Sleep is integral to life. Although insufficient or disrupted sleep increases the risk of multiple pathological conditions, including cardiovascular disease, we know little about the cellular and molecular mechanisms by which sleep maintains cardiovascular health. Here we report that sleep regulates haematopoiesis and protects against atherosclerosis in mice. We show that mice subjected to sleep fragmentation produce more Ly-6C<sup>hi</sup> monocytes, develop larger atherosclerotic lesions and produce less hypocretin—a stimulatory and wake-promoting neuropeptide—in the lateral hypothalamus. Hypocretin controls myelopoiesis by restricting the production of CSF1 by hypocretin-receptor-expressing pre-neutrophils in the bone marrow. Whereas hypocretin-null and haematopoietic hypocretin-receptor-null mice develop mononcytosis and accelerated atherosclerosis, sleep-fragmented mice with either haematopoietic CSF1 deficiency or hypocretin supplementation have reduced numbers of circulating monocytes and smaller atherosclerotic lesions. Together, these results identify a neuro-immune axis that links sleep to haematopoiesis and atherosclerosis.

Poor or insufficient sleep is an increasingly important public health issue, as nearly half of adults in the United States sleep fewer than the recommended seven to eight hours per day. Lack of sleep increases risk of obesity, diabetes, cancer and cardiovascular disease, but we know little about the underlying mechanisms that link sleep to disease.

To investigate how sleep might protect against cardiovascular disease, we subjected atherosclerosis-prone Apo<sup>e−/−</sup> mice to chronic sleep fragmentation (SF) (Extended Data Fig. 1a and Supplementary Video 1). We found no changes in body weight, plasma cholesterol or glucose tolerance (Extended Data Fig. 1b–e); however, mice developed progressively larger atherosclerotic lesions compared to controls (Fig. 1a and Extended Data Fig. 1f–h). Not only did lesion volume increase in SF mice (Fig. 1b), but aortas from SF mice contained more Ly-6C<sup>hi</sup> monocytes, neutrophils and macrophages (Fig. 1c); a change that did not result from increased proliferation of macrophages (Extended Data Fig. 1i).

Leukocytosis is predictive of cardiovascular disease. Although myeloid cell numbers in the blood of mice fluctuated according to a circadian pattern with a peak at zeitgeber time (ZT)5 and a nadir at ZT14 (Fig. 1d), mice subjected to sleep fragmentation had significantly more circulating Ly-6C<sup>hi</sup> monocytes and neutrophils during the light period. Rhythmlicity analysis revealed that the circadian amplitude was increased (0.7 ± 0.16 × 10<sup>6</sup> compared to 1.5 ± 0.15 × 10<sup>6</sup>) in control and SF mice, respectively. P = 0.02, for Ly-6C<sup>hi</sup> monocytes and 3.6 ± 0.17 × 10<sup>5</sup> compared to 5.1 ± 0.11 × 10<sup>5</sup> in control and SF mice, respectively. P = 0.13, for neutrophils, but the period and phase were unaltered. Sleep fragmentation did not change lymphocyte numbers (Extended Data Fig. 1j–l). To understand the influence of sleep on circadian leukocyte migration to tissues, we profiled leukocytes in various organs at ZT3 and ZT14. Both control and SF mice had elevated Ly-6C<sup>hi</sup> monocyte and neutrophil levels in various tissues during the dark period, and these increases were higher in SF mice (Extended Data Fig. 2). These observations align with human studies that have linked sleep curtailment or interruption with leukocyte numbers.

Next, we focused on haematopoiesis. In Apo<sup>e−/−</sup> mice, sleep fragmentation increased proliferation of lineage (Lin)–Kit<sup>−/−</sup> (LSK) haematopoietic progenitors in the bone marrow, and this increase corresponded to an approximately twofold-higher number of bone marrow LSK cells (Fig. 1e) and other progenitor subsets (Extended Data Fig. 3a). The spleens of SF mice contained more LSK cells and granulocyte–macrophage progenitors, which indicates heightened extramedullary haematopoiesis (Extended Data Fig. 3b). Sleep fragmentation promoted myelopoiesis not only in Apo<sup>e−/−</sup> mice that were fed a high-fat diet, but also in C57BL/6 mice fed a Chow diet (Extended Data Fig. 3c). Together, these data show that sleep fragmentation boosts myeloid-biased haematopoiesis.

Mice subjected to sleep fragmentation had normal bone structure (Extended Data Fig. 4a, b) and leukocytosis persisted even after prolonged treatment with antibiotics (Extended Data Fig. 4c), suggesting that enhanced myelopoiesis was not driven by either physical alterations to the bone or the microbiome, respectively. Because stress activates the sympathetic nervous system, which can heighten haematopoiesis, we wondered whether sleep-fragmentation-induced myelopoiesis similarly depended on activation of the sympathetic nervous system, but found no evidence for such a mechanism (Extended Data Fig. 5a–d). Nevertheless, SF mice were more anxious (Extended Data Fig. 5e–g), which demonstrates that mice do not easily habituate to sleep fragmentation.

We then focused on the hypothalamus and, specifically, on expression of transcripts that encode sleep-regulating proteins (Extended Data Fig. 5h–j). Sleep fragmentation decreased expression of hypocretin (Hcrt, also known as orexin) in the hypothalamus (Fig. 1f–h), correlating with reduced levels of the isoform hypocretin-1 in plasma and bone marrow (Fig. 1i). Hypocretin deficient hypothalamic hypocretin reduced gradually and correlated inversely with leukocytosis (Extended Data Fig. 6a). We did not see alterations in dynorphin, a co-transmitter of hypocretin<sup>15</sup> (Extended Data Fig. 6b–e) or any evidence for death of hypocretin-producing neurons (Extended Data Fig. 6f), which suggests that specific repression of hypocretin occurred, potentially mediated by neuropeptide Y<sup>16</sup> (Extended Data Fig. 5h). Furthermore, sleep recovery following sleep fragmentation restored hypothalamic hypocretin content and normalized myelopoiesis (Extended Data Fig. 6g–i). These data intrigued us, because hypocretin mediates metabolism, sleep and appetite<sup>17</sup> and autoimmune destruction of hypocretin neurons causes

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narcolepsy and a pro-inflammatory immune signature. In keeping with the function of hypocretin in the promotion of appetite, SF mice consumed less food than controls (Extended Data Fig. 5k).

In humans, reduced plasma hypocretin is associated with risk of myocardial infarction, heart failure and obesity. Similarly, studies suggest that patients with narcolepsy have heightened risk of heart disease. In mice, deleting hypocretin severely fragments sleep-wake cycles, causes cataplexy and promotes diet-induced obesity. Moreover, deleting the hypocretin receptor-2 worsens healing after myocardial infarction. We therefore profiled leukocytes in hypocretin-null (Hcrtr−/−) mice and found a higher number of Ly-6Chigh monocytes and neutrophils in the blood, spleen and bone marrow relative to wild-type controls (Fig. 2a, b and Extended Data Fig. 7). Rhythmicity analysis of Hcrtr−/− mouse blood revealed that Ly-6Chigh monocytes and neutrophils had elevated circadian amplitudes (1.8 ± 0.32 × 10^5 compared to 3.7 ± 0.55 × 10^4 in wild-type and Hcrtr−/− mice, respectively, P = 0.02, for Ly-6Chigh monocytes and 4.1 ± 0.69 × 10^5 compared to 8.1 ± 0.11 × 10^5, in wild-type and Hcrtr−/− mice, respectively, P = 0.03, for neutrophils), but that the period and phase were unchanged. Bone marrow from Hcrtr−/− mice had more haematopoietic progenitors along with increased proliferation of LSK cells (Fig. 2c and Extended Data Fig. 7). As in the SF mice, accelerated haematopoiesis in hypocretin-deficient mice did not appear to depend on the microbiome (Extended Data Fig. 4d). These results suggest that sleep regulates haematopoiesis through hypocretin.

Next, we tested whether hypocretin can affect haematopoiesis and atherosclerosis. We found that the hypothalamus produced nearly all of the hypocretin (Extended Data Fig. 8a, b). Sixteen weeks of sleep fragmentation did not alter hypocretin production in the bone and bone marrow (Extended Data Fig. 8c), which indicates that the hypothalamus was the relevant source affected by sleep. Indeed, we found substantial concentrations of the neuropeptide in the cerebrospinal fluid, plasma and bone marrow fluid of wild-type but not Hcrtr−/− mice, and we detected high levels of HCRT-I in the plasma and bone marrow fluid of Hcrtr−/− mice after injecting HCRT-I into the cerebrospinal fluid of the cisterna magna (Extended Data Fig. 8d, e). Consequently, we generated chimeric mice lacking hypocretin production in either the non-haematopoietic compartment (including the hypothalamus) or haematopoietic cells (Fig. 2d), and found heightened haematopoiesis in Hcrtr−/− mice containing wild-type bone marrow cells (Fig. 2e). We also placed Hcrtr−/− mice in parabiosis with wild-type mice (Fig. 2f), noting that wild-type-partnered Hcrtr−/− mice had suppressed haematopoiesis compared to Hcrtr−/−-partnered Hcrtr−/− mice (Fig. 2g). These data suggest that hypothalamus-produced hypocretin can enter the circulation to affect haematopoiesis in the distal bone marrow. To determine whether hypocretin deficiency aggravates atherosclerosis, we generated Hcrtr−/− Apoe−/− mice, which had larger lesions with more aortic leukocytes than did Apoe−/− controls (Fig. 2h, i).

Having identified a link between hypocretin, haematopoiesis and atherosclerosis, we examined the underlying mechanism.
Although hypocretin receptor-1 (Hcrtr1) and hypocretin receptor-2 (Hcrtr2) expression was the highest in the hypothalamus, we detected Hcrtr1 in multiple tissues, including the bone marrow (Extended Data Fig. 8f, g). However, adding hypocretin to granulocyte–macrophage colony-forming cultures had no effect on haematopoiesis in vitro (Extended Data Fig. 8h). We therefore sorted 14 different cell types from the bone marrow and found that neutrophils—but not other cells—expressed Hcrtr1 mRNA (Fig. 3a) and HCRT1 protein (Extended Data Fig. 8i). We next sorted neutrophils by maturation stage

### Figures

**Fig. 2 | Hypocretin suppresses haematopoiesis and atherosclerosis.**

**Assessment of haematopoiesis and atherosclerosis in Hcrtr1−/− mice.**

a, Quantification of Ly-6Chigh monocytes (a) and neutrophils (b) in the blood of Hcrtr1−/− and wild-type mice over 24 h (n = 3 mice per group).

**P** < 0.01, ***P** < 0.001, two-way ANOVA.

c, Quantification of bone marrow LSK cells and BrdU incorporation in wild-type and Hcrtr1−/− mice (for LSK cells per leg; n = 7 wild-type mice and n = 10 Hcrtr1−/− mice; for proliferation assays, n = 4 mice per group).

**D** Schematic of chimeric models. e, Assessment of blood Ly-6Chigh monocytes and neutrophils, bone marrow LSK cells and BrdU incorporation in chimeric mice (n = 4 WT;bmWT mice; n = 3 Hcrtr1−/−;bmHcrtr1−/− mice; n = 4 WT;bmHcrtr1−/− mice; n = 5 Hcrtr1−/−;bmWT mice). **P** < 0.05, **P** < 0.01, ***P** < 0.001, one-way ANOVA.

**f** Schematic of parabiosis models. g, Quantification of LSK cells and BrdU incorporation in the bone marrow of parabiosis mice (n = 4 per group). **P** < 0.05, **P** < 0.01, one-way ANOVA.

h, Images of cross-sections of aortic roots stained with oil red O and quantification of atherosclerosis in Apoe−/− mice and Hcrtr1−/− Apoe−/− mice fed a high-fat diet for 16 weeks (n = 7 Apoe−/− mice; n = 5 Hcrtr1−/− Apoe−/− mice). i, Aortic myeloid cells in Apoe−/− and Hcrtr1−/− Apoe−/− mice (for Ly-6Chigh monocytes, n = 10 mice per group; for neutrophils, n = 11 Apoe−/− mice and n = 9 Hcrtr1−/− Apoe−/− mice; for macrophages, n = 11 Apoe−/− mice and n = 10 Hcrtr1−/− Apoe−/− mice). Data are mean ± s.e.m., **P** < 0.05, **P** < 0.01, ***P** < 0.001, two-tailed Mann–Whitney U-tests unless otherwise indicated.

**Fig. 3 | Hypocretin controls production of CSF1 by pre-neutrophils in the bone marrow.**

Hcrtr1 mRNA in cells sorted from bone marrow (n = 4 mice except neutrophils, n = 7 mice). CMPS, common myeloid progenitors; GMPs, granulocyte–macrophage progenitors; MDPs, monocyte–dendritic cell progenitors. b, Hcrtr1 mRNA expression in bone marrow and blood neutrophil populations (n = 4 mice). c, Flow cytometry plot of HCRTR1+ pre-neutrophils in the bone marrow of wild-type mice transplanted with Hcrtr1GFP/GFP bone marrow. d, CSF1 expression in sorted bone marrow cells (for Ly-6Chigh monocytes, B cells and other leukocytes, n = 3 mice; for neutrophils and CD45+ cells, n = 5 mice).

f, CSF1 expression in sorted bone marrow neutrophil populations (n = 4 mice).

