LECT2 drives haematopoietic stem cell expansion and mobilization via regulating the macrophages and osteolineage cells

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Haematopoietic stem cells (HSCs) can differentiate into cells of all lineages in the blood. However, the mechanisms by which cytokines in the blood affect HSC homeostasis remain largely unknown. Here we show that leukocyte cell-derived chemotaxin 2 (LECT2), a multifunctional cytokine, induces HSC expansion and mobilization. Recombinant LECT2 administration results in HSC expansion in the bone marrow and mobilization to the blood via CD209a. The effect of LECT2 on HSCs is reduced after specific depletion of macrophages or reduction of osteolineage cells. LECT2 treatment reduces the tumour necrosis factor (TNF) expression in macrophages and osteolineage cells. In TNF knockout mice, the effect of LECT2 on HSCs is reduced. Moreover, LECT2 induces HSC mobilization in irradiated mice, while granulocyte colony-stimulating factor does not. Our results illustrate that LECT2 is an extramedullar cytokine that contributes to HSC homeostasis and may be useful to induce HSC mobilization.

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Hematopoietic stem cells (HSCs) are used in clinical transplantation protocols for the treatment of a wide variety of immune-related diseases. HSCs are the bone marrow (BM), and HSCs can be obtained from the peripheral blood, following mobilization procedures. HSC expansion and mobilization are regulated by BM niche cells, including osteolineage cells (mature osteoblasts and osteoblast progenitors), macrophages, osteoclasts, endothelial cells, neutrophils, and mesenchymal stem and stromal cells. These BM niche cells can secrete a variety of growth factors or cytokines that affect HSC function, for example, osteolineage cells produce granulocyte colony-stimulating factor (G-CSF), the stromal cells that surround HSCs release stem cell factor, and endothelial cells produce E-selectin ligand to regulate HSC proliferation. Although HSCs can produce all immune cell lineages in the blood, it is unclear whether signals from the peripheral blood, following mobilization procedures, affect HSC function.

In this study, we report a previously unknown role of LECT2 in HSC homeostasis and the BM microenvironment. We determine that LECT2 is a novel candidate gene responsible for HSC expansion and mobilization via interacting with CD209a in the BM cells of 3-day LECT2-treated mice was 3.12-fold of that of PBS-treated mice (Fig. 2c). Furthermore, the RU value in the BM cells of 3-day LECT2-treated mice was 3.12-fold of that of PBS-treated mice (Fig. 2g).

Because HSCs participate in the inflammatory response during sepsis and LECT2 administration promotes septic mouse survival, we further evaluated the effect of LECT2-treated HSCs on sepsis. Lin^c-Kit^+ cells from the BM of LECT2-treated mice promoted survival and decreased the bacterial burden in septic mice (Supplementary Fig. 2a,b). These results reveal that LECT2 regulates HSC expansion, mobilization and transplantation efficiency.

**Results**

**LECT2 enhances HSC expansion and mobilization.** We first investigated the relationship between LECT2 expression and HSC number in the blood of humans in steady state. The number of HSCs was positively correlated with plasma LECT2 levels in humans (Fig. 1a). The effect of recombinant LECT2 on mouse HSCs was evaluated (Fig. 1b). The number of colony-forming unit cells (CFU-Cs), white blood cells (WBCs) and Lin^−Sca-1^+c-Kit^+ (LSK) cells in the blood increased after LECT2 treatment for 5 days (Fig. 1c,d). Moreover, the LECT2 treatment also enhanced the efficiency of reconstituting cells from the BM of LECT2-treated mice for 3 days (Fig. 1e). Kinetic studies demonstrated that LECT2 increased the number of LSK cells in the blood at 4 and 5 days after treatment, but not at earlier time points (Fig. 1f). This increase of LSK cell number in LECT2-treated mice was accompanied by the increased proliferation of LSK cells (Fig. 1g,h). LECT2 treatment for 3 days also increased the number of BM long-term HSCs (LT-HSCs, LSK CD34^−Flk2^− cells), short-term HSCs (ST-HSCs, LSK CD34^+Flk2^− cells) and lymphoid-primed multipotent progenitors (LMPPs, LSK CD34^−Flk2^+ cells; Fig. 1i). Furthermore, the number of CFU-Cs, LSK cells in the blood and LSK cells in the BM decreased in LECT2 KO mice (Fig. 1j–l).

Because the phenotype (LSK Flt3^−CD34^−) by flow cytometry is not always reliable when mice are stimulated by an exogenous agent, we further measured the repopulating activity of LSK cells from the BM of mice treated with LECT2 or PBS for 3 days by monitoring animal survival for 60 days after transplantation with LSK cells. The frequency of reconstituting cells was 1 per 194.1 LSK cells in PBS-treated mice and 1 per 77.3 LSK cells in LECT2-treated mice (Fig. 2a). We further evaluated their reconstitution efficiency using a competitive repopulating assay. The frequency of competitive repopulating units (CRUs) was 1 per 18.4 LSK cells in PBS-treated mice and 1 per 12.0 LSK cells in LECT2-treated mice (Fig. 2b). Normal HSC self-renewal is important for the maintenance of haematopoiesis. We further examined the extent of the repopulation of the HSCs and the different lineages in the initial 1:1 ratio of LSK cells from the BM of LECT2-treated C57BL/6 (CD45.2) mice and B6.SJL (CD45.1) mice. We observed higher frequencies of HSCs after the first transplantation with LSK cells from the BM of LECT2-treated mice for 3 days compared with PBS-treated mice (Fig. 2c, 2e).

Because LECT2 mediates the effect of LECT2 on HSC homeostasis, LECT2 can interact with two receptors, CD209a (ref. 12) and c-MET. We next determined which receptor mediates the effect of LECT2 on HSC homeostasis (Supplementary Fig. 3a). CD209a blockade, but not c-MET inhibition, reduced the effect of LECT2 on the HSC number in the blood and BM (Supplementary Fig. 3b–g). There was no change on HSC number in the blood and BM after c-MET inhibitor treatment alone (Supplementary Fig. 3b,d,e).

The LECT2 receptor was investigated in CD209a KO mice. The effect of LECT2 on HSC homeostasis was abolished in CD209a KO mice (Fig. 3a–d). Moreover, both the number of HSCs and plasma LECT2 levels were reduced in CD209a KO mice (Fig. 3a–e). Anti-LECT2 IgG reduced the number of LSK cells in the blood of the wild type (WT) but not CD209a KO mice (Fig. 3f). However, LECT2 messenger RNA (mRNA) could not be detected in the BM transcriptome, suggesting that LECT2 is an extramedullar cytokine. These data suggest that CD209a mediates the effects of both endogenous and exogenous LECT2 on HSCs.

We also investigated the potential role of LECT2/CD209a signal in regulating HSC regeneration after 5-fluorouracil (5FU)
**Figure 1 | LECT2 increases the mobilization and expansion of HSCs and their transplantation potential.** (a) The correlation between human plasma LECT2 levels and the number of CD34+ CD38− CD90+ Lin− cells in the blood (Pearson correlation coefficient = 0.624, P < 0.001; n = 31). (b) The protocol used to evaluate the effect of LECT2 on HSC homeostasis. (c) The number of CFU-Cs per millilitre of mouse blood; n = 10. (d) The number of WBCs and LSK cells in the blood. (e) The number of WBCs and LSK cells in BM. (f) The number of LSK cells in the blood at different time points after LECT2 administration. (g, h) Percentage of quiescent G0 (g) and BrdU+ (h) LSK cells in the BM of LECT2-treated mice. (i) Effects of LECT2 on the number of LK cells, LSK cells, LT-HSCs, ST-HSCs and LMPPs in BM. n = 5. (j–l) The number of CFU-Cs (j), WBCs and LSK cells (k) in the blood and the number of WBCs and LSK cells (l) in the BM of LECT2 KO mice; n = 5. The small black dots in histograms are data points. The data represent means ± s.e.m. The data are representative of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using Pearson’s correlation coefficient analysis (a) and one-way analysis of variance (ANOVA) (c–l).
The data represent means determined by flow cytometry in peripheral blood 20 weeks after competitive transplantation with BM cells of 3-day LECT2 treated mice; and ST-HSCs at 20 weeks after second competitive transplantation with LSK cells.

CD209a. CD209a regeneration during 5FU stress. suggest that LECT2/CD209a signal participates in HSC upregulated (Supplementary Fig. 4f,g). Furthermore, LECT2 The expression levels of LECT2 and CD209a were downregulated (Supplementary Fig. 4h–i). These data suggest that LECT2/CD209a signal participates in HSC regeneration during 5FU stress.

We further aimed to identify the cellular localization of LECT2 and CD209a and assessed the role of macrophages and osteolineage cells in the effect of LECT2. We further investigated whether macrophages and osteolineage cells contribute to the effect of LECT2 on HSCs. Diphtheria toxin (DT) treatment in CD169DTR+ mice has been used to deplete macrophages. The number of CD169+ cells decreased in DT-treated CD169DTR+ mice compared with WT mice (Fig. 4a). After LECT2 treatment, the number of LSK cells in the BM and blood of DT-treated CD169DTR+ mice decreased compared with WT mice (Fig. 4b,c). In DT-treated CD169DTR+ mice, the number of LSK cells in the blood slightly increased after LECT2 treatment (Fig. 4c). LSK cells were measured after macrophage depletion by the administration of clodronate liposome. After LECT2 treatment, the number of LSK cells in the BM and blood of clodronate liposome-treated mice decreased compared with PBS liposome-treated mice (Fig. 4d–f).

To assess the role of osteolineage cells in the effect of LECT2 on HSCs, biglycan (Bgn) KO mice, in which osteolineage cells are reduced, were used. The number of LSK cells in the BM and blood decreased in Bgn−/− mice compared with WT mice after LECT2 treatment (Fig. 5a–c). Strontium chloride (SrCl2) has been used to increase osteolineage cells31, and we used SrCl2 to confirm the role of osteolineage cells in the LECT2 effect. The number of LSK cells in the SrCl2-treated mice increased compared with the PBS-treated mice after LECT2 treatment (Supplementary Fig. 5).

**Figure 2 | LECT2 increases the transplantation potential of BM LSK cells.** (a) The survival rate was analysed following transplantation with LSK cells from the BM of 3-day LECT2 treated mice; n = 15 each point. (b) Competitive repopulation unit (CRU) content within each group of mice competitively transplanted with LSK cells at each dose; n = 15 each point. Horizontal dashed line, 37% of recipient mice failed to engraft. Vertical dashed lines, various CRU frequencies for each treatment. (c) The percentages of donor LSK cells, LT-HSCs, and ST-HSCs in BM at 20 weeks after first competitive transplantation with PBS- or LECT2-treated mice are shown; n = 5. (d) The percentages of B cells (B220+) and myeloid cells (CD11b+) in the peripheral blood of the chimaeric mice competitively transplanted with LSK cells; n = 6. (e) Repopulating units based on donor chimaerism determined by flow cytometry in peripheral blood 20 weeks after competitive transplantation with LSK cells; n = 6. (f) The percentages of donor LSK cells, LT-HSCs, and ST-HSCs at 20 weeks after second competitive transplantation with LSK cells, n = 5. (g) Repopulating units (RU) based on donor chimaerism determined by flow cytometry in peripheral blood 20 weeks after competitive transplantation with BM cells of 3-day LECT2 treated mice; n = 5. The data represent means ± s.e.m. The data are representative of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using Poisson’s statistic (a,b) and one-way ANOVA (c–g).
**Figure 3 | CD209a in BM niche cells mediates the effect of LECT2 on HSC mobilization and expansion.** (a,b) The number of LSK cells in the blood (a) and BM (b) of WT and CD209a KO mice after PBS or LECT2 treatment. (c) The number of CFU-Cs in the blood of WT and CD209a KO mice. (d) The number of LK cells, LSK cells, LT-HSCs, ST-HSCs and LMPPs in the BM of LECT2-treated CD209a KO mice; n = 4. (e) LECT2 protein levels in WT and CD209a KO mice; n = 6. (f) LSK cell number in WT and CD209a KO mice after iso IgG or anti-LECT2 IgG treatment. (g) The mRNA levels of BM cell markers in CD209a+ BM cells relative to the level in steady-state BM cells. CD169, a marker of macrophages; RunX2, a marker of osteolineage cells; nestin, a marker of mesenchymal stem or stromal cells; calcitonin receptor (CTR), a marker of osteoclasts; CD31, a marker of endothelial cells; myeloperoxidase (MPO), a marker of neutrophils. (h) A representative histogram of the percentage of CD209a-positive cells within LSK cells, macrophages and osteolineage cells in the steady-state condition; n = 4. The small black dots in histograms are data points. The data represent means ± s.e.m. The data are representative of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using one-way ANOVA.

**Figure 4 | Macrophages mediate the effect of LECT2 on HSC expansion and mobilization.** (a) The number of CD169+ cells in WT mice and WT or CD169DTR/+ mice injected with DT. (b) The number of LSK cells in the BM of WT or CD169DTR/+ mice after PBS or LECT2 treatment. (c) The number of LSK cells in the blood of WT or CD169DTR/+ mice after PBS or LECT2 treatment. (d) Effect of PBS liposomes (PBS-Lip) and clodronate liposomes (Clo-Lip) on macrophage number. (e) Effect of LECT2 on the number of BM LSK cells after macrophage depletion. (f) Effect of LECT2 on the number of blood LSK cells after macrophage depletion; n = 4. The small black dots in histograms are data points. The data represent means ± s.e.m. The data are representative of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using one-way ANOVA.
inhibitory factor. TNF mediated the downregulation of CCL3 expression by LECT2 in the macrophages (Supplementary Fig. 7a), suggesting that TNF mediates the effect of LECT2/CD209a signal on CCL3 downregulation. Therefore, we hypothesized that TNF play important roles in LECT2/CD209a signal on HSCs in the BM. The expression levels of TNF were not upregulated in the BM of CD209a KO mice after LECT2 treatment (Supplementary Fig. 7b). Because CD209a was mainly expressed in macrophages and osteolineage cells (Fig. 3h), we further investigated the effect of LECT2/CD209a signal on TNF expression levels in vitro. TNF levels in both osteolineage cells and macrophages were downregulated after LECT2 treatment (Supplementary Fig. 7c).

**Figure 6 | TNF mediates the effect of LECT2 on HSC expansion and mobilization.** (a) The protocol for TNF and LECT2 treatment. (b) The number of LSK cells in the BM of WT mice treated with LECT2 combined with TNF. (c) The number of LSK cells in the blood of mice treated with LECT2 combined with TNF. n = 5. (d,e) LSK cells in the BM and blood of TNF KO mice after LECT2 treatment; n = 6. The data represent means ± s.e.m. The data are representative of two (b,c) and three (d,e) independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using one-way ANOVA.

Furthermore, BM cells from CD209a KO mice were injected intravenously (i.v.) into lethally irradiated (9 Gy) WT mice that expressed CD209a in osteolineage cells but not in macrophages (Fig. 5d). In these mice, LECT2 still led to the increase of LSK cell number in the BM and blood, but only a 1.3-fold and 3.1-fold increase in the BM and blood, respectively (Fig. 5e,f).

**Figure 5 | Osteolineage cells partly mediate the effect of LECT2 on HSC expansion and mobilization.** (a) Representative tissue expression of OPN in the BM of WT and Bgn KO mice. (b) The number of LSK cells in the BM of WT or Bgn KO mice after PBS or LECT2 treatment. (c) The number of LSK cells in the blood of WT or Bgn KO mice after PBS or LECT2 treatment. (d) The protocol used to produce chimaeras of WT mice with BM cells from CD209a KO mice. (e) Effect of LECT2 on LSK cell number in the BM of WT mice with BM cells from CD209a KO mice. (f) Effect of LECT2 on LSK cell number in the blood of WT mice with BM cells from CD209a KO mice. n = 4. The small black dots in histograms are data points. The data represent means ± s.e.m. The data are representative of two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 using one-way ANOVA.

**TNF secretion is suppressed by the LECT2 signal.** Because LSK cells did not express CD209a (Fig. 3h), we hypothesized that secreted soluble factors from macrophages or osteolineage cells might mediate the effect of LECT2. The data from transcriptome sequencing revealed that several cytokines were downregulated in the BM after LECT2 treatment (Supplementary Fig. 6a). Moreover, the protein levels of TNF and CC motif chemokine ligand (CCL) were downregulated to < 25% of the control levels in the BM supernatant of LECT2-treated mice (Supplementary Fig. 6b)

TNF plays an important role in regulating HSC expansion and emergence. CCL3 has also been identified as a stem cell
mediated by TNF (Fig. 7c,d). LECT2 slightly upregulated total SDF-1 in the BM and did not alter the expression of total SDF-1 in the plasma (Fig. 7e,f). Furthermore, LECT2 treatment led to the downregulation of intact SDF-1 (1–68; Fig. 7i). CD26 activity in the BM (Fig. 7i). In LECT2-treated mice, total SDF-1 levels did not change after diprotin A or TNF treatment, whereas intact SDF-1 was upregulated after diprotin A or TNF treatment (Fig. 7i). These results suggest that LECT2 increases CD26 activity to reduce intact SDF-1 via downregulation of TNF. The number of LSK cells increased in the blood after intra-marrow injection of SDF-1 (3–68) compared with SDF-1 (1–68) (Fig. 7j).

The effects of G-CSF and LECT2 on HSC homeostasis. G-CSF is the most widely used HSC-mobilizing agent in clinical settings, G-CSF and LECT2 did not have a synergistic effect on HSC expansion and mobilization (Fig. 8a–e). To further investigate the differences in HSC mobilization due to LECT2 and G-CSF in detail, LT-HSCs and LMPPs in the blood were both measured. The number of LT-HSCs and LMPPs in the blood both increased after LECT2 or G-CSF treatment (Fig. 8f). Furthermore, the effect of G-CSF on LSK cell mobilization was completely lost 20 days after irradiation (6 Gy; Fig. 8g). Twenty days after irradiation, the effect of LECT2 on HSC expansion and mobilization in mice was evaluated. LECT2 increased the number of LSK cells and WBCs in the BM of irradiated mice (Fig. 8h). LECT2 also markedly increased the number of CFU-Cs, LSK cells and WBCs in the blood of irradiated mice (Fig. 8i,j). By contrast, G-CSF did not induce a change in the number of LSK cells in the
irradiated mice (Fig. 8h–j). To further determine whether the effects of LECT2 and G-CSF were independent, we administered G-CSF to CD209a KO mice and LECT2 to G-CSF receptor (G-CSFR) KO mice. In CD209a KO mice, G-CSF treatment markedly increased the number of LSK cells in the blood, whereas LECT2 treatment had no effect on LSK cell mobilization (Fig. 8k). In G-CSFR KO mice, LECT2 treatment markedly increased the number of LSK cells in the blood, whereas G-CSF treatment had no effect on LSK cell mobilization (Fig. 8l). Moreover, mRNA expression of G-CSF in the BM was not altered by LECT2 treatment (Fig. 8m). LECT2 mRNA expression was not detected in the BM after G-CSF treatment.

We further analysed the number of reconstituting HSCs mobilized per ml blood by LECT2 and G-CSF. A RU value per ml blood from LECT2-treated mice was 1.86-fold of that of G-CSF-treated mice (Fig. 9a). Using limiting dilution competitive transplantation assays, we could demonstrate that LECT2 mobilized more CRUs than G-CSF (Fig. 9b). We further investigated the effect of G-CSF on the LECT2 downstream signal, target cells and TNF. LECT2 treatment increased the number of macrophages but did not change the number of osteolineage cells, whereas G-CSF downregulated both macrophages (F4/80+CD169+) and osteolineage cells (CD45−Ter119−CD31−Sca-1−CD51+) in the BM (Fig. 9c,d). Both LECT2 and G-CSF downregulated TNF production in the BM (Fig. 9e). However, BM TNF level in LECT2-treated mice was lower than that in G-CSF-treated mice after 5 days of administration (Fig. 9e). Moreover, the marked effect of G-CSF on the expansion and mobilization of LSK cells was maintained in TNF KO mice (Fig. 9f,g). These results reveal that the effects of LECT2 and G-CSF on the BM niche are different.

Discussion

In the present study, we demonstrated that recombinant LECT2 injection induced HSC expansion and mobilization. In addition, we determined that CD209a, the LECT2 receptor, is mainly expressed in macrophages and osteolineage cells, which mediate the effect of LECT2 on HSC homeostasis. The number of CFU-Cs and LSK cells both decreased in LECT2 KO mice, supporting the connection between HSC homeostasis and LECT2. LECT2 mRNA was not detected in BM in our transcriptome analysis. A previous study showed that LECT2 is secreted by the liver into the blood,11, therefore, LECT2 is an extramedullary cytokine for HSC regulation. Plasma LECT2 levels are downregulated in septic...
Flow cytomter analysis showed that LECT2 treatment increased the number of LSK cells, LT-HSCs and ST-HSCs in the BM of mice. Because some exogenous agents could change the expression of HSC markers, we further detected stem cell activity and number (RUs and CRUs) of LSK cells from the BM of LECT2-treated mice. Both the stem cell activity and number of LSK cells from the BM of LECT2-treated mice increased compared with PBS-treated mice, suggesting that LECT2 induces the expansion of HSCs in the BM. Limiting dilution competitive transplantation assays with LECT2-mobilized blood showed the presence of CRUs. Therefore, the LECT2 induces the mobilization of true HSCs, which is important when clinical transplantation of LECT2-mobilized HSCs is planned. Moreover, the number of CRUs in per ml blood of LECT2-treated mice was higher than G-CSF-treated mice. Since the number of LSK cells in the blood of G-CSF- and LECT2-treated mice was similar, the cells with the LSK phenotype mobilized by G-CSF may have reduced hap hematopoietic potential, which is consistent with previous findings demonstrating G-CSF-mobilized LSK cells have impaired engraftment potential.

HSC expansion and mobilization after LECT2 treatment were downregulated in both CD169<sup>DTR</sup>/+ mice treated with DT and Bgn<sup>−/−</sup> mice compared with WT mice. The effect of LECT2 on HSC homeostasis was reduced but not completely impaired in irritated mice transplanted with CD209a-deficient HSCs, in which osteolineage cells but not macrophages expressed CD209a. This result suggests that osteolineage cells participate in the effect of LECT2 on HSC homeostasis. Osteolineage cells play an important role in BM niches to regulate HSC homeostasis<sup>3,38</sup>. Some hematopoietic growth factors are produced by osteolineage cells, such as G-CSF, a hepatocyte growth factor<sup>40</sup>. In the present study, we observed that TNF was also produced by osteolineage cells to suppress HSC expansion and mobilization. Our data contribute to the elucidation of the complex signals from osteolineage cells that regulate HSC homeostasis.

Macrophages play an important role in HSC expansion and mobilization in the BM niche. Macrophages are pivotal to maintaining the BM niche and regulating the egress of HSCs into the blood<sup>4,42</sup>. The depletion of CD169<sup>+</sup> macrophages induces HSC egress by regulating SDF-1 expression<sup>20</sup>. α-Smooth muscle actin<sup>+</sup> macrophages secrete prostaglandin E<sub>2</sub> to prevent HSC exhaustion<sup>21</sup>. The liver X receptor in macrophages is essential for the rhythmic egress of HSCs into the circulation<sup>43</sup>. We observed that the effect of LECT2 was reduced in CD169<sup>DTR</sup>/+ mice treated with DT compared with WT mice, confirming the important role of macrophages in HSC homeostasis. Moreover, although macrophage depletion induced HSC mobilization, LECT2 also induced HSC mobilization but increased the macrophage number in BM. Therefore, the specific factor secreted by macrophages but not the macrophage number is the essential factor in regulating HSC homeostasis. Transcriptome analysis revealed that TNF, which is constitutively expressed in BM, was markedly downregulated after LECT2 treatment. We subsequently confirmed that TNF mediated the effect of LECT2 on HSC homeostasis. In addition to TNF, oncostatin M was recently suggested to be a macrophage-derived factor mediating HSC function<sup>44</sup>. Thus, macrophages in BM appear to express a variety of signals for regulating HSC homeostasis.

Macrophages are first identified as the source of TNF after endotoxin activation<sup>45</sup>. TNF is a repressor of HSC activity in BM and may be a mediator of BM failure syndromes after inflammatory diseases<sup>35</sup>. We observed that macrophages and osteolineage cells in BM produced TNF at a concentration of 1 ng ml<sup>−1</sup> in the supernatant and that TNF mediated the effect of
LEC2 on HSC homeostasis. Therefore, TNF is a HSC regulator not only under inflammatory conditions but also under steady-state conditions. Although we observed that TNF downregulation contributed to HSC homeostasis, the HSCs in the BM of TNF KO mice were similar to those in WT mice. Furthermore, the percentages of HSCs in the BM of the two types of TNF receptor KO mice are similar to those in WT mice. Normal HSC number in mice that are deficient in TNF or its receptors may result from TNF superfamily of TNF proteins consisting of 19 members and 29 receptors. In the present study, in TNF KO mice, the effect of LECT2 on HSC homeostasis was markedly reduced, suggesting that TNF is the specific downstream target of LECT2 signal. These data illustrate a new pathway for LECT2 signal regulating HSC homeostasis via regulation of the pro-inflammatory cytokine TNF in the absence of infection.

In the present study, LECT2 decreased CXCR4 expression in LSK cells and increased CD26 activity to cleave intact SDF-1 to SDF-1 (3–68). G-CSF increases plasma SDF-1 and surface expression of CXCR4 in HSCs. The CXCR4–SDF-1 axis plays a unique role in HSC migration. In the steady state, macrophages and osteolineage cells secrete TNF in BM. TNF downregulation causes HSC expansion. After LECT2 stimulation, TNF secretion from CD209a+ macrophages and osteolineage cells is downregulated in BM. TNF downregulation results in the activation of CD26, which cleaves SDF-1 to SDF-1 (3–68). The SDF-1 (3–68) in BM increases HSC egress to the blood. TNF downregulation also reduces CXCR4 expression in HSCs to enhance HSC egress. Moreover, TNF downregulation causes HSC expansion.

In the present study, LECT2 decreased CXCR4 expression in LSK cells and increased CD26 activity to cleave intact SDF-1 to SDF-1 (3–68). G-CSF increases plasma SDF-1 and surface expression of CXCR4 in HSCs. The mechanisms of HSC mobilization appear to be different between LECT2 and G-CSF. In G-CSF-induced mobilization, SDF-1 is higher in the plasma than in the BM supernatant. High CXCR4-expressing HSCs are mobilized to the blood. In LECT2-induced mobilization, SDF-1 is also higher in the BM supernatant than in the plasma. The downregulation of CXCR4 in LSK cells after LECT2 treatment mobilized HSCs to blood. Moreover, SDF-1 (3–68) acts as an antagonist of the SDF-1–CXCR4 axis, resulting in a reduction of the migratory response to normal SDF-1 (ref. 36). We observed that SDF-1 (3–68) injection in BM enhanced HSC mobilization. These results demonstrate that LECT2 has different effects on the SDF-1–CXCR4 axis compared with G-CSF.

As a BM niche-derived cytokine, G-CSF may fail to mobilize HSCs in some patients due to several factors, such as prior myelosuppressive chemotherapy, prior radiotherapy and diabetes. This failure may be due to severe G-CSF-induced alterations of the BM microenvironment. As an extra-medullary cytokine, the HSC-mobilizing effect of LECT2 is similar to that of G-CSF in healthy mice. However, in contrast to the loss of the HSC-mobilizing effect of G-CSF in irradiated mice, LECT2 still induces HSC expansion and mobilization in irradiated mice. LECT2 increased the number of macrophages in our study, whereas G-CSF reduced the number of macrophages in BM. Macrophage proliferation by LECT2 induction may enhance HSC expansion and mobilization under myelosuppressive conditions. Furthermore, LECT2 in combination with AMD3100, a CXCR4 antagonist of HSC mobilization, exhibited a stronger effect on HSC mobilization compared with LECT2 alone. Therefore, LECT2 may represent a potential HSC-mobilizing agent in patients who do not exhibit mobilization with G-CSF treatment.

In summary, our study reveals the potential value of the clinical application of LECT2 for HSC mobilization. LECT2 can regulate HSC expansion and mobilization by modulating TNF expression in CD209a-expressing osteolineage cells and macrophages (Fig. 10). Because LECT2 is a cytokine that originates in the blood and has been implicated in a variety of immune disorders, our study suggests that immune signals from the blood control HSC expansion and mobilization.

**Methods**

**Mice.** Six- to eight-week-old male C57BL/6 mice were purchased from the Zhejiang Province Experimental Animal Center. All injections and measurements were performed in males unless otherwise indicated. B6.SJL-NOD-SCID and C3H/HeJ mice were purchased from Charles River Laboratories (Beijing, China). B6 background CD209a KO mice (B6.FVB-Cd209atm1.1.Cfg/Muucd) were purchased from the Mutant Mouse Resource Regional Center (University of Missouri, Columbia, MO). CD209a KO mice were used in the experiments, and their wild-type CD209a+/- littermates were used as controls. CD169-diphtheria toxin receptor (DTR) heterozygous (CD169DTR+/-) mice on a C57BL/6 background, which were generated with a DTR complementary DNA were bred in-house by crossing the CD169DTR/DTR mice with C57BL/6 mice. Bng-1/0 mice (B6;129S4(C3)Bngtm1Mfy/Mmhy) were obtained from Mutant Mouse Resource Regional Center. TNF KO mice (B6;129S-Tnf1tm1Gkl/) and G-CSF KO mice (B6.129X1(Cq)-CsDrtml1Link/) were obtained from Jackson Laboratories (Bar Harbor, ME). A mouse sepsis model was established by intraperitoneally (i.p.) injection of S. pneumoniae and Pseudomonas aeruginosa (ATCC 27853). Survival was monitored once per 12-h period for 96 h. For bacterial clearance analysis, liver, spleen and blood samples were taken from mice after killing. Necropsies of tissues and blood were then serially diluted to determine colony-forming units. The experimental conditions and procedures were approved by the Ningbo University Institutional Animal Care and Use Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

To generate LECT2 KO mice, single guide RNA (sgRNA) for clustered regularly interspaced short palindromic repeats ligase 3 (CRISPR/Cas9) gene editing were designed on the website developed by Feng Zhang Lab (http://crispr.mit.edu/). The template for in vitro transcription of sgRNA was amplified using forward primer contain T7 promoter and sgRNA targeting LECT2 (sgrNA: 5'-TAATACGACCTTATACAGGGAGCCATATGGTCACACGGTCAGTTTAGCGCTAGAA-3') and reverse primer (5'-AAAACGACCACTGCGGT-3'). Zygotes of C57BL/6J mouse strains were used for microinjection and
transplanted to pseudopregnant recipient mice. In vitro transcribed Cas9 RNA (100 ng µL⁻¹) and sgRNA (50 ng µL⁻¹) were injected into the zygotes. Oviduct transfers were performed on the second day. To analyse the genotype, genomic DNA was extracted with 50 mM sodium hydroxide from the LECT2 KO mice and PCR amplified using the forward and reverse primers (5'-CATACGGCAGG ACTATGTGT-3', R'-CTAGATCAGCTGCCAGACAGAC-3'). With expected product size of 377 bp for the amplimer, PCR products were then submitted for Sanger sequencing.

**Humans.** This study was approved by the Ethics Committee of Ningbo University. Informed consent was obtained according to the Declaration of Helsinki. Human peripheral blood mononuclear cells were obtained from 31 healthy subjects (Ningbo No. 2 Hospital). The Ethics Committee of Ningbo No. 2 Hospital approved the use of discarded human blood protocol (kYLL2013002). All subjects gave informed consent prior to blood collection.

**Reagents.** The recombinant LECT2 proteins were produced from Chinese hamster ovary (CHO) cells. The mouse LECT2 cDNA fragment was cloned into the EcoRI and KpnI site of an expression vector pcDL-Sr296 provided by Biovector Science Lab to generate a LECT2 expression plasmid named pcDL-Sr296-mLECT2. CHO cells transfected with pcDL-Sr296-mLECT2 were maintained in Eagle’s minimal essential medium supplemented with 10% fetal bovine serum. Recombinant LECT2 protein was purified from the culture fluids of CHO cells. Endotoxin in the recombinant proteins was less than 0.1 EU mg⁻¹ after toxin removal with an endotoxin-removal column (Pierce). LECT2 or G-CSF (Filgastrim, Amgen) was sc injected at a dose of 300 µg kg⁻¹ body weight (once a day for 5 days). The mice were killed 4 h after the final injection. For kinetic studies, the number of nucleated blood was analysed at 1, 2, 4, 8, 12, 24 h, Day 1, 4, 5 after LECT2 injection. 5FU (Sigma-Aldrich) was injected (i.v.) 150 mg kg⁻¹ body weight. In combination with LECT2 administration, anti-G-CSF IgGs and anti-G-CSFR IgGs (R&D Systems) were i.p. administered (300 µg kg⁻¹ body weight daily) for 5 consecutive days. For the G-CSF and LECT2 comparison, mice were exposed to 8 Gy (Cammacell-40, Atomic Energy of Canada Ltd). Mice were separated at 30, 25 or 20 h before the G-CSF or LECT2 treatment. A C-MET inhibitor (PHA-665752, Pfizer, 7.5 mg kg⁻¹) was injected (i.v.) twice per day on the first and fourth days, and the mice were killed 4 h after the last injection. The CD169DTR/- mice were injected (i.v.) with diphtheria toxin (10 µg kg⁻¹) body weight on the first and fourth days, and the mice were killed 4 h after the last injection. 5FU (Sigma-Aldrich) was injected (i.v.) once (150 mg kg⁻¹ body weight). In combination with LECT2 administration, anti-G-CSF IgGs and anti-G-CSFR IgGs (R&D Systems) were i.p. administered (300 µg kg⁻¹ body weight daily) for 5 consecutive days. For the G-CSF and LECT2 comparison, mice were exposed to 8 Gy (Cammacell-40, Atomic Energy of Canada Ltd). Mice were separated at 30, 25 or 20 h before the G-CSF or LECT2 treatment. To analyse osteolineage cell function, the mice were administered SrCl₂ (4 mmol kg⁻¹ per day, Sigma) via their drinking water for 12 weeks. For LECT2 neutralization, the mice received intraperitoneal injections of a polyclonal anti-LECT2 IgG (100 µg mg⁻¹ per mouse weight per day). Flow cytometry

**Immunofluorescence.** The femurs were isolated from the mice, post-fixed in 4% paraformaldehyde at 4 °C overnight, and decalcified in 10% EDTA at 4 °C for 1 week. Longitudinal sections of the femoral bones were prepared using a cryostat (CM1950, Leica). The sections were rinsed three times with PBS and blocked in 2% BSA/PBS for 30 min. The slides were incubated overnight at 4 °C with polyclonal anti-osteopontin (Santa Cruz Biotechnology) (1:50) and rinsed three times with PBS. The slides were incubated with anti-goat-igG (1:100) for 45 min at room temperature after the primary antibody incubation. The coverslips were mounted with SlowFade Gold Antifade reagent supplemented with 4.6-diamino-2-phenylindole dihydrochloride (DAPI) nuclear stain (Invitrogen). Confocal images were captured using a Zeiss LSM 780 microscope (Carl Zeiss).

**CFU assays.** The peripheral blood mononuclear cells were collected from the mice using standard techniques. We plated 10 µl of blood in 2.5 ml methylcellulose medium supplemented with a cocktail of recombinant cytokines (MethoCult 3434; STEM CELL Technologies). After 7 days in culture, the number of colonies per dish was counted.

**Real-time quantitative PCR.** The CD209a⁺ BM cells were sorted using the MoFlo XDP cell sorter (Beckman Coulter). The total RNA was extracted and purified from the steady-state or CD209a⁺ BM cells using RNAsio reagents (TaKaRa). For the analysis of survival after transplantation, the pCAGG-neo expression vector (TaKaRa) was used. To observe the transcription of the cDNAs, we synthesized gene-specific primers for the following genes: CCR2, 5'-AATTTGTGGTTCGAGATGATG-3'; CCL2, 5'-TCCTCGTTTGATTCGCTGCTC-3'; CCL5, 5'-GTCGTCCTTGCTTCC-3'; CCR5, 5'-GACAGGAATTATAGG-3'. The PCR products were then expected product sizes of 377 bp for the amplicon. The primers were designed using standard techniques. We plated 10 µl of blood in 2.5 ml methylcellulose medium supplemented with a cocktail of recombinant cytokines (MethoCult 3434; STEM CELL Technologies). After 7 days in culture, the number of colonies per dish was counted.

**Cell isolation and culture.** The osteolineage cells were isolated53. LSK cells were sorted from the BM of the mice that had been injected (i.v.) into Pseudomomas aeruginosa-infected mice. For the in vitro studies, the following reagents were used: TNF (10 ng ml⁻¹), etanercept (Enbrel, Pfizer, 10 µm). For the in vivo studies, the following reagents were used: TNF (10 ng ml⁻¹), etanercept (Enbrel, Pfizer, 10 µm).

**Transplantation experiments.** For the analysis of survival after transplantation, LSK cells were sorted using the MoFlo XDP cell sorter (Beckman Coulter) and Lin⁻ c-kit⁺ cells were MACs (Milteny Biotech) sorted from BM of mice that had been treated with PBS or LECT2 for 3 days. The LSK cells were injected (i.v.) into lethally irradiated (9 Gy) mice. In the sepsis model, 1 × 10⁵ Lin⁻ c-kit⁺ cells were injected (i.v.) into Pseudomomas aeruginosa-infected mice. For competitive bone marrow transplantation, the congenic Ly5.1/Ly5.2 system was used53. LSK cells were sorted from the BM of the mice that had been treated with PBS or LECT2 for 3 days. The LSK cells were injected (i.v.) into lethally irradiated (9 Gy) mice. In the sepsis model, 1 × 10⁵ Lin⁻ c-kit⁺ cells were sorted from the BM of the mice that had been treated with PBS or LECT2 for 3 days. The LSK cells were injected (i.v.) into lethally irradiated (9 Gy) mice. In the sepsis model, 1 × 10⁵ Lin⁻ c-kit⁺ cells were sorted from the BM of the mice that had been treated with PBS or LECT2 for 3 days. The LSK cells were injected (i.v.) into lethally irradiated (9 Gy) mice.

**Cell survival analysis.** The cell survival was quantified by the trypan blue exclusion. Cell proliferation was determined by measuring 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) [Promega] and 5-carboxyfluorescein diacetate (CFDA) [Invitrogen] staining. The cell viability was determined by measuring the relative photoluminescence (PicoGreen, Invitrogen) of DNA content in the cell nuclei. The cell proliferation was determined by measuring the relative photoluminescence (PicoGreen, Invitrogen) of DNA content in the cell nuclei. The cell proliferation was determined by measuring the relative photoluminescence (PicoGreen, Invitrogen) of DNA content in the cell nuclei.
For the second transplantation, CD45.2– LSK cells were sorted from BM isolated from the chimaeras of the first transplantation and transplanted as above. For the analysis of osteocline cell role, BM cells (5 × 10^6) were obtained from CD209a KO mice. BM cells (5 × 10^6) were injected (i.v.) into lethally irradiated (9 Gy) WT mice. The analysis of LSK cells in BM was performed 8 weeks after reconstitution.

For the mobilized blood cell transplantations, the lethally irradiated B6.SJL (CD45.1) mice were transplanted with 20 μl blood from six pooled mobilized C57Bl/6 mice (CD45.2) in competition with 2 × 10^6 BM cells from the SJL mice (CD45.1). Moreover, the lethally irradiated recipient B6.SJL mice (CD45.1) were also transplanted with 2 × 10^6 competitive whole BM cells from untreated B6.SJL mice (CD45.1) mixed with 5–80 μl blood from six pooled mobilized C57Bl/6 mice (CD45.2). Reconstitution of donor peripheral blood was further employed to estimate the RU and CRU values.

RU values were calculated according to Harrison’s method. Each RU represents the repopulating activity of 1 × 10^6 BM cells per mouse. The RU formula can be rearranged as: donor RU = % donor × C/(100 – % donor), C is the number of competing BM RU co-transplanted with the donor cells and C = 2 for 2 × 10^5 competitor BM cells. Limiting dilution analysis was performed. After 20 weeks, the relative contribution of CD45.2 to total CD45.2 was established by flow cytometric analysis. Animals with >1% donor contribution were considered positive for donor cell engraftment. CRU frequency was calculated using L-Calc software (Stem Cell Technologies).

**NOD-SCID mice.** The NOD-SCID mice (NOD/LtSzPrKdcsid/PrKdcsid63) were sublethally irradiated with 3.75 Gy and injected (i.v.) with 2 × 10^6 NATURE COMMUNICATIONS | DOI: 10.1038/ncomms12719 | www.nature.com/naturecommunications

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consecutive days, together with LECT2, and killed for analysis 3 h following the last injection (i.p.) for three days. The NOD-SCID mice were given two daily doses of BrdU (3.3 mg per mouse) by injection (i.p.) for three days. The library was prepared using an Illumina TruSeq RNA sample prep kit. After mice treated with LECT2 for 0, 3 and 5 days, the animals were performed by an observer blinded to experimental conditions. We calculated the correlations between data sets using Pearson’s correlation coefficient and the SPSS (version 13.0) software. We analysed the observed effect sizes. The mice used in the experiments were randomly chosen by one-way ANOVA. When variances were significantly different (P < 0.05), a Student’s t-test was evaluated using the Mann–Whitney U-test (two-tailed). * * * and ** ** ** represent P values <0.05, 0.01 and 0.001, respectively.

**Data availability.** RNA Sequencing data have been deposited in Gene Expression Omnibus (GEO) under accession number no. GSE70014. The authors declare that the remaining data are contained within the Article and Supplementary Information files or available from the corresponding author upon request.

References
1. Kurzäke, D. R. Hematopoietic stem cell transplantation for HIV cure. J. Clin. Transl. 12, 432–437 (2016).
2. Sorrentino, B. P. Clinical strategies for expansion of haematopoietic stem cells. Nat. Rev. Immunol. 4, 878–888 (2004).
3. Morrison, S. J. & Scadden, D. T. The bone marrow niche for haematopoietic stem cells. Nature 505, 327–334 (2014).
4. Golan, K. et al. S1P promotes murine progenitor cell egress and mobilization via S1P1-mediated ROS signaling and SDF-1 release. Blood 119, 2478–2488 (2012).
5. Dar, A. et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and cachectolamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. Leukemia 25, 1286–1296 (2011).
6. Kfoury, Y. & Scadden, D. T. Mesenchymal cell contributions to the stem cell niche. Cell Stem Cell 16, 239–253 (2015).
7. Calvi, L. M. & Link, D. C. The hematopoietic stem cell niche in homeostasis and disease. Blood 126, 2443–2451 (2015).
8. Petti, I. et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat. Immunol. 3, 687–694 (2002).
9. Siegemund, S. et al. IP3 3-kinase B controls hematopoietic stem cell homeostasis and prevents lethal hematopoietic failure in mice. Blood 125, 2786–2797 (2015).
10. Leiva, M., Quintana, J. A., Ligos, J. M. & Hidalgo, A. Haematopoietic ESL-1 enables stem cell proliferation in the bone marrow by limiting TGFβ availability. Nat. Commun. 7, 10222 (2016).
11. Yamagoe, S. et al. Purification and primary amino acid sequence of a novel neutrophil chemotactic factor LECT2. Immunol. Lett. 52, 9–13 (1996).
12. Lu, X. J. et al. LECT2 protects mice against bacterial sepsis by activating macrophages via the CD209a receptor. J. Exp. Med. 210, 5–13 (2013).
13. Lan, F. et al. LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance. Diabetes 63, 1649–1664 (2014).
14. Comenzoni, R. L. LECT2 makes the amyloid list. Br. J. Haematol. 153, 449–452 (2011).
15. Said, S. M. et al. Characterization and outcomes of renal leukocyte chemotactic factor 2-associated amyloidosis. Kidney Int. 86, 370–377 (2015).
16. Ong, H. T. et al. The tumor suppressor function of LECT2 in human hepatocellular carcinoma makes it a potential therapeutic target. Cancer Gene Ther. 18, 399–406 (2011).
17. Park, C. G. et al. Five mouse homologues of the human dendritic cell C-type lectin, DC-SIGN/CD209. J. Immunol. 173, 1283–1290 (2004).
18. Wang, Y. S. et al. A dominant complement fixation pathway for pneumococcal polysaccharides initiated by SIGN-R1 interacting with C1q. Cell 125, 47–58 (2006).
19. Cheong, C. et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209– dendritic cells for immune T cell areas. Cell 143, 416–429 (2010).
20. Chow, A. et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J. Exp. Med. 208, 261–271 (2011).
21. Ludin, A. et al. Monocytes-macrophages that express α-smooth muscle actin preserve primitive hematopoietic cells in the bone marrow. Nat. Immunol. 13, 1072–1082 (2012).
22. Essers, M. A. et al. IFNα activates dormant haematopoietic stem cells in vivo. Nature 458, 904–908 (2009).
23. Sato, T., Laver, J. H. & Ogawa, M. Reversible expression of CD34 by murine hematopoietic stem cells. Blood 113, 2548–2554 (1999).
24. Ogawa, M. et al. CD34 expression by murine hematopoietic stem cells. Developmental changes and kinetic alterations. Annu. NY Acad. Sci. 938, 139–145 (2001).
25. Zon, L. I. Intrinsic and extrinsic control of haematopoietic stem-cell self-renewal. Nature 453, 306–313 (2008).
26. Yu, H. et al. Downregulation of Prdm16 mRNA is a specific antileukemic mechanism during HOXB4-mediated HSC expansion in vivo. Blood 124, 1737–1747 (2014).
27. King, K. Y. & Goodell, M. A. Inflammatory modulation of HSCs viewing the HSC as a foundation for the immune response. Nat. Rev. Immunol. 11, 685–692 (2011).
28. Chen, C. K. et al. Leukocyte cell-derived chemotaxin 2 antagonizes MET receptor activation to suppress hepatocellular carcinoma vascular invasion by promoting osteoskinectin phosphatase 1B recruitment. Hepatology 59, 974–985 (2014).
29. Zhao, M. et al. Megakaryocytes maintain hematopoietic quiescence and promote post-injury regeneration of hematopoietic stem cells. Nat. Med. 20, 1321–1326 (2014).
30. Xu, T. et al. Targeted disruption of the biglycan gene leads to an osteoporosis-like phenotype in mice. Nat. Genet. 20, 78–82 (1998).
31. Lymperi, S. et al. Stromatin promotes some osteoblasts without increasing hematopoietic stem cells. Blood 111, 1173–1181 (2008).
32. Espin-Palazon, R. et al. Proinflammatory signaling regulates hematopoietic stem cell emergence. Cell 159, 1070–1085 (2014).
33. Pronk, C. J., Velby, O. P., Bryder, D. & Jacobsen, S. E. Tumor necrosis factor restricts hematopoietic stem cell activity in mice: involvement of two distinct receptors. J. Exp. Med. 208, 1563–1570 (2011).

34. Graham, G. J. et al. Identification and characterization of an inhibitor of haemopoietic stem cell proliferation. Nature 344, 442–444 (1990).

35. Christopherson, 2nd K. W., Hangoc, G., Mantel, C. R. & Broxmeyer, H. E. Modulation of hematopoietic stem cell homing and engraftment by CD26. Science 305, 1000–1003 (2004).

36. Christopherson, 2nd K. W., Cooper, S. & Broxmeyer, H. E. Cell surface peptidase CD26/DPP4 mediates G-CSF mobilization of mouse progenitor cells. Blood 101, 4680–4686 (2003).

37. Cao, B. et al. Therapeutic targeting and rapid mobilization of endosteal HSC using a small molecule integrin antagonist. Nat. Commun. 7, 11007 (2016).

38. Calvi, L. M. et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425, 841–846 (2003).

39. Taichman, R. S. & Emerson, S. Hematopoietic stem cell expansion and mobilization by G-CSF in mice. Exp. Hematol. 44, 207–212 (2015).

40. Aggarwal, B. B. Signalling pathways of the TNF superfamily: a double-edged sword. Nat. Rev. Immunol. 3, 745–756 (2003).

41. To, L. B., Levesque, J. P. & Herbert, K. E. How I treat patients who mobilize hematopoietic stem cells poorly. Blood 118, 4530–4540 (2011).

42. Gotter, R. et al. Impact of different strategies of second-line stem cell harvest on the outcome of autologous transplantation in poor peripheral blood stem cell mobilizers. Bone Marrow Transplant. 36, 847–853 (2005).

43. Fadini, G. P. et al. Diabetes mellitus limits stem cell mobilization following G-CSF but not plerixafor. Diabetes 64, 2957–2968 (2015).

44. Carswell, E. A. et al. An endotoxin-induced serum factor that causes necrosis of tumors. Proc. Natl Acad. Sci. USA 72, 3666–3670 (1975).

45. Saito, M. et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood 116, 4815–4828 (2010).

46. Jacobsen, R. N. et al. Fms-like tyrosine kinase 3 (Flt3) ligand depletes erythroid island macrophages and blocks mural erythropoiesis in the mouse. Exp. Hematol. 44, 207–212 (2015).

47. Alibino, M. et al. Bone marrow macrophages contribute to diabetic stem cell mobilopathy by producing Oncostatin M. Diabetes 64, 2957–2968 (2015).

48. Fadini, G. P. Diabetes mellitus limits stem cell mobilization following G-CSF but not plerixafor. Diabetes 64, 2957–2968 (2015).

49. Christopher, M. J., Rao, M., Liu, F., Woloszynek, J. R. & Link, D. C. Expression of the G-CSF receptor in mononcic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. J. Exp. Med. 208, 251–260 (2011).

50. Miyake, Y. et al. Critical role of macrophages in the marginal zone in the suppression of immune responses to apoptotic cell-associated antigens. J. Clin. Invest. 117, 2268–2278 (2007).

51. Saito, M. et al. Diphtheria toxin receptor-mediated conditional and targeted cell ablation in transgenic mice. Nat. Biotechnol. 19, 746–750 (2001).

52. Gur-Cohen, S. et al. PAR1 signaling regulates the retention and recruitment of EPCR-expressing bone marrow hematopoietic stem cells. Nat. Med 21, 1307–1317 (2015).