Bone marrow adipocytes promote the regeneration of stem cells and haematopoiesis by secreting SCF

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Endothelial cells and leptin receptor+ (LepR+) stromal cells are critical sources of haematopoietic stem cell (HSC) niche factors, including stem cell factor (SCF), in bone marrow. After irradiation or chemotherapy, these cells are depleted while adipocytes become abundant. We discovered that bone marrow adipocytes synthesize SCF. They arise from Adipoq-Cre/ER + progenitors, which represent ~5% of LepR+ cells, and proliferate after irradiation. SCF deletion using Adipoq-Cre/ER inhibited haematopoietic regeneration after irradiation or 5-fluorouracil treatment, depleting HSCs and reducing mouse survival. SCF from LepR+ cells, but not endothelial, haematopoietic or osteoblastic cells, also promoted regeneration. In non-irradiated mice, SCF deletion using Adipoq-Cre/ER did not affect HSC frequency in long bones, which have few adipocytes, but depleted HSCs in tail vertebrae, which have abundant adipocytes. A-ZIP/F1 ‘fatless’ mice exhibited delayed haematopoietic regeneration in long bones but not in tail vertebrae, where adipocytes inhibited vascularization. Adipocytes are a niche component that promotes haematopoietic regeneration.

HSCs reside in a perivascular niche in the bone marrow created partly by endothelial cells and leptin receptor+ (LepR+) stromal cells. Approximately 80% of dividing and non-dividing HSCs in the bone marrow are adjacent to sinusoidal blood vessels, while another 10% are near arterioles and 10% are near transition zone vessels. More than 90% of the cells that express high levels of SCF and Cxcl12 in normal young adult bone marrow are LepR+, while endothelial cells express much lower levels of SCF and Cxcl12 (ref. 4). Conditional deletion of SCF or Cxcl12 from LepR+ cells or endothelial cells depletes HSCs. Deletion of SCF from LepR+ cells and endothelial cells in the same mice eliminates all quiescent and serially transplantable HSCs from adult bone marrow. The niche cells we identified on the basis of LepR expression have also been identified by others on the basis of their expression of high levels of Cxcl12 (refs 7–9), low levels of the Nestin-GFP transgene and PDGFRα (refs 4, 12) and Prx1-Cre.

Skeletal stem cells (SSCs) in the bone marrow contribute to the HSC niche and LepR+ cells include the SSCs that give rise to nearly all of the fibroblast colony-forming cells as well as most of the osteoblasts and adipocytes that form in adult bone marrow. While restricted adipocyte progenitors have been characterized in fat depots outside of the bone marrow, the identity of adipocyte progenitors in the bone marrow remains uncertain. Additional cell types, including Schwann cells, nerve fibres, macrophages and megakaryocytes, also directly or indirectly regulate the niche.

Adipocytes are rare in the marrow of most young adult mouse bones, but dramatically increase in frequency during ageing and after myeloablation. HSC frequency is lower in tail vertebrae, where adipocytes are abundant, than in thoracic vertebrae, where adipocytes are rare. Expression of the dominant-negative A-ZIP/F protein under the Fdh promoter reduces adipogenesis in mice and increases HSC frequency in tail vertebrae, accelerating haematopoietic recovery after irradiation. These data suggested that bone marrow adipocytes negatively regulate HSC function and haematopoietic recovery, although it remains unclear whether this reflects a direct effect on HSCs or an indirect effect on the niche. A-ZIP/F1 mice also exhibit changes in angiogenesis and regeneration of bone marrow sinusoids is critical for haematopoietic regeneration after irradiation.

Irradiation and chemotherapy not only deplete HSCs but also disrupt their niche by destroying sinusoidal blood vessels and depleting stromal cells. Niche regeneration is necessary for regeneration of HSCs and haematopoiesis. Denervation with 6-hydroxydopamine does not alter normal haematopoiesis but significantly inhibits regeneration after irradiation. Here we report that bone marrow adipocytes, but not adipocytes in peritoneal fat pads, express a high

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Received 18 September 2016; accepted 12 June 2017; published online 17 July 2017; DOI: 10.1038/ncb3570
level of Scf and that Scf from these cells promotes the regeneration of HSCs and haematopoiesis after irradiation or 5-fluorouracil (5-FU) treatment. Our results also reveal differences in adipocyte function among bones as adipocytes in tail vertebrae, but not long bones, inhibit bone marrow vascularization. The net result is that adipocytes in long bones promote haematopoietic recovery after irradiation while in caudal vertebrae they inhibit haematopoietic regeneration despite being an important source of SCF in both locations.

RESULTS

Irradiation changes the bone marrow stroma

Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice were irradiated and transplanted with one million bone marrow cells for radioprotection. As expected, the numbers of bone marrow cells, blood cells and Lineage<sup>-</sup>Sca-1<sup>-</sup>c-kit<sup>+</sup> (LSK) stem/progenitor cells substantially declined two weeks after irradiation but rebounded to normal or near normal levels by four weeks after irradiation (Fig. 1a–e and Supplementary Fig. 1a–e). Consistent with prior studies<sup>38,40</sup>, sinusoids were also reduced in number and substantially dilated two weeks after irradiation but largely recovered in number and morphology by four weeks after irradiation (Fig. 1f–i). We did not observe changes in the number or morphology of arterioles after irradiation (Fig. 1f–h)). Consistent with the damage to sinusoids, the numbers of VE-cadherin<sup>+</sup> endothelial cells (Fig. 1k) and Tomato<sup>+</sup> stromal cells (Fig. 1l) declined two weeks after irradiation but then partially recovered by 4 weeks after irradiation.

In contrast to the flow cytometry data, the number of Tomato<sup>+</sup> stromal cells after irradiation did not appear markedly different than in non-irradiated controls in sections (Fig. 1f–h). This appeared to be due to the differentiation of LepR<sup>+</sup> cells into perilipin<sup>+</sup> adipocytes, which are visible in sections but are too large and fragile to detect by flow cytometry. Adipocytes are Tomato<sup>+</sup> in these mice because they derive from LepR<sup>+</sup> cells<sup>2</sup> (Supplementary Fig. 1f). Adipocytes were rare in femurs from non-irradiated mice but became abundant two weeks after irradiation before modestly declining in number by 4 weeks after irradiation (Fig. 1m–p).

LepR<sup>+</sup> stromal cells and adipocytes are the primary sources of Scf after irradiation

In 2-month-old Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice, Scf-GFP was expressed at a high level by Tomato<sup>+</sup>CD45<sup>-</sup>Ter119<sup>-</sup> stromal cells (Fig. 2a) and at a low level by VE-cadherin<sup>+</sup>CD45<sup>-</sup>Ter119<sup>-</sup> endothelial cells (Fig. 2b). Irradiation did not appear to affect the percentage of endothelial cells or LepR<sup>+</sup> cells that expressed Scf-GFP or the level of expression (Fig. 2a,b). Most Scf<sup>-GFP<sup>high</sup></sup> cells were Tomato<sup>+</sup> in the bone marrow of Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice before and after irradiation (Supplementary Fig. 2a).

Tomato<sup>+</sup> cells were associated with sinusoids as well as large- and small-diameter arterioles throughout the bone marrow of Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (Fig. 2c). The Tomato<sup>+</sup> stromal cells around sinusoids and small-diameter arterioles were uniformly positive for Scf-GFP (Fig. 2c, arrows). The Tomato<sup>+</sup> stromal cells around large-diameter arterioles near the centre of the bone marrow were negative for Scf-GFP (Fig. 2c, arrowheads). Two weeks after irradiation, Scf-GFP<sup>+</sup> Tomato<sup>+</sup> stromal cells remained primarily around sinusoids and small-diameter arterioles (Fig. 2d). Scf-GFP expression was not detected in haematopoietic cells or in osteoblasts/osteocytes of 2-month-old Col1a1<sup>2.3-cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice, with or without irradiation (Supplementary Fig. 2b–e).

We confirmed the identity of adipocytes by staining with an antibody against perilipin, a protein that coats lipid droplets in the cytoplasm<sup>42</sup>. In non-irradiated 2-month-old mice, perilipin<sup>+</sup> adipocytes were rare (Fig. 1n) but always positive for Scf-GFP (Fig. 2e; Supplementary Fig. 3a). Although these cells derive from LepR<sup>+</sup> cells<sup>4</sup>, perilipin<sup>+</sup> adipocytes were negative for anti-Lepr antibody staining (Fig. 2g). Two weeks after irradiation, the numbers of perilipin<sup>+</sup> adipocytes in bone marrow sections increased dramatically and these cells remained Scf-GFP<sup>+</sup> (Fig. 2f). Note that although perilipin-positive cells always stained positively for Scf-GFP, the staining did not overlap in all optical sections (1 μm thick) because Scf-GFP is present throughout the cytoplasm while perilipin is not<sup>42</sup>. Since adipocytes arise from LepR<sup>+</sup> cells in the bone marrow, Scf-GFP overlapped with Tomato in perilipin<sup>+</sup> adipocytes in Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (Supplementary Fig. 2f,g).

Quantitative PCR with reverse transcription showed that Scf was expressed by LepR<sup>+</sup> stromal cells at high levels and in VE-cadherin<sup>+</sup> endothelial cells at low levels, but not by CD45<sup>-</sup>/Ter119<sup>-</sup> haematopoietic cells or Col1a1<sup>2.3-GFP</sup> osteoblasts, in irradiated or non-irradiated bone marrow (Fig. 2h). Since adipocytes cannot be isolated by flow cytometry, we isolated them by collecting the floating cells after enzymatic dissociation of bone marrow cells. Adipocytes from irradiated and non-irradiated bone marrow, but not from intraperitoneal fat pads, expressed Scf at a level comparable to LepR<sup>+</sup> stromal cells (Fig. 2h). We confirmed adipocyte purity by also assessing leptin (a marker of adipocytes but not LepR<sup>+</sup> stromal cells) and full-length Lepr (a marker of LepR<sup>+</sup> cells but not adipocytes) (Supplementary Fig. 3b,c). Two other adipocyte markers, perilipin and FABP4, were also abundant in adipocytes but absent from LepR<sup>+</sup> stromal cells (Supplementary Fig. 3e,f). Adiponectin (Adipoq), a marker of adipocytes and their progenitors, was detected in adipocytes and at a lower level in LepR<sup>+</sup> cells (Supplementary Fig. 3d).

Human bone marrow from 8- to 17-year-old donors contained large numbers of adipocytes (Supplementary Fig. 3g) and Scf transcripts were 52 ± 11-fold enriched in human bone marrow adipocytes as compared with bone marrow mononuclear cells (Fig. 2i).

Scf from osteoblastic, haematopoietic and endothelial cells is not required for regeneration

We conditionally deleted Scf from osteoblasts using Col1a1<sup>2.3-Cre<sup>1,43</sup></sup> (Supplementary Fig. 2d). Non-irradiated adult Col1a1<sup>2.3-cre</sup>; Scf<sup>GFP<sup>/+</sup></sup> mice had normal blood cell counts and normal numbers of total bone marrow cells, LSK cells and CD150<sup>-</sup>CD48<sup>-</sup>Lineage<sup>-</sup>Sca-1<sup>-</sup>c-kit<sup>+</sup> HSCs<sup>44</sup> in the bone marrow (Supplementary Fig. 4a-f). Irradiated Col1a1<sup>2.3-cre</sup>; Scf<sup>GFP<sup>/+</sup></sup> mice transplanted with wild-type bone marrow cells also did not significantly differ from irradiated and transplanted control mice at 2 or 4 weeks after irradiation with respect to blood cell counts or numbers of total bone marrow cells, LSK cells or HSCs in the bone marrow (Supplementary Fig. 4a-f). Bone marrow cells competitively transplanted from these Col1a1<sup>2.3-cre</sup>; Scf<sup>GFP<sup>/+</sup></sup> recipients at 4 weeks after irradiation gave similar levels of donor cell reconstitution in all lineages as compared with bone marrow cells from control recipients (Supplementary Fig. 4g). Therefore, Scf expression by osteoblasts is dispensable for haematopoietic regeneration.

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Figure 1 Irradiation disrupted sinusoids and depleted HSCs, endothelial cells, and LepR+ stromal cells while dramatically increasing adipocytes in the bone marrow. (a-p) One million bone marrow cells from wild-type mice were transplanted into irradiated wild-type (a-e,m-p) or Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> (f-i) mice. Statistical significance was assessed using repeated measures one-way ANOVAs with Geisser-Greenhouse sphericity corrections along with Tukey’s multiple comparisons tests (a-e,i-m). Asterisks indicate statistical significance relative to control (Con) while hash symbols indicate statistical significance of differences between 2 and 4 weeks (2wk and 4wk) after irradiation (* or # P<0.05, ** or ## P<0.01, ### or ### P<0.001). All data represent mean ± s.d. (a-e) Flow cytometric analysis of mechanically dissociated bone marrow cells revealed significant reductions in bone marrow cellularity (a) and the numbers of Lineage<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>−</sup> (LSK) cells (b), CD150<sup>−</sup>CD48<sup>−</sup>Lineage<sup>−</sup>Sca-1<sup>−</sup>c-kit<sup>−</sup> HSCs (c), Mac1<sup>−</sup>Gr-1<sup>−</sup> myeloid cells (d) and Ter119<sup>+</sup> erythroid cells (e) at 2 and/or 4 weeks after irradiation as compared with non-irradiated control mice. Cell numbers reflect two femurs and two tibias per mouse (n=5 mice per treatment from 5 independent experiments). (f-h) Confocal imaging of thin femur sections from non-irradiated Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice (control, f) or at 2 weeks (g) or 4 weeks (h) after irradiation and bone marrow transplantation. Arrows indicate sinusoidal blood vessels and arrowheads indicate arterioles. (i,j) Flow cytometric analysis of enzymatically dissociated bone marrow cells from Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice revealed significant reductions in the numbers of VE-cadherin<sup>+</sup> laminin<sup>+</sup> sinusoids (i) and VE-cadherin<sup>+</sup> laminin<sup>+</sup> arterioles (j) were quantified in sections (n=5 mice per condition from 3 independent experiments). (k,l) Flow cytometric analysis of enzymatically dissociated bone marrow cells from Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice revealed significant reductions in the numbers of VE-cadherin<sup>+</sup> endothelial cells (k) and Tomato<sup>+</sup> stromal cells (l) after irradiation (n=4 mice per condition from 4 independent experiments). (m) Adipocyte numbers in thick femur sections (50 μm) from non-irradiated mice (Con) or mice at 2 or 4 weeks after irradiation (n=5 mice per condition from 3 independent experiments). (n-p) Whole-mount imaging of thick femur sections (50 μm) from non-irradiated mice (Control, n) or mice 2 (o) or 4 (p) weeks after irradiation and bone marrow transplantation. Adipocytes were identified on the basis of anti-perilipin staining (n=6 mice per condition from 3 independent experiments).

Conditional deletion of Sce from haematopoietic cells using Vav1-Cre also did not affect blood cell counts, LSK cells or HSCs in the bone marrow or non-irradiated (Supplementary Fig. 4h-m) or irradiated mice (Supplementary Fig. 4h-m). Competitive secondary transplantation at 4 weeks after irradiation found no differences in the reconstituting capacity of Vav1-cre; Sce<sup>GFP/β</sup> versus control bone...
Figure 2  Scf was highly expressed by LepR⁺ stromal cells and adipocytes in the bone marrow before and after irradiation. (a–f) One million whole bone marrow cells from wild-type mice were transplanted into irradiated LepR⁺; R26Gf-reporter; Scf⁺/− (a–d) or Scf−/− (e,f) mice. (a,b) Flow cytometric analysis of enzymatically dissociated bone marrow cells from LepR⁺; R26Gf-reporter; Scf⁺/− mice showed that Scf-GFP was expressed at a high level by LepR⁺ stromal cells (a) and at a low level by endothelial cells (b) in non-irradiated mice (Control) and at 2 weeks after irradiation and bone marrow transplantation (representative results from 3 independent experiments). (c,d) Representative femur diaphysis sections showed Scf-GFP expression by Tomato⁺ stromal cells in the bone marrow of LepR⁺; R26Gf-reporter; Scf⁺/− mice that were not irradiated (Control, c) or at 2 weeks after irradiation and bone marrow transplantation (d). Tomato⁺ cells around small arterioles and sinusoids (arrows) expressed Scf-GFP while Tomato⁺ cells around large arterioles (arrowheads) did not (representative results from 3 independent experiments). (e,f) Representative femur metaphysis sections showed Scf-GFP expression by perilipin⁺ adipocytes in non-irradiated mice (Control, e) and at 2 weeks after irradiation and bone marrow transplantation (f) (representative results from 6 independent experiments). Note that the subcellular distributions of perilipin and GFP differ. See Supplementary Fig. 2e for serial optical sections showing Scf-GFP expression by a perilipin⁺ adipocyte. (g) Confocal imaging of thin femur sections from non-irradiated Scf⁺/− mice co-stained with anti-LepR and anti-perilipin antibodies. LepR⁺ cells were Scf-GFP⁺ but perilipin-negative (arrows). Perilipin⁺ cells were Scf-GFP⁺ but LepR-negative (arrowheads; representative results from 3 independent experiments). (h,i) Quantitative real-time PCR analysis of Scf transcript levels (normalized to β-actin) in CD45⁺/Ter119⁺ haematopoietic cells (Haema), Col1a1⁺/2.3-GFP⁺ osteoblasts (Osteo), VE-cadherin⁺ endothelial cells (Endo), Tomato⁺CD45⁺/Ter119⁻ bone marrow stromal cells from LepR⁺; R26Gf-reporter (LepR⁺), bone marrow adipocytes (Adip-BM) and intraperitoneal adipocytes (Adip-IP) relative to unfractionated bone marrow cells in mouse (h) and human bone marrow (i). The Scf transcript level in unfractionated bone marrow cells was normalized to 1. Data represent mean ± s.d. (n=3 mice (h) and n=3 human (i) samples, each from 3 independent experiments).
mature bone marrow cells (Supplementary Fig. 4g). Scf expression by haematopoietic cells is also dispensable for haematopoietic regeneration.

