nature research

Corresponding author(s): Filip Swirski

Last updated by author(s): Dec 20, 2018

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 Authors & Referees 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	BD FACSDiVa software was used to collect data from flow cytometry; NPDview2 and Nanozoomer 2.0Rs were used to collect histology data.
Data analysis	Flow cytometric analyses were performed with FlowJo software (FlowJo 8.7.2); NPDviewview2 software was used to analyze histology data; Statistical analyses were performed with GraphPad Prism 7.0 software

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/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

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files.

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

Life sciences study design

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

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

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-CD45 (BioLegend, clone30-F11, Cat#103147, Lot#B243834), anti-CD45.1 (BioLegend, clone A20, Cat#110708), anti-CD45.2 (BioLegend, clone 104, Cat#109802), anti-CD3 (BioLegend, clone 17A2, Cat#100206), anti-CD90.2 (BioLegend, clone 53-2.1, Cat#105308, Lot#B260050), anti-CD19 (BioLegend, clone 6D5, Cat#115508, Lot#B226581), anti-B220 (BD Biosciences, clone RA3-6B2, Cat#553089, Lot#6012954), anti-NK1.1 BioLegend, clone PK136, Cat#108708), anti-Ly6G (BioLegend, clone 1A8, Cat127614#, Lot#B259670), anti-Ly6C (BioLegend, AL-21, Cat#128006, Lot#B247728), anti-MHCII (BioLegend, clone M5/114.152, Cat#107602, Lot#B217859), anti-F4/80 (Biolegend, clone BM8, Cat#123114, Lot#B237342), anti-CD11b (BioLegend, clone M1/70, Cat#101226, Lot#B238268), anti-CD115 (BioLegend, clone AFS98, Cat#135517, Lot#B265220), anti-Ter119 (BioLegend, clone TER-119, Cat#116208, Lot#B220899), anti-CD34 (eBioscience, clone RAM34, Cat#11-0341-85, Lot#E00265-1634), anti-CD49b (BioLegend, clone DX5, Cat#1089008, Lot#B258302), ant-CD11c (BioLegend, clone N418, Cat#117310, Lot#B206713), anti-IL7Rα (BioLegend, clone SB/199, Cat#121112, Lot#B189668), anti-CD16/32 (BioLegend, clone 93, Cat#101324, Lot#B250025), anti-CD150 (BioLegend, clone TC15-12F12.2, Cat#115922, Lot#B220585), anti-cKit (BioLegend, clone 2B8, Cat#105814, Lot#B252918), anti-CD135 (BioLegend, clone A2F10, Cat#135310, Lot#B234045), anti-CD48 (BioLegend, clone HM48-1, Cat#103426, Lot#B236445), anti-Sca1 (BioLegend, clone D7, Cat#108126, Lot#B234288), anti-CD8 (BD Bioscience, clone 53-6.7, Cat#553035, Lot#2296946), anti-CD4 (BioLegend, clone GK1.5, Cat#100428, Lot#B237336), anti-SiglecF (BD Pharmingen, clone E50-2440, Cat#562680, Lot#7054789), anti-CXCR4 (Invitrogen, clone 2B11, Cat#12-9991-81, Lot#B251481), anti-CXCR2 (BioLegend, clone SA044G4, Cat#149307, Lot#B251481), anti-BrdU (eBioscience, clone BU20A, Cat#17-5071-42, Lot#4319920). All antibodies were used in a 1:700 dilution.
Validation	These antibodies were all used for flow cytometry on mice. Antibody validations were performed by antibody suppliers per quality assurance literature provided by each supplier.

Animals and other organisms

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

Laboratory animals	C57BL/6J (wild type, WT), Apoetm1Unc (Apoe−/−), Ldlrtm1Her/J (Ldlr−/−) and Csf1op (Csf1−/−) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Hcrt−/− mice were kindly provided by Dr. Thomas Scammell (Professor of Neurology, Division of Sleep Science, Harvard Medical School) and bred in-house. HcrtR1GFP/GFP mice were kindly provided by Dr. Anne Vassalli (Department of Physiology, University of Lausanne). Stromal cell reporter mice Nestin-GFP, LeptinRcre-R26-EYFP, and OCN-GFPtopaz− were bred in-house. Age- and sex-matched animals were used at 8–12 weeks of age. For experiments on Apoe-/- and Ldlr-/- mice females were used. In all other experiments both males and females were used.

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 protocols were approved by the Animal Review Committee at the Massachusetts General Hospital

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	Peripheral blood was collected by retro-orbital bleeding and red blood cells were lysed in RBC lysis buffer (Biolegend, San Diego, CA). Aortas, lung, liver and heart were excised after PBS (Thermo Fisher Scientific, Waltham, MA) perfusion, minced and digested with 450 U/ml collagenase I, 125 U/ml collagenase XI, 60 U/ml DNase I and 60 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO) in PBS for 20 minutes (liver), 40 minutes (aorta) or 1hr (heart and lung) at 37°C. Spleens were crushed through a 40mm cell strained and red blood cells were lysed with RBC lysis buffer. Bone marrow cells were collected by flushing bones with PBS after which a single cell suspension was created by passing cells through a 26-gage needle and red blood cells were lysed with RBC lysis buffer.
Instrument	Data were acquired on a LSRII and a Area II
Software	DIVA and FlowJo
Cell population abundance	Post sort, cell abundance was sufficient for down stream applications. After sorting, a small fraction of the sorted cells were run through Aria II and the same gating strategy was applied to check the purity of sorted cell populations. A general purity of higher than 95% were achieved for all the sorted population.
Gating strategy	FSC/SSC gating was used to exclude dead cells and debris followed by FSCA/FSCH to select singlets. Viable cells were identified as unstained with Zombie Aqua (Biolegend, CA). Cells were identified as (i) Ly6Chigh monocytes (CD45+Lin1–CD11b+CD115+F4/80–Ly-6Chigh), (ii) neutrophils (CD45+Lin1–CD11b+Ly-6G+F4/80–), (iii) macrophages (CD45+Lin1–CD11b+F4/80+Ly-6Clow), (iv) B-cells (CD45+B220+CD19+F4/80–CD11b–), (v) CD4 T-cells (CD45+CD3+CD90+CD4+CD11b–F4/80–), (vi) CD8 T-cells (CD45+CD3+CD90+CD8+CD11b–F4/80–), (vii) LSKs (CD45+Lin2–cKit+Sca1+), (viii) MPP4 (CD45+Lin2–cKit+Sca1+CD135+CD150–), (ix) MPP3 (CD45+Lin2–cKit+Sca1+CD135+CD150–CD48+), (x) MPP2 (CD45+Lin2–cKit+Sca1+CD135+CD150+CD48+), (xi) short-term hematopoietic stem cell (StHSC, CD45+Lin2–cKit+Sca1+CD135+CD150–CD48-), (xii) LtHSC (CD45+Lin2–cKit+Sca1+CD135+CD150+CD48-), (xiii) common myeloid progenitor (CMP, CD45+Lin2–cKit+Sca1–CD34+CD16/32mid), (xiv) granulocyte/macrophage progenitor (GMP, CD45+Lin2–cKit+Sca1–CD34+CD16/32highCD115–), (xv) monocyte-dendritic cell progenitor (MDP, CD45+Lin2–cKit+Sca1–CD34+CD16/32highCD115+), for neutrophils populations (CD45+CD3-CD90.2-CD19-NK1.1-SiglecF-CD115-cKitlowCD11b+Gr-1+) (xvi) pre-neutrophils were then separated as cKitintCXCR4+CXCR2-, (xvii) immature neutrophils as ckit-CXCR4-CXCR2-, and (xviii) mature neutrophils as ckit-CXCR4-CXCR2+Ly6G+. Lineages were defined as Lin1: CD3, CD90.2, CD19, NK1.1, Ter119 and Lin2: B220, CD19, CD49b, Ter119, CD90.2, CD11b, CD11c, Ly-6G, IL7Rα. Osteolineage cells (OCN+) cells were identified as CD45–Ter119–CD31–GFP+ from the bone fraction of OCN-GFP mice. Leptin receptor (LepR+) cells were identified as CD45–Ter119–CD31–YFP+ form the bone marrow fraction of LeptinRcre-R26-EYFP. Nestin+ cells were identified as CD45–Ter119–CD31–GFP+ form the bone and bone marrow fraction of Nestin-GFP mice. Endothelial cells were identified as CD45–Ter119–CD31+ of the bone marrow fraction of LeptinRcre-R26-EYFP mice.

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