1.1
Klhl3	0.44	0.54	2.68	1.58	2.61	1.55	-1.1
Plxnc1	0.65	0.79	23.62	13.81	5.18	4.13	-1.1
Tnfaip8l1	0.78	1.04	5.97	3.86	2.94	1.89	-1.1
Nos3	1.14	1.14	47.99	23.36	5.4	4.36	-1.0
Pde2a	0.65	0.91	11.93	8.17	4.2	3.17	-1.0
Npr1	2.67	3.49	23.37	15	3.13	2.1	-1.0
Sash3	0.16	0.27	1.24	1.02	2.95	1.92	-1.0
Tnnt2	0.87	1.2	15.32	10.4	4.14	3.12	-1.0
Vwa7	0.82	1.18	8.01	5.71	3.29	2.27	-1.0
Flt4	4.65	4.37	30.13	14.02	2.7	1.68	-1.0
Shank3	8.93	10.91	36.86	22.34	2.05	1.03	-1.0
Prkcdbp	0.56	0.36	8.81	2.82	3.98	2.97	-1.0
Alox5ap	0.47	0.47	5.05	2.51	3.43	2.42	-1.0
Coro2b	1.16	1.9	11.81	9.61	3.35	2.34	-1.0
Lyl1	1.59	2.71	13.51	11.45	3.09	2.08	-1.0
Rin3	2.15	2.85	17.15	11.34	3	1.99	-1.0
Ppm1j	3.09	3.95	14.55	9.28	2.24	1.23	-1.0
Rasip1	2.01	2.9	78.71	56.8	5.29	4.29	-1.0
Ptpre	0.93	1.64	4.95	4.4	2.41	1.42	-1.0
Tek	1.97	3.4	150.39	131.46	6.25	5.27	-1.0
Serpinb1a	0.11	0.18	2.68	2.23	4.61	3.63	-1.0
Cpt1a	0.5	0.37	3.87	1.45	2.95	1.97	-1.0
Nfkbie	1.62	2.56	11.02	8.86	2.77	1.79	-1.0
Cd200r1	0.11	0.18	1.83	1.53	4.06	3.09	-1.0
Acvrl1	0.8	1.08	37.77	26.11	5.56	4.6	-1.0
Col18a1	19.11	25.6	354.59	243.34	4.21	3.25	-1.0
Ramp3	0.38	0.48	4.46	2.9	3.55	2.59	-1.0

Supplementary Table 5.3 – Deregulation of genes between Flk1+ cells and HE1 in Sp1$^{del/del}$ cells

Genes that are differentially expressed in WT cells between differentiation stages Flk1+ and HE1 cells were identified (at least two-fold changed) and the expression levels compared to that in Sp1$^{del/del}$ cells. The FPKM values in each cell type are shown, as well as the respective fold change (FC, log2 value) between ESC and Flk1+ stages, and the fold change between these values in WT and Sp1$^{del/del}$ cells. Cluster 1 and 12 show genes that are unchanged between WT and Sp1$^{del/del}$ cells (not shown). No genes were identified as downregulated in mutant/upregulated in WT (cluster 6).

Supplementary Table 5.4

Sp1$^{del/del}$ HE1 – HE2
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rfx6	1.867	1.592	0.112	0.187	-4.06	-3.09	1.0
Postn	87.776	122.93	18.692	50.773	-2.23	-1.28	1.0
Rnase4	2.278	2.514	0.51	1.144	-2.16	-1.14	1.0

Sp1$^{del/del}$ HE1 – HE2
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Tph1	2.078	1.245	0.423	0.812	-2.3	-0.62	1.7
Plet1	1.097	1.261	0.403	1.123	-1.44	-0.17	1.3
Bicc1	1.276	1.351	0.514	1.216	-1.31	-0.15	1.2
Sbsn	1.516	1.182	0.737	1.264	-1.04	0.1	1.1
Clec1a	3.814	2.241	1.949	2.529	-0.97	0.17	1.1
H2-T9	3.618	3.441	1.877	3.841	-0.95	0.16	1.1
Fam19a5	1.342	1.435	0.671	1.513	-1	0.08	1.1
Ntf3	1.165	1.07	0.394	0.748	-1.56	-0.52	1.0
Dync1i1	2.872	2.148	0.932	1.413	-1.62	-0.6	1.0
Sytl2	1.404	1.498	0.405	0.85	-1.79	-0.82	1.0
Ppp1r14a	2.158	1.597	1.065	1.547	-1.02	-0.05	1.0
Arhgap8	2.71	1.076	1.004	0.744	-1.43	-0.53	0.9
Ly6a	10.368	5.16	4.277	3.686	-1.28	-0.49	0.8
Mycl	1.445	1.361	0.72	1.167	-1.01	-0.22	0.8
Gnal	1.056	1.29	0.464	0.973	-1.19	-0.41	0.8
Sdcbp2	2.657	3.12	1.216	2.365	-1.13	-0.4	0.7
Klf14	2.821	2.303	1.302	1.751	-1.12	-0.4	0.7
Adgrf5	23.992	16.937	9.874	11.332	-1.28	-0.58	0.7
Plac1	19.664	17.07	7.583	10.68	-1.37	-0.68	0.7
Ly75	2.048	1.658	0.842	1.092	-1.28	-0.6	0.7
Nucb2	15.538	13.835	7.315	10.306	-1.09	-0.42	0.7
Tnnt2	15.225	10.298	7.537	8.069	-1.01	-0.35	0.7
Slc1a2	9.602	8.782	3.748	5.379	-1.36	-0.71	0.7
Cnnm2	7.58	5.286	3.322	3.605	-1.19	-0.55	0.6
Rnd1	3.346	3.778	1.164	2.032	-1.52	-0.89	0.6
Serpine1	25.767	28.234	10.283	17.259	-1.33	-0.71	0.6
Fosb	16.11	14.495	8.164	11.267	-0.98	-0.36	0.6
Sorcs2	3.544	1.714	1.378	1.008	-1.36	-0.77	0.6
Rgn	1.005	1.162	0.424	0.735	-1.25	-0.66	0.6
Serpinb6b	8.818	4.988	3.174	2.7	-1.47	-0.89	0.6
Chrna7	2.654	3.942	1.101	2.44	-1.27	-0.69	0.6
Syne2	14.443	12.804	6.212	8.234	-1.22	-0.64	0.6
Edn1	56.908	92.285	21.273	51.064	-1.42	-0.85	0.6
Ror1	3.402	2.585	1.199	1.351	-1.5	-0.94	0.6
Dock4	11.48	14.357	5.897	10.833	-0.96	-0.41	0.6
Cd109	25.675	22.989	9.884	12.839	-1.38	-0.84	0.5
Etv1	1.641	1.221	0.693	0.751	-1.24	-0.7	0.5
Pdgfrl	1.233	2.132	0.504	1.259	-1.29	-0.76	0.5
Mcam	69.278	62.717	31.105	40.332	-1.16	-0.64	0.5
Stab1	29.388	24.229	14.406	17.011	-1.03	-0.51	0.5
Tnks1bp1	14.447	7.215	7.106	5.087	-1.02	-0.5	0.5
Dennd5b	29.769	26.987	12.295	15.764	-1.28	-0.78	0.5
Olfml1	2.162	2.209	0.969	1.399	-1.16	-0.66	0.5

Sp1$^{del/del}$ HE1 – HE2
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Dkk1	1.48	1.986	0.698	0.326	-1.08	-2.61	-1.5
Cited1	2.882	3.2	1.391	0.589	-1.05	-2.44	-1.4
Adora1	1.864	3.068	0.513	0.328	-1.86	-3.23	-1.4
Fabp3	18.142	34.345	5.213	4.092	-1.8	-3.07	-1.3
Pth1r	1.105	2.834	0.373	0.4	-1.57	-2.82	-1.3
Shisa4	3.133	2.339	1.209	0.41	-1.37	-2.51	-1.1
Efna3	3.338	2.775	1.625	0.62	-1.04	-2.16	-1.1
Pdyn	3.715	8.655	0.687	0.765	-2.43	-3.5	-1.1
Tnnt3	3.22	2.682	1.445	0.577	-1.16	-2.22	-1.1
Rai2	1.353	1.023	0.618	0.225	-1.13	-2.18	-1.1
Mertk	1.258	2.376	0.467	0.43	-1.43	-2.47	-1.0
Lsr	9.053	7.2	4.314	1.781	-1.07	-2.02	-1.0

