Stem Cell Reports
Article

OPEN ACCESS

Age-Associated Decrease of the Histone Methyltransferase SUV39H1 in HSC Perturbs Heterochromatin and B Lymphoid Differentiation

Dounia Djeghloul,[1] Klaudia Kuranda,[1,8] Isabelle Kuzniak,[1] Daniela Barbieri,[2] Irina Naguibneva,[3] Caroline Choisy,[1] Jean-Christophe Bories,[1] Christine Dosquet,[4] Marika Pla,[4] Valérie Vanneaux,[5] Gérard Socié,[6] Françoise Porteu,[2] David Garrick,[1,7] and Michele Goodhardt[1,7,*]

[1]INSERM UMRS-1126, Institut Universitaire d'Hématologie, Université Paris Diderot, Paris, France
[2]INSERM UMRS-1170, Gustave Roussy Cancer Campus, Université Paris Sud - Université Paris-Saclay, Villejuif, France
[3]INSERM UMRS-967, Institut de Radiobiologie Cellulaire et Moléculaire, Commissariat à l'Energie Atomique, Fontenay-aux-Roses, France
[4]INSERM UMRS-1131, Institut Universitaire d'Hématologie, Université Paris Diderot, Paris, France
[5]AP-HP Unité de Thérapie Cellulaire, Centre d'Investigation Clinique en Biothérapie Cellulaire and INSERM UMRS-1160, Université Paris Diderot, Paris, France
[6]AP-HP Hematology Transplantation and INSERM UMRS-1160, Université Paris Diderot, Paris, France
[7]Co-senior author
[8]Present address: INSERM U974, Université Pierre et Marie Curie, Paris, France
*Correspondence: michele.goodhardt@univ-paris-diderot.fr
http://dx.doi.org/10.1016/j.stemcr.2016.05.007

SUMMARY

The capacity of hematopoietic stem cells (HSC) to generate B lymphocytes declines with age, contributing to impaired immune function in the elderly. Here we show that the histone methyltransferase SUV39H1 plays an important role in human B lymphoid differentiation and that expression of SUV39H1 decreases with age in both human and mouse HSC, leading to a global reduction in H3K9 trimethylation and perturbed heterochromatin function. Further, we demonstrate that SUV39H1 is a target of microRNA miR-125b, a known regulator of HSC function, and that expression of miR-125b increases with age in human HSC. Overexpression of miR-125b and inhibition of SUV39H1 in young HSC induced loss of B cell potential. Conversely, both inhibition of miR-125 and enforced expression of SUV39H1 improved the capacity of HSC from elderly individuals to generate B cells. Our findings highlight the importance of heterochromatin regulation in HSC aging and B lymphopoiesis.

INTRODUCTION

All myeloid and lymphoid blood cell lineages are continually replenished throughout adult life from a reservoir of rare multipotent hematopoietic stem cells (HSC) residing in the bone marrow. Studies in both humans and mice have shown that HSC are not constant throughout life (Chambers et al., 2007; Dykstra et al., 2011; Flach et al., 2014; Kuranda et al., 2011; Lescale et al., 2010; Pang et al., 2011; Rossi et al., 2005). There is an increase in the number of phenotypically defined HSC with age, but the stem cells that accumulate exhibit a diminished long-term reconstitution potential as well as a cell-intrinsic reduction in their capacity to generate immune-competent B lymphocytes, leading to a myeloid-biased differentiation output. This age-associated skewing of HSC differentiation potential from lymphoid to myeloid lineages, and the resultant decreased output of naive B cells, leads to a decline in antibody diversity and is believed to contribute to the general depletion of immune function observed in the elderly (reviewed in Denkinger et al., 2015). HSC aging is driven by both cell-extrinsic alterations in the stem cell niche and systemic signals, as well as changes intrinsic to the stem cells themselves (reviewed in Garrick et al., 2015; Geiger et al., 2013), including widespread changes in gene-expression patterns (Chambers et al., 2007; Flach et al., 2014; Pang et al., 2011; Rossi et al., 2005; Sun et al., 2014).

While the molecular triggers for these transcriptomic and functional changes are still incompletely understood, recent studies in mouse HSC have demonstrated that aging is associated with alterations in the DNA methylation and histone modification profiles (Beerman et al., 2013; Sun et al., 2014), suggesting that disruption of the normal epigenetic state is an important factor in the aging HSC phenotype. One key component of the epigenetic landscape is the formation of domains of heterochromatin. These regions of compacted and transcriptionally repressive chromatin are critical for diverse aspects of nuclear biology, including the regulation of gene-expression patterns, the transcriptional silencing of genomic repeats, and the maintenance of genome stability, as well as normal centromere and telomere function (Bulut-Karslioglu et al., 2014; Grewal and Jia, 2007; Peters et al., 2001; Schoeftner and Blasco, 2009). One of the principal enzymes involved in the formation of heterochromatin is SUV39H, a family of two histone methyltransferases (SUV39H1/KMT1A and SUV39H2/KMT1B) that catalyze tri-methylation of lysine 9 of histone H3 (H3K9me3) (Peters et al., 2001). The H3K9me3 histone modification is recognized and bound by members of the heterochromatin protein 1 (HP1) family (Lachner et al., 2001), critical adaptor proteins that coordinate chromatin compaction by

undergoing self-association as well as recruiting histone deacetylases, DNA methyltransferases, and structural RNAs (reviewed in Maison and Almouzni, 2004). Consistent with a crucial role for heterochromatin during differentiation and development, it has been shown that SUV39H1-mediated H3K9me3 regulates lineage commitment during early mouse development by repressing lineage-inappropriate genes (Alder et al., 2010) and that depletion of SUV39H gives rise to pre- and postnatal developmental defects and lethality in mice (Peters et al., 2001). Accumulating evidence suggests that SUV39H may also regulate various aspects of hematopoiesis. The SUV39H1/HP1 regulatory axis is important to maintain cellular fate following commitment to the T helper 2 (T_H2) lymphocyte lineage (Allan et al., 2012). Further, deletion of *Suv39h* in mice leads to the development of late-onset B cell lymphomas (Peters et al., 2001), while overexpression of SUV39H leads to impaired erythroid differentiation (Czvitkovich et al., 2001). However, at present the role of SUV39H and heterochromatin structure in HSC aging and the regulation of differentiation potential has not been investigated directly.

In this study we show that SUV39H1 plays an important role in the differentiation of human HSC toward the B cell lineage. Expression of SUV39H1 in HSC declines with age, leading to relaxation of heterochromatin and derepression of genomic repeat elements. The age-associated decrease in SUV39H1 in human HSC correlates with an increase in expression of miR-125b, a microRNA (miRNA) which has been shown previously to target SUV39H1 and is a key regulator of HSC self-renewal and differentiation potential. Both inhibition of miR-125 and overexpression of SUV39H1 improved the B cell output of HSC from elderly individuals. Our findings support previous studies implicating the loss of heterochromatin as a key hallmark of aging and suggest that this axis may be targeted to improve HSC function with age.

RESULTS

SUV39H1 Is Highly Expressed in Human HSC and Is Important during B Cell Generation

The SUV39H family comprises two members, SUV39H1 and SUV39H2. SUV39H1 is the predominant form expressed in both human cord blood and bone marrow $CD34^+CD38^-$ HSC, as has previously been observed in mouse long-term repopulating HSC (LT-HSC) (Figures S1A and S1B; Sun et al., 2014). Analysis of *SUV39H1* expression during human hematopoietic differentiation revealed that *SUV39H1* is highly expressed in the primitive HSC compartment and that its expression decreases during myeloid differentiation, but is maintained in bone marrow B lymphoid cells (Figure 1A). To investigate the role of SUV39H1 during HSC differentiation, we treated bone marrow $CD34^+$ progenitors with chaetocin, an inhibitor of SUV39H (Greiner et al., 2005), and analyzed the effects on B lymphoid and myeloid output in in vitro differentiation assays. Treatment with chaetocin induced a significant decrease in the global levels of H3K9me3 in $CD34^+CD38^-$ HSC as assessed by fluorescence-activated cell sorting (FACS) analysis, while histone H3 acetylation (H3ac) levels remained unchanged, showing that SUV39H1 is important for the maintenance of H3K9me3 levels in HSC (Figures 1B and S2A). We found that chaetocin decreased the generation of B lineage cells following in vitro differentiation, whereas the production of myeloid colonies was not affected (Figure 1C). Treatment with chaetocin (10–100 μM) of $CD34^+CD38^-$ HSC, $CD34^+CD33^+$ myeloid progenitors, or $CD34^+CD19^+$ B lymphoid progenitors did not lead to increased apoptosis as measured by Annexin V staining, indicating that loss of B cell generation observed in these differentiation assays was not due to treatment-related toxicity (data not shown). As chaetocin may also inhibit the activity of other histone lysine methyltransferases, to confirm that the decline in B cell differentiation was indeed due to an effect on SUV39H1, human $CD34^+$ stem and progenitor cells were transfected with small interfering RNA (siRNA) targeting *SUV39H1*, or non-silencing control (Figures 1D and S2B–S2D). Knockdown of *SUV39H1* mRNA and protein relative to control-treated cells was confirmed by qRT-PCR and FACS analysis, respectively (Figures S2C and S2D). In vitro differentiation assays performed on siRNA-transfected cells revealed that knockdown of SUV39H1 specifically decreased B cell differentiation, without affecting myeloid output (Figure 1D), similar to the result observed with chaetocin. In contrast, no effect on B cell differentiation was observed using the same concentration range of the control, non-targeting siRNA (Figure S2E). Taken together, these results indicate that SUV39H1 is highly expressed in HSC and is an important factor for human B lymphopoiesis.

