nature research

Corresponding author(s): Tony Wyss-Coray

Last updated by author(s): Jan 20, 2020

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	FACS Diva (BD), SH800S (Sony), ZEN Black (Zeiss), Imaris (Bitplane), ImageStudio (LI-COR), Inveon microPET-CT (Siemens), Cobra II γ counter (Packard-Perkin Elmer), GenePix Pro7 software (Molecular Devices), NDP.view2 (Hamamatsu), SkanIT Fisher Scientific), and Typhoon 9410 ImageQuant (Perkin Elmer).
Data analysis	Cytobank, FlowJo (Treestar), Prism 7 (GraphPad), R (DESeq2, ggplot2, tidyverse), ImageJ/ Fiji (NIH), GeneAnalytics, Excel (Microsoft), MaxQuant (Max Planck), Perseus (Max Planck), VivoQuant (v4.0, inviCRO), STAR, bcl2fastq, HTSEQ (v0.6.1p1), and Cell Ranger (10X Genomics).

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

Raw sequencing data will be deposited in NCBI GEO. Raw and summarized single-cell RNA-seq (Supp Table 2, 3, and 5) are provided.

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 power analyses were used to predetermine sample sizes. However, sample sizes were informed by prior literature using similar experimental paradigms that yielded interpretable results and the lab's previous experience.
Data exclusions	None.
Replication	For in vivo experiments, biological replicates as well as independent cohorts of mice were used. ALPL inhibition and RNA-seq data were not replicated in independent experiments due to resource restrictions. All other data were successfully replicated in at least two independent experiments as stated in figure legends.
Randomization	For ALPL inhibition studies, aged mice were randomized into 2 groups using a random list generator. For other studies, cages of young and aged mice of similar stays at the veterinary facility were allocated at random.
Blinding	All immunohistochemical analyses were performed by a blinded observer. In general, experimenters were blinded to group allocation during data acquisition and analysis.

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	Flow cytometry: rat anti-CD31-PE/CF594 (1:100, clone MEC 13.3, BD, cat. No. 563616), rat anti-CD45-PE/Cy7 (1:200, clone 30-F11, Biolegend, cat. no. 103114), rat anti ACSA-2-PE (1:200, clone IH3-18A3, Miltenyi, cat. no. 130-102-365), mouse anti-NeuN-PE (1:100, clone A60, Millipore Sigma, cat. no. FCMAB317PE), and rat anti-CD90.2-FITC (1:100, clone 30-H12, Biolegend, cat. no. 105305) Immunostaining primary antibodies: goat monoclonal anti-CD31 (1:100, AF3628, R&D), Fluorescein-labeled Lectin (1:200, Vector Laboratories), rabbit monoclonal anti-Aquaporin 4 (1:500, AB2218, Millipore Sigma), rat anti-CD13 (1:100, MCA2183EL, Bio-Rad), goat anti-Alpl/ ALPL (1:100, AF2909, R&D), mouse anti-NeuN (1:400, MAB377, Millipore), goat anti-albumin (1:100, NB600, Novus), rabbit anti-transferrin (1:100, ab82411, Abcam or 1:100, AF3987, R&D), rabbit anti-Collagen I (1:100, ab21286, Abcam), goat anti-Iba1 (1:500, ab5076, Abcam), rabbit anti-MFSD2A (1:300, gift from Chenghua Gu), rat anti-TFRC (1:100, Novus, NB100-64979), and rabbit anti-Phospho-Stat3 (1:100, 9145S, CST). Immunostaining secondary antibodies: Alexa Fluor 488 donkey anti-goat-IgG (1:250; Invitrogen, A-11055); Alexa Fluor 488 donkey anti-rat-IgG (1:250; Invitrogen, A-21208); Alexa Fluor 555 donkey anti-mouse-IgG (1:250; Invitrogen, A-31570); Alexa Fluor 555 donkey anti-goat-IgG (1:250; Invitrogen, A-21432); Alexa Fluor 647 donkey anti-mouse-IgG (1:250; Invitrogen, A-31571); Alexa Fluor 647 donkey anti-rabbit-IgG (1:250; Invitrogen, A-31573); Alexa Fluor 647 donkey anti-goat-IgG (1:250; Invitrogen, A-21447); Alexa Fluor 647 donkey anti-rabbit-IgG (1:250; Invitrogen, A-31573); and Alexa Fluor 647 donkey anti-goat-IgG (1:250; Invitrogen, A-21447).
Validation	All antibodies were validated for the indicated species and applications by the manufacturer.

Animals and other organisms

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

Laboratory animals	C57Bl/6 male mice, aged (20-24 months from NIA rodent colony), young (3 months from Charles River or Jackson Labs)
Wild animals	This study did not involve wild animals.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	Institutional Animal Care and Use Committee at Stanford University

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	For immunostaining, cells were passed through a 100 micron strainer, blocked for 10 minutes on ice with mouse Fc-blocking reagent (BD), and stained for 30 minutes in PBS supplemented with 1% bovine serum albumin.
Instrument	FACS: BD FACSAria III Analysis: BD LSRFortessa
Software	BD FACS Diva, Cytobank
Cell population abundance	For analysis of plasma uptake, at least 1,000 cells of the population of interest were analyzed. Using plate-based scRNA-seq, 745 brain endothelial cells were analyzed for plasma uptake. Using droplet-based scRNA-seq, 8,637 brain endothelial cells were analyzed after ALPL inhibition.
Gating strategy	Positive and negative gates were set using unstained and fluorescence minus one (FMO) background intensity controls. Fluorophores were chosen to minimize spectral overlap.

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