nature portfolio

Corresponding author(s): Christoph A. Thaiss

Last updated by author(s): Dec 23, 2025

Reporting Summary

Nature Portfolio 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 Portfolio 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	Fastq files were generated by MiSeq and NextSeq machines with the software available on these machines.
Data analysis	Fastq files were processed using QIIME 2 (2021.2.0), Kallisto (0.46.0), and Sunbeam (v2.1.1). Downstream analysis was performed in RStudio (R v4.2) using the following packages: tidyverse (v2.0.0), phyloseq (1.42.0), vegan (v2.6.4), DESeq2 (v1.32.0).

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw sequencing data for this study are available under accession numbers GSE307834, GSE307836, and GSE307838.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Three or more mice per group were used in each experiment. Sample sizes were chosen based on the minimal number of experiments needed to reach statistical significance in pilot experiments.
Data exclusions	No data were excluded
Replication	Besides the longitudinal aging experiment, all the findings were replicated at least twice.
Randomization	For cohousing, mice were randomly assigned to each group, otherwise no randomization was performed. Whenever possible, groups were sex-matched with the exception of some transgenic mice due to breeding efficiency.
Blinding	When possible, behavioral experimenter was blinded to treatment and group. However, NIA mice have a tail tattoo and appear visibly older and could be identified visually as such.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	GFAP (Dako GA524, 1:25k) cFos (CST 2250, Lot 12, 1:1,500)

NeuN (Millipore 377, Lot 3248401, 1:500)
Iba1 (Wako 016-26461, Lot WTN4536, 1:500)
BrdU (Abcam 6326, Lot GR3365969-5, 1:500)
CD45 (clone 30-F11, BioLegend 103106 and 103108, 1:100)
CD45.1 (clone A20, BioLegend 110706, 1:100)
CD45.2 (clone 104, BioLegend 109814, 1:100),
TCRb (clone H57-597, BioLegend 109210, 1:100)
CD4 (clone GK1.5, BioLegend 100412, 1:100)
CD8a (clone 53-6.7, BioLegend 100706, 1:100)
NK1.1 (clone PK136, BioLegend 108739, 1:100)

Validation

Anti-GFAP rabbit polyclonal, Dako GA524. Discontinued.
https://www.citeab.com/antibodies/3383210-ga524-glial-fibrillary-acidic-protein
Anti-cFos rabbit monoclonal, CST 2250. Validated for IF in mouse tissue.
https://www.citeab.com/antibodies/123097-2250-c-fos-9f6-rabbit-mab
Anti-NeuN mouse monoclonal, Millipore 377. Validated for IF in mouse tissue.
https://www.citeab.com/antibodies/226230-mab377-anti-neun-antibody-clone-a60
Anti-Iba1 rabbit polyclonal, Wako 016-26461. Validated for IF in mouse tissue.
https://www.citeab.com/antibodies/19287285-019-19741-anti-iba1-rabbit-for-immunocytochemistr
Anti-BrdU rat monoclonal, Abcam 6326. Validated for IF in mouse tissue.
https://www.citeab.com/antibodies/714967-ab6326-anti-brdu-antibody-bu1-75-icr1-prolifera
Anti-CD4 rat monoclonal, BE0003. Validated for in vivo CD4+ T cell depletion in mice.
https://www.citeab.com/antibodies/7570486-be0003-invivomab-anti-mouse-cd4
Anti-CD8a rat monoclonal, BE0061. Validated for in vivo CD8+ T cell depletion in mice.
https://www.citeab.com/antibodies/7570533-be0061-invivomab-anti-mouse-cd8
Anti-NK1.1 mouse monoclonal, BE0036. Validated for in vivo NK cell depletion in mice.
https://www.citeab.com/antibodies/7570514-be0036-invivomab-anti-mouse-nk1-1
Anti-Ly6G rat monoclonal, BP0075-01. Validated for in vivo neutrophil depletion in mice. Citation PMID: 32488020.
https://www.citeab.com/antibodies/12766748-bp0075-1-invivomab-anti-mouse-ly6g
Anti-CD16/32 rat monoclonal, BioLegend 156604. Validated for blocking non-specific IgG binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/6999339-156604-trustain-fcx-plus-anti-mouse-cd16-32-s170
Anti-CD45 (clone 30-F11) rat monoclonal, BioLegend 103106 and 103108. Validated for CD45 binding (both CD45.1 and CD45.2) in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517989-103106-pe-anti-mouse-cd45-30-f11-monoclonal
Anti-CD45.1 (clone A20) mouse monoclonal, BioLegend 110706. Validated for CD45.1 without CD45.2 binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517714-110706-fitc-anti-mouse-cd45-1-a20-monoclonal
Anti-CD45.2 (clone 104) mouse monoclonal, BioLegend 109814. Validated for CD45.2 without CD45.1 binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517625-109814-apc-anti-mouse-cd45-2-104-monoclonal
Anti-TCRb (clone H57-597) Armenian hamster monoclonal, BioLegend 109210. Validated for TCR binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517570-109210-pe-cyanine5-anti-mouse-tcr-chain-h57-597
Anti-CD4 (clone GK1.5) rat monoclonal, BioLegend 100412. Validated for CD4 binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517114-100412-apc-anti-mouse-cd4-gk1-5-monoclonal
Anti-CD8a (clone 53-6.7) rat monoclonal, BioLegend 100706. Validated for CD8a binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/517201-100706-fitc-anti-mouse-cd8a-53-6-7-monoclonal
Anti-NK1.1 (clone PK136) mouse monoclonal, BioLegend 108739. Validated for NK1.1 binding in mouse cells for flow cytometry.
https://www.citeab.com/antibodies/1483796-108739-brilliant-violet-605-anti-mouse-nk-1-1-pk13