Decreased SUV39H1 and Global Levels of H3K9me3 in HSC with Age

Aging is associated with functional changes in the HSC compartment and, in particular, the capacity of HSC to generate B cells is decreased with age in both mice and humans (Dykstra et al., 2011; Kuranda et al., 2011; Lescale et al., 2010; Pang et al., 2011; Rossi et al., 2005). Our observations that SUV39H1 is highly expressed in the HSC compartment and is required for B cell differentiation prompted us to investigate whether expression of this factor is altered with age. We found that expression of *SUV39H1* is significantly decreased in human $CD34^+CD38^-$ HSCs isolated from the

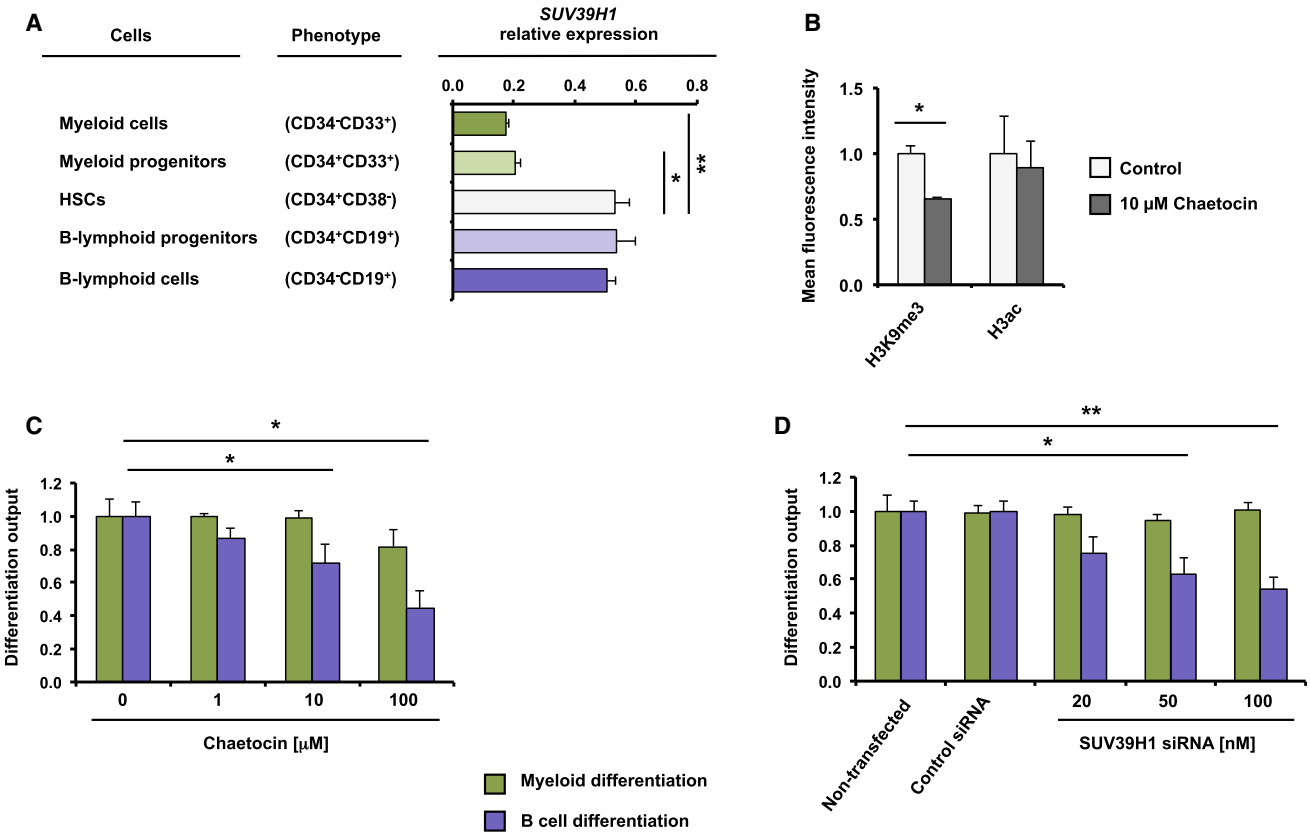


Figure 1. The Histone Methyltransferase SUV39H1 Is Critical for B Lymphoid but Not Myeloid Differentiation

(A) qRT-PCR analysis of the expression of *SUV39H1* in the populations indicated, isolated from human bone marrow. Values shown are normalized to *HPRT1* expression (mean + SD; $n \geq 4$ for each population).

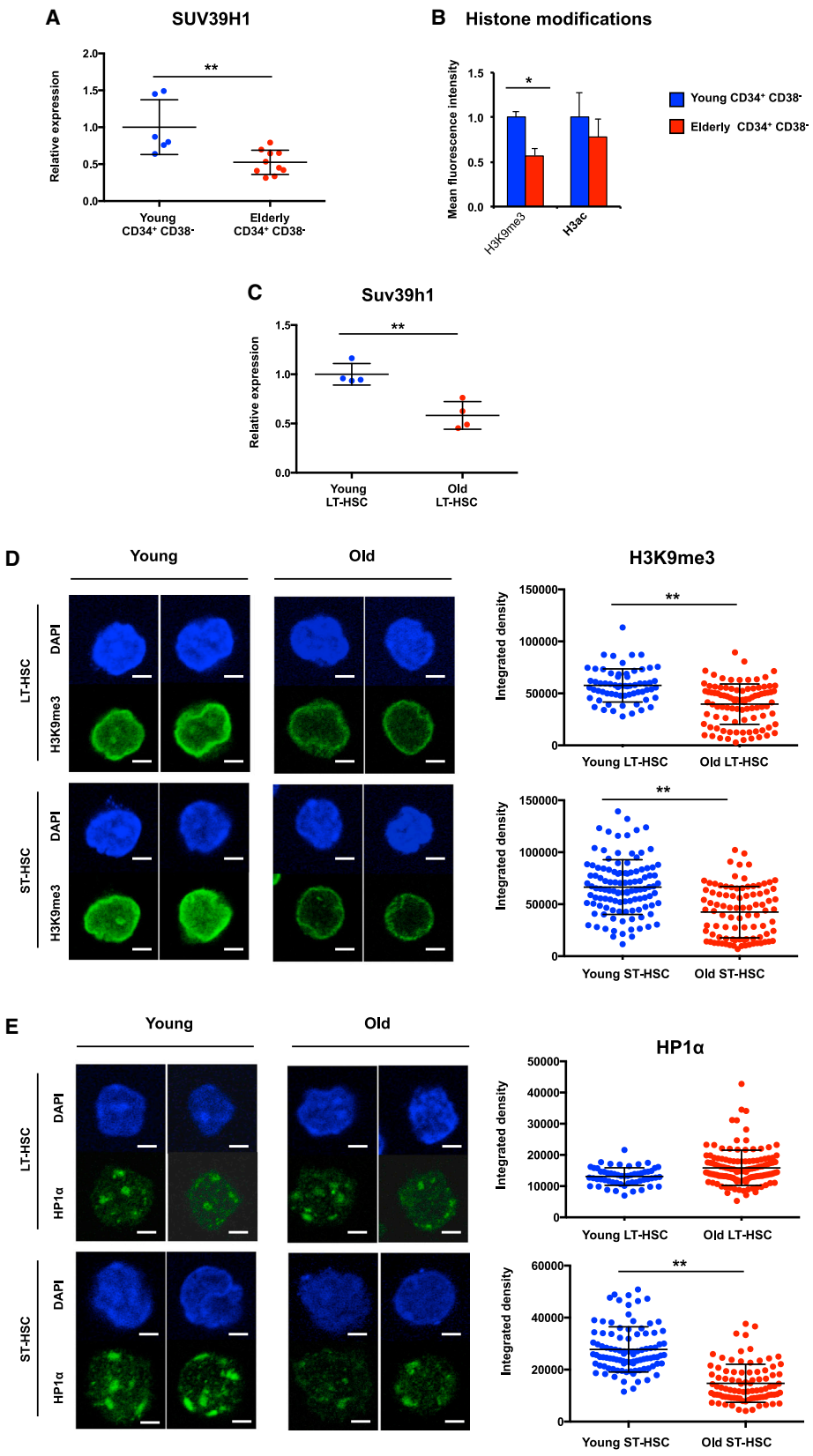
(B) FACS analysis of histone H3K9me3 and H3ac in $CD34^+CD38^-$ HSC following treatment of $CD34^+$ cells with 10 μM chaetocin or carrier (DMSO) for 2 hr (mean + SD; $n \geq 4$). Results are normalized with respect to the mean fluorescence intensity of control-treated cells.

(C) In vitro differentiation of control or chaetocin-treated $CD34^+$ cells. B cell differentiation was measured as the percentage of wells containing B lymphocytes ($CD45^+CD19^+CD33^-CD34^-$) 4 weeks after plating limiting numbers of $CD34^+$ cells on MS5 stromal layers. Myeloid differentiation is expressed as the total number of colonies observed after 10 days in methylcellulose cultures. Results are normalized with respect to untreated cells (mean + SEM; $n \geq 3$).

(D) In vitro differentiation of $CD34^+$ cells transfected with SUV39H1 siRNA or non-targeting control. B lymphoid and myeloid differentiation was assessed as described in (C) (mean + SEM; $n \geq 3$).

*$p < 0.05$; **$p < 0.01$. See also Figures S1 and S2.

bone marrow of elderly individuals (>70 years) relative to the level observed in the same population from young individuals (<35 years) (Figures 2A, S3A, and S3B). In contrast to *SUV39H1*, expression of *SETDB1/KMT1E*, a methyltransferase which also catalyzes H3K9me3 (Schultz et al., 2002) did not change with age in HSC (Figure S3B). In order to investigate whether the observed decrease of SUV39H1 affected global levels of H3K9me3, we measured this modification by intracellular FACS in $CD34^+38^-$ HSC isolated from young and old individuals. We found that aging was associated with a reduction in the levels of H3K9me3, while no change was observed in the global levels of H3ac (Figure 2B). Consistent with these findings in human HSCs, we observed a similar decline with age in the expression of *Suv39h1* in both the mouse $Lin^-SCA1^+c\text{-}KIT^+$ (LSK) population of hematopoietic stem and progenitor cells, as well as in the more primitive LT-HSC ($Lin^-SCA1^+c\text{-}KIT^+FLT3^-CD34^-CD150^+$) (Figures 2C and S3C). Within the murine HSC compartment, reduced expression of *Suv39h1* was associated with a decrease in the global levels of H3K9me3 as assessed by immunofluorescence in LT-HSC and short-term repopulating HSC (ST-HSC; $Lin^-SCA1^+c\text{-}KIT^+FLT3^-CD34^+$) (Figure 2D). In ST-HSC, where a higher proportion of cells have exited quiescence (Wilson et al., 2008), we also observed a decrease with age in the level of the heterochromatin-binding protein HP1α, suggesting that downstream



(legend on next page)

changes in chromatin are more strongly detected once the stem cells start cycling (Figure 2E). These results demonstrate that aging is associated with reduced SUV39H1 expression and decreased levels of H3K9me3 in the HSC compartment, indicative of perturbed heterochromatin.

