The transcription factor Foxm1 is essential for the quiescence and maintenance of hematopoietic stem cells

Yu Hou1, Wen Li1, Yue Sheng1, Liping Li1,2, Yong Huang3, Zhonghui Zhang1, Tongyu Zhu2, David Peace1, John G Quigley1, Wenshu Wu1, You-yang Zhao4 & Zhijian Qian1

Foxm1 is known as a typical proliferation-associated transcription factor. Here we found that Foxm1 was essential for maintenance of the quiescence and self-renewal capacity of hematopoietic stem cells (HSCs) in vivo in mice. Reducing expression of FOXM1 also decreased the quiescence of human CD34+ HSCs and progenitor cells, and its downregulation was associated with a subset of myelodysplastic syndrome (MDS). Mechanistically, Foxm1 directly bound to the promoter region of the gene encoding the receptor Nurr1 (Nr4a2; called ‘Nurr1’ here), inducing transcription, while forced expression of Nurr1 reversed the loss of quiescence observed in Foxm1-deficient cells in vivo. Thus, our studies reveal a previously unrecognized role for Foxm1 as a critical regulator of the quiescence and self-renewal of HSCs mediated at least in part by control of Nurr1 expression.

Hematopoietic stem cells (HSCs) have the ability to self-renew and differentiate into all blood cell lineages and are critical for maintaining the homeostasis of the hematopoietic system. HSCs exist predominantly in a quiescent state, which is critical for preservation of self-renewal capacity and enablement of life-long hematopoiesis. Elucidating the molecular regulation of HSC quiescence should increase the understanding of mechanisms important for tissue regeneration and might indicate how these become dysregulated in pathological conditions. The quiescent state of HSCs is tightly controlled by both intrinsic molecular mechanisms and extrinsic signals from the microenvironment. Several cell-cycle regulators, as well as molecules with functions in the regulation of oxidative stress, transcriptional regulation of hematopoiesis, or chromatin modification, have been shown to regulate HSC quiescence by intrinsic mechanisms.

Foxm1 belongs to the large family of Fox (‘Forkhead box’) transcription factors. It is a key regulator of aspects of the transition from G1 phase to S phase in the cell cycle, progression through S phase, transition from G2 phase to M phase, and progression through M phase, and is critical for DNA replication, mitosis and genomic stability. Foxm1 has pleiotropic roles during embryonic development and tissue regeneration after injury. Foxm1 is broadly expressed in embryonic tissues, while its expression in adult mice is restricted to the thymus, thymus and intestinal crypts. However, Foxm1 expression is reactivated after organ injury. Studies have demonstrated that Foxm1 has a role in the proliferation of hepatocytes and pancreatic endocrine cells during liver and pancreatic regeneration. Consistent with a critical role for Foxm1 in cell-cycle progression, increased expression of FOXM1 has been found in various human tumors, including lung cancer, breast cancer, liver cancer, glioblastoma and pancreatic cancer. Collectively, Foxm1 has been considered a proliferation-specific transcription factor required for cellular proliferation in various tissues. However, little is known of the function of Foxm1 during hematopoiesis. Deletion of Foxm1 during T cell lymphopoiesis reduces the proliferation of early thymocytes and activates mature T cells but does not affect T cell differentiation, while deletion of Foxm1 in the myeloid lineage does not affect the proliferation or differentiation of myeloid cells. Notably, the effects of loss of Foxm1 in HSCs or hematopoietic progenitor cells (HPCs) have not been examined.

Here we investigated the function of Foxm1 in HSCs and HPCs through the use of mouse models with conditional deficiency in Foxm1. We found that loss of Foxm1 reduced the frequency of quiescent HSCs and increased the proliferation of both HSCs and HPCs but did not affect the differentiation of HSCs or HPCs. As a consequence, Foxm1-deficient HSCs had significantly lower self-renewal capacity than that of Foxm1-sufficient HSCs. Mechanistically, loss of Foxm1 induced downregulation of cyclin-dependent kinase inhibitors, including p21 and p27, by directly suppressing the expression of the gene encoding Nurr1 (Nr4a2; called ‘Nurr1’ here), a critical regulator of HSC quiescence. Notably, reducing expression of FOXM1 in human CD34+ primitive hematopoietic cells also decreased quiescence, and database analysis revealed that the expression of both FOXM1 and NURR1 was significantly downregulated in CD34+ cells from a subset of patients with myelodysplastic syndrome (MDS). Together our data provide evidence that Foxm1 is a critical regulator of the quiescence and self-renewal capacity of HSCs through Nurr1-mediated pathways.

1Department of Medicine and Cancer Research Center, The University of Illinois Hospital and Health Sciences System, Chicago, Illinois, USA. 2Fudan University ZhongShan Hospital, Shanghai, China. 3Section of Gastroenterology, Department of Medicine, The University of Chicago, Chicago, Illinois, USA. 4Department of Pharmacology, The University of Illinois Hospital and Health Sciences System, Chicago, Illinois, USA. Correspondence should be addressed to Z.Q. (zjqian@uic.edu).
RESULTS
Altered hematopoiesis in Foxm1-deficient mice
A function for Foxm1 in HSCs and HPCs has not been reported before, to our knowledge. To determine its role, we characterized the expression of Foxm1 in subsets of primitive and mature bone marrow (BM) cells. Foxm1 had higher expression in primitive hematopoietic cells than in differentiated cells, including mature Mac-1+Gr-1+ myeloid cells, B220+ B cells, CD71+Ter119+ erythroblasts, and CD4+ or CD8+ T cells (Fig. 1a). Notably, Foxm1 had higher expression in long-term HSCs (LT-HSCs; Lin−Sca-1+ c-Kit+CD48−CD150+) than in LSK (Lin−Sca-1+ c-Kit+) cells or HPCs (Lin−c-Kit−Sca-1−), which suggested an important role for Foxm1 in HSCs.

