nature portfolio

Corresponding author(s): Duanqing Pei

Last updated by author(s): Mar 27, 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	Cells were sequenced using NovaSeq 6000 and corresponding Illumina commercial software.
Data analysis	The quality control of FASTQ data was implemented with fastp (v0.20.0). The sequence of Read1 was trimmed to 28bp and Read2 was trimmed to 98bp using TrimGalore-0.6.5. Trimmed reads were mapping to mouse genome reference of gencode.vM21 using STAR-2.7.0f with settings '--soloType Droplet --soloFeatures Gene --soloCBwhitelist 3M-february-2018.txt --soloCBstart 1 --soloCBlen 16 --soloUMIstart 17 --soloUMIlen 12 --soloBarcodeReadLength 0'. The 3M-february-2018.txt is the barcode whitelist provided by Cell Ranger v3. Data were analyzed on the Python (v3.10.13) platform using Scanpy v1.9.1, pandas v1.5.3, NumPy v1.26.3, SciPy v1.11.4, Matplotlib v3.7.2, liana v1.7.1, palantir v1.0.1, harmonyTS v0.1.5, scvelo v0.2.5, moscot v0.3.0, wot v1.0.8post2, fitsne v1.2.1, and pyscenic v0.12.1. Data were analyzed on the R (4.3.2) platform using CellChat v1.6.1, monolce3 v1.3.1 and slingshot v2.6.0 The code used for this study is available in the GitHub repository: https://github.com/JiekaiLab/mdCLA.

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 raw sequence data reported in this study have been deposited in the Genome Sequence Archive (GSA) in National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences (GAS: CRA003171 and CRA039992) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.
The processed data reported in this study have been deposited in the OMIX, China National Center for Bioinformation, Beijing Institute of Genomics, Chinese Academy of Sciences (https://ngdc.cncb.ac.cn/omix: accession number OMIX014646).

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	N/A - This study did not involve human samples
Reporting on race, ethnicity, or other socially relevant groupings	N/A - This study did not involve human samples
Population characteristics	N/A - This study did not involve human samples
Recruitment	N/A - This study did not involve human samples
Ethics oversight	N/A - This study did not involve human samples

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	Single-cell RNA sequencing was performed on 322 libraries derived from 351 embryos. The number of embryos sampled at each time point was determined via sample size estimation based on the negative binomial model (https://satijalab.org/howmanycells/). Briefly, we estimated that 10–200 distinct cell types are present at each time point, with the rarest cell type accounting for a relative abundance of 0.001. To ensure the recovery of at least 10 cells for each cell type in the final dataset, we first calculated the total number of cells required per time point. Additionally, considering that each single-cell library prepared with the 10x Genomics platform can capture approximately 10,000 cells, we further estimated the number of embryos needed for each time point accordingly. This sampling strategy ensures adequate representation of all cell populations, including low-abundance cell types.
Data exclusions	No data were excluded from the analyses.
Replication	More than 3 biological replicates (i.e. embryo pools) were generated for E1.5-E7.0 and E19.0 and two biological replicates for E7.5-E18.5 including one male and one female embryo except for E13.5 (only female) and E16.0 (only male). Immunofluorescence experiments were independently repeated as indicated in the figure legends. For Fig. 5d, experiments were performed in two independent biological replicates, and multiple tissue sections from different levels were analyzed in each experiment, yielding similar results. For Fig. 5g, representative images are shown, with additional sections from different tissue levels and an independent biological replicate provided in Extended Data Fig. 10, all showing similar results. For Fig. 5h, images are representative of a single experiment, and similar staining patterns were observed across multiple fields of view. For datasets with n < 3 in Extended Data Fig10c., no statistical analyses were performed, and no error bars or significance annotations are shown.
Randomization	Data collection and experimental conditions were organized in a randomized manner to minimize bias.

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
☒	☐ 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	anti-TUB (Sino Biological, 102415-T08); anti-FSH (Abcam, ab281562; monoclonal, clone EPR24439-141)
Validation	The primary antibodies used for immunostaining were anti-TUB (Sino Biological, 102415-T08) and anti-FSH (Abcam, ab281562; monoclonal, clone EPR24439-141), both of which were rabbit-derived and used at a dilution of 1:200. The secondary antibody was Alexa Fluor™ 568-conjugated donkey anti-rabbit IgG (H+L), highly cross-adsorbed, used at a dilution of 1:500.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Mus musculus C57BL/6 at E1.5, E2.0, E2.5, E3.0, E3.5, E4.0, E4.5, E5.0, E5.5, E6.0, E6.5, E7.0, E7.5, E7.75, E8.0, E8.5, E9.0, E9.5, E10.0, E10.5, E11.0, E11.5, E12.0, E12.5, E13.0, E13.5, E14.0, E14.5, E15.0, E15.5, E16.0, E16.5, E17.0, E17.5, E18.0, E18.5, E19.0, samples from each time point include male and female. Tub cKO mice on a C57 background were used, and the tissue images presented in the study were obtained from male mice in both the control and experimental groups. All mice were housed in a specific pathogen-free (SPF) facility under controlled environmental conditions, with a 12 h light/12 h dark cycle (lights on at 07:00 and off at 19:00), at an ambient temperature of 22 ± 2°C and relative humidity of 50%–60%. Animals were provided with standard chow and water ad libitum.
Wild animals	The study did not involve wild animals.
Reporting on sex	The findings of study apply to both sexes. The study design did not specifically focus on a single sex. Sex was determined by morphological observation at late embryonic stages (e.g., E19.0). Subsequently, a classifier based on sex-associated genes was constructed to predict sex in earlier embryos. The predicted sex information is provided in the cell annotation metadata (available for download under OMIX014646). Embryonic development is conserved between sexes, with the exception of reproductive organ development. Therefore, sex-specific analyses were unnecessary.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were performed according to the Animal Protection Guidelines of Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences (N2022019).

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

Plants

Seed stocks	N/A - This study did not involve palnt samples
Novel plant genotypes	N/A - This study did not involve palnt samples
Authentication	N/A - This study did not involve palnt samples