Sp1$^{del/del}$ HE1 – HE2
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Hist1h4j	2.092	1.366	3.506	0.498	0.74	-1.46	-2.2
Gm20594	1.467	5.677	1.865	1.84	0.35	-1.63	-2.0
Spata5l1	1.433	1.779	2.132	0.851	0.57	-1.06	-1.6
Acta1	1.441	6.938	1.02	1.651	-0.5	-2.07	-1.6
Fah	4.917	1.249	4.438	0.378	-0.15	-1.72	-1.6
Tmem51	1.595	1.094	1.062	0.256	-0.59	-2.1	-1.5
Samd12	1.391	4.877	1.089	1.478	-0.35	-1.72	-1.4
Mocos	1.096	2.031	0.647	0.485	-0.76	-2.07	-1.3
Hoxd9	2.365	1.468	1.402	0.378	-0.75	-1.96	-1.2
B4galnt1	1.288	1.478	1.432	0.719	0.15	-1.04	-1.2
Pard6g	5.07	8.042	4.321	3.067	-0.23	-1.39	-1.2
Tmem243	2.145	4.367	2.159	1.997	0.01	-1.13	-1.1
Bend5	2.647	4.485	2.313	1.799	-0.19	-1.32	-1.1
Slc6a15	1.525	3.215	0.892	0.872	-0.77	-1.88	-1.1
Abhd14b	2.175	2.18	1.367	0.634	-0.67	-1.78	-1.1
Lrpap1	36.02	39.63	23.09	12.05	-0.64	-1.72	-1.1
Pdgfa	4.28	7.572	2.424	2.074	-0.82	-1.87	-1.1
Snai1	1.751	2.066	1.339	0.778	-0.39	-1.41	-1.0
Rac3	2.047	3.814	1.563	1.465	-0.39	-1.38	-1.0
Fam213b	3.71	1.489	2.775	0.57	-0.42	-1.39	-1.0
Slc16a1	41.527	67.704	34.863	29.862	-0.25	-1.18	-0.9
Phyh	2.47	2.848	2.061	1.254	-0.26	-1.18	-0.9
Nppb	2.041	5.6	1.256	1.829	-0.7	-1.61	-0.9
Mob3b	2.18	3.699	1.38	1.257	-0.66	-1.56	-0.9
Gatsl3	4.157	3.537	2.28	1.059	-0.87	-1.74	-0.9
Zfp52	1.986	2.399	1.503	1	-0.4	-1.26	-0.9
Llgl2	7.075	8.957	4.141	2.937	-0.77	-1.61	-0.8
Hpcal1	18.707	17.864	15.038	8.081	-0.31	-1.14	-0.8
Moap1	2.943	5.964	2.532	2.899	-0.22	-1.04	-0.8
Agpat2	4.528	2.587	3.98	1.297	-0.19	-1	-0.8
Nodal	2.552	1.645	1.543	0.572	-0.73	-1.52	-0.8
Garnl3	1.539	2.231	0.803	0.678	-0.94	-1.72	-0.8
Nupr1	1.551	4.496	1.113	1.883	-0.48	-1.26	-0.8
Cdh1	2.143	4.244	1.172	1.358	-0.87	-1.64	-0.8
Sptb	6.81	7.014	4.03	2.448	-0.76	-1.52	-0.8
Tmc7	1.237	2.165	0.806	0.839	-0.62	-1.37	-0.8
Efna2	1.332	1.674	0.905	0.675	-0.56	-1.31	-0.8
Ddit4l	2.599	1.61	2	0.736	-0.38	-1.13	-0.8
Dll1	1.1	1.234	0.865	0.579	-0.35	-1.09	-0.7
Tgfb3	1.19	1.411	0.949	0.672	-0.33	-1.07	-0.7
Mapk8ip1	2.11	3.113	1.414	1.258	-0.58	-1.31	-0.7

Sp1$^{del/del}$ HE1 – HE2
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cd302	2.274	2.12	1.699	0.959	-0.42	-1.14	-0.7
Mfap2	2.007	4.621	1.274	1.803	-0.66	-1.36	-0.7
1700007K13Rik	2.431	6.379	1.31	2.137	-0.89	-1.58	-0.7
Gria4	1.326	3.223	1.022	1.543	-0.38	-1.06	-0.7
Dact2	1.943	1.602	1.201	0.625	-0.69	-1.36	-0.7
1110008P14Rik	2.312	3.206	1.589	1.383	-0.54	-1.21	-0.7
Pvrl1	1.427	1.755	0.996	0.767	-0.52	-1.19	-0.7
Mtcl1	1.865	2.226	1.161	0.879	-0.68	-1.34	-0.7
Sall4	16.016	20.791	10.791	8.838	-0.57	-1.23	-0.7
Prr5	9.728	6.861	6.597	2.936	-0.56	-1.22	-0.7
Ano10	23.867	7.702	13.613	2.807	-0.81	-1.46	-0.7
Nr1d1	1.416	3.262	1.057	1.555	-0.42	-1.07	-0.7
Hspbap1	2.194	2.841	1.69	1.405	-0.38	-1.02	-0.6
Shb	4.24	5.09	2.312	1.794	-0.87	-1.5	-0.6
Tnfrsf19	1.037	2.306	0.645	0.925	-0.69	-1.32	-0.6
Twist1	5.133	4.914	3.204	1.986	-0.68	-1.31	-0.6
B3galnt1	2.926	4.825	1.984	2.115	-0.56	-1.19	-0.6
Slc9a3r1	14.16	13.29	9.937	6.049	-0.51	-1.14	-0.6
Kcnip3	2.716	2.614	2.004	1.247	-0.44	-1.07	-0.6
Hand1	9.998	14.797	5.219	5.07	-0.94	-1.55	-0.6
Prrx2	1.753	1.929	1.145	0.83	-0.61	-1.22	-0.6
Dpf1	1.101	2.054	0.634	0.79	-0.8	-1.38	-0.6
Pik3ip1	2.129	2.915	1.566	1.435	-0.44	-1.02	-0.6
Angpt2	1.398	2.868	0.761	1.047	-0.88	-1.45	-0.6
Htra1	1.396	2.203	1	1.065	-0.48	-1.05	-0.6
Hid1	2.634	2.003	1.996	1.032	-0.4	-0.96	-0.6
Rtn1	1.888	2.572	1.009	0.942	-0.9	-1.45	-0.6
Abcb1b	4.215	4.677	2.278	1.729	-0.89	-1.44	-0.6
Bdh1	2.424	1.614	1.368	0.619	-0.83	-1.38	-0.6
Plekha7	6.967	7.588	4.485	3.331	-0.64	-1.19	-0.6
Fas	1.654	2.043	1.159	0.983	-0.51	-1.06	-0.6
Enox1	1.53	3.246	1.135	1.643	-0.43	-0.98	-0.6
Wwc1	1.991	1.912	1.497	0.986	-0.41	-0.96	-0.6
Plekhf1	6.667	7.065	3.655	2.664	-0.87	-1.41	-0.5
Mdk	149.04	84.772	105.106	41.22	-0.5	-1.04	-0.5
Tob1	6.471	7.282	4.088	3.193	-0.66	-1.19	-0.5
Fam124a	1.712	2.221	1.21	1.089	-0.5	-1.03	-0.5
Dact1	3.01	2.14	1.647	0.819	-0.87	-1.39	-0.5
Tppp3	5.424	5.626	3.995	2.897	-0.44	-0.96	-0.5
Lbh	3.658	4.414	2.058	1.758	-0.83	-1.33	-0.5
Dnajb2	5.489	5.914	3.569	2.729	-0.62	-1.12	-0.5
Gm7694	1.127	1.826	0.749	0.855	-0.59	-1.09	-0.5

Sp1$^{del/del}$ HE1 – HE2
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rnaset2a	1.87	0.60	1.72	2.09	-0.13	1.80	1.9
Pygo1	1.23	0.63	1.05	1.67	-0.23	1.41	1.6
Gstk1	6.85	0.55	7.05	1.75	0.04	1.67	1.6
Gnrh1	2.73	1.79	2.66	4.77	-0.03	1.42	1.5
Gm20604	3.37	1.37	3.65	4.00	0.12	1.55	1.4
Ccrl2	1.44	0.40	1.70	1.20	0.24	1.59	1.4
Tspan15	1.16	0.37	1.28	1.00	0.14	1.44	1.3
Npff	2.19	1.65	1.90	3.56	-0.20	1.10	1.3
Tnfrsf14	1.97	0.69	3.68	3.08	0.90	2.16	1.3
Mylpf	1.31	0.67	1.69	2.01	0.37	1.59	1.2
Klk8	3.50	0.46	6.21	1.89	0.83	2.03	1.2

