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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☒	☐	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used except for Illumina RTA basecalling.
Data analysis	Common, freely available DNA sequencing data analysis software was used to analyze data, as described in Methods: bcl2fastq/v2.16, python/v2.7.13, deML(https://github.com/grenaud/deML), trim_galore/v0.4.1, STAR/v 2.5.2b, R/3.5.0, Monocle2/v2.6.0, scanpy/v1.0, scrublet/v0.1, EnrichR/v1.0, UMAP/v0.3.2, reticulate/v1.10, Monocle3/alpha Scripts for processing sci-RNA-seq3 sequencing were written in python and R with code available at https://github.com/JunyueC/sci-RNA-seq3_pipeline. Trajectory analysis is done with Monocle3 with setup instructions and tutorial available at http://cole-trapnell-lab.github.io/monocle-release/monocle3/.

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/reviewers upon request. 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

sci-RNA-seq3 protocol and all data are made freely available, including through a cell type wiki to facilitate their ongoing annotation by the developmental biology research community (http://atlas.gs.washington.edu/mouse-rna/). The data generated by this study can be downloaded in raw and processed forms from the NCBI Gene Expression Omnibus (GSE119945).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No explicit calculations were performed to determine sample size. Rather, we aim to capture at least three male and female embryos at each development stage, thus we includes 10 to 15 embryos from at least three independent litters per development stage from E9.5 to E13.5.
Data exclusions	No data were excluded from the study.
Replication	We isolated nuclei from 61 individual whole embryos and performed sci-RNA-seq3. We performed 15 replicates at E9.5, 11 replicates at E10.5, 13 replicates at E11.5, 10 replicates at E12.5, and 12 replicates at E13.5. All attempts at replication were successful.
Randomization	Embryos in experiment were randomized before nuclei extraction. Nuclei derived from each embryo were deposited to different wells during the first round of indexing, such that the RNA-seq profiles of individual nuclei could be linked to the embryos from which they were derived. Nuclei from the same developmental stage were deposited to different indexing plates to control covariants of first round of reaction. After the first round indexing, nuclei from all embryos were pooled and randomly redistributed across four plates for second round reactions.
Blinding	Investigators were blinded to group allocation during data collection and analysis: embryo collection and sci-RNA-seq3 analysis were performed by two different researchers.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T and NIH/3T3 cells were from ATCC
Authentication	None of the cell lines were authenticated.

Mycoplasma contamination	Cell lines were not tested for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	We collected mouse embryos (C57BL/6 , male and female) at E9.5, E10.5, E11.5, E12.5 and E13.5.
Wild animals	Study did not involve wild animals.
Field-collected samples	Study did not involve field-collected samples.