nature research

NN-RS72393B
Corresponding author(s): Andrew E Jaffe
Keri Martinowich
Last updated by author(s): Dec 1, 2020

Reporting Summary

Nature Research 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 Research 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	The Space Ranger tool from 10x Genomics was used to process the raw sequencing reads and histology imaging data
Data analysis	The code used for this project is available at https://github.com/LieberInstitute/HumanPilot and archived through Zenodo at https://doi.org/10.5281/zenodo.3691916. The software developed as part of this project, spatialLIBD, is available through Bioconductor at bioconductor.org/packages/spatialLIBD and GitHub at https://github.com/LieberInstitute/spatialLIBD. Here's a list of software used: * SpaceRanger version 1.0.0 from 10X Genomics (uses STAR v2.5.1b) with refdata-cellranger-GRCh38-3.0.0 annotation files. * rgb2lab and imsegkmeans from MATLAB version R2019a. * SingleCellExperiment (1.8.0), scran (1.14.5), scater (1.14.3), spatialLIBD (0.99.0), shiny (1.4.0), plotly (4.9.2), igraph (1.2.4.1), limma (3.42.0), BiocSingular (1.2.2), and uwot (0.1.5) R software packages matching Bioconductor version 3.10 from R 3.6. Version numbers extracted from scripts such as https://github.com/LieberInstitute/HumanPilot/blob/master/Analysis/Layer_Guesses/layer_specificity.R#L1724. * Zen version "Black" https://www.zeiss.com/content/dam/Microscopy/Downloads/Pdf/FAQs/zen-version-investigator.pdf. * dotdotdot from git commit with hash ID 4e1350b from https://github.com/LieberInstitute/dotdotdot/tree/4e1350b027f5457f2e96e562cfa80c6752461478. * Cell Ranger version 3.0.2 from 10X Genomics. * LDSC version 1.0.1. * MAGMA version 1.07b. * SpatialDE version 1.1.0 with Python v3.8.0.

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Processed data is publicly available from the Bioconductor package spatialLIBD http://bioconductor.org/packages/spatialLIBD and https://github.com/LieberInstitute/spatialLIBD.

The raw data is publicly available from the Globus endpoint "jhpce#HumanPilot10x" that is also listed at http://research.libd.org/globus/. The raw data provided through Globus includes all the FASTQ files and raw image files. External data used in this project is detailed under "Methods: snRNA-seq spatial registration" as well as "Methods: Clinical gene set enrichment analyses".

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 methods were used to pre-determine sample sizes, but our sample sizes are greater than previous publications with the earlier Spatial Transcriptomics technology that preceded Visium (DOIs: 10.1126/science.aaf2403 and 10.1126/science.aav9776). Three high quality donors similar in age and primary diagnosis (all three are neurotypical controls) were selected for this study. Two are males and one is a female, with age at time of death from 30 to 46 years of age.
Data exclusions	No data was excluded from the analyses. All aligned RNA-seq reads were used and all Visium spots were used, as typical scRNA-seq quality control metrics were related to the DLPFC layers.
Replication	Spatially-adjacent replicates were obtained for all donor brains. Furthermore, for each donor, we obtained two pairs of spatially-adjacent replicates at a 300 micron distance between the pairs as shown in Figure 1. We verified that the spatial replicates samples clustered with each other as described in the Methods.
Randomization	Samples were not randomized. We used the "block" argument in the analysis methods from scran for the 6 pairs of spatially-adjacent replicates as explained in the Methods: spot-level data processing. Given the sample size (3 donors) we did not adjust statistically for covariates such as sex and age.
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

Note that full information on the approval of the study protocol must also be provided in the manuscript.