Decreased Expression of SUV39H1 in HSC Results in Derepression of Genomic Repeats with Age

To investigate the effects of these age-associated changes on heterochromatin function in HSC, we analyzed the expression of a number of genomic repetitive elements which are normally repressed by the H3K9me3/HP1 heterochromatic axis (Sharma et al., 2012). In human HSC, aging was associated with increased expression of several of these repeat elements, including Satellite II tandem pericentromeric repeats as well as certain dispersed human endogenous retrovirus elements (*HERV-K/env102* and *HERV-H/env62*) (Figure 3A). Consistent with these results, we found that several mouse endogenous retroviruses and interspersed repeats previously shown to be controlled by Suv39h-mediated H3K9me3 in embryonic stem cells (particularly active LINE1 family members *L1_A* and *L1_Tf* as well as intracisternal A-type particles [*IAP*]) (Bulut-Karslioglu et al., 2014) were also upregulated with age in murine LSK cells (Figure 3B). The transcriptional derepression of interspersed repeats has also been reported previously in LT-HSC of old mice (Sun et al., 2014). Similar to these changes observed with age, expression of *LINE1* and *IAP* elements was also upregulated in LSK cells of Suv39h1^{null} mice (Figures 3C and S4A), indicating that SUV39H1-mediated H3K9me3 is indeed required for the normal transcriptional repression of these repeats in the HSC compartment. Consistent with previous observations, no significant differences were observed between the B cell compartments of Suv39h1^{null} and wild-type (WT) mice under homeostatic conditions (data not shown and Allan et al., 2012; Braig et al., 2005; Peters et al., 2001). However, competitive transplantation experiments revealed that Suv39h1^{null} bone marrow progenitors exhibited a myeloid-biased engraftment potential, a characteristic of aged HSC (Figure S4B). Interestingly, Suv39h1^{null} LSK cells also showed elevated expression of the *Selp* gene encoding P-SELECTIN, a hallmark gene whose expression is increased with age in both human (Pang et al., 2011) and mouse HSC (Flach et al., 2014; Rossi et al., 2005; Sun et al., 2014) (Figure S4C). These results support the idea that the age-associated decrease in expression of SUV39H1 in HSC leads to relaxation of heterochromatin structure and to transcriptional derepression, including upregulation of certain genomic repeat elements.

miR125b Increases with Age and Targets SUV39H1 in HSC

We next investigated the mechanism responsible for the age-associated decline in expression of *SUV39H1*. miRNAs are known to play important roles in controlling hematopoietic differentiation programs (Baltimore et al., 2008) and have more recently been implicated in HSC aging (Mehta et al., 2015). We used miRWalk2.0 (Dweep et al., 2011) to search for miRNAs that are predicted to target the human *SUV39H1* transcript. Among the most significant hits were members of the miR-125 family (miR-125a and miR-125b), which are predicted to bind to a perfect 8-mer target site that has been evolutionarily conserved within the 3′ UTR of the *SUV39H1* transcript (Figure 4A). This was of particular interest, since members of this family have been shown to be critical regulators of HSC self-renewal and differentiation potential in mice (Guo et al., 2010; O'Connell et al., 2010; Ooi et al., 2010). Notably, overexpression of miR-125 has been reported to lead to a myeloid-biased differentiation of HSC, similar to that observed with age (Gerrits et al., 2012; Guo et al., 2010; O'Connell et al., 2010; Wojtowicz et al., 2014). The miR-125 family consists of three mature miRNAs (miR-125b-1, miR-125b-2, and miR-125a), which

Figure 2. Age-Related Decrease in SUV39H1 Expression and Histone H3K9me3 Levels in Human and Mouse HSC

(A) qRT-PCR analysis of the expression of *SUV39H1* in CD34^{+}CD38^{-} HSC pooled from bone marrows of young (<35 years) and elderly (>70 years) individuals. Expression relative to *ACTB* was normalized to the mean value observed in HSC from young individuals (mean ± SD; n ≥ 6, with each sample comprising a pool of two to four individuals).

(B) FACS analysis of H3K9me3 and H3ac in CD34^{+}CD38^{-} HSC isolated from young (<35 years) and elderly (>70 years) individuals (mean + SD; n ≥ 4).

(C) qRT-PCR analysis of the expression of *Suv39h1* in LT-HSC isolated from bone marrows of young (2–3 months) and old (20–22 months) C57BL/6 mice. Expression relative to *Actb* was normalized to the mean value observed in young mice (mean ± SD; n ≥ 4, with each sample comprising a pool of four young or old mice).

(D and E) Immunofluorescence analysis of H3K9me3 (D) and HP1α (E) in LT-HSC and ST-HSC isolated from bone marrows of young and old mice. The left panel shows representative images (scale bars, 2 μm) and the graphs in the right panel show background-corrected total cell fluorescence (integrated density) of a minimum of 60 cells (bars indicate mean ± SD). The result is a representative of two independent experiments.

*p < 0.05; **p < 0.01. See also Figure S3.

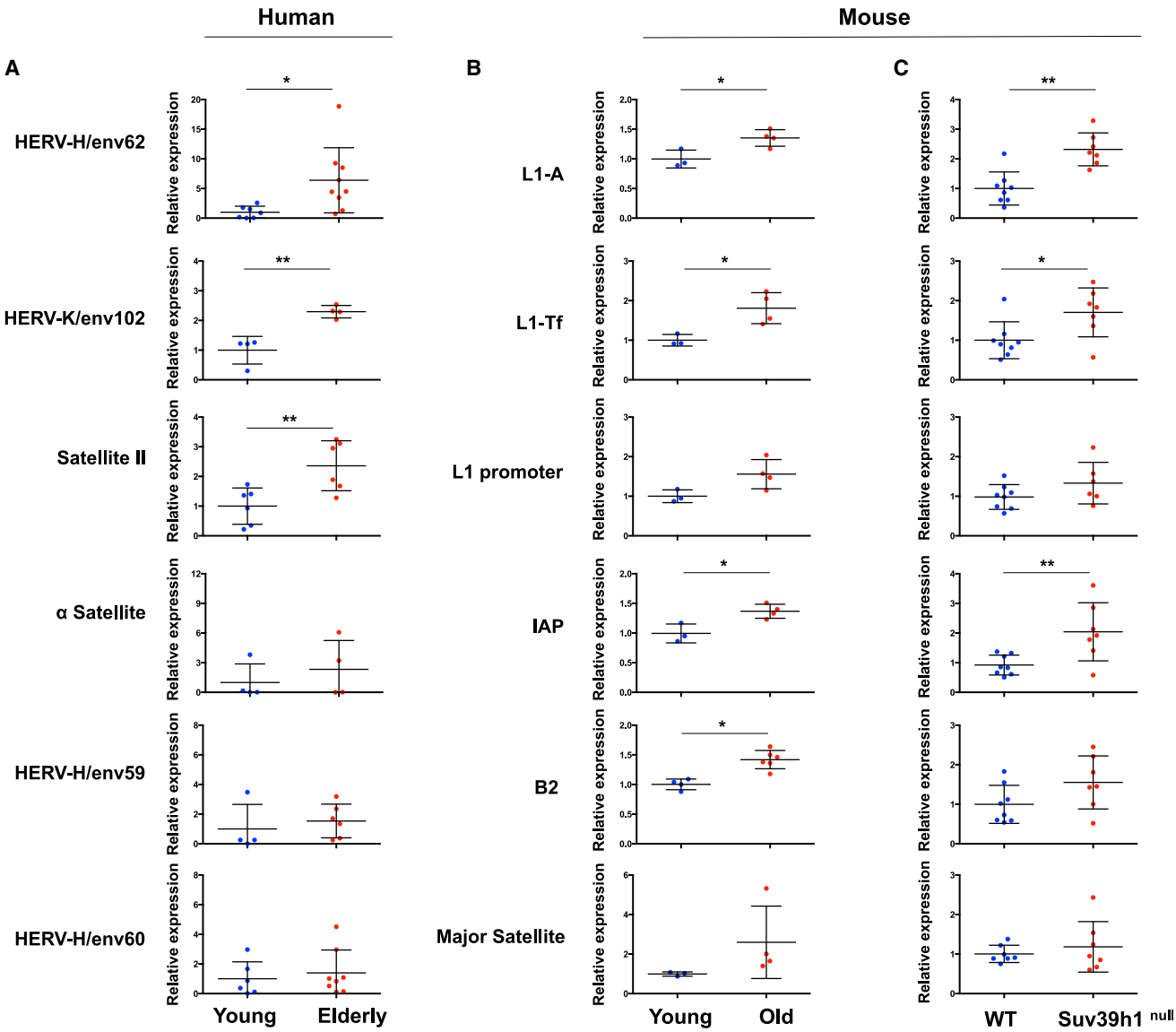


Figure 3. Expression of Genomic Repeat Elements in HSC Is Upregulated with Age and in the Absence of SUV39H1

(A) qRT-PCR analysis of the expression of interspersed (*HERV-H/env62*, *HERV-K/env102*, *HERV-H/env59*, *HERV-H/env60*) and pericentromeric (*Satellite II*, *α Satellite*) repetitive elements in $CD34^{+}CD38^{-}$ HSC from young (<35 years) and elderly (>70 years) individuals. For each repeat, expression relative to *RPLP0* has been normalized to the mean value observed in young individuals (mean ± SD; $n \geq 4$).

(B) qRT-PCR analysis of the expression of *LINE1* (L1-A, L1-Tf, and L1-prom), *IAP* and *SINE B2* (B2) elements and major satellite repeats in LSK cells isolated from bone marrows of young (2–3 months) and old (20–22 months) C57BL/6 mice. Expression of each repeat relative to *Actb* has been normalized to the mean value observed in LSK of young mice (mean ± SD; $n \geq 3$ individual mice).

