

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](#).

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

BD FACS Diva 8

Data analysis

FlowJo 10, Prism 7, SnapGene 4, Extreme Limiting Dilution Assay (ELDA) software (<http://bioinf.wehi.edu.au/software/elda/>)

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 upon request. 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

All graphed datasets can be found in the Supplementary Source Data Files. All datasets available upon request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

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

Sample size	No statistical methods were used to predetermine the experimental sample size.
Data exclusions	No data were excluded.
Replication	For all figures, multiple independent experiments were performed and all attempts at replicating observation as described in the manuscript were successful.
Randomization	Recipient mice were randomly selected for transplantation groups.
Blinding	Blinding was not performed.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used

Biotin anti-mouse CD4 eBioscience Cat#13-0042-85 (1:2800)
 Biotin anti-mouse CD8 eBioscience Cat# 13-0081-86 (1:2800)
 Biotin anti-mouse CD45R/B220 eBioscience Cat# 13-0452-85 (1:1400)
 Biotin anti-mouse TER-119 eBioscience Cat# 13-5921-85 (1:700)
 Biotin anti-mouse Ly-6G/Ly-6C (RB6-8C5) eBioscience Cat# 13-5931-85 (1:700)
 Biotin anti-mouse CD127 (A7R34) eBioscience Cat# 13-1271-85 (1:1400)
 APC anti-mouse c-Kit (2B8) eBioscience Cat# 17-1171-83 (1:100)
 PE-Cy7 anti-mouse c-Kit (2B6) Biolegend Cat# 105814 (1:100)
 FITC anti-mouse CD34 (RAM34) eBioscience Cat# 11-0341-85 (1:100)
 PE-Cy7 anti-mouse CD150 (TC15-12F12.2) BioLegend Cat# 115914 (1:350)
 PE anti-mouse-Ly-6A/E (Sca-1) (D7) BioLegend Cat# 122508 (1:700)
 PE anti-mouse-Ly-6A/E (Sca-1) (D7) eBioscience Cat# 12-5981-83 (1:700)
 FITC anti-mouseLy-6A/E (Sca-1) (D7) Biolegend Cat# 108105 (1:100)
 Streptavidin-APC/eFluor780 eBioscience Cat# 47-4317-82 (1:700)
 PE-Cy7 anti-mouse CD45.1 BioLegend Cat# 110730 (1:500)
 BrilliantViolet421 anti-mouse CD45.2 (104) BioLegend Cat# 109832 (1:400)
 eFluor450 anti-mouse CD45.2 (104) eBioscience Cat# 48-0454-82 (1:500)
 FITC anti-mouse Ly-6G/Ly-6C (RB6-8C5) eBioscience Cat# 11-5931-85 (1:2000)
 FITC anti-mouse CD11b (M1/70) eBioscience Cat# 11-0112-41 (1:2000)
 PE anti-mouse Ly-6G/Ly-6C (RB6-8C5) eBioscience Cat# 12-5931-82 (1:2000)
 PE anti-mouse CD11b (M1/70) eBioscience Cat# 12-0112-82 (1:2000)
 APC-eFluor780 anti-mouse CD45R (RA3-6B2) eBioscience Cat# 17-0452-83 (1:1000)
 APC anti-mouse CD4 (RM4-5) BioLegend Cat# 100516 (1:2000)
 APC anti-mouse CD8 (53-6.7) eBioscience Cat# 17-0081-83 (1:2000)

Pacific Blue anti-mouse Ter119 (TER119) eBioscience Cat# 48-5921-82 (1:100)
 PE-Cy7 anti-mouse Ly-6G/Ly-6C (RB6-8C5) eBioscience Cat# 25-5931-82 (1:1000)
 FITC anti-mouse CD127 (A7R34) eBioscience Cat# 11-1271-85 (1:100)
 PE anti-mouse FceR1 (MAR-1) eBioscience Cat# 12-5898-81 (1:800)
 BrilliantViolet421 anti-mouse CD135 (A2F10) Biolegend Cat# 135313 (1:200)
 PE anti-mouse CD11a (M17/4) eBioscience Cat# 12-0111-081 (1:800)
 APC anti-mouse CD201 (eBio1560) eBiosciences Cat# 17-2012-82 (1:400)
 Pacific Blue anti-mouse CD48 (BCM1) Biolegend Cat# 103418 (1:200)
 APC anti-mouse ESAM (1G8/ESAM) Biolegend Cat# 136207 (1:400)
 APC anti-human CD34 (8G12) BD Cat# 560940 (1:10)
 PE anti-human CD38 (HB7) BD Cat# 347687 (1:10)
 FITC anti-human CD90 (5E10) Biolegend Cat# 328113 (1:10)
 APC-Cy7 anti-human CD49f (GoH3) Biolegend Cat# 313611 (1:10)
 V450 anti-human CD45 (HI30) BD Cat# 560367 (1:10)
 AlexaFluor488 anti-phospho-histone H2A.X (20E3) Cell Signaling Technology Cat# 9719S (1:200)

Validation

All antibodies were validated by manufacturers for the applications and species used in this study. See manufacturers websites for validation statements (www.biolegend.com; www.thermofisher.com/us/en/home/life-science/antibodies/ebioscience ; www.bdbiosciences.com; www.cellsignal.com).

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

All mice were 8-12 weeks when experiments were started. Male and female mice were used in this study.
 C57BL/6 mice - Jackson Laboratories (000664), Japan SLC, or Sankyo-Lab Service
 PepBoyJ mice - Jackson Laboratories (002014)
 TLR2-knockout mice - Jackson Laboratories (004650)
 TLR4-knockout mice - Jackson Laboratories (007227)
 NOD/Scid (NOD.Cg-Prkdcscid/SzJ) mice - Jackson Laboratories (001303)
 NOG (NOD.Cg-Prkdcscid Il2rgTm1Sug/ShiJic) mice - In Vivo Sciences Inc

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve field-collected samples

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

Bone marrow, peripheral blood, and in vitro cell cultures were prepared into a single cell suspension in PBS. Bone marrow cells were cKit-enriched using a MACS LS column (Miltenyi). Red blood cell lysis using aqueous 140 mM ammonium chloride was performed on peripheral blood cells. Cells were filters (40uM) before FACS.

Instrument

BD FACS ArialI, BD LSRFortessa, BD FACS Canto.

Software

FACS Diva for data collection, FlowJo for data analysis.

Cell population abundance

FACS machine cell sorting efficiency was confirmed by flow cytometric analysis of post-sorted cells.

Gating strategy

FSC-A/SSC-A for mononuclear cells, FSC-H/FSC-W followed by SSC-H/SSC-W for singlets, PI/Sca1 for PI- live cells, Lineage-cocktail/cKit for Lin- cells, Sca1/CD34 for CD34-/lo Lin-, cKit/Sca1 for CD34-/lo cKit+ Sca1+ Lin-, CD150/blank channel for CD150+ CD34-/lo Kit+ Sca1+ Lin-. See Extended Data Figure 1b for details. For peripheral blood analysis, live cells were gated based on positive and negative markers.

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