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The Methyltransferase Setd8 is Essential for Erythroblast Survival and Maturation

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Summary

Erythropoiesis is a highly regulated process that generates enucleate red blood cells from committed erythroid progenitors. Chromatin condensation culminating in enucleation is a defining feature of this process. Setd8 is the sole enzyme that can mono-methylate histone H4, lysine 20 and is highly expressed in erythroblasts compared to most other cell types. Erythroid Setd8 deletion results in embryonic lethality from severe anemia due to impaired erythroblast survival and proliferation. Setd8 protein levels are also uniquely regulated in erythroblasts suggesting a cell type-specific role for Setd8 during terminal maturation. Consistent with this hypothesis, Setd8 / erythroblasts have profound defects in transcriptional repression, chromatin condensation, and heterochromatin accumulation. Together these results suggest that Setd8, used by most cells to promote mitotic chromatin condensation, is an essential aspect of the transcriptional repression and chromatin condensation that are hallmarks of terminal erythroid maturation.

Graphical abstract

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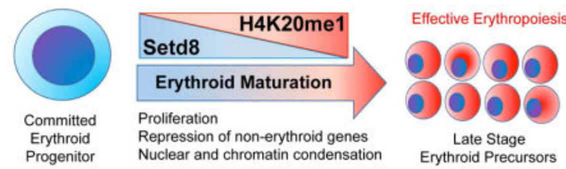

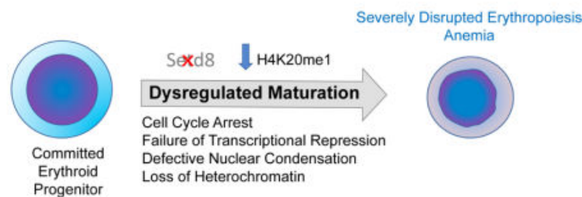

Keywords

Setd8; H4K20me1; erythropoiesis; chromatin condensation

Introduction

Erythropoiesis is an essential process that generates enucleate erythrocytes from committed progenitor cells. The terminal maturation of erythroblasts is characterized by decreasing cell size, hemoglobin accumulation, and progressive nuclear condensation that culminates in enucleation. Cell cycle progression and terminal maturation are inexorably linked in erythroblasts; the number of erythroblast cell divisions has been linked with both the number and size of their enucleate progeny.(Sankaran et al., 2012) This may be in part to meet the body's staggering demand for erythrocytes; ~80% of cells in the human body are erythrocytes,(Sender et al., 2016) with a limited lifespan of 120 days. Chromatin condensation is also an essential feature of erythropoiesis, with disruption of chromatin condensation associated with inherited anemias (Orkin and Nathan, 2009) and myelodysplastic syndrome.(Vardiman et al., 2002) While several studies have identified a role for histone deacetylation, (Ji et al., 2010; Popova et al., 2009) and selective nuclear export of histone proteins,(Hattangadi et al., 2014; Zhao et al., 2016) the mechanisms that underlie erythroblast chromatin condensation are incompletely understood. The role of histone methylation and cell cycle regulators in promoting chromatin condensation during erythropoiesis has been largely unexplored.

In mammals, Setd8 (also known as PRSet7 and Set8) is the sole enzyme that can mono-methylate histone H4, lysine 20 (H4K20me1). (Nishioka et al., 2002; Oda et al., 2009) Setd8 is essential for mammalian development, since targeted disruption of Setd8 and subsequent loss of H4K20me1 results in pre-implantation lethality.(Oda et al., 2009) Setd8 is important for cell cycle progression, chromatin condensation, and genomic stability, (Oda et al., 2009) and can modify non-histone proteins, including p53.(Shi et al., 2007) Setd8 and H4K20me1 are unique among epigenetic modifiers in that they are regulated in a cell cycle-specific

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manner. The expression of Setd8 peaks in G2/M and reaches a nadir during S-phase due to ubiquitin-dependent destruction.(Abbas et al., 2010; Centore et al., 2010) The abundance of H4K20me1 mirrors the expression pattern of Setd8, peaking in G2/M where it promotes condensation of mitotic chromosomes,(Abbas et al., 2010; Oda et al., 2009) and dropping to low levels during S-phase due to removal by the histone demethylase PHF8.(Liu et al., 2010) In cell lines, accumulation of H4K20me1 during S-phase, either due to forced Setd8 expression or disruption of PHF8 activity, results in aberrant chromatin condensation. (Centore et al., 2010; Liu et al., 2010) Relatively little is known about cell type-specific roles of Setd8, as Setd8 null cells have been difficult to generate and analyze. In this report we investigate the hypothesis that Setd8 has an essential function during erythropoiesis.

Results

Setd8 is essential for mammalian erythropoiesis

Setd8 is expressed highly in CD71+ erythroblasts compared to other cell types (Fig 1A, Figure S1A–B). To delineate the function of Setd8 during erythropoiesis we crossed mice with flox alleles surrounding the methyltransferase domain of Setd8 (Setd8 fl/fl;(Oda et al., 2009)) with mice that express a Cre Recombinase GFP fusion protein under the direction of the endogenous erythropoietin receptor promoter (ErGFPCre).(Heinrich et al., 2004) No live Setd8 fl/fl; ErGFPCre (herein Setd8 /) pups were obtained. Examination of timed embryos revealed normal blood islands at E8.5 (Embryonic Day 8.5) the approximate onset of Cre-recombinase expression, with visible anemia starting at E9.5, and death from anemia by E12.5 (Fig. 1B). Cells isolated from Setd8 / embryos had a diminished ability to form primitive erythroid colonies (Fig. S1D), suggesting primitive erythropoiesis is impaired beginning at the EryP-CFC. As expected with an erythroid-specific Setd8 deletion, Setd8 / embryos had similar numbers of erythroid-myeloid progenitors as littermate controls (Fig. S1E). Erythroblasts from Setd8 / embryos expressed the GFP-Cre fusion protein and had excision of exon 7 of the Setd8 gene, which encodes the SET domain responsible for methyltransferase activity (Fig. S2A–C).

