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Corresponding author(s): Maura B Dupont

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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	Sequencing data were obtained from the Illumina NovaSeq 6000 and were pre-processed using 10X CellRanger (v2.0.0) pipeline with the chemistry parameter set to Single Cell Multiome ATAC + GEX v1, SpaceRanger (v1.3.0) pipeline with chemistry set to Spatial 3' for Visium v1, and SpaceRanger (v3.1.2) pipeline with chemistry set to Spatial Probe-based assay for Visium v2. Spatial data were collected using Visium v1 (6.5x6.5 mm capture area, poly(A)-based) and Visium v2 (11x11 mm capture area, probe based, 18,000 transcripts; 10X Genomics). Validation of relevant gene co-expression was completed using Multiplex and Duplex RNAScope (ACDBio), as well as immunofluorsecence and immunohistochemistry. Stereology was performed using Stereo Investigator and Microfile (MBF Inc). We used shotgun proteomics liquid chromatography with tandem mass spectrometry (LC/MS/MS), and the software Rosetta Elucidator Protein Expression Analysis System (V. 4.0.0.2.13, PerkinElmer, Boston, MA).
Data analysis	Custom scripts were written in Python 3.12.6: scanpy 1.10.4, scvelo 0.3.3, pyscenic 0.12.1, pysam 0.23.0; macs3 3.0.2, palantir 1.4.1, velocyto 0.17.17, Tobias 0.17.1, pyBigWig 0.3.24, gseapy 1.1.11, Samtools v1.9, UROPA v4.0.3. R packages: Seurat_5.4.0 , Signac_1.16.0, SeuratDisk_0.0.0.9021, BPCells_0.3.0, SeuratObject_5.3.0, scDblFinder_1.20.2, SoupX_1.6.2, SingleCellExperiment_1.28.1, GenomicRanges_1.58.0, harmony_1.2.4, edgeR_4.4.2, scater_1.34.1, limma_3.62.2, glmmTMB_1.1.13, spatialLIBD_1.20.1, scuttle_1.18.0, scran_1.36.0, apeglm_1.30.0, DESeq2_1.48.2, car_3.1-3, org.HS.eg.db_3.21.0, clusterProfiler_4.16.0, ReactomePA v1.52.0, clusterProfiler v4.16.0, Monocle3 v1.4.26. All scripts used can be found at https://github.com/mauibol/dupont-lab-mdd. Stereology was performed using Stereo Investigator (MBF Inc.).

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

We deposited the processed Seurat objects in Zenodo (https://doi.org/10.5281/zenodo.21041190) because this preserves the complete processed dataset, including the count matrices, cell-level metadata, dimensionality reductions, and analysis results in a single file. This allows users to directly reproduce the analyses presented in the manuscript without repeating the computational preprocessing steps that would be necessary if sharing raw data.

The datasets for this study, including mass spectroscopy data, are available in the Supplementary Tables, and complete raw data are archived in our servers and available upon request.

Clinical and sociodemographic data on the subjects included in this study are shown in Supplementary Table 1, and the complete dataset for each subject will be available upon request. Because the data we collect are sensitive in nature, we carefully ensure that appropriate privacy safeguards are in place. All data will be stripped of identifiers prior to release for sharing.

In addition, we will provide relevant protocols and published genetic and phenotypic data upon request. Material transfer agreements will be made with no more restrictive terms than in the Simple Letter Agreement (SLA) or the Uniform Biological Materials Transfer Agreement (UBMTA) and without reach through requirements.

Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remains widely available to the research community in accordance with the NIH Principles and Guidelines.

