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<br>*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<br>*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 | Short-read libraries were sequenced on an Illumina NovaSeq6000. Long-read libraries were sequenced on a PacBio Sequel II. |
| Data analysis | Short-read sequencing analysis: Cell Ranger (v7.1.0) to demultiplex and align to the pre-mRNA reference, Seurat (v4.3) to identify cell types and perform DEG analysis. WebGestalt for gene ontology analysis.<br>Long-read sequencing analysis: ccs (v6.4.0) to generate HIFI reads, skera (v0.1.0) to split into S-reads, lima (v2.7.1) to orient and remove primers, isoseq (v3.8.2) to remove barcodes, cDNA_Cupcake (v29.0) to collapse reads into unique isoforms, modified SQANTI3 (https://github.com/christine-liu/SQANTI3/tree/SQANTICL) for isoform annotation, isoSeQL (https://github.com/christine-liu/isoSeQL/) for comparing isoforms across samples. |

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

Fastq files from short-read sequencing and bam files from long-read sequencing have been submitted to the European Genome-Phenome Archive (EGA). For privacy reasons, these data are access controlled and can be obtained after signing a Data Usage Agreement. The accession code will be provided during the proof process.

## 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 obtained frozen human brain samples from several different brain banks - 8 female samples, and 7 male samples. PCA and UMAP visualizations indicated that age, RIN, and sex did not drive clustering or changes in proportions of cell types present in each sample. We used samples from both sexes in the analysis and did not perform a separate analysis comparing only female or only male samples due to the small sample size upon subsetting by sex and disease. |
| Reporting on race, ethnicity, or other socially relevant groupings | We were not able to obtain information about race/ethnicity for every sample included in this study. We attempted to match the groups as much as possible but were limited by the availability of high RIN samples. |
| Population characteristics | Samples were matched for age, sex, RNA integrity number (RIN). Samples ranged in age from 55-95 years old and all had a RIN greater than 6.0 |
| Recruitment | Fifteen prefrontal cortices were selected from various brain bank sources (8 female and 7 male donors. The sources are: Emory University (n=9), UK Imperial (n=3), Southwest Neurodegenerative Diseases Brain Bank (n=2), Pittsburgh Neurodegenerative Diseases Brain Bank (n=1), and Sepulveda Neurodegenerative Diseases Brain Bank (n=1). Human research participants were not involved in this study. |
| Ethics oversight | MTAs were approved by the respective brain banks and Sanford Burnham Prebys Medical Discovery Institute. |

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 statistical method were used to determine sample size. We chose samples based on criteria for suitability for long-read RNA-sequencing which requires higher quality RNA to ensure less fragmentation. Our data suggest that while our sample size was sufficient to detect some significant changes, examining isoforms with lower expression may require a larger sample size. |
| Data exclusions | No data were excluded. |
| Replication | Experiments to replicate findings were not performed nor included in this manuscript. |
| Randomization | Samples were matched for age, sex, and RIN across experimental groups (AD and control). They were randomized into different batches for nuclei isolation, library preparation, and sequencing. |
| Blinding | Scientists were not blinded during analysis. |

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