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ORIGINAL ARTICLE

Lysine-specific demethylase 1 restricts hematopoietic progenitor proliferation and is essential for terminal differentiation

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Lysine (K)-specific demethylase 1A (LSD1/KDM1A) has been identified as a potential therapeutic target in solid cancers and more recently in acute myeloid leukemia. However, the potential side effects of a LSD1-inhibitory therapy remain elusive. Here, we show, with a newly established conditional *in vivo* knockdown model, that LSD1 represents a central regulator of hematopoietic stem and progenitor cells. LSD1 knockdown (LSD1-kd) expanded progenitor numbers by enhancing their proliferative behavior. LSD1-kd led to an extensive expansion of granulomonocytic, erythroid and megakaryocytic progenitors. In contrast, terminal granulopoiesis, erythropoiesis and platelet production were severely inhibited. The only exception was monopoiesis, which was promoted by LSD1 deficiency. Importantly, we showed that peripheral blood granulocytopenia, monocytosis, anemia and thrombocytopenia were reversible after LSD1-kd termination. Extramedullary splenic hematopoiesis contributed to the phenotypic reversion, and progenitor populations remained expanded. LSD1-kd was associated with the upregulation of key hematopoietic genes, including *Gfi1b*, *Hoxa9* and *Meis1*, which are known regulators of the HSC/progenitor compartment. We also demonstrated that LSD1-kd abrogated Gfi1b-negative autoregulation by crossing LSD1-kd with Gfi1b:GFP mice. Taken together, our findings distinguish LSD1 as a critical regulator of hematopoiesis and point to severe, but reversible, side effects of a LSD1-targeted therapy.

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INTRODUCTION

Hematopoiesis is a tightly regulated process responsible for the production of mature blood cells. Covalent histone modifications including methylation and acetylation have been shown to have a central role in controlling hematopoiesis. Enzymes with opposing activities dynamically regulate histone modifications. Examples for opposing enzymes are the mixed-lineage leukemia group histone SET-domain methyl transferases and the JARID1 group and LSD1 histone demethylases, which specifically modify histone H3 Lysine 4 (H3K4).[1]

LSD1, the first histone demethylase to be identified,[2] is essential for normal embryonic development[3] and for regulating ES cell self-renewal versus differentiation.[4,5] To our knowledge, the role of LSD1 in adult stem cell maintenance has not yet been addressed. LSD1 specifically demethylates di- and mono-methylated histone H3K4 or H3K9 residues resulting in context-dependent transcriptional repression or activation, respectively.[2,6,7] The methylation status of the H3K4 and H3K27 residues, composing a so-called bivalent domain, in hematopoietic stem and progenitor cells was shown to be capable of restraining lineage-specific gene expression, while exposing the locus to rapid induction.[8–10] Recent *in vitro* studies have demonstrated that the LSD1 H3K4 demethylating activity is directed to specific genes by the key hematopoietic transcription factors, SCL (also called Tal-1) and Gfi1b. Moreover, *in vitro* knockdown of LSD1 disturbed hematopoietic differentiation of several cell lines.[11,12]

We and others have previously demonstrated LSD1 involvement in cancer. High LSD1 expression in primary neuroblastomas was associated with an aggressive clinical course, and pharmacological LSD1 inhibition resulted in reduced neuroblastoma growth in a xenograft model.[13] High LSD1 expression has also been reported for early relapsed prostate cancer, estrogen receptor-negative breast cancer and malignant sarcomas.[6,14–16] Strikingly, two recent studies report a central role of LSD1 in acute myeloid leukemia (AML).[17,18] These data identify LSD1 as a promising target for anticancer therapy. A prerequisite to further develop a LSD1-inhibitory therapy is to determine potential side effects.

To understand the *in vivo* role of LSD1 in adult hematopoiesis and its potential function in malignancies, we developed a conditional LSD1 knockdown mouse model (shLSD1) based on the combination of a TET-ON system with RNA interference technology. This technical approach is capable of facilitating *in vivo* doxycyline-dependent loss-of-function.[19–21] Here, we used this newly established model to investigate the impact of LSD1-kd on adult hematopoiesis.

METHODS

Mice

The transgenic shLSD1 mouse model was generated as outlined in Figure 1a (TaconicArtemis, Cologne, Germany). Genotyping details are specified in the Supplementary Methods. To achieve LSD1-kd *in vivo*, 8 mg

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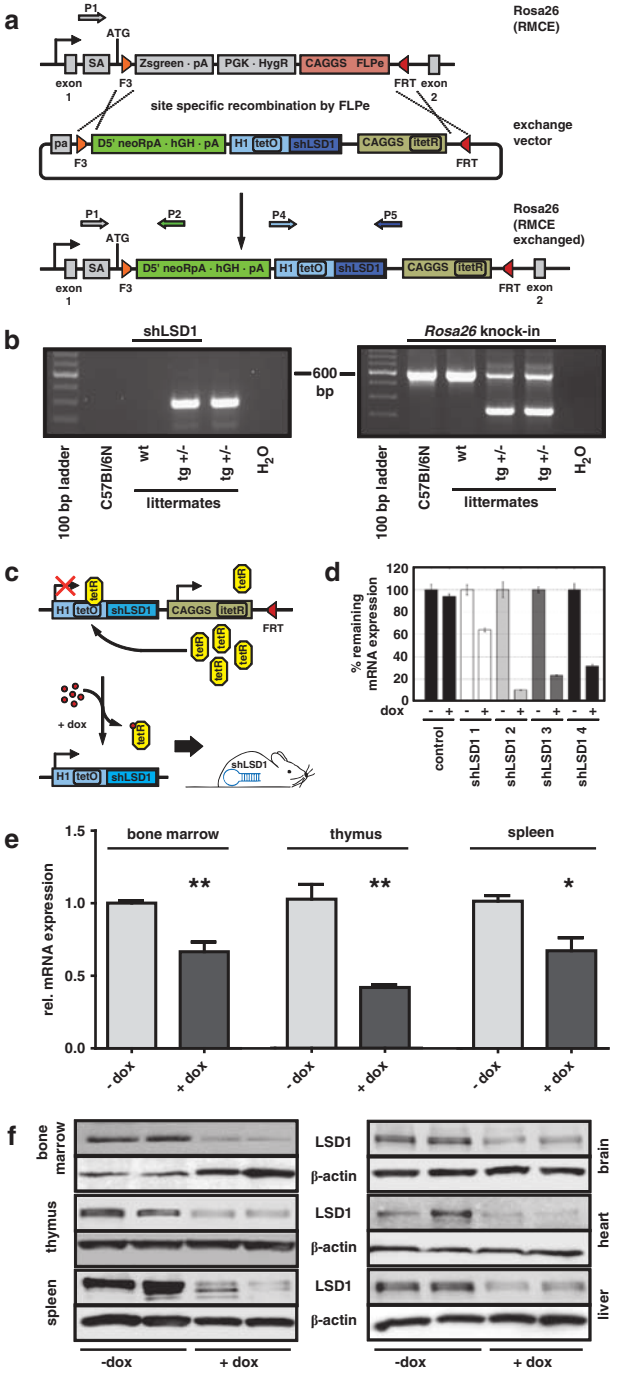
doxycycline (dox, Doxycyclin-Ratiopharm, ratiopharm, Ulm, Germany) or 0.9% NaCl solution (Braun, Melsungen, Germany) as a control were administered to shLSD1 mice (7–13 weeks old) by daily orogastric gavage. Gfi1b:GFP mice were crossed to shLSD1 mice and genotyped as previously described.[22] Mouse experiments were carried out in accordance with the German Animal Welfare Act and approved by the local animal ethics committees.

Additional methods are specified in the Supplementary Information section.

RESULTS

The shLSD1 mouse model facilitates conditional LSD1 knockdown *in vivo*

To generate a model that allows conditional LSD1 loss-of-function studies *in vivo*, four different TET-ON LSD1 short hairpin RNA (shLSD1) expression constructs encompassing different short hairpinRNAs against LSD1 were generated. These constructs were targeted into a pre-engineered *Rosa26* locus within ES cells by recombinase-mediated cassette exchange technology[21] (Figure 1a). Targeted ES-cell clones and mice were genotyped using Southern blotting (data not shown), *Rosa26* knock-in PCR[23] or shLSD1-specific PCR genotyping (Figure 1b). In the absence of dox, the transcription of LSD1-specific shRNA (shLSD1) in the construct is blocked by the tet repressor. Dox exposure removes the tet repressor from the tet-operator sequences allowing shLSD1 expression (Figure 1c). ES cell clones with the different shLSD1 constructs were treated with dox *in vitro*. The clone with shLSD1 construct 2 was most efficienct at LSD1 messenger RNA (mRNA) knockdown (Figure 1d). This clone was used to generate shLSD1 knockdown mice. From the offspring, one male mouse with shLSD1 germ-line transmission was used to establish a colony. Compared with control treatment, Dox treatment significantly decreased LSD1 mRNA expression in hematopoietic organs (Figure 1e) and LSD1 protein expression in the hematopoietic organs, brain, heart and liver (Figure 1f), confirming *in vivo* inducible LSD1-kd activity.



LSD1 knockdown results in expansion of hematopoietic stem and progenitor cells

To understand LSD1 histone demethylase functionality in bone marrow HSC and progenitor regulation, we analyzed the HSC compartment of dox-treated shLSD1 mice compared with controls by flow cytometry (Figure 2a). Strikingly, LSD1-kd for 4 weeks caused extensive expansion of progenitors and HSCs, and this expansion was most pronounced in the most immature LSK $CD48^-CD150^+$ compartment (Figures 2b and c). However, the LSD1-kd progenitor expansion was not associated with a significant increase of marrow cellularity ($-$ dox ($n=5$): $6.4 \pm 1.1 \times 10^7$, $+$ dox ($n=4$): $7.5 \pm 1.2 \times 10^7$ per tibiae and femora, $P=0.22$). To assess whether expanded LSD1-kd progenitors and HSCs are mobilized, we analyzed peripheral blood for the presence of $c\text{-}kit^+ lineage^- Sca\text{-}1^-$ progenitor and LSK HSC cells (Figure 2d). Circulating progenitors and LSK HSCs were increased by more than 100-fold and 250-fold in LSD1-kd blood, respectively (Figure 2e). In contrast to this dramatic rise of blood HSCs and progenitors, white blood cells were only slightly increased ($-$ dox, $5.4 \pm 1.3/\mu l$; $+$ dox, $12.3 \pm 3.0/\mu l$; $P=0.01$) in LSD1-kd blood. The most likely cause of HSC expansion was altered proliferative behavior. Thus, ki67 and Hoechst staining were used to assign cells to G_0, G_1 or $S/G_2/M$ cell cycle phases[24,25] (Figure 2f). The G_0

Figure 1. Conditional LSD1 knockdown *in vivo*. (**a**) Schematic representation of the generation of the transgenic shLSD1 targeting construct. Four different LSD1-specific shRNAs were cloned into a tetracycline-regulatable construct and targeted to the *Rosa26* locus of ES cells by recombinase-mediated cassette exchange (RMCE). Locations of genotyping primers used are indicated. (**b**) Targeted alleles were identified by standard *Rosa26* knock-in (Primers P1, P2 (P3 not shown, binding *Rosa26* wild-type allele)) and specific shLSD1 (P4, P5) genotyping PCR protocols. (**c**) Schematic drawing of the TET-ON system used to induce the LSD1-specific shRNA *in vivo*. (**d**) *In vitro* knockdown efficiency (after 1 μg/ml dox for 72 h) of the four different shLSD1 constructs targeted into ES cells. Residual LSD1 mRNA expression was analyzed by real-time PCR, and the ES clone with the highest knockdown efficiency (shLSD1 2) was used to generate shLSD1 mice. (**e**) *In vivo* hematopoietic LSD1-kd was achieved in bone marrow, thymus and spleen by daily orogastric dox application (8 mg, $n=4$ mice) compared with control (0.9% NaCl solution, $n=4$ mice) for 9 days. (**f**) Dox-induced knockdown of LSD1 protein expression in tissues after 9 days of dox ($+$ dox) or control ($-$ dox) treatment of shLSD1 mice was assessed by western blotting. β-actin was used as a loading control. $^*P<0.05$; $^{**}P<0.01$.

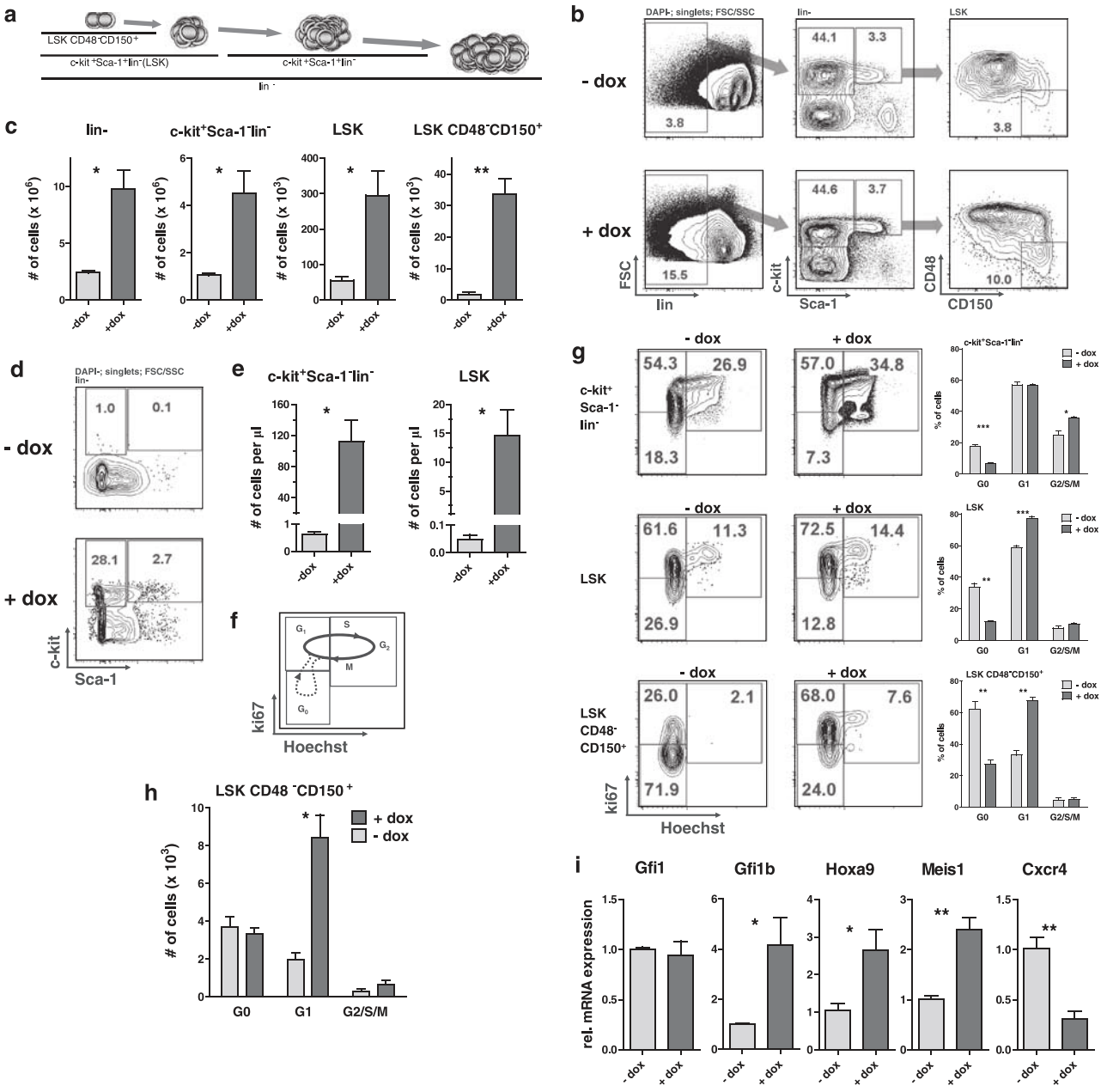


Figure 2. LSD1 knockdown leads to an expansion of the hematopoietic stem and progenitor compartment. (**a**) Hierarchy and surface marker characteristics of progenitor and HSC populations analyzed. Rare long-term repopulating HSCs are characterized by their lineage$^-$Sca-1$^+$ c-kit$^+$ (LSK) CD48$^-$CD150$^+$ surface marker phenotype while the LSK, the c-kit$^+$lineage$^-$Sca-1$^-$ and lineage$^-$ cell populations define hierarchical stem and progenitor compartments with decreasing self-renewal potential. (**b**) Representative plots and (**c**) quantification of bone marrow progenitors (lin$^-$ and c-kit$^+$Sca-1$^-$lin$^-$) and HSCs (LSK and LSK CD48$^-$CD150$^+$) from shLSD1 mice after 27 days of control ($-$dox, $n=5$) or dox ($+$dox, $n=4$) treatment. (**d**) Representative plots and (**e**) quantification of circulating c-kit$^+$Sca-1$^-$lin$^-$ progenitors and LSK HSCs in the peripheral blood from shLSD1 mice treated 27 days with vehicle ($-$dox, $n=5$) or dox ($+$dox, $n=4$). (**f**) Experimental flow cytometric strategy to define the cell cycle status of progenitor and HSC populations using Hoechst and intracellular ki67 staining. (**g**) Representative cell cycle plots and corresponding quantification of cell cycle distributions (bone marrow progenitor and HSC populations; shLSD1 mice, 9 days treatment; $-$dox, $n=4$; $+$dox, $n=4$). (**h**) Absolute bone marrow cell numbers of G_0, G_1 and $S/G_2/M$ HSCs (LSK CD48$^-$CD150$^+$). (**i**) Real-time PCR expression data (bone marrow) of genes regulating the HSC and progenitor pool size (shLSD1 mice; 9 days treatment; $-$dox, $n=4$; $+$dox, $n=4$). $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

HSC-fraction contains the majority of the stem cell activity.[24] To capture the early impact of LSD1-kd on HSC cycling, we treated mice for 9 days with dox. Even this relatively short LSD1-kd induced a 2- to 5-fold expansion of HSC/progenitor cells (data not shown). As expected, the more immature the cell population, the larger the proportion of quiescent cells (% G_0 cells). Strikingly, LSD1-kd consistently reduced the proportion of quiescent G_0 cells throughout all HSC/progenitor populations analyzed by more than 50%. Concordantly, the proportion of LSK and LSK CD48$^-$CD150$^+$ cells actively entering the cell cycle (G_1) increased significantly. In line with a previous report that the G_0–G_1 transition is prolonged in HSCs,[26] the proportion of $S/G_2/M$ cells within the LSK and LSK CD48$^-$CD150$^+$ populations were relatively low. The prolonged transition may be the reason why LSD1-kd did not significantly

affect the proportion of actively proliferating cells. LSD1-kd did result in a significant proportional increase of c-kit$^+$lineage$^-$ Sca-1$^-$ progenitors in the S/G_2/M phase (Figure 2g). The absolute number of LSK CD48$^-$CD150$^+$ cells in G_0, following LSD1-kd was determined to resolve whether the relative decrease in the G_0 fraction was genuinely associated with a loss of quiescent HSCs. Intriguingly, the absolute number of marrow LSK CD48$^-$ CD150$^+$ cells in G_0 was unaffected by LSD1-kd, but the number of LSK CD48$^-$CD150$^+$ cells in G_1 was remarkably increased (Figure 2h).

To elucidate how LSD1 deficiency mediates the expansion of more mature HSCs and hematopoietic progenitors, we analyzed expression of important regulatory genes in the bone marrow after 9 days of LSD1-kd. LSD1 has been previously shown to mediate the repressor activity of the Gfi1 and Gfi1b transcription factors in cell lines, and especially Gfi1b autoregulation.[11] Consistent with these *in vitro* findings, we observed more than a 4-fold de-repression of Gfi1b in dox-treated shLSD1 mice (Figure 2i). Gfi1 restricts progenitor expansion via *HoxA9* and *Meis1* gene repression,[28] which is likely mediated by LSD1 since LSD1-kd upregulated *HoxA9* and *Meis1* (Figure 2i). The downregulation of *CXCR4* (Figure 2i) that we observed could explain the diminished bone marrow retention of LSD1-kd progenitors and HSCs.[29] Taken together, *in vivo* LSD1-kd results in the expansion and mobilization of hematopoetic progenitors and stem cells in association with the upregulation of central hematopoietic regulatory genes. Despite the dramatic increase of more mature HSC and progenitor cell numbers, the size of the dormant HSC pool remained unaffected by LSD1 depletion.

LSD1 knockdown enhances monopoiesis while perturbing terminal granulopoiesis

Cytospin preparations of bone marrow from LSD1-kd mice were analyzed to study progenitor cell alterations after knockdown. As expected, LSD1-kd bone marrow contained a high proportion of blast–like cells. Strikingly, maturing granulocytes with banded or segmented nuclei were completely absent (Figure 3a). This prompted us to analyze LSD1-kd granulomonopoiesis in more detail. Quantification of granulomonocytic progenitors (GMPs) using high-resolution cytometry[30] (Supplementary Figure S1) revealed that LSD1-kd increased GMPs by twofold above controls. We assessed myeloid progenitors, which when committed to the monocytic/dendritic cell lineage (MDP) express the M-CSF receptor (CD115).[31] MDPs were increased threefold in LSD1-kd marrow (Figure 3b). We also assessed non-lymphoid/-erythroid terminal granulocytes (CD11b$^+$Ly6G$^+$) and monocytes (CD11b$^+$ Ly6G$^-$CD115$^+$, Figure 3c). We confirmed the morphological finding from bone marrow cytospins that LSD1-kd almost completely disrupted granulopoiesis in the bone marrow (Figure 3c). Both mature CD11b$^+$Ly6G^{high} granulocytes and immature CD11b$^+$Ly6G^{intm} granulocytic cells were significantly reduced in LSD1-kd bone marrow (Figures 3c and d). In contrast, CD11b$^+$Ly6G$^-$CD115$^+$ monocytic cells were expanded by more than sixfold in LSD1-kd marrow (Figure 3d). As expected, LSD1-kd blood smears (Supplementary Figure S2) and flow cytometric analyses (Figure 3e) revealed monocytic cells as the predominating mature myeloid blood cell type. Additionally, abnormal leukocytes in LSD1-kd blood smears frequently exhibited bilobular, pelgroid and pleomorphic nuclei (Supplementary Figure S2). The depletion of CD11b$^+$Ly6G^{high} granulocytes in the blood did not seem to be as prominent as that observed in bone marrow, and the number of immature CD11b$^+$Ly6G^{intm} granulocytes was elevated in LSD1-kd blood, suggesting premature exit from the bone marrow or extramedullary granulopoiesis (Figures 3e and f). We also assessed LSD1-kd bone marrow cells *ex vivo* in myeloid colony-formation assays, and observed a decreased granulocytic and enhanced monocytic contribution to myeloid colony formation (Supplementary Figure S3). In line with the almost complete depletion of granulocytic cells in LSD1-kd bone marrow, LSD1-kd also significantly downregulated the expression of genes preferentially expressed in granulocytes (Figure 3g). Taken together, the data demonstrate that LSD1 is required for terminal granulopoiesis and that LSD1 has an important role in the granulocytic versus monocytic lineage decision.

Expanded early and inhibited terminal erythropoiesis in LSD1 knockdown mice

As the hematopoietic regulators, SCL and Gfi1b, are known to interact with LSD1 *in vitro* and perform essential regulatory functions in erythropoiesis,[27,32] we analyzed erythropoiesis in shLSD1 mice in more detail. We examined nine sequential stages of erythropoiesis using a combination of previously established markers (Supplementary Figure S4A). The PreMegE (1), PreCFU-E (erythroid colony-forming unit progenitors) (2) and CFU-E (3) early erythroid progenitors were studied according to the strategy described by the Bryder group[30] (Supplementary Figure S1). To achieve high resolution in separating stages (4) through (9) of terminal erythropoiesis, we assessed c-kit[33] and CD44[34] markers along side the widely used combination of CD71 and Ter119.[35] We used the membrane-permeant SYTO16 nucleic acid dye to assess enucleation[36] (Supplementary Figure S4A). LSD1-kd caused an expansion of PreMegE (1), PreCFU-E (2) and, most strikingly, phenotypic CFU-E (3) progenitors (Figures 4a and b). This erythroid expansion was sustained at the proerythroblast stages of ProE (4–5); however, subsequent marrow erythroid precursor stages (6–7) were relatively underrepresented (Figure 4c). We derived fold-change data to compare successive terminal erythroid developmental stages. ProE cells, both positive and negative for c-kit expression, were expanded in the bone marrow from LSD1-kd mice, while basophilic, polychromatic and orthochromatic erythroblast numbers were unaltered (Figure 4d). In contrast, the numbers of Ter119$^+$SYTO19$^-$CD44$^+$CD71$^+$ bone marrow reticulocytes and blood erythrocytes were significantly reduced in LSD1-kd mice (Figure 4d). These data suggested expansion of early erythroblasts in an effort to compensate for deficient terminal erythropoiesis between the basophilic (6) to orthochromatic (8) stages. Splenic stress erythropoiesis was implied by the splenomegaly observed in LSD1-kd mice (Figure 4e). In fact, splenic erythropoiesis was dramatically augmented. Mirroring our results from LSD1-kd bone marrow, enhancement of erythropoiesis in the spleen declined after the c-kit$^+$ ProE stage was reached (Figure 4d). Even short-term LSD1-kd (9 days) produced similar results, though not as profoundly (Supplementary Figure S4B). Insufficient terminal erythropoiesis was further underscored by the presence of circulating nucleated erythroblasts in LSD1-kd peripheral blood, possibly due to deficient enucleation (Figure 4f). Finally, CFU-E colony formation was diminished, reflecting deficient erythropoiesis *in vivo* (Supplementary Figure S5).

We next addressed the possible mechanism behind defective erythropoiesis after LSD1-kd. A drop in cellular Ter119 expression was observed in addition to suppressed erythroid development in the bone marrow of LSD1-kd mice (Figure 4g). Interestingly, lower Ter119 expression is a feature of SCL-deficient erythropoiesis,[37] suggesting cooperation between SCL and LSD1 in regulating Ter119. Even more prominent was the decrease of CD105/endoglin-expression on LSD1-kd CFU-E progenitor cells. Remarkably, LSD1 seems to be differentially involved in CD105 regulation, because CD105 expression remained unchanged in HSCs (Figures 4a and g). One critical gene regulating survival of maturing erythroblasts is *Bcl-x_L*.[38] *Bcl-x_L* expression was significantly reduced in LSD1-kd bone marrow, providing a possible explanation for the decline in terminal erythropoiesis in LSD1-kd mice (Figure 4h). Perturbed