Primitive erythroblasts emerge from the yolk sac at E8-E8.5, and mature and enucleate in the circulation as a semi-synchronous cohort.(Kingsley et al., 2004) The rapid expansion of these cells supports the growing embryo until E12.5–E13.5, when the fetal liver becomes the major source of erythropoiesis.(McGrath et al., 2011) The Setd8 / cells did not undergo the semi synchronous phenotypic maturation that normally occurs between E9.5 and E11.5 (Fig. 1C) or the rapid expansion that is necessary for embryonic viability (Fig 1D). Intriguingly, occasional large, enucleate erythrocytes were noted on cytospins from E11.5 Setd8 / embryos, (Fig. 1C) several days before enucleate primitive or definitive erythrocytes are expected to be in circulation (Kingsley et al., 2004). Despite their abnormal morphology, the Setd8 / erythroblasts did have changes in cell surface phenotype consistent with progressive maturation, with a higher proportion of E10.5 Setd8 / erythroblasts demonstrating a mature cell surface phenotype (Ter119 high, CD71 neg) than Setd8 /+ controls ($p<0.05$; Fig 1E). Together, these results demonstrate that Setd8 is required for normal erythropoiesis and that Setd8 deletion dysregulates, but does not arrest, erythroid maturation.

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Setd8 is essential for erythroblast survival and proliferation

To determine the cause of the severe anemia, we assessed for apoptosis using Annexin V staining. Setd8 / embryos had an approximately 10-fold increase in the frequency of apoptotic erythroblasts (CD71+ Ter119+ Annexin V+ Dapi negative) compared to Setd8 /+ controls ($p<0.05$; Fig. 1F). As Setd8 is essential for cell cycle progression and the primitive erythroid lineage needs to expand 10-fold from E9.5 to E11.5 to support the growing embryo,(Malik et al., 2013) we hypothesized that abnormalities in cell cycle progression were also contributing to the profound anemia in the Setd8 / embryos. Consistent with this hypothesis, ex-vivo BrdU incorporation assays demonstrated a significant accumulation of Setd8 / erythroblasts in S-phase compared to Setd8 /+ control (90% v 65%, $p<0.05$, Fig. 1G). Together, these data suggest that increased apoptosis and defects in cell cycle progression prevented the Set8 / erythroblasts from undergoing the rapid and extensive expansion necessary to support embryonic viability.

Setd8 / cells have a failure of transcriptional repression but express erythroid genes at levels similar to controls

To gain insights into the mechanisms underlying the Setd8 / phenotype, we compared global gene expression in sorted populations of CD71+, Ter119+ erythroblasts isolated from the fetal blood of E10.5 Setd8 / and littermate control (Setd8 /+ and Setd8 +/+) embryos. We identified 345 genes as differentially expressed (FDR<0.05; Fig. 2A, Table S1). The vast majority of differentially expressed genes (340/345) were expressed at higher levels in the Setd8 / erythroblasts compared to controls, (Fig. 2A, Fig S3A) suggesting that Setd8 loss impairs the widespread transcriptional repression that is associated with terminal erythroid maturation. Consistent with previous reports, Setd8 / erythroblasts failed to silence the master transcriptional regulator GATA2 (DeVilbiss et al., 2015; Malik et al., 2015).

As Setd8 has previously been implicated as a GATA1 co-repressor (Devilbiss et al., 2013) we examined the impact of Setd8 disruption on the transcriptional repression that normally occurs during the transition from proerythroblast to basophilic erythroblast. We used the ErythronDB (Kingsley et al., 2013) to identify 1901 genes whose expression is a minimum of 1.5-fold higher in primitive proerythroblasts than in basophilic erythroblasts. We then compared those 1901 genes to the 340 genes expressed at higher levels in Setd8 / erythroblasts compared to controls. Approximately 1/3 of the genes expressed at higher levels in Setd8 / erythroblasts are normally repressed during the proerythroblast to basophilic erythroblast transition (111/340; 32%, Fig. S3B, Table S2). Among the 111 genes are important transcriptional regulators that are normally silenced with maturation including Gata2, Hhex, and Jun. Intriguingly, key erythroid genes such as Gata1, Tal1, Klf1, and Hbb-y are expressed at similar levels in Setd8 / and control cells (Fig. 2B), suggesting that the Setd8 / erythroblasts are able to express key aspects of the erythroid differentiation program, despite their defect in transcriptional repression.

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Setd8 / cells have activation of p53-target genes, but are not rescued by concomitant p53 deletion

Ingenuity Pathway Analyses and Gene Set Enrichment Analyses revealed significant enrichment for the p53-signaling pathway (Figs 2C and 2D). In addition, Setd8 / erythroblasts have many of hallmarks of p53 activation, including cell cycle abnormalities (Fig. 1G) and accumulation of DNA damage (Fig. S1F), and Setd8 has been implicated as a direct regulator of p53. (Shi et al., 2007) We therefore sought to determine the extent to which p53 activation contributes to the Setd8 phenotype. To that end, we deleted p53 in Setd8 / erythroblasts utilizing mice that harbor floxed p53 alleles (Jonkers et al., 2001) (Fig. S3C–E). Regardless of p53 genotype, the Setd8 / embryos were profoundly anemic (Figs. 2E and F) and the Setd8 / erythroblasts had phenotypic abnormalities and high rates of apoptosis (Fig 2E lower panel, Fig S3F). These data are consistent with a recent study in epithelial stem cells (Driskell et al., 2015), and strongly suggest an essential role for Setd8 in erythroid proliferation and survival that is independent of the p53 pathway.

