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Corresponding author(s): NCOMMS-24-69600

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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	Not applicable.
Data analysis	Single-nucleus sequencing (10X Genomics), Slide-seq (Curio Seeker), and MERFISH (Vizgen) datasets were preprocessed and analyzed using Seurat v5. Slide-seq deconvolution was performed using the RCTD wrapper in Seurat from Cable et al. 2023. Differential expression was performed using the MAST wrapper in Seurat from Finak et al. 2015. RNA velocity was performed using Velocyto and scVelo from La Manno et al. 2018 and Bergen et al. 2020. Relevant codes used in this manuscript are provided in Github (https://github.com/annaminyifeng/Molecular-Cartography-of-DS-Brain). Reproducible results for the microenvironment analysis can also be accessed via Code Ocean at DOI 10.24433/CO.4591687.v2.

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 files for human single-nucleus sequencing, Slide-seq, and MERFISH have been deposited in GEO under the accession codes GSE280175 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280175], GSE280170 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280170], and GSE280177 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE280177], respectively. Proteomics data has been deposited as a complete submission to the MassIVE repository (https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp) and assigned the accession number MSV000096108. The dataset is currently available for reviewers at ftp://MSV000096108@massive.ucsd.edu. Please login with username MSV000096108_reviewer; password: DownHuman. The dataset will be made public upon acceptance of the manuscript. Processed datasets are available from the authors with reasonable request. Source data are provided with this paper.

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	The genotype for each prenatal human sample used in Slide-seq, single-nucleus sequencing, and proteomics was confirmed through karyotyping and is detailed in Supplementary Table 1. Sex was not taken into consideration for this manuscript.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Research agreement from Clinical Trials Ontario (Study # 3227)

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 for Slide-seq spatial transcriptomics was n=3 per condition (DS and euploid). Sample size for single-nucleus sequencing was n=5 per condition (DS and euploid). Sample size for proteomics was n=4 per condition (DS and euploid). Sample sizes for the aforementioned experiments were determined based on the standard practices in the field and availability of human brain tissue. Sample size for MERFISH spatial transcriptomics was n=3 per condition (Ts65Dn and euploid) and age (postnatal and 6 months). This sample size was determined according to standard practices in the field and the observed variability among biological replicates, as there is no established method for power calculation in spatial transcriptomics.
Data exclusions	One control sample was excluded from the proteomics analysis due to substantial variability compared to the other control samples.
Replication	Data from Slide-seq, single-nucleus sequencing, proteomics, and MERFISH experiments were obtained from three, five, four, and three replicates, respectively. The findings were consistent across replicates.
Randomization	Human samples were selected based on their karyotype and age-matched for the Slide-seq, single-nucleus sequencing, and proteomics datasets. For MERFISH, mice were randomly assigned to experimental groups to avoid selection bias, with all groups age-matched and balanced for weight.
Blinding	Investigators were not blinded to sample identity for any of the datasets. However, the individuals responsible for the technical execution of each experiment were independent from those analyzing the data. All immunohistochemistry analyses were conducted in a blinded manner.

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: The primary antibodies used for IF were goat anti-SOX2 (1:200, Abcam, catalog #Af2018), rabbit anti-LMNB1 (1:150, Abcam, #Ab16048), rabbit anti-LINE1 ORF1p antibody (1:50, Abcam, #Ab216324), mouse p53 antibody (1:200, Santa Cruz, #sc-126), mouse anti-NeuN antibody (1:200, Millipore, #MAB377), and goat ROBO1 antibody (1:100, Thermo Fisher, #PA5-18460). The secondary antibodies used for IF were Alexa Fluor 488 donkey anti-goat IgG (1:500, ThermoFisher, catalog #A-31573), Alexa Fluor 555 donkey anti-mouse IgG (1:500, ThermoFisher, catalog #A-31570), Alexa Fluor 647 donkey anti-rabbit (1:500, ThermoFisher, catalog #A-31573).

Validation: No antibody validation data is provided in this manuscript.

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, mosiacism, off-target gene editing) were examined.*