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Except where noted, young female (8 weeks) and male (4 weeks) C57BL/6 mice were obtained from The Jackson Laboratory and old mice (18-20 months) were acquired from the NIA. Germ-free C57BL/6 mice were maintained in sterile isolators at the University of Pennsylvania Gnotobiotic Animal Facility. The following mouse strains were used in manuscript and were purchased from The Jackson Laboratory: C57BL/6J (000664), CD45.1 (002014), DBA/2J (000671), Trpv1Cre (017769), Snap25-GCaMP6s (025111), DTA (009669), Phox2bCre (016223), Phox2bFlpO (022407), Il1rfl/fl (028398), Ccr2-/- (004999), Nlrp3-/- (021302), Tnfr-/- (003243), Cre-dependent hM4Di (026219), Cre/FlpO-dependent hM4Di (029040), and Ai65 (Cre/FlpO-dependent tdTomato) (021875). Gpr84-/- mice were a kind gift from Ikuo Kimura. Animals were housed in ULAR facilities at the University of Pennsylvania under 7am-7pm light-dark cycles, 20-25C, and 30-70% humidity.
Wild animals	No wild animals were used
Reporting on sex	In initial cohousing experiments, female mice were used to avoid fighting with non-littermates. However, the phenotype was confirmed in male mice as well. Otherwise, experiments were performed in both male and female mice.
Field-collected samples	No field collected samples were used
Ethics oversight	All animal procedures were performed in accordance with protocols approved by the Institutional Animal Care and Usage Committee at the Perelman School of Medicine at the University of Pennsylvania (protocol # 806361 and #805984).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	The ilial lumen was flushed with cold PBS, opened longitudinally, and cut into 1 cm pieces. Epithelial cells were removed by incubating in dissociation buffer (3% fetal bovine serum (FBS) (Corning, 35-010-CV) and 10 mM EDTA in Hank's Balanced Salt Solution (HBSS)) at 4°C with shaking for 30 minutes. After removing epithelial cells by vortexing and straining through a 70 m filter, the remaining intestinal pieces were washed thoroughly in cold PBS and then digested in HBSS containing 0.1 mg/ml Liberase (Sigma-Aldrich, 05401151001) and 1 mg/ml DNase (ROC, 10104159001) for 20 minutes at 37°C shaking at 180 rpm. The digested cells were washed with PBS and passed through a 70 µm cell strainer. Blood leukocytes were collected via cardiac puncture and red blood cells lysed with ACK Lysing Buffer (Quality Biological, 118-156-101).
Instrument	LSR II, BD Biosciences
Software	All samples were analyzed with FlowJo 10.9.0 (Tree Star Inc, Ashland, OR, USA).
Cell population abundance	Sorting was not performed
Gating strategy	Gating strategy is provided in Extended Data Fig. 9

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