To investigate the in vivo function of Foxm1 in normal hematopoiesis, we crossed mice withloxP-flanked Foxm1 alleles (Foxm1fl/fl),11 with Tie2-Cre mice, in which a transgene encoding Cre recombinase is expressed in endothelial cells and HSCs under the control of the Tie2 promoter18,19, to generate control Foxm1/fl/fl mice and Foxm1flo/fl/Tie2-Cre mice, with conditional deletion of Foxm1 (called ‘Foxm1-CKO mice’ here). We confirmed high efficiency of Foxm1 deletion in BM and LSK cells by semiquantitative PCR analysis of genomic DNA isolated from BM cells (Supplementary Fig. 1a) and LSK cells (Fig. 1b) from Foxm1−CKO and Foxm1fl/fl mice. Accordingly, quantitative PCR analysis showed only trace amounts of Foxm1 mRNA in BM cells (Supplementary Fig. 1b) and LSK cells (Fig. 1c) from Foxm1-CKO mice. We analyzed the key hematopoietic parameters in these mice at 6 weeks of age. Foxm1-CKO mice had considerably fewer white blood cells, neutrophils, lymphocytes, monocytes and platelets than did their Foxm1fl/fl littermates (Fig. 1d). The total number of BM cells was lower in Foxm1-CKO mice than in their Foxm1fl/fl control littermates (Fig. 1e), with some Foxm1−CKO mice showing distinctly hypocellular BM (Supplementary Fig. 1c). Likewise, flow cytometry revealed that the total number of mature myeloid cells, B cells, CD41+ megakaryocytes and F4/80+ macrophages was also lower in BM from Foxm1−CKO mice than in that of their Foxm1fl/fl control littermates (Supplementary Fig. 2a). However, the frequency of these mature blood cells in BM was similar for Foxm1−CKO mice and Foxm1−CKO control mice (Supplementary Fig. 2b–e), which suggested that loss of Foxm1 did not affect the differentiation of myeloid cells or B cells under homeostatic conditions in young mice. Loss of Foxm1 did not interfere with differentiation of the erythroid lineage. We detected a similar frequency of erythroid blasts in four stages of mouse erythroid differentiation20 in both the BM and spleen of Foxm1−CKO mice and Foxm1−CKO control mice (Supplementary Fig. 2f–i). Thus, our data indicated that deletion of Foxm1 led to ineffective hematopoiesis but did not affect the differentiation of mature blood cells.

Cell-intrinsic control of HSC and HPC pools by Foxm1
During normal hematopoiesis, LT-HSCs have ability to self-renew, with the potential for differentiation into common myeloid progenitors (CMPs) and more-committed myeloid progenitor cells, including granulocyte-monoctye progenitors (GMPs) and megakaryocyte-erythroid progenitors (MEPs)21. We hypothesized that the abnormal hematopoiesis observed in Foxm1−CKO mice was probably a consequence of an alteration in HSCs and/or HPCs. We thus examined these compartments by flow cytometry. We observed that both the frequency and the total number of LSK cells, a stem cell–enriched population, were significantly lower, by fivefold to sixfold, in Foxm1−CKO mice than in Foxm1fl/fl control mice (Fig. 2a,b). In addition, the total number of CD34+ LSK cells or LT-HSCs was also significantly lower in Foxm1−CKO mice (Fig. 2a,b). We also noted that both the frequency of HPCs and total number of HPCs were 50% lower in Foxm1−CKO mice (Fig. 2a,d).

We next characterized myeloid progenitor cells in Foxm1−CKO and Foxm1fl/fl mice. The frequency of CMPs was significantly lower in Foxm1−CKO mice than in Foxm1fl/fl mice, while Foxm1−CKO and Foxm1−CKO mice had a similar frequency of GMPs and MEPs cells in vivo (Fig. 2c). However, the total number of all these subsets of myeloid progenitors was lower in Foxm1−CKO mice than in Foxm1fl/fl mice (Fig. 2d). A pool of lymphoid-primed multipotent progenitors (LMPPs; Lin−Sca-1+ c-Kit+Flt3+) has been identified with the ability to generate B cells, T cells and monocytes22. Foxm1−CKO mice had a significantly lower absolute number of LMPPs than did Foxm1fl/fl mice (Fig. 2e). We determined the frequency of myeloid progenitors in BM from Foxm1−CKO and Foxm1fl/fl mice by in vitro colony-forming unit

Figure 1 Foxm1 loss leads to abnormal hematopoiesis. (a) Quantitative RT-PCR analysis of Foxm1 in hematopoietic cells from wild-type BM; results were normalized to expression of the control gene Actb and are presented relative to those of HSCs, set as 1. (b) Semiquantitative PCR analysis of the deletion of Foxm1 (Foxm1fl/fl) and theloxP-flanked Foxm1 allele (Foxm1fl/flo) among genomic DNA in BM LSK cells from Foxm1fl/fl and Foxm1−CKO mice. Left margin (in parentheses), molecular size, in base pairs (bp). (c) Quantitative RT-PCR analysis of Foxm1 mRNA in BM LSK cells from Foxm1fl/fl and Foxm1−CKO mice; results were normalized as in a and are presented relative to those of Foxm1fl/fl cells, set as 1. (d) Absolute number of white blood cells (WBC), neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO) and basophils (BA) (left), as well as platelets (PLT) and red blood cells (RBC) (middle), and concentration of hemoglobin (hb), in peripheral blood from 6-week-old Foxm1−CKO and Foxm1fl/fl mice. (e) Total BM cells in Foxm1−CKO and Foxm1−CKO mice. * P < 0.05, ** P < 0.005 and *** P < 0.0005 (two-tailed Student’s t-test). Data are from two independent experiments (a: mean ± s.d. of n = 3 mice per genotype in each) or are representative of three experiments (b), three independent experiments (c), three independent experiments (d,e: mean ± s.d. of n = 3 mice per genotype in each) or two independent experiments (d,e: mean ± s.d. of n = 5 or n = 9 (e) mice per genotype).
assays. We plated a similar number of BM cells isolated from Foxm1fl/fl or Foxm1-CKO mice in methylcellulose medium containing interleukin 3 (IL-3), IL-6, stem-cell factor and erythropoietin and cultured the cells for 10–12 d. Foxm1-deficient BM cells (from Foxm1-CKO mice) gave rise to markedly lower numbers of total colony-forming units, as well as colony-forming units of a variety of myeloid precursors, including granulocyte-erythroid-macrophage-megakaryocyte colonies, granulocyte-macrophage colonies, granulocyte colonies, macrophage colonies and burst-forming unit-erythroid colonies (BFU-E) and total colony-forming cells (CFU) 10–12 d after plating of Foxm1fl/fl and Foxm1-CKO BM. Total BM cells, LT-HSCs and CD34− LSK cells (defined as in a) in BM from 6- to 8-week-old Foxm1fl/fl and Foxm1-CKO mice 3 weeks after injection of poly(I:C). Total HPCs (Lin−Sca-1−c-Kit+), CMPs (CD34pos–loCD16/32int), GMPs (CD34+CD16/32−) and MEPs (CD34−CD16/32−) (left) and LMPPs (Flk2+) (right) in BM from mice as in g. *P < 0.05, **P < 0.005 and ***P < 0.0005 (two-tailed Student’s t-test). Data are representative of three experiments (a,c) or are from three experiments (b,d,e); mean ± s.d. of n = 6–7 mice per genotype or two experiments (f–h; mean ± s.d. of n = 3 mice per genotype).

Figure 2 Marked decrease in the size of HSC and HPC pools in Foxm1-deficient mice. (a) Flow cytometry of BM cells from Foxm1fl/fl and Foxm1-CKO mice. Numbers adjacent to outlined areas indicate percent c-Kit+Sca-1− cells (HPCs) or c-Kit+Sca-1− cells (LSK cells) among Lin− cells (left), or CD34− LSK cells (middle) or CD34− cells (right) among LSK cells. SS Log side scatter (log value). (b) Total LSK cells, CD34− LSK cells and LT-HSCs in BM from 6- to 8-week-old Foxm1fl/fl and Foxm1-CKO mice. Numbers adjacent to outlined areas indicate percent CD16/32−CD34− cells (bottom left), CD16/32+CD34− cells (GMPs) (top right) or CD16/32−CD34− cells (CMPs) (bottom right) among LSK cells (left), or Flk2+ cells (LMPPs) (right) among LSK cells (right). (d,e) Total HPCs, CMPs, GMPs and MEPs (d) and LMPPs (e) in BM from 6- to 8-week-old Foxm1fl/fl and Foxm1-CKO mice. (f) In vitro assay of the colony-forming units of granulocyte, erythroid, macrophage, megakaryocyte colonies (GEMM), granulocyte-macrophage colonies (GM), granulocyte colonies (G), macrophage colonies (M), burst-forming unit-erythroid colonies (BFU-E) and total colony-forming cells (CFU) 10–12 d after plating of Foxm1fl/fl and Foxm1-CKO BM. (g) Total BM cells, LT-HSCs and CD34− LSK cells (defined as in a) in BM from 6- to 8-week-old Foxm1fl/fl and Foxm1-CKO mice 3 weeks after injection of poly(I:C). Total HPCs (Lin−Sca-1−c-Kit+), CMPs (CD34pos–loCD16/32int), GMPs (CD34−CD16/32−) and MEPs (CD34−CD16/32−) (left) and LMPPs (Flk2+ LSK cells) (right) in BM from mice as in g. *P < 0.05, **P < 0.005 and ***P < 0.0005 (two-tailed Student’s t-test). Data are representative of three experiments (a,c) or are from three experiments (b,d,e); mean ± s.d. of n = 6–7 mice per genotype or two experiments (f–h; mean ± s.d. of n = 3 mice per genotype).

Endothelial cells constitute a critical niche for HSCs23–25. To rule out the possibility that the endothelial niche that HSCs are exposed to during development caused the observed hematopoietic defects in Foxm1-CKO mice, we crossed Foxm1fl/fl mice with Mxl1-Cre mice (which express Cre under the control of the interferon-inducible Mxl1 promoter) to generate Foxm1fl/flMxl1-Cre mice, in which deletion of Foxm1 in the hematopoietic compartments is efficiently induced in adult mice by intraperitoneal injection of the interferon-α inducer polyinosinic-polycytidylic acid (poly(I:C)) into adult mice (Supplementary Fig. 3a,b). Following induction of the deletion of Foxm1, Foxm1fl/flMxl1-Cre mice had a reduced number of total BM cells (Supplementary Fig. 3c) and developed a defect in the HSC compartment similar to that observed in Foxm1-CKO mice (Fig. 2g,h). Foxm1fl/flMxl1-Cre mice had abnormal hematopoiesis (Supplementary Table 1) characterized by significantly fewer total LSK cells, CD34− LSK cells and LT-HSCs than the number of those cells in Foxm1fl/fl mice (Fig. 2g) and a concomitant reductions in the number of HPCs, CMPs and LMPPs (Fig. 2h,i). Together these data suggested that loss of Foxm1 significantly interfered with the maintenance of adult HSCs and early HPCs during normal hematopoiesis and that the defect seemed to be independent of the endothelial niche.

However, Cre expressed from the Mxl1-Cre transgene also induces gene deletion in non-hematopoietic cells, including the BM stromal compartment and liver29. To rule out the possibility that the effects of Foxm1 deletion on HSCs and HPCs were dependent on the BM microenvironment, we transplanted BM cells from Foxm1fl/flMxl1-Cre or Foxm1fl/fl mice (CD45.2+) into lethally irradiated wild-type syngeneic recipient mice (CD45.1+) to generate Foxm1fl/flMxl1-Cre or Foxm1fl/fl chimeric mice. Foxm1fl/flMxl1-Cre and Foxm1fl/fl BM cells resulted in similar engraftment efficiency, with more than 95% of BM cells in recipient mice replaced with BM cells from donor mice (Supplementary Fig. 4a). We induced deletion of Foxm1 in the chimeric Foxm1fl/flMxl1-Cre mice by injection of poly(I:C) 6 weeks after transplantation and, 2 months later, we assessed the HSC and...
Figure 3 Depletion of Foxm1 decreases the self-renewal capacity of HSCs. (a) Survival of Foxm1fl/fl and Foxm1-CKO mice (n = 12 per genotype) after multiple injections (upward arrows) of 5-FU (50 mg per kg body weight), presented as Kaplan-Meier curves. P < 0.0001 (Mantel-Cox test). (b) Total BM cells (b) and Lin−Sca-1+cells (c) in Foxm1fl/fl and Foxm1-CKO mice before (day 0) and on days 4, 7 and 10 after injection of 5-FU (200 mg per kg body weight). *P < 0.05 (two tailed Student’s t-test). (d) Survival of recipient mice (n = 10) after transplantation of BM cells from Foxm1-CKO and Foxm1fl/fl mice that had undergone primary transplantation (Kaplan-Meier curves). P = 0.0001 (Mantel-Cox test). Data are from one experiment (a,d) or three experiments (b,c); mean ± s.d. of n = 3 mice per genotype).

HPC compartments by flow cytometry. The number of total LSK cells, CD34+ LSK cells and LT-HSCs was significantly lower in Foxm1fl/flMx1-cre chimeric mice than Foxm1fl/fl chimeric control mice (Supplementary Fig. 4b). In addition, the number of HPCs and CMPs was also significantly lower in the Foxm1fl/flMx1-cre chimeric mice (Supplementary Fig. 4c). In summary, our results indicated that Foxm1 was an intrinsic regulator of HSCs and HPCs.

Loss of Foxm1 impairs the long-term self-renewal of HSCs
To further investigate the effects of the deletion of Foxm1 on hematopoietic reconstitution in situ in primary mice, we gave Foxm1-CKO and Foxm1fl/fl mice weekly injections of the cell cycle–dependent myelotoxic agent 5-fluorouracil (5-FU)27, which kills proliferating cells, including HPCs, and thus stimulates HSCs to proliferate and replenish the hematopoietic system. We monitored the treated mice for up to 3 weeks. 80% of Foxm1-1-deficient mice succumbed after the second injection of 5-FU, and all remaining mice died after a third injection of 5-FU (Fig. 3a). In contrast, more than 90% of control mice were still alive after the third injection of 5-FU (Fig. 3a). Analysis of BM cells and HPCs from another cohort of 5-FU-treated Foxm1-CKO and Foxm1fl/fl mice indicated that Foxm1-deficient HSCs failed to efficiently replenish BM cells and HPCs (Fig. 3b,c).

To further evaluate the long-term self-renewal capacity of Foxm1-deficient HSCs, we performed serial BM-transplantation assays. We obtained BM cells (5 × 10^6) from two to three Foxm1-CKO or Foxm1fl/fl mice and serially transplanted the cells into lethally irradiated wild-type recipients. While Foxm1fl/fl HSCs ceased to reconstitute the lethally irradiated mice after the fourth transplantation cycle, Foxm1-CKO HSCs failed to reconstitute hematopoiesis after secondary transplantation (Fig. 3d), which suggested that Foxm1-deficient HSCs had a considerable defect in their self-renewal capacity.

Next we assessed the reconstituting capacity of Foxm1-deficient HSCs in a competitive environment by performing competitive serial transplantation assays. We transplanted an equal number of BM cells or sorted LT-HSCs from Foxm1-CKO mice or their Foxm1fl/fl control littersmates (all CD45.2+ into lethally irradiated Ly5.1+ (CD45.1+) recipient mice together with competitor BM cells from Ly5.1+ (CD45.1+CD45.2+) C57BL/6 mice. At 4 months after transplantation, we obtained BM cells from a cohort of mice that had undergone primary transplantation and transplanted equal numbers of those cells into a second set of lethally irradiated mice. We assessed donor-derived (CD45.2+) peripheral blood cells and competitor-derived peripheral blood cells by flow cytometry every month after transplantation. Of note, un fractionated Foxm1-deficient BM cells showed...
a progressive repopulation defect, with 80% less repopulation than that of Foxm1fl/fl cells at 1 month after primary transplantation and 90% less repopulation at 4 months (Fig. 4a, left). Purified Foxm1-deficient LT-HSCs also showed a progressive decrease in repopulation capacity, with 25% less repopulation than that of Foxm1fl/fl LT-HSCs at 1 month after transplantation and 90% less at 4 months (Fig. 4a). Peripheral blood cells derived from Foxm1-deficient BM cells and LT-HSCs were barely detectable at 4 months after the second transplantation (Fig. 4a).

We also performed competitive repopulation assays with BM cells from Foxm1fl/flMx1-Cre and Foxm1fl/fl mice. Without induction of Foxm1 deletion by poly(I:C), Foxm1fl/flMx1-Cre and Foxm1fl/fl BM cells had a similar repopulation capacity (Fig. 4b). Delayed deletion of Foxm1 induced at 6 weeks after transplantation consistently resulted in a progressive decrease in HSC repopulation capacity (Fig. 4b). Flow cytometry of total mature cells (Fig. 4c) and of the myeloid, B cell and T cell lineages in peripheral blood (Supplementary Fig. 5a–c) revealed that the repopulation of all three lineages was impaired. Additionally, immature BM cells derived from Foxm1-deficient donor cells were barely detectable at 4 months after the second transplantation (Fig. 4d and Supplementary Fig. 5d,e), indicative of an HSC defect. Consistent with the data obtained with Foxm1-CKO mice, loss of Foxm1 did not affect lineage differentiation during the repopulation of hematopoietic cells in recipient mice (Supplementary Fig. 5f). In summary, these studies provided evidence that loss of Foxm1 exerted a negative effect on the long-term regenerative capacity of HSCs.

**Foxm1 deficiency increases the cycling of HSCs and HPCs**

Published findings indicate that Foxm1 has a role in the G1-to-S cell-cycle transition and also contributes to regulation of the G2-to-M transition in mammalian cells. As Foxm1 is a critical cell-cycle regulator, we investigated whether loss of Foxm1 would lead to cell-cycle defects in HSCs and HPCs. We observed a greater accumulation of cells in the S phase and G2-M phase among Foxm1-deficient HPCs, LSK cells and LT-HSCs (from Foxm1-CKO mice) than among their counterparts from control (Foxm1fl/fl) mice (Fig. 5a–c). To determine whether the accumulation of cells in the S and G2-M phases among Foxm1-deficient LSK cells and LT-HSCs was due to the block of the cell cycle in S phase and the G2-M transition or to an overall increase in cycling cells, we assessed in vivo incorporation of the thymidine analog BrdU to investigate the cell-cycle kinetics of LSK cells and LT-HSCs from Foxm1-CKO and Foxm1fl/fl mice. While ~13% of Foxm1fl/fl LSK cells (and ~5% of Foxm1fl/fl LT-HSCs) incorporated BrdU, ~50% of the Foxm1-CKO LSK cells (and ~35% of Foxm1-CKO LT-HSCs) were BrdU+, which indicated that the absence of Foxm1 enhanced the proliferation of LT-HSCs and LSK cells. In addition, loss of Foxm1 also led to the accumulation of cells at the G2-M transition among LT-HSCs and LSK cells (Fig. 5d,e). Collectively, our studies suggested that Foxm1 regulated the cell cycle in a context-dependent manner.

As quiescence is crucial for the function of HSCs, we next determined the fraction of the stem cell–enriched population (LSK cells) in G0 by assessing RNA and DNA content through the use of staining...
with the RNA-binding red fluorescent dye pyronin Y and DNA-binding blue fluorescent stain Hoechst 33342, as described. The proportion of quiescent cells was threefold lower among Foxm1-deficient (Foxm1-CKO) LSK cells than among Foxm1-sufficient (Foxm1fl/fl) LSK cells (Fig. 5f), which suggested that Foxm1 deficiency regulated the G0 phase in HSCs. Similarly, deletion of Foxm1 induced by Cre expressed from Mx1-Cre resulted in a loss of G0 maintenance in LSK cells and defects in cell-cycle progression in HSCs, LSK cells and HPCs in both primary and chimera mice (Supplementary Fig. 6). Thus, these data suggested that Foxm1 was critical for the maintenance of HSC quiescence and that in its absence, HSCs more easily entered the cell cycle.

**Foxm1 loss reduces the survival of HSCs and HPCs under stress**

As cell survival contributes to the maintenance of HSCs and HPCs, we examined Foxm1-deficient and control HSCs and HPCs for evidence of apoptosis by staining cells with annexin V. We found that Foxm1 loss did not affect the frequency of apoptosis of LSK cells and HPCs from Foxm1-CKO mice (Supplementary Fig. 7a). Notably, we found that the frequency of apoptosis was significantly higher in LSK cells and HPCs from Foxm1-CKO and Foxm1fl/fl Mx1-Cre chimera mice than in those from Foxm1fl/fl chimera mice (Supplementary Fig. 7b,c). As transplantation of HSCs exposes HSCs and HPCs to various stresses, our studies suggested that loss of Foxm1 reduced the survival of HSCs and HPCs under stress.

