

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 (<i>n</i>) 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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☐ | ☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☐ | ☒ 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 <i>d</i> , Pearson's <i>r</i>), 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	Image acquisition: Zeiss Zen Black software (SP2) was used to capture images of hybridisation chain reaction in situ experiments on temporal cortex tissue sections. The EVOS M5000 software was used to capture Images of H&E stained temporal cortex tissue sections.
Data analysis	We used publicly available software for all analyses. Names and versions for all the software used are as follows: 10X Genomics Cell Ranger (V.6.1.1) R (V.4.2.0) Seurat (V.2.0) DoubletFinder (V3.0) DoubletDecon (V1.1.5) Scrublet (V0.2) SCP package (V0.4.8) DESeq2 (V1.40.1) Psupertime (V0.2.6) GSEA (V.2023.1) dittoSeq (V1.13.1) NS-Forest tool (v2.0) gProfiler (2023-09-14 build) Microsoft Image Composite Editor (V.2.0.3) 10X Genomics Loupe Browser (V4.0) 10X Space Ranger (V1.3.0) MATLAB (R2019a)

Vistoseg (V1)
 Cell2location (V.0.7a0)
 BayesSpace (V1.5.1)
 VIZGEN MERSCOPE Vizualizer software (V.2.3.3330.0)
 ggplot2 (V3.4.2)
 ShinyCell (V2.1.0)
 Microsoft Excel (V.16.54)

All scripts used to analyse the data are indicated as numbered files in the methods section and are available on Zeondo: <https://zenodo.org/doi/10.5281/zenodo.13321264>

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](#)

As the data from this study contain personal information from living donors, the raw sequence files and analysed data files (integrated and annotated Seurat Object, Cell2location trained model, Visium image files) for snRNA-seq and Visium experiments will be available through the Human Cell Atlas Data repository (https://url.za.m.mimecastprotect.com/s/AVW_C0go6LfGP0oLOTwfYC91ndy) as part of the “managed access” tier, once data curation is complete and a data use oversight system (DUOS) ID has been assigned. Potential users will be able to request access to the data by following the instructions on the Human Cell Atlas Data Portal website: <https://data.humancellatlas.org/guides/requesting-access-to-controlled-access-data>. A ShinyApp (https://hockman-lab.shinyapps.io/shiny_app/) for exploration of the annotated snRNA-seq data is available. Source data for in situ hybridisation images are provided with this publication.

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	Data on the biological sex of the donors, obtained from their clinical records or from previously published data for publicly available datasets, is given in Extended Data Table 1 (n female= 8, n male= 4) . Data on gender was not collected.
Reporting on race, ethnicity, or other socially relevant groupings	Data on the ancestry of the South African donors (i.e. whether they are black or white South African) is given in Extended Data Table 1. This data was determined by the neurosurgeons the Red Cross War Memorial Children’s Hospital and Constantiaberg Mediclinic (Cape Town, South Africa). The “ancestry” descriptors in Supplementary Table 1 were used solely to ensure that the indicated population cohorts are represented in the Human Cell Atlas Data repository. These descriptors were not used in participant accrual, study design, data analysis, or data interpretation.
Population characteristics	Data on the age (range: 4-50 years) and diagnosis of the donors (cause of epilepsy), obtained from their clinical records, is given in Extended Data Table 1. Genetic information is not known.
Recruitment	Neurosurgeons at the Red Cross War Memorial Children’s Hospital and Constantiaberg Mediclinic (Cape Town, South Africa) obtained informed written consent from donors for sample collection for use in research studies prior to surgery to conduct temporal lobe resections. We are not aware of any self-selection bias that would impact the results of the study.
Ethics oversight	Ethical approval was granted for the collection and use of paediatric and adult human brain tissue by the University of Cape Town Human Research Ethics Committee (UCT HREC REF 016/2018; sub-studies 146/2022 and 147/2022)

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	No sample size calculations were performed. Sample size was determined by the availability of samples for the given experiment. The sample size is in line with similar single cell studies using human tissue (e.g. PMID: 34239132, PMID: 37443107)
Data exclusions	Nuclei-level filtering was performed to remove poor quality nuclei according to their number of UMIs (nUMIs) detected, number of genes

Data exclusions	detected (nGene), number of genes detected per UMI (log10GenesPerUMI), and the fraction of mitochondrial read counts to total read counts (mitoRatio). Nuclei that met the following criteria were retained: nUMI > 500, nGene > 250, log10GenesPerUMI > 0.8 and mitoRatio < 0.2. Gene-level filtering was performed to remove genes that had zero counts in all nuclei, remove genes expressed in fewer than 10 nuclei, and remove mitochondrial genes from the gene by cell counts matrix. These criteria were based on the Harvard Chan Bioinformatics Core scRNA-seq analysis pipeline (https://hbctraining.github.io/scRNA-seq_online/). Three doublet removal tools namely DoubletFinder, DoubletDecon, and Scrublet) were used to identify doublets for each dataset individually. The sample-specific parameters of each of the tools were adjusted according to the specified guidelines. To achieve a balance between the false positive and false negative rate of the different doublet detection tools, all doublets identified by DoubletFinder as well as the intersection of the doublets identified by DoubletDecon and Scrublet, were removed. Nuclei with discordant level 1 and level 2 annotations were removed from the analyses.
Replication	Our single nucleus (sn)RNA-seq analysis was performed on samples obtained from 6 paediatric and 6 adult samples. For a subset of samples, we generated technical replicate snRNA-seq libraries and showed that our annotation approach gave consistent results across the technical replicates (see Extended Data Fig. 2). We validated our annotation through correlation analysis to the existing middle temporal gyrus dataset (PMID: 31435019) (see Fig. 1e). We further validated this annotation through comparison to 10x Genomics Visium Spatial Gene Expression datasets for two of our datasets, with two technical replicate libraries each (See Fig. 2). Results of the Visium analyses were validated with MERFISH analysis (see Extended Data Fig. 8). Results of the differential expression analysis performed on snRNA-seq samples were also validated through comparison to the 10x Genomics Visium Spatial Gene Expression datasets (See Supplementary Fig. 2)
Randomization	Donor samples were assigned to either paediatric or adult group based on their age (<18 = paediatric).
Blinding	The donor specimens were de-identified and assigned a unique numerical code. Researchers conducting the statistical analyses had access to basic information about donors (e.g. age, diagnosis) as well as the unique numerical code assigned to each donor. Researchers were not blinded to group allocations as knowledge of the age of the sample donor was necessary for the experimental design.

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

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.