(C) qRT-PCR analysis of the same genomic repeats as in (B) in LSK cells isolated from bone marrows of $Suv39h1^{null}$ mice (n = 7) and WT littermates (n = 8) (aged 4–6 months). Expression of each repeat relative to *Actb* has been normalized to the mean value observed in LSK of WT mice (mean ± SD).

*p < 0.05; **p < 0.01. See also Figure S4.

share an identical seed sequence and exert similar effects on HSC function (Wojtowicz et al., 2014). We found that the level of mature miR-125b is significantly increased in $CD34^{+}CD38^{-}$ HSC from elderly (>70 years) as compared with young (<35 years) individuals, a result that was confirmed with two independent real-time PCR assays (Figures 4B and S5A). We also observed a significant increase in the immature primary miRNA transcript pri-miR-125b-2, but not pri-miR-125b-1 (Figures 4B and S5B). An increase was also observed in the levels of mature

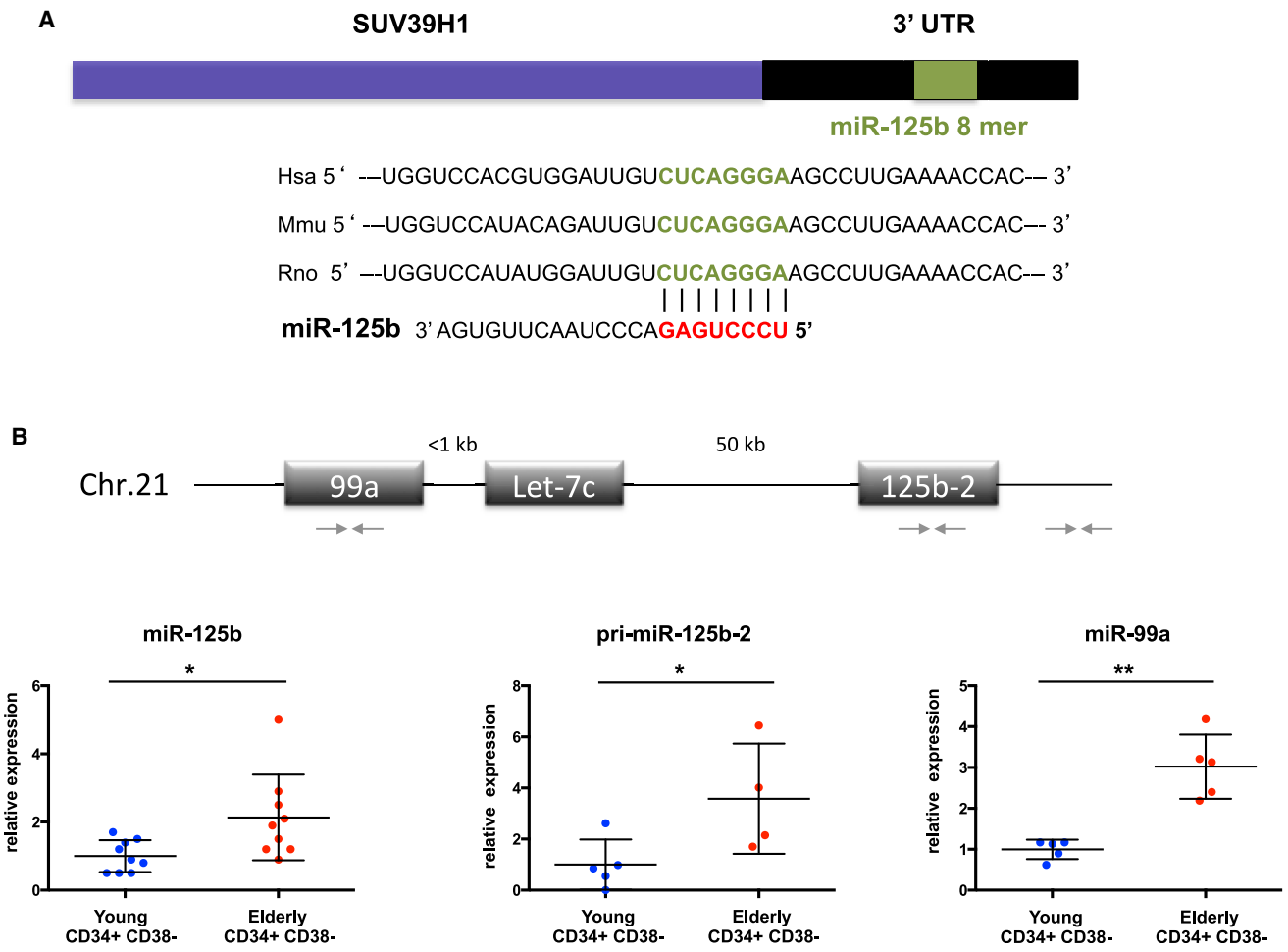


Figure 4. Expression of miR-125b Increases with Age in Human HSC

(A) Schematic of the 3′ UTR of the *SUV39H1* mRNA in human (Hsa), mouse (Mmu), and rat (Rno), showing the conserved miR-125 target sequence (green). Below is shown the sequence of the mature miR-125b (adapted from www.targetscan.org).

(B) Top: Genomic organization (not to scale) of the miRNA cluster containing miR-125b-2 (human chromosome 21q21). The positions of the amplicons used to detect mature miR-99a, mature miR-125b, and pri-miR-125b-2 are indicated. Bottom: qRT-PCR analysis of mature miR-125b (left panel), pri-miR-125b-2 (middle panel), and mature miR-99a (right panel) in $CD34^{+}CD38^{-}$ HSC isolated from bone marrows of young (<35 years) and elderly (>70 years) individuals. Expression of mature miR-125b and −99a (relative to *U6*) and pri-miR-125b-2 (relative to *ACTB*) was normalized to the mean value observed in HSC from young individuals (mean ± SD; $n \geq 5$).

*p < 0.05; **p < 0.01. See also Figure S5.

miR-99a, which is part of a tricistronic cassette with miR-125b-2 and let-7c on human chromosome 21 (Emmrich et al., 2014), suggesting that the entire miR-125b-2 tricistronic cluster is upregulated with age in human HSC (Figure 4B). We also observed an age-associated increase in the level of mature miR-125a, although this miRNA appears to be expressed at lower levels in $CD34^{+}CD38^{-}$ HSC (Figures S5C and S5D). These findings, therefore, reveal an age-associated increase in the level of miR-125 miRNA in human HSC.

To test whether SUV39H1 is indeed a target of miR-125, we initially ectopically expressed miR-125b in HeLa cells. Transfection of HeLa cells with a miR-125b mimic, a double-stranded RNA oligonucleotide mimicking the mature miRNA, resulted in downregulation of SUV39H1 mRNA and protein (Figures 5A–5C). We also observed reduced *SUV39H1* expression and decreased enrichment of H3K9me3 at pericentromeric (SAT II) and dispersed (HERV/env102) genomic repeats following lentiviral-mediated expression of miR-125b in HeLa cells (Figures 5D–5F). Consistent with these findings, SUV39H1 has also been shown to be regulated by miR-125b in murine muscle cells (Villeneuve et al., 2010) and human hepatocellular carcinomas (Fan et al., 2013). To investigate

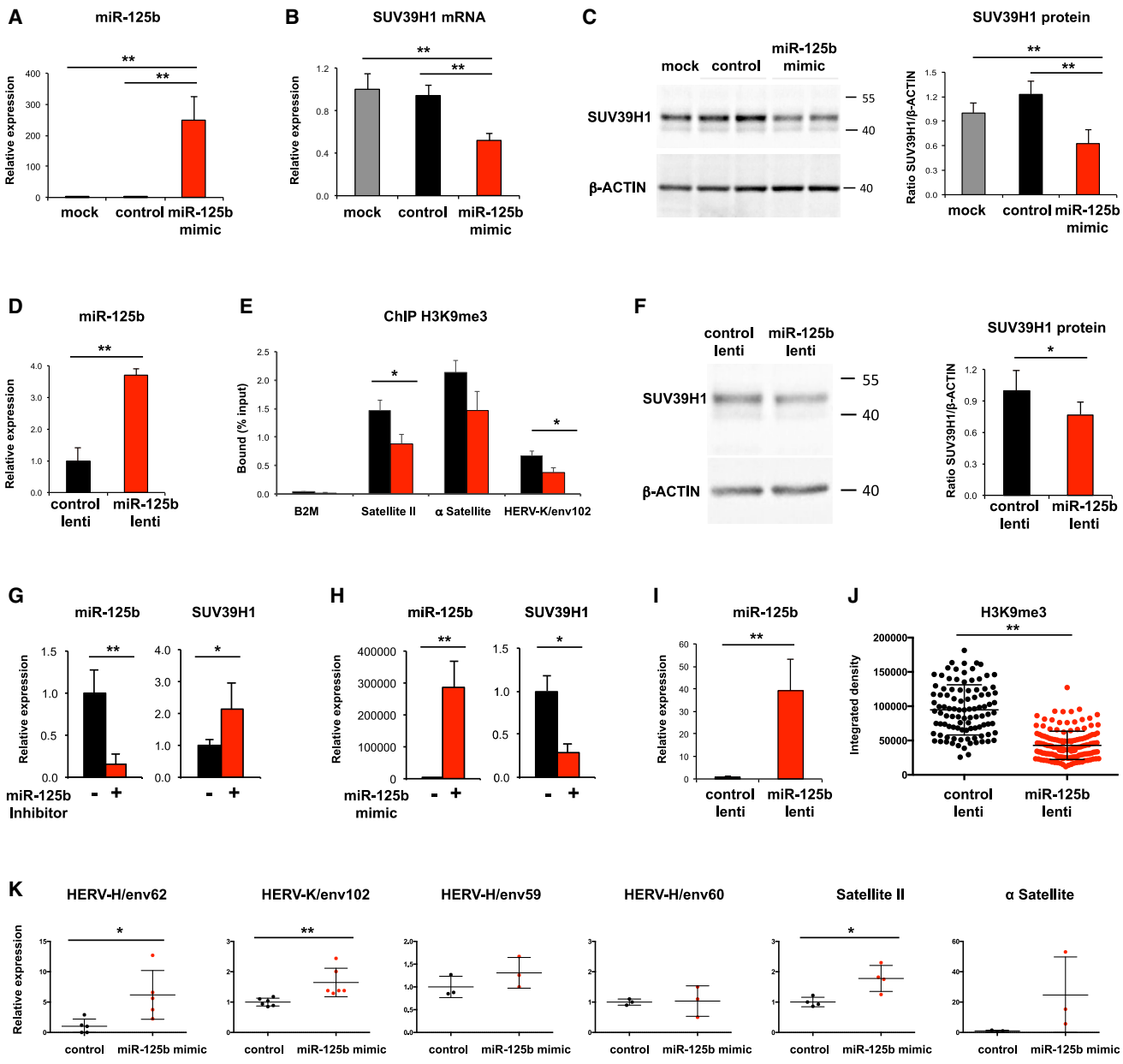


Figure 5. SUV39H1 Is a Target of miR-125b

(A–C) HeLa cells were either mock transfected (gray) or transfected with control mimic (black) or miR-125b mimic (red) for 30 hr. qRT-PCR analysis of mature miR-125b (A) and *SUV39H1* mRNA (B) (mean + SD; n = 4). (C) Western blot analysis of SUV39H1 protein in whole-cell extracts. Left: representative blot. Right: Quantification of SUV39H1 protein relative to β-ACTIN and normalized to the mean value observed in mock-transfected cells (mean + SD; $n \geq 5$).