**Foxm1 deficiency affects multiple pathways in HSCs**

To identify Foxm1-dependent genes and the molecular pathways involved in the regulation of HSC function, we performed global gene-expression profiling of LT-HSCs isolated from 2-month-old Foxm1-CKO mice and Foxm1fl/fl control mice. We found that 70 genes had over-twofold differential expression in Foxm1-CKO HSCs relative to their expression in Foxm1fl/fl HSCs (Fig. 6a and Supplementary Table 2). The differentially expressed genes were classified into seven functional groups, including genes encoding products with nucleic acid–binding transcription factor activity, binding activity, catalytic activity and structural activity (Fig. 6b). Accumulating evidence suggests a regulatory function for ribosomes in gene expression. Deregulation of protein synthesis by altering ribosome function impairs HSF function. Notably, genes encoding several ribosomal proteins, including Rpl38, Ultp44a, Rpl29, Mrpl24 and Rpl21, had significantly higher expression in HSCs lacking Foxm1 (Foxm1-CKO) than in Foxm1-sufficient (Foxm1fl/fl) control HSCs (Supplementary Table 2). Next, we used gene-set–enrichment analysis to determine whether an a priori–defined set of genes showed significantly different expression in HSCs in the absence of Foxm1 than in the presence of Foxm1 by analyzing a data set with 20,000 transcripts. This analysis revealed that the set of genes downregulated in Foxm1-deficient HSCs showed enrichment for a gene set encoding products associated with cytokine signaling in the immune system (Fig. 6c, left). Notably, genes targeted by AML1 (Runx1), a critical regulator of HSC self-renewal, were also downregulated in Foxm1-deficient HSCs (Fig. 6c, right). Consistent with the observed increase in cycling HSCs in Foxm1-deficient mice, the set of genes upregulated in Foxm1-deficient HSCs showed enrichment for a gene set encoding products associated with cell-cycle progression through S phase (Fig. 6d, left). E2F1 is a positive regulator of G1-S of the cell cycle, and the set of genes upregulated in Foxm1-deficient HSCs showed enrichment for genes targeted by E2F1 (Fig. 6d, right), which provided additional evidence for enhanced cell cycling in Foxm1-deficient HSCs. Thus, these Foxm1 deficiency–mediated transcriptional changes indicated that the absence of Foxm1 perturbed multiple stem cell–maintenance mechanisms.

**Nurr1 mediates Foxm1 function in regulating HSC quiescence**

Among the genes significantly downregulated in Foxm1-deficient HSCs (Fig. 6a), the nuclear receptor Nur1 stood out due to its important role in the maintenance of HSC quiescence. Loss of a single allele of Nur1 is sufficient to induce HSCs to enter into the cell cycle and proliferate. This effect is associated with the downregulation of genes encoding cyclin-dependent kinase inhibitors, including p21 and p27, in Nur1-null HSCs. Notably, our expression profiling data (confirmed by quantitative RT-PCR; Fig. 6e) revealed that the absence of Foxm1 in HSCs correlated with downregulation of the
Figure 7 Forced expression of Nurr1 ‘rescues’ Foxm1 deletion–induced loss of quiescence in vivo. (a) Mouse Nurr1 upstream promoter region (top), including predicted Foxm1-binding regions (site 1 and site 2), as well as wild-type and mutated (Mut) Nurr1 promoter luciferase (Luc) constructs (core motif TTATTTTGGG mutated (red underline) to CCACCCGCCC); arrow (top right) indicates transcription start site (TSS). pGL3, plasmid. (b,c) Luciferase activity in 293T cells transfected with the wild-type (WT) (b) or mutated (Mut) (c) Nurr1 promoter luciferase construct in a plus empty vector (EV) or vector for the expression of Foxm1 (key); results are presented relative to those of cells transfected with the luciferase construct and empty vector. (d) Chromatin-immunoprecipitation assay of the binding of endogenous Foxm1 to the upstream region of Nurr1 (site 1 and site 2) or to a control site downstream of Ccnb1 (Ctrl), in Lin−/BM cells; IgG serves as a negative control. (e) Flow cytometry (as in Fig. 5f) of LSK cells from lethally irradiated recipient mice reconstituted with Foxm1+/+ or Foxm1−/−/Mxl1-Cre BM cells (key) transduced with control vector (CV) or Nurr1-expressing vector (Nurr1) (numbers in quadrants as in Fig. 5f). (f) Summary of cell-cycle status in e. (g) Total LSK cells and HSCs from mice as in e. **P < 0.01, ***P < 0.001 and ****P < 0.0005 (two-tailed Student’s t-test). Data are from three experiments (b–d; mean ± s.d. of n = 3 mice) or two experiments (e.g.; mean ± s.d. of n = 5 mice per genotype) or are representative of two experiments (e).

To determine whether Foxm1 modulates Nurr1 expression through direct transcriptional activation, we searched for the consensus Foxm1-binding site T(G/A)TTT(G/A)TT (where ‘G’ or ‘A’ means G or A)58 in the proximal promoter region of Nurr1. We identified two putative Foxm1-binding sites upstream of the Nurr1 transcription start site (Fig. 7a). We next performed dual-luciferase reporter assays with cells expressing the wild-type Nurr1 promoter or a promoter with mutation of the predicted Foxm1-binding sites. The luciferase activity of a construct containing the wild-type binding site 1 was activated by Foxm1 expression, but a construct containing a mutated binding site 1 or a construct containing binding site 2 alone did not (Fig. 7b,c); this suggested that the integrity of consensus site 1 in the Nurr1 promoter was required for Foxm1-mediated activation of Nurr1 expression. A chromatin-immunoprecipitation assay of LIN−/BM cells revealed that Foxm1 directly bound to site 1 but not site 2 of the Nurr1 promoter (Fig. 7d).

To determine whether downregulation of Nurr1 was critical for the loss of quiescence seen in Foxm1-deficient LK cells, we assessed the effect of enforced expression of Nurr1 on the quiescence of Foxm1+/−/Mxl1-Cre LK cells in vitro. In these experiments, Nurr1 was expressed under control of a modified tetracycline-responsive promoter59, in a lentivirus vector that allows doxycycline-controlled gene expression. We infected Foxm1+/− and Foxm1+/−/Mxl1-Cre BM cells with empty lentivirus expression vector or vector containing Nurr1 and transplanted the cells into the lethally irradiated recipient mice to generate chimeric mice. We induced Foxm1 depletion and expression of Nurr1 in these mice by treatment with poly(I:C) and doxycycline, respectively, at 6 weeks after transplantation. We confirmed induction of Nurr1 expression in BM cells from chimeric mice by immunoblot analysis (Supplementary Fig. 8a). Notably, the frequency of quiescent cells was significantly greater among Nurr1−/− transduced Foxm1+/−/Mxl1-Cre LK cells than among empty vector–transduced Foxm1+/+ control LK cells in chimeric mice (Fig. 7e,f). The frequency of LK cells and LT-HSCs was higher among BM cells from Nurr1−/− transduced Foxm1+/−/Mxl1-Cre chimeric mice than among empty vector–transduced Foxm1+/+/Mxl1-Cre chimeric mice, even 2 weeks after the induction of Nurr1 expression (Fig. 7g); this indicated that Nurr1 expression prevented the depletion of LT-HSCs and LK cells induced by the absence of Foxm1. In addition, the genes encoding p21 and p27 were upregulated by Nurr1 expression, which reversed the downregulation of the expression of these genes in Foxm1−/− LK cells (Supplementary Fig. 8b). Together these data suggested that Nurr1 insufficiency mediated Foxm1 depletion–induced loss of quiescence in LK cells.

