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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.	snDrop-seq was performed at least 2-times per individual and per region (6 individuals total). scTHSseq was performed for a single individual for each region, with each run capable of generating sufficient sampling. Final dataset number was determined by quality filtering metrics described in the methods

2. Data exclusions

Describe any data exclusions.	Low quality data sets were excluded based on parameters outlined in the methods section (e.g. low read mapping).

3. Replication

Describe whether the experimental findings were reliably reproduced.	All attempts at replication were successful

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.	All data sets for each brain region (snDrop-seq or scTHS-seq) were combined and clustered using an unbiased approach

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.	Individual data set clustering was performed using an unbiased and unsupervised approach prior to cell type assignments

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	All software used in this study has been described in published literature (e.g. Seurat, Destiny, Monocle, etc...) or are custom and available on Github (e.g. Pagoda2). Custom code needed to repeat analyses has also been made available on Github, as documented in the Methods section.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	The Tn5059 hyperactive transposase was acquired through a collaboration with Illumina, however, the mutations and enzyme production methods needed to generate this enzyme have been published and are referenced in the Methods. All other reagents are commercially available

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	All antibody stains were obtained from the human protein atlas (http://www.proteinatlas.org) which provides antibody validation and availability information

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	293T and NIH/3T3 cell lines (species mixing snDrop-seq experiment) were from ATCC
b. Describe the method of cell line authentication used.	Genomic mapping of RNA-seq reads
c. Report whether the cell lines were tested for mycoplasma contamination.	Cell lines were not tested for mycoplasma contamination
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in this study

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	No human research participants were used in this study