

©Copyright 2025

Elizabeth Bonner

Dissecting the role of CDK8 mis-splicing in SF3B1-mutant MDS

Elizabeth Bonner

A dissertation

submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington

2025

Reading Committee:

Stanley C. Lee, Chair

Robert Bradley

Janis Abkowitz

Program Authorized to Offer Degree:

Molecular and Cellular Biology

University of Washington

Abstract

Dissecting the role of CDK8 mis-splicing in *SF3B1*-mutant MDS

Elizabeth Bonner

Chair of the Supervisory Committee:

Stanley C. Lee

Department of Laboratory Medicine and Pathology

Somatic *SF3B1* mutations are believed to arise early in the pathogenesis of clonal myeloid disorders such as myelodysplastic syndromes (MDS). Studies show that *SF3B1* mutations bias hematopoietic stem and progenitor cells (HSPCs) toward the myeloid and erythroid lineages and impair terminal erythroid maturation. However, a direct mechanistic link between mutant SF3B1 and cell fate choice in primitive HSPCs has yet to be established. Recent studies suggest that mutant SF3B1 alters transcription via impaired transcriptional elongation and lead to altered transcriptional cell states of primitive progenitors. Given the central role of transcriptional regulators in modulating lineage-specific gene expression programs and cell fate determination in HSPCs, we hypothesize that mutant SF3B1 promotes aberrant splicing of core transcriptional cofactors, which, in turn, disrupt gene regulatory networks and drive biased hematopoietic differentiation. To investigate this, we analyzed *SF3B1*-mutant MDS patient data and identified CDK8 as a candidate driver. Using primary CD34⁺ HSPCs and preclinical modeling our study identifies CDK8 as an important regulator of HSPC homeostasis and cell fate determination in

SF3B1-mutant MDS. CDK8 depletion not only biases HSPCs towards myelomonocytic and erythroid lineages but also mirrors phenotypes observed in *SF3B1*-mutant MDS. This finding directly connects an *SF3B1*-mutant splicing event to skewed hematopoietic differentiation, shedding new light on MDS etiology.

Table of Contents

<i>List of Figures</i>	3
<i>Acknowledgements</i>	4
<i>Dedication</i>	7
<i>Chapter 1. Introduction</i>	8
1.1 Myelodysplastic syndromes	8
1.2 Genomics of MDS	9
1.3 Current treatment and management of MDS	10
1.4 Molecular Characteristics of <i>SF3B1</i> mutations	12
1.5 Transcriptional characteristics of <i>SF3B1</i> mutations	14
1.6 Pathology of <i>SF3B1</i> mutations	16
1.7 CDK8 and Mediator Complex	17
1.8 CDK8 Regulates Transcription	18
1.9 CDK8 and Cancer	19
1.10 CDK8 and inflammation	21
<i>Chapter 2. MDS-associated SF3B1 mutations promote aberrant fate choice of hematopoietic stem cell via mis-splicing of mediator kinase module component CDK8</i>	23
2.1 Abstract	23
2.2 Introduction	24
2.3 Results	26
2.3.1 Focused RNA-seq analysis of transcriptional cofactors identifies recurrent mis-splicing of CDK8 in <i>SF3B1</i> -mutant MDS patients	26
2.3.2 Mutant SF3B1-induced mis-splicing of CDK8 results in loss of expression via nonsense-mediated decay	27
2.3.3 Loss of CDK8 causes defective hematopoietic stem and progenitor cell function and multi-lineage differentiation <i>in vitro</i> and <i>in vivo</i>	28
2.3.4 CDK8 loss deregulates homeostatic- and lineage-specific transcriptional pathways in HSPCs	30
2.3.5 Restoration of CDK8 partially rescues erythroid differentiation defects in <i>SF3B1</i> -mutant iPSC model ..	31
2.3.6 Discussion	32
2.4 Figures and Legends	35
2.6 Supplemental Figures and Legends	42
2.7 Methods	51
2.7 Tables	59

2.7.1 GO Transcriptional Cofactors.....	59
2.7.2 Differentially expressed genes.....	60
2.7.3 Reactome Analysis	61
2.7.4 Primers	61
2.7.5 Antibodies.....	61
2.8 Acknowledgements	62
2.9 Authorship.....	62
<i>Chapter 3. Discussion</i>	<i>63</i>
<i>References</i>	<i>70</i>

List of Figures

Figure 2.5.1 RNA sequencing reveals CDK8 deregulation in <i>SF3B1</i> mutant MDS patient samples and isogenic cell lines.	36
Figure 2.5.2 Loss of CDK8 alters human HSPC differentiation.	37
Figure 2.5.3 Homeostatic and lineage pathways are deregulated by CDK8 loss	39
Figure 2.5.4 CDK8 re-expression rescues early erythroid differentiation	40
Figure 2.6.S1 Related to Figure 2.5.1	42
Figure 2.6.S2 Related to Figure 2.5.2	44
Figure 2.6.S3 Related to Figure 2.5.2	47
Figure 2.6.S4 Related to Figure 2.5.3	48
Figure 2.6.S5 Related to Figure 2.5.4	49
Figure 2.6.S6 Related to Discussion	51

Acknowledgements

I want to begin this section by thanking all my teachers, from high school on. There are so many people who saw something in me, even when I couldn't, who supported my curiosity, saw my struggle and continued to believe in me. Thank you to Fleur Ferro, who showed me that women in science do not fit cookie-cutter molds, and Dr. Nancy Lorenzon, who sparked my deep love of molecular biology and answered so many questions after class.

The work today would not have been possible without the people who took a chance on me in science. Thank you to Drs. Matthew Galbraith and Joaquin Espinosa, who took me in without any experience and taught me so much about the daily ins and outs of the scientific process. It is thanks to your guidance and training that I was able to do much of the work here today. I would also like to acknowledge Dr. Sujatha Jagannathan, for teaching me what compassionate mentorship in science looks like, inspired me to straddle the world of *in vitro* and *in silico*, and for creating the space for me to pursue graduate school. I do not know if I would have ever received an acceptance letter without her help.

I want to thank the Molecular and Cellular Biology (MCB) program for welcoming me. I have been provided so many amazing opportunities to grow as a scientist, a mentor, a leader and a community member. I quite literally would not be here without the support of MCB. And a special thank you to Maia Low. Your love and care for each student who walks through the orientation doors ensures that we all know we have a place in this community. MCB would not be what it is without you.

Thank you to my thesis committee. We formed in times of uncertainty, and we are ending in the same. I am well aware of the volumes of work that each of you have when you walk through the work doors each day and am so grateful that every one of you has taken some of that time to

guide me through the PhD process. I also want to take a moment to thank Dr. Beverly Torok-Storb, who is not here to see me finish, but was so keen to help me start. Dr. Bev. was the truest definition of a rebel, who did things the way she saw best, and a staunch advocate for everyone's place in science. Her legacy will deservedly outlive us all.

To Dr. Stanley Lee, and the Stanley Lee Lab. There are no words. It has been an absolute honor to pursue science with every one of you. Stan, you are the best graduate student mentor anyone could ask for. I am so lucky to have been your first graduate student. We have learned together in this process, and I wouldn't trade a moment of it. Thank you for teaching me how to be a scientist, for creating space when my life got tough, for being honest, providing balance, and fostering the community that is your beautiful, brilliant lab. I have learned so much in these last 6 years. My wins are yours too. Thank you.

I want to thank my family. When I turned 26 my father said "Elizabeth, the only thing you can't get back in life is time" – the catalyst for this whole journey. My father decided to go back for his PhD shortly after my mother passed. He was a middle-aged single parent of two children. He showed me that anything is possible, you just have to try. He is forever an example of grit, that sometimes just putting one foot in front of the other is enough. Thank you to my brother, who taught me what compassion is, and that people are many things over the course of a life. I am so immeasurably proud of you and the life you have created. Thank you to my stepmother, who is a force. She has supported me in more ways than can be mentioned and provides a constant example of what strength and perseverance looks like.

Most importantly I want to thank my husband. Pete Snodgrass, you have stood by me through all the late nights, the lonely weekends, the bad moods, everything. You celebrated my wins, mourned my losses and sacrificed so much so I could get to this finish line. You balance me

out and remind me that I am more than my work. You help me create the space to recover, so I can start again fresh the next day. I could not have done this without you in my corner. It is an honor to walk through life with you by my side.

I am sure there are many more I could acknowledge here, Dr. David Shechner, Marlana Kaplan, Michael Donahue and Glenna Norvel, Allison Coombs, Amy Spens and Hannah Lewis, Tuna the Cat... The list goes on. Thank you all.

Dedication

This work is written in the memory of and dedicated to two amazing women who believed in me with every ounce of their beings but did not live long enough to see its completion. To my mother, Alice-Marie Bonner, whose courage and bravery saw her through countless adventures despite the limitations her body imposed, and Annie Brumbaugh, a woman who forged her own path with grace and elegance, thank you for teaching me the true meaning of resilience, intelligence and grace. I would not have gotten through this process in the absence of your shining examples.

Chapter 1. Introduction

1.1 Myelodysplastic syndromes

Healthy hematopoiesis is a tightly regulated process, in which polyclonal hematopoietic stem cells (HSCs) must carefully coordinate self-renewal and balanced lineage differentiation to sustain stem cell pools and ensure continuous blood cell production. However, throughout the course of aging, cell intrinsic and extrinsic factors can shift this balance, resulting in clonal expansion, a state known as clonal hematopoiesis of indeterminate potential (CHIP). CHIP is characterized as the clonal expansion of HSPCs, bearing somatic mutations in genes associated with but lacking hematologic malignancy or cytopenia.¹ Approximately 10% of individuals over the age of 65 harbor CHIP-related mutations. Analysis of The Cancer Genome Atlas (TCGA) and the Swedish patient cohort identified mutations in genes associated with MDS, predominantly *TET2*, *DNMT3A*, and *ASXL1* (80%), as well as lower frequencies of *JAK2*, *TP53*, *PPM1D*, *SRSF2* and *SF3B1*.²⁻⁴ While most individuals will never progress to overt disease, certain CHIP mutations are associated with increased risks of coronary artery disease, ischemic stroke, and all-cause mortality, likely reflecting broader systemic consequences of clonal expansion.^{3,5-7} CHIP is widely regarded as both a sequela of and driven by inflammation.^{3,5} Research has shown that, individuals with CHIP have higher concentrations of proinflammatory cytokines, such as IL-6, MCP-1, and TNF α ,^{8,9} and individual mutations are associated with unique cytokine profiles. As an example, *TET2* mutations are associated with increased IL-1 β , whereas *SF3B1* mutations are associated with increased IL-18¹⁰. Given the shared mutational spectrum and inflammatory milieu, CHIP is increasingly recognized as a precursor state to clonal myeloid neoplasms such as myelodysplastic syndromes (MDS).

MDS is distinguished from indolent hematopoietic proliferation by the presence of morphologic dysplasia of ~10% of any lineage and characterized by cytopenia profile and burden, cytogenetic abnormalities and more recently somatic mutations.¹¹⁻¹⁵ It is estimated that 10,000 new cases of MDS are diagnosed annually in the United States, with ~86% occurring in patients 60 years or older, with the highest incidence in white males. The median 5 year survival time post diagnosis is 29%, and can vary widely depending on external factors such as transfusion dependence, comorbidities and socioeconomic status, and internal factors such as blast percentage, cytopenia number and cytogenetics.¹⁶ Clinically, MDS is characterized from very low to high risk, based on the extent of blast accumulation and cytopenia burden, with an increasing likelihood of transformation to acute myeloid leukemia (AML) along the spectrum. Traditionally classification relied on morphological and cytogenetic criteria, with karyotypic abnormalities in up to 70% of individuals. However, with the advent of next generation sequencing a more in-depth array of loss-of-function, gain-of-function and neomorphic mutations have been incorporated to better reflect clinical presentation.^{13,17-20}

1.2 Genomics of MDS

Somatic mutations associated with MDS may arise individually or as multiple lesions, occurring either within the same clonal population or across distinct subclones. Variant allele frequency (VAF) serves as a proxy for clonal architecture, distinguishing dominant from subclonal events.^{19,20} The co-occurrence of mutations within individual cells appears to follow a non-random pattern and significantly influences clinical outcomes and disease risk.^{19,21-27} In terms of genetic pathways frequently disrupted in MDS, approximately 30-50% of individuals present with lesions disrupting factors involved in epigenetic regulation (chromatin modification and DNA methylation), 30% in genes involved in transcriptional regulation and over 60% in genes encoding

splicing factors.¹⁹ Surprisingly, while many mutations are associated with MDS development, only a small subset of driver mutations (*SF3B1*, *SRSF2*, *TET2*, *DNMT3A*, *ASXL1*, and *RUNX1*) are mutated in >10% of patients, likely indicating their importance in pathologic advancement.²⁰ While no one pathway indicates clinical course, individual and combinatorial lesions have prognostic and predictive value. As an example, mutations in genes encoding members of the spliceosome are the most frequent occurrence in MDS, however not all present with the same outcome. Mutations in *SRSF2* and *U2AF1* are associated with lower overall survival and higher incidence of transformation to AML, while *SF3B1* mutations, the most frequently mutated gene in MDS, are generally associated with favorable overall survival, and low incidence of transformation.^{23,25,28-32}

1.3 Current treatment and management of MDS

Therapeutic intervention for MDS is based on risk classification and focuses on maintenance therapy to assuage symptoms from cytopenia, prolonging overall survival and reducing transformation risk. To date, the only potentially curative therapy is allogeneic bone marrow transplantation, not available to most patients due to donor availability and age associated comorbidities. For high risk individuals, hypomethylating agents (HMAs) have been the standard of care since the early 2000s.³³ However, despite wide-spread application, clinical response to HMAs such as azacitidine and decitabine remains low with a maximum response rate of ~50%. Recent clinical trials pairing HMAs with a host of other therapeutics such as HDAC inhibitors, immunomodulatory drugs, TIM-3 monoclonal antibodies and BCL-2 inhibitors have yielded little respite.³⁴⁻⁴⁷

For low-risk disease, maintenance therapy usually consists of blood transfusions and erythroid stimulating agents to reduce cytopenia burden. However repeated blood transfusions leads to iron overload, forcing the need for chelation therapy and erythropoiesis stimulating agents only present a clinical response rate of ~40%.⁴⁸⁻⁵¹ To overcome transfusion-dependence, recent clinical trials using Luspatercept, a TGF- β ligand trap, which reduces SMAD2/3 signaling, has shown some promise. In a phase III clinical trial, 68% of treated patients saw an erythroid response and 38% of treated patients with low risk MDS achieved transfusion independence.⁵² Additionally, a retrospective analysis of low-risk MDS patients treated with Luspatercept showed that 84% of *SF3B1*-mutant patients achieved transfusion independence.⁵³

Given the high frequency of splicing mutations across risk stratification, numerous spliceosome-targeting drugs have been explored. Preclinical studies in hematologic malignancy with and without splicing mutations showed promising results.⁵⁴⁻⁵⁷ In a Phase 1 dose-escalation study, splicing-factor mutant MDS patients received the *SF3B1* inhibitor H3B-8800 with mild results; while no clinical response was achieved 14% of patients had reduced transfusion requirements.^{55,56} Efforts have also been made in targeting splicing effector kinases. Currently, clinical trials are underway for the treatment of high-risk MDS and AML using the CLK inhibitor CTX-712. CLK proteins collaborate with SRPKs to phosphorylate SR proteins to modulate alternative splicing.⁵⁸ These trials were initiated based on data showing that *SRSF2* mutant MDS/AML patient-derived xenograft (PDX) models had significant treatment response, with many mice achieving complete remission.⁵⁹

Given the frequent occurrence of *SF3B1* mutations across the spectrum from CHIP to MDS, and their distinct clinical implications, a deeper mechanistic understanding of *SF3B1*-driven disease is needed. *SF3B1* is the most frequently mutated splicing factor in MDS, highlighting its

central role in disease pathogenesis. Although *SF3B1* mutations are often associated with a favorable prognosis compared to other splicing factor mutations, their widespread occurrence suggests a fundamental role in the initiation and progression of dysplastic hematopoiesis. Current therapeutic strategies may relieve symptoms arising from dysplastic hematopoiesis, but do not address the fundamental changes occurring in the early stem cell populations. Therefore, elucidating how *SF3B1* mutations alter RNA splicing, disrupt hematopoietic homeostasis, and contribute to disease evolution is essential. Such insight will be critical not only for understanding MDS biology but also for guiding the development of more effective, mutation-specific therapeutic interventions.

1.4 Molecular Characteristics of *SF3B1* mutations

SF3B1 is a member of the U2 small nuclear ribonucleoprotein (snRNP) complex, a core member of the spliceosome. In splicing, SF3B1 facilitates U2 snRNP binding at the second step of spliceosome formation, where sequence features in the pre-mRNA, such as the branch point, polypyrimidine track and a highly conserved intronic adenosine facilitate duplex formation in an ATP-dependent manner. This binding causes the adenosine to bulge, exposing its 2' OH group and positioning it for nucleophilic attack by the 5' splice site.⁶⁰ Structurally, SF3B1 consists of a N-terminal U2AF binding motif, which helps facilitate spliceosome assembly, and a C-terminal region consisting of 22 non-identical HEAT domains responsible for interaction with the branch point.⁶¹

Cancer-associated mutations in *SF3B1* are heterozygous and occur in hot spot regions within the C-terminal HEAT domains, with MDS-associated mutations largely accumulating in domains 4-7.⁶²⁻⁶⁶ Structural analysis of SF3B1 indicates that the location of these mutations fall

on the edge of the HEAT repeat helices at a periodicity of ~40 nucleotides.⁶⁶ These mutations confer neomorphic activity to SF3B1, leading to the use of non-canonical branch point sequences that results in the use of cryptic 3' splice sites clustering 12-24 nucleotides upstream of the canonical 3' AG dinucleotide.⁶² As a result, *SF3B1* mutations lead to widespread disruptions in the splicing landscape, marked by a significant rise in mis-spliced transcripts, predominantly in the form of alternative 3' splice site usage (A3SS) and retained introns (RI), giving rise to transcripts which are either degraded by cellular quality control mechanisms or aberrant mRNA isoforms.

Despite the well-characterized splicing alterations associated with *SF3B1* mutations, the underlying mechanisms driving these changes remain incompletely defined. The mutation-specific nature of these splicing events suggests that the affected regions of SF3B1 are functionally important for mediating structural changes and interactions with RNA or protein partners.^{67,68} Prior studies have demonstrated that *SF3B1* mutations alter RNA-binding affinity, shifting sequence preference from thymine-rich to adenine-rich sequences.⁶⁹ Additionally, splice site selection may depend on the presence of specific NAG trinucleotides and the local RNA secondary structure or flexibility.⁷⁰ Many cryptic 3' splice sites utilized in mutant cells are believed to be embedded within RNA secondary structures that are inaccessible to wild-type SF3B1.⁷¹ Beyond RNA sequence and structure, *SF3B1* mutations may perturb interactions with other spliceosomal components, such as DDX42 and SUGP1. Knockout experiments have shown that loss of either factor partially recapitulates the aberrant splicing patterns seen in *SF3B1*-mutant cells, while overexpression partially rescues normal splicing.^{72,73} Furthermore, these effects may be compounded by mutant SF3B1's persistent association with GPATCH8, as evidenced by the partial rescue of mis-splicing following GPATCH8 knockout in mutant cells.⁷⁴

In addition to changes in splicing, *SF3B1* mutations appear to perturb the transcriptional machinery itself. A recent study by Boddu *et al.* demonstrated that *SF3B1*-mutant cell lines exhibit global redistribution of RNA Polymerase II (RNAPII), shifting it from transcriptional start sites (TSS) into gene bodies.⁷⁵ This redistribution is thought to result from impaired recruitment of transcriptional elongation factors, such as P-TEFb, and defective spliceosome complex transitions, both consequences of disrupted SF3B1 function. These findings underscore the multifaceted impact of *SF3B1* mutations, extending beyond splicing to include profound alterations in transcriptional regulation and chromatin dynamics—thereby contributing to the complex pathogenesis of MDS.⁷⁵

1.5 Transcriptional characteristics of *SF3B1* mutations

Significant efforts in the field have been directed toward identifying mis-splicing events that contribute to the pathogenesis of MDS. One well-characterized example is the aberrant splicing of the mitochondrial iron transporter *ABCB7*, which results in transcript degradation due to the inclusion of 22 base pairs of frame-shifting intronic sequence.⁷⁶ Loss of *ABCB7* impairs erythroid differentiation of CD34⁺ HSPCs,⁷⁷ and has been directly implicated in ring sideroblast formation through studies using human-derived erythroid progenitor (HuDEP2) and induced pluripotent stem cell (iPSC) models.^{77,78} Similarly, A3SS-mediated mis-splicing of *MAP3K7* leads to its downregulation, resulting in a premature decreased in *GATA1* expression. This dysregulation drives an expansion of immature erythroid progenitors that ultimately undergo apoptosis prior to terminal differentiation.^{79,80} Together, these findings provide a mechanistic link between *SF3B1* mutation-induced aberrant splicing and the erythroid phenotypes observed in MDS patients.

The consequences of SF3B1-mutant mis-splicing extend beyond defects in erythroid differentiation. Studies have identified aberrant splicing of genes involved in metabolic regulation, such as COASY in MDS,⁸¹ and multiple targets in uveal melanoma.⁸² Additionally, *SF3B1* mutations contribute to hyperactive inflammatory signaling, including enhanced NF-κB activity via mis-splicing of MAP3K7,⁸³ and dysregulated toll-like receptor (TLR) signaling through mis-splicing of IRAK4.⁸⁴ Aberrant splicing has also been observed in SF3B1's own transcript,⁸⁵ as well as in genes involved in RNA export.⁸⁵ Importantly, several mis-splicing events are emerging as potential therapeutic vulnerabilities – small molecule inhibition targeting mis-spliced UBA1⁸⁶ and T cell-based therapies directed at neoantigens arising from aberrant splicing⁸⁷ represent promising strategies in *SF3B1*-mutant malignancies.

Transcriptomic analyses have revealed widespread deregulation in *SF3B1*-mutant cells. Studies have consistently demonstrated that pathways related to DNA damage repair are significantly downregulated in *SF3B1*-mutant CD34⁺ HSPCs. Additionally, inflammatory signaling pathways are upregulated, mirroring the pro-inflammatory bone marrow environment and clonal outgrowth that typifies MDS pathophysiology.^{88,89} Recent single-cell analyses has provided even greater resolution of the transcriptional landscape in *SF3B1*-mutant HSPCs. In two independent studies—one involving primary MDS patient samples and another employing CRISPR-engineered CD34⁺ umbilical cord blood cells (UCB)—*SF3B1*-mutant transcriptional cell states were found to accumulate predominantly within early HSPC, myeloid, and erythroid progenitor compartments. These findings suggest a link between the expansion of erythroid precursors in the bone marrow and altered transcriptional programs driven by *SF3B1* mutation. In particular, both studies reported suppression of cell cycle-related gene expression and a concomitant upregulation of translation-associated pathways within the erythroid lineage.^{90,91}

1.6 Pathology of *SF3B1* mutations

SF3B1 mutations occur in ~25-30% of patients with MDS, and in ~80% of individuals with ring sideroblasts (RS) – abnormal erythroid precursors characterized by perinuclear iron-laden mitochondria– making *SF3B1* one of the most frequently mutated genes in this disease.^{25,76,92} Phenotypically, *SF3B1* mutant MDS patients present with bone marrow hypercellularity, apoptotic erythroblasts, RS, and iron overload and are categorized as a unique subgrouping.^{15,92-94} VAF analysis of patients has shown these mutations largely exists in the dominant clonal population indicating *SF3B1* mutations drive pathogenesis.²⁰ When looking at the co-mutational pattern with other common MDS mutations, *SF3B1* mutations occur in a mutually exclusive manner with other splicing factor mutations and in a non-random manner with other common MDS associated mutations like *DNMT3A*.⁹⁵ Additionally, *SF3B1* mutations have been detected, at significance in other hematologic malignancies such as CLL, where mutations occur mostly as a secondary hit and patients have poor clinical outcomes,^{63,96,97} and in solid tumors such as uveal melanoma (~25%), breast and pancreatic cancers.^{19,68,98-100}

Despite the colossal effort to understand the pathogenesis of *SF3B1*-mutant MDS, the field still lacks a clear understanding as to how *SF3B1* mutations disrupt early HSPCs to drive clonal outgrowth and polarize differentiation. In culture, *SF3B1* mutant cells grow slower and senesce, yet in the bone marrow these clones have a competitive advantage. So how does this mutation provide an advantage in for clonal outgrowth in CHIP? Why do *SF3B1*-mutant cell transcriptional states accumulate in early progenitor compartments? How do *SF3B1* mutations promote differentiation along erythroid axis?

