

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	CFX384 RealTime System Nikon SMZ18 Nikon ECLIPSE Ti Olympus FV3000 Leica TCS-SP8 Leica Stellaris 8 Cell Ranger 5.0.1
Data analysis	Graphpad Prism 10.1.2 R version 4.3.2 Seurat 4.3.0.1 ClusterProfiler 4.10.0 FastQC 0.11.9 Trimmomatic 0.39 featureCounts 2.0.6 STAR 2.7.10b pheatmap 1.0.12 Monocle 2.30.0 Monocle3 1.3.1

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](#)

Sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE287909 (scRNAseq) and GSE287908 (bulk RNA-seq).

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

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

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](https://www.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

Sample sizes were determined based on preliminary experiments, commonly used sample sizes in comparable publications within this field. We independently performed all experiments at least 3 times, allowing to judge the degree of variability between replicates. The sample sizes and number of repeats are defined in each figure legends.

Data exclusions

No data was excluded from the analysis.

Replication

All experiments presented here been repeated several times with similar results, the number of repeated experiments is indicated in the figure legends.

Randomization

For chimeric experiments, mice and embryos were randomly distributed amongst groups at the start of each experiment. For in vitro experiments using cells, cells were randomly assigned to either experiment or control groups.

Blinding

Blinding was not used in this study.

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

Primary antibodies:

Rabbit Anti-Oct4 Monoclonal Antibody, Abcam; Cat#ab181557; RRID:AB_2687916; Clone:EPR17929; 1:500
 Mouse Anti-Cdx2 Monoclonal Antibody, BioGenex; Cat#MU392A-UC; RRID:AB_3101998; Clone:CDX2-88; 1:200
 Rabbit Anti-CDX2 Monoclonal Antibody, Abcam; Cat#ab76541; RRID:AB_1523334; Clone:EPR2764Y; 1:500
 Goat Anti-Human Sox17 Polyclonal Antibody; R and D Systems; Cat#AF1924; RRID:AB_355060; 1:200
 Rabbit Anti-AP2 gamma/TFAP2C Monoclonal Antibody; Abcam; Cat#ab218107; RRID:AB_2891087; Clone:EPR20331; 1:500
 Rabbit Anti-Brachyury / Bry Monoclonal Antibody; Abcam; Cat#ab209665; RRID:AB_2750925; Clone: EPR18113; 1:500
 Rabbit MuERV-L-Gag Polyclonal Antibody; Epigentek; Cat#A-2801; RRID:AB_3097813; 1:500
 Rabbit Anti-TBR2 / Eomes Monoclonal Antibody; Abcam; Cat#ab183991; RRID:AB_2721040; Clone: EPR19012; 1:500
 Gost anti-tdTomato Polyclonal Antibody; SICGEN; Cat#AB8181; RRID:AB_2722750; 1:2000
 Rabbit Anti-Cytokeratin 8 Monoclonal Antibody; Abcam; Cat#ab53280; RRID:AB_869901; Clone: EP1628Y; 1:500
 Rabbit Anti-Zscan4 Antibody Polyclonal Antibody; Millipore; Cat#AB4340; RRID:AB_2827621; 1:5000
 Rabbit Anti-RUNX1 / AML1 Monoclonal Antibody; Abcam; Cat#ab92336; RRID:AB_2049267; Clone: EPR3099; 1:200
 Rabbit Anti-FOXA2 Monoclonal Antibody; Abcam; Cat#ab108422; RRID:AB_11157157; Clone: EPR4466; 1:200
 Goat Anti-Cerberus 1 Polyclonal Antibody; R and D Systems; Cat#AF1986; RRID:AB_2077219; 1:200
 Mouse Anti-Human LRH-1/NR5A2 Monoclonal Antibody; R and D Systems; Cat#PP-H2325; RRID:AB_2154053; Clone:# H2325; 1:200
 Rabbit Anti-SOX2 Polyclonal Antibody; MilliporeSigma; Cat#AB5603; RRID:AB_2286686; 1:200
 Goat Anti-TFAP2C Polyclonal Antibody; R and D Systems; Cat#AF5059; RRID:AB_2255891; 1:200
 Goat anti-OTX2 Polyclonal Antibody; R and D Systems; Cat#AF1979; RRID:AB_2157172; 1:200
 Mouse anti-MHC-II Monoclonal Antibody; R and D Systems; Cat#MAB4470; RRID:AB_1293549; 1:500
 Goat anti-SOX1 Polyclonal Antibody; R and D Systems; Cat#AF3369; RRID:AB_2239879; 1:500

Secondary antibodies:

Cy3-AffiniPure Donkey Anti-Mouse IgG (H+L); Jackson ImmunoResearch Labs; Cat#715-165-151; RRID:AB_2315777; 1:200
 Alexa Fluor 488-AffiniPure Donkey Anti-Mouse IgG (H+L); Jackson ImmunoResearch Labs; Cat#715-545-150; RRID:AB_2340846; 1:200
 Alexa Fluor 647-AffiniPure Donkey Anti-Rabbit IgG (H+L); Jackson ImmunoResearch Labs; Cat#711-605-152; RRID:AB_2492288; 1:200
 Alexa Fluor 488-AffiniPure Donkey Anti-Rabbit IgG (H+L); Jackson ImmunoResearch Labs; Cat#711-545-152; RRID:AB_2313584; 1:200
 Cy3-AffiniPure Donkey Anti-Goat IgG (H+L); Jackson ImmunoResearch Labs; Cat#705-165-147; RRID:AB_2307351; 1:200
 Alexa Fluor 488-AffiniPure Donkey Anti-Goat IgG (H+L); Jackson ImmunoResearch Labs; Cat#705-545-147; RRID:AB_2336933; 1:200
 Alexa Fluor 647-AffiniPure Donkey Anti-Goat IgG (H+L); Jackson ImmunoResearch Labs; Cat#705-605-147; RRID:AB_2340437; 1:200

Validation

All the antibodies have been validated by the company that sells them. Details of the validation statement, antibody profiles and relevant citations can be found on the manufacturer's website. In addition to that, we validated all antibodies used in this study by immunofluorescence in natural mouse embryos and cells, and cross-checked them against their well-described expression pattern in reported studies.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Cell lines of EPSCs, mc6-1/Mu-mClover3-10# and C1-EPS-20# were used to generate S1-S8 embryoids. Both cell lines were generated in-house and cultured in LCDM condition.

Authentication

All cell lines in this study were authenticated via gene expression analysis.

Mycoplasma contamination

All cultured cells were tested negative for mycoplasma contamination by PCR.

Commonly misidentified lines
(See [ICLAC](#) register)

No commonly misidentified lines were used in this study.

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	Mice was used in this study. The male and female ICR mice were 6-8 weeks old. The mice were housed with a 12 hr light/dark cycle between 06:00 and 18:00 in a temperature controlled room ($22 \pm 1^\circ\text{C}$) with free access to water and food.
Wild animals	This study did not involve wild animals.
Reporting on sex	Both male and female embryos were used in this study; the embryos were not genotyped to determine the sex.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	The Ethics Committee of Peking University Health Science Center approved the protocols used in this study.

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

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>