Erythroblasts express Setd8 and accumulate H4K20me1 throughout the cell cycle

Setd8 is expressed throughout erythroid maturation, with robust expression at the polychromatic erythroblast stage when erythroblasts are actively condensing their chromatin, and expression declining at the orthochromatic stage when chromatin condensation has been largely completed (Fig 3A, S1C). The level of H4K20me1, which promotes a condensed chromatin structure, appears highest in late-stage (polychromatic and orthochromatic) erythroblasts (Fig 3A).

Maturing erythroblasts express Setd8 at levels significantly higher than other cell types, but express PHF8 at basal levels (Fig. S1B–C). We hypothesized that the robust expression of Setd8 coupled with basal levels of PHF8 would be associated with H4K20me1 accumulation throughout the cell cycle and may contribute to the chromatin condensation that is characteristic of terminal erythroid maturation. To test our hypothesis, we assessed Setd8 and H4K20me1 protein levels in primary hematopoietic progenitors (Kit+, CD71 neg, Ter119 neg), primitive erythroblasts from E10.5 embryonic blood (CD71+, Ter119+), and definitive erythroblasts isolated from murine bone marrow (CD71+, Ter119+), that were sorted into cell cycle fractions based on DNA content. In the hematopoietic progenitors, Setd8 followed the expected pattern of expression, with levels that peaked in G2/M (Fig. 3B). The level of H4K20me1 did not appear to oscillate significantly during the cell cycle as previously reported, however enriching for specific cell cycle fractions using DNA content may not have sufficient resolution to identify the nadir of H4K20me1 levels. In primary E10.5 primitive embryonic erythroblasts Setd8 was expressed throughout the cell cycle, with similar levels G1/G0, S, and G2/M (Fig. 3C). Definitive erythroblasts had a pattern of Setd8 expression similar to primitive erythroblasts with robust Setd8 expression in all cell cycle fractions (Fig. 3D). These results suggest that Setd8 expression is uniquely regulated in erythroid cells, and further suggest a role for Setd8 during terminal erythroid maturation.

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Setd8 is essential for erythroblast chromatin condensation and heterochromatin accumulation in maturing erythroblasts

Decreasing cell and nuclear size are cardinal features of terminal erythroid maturation. We used imaging flow cytometric analyses to quantify cell and nuclear size in Setd8 / and littermate control erythroblasts. The Setd8 / erythroblasts had a significantly larger cell and nuclear area than controls (Figs. 4A and S4A). The defect in nuclear condensation was particularly striking; the Setd8 / cells had a nuclear area nearly twice that of controls at E11.5 (Fig. 4A), suggesting a defect in chromatin condensation. Setd8 /+ erythroblasts had a similar cell area, but a significantly larger nuclear area than Setd8 (+/+) cells, (Fig 4A) further implicating Setd8 in the chromatin condensation that occurs during terminal erythroid maturation.

To obtain a more detailed assessment of the chromatin in Setd8 / erythroblasts we performed transmission electron microscopy (TEM) on cells from E9.5 and E11.5 embryos. Chromatin condensation was significantly impaired in the Setd8 / erythroblasts compared to controls at both time points (Fig. 4B and S4B–C). Unexpectedly, the Setd8 / cells contained only a small amount of heterochromatin that was localized to the nuclear periphery. The Setd8 / cells also had a “ruffled” appearance of the nuclear membrane at E9.5 that progressed to a severe disruption of the nuclear membrane by E11.5 (Figs. 4B and S4B–C). Notably, both the Setd8 / and control erythroblasts have lost the majority of their mitochondria at E11.5 (Fig.4B and S4B–C), as is expected with normal erythroblast maturation (Malik et al., 2013) and the appearance of the Setd8 / cells is not consistent with apoptosis, which is typically characterized by cell shrinkage and chromatin condensation (Tinari et al., 2008).

To further characterize the chromatin of the Setd8 / cells, we performed confocal microscopy. As expected, H4K20me1 staining was nearly absent in the Set8 / cells but robust in littermate controls (Fig 4C). Consistent with the TEM studies, staining for both H3K9me2 (dimethylated histone H3, lysine 9) and HP1 (heterochromatin protein 1) in the Setd8 / cells was largely relegated to the nuclear peripherally with a paucity of central staining compared to controls (Fig 4C). Together, these data suggest a defect in chromatin condensation and heterochromatin accumulation in the Setd8 / cells.

Discussion

Setd8 is a broadly expressed methyltransferase that contributes to a number of essential cellular processes. In this report, we demonstrate that Setd8 is essential for erythropoiesis, with erythroid Setd8 deletion resulting in early embryonic lethality due to severe anemia. Underlying the anemia were increased rates of apoptosis and profound defects in cell cycle progression. While Setd8 is known to be important for many pan-cellular processes, information regarding cell type-specific Setd8 functions is lacking. We demonstrate that Setd8, used by most cells to promote mitotic chromatin condensation, is an essential aspect of the chromatin condensation and transcriptional repression that are hallmarks of terminal erythroid maturation.