Herein, we have identified mis-splicing of CDK8 as a potential mechanism by which *SF3B1* mutations lead to clonal outgrowth of CD34⁺ HSPCs. Our work shows that, mutant SF3B1-mediated A3SS usage deregulates CDK8 to promote differentiation along the myeloid and erythroid axis, potentially mediated through transcriptional deregulation of key signaling intermediate and lineage commitment genes. (Discussed in Chapter 2)

1.7 CDK8 and Mediator Complex

CDK8 is a member of the CDK8 mediator kinase module (CKM) comprised of CDK8, Med12, Med13 and cyclin C (CCNC). The CKM functions as an accessory module of the 1.2 MDa Mediator complex (referred to Mediator hereafter) and is present only in a sub-population of Mediator complexes. Although functionally conserved between yeast and humans, several human CKM components—with the exception of CCNC—have evolved paralogues (CDK19, MED12L, and MED13L) through gene duplication. These paralogues associate with the CKM in a mutually exclusive manner, resulting in the formation of up to eight distinct CKM assemblies.¹⁰¹

Mediator is a master regulator of transcriptional activation in eukaryotes, where it forms a bridge between enhancer bound transcription factors and RNAPII.¹⁰²⁻¹⁰⁵ Mediator consists of 26 subunits organized into 4 distinct structural models – the head, middle, tail and CKM.^{106,107} Although Mediator is ubiquitously expressed at low levels across all eukaryotic cell types, it is essential for transcriptional activation through its coordinated interactions with gene-specific transcription factors, RNAPII, and the general transcription factors TFIIB, TFIID, TFIIE, and TFIIH at transcription start sites.¹⁰⁵ Mediator undergoes conformational rearrangements upon binding to DNA-associated transcription factors which enable stable interactions with RNAPII and, in concert with TFIID, facilitates assembly of the pre-initiation complex (PIC). This spatial

organization is critical for positioning CDK7, a component of TFIIH, to phosphorylate the C-terminal domain (CTD) of RNAPII — a prerequisite for productive transcription initiation.¹⁰⁸ In addition to transcriptional initiation, Mediator has also been shown to modulate transcriptional factor residency^{108,109}, participate in RNAPII reinitiation following pausing,¹¹⁰ and may be important for facilitating molecular condensate formation at enhancer and super enhancer sites^{111,112} making Mediator function critical for tight regulation of transcriptional output. Adding another layer of transcriptional fine tuning is the association and phosphorylation of transcriptional proteins by the CKM.

1.8 CDK8 Regulates Transcription

Unlike other CDKs, which rely on phosphorylation of their T-loop for functional activation, CDK8 kinase activation results from MED12 activation helix interaction, specifically residue E33.¹¹³ In yeast, CKM was initially shown to inhibit transcriptional initiation, where association of CKM to Mediator occludes interaction with RNAPII,^{114,115} or through synergistic interaction with GCN5L to modify histones (H3S10P/K14Ac).¹¹⁶ However, in human cells, the role for CDK8 and the CKM have expanded, where the CKM has been shown to both activate and repress transcription in a pathway specific manner either through direct phosphorylation of RNAPII CTD¹¹⁷ or other members of the transcriptional machinery. Additionally, CDK8 has been shown to play Mediator independent roles in transduction of signaling pathways, and to have important functions independent of its kinase activity.

The first papers to characterize CDK8's functions as a transcriptional activator in human cells were published in the early 2000's by Donner *et al.* In a 2007 study, Donner showed that CDK8 is required for sustained transcription of the p53 target gene p21 in HCT116 colorectal

carcinoma cells upon treatment with the p53 activating compound Nutlin3, but was not recruited with transient activation by ultraviolet light.¹¹⁸ In follow up work, Donner *et al.* demonstrated that the CKM regulates precise steps in the assembly of the super elongation complex (recruitment of CDK7, P-TEFb and BRD4) required for transcriptional activation of genes within the serum response network (*FOS*, *JUN*, *FOSB*, *JUNB*, *EGR1-3*).¹¹⁹ Since these seminal publications, a plethora of work describing CDK8's role as a transcriptional modulator for stimulus responsive genes have been published.

1.9 CDK8 and Cancer

In many solid tumors, CDK8 functions as an oncogene by colocalizing with oncogenic transcription factors to promote transcriptional activation. Among its most well-characterized roles is the activation of MYC target genes. In colorectal carcinoma, CDK8 plays a pivotal role in upregulating the expression of MYC and pluripotency-associated transcription factors, including OCT4, NANOG, and SOX2—factors essential for maintaining pluripotency in murine embryonic stem cells. This transcriptional program is hijacked in cancer to drive tumor cell dedifferentiation and proliferation.¹²⁰ CDK8-mediated MYC activation has also been implicated in the hyperactivation of the β -catenin signaling pathway, a key driver of colorectal cell transformation.¹²¹ Furthermore, CDK8-dependent transcriptional activation has been shown to promote the hypoxia-inducible factor 1-alpha (HIF1 α) response network, and glycolytic gene expression, thereby supporting sustained proliferation and the metabolic reprogramming characteristic of the Warburg effect.^{122,123}

In addition to its role in regulating RNAPII transcriptional activation through associating with core transcriptional machinery, CDK8 has been shown to regulate signal transduction

independent of its functions with Mediator. Phosphorylation of the Notch intracellular domain (ICD) to mediate turnover is an important regulatory mechanism to fine tune transcriptional bursting in many developmental pathways. CDK8 has been shown to colocalize with Notch ICD to promoters, where it phosphorylates the Notch ICD transactivation domain, mediating its degradation by the ubiquitin ligase FBW7 to facilitate turnover.¹²⁴ Additionally, CDK8 has also been shown to play a mediator independent role in STAT1 signaling upon interferon stimulation. Upon translocation to the nucleus, STAT1 proteins recruit CDK8 to promoters, where STAT transactivation domain phosphorylation promotes activation of interferon inducible genes.¹²⁵ Similarly, in the TGF- β pathway, CDK8 phosphorylates receptor-regulated Smads, such as Smad2 and Smad3, enhancing their transcriptional activity and contributing to context-specific gene regulation.¹²⁶

Given its ability to integrate multiple signaling pathways and its role in oncogenesis, CDK8 has garnered some attention for its role in hematopoietic disease. Using the BCR-ABL1+ murine model to investigate CDK8's role in B-cell acute lymphoblastic leukemia (B-ALL), Mezl *et al* discovered that, while CDK8 was not necessary for leukemic transformation, knock-out of CDK8 supported leukemic maintenance, likely through activation of the mTOR pathway.¹²⁷ Surprisingly, this phenomenon was kinase independent, as kinase inhibitor treatment had no phenotype. Conversely, pharmacological inhibition of CDK8 by the natural product cortistatin A in several leukemia cell lines resulted in decreased cell proliferation and a significant upregulation of genes associated with super-enhancers.¹¹² In fact, in the last 5 years a wealth of drug development studies have been performed purporting CDK8 as a therapeutic vulnerability for the treatment of leukemia,¹²⁸⁻¹³² though the mechanism underlying efficacy is not well defined.

1.10 CDK8 and inflammation

CDK8 functions as a critical regulator of inflammatory signaling across lymphoid and myeloid lineages, primarily through modulation of STAT and NF- κ B signaling cascades. CDK8 has been shown to mediate NF- κ B-induced transcription, whereby CDK8/NF- κ B colocalization to promoters facilitates transcriptional initiation through CDK8-mediated phosphorylation of RNAPII CTD.¹³³ In RPMI8226 B lymphocytes, CDK8 knockdown attenuates TLR9-induced transcription of pro-inflammatory genes via reduced NF- κ B and C/EBP β activity.^{134,135} Pharmacologic inhibition of CDK8 in murine CD4⁺ T cells with CCT251921 enhances TGF- β -driven SMAD2/3 signaling while suppressing IFN- γ /STAT1, thereby promoting regulatory T cell differentiation.¹³⁶ In myeloid cells, CDK8 inhibitors such as BRD10330 and BRD6989 elevate IL-10 production and M2 macrophage polarization, respectively, through suppression of STAT1/2 signaling, upregulation of AP-1 activity, and enhancement of IL-4-induced STAT6 phosphorylation and p38 MAPK-dependent C/EBP β expression.^{137,138}

CDK8 has been implicated in the epigenetic repression of inflammatory gene expression. Tsutsui *et al* demonstrated that the CKM regulates C/EBP β -dependent transcription through targeted histone and DNA modifications. Using mass spectrometry, *in vitro* methylation assays, and gene expression analyses, they showed that the CKM recruits the PRMT5/MEP50 complex to promoters of C/EBP β target genes, catalyzing symmetric dimethylation of histone H4 at arginine 3 (H4R3me2s). This histone mark facilitates the recruitment of DNMT3A, leading to *de novo* DNA methylation and reduced assembly of the pre-initiation complex at these loci. Functionally, this cascade attenuates transcriptional induction of inflammatory genes, including IL8 and TNF α , in response to inflammatory stimuli.¹³⁹

Given the convergence of aberrantly activated signaling pathways observed in *SF3B1*-mutant cells—particularly those involving MYC and NF- κ B, both of which are under transcriptional control mediated by CDK8—and the established role of CDK8 in recruiting P-TEFb to drive productive transcription elongation, there is a strong rationale to explore CDK8 as a critical mediator of SF3B1-driven pathology. *SF3B1* mutations are known to disrupt splicing of key regulators of transcription, our data suggest that CDK8 itself is a target of such mis-splicing. Moreover, CDK8 plays essential regulatory roles in pathways governing homeostasis, metabolic signaling, serum response, and inflammation—many of which are perturbed in MDS. Taken together, we hypothesize that SF3B1-mediated mis-splicing leads to loss of CDK8, which in turn compromises transcriptional control of differentiation and self-renewal programs in HSPCs. This disruption may promote clonal expansion and disrupt differentiation, contributing to disease progression in MDS. These mechanistic intersections position CDK8 as a compelling node of etiology.

In this dissertation I will address the outstanding question:

How do *SF3B1* mutations disrupt cell fate choice in early HSPCs?

In chapter 2, we use bioinformatic analysis of publicly available patient data to identify CDK8 as a target of *SF3B1*-mutant mediated mis-splicing. Using primary CD34⁺ cell culture and xenotransplantation models, we test the hypothesis that mis-splicing of CDK8 disrupts early HSPC cell fate choice. Using RNA sequencing, we identify candidate transcriptional pathways which may be altering HSPC differentiation resulting from CDK8 deregulation. Finally, we validate CDK8's role in mutant *SF3B1* differentiation phenotypes using immortalized iPSCs.

Chapter 2. MDS-associated *SF3B1* mutations promote aberrant fate choice of hematopoietic stem cell via mis-splicing of mediator kinase module component CDK8

Bonner E. A., Hsueh T., Song A., Arriaga-Gomez E. A., Venkataraman R., Sinha S., Nguyen E., Ferrell P. B., Welner R., Stirewalt D. L., Doulatov S., Lee S. L. (2025)

2.1 Abstract

Mutations in the RNA splicing factor *SF3B1* are among the most common in MDS and are strongly associated with MDS with ring sideroblasts (MDS-RS). While aberrant splicing of terminal erythroid regulators has been implicated in MDS pathogenesis, the impact of *SF3B1* mutations on early hematopoietic progenitor function remains unclear. Here, we identify CDK8, a key kinase of the mediator complex involved in transcriptional regulation, as a recurrent mis-spliced target in *SF3B1*-mutant MDS. Mutant *SF3B1* induces cryptic 3' splice site selection in CDK8, leading to loss of CDK8 mRNA and protein. Using primary human HSPCs, our study identifies CDK8 as an important regulator of HSPC homeostasis and cell fate determination. CDK8 depletion results in expanded primitive HSPCs and shifts differentiation toward the myeloid and erythroid lineages, mirroring phenotypes observed in *SF3B1*-mutant MDS, and functional rescue of CDK8 rescues early erythroid phenotypes in *SF3B1*-mutant cells. These findings implicate CDK8 mis-splicing as a mechanistic driver of altered progenitor fate and dysplasia in *SF3B1*-mutant MDS, linking aberrant splicing to transcriptional dysregulation and hematopoietic lineage commitment.

2.2 Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of age-related clonal myeloid disorders characterized by cytopenia, morphologic dysplasia, and an increased risk of developing secondary acute myeloid leukemia (AML). The etiology of MDS can be broadly attributed to the complex interplay between genetic, epigenetic and environmental factors that cause functional abnormalities in bone marrow resident hematopoietic stem cells (HSCs), leading to clonal outgrowth and ineffective hematopoiesis. Somatic mutations in the RNA splicing factor *SF3B1* are highly prevalent in MDS, occurring in ~25% of patients, and in over 70% of patients with ring sideroblasts (MDS-RS).^{30,92,140-143} *SF3B1* is a member of the U2 small nuclear ribonucleoprotein (U2 snRNP) complex, which is responsible for intronic branchpoint recognition, an essential step in pre-mRNA splicing. MDS-associated *SF3B1* mutations occur in hot-spot regions within the heat domains, and confer neomorphic activity that commonly results in cryptic branchpoint selection and the preferential usage of intron-proximal 3' splice sites.^{64,66,144-147} Aberrant splicing of transcripts such as *ABCB7*, *TMEM14C*, *MAP3K7*, *EFRE* and *PPOX* by mutant *SF3B1* have been directly linked to impaired terminal erythroid maturation, deregulation of iron homeostasis and ring sideroblast formation, which are pathognomonic features of MDS-RS.^{78,80,145,148,149}

In addition to alterations in cellular splicing repertoires, RNA sequencing has demonstrated that *SF3B1* mutations cause alterations to the global transcriptional landscape of hematopoietic stem and progenitor cells (HSPCs). Single cell transcriptomic analysis of *SF3B1*-mutant CD34⁺ HSPCs shows that the cell transcriptional states of mutant HSPCs largely accumulate in myeloid and erythroid progenitor compartments and may be primed for preferential differentiation along the erythroid lineage.^{90,150} While many individual mis-spliced targets have been linked to deficits in late erythroid development, how mutant *SF3B1* disrupts the function of early hematopoietic

progenitors remains unclear. Because a vast body of evidence has shown that lineage commitment is heavily reliant on appropriate gene expression to facilitate hematopoietic differentiation,¹⁵¹⁻¹⁵⁵ we hypothesize that *SF3B1* mutations may be disrupting transcriptional cofactors responsible for fine tuning gene expression.

In this study, we discovered that CDK8, a member of the mediator kinase module, is consistently mis-spliced in *SF3B1*-mutant MDS. CDK8 is the catalytic component of mediator kinase module, an accessory subunit of mediator complex that bridges transcription factors with the RNA polymerase II (RNAPII) machinery and integrates various cellular signals to modulate transcriptional output.¹¹⁰ Initially thought to function as a transcriptional repressor through phosphorylation of the RNAPII C-terminal domain,^{114,115} published work indicates CDK8 functions as a transcriptional activator in the hypoxia response network and p53 pathways.^{118,122} Additionally, CDK8 kinase activity has shown to be an important modulator of various signaling pathways such WNT, TGF β , and INF γ signal cascades by phosphorylating downstream effectors such as c-MYC, SMADs, STAT1 and STAT3.^{121,125,156-161} Given the pleiotropic roles of CDK8 as a transcriptional regulator, we investigated how MDS-associated *SF3B1* mutations promote CDK8 deregulation and its impact on dysplastic hematopoietic differentiation in MDS.

2.3 Results

2.3.1 Focused RNA-seq analysis of transcriptional cofactors identifies recurrent mis-splicing of CDK8 in *SF3B1*-mutant MDS patients

To identify candidate transcriptional co-factors which undergo mutant SF3B1-mediated aberrant splicing, we performed splicing analysis¹⁶² using RNA sequencing data from isogenic leukemia lines (K562 *SF3B1*-WT, K562 *SF3B1*-K700E, and HNT34, a leukemia cell line that harbors an endogenous *SF3B1*-K700E mutation), as well as publicly available MDS patient samples with and without *SF3B1* mutations.^{163,164} We curated a list of 162 transcriptional co-factors using gene ontology^{165,166} (**Table 2.7.1**) and looked for significant splicing events from our cohort that followed a set of criteria: 1) The splicing event must be enriched in *SF3B1* mutant cells compared to wild-type (WT), 2) the splicing event deregulates transcript stability, 3) mis-splicing must occur across different SF3B1 mutations, 4) the splicing event is exclusive to *SF3B1* mutations. Using a delta percent spliced-in (dPSI) cut off of 0.08 and FDR <0.05, our analysis showed that, of the transcripts predicted to undergo alternative 3' splice site usage, members of the mediator complex are highly enriched, including MED13L, MED6 and CDK8 (**Figure 2.5.1A**). Because alternative splice site usage can lead to either stable alternative isoform production or can introduce a premature termination codon, we next asked if any of the transcriptional co-factors also displayed changes in transcript level by RNA sequencing (**Figure 2.5.1B**). We performed differential gene expression analysis using DESeq2¹⁶⁷ and found that, among the 20 predicted mis-splicing events, only three transcripts (CDK8, TAF6L, TAF2) showed significant changes in expression (adjusted p-value <0.05). Notably, CDK8 was the only mediator complex member with significantly altered transcript levels. To determine if changes in CDK8 mRNA levels were specific

to the *SF3B1*-K700E mutation, we performed differential expression analysis on a larger cohort of MDS patients with and without *SF3B1* mutations,^{163,168-170} healthy bone marrow and *SF3B1*-WT (K562) and *SF3B1*-K700E (K562, HNT34) cell lines (**Figure 2.5.1C**). While TAF6L displayed a greater decrease in mRNA expression in *SF3B1*-mutant samples, alternative 3' splice site usage in both patient samples and cell lines is significantly enriched in the *SF3B1*-WT cells (**Figure 2.5.1A**, **Figure 2.6.S1A**), which did not meet our criteria. Similarly, the mis-splicing of MED13L, TAF2, and MED6 in *SF3B1*-mutant cells did not meet our criteria, either due to a lack of significant transcript downregulation or because the resulting splice variants generated stable mRNA isoforms (**Figure 2.6.S1B-H**).

Due to alternative 3' splice site usage, CDK8 is predicted to include 14-base pairs of intronic sequence proximal to exon 8. To confirm this, we examined CDK8 read coverage in the Integrated Genome Viewer¹⁷¹ (IGV) and observed intronic inclusion exclusively in samples from *SF3B1*-mutant cells (**Figure 2.5.1D**). Additionally, we did not observe CDK8 mis-splicing in other common spliceosome mutations (**Figure 2.6.S1I**) Taken together, these data indicate that *SF3B1* mutations affect only a small subset of transcriptional co-factors, and among those, CDK8 is the only one consistently showing decreased mRNA transcript levels across different *SF3B1*-mutant contexts. (**Figure 2.6.S1A-D**)

2.3.2 Mutant SF3B1-induced mis-splicing of CDK8 results in loss of expression via nonsense-mediated decay

We next sought to validate CDK8 mis-splicing in *SF3B1*-mutant MDS patient samples and cells lines. We designed PCR primers which spanned exon-exon boundaries around CDK8 exon 8

and performed RT-PCR followed by native polyacrylamide gel electrophoresis (PAGE). We could detect the 14 base-pair intronic sequence inclusion in *SF3B1*-mutant but not *SF3B1*-WT isogenic cell lines (**Figure 2.5.1E**). We then wanted to determine if reductions in CDK8 mRNA corresponded to a reduction of CDK8 at the protein level. We performed western blot in *SF3B1*-mutant cell lines and found that CDK8 protein was reduced in SF3B1 mutant samples but not their WT counterparts (**Figure 2.5.1F**). The inclusion of 14 base pairs of intronic sequence is predicted to lead to a frame shift, leading to reduction of CDK8 mRNA by the quality surveillance pathway nonsense-mediated decay (NMD). To verify this prediction, we treated cells with a NMD inhibitor, NMDi-14, and performed qPCR in isogenic *SF3B1*-mutant cell lines. We found that NMDi-14 treatment restored CDK8 mRNA levels in *SF3B1*-mutant cells but not in *SF3B1*-WT cells (**Figure 2.5.1G**). Together, these data verify that CDK8 is mis-spliced by mutant SF3B1 leading to reduction of CDK8 mRNA and protein due to mRNA degradation by NMD.

2.3.3 Loss of CDK8 causes defective hematopoietic stem and progenitor cell function and multi-lineage differentiation *in vitro* and *in vivo*

Phenotypically, *SF3B1*-mutant MDS patients present with an expansion of erythroblasts and single cell transcriptomic data has indicated that *SF3B1*-mutant HSPCs have an accumulation of cell states resembling early erythroid and myeloid precursors.^{78,90,150,172,173} To determine if CDK8 loss is contributing to the shift in frequency of early myeloid and erythroid progenitors, we used a dual-guide RNA-based CRISPR/Cas9 system¹⁷⁴ to target CDK8 (CDK8^{KO}) or the AAVS1 (NTC) locus as a negative control in human CD34⁺ HSPCs (**Figure 2.6.S2A-C**). To monitor differentiation along a single lineage, we used GCSF-mobilized healthy adult CD34⁺ cells and

performed liquid culture differentiation along the myeloid or erythroid axis. We found that CDK8^{KO} HSPCs produced a higher percentage of CD13⁺CD14⁺ myeloid cells, and CD71⁺CD235a⁺ erythroid cells compared to NTC cells (**Figure 2.5.2A-B**). To monitor multilineage differentiation of early progenitors, we generated CDK8^{KO} cells from umbilical cord blood-derived CD34⁺ HSPCs (UCB) and performed colony-forming unit (CFU) assays (**Figure 2.5.2C**). Our data showed that CDK8^{KO} HSPCs generated significantly more colonies, driven by a marked increase in CFU-Granulocyte/Macrophage (CFU-G/M) colonies. While CDK8 knock-out did not produce a significant increase in the number of early burst-forming erythroid colonies (BFU-E), we found that when we collected total colonies at the end of the experiment, CDK8^{KO} colonies produced much redder pellets (**Figure 2.5.2D**). Together, these results indicate that CDK8 loss leads to increased differentiation along the myeloid and erythroid axes and supports our hypothesis that CDK8 mis-splicing may be contributing to the expansion of early erythroid and myeloid progenitors in *SF3B1*-mutant MDS.

To determine the long-term *in vivo* consequences of CDK8 loss in hematopoiesis, we used the same CRISPR/Cas9 system to knockout CDK8 in UCB CD34⁺ cells followed by xenotransplantation in NOD/SCID/ILR2 γ -null (NSG) mice (**Figure 2.6.S3A**). At terminus (20 weeks post xenotransplantation), we harvested spleen, bone marrow and peripheral blood to assess lineage composition and confirm knock-down in bone marrow CD45⁺ cells (**Figure 2.6.S2C-D**). CDK8^{KO} UCB mice had a mild increase in CD33⁺ myeloid cells in the blood and bone marrow (**Supplemental Fig. S3C-E**). There were no significant differences between the level of human cell chimerism (%CD45⁺) in the bone marrow mononuclear cell fraction of mice transplanted with AAVS1^{KO} or CDK8^{KO} HSPCs (**Figure 2.6.S3F**). Interestingly, we observed a significant increase CD14⁺CD16⁺ intermediate monocytes in the bone marrow (**Figure 2.5.2E**). Further examination

of the bone marrow HSPC fraction (defined by CD45⁺lineage⁻CD34⁺) revealed there was no significant increase in the total number of HSPCs between AAVS1^{KO} and CDK8^{KO} groups (**Figure 2.5.2F**). Interestingly, we observed a mild but significant increase in the proportions of the more immature progenitors (defined by CD45⁺lineage⁻CD34⁺CD38⁻) and lymphoid-primed multipotent progenitors (LMPP, defined by CD45⁺lineage⁻CD34⁺CD38⁻CD45RA⁺CD90⁻) (**Figure 2.5.2G-H, Figure 2.6.S3G**). When we looked within the more differentiated myeloid progenitor fraction (defined by CD45⁺lineage⁻CD34⁺CD38⁺), we observed a significant decrease in classically defined immunophenotypic megakaryocyte-erythroid progenitors (MEPs; CD45⁺lineage⁻CD34⁺CD38⁺CD45RA⁻CD123⁻) and granulocyte-macrophage progenitors (GMPs; CD45⁺lineage⁻CD34⁺CD38⁺CD45RA⁺CD123⁺) (**Figure 2.5.2I**). While we do not see an expansion in immunophenotypic MEPs or GMPs, we observed a significant expansion in a distinct subset of cells within the CD34⁺CD38⁺ compartment that express CD71 (**Figure 2.5.2J**), a population with the potential to differentiate into both erythroid and granulocytic lineages.^{175,176} Our *in vivo* data indicates that loss of CDK8 leads to an expansion of specific primitive HSPC compartments that skew towards the erythroid and granulocytic/myeloid differentiation trajectories.

2.3.4 CDK8 loss deregulates homeostatic- and lineage-specific transcriptional pathways in HSPCs

Because CDK8 functions as transcriptional modulator, we next sought to determine the changes resulting from CDK8 loss in UCB CD34⁺ HSPCs. We performed RNA sequencing in CD34⁺ UCB cells treated with 2 independent short-hairpin RNAs (shRNAs) targeting CDK8 (shCDK8) or a non-targeting control (shNTC) (**Figure 2.5.3A, Figure 2.6.S4A-C**). We performed

differential expression analysis (DESeq2) and filtered for significant ($\text{padj} < 0.05$) changes shared across both hairpins (**Figure 2.5.3B**, solid dots). We found that CDK8-deficient CD34⁺ cells had increased expression of known markers of myeloid differentiation (CD14, CECAM6, S100A8, S100A9), and decreased expression in signaling intermediates of key intracellular signaling pathways including the MAP kinase signaling (FOS, FOSB, JUN, DUSP2), JAK/STAT pathway (SOCS3) and GPCR signaling (RGS1, RGS2) pathways (**Figure 2.5.3A**, **Figure 2.6.S4D**). We then asked if we could detect similar patterns of deregulation in our MDS patient cohort. We found that most of the transcripts deregulated in CDK8-deficient CD34⁺ HSPCs showed a similar expression pattern in *SF3B1*-mutant MDS patients (**Figure 2.5.3C**). Taken together, these results indicate that CDK8 loss is leading to deregulation of several important transcriptional networks, likely resulting in the upregulation of genes associated with lineage differentiation.

