nature portfolio

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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	A custom Python script was used to operate the microscope used in this study and is available upon request.
Data analysis	The following software was used to process raw microscopy datasets: B3D compression filter for HDF5 files (https://github.com/ome/B3D), BigStitcher (0.8.2), FalseColor Python (custom Python package: https://github.com/serrob23/falsecolor), Bead PSF Computation (custom Python package: https://github.com/LiuBiophotonicsLab/Bead_PSF_computation), and FixImage3D (custom Python package: https://github.com/LiuBiophotonicsLab/FixImage3D). The following software was used to view and analyze Path3D datasets: FIJI (2.3.0), Napari (0.4.14), Imaris (9.1.2), Avia (12.0.0), QuPath (0.2.3), and a custom web viewer developed by Ground Truth Labs (UK).

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

> Due to the large size of the imaging datasets shown in this manuscript, the datasets are not available in a public repository. They are available from the authors upon request.

Human research participants

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

Reporting on sex and gender	Information on sex and gender was not collected. Data is provided to show example results from this protocol, and no biological conclusions are made.
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	The human specimens shown in Fig. S1b were obtained with approval from the University of Washington IRB and with informed consent from patients. All other human tissues shown in this manuscript were de-identified specimens provided by an institutional tissue bank and were not subject to IRB approval at the University of Washington.

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	N/A
Data exclusions	N/A
Replication	N/A
Randomization	N/A
Blinding	N/A

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

Antibodies

Antibodies used	Fig. S1c: Rat IgG2a CD31 monocolonal antibody, AlexaFluor 647 (clone 390; ThermoFisher Scientific catalog # A14716)
Validation	Testing data is provided on the manufacturer's website, no additional validation was performed for this study: https://www.thermofisher.com/antibody/product/CD31-Antibody-clone-390-Monoclonal/A14716

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	The mouse used in Fig. S1c is a young C57BL/6N from the Jackson Laboratory. Stain and age information was not collected for the animal tissue shown in Fig. S8b, which is shown to illustrate procedures only.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex information was not collected. Data is provided to show example procedures/results from this protocol, and no biological conclusions are made.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	The animal tissues shown in Fig. S1c were collected with approval by the Animal Care and Use Committee at the Indiana University School of Medicine and in accordance with National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The animal tissues shown in Fig. S8b were collected from already-euthanized animals donated by an institutional animal facility at the University of Washington and not subject to IACUC approval.

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