In contrast to nearly all other tissues, the anatomy of cell differentiation in the bone marrow remains unknown. This is owing to a lack of strategies for examining myelopoiesis—the differentiation of myeloid progenitors into a large variety of innate immune cells—in situ in the bone marrow. Such strategies are required to understand differentiation and lineage-commitment decisions, and to define how spatial organizing cues inform tissue function. Here we develop approaches for imaging myelopoiesis in mice, and generate atlases showing the differentiation of granulocytes, monocytes and dendritic cells. The generation of granulocytes and dendritic cells—monocytes localizes to different blood-vessel structures known as sinusoids, and displays lineage-specific spatial and clonal architectures. Acute systemic infection with *Listeria monocytogenes* induces lineage-specific progenitor clusters to undergo increased self-renewal of progenitors, but the different lineages remain spatially separated. Monocyte–dendritic cell progenitors (MDPs) map with nonclassical monocytes and conventional dendritic cells; these localize to a subset of blood vessels expressing a major regulator of myelopoiesis, colony-stimulating factor 1 (CSF1, also known as M-CSF). Specific deletion of Csf1 in endothelium disrupts the architecture around MDPs and their localization to sinusoids. Subsequently, there are fewer MDPs and their ability to differentiate is reduced, leading to a loss of nonclassical monocytes and dendritic cells during both homeostasis and infection. These data indicate that local cues produced by distinct blood vessels are responsible for the spatial organization of definitive blood cell differentiation.

In the bone marrow, haematopoietic stem cells (HSCs) and multipotent progenitor cells differentiate into lineage-specific progenitors that then generate all major lineages of blood cells, including red blood cells and immune cells. Single-cell RNA sequencing (scRNAseq) and lineage-tracing analyses have provided key insights into the regulation and pathways of differentiation, but require destruction of the organization of the tissue. With few exceptions, most of our knowledge of the anatomy of blood differentiation still derives from classical studies using light or electron microscopy. Because most myeloid cells cannot be uniquely identified on the basis of morphology, the anatomy of myelopoiesis and the structures that support it remain unknown.

### Strategies for imaging myelopoiesis

During myelopoiesis, common myeloid progenitors (CMPs) generate monocyte–dendritic cell progenitors (MDPs) and granulocyte–monocyte progenitors (GMPs). MDPs further differentiate into monocyte progenitors expressing low levels of Gfi1 (Gfi1lo cells), which in turn generate Ly6Ch and Ly6Cl monocytes as well as common dendritic cell progenitors (CDPs), the latter ultimately producing dendritic cells. GMPs also generate monocytes via Gfi1hi monocyte progenitors, and neutrophils via granulocyte progenitors (GPs, also known as proNeu cells) (Fig. 1a). Gfi1hi and Gfi1lo monocyte progenitors share cell-surface markers and can be distinguished only transcriptionally.

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**Article**

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or using Gfi1 reporter mice. We refer here to the population containing both as monocyte progenitors (MOPs).

To examine myelopoiesis in situ, we developed three antibody stains (Fig. 1a, shaded areas). The first stain combined two published approaches to simultaneously detect MDPs, GMPs, MOPs and GPs (Fig. 1a–f). These progenitors showed the same frequencies and colony-forming potential (Fig. 1c, d) as those detected by established fluorescence-activated cell sorting (FACS) stains, indicating that they label the same populations. We observed identical frequencies when using imaging or FACS (Fig. 1e, f), showing that imaging detected that entire population of bone marrow progenitors. The use of Ly6C additionally allowed arteriole visualization (Extended Data Fig. 1c).

For the second stain, we replaced the lineage panel and the anti-CD16/32 monoclonal antibodies with anti-CD11b and anti-Ly6G antibodies, enabling simultaneous detection of MDPs, GMPs, MOPs and GPs (Fig. 1a–f). These progenitors showed the same frequencies and colony-forming potential (Fig. 1c, d) as those detected by established fluorescence-activated cell sorting (FACS) stains, indicating that they label the same populations. We observed identical frequencies when using imaging or FACS (Fig. 1e, f), showing that imaging detected that entire population of bone marrow progenitors. The use of Ly6C additionally allowed arteriole visualization (Extended Data Fig. 1c).

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Ly6Cl0 monocytes are closer to each other than to random cells (Fig. 2a, Extended Data Fig. 3g), generating areas rich in Ly6Cl0 monocytes that are selectively enriched near MDPs (Fig. 2a, h, i and Supplementary Video 6, 7). In agreement with previous studies23,24, we found that cDCs are also enriched in specific regions (Fig. 2a, Extended Data Fig. 3h). cDCs also map with MDPs (Fig. 2a, h, i and Supplementary Video 7). These results suggested that MDPs might cluster with their progeny. Fate mapping of MDPs was difficult, as only 2 out of 125 MDPs analysed in 9 sternum segments were confetti-labelled. These showed no clonal relationship with surrounding cDCs or Ly6Cl0 monocytes (Fig. 2j, Extended Data Fig. 3i).
Together with the observation that the Ly6C<sup>lo</sup> monocytes and cDCs are not clonally related to each other (Extended Data Fig. 3j, k), these findings indicate that Ly6C<sup>lo</sup> monocytes and cDCs are produced elsewhere and then recruited near to MDPCs. These results indicate that the granulocyte and monocyt–dendritic cell lineages have distinct spatial and clonal architectures. We note that these architectures are maintained between different samples and types of sections (Extended Data Fig. 4a–g).

**Spatial segregation of myelopoiesis**

Granulocyte progenitors identify areas of granulopoiesis, and MDPCs localize with Ly6C<sup>lo</sup> monocytes and cDCs are farther apart from each other than predicted from random distributions (Extended Data Fig. 5a–c), showing the segregation of myeloid lineages to different locations in the bone marrow.

Sinusoids, arterioles and the endosteum organize the bone marrow by secreting cytokines<sup>25</sup>. Imaging analyses demonstrate that MDPCs, MOPs and cDCs are farther away from arterioles, show no specific localization with the endosteum, and are closer to sinusoids when compared with random progenitors (Fig. 3a, b, Extended Data Fig. 5d, e and Supplementary Videos 8–10). We note that because MDPCs are rare cells, they sometimes appear to concentrate towards one end of the sterna in some images (Fig. 3a, b), but they are equally distributed through the sternum (Extended Data Fig. 5f). As sinusoids are a niche for HSCs<sup>25</sup>, we mapped the distances from MDPCs—or a mixed population of GPs and MOPs—to HSCs, and found no differences when compared with random cells (Extended Data Fig. 5g–j). Together these data indicate that myeloid progenitors do not reside in HSC niches and that sinusoids are the site of myelopoiesis. Therefore, as the differentiation of granulocytes and of monocytes/dendritic cells do not overlap, it is possible that different sinusoids regulate specific myeloid lineages.

**CSF1<sup>+</sup> vessels organize myelopoiesis**

We reclustered and analysed published scRNAseq data sets<sup>26,27</sup> (Extended Data Fig. 6a–I) and found that LepR<sup>+</sup> perisinusoidal cells from cDCs to the closest CSF1<sup>+</sup> vessel (n = 442 cDCs from a total of 4 sections of 3 wild-type mice). Distance from MDPCs to the closest vessel (n = 44 MDPCs from a total 5 sections from 3 control mice; n = 29 MDPCs from a total of 5 sections from 3 Csf1<sup>ΔEC</sup> mice). Distance from MDPCs to the closest Ly6C<sup>+</sup> monocyte or cDC. For MDPC-Ly6C<sup>+</sup> monocytes, n = 37 MDPCs from a total of 4 sections from 3 control mice; n = 18 MDPCs from a total of 4 sections from 3 Csf1<sup>ΔEC</sup> mice. For MDPC-cDCs, n = 47 MDPCs from a total of 6 sections from 3 control mice, and n = 47 MDPCs from a total of 9 sections from 3 Csf1<sup>ΔEC</sup> mice. Unless indicated, one dot represents one cell. Progenitor dots are three times, and cDCs are two times, the size of the relevant cell. Scale bars, 200 μm. Statistical differences were calculated using two-tailed Student’s t-tests; P-values are shown.

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Effect of infection

We used *L. monocytogenes* to investigate stress-induced myelopoiesis. In agreement with previous studies, we found a loss of myeloid progenitors and mature cells two days after infection, followed by a massive expansion of cDCs (Fig. 4a, Extended Data Fig. 9a). Even during stress, GPs, MOPs and MDPs remained spatially segregated (Fig. 4b, Extended Data Fig. 9b). Whereas MDPs were always found as single cells, GPs and MOPs recovered via homogeneous clusters of 5–14 GPs or 4–11 MOPs at day 4 that mostly disaggregated by day 6 as single cells, GPs and MOPs recovered via homogeneous clusters (Fig. 4b, Extended Data Fig. 9b). Whereas MDPs were always found a massive expansion of cDCs (Fig. 4a, Extended Data Fig. 9a). Even in this setting of accelerated myelopoiesis, neither confetti-labelled MOPs nor confetti-labelled MDPs were clonally related to surrounding cells (Extended Data Fig. 9k–m), indicating that migration away from their progenitors is an intrinsic feature of monocytes and dendritic cells. Myeloid progenitors still mapped to sinusoids, but farther away as compared with uninfected mice (Fig. 4d, e). In agreement with this, we found reduced expression of Csf1—which causes MDP to localize to sinusoids (Fig. 3h)—in endothelial cells from infected mice (Extended Data Fig. 9n). This prevented the detection of CSF1* vessels. Loss of endothelial-derived CSF1 did not cause further relocation of MDPs away from sinusoids, but did disrupt the close localization of MDPs with Ly6C* monocytes and cDCs, and led to reduced recovery of MDPs and downstream progenitors and cDCs, with other myeloid cells being less affected (Fig. 4f, g, Extended Data Fig. 9o–r). These results indicate that endothelial-derived CSF1 is required for emergency production of dendritic cells in response to infection. Extended Data Fig. 10 summarizes our findings.

Discussion

The anatomy of blood cell differentiation in the bone marrow has hitherto remained largely unknown. Here we mapped myelopoiesis...
in situ and assessed clonal relationships between myeloid progenitors and surrounding cells. We found that myelopoiesis is spatially organized, with the generation of granulocytes and of monocytes–dendritic cells taking place in different sinusoids and displaying lineage-specific spatial and clonal architectures. Sinusoids expressing CSF1 provide a unique niche that regulates MDPs, monocytes and DCs and is required for normal and stress-induced production of dendritic cells. Thus, local cues produced by distinct blood vessels spatially organize and control myeloid differentiation. Our findings provide a new conceptual framework for understanding differentiation, dissecting haematopoiesis during disease, and designing organ systems capable of producing multilineage blood cells ex vivo.

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/s41586-021-03201-2.

1. Stanley, E. R. & Chitu, V. CSF-1 receptor signaling in myeloid cells. Cold Spring Harbor Perspect Biol. 6, a021857 (2014).
2. Olsson, A. et al. Single-cell analysis of mixed-lineage states leading to a binary cell fate choice. Nature 537, 698–702 (2016).
3. Nestorowa, S. et al. A single-cell resolution map of mouse hematopoietic stem and progenitor cell differentiation. Blood 128, e20–e31 (2016).
4. Tusi, B. K. et al. Population snapshots predict early hematopoietic and erythroid hierarchies. Nature 555, 54–60 (2018).
5. Sun, J. et al. Clonal dynamics of native hematopoiesis. Nature 514, 322–327 (2014).
6. Busch, K. et al. Fundamental properties of unperturbed hematopoiesis from stem cells in vivo. Nature 518, 542–546 (2015).
7. Upadhyaya, S. et al. Kinetics of adult hematopoietic stem cell differentiation in vivo. J. Exp. Med. 215, 2815–2832 (2018).
8. Weiss, L. The structure of bone marrow. Functional interrelationships of vascular and hematopoietic compartments in experimental hemolytic anemia: an electron microscopic study. J. Morphol. 117, 467-537 (1965).
9. Mohandas, N. & Prenant, M. Three-dimensional model of bone marrow. Blood 51, 633–643 (1978).
10. Weston, H. & Bainton, D. F. Association of alkaline phosphatase-positive reticulum cells in bone marrow with granulocytic precursors. J. Exp. Med. 150, 919–937 (1979).
11. Charbord, P., Tavian, M., Humeau, L. & Peault, B. Early ontogeny of the human marrow from long bones: an immunohistochemical study of hematopoiesis and its microenvironment. Blood 87, 4109–4119 (1996).
12. Ding, L. & Morrison, S. J. Haematopoietic stem cell and early lymphoid progenitors occupy distinct bone marrow niches. Nature 495, 231–235 (2013).
13. Cordeiro Gomes, A. et al. Hematopoietic stem cell niches produce lineage-instructive signals to control multipotent progenitor differentiation. Immunity 45, 1219–1231 (2016).
14. Hérauld, A. et al. Myeloid progenitor cluster formation drives emergency and leukemic myelopoiesis. Nature 544, 53–58 (2017).
15. Comazzetto, S. et al. Restricted hematopoietic progenitors and erythropoiesis require SCF from leptin receptor+ niche cells in the bone marrow. Cell Stem Cell 24, 477–486 (2019).
16. Yanez, A. et al. Granulocyte-monocyte progenitors and monocyte-dendritic cell progenitors independently produce functionally distinct monocytes. Immunity 47, 890–902 (2017).
17. Evrard, M. et al. Developmental analysis of bone marrow neutrophils reveals populations specialized in expansion, trafficking, and effector functions. Immunity 48, 364–379 (2018).
18. Kwok, I. et al. Combinatorial single-cell analyses of granulocyte-monocyte progenitor heterogeneity reveals an early uni-potent neutrophil progenitor. Immunity 53, 303–318 (2020).
19. Hettinger, J. et al. Origin of monocytes and macrophages in a committed progenitor. Nat. Immunol. 14, 821–830 (2013).
20. Yona, S. et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 38, 79–91 (2013).
21. Snippert, H. J. et al. Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells. Cell 143, 134–144 (2010).
22. Ruzankina, Y. et al. Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. Cell Stem Cell 1, 123–126 (2007).
23. Sapozhnikov, A. et al. Pervascular clusters of dendritic cells provide critical survival signals to B cells in bone marrow niches. Nat. Immunol. 9, 388–395 (2008).
24. Zhang, B. et al. Bone marrow dendritic cells regulate hematopoietic stem/progenitor cell trafficking. J. Clin. Invest. 129, 2920–2931 (2019).
25. Wei, Q. & Frenette, P. S. Niches for hematopoietic stem cells and their progeny. Immunity 48, 632–648 (2018).
26. Tikhonova, A. N. et al. The bone marrow microenvironment at single-cell resolution. Nature 569, 222–228 (2019).
27. Baryawno, N. et al. A cellular taxonomy of the bone marrow stroma in homeostasis and leukemia. Cell 177, 1915–1932 (2019).
28. Mossadegh-Keller, N. et al. M-CSF instructs myeloid lineage fate in single hematopoietic stem cells. Nature 497, 239–243 (2013).
29. Serbina, N. V., Hohl, T. M., Cherny, M. & Pamer, E. G. Selective expansion of the monocytic lineage directed by bacterial infection. J. Immunol. 183, 1900–1910 (2009).
30. Venkatasubramanian, M., Chetal, K., Schnell, D. J., Atluri, G. & Salomonis, N. Resolving single-cell heterogeneity from hundreds of thousands of cells through sequential hybrid clustering and NMF. Bioinformatics 36, 3773–3780 (2020).
Methods

Mice
C57BL/6j–Pptcr<sup>+</sup> (CD45.2<sup>+</sup>), B6. SJL–Pptcr<sup>Pepc</sup>/BoyJ (CD45.1<sup>+</sup>), B6.129–Tg(Cdh5<sup>-cre</sup>)Sp/J, B6.129(Cg)–Lep<sup>cre</sup>Fl/Fl (Lep<sup>-cre</sup>), B6. C57BL/6–Cdh5<sup>-cre</sup>Fl/Fl (Lep<sup>-cre</sup>)<sup>+</sup>, B6. C57BL/6–Cdh5<sup>-cre</sup>Fl/Fl (Lep<sup>-cre</sup>)<sup>-</sup> (CX3CR1–GFP<sup>+</sup>) mice were obtained from the Jackson Laboratory. Nestin–gfp<sup>+</sup> mice were provided by P.S. Frenette. Csf1<sup>fl/fl</sup> and Gfi1<sup> knockout</sup> mice have been described as previously<sup>33,34</sup>. To generate Csf1<sup>fl/fl</sup> mice, we first bred Csf1<sup>fl/fl</sup> mice with Ubc–cre<sup>ERT2</sup> mice to generate Ubc–cre<sup>ERT2</sup>:Csf1<sup>fl/fl</sup> mice. These were treated with tamoxifen, leading to deletion of the Csf1<sup>fl/fl</sup> allele in the germline. Tamoxifen-treated Ubc–cre<sup>ERT2</sup>:Csf1<sup>fl/fl</sup> mice were then bred with wild-type mice to generate Csf1<sup>−/−</sup> mice. We mated Csf1<sup>−/−</sup> mice with Cdh5–cre<sup>ERT2</sup>:Csf1<sup>fl/fl</sup> and LepR–cre:Csf1<sup>fl/fl</sup> mice to generate Cdh5<sup>-cre</sup>:Csf1<sup>fl/fl</sup> and LepR<sup>-cre</sup>:Csf1<sup>fl/fl</sup> mice. Mice received water and food ad libitum. All experiments, except confetti fate mapping, were performed with 8–14-week-old female and male mice (Mus musculus).

Mice were housed at the vivarium at Cincinnati Children’s Hospital Medical Center under a 14 h light:10 h darkness schedule, 30–70% humidity and at 22.2 ± 1.1 °C. All animal experiments followed all relevant guidelines and regulations and were approved by the Animal Care Committee of Cincinnati Children’s Hospital Medical Center.

Tamoxifen treatment
Six-week-old B6.129P–Gt(Rosa)26Sor<sup>CAG-Brainbow2.1Cle</sup> (confetti)<sup>33</sup> mice were treated with two pulses of tamoxifen in their diet (0.4 g of tamoxifen citrate per kilogram of rodent diet; Envigo). Each pulse was 2 weeks long, and pulses were 2 weeks apart. Because GPS, MDPs and MOPS do not persist in vivo for longer than two weeks<sup>35,36</sup>, we chased the mice for 12 weeks to ensure that all of the structures originated from upstream progenitors.

Infection with L. monocytogenes and antibiotic treatment
For infection, the wild-type L. monocytogenes strain 10403s was grown overnight, and then back-diluted to early log-phase growth (OD<sub>600</sub> 0.1) in brain–heart infusion (BHI) medium (Difco), washed and diluted in sterile saline, and injected intravenously into mice (1 × 10<sup>4</sup> CFU per 200 microlitres per mouse). CsF1<sup>−/−</sup> heterozygous mice (CsF1<sup>−/+</sup> and controls) did not survive this dosage and were thus treated by supplementing the drinking water with ampicillin (2 mg ml<sup>−1</sup>) 48 h after infection. This rescued death in 50% of the mice.

Collection of bone marrow and peripheral blood
Mice were euthanized by inhalation of isoflurane followed by cervical dislocation. Bone marrow cells were harvested by flushing bones with 1 ml of ice-cold PEB buffer (2 mM EDTA and 0.5% bovine serum albumin in phosphate-buffered saline (PBS)). Blood was collected from the retro-orbital venous sinus in tubes containing EDTA. Red blood cells (RBCs) in peripheral blood were lysed by adding 1 ml of RBC lysis buffer (150 mM NH<sub>4</sub>Cl, 10 mM Na<sub>2</sub>CO<sub>3</sub> and 0.1 mM EDTA). Cells were immediately decanted by centrifugation, resuspended in ice-cold PEB and used in subsequent assays. We used CountBright absolute counting beads (Thermo Fisher Scientific, catalogue number C36950) to count bone marrow cells and blood cells in a BD LSR II or BD Fortessa (BD Biosciences) flow cytometer according to the manufacturer’s protocol.

Isolation of bone marrow stromal cells
Digestion of bone marrow was performed as described<sup>34</sup>. On the basis of the distribution of forward scatter (FSC) and side scatter (SSC), and on the exclusion of 4′,6-diamidino-2-phenylindole (DAPI; Sigma), Antibiotics used were: B220 (clone RA3–682), CD3 (clone I45-2C11), CD8 (clone 33–6.7), CD11b (clone MI/70), CD11c (clone N418), CD16/32 (clone 93), CD24 (clone 30–F1), CD31 (clone A20), CD41 (clone MWReg30), CD45 (clone 30–F11), CD45.1 (clone A20), CD45.2 (clone 104), CD48 (clone HM48-1), CD105 (clone MJ/17), CD115 (clone AF598), CD135 (clone A2F10), CD144 (clone BV13), CD150 (clone TC15-12F2.12), CD169 (clone 3D6.112), CD172ax (clone P94), F4/80 (clone BMS5), Gr1 (clone RB6–8C5), Ly6c (clone HK1.1), Ly6–G (clone 1A8), Sca-1 (clone D7), Ter119 (clone TER-119), MHC II (clone M5/114.15.2), from BioLegend; and CD34 (clone RAM34), CD147 (clone 2B8) and Siglec-H (clone eBio440c) from BioLegend or Thermo Fisher Scientific. Data were analysed with FlowJo (Tree Star). Gating strategies for most analyses are shown in the main figures or Extended Data figures. CDPs<sup>33</sup>, pre-DCs<sup>36</sup>, cDC1s and CDC2s (ref. 31), CMPs<sup>36</sup> and macrophages<sup>37</sup> were gated as described.

Preparation and analysis of cytoospin experiments
FACS-purified cells were decanted onto 30 slides using a Cytospin 4 cytocentrifuge (Thermo Fisher Scientific) according to the manufacturer’s instructions. For histology analyses, slides were stained with Camco Stain Pak (Cambridge Diagnostics) according to the manufacturer’s instructions. This kit gives results similar to those of a Wright–Giemsa stain. Slides were analysed on Zeiss AX10 inverted microscope (Carl Zeiss, Oberkochen, Germany).

CFU assay
The indicated numbers (Figs. 1c, 3e, Extended Data Figs. 1g, 7d, 8b, d) of FACS-purified cells were seeded into methylcellulose culture medium (StemCell Technologies, catalogue number M3534), plated in 35-mm dishes and incubated at 37 °C in 5% CO<sub>2</sub>, with at least 95% humidity for 7–10 days. Colonies were identified and counted on the basis of cluster size and cell morphology using a Nikon Eclipse Ti inverted microscope (Nikon Instruments, NY, USA).

RNA isolation and qPCR
RNA was isolated using a Dynabeads mRNA direct kit (Thermo Fisher Scientific, catalogue number 61011) according to the manufacturer’s protocol. Complementary DNA was synthesized using RNA to cDNA EcoDry premix (Takara, catalogue number 95054) in an ABI Prism 7900HT sequence detection system (Applied Biosystems). Results were analysed using SDS 2.4 software (Applied Biosystems). Primers were as follows: CSF1 forward, 5′-ATGACGAGGATGTCCAAGG-3′; CSF1 reverse, 5′-TCCATTTCACATCTGTTGCA-3′; IRF8 forward, 5′-AGACCATGTTCCGTATTCCCC-3′, IRF8 reverse, 5′-CACAGGGTACCTCGTCTCC-3′; GFI1 forward, 5′-AGAGCCGCGACGCTAC-3′; GFI1 reverse, 5′-GGCCCTATTATTGACTGCC-3′; mGAPDH forward, 5′-TGTGCGTCGTGTAGTCTGA-3′; mGAPDH reverse, 5′-CCTGCTTACACCCTTCTGGA-3′.

Competitive reconstitution assays
Recipient mice were lethally conditioned with a total irradiation dose of 1,175 rads (700 rads plus 475 rads, 3h apart). Next, 1 × 10<sup>5</sup> bone marrow cells from control or conditional knockout mice were mixed with 1 × 10<sup>4</sup> CD45.1<sup>+</sup> competitor bone marrow cells and transplanted by injection into the tail vein of CD45.1<sup>+</sup> recipients.

Whole-mount immunostaining
In some cases, we injected mice retro-orbitally with 10 μg of Alexa Fluor 647 anti-mouse CD31 antibody (BioLegend, catalogue number 110724) and 10 μg of Alexa Fluor 647 anti-mouse CD144 antibody (BioLegend,
catalogue number 138006) 10 min before euthanasia in order to visualize the vasculature of the bone marrow as described. Whole-mount sternum staining has been described before. Sterna were processed immediately after euthanasia. After dissection, we removed all connective tissue by gentle scraping with a blade. Fragments with bone marrow cavity were dissected and sectioned along the sagittal or coronal plane to expose the bone marrow as described. Each half of the sternum was fixated in 4% paraformaldehyde (PFA; Electron Microscopy Sciences, catalogue number 15710) in Dulbecco’s PBS (DPBS) for 3 h. Each fragment was washed three times with DPBS, and then blocked for 1 h in DPBS containing 10% (w/v) goat serum (Sigma-Aldrich, catalogue number G9023). We stained each sample with 100 μl staining buffer (2% goat serum in DPBS and the indicated antibodies. For each immunofluorescence image (Fig. 1f, h, i, Extended Data Figs. 1c, i, n, p, o, 2b, d, f, h, j, 3e, i, j, k, 5a, d, g, i, 8k, m, 9c, f, k, m), we show the individual fluorescence channels and indicate which antibody was used for detection. All steps were performed at 4 °C.

Detection of CSF1
After fixation and blocking as above, sternae were stained with 100 μl of staining buffer containing anti-CSF1 antibody (Thermo Fisher Scientific, catalogue number PAS-95279; 1:200 dilution), anti-MHC II antibody (BioLegend, catalogue number 107618) and 2% goat serum in DPBS at 4 °C for 12 h. Each sternum was washed thrice with DPBS and then incubated with Superboost anti–rabbit antibody conjugated to horseradish peroxidase (HRP) (Invitrogen, catalogue number B40943) in DPBS at room temperature for 1 h. After washing three times, each sternum was developed by incubation in 100 μl of tyramide solution (Invitrogen, catalogue number B40943) for 8 min. Sterna were washed three more times in DPBS and used for confocal analyses.

Confocal imaging
For upright confocal imaging, we glued the periosteum to plastic plates, positioning the exposed marrow facing up. Each sternum fragment was 300–μm deep (from the exposed bone marrow surface to the external surface of the periosteum). All fluorophores can be confidently detected to a depth of 35 μm, but we imaged to a depth of 250 μm to ensure that the sternum was fully covered even if the bone marrow surface was not completely parallel to the surface of the plate. We used a Nikon A1R GaAsP multiphoton upright confocal microscope. Specifications for this microscope were as follows: diode lasers with wavelengths of 405 nm, 488 nm, 561 nm and 647 nm; Coherent Chameleon II Ti:Sapphire infrared laser; tunable from 700 nm to 1,000 nm; fully encoded scanning XY motorized stage; Piezo-Z nosepiece for high-speed Z-stack acquisition (100 μm s⁻¹); resonant and galvanometric scanners; four high-quantum-efficiency, low-noise Hamamatsu photomultiplier tubes; a transmitted photomultiplier tube for transmitted light, and second harmonic generation to detect bone. These images were stitched together using Nikon Elements. Stitched whole-bone images were further processed by an artificial intelligence algorithm (Denoise, AI) for noise removal using Nikon Elements. For confetti analyses, we used a Nikon A1R LUN-V inverted confocal microscope. Specifications for the Nikon A1R LUN-V inverted confocal microscope used for inverted confocal sampling: 405 nm, 445 nm, 488 nm, 514 nm, 561 nm and 647 nm diode lasers with fully encoded scanning XY motorized stage; Piezo-Z stage insert for high-speed Z-stack acquisition (100 μm s⁻¹), resonant and galvanometric scanners, two high-quantum-efficiency, low-noise Hamamatsu photomultiplier tubes, a transmitted photomultiplier tube for transmitted light, and two very-high-quantum-efficiency GaAsP photomultiplier tubes for overall detection at 400–820 nm. We used a 40× Apo, 1.15 NA, LWD, differential interference contrast (DIC) water-immersion objective with a working distance of 0.59 mm for high-power imaging. Images were taken using the resonant scanner with line averaging of 8×, a pixel resolution of 1,024 × 1,024, a z-step of 0.5 μm, and a pinhole at 50 μm. We also used a 20× Apo, 0.95 NA, LWD water-immersion objective with a working distance of 0.95 mm. Images were taken using a resonant scanner with line averaging of 8×, a pixel resolution of 1,024 × 1,024, and a z-step of 2 μm.

Image and distance analyses
We used Nikon Elements software (version 5.20.02), Imaris x64 software (version 9.5) and Matlab software (version 2018a) installed in a HP Z4 64-bit workstation with Dual Intel Xeon Processor W-2145, 128 Gb ECC-RAM, and an 8 Gb NVIDIA Quadro RTX5000 graphics card for all our analyses. We analysed only images for which the whole sternum fragment (six to eight images) was successfully imaged. These images were stitched together using Nikon Elements. Stained whole-bone images were further processed by an artificial intelligence algorithm (Denoise, AI) for noise removal using Nikon Elements. For confetti analyses, we used Nikon Elements. We adjusted the brightness and contrast of each channel to be able to detect negative, dim and bright cells for each fluorescent signal. We used Imaris to replace each cell with a colour-coded sphere and obtain its X, Y and Z coordinates. We used Imaris to create surfaces for sinusoids, arterioles and the endosteal surface. For each cell type, we measured the diameter of 50–150 cells to obtain the mean diameter (HSCs, 8.84 ± 1.56 μm; GMPs, 12.68 ± 1.52 μm; MDPs, 12.13 ± 1.19 μm; GPs, 11.70 ± 0.99 μm; MOPs, 11.49 ± 1.25 μm; PN1s, 10.21 ± 0.64 μm; INs, 8.72 ± 0.73 μm; MNS, 8.10 ± 1.09 μm; Ly6C⁺ monocytes, 9.25 ± 0.86 μm; Ly6C⁻ monocytes, 9.30 ± 1.17 μm; cDCs, 12.33 ± 2.69 μm). As cDCs have a significantly higher diameter than other cell types, we used cDCs to define the cell radius and then used this radius to define the cell volume. We used Imaris to measure the distance from each cell to the closest vascular structure or the endosteum, and then subtracted the mean radius for each cell type. To quantify cell-to-cell distance, we exported the coordinates of the cells of interest to Matlab, and then used an algorithm to quantify the distance from the centre of each cell to the centre of all other cells. We then subtracted the mean radius of each cell from these values.

Confetti fate mapping
In the confetti model, GFP is detected in the nucleus whereas RFP is expressed in the cytoplasm. By using antibodies conjugated to fluorochromes (Alexa Fluor 488 and phycoerythrin (PE)) that spectrally overlap with GFP or RFP but that stain only the membrane, we could distinguish Ly6C⁺ GFP cells from GFP⁺ cells and CD11b/Ly6C⁺ RFP⁺ cells from RFP⁺ cells. This allowed us to examine the relationships between GFP⁻ or RFP⁻ labelled cells. As we could not distinguish the membrane signal from the nuclear/cytoplasmic signal in GFP⁺ or RFP⁺ cells, we discarded these from our analyses.

Random simulations
We stained and imaged a sternum fragment with anti-CD45 and anti-Ter119 antibodies to detect haematopoietic cells; anti-CD31, anti-CD144 and anti-Scal antibodies to detect sinusoids and arterioles; and second harmonic generation to detect bone. These images were processed as above to obtain the coordinates of all haematopoietic
cells (59,659 cells), vessels and bone in the sternum. We then used Research Randomizer \(^\text{a}\) to randomly select dots representing each type of myeloid cell at the same frequencies found in vivo through the bone marrow cavity, and measured the distances between these random cells or with vessels and bone as above. Each random simulation was repeated 100–200 times.

To generate random distributions in fate-mapping experiments using confetti mice, we first obtained the coordinates and confetti colour for each type of myeloid cell in each section analysed. Then we used Research Randomizer \(^\text{a}\) to randomly place confetti-labelled cells—at the same frequencies found in vivo—in the positions occupied by the myeloid cells in each section. We then measured the distances between these random cells. Each random simulation was repeated 100–200 times.

**Total cell frequencies in sternum sections**

We compared the number of each type of myeloid cell detected in each sternum section with the average number of haematopoietic cells (59,659 cells) detected by anti-CD45 and anti-Ter119 staining in a whole mounted sternum section.

**Stromal UMAP analysis**

To identify diverse stromal, haematopoietic and other cell populations, we reanalysed 19 independent 10X Genomics captures from two complementary stromal bone marrow scRNAseq data sets (GSE128423; ref. \(^\text{27}\), and GSE108891; ref. \(^\text{28}\)). For GSE108891, samples were reprocessed from the original FASTQ files, consistent with GSE128423 (Cell Ranger software). The merged counts files from these data were scaled, normalized and subjected to an unsupervised analysis with the ICGS software package AltAnalyse (version 2.1.4). This analysis identified 26 transcriptionally distinct cell populations in 9,165 cells from the data set from ref. \(^\text{28}\), and 46 preliminary cell populations in 89,007 cells in the data set from ref. \(^\text{27}\). Preliminary annotations were produced using the ICGS2 BioMarker database, which includes marker genes from more than 1,000 previously defined cell populations. To further annotate these populations and identify subclusters on the basis of prior knowledge, we carried out a secondary analysis using the supervised classification tool cell Harmony \(^\text{40}\), comparing all cells with reference haematopoietic (GSE120409; ref. \(^\text{27}\)) and sorted stromal (GSE108891; ref. \(^\text{28}\)) populations, resulting in 61 final annotated cell populations for the data set from ref. \(^\text{27}\). Visualization of clusters and marker genes was performed using UMAP visualization in AltAnalyse. To allow others to explore side-by-side population-specific markers in these two data sets, we provide an online viewer to query these and other genes in both data sets (http://www.altaanalyse.org/Marrow-Stromal.html).

**Statistics and reproducibility**

In graphs that quantify cells in different mice, we indicate the means; each dot corresponds to one mouse. Statistical differences were calculated using two-tailed Student’s \(t\) tests, and each mouse was considered biologically independent. In graphs that show distances between cells and structures, each dot corresponds to one cell and the horizontal bar indicates the median. Statistical differences were calculated using two-tailed Student’s \(t\) tests and each cell was considered biologically independent.

For cluster analyses, we benchmarked \(k\)-means \(^\text{41}\) and used spectral clustering approaches such as DBSCAN \(^\text{42}\). We validated the clusters by sweeping the parameters on each algorithm, and found an optimal clustering with \(k\)-means and by setting a minimum total count of five PN/IN cells in each cluster. We used the \(k\)-means++ (ref. \(^\text{43}\)) for centroid seeds with a three-dimensional Euclidean distance metric. The computed optimal clusters are characterized by the lower sum of within-PN/IN cluster distances and minimal sum-of-squares point-to-cluster-centroid distances.

Numerous figure panels show images that are representative of several independent experiments, as follows: Fig. 1f, \(n\) = a total of 4 mice in 4 experiments; Fig. 1h, \(n\) = a total of 16 mice in 12 experiments; Fig. 1l, \(n\) = a total of 12 mice in 11 experiments; Fig. 2a, \(n\) = a total of 4 mice in 3 experiments for progenitors, a total of 4 mice in 3 experiments for granulopoiesis, and a total of 6 mice in 6 experiments for monopoiesis; Fig. 2b, \(n\) = a total of 16 mice in 12 experiments; Fig. 2d, \(n\) = a total of 3 mice in 3 experiments; Fig. 2f, \(n\) = a total of 3 mice in 3 experiments; Fig. 2h, \(n\) = a total of 12 mice in 11 experiments; Fig. 2j, \(n\) = a total of 3 mice in 3 experiments; Fig. 3a, \(n\) = a total of 6 mice in 6 experiments; Fig. 3f, \(n\) = a total of 3 mice in 3 experiments; Fig. 4b, \(n\) = a total of 3 mice in 3 experiments for each time point; Fig. 4c, \(n\) = a total of 2 mice in 2 experiments; Fig. 4d, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 1c, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 1l, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 1p, \(n\) = a total of 3 mice in 2 experiments; Extended Data Fig. 2f, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 3b, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 3e, \(n\) = a total of 3 mice in three experiments; Extended data Fig. 3k, \(n\) = total of 3 mice in 3 experiments; Extended Data Fig. 4d, \(n\) = a total of 3 mice in 3 experiments for GP PN IN coronal sections, \(n\) = a total of 2 mice in 2 experiments for GP PN IN sagittal sections; Extended Data Fig. 4f, \(n\) = a total of 4 mice in 4 experiments for MDPLy6C\(^{+}\) monocyte cDC coronal sections, \(n\) = a total of 2 mice in 2 experiments for MDPLy6C\(^{+}\) monocyte cDC sagittal sections; Extended Data Fig. 5a, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 5b, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 5d, \(n\) = a total of 6 mice in 6 experiments; Extended Data Fig. 5f, \(n\) = a total of 26 fragments in 25 experiments; Extended Data Fig. 5g, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 5i, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 5j, \(n\) = a total of 3 mice in 3 experiments for HSCs and MDPs and \(n\) = a total of 3 mice in 3 experiments for HSCs, GPs and MOPs; Extended Data Fig. 8k, \(n\) = a total of 3 mice in 3 experiments for anti-CSF1 staining and \(n\) = a total of 3 mice in 3 experiments for isotype control; Extended Data Fig. 8l, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 8m, \(n\) = a total of 3 mice in 3 experiments; Extended Data Fig. 8p, \(n\) = a total of 3 mice in 3 experiments for control and \(n\) = a total of 3 mice in 3 experiments for CSF1\(^{−/−}\); Extended Data Fig. 8q, \(n\) = a total of 3 mice in 3 experiments for control and \(n\) = a total of 3 mice in 3 experiments for CSF1\(^{−/−}\); Extended Data Fig. 9f, \(n\) = a total of 2 mice in 2 experiments; Extended Data Fig. 9i, \(n\) = a total of 2 mice in 2 experiments; Extended Data Fig. 9k, \(n\) = a total of 2 mice in 2 experiments; Extended Data Fig. 9m, \(n\) = a total of 2 mice in 2 experiments; Extended Data Fig. 9p, \(n\) = a total of 3 mice in 3 experiments for control and \(n\) = a total of 3 mice in 3 experiments for CSF1\(^{−/−}\).

**Data reporting**

Sample sizes were chosen on the basis of previous studies. No statistical methods were used to predetermine sample size. All mice were included in the analyses. Mice were randomly allocated to the different groups on the basis of cage, genotype and litter size. For all experiments, we aimed to have the same number of mice in the control and experimental groups. Most experiments were carried out with unperturbed wild-type mice and thus these samples were not blinded. The phenotypes of mice with conditional deletion of CSF1 in endothelial cells or after L. monocytogenes infection were obvious in all imaging analyses, making blinding moot.

**Reporting summary**

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

Source data for quantities described in the text or shown in graphs plotted in Figs. 1–4 and Extended Data Figs. 1–5, 7–9 are available with the manuscript. scRNAseq data shown in Fig. 3c and Extended Data Fig. 6 are reanalyses of published data sets: GSE128423 (ref. 27) and GSE108891 (ref. 28). These scRNAseq data underwent a preliminary annotation using the ICGS2 BioMarker database40, followed by a secondary analysis using the supervised classification tool cellHarmony40, comparing all cells to reference haematopoietic cells44 (GSE120409)44. Supplementary Table 2 shows the different ICGS2 marker genes and cellHarmony barcode assignments for the different cell clusters identified. Supplementary Table 1 lists all of the reagents and other resources used in the experiments described. Source data are provided with this paper.

31. Harris, S. E. et al. Meox2Cre-mediated disruption of CSF-1 leads to osteopetrosis and osteocyte defects. Bone 50, 42–53 (2012).
32. Thambirajah, R. et al. GFI1 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSLD1. Nat. Cell Biol. 18, 21–32 (2016).
33. Liu, Z. et al. Fate mapping via Ms4a3-expression history traces monocyte-derived cells. Cell 178, 1509–1525 (2019).
34. Bowers, E. et al. Granulocyte-derived TNFα promotes vascular and hematopoietic regeneration in the bone marrow. Nat. Med. 24, 95–102 (2018).
35. Schlitzer, A. et al. Identification of CD1c1- and CD2-negative DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat. Immunol. 16, 718–728 (2015).
36. Akashi, K., Traver, D., Miyamoto, T. & Weissman, I. L. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404, 103–107 (2000).
37. Chow, A. et al. CD169+ macrophages provide a niche promoting erythropoiesis under homeostasis and stress. Nat. Med. 19, 429–436 (2013).
38. Takaku, T. et al. Hematopoiesis in 3 dimensions: human and murine bone marrow architecture visualized by confocal microscopy. Blood 116, e41–e55 (2010).
39. Urbaniak, G. C. & Plous, S. Research Randomizer (version 4.0). http://www.randomizer.org/ (2013).
40. DePasquale, E. A. K. et al. cellHarmony: cell-level matching and holistic comparison of single-cell transcriptomes. Nucleic Acids Res. 47, e138 (2019).
41. Jain, A. K., Murty, M. N. & Flynn, P. J. Data clustering: a review. ACM Comput. Surv. 31, 264–323 (1999).
42. Ester, M., Kriegel, H., Sander, J. & Xiaowei, X. A density-based algorithm for discovering clusters in large spatial databases with noise. In Proc. 2nd Intl Conf. Knowledge Discovery Data Mining. 96, 226–231 (1996).
43. Arthur, D. & Vassilvitskii, S. k-means++: the advantages of careful seeding. In Proc. 18th Annual Acm-Siam Symp. Discrete Algorithms (Soc. Industry and Applied Mathematics) 1027–1035 (Philadelphia, PA, 2007).
44. Muench, D. E. et al. Mouse models of neutropenia reveal progenitor-stage-specific defects. Nature 582, 109–114 (2020).
45. Pinho, S. et al. PDGF-Rα and CD51 mark human Nestin+ sphere-forming mesenchymal stem cells capable of hematopoietic progenitor cell expansion. J. Exp. Med. 210, 1351–1367 (2013).

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Author contributions D.L. conceptualized and managed the study. D.L., J.Z., H.L.G., N.S., J.D.E., J.M.K., A.D., S.S.W. and Q.W. designed experiments. J.Z. and Q.W. developed all of the stains and generated the infected mice with L. monocytogenes infection. N.S., V.B.S.P. and L.F.H. performed bioinformatics analyses. D.L., J.Z. and Q.W. performed FACS analyses. J.X.J., A.S., M.M. and B.W. mapped haematopoietic cells for random simulations. G.P. and J.M.K. helped in designing granulopoiesis experiments and reviewing the manuscript. We also thank A. Hidalgo for feedback on the manuscript. We are grateful to L. G. Ng, J. Kwok and K. Leong for help in designing granulopoiesis experiments and reviewing the manuscript. We also thank the Confocal Imaging Core, the Research Flow Cytometry Core and the Veterinary Services at the University of Michigan and Cincinnati Children’s Medical Center for experimental and technical assistance. This work was partially supported by the National Heart Lung and Blood Institute (grants RO1HL122661 to H.L.G. and RO3HL136529 to D.L.) V.B.S.P. is supported by National Institutes of Health (NIH)/National Center for Advancing Translational Sciences (NCATS) grant U2CTR002818, NIH/National Heart, Lung and Blood Institute (NHLBI) grant U24HL148865, and NIH/National Institute of Allergy and Infectious Diseases (NIAID) grant U01AI150748. N.S. is supported by the Cincinnati Pediatric Cell Atlas Center. J.F.H. is supported by the Department of Defense (DOD) through a Peer Reviewed Cancer Research Program (PRCPR) award, W81XWH-20-1-0870(NCA191188). S.S.W. is supported by the NIH through grants R01AI120202, R01AI124657 and DP1AI131080 and by the Howard Hughes Medical Institute (HHMI) Faculty Scholar’s program, the March of Dimes Ohio Collaborative for Prematurity Research, and a Burroughs Wellcome Fund Investigator in Pathogenesis Award. J.X.J. is supported by NIH/National Institute of Aging (NIA) grant AG045040 and a Welch Foundation Grant, AQ-1507. A.S. is supported by grant T32 AI08795/NIH from the NIH Department of Health and Human Services (HHS) of the U.S. Data were generated using an SH800 cell sorter funded by NIH grant S10/OD023410.

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Additional information

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Validation of stains to detect myeloid cells. a, FACS plots showing the gating strategy used to identify MDPs and MOPs (as described in ref. 19). BM, bone marrow. b, Gating strategy used to identify GMPs, GPs and MOPs (as described in ref. 16). The lineage panel contains antibodies against Ly6G, CD11b, Ter119, B220 and CD3. c, Images showing that Ly6C labels arterioles, detected as either CD31+CD144brightSca1bright in wild-type mice or CD31+CD144brightNestinbright structures in Nestin–GFP mice; the histograms show quantifications demonstrating that all Sca1+ arterioles or Nestin–GFPbright arterioles are also Ly6C+. Scale bars, 50 μm. d, e, FACS plots (d) showing that only the CD11b+ gate contains CD117+CD115+ or CD117+Ly6C+ cells, and histogram (e) showing that GPs and MOPs are the only Ly6C+ cells in the Lin−CD117+ gate. Together these data indicate that CD11b alone can be used to replace the lineage panel to exclude contamination of mature cells when detecting MDPs, MOPs and GPs. f, Cell numbers per femur detected by FACS of the indicated progenitors using previously described strategies 16,19 (white) or that described in Fig. 1g (purple). g, Colony-forming activity (green, MDPs; orange, MOPs; red, GPs) of the indicated progenitors using previously described strategies 16,19 (diamonds) or the one described in Fig. 1g (circles). For f, g, n = a total of three mice in two experiments. h, Frequency of total BM cells for each of the indicated populations in sternum when detected by FACS (white) or imaging (orange). n = 3. i, Representative image showing that CD11b+CD117+CD115+Ly6C+ MDPs and CD11b+CD117+CD115+Ly6C+ MOPs are GFP+ in Cx3cr1–gfp mice. Scale bar, 10 μm. j, qPCR results showing Gfi1 and Irf8 expression (relative to Gapdh expression) in FACS-purified GPs or MOPs; n = a total of six mice in three experiments. k, FACS analyses in Gfi1–tdTomato mice showing differential tdTomato expression in GPs and MOPs in Gfi1–tdTomato mice. l, Quantification of promyelocytes (PMs), myelocytes (MCs), metamyelocytes (MMs), banded cells (BCs) and polymorphonucleated neutrophils (PMNs) in cytospin preparations of FACS-purified PMs, MCs and MMs. n = a total of two mice. Scale bar, 10 μm. m, The stains require discrimination of CD16/32, CD117 and Ly6G bright and dim cells. The panels show the gating strategy, experimental design, and quantification of frequencies of decanted CD16/32, CD117 and Ly6G bright and dim cells or PMs, MCs and MMs when compared with frequencies obtained by FACS before cytospinning. Each dot represents one image field from two experiments. n, o, Dendritic cells can be imaged as reticulated CX3CR1–GFP+ or CX3CR1–GFP+ MHC II+ cells in Cx3cr1–gfp reporter mice 23,24. The images and histograms show that all reticulated GFP+ cells were also MHC II+ and CD11c+, indicating that MHC II expression and cell shape are sufficient to unambiguously identify dendritic cells and distinguish them from macrophages that are CXCR1–GFP−CD11c− cells 24; n = a total of three mice. Scale bar, 10 μm. p, Image and histogram showing that CX3CR1–GFP+ MHCII+ dendritic cells are conventional dendritic cells, as they are CD11b+ but do not express B220 or CD8. Scale bar, 10 μm. n = a total of three mice. q, FACS gating strategy for isolation and imaging of the indicated cells. Dendritic cells are detected as MHCII+ reticulated cells in imaging analyses. In all bar graphs, one dot corresponds to one mouse. Statistical differences were calculated using two-tailed Student’s t-tests; P values are shown.
Extended Data Fig. 2 | Strategies to map myelopoiesis in whole mounted sternum. a, Scheme showing the experimental pipeline to identify myeloid cells, obtain the X, Y and Z coordinates, and replace each myeloid cell with a colour-coded sphere centred on the cell to better visualize differentiation and to generate random distributions. Scale bar, 200 μm. b, Histograms showing the observed distribution of distances from each GMP (blue), MDP (green), MOP (orange) and GP (red) or random cell (white) to the closest indicated cell (n = 86 GMPs from 4 sternum sections of 4 mice; n = 243 MDPs from a total of 23 sternum sections of 15 mice; n = 458 MOPs from a total of 11 sternum sections of 11 mice; n = 338 GPs from a total of 15 sternum sections of 12 mice). c, Left, XY graphs showing the location of GPs, PNs and INs in mouse sternum sections; centre, the different colour-coded PN/IN clusters identified using the k-means algorithm; and right, PN/IN clusters containing (pink) or not containing (blue) GPs within the cluster. d, Number of PNs and INs in each type of cluster. n = 1,443 PNs from a total of 3 sternum sections from 3 mice in clusters with GPs; n = 1,050 PNs from a total of 3 sternum sections from 3 mice in clusters without GPs; n = 880 INs from a total of 3 sternum sections from 3 mice in clusters with GPs; n = 866 PNs from a total of 3 sternum sections from 3 mice in clusters without GPs. e, Experimental design, representative image, and histogram showing the percentage of CFP-, GFP-, RFP- and YFP-positive cells in fate-mapping experiments using Ubc–creERT2:confetti mice. Scale bar, 10 μm. Each dot represents one sternum segment from a total of three confetti mice. TAM, tamoxifen. f, In the confetti model, GFP is detected in the nucleus whereas RFP is expressed in the cytoplasm. The images show that by using antibodies conjugated to fluorochromes (Alexa Fluor488 and phycoerythrin (PE)) that spectrally overlap with GFP or RFP but that stain only the membrane, we could distinguish CD11b–Alexa488–GFP−, Ly6C–Alexa488–GFP− cells from GFP+ cells and CD115–PE–RFP+, Ly6C–PE–RFP+ from RFP+ cells. This allowed us to examine the relationships between YFP- or CFP-labelled cells. As we could not distinguish the membrane signal from the nuclear/cytoplasmic signal in GFP+ or RFP+ cells, these were discarded from the analyses. Scale bar, 10 μm. ‘α’ refers to an antibody against the indicated molecule. g, Percentage of PN clusters with at least one confetti-labelled PN that are oligoclonal (containing cells with at least two different origins: CFP+, YFP+, or no confetti label). Each dot represents one sternum segment from a total of three confetti mice. Statistical differences were calculated using two-tailed Student’s t-tests; P values are shown. Ob, observed; Rd, random.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Confetti analyses of the differentiation of monocytes and dendritic cells. a, Histograms showing the observed (colour) and random (white) distribution of distances from each MOP to the closest indicated cells (MOP–Ly6C\(^+\) monocyte, 137 MOPs from a total of 3 sternum sections from 3 mice; MOP–Ly6C\(^-\) monocyte, 171 MOPs from a total of 4 sternum sections from 4 mice; MOP–cDCs, 200 MOPs from a total of 5 sternum sections from 4 mice). b, Maps showing the location of Gfi1\(^+\) and Gfi1\(^-\) MOPs, MDPs, Ly6C\(^+\) monocytes and Ly6C\(^+\) monocytes in a sternum segment from Gfi1–tomato mice. Scale bar, 200 μm. c, Number of Gfi1\(^+\) and Gfi1\(^-\) MOPs per sternum segment. Each dot represents one sternum segment from three Gfi1–tomato mice in two experiments. d, Histograms showing the observed (colour) and random (white) distribution of distances from each Gfi1\(^+\) MOP (orange) and Gfi1\(^-\) MOP (purple) to the closest indicated cells. n = 113 Gfi1\(^+\) MOPs and n = 72 Gfi1\(^-\) MOPs from a total of 3 sternum segments from 3 mice. e, Representative image showing the lack of contribution of a confetti-labelled MOP to surrounding monocytes. Tracked cells are YFP\(^+\), CFP\(^+\) or unlabelled CD117\(^+\) CD115\(^+\) Ly6C\(^+\) MOPs, CD117\(^+\) CD115\(^+\) Ly6C\(^-\) monocytes and CD117\(^+\) CD115\(^+\) Ly6C\(^+\) monocytes (bottom) found within the indicated distances to the closest CFP\(^+\) MOP cell. Each dot represents one CFP\(^+\) MOP (n = 9 MOPs from a total of 6 sternum segments from 4 confetti mice). f, Histograms showing the observed (colour) and random (white) distribution of distances from each Ly6C\(^+\) monocyte (yellow) and cDC (pink) to the six closest indicated neighbour cells. n = 1,603 Ly6C\(^+\) monocytes from a total of 3 sternum segments from 3 mice; n = 1,228 cDCs from a total of 6 sternum segments from 6 mice. i, Images showing the lack of contribution of a CFP\(^+\) MDP to surrounding Ly6C\(^+\) monocytes. Tracked cells are YFP\(^+\), CFP\(^+\) or unlabelled CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^+\) MDPs, CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^-\) MOPs, CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^+\) monocytes and CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^-\) monocytes. Scale bar, 20 μm. j, Images showing a lack of association between confetti-labelled Ly6C\(^+\) and Ly6C\(^-\) monocytes. Tracked cells are YFP\(^+\), CFP\(^+\) or unlabelled CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^+\) monocytes and CD117\(^+\) CD115\(^+\) CD11b\(^+\) Ly6C\(^-\) monocytes. Scale bars for main and zoomed-in images, 40 μm and 10 μm respectively. The histograms show the observed (colour) and random (white) distribution of distances from each confetti-labelled Ly6C\(^+\) (blue) or Ly6C\(^-\) (yellow) monocytes to the closest Ly6C\(^+\) or Ly6C\(^-\) monocyte in the same colour. n = 48 confetti-labelled Ly6C\(^+\) monocytes, and n = 32 confetti-labelled Ly6C\(^-\) monocytes, from a total of 3 sternum sections from 3 mice. k, Images showing a lack of association between confetti labelled cDCs. Tracked cells are RFP\(^+\), GFP\(^+\), YFP\(^+\), CFP\(^+\) or unlabelled MCH II\(^+\) reticulated cDCs. Scale bars for main and zoomed-in images, 40 μm and 10 μm respectively. The histogram shows the observed (colour) and random (white) distribution of distances from each confetti-labelled cDC (pink) to the closest cDC in the same colour. n = 80 confetti-labelled cDCs from a total of 3 sternum sections from 3 mice. Unless otherwise indicated, for all graphs one dot corresponds to one cell. Horizontal blue bars indicate the median distance. Statistical differences were calculated using two-tailed Student’s t-tests; P values are shown.
Extended Data Fig. 4 | See next page for caption.
**Extended Data Fig. 4 | The architecture of myelopoiesis is similar in different sternum sections.**

**a.** Observed distributions of distances for individual sternum sections (Ob), pooled values, or random (Rd) simulations for the data shown in Extended Data Fig. 2b. For distance to GMPs, n = 25, 22, 19, 20 GMPs per sternum section from a total of 4 sternum sections in 4 mice; n = 11, 12, 11, 10 MDPs per sternum section from a total of 4 sternum sections in 4 mice; n = 40, 37, 42, 46 MOPs per sternum section from a total of 4 sternum sections in 4 mice; n = 19, 21, 16, 26 GPs per sternum section from a total of 4 sternum sections in 4 mice. For distance to MDPs, n = 25, 22, 19, 20 GMPs per sternum section from a total of 4 sternum sections in 4 mice; n = 13, 14, 11, 9, 7, 14, 9, 11, 12, 10, 11, 13, 11 MDPs per sternum section from a total of 16 sternum sections in 16 mice; n = 40, 37, 42, 46 MOPs per sternum section from a total of 4 sternum sections in 4 mice; n = 22, 24, 28, 23, 16, 24, 18, 22, 21, 17, 26, 25, 20 GPs per sternum section from a total of 14 sternum sections in 14 mice. For distance to MOPs, n = 25, 22, 19, 20 GMPs per sternum section from a total of 4 sternum sections in 4 mice; n = 9, 11, 12, 10, 11, 13, 11, 10, 14, 11, 13, 10, 9, 11, 10, 14 MDPs per sternum section from a total of 16 sternum sections in 16 mice; n = 39, 49, 38, 33, 44, 36, 37, 44 MOPs per sternum section from a total of 8 sternum sections in 8 mice; n = 22, 24, 28, 23, 16, 24, 18, 22, 21, 17, 26, 25, 20 GPs per sternum section from a total of 13 sternum sections in 13 mice. For distance to GPs, n = 25, 22, 19, 20 GMPs per sternum section from a total of 4 sternum sections in 4 mice; n = 15, 12, 11, 10, 15, 9, 12, 11, 12, 11, 13, 10, 9, 11, 10, 15 MDPs per sternum section from a total of 16 sternum sections in 16 mice; n = 40, 37, 42, 46, 39, 39, 49, 53, 38, 34 MOPs per sternum section from a total of 10 sternum sections in 10 mice; n = 24, 23, 23, 28, 29, 22, 26, 17, 26 GPs per sternum section from a total of 9 sternum sections in 9 mice. **b, c.** As for **a**, but corresponding to the data shown in Fig. 2c, i. n = 24, 23, 28 GPs per sternum section from a total of 3 sternum sections in 3 mice. For MDPs to Ly6C\(^{hi}\) and Ly6C\(^{lo}\) monocytes, n = 11, 14, 8, 9, 9, 16 MDPs per sternum section from a total of 6 sternum sections in 4 mice. For MDPs to cDCs, n = 13, 14, 12, 9, 16, 15, 10, 10, 15 MDPs per sternum section from a total of 11 sternum sections in 6 mice. **d, e.** Throughout the paper, we have indistinctly used coronal and sagittal sternum sections. The maps (**d**) show granulopoiesis in coronal and sagittal sternum sections shown or analysed in Fig. 2a or Fig. 2c; the histograms show (**e**) observed and random distributions of distances for the indicated cells in each section or the pooled data for each type of section. n = 23 and 28 GPs in coronal sections and n = 24 and 23 GPs in sagittal sections. Scale bar, 200 μm. **f, g.** Representative maps showing the differentiation of monocytes and dendritic cells (**f**), comparing coronal and sagittal sternum sections with those shown or analysed in Fig. 2a, i; and observed and random distributions (**g**) of distances for the indicated cells in coronal or sagittal sections. n = 11 or 10 MDPs, n = 390 or 334 Ly6C\(^{hi}\) monocytes, and n = 218 or 258 cDCs in coronal sections; n = 9 or 10 MDPs, n = 419 or 380 Ly6C\(^{lo}\) monocytes, and n = 183 or 159 cDCs in sagittal sections. Scale bar, 200 μm. Statistical differences were calculated using two-tailed Student’s t-tests; P-values are shown.
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | Interaction of myeloid progenitors with the microenvironment and with HSCs. a, Representative images showing the simultaneous detection of PNs, INs, Ly6C<sup>lo</sup> monocytes and cDCs. Scale bar, 10 μm. b, Map showing the location of the indicated cells in the bone marrow. Each dot corresponds to one cell. Note that the radius of each dot is two times the average radius of the cell. Scale bar, 200 μm. c, Histograms showing the distance from each Ly6C<sup>lo</sup> monocyte (yellow dots) or cDC (pink dots) and their random simulation (white dots) to the closest indicated cell (Ly6C<sup>lo</sup> to PN/IN, n = 500 Ly6C<sup>lo</sup> monocytes; cDC to PN/IN, n = 727 cDCs; Ly6C<sup>lo</sup> to cDC, n = 1,322 Ly6C<sup>lo</sup> monocytes; from a total of 3 sternum sections from 3 mice). d, High-power images showing the relative positions of MDPs, MOPs, GPs and sinusoids. Scale bar, 10 μm. e, Histograms showing the distance from each MDP (green dots), MOP (orange dots), GP (red dots) or random distribution (white dots) to the closest indicated structure (for distances to arterioles, n = 62 MDPs from a total of 6 sternum sections of 6 mice; n = 218 MOPs and n = 114 GPs from a total of 5 sternum sections from 5 mice; for distances to endosteal surface, n = 98 MDPs, n = 410 MOPs, n = 217 GPs, from a total of 9 sternum from 6 mice). f, Representative images of multiple sternum segments, showing that MDPs are evenly distributed through the bone marrow, consistent with their sinusoidal location. Scale bars, 200 μm. g, Representative images showing the detection of HSCs and MDPs in a single stain. Scale bar, 10 μm. h, Quantification of MDP and Lin<sup>−</sup> CD117<sup>+</sup> CD48<sup>−</sup> CD41<sup>dim</sup> CD150<sup>+</sup> HSCs in femurs by FACS (white) or imaging (orange) analyses. Each dot corresponds to one mouse femur or sternum image. n = 4. i, Representative images showing detection of HSCs and a population containing CD117<sup>+</sup> Ly6C<sup>+</sup> GPs and MOP in a single stain. Scale bar, 10 μm. j, Maps and histograms showing the relationships between HSCs and MDPs or GPs/MOPs in the bone marrow. In the map, the dot radius is three times the average cell radius. Scale bar, 200 μm. (n = 35 MDPs from a total of 4 sternum sections from 3 mice, and n = 191 GPs and MOPs from a total of 3 sternum sections from 3 mice.) Unless otherwise indicated, for all graphs one dot corresponds to one cell. Horizontal blue bars indicate the median distance. Statistical differences were calculated using two-tailed Student’s t test; P values are shown.
Extended Data Fig. 6 | Broad comparison of stromal bone marrow compartments by scRNAseq. Comparative analyses of two previously described scRNAseq data sets profiling stromal and haematopoietic cell populations in bone marrow (9,165 cells from ref. 26 and 89,007 cells from ref. 27).

a–c, Predictions of cell populations displayed on a UMAP plot from an unsupervised analysis of the two separate scRNAseq data sets (using ICGS version 2). Distinct captures are denoted by the gating strategy (col2.3, niche-col2.3, niche-LepR+ and niche-VEcad+). Populations are denoted as haematopoietic or stromal/mesenchymal on the basis of previously defined signatures of marker genes from scRNAseq cell populations (ICGS; see cluster labels in each panel).

d–l, Relative expression of marker genes from ICGS2-identified cell populations (identified in both data sets), projected onto the two UMAP plots to verify cell identity (gene expression is shown relative to capture strategy). Supplementary Table 2 shows the different ICGS2 marker genes and cellHarmony barcode assignments for the different cell clusters identified.
Extended Data Fig. 7 | CSF1 from LepR+ cells is dispensable for myelopoiesis. a, qPCR analyses showing Csf1 mRNA levels (relative to Gapdh) in Nestin–GFP<sup>dim</sup> perivascular cells (which largely overlap with LepR<sup>+</sup> perivascular cells<sup>45</sup>). n = a total of four control mice and n = total of four Csf1<sup>ΔLepR</sup> mice in four experiments. b, Number of bone marrow cells or the indicated cell populations in the femur of control or Csf1<sup>ΔLepR</sup> mice. n = a total of six control and n = a total of eight Csf1<sup>ΔLepR</sup> mice in six experiments. c, Colony-forming activity (blue, GMPs; green, MDP; orange, MOPs; red, GPs) of the indicated progenitors, FACS-purified from control (diamonds, n = 5) or Csf1<sup>ΔLepR</sup> (circles, n = 5) mice in 4 experiments. d, Number of the indicated populations in the blood of control or Csf1<sup>ΔLepR</sup> mice. In all panels, one dot equals one mouse. n = a total of six control and n = a total of eight Csf1<sup>ΔLepR</sup> mice in six experiments. Statistical differences were calculated using two-tailed Student’s t-tests; P values are shown.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | CSF1 from a subset of endothelial cells is necessary for the generation of dendritic cells. a, qPCR analyses showing Csf1 mRNA levels (normalized to endothelial control, Ctrl) in FACS-purified endothelial cells and the indicated haematopoietic cells in control or Csf1ΔEC mice; n = a total of four control and n = a total of four Csf1ΔEC mice in four experiments. Note that although Cdh5-cre also recombines in subsets of haematopoietic cells, it does not affect Csf1 expression in these cells. (n.d., none detected; n = a total of three control and n = a total of three Csf1 ΔEC mice in three experiments.)

EOS, eosinophil; Ery, erythrocyte. b, Colony-forming activity (blue, GMPs; green, MDPs; orange, MOP; red, GPs) of the indicated progenitors FACS-purified from control (diamonds, n = 5) or Csf1ΔEC (circles, n = 5) mice in four experiments. c, d, Number (c) and CFU-M activity (d) of GMPs or MOPs from control or Csf1ΔEC mice. n = a total of six control and n = a total of six Csf1ΔEC mice in five experiments. e, Number of Ly6Clo and Ly6Chi monocytes in the bone marrow and peripheral blood of control or Csf1ΔEC mice. n = a total of eight control and n = a total of eight Csf1ΔEC mice in six experiments. f, h, Number of bone marrow cells from the indicated populations in the femur or blood of control or Csf1ΔEC mice. n = a total of eight control and n = a total of eight Csf1ΔEC mice in six experiments. i, Volume and number of vessels in sternum sections of control or Csf1ΔEC mice. Each dot represents one sternum segment, from three control and three Csf1ΔEC mice. j, Percentages of the indicated CD45.2+ cells in the blood of lethally irradiated Cdot control or Csf1ΔEC mice. Each dot represents one mouse. k, Representative images showing anti-CSF1 or isotype control stains in the bone marrow of wild-type mice. Scale bars, 200 μm and 10 μm. l, Map of CSF1+ and CSF1− vessels and cDCs in Csf1ΔEC mice. Scale bar, 200 μm. m, High-power images showing a CSF1+ vessel and cDCs (pink dots) in control mice. The radius of the dot is two times the average cDC radius. Scale bar, 20 μm. n, Number of cDCs found within the indicated distances of CSF1+ and CSF1− vessels in wild-type (n = 76 CSF1+ vessels and n = 520 CSF1− vessels in a total of 4 sternum sections from 3 wild-type mice). o, Histograms showing the distance from each cDC to the closest sinusoid in control or Csf1ΔEC mice (n = 451 cDCs in a total of 2 sternum sections from 2 control mice; n = 343 cDCs in a total of 3 sternum sections from 3 Csf1ΔEC mice). p, Maps showing the relocation of MDPs away from sinusoids in Csf1ΔEC mice. Scale bars, 200 μm and 10 μm. The radius of the dots is three times (left) or one times (right) the average radius of the MDP.

q, Maps showing the distribution of MDPs, Ly6Clo monocytes and cDCs in the sternum of control or Csf1ΔEC mice. Scale bar, 200 μm. The radius of the dot is three times (for MDPs) or two times (for all other cells) the average radius of the replaced cell. r, Histograms showing the distribution of distances from each MDP to the six closest Ly6Clo monocytes or cDCs in control or Csf1ΔEC mice. (For MDPs to Ly6Clo monocytes, n = 37 MDPs from a total of 4 sternum sections from 3 control mice; n = 18 MDPs from a total of 4 sternum sections from 3 Csf1ΔEC mice. For MDPs to cDCs, n = 47 MDPs from a total of 6 sternum sections from 3 control mice; n = 47 MDPs from a total of 9 sternum sections from 3 Csf1ΔEC mice.) Unless otherwise indicated, for a–i each dot corresponds to one mouse; for j–r each dot corresponds to one cell. Statistical differences were calculated using two-tailed Student’s t-tests; P values are shown.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Changes in progenitor localization after infection.

a, Average percentage of the indicated cells per femur (normalized to numbers at day 0) at the indicated time points after infection of wild-type mice with *L. monocytogenes*. (n = 6 mice for days 0 and 2; n = 3 mice for day 4; n = 4 mice for day 6; n = 3 mice for day 8.) b, Histograms showing the distribution of distances from each MDP (green, n = 243 MDP from a total of 23 sternum sections from 15 uninfected wild-type mice and n = 37 MDPs from a total of 3 sternum sections from 3 wild-type mice 4 days after infection with *L. monocytogenes*), MOP (orange, n = 458 MOP from a total of 11 sternum sections from 11 uninfected wild-type mice and n = 218 GPs from a total of 3 sternum sections from 3 wild-type mice 4 days after infection with *L. monocytogenes*) or GP (red, n = 338 GPs from a total of 15 sternum sections from 12 uninfected wild-type mice and n = 218 GPs from a total of 3 sternum sections from 3 wild-type mice 4 days after infection with *L. monocytogenes*). The values for day 0 (d0) are the same as shown in Fig. 2g, i.

c, Number of GPs or MOPs per cluster for the sternum sections analysed in b. Each dot represents a cluster; n = 17 GP clusters and n = 15 MOP clusters from 3 wild-type mice 4 days after infection with *L. monocytogenes*. d, Experimental design for in vivo fate mapping using Ubc−creERT2-confetti mice. e, Percentage of GP or MOP clusters with at least one confetti-labelled GP or MOP that are monoclonal (all cells in the cluster are labelled in the same confetti colour) or oligoclonal (containing cells with at least two different origins: CFP−, YFP−, or no confetti label). Each dot represents a GP or MOP cluster from a total of three sternum segments from two confetti mice in two experiments four days after infection with *L. monocytogenes*. f, Representative images showing a cluster composed of MDP-derived Gfi1 hi MOPs and GMP-derived Gfi1 hi MOPs in *Gfi1-tomato* mice four days after infection with *L. monocytogenes*. Scale bar, 10 μm. g, Percentage of GMP-derived Gfi1 lo MOPs (orange dots) or MDP-derived Gfi1 lo MOPs (purple dots) per cluster (each dot represents one cluster; n = 15 clusters in a total of 3 sternum sections from two *Gfi1-tomato* mice four days after infection with *L. monocytogenes*).

h, Quantification of CD117 expression in PNs of wild-type mice at the indicated time points after infection. (n = 6 mice for day 0; n = 8 mice for days 2 and 4; n = 5 mice for day 6; n = 4 mice for day 8 in total 8 experiments). One dot indicates one mouse. i, Map showing the location of Gfi1 hi and Gfi1 lo MOPs, MDPs, and Ly6C hi and Ly6C lo monocytes in a sternum segment from *Gfi1-tomato* mice four days after infection. Scale bar, 200 μm. j, Histograms showing the distribution of distances from each Gfi1 hi (orange) and Gfi1 lo (purple) MOP and MDP (green) to the indicated cells in uninfected wild-type mice or four days after infection with *L. monocytogenes*. The values for day 0 (d0) are the same as shown in Fig. 2g, i.

(n = 113 Gfi1 hi MOP and n = 72 Gfi1 lo MOPs from a total of 3 sternum sections from 3 uninfected *Gfi1-tomato* mice; n = 155 Gfi1 hi MOP and n = 144 Gfi1 lo MOP from total 3 sternum sections of 2 *Gfi1-tomato* mice 4 days after infection with *L. monocytogenes*; MDP to Ly6C hi monocyte, n = 67 MDPs from a total of 6 sternum sections from 4 mice; MDP to Ly6C lo monocyte, n = 67 MDPs from a total of 6 sternum sections from 4 wild-type uninfected mice; MDP to cDC, n = 139 MDPs from a total of 11 sternum sections from 6 wild-type uninfected mice; and n = 32 MDPs from a total of 3 sternum sections from 3 wild-type mice 4 days after infection with *L. monocytogenes*.) k, Representative image showing lack of contribution of a confetti-labelled MOP. Tracked cells are YFP+, CFP+ or unlabelled CD117+CD115+CD11b+Ly6C+MOPs, CD117+CD115+CD11b+Ly6C hi monocytes and CD117+CD115+CD11b+Ly6C lo monocytes. Scale bar, 10 μm. l, Quantification of cell numbers for CFP− (white) and CFP+ (blue) Ly6C hi monocytes (left) or Ly6C lo monocytes (right) found within the indicated distances to the closest CFP+ MOP cell in confetti mice four days after infection with *L. monocytogenes*. Each dot represents one CFP+ MOP from a total of eight sternum segments from two confetti mice in two experiments four days after infection. m, Representative images showing the lack of contribution of a confetti-labelled MDP to surrounding monocytes. Tracked cells are YFP+, CFP− or unlabelled CD117+CD115+CD11b+Ly6C+MOPs, CD117+CD115+CD11b+Ly6C hi MOPs, CD117+CD115+CD11b+Ly6C lo monocytes and CD117+CD115+CD11b+Ly6C hi monocytes. Scale bar, 20 μm. n, qPCR analyses showing Csf1mRNA levels (normalized to non-infected) in bone marrow endothelial cells FACS-purified from wild-type mice in the steady-state or four days after infection. n = a total of three uninfected mice and n = a total of three infected mice in two experiments.

o, Histogram showing the distance from each MDP to the closest sinusoid in control (pool of *Cre:Csf1 ΔEC*, *Csf1 fl/fl* and *Csf1 ΔEC*) or *Csf1 ΔEC* mice four days after infection. n = 36 MDPs from a total of 4 sternum sections from 3 control mice and n = 36 MDPs from a total of 4 sternum sections from 3 *Csf1 ΔEC* mice.

p, q, Maps (p) showing the location of the indicated cells; and histogram (q) showing the distance from each MDP to the closest Ly6C+ monocyte and cDC in control or *Csf1 ΔEC* mice four days after infection. n = 51 MDPs from a total of 3 sternum sections of 3 control mice and n = 29 MDP from total 3 sternum sections of 3 *Csf1 ΔEC* mice. r, Number of the indicated cells per femur in control or *Csf1 ΔEC* mice 4 days after infection. Each dot indicates one mouse. n = 3 control and n = 3 *Csf1 ΔEC* mice. Unless otherwise indicated, one dot represents one cell. Statistical differences were calculated using two-tailed Student’s *t*-tests; *P*-values are shown.
Extended Data Fig. 10 | Architecture of myelopoiesis at steady state and after infection. The models show the spatial distributions of, and average distances between, the indicated cells at steady state and four days after infection with *L. monocytogenes*. 

Serial GP recruitment to the cluster with each GP contributing 2 to 8 PN.
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- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted. Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- 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: Nikon NIS Elements software (5.20.02) for imaging; FACSDiva v8.0.1 for flow cytometry.

Data analysis: Imaris x64 software (9.5) for image analysis, FlowJo v10 for flow cytometry, Matlab software (2018a) for distance analysis, GraphPad Prism v8 for statistics.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Source Data for quantifications described in the text or shown in graphs plotted in Figs. 1-4 and Extended Data Figs. 1-9 are available with the manuscript. scRNAseq data shown in Figure 3c and Extended Data Figure 6 are reanalyses of published datasets: (GSE128423 URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128423) and (GSE108891 URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE108891). These were preliminary annotated based on ICGS2 BioMarker database, followed by a secondary analysis using the supervised classification tool cellHarmony, comparing all cells to reference hematopoietic (GSE120409 URL: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE120409). Supplementary Table 1 shows the different ICGS2 marker genes and Cell barcode assignments for the difference cell clusters identified.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

**Sample size**
Sample sizes were chosen based on previous studies. No statistical methods were used to predetermine sample size. All mice were included in the analyses. Mice were randomly allocated to the different groups on the basis of cage, genotype, and litter size. For all experiments, we aimed to have the same number of mice in the control and experimental groups.

**Data exclusions**
No data were excluded.

**Replication**
Each experiment was replicated at least two to three times on different dates. All attempts at replication were successful.

**Randomization**
No statistical methods were used to predetermine sample size. All mice were included in the analyses. Mice were randomly allocated to the different groups on the basis of cage, genotype, and litter size. For all experiments, we aimed to have the same number of mice in the control and experimental groups.

**Blinding**
Most experiments were performed in unperturbed wild-type mice and thus these samples were not blinded. The phenotypes of mice with conditional deletion of Csf1 in endothelial cells or after L. monocytogenes infection were obvious in all imaging analyses making blinding moot.

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

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

### Methods

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

### Antibodies

For flow cytometry:
- B220-APCCy7, BioLegend, catalog number 103224;
- B220-AF488, BioLegend, catalog number 103225;
- B220-biotin labeled, BioLegend, catalog number 103204;
- CD3-biotin labeled, BioLegend, catalog number 100304;
- CD3-PE, BioLegend, catalog number 100307;
- CD3-AF488, BioLegend, catalog number 100321;
- CD3-AF647, BioLegend, catalog number 100322;
- CD8-biotin labeled, BioLegend, catalog number 100704;
- CD8-PECy7, BioLegend, catalog number 100722;
- CD11b-biotin labeled, BioLegend, catalog number 101204;
- CD11b-PECy7, BioLegend, catalog number 101216;
- CD11b-AF488, BioLegend, catalog number 101217;
- CD11b-AF647, BioLegend, catalog number 101218;
- CD11c-FITC, BioLegend, catalog number 117305;
- CD11c-PE, BioLegend, catalog number 117307;
- CD16/32-APCCy7, BioLegend, catalog number 101328;
- CD24-PE, BioLegend, catalog number 138503;
CD31-PE, BioLegend, catalog number 102507;
CD31-AF647, BioLegend, catalog number 102516;
CD34-PEC, Thermo Fisher Scientific, catalog number 11-0341-85;
CD34-efluor 660, Thermo Fisher Scientific, catalog number 50-0341-82;
CD41-FTC, BioLegend, catalog number 133904;
CD41-BV605, BioLegend, catalog number 133921;
CD41-biotin labeled, BioLegend, catalog number 133930;
CD45-PE, BioLegend, catalog number 103106;
CD45.1-PE, BioLegend, catalog number 110708;
CD45.1-APC, BioLegend, catalog number 110714;
CD45-2-FTC, BioLegend, catalog number 109806;
CD45-2-APC, BioLegend, catalog number 109814;
CD45-2-AF700, BioLegend, catalog number 109822;
CD48-AF488, BioLegend, catalog number 103414;
CD48-AF647, BioLegend, catalog number 103416;
CD105-PECy7, BioLegend, catalog number 120410;
CD115-PE, BioLegend, catalog number 135506;
CD115-AF488, BioLegend, catalog number 135512;
CD115-BV421, BioLegend, catalog number 135513;
CD117-AF488, BioLegend, catalog number 105816;
CD117-APC, BioLegend, catalog number 105821;
CD172-α-AF700, BioLegend, catalog number 144022;
CD172-α-PECy7, BioLegend, catalog number 144027;
CD117-AF488, BioLegend, catalog number 123122;
Gr1-biotin labeled, BioLegend, catalog number 108404;
MHC I-biotin labeled, BioLegend, catalog number 107603;
MHC II-APC, BioLegend, catalog number 107618;
Ly6C-biotin labeled, BioLegend, catalog number 128004;
Ly6C-PECy7, BioLegend, catalog number 128010;
Ly6C-AF647, BioLegend, catalog number 128022;
Ly6C-PerCP, BioLegend, catalog number 128028;
Ly6C-AF700, BioLegend, catalog number 128023;
Ly6G-biotin labeled, BioLegend, catalog number 127604;
Ly6G-PerPCy5.5, BioLegend, catalog number 127616;
Ly6G-PECy7, BioLegend, catalog number 127617;
Ly6G-AF488, BioLegend, catalog number 127626;
Ly6G-BV605, BioLegend, catalog number 127639;
Sca1-FTC, BioLegend, catalog number 108106;
Sca1-PECy7, BioLegend, catalog number 108114;
Siglec H-FTC, Thermo Fisher Scientific, catalog number 11-0333-82;
Ter119-biotin labeled, BioLegend, catalog number 116204;
Ter119-APC, BioLegend, catalog number 116215;
Ter119-AF647, BioLegend, catalog number 116218;
Ter119-AF700, BioLegend, catalog number 116220;
Streptavidin-FTC, BioLegend, catalog number 405202;
Streptavidin-APC, BioLegend, catalog number 405203;
Streptavidin-PerCP/Cy5.5, BioLegend, catalog number 405214;
Streptavidin-Pacific Orange, Thermo Fisher Scientific, catalog number 542365;

For imaging:
B220-biotin labeled, BioLegend, catalog number 101204;
B220-AF647, BioLegend, catalog number 101204;
B220-biotin labeled, BioLegend, catalog number 101204;
B220-AF647, BioLegend, catalog number 101204;
B220-AF700, BioLegend, catalog number 101204;
Validation

Antibodies were validated by comparing their staining pattern on BM cells by FACS. For imaging experiments each antibody was validated by testing that the frequency of cells stained matched the one obtained in FACS analyses. The exception was the a-CSF1 (M-CSF) antibody for which we tested specificity by confirming lack of signal in vessels of Cdh5-cre:csf1fl/fl mice. All flow cytometry used antibodies were validated by the source company.

Animals and other organisms

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

Laboratory animals

- C57BL/6-J-Ptprcb (CD45.2+) mice; B6.SJL-PtprcaPepcb/Boyj (CD45.1+) mice, JAX: 002014; Csf1fl/fl mice (reference 1); Nestin-gfp mice (reference 2); B6;129-Tg(Cdh5-cre)1Spe/J, JAX: 017968; B6.129(Cg)-Leprtm2(cre)Rck/J (LepR-cre), JAX: 008320; B6.Cg-Ndor1Tg(UBC-cre/ERT2)1Ejb/J, JAX: 007001; B6.129P2(Cg)-Cx3cr1tm1Litt/J (CX3CR1-GFP), JAX:005582; B6.129P2-Gt(Rosa)26Sortm1(CAG-Brainbow2.1)Cle (confetti) JAX:017492; Gfi1Tomato mice (reference 3). All experiments except confetti fate mapping were performed in 8 to 14 weeks old male and female mice (Mus musculus). Mice were housed at the vivarium at Cincinnati Children’s Hospital Medical Center under a 14h light: 10h darkness schedule, 30-70% humidity and at 22.2±1.1ºC. All animal experiments followed all relevant guidelines and regulations and were approved by the Animal Care Committee of Cincinnati Children’s Hospital Medical Center.

1. Harris, S. E. et al. Meox2Cre-mediated disruption of CSF-1 leads to osteopetrosis and osteocyte defects. Bone 50, 42-53, doi:10.1016/j.bone.2011.09.038 (2012).
2. Kunisaki, Y. et al. Arteriolar niches maintain haematopoietic stem cell quiescence. Nature 502, 637-643, doi:10.1038/nature12612 (2013).
3. Thambyrajah, R. et al. GFI1 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. Nat Cell Biol 18, 21-32, doi:10.1038/ncb3276 (2016).

Wild animals

No wild animals were involved in our study.

Field-collected samples

No field-collected samples were involved in our study.

Ethics oversight

All studies were approved by the Animal Care Committee of Cincinnati Children’s Hospital Medical Center.

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

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
Mice were euthanized by isoflurane inhalation followed by cervical dislocation. Bone marrow cells were harvested by flushing bones with 1 ml of ice-cold PEB buffer (2 mM EDTA and 0.5% bovine serum albumin in PBS). Blood was collected from the retro-orbital venous sinus in tubes containing EDTA. Red blood cells in peripheral blood were lysed by the addition of 1 ml of RBC lysis buffer (150 mM NH4Cl, 10 mM NaCO3 and 0.1 mM EDTA). Cells were immediately decanted by centrifugation, resuspended in ice-cold PEB. Cells were stained under dark for 30 min in PEB buffer containing antibodies, washed thrice with ice-cold PBS.

Instrument
BD LSR II (BD Biosciences) instrument, BD FACS Aria II sorter, SH800S (Sony) cell sorter.

Software
FACSDiva software (BD Biosciences) for data collection and FlowJo (Tree Star) for data analysis.

Cell population abundance
Freshly sorted cells were examined by the same FACS sorter again and the purity is >95%.

Gating strategy
In all experiments, debris were excluded by using Forward scatter/Side scatter (FSC/SSC). Doublets were excluded by double forward (FSC-A and FSC-W), and side scatter (SSC-A and SSC-H). Dead cells were excluded as DAPI+ cells. BM HSC, MPP, CMP were gated as previously described[1, 2]. Briefly, BM HSC are Lineage-CD117+Sca1+CD150+CD105+, BM MPP are CD117+Sca1+CD150-CD105-, BM CMP are Lineage-CD117+Sca1-CD34+CD16/32-. BM macrophages were gated as B220-CD4-CD117-CD170-Ter119-Ly6G-CD115-F4/80+CD169+. BM Pre-DC were gated as Lineage-MHC II-CD11c+CD135+CD172a+CD172b-. BM cDC1 were gated as Lineage-Siglec H-B220-MHC II+CD24+CD172a+. Both in the BM and peripheral blood, B cells and T cells were gated as B220+ or CD3+ cells, respectively. In the peripheral blood, neutrophils and monocytes were gated as Ly6G+ or CD115+ cells, respectively. Gating strategies for other cells were described in the manuscript and figures.

1. Pronk, C.J., et al., Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. Cell Stem Cell, 2007. 1(4): p. 428-42.
2. Akashi, K., et al., A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature, 2000. 404(6774): p. 193-7.
3. Chow, A. et al. CD169(+) macrophages provide a niche promoting erythropoiesis under homeostasis and stress. Nat Med 19, 429-436, doi:10.1038/nm.3057 (2013).
4. Liu, Z. et al. Fate Mapping via Ms4a3-Expression History Traces Monocyte-Derived Cells. Cell 178, 1509-1525 e1519, doi:10.1016/j.cell.2019.08.009 (2019).
5. Schlitzer, A. et al. Identification of cDC1- and cDC2-committed DC progenitors reveals early lineage priming at the common DC progenitor stage in the bone marrow. Nat Immunol 16, 718-728, doi:10.1038/ni.3200 (2015).

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