Hcrtr1 production by pre-neutrophils sorted from wild-type mice and exposed to lipopolysaccharides (LPS) and/or HCRT1− (for untreated and HCRT1−, n = 4 mice per group; for LPS, and LPS and HCRT1−, n = 6 mice per group). **P** < 0.05, ***P** < 0.001, one-way ANOVA.

g, CSF1 production by pre-neutrophils sorted from wild-type mice and exposed to lipopolysaccharides (LPS) and/or HCRT1− (for untreated and HCRT1−, n = 4 mice per group; for LPS, and LPS and HCRT1−, n = 6 mice per group). **P** < 0.05, ***P** < 0.001, one-way ANOVA.

h, Concentration of CSF1 in the bone marrow of Apoe−/− SF mice and Hcrtr1−/− mice (n = 4 Apoe−/− mice; n = 6 Apoe−/− SF mice; n = 4 wild-type mice; n = 7 Hcrtr1−/− mice). i, Quantification of blood Ly-6Chigh monocytes over 24 h in WT;bmHcrtr1−/− mice (n = 3 per group). **P** < 0.001, two-way ANOVA. j, Quantification of bone marrow LSK cells and proliferation of LSK cells in wild-type mice transplanted with wild-type or Hcrtr1GFP/GFP bone marrow cells (for LSK cells per leg; n = 5 mice per group; for proliferation assays, n = 4 WT;bmWT mice and n = 5 WT;bmHcrtr1GFP/GFP mice).

k, Concentration of CSF1 in the bone marrow of wild-type mice transplanted with wild-type or Hcrtr1GFP/GFP bone marrow cells (n = 4 WT;bmWT mice; n = 5 WT;bmHcrtr1GFP/GFP mice). l, Images of cross-sections of aortic roots stained with oil red O and quantification of atherosclerosis in Ldlr−/− mice transplanted with wild-type or Hcrtr1GFP/GFP bone marrow cells and fed a high-cholesterol diet for 12 weeks (n = 8 Ldlr−/−;bmWT mice; n = 9 Ldlr−/−;bmHcrtr1GFP/GFP mice). Data are mean ± s.e.m., **P** < 0.05, ***P** < 0.001, two-tailed Mann–Whitney U-tests unless otherwise indicated. ND, not detected.
pre-neutrophils as GFP+ and thus able to express the receptor (Fig. 3c and Extended Data Fig. 9b).

The observation that bone marrow CXCRI4+CXCRI2− pre-neutrophils expressed Hcrt1 was notable, because these cells reside in close proximity to haematopoietic progenitors. Moreover, neutrophils produce substantial amounts of colony stimulating factor-1 (CSF1), which promotes bone marrow myeloid-biased haematopoiesis. In part because pre-neutrophils sorted from wild-type mice expressed less Csf1 compared to mature neutrophils (Fig. 3e), we tested whether hypocretin can control haematopoiesis through HCRT1+ pre-neutrophil-derived CSF1. In vitro, hypocretin limited the capacity of pre-neutrophils to produce CSF1 in response to lipopolysaccharides (Fig. 3f and Extended Data Fig. 9c). Ex vivo, pre-neutrophils and neutrophils sorted from Hcrt−/− mice contained more Csf1 mRNA and secreted more CSF1 protein than cells sorted from wild-type mice (Fig. 3g and Extended Data Fig. 9d). In vivo, we found increased levels of CSF1 in the bone marrow of Hcrt−/− and Apoe−/− mice subjected to sleep fragmentation (Fig. 3h). Indeed, sleep fragmentation increased bone marrow CSF1 production without substantially changing the expression of other growth factors (Extended Data Fig. 9e).

Because bone marrow non-haematopoietic cells do not express HCRT1 (Fig. 3a) but produce CSF1 (Fig. 3d), we asked whether hypocretin-mediated control of leukocyte-derived CSF1 is important. First, we lethally irradiated wild-type mice and transplanted them with bone marrow cells from either wild-type or Hcrt1−/− knockout reporter (Hcrt1GFP/GFP) mice. Chimaeras with Hcrt1GFP/GFP bone marrow developed monocytosis (Fig. 3i), along with an increased number of LSK cells, increased proliferation of LSK cells (Fig. 3j) and increased levels of CSF1 in the bone marrow (Fig. 3k). Second, we transplanted Ldlr−/− mice with Hcrt1GFP/GFP bone marrow and noted augmented atherosclerosis (Fig. 3l). Third, we lethally irradiated wild-type or Hcrt−/− mice and transplanted them with bone marrow cells from either wild-type or Csf1−/− mice (Extended Data Fig. 10a).

As expected, wild-type mice had relatively few Ly-6Chigh monocytes regardless of bone marrow source. However, whereas Hcrt−/− mice reconstituted with wild-type bone marrow cells developed leukocytosis, Hcrt−/− mice reconstituted with Csf1−/− bone marrow cells had relatively few Ly-6Chigh monocytes, limited haematopoietic progenitors and proliferation, and reduced concentrations of CSF1 (Extended Data Fig. 10b–f). Notably, neutrophil numbers remained high in
Hcrt−/− mice reconstituted with Csf1−/− bone marrow, which indicates that neutrophilia in Hcrt−/− mice was not directly related to the interactions between HCRT and leukocyte control of Csf1, an observation we confirmed in wild-type (WT) mice reconstituted with bone marrow (bm) from Hcrt1−/−/GFP mice (hereafter WT;bmHcrt1−/−/GFP/GFP mice) (Extended Data Fig. 9f). Fourth, we induced atherogenesis in wild-type and Hcrt−/− chimaeras reconstituted with either wild-type or Csf1−/− bone marrow by overexpressing PCSK9 and feeding the mice a high-cholesterol diet. We found that Hcrt−/− bm WT mice developed larger lesions than Hcrt−/− bm Csf1−/− mice (Extended Data Fig. 10g–i). These data agree with the hypothesis that hypocretin controls mononucytsis and atherosclerosis by mediating Csf1 production by pre-neutrophils.

Finally, we sought to explore the role of hypocretin in atherosclerosis and sleep fragmentation. We generated Ldrl−/− mice with either wild-type or Csf1−/− bone marrow, and subjected the chimaeras to sleep fragmentation (Fig. 4a). Ldrl−/− mice with wild-type bone marrow that were subjected to sleep fragmentation developed mononcytosis through increased haematopoiesis, increased proliferation of LSK cells and increased levels of Csf1 in the bone marrow—processes that together led to bigger atheroescerotic lesions—whereas the absence of bone marrow-derived Csf1 reduced these parameters in SF mice (Fig. 4b–e). Moreover, we delivered hypocretin to the periphery of Apc−/− mice subject to sleep fragmentation (Fig. 4f). Compared to controls, Apc−/− mice subject to sleep fragmentation that received hypocretin had fewer monocytos and neutrophils in the blood (Fig. 4g), reduced proliferation and a reduction in the number of LSK cells in the bone marrow (Fig. 4h), lower levels of Csf1 in the bone marrow (Fig. 4i) and smaller lesions (Fig. 4j). These results demonstrate that hypocretin loss during sleep fragmentation aggravates haematopoiesis and atherosclerosis.

Our data indicate that sleep protects against atherosclerosis. Undisturbed sleep maintains proper hypothalamic release of hypocretin, which limits pre-neutrophil Csf1 in the bone marrow, thereby curtailing haematopoiesis and atherosclerosis. This neuro-immune axis directly connects sleep to immune function and cardiovascular disease (Fig. 4k).

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Flow cytometry. Single-cell suspensions were stained in PBS supplemented with 2% FCS and 0.5% BSA. The following monoclonal antibodies were used for flow cytometry analyses: anti-CD45 (BioLegend, clone 30-F11, 103147, lot B243834), anti-CD45.1 (BioLegend, clone A20, 110708), anti-CD45.2 (BioLegend, clone 104, 109802), anti-CD3 (BioLegend, clone I7A2, 100206), anti-CD90.2 (BioLegend, clone 53-2-1, 105308, lot B266050), anti-CD19 (BioLegend, clone DB1, 115508, lot B265260), anti-B20 (BD Biosciences, clone RA3-6B2, 55309, lot 6012994), anti-NK1.1 (BioLegend, clone PK1D6, 108708), anti-Ly-6G (BioLegend, clone 1A8, 127614, lot B259670), anti-Ly-6C (BioLegend, clone AL-2, 121006, lot B247728), anti-MHCII (BioLegend, clone M5/114.152, 107602, lot B217849), anti-F4/80 (Biolegend, clone BM8, 123114, lot B237342), anti-CD11b (BioLegend, clone M1/70, 101226, lot B238268), anti-CD11b (BioLegend, clone AF58, 135517, lot B265520), anti-Ter119 (BioLegend, clone TER119, 116208, lot B2028899), anti-CD34 (biocytin, clone RAM34, 11-0341-85, lot E00263-1634), anti-CD49f (BioLegend, clone DX5, 1089008, lot B258302), anti-CD11c (BioLegend, clone N418, 117310, lot B206713), anti-IL-7Rα (BioLegend, clone SB-199, 121112, lot B189668), anti-CD16/32 (BioLegend, clone 93, 101324, lot B250025), anti-CD150 (BioLegend, clone TC15-12F12, 115922, lot B220585), anti-Kit (BioLegend, clone B8, 105814, lot B252918), anti-CD135 (BioLegend, clone A2F10, 135510, lot B234045), anti-CD48 (BioLegend, clone HM48-1, 103426, lot B236445), anti-Scal (BioLegend, clone D7, 108126, lot B234288), anti-CD8 (BD Biosciences, clone 53-6.7, 553035, lot 2299646), anti-CD4 (BioLegend, clone GK1.5, 100428, lot B273363), anti-SiglecF (BD PharMingen, clone E5-2440, 562680, lot 7057489,), anti-CCXCR4 (Invitrogen, clone 2B11, 12-9991-91, lot B251481), anti-CCXCR2 (BD Biosciences, clone 4A4G4, 149307, lot B251481), anti-Brdu (biocytin, clone BU200-1, 118771, lot 129208), antibodies were used in a 1:700 dilution. Cells were identified as uninfected with Zombie Aqua (BioLegend). Cells were identified as (1) Ly-6Chigh monocytes (CD45+Lin1+CD11b+CD115+F4/80−Ly-6C+), (2) neutrophils (CD45+Lin1+CD11b−Ly-6G+F4/80+), (3) macrophages (CD45+Lin1+CD11b−F4/80−Ly-6C+), (4) B220+CD20+CD45+Lin1−F4/80−CD11b−, (5) CD4 T cells (CD45+CD3+CD69−CD4−CD11b−F4/80−), (6) CD8 T cells (CD45+CD3−CD69−CD8−CD11b−F4/80−), (7) LSK cells (CD45+Lin1−Kit+Scal+), (8) multipotent progenitor (MP4) (CD45+Lin1−Kit+Scal+CD135−CD150+), (9) MP3 (CD45+Lin1−Kit+Scal+CD135−CD150−), (10) MP2 (CD45+Lin1−Kit+Scal−CD135−CD150+), (11) long-term haematopoietic stem cells (CD45+Lin1−Kit+Scal−CD135+CD150+), (12) long-term haematopoietic stem cells (CD45+Lin1−Kit+Scal−CD135+CD150−), (13) common myeloid progenitor (CD45+Lin1−Kit+Scal+CD34+CD163+), (14) granulocyte–macrophage progenitor (CD45+Lin1−Kit+Scal+CD34+CD163+CD115+), (15) monocyte–dendritic cell progenitor (CD45+Lin1−Kit+Scal+CD163+CD115+); for neutrophil populations (CD45+CD3−CD69−CD4−CD11b−F4/80−), antibodies were used in a 1:700 dilution. Lineages were defined as Lin1: CD3+CD49f+CD11b−CD11c−, (16) pre-neutrophils were then separated as Kit−CCXCR4+CCXCR2−, (17) immature neutrophils as Kit−CCXCR4+CCXCR2+, (18) mature neutrophils as Kit−CCXCR4−CCXCR2−Ly-6G−. Lineages were defined as Lin1: CD3+CD49f+CD11b−CD11c−, (19) CD19+CD49f+CD11b−CD11c−, (20) CD11c−Ly-6G−IL-7Rα. Osteoclineage cells (OCN+ cells) were identified as CD45+Ter119+CD31+ GFP− from the bone fraction of OCN−GFP mice. Lepin receptor (Lepr+) cells were identified as CD45+Ter119+CD31+ YFP+ forming the bone marrow fraction of Lepr−control. iBrdU incorporation. To assess cell proliferation, 1 mg BrdU was injected intra-peritoneally 2 h before euthanasia. A BrdU flow kit (BD Biosciences) was used to stain BrdU+ cells.

RNA and protein assays. PCR. Total RNA was isolated using the RNeasy Mini Kit (Qiagen) or the NucleoSpin RNA XS kit (Takara Bio) according to the manufacturer’s instructions. RNase-free DNase Set (Qiagen) was used for DNA digestion during RNA purification. RNA quantity and quality were assessed by Nanodrop Bradford dye (BioRad) and 1% Agilent Technologies) on the Agilent 2100 Bioanalyzer for RNA of fluorescence-activated cell sorting (FACS)-purified cells. cDNA was generated from 1 μg of total RNA sample using the High capacity CDNA Revers Transcription Kit (Applied Biosystems). Quantitative real-time TaqMan PCR was performed using the following TaqMan primers (Applied Biosystems): Actb (Mm00607939_s1), Hrt (Mm01964030_s1), Htr1 (Mm01187576_m1), Htr2r (Mm01793512_m1), Csf1 (Mm00342886_m1), Pmch (Mm0124886_g1), Tph2 (Mm05577155_m1), Agrp (Mm00475829_g1), Galp (Mm00626153_m1), Ghrl (Mm00612524_m1), Gad1 (Mm00475537_m1), Gad2 (Mm00475537_m1), Ntsr1 (Mm00410181_m1), Clock (Mm00455950_m1), Arntl2 (Mm05549497_m1), Nr1d2 (Mm01310356_g1), Csf2 (Mm01290662_m1), Il1b (Mm00434228_m1), Cld2 (Mm00441242_m1), Ixb (Mm00461901_m1), Mpo (Mm01289424_m1), Prdx2 (Mm01259495_m1).
Apoptotic cells were investigated using the In situ Cell Death using a motorized fluorescence microscope Olympus BX63 and processed by FIJI. Conjugated hypocretin antibody (clone D6G9T, Cell Signaling) and CSF1 (M-CSF) were measured using an ELISA kit (Boster Biological Technology) according to the manufacturer’s instructions. Anti-hypocretin receptor-1 antibody (ab68718, Abcam) and anti-β-actin antibody (clone 13E5, Cell Signalling) were used.

Cell culture. For cell-culture experiments, cells were cultured in complete medium (RPMI-1640 supplemented with 10% FBS, 2 mM l-glutamine, 100 U ml⁻¹ penicillin and streptomycin, 10 mM HEPES, 50 μM 2-mercaptoethanol, 1 mM sodium pyruvate and l-α nonessential amino acids) and kept in a humidified 5% CO₂ incubator at 37°C. Neutrophils were sorted with a FACS Aria II and 5 × 10⁵ cells were seeded into 48-well plates in 0.5 ml medium. Cells were then pre-incubated with 0.1 μM hypocretin-1 for 3 h followed by co-incubation with 20 ng ml⁻¹ LPS for 3 h as indicated. Cells and medium were collected for downstream analysis.

Myeloid colony-forming unit assay. Single-cell suspensions from bone marrow were prepared and 2 × 10⁵ cells were plated in triplicates in complete methycellulose medium (MethoCult GF M3534) with or without hypocretin-1 according to the manufacturer’s instructions. Colony-forming units were counted after 11 days in a 5% CO₂ incubator at 37°C.

Histology and μCT. Aortas. Aortic roots were dissected and embedded in Tissue-Tek OCT compound (Sakura Finetek), frozen in 2-methylbutane (Fisher Scientific) cooled with dry ice and sectioned into 6-μm slices. To compare lesion size among the groups, sections that captured the maximum lesion area were used. To measure lesion volume, sections were collected beginning at the first appearance of the lesion area was quantified by measuring the atherosclerotic plaque of the intima—media area. Brains were sectioned coronally and 6-μm slices. To compare lesion size among the groups, sections that captured the maximum lesion area were used. To measure lesion volume, sections were collected beginning at the first appearance of the lesion area was quantified by measuring the atherosclerotic plaque of the intima—media area. Brains were sectioned coronally and 6-μm slices. To compare lesion size among the groups, sections that captured the maximum lesion area were used. To measure lesion volume, sections were collected beginning at the first appearance of the lesion area was quantified by measuring the atherosclerotic plaque of the intima—media area. Brains were sectioned coronally and 6-μm slices.

Bone. Femurs were collected and fixed in 4% paraformaldehyde overnight and then scanned by μCT at the MGH Center for Skeletal Research Core (NIH3P01AR066261). After scanning, femurs were decalcified in 0.375 M EDTA in PBS for 10 days before paraffin-embedding. Sections were cut and stained with anti-tyrosine hydroxylase antibody (Millipore, AB152).

Behaviour phenotyping. Open field test. Mice were individually placed in a white box (50 cm × 50 cm) for 5 min with ambient lighting and videos were recorded. The time the mouse spent within 5 cm of the edge of the box was quantified.

Light–dark box test. Mice were placed in a box in which the floor (25 cm × 25 cm) was black and blocked from light, while the other half was white and exposed to ambient lighting. A dividing wall with a small hole separated the two halves of the box but allowed mice to move freely between the two halves. Videos were recorded for 5 min and the time the mice spent in the white box was quantified.

Y-maze. Mice were allowed to explore two arms of the Y-maze for 5 min before being returned to their home cage. Twenty minutes later, mice were returned to the Y-maze and allowed to explore all three arms for 5 min while being recorded. The time spent in the new arm was quantified. Quantification of behavioural phenotypes was done with ImageJ software.

Statistics. Results are shown as mean ± s.e.m. Statistical tests included unpaired, two-tailed non-parametric Mann–Whitney U-tests (when Gaussian distribution was not assumed). For multiple comparisons, a non-parametric multiple-comparisons test comparing the mean rank of each group (when Gaussian distribution was not assumed) was used, or one- or two-way ANOVAs followed by Turkey’s test were used. To analyse circadian rhythmicity, cosinor analysis was performed with the following equation: Y = baseline + amplitude × cos(frequency × X + phase-shift). P values of 0.05 or less were considered to denote significance.

Data availability
All relevant data are included in the paper and its Supplementary Information. Source Data for Figs. 1–4 are available in the online version of the paper.
Extended Data Fig. 1 | Effects of sleep fragmentation on metabolic and cellular parameters. a, Image of a sleep fragmentation cage. b, Body weight (n = 10 per group). c, Plasma cholesterol at ZT3 (n = 5 per group). d, Plasma glucose at ZT3 (n = 5 per group). e, Glucose tolerance test (GTT) beginning at ZT3 (light period) and ZT12 (dark period) (n = 4 per group). f–h, Apoe−/− mice were placed in sleep fragmentation chambers where the sweep bar operated during the dark period (ZT12–0) when mice are normally awake. Control mice were maintained in sleep fragmentation chambers with a stationary sweep bar. f, Assessment of atherosclerosis and lesion area (n = 5 per group). g, Assessment of blood Ly-6C^hi monocytes and neutrophils (n = 5 per group). b, Assessment of bone marrow LSK cells and proliferation (n = 5 per group). i, Aortic macrophage proliferation in Apoe−/− and Apoe−/− SF mice after 16 weeks of sleep fragmentation at ZT3 and ZT14 (n = 5 Apoe−/− mice; n = 4 Apoe−/− SF mice). j, Quantification of B cells, CD4^+ T cells and CD8^+ T cells in the blood of Apoe−/− and Apoe−/− SF mice at ZT3 (n = 10 Apoe−/− mice; for B and CD4 T cells, n = 6 Apoe−/− SF mice and for CD8 T cells, n = 7 Apoe−/− SF mice). k, Quantification of B cells, CD4^+ T cells and CD8^+ T cells in the spleen of Apoe−/− and Apoe−/− SF mice at ZT3 (n = 10 Apoe−/− mice; n = 7 Apoe−/− SF mice). l, Quantification of B cells in the bone marrow of Apoe−/− and Apoe−/− SF mice at ZT3 (n = 10 Apoe−/− mice; n = 7 Apoe−/− SF mice). Data are mean ± s.e.m.
Extended Data Fig. 2 | Sleep and circadian migration of leukocytes.

a–d. Quantification of Ly-6C<sup>high</sup> monocytes and neutrophils at ZT3 and ZT14 in the spleen (a), bone marrow (b), lung (c) and liver (d) of Apoe<sup>−/−</sup> mice and Apoe<sup>−/−</sup> SF mice after 16 weeks of sleep fragmentation. Group sizes are indicated in the figure. Data are mean ± s.e.m., *P < 0.05, **P < 0.01, two-way ANOVA.
Extended Data Fig. 3 | Sleep-mediated haematopoiesis and extramedullary haematopoiesis. a, Gating strategy and quantification of haematopoietic progenitor cells at ZT3 in the bone marrow (n = 10 Apoe−−/− mice, except for GMPs, n = 11 mice; n = 10 Apoe−−/− SF mice, except MPP3 and MPP4, n = 9 mice). LtHSCs, long-term haematopoietic stem cells; StHSC, short-term haematopoietic stem cells. b, Gating strategy and quantification of haematopoietic progenitor cells at ZT3 in the spleen (n = 9 Apoe−−/− mice, except CMPs, n = 10 mice; n = 9 Apoe−−/− SF mice). c, C57BL/6 wild-type mice that were fed a regular chow diet were subjected to sleep fragmentation for 16 weeks after which Ly-6C^{hi} monocytes, neutrophils and LSK cells were quantified at ZT3 (n = 8 wild-type mice, except n = 9 mice for spleen Ly-6C^{hi} monocytes, n = 5 mice for bone marrow neutrophils, n = 10 mice for bone marrow LSK cells; n = 4 wild-type SF mice, except n = 9 mice for bone marrow LSK cells). Data are mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed Mann–Whitney U-tests.
Extended Data Fig. 4 | Sleep fragmentation does not alter bone structure and does not depend on the microbiome. a, b, µCT analysis of trabecular (a) and cortical bone structure (b) of Apoe⁻/⁻ mice and Apoe⁻/⁻ mice after 16 weeks of sleep fragmentation. The bone volume fraction (BV/TV), bone mineral density (BMD), trabecular number (Tb.N), trabecular thickness (Tb.Th), structural model index (SMI), cortical tissue mineral density (Ct.TMD), cortical area (Ct.Ar), total area (T.Ar), cortical thickness (Ct.Th) and cortical porosity (Ct.Porosity) were analysed (n = 9 per group). c, d, Analysis of leukocytosis in SF (c) and Hcrt⁻/⁻ (d) mice at ZT3 after receiving a cocktail of antibiotics in drinking water for 4 weeks (n = 3 per group). Data are mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, two-tailed Mann–Whitney U-tests.
Extended Data Fig. 5  | See next page for caption.
Extended Data Fig. 5 | Sleep fragmentation does not activate the peripheral sympathetic nervous system but has effects on hypothalamic gene transcription and food consumption. a, Plasma corticosterone levels in Apoe−/− mice and Apoe−/− mice after 16 weeks of sleep fragmentation at ZT3 and ZT14 (n = 4 mice per group, except n = 5 Apoe−/− mice at ZT3). b, Systolic and diastolic blood pressure at ZT3 (n = 4 mice per group). c, Immunohistochemical analysis and quantification of tyrosine hydroxylase (TH) staining in the bone marrow of Apoe−/− mice, Apoe−/− SF mice and Apoe−/− mice subjected to 3 weeks of chronic variable stress (n = 4 Apoe−/− mice; n = 4 Apoe−/− SF mice; n = 3 Apoe−/− stressed mice). d, Quantification at ZT3 of blood Ly-6C high monocytes and neutrophils, bone marrow LSK cells and proliferation in Apoe−/− mice and Apoe−/− SF mice after antagonism of the β3 receptor for 4 weeks (n = 3 Apoe−/− mice treated with β3 blocker; n = 4 Apoe−/− SF mice treated with β3 blocker). e, Quantification of time in outer zone during open field test (n = 9 Apoe−/− mice; n = 8 Apoe−/− SF mice). f, Quantification of time spent in light box during light–dark box test (n = 6 per group). g, Quantification of time in new arm during Y-maze test (n = 8 Apoe−/− mice; n = 5 Apoe−/− SF mice). h, Analysis of neuropeptide expression in the hypothalamus at ZT3 (n = 5 Apoe−/− mice, except n = 6 for Pmch, Tph2, Gad1 and Npy; n = 5 Apoe−/− SF mice, except n = 4 for Npy). i, Neuropeptide receptor expression in the hypothalamus at ZT3 (n = 6 Apoe−/− mice, except Hcrtr1, n = 10 mice; n = 5 Apoe−/− SF mice, except Hcrtr2, n = 6 mice). j, Circadian gene expression in the hypothalamus at ZT3 and ZT14 (n = 3 Apoe−/− mice; n = 4 Apoe−/− SF mice). k, Mouse food consumption during the course of sleep fragmentation (n = 4 Apoe−/− mice at ZT3, except n = 6 Apoe−/− mice on HFD for 16 weeks at ZT3; n = 4 Apoe−/− SF mice at ZT3, except n = 6 Apoe−/− SF mice on HFD for 16 weeks at ZT3; n = 4 Apoe−/− mice at ZT14, except n = 4 Apoe−/− mice on HFD for 10 weeks at ZT14 and n = 6 Apoe−/− mice on HFD for 16 weeks at ZT14; n = 5 Apoe−/− SF mice at ZT14, except n = 4 Apoe−/− SF mice on HFD for 10 weeks at ZT14 and n = 6 Apoe−/− SF mice on HFD for 16 weeks at ZT14). Data are mean ± s.e.m., *P < 0.05, **P < 0.01, two-tailed Mann–Whitney U-tests. ns, not significant.
Extended Data Fig. 6 | Hypothalamic expression of hypocretin and dynorphin. a, Hypothalamic expression of hypocretin and quantification of blood Ly-6C<sup>hi</sup> monocytes and neutrophils in Apoe<sup>−/−</sup> mice after 6, 8 and 12 weeks of sleep fragmentation (for Hcrt, n = 4 Apoe<sup>−/−</sup> mice, except n = 5 for Apoe<sup>−/−</sup> SF mice; for blood cells at 6 weeks, n = 5 mice per group; for blood cells at 8 weeks, n = 4 mice per group; for blood cells at 12 weeks, n = 9 mice per group). b, Sections of the hypothalamus stained for dynorphin and hypocretin. c, Quantification of hypothalamic dynorphin<sup>+</sup> cells per high-powered field of view (n = 5 Apoe<sup>−/−</sup> mice; n = 4 Apoe<sup>−/−</sup> SF mice, of two independent experiments). d, e, Dynorphin (Pdny) mRNA expression in the hypothalamus of SF mice (d; n = 6 Apoe<sup>−/−</sup> mice; n = 5 Apoe<sup>−/−</sup> SF mice) and Hcrt<sup>−/−</sup> mice (e; n = 4 wild-type mice; n = 5 Hcrt<sup>−/−</sup> mice). f, TUNEL staining of hypothalamic sections from Apoe<sup>−/−</sup> and Apoe<sup>−/−</sup> SF mice (representative of four biological replicates) along with a positive control of TUNEL-stained myocardium 1 day after myocardial infarction (MI) (n = 1). g-i, Apoe<sup>−/−</sup> mice were sleep-fragmented for 16 weeks then allowed to recover and sleep normally for 10 weeks. Control mice slept normally for 26 weeks. g, Analysis of hypothalamic hypocretin expression (n = 5 Apoe<sup>−/−</sup> mice; n = 4 Apoe<sup>−/−</sup> SF mice). h, Blood Ly-6C<sup>hi</sup> monocytes and neutrophils (n = 5 mice per group). i, Quantification of bone marrow LSK cells and proliferation of LSK cells (n = 5 mice per group). Data are mean ± s.e.m., *P < 0.05, **P < 0.01, two-tailed Mann–Whitney U-tests.
Extended Data Fig. 7 | Haematopoiesis in hypocretin-deficient mice. a–c. Quantification of the number of leukocytes in wild-type and Hcrt−/− mice at ZT3 in blood (a; n = 5 mice per group), spleen (b; for Ly-6C^hi^ monocytes and neutrophils, n = 7 wild-type mice and n = 8 Hcrt−/− mice; for B, CD8^+^ and CD4^+^ T cells, n = 5 wild-type mice and n = 6 Hcrt−/− mice) and bone marrow (c; for Ly-6C^hi^ monocytes and neutrophils, n = 7 wild-type mice and n = 8 Hcrt−/− mice; for B and T cells, n = 5 wild-type mice and n = 6 Hcrt−/− mice; for CMPs, GMPs and MDPs, n = 7 wild-type mice and n = 8 Hcrt−/− mice; for LSK populations, n = 5 wild-type mice and n = 6 Hcrt−/− mice). Data are mean ± s.e.m., **P < 0.01, ***P < 0.001, two-tailed Mann–Whitney U-tests.
Extended Data Fig. 8 | Hypocretin and hypocretin receptor-1 expression and production. a, b, Relative Hcrt mRNA expression in tissues (a; n = 3) and sorted bone marrow cells (b; n = 4). c, Hcrt expression in the bone marrow and bone in Apoe−/− mice and in Apoe−/− mice subjected to sleep fragmentation for 16 weeks (n = 5 Apoe−/− mice; n = 4 Apoe−/− SF mice). d, Hypocretin-1 protein levels in cerebrospinal fluid (CSF), plasma and bone marrow (BM) fluid of wild-type and Hcrt−/− mice (n = 4 mice per group). e, Hypocretin-1 protein levels in the plasma and bone marrow fluid of Hcrt−/− mice 3 h after intra-cisterna magna (i.c.m.) injection of HCRT-1 or PBS. (n = 4 mice per group). f, Relative Hcrtr1 mRNA expression in tissues (n = 4 mice, except aorta and spleen, n = 3). g, Hcrtr2 expression in sorted bone marrow cells (n = 4 mice). h, Granulocyte–macrophage colony forming units (CFU-GM) from bone marrow cells of wild-type mice exposed to hypocretin-1 ex vivo in culture medium (n = 3 per group). i, Assessment of hypocretin receptor-1 protein in hypothalamus and sorted bone marrow neutrophils by western blot. Data are mean ± s.e.m.
Extended Data Fig. 9 | Hypocretin, bone marrow neutrophils and HCRTR1. a, Flow cytometry gating strategy for bone marrow pre-neutrophils, immature neutrophils and mature neutrophils. b, HCRTR1 (GFP) in bone marrow and blood neutrophils from WT;bmHcrtr1<sup>GFP/GFP</sup> mice. c, mRNA expression in cultured bone marrow pre-neutrophils exposed to LPS and/or HCRT-1 (for untreated n = 3 mice, except Mpo, n = 6 mice; for HCRT-1, n = 3 mice, expect Csf1, n = 4 mice and Mpo, n = 6 mice; for LPS, n = 3 mice except Csf1, n = 7 mice and Mpo, n = 6 mice; for LPS and HCRT-1, n = 3 mice, except Csf1, n = 11 mice, Csf2, n = 4 mice and Mpo, n = 6 mice). d, Csf1 expression in sorted bone marrow cells of wild-type and Hert<sup>−/−</sup> mice (n = 5 wild-type mice; n = 6 Hert<sup>−/−</sup> mice). e, Analysis of mRNA transcript expression in bone marrow leukocytes of Apoe<sup>−/−</sup> mice after 16 weeks of sleep fragmentation (for Apoe<sup>−/−</sup>, n = 5 mice, except Il10, Il34, Cxcl12, Csf3, n = 4 mice and Csf1, n = 9 mice; for Apoe<sup>−/−</sup>-SF mice, n = 6 mice, except Il5, Il13, Il6, Il34, Cxcl12, Csf3, n = 5, Il10, n = 5 mice and Csf1, n = 12). nd, not detected. f, Blood neutrophils in WT;bmHcrtr1<sup>GFP/GFP</sup> mice over 24 h (n = 3 per group). Data presented as mean ± s.e.m., **P < 0.01, one-way ANOVA.
Extended Data Fig. 10 | Haematopoietic CSF1 deletion protects against haematopoiesis and atherosclerosis in hypocretin-deficient mice.

a, Schematic of chimeric models. b, c, Quantification of Ly-6Chi monocytes and neutrophils in blood (b, n = 4 WT;bmWT mice; n = 6 Hct−/−;bmWT mice; n = 3 WT;bmCsf1−/− mice; n = 5 Hct−/−;bmCsf1−/− mice) and bone marrow (c; n = 4 WT;bmWT mice; n = 6 Hct−/−;bmWT mice; n = 3 WT;bmCsf1−/− mice; n = 5 Hct−/−;bmCsf1−/− mice). d, Quantification of the number of LSK cells (n = 4 WT;bmWT mice; n = 6 Hct−/−;bmWT mice; n = 3 WT;bmCsf1−/− mice; n = 5 Hct−/−;bmCsf1−/− mice) and proliferation (n = 4 WT;bmWT mice; n = 4 Hct−/−;bmWT mice; n = 3 WT;bmCsf1−/− mice; n = 5 Hct−/−;bmCsf1−/− mice) in bone marrow. e, Quantification of CMPs, GMPs and MDPs in chimeric mice (n = 4 WT;bmWT mice; n = 6 Hct−/−;bmWT mice; n = 3 WT;bmCsf1−/− mice; n = 5 Hct−/−;bmCsf1−/− mice). f, Bone marrow CSF1 levels (n = 4 WT;bmWT mice; n = 8 Hct−/−;bmWT mice; n = 4 WT;bmCsf1−/− mice; n = 7 Hct−/−;bmCsf1−/− mice). g, Schematic of chimeric models receiving Adv-PCSK9 and fed a HCD for 12 weeks. h, Plasma cholesterol levels (n = 7 WT;bmWT mice; n = 10 Hct−/−;bmWT mice; n = 5 WT;bmCsf1−/− mice; n = 6 Hct−/−;bmCsf1−/− mice). i, Images of cross-sections of aortic roots stained with oil red O and quantification of atherosclerosis in chimeric mice (n = 7 WT;bmWT mice; n = 9 Hct−/−;bmWT mice; n = 5 WT;bmCsf1−/− mice; n = 6 Hct−/−;bmCsf1−/− mice). Data are mean ± s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, one-way ANOVA.
Reporting Summary

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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  BD FACSDiVa software was used to collect data from flow cytometry; NPDview2 and Nanozoomer 2.0Rs were used to collect histology data.

Data analysis  Flow cytometric analyses were performed with FlowJo software (FlowJo 8.7.2); NPDviewview2 software was used to analyze histology data; Statistical analyses were performed with GraphPad Prism 7.0 software

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

Sample size | Power calculations were performed to determine sample size.
--- | ---
Data exclusions | No data were excluded from analysis.
Replication | All attempts at replication were successful. Findings were replicated in at least three biologically independent samples each.
Randomization | Where appropriate, the mice were selected at random. Otherwise, animals were placed into separate groups according to their genotype.
Blinding | Where possible, groups were blinded

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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**

| n/a | Involved in the study |
| --- | --- |
| ☑️ | Antibodies |
| ☑️ | Eukaryotic cell lines |
| ☑️ | Palaeontology |
| ☑️ | Animals and other organisms |
| ☑️ | Human research participants |
| ☑️ | Clinical data |

**Methods**

| n/a | Involved in the study |
| --- | --- |
| ☑️ | ChIP-seq |
| ☑️ | Flow cytometry |
| ☑️ | MRI-based neuroimaging |

**Antibodies**

| | |
| --- | --- |
| | anti-CD45 (BioLegend, clone30-F11, Cat#103147, Lot#B2438334), anti-CD45.1 (BioLegend, clone A20, Cat#110708), anti-CD45.2 (BioLegend, clone 104, Cat#109802), anti-CD3 (BioLegend, clone 17A2, Cat#100206), anti-CD90.2 (BioLegend, clone 53-2-1, Cat#105308, Lot#B260050), anti-CD19 (BioLegend, clone M5/114.152, Cat#105802, Lot#B217869), anti-CD11b (BioLegend, clone M1/70, Cat#101226, Lot#B238268), anti-CD11c (BioLegend, clone AF598, Cat#135517, Lot#B265220), anti-Ter119 (BioLegend, clone TER-119, Cat#116208, Lot#B220899), anti-CD34 (eBioscience, clone RAM34, Cat#11-0341-85, Lot#E0026-1634), anti-CD49b (BioLegend, clone DX5, Cat#108908, Lot#B258302), anti-CD11c (BioLegend, clone N418, Cat#117390, Lot#B206713), anti-IL7Rα (BioLegend, clone SIB2, Cat#121112, Lot#B189668), anti-CD16/32 (BioLegend, clone 93, Cat#101324, Lot#B250025), anti-CD150 (BioLegend, clone TC15-12F12, Cat#115922, Lot#B220895), anti-CD71 (BioLegend, clone 288, Cat#105814, Lot#B252918), anti-CD135 (BioLegend, clone A2F10, Cat#135310, Lot#B234045), anti-CD48 (BioLegend, clone HM48-1, Cat#103426, Lot#B236445), anti-Sca1 (BioLegend, clone D7, Cat#108126, Lot#B234288), anti-CD8 (BD Bioscience, clone 53-6.7, Cat#100428, Lot#B237336), anti-SiglecF (BD Pharmingen, clone 55-2440, Cat#17-9991-81, Lot#B251481), anti-CXCR2 (BioLegend, clone SA04A4, Cat#149307, Lot#B251481), anti-BrdU (eBioscience, clone BU20A, Cat#17-5071-42, Lot#F419920). All antibodies were used in a 1:700 dilution.

**Validation**

These antibodies were all used for flow cytometry on mice. Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier.

**Animals and other organisms**

Policy information about [studies involving animals: ARRIVE guidelines](https:// ARRIVEguidelines.org) recommended for reporting animal research

**Laboratory animals**

| | |
| --- | --- |
| CS7Bl/6J (wild type, WT), Apomt1Unc (Apoe/-), Ldrtm1Her/l (Ldlr/-) and Csf1op (Csf1-/-) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Hcrt/- mice were kindly provided by Dr. Thomas Scammell (Professor of Neurology, Division of Sleep Science, Harvard Medical School) and bred in-house. HcrtR1GFP/GFP mice were kindly provided by Dr. Anne Vassalli (Department of Physiology, University of Lausanne). Stromal cell reporter mice Nestin-GFP, LeptinRcre-R26-EYFP, and OCN-GFPtopaz were bred in-house. Age- and sex-matched animals were used at 8–12 weeks of age. For experiments on Apoe/- and Ldlr/- mice females were used. In all other experiments both males and females were used. |
Flow Cytometry

Plots

Confirm that:

☐ 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

Peripheral blood was collected by retro-orbital bleeding and red blood cells were lysed in RBC lysis buffer (Biolegend, San Diego, CA). Aortas, lung, liver and heart were excised after PBS (Thermo Fisher Scientific, Waltham, MA) perfusion, minced and digested with 450 U/ml collagenase I, 125 U/ml collagenase XI, 60 U/ml DNase I and 60 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO) in PBS for 20 minutes (liver), 40 minutes (aorta) or 1hr (heart and lung) at 37°C. Spleens were crushed through a 40mm cell strained and red blood cells were lysed with RBC lysis buffer. Bone marrow cells were collected by flushing bones with PBS after which a single cell suspension was created by passing cells through a 26-gage needle and red blood cells were lysed with RBC lysis buffer.

Instrument

Data were acquired on a LSRll and a Area II

Software

DIVA and FlowJo

Cell population abundance

Post sort, cell abundance was sufficient for down stream applications. After sorting, a small fraction of the sorted cells were run through Aria II and the same gating strategy was applied to check the purity of sorted cell populations. A general purity of higher than 95% were achieved for all the sorted population.

Gating strategy

FSC/SSC gating was used to exclude dead cells and debris followed by FSCA/FSCB to select singlets. Viable cells were identified as unstained with Zombie Aqua (Biolegend, CA). Cells were identified as (i) Ly6Chigh monocytes (CD45+Lin1–CD11b+CD115+F4/80–Ly6Chigh), (ii) neutrophils (CD45+Lin1–CD11b+Ly-6G+F4/80–), (iii) macrophages (CD45+Lin1–CD11b+F4/80+Ly-6Chigh), (iv) B cells (CD45+B220+CD19+F4/80–CD11b–), (v) CD4 T-cells (CD45+CD3+CD90+CD4+CD11b–F4/80–), (vi) CD8 T-cells (CD45+CD3+CD90+CD8+CD11b–F4/80–), (vii) LSKs (CD45+Lin2–cKit+Sca1+CD135+CD150–), (viii) MPP4 (CD45+Lin2–cKit+Sca1+CD135+CD150+CD48–), (ix) short-term hematopoietic stem cell (ST-HSC, CD45+Lin2–cKit+Sca1+CD135+CD150–CD48–), (x) MPP2 (CD45+Lin2–cKit+Sca1+CD135+CD150+CD48–), (xi) monocyte/macrophage progenitor (GMP, CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xii) granulocyte/macrophage progenitor (GMP, CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xiii) common myeloid progenitor (CMP, CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xiv) monocyte/dendritic cell progenitor (MDP, CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xv) osteoclast progenitor (OCP, CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xvi) pre-neutrophils were then separated as cKit+CXCR4+CXCR2–, (xvii) immature neutrophils as cKit–CXCR4+CXCR2–, and (xviii) mature neutrophils as cKit–CXCR4+CXCR2+Ly6G+. Lineages were defined as Lin1: CD3, CD90.2, CD11b, CD11c, Ly-6G, IL7Rα. Osteolineage cells (OCN+) cells were identified as CD45–Ter119–CD31–GFP+ from the bone fraction of OCN-GFP mice. Leptin receptor (LepR+) cells were identified as CD45–Ter119–CD31–YFP+ from the bone marrow fraction of LeptinRcre-R26-EYFP. Nestin+ cells were identified as CD45–Ter119–CD31–GFP+ form the bone and bone marrow fraction of Nestin-GFP mice. Endothelial cells were identified as CD45–Ter119–CD31+ of the bone marrow fraction of LeptinRcre-R26-EYFP mice.

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