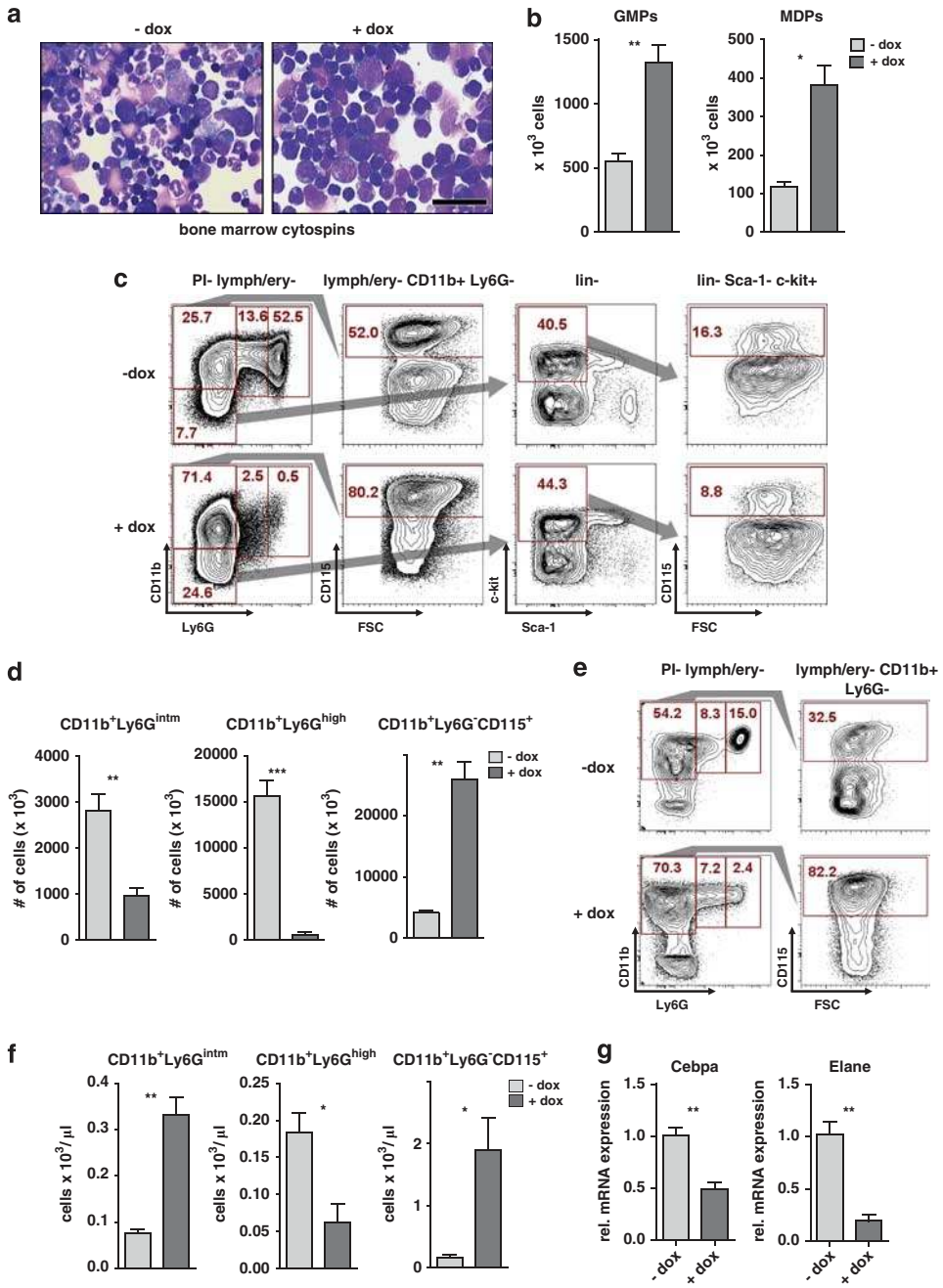


Figure 3. LSD1 knockdown interferes with granulocytic differentiation but promotes the generation of monocytic cells. Analysis of the myeloid compartment in bone marrow (**a**–**d**) and peripheral blood (**e**, **f**) from dox-treated shLSD1 mice (+dox, $n=4$, 27 days) compared with controls (−dox, $n=5$, 27 days). (**a**) Representative images of bone marrow cytospin preparations revealing the absence of terminally differentiated granulocytic cells in LSD1-kd bone marrow. Scale bar, 30 µm. (**b**) Absolute expansion of bone marrow GMPs (defined as illustrated in Supplementary Figure S1) and MDPs (as shown in (**c**)) in LSD1-kd bone marrow. (**c**) Representative plots analyzing the non-lymphoid/erythroid (lymph/ery-) myeloid compartment for monocytic ($CD11b^+Ly6G^-CD115^+$), immature ($CD11b^+Ly6G^{intm}$) and mature ($CD11b^+Ly6G^{high}$) granulocytic cells. MDPs ($c\text{-}kit^+Sca\text{-}1^-lin^-CD115^+$) were defined in parallel in the same stain. (**d**) Quantification of the absolute myeloid cell numbers in bone marrow, demonstrating the simultaneous dramatic drop in terminally differentiated granulocytic cells while monocytic cells increased in LSD1-kd mice. (**e**) Representative analysis of monocytes and immature and mature granulocytes in peripheral blood from LSD1-kd mice. (**f**) Numbers of monocytic and immature granulocytic cells increased in the peripheral blood from LSD1-kd mice, while the number of circulating mature granulocytic cells declined. (**g**) Real-time PCR expression analysis of neutrophil elastase (*Elane*) and C/EBPα (*Cebpa*) (bone marrow, 9 days treatment, −dox, $n=4$ mice; +dox, $n=4$ mice). $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

erythropoiesis in the LSD1-kd bone marrow and spleen resulted anemia (Figure 4i). Next, we studied whether rapidly increasing serum erythropoietin levels could be responsible for the CFU-E and ProE expansion observed after short-term (9 days) dox-treatment of shLSD1 mice. Indeed, we measured a sevenfold increase in the serum erythropoietin in LSD1-depleted mice (Figure 4j). In summary, LSD1-kd resulted in anemia caused by perturbed terminal erythropoiesis, and expansion of early erythroid progenitors was at least in part erythropoietin-driven.

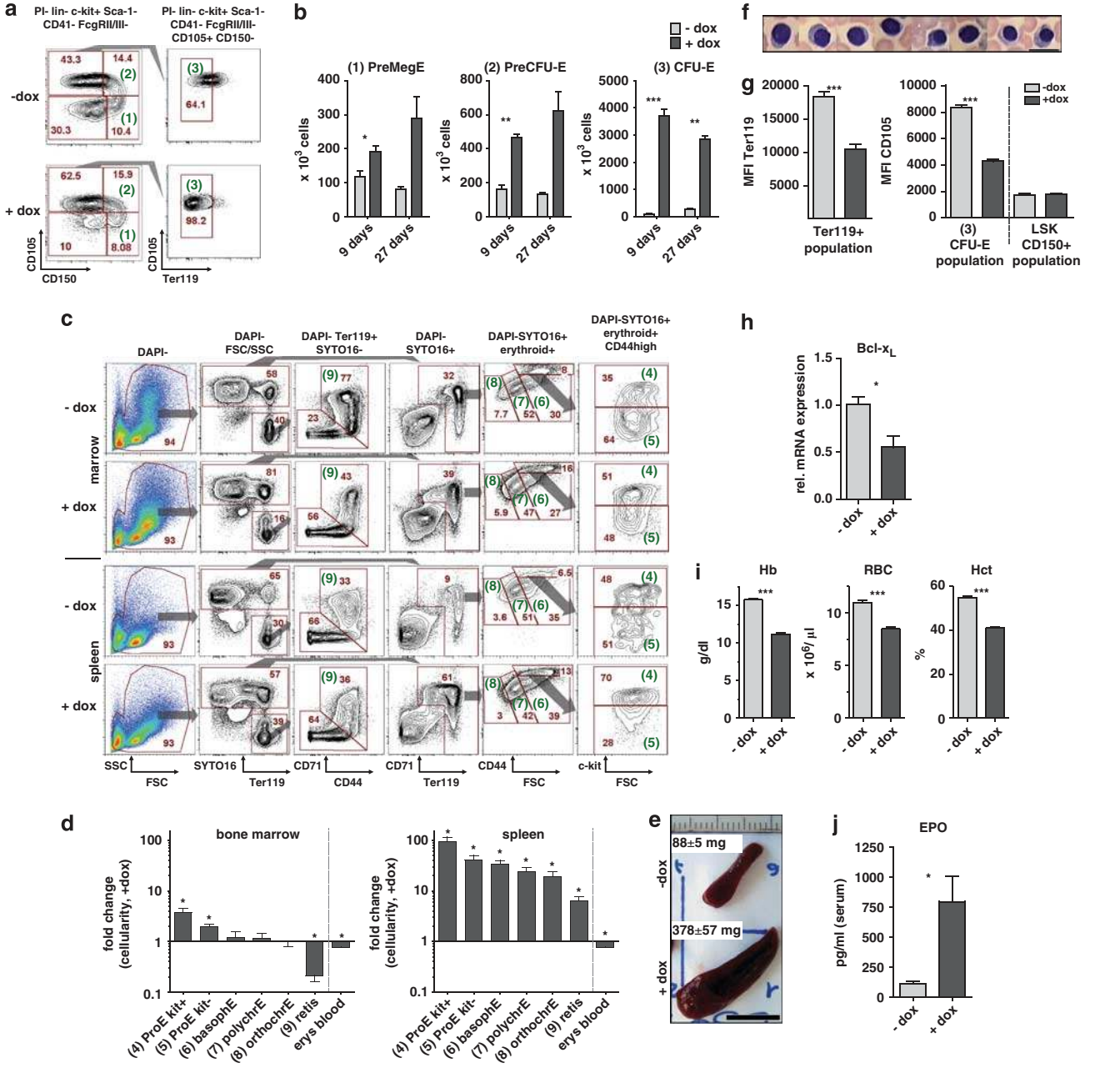


Figure 4. LSD1 knockdown causes anemia by interfering with terminal erythropoiesis. (**a**) Early erythroid progenitors were analyzed by their specific immunophenotype (Supplementary Figure S4A), according to the gating strategy outlined in Supplementary Figure S1. Representative plots displaying PreMegE (1), PreCFU-E (2) and CFU-E (3) populations are shown. (**b**) Quantitative evaluation of PreMegE, PreCFU-E and CFU-E cells in bone marrow of shLSD1 mice after 9 (− dox, $n=4$; + dox, $n=4$) and 27 days (− dox, $n=5$; + dox, $n=3$) of treatment. (**c**) Terminal erythropoiesis from c-kit$^+$ proerythroblasts onwards was assessed by staining bone marrow and spleen with the cell-permeable SYTO16 nucleic acid dye, DAPI and antibodies against Ter119, CD71, CD44 and c-kit (definition of subsets: Supplementary Figure S4A). Representative plots are shown. (**d**) Normalized cellularity data of dox treated shLSD1 mice in relation to control data (27 days treatment; − dox, $n=5$; + dox, $n=4$). (**e**) Spleens of shLSD1-kd mice were enlarged compared with control mice after 27 days of treatment (weight, mean ± s.e.m., $P=0.014$; − dox, $n=5$; + dox, $n=4$). Scale bar, 1 cm. (**f**) Orthochromatic erythroblasts were detectable in peripheral blood smears from LSD1-kd mice after 27 days of dox treatment. Scale bar, 10 µm. (**g**) Downregulation of Ter119 (assessed as median fluorescence intensity) on LSD1-kd erythroblasts (bone marrow Ter119$^+$ cells). Downregulation of CD105 on bone marrow LSD1-kd phenotypic CFU-E cells in contrast to unaltered CD105 expression on LSK CD150$^+$ HSCs (CFU-E gating strategy as shown in Supplementary Figure S1; 27 days treatment; − dox, $n=5$; + dox, $n=4$). (**h**) Decreased bone marrow expression of the *Bcl-x$_L$* erythroid pro-survival factor (real-time PCR; shLSD1 mice; 9 days treatment, − dox, $n=4$; + dox, $n=4$). (**i**) Peripheral blood erythroid parameters (27 days treatment, − dox, $n=5$; + dox, $n=4$). (**j**) Erythropoetin serum levels (9 days treatment, − dox, $n=8$; + dox, $n=8$). *$P<0.05$; **$P<0.01$; ***$P<0.001$.

LSD1 knockdown triggers dysmorphic megakaryocytes that produce insufficient numbers of large platelets

Both erythropoietic and megakaryopoietic lineages originate from a bipotent progenitor population, which was assayed as PreMegE (Supplementary Figure S1). Because LSD1-kd expanded this population (Figure 4b), we further analyzed lineage-specific megakaryocytic progenitors (MkP, Supplementary Figure S1). Similar to the progenitors of the erythroid and granulomonocytic

lineages, MkPs were significantly expanded in LSD1-kd bone marrow (Figures 5a and b). Strikingly, LSD1-kd increased the number of megakaryocytes in the bone marrow by more than sevenfold (Figures 5c and d). Megakaryocytes from control bone marrow exhibited the expected morphological features under light microscopy, including a single, lobulated nucleus surrounded by broad cytoplasm. Contrastingly, megakaryocytes from LSD1-kd bone marrow displayed marginalized hyperlobulated nuclei with a reduced cytoplasmic volume that appeared more basophilic. Some of the LSD1-kd megakaryocytes contained nuclei whose lobes appeared completely separated from each other whereas the nuclear lobes of others appeared to be connected by thin bridges of nucleoplasm (Figure 5e). Under electron microscopy, LSD1-kd megakaryocytes exhibited a dysmorphic demarcation system, a lack of α-granules, an accumulation of perinuclear mitochondria and nuclear protrusions resembling the nucleoplasm bridges observed in the cytospin preparations (Supplementary Figure S6). LSD1-kd peripheral blood platelet counts dropped to 10% of controls and the mean LSD1-kd platelet volume rose (Figure 5f). We compared serum levels of thrombopoietin, the central regulator of thrombopoiesis, in LSD1-kd and control mice. Serum thrombopoietin levels are primarily regulated by receptor-mediated catabolism.[39] Thrombopoietin serum levels were lower in LSD1-kd than in control mice (Figure 5g). This may reflect an increase in c-mpl receptors associated with the dramatic expansion of the marrow megakaryocyte pool. Taken together, LSD1-kd expanded megakaryocytic progenitors, and developing megakaryocytes were dysmorphic and incapable of producing normal platelet numbers.