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Stark differences in the phenotype observed in Setd8 / erythroblasts and the phenotypes observed following Setd8 deletion in hepatocytes and murine embryonic stem cells supports an erythroid-specific function of Setd8. In contrast to Setd8 / erythroblasts, Setd8-null hepatocytes appeared to be arrested in G2/M and had TEM changes consistent with necrosis. (Nikolaou et al., 2015) Setd8 deletion in murine embryonic stem cells also had a phenotype distinct from that in erythroblasts, resulting in chromatin decondensation without an increase in nuclear volume. (Oda et al., 2009) The increased nuclear size in both the Setd8 / and Setd8 /+ erythroblasts, and impaired chromatin condensation in the Setd8 / erythroblasts suggest that Setd8 is important for chromatin condensation during terminal erythroid maturation.

The precise mechanisms by which Setd8 promotes chromatin condensation and regulates gene expression in erythroblasts remain elusive, however forced expression of Setd8 during S-phase of non-erythroid cells results in aberrant pre-mitotic chromatin compaction,(Centore et al., 2010; Liu et al., 2010) suggesting that robust expression of Setd8 throughout the erythroblast cell cycle may be contributing to chromatin condensation during terminal maturation. Mono-methylation of H4K20 can promote chromatin compaction via multiple mechanisms including progressive methylation to H4K20me3 and interaction with factors such as L3MBTL1 and the Condensin II complex that actively promote chromatin compaction. (Liu et al., 2010; Trojer et al., 2007) Mono-methylation of H4K20 may also impact post-translational modifications at neighboring lysine residues on the H4 tail, with some studies suggesting that H4K16Ac and H4K20me1 are competing marks (Nishioka et al., 2002). As histone deacetylation is necessary for chromatin condensation to occur during terminal erythroid maturation, these data suggest a model where histone H4 deacetylation and H4K20me1 accumulation occur in tandem to facilitate chromatin condensation in maturing erythroblasts.

The maturation of the Setd8 / erythroblasts is profoundly dysregulated. Although the Setd8 / erythroblasts had changes in cell surface marker expression consistent with progressive maturation, expressed erythroid genes such as GATA1 at levels similar to controls, and shed their mitochondria, they also had significant phenotypic abnormalities that were accompanied by defects in transcriptional repression, chromatin condensation, and heterochromatin accumulation. Data regarding the function of Setd8 and H4K20me1 in heterochromatin formation and maintenance are limited. Mono-methylation of histone H3 lysine 9 (H3K9) is known to play a critical role in heterochromatin formation and intriguingly, simultaneous knockdown of PRMD3 and PRDM16, enzymes that mono-methylate H3K9, results in a phenotype similar to the Setd8 / cells (Pinheiro et al., 2012). The dramatic failure of Sed8 / erythroblasts to accumulate heterochromatin is unlikely to be solely due to dysregulated maturation and suggests that there may be a pathway for heterochromatin formation that is dependent on Setd8. As heterochromatin accumulation is a common feature of cellular differentiation,(Grigoryev et al., 2006) such a pathway would have broad implications across multiple cell types and biologic processes.

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Experimental Procedures

Generation of Timed Embryos

The University of Rochester's Committee on Animal Resources approved all experiments utilizing mice (UCAR 101396). Mice of the appropriate genotype were bred overnight and vaginal plugs checked after 12 hours (Embryonic Day 0.5; E0.5). At appropriate time points for analyses, the pregnant dam was anesthetized and sacrificed via cervical dislocation and the embryos dissected for further analyses. Additional details are available in supplemental methods.

Flow Cytometric Analyses

For analyses of cell and nuclear size, erythroblasts were stained with anti-CD71 (eBioscience), anti-Ter119 (eBioscience) and DRAQ5 (eBioscience) and run on the ImageStream X (Amnis). The data was analyzed with IDEAS 6.0 software (Amnis) as previously published. (Malik et al., 2015) Additional details on FACS analyses are in supplemental methods.

RNA sequencing and bioinformatics analyses

CD71+Ter119+ erythroblasts were isolated from E10.5 fetal blood via fluorescent cell sorting on a FACSAria II (BD Biosciences) and subjected to RNA sequencing as previously published(Malik et al., 2015).fig Please see supplemental methods for details.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and Notes

Abbas T, Shibata E, Park J, Jha S, Karnani N, Dutta A. 2010; CRL4(Cdt2) regulates cell proliferation and histone gene expression by targeting PR-Set7/Set8 for degradation. Molecular cell. 40:9–21. [PubMed: 20932471]

Centore RC, Havens CG, Manning AL, Li JM, Flynn RL, Tse A, Jin J, Dyson NJ, Walter JC, Zou L. 2010; CRL4(Cdt2)-mediated destruction of the histone methyltransferase Set8 prevents premature chromatin compaction in S phase. Molecular cell. 40:22–33. [PubMed: 20932472]

Devilbiss AW, Boyer ME, Bresnick EH. 2013; Establishing a hematopoietic genetic network through locus-specific integration of chromatin regulators. Proceedings of the National Academy of Sciences of the United States of America. 110:E3398–3407. [PubMed: 23959865]

DeVilbiss AW, Sanalkumar R, Hall BD, Katsumura KR, de Andrade IF, Bresnick EH. 2015; Epigenetic Determinants of Erythropoiesis: Role of the Histone Methyltransferase SetD8 in Promoting Erythroid Cell Maturation and Survival. Molecular and cellular biology. 35:2073–2087. [PubMed: 25855754]

Driskell I, Oezteurk-Winder F, Humphreys P, Frye M. 2015; Genetically induced cell death in bulge stem cells reveals their redundancy for hair and epidermal regeneration. Stem cells. 33:988–998. [PubMed: 25447755]

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Grigoryev SA, Bulynko YA, Popova EY. 2006; The end adjusts the means: heterochromatin remodelling during terminal cell differentiation. Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology. 14:53–69.