Non-irradiated Tie2-cre; Scf<sup>GFP/fl</sup> mice had normal blood cell counts (Fig. 3a–c) but significantly fewer LSK cells, and HSCs as compared with non-irradiated control mice (Fig. 3d–f). In contrast, at 2 or 4 weeks after irradiation we observed no significant difference between control mice reconstituted with control bone marrow cells versus Tie2-cre; Scf<sup>GFP/fl</sup> mice reconstituted by control bone marrow in terms of blood cell counts, or the numbers of total cells, LSK cells or HSCs in the bone marrow (Fig. 3a–f). Bone marrow cells competitively transplanted from these Tie2-cre; Scf<sup>GFP/fl</sup> recipients at 4 weeks after irradiation gave similar levels of donor cell reconstitution in all lineages as compared with bone marrow cells from control recipients (Fig. 3g). Therefore, the low level of SCF produced by endothelial cells contributes to HSC maintenance and haematopoiesis in normal adult bone marrow but is dispensable for haematopoietic regeneration after irradiation.

**Scf from LepR<sup>+</sup> cells promotes haematopoietic regeneration**

In non-irradiated bone marrow from 2-month-old Lepr<sup>cre</sup>; R26<sup>DTomato</sup> mice, more than 95% of Tomato<sup>+</sup> cells (Supplementary Fig. 5a,d) as well as cells that express high levels of Scf-GFP stain positively with an

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**Figure 3** Scf from LepR<sup>+</sup> stromal cells, but not endothelial cells, is necessary for haematopoietic regeneration and mouse survival after irradiation. (a–f) White blood cell (a), red blood cell (b) and platelet counts (c), as well as bone marrow (two femurs and two tibias per mouse) cellularity (d) and numbers of Lineage-Scf<sup>GFP/fl</sup> cells (e) and CD150<sup>+</sup>CD48<sup>+</sup>Lineage-Scf<sup>GFP/fl</sup> cells (f) from paired Tie2-cre; Scf<sup>GFP/fl</sup> mice and Scf<sup>GFP/fl</sup> controls that were non-irradiated (Con) or analysed 2 or 4 weeks after irradiation and bone marrow transplantation. n = 5 mice per genotype per condition from 3 independent experiments. HSCs could not be detected at 2 weeks after irradiation. Two-way ANOVAs with Sidak’s multiple comparisons tests (a–e) or two-tailed Student’s t-tests with Holm–Sidak’s multiple comparisons test (f) were used to assess differences between Tie2-cre; Scf<sup>GFP/fl</sup> and Scf<sup>GFP/fl</sup> controls that were non-irradiated (Con) or analysed at 2 or 4 weeks after irradiation and bone marrow transplantation (n = 5 mice per genotype per condition from 3 independent experiments). Differences were assessed using two-way ANOVAs with Sidak’s multiple comparisons tests. (h–m) White blood cell (h), red blood cell (i) and platelet counts (j), as well as bone marrow cellularity (k) and numbers of LSK cells (l) and HSCs (m) from paired Lepr-cre; Scf<sup>GFP/fl</sup> mice and Scf<sup>GFP/fl</sup> controls that were non-irradiated (Con) or analysed at 2 or 4 weeks after irradiation and bone marrow transplantation (n = 5 mice per genotype per condition from 3 independent experiments). Two-way ANOVAs with Sidak’s multiple comparisons tests (h–l) or two-tailed Student’s t-tests with Holm–Sidak’s multiple comparisons test (m) were used to assess the statistical significance of differences between Lepr-cre; Scf<sup>GFP/fl</sup> and Scf<sup>GFP/fl</sup> mice. Differences were assessed using two-way ANOVAs with Sidak’s multiple comparisons test (P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001). (o) Mouse survival after irradiation and transplantation of 2 × 10<sup>6</sup> whole bone marrow cells (n = 20 mice per genotype per condition from 5 independent experiments). The Gehan–Breslow–Wilcoxon test was used to assess statistical significance. All data represent mean ± s.d.
antibody against LepR (Fig. 2g). At 2 and 4 weeks after irradiation, the accumulation of adipocytes (Fig. 1o) that derive from LepR+ cells (Supplementary Fig. 1f) reduced the proportion of Tomato+ bone marrow cells that stained positively for anti-LepR antibody as differentiation to adipocytes abolished LepR expression (Supplementary Fig. 5a–d).

Non-irradiated Leprcre/+; ScfGFP/fl mice had normal blood cell counts (Fig. 3h–i) but significantly lower bone marrow cellularity (Fig. 3k) and numbers of LSK cells (Fig. 3l) and HSCs (Fig. 3m) as compared with non-irradiated control mice. To test whether LepR+ cells and their progeny promote haematopoietic regeneration after irradiation we transplanted Leprcre/+; ScfGFP/fl and ScfGFP/fl controls with wild-type bone marrow cells and compared haematopoietic reconstitution. Leprcre/+; ScfGFP/fl recipients had much lower levels of SCF in the bone marrow as compared with control mice at 2 weeks after irradiation (Supplementary Fig. 5e). At 2 and 4 weeks after irradiation, Leprcre/+; ScfGFP/fl mice had significantly lower white (Fig. 3h) and red blood cell counts (Fig. 3i) as well as bone marrow cellularity (Fig. 3k) and numbers of LSK cells (Fig. 3l) and HSCs (Fig. 3m) as compared with control mice. Competitive secondary transplantation of bone marrow cells from these recipient mice confirmed the significantly reduced reconstituting activity of bone marrow cells from Leprcre/+; ScfGFP/fl as compared with control recipients (Fig. 3n). Significantly more Leprcre/+; ScfGFP/fl recipients died after irradiation and transplantation of 2 × 10^5 bone marrow cells as compared with control recipients (Fig. 3o).

**Adipoq-Cre/ER expression identifies adipocyte progenitors**

Adiponectin-Cre/ER (Adipoq-Cre/ER), which recombines in adipocytes in white adipose depots35,46, also recombined in bone marrow adipocytes. Four weeks after gavaging 6-week-old Adipoqcre/ER; R26tdTomato mice with tamoxifen, Tomato was expressed in 0.017 ± 0.00088% of bone marrow cells (Fig. 4a), including 93 ± 5% of perilipin+ adipocytes (Fig. 4b). Virtually all of the Tomato expression in the bone marrow outside of adipocytes was in a subset of LepR+ stromal cells: 5.9 ± 3.1% of LepR+ cells in Adipoq-Cre/ER; R26tdTomato bone marrow were Tomato+ (Fig. 4c). We did not detect Tomato expression in haematopoietic cells (Fig. 4a), VE-cadherin+ endothelial cells (Fig. 4b,e) or osteoblasts (Fig. 4d). Thus, Adipoq-Cre/ER recombines in Scf-GFP-expressing adipocytes in the bone marrow as well as in a small subset of LepR+ stromal cells (Fig. 4e,f,g).

At four weeks after tamoxifen treatment, both LepR+Tomato+ cells and LepR+Tomato+ cells from Adipoq-Cre/ER; R26tdTomato mice formed CPU-F colonies (Fig. 4h), and differentiated into oil-red-O- adipocytes and alizarin-red osteoblasts (Fig. 4i). However, LepR+Tomato+ cells spontaneously formed many more perilipin+ adipocytes as compared with LepR+Tomato+ cells (Fig. 4j and Supplementary Fig. 6a,b).

To determine the fate of Adipoq-Cre/ER+ cells in vivo, we treated 6-week-old Adipoq-Cre/ER; R26tdTomato mice with tamoxifen and then analysed femur bone marrow 2 to 24 weeks later. Two weeks after tamoxifen, Tomato+ stromal cells accounted for only 0.009 ± 0.0004% of enzymatically dissociated bone marrow cells (Fig. 5a), including 3.5 ± 2.3% of LepR+ cells (Fig. 5b), and 95 ± 2.3% of perilipin+ adipocytes (Fig. 5c), which were rare in femur bone marrow (Fig. 5d). The percentages of bone marrow cells and LepR+ cells that were Tomato+, as well as the numbers of adipocytes in the bone marrow, all increased over time. By 16 weeks after tamoxifen treatment, Tomato+ stromal cells accounted for 0.07 ± 0.02% of bone marrow cells (Fig. 5a), including 23 ± 5.9% of LepR+ cells (Fig. 5b), and 78 ± 11% of perilipin+ adipocytes (Fig. 5c). In normal adult mice, Adipoq-Cre/ER+ LepR+ stromal cells thus expand in number and give rise to increasing numbers of adipocytes over time (Fig. 5d). Although Adipoq-Cre/ER+ cells had the potential to form bone in culture, they rarely formed osteoblasts in vivo (Fig. 5e,f). This suggests these cells are specified to form adipocytes in vivo.

The percentage of Tomato+ adipocytes modestly declined between 2 and 24 weeks after tamoxifen treatment (Fig. 5c). This suggests that Adipoq-Cre/ER+ adipocyte progenitors were replenished by LepR+Adipoq-Cre/ER+ SSCs as they differentiated into adipocytes.

We treated Adipoq-Cre/ER; R26tdTomato mice with tamoxifen 2 weeks before irradiation and bone marrow transplantation. Two weeks after irradiation, LepR+Tomato+ stromal cells and LepR+Tomato+ stromal cells were dramatically depleted in the bone marrow (Fig. 5g,h), partly as a result of cell death (Fig. 5i). The numbers of these cells in the bone marrow increased between 2 and 4 weeks after irradiation but remained significantly lower than in non-irradiated bone marrow (Fig. 5g,h). In contrast, the number of LepR-negative adipocytes dramatically increased after irradiation (Fig. 5k).

In normal young adult mice, adipocyte progenitors were quiescent (Fig. 5m). However, 2 weeks after irradiation, Tomato+ adipocyte progenitors had gone into cycle (Fig. 5m,n). Tomato+ cells from Leprcre/+; R26tdTomato mice were also quiescent in normal young adult mice but went into cycle after irradiation (Fig. 5m,n).

**Bone marrow adipocytes are an important source of Scf**

Deletion of Scf with Adipoq-Cre/ER had little effect on SCF protein levels in non-irradiated bone marrow but substantially reduced SCF levels in irradiated bone marrow (Supplementary Fig. 5e). Non-irradiated 3-month-old Adipoq-Cre/ER; ScfGFP/fl mice at 4 weeks after tamoxifen administration had normal blood cell counts (Fig. 6a–c), normal bone marrow cellularity (Fig. 6d) and normal numbers of LSK cells (Fig. 6e) and HSCs (Fig. 6f) in femur and tibia bone marrow. In contrast, by 4 weeks after irradiation and transplantation of wild-type bone marrow cells, Adipoq-Cre/ER; ScfGFP/fl mice had significantly lower red blood cell counts (Fig. 6b), total bone marrow cells (Fig. 6d), LSK cells (Fig. 6e) and HSCs (Fig. 6f) in their femurs and tibias as compared with irradiated ScfGFP/fl controls.

Bone marrow cells from the femurs and tibias of non-irradiated Adipoq-Cre/ER; ScfGFP/fl mice gave similar levels of donor cell reconstitution as non-irradiated control bone marrow cells following transplantation into irradiated mice (Fig. 6g). However, bone marrow cells from Adipoq-Cre/ER; ScfGFP/fl recipient mice at 4 weeks after irradiation gave significantly lower levels of donor cell reconstitution as compared with bone marrow cells from ScfGFP/fl recipient mice (Fig. 6h). A significantly higher proportion of Adipoq-Cre/ER; ScfGFP/fl mice died after irradiation and bone marrow transplantation as compared with control mice (Fig. 6i). Therefore, Scf from adipocytes promotes haematopoietic regeneration and mouse survival after irradiation.

Although SCF acts directly on HSCs to promote c-kit receptor signalling47,48, Scf deletion could potentially also indirectly affect HSCs as a result of changes in stromal cells; however, few
megakaryocytes or endothelial cells expressed c-kit (Supplementary Fig. 7a–d) and Scf deletion using Adipoq-CreER did not significantly affect the morphologies or frequencies of megakaryocytes, sinusoids or arterioles in the bone marrow at 4 weeks after irradiation (Supplementary Fig. 7e–h).

We also induced myeloablation by treating with 5-FU. Twelve days later we observed abundant perilipin+ adipocytes throughout the bone marrow (Supplementary Fig. 6e). Adipoq-cre/ER; ScfGFP/β mice had significantly fewer bone marrow cells (Fig. 6j), LSK cells (Fig. 6k) and HSCs (Fig. 6l; HSC markers are effective 12 days after 5-FU treatment, Supplementary Fig. 6e). Only 5.9 ± 3.1% of LepR+ cells were Tomato+ in the bone marrow of Adipoq-cre/ER; R26 conditional mice at 4 weeks after tamoxifen administration. While not detected by flow cytometry, perilipin+ adipocytes in bone marrow sections from Adipoq-cre/ER; R26ScfGFP mice were consistently positive for Scf-GFP and Tomato. Percentages of LepR–Tomato+ or LepR–Tomato+ stromal cells from Adipoq-cre/ER; R26ScfGFP mice that formed CFU-F colonies in culture were 0.017 ± 0.008% of bone marrow cells were Tomato+ in enzymatically dissociated bone marrow cells from Adipoq-cre/ER; R26tdTomato mice at 4 weeks after tamoxifen administration. (b) Adipoq-cre/ER; R26tdTomato mice exhibited Tomato expression by perilipin+ adipocytes (arrowheads) and a subset of perilipin-negative stromal cells (arrows) in the bone marrow. (c) Only 5.9 ± 3.1% of LepR+ stromal cells were Tomato+ in the bone marrow of Adipoq-cre/ER; R26ScfGFP mice at 4 weeks after tamoxifen administration. (d) Tomato expression was rarely detected in bone-lining GFP+ osteoblasts from Adipoq-cre/ER; R26GFP mice. (e) Tomato expression was not detected in VE-cadherin+CD45/Ter119– endothelial cells in the bone marrow of Adipoq-cre/ER; R26tdTomato mice at 4 weeks after tamoxifen administration. (f) Only 6.2 ± 2.9% of Scf-GFP+ stromal cells were Tomato+ in the bone marrow of Adipoq-cre/ER; R26GFP mice at 4 weeks after tamoxifen administration. (g) While not detected by flow cytometry, perilipin+ adipocytes in bone marrow sections from Adipoq-cre/ER; R26GFP mice were consistently positive for Scf-GFP and Tomato. (h) Percentages of LepR–Tomato+ or LepR–Tomato+ stromal cells from Adipoq-cre/ER; R26GFP mice that formed CFU-F colonies in culture. (i) Percentage of CFU-F colonies that contained oil-red-O+ adipocytes or alizarin-red-S+ osteoblastic cells. (j) The average numbers of perilipin+ adipocytes or alkaline phosphatase (ALP) osteogenic cells that spontaneously differentiated per osteoblasts from 3 mice; 3 independent experiments. A two-way ANOVA with Sidák’s multiple comparisons test was used to assess statistical significance. *P < 0.01. All data represent mean ± s.d. and representative images from n = 3 mice in 3 independent experiments.
Figure 5  Adipoq-Cre/ER⁺ bone marrow stromal cells form adipocytes, but rarely osteoblasts, in vivo. (a–c) Percentages of whole bone marrow (WBM) cells (a; by flow cytometry), LepR⁺ stromal cells (b; by flow cytometry), or adipocytes (c; by microscopy in sections) that were Tomato⁺ in Adipoq-Cre/ER; R26tdTomato mice at 2–24 weeks after tamoxifen administration. (d) Numbers of adipocytes per section. Two-way ANOVAs with Sidak’s multiple comparisons tests were used to assess differences among consecutive ages. (e,f) Four months after tamoxifen treatment, Adipoq-Cre/ER; R26tdTomato; Col1a1*2.3-GFP mice had only rare GFP⁺ osteoblasts that were Tomato⁺ in bone marrow sections (e) or by flow cytometry (f). (g,h) Based on flow cytometric analysis, the numbers of LepR⁺Tomato⁺ cells (g) and LepR⁺Tomato⁻ cells (h) declined in Adipoq-Cre/ER; R26tdTomato mice after irradiation and bone marrow transplantation. Differences between non-irradiated (Con) and irradiated mice (at 2 or 4 weeks) were assessed by one-way ANOVAs with Dunnett’s multiple comparisons tests (data from g were log₂-transformed). (i) Percentages of LepR⁺Tomato⁺ or LepR⁺Tomato⁻ cells that were also DAPI⁺ Annexin V⁺ in enzymatically dissociated bone marrow cells from Adipoq-Cre/ER; R26tdTomato mice one day after irradiation. A two-way ANOVA with Sidak’s multiple comparisons test was used to assess differences among treatments. (j,k) The percentages of LepR⁺ cells that were Tomato⁺ declined in Adipoq-Cre/ER; R26tdTomato mice after irradiation. In contrast, the number of Tomato⁺ adipocytes in femur sections increased after irradiation (k). The statistical significance of differences between non-irradiated and irradiated mice was measured using one-way ANOVAs with Dunnett’s multiple comparisons tests (data of k were log₂-transformed). (l) The vast majority of bone marrow adipocytes were Tomato⁺ in non-irradiated and irradiated Adipoq-Cre/ER; R26tdTomato mice. (m,n) Hoechst staining and BrdU incorporation (14 day pulse, n) by Tomato⁺ bone marrow stromal cells in Adipoq-Cre/ER; R26tdTomato mice or LepCrem; R26tdTomato mice that were non-irradiated (Con) or at 2 weeks after irradiation and bone marrow transplantation. Two-way repeated measures ANOVAs with Sidak’s multiple comparisons tests were used to assess differences among treatments. All data represent mean ± s.d. from n=5 mice per time point from 3 independent experiments. For all panels *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 6 Scf from adipocytes is required for the regeneration of HSCs and haematopoiesis and mouse survival after irradiation. (a–f) One million WBM cells were transplanted into irradiated Adipoq-cre/ER; ScfGFP/fl mice or ScfGFP/fl controls 2 weeks after tamoxifen treatment. Non-irradiated Adipoq-cre/ER; ScfGFP/fl mice and ScfGFP/fl controls mice were treated with tamoxifen 4 weeks before analysis. (a–f) White blood cell (a), red blood cell (b) and platelet counts (c), as well as WBM cellularity (d) and numbers of LSK cells (e) and HSCs (f) from Adipoq-cre/ER; ScfGFP/fl and ScfGFP/fl mice that were non-irradiated (Con) or analysed at 2 or 4 weeks after irradiation and bone marrow transplantation. n=5 mice per genotype per condition from 3 independent experiments. Statistical significance was assessed using two-way repeated measures ANOVAs (a–f) 10^6 donor WBM cells from the indicated primary recipient mice were transplanted 4 weeks after irradiation along with equal numbers of recipient WBM cells into irradiated secondary recipient mice (n=7 mice per genotype). A Gehan–Breslow–Wilcoxon test was used to assess statistical significance in (i) and (m). All data represent mean ± s.d. For all panels *P < 0.05, **P < 0.01, ***P < 0.001.
Figure 7 *Sf* from adipocytes is required for normal HSC frequency and haematopoiesis in the caudal vertebrae of normal young adult mice. (a) Whole-mount imaging of cross-sections of a caudal vertebra showing large numbers of adipocytes in the bone marrow (representative image from 3 independent experiments performed on CA1–CA5 vertebrae). (b) The frequency of LepR* stromal cells in bone marrow from femurs and tibia versus caudal vertebrae (CA1–CA5). Data represent mean ± s.d. from *n*=3 mice in 3 independent experiments. A two-tailed Student’s *t*-test was used to assess the statistical significance between genotypes (data were log2 transformed; **P < 0.01). (c–e) Adipoq-cre/ER recombined in nearly all adipocytes (c,d) but only in a small subset of LepR* stromal cells (e) four weeks after tamoxifen treatment. Data represent mean ± s.d. from *n*=3 mice in 3 independent experiments. (f) Adipocytes were abundant in all caudal vertebrae (proximal = CA1–CA5; middle = CA6–CA10; distal = CA11–CA16; representative images from 3 independent experiments). (g) Bone marrow vascularity progressively declined in caudal vertebrae (representative images from 3 independent experiments). (h–k) Endothelial cell frequency (h), LepR* cell frequency (i), total bone marrow cellularity (j) and HSC frequency (k) in caudal vertebrae from Adipoq-cre/ER; *Sf*GFP/fl mice and *Sf*GFP/fl controls that had been administered tamoxifen 4 weeks earlier. Data represent mean ± s.d. from *n*=5 mice per treatment in 3 independent experiments. Paired, two-tailed Student’s *t*-tests with Holm–Sidak’s multiple comparisons tests were used to assess statistical significance for j and k (**P < 0.05). (l) Competitive reconstitution assay in which 5 × 10^5 donor bone marrow cells from middle caudal vertebrae were transplanted along with equal numbers of recipient bone marrow cells into irradiated recipient mice. All data represent mean ± s.d. (n=10 recipient mice per genotype from 3 independent experiments). The statistical significance of differences was assessed using two-way ANOVAs with Sidak’s multiple comparisons tests (**P < 0.05, ***P < 0.01, ****P < 0.001).

*Sf* from adipocytes promotes HSC maintenance in caudal vertebrae

Differences in the numbers of adipocytes in different bones might create regional differences in the cellular composition of the HSC niche. We confirmed that perilipin+ adipocytes were more abundant

ref. 49) as compared with *Sf*GFP/fl control mice 12 days after 5-FU treatment. Significantly more Adipoq-cre/ER; *Sf*GFP/fl mice died after two doses of 5-FU as compared with control mice (Fig. 6m). SCF synthesized by adipocytes thus promotes haematopoietic regeneration after 5-FU treatment.
A-ZIP/F1 ‘fatless’ mice exhibit delayed haematopoietic regeneration in the femur but accelerated regeneration of haematopoesis and vasculature in caudal vertebrae. All data in a–n represent mean ± s.d. from n=5 mice (Con and 4wk) or n=8 mice (2wk) per genotype per treatment from 3 independent experiments performed on leg bones (femurs + tibia; a–g) or caudal vertebrae (CA6–CA10, h–n) from non-irradiated control mice (Con) or mice at 2 or 4 weeks after irradiation and bone marrow transplantation. Two-way ANOVAs (b–e) or repeated measures two-way ANOVAs (a and f–n) with Sidak’s multiple comparisons tests were used to assess the statistical significance of differences between control and A-ZIP/F1 mice (*P < 0.05, **P < 0.01, ***P < 0.001). Data in some panels (a, b, e–n) were log-transformed prior to performing these statistical tests because the data showed unequal variance among groups. (a) Perilipin+ adipocytes in thin femur sections. (b–g) Numbers of total bone marrow cells (b), HSCs (c), LSK cells (d), LK cells (e), LepR+ stromal cells (f) and VE-cadherin+ endothelial cells (g) in two femurs and two tibias. (h) Perilipin+ adipocytes in thin caudal vertebra sections. (i–n) Numbers of total bone marrow cells (i), HSCs (j), LSK cells (k), LK cells (l), LepR+ stromal cells (m) and VE-cadherin+ endothelial cells (n) per caudal vertebra. (o–q) Confocal imaging of thin femur sections from control and A-ZIP/F1 mice that were non-irradiated (Con) or analysed at 2 or 4 weeks after irradiation and bone marrow transplantation. (Images are representative of 3 mice per genotype per condition from 3 independent experiments.) (r–t) Confocal imaging of thin caudal vertebra sections from control and A-ZIP/F1 mice that were non-irradiated (Con) or analysed at 2 or 4 weeks after irradiation and bone marrow transplantation. (n=3 mice per genotype per condition from 3 independent experiments).
in caudal vertebrae than in femurs and tibias from normal young adult mice (compare Fig. 7a with 1n). The frequency of LepR+ stromal cells was significantly lower in bone marrow from caudal vertebrae as compared with femurs and tibia (Fig. 7b). Adipoq-Cre/ER recombined in 89 ± 4.3% of adipocytes (Fig. 7c,d) but only in 4.3 ± 2.2% of LepR+ stromal cells in caudal vertebrae (Fig. 7e).

Vascularization (Fig. 7g), endothelial cell frequency (Fig. 7h), LepR+ cell frequency (Fig. 7i), total bone marrow cellularity (Fig. 7j) and HSC frequency (Fig. 7k) all progressively declined in distal (CA11–CA15) as compared with proximal (CA1–CA5) caudal vertebrae. To test whether HSCs depend on SCF from adipocytes in these bones we treated 2-month-old Adipoq-cre/ER; Scf<sup>gfp/+</sup> mice with tamoxifen. Four weeks later, Adipoq-cre/ER; Scf<sup>gfp/+</sup> mice had significantly reduced total bone marrow cells (Fig. 7j) and HSCs (Fig. 7k) relative to control mice in all caudal vertebrae. The femurs and tibias of the same Adipoq-cre/ER; Scf<sup>gfp/+</sup> mice did not show any haematopoietic defects. Bone marrow cells from middle caudal vertebrae of Adipoq-cre/ER; Scf<sup>gfp/+</sup> mice gave significantly lower levels of donor cell reconstitution in all lineages as compared with control bone marrow cells (Fig. 7l). HSC maintenance thus depends on SCF from adipocytes in caudal vertebrae, but not long bones, of non-irradiated young adult mice.

**Positive and negative effects of adipocytes on regeneration in different bones**

We ablated adipocytes by treating Adipoq-cre/ER; R26<sup>tdTomato</sup>; R26<sup>tdTP</sup> mice with tamoxifen and then diphtheria toxin. Diphtheria toxin initially eliminated most Tomato<sup>+</sup> cells from the bone marrow but adipocytes were rapidly regenerated from unrecombined Tomato-negative progenitors (presumably LepR<sup>+</sup> cells) within 14 days (Supplementary Fig. 7i–k). This was not, therefore, an effective way to test whether adipocytes promote haematopoietic regeneration.

Instead, we examined A-ZIP/F1 mice, which constitutively inhibit the differentiation of adipocytes. As expected<sup>29</sup>, these mice had modestly but significantly fewer adipocytes in their long bones (femurs-tibias; Fig. 8a) relative to littermate controls. In non-irradiated long bones, A-ZIP/F1 mice had normal numbers of bone marrow cells (Fig. 8b), HSCs (Fig. 8c), LSK cells (Fig. 8d) and LK cells (Fig. 8e). However, two weeks after irradiation, A-ZIP/F1 mice had significantly fewer total bone marrow cells (Fig. 8b), and LSK cells (Fig. 8d) as well as a trend toward reduced HSC numbers (P = 0.057) in long bones as compared with control mice. In non-irradiated long bones, adipocytes are rare and do not have a significant source of SCF but after irradiation they become abundant and promote regeneration.

Consistent with the results from ref. 29, non-irradiated caudal vertebrae (CA6–CA10) from A-ZIP/F1 mice had substantially reduced numbers of adipocytes (Fig. 8h) accompanied by increased numbers of total bone marrow cells (Fig. 8i), HSCs (Fig. 8j), LSK cells (Fig. 8k) and LK cells (Fig. 8l) as compared with littermate controls. By 4 weeks after irradiation, A-ZIP/F1 mice had significantly increased numbers of total bone marrow cells (Fig. 8i), HSCs (Fig. 8j), LSK cells (Fig. 8k) and LK cells (Fig. 8l) in caudal vertebrae. Adipocytes therefore negatively regulate HSC frequency and haematopoietic regeneration in tail vertebrae.

In young adult long bones, the vasculature (Fig. 8o–q) and the numbers of LepR<sup>+</sup> cells (Fig. 8f) and endothelial cells (Fig. 8g) appeared normal in non-irradiated A-ZIP/F1 mice and appeared to regenerate normally at 2 and 4 weeks after irradiation. In contrast, in non-irradiated caudal vertebra bone marrow, the vasculature was sparse relative to femur bone marrow (compare controls in Fig. 8r with 8o). With and without irradiation, caudal vertebrae in the A-ZIP/F1 mice had more sinusoidal blood vessels (Fig. 8r–t), LepR<sup>+</sup> cells (Fig. 8m) and endothelial cells (Fig. 8n) as compared with littermate controls. This suggests that adipocytes negatively regulated the vascularization of the bone marrow in caudal vertebrae in a way that was not observed in long bones. The improved regeneration in the caudal vertebrae of A-ZIP/F1 mice correlated with increased vascularity. It is not clear whether this reflects only a developmental effect or whether there is an ongoing effect of adipocytes on the vasculature of caudal vertebrae in adult bone marrow. Overall, the data indicate that adipocytes promote HSC maintenance and haematopoietic regeneration by secreting SCF but that in adipocyte-rich caudal vertebrae there is also an inhibition of bone marrow vascularization that has independent negative effects on HSC frequency and haematopoietic regeneration.

**DISCUSSION**

The long-standing clinical observation that adipocyte-rich yellow marrow is associated with reduced haematopoiesis in humans has commonly been interpreted to reflect a negative effect of adipocytes on haematopoiesis; however, our data suggest the opposite—that adipogenesis is an emergency response that promotes increased haematopoiesis in response to cytopenia. Adipogenesis is probably a faster way of increasing the production of HSC niche factors as compared with the construction of new perivasculare niches, which would involve the generation of new blood vessels. Nonetheless, there are context-dependent differences in adipocyte function between bone marrow compartments (long bones versus caudal vertebrae) and there may also be differences between humans and mice.

When adipocytes have been co-cultured with haematopoietic cells, they have been reported to have a wide range of positive and negative effects on haematopoietic progenitors and haematopoiesis<sup>50–53</sup>. Beyond SCF, bone marrow adipocytes also synthesize adiponectin, which promotes HSC regeneration after irradiation by promoting HSC proliferation<sup>54</sup>, as well as leptin<sup>55</sup>, which promotes adipogenesis and inhibits osteogenesis by SSCs in the bone marrow<sup>56</sup>. Adipocytes probably secrete multiple factors that influence the function of HSCs and stromal cells in the bone marrow. Our results indicate that the sum of these effects promotes haematopoietic regeneration in most bones while inhibiting haematopoietic regeneration in caudal vertebrae.

**METHODS**

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of this paper.

Note: Supplementary Information is available in the online version of the paper.

**ACKNOWLEDGEMENTS**

S.J.M. is a Howard Hughes Medical Institute Investigator, the Mary McDermott Cook Chair in Pediatric Genetics, the Kathryn and Gene Bishop Distinguished Chair in Pediatric Research, the director of the Hamon Laboratory for Stem Cells and Cancer, and a Cancer Prevention and Research Institute of Texas Scholar. B.O.Z. was supported by a fellowship from the Leukemia and Lymphoma Society and the Thousand Talents Plan-Youth in China. This work was funded by the National...
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METHODS

Mice. Mice were generally maintained on a C57BL/6 background, including Lepr<sup>tm</sup> (ref. 57), Tie2cre (ref. 58), Vav1cre (ref. 59), Col2a1<sup>2.3Cre</sup> (ref. 43), Adipoqcre/ER (ref. 45), Sfn<sup>tm</sup> (ref. 1), Sfn<sup>Cre</sup> (ref. 1), R26<sup>cre</sup> (ref. 60), R26<sup>tm</sup> (ref. 61), Pdhr4<sup>cre</sup> (ref. 62) and Col1a1<sup>2.3Cre</sup>-GFP (ref. 63). A ZIP/F1 mice were maintained on a FVB.C57BL/6 hybrid background. To induce CreER activity in Adipoqcre/ER mice, ~6-week-old mice were gavaged with 1 mg tamoxifen (Sigma), dissolved in corn oil, daily for 3 consecutive days. To induce myeloablation by 5-FU, mice were intraperitoneally administered one or two doses of 5-FU (8 days apart; 150 mg kg<sup>-1</sup> d<sup>-1</sup>). All mice were housed in the Animal Resource Center at UT Southwestern Medical Center (UTSW) although some experiments were completed in the Animal Facility at the Shanghai Institute of Biochemistry and Cell Biology (SIBCB). All procedures were approved by the Institutional Animal Care and Use Committees of UTSW and SIBCB and all animal experiments were done in compliance with ethical guidelines and the approved protocols.

Human bone marrow specimens. Patient samples were collected during standard surgical procedures after obtaining written informed consent approved by the Institutional Review Board of Texas Scottish Rite Hospital for Children (approval number IRB STU092211-034) and all experiments involving human samples were done in compliance with ethical guidelines and the approved protocols. Bone marrow was harvested from the tibia (n = 1) or femur (n = 2) of paediatric patients (8 to 17 year-old) and transferred to cold PBS on ice.

Irradiation and haematopoietic regeneration. Adult mice were given two doses of 540 rats (total 1,080 rats) at least 2 h apart using an XRAD 320 X-ray irradiator (Precision X-Ray). One million bone marrow cells from wild-type or Uck-GFP mice were injected into the retro-orbital venous sinus of anaesthetized control or mutant mice for radioprotection. In most experiments recipient mice were maintained on antibiotic water (neomycin sulphate 1.11 g l<sup>-1</sup> and polymyxin B 0.121 g l<sup>-1</sup>) for at least 14 days after transplantation, and then switched to regular water.

Long-term competitive reconstitution assay. Adult recipient mice were irradiated using an XRAD 320 X-ray irradiator with two doses of 540 rad (total 1,080 rad) delivered at least 2 h apart. Cells were transplanted intravenously into the retro-orbital venous sinus of anaesthetized mice. For competitive reconstitution assays we transplanted 3 × 10<sup>5</sup> donor bone marrow cells along with 3 × 10<sup>5</sup> recipient bone marrow cells. For secondary transplantation assays we transplanted 10<sup>5</sup> donor bone marrow cells from primary recipients along with 10<sup>5</sup> compromised (previously transplanted, recipient-type) bone marrow cells. Mice were maintained on antibiotic water (neomycin sulphate 1.11 g l<sup>-1</sup> and polymyxin B 0.121 g l<sup>-1</sup>) for 14 days and then switched to regular water. Recipient mice were periodically bled to assess the level of donor-derived blood cells, including myeloid, B and T cells for 16 weeks after transplantation.

Data were analysed by FlowJo (Tree Star) software.

Quantitative real-time PCR. Cells were sorted directly into Trizol (Life Technologies). We isolated bone marrow adipocytes as described in a prior study<sup>48</sup>; bone marrow from 6 ~ 8 month-old mice or recipient mice at 14 days after irradiation was digested as described above. Floating cells were transferred to a new tube, twice washed with HBSS and then transferred into Trizol. Total RNA was extracted according to the manufacturer's instructions. RNA was reverse transcribed using SuperScript III Reverse Transcriptase (Life Technologies). Quantitative real-time PCR was performed using SYBR green on a LightCycler 480 (Roche). β-actin was used to normalize the RNA content of samples. Primers used in this study were Sf: 5′-GCCGACAAACTCAGTCTTACTCTGTA-3′ and 5′-CAAATTGTTGTGTACGTCATTG-3′; Ob-Rb: 5′-GATGTTCCAAAACC CAAAGAA-5′ and 5′-TTCTGCAGTCTGGTAAAGAAAAGA-3′; leptin: 5′-GTTGGTG CTTGGTGCTAGAT-5′ and 5′-TTGATGAGTAGCACAAGGTG-3′; peri-5-G GCTGTGACGACAAAAC-3′ and 5′-GAGATGGTGCTGCCATGAC-3′; Adipoq: 5′- TGTCCCTCTTATTCGTCGACA-5′ and 5′-CCAAGCTCGAAGATCTCTT-3′; Fabp4: 5′-TGCTGATGAAATCACC-5′ and 5′-GTTGACATTCTTCATC-3′; β-actin: 5′-GCTTCTTTCTGAGGCTCTTT-3′ and 5′-CTCTCGATCATGTCGTAG-3′; human Gapdh: 5′-AAGGCTCATTCTCAGTGGAA-3′ and 5′-TGCAAAATGGTCTGTTGAGG-3′.

Bone sectioning, immunostaining and confocal imaging. Freshly dissected bones were fixed in 4% paraformaldehyde overnight followed by 3-day decalcification in 10% EDTA. Bones were sectioned (5 μm for thin sections and 50 μm for thick sections) using the CryoLake tape-transfer system (Intrumedics). Sections were blocked in PBS with 10% horse serum for 1 h and then stained overnight with chicken anti-GFP (Aves, 1:1,000), anti-CD41 APC (eBioscience, Bio-entreeWReg30, 1:200), goat anti-rabbit (Jackson, 1:400), rabbit anti-perilipin (Sigma, 1:1,000) and/or rabbit anti-laminin (Abcam, 1:400) antibodies. Donkey-anti-goat Alexa Fluor 647 and Donkey-anti-rabbit Alexa Fluor 488 were used as secondary antibodies (Life Technologies, 1:400). Slides were mounted with anti-fade prolong gold (Life Technologies) and images were acquired with a Zeiss LSM780 confocal microscope.

Western blot. Bone marrow was flushed out of the bone and then dissociated in 66% trichloroacetic acid (TCA) in water. Extracts were incubated on ice for at least 15 min and centrifuged at 16,100g at 4 °C for 10 min. Precipitates were washed in acetonitrile twice and then dried pellets were solubilized in 9 M urea, 2% Triton X-100 and 1% dithiothreitol. Samples were separated on 4–12% Bis-Tris polyacrylamide gels (Invitrogen) and transferred to PVDF membrane (Millipore). The blots were incubated with primary antibodies overnight at 4 °C and then with secondary antibodies. Blots were developed with the SuperSignal West Femtomololuminescence kit (Thermo Scientific). Primary antibodies used: rabbit-anti-SCF (Abcam, catalogue number ab64677, 1:1,000) and mouse-anti-actin (Santa Cruz, clone AC-15, 1:2,000,000).

Statistics and reproducibility. Panels generally represent multiple independent experiments performed on different days with different mice. Sample sizes were not pre-determined on the basis of the statistical power calculations. No formal randomization techniques were used but mice were allocated to experiments randomly and samples were processed in an arbitrary order. No blinding was performed. No animals were excluded from analysis. Variation was always indicated using standard deviation. For analysis of the statistical significance of differences between two groups we generally performed two-tailed Student's test followed by Holm–Sidak's multiple comparisons tests. When samples were effectively matched, paired two-tailed t-tests were used. For analysis of the statistical
significance of differences among more than two groups, we performed one-way ANOVAs with Dunnett's multiple comparisons tests. When samples were effectively matched, we performed repeated measures one-way ANOVAs with the Geisser–Greenhouse method for sphericity corrections and Tukey's multiple comparisons tests. To assess the statistical significance of differences among multiple groups when the experimental design involved multiple conditions, such as time points or cell types in addition to differences in genotypes, we performed two-way ANOVAs with Sidak's multiple comparisons tests. When samples were effectively matched, we performed repeated measures two-way ANOVAs with Sidak's multiple comparisons tests. Normality was tested using the Shapiro–Wilk tests or the D'Agostino–Pearson omnibus tests when sample sizes were sufficient. Most data were normally distributed. When they were not, data were log₂-transformed prior to statistical testing. Variability within groups was tested using Levine's median test prior to performing t-tests or one-way ANOVAs. Other than in Fig. 8, we rarely observed significant differences in variability within groups. When there were significant differences in variability within treatments, data were log₂-transformed and tested again for normality and variability prior to statistical testing. For one-way repeated measures ANOVAs, sphericity was not assumed, and it was corrected using the Geisser–Greenhouse method. To assess the statistical significance of differences in survival, we performed Gehan–Breslow–Wilcoxon tests. All statistical tests were performed using GraphPad with Prism7, following its Statistics Guide.

Data availability. Source data for Figs 1k,l and 7b and Supplementary Fig. 3b–f have been provided as Supplementary Table 1. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Supplementary Figure 1 Irradiation depletes bone marrow hematopoiesis. One million mechanically dissociated bone marrow cells from wild-type mice were transplanted into irradiated wild-type mice. The statistical significance of differences among treatments was assessed using repeated measures one-way ANOVAs with the Geisser-Greenhouse method for sphericity correction and Tukey’s multiple comparisons tests (a-e). * indicates statistical significance relative to non-irradiated controls (con) while # indicates statistical significance between 2 and 4 weeks after irradiation (*P<0.05, ** or ## P<0.01, *** P<0.001). All data represent mean±SD from two femurs and two tibias in n=5 mice/genotype/condition from 5 independent experiments. (a-e) Numbers of B (a) and T (b) cells in the bone marrow as well as WBC (c), RBC (d), and platelet (e) counts in non-irradiated control (Con) mice as well as mice at 2 and 4 weeks after irradiation and bone marrow transplantation. (f) Confocal imaging showed that perilipin+ adipocytes were uniformly Tomato+ in Leprcre; R26tdTomato mice 2 weeks after irradiation and bone marrow transplantation (representative image from 3 independent experiments).
Supplementary Figure 2  Scf was expressed by adipocytes but not by hematopoietic cells or osteoblasts in the bone marrow of non-irradiated or irradiated mice. (a) Virtually all Scf-GFP<sup>+</sup> cells were Tomato<sup>+</sup> in the bone marrow of irradiated and non-irradiated Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (representative plots from 3 independent experiments). (b) Scf-GFP was not expressed by CD45<sup>+</sup> or Ter119<sup>+</sup> hematopoietic cells in the bone marrow of non-irradiated or irradiated Scf<sup>GFP</sup> mice (representative plots from 3 independent experiments). (c) Tomato<sup>+</sup> osteoblasts did not express Scf-GFP in non-irradiated or irradiated Col1a1*2.3-cre; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (representative plots from 3 independent experiments). (d, e) Scf-GFP was not detectably expressed by Tomato<sup>+</sup> osteoblasts or osteocytes in femur sections from non-irradiated or irradiated Col1a1*2.3-cre; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (representative images from 3 independent experiments). (f, g) Scf-GFP and Tomato were expressed by perilipin<sup>+</sup> adipocytes in the bone marrow of normal Lepr<sup>cre</sup>; R26<sup>tdTomato</sup>; Scf<sup>GFP</sup> mice (f) as well as 2 weeks after irradiation and wild-type bone marrow transplantation (g; representative images from 3 independent experiments).
Supplementary Figure 3  Scf is expressed by bone marrow adipocytes. (a) Confocal imaging showed five consecutive optical images (each 1.5µm) demonstrating the expression of Scf-GFP in perilipin+ adipocytes from non-irradiated mice (representative images from 3 independent experiments). (b-f) Quantitative RT-PCR analysis of leptin (b), Ob-Rb (c), Adipoq (d), perilipin (e) and FABP4 (f) transcript levels (normalized to b-Actin) in LepR+ stromal cells (LepR+), bone marrow adipocytes (Adip-BM) and intraperitoneal adipocytes (Adip-IP) relative to unfractionated whole bone marrow cells (WBM). The transcript levels in WBM were normalized to 1. All data represent mean±SD from n=3 mice in 3 independent experiments. One-way ANOVAs with Tukey’s multiple comparisons tests were used to assess statistical significance (*, p<0.05; **, p<0.01; ***, p<0.001). (g) Abundant adipocytes in human tibia bone marrow sections from an 8 year-old donor (representative image from 3 independent experiments).
**Supplementary Figure 4** Scf from osteoblasts and hematopoietic cells is dispensable for the regeneration of HSCs and hematopoiesis after irradiation. (a-f) White blood cell (a) red blood cell (b), and platelet counts (c), as well as bone marrow cellularity (d) and numbers of LSK cells (e) and HSCs (f) from paired *Col1a1*2.3-cre; *Scf*GFP/fl mice and *Scf*GFP/fl controls that were non-irradiated (Con) or analyzed at 2 or 4 weeks after irradiation and bone marrow transplantation (n=5 mice/genotype/condition from 3 independent experiments). HSCs could not be detected at 2 weeks after irradiation. Two-way ANOVAs with Sidak’s multiple comparisons tests (a-e) or two-tailed Student’s t-tests with Holm-Sidak’s multiple comparisons test (f) were used to assess the significance of differences between *Col1a1*2.3-cre; *Scf*GFP/fl mice and *Scf*GFP/fl controls. (g) Competitive reconstitution assay in which 10^6 donor bone marrow cells from the indicated primary recipient mice were transplanted 4 weeks after irradiation along with recipient-type competitor cells into irradiated secondary recipient mice (n=12 recipient mice/genotype from 3 independent experiments). The statistical significance of differences was assessed using two-way repeated measures ANOVAs with Sidak’s multiple comparisons tests. (h-m) White blood cell (h) red blood cell (i), and platelet counts (j), as well as bone marrow cellularity (k) and numbers of LSK cells (l) and HSCs (m) from paired *Vav1*-cre; *Scf*GFP/fl mice and *Scf*GFP/fl controls that were non-irradiated (Con) or analyzed at 2 or 4 weeks after irradiation and bone marrow transplantation (n=5 mice/genotype/condition from 3 independent experiments). Two-way ANOVAs with Sidak’s multiple comparisons tests (h-l) or two-tailed Student’s t-tests with Holm-Sidak’s multiple comparisons test (m) were used to assess the significance of differences between *Vav1*-cre; *Scf*GFP/fl and *Scf*GFP/fl mice. (n) Competitive reconstitution assay in which 10^6 donor bone marrow cells from the indicated primary recipient mice were transplanted 4 weeks after irradiation along with recipient-type competitor cells into irradiated secondary recipient mice (n=12 recipient mice/genotype from 3 independent experiments). The statistical significance of differences was assessed using two-way repeated measures ANOVAs with Sidak’s multiple comparisons tests. All data in this figure represent mean±SD.
Supplementary Figure 5 Percentage of LepR+ cells in Tomato+ bone marrow cells from Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice dropped after irradiation. (a-c) Confocal imaging of thin femur sections from Lepr<sup>cre</sup>, R26<sup>tdTomato</sup> mice co-stained with anti-LepR and anti-perilipin antibodies. (representative results from 3 independent experiments). (d) Quantification of the percentage of LepR+ cells in Tomato+ bone marrow cells from Lepr<sup>cre</sup>; R26<sup>tdTomato</sup> mice dropped after irradiation. Data represent mean±SD from n=3 mice in 3 independent experiments. A one-way ANOVA with Tukey’s multiple comparisons test was used to assess statistical significance (*P<0.05, *** or ### P<0.001). (e) Western blot of SCF protein levels in the bone marrow of Adipoq-cre/ER; Scf<sup>GFP/fl</sup> mice, Lepr<sup>cre</sup>; Scf<sup>GFP/fl</sup> mice, and Scf<sup>GFP/fl</sup> controls, either non-irradiated (Con) or 2 weeks after irradiation and transplantation of wild-type bone marrow cells (n=3 mice/genotype from 3 independent experiments).
Supplementary Figure 6 *Adipoq-Cre/ER* CFU-F had increased adipogenic activity as compared to *Adipoq-Cre/ER* CFU-F in culture. (a, b) Representative images of CFU-F colonies derived from *Tomato* (a) or *Tomato*+ bone marrow stromal cells (b) from *Adipoq-Cre/ER; R26<sup>tdTomato</sup>* mice. The colonies were stained with anti-perilipin antibody after culturing in DMEM plus 20% fetal bovine serum for one week. The average number of perilipin<sup>+</sup> adipocytes that spontaneously differentiated per CFU-F colony was quantified in Fig. 4j. (c, d) Most bone marrow adipocytes that formed after irradiation were *Tomato*+ in *Adipoq-Cre/ER; R26<sup>tdTomato</sup>* mice that had been administered tamoxifen 2 weeks before irradiation (n=4 mice/time point from 3 independent experiments). (e) Whole-mount imaging of a thick femur section 12 days after 5-FU treatment (representative image from n=3 mice from 3 independent experiments).
Supplementary Figure 7 We did not detect any effect of Scf deletion on the morphology or frequency of megakaryocytes, endothelial cells, or blood vessels in the bone marrow. (a, b) Few CD41\(^+\) megakaryocyte lineage cells expressed c-kit in mechanically dissociated bone marrow cells (a) or in femur sections (b; results are representative of n=3 mice analyzed in 3 independent experiments). (c, d) Few endothelial cells expressed c-kit in enzymatically dissociated bone marrow cells (c) or in blood vessels identified in bone marrow sections by laminin staining (d; n=3 mice from 3 independent experiments). (e-h) The morphologies and frequencies of megakaryocytes, endothelial cells, and blood vessels in the bone marrow did not significantly differ between Adipoq-cre/ER; Scf\(^{GFP/}\) mice (mut) and Scf\(^{GFP/}\) controls (con) at 4 weeks after irradiation and bone marrow transplantation. Two-tailed Student’s t-tests were used to assess the significance of differences between genotypes but the differences were not statistically significant (n=5 mice per genotype analyzed in 3 independent experiments). (i-k) Diphtheria toxin ablated Tomato\(^+\) cells in the bone marrow of Adipoq-cre/ER; R26\(^{iDTR}\); R26\(^{tdTomato}\) mice at 3 days after treatment but adipocytes regenerated from unrecombined Tomato negative progenitors within 14 days after treatment (n=5 mice per genotype analyzed in 3 independent experiments). All data in this figure represent mean±SD.
Supplementary Figure 8 Unprocessed scans of western-blot from Supplementary Figure 5e.
Supplementary Table Legends

Supplementary Table 1 Source data.
Life Sciences Reporting Summary

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1. Sample size
   Describe how sample size was determined.

   Samples sizes were not pre-determined based on statistical power calculations.

2. Data exclusions
   Describe any data exclusions.

   No animals were excluded.

3. Replication
   Describe whether the experimental findings were reliably reproduced.

   Similar results were consistently observed in multiple independent experiments

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.

   No formal randomization techniques was used but mice were allocated to experiments randomly and samples were processed in an arbitrary order.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   No blinding was used.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

   n/a Confirmed

   ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

   ☐ ☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.

   ☐ ☒ A statement indicating how many times each experiment was replicated

   ☐ ☐ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

   ☐ ☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons

   ☒ ☒ The test results (e.g. p values) given as exact values whenever possible and with confidence intervals noted

   ☐ ☒ A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

   ☐ ☒ Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.

7. Software
   Describe the software used to analyze the data in this study.

   GraphPad Prism v7.0
Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There is no restriction on availability of the materials in this study.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies used in flow cytometry were anti-CD45.2 (Biolegend, 109806, clone 104), anti-CD45.1 (Biolegend, 110716, clone A20), anti-Gr1 (eBioscience, 25-5931, clone RB6-8C5), anti-Mac-1 (Biolegend, 101212, clone M1/70), anti-8220 (eBioscience, 15-0452, clone RA3-6B2), and anti-CD3 (Biolegend, 100206, clone KT31.1), anti-CD150 (Biolegend, 115904, clone TC15-12F12.2), anti-CD48 (Biolegend, 103412, clone HM48-1), anti-Sca1 (Biolegend, 122524, clone E13-161.7), anti-c-kit (eBioscience, 47-1171, clone 2B8), anti-Ter119 (Biolegend, 116206, clone TER-119), anti-CD2 (Biolegend, 100105, clone RM2-5), anti-CD5 (Biolegend, 100606, clone S3-7.3) and anti-CD8 (Biolegend, 100706, clone S3-6.7), anti-CD45 (Biolegend, 113112, clone 30F-11), anti-CD31 (eBioscience, 17-0311, clone 390) and anti-LepR (R&D Systems, BAF497).

Antibodies used in Western-blot were rabbit-anti-SCF (Abcam, ab64677) and mouse-anti-beta-Actin (Santa Cruz, sc-69879, clone AC-15). Antibodies used in immunostaining were chicken-anti-GFP (Aves, GFP-1020), anti-CD41-APC (eBioscience, 17-0411, clone eBioMWReg30), goat-anti-c-kit (R&D, AF1356), rabbit-anti-perilipin (Sigma, P1873) and rabbit-anti-laminin (Abcam, ab11575). Donkey-anti-rabbit Alexa Fluor 555 (Life Technologies, A-31572), donkey-anti-chicken Alexa Fluor 488 (Life Technologies, A-21447) and Donkey-anti-goat Alexa Fluor 647 (Life Technologies, A-21447) were used as secondary antibodies.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No cell lines were used in our study.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice were generally maintained on a C57BL/6 background, including Lepr-cre, Tie2-cre, Vav1-cre, Col1a1*2.3-cre, Adipoq-cre/ER, ScfGFP, ScfIfl, R26tdTomato, R26DTR, Fabp4-cre, and Col1a1*2.3-GFP. A-ZIP/F1 mice were maintained on a FVB.C57BL/6 hybrid background. Unless indicated in the text, mice were 2-4 months of age, including both males and females. All mice were housed in the Animal Resource Center at UT Southwestern Medical Center (UTSW) though some experiments were completed in the Animal Facility at the Shanghai Institute of Biochemistry and Cell Biology (SIBCB). All procedures were approved by the Institutional Animal Care and Use Committees of UTSW and SIBCB.
12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Patient samples were collected during standard surgical procedures after obtaining written informed consent, approved by the Institutional Review Board of Texas Scottish Rite Hospital for Children (approval number IRB STU092011-034). Bone marrow was harvested from the tibia (n=1) or femur (n=2) of pediatric patients (8 to 17 year-old) and transferred to cold PBS on ice.
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

- **Data presentation**
  
  For all flow cytometry data, confirm that:
  
  1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  2. 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).
  3. All plots are contour plots with outliers or pseudocolor plots.
  4. A numerical value for number of cells or percentage (with statistics) is provided.

- **Methodological details**
  
  5. Describe the sample preparation. 
     
     Samples were fresh mouse bone marrow cells. Please see the methods for details.
  6. Identify the instrument used for data collection. 
     
     BD Aria II or LSR II
  7. Describe the software used to collect and analyze the flow cytometry data. 
     
     FlowJo v10.0
  8. Describe the abundance of the relevant cell populations within post-sort fractions. 
     
     The frequencies of the key cell populations are shown in the figures. Adipoq-creER+ adipocyte progenitors represented 0.017% of bone marrow cells (Figure 4a shows the gates and frequency data). LepR+ cells represented 0.32% of bone marrow cells (see Figure 4c for gates and frequency). Scf-GFP+ bone marrow stromal cells represented 0.34% of bone marrow cells (see Figure 4f for gates and frequency). The gates used to identify other cell populations, including VE-cadherin+ endothelial cells (Figure 2b) and osteoblasts (Figure 5f) are shown in the relevant figures.
  9. Describe the gating strategy used. 
     
     FSC/SSC gates and viability dye staining of the starting cell population was used to identify live cells with appropriate light scatter characteristics. Boundaries between positive and negative staining cell populations were defined by using fluorescence minus one controls.

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