2.3.5 Restoration of CDK8 partially rescues erythroid differentiation defects in *SF3B1*-mutant iPSC model

Next, we sought to formally demonstrate the causal link between the phenotypic consequences of CDK8 loss to *SF3B1* mutations in MDS. To address this, we used a previously established isogenic pair of induced pluripotent stem cell-derived hematopoietic progenitor cell lines (iPSC-HPCs) generated from an MDS-RS patients with an *SF3B1*-G742D mutation¹⁷⁷ (**Figure 2.6.S5A**). We also observed in the iPSC-HPC model that CDK8 is robustly mis-spliced and downregulated at both the mRNA and protein level in *SF3B1*-mutant cells relative to their *SF3B1*-WT isogenic counterparts (**Figure 2.5.4B-C**, **Figure 2.6.S5B**). Consistent with prior erythroid differentiation data, we observed that *SF3B1*-mutant iPSC-HPCs have a sharp increase in early erythroid cells

(CD71⁺CD235a⁻). We therefore hypothesized that this early expansion of erythroid progenitors may be due to loss of CDK8. To determine if restoration of CDK8 could rescue erythroid differentiation defects in *SF3B1*-mutant cells, we stably introduced CDK8 cDNA or an empty vector (EV) control in *SF3B1*-mutant iPSC-HPCs and performed erythroid differentiation (**Figure 2.5.4A & 4D**). Compared to EV-transduced *SF3B1*-mutant cells, re-expression of CDK8 significantly reduced the expansion of CD71⁺CD235a⁻ cells (**Figure 2.5.4E & 4G**). Surprisingly, as differentiation continued, re-expression of CDK8 in this model continued to repress expansion of erythroid cells (**Figure 2.5.4F & 4H**). Taken together, our data indicates that the aberrant erythroid expansion and differentiation phenotype characteristic of *SF3B1* mutations in MDS-RS is partly mediated through mis-splicing and the subsequent loss of CDK8 function.

2.3.6 Discussion

SF3B1 mutations are purported as functional drivers of expansion in clonal hematopoiesis and MDS. However, despite extensive research, the mechanistic understanding of how splicing defects confer a competitive advantage and skew early HSPC cell fate choice remain elusive. Here, we have utilized publicly available patient transcriptomics data,^{163,168-170} *ex vivo* and *in vivo* models to identify CDK8 mis-splicing as a potential mechanism governing impaired hematopoiesis. Our findings highlight CDK8 as a critical effector in *SF3B1*-mutated MDS and elucidated its importance in regulating proper hematopoietic differentiation.

SF3B1 is one of the most commonly mutated genes and the most frequently mutated spliceosome component in MDS and is associated with widespread splicing deregulation.^{30,62,64,66,71,76,92,140-143,145-147,168,178} Here, we identify CDK8 as a recurrent mis-splicing

target in *SF3B1*-mutant MDS patients across a range of hotspot mutations. Mis-splicing led to deregulation of CDK8 transcript levels in mutant cells due to the introduction of a premature termination codon, resulting in CDK8 degradation by the quality surveillance pathway nonsense mediated decay.

Previous studies have linked *SF3B1* mutations to impaired terminal erythroid differentiation, iron metabolism and the formation of ring sideroblasts,^{76,78,80,168} but a mechanistic link between clonal advantage and early fate choice of HSPCs had not been made. Here, we show that, as a result of CDK8 loss, primitive HSPCs compartments expand *in vivo* and may preferentially differentiate along the myeloid and erythroid axes *in vitro* and *in vivo*. This skewed differentiation parallels the expansion of immature erythroid and myeloid precursors observed in *SF3B1*-mutant MDS patients and from *SF3B1*-mutant HSPCs from single cell transcriptomic studies. Thus, our findings provide a mechanistic link between *SF3B1* mutations and disrupted early HPSC fate via impaired CDK8-mediated transcriptional control.

CDK8 is well known as a context-specific transcriptional regulator in solid tumors, influencing pathways like WNT, p53 and STAT signaling.^{121,125,156-161} Using RNA sequencing, our study identifies CDK8 as an important regulator of myeloid marker gene expression and indicates that deregulation of MYC and STAT pathways may be driving altered HSPC cell fate choice in *SF3B1*-mutant cells. Further supporting our findings, published work has shown that both MYC and JAK/STAT pathways play important regulatory roles in HSPC self-renewal, lineage commitment, and leukemogenic transformation.¹⁷⁹⁻¹⁸⁵

SF3B1-mutant MDS is generally considered low-risk, with current treatments focused on managing anemia. Our Data shows that reintroduction of CDK8 rescues erythroid abnormalities in *SF3B1*-mutant cell models and suggests a targeted approach to correcting differentiation defects

may be a therapeutic viability, potentially through the use of splice correcting antisense oligonucleotides (ASOs). Additionally, the observation that CDK8 loss enhances erythroid output underscores its therapeutic relevance, particularly given the abundance of available CDK8 kinase inhibitors that could be repurposed to promote erythropoiesis in other anemic conditions.

2.4 Figures and Legends

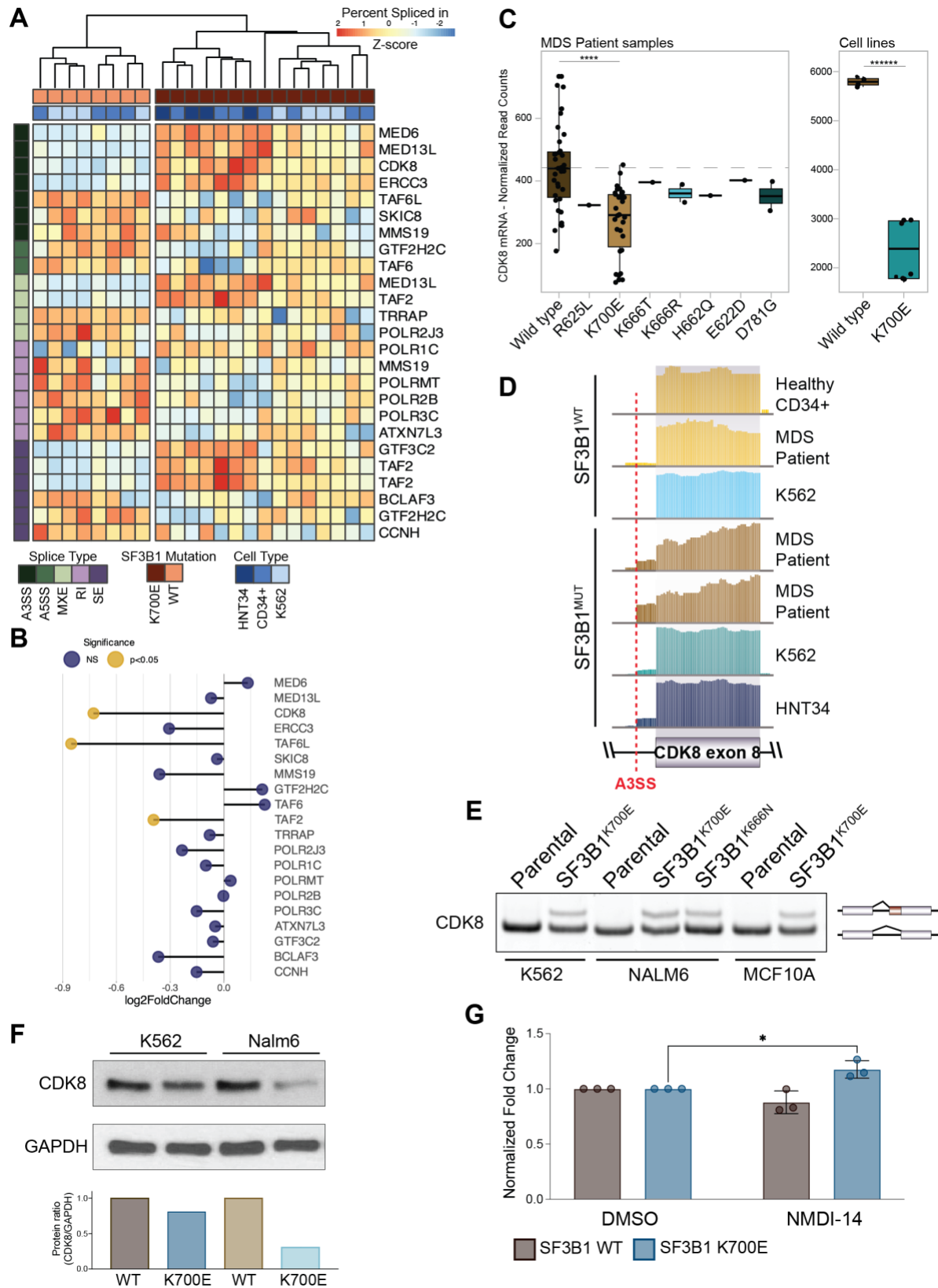


Figure 2.5.1 RNA sequencing reveals CDK8 deregulation in *SF3B1* mutant MDS patient samples and isogenic cell lines.

(A) Unsupervised clustering (columns) of significant (compared to WT MDS and healthy controls) mis-spliced transcriptional cofactors (read count > 10, |PSI| > 0.08, FDR < 0.05, rows) from publicly available *SF3B1*-mutant MDS patient RNA sequencing data and isogenic cell lines (K562, HNT34). **(B)** Lollipop plot of the Log₂ fold change (DESeq2) of significantly mis-spliced transcriptional co-factors. Yellow, adjusted pvalue < 0.05, blue, adjusted pvalue ≥ 0.05. **(C)** Normalized read counts (DESeq2) of CDK8 mRNA from publicly available data. Dashed line = mean read count of *SF3B1* WT samples. **(D)** IGV plots of the mis-spliced region of CDK8 in *SF3B1* WT and *SF3B1*-mutant MDS patient samples and cell lines. Y-axis, absolute read count. **(E)** RT-PCR and native PAGE of 158 bp surrounding the alternative 3' splice site usage in CDK8 mRNA collected from wildtype and *SF3B1*-mutant cell lines (right). **(F)** Western blot (top) and quantification (bottom) of CDK8 protein levels from wild type and SF3B1 mutant cell lines. Western blot was quantified and protein ratio (relative to loading control, ImageJ) was plotted (bottom) **(G)** qPCR mean ± SD of isogenic K562 cell lines treated with NMDI-14 (n=3). Treated sample were normalized to untreated controls. *, p < 0.05, 2-way Anova.

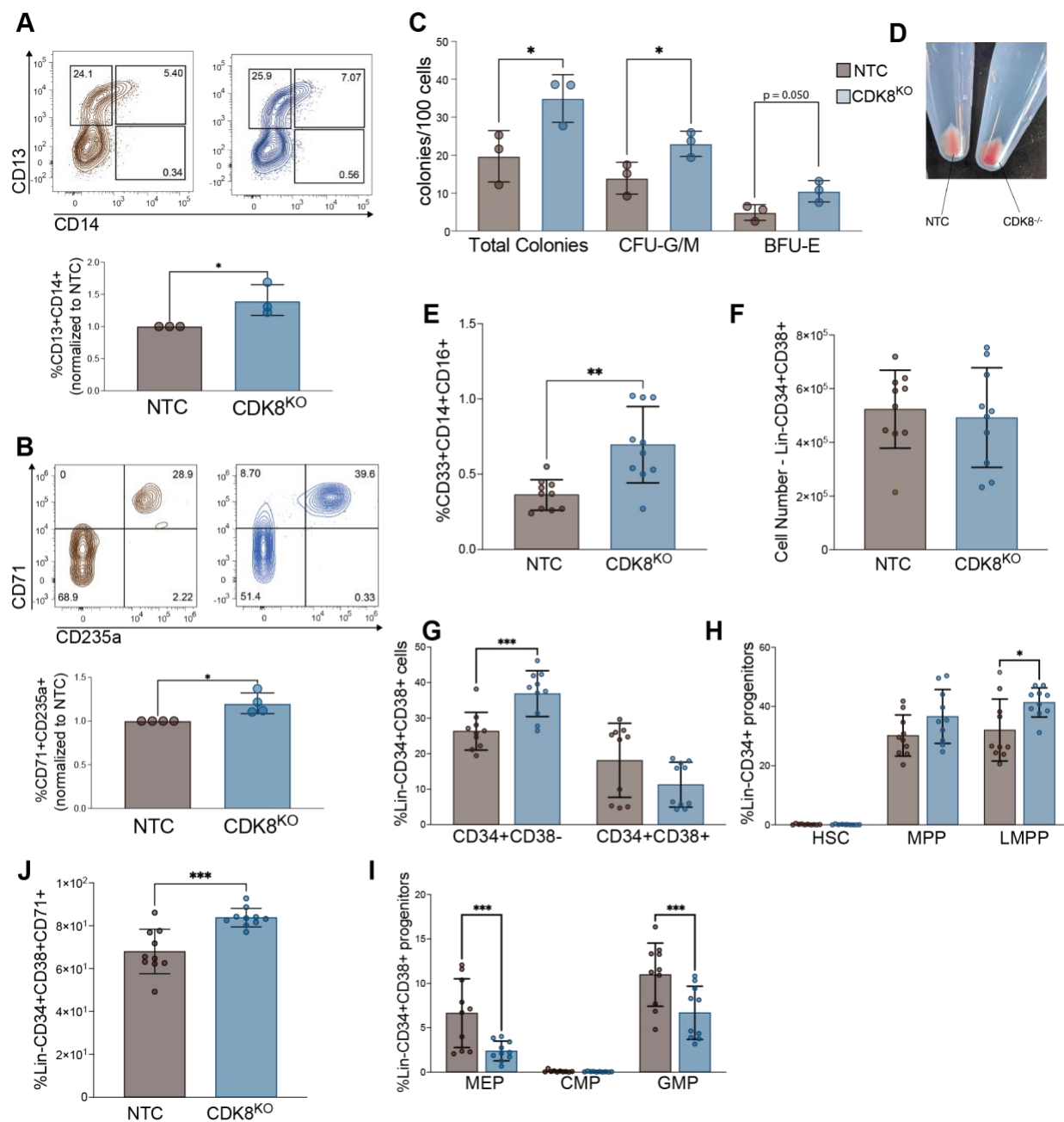


Figure 2.5.2 Loss of CDK8 alters human HSPC differentiation.

(A) Representative flow plots (top) and mean $\pm$ SD (bottom) of CD13⁺CD14⁺ myelo-monocytic cells produced by GCSF-mobilized CD34⁺ cells electroporated with AAVS1 or CDK8 guide RNAs. (B) Representative flow plots (top) and mean $\pm$ SD (bottom) of CD71⁺CD235a⁺ erythroid cells produced by GCSF-mobilized CD34⁺ cells electroporated with AAVS1 or CDK8 guide RNAs. Data in (A, B) are

normalized to non-targeting control (AAVS1). **(C)** Number of colony forming units (CFU) and the types of colonies produced (G/M, granulocyte/macrophage progenitors, BFU-E, burst-forming unit – erythroid). Data are represented as CFU number per 100 plated CD34⁺ UCB cells electroporated with AAVS1 or CDK8 guide RNAs. **(D)** Representative image of CFU colony pellet following collection at assay termination. *, $p < 0.05$, unpaired two-tailed student's t-test. **(E)** Percentage of human CD45⁺CD33⁺CD14⁺CD16⁺ intermediate monocytes in the bone marrow of mice (mean $\pm$ SD). **(F)** Viable cell number of lineage negative CD34⁺CD38⁺ cells in the bone marrow. **(G)** Percentage lineage negative CD34⁺CD38⁻ cells in bone marrow (mean $\pm$ SD). **(H)** Percentage Lin⁻CD34⁺CD38⁻ of hematopoietic stem cells (HSC, CD90⁺CD45RA⁻), multipotent progenitors (MPP, CD90⁻CD45RA⁻), and lymphoid myeloid primed progenitors (LMPP, CD90⁻CD45RA⁺) cells in bone marrow (mean $\pm$ SD). **(I)** Percentage Lin⁻CD34⁺CD38⁺ megakaryocyte erythroid progenitors (MEP, CD45RA⁻CD123⁻), common myeloid progenitors (CMP, CD45RA⁻CD123⁺), and granulocyte macrophage progenitors (GMP, CD45RA⁺CD123⁺) in the bone marrow. **(J)** Viable cell number of Lin⁻CD34⁺CD38⁺CD71⁺ cells in the bone marrow.

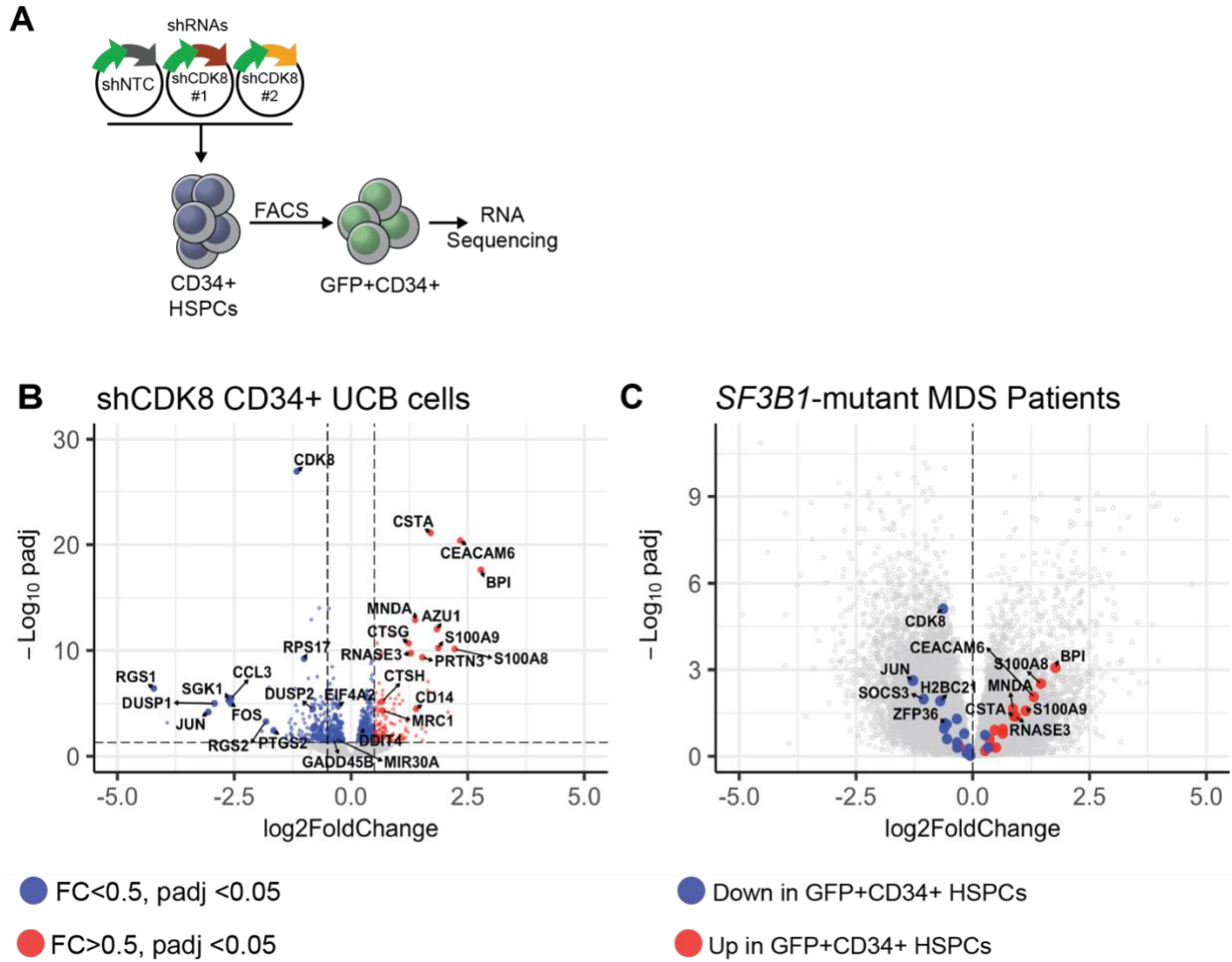


Figure 2.5.3 Homeostatic and lineage pathways are deregulated by CDK8 loss

(A) Experimental overview for RNA sequencing experiment. (B) Volcano plot representation of differentially expressed genes from CDK8 knock-down cells compared to non-targeting control, showing the magnitude of difference (log₂ fold change, x-axis) and significance (-log₁₀(adjusted p-value), y-axis). Red dots, adjusted p-value < 0.05, LFC > 0.5, blue dots, adjusted p-value < 0.05, LFC < 0.5, solid dots, significant genes across both shRNAs. Dashed lines mark the cutoff for significance (horizontal, adjusted p-value < 0.05) and fold change (vertical, -0.5 < LFC > 0.5). (C) Volcano plot representation of differentially expressed genes from SF3B1-mutant MDS patient samples compared to SF3B1-WT MDS and healthy controls. Dots, genes shown to be significantly up (red) or down (blue) in CDK8 knockdown CD34⁺ HSPCs, grey circles, all other genes.

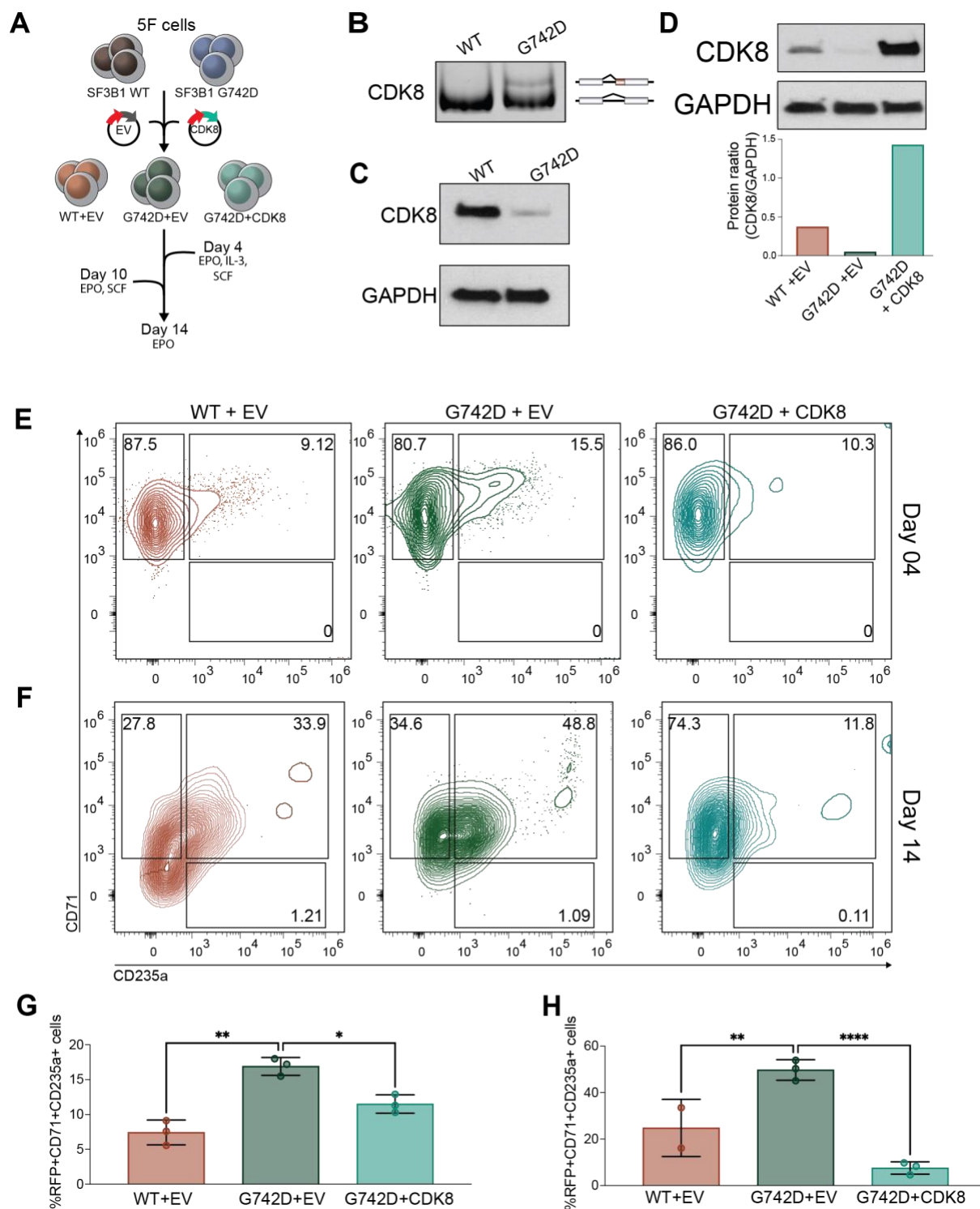


Figure 2.5.4 CDK8 re-expression rescues early erythroid differentiation

(A) Experimental overview for erythroid differentiation assay using the iPSC-HPC model. (B) RT-PCR and native PAGE of 158 bp surrounding the alternative 3' splice site usage in CDK8 mRNA collected from

wildtype (WT) and SF3B1 G742D mutant iPSC-HPCs. **(C)** Western blot of CDK8 protein levels from SF3B1 wildtype and mutant iPSC-HPCs. **(D)** Western blot (top) and quantification of iPSC-HPCs transduced with lentiviral particles containing empty vector (EV), or full length CDK8 cDNA. Western blot was quantified and protein ratio (relative to loading control, ImageJ) was plotted (bottom). **(E)** Representative flow plots and **(G)** mean $\pm$ SD (bottom) of CD71⁺CD235a⁺ iPSC-HPCs transduced with CDK8 cDNA or EV control on day 4 of erythroid differentiation. **(F)** Representative flow plots and **(H)** mean $\pm$ SD (bottom) of CD71⁺CD235a⁺ iPSC-HPCs transduced with CDK8 WT cDNA or EV control on day 13 of erythroid differentiation. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$, ****, $p < 0.0001$ ordinary one-way Anova.

2.6 Supplemental Figures and Legends

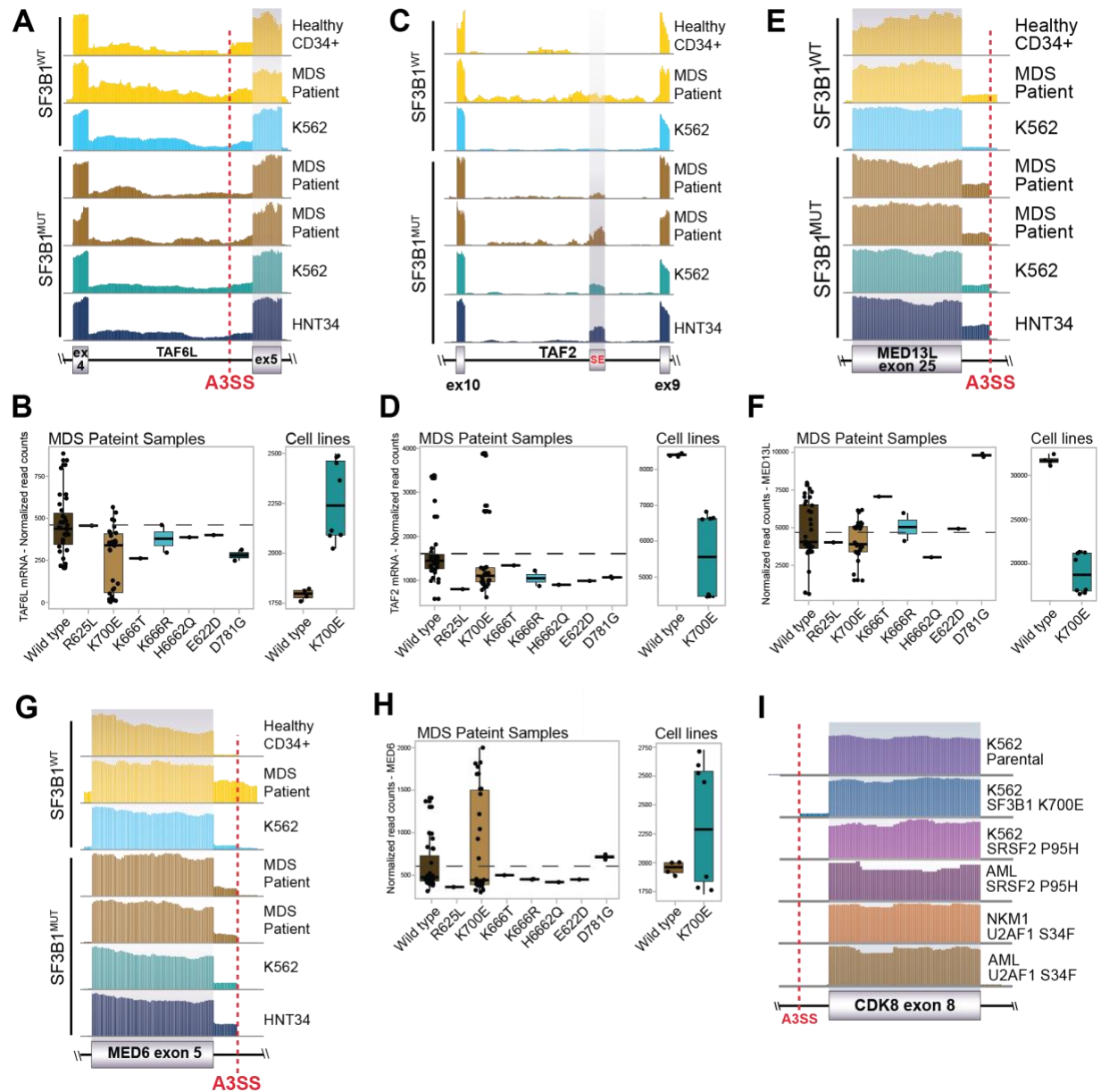


Figure 2.6.S1 Related to Figure 2.5.1

(A) IGV plots of the alternative 3' splice site (A3SS) between exon 4 and exon 5 in TAF6L, Y-axis, absolute read count. (B) Normalized read counts (DESeq2) of TAF6L mRNA in *SF3B1*-mutant MDS patient samples and cell lines, dashed line marks the mean counts of the *SF3B1*-WT patient samples. (C) IGV plots of the skipped exon (SE) between exon 9 and exon 10 in TAF2, Y-axis, absolute read count. (D) Normalized read counts (DESeq2) of TAF2 mRNA in *SF3B1*-mutant MDS patient samples and cell lines, dashed line marks

the mean counts of the *SF3B1*-WT patient samples. **(E)** IGV plots of the A3SS between in exon 17 of MED13L, Y-axis, absolute read count. **(F)** Normalized read counts (DESeq2) of MED13L mRNA in *SF3B1*-mutant MDS patient samples and cell lines, dashed line marks the mean counts of the *SF3B1*-WT patient samples. **(G)** IGV plots of the A3SS between in exon 5 of MED6, Y-axis, absolute read count. **(H)** Normalized read counts (DESeq2) of MED6 mRNA in *SF3B1*-mutant MDS patient samples and cell lines, dashed line marks the mean counts of the *SF3B1*-WT patient samples. **(I)** IGV plots of the A3SS in CDK8 exon 8 in AML patient and cell lines with different splicing mutations (*SF3B1* K700E, *SRSF2* P95H, *U2AF1* S34F), Y-axis, absolute read count.

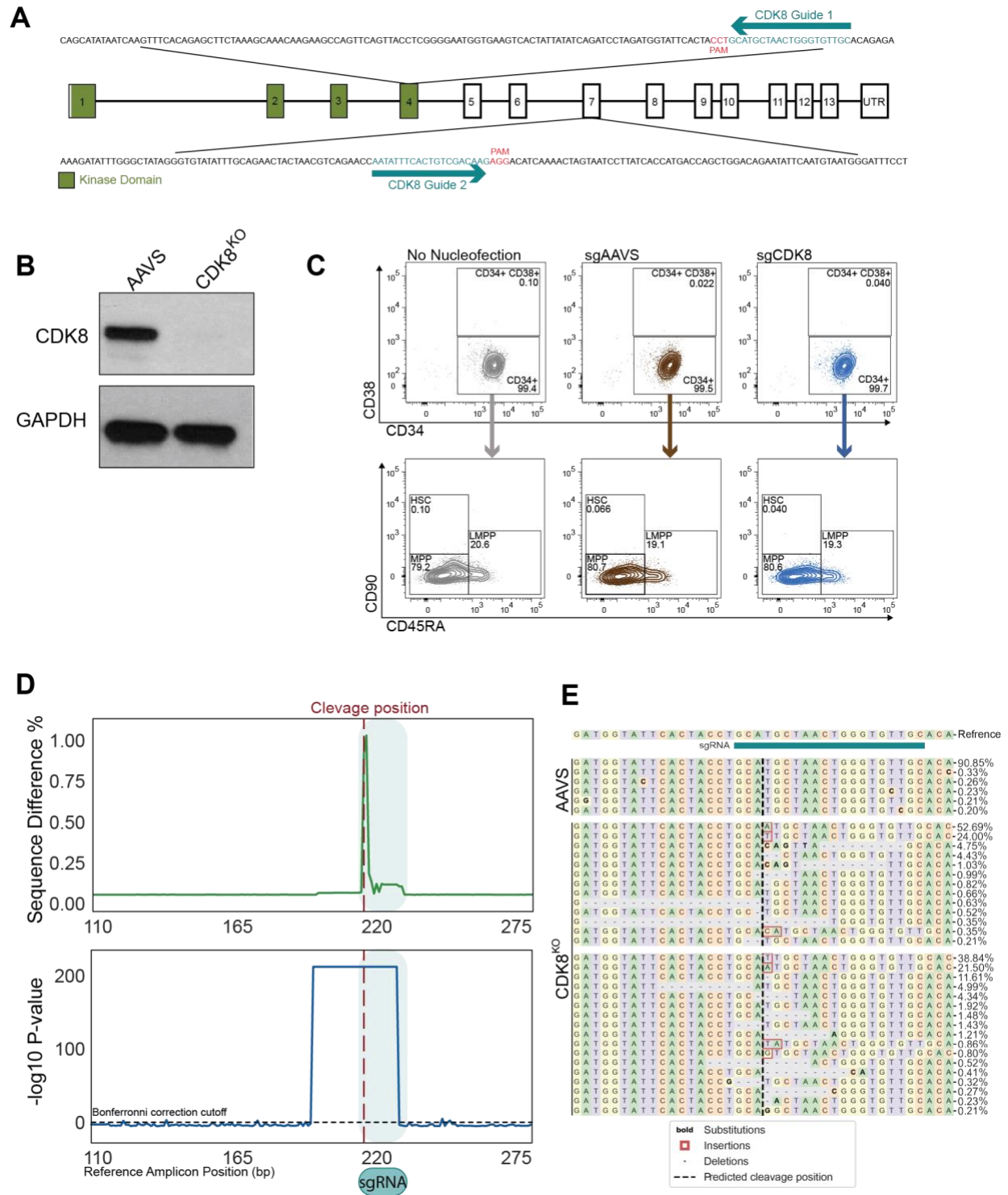


Figure 2.6.S2 Related to Figure 2.5.2

(A) Diagram of sgRNA's targeting CDK8. (B) Analysis (CRISPResso) of editing efficiency surrounding CDK8 guide RNA 2 (sgRNA2) of bone marrow CD45⁺ cells collected from 3 mice at 20 weeks post-

transplant. Top – percent sequence difference (green line), Bottom – $-\log_{10}$ of the p value for the edits at each position (blue line). Dashed black line – Bonferroni cutoff correction. Percent sequence difference and p value calculated compared to the reference sequence (ENSG00000132964). Red dashed line – predicted cleavage position. **(C)** Sequence alignments from amplicon sequencing surrounding CDK8 sgRNA2 of bone marrow CD45⁺ cells collected from 3 mice at 20 weeks post-transplant and percentage of each alignment of total reads. Dashed black line – predicted cut site. **(D)** Representative flow plots from CD34⁺ UCB cells 24 hours post nucleofection with non-targeting (sgAAVS, brown), or CDK8 (sgCDK8, blue) guides or untreated cells (grey). **(E)** Western blot of CD34⁺ UCB cells 5 days post nucleofection.

Figure 2.6.S3 Related to Figure 2.5.2

(A) Experimental overview of xenotransplantation assays. **(B)** Mature human (Ter119⁺CD45⁺) hematopoietic lineage composition (B cells – CD19⁺, Myeloid – CD33⁺, T cells – CD3⁺) in the peripheral blood of mice over 16 weeks. **(C)** Percent peripheral blood mature human hematopoietic cells at 20 weeks post transplantation. **(D)** Percent mature human hematopoietic cells at 20 weeks in the spleen post transplantation. **(E)** Percent mature human hematopoietic cells at 20 weeks in the bone marrow post transplantation. **(F)** Percent human chimerism (human CD45⁺) in peripheral blood (red), spleen (green), and bone marrow (blue). **(G)** Representative flow plots and gating strategy for progenitor populations in the bone marrow at 20 weeks (AAVS^{KO} – brown, CDK8^{KO} – blue)

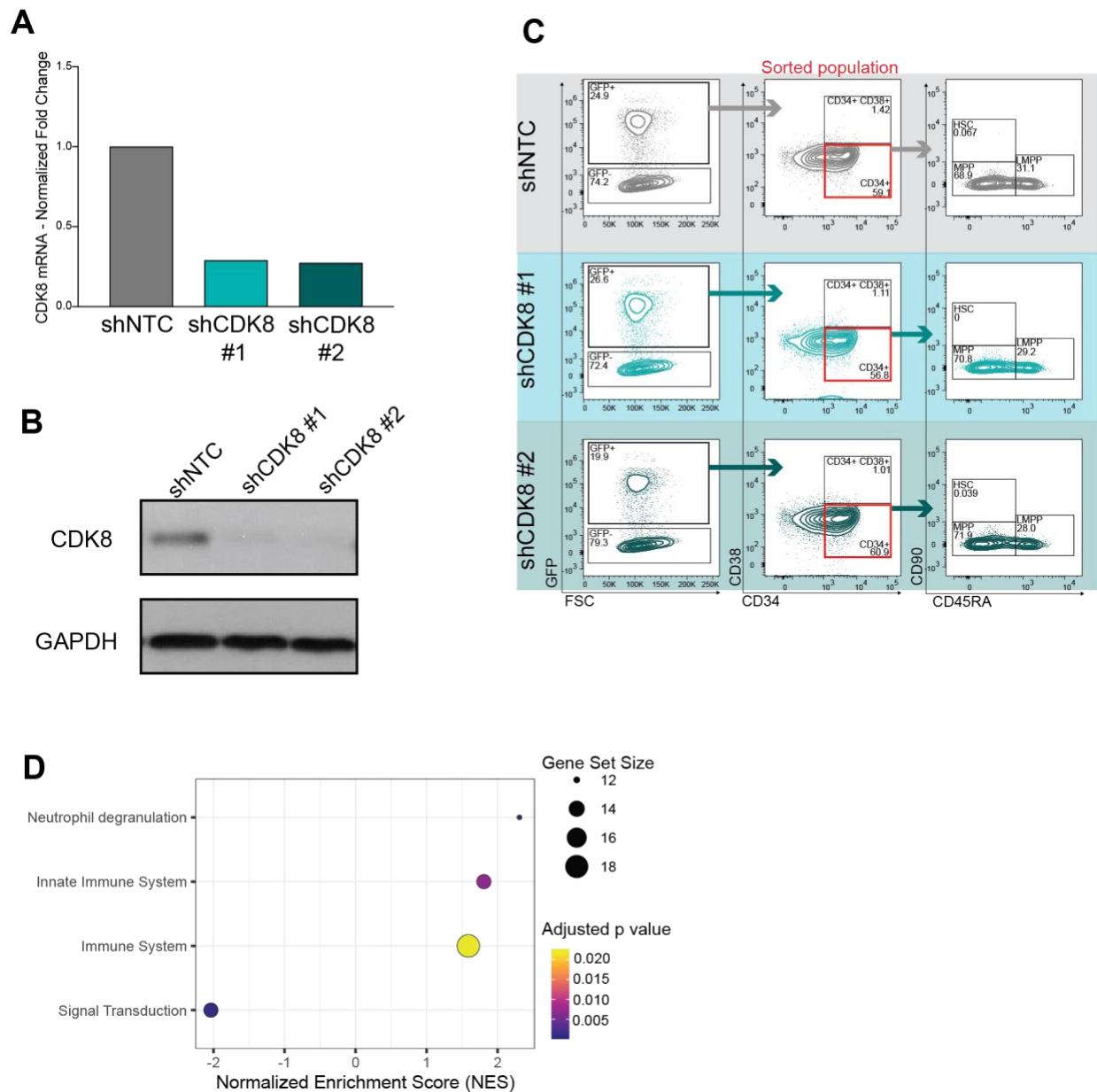


Figure 2.6.S4 Related to Figure 2.5.3

(A) CDK8 qPCR from CD34⁺ UCB cells treated with viral particles containing non targeting (shNTC) or CDK8 (shCDK8) shRNAs. Data are plotted as the ratio with 18s RNA, normalized to shNTC. (B) Western blot from CD34⁺ UCB cells treated with shNTC or shCDK8. (C) Gating strategy and representative flow plots of UCB CD34⁺ cells treated with shRNAs. Red box – sorted population. (D) Reactome analysis¹⁸⁶ of CDK8^{KO} CD34⁺ UCB cells, showing significant (Benjamini Hochberg corrected $p < 0.05$) pathways. X-axis, normalized enrichment score (NES). For corresponding genes see **Table 2.7.3**

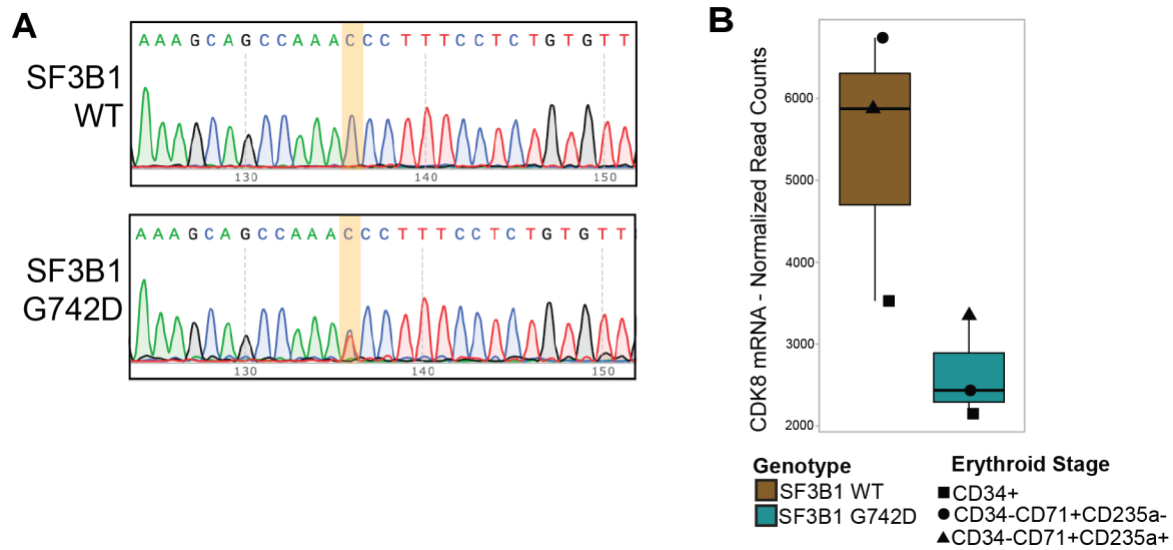


Figure 2.6.S5 Related to Figure 2.5.4

(A) Sanger sequencing traces from WT (top) and SF3B1 mutant (bottom) iPSC-HPCs. Yellow bar highlights the C>A mutation encoding the G742D mutation. (B) Normalized read counts (DESeq2) of CDK8 mRNA from published RNA-seq data of SF3B1-mutant iPSC-HPCs in erythroid differentiation conditions (n=1/condition, 3 conditions total). Erythroid stage is indicated by point shape (circle = early, triangle = late, square = undifferentiated).

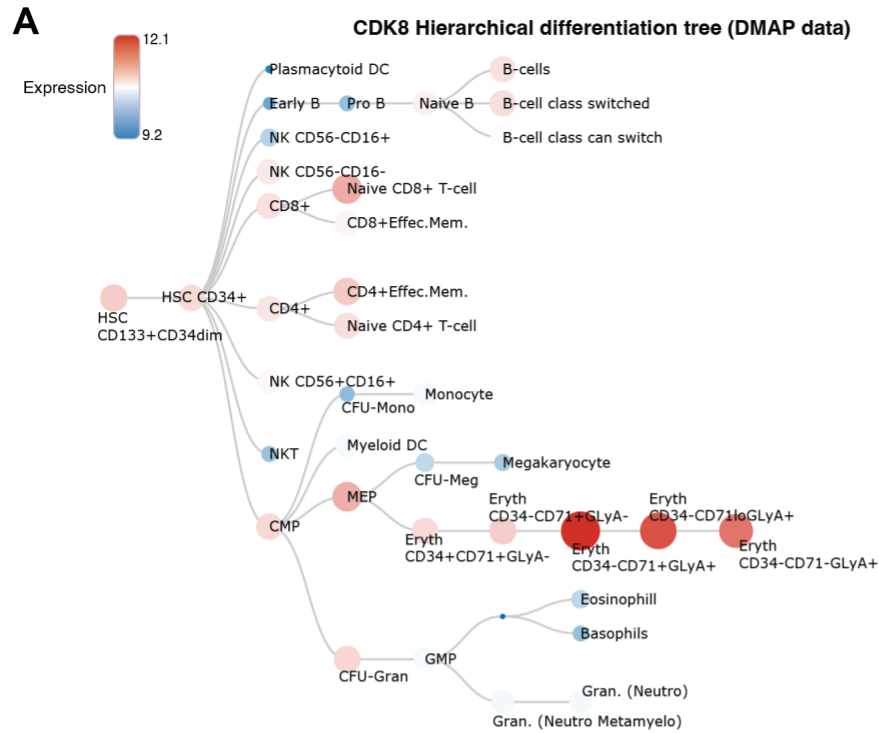
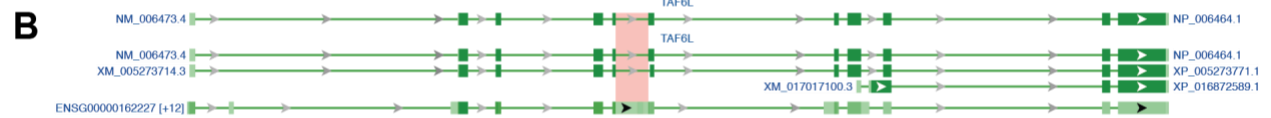
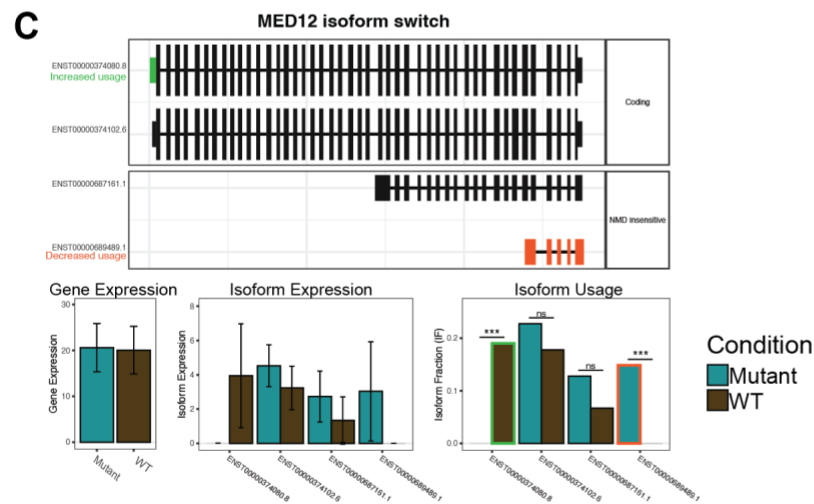
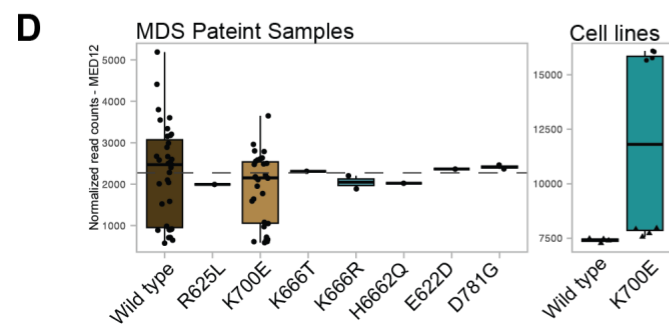


Figure 2.6.S6 Related to Discussion

(A) CDK8 expression across hematopoietic cell populations – generated using BloodSpot¹⁸⁷ analysis of Differentiation Map (DMAP) data¹⁸⁸. (B) Diagram of annotated and predicted isoforms of TAF6L from NCBI Genome Data Viewer (<https://www.ncbi.nlm.nih.gov/gdv/>). (C) Significant (Isoform fraction > 10%) isoforms of MED12 expressed by *SF3B1*-mutant samples compared to WT controls. Top – diagram of significant isoforms (Increased usage in WT–green, Increased usage in *SF3B1*-mutant–orange). Bottom Left – total RNA expression, y-axis = TPM of total MED12 mRNA. Bottom Center – Isoform TPM in *SF3B1*-mutant and *SF3B1*-WT samples, y-axis = isoform TPM. Bottom Right – Isoform usage *SF3B1*-mutant and *SF3B1*-WT samples, y-axis = isoform TPM/Total TPM.

2.7 Methods

Antibodies and oligos

All primers used in this study are available in **Tables 2.7.4**. All antibody information is available in **Tables 2.7.5**.

Cell Culture

Human umbilical cord blood CD34⁺ cells (STEMCELL Technologies) and GCSF-mobilized adult CD34⁺ cells (Fred Hutch Co-Operative Center for Excellence on Hematology (CCEH)) were thawed according to the manufacturer's instructions. Cells were cultured in SFEM-II (STEMCELL Technologies) supplemented with recombinant human cytokines (50 ng/mL SCF, 50 ng/mL FLT3L, 50 ng/mL TPO; all from Peprotech), 10 µg/mL LDL (STEMCELL Technologies), 500 nM SR1 (Selleck Chem.), 1 µM UM729 (STEMCELL Technologies) for 24 hours prior to nucleofection. K562, 293T, HNT34, and Nalm6 cells were cultured in base media

(IMDM, DMEM, and RPMI, respectively) supplemented with penicillin-streptomycin (100 U/mL, Gibco, No. 15140122), Glutamax (1%, Gibco, No. 35050061) and heat-inactivated FBS (10%: K562, 293T, NALM6, 20%: HNT34). The MDS-RS patient derived *SF3B1*-G742D and isogenic *SF3B1*-WT iPSC-derived hematopoietic progenitors (iPSC-HPCs) were generated and maintained as previously described.¹⁸⁹ Briefly, cells were maintained in SFEM-II (STEMCELL Technologies) supplemented with penicillin-streptomycin (100 U/mL, Gibco, No. 15140122), doxycycline (2 µg/mL) and cytokines (10 ng/mL IL-3, 50 ng/mL IL-6, 50 ng/mL TPO, 50 ng/mL SCF, 50 ng/mL FLT3L). Cells were washed and replated at a density of 5-7x10⁵ cells/ mL every 3 days and not allowed to exceed 1.5x10⁶ cells/mL. The iPSC-HPCs were maintained in culture for no more than 7 weeks. All cell lines were maintained in a 37°C/5% CO₂ incubator.

Gene Editing

Nucleofection of CD34⁺ HSPCs was carried out as previously described¹⁹⁰ using the P3 4D Nucleofector kit (Lonza). Briefly, 24 hours post-thaw, 2-4 x10⁵ cells were collected and rinsed in PBS and re-suspended in 20 µL nucleofection media consisting of 2.5 pmol/µL Sp.Cas9 (Aldeveron), 2.5 pmol/µL guide RNA (1.25 pmol/µl each guide, Synthego), in nucleofector solution supplemented with buffer P3. Cells were nucleofected in the 4D nucleofector (Lonza) using program DS 150 for buffer P3. Cells were recovered in 200 µL complete SFEM-II (see above) and allowed to recover for 24 hours prior to use. For applications that required greater cell numbers, multiple nucleofection reactions were performed and subsequently mixed post nucleofection. Guides in **Table 2.7.4**.

Lentivirus production

Lentiviral particle production was performed using low-passage 293T cells (Takara ClonTech). Cells were seeded in 10 cm tissue culture dishes at a density of 3.2×10^4 cells/cm² in 10 mL complete DMEM (see above). 2 hours prior to transfection media was removed and replaced with 8 mL complete DMEM (see above). Transfection was performed 24 hours post plating by adding transfection master mix. To make the transfection master mix 12 µg of viral plasmid, and 12 µg of packaging viral mix (3:1 ratio of psPAX2:VSVG) was added to 2.34 mL OptiMEM (Gibco). 30 µg of polyethylenimine (Polysciences, 23966-100) was added to the media/plasmid solution and vortexed for 30 seconds. Transfection master mix was allowed to sit at room temperature for 20 minutes prior to adding it to 293T cells, drop-wise. Complete media change was performed 16 hours post-transfection by removing media/transfection mix and replaced with 10.5 mL of complete DMEM. Supernatants containing lentiviral particles were harvested 72 hours post transfection, snap-frozen in liquid nitrogen, and stored at -80°C. To concentrate lentiviral particles, fresh viral supernatant was first filtered of cell debris in using Millex -GP syringe filters (Millipore, SLGVM33RS), and concentrated using Amicon Ultra-15 centrifugal filter units (Millipore, UFC905024), snap frozen and stored at -80°C

Immunoblotting

Cells were lysed in RIPA buffer (Invitrogen, CAT#89900) supplemented with protease inhibitors (ThermoFisher, CAT#78446) and sonicated (Bioruptor UCD-200, Diagenode) for 5 minutes in 30 second on/off cycles on medium setting. Protein concentrations were normalized using a Pierce BCA protein assay kit (Thermo Fisher Scientific CAT#23227). 20 ng of protein lysate was mixed with 6XSDS loading buffer (0.3M Tris-HCL, 10% SDS, 60% Glycerol, 6% 2-mercaptoethanol, 1% bromophenol blue), loaded in pre-cast SDS acrylamide gels (BioRad,

CAT#4561084) and run at 90V. Blots were transferred using the Bio-Rad turbo transfer apparatus following manufacturers recommendation. Membranes were blocked in Tris-buffered saline with 0.05% Tween-20 (TBS-T) and 5% milk for 1h at room temperature and immunoblotted with primary antibodies overnight at 4°C. Antibodies are listed in **Table 2.7.5**. Membranes were washed 3 times with TBS-T and incubated for 1 h at room temperature with secondary antibodies conjugated to horseradish peroxidase. Blots were imaged using ECL Substrate (BioRad, CAT#1705062) using AFP mini-medical 90 film processor.

RT-PCR

200 ng Trizol extracted RNA was used to make cDNA using iScript (BioRad, CAT# 1708891). PCR was performed using GoTAQ (Promega, CAT#M7123). PCR products were run using the Protean II xi electrophoresis tank (BioRad) in a 12% native polyacrylamide gel (BioRad, CAT#1610156) in 1XTBE (Fisher Chemical, SO-0016) at 45V for 48 hours at 4°C and imaged using ethidium bromide (Fisher Scientific, CAT#BP1302-10) on a Gel Doc XR (Bio-Rad). Primers are listed in **Table 2.7.4**.

Flow Cytometry

Cells were harvested and washed in cold FACS buffer (1XPBS, 2%FBS). Cells were resuspended in 100 µL staining buffer (FACs buffer with a 1:200 dilution of each antibody) and incubated in the dark at 4C for 20 minutes. Staining buffer was washed by adding 1 mL FACS buffer and resuspended in 100-300 µL FACS buffer with 1:500 dilution dapi (Millipore, 10236276001). Flow cytometry was performed on the BD FACSymphony A5. Antibodies are listed in **Table 2.7.5**.

Animals

All animal experiments were performed with approval by and in accordance with Fred Hutchinson Cancer Center (FHCC) Institutional Animal Care and Use Committees guidelines. Animal experiments were performed within the FHCC Comparative Medicine facility. For xenotransplantation experiments, 6–7-week-old female NOD.Cg-*Prdkc*^{SCID} *Il2rγ*^{-/-} (NSG) mice (Fred Hutch Comparative Medicine Shared Resources) were used. NSG mice were sub-lethally irradiated (2.0 cGy) 24 hours prior to transplantation. Twenty-four hours post recovery from nucleofection, $\sim 4\text{--}8 \times 10^4$ cells were transplanted intravenously via tail vein injection. Peripheral blood was monitored by flow cytometry every 4 weeks to track human cell chimerism. At 20 weeks post-xenotransplantation, hematopoietic organs (bone marrow, spleen and peripheral blood) were harvested and analyzed by flow cytometry using antibodies against CD45, CD34, CD38, CD33, CD19, CD71, CD45RA, CD90, CD123, and CD3.

***Ex vivo* differentiation assays**

For colony-forming unit (CFU) assays, umbilical cord blood-derived CD34⁺ HSPCs were plated 24 hours post nucleofection in 1 mL MethoCult H4034 (STEMCELL Technologies). Cells were plated at a density of 500 cells/mL in triplicate plates. Colonies were counted and scored 11 days post-plating.

For myeloid differentiation assays, healthy adult CD34⁺ HSPCs were electroporated and plated at a density of 2.5×10^4 cells/well in 96-well U-bottom plates in SFEM-II containing Myeloid II Expansion supplement (STEMCELL Technologies). Differentiation was monitored by flow cytometry for expression of CD14 and CD13.

Erythroid differentiation was performed using healthy adult CD34⁺ HSPCs following previously described protocols.¹⁹¹ Briefly, 1×10^5 cells were plated in 1 mL of expansion media

(SFEM supplemented with 100 ng/mL FLT3 ligand, 100ng/mL SCF, 5 ng/mL IL-3, 20 ng/mL IL-6) for 3 days. On day 4 cells were plated at a density of 1×10^5 cells/ mL in Stage 1 media (IMDM, 15% FBS, 2 mM glutamine, 1% BSA, 500 μ g/mL holo-transferrin, 10 μ g/mL insulin, 100 ng/mL SCF, 5 ng/mL IL3, 6 U/mL EPO) and cultured for 5 days. On day 9 cells were plated at a density of 2×10^5 cells/ mL in Stage 2 media (IMDM, 15% FBS, 2 mM glutamine, 1% BSA, 500 μ g/mL holo-transferrin, 10 μ g/mL insulin, 100 ng/mL SCF, 6 U/mL EPO). On day 14 cells were seeded at 3×10^5 cells/mL in Stage 3 media (IMDM, 15% FBS, 2 mM glutamine, 1% BSA, 500 μ g/mL holo-transferrin, 10 μ g/mL insulin, 2 U/mL EPO). The appropriate media was supplemented in the middle of each stage to maintain cells at a density below 2×10^6 cells/mL. Differentiation was monitored by flow cytometry for expression of CD71 and CD235a

RNA Sequencing

For cultured cell lines total RNA was isolated from 5×10^6 K562 SF3B1^{WT}, K562 SF3B1^{K700E} or HNT34 cells using TRIzol according to the manufacturer's instruction. For CDK8 knock-down UCB derived CD34⁺ cells, $0.2\text{--}3 \times 10^6$ CD34⁺ GFP⁺ cells were sorted using the Symphony S6 sorter (BD Biosciences), followed by RNA isolation by Trizol. RNA quality was determined using Agilent RNA 6000 Nano chip on the Agilent 4200 TapeStation and quantification was performed using NanoDrop spectrophotometer (Thermo Fisher Scientific). Total RNA samples with RNA integrity number (RIN) >8 was selected for Illumina sequencing library preparation. One microgram of DNA-free total RNA was used to prepare the library using Ultra II Directional RNA Library Prep Kit (New England Biolabs, No. E7760L) following manufacturer's instructions. Paired end sequencing (150 bp) of the libraries were performed using an Illumina NovaSeq SP platform.

Amplicon Sequencing

Human CD45⁺ cells were isolated from murine bone marrow 20 weeks post-transplant using the BD Symphony S6 sorter (BD Biosciences). DNA was extracted using the Qiagen DNeasy blood and tissue kit (CAT#69504). PCR was performed using Platinum II high fidelity PCR master mix (Invitrogen, CAT#14000013). PCR products were precipitated overnight using ethanol precipitation (https://projects.iq.harvard.edu/files/hlallab/files/ethanol-precipitation-of-rna_hla.pdf) and submitted for sequencing at Azetna following submission guidelines.

Bioinformatic Analysis

For RNA sequencing, raw fastq files were trimmed and quality was assessed using TrimGalore (<https://github.com/FelixKrueger/TrimGalore>). Trimmed fastq files were aligned (GRCh38, Gencode Release 48) with STAR¹⁹² using `--quantMode GeneCounts`. For publicly available MDS patient data batch correction was performed using limma¹⁹³ and differential expression for all datasets was performed using DESeq2¹⁹⁴ comparing wild type to mutant samples. Significant changes were determined using a cutoff of $|\text{Log}_2 \text{ fold change}| \geq 0.5$ and p-adjusted value ≤ 0.05 . For splicing analysis reads were aligned (GRCh38, Gencode Release 48) using STAR along with soft-clipping and splicing changes were quantified using RMATS-turbo 4.1.1¹⁹⁵ with the novel splice site detection feature enabled.

To generate heat map plots of publicly available data, .JC files were imported using maser (<https://github.com/DiogoVeiga/maser>) and significant splicing events were determined (minimum read count =10, $|\text{PSI}| \geq 0.05$, FDR <0.05). A list of 162 transcriptional cofactors was

curated from gene ontology (<https://geneontology.org/>) and filtered for unique entries. Significant splicing events were filtered using the unique transcriptional cofactor list. Z scores were calculated for splicing events within each data set to control for batch effects and unsupervised clustering was performed across all samples within each splice type.

For amplicon sequencing analysis, quality and trimming was performed on raw fastq files (see above). Reads were aligned to CDK8 reference sequence (NCBI NC_000013.11) and analyzed using CRISPResso¹⁹⁶ using a window of 50 nucleotides surrounding the guide.

To perform isoform analysis, trimmed fastq files were aligned using Kallisto¹⁹⁷ (GRCh38, Gencode Release 43). To determine novel isoforms, alignments were imported into R using IsoformSwitchAnalyzeR^{198,199} and significant (gene expression cutoff =1, isoform cutoff =0) isoform changes were analyzed and plotted.

2.7 Tables

2.7.1 GO Transcriptional Cofactors

GO_term	Gene	Description	GO_term	Gene	Description
GO:0000126	BRF1	transcription factor TFIIB complex	GO:0016591	ATXN7	RNA polymerase II, holoenzyme
GO:0000126	BRF2	transcription factor TFIIB complex	GO:0016591	TRRAP	RNA polymerase II, holoenzyme
GO:0000126	BDP1	transcription factor TFIIB complex	GO:0016591	SUPT3H	RNA polymerase II, holoenzyme
GO:0000127	GTF3C1	transcription factor TFIIC complex	GO:0016591	CTR9	RNA polymerase II, holoenzyme
GO:0000127	GTF3C2	transcription factor TFIIC complex	GO:0016591	TADA3	RNA polymerase II, holoenzyme
GO:0000127	GTF3C5	transcription factor TFIIC complex	GO:0016591	RTF1	RNA polymerase II, holoenzyme
GO:0000127	GTF3C4	transcription factor TFIIC complex	GO:0016591	USP22	RNA polymerase II, holoenzyme
GO:0000127	GTF3C3	transcription factor TFIIC complex	GO:0016591	TAF5L	RNA polymerase II, holoenzyme
GO:0000127	GTF3C6	transcription factor TFIIC complex	GO:0016591	PAF1	RNA polymerase II, holoenzyme
GO:0005665	POLR2A	RNA polymerase II, core complex	GO:0016591	ENY2	RNA polymerase II, holoenzyme
GO:0005665	POLR2B	RNA polymerase II, core complex	GO:0016591	ATXN7L3	RNA polymerase II, holoenzyme
GO:0005665	POLR2C	RNA polymerase II, core complex	GO:0016591	CDC73	RNA polymerase II, holoenzyme
GO:0005665	POLR2D	RNA polymerase II, core complex	GO:0016591	SKIC8	RNA polymerase II, holoenzyme
GO:0005665	POLR2E	RNA polymerase II, core complex	GO:0016591	LEO1	RNA polymerase II, holoenzyme
GO:0005665	POLR2F	RNA polymerase II, core complex	GO:0016591	MYZAP	RNA polymerase II, holoenzyme
GO:0005665	POLR2G	RNA polymerase II, core complex	GO:0016592	CCNC	mediator complex
GO:0005665	POLR2H	RNA polymerase II, core complex	GO:0016592	CDK8	mediator complex
GO:0005665	POLR2I	RNA polymerase II, core complex	GO:0016592	MED1	mediator complex
GO:0005665	POLR2J	RNA polymerase II, core complex	GO:0016592	MED22	mediator complex
GO:0005665	POLR2K	RNA polymerase II, core complex	GO:0016592	MED14	mediator complex
GO:0005665	POLR2L	RNA polymerase II, core complex	GO:0016592	MED21	mediator complex
GO:0005665	POLR2M	RNA polymerase II, core complex	GO:0016592	MED23	mediator complex
GO:0005665	POLR2J2	RNA polymerase II, core complex	GO:0016592	MED17	mediator complex
GO:0005665	POLR2J3	RNA polymerase II, core complex	GO:0016592	MED26	mediator complex
GO:0005669	ERCC2	transcription factor TFIID complex	GO:0016592	MED27	mediator complex
GO:0005669	ERCC3	transcription factor TFIID complex	GO:0016592	MED7	mediator complex
GO:0005669	GTF2A1	transcription factor TFIID complex	GO:0016592	MED20	mediator complex
GO:0005669	GTF2A2	transcription factor TFIID complex	GO:0016592	BCLAF1	mediator complex
GO:0005669	GTF2B	transcription factor TFIID complex	GO:0016592	MED24	mediator complex
GO:0005669	GTF2E1	transcription factor TFIID complex	GO:0016592	THRAP3	mediator complex
GO:0005669	GTF2E2	transcription factor TFIID complex	GO:0016592	MED12	mediator complex
GO:0005669	GTF2F1	transcription factor TFIID complex	GO:0016592	MED13	mediator complex
GO:0005669	GTF2H2	transcription factor TFIID complex	GO:0016592	MED6	mediator complex
GO:0005669	GTF2H3	transcription factor TFIID complex	GO:0016592	MED16	mediator complex
GO:0005669	GTF2H4	transcription factor TFIID complex	GO:0016592	MED13L	mediator complex
GO:0005669	TAF1	transcription factor TFIID complex	GO:0016592	MED4	mediator complex
GO:0005669	TAF2	transcription factor TFIID complex	GO:0016592	MED31	mediator complex
GO:0005669	TAF4	transcription factor TFIID complex	GO:0016592	MED18	mediator complex
GO:0005669	TAF4B	transcription factor TFIID complex	GO:0016592	MED9	mediator complex
GO:0005669	TAF5	transcription factor TFIID complex	GO:0016592	MED29	mediator complex
GO:0005669	TAF6	transcription factor TFIID complex	GO:0016592	MED28	mediator complex
GO:0005669	TAF7	transcription factor TFIID complex	GO:0016592	MED25	mediator complex
GO:0005669	TAF9	transcription factor TFIID complex	GO:0016592	MED10	mediator complex
GO:0005669	TAF10	transcription factor TFIID complex	GO:0016592	HAVCR2	mediator complex
GO:0005669	TAF11	transcription factor TFIID complex	GO:0016592	MED30	mediator complex
GO:0005669	TAF12	transcription factor TFIID complex	GO:0016592	MED8	mediator complex
GO:0005669	TAF13	transcription factor TFIID complex	GO:0016592	MED12L	mediator complex
GO:0005669	TBP	transcription factor TFIID complex	GO:0016592	PPARGC1B	mediator complex
GO:0005669	TCEA1	transcription factor TFIID complex	GO:0016592	MED19	mediator complex
GO:0005669	TAF6L	transcription factor TFIID complex	GO:0016592	BCLAF3	mediator complex
GO:0005669	TAF9B	transcription factor TFIID complex	GO:0016592	MED11	mediator complex
GO:0005669	TAF7L	transcription factor TFIID complex	GO:0030880	POLR3D	RNA polymerase complex
GO:0005669	PAAF1	transcription factor TFIID complex	GO:0030880	MCM3	RNA polymerase complex
GO:0005669	TAF3	transcription factor TFIID complex	GO:0030880	POLA1	RNA polymerase complex

GO:0005669	TAF8	transcription factor TFIID complex	GO:0030880	POLRMT	RNA polymerase complex
GO:0005669	TAF1L	transcription factor TFIID complex	GO:0030880	PRIM1	RNA polymerase complex
GO:0005669	TAF11L2	transcription factor TFIID complex	GO:0030880	PRIM2	RNA polymerase complex
GO:0005669	TAF11L4	transcription factor TFIID complex	GO:0030880	TERT	RNA polymerase complex
GO:0005669	TAF11L6	transcription factor TFIID complex	GO:0030880	POLR1C	RNA polymerase complex
GO:0005669	GTF2H5	transcription factor TFIID complex	GO:0030880	POLR3F	RNA polymerase complex
GO:0005669	TAF11L3	transcription factor TFIID complex	GO:0030880	POLR3G	RNA polymerase complex
GO:0005669	TAF11L7	transcription factor TFIID complex	GO:0030880	POLR3C	RNA polymerase complex
GO:0005669	TAF11L8	transcription factor TFIID complex	GO:0030880	POLR1G	RNA polymerase complex
GO:0005669	TAF11L10	transcription factor TFIID complex	GO:0030880	POLR3A	RNA polymerase complex
GO:0005669	TAF11L12	transcription factor TFIID complex	GO:0030880	POLA2	RNA polymerase complex
GO:0005669	TAF11L14	transcription factor TFIID complex	GO:0030880	POLR1A	RNA polymerase complex
GO:0005669	TAF11L9	transcription factor TFIID complex	GO:0030880	RPAP1	RNA polymerase complex
GO:0005669	TAF11L11	transcription factor TFIID complex	GO:0030880	CRCP	RNA polymerase complex
GO:0005669	TAF11L13	transcription factor TFIID complex	GO:0030880	POLR1H	RNA polymerase complex
GO:0005672	TBPL1	transcription factor TFIIA complex	GO:0030880	POLR1D	RNA polymerase complex
GO:0005672	GTF2A1L	transcription factor TFIIA complex	GO:0030880	POLR3K	RNA polymerase complex
GO:0005674	GTF2F2	transcription factor TFIIIF complex	GO:0030880	POLR3B	RNA polymerase complex
GO:0005675	CCNH	transcription factor TFIIH holo complex	GO:0030880	POLR3E	RNA polymerase complex
GO:0005675	CDK7	transcription factor TFIIH holo complex	GO:0030880	ZNFX1	RNA polymerase complex
GO:0005675	GTF2H1	transcription factor TFIIH holo complex	GO:0030880	POLR1E	RNA polymerase complex
GO:0005675	MNAT1	transcription factor TFIIH holo complex	GO:0030880	POLR1B	RNA polymerase complex
GO:0005675	MMS19	transcription factor TFIIH holo complex	GO:0030880	POLR3GL	RNA polymerase complex
GO:0005675	GTF2H2C	transcription factor TFIIH holo complex	GO:0030880	TENT2	RNA polymerase complex
GO:0005675	GTF2H2C_2	transcription factor TFIIH holo complex	GO:0030880	POLR3H	RNA polymerase complex
GO:0016591	KAT2A	RNA polymerase II, holoenzyme	GO:0030880	PRIMPOL	RNA polymerase complex
GO:0016591	PEX2	RNA polymerase II, holoenzyme	GO:0030880	POLR1F	RNA polymerase complex

2.7.2 Differentially expressed genes

ensID	baseMean	log2FoldChange	lfcSE	padj	Symbol
ENSG00000125740	1878.7826136516646	-5.17420266	0.7470188559797255	2.335289529292566e-9	FOSB
ENSG00000090104	825.7795585631396	-4.220729611	0.6905700804334852	3.716430110005118e-7	RGS1
ENSG00000177606	2489.458889632369	-3.056824789	0.6124597028949723	6.596096477997943e-5	JUN
ENSG00000120129	2770.765503378519	-2.926330941	0.5393793503994113	1.0097593714401976e-5	DUSP1
ENSG00000118515	1074.3584376154172	-2.623042227	0.47037505297972076	5.0441303397957194e-6	SGK1
ENSG00000170345	12930.647574938048	-2.577293336	0.47400244126348723	9.651933776434896e-6	FOS
ENSG00000277632	55.58257408257569	-2.565155666	0.4515637390236164	3.307975186538853e-6	CCL3
ENSG00000116741	1433.0923605116338	-1.822042176	0.4064934013395392	5.234732919821925e-4	RGS2
ENSG00000073756	521.0046434285538	-1.65754851	0.42593862196815846	0.00385498	PTGS2
ENSG00000132964	723.9034206792483	-1.158014797	0.09897008	1.026771656574397e-27	CDK8
ENSG00000182774	357.35341636638356	-1.001460542	0.1403347622359276	5.990730233884701e-10	RPS17
ENSG00000158050	413.03934302955196	-0.812058189	0.1575343497445572	3.298068734523109e-5	DUSP2
ENSG00000184557	280.7575681007912	-0.789858827	0.1891523802310188	0.00158093	SOCS3
ENSG00000128016	1898.3105918540857	-0.777887323	0.22401595963527565	0.0127953	ZFP36
ENSG00000184678	359.9573145078734	-0.664709732	0.17829422469046857	0.00631415	H2BC21
ENSG00000184205	654.0715317830264	-0.484370032	0.12320497079698715	0.00342791	TSPYL2
ENSG00000099860	607.4976294	-0.369946331	0.11748194978571683	0.028755822642934987	GADD45B
ENSG00000115875	8973.869337	-0.334925735	0.07139787	2.373606844050005e-4	SRSF7
ENSG00000207827	1563.074527635818	-0.329798714	0.10118088359312803	0.02231193	MIR30A
ENSG00000156976	17060.708773729457	-0.275448581	0.052114514503249096	1.8179230097703505e-5	EIF4A2
ENSG00000255717	7070.889591	-0.220936579	0.05039677	7.383180207228241e-4	SNHG1
ENSG00000168209	6897.294557354342	0.2055778585950733	0.05174286	0.00307217	DDIT4
ENSG00000103811	757.7166385827011	0.671705084	0.12147521099414889	6.280113701983998e-6	CTSH
ENSG00000260314	819.9228977079372	0.685285848	0.1350038229889804	4.7403004466840905e-5	MRC1
ENSG00000100448	5187.598726877983	1.2311566005856651	0.16077495937894287	2.0982368537212392e-11	CTSG
ENSG00000169397	363.22106475704595	1.2792299336010846	0.17424053206809356	1.8016521270311612e-10	RNASE3
ENSG00000163563	402.48945732314496	1.3736638093452906	0.16469517583348636	1.2584608647544614e-13	MNDA

ENSG00000170458	120.64453356979837	1.3947519420769008	0.2701091907862268	3.1968117020644744e-5	CD14
ENSG00000196415	13659.607819159079	1.5286750177435138	0.2127039003237495	4.4850703323591617e-10	PRTN3
ENSG00000121552	351.0193224991847	1.709440178310159	0.16356745480875684	7.848916126556517e-22	CSTA
ENSG00000172232	9732.000277510488	1.8436254373650547	0.2285143462335916	9.678817394728107e-13	AZU1
ENSG00000163220	159.81529791352145	1.872561885677348	0.24916297584503833	5.7573270515701774e-11	S100A9
ENSG00000143546	113.08487904163589	2.226735497604151	0.29775919177314336	7.190558135063182e-11	S100A8
ENSG00000086548	196.9488889123964	2.3442469387556697	0.22841957185801529	4.2033827264413034e-21	CEACAM6
ENSG00000197561	6010.374727727248	2.498734061804507	0.19410165750687633	1.03271413028086e-33	ELANE
ENSG00000101425	454.1260027613963	2.7829060405792463	0.2895722247965788	2.3467319553399878e-18	BPI
ENSG00000206249	56.12894316	3.577563261618314	0.4836271070151829	1.2531334809738146e-10	
ENSG00000214389	1391.168138637989	6.251596438128567	1.2547107956827572	6.765357734341583e-5	RPS3AP26

2.7.3 Reactome Analysis

ID	Description	NES	p.adjust	symbol_enrichment
R-HSA-6798695	Neutrophil degranulation	2.35456467	4.76E-05	BPI/ELANE/CEACAM6/S100A8/S100A9/AZU1 /PRTN3/CD14/MNDA/RNASE3/CTSG
R-HSA-162582	Signal Transduction	-2.011950239	1.44E-03	SOCS3/DUSP2/CDK8/RGS2/CCL3/ FOS/SGK1/DUSP1/JUN/RGS1/FOSB
R-HSA-168249	Innate Immune System	1.834870044	1.01E-02	BPI/ELANE/CEACAM6/S100A8/S100A9/AZU1/ PRTN3/CD14/MNDA/RNASE3/CTSG
R-HSA-168256	Immune System	1.620583475	2.46E-02	BPI/ELANE/CEACAM6/S100A8/S100A9/AZU1/PRTN3/CD14/ MNDA/RNASE3/CTSG/MRC1/CTSH

2.7.4 Primers

Primer Name	Sequence (5'-3')	Application
CDK8_99514_F	TGGGAATCCCTGAGTGTTCCT	Amplicon Sequencing
CDK8_99830_R	CAGGCCTACATATAGCACTGGT	Amplicon Sequencing
CDK8_Guide1	AATATTTCACTGTCGACAAG	Guide RNA
CDK8_Guide2	GCATGCTAACTGGGTGTTGC	Guide RNA
CDK8_ex6-7_F	TTGATATTTGGGCTATAGGGTGT	RT-PCR
CDK8_ex9-10_R	TTCTGAAGCAAGTGAATGC	RT-PCR
AAVS_Guide1	TTCTGGGAGAGGGTAGCGCA	Guide RNA
AAVS_Guide2	GAGATGGCTCCAGGAAATGG	Guide RNA

2.7.5 Antibodies

Antigen	clone	supplier	application
CD117	104D2	Biolegend	Flow Cytometry
CD123	6H6	Biolegend	Flow Cytometry
CD13	WM15	Biolegend	Flow Cytometry
CD14	M5E2	Biolegend	Flow Cytometry
CD16	3G8	Biolegend	Flow Cytometry
CD19	H1B19	Biolegend	Flow Cytometry
CD235a	HIR2	Biolegend	Flow Cytometry
CD33	WM53	Biolegend	Flow Cytometry
CD34	581	Biolegend	Flow Cytometry

CD38	HB7	Biolegend	Flow Cytometry
CD45	2D1	Biolegend	Flow Cytometry
CD45	A20	Biolegend	Flow Cytometry
Cd45.1	30-F11	Biolegend	Flow Cytometry
CD45RA	HI100	Biolegend	Flow Cytometry
CD71	CY1G4	Biolegend	Flow Cytometry
CD90	5E10	Biolegend	Flow Cytometry
CDK8	P455	Cell Signal Technologies	Western Blot
GAPDH	1D4	Novus Biologicals	Western Blot
hCD3	SK7	BD Biosciences	Flow Cytometry
Streptavadin		Biolegend	Flow Cytometry
Ter119	TER-119	Biolegend	Flow Cytometry

2.8 Acknowledgements

We thank Sean Bennette for counsel on bioinformatics, Anthony Rongvaux and Stefan Radtke on experiments involving immunocompromised mice, Beverly Torok-Storb for her mentorship, Daniel Kupperts for advice on gene editing, Laura Baquerro-Galves for advice on iPSC-HPC culture, and the Subramanaian lab and Tapscott lab at Fred Hutch for equipment loan. We would like to also acknowledge the Fred Hutch Cancer Center Flow Cytometry, Genomics, and Comparative Medicine cores for their assistance. A special thank you to the mice, who make an immeasurable contribution to science.

2.9 Authorship

Contribution: E.A.B., S.C.L. designed the study; E.A.B, C.T.H., A.S., E.A.G, R.V, S.S., E.N., performed experiments; E.A.B. and S.C.L analyzed and interpreted data; S.D.D. and D.L.S. provided samples; E.A.B., and S.C.L. wrote the manuscript

Chapter 3. Discussion

While our data provides a clear and novel role for CDK8 in the control of hematopoietic differentiation, there are still many avenues left unexplored. How does loss of CDK8 drive dysplastic erythropoiesis and immature HSPC expansion? Is loss of CDK8 contributing to alterations in transcriptional initiation and elongation in *SF3B1*-mutant cells? Our work shows that *SF3B1* mutations also disrupt other members of the CKM and transcriptional machinery. Are these changes synergistic or is loss of CDK8 sufficient to drive phenotypic changes?

Our *in vivo* data indicates that mice xenotransplanted with CDK8^{KO} UCB CD34⁺ cells have an expanded CD34⁺CD38⁺CD71⁺ population in the bone marrow (**Figure 2.5.2J**). Are these functionally differentiating or is this the result of upregulated expression of the gene encoding this transmembrane protein (TFRC)? Several publications have indicated that upregulation of TFRC can occur through the MYC, HIF-1, and mTOR pathways under various physiological conditions²⁰⁰⁻²⁰² – pathways shown to be regulated by CDK8. One hypothesis is that, in absence of CDK8, one of these transcriptional pathways are driving overexpression of CD71 leading to false identification of erythroid progenitor cells due to increased presence of CD71.

Conversely, upregulation of CD71 could be a result of pernicious differentiation related to deregulation of the GATA1/STAT5 signaling axis. Publications using cultured cell lines and CB HSPCs have demonstrated that these pathways are intrinsically linked, where STAT5 has been shown to promote expression of GATA1 and GATA1 driven differentiation relies on STAT5 activity.^{203,204} STAT5 is activated by phosphorylation to promote heterodimer formation and DNA binding.²⁰⁵ *In vitro* and *in vivo* studies using the CDK8 kinase inhibitor Sel120-34A have indicated that STAT5 is a phosphorylation target of CDK8.²⁰⁶ Based on these data, another potential hypothesis is that expanded erythroid differentiation is initiated by deregulation of the

STAT5/GATA1 signaling axis due to changes in STAT5 phosphorylation induced by mutant SF3B1 mis-splicing of CDK8.

Analysis of Differentiation Map (DMAP) data¹⁸⁸ shows that CDK8 expression is highest in cells of the erythroid lineage (**Figure 2.6.S6A**) indicating that CDK8 likely plays a role in promoting differentiation. Addressing these questions will not only provide deeper understanding into the mechanisms driving dysplastic erythroid development in *SF3B1*-mutant MDS but will elucidate a novel role for CDK8 in the context of erythroid differentiation.

Our xenotransplantation data indicates that loss of CDK8 leads to increased numbers/proportion of CD34⁺CD38⁻ HSPCs in the bone marrow, although the underlying mechanism remains unclear. CDK8 has been shown to play an important regulatory role in many key pathways involved in pluripotency and homeostasis. Indeed, in our data we find that loss of CDK8 reduces HSPC expression of *FOS*, *FOSB*, and *JUN* (**Figure 2.5.3A, Table 2.7.1**)—members of the AP-1 transcription factor family. The AP-1 transcription factor family consists of the FOS, Jun and ATF and MAF gene families, which bind as either hetero- or homo-dimers to distal genomic enhancer regions to modulate cellular transcriptional responses to external stimuli.²⁰⁷ AP1-mediated transcription has been shown to play multiple roles in hematopoietic differentiation.²⁰⁸ Additionally, single cell ATAC-seq in murine HSCs and MPPs has shown that AP-1 binding motifs are enriched in open chromatin clusters associated with HSPC identity,²⁰⁹ further highlighting their importance in mediating differentiation. Given the established role of AP-1 in regulating hematopoietic differentiation, one hypothesis is that CDK8 loss impairs the ability of these cells to initiate the transcriptional programs required for proper differentiation, resulting in the accumulation of early HSPCs in the bone marrow.

Published studies have demonstrated that aging induces a pro-inflammatory shift in the bone marrow microenvironment, characterized by elevated levels of specific cytokines such as TNF α , IL-6, MCP-1, and—in the context of *SF3B1* mutations—IL-18.^{8,9} This inflammatory milieu is strongly associated with the development of CHIP and its progression to MDS, where somatic mutations confer a selective advantage in this hostile environment over non-mutated hematopoietic clones. Notably, mutations in DNMT3A and TET2 have been shown to promote inflammatory resistance, facilitating clonal expansion under these conditions.²¹⁰⁻²¹³ However, the mechanisms by which *SF3B1* mutations might drive similar clonal dominance remain poorly understood. Given CDK8's established role in mediating inflammatory responses—particularly through the NF- κ B and STAT signaling pathways^{134-139,214}—and the fact that many of the age-associated cytokines signal through these cascades,²¹⁵⁻²¹⁷ it is plausible that CDK8 loss may confer a selective or proliferative advantage in *SF3B1*-mutant cells. While further investigation is needed to delineate the specific contribution of CDK8 to inflammatory signaling in this context, a deeper understanding of this regulatory axis could shed light on the mechanisms driving *SF3B1*-mutant clonal outgrowth and MDS pathogenesis.

In addition to alterations in gene expression, loss of CDK8 may, in part, be driving *SF3B1*-mutant defects observed in the transcriptional machinery. Published work has shown that *SF3B1* mutations result in global redistribution of RNAPII to gene bodies, hypothesized to occur partially resulting from impaired P-TEFb recruitment.⁷⁵ Multidimensional protein identification technology (MudPIT) has shown that CDK9 – the catalytic subunit of P-TEFb, regularly co-purifies with CDK8 and biochemical assays indicate that P-TEFb preferentially associates with CKM-containing Mediator complex, suggesting a direct physical basis for CDK8-mediated recruitment of P-TEFb.¹¹⁹ These data lead to the hypothesis that loss of CDK8 may be disrupting the

recruitment or function of the P-TEFb complex, contributing to transcriptional defects observed in *SF3B1*-mutant cells. Whether this is a global effect or, more likely, occurring at specific subsets of genes is unclear and warrants further investigation.

Finally, we have discovered that, in addition to CDK8, other transcriptional cofactors and members of the CKM undergo alternative or aberrant splicing indicating extensive deregulation of the CKM and transcriptional machinery by mutant *SF3B1*. Our splicing and differential gene expression analysis shows that *SF3B1* mutations cause exonization of 149 bps of intronic sequence between exon 9 and exon 10 of TAF2, a frame shifting event which likely results in TAF2 degradation by NMD, indicated by a significant reduction in TAF2 transcript level (**Figure 2.5.1B**, **Figure 2.6.S1A**). TAF2 is the second largest subunit of the TFIID complex, responsible for recognition, interaction and stabilization of TFIID on a subset of core promoter elements.²¹⁸ ChIP-seq analysis has revealed that in cultured human cell lines, TAF2 binding is enriched at promoters for RNA binding and ribosome biogenesis genes and knock down significantly reduces oncogenic proliferation of solid tumor cell lines but does not alter global transcription.²¹⁹ Additionally, TAF2 has been shown to localize to nuclear speckles, where it associated with RNA binding proteins, including members of the U2 snRNP.²²⁰ Given these data coupled with other publications purporting altered association of U2 snRNP members in *SF3B1*-mutant cells, further investigation of TAF2 mis-splicing may provide another layer of mechanistic insight into *SF3B1*-mutant mis-splicing.

In addition to TAF2, our splicing analysis shows that TAF6L also undergoes *SF3B1* mutant-mediated aberrant splicing. Our prediction indicates that *SF3B1* mutations result in loss of 14 base pairs of exonic sequence because of A3SS usage. However, when looking at TAF6L IGV tracks, this splicing event becomes less well defined. Upon further investigation in the NCBI genome

browser, it appears this event might occur in an annotated intron retention (RI) event or is a predicted splice variant (**Figure 2.6.S6B**). Regardless, given the dramatic reduction in mRNA abundance, it is likely that this event is disrupting transcript stability. TAF6L is part of the core module of the SAGA complex, the structural module responsible for recruitment and assembly of other transcriptional complexes (PIC, TFIID, TBP).¹¹⁰ Additionally, cryo-EM studies have shown that, in metazoans, TAF6L's heat repeats bind U2 snRNP proteins SF3B3 and SF3B5, effectively bridging SAGA to RNA processing machinery.²²¹ In the context of the “intron-loop” model of co-transcriptional splicing, where RNAPII remains stalled at 5' splice sites until catalytic B complex formation²²², these data indicate that loss of TAF6L might also be contributing to RNAPII redistribution in *SF3B1*-mutant cells and merits further investigation.

MED13L, an orthologue of MED13, sharing 50% identity, has been shown to structurally link CDK8, MED12, and CCNC.^{102,108} Mis-splicing of MED13L is predicted to result in 36 bps of in-frame intronic sequence occurring just before exon 25 in the MED13L transcript resulting in alternative isoform production (**Figure 2.6.S1E**). Structural analysis of MED13L indicate that this insertion likely occurs in MED13L's PIWI domain¹⁰⁸ – a domain generally associated with nucleic acid binding. While little is known about MED13L, mis-sense change of function mutations are linked to developmental defects in the brain.²²³ This glaring hole in our understanding of MED13L's function independent of its orthologue presents an exciting avenue of exploration. How does mis-splicing alter protein structure and in turn MED13L function. Are there phenotypic consequences to blood cells resulting from this aberrant splice variant? While beyond the scope of this project, understanding the ramifications of MED13L mis-splicing may provide deeper insight into the functional role of MED13L on both a molecular and cellular level.

In addition to mis-splicing of MED13L, we have also discovered that MED12 undergoes alternative isoform usage in the presence of *SF3B1* mutations. Our analysis has shown that, while overall expression of MED12 mRNA remains unchanged, that mutant cells significantly downregulate an isoform with a longer 5' untranslated region and up regulate an N-terminally truncated, but stable (predicted) MED12 isoform (**Figure 2.6.S6C**). MED12 is the kinase activating component of the CKM and has been shown to interact with all other members of the CKM.²²⁴ In addition to its role in mediating CDK8 kinase activity, MED12 has been shown to act independently of the CKM to stabilize p300 binding at HSPC lineage specific enhancers²²⁵ and mutations have been implicated as both prognostic markers and pathologic drivers of leukemia.²²⁶ Changes in 5' UTR length have a host of regulatory implications which can impact secondary structure, translation, and stability of the mRNA.^{227,228} Given that overall, mRNA levels of MED12 remain unchanged in *SF3B1*-mutant cells (**Figure 2.6.S6D**) and we see no changes in splicing, decreased 5' UTR length might be affecting protein translation, whether through alternative start codon or translational repression remains unclear. Additionally, both the presence of and increase in a N-terminally truncated isoform with coding potential is evocative. Could this be an undiscovered microprotein with some regulatory effect on the CKM or Mediator? Or is this a non-coding RNA with some yet to be described function? Given the important role MED12 plays in regulating CDK8 kinase activity and its implication in HSPC homeostasis and disease, an understanding of the functional role of alternative MED12 isoform usage begs further investigation.

Together, our findings uncover a novel and multifaceted role for CDK8 in regulating hematopoietic differentiation. The observed expansion of CD71⁺ erythroid-like populations and primitive HSPCs upon CDK8 loss, coupled with alterations in key transcriptional regulators, suggests that CDK8 is essential for orchestrating the transcriptional programs governing lineage

commitment. Furthermore, our data point to widespread disruption of the CKM in *SF3B1*-mutant cells, including aberrant splicing of MED12 and MED13L, which may further destabilize transcriptional regulation and contribute to disease pathogenesis. While this work provides foundational insight into the interplay between CDK8, transcriptional control, and splicing in hematopoiesis, critical questions remain. Elucidating the specific downstream pathways and isoform-specific functions will be key to understanding how transcriptional cofactor deregulation and *SF3B1* mutations converge to drive hematologic malignancy—and may ultimately inform targeted therapeutic strategies.

References

1. Watson CJ, Papula AL, Poon GYP, et al. The evolutionary dynamics and fitness landscape of clonal hematopoiesis. *Science*. 2020-03-27;367(6485)doi:10.1126/science.aay9333
2. Loh P-R, Genovese G, Handsaker RE, et al. Insights about clonal hematopoiesis from 8,342 mosaic chromosomal alterations. *Nature*. 2018 Jul 11;559(7714)doi:10.1038/s41586-018-0321-x
3. S J, P F, J F, et al. Age-related clonal hematopoiesis associated with adverse outcomes - PubMed. *The New England journal of medicine*. 12/25/2014;371(26)doi:10.1056/NEJMoA1408617
4. Xie M, Lu C, Wang J, et al. Age-related cancer mutations associated with clonal hematopoietic expansion. *Nature medicine*. 2014 Oct 19;20(12)doi:10.1038/nm.3733
5. Genovese G, Kähler AK, Handsaker RE, et al. Clonal Hematopoiesis and Blood-Cancer Risk Inferred from Blood DNA Sequence. *The New England journal of medicine*. 2014 Nov 26;371(26)doi:10.1056/NEJMoA1409405
6. RL B, L B, RL L. Clonal Hematopoiesis and Evolution to Hematopoietic Malignancies - PubMed. *Cell stem cell*. 02/01/2018;22(2)doi:10.1016/j.stem.2018.01.011
7. S J, P N, AJ S, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease - PubMed. *The New England journal of medicine*. 07/13/2017;377(2)doi:10.1056/NEJMoA1701719
8. AG B, JS W, SK N, et al. Inherited causes of clonal haematopoiesis in 97,691 whole genomes - PubMed. *Nature*. 2020 Oct;586(7831)doi:10.1038/s41586-020-2819-2
9. EK C, T I, S Y, et al. Comorbid and inflammatory characteristics of genetic subtypes of clonal hematopoiesis - PubMed. *Blood advances*. 08/27/2019;3(16)doi:10.1182/bloodadvances.2018024729
10. Nathan DI, Dougherty M, Bhatta M, Mascarenhas J, Marcellino BK. Clonal hematopoiesis and inflammation: A review of mechanisms and clinical implications. *Critical Reviews in Oncology/Hematology*. 2023/12/01;192doi:10.1016/j.critrevonc.2023.104187
11. Nimer SD. Myelodysplastic syndromes. *Blood*. 2008/05/15;111(10)doi:10.1182/blood-2007-08-078139
12. Ogawa S. Genetics of MDS. *Blood*. 2019/03/07;133(10)doi:10.1182/blood-2018-10-844621
13. Tefferi A, Vardiman JW. Myelodysplastic Syndromes. *New England Journal of Medicine*. 2009-11-05;361(19)doi:10.1056/NEJMra0902908
14. Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellström-Lindberg E. The WHO classification of MDS does make a difference. *Blood*. 2004/05/01;103(9)doi:10.1182/blood-2003-06-2124
15. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* 2022 36:7. 2022-06-22;36(7)doi:10.1038/s41375-022-01613-1
16. Ma X. Epidemiology of Myelodysplastic Syndromes. *The American Journal of Medicine*. 2012 Jul;125(7 Suppl)doi:10.1016/j.amjmed.2012.04.014
17. Haase D, Germing U, Schanz J, et al. New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: evidence from a core dataset of 2124 patients. *Blood*. 2007/12/15;110(13)doi:10.1182/blood-2007-03-082404

18. Haase D, Haase D. Cytogenetic features in myelodysplastic syndromes. *Annals of Hematology* 2008 87:7. 2008-04-15;87(7)doi:10.1007/s00277-008-0483-y
19. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* 2014 28:2. 2013/11// 2013;28(2):241-247. doi:10.1038/leu.2013.336
20. Papaemmanuil E, Consortium obotCMDwgotICG, Gerstung M, et al. Clinical and biological implications of driver mutations in myelodysplastic syndromes. *Blood*. 2013/11// 2013;122(22):3616-3627. doi:10.1182/BLOOD-2013-08-518886
21. Dukenik DB, Soong D, Scarpa F, et al. Distinct SF3B1 Allele HEAT Repeat Location Is Associated with Co-Occurring Mutation Patterns in MDS. *Blood*. 2023/11/02;142(Supplement 1)doi:10.1182/blood-2023-181191
22. Jain T, Ware AD, Dalton WB, et al. Co-occurring mutations in ASXL1, SRSF2, and SETBP1 define a subset of myelodysplastic/ myeloproliferative neoplasm with neutrophilia. *Leukemia Research*. 2023/08/01;131doi:10.1016/j.leukres.2023.107345
23. Janusz K, Izquierdo MM, Cadenas FL, et al. Clinical, biological, and prognostic implications of SF3B1 co-occurrence mutations in very low/low- and intermediate-risk MDS patients. *Annals of Hematology* 2021 100:8. 2021-01-06;100(8)doi:10.1007/s00277-020-04360-4
24. M L, F W, Y Z, et al. Gene mutation spectrum of patients with myelodysplastic syndrome and progression to acute myeloid leukemia - PubMed. *International journal of hematologic oncology*. 06/22/2021;10(2)doi:10.2217/ijh-2021-0002
25. Malcovati L, Stevenson K, Papaemmanuil E, et al. SF3B1-mutant MDS as a distinct disease subtype: a proposal from the International Working Group for the Prognosis of MDS. *Blood*. 2020/7// 2020;136(2):157-170. doi:10.1182/BLOOD.2020004850
26. Martín-Izquierdo M, Abáigar M, Hernández-Sánchez JM, et al. Co-occurrence of cohesin complex and Ras signaling mutations during progression from myelodysplastic syndromes to secondary acute myeloid leukemia. *Haematologica*. 2020 Jul 16;106(8)doi:10.3324/haematol.2020.248807
27. Todisco G, Creignou M, Galli A, et al. Co-mutation pattern, clonal hierarchy, and clone size concur to determine disease phenotype of SRSF2P95-mutated neoplasms. *Leukemia* 2020 35:8. 2020-12-21;35(8)doi:10.1038/s41375-020-01106-z
28. Cockey SG, Zhang H, Hussaini M, et al. Molecular landscape and clinical outcome of SRSF2/TET2 Co-mutated myeloid neoplasms. *Leukemia & Lymphoma*. 2025-2-23;66(3)doi:10.1080/10428194.2024.2432581
29. Li B, Liu J, Jia Y, et al. Clinical Features and Biological Implications of U2AF1 Mutations in Myelodysplastic Syndromes. *Blood*. 2017/12/07;130(Supplement 1)doi:10.1182/blood.V130.Suppl_1.586.586
30. Malcovati L, Papaemmanuil E, Bowen DT, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2011/12// 2011;118(24):6239-6246. doi:10.1182/BLOOD-2011-09-377275
31. Thol F, Kade S, Schlarman C, et al. Frequency and prognostic impact of mutations in SRSF2, U2AF1, and ZRSR2 in patients with myelodysplastic syndromes. *Blood*. 2012/04/12;119(15)doi:10.1182/blood-2011-12-399337
32. Wu S-J, Kuo Y-Y, Hou H-A, et al. The clinical implication of SRSF2 mutation in patients with myelodysplastic syndrome and its stability during disease evolution. *Blood*. 2012/10/11;120(15)doi:10.1182/blood-2012-02-412296

33. Ball B, Zeidan A, Gore SD, Prebet T. Hypomethylating agent combination strategies in myelodysplastic syndromes: Hopes and shortcomings. *Leukemia & lymphoma*. 2016 Sep 21;58(5)doi:10.1080/10428194.2016.1228927
34. AM Z, A G, MA S, et al. STIMULUS-MDS2 design and rationale: a phase III trial with the anti-TIM-3 sabatolimab (MBG453) + azacitidine in higher risk MDS and CMML-2 - PubMed. *Future oncology (London, England)*. 2023 Mar;19(9)doi:10.2217/fon-2022-1237
35. AM Z, K A, O R, et al. Sabatolimab plus hypomethylating agents in previously untreated patients with higher-risk myelodysplastic syndromes (STIMULUS-MDS1): a randomised, double-blind, placebo-controlled, phase 2 trial - PubMed. *The Lancet Haematology*. 2024 Jan;11(1)doi:10.1016/S2352-3026(23)00333-2
36. DA S, AE D, G G-M, et al. Eprenetapopt (APR-246) and Azacitidine in TP53-Mutant Myelodysplastic Syndromes - PubMed. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 05/10/2021;39(14)doi:10.1200/JCO.20.02341
37. Daver NG, Vyas P, Huls GA, et al. The ENHANCE-3 study: venetoclax and azacitidine plus magrolimab or placebo for untreated AML unfit for intensive therapy. *Blood*. 2025;doi:10.1182/blood.2024027506
38. DeZern AE, Thepot S, De Botton S, et al. Pivotal Results of SELECT-MDS-1 Phase 3 Study of Tamibarotene with Azacitidine in Newly Diagnosed Higher-Risk MDS. *Blood Advances*. 2025;doi:10.1182/bloodadvances.2025016229
39. Garcia-Manero G, Al-Kali A, Baer MR, et al. INSPIRE: A randomized phase III trial of intravenous rigosertib in patients with higher-risk myelodysplastic syndromes (HR-MDS) after failure of hypomethylating agents (HMAs)—Study design informed by subgroup analyses of ONTIME. *Journal of Clinical Oncology*. 2016-5-20;34(15_suppl)doi:10.1200/JCO.2016.34.15_suppl.TPS7077
40. L A, L G, VA D, et al. Pevonedistat plus azacitidine vs azacitidine alone in higher-risk MDS/chronic myelomonocytic leukemia or low-blast-percentage AML - PubMed. *Blood advances*. 09/13/2022;6(17)doi:10.1182/bloodadvances.2022007334
41. L A, N D, A G-B, et al. A randomised phase II study of azacitidine (AZA) alone or with Lenalidomide (LEN), Valproic acid (VPA) or Idarubicin (IDA) in higher-Risk MDS or low blast AML: GFM's "pick a winner" trial, with the impact of somatic mutations - PubMed. *British journal of haematology*. 2022 Aug;198(3)doi:10.1111/bjh.18193
42. MA S, J W, A R, et al. Randomized phase 2 trial of pevonedistat plus azacitidine versus azacitidine for higher-risk MDS/CMML or low-blast AML - PubMed. *Leukemia*. 2021 Jul;35(7)doi:10.1038/s41375-021-01125-4
43. MA S, M O, AF L, et al. Randomized Phase II Study of Azacitidine Alone or in Combination With Lenalidomide or With Vorinostat in Higher-Risk Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia: North American Intergroup Study SWOG S1117 - PubMed. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 08/20/2017;35(24)doi:10.1200/JCO.2015.66.2510
44. PM R, J C, CD C, et al. Next-generation sequencing of baseline genetic mutations and outcomes of eltrombopag and azacitidine therapy in patients with myelodysplastic syndromes and thrombocytopenia: Data from the SUPPORT clinical trial - PubMed. *EJHaem*. 05/22/2023;4(3)doi:10.1002/jha2.694
45. Pophali P, Cheloni G, Stone RM, et al. Randomized Phase II Trial of Dendritic Cell/AML Fusion Cell Vaccination Compared to Standard of Care Therapy in AML CR1. *Blood*. 2024/11/05;144(Supplement 1)doi:10.1182/blood-2024-200876

46. Tiong IS, Stevenson WS, Wall M, et al. Favorable Outcomes of DDX41-Mutated MDS and Low Blast Count AML Treated with Azacitidine with or without Lenalidomide (ALLG MDS4 Trial). *Blood*. 2022/11/15;140(Supplement 1)doi:10.1182/blood-2022-162880
47. Zeidan AM, Garcia JS, Fenaux P, et al. Phase 3 VERONA study of venetoclax with azacitidine to assess change in complete remission and overall survival in treatment-naïve higher-risk myelodysplastic syndromes. *Journal of Clinical Oncology*. 2021-05-20;39(15_suppl)doi:10.1200/JCO.2021.39.15_suppl.TPS7054
48. Angelucci E, Li J, Greenberg P, et al. Iron Chelation in Transfusion-Dependent Patients With Low- to Intermediate-1-Risk Myelodysplastic Syndromes. *Annals of Internal Medicine*. 2020-03-24;172(8)doi:10.7326/M19-0916
49. Fenaux P, Santini V, Spiriti MAA, et al. A phase 3 randomized, placebo-controlled study assessing the efficacy and safety of epoetin- α in anemic patients with low-risk MDS. *Leukemia* 2018 32:12. 2018-03-30;32(12)doi:10.1038/s41375-018-0118-9
50. Jädersten M, Montgomery SM, Dybedal I, Porwit-MacDonald A, Hellström-Lindberg E. Long-term outcome of treatment of anemia in MDS with erythropoietin and G-CSF. *Blood*. 2005/08/01;106(3)doi:10.1182/blood-2004-10-3872
51. Platzbecker U, Symeonidis A, Oliva EN, et al. A phase 3 randomized placebo-controlled trial of darbepoetin alfa in patients with anemia and lower-risk myelodysplastic syndromes. *Leukemia* 2017 31:9. 2017-06-19;31(9)doi:10.1038/leu.2017.192
52. Fenaux P, Platzbecker U, Mufti GJ, et al. Luspatercept in Patients with Lower-Risk Myelodysplastic Syndromes. *New England Journal of Medicine*. 2020-01-09;382(2)doi:10.1056/NEJMoa1908892
53. Mukherjee S, Brown-Bickerstaff C, Falkenstein A, et al. Treatment patterns and outcomes with luspatercept in patients with lower-risk myelodysplastic syndromes: A retrospective US cohort analysis. *HemaSphere*. 2024/01/01;8(1)doi:10.1002/hem3.38
54. Lee SCW, Dvinge H, Kim E, et al. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. *Nature Medicine* 2016 22:6. 2016/5// 2016;22(6):672-678. doi:10.1038/NM.4097
55. Steensma DP, Wermke M, Klimek VM, et al. Results of a Clinical Trial of H3B-8800, a Splicing Modulator, in Patients with Myelodysplastic Syndromes (MDS), Acute Myeloid Leukemia (AML) or Chronic Myelomonocytic Leukemia (CMML). *Blood*. 2019/11// 2019;134(Supplement_1):673-673. doi:10.1182/BLOOD-2019-123854
56. Steensma DP, Wermke M, Klimek VM, et al. Phase I First-in-Human Dose Escalation Study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. *Leukemia* 2021 35:12. 2021/6// 2021;35(12):3542-3550. doi:10.1038/s41375-021-01328-9
57. Seiler M, Yoshimi A, Darman R, et al. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. *Nature medicine*. 2018/5// 2018;24(4):497-504. doi:10.1038/nm.4493
58. Bonner EA, Lee SC. Therapeutic Targeting of RNA Splicing in Cancer. *Genes*. 2023/6// 2023;14(7):1378-1378. doi:10.3390/genes14071378
59. Yoda A, Morishita D, Ochi Y, et al. CTX-712, a Novel Clk Inhibitor Targeting Myeloid Neoplasms with SRSF2 Mutation. *Blood*. 2021/11/23;138(Supplement 1)doi:10.1182/blood-2021-148205
60. Matera AG, Wang Z, Matera AG, Wang Z. A day in the life of the spliceosome. *Nature Reviews Molecular Cell Biology* 2014 15:2. 2014-01-23;15(2)doi:10.1038/nrm3742

61. Gozani O, Potashkin J, Reed R. A Potential Role for U2AF-SAP 155 Interactions in Recruiting U2 snRNP to the Branch Site. *Molecular and Cellular Biology*. 1998-8-1;18(8)doi:10.1128/MCB.18.8.4752
62. Alsafadi S, Houy A, Battistella A, et al. Cancer-associated SF3B1 mutations affect alternative splicing by promoting alternative branchpoint usage. *Nature Communications*. 2016/2// 2016;7doi:10.1038/ncomms10615
63. Quesada V, Conde L, Villamor N, et al. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nature Genetics* 2011 44:1. 2011-12-11;44(1)doi:10.1038/ng.1032
64. Darman Rachel B, Seiler M, Agrawal Anant A, et al. Cancer-Associated SF3B1 Hotspot Mutations Induce Cryptic 3' Splice Site Selection through Use of a Different Branch Point. *Cell Reports*. 2015/11// 2015;13(5):1033-1045. doi:10.1016/J.CELREP.2015.09.053
65. DeBoever C, Ghia EM, Shepard PJ, et al. Transcriptome Sequencing Reveals Potential Mechanism of Cryptic 3' Splice Site Selection in SF3B1-mutated Cancers. *PLOS Computational Biology*. Mar 13, 2015;11(3)doi:10.1371/journal.pcbi.1004105
66. Seiler M, Peng S, Agrawal AA, et al. Somatic Mutational Landscape of Splicing Factor Genes and Their Functional Consequences across 33 Cancer Types. *Cell Reports*. 2018/4// 2018;23(1):282-296.e4. doi:10.1016/j.celrep.2018.01.088
67. Cretu C, Schmitzová J, Ponce-Salvatierra A, et al. Molecular Architecture of SF3b and Structural Consequences of Its Cancer-Related Mutations. *Molecular Cell*. 2016/10/20;64(2)doi:10.1016/j.molcel.2016.08.036
68. Seiler M, Peng S, Agrawal AA, et al. Somatic Mutational Landscape of Splicing Factor Genes and Their Functional Consequences across 33 Cancer Types. *Cell reports*. 2018 Apr 3;23(1)doi:10.1016/j.celrep.2018.01.088
69. Mupo A, Seiler M, Sathiaselan V, et al. Hemopoietic-specific Sf3b1-K700E knock-in mice display the splicing defect seen in human MDS but develop anemia without ring sideroblasts. *Leukemia* 2017 31:3. 2016/9// 2016;31(3):720-727. doi:10.1038/leu.2016.251
70. Herbert A, Hatfield A, Randazza A, Miyamoto V, Palmer K, Lackey L. Precursor RNA structural patterns at SF3B1 mutation sensitive cryptic 3' splice sites. *bioRxiv*. 2025-02-22;doi:10.1101/2025.02.19.638873
71. AK K, O R, AK G, et al. Cancer-associated SF3B1 mutants recognize otherwise inaccessible cryptic 3' splice sites within RNA secondary structures - PubMed. *Oncogene*. 02/23/2017;36(8)doi:10.1038/onc.2016.279
72. B Z, Z L, R Q, et al. Cancer-associated mutations in SF3B1 disrupt the interaction between SF3B1 and DDX42 - PubMed. *Journal of biochemistry*. 07/25/2022;172(2)doi:10.1093/jb/mvac049
73. J Z, AM A, YK L, et al. Disease-Causing Mutations in SF3B1 Alter Splicing by Disrupting Interaction with SUGP1 - PubMed. *Molecular cell*. 10/03/2019;76(1)doi:10.1016/j.molcel.2019.07.017
74. S B, JMB P, LB G, et al. GPATCH8 modulates mutant SF3B1 mis-splicing and pathogenicity in hematologic malignancies - PubMed. *Molecular cell*. 05/16/2024;84(10)doi:10.1016/j.molcel.2024.04.006
75. PC B, AK G, R R, et al. Transcription elongation defects link oncogenic SF3B1 mutations to targetable alterations in chromatin landscape - PubMed. *Molecular cell*. 04/18/2024;84(8)doi:10.1016/j.molcel.2024.02.032

76. Dolatshad H, Pellagatti A, Liberante FG, et al. Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. *Leukemia* 2016 30:12. 2016-05-23;30(12)doi:10.1038/leu.2016.149
77. Ochi T, Fujiwara T, Ono K, et al. Exploring the mechanistic link between SF3B1 mutation and ring sideroblast formation in myelodysplastic syndrome. *Scientific Reports* 2022 12:1. 2022-08-26;12(1)doi:10.1038/s41598-022-18921-2
78. Clough CA, Pangallo J, Sarchi M, et al. Coordinated missplicing of TMEM14C and ABCB7 causes ring sideroblast formation in SF3B1-mutant myelodysplastic syndrome. *Blood*. 2022/3// 2022;139(13):2038-2049. doi:10.1182/BLOOD.2021012652
79. Li Z, Zhao B, Shi Y, Liang Y, Qian R, Wan Y. Characterization of the aberrant splicing of MAP3K7 induced by cancer-associated SF3B1 mutation. *The Journal of Biochemistry*. 2021/09/22;170(1)doi:10.1093/jb/mvab023
80. Lieu YK, Liu Z, Ali AM, et al. SF3B1 mutant-induced missplicing of MAP3K7 causes anemia in myelodysplastic syndromes. *Proceedings of the National Academy of Sciences*. 2021-12-20;119(1)doi:10.1073/pnas.2111703119
81. Mian SA, Philippe C, Maniati E, et al. Vitamin B5 and succinyl-CoA improve ineffective erythropoiesis in SF3B1 mutated myelodysplasia. *Science translational medicine*. 2023 Mar 1;15(685)doi:10.1126/scitranslmed.abn5135
82. R V-N, M T, C C, et al. Glycolysis Dependency as a Hallmark of SF3B1-Mutated Cells - PubMed. *Cancers*. 04/24/2022;14(9)doi:10.3390/cancers14092113
83. Lee SC-W, North K, Kim E, et al. Synthetic Lethal and Convergent Biological Effects of Cancer-Associated Spliceosomal Gene Mutations. *Cancer Cell*. 2018/08/13;34(2)doi:10.1016/j.ccell.2018.07.003
84. Choudhary GS, Smith MA, Pellagatti A, et al. SF3B1 Mutations Induce Oncogenic IRAK4 Isoforms and Activate Targetable Innate Immune Pathways in MDS and AML. *Blood*. 2019/11/13;134(Supplement_1)doi:10.1182/blood-2019-124458
85. G L, B Z, Y S, Y W. Cancer-associated SF3B1 mutations inhibit mRNA nuclear export by disrupting SF3B1-THOC5 interactions - PubMed. *Journal of biochemistry*. 12/02/2024;176(6)doi:10.1093/jb/mvae061
86. Thier J, Hofmann S, Kirchhof KM, et al. SF3B1-mutant mis-splicing of UBA1 confers a targetable therapeutic vulnerability through UBA1 inhibition. *bioRxiv*. 2024-08-28;doi:10.1101/2024.08.28.610114
87. Biernacki MA, Lok J, Foster KA, et al. SF3B1K700E neoantigen is a CD8+ T-cell target shared across human myeloid neoplasms. *Cancer Immunology Research*. 2025;doi:10.1158/2326-6066.CIR-24-0091
88. Liu Z, Yoshimi A, Wang J, et al. Mutations in the RNA Splicing Factor SF3B1 Promote Tumorigenesis through MYC Stabilization. *Cancer Discovery*. 2020/06/01;10(6)doi:10.1158/2159-8290.CD-19-1330
89. Pollyea DA, Kim HM, Stevens BM, et al. MDS-associated SF3B1 mutations enhance proinflammatory gene expression in patient blast cells. *Journal of Leukocyte Biology*. 2021/06/29;110(1)doi:10.1002/JLB.6AB0520-318RR
90. Cortés-López M, Chamely P, Hawkins AG, et al. Single-cell multi-omics defines the cell-type-specific impact of splicing aberrations in human hematopoietic clonal outgrowths. *Cell Stem Cell*. 2023/09/07;30(9)doi:10.1016/j.stem.2023.07.012

91. M S, CA C, EI C, et al. Mis-splicing of Mitotic Regulators Sensitizes SF3B1-Mutated Human HSCs to CHK1 Inhibition - PubMed. *Blood cancer discovery*. 09/03/2024;5(5)doi:10.1158/2643-3230.BCD-23-0230
92. Papaemmanuil E, Cazzola M, Boulton J, et al. Somatic SF3B1 Mutation in Myelodysplasia with Ring Sideroblasts. *The New England journal of medicine*. 2011/10//2011;365(15):1384-1384. doi:10.1056/NEJMOA1103283
93. Zhu Y, Li X, Chang C, et al. SF3B1-mutated myelodysplastic syndrome with ring sideroblasts harbors more severe iron overload and corresponding over-erythropoiesis. *Leukemia Research*. 2016/05/01;44doi:10.1016/j.leukres.2016.02.011
94. Ambaglio I, Malcovati L, Papaemmanuil E, et al. Inappropriately low hepcidin levels in patients with myelodysplastic syndrome carrying a somatic mutation of SF3B1. *Haematologica*. 2013/03/01;98(3)doi:10.3324/haematol.2012.077446
95. Inoue D, Bradley RK, Abdel-Wahab O. Spliceosomal gene mutations in myelodysplasia: molecular links to clonal abnormalities of hematopoiesis. *Genes & Development*. 2016/5//2016;30(9):989-1001. doi:10.1101/GAD.278424.116
96. D R, A B, V S, et al. Mutations of the SF3B1 splicing factor in chronic lymphocytic leukemia: association with progression and fludarabine-refractoriness - PubMed. *Blood*. 12/22/2011;118(26)doi:10.1182/blood-2011-08-373159
97. L W, MS L, Y W, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia - PubMed. *The New England journal of medicine*. 12/29/2011;365(26)doi:10.1056/NEJMoa1109016
98. Biankin AV, Waddell N, Kassahn KS, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. *Nature* 2012 491:7424. 2012-10-24;491(7424)doi:10.1038/nature11547
99. Martelotto LG, Filippa MRD, Ng CK, et al. Genomic landscape of adenoid cystic carcinoma of the breast. *The Journal of Pathology*. 2015/10/01;237(2)doi:10.1002/path.4573
100. Harbour JW, Roberson EDO, Anbunathan H, et al. Recurrent mutations at codon 625 of the splicing factor SF3B1 in uveal melanoma. *Nature Genetics* 2013 45:2. 2013-01-13;45(2)doi:10.1038/ng.2523
101. Urh M, <https://independent.academia.edu/MarjetaUrh>. Mutual Exclusivity of MED12/MED12L, MED13/13L, and CDK8/19 Paralogues Revealed within the CDK-Mediator Kinase Module. *Journal of Proteomics & Bioinformatics*. 2013/01/01;01(S2)doi:10.4172/JPB.S2-004
102. Luyties O, Taatjes DJ. The Mediator kinase module: an interface between cell signaling and transcription. *Trends in Biochemical Sciences*. 2022/04/01;47(4)doi:10.1016/j.tibs.2022.01.002
103. Malik S, Roeder RG. Dynamic regulation of pol II transcription by the mammalian Mediator complex. *Trends in Biochemical Sciences*. 2005/05/01;30(5)doi:10.1016/j.tibs.2005.03.009
104. Malik S, Roeder RG, Malik S, Roeder RG. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. *Nature Reviews Genetics* 2010 11:11. 2010-10-13;11(11)doi:10.1038/nrg2901
105. Taatjes DJ. The human Mediator complex: a versatile, genome-wide regulator of transcription. *Trends in Biochemical Sciences*. 2010/06/01;35(6)doi:10.1016/j.tibs.2010.02.004
106. Dotson MR, Yuan CX, Roeder RG, et al. Structural organization of yeast and mammalian

- mediator complexes. *Proceedings of the National Academy of Sciences*. 2000-12-19;97(26)doi:10.1073/pnas.260489497
107. FJ A, YW J, LC M, CM G, RD K. Conserved structures of mediator and RNA polymerase II holoenzyme - PubMed. *Science (New York, NY)*. 02/12/1999;283(5404)doi:10.1126/science.283.5404.985
108. X C, X Y, J L, et al. Structures of the human Mediator and Mediator-bound preinitiation complex - PubMed. *Science (New York, NY)*. 06/04/2021;372(6546)doi:10.1126/science.abg0635
109. A E, JF M. How do DNA-bound proteins leave their binding sites? The role of facilitated dissociation - PubMed. *Current opinion in chemical biology*. 2019 Dec;53doi:10.1016/j.cbpa.2019.08.007
110. Richter WF, Nayak S, Iwasa J, et al. The Mediator complex as a master regulator of transcription by RNA polymerase II. *Nature Reviews Molecular Cell Biology* 2022 23:11. 2022-06-20;23(11)doi:10.1038/s41580-022-00498-3
111. AV Z, A DA, JE H, et al. Mediator Condensates Localize Signaling Factors to Key Cell Identity Genes - PubMed. *Molecular cell*. 12/05/2019;76(5)doi:10.1016/j.molcel.2019.08.016
112. Pelish HE, Liao BB, Nitulescu II, et al. Mediator kinase inhibition further activates super-enhancer-associated genes in AML. *Nature*. 2015/10// 2015;526(7572):273-276. doi:10.1038/NATURE14904
113. Klatt F, Leitner A, Kim IV, et al. A precisely positioned MED12 activation helix stimulates CDK8 kinase activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2020/2// 2020;117(6):2894-2905. doi:10.1073/pnas.1917635117
114. H E, V B, M L, et al. The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II - PubMed. *Proceedings of the National Academy of Sciences of the United States of America*. 10/24/2006;103(43)doi:10.1073/pnas.0607483103
115. Knuesel MT, Meyer KD, Donner AJ, Espinosa JM, Taatjes DJ. The Human CDK8 Subcomplex Is a Histone Kinase That Requires Med12 for Activity and Can Function Independently of Mediator. *Molecular and Cellular Biology*. 2009/2// 2009;29(3):650-661. doi:10.1128/mcb.00993-08
116. KD M, AJ D, MT K, AG Y, JM E, DJ T. Cooperative activity of cdk8 and GCN5L within Mediator directs tandem phosphoacetylation of histone H3 - PubMed. *The EMBO journal*. 05/21/2008;27(10)doi:10.1038/emboj.2008.78
117. Rickert P, Seghezzi W, Shanahan F, Cho H, Lees E. Cyclin C/CDK8 is a novel CTD kinase associated with RNA polymerase II. *Oncogene*. Jun 20 1996;12(12):2631-40.
118. Donner AJ, Szostek S, Hoover JM, Espinosa JM. CDK8 Is a Stimulus-Specific Positive Coregulator of p53 Target Genes. *Molecular Cell*. 2007;27(1):121-133. doi:<https://doi.org/10.1016/j.molcel.2007.05.026>
119. Donner AJ, Ebmeier CC, Taatjes DJ, Espinosa JM. CDK8 is a positive regulator of transcriptional elongation within the serum response network. *Nature structural & molecular biology*. 2010/2// 2010;17(2):194-194. doi:10.1038/NSMB.1752
120. Adler AS, McClelland ML, Truong T, et al. CDK8 maintains tumor dedifferentiation and embryonic stem cell pluripotency. *Cancer Research*. 2012/4// 2012;72(8):2129-2139. doi:10.1158/0008-5472.CAN-11-3886
121. Firestein R, Bass AJ, Kim SY, et al. CDK8 is a colorectal cancer oncogene that regulates β -catenin activity. *Nature* 2008 455:7212. 2008-09-14;455(7212)doi:10.1038/nature07179

