nature research

Corresponding author(s): Charles Chan

Last updated by author(s): May 20, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	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	Bruker Skyscan 1276 (Bruker Preclinical Imaging). Lago-X scanner (Spectral Instruments Imaging). A custom delaminator maintained by the R.H. Dauskardt laboratory for mechanical strength testing (Stanford, CA). Flow cytometry was performed on FACS Aria II (BD Biosciences). Spectrometric measurements were conducted on a Ultraspec 2100 UV/Visible Spectrophotometer (Biochrom, Harvard Bioscience).
Data analysis	Statistical analysis was performed with Prism 9 (GraphPad); ImageJ v1.48 (NIH, http://imagej.nih.gov/ij/); MicroCT analysis with CTAn v1.17.7.2 and CTvox software v3.3.0 (Bruker); FACS analysis was conducted using FlowJo v10 software; Microarrays were scanned with a Gene Chip Scanner 3000 (Affymetrix) running GCOS 1.1.1 software; Bulk RNA-sequencing raw FASTQ reads were aligned to the GENCODE vM20 mouse reference transcripts (GRCm38.p6) with Salmon v0.12.0. Salmon results were merged into a single gene-level transcripts per million (TPM) matrix using the R package tximport v1.10.1; Single RNA-sequencing data was demultiplexed using bcl2fastq2 2.18 (Illumina). Raw reads were further processed using skewer v0.2.2. Mapping of reads with STAR v2.6.1d. Gene counts wer calculated using RSEM 1.3.1plus. Data was explored and plots were generated using the Scanpy package v1.8.0 with UMAP v0.4.6. and leidenalg v0.8.2.; 10X Genomics single cell RNA-sequencing data were UMI-collapsed with the Cellranger toolkit version 4.0.0 (10X Genomics Inc)

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 files are provided with this paper. All sequencing data have been submitted to repositories and are available online. Single cell RNA-sequencing data is available from the NCBI Gene expression Omnibus with GEO Accessions GSE161946 and GSE172149. Bulk RNA-sequencing data has been deposited under GSE166441 and Microarray data is publicly accessible as previously published under GSE34723 as well as in the GEXC database under https://gexc.riken.jp/models/2399 and https://gexc.riken.jp/models/2400.

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	No statistical test was used to pre-determine the sample sizes. Sample sizes were based on experience from previous work with assays performed. For assays with commonly high variability we typically used n≥5 and for assays with commonly low variability we typically used n<5.
Data exclusions	Animals that unexpectedly became morbid during the course of parabiosis and hematopoietic reconstitution experiments were excluded. Only animals that survived to final timepoints of experiments were included. During processing of single cell genomic data filtering of low quality cells was applied as described.
Replication	All data presented are biological replicates unless otherwise stated in the figure legends. Each experimental finding was reproduced in at least two independent experiments.
Randomization	Animals were allocated randomly into the different experimental group.
Blinding	Investigators were not blinded during data collection and analysis. Controls and samples were treated equally.

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

Antibodies used	Antibodies (ThermoFisher) against CD45 (Cat#: 15-0451; 1:200), Ter119 (Cat#: 15-5921; 1:200), CD51 (Cat#: 12-0512; 1:50), Tie2 (14-5987; 1:20), Thy1.1 (Cat#: 47-0900; 1:100), Thy1.2 (Cat#: 47-0902; 1:100), 6C3 (Cat#: 17-5891; 1:100), CD49f (Cat#: 11-0495; 1:100), CD105 (Cat#: 13-1051; 1:50), and Streptavidin-PE-Cy7 conjugate (Cat#: 25-4317-82; 1:100). Lineage [(CD3 (Cat#: 15-0031; 1:20), CD4 (Cat#: 15-0041; 1:20), CD8 (Cat#: 15-0081; 1:20), B220 (Cat#: 15-0452; 1:20), Gr-1 (Cat#: 15-9668; 1:20), Mac1 (Cat#: 15-0112; 1:20), Ter119 (Cat#: 15-5921; 1:20)], Mac1 (Cat#: 56-0112-82; 1:50), cKit (Cat#: A15423; 1:50), Sca1 (Biolegend; Cat#: 108120; 1:50), Flk2/Flt3 (Cat#46-1351-82; 1:50), CD16/32 (Cat#: 12-0161-82; 1:50), CD127 (Biolegend; Cat#:135035; 1:50), Slam/

CD150 (Cat#: 12-1502; 1:50), CD34 (Cat#:50-0341; 1:50), and CD200 (Cat#: MA5-17980; 1:100) and Rat IgG2a kappa Isotype Control (Cat#: 11-4321-80; 1:100).

Validation

All antibodies were used for flow cytometry and are validated, commercially available products that addtionally have been validated in previously published studies (e.g. PMID: 29748647 & PMID: 15967997).

Anti-mouse CD45 (Cat#: 15-0451), CD45 Monoclonal Antibody (30-F11), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD45
Anti-mouse Ter119 (Cat#: 15-5921), TER-119 Monoclonal Antibody (TER-119), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse Ter119
Anti-mouse CD51 (Cat#: 12-0512), CD51 (Integrin alpha V) Monoclonal Antibody (RMV-7), PE, eBioscience™, reported for flow cytometry recognizing mouse CD51
Anti-mouse Tie2 (Cat#: 14-5987), CD202b (TIE2) Monoclonal Antibody (TEK4), eBioscience™, reported for flow cytometry recognizing mouse Tie2
Anti-mouse Thy1.1 (Cat#: 47-0900), CD90.1 (Thy-1.1) Monoclonal Antibody (HIS51), APC-eFluor 780, eBioscience™, reported for flow cytometry recognizing mouse CD90.1
Anti-mouse Thy1.2 (Cat#: 47-0902), CD90.2 (Thy-1.2) Monoclonal Antibody (53-2.1), APC-eFluor 780, eBioscience™, reported for flow cytometry recognizing mouse CD90.2
Anti-mouse 6C3 (Cat#: 17-5891), CD249 (BP-1) Monoclonal Antibody (6C3), APC, eBioscience™, reported for flow cytometry recognizing mouse CD249
Anti-mouse CD49f (Cat#: 11-0495), CD49f (Integrin alpha 6) Monoclonal Antibody (eBioGoH3 (GoH3)), FITC, eBioscience™, reported for flow cytometry recognizing mouse CD49f
Anti-mouse CD105 (Cat#: 13-1051), CD105 (Endoglin) Monoclonal Antibody (MJ7/18), Biotin, eBioscience™, reported for flow cytometry recognizing mouse CD105
Anti-mouse Streptavidin-PE-Cy7 (Cat#: 25-4317-82), eBioscience™ Streptavidin PE-Cyanine7 Conjugate, reported for flow cytometry recognizing Biotin
Anti-mouse CD3 (Cat#: 15-0031), CD3e Monoclonal Antibody (145-2C11), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD3e
Anti-mouse CD4 (Cat#: 15-0041), CD4 Monoclonal Antibody (GK1.5), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD4
Anti-mouse CD8 (Cat#: 15-0081), CD8a Monoclonal Antibody (53-6.7), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD48a
Anti-mouse B220 (Cat#: 15-0452), CD45R (B220) Monoclonal Antibody (RA3-6B2), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD45R
Anti-mouse Gr-1 (Cat#: 15-9668), Ly-6G Monoclonal Antibody (1A8-Ly6g), PE-Cyanine5, eBioscience, reported for flow cytometry recognizing mouse Ly-6G
Anti-mouse Mac1 (Cat#: 15-0112), CD11b Monoclonal Antibody (M1/70), PE-Cyanine5, eBioscience™, reported for flow cytometry recognizing mouse CD11b
Anti-mouse Mac1 (Cat#: 56-0112-82), CD11b Monoclonal Antibody (M1/70), Alexa Fluor 700, eBioscience™, reported for flow cytometry recognizing mouse CD11b
Anti-mouse cKit (Cat#: A15423), c-Kit Monoclonal Antibody (2B8), APC-Cyanine7, ThermoFisher, reported for flow cytometry recognizing mouse c-Kit
Anti-mouse Sca1 (Cat#: 108120), Pacific Blue™ anti-mouse Ly-6A/E (Sca-1) Antibody, BioLegend, reported for flow cytometry recognizing mouse Ly-6A/E
Anti-mouse Slam/CD150 (Cat#: 12-1502), CD150 Monoclonal Antibody (mShad150), PE, eBioscience™, reported for flow cytometry recognizing mouse CD150
Anti-mouse Flk2/Flt3 (Cat#46-1351-82), CD135 (Flt3) Monoclonal Antibody (A2F10), PerCP-eFluor 710, eBioscience™, reported for flow cytometry recognizing mouse CD135
Anti-mouse CD16/32 (Cat#: 12-0161-82), CD16/CD32 Monoclonal Antibody (93), PE, eBioscience™, reported for flow cytometry recognizing mouse CD16/32
Anti-mouse CD127 (Cat#:135035), Brilliant Violet 711™ anti-mouse CD127 (IL-7Rα) Antibody, BioLegend, reported for flow cytometry recognizing mouse CD127
Anti-mouse CD34 (Cat#:50-0341), CD34 Monoclonal Antibody (RAM34), eFluor 660, eBioscience™, reported for flow cytometry recognizing mouse CD34
Anti-mouse CD200 (Cat#: MA5-17980), CD200 Monoclonal Antibody (OX90), FITC, ThermoFisher, reported for flow cytometry recognizing mouse CD200
Rat IgG2a kappa Isotype Control (Cat#: 11-4321-80), Rat IgG2a kappa Isotype Control (eBR2a), FITC, eBioscience™, reported for flow cytometry recognizing rat IgG2a kappa

Animals and other organisms

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

Laboratory animals

Mice were maintained at the Stanford University Research Animal Facility in accordance with Stanford University guidelines. Animals were given food and water ad libitum and housed in temperature-, moisture-, and light-controlled (12h light/dark cycle) micro-insulators. Postnatal Day-3 (0-month-old), 2-month-old, 12-month-old, and 24-month-old male C57BL/6 or β Actin-CreERT/Rainbow mice were used to examine skeletal aging. 15 month-old female and male CSF1-KO+/+ and CSF1-KO+/- mice were used as described. 2-month-old male immunodeficient NSG mice were used in renal capsule transplant experiments.

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

Ethics oversight

All animal experiments were conducted under approved protocols by Stanford's Administrative Panel on Laboratory Animal Care.

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	Detailed sample preparation protocol is provided in methods section of the manuscript.
Instrument	Flow cytometry was performed on FACS Aria II (BD Biosciences). Gating schemes were established with fluorescence-minus-one (FMO: staining with all fluorophores except one) controls and negative propidium iodide (PI) (Sigma-Aldrich, Cat#P4170) staining (1 mg/ml) was used as a measure for cell viability.
Software	FlowJo v10 was used to analyze FACS data.
Cell population abundance	Quantification of cell populations are provided as total number of tissue or percentage of reference population as described in the figure and figure legends.
Gating strategy	Gating Strategy is provided in diagrams in manuscript.

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