Sp1$^{del/del}$ HE1 – HE2
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Nr4a2	2.36	1.41	2.54	3.45	0.10	1.29	1.2
Nsun3	2.42	1.59	2.15	3.08	-0.17	0.95	1.1
Slc30a3	3.30	1.52	3.27	3.11	-0.01	1.04	1.1
Trim30a	1.45	0.96	2.53	3.30	0.80	1.79	1.0
Nat2	2.00	0.69	2.17	1.48	0.12	1.10	1.0
Zfp667	1.56	1.04	1.71	2.20	0.14	1.09	1.0
Nfkbid	1.03	0.56	1.82	1.82	0.81	1.71	0.9
Naip7	2.48	0.84	4.40	2.69	0.82	1.68	0.9
Rwdd3	1.12	0.99	1.66	2.62	0.56	1.41	0.9
H2-T23	0.77	2.05	1.10	5.21	0.51	1.35	0.8
Aplnr	80.87	33.17	103.56	75.56	0.36	1.19	0.8
Pon3	4.29	0.93	6.66	2.54	0.63	1.45	0.8
Rasgrp4	0.69	0.56	1.11	1.56	0.69	1.48	0.8
Slc15a2	3.94	2.49	5.85	6.37	0.57	1.36	0.8
Gfi1	4.59	2.75	5.18	5.35	0.17	0.96	0.8
Rad9b	1.30	0.98	1.45	1.89	0.16	0.95	0.8
Tcea2	4.12	3.26	5.32	7.19	0.37	1.14	0.8
Slc26a6	1.18	0.98	1.47	2.08	0.32	1.08	0.8
Ltc4s	8.09	2.68	11.95	6.65	0.56	1.31	0.8
Adamtsl2	1.05	0.76	1.22	1.49	0.22	0.97	0.8
2610044O15Rik8	1.40	1.00	1.91	2.27	0.45	1.19	0.7
Ifi35	3.51	1.80	5.79	4.93	0.72	1.45	0.7
Tmc6	6.56	2.12	9.81	5.16	0.58	1.29	0.7
Naip6	1.07	0.61	1.78	1.63	0.74	1.43	0.7
Atp2b4	14.83	7.36	19.51	15.42	0.40	1.07	0.7
Pdk2	1.22	0.79	1.82	1.86	0.58	1.24	0.7
Cmpk2	2.50	1.53	4.43	4.24	0.82	1.47	0.7
Lcp1	13.89	10.67	20.93	25.24	0.59	1.24	0.7
Ccdc60	2.20	1.71	3.01	3.67	0.45	1.10	0.7
Tst	1.92	0.81	2.55	1.68	0.40	1.05	0.7
Naip5	2.00	1.04	2.95	2.40	0.56	1.20	0.6
Ankrd37	1.26	1.27	2.13	3.32	0.76	1.39	0.6
Elovl7	3.62	1.91	5.19	4.24	0.52	1.15	0.6
Tmem176a	21.87	10.03	28.09	19.99	0.36	0.99	0.6
Zcwpw1	1.33	1.49	2.31	4.01	0.80	1.42	0.6
Ccdc57	1.10	0.75	1.85	1.93	0.74	1.36	0.6
Frat2	3.27	2.08	4.23	4.12	0.37	0.99	0.6
Arap1	6.75	2.95	10.79	7.21	0.68	1.29	0.6
Rad51b	2.35	1.66	3.37	3.65	0.52	1.13	0.6
Gm13889	3.46	2.73	4.58	5.51	0.40	1.01	0.6
Lepr	0.75	0.57	1.37	1.58	0.87	1.47	0.6
Tmem177	2.40	0.83	3.64	1.90	0.60	1.20	0.6
Pcbd1	3.32	1.79	4.64	3.72	0.48	1.05	0.6
Fam111a	14.95	10.95	28.68	31.06	0.94	1.50	0.6
Smim20	11.42	0.86	17.73	1.98	0.64	1.20	0.6
Sh2d4b	3.00	3.81	4.06	7.63	0.44	1.00	0.6
Hif3a	4.00	2.04	7.04	5.27	0.82	1.37	0.6
Lgals3bp	4.55	4.44	7.86	11.29	0.79	1.34	0.6
Mthfsl	1.60	0.49	2.49	1.12	0.64	1.19	0.6
Fancf	1.28	0.67	1.87	1.40	0.54	1.08	0.5
Zfp784	0.69	0.80	1.14	1.90	0.71	1.24	0.5
Hacl1	3.19	1.46	4.80	3.14	0.59	1.11	0.5
Atp8a1	4.51	4.01	7.46	9.46	0.73	1.24	0.5
Accs	3.34	1.88	5.17	4.16	0.63	1.14	0.5
Abca7	5.52	3.53	8.12	7.40	0.56	1.07	0.5
Iqgap2	17.57	14.45	24.18	28.23	0.46	0.97	0.5
Trim21	2.59	0.70	3.55	1.37	0.45	0.96	0.5
Kif24	2.79	1.68	4.93	4.20	0.82	1.32	0.5

Sp1$^{del/del}$ HE1 – HE2
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cd52	0.23	0.00	4.34	2.23	4.23	11.12	6.9
Rgs18	0.06	0.00	1.09	1.65	4.11	10.69	6.6
Cst7	0.06	0.00	2.31	2.72	5.27	11.41	6.1
Kcne3	0.77	0.05	1.73	1.12	1.17	4.54	3.4
Ccl3	0.32	0.09	6.45	8.57	4.34	6.53	2.2
Hbb-b1	0.60	0.23	2.52	2.93	2.08	3.69	1.6
Hmha1	5.37	0.85	18.18	8.41	1.76	3.30	1.5
Mpl	0.55	0.13	14.11	9.48	4.69	6.19	1.5
Cd200r4	0.06	0.04	1.08	2.11	4.22	5.72	1.5
I830077J02Rik	2.15	0.84	8.53	9.49	1.99	3.49	1.5
P2rx1	1.19	0.18	32.40	12.19	4.76	6.11	1.4
Mgst2	0.35	0.25	3.06	5.54	3.15	4.48	1.3
Slfn5	0.29	0.26	1.01	2.28	1.80	3.13	1.3
Kcnj5	0.40	0.10	2.56	1.56	2.67	3.94	1.3
Plek	1.59	0.86	50.29	64.46	4.98	6.22	1.2
H2-T24	0.74	0.90	1.49	4.13	1.02	2.20	1.2
Ltf	0.10	0.05	1.47	1.52	3.87	5.04	1.2
Mfng	3.01	0.46	19.67	6.83	2.71	3.88	1.2
Lax1	0.53	0.25	3.05	3.06	2.53	3.63	1.1
Bcl6	0.22	0.13	1.07	1.29	2.27	3.30	1.0
Khk	2.22	1.39	4.38	5.52	0.98	1.99	1.0
Coro1a	0.50	0.11	9.03	4.08	4.18	5.16	1.0
Lrfn1	1.02	0.37	2.64	1.91	1.38	2.36	1.0
Il10ra	0.25	0.12	1.12	1.03	2.17	3.14	1.0
Cd200r1	1.73	1.43	7.61	12.37	2.14	3.11	1.0

Sp1$^{del/del}$ HE1 – HE2
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Tsacc	1.023	2.319	2.631	1.98	1.36	-0.23	-1.6
Ifitm1	5.927	6.724	14.178	5.416	1.26	-0.31	-1.6
2300009A05Rik	0.998	1.328	2.926	1.332	1.55	0.001	-1.5
Gm5617	1.071	1.115	2.543	1.105	1.25	-0.01	-1.3
S100a4	4.292	5.925	9.947	6.065	1.21	0.03	-1.2
Ctsc	1.827	5.529	4.41	6.154	1.27	0.15	-1.1
Hsd3b1	0.469	0.856	1.612	1.448	1.78	0.76	-1.0
Rab32	0.879	1.772	2.526	2.583	1.52	0.54	-1.0
Myl6b	0.365	0.627	1.263	1.2	1.79	0.94	-0.9
Dhrs11	2.679	5.585	6.694	7.815	1.32	0.48	-0.8
Smim1	2.206	1.571	5.093	2.05	1.21	0.38	-0.8
Hist1h4i	60.118	45.342	121.55	52.043	1.02	0.2	-0.8
Amd2	13.04	22.563	25.299	24.885	0.96	0.14	-0.8
Tuba4a	1.053	1.317	2.57	1.861	1.29	0.5	-0.8
Hist1h2bj	24.003	24.221	52.141	31.134	1.12	0.36	-0.8
Dctd	10.205	10.75	20.395	12.8	1	0.25	-0.8
Ptprj	2	3.711	4.1	4.554	1.04	0.3	-0.7
Pak1	2.673	1.927	5.471	2.373	1.03	0.3	-0.7
Pip5k1b	0.434	0.65	1.134	1.035	1.39	0.67	-0.7
Tfap4	3.109	3.313	7.173	4.643	1.21	0.49	-0.7
Fuz	1.734	2.216	4.024	3.127	1.21	0.5	-0.7
Pik3cd	1.344	1.961	3.059	2.757	1.19	0.49	-0.7
Ttc32	2.164	3.506	4.744	4.724	1.13	0.43	-0.7
Ppif	10.683	9.296	23.674	12.776	1.15	0.46	-0.7
Susd1	3.508	4.064	10.536	7.663	1.59	0.92	-0.7
Rbks	1.63	1.821	3.452	2.464	1.08	0.44	-0.6
Mylk3	0.81	0.982	2.251	1.76	1.47	0.84	-0.6
Metrn	2.299	1.831	4.831	2.513	1.07	0.46	-0.6

Sp1$^{del/del}$ HE1 – HE2
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rpia	4.124	5.165	11.798	9.793	1.52	0.92	-0.6
Abhd11	8.991	17.736	21.475	28.198	1.26	0.67	-0.6
Cyb5rl	1.147	1.4	2.506	2.041	1.13	0.54	-0.6
Helb	2.087	2.68	4.361	3.755	1.06	0.49	-0.6
Srl	1.481	2.937	4.004	5.463	1.43	0.9	-0.5
Ifi27	0.44	0.708	1.038	1.164	1.24	0.72	-0.5
Gm13157	2.217	3.576	4.346	4.88	0.97	0.45	-0.5
Hist2h3b	12.624	8.195	24.939	11.322	0.98	0.47	-0.5
Wdr12	16.914	18.213	32.629	24.849	0.95	0.45	-0.5

Sp1$^{del/del}$ HE1 – HE2
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Myo1f	0.015	0.126	2.74	1.857	7.51	3.88	-3.6
Evi2b	0.011	0.096	1.576	2.102	7.16	4.45	-2.7
Tyrobp	0.25	0.519	18.149	9.177	6.18	4.14	-2.0
Hba-a1	0.18	0.345	7.575	4.192	5.4	3.6	-1.8
Spi1	0.236	0.453	14.668	9.856	5.96	4.44	-1.5
Cxcr3	0.083	0.247	1.319	1.377	3.99	2.48	-1.5
Fgf3	0.518	0.536	4.412	1.722	3.09	1.68	-1.4
Spa17	0.272	0.487	2.242	1.621	3.04	1.73	-1.3
6030468B19Rik	0.078	0.192	1.283	1.296	4.04	2.75	-1.3
Fgl2	0.041	0.143	1.569	2.289	5.26	4	-1.3
Pdzk1ip1	0.187	0.265	2.201	1.307	3.56	2.3	-1.3
Nfam1	0.162	0.297	1.422	1.11	3.13	1.9	-1.2
Fam105a	0.26	0.443	2.119	1.743	3.03	1.98	-1.1
Snca	0.316	0.565	2.341	2.068	2.89	1.87	-1.0

Supplementary Table 5.4 – Gene expression deregulation between HE1 and HE2 stages in Sp1$^{del/del}$ cells

Genes that were differentially expressed between HE1 and HE2 (at least two fold changed) were identified. The genes that were differently differentially expressed in Sp1$^{del/del}$ cells were selected and grouped into clusters based on how the expression changes. The expression level (FPKM) in each cell type is shown, as well as the log2 fold change between stages in WT or Sp1$^{del/del}$ cells, and the difference between the fold changes. Cluster 1 and 12 represent genes that are not changed between WT and Sp1$^{del/del}$ cells, hence are not shown. No genes were found in clusters 6 (downregulated in mutant/upregulated in WT) and 9 (upregulated in mutant/downregulated in WT).

