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.

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
NIS Elements (Nikon Corporation), ZEN 2010 (Zeiss AG), QuantStudio 6(Applied Biosystems), NanoDrop 2000/2000c (Thermo Scientific)

Data analysis
Fiji (v2.0.0-rc-69/1.52p) and custom plugins for image processing from Bioimaging Core Facility (BIOP, EPFL), Microsoft Excel 2011 (v14.5.5.) (Microsoft Corporation), GraphPad Prism (v9.1.2), Cutadapt (v2.1) , Seurat (v3.1)

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/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

All data regarding image processing/quantification and RNA-sequencing analysis are available upon request.

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
Life sciences study design

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

| Sample size | For EPI and TSC aggregates generated on microwells at 72 h, the sample size was >100. After transferring aggregates to 96 well plates, in general, the experiment design was to set 4 conditions with 24 aggregates per condition. |
| Data exclusions | EpITs embryos that accidentally contain more than one EPI or TSC aggregate were excluded from the analysis. Also, EpITs embryos lost during medium exchange were excluded. |
| Replication | All experiments were successfully replicated at least 3 times with similar results. |
| Randomization | EPI and TSC aggregates with rounded morphology and no clear sign of apoptosis were picked and transferred together to form EpITs embryos. |
| Blinding | Blinding was not performed. |

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 |
| ☒ | Animals and other organisms |
| | Clinical data |
| | Human research participants |

### Methods

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

### Antibodies

Primary antibodies used in this study:

- **anti-E-cadherin Rabbit** 1:500 #24E10 Cell Signaling Technology
- **anti-Sox2 Rabbit** 1:400 #ab97959 Abcam
- **anti-Sox1 Goat** 1:50 #af3369 R&D Systems
- **anti-Ohx2 Goat** 1:25 #af1979 R&D Systems
- **anti-Brachyury Goat** 1:300 #sc-17745 (C-19) Santa Cruz
- **anti-Brachyury Rabbit** 1:100 #ab209665 Abcam
- **anti-Oct4 Mouse** 1:200 #sc-5270 (C-10) Santa Cruz
- **anti-Nanog Rat** 1:300 #14-5761-80 ThermoFisher
- **anti-Cdx2 Rabbit** 1:200 #ab76541 Abcam
- **anti-Eomes Rabbit** 1:200 #ab23345 Abcam
- **anti-pAKC Mouse** 1:100 #sc-17781 (H-1) Santa Cruz
- **anti-Pax6 Rabbit** 1:100 #901301 (Poly19013) BioLegend
- **anti-Six1 Rabbit** 1:200 #12891S (DA48K) Cell Signaling Technology
- **anti-Podocalyxin Rat** 1:200 #MAB1556 (192703) R&D systems
- **anti-Par6 Mouse** 1:100 #sc-166405 (B-10) Santa Cruz
- **anti-Tuj1 Rabbit** 1:400 #ab18207 Abcam
- **anti-Dppa3 Mouse** 1:100 #AF2566-SP R&D systems
- **anti-Foxa2 Rabbit** 1:200 #ab23345 Abcam
- **anti-Incretin Mouse** 1:100 #AF1356-SP R&D systems
- **anti-Dppa3 Mouse** 1:100 #AF2566-SP R&D systems
- **anti-Eomes Rabbit** 1:200 #ab23345 Abcam
- **anti-Sox17 Rabbit** 1:30 #af1979 R&D Systems
- **anti-Brachyury Goat** 1:300 #sc-17745 (C-19) Santa Cruz
- **anti-Brachyury Rabbit** 1:200 #ab209665 Abcam
- **anti-Otx2 Goat** 1:25 #af1979 R&D Systems

**Validation**

- Anti-E-cadherin antibody was validated by the manufacturer for immunostaining of MCF7 cells.
- Sox1 antibody was validated by the manufacturer for immunostaining of Mouse Cortical Stem Cells.
- Oct4 antibody was validated by the manufacturer for immunostaining of Ntera-2 Human Cell Line.
- Nanog antibody was validated by the manufacturer for immunostaining of F9 Cell Line.
- Eomes antibody was validated by the manufacturer for immunostaining of adult mouse SVZ (subventricular zone).
- pAKC antibody was validated by the manufacturer for immunostaining of SW480 cells.
- Pax6 antibody was validated by Milad Riazifar (U.C. Irvine) for immunostaining of iPSC derived neural rosettes.
- Six1 antibody was validated by the manufacturer for immunostaining of NSC-34 cells.
- Tuj1 antibody was validated by the manufacturer for immunostaining of SK-N-SH (Human neuroblastoma cell line) cells.
- Dppa3 antibody was validated by the manufacturer for immunostaining of mouse ovary tissue.
- Foxa2 antibody was validated by the manufacturer for immunostaining of HT-29 cells.
- Tifap2c antibody was validated by the manufacturer for immunostaining of HeLa cells.
- Eya1 antibody was validated by the manufacturer for immunostaining of RH-30 cells.
- Lamin antibody was validated by the manufacturer for immunostaining of LS174T cells.
- Frhnecin antibody was validated by Kunnen et al., 2017 for immunostaining of Proximal Tubular Epithelial Cells (PTECs).
- mCherry antibody was validated by the manufacturer for immunostaining of mCherry transduced U2OS cells.
**Eukaryotic cell lines**

Policy information about cell lines

| Cell line source(s)                  | SBR ES cell line was generated and provided by David Suter Lab (Deluz et. al., 2016) |
|--------------------------------------|-------------------------------------------------------------------------------------|
|                                      | TLC:mCherry line was generated by Ferrer Vaquer et. al., 2010 and provided by Alfonso Martinez-Arias. |
| Authentication                       | AR8:mCherry line was generated by Serup et. al., 2012 and provided by Alfonso Martinez-Arias. |
|                                      | TS:GFP cells was generated by Tanaka et. al., 1998 and provided by Christian Schrotter. |
| Mycoplasma contamination              | SBR, TLC:mCherry and AR8:mCherry cell lines were authenticated by PCR genotyping following gene targeting. |
| Commonly misidentified lines (See ICLAC register) | TS:GFP cell line was authenticated in Tanaka et. al., Science, 1998. The authentication was done by chimera formation and GFP detection in only extraembryonic tissues. |

All cell lines were tested regularly and confirmed free of mycoplasma with in house mycoplasma test and MYCOPLASMACHECK service from GATC.

No cell lines used in this study are in the data-base of commonly misidentified cell lines.