(D–F) HeLa cells were transduced with a control empty lentiviral vector (expressing mCherry alone, black) or a vector expressing miR-125b and mCherry (red) for 5–6 days. (D) qRT-PCR analysis of mature miR-125b in mCherry$^+$ HeLa cells (mean + SD; n = 3). (E) ChIP experiments with anti-H3K9me3 antibody in mCherry$^+$ HeLa cells. Enrichment (bound as a percentage of input) was measured by qPCR at Satellite II and α Satellite tandem repeats, at HERV-K/env102, and at the promoter of the constitutively expressed β2 microglobulin gene (B2M) as negative control (mean + SEM; n = 6). (F) Western blot analysis of SUV39H1 protein in whole-cell extracts from mCherry$^+$ HeLa cells. Left: Representative blot. Right: Quantification of SUV39H1 protein relative to β-ACTIN and normalized to the mean value observed in control-transduced cells (mean + SD; n = 6).

(G and H) qRT-PCR analysis of the expression of *SUV39H1* and mature miR-125b in (G) CD34$^+$CD38$^-$ HSC from elderly individuals (>70 years) treated with miR-125b inhibitor for 24 hr or (H) in cord blood CD34$^+$CD38$^-$ HSC treated with miR-125b mimic for 24 hr (mean + SD; $n \geq 4$).

(legend continued on next page)

whether miR-125b also regulates SUV39H1 in HSC, $CD34^+CD38^-$ HSC from elderly individuals were treated with a miR-125b inhibitor, a chemically modified single-stranded oligonucleotide that interacts with mature miR-125b. Treatment of aged HSC with the inhibitor resulted in upregulation of *SUV39H1* transcripts indicating that *SUV39H1* is a target of miR-125b in human HSC (Figure 5G). This was confirmed in a complementary set of experiments where cord blood $CD34^+CD38^-$ HSC, which express high levels of *SUV39H1* (Figure S1A), were transfected with the miR-125b mimic. After 24 hr with the miR-125b mimic, expression of *SUV39H1* was significantly depleted (Figure 5H). Furthermore, overexpression of miR-125b in $CD34^+CD38^-$ HSC led to a decrease in levels of H3K9me3 (Figures 5I and 5J), as well as increased expression of a subset of genomic repeats (Figure 5K), indicating a disruption of the normally repressive heterochromatin structure at these elements. Interestingly, the repeat elements that responded to the miR-125b mimic (*HERV-H/env62* and *HERV-K/env102* and *Satellite II*) were the same as those whose expression increased with age in human HSC (Figure 3A). These results demonstrate that SUV39H1 is targeted by miR-125b in HSC and that the levels of this miRNA impact upon HSC heterochromatin.

miR125b Regulates the B Cell Potential of Human HSC

The above experiments indicate that overexpression of mir-125b causes similar changes in heterochromatin as those occurring in aged HSC. To examine the effects of miR-125b on the differentiation potential of human HSC, we initially transduced cord blood $CD34^+CD38^-$ HSC with control or miR-125b-expressing lentiviruses, and the cells were plated in in vitro differentiation cultures to assess B lymphoid and myeloid output. Overexpression of miR-125b resulted in a significant decrease in the generation of $CD19^+$ B cells and an increase in $CD33^+$ myeloid cells (Figures 6A and 6B), resulting in an increased ratio of myeloid to B lymphoid progeny in the assay (Figure 6C). No changes in differentiation were observed in the untransduced population (Figures S6A and S6B). These experiments indicate that, as with age, overexpression of miR-125b in human HSC leads to decreased B lymphopoiesis and a myeloid-biased differentiation output.

Inhibition of miR-125 or Overexpression of SUV39H1 Enhances B Lymphoid Differentiation Capacity of Aged HSC

Finally we investigated whether inhibition of miR-125b or overexpression of SUV39H1 in HSC from elderly individuals improved their capacity to generate B lymphocytes. We first transduced $CD34^+CD38^-$ HSC purified from the bone marrow of elderly individuals (>70 years) with a miR-125 "sponge" lentivirus, which expresses a transcript containing four target sites for miR-125, or a control lentivirus containing four scrambled target sites. In vitro differentiation analysis of transduced cells revealed that expression of the miR-125 sponge in aged HSC resulted in a significant increase in the output of $CD19^+$ B cells and a reduction in the myeloid:lymphoid ratio (Figures 7A and 7B). No changes were observed in the untransduced population (Figures S6C and S6D). We next examined the effect of SUV39H1 overexpression on the differentiation potential of aged HSC. For these experiments, we generated a lentiviral construct expressing human SUV39H1 together with GFP. This lentivirus restored the WT concentration of H3K9me3 at pericentromeric heterochromatin when transduced into mouse embryonic fibroblasts lacking SUV39H1/2 (Figure S7). $CD34^+CD38^-$ HSC isolated from the bone marrow of elderly individuals were transduced with the empty control (expressing GFP alone) or SUV39H1 lentivirus, then differentiated in vitro on MS5 stromal cells as above. Similar to the results obtained following inhibition of miR-125, expression of SUV39H1 in aged HSC increased the generation of $CD19^+$ B cells and resulted in an overall decrease in the myeloid:lymphoid differentiation output (Figures 7C and 7D). Again, no changes were observed in untransduced cells (Figures S6E and S6F). These experiments show that increasing the expression of SUV39H1 or inhibition of miR-125 enhances the capacity of HSC from elderly individuals to generate B cells and attenuates the age-associated myeloid bias. These findings support the idea that repression of SUV39H1 by miR-125 and perturbed heterochromatin regulation are important factors contributing to the decline in B cell potential of HSC with age.

(I–J) Cord blood $CD34^+CD38^-$ HSC were transduced with the control (black) or miR-125b (red) lentiviral vector for 72 hr. (I) qRT-PCR analysis of mature miR-125b in $mCherry^+$ cells (mean + SD; n = 6). (J) Immunofluorescence analysis of H3K9me3 in $mCherry^+$ cells. Results show total cell fluorescence (integrated density) of a minimum of 100 cells (mean ± SD from two independent experiments).

(K) qRT-PCR analysis of the expression of human endogenous retroviral repeats (*HERV-H/env62*, *HERV-K/env102*, *HERV-H/env59*, *HERV-H/env60*) and pericentromeric repeats (*Satellite II* and *α Satellite*) in cord blood $CD34^+CD38^-$ HSC treated with miR-125b mimic or control for 24 hr. For each repeat, expression relative to *RPLP0* has been normalized to the mean value observed in control (mean ± SD; $n \geq 3$).

qRT-PCR results in (A), (B), (D), (G), (H), and (I) are expressed relative to *U6* for miR-125b or *ACTB* for *SUV39H1* and are normalized to the mean value observed in control cells. *$p < 0.05$; **$p < 0.01$.

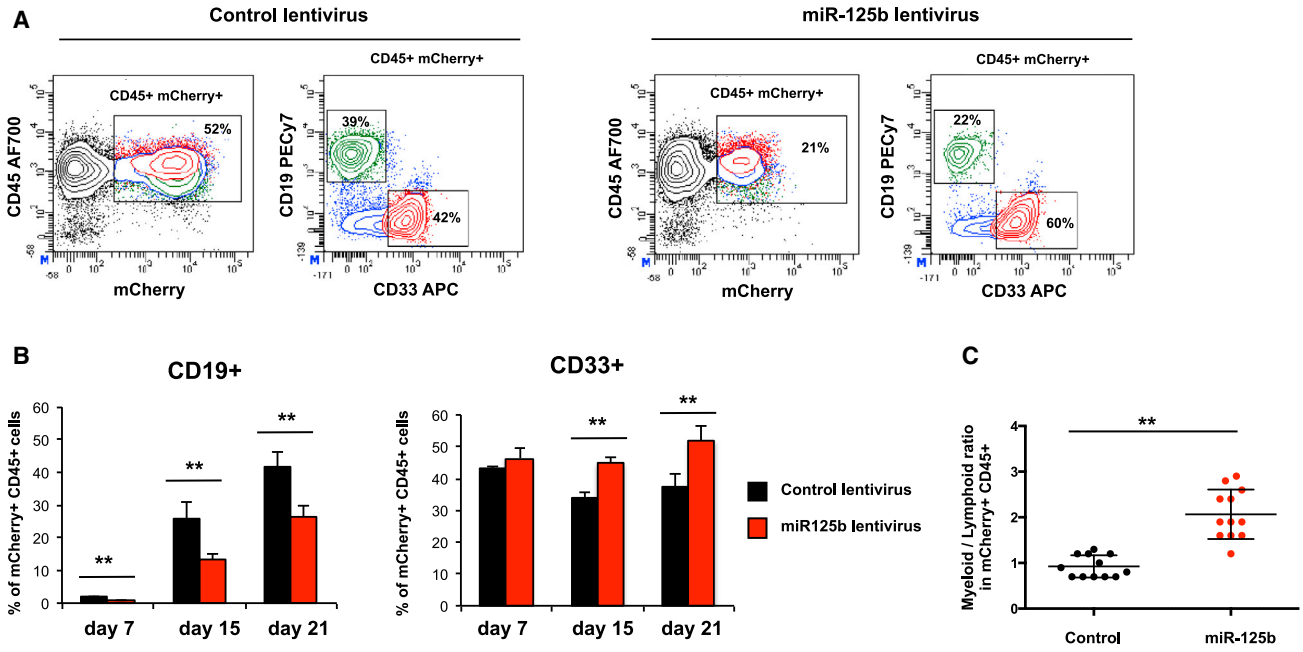


Figure 6. Expression of miR-125b Decreases B Cell Output in Human HSC

(A) Gating strategy and representative flow cytometric profile of progeny derived from cord blood $CD34^{+}CD38^{-}$ HSC transduced with an empty control lentivirus (expressing mCherry alone) or a lentivirus expressing miR-125b and mCherry and differentiated for 21 days on MS5 stromal cells. Cells were gated initially on expression of hCD45 and mCherry (expressed as a percentage of total live cells). B cells ($CD19^{+}$) and myeloid cells ($CD33^{+}$) are expressed as a percentage of cells within the $CD45^{+}mCherry^{+}$ gate.