Downregulation of FOXM1 in a subset of patients with MDS

To determine the function of Foxm1 in the cell-cycle regulation of human HSCs and HPCs, we investigated whether downregulation of FOXM1 expression in CD34+ hematopoietic cells from human umbilical cord blood would lead to the same perturbations in the cell cycle that we had observed in Foxm1-null mice. We downregulated FOXM1 expression in CD34+ cells from human cord blood through the use of short hairpin RNA (shRNA). Consistent with published results48, a large number of CD34+ HSCs were quiescent after several days of in vitro culture (Fig. 8a,b). We observed more proliferation (frequency of cells in S phase) and fewer quiescent cells (frequency of cells in G0 phase) when FOXM1 expression was reduced by shRNA (Fig. 8a,b). Thus, decreasing FOXM1 expression in these human cells reduced quiescence. Decreased HSC function contributes to the development of MDS, which is a clonal HSC disorder characterized by infective hematopoiesis41. By analyzing a published set of microarray data of CD34+ HSCs and HPCs from 183 patients with MDS with various cytogenetic abnormalities and 17 healthy control subjects42,
we found that FOXM1 and NURR1 were downregulated in CD34+ cells from patients with MDS with deletion of chromosome 5q relative to their expression in cells from healthy control subjects (Fig. 8c).

Notably, reducing FOXM1 expression via shRNA in human primary CD34+ cells led to downregulation of NURR1 (Fig. 8d). Our findings raised the possibility of a critical role for a Foxm1-mediated pathway in the pathogenesis of MDS with deletion of chromosome 5q via deregulation of HSC function in patients with this deletion.

**DISCUSSION**

In this study, we have identified a previously unrecognized role for Foxm1 in the maintenance of HSCs and HPCs. Using mouse models with conditional deletion of Foxm1, we have shown that Foxm1 is essential for maintaining the quiescence and self-renewal of HSCs in vivo and that it intrinsically regulated the proliferation of HSCs and HPCs but not their differentiation. We also have identified Nurr1 as an additional downstream effector of Foxm1 that was directly activated by Foxm1 and was able to mediate the function of Foxm1 in regulating HSC quiescence.

Foxm1 has been consistently reported as a ‘pro-proliferation’ factor in proliferating cells through its promotion of progression through the cell cycle. However, some studies have suggested a role for Foxm1 in the self-renewal of stem cells. Foxm1 is involved in maintaining the pluripotency of embryonic stem cells. Interestingly, deletion of Foxm1 in neural cortical stem cells and/or progenitor cells from cortical tissue at embryonic day 14.5 reduces their formation of the neurosphere in vitro, which indicates a possible role for Foxm1 in the self-renewal of stem cells and/or progenitor cells. Foxm1 also regulates the self-renewal of glioma-initiating cells in vitro by promoting localization of β-catenin to the nucleus (and thus activation of its target genes).

However, the in vivo role of Foxm1 in adult stem cells has not been characterized before, to our knowledge. Our findings have provided in vivo evidence that Foxm1 seems to have divergent functions in HSCs. We observed that inducing the loss of Foxm1 in HSCs of either embryos or adults (via either Tie2-Cre or Mxi1-Cre, respectively) had an almost identical effect on the function of HSCs and HPCs, which suggested that Foxm1 intrinsically regulates the self-renewal and quiescence of HSCs in adults. Foxm1 is required for injury-induced regeneration of the liver, lung, and pancreas in adults. Our findings raise the possibility that the role of Foxm1 in tissue regeneration might be mediated in part by its effect on relevant tissue-specific stem cells.

Published studies have shown that Foxm1 stimulates proliferation by promoting entry into the S and M phases of the cell cycle. In contrast, we observed here that Foxm1 increased the proliferation of HSCs and HPCs. In line with our observation that loss of Foxm1 promoted the proliferation of HSCs and HPCs, our expression profiling data showed that genes that are targets of E2F1, as well as other genes encoding products that promote transition through the S phase, were significantly upregulated when Foxm1 was absent in HSCs, which provided molecular evidence for the observed promotion of the progression of HSCs through S phase. Moreover, loss of Foxm1 led to downregulation of Nurr1 and genes encoding cyclin-dependent kinase inhibitors, including p21 and p27. Of interest, published studies have shown that loss of even a single allele of Nurr1 markedly impairs the quiescence of HSCs and their capacity for self-renewal and is associated with downregulation of the genes encoding p21 and p27 in HSCs. We found that ectopic expression of Nurr1 reversed the downregulation of the genes encoding p21 and p27 induced by deletion of Foxm1, which suggested that these effects are probably mediated by Nurr1. Finally, our studies indicated that Foxm1 activated Nurr1 expression by directly binding its promoter and that forced expression of Nurr1 reversed the loss of quiescence in Foxm1-deficient populations. Nonetheless, analysis of global gene expression in Foxm1-deficient and Foxm1-sufficient LT-HSCs suggested that Foxm1 regulates the function of HSCs through multiple molecular pathways. Additional studies are needed to determine whether the other downstream pathways are important for mediating Foxm1 function in HSCs.

Several known downstream targets of Foxm1, including Sox2 and Bmi1, seem to be important for the self-renewal of neural stem cells. However, we did not observe changes in the expression of Sox2 or Bmi1 in Foxm1-deficient HSCs relative to their expression in control HSCs. In addition, we did not detect significant changes in Foxm1-deficient HSCs in the expression of genes that are targets of β-catenin (regulated by Foxm1 in glioma-initiating cells). Thus, we conclude that Foxm1 regulates gene expression in a tissue-specific manner, perhaps to confer onto Foxm1 its divergent functions in various tissues.

The role of Foxm1 in the regulation of apoptosis is controversial. We found that depletion of Foxm1 did not affect the apoptosis of HSCs or HPCs at steady state. However, the absence of Foxm1 induced apoptosis of HSCs and HPCs upon transplantation into chimeric mice, a condition that exposes HSCs and HPCs to various stresses. These data indicate a role for Foxm1 in maintaining the survival of HSC or HPCs under conditions of cellular stress.
The role of Foxm1 in MDS is unknown. By analyzing public database sources, we found that the gene encoding Foxm1 and its downstream target NURR1 were both significantly downregulated in a subset of patients with MDS. More notably, reduced expression of FOXM1 promotes loss of quiescence and downregulated NURR1 expression in human primary CD34+ HSCs and/or HPCs. Thus, our data indicate a critical role for Foxm1 in maintaining human HSCs and/or HPCs and that deregulation of FOXM1 expression might contribute to human hematological malignant diseases such as MDS by affecting the function of HSCs and/or HPCs. In summary, we have provided functional and molecular evidence that Foxm1 acts a critical regulator of self-renewal of adult HSCs through control of HSC quiescence and have provided evidence in support of a tissue-specific multi-faceted role for Foxm1 in cell-cycle regulation.

METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. GEO: gene-expression data for HSCs in Foxm1−/− and Foxm1−/−KO mice, GSE62360.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

ACKNOWLEDGMENTS

We thank M.A. Goodell (Baylor College of Medicine) for the plasmid MSCV-FlagNurr1. Supported by the US National Institutes of Health (ROI CA140979 to Z.Q.).

AUTHOR CONTRIBUTIONS

Y.Ho., W.L., Y.S., L.L., Y.Hu. and Z.Z. performed research; T.Z., D.P., J.G.Q., V.P., M.C., H.M., Z.Q., M.A. and A.F. analyzed data; A.F., Z.Q., M.A. and T.Z. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Wilson, A. et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell 135, 1118–1129 (2008).
2. Reya, T., Morrison, S.J., Clarke, M.F. & Weissman, I.L. Stem cells, cancer, and stem cell theory. Nature 414, 105–111 (2001).
3. Cheung, T.H. & Rando, T.A. Molecular regulation of stem cell quiescence. Nat. Rev. Mol. Cell Biol. 14, 329–340 (2013).
4. Pietras, E.M., Warr, M.R. & Passegue, E. Cell cycle regulation in hematopoietic stem cells. J. Cell Biol. 195, 709–720 (2011).
5. Kalin, T.V., Ustiyan, V.A. & Kalinichenko, V.V. Multiple faces of FoxM1 transcription factor is essential for hepatocyte DNA replication and mitosis during transcription factor function, target genes, mouse models, and normal biological roles. Adv. Cancer Res. 118, 97–398 (2013).
ONLINE METHODS

Mice and blood cell counts. For conditional deletion of Foxm1 in vivo, Foxm1fl/fl mice were mated to Tie2-Cre or Mx1-Cre transgenic mice to generate Foxm1-CKO or Foxm1fl/flMx1-Cre mice. Mx1-Cre expression was induced by three intraperitoneal injections of 6–10 µg (poly(I:C)) (GE Healthcare) per gram of body weight every second day for a total of three injections. Times after poly(I:C) injection are ‘counted’ from the third poly(I:C) injection. Peripheral blood samples were collected from the tail vein into tubes containing EDTA. Complete blood counts and differentials were obtained with a Hemavet 950FS (Drew Scientific). All animal research was approved by the University of Illinois at Chicago Institutional Animal Care and Use Committee.

Flow cytometry. Single-cell suspensions were prepared from BM (femurs and tibiae), spleen, thymus and peripheral blood. Red cells were lysed with ammonium-chloride-potassium (ACK) buffer. Cells were incubated for 20 min on ice with antibodies. The following fluorochrome- or biotin-conjugated antibodies to mouse molecules were used (all from eBioscience, except allotypochocyanin-conjugated anti-CD150 (from BioLegend); all antibodies identified in Supplementary Table 3): anti-Gr-1, anti-Ter119, anti-B220, anti-CD19, anti-IL-7R and anti-CD3, for lineage markers; streptavidin-fluorescein isothiocyanate (PE)-indocarbocyanine (Cy5), PE-anti-Sca-1, allotypochocyanin-indocarbocyanine (Cy5)-anti-c-Kit, PE-Cy7-anti-CD48 and allotypochocyanin-anti-CD150, for analysis of HSCs, LSK cells and HPCs; streptavidin-allophycocyanin-Cy7, PE-anti-Sca-1, allotypochocyanin-anti-c-Kit and PE-Cy5-anti-Flt3, for analysis of LMPs; streptavidin-allophycocyanin-Cy7, PE-anti-Sca-1, allotypochocyanin-anti-c-Kit, PE-Cy7-anti-CD16/32 and eFluor 450-anti-CD34, for analysis of CMPs, GMPs, MEPs8,48, PE-anti-B220 and allotypochocyanin-IgM, for analysis of B cells; PE-anti-Gr-1 and allophycocyanin-anti-Mac-1, for analysis of myeloid cells; allotypochocyanin-anti-Ter119 and PE-anti-CD71, for analysis of erythroid cells; anti-CD4 and anti-CD8, for analysis of mature T cells; PE-Cy7-anti-CD41 and allophycocyanin-anti-c-Kit, for analysis of megakaryocytes; PE-anti-F4/80 and allophycocyanin-anti-MAC, for analysis of macrophages; and allophycocyanin-anti-CD45.2 or fluororescin isothiocyanate–anti-CD45.2 and PE-anti-CD45.1, for identification of donor cells in recipient mice.

For cell-cycle analysis, 5 × 10^5 BM cells were stained for the identification of HSCs, then cells were fixed with 1% formaldehyde in PBS, treated with 0.1% Triton X-100, stained with 5 µg/ml DAPI and analyzed on a Gallios flow cytometer. For cell-cycle analysis with Hoechst 33342 and Pyronin Y, cells were stained with ‘cocktail’ of antibodies to lineage markers (Supplementary Table 3), and then the cells were stained with streptavidin-allophycocyanin-Cy7, fluororescin isothiocyanate–anti-Sca-1 and allophycocyanin-anti-c-Kit. For the detection of apoptosis, BM cells were stained with antibody conjugates, annexin V and DAPI.

Colony-forming assays. 6 × 10^3 BM cells from Foxm1fl/fl or Foxm1-CKO mice were plated in triplicate in 35-mm tissue culture dishes containing Mouse Methylcellulose Complete Media (HSC007; R&D Systems). After 10 d mice were plated in triplicate in 35-mm tissue culture dishes containing for analysis of macrophages; and allophycocyanin-Cy7–anti-CD45.2, for analysis of megakaryocytes, granulocyte-macrophage colonies, granulocyte colonies, macrophage colonies, and burst-forming unit–erythroid colonies were viewed with an inverted microscope and assigned scores.

BM-transplantation assays. For competitive reconstitution assays, total BM cells (2.5 × 10^6) or sorted CD150^+CD48^- LSK cells (2 × 10^5) from Foxm1-CKO or Foxm1fl/flMx1-Cre mice or their corresponding control littermates (CD45.2^+) were transplanted into lethally irradiated Ly5.1^+ (CD45.1^+) recipient mice together with competitor BM cells (2.5 × 10^6) for BM cell analysis or (0.4 × 10^6) for HSC analysis from Ly5.1^+ C57BL/6 mice (CD45.1^+CD45.2^+). For second transplantation, BM cells (2 × 10^6) were transferred to a second set of lethally irradiated mice. For BM-transplantation assays, BM cells (5 × 10^6) from Foxm1fl/fl or Foxm1fl/flMx1-Cre were transplanted into lethally irradiated Ly5.1^+ mice. For serial transplantation analysis, BM cells (1 × 10^6) were obtained from recipient at 4 months after first transplantation and were transferred to a second set of lethally irradiated mice.

Microarray and bioinformatics analysis. Total RNA was extracted from sorted LT-HSC cells (CD150^+CD48^- LSK) from 2-month-old Foxm1fl/fl and Foxm1-CKO mice with and RNeasy Micro Kit (Qiagen) and was amplified with the Ovation Pico WTA System V2 (NuGEN Technologies). Labeled cDNA was hybridized to a Mouse Gene 1.0 ST Array (Affymetrix). Chip quality was analyzed and determined with Affymetrix GCGOS software. All RNA samples and arrays used in this study passed the established quality criteria. The raw data were normalized with the R/Bioconductor software package affy49. Gene-set–enrichment analysis was performed with GSEA v2.0 software (available from the Broad Institute).

RNA extraction and quantitative RT-PCR analysis. Total RNA was isolated from 150 mg per kg body weight, once per week for 3 weeks, and the survival of individual mice was monitored daily.

BrdU-incorporation assay. Mice were given intraperitoneal injection of 100 µl of 10 mg/ml BrdU (Sigma) 24 h before being killed for analysis. BM cells were stained with fluorochrome-conjugated antibodies (Supplementary Table 3), followed by fixation and permeabilization with Cytofix/Cytoperm (BD Biosciences), treatment with DNase I (Sigma), and staining with a BrdU–specific antibody (Bu20a; eBioscience) according to the instruction manual of the BrdU flow kit (BD Pharmingen). The cells were analyzed by flow cytometry with a CyAn ADP flow cytometer.

Chip assay. BM cells from wild-type mice were fixed for 10 min at 37 °C with 1% formaldehyde, the reaction was stopped by the addition of glycine to a final concentration of 125 mM, followed by incubation for 5 min at 25 °C. Cells were washed two times with cold PBS and were lysed in Szak RIPA buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, 5 and mM EDTA) with a protease inhibitor cocktail. After cell lysis, crosslinked chromatin was sheared with a sonicator. Then, 5 µg of anti-Foxm1 (K-19; Santa Cruz Biotechnology) or control IgG (sc-2027; Santa Cruz Biotechnology) were used for immunoprecipitation. After elution of DNA from precipitated immunocomplexes, quantitative real-time PCR analysis was performed with specific primers (sequences, Supplementary Table 4) for amplification of site 1 and site 2 in the Nurrl promoter region, and a control site 8 kilobases downstream of the final exon of Ccbl1 (encoding cyclin B1).

Luciferase reporter assay. Two putative Foxm1-binding sites were identified by searching of the consensus Foxm1-binding site [T(G/A)TTT(G/A)TTT] upstream of the Nurrl transcription start site. The corresponding genomic region that included these putative binding sites was amplified by PCR from wild-type C57BL/6 mouse genomic DNA (primer sequences, Supplementary Table 4), then was subsequently subcloned into a pGL3-Basic luciferase reporter vector and called ‘Site 1-pGL3’ or ‘Site 2-pGL3’. ‘Mut-Site 1-pGL3’ was generated by site-directed mutagenesis of the Foxm1-binding site in Site 1-pGL3. Then, the raw data were normalized with the R/Bioconductor software package affy49. Gene-set–enrichment analysis was performed with GSEA v2.0 software (available from the Broad Institute).
The mononuclear cells at the interface were collected and washed with PBS containing 2% FBS once at 300g for 10 min and twice at 200g for 10 min. CD34+ cells were isolated from the mononuclear population by positive selection with a CD34 MicroBead Kit according to the manufacturer’s instructions (Miltenyi Biotec). To obtain high purity, the cells were first passed through the LS column and then through the MS column (Miltenyi Biotec). The purity of CD34+ cells was above 90%, as determined by flow cytometry.

**Lentiviral constructs and packaging.** For generation of the doxycycline-inducible Flag-tagged Nurr1 expression vector, Nurr1 cDNA was obtained by PCR and was cloned into the plasmid pLVX-Tight-Puro (PT3996-5; Clontech). Correct cloning of Nurr1 was confirmed by DNA sequencing. pLVX-Tight-Puro is a lentiviral expression vector that allows tightly regulated, doxycycline-controlled expression of a gene of interest. To generate the vector for the expression of Foxm1-specific shRNA, we first constructed the pLKO.1maxGFP2aPuro vector by cloning a maxGFP2aPuro fragment into the BamHI and NsiI sites of the vector pLKO.1. Then, we cloned Foxm1-specific shRNA (1 or 2) into the vector pLKO.1maxGFP2aPuro (primer sequences, Supplementary Table 4).

For lentivirus production, 293T cells were transfected with the helper plasmid pCMV∆R8.91 and pMD.G through the use of Lipofectamine 3000. The medium was replaced with fresh medium 12 h after transfection. The culture supernatants were collected at 36 h after transfection and were concentrated by ultracentrifugation in a Beckman Optima L-90K ultracentrifuge using a SW32 Ti rotor at 100,000g for 2.5 h, 4°C. Virus pellet was resuspended in fresh medium, and stored at −80°C until use.

**Lentiviral infection of cells.** Human CD34+ cells were cultured in StemSpam serum-free expansion medium (09650; Stemcell Technologies) with 10 ng/ml human stem-cell factor, 20 ng/ml human thrombopoietin, 10 ng/ml fibroblast growth factor 1, 500 ng/ml angiopoietin and 10 ng/ml heparin as described.

For lentiviral infection of human CD34+ cells, lentiviral stock was added to CD34+ cells and then cells were transferred onto 96-well plates coated with RetroNectin (Takara Mirus). After incubation overnight at 37°C, supernatants were removed, and cells were washed with PBS and then resuspended in fresh medium. Similar methods were used for infection of mouse Lin−BM cells. BM cells were collected from Foxm1fl/fl and Foxm1fl/fl Mx1-Cre mice, and samples were depleted of Lin+ cells through the use of Dynabeads (Life Technologies).

**Statistical analysis.** Statistical significance was calculated with the two-tailed Student’s t-test. P values of <0.05 were considered statistically significant. Sample sizes were determined empirically to ensure sufficient statistical power. No samples were excluded specifically from analysis. No inclusion and exclusion criteria for samples were used to predetermine the size of experiments.

47. Qian, Z., Chen, L., Fernald, A.A., Williams, B.O. & Le Beau, M.M. A critical role for Apc in hematopoietic stem and progenitor cell survival. *J. Exp. Med.* **205**, 2163–2175 (2008).
48. Hou, Y. et al. FHL2 regulates hematopoietic stem cell functions under stress conditions. *Leukemia* **3**, 615–624 (2014).
49. Irizarry, R.A. et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4**, 249–264 (2003).
50. Zhang, C.C., Kaba, M., Iizuka, S., Huynh, H. & Ladish, H.F. Angiopoietin-like 5 and IGFBP2 stimulate ex vivo expansion of human cord blood hematopoietic stem cells as assayed by NOD/SCID transplantation. *Blood* **111**, 3415–3423 (2008).