Stage-specific requirement for Mettl3-dependent m^6^A mRNA methylation during haematopoietic stem cell differentiation

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Haematopoietic stem cells (HSCs) maintain balanced self-renewal and differentiation, but how these functions are precisely regulated is not fully understood. N^6^-methyladenosine (m^6^A) messenger RNA methylation has emerged as an important mode of epitranscriptional gene expression regulation affecting many biological processes. We show that deletion of the m^6^A methyltransferase Mettl3 from the adult haematopoietic system led to an accumulation of HSCs in the bone marrow and a marked reduction of reconstitution potential due to a blockage of HSC differentiation. Interestingly, deleting Mettl3 from myeloid cells using Lysm-cre did not impact myeloid cell number or function. RNA sequencing revealed 2,073 genes with significant m^6^A modifications in HSCs. Myc was identified as a direct target of m^6^A in HSCs. Mettl3-deficient HSCs failed to upregulate MYC expression following stimulation to differentiate and enforced expression of Myc rescued differentiation defects of Mettl3-deficient HSCs. Our results reveal a key role of m^6^A in governing HSC differentiation.
**Fig. 1 | Loss of Mettl3 leads to the accumulation of HSCs and perturbed haematopoiesis.**

**a.** White blood cell (a) and peripheral blood platelet (b) counts from pIpC-treated control and Mxl-cre; Mettl3<sup>fl/fl</sup> mice (10–14 d after treatment, n = 7 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months after treatment, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months after treatment, n = 3 control and 4 Mxl-cre; Mettl3<sup>fl/fl</sup>). c. Bone marrow cellularity per hindlimb (10–14 d, n = 8 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 5 control and 6 Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). d, Representative images of the spleens from Mxl-cre; Mettl3<sup>fl/fl</sup> and control mice 10 d and 3 months after pIpC treatment. e. Spleen cellularity (10–14 d, n = 8 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 5 control and 6 Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). f. Spleen HSC frequency (10–14 d, n = 6 control and 5 Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 6 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). g. Frequencies of bone marrow LSK progenitors (10–14 d, n = 7 control and 6 Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 6 control and 7 Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). h. Frequency of bone marrow HSCs (10–14 d, n = 7 control and 6 Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 6 control and 7 Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). i, Fold increase in Mxl-cre; Mettl3<sup>fl/fl</sup> bone marrow HSC or MPP frequencies compared with control littermate frequencies at the indicated times after pIpC treatment (10–14 d, n = 6; 2–3 months, n = 7; and 4 months, n = 4). j. Frequencies of mature cell populations in the bone marrow (10–14 d, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; 2–3 months, n = 5 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>; and 4 months, n = 4 for both control and Mxl-cre; Mettl3<sup>fl/fl</sup>). k. Frequency of megakaryocyte progenitor (Lin<sup>−</sup>Scal<sup>−</sup>ckit<sup>−</sup>CD150<sup>−</sup>CD41<sup>+</sup>) cells in the bone marrow >10 d after pIpC treatment (n = 5 control and 6 Mxl-cre; Mettl3<sup>fl/fl</sup>). All of the samples were from biologically independent mice. Values are shown as individual points with the mean ± s.d. The P values were determined by unpaired two-sided Student’s t-tests. Con, control; ∆/∆, pIpC-treated Mxl-cre; Mettl3<sup>fl/fl</sup> and d, days; m, months.
DELETION OF METTL3 LEADS TO A SIGNIFICANT REDUCTION IN BONE MARROW CELLularity (Fig. 1c), but not spleen cellularity 10–14 d after the last plpC injection (Fig. 1d,e). However, by 2–4 months after the last plpC injection, in addition to a significant reduction in bone marrow cellularity, the spleen size and cellularity were significantly increased with a distortion of cell-type distribution (Fig. 1c–e and Supplementary Fig. 2c). The spleens contained more HSCs in Mx1-cre; Mettl3fl/fl mice compared with controls (Fig. 1f). These data are suggestive of extramedullary haematopoiesis after the loss of m6A.

In the bone marrow, Lin-Scal+Kit+ (LSK) haematopoietic progenitors (Fig. 1g) and HSCs (Fig. 1h) were significantly increased at all of the time points examined. The HSC pool uniquely expanded over time from 10–14 d to 4 months after the last plpC injection, progressing from a 3-fold to a 17-fold increase in HSC frequency (Fig. 1h and Supplementary Fig. 2d,e). In contrast, the frequency of CD150+CD48−Lin−sorted multipotent progenitors (MPPs) was not significantly increased, whereas the frequency of CD150−CD48−LSK progenitors was only modestly increased (Fig. 1i and Supplementary Fig. 2f). The frequency of CD150+CD48−LSK megakaryocyte-skewed progenitors was significantly increased (Supplementary Fig. 2f), suggesting that there is also an effect on the megakaryocyte lineage. Thus, at the top of the haematopoietic hierarchy, the loss of m6A preferentially leads to HSC accumulation.

We also examined other haematopoietic progenitors in the bone marrow. These included Lin-Scal-εcKitεFlt3εIL7Rαε common lymphoid progenitors, CD34εFcεRεLin-ScalεcKitε common myeloid progenitors, CD34εFcεRεLin-ScalεcKitεgranulocyte/macrophage progenitors and CD34εFcεRεLin-ScalεcKitε megakaryocyte/erythroid progenitors. The frequencies of these haematopoietic progenitors were unchanged in Mx1-cre; Mettl3fl/fl mice compared with controls until four months after the last plpC treatment when there was a modest decrease in the granulocyte/macrophage progenitor and a modest increase in megakaryocytic/erythroid progenitor frequencies (Supplementary Fig. 2g). There was a significant increase in Lin-ScalεcKitεCD150εCD41+ megakaryocyte progenitors and CD41+ megakaryocyte cells in the bone marrow (Fig. 1j,k), suggesting that the reduction in platelet counts (Fig. 1b) is due to a differentiation defect in the megakaryocyte lineage. These data suggest that a loss of Mettl3 preferentially impacts HSCs, with effects on the megakaryocyte lineage.

Deletion of Mettl3 preferentially blocks HSC differentiation. Haematopoietic stem cells from plpC-injected Mx1-cre; Mettl3fl/fl mice did not incorporate more BrdU (Fig. 2a) but exhibited a slight increase in cell death (Fig. 2b), suggesting that the accumulation of HSCs may be the result of blocked differentiation rather than enhanced self-renewal. The Mettl3-deficient HSCs had reduced levels of reactant oxygen species or yH2Axs staining (Supplementary Fig. 2h,i). Consistent with an HSC-differentiation defect, plpC-injected Mx1-cre; Mettl3fl/fl mice had lower survival rates compared with controls when challenged with a myeloablative agent, 5-fluorouracil (Fig. 2c).

We performed methylcellulose assays to directly assess the differentiation potential of Mettl3-deficient HSCs. Single HSCs from plpC-treated Mx1-cre; Mettl3fl/fl or control mice were directly sorted into methylcellulose, which supports myelo-erythroid differentiation in vitro23,24. Significantly fewer Mettl3-deficient HSCs formed colonies compared with control HSCs, particularly multipotent granulocyte, erythrocyte, monocyte and megakaryocyte colonies (CFU-GEMM) (Fig. 2d). The vast majority of the colonies formed by Mettl3-deficient HSCs were significantly smaller compared with those formed by control HSCs (Fig. 2e and Supplementary Fig. 3a). Flow cytometry analyses on cells from these colonies showed that Mettl3-deficient HSCs failed to differentiate, whereas control HSCs readily differentiated into Gr1+ and Mac1+ myeloid cells (Fig. 2f,h). A few colonies formed by Mettl3-deficient HSCs were of normal size and genotyping revealed that these colonies had escaped complete Mettl3 deletion (Supplementary Fig. 3bc). Mettl3-deficient HSCs in spleens also failed to differentiate (Supplementary Fig. 3d).

In contrast, when Mettl3-deficient whole bone marrow cells were plated into methylcellulose, the colonies that formed were similar in number, size and type compared with controls (Fig. 2g). We further plated sorted Mettl3-deficient restricted progenitor populations (LSK, CD150−CD48−LSK MPP, CD150−CD48−LSK and CD150+CD48−LSK) into methylcellulose. They formed normal numbers of colonies, similar in size and type compared with controls (Supplementary Fig. 3e). Flow cytometry analysis confirmed that these colonies contained normal numbers of differentiated cells (Fig. 2h and Supplementary Fig. 3f). Together, these data suggest that a loss of Mettl3 preferentially leads to a differentiation block in HSCs but not in restricted progenitors in vitro.

Deletion of Mettl3 leads to cell-intrinsic HSC reconstitution and differentiation defects in vivo. To test whether a loss of Mettl3 leads to HSC defects in vivo, we performed competitive reconstitution assays by transplanting 500,000 bone marrow cells from either Mx1-cre; Mettl3fl/fl or control mice 10 d after the last plpC injection along with 500,000 competing wild-type bone marrow cells into lethally irradiated recipient mice. The Mettl3-deficient bone marrow cells contributed significantly lower levels of overall, myeloid, B and T lineage cells in peripheral blood and a trend towards lower levels of HSCs in bone marrow compared with controls (Fig. 3a and Supplementary Fig. 4a). Genotyping analysis of sorted residual donor-derived peripheral blood cells demonstrated that these residual cells escaped complete Mettl3 deletion (Supplementary Fig. 4b). To specifically test whether HSCs were functionally defective, we sorted and transplanted Mettl3-deficient and control HSCs along with wild-type competing bone marrow cells into lethally irradiated mice. Although control HSCs readily reconstituted the recipient mice in all major haematopoietic lineages, Mettl3-deficient HSCs failed to give any discernable reconstitution (Fig. 3a and Supplementary Fig. 4c). These data suggest that Mettl3-deficient HSCs are defective in reconstituting recipient mice.

To determine whether Mettl3 is required cell-intrinsically for HSC function, we transplanted either Mx1-cre; Mettl3fl/fl or control bone marrow cells, without plpC treatment, along with wild-type competitor bone marrow cells into irradiated recipient mice. We waited at least eight weeks after transplantation for stable bone marrow chimaerism to be established. Mettl3 was then deleted by plpC injection and the donor-derived peripheral blood cells were monitored over time. The overall reconstitution by Mettl3-deficient bone marrow cells was significantly lower than controls (Fig. 3c). The reconstitution defects were mainly in the myeloid and B lineages, but not in the T cell lineages (Fig. 3c), which is consistent with the slow turnover kinetics of T cells in adults in vivo. After 16 weeks, the recipient mice were killed and analysed for the frequencies of donor-derived bone marrow HSCs and haematopoietic progenitors. Mettl3-deficient HSCs were present at similar levels compared with control HSCs (Fig. 3d), suggesting that HSC self-renewal is largely normal. However, these HSCs generated gradually fewer mature haematopoietic cells along the differentiation hierarchy (Fig. 3d). Consistent with the depletion of donor-derived myeloid cells in peripheral blood (Fig. 3c), very few common myeloid progenitors, granulocyte/macrophage progenitors and megakaryocytic/erythroid progenitors were derived from Mettl3-deficient HSCs (Fig. 3d). Genotyping of sorted donor-derived cells in recipient mice showed that these residual cells escaped complete Mettl3 deletion (Supplementary Fig. 4d). These data suggest that Mettl3 is required for HSC differentiation in vivo.
Fig. 2 | Mettl3 is essential for adult HSC differentiation in vitro. a, Frequency of BrdU+ HSCs 10–14 d after plpC treatment (n = 4 for both control and Mxl-cre; Mettl3fl/fl). b, Frequency of DAPI+AnnexinV+ HSC, MPP and LSK cell populations 10–14 d after plpC treatment (n = 6 for both control and Mxl-cre; Mettl3fl/fl). c, Kaplan–Meyer survival analysis of control and Mxl-cre; Mettl3fl/fl mice treated with weekly 5-fluorouracil injections 10 d after the last dose of plpC. The P value was determined by a log-rank test (n = 4 for both control and Mxl-cre; Mettl3fl/fl). d, Frequency of colony formation from single sorted HSCs. The colony-forming units (CFUs) were scored as granulocyte, erythrocyte, monocyte and megakaryocyte (GEMM), macrophage (GM), megakaryocyte (Mk) or erythrocyte (E) colonies (n = 7 for both control and Mxl-cre; Mettl3fl/fl). e, Representative image of CFU-GM from single HSCs from control (left) and Mettl3-deleted (right) single HSCs; the genotypes were confirmed by PCR. Scale bars, 400 µm. f, Representative flow cytometry histograms of mature myeloid cell surface markers Gr1 and Mac1 on HSC-derived colony cells from plpC-treated control and Mxl-cre; Mettl3fl/fl mice. g, Number of colonies formed from 10,000 bone marrow cells, scored as CFU-GEMM, CFU-GM, CFU-E or CFU-Mk (n = 5 for both control and Mxl-cre; Mettl3fl/fl). h, Flow cytometry quantification of the expression of the cell surface markers Gr1 and Mac1 on HSC and LSK colony cells (HSC, n = 8 control and 6 Mxl-cre; Mettl3fl/fl; LSK, n = 6 for both control and Mxl-cre; Mettl3fl/fl). All of the samples were from biologically independent animals. Values are shown as individual points with the mean ± s.d. The P values were determined by unpaired two-sided Student’s t-tests. Con, control; Δ/Δ, plpC-treated Mxl-cre; Mettl3fl/fl.

We also conditionally deleted Mettl3 from haematopoietic cells using Rosa26-creER<sup>25</sup>. We non-competitively transplanted bone marrow cells from Rosa26-creER; Mettl3<sup>fl/fl</sup> mice into irradiated recipient mice and then deleted Mettl3 by administering tamoxifen. The deletion of Mettl3 led to a significantly higher frequency of HSCs in the recipient mice (Supplementary Fig. 4e) recapitulating the Mxl-cre; Mettl3<sup>fl/fl</sup> model (Fig. 1h). We also competitively transplanted Rosa26-creER; Mettl3<sup>fl/fl</sup> bone marrow cells together with recipient-type bone marrow cells and waited at least eight weeks after transplantation for stable bone marrow chimaerism before administrating tamoxifen. Tamoxifen-induced deletion of Mettl3 also led to a significant reduction in reconstitution activity (Supplementary Fig. 4f), similar to Mxl-cre; Mettl3<sup>fl/fl</sup> mice (Fig. 3c).

Mettl3 is not required for myelopoiesis. To directly test whether the observed predominant myeloid lineage reconstitution defect (Fig. 3c) was a consequence of a loss of Mettl3 in myeloid cells, we conditionally deleted Mettl3 from myeloid cells by generating Lysm-cre; Mettl3<sup>fl/fl</sup> mice. Consistent with previous reports<sup>26</sup>, Lysm-cre efficiently recombined in bone marrow myeloid cells (Supplementary Fig. 4g). Six- to eight-week-old Lysm-cre; Mettl3<sup>fl/fl</sup> mice had normal peripheral blood counts (Fig. 4a,b, and Supplementary Fig. 4h), bone marrow and spleen cellularity (Fig. 4c and Supplementary Fig. 4i), and frequencies of myeloid cells in the bone marrow and spleens (Fig. 4d).

Mettl3-deficient bone marrow myeloid cells from Lysm-cre; Mettl3<sup>fl/fl</sup> mice formed macrophages with normal morphology (Supplementary Fig. 4j). When challenged with lipopolysaccharide, these macrophages showed normal upregulation of the inflammatory cytokines Tnfα, Il1b and Il6 (Fig. 4e). Mettl3-deficient bone marrow-derived macrophages also displayed a robust engulfing activity, similar to controls (Fig. 4f). These data suggest that Mettl3
Fig. 3 | Loss of Mettl3 disrupts HSC differentiation in vivo. a, Schematic of the competitive transplantation (top). 500,000 donor whole bone marrow cells from plpC-treated Mx1-cre; Mettl3fl/fl or Mettl3Δ/Δ controls with 500,000 competitor cells were transplanted into recipient mice. The multi-lineage chimera levels in peripheral blood were assessed for up to 16 weeks after transplantation (bottom; n = 7 control and 8 Mx1-cre; Mettl3Δ/Δ; the samples were from independent recipients of 2 independent donor pairs from 2 experiments). b, Competitive transplantation of 50 sorted HSCs from plpC-treated Mx1-cre; Mettl3fl/fl or control mice with 300,000 competitor cells. The multi-lineage chimaera levels in peripheral blood were assessed for up to 16 weeks after transplantation (n = 9 for both controls and Mx1-cre; Mettl3Δ/Δ; the samples were from independent recipients of 2 independent donor pairs from 2 experiments). c, Donor whole bone marrow cells (500,000) from untreated Mx1-cre; Mettl3fl/fl or controls with 500,000 competitor cells were transplanted into lethally irradiated recipient mice. The recipients were treated with plpC after stable peripheral blood chimaera was established (top). The multi-lineage peripheral blood chimaera levels are shown as the percentage of the original chimera level up to 16 weeks after plpC treatment (bottom; n = 11 control and 12 Mx1-cre; Mettl3Δ/Δ; the samples were from independent recipients of 3 independent donor pairs from 3 experiments). d, Chimaera levels of the indicated bone marrow cell populations 20 weeks after plpC treatment from c (n = 9 for both control and Mx1-cre; Mettl3Δ/Δ; the samples were from independent recipients of 3 independent donor pairs from 3 experiments). CMP, common myeloid progenitors; GMP, granulocyte/monocyte progenitors; MEP, megakaryocyte/erythroid progenitors; WBM, whole bone marrow. Values are shown as individual points with the mean ± s.d. The P values were determined by unpaired two-sided Student’s t-tests. Con, control; Δ/Δ, plpC-treated Mx1-cre; Mettl3Δ/Δ.
Fig. 4 | Loss of Mettl3 has no impact on myeloid cell maintenance or function. 

(a) White blood cell counts in LysM-cre;Mettl3<sup>fl/fl</sup> mice (n = 6 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). (b) Frequency of differential white blood cell counts in LysM-cre;Mettl3<sup>fl/fl</sup> mice (n = 6 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). (c) Bone marrow cellularity (n = 5 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). (d) Frequency of neutrophils (Mac1<sup>+</sup>/Gr1<sup>+</sup>) and macrophages (Mac1<sup>+</sup>/F4/80<sup>+</sup>) in the bone marrow (left) and spleen (right), as determined by flow cytometry (n = 5 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). (e) Analysis by qPCR of the cytokine expression of unstimulated and lipopolysaccharide-stimulated bone marrow macrophages (n = 3 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). (f) Representative histogram (left) and quantification (middle and right) of bone marrow macrophage phagocytosis of fluorescent-labelled beads (n = 3 for both control and LysM-cre;Mettl3<sup>fl/fl</sup>). The gray curve denotes the background staining (4°C incubation). MFI, mean fluorescence intensity. All of the samples were from biologically independent animals. Values are shown as individual points with the mean ± s.d. The P values were determined by unpaired two-sided Student’s t-tests. Con, control; Δ/Δ, LysM-cre;Mettl3<sup>fl/fl</sup>.</p>

Identification of m<sup>A</sup> targets in HSCs. To identify direct m<sup>A</sup> targets in rare HSCs, we developed an m<sup>A</sup>-tagged mRNA immunoprecipitation sequencing (meRIP-seq) method to analyse the m<sup>A</sup> methylome of 2,000 freshly sorted wild-type HSCs from six- to eight-week-old mice (Supplementary Fig. 5a,b). Compared with the unbound fraction, 2,073 transcripts (false discovery rate (FDR) < 0.05 and fold > 2) were significantly enriched in the m<sup>A</sup> antibody-bound fraction and were defined as high-confidence m<sup>A</sup> targets (Fig. 5a,b and Supplementary Table 1). This represented only about 9% of the genes expressed in the input mRNA fraction, suggesting that m<sup>A</sup> preferentially marks a subset of genes in HSCs. We also performed meRIP-seq on Mettl3-deficient HSCs from Mx1-cre; Mettl3<sup>fl/fl</sup> mice 10 d after piPc administration. The enrichment of 995 transcripts (approximately 48% of the high-confidence m<sup>A</sup> targets) was significantly reduced (P < 0.05) in Mettl3-deficient HSCs,
**Fig. 5 | Identification of m^6A methylation targets in HSCs.**

**a.** Heat map of the transcript abundance of the input, m^6A-tagged and unbound fractions. A total of 5,676 genes with log2[^read per kilobase per million reads, RPKM^] ≥ 5 in ≥ 4 samples and s.d. for log2[^RPKM^] ≥ 0.6 are shown. Colours correspond to mean-centered log2[^RPKM^] values −3 to 3.

**b.** Volcano plot of the meRIP-seq transcript expression differences between m^6A-tagged and unbound fractions in HSCs. The dashed vertical and horizontal lines indicate the filtering criteria (log2[^fold change^] > 1 and FDR < 0.05). The orange dots represent 2,621 transcripts showing statistically significant differences between the bound and unbound fractions, of which 2,073 are significantly enriched in the m^6A-tagged fraction.

**c.** Data from meRIP-seq showing that the transcripts of some known HSC regulators are m^6A-tagged in wild-type HSCs and this methylation is largely eliminated in Mx1-cre; Mettl3^fl/fl^ (Δ/Δ) HSCs 10 d after Cre induction. Data were from n = 3 biologically independent samples for control and Mx1-cre; Mettl3^fl/fl^. Values are shown as individual points with the mean ± s.d.

**d.** GSEA plots showing that Mettl3-deficient HSCs lose quiescent non-mobilized undifferentiated HSC gene signature (top) and bone marrow HSC gene signature (bottom), as determined by RNA-seq profiling. Analysis was completed on a gene list ranked by log10 FDR and fold-change sign, with enrichment determined after 1,000 permutations. Data were from n = 3 biologically independent samples for both the control and Mx1-cre; Mettl3^fl/fl^. The statistics were computed using GSEA and controlled for multiple comparisons by FDR.

**e.** Cumulative distribution of log2 gene expression ratios of Mettl3-deficient or control mice. The genes were categorised as m^6A and non-m^6A by meRIP-seq. Inset, the box plot of the log2 fold change in expression of non-m^6A and m^6A targets in HSCs. The plot displays the mean, standard deviation and interquartile range. The P value was determined by a two-sided Kolmogorov-Smirnov test. All sequencing data were from n = 3 biologically independent animals for both the control and Mx1-cre; Mettl3^fl/fl^. Con, control; Δ/Δ, pIpC-treated Mx1-cre; Mettl3^fl/fl^.
suggesting that the m^A modification of these targets depended acutely on METTL3 (Supplementary Fig. 5c,d). The lack of complete elimination of m^A may be due to residue METTL3 protein, slow turnover of some target mRNAs or antibody detection of the cap region m^A, which, unlike m^A, is known to not be regulated by Mettl3 but is also detected by the anti-m^A antibodies used.  

Gene ontology analysis of the m^A-tagged mRNA demonstrated an enrichment of genes related to nucleic acid metabolism, cell cycle regulation, transcription, RNA binding, cellular stress responses, immune system development and haematopoiesis (Supplementary Fig. 5e). Supporting feed-forward regulation, several genes encoding components of the m^A pathway, including WTAP and m^A-reader proteins, were also targets of m^A (Supplementary Fig. 5f). Consistent with the differentiation defects observed in Mettl3-deficient HSCs (Figs. 1–1), we observed significant enrichment of genes associated with haematopoietic differentiation.
Together, these data suggest that although m6A may directly control Mettl3 had slightly increased abundance in m6A targets were upregulated in Supplementary Table 2). Only seven genes showed significant control HSCs after stringent analysis with correction for multiple 384 upregulated genes in -deficient HSCs, 95 were m6A tar- global mRNA levels.