(B) Quantification of the populations shown in (A) at day 7, 15, and 21 of differentiation (mean + SD; n = 6 [day 7] or 12 [days 15 and 21] from two independent experiments).

(C) Ratio of myeloid to B lymphoid cells within the $mCherry^{+}CD45^{+}$ population at day 21 of differentiation (mean ± SD; n = 12 from two independent experiments).

**p < 0.01. See also Figures S6A and S6B.

DISCUSSION

Loss of heterochromatin structure and its associated histone mark H3K9me3 have been observed during aging in many cell types and diverse organisms, leading to the proposal that a decline in heterochromatin integrity may be a common feature of aging (Tsurumi and Li, 2012). That age-associated decline of heterochromatin is likely to be functionally important in driving the aging phenotype in mammals is strongly supported by the observation that global loss of heterochromatin markers is a characteristic feature of the human premature aging disorders, Hutchinson-Gilford progeria and Werner syndromes (Shumaker et al., 2006; Zhang et al., 2015). Furthermore, in model organisms, it has been shown that the heterochromatin protein HP1α and the regulation of heterochromatin formation control life span (Larson et al., 2012). Heterochromatin has recently been shown to play a key role in the regulation of HSC differentiation (Ugarte et al., 2015); however, little was known about the contribution of H3K9me3 and heterochromatin structure to HSC aging. In this study, we show that expression of SUV39H1, a critical factor in heterochromatin formation, decreases with age in both human and mouse HSC, leading to widespread changes in HSC heterochromatin state, and contributing to the decline in B cell potential observed with advancing age. Consistent with our observations in HSC, reduced expression of SUV39H1 and a decrease in global H3K9me3 have also been observed recently during physiological aging both in human mesenchymal stem cells and in immune organs of the rat (Sidler et al., 2013; Zhang et al., 2015). Overall, our observations of decreased expression of SUV39H1 and perturbed HSC heterochromatin with age support recent studies implicating epigenetic change as an important driver of the decline in HSC function during aging (Beerman et al., 2013; Sun et al., 2014).

At present the molecular pathways affected by the decrease in SUV39H1 and heterochromatin, which contribute to the age-associated changes in HSC function, are not known. As a critical factor in the establishment of heterochromatin, SUV39H1 plays an important role in the maintenance of genome stability. Indeed, hotspots of

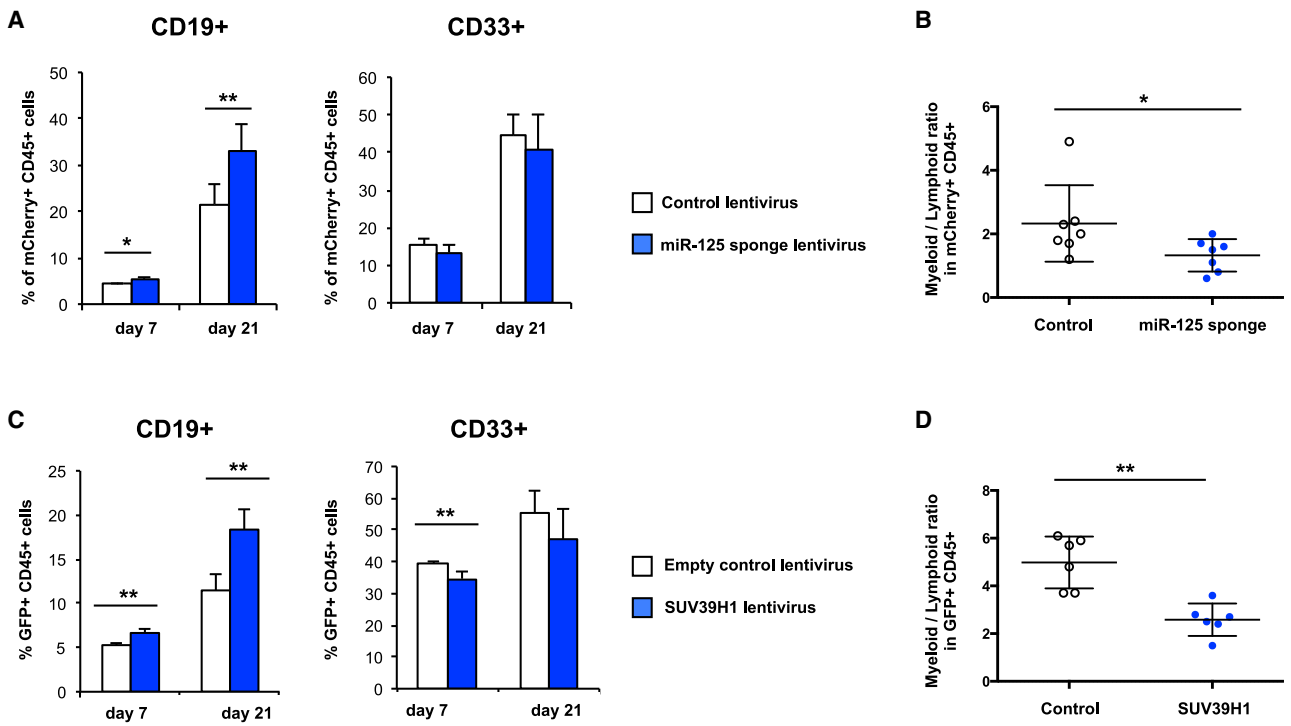


Figure 7. Expression of a miR-125 Sponge and SUV39H1 in Aged HSC Increases B Cell Output and Alleviates Age-Associated Myeloid Bias

(A) Quantification by FACS of B cells ($CD19^+$) and myeloid cells ($CD33^+$) within the $mCherry^+CD45^+$ population at day 7 and 21 of differentiation of $CD34^+CD38^-$ HSC from elderly individuals transduced with the control lentivirus or lentivirus expressing a miR-125 sponge (mean + SD; n = 5 [day 7] and n = 7 [day 21] from three independent experiments).

(B) Ratio of myeloid to B lymphoid cells within the $mCherry^+CD45^+$ population at day 21 of the differentiation in (A) (mean ± SD; n = 7 from three independent experiments).

(C) Quantification by FACS of B cells ($CD19^+$) and myeloid cells ($CD33^+$) within the GFP^+CD45^+ population at day 7 and 21 of differentiation of $CD34^+CD38^-$ HSC from elderly individuals transduced with empty control- or SUV39H1-lentivirus (mean + SD; n = 6 from three independent experiments).

(D) Ratio of myeloid to B lymphoid cells within the GFP^+CD45^+ population at day 21 of the differentiation in (C) (mean ± SD; n = 6 from three independent experiments). For all panels, FACS gating and quantification were carried out as in Figure 6.

*$p < 0.05$; **$p < 0.01$. See also Figures S6C–S6F and S7.

chromosomal translocation breakpoints associated with hematological malignancy are characterized by depleted levels of H3K9me3 in human $CD34^+$ cells, and SUV39H1-mediated deposition of this histone mark has been shown to directly protect against the formation of DNA double-strand breaks (Burman et al., 2015). Thus, the age-associated decline in SUV39H1 shown here could contribute to the accumulation of double-strand breaks observed in HSC from elderly individuals and predispose hematopoietic progenitors to increased translocations and malignant transformation (Rube et al., 2011; Yahata et al., 2011). In this study, we also observed a derepression of repeat and retroviral elements, which could also contribute to genomic instability (Belgnaoui et al., 2006; Kines et al., 2014), and indeed a breakdown in the normal transcriptional repression of retrotransposable elements is emerging as a common characteristic of aging (Gorbunova et al., 2014). Interestingly, recent evidence suggests that B cell differentiation is particularly sensitive to transcriptional derepression of endogenous retroviruses (Collins et al., 2015). Activated genomic repeats can impact upon the expression of neighboring genes through the formation of chimeric transcripts (Cruickshanks and Tufarelli, 2009; Nigumann et al., 2002). Indeed enhanced expression of genes proximal to transcriptionally reactivated repeat elements have been reported both in murine HSC (Sun et al., 2014) and proB (Collins et al., 2015) cells, indicating that derepression of repeat elements can lead to dysregulation of gene-expression programs. Finally, reduced expression of SUV39H1 could also contribute directly to the widespread transcriptomic changes that have been observed in HSC with age (Pang et al., 2011; Rossi et al., 2005; Sun et al., 2014) by the upregulation of genes

normally silenced by facultative heterochromatin. This idea is supported by our findings that the expression of *Selp*, one of the most strongly upregulated genes with age in HSC, is also increased in the hematopoietic progenitors of Suv39h1^{null} mice.

