Sexually dimorphic mammalian tissues, including sexual organs and the brain, contain stem cells that are directly or indirectly regulated by sex hormones\(^1\)–\(^4\). An important question is whether stem cells also exhibit sex differences in physiological function and hormonal regulation in tissues that do not show sex-specific morphological differences. The terminal differentiation and function of some haematopoietic cells are regulated by sex hormones\(^5\)–\(^9\), but haematopoietic stem-cell function is thought to be similar in both sexes. Here we show that mouse haematopoietic stem cells exhibit sex differences in cell-cycle regulation by oestrogen. Haematopoietic stem cells in female mice divide significantly more frequently than in male mice. This difference depends on the ovaries but not the testes. Administration of oestradiol, a hormone produced mainly in the ovaries, increased haematopoietic stem-cell division in males and females. Oestrogen levels increased during pregnancy, increasing haematopoietic stem-cell division, haematopoietic stem-cell frequency, cellularity, and erythropoiesis in the spleen. Haematopoietic stem cells expressed high levels of oestrogen receptor-\(a\) (ER\(a\)). Conditional deletion of ER\(a\) from haematopoietic stem cells reduced haematopoietic stem-cell division in female, but not male, mice and attenuated the increases in haematopoietic stem-cell division, haematopoietic stem-cell frequency, and erythropoiesis during pregnancy. Oestrogen/ER\(a\) signalling promotes haematopoietic stem-cell self-renewal, expanding splenic haematopoietic stem cells and erythropoiesis during pregnancy.

A fundamental question in stem-cell biology concerns the extent to which stem cells are regulated by long-range signals to ensure that stem-cell function within individual tissues is integrated with the overall physiological state\(^1\). For example, stem cells in the intestine, central nervous system and germ line are regulated by insulin and nutritional status\(^1\)–\(^5\). Among haematopoietic cells oestrogen regulates proliferation, survival, differentiation and cytokine production by lymphoid and myeloid cells\(^1\)–\(^3\),\(^18\),\(^19\), and induces apoptosis in erythroid cells by inhibiting GATA1 (refs 20, 21). This raises the question of whether some haematopoietic cells are regulated by sex hormones\(^7\)–\(^10\), but logical differences. The terminal differentiation and function of haematopoietic stem cells or MPPs\(^2\)–\(^2\) or in the percentage of bone marrow cells that incorporated BrdU while remaining in the haematopoietic stem-cell pool, haematopoietic stem cells undergo more frequent self-renewing divisions in female mice than in male mice.

To test this using an independent approach we treated 4–6-week-old Rosa26-rtTA; tetO-H2B-GFP mice\(^2\) with doxycycline for 6 weeks to induce histone H2B–GFP expression and then chased for 12 weeks without doxycycline to assess the rate of H2B–GFP dilution as a result of cell division. After 6 weeks of doxycycline treatment, haematopoietic stem cells, MPPs and whole bone marrow (WBM) cells in male and female mice were strongly and uniformly labelled with H2B–GFP (Fig. 1e). However, after the 12-week chase almost all bone marrow cells lost H2B–GFP expression in male and female mice (Fig. 1e, f). As expected\(^2\)–\(^2\), haematopoietic stem cells and MPPs retained substantial frequencies of H2B–GFP\(^a\) cells that were relatively quiescent during the chase period (Fig. 1e, f). Consistent with the higher rate of BrdU incorporation in female haematopoietic stem cells, significantly (\(P < 0.005\)) lower percentages of haematopoietic stem cells and MPPs retained high levels of H2B–GFP in female as compared to male mice (Fig. 1e, f). Haematopoietic stem cells and MPPs thus divide more frequently in female as compared to male mice.

Ovariectomy, but not castration, significantly reduced the percentage of haematopoietic stem cells and MPPs that incorporated a 10-day pulse of BrdU (Fig. 2a). Indeed, ovariectomy reduced haematopoietic stem-cell and MPP division in females to male levels (Fig. 2a). Castration or ovariectomy did not affect the numbers of haematopoietic stem cells or MPPs in the bone marrow (Extended Data Fig. 1a) and produced only minor changes in the gross lineage composition of bone marrow cells (Extended Data Fig. 1b). The rate of haematopoietic stem-cell division in female mice is therefore increased by signals from the ovary.

To test whether female sex hormones can affect haematopoietic stem-cell cycling we administered oestradiol (E2; 2 \(\mu\)g day\(^{-1}\)), progesterone (P; 1 mg day\(^{-1}\)), or oestradiol with progesterone (E2+P) to young adult male and female mice for 1 week along with BrdU for the last 3 days. This significantly increased oestrogen and/or progesterone levels in both male and female mice (Extended Data Fig. 3a, b) without exceeding the physiological levels observed during pregnancy. These treatments did not affect bone marrow or spleen cellularity (Fig. 2b) or haematopoietic stem-cell frequency (Fig. 2c), but E2 induced erythropoiesis in the spleen (Extended Data Fig. 2d). Treatment with E2 or E2+P, but not P alone, significantly increased BrdU incorporation by haematopoietic stem cells, but not by unfractionated bone-marrow cells, in both male and female mice (Fig. 2d).

E2 treatment increased BrdU incorporation by haematopoietic stem cells in castrated and ovariectomized mice, indicating that E2 acts independently of the gonads (Fig. 2e). E2 treatment also increased the frequency of Ki67\(^+\) haematopoietic stem cells (Fig. 2f and Extended Data Fig. 4a). E2 treatment significantly reduced the frequency of haematopoietic stem cells that retained H2B–GFP in Rosa26-rtTA; tetO-H2B-GFP mice (Extended Data Fig. 4b, c). In contrast, treatment with dihydrotestosterone did not affect BrdU incorporation by male or female haematopoietic stem cells (Fig. 2g) or haematopoietic stem-cell frequency in bone marrow (Fig. 2h).
Figure 1 | Haematopoietic stem cells divide more frequently in female mice than in male mice. a–c, The frequency of haematopoietic stem cells (HSCs) and MPPs in the bone marrow (a), the total numbers of haematopoietic stem cells and MPPs in two femurs and tibias (b), and the numbers of haematopoietic stem cells and MPPs per gram of body mass (c) did not differ between young adult male and female mice. d, BrdU incorporation into whole bone marrow (WBM) cells, haematopoietic stem cells and MPPs during a 10-day pulse (a–d, n = 5 per group in five independent experiments). e, H2B–GFP intensity immediately after a 6-week pulse of doxycycline in Col1A1-H2B-GFP; Rosa26-M2-nTAg mice (left) or after a 12-week chase without doxycycline (right). The percentages of WBM cells, haematopoietic stem cells and MPPs that retained H2B–GFP (4 males and 3 females in 3 independent experiments). All data represent mean ± standard deviation; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test.

Consistent with the observation that oestrogen induces apoptosis in erythroid progenitors, we observed an increased frequency of annexin V–Ter119+ cells in female as compared to male bone marrow (Extended Data Fig. 5a). This appeared to be offset by increased generation of megakaryocyte–erythroid progenitors (MEPs) in female mice (Extended Data Fig. 5b). Neither MEPs nor Ter119+ cells exhibited differences in cell-cycle distribution between males and females (Extended Data Fig. 5c). Given that MEPs may arise directly from the asymmetric division of HSCs, these observations raise the possibility that the increased frequency of MEPs in female mice reflects increased asymmetric self-renewal of female haematopoietic stem cells in response to oestrogen.

We treated mice for 14 days with the aromatase inhibitor anastrozole, which reduces oestrogen levels. Anastrozole did not significantly affect bone marrow cellularity (Fig. 3a) or lineage composition (Extended Data Fig. 6a), but slightly reduced haematopoietic stem-cell frequency in female mice (Fig. 3b). Anastrozole did not significantly affect BrdU incorporation (during the last 10 days of anastrozole) by whole bone marrow cells or MPPs in male or female mice, but did significantly reduce BrdU incorporation by female haematopoietic stem cells (P < 0.05, Fig. 3c). Treatment with the progesterone receptor antagonist RU486 had no effect on bone marrow or spleen cellularity, haematopoietic stem-cell frequency, or BrdU incorporation (Extended Data Fig. 3d–f). These results indicated that endogenous oestrogen increases haematopoietic stem-cell division in female mice.

Haematopoietic stem cells and MPPs from male and female mice expressed high levels of oestrogen receptor-α (ERα; encoded by Esr1) (Fig. 3d, e). However, haematopoietic stem cells expressed little or no ERβ (encoded by Esr2), progesterone receptor (Pgr), or androgen receptor (Ar) (Fig. 3d). To assess the roles of ERα and ERβ in haematopoietic-stem-cell regulation we treated male mice with the ERα agonist propylpyrazoletriol (PPT) or the ERβ agonist diarylpropionitrile (DPN) for 2 weeks along with BrdU for the last 10 days. PPT and DPN did not affect bone marrow or spleen cellularity, or the frequencies of haematopoietic stem cells and MPPs (Extended Data Fig. 7a, b). PPT, but not DPN, significantly increased erythropoiesis in the bone marrow and spleen (Extended Data Fig. 7c) as well as BrdU incorporation by haematopoietic stem cells (Extended Data Fig. 7d), indicating that oestrogen effects on haematopoietic stem cells are mediated mainly by ERα. Consistent with this conclusion, germline Esr1-deficient mice of both sexes had normal bone marrow cellularity and lineage composition.

Figure 2 | Increased haematopoietic stem-cell division in female mice depends on the ovaries and is stimulated by oestriadiol. a, Effect of castration (cast) or ovariectomy (ovx) on the rates of division by WBM cells, haematopoietic stem cells, or MPPs (3 sham and 4 gonadectomized mice in 3 independent experiments). b, c, Administering oestriadiol (E2), progesterone (P), or both (E2+P) for 1 week did not affect the number of bone marrow cells or splenocytes, or haematopoietic stem-cell frequency in bone marrow (c). Oil, corn oil. d, Administering E2 or E2+P significantly increased haematopoietic stem-cell division in male and female mice (b–d, n = 3 mice per treatment in three independent experiments). e, Administering E2 to castrated or ovariectomized mice significantly increased haematopoietic stem-cell division by BrdU incorporation (n = 5). f, Administering E2 to male mice increased the frequency of haematopoietic stem cells positive for Kae7 (n = 3). g, h, Administering dihydrotestosterone (DHT) for 7 days did not affect haematopoietic stem-cell division or haematopoietic stem-cell frequency (n = 4 mice per treatment in 4 independent experiments). Data represent mean ± standard deviation; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test. NS, not significant.
Figure 3 | Oestradiol–ERα signalling promotes haematopoietic stem-cell division in female mice. a, b, Bone marrow cellularity (a) and haematopoietic stem-cell and MPP frequency (b) in mice administered the aromatase inhibitor anastrozole (A) or vehicle (PBS, abbreviated V) for 2 weeks. c, BrdU incorporation (10-day pulse) by WBM cells, haematopoietic stem cells, or MPPs in male or female mice treated with anastrozole or vehicle (a–c, 4 PBS-treated and 6 anastrozole-treated mice in 4 independent experiments). d, qRT–PCR revealed that haematopoietic stem cells and MPPs from female and male mice expressed greatly elevated levels of Esr1 (which encodes ERα) but not Esr2 (ERβ), Pgr (progesterone receptor), or Ar (androgen receptor) relative to male WBM (*P < 0.05 between HSC/MPP and WBM; #P < 0.05 between male and female). Expression levels were normalized to β-actin. e, Immunofluorescence for ERα in haematopoietic stem cells (d, e, n = 3 mice from 3 experiments). Scale bar, 4 μm. f, BrdU incorporation (10-day pulse) by WBM cells, haematopoietic stem cells and MPPs in male and female mice (−/−, Esr1-deficient; +/+; littermate controls, f–h, n = 3 mice per group in 3 independent experiments). g, Conditional deletion of Esr1 in female Mx1-cre; Esr1fl/fl mice reduced BrdU incorporation into haematopoietic stem cells (Cre−, Esr1fl/fl; Mx1, Mx1-cre; Esr1fl/fl, n = 3). h, i, Conditional deletion of Esr1 in male Mx1-cre; Esr1fl/fl mice (b) or Vav1-cre; Esr1fl/fl mice (i) rendered haematopoietic stem cells insensitive to exogenous oestrogen (b, i, n = 3 mice per group in 2 independent experiments). j, E2 treatment of mice competitively reconstituted with WBM cells from wild-type and Vav1-cre; Esr1fl/fl mice increased BrdU incorporation by wild-type haematopoietic stem cells but not Esr1-deficient haematopoietic stem cells (3 oil-treated and 4 E2-treated mice in 2 independent experiments). k, Effect of E2 on haematopoietic stem cells freshly added to culture (serum-free, phenol-red-free medium with E2 or vehicle for 3 days; BrdU for 1 h; n = 3 mice in 2 independent experiments). All data represent mean ± standard deviation; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test.

(Extended Data Fig. 6b, d), as well as normal haematopoietic stem-cell and MPP frequency (Extended Data Fig. 6c), but significantly reduced BrdU incorporation into haematopoietic stem cells in female but not male mice (Fig. 3f).

To test whether ERα acts autonomously in haematopoietic stem cells we conditionally deleted Esr1 from haematopoietic cells. Mx1-cre; Esr1

Figure 4 | Increased haematopoietic stem-cell division, haematopoietic stem-cell frequency, and erythropoiesis in the spleen during pregnancy depend on ERα signalling in haematopoietic cells. a, Splenic and bone marrow cellularity. Pregnant mice were on day 14.5 of gestation. b, Pregnant mice had significantly increased Mac1/Gr1⁺ myeloid cells, Ter119⁺ erythroid cells, and overall cellularity in the spleen, but reduced bone marrow B220⁺ B cells. The increase in splenic erythropoiesis required ERα expression by haematopoietic cells. c, Haematopoietic stem-cell frequency in the bone marrow and spleen. d, In pregnant mice the rate of BrdU incorporation (24-h pulse) significantly increased in whole bone marrow (WBM) cells, bone marrow haematopoietic stem cells, and spleen haematopoietic stem cells and depended on ERα expression by haematopoietic cells (a–d, 9 non-pregnant, 7 pregnant Esr1+/+, and 6 pregnant Mx1-cre; Esr1fl/fl mice in 9 independent experiments). e, Serum E2 levels in mice (21 non-pregnant and 9 pregnant mice from 6 independent experiments). All data represent mean ± standard deviation; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test.

Esr1fl/fl mice and Esr1+/+ controls were treated with polyinosinic-polycytidylic acid (poly(I:C); four doses of 10 μg per 20 g body mass per day over 8 days) to induce Mxl-1 expression, then 19–21 days after poly(I:C) treatment we pulsed with BrdU for 10 days. Conditional deletion of Esr1 from haematopoietic cells significantly reduced BrdU incorporation into haematopoietic stem cells in female, but not male, mice (Fig. 3g).

Seven days of E2 significantly increased BrdU incorporation (3-day pulse) by haematopoietic stem cells from Esr1+/+ controls but not Mxl1-cre; Esr1fl/fl mice (Fig. 3h). Similar results were obtained using Vav1-cre; Esr1fl/fl mice (Fig. 3i), indicating that Esr1-deficient haematopoietic stem cells are not capable of responding to exogenous oestrogen.

To test whether E2 acts directly on haematopoietic stem cells, we competitively transplanted 10⁶ CD45.2⁺ Vav1-cre; Esr1fl/fl bone marrow cells along with 10⁶ CD45.1⁺ bone marrow cells into irradiated mice. Fifteen weeks later we treated the mice with either E2 or vehicle for 7 days along with BrdU for the last 3 days. E2 treatment did not significantly affect BrdU incorporation by wild-type or Esr1-deficient bone marrow cells (Fig. 3j). E2 treatment did significantly increase BrdU incorporation by wild-type haematopoietic stem cells but not by Esr1-deficient haematopoietic stem cells in the same mice (Fig. 3j). This demonstrates that E2 acts directly on haematopoietic stem cells, rather than acting indirectly by stimulating secondary signals from other haematopoietic stem cells. Consistent with this, addition of E2 to cultured haematopoietic stem cells significantly increased BrdU incorporation by wild-type haematopoietic stem cells from male and female mice but not Esr1-deficient haematopoietic stem cells (Fig. 3k).
Gene set enrichment analysis (GSEA) revealed significant enrichment of cell-cycle genes and genes with E2F1 motifs in haematopoietic stem cells from mice treated with E2 for 1 week (Extended Data Fig. 8a, b). ERx signalling may therefore promote haematopoietic stem-cell division by activating E2Fs.

Oestrogen levels increase in females during ovulation and pregnancy24. Relative to normal female mice, pregnant mice exhibited significantly increased cellularity, erythropoiesis and myelopoiesis in the spleen (Fig. 4a, b) as well as more haematopoietic stem cells in the bone marrow and spleen (Fig. 4c). A 24-h pulse of BrdU to pregnant mothers on day 13.5 of gestation revealed significant increases in proliferation among haematopoietic stem cells, whole bone marrow cells and splenocytes in pregnant as compared to normal female mice (Fig. 4a, d). As expected24, serum E2 levels increased significantly in pregnant as compared to control mice (Fig. 4e).

Deletion of Esr1 from haematopoietic cells in Mx1-cre; Esr1Δ/Δ mice significantly reduced cellularity (Fig. 4a), erythropoiesis (Fig. 4b) and haematopoietic stem-cell numbers (Fig. 4c) in the spleens of pregnant mice relative to pregnant Esr1+/Δ controls. Deletion of Esr1 from haematopoietic cells also significantly reduced BrdU incorporation into haematopoietic stem cells in the bone marrow and spleen of pregnant mice (Fig. 4d). Esr1 deletion from haematopoietic cells in pregnant mice did not block the increase in haematopoietic stem-cell frequency in the bone marrow but nearly eliminated the increase in haematopoietic stem-cell frequency in the spleen (Fig. 4c). This indicates that oestrogen is not the only factor that increases haematopoietic stem-cell activity in pregnant mice but that it is critical for the mobilization of proliferating haematopoietic stem cells to the spleen and for the expansion of splenic erythropoiesis.

The increase in spleen cellularity and erythropoiesis during pregnancy may also occur in humans, which exhibit increased spleen size during pregnancy25,30. There may be many unexplored mechanisms by which systemic signals modulate the function of stem cells within individual tissues in response to physiological change.

METHODS SUMMARY

Specific mouse alleles used in this study are referenced in Methods. Mice were housed in AAALAC-accredited, specific-pathogen-free animal care facilities at the University of Michigan (UM), Baylor College of Medicine (BCM), or UT Southwestern Medical Center (UTSW). All procedures were approved by the UM, BCM and UTSW Institutional Animal Care and Use Committees. For hormonal treatment, mice were injected subcutaneously with 100 µl of corn oil containing 2 µg oestradiol (Sigma), 1 mg progesterone (Sigma) or 100 µg of dihydrotestosterone (Steroids). 50 µg of anastrozole (Sigma) dissolved in PBS was given intraperitoneally. RU486, PPT and DPN (all from Sigma) dissolved in corn oil were administered subcutaneously at 5 mg kg⁻¹. RU486, PPT and DPN (all from Sigma) dissolved in corn oil were administered subcutaneously to pregnant mice during pregnancy but that it is critical for the mobilization of

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions D.N., H.O. and B.P.L. designed and performed most experiments. G.R.W., A.K., N.R., Y.S. and M.T. performed some experiments with D.N. D.N. and S.J.M. analysed results and wrote the manuscript.

Author Information Microarray data have been deposited to the Gene Expression Omnibus under accession number GSE52711. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to D.N. (nakada@bcm.tmc.edu) or S.J.M. (SmithGroup@baylorwestern.edu).
METHODS

Mice. The mouse alleles used in this study were Rosa26-CAG-rtTA/tetO-H2B-GFP (ref. 23), germline Era-deficient30, Mxi-1 (ref. 32), Vav-1 (ref. 33), and Erafl (ref. 34). Most studies of haematopoietic stem–cell function and cycling used young adult C57BL/6J-Tg(cre)1Cui (CD45.1) mice (8–12 weeks of age). C57BL/6J- Thy-1.2 (CD45.1) mice were used in transplantation experiments. Mice were housed in AAALAC-accredited, specific-pathogen-free animal care facilities at the University of Michigan (UM), at Baylor College of Medicine (BCM), or UT Southwestern Medical Center (UTSW). All procedures were approved by the UM, BCM and UTSW Institutional Animal Care and Use Committees.

Hormone and poly(I:C) treatments. Mice were injected subcutaneously with 100 μl of estradiol (Sigma) or progesterone (Sigma) and/or 1 mg prostaglandin E2 (Sigma). 100 μg of dihydrotestosterone (Steraloids) in corn oil was administered subcutaneously30. 50 μg of anastrozole (Sigma) dissolved in PBS was given intraperitoneally. RU486, PPT and DPN (all from Sigma) dissolved in corn oil were administered subcutaneously at 5 mg kg−1. Poly (IC) (Amersham) was re-suspended in PBS at 50 mg ml−1, and mice were injected intraperitoneally with 0.5 mg kg−1 of body mass every other day for 6 days. Note that the biological effect of poly(I:C) varies with polymer length and manufacturer such that doses must be optimized with each batch to obtain complete recombination without inducing haematopoietic stem-cell cycling. Females were mated with male mice 1 week after the last injection.

Statistical methods. Multiple independent experiments were performed to verify the reproducibility of all experimental findings. Group data always represent mean ± standard deviation. Unless otherwise indicated, two-tailed Student’s t-tests were used to assess statistical significance. No randomization or blinding was used in any experiments. Experimental mice were not excluded from analysis in any experiments. Sample sizes were selected on the basis of previous experience with the degree of variance in each assay.

Cell-cycle analysis. BrdU incorporation in vivo was measured by flow cytometry using the APC BrdU Flow Kit (BD Biosciences). Mice were given an intraperitoneal injection of 1 mg of BrdU (Sigma) per 6 g of body mass in PBS and maintained on 1 mg ml−1 BrdU in the drinking water for up to 10 days.

Flow cytometry and haematopoietic stem-cell isolation. Bone marrow cells were either flushed from the long bones (tibias and femurs) or isolated by crushing the long bones (tibias and femurs), pelvic bones and vertebrae with mortar and pestle in Hank’s buffered salt solution (HBSS) without calcium and magnesium, supplemented with 2% heat-inactivated bovine serum (Gibco). Cells were trituration and filtered through nylon screen (100 μm, Sefar America) or a 40 μm cell strainer (Fisher Scientific) to obtain a single-cell suspension. For isolation of CD150+CD48−Lin−Sca−1−c−kit− haematopoietic stem cells, bone marrow cells were incubated with PE–Cy5-conjugated anti-CD150 (TC15-12F12.2, BioLegend), PE-conjugated anti-CD48 (HM48-1, BioLegend), APC-conjugated anti-Sca−1 (Ly6A/E; E13-6.7), and biotin-conjugated anti-c–kit (2B8) antibody, in addition to antibodies against the following FITC-conjugated lineage markers: CD41 (M12/16, BD Biosciences), Ter119, B220 (6B2), Gr1 (8C5), CD2 (RM2-5), CD3 (KT31.1) and CD8 (53-6.7). Biotin-conjugated anti-c–kit (2B8) antibody, in addition to antibodies against the following PE-conjugated lineage markers: CD11b (M1/70, BD Biosciences), PE-conjugated anti-CD34 (MWReg30; BD Biosciences), TER119, B220 (6B2, Gr1 (8C5), CD2 (RM2-5), CD3 (KT31.1) and CD8 (53-6.7). Unless otherwise noted, antibodies were obtained from Biolegend, BD Biosciences, or eBiosciences. PE-conjugated anti-IL-7R (A7R34; eBiosciences), PE-Cy7-conjugated anti-Gr1 antibodies. Annexin-V staining was performed using Annexin-V APC (BD Biosciences). Flow cytometry was performed with FACSari II, FACSCanito II, LSR II, or LSRfortessa flow-cytometers (BD Biosciences).

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Castration. An incision was made in the scrotum and the testis and attached testicular fat pads were pulled out of the incision. Spermatochords were individually ligated with absorbable sutures (4–0 chromic gut), then excised, and then 1–3 non-absorbable sutures (3–0 Tevdek II) were used to close the skin.

Ovariectomy. The skin around the dorsal midline caudal to the posterior borders of the ribs was shaved and an incision was made to expose the ovaries on each side. The ovaries were isolated, ligated with absorbable sutures (4–0 chromic gut) and excised, and then 3–4 non-absorbable sutures (3–0 Tevdek II) were used to close the skin. Sham-treated mice underwent similar surgeries except that the gonads were left intact. All animals were allowed to recover for 2 weeks before BrdU was administered.

Cell-cycle analysis of haematopoietic stem cells from competitively reconstituted mice. Adult recipient mice (CD45.1) were irradiated with an X-ray source delivering approximately 300 rad min−1 in two equal doses of 540 rad, delivered at 2 h apart. 104 whole bone marrow cells from CD45.2 Vav1-cre Erafl30 mice were transplanted along with 105 CD45.1 whole bone marrow cells from wild-type mice into the retro-orbital venous sinus of anesthetized recipients. Fifteen weeks after transplantation, recipient mice were either treated with oil or 2 μg oestradiol for 7 days. Mice were administered BrdU continuously for the last 3 days of oestradiol treatment. BrdU incorporation into haematopoietic stem cells was analysed by flow cytometry as described above.

Measurement of serum hormone concentration. Whole blood samples were collected and allowed to clot at room temperature for 90 min before being centrifuged at 2000 g for 15 min at room temperature. Serum samples were analysed for oestradiol and progesterone levels at the University of Virginia Center for Research in Reproduction.

Quantitative real-time (reverse transcription) PCR. Haematopoietic stem cells and other haematopoietic cells were sorted into Trizol (Life Technologies) and RNA was isolated according to the manufacturer’s instructions. cDNA was made with random primers and SuperScript III reverse transcriptase (Life Technologies). Quantitative PCR was performed using a LightCycler 480 (Roche Applied Science) or Via7 Real-Time PCR System (Life Technologies). Each sample was normalized to β-actin. Primers to quantify cDNA levels were Esr1 forward, 5′-CTTCTTGA CCCTTCACTGAGGC-3′, Esr1 reverse, 5′-GGCGGCGAATTCAAGGTGC-3′; E2f3 forward, 5′-CCGCGCTTGTTACTTCAAGG-3′, E2f3 reverse, 5′-GTCACTACGATTGCTGCGT-3′, Pgr forward, 5′-CCGCGCCCGTCGTACC-3′, Pgr reverse, 5′-GAAGAGGGACGCGCTCCCC-3′, Ar forward, 5′-GGTGTGCGCCAGCATGAC-3′, Ar reverse, 5′-GGGCTGATCATGCGGCTCTGAG-3′.

Microarray analysis. Groups of three male and three female mice were treated with either E2 (2 μg per day) or vehicle (oil) for one week. Haematopoietic stem cells were sorted into Trizol and RNA purified according to the manufacturer’s instructions. Vav-1-transduced RNA samples were amplified and biotinylated using the Nugen Ovation Pico WTA V2 system and the Encore Biotin Module (Nugen Technologies). Biotinylated samples were hybridized to Affymetrix Mouse Gene 1.0 ST Arrays (Affymetrix) by the Baylor College of Medicine Genomic and RNA profiling core. dChip software27 was used to calculate normalized expression values for each array. Gene set enrichment analysis was performed as described previously30.

Haematopoietic stem-cell culture and in vitro BrdU incorporation. Haematopoietic stem cells were sorted directly into serum-free, phenol-red-free medium (X-Vivo 15, Lonza) supplemented with 50 ng ml−1 of SCF and 50 ng ml−1 TPO (both from Peprotech), with or without 100 nM oestradiol, and cultured for 3 days. BrdU (10 μM final concentration) was added for an hour before cells were cytospun to a slide. Slides were fixed with cold methanol for 5 min at ~20 °C, then washed with PBS containing 0.01% NP-40 and treated with 2N HCl for 15 min. Slides were blocked in PBS containing 4% goat serum, 4 mg ml−1 BSA, and 0.1% NP-40 followed by staining overnight at 4 °C with antibody against BrdU (BU17/5, 1:100, Abcam) as described previously30.
Immunostaining. Sorted haematopoietic stem cells were fixed with methanol and stained overnight at 4°C with antibodies against ERα (MC-20, 1:500, Santa Cruz Biotechnology) diluted in PBS containing 4% goat serum, 4 mg ml\(^{-1}\) BSA and 0.1% NP-40. Primary antibody staining was developed with secondary antibodies conjugated to Alexa fluor 488 together with DAPI (2 µg ml\(^{-1}\)). Slides were analysed on a Leica DMI6000 fluorescence microscope.

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Extended Data Figure 1 | Castration or ovariectomy modestly increased the numbers of B cells in the bone marrow without affecting the numbers of haematopoietic stem cells or MPPs. a, Castration (cast) or ovariectomy (ovx) did not significantly affect the numbers of haematopoietic stem cells or MPPs in the bone marrow (femurs and tibias). b, Castration or ovariectomy significantly increased the numbers of B220⁺ B cells in the bone marrow but did not affect the numbers of Mac1⁺/Gr1⁺ myeloid cells, CD3⁺ T cells, or Ter119⁺ erythroid cells in the bone marrow or spleen. 3 sham and 4 gonadectomized mice used in 3 independent experiments. All data represent mean ± standard deviation; *P < 0.05 by Student’s t-test.
Extended Data Figure 2 | Administration of oestradiol (E2) to mice induced erythropoiesis in the spleen. a–c, Treatment of male and female mice for 1 week with E2, with or without P, did not affect the numbers of Mac1⁻/Gr1⁻ myeloid cells, B220⁻ B cells, or CD3⁺ T cells in the bone marrow or spleen of either sex. d, E2 and E2+P treatment did significantly increase the number of Ter119⁺ erythroid cells in the spleen of male mice, and E2 treatment significantly increased the number of Ter119⁺ erythroid cells in the spleen of female mice. n = 3 mice per treatment in 3 independent experiments. All data represent mean ± standard deviation; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test comparing each treatment to vehicle.
Extended Data Figure 3 | Administration of exogenous oestrogen and progesterone significantly increased serum oestrogen and progesterone levels in mice but progesterone did not affect haematopoietic stem-cell division in vivo. a, Oestradiol treatment significantly increased serum oestradiol levels in male and female mice but the increased levels remained within the physiological range, similar to levels observed during pregnancy (see Fig. 4e) (male oil, 22; male E2, 20; female oil, 33; female E2, 14 mice used in 8 independent experiments). b, Progesterone treatment significantly increased serum progesterone levels in male and female mice (n = 3 mice per treatment in 3 independent experiments). Note that this did not affect bone marrow or spleen cellularity, haematopoietic stem-cell frequency, or haematopoietic stem-cell division (Fig. 2b–d). c, Esr1-deficient mice had normal levels of serum progesterone (n = 3 mice per group in 3 independent experiments). d–f, Administration of a progesterone receptor antagonist, RU486 (RU), did not affect bone marrow or spleen cellularity (d), haematopoietic stem-cell frequency in the bone marrow (e), or the division of haematopoietic stem cells, MPPs, or WBM cells (f). All data represent mean ± standard deviation from 3 independent experiments, except as indicated above; *P < 0.05; **P < 0.005; ***P < 0.0005 by Student’s t-test comparing each treatment to vehicle (oil).
Extended Data Figure 4 | Oestrogen treatment increased the frequency of Ki67⁺ cycling haematopoietic stem cells. a, Administration of oestrogen (E2) significantly increased the frequency of haematopoietic stem cells in G1 phase of the cell cycle, and reduced the frequency of haematopoietic stem cells in G0 phase of the cell cycle based on Ki67/propidium iodide staining (n = 3 mice per treatment in 3 independent experiments). b, c, Col1A1-H2B-GFP; Rosa26-M2-rtTA mice pulsed with doxycycline for 6 weeks to induce H2B–GFP expression were treated with oil (blue histogram) or E2 (red histogram) for 2 weeks without doxycycline. E2 treatment significantly increased the rate of haematopoietic stem-cell division as indicated by the reduced frequency of GFP<sup>hi</sup> quiescent haematopoietic stem cells and the increased frequency of GFP<sup>lo</sup> moderately cycling haematopoietic stem cells (5 oil-treated and 4 E2-treated mice used in 3 independent experiments). All data represent mean ± standard deviation; *P < 0.05 by Student’s t-test comparing each treatment to vehicle (oil).
Extended Data Figure 5 | Female mice have increased frequencies of megakaryocyte-erythroid progenitors (MEPs) and apoptotic Ter119<sup>−</sup> cells relative to male mice. a, Annexin-V staining of the indicated cell populations in the bone marrow of male and female mice revealed a significantly increased frequency of apoptotic Annexin-Y<sup>−</sup> cells among Ter119<sup>−</sup> erythroid progenitors in female mice. b, Female mice had a significantly increased frequency of CD34<sup>−</sup>CD16/32<sup>−</sup>Lin<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>−</sup> MEPs, but no significant differences in the frequencies of CD34<sup>−</sup>CD16/32<sup>−</sup>Lin<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>−</sup> CMPs, CD34<sup>−</sup>CD16/32<sup>−</sup>Lin<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>−</sup> GMPs, or Flt3<sup>−</sup>IL-7R<sup>−</sup>Lin<sup>−</sup>Sca-1<sup>lo</sup>c-kit<sup>lo</sup> CLPs. c, None of the restricted progenitors or differentiated cells displayed differences in cell-cycle status between male and female mice (a–c, n = 5 mice per group in three independent experiments). Data represent mean ± standard deviation; *P < 0.05; **P < 0.005; by Student’s t-test comparing each treatment between sexes.
Extended Data Figure 6 | Inhibiting oestrogen signalling by anastrozole treatment or Esr1 deficiency did not affect the numbers of haematopoietic cells in the bone marrow or spleen. a, Administration of anastrozole (Ana) to mice for 2 weeks did not significantly affect the number of Ter119− erythroid cells, CD3+ T cells, B220− B cells, or Mac1+/Gr1− myeloid cells in the bone marrow or spleen (4 PBS-treated and 6 anastrozole-treated mice were used in 4 independent experiments). b, c, Esr1 deficiency did not significantly affect bone marrow cellularity (b) or the frequencies of haematopoietic stem cells or MPPs (c) in either sex. d, Esr1 deficiency did not significantly affect the numbers of Ter119− erythroid cells, CD3+ T cells, B220− B cells, or Mac1+/Gr1− myeloid cells in the bone marrow or spleen of normal mice. −/− indicates Esr1− deficient mice and +/+ indicates wild-type littermate control mice (b–d, n = 3 mice per group in 3 independent experiments). All data represent mean ± standard deviation.
Extended Data Figure 7 | Pharmacological ERα activation, but not ERβ activation, is sufficient to promote haematopoietic stem-cell division. Male mice (n = 5 mice per treatment in 3 independent experiments) were treated with oil, ERα agonist PPT, or ERβ agonist DPN for 14 days then pulsed with BrdU for 10 days, beginning on the fourth day of hormone treatment. a, b, PPT or DPN treatment did not significantly affect the cellularity of bone marrow or spleen (a), or the frequencies of haematopoietic stem cells or MPPs in bone marrow (b). c, PPT treatment, but not DPN treatment, significantly increased erythropoiesis in bone marrow and spleen. d, PPT significantly increased the division rates of haematopoietic stem cells and MPPs, but DPN failed to do so, suggesting that ERα activation, but not ERβ activation, promotes haematopoietic stem cell division. Data represent mean ± standard deviation; ***P < 0.0005 by Student’s t-test comparing each treatment to vehicle (oil).
Extended Data Figure 8 | E2 treatment changes haematopoietic stem-cell gene expression profile in a manner consistent with increased cell division. 

a, b, Gene set enrichment analysis revealed that haematopoietic stem cells from mice treated with E2 exhibited significant enrichment in the expression of genes involved in cell cycling (a). We also observed a significant enrichment in the expression of genes with E2F1 motifs in haematopoietic stem cells from E2-treated mice, consistent with the role of E2Fs in cell-cycle control (b). n = 3 mice per treatment of each sex were used to isolate independent aliquots of RNA from haematopoietic stem cells for gene expression profiling.