Adiponectin receptors sustain haematopoietic stem cells throughout adulthood by protecting them from inflammation

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How are haematopoietic stem cells (HSCs) protected from inflammation, which increases with age and can deplete HSCs? Adiponectin, an anti-inflammatory factor that is not required for HSC function or haematopoiesis, promotes stem/progenitor cell proliferation after bacterial infection and myeloablation. Adiponectin binds two receptors, AdipoR1 and AdipoR2, which have ceramidase activity that increases upon adiponectin binding. Here we found that adiponectin receptors are non-cell-autonomously required in haematopoietic cells to promote HSC quiescence and self-renewal. Adiponectin receptor signalling suppresses inflammatory cytokine expression by myeloid cells and T cells, including interferon-γ and tumour necrosis factor. Without adiponectin receptors, the levels of these factors increase, chronically activating HSCs, reducing their self-renewal potential and depleting them during ageing. Pathogen infection accelerates this loss of HSC self-renewal potential. Blocking interferon-γ or tumour necrosis factor signalling partially rescues these effects. Adiponectin receptors are thus required in immune cells to sustain HSC quiescence and to prevent premature HSC depletion by reducing inflammation.

Adiponectin is a circulating factor that suppresses inflammation and regulates glucose and fatty acid metabolism in multiple tissues¹⁻⁴. Adiponectin is synthesized by adipocytes throughout the body⁵⁻⁷ and by leptin receptor (LepR)⁺ stromal cells in the bone marrow⁸–¹¹. Adiponectin suppresses the activation of macrophages¹²–¹⁴, natural killer (NK) cells¹⁵ and T cells¹⁶ through multiple mechanisms, reducing their production of inflammatory factors, including interferon-γ (IFNγ)¹⁷ and tumour necrosis factor (TNF)¹²–¹⁴,¹⁷. Adiponectin deficiency has no effect on haematopoietic stem cells (HSCs) or haematopoiesis in the bone marrow of specific-pathogen-free mice, but after bacterial infection, adiponectin promotes haematopoietic progenitor proliferation by suppressing TNF expression¹⁷. Adiponectin also promotes HSC proliferation after myeloablation²⁻³,⁷–⁹,¹⁷.

Adiponectin has two ubiquitously expressed receptors with enzymatic activity¹²–¹⁴, AdipoR1 and AdipoR2²⁻³, and a third receptor that binds adiponectin but lacks an intracellular signalling domain, Tcad²¹. AdipoR1 and AdipoR2 have constitutive ceramidase activity that increases upon adiponectin binding¹²,¹⁸. They convert ceramide to sphingosine and sphingosine-1-phosphate²², which regulates HSC proliferation, migration and survival²³–²⁶. AdipoR1/R2 deficiency causes more severe insulin resistance and glucose intolerance than adiponectin deficiency²⁴, perhaps because the receptors signal even in the absence of adiponectin²⁴,²⁵. This raises the possibility that AdipoR1/R2 may be necessary to regulate aspects of HSC function that do not necessarily require adiponectin.

The bone marrow becomes more inflammatory during ageing²⁷–³⁰. Inflammatory factors promote HSC activation, and chronic inflammation promotes HSC depletion³¹–³⁶. However, HSCs remain mainly quiescent³⁷ and increase in number with age in most mouse strains³⁸. This suggests the existence of mechanisms to protect HSCs from chronic inflammation in adult bone marrow. Regulatory T cells protect HSCs from immune cells after allogeneic transplantation³⁹,⁴⁰. In this Article, we investigate whether there are factors that protect HSCs from immune cells and sustain HSC quiescence in normal adult bone marrow.

Results
Adiponectin receptors promote HSC quiescence. AdipoR1 and AdipoR2 are ubiquitously expressed by haematopoietic cells¹²,³¹,³⁴ (Fig. 1a,b) while Tcad was not detected (Fig. 1c). To test if adiponectin receptors are required by haematopoietic cells, we generated Vav1-Cre; AdipoR1fl/fl; AdipoR2fl/fl mice. Vav1-Cre; AdipoR1fl/fl; AdipoR2fl/fl mice were born at expected Mendelian frequencies (Extended Data Fig. 1a) and were grossly normal in size and appearance (Extended Data Fig. 1b,c). Vav1-Cre itself did not have any effect on bone marrow or spleen cellularity, blood cell counts or the frequencies of HSCs, multipotent progenitors (MPPs) or restricted haematopoietic progenitors in the bone marrow or spleen (Extended Data Fig. 1d–m). At 8–10 weeks of age, Vav1-Cre; AdipoR1fl/fl; AdipoR2fl/fl mice had normal bone marrow and thymus cellularity, but increased spleen cellularity (Fig. 1d) and normal blood cell counts (Fig. 1c) relative to AdipoR1fl/fl;AdipoR2fl/fl controls. In the bone marrow, Vav1-Cre; AdipoR1fl/fl; AdipoR2fl/fl mice did not significantly differ from AdipoR1fl/fl; AdipoR2fl/fl controls in the frequencies of HSCs (Fig. 1f), MPPs (Fig. 1g), common myeloid progenitors (CMPs), granulocyte–macrophage progenitors (GMPs) or megakaryocyte–erythroid progenitors (MEPs) (Fig. 1i), although they did have a modest increase in the frequency of Lineage⁻Sca¹⁻kit⁻CD48⁺ haematopoietic progenitor cells (HPCs) (Fig. 1h), Vav1-Cre; AdipoR1fl/fl; AdipoR2fl/fl mice also did not significantly differ from AdipoR1fl/fl; AdipoR2fl/fl controls in the frequencies of CD3⁺
Adiponectin receptors promote HSC quiescence. **a–c.** RNA-seq analysis of the adiponectin receptors Adipor1, Adipor2 and Tcad in haematopoietic stem and progenitor cell populations (n = 3 mice per genotype in one experiment). **d.** The number of cells in one femur and one tibia, the thymus and the spleen of 8–10-week-old Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl control mice (n = 12 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 13 control mice in six independent experiments for bone marrow and spleen, n = 3 mice per genotype in one experiment for thymus). **e.** White blood cell, red blood cell and platelet counts in the blood of 8–10-week-old Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control mice (n = 16 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 17 control mice in seven independent experiments). **f–j.** The frequencies of HSCs (i), MPPs (g), HPCs (h), CMPs, GMPs, MEPs (i) and differentiated T, B, myeloid and erythroid cells (j) in bone marrow cells from one femur and one tibia of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control mice (n = 12 (f–h) or n = 13 (i–j) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 13 control mice in six independent experiments). **k–o.** The frequencies of HSCs (k), MPPs (l), HPCs (m), CMPs, GMPs, MEPs (n) and differentiated T, B, myeloid and erythroid cells (o) in the spleens of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control mice (n = 12 (k–m) or n = 13 (n,o) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 13 control mice in six independent experiments). **p–r.** The percentage of HSCs (p), HPCs (q) and LK myeloid progenitors (r) that incorporated a 72 h (p) or 2 h (q and r) pulse of BrdU in Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control mice (n = 4 mice per genotype for HSCs; n = 5 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 13 control mice for HPCs and LKs in two independent experiments for each cell population). All data represent mean ± standard deviation, and each dot reflects a different mouse. Statistical significance was assessed using Mann–Whitney tests followed by the Holm–Sidak’s multiple comparisons adjustment (d–o), a Student’s t-test (p) or matched samples two-way ANOVAs followed by Sidak’s multiple comparisons adjustments (q and r). All statistical tests were two-sided. Source numerical data are in the source data files.
Adiponectin receptors maintain HSC function. To test if adiponectin receptors promote HSC function, we performed competitive reconstitution assays in irradiated mice. Young adult (8–10 weeks old) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl bone marrow cells gave significantly lower levels of donor cell reconstitution compared with control donor cells in primary recipient mice, although all recipients were long-term multilineage reconstituted by donor cells (Fig. 2a). Upon serial transplantation into secondary and tertiary recipient mice, Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl donor cells continued to give significantly lower levels of reconstitution (Fig. 2b,c). In tertiary recipient mice, control cells gave long-term multilineage reconstitution in all 20 recipients but Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl cells gave long-term multilineage reconstitution in only 5 of 20 recipients (Fig. 2d). Tertiary recipients of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl cells had significantly lower frequencies of donor HSCs, MPPs, HPCs and LK myeloid progenitors compared with recipients of control cells (Fig. 2e). AdipoR1/R2 deficiency, therefore, reduced HSC proliferation and the induction of extramedullary haematopoiesis in young adult mice.

Adiponectin receptors protect HSCs from inflammation. To assess the mechanism by which adiponectin receptors preserve HSC function, we sequenced RNA from HSCs and HPCs from Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control bone marrow. Gene set enrichment analysis showed that four of the seven pathways that were significantly enriched (false discovery rate (FDR) <0.05 and normalized enrichment score (NES) >1.5) in AdipoR1/R2-deficient HSCs compared with control HSCs were related to interferon signalling (Fig. 3a). The other three pathways were related to cell cycle progression, consistent with the increased division of AdipoR1/R2-deficient HSCs (Fig. 1p). These results suggest that adiponectin receptors are required in haematopoietic cells to protect bone marrow HSCs from inflammation. None of these pathways, nor any other pathways, significantly differed between Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control HPCs. Restricted progenitors, thus, did not exhibit an interferon response. This is consistent with the observation that HSCs are more sensitive to inflammatory cytokines than restricted progenitors and differentiated cells.

Consistent with the interferon response in AdipoR1/R2-deficient HSCs, the levels of IFNγ, TNF and interleukin-6 (IL6) were all significantly elevated in the blood plasma of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice compared with control mice (Fig. 3b). We did not detect significant increases in the levels of IFNα, IFNβ or IL1β (Fig. 3b). We also did not detect significantly increased levels of T cells or CD71+Ter119+ erythrocyte precursors in the bone marrow but had a lower frequency of B220+ B cells and a higher frequency of Mac-1+Gr-1+ myeloid cells (Fig. 1j). The markers used to identify each cell population are listed in Supplementary Table 1, and the gates used to isolate the cells by flow cytometry are shown in Extended Data Fig. 2.

In contrast to the bone marrow, Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice exhibited splenic extramedullary haematopoiesis with increased numbers of erythrocytoid cells, myeloid cells and megakaryocytes throughout the red pulp (Extended Data Fig. 2d) that was not evident in AdipoR1fl/fl; Adipor2fl/fl controls (Fig. 1p). We did not observe any difference in the rate of proliferation of HPCs or Lineage-c-kit+ (LK) myeloid progenitors in the bone marrow of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and Adipor1fl/fl; Adipor2fl/fl controls (Fig. 1q,r). Adiponectin receptors, therefore, reduced HSC proliferation and the induction of extramedullary haematopoiesis in young adult mice.

Adiponectin receptors are required to sustain HSC function. a. Donor CD45+ cells and T, B and myeloid cells from the blood of mice competitively transplanted with donor bone marrow cells from Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice produced fewer granulocyte, erythrocyte, macrophage and megakaryocyte colony-forming units (CFUs) compared with control bone marrow cells (Extended Data Fig. 3c); however, we observed no differences between Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control bone marrow cells in the frequencies of more mature colonies, including granulocyte–macrophage CFUs, granulocyte CFUs, macrophage CFUs and erythroid burst-forming units (Extended Data Fig. 3c). This suggests that HSCs were more dependent upon adiponectin receptors than restricted progenitors.

We also performed competitive transplant experiments with bone marrow cells obtained from Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and Adipor1fl/fl; Adipor2fl/fl controls that had been treated with polyninosine–polycytidine (pLpC) to induce Cre expression at 6 weeks of age. Again, we observed significantly lower levels of donor cell reconstitution by Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl cells compared with Adipor1fl/fl; Adipor2fl/fl controls in primary, secondary and tertiary recipient mice (Fig. 2f and Extended Data Fig. 3d–e). In tertiary recipient mice, the control cells gave long-term multilineage reconstitution in 12 of 17 recipients but Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl cells gave long-term multilineage reconstitution in only 1 of 8 recipients (Fig. 2g). Tertiary recipients of Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl cells had significantly lower frequencies of donor HSCs, MPPs, HPCs and LK cells compared with recipients of control cells (Fig. 2h). This confirmed that AdipoR1/R2 deficiency reduced HSC self-renewal potential.
inflammatory factors in spleen lysates (Extended Data Fig. 4a). Adiponectin receptors in haematopoietic cells thus systemically suppressed the levels of multiple pro-inflammatory cytokines. To determine the cellular sources of IFNγ and TNF, we performed quantitative reverse-transcription polymerase chain reaction (qRT–PCR) on whole bone marrow cells, HSCs, restricted...
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chronic exposure to elevated levels of inflammatory cytokines in spleens of mice transplanted with AdipoR1/R2 or secondary recipient mice (Fig. 3h), confirming that adiponectin cells and NK cells (Fig. 3c–f). CD4 populations in which we detected Ifng or Tfng expression by these cells (Extended Data Fig. 4f). Myeloid and lymphoid cells thus increased their expression of inflammatory cytokines in the bone marrow of AdipoR1/R2-deficient mice.

Adiponectin receptors non-cell-autonomously protect HSCs. These results raised the possibility that adiponectin receptors maintain the quiescence and self-renewal potential of HSCs by acting within immune cells to suppress their production of inflammatory cytokines. If so, the reduced reconstituting activity of HSCs from Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice may have reflected chronic exposure to elevated levels of inflammatory cytokines in the donor mice before transplantation. To test this, we transplanted equal numbers of Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl and control bone marrow cells into irradiated mice and then administered plpC to induce reduction 6 weeks after transplantation. In this experiment, if adiponectin receptors are non-autonomously required in HSCs, we would expect donor cell reconstitution by Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl cells to decline after plpC treatment. Conversely, if adiponectin receptors are non-autonomously required in immune cells to sustain HSC function, we would not expect any effect of post-transplant plpC treatment on donor cell reconstitution because both donor and competitor HSCs in the recipient mice would be exposed to the same inflammatory factors. In fact, we observed no effect of post-transplant plpC treatment on donor cell reconstitution by Mx1-Cre; Adipor1fl/fl; Adipor2fl/fl cells in either primary (Fig. 3g) or secondary recipient mice (Fig. 3h), confirming that adiponectin receptors act non-cell-autonomously to promote HSC function.

To independently test this, we also examined the frequencies of AdipoR1/R2-deficient donor and wild-type competitor HSCs in the spleens of mice transplanted with Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control bone marrow cells. If adiponectin receptors act cell-autonomously within HSCs to suppress mobilization and extramedullary haematopoiesis, we would expect a higher frequency of AdipoR1/R2-deficient donor HSCs compared with wild-type competitor HSCs in the spleens of these mice. Conversely, if adiponectin receptors act non-cell-autonomously to promote HSC quiescence, then we would not expect to observe any difference in the frequencies of AdipoR1/R2-deficient donor and wild-type competitor HSCs in the spleens as HSCs of both genotypes would be exposed to the same inflammatory factors. In fact, we observed no difference in the frequency of AdipoR1/R2-deficient donor compared with wild-type competitor HSCs in the spleens (Extended Data Fig. 4g). When we transplanted control donor HSCs instead of AdipoR1/R2-deficient donor HSCs, we observed lower overall frequencies of HSCs in the spleen, confirming that AdipoR1/R2-deficient cells promoted HSC mobilization in these experiments (Extended Data Fig. 4g). These results further demonstrate that adiponectin receptors act non-cell-autonomously to suppress mobilization and extramedullary haematopoiesis.

IFN and TNF impair HSC function in the absence of AdipoR1/R2. To test if IFNγ contributed to the defects in HSC function in Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice we tested if we could restore normal haematopoiesis by deleting interferon γ receptor 1 (Ifngr1) from haematopoietic cells. Consistent with a prior study, Ifngr1 deficiency alone had no effect on bone marrow or spleen cellularity (Extended Data Fig. 5a) or on the frequencies of HSCs, MPPs, restricted progenitors or differentiated haematopoietic cells in the bone marrow (Extended Data Fig. 5b–d) or spleen (Extended Data Fig. 5e–g). Most haematopoietic parameters in the bone marrow of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Ifngr1fl/fl and Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice did not significantly differ (Extended Data Fig. Si–o); however, Ifngr1 deficiency did significantly rescue the increased frequency of HPCs in the bone marrow of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice (Extended Data Fig. 5k) as well as the frequencies of MPPs, HPCs, Mac-1+Gr-1+ myeloid cells and CD71+Ter119+ erythroid cells in the spleens (Fig. 4c–h). We observed trends towards reduced splenic cellularity (Fig. 4a) and HSC frequency (Fig. 4b) in Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Ifngr1fl/fl mice compared with Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice. Ifngr1 deficiency thus partially rescued multiple haematopoietic changes in Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice.

Ifngr1 deficiency, by itself, did not affect HSC proliferation (Extended Data Fig. 5h) but partially rescued the increased division of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl HSCs compared with control HSCs (Fig. 4i). Ifngr1 deficiency also partially rescued the reconstituting capacity of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl bone marrow cells upon competitive transplantation into irradiated mice (Fig. 4j). The defects in HSC function in AdipoR1/R2-deficient mice were thus caused partly by increased IFNγ receptor signalling.

We also tested whether TNF contributed to the haematopoietic changes in Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice. TNF has both positive and negative effects on HSC function. We found that, by itself, Tnf deficiency had limited effects on bone marrow and spleen haematopoiesis (Extended Data Fig. 6a–g). The frequencies of most HSC and restricted progenitor populations in...
the bone marrow and spleen were unaffected, but *Tnf* deficiency modestly reduced bone marrow and spleen cellularity (Extended Data Fig. 6a) as well as the frequencies of MEPs, GMPs and erythroid progenitors in the bone marrow (Extended Data Fig. 6c,d). Most haematopoietic parameters in the bone marrow of *Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Tnf−/−* and *Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl* mice did not significantly differ (Extended Data Fig. 6i–o). However, *Tnf* deficiency significantly rescued most of the changes in splenic haematopoiesis in *AdipoR1/R2*-deficient mice compared with control mice, including the increases in spleen cellularity.
Fig. 4 | Adiponectin receptors promote HSC function by reducing IFNγ signalling. a, The number of cells in one femur and one tibia or in the spleen of Vav1-Cre; Adipor1−/−; Adipor2−/−, Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− or control mice (n = 12 Vav1-Cre; Adipor1−/−; Adipor2−/− mice, n = 10 Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− mice and n = 20 control mice in five independent experiments). b–h, The frequencies of HSCs (b), MPPs (c), HPCs (d), CMPs (e), MEPs (f), GMPs (g) or differentiated T, B, myeloid and erythroid cells (h) in the spleen of Vav1-Cre; Adipor1−/−; Adipor2−/− or Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− or control mice (n = 15 Vav1-Cre; Adipor1−/−; Adipor2−/− mice, n = 12 Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− mice and n = 20 (b–d) or n = 21 (e–h) control mice in five independent experiments). i, The percentage of HSCs that incorporated a 72 h pulse of BrdU (n = 19 recipients from four donors) in four independent experiments. j, Donor CD45+ cells and T, B and myeloid cells from the blood of mice competitively transplanted with Vav1-Cre; Adipor1−/−; Adipor2−/− (n = 19 recipients from five donors), Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− (n = 18 recipients from five donors) or control donor bone marrow cells (n = 19 recipients from four donors) in four independent experiments. All data represent mean ± standard deviation, and each dot reflects a different mouse. Statistical significance was assessed using one-way ANOVAs followed by Tukey’s multiple comparisons adjustments (a, d–f, h and i), Kruskal–Wallis tests followed by Dunn’s multiple comparisons adjustments (b, g and h), Welch’s one-way ANOVAs followed by Dunnett’s T3 multiple comparisons adjustments (c and h) or nparLD tests followed by FDR multiple comparisons adjustments (j). All statistical tests were two-sided. Source numerical data are available in the source data files.

(Fig. 5a) and the frequencies of MPPs, HPCs, CMPs, MEPs, Mac-1+Gr-1+ myeloid cells and CD71+Ter119+ erythroid cells (Fig. 5c–h). We observed a trend towards reduced HSC frequency in the spleen (Fig. 5b). Tnf deficiency also significantly rescued the decline in B-cell frequency in the spleens of AdipoR1/R2-deficient mice compared with control mice (Fig. 5h). Elevated TNF levels thus contributed to the induction of extramedullary haematopoiesis in AdipoR1/R2-deficient mice.

By itself, Tnf deficiency had no effect on the rate of HSC cell division (Extended Data Fig. 6h), but Tnf deficiency partially rescued the increased division of AdipoR1/R2-deficient HSCs (Fig. 5i). Tnf deficiency also partially rescued the reconstituting capacity of AdipoR1/R2-deficient bone marrow cells upon competitive transplantation into irradiated mice (Fig. 5i). The defects in bone marrow HSC function in AdipoR1/R2-deficient mice were thus caused partly by increased TNF levels.

As IFNγ and TNF regulate each other's expression49–48, we tested whether there is cross-regulation of these cytokines in AdipoR1/R2-deficient mice. We observed a significant reduction in TNF levels in Vav1-Cre; Adipor1−/−; Adipor2−/−; Ifngr1−/− mice compared with Vav1-Cre; Adipor1−/−; Adipor2−/− mice (Extended Data Fig. 6p). This indicates that IFNγ signalling promotes TNF expression in AdipoR1/R2-deficient mice. However, given that Tnf deficiency gave a stronger rescue than Ifngr1 deficiency of some of the extramedullary haematopoiesis phenotypes in AdipoR1/R2-deficient mice, there may be IFNγ-dependent as well as IFNγ-independent effects of TNF.

We also performed IFNγ enzyme-linked immunosorbent assays (ELISAs) on the serum of control, Vav1-Cre; Adipor1−/−; Adipor2−/− and Vav1-Cre; Adipor1−/−; Adipor2−/−; Tnf−/− mice. We found no statistically significant differences in IFNγ levels between AdipoR1/R2/Tnf-deficient and AdipoR1/R2-deficient mice, although there was a trend towards increased IFNγ levels in a subset of the AdipoR1/R2/Tnf-deficient mice (Extended Data Fig. 6q). This suggested that TNF negatively regulated IFNγ levels in certain circumstances, as has been reported48.

Our results are thus consistent with the known cross-talk between these inflammatory cytokines. Beyond TNF and IFNγ,
Adiponectin receptors protect HSCs from pathogen infection. To test if pathogen infection accelerates the loss of HSC self-renewal potential in the absence of adiponectin receptors, we gave 8–10-week-old Vav1-Cre; Adipor1–/–, Adipor2–/– mice or control mice sepsis by intraperitoneally injecting caecal contents from wild-type mice. Forty-eight hours later, we competitively
**Fig. 6** | Adiponectin receptors are required to sustain HSCs during ageing. 
**a,b.** The number of cells in one femur and one tibia or in the spleen of 5–6-month-old (a) or 19–24-month-old (b) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl control mice (n = 7 (a) or n = 5 (b) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 10 (a) or n = 6 (b) control mice in two independent experiments for each age group). c,d. The frequencies of bone marrow HSCs in 5–6-month-old (c) and 19–24-month-old (d) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl control mice (n = 7 (c) or n = 5 (d) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 10 (c) or n = 6 (d) control mice in two independent experiments for each age group). e. The percentage of HSCs from 19–24-month-old mice that incorporated a 72h pulse of BrdU (n = 6 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 5 control mice in two independent experiments). f–i. The frequencies of MPPs (f and h) and HPCs (g and i) in the bone marrow from 5–6-month-old (f and g) or 19–24-month-old (h and i) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl control mice (n = 7 (f and g) or n = 5 (h and i) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 10 (f and g) or n = 6 (h and i) control mice in two independent experiments for each age group). j. Donor cells from the blood of mice competitively transplanted with bone marrow cells from 5–6-month-old Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl (n = 11 recipients) or control (n = 11 recipients) mice (three donors per genotype in three independent experiments). k. Donor cells from the blood of mice competitively transplanted with bone marrow cells from 15-month-old Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl (n = 15 recipients) or control (n = 14 recipients) mice (three donors per genotype in three independent experiments). l. Secondary recipients of bone marrow cells from the primary recipients in (k) (n = 17 recipients from four Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl donors and n = 14 recipients from three control donors in three independent experiments). All data represent mean ± standard deviation, and each dot reflects a different mouse. Statistical significance was assessed using matched samples two-way ANOVAs followed by Sidak’s multiple comparisons adjustments (a and b), Mann–Whitney tests (c,d and f–i), or a Student’s t-test (e). All statistical tests were two-sided. Source numerical data are available in the source data files.
transplanted bone marrow cells from septic AdipoR1/R2-deficient or control donor mice into irradiated recipients. AdipoR1/R2-deficient bone marrow cells from septic donor mice gave significantly lower levels of reconstitution than bone marrow cells from septic control mice (Fig. 5k). All recipients of septic control cells were long-term multilineage reconstituted by donor cells (15/15), whereas only some recipients of septic AdipoR1/R2-deficient cells were long-term multilineage reconstituted (11/15) (Fig. 5k). In contrast, all (29/29) recipients of AdipoR1/R2-deficient bone marrow cells from young non-septic donors were long-term multilineage reconstituted (Fig. 2a). This suggests that acute pathogen infection accelerates the loss of HSC self-renewal potential in the absence of adiponectin receptors.

**Adiponectin receptors sustain HSCs during ageing.** To test if adiponectin receptors are required to preserve HSC function during ageing, we examined 5–6-month-old and 19–24-month-old mice. Bone marrow and spleen cellularity did not significantly differ between Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and Adipor2fl/fl controls in either 5–6-month-old (Fig. 6a) or 19–24-month-old (Fig. 6b) mice. In contrast to 2-month-old mice, in which HSC frequency was normal in the bone marrow (Fig. 1f), HSCs were depleted from the bone marrow of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice compared with controls at 5–6 months (Fig. 6c) and 19–24 months (Fig. 6d) of age. The rate of HSC division was substantially increased in the bone marrow of 19–24-month-old Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice compared with controls (Fig. 6e). HSCs appeared to be more dependent upon adiponectin receptor function than restricted haematopoietic progenitors as MPPs, HPCs, CMPs, MEPs, GMPs and differentiated haematopoietic cells did not significantly differ between Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and Adipor2fl/fl mice and controls in either 5–6-month-old (Fig. 6f,g and Extended Data Fig. 7a,b) or 19–24-month-old (Fig. 6h,i and Extended Data Fig. 7c,d) mice.

We observed no significant differences in the spleens of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice versus Adipor1fl/fl; Adipor2fl/fl controls in terms of the frequencies of HSCs, MPPs, HPCs, CMPs, MEPs or GMPs at either 5–6 months (Extended Data Fig. 7e–h) or 19–24 months (Extended Data Fig. 7i–m) of age. As in 2-month-old mice, the spleens of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice continued to have higher frequencies of myeloid and erythroid cells and a lower frequency of T cells compared with controls at 5–6 months of age (Extended Data Fig. 7i) and a higher frequency of myeloid cells at 19–24 months of age (Extended Data Fig. 7n).

To test if adiponectin receptors are required to sustain HSC function during ageing, we performed competitive transplants into irradiated recipients. Bone marrow cells from 5–6-month-old and 15-month-old (Fig. 6f) Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice gave significantly lower levels of donor cell reconstitution in all lineages compared with bone marrow cells from age-matched controls. We performed secondary transplants of bone marrow cells from primary recipients of 15-month-old bone marrow and found that while control cells gave long-term multilineage reconstitution in most (9 of 14) secondary recipients, AdipoR1/R2-deficient donor cells did not give long-term multilineage reconstitution in any (0 of 17) secondary recipients (Fig. 6l). The self-renewal potential of HSCs, therefore, declined at an accelerated rate during adulthood in the absence of adiponectin receptors in haematopoietic cells.

**Discussion**

Adiponectin receptors are thus, non-cell-autonomously required to preserve the frequency, quiescence and self-renewal potential of HSCs throughout adulthood. They act by suppressing the production of inflammatory cytokines, including TNF and IFNγ, by immune cells. In the absence of adiponectin receptors, the levels of these inflammatory cytokines increase and drive HSCs into cycle, chronically activating them, reducing their self-renewal potential and depleting HSCs during ageing. Adiponectin receptors were not required for the maintenance or function of restricted progenitors in the bone marrow, suggesting that HSCs were preferentially sensitive to this inflammatory environment. HSCs are known to be more sensitive to inflammatory cytokines than restricted haematopoietic progenitors, a mechanism that may protect HSCs from viral infection42. Our data suggest that the cost of this hypersensitivity is increased dependence upon adiponectin receptors in immune cells to protect HSCs from inflammation.

While we found that adiponectin receptors were required to maintain HSC quiescence and HSC frequency, deficiency of the ligand, adiponectin, does not affect HSC quiescence or HSC frequency in specific-pathogen-free mice43. Moreover, adiponectin modulates HSC function after myeloablation or bacterial infection by promoting the proliferation of HSCs and restricted progenitors44, rather than promoting quiescence. The differences in phenotype between adiponectin receptor and adiponectin mutant mice might reflect ligand-independent signalling by the adiponectin receptors45–48 or the influence of a different ligand49. It is also possible that increased HSC division or impaired HSC function would be observed in adiponectin-deficient mice if these mice were aged or housed in conventional colonies that are not pathogen free. Either way, our data make the broader point that inflammation is actively suppressed in the adult haematopoietic system and that this is necessary for the maintenance and quiescence of HSCs throughout adulthood.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-022-00909-9.

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Methods

Mice. All mouse experiments complied with all relevant ethical regulations and were performed according to protocols approved by the Institutional Animal Care and Use Committees at University of Texas Southwestern Medical Center (protocols 2019-1026, F18 and 102427). Mx1-Cre, Adipor1fl/fl and Adipor2fl/fl mice were obtained from Gerard Karsenty. All mice were backcrossed at least four times onto a C57BL/6j background. To induce Cre expression, 6-week-old Mx1-Cre, Adipor1fl/fl and Adipor2fl/fl mice were injected with five intraperitoneal injections of 20 μg poly(IC) (in PBS) every other day (GE Healthcare). Mx1-Cre mice were analysed at least 2 weeks after the last plpC dose. All other mice were analysed between 7 and 11 weeks of age unless otherwise indicated. Male and female mice were used in all studies. C57BL/6j, Ka-Thy-1.1, Mx1-Cre, Ka-Thy-1.2 and CD1 were used as recipients or as a source of competitor bone marrow cells in competitive transplantation experiments. To induce secondary engraftment, mice were intraperitoneally injected with 15 mg of caecal contents from wild-type mice and bone marrow cells were collected 48 h later for competitive transplantation into irradiated recipients55. Mice were housed at the Animal Resource Center at the University of Texas Southwestern Medical Center in AAALAC-accredited, specific-pathogen-free animal facilities under a 12:12 h lightdark cycle with a temperature of 18–24°C and humidity of 35–60%.

Primary cell culture. To assess the frequencies of CFUs, 1.5 × 104 whole bone marrow cells were plated per well in Methocult GM M3434 medium supplemented with 10 ng ml−1 of thrombopoietin and 1 μg penicillin-streptomycin in six-well plates (three wells per sample). Colonies were counted after 10–12 days of an in vitro culture. To assess the colony forming capacity of HSCs, individual HSCs were sorted by flow cytometry, one cell per well, into 96-well plates containing Methocult GM M3434 medium supplemented with 10 ng ml−1 of TPO and 1 μg penicillin-streptomycin. HSC colonies were counted and the colony diameter was measured after 10–12 days using an inverted microscope.

Flow cytometric analysis and sorting of haematopoietic cells. Bone marrow was flushed from one tibia and one femur using staining medium (Ca2+- and Mg2+-free Hank's balanced salt solution (HBSS) supplemented with 2% heat-inactivated bovine serum). Spleens and thymuses were mechanically dissected by crushing them between two glass slides. The cells were dissociated into a single cell suspension by gentle pipetting through a 25-gauge needle and then filtering through 70-μm nylon mesh. Cell counts were performed, and stained with antibodies to incubate cell suspensions on ice for 30 min. For analysis of HSCs, cells were stained with fluorochrome-conjugated antibodies against lineage markers (CD2 (RM2-5, Tonbo #35-0021), CD3 (17A2, Biolegend #100204), CD5 (53-73, Biolegend #100668), CD8a (53-67, Biolegend #35-0081), Gr1 (RB6-8C5, Biolegend #108406), Ter119 (TER-119, Biolegend #116206) and B220 (RA3-6B2, Biolegend #103206)), c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD45 (T12-15-12F12.2, Biolegend #115904). To sort NK cells and T cells, cells were stained with antibodies against lineage markers, c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD150 (TC15-12F12.2, Biolegend #115904). To sort LK cells, cells were stained with antibodies against lineage markers, c-kit, Sca1, CD150 and c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD150 (T12-15-12F12.2, Biolegend #115904) and c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD45 (T12-15-12F12.2, Biolegend #115904). To sort MK cells, cells were stained with antibodies against lineage markers, c-kit, Sca1, CD150 and c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD45 (T12-15-12F12.2, Biolegend #115904) and c-kit (2B8, Thermo #47-1171-82), Sc1 (D7, Thermo #45-5981-82), CD45 (T12-15-12F12.2, Biolegend #115904). For analysis of restricted progenitors, cells were stained with fluorochrome-conjugated antibodies against lineage markers, c-kit, Sca1, CD150 (CD16/20, Biolegend #123002), CD45 (53-73, Biolegend #100668), CD8a (53-67, Biolegend #35-0081), Gr1 (RB6-8C5, Biolegend #108406), Ter119 (TER-119, Biolegend #116206) and B220 (RA3-6B2, Biolegend #103206)). For analysis of differentiated cells, cells were stained with fluorochrome-conjugated antibodies against Mac-1 (M1/70, Biolegend #135312), TER119 (TER-119, Biolegend #116206) and B220 (RA3-6B2, Biolegend #103206) or LK cells were analysed using a FACSAria II or a FACSAria Fusion cytometer. Dead cells were identified and gated out of all analyses by including 1 μg ml−1 of 4′,6-diamidino-2-phenylindole (DAPI) or propidium iodide in the staining medium used to resuspend cells for flow cytometry. Flow cytometry data were analysed using FlowJo (BD Biosciences). The markers used to identify each population in this study are summarized in Supplementary Table 1 and the gates used to identify these populations by flow cytometry are shown in Extended Data Fig. 1. All antibodies were used at 1:400 dilution. For analysis of differentiated cells, cells were stained with fluorochrome-conjugated antibodies against lineage markers, c-kit, Sca1, CD150 (CD16/20, Biolegend #123002), CD45 (53-73, Biolegend #100668), CD8a (53-67, Biolegend #35-0081), Gr1 (RB6-8C5, Biolegend #108406), Ter119 (TER-119, Biolegend #116206) and B220 (RA3-6B2, Biolegend #103206). For analysis of differentiated cells, cells were stained with fluorochrome-conjugated antibodies against Mac-1 (M1/70, Biolegend #135312), TER119 (TER-119, Biolegend #116206) and B220 (RA3-6B2, Biolegend #103206).

RNA-seq library preparation and data analysis. In total, 5,000–15,000 cells were sorted into three 0.5 ml of RLT buffer (Qiagen RNAeasy Micro kit) and purified according to the manufacturer’s instructions. RNA was reverse transcribed using Scriptscript Reverse Transcription Supermix (Bio-Rad). The primers used for quantitative PCR (qPCR) analysis included Il1f1 (3′-CAA-GGA-GGA-GGA-AAG-ATG-3′ and 5′-TCC-TGG-AGC-ACC-GTG-3′), Irf5 (3′-TGC-ACC-TCC-GAA-5′ and 5′-ATG-GAC-CCA-TAC-GGC-3′), Irf7 (3′-TTC-CCT-GGC-GAT-3′ and 5′-ATC-TGG-TCG-TTC-ACC-ACT-3′) and Actb (3′-TCT-TTC-TGA-GTT-3′ and 5′-GGT-GAG-CTG-3′). Transcript levels were normalized to Actb and fold change was calculated using the ΔCt method.

Measurement of inflammatory cytokines by ELISA. Plasma was obtained from the blood after centrifugation at 900 g for 10 min. Spleens were lysed in protein lyssis buffer (100 mM Tris, 150 mM NaCl, 1 mM EDTA, 1 mM EDTA, 1% Triton X-100). Inflammatory cytokines were measured using Biolegend Legend Max (IFNγ, IL10, TNF and IL6), Biolegend ELISA Max Deluxe (IL12p70) and PBL high-sensitivity (IFNγ) mouse ELISA kits according to the manufacturer’s instructions.

RNA extraction and real-time qPCR. For RNA extraction from sorted haematopoietic cells, 5,000–15,000 cells were sorted into 300 μl of RLT buffer (Qiagen RNAeasy Micro kit) and purified according to the manufacturer’s instructions. RNA was reverse transcribed using Scriptscript Reverse Transcription Superscript (Bio-Rad). The primers used for quantitative PCR (qPCR) analysis included Il1f1 (3′-CAA-GGA-GGA-GGA-AAG-ATG-3′ and 5′-TCC-TGG-AGC-ACC-GTG-3′), Irf5 (3′-TGC-ACC-TCC-GAA-5′ and 5′-ATG-GAC-CCA-TAC-GGC-3′), Irf7 (3′-TTC-CCT-GGC-GAT-3′ and 5′-ATC-TGG-TCG-TTC-ACC-ACT-3′) and Actb (3′-TCT-TTC-TGA-GTT-3′ and 5′-GGT-GAG-CTG-3′). Transcript levels were normalized to Actb and fold change was calculated using the ΔCt method.
were analysed with workflows based on the Tuxedo protocol\textsuperscript{54}. The quality of RNA-seq raw reads was checked using FastQC 0.11. Raw reads were trimmed using TrimmGalore 0.6 and mapped to the Ensembl GRCm38 mouse reference genome with TopHat2. Mapped reads were quality filtered using SAMtools 1.9 to keep uniquely mapped reads only and quantified using Cufflinks 2. Quantified mapped reads were normalized to fragments per 1,000 exonic bases per million mapped reads (FPKMs) and gene expression levels were measured as FPKMs using Cuffnorm. Differential expression was assessed using Cuffdiff from the Cufflinks software suite. Gene set enrichment analyses were performed using GSEA4 (refs. \textsuperscript{57},\textsuperscript{58}).

Statistical methods. In each type of experiment, multiple mice were tested in multiple independent experiments performed on different days. Mice were allocated to experiments randomly and samples processed in an arbitrary order, but formal randomization techniques were not used. No formal blinding was applied when performing the experiments or analysing the data. Data samples were not pre-determined on the basis of statistical power calculations but were based on our experience with these assays. The same samples were not repeatedly measured. No data were excluded. Different replicates typically reflected samples obtained from different mice. In multiple figure panels, we either observed no differences among treatments or similar differences were observed in multiple figure panels.

Before analysing the statistical significance of differences among groups, we tested whether data were normally distributed and whether variance was similar among groups. To test for normality, we performed Shapiro–Wilk tests when $3 \leq n \leq 20$ or D’Agostino Omnibus tests when $n \geq 20$. To test whether variability significantly differed among groups, we performed F-tests (for experiments with two groups) or Levene’s median tests (for experiments with more than two groups). When the data significantly deviated from normality or variability significantly differed among groups, we log transformed the data and tested again for normality and variability. If the transformed data no longer significantly deviated from normality and equal variability, we performed parametric tests on the transformed data. If log transformation was not possible or the transformed data still significantly deviated from normality or equal variability, we performed non-parametric tests on the non-transformed data. The dagoTest and shapiroTest functions of the EBasics package were used to perform the normality tests, and the leveTest function of the car package was used to perform the Levene’s median test for variances.

When data or log-transformed data were normal and equally variable, statistical analyses were performed using Student’s $t$-tests (when there were two groups), one-way analyses of variance (ANOVA) (when there were more than two groups) or two-way ANOVAs or matched samples two-way ANOVAs (when there were two or more groups with multiple tissues or cell populations). When the data or log-transformed data were normal but unequally variable, statistical analyses were performed using Welch’s $t$-tests (when there were two groups) or Welch’s one-way ANOVAs (when there were more than two groups). When the data were not normally distributed, statistical analysis was performed using Mann–Whitney tests (when there were two groups), Kruskal–Wallis tests (when there were more than two groups or nparLD tests\textsuperscript{14} (when there were two or more groups measured at multiple timepoints). Fisher’s exact tests were used to compare the fractions of mice that were long-term multilineage reconstituted by donor cells in transplantation assays. Multinomial logistic regression analysis was used to compare HSC colony sizes. $P$ values from multiple comparisons were adjusted using Tukey’s (when there were more than two groups and all the comparisons were of interest) or Sidak’s method (when there were more than two groups and planned comparisons) after ANOVAs, Dunnett’s $T_3$ method after Welch’s one-way ANOVAs, Dunn’s method after Kruskal–Wallis tests or Bonferroni–Hochberg’s $T_3$ method after nparLD tests. Holm–Sidak’s method was used to adjust comparisons involving multiple cell populations with two groups after multiple Student’s $t$-tests or Mann–Whitney tests. All statistical tests were two-sided. All data represent mean ± standard deviation. Statistical tests were performed using GraphPad Prism V9.1.0 or R 4.0.2.

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

Data availability RNA-seq data associated with this paper are accessible in the NCBI Sequence Read Archive, BioProjects PRJNA699997 (associated with Fig. 1a–c), PRJNA723963 (associated with Fig. 5a) and PRJNA765672 (associated with Extended Data Fig. 4f). All other data supporting the findings of this study are available from the corresponding author on reasonable request. Source data are provided with this paper.

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Author contributions C.E.M. and S.J.M. conceived the project, and designed and interpreted experiments. C.E.M. performed most of the experiments. E.C.J. and R.B.J discussed experiments and helped interpret data. E.C.J. performed the stromal cell analysis. A.M.S. processed samples for flow cytometric analysis in Fig. 2. E.M.C. processed samples for flow cytometric analysis in Fig. 4. C.D.S. and M.A.A. performed ELISAs in Fig. 3 and processed samples for flow cytometric analysis in Fig. 6. A.M.S., E.M.C. and M.A.A. analysed blood samples from transplanted mice. G.M.C. performed histopathological analysis of spleen sections. Z.Z. performed bioinformatic and statistical analyses. C.E.M. and S.J.M. wrote the manuscript.

Competing interests The authors declare no competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41556-022-00999-9. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41556-022-00999-9. Correspondence and requests for materials should be addressed to Sean J. Morrison.

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Extended Data Fig. 1 | Adiponectin receptor deficient mice are born in normal numbers and are normal in size (related to Fig. 1). a-c. Vav1-Cre; Adipor1^tm^; Adipor2^tm^ mice were born at the expected mendelian frequencies (a) and were grossly normal in size (b) and appearance (c) (n = 4 female and n = 8 male Vav1-Cre; Adipor1^tm^; Adipor2^tm^ mice and n = 10 female and n = 3 male control mice in one experiment (b)). d. The number of bone marrow cells in one femur and one tibia or in the spleen of 8–10-week-old Vav1-Cre and control mice (five mice per genotype in one experiment). e. White blood cell, red blood cell, and platelet counts in the blood of 8–10-week-old Vav1-Cre and control mice (five mice per genotype in one experiment). f-i. The frequencies of HSCs (f), MPPs (g), HPCs (h), CMPs, MEPs, and GMPs (i) in bone marrow from one femur and one tibia of Vav1-Cre and control mice (five mice per genotype in one experiment). j-m. The frequencies of HSCs (j), MPPs (k), HPCs (l), CMPs, MEPs, and GMPs (m) in splenocytes from Vav1-Cre and control mice (five mice per genotype in one experiment). All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using Mann-Whitney tests followed by Holm-Sidak’s multiple comparisons adjustments (b and d-e) or Student’s t-tests followed by Holm-Sidak’s multiple comparisons adjustments (f-m). All statistical tests were two-sided. Source numerical data are available in the source data files.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Flow cytometry gating strategy for the isolation of hematopoietic stem cells, progenitor cells, and differentiated cells (related to Figs. 1–6). a–c. Representative flow cytometry gates used to identify hematopoietic stem and progenitor cells (a–b), NK cells, CD4+ T cells, and CD8+ T cells (c) in the bone marrow. The markers used to identify each of the cell populations characterized in this study are listed in Supplementary Table 1. d. Hematoxylin and Eosin stained sections from the spleens of Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl and control mice. In Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice the spleens were enlarged (Fig. 1d) and the red pulp was expanded throughout the spleen with increased numbers of myeloid, erythroid, and megakaryocytic cells. In control spleens there was limited extramedullary hematopoiesis confined to the subcapsular region. Representative images from three mice per genotype in one experiment are shown.
Extended Data Fig. 3 | Adiponectin receptors are required to sustain HSC function (related to Fig. 2). a–b. The percentages of HSCs that formed colonies (n = 6 mice per genotype in two independent experiments) (a) and colony size (n = 3 mice per genotype in one experiment) (b) from Vav1-Cre; Adipor1floflo; Adipor2floflo and control mice. c. The percentage of whole bone marrow cells that formed CFU-GEMM, CFU-GM, CFU-G, CFU-M, and BFU-E colonies (n = 3 mice per genotype in one experiment). d. Donor CD45+ cells, T, B, and myeloid cells from the blood of mice competitively transplanted with donor bone marrow cells from Mx1-Cre; Adipor1floflo; Adipor2floflo (n = 27 recipients) or control (n = 25 recipients) mice (five donors per genotype in five independent experiments). e. Secondary recipients of bone marrow cells from the mice in (d) (n = 25 recipients from six Mx1-Cre; Adipor1floflo; Adipor2floflo donors and n = 24 recipients from five control donors in five independent experiments). All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using Mann-Whitney tests (d–e), a Student’s t-test (a), a multinomial logistic regression (b), and Student’s t-tests followed by Holm-Sidak’s multiple comparisons adjustments (c), or Mann-Whitney tests (d–e). All statistical tests were two-sided. Source numerical data are available in the source data files.
Extended Data Fig. 4 | Adiponectin receptors suppress the production of inflammatory factors (related to Fig. 3). a. ELISA analysis of inflammatory cytokines in spleen lysates from Vav1-Cre; Adipor1<sup>fl/fl</sup>; Adipor2<sup>fl/fl</sup> and control mice (spleen lysates from 16 (IFNγ, TNF, IL6, and IL1β) or 13 (IFNα and IFNβ) Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> mice and 19 (IFNγ, TNF, IL6, and IL1β) or 14 (IFNα and IFNβ) control mice in two independent ELISAs per cytokine). b. qRT-PCR analysis of Tnf transcript levels in sorted bone marrow cells. Data are normalized to transcript levels in wildtype whole bone marrow (WBM) cells (cells sorted from six (WBM), three (HSC, HPC, CMP, MEP, GMP, T-cells, B-cells, and erythroid progenitors), or five (myeloid cells) Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> mice and from seven (WBM), three (HSC, HPC, CMP, MEP, GMP, and erythroid progenitors), or four (T-cells, B-cells, and myeloid cells) control mice in one experiment). c. qRT-PCR analysis of Tnf transcripts in sorted CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, or NK cells from the bone marrow (cells sorted from three mice per genotype in one experiment). d. qRT-PCR analysis of Tnf transcripts in sorted macrophages, inflammatory monocytes, and neutrophils from the bone marrow. Data are normalized to transcript levels in wildtype WBM (cells sorted from seven mice per genotype in two independent experiments). e. The frequency of LepR<sup>+</sup> stromal cells in bone marrow from 8-10 week old Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> and control mice (n = 7 mice per genotype in two independent experiments). f. RNAseq analysis of transcripts for Ifng and Tnf in Lep<sup>+</sup> cells from 8-10 week old Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> and control mice (n = 3 mice per genotype one experiment). g. The frequencies of donor and competitor HSCs in the spleens of recipient mice co-transplanted with Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> or control donor cells and wildtype competitor cells 16 weeks after transplantation (n = 19 recipients from Vav1-Cre; Adipor<sup>fl/fl</sup>; Adipor<sup>fl/fl</sup> donors and n = 20 recipients from control donors in four independent experiments). All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using Student’s t-tests followed by Holm-Sidak’s multiple comparisons adjustments (a-b and d), a matched samples two-way ANOVA followed by Sidak’s multiple comparisons adjustment (c), Student’s t-tests (e), or Mann-Whitney tests followed by Holm-Sidak’s multiple comparisons adjustments (a and g). All statistical tests were two-sided. Source numerical data are available in the source data files.
Extended Data Fig. 5 | Adiponectin receptors promote HSC function by reducing IFNγ receptor signaling (related to Fig. 4). a. Bone marrow (one femur and one tibia) and spleen cellularity in Vav1-Cre; Ifngr1<sup>fl/fl</sup> and control mice (n = 7 Vav1-Cre; Ifngr<sup>fl/fl</sup> mice and n = 6 control mice in three independent experiments). b–d. The frequencies of HSCs, MPPs, HPCs (b), CMPs, MEPs, GMPs (c), and differentiated T, B, myeloid, and erythroid cells (d) in the bone marrow of Vav1-Cre; Ifngr1<sup>fl/fl</sup> and control mice (n = 7 Vav1-Cre; Ifngr<sup>fl/fl</sup> mice and n = 6 control mice in three independent experiments). e–g. The frequencies of HSCs, MPPs, HPCs (e), CMPs, MEPs, GMPs (f), and differentiated cells (g) in the spleens of Vav1-Cre; Ifngr1<sup>fl/fl</sup> and control mice (n = 7 Vav1-Cre; Ifngr<sup>fl/fl</sup> mice and n = 6 control mice in three independent experiments). h. The percentage of HSCs that incorporated a 72 hour pulse of BrdU (n = 3 Vav1-Cre; Ifngr1<sup>fl/fl</sup> mice and n = 4 control mice one experiment). i–o. The frequencies of HSCs (i), MPPs (j), HPCs (k), CMPs (l), MEPs (m), GMPs (n) and differentiated cells (o) in the bone marrow of control, Vav1-Cre; Adipor<sup>1<sup>/</sup>fl/fl</sup>; Adipor<sup>2<sup>/</sup>fl/fl</sup> and Vav1-Cre; Adipor<sup>1<sup>/</sup>fl/fl</sup>; Adipor<sup>2<sup>/</sup>fl/fl</sup>; Ifngr<sup>fl/fl</sup> mice (n = 15 Vav1-Cre; Adipor<sup>1<sup>/</sup>fl/fl</sup>; Adipor<sup>2<sup>/</sup>fl/fl</sup> mice, n = 13 Vav1-Cre; Adipor<sup>1<sup>/</sup>fl/fl</sup>; Adipor<sup>2<sup>/</sup>fl/fl</sup>; Ifngr<sup>fl/fl</sup> mice and n = 21 control mice in five independent experiments). All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using a matched samples two-way ANOVA followed by Sidak’s multiple comparisons adjustment (a), Mann-Whitney tests followed by Holm-Sidak’s multiple comparisons adjustments (b–g), a Student’s t-test (h), or one-way ANOVAs followed by Tukey’s multiple comparisons adjustments (i–o). All statistical tests were two-sided. Source numerical data are available in the source data files.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Adiponectin receptors promote HSC function by reducing TNF levels (related to Fig. 5). a. Bone marrow (one femur and one tibia) and spleen cellularity in Tnf deficient and control mice (n = 5 Tnf−/− mice and n = 7 control mice in four independent experiments). b-d. The frequencies of HSCs, MPPs, HPCs (b), CMPs, MEPs, GMPs (c) and differentiated T, B, myeloid, and erythroid cells (d), in the bone marrow of Tnf deficient and control mice (n = 5 Tnf−/− mice and n = 7 control mice in four independent experiments). e-g. The frequencies of HSCs, MPPs, HPCs (e), CMPs, MEPs, GMPs (f) and differentiated cells (g), in the spleens of Tnf deficient and control mice (n = 5 (e and g) or n = 4 (f) Tnf−/− mice and n = 7 control mice in four independent experiments). h. The percentage of HSCs that incorporated a 72 hour pulse of BrdU (n = 4 Tnf−/− mice and n = 9 control mice in two independent experiments) i-o. The frequencies of HSCs (i), MPPs (j), HPCs (k), CMPs (l), MEPs (m), GMPs (n) and differentiated cells (o) in the bone marrow of control, Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl, and Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Tnf−/− mice (n = 12 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Tnf−/− mice, n = 11 Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl; Tnf−/− mice and n = 12 control mice in five independent experiments). p-q. ELISA of TNF (p) or INFγ (q) in blood plasma from Vav1-Cre; Adipor1fl/fl; Adipor2fl/fl mice and n = 12 control mice in one ELISA. All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using a matched samples two-way ANOVA followed by Sidak’s multiple comparisons adjustment (a), Student’s t-tests (b-h) followed by Holm-Sidak’s multiple comparisons adjustments (b-g), one-way ANOVAs followed by Tukey’s multiple comparisons adjustments (i-o and q), a Welch’s one-way ANOVA followed by Dunnett’s T3 multiple comparisons adjustment (o), or a Kruskal-Wallis test followed by Dunn’s multiple comparisons adjustment (a and p). All statistical tests were two-sided. Source numerical data are available in the source data files.
Extended Data Fig. 7 | Adiponectin receptor function reduces the frequencies of splenic myeloid cells in 5–6 and 19–24 month old mice (related to Fig. 6). a–d. The frequencies of CMPs, MEPs, GMPs (a, c), differentiated T, B, myeloid, and erythroid cells (b, d) in the bone marrow from 5–6 month old (a–b) or 19–24 month old (c–d) Vav1-Cre; Adipor1ΔΔ; Adipor2ΔΔ or control mice (n = 7 (a–b) or n = 5 (c–d) Vav1-Cre; Adipor1ΔΔ; Adipor2ΔΔ mice and n = 10 (a–b) or n = 6 (c–d) control mice in two independent experiments per age group). e–n. The frequencies of HSCs (e, j), MPPs (f, k), HPCs (g, l), CMPs, MEPs, GMPs (h, m), and differentiated T, B, myeloid, and erythroid cells (i, n) in the spleens of 5–6 month-old (e–i) and 19–24 month-old (j–n) Vav1-Cre; Adipor1ΔΔ; Adipor2ΔΔ or control mice (n = 7 (e–i) or n = 5 (j–n) Vav1-Cre; Adipor1ΔΔ; Adipor2ΔΔ mice and n = 10 (e–i) or n = 6 (j–n) control mice in two independent experiments per age group). All data represent mean ± standard deviation and each dot reflects a different mouse. Statistical significance was assessed using Mann-Whitney tests followed by Holm-Sidak’s multiple comparisons adjustments (a–n). All statistical tests were two-sided. Source numerical data are available in the source data files.
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☐ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection Flow cytometry data were collected using BD FACSDiva 9.0

Data analysis Graphpad Prism 9, R 4.0.2, Ibasics 3042.89.1, car 3.0, and nparLD 2.1 packages were used for statistical analysis. FlowJo v10 (BD) was used to analyze flow cytometry data. RNAseq data were analyzed with workflows based on the Tuxedo protocol. The quality of RNA-seq raw reads was checked using FastQC 0.11. Raw reads were trimmed using TrimGalore 0.6 and mapped to the Ensembl GRCm38 mouse reference genome using TopHat2 with Bowtie2. Mapped reads were quality-filtered using SAMtools 1.9 to keep uniquely mapped reads only and quantified using Cufflinks 2. Quantified mapped reads were normalized to fragments per 1000 exonic bases per million mapped reads (FPKM) and gene expression levels were measured as FPKMs using Cuffnorm. Differential expression was assessed using Cuffdiff from the Cufflinks software suite. Gene set enrichment analyses were performed using GSEA4.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source data files are provided with this paper. RNA sequencing data associated with this paper are accessible in the NCBI Sequence Read Archive (SRA), BioProjects PRJNA699097 (associated with Figure 1 a-c), PRJNA729963 (associated with Figure 3a), and PRJNA765672 (associated with Extended Data Figure 4f).
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**  
Samples sizes were not pre-determined based on statistical power calculations but were based on our experience with these assays. For assays that in our experience tend to have low variability (like assays of HSC and progenitor frequencies by flow cytometry) we tended to use n<10 and for assays that tend to have high variability (like reconstitution assays) we used n>10 (e.g. Nature 591: 438-44 2021, Nature 549: 476-81, 2017, and Nature 527: 466-71 2015).

**Data exclusions**  
No data were excluded

**Replication**  
The experimental findings were reproduced in multiple independent experiments. The number of independent experiments and biological replicates for each data panel are indicated in the figure or figure legend. Data shown in the figures represent the aggregate of all independent experiments in the majority of cases. Data shown in a minority of panels are from a representative experiment (e.g. for H&E staining) and in those cases this was noted and the number of experiments that confirmed the representative experiment is also indicated in the figure legend.

**Randomization**  
No formal randomization techniques were used; however samples were allocated randomly to experiments and processed in an arbitrary order.

**Blinding**  
Formal blinding techniques were not used; however, in most experiments that involved the analysis of cell populations by flow cytometry, the samples were processed and analyzed in an arbitrary order without knowledge of mouse genotypes. The genotypes of samples was only revealed when the measurements were entered into a spreadsheet to analyze whether there were differences among treatments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

|Materials & experimental systems|Methods|
|---|---|
|n/a|n/a|
|☒ |☒ |
|Involved in the study|Involved in the study|
|Antibodies|ChiP-seq|
|Eukaryotic cell lines|Flow cytometry|
|Palaeontology and archaeology|MRI-based neuroimaging|
|Animals and other organisms| |
|Human research participants| |
|Clinical data| |
|Dual use research of concern| |

**Antibodies**

The following antibodies were used in this study

- α-mouse CD2 FITC, Tonbo Biosciences, Clone RM2-5, Cat. #50-0021, Lot: C0021121118354, 1:400, Flow cytometry
- α-mouse CD2 PE, Tonbo Biosciences, Clone RM2-5, Cat. #50-0021, Lot: C0021022119503, 1:400, Flow cytometry
- α-mouse CD3 FITC, Biolegend, Clone 17A2, Cat. #100204, Lot: B291431, 1:400, Flow cytometry
- α-mouse CD3 PE, Biolegend, Clone 17A2, Cat #100206, Lot: B292677, 1:400, Flow cytometry
- α-mouse CD4 PE-Cy7, BioLegend, Clone GK1.5, Cat #100422, Lot: B288470, 1:400, Flow cytometry
- α-mouse CD5 FITC, Biolegend, Clone 53-7.3, Cat #100606, Lot: B295397, 1:400, Flow cytometry
- α-mouse CD5 PE, Biolegend, Clone 53-7.3, Cat #100608, Lot: B283693, 1:400, Flow cytometry
α-mouse CD8a FITC, Thermo Fisher Scientific, Clone S3-6.7, Cat #35-0081, Lot: C008101249354, 1:400, Flow cytometry
α-mouse CD8a PE, Thermo Fisher Scientific, Clone S3-6.7, Cat #50-0081, Lot: C0081012220504, 1:400, Flow cytometry
α-mouse Ter119 FITC, Biolegend, Clone TER-119, Cat #116206, Lot: B272256, 1:400, Flow cytometry
α-mouse Ter119 PE, Biolegend, Clone TER-119, Cat #116208, Lot: B276503, 1:400, Flow cytometry
α-mouse Ter119 APC, Thermo Fisher Scientific, Clone TER-119, Cat #20-5921, Lot: C5921040320203, 1:400, Flow cytometry
α-mouse Gr1 FITC, Biolegend, Clone RB6-8C5, Cat #108406, Lot: 108406, 1:400, Flow cytometry
α-mouse Gr1 PE, Biolegend, Clone R66-8C5, Cat #108408, Lot: B275223, 1:400, Flow cytometry
α-mouse Gr1 PE-Cy7, Thermo Fisher Scientific, Clone RB6-8C5, Cat #60-5931, Lot: C593102919603, 1:400, Flow cytometry
α-human/mouse B220 FITC, Biolegend, Clone RA3-6B2, Cat #103206, Lot: B277574, 1:400, Flow cytometry
α-human/mouse B220 PE, Thermo Fisher Scientific, Clone RA3-6B2, Cat #50-0452, Lot: C0452082418503, 1:400, Flow cytometry
α-human/mouse B220 APC, Thermo Fisher Scientific, Clone RA3-6B2, Cat #20-0452, Lot: C0452100418203, 1:400, Flow cytometry
α-human/mouse B220 PerCp-Cy5.5, Thermo Fisher Scientific, Clone RA3-6B2, Cat #65-0452, Lot: C045206619653, 1:400, Flow cytometry
α-mouse Mac-1 (CD11b) APC-eFluor780, Thermo Fisher Scientific, Clone M1/70, Cat #47-0112-82, Lot: 2011193, 1:400, Flow cytometry
α-mouse CD71 FITC, Thermo Fisher Scientific, Clone R17217, Cat #11-0711-82, Lot: 2213055, 1:400, Flow cytometry
α-mouse NK1.1 APC, Thermo Fisher Scientific, Clone PK136, Cat #20-5941, Lot: C5941122118203, 1:400, Flow cytometry
α-mouse CD45.1 FITC, Biolegend, Clone A20, Cat #110706, Lot: B249479, 1:400, Flow cytometry
α-mouse CD45.2 violetFluor 450, Thermo Fisher Scientific, Clone 104, Cat #75-0454, Lot: C0454091819763, 1:400, Flow cytometry
α-mouse CD45 APC, Thermo Fisher Scientific, Clone 30-F11, Cat #20-0451, Lot: C0451070520203, 1:200, Flow cytometry
α-mouse CD150 PE, Biolegend, Clone TC15-12F12.2, Cat #115904, Lot: B270365, 1:400, Flow cytometry
α-mouse CD150 APC, Biolegend, Clone TC15-12F12.2, Cat# 115910, Lot: B283740, 1:400, Flow cytometry
α-mouse CD48 APC, Thermo Fisher Scientific, Clone HM48-1, Cat# 17-0481-82, Lot: 2040759, 1:400, Flow cytometry
α-mouse CD48 Alexa Fluor 700, Biolegend, Clone HM48-1, Cat# 103426, Lot: B279067, 1:400, Flow cytometry
α-mouse Sca1 PerCp-Cy5.5, Thermo Fisher Scientific, Clone D7, Cat# 45-5981-82, Lot: 2162718, 1:400, Flow cytometry
α-mouse Sca1 PE-Cy7, Biolegend, Clone E13-161.7, Cat# 122514, Lot: B280435, 1:400, Flow cytometry
α-mouse c-kit APC-eFluor780, Thermo Fisher Scientific, Clone 288, Cat# 47-1171-82, Lot: 2261910, 1:400, Flow cytometry
α-mouse CD16/32 (FcγRIII/I) Alexa Fluor 700, Thermo Fisher Scientific, Clone 93, Cat# 56-0161-82, Lot: 2116877, 1:400, Flow cytometry
α-mouse CD34 FITC, Thermo Fisher Scientific, Clone RAM34, Cat# 11-0341-82, Lot: 2112197, 1:400, Flow cytometry
α-mouse CD135 PE-Cy5, Biolegend, Clone A2F10, Cat# 135312, Lot: B288264, 1:400, Flow cytometry
α-mouse Ly6g APC, Thermo Fisher Scientific, Clone 1A8, Cat# 20-1276-U100, Lot: C127601239203, 1:400, Flow cytometry
α-mouse Ly6c Alexa Fluor 700, Biolegend, Clone HK1.4, Cat# 128024, Lot: B318988, 1:400, Flow cytometry
α-mouse CD11S PerCp-Cy5.5, Biolegend, Clone AF598, Cat# 135526, Lot: B291992, 1:400, Flow cytometry
α-mouse F4/80 PE, Biolegend, Clone BM8, Cat# 123110, Lot: B223150, 1:400, Flow cytometry
α-mouse LepR, Thermo Fisher Scientific, Clone BAF497, Cat# BAF497, Lot: BTV021071, 1:100, Flow cytometry
α-mouse CD144 e660, eBioscience, Clone BV13, Cat# 50-1441-82, Lot: 2289519, 1:11, Flow cytometry

Validation

All antibodies are commercially available and have been validated in previously published studies (e.g. Cell Stem Cell 13: 102-116 [2013]).

α-mouse CD2. This monoclonal antibody recognizes CD2. https://tonbobo.com/products/fltc-anti-mouse-cd2-rm2-5
α-mouse CD3. This monoclonal antibody recognizes CD3.  https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd3-antibody-45

α-mouse CD4. This monoclonal antibody recognizes CD4.  https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd4-antibody-1919

α-mouse CD5. This monoclonal antibody recognizes CD5.  https://www.biolegend.com/en-us/search-results/fitc-anti-mouse-cd5-antibody-159

α-mouse CD8a. This monoclonal antibody recognizes CD8.  https://tonbobio.com/products/fitc-anti-mouse-cd8a-s3-6-7.

α-mouse Ter119. This monoclonal antibody recognizes Ter119.  https://www.biolegend.com/en-us/products/fitc-anti-mouse-ter-119-erythroid-cells-antibody-1865

α-mouse Gr1. This monoclonal antibody recognizes Gr1.  https://www.biolegend.com/en-us/products/fitc-anti-mouse-ly-6g-ly-6c-gr-1-antibody-458

α-human/mouse B220. This monoclonal antibody recognizes B220.  https://www.biolegend.com/en-us/products/fitc-anti-mouse-human-cd45r-b220-antibody-445

α-mouse Mac-1 (CD11b). This monoclonal antibody recognizes Mac-1.  https://www.thermofisher.com/antibody/product/CD11b-Antibody-clone-M1-70-Monoclonal/47-0112-82

α-mouse CD71. This monoclonal antibody recognizes CD71.  https://www.thermofisher.com/antibody/product/CD71-Transferrin-Receptor-Antibody-clone-R17217-R17-217-1-4-Monoclonal/11-0711-82

α-mouse NK1.1. This monoclonal antibody recognizes NK1.1.  https://tonbobio.com/products/apc-anti-mouse-nk1-1-cd161-pk136.

α-mouse CD45.1. This monoclonal antibody recognizes CD45.1  https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-1-antibody-198.

α-mouse CD45.2. This monoclonal antibody recognizes CD45.2.  https://tonbobio.com/products/violetfluor-450-anti-mouse-cd45-2-104

α-mouse CD45 APC. This monoclonal antibody recognizes CD45.  https://tonbobio.com/products/apc-anti-mouse-cd45-30-f11

α-mouse CD150. This monoclonal antibody recognizes CD150.  https://www.biolegend.com/en-us/products/pe-anti-mouse-cd150-slam-antibody-13697Group0-BL10572

α-mouse CD48. This monoclonal antibody recognizes CD48.  https://www.thermofisher.com/antibody/product/CD48-Antibody-clone-HM48-1-Monoclonal/17-0481-82

α-mouse Sca1. This monoclonal antibody recognize Sca1.  https://www.thermofisher.com/antibody/product/Ly-6A-E-Sca-1-Antibody-clone-07-Monoclonal/45.5981.82

α-mouse c-kit. This monoclonal antibody recognizes c-kit.  https://www.thermofisher.com/antibody/product/CD117-c-Kit-Antibody-clone-298-Monoclonal/47-1171-82.

α-mouse CD16/32. This monoclonal antibody recognizes CD16/32.  https://www.thermofisher.com/antibody/product/CD16-CD32-Antibody-clone-93-Monoclonal/56-0161-82

α-mouse CD34. This monoclonal antibody recognizes CD34.  https://www.thermofisher.com/antibody/product/CD34-Antibody-clone-RAM34-Monoclonal/11-0341-82

α-mouse CD135. This monoclonal antibody recognizes CD135.  https://www.biolegend.com/en-gb/products/pe-cyanine5-anti-mouse-cd135-antibody-630079851

α-mouse Ly6g. This monoclonal antibody recognizes Ly6g.  https://tonbobio.com/products/apc-anti-mouse-ly-6g-1a8

α-mouse Ly6c. This monoclonal antibody recognizes Ly6c.  https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-ly-6c-antibody-6757

α-mouse CD115. This antibody recognizes CD115.  https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd115-csf-1r-antibody-12377

α-mouse F4/80. This monoclonal antibody recognizes F4/80.  https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-antibody-4068

α-mouse LepR. This monoclonal antibody recognizes LepR.  https://fishersci.com/shop/products/anti-mouse-leptin-r-bisintypolyclonal-r-d-systems/BAF497#?keyword=

α-mouse CD144. This monoclonal antibody recognizes CD144.  https://www.thermofisher.com/antibody/product/CD144-VE-cadherin-Antibody-clone-eBio8V13-BV13-Monoclonal/50-1441-82
**Animals and other organisms**

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| **Laboratory animals** | Two-month to 24-month-old C57BL/Ka mice were used. Both male and female mice were used. |
|------------------------|-----------------------------------------------------------------------------------------------|
| **Wild animals**       | No wild animals were used.                                                                      |
| **Field-collected samples** | No field collected samples were used.                                                            |
| **Ethics oversight**   | All mouse experiments complied with all relevant ethical regulations and were performed according to protocols approved by the Institutional Animal Care and Use Committees at University of Texas Southwestern Medical Center (protocol 2019-102632-G). |

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

**Flow Cytometry**

**Plots**

- The axis labels state the marker and fluorochrome used (e.g., CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

**Methodology**

**Sample preparation**

Bone marrow was flushed from one tibia and one femur using staining medium (Ca²⁺ and Mg²⁺-free HBSS supplemented with 2% heat inactivated bovine serum). Spleens and thymuses were mechanically dissociated by crushing them between two glass slides. The cells were dissociated into a single cell suspension by gently passing them through a 25-gauge needle and then filtering through 70-µm nylon mesh.

**Instrument**

Samples were analyzed on a FACS Canto RUO (BD Biosciences), FACS Aria II (BD Biosciences), or a FACS Aria Fusion (BD Biosciences) cytometer. Samples were sorted using FACS Aria II (BD Biosciences), or a FACS Aria Fusion (BD Biosciences) cytometer.

**Software**

BD FACSDiva 8.0, FlowJo v10

**Cell population abundance**

Cells were sorted in yield mode and then re-sorted in purity mode in every experiment. The abundance of the relevant cell populations within post-sort fractions was almost 100% in pilot experiments. The abundance could not be tested after every experiment because cells were usually sorted directly into lysis buffer.

**Gating strategy**

The markers used to identify hematopoietic stem and progenitor cells in this study are summarized in Extended Data Table 1 and the gates used to identify these populations by flow cytometry are shown in Extended Data Figure 2. To eliminate dead cells from sorts and analyses, 1 µg/ml, 4’,6-diamidino-2-phenylindole (DAPI) or propidium iodide was included in staining medium used to resuspend cells for flow cytometry.

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