The transcriptional regulation by MYSM1 in hematopoietic stem and progenitor cells

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List of Abbreviations

AML	Acute myeloid leukemia
CBP	CREB-binding protein
cDC	Conventional dendritic cell
cDNA	Complementary DNA
ChIP-seq	Chromatin immunoprecipitation sequencing
CLP	Common lymphoid progenitor
CMP	Common myeloid progenitor
CPM	Counts per million
DBA	Diamond-Blackfan anemia
DC	Dendritic cell
DN	Double negative
DNA	Deoxyribonucleic acid
DP	Double positive
DR	Diabetic retinopathy
DUB	Deubiquitinase
eADA	Erythrocyte adenosine deaminase
eEF1G	Eukaryotic translation elongation factor 1 gamma
FDR	False discovery rate
Flt3	FMS-like tyrosine kinase 3
GMP	Granulocyte/macrophage progenitor
GO	Gene ontology
GSEA	Gene set enrichment analysis
H2AK119ub	Monoubiquitinated histone H2A lysine 119
H3K27ac	Acetylated histone H3 lysine 27
HSC	Hematopoietic stem cell
HSPC	Hematopoietic stem and progenitor cell
iPSC	Induced pluripotent stem cells
IBMFS	Inherited bone marrow failure syndrome
LSK	Lin-Sca1+cKit+
LT-HSC	Long-term hematopoietic stem cell
MDM2	Mouse double minute 2
MEP	Megakaryocyte/erythrocyte progenitor
MFI	Mean fluorescence intensity
MPP	Multipotent progenitor
mRNA	Messenger ribonucleic acid
mRNA	Messenger RNA
MYSM1	Myb-like, Swirm and MPN domain-containing protein 1
NK	Natural killer
OPP	O-propargyl-puromycin
PCAF	p300/CBP-associated factor
PcG	Polycomb Group proteins

pDC	Plasmacytoid DC
PRC	Polycomb Repressive Complex
PUMA	p53 upregulated modulator of apoptosis
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
RP	Ribosomal protein
RP-gene	Ribosomal protein-encoding gene
RPL	Large subunit ribosomal protein
RPS	Small subunit ribosomal protein
rRNA	Ribosomal ribonucleic acid
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SDS	Schwachman-Diamond syndrome
shRNA	Short hairpin RNA
ST-HSC	Short-term hematopoietic stem cell
TF	Transcription factor
TSS	Transcription start site
USP	Ubiquitin specific protease

Abstract

Myb-like, Swirm and MPN domain-containing protein 1 (MYSM1) is a transcriptional regulator essential to the functions of hematopoietic stem cells (HSCs) in both human and mouse. Previous studies show that in *Mysm1*$^{-/-}$ mice, the p53-regulated stress-response is induced and leads to HSC dysfunction and depletion of downstream progenitors and mature blood cells. In *Mysm1*$^{-/-}$*p53*$^{-/-}$ mice, these phenotypes revert to normal, indicating an antagonistic role of MYSM1 to p53. It is established that the HSC dysfunction in *Mysm1*-deficiency is driven by p53. However, the mechanism leading to p53 activation in *Mysm1*-deficiency remains unknown. In this study, we present the first data set describing the transcriptomic alterations in *Mysm1*$^{-/-}$ mouse HSCs and multipotent progenitors (MPPs) using RNA-Seq and the genome-wide binding pattern of MYSM1 in mouse hematopoietic progenitor cell lines using ChIP-Seq. I analyzed and identified profound changes in the transcriptome of *Mysm1*-deficient mice, including a group of downregulated translation-related and ribosomal protein-encoding genes (*RP-genes*). I showed that MYSM1 binds to the promoters of these genes and orchestrates the induction of transcriptional activation marker H3K27ac, suggesting directly regulation of these genes by MYSM1. I validated these findings use ChIP-qPCR. Furthermore, I showed that the expressions of the *RP-genes* are independent of p53 activation in *Mysm1*-deficiency and examined the roles of transcriptional regulators PRC1 and c-MYC in regulating *RP-gene* expressions. Overall, this work provides insight into the transcriptional control by MYSM1 in hematopoietic stem and progenitor cells and advances our knowledge of bone marrow failure in human *MYSM1*-deficiency.

Résumé

La protéine MYSM1 (*Myb-like, Swirm and MPN domain-containing protein 1*) est un régulateur transcriptionnel essentiel aux fonctions des cellules souches hématopoïétiques (CSH), autant chez l'homme que chez la souris. Des études antérieures ont démontré que chez les souris *Mysm1*$^{-/-}$, l'absence de MYSM1 induit une réponse au stress régulée par p53, menant à une dysfonction des CSH, à l'épuisement des cellules progénitrices qui se retrouvent en aval des CSH, et à une déplétion des cellules sanguines matures. Chez les souris *Mysm1*$^{-/-}$*p53*$^{-/-}$, ces phénotypes reviennent à la normale, signalant que MYSM1 agit en antagoniste vis-à-vis p53. Il est déjà bien établi que dans le contexte d'une déficience en *Mysm1,* les dysfonctions observées auprès des CSH sont provoquées par p53. Cependant, le mécanisme précis conduisant à l'activation de p53 en l'absence de *Mysm1* demeure inconnu. Nous présentons dans cette étude un premier ensemble de données qui révèle à l'aide du séquençage de l'ARN les altérations transcriptomiques des CSH et des cellules progénitrices multipotentes (MPP) issues de souris *Mysm1*$^{-/-}$. De plus, cet ensemble de données nous a permis de noter les positions génomiques précises où peuvent se lier la protéine MYSM1 à l'échelle du génome de la souris, à partir de lignées de cellules progénitrices hématopoïétiques soumises à la méthode ChIP-Seq. En ayant effectué ces analyses, j'ai identifié d'importants changements dans le transcriptome des souris déficientes en *Mysm1*, notamment l'expression réduite d'une famille de gènes qui encode diverses sous-unités de protéines liées à la traduction et au ribosome (gènes *RP*). J'ai démontré que MYSM1 se lie aux promoteurs de ces gènes *RP* pour ensuite induire le marqueur d'activation transcriptionnelle H3K27ac, ce qui suggère que MYSM contribue à la régulation de ces gènes de façon directe. J'ai validé ces résultats en utilisant la méthode ChIP-qPCR. De plus, j'ai découvert que l'expression des gènes *RP* est indépendante de l'activation de p53 dans le contexte d'une déficience en *Mysm1*, et j'ai examiné le rôle des régulateurs transcriptionnels PRC1 et c-MYC dans le contrôle de l'expression des gènes *RP*. Globalement, ce travail nous permet de mieux comprendre l'important rôle de MYSM1 dans la régulation transcriptionnelle des cellules souches, des cellules progénitrices hématopoïétiques, et de l'insuffisance de la moelle osseuse observée en cas de déficience humaine en *MYSM1*.

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List of Tables and Figures

Preface

This thesis includes content from the article "*MYSM1 maintains ribosomal protein gene expression in hematopoietic stem cells to prevent hematopoietic dysfunction*" published in The Journal of Clinical Investigation Insight (JCI Insight) on July 9th, 2020 of which I am the co-first author.

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Thesis contributions:

The design and supervision of the experiments in this thesis were by A. Nijnik and D. Langlais. The development of the *Mysm1*-deficient and the *Mysm1*$^{-/-}$*Puma*$^{-/-}$ mouse lines and the preparation of RNA-Seq and ChIP-Seq samples were done by J. Petrov and J. Belle. The sequencing of the RNA-Seq and ChIP-Seq was done by D. Neyret and M.

Rondeau from the Molecular Biology and Functional Genomics Facility at the Institut de Recherches Cliniques de Montréal (IRCM). The results from experiments performed by lab members were indicated in the text. A. Fiore performed the RT-qPCR and ChIP-qPCR experiments displayed in Figure 5D-E and 8D and designed some of the ChIP-qPCR primers of *RP-genes* listed in Table 4. A. Fiore, C. Feng, and Y. Lin performed the experiments outlined in the supplemental figures. The editorial of the thesis was done by A. Nijnik and D. Langlais. My (H. Wang) original contributions to this thesis are displayed as figures in Chapter 4 (except for Figures 5D-E, 8D by A. Fiore). I performed the bioinformatics analyses on the RNA-Seq and ChIP-Seq data sets, created the figures, and conducted the ChIP-qPCR experiments on MYSM1, RING1B, and YY1 with guidance and supervision from A. Nijnik and D. Langlais.

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Chapter 1 Literature Review:

Hematopoietic stem and progenitor cells, the regulation of hematopoiesis, and MYSM1.

1.1 Hematopoiesis

1.1.1 Introduction to hematopoiesis

The first appearance of a stem-like cell population in literature is from Ernst Haeckel's publication in 1889 describing the concept of a *stamzelle*, an undifferentiated cell that can produce many cell types in the body[1]. It took some time for the scientific community to characterize this *stamzelle* population and identify one that can differentiate into red and white blood cells in normal and leukemic conditions[2]. In the 1960s, James Till and Ernest McCulloch for the first time provided the proof of stem cells that, upon transplantation, proliferate and form macroscopic colonies[3,4]. In 1988, the purification of mouse hematopoietic stem cells was made possible by the use of phenotypic markers and fluorescence-activated cell sorter (FACS)[5]. Isolated Thy-1^{lo}Lin^{-}Sca-1^{+} bone marrow cells were shown to be more capable in rescuing lethally irradiated mice than unsorted bone marrow cells and as few as 30 sorted bone marrow cells could rescue 50 percent of the mice[5]. Since then, studies gradually shaped the current view of hematopoiesis and defined hematopoietic stem cells (HSC) as stem cells that exhibit self-renewal and multipotency properties, giving rise to all blood cell types[6].

Hematopoiesis is the process of generating all blood cell types and is essential for vertebrates[7]. A healthy human adult generates approximately 10^{11}-10^{12} blood cells every day through hematopoiesis[8]. These cells constitute the various parts of the blood, the innate and adaptive immunities, and so on. Despite having diverse characteristics and functions, these blood cells originate from the same stem cell, the HSC, that resides in the bone marrow. HSCs, accounting for only 0.01% of total nucleated bone marrow cells in an adult mouse[9], undergo two methods of cell division, the symmetrical cell division and the asymmetrical cell division. Symmetrical cell division enables an HSC to generate two identical daughter HSCs, whereas asymmetrical cell division generates a daughter HSC and a more differentiated progeny. Through these two methods of cell division, HSCs maintain their population and replenish the blood with a variety of differentiated progenies.

1.1.2 The classical model of hematopoiesis

The classical model of hematopoiesis is depicted as a hierarchy with HSCs at the top and branching into the various progenitors and differentiated cells (Figure 1)[6,10,11]. HSCs first differentiate into multipotent progenitors (MPP) as they lose their self-renewal ability. Then, a branching point occurs as MPPs differentiate into either common lymphoid or common myeloid progenitors. The common lymphoid progenitors (CLP) divide multi-laterally into progenitors of natural killer (NK) cells, T cells, B cells, and lymphoid lineage derived dendritic cells (DC). The common myeloid progenitors (CMP) give rise to granulocyte/macrophage progenitors (GMP) and megakaryocyte/erythrocyte progenitors (MEP). The GMPs develop into myeloid lineage derived DCs, monocytes, and myeloblasts. Monocytes can differentiate into macrophages and monocyte-derived DCs. Myeloblasts give rise to neutrophils, eosinophils, and basophils. The MEPs develop into erythrocytes and megakaryocytes, which then develop into platelets. Overall, the classical model of hematopoiesis depicts the relationships between the hematopoietic progenitors and their progenies.

Recently, controversies revolve around the classical model of hematopoiesis, highlighting its poor representation of the heterogeneity and plasticity within hematopoiesis. Several studies suggested a continuum model that better demonstrates the gradual process of hematopoietic progenitors in acquiring lineage biases without passing through discrete populations[6,11]. MPPs, for example, are characterized as heterogenous with lineage-primed subpopulations but there still exist the possibilities for a lineage-primed MPP to commit to an alternative fate[11-13]. In contrast, several other high-resolution transcriptomic and transplantation studies suggested that the progenitors may be more lineage-restricted than we think and are pre-programmed before their differentiation[14]. Thus, hematopoiesis is a complex process following either a step-wise or a gradual developmental timeline but nevertheless gives rise to the mature cell types in the blood.

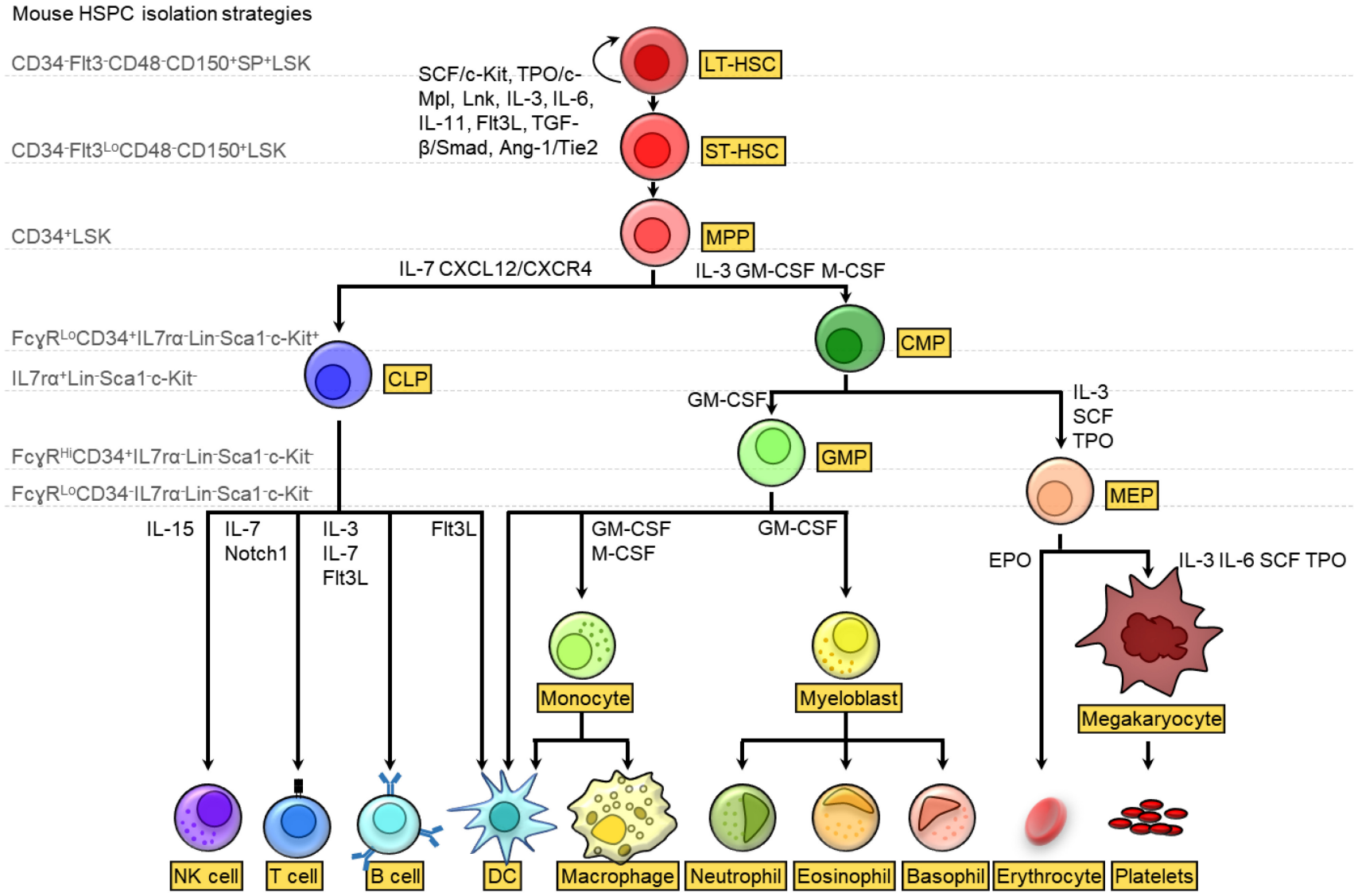


Figure 1. Model of hematopoiesis

1.1.3 Heterogeneities within the HSC and MPP populations

With advances in cell sorting technology, the population previously defined as HSCs is now considered as molecularly and functionally heterogenous[6]. Within this population, HSCs that provide bone marrow reconstitution in a primary round of transplantation and at least a secondary transplantation are characterized as long-term HSCs (LT-HSC)[15-19]. HSCs that can only reconstitute in a primary round of transplantation are grouped as short-term HSCs (ST-HSC) and MPPs based on the length and robustness of the reconstitution[15-19]. LT-HSCs are mostly maintained in quiescence, are multipotent, and have self-renewal potential[10]. Through asymmetric cell division, LT-HSCs give rise to ST-HSCs with reduced self-renewal potential[10]. As ST-HSCs further differentiate, they lose their ability to self-renew and become MPPs[10].

Heterogeneity also exists within the MPP population. MPPs are subdivided into four subpopulations based on their eventual cell fates[12,13,20]. The MPP1 subpopulation is the most stem-like, exhibits short-term self-renewal ability[12], and has similar transcriptomic and proteomic characteristics as ST-HSCs[13]. The MPP2 and MPP3 subpopulations are myeloid lineage biased and the MPP4 subpopulation is lymphoid lineage primed[12,13].

1.1.4 Strategies to isolate mouse hematopoietic stem and progenitors

Hematopoietic lineage cells can be purified based on their cell surface markers using a fluorescence-activated cell sorter (FACS) (Table 1). A combination of low level of lineage marker (Lin^-) expression and high level of Sca1 and c-Kit expression ($Lin^-Sca1^+c\text{-}Kit^+$ or LSK) can separate mouse HSCs and MPPs from more differentiated cells[10,12,13,15,17,19-22]. Within the sorted LSK population, LT-HSCs can be isolated using gates such as $CD34^-Flt3^-$, $CD48^-CD150^+$, and Hoechst-effluxing side population (SP^+)[10,12,13,15,19,20,22]. ST-HSCs share similar surface markers as LT-HSCs except for a slight increase in the level of FMS-like tyrosine kinase 3 (Flt3) expression ($CD34^-Flt3^{Lo}CD48^-CD150^+LSK$)[10,20,23]. MPPs can be distinguished from the HSCs based on high levels of CD34 expression ($CD34^+LSK$)[10,12,13,15,19,21,22]. MPPs are further characterized into the four lineage biased subpopulations based on the expressions of Flt3, CD48, and

CD150: MPP1 (Flt3$^-$CD48$^-$CD150$^+$), MPP2 (Flt3$^-$CD48$^+$CD150$^+$), MPP3 (Flt3$^-$CD48$^+$CD150$^-$), and MPP4 (Flt3$^+$CD48$^+$CD150$^-$) [12,13,20,21].

MPPs give rise to lineage-restricted progenitors, CLPs and CMPs. CLPs are identified by low levels of Lin, cKit and Sca1 expression, and high level of Interleukin 7 receptor α chain (IL7rα) expression, which interacts with IL7 during T and B cell development[22,24]. CMPs are identified by Lin$^-$Sca1$^-$c-Kit$^+$, high levels of CD34 expression, and low levels of IL7rα and Fcɣ receptor II/III (FcɣR) expression, which is an important receptor for myelomonocytic cells[22,25]. Rapid differentiation of the CMPs gives rise to GMPs and MEPs. The GMPs are FcɣR^{Hi}CD34$^+$ and the MEPs are FcɣR^{Lo}CD34$^-$[22,25]. From this point, the myeloid lineage progenitors develop into their mature cell types, including monocytes, neutrophils, eosinophils, basophils, erythrocytes, and platelets.

Table 1. Markers for isolating mouse hematopoietic stem and progenitor cells.

Cell types	Cell surface markers	References
LT-HSC	CD34$^-$Flt3$^-$CD48$^-$CD150$^+$SP$^+$LSK	10,12,13,15,19-22
ST-HSC	CD34$^-$Flt3^{Lo}CD48$^-$CD150$^+$LSK	10,20,21,23
MPP1	CD34$^+$Flt3^{Lo}CD48$^-$CD150$^+$LSK	12,13,20,21
MPP2	CD34$^+$Flt3^{Lo}CD48$^+$CD150$^+$LSK	12,13,20,21
MPP3	CD34$^+$Flt3^{Lo}CD48$^+$CD150$^-$LSK	12,13,20,21
MPP4	CD34$^+$Flt3$^+$CD48$^+$CD150$^-$LSK	12,13,20,21
CLP	IL7rα$^+$Lin$^-$Sca1^{lo}c-Kit^{lo}	22,24
CMP	FcɣR^{Lo}CD34$^+$IL7rα$^-$Lin$^-$Sca1$^-$c-Kit$^+$	22,25
MEP	FcɣR^{Lo}CD34$^-$IL7rα$^-$Lin$^-$Sca1$^-$c-Kit$^+$	22,25
GMP	FcɣR^{Hi}CD34$^+$IL7rα$^-$Lin$^-$Sca1$^-$c-Kit$^+$	22,25

LSK= Lin$^-$Sca1$^+$c-Kit$^+$

1.2 Factors regulating hematopoiesis

Most mature blood cells are short-lived and depend on their stem and progenitor cells for continual supplies. A common misunderstanding is that hematopoietic stem cells (HSC), at the top of the hematopoietic hierarchy, must be constantly proliferating and differentiating to replenish the rapid turn-over of mature blood cells. Surprisingly, most

HSCs are found in the quiescent G_0 phase of the cell cycle under homeostatic conditions. The onset of HSC differentiation is precisely regulated by multiple factors including cytokines, transcription factors, and bone marrow niche[26-28].

1.2.1 Cytokines in the regulation of hematopoiesis

The self-renewal, differentiation, quiescence maintenance, and stress-response of HSCs are all tightly regulated. Two ligand/receptor pairs, which are Stem Cell Factor (SCF)/c-Kit and Thrombopoietin (TPO)/c-Mpl, are found to be essential for HSC self-renewal[26]. The cytokine receptors c-Kit and c-Mpl are expressed on all HSCs and bind to their ligands SCF and TPO, respectively, to activate downstream signalling promoting HSC self-renewal[26,29-32]. An intracellular scaffold protein Lnk, on the other hand, is a negative regulator of HSC self-renewal through inhibiting the signalling of cytokines SCF, TPO, erythropoietin (EPO), Interleukin 3 (IL-3), and IL-7[26]. Other cytokines, such as IL-3, IL-6, IL-11, and Flt3 ligand (Flt3L), are also shown to promote HSC survival and proliferation[26]. However, their roles may be redundant[26]. The TGF-β/Smad and Angiopoietin-1 (Ang-1)/Tie2 signalling pathways are responsible for maintaining HSC quiescence[26,33]. In response to non-homeostatic events such as acute blood loss, HSCs are activated partially by the PI3K/AKT/mTORC1 signalling pathway to replenish the mature blood cells[34].

The formation of the common lymphoid progenitors (CLP) from multipotent progenitors (MPP) requires cytokine IL-7 and chemokine/receptor pair CXCL12/CXCR4[35-37]. Subsequently, the differentiation from CLPs is regulated by cytokines IL-3, IL-7, and Flt3L to B cell progenitors[38-43], by IL-7 and Notch1 signalling to T cell progenitors[35,38,39,44-47], by IL-15 to NK cell progenitors[48-50], and by Flt3L to lymphoid-derived dendritic cells (DC) progenitors[51-54].

The differentiation from MPPs to common myeloid progenitors (CMP) is mediated by IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and macrophage-CSF (M-CSF)[55-59]. Within the myeloid lineage, CMPs differentiate into granulocyte/macrophage progenitors (GMP) with the help of GM-CSF[11,60]. GMPs receive further stimulation by GM-CSF and M-CSF to differentiate into monocytes[59,60], and by GM-CSF to differentiate into myeloblasts, which give rise to neutrophils, eosinophils, and

basophils[59,61]. On the other branch, CMPs differentiate into megakaryocyte/erythroid progenitors (MEP) with the help of cytokines IL-3, SCF, and TPO[62-68]. MEPs further differentiate into erythrocytes by EPO stimulation[69,70], and into megakaryocytes and then platelets by IL-3, IL-6, SCF, and TPO stimulation [62-68]. CMPs can give rise to myeloid-derived DC progenitors by Flt3L, GM-CSF, TNF-α stimulation[53,54,71].

Although extensive studies have characterized the complex cytokine network regulating different stages of hematopoiesis, our understanding is still developing and being reshaped by advances in transcriptomic, proteomic, and metabolomic studies[57,72,73]. Some cytokine regulations, such as IL-7 in the lymphopoiesis and TPO in thrombopoiesis, are shown to be indispensable, whereas others have been suggested to play supportive instead of instructive roles in hematopoiesis[57].

1.2.2 Transcription factors in the regulation of hematopoiesis

Transcription factors (TF), which are proteins involved in transcriptional regulation of genes, play important roles in all stages of hematopoiesis (Table 2). FLI1[74], LMO2[75], MYB[76], RUNX1[77], and SCL/TAL1[78-80] are required to establish definitive hematopoiesis and HSCs during embryogenesis. ERG[81-83], FLI1[84], GATA2[85], and many other TFs regulate the different functions of HSCs, including quiescence, self-renewal, and differentiation. In both lymphoid and myeloid lineages, the lineage specification, differentiation, and maturation are regulated by TFs. The list of TFs regulating the different stages of hematopoiesis is not exhaustive and many more are to be consolidated.

Many TFs were shown to regulate the same stage of hematopoietic development and they interacted with each other in a complex regulatory network. To better understand the combinatory control exerted by the different TFs in hematopoiesis, characterizations of multiple TFs at the same time and in the same cell type were initiated in 2010[86-88]. Several studies had characterized the genome-wide binding profiles of multiple TFs essential to hematopoiesis using the chromatin immunoprecipitation sequencing (ChIP-Seq) technology in the HSPC cell line HPC7[86-88]. The TFs were largely selected from three groups, which are an HSPC specific TF group including ERG, FLI1, GATA2, GFI1B, LDB1, LMO2, LYL1, MEIS1, MYB, RUNX1, and SCL/TAL1, a myeloid/generic TF group composed of C/EBPα, C/EBPβ, c-FOS, c-JUN, c-MYC, E2F4, EGR1, ELF1, ETO2, MAX,

NFE2, p53, pSTAT1, PU.1, and STAT3, and a structural TF group comprised on CTCF and RAD21[88]. ChIP-Seq on histone markers H2AK5ac, H3K4me3, and H3K36me3 were performed in parallel to provide descriptions of the chromatin status. From these large-scale studies, a heptad of TFs from the HSPC specific group, including ERG, FLI1, GATA2, LMO2, LYL1, RUNX1, and SCL/TAL1, was identified to work conjunctionally in the regulation of hematopoiesis[87]. Another set of TFs from the myeloid/generic group including c-JUN, c-MYC, E2F4, EGR1, ELF1, ETO2, MAX, and STAT3, exhibited similar binding preferences and were proposed to involve in TF partnering[88]. Furthermore, known protein-protein interactions, such as FLI1/GATA2 and c-FOS/c-JUN, were identified in the studies and novel interactions between TFs were proposed[88]. However, due to the large-scale of the studies, the profiles of many TFs and their proposed interactions remain to be further examined. Nevertheless, these studies provided a solid foundation for future studies investigating the roles of novel TFs in the combinatorial regulation of hematopoiesis.

Table 2. Transcriptional regulators of hematopoiesis.

	TFs	Role(s) in HSPCs	Role(s) in the lymphoid lineage	Role(s) in the myeloid lineage
HSPC specific	**ERG**	Promotes HSC maintenance over differentiation[81-83].	Dispensable for lymphoid lineage development[83], except for B cells[89].	Dispensable for myeloid lineage development[83].
	FLI1	Establishes definitive hematopoiesis[74], and regulates HSC maintenance with ERG[84].	Regulates development of B cells[90] and NK cells[91].	Regulates development of granulocytes[91,92], monocytes[92,93], megakaryocyte[94-96], and DCs[92].
	GATA2	Regulates HSC maintenance[85], but not differentiation or self-renewal[25,97]	Not required for the development of lymphoid-lineage derived DCs[98].	Promotes HSC differentiation into myeloid progenitors[25,93,99], development of mast cells[25,100,101], and myeloid-lineage derived DCs[98].
	GFI1/ GFI1B	GFI1 regulates HSC quiescence and localization[102].	GFI1 regulates development of B and T cells[103].	GFI1B regulates development of megakaryocytes, and erythrocytes[103]. GFI1 regulates development of DCs, macrophages, and granulocytes[103].
	LDB1	Regulates HSC maintenance[104].	Not reported.	Negatively regulates erythroid differentiation[105,106].
	LMO2	Establishes and maintains definitive hematopoiesis[75].	Regulates T cell differentiation in T cell neoplasia[75], and induces stem-like features in T cell progenitors[107].	Negatively regulates erythroid differentiation[105].
	LYL1	Regulates HSC maintenance[108]. Overlaps in function with SCL[108].	Required for B cell differentiation[109].	Not reported.
	MEIS1	Regulates HSC maintenance[110].	Not reported.	Regulates development of CMPs, MEPs, megakaryocytes and erythrocytes[110].
	MYB	Establishes definitive hematopoiesis[76], and regulates HSC self-renewal[25,111].	Regulates development of B and T cells[25,112].	Regulates development of granulocytes[113] and erythrocytes[112].
	RUNX1	Establishes definitive hematopoiesis[77], and regulates HSC quiescence[114].	Regulates B cell lineage specification and transition from pre-pro B to pro-B cells via *Ebf1*[115-117], and regulates T cell differentiation and maturation[118-122],	Regulates development of monocytes[93] and DCs[123], and mediates maturation of granulocytes[124] and megakaryocytes[125].
	SCL/ TAL1	Establishes definitive hematopoiesis[78-80], regulates HSC maintenance[25,126,127].	Not reported.	Regulates monocyte progenitor proliferation[25,127,128], and development of mast cells[129], erythrocytes[130-132], and megakaryocytes[130]
Myeloid/generic	**C/EBPα**	Regulates HSC self-renewal, maintenance, and differentiation into CMPs[25,133].	Promotes progression of CLPs to B cells[134].	Regulates development of CMPs and GMPs[25,133], steady-state granulopoiesis, and differentiation of neutrophils[135,136].
	C/EBPβ	Not reported.	Mediates B cell survival and proliferation[137].	Regulates emergency granulopoiesis[138-140].
	c-FOS	Not reported.	Mediates B cell terminal differentiation[141].	Regulates myeloid cell survival and differentiation by dimerizing with c-JUN[142-144].

	TFs	Role(s) in HSPCs	Role(s) in the lymphoid lineage	Role(s) in the myeloid lineage
	c-JUN	Not reported.	Mediates B cell terminal differentiation[141].	Regulates myeloid cell development by dimerizing with c-JUN[142-144], co-activates PU.1 and M-CSF transcription in myeloid lineage[145-147].
	c-MYC	Regulates HSC self-renewal[148] and differentiation into CMPs and CLPs[148].	Regulates development of B cells[149,150], activated T cells[151,152], and immature NK cells[152].	Promotes M2 macrophage pro-tumour programs[152-154].
	E2F4	Regulates HSC differentiation into CLPs[155].	Regulates development of lymphocytes[155].	Regulates erythropoiesis[155-157].
	EGR1	Regulates HSC proliferation and localization[158].	Mediates development of B cells[159] and T cells[160].	Regulates differentiation of myeloid lineage cells[161,162], but dispensable in macrophages[163].
	ELF1	Regulates genes important for hematopoiesis (*Scl*, *Fli*-1, *Lyl-1*, *Runx1* and *Lmo2*)[164].	Regulates development of natural killer T cells[165].	Regulates terminal erythropoiesis[164].
	ETO2	Regulates HSC self-renewal[166,167]	Not reported.	Mediates development of erythroid precursors[131].
	MAX	Dimerizes with c-MYC to function.	Dimerizes with c-MYC to function.	Dimerizes with c-MYC to function.
	NFE2	Regulates HSC self-renewal[168].	Not reported.	Regulates megakaryocyte maturation and platelet production[25,169-171].
	p53	Regulates HSC quiescence and self-renewal[172].	Important during stress-response.	Important during stress-response.
	pSTAT1	STAT1 signalling in normal hematopoiesis[173].	STAT1 signalling in T cell development[174].	Regulates erythropoiesis[175].
	PU.1	Required for HSC self-renewal, quiescence[176], and differentiation into CMPs and CLPS[25,177].	Regulates development of CLPs[177], NK cells[178,179], and early B cells[177,180,181]. Inhibits T cell maturation[182,183] and represses lymphoid-lineage derived DCs[184].	Regulates development of CMPs, monocytes, and granulocytes[93,177,180]. Represses mast cell production[101]. Maintains erythroid progenitors[185,186] and promotes myeloid-lineage derived DCs[184].
	STAT3	Regulates HSC self-renewal[187-189]	Regulates development of B cells[190,191] and T cells[191-193]. Mediates DC lineage commitment from CLPs[194].	Regulates emergency granulopoiesis[195], functions of neutrophils[195,196], and megakaryopoiesis[197]. Mediates DC lineage commitment from CMPs[194].
Structural	**CTCF**	Mediates HSC quiescence[198,199].	Represses premature differentiation of plasma cells[200,201] and promotes differentiation of effector T cells[202].	Regulates DC differentiation and maturation[203].
	RAD21	Negatively regulates HSC self-renewal[204].	Mediates B cell maturation[205], and T cell differentiation[206].	Not reported.

Abbreviations: HSC=hematopoietic stem cell, CMP=common myeloid progenitor, CLP=common lymphoid progenitor, NK cell=natural killer cell, DC=dendritic cell.

1.2.3 Histone modifications in the regulation of hematopoiesis

Histone modifications are well-characterized mechanisms regulating gene expression without altering the genetic code. Histones are a group of positively-charged proteins termed H1, H2A, H2B, H3, and H4, and are critical in the packaging of DNA in eukaryotes[207]. Two copies of histone H2A, H2B, H3, and H4 form a histone octamer and strongly adhere to the negatively-charged DNA and form structures called nucleosomes[207]. Histone H1 is a linker protein that stabilizes the DNA between nucleosomes[207]. Nucleosomes fold into chromatin fibers and then coil into the chromatid of a chromosome[207]. Histones can undergo various types of post-translational modifications on their exposed N-terminal tails, altering their interactions with the DNA[208]. Modifications that disrupt the interaction with DNA cause nucleosomes to unwrap and form euchromatin, which is an accessible region of the chromatin that transcription factors bind to and activate gene expression[208]. On the other hand, modifications that strengthen the interaction with DNA create tightly packed heterochromatin and disable any access of transcriptional factors to DNA, leading to gene silencing[208]. Thus, histone modifications are mechanisms regulating gene expression.

Several types of histone modifications have been discovered, including acetylation, ubiquitination, methylation, and others[208]. Acetylation occurs on histones H3 and H4 and adds a negative charge to lysine residue, freeing the tightly wound DNA and leading to chromatin opening[208]. For example, the acetylation of lysine 27 on histone H3 tail (H3K27ac) marks regions of the chromatin with active gene promoters and enhancers[208]. Ubiquitination occurs at the lysine substrates on histone H2A and H2B and their effects are contrasting[209,210]. Mono-ubiquitination of lysine 119 on histone H2A (H2AK119ub) leads to repression of gene activity, whereas mono-ubiquitination of lysine 120 on histone H2B (H2BK120ub) leads to transcriptional activation[209,210]. On the other hand, methylation of different substrates and the level of methylation (mono-, di-, or tri-methylated) lead to induction or repression of transcription activities[208], adding another level of complexity to histone modifications[208,211]. Other than these modifications, histone phosphorylation[208], GlcNacylation[212], citrullination[213], and isomerization[214] have also been characterized that lead to different statuses of transcription. Together, histone modifications control the DNA accessibility to transcriptional regulatory programs.

Hematopoiesis, a process by which the HSCs give rise to the various mature cell types in the blood, is fundamentally orchestrated by alterations in gene expression. Transcription factors, which direct these gene expression changes, are strongly influenced by the chromatin status of their target genes[215,216]. Histone modifications, which lead to the opening or closing of the chromatin, are involved in enabling cell-specific gene expression during hematopoiesis[215,216]. Mutations in the genes encoding for histone-modifying enzymes lead to hematopoietic dysfunctions and improper lineage development[215,216]. For example, Polycomb Group (PcG) protein complexes are histone-modifying proteins essential to normal hematopoiesis. Two major PcG protein complexes, termed Polycomb Repressive Complex 1 (PRC1) and PRC2, play significant roles in transcriptional regulation during hematopoiesis, including the mono-ubiquitination of histone H2A K119 and the methylation of histone H3 K27, respectively[217]. Mutation in *Bmi1*, a gene encoding for a component of PRC1, leads to improper HSC maintenance and the development of lymphoma[215,218]. Mutation in *Ezh2*, a gene encoding for a component of PRC2 essential for B cell development[219], leads to improper gene silencing and the development of acute and chronic myeloid malignancies[220-223]. Histone acetyltransferase CBP is recruited by GATA1 to stimulate the transcription of the β-globin locus during erythropoiesis[215,224], and histone acetyltransferase P300/CBP-associated factor (PCAF) is recruited by oncoprotein SCL/TAL1 to mediate the transcription of SCL/TAL1-directed genes in T-cell acute lymphoblastic leukemia[215,225]. These examples represent only a small portion of histone-modifying enzymes that are critical in normal hematopoiesis. Overall, histone modifications are mechanisms regulating gene expression and serve a pivotal role in hematopoiesis.

1.2.4 The bone marrow niche in the regulation of hematopoiesis

Other than cytokines and transcription factors, the bone marrow microenvironment, or the niche, plays an essential role in hematopoiesis[26,28,226-230]. The initial identification of a bone marrow niche occurred when mutations of c-Kit and stem cell factor (SCF), at the time referred to as the *W* and *Sl* gene loci, were discovered in mice[26,231,232]. These mutant mice exhibited similar phenotypes including anemia and deficiencies in mast cells, melanocytes, and germ cells[26,233-239]. Transplantation of wildtype bone marrow cells into

the c-Kit mutant mice restored their hematopoiesis while into the SCF mutant mice did not[240-243]. Furthermore, transplantation of bone marrow cells from the c-Kit mutant mice into wildtype or SCF mutant mice did not reconstitute hematopoiesis, whereas transplantation of bone marrow cells from the SCF mutant mice into wildtype or c-Kit mutant mice showed full reconstitution[240-243]. The receptor tyrosine kinase c-Kit is expressed on all HSCs[26,29-31], but its ligand SCF is only produced from non-hematopoietic endothelial cells and leptin receptor$^+$ (Lepr$^+$) stromal cells surrounding the HSCs[15,244-248]. Thus, the requirement of SCF in hematopoiesis suggests the existence and necessity of a bone marrow niche.

Further characterization of the bone marrow niche revealed important roles of several cell populations in the regulation of HSCs and hematopoiesis[28,247]. The bone marrow vasculature is important for the formation of HSCs during embryogenesis. The endothelial cells produce factors promoting hematopoiesis, such as G-CSF, GM-CSF, M-CSF, SCF, IL6, Flt3L, and adhesion molecules[28]. Mesenchymal stromal cell populations, including CXCL12-abundant reticular (CAR) cells, Lepr$^+$ cells, and Nestin-GFP$^+$ cells, produce factors such as Ang-1, SCF, and CXCL12 to promote HSC maintenance and retention in the bone marrow[28,247,249-254]. Osteoblasts support HSC growth by producing TPO and CXCL12[28,32,247,255-259]. Spindle-shaped N-cadherin$^+$ osteoblastic cells (SNO) provide a region of localization and attachment for the HSCs[28,246,247]. Glial cells promote HSC quiescence by activating TGF-β signalling[33,260,261], and adipocytes negatively regulate HSC maintenance[28,247,262]. The non-hematopoietic cell populations in the bone marrow niche serve important roles in regulating HSC development and hematopoiesis.

Overall, hematopoiesis is not an isolated system that gives rise to the various blood cells on its own but requires a complex network of instructive, supportive, and inhibitory cues from cytokines, transcription factors, and other cells in the bone marrow niche.

1.3 Myb-like, Swirm and MPN domain-containing protein 1 (MYSM1)

The Myb-like, Swirm and MPN domain-containing protein 1 (MYSM1) is a transcriptional regulator and chromatin interacting protein. MYSM1 functions in regulating the expressions of genes important in different stages of hematopoiesis[20,263-267], removing

ubiquitin from histone H2A lysine 119 (H2AK119)[263,267,268] and other substrates[267,269,270], and performing other non-catalytic activities[267].

1.3.1 The structure and function of MYSM1

The full-length MYSM1 protein in human is composed of 828 amino acids (819aa in mouse) and contains an N-terminal SANT domain, a SWIRM domain, and a C-terminal JAMM/MPN+ domain[263].

The MYSM1 SANT domain contains three α-helices and shares structural similarity with the DNA-binding domain of transcription factor c-MYB[263]. NMR chemical shift perturbation experiment determined the location of seven DNA-binding residues on the last helix of the MYSM1 SANT domain, suggesting DNA-binding activities[263,271]. Furthermore, the MYSM1 SANT domain was shown to bind to DNA *in vitro*[263].

The MYSM1 SWIRM domain contains five α-helices. Unlike the SWIRM domain on other proteins, the MYSM1 SWIRM domain does not exhibit or enhance DNA-binding activity[263]. Currently, the MYSM1 SWIRM domain is suggested to assist in chromatin-related activity[263]. However, its function remains to be characterized.

The MYSM1 JAMM/MPN+ domain is a metalloprotease-catalytic domain targeting ubiquitin. MYSM1 is a deubiquitinase for monoubiquitinated lysine 119 of histone H2A (H2AK119ub)[263,268], for K63-polyubiquitin chains on cytosolic substrates TRAF3, TRAF6, and RIP2[269,270], and possibly for M1 and K27 polyubiquitin chains on RIP2[270]. The multiplex deubiquitination roles of MYSM1 prompt us that the complete list of substrates where MYSM1 exerts its catalytic activity remains to be defined.

Histone H2AK119ub is a well-characterized substrate for MYSM1 deubiquitinase activity. Monoubiquitinating histone H2AK119 marks genes for long-term transcriptional repression during development and is primarily ubiquitinated by the Polycomb Repressive Complex 1 (PRC1) and PRC1 component proteins RING1A and RING1B[272-274]. In terms of deubiquitinating H2AK119, a collection of deubiquitinases including MYSM1, BAP1, USP3, USP12, USP16, USP22, and USP44 can perform this function, leading to activation of gene expression[209]. Currently, the network controlling the specific cell type and developmental stage in which these deubiquitinases function remains poorly defined.

In terms of interaction with other proteins, MYSM1 has been shown to interact *in vitro* with hematopoietic transcription factors (TF) E2A[264], PU.1[266,275], GATA2[20], RUNX1[20], c-MYC[276], and NFIL3[265], cell fate regulator p53[277], histone acetyltransferase P300/CBP-associated factor (PCAF)[268], and chromatin remodelling complex components BRM and BRG1[264]. MYSM1 mediates the recruitment of the hematopoietic TFs to their target genes[20,264-266,275,276], represses the p53 function to promote cell survival[277], and orchestrates chromatin remodelling to create favorable sites for TF recruitment[268]. Through these mechanisms, MYSM1 regulates gene expression.

Overall, MYSM1 functions as a deubiquitinase and a regulator of gene expression.

1.3.2 *MYSM1* mutations in human result in a bone marrow failure syndrome

MYSM1-deficiency is a rare autosomal recessive disorder that is frequently categorized under Inherited Bone Marrow Failure Syndromes (IBMFS), a group of rare genetic blood disorders characterized by bone marrow failure and high chances of developing aplastic anemia and leukemia[278-284]. Patients with homozygous *MYSM1* mutations developed hematopoietic defects and physical dysmorphisms[278-281]. Due to the rarity of this syndrome, only five studies have reported a total of seven patients with homozygous mutations of *MYSM1* to date (Table 3)[278-281,285].

Three sites of *MYSM1* mutations were described in the seven patients. Five of the seven patients had a nonsense mutation at nucleotide position 1168 (c.1168G>T) of *MYSM1*, leading to a premature stop codon p.E390* in the MYSM1 SWIRM domain[278,280,281]. Another patient had a mutation at nucleotide position 1967 (c.1967A>G), translating to an amino acid substitution p.H656R in the highly conserved region of MYSM1 JAMM/MPN+ domain, compromising the MYSM1 deubiquitination activities[279]. Another patient had a mutation at nucleotide position 869 (c. 869C>G), leading to a nonsense mutation p.S290* in a region in between MYSM1 SANT and SWIRM domains[285].

Despite the different sites of the *MYSM1* mutation, the patients shared many similar phenotypes and hematopoietic defects (Table 3). They all developed severe neonatal anemia with low hemoglobin levels, leukopenia, thrombocytopenia, reduced erythropoiesis, varied degrees of lymphopenia with significant B cell deficiency, and had

hypocellular bone marrow (Table 3)[278-281,285]. Most patients experienced recurrent infections at a young age and had short stature, dysmorphisms of the face, limbs, bones, and skin, and neurodevelopmental delays[278-281,285]. In terms of treatment, most patients required monthly blood transfusions to treat the anemia and enrolled in allogeneic hematopoietic stem cell transplantation to treat *MYSM1*-deficiency[278-281,285]. One patient, surprisingly, experienced a spontaneous reversion, also known as a back mutation, of the *MYSM1* mutation and the hematopoiesis of the patient was subsequently restored, suggesting a link between *MYSM1*-deficiency and the observed hematopoietic defects[279]. Together, these case reports emphasize the drastic effects of *MYSM1*-deficiency and suggest the importance of MYSM1 in normal hematopoiesis and development.

In addition to these case reports, other studies had also proposed links between human *MYSM1* mutations and diseases. Diamond-Blackfan Anemia (DBA) is a rare type of IBMFS that occurs in 1 out of 100,000-200,000 births and causes normochromic macrocytic anemia within the first year of life[286-290]. 19 ribosomal protein-encoding genes (*RP-genes*), *GATA1*, and *TSR2* have been linked to the cause of DBA and mutations in any of these 21 genes are haploinsufficient[288,290-294]. In an exome sequencing study conducted on 472 patients diagnosed with DBA in North America and Europe, the majority of the patients had mutations in at least one of the 21 DBA-causing genes[289]. However, one patient did not have any mutation in the 21 genes but was identified to have a *MYSM1* mutation at the nucleotide position 1432 (c.1432G>A, p.R478*)[289]. Despite showing many DBA-like symptoms including early childhood transfusion-dependent anemia, it was suggested that the patient might have received a misdiagnosis of DBA[289]. This study highlights the similarity between patients with *MYSM1*-deficiency and DBA.

Diabetic retinopathy (DR) is a common complication in type II diabetes mellitus (T2D) patients caused by alteration of glucose metabolism, leading to new-onset blindness[295-298]. Recent studies suggested that there might be a genetic factor making the T2D patients more susceptible to DR[299-302]. In a study of 749 Han Chinese T2D patients residing in Taiwan, two SNPs in the intronic region of *MYSM1* (Chr1:g.59162148C>T and Chr1:g.59153010G>A) were linked to the increased risk of

DR[298]. However, due to the limited sample size, further investigation is required to associate the *MYSM1* mutations to DR.

Overall, human *MYSM1*-deficiency is a rare autosomal recessive disorder and patients developed hematopoietic defects and physical dysmorphisms. Due to the limited number of human *MYSM1*-deficiency cases reported, the mechanisms leading to the phenotypes observed in human *MYSM1*-deficiency remain poorly characterized.

As human and mouse MYSM1 share high protein homology (identity = 87%)[267] and mouse models have been used frequently to study the human hematopoietic stem cell biology[303,304], studying mouse models of *MYSM1*-deficiency can help to elucidate significant insight into the hematopoietic dysfunction observed in human.

Table 3. Clinical features of patients with MYSM1-deficiency.

Table adapted from Bahrami *et al.*[280] and updated based on recent publications.

Features	Alsultan *et al.*[278]		Le Guen *et al.*[279]	Bahrami *et al.*[280]		Nanda *et al.*[281]	Belle *et al.*[285]
MYSM1 Mutation	c.1168G>T, p.E390*		c.1967A>G, p.H656R	c.1168G>T, p.E390*		c.1168G>T, p.E390*	c.869C>G, p.S290*
Birth	Not indicated	Not indicated	At term	Preterm 33 weeks	Preterm 33 weeks	Not indicated	38 weeks
Anemia/ Hemoglobin	7.5g/dL (6-month)	4.4g/dL (15-month)	5g/dL (at birth)	3g/dL (at birth)	2g/dL (at birth)	8.8-9.5g/dL (5-year)	8.1g/dL (at birth)
Leukopenia	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Thrombo-cytopenia	No	Yes	No	Yes	No	Yes	Yes
Bone marrow cellularity	Hypocellular	Hypocellular	Hypocellular	Hypocellular	Hypocellular	Hypocellular	Moderately hypocellular
Lymphopenia	Yes	Yes	Yes	Yes	Yes	Yes	No
B cell	Deficient	Deficient	Deficient, pre-B to immature B transition blockage	Deficient	Deficient	Deficient	Deficient
T cell	Normal	Normal	Deficient	Moderately deficient	Moderately deficient	Normal	Normal
NK cell	Deficient	Deficient	Deficient	Normal	Moderately deficient	Normal	Normal
Monocyte	Not indicated	Not indicated	Reduced progenitors	Not indicated	Not indicated	Not indicated	Reduced
Erythropoiesis	Reduced	Functional, dysplastic precursors	Reduced and deficient precursors	Dysplastic precursors	Dysplastic precursors	Reduced and dysplastic	Reduced
Granulopoiesis	Reduced	Reduced	Normal maturation but polymorpho-nuclear neutrophils	Dysplastic, moderate neutropenia	Dysplastic, moderate neutropenia	Normal	Neutropenia
Megakaryo-poiesis	Not indicated	Reduced and dysplastic	Not indicated	Dysplastic	Dysplastic	Reduced	Not indicated

Features	Alsultan *et al.*[278]		Le Guen *et al.*[279]	Bahrami *et al.*[280]		Nanda *et al.*[281]	Belle *et al.*[285]
Physical dysmorphism	Normal growth parameters, facial dysmorphism, and lack of hepatosplenomegaly.	Normal growth parameters. Similar physical examination and course of disease as the sibling.	Short stature, overt microcephaly, choanal atresia, bilateral deafness due to agenesis of the cochlea-vestibular nerves.	Short stature, thoracic asymmetry, short left humerus, short metacarpal bone, dry skin, trigonocephaly, midface hypoplasia, gingiva hyperplasia, bilateral cataracts, and neuro-developmental delay with reduced cerebral volume.	Short stature, rhizomelic shortening of arms, short fingers, bilateral protrusions on collar bones, dry skin, eczema, accessory papilla of the breast, noncompaction cardiomyopathy, midface hypoplasia, gingiva hyperplasia, delayed dentition, and neuro-developmental delay with reduced cerebral volume.	Short stature, coarse facial features. Trivial tricuspid regurgitation, developmental delay, intellectual disability, and recurrent chest infections. Neutrophilic panniculitis.	Short stature, midface hypoplasia, round face, almond-shaped eyes, discrete hypertelorism, laterally increasing lid axes, broad and depressed nasal bridge, short nose, long smooth philtrum, rhizomelic shortening of arms, brachydactyly, male infantile genital.
Treatment	Monthly blood transfusions between 5 and 9-month of age.	Monthly blood transfusions between 15 and 33-month of age.	Intravenous immunoglobulin replacement and co-trimoxazole prophylaxis after birth to prevent infection. Spontaneous *in vivo* reversion of the *MYSM1*-mutation.	Blood transfusions after birth. Intravenous antibiotics and co-trimoxazole prophylaxis to treat recurrent infection. Allogeneic hematopoietic stem cell transplantation at 42-month.	Cardiopulmonary resuscitation and blood transfusions after birth. Co-trimoxazole prophylaxis to treat infections. Allogeneic hematopoietic stem cell transplantation at 23-month.	Hematopoietic stem cell transplantation.	Blood transfusion started at 3 weeks of age and continued every 5-6 weeks. Allogenic hematopoietic stem cell transplantation.

1.3.3 MYSM1 and the p53 stress-response pathway

Most studies to date on the hematopoietic dysfunction in *MYSM1*-deficiency were conducted using mouse models[267]. *Mysm1*-deficient mice exhibited many similar phenotypes that were also observed in human, including small body size, abnormal physical development, anemia, lymphopenia, and profound B and NK cell deficiencies[20,264,265,305,306], suggesting that the study of mouse *Mysm1*-deficiency may help to elucidate insight into the human *MYSM1*-deficiency.

The master regulator of cell fate, p53, senses cellular stress and mediates stress-responses including cell cycle arrest, cellular senescence, DNA repair, and apoptosis when necessary. In *Mysm1*-deficient mice, p53 and its stress-response genes were activated in most hematopoietic cell types, leading to the loss of HSCs, MPPs, CLPs, and lymphoid lineage cells due to apoptosis[277,305,307-311]. The deletion of p53 in the *Mysm1*-deficient mice (*Mysm1*$^{-/-}$*p53*$^{-/-}$ double-knockout) restored normal phenotypes including body weight and size, skeletal and facial morphology[309], cellularity in the bone marrow and lymphoid organs[308,309], and importantly the hematopoietic system[309]. The *Mysm1*$^{-/-}$ *p53*$^{-/-}$ mice supported normal quiescence of the HSCs, proper development of the lymphoid lineage cells, and their HSCs were able to reconstitute the bone marrow following transplantation[309], suggesting a restoration of normal HSC functions. Together, these suggest that p53 activation is the common mechanism causing the hematopoietic dysfunctions in response to *Mysm1*-deficiency.

To further identify the mediating pathway following p53 activation, *Bbc3* (p53 upregulated modulator of apoptosis, PUMA) or *Cdkn1a* (p21), which are two major p53-induced mediators of cell apoptosis and cell cycle arrest, were deleted in *Mysm1*-deficient mice[277]. The deletion of *Cdkn1a* (p21) in *Mysm1*-deficient mice did not result in the restoration of normal phenotypes and the mice still had high risks of embryonic and neonatal mortality[277], suggesting that p21-mediated apoptosis is not induced following p53 activation in *Mysm1*-deficiency. On the other hand, the deletion of *Bbc3* (PUMA) in *Mysm1*-deficient mice rescued the physical abnormalities and partially restored the bone marrow cellularity, the lymphoid-primed multipotent progenitors (MPP4), and the myeloid-lineage cells[277], suggesting PUMA-mediated apoptosis is a pathway activated following p53 activation in *Mysm1*-deficiency. However, persistent p53 protein elevation, loss of

HSC quiescence, and lymphopenia were still identified in the *Mysm1*$^{-/-}$*Puma*$^{-/-}$ mice[277], indicating that PUMA-mediated apoptosis is not the only activated mediator. In conclusion, the apoptotic pathway mediated by PUMA, instead of p21, is induced following p53 activation in *Mysm1*-deficiency, partially driving the hematopoietic dysfunction.

To investigate the molecular mechanism between *Mysm1*-deficiency and p53 activation, co-immunoprecipitation experiments suggested interactions between MYSM1 and p53 in hematopoietic cells. ChIP-qPCR experiments showed that MYSM1 and p53 independently bound to the promoters of the p53 stress-response genes *Bbc3* (PUMA) and *Cdkn1a* (p21)[277], although the co-binding of the two regulators to the promoters remains unclear. The knockdown of MYSM1 (*shMysm1*) in a pro-B cell line Ba/F3 resulted in increased p53 binding at the *Bbc3* (PUMA) promoter and elevated levels of H3K27ac and H3K4me3 at both *Bbc3* (PUMA) and *Cdkn1a* (p21) promoters, suggesting a repressive role of MYSM1 in the expression of p53 stress-response genes[277]. Altogether these findings suggested potential interactions between MYSM1 and p53 in regulating hematopoiesis. However, these findings were restricted to a few selected sites of interest and thus cannot rule out other possible mechanisms contributing to the hematopoietic dysfunctions in *Mysm1*-deficiency.

Overall, the transcriptional regulator p53 is activated following *Mysm1*-deficiency, leading to hematopoietic cell apoptosis mediated by PUMA and possibly other pathways. Currently, although possible interactions between MYSM1 and p53 were suggested, the mechanism leading to p53 activation in *Mysm1*-deficiency remains undefined.

1.3.4 MYSM1 and carcinogenicity

In several recent studies, the role of MYSM1 in cancer has been explored in both the hematopoietic tissue and other body tissues[267]. These studies were reviewed in our recent work[267] and briefly summarized here. In the hematopoietic system, the loss of *Mysm1* in mice resulted in the development of thymic lymphomas at 6 to 9 months[309], although the cause of the tumours remains unclear. As *Mysm1*-deficiency was reported to lead to p53 activation in hematopoietic cells[277,305,308,309] and the tumours found in the *Mysm1*-deficient mice resemble the ones found in *p53*-deficient mice[312], they suggest that the *Mysm1*-deficient mice may have developed sporadic mutations in the p53-stress

response pathway[313]. However, further studies are needed to confirm this hypothesis and investigate the underlying mechanism.

In tissues outside of the hematopoietic system, MYSM1 was suggested to be a positive regulator of cancer progression. MYSM1 was found to alter the transcriptional programs and regulate the activation of androgen receptor-dependent genes in human prostate cancer cell lines[268]. MYSM1 expression was also elevated in human melanomas compared to normal melanocytes, and the silencing of *Mysm1* in murine melanoma cell lines significantly reduced the proliferation and survival of these cells[314], suggesting MYSM1 as a potential target for treating tumours. Additionally, an elevated level of MYSM1 expression was found in human colorectal cancers compared to adjacent normal mucosal cells and the presence of MYSM1 in the carcinomas was positively correlated with tumour status, metastasis, and clinical stage[315]. Furthermore, patients with positive MYSM1 expression in the tumours showed poor survival compared to patients without expression[315]. These findings suggest MYSM1 as a positive marker for tumour progression. However, the mechanism underlying these findings remain to be explored.

In human patients with *Mysm1*-deficiency syndromes, the development of tumour was not reported[278-281,285]. However, this could be attributed to the young age and the rarity of the patients. Overall, MYSM1 was identified in several studies of human and murine cancers, but the role played by MYSM1 in carcinogenicity remains to be further explored.

1.3.5 MYSM1 is crucial for the normal functions of hematopoiesis in mice.

Human and mouse MYSM1 share high protein homology (identity = 87%)[267]. Due to the limited number of human *MYSM1*-deficiency cases reported, most studies of MYSM1 to date were conducted using mouse models. Comparing to human, *Mysm1*-deficient mice share many similar phenotypes including small body size, abnormal physical development, anemia, lymphopenia, and profound B and NK cell deficiencies[20,264,265,305,306]. The high protein homology and phenotypic similarities between human and mouse MYSM1 suggest that the study of mouse *Mysm1*-deficiency may help to elucidate insight into the human *MYSM1*-deficiency.

1.3.5.1 The effects of *Mysm1*-deficiency on mouse HSCs and MPPs

Mysm1-deficiency has a significant impact on the normal function of hematopoietic stem and progenitor cells (HSPC). The *Mysm1* gene expression is higher in hematopoietic stem cells (HSC) than in any other hematopoietic cell type[20]. Mice with *Mysm1*-deficiency developed hypocellular bone marrow[20,305]. The *Mysm1*-deficient HSCs lost quiescence and entered into the G1-S-G2 phases of the cell cycle, continuing to proliferate until the reserve was exhausted[20,305,306]. The long-term HSCs lost their ability to reconstitute bone marrow following transplantation and the HSPCs had increased p53-activation, oxidative stress, DNA damage, and apoptosis[20,305,306]. The multipotent progenitors (MPPs), particularly the lymphoid-primed MPP4 subpopulation, and the common lymphoid progenitors (CLP) were severely depleted due to apoptosis[20,305]. These observations characterize the detrimental effects of *Mysm1*-deficiency on the HSPCs, leading to loss of HSC quiescence and depletion of MPPs and CLPs.

Mechanistically, MYSM1 regulates the HSPCs partially through the transcriptional activation of the *Gfi1* gene, which encodes for the Growth Factor Independent 1 (GFI1) transcription factor. GFI1 regulates HSC quiescence, B and T cell differentiation, and development of DCs, macrophages, and granulocytes[20,102,103]. MYSM1 bound to the regulatory region of *Gfi1* and was shown to interact with other hematopoietic transcription factors, including SCL/TAL1, PU.1, RUNX1, and GATA2 to regulate *Gfi1* expression[316]. In addition, MYSM1 deubiquitinated histone H2A at the promoter of *Gfi1* and facilitates the removal of repressive marker H3K27me3 [20], leading to transcriptional activation of the gene. Therefore, MYSM1 is proposed to regulate HSC partially through the induction of *Gfi1* expression.

Overall, the effects of *Mysm1*-deficiency in HSPCs include loss of HSC quiescence and depletion of lymphoid lineage progenitors. MYSM1 mediates HSC functions partially through the regulation of *Gfi1* expression, a gene encoding for essential hematopoietic transcription factor GFI1. However, it is important to keep in mind that these findings were based on limited analyses of the transcriptional changes and tested only selected sites of interests for MYSM1 binding. The full transcriptome of *Mysm1*-deficient cells and the genome-wide binding profile of MYSM1 have not been characterized to date.

1.3.5.2 Lymphoid lineage cells, including B, T, and NK cells, are severely depleted in *Mysm1*-deficiency

Lymphoid lineage cells, largely composed of B, T, and natural killer (NK) cells, are important in host immunity. In human *MYSM1*-deficiency, varied degrees of lymphopenia and profound B cell deficiency were described[278-281,285]. In mouse *Mysm1*-deficiency, the depletion of lymphoid lineage cells starts in the lymphoid-primed MPP4 and CLPs[20,305] and results in lymphopenia and severe B and NK cell deficiencies[265,305].

MYSM1 is essential for B cell development. The depletion of the B cell populations was reported in both human patients and mouse models of *MYSM1*-deficiency[264,278,279,285]. In human *MYSM1*-deficiency, a significant B cell deficiency and a blockage between the pre-B and immature B cell transition were identified (Table 3)[279]. *Mysm1*-deficient mice also had profound B cell deficiency, and particularly in pre-B and pro-B cell stages[264]. The B cell depletion in *Mysm1*-deficient mice is linked to the role of MYSM1 in inducing the expression of genes encoding for essential early B cell development transcription factors[264]. The expressions of genes including *Ebf1*, *Pax5*, *Vpreb1*, *Igll1*, *Cd79a*, and *Cd79b* were reduced in *Mysm1*-deficient B cell progenitors and pre-pro-B cells[264]. Particularly, MYSM1 was suggested to regulate the expression of *Ebf1* through binding to its promoter, assisting in the local recruitment of transcription factor E2A and chromatin-remodelling proteins BRM and BRG-1, repressing the recruitment of PRC1 complex, orchestrating histone modifications at the *Ebf1* promoter including deubiquitination of histone H2AK119, induction of histone H3K4me3, and removal of histone H3K27me3 [264]. In *Mysm1*$^{-/-}$*p53*$^{-/-}$ mice, the B cell populations are restored to normal levels[309]. Overall, MYSM1 is intrinsic for early B cell development and regulates the expression of the *Ebf1* gene, which encodes for an early B cell development TF.

T cell development is profoundly impacted by mouse *Mysm1*-deficiency and to a lesser degree in human patients. MYSM1 is constitutively expressed at different stages of T cell development including double-negative (DN) 1-4, double-positive (DP), single-positive (SP) CD4$^+$, and SP CD8$^+$ thymocytes[308]. In mouse *Mysm1*-deficiency, thymic cellularity was drastically decreased and the DN2-3, DP, SP CD4$^+$, and SP CD8$^+$ thymocytes were depleted[305,308,310]. The lymphoid-primed MPP4 and early T cell progenitors were significantly depleted[20,305,308]. The transition between DN1 and DN2-3

thymocytes was partially blocked[308,310] and mature T cells were defective in responding to *in vitro* stimulation[305,308], suggesting a defective early development and maturation of T cells in *Mysm1*-deficiency. Apoptosis and necrosis were induced in both bone marrow and thymus[305,308]. The transcription factor p53 is known as a master regulator of cell fate and is involved in mediating cellular apoptosis. Increased expressions of *Cdkn2a*, which encodes for a p53-activating protein $p19^{ARF}$, and p53 stress-response genes, including *Bax*, *Cdkn1a* (p21), *Bbc3* (PUMA), and *Pmaip1* (NOXA), were observed in the *Mysm1*-deficient T cells[308]. The deletion of p53 in the *Mysm1*-deficient mice restored the T cell numbers[308]. Together, these suggest that MYSM1 is essential for T cell lineage commitment and early development.

Natural killer (NK) cells are cytotoxic lymphocytes important in host innate immunity targeting virus-infected cells and tumour cells. NK cells originate from the CLPs and develop into NK progenitors (NKP, $CD122^{+}NK1.1^{-}DX5^{-}$), immature NK cells (iNK, $CD122^{+}NK1.1^{+}\ DX5^{-}$), and mature NK cells (mNK, $CD122^{+}NK1.1^{+}DX5^{+}$) [265,317,318]. Both human and mouse *MYSM1*-deficiency reported depletion of the NK cells (Table 3)[265,278-280]. Both *Mysm1*-deficient mouse bone marrow and spleen developed normal levels of NK lineage-primed CLPs, NKPs, and iNKs but had a drastic depletion of mNKs[265], demonstrating that NK cell lineage commitment and development up to the iNK stage were not compromised. *Mysm1*-deficient NK cells expressed reduced levels of maturation markers than wildtype control and the transplantation of *Mysm1*-deficient NKPs did not reconstitute mNKs in the recipient mice[265], highlighting a defective NK cell maturation in *Mysm1*-deficiency. Furthermore, forced expression of MYSM1 in *Mysm1*-deficient NKPs restored their ability to generate mNKs *in vitro*, showing that MYSM1 plays an essential role in the maturation of NK cells[*265*]. ID2 and NFIL3 are transcription factors required for NK cell maturation[319,320]. MYSM1 binds and recruits NFIL3 to the *Id2* gene promoter[265,320]. *Mysm1*-deficient mNKs had significantly reduced *Id2* expression, loss of NFIL3 recruitment, increased binding of PRC complex proteins, and induced level of repressive histone markers H2AK119ub and H3K27me3 enrichment at the *Id2* gene promoter, suggesting a regulatory role of MYSM1 on *Id2* expression in NK cells. Overall, MYSM1 has an intrinsic role in NK cell maturation and regulates the expression of *Id2*, which encodes for an essential to NK cell maturation TF.

In conclusion, human and mouse *MYSM1*-deficiency have many similar developmental defects in the lymphoid lineage. In mouse, MYSM1 is important to the development of B, T, and NK cells, and regulates the expressions of important developmental genes *Ebf1* in B cells and *Id2* in NK cells. It is necessary to keep in mind that the *Ebf1* and *Id2* regulatory regions only represent selected sites of interest and no genome-wide binding profile of MYSM1 has been characterized to date.

1.3.5.3 Myeloid dendritic cells, but not macrophages or granulocytes depend on MYSM1 for differentiation and fate determination

The effects of *MYSM1*-deficiency are more profound in the lymphoid lineage. Nevertheless, the myeloid lineage still experienced impacts from *MYSM1*-deficiency[20,266,277,305,307,309] as some of the human patients reported dysplastic granulopoiesis, erythropoiesis, and megakaryopoiesis (Table 3)[278-281,285]. In mouse *Mysm1*-deficiency, the most significant effect was observed in the development of Dendritic cells (DC) derived from both the lymphoid and myeloid lineages[266].

DCs have vast functions in host immunity ranging from antigen presentation to cytokine production. DCs are classified as either conventional dendritic cells (cDC) or plasmacytoid DC (pDC) based on their differentiation from the lymphoid or the myeloid lineage, respectively[266,321,322]. *Mysm1*-deficient mice had significant depletion of splenic cDCs and pDCs, and DC-committed precursors including common DC progenitors (CDP), migratory DC precursors (MDP), and pre-cDC[266].

The *Mysm1*-deficient bone marrow lost the ability to reconstitute DCs in transplantation and forced expression of MYSM1 in the bone marrow restored the ability, suggesting a critical role of MYSM1 in DC development[266]. In the steady-state, the development of both cDCs and pDCs requires Flt3 ligand (Flt3L)/Flt3 receptor signalling[52,53,323-325]. MYSM1 binding was confirmed to the *Flt3* promoter[266]. *Mysm1*-deficiency resulted in significantly reduced expressions of *Flt3* in CMPs and DC precursors, increased repressive histone markers H2AK119ub and H3K27me3 enrichment, and lost recruitment of an important DC development regulator PU.1[326-329], to the *Flt3* promoter[266]. Overall, MYSM1 is important for DC development and regulates the expression of *Flt3* in DCs.

Other cells in the myeloid lineage such as erythrocytes and megakaryocytes are mostly Flt3$^-$ and not highly dependent on MYSM1 for development[20,266,277,305,307,309]. Nevertheless, a human patient with MYSM1 homozygous mutation (c.1168G>T, p.E390*) developed Neutrophilic Panniculitis (NP), a condition characterized by subcutaneous nodular eruptions and neutrophilic infiltration in the subcutaneous fat, along with the other phenotypes of *MYSM1*-deficiency[281]. NP represents a completely novel phenotypic characteristic of *MYSM1*-deficiency and invites for further investigations into the previously uncharacterized role of MYSM1 in neutrophil development. MYSM1 was also suggested to promote the survival and polarization of macrophages *in vitro*[311] as mouse *Mysm1*-deficiency resulted in decreased numbers of macrophages[20,266,311], although this could be attributed to the reduced bone marrow cellularity.

Overall, mild effects of *Mysm1*-deficiency are observed in the myeloid lineage, except during the development of Flt3-dependent DCs, and possibly the development of neutrophils and macrophages. MYSM1 regulates the transcription of *Flt3* and mediates the recruitment of other transcriptional regulators in DCs. However, the myeloid lineage cells are less characterized than the lymphoid lineage in *MYSM1*-deficiency.

In summary, MYSM1 is essential for the normal function and maintenance of hematopoietic stem cells, the development of lymphoid lineage cells, and the development of some myeloid lineage cells. Previous studies have established that the activation of p53 and its stress-responses, such as the PUMA-mediated apoptosis, are the main mechanisms causing dysfunctions in *Mysm1*-deficient hematopoietic stem and progenitor cells (HSPC). However, the mechanistic link between *Mysm1*-deficiency and p53 activation remains undefined. In addition, although MYSM1 was shown to regulate the expressions of genes such as *Gfi1*, *Ebf1*, *Id2*, and *Flt3* in different hematopoietic cells, the full transcriptional control by MYSM1 and the transcriptome of *Mysm1*-deficient HSPCs are yet to be characterized. In this study, we will characterize the transcriptional control by MYSM1 in HSPCs and propose the mechanism linking *Mysm1*-deficiency to p53 activation.

Chapter 2 Rationale and Objectives

2.1 Rationale and Hypothesis

MYSM1 is a regulator of gene expression and a histone H2A deubiquitinase. MYSM1 is essential to the function of hematopoietic stem cells (HSC) and the process of hematopoiesis. *Mysm1*-deficiency in mice results in the loss of HSC quiescence, reduced numbers of multipotent progenitors (MPP) and cells in the myeloid lineage, and severe depletion of the cells in the lymphoid lineage, including common lymphoid progenitors (CLP), B and T cells. The p53 stress-response pathway is activated in these affected cells. The deletion of p53 in *Mysm1*-deficient mouse restores the numbers of MPPs, downstream CLPs, B and T cells, indicating p53 as the main stress-response pathway following *Mysm1*-deficiency. However, the mechanism linking *Mysm1*-deficiency to the activation of p53 remains unknown. Our lab is the first to generate RNA-Seq data on *Mysm1*-deficient and control mouse HSCs and MPPs, and ChIP-Seq data on MYSM1 and p53 binding sites in HPC7 and Ba/F3 cell lines. The analysis of these data in my current work provides insight into the transcriptomic changes in *Mysm1*-deficient HSCs and MPPs, the genome-wide binding patterns of MYSM1 and p53 in the two cell lines, and the genes directly regulated by MYSM1 from the consolidation of these two data sets. Here, I propose two hypotheses that MYSM1 directly interacts and antagonizes p53 in the regulation of gene expression, or MYSM1 regulates an intermediate factor which then signals the p53 activity.

2.2 Objectives

Aim 1 - Identify the transcriptomic changes in *Mysm1*-deficient mouse HSCs and MPPs (RNA-Seq).

Aim 2 - Characterize the genome-wide binding pattern of MYSM1 and identify potential co-localizing TFs near MYSM1 binding sites (ChIP-Seq).

Aim 3 - Identify genes directly regulated by MYSM1 and investigate the mechanism linking *Mysm1*-deficiency to p53 activation (RNA-Seq and ChIP-Seq).

Chapter 3 Materials and Methods

3.1 Mouse lines

The *Mysm1*$^{tm1a(KOMP)WTSI}$ mouse line, hereby referred to as *Mysm1*$^{-/-}$, was generated as previously described in the C57BL/6 genetic background and showed over 100-fold reduction in *Mysm1* levels[305,330]. The p53-knockout and PUMA/Bbc3-knockout lines were obtained from The Jackson Laboratory (JAX002101, JAX011067). The inducible knockout mouse line *Mysm1*$^{fl/fl}$ was generated by crossing the *Mysm1*$^{-/-}$ mice to a mouse line with ubiquitous expression of Flp recombinase Gt(ROSA)26Sor$^{tm1(FLP1)Dym/Wtsi}$ as described[307,331]. The line was then crossed to the Gt(ROSA)26Sor$^{tm1(cre/ERT2)}$ line, generating *Mysm1*$^{fl/fl}$CreERT2 mice for tamoxifen-induced *Mysm1* deletion[307]. For the induction of *Mysm1* deletion, 8 doses of intraperitoneal injections of tamoxifen (Sigma T5648) in sterilized corn oil at 0.15mg/gram per injection were administered over 16 days. Successful deletion of *Mysm1* exon 3 was validated by genotyping of the genomic DNA from hematopoietic and lymphoid organs, and the loss of *Mysm1* expression was confirmed by RT-qPCR with Lin^{-}cKit^{+}Sca1^{+} HSC/MPP cells from the mouse bone marrow[277,307]. *Mysm1*$^{fl/+}$CreERT2 mice undergoing identical tamoxifen treatment and *Mysm1*^{fl} mice with corn-oil only treatment served as controls. The mice were maintained under specific pathogen-free conditions. All experiments were conducted following the guidelines of the Canadian Council on Animal Care and the protocol AUP-2011-6029 approved by the McGill Animal Care Committee.

3.2 RNA-Sequencing

Bone marrow cells were flushed in PBS supplemented with 0.1% BSA and 2mM EDTA, filtered through 40µm cell-strainers, and subjected to red blood cell lysis in ACK buffer (0.15M NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA). The samples were stained with biotin anti-mouse Lineage Panel (BioLegend), PE-Cy7 cKit (clone 2B8, BioLegend), APC-Cy7 Sca1 (D7, BioLegend), FITC CD48 (HM48-1, eBioscience), APC CD150 (TC15-12F12.2, BioLegend), PE Flt3 (A2F10, eBioscience), Brilliant Violet 421 CD34 (RAM34, BD Biosciences), and streptavidin-PECy5 (BioLegend). DAPI was added immediately before sorting for dead cell exclusion. Cell sorting was performed as previously described[277] using FACSAria and analyzed with FACS Diva software (BD Biosciences).

The preparation of RNA-Seq samples was as previously described[277]. RNA was isolated using the MagMax total RNA kit (Ambion, Life Technologies), and quality assessed using Bioanalyzer RNA Pico chips (Agilent). rRNA depletion and library preparation were performed using the SMARTer Stranded RNA-Seq kit (Takara Clontech). The libraries were sequenced on an Illumina HiSeq 2500 sequencer in paired-end 50bp configuration. The quality of the sequenced reads was checked using the FastQC tool (Babraham Bioinformatics), and low-quality bases were trimmed using Trimmomatic v.0.33[332]. The trimmed reads were mapped to the mouse UCSC mm9 genome using TopHat v.2.0.9 in conjunction with Bowtie v.1.0.0 algorithms[333-335]. Gene expression was measured by counting the number of uniquely mapped reads using featureCounts[336]. Genes expressed at least 5 counts per million reads (CPM) in at least 3 samples were kept and quantile normalized using the preprocessCore package to remove batch effects[337]. TMM normalization and differential gene expression analyses were conducted using the edgeR Bioconductor package[338-340]. Dimension reduction analysis was performed using Partial Least Square regression method[341]. Pairwise comparisons were performed between samples from different genotypes. Genes with changes in expression ≥1.5 folds and Benjamini-Hochberg adjusted *p* values ≤0.01 were marked as significant. For data visualization in the Integrative Genomics Viewer (IGV)[342], replicates with the same genotype were merged and bigwig files were generated using the genomeCoverageBed and wigToBigWig tools scaled per 10 million reads mapped to exons. Gene Ontology (GO) analyses on differentially expressed gene clusters were performed with DAVID Bioinformatics Resources 6.8[343]. Gene Set Enrichment Analysis (GSEA) was performed in command-line using MSigDB v6.0 with default configuration and permutation within gene sets[344,345].

3.3 Cell culture

The mouse Pro-B cell line Ba/F3 was maintained in the exponential growth phase (0.5-2x10^6 cells/mL) in media composed of RPMI-1640 (Wisent), 10% Fetal Calf Serum (FCS, Wisent), 2mM L-Glutamine, 100µg/mL streptomycin, 100U/mL penicillin (Wisent), and 5% WEHI conditioned media for IL-3. The mouse multipotent hematopoietic progenitor cell line HPC7, derived from murine ES-cells via constitutive expression of LIM-

homeobox gene LH2, was provided by Prof. Leif Carlsson (Umea Center for Molecular Medicine, Sweden)[87,88,346].The HPC7 cells were maintained in the exponential growth phase (0.5-2x10^6 cells/mL) in media composed of IMDM (Life Technologies), 10% FCS (Gibco, Life Technologies), 100µg/mL streptomycin, 100U/mL penicillin (Wisent), 7.48x10^{-5}M MTG (M6145, Sigma-Aldrich), and 100ng/mL murine SCF (Shenandoah Biotechnology, Cedarlane).

The shRNA knockdown of *Mysm1* (*shMysm1*) and control Firefly Luciferase shRNA (*shFF*) Ba/F3 cell lines were previously described[277] and produced through retroviral transduction of the cells with pMSCV-Puro-IRES-GFP shRNA vectors (Addgene)[347]. Stable expression of triple-FLAG-tagged MYSM1 in Ba/F3 and HPC7 cell lines were generated using a similar protocol as described[277]. Both cell lines were maintained in 2µg/mL puromycin selection (Wisent).

3.4 Chromatin Immunoprecipitation (ChIP)

ChIP was performed as described[348], with minor modifications. Cells were maintained in the exponential growth phase (0.5-2x10^6 cells/mL) before fixing with 1% formaldehyde in the culture media for 10 minutes at room temperature. 0.125M glycine was added to stop the fixation. Nuclei were extracted with 5 minutes lysis in 0.25% Triton buffer, follower by 30 minutes in 20mM NaCl buffer. Nuclei were resuspended in sonication buffer and sonicated for twelve cycles of 30 seconds sonication using a digital sonifier (Branson Ultrasonics) at 80%, and 30 seconds rest in cooled circulating water.

Beads were prepared overnight with 40µL of Dynabeads Protein G (Invitrogen, Life Technologies) conjugated with 3-5µg of antibodies. The antibodies used were anti-Flag M2 (Sigma, F1804), anti-H3K27ac (Abcam, ab4729), and anti-H2AK119ub (Cell Signaling Technology, D27C4). Immunoprecipitation was performed by overnight incubation of antibody-bead matrices with sonicated chromatin from 5X10^6 cells. For MYSM1-FLAG ChIP, 6 washes were performed with low stringency buffers, while for p53 and histone ChIP, 4 washes of medium stringency buffers were performed. Samples were de-crosslinked by overnight incubation at 65°C in 1% SDS buffer and followed by RNase A and Proteinase K enzymatic treatments. ChIP DNA was purified with the Qiaquick PCR Cleanup kit (Qiagen).

Due to the lack of a ChIP-grade anti-MYSM1 antibody, the N-terminus of MYSM1 was tagged with triple-FLAG in the Ba/F3 and HPC7 cell lines. Importantly, our ChIP-Seq and ChIP-qPCR datasets demonstrate that the DNA-binding activity of MYSM1 is preserved after FLAG-tagging of the N-terminus, even though this site is near the MYSM1 SANT DNA-binding domain[263,271]. The specificity of the MYSM1 ChIP signal at *RP-gene* promoters is further supported by the following observations: 1) no ChIP signal was observed when an isotype control antibody was used instead of anti-FLAG antibody; 2) ChIP signal was specific to the MYSM1 FLAG-expressing cells and not observed in the corresponding wildtype cell lines; 3) ChIP signal at *RP-gene* promoters was specific to cells expressing MYSM1-FLAG and was not observed in Ba/F3 cells expressing another FLAG-tagged deubiquitinase BAP1 (data not shown). Nevertheless, due to lack of ChIP-grade MYSM1-specific antibodies, it is not possible to fully rule out that some differences may exist in the DNA binding activities of wildtype and triple-FLAG tagged MYSM1 proteins. The development of ChIP-grade anti-MYSM1 antibodies will facilitate further validation of our ChIP-Seq and ChIP-qPCR datasets in the future directions of this project.

ChIP enrichment was quantified using qPCR analysis, with primer sequences listed below. All C_T values were normalized to those of the pro-opiomelanocortin (*Pomc*) gene, which serves as a negative binding region. Enrichment was calculated relative to input DNA for MYSM1-FLAG ChIP, and to total histone H3 for histone marker ChIP.

Table 4. ChIP-qPCR primers

A lab member and I collectively designed the primer sequences.

Target Region	Forward Sequence	Reverse Sequence
Rps3 98 bp downstream (Chr7:106,631,961-106,632,121)	aatacacaatctacggccatcc	agatttccaagaagaggaaggtaag
Rps10 199 bp downstream (Chr17:27,771,920-27,771,988)	gtggccttcaaactcctctc	actcagagtcgactgaagaaga
Rps24 0 bp upstream TSS (5'UTR) (Chr14:25,309,903-25,310,020)	cttgcgcgttgatatgattgg	gataagcgacggatagtgctg
Rpl7 141 bp downstream (Chr1:16,094,250-16,094,373)	ctcagtttgctcctggtactg	tgtatctgagtgtagcctgga

Rpl9 8 bp upstream (Chr5:65,782,562-65,782,678)	caaacagaggatgggttcagatt	gccctgacggattacaagaac
Rpl11 70 bp upstream (Chr4:135,609,214-135,609,356)	cggatggagacggatgaaag	ctcgtttgtctgcctagaagaa
Rpl13 18 bp upstream (Chr8:125,626,232-125,626,358)	cacttccctttcgcctgattt	ggcagagactcacctcctatac
Eef1g 197 bp downstream (Chr19:9,041,728-9,041,874)	gctccggtgattagggtcac	ctccaggccctagaaaccat
POMC 744 bp downstream (Chr12:3,953,603-3,955,695)	aggcagatggacgcacataggtaa	tccacttagaactggacagaggct

3.5 ChIP-Sequencing (ChIP-Seq)

ChIP-Seq libraries were prepared using the Illumina TruSeq kit and sequenced on an Illumina HiSeq 2500 sequencer. The input DNA from the same cells were sequenced as negative control. The sequencing reads were mapped to the UCSC mouse mm9 genome with Bowtie 1.0.0[334]. Chromatin binding sites were identified using peak detection algorithm MACS1.4.1[349], by comparing read enrichment against control input DNA from the same cells. Normalized sequence read density profiles (bigwig) were generated with Homer tool[350] and visualized on IGV[342]. Gene ontology (GO) and disease ontology enrichment analyses on genes associated with MYSM1 ChIP-Seq binding clusters were performed on GREAT 3.0.0 with basal plus extension option, searching for genes within 2kb upstream, 2kb downstream, and 200kb in distal to the binding sites[351].

3.6 RNA-Seq/ChIP-Seq consolidation

Full gene annotations with transcription start site (TSS) locations were obtained from the UCSC mouse mm9 genome. An in-house Python script was developed to load the locations of the MYSM1 binding sites and the TSS locations of dysregulated genes, and search for gene TSS located within a specific distance to each MYSM1 binding site.

Chapter 4 Results

To gain insight into the transcriptional regulation by MYSM1, our lab performed RNA sequencing (RNA-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq) before the start of my project. The RNA-Seq experiment characterized the transcriptomic profiles of *Mysm1*-deficient and wildtype control mouse hematopoietic stem cells (HSC) and multipotent progenitors (MPP). The ChIP-Seq experiment examined transcription factors MYSM1 and p53, and histone markers H3K27ac and H2AK119ub in both HPC7 and Ba/F3 cell lines. HPC7 is a cell line for early hematopoiesis and is used extensively in studies investigating the transcriptional network regulating hematopoiesis[87,88]. Ba/F3 is a cell line for pro-B cells and represents one of the severely depleted cell populations in both human and mouse *Mysm1*-deficiency[264,277,307,309]. Both the RNA-Seq and ChIP-Seq data sets represent the first characterization of MYSM1 using genome-wide sequencing and provide valuable characterizations of the transcriptional regulation by MYSM1 in hematopoiesis.

4.1 Profound gene dysregulations in *Mysm1*-deficient HSCs and MPPs.

Our lab and other groups had established that MYSM1 is essential for the functions of hematopoietic stem and progenitor cells (HSPC)[20,277,305-309]. To investigate the mechanisms driving hematopoietic failure in *Mysm1*-deficiency, RNA-Seq was performed on *Mysm1*-deficient and control mouse HSC, MPP1, and MPP2 cells[12,21]. Bone marrow cells were collected from *Mysm1*$^{fl/fl}$CreERT2 mice following tamoxifen-induced *Mysm1*-deletion and compared against cells from tamoxifen-treated *Mysm1*$^{fl/+}$CreERT2 and corn-oil vehicle-treated *Mysm1*^{fl} mice (Figure 2A). The use of an inducible model allowed us to focus on the direct effects of *Mysm1*-deletion in HSPCs without the complex developmental phenotypes reported in constitutive *Mysm1*-deficiency[307].

To identify the transcriptomic changes in *Mysm1*-deficiency, the RNA-Seq data set was analyzed with dimension reduction and differential gene expression analyses. Dimension reduction analysis showed clear segregation of cell types (Principal Component 1, 42.6% variability) and genotypes (PC2, 12% variability), highlighting a major transcriptomic variation in *Mysm1*-deficiency (Figure 2B). Importantly, samples from the tamoxifen-treated *Mysm1*$^{fl/+}$CreERT2 and corn-oil vehicle-treated *Mysm1*^{fl} mice clustered together, indicating that the tamoxifen treatment and the CreERT2-transgene

did not impact the transcriptome. These control samples were therefore grouped as the wildtype (*Mysm1*^{WT}) for subsequent analyses. Differential gene expression analysis compared *Mysm1*$^{\Delta/\Delta}$ with *Mysm1*^{WT} samples within each cell type and identified a total of 702 genes significantly dysregulated in HSC, MPP1, and MPP2 cells with fold change ≥ 1.5 and false discovery rate ≤ 0.01. More specifically, *Mysm1*$^{\Delta/\Delta}$ HSCs had the most profound transcriptomic change of all three cell types with 357 upregulated and 239 downregulated genes. *Mysm1*$^{\Delta/\Delta}$ MPPs had less degree of dysregulation with 44 upregulated and 67 downregulated genes in MPP1, and 88 upregulated and 19 downregulated genes in MPP2. Also, the average fold change of the dysregulated genes in the HSCs was higher than that in the MPPs (Figure 2C), suggesting that MYSM1 plays a more prominent role in the transcriptional regulation of HSCs than MPPs.

4.2 Transcriptional alterations in *Mysm1*-deficient HSCs and MPPs

To examine the biological functions associated with the gene dysregulation in *Mysm1*-deficiency, gene set enrichment analyses (GSEA) were performed searching for over-representations of *a priori* defined gene sets[344,345]. GSEA of *Mysm1*$^{\Delta/\Delta}$ HSCs showed upregulation of genes involved in the cell cycle, DNA replication, and DNA packaging processes (Figure 2D), which are in agreement with the previously reported loss of HSC quiescence in *Mysm1*-deficiency[20,277]. On the other hand, GSEA of *Mysm1*$^{\Delta/\Delta}$ MPP2s showed upregulation of genes involved in the p53 pathway and apoptosis, reflecting the previously described p53 activation in *Mysm1*-deficiency[277,308,309]. Thus, the transcriptomic signatures differ in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs, corresponding to their previously described phenotypes. Importantly, the GSEA of *Mysm1*$^{\Delta/\Delta}$ HSCs revealed a previously unknown downregulation of genes encoding for translational and ribosomal proteins (Figure 2D).

To investigate the gene dysregulation in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs in detail, hierarchical clustering of the 702 dysregulated genes was performed and generated four clusters based on the pattern of expression (Figure 2E). Gene ontology (GO) analyses of each gene cluster were performed to identify enriched biological processes (Figure 2F). Gene clusters III and IV consisted of 45 genes upregulated in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs and 52 genes downregulated in *Mysm1*$^{\Delta/\Delta}$ MPP2s, respectively. Due to the small cluster

sizes, the detection of significant enrichment terms was limited. Nevertheless, cluster III included genes participating in the regulation of apoptosis and cell cycle arrest, such as *Cdkn1a*, *Ccnd1*, *P2rx7*, *Blc2l1*, and *Zmat3*, and cluster IV was enriched in genes functioning in antigen processing and presentation, and the immune system (Figure 2F). Gene cluster II consisted of 348 genes upregulated in *Mysm1*$^{\Delta/\Delta}$ HSCs, MPP1s, and, to a lesser extent in MPP2s. The early expressions of these genes involved in cell cycle-related GO categories in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPP1s (Figure 2F), is consistent with previous reports demonstrating that *Mysm1*-deficient mouse HSCs lose their quiescence and upregulate cell cycle genes[20,305]. Most importantly, cluster I comprised 257 genes downregulated in *Mysm1*$^{\Delta/\Delta}$ cells, with the most significant downregulation in the *Mysm1*$^{\Delta/\Delta}$ HSCs (Figure 2E). As MYSM1 was previously described to show transcriptional activation activities[20,263-266], the downregulation of gene expression was expected to be the direct result of *Mysm1*-deficiency. Cluster I included genes participating in translational and ribosomal assembly processes, and strikingly, contained 35 ribosomal protein-encoding genes (*RP-genes*) and 2 translation factor encoding genes, *Eif4a2* and *Eef1g* (Figure 2F). Overall, *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs exhibited upregulation of cell cycle and cellular apoptosis genes, and downregulation of genes involved in the translational machinery.

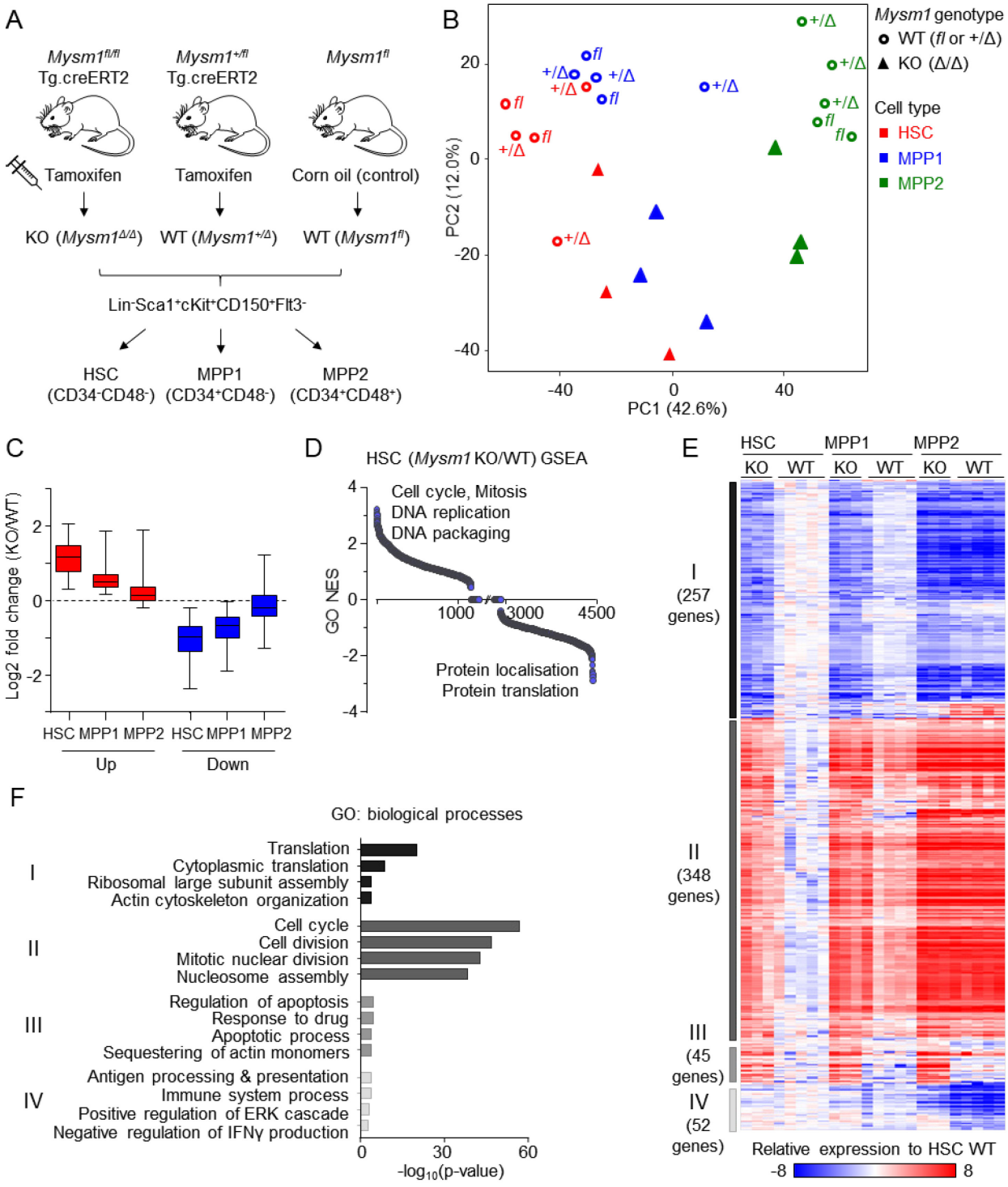


Figure 2. RNA-Seq analysis of the transcriptome of *Mysm1*-deficient HSC and MPPs.

(A) Schematic representation of the inducible *Mysm1*-deletion mouse model and the gating strategies for HSC, MPP1, and MPP2 cells. The KO group is composed of *Mysm1*$^{fl/fl}$CreERT2 mice following tamoxifen-induced *Mysm1*-deletion. The WT group is

composed of tamoxifen-treated *Mysm1*$^{fl/+}$CreERT2 and corn-oil vehicle-treated *Mysm1*^{fl} mice. **(B)** Partial Least Square Regression graph demonstrates the gene expression profiles of each RNA-Seq sample: differences between cell types (Principal Component 1, 42.6% variability) and genotypes (PC2, 12% variability). **(C)** Fold changes of 702 significantly dysregulated genes in *Mysm1*-deficient HSCs and MPPs show the most significant transcriptional changes in HSCs. **(D)** Normalized enrichment scores (NES) of 4,437 pre-established biological processes expression signatures used in the gene set enrichment analysis. **(E)** Heat map displaying 702 significantly dysregulated genes when comparing KO to WT expression levels. The significance threshold is fold change ≥ 1.5 and False Discovery Rate (FDR) ≤0.01. Relative expressions to the average of HSC WT group are used to generate the heat map. Hierarchical Clustering of the genes is performed, using Pearson correlation and average linkage, to generate the four gene clusters. **(F)** Gene ontology (GO) enrichment analysis on genes from the four clusters described in 2D. The top four enriched biological process terms are displayed.

4.3 MYSM1 maintains *RP-gene* expression in HSPCs

As mutations in many *RP-genes* lead to hematopoietic defects[352], we were prompted to examine further the transcriptional changes of *RP-genes*. Using gene set enrichment analyses (GSEA), we searched for the expressions of 80 *RP-genes* and observed significant downregulation of *RP-genes* in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs (Figure 3A-B). In particular, heterozygous coding mutations in 19 *RP-genes,* including *Rpl5*, *Rpl11*, and *Rps19*, have been reported to cause a rare type of Inherited Bone Marrow Failure Syndrome (IBMFS), known as Diamond-Blackfan Anemia (DBA)[288,290-294]. GSEA of these 19 DBA-causing *RP-genes* again showed significant downregulation in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs (Figure 3C-D), suggesting the significance of ribosomopathies in *Mysm1*$^{\Delta/\Delta}$ hematopoietic defects. Together, the expressions of *RP-genes* were downregulated in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs.

Interestingly, there was a downward trend in the expressions of *RP-genes* in the *Mysm1*^{WT} HSCs relative to *Mysm1*^{WT} MPP1 and MPP2 cells. To reduce the effect of confounders, such as the sequencing library sizes, values of quantile normalized count

per million (CPM) were used to generate the *RP-gene* expression boxplots and compare *Mysm1*^{WT} HSCs and MPPs (Figure 3B and 3D), with the results further supporting the elevated expression of *RP-genes* in HSCs. This observation is surprising given the quiescent state and low metabolic activity of HSCs[353], but consistent with some previous reports[354]. Previous studies also indicated that the protein synthesis rate is HSCs needs to be stringently regulated to maintain normal cell function[355,356], and the relatively high expression for *RP-genes* in HSCs may be one of the adaptations to allow for this regulation.

As p53 is the master regulator of cell fate, controlling HSC maintenance[172] and mediating HSC dysfunctions in *Mysm1*-deficiency[277,308,309], we examined the expression of p53 target genes in the RNA-Seq. GSEA of p53 target genes confirmed their progressive upregulation from the *Mysm1*$^{\Delta/\Delta}$ HSCs to MPP2s (Figure 3E). It is noteworthy that the expression of *Mysm1* was higher in the *Mysm1*^{WT} HSCs than in the MPPs (Figure 3F) and coincidentally, the *RP-genes* were more profoundly downregulated in the *Mysm1*$^{\Delta/\Delta}$ HSCs, suggesting a direct functional link between MYSM1 and *RP-gene* expressions. The progressive upregulation of p53 target genes from *Mysm1*$^{\Delta/\Delta}$ HSCs to MPP2s, on the contrary, suggests that they are induced in an indirect manner responding to the increased cellular stress.

In summary, we identified significant transcriptomic changes in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs and particularly in genes leading to the loss of HSC quiescence[20,306,308] and activation of p53 stress-response[277,308,309], in agreement with previously reported phenotypes of *Mysm1*-deficiency. We also present the first report on the broad downregulation of *RP-genes* and genes encoding for translational machinery in *Mysm1*$^{\Delta/\Delta}$ HSCs.

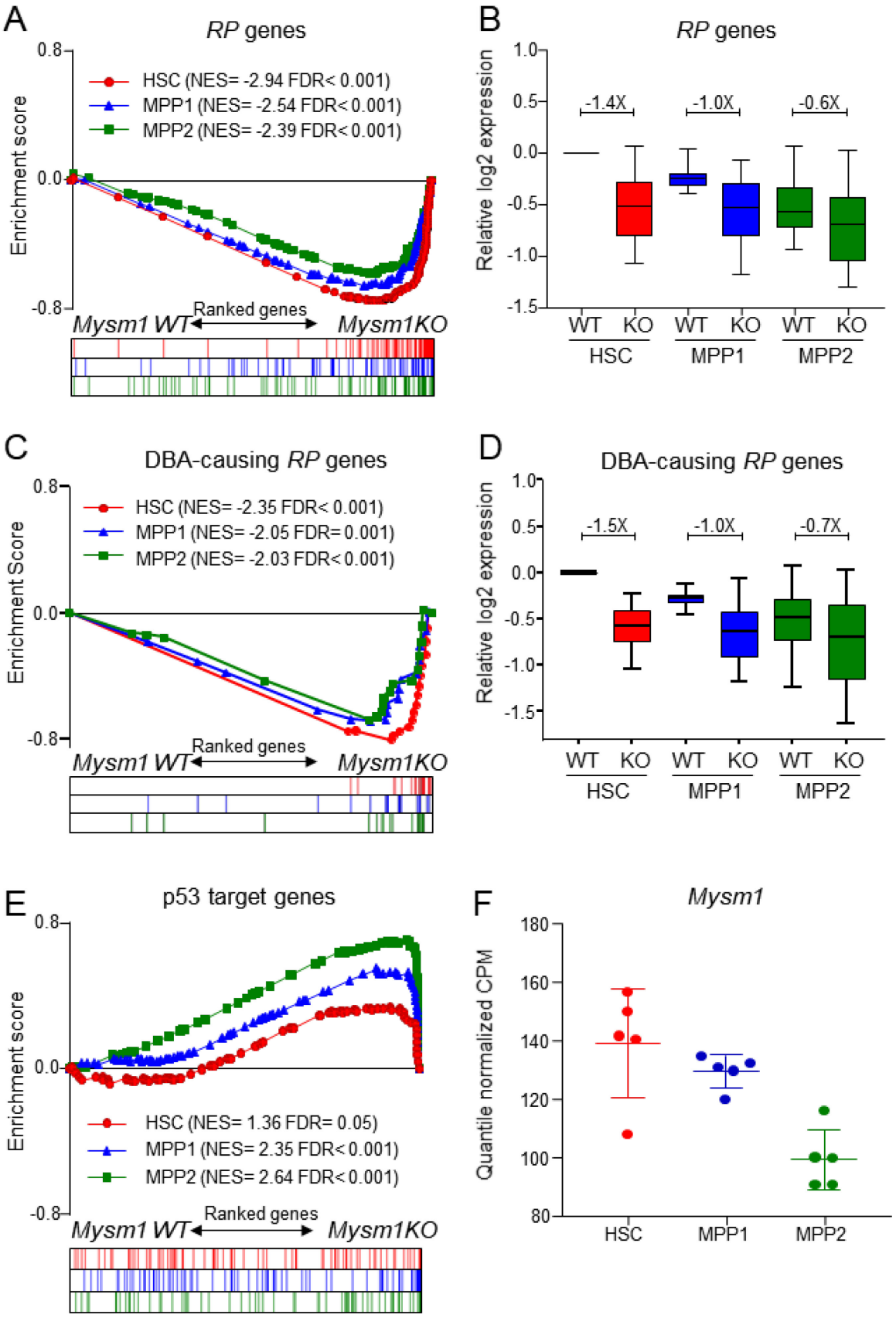

A
RP genes
Enrichment score
HSC (NES= -2.94 FDR< 0.001)
MPP1 (NES= -2.54 FDR< 0.001)
MPP2 (NES= -2.39 FDR< 0.001)
Mysm1 WT
Ranked genes
Mysm1KO
B
RP genes
Relative log2 expression
-1.4X
-1.0X
-0.6X
WT KO WT KO WT KO
HSC MPP1 MPP2
C
DBA-causing RP genes
Enrichment Score
HSC (NES= -2.35 FDR< 0.001)
MPP1 (NES= -2.05 FDR= 0.001)
MPP2 (NES= -2.03 FDR< 0.001)
Mysm1 WT
Ranked genes
Mysm1KO
D
DBA-causing RP genes
Relative log2 expression
-1.5X
-1.0X
-0.7X
WT KO WT KO WT KO
HSC MPP1 MPP2
E
p53 target genes
Enrichment score
HSC (NES= 1.36 FDR= 0.05)
MPP1 (NES= 2.35 FDR< 0.001)
MPP2 (NES= 2.64 FDR< 0.001)
Mysm1 WT
Ranked genes
Mysm1KO
F
Mysm1
Quantile normalized CPM
HSC MPP1 MPP2

Figure 3. Expressions of *RP-genes* and p53-target genes in *Mysm1*-deficient HSCs and MPPs.

(A) Gene Set Enrichment Analysis (GSEA) ranked and demonstrated reduced expression of 80 ribosomal protein (*RP*) genes in *Mysm1*-deficient cells among 10,769 genes detected in HSCs and MPPs in the RNA-Seq dataset. Each relevant gene is represented by a dot and a vertical bar below. **(B)** Boxplots showing reduced expression of *RP-genes* in *Mysm1*-deficient HSCs and MPPs. **(C)** Gene Set Enrichment Analysis (GSEA) showing reduced expression of DBA-linked *RP-genes* in *Mysm1*-deficient HSC and MPP cells relative to wildtype. GSEA ranked and demonstrated reduced expression of 19 DBA-causing *RP-genes* in *Mysm1*-deficient cells among 10,769 genes detected in HSCs and MPPs in the RNA-Seq dataset. Each relevant gene is represented by a dot and a vertical bar below. **(D)** Boxplots showing reduced expression of DBA-causing *RP-genes* in *Mysm1*-deficient HSCs and MPPs relative to wildtype. **(E)** GSEA demonstrating the upregulation of p53-target genes in *Mysm1*-deficient HSC, MPP1 and MPP2 cells relative to the corresponding control cells, with increasing upregulation along with the HSC to MPP1 to MPP2 differentiation axis. **(F)** The expression of *Mysm1* is reduced along with the development of wildtype HSC to MPP2 cells.

4.4 MYSM1 binds to *RP-gene* promoters

To identify the genomic regions directly regulated by MYSM1, we mapped MYSM1 DNA binding sites by ChIP-Seq. Due to the very limited numbers of primary mouse HSCs, the experiments were performed in the hematopoietic progenitor cell lines HPC7 and Ba/F3. HPC7 is a cell line for early hematopoiesis that was used extensively in studies investigating the transcriptional network regulating hematopoiesis[87,88]. Ba/F3 is a cell line for pro-B cells and represents one of the severely depleted cell populations in both human and mouse *Mysm1*-deficiency[264,277,307,309]. Analysis of the MYSM1 ChIP-Seq samples identified a total of 2099 MYSM1 DNA binding sites from the two cell lines, with most of the sites shared between the two cell lines. To further characterize the genomic binding pattern of MYSM1, the 2099 MYSM1 binding sites were ordered based on their distance to the nearest gene transcription start site (TSS). A bimodal distribution pattern of MYSM1

binding sites was observed, with a distinct group of sites gene-proximal (≤ 1kb to TSS, 102 sites) and the other sites gene-distal (> 1kb to TSS, 1997 sites) (Figure 4A).

Characterization of the chromatin status was performed in parallel to the MYSM1 ChIP-Seq, enabling interpretation of the epigenetic context at MYSM1 binding sites in HPC7 and Ba/F3 cells. H3K27ac, a marker of active promoters and enhancers, and H2AK119ub, a repressive marker and a known target of MYSM1[209,268], were analyzed by ChIP-Seq. This revealed the absence of H3K27ac and high level of H2AK119ub enrichment at the gene-distal MYSM1 binding sites, suggesting that these are mostly transcriptionally inactive sites (Figure 4A-C). In contrast, high levels of H3K27ac and low H2AK119ub enrichment were observed at the gene-proximal MYSM1 binding sites, indicating regions of active transcription (Figure 4A-C).

We have further performed gene ontology and disease ontology enrichment analyses for the genes bound in proximity to MYSM1 binding sites using the GREAT software[351]. The 102 MYSM1 gene-proximal sites were highly enriched for the related ontology terms “translation” and “ribosome biogenesis” (Figure 4D), and the corresponding disease ontology terms “congenital hypoplastic anemia” and “Diamond-Blackfan anemia” (Figure 4E). The 1977 MYSM1 gene-distal sites were not significantly enriched in any ontology terms, highlighting the challenge to infer a role for intergenic MYSM1 binding. Overall, these data provide further evidence for the regulation of genes encoding ribosomal proteins and other translational machinery by MYSM1.

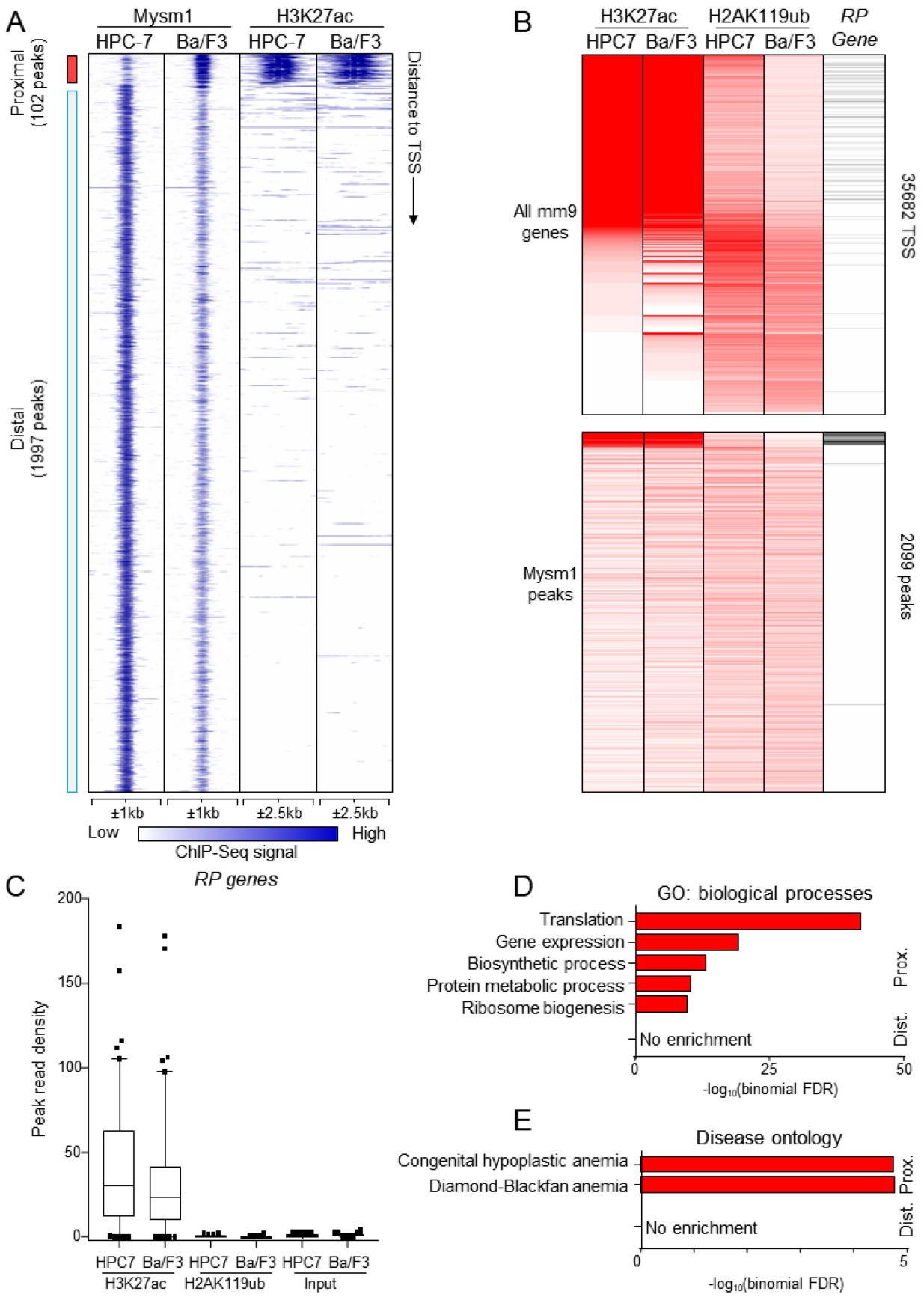

A
Mysm1
H3K27ac
HPC-7
Ba/F3
HPC-7
Ba/F3
Proximal (102 peaks)
Distal (1997 peaks)
Distance to TSS
±1kb
±1kb
±2.5kb
±2.5kb
Low
High
ChIP-Seq signal
B
H3K27ac
H2AK119ub
RP Gene
HPC7
Ba/F3
HPC7
Ba/F3
All mm9 genes
35682 TSS
Mysm1 peaks
2099 peaks
C
RP genes
Peak read density
200
150
100
50
0
HPC7
Ba/F3
HPC7
Ba/F3
HPC7
Ba/F3
H3K27ac
H2AK119ub
Input
D
GO: biological processes
Translation
Gene expression
Biosynthetic process
Protein metabolic process
Ribosome biogenesis
No enrichment
Prox.
Dist.
0
25
50
-log10(binomial FDR)
E
Disease ontology
Congenital hypoplastic anemia
Diamond-Blackfan anemia
No enrichment
Prox.
Dist.
0
5
-log10(binomial FDR)

Figure 4. ChIP-Seq reveals MYSM1 binding at *RP-gene* promoters.

(A) Heat map showing the intensities of MYSM1 and H3K27ac around 2,099 MYSM1 binding sites identified in ChIP-Seq data from HPC7 and Ba/F3 cells. The sites are ranked based on their distances to the nearest gene transcriptional start site (TSS). The gene-proximal sites are defined as having the nearest gene TSS within 1kb. The gene-distal sites are defined as having the nearest gene TSS further than 1kb. **(B)** Heat map showing the intensities of H3K27ac and H2AK119ub around all 35,682 mm9 gene transcriptional start sites (TSS) and 2,099 MYSM1 binding sites. The sites around mm9 gene TSS are ranked based on the level of H3K27ac enrichment in HPC7 cells. The sites around MYSM1 binding sites are ranked based on their distance to the nearest gene TSS. **(C)** Box plot displays the read densities of H3K27ac and H2AK119ub peaks around each *RP-gene* in HPC7 and Ba/F3 cells. **(D-E)** Gene Ontology and Disease Ontology analyses on the nearest genes to each MYSM1 binding site, performed using the GREAT software. The -log10(binomial FDR) value for each term is plotted.

4.5 MYSM1 regulates *RP-gene* expression

To identify the genes directly regulated by MYSM1, the RNA-Seq and ChIP-Seq data were consolidated, searching for genes that had both nearby MYSM1 binding sites and dysregulation in *Mysm1*-deficient cells. The cluster II, III, and IV dysregulated genes, which were enriched in cell cycle-related, apoptotic, and immune processes, respectively (Figure 2E-F), had no nearby MYSM1 binding site (Figure 5A), suggesting that their dysregulations in *Mysm1*-deficiency occur through an indirect mechanism. Importantly, many of the cluster I downregulated genes, which were enriched in translational and *RP* genes, had MYSM1 binding sites within 1kb to their TSS, indicating their expression is likely directly regulated by MYSM1 (Figure 5A). More specifically, 31 *RP-genes*, including 9 DBA-causing *RP-genes*, and the gene encoding for elongation factor 1-gamma (*Eef1g*) were among the cluster I genes that were likely directly regulated by MYSM1. These genes accounted for 35% of gene-proximal MYSM1 binding sites and their promoters were marked with high levels of H3K27ac and low H2AK119ub enrichment (Figure 5A, 4B-C), indicating their active transcription. Thus, MYSM1 is suggested to directly regulate the expressions of many *RP-genes* in HSPCs.

We provide genomic visualizations of some downregulated genes, including the *Eef1g* gene, three DBA-causing *RP-genes* including *Rps10*, *Rps24*, and *Rpl11*, and five other *RP-genes* including *Rps3*, *Rps14*, *Rpl7*, *Rpl9*, and *Rpl13* using IGV[342]. These genes showed evident MYSM1 binding at their promoters and were downregulated in *Mysm1*$^{\Delta/\Delta}$ HSCs and MPPs (Figure 5B). The binding of MYSM1 to the promoters of these genes was independently validated by ChIP-qPCR in the FLAG-MYSM1 Ba/F3 lines (Figure 5C). Moreover, we have confirmed a reduction in gene expressions for those MYSM1-target genes by using RT-qPCR in shRNA *Mysm1*-knockdown (*shMysm1*) Ba/F3 cells in comparison with control cells expressing an off-target shRNA against firefly luciferase (*shFF*) (Figure 5D, data obtained from the lab). The recruitment of MYSM1 to the promoter of *RP-genes* together with their downregulation both *in vitro* and *in vivo* due to MYSM1 loss, supports the direct role of MYSM1 in their transcriptional regulation.

To provide further information on MYSM1 regulation of *RP-gene* expressions, histone modifications H3K27ac and H2AK119ub at the MYSM1-regulated *RP-gene* promoters were analyzed by ChIP-qPCR in the knockdown *shMysm1* and control *shFF* Ba/F3 cells (Figure 5E, data obtained from the lab). We focused on the previously selected DBA-causing *RP-genes* and *Eef1g* and confirmed that their gene promoters had a very low level of H2AK119ub enrichment in both *shMysm1* and *shFF* cells (data not shown) and reduced level of H3K27ac enrichment in the *shMysm1* cells (Figure 5E). The constitutively low level of H2AK119ub suggests that MYSM1 regulates these genes through mechanisms other than deubiquitination, consistent with the current view that H2AK119ub only marks long-term gene silencing and is not induced in housekeeping genes such as the *RP-genes*[272-274]. In contrast, the reduced level of H3K27ac in the *shMysm1* cells confirms the reduction in transcriptional activation of *RP-genes* following MYSM1 loss. These results suggest that the regulation of *RP-gene* expressions by MYSM1 is through the induction or maintenance of active transcription histone marker rather than through the deubiquitination of H2AK119ub at *RP-gene* promoter.

In summary, we are the first to characterize the genome-wide binding sites of MYSM1 in two murine hematopoietic progenitor cell lines. Importantly, we identified a group of *RP-genes* and *Eef1g* directly regulated by MYSM1.

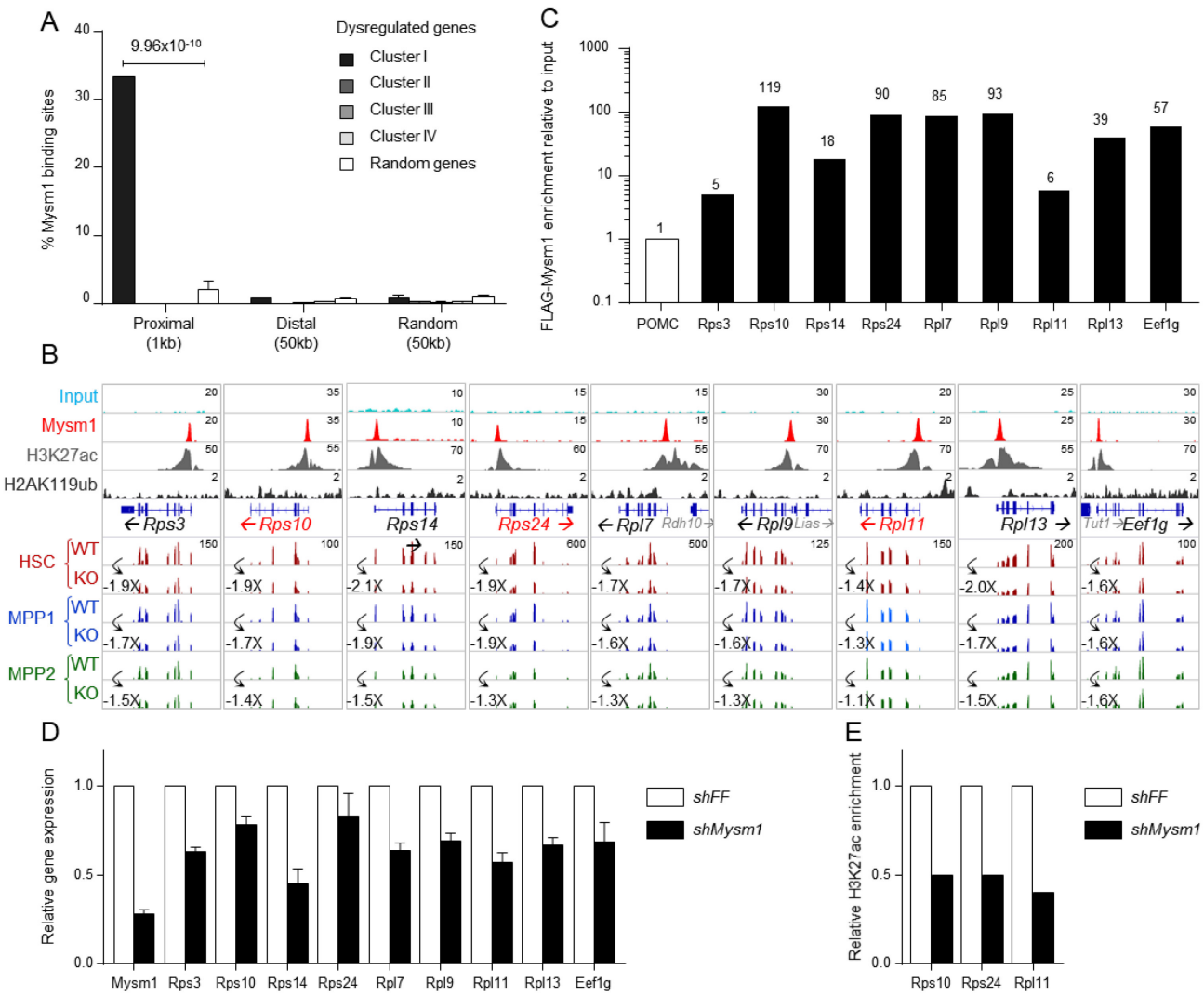


Figure 5. Validation of the direct role of MYSM1 in the regulation of *RP-gene* expression.

(A) Consolidation of ChIP-Seq and RNA-Seq datasets showing that gene-proximal MYSM1-binding sites preferentially locate near Cluster I genes dysregulated in *Mysm1*-deficient HSC/MPP cells. The percentages of MYSM1 binding sites that have at least one significantly dysregulated gene TSS within the indicated search window are plotted. The random genes cluster consists of ten groups of 300 genes randomly selected from 10,169 expressed genes. The random binding sites consist of ten groups of 2000 genomic locations randomly selected from the mm9 genome. A binding site with more than one gene TSS from the same gene cluster is counted only once. Fisher Exact Test is used to calculate the *p*-value. **(B)** Genomic snapshots of select dysregulated genes. ChIP-Seq

tracks of input DNA, MYSM1, H3K27ac, and H2AK119ub are shown on the top four lanes. The gene feature track is shown in the middle. Averaged RNA-Seq tracks are in the bottom six lanes, with fold changes comparing expression levels in *Mysm1*^{WT} and *Mysm1*$^{\Delta/\Delta}$ samples indicated for each cell type. The maximum data range of each track is indicated at the top-right corner of the track. **(C)** Enrichment of MYSM1 at *RP-gene* promoter sites is validated with ChIP-qPCRs in MYSM1-FLAG Ba/F3 hematopoietic progenitor cells. **(D)** Downregulation of *RP-gene* expression in knockdown *shMysm1* Ba/F3 cells relative to control *shFF* Ba/F3 cells, validated with RT-qPCRs. **(E)** Representative H3K27ac ChIP-qPCRs showing reduced relative enrichments in knockdown *shMysm1* Ba/F3 cells relative to control *shFF* Ba/F3 cells. Data in **(C-E)** is representative of two or more independent experiments. Data in **(D-E)** are obtained from a colleague in the lab.

4.6 MYSM1-regulated *RP-gene* expression is independent of p53

Previous reports have established that the hematopoietic failure in *Mysm1*-deficiency is mediated by the activation of p53 and the induction of its downstream apoptotic mediator PUMA[277,308,309]. The deletion of p53 in the *Mysm1*-deficient mice fully rescues the HSC functions and lymphocyte development[309] and the deletion of PUMA partially rescues the progenitor numbers[277]. Here, we investigated the relationship between the p53-induced PUMA-pathway and the *RP-gene* downregulation in *Mysm1*-deficiency.

To study whether p53-induced PUMA-pathway contributes to the *RP-gene* downregulation in *Mysm1*-deficiency, RNA-Seq on the combined HSC/MPP1/MPP2 populations (gated by Lin$^-$Sca1$^+$cKit$^+$, LSK, and CD150$^+$) from *Mysm1*$^{-/-}$*Puma*$^{-/-}$ and control mouse bone marrow was performed. This data set was analyzed collectively with our previously published RNA-Seq data set on the MPP3/MPP4 populations (gated by LSKCD150$^-$) from the same mice[277] and used the same analysis pipeline already described in this study. Dimension reduction analysis showed clear segregation of the cell types (PC1, 41.7% variability) and between *Mysm1*-deficient and *Mysm1*-wildtype samples (PC2, 12.4% variability) (Figure 6A). The *Mysm1*$^{+/+}$*Puma*$^{-/-}$ samples grouped

with the wildtype control, suggesting that the major transcriptomic variation was contributed by *Mysm1*-deletion. Hierarchical clustering of the 770 significantly dysregulated genes (FC ≥1.5 and FDR≤0.01) in mutant cells relative to wildtype control cells arranged the genes into 8 clusters (Figure 6B). The activation of p53 was a major signature in *Mysm1*-deficient cells and persisted in *Mysm1*$^{-/-}$*Puma*$^{-/-}$ samples, consistent with our previous report[277], with cluster 1 including the hallmark p53 target genes *Cdkn1a* (p21), *Pmaip1* (NOXA), and *Bax* (Figure 6B). Strikingly, the *RP-genes*, *Eef1g*, and genes encoding for other translational machinery proteins made up more than half of cluster 4 and were still downregulated in *Mysm1*$^{-/-}$*Puma*$^{-/-}$ samples of both CD150^{+} and CD150^{-} LSK bone marrow cells. GSEA performed on all 80 *RP-genes* further confirmed the broad downregulation of *RP-genes* in *Mysm1*-deficient HSPCs regardless of *Puma*-deletion (Figure 6C-D). Thus, *RP-gene* downregulation is independent of PUMA and not a result of the induction of apoptosis.

In addition to the MYSM1 ChIP-Seq, we performed p53 ChIP-Seq in the HPC7 and Ba/F3 cell lines and identified a total of 5132 binding sites. Comparing the binding sites between the MYSM1 and p53, only 3 sites were shared. These 3 sites were in an intronic region of the Histocompatibility 13 (*H13*) gene, a high genomic background region suggesting of residual false positive peaks, and an intergenic region having no gene feature within 500kb. Overall, the lack of MYSM1 and p53 co-binding at genomic sites suggest that the direct MYSM1-dependent regulation of *RP-genes* is independent of the recruitment of p53.

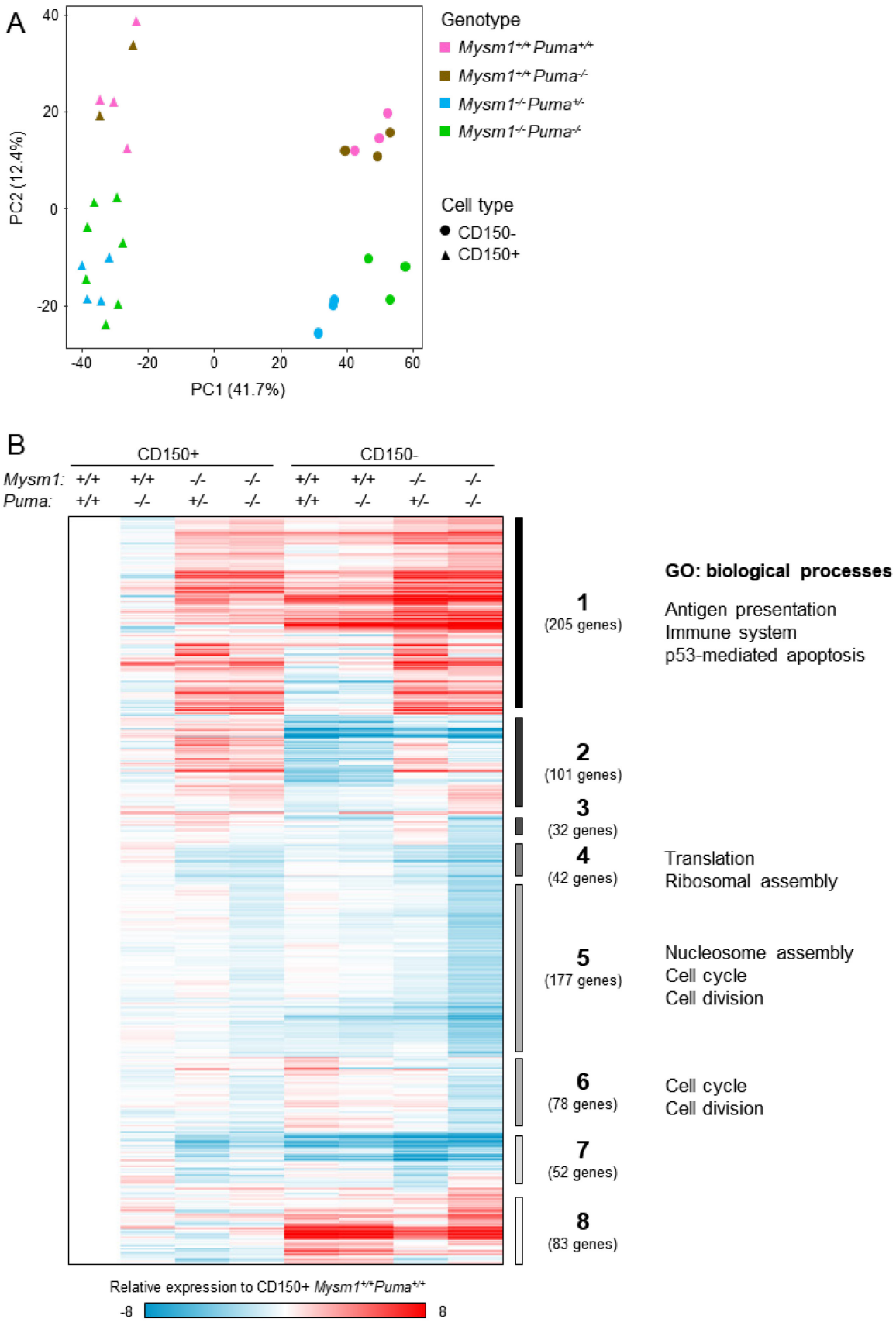

A
PC2 (12.4%)
PC1 (41.7%)
Genotype
Mysm1+/+ Puma+/+
Mysm1+/+ Puma-/-
Mysm1-/- Puma+/-
Mysm1-/- Puma-/-
Cell type
CD150-
CD150+
B
CD150+
CD150-
Mysm1:
Puma:
1 (205 genes)
2 (101 genes)
3 (32 genes)
4 (42 genes)
5 (177 genes)
6 (78 genes)
7 (52 genes)
8 (83 genes)
GO: biological processes
Antigen presentation
Immune system
p53-mediated apoptosis
Translation
Ribosomal assembly
Nucleosome assembly
Cell cycle
Cell division
Cell cycle
Cell division
Relative expression to CD150+ Mysm1+/+Puma+/+
-8
8

Figure 6. RNA-Seq analysis of the transcriptome of *Mysm1*- and *Puma*-deficient HSPCs.

(A) Partial Least Squares Regression graph of the transcriptome profiles of $CD150^{+}$ and $CD150^{-}$ LSK hematopoietic stem and progenitor cells, isolated from *Mysm1*-deficient *Mysm1*$^{-/-}$*Puma*$^{+/-}$ and *Mysm1*$^{-/-}$*Puma*$^{-/-}$, and control wildtype and *Puma*$^{-/-}$ mice. The samples are segregated by cell type on the principal component 1 (PC1) and by *Mysm1* genotype on PC2. **(B)** Heat map displaying 770 genes significantly dysregulated in *Mysm1*-deficient relative to wildtype control cells for each cell-type. The threshold for significance is fold change ≥1.5 and False Discovery Rate (FDR) ≤0.01. Relative expression to the average of wildtype CD150+ LSK group is used to generate the heat map. Hierarchical clustering using Pearson correlation and complete linkage is performed to generate the gene clusters (Clusters 1-8). Significantly enriched gene ontology (GO) terms for select gene clusters are shown.

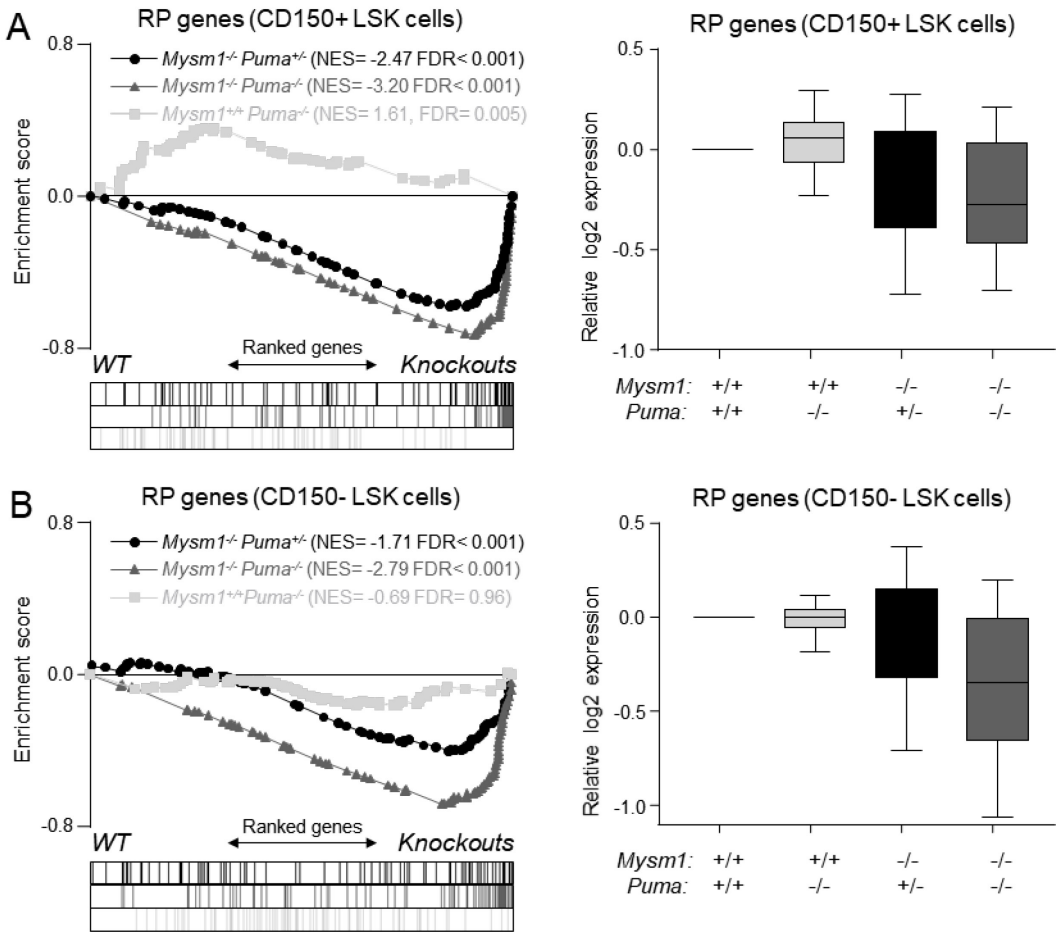


Figure 7. *RP-gene* downregulation is independent of PUMA and not a result of the induction of apoptosis

(A-B) Gene Set Enrichment Analyses (GSEA) demonstrate reduced expression of 80 *RP-genes* in *Mysm1*$^{-/-}$*Puma*$^{+/-}$ and *Mysm1*$^{-/-}$*Puma*$^{-/-}$ hematopoietic stem and progenitor cells (CD150^{+} LSK in **A** and CD150^{-} LSK in **B**) relative to wildtype control cells; 10,987 genes expressed in the RNA-Seq dataset are ranked based on signal-to-noise ratio and each relevant *RP-gene* represented by a dot and a vertical bar below. Boxplots of relative Log2 expression of the *RP-genes* also show downregulation in *Mysm1*$^{-/-}$*Puma*$^{+/-}$ and *Mysm1*$^{-/-}$ *Puma*$^{-/-}$ cells relative to control cells.

4.7 MYSM1 may interact with several transcriptional regulators to control *RP-gene* expression

Since p53 is not co-recruited to the promoters of the *RP-genes*, we sought to identify other potential MYSM1 co-binding factors. To this end, we performed *de novo*

motif analysis on all MYSM1 binding sites to identify regulatory elements, or motifs, that were enriched relative to the background[350]. Motifs enriched in the gene-proximal MYSM1 binding sites included zinc finger domain-containing Yin Yang (YY) proteins, ETS domain-containing proteins, and RE1-Silencing Transcription factor (REST). Motifs enriched in gene-distal MYSM1 binding sites were best matched to Forkhead proteins, Gata proteins, and ZBTB33 (Table 5). The distinct enrichment of motifs between the gene-proximal and gene-distal binding sites suggests that MYSM1 may interact with different complexes in the regulation of gene expression.

Table 5. *de novo* motifs enriched for MYSM1 binding sites

Peaks	*de novo* motifs	Frequency	Best match known motifs
All		50.14%	Zfp187, Egr1-4
		48.22%	Hoxb9, TATA-box
		50.00%	Sox family, HMG-box
Proximal		41.30%	YY1-2, Zinc-finger
		43.48%	NeuroD1, ETS domain
		8.70%	REST
Distal		53.13%	FoxL2, Forkhead
		53.28%	Gata proteins
		53.13%	ZBTB33

Polycomb Group proteins (PcG) are essential during mammalian development and differentiation. Two PcG protein complexes, the Polycomb Repressive Complex 1 (PRC1) and PRC2, regulate the transcriptional silencing of genes during development through the mono-ubiquitination of histone H2A K119 and the methylation of histone H3 K27, respectively[217]. YY1 and YY2 are proteins that interact and recruit PRC proteins to their sites of function[357,358], and are the top enriched motifs in the gene-proximal MYSM1

binding sites (Table 5). To examine whether the PRC proteins or YY proteins are recruited to the MYSM1-regulated *RP-gene* promoters, ChIP-qPCR was performed on RING1B, the main catalytic component of the PRC1 complex, and YY1 in Ba/F3 cells. Unfortunately, we were not able to identify a working ChIP-grade anti-YY1 antibody. On the other hand, the binding of RING1B to the promoters of the selected *RP-genes* was confirmed, suggesting that the PRC1 complex may play a role in the regulation of these *RP-genes* (Figure 6B). To further investigate whether RING1B is recruited by MYSM1, we examined the levels of RING1B binding to the *RP-gene* promoters using the knockdown *shMysm1* and control *shFF* Ba/F3 cells. The levels of RING1B binding, unfortunately, were not affected by the loss of MYSM1 (Figure 6C). Together, the PRC1 complex may play a role in the regulation of *RP-gene* expressions but its recruitment is independent of MYSM1.

A collection of transcription factors important to hematopoiesis has been extensively studied and the corresponding ChIP-Seq data sets in the HPC7 cells were available for re-analysis[86-88]. To further explore the potential interacting TFs with MYSM1, we measured the binding intensities of these TFs at the MYSM1 binding sites and performed a Pearson correlation analysis. Due to the weaker gene-proximal MYSM1 binding in the HPC7 cells, no trend was observed between MYSM1 in HPC7 cells and the other TFs. However, some TF binding intensities showed trends of co-binding between MYSM1 in Ba/F3 cells and c-MYC (r=0.3), Max (r=0.2), ETO2 (r=0.4), and STAT3 (r=0.3) in the HPC7 cells (Figure 7A); while considering those potential MYSM1-interacting partners, it must be reminded that the data were from different cell types. The transcription factor c-MYC is an important regulator of *RP-gene* expression[359], HSC self-renewal and differentiation[148], and lymphoid and myeloid lineage development (Table 2)[149-154]. As c-MYC showed one of the stronger correlations in this analysis, we decided to investigate its possible recruitment to MYSM1 binding sites. We performed a c-MYC ChIP experiment and assessing the enrichment at the selected MYSM1-regulated *RP-gene* promoters by qPCR. The binding of c-MYC to these *RP-gene* promoters was confirmed in the Ba/F3 cells (Figure 7B, data obtained from the lab). Further experiments investigating the co-recruitment of MYSM1 and c-MYC are necessary to confirm the interaction of the two transcriptional regulators, but these results indicate that c-MYC may co-localize with MYSM1 to the *RP-gene* promoters.

In summary, several transcription factors, including PRC1 complex, c-MYC, and others, have been suggested bioinformatically to co-localize with MYSM1 in the transcriptional regulation of *RP-genes*. However, further experiments are required to validate these interactions at *RP-gene* promoters.

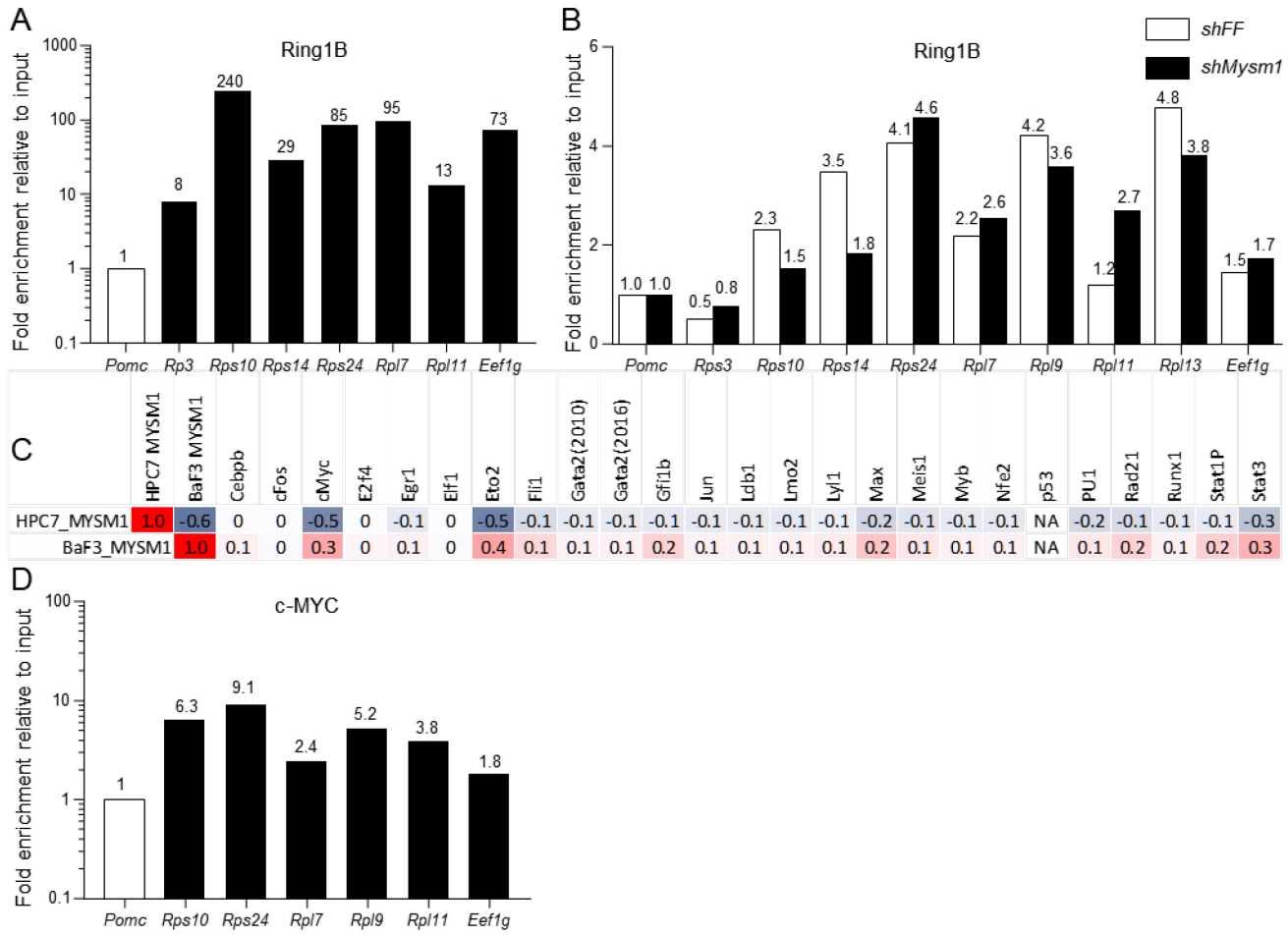


Figure 8. RING1B and c-MYC bind to *RP-gene* promoters.

(A) Enrichment of RING1B at *RP-gene* promoter sites is validated with ChIP-qPCRs in MYSM1-FLAG Ba/F3 hematopoietic progenitor cells. **(B)** Enrichment of RING1B at *RP-gene* promoter sites, validated with ChIP-qPCRs showing reduced relative enrichments in knockdown *shMysm1* Ba/F3 cells relative to control *shFF* Ba/F3 cells. **(C)** Pearson correlation analysis of the binding intensities of various TFs at MYSM1 binding sites. The coefficient *r* values are displayed. **(D)** Enrichment of c-MYC at *RP-gene* promoter sites is validated with ChIP-qPCRs in MYSM1-FLAG Ba/F3 hematopoietic progenitor cells. Data in **(D)** is obtained from a colleague in the lab.

Chapter 5 Discussion

The project presents the first characterization of the transcriptome in *Mysm1*-deficient hematopoietic stem and progenitor cells (HSPC) and the genome-wide binding pattern of MYSM1 in hematopoietic progenitor cell lines, providing significant insight into the transcriptional regulation by MYSM1 in mouse HSPCs. The *Mysm1*-deficient HSPCs have profound transcriptomic alterations from the wildtype controls, including upregulation of cell cycle-related and apoptotic genes and downregulation of translational and ribosomal biogenesis-related genes. The upregulation of cell cycle-related and apoptotic genes is consistent with the observed phenotypic loss of HSC quiescence and hematopoietic failure previously reported in *Mysm1*-deficiency[20,277,308,309]. Importantly, *Mysm1*-deficiency leads to a previously unknown downregulation of many ribosomal protein-encoding genes (*RP-genes*) in the HSPCs. MYSM1 binds to the promoters of these *RP-genes* and orchestrates the induction of transcriptional activation histone marker H3K27ac at their promoters, suggesting a direct regulatory role of MYSM1 on the expression of these *RP-genes*. Furthermore, the downregulation of *RP-gene* expression in *Mysm1*-deficient HSPCs is independent of p53 activation, indicating that it is not a result of the induction of apoptosis. Other hematopoietic transcriptional regulators, including PRC1 and c-MYC, are proposed to regulate the *RP-gene* expressions in cooperation with MYSM1. Overall, this project provides insight into the HSPC transcriptional programme regulated by MYSM1 and proposes that MYSM1 directly regulates the expression of a set of *RP-genes*.

In this study, we showed that MYSM1 regulates *RP-gene* expressions in the HSPCs. However, the mechanism of the MYSM1 regulation remains unclear. The function of MYSM1 as a deubiquitinase has been extensively studied[263,268-270] and linked to interactions with other transcriptional and epigenetic regulators. The histone H2AK119ub, which is a substrate of MYSM1 deubiquitination[209,268], is mono-ubiquitinated by PRC1 during long-term gene silencing[272-274]. At different stages of hematopoiesis, MYSM1 was shown to deubiquitinate histone H2AK119 at the promoters of *Gfi1*, *Ebf1*, *Id2*, and *Flt3* genes, which encode for essential hematopoietic transcription factors[20,263-266]. In this study, we observed very low levels of H2AK119ub at the promoters of *RP-genes* (Figure 4B-C), consistent with the high transcriptional activity of these genes. The loss of MYSM1 in Ba/F3 cells (*shMysm1*) did not increase H2AK119ub levels (data not

shown) but led to reduction of H3K27ac levels at the *RP-gene* promoters (Figure 5E, data obtained from the lab), suggesting that MYSM1 regulates *RP-genes* through mechanisms other than the deubiquitination of H2AK119. Interestingly, MYSM1 is known to interact with histone acetylase PCAF, proposing a potential mechanism for regulating H3K27ac[268]. Other histone H2A deubiquitinases, such as BAP1, USP3, and USP16, regulate transcription through diverse mechanisms, ranging from deubiquitinating H2AK119 to targeting transcriptional regulators[209,360-362]. Together, these suggest that the mechanism of MYSM1 action can be complex and context-specific.

Previous studies showed that MYSM1 was bound to the promoters of *Gfi1* in HSCs[20] and *Ebf1* in B cell progenitors[264] using ChIP-qPCR and both genes encode for transcription factors important in the respective hematopoietic stages, highlighting the significance of MYSM1 in the transcriptional regulation of these genes. As our MYSM1 ChIP-Seq was performed in cell lines representing these two primary cell types, we examined the binding of MYSM1 in the promoters of these two genes. However, the binding of MYSM1 in these promoters was not confirmed by our ChIP-Seq (data not shown). A wide range of reasons can help to explain this discrepancy, including the subjectivity of ChIP-qPCR primer design, low input of DNA sequenced in ChIP-Seq resulting in the identification of only top binding sites, or potential inconsistency between cell lines and primary cells used in the studies. Observing this discrepancy, we validated the MYSM1 binding sites identified in our ChIP-Seq using independent experiments. The MYSM1 binding sites identified at the promoters of *RP-genes* were confirmed using independent ChIP-qPCR experiments in MYSM1-FLAG Ba/F3 cells (Figure 5C), adding a level of certainty to our findings.

RP-genes encode for ribosomal proteins that make up the ribosome and are conserved across most life forms[363], including human and mouse[364]. Currently, over 80 *RP-genes* are identified in human and mouse. The assembly of the ribosome, which is essential for the translation process, is the most energy-demanding cellular activity and requires the coordinated action of all three RNA polymerases[364,365]. Ribosomal assembly begins in the nucleolus, which is a region in the nucleus and the main site for ribosomal biogenesis, with the synthesis of a precursor rRNA transcript (47S precursor rRNA) by RNA Polymerase I and then this transcript is processed into the 18S, 5.8S, and 28S

rRNAs[364]. Concurrently, *RP-genes* are transcribed by the RNA Polymerase II in the nucleus, translated in the cytoplasm, and then migrated to the nucleolus[364]. In addition, a 5S rRNA is transcribed by RNA Polymerase III in the nucleus and migrates to the nucleolus[364]. Together in the nucleolus, a 90S pre-ribosome is assembled using the rRNAs and ribosomal proteins and gives rise to precursors of the 40S and 60S ribosomal subunits after extensive processing[364]. The 40S and 60S subunits are then exported to the cytoplasm for final maturation before assembling into a ribosome for protein translation[364]. Together, the ribosomal biogenesis is a highly-demanded and tightly-regulated process requiring proper *RP-genes* expression.

Ribosomal biogenesis is critical for normal translation and protein synthesis in all cells, including the HSCs[355,356]. In this study, we identified a set of *RP-genes* downregulated in the *Mysm1*-deficient HSPCs (Figure 3A-B) and proposed that this broad downregulation contributed to the hematopoietic dysfunction in *Mysm1*-deficiency. Investigations of the ribosomal biogenesis and protein synthesis rates in *Mysm1*-deficiency were performed by other lab members[285]. Knockdown *shMysm1* Ba/F3 cells treated with inhibitors of rRNA synthesis (actinomycin D) and protein translation (cycloheximide) showed increased levels of cell death compared to control *shFF* cells treated with the same inhibitors (Supplemental Figure 1A-B, data obtained from the lab), suggesting that loss of MYSM1 makes the cells more sensitive to inhibition of ribosomal biogenesis and translation. Furthermore, HSCs from *Mysm1*$^{-/-}$ mice administered with O-propargyl-puromycin (OPP)[355,366], showed reduced protein synthesis rates than HSCs from wildtype control mice administered with OPP (Supplemental Figure 1C-D, data obtained from the lab). Together, these findings show that the broad downregulation of *RP-gene* serves as a mediator of hematopoietic dysfunction in *Mysm1*-deficiency.

The link between ribosomal biogenesis stress and activation of p53 has been well-established in previous studies. Ribosomal biogenesis is tightly regulated and subjected to intensive quality control checks[367]. Under homeostatic conditions, ribosomal proteins assemble with the rRNAs into the 40S and 60S ribosome in the nucleolus and the activity of p53 is maintained at baseline levels, subjected to mouse double minute (MDM2)-mediated ubiquitination and degradation[367]. Nucleolar stress, or ribosomal biogenesis stress, occurs when there are disruptions to proper ribosomal biogenesis, such as

defective synthesis of ribosomal proteins and rRNAs, UV and gamma radiation, oncogenes, nutrient and growth factor deprivation, hypoxia, and genotoxic agents[367-369]. Under ribosomal biogenesis stress, some ribosomal proteins, such as RPL5[370,371], RPL11[371,372], RPL23[371], and RPS7[373], enter the nucleoplasm to interact with MDM2, releasing p53 from degradation[367,369]. The accumulation of p53 leads to activation of its stress-response genes, which initiate processes such as cell cycle arrest and apoptosis[367,369]. In addition to the regulation through MDM2, RPL26 induces p53 production by associating with the p53 mRNA and augmenting its translation[374]. In the context of hematopoiesis, previous studies have well-documented the activation of p53 and its stress-responses in *Mysm1*-deficient HSCs[277,308,309]. In this current study, we report a broad downregulation in the expressions of 35 *RP-genes* for at least 1.5-fold in the *Mysm1*-deficient HSPCs (Figure 3A-B). Others in the lab also demonstrated significantly reduced translation and protein synthesis rates in the *Mysm1*-deficient HSPCs (Supplemental Figure 1A-D, data obtained from the lab), highlighting the significance of the ribosome biogenesis stress. Further characterization showed persistent downregulation of *RP-genes* in the HSPCs collected from mice deficient of both MYSM1 and p53, and not from mice deficient of p53 (Supplemental Figure 2, data obtained from the lab). The downregulation was also observed in the RNA-Seq performed using HSPCs from mice deficient of both MYSM1 and PUMA, the major p53-induced mediator of apoptosis, and not from mice deficient of PUMA (Figure 7). In future work, the impact of *Mysm1*-deficiency on ribosomal biogenesis and function can be further studied. This work may include exploring the effect of the downregulation in *RP-gene* expression in the *Mysm1*-deficient HSCs on RP protein levels, directly measured using the single-cell Western blot technology, which visualizes and measures protein expression in single cells. Ribosome function can be further assessed by performing polysome profiling, to evaluate the alterations in protein translational activity in *Mysm1*-deficient and wildtype HSCs. The link between ribosomal stress and the cause of p53 activation can be solidified by co-immunoprecipitating MDM2 and RP proteins such as RPL11 and RPL5 in wildtype and *Mysm1*-deficient hematopoietic cells. This will test whether *Mysm1*-deficiency triggers the formation of the MDM2-RP complex, which is known to lead to p53 activation in other systems with disruption in ribosome biogenesis and function[370-373]. Together with

the established role of ribosomal biogenesis stress activating p53 and our finding that the *RP-gene* downregulation is independent of p53 activation, we propose that the *RP-gene* downregulation, which causes ribosomal biogenesis stress, in *Mysm1*-deficient HSPCs may lead to the activation of p53 and its stress-response.

The transcription factor c-MYC, which serves essential regulatory functions in hematopoiesis and cell cycle progression, is important to ribosomal biogenesis. Previous studies established that c-MYC mediates several critical processes in ribosomal biogenesis, including the production of rRNA and co-factors, synthesis of ribosomal proteins, and export of ribosomal subunits[359,367]. On the other hand, during ribosomal biogenesis stress, several ribosomal proteins, namely RPL5, RPL11, and RPS14, interact and negatively regulate c-MYC by repressing c-MYC synthesis, inhibiting c-MYC-regulated transcription, and preventing interaction between c-MYC and its heterodimeric partner MAX[367]. Through these mechanisms, ribosomal proteins suppress the c-MYC-induced cell cycle progression during ribosomal biogenesis stress. In our study, we showed trends of c-MYC (r=0.3) and its heterodimeric partner MAX (r=0.2) recruitment to the gene-proximal MYSM1 binding sites, mainly to the *RP-genes* promoters (Figure 7A). We confirmed the binding of c-MYC at selected *RP-genes* promoters in Ba/F3 cells using ChIP-qPCR (Figure 8D, data obtained from the lab), suggesting that c-MYC may be a potential co-binding factor of MYSM1 in the regulation of *RP-gene* expression. However, this preliminary result requires further investigation of the interaction between c-MYC and MYSM1 and particularly the levels of c-MYC recruitment with and without MYSM1. Together, we propose that c-MYC is a potential co-regulator of *RP-genes* with MYSM1.

Diseases caused by abnormal ribosomal biogenesis are known as ribosomopathies. They are rare disorders and display varied phenotypes in human, with Diamond-Blackfan anemia (DBA) as the most characterized ribosomopathy[289,352]. DBA is a rare Inherited Bone Marrow Failure Syndrome (IBMFS) caused by heterozygous coding mutations in 19 *RP-genes*, *TSR2*, and *GATA1*. DBA presents a characteristic normochromic macrocytic anemia and shares many overlapping features with other ribosomopathies, such as hematopoietic dysfunction (5q-myelodysplastic syndrome, Dyskeratosis congenita and Shwachman-Diamond syndrome) and skeletal defects (Treacher Collins syndrome, Bowen-Conradi syndrome and RPS23-related

ribosomopathy)[289,352,364]. Mechanistically, ribosomopathies are caused by mutations in the *RP-genes* or ribosomal biogenesis factors, causing ribosomal biogenesis stress and leading to subsequent activation of p53 stress-responses[364]. The varied levels of tissue-specific activation of p53 and its stress-responses, such as cell cycle arrest and apoptosis, compromise the development of cells in specific tissues[364]. *MYSM1*-deficiency shared many characteristics of ribosomopathies, including early childhood anemia (DBA and 5q-myelodysplastic syndrome), growth retardation (Roberts syndrome, Bowen-Conradi syndrome, and Cartilage-hair hypoplasia), physical dysmorphism (DBA and RPS23-related ribosomopathy), and neurodevelopmental defects (RPS23-related ribosomopathy, Treacher Collins syndrome, and Roberts syndrome)[364]. Importantly, we showed that *Mysm1*-deficient mice had reduced translation and protein synthesis rates (Supplemental Figure 1, data obtained from the lab). We further characterized a human patient with *MYSM1* mutation (c.869C>G, p.S290*)[285], and identified reduced translation and protein synthesis rates (Supplemental Figure 3A, data obtained from the lab). Additionally, the human patient showed elevated Erythrocyte Adenosine Deaminase (eADA, 1366 IU/1 EC)[285], which is characteristically seen in DBA[287,375]. Altogether the overlapping phenotypes and our findings in the human patient suggest that *MYSM1*-deficiency is a type of ribosomopathy. Furthermore, as significant numbers of DBA and Schwachman-Diamond syndrome (SDS) patients do not carry mutations in the established ribosomopathy-causing genes[289,352,376], *MYSM1* mutation may be a target for screening in the diagnosis of ribosomopathy.

In summary, this project presents the first characterization of *Mysm1*-deficient HSPC transcriptome and the genome-wide binding profile of MYSM1 in hematopoietic progenitor cell lines, revealing a previously unknown downregulation of *RP-genes* in the *Mysm1*-deficient HSPCs and demonstrating that MYSM1 directly regulates these *RP-genes*. Further, this study showed that the *RP-gene* downregulation in *Mysm1*-deficiency is independent of the p53 activation and induction of apoptosis, providing novel insight into the consequences of *Mysm1*-deficiency. Altogether with previous reports that defective *RP-gene* expression activates p53, our findings propose that *RP-gene* downregulation is a mechanism linking *Mysm1*-deficiency to p53 activation.

Chapter 6 Conclusion and Future Directions

In terms of future work, our data suggested that MYSM1 regulates *RP-genes* in HSCs and MPPs. However, the mechanism in which MYSM1 regulates *RP-genes* remains unclear. Further investigation into the promoter accessibility with ATAC-Seq in *Mysm1*-KO and *Mysm1*-WT primary mouse HSPCs may help to elucidate the mechanism.

The deubiquitinating activity of MYSM1 has been widely reported in previous studies[263,267,268]. In our study, however, we did not observe an increase in the H2AK119ub enrichment in *shMysm1* Ba/F3 cells, either globally or specifically at the known MYSM1 binding sites near *RP-gene* promoters (data not shown), suggesting that the mechanism of *RP-gene* regulation by MYSM1 may not be through the deubiquitination of histone H2A K119. The role of MYSM1 catalytic activity in the regulation of *RP-gene* expression may be further investigated with over-expression of full-length *Mysm1* or *Mysm1* that has an inactivating mutation in the catalytic JAMM/MPN+ domain in *shMysm1* cells, measuring and comparing the effect on *RP-gene* expression. In addition, our lab has developed a mouse line that expresses the p.D660N mutant form of MYSM1 protein, which is in the catalytic domain and renders the protein catalytically inactive[268]. Studying the phenotype and the regulation of *RP-gene* expression in the HSCs of these mice can provide valuable information on how MYSM1 regulates *RP-gene* expression and whether its deubiquitinase catalytic activity is required for this regulation.

MYSM1 induces the acetylation of H3K27, which is associated with chromatin opening, at the promoters of *RP-genes* in HSPCs. As the mechanism of MYSM1 transcriptional regulation on *RP-genes* is unclear, the investigation into the interactions between MYSM1 and histone acetylases, such as PCAF[268], in HSPCs may reveal novel insight.

Another mechanism of the transcriptional regulation by MYSM1 may be through the recruitment of co-factors. We suggested possible co-recruitment of PRC1, c-MYC (r=0.3), Max (r=0.2), ETO2 (r=0.4), and STAT3 (r=0.3) with MYSM1 to the *RP-gene* promoters and confirmed the binding of PRC1 and c-MYC to the selected MYSM1-regulated *RP-gene* promoters using ChIP-qPCR. Similar ChIP-qPCR experiments can be performed to examine the recruitment of other factors. Furthermore, co-immunoprecipitation (co-IP) and sequential-ChIP-qPCR experiments can be performed to assess the co-recruitment. During my study, I have attempted co-IP and sequential-

ChIP-qPCR assessing the possible co-recruitment of MYSM1 and c-MYC to the *RP-gene* promoters in Ba/F3 cells[377-382]. However, the results were inconclusive due to poor conditioning of the DNA after the first ChIP.

Induced pluripotent stem cells (iPSC) derived from the MYSM1-deficiency (c.869C>G, p.S290*) patient[285] and controls are currently being developed in the lab. They can give significant insight into the role of MYSM1 in humans, however, their analysis is beyond the scope of this manuscript.

Altogether, the findings reported in this thesis demonstrate the transcriptional regulatory role of MYSM1 in hematopoietic stem and progenitor cells and identified a mechanism leading to p53 activation, which in turn drives HSC dysfunctions, in *Mysm1*-deficiency.

Appendix I: References

1 Haeckel, E. H. P. A. Natürliche schöpfungsgeschichte : Gemeinverständliche wissenschaftliche vorträge über die entwickelungslehre im allgemeinen und diejenige von Darwin, Goethe und Lamarck im besonderen. (1889).
2 Pappenheim, A. Ueber Entwickelung und Ausbildung der Erythroblasten. *Archiv für Pathologische Anatomie und Physiologie und für Klinische Medicin* **145**, 587-643, doi:10.1007/bf01969901 (1896).
3 Till, J. E. & Mc, C. E. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res* **14**, 213-222 (1961).
4 Becker, A. J., Mc, C. E. & Till, J. E. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* **197**, 452-454, doi:10.1038/197452a0 (1963).
5 Spangrude, G. J., Heimfeld, S. & Weissman, I. L. Purification and characterization of mouse hematopoietic stem cells. *Science* **241**, 58-62, doi:10.1126/science.2898810 (1988).
6 Laurenti, E. & Gottgens, B. From haematopoietic stem cells to complex differentiation landscapes. *Nature* **553**, 418-426, doi:10.1038/nature25022 (2018).
7 Jagannathan-Bogdan, M. & Zon, L. I. Hematopoiesis. *Development* **140**, 2463-2467, doi:10.1242/dev.083147 (2013).
8 Ogawa, M. Differentiation and proliferation of hematopoietic stem cells. *Blood* **81**, 2844-2853 (1993).
9 Challen, G. A., Boles, N., Lin, K. K. & Goodell, M. A. Mouse hematopoietic stem cell identification and analysis. *Cytometry A* **75**, 14-24, doi:10.1002/cyto.a.20674 (2009).
10 Sharpless, N. E. & DePinho, R. A. How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* **8**, 703-713, doi:10.1038/nrm2241 (2007).
11 Cheng, H., Zheng, Z. & Cheng, T. New paradigms on hematopoietic stem cell differentiation. *Protein Cell* **11**, 34-44, doi:10.1007/s13238-019-0633-0 (2020).
12 Cabezas-Wallscheid, N. *et al.* Identification of regulatory networks in HSCs and their immediate progeny via integrated proteome, transcriptome, and DNA methylome analysis. *Cell Stem Cell* **15**, 507-522, doi:10.1016/j.stem.2014.07.005 (2014).
13 Pietras, E. M. *et al.* Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. *Cell Stem Cell* **17**, 35-46, doi:10.1016/j.stem.2015.05.003 (2015).
14 Mercier, F. E. & Scadden, D. T. Not All Created Equal: Lineage Hard-Wiring in the Production of Blood. *Cell* **163**, 1568-1570, doi:10.1016/j.cell.2015.12.013 (2015).
15 Kiel, M. J. *et al.* SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121**, 1109-1121, doi:10.1016/j.cell.2005.05.026 (2005).
16 Majeti, R., Park, C. Y. & Weissman, I. L. Identification of a hierarchy of multipotent hematopoietic progenitors in human cord blood. *Cell Stem Cell* **1**, 635-645, doi:10.1016/j.stem.2007.10.001 (2007).

17 Kent, D. G. *et al.* Prospective isolation and molecular characterization of hematopoietic stem cells with durable self-renewal potential. *Blood* **113**, 6342-6350, doi:10.1182/blood-2008-12-192054 (2009).
18 Notta, F. *et al.* Isolation of single human hematopoietic stem cells capable of long-term multilineage engraftment. *Science* **333**, 218-221, doi:10.1126/science.1201219 (2011).
19 Oguro, H., Ding, L. & Morrison, S. J. SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors. *Cell Stem Cell* **13**, 102-116, doi:10.1016/j.stem.2013.05.014 (2013).
20 Wang, T. *et al.* The control of hematopoietic stem cell maintenance, self-renewal, and differentiation by Mysm1-mediated epigenetic regulation. *Blood* **122**, 2812-2822, doi:10.1182/blood-2013-03-489641 (2013).
21 Wilson, A. *et al.* Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* **135**, 1118-1129, doi:10.1016/j.cell.2008.10.048 (2008).
22 Mayle, A., Luo, M., Jeong, M. & Goodell, M. A. Flow cytometry analysis of murine hematopoietic stem cells. *Cytometry A* **83**, 27-37, doi:10.1002/cyto.a.22093 (2013).
23 Mooney, C. J., Cunningham, A., Tsapogas, P., Toellner, K. M. & Brown, G. Selective Expression of Flt3 within the Mouse Hematopoietic Stem Cell Compartment. *Int J Mol Sci* **18**, doi:10.3390/ijms18051037 (2017).
24 Kondo, M., Weissman, I. L. & Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* **91**, 661-672, doi:10.1016/s0092-8674(00)80453-5 (1997).
25 Akashi, K., Traver, D., Miyamoto, T. & Weissman, I. L. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* **404**, 193-197, doi:10.1038/35004599 (2000).
26 Seita, J. & Weissman, I. L. Hematopoietic stem cell: self-renewal versus differentiation. *Wiley Interdiscip Rev Syst Biol Med* **2**, 640-653, doi:10.1002/wsbm.86 (2010).
27 Sachs, L. & Lotem, J. The network of hematopoietic cytokines. *Proc Soc Exp Biol Med* **206**, 170-175, doi:10.3181/00379727-206-43736 (1994).
28 Mercier, F. E., Ragu, C. & Scadden, D. T. The bone marrow at the crossroads of blood and immunity. *Nat Rev Immunol* **12**, 49-60, doi:10.1038/nri3132 (2011).
29 Broudy, V. C. Stem cell factor and hematopoiesis. *Blood* **90**, 1345-1364 (1997).
30 Edling, C. E. & Hallberg, B. c-Kit--a hematopoietic cell essential receptor tyrosine kinase. *Int J Biochem Cell Biol* **39**, 1995-1998, doi:10.1016/j.biocel.2006.12.005 (2007).
31 Wang, Z. & Ema, H. Mechanisms of self-renewal in hematopoietic stem cells. *Int J Hematol* **103**, 498-509, doi:10.1007/s12185-015-1919-5 (2016).
32 Yoshihara, H. *et al.* Thrombopoietin/MPL signaling regulates hematopoietic stem cell quiescence and interaction with the osteoblastic niche. *Cell Stem Cell* **1**, 685-697, doi:10.1016/j.stem.2007.10.020 (2007).
33 Blank, U. & Karlsson, S. TGF-beta signaling in the control of hematopoietic stem cells. *Blood* **125**, 3542-3550, doi:10.1182/blood-2014-12-618090 (2015).

34 Baumgartner, C. *et al.* An ERK-Dependent Feedback Mechanism Prevents Hematopoietic Stem Cell Exhaustion. *Cell Stem Cell* **22**, 879-892 e876, doi:10.1016/j.stem.2018.05.003 (2018).
35 Wang, H. & Spangrude, G. J. Aspects of early lymphoid commitment. *Curr Opin Hematol* **10**, 203-207, doi:10.1097/00062752-200305000-00002 (2003).
36 Cordeiro Gomes, A. *et al.* Hematopoietic Stem Cell Niches Produce Lineage-Instructive Signals to Control Multipotent Progenitor Differentiation. *Immunity* **45**, 1219-1231, doi:10.1016/j.immuni.2016.11.004 (2016).
37 Plumb, A. W. *et al.* Interleukin-7 in the transition of bone marrow progenitors to the thymus. *Immunol Cell Biol* **95**, 916-924, doi:10.1038/icb.2017.68 (2017).
38 Peschon, J. J. *et al.* Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J Exp Med* **180**, 1955-1960, doi:10.1084/jem.180.5.1955 (1994).
39 von Freeden-Jeffry, U. *et al.* Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J Exp Med* **181**, 1519-1526, doi:10.1084/jem.181.4.1519 (1995).
40 Crooks, G. M., Hao, Q. L., Petersen, D., Barsky, L. W. & Bockstoce, D. IL-3 increases production of B lymphoid progenitors from human CD34+CD38- cells. *J Immunol* **165**, 2382-2389, doi:10.4049/jimmunol.165.5.2382 (2000).
41 Northrup, D. L. & Allman, D. Transcriptional regulation of early B cell development. *Immunol Res* **42**, 106-117, doi:10.1007/s12026-008-8043-z (2008).
42 Kikuchi, K., Kasai, H., Watanabe, A., Lai, A. Y. & Kondo, M. IL-7 specifies B cell fate at the common lymphoid progenitor to pre-proB transition stage by maintaining early B cell factor expression. *J Immunol* **181**, 383-392, doi:10.4049/jimmunol.181.1.383 (2008).
43 Klein, F. *et al.* Accumulation of Multipotent Hematopoietic Progenitors in Peripheral Lymphoid Organs of Mice Over-expressing Interleukin-7 and Flt3-Ligand. *Front Immunol* **9**, 2258, doi:10.3389/fimmu.2018.02258 (2018).
44 Pui, J. C. *et al.* Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* **11**, 299-308, doi:10.1016/s1074-7613(00)80105-3 (1999).
45 Radtke, F. *et al.* Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* **10**, 547-558, doi:10.1016/s1074-7613(00)80054-0 (1999).
46 Gonzalez-Garcia, S., Garcia-Peydro, M., Alcain, J. & Toribio, M. L. Notch1 and IL-7 receptor signalling in early T-cell development and leukaemia. *Curr Top Microbiol Immunol* **360**, 47-73, doi:10.1007/82_2012_231 (2012).
47 Mercier, F. *et al.* Persistent human immunodeficiency virus-1 antigenaemia affects the expression of interleukin-7Ralpha on central and effector memory CD4+ and CD8+ T cell subsets. *Clin Exp Immunol* **152**, 72-80, doi:10.1111/j.1365-2249.2008.03610.x (2008).
48 Puzanov, I. J., Bennett, M. & Kumar, V. IL-15 can substitute for the marrow microenvironment in the differentiation of natural killer cells. *J Immunol* **157**, 4282-4285 (1996).

49 Mrozek, E., Anderson, P. & Caligiuri, M. A. Role of interleukin-15 in the development of human CD56+ natural killer cells from CD34+ hematopoietic progenitor cells. *Blood* **87**, 2632-2640 (1996).
50 Williams, N. S. *et al.* Generation of lytic natural killer 1.1+, Ly-49- cells from multipotential murine bone marrow progenitors in a stroma-free culture: definition of cytokine requirements and developmental intermediates. *J Exp Med* **186**, 1609-1614, doi:10.1084/jem.186.9.1609 (1997).
51 Saunders, D. *et al.* Dendritic cell development in culture from thymic precursor cells in the absence of granulocyte/macrophage colony-stimulating factor. *J Exp Med* **184**, 2185-2196, doi:10.1084/jem.184.6.2185 (1996).
52 Brasel, K., De Smedt, T., Smith, J. L. & Maliszewski, C. R. Generation of murine dendritic cells from flt3-ligand-supplemented bone marrow cultures. *Blood* **96**, 3029-3039 (2000).
53 Gilliet, M. *et al.* The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. *J Exp Med* **195**, 953-958, doi:10.1084/jem.20020045 (2002).
54 Karsunky, H., Merad, M., Cozzio, A., Weissman, I. L. & Manz, M. G. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. *J Exp Med* **198**, 305-313, doi:10.1084/jem.20030323 (2003).
55 Kindler, V. *et al.* Stimulation of hematopoiesis in vivo by recombinant bacterial murine interleukin 3. *Proc Natl Acad Sci U S A* **83**, 1001-1005, doi:10.1073/pnas.83.4.1001 (1986).
56 Stanley, E. R. *et al.* Biology and action of colony--stimulating factor-1. *Mol Reprod Dev* **46**, 4-10, doi:10.1002/(SICI)1098-2795(199701)46:1<4::AID-MRD2>3.0.CO;2-V (1997).
57 Kondo, M. Lymphoid and myeloid lineage commitment in multipotent hematopoietic progenitors. *Immunol Rev* **238**, 37-46, doi:10.1111/j.1600-065X.2010.00963.x (2010).
58 Griseri, T., McKenzie, B. S., Schiering, C. & Powrie, F. Dysregulated hematopoietic stem and progenitor cell activity promotes interleukin-23-driven chronic intestinal inflammation. *Immunity* **37**, 1116-1129, doi:10.1016/j.immuni.2012.08.025 (2012).
59 Gupta, D., Shah, H. P., Malu, K., Berliner, N. & Gaines, P. Differentiation and characterization of myeloid cells. *Curr Protoc Immunol* **104**, 22F 25 21-22F 25 28, doi:10.1002/0471142735.im22f05s104 (2014).
60 Ushach, I. & Zlotnik, A. Biological role of granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) on cells of the myeloid lineage. *J Leukoc Biol* **100**, 481-489, doi:10.1189/jlb.3RU0316-144R (2016).
61 Cannistra, S. A., Koenigsmann, M., DiCarlo, J., Groshek, P. & Griffin, J. D. Differentiation-associated expression of two functionally distinct classes of granulocyte-macrophage colony-stimulating factor receptors by human myeloid cells. *J Biol Chem* **265**, 12656-12663 (1990).

62 Mayani, H., Dragowska, W. & Lansdorp, P. M. Cytokine-induced selective expansion and maturation of erythroid versus myeloid progenitors from purified cord blood precursor cells. *Blood* **81**, 3252-3258 (1993).
63 Kaushansky, K. The mpl ligand: molecular and cellular biology of the critical regulator of megakaryocyte development. *Stem Cells* **12 Suppl 1**, 91-96; discussion 96-97 (1994).
64 Kaushansky, K. *et al.* Promotion of megakaryocyte progenitor expansion and differentiation by the c-Mpl ligand thrombopoietin. *Nature* **369**, 568-571, doi:10.1038/369568a0 (1994).
65 Bartley, T. D. *et al.* Identification and cloning of a megakaryocyte growth and development factor that is a ligand for the cytokine receptor Mpl. *Cell* **77**, 1117-1124, doi:10.1016/0092-8674(94)90450-2 (1994).
66 Lok, S. *et al.* Cloning and expression of murine thrombopoietin cDNA and stimulation of platelet production in vivo. *Nature* **369**, 565-568, doi:10.1038/369565a0 (1994).
67 de Sauvage, F. J. *et al.* Stimulation of megakaryocytopoiesis and thrombopoiesis by the c-Mpl ligand. *Nature* **369**, 533-538, doi:10.1038/369533a0 (1994).
68 Noetzli, L. J., French, S. L. & Machlus, K. R. New Insights Into the Differentiation of Megakaryocytes From Hematopoietic Progenitors. *Arterioscler Thromb Vasc Biol* **39**, 1288-1300, doi:10.1161/ATVBAHA.119.312129 (2019).
69 Fried, W. Erythropoietin and erythropoiesis. *Exp Hematol* **37**, 1007-1015, doi:10.1016/j.exphem.2009.05.010 (2009).
70 Hedley, B. D., Allan, A. L. & Xenocostas, A. The role of erythropoietin and erythropoiesis-stimulating agents in tumor progression. *Clin Cancer Res* **17**, 6373-6380, doi:10.1158/1078-0432.CCR-10-2577 (2011).
71 Inaba, K. *et al.* Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* **176**, 1693-1702, doi:10.1084/jem.176.6.1693 (1992).
72 Schuettpelz, L. G. & Link, D. C. Regulation of hematopoietic stem cell activity by inflammation. *Front Immunol* **4**, 204, doi:10.3389/fimmu.2013.00204 (2013).
73 Mirantes, C., Passegue, E. & Pietras, E. M. Pro-inflammatory cytokines: emerging players regulating HSC function in normal and diseased hematopoiesis. *Exp Cell Res* **329**, 248-254, doi:10.1016/j.yexcr.2014.08.017 (2014).
74 Pimanda, J. E. *et al.* Gata2, Fli1, and Scl form a recursively wired gene-regulatory circuit during early hematopoietic development. *Proc Natl Acad Sci U S A* **104**, 17692-17697, doi:10.1073/pnas.0707045104 (2007).
75 Chambers, J. & Rabbitts, T. H. LMO2 at 25 years: a paradigm of chromosomal translocation proteins. *Open Biol* **5**, 150062, doi:10.1098/rsob.150062 (2015).
76 Soza-Ried, C., Hess, I., Netuschil, N., Schorpp, M. & Boehm, T. Essential role of c-myb in definitive hematopoiesis is evolutionarily conserved. *Proc Natl Acad Sci U S A* **107**, 17304-17308, doi:10.1073/pnas.1004640107 (2010).
77 Okuda, T., Nishimura, M., Nakao, M. & Fujita, Y. RUNX1/AML1: a central player in hematopoiesis. *Int J Hematol* **74**, 252-257, doi:10.1007/BF02982057 (2001).
78 Robb, L. *et al.* The scl gene product is required for the generation of all hematopoietic lineages in the adult mouse. *EMBO J* **15**, 4123-4129 (1996).

79 Porcher, C. *et al.* The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell* **86**, 47-57, doi:10.1016/s0092-8674(00)80076-8 (1996).
80 Mikkola, H. K. *et al.* Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. *Nature* **421**, 547-551, doi:10.1038/nature01345 (2003).
81 Loughran, S. J. *et al.* The transcription factor Erg is essential for definitive hematopoiesis and the function of adult hematopoietic stem cells. *Nat Immunol* **9**, 810-819, doi:10.1038/ni.1617 (2008).
82 Taoudi, S. *et al.* ERG dependence distinguishes developmental control of hematopoietic stem cell maintenance from hematopoietic specification. *Genes Dev* **25**, 251-262, doi:10.1101/gad.2009211 (2011).
83 Knudsen, K. J. *et al.* ERG promotes the maintenance of hematopoietic stem cells by restricting their differentiation. *Genes Dev* **29**, 1915-1929, doi:10.1101/gad.268409.115 (2015).
84 Kruse, E. A. *et al.* Dual requirement for the ETS transcription factors Fli-1 and Erg in hematopoietic stem cells and the megakaryocyte lineage. *Proc Natl Acad Sci U S A* **106**, 13814-13819, doi:10.1073/pnas.0906556106 (2009).
85 Rodrigues, N. P., Tipping, A. J., Wang, Z. & Enver, T. GATA-2 mediated regulation of normal hematopoietic stem/progenitor cell function, myelodysplasia and myeloid leukemia. *Int J Biochem Cell Biol* **44**, 457-460, doi:10.1016/j.biocel.2011.12.004 (2012).
86 Wilson, N. K. *et al.* The transcriptional program controlled by the stem cell leukemia gene Scl/Tal1 during early embryonic hematopoietic development. *Blood* **113**, 5456-5465, doi:10.1182/blood-2009-01-200048 (2009).
87 Wilson, N. K. *et al.* Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. *Cell Stem Cell* **7**, 532-544, doi:10.1016/j.stem.2010.07.016 (2010).
88 Wilson, N. K. *et al.* Integrated genome-scale analysis of the transcriptional regulatory landscape in a blood stem/progenitor cell model. *Blood* **127**, e12-23, doi:10.1182/blood-2015-10-677393 (2016).
89 Sondergaard, E. *et al.* ERG Controls B Cell Development by Promoting Igh V-to-DJ Recombination. *Cell Rep* **29**, 2756-2769 e2756, doi:10.1016/j.celrep.2019.10.098 (2019).
90 Zhang, X. K. *et al.* The transcription factor Fli-1 modulates marginal zone and follicular B cell development in mice. *J Immunol* **181**, 1644-1654, doi:10.4049/jimmunol.181.3.1644 (2008).
91 Masuya, M. *et al.* Dysregulation of granulocyte, erythrocyte, and NK cell lineages in Fli-1 gene-targeted mice. *Blood* **105**, 95-102, doi:10.1182/blood-2003-12-4345 (2005).
92 Suzuki, E. *et al.* The transcription factor Fli-1 regulates monocyte, macrophage and dendritic cell development in mice. *Immunology* **139**, 318-327, doi:10.1111/imm.12070 (2013).
93 Zhu, Y. P., Thomas, G. D. & Hedrick, C. C. 2014 Jeffrey M. Hoeg Award Lecture: Transcriptional Control of Monocyte Development. *Arterioscler Thromb Vasc Biol* **36**, 1722-1733, doi:10.1161/ATVBAHA.116.304054 (2016).

94 Athanasiou, M. *et al.* Increased expression of the ETS-related transcription factor FLI-1/ERGB correlates with and can induce the megakaryocytic phenotype. *Cell Growth Differ* **7**, 1525-1534 (1996).
95 Jackers, P., Szalai, G., Moussa, O. & Watson, D. K. Ets-dependent regulation of target gene expression during megakaryopoiesis. *J Biol Chem* **279**, 52183-52190, doi:10.1074/jbc.M407489200 (2004).
96 Szalai, G., LaRue, A. C. & Watson, D. K. Molecular mechanisms of megakaryopoiesis. *Cell Mol Life Sci* **63**, 2460-2476, doi:10.1007/s00018-006-6190-8 (2006).
97 Rodrigues, N. P. *et al.* Haploinsufficiency of GATA-2 perturbs adult hematopoietic stem-cell homeostasis. *Blood* **106**, 477-484, doi:10.1182/blood-2004-08-2989 (2005).
98 Onodera, K. *et al.* GATA2 regulates dendritic cell differentiation. *Blood* **128**, 508-518, doi:10.1182/blood-2016-02-698118 (2016).
99 Nandakumar, S. K. *et al.* Low-level GATA2 overexpression promotes myeloid progenitor self-renewal and blocks lymphoid differentiation in mice. *Exp Hematol* **43**, 565-577 e561-510, doi:10.1016/j.exphem.2015.04.002 (2015).
100 Tsai, F. Y. & Orkin, S. H. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood* **89**, 3636-3643 (1997).
101 Walsh, J. C. *et al.* Cooperative and antagonistic interplay between PU.1 and GATA-2 in the specification of myeloid cell fates. *Immunity* **17**, 665-676, doi:10.1016/s1074-7613(02)00452-1 (2002).
102 Hock, H. *et al.* Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. *Nature* **431**, 1002-1007, doi:10.1038/nature02994 (2004).
103 van der Meer, L. T., Jansen, J. H. & van der Reijden, B. A. Gfi1 and Gfi1b: key regulators of hematopoiesis. *Leukemia* **24**, 1834-1843, doi:10.1038/leu.2010.195 (2010).
104 Li, L. *et al.* Nuclear adaptor Ldb1 regulates a transcriptional program essential for the maintenance of hematopoietic stem cells. *Nat Immunol* **12**, 129-136, doi:10.1038/ni.1978 (2011).
105 Visvader, J. E., Mao, X., Fujiwara, Y., Hahm, K. & Orkin, S. H. The LIM-domain binding protein Ldb1 and its partner LMO2 act as negative regulators of erythroid differentiation. *Proc Natl Acad Sci U S A* **94**, 13707-13712, doi:10.1073/pnas.94.25.13707 (1997).
106 Li, L. *et al.* A requirement for Lim domain binding protein 1 in erythropoiesis. *J Exp Med* **207**, 2543-2550, doi:10.1084/jem.20100504 (2010).
107 Cleveland, S. M. *et al.* Lmo2 induces hematopoietic stem cell-like features in T-cell progenitor cells prior to leukemia. *Stem Cells* **31**, 882-894, doi:10.1002/stem.1345 (2013).
108 Souroullas, G. P., Salmon, J. M., Sablitzky, F., Curtis, D. J. & Goodell, M. A. Adult hematopoietic stem and progenitor cells require either Lyl1 or Scl for survival. *Cell Stem Cell* **4**, 180-186, doi:10.1016/j.stem.2009.01.001 (2009).

109 Capron, C. *et al.* The SCL relative LYL-1 is required for fetal and adult hematopoietic stem cell function and B-cell differentiation. *Blood* **107**, 4678-4686, doi:10.1182/blood-2005-08-3145 (2006).
110 Miller, M. E., Rosten, P., Lemieux, M. E., Lai, C. & Humphries, R. K. Meis1 Is Required for Adult Mouse Erythropoiesis, Megakaryopoiesis and Hematopoietic Stem Cell Expansion. *PLoS One* **11**, e0151584, doi:10.1371/journal.pone.0151584 (2016).
111 Lieu, Y. K. & Reddy, E. P. Conditional c-myb knockout in adult hematopoietic stem cells leads to loss of self-renewal due to impaired proliferation and accelerated differentiation. *Proc Natl Acad Sci U S A* **106**, 21689-21694, doi:10.1073/pnas.0907623106 (2009).
112 Wang, X., Angelis, N. & Thein, S. L. MYB - A regulatory factor in hematopoiesis. *Gene* **665**, 6-17, doi:10.1016/j.gene.2018.04.065 (2018).
113 Friedman, A. D. Transcriptional regulation of granulocyte and monocyte development. *Oncogene* **21**, 3377-3390, doi:10.1038/sj.onc.1205324 (2002).
114 Ichikawa, M. *et al.* AML1/Runx1 negatively regulates quiescent hematopoietic stem cells in adult hematopoiesis. *J Immunol* **180**, 4402-4408, doi:10.4049/jimmunol.180.7.4402 (2008).
115 Blyth, K. *et al.* Runx1 promotes B-cell survival and lymphoma development. *Blood Cells Mol Dis* **43**, 12-19, doi:10.1016/j.bcmd.2009.01.013 (2009).
116 Seo, W., Ikawa, T., Kawamoto, H. & Taniuchi, I. Runx1-Cbfbeta facilitates early B lymphocyte development by regulating expression of Ebf1. *J Exp Med* **209**, 1255-1262, doi:10.1084/jem.20112745 (2012).
117 Niebuhr, B. *et al.* Runx1 is essential at two stages of early murine B-cell development. *Blood* **122**, 413-423, doi:10.1182/blood-2013-01-480244 (2013).
118 Taniuchi, I. *et al.* Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. *Cell* **111**, 621-633, doi:10.1016/s0092-8674(02)01111-x (2002).
119 Komine, O. *et al.* The Runx1 transcription factor inhibits the differentiation of naive CD4+ T cells into the Th2 lineage by repressing GATA3 expression. *J Exp Med* **198**, 51-61, doi:10.1084/jem.20021200 (2003).
120 Woolf, E. *et al.* Runx3 and Runx1 are required for CD8 T cell development during thymopoiesis. *Proc Natl Acad Sci U S A* **100**, 7731-7736, doi:10.1073/pnas.1232420100 (2003).
121 Egawa, T., Tillman, R. E., Naoe, Y., Taniuchi, I. & Littman, D. R. The role of the Runx transcription factors in thymocyte differentiation and in homeostasis of naive T cells. *J Exp Med* **204**, 1945-1957, doi:10.1084/jem.20070133 (2007).
122 Hsu, F. C. *et al.* An Essential Role for the Transcription Factor Runx1 in T Cell Maturation. *Sci Rep* **6**, 23533, doi:10.1038/srep23533 (2016).
123 Satpathy, A. T. *et al.* Runx1 and Cbfbeta regulate the development of Flt3+ dendritic cell progenitors and restrict myeloproliferative disorder. *Blood* **123**, 2968-2977, doi:10.1182/blood-2013-11-539643 (2014).
124 Ng, K. P. *et al.* Runx1 deficiency permits granulocyte lineage commitment but impairs subsequent maturation. *Oncogenesis* **2**, e78, doi:10.1038/oncsis.2013.41 (2013).

125 Pencovich, N. *et al.* Cell-autonomous function of Runx1 transcriptionally regulates mouse megakaryocytic maturation. *PLoS One* **8**, e64248, doi:10.1371/journal.pone.0064248 (2013).
126 Lecuyer, E. & Hoang, T. SCL: from the origin of hematopoiesis to stem cells and leukemia. *Exp Hematol* **32**, 11-24, doi:10.1016/j.exphem.2003.10.010 (2004).
127 Porcher, C., Chagraoui, H. & Kristiansen, M. S. SCL/TAL1: a multifaceted regulator from blood development to disease. *Blood* **129**, 2051-2060, doi:10.1182/blood-2016-12-754051 (2017).
128 Dey, S., Curtis, D. J., Jane, S. M. & Brandt, S. J. The TAL1/SCL transcription factor regulates cell cycle progression and proliferation in differentiating murine bone marrow monocyte precursors. *Mol Cell Biol* **30**, 2181-2192, doi:10.1128/MCB.01441-09 (2010).
129 Salmon, J. M. *et al.* Aberrant mast-cell differentiation in mice lacking the stem-cell leukemia gene. *Blood* **110**, 3573-3581, doi:10.1182/blood-2006-10-053124 (2007).
130 Hall, M. A. *et al.* The critical regulator of embryonic hematopoiesis, SCL, is vital in the adult for megakaryopoiesis, erythropoiesis, and lineage choice in CFU-S12. *Proc Natl Acad Sci U S A* **100**, 992-997, doi:10.1073/pnas.0237324100 (2003).
131 Goardon, N. *et al.* ETO2 coordinates cellular proliferation and differentiation during erythropoiesis. *EMBO J* **25**, 357-366, doi:10.1038/sj.emboj.7600934 (2006).
132 Rogers, H. *et al.* T-cell acute leukemia 1 (TAL1) regulation of erythropoietin receptor and association with excessive erythrocytosis. *J Biol Chem* **287**, 36720-36731, doi:10.1074/jbc.M112.378398 (2012).
133 Hasemann, M. S. *et al.* C/EBPalpha is required for long-term self-renewal and lineage priming of hematopoietic stem cells and for the maintenance of epigenetic configurations in multipotent progenitors. *PLoS Genet* **10**, e1004079, doi:10.1371/journal.pgen.1004079 (2014).
134 Guo, H., Barberi, T., Suresh, R. & Friedman, A. D. Progression from the Common Lymphoid Progenitor to B/Myeloid PreproB and ProB Precursors during B Lymphopoiesis Requires C/EBPalpha. *J Immunol* **201**, 1692-1704, doi:10.4049/jimmunol.1800244 (2018).
135 Ma, O., Hong, S., Guo, H., Ghiaur, G. & Friedman, A. D. Granulopoiesis requires increased C/EBPalpha compared to monopoiesis, correlated with elevated Cebpa in immature G-CSF receptor versus M-CSF receptor expressing cells. *PLoS One* **9**, e95784, doi:10.1371/journal.pone.0095784 (2014).
136 Avellino, R. *et al.* An autonomous CEBPA enhancer specific for myeloid-lineage priming and neutrophilic differentiation. *Blood* **127**, 2991-3003, doi:10.1182/blood-2016-01-695759 (2016).
137 Pal, R. *et al.* C/EBPbeta regulates transcription factors critical for proliferation and survival of multiple myeloma cells. *Blood* **114**, 3890-3898, doi:10.1182/blood-2009-01-201111 (2009).
138 Hirai, H. *et al.* C/EBPbeta is required for 'emergency' granulopoiesis. *Nat Immunol* **7**, 732-739, doi:10.1038/ni1354 (2006).

139 Akagi, T. *et al.* Impaired response to GM-CSF and G-CSF, and enhanced apoptosis in C/EBPbeta-deficient hematopoietic cells. *Blood* **111**, 2999-3004, doi:10.1182/blood-2007-04-087213 (2008).
140 Satake, S. *et al.* C/EBPbeta is involved in the amplification of early granulocyte precursors during candidemia-induced "emergency" granulopoiesis. *J Immunol* **189**, 4546-4555, doi:10.4049/jimmunol.1103007 (2012).
141 Ohkubo, Y. *et al.* A role for c-fos/activator protein 1 in B lymphocyte terminal differentiation. *J Immunol* **174**, 7703-7710, doi:10.4049/jimmunol.174.12.7703 (2005).
142 Lord, K. A., Abdollahi, A., Hoffman-Liebermann, B. & Liebermann, D. A. Proto-oncogenes of the fos/jun family of transcription factors are positive regulators of myeloid differentiation. *Mol Cell Biol* **13**, 841-851, doi:10.1128/mcb.13.2.841 (1993).
143 Liebermann, D. A., Gregory, B. & Hoffman, B. AP-1 (Fos/Jun) transcription factors in hematopoietic differentiation and apoptosis. *Int J Oncol* **12**, 685-700, doi:10.3892/ijo.12.3.685 (1998).
144 Shafarenko, M., Amanullah, A., Gregory, B., Liebermann, D. A. & Hoffman, B. Fos modulates myeloid cell survival and differentiation and partially abrogates the c-Myc block in terminal myeloid differentiation. *Blood* **103**, 4259-4267, doi:10.1182/blood-2002-09-2704 (2004).
145 Behre, G. *et al.* c-Jun is a JNK-independent coactivator of the PU.1 transcription factor. *J Biol Chem* **274**, 4939-4946, doi:10.1074/jbc.274.8.4939 (1999).
146 Cai, D. H. *et al.* C/EBP alpha:AP-1 leucine zipper heterodimers bind novel DNA elements, activate the PU.1 promoter and direct monocyte lineage commitment more potently than C/EBP alpha homodimers or AP-1. *Oncogene* **27**, 2772-2779, doi:10.1038/sj.onc.1210940 (2008).
147 Xiao, X. *et al.* Targeting JNK pathway promotes human hematopoietic stem cell expansion. *Cell Discov* **5**, 2, doi:10.1038/s41421-018-0072-8 (2019).
148 Wilson, A. *et al.* c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev* **18**, 2747-2763, doi:10.1101/gad.313104 (2004).
149 de Alboran, I. M. *et al.* Analysis of C-MYC function in normal cells via conditional gene-targeted mutation. *Immunity* **14**, 45-55, doi:10.1016/s1074-7613(01)00088-7 (2001).
150 Cattoretti, G. MYC expression and distribution in normal mature lymphoid cells. *J Pathol* **229**, 430-440, doi:10.1002/path.4141 (2013).
151 Chou, C. & Egawa, T. Myc or no Myc, that is the question. *EMBO J* **34**, 1990-1991, doi:10.15252/embj.201592267 (2015).
152 Gnanaprakasam, J. N. & Wang, R. MYC in Regulating Immunity: Metabolism and Beyond. *Genes (Basel)* **8**, doi:10.3390/genes8030088 (2017).
153 Pello, O. M. *et al.* In vivo inhibition of c-MYC in myeloid cells impairs tumor-associated macrophage maturation and pro-tumoral activities. *PLoS One* **7**, e45399, doi:10.1371/journal.pone.0045399 (2012).
154 Pello, O. M. *et al.* Role of c-MYC in alternative activation of human macrophages and tumor-associated macrophage biology. *Blood* **119**, 411-421, doi:10.1182/blood-2011-02-339911 (2012).

155 Enos, M. E., Bancos, S. A., Bushnell, T. & Crispe, I. N. E2F4 modulates differentiation and gene expression in hematopoietic progenitor cells during commitment to the lymphoid lineage. *J Immunol* **180**, 3699-3707, doi:10.4049/jimmunol.180.6.3699 (2008).
156 Humbert, P. O. *et al.* E2F4 is essential for normal erythrocyte maturation and neonatal viability. *Mol Cell* **6**, 281-291, doi:10.1016/s1097-2765(00)00029-0 (2000).
157 Kinross, K. M., Clark, A. J., Iazzolino, R. M. & Humbert, P. O. E2f4 regulates fetal erythropoiesis through the promotion of cellular proliferation. *Blood* **108**, 886-895, doi:10.1182/blood-2005-09-008656 (2006).
158 Min, I. M. *et al.* The transcription factor EGR1 controls both the proliferation and localization of hematopoietic stem cells. *Cell Stem Cell* **2**, 380-391, doi:10.1016/j.stem.2008.01.015 (2008).
159 Dinkel, A. *et al.* The transcription factor early growth response 1 (Egr-1) advances differentiation of pre-B and immature B cells. *J Exp Med* **188**, 2215-2224, doi:10.1084/jem.188.12.2215 (1998).
160 Bettini, M., Xi, H., Milbrandt, J. & Kersh, G. J. Thymocyte development in early growth response gene 1-deficient mice. *J Immunol* **169**, 1713-1720, doi:10.4049/jimmunol.169.4.1713 (2002).
161 Krishnaraju, K., Hoffman, B. & Liebermann, D. A. Early growth response gene 1 stimulates development of hematopoietic progenitor cells along the macrophage lineage at the expense of the granulocyte and erythroid lineages. *Blood* **97**, 1298-1305, doi:10.1182/blood.v97.5.1298 (2001).
162 Gibbs, J. D., Liebermann, D. A. & Hoffman, B. Egr-1 abrogates the E2F-1 block in terminal myeloid differentiation and suppresses leukemia. *Oncogene* **27**, 98-106, doi:10.1038/sj.onc.1210627 (2008).
163 Carter, J. H. & Tourtellotte, W. G. Early growth response transcriptional regulators are dispensable for macrophage differentiation. *J Immunol* **178**, 3038-3047, doi:10.4049/jimmunol.178.5.3038 (2007).
164 Calero-Nieto, F. J. *et al.* Transcriptional regulation of Elf-1: locus-wide analysis reveals four distinct promoters, a tissue-specific enhancer, control by PU.1 and the importance of Elf-1 downregulation for erythroid maturation. *Nucleic Acids Res* **38**, 6363-6374, doi:10.1093/nar/gkq490 (2010).
165 Choi, H. J. *et al.* Differential requirements for the Ets transcription factor Elf-1 in the development of NKT cells and NK cells. *Blood* **117**, 1880-1887, doi:10.1182/blood-2010-09-309468 (2011).
166 Barakat, S., Lambert, J., Sauvageau, G. & Hoang, T. ETO2 Controls Hematopoietic Stem Cell Expansion. *Blood* **114**, 396-396, doi:10.1182/blood.V114.22.396.396 (2009).
167 Steinauer, N. *et al.* ETO2 Regulates Cell-Fate Genes and Controls Relapse in Acute Myeloid Leukemia. *Blood* **130**, 3808-3808, doi:10.1182/blood.V130.Suppl_1.3808.3808 (2017).
168 Di Tullio, A., Passaro, D., Rouault-Pierre, K., Purewal, S. & Bonnet, D. Nuclear Factor Erythroid 2 Regulates Human HSC Self-Renewal and T Cell Differentiation by Preventing NOTCH1 Activation. *Stem Cell Reports* **9**, 5-11, doi:10.1016/j.stemcr.2017.05.027 (2017).

169 Shivdasani, R. A. *et al.* Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell* **81**, 695-704, doi:10.1016/0092-8674(95)90531-6 (1995).
170 Shivdasani, R. A. The role of transcription factor NF-E2 in megakaryocyte maturation and platelet production. *Stem Cells* **14 Suppl 1**, 112-115, doi:10.1002/stem.5530140714 (1996).
171 Gasiorek, J. J. & Blank, V. Regulation and function of the NFE2 transcription factor in hematopoietic and non-hematopoietic cells. *Cell Mol Life Sci* **72**, 2323-2335, doi:10.1007/s00018-015-1866-6 (2015).
172 Asai, T., Liu, Y., Bae, N. & Nimer, S. D. The p53 tumor suppressor protein regulates hematopoietic stem cell fate. *J Cell Physiol* **226**, 2215-2221, doi:10.1002/jcp.22561 (2011).
173 Dorritie, K. A., McCubrey, J. A. & Johnson, D. E. STAT transcription factors in hematopoiesis and leukemogenesis: opportunities for therapeutic intervention. *Leukemia* **28**, 248-257, doi:10.1038/leu.2013.192 (2014).
174 Quigley, M., Huang, X. & Yang, Y. STAT1 signaling in CD8 T cells is required for their clonal expansion and memory formation following viral infection in vivo. *J Immunol* **180**, 2158-2164, doi:10.4049/jimmunol.180.4.2158 (2008).
175 Halupa, A. *et al.* A novel role for STAT1 in regulating murine erythropoiesis: deletion of STAT1 results in overall reduction of erythroid progenitors and alters their distribution. *Blood* **105**, 552-561, doi:10.1182/blood-2003-09-3237 (2005).
176 Staber, P. B. *et al.* Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells. *Mol Cell* **49**, 934-946, doi:10.1016/j.molcel.2013.01.007 (2013).
177 Iwasaki, H. *et al.* Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation. *Blood* **106**, 1590-1600, doi:10.1182/blood-2005-03-0860 (2005).
178 Colucci, F. *et al.* Differential requirement for the transcription factor PU.1 in the generation of natural killer cells versus B and T cells. *Blood* **97**, 2625-2632, doi:10.1182/blood.v97.9.2625 (2001).
179 Luevano, M., Madrigal, A. & Saudemont, A. Transcription factors involved in the regulation of natural killer cell development and function: an update. *Front Immunol* **3**, 319, doi:10.3389/fimmu.2012.00319 (2012).
180 Klemsz, M. J., McKercher, S. R., Celada, A., Van Beveren, C. & Maki, R. A. The macrophage and B cell-specific transcription factor PU.1 is related to the ets oncogene. *Cell* **61**, 113-124, doi:10.1016/0092-8674(90)90219-5 (1990).
181 Oikawa, T. *et al.* The role of Ets family transcription factor PU.1 in hematopoietic cell differentiation, proliferation and apoptosis. *Cell Death Differ* **6**, 599-608, doi:10.1038/sj.cdd.4400534 (1999).
182 Anderson, M. K., Weiss, A. H., Hernandez-Hoyos, G., Dionne, C. J. & Rothenberg, E. V. Constitutive expression of PU.1 in fetal hematopoietic progenitors blocks T cell development at the pro-T cell stage. *Immunity* **16**, 285-296, doi:10.1016/s1074-7613(02)00277-7 (2002).
183 Rothenberg, E. V., Hosokawa, H. & Ungerback, J. Mechanisms of Action of Hematopoietic Transcription Factor PU.1 in Initiation of T-Cell Development. *Front Immunol* **10**, 228, doi:10.3389/fimmu.2019.00228 (2019).

184 Chopin, M. *et al.* Transcription Factor PU.1 Promotes Conventional Dendritic Cell Identity and Function via Induction of Transcriptional Regulator DC-SCRIPT. *Immunity* **50**, 77-90 e75, doi:10.1016/j.immuni.2018.11.010 (2019).
185 Fisher, R. C. *et al.* PU.1 supports proliferation of immature erythroid progenitors. *Leuk Res* **28**, 83-89, doi:10.1016/s0145-2126(03)00178-4 (2004).
186 Back, J., Dierich, A., Bronn, C., Kastner, P. & Chan, S. PU.1 determines the self-renewal capacity of erythroid progenitor cells. *Blood* **103**, 3615-3623, doi:10.1182/blood-2003-11-4089 (2004).
187 Chung, Y.-J., Kim, T.-M., Eaves, C. & Oh, I.-H. Role of STAT3 for hematopoietic stem cells. *International Journal of Hematology* **76**, 152-152, doi:10.1007/bf03165231 (2002).
188 Oh, I. H. & Eaves, C. J. Overexpression of a dominant negative form of STAT3 selectively impairs hematopoietic stem cell activity. *Oncogene* **21**, 4778-4787, doi:10.1038/sj.onc.1205592 (2002).
189 Mantel, C. *et al.* Mouse hematopoietic cell-targeted STAT3 deletion: stem/progenitor cell defects, mitochondrial dysfunction, ROS overproduction, and a rapid aging-like phenotype. *Blood* **120**, 2589-2599, doi:10.1182/blood-2012-01-404004 (2012).
190 Ding, C. *et al.* STAT3 Signaling in B Cells Is Critical for Germinal Center Maintenance and Contributes to the Pathogenesis of Murine Models of Lupus. *J Immunol* **196**, 4477-4486, doi:10.4049/jimmunol.1502043 (2016).
191 Deenick, E. K., Pelham, S. J., Kane, A. & Ma, C. S. Signal Transducer and Activator of Transcription 3 Control of Human T and B Cell Responses. *Front Immunol* **9**, 168, doi:10.3389/fimmu.2018.00168 (2018).
192 Oh, H. M. *et al.* STAT3 protein promotes T-cell survival and inhibits interleukin-2 production through up-regulation of Class O Forkhead transcription factors. *J Biol Chem* **286**, 30888-30897, doi:10.1074/jbc.M111.253500 (2011).
193 Siegel, A. M. *et al.* A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. *Immunity* **35**, 806-818, doi:10.1016/j.immuni.2011.09.016 (2011).
194 Laouar, Y., Welte, T., Fu, X.-Y. & Flavell, R. A. STAT3 Is Required for Flt3L-Dependent Dendritic Cell Differentiation. *Immunity* **19**, 903-912, doi:10.1016/s1074-7613(03)00332-7 (2003).
195 Panopoulos, A. D. *et al.* STAT3 governs distinct pathways in emergency granulopoiesis and mature neutrophils. *Blood* **108**, 3682-3690, doi:10.1182/blood-2006-02-003012 (2006).
196 Nguyen-Jackson, H., Panopoulos, A. D., Zhang, H., Li, H. S. & Watowich, S. S. STAT3 controls the neutrophil migratory response to CXCR2 ligands by direct activation of G-CSF-induced CXCR2 expression and via modulation of CXCR2 signal transduction. *Blood* **115**, 3354-3363, doi:10.1182/blood-2009-08-240317 (2010).
197 Miyakawa, Y. *et al.* Thrombopoietin induces tyrosine phosphorylation of Stat3 and Stat5 in human blood platelets. *Blood* **87**, 439-446 (1996).
198 Takayama, N. *et al.* Chromatin Accessibility Identifies CTCF As a Gatekeeper of Stemness Functions in Human Hematopoietic Development. *Blood* **128**, 3873-3873, doi:10.1182/blood.V128.22.3873.3873 (2016).

199 Kim, T. G. *et al.* CCCTC-binding factor is essential to the maintenance and quiescence of hematopoietic stem cells in mice. *Exp Mol Med* **49**, e371, doi:10.1038/emm.2017.124 (2017).
200 Majumder, P., Scharer, C. D., Choi, N. M. & Boss, J. M. B cell differentiation is associated with reprogramming the CCCTC binding factor-dependent chromatin architecture of the murine MHC class II locus. *J Immunol* **192**, 3925-3935, doi:10.4049/jimmunol.1303205 (2014).
201 Perez-Garcia, A. *et al.* CTCF orchestrates the germinal centre transcriptional program and prevents premature plasma cell differentiation. *Nat Commun* **8**, 16067, doi:10.1038/ncomms16067 (2017).
202 Quon, S. J. *et al.* CTCF is necessary for CD8⁺ effector T cell differentiation. *The Journal of Immunology* **202**, 125.126-125.126 (2019).
203 Koesters, C. *et al.* Regulation of dendritic cell differentiation and subset distribution by the zinc finger protein CTCF. *Immunol Lett* **109**, 165-174, doi:10.1016/j.imlet.2007.02.006 (2007).
204 Fisher, J. B. *et al.* The cohesin subunit Rad21 is a negative regulator of hematopoietic self-renewal through epigenetic repression of Hoxa7 and Hoxa9. *Leukemia* **31**, 712-719, doi:10.1038/leu.2016.240 (2017).
205 Gunal-Sadik, G. *et al.* Stage-specific binding profiles of cohesin in resting and activated B lymphocytes suggest a role for cohesin in immunoglobulin class switching and maturation. *PLoS One* **9**, e111748, doi:10.1371/journal.pone.0111748 (2014).
206 Seitan, V. C. *et al.* A role for cohesin in T-cell-receptor rearrangement and thymocyte differentiation. *Nature* **476**, 467-471, doi:10.1038/nature10312 (2011).
207 Kornberg, R. D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**, 285-294, doi:10.1016/s0092-8674(00)81958-3 (1999).
208 Bannister, A. J. & Kouzarides, T. Regulation of chromatin by histone modifications. *Cell Res* **21**, 381-395, doi:10.1038/cr.2011.22 (2011).
209 Belle, J. I. & Nijnik, A. H2A-DUBbing the mammalian epigenome: expanding frontiers for histone H2A deubiquitinating enzymes in cell biology and physiology. *Int J Biochem Cell Biol* **50**, 161-174, doi:10.1016/j.biocel.2014.03.004 (2014).
210 Cao, J. & Yan, Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. *Front Oncol* **2**, 26, doi:10.3389/fonc.2012.00026 (2012).
211 Greer, E. L. & Shi, Y. Histone methylation: a dynamic mark in health, disease and inheritance. *Nat Rev Genet* **13**, 343-357, doi:10.1038/nrg3173 (2012).
212 Dehennaut, V., Leprince, D. & Lefebvre, T. O-GlcNAcylation, an Epigenetic Mark. Focus on the Histone Code, TET Family Proteins, and Polycomb Group Proteins. *Front Endocrinol (Lausanne)* **5**, 155, doi:10.3389/fendo.2014.00155 (2014).
213 Christophorou, M. A. *et al.* Citrullination regulates pluripotency and histone H1 binding to chromatin. *Nature* **507**, 104-108, doi:10.1038/nature12942 (2014).
214 Sadakierska-Chudy, A. & Filip, M. A comprehensive view of the epigenetic landscape. Part II: Histone post-translational modification, nucleosome level, and chromatin regulation by ncRNAs. *Neurotox Res* **27**, 172-197, doi:10.1007/s12640-014-9508-6 (2015).

215 Rice, K. L., Hormaeche, I. & Licht, J. D. Epigenetic regulation of normal and malignant hematopoiesis. *Oncogene* **26**, 6697-6714, doi:10.1038/sj.onc.1210755 (2007).
216 Butler, J. S. & Dent, S. Y. The role of chromatin modifiers in normal and malignant hematopoiesis. *Blood* **121**, 3076-3084, doi:10.1182/blood-2012-10-451237 (2013).
217 Di Croce, L. & Helin, K. Transcriptional regulation by Polycomb group proteins. *Nat Struct Mol Biol* **20**, 1147-1155, doi:10.1038/nsmb.2669 (2013).
218 Martin-Perez, D., Piris, M. A. & Sanchez-Beato, M. Polycomb proteins in hematologic malignancies. *Blood* **116**, 5465-5475, doi:10.1182/blood-2010-05-267096 (2010).
219 Su, I. H. *et al.* Ezh2 controls B cell development through histone H3 methylation and Igh rearrangement. *Nat Immunol* **4**, 124-131, doi:10.1038/ni876 (2003).
220 Ernst, T. *et al.* Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet* **42**, 722-726, doi:10.1038/ng.621 (2010).
221 Nikoloski, G. *et al.* Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet* **42**, 665-667, doi:10.1038/ng.620 (2010).
222 Jankowska, A. M. *et al.* Mutational spectrum analysis of chronic myelomonocytic leukemia includes genes associated with epigenetic regulation: UTX, EZH2, and DNMT3A. *Blood* **118**, 3932-3941, doi:10.1182/blood-2010-10-311019 (2011).
223 Kim, K. H. & Roberts, C. W. Targeting EZH2 in cancer. *Nat Med* **22**, 128-134, doi:10.1038/nm.4036 (2016).
224 Letting, D. L., Rakowski, C., Weiss, M. J. & Blobel, G. A. Formation of a tissue-specific histone acetylation pattern by the hematopoietic transcription factor GATA-1. *Mol Cell Biol* **23**, 1334-1340, doi:10.1128/mcb.23.4.1334-1340.2003 (2003).
225 Huang, S., Qiu, Y., Stein, R. W. & Brandt, S. J. p300 functions as a transcriptional coactivator for the TAL1/SCL oncoprotein. *Oncogene* **18**, 4958-4967, doi:10.1038/sj.onc.1202889 (1999).
226 Purton, L. E. & Scadden, D. T. in *StemBook* (2008).
227 Morrison, S. J. & Scadden, D. T. The bone marrow niche for haematopoietic stem cells. *Nature* **505**, 327-334, doi:10.1038/nature12984 (2014).
228 Scadden, D. T. Nice neighborhood: emerging concepts of the stem cell niche. *Cell* **157**, 41-50, doi:10.1016/j.cell.2014.02.013 (2014).
229 Kfoury, Y. & Scadden, D. T. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* **16**, 239-253, doi:10.1016/j.stem.2015.02.019 (2015).
230 Yu, V. W. & Scadden, D. T. Hematopoietic Stem Cell and Its Bone Marrow Niche. *Curr Top Dev Biol* **118**, 21-44, doi:10.1016/bs.ctdb.2016.01.009 (2016).
231 Russell, E. S. Analysis of pleiotropism at the W-locus in the mouse; relationship between the effects of W and Wv substitution on hair pigmentation and on erythrocytes. *Genetics* **34**, 708-723 (1949).
232 Sarvella, P. A. & Russell, L. B. Steel, a New Dominant Gene in the House Mouse. *Journal of Heredity* **47**, 123-128, doi:10.1093/oxfordjournals.jhered.a106620 (1956).

233 Bernstein, S. E., Russell, E. S. & Keighley, G. TWO HEREDITARY MOUSE ANEMIAS (Sl/Sldand W/Wv) DEFICIENT IN RESPONSE TO ERYTHROPOIETIN. *Annals of the New York Academy of Sciences* **149**, 475-485, doi:10.1111/j.1749-6632.1968.tb15186.x (1968).
234 Ebbe, S., Phalen, E. & Stohlman, F., Jr. Abnormalities of megakaryocytes in S1-S1d mice. *Blood* **42**, 865-871 (1973).
235 Ebbe, S., Phalen, E. & Stohlman, F., Jr. Abnormalities of megakaryocytes in W-WV mice. *Blood* **42**, 857-864 (1973).
236 Ruscetti, F. W., Boggs, D. R., Torok, B. J. & Boggs, S. S. Reduced blood and marrow neutrophils and granulocytic colony-forming cells in S1/S1d mice. *Proc Soc Exp Biol Med* **152**, 398-402, doi:10.3181/00379727-152-39405 (1976).
237 Kitamura, Y., Go, S. & Hatanaka, K. Decrease of mast cells in W/Wv mice and their increase by bone marrow transplantation. *Blood* **52**, 447-452 (1978).
238 Kitamura, Y. & Go, S. Decreased production of mast cells in S1/S1d anemic mice. *Blood* **53**, 492-497 (1979).
239 Geissler, E. N. & Russell, E. S. Analysis of the hematopoietic effects of new dominant spotting (W) mutations of the mouse. II. Effects on mast cell development. *Exp Hematol* **11**, 461-466 (1983).
240 Mayer, T. C. & Green, M. C. An experimental analysis of the pigment defect caused by mutations at the W and S1 loci in mice. *Dev Biol* **18**, 62-75, doi:10.1016/0012-1606(68)90023-7 (1968).
241 Russell, E. S. & Bernstein, S. E. Proof of whole-cell implant in therapy of W-series anemia. *Arch Biochem Biophys* **125**, 594-597, doi:10.1016/0003-9861(68)90617-6 (1968).
242 Jarboe, D. L. & Huff, T. F. The mast cell-committed progenitor. II. W/Wv mice do not make mast cell-committed progenitors and S1/S1d fibroblasts do not support development of normal mast cell-committed progenitors. *J Immunol* **142**, 2418-2423 (1989).
243 Fujita, J., Onoue, H., Ebi, Y., Nakayama, H. & Kanakura, Y. In vitro duplication and in vivo cure of mast-cell deficiency of Sl/Sld mutant mice by cloned 3T3 fibroblasts. *Proc Natl Acad Sci U S A* **86**, 2888-2891, doi:10.1073/pnas.86.8.2888 (1989).
244 Whitlock, C. A., Tidmarsh, G. F., Muller-Sieburg, C. & Weissman, I. L. Bone marrow stromal cell lines with lymphopoietic activity express high levels of a pre-B neoplasia-associated molecule. *Cell* **48**, 1009-1021, doi:10.1016/0092-8674(87)90709-4 (1987).
245 Zsebo, K. M. *et al.* Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell* **63**, 213-224, doi:10.1016/0092-8674(90)90302-u (1990).
246 Zhang, J. *et al.* Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* **425**, 836-841, doi:10.1038/nature02041 (2003).
247 Anthony, B. A. & Link, D. C. Regulation of hematopoietic stem cells by bone marrow stromal cells. *Trends Immunol* **35**, 32-37, doi:10.1016/j.it.2013.10.002 (2014).
248 Xu, C. *et al.* Stem cell factor is selectively secreted by arterial endothelial cells in bone marrow. *Nat Commun* **9**, 2449, doi:10.1038/s41467-018-04726-3 (2018).

249 Sugiyama, T., Kohara, H., Noda, M. & Nagasawa, T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* **25**, 977-988, doi:10.1016/j.immuni.2006.10.016 (2006).
250 Battiwalla, M. & Hematti, P. Mesenchymal stem cells in hematopoietic stem cell transplantation. *Cytotherapy* **11**, 503-515, doi:10.1080/14653240903193806 (2009).
251 Mendez-Ferrer, S. *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* **466**, 829-834, doi:10.1038/nature09262 (2010).
252 Omatsu, Y. *et al.* The essential functions of adipo-osteogenic progenitors as the hematopoietic stem and progenitor cell niche. *Immunity* **33**, 387-399, doi:10.1016/j.immuni.2010.08.017 (2010).
253 Ding, L., Saunders, T. L., Enikolopov, G. & Morrison, S. J. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature* **481**, 457-462, doi:10.1038/nature10783 (2012).
254 Greenbaum, A. *et al.* CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature* **495**, 227-230, doi:10.1038/nature11926 (2013).
255 Taichman, R. S., Reilly, M. J. & Emerson, S. G. Osteoblasts and The Hematopoietic Microenvironment. *Hematology* **4**, 421-426, doi:10.1080/10245332.1999.11746468 (1999).
256 Calvi, L. M. *et al.* Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* **425**, 841-846, doi:10.1038/nature02040 (2003).
257 Jung, Y. *et al.* Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. *Bone* **38**, 497-508, doi:10.1016/j.bone.2005.10.003 (2006).
258 Qian, H. *et al.* Critical role of thrombopoietin in maintaining adult quiescent hematopoietic stem cells. *Cell Stem Cell* **1**, 671-684, doi:10.1016/j.stem.2007.10.008 (2007).
259 Wu, J. Y., Scadden, D. T. & Kronenberg, H. M. Role of the osteoblast lineage in the bone marrow hematopoietic niches. *J Bone Miner Res* **24**, 759-764, doi:10.1359/jbmr.090225 (2009).
260 Yamazaki, S. *et al.* TGF-beta as a candidate bone marrow niche signal to induce hematopoietic stem cell hibernation. *Blood* **113**, 1250-1256, doi:10.1182/blood-2008-04-146480 (2009).
261 Yamazaki, S. *et al.* Nonmyelinating Schwann cells maintain hematopoietic stem cell hibernation in the bone marrow niche. *Cell* **147**, 1146-1158, doi:10.1016/j.cell.2011.09.053 (2011).
262 Naveiras, O. *et al.* Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature* **460**, 259-263, doi:10.1038/nature08099 (2009).
263 Yoneyama, M. *et al.* Structural and functional differences of SWIRM domain subtypes. *J Mol Biol* **369**, 222-238, doi:10.1016/j.jmb.2007.03.027 (2007).

264 Jiang, X. X. *et al.* Control of B cell development by the histone H2A deubiquitinase MYSM1. *Immunity* **35**, 883-896, doi:10.1016/j.immuni.2011.11.010 (2011).
265 Nandakumar, V., Chou, Y., Zang, L., Huang, X. F. & Chen, S. Y. Epigenetic control of natural killer cell maturation by histone H2A deubiquitinase, MYSM1. *Proc Natl Acad Sci U S A* **110**, E3927-3936, doi:10.1073/pnas.1308888110 (2013).
266 Won, H. *et al.* Epigenetic control of dendritic cell development and fate determination of common myeloid progenitor by Mysm1. *Blood* **124**, 2647-2656, doi:10.1182/blood-2013-10-534313 (2014).
267 Fiore, A. *et al.* Deubiquitinase MYSM1 in the Hematopoietic System and beyond: A Current Review. *Int J Mol Sci* **21**, 3007, doi:10.3390/ijms21083007 (2020).
268 Zhu, P. *et al.* A histone H2A deubiquitinase complex coordinating histone acetylation and H1 dissociation in transcriptional regulation. *Mol Cell* **27**, 609-621, doi:10.1016/j.molcel.2007.07.024 (2007).
269 Panda, S., Nilsson, J. A. & Gekara, N. O. Deubiquitinase MYSM1 Regulates Innate Immunity through Inactivation of TRAF3 and TRAF6 Complexes. *Immunity* **43**, 647-659, doi:10.1016/j.immuni.2015.09.010 (2015).
270 Panda, S. & Gekara, N. O. The deubiquitinase MYSM1 dampens NOD2-mediated inflammation and tissue damage by inactivating the RIP2 complex. *Nat Commun* **9**, 4654, doi:10.1038/s41467-018-07016-0 (2018).
271 Williamson, M. P. Using chemical shift perturbation to characterise ligand binding. *Prog Nucl Magn Reson Spectrosc* **73**, 1-16, doi:10.1016/j.pnmrs.2013.02.001 (2013).
272 Wang, H. *et al.* Role of histone H2A ubiquitination in Polycomb silencing. *Nature* **431**, 873-878, doi:10.1038/nature02985 (2004).
273 Steffen, P. A. & Ringrose, L. What are memories made of? How Polycomb and Trithorax proteins mediate epigenetic memory. *Nat Rev Mol Cell Biol* **15**, 340-356, doi:10.1038/nrm3789 (2014).
274 Vidal, M. & Starowicz, K. Polycomb complexes PRC1 and their function in hematopoiesis. *Exp Hematol* **48**, 12-31, doi:10.1016/j.exphem.2016.12.006 (2017).
275 Jiang, X. X. *et al.* Epigenetic Regulation of Antibody Responses by the Histone H2A Deubiquitinase MYSM1. *Sci Rep* **5**, 13755, doi:10.1038/srep13755 (2015).
276 Jiang, X. X. *et al.* MYSM1/miR-150/FLT3 inhibits B1a cell proliferation. *Oncotarget* **7**, 68086-68096, doi:10.18632/oncotarget.11738 (2016).
277 Belle, J. I. *et al.* Repression of p53-target gene Bbc3/PUMA by MYSM1 is essential for the survival of hematopoietic multipotent progenitors and contributes to stem cell maintenance. *Cell Death Differ* **23**, 759-775, doi:10.1038/cdd.2015.140 (2016).
278 Alsultan, A., Shamseldin, H. E., Osman, M. E., Aljabri, M. & Alkuraya, F. S. MYSM1 is mutated in a family with transient transfusion-dependent anemia, mild thrombocytopenia, and low NK- and B-cell counts. *Blood* **122**, 3844-3845, doi:10.1182/blood-2013-09-527127 (2013).
279 Le Guen, T. *et al.* An in vivo genetic reversion highlights the crucial role of Myb-Like, SWIRM, and MPN domains 1 (MYSM1) in human hematopoiesis and

lymphocyte differentiation. *J Allergy Clin Immunol* **136**, 1619-1626 e1615, doi:10.1016/j.jaci.2015.06.008 (2015).
280 Bahrami, E. *et al.* Myb-like, SWIRM, and MPN domains 1 (MYSM1) deficiency: Genotoxic stress-associated bone marrow failure and developmental aberrations. *J Allergy Clin Immunol* **140**, 1112-1119, doi:10.1016/j.jaci.2016.10.053 (2017).
281 Nanda, A. *et al.* Neutrophilic Panniculitis in a child with MYSM1 deficiency. *Pediatr Dermatol* **36**, 258-259, doi:10.1111/pde.13757 (2019).
282 Dokal, I. & Vulliamy, T. Inherited bone marrow failure syndromes. *Haematologica* **95**, 1236-1240, doi:10.3324/haematol.2010.025619 (2010).
283 Chirnomas, S. D. & Kupfer, G. M. The inherited bone marrow failure syndromes. *Pediatr Clin North Am* **60**, 1291-1310, doi:10.1016/j.pcl.2013.09.007 (2013).
284 Alter, B. P. Inherited bone marrow failure syndromes: considerations pre- and posttransplant. *Blood* **130**, 2257-2264, doi:10.1182/blood-2017-05-781799 (2017).
285 Belle, J. I. *et al.* MYSM1 maintains ribosomal protein gene expression in hematopoietic stem cells to prevent hematopoietic dysfunction. *JCI Insight* **5**, doi:10.1172/jci.insight.125690 (2020).
286 Diamond, L. K., Wang, W. C. & Alter, B. P. Congenital hypoplastic anemia. *Adv Pediatr* **22**, 349-378 (1976).
287 Vlachos, A. *et al.* Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* **142**, 859-876, doi:10.1111/j.1365-2141.2008.07269.x (2008).
288 Boria, I. *et al.* The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update. *Hum Mutat* **31**, 1269-1279, doi:10.1002/humu.21383 (2010).
289 Ulirsch, J. C. *et al.* The Genetic Landscape of Diamond-Blackfan Anemia. *Am J Hum Genet* **103**, 930-947, doi:10.1016/j.ajhg.2018.10.027 (2018).
290 Clinton, C. & Gazda, H. T. in *GeneReviews((R))* (eds M. P. Adam *et al.*) (1993).
291 Draptchinskaia, N. *et al.* The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* **21**, 169-175, doi:10.1038/5951 (1999).
292 Klar, J., Khalfallah, A., Arzoo, P. S., Gazda, H. T. & Dahl, N. Recurrent GATA1 mutations in Diamond-Blackfan anaemia. *Br J Haematol* **166**, 949-951, doi:10.1111/bjh.12919 (2014).
293 Gripp, K. W. *et al.* Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. *Am J Med Genet A* **164A**, 2240-2249, doi:10.1002/ajmg.a.36633 (2014).
294 Khajuria, R. K. *et al.* Ribosome Levels Selectively Regulate Translation and Lineage Commitment in Human Hematopoiesis. *Cell* **173**, 90-103 e119, doi:10.1016/j.cell.2018.02.036 (2018).
295 Stratton, I. M. *et al.* Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* **321**, 405-412, doi:10.1136/bmj.321.7258.405 (2000).
296 Brownlee, M. Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813-820, doi:10.1038/414813a (2001).
297 Caldwell, R. B. *et al.* Vascular endothelial growth factor and diabetic retinopathy: pathophysiological mechanisms and treatment perspectives. *Diabetes Metab Res Rev* **19**, 442-455, doi:10.1002/dmrr.415 (2003).

298 Huang, Y. C. *et al.* Genome-wide association study of diabetic retinopathy in a Taiwanese population. *Ophthalmology* **118**, 642-648, doi:10.1016/j.ophtha.2010.07.020 (2011).
299 Sharma, A., Valle, M. L., Beveridge, C., Liu, Y. & Sharma, S. Unraveling the role of genetics in the pathogenesis of diabetic retinopathy. *Eye (Lond)* **33**, 534-541, doi:10.1038/s41433-019-0337-y (2019).
300 Han, J., Lando, L., Skowronska-Krawczyk, D. & Chao, D. L. Genetics of Diabetic Retinopathy. *Curr Diab Rep* **19**, 67, doi:10.1007/s11892-019-1186-6 (2019).
301 Fan, W. Y. *et al.* Association of candidate gene polymorphisms with diabetic retinopathy in Chinese patients with type 2 diabetes. *Int J Ophthalmol* **13**, 301-308, doi:10.18240/ijo.2020.02.15 (2020).
302 Cabrera, A. P. *et al.* Do Genomic Factors Play a Role in Diabetic Retinopathy? *J Clin Med* **9**, doi:10.3390/jcm9010216 (2020).
303 Sykes, S. M. & Scadden, D. T. Modeling human hematopoietic stem cell biology in the mouse. *Semin Hematol* **50**, 92-100, doi:10.1053/j.seminhematol.2013.03.029 (2013).
304 Schmitt, C. E., Lizama, C. O. & Zovein, A. C. From transplantation to transgenics: mouse models of developmental hematopoiesis. *Exp Hematol* **42**, 707-716, doi:10.1016/j.exphem.2014.06.008 (2014).
305 Nijnik, A. *et al.* The critical role of histone H2A-deubiquitinase Mysm1 in hematopoiesis and lymphocyte differentiation. *Blood* **119**, 1370-1379, doi:10.1182/blood-2011-05-352666 (2012).
306 Huo, Y. *et al.* MYSM1 Is Essential for Maintaining Hematopoietic Stem Cell (HSC) Quiescence and Survival. *Med Sci Monit* **24**, 2541-2549, doi:10.12659/msm.906876 (2018).
307 Forster, M. *et al.* Deubiquitinase MYSM1 Is Essential for Normal Fetal Liver Hematopoiesis and for the Maintenance of Hematopoietic Stem Cells in Adult Bone Marrow. *Stem Cells Dev* **24**, 1865-1877, doi:10.1089/scd.2015.0058 (2015).
308 Gatzka, M. *et al.* Interplay of H2A deubiquitinase 2A-DUB/Mysm1 and the p19(ARF)/p53 axis in hematopoiesis, early T-cell development and tissue differentiation. *Cell Death Differ* **22**, 1451-1462, doi:10.1038/cdd.2014.231 (2015).
309 Belle, J. I. *et al.* p53 mediates loss of hematopoietic stem cell function and lymphopenia in Mysm1 deficiency. *Blood* **125**, 2344-2348, doi:10.1182/blood-2014-05-574111 (2015).
310 Forster, M. *et al.* A role for the histone H2A deubiquitinase MYSM1 in maintenance of CD8(+) T cells. *Immunology* **151**, 110-121, doi:10.1111/imm.12710 (2017).
311 Zhao, X. *et al.* Deubiquitinase Mysm1 regulates macrophage survival and polarization. *Mol Biol Rep* **45**, 2393-2401, doi:10.1007/s11033-018-4405-3 (2018).
312 Donehower, L. A. *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221, doi:10.1038/356215a0 (1992).

313 Martins, V. C. *et al.* Cell competition is a tumour suppressor mechanism in the thymus. *Nature* **509**, 465-470, doi:10.1038/nature13317 (2014).
314 Wilms, C. *et al.* MYSM1/2A-DUB is an epigenetic regulator in human melanoma and contributes to tumor cell growth. *Oncotarget* **8**, 67287-67299, doi:10.18632/oncotarget.18617 (2017).
315 Li, Y., Li, J., Liu, H., Liu, Y. & Cui, B. Expression of MYSM1 is associated with tumor progression in colorectal cancer. *PLoS One* **12**, e0177235, doi:10.1371/journal.pone.0177235 (2017).
316 Wilson, N. K. *et al.* Gfi1 expression is controlled by five distinct regulatory regions spread over 100 kilobases, with Scl/Tal1, Gata2, PU.1, Erg, Meis1, and Runx1 acting as upstream regulators in early hematopoietic cells. *Mol Cell Biol* **30**, 3853-3863, doi:10.1128/MCB.00032-10 (2010).
317 Rosmaraki, E. E. *et al.* Identification of committed NK cell progenitors in adult murine bone marrow. *Eur J Immunol* **31**, 1900-1909, doi:10.1002/1521-4141(200106)31:6<1900::aid-immu1900>3.0.co;2-m (2001).
318 Kim, S. *et al.* In vivo developmental stages in murine natural killer cell maturation. *Nat Immunol* **3**, 523-528, doi:10.1038/ni796 (2002).
319 Boos, M. D., Yokota, Y., Eberl, G. & Kee, B. L. Mature natural killer cell and lymphoid tissue-inducing cell development requires Id2-mediated suppression of E protein activity. *J Exp Med* **204**, 1119-1130, doi:10.1084/jem.20061959 (2007).
320 Gascoyne, D. M. *et al.* The basic leucine zipper transcription factor E4BP4 is essential for natural killer cell development. *Nat Immunol* **10**, 1118-1124, doi:10.1038/ni.1787 (2009).
321 McKenna, K., Beignon, A. S. & Bhardwaj, N. Plasmacytoid dendritic cells: linking innate and adaptive immunity. *J Virol* **79**, 17-27, doi:10.1128/JVI.79.1.17-27.2005 (2005).
322 Ishikawa, F. *et al.* The developmental program of human dendritic cells is operated independently of conventional myeloid and lymphoid pathways. *Blood* **110**, 3591-3660, doi:10.1182/blood-2007-02-071613 (2007).
323 Vremec, D. *et al.* The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. *Eur J Immunol* **27**, 40-44, doi:10.1002/eji.1830270107 (1997).
324 D'Amico, A. & Wu, L. The early progenitors of mouse dendritic cells and plasmacytoid predendritic cells are within the bone marrow hemopoietic precursors expressing Flt3. *J Exp Med* **198**, 293-303, doi:10.1084/jem.20030107 (2003).
325 Xu, Y., Zhan, Y., Lew, A. M., Naik, S. H. & Kershaw, M. H. Differential development of murine dendritic cells by GM-CSF versus Flt3 ligand has implications for inflammation and trafficking. *J Immunol* **179**, 7577-7584, doi:10.4049/jimmunol.179.11.7577 (2007).
326 Zhang, D. E., Hetherington, C. J., Chen, H. M. & Tenen, D. G. The macrophage transcription factor PU.1 directs tissue-specific expression of the macrophage colony-stimulating factor receptor. *Mol Cell Biol* **14**, 373-381, doi:10.1128/mcb.14.1.373 (1994).

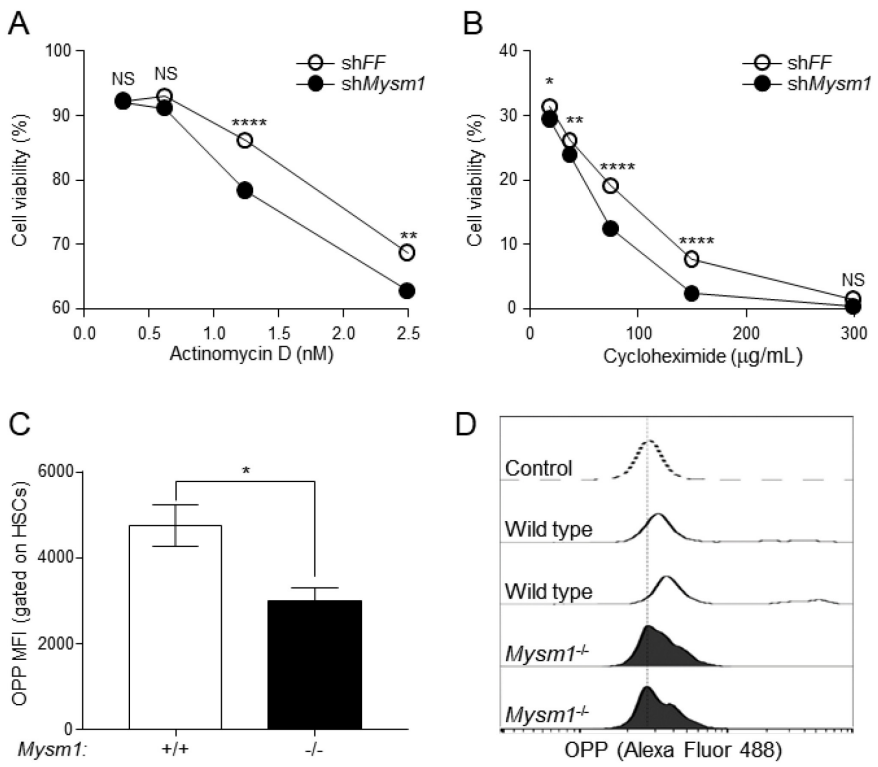
327 Hohaus, S. *et al.* PU.1 (Spi-1) and C/EBP alpha regulate expression of the granulocyte-macrophage colony-stimulating factor receptor alpha gene. *Mol Cell Biol* **15**, 5830-5845, doi:10.1128/mcb.15.10.5830 (1995).
328 Anderson, K. L. *et al.* Transcription factor PU.1 is necessary for development of thymic and myeloid progenitor-derived dendritic cells. *J Immunol* **164**, 1855-1861, doi:10.4049/jimmunol.164.4.1855 (2000).
329 Kastner, P. & Chan, S. PU.1: a crucial and versatile player in hematopoiesis and leukemia. *Int J Biochem Cell Biol* **40**, 22-27, doi:10.1016/j.biocel.2007.01.026 (2008).
330 Skarnes, W. C. *et al.* A conditional knockout resource for the genome-wide study of mouse gene function. *Nature* **474**, 337-342, doi:10.1038/nature10163 (2011).
331 Farley, F. W., Soriano, P., Steffen, L. S. & Dymecki, S. M. Widespread recombinase expression using FLPeR (flipper) mice. *Genesis* **28**, 106-110 (2000).
332 Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120, doi:10.1093/bioinformatics/btu170 (2014).
333 Kim, D. *et al.* TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* **14**, R36, doi:10.1186/gb-2013-14-4-r36 (2013).
334 Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **10**, R25, doi:10.1186/gb-2009-10-3-r25 (2009).
335 Trapnell, C., Pachter, L. & Salzberg, S. L. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics* **25**, 1105-1111, doi:10.1093/bioinformatics/btp120 (2009).
336 Liao, Y., Smyth, G. K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-930, doi:10.1093/bioinformatics/btt656 (2014).
337 Bolstad, B. preprocessCore: A collection of pre-processing functions. R package version 1.44.0. *https://githubcom/bmbolstad/preprocessCore* (2018).
338 Robinson, M. D. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol* **11**, R25, doi:10.1186/gb-2010-11-3-r25 (2010).
339 Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140, doi:10.1093/bioinformatics/btp616 (2010).
340 McCarthy, D. J., Chen, Y. & Smyth, G. K. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res* **40**, 4288-4297, doi:10.1093/nar/gks042 (2012).
341 Mevik, B.-H. & Wehrens, R. ThepIsPackage: Principal Component and Partial Least Squares Regression inR. *Journal of Statistical Software* **18**, 23, doi:10.18637/jss.v018.i02 (2007).
342 Thorvaldsdottir, H., Robinson, J. T. & Mesirov, J. P. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. *Brief Bioinform* **14**, 178-192, doi:10.1093/bib/bbs017 (2013).

343 Huang, D. W. *et al.* The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. *Genome Biol* **8**, R183, doi:10.1186/gb-2007-8-9-r183 (2007).
344 Mootha, V. K. *et al.* PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet* **34**, 267-273, doi:10.1038/ng1180 (2003).
345 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545-15550, doi:10.1073/pnas.0506580102 (2005).
346 Pinto do, O. P., Kolterud, A. & Carlsson, L. Expression of the LIM-homeobox gene LH2 generates immortalized steel factor-dependent multipotent hematopoietic precursors. *EMBO J* **17**, 5744-5756, doi:10.1093/emboj/17.19.5744 (1998).
347 Mavrakis, K. J. *et al.* Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. *Nat Cell Biol* **12**, 372-379, doi:10.1038/ncb2037 (2010).
348 Langlais, D., Couture, C., Balsalobre, A. & Drouin, J. The Stat3/GR interaction code: predictive value of direct/indirect DNA recruitment for transcription outcome. *Mol Cell* **47**, 38-49, doi:10.1016/j.molcel.2012.04.021 (2012).
349 Zhang, Y. *et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**, R137, doi:10.1186/gb-2008-9-9-r137 (2008).
350 Heinz, S. *et al.* Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* **38**, 576-589, doi:10.1016/j.molcel.2010.05.004 (2010).
351 McLean, C. Y. *et al.* GREAT improves functional interpretation of cis-regulatory regions. *Nat Biotechnol* **28**, 495-501, doi:10.1038/nbt.1630 (2010).
352 Da Costa, L., Narla, A. & Mohandas, N. An update on the pathogenesis and diagnosis of Diamond-Blackfan anemia. *F1000Res* **7**, doi:10.12688/f1000research.15542.1 (2018).
353 Suda, T., Takubo, K. & Semenza, G. L. Metabolic regulation of hematopoietic stem cells in the hypoxic niche. *Cell Stem Cell* **9**, 298-310, doi:10.1016/j.stem.2011.09.010 (2011).
354 Huttmann, A. *et al.* Gene expression profiles in murine hematopoietic stem cells revisited: analysis of cDNA libraries reveals high levels of translational and metabolic activities. *Stem Cells* **24**, 1719-1727, doi:10.1634/stemcells.2005-0486 (2006).
355 Signer, R. A., Magee, J. A., Salic, A. & Morrison, S. J. Haematopoietic stem cells require a highly regulated protein synthesis rate. *Nature* **509**, 49-54, doi:10.1038/nature13035 (2014).
356 Barna, M. & Ruggero, D. Tailor made protein synthesis for HSCs. *Cell Stem Cell* **14**, 423-424, doi:10.1016/j.stem.2014.03.011 (2014).
357 Basu, A., Wilkinson, F. H., Colavita, K., Fennelly, C. & Atchison, M. L. YY1 DNA binding and interaction with YAF2 is essential for Polycomb recruitment. *Nucleic Acids Res* **42**, 2208-2223, doi:10.1093/nar/gkt1187 (2014).

358 Lu, Z. *et al.* Polycomb Group Protein YY1 Is an Essential Regulator of Hematopoietic Stem Cell Quiescence. *Cell Rep* **22**, 1545-1559, doi:10.1016/j.celrep.2018.01.026 (2018).
359 van Riggelen, J., Yetil, A. & Felsher, D. W. MYC as a regulator of ribosome biogenesis and protein synthesis. *Nat Rev Cancer* **10**, 301-309, doi:10.1038/nrc2819 (2010).
360 Dey, A. *et al.* Loss of the tumor suppressor BAP1 causes myeloid transformation. *Science* **337**, 1541-1546, doi:10.1126/science.1221711 (2012).
361 Lancini, C. *et al.* Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells. *J Exp Med* **211**, 1759-1777, doi:10.1084/jem.20131436 (2014).
362 Gu, Y. *et al.* The histone H2A deubiquitinase Usp16 regulates hematopoiesis and hematopoietic stem cell function. *Proc Natl Acad Sci U S A* **113**, E51-60, doi:10.1073/pnas.1517041113 (2016).
363 Ban, N. *et al.* A new system for naming ribosomal proteins. *Curr Opin Struct Biol* **24**, 165-169, doi:10.1016/j.sbi.2014.01.002 (2014).
364 Bohnsack, K. E. & Bohnsack, M. T. Uncovering the assembly pathway of human ribosomes and its emerging links to disease. *EMBO J* **38**, e100278, doi:10.15252/embj.2018100278 (2019).
365 Thomson, E., Ferreira-Cerca, S. & Hurt, E. Eukaryotic ribosome biogenesis at a glance. *J Cell Sci* **126**, 4815-4821, doi:10.1242/jcs.111948 (2013).
366 Liu, J., Xu, Y., Stoleru, D. & Salic, A. Imaging protein synthesis in cells and tissues with an alkyne analog of puromycin. *Proc Natl Acad Sci U S A* **109**, 413-418, doi:10.1073/pnas.1111561108 (2012).
367 Russo, A. & Russo, G. Ribosomal Proteins Control or Bypass p53 during Nucleolar Stress. *Int J Mol Sci* **18**, doi:10.3390/ijms18010140 (2017).
368 Boulon, S., Westman, B. J., Hutten, S., Boisvert, F. M. & Lamond, A. I. The nucleolus under stress. *Mol Cell* **40**, 216-227, doi:10.1016/j.molcel.2010.09.024 (2010).
369 Golomb, L., Volarevic, S. & Oren, M. p53 and ribosome biogenesis stress: the essentials. *FEBS Lett* **588**, 2571-2579, doi:10.1016/j.febslet.2014.04.014 (2014).
370 Dai, M. S. & Lu, H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. *J Biol Chem* **279**, 44475-44482, doi:10.1074/jbc.M403722200 (2004).
371 Fumagalli, S., Ivanenkov, V. V., Teng, T. & Thomas, G. Suprainduction of p53 by disruption of 40S and 60S ribosome biogenesis leads to the activation of a novel G2/M checkpoint. *Genes Dev* **26**, 1028-1040, doi:10.1101/gad.189951.112 (2012).
372 Bhat, K. P., Itahana, K., Jin, A. & Zhang, Y. Essential role of ribosomal protein L11 in mediating growth inhibition-induced p53 activation. *EMBO J* **23**, 2402-2412, doi:10.1038/sj.emboj.7600247 (2004).
373 Zhu, Y. *et al.* Ribosomal protein S7 is both a regulator and a substrate of MDM2. *Mol Cell* **35**, 316-326, doi:10.1016/j.molcel.2009.07.014 (2009).
374 Ofir-Rosenfeld, Y., Boggs, K., Michael, D., Kastan, M. B. & Oren, M. Mdm2 regulates p53 mRNA translation through inhibitory interactions with ribosomal protein L26. *Mol Cell* **32**, 180-189, doi:10.1016/j.molcel.2008.08.031 (2008).

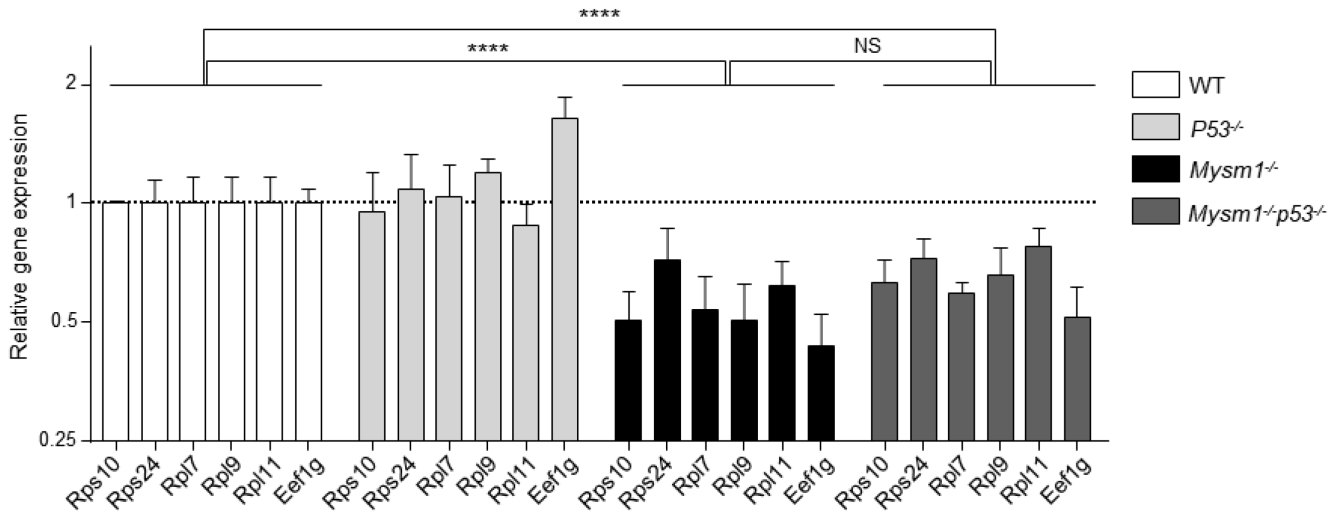
375 Fargo, J. H. *et al.* Erythrocyte adenosine deaminase: diagnostic value for Diamond-Blackfan anaemia. *Br J Haematol* **160**, 547-554, doi:10.1111/bjh.12167 (2013).
376 Narla, A. & Ebert, B. L. Ribosomopathies: human disorders of ribosome dysfunction. *Blood* **115**, 3196-3205, doi:10.1182/blood-2009-10-178129 (2010).
377 Langlais, D., Couture, C., Balsalobre, A. & Drouin, J. Regulatory network analyses reveal genome-wide potentiation of LIF signaling by glucocorticoids and define an innate cell defense response. *PLoS Genet* **4**, e1000224, doi:10.1371/journal.pgen.1000224 (2008).
378 Medeiros, R. B. *et al.* Novel sequential ChIP and simplified basic ChIP protocols for promoter co-occupancy and target gene identification in human embryonic stem cells. *BMC Biotechnol* **9**, 59, doi:10.1186/1472-6750-9-59 (2009).
379 Furlan-Magaril, M., Rincon-Arano, H. & Recillas-Targa, F. Sequential chromatin immunoprecipitation protocol: ChIP-reChIP. *Methods Mol Biol* **543**, 253-266, doi:10.1007/978-1-60327-015-1_17 (2009).
380 Tikhanovich, I. *et al.* Arginine methylation regulates c-Myc-dependent transcription by altering promoter recruitment of the acetyltransferase p300. *J Biol Chem* **292**, 13333-13344, doi:10.1074/jbc.M117.797928 (2017).
381 Desvoyes, B., Sequeira-Mendes, J., Vergara, Z., Madeira, S. & Gutierrez, C. Sequential ChIP Protocol for Profiling Bivalent Epigenetic Modifications (ReChIP). *Methods Mol Biol* **1675**, 83-97, doi:10.1007/978-1-4939-7318-7_6 (2018).
382 Beischlag, T. V., Prefontaine, G. G. & Hankinson, O. ChIP-re-ChIP: Co-occupancy Analysis by Sequential Chromatin Immunoprecipitation. *Methods Mol Biol* **1689**, 103-112, doi:10.1007/978-1-4939-7380-4_9 (2018).

Appendix II: Supplemental Figures



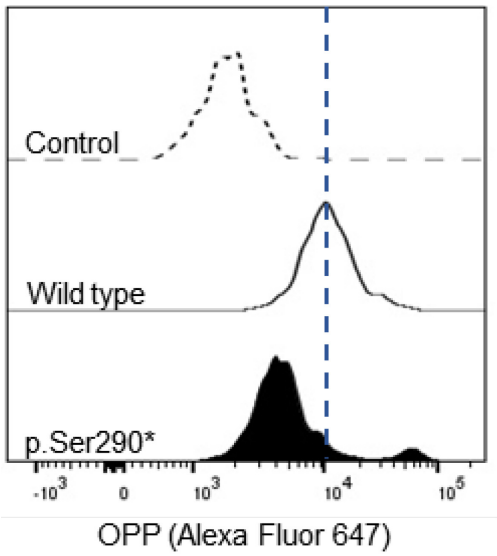
Supplemental Figure 1. Hypersensitivity to ribosomal stress and reduction in protein synthesis rates

(A-B) *Mysm1*-knockdown Ba/F3 cells are hypersensitive to ribosomal and translational stress. Control sh*FF* and knockdown sh*Mysm1* cells were treated with **(A)** actinomycin D or **(B)** cycloheximide across of a range of concentrations for 48 hours, and cell viability measured by flow cytometry. The data is from three *shMysm1* and three *shFF* lines, each analyzed in duplicate and was reproduced in two independent experiments. Statistical comparisons using 2-way ANOVA with Sidak's post-hoc test; * $p<0.05$, ** $p<0.01$, **** $p<0.0001$, NS - non-significant. **(C-D)** Reduction in the protein synthesis rate in *Mysm1*-/- primary HSCs, measured using the *in vivo* OPP-incorporation method. HSCs gated as live Lin- cKit+Sca1+CD150+CD34-Flt3-. **(C)** Bar chart comparing the OPP incorporation levels in wildtype and *Mysm1*-/- HSCs. Four mice were analyzed per genotype in total in two independent experiments; means ± SEM are presented; statistical comparison using Student's *t*-test; * $p<0.05$. **(D)** Representative flow cytometry histograms showing OPP-incorporation in HSCs from two wildtype and two *Mysm1*-/- mice. The control histogram represents a mouse that received DMSO vehicle control, instead of the OPP injection. This data is obtained from a colleague in the lab.



Supplemental Figure 2. MYSM1-dependent regulation of ribosomal protein genes is independent of p53

Downregulation of *RP-gene* expression in *Mysm1*-/- and *Mysm1*-/-*p53*-/- hematopoietic progenitor cells; FACS-sorted CD150- LSK cells from 3-5 mice per group were analyzed by RT-qPCR. Statistical comparisons use two-way ANOVA; ****p<0.0001. This data is obtained from a colleague in the lab.



Supplemental Figure 3. Reduced protein synthesis rate in a human patient with *MYSM1*-deficiency (c.869C>G, p.S290*)

Representative flow cytometry histograms showing the reduction in protein synthesis rate in the human patient with *MYSM1*-deficiency (c.869C>G, p.S290*)[285]; the plots are gated on $Lin^{-}CD38^{+}CD34^{-}$ hematopoietic progenitor cells. Protein synthesis rate is measured using the O-propargyl puromycin (OPP) incorporation method; control samples represent OPP-untreated but stained cells. This data is obtained from a colleague in the lab.