Supplementary Table 5.5

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 2: less downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cyyr1	3.04	2.99	0.02	0.12	-7.66	-4.63	3.0
Ly6h	4.43	1.84	0.13	0.37	-5.15	-2.31	2.8
Pirt	1.52	1.54	0.03	0.19	-5.87	-3.04	2.8
Galnt18	2.83	2.04	0.12	0.54	-4.51	-1.92	2.6
Nr5a2	1.48	1.30	0.01	0.04	-7.36	-5.21	2.1
Serpina3i	2.10	2.64	0.08	0.33	-4.72	-3.02	1.7
Cxcl1	2.17	2.67	0.14	0.53	-3.94	-2.34	1.6
Tppp	2.52	1.16	0.29	0.39	-3.13	-1.57	1.6
Ly6c1	5.51	5.42	0.70	1.87	-2.98	-1.54	1.4
Lyve1	14.20	8.50	2.14	3.17	-2.73	-1.42	1.3
Dupd1	1.33	1.56	0.08	0.22	-4.13	-2.84	1.3
Ramp3	3.69	3.09	0.06	0.12	-5.99	-4.71	1.3
Slc2a12	2.08	1.10	0.28	0.33	-2.90	-1.74	1.2
Sh3gl3	6.75	11.33	0.20	0.75	-5.06	-3.92	1.1
Il27ra	2.77	2.70	0.56	1.07	-2.32	-1.34	1.0

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rsad2	6.55	5.96	1.73	5.52	-1.92	-0.11	1.8
Vwf	8.54	19.17	1.87	14.23	-2.19	-0.43	1.8
Wt1	2.46	1.84	0.51	1.24	-2.26	-0.57	1.7
Trpc6	1.55	2.48	0.55	2.59	-1.50	0.06	1.6
Pi16	7.96	4.62	2.69	4.22	-1.57	-0.13	1.4
Parvb	11.95	9.64	4.66	9.98	-1.36	0.05	1.4
Bcl11a	2.33	1.23	0.92	1.14	-1.35	-0.10	1.2
Sorcs2	1.38	1.01	0.35	0.59	-1.99	-0.77	1.2
Naip7	4.40	2.69	1.53	2.16	-1.52	-0.32	1.2
Wscd1	3.89	3.36	1.69	3.26	-1.21	-0.04	1.2
Ccm2l	12.47	11.70	3.45	7.07	-1.85	-0.73	1.1
Ly6a	4.28	3.69	1.71	3.19	-1.32	-0.21	1.1
Pon3	6.66	2.54	1.69	1.39	-1.98	-0.87	1.1
Etv2	2.82	1.63	1.41	1.73	-1.00	0.09	1.1
Bst2	11.53	14.42	5.63	14.86	-1.03	0.04	1.1
Capn5	23.88	13.43	7.04	8.22	-1.76	-0.71	1.1
Ocln	2.55	2.22	0.73	1.24	-1.81	-0.83	1.0
Naip5	2.95	2.40	0.90	1.41	-1.72	-0.77	1.0
Akr1c12	1.93	1.35	0.93	1.24	-1.06	-0.12	0.9
Efhc1	1.91	1.47	0.69	1.00	-1.47	-0.56	0.9
Lgals9	79.16	34.27	40.12	32.28	-0.98	-0.09	0.9
Rab6b	1.49	1.62	0.46	0.93	-1.68	-0.80	0.9
Casp4	2.26	1.87	0.74	1.12	-1.62	-0.74	0.9
Robo3	3.59	8.04	1.31	5.39	-1.45	-0.58	0.9
Gnrh1	2.66	4.77	1.10	3.55	-1.28	-0.42	0.9
Vamp5	7.89	5.85	3.10	4.03	-1.35	-0.54	0.8
Gbp9	1.57	2.45	0.52	1.40	-1.60	-0.81	0.8
Scn3b	1.77	1.34	0.64	0.81	-1.48	-0.72	0.8
Nfkbie	8.47	6.33	3.88	4.81	-1.13	-0.40	0.7
Spata1	1.51	2.88	0.63	1.98	-1.27	-0.54	0.7
6430548M08Rik	1.05	1.03	0.36	0.57	-1.56	-0.84	0.7
Cdkn1c	12.51	10.11	4.83	6.33	-1.37	-0.68	0.7
Smtnl2	54.75	28.79	24.22	20.46	-1.18	-0.49	0.7
Glcci1	1.28	2.23	0.65	1.78	-0.98	-0.33	0.7
Cdc42ep1	48.69	27.31	17.21	15.00	-1.50	-0.86	0.6
Tmem86a	1.70	2.03	0.86	1.58	-0.98	-0.36	0.6
Tnfaip8l1	5.62	3.55	2.21	2.13	-1.35	-0.74	0.6
Sult5a1	1.71	1.06	0.80	0.76	-1.09	-0.48	0.6
Nefl	16.05	15.51	6.41	9.40	-1.33	-0.72	0.6
Gfi1	5.18	5.35	2.03	3.16	-1.35	-0.76	0.6

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 3: not downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Cnr2	8.36	9.57	3.45	5.91	-1.28	-0.70	0.6
Xkr6	1.17	1.28	0.60	0.98	-0.97	-0.39	0.6
Sapcd1	4.60	2.52	2.04	1.62	-1.17	-0.64	0.5
Plxnc1	24.29	19.45	10.02	11.58	-1.28	-0.75	0.5
Rcsd1	11.85	6.87	5.74	4.76	-1.05	-0.53	0.5

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 4: more downregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Gja5	8.34	1.68	0.25	0.01	-5.07	-8.39	-3.3
Sncg	2.22	5.46	0.98	0.66	-1.17	-3.05	-1.9
Stc1	1.69	2.21	0.08	0.03	-4.36	-6.16	-1.8
Adrb3	1.55	2.48	0.46	0.24	-1.75	-3.35	-1.6
Gpm6a	5.83	9.91	0.74	0.42	-2.98	-4.57	-1.6
Slc26a10	5.83	4.04	0.39	0.10	-3.89	-5.35	-1.5
Klf14	1.30	1.75	0.65	0.32	-1.01	-2.44	-1.4
Slc1a2	3.75	5.38	0.20	0.11	-4.26	-5.59	-1.3
Plekhh1	3.19	4.09	1.29	0.67	-1.30	-2.61	-1.3
Akr1cl	1.18	3.33	0.15	0.17	-3.02	-4.32	-1.3
Kctd12b	2.90	5.45	0.58	0.44	-2.33	-3.62	-1.3
Nts	3.09	8.89	0.14	0.17	-4.48	-5.70	-1.2
Rerg	1.63	1.66	0.51	0.23	-1.67	-2.85	-1.2
Ctsh	3.52	3.39	0.58	0.26	-2.59	-3.73	-1.1
BC028528	19.70	25.00	6.22	3.60	-1.66	-2.79	-1.1
Igfbp3	12.72	14.38	3.81	1.97	-1.74	-2.87	-1.1
Hoxb2	2.37	1.72	0.65	0.22	-1.87	-2.97	-1.1
Nfib	5.91	8.21	0.36	0.24	-4.02	-5.11	-1.1
Hoxb5	1.29	1.04	0.07	0.03	-4.29	-5.37	-1.1
Dennd5b	12.30	15.76	2.05	1.27	-2.58	-3.63	-1.1
Grid1	1.73	1.83	0.33	0.17	-2.38	-3.43	-1.1
Eva1a	1.78	1.80	0.20	0.10	-3.15	-4.20	-1.0
Thsd7a	3.43	5.77	0.81	0.68	-2.07	-3.08	-1.0
Ccnd1	24.62	39.80	11.96	9.68	-1.04	-2.04	-1.0
Sost	5.93	9.92	0.16	0.13	-5.25	-6.23	-1.0
Filip1l	10.83	21.64	5.46	5.54	-0.99	-1.97	-1.0
Cd109	9.88	12.84	0.99	0.66	-3.32	-4.29	-1.0
Edn1	21.27	51.06	1.87	2.30	-3.51	-4.47	-1.0
Cobll1	11.22	17.21	5.68	4.50	-0.98	-1.94	-1.0

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Ppp1r14a	1.07	1.55	1.11	0.21	0.06	-2.92	-3.0
Cav1	5.33	14.98	4.34	3.68	-0.30	-2.03	-1.7
Plac1	7.58	10.68	3.97	1.77	-0.93	-2.59	-1.7
Gm20594	1.87	1.84	2.32	0.74	0.32	-1.32	-1.6
2200002D01Rik	4.39	2.73	5.66	1.22	0.37	-1.16	-1.5
Awat2	1.16	1.99	0.95	0.59	-0.29	-1.77	-1.5
Postn	18.69	50.77	19.29	18.80	0.05	-1.43	-1.5
Ly96	1.12	2.00	1.18	0.77	0.07	-1.37	-1.4
Aff2	1.49	2.89	1.55	1.13	0.05	-1.35	-1.4
Apln	2.14	4.48	1.63	1.30	-0.40	-1.78	-1.4
Rspo3	24.66	24.21	13.61	5.25	-0.86	-2.21	-1.3

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Samd5	2.17	3.29	2.43	1.48	0.17	-1.15	-1.3
Zim1	7.30	17.90	4.87	4.85	-0.59	-1.88	-1.3
Il17rd	1.91	3.19	1.50	1.04	-0.35	-1.62	-1.3
Snai2	1.54	1.99	1.79	0.97	0.22	-1.03	-1.3
Nudt7	5.82	2.03	6.64	1.04	0.19	-0.97	-1.2
Cdkl1	3.12	6.34	1.69	1.55	-0.88	-2.03	-1.1
Anxa1	39.44	68.46	43.43	34.47	0.14	-0.99	-1.1
Tcf7l1	2.21	2.21	1.26	0.58	-0.80	-1.93	-1.1
Nrk	3.89	4.47	3.91	2.08	0.01	-1.11	-1.1
Uprt	7.64	11.41	4.46	3.11	-0.78	-1.88	-1.1
Rnd1	1.16	2.03	0.96	0.79	-0.27	-1.37	-1.1
Zfp462	7.31	11.54	4.36	3.24	-0.74	-1.83	-1.1
Chrna7	1.10	2.44	0.58	0.61	-0.92	-2.00	-1.1
Arhgap42	2.70	3.14	1.44	0.80	-0.91	-1.98	-1.1
Zbtb20	1.16	2.95	1.03	1.26	-0.17	-1.23	-1.1
Map1b	16.03	26.71	8.46	6.94	-0.92	-1.94	-1.0
Btg3	2.70	3.92	2.77	1.99	0.04	-0.98	-1.0
Cdh2	28.35	43.43	22.55	17.14	-0.33	-1.34	-1.0
Gata3	1.18	1.46	0.88	0.55	-0.41	-1.42	-1.0
Abtb2	2.68	2.83	1.99	1.05	-0.43	-1.43	-1.0
Moap1	2.53	2.90	2.55	1.47	0.01	-0.98	-1.0
Hs6st2	13.22	15.84	10.57	6.39	-0.32	-1.31	-1.0
Nup62cl	10.00	11.90	9.39	5.72	-0.09	-1.06	-1.0
Flrt3	4.37	8.77	4.00	4.11	-0.13	-1.09	-1.0
Zfp37	2.04	5.39	1.44	1.99	-0.50	-1.44	-0.9
Nr1d1	1.06	1.56	1.03	0.79	-0.04	-0.98	-0.9
Greb1l	6.15	7.66	4.62	3.02	-0.41	-1.34	-0.9
Stx1a	2.00	2.76	1.58	1.15	-0.34	-1.26	-0.9
Samd4	3.95	7.26	3.18	3.08	-0.31	-1.24	-0.9
Bche	1.19	2.15	1.03	0.99	-0.21	-1.12	-0.9
Smarca1	2.05	3.40	1.98	1.75	-0.05	-0.96	-0.9
Gjc1	5.48	6.70	3.33	2.16	-0.72	-1.63	-0.9
Colec12	9.89	12.77	6.46	4.49	-0.61	-1.51	-0.9
Fbxl22	1.04	1.51	0.86	0.67	-0.29	-1.17	-0.9
Steap1	11.83	12.52	6.38	3.66	-0.89	-1.78	-0.9
Wbp5	124.37	208.57	103.56	94.79	-0.26	-1.14	-0.9
Tyro3	6.50	5.91	5.32	2.66	-0.29	-1.15	-0.9
Lef1	3.99	4.79	2.62	1.74	-0.60	-1.46	-0.9
Cyb561	1.80	1.60	1.31	0.64	-0.46	-1.32	-0.9
Tead1	14.49	23.41	11.03	9.92	-0.39	-1.24	-0.8
Ston1	10.60	14.66	8.00	6.23	-0.41	-1.23	-0.8
Tnc	17.35	19.34	15.51	9.80	-0.16	-0.98	-0.8
Fndc3c1	19.29	21.11	14.97	9.30	-0.37	-1.18	-0.8
Sestd1	5.45	6.49	3.31	2.24	-0.72	-1.53	-0.8
Nhs	4.95	8.58	2.88	2.84	-0.78	-1.59	-0.8
D16Ertd472e	3.68	4.81	3.14	2.36	-0.23	-1.03	-0.8
Pcdhgb5	3.56	4.20	1.88	1.28	-0.92	-1.72	-0.8
Ext1	20.31	28.37	13.73	11.10	-0.57	-1.35	-0.8
Msi1	1.33	1.46	1.01	0.64	-0.41	-1.19	-0.8
Palld	13.39	23.02	11.16	11.24	-0.26	-1.03	-0.8
Mpped2	2.28	2.19	1.52	0.86	-0.59	-1.36	-0.8
Tmem245	8.56	13.82	6.98	6.67	-0.29	-1.05	-0.8
Zdbf2	9.43	12.12	7.64	5.82	-0.30	-1.06	-0.8
Cyr61	62.90	120.81	50.77	57.89	-0.31	-1.06	-0.8
Exoc3l4	9.11	9.30	6.01	3.66	-0.60	-1.35	-0.7
Nes	27.61	34.98	18.27	13.81	-0.60	-1.34	-0.7
S100a11	41.01	65.41	34.72	33.14	-0.24	-0.98	-0.7
Elovl6	25.69	30.89	21.29	15.56	-0.27	-0.99	-0.7
Gldc	11.88	11.36	9.41	5.47	-0.34	-1.05	-0.7
Pls3	31.61	55.87	23.18	24.97	-0.45	-1.16	-0.7

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 5: downregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Efna4	4.88	4.66	3.81	2.22	-0.36	-1.07	-0.7
Ccdc80	9.48	10.85	7.48	5.24	-0.34	-1.05	-0.7
Cald1	76.05	115.14	53.54	49.70	-0.51	-1.21	-0.7
H2afy2	9.32	12.48	6.08	5.02	-0.62	-1.31	-0.7
Fndc3b	13.63	16.24	7.20	5.32	-0.92	-1.61	-0.7
Bcar3	4.89	6.57	2.81	2.35	-0.80	-1.48	-0.7
Hmgn3	3.87	5.22	2.81	2.36	-0.46	-1.14	-0.7
Phldb2	17.96	24.58	10.31	8.78	-0.80	-1.49	-0.7
Anxa3	49.70	38.85	37.70	18.40	-0.40	-1.08	-0.7
Aim2	6.63	18.74	4.60	8.16	-0.53	-1.20	-0.7
Zfyve9	9.38	9.40	6.89	4.34	-0.44	-1.11	-0.7
Mum1l1	14.85	23.03	11.70	11.43	-0.34	-1.01	-0.7
Unc5b	32.88	34.32	17.37	11.45	-0.92	-1.58	-0.7
Pcdhgb4	3.50	4.79	1.95	1.69	-0.85	-1.51	-0.7
Etv4	1.31	1.43	0.98	0.68	-0.42	-1.08	-0.7
Slc39a14	7.97	11.23	5.43	4.87	-0.55	-1.21	-0.7
Col1a2	16.59	10.80	11.86	4.92	-0.48	-1.13	-0.6
Tulp4	8.38	12.13	4.53	4.20	-0.89	-1.53	-0.6
Tbx15	1.01	1.08	0.74	0.51	-0.45	-1.09	-0.6
Usp43	1.19	2.22	0.62	0.75	-0.93	-1.57	-0.6
Igf2bp2	35.37	28.54	25.77	13.41	-0.46	-1.09	-0.6
Dmpk	1.79	1.90	1.41	0.97	-0.35	-0.98	-0.6
Timp1	8.15	2.87	5.97	1.36	-0.45	-1.08	-0.6
Arhgap32	2.60	3.75	1.84	1.72	-0.50	-1.13	-0.6
Fam101b	3.31	5.12	2.42	2.44	-0.45	-1.07	-0.6
Lrp4	5.80	7.68	4.28	3.69	-0.44	-1.06	-0.6
Fzd3	4.27	4.96	2.59	1.96	-0.72	-1.34	-0.6
Tmem47	6.77	10.28	3.67	3.65	-0.88	-1.49	-0.6
Stk39	5.94	6.72	3.24	2.42	-0.87	-1.47	-0.6
Met	1.33	2.13	0.73	0.77	-0.87	-1.47	-0.6
Atp2b1	30.14	46.64	19.03	19.54	-0.66	-1.25	-0.6
Gprc5a	3.12	4.32	1.88	1.73	-0.73	-1.32	-0.6
Bmp2	20.64	21.87	13.84	9.75	-0.58	-1.17	-0.6
Zfp383	2.14	2.89	1.59	1.43	-0.43	-1.02	-0.6
Sema5a	1.54	1.36	1.17	0.69	-0.40	-0.98	-0.6
Ctif	3.83	5.50	2.29	2.21	-0.74	-1.32	-0.6
Mxra7	5.46	4.90	3.57	2.15	-0.61	-1.19	-0.6
D830031N03Rik	16.77	22.79	11.39	10.40	-0.56	-1.13	-0.6
Rep15	2.64	3.64	1.46	1.35	-0.86	-1.43	-0.6
Hip1	15.40	17.33	8.88	6.74	-0.79	-1.36	-0.6
Rassf3	20.34	19.51	13.46	8.74	-0.60	-1.16	-0.6
Sntb2	6.41	6.22	4.09	2.69	-0.65	-1.21	-0.6
Rock2	23.88	33.35	14.14	13.46	-0.76	-1.31	-0.6
Akap2	20.16	29.00	11.91	11.68	-0.76	-1.31	-0.6
Chst7	1.86	2.16	0.97	0.77	-0.94	-1.49	-0.6
Arl5b	11.04	14.93	5.96	5.50	-0.89	-1.44	-0.6
Erbb2	2.97	2.97	1.89	1.29	-0.65	-1.20	-0.5
Ppp2r3a	5.14	7.59	3.59	3.64	-0.52	-1.06	-0.5
Itgav	23.91	30.61	16.56	14.56	-0.53	-1.07	-0.5
Col5a2	1.64	4.79	1.20	2.42	-0.45	-0.99	-0.5
Slc12a2	12.23	20.56	8.35	9.66	-0.55	-1.09	-0.5
Ermp1	12.26	15.17	6.99	5.99	-0.81	-1.34	-0.5
Parva	31.90	38.53	18.01	15.08	-0.82	-1.35	-0.5
Rcbtb1	29.79	31.18	17.63	12.82	-0.76	-1.28	-0.5
Nexn	1.68	4.04	1.20	2.01	-0.49	-1.01	-0.5
Zfp516	10.12	10.62	6.44	4.72	-0.65	-1.17	-0.5
Tead4	4.90	5.68	2.65	2.15	-0.89	-1.40	-0.5
Dlg5	10.03	8.92	7.34	4.59	-0.45	-0.96	-0.5
Macf1	73.99	110.31	40.24	42.17	-0.88	-1.39	-0.5
Dab2	10.11	14.17	5.52	5.47	-0.87	-1.37	-0.5