Reversion of LSD1 knockdown leads to the normalization of peripheral blood parameters

LSD1 has been shown to contribute to the regulation of ES cell multipotency and differentiation by controlling the methylation status of H3K4.[4,5] Similarly, the H3K4 methylation status of promoter regions was reported to regulate hematopoietic stem and progenitor multilineage potential and differentiation.[8–10] Thus, it is likely that LSD1-kd disturbs hematopoiesis by interfering with the tightly regulated H3K4 methylation status of genes critical for early events in hematopoietic stem and progenitor cell differentiation. Because H3K4 demethylation activity at these promoter regions should be reestablished upon reinstatement of LSD1 expression, we were interested whether normal hematopoiesis returns upon normalization of LSD1 levels. We longitudinally examined peripheral blood parameters in 8 shLSD1 mice before and after 20 days of dox treatment and for 39 days following cessation of treatment. A blood cell count and one flow cytometric stain allowed the parallel quantification of HSCs, progenitors, monocytic cells and granulocytes. As expected, LSD1-kd resulted in mobilization of stem and progenitor cells in the peripheral blood. Interestingly, one week after cessation of dox treatment, blood progenitor levels had dropped but HSC levels had risen (Figures 6a and b). Circulating granulocyte levels remained low for a week (d28), then rapidly recovered (d32), whereas the return of elevated monocyte levels to baseline levels took more than 12 days (Figures 6a and c). Recovery from anemia after termination of dox treatment took more than 3 weeks (Figure 6d). Remarkably, platelets increased to baseline levels within 7 days. Platelet levels continued to increase after reaching the baseline level, and were elevated sevenfold, 4 days after reaching baseline (rebound thrombocytosis). Subsequently, platelet levels returned to slightly above baseline levels (>5 weeks after dox termination). Return of the mean platelet volume to baseline levels took more than 3 weeks (Figure 6e). Large platelet aggregates were present in peripheral blood smears during rebound thrombocytosis (Figure 6f). We intended to determine whether the expanded progenitor and stem cell populations in the bone marrow had returned to normal levels on day 59, when all measured blood parameters had returned to baseline levels or slightly above. Wild-type littermates treated with dox (d1–d20) in parallel were used as controls. In contrast to peripheral blood values, bone marrow progenitor and stem cell levels did not normalize and remained significantly expanded 39 days after terminating dox treatment (Figure 6g). Because splenomegaly was prominent in dox-treated shLSD1 mice (Figure 6h), specific subtyping of expanded progenitors was also carried out for the spleen. Strikingly, expansion of CFU-E progenitors almost entirely accounted for total progenitor expansion in LSD1-kd bone marrow, whereas, MkP and GMP progenitors were expanded in addition to CFU-E progenitors in the spleen (Figure 6i). These data infer that splenic hematopoiesis significantly contributed to the recovery from thrombocytopenia, granulocytopenia and anemia induced by LSD1 depletion. The prolonged expansion of both bone marrow and splenic erythroid progenitors that we observed implies that reestablishing normal H3K4 methylation after reversing LSD1-kd is a slow process.

LSD1 knockdown leads to the de-repression of Gfi1b in hematopoietic stem and progenitor cells

By crossing shLSD1 mice to Gfi1b:GFP knock-in mice,[22] we aimed to demonstrate de-repression of essential hematopoietic regulatory genes in hematopoietic stem and progenitor cells by *in vivo* LSD1 depletion. *Gfi1b* is the ideal gene to study this issue, because *Gfi1b* expression was shown to be negatively controlled by autoregulation via LSD1 *in vitro*.[11] *Gfi1b* expression was also shown to be highest in HSCs and megakaryoerythroid progenitors in Gfi1b:GFP mice, and GFP expression directly correlated with *Gfi1b* expression.[22,40] We compared GFP expression in bone marrow progenitor cells from dox-treated shLSD1;Gfi1b:GFP and dox-treated littermate Gfi1b:GFP control mice. Strikingly, LSD1-kd abolished the dichotomy of GFP expression reported for Gfi1b-GFP c-kit$^+$ Sca-1$^-$ lin$^-$ progenitor cells[22] and resulted in an excessive GFP upregulation. LSD1-kd also induced the appearance of a GFP^{high} population in LSK cells. Similarly, GFP expression also increased in the most immature LSK CD48$^-$CD150$^+$ HSC population (Figure 7a). We also confirmed that GFP expression was amplified in the expanded LSD1-kd MkP and CFU-E populations (Figure 7b). *Gfi1b* expression is normally downregulated during terminal erythropoiesis.[22] Thus, downregulation of GFP and the erythroblast immaturity marker, CD44,[34] were expected to proceed in parallel. Remarkably, GFP downregulation proceeded in parallel with decreasing CD44 expression in LSD1-kd erythroblasts, but from a higher level of GFP expression (Figure 7c). These data demonstrate that loss of LSD1 function *in vivo* leads to overexpression of the critical hematopoietic regulator, Gfi1b, via de-repression. Taken together, LSD1-kd caused deregulation of critical hematopoietic genes leading to severe alterations in hematopoiesis (Figure 7d). We excluded that dox alone affected the hematopoietic parameters, analyzed by comparing dox- and control-treated wild-type mice (Supplementary Figure S7). Moreover, we demonstrated that the abnormalities described for LSD1-kd mice are intrinsic to the hematopoietic system by transplanting shLSD1 bone marrow into wild-type recipients (Supplementary Figure S8).

DISCUSSION

Previous *in vitro* studies suggested an important function for LSD1 in hematopoiesis.[11,12,41] A constitutive LSD1-knockout model was embryonic lethal, precluding the analysis of LSD1 function within adult organ systems.[3] To investigate LSD1 function in adult mice, we generated a stable conditional LSD1-kd model (shLSD1), using reversible RNA-interference technology. This LSD1-kd approach does not require tedious breeding strategies and has previously been

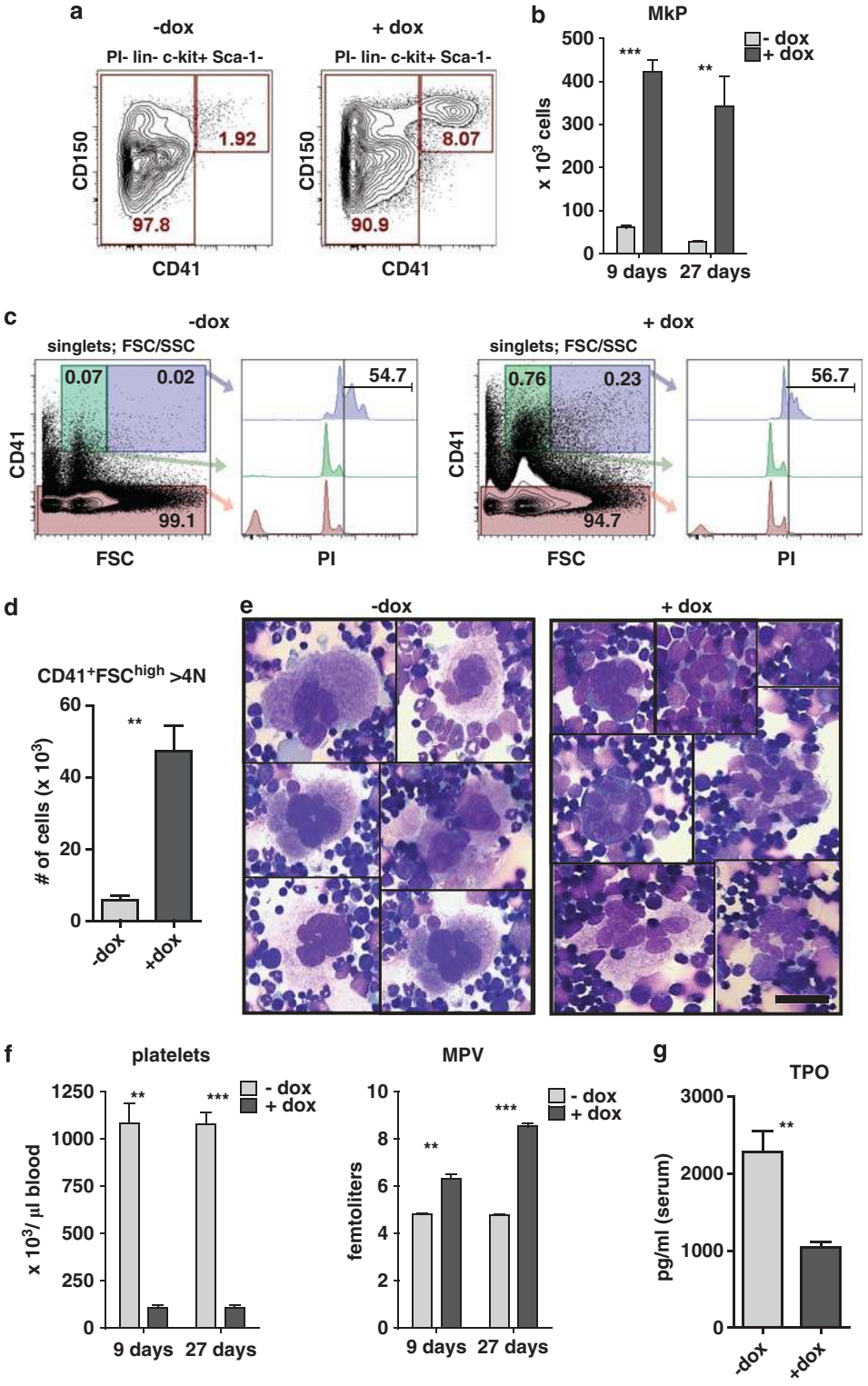


Figure 5. Induced LSD1 knockdown leads to severe macrothrombocytopenia associated with extensive expansion of bone marrow megakaryocytes. (**a**) Representative flow cytometric characterization of MkPs of dox treated compared with control treated shLSD1 mice (27 days). (**b**) Rapid and sustained expansion of bone marrow MkPs in LSD1-kd mice compared with controls after 9 (− dox, $n=4$; + dox, $n=4$) and 27 days (− dox, $n=5$; + dox, $n=3$) of treatment. (**c**) Representative analysis of polyploid (>4N) megakaryocytes (blue, $CD41^+FSC^{high}$) in comparison to smaller presumably megakaryocytic precursors (green, $CD41^+FSC^{low}$) and non-megakaryocytic total bone marrow cells (red, $CD41^-$). (**d**) Substantial increase in megakaryocyte numbers (defined as $CD41^+FSC^{high}$>4N) in LSD1-kd bone marrow compared with controls (cell numbers per tibiae, 9 days treatment, − dox, $n=4$; + dox, $n=4$). (**e**) Representative images of bone marrow cytospin preparations showing abnormal megakaryocyte morphology in LSD1-kd mice compared with controls (27 days of treatment). Black scale bar, 30 µm. (**f**) Peripheral blood analysis revealed macrothromobocytopenia in shLSD1-kd mice after 9 (− dox, $n=4$; + dox, $n=4$) and 27 days (− dox, $n=5$; + dox, $n=4$) of treatment (MPV, mean platelet volume). (**g**) Decreased thrombopoetin (TPO) plasma levels in LSD1-kd mice compared with controls (9 days treatment, − dox, $n=8$; + dox, $n=8$). $^{**}P<0.01$; $^{***}P<0.001$.

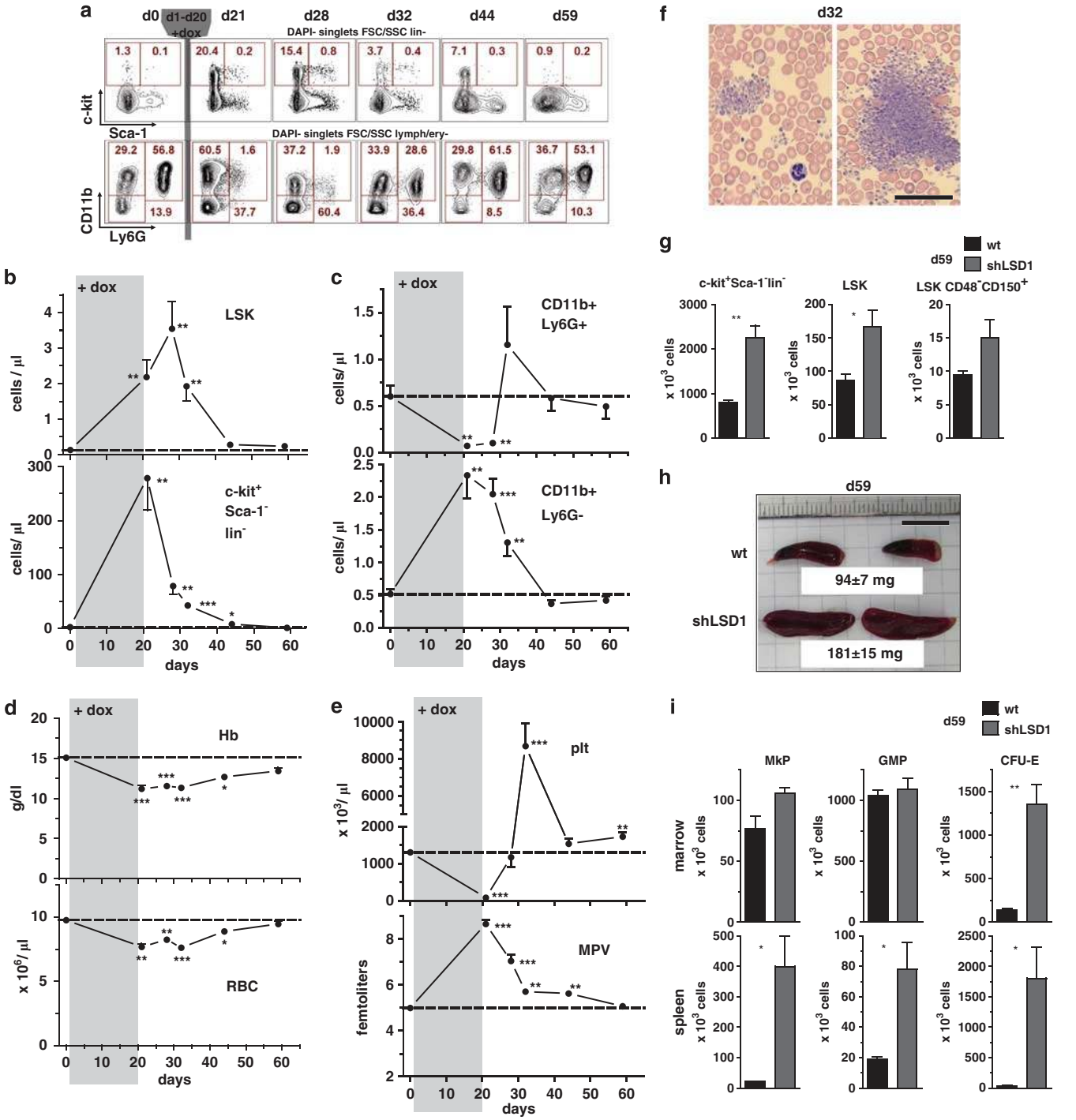
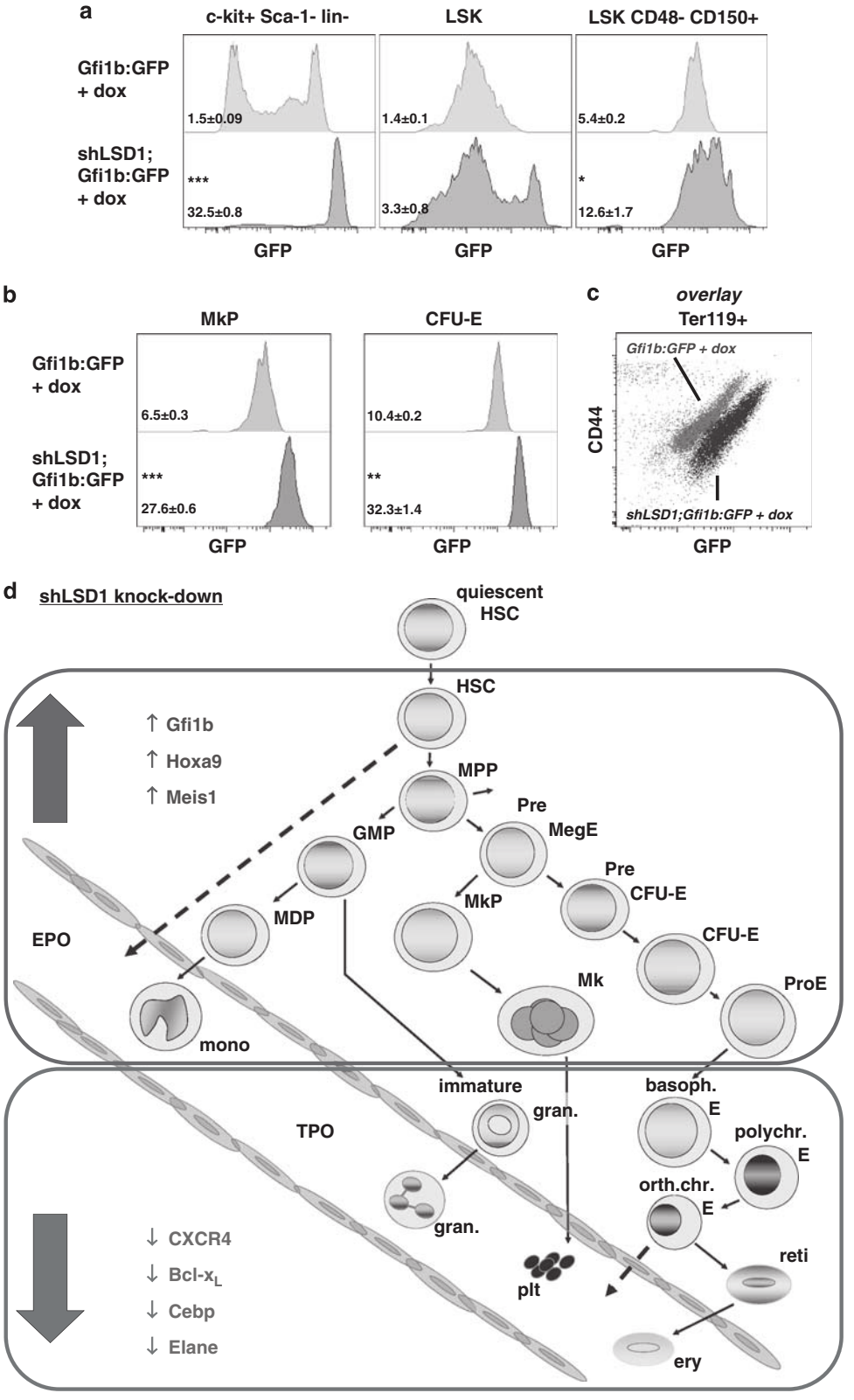


Figure 6. Reversion of LSD1 knockdown leads to recovery of peripheral blood abnormalities through sustained extramedullary hematopoiesis. (**a**) Peripheral blood parameters were longitudinally analyzed at different time points before (day(d) 0) and after (d21, d28, d32, d44 and d59) dox treatment (d1–d20; $n = 8$ shLSD1 mice). Peripheral blood count and the parallel evaluation of HSCs (LSK), progenitors (c-kit$^+$Sca-1$^-$lin$^-$), granulocytes (CD11b$^+$Ly6G$^+$lymph$^-$) and monocytes (CD11b$^+$Ly6G$^-$lymph$^-$), using flow cytometry was performed per time point and mouse. The longitudinal flow cytometry data of a representative shLSD1 mouse are shown. Longitudinal peripheral blood quantification of (**b**) HSCs, progenitors, (**c**) granulocytes, monocytes, (**d**) hemoglobin (Hb), red blood cells (RBCs), (**e**) platelets (plt) and mean platelet volume (MPV) is shown. (**b**–**e**) Statistical analysis was performed by paired analysis in relation to baseline values. (**f**) Representative blood smears from shLSD1 mice with rebound thrombocytosis on day 32 (12 days post-dox cessation). Thrombocytosis was the probable cause of death of $n = 2$ shLSD1 mice during the blood taking procedure, due to cerebral thromboembolism. Scale bar, 30 µm. (**g**) On day 59, when all measured blood parameters had reached or exceeded baseline levels, shLSD1 bone marrow progenitor and stem cells ($n = 6$ mice) were analyzed in comparison to wild-type littermates (wt; $n = 3$) that received the identical dox treatment (d1–d20). (**h**) In spite of LSD1-kd reversion (no dox treatment for 39 days), LSD1-kd splenomegaly was still evident (shLSD1 mice, $n = 6$; wt, $n = 3$; weight, mean ± s.e.m.; $P = 0.001$). Scale bar, 1 cm. (**i**) Analysis of hematopoietic progenitors revealed MkP and GMP numbers in the bone marrow from shLSD1 mice close to the those of wild-type controls. However, CFU-E numbers in both bone marrow and spleen and MkP and GMP numbers in only the spleen were significantly increased. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

used to establish efficient loss-of-function models.[19–21] This system offers two primary advantages: (1) the knockdown can be rescued at an arbitrary time point and (2) this system presumably models pharmacological inhibition more closely than genetic mutagenesis. This is particularly relevant, because previous studies established LSD1 as a potential drug target for cancer treatment.[13–18,42]

Continuous dox treatment of shLSD1 mice resulted in dramatic changes within different hematopoietic compartments. Mechanistically, enhanced expression of hematopoietic regulatory genes in these mice is presumably caused by the lack of LSD1 repressive activity at H3K4 residues in the gene promoters. Most strikingly, hematopoietic abnormalities in LSD1-kd mice



phenocopied alterations observed in Gfi1 and Gfi1b loss-of-function models. To some extent, this was expected because LSD1 was shown to be a major facilitator of Gfi-mediated transcriptional repression.[11] However, ChIP-on-chip experiments using an erythroid cell line showed that only 21% of LSD1 transcriptional targets were co-occupied by Gfi1b,[11] indicating that LSD1 probably regulates a number other targets independently of Gfi1b. This same study also reported that LSD1-kd in hematopoietic cell lines inhibited erythroid differentiation, which is in concordance with the deficiency, we observed in terminal erythroid differentiation in LSD1-kd mice. The inhibition of megakaryocytic differentiation induced by LSD1-kd *in vitro* only partially reflects our *in vivo* findings because LSD1-kd inhibited gpIIb/CD41 expression in the megakaryoblastic cell line, L8057, whereas we detected normal gpIIb/CD41 expression by murine LSD1-kd megakaryocytes. Saleque *et al.* described enhanced granulocytic differentiation after *in vitro* LSD1-kd,[11] which contrasts our finding of almost completely abolished terminal granulopoiesis upon *in vivo* LSD1-kd. Intriguingly, a hallmark of Gfi1-null mice is inhibited granulocytic differentiation together with enhanced monocytic differentiation.[43] Gfi1-null mice further display myeloid progenitor expansion associated with Hoxa9 overexpression,[28] similar to our LSD1-kd model. As Gfi1 and LSD1 are known to directly interact[11] and LSD1-kd mice mirror myeloid abnormalities of Gfi1-deficient mice, LSD1 presumably represents a key mediator of Gfi1 function in myelopoiesis.

It was shown that Gfi1 and Gfi1b are both negative regulators of HSC proliferation.[43] However, Gfi1-deficient HSCs displayed impaired self-renewal capacity in competitive repopulation assays whereas the self-renewal of Gfi1b-deficient HSCs remained unaltered.[40] Interestingly, conditional deletion of Gfi1b resulted in expanded phenotypic HSCs, which parallels our findings in LSD1-kd mice. We observed decreased G_0 and increased G_1 cell cycle fractions within LSD1-kd HSCs. However, in consideration of increased HSC numbers, we show that the absolute number of dormant G_0 HSCs in LSD1-kd bone marrow remained unaffected. Maybe LSD1 is not expressed in these dormant cells containing the majority of stem cell activity within the murine bone marrow.[24] The quiescent G_0 HSC compartment of Gfi1b-deficient HSCs was not directly assayed, but in competitive repopulation assays, the multilineage-repopulation capacity of Gfi1b-deficent HSCs was comparable to controls.[40] This suggests that neither the loss of Gfi1b nor LSD1 function expands the dormant long-term repopulating HSC compartment. In contrast, we show HSC and progenitor compartments hierarchically downstream to be dramatically increased in LSD1-kd mice. Detailed data regarding adult progenitor compartments in the Gfi1b loss-of-function context have not yet been published. Nevertheless, the severe thrombocytopenia and anemia of Gfi1b-null mice[40] reflect our findings in LSD1-kd mice. Also in concordance with our findings in adult LSD1-kd mice, analysis of the constitutive Gfi1b-null embryos revealed that Gfi1b is a key regulator of megakaryopoiesis and definitive erythropoiesis.[27] Taken together, these remarkable similarities between the Gfi1b-null and LSD1-kd phenotypes imply that LSD1 cooperates with Gfi1b *in vivo* to control stem/progenitors, megakaryo- and erythropoiesis.

Here, we demonstrated that LSD1-kd dramatically potentiates the size of lineage-committed progenitor compartments. This particularly applied to the erythroid and megakaryocytic progenitors. This expansion is probably at least partly driven by the effort to counteract the LSD1-kd terminal differentiation defect (for example, by elevating erythropoietin levels). However, the rapidity and extent of progenitor expansion suggest that LSD1-kd has a cell-intrinsic impact on progenitor pool size. To confirm that LSD1 deficiency is associated with deregulated expression of key genes, we crossed shLSD1 and Gfi1b:GFP mice, allowing us to directly trace Gfi1b expression within specific populations. Negative autoregulation of Gfi1b was shown to be mediated by LSD1 and associated with an altered H3K4 methylation status *in vitro*.[11] Strikingly, Gfi1b expression was deregulated in the entire LSD1-kd c-kit$^+$ Sca-1$^-$ lin$^-$ progenitor compartment. Enforced expression of a Gfi1b mutant lacking its LSD1-binding SNAG domain enhanced early erythoid progenitor proliferation,[11,44] implying that elevated Gfi1b expression might well be responsible for the expanded erythroid progenitor compartment in LSD1-kd mice.

Two recent studies used pharmacologic inhibitors to validate LSD1 as a target for AML therapy.[17,18] Schenk *et al.*[17] used the approved antidepressant drug tranylcypromin (TCP) while Harris *et al.*[18] used recently developed, more potent and more LSD1-specific TCP analogs. Inhibition of LSD1 enzymatic activity by the novel TCP analogs was up to 160-fold greater than the inhibition by TCP. In line with these pharmacologic data, Harris *et al.*[18] noted that leukemic mice treated with the novel TCP analogs were anemic and thrombocytopenic whereas Schenk *et al.*[17] treating human AML xenografted mice with TCP did not report the occurrence of hematologic side effects. We observed anemia and thrombocytopenia in our LSD1-kd model. Therefore, the LSD1 activity decline in our transgenic RNA interference model is probably stronger than the decline of LSD1 activity achieved with TCP and might be more comparable to that achieved by potent LSD1 inhibitors. This notion is further supported by the repressed *in vitro* erythroid colony formation induced by the potent TCP analogs.[18] However, in contrast to our findings granulomonocytic colony formation was reported to be unaltered by TCP analog exposure. Perhaps a monocytic lineage bias of granulomonocytic colonies was overseen because morphological or immunophenotypic colony analyses on the cellular level were not performed.[18]

Our data demonstrate that in normal development, LSD1 restrains progenitor proliferation and is required to foster differentiation presumably by repressing genes not affiliated with the respective lineage. The LSD1 depletion-induced progenitor hyperplasia associated with dysplastic morphological alterations and peripheral multi-lineage cytopenia is reminiscent of myelodysplastic syndrome (MDS). To our knowledge, LSD1 mutations or altered expression levels have not yet been reported in MDS patients. It will be interesting to investigate whether MDS

Figure 7. LSD1 knockdown causes deregulated activity of the *Gfi1b* locus in hematopoietic progenitors and stem cells. GFP expression of (**a**) stem and progenitor and (**b**) MkP, phenotypic CFU-E and (**c**) Ter119$^+$ populations was analyzed in dox-treated (15 days) shLSD1;Gfi1b:GFP mice ($n=4$) in comparison to dox-treated control Gfi1b:GFP mice ($n=4$). Numbers displayed on plots represent median fluorescence intensity values ($\pm$ s.e.m.). (**d**) Schematic drawing summarizing the impact of conditional LSD1-kd on hematopoiesis. LSD1-kd resulted in disturbed expression of crucial hematopoietic regulatory genes associated with a striking stem/progenitor expansion. However, quiescent long-term repopulating HSCs remained unaltered. Immature as well as lineage-restricted progenitors contributed to the expansion. With the exception of monopoiesis, terminal differentiation of the granulocytic, megakaryocytic and erythroid lineages were severely perturbed. Arrows with solid lines, precursor-product relationship; Arrows with dashed lines, egress of cells into the peripheral blood. MPP, multipotent progenitors; MDP, monocytic/dendritic progenitors; mono, monocytes; PreMegE, pre-megakaryoerythroid progenitors; PreCFU-E, pre-erythroid colony-forming unit progenitors; ProE, proerythroblasts; Mk, megakaryocytes; EPO, erythropoietin; TPO, thrombopoetin; gran., granulocytes; basoph. E, basophilic erythroblasts; polychr., polychromatic; orth.chr., orthochromatic; reti, reticulocytes; ery, erythrocytes; plt, platelets. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

pathogenesis is associated with LSD1 abnormalities in future studies. Decreased LSD1 activity might be involved in early leukemogenesis because LSD1 depletion resulted in myeloid progenitor expansion by deregulating HoxA9 and Meis1. However, two recent reports identify enhanced LSD1 function in AML as an important component of the malignant leukemic program.[17,18] Thus, a shift from low-to-high LSD1 expression might occur during the transition of MDS to overt AML. LSD1 likely contributes to the malignant phenotype of AML cells by mediating aberrant gene repression[17] or by maintaining the oncogenic program by cooperating with oncogenic fusion proteins such as mixed-lineage leukemia–AF9.[18] Strikingly, LSD1-inhibitor treatment facilitated leukemic blast differentiation with subsequent apoptosis.[17,18] As our results indicate that stronger pharmacological LSD1 inhibition is likely to cause severe hematological toxicity, beneficial therapeutic effects of LSD1 inhibitors will have to be balanced against hematologically toxic effects. Finally, we showed that LSD1-kd led to peripheral blood HSC mobilization suggesting that LSD1 inhibitors could represent an alternative option for patients with suboptimal HSC mobilization, using classical mobilizing agents.