Hattangadi SM, Martinez-Morilla S, Patterson HC, Shi J, Burke K, Avila-Figueroa A, Venkatesan S, Wang J, Paulsen K, Gorlich D, et al. 2014; Histones to the cytosol: exportin 7 is essential for normal terminal erythroid nuclear maturation. Blood. 124:1931–1940. [PubMed: 25092175]

Heinrich AC, Pelanda R, Klingmuller U. 2004; A mouse model for visualization and conditional mutations in the erythroid lineage. Blood. 104:659–666. [PubMed: 15090451]

Ji P, Yeh V, Ramirez T, Murata-Hori M, Lodish HF. 2010; Histone deacetylase 2 is required for chromatin condensation and subsequent enucleation of cultured mouse fetal erythroblasts. Haematologica. 95:2013–2021. [PubMed: 20823130]

Jonkers J, Meuwissen R, van der Gulden H, Peterse H, van der Valk M, Berns A. 2001; Synergistic tumor suppressor activity of BRCA2 and p53 in a conditional mouse model for breast cancer. Nature genetics. 29:418–425. [PubMed: 11694875]

Kingsley PD, Greenfest-Allen E, Frame JM, Bushnell TP, Malik J, McGrath KE, Stoeckert CJ, Palis J. 2013; Ontogeny of erythroid gene expression. Blood. 121:e5–e13. [PubMed: 23243273]

Kingsley PD, Malik J, Fantauzzo KA, Palis J. 2004; Yolk sac-derived primitive erythroblasts enucleate during mammalian embryogenesis. Blood. 104:19–25. [PubMed: 15031208]

Liu W, Tanasa B, Tyurina OV, Zhou TY, Gassmann R, Liu WT, Ohgi KA, Benner C, Garcia-Bassets I, Aggarwal AK, et al. 2010; PHF8 mediates histone H4 lysine 20 demethylation events involved in cell cycle progression. Nature. 466:508–512. [PubMed: 20622854]

Malik J, Getman M, Steiner LA. 2015The histone methyltransferase Setd8 represses Gata2 expression and regulates erythroid maturation. Molecular and cellular biology.

Malik J, Kim AR, Tyre KA, Cherukuri AR, Palis J. 2013; Erythropoietin critically regulates the terminal maturation of murine and human primitive erythroblasts. Haematologica. 98:1778–1787. [PubMed: 23894012]

McGrath KE, Frame JM, Fromm GJ, Koniski AD, Kingsley PD, Little J, Bulger M, Palis J. 2011; A transient definitive erythroid lineage with unique regulation of the beta-globin locus in the mammalian embryo. Blood. 117:4600–4608. [PubMed: 21378272]

Nikolaou KC, Moulos P, Chalepakis G, Hatzis P, Oda H, Reinberg D, Talianidis I. 2015; Spontaneous development of hepatocellular carcinoma with cancer stem cell properties in PR-SET7-deficient livers. The EMBO journal. 34:430–447. [PubMed: 25515659]

Nishioka K, Rice JC, Sarma K, Erdjument-Bromage H, Werner J, Wang Y, Chuikov S, Valenzuela P, Tempst P, Steward R, et al. 2002; PR-Set7 is a nucleosome-specific methyltransferase that modifies lysine 20 of histone H4 and is associated with silent chromatin. Molecular cell. 9:1201–1213. [PubMed: 12086618]

Oda H, Okamoto I, Murphy N, Chu J, Price SM, Shen MM, Torres-Padilla ME, Heard E, Reinberg D. 2009; Monomethylation of histone H4-lysine 20 is involved in chromosome structure and stability and is essential for mouse development. Molecular and cellular biology. 29:2278–2295. [PubMed: 19223465]

Orkin, SH, Nathan, DG. Nathan and Oski's hematology of infancy and childhood. 7th. Philadelphia: Saunders/Elsevier; 2009.

Pinheiro I, Margueron R, Shukeir N, Eisold M, Fritzsch C, Richter FM, Mittler G, Genoud C, Goyama S, Kurokawa M, et al. 2012; Prdm3 and Prdm16 are H3K9me1 methyltransferases required for mammalian heterochromatin integrity. Cell. 150:948–960. [PubMed: 22939622]

Popova EY, Krauss SW, Short SA, Lee G, Villalobos J, Etzell J, Koury MJ, Ney PA, Chasis JA, Grigoryev SA. 2009; Chromatin condensation in terminally differentiating mouse erythroblasts does not involve special architectural proteins but depends on histone deacetylation. Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology. 17:47–64.

Sankaran VG, Ludwig LS, Sicinska E, Xu J, Bauer DE, Eng JC, Patterson HC, Metcalf RA, Natkunam Y, Orkin SH, et al. 2012; Cyclin D3 coordinates the cell cycle during differentiation to regulate erythrocyte size and number. Genes & development. 26:2075–2087. [PubMed: 22929040]

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Sender R, Fuchs S, Milo R. 2016; Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. Cell. 164:337–340. [PubMed: 26824647]

Shi X, Kachirskaia I, Yamaguchi H, West LE, Wen H, Wang EW, Dutta S, Appella E, Gozani O. 2007; Modulation of p53 function by SET8-mediated methylation at lysine 382. Molecular cell. 27:636–646. [PubMed: 17707234]

Tinari A, Giammarioli AM, Manganelli V, Ciarlo L, Malorni W. 2008; Analyzing morphological and ultrastructural features in cell death. Methods in enzymology. 442:1–26. [PubMed: 18662562]

Trojer P, Li G, Sims RJ 3rd, Vaquero A, Kalakonda N, Boccuni P, Lee D, Erdjument-Bromage H, Tempst P, Nimer SD, et al. 2007; L3MBTL1, a histone-methylation-dependent chromatin lock. Cell. 129:915–928. [PubMed: 17540172]

Vardiman JW, Harris NL, Brunning RD. 2002; The World Health Organization (WHO) classification of the myeloid neoplasms. Blood. 100:2292–2302. [PubMed: 12239137]

Zhao B, Mei Y, Schipma MJ, Roth EW, Bleher R, Rappoport JZ, Wickrema A, Yang J, Ji P. 2016; Nuclear Condensation during Mouse Erythropoiesis Requires Caspase-3-Mediated Nuclear Opening. Developmental cell. 36:498–510. [PubMed: 26954545]

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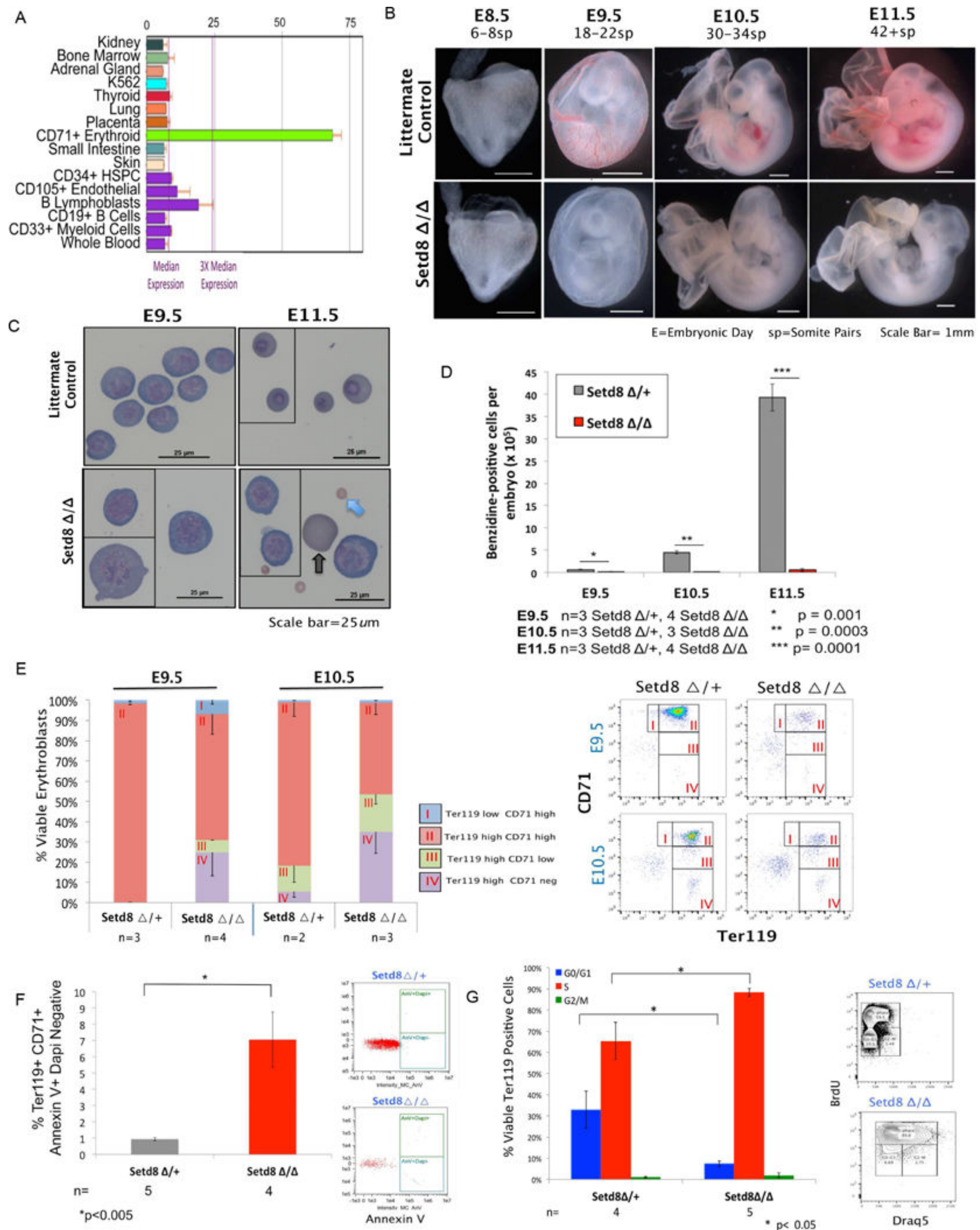

Fig. 1. Setd8 is essential for mammalian erythropoiesis

(A) mRNA expression in various human cell types shown. Data from adapted from Biogps.org (Wu et al 2009) **(B)** Setd8 / and littermate control embryos. **(C)** Cytospins from Setd8 / and littermate control embryos. Blue arrow denotes maternal erythrocyte. Black arrow denotes enucleate fetal erythrocyte. **(D)** Benzidine counts from the fetal blood of Setd8 / and Setd8 /+ littermate controls. **(E)** Quantification of CD71 and Ter119 staining from the fetal blood of E9.5 and E10.5 Setd8 / and Setd8 /+ embryos. Representative flow cytometry plots shown in right panel. **(F)** Annexin V staining of Setd8

/ and control erythroblasts at E9.5. Representative flow plots are shown in right panel. **(G)** Cell cycle analyses of Setd8 / and control erythroblasts by BrdU incorporation. Representative flow plots are shown in right panel. p-values determined by Students t-test. Error bars represent standard error of the mean.

