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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	scRNA-seq data was collected using the 10x Genomics Chromium Single Cell 3' v3 kit. Sequencing was performed on the Illumina NovaSeq6000. Flow cytometry data were acquired on a BD FACSAria II or FACSAria Fusion running FACSDiva v8 software. Resolve spatial transcriptomics data were acquired using the Molecular Cartography platform.
Data analysis	Raw scRNA-seq fastq files were aligned to the mouse reference (mm10/gencode.vM23) using the 10x Genomics CellRanger v6.0.0 pipeline. scRNA-seq iterative clustering and differential gene expression analysis was performed in R using the scrattch.bigcat package (https://github.com/AllenInstitute/scrattch.bigcat), v0.0.1, which also contains many functions to visualize the data together with the scrattch.vis package (https://github.com/AllenInstitute/scrattch.vis). Age-associated DE genes were calculated using the R package MAST v1.20.0 (https://github.com/RGLab/MAST), and compared with the DE genes computed at the pseudo-bulk level using edgeR v3.32.1 (https://bioconductor.org/packages/release/bioc/html/edgeR.html). Augur v1.0.3 (https://github.com/neurorestore/Augur) was used to prioritize cell types at subclass and supertype levels that are most responsive to age-associated effects. Gene ontology (GO) term enrichment was performed using the R package gprofiler2, v0.2.2. Raw Resolve data were segmented using a combination of open-source software Cellpose v2.1.0 (https://www.cellpose.org/) and Baysor v0.6.2 (https://github.com/kharchenkolab/Baysor). Filtering of low quality cells and mapping to scRNASeq taxonomy was performed using custom made scripts in R. Additional downstream analysis was conducted using custom scripts in R. All the analysis scripts used in this study, including examples using the tools mentioned above, are available at https://github.com/AllenInstitute/mouse_aging_scRNAseq.

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

Primary and processed single cell RNA seq data have been deposited to the Neuroscience Multi-omic Data Archive (NeMO), https://nemoarchive.org/, and can be accessed at https://assets.nemoarchive.org/dat-61kfys3.
Primary spatial transcriptomics data have been deposited to Brain Image Library (BIL), https://www.brainimagelibrary.org/, and can be accessed at https://doi.org/10.35077/g.1157.

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	This study does not involve human research participants.
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	Sample size calculations were not performed. For scRNA-seq data and spatial transcriptomics data, sample size was determined based on extensive prior experience using these technologies in brain and resource availability, with balance in sex and age groups. The scRNA-seq data for each ROI were generated from 10-30 independent samples.
Data exclusions	Cells from scRNA-seq data and segmented cells from Resolve data that did not pass the QC processes described in Methods and supplementary tables were excluded.
Replication	The scRNA-seq data were generated from a total of 284 libraries from 96 animals, with multiple animals used for each of the 16 ROIs at each of the aged and adult age groups. The scRNA-seq data were obtained from at least two males and two females per ROI per age group, except for ROIs ACA (3M 1F in the adult group), PL-ILA-ORB (2M 1F in the adult group), and STRv (5M 1F in the adult group). The Resolve data were generated from at least 2 biological replicates per ROI per sex per age group.
Randomization	Randomization of animals to different groups was not relevant to our study design. There were no experimental vs. control groups.
Blinding	Experiments were not blinded in this study as all results presented are based on quantitative analysis and not subject to human biases.

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

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	Laboratory animals Mus musculus, males and females, wild-type or Cre transgenic, in the C57BL/6J strain, in two age groups - adult (P53-P69) and aged (P540-P553).
Wild animals	This study did not involve wild animals.
Reporting on sex	For each brain ROI and each age, both male and female mice were used to collect scRNA-seq data.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All experimental procedures related to the use of mice were approved by the Institutional Animal Care and Use Committee of the Allen Institute for Brain Science, in accordance with NIH guidelines.

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

Plants

Seed stocks	This study does not involve plants.
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	Sample preparation was done according to protocols: Allen Institute for Brain Science 2021, Slice Preparation with Tissue Dissociation - Mouse Protocol. protocols.io https://dx.doi.org/10.17504/protocols.io.bq6wmzfe; and Allen Institute for Brain Science 2020, FACS Single Cell Sorting. protocols.io https://dx.doi.org/10.17504/protocols.io.be4cjgsw. Single cells were isolated by adapting previously described procedures. The brain was dissected, submerged in ACSF, embedded in 2% agarose, and sliced into 350-µm coronal sections on a compresstome (Precisionary Instruments). Block-face images were captured during slicing. Regions of interest (ROIs) were then microdissected from the slices and dissociated into single cells as previously described. Fluorescent images of each slice before and after ROI dissection were taken at the

dissection microscope. These images were used to document the precise location of the ROIs using annotated coronal plates of CCFv3 as reference.

Dissected tissue pieces were digested with 30 U/ml papain (Worthington PAP2) in ACSF for 30 minutes at 30°C. Due to the short incubation period in a dry oven, we set the oven temperature to 35°C to compensate for the indirect heat exchange, with a target solution temperature of 30°C. Enzymatic digestion was quenched by exchanging the papain solution three times with quenching buffer (ACSF with 1% FBS and 0.2% BSA). Samples were incubated on ice for 5 minutes before trituration. The tissue pieces in the quenching buffer were triturated through a fire-polished pipette with 600-µm diameter opening approximately 20 times. The tissue pieces were allowed to settle and the supernatant, which now contained suspended single cells, was transferred to a new tube. Fresh quenching buffer was added to the settled tissue pieces, and trituration and supernatant transfer were repeated using 300-µm and 150-µm fire polished pipettes. The single cell suspension was passed through a 70-µm filter into a 15-ml conical tube with 500 µl of high BSA buffer (ACSF with 1% FBS and 1% BSA) at the bottom to help cushion the cells during centrifugation at 100 x g in a swinging bucket centrifuge for 10 minutes. The supernatant was discarded, and the cell pellet was resuspended in the quenching buffer. We collected 1,508,284 cells without performing FACS. The concentration of the resuspended cells was quantified, and cells were immediately loaded onto the 10x Genomics Chromium controller.

To enrich for neurons or live cells, cells were collected by fluorescence-activated cell sorting (FACS) (BD FACSAria II or FACSAria Fusion, running FACSDiva v8 software) using a 130-µm nozzle. Neuronal enrichment was achieved by sorting RFP+ cells from Snap25-IRES2-Cre/wt;Ai14/wt transgenic mice counterstained with DAPI to exclude dead or dying cells. To enrich for live cells, samples were stained and sorted for Hoechst+ or Calcein+/Hoechst+ signal. Cells were prepared for sorting by passing the suspension through a 70-µm filter. Sorting strategy was as previously described (Tasic et al 2018). 30,000 cells were sorted within 10 minutes into a tube containing 500 µl of quenching buffer. We found that sorting more cells into one tube diluted the ACSF in the collection buffer, causing cell death. We also observed decreased cell viability for longer sorts.

Instrument

FACSAria II or FACSAria Fusion

Software

FACSDiva v8

Cell population abundance

Abundance of sorted cell populations for 10x scRNA-seq were determined by hemocytometer post-FACS.

Gating strategy

For sorting RFP+ cells, the morphology gate (SSC-A vs FSC-A) included all events that passed FSC threshold to allow profiling of all possible RFP+ cells. SC-FSC and SC-SSC were used to exclude doublets, and RFP+ cells were sorted from the rest of the cells based on the RFP+ DAPI- phenotype. Gating strategy for RFP+ mouse neurons is described in more detail here: Allen Institute for Brain Science 2020, FACS Single Cell Sorting, protocols.io https://dx.doi.org/10.17504/protocols.io.be4cjgsw.

Live cells were sorted using a similar strategy. The morphology gate included all events that passed FSC threshold to allow profiling of all possible live cells. SC-FSC and SC-SSC were used to exclude doublets, and live cells were sorted from the rest of the cell population based on the Hoechst+ or Calcein+/Hoechst+ phenotype.

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