In summary, the newly established shLSD1 mouse model and the dramatic hematopoietic sequelae of LSD1-kd reported here warrant further studies of LSD1 function in both normal hematopoieis and in hematological malignancies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Rice KL, Hormaeche I, Licht JD. Epigenetic regulation of normal and malignant hematopoiesis. *Oncogene* 2007; **26**: 6697–6714.
2 Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA *et al.* Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell* 2004; **119**: 941–953.
3 Wang J, Scully K, Zhu X, Cai L, Zhang J, Prefontaine GG *et al.* Opposing LSD1 complexes function in developmental gene activation and repression programmes. *Nature* 2007; **446**: 882–887.
4 Whyte WA, Bilodeau S, Orlando DA, Hoke HA, Frampton GM, Foster CT *et al.* Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. *Nature* 2012; **482**: 221–225.
5 Adamo A, Sese B, Boue S, Castano J, Paramonov I, Barrero MJ *et al.* LSD1 regulates the balance between self-renewal and differentiation in human embryonic stem cells. *Nat Cell Biol* 2011; **13**: 652–659.
6 Metzger E, Wissmann M, Yin N, Muller JM, Schneider R, Peters AH *et al.* LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. *Nature* 2005; **437**: 436–439.
7 Wissmann M, Yin N, Muller JM, Greschik H, Fodor BD, Jenuwein T *et al.* Cooperative demethylation by JMJD2C and LSD1 promotes androgen receptor-dependent gene expression. *Nat Cell Biol* 2007; **9**: 347–353.
8 Cui K, Zang C, Roh TY, Schones DE, Childs RW, Peng W *et al.* Chromatin signatures in multipotent human hematopoietic stem cells indicate the fate of bivalent genes during differentiation. *Cell Stem Cell* 2009; **4**: 80–93.
9 Weishaupt H, Sigvardsson M, Attema JL. Epigenetic chromatin states uniquely define the developmental plasticity of murine hematopoietic stem cells. *Blood* 2010; **115**: 247–256.
10 Orford K, Kharchenko P, Lai W, Dao MC, Worhunsky DJ, Ferro A *et al.* Differential H3K4 methylation identifies developmentally poised hematopoietic genes. *Dev Cell* 2008; **14**: 798–809.
11 Saleque S, Kim J, Rooke HM, Orkin SH. Epigenetic regulation of hematopoietic differentiation by Gfi-1 and Gfi-1b is mediated by the cofactors CoREST and LSD1. *Mol Cell* 2007; **27**: 562–572.
12 Hu X, Li X, Valverde K, Fu X, Noguchi C, Qiu Y *et al.* LSD1-mediated epigenetic modification is required for TAL1 function and hematopoiesis. *Proc Natl Acad Sci USA* 2009; **106**: 10141–10146.
13 Schulte JH, Lim S, Schramm A, Friedrichs N, Koster J, Versteeg R *et al.* Lysine-specific demethylase 1 is strongly expressed in poorly differentiated neuroblastoma: implications for therapy. *Cancer Res* 2009; **69**: 2065–2071.
14 Kahl P, Gullotti L, Heukamp LC, Wolf S, Friedrichs N, Vorreuther R *et al.* Androgen receptor coactivators lysine-specific histone demethylase 1 and four and a half LIM domain protein 2 predict risk of prostate cancer recurrence. *Cancer Res* 2006; **66**: 11341–11347.
15 Lim S, Janzer A, Becker A, Zimmer A, Schule R, Buettner R *et al.* Lysine-specific demethylase 1 (LSD1) is highly expressed in ER-negative breast cancers and a biomarker predicting aggressive biology. *Carcinogenesis* 2010; **31**: 512–520.
16 Schildhaus HU, Riegel R, Hartmann W, Steiner S, Wardelmann E, Merkelbach-Bruse S *et al.* Lysine-specific demethylase 1 is highly expressed in solitary fibrous tumors, synovial sarcomas, rhabdomyosarcomas, desmoplastic small round cell tumors, and malignant peripheral nerve sheath tumors. *Hum Pathol* 2011; **42**: 1667–1675.
17 Schenk T, Chen WC, Gollner S, Howell L, Jin L, Hebestreit K *et al.* Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia. *Nat Med* 2012; **18**: 605–611.
18 Harris WJ, Huang X, Lynch JT, Spencer GJ, Hitchin JR, Li Y *et al.* The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer Cell* 2012; **21**: 473–487.
19 He G, Luo W, Li P, Remmers C, Netzer WJ, Hendrick J *et al.* Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease. *Nature* 2010; **467**: 95–98.
20 Acehan D, Vaz F, Houtkooper RH, James J, Moore V, Tokunaga C *et al.* Cardiac and skeletal muscle defects in a mouse model of human Barth syndrome. *J Biol Chem* 2011; **286**: 899–908.
21 Seibler J, Kleinridders A, Kuter-Luks B, Niehaves S, Bruning JC, Schwenk F. Reversible gene knockdown in mice using a tight, inducible shRNA expression system. *Nucleic Acids Res* 2007; **35**: e54.
22 Vassen L, Okayama T, Moroy T. Gfi1b:green fluorescent protein knock-in mice reveal a dynamic expression pattern of Gfi1b during hematopoiesis that is largely complementary to Gfi1. *Blood* 2007; **109**: 2356–2364.
23 Soriano P. Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 1999; **21**: 70–71.
24 Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M *et al.* Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* 2008; **135**: 1118–1129.
25 Wilson A, Murphy MJ, Oskarsson T, Kaloulis K, Bettess MD, Oser GM *et al.* c-Myc controls the balance between hematopoietic stem cell self-renewal and differentiation. *Genes Dev* 2004; **18**: 2747–2763.
26 Nygren JM, Bryder D, Jacobsen SE. Prolonged cell cycle transit is a defining and developmentally conserved hemopoietic stem cell property. *J Immunol* 2006; **177**: 201–208.
27 Saleque S, Cameron S, Orkin SH. The zinc-finger proto-oncogene Gfi-1b is essential for development of the erythroid and megakaryocytic lineages. *Genes Dev* 2002; **16**: 301–306.
28 Horman SR, Velu CS, Chaubey A, Bourdeau T, Zhu J, Paul WE *et al.* Gfi1 integrates progenitor versus granulocytic transcriptional programming. *Blood* 2009; **113**: 5466–5475.
29 Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 2006; **25**: 977–988.
30 Pronk CJ, Rossi DJ, Mansson R, Attema JL, Norddahl GL, Chan CK *et al.* Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell* 2007; **1**: 428–442.
31 Waskow C, Liu K, Darrasse-Jeze G, Guermonprez P, Ginhoux F, Merad M *et al.* The receptor tyrosine kinase Flt3 is required for dendritic cell development in peripheral lymphoid tissues. *Nat Immunol* 2008; **9**: 676–683.
32 Hall MA, Curtis DJ, Metcalf D, Elefanty AG, Sourris K, Robb L *et al.* The critical regulator of embryonic hematopoiesis, SCL, is vital in the adult for megakaryopoiesis, erythropoiesis, and lineage choice in CFU-S12. *Proc Natl Acad Sci USA* 2003; **100**: 992–997.
33 Liu Y, Pop R, Sadegh C, Brugnara C, Haase VH, Socolovsky M. Suppression of Fas-FasL coexpression by erythropoietin mediates erythroblast expansion during the erythropoietic stress response in vivo. *Blood* 2006; **108**: 123–133.
34 Chen K, Liu J, Heck S, Chasis JA, An X, Mohandas N. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane

protein expression during erythropoiesis. *Proc Natl Acad Sci USA* 2009; **106**: 17413–17418.
35 Socolovsky M, Nam H, Fleming MD, Haase VH, Brugnara C, Lodish HF. Ineffective erythropoiesis in Stat5a(−/−)5b(−/−) mice due to decreased survival of early erythroblasts. *Blood* 2001; **98**: 3261–3273.
36 Boles NC, Peddibhotla S, Chen AJ, Goodell MA, Rosen JM. Chk1 haploinsufficiency results in anemia and defective erythropoiesis. *PLoS One* 2010; **5**: e8581.
37 Hall MA, Slater NJ, Begley CG, Salmon JM, Van Stekelenburg LJ, McCormack MP *et al.* Functional but abnormal adult erythropoiesis in the absence of the stem cell leukemia gene. *Mol Cell Biol* 2005; **25**: 6355–6362.
38 Wagner KU, Claudio E, Rucker III EB, Riedlinger G, Broussard C, Schwartzberg PL *et al.* Conditional deletion of the Bcl-x gene from erythroid cells results in hemolytic anemia and profound splenomegaly. *Development* 2000; **127**: 4949–4958.
39 Kuter DJ, Rosenberg RD. The reciprocal relationship of thrombopoietin (c-Mpl ligand) to changes in the platelet mass during busulfan-induced thrombocytopenia in the rabbit. *Blood* 1995; **85**: 2720–2730.
40 Khandanpour C, Sharif-Askari E, Vassen L, Gaudreau MC, Zhu J, Paul WE *et al.* Evidence that growth factor independence 1b regulates dormancy and peripheral blood mobilization of hematopoietic stem cells. *Blood* 2010; **116**: 5149–5161.
41 Laurent B, Randrianarison-Huetz V, Kadri Z, Romeo PH, Porteu F, Dumenil D. Gfi-1B promoter remains associated with active chromatin marks throughout erythroid differentiation of human primary progenitor cells. *Stem Cells* 2009; **27**: 2153–2162.
42 Metzger E, Muller JM, Ferrari S, Buettner R, Schule R. A novel inducible transactivation domain in the androgen receptor: implications for PRK in prostate cancer. *EMBO J* 2003; **22**: 270–280.
43 van der Meer LT, Jansen JH, van der Reijden BA. Gfi1 and Gfi1b: key regulators of hematopoiesis. *Leukemia* 2010; **24**: 1834–1843.
44 Osawa M, Yamaguchi T, Nakamura Y, Kaneko S, Onodera M, Sawada K *et al.* Erythroid expansion mediated by the Gfi-1B zinc finger protein: role in normal hematopoiesis. *Blood* 2002; **100**: 2769–2777.

Supplementary Information accompanies the paper on the Leukemia website (http://www.nature.com/leu)