In this study, we further show that expression of SUV39H1 in HSC is controlled by the highly conserved miR-125 miRNA family. While previously shown to be important regulators of HSC self-renewal and differentiation potential (O'Connell et al., 2010; Ooi et al., 2010), the contribution of miR-125 members to age-associated changes in the hematopoietic system had not previously been investigated. We found that the expression of miR-125b is increased with age in HSC and that inhibition of miR-125b leads to increased SUV39H1 expression and attenuates the myeloid-biased differentiation potential of HSC from elderly individuals. miRNAs play an important role in the regulation of lifespan and aging in model organisms and a growing body of evidence indicates that they may also act as regulators of aging pathways in mammalian tissues (reviewed in Smith-Vikos and Slack, 2012). Indeed a recent study has shown that the miR-212/132 cluster is upregulated with age in mouse HSC and that this miRNA can affect HSC function with age via the regulation of the transcription factor FOXO3 (Mehta et al., 2015). As well as impacting on expression of SUV39H1, overexpression of the miR-125b-2 tricistron in HSC has been shown to suppress signaling through the transforming growth factor β (TGFβ) pathway (Emmrich et al., 2014). TGFβ signaling is a critical regulator of HSC homeostasis and quiescence (Blank and Karlsson, 2015) and, interestingly, reduced TGFβ signaling is a prominent feature of aging in mouse HSC (Sun et al., 2014). As well as playing an important role during normal hematopoiesis, miR-125 has also been implicated as an oncomir in a number of myeloid and lymphoid leukemias (Shaham et al., 2012). Thus, the age-associated increase in miR-125 observed in this study may also contribute to the enhanced susceptibility to hematological malignancies observed in the elderly (Sant et al., 2010). At present, the mechanism driving upregulation of miR-125 with age in HSC is not known and will require further investigation. Interestingly, HSC aging is associated with increased levels of both homeobox factors (Pang et al., 2011; Sun et al., 2014) and nuclear NF-κB (Chambers et al., 2007), two transcription factors that have been shown to be activators of miR-125b expression (Emmrich et al., 2014; Tan et al., 2012).

In summary, our study has characterized an axis in which age-associated increase in expression of miR-125b contributes to a decline in the B cell potential of human HSC via an effect on SUV39H1 and HSC heterochromatin. These findings add to a growing body of evidence demonstrating that epigenetic change, and in particular a disruption of heterochromatin integrity, are a general feature of age-associated tissue decline. The implication of miR-125 and SUV39H1 in the aging HSC phenotype presents the possibility of using miR antagonists and epigenetic modifiers as potential therapeutic approaches to address the decline in immune function observed in the elderly.

EXPERIMENTAL PROCEDURES

Samples

Cord blood and human bone marrow samples from young (18–35 years) and elderly (70–98 years) individuals were obtained from Hôpital Saint Louis and Hôpital Lariboisière, Paris. Young (2–3 months) and old (20–24 months) C57BL/6 male mice were purchased from Janvier. Suv39h1^{null} mice (Peters et al., 2001) were obtained from S. Amigorena (Institute Curie). Details of cell labeling, FACS sorting, and analysis are described in the Supplemental Experimental Procedures.

In Vitro Differentiation Assays

B lymphoid and myeloid differentiation assays were performed as described in Kuranda et al. (2011) and in the Supplemental Experimental Procedures.

Immunofluorescence Staining

Immunofluorescence staining of human and murine HSC was performed with anti-H3K9me3 (07-523, Millipore) or anti-HP1α (MAB3584, Millipore) antibodies as described in the Supplemental Experimental Procedures.

Statistics

All data are expressed as means and SD except where stated otherwise. All statistical significance of differences was measured by unpaired two-tailed Student's t test (*$p < 0.05$; **$p < 0.01$). Unless stated otherwise, n indicates the number of independent experiments performed on HSC isolated from different pools of two to four individual mouse or human bone marrow samples.

Study Approval

Ethical approval for the use of human samples was granted by Institutional Review Board number 00006477 of Paris North Hospitals (approval no. 13-052) and samples were obtained after informed consent. All animal experiments were performed at the animal facility of the Institut Universitaire d'Hématologie (no. B75-10-08) in accordance with the guidelines of the Institutional Animal Care Committee.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, seven figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.stemcr.2016.05.007.

AUTHOR CONTRIBUTIONS

D.D. and D.G. designed and conducted the experiments, analyzed the data, and wrote the manuscript. K.K., I.K., D.B., C.C., and M.P. conducted the experiments. I.N. and F.P. provided reagents, and assisted in the study design and the writing of the paper. J.C.B. and C.D. provided scientific advice and support. V.V. and G.S. provided advice and clinical samples. M.G. designed the study and wrote the manuscript.

ACKNOWLEDGMENTS

We thank Drs T. Jenuwein and S. Amigorena for Suv39h1^{null} mice, Dr C. Muchardt for iMEFs, and Drs H. Raslova and D. Bluteau for the pRRL-EF1α-MCS-PGK-GFP lentiviral vector. We are grateful to Dr D. Bonnet for critical reading of the manuscript. The authors also wish to thank clinicians of the Service Hématologie-Greffe of Hôpital Saint Louis and Chirurgie Orthopédique of Hôpital Lariboisière as well as the Fondation Générale de Santé and members of the Unité de Thérapie Cellulaire, Hôpital Saint Louis for human samples. We are also grateful to the Pole Cytométrie of the IUH for help with FACS sorting, to A. Biffi for help with analysis of Suv39h1^{null} mice, and to L. Renou for preparation of miR-125b lentiviral vectors. M.G. was supported by grants from INSERM, the Agence de la Biomedecine, and the Fondation de France. D.G. was supported by a Marie-Curie Intra-European Fellowship. F.P. was supported by grants from INSERM and the Ligue Contre le Cancer Comité d'Ile de France. D.D. is recipient of a fellowship from the Fondation de France and D.B. is recipient of a fellowship from the Ligue Nationale Contre le Cancer.

Received: December 28, 2015
Revised: May 13, 2016
Accepted: May 16, 2016
Published: June 14, 2016

REFERENCES

Alder, O., Lavial, F., Helness, A., Brookes, E., Pinho, S., Chandrashekran, A., Arnaud, P., Pombo, A., O'Neill, L., and Azuara, V. (2010). Ring1B and Suv39h1 delineate distinct chromatin states at bivalent genes during early mouse lineage commitment. Development *137*, 2483–2492.

Allan, R.S., Zueva, E., Cammas, F., Schreiber, H.A., Masson, V., Belz, G.T., Roche, D., Maison, C., Quivy, J.P., Almouzni, G., and Amigorena, S. (2012). An epigenetic silencing pathway controlling T helper 2 cell lineage commitment. Nature *487*, 249–253.

Baltimore, D., Boldin, M.P., O'Connell, R.M., Rao, D.S., and Taganov, K.D. (2008). MicroRNAs: new regulators of immune cell development and function. Nat. Immunol. *9*, 839–845.

Beerman, I., Bock, C., Garrison, B.S., Smith, Z.D., Gu, H., Meissner, A., and Rossi, D.J. (2013). Proliferation-dependent alterations of the DNA methylation landscape underlie hematopoietic stem cell aging. Cell Stem Cell *12*, 413–425.

Belgnaoui, S.M., Gosden, R.G., Semmes, O.J., and Haoudi, A. (2006). Human LINE-1 retrotransposon induces DNA damage and apoptosis in cancer cells. Cancer Cell Int. *6*, 13.

Blank, U., and Karlsson, S. (2015). TGF-beta signaling in the control of hematopoietic stem cells. Blood *125*, 3542–3550.

Braig, M., Lee, S., Loddenkemper, C., Rudolph, C., Peters, A.H., Schlegelberger, B., Stein, H., Dorken, B., Jenuwein, T., and Schmitt, C.A. (2005). Oncogene-induced senescence as an initial barrier in lymphoma development. Nature *436*, 660–665.

Bulut-Karslioglu, A., De La Rosa-Velazquez, I.A., Ramirez, F., Barenboim, M., Onishi-Seebacher, M., Arand, J., Galan, C., Winter, G.E., Engist, B., Gerle, B., et al. (2014). Suv39h-dependent H3K9me3 marks intact retrotransposons and silences LINE elements in mouse embryonic stem cells. Mol. Cell *55*, 277–290.

Burman, B., Zhang, Z.Z., Pegoraro, G., Lieb, J.D., and Misteli, T. (2015). Histone modifications predispose genome regions to breakage and translocation. Genes Dev. *29*, 1393–1402.

Chambers, S.M., Shaw, C.A., Gatza, C., Fisk, C.J., Donehower, L.A., and Goodell, M.A. (2007). Aging hematopoietic stem cells decline in function and exhibit epigenetic dysregulation. PLoS Biol. *5*, e201.

Collins, P.L., Kyle, K.E., Egawa, T., Shinkai, Y., and Oltz, E.M. (2015). The histone methyltransferase SETDB1 represses endogenous and exogenous retroviruses in B lymphocytes. Proc. Natl. Acad. Sci. USA *112*, 8367–8372.

Cruickshanks, H.A., and Tufarelli, C. (2009). Isolation of cancer-specific chimeric transcripts induced by hypomethylation of the LINE-1 antisense promoter. Genomics *94*, 397–406.

Czvitkovich, S., Sauer, S., Peters, A.H., Deiner, E., Wolf, A., Laible, G., Opravil, S., Beug, H., and Jenuwein, T. (2001). Over-expression of the SUV39H1 histone methyltransferase induces altered proliferation and differentiation in transgenic mice. Mech. Dev. *107*, 141–153.

Denkinger, M.D., Leins, H., Schirmbeck, R., Florian, M.C., and Geiger, H. (2015). HSC aging and senescent immune remodeling. Trends Immunol. *36*, 815–824.

Dweep, H., Sticht, C., Pandey, P., and Gretz, N. (2011). miRWalk–database: prediction of possible miRNA binding sites by "walking" the genes of three genomes. J. Biomed. Inform. *44*, 839–847.

Dykstra, B., Olthof, S., Schreuder, J., Ritsema, M., and de Haan, G. (2011). Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J. Exp. Med. *208*, 2691–2703.

Emmrich, S., Rasche, M., Schoning, J., Reimer, C., Keihani, S., Maroz, A., Xie, Y., Li, Z., Schambach, A., Reinhardt, D., and Klusmann, J.H. (2014). miR-99a/100~125b tricistrons regulate hematopoietic stem and progenitor cell homeostasis by shifting the balance between TGFbeta and Wnt signaling. Genes Dev. *28*, 858–874.

Fan, D.N., Tsang, F.H., Tam, A.H., Au, S.L., Wong, C.C., Wei, L., Lee, J.M., He, X., Ng, I.O., and Wong, C.M. (2013). Histone lysine methyltransferase, suppressor of variegation 3-9 homolog 1, promotes hepatocellular carcinoma progression and is negatively regulated by microRNA-125b. Hepatology *57*, 637–647.

Flach, J., Bakker, S.T., Mohrin, M., Conroy, P.C., Pietras, E.M., Reynaud, D., Alvarez, S., Diolaiti, M.E., Ugarte, F., Forsberg, E.C., et al. (2014). Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. Nature *512*, 198–202.

Garrick, D., Djeghloul, D., Kuranda, K., and Goodhardt, M. (2015). Aging of human haematopoietic stem cells. In Stem Cell Aging: Mechanisms, Consequences, Rejuvenation, H. Geiger, H. Jasper, and M.-C. Florian, eds. (Springer), pp. 127–148.

Geiger, H., de Haan, G., and Florian, M.C. (2013). The ageing haematopoietic stem cell compartment. Nat. Rev. Immunol. *13*, 376–389.

Gerrits, A., Walasek, M.A., Olthof, S., Weersing, E., Ritsema, M., Zwart, E., van Os, R., Bystrykh, L.V., and de Haan, G. (2012). Genetic screen identifies microRNA cluster 99b/let-7e/125a as a regulator of primitive hematopoietic cells. Blood *119*, 377–387.

Gorbunova, V., Boeke, J.D., Helfand, S.L., and Sedivy, J.M. (2014). Human genomics. Sleeping dogs of the genome. Science *346*, 1187–1188.

Greiner, D., Bonaldi, T., Eskeland, R., Roemer, E., and Imhof, A. (2005). Identification of a specific inhibitor of the histone methyltransferase SU(VAR)3-9. Nat. Chem. Biol. *1*, 143–145.

Grewal, S.I., and Jia, S. (2007). Heterochromatin revisited. Nat. Rev. Genet. *8*, 35–46.

Guo, S., Lu, J., Schlanger, R., Zhang, H., Wang, J.Y., Fox, M.C., Purton, L.E., Fleming, H.H., Cobb, B., Merkenschlager, M., et al. (2010). MicroRNA miR-125a controls hematopoietic stem cell number. Proc. Natl. Acad. Sci. USA *107*, 14229–14234.

Kines, K.J., Sokolowski, M., deHaro, D.L., Christian, C.M., and Belancio, V.P. (2014). Potential for genomic instability associated with retrotranspositionally-incompetent L1 loci. Nucleic Acids Res. *42*, 10488–10502.

Kuranda, K., Vargaftig, J., de la Rochere, P., Dosquet, C., Charron, D., Bardin, F., Tonnelle, C., Bonnet, D., and Goodhardt, M. (2011). Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell *10*, 542–546.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature *410*, 116–120.

Larson, K., Yan, S.J., Tsurumi, A., Liu, J., Zhou, J., Gaur, K., Guo, D., Eickbush, T.H., and Li, W.X. (2012). Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis. PLoS Genet. *8*, e1002473.

Lescale, C., Dias, S., Maes, J., Cumano, A., Szabo, P., Charron, D., Weksler, M.E., Dosquet, C., Vieira, P., and Goodhardt, M. (2010). Reduced EBF expression underlies loss of B-cell potential of hematopoietic progenitors with age. Aging Cell *9*, 410–419.

Maison, C., and Almouzni, G. (2004). HP1 and the dynamics of heterochromatin maintenance. Nat. Rev. Mol. Cell Biol. *5*, 296–304.

Mehta, A., Zhao, J.L., Sinha, N., Marinov, G.K., Mann, M., Kowalczyk, M.S., Galimidi, R.P., Du, X., Erikci, E., Regev, A., et al. (2015). The MicroRNA-132 and MicroRNA-212 cluster regulates hematopoietic stem cell maintenance and survival with age by buffering FOXO3 expression. Immunity *42*, 1021–1032.

Nigumann, P., Redik, K., Matlik, K., and Speek, M. (2002). Many human genes are transcribed from the antisense promoter of L1 retrotransposon. Genomics *79*, 628–634.

O'Connell, R.M., Chaudhuri, A.A., Rao, D.S., Gibson, W.S., Balazs, A.B., and Baltimore, D. (2010). MicroRNAs enriched in hematopoietic stem cells differentially regulate long-term hematopoietic output. Proc. Natl. Acad. Sci. USA *107*, 14235–14240.

Ooi, A.G., Sahoo, D., Adorno, M., Wang, Y., Weissman, I.L., and Park, C.Y. (2010). MicroRNA-125b expands hematopoietic stem cells and enriches for the lymphoid-balanced and lymphoid-biased subsets. Proc. Natl. Acad. Sci. USA *107*, 21505–21510.

Pang, W.W., Price, E.A., Sahoo, D., Beerman, I., Maloney, W.J., Rossi, D.J., Schrier, S.L., and Weissman, I.L. (2011). Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc. Natl. Acad. Sci. USA *108*, 20012–20017.

Peters, A.H., O'Carroll, D., Scherthan, H., Mechtler, K., Sauer, S., Schofer, C., Weipoltshammer, K., Pagani, M., Lachner, M., Kohlmaier, A., et al. (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. Cell *107*, 323–337.

Rossi, D.J., Bryder, D., Zahn, J.M., Ahlenius, H., Sonu, R., Wagers, A.J., and Weissman, I.L. (2005). Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc. Natl. Acad. Sci. USA *102*, 9194–9199.

Rube, C.E., Fricke, A., Widmann, T.A., Furst, T., Madry, H., Pfreundschuh, M., and Rube, C. (2011). Accumulation of DNA damage in hematopoietic stem and progenitor cells during human aging. PLoS One *6*, e17487.

Sant, M., Allemani, C., Tereanu, C., De Angelis, R., Capocaccia, R., Visser, O., Marcos-Gragera, R., Maynadie, M., Simonetti, A., Lutz, J.M., and Berrino, F. (2010). Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood *116*, 3724–3734.

Schoeftner, S., and Blasco, M.A. (2009). A 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. EMBO J. *28*, 2323–2336.

Schultz, D.C., Ayyanathan, K., Negorev, D., Maul, G.G., and Rauscher, F.J., 3rd. (2002). SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev. *16*, 919–932.

Shaham, L., Binder, V., Gefen, N., Borkhardt, A., and Izraeli, S. (2012). MiR-125 in normal and malignant hematopoiesis. Leukemia *26*, 2011–2018.

Sharma, P., Azebi, S., England, P., Christensen, T., Moller-Larsen, A., Petersen, T., Batsche, E., and Muchardt, C. (2012). Citrullination of histone H3 interferes with HP1-mediated transcriptional repression. PLoS Genet. *8*, e1002934.

Shumaker, D.K., Dechat, T., Kohlmaier, A., Adam, S.A., Bozovsky, M.R., Erdos, M.R., Eriksson, M., Goldman, A.E., Khuon, S., Collins, F.S., et al. (2006). Mutant nuclear lamin A leads to progressive alterations of epigenetic control in premature aging. Proc. Natl. Acad. Sci. USA *103*, 8703–8708.

Sidler, C., Woycicki, R., Ilnytskyy, Y., Metz, G., Kovalchuk, I., and Kovalchuk, O. (2013). Immunosenescence is associated with altered gene expression and epigenetic regulation in primary and secondary immune organs. Front Genet. *4*, 211.

Smith-Vikos, T., and Slack, F.J. (2012). MicroRNAs and their roles in aging. J. Cell Sci. *125*, 7–17.

Sun, D., Luo, M., Jeong, M., Rodriguez, B., Xia, Z., Hannah, R., Wang, H., Le, T., Faull, K.F., Chen, R., et al. (2014). Epigenomic profiling of young and aged HSCs reveals concerted changes during aging that reinforce self-renewal. Cell Stem Cell *14*, 673–688.

Tan, G., Niu, J., Shi, Y., Ouyang, H., and Wu, Z.H. (2012). NF-kappaB-dependent microRNA-125b up-regulation promotes cell survival by targeting p38alpha upon ultraviolet radiation. J. Biol. Chem. *287*, 33036–33047.

Tsurumi, A., and Li, W.X. (2012). Global heterochromatin loss: a unifying theory of aging? Epigenetics *7*, 680–688.

Ugarte, F., Sousae, R., Cinquin, B., Martin, E.W., Krietsch, J., Sanchez, G., Inman, M., Tsang, H., Warr, M., Passegue, E., et al. (2015). Progressive chromatin condensation and H3K9 methylation regulate the differentiation of embryonic and hematopoietic stem cells. Stem Cell Rep. *5*, 728–740.

Villeneuve, L.M., Kato, M., Reddy, M.A., Wang, M., Lanting, L., and Natarajan, R. (2010). Enhanced levels of microRNA-125b in vascular smooth muscle cells of diabetic db/db mice lead to increased inflammatory gene expression by targeting the histone methyltransferase Suv39h1. Diabetes *59*, 2904–2915.

Wilson, A., Laurenti, E., Oser, G., van der Wath, R.C., Blanco-Bose, W., Jaworski, M., Offner, S., Dunant, C.F., Eshkind, L., Bockamp, E., et al. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell *135*, 1118–1129.

Wojtowicz, E.E., Walasek, M.A., Broekhuis, M.J., Weersing, E., Ritsema, M., Ausema, A., Bystrykh, L.V., and de Haan, G. (2014). MicroRNA-125 family members exert a similar role in the regulation of murine hematopoiesis. Exp. Hematol. *42*, 909–918.e1.

Yahata, T., Takanashi, T., Muguruma, Y., Ibrahim, A.A., Matsuzawa, H., Uno, T., Sheng, Y., Onizuka, M., Ito, M., Kato, S., and Ando, K. (2011). Accumulation of oxidative DNA damage restricts the self-renewal capacity of human hematopoietic stem cells. Blood *118*, 2941–2950.

Zhang, W., Li, J., Suzuki, K., Qu, J., Wang, P., Zhou, J., Liu, X., Ren, R., Xu, X., Ocampo, A., et al. (2015). Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. Science *348*, 1160–1163.