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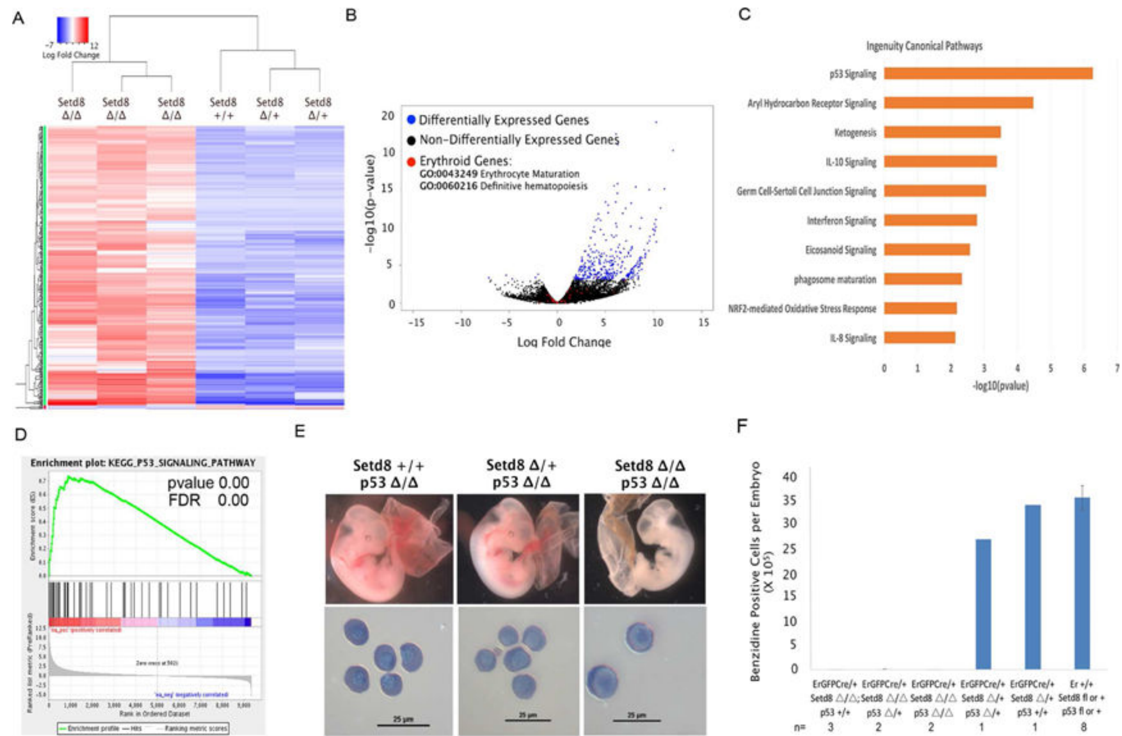

Fig. 2. Setd8 deletion results in dysregulated gene expression and is not rescued by p53 deletion

(A) Heat map of gene expression in Setd8 / and littermate control erythroblasts. **(B)** Volcano plot, with differentially expressed genes in blue. Erythroid genes, identified by GO terms "erythrocyte maturation" and "definitive hematopoiesis," are in red. **(C)** Ingenuity pathway analyses of the differentially expressed genes. **(D)** Gene set enrichment analyses of differentially expressed genes. **(E)** Photographs of embryos with genotype indicated. Bottom panel represents corresponding cytospin. **(F)** Benzidine counts from Setd8 / and control embryos, with varying p53 genotype.

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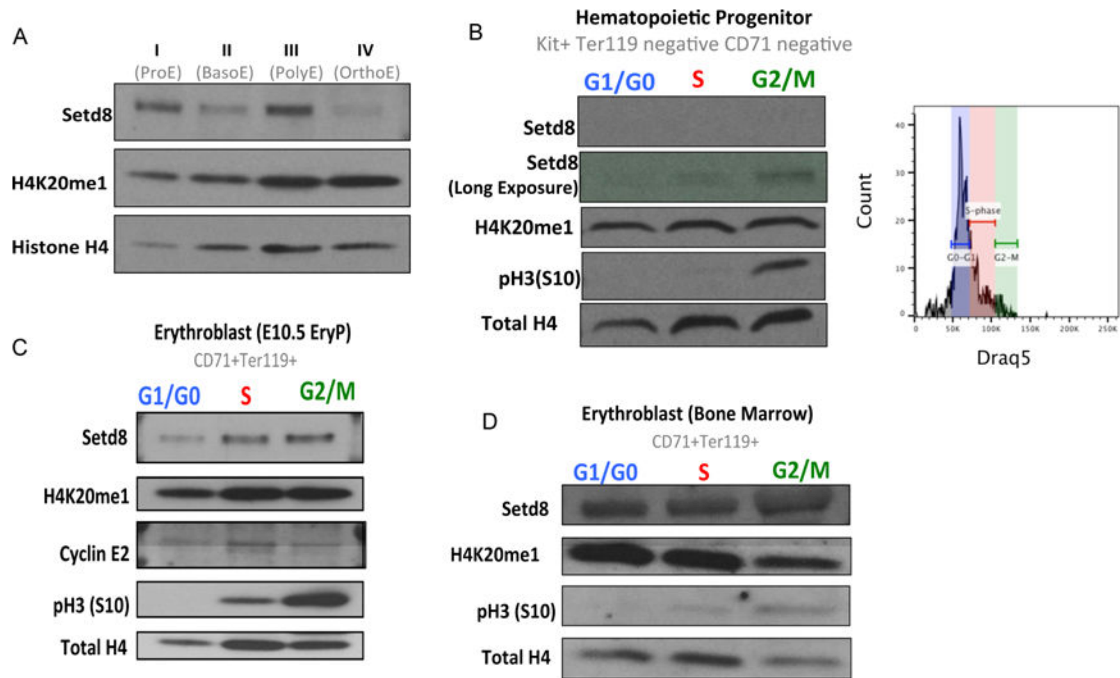

Fig. 3. Setd8 expression in primary erythroblasts

(A) Setd8 protein levels in the indicated populations of maturing erythroblasts. **(B)** Setd8 and H4K20me1 levels in hematopoietic progenitors sorted into cell cycle fractions by DNA content. Representative histogram from cell sorting is shown in the right panel. **(C)** Setd8 and H4K20me1 levels in E10.5 primitive erythroblasts sorted into cell cycle fractions by DNA content. **(D)** Setd8 and H4K20me1 levels in definitive erythroblasts sorted into cell cycle fractions by DNA content. For all blots, histone H4 is used as a loading control. Where indicated, phospho histone H3 is used to confirm enrichment of the G2/M population and cyclin E2 is used to confirm enrichment of the S phase population.

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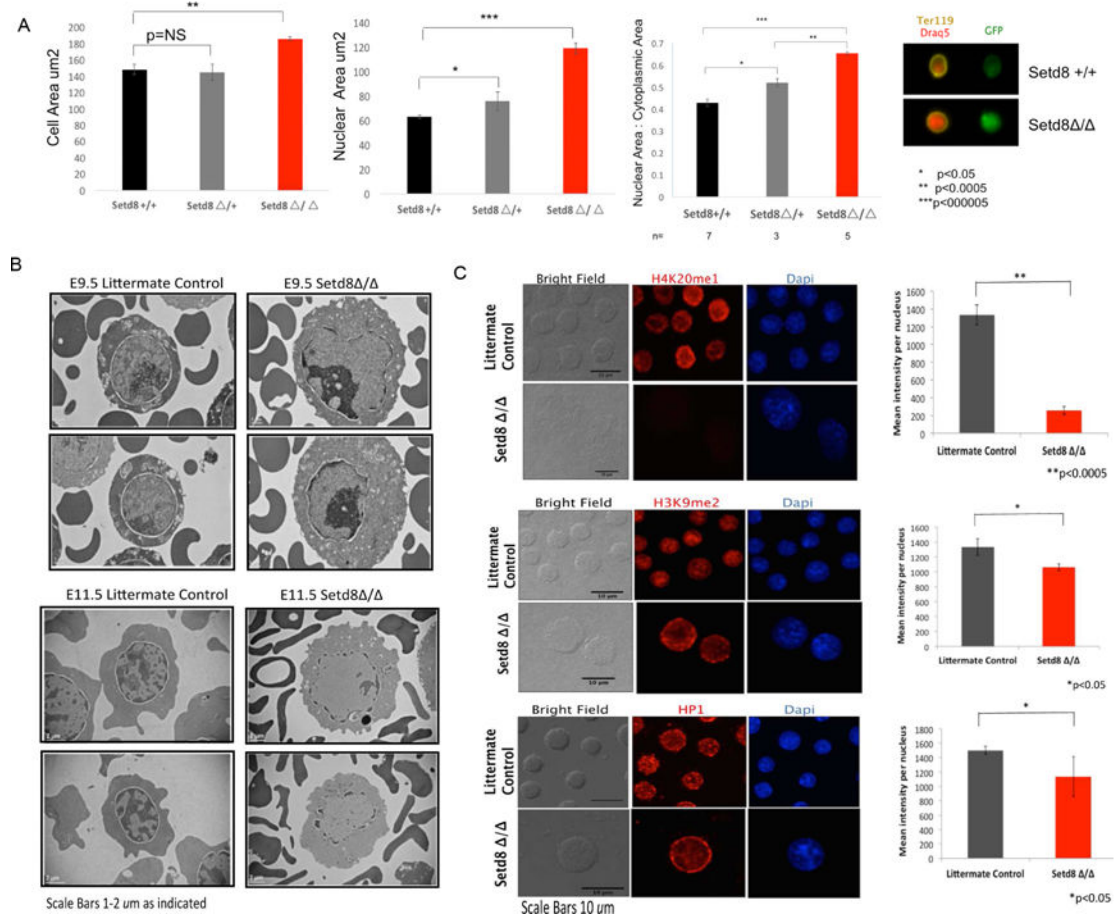

Fig. 4. Setd8 / erythroblasts have a profound deficit in chromatin condensation and accumulation of heterochromatin

(**A**) Quantification of cell and nuclear area of Ter119+ Setd8 / , Setd8 /+, and Setd8 +/+ erythroblasts by imaging flow cytometric analyses. Representative cell images are shown in the right panel. Error bars represent standard error of the mean. (**B**) Transmission electron microscopy (TEM) of Setd8 / and control erythroblasts at E9.5 and E11.5. Background cells in TEM images represent maternal erythrocytes used as carriers during preparation of embryonic cells for TEM. (**C**) Confocal microscopy with staining for H4K20me1, H3K9me2, and HP1. Top panels: cells from littermate control embryos. Bottom panels: cells from Setd8 / embryos. Quantification is shown in the right panel. Error bars represent SEM.