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Hist1h4j	3.51	0.50	3.82	3.02	0.12	2.60	2.5
Flywch2	0.86	0.23	1.23	1.34	0.51	2.57	2.1
Apoc1	2.91	0.17	4.99	1.20	0.78	2.80	2.0
Gnaz	3.83	3.58	5.85	20.01	0.61	2.48	1.9
Tnni1	13.60	7.35	15.45	28.16	0.18	1.94	1.8
Clec1b	12.86	12.78	11.08	36.08	-0.22	1.50	1.7
Mrvi1	6.22	6.11	7.52	24.19	0.27	1.99	1.7
Fermt3	52.29	20.55	76.19	91.12	0.54	2.15	1.6
F2rl2	5.36	5.56	6.93	20.74	0.37	1.90	1.5
Acy3	4.26	0.87	4.12	2.31	-0.05	1.41	1.5
Abhd14b	1.37	0.63	1.09	1.39	-0.32	1.13	1.4
Clu	38.70	42.74	36.51	105.73	-0.08	1.31	1.4
Fbxl15	1.29	0.63	1.33	1.69	0.05	1.42	1.4
Optn	11.51	5.36	8.83	10.62	-0.38	0.99	1.4
Lat	4.44	2.66	4.05	6.26	-0.13	1.23	1.4
Rtn4r	0.89	0.34	1.55	1.51	0.80	2.15	1.4
Hist1h4b	94.79	37.57	124.09	123.70	0.39	1.72	1.3
Tspan32	45.39	14.78	86.56	70.50	0.93	2.25	1.3
Unc13d	5.98	2.93	7.63	9.32	0.35	1.67	1.3
Prkcq	3.96	3.66	7.34	16.87	0.89	2.20	1.3
Tspo	12.23	1.61	22.46	7.34	0.88	2.19	1.3
Cyba	32.43	6.08	36.86	17.02	0.18	1.48	1.3
Tmem163	1.75	0.90	2.22	2.80	0.35	1.64	1.3
Fah	4.44	0.38	7.79	1.63	0.81	2.11	1.3
Tmcc2	7.56	5.47	7.30	12.94	-0.05	1.24	1.3
Gpt	1.45	0.72	1.27	1.52	-0.19	1.08	1.3
Hist1h2ae	22.09	10.04	29.03	31.55	0.39	1.65	1.3
Gucy1a3	3.38	2.18	6.14	9.42	0.86	2.11	1.3
Emp3	43.50	9.03	57.26	28.28	0.40	1.65	1.3
Tap1	2.18	1.24	3.89	5.18	0.84	2.07	1.2
Zfp296	0.87	0.30	1.32	1.04	0.59	1.82	1.2
Ccrl2	1.70	1.20	1.86	3.08	0.13	1.36	1.2
Stx11	1.33	0.96	2.17	3.64	0.70	1.92	1.2
Dand5	1.34	0.61	1.68	1.78	0.32	1.54	1.2
Spa17	2.24	1.62	2.13	3.58	-0.07	1.14	1.2
Helz2	1.08	0.38	1.56	1.27	0.53	1.74	1.2
Dennd2d	1.19	0.68	1.72	2.25	0.54	1.74	1.2
Tmem40	0.77	0.47	1.46	1.98	0.92	2.08	1.2
Anxa11	10.40	3.82	9.50	7.76	-0.13	1.02	1.2
S100a4	9.95	6.07	14.16	19.10	0.51	1.66	1.1
Abcg1	3.41	3.10	3.73	7.49	0.13	1.27	1.1
Hist1h4h	289.43	136.47	349.72	356.46	0.27	1.39	1.1
Tap2	0.94	0.79	1.49	2.67	0.66	1.77	1.1
Dapk2	3.98	2.28	5.41	6.63	0.44	1.54	1.1
P2rx1	32.40	12.19	55.85	44.65	0.79	1.87	1.1
Nqo1	11.20	8.96	19.50	32.76	0.80	1.87	1.1
Gm5617	2.54	1.11	3.09	2.81	0.28	1.34	1.1
Hist1h4i	121.55	52.04	146.54	130.99	0.27	1.33	1.1
Gp1ba	4.08	4.56	6.04	14.04	0.57	1.62	1.1
S100a1	16.67	7.80	21.12	20.52	0.34	1.40	1.1
Xaf1	0.89	1.20	1.13	3.16	0.35	1.40	1.1
Ptp4a3	17.97	11.68	28.43	37.64	0.66	1.69	1.0
Hist1h4k	180.46	95.58	229.77	246.00	0.35	1.36	1.0
Hist4h4	393.67	198.12	468.07	472.82	0.25	1.25	1.0
Dennd3	1.25	0.50	1.58	1.27	0.35	1.35	1.0
Uba7	6.60	5.63	11.05	18.79	0.74	1.74	1.0
2310030G06Rik	1.19	0.80	1.63	2.17	0.45	1.45	1.0
Ppp1r14c	1.69	1.52	3.13	5.55	0.89	1.87	1.0

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Hist1h4c	300.54	160.65	354.49	372.00	0.24	1.21	1.0
Hist1h4a	267.84	123.47	315.62	284.09	0.24	1.20	1.0
Ap5b1	1.48	0.70	2.78	2.56	0.91	1.87	1.0
Hist3h2bb-ps	7.78	4.20	10.02	10.51	0.36	1.33	1.0
Serpina3f	0.78	0.72	1.27	2.26	0.70	1.66	1.0
Stxbp2	27.22	10.77	41.03	31.27	0.59	1.54	0.9
Ndrg2	3.39	1.73	3.57	3.49	0.07	1.01	0.9
Hist1h2ak	90.59	43.58	126.08	116.38	0.48	1.42	0.9
Slc45a4	6.28	3.69	8.39	9.44	0.42	1.36	0.9
Hist1h2an	13.49	7.22	15.20	15.49	0.17	1.10	0.9
Mblac1	2.12	1.12	2.36	2.37	0.16	1.09	0.9
Spata5l1	2.13	0.85	2.42	1.82	0.18	1.10	0.9
Pde5a	3.22	3.89	5.19	11.80	0.69	1.60	0.9
Dennd2c	6.37	5.35	7.22	11.38	0.18	1.09	0.9
Hist2h2ac	59.74	25.14	71.84	56.67	0.27	1.17	0.9
Ube2l6	6.49	4.08	7.16	8.22	0.14	1.01	0.9
Ufsp1	2.29	2.21	4.35	7.61	0.93	1.78	0.9
Hist1h2ad	23.96	15.89	33.63	40.35	0.49	1.34	0.9
Blvrb	22.58	13.54	41.16	44.61	0.87	1.72	0.9
Fkbpl	4.67	3.19	5.36	6.63	0.20	1.05	0.9
Fam65c	2.48	2.41	3.46	6.04	0.48	1.33	0.8
Parp12	1.52	1.86	2.31	5.08	0.61	1.45	0.8
Hist1h4f	408.15	222.88	515.58	502.49	0.34	1.17	0.8
Mpl	14.11	9.48	24.91	29.74	0.82	1.65	0.8
Hist1h2ab	133.63	75.39	163.23	163.23	0.29	1.11	0.8
Alox5ap	46.34	24.76	82.36	77.68	0.83	1.65	0.8
Vps33b	11.33	9.78	14.73	22.46	0.38	1.20	0.8
Hist1h2af	22.84	12.20	32.10	30.25	0.49	1.31	0.8
Capg	13.16	4.22	22.27	12.56	0.76	1.57	0.8
Tbc1d10c	1.67	1.64	2.27	3.89	0.44	1.25	0.8
2300009A05Rik	2.93	1.33	4.70	3.75	0.68	1.49	0.8
Fam210b	6.59	3.88	7.68	7.91	0.22	1.03	0.8
Hist2h3b	24.94	11.32	33.51	26.49	0.43	1.23	0.8
Inafm2	4.14	3.59	6.50	9.80	0.65	1.45	0.8
Itgb3	132.96	96.82	182.40	229.77	0.46	1.25	0.8
Hist1h2bh	231.52	137.99	307.81	317.22	0.41	1.20	0.8
Fam132a	2.96	0.71	3.86	1.60	0.38	1.17	0.8
Ckb	23.82	18.95	28.29	38.83	0.25	1.03	0.8
Dscc1	7.03	4.26	8.49	8.87	0.27	1.06	0.8
Il15	1.16	0.88	1.56	2.04	0.43	1.21	0.8
Draxin	1.98	2.07	2.48	4.44	0.32	1.10	0.8
Dtx3l	2.56	2.80	3.50	6.56	0.45	1.23	0.8
Hist1h3h	71.24	39.83	86.94	83.16	0.29	1.06	0.8
Lrrc29	2.78	2.73	3.50	5.88	0.33	1.11	0.8
Hist1h3f	78.71	39.94	114.33	99.05	0.54	1.31	0.8
Mettl18	6.67	4.19	8.31	8.86	0.32	1.08	0.8
Tmem86b	2.94	2.20	3.52	4.46	0.26	1.02	0.8
Hist1h4d	767.39	427.87	949.76	891.81	0.31	1.06	0.8
Arrdc1	12.39	4.96	16.42	11.02	0.41	1.15	0.7
Ada	1.94	0.95	2.57	2.11	0.41	1.16	0.7
Isg20	1.12	2.05	1.87	5.74	0.74	1.49	0.7
Mpst	6.71	7.29	8.30	15.10	0.31	1.05	0.7
Trim21	3.55	1.37	5.70	3.67	0.68	1.42	0.7
Syngr1	14.79	9.96	23.92	26.85	0.69	1.43	0.7
Smagp	53.74	30.96	72.01	68.88	0.42	1.15	0.7
Sp140	1.82	0.97	3.17	2.80	0.80	1.53	0.7
Hist1h2bj	52.14	31.13	64.08	63.47	0.30	1.03	0.7
Dok2	22.35	23.93	39.79	70.63	0.83	1.56	0.7
Cox7a1	4.53	5.78	6.80	14.24	0.59	1.30	0.7
Hist1h2ag	31.92	16.74	40.78	34.99	0.35	1.06	0.7

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Arap1	10.79	7.21	13.94	15.23	0.37	1.08	0.7
Ccbl1	2.80	1.95	4.07	4.62	0.54	1.25	0.7
Cuedc1	8.39	8.85	10.15	17.44	0.27	0.98	0.7
Hist1h3c	215.25	120.98	265.96	243.25	0.31	1.01	0.7
Hist1h3b	259.72	161.70	311.98	315.62	0.26	0.96	0.7
Hist1h2ah	5.66	3.62	7.41	7.70	0.39	1.09	0.7
Tgfb1	50.54	33.37	69.47	74.33	0.46	1.16	0.7
Cenpw	9.57	7.01	13.54	15.98	0.50	1.19	0.7
Trim30a	2.53	3.30	3.76	7.89	0.57	1.26	0.7
Cryl1	3.33	2.60	4.04	5.09	0.28	0.97	0.7
Tnfsf9	2.94	2.19	3.55	4.25	0.27	0.96	0.7
Glrx	5.43	3.16	8.66	8.09	0.67	1.36	0.7
Hist2h4	148.16	82.08	188.94	168.25	0.35	1.04	0.7
Ank3	9.40	10.22	15.37	26.81	0.71	1.39	0.7
Ucp2	27.09	8.30	40.38	19.85	0.58	1.26	0.7
Hist1h3e	265.96	155.28	358.25	335.63	0.43	1.11	0.7
Fam117a	4.34	2.99	7.10	7.84	0.71	1.39	0.7
Rasgef1b	12.30	10.93	18.31	26.09	0.57	1.25	0.7
Hist1h2bk	151.83	86.94	205.89	188.41	0.44	1.12	0.7
Hspa2	2.80	3.52	3.68	7.39	0.40	1.07	0.7
Dap	19.38	14.54	24.62	29.45	0.35	1.02	0.7
Nedd4l	3.11	2.65	4.52	6.11	0.54	1.21	0.7
Ehbp1l1	18.60	9.93	32.48	27.50	0.80	1.47	0.7
I830077J02Rik	8.53	9.49	13.57	23.85	0.67	1.33	0.7
Ocel1	2.31	1.66	3.16	3.59	0.45	1.11	0.7
Hist1h3g	96.40	51.20	127.60	106.89	0.40	1.06	0.7
Prodh	18.31	10.88	24.25	22.62	0.40	1.06	0.7
Rbfa	26.26	5.81	38.73	13.39	0.56	1.21	0.6
Ifit2	2.54	4.86	3.72	11.12	0.55	1.19	0.6
Hist1h2ac	47.27	31.82	61.30	63.83	0.37	1.00	0.6
Ccbl2	1.33	0.66	1.94	1.47	0.54	1.17	0.6
Stat1	9.35	10.34	14.23	24.30	0.61	1.23	0.6
Plscr1	31.32	23.11	50.09	56.64	0.68	1.29	0.6
Aldh9a1	32.53	18.69	42.75	37.61	0.39	1.01	0.6
Oxld1	4.66	3.17	6.02	6.27	0.37	0.98	0.6
Fbxl8	0.97	0.69	1.26	1.37	0.38	0.99	0.6
Ubash3b	37.74	37.21	48.10	72.39	0.35	0.96	0.6
Unc119	9.80	6.03	12.49	11.66	0.35	0.95	0.6
H2-T23	1.10	5.21	1.69	12.16	0.62	1.22	0.6
F10	3.97	4.01	6.57	10.05	0.73	1.32	0.6
Tmem179b	21.35	19.05	28.71	38.73	0.43	1.02	0.6
Ccdc8	15.33	5.17	22.51	11.47	0.55	1.15	0.6
Orai2	4.22	4.27	8.07	12.31	0.93	1.53	0.6
Rasgrp2	9.33	8.67	17.79	24.90	0.93	1.52	0.6
Myd88	11.48	6.35	17.42	14.49	0.60	1.19	0.6
Slfn2	6.08	6.25	10.88	16.80	0.84	1.43	0.6
Hps6	3.60	1.56	5.04	3.28	0.48	1.07	0.6
Crlf2	3.46	1.92	5.02	4.17	0.54	1.12	0.6
Prrt4	4.76	3.97	7.18	8.98	0.59	1.18	0.6
Pnpo	35.51	28.99	55.73	68.19	0.65	1.23	0.6
Tnnt1	1.76	2.46	2.63	5.48	0.58	1.16	0.6
Hist1h2bc	232.45	140.14	305.98	275.07	0.40	0.97	0.6
AI467606	9.17	5.90	14.80	14.14	0.69	1.26	0.6
Mknk1	15.89	11.32	28.43	30.02	0.84	1.41	0.6
Gypc	35.04	28.93	56.44	69.08	0.69	1.26	0.6
Rbm43	11.03	12.04	15.51	25.06	0.49	1.06	0.6
Pts	20.75	17.10	28.98	35.28	0.48	1.04	0.6
Hist1h2ai	53.78	34.30	72.12	67.70	0.42	0.98	0.6
Fam58b	11.85	2.31	15.79	4.52	0.41	0.97	0.6
Man2b2	6.62	6.50	9.25	13.34	0.48	1.04	0.6

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 7: upregulated in mutant/no change in WT

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Fech	22.68	16.68	38.96	42.06	0.78	1.33	0.6
Susd1	10.54	7.66	18.98	20.22	0.85	1.40	0.6
Cml1	0.80	0.52	1.14	1.07	0.50	1.05	0.5
Parp9	2.96	3.98	4.11	8.09	0.47	1.02	0.5
Kalrn	2.92	2.80	4.17	5.84	0.51	1.06	0.5
Rab27a	10.27	5.48	16.43	12.80	0.68	1.22	0.5
Diap1	29.26	26.03	49.55	64.21	0.76	1.30	0.5
Hist1h2bg	275.97	210.24	382.38	423.66	0.47	1.01	0.5
Rltpr	1.11	0.44	2.03	1.17	0.87	1.41	0.5
Cnst	7.13	7.48	9.62	14.62	0.43	0.97	0.5
Prorsd1	3.82	2.21	6.33	5.31	0.73	1.26	0.5
Smim3	39.06	32.18	63.35	75.32	0.70	1.23	0.5
Lrwd1	23.43	16.19	34.56	34.15	0.56	1.08	0.5
Nme3	8.79	5.87	15.72	15.00	0.84	1.35	0.5
Hmbs	50.48	37.15	78.11	81.98	0.63	1.14	0.5
Gusb	23.87	16.11	37.05	35.61	0.63	1.14	0.5
Bola1	6.91	3.49	11.34	8.11	0.71	1.22	0.5
Marveld2	0.63	0.42	1.07	1.02	0.77	1.27	0.5

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Npy	0.70	0.10	2.35	9.07	1.74	6.58	4.8
Hbq1b	0.66	0.07	2.21	3.39	1.75	5.56	3.8
Trim10	0.45	0.13	2.26	3.22	2.34	4.65	2.3
Gp5	0.99	1.10	2.81	15.44	1.51	3.82	2.3
Itln1	0.26	0.12	1.02	2.18	1.96	4.22	2.3
Gp9	0.85	0.56	3.48	10.43	2.04	4.21	2.2
Tubb1	4.00	2.78	18.41	53.41	2.20	4.26	2.1
Gp1bb	0.40	0.28	2.16	5.84	2.44	4.41	2.0
Ptger3	0.40	0.09	2.27	1.84	2.51	4.41	1.9
Unc93b1	1.59	0.34	6.19	4.82	1.96	3.84	1.9
Kcnj5	2.56	1.56	5.51	11.05	1.10	2.82	1.7
Il1b	0.30	0.11	1.39	1.58	2.19	3.91	1.7
Pf4	1.28	1.95	3.09	15.30	1.27	2.97	1.7
E130309D14Rik	0.66	0.18	2.04	1.72	1.62	3.27	1.7
Il10ra	1.12	1.03	5.27	14.59	2.24	3.83	1.6
Ctrl	0.81	0.17	1.88	1.19	1.21	2.78	1.6
Pkd1l3	1.36	1.84	3.07	12.01	1.17	2.70	1.5
Milr1	0.33	0.19	1.21	1.97	1.87	3.36	1.5
Csf2rb2	4.94	6.27	10.28	35.52	1.06	2.50	1.4
Selenbp1	1.92	0.43	4.64	2.79	1.27	2.70	1.4
Ajap1	0.77	0.39	2.91	4.00	1.92	3.35	1.4
Mcf2l	0.40	0.22	1.02	1.49	1.36	2.78	1.4
G6b	0.31	0.64	1.31	7.12	2.06	3.48	1.4
Colec11	0.90	1.46	1.75	7.32	0.97	2.32	1.4
Fam110c	1.02	0.31	2.70	2.12	1.41	2.75	1.3
Treml2	2.68	1.27	12.85	15.38	2.26	3.60	1.3
Proser2	0.34	0.30	1.08	2.38	1.66	2.99	1.3
Slc14a1	0.20	0.07	2.41	1.91	3.56	4.88	1.3
Irgm2	1.22	0.72	2.89	4.23	1.25	2.55	1.3
Clec4n	0.65	0.29	3.45	3.74	2.40	3.70	1.3
AF251705	0.73	0.37	2.85	3.48	1.96	3.25	1.3
Igsf6	0.26	0.10	2.16	2.09	3.06	4.36	1.3
Soat2	1.42	1.33	3.51	8.02	1.30	2.60	1.3
Atp2a3	23.80	7.83	56.53	45.36	1.25	2.53	1.3
Matn1	0.69	0.32	3.47	3.92	2.34	3.62	1.3

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 8: more upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Tyrobp	18.15	9.18	89.95	110.66	2.31	3.59	1.3
Mylk3	2.25	1.76	7.67	14.52	1.77	3.04	1.3
Epor	3.79	1.65	11.35	11.90	1.58	2.85	1.3
Btk	1.65	1.21	7.20	12.66	2.13	3.39	1.3
Pira11	0.17	0.07	1.50	1.50	3.15	4.40	1.3
Hsd3b1	1.61	1.45	6.04	12.87	1.91	3.15	1.2
Grtp1	0.59	0.31	1.63	2.06	1.48	2.72	1.2
Tnfrsf1b	1.06	0.24	3.75	1.97	1.82	3.05	1.2
BC035044	0.71	0.39	2.10	2.71	1.56	2.79	1.2
Coro2a	0.74	0.27	5.40	4.61	2.86	4.09	1.2
Chchd10	2.44	0.61	5.13	3.01	1.07	2.30	1.2
Aqp8	2.53	1.87	8.72	14.79	1.79	2.98	1.2
Alas2	9.27	3.43	21.04	17.72	1.18	2.37	1.2
Tbxas1	0.27	0.31	1.92	4.91	2.83	4.00	1.2
Tgm1	2.30	0.45	6.35	2.79	1.46	2.63	1.2
Pstpip2	1.59	0.91	7.87	10.03	2.31	3.46	1.2
Rab37	0.92	0.67	4.57	7.33	2.31	3.46	1.2
Rgs1	0.33	0.30	1.83	3.68	2.47	3.61	1.1
Gata1	4.61	3.21	31.72	48.72	2.78	3.92	1.1
Plcb2	1.20	0.91	4.74	7.79	1.98	3.10	1.1
Ak8	0.47	0.39	1.14	2.03	1.28	2.38	1.1
Tmod1	1.28	0.71	8.79	10.43	2.78	3.88	1.1
Sla2	5.97	3.66	12.07	15.73	1.02	2.10	1.1
Il1r2	0.61	0.36	2.64	3.29	2.11	3.19	1.1
Map3k6	1.08	0.37	2.42	1.74	1.17	2.24	1.1
Smim1	5.09	2.05	14.19	12.04	1.48	2.55	1.1
Ubash3a	5.42	3.17	14.09	17.36	1.38	2.45	1.1
Hmha1	18.18	8.41	38.08	36.74	1.07	2.13	1.1
Trim58	0.51	0.41	2.02	3.38	1.98	3.04	1.1
Acp5	0.70	0.44	3.69	4.84	2.40	3.46	1.1
Syk	13.00	9.00	26.51	38.07	1.03	2.08	1.1
Samd14	3.79	2.13	15.72	18.32	2.05	3.10	1.0
Slc38a5	0.22	0.14	2.21	2.85	3.32	4.37	1.0
Rgs10	19.12	14.37	56.49	87.40	1.56	2.60	1.0
Mfsd2b	8.30	4.95	21.40	26.11	1.37	2.40	1.0
Fam109b	5.05	1.95	14.85	11.72	1.56	2.59	1.0
Ttc39a	1.82	0.62	4.16	2.91	1.19	2.22	1.0
Tnni2	1.00	0.80	4.03	6.58	2.01	3.03	1.0
Ifit3	0.99	2.19	1.94	8.82	0.98	2.01	1.0
Siglecf	1.07	0.91	6.60	11.36	2.63	3.65	1.0
Rgs18	1.09	1.65	4.59	14.10	2.08	3.10	1.0
Susd3	2.72	0.83	8.12	5.01	1.58	2.59	1.0
Gfi1b	21.92	16.50	53.34	80.77	1.28	2.29	1.0
Csf2rb	27.05	27.74	73.13	149.04	1.43	2.43	1.0
1110008P14Rik	1.59	1.38	3.75	6.45	1.24	2.22	1.0
Lst1	2.75	1.99	7.37	10.51	1.42	2.40	1.0
Alox5	6.51	2.99	26.11	23.68	2.00	2.98	1.0
Zbtb16	1.22	0.43	7.82	5.46	2.68	3.66	1.0
Sptb	4.03	2.45	15.62	18.39	1.95	2.91	1.0
Mpp2	2.22	0.81	6.32	4.49	1.51	2.46	1.0

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rps27	6.33	5.49	39.85	7.30	2.66	0.41	-2.2

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 10: not upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Lars2	40.95	54.22	153.32	65.57	1.90	0.27	-1.6
Lypd6	1.17	1.98	2.38	1.46	1.02	-0.44	-1.5
Fam209	0.39	0.91	1.17	1.05	1.60	0.21	-1.4
Pdgfrl	0.50	1.26	1.15	1.34	1.19	0.09	-1.1
Sbsn	0.74	1.26	1.45	1.18	0.97	-0.10	-1.1
Stmn2	1.36	3.23	2.74	3.19	1.01	-0.01	-1.0
Cbln1	0.64	1.41	1.43	1.56	1.16	0.15	-1.0
Jmjd7	0.50	1.63	1.36	2.27	1.45	0.48	-1.0
Adrb2	0.99	0.86	2.34	1.06	1.24	0.30	-0.9
Mfap4	0.93	0.91	2.33	1.20	1.33	0.41	-0.9
Lgr5	1.01	1.86	2.25	2.31	1.15	0.31	-0.8
Efemp1	0.70	0.92	1.37	1.05	0.96	0.18	-0.8
Myl7	0.65	0.79	1.61	1.17	1.31	0.57	-0.7
Glipr1	4.17	8.99	9.02	11.67	1.11	0.38	-0.7
Asb12	1.63	2.69	3.33	3.36	1.04	0.32	-0.7
Six4	1.88	2.39	3.73	2.90	0.99	0.28	-0.7
Itgb5	3.16	2.94	6.96	3.98	1.14	0.44	-0.7
Nog	1.95	2.29	4.08	2.95	1.06	0.37	-0.7
Rnf128	4.15	6.87	9.43	9.76	1.18	0.51	-0.7
Calca	0.48	0.97	1.22	1.61	1.34	0.73	-0.6
Emid1	0.62	1.05	1.54	1.70	1.31	0.70	-0.6
Ifi30	2.37	3.48	5.46	5.28	1.21	0.60	-0.6
Mlkl	0.85	1.30	1.91	1.94	1.17	0.58	-0.6
Arhgdig	1.55	2.84	3.02	3.68	0.96	0.37	-0.6
Pdyn	0.69	0.77	1.42	1.06	1.05	0.47	-0.6
Tdrp	3.33	3.39	7.57	5.24	1.19	0.63	-0.6
Setbp1	1.26	2.15	2.88	3.36	1.19	0.64	-0.6
Adrbk2	0.62	1.19	1.30	1.72	1.08	0.53	-0.5
Pdgfrb	5.75	5.13	11.25	6.97	0.97	0.44	-0.5
Tpd52	4.71	7.96	9.70	11.50	1.04	0.53	-0.5

Sp1$^{del/del}$ HE2 – Progenitors
Cluster 11: less upregulated in mutant

Gene	ES WT FPKM	ES Sp1$^{del/del}$ FPKM	Flk1+ WT FPKM	Flk1+ Sp1$^{del/del}$ FPKM	WT ES/Flk log2 FC	Sp1$^{del/del}$ ES/Flk log2 FC	WT FC/Sp1$^{del/del}$ FC
Rtp4	0.11	1.01	1.48	3.87	3.72	1.95	-1.8
Bex6	0.13	0.34	2.76	2.88	4.41	3.10	-1.3
Bcl2a1d	0.14	0.26	2.73	2.23	4.32	3.11	-1.2
Ltb4r1	0.17	0.47	1.50	2.14	3.17	2.18	-1.0

Supplementary Table 5.5 – Gene deregulation between HE2 and haematopoietic progenitors in Sp1$^{del/del}$ cells

Genes that were differentially expressed between HE2 and progenitors (at least two fold changed) were identified and compared to the expression in Sp1$^{del/del}$ cells. The genes that were differently differentially expressed were grouped into clusters based on how the expression changes. The expression level (FPKM) in each cell type is shown, as well as the log2 fold change between stages in WT or Sp1$^{del/del}$ cells, and the difference between the fold changes. Cluster 1 and 12 represent genes that are not changed between WT and Sp1$^{del/del}$ cells, hence are not shown. No genes were found in clusters 6 (downregulated in mutant/upregulated in WT) and 9 (upregulated in mutant/downregulated in WT).

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