nature portfolio

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Last updated by author(s): 1/9/2026

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	FACSDiva software, version 8.0.2 (BD Biosciences)
Data analysis	All the source codes used in this study are available via GitHub: https://github.com/DiseaseNeuroGenomics/DLPFC_Lifespan_Atlas. The following software were used for data analysis: CellBender v0.2.1, cellSNP v1.2.0, crumblr v0.99.6, decoupler, decoupleR v2.9.7, DiffCircaPipeline v0.0.0.9000, dplyr v1.1.4, dreamlet v1.1.17, enrichR v3.2, ggplot2 v4.0.0, Harmony v0.1, MAGMA v1.08b, mashr v0.2.79, Monocle3 v1.0.0, Pegasus v1.7.0, Pegasus v1.8.1, QTLtools-mbv v1.3, Scanpy v1.9.1, Scanpy v1.9.3, scANVI v0.20.3, scDRS v1.0.1, Scrublet v0.2.3, scvi-tools v1.2.0, spatialLIBD v1.19.11, STARsolo v2.7.9a, stats , STRING-db v 12.0, variancePartition v1.33.11, Vireo v0.5.8.

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 DLPFC lifespan single-nucleus RNA-sequencing (snRNA-seq) data generated in this study are available via Synapse under accession syn52396927, as part of the PsychAD Study (study accession syn60084804). Sequencing reads were aligned and quantified against the human reference genome GRCh38 (hg38). Processed data and analysis outputs are accessible through the AD Knowledge Portal (https://adknowledgeportal.org), which hosts data generated by the Accelerating Medicines Partnership – Alzheimer's Disease (AMP-AD) Target Discovery Program and other National Institute on Aging–supported initiatives. Data are shared without a publication embargo on secondary use and are available for research purposes in accordance with portal data access and attribution policies (https://adknowledgeportal.synapse.org/Data%20Access). Access to the datasets described in this manuscript is provided via https://doi.org/10.7303/syn52396927. These data are distributed under controlled-use conditions to protect participant privacy; access requires completion of a data use agreement. A separate data descriptor manuscript details sample collection and data processing. Interactive visualization of the single-cell data is available through CELLxGENE (https://cellxgene.cziscience.com/e/4442d412-91cb-4261-acca-8adf5fa04c11.cxg/).

Publicly available snRNA-seq datasets used as integrated lifespan replication cohorts include: Herring et al. (GEO accession GSE168408) accessed via Gene Expression Omnibus; Ling et al. accessed through the Neuroscience Multi-omic Data Archive (NeMO; https://assets.nemoarchive.org/dat-bmx7s1t); and Emani et al. accessed via Synapse (syn47700762). Public Visium spatial transcriptomics data from Huuki-Myers et al. were accessed using the spatialLIBD package.

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	Sex was included as a covariate in all statistical models. For samples with genotype data, we confirmed the match between one's self-reported and genetically inferred sex.
Reporting on race, ethnicity, or other socially relevant groupings	Race and ethnicity information was taken from clinical metadata (European (n = 158) followed by African (n = 95), American (n = 26), East Asian and South Asian (n=5)) provided by the respective brain banks that contributing to this study. Race and ethnicity were not used as exclusion criteria and were not included as covariates in statistical analyses.
Population characteristics	Brain tissue specimens were obtained from the NIMH-IRP Human Brain Collection Core (HBCC; 172 samples, ages 0.2–85 years) and the Mount Sinai NIH Neurobiobank (MSSM; 112 samples, ages 20–97 years). In total, 284 neurotypical controls aged 0–97 years from the PsychAD dataset were included. The majority of donors were European descent (n=158), followed by African (n=95), American (n=26), and East/South Asian (n=5). Donors were stratified into four age groups: developmental (0–19 years), young adulthood (20–39 years), middle adulthood (40–59 years), and late adulthood (≥60 years).
Recruitment	All brain specimens were obtained through informed consent via brain donation programs at the respective organizations.
Ethics oversight	All neuropsychological, diagnostic, and autopsy protocols were approved by the Mount Sinai and JJ Peters VA Medical Center Institutional Review Boards.

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 formal sample-size calculation was performed. The study analyzed all available neurotypical human prefrontal cortex specimens in the PsychAD dataset. In total, 284 donors aged 0–97 years were included (172 from the NIMH Intramural Research Program Human Brain Collection Core and 112 from the Mount Sinai NIH Neurobiobank). 1,307,674 single-nucleus transcriptomes were retained across major neuronal and glial cell types. Donors were stratified into four age groups (developmental, young adulthood, middle adulthood, and late adulthood), each comprising at least 50 individuals.
Data exclusions	No data were excluded from the analysis.

Replication	Technical replicates were designed to improve the reproducibility of the experimental findings where applicable. For snRNA-seq data, each donor had two technical replicates. The statistical method was applied with this study design in mind to partition the inter-individual differences from within-individual technical differences.
Randomization	All the brain specimens used in our study were acquired through brain donation programs facilitated by the involved institutions. The availability of these specimens was subject to chance, with no selection bias in the procurement process.
Blinding	The investigators were blinded to allocation of brain specimens.

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	TotalSeq-A nuclear hashing antibodies were obtained from BioLegend. Samples were processed in batches of 6 samples and 2M nuclei from each sample were labeled with 1µl of a unique TotalSeq-A antibody, in accordance with manufacturer's instructions. No two samples within a batch were labeled with the same TotalSeq-A antibody. A total of 10 different TotalSeq-A antibodies were used across the study (BioLegend Cat no. #1 682205, #2 682207, #3 682209, #4 682211, #5 682213, #6 852215, #7 682217, #8 682219, #9 682221 & #10 682223).
Validation	The specificity of the nuclei hashing antibody has been cited in numerous publications, including PMID: 2661560, PMID: 2661560 and PMID: 11448990, while the TotalSeq product has been cited several times, including PMID: 36071158 and PMID: 38781388.

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Flow cytometry was utilized to remove debris and to generate heterogenous populations of nuclei representative of each tissue specimen. Frozen tissue was homogenized in lysis buffer and subjected to sucrose gradient ultracentrifugation . The resulting nuclei pellet was reuspended in PBS and stained with 7-Aminoactinomycin D (7-AAD) (Invitrogen) to facilitate isolation using a FACSAria flow cytometer (BD Biosciences).
Instrument	FACSAria Flow cytometer (BD Biosciences)
Software	FACSDiva software, version 8.0.2 (BD Biosciences)
Cell population abundance	Post FANS, sorted nuclei were quantified using a Countess II cell counter (Life Technologies).
Gating strategy	Forward and side scatter were utilized to differentiate single nuclei from debris. 7-AAD staining was utilized to isolate individual nuclei.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.