

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

No genome or transcriptome sequencing data was used in this study, so no computer code about data preparation was available in this article.

Data analysis

-qRT-Primers, designed with Primer3web (version: 4.1.0) and SnapGene (version: 2.3.2).
-Statistical analyses, analyzed with:
GraphPad Prism (version: 7.0)
Microsoft Excel
OriginLab (version: 8.6.0)
-Analysis of 96.96 single-cell quantitative PCR, analyzed with:
BioMark Data Collection (version: 4.2.2)
Real-Time PCR Analysis (version: 4.3.1)
Singular Analysis Toolset Software (version: 3.6.2)
R (version: 3.3.3 and 3.4.3)
Fiji (Version: 1.52e)

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

Source data for single-cell quantitative PCR experiments (Fig. 1h, 5g, 5i, 5j, 6d-g and 6i; Supplementary Fig. 9f, 9g, 10a and 10b) and the gene list with corresponding sequences (Supplementary Fig. 10a) have been provided in Supplementary Data1. Qualifications of the data (Fig. 1i, 2h, 2i, 3k, 4c, 4f, 5e and 5f; Supplementary Fig. 2d, 3c, 4g, 4h, 8d and 12f) and embryo transplantation data (Fig. 7b, 7c, 7m and 7n; Supplementary Fig. 11f, 12a) have been provided in Source Data. The data supporting the findings of this study are available from the corresponding author on reasonable 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

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	Sample size was determined based on our previous experience and the work of other groups using embryos and stem cells as experimental materials.
Data exclusions	In single-cell gene expression analysis, samples with low or absent expression levels of endogenous control genes were excluded from subsequent analysis.
Replication	All the experimental data was replicated at least in two independent experiments.
Randomization	Samples were randomly allocated to control and experimental groups (method section).
Blinding	The investigators were not blinded to group allocation.

Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

Ecological, evolutionary & environmental sciences study design

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

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data collection	Describe the data collection procedure, including who recorded the data and how.
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.
Did the study involve field work?	☐ Yes ☒ No

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
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

The following antibodies were used:

Mouse mAB anti Oct 3/4, Santa Cruz Biotechnology, SC-5279, 1:50
 Mouse mAB anti aPKC, Santa Cruz Biotechnology, sc-17781, 1:100
 Rat mAB anti Podocalyxin, R&D Systems, MAB1556, 1:400
 Rat mAB anti E-cadherin, Santa Cruz Biotechnology, SC-59778, 1:100
 Goat pAB anti Gata4, Santa Cruz Biotechnology, sc-1237, 1:200
 Rabbit pAB anti Laminin, Sigma, L-9393, 1:500
 Rabbit mAB anti Cleaved caspase-3, Cell Signaling Technologies, #9664s, 1:200
 Rabbit pAB anti Cyclooxygenase 2, Abcam, ab15191, 1:100
 Mouse mAB anti Placental lactogen I, Santa Cruz Biotechnology, sc-376436, 1:50
 Rabbit pAB anti-wide spectrum Cytokeratin antibody, Abcam, ab9377, 1:100
 Mouse mAB anti Cdx2, BioGenex, MU392A-UC, 1:100
 Mouse pAB anti Cripto, Cell Signaling Technologies, #2818, 1:200
 Alexa 488 (Rabbit anti-rat), Thermofisher scientific, A21210, 1:500

Alexa 488 (Rabbit anti-mouse), Thermofisher scientific, A21042, 1:500
 Alexa 488 (Goat anti-mouse), Thermofisher scientific, A11001, 1:500
 Alexa 488 (Donkey anti-goat), Thermofisher scientific, A11055, 1:500
 Alexa 488 (Goat anti-rabbit), Thermofisher scientific, A11008, 1:500
 Alexa 488 (Donkey anti-rabbit), Thermofisher scientific, A21206, 1:500
 Alexa 594 (Goat anti-mouse), Thermofisher scientific, A11005, 1:500
 Alexa 594 (Donkey anti-mouse), Thermofisher scientific, A21203, 1:500
 Alexa 594 (Donkey anti-rabbit), Thermofisher scientific, A21207, 1:500
 Alexa 594 (Goat anti-rat), Thermofisher scientific, A11007, 1:500
 Alexa 647 (Donkey anti-rabbit), Thermofisher scientific, A31573, 1:500

Validation

The subcellular localization of the proteins analyzed in this study has been previously reported in mouse embryo/stem cell studies.
 Oct4: it specifically stained the epiblast at all stages tested, as expected (Science 356, doi:10.1126/science.aal1810)
 a-PKC: it specifically enriched in PrE precursors as reported and expected (Nature 536 doi: 10.1038/nature18958.)
 Podocalyxin: it correctly stained polarized lumen in the embryo as reported elