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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was not predetermined, as a default we tried to have the same number of mice in each group and analyze at least 2-3 mice per experiment.

2. Data exclusions

Describe any data exclusions.

No data were excluded from these analyses

3. Replication

Describe whether the experimental findings were reliably reproduced.

Each experiment was repeated at least 2-3 times as indicated in each panel. Experimental findings were reliably reproduced between experiments.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomly allocated to each group.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

For all experiments, at the time of analyses, the investigator was blinded to the group allocation. The only exception was the blood vessel quantification in 3D reconstructions of sternal segments.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Flow cytometry samples were analyzed using FACSDiva Software (version 8.0). Graphs were prepared using Graphpad Prism (version 7.00) or Microsoft Excel (version 14.0.718.5000 (64-bit)). Statistical analyses were performed in Microsoft Excel. Images were acquired using Leica Application Suite Advanced Fluorescence software, postprocessed using Fiji software (ImageJ 1.51p) and assembled in Microsoft PowerPoint ((version 14.0.718.5000 (64-bit))). qPCR data was analyzed using SDS 2.4 software.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials were used

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies used were against B220 (clone RA3-6B2, Cat No: 103224), CD3 (clone 145-2C11, Cat No:100304), CD4 (clone GK1.5, Cat No:100422), CD8 (clone 53-6.7, Cat No:100722), CD11b (clone M1/70, Cat No:101216 or 101204), CD16/32 (clone 93, Cat No:101328), CD19 (clone 6D5, Cat No: 115508), CD31 (clone A20, Cat No:110724), CD41 (clone MWReg30, Cat No:133921 or clone D7, Cat No: 108104), CD45 (clone 30-F11, Cat No:103116), CD45.1 (clone A20, Cat No:110723 or 110708), CD45.2 (clone 104, Cat No:109845, 109823 or 109814), CD105 (clone MJ7/18, Cat No:120410), CD115 (clone AFS98, Cat No:135506 or 135513), CD144 (clone BV13, Cat No: 138006), CD150 (clone TC15-12F12.2, Cat No:115904), F4/80 (clone BM8, Cat No:123122), Gr1 (clone RB6-8C5, Cat No: 108406 or 108404), Ly6-G (clone 1A8, Cat No: 127625), Sca-1 (clone D7, Cat No:108106), Secondary antibody (Goat anti-rat IgG, clone Poly4054, Cat No: 405418), and Ter119 (clone TER-119, Cat No:116220), all from Biolegend, CD117 (clone 2B8, Cat No:105828 and 105833 from Biolegend or 562417 from BD Biosciences), Ki67 (clone SolA15, Cat No:50-5698-82 from ThermoFisher Scientific), Ly6G (clone 1A8, or Cat No: BP0075-1 from Bioxcell) or isotype control (clone 2A3, Cat No: BP0089 from Bioxcell), TNF-alpha (clone MP6-XT22. Cat No: 506303). In addition to manufacturer's assurances the antibodies were validated by staining WT bone marrow cells and determining that the stained cells presented the appropriate FACS profiles and frequencies described in the literature. The TNF-alpha antibody was validated by staining cells from Tnfa-/- mice. Lot numbers were not recorded.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No eukaryotic cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

C57BL/6J (CD45.2+) and B6.SJL-Ptprca Pepcb/BoyJ (B6.SJL, CD45.1+) were purchased from the Jackson laboratory and bred in house. C57BL/6j:B6.SJL hybrids (CD45.2+:CD45.1+) were generated by breeding C57BL/6J with B6.SJL mice. Ubc-gfp mice (ref 24), Mrp8-cre-IRES-gfp (Mrp8-cre) (ref 28), iDTR (ref 30), Tnfa-/- (ref 49) mice were originally purchased from the Jackson laboratory. Tnfr1-/-:Tnfr2-/- (ref 50) mice were also purchased from the Jackson laboratory and then backcrossed for four additional generation into C57BL/6J background and then bred in house. Nestin-gfp mice (ref 21,36) were a gift from Paul S. Frenette. All experiments were performed in 8-14 week old male mice. All mice were housed at the facility managed by the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan. Experiments were approved by the Institutional Animal Care and Use Committee at the University of Michigan.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

No human patients

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.

Bone marrow isolation.
Mice were euthanized by isoflurane overdose. Bone marrow was harvested by flushing mouse long bones with 1 ml of ice-cold PEB (2mM EDTA 0.5% Bovine serum albumin in PBS). Red blood cells were lysed once by adding 1 mL of RBC Lysis Buffer (NH_4Cl 150mM, $NaCO_3$ 10mM, EDTA 0.1mM). Cells were immediately decanted by centrifugation, resuspended in ice-cold PEB and used in subsequent assays. Due to the known effect of circadian rhythm effects in granulocytes and HSC (refs 12,51-53) we performed BM harvest at zeitgeber times 3-6 in mice under standard (12h light:12h dark) cycles.

Peripheral blood analyses.
Blood was collected from the facial vein in tubes containing EDTA. White blood cell (WBC), red blood cell (RBC) and platelet counts were obtained using an Advia Counter (Siemens) or a Hemavet 950 (Drew Scientific). Prior to flow cytometry staining and analyses, red blood cells in peripheral blood were lysed once by adding 1 mL of RBC Lysis Buffer (NH_4Cl 150mM, $NaCO_3$ 10mM, EDTA 0.1mM). Cells were immediately decanted by centrifugation, resuspended in ice-cold PEB and used in subsequent assays.

Collagenase/Dispase digestion
To purify the stromal cell fraction of the bone marrow (including endothelial cells) we used a modified version of the serial digestion protocol developed by the Simmons laboratory54. Digestion buffer was made using 2 mg/ml Collagenase Type IV (Gibco, 17104-019) and 3 mg/ml Dispase (Gibco, 17105-041) dissolved in room temperature PBS. We harvested the BM by flushing a tibia with 1 ml of digestion buffer into a 5 ml polypropylene snap-cap tube containing another 1 ml of digestion buffer. We mixed the tubes vigorously by hand and incubated at 37°C for 5-7 minutes. Following the first incubation, the tubes were mixed vigorously by hand and then placed back at 37°C for another 5-7 minutes. After this second incubation we collected the supernatant taking care of leaving any macroscopic clumps in the tube. We transferred the digested cells to a tube containing 5 ml of ice-cold PEB. Then we added one ml of digestion buffer to the snap-cap tubes and the process above repeated until all macroscopic pieces of bone marrow had been digested. The red

blood cells were lysed once using RBC Lysis Buffer, filtered through a 100 µm filter (Greiner Bio-one, 542-000), and then immediately spun down in the centrifuge. The cells were resuspended in 1 ml of ice-cold PEB and used for subsequent analyses.
Cells were stained for 30 minutes in PEB buffer and analyzed in one of the instruments indicated below.

6. Identify the instrument used for data collection.

Data was collected in a BD LSRFortessa (BD Biosciences). Cells were FACS-purified using a BD FACS Aria II or a Synergy SY3200 Cell sorter (Sony)

7. Describe the software used to collect and analyze the flow cytometry data.

Samples were analyzed using BD FacsDiva version 8.0

8. Describe the abundance of the relevant cell populations within post-sort fractions.

Sorting efficiency was quantified by reanalyzing the sorted cells in one of the instruments above to ensure more than 95% purity

9. Describe the gating strategy used.

Gating based on FSC area vs width and SSC area vs height and DAPI viability dye staining were used to gate live, single, cells. Gates were drawn as shown in the relevant panels or as described in Nat Med. 2014 Nov;20(11):1315-20. Positive and negative populations were defined by using fluorescence minus one controls, or staining cells purified from mice lacking the detected protein or appropriate positive and negative controls.

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