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	We included men and women. Sex and gender information was collected from clinical charts, autopsy reports, and interviews of next of kin.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity were assigned based on autopsy reports. The sample includes people with ancestral origin from Africa, Europe, and the Americas. Information was collected from clinical charts, autopsy reports, and interviews of next of kin. Subject information can be found in Supplementary Table 1 which was submitted as Data Set, and in Extended Data Table 1.
Population characteristics	De-identified clinical and demographic information on all subjects is described in Supplementary Table 1 which was submitted as Data Set, and in Extended Data Table 1.
Recruitment	Informed consent was obtained from the next of kin that agreed to donate the brain of the deceased.
Ethics oversight	Brain tissue was obtained with approval from the Institutional Review Boards (IRB) of the New York State Psychiatric Institute, the Human Brain Collection Core of the National Institute of Mental Health, and the Institute of forensic medicine, of the Ss. Cyril and Methodius University of Skopje, North Macedonia, and with written informed consent from subjects' next of kin for brain donation and clinical and sociodemographic data collection. The IRB determined that postmortem brain collection and research does not constitute human subject research as it only involves biological samples from deceased individuals.

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	The total sample size was 123 subjects, and smaller sub-samples were used for the different omics studies and for the validation experiments. We performed power analysis based on findings from previous studies and established the sample size needed to have enough statistical power.
Data exclusions	We excluded low quality nuclei with low UMIs and genes expressed, along with multiplets. Genes and peaks expressed in less than 0.01% of

Data exclusions	cells that passed QC were removed. For chromatin accessibility data, we followed the Signac workflow for merging multiple objects. Peaks on nonstandard chromosomes and in blacklist regions identified using the ENCODE project archived blacklist were removed. We excluded Visium spots with less than 500 genes/spot. Occasional folds in the tissue were labeled as such and excluded from downstream analysis. Proteomics quality control indices included peptide count above two for considering a protein a good candidate. No data were excluded for quantifications of histological experiments.
Replication	For each single-nuclei multiome sample, we had 1-3 technical replicates. For Visium we had 1-2 biological replicate per subject. For Proteomics we had 2 biological replicated per subject. See Methods and Supplementary Tables which were submitted as Data Sets.
Randomization	Not applicable
Blinding	Experimenters preparing tissue, extracting nuclei and preparing libraries for snMultiome and Visium v1 and v2, and performing validation experiments, participated in selecting the cases based on clinical characteristics and RIN score. People performing sequencing, proteomics, and stereology were blind to the group assignment of the cases.

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 following primary antibodies were used in the study: = for immunohistochemistry: - anti-nestin mouse monoclonal, 1:8,000, Chemicon Cat. No. ST1111-100UL; - anti-Ki67 Novocastra mouse monoclonal 1:1 solution Leica Biosystems Cat. No. NCL-L-Ki67-MM1; - anti-doublecortin guinea pig polyclonal, 1:30,000, Sigma Aldrich Cat. No. AB2353; - anti-NeuN mouse monoclonal, 1:100,000, Clone A60, MilliporeSigma Cat. No. MAB377. = for immunofluorescence: - antibodies (anti-doublecortin guinea pig, 1:1,000, Millipore Cat. No. AB2253; - anti-NeuN rabbit monoclonal, Sigma-Aldrich Cat. No. MABN140. The following secondary antibodies were used: = for immunohistochemistry: - goat anti-guinea pig biotin-conjugated secondary antibody (Vector Laboratories Cat. No. PI23227) - horse anti-mouse biotin-conjugated secondary antibody (Vector Laboratories Cat. No. BA-2000) = for immunofluorescence: - Alexa Fluor 594 goat anti- guinea pig (1:500, Jackson ImmunoResearch Cat. No. 106-585-003) - Alexa Fluor 488 goat anti-rabbit (1:500, Jackson ImmunoResearch Cat. No. 111-545-003)
Validation	For immunostaining (immunohistochemistry and immunofluorescence) validation studies, we performed assays without primary antibodies, to assure no unspecific staining. For Multiplex RNAscope validation studies, we used ACDbio probes for POSTN in Channel 1 (Cat No. 409181), BHLHE22 in Channel 2 (Cat No. 448351-C2), PROX1 in Channel 3 (Cat No. 530241-C3). For Duplex RNAscope we used probes for TUBB3 in Channel 1 (Cat No. 318901) and DCX in Channel 2 (489551-C2), following the ACDbio protocols (Multiplex User Manual 323100 Rev C and Duplex User Manual 322500) which also included positive and negative control assays using positive and negative probes.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable