Several cell types have been proposed to create niches for haematopoietic stem cells (HSCs). However, the expression patterns of HSC maintenance factors have not been systematically studied and no such factor has been conditionally deleted from any candidate niche cell. Thus, the cellular sources of these factors are undetermined. Stem cell factor (SCF; also known as KITL) is a key niche component that maintains HSCs. Here, using Scf conditional knockout mice, we found that Scf was primarily expressed by perivascular cells throughout the bone marrow. HSC frequency and function were not affected when Scf was conditionally deleted from haematopoietic cells, osteoblasts, nestin−cre− or nestin−creER−expressing cells. However, HSCs were depleted from bone marrow when Scf was deleted from endothelial cells or leptin receptor (Lepr)−expressing perivascular stromal cells. Most HSCs were lost when Scf was deleted from both endothelial and Lepr−expressing perivascular cells. Thus, HSCs reside in a perivascular niche in which multiple cell types express factors that promote HSC maintenance.

**Scf is expressed by perivascular cells**

We generated Scf−/− knock-in mice by inserting enhanced green fluorescent protein (gfp) into the endogenous Scf locus (Supplementary Fig. 1a−c). Scf−/− mice died perinatally (Fig. 1a and Supplementary Fig. 1f, g) with severe anaemia (Fig. 1b and Supplementary Fig. 2c), as observed in mice with a strong loss of SCF/c-Kit function21. By quantitative reverse transcription–polymerase chain reaction (qRT–PCR), Scf transcripts were nearly undetectable in Scf−/− newborn liver (Fig. 1c).

The overall cellularity of the newborn liver was reduced about twofold in Scf−/− and about fivefold in Scf−/− mutant mice compared to Scf+/+ controls (Fig. 1d). The frequency of HSCs (CD150+ CD48− CD41− Sca1+cKit+ cells) in the newborn liver was reduced about eightfold in Scf−/− mutant mice compared to littermate Scf+/+ or Scf+/− controls (Fig. 1e). Consistent with this, newborn Scf−/− liver cells gave significantly lower levels of donor cell reconstitution in irradiated mice compared to Scf−/− or Scf+/− controls (Fig. 1f and Supplementary Fig. 2d). Scf−/− mice therefore have a severe loss of Scf function.

Using flow cytometry we determined that only rare (0.027 ± 0.0099%, mean ± standard deviation (s.d.)) enzymatically dissociated bone marrow cells were positive for GFP. The actual frequency of GFP− cells in the bone marrow may be somewhat higher as our dissociation conditions may not have recovered all of the GFP− cells. These GFP− cells were negative for CD45 and Ter119, indicating a non-haematopoietic source of SCF (Fig. 1g). Endogenous Scf transcripts were highly enriched in GFP− stromal cells and highly depleted in GFP+ stromal cells (Supplementary Fig. 2f, g), suggesting that GFP expression faithfully reflected endogenous Scf expression.

GFP was mainly expressed by cells surrounding sinusoids throughout the Scf−/− bone marrow, with some expression by cells surrounding venuoles and arterioles (Fig. 1h–m and Supplementary Fig. 2h, i).
Student’s expression profiling. Several mesenchymal stem/stromal cell markers, (Fig. 1n). Perivascular stromal and endothelial cells therefore blast markers in either the diaphysis (Fig. 1k–m) or trabecular bone detect any GFP expression by bone-lining cells that expressed osteo-

perivascular stromal cells express o–q and Supplementary Fig. 2i), suggesting that both endothelial and GFP partially overlapped with endothelial marker staining (Fig. 1h–j, 1o–q). Mice homozygous for the germline recombined allele of Scf—Scf—were perinatally lethal and anaemic (Fig. 2a), similar to other Scf-deficient mice (Fig. 1a)17. Recombination of the Scf allele therefore led to a strong loss of SCF function. We were unable to amplify Scf transcripts by PCR from the liver of Scf/—newborns (Fig. 2b).

We generated Ubc-creER; ScfΔ/Δ mice to ubiquitously delete Scf upon tamoxifen administration. We administered tamoxifen-containing chow to Ubc-creER; ScfΔ/Δ mice and littermate controls for 1–2 months beginning at 8 weeks of age, and then killed them for analysis. Some of the mice became anaemic and ill during tamoxifen administration. The Ubc-creER; ScfΔ/Δ mice had significantly lower red blood cell counts than controls (Fig. 2c) and a trend towards lower

**Scf is required by adult HSCs**

We generated a floxed allele of Scf (ScfΔ) to conditionally delete Scf from candidate niche cells (Supplementary Fig. 3a–c). Mice homozygous for the germline recombined allele of Scf—ScfΔ—were perinatally lethal and anaemic (Fig. 2a) and did not survive to adulthood. Recombination of the ScfΔ allele therefore led to a strong loss of SCF function. We were unable to amplify Scf transcripts by PCR from the liver of ScfΔ/Δ newborns (Fig. 2b).

We generated Ubc-creER; ScfΔ/Δ mice to ubiquitously delete Scf upon tamoxifen administration. We administered tamoxifen-containing chow to Ubc-creER; ScfΔ/Δ mice and littermate controls for 1–2 months beginning at 8 weeks of age, and then killed them for analysis. Some of the mice became anaemic and ill during tamoxifen administration. The Ubc-creER; ScfΔ/Δ mice had significantly lower red blood cell counts than controls (Fig. 2c) and a trend towards lower

Figure 1 | ScfΔ is a strong loss-of-function allele and Scf is primarily expressed by perivascular cells in the bone marrow. a, b, ScfΔ/Δ homozygous mice died perinatally and were severely anaemic (n = 4–20). RBC, red blood cell. c, Scf transcripts in livers from newborn mice by qRT–PCR (n = 3). d, e, Newborn liver cellularity and HSC frequency (n = 4). f, Irradiated mice (CD45.1+) were transplanted with 3 × 10² newborn liver cells from ScfΔ/Δ, ScfΔ/Δ or ScfΔ/Δ donor (CD45.2+) mice along with 3 × 10² recipient (CD45.1+) bone marrow cells (3–4 experiments with 13–18 mice per genotype). g, Scf-GFP was expressed by rare non-haematopoietic stromal cells (n = 8). h–j, GFP was primarily expressed by perivascular cells in the bone marrow of ScfΔ/Δ mice. Endothelial cells were stained with an anti-endoglin antibody. k–n, GFP was not detected in bone-lining osteoblast lineage cells (osteopontin) in the diaphysis (k–m) or in trabecular bone (n). o–q, Higher magnification images of a sinusoid. r–u. A CD150+CD48+ candidate HSC (arrow) localized adjacent to a GFP-expressing perivascular cell. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; in blue). All data represent mean ± s.d. Two-tailed Student’s t-tests were used to assess statistical significance. **P < 0.01, ***P < 0.001. Scale bars in j, m and n are 50 μm. Scale bars in q and u are 20 μm.

GFP partially overlapped with endothelial marker staining (Fig. 1h–j, o–q and Supplementary Fig. 2i), suggesting that both endothelial and perivascular stromal cells express Scf. In contrast, GFP was not concentrated near the endosteme (Supplementary Fig. 2h) and we did not detect any GFP expression by bone-lining cells that expressed osteo-
blast markers in either the diaphysis (Fig. 1k–m) or trabecular bone (Fig. 1n). Perivascular stromal and endothelial cells therefore appeared to represent the major sources of SCF in bone marrow.

We isolated Scf-GFP+ cells by flow cytometry and performed gene expression profiling. Several mesenchymal stem/stromal cell markers, including Cxcl12, alkaline phosphatase, Vcam1, Pdgfra and Pdgfrb were highly elevated in Scf-GFP+ cells relative to whole bone marrow cells (Supplementary Table 1). This indicates that Scf-GFP+ cells included mesenchymal stem/stromal cells27 and Cxcl12-expressing perivascular stromal cells10. Nestin was not detected in Scf-GFP perivascular cells (Supplementary Table 1).

As we observed previously16,18, CD150+CD48+ lineage–candidate HSCs were mainly found adjacent to sinusoidal blood vessels throughout the bone marrow. Sixty-five per cent (47/73) of all CD150+CD48+ lineage–candidate stromal cells were immediately adjacent to GFP-expressing stromal cells (Fig. 1r–u). Almost all of the remaining cells (30%; 22/73) were within five cell diameters of GFP-expressing cells. This suggests that Scf-GFP-expressing cells form a perivascular niche for HSCs.

**Scf is required by adult HSCs**

We generated a floxed allele of Scf (ScfΔ) to conditionally delete Scf from candidate niche cells (Supplementary Fig. 3a–c). Mice homozygous for the germline recombined allele of Scf—ScfΔ—were perinatally lethal and anaemic (Fig. 2a), similar to other Scf-deficient mice (Fig. 1a)17. Recombination of the ScfΔ allele therefore led to a strong loss of SCF function. We were unable to amplify Scf transcripts by PCR from the liver of ScfΔ/Δ newborns (Fig. 2b).

We generated Ubc-creER; ScfΔ/Δ mice to ubiquitously delete Scf upon tamoxifen administration. We administered tamoxifen-containing chow to Ubc-creER; ScfΔ/Δ mice and littermate controls for 1–2 months beginning at 8 weeks of age, and then killed them for analysis. Some of the mice became anaemic and ill during tamoxifen administration. The Ubc-creER; ScfΔ/Δ mice had significantly lower red blood cell counts than controls (Fig. 2c) and a trend towards lower

Figure 2 | Scfs required for adult HSC maintenance. a, Homozygous ScfΔ/Δ mutant mice generated from germline recombination of the ScfΔ allele were perinatally lethal and anaemic. b, Scf transcripts amplified by RT–PCR from the livers of newborn mice. c, Global deletion of Scf in Ubc-creER; ScfΔ/Δ mice led to anaemia (n = 5–6). d, e, Global deletion of Scf in adult mice significantly reduced cellularity and HSC frequency in bone marrow (two femurs and two tibias) and spleen (n = 8–10). BM, bone marrow. f, To perform a limited dilution analysis21, three doses of donor bone marrow cells were competitively transplanted into irradiated mice. ELDA software (http://bioinf.wehi.edu.au/software/elda/) was used to calculate HSC frequency and assess statistical significance (two experiments). g, 3 × 10³ donor bone marrow cells were transplanted with 3 × 10³ recipient bone marrow cells into irradiated recipient mice (three experiments with a total of 12–14 recipients per genotype). h, HSCs did not express Scf-GFP by flow cytometry. Δ, recombined ScfΔ allele; +, wild-type allele of Scf. All data represent mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001.
white blood cell and platelet counts (Supplementary Fig. 3d). UbcreER; ScfΔβ2/Δβ2 mice exhibited approximately twofold reductions in the overall cellularity of bone marrow and spleen compared to controls (Fig. 2d).

CD150⁺ CD48⁻ Lin⁻ Sca1⁺ c-Kit⁺ HSCs were also depleted in the bone marrow and spleen of UbcreER; ScfΔβ2/Δβ2 mice compared to controls treated concurrently with tamoxifen (Fig. 2e). Limit dilution analysis demonstrated that long-term multilineage reconstituting cells were 3.5-fold less frequent in the bone marrow of UbcreER; ScfΔβ2/Δβ2 mice compared to controls upon transplantation into irradiated mice (Fig. 2f). Bone marrow cells from UbcreER; ScfΔβ2/Δβ2 mice gave significantly lower levels of donor cell reconstitution in irradiated mice (Fig. 2g). These data confirmed that SCF is required for HSC maintenance in adult mice.

CD150⁺ CD48⁻ Lin⁻ Sca1⁺ c-Kit⁺ HSCs from ScfΔβ2/+ mice did not express GFP by flow cytometry (Fig. 2h). This is consistent with prior studies in suggesting that Scf non-cell autonomously regulates HSC maintenance. To test the role of other haematopoietic cells we conditionally deleted Scf using Vav1-cre. As expected, Vav1-cre recombined a conditional loxP-EYFP reporter in virtually all HSCs, CD45+ and Ter119+ haematopoietic cells (Fig. 3a and Supplementary Fig. 4a). Eight-week-old Vav1-cre; ScfΔβ2/+ mice exhibited normal blood cell counts, bone marrow composition (Supplementary Fig. 4b, c), and bone marrow and spleen cellularity (Fig. 3b). ScfΔβ2/Δβ2 heterozygous mice exhibited a twofold decline in the frequency of CD150⁺ CD48⁻ Lin⁻ Sca1⁺ c-Kit⁺ HSCs relative to wild-type littermates. However, deletion of the second allele of Scf from haematopoietic cells in Vav1-cre; ScfΔβ2/+ mice did not further reduce HSC frequency in the bone marrow or spleen (Fig. 3c). Bone marrow cells from adult Vav1-cre; ScfΔβ2/+ mice had a normal capacity to reconstitute irradiated mice (Fig. 3d and Supplementary Fig. 4d) and to form colonies in methylcellulose (Supplementary Fig. 4e, f). Therefore, Scf expression by haematopoietic cells is not required for HSC maintenance in adult bone marrow.

HSCs do not require SCF from osteoblasts

Col2.3-Cre recombines genes in fetal and postnatal osteoblasts.10 Consistent with this, we found strong enhanced yellow fluorescent protein (EYFP) expression among bone-lining cells in Col2.3-cre; loxP-EYFP mice (Fig. 3e). To test whether osteoblasts produce SCF for HSC maintenance, we analysed 8-week-old Col2.3-cre; ScfΔβ2/+ mice. Col2.3-cre; ScfΔβ2/+ mice had normal blood counts (Supplementary Fig. 5a), normal lineage composition in the bone marrow and spleen (Supplementary Fig. 5b) and normal bone marrow and spleen cellularity (Fig. 3g). Although ScfΔβ2/Δβ2 germline heterozygous mice exhibited a twofold decline in the frequency of CD150⁺ CD48⁻ Lin⁻ Sca1⁺ c-Kit⁺ HSCs relative to wild-type littermates, conditional deletion of the second allele of Scf from osteoblasts in Col2.3-cre; ScfΔβ2/Δβ2 mice did not further reduce HSC frequency in the bone marrow or spleen (Fig. 3h). Bone marrow cells from Col2.3-cre; ScfΔβ2/Δβ2 mice had a normal capacity to reconstitute irradiated mice (Fig. 3i and Supplementary Fig. 5c) and to form colonies in methylcellulose (Supplementary Fig. 5d, e). Therefore, Scf expression by osteoblasts is not required for HSC maintenance in adult bone marrow.

HSCs do not require SCF from nestin⁺ cells

In nestin-cre; loxP-EYFP mice we found rare EYFP-expressing perivascular stromal cells around larger blood vessels, not sinusoids, in the bone marrow (Fig. 3f). These cells exhibited a very different distribution than Scf-expressing cells (compare Fig. 3f to Fig. 1h–m and Supplementary Fig. 2h, i). Eight-week-old nestin-cre; ScfΔβ2/+ mice had normal blood cell counts (Supplementary Fig. 6b), normal lineage composition and cellularity in the bone marrow and spleen (Supplementary Fig. 6c and Fig. 3j). Comparing nestin-cre; ScfΔβ2/Δβ2 mutants with ScfΔβ2/+ controls, deletion of Scf from nestin-cre-expressing cells did not reduce HSC frequency in the bone marrow (Fig. 3k).

Figure 3 | SCF from haematopoietic cells, osteoblasts and nestin-Cre-expressing stromal cells is not required for HSC maintenance. a, Vav1-Cre recombined the loxP-EYFP reporter in virtually all HSCs, CD45+ and Ter119+ haematopoietic cells. b, c, Deletion of Scf from haematopoietic cells did not significantly affect bone marrow or spleen cellularity or HSC frequency (n = 4). d, A competitive reconstitution assay with Vav1-cre; ScfΔβ2/Δβ2 and ScfΔβ2/+ bone marrow cells (2 experiments with a total of 10 recipients per genotype). e, Col2.3-Cre recombined the loxP-EYFP reporter in bone-lining osteoblast lineage cells. f, Nestin-Cre recombined the loxP-EYFP reporter in rare stromal cells around larger blood vessels. g, h, Bone marrow and spleen cellularity (g) and HSC frequency (h) in Col2.3-cre; ScfΔβ2/+ mice relative to controls (n = 5–6). i, A competitive reconstitution assay with Col2.3-cre; ScfΔβ2/Δβ2 and ScfΔβ2/+ bone marrow cells (3–5 experiments with a total of 14–22 recipients per genotype). j, k, Bone marrow and spleen cellularity (j) and HSC frequency (k) in nestin-cre; ScfΔβ2/+ mice relative to controls (n = 5–7). l, 1 × 10⁵ donor bone marrow cells from nestin-cre; ScfΔβ2/+ and ScfΔβ2/+ mice gave similar levels of donor cell reconstitution in irradiated mice. Reconstitution levels from ScfΔβ2/+ cells were modestly but significantly lower (3–5 experiments with a total of 14–24 recipient mice per genotype). a, h, i, k, l, Reconstituted EYFP⁺ donor cells were identified by flow cytometry (EYFP-GFP-FACS). b, c, d, e, g, h, i, j, k, l, Data represent mean ± s.d. NS, not significant. *P < 0.05, **P < 0.01, ***P < 0.001.
Nestin-cre; Scf−/− mice did exhibit a significant decline in HSC frequency in the spleen (Fig. 3k), raising the possibility that nestin-cre-expressing cells are a component of the HSC niche in the spleen. Bone marrow cells from adult nestin-cre; Scf−/− mice had a normal capacity to reconstitute irradiated mice (Fig. 3l and Supplementary Fig. 9a–c). Nestin-cre ER; Scf−/− mice also did not affect haematopoiesis, HSC frequency, or reconstituting capacity in irradiated mice (Supplementary Fig. 7). Therefore, Scf expression by nestin-cre-expressing or nestin-cre ER-expressing perivascular cells is not required for the maintenance of HSCs in adult bone marrow.

Because nestin–GFP-expressing bone marrow cells express Scf+2, we independently characterized nestin–GFP expression. Consistent with the prior report11, we observed strong nestin–GFP staining along larger vessels in the bone marrow (Supplementary Fig. 8; see supplementary figure 1 from ref. 12). Nestin–GFP was also observed in perisinusoidal stromal cells in a pattern that resembled Scf-GFP+ expression (Supplementary Fig. 8a). This appeared to be different from the nestin-cre expression pattern, which we detected only around larger blood vessels in the bone marrow (Fig. 3f). In nestin-Cherry and nestin-GFP double transgenic mice we detected nestin-Cherry expression around larger vessels but not around sinusoids, whereas nestin–GFP was detected around both (Supplementary Fig. 8). Thus, different nestin transgenes seem to be expressed by different subpopulations of perivascular stromal cells. Nestin-GFP appears to exhibit more expression in perisinusoidal stromal cells than other nestin transgenes. Our data are therefore consistent with the possibility that nestin–GFP-expressing stromal cells contribute to the HSC niche as previously suggested12, even though conditional deletion of Scf with nestin-cre and nestin-cre ER did not affect HSC frequency.

**HSCs require SCF from endothelial cells**

We conditionally deleted Scf from endothelial cells using Tie2-cre1. Tie2-Cre recombinated in endothelial (Fig. 4a) and haematopoietic cells (Fig. 4b) but not in mesenchymal stem/stromal cells from the bone marrow (Supplementary Fig. 9d, e). Because haematopoietic cells do not express Scf (Fig. 1g and Fig. 2i) and conditional deletion of Scf from haematopoietic cells did not affect HSC frequency (Fig. 3a–d), the use of Tie2-cre allowed us to test whether SCF expression by endodtheial cells regulates HSC frequency.

Eight-week-old Tie2-cre; Scf−/− mice exhibit normal blood cell counts (data not shown), bone marrow and spleen cellularity (Fig. 4c). However, the frequency of CD150+ CD48− Lin− Sca1+c-Kit+ HSCs in the bone marrow was significantly reduced in Tie2-cre; Scf−/− relative to controls (Fig. 4d). Consistent with this, 300,000 bone marrow cells from Tie2-cre; Scf−/− mice gave significantly lower levels of donor reconstitution upon transplantation into irradiated mice (Fig. 4g). In 4 independent experiments, 24 of 25 recipients of Scf+/− cells, 15 of 15 recipients of Scf+/− cells, and only 7 of 21 recipients of Tie2-cre; Scf−/− mice were long-term multilineage reconstituted. By Poisson statistics this corresponds to an HSC frequency in control bone marrow of at least 1/93,200 cells but only 1/739,900 in Tie2-cre; Scf−/− mice. Endothelial cells are therefore an important source of SCF for HSC maintenance.

The HSC depletion in Tie2-cre; Scf−/− mice probably reflects an ongoing need for SCF expression by endothelial cells in adult bone marrow, because when HSCs are depleted as a consequence of reduced SCF/c-Kit signalling, HSC frequencies return to normal levels upon restoration of normal SCF/c-Kit signalling19,21. Nonetheless, we also examined whether SCF expression by endothelial cells during development is required by HSCs. We found a 1.7–2.1-fold reduction in HSC frequency in the liver of newborn Tie2-cre; Scf−/− mice (Fig. 4e) and a twofold reduction in HSC frequency in the bone marrow of 1-month-old Tie2-cre; Scf−/− mice (Fig. 4f) relative to Scf+/− and Scf+/+ controls. The magnitude of HSC depletion in adult bone marrow seemed to increase, as we found a 2.7-fold and 5.2-fold reduction in HSC frequency in the bone marrow of 8-week-old Tie2-cre; Scf−/− mice relative to Scf+/− and Scf+/+ controls, respectively (Fig. 4d). These data suggest that ongoing SCF expression by endothelial cells in adult bone marrow contributes to HSC maintenance; however, HSC depletion in adult bone marrow may reflect a loss of SCF expression by endothelial cells during development.

**HSCs require SCF from perivascular cells**

We found that Lepr is highly restricted in its expression within the bone marrow to Scf-GFP-expressing perivascular stromal cells (Supplementary Table 1). Consistent with this, Lepr-cre;loxP-EYFP mice exhibited EYFP expression in perivascular stromal cells (Fig. 5b, e) but not in haematopoietic cells (Fig. 5b, c, e), bone-lining cells (Fig. 5c), or endothelial cells (Fig. 5d).

Consistent with the gene expression profile of Scf-GFP+ cells (Supplementary Table 1), EYFP+ cells from Lepr-cre;loxP-EYFP mice did not detectably express nestin but did express mesenchymal stem/stromal cell markers including Cxcl12, alkaline phosphatase, PDGFRα and PDGFRβ (Supplementary Fig. 9a–c). These data indicate a mesenchymal identity for the Lepr-expressing stromal cells; however, the lack of EYFP expression in bone-lining cells from Lepr-cre;loxP-EYFP mice suggests that the Lepr-expressing perivascular cells did not give rise to osteoblasts during normal development. Future studies will be required to assess the relationship between Lepr-expressing perivascular cells, mesenchymal stem cells, and other perivascular stromal cells.

Bone marrow cellularity was significantly reduced in Lepr-cre; Scf+/&Scf−/− mice compared to Scf+/− controls, but not compared to Scf+/&Scf−/− controls (Fig. 5g). Spleen size (Fig. 5f) and cellularity were significantly increased in Lepr-cre; Scf+/&Scf−/− mice (Fig. 5g). Sections through the spleen revealed increased extramedullary haematopoiensis in Lepr-cre; Scf+/&Scf−/− mice (data not shown). The frequency of CD150+ CD48− Lin− Sca1+c-Kit+ HSCs was significantly reduced in the bone marrow of Lepr-cre; Scf+/&Scf−/− mice, but significantly increased in the spleen (Fig. 5h). The total number of bone marrow and spleen HSCs...
Lepr-cre-expressing perivascular stromal cells depletes HSCs from adult bone marrow. The frequency of GFP+ cells in the bone marrow of Lepr-cre; Scf+/- mice did not significantly differ from Scf+/+ controls (Supplementary Fig. 10), suggesting that Scf deletion did not lead to the death of Lepr-expressing cells.

The HSC depletion observed in Lepr-cre; Scf+/- mice did not reflect a developmental effect of SCF expression by Lepr-cre-expressing cells as no HSC depletion was detected in the liver of newborn Lepr-cre; Scf+/- mice (Fig. 5k). Furthermore, the magnitude of the HSC depletion increased with time in the adult bone marrow (Fig. 5h, i).

To test whether deletion of Scf from endothelial and Lepr-cre-expressing perivascular cells has additive effects on HSC depletion we analysed 8-week-old Tie2-cre; Lepr-cre; Scf+/- mice. Bone marrow cellularity was significantly reduced in Tie2-cre; Lepr-cre; Scf+/- mice compared to Tie2-cre; Scf+/+ and Lepr-cre; Scf+/+ mice (Fig. 5m). Spleen cellularity was significantly increased in Tie2-cre; Lepr-cre; Scf+/- mice compared to Scf+/+ and Tie2-cre; Scf+/+ mice (Fig. 5n). The frequency and absolute number of HSCs in the bone marrow of Tie2-cre; Lepr-cre; Scf+/- mice was less than 5% of wild-type levels (Fig. 5m–o). This suggests that endothelial and Lepr-cre-expressing perivascular stromal cells are the major sources of SCF for HSC maintenance in normal adult bone marrow and that deletion of Scf from each cell population has additive effects on HSC depletion.

qRT-PCR revealed that endothelial and Lepr-cre-expressing perivascular cells expressed both long and short splice isoforms of Scf, rendering both cell types capable of expressing membrane-bound and soluble SCF (Supplementary Fig. 11). The levels of both isoforms of Scf in the two cell populations were significantly higher than in whole bone marrow cells, although Lepr-cre-expressing cells expressed much higher levels of both isoforms compared to endothelial cells (Supplementary Fig. 11).

Discussion

Our data demonstrate that HSCs reside in a perivascular niche in which endothelial and Lepr-expressing perivascular stromal cells are two functionally important components of the niche (Supplementary Fig. 12). The simplest interpretation of our data is that both cell types produce SCF for the maintenance of HSCs in adult bone marrow; however, endothelial cells also produce SCF for HSC maintenance/expansion during development so it is possible that the depletion of bone marrow HSCs in adult Tie2-cre; Scf+/- mice reflects a developmental effect of endothelial SCF. Endothelial cells and perivascular stromal cells are probably not the only components of the HSC niche, as other cell types probably contribute through mechanisms other than SCF production (for example, refs 32, 33).

Lepr-cre-expressing stromal cells did not express endogenous nestin (Supplementary Fig. 9c). Nestin-cre- or nestin-creER-mediated deletion of Scf did not deplete HSCs (Fig. 3j–1 and Supplementary Figs 6 and 7). However, Lepr-cre-expressing perisinusoidal cells do partially overlap with nestin-GFP-expressing perivascular cells (Supplementary Fig. 8 and Supplementary Fig. 9a–c). The Lepr-cre-expressing stromal cells therefore include stromal cells that express certain nestin transgenes, consistent with previous work14, and may also include Cxcl12-abundant reticular cells10. Perivascular stromal cells are probably heterogeneous and may include multiple cell types that contribute to HSC maintenance through different mechanisms.

Although we have partially characterized the bone marrow niche for HSCs in adult mice under homeostatic conditions, other studies will be required to functionally characterize HSC niches in other haematopoietic tissues and after haematopoietic stress.

METHODS SUMMARY

Targeting vectors for making Scf+/- and Scf+/- mice were constructed by recombining9. The Frt-flanked Neo cassette was removed by mating with Flpe

Figure 5 | Deletion of Scf from Lepr-cre-expressing perivascular stromal cells depletes HSCs in the bone marrow. a–c, Lepr-cre recombinated the loxp-EYFP reporter in perisinusoidal stromal cells in the bone marrow but not in bone-lining or haematopoietic cells. d, Lepr-cre did not recombine in Vcadherin+ endothelial cells. e, 0.013 ± 0.009% (mean ± s.d.; n = 3) of bone marrow cells from Lepr-cre; loxp-EYFP mice were EYFP+. f, g, Bone marrow and spleen cellularity (g) (n = 4–7). h, HSC frequency (n = 4–7). i, Total HSC numbers (including bone marrow and spleen) in Lepr-cre; Scf-loxP mice (n = 4–7). j, Limit dilution analysis of the frequency of long-term multilineage reconstituting cells in the bone marrow of Lepr-cre; Scf-loxP mice relative to controls (two experiments). k, HSC frequency in the newborn liver (n = 4–11). l, HSC frequency in 1-month-old Lepr-cre; Scf-loxP mice and controls (n = 3–6). m, Tie2-cre; Lepr-cre; Scf-loxP mice had significantly reduced bone marrow cellularity and increased spleen cellularity compared to Scf+/+ or Tie2-cre; Scf-loxP controls (n = 4–11). n, Deletion of Scf from endothelial and perivascular stromal cells in Tie2-cre; Lepr-cre; Scf-loxP mice greatly depleted HSCs from adult bone marrow (n = 4–11). o, Total HSC number was significantly reduced in Tie2-cre; Lepr-cre; Scf-loxP mice compared to Tie2-cre; Scf+/- or Lepr-cre; Scf+/- mice (n = 4–11). g or gfp, Scf+/- allele; f, Scf+/- allele. Scale bars are 50 μm. All data represent mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001.
mice. ScfR and ScfY mice were backcrossed onto a C57BL/Ka background before analysis. Mice used in this study included Ubc-creER2, CMV-cre2, Vav1-cre2, nestin-cre2, Tie2-cre2, Lepr-cre2 and Ipox-EPP2 (all from the Jackson Laboratory), Col2a-cre2, nestin-creER2 and nestin-GFP2. All were maintained on a C57BL/Ka background. Unless otherwise indicated, data always reflect mean ± s.d. and two-tailed Student’s t-tests were used to assess statistical significance (*P < 0.05, **P < 0.01, ***P < 0.001).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information The microarray data were deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE33158. 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 this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to S.J.M. (Sean.Morrison@UTSouthwestern.edu).
METHODS

Mice. Targeting vectors for making Scf<sup>fl/fl</sup> and Scf<sup>cre</sup> mice were constructed by recombineering<sup>30</sup>. Linearized targeting vector was electroporated into W4 (Scf<sup>fl/fl</sup>) or Bruce4 (Scf<sup>cre</sup>) embryonic stem (ES) cells. Positive clones were identified by Southern blotting and injected into B6 or C57BL/6-Tyr<sup>2i</sup> blastocysts. Chimaeric mice were bred with B6 or C57BL/6-Tyr<sup>2i</sup> mice to obtain germine transmission. The Flrt-flanked Neo cassette was subsequently removed by mating with Flpe mice<sup>33</sup>. Scf<sup>fl/fl</sup> mice were backcrossed at least five times and Scf<sup>cre</sup> mice were backcrossed at least three times onto a C57BL/Ka background before analysis. Note that Scf<sup>fl/fl</sup> mice were backcrossed fewer times before analysis because Bruce4 ES cells were from a substantially C57BL/6 background.<sup>30</sup> Nestin<sup>−/−</sup>-CATTGTATGGGATCTGATCTGG-3<sup>′</sup> was obtained from the Jackson Laboratory and maintained by crossing with C57BL/Ka mice. Col2-<sup>cre</sup> mice on a C57BL/6 background were provided by B. Cream and F. Liu<sup>30</sup>. Nestin<sup>−</sup>-CRE mice were obtained from G. Fehoss and backcrossed onto a C57BL/Ka background<sup>4</sup>. Nestin-GFP mice were as previously described<sup>30</sup>. C57BL/6-SI (CD45.1) mice were used as recipients in transplantation experiments. All mice were housed in the Unit for Laboratory Animal Medicine at the University of Michigan. All protocols were approved by the University of Michigan Committee on the Use and Care of Animals.<sup>8</sup> Unless otherwise indicated, data are mean ± s.d. and two-tailed Student’s t-tests were used to assess statistical significance (P<0.05, **P<0.01, ***P<0.001).

Genotyping PCR. Primers for genotyping Scf<sup>fl/fl</sup> were: OLD291, 5′-CCGCC AGCTCTGTTAATTTCG-3′; OLD292, 5′-CCGCAACCTGTAAGTGTGG-3′; and OLD360, 5′-AAGCCTCAGTCCATGGG-3′. Primers for genotyping Scf<sup>cre</sup> were: OLD301, 5′-GGAAAAGAACAAGTGAAGTC-3′; and OLD302, 5′-GTCGCCAGCAAGCTCACCCAG-3′. Primers for genotyping C57BL-KA were: OLD309, 5′-GGAAGAAGAAGAAGATGGT-3′; and OLD304, 5′-AAGG GGAAAGCCTGCGTC-3′. Primers for genotyping Tie2-<sup>cre</sup> were: OLD438, 5′-CTTGTGGCTCAGGACCAATG-3′; and OLD435, 5′-GGCAGA TTGGTGATGGGTC-3′. Primers for genotyping Lep<sup>cre</sup>-<sup>fl/f</sup> were: OLD344, 5′-CATTTGTATGGAATGCTATGG-3′; and OLD435, 5′-GGCAATTTTG GTTACCGTG-3′.

Tamoxifen administration. Tamoxifen citrate (Sigma or Spectrual Chemical) was administered in chow at 400 mg kg<sup>−1</sup> with 5% sucrose (Harlan). Mice were fed tamoxifen chow for 1–5 months before being analysed.

Long-term competitive reconstitution assay and limit dilution assay. Adult recipient mice were lethally irradiated by a Cincinnati 137 GammaCell<sup>40</sup> Irradiator (MDS Nordita) at 300 rad per minute with two doses of 540 rad (total 1,080 rad) delivered at 2 h apart. Cells were transplanted by retro-orbital venous sinus injection of anesthetized mice. 3×10<sup>6</sup> fetal liver or bone marrow cells were transplanted along with 3×10<sup>5</sup> recipient bone marrow cells into each irradiated mouse unless otherwise indicated. Mice were maintained on antibiotic water (<sup>©</sup>2012 Macmillan Publishers Limited. All rights reserved)