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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	Leica LAS-X microscope imaging software was used during data acquisition. Huygens Essential 21.04 was used for image deconvolution
Data analysis	MATLAB R2019a was used for imaging analysis of in situ sequencing images. Python (Version 3.8) and R (version 4.1) was used for data analysis. Clustermap (Version 0.0.1, https://github.com/wanglab-broad/ClusterMap) was used for cell segmentation. GO network diagrams are plotted using CytoScape (Version 3.9.1). The code and demos of the CAST have been deposited to Zenodo (https://zenodo.org/records/12215315) and Github (https://github.com/wanglab-broad/CAST). The implementation of CAST, as well as the tutorials, is available in the pipeline files. Versions of R packages: gprofiler2, 0.2.1; Seurat, 4.0.3; ComplexHeatmap, 2.10.0; clusterProfiler, 3.18.1. Versions of other Python packages used in analysis: squidpy, 1.2.2; matplotlib, 3.5.2; seaborn, 0.11.2; scikit-learn, 1.1.0; h5py, 3.6.0; statsmodels, 0.13.2; tqdm, 4.64.0; geopandas, 0.10.2; Rtree, 1.0.0; scanpy, 1.9.1; libpysal, 4.6.2; ipython, 8.3.0; jupyterlab, 3.4.2; jupyter, 1.0.0; numpy, 1.21.6; pandas, 1.4.2; pytorch,1.11.0; torchvision, 0.12.0; torchaudio, 0.11.0; cudatoolkit, 11.3; dgl-cuda11.3, 0.9.1.

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

The RIBOmap and STARmap datasets are available from (RIBOmap_mouse1, STARmap_mouse1 and RIBOmap_mouse2: https://singlecell.broadinstitute.org/single_cell/study/SCP1835; STARmap_mouse2: https://singlecell.broadinstitute.org/single_cell/study/SCP2203). The AD STARmap PLUS dataset is publicly available at (https://singlecell.broadinstitute.org/single_cell/study/SCP1375/).
The mouse brain atlas dataset used is available at (https://singlecell.broadinstitute.org/single_cell/study/SCP1830).
Two Visium datasets (Mouse Brain Coronal Section 1 (FFPE) and Mouse Brain Coronal Section 2 (FFPE)) used are available from:
https://www.10xgenomics.com/resources/datasets/mouse-brain-coronal-section-1-ffpe-2-standard
https://www.10xgenomics.com/resources/datasets/mouse-brain-coronal-section-2-ffpe-2-standard
The MERFISH dataset used (co1_slice37 in co1_sample13) is available from:
https://doi.brainimagelibrary.org/doi/10.35077/act-bag
The Slide-seq dataset used (slice042) is available from:
https://docs.braincelldata.org/downloads/index.html
The two Stereo-seq MOSTA datasets used (E16.5_E2S5 and E16.5_E2S6) are available from:
https://db.cngb.org/stomics/mosta/download/

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Human research is not relavant to this study.
Population characteristics	Human research is not relavant to this study.
Recruitment	Human research is not relavant to this study.
Ethics oversight	Human research is not relavant to this study.

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	AD STARmap PLUS dataset contains 4 diseased samples (TauPS2APP mouse model; 2 13-month mice and 2 8-month mice) and 4 control samples (age-matched wild-type mouse, 2 13-month mice and 2 8-month mice). The sample size for each sample: S1, n = 9,803; S2, n = 8,506; S3, n = 9,428; S4, n = 8,034; S5, n = 8,202; S6, n = 8,186; S7, n = 9,634; S8, n = 10,372; simulated sample S1', n = 8,789. In delta-sample spatial analysis, the 8 samples are grouped into two comparisons: 8-month disease versus control (8-mos) and 13-month disease versus control (13-mos). The RIBOmap dataset contains 4 brain slice from 2 mouse individuals. The sample size for each sample: STARmap_mouse1, n = 59,165; RIBOmap_mouse1, n = 58,692; STARmap_mouse2, n = 44,751; RIBOmap_mouse2, n = 60,481. For each statistical test, we describe the sample size in the figures or legends.
Data exclusions	In the delta-sample analysis, we filtered the small plaques with the area less than 300 pixels in the initial image.
Replication	We used 4 control samples and 4 diseased samples for conclusions in AD STARmap PLUS dataset (Fig. 4 and Extended Data Fig. 5, Supplementary Fig. 7). For scRTE analysis in the mouse brain, we used samples from 2 mouse individuals as biological replicates(Fig. 6, Extended Fig. 8-10). All findings or major discoveries can be reproduced and be found in different replicates.
Randomization	For CAST benchmark (Extended Data Fig. 1e, Supplementary Fig. 1,5), we performed 10 replicates using randomization (random seeds are generated based on the system time). In AD STARmap PLUS dataset analysis, we use averaged values of two normal samples for each

comparisons (13-mos or 8-mos) as control sample profiles.
In scRTE analysis, the randomization is not applicable since each mouse only contains one STARmap and one RIBOmap dataset.

Blinding

Blinding is not applicable to this study because the different groups of the STARmap or RIBOmap datasets were known throughout the experiments, 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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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