Prompted by the observations that Myc and blockage of differentiation), we investigated whether Myc is a major determinant of leads to a loss of HSC identity without drastic Deletion of Mettl3 leads to a loss of HSC identity without drastic alterations of the transcriptome. To further understand the molec- ular mechanisms underpinning the functional defects of Mettl3-deficient HSCs, we performed RNA sequencing (RNA-seq) analysis on Mettl3-deficient HSCs using Smart-seq2 (ref. 2). Significant differences in expression were observed for 830 genes (P < 0.05; Supplementary Table 2). Only seven genes showed significant expression differences (FDR < 0.05) between Mettl3-deficient and control HSCs after stringent analysis with correction for multiple comparisons (Supplementary Fig. 6a and Supplementary Table 2). As expected, Mettl3 was one of the most significantly downregulated genes in Mettl3-deficient HSCs (Supplementary Fig. 6a). Gene set enrichment analysis (GSEA)25 showed that Mettl3 defi- ciency predominantly led to a loss of expression of HSC-identity-associated genes defined by previous publications29–31 (Fig. 5d and Supplementary Fig. 6b). These data suggest that METTL3-mediated m6A modestly altered HSC identity without profoundly impacting global mRNA levels.

It has been demonstrated that m6A negatively regulates target mRNA stability in several cell types. We thus assessed whether m6A targets were upregulated in Mettl3-deficient HSCs. Of the 384 upregulated genes in Mettl3-deficient HSCs, 95 were m6A tar- gets (P < 0.05 and fold change > 1.2; Supplementary Table 2 and Supplementary Fig. 6c). However, none of the seven high-confi- dence differentially expressed genes (FDR < 0.05; Supplementary Table 1 and Supplementary Fig. 6a) were high-confidence m6A tar- gets. Compared with non-m6A targets, the transcripts of m6A targets had slightly increased abundance in Mettl3-deficient HSCs (Fig. 5e). Together, these data suggest that although m6A may directly control the transcript levels of some genes, it predominantly regulates target mRNA post-transcriptionally in HSCs.

Myc is a direct target of m6A in HSCs. It has been shown that Myc protein levels are very low in HSCs and are upregulated following differentiation through post-transcriptional mechanisms. Furthermore, deletion of Myc blocks HSC differentiation, whereas higher levels of MYC protein promote HSC differentiation. Prompted by the observations that Myc is a major determinant of HSC differentiation and the phenotypic similarity between Myc and Mettl3 conditional knockout mice (accumulation of HSCs and blockage of differentiation), we investigated whether Myc is a direct target of m6A in HSCs. Our meRIP-seq analyses revealed that Myc was one of the top candidates highly enriched in m6A antibody-bound fraction compared with the unbound fraction (~30 fold enrichment, FDR = 3.96 × 10−15; Fig. 5b,c) in a Mettl3-dependent manner (Fig. 5c and Supplementary Fig. 5c,d). We confirmed these meRIP-seq data using qPCR on independent samples (Fig. 6a). Interestingly, Myc transcript levels were not significantly changed in Mettl3-deficient HSCs compared with controls (Supplementary Fig. 6d). Using several previously published Myc-target gene sets28,29, GSEA revealed a significant decrease in Myc-target gene signatures in Mettl3-deficient HSCs (Fig. 6b and Supplementary Fig. 6e), suggesting that m6A may primarily regulate Myc mRNA translation.

In vivo Myc protein levels can be directly measured by a knockin Myc–green fluorescent protein (GFP) translational reporter30. To assess the impact of Mettl3 deletion on the level of Myc protein in HSCs, we generated Mx1-cre; Mettl3fl/fl; Myc–GFP mice. Consistent with previous publications28,31, HSCs expressed low levels of MYC–GFP compared with haematopoietic progenitors at steady state (Fig. 6c). The deletion of Mettl3 led to a modestly significant reduction of MYC–GFP in HSCs (Fig. 6c). Remarkably, following activation to differentiate, Mettl3-deficient HSCs failed to upregulate MYC–GFP, whereas control HSCs could readily do so (Fig. 6d). Mettl3 deletion did not lead to changes in HSC Myc transcript levels under the activation condition (Supplementary Fig. 6d). Given that m6A pri- marily regulates target mRNA stability and translation32,33, these data suggest that m6A is required for Myc mRNA translation in HSCs, particularly following differentiation.

We tested whether forced expression of Myc can rescue the HSC differentiation defects caused by the deletion of Mettl3. We trans- duced Mettl3-deficient HSCs with Myc-expressing or control retroviruses (Fig. 6e and Supplementary Fig. 6f) and assessed their differentiation potential in vitro. Forced expression of Myc signifi- cantly rescued the differentiation defect of Mettl3-deficient HSCs compared with controls (Fig. 6e). These data indicate that Myc is one of the major functional targets of m6A in HSCs.

Discussion
We demonstrate that m6A is preferentially required for adult HSC differentiation in vivo and in vitro, but not mature myeloid cell maintenance or function. These results demonstrate that m6A is not a general cellular housekeeping mechanism, but rather a modification with specific roles in HSCs. Our results are in con- trast to reports that show that m6A inhibits the differentiation of CD34+ human haematopoietic progenitor cells in vitro34,35. As those experiments were performed using shRNAs on cells of mixed populations of HSCs and progenitors in vitro, it is not clear what the effects on human HSCs are in vivo. Notably, we observed differences between HSCs and progenitors in their response to Mettl3 deletion (Fig. 2 and Supplementary Fig. 3), suggesting that the roles of m6A in HSCs and progenitors need to be assessed independently. Consistent with our data, Yao et al. recently showed that the conditional deletion of Mettl3 led to an accumulation of HSCs in the bone marrow while our paper was under review36.

In contrast to its role in enabling HSC differentiation, m6A inhibits differentiation in acute myeloid leukaemia cells18,19,40. This surprising difference may reflect distinct mechanisms of action for m6A in leukaemic cells and HSCs, and suggests that it may be possible to develop m6A-targeted therapies against leukaemia cells without damaging HSC function. More work is needed to eluci- date the differences between the role of m6A in leukaemic cells and in HSCs.

We found that HSC self-renewal and quiescence is largely intact after Mettl3 deletion (Figs. 2a and 3d). Thus, it appears that m6A specif- ically regulates HSC differentiation. Epitranscriptional regulation mediated by m6A in HSCs may provide a fast and flexible mecha- nism to control mature blood cell output, allowing the organism to adapt to ever-changing physiological demands. Mettl3-deficient HSCs failed to establish bone marrow chimera in transplantation (Supplementary Fig. 4a,c), suggesting that m6A may regulate HSC homing and/or engraftment, which will be of interest in future fur- ther investigations.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41556-019-0318-1.

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Author contributions
H.L., J.J.L. and C.Z. performed all of the experiments. S.B., Y.Q. and C.Z. performed the bioinformatics analysis on all sequencing data. S.G. and J.H.H. generated and validated the Mettl3 strain and assisted with gene expression analysis and the development of the project. H.L. and J.D. designed the experiments, interpreted the results and wrote the manuscript with input from J.H.H. and C.Z. L.D. supervised the project.

Competing interests
The authors declare no competing interests.

Additional information
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Methods

Mice. Mx1-cre\(^{1,2}\), Lysm-cre\(^{3}\) and MyC-GFP\(^{4}\) mice were obtained from the Jackson Laboratory and maintained on a C57BL/6J background. Rosa26-creER\(^{2}\) mice were generated and provided by T. Ludwig at Columbia University. Mettl3\(^{5}\) mice were generated by electrotransfection of a targeting vector (obtained from the Knockout Mouse Project Repository) into V6.5 embryonic stem cells. A correctly targeted embryonic stem cell clone, validated by PCR and Southern blot analysis, was identified and injected into BDF1 blastocysts and chimaeric mice were generated. The chimaeric mice were bred with C57BL/6 mice to obtain germline transmission. The resulting mice were crossed with Flp mouse\(^{6}\) to remove the Neo cassette. These mice were backcrossed at least six times onto a C57BL/6J background before analysis. To induce Cre expression in Mx1-cre, Mettl3\(^{5}\) mice, six- to eight-week-old animals were intraperitoneally injected with 10\(\mu\)g plpG (GE or InvivoGen) in PBS every other day for five injections. The mice were analysed 10–14 d, 2–3 months or 4 months after the final injection, as indicated in each experiment. To induce Cre expression in Rosa26-creER\(^{2}\); Mettl3\(^{5}\) mice, the recipients were given 1\(\mu\)g tamoxifen in 50\(\mu\)l corn oil by gavage every other day for five doses. All of the mice were housed in specific pathogen-free, free for the Assessment and Accreditation of Laboratory Animal Care-approved facilities at the Columbia University Medical Center. All protocols were approved by the Institute Animal Care and Use Committee of Columbia University. The study is compliant with all relevant ethical regulations regarding animal research.

Genotyping PCR primers. The following primers were used for genotyping: GTTGATGAAATTACGACAACTGTGGTTGA and GTAAGAATGTTGAATTGCTCGTA for the Mettl3\(^{5}\) allele; GAGTATTGCTTCTACATTCCCTACC and CAGAAGCCCATCTCCAGGGTG for the Mettl3 deletion allele.

Administration of 5-fluorouracil. For 5-fluorouracil (Sigma-Aldrich) treatment, Mx1-cre, Mettl3\(^{5}\) and littermate control mice were treated with plpG as previously described. Ten days after the last plpG injection, the mice were intraperitoneally injected with 150\(\mu\)g\(^{-1}\) 5-fluorouracil weekly until death or experimental conclusion.

Complete blood counts. Peripheral blood was collected by tail-vein or cardiac puncture into EDTA coated capillary tubes. The blood samples were analysed by Genysis (Oxford Science Inc).

Flow cytometry. Bone marrow cells were isolated by flushing the long bones or by crushing the long bones, pelvis and vertebrae with a mortar and pestle in Ca\(^{2+}\)– and Mg\(^{2+}\)-free HBSS medium with 2% heat-inactivated bovine serum. Splenic cells were obtained by crushing the spleens between two glass slides. The splenic and bone marrow cells were separated several times through a 25\(\mu\)m needle and filtered through 70-\(\mu\)m nylon mesh. The following antibodies were used to perform FSC staining: lineage markers anti-CD45 (2B8); anti-CD48 (HM48-1) and anti-CD150 (TC15-12F12.2). In some experiment, anti-CD2 (RM2-5), anti-CD3 (17A2), anti-CD5 (53-7.3), anti-CD8a (53-6.7), anti-B220 (6B2), anti-Gr1 (8C5) and anti-Mac1 (M1/70) were used to stain cells.

Cell cycle, DNA damage, cell death and reactive oxygen species analysis. For BrdU incorporation analysis, mice were intraperitoneally injected with 0.1\(\mu\)g kg\(^{-1}\) BrdU and maintained on 0.5 mg ml\(^{-1}\) BrdU water for 5 d before analysis. The frequency of BrdU\(^{+}\) cells was determined by flow cytometry using an APC BrdU flow kit (BD Biosciences) as per the manufacturer’s instructions. To measure \(\gamma\)H2AX levels, the cells were stained for SLAM markers, fixed and permeabilized (BD Biosciences BrdU flow kit) and stained with anti-\(\gamma\)H2AX (Bioplex, clone 2F3), followed by flow cytometry. Annexin V staining was performed using the Annexin V apoptosis detection kit APC (eBioscience) as per the manufacturer’s instructions. Alternatively, reactive oxygen species levels were measured by DCFH-DA (29-79-dichlorofluorescin diacetate; Thermofisher Scientific). After antibody staining, the cells were incubated with 10\(\mu\)M DCFDA for 15 min at 37\(^\circ\)C, followed by flow cytometry.

Long-term competitive reconstitution assay. Adult recipient mice were lethally irradiated by a caesium 137 irradiator (IL Shepherd and Associates) at 300 rad min\(^{-1}\) with two doses of 540 rad (total, 1,080 rad) delivered at least 2 h apart. Cells were transplanted by retro-orbital venous sinus injection of anaesthetized mice. Mice were transplanted with either 5\(^{10}\) donor and 5\(^{10}\) competitor bone marrow cells or 50 sorted donor HSCs and 3\(^{10}\) competitor bone marrow cells, as indicated. The donor mice were either untreated or killed 10 d after plpG treatment, as indicated. The recipient mice were temporarily maintained on antibiotic water (0.17\(g\)\(^{-1}\) Baytril) for 14 d after transplantation. Peripheral blood from recipient mice was analysed by flow cytometry at the indicated time points for at least 16 weeks after transplantation to assess the level of donor-derived blood lineages (donor chimaeraism), including myeloid, B and T cells. For the post-transplantation Cre-induction experiments, the peripheral blood of the recipients was analysed over a minimum of two time points at least six to eight weeks after transplantation to ensure stable engraftment before treatment with 5-Fluoro-2’-deoxyuridine (5-FUDR).

Colonization by unbound and m6A-bound fraction RNA was reverse transcribed using Superscript III (Invitrogen). Quantitative real-time PCR was run using SYBR green on a CFX Connect (Biorad) system. The RNA content of samples was normalized to \(\beta\)-actin. The primer sequences were: \(\beta\)-actin, CACTTCTTCCACCTTCTCCTITT; CTCTTGGCATCTGACCA; Mettl3, AAGGAGCCGGCTAAGAAGTC and TCTACGGCTTTTCTGACACTCT; \(\beta\)-actin, TTGCTCTTCTCTAGAGTTCCT; Infia, GGAAGTGGAAAAAGGAGACTCT and GACAGAGAAGGAGGCTGACAG; IL6, CCACTTGGTCTTCTGAGACT; INFIA, AGA ACTGAGAGGAGGAGAC; CTACCTGGTGTTTCTGCACACC and EGF, AGAAGAGGAGGAGGAGAC; CAGGAGAAGGAGGAGGAGAC; and TGTGTTGTGTTCTGCTCCTGTT.

MeRIP-qPCR. Poly(A)+ RNA was isolated from whole bone marrow cells or sorted HSCs using Dynabeads Oligo(dT)\(_{25}\) magnetic beads (Thermo Fisher Scientific) according to the manufacturer’s instructions. Anti-\(\beta\)-actin antibody (1:250; Synaptic Systems) was pre-bound to Protein A/G magnetic beads (Millipore) in IP buffer (20 mM Tris pH 7.5, 140 mM NaCl, 1% NP-40 and 2 mM EDTA) for 1 h. The sample RNA was incubated with antibody-bound Protein A/G beads for 2 h at 4\(^\circ\)C. The samples were washed twice in low-salt wash buffer (10 mM Tris pH 7.5 and 5 mM EDTA), twice with high-salt wash buffer (20 mM Tris pH 7.5, 1 M NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1 mM EDTA) and twice with RIPA buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and 1 mM EDTA). All of the wash solutions for each sample were collected as the ‘unbound’ fraction. RNA was isolated from the beads by incubating with 50 \(\mu\)l 20 mM N\(_{2}\)-methyladenosine 5-monophosphate sodium salt (Sigma-Aldrich) for 1 h at 4\(^\circ\)C. Following ethanol precipitation, the input, unbound and m6A-bound fraction RNA was reverse transcribed using Superscript III (Invitrogen) with random hexamers. Enrichment of m6A-containing
transcripts was determined by quantitative PCR relative to \textit{Rplp0} expression. The primer sequences for \textit{Rplp0} were \texttt{GATGGGCAACTGTAACGTGACTG} and \texttt{CTGGGCTCCTCCTTTGGAATG}.

**RNA-seq and meRIP-seq.** For RNA-seq, libraries were prepared according to the Smart-seq2 protocol as previously described\(^2\) from 150 sorted HSCs from \textit{Mx1-cre}; \textit{Mettl3}\(^{-}\text{fl}/\text{fl}\) or control mice 10 d after \textit{pipC} treatment. Three control and four \textit{Mx1-cre}; \textit{Mettl3}\(^{-}\text{fl}/\text{fl}\) biological replicate samples were sequenced using an Illumina HiSeq 2500 with paired-end 100-bp read length.

For meRIP-seq, poly(A\(^+\)) RNA was isolated from 3,000 sorted HSCs from \textit{C57BL/6j} or \textit{Mx1-cre}; \textit{Mettl3}\(^{-}\text{fl}/\text{fl}\) mice 10 d after \textit{pipC} injection using Dynabeads Oligo-(dT)\(^{25}\) magnetic beads (Thermo Fisher Scientific) according to the manufacturer's instructions. Anti-m\(^{6}\)A antibody (1.25\(\mu\)g) or rabbit IgG (Jackson ImmunoResearch) was pre-bound to Protein A/G magnetic beads (Millipore) in IP buffer for 1 h. The sample RNA was incubated with antibody-bound Protein A/G beads for 2 h at 4 °C. The samples were washed twice in low-salt wash buffer, twice with high-salt wash buffer and twice with RIPA buffer. All of the wash solutions for each sample were collected as the unbound fraction. The RNA was eluted from the beads by incubating with 50\(\mu\)l 20 mM \(N\(^{6}\)\)-methyladenosine 5-monophosphate sodium salt (Sigma) for 1 h at 4 °C. Following ethanol precipitation, RNA from input, m\(^{6}\)A-antibody unbound, m\(^{6}\)A-antibody eluted and IgG-eluted fractions was reverse transcribed, amplified, tagmented and indexed according to the Smart-seq2 protocol\(^2\). Three biological replicate samples were sequenced using an Illumina HiSeq 2500 or NextSeq 500 with paired-end 100 bp or 150 bp read length.

**Analysis of RNA-seq and meRIP-seq data** was performed using the Quantas pipeline (https://zhanglab.c2b2.columbia.edu/index.php/Quantas)\(^3\) with minor modifications. In brief, raw reads were mapped to the mouse genome and an exon-junction database using OLeGo\(^4\). Due to the low input during library preparation, potential PCR duplicates were removed using Pcard (http://broadinstitute.github.io/pcard). Exonic and junction reads were then counted to estimate transcript abundance (RPKM) and perform differential expression analysis using edgeR\(^5\).

Cumulative distribution analysis of RNA-seq log\(_{2}\) fold changes in expression between \textit{Mettl3}\(^{-}\)-depleted and control HSCs was performed in R using the \texttt{ecdf} function. Groups were defined as m\(^{6}\)A (meRIP-seq FDR < 0.05, fold change > 2) or non-m\(^{6}\)A (the remainder of the genes), excluding genes with RNA-seq RPKM < 1 for both \textit{Mettl3}-depleted and control HSCs. Significance of difference between the cumulative distribution curves was determined using the Kolmogorov–Smirnov test.

**Statistics and reproducibility.** All experiments were independently repeated on biological replicates with similar results. The exact sample sizes for each experiment are indicated in the figure legends. All statistics comparing two groups used two-sided Student’s \(t\)-tests. The Kaplan–Meier estimation and two-sided log-rank test were used to compare survival differences between mouse groups. Statistical analyses were performed with Microsoft Excel or GraphPad Prism\(^7\). The statistical source data are included in Supplementary Table 3.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** Sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE123527. Source data have been provided as Supplementary Table 3. All other data supporting the findings of this study are available from the corresponding authors on reasonable request.

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RNAseq data from this study have been deposited to GEO with Access number GSE123527. DNA gel raw image data were included in the Supplementary Figure 7. Statistical source data were included in the Supplementary Table 3.

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| Sample size | Sample sizes were not pre-determined based on statistical power calculations. The sizes were determined based on previous published studies, experimental experience and knowledge. |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | No data were excluded.                                                                                                                                                                           |
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| Eukaryotic cell lines | | Flow cytometry |
| Palaeontology | | MRI-based neuroimaging |
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| Clinical data | | |

Antibodies

The following antibodies were used for flow cytometry:

- CD34 FITC 1:100 BD Pharmingen 560238 RAM34
- CD45.2 FITC 1:400 BioLegend 109806 104
- CD45R FITC 1:400 BioLegend 103206 RA3-6B2
- CD2 FITC 1:200 Invitrogen 1934090 RM2-5
- CD5 FITC 1:400 BioLegend 100606 5.3-7.3
- TER119 FITC 1:200 BioLegend 116206 TER-119
- CD8A FITC 1:400 BioLegend 100706 53-6.7
- CD3 FITC 1:200 BioLegend 100204 17A2
- CD41 FITC 1:200 BioLegend 133904 MWREG30
- GR1 FITC 1:400 BioLegend 108406 RB6-BC5
- CD4.2 PE 1:400 BioLegend 109808 104
- CD45R PE 1:400 BioLegend 103207 RA3-6B2
- CD2 PE 1:200 BioLegend 100108 RM2-5
- CD5 PE 1:400 BioLegend 100607 5.3-7.3
- TER119 PE 1:200 BioLegend 116208 TER-119
- CD8A PE 1:400 BioLegend 100708 53-6.7
- CD3 PE 1:200 BioLegend 100206 17A2
- CD41 PE 1:200 BioLegend 133905 MWREG30
- GR1 PE 1:400 BioLegend 108408 RB6-BC5
- CD48 PE 1:200 BioLegend 103405 HM48-1
- CD150 PE 1:200 BioLegend 115904 TC15-12F112.2
- CD150 APC 1:200 BioLegend 115909 TC15-12F112.2
- CD48 APC 1:200 BioLegend 103412 HM48-1
- CD16/32 APC 1:400 BioLegend 101325 93
- CD11B APC 1:400 BioLegend 101212 M1/70
- CD150 PE-CYS 1:200 BioLegend 115912 TC15-12F112.2
- CD45R PE-CYS 1:400 BioLegend 103210 RA3-6B2
- CD155 PE-CYS 1:100 BioLegend 135312 A2F10
- CD11B PE-CY7 1:400 BioLegend 101216 M1/70
- GR1 PE-CY7 1:400 BioLegend 108416 RB6-BC5
- CD45.1 PE-CY7 1:200 BioLegend 110729 A20
Validation

They are all from commercial sources (BD Pharmingen, Biolegend or Invitrogen) and are widely used in the field. They are all validated by the manufacture on flow cytometry experiments.

Animals and other organisms

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Laboratory animals

Mx1-cre, Lysm-cre, Rosa26-creER, Myc-GFP, Rosa-loxpMyc and Mettl3 fl mice. All of the mice were on B6 background. Both adult males and females (~2 month old) are used in experiments.

Wild animals

No wild animals were used in this study.

Field-collected samples

No field-collected samples were used in this study.

Ethics oversight

All experiments were approved by Columbia University Institutional Animal Care and Use Committee under protocol AC-AAAP7405.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☑️ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☑️ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☑️ All plots are contour plots with outliers or pseudocolor plots.

☑️ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Bone marrow cells were isolated by flushing the long bones or by crushing the long bones, pelvis, and vertebrae with mortar and pestle in Ca2+ free and Mg2+ free HBSS with 2% heat-inactivated bovine serum. Splenic cells were obtained by crushing the spleens between two glass slides. The splenic and bone marrow cells were passed through a 25G needle several times and filtered through 70um nylon mesh.

Instrument

Samples were run on FACSAria II, BD LSR II, FACSCanto or FACSCelesta flow cytometers.

Software

FACSDiva v8.0.2 (BD) was used for data collection. FlowJo v10 (Tree Star) software was used for data analysis.

Cell population abundance

The HSC frequency is about 0.007% in wild type bone marrow. It goes up to about 0.1% (14 fold higher in mutants).

Gating strategy

The gating strategy is illustrated in the figures (Supplementary Fig 2 and Supplementary Fig 6).

☑️ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.