122. Galbraith MD, Allen MA, Bensard CL, et al. HIF1A Employs CDK8-Mediator to Stimulate RNAPII Elongation in Response to Hypoxia. *Cell*. 2013/6// 2013;153(6):1327-1339. doi:10.1016/J.CELL.2013.04.048
123. Galbraith MD, Andrysik Z, Pandey A, et al. CDK8 Kinase Activity Promotes Glycolysis. *Cell Reports*. 2017;21(6):1495-1506. doi:<https://doi.org/10.1016/j.celrep.2017.10.058>
124. CJ F, JB W, KA J. Mastermind recruits CycC:CDK8 to phosphorylate the Notch ICD and coordinate activation with turnover - PubMed. *Molecular cell*. 11/19/2004;16(4)doi:10.1016/j.molcel.2004.10.014
125. Bancerek J, Poss ZC, Steinparzer I, et al. CDK8 Kinase Phosphorylates Transcription Factor STAT1 to Selectively Regulate the Interferon Response. *Immunity*. 2013/2// 2013;38(2):250-262. doi:10.1016/J.IMMUNI.2012.10.017
126. C A, AI Z, Q X, et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways - PubMed. *Cell*. 11/13/2009;139(4)doi:10.1016/j.cell.2009.09.035
127. Menzl I, Zhang T, Berger-Becvar A, et al. A kinase-independent role for CDK8 in BCR-ABL1+ leukemia. *Nature Communications*. 2019/12// 2019;10(1):1-15. doi:10.1038/s41467-019-12656-x
128. Khamidullina AI, Yastrebova MA, Bruter AV, et al. CDK8/19 inhibition attenuates G1 arrest induced by BCR-ABL antagonists and accelerates death of chronic myelogenous leukemia cells. *Cell Death Discovery* 2025 11:1. 2025-02-15;11(1)doi:10.1038/s41420-025-02339-6
129. Mazan M, Pakulska U, Moszyńska A, et al. Targeting CDK8/CDK19 to Disrupt Leukemic Stem Cell-like Population in Acute Myeloid Leukemia: Exploring RVU120 As a Promising Frontline Therapy. *Blood*. 2023/11/02;142(Supplement 1)doi:10.1182/blood-2023-188866
130. Piya S, Ma H, Piya S, et al. Inhibition of Cyclin Dependent Kinase 8(CDK8): A Novel Approach to Target the Leukemia Initiating Cells (LICs) in T-Cell Acute Lymphoblastic Leukaemia (T-ALL). *Blood*. 2021/11/23;138(Supplement 1)doi:10.1182/blood-2021-148853
131. XX Z, YY Y, X M, et al. Discovery of a novel oral type I CDK8 inhibitor against acute myeloid leukemia - PubMed. *European journal of medicinal chemistry*. 05/05/2023;251doi:10.1016/j.ejmech.2023.115214
132. Y L, Y L, J W, et al. Discovery of Potent, Selective, and Orally Bioavailable Small-Molecule Inhibitors of CDK8 for the Treatment of Cancer - PubMed. *Journal of medicinal chemistry*. 04/27/2023;66(8)doi:10.1021/acs.jmedchem.2c01718
133. Chen M, Liang J, Ji H, et al. CDK8/19 Mediator kinases potentiate induction of transcription by NFκB. *Proceedings of the National Academy of Sciences of the United States of America*. 2017/9// 2017;114(38):10208-10213. doi:10.1073/pnas.1710467114
134. Sameer AS, Nissar S. Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. *BioMed Research International*. 2021 Sep 12;2021(1)doi:10.1155/2021/1157023
135. Yamamoto S, Hagihara T, Horiuchi Y, et al. Mediator cyclin-dependent kinases upregulate transcription of inflammatory genes in cooperation with NF-κB and C/EBPβ on stimulation of Toll-like receptor 9. *Genes to Cells*. 2017/03/01;22(3)doi:10.1111/gtc.12475
136. Guo Z, Wang G, Lv Y, Wan YY, Zheng J. Frontiers | Inhibition of Cdk8/Cdk19 Activity Promotes Treg Cell Differentiation and Suppresses Autoimmune Diseases. *Frontiers in Immunology*. 2019/08/20;10doi:10.3389/fimmu.2019.01988
137. Johannessen L, Sundberg TB, O'Connell DJ, et al. Small-molecule studies identify CDK8 as a regulator of IL-10 in myeloid cells. *Nature Chemical Biology* 2017 13:10. 2017-08-14;13(10)doi:10.1038/nchembio.2458

138. Mizuno N, Shiga S, Tanaka Y, Kimura T, Yanagawa Y. CDK8/19 inhibitor enhances arginase-1 expression in macrophages via STAT6 and p38 MAPK activation. *European Journal of Pharmacology*. 2024/09/15;979doi:10.1016/j.ejphar.2024.176852
139. Tsutsui T, Fukasawa R, Shinmyozu K, et al. Mediator Complex Recruits Epigenetic Regulators via Its Two Cyclin-dependent Kinase Subunits to Repress Transcription of Immune Response Genes *. *Journal of Biological Chemistry*. 2013/07/19;288(29)doi:10.1074/jbc.M113.486746
140. Patnaik MM, Lasho TL, Hodnefield JM, et al. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. *Blood*. 2011/1// 2011;119(2):569-569. doi:10.1182/BLOOD-2011-09-377994
141. Visconte V, Makishima H, Jankowska A, et al. SF3B1, a splicing factor is frequently mutated in refractory anemia with ring sideroblasts. *Leukemia*. 2012;26(3):542-545. doi:10.1038/LEU.2011.232
142. Yoshida K, Sanada M, Shiraishi Y, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. *Chang Gung Memorial Hospital*. 2011;105doi:10.1038/nature10496
143. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014/2// 2014;28(2):241-247. doi:10.1038/leu.2013.336
144. Alsafadi S, Houy A, Battistella A, et al. Cancer-associated SF3B1 mutations affect alternative splicing by promoting alternative branchpoint usage. *Nature Communications*. 2016/2// 2016;7(1):1-12. doi:10.1038/ncomms10615
145. Dolatshad H, Pellagatti A, Fernandez-Mercado M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. *Leukemia*. 2015/5// 2015;29(5):1092-1103. doi:10.1038/leu.2014.331
146. Inoue D, Chew GL, Liu B, et al. Spliceosomal disruption of the non-canonical BAF complex in cancer. *Nature*. 2019/10// 2019;574(7778):432-436. doi:10.1038/s41586-019-1646-9
147. Kesarwani AK, Ramirez O, Gupta AK, et al. Cancer-associated SF3B1 mutants recognize otherwise inaccessible cryptic 3' splice sites within RNA secondary structures. *Oncogene*. 2017/2// 2017;36(8):1123-1133. doi:10.1038/onc.2016.279
148. H D, A P, Fg L, et al. Cryptic splicing events in the iron transporter ABCB7 and other key target genes in SF3B1-mutant myelodysplastic syndromes. *Leukemia*. 2016/12// 2016;30(12):2322-2331. doi:10.1038/LEU.2016.149
149. S B, AS A, C L, et al. A variant erythroferrone disrupts iron homeostasis in SF3B1-mutated myelodysplastic syndrome - PubMed. *Science translational medicine*. 07/10/2019;11(500)doi:10.1126/scitranslmed.aav5467
150. Benbarche S, Pineda JMB, Galvis LB, et al. GPATCH8 modulates mutant SF3B1 mis-splicing and pathogenicity in hematologic malignancies. *Molecular Cell*. 2024/5// 2024;84(10):1886-1903.e10. doi:10.1016/j.molcel.2024.04.006
151. Antoniani C, Romano O, Miccio A. Concise Review: Epigenetic Regulation of Hematopoiesis: Biological Insights and Therapeutic Applications. *STEM CELLS Translational Medicine*. 2017/12/01;6(12)doi:10.1002/sctm.17-0192
152. Godini R, Yan J, Chopin M. Frontiers | Transcriptional regulatory logic orchestrating lymphoid and myeloid cell fate decisions. *Frontiers in Immunology*. 2025/05/29;16doi:10.3389/fimmu.2025.1544483

153. Göttgens B. Regulatory network control of blood stem cells. *Blood*. 2015/04/23;125(17)doi:10.1182/blood-2014-08-570226
154. M L, R P. Global genetic regulatory networks controlling hematopoietic cell fates - PubMed. *Current opinion in hematology*. 2006 Jul;13(4)doi:10.1097/01.moh.0000231419.15654.7f
155. P L, J M P, D W L, H S. Gene regulatory networks directing myeloid and lymphoid cell fates within the immune system - PubMed. *Seminars in immunology*. 2008 Aug;20(4)doi:10.1016/j.smim.2008.08.003
156. Morris EJ, Ji J-Y, Yang F, et al. E2F1 represses β -catenin transcription and is antagonized by both pRB and CDK8. *Nature* 2008 455:7212. 2008-09-14;455(7212)doi:10.1038/nature07310
157. J M-F, L W, E P, et al. CDK8 Fine-Tunes IL-6 Transcriptional Activities by Limiting STAT3 Resident Time at the Gene Loci. *Cell reports*. 2020/12// 2020;33(12)doi:10.1016/J.CELREP.2020.108545
158. Johannessen L, Sundberg TB, O'Connell DJ, et al. Small-molecule studies identify CDK8 as a regulator of IL-10 in myeloid cells. *Nature chemical biology*. 2017/10// 2017;13(10):1102-1102. doi:10.1038/NCHEMBIO.2458
159. Martinez-Fabregas J, Wang L, Pohler E, et al. CDK8 Fine-Tunes IL-6 Transcriptional Activities by Limiting STAT3 Resident Time at the Gene Loci. *Cell Reports*. 2020/12// 2020;33(12):108545-108545. doi:10.1016/J.CELREP.2020.108545
160. Rzymiski T, Mikula M, Żylkiewicz E, et al. SEL120-34A is a novel CDK8 inhibitor active in AML cells with high levels of serine phosphorylation of STAT1 and STAT5 transactivation domains. *Oncotarget*. 2017;8(20):33779-33779. doi:10.18632/ONCOTARGET.16810
161. Yamamoto S, Hagihara T, Horiuchi Y, et al. Mediator cyclin-dependent kinases upregulate transcription of inflammatory genes in cooperation with NF- κ B and C/EBP β on stimulation of Toll-like receptor 9. *Genes to Cells*. 2017/3// 2017;22(3):265-276. doi:10.1111/gtc.12475
162. Shen S, Park JW, Lu ZX, et al. rMATS: Robust and flexible detection of differential alternative splicing from replicate RNA-Seq data. *Proceedings of the National Academy of Sciences of the United States of America*. 2014/12// 2014;111(51):E5593-E5601. doi:10.1073/PNAS.1419161111/SUPPL_FILE/PNAS.1419161111.SD05.XLS
163. Obeng EA, Chappell RJ, Seiler M, et al. Physiologic expression of SF3B1K700E causes impaired erythropoiesis, aberrant splicing, and sensitivity to pharmacologic spliceosome modulation. *Cancer cell*. 2016/9// 2016;30(3):404-404. doi:10.1016/J.CCELL.2016.08.006
164. Sarchi M, Clough CA, Crosse EI, et al. Mis-splicing of Mitotic Regulators Sensitizes SF3B1-Mutated Human HSCs to CHK1 Inhibition. *Blood cancer discovery*. 2024/9// 2024;5(5):353-370. doi:10.1158/2643-3230.BCD-23-0230
165. Ashburner M, Ball CA, Blake JA, et al. Gene Ontology: tool for the unification of biology. *Nature genetics*. 2000/5// 2000;25(1):25-25. doi:10.1038/75556
166. Consortium TGO, Aleksander SA, Balhoff J, et al. The Gene Ontology knowledgebase in 2023. *Genetics*. 2023/5// 2023;224(1)doi:10.1093/GENETICS/IYAD031
167. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*. 2014/12// 2014;15(12):1-21. doi:10.1186/S13059-014-0550-8/FIGURES/9
168. Dolatshad H, Pellagatti A, Fernandez-Mercado M, et al. Disruption of SF3B1 results in deregulated expression and splicing of key genes and pathways in myelodysplastic syndrome hematopoietic stem and progenitor cells. *Leukemia* 2015 29:5. 2014/11// 2014;29(5):1092-1103. doi:10.1038/leu.2014.331

169. Zhang J, Ali AM, Lieu YK, Raza A, Mukherjee S, Manley Correspondence JL. Disease-Causing Mutations in SF3B1 Alter Splicing by Disrupting Interaction with SUGP1. *Molecular Cell*. 2019;76:82-95.e7. doi:10.1016/j.molcel.2019.07.017
170. Pollyea DA, Kim HM, Stevens BM, et al. MDS-associated SF3B1 mutations enhance proinflammatory gene expression in patient blast cells. *Journal of Leukocyte Biology*. 2020/11// 2020;JLB.6AB0520-318RR. doi:10.1002/JLB.6AB0520-318RR
171. Robinson JT, Thorvaldsdóttir H, Winckler W, et al. Integrative Genomics Viewer. *Nature biotechnology*. 2011 Jan;29(1)doi:10.1038/nbt.1754
172. Banerjee T, Calvi LM, Becker MW, Liesveld JL. Flaming and fanning: The Spectrum of inflammatory influences in myelodysplastic syndromes. *Blood reviews*. 2019/7// 2019;36:57-57. doi:10.1016/J.BLRE.2019.04.004
173. Moura PL, Mortera-Blanco T, Hofman IJ, et al. Erythroid Differentiation Enhances RNA Mis-Splicing in SF3B1-Mutant Myelodysplastic Syndromes with Ring Sideroblasts. *Cancer Research*. 2024/01/15;84(2)doi:10.1158/0008-5472.CAN-23-3038
174. Kupperts DA, Linton J, Espinosa SO, McKenna KM, Rongvaux A, Paddison PJ. Gene knock-outs in human CD34+ hematopoietic stem and progenitor cells and in the human immune system of mice. *PloS one*. 2023/6// 2023;18(6)doi:10.1371/JOURNAL.PONE.0287052
175. Chen K, Liu J, Heck S, Chasis JA, An X, Mohandas N. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. *Proceedings of the National Academy of Sciences of the United States of America*. 2009 Sep 28;106(41)doi:10.1073/pnas.0909296106
176. HQ D, T E, MA M, et al. Coexpression of CD71 and CD117 Identifies an Early Unipotent Neutrophil Progenitor Population in Human Bone Marrow - PubMed. *Immunity*. 08/18/2020;53(2)doi:10.1016/j.immuni.2020.07.017
177. Hsu J, Reilly A, Hayes BJ, et al. Reprogramming identifies functionally distinct stages of clonal evolution in myelodysplastic syndromes. *Blood*. 2019/07/11;134(2)doi:10.1182/blood.2018884338
178. Pellagatti A, Armstrong RN, Steeples V, et al. Impact of spliceosome mutations on RNA splicing in myelodysplasia: dysregulated genes/pathways and clinical associations. *Blood*. 2018/9// 2018;132(12):1225-1240. doi:10.1182/BLOOD-2018-04-843771
179. MJ W, X W, HP B, et al. Maintenance of hematopoietic stem cells by tyrosine-unphosphorylated STAT5 and JAK inhibition - PubMed. *Blood advances*. 01/28/2025;9(2)doi:10.1182/bloodadvances.2024014046
180. Johnson CS, Williams M, Sham K, et al. Adaptation to ex vivo culture reduces human hematopoietic stem cell activity independently of the cell cycle. *Blood*. 2024/08/15;144(7)doi:10.1182/blood.2023021426
181. Ng AP. Hit the road JAK, don't P-STAT, stem more! *Blood*. 2024/08/15;144(7)doi:10.1182/blood.2024025441
182. Fasouli ES, Katsantoni E. Frontiers | JAK-STAT in Early Hematopoiesis and Leukemia. *Frontiers in Cell and Developmental Biology*. 2021/05/14;9doi:10.3389/fcell.2021.669363
183. A W, MJ M, T O, et al. c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation - PubMed. *Genes & development*. 11/15/2004;18(22)doi:10.1101/gad.313104
184. Rodríguez A, Zhang K, Färkkilä A, et al. MYC Promotes Bone Marrow Stem Cell Dysfunction in Fanconi Anemia. *Cell Stem Cell*. 2021/01/07;28(1)doi:10.1016/j.stem.2020.09.004

185. Laurenti E, Varnum-Finney B, Wilson A, et al. Hematopoietic Stem Cell Function and Survival Depend on c-Myc and N-Myc Activity. *Cell stem cell*. 2008 Dec 4;3(6)doi:10.1016/j.stem.2008.09.005
186. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics* 2013 14:1. 2013-04-15;14(1)doi:10.1186/1471-2105-14-128
187. MH G, GS D, M P, et al. BloodSpot 3.0: a database of gene and protein expression data in normal and malignant haematopoiesis - PubMed. *Nucleic acids research*. 01/05/2024;52(D1)doi:10.1093/nar/gkad993
188. Novershtern N, Subramanian A, Lawton LN, et al. Densely Interconnected Transcriptional Circuits Control Cell States in Human Hematopoiesis. *Cell*. 2011/01/21;144(2)doi:10.1016/j.cell.2011.01.004
189. Clough CA, Pangallo J, Sarchi M, et al. Coordinated missplicing of TMEM14C and ABCB7 causes ring sideroblast formation in SF3B1-mutant myelodysplastic syndrome. *Blood*. 2022/03/31;139(13)doi:10.1182/blood.2021012652
190. Kupperts DA, Linton J, Espinosa SO, McKenna KM, Rongvaux A, Paddison PJ. Gene knock-outs in human CD34+ hematopoietic stem and progenitor cells and in the human immune system of mice. *PLOS ONE*. Jun 28, 2023;18(6)doi:10.1371/journal.pone.0287052
191. HY L, X G, MI B, et al. PPAR- α and glucocorticoid receptor synergize to promote erythroid progenitor self-renewal - PubMed. *Nature*. 06/25/2015;522(7557)doi:10.1038/nature14326
192. AD, CAD, F S, et al. STAR: ultrafast universal RNA-seq aligner - PubMed. *Bioinformatics (Oxford, England)*. 01/01/2013;29(1)doi:10.1093/bioinformatics/bts635
193. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*. 2015/04/20;43(7)doi:10.1093/nar/gkv007
194. Love MI, Huber W, Anders S, Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 2014 15:12. 2014-12-05;15(12)doi:10.1186/s13059-014-0550-8
195. Wang Y, Xie Z, Kutschera E, et al. rMATS-turbo: an efficient and flexible computational tool for alternative splicing analysis of large-scale RNA-seq data. *Nature Protocols* 2024 19:4. 2024-02-23;19(4)doi:10.1038/s41596-023-00944-2
196. Clement K, Rees H, Canver MC, et al. CRISPResso2 provides accurate and rapid genome editing sequence analysis. *Nature Biotechnology* 2019 37:3. 2019-02-26;37(3)doi:10.1038/s41587-019-0032-3
197. Bray NL, Pimentel H, Melsted P, et al. Near-optimal probabilistic RNA-seq quantification. *Nature Biotechnology* 2016 34:5. 2016-04-04;34(5)doi:10.1038/nbt.3519
198. Vitting-Seerup K, Sandelin A. The Landscape of Isoform Switches in Human Cancers. *Molecular Cancer Research*. 2017/09/01;15(9)doi:10.1158/1541-7786.MCR-16-0459
199. Vitting-Seerup K, Sandelin A. IsoformSwitchAnalyzeR: analysis of changes in genome-wide patterns of alternative splicing and its functional consequences. *Bioinformatics*. 2019/11/01;35(21)doi:10.1093/bioinformatics/btz247
200. O'Donnell KA, Yu D, Zeller KI, et al. Activation of Transferrin Receptor 1 by c-Myc Enhances Cellular Proliferation and Tumorigenesis. *Molecular and Cellular Biology*. 2006 Mar;26(6)doi:10.1128/MCB.26.6.2373-2386.2006
201. Tacchini L, Gammella E, Ponti CD, Recalcati S, Cairo G. Role of HIF-1 and NF- κ B Transcription Factors in the Modulation of Transferrin Receptor by Inflammatory and Anti-

inflammatory Signals. *The Journal of Biological Chemistry*. 2008 Jul 25;283(30)doi:10.1074/jbc.M800365200

202. Yu X, Guo Q, Zhang H, Wang X, Han Y, Yang Z. Hypoxia-inducible factor-1 α can reverse the Adriamycin resistance of breast cancer adjuvant chemotherapy by upregulating transferrin receptor and activating ferroptosis. *The FASEB Journal*. 2024 Aug 9;38(15)doi:10.1096/fj.202401119R

203. AT W, E V, JJ S. Down-regulation of GATA1 uncouples STAT5-induced erythroid differentiation from stem/progenitor cell proliferation - PubMed. *Blood*. 06/03/2010;115(22)doi:10.1182/blood-2009-10-250894

204. M M, M Y, H H, et al. Growth hormone is involved in GATA1 gene expression via STAT5B in human erythroleukemia and monocytic cell lines - PubMed. *Blood cells, molecules & diseases*. 2025 Feb;110doi:10.1016/j.bcmd.2024.102894

205. Smith MR, Satter LRF, Vargas-Hernández A. Frontiers | STAT5b: A master regulator of key biological pathways. *Frontiers in Immunology*. 2023/01/23;13doi:10.3389/fimmu.2022.1025373

206. T R, M M, E Ž, et al. SEL120-34A is a novel CDK8 inhibitor active in AML cells with high levels of serine phosphorylation of STAT1 and STAT5 transactivation domains - PubMed. *Oncotarget*. 05/16/2017;8(20)doi:10.18632/oncotarget.16810

207. Bejjani F, Evanno E, Zibara K, Piechaczyk M, Jariel-Encontre I. The AP-1 transcriptional complex: Local switch or remote command? *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer*. 2019/08/01;1872(1)doi:10.1016/j.bbcan.2019.04.003

208. DA L, B G, B H. AP-1 (Fos/Jun) transcription factors in hematopoietic differentiation and apoptosis - PubMed. *International journal of oncology*. 1998 Mar;12(3)doi:10.3892/ijo.12.3.685

209. External signals regulate continuous transcriptional states in hematopoietic stem cells. *eLife*. 2021-12-23;10doi:10.7554/eLife.66512

210. McClatchy J, Strogantsev R, Wolfe E, et al. Clonal hematopoiesis related TET2 loss-of-function impedes IL1 β -mediated epigenetic reprogramming in hematopoietic stem and progenitor cells. *Nature Communications* 2023 14:1. 2023-12-07;14(1)doi:10.1038/s41467-023-43697-y

211. Shumliakivska M, Luxán G, Hemmerling I, et al. DNMT3A clonal hematopoiesis-driver mutations induce cardiac fibrosis by paracrine activation of fibroblasts. *Nature Communications* 2023 15:1. 2024-01-19;15(1)doi:10.1038/s41467-023-43003-w

212. SO A, R B, RA W, MJ R. An inflammatory environment containing TNF α favors Tet2-mutant clonal hematopoiesis - PubMed. *Experimental hematology*. 2018 Mar;59doi:10.1016/j.exphem.2017.11.002

213. Wang H, Divaris K, Pan B, et al. Clonal hematopoiesis driven by mutated DNMT3A promotes inflammatory bone loss. *Cell*. 2024 Jun 4;187(14)doi:10.1016/j.cell.2024.05.003

214. Martinez-Fabregas J, Wang L, Pohler E, et al. CDK8 Fine-Tunes IL-6 Transcriptional Activities by Limiting STAT3 Resident Time at the Gene Loci. *Cell Reports*. 2020/12/22;33(12)doi:10.1016/j.celrep.2020.108545

215. Alboni S, Cervia D, Sugama S, Conti B. Interleukin 18 in the CNS. *Journal of Neuroinflammation*. 2010 Jan 29;7(1)doi:10.1186/1742-2094-7-9

216. Kamimura D, Ishihara K, Hirano T. IL-6 signal transduction and its physiological roles: the signal orche. 2003;doi:10.1007/s10254-003-0012-2

217. Singh S, Anshita D, Ravichandiran V. MCP-1: Function, regulation, and involvement in disease. *International Immunopharmacology*. 2021 May 20;101doi:10.1016/j.intimp.2021.107598

218. Papai G, Weil PA, Schultz P. New insights into the function of transcription factor TFIID from recent structural studies. *Current opinion in genetics & development*. 2011 Mar 21;21(2)doi:10.1016/j.gde.2011.01.009
219. Cheng I-H, Pi W-C, Hsu C-H, et al. TAF2, within the TFIID complex, regulates the expression of a subset of protein-coding genes. *Cell Death Discovery* 2024 10:1. 2024-05-21;10(1)doi:10.1038/s41420-024-02017-z
220. Bhuiyan T, Arecco N, Sanchez PKM, et al. TAF2 condensation in nuclear speckles links basal transcription factor TFIID to RNA splicing factors. *Cell Reports*. 2025/05/27;44(5)doi:10.1016/j.celrep.2025.115616
221. Zhang Y, Yin C, Yin Y, et al. Cryo-EM structure of human SAGA transcriptional coactivator complex. *Cell Discovery*. 2022 Nov 22;8(1)doi:10.1038/s41421-022-00489-w
222. AM M, N A, B EK, A A. Role for gene looping in intron-mediated enhancement of transcription - PubMed. *Proceedings of the National Academy of Sciences of the United States of America*. 05/29/2012;109(22)doi:10.1073/pnas.1112400109
223. Hamada N, Iwamoto I, Nagata K-i. MED13L and its disease-associated variants influence the dendritic development of cerebral cortical neurons in the mammalian brain. *Journal of Neurochemistry*. 2023/05/01;165(3)doi:10.1111/jnc.15783
224. Chao T-C, Chen S-F, Kim HJ, et al. Structural basis of the human transcriptional Mediator regulated by its dissociable kinase module. *Molecular Cell*. 2024/10/17;84(20)doi:10.1016/j.molcel.2024.09.001
225. Aranda-Orgilles B, Saldaña-Meyer R, Wang E, et al. MED12 Regulates HSC-Specific Enhancers Independently of Mediator Kinase Activity to Control Hematopoiesis. *Cell Stem Cell*. 2016/12/01;19(6)doi:10.1016/j.stem.2016.08.004
226. Zhang S, O'Regan R, Xu W. The Emerging Role of Mediator Complex Subunit 12 in Tumorigenesis and Response to Chemotherapeutics. *Cancer*. 2019 Dec 23;126(5)doi:10.1002/cncr.32672
227. E R, A M, O D, N S, M R. Structural Features of 5' Untranslated Region in Translational Control of Eukaryotes - PubMed. *International journal of molecular sciences*. 02/25/2025;26(5)doi:10.3390/ijms26051979
228. K L, R D, M B. Functional 5' UTR mRNA structures in eukaryotic translation regulation and how to find them - PubMed. *Nature reviews Molecular cell biology*. 2018 Mar;19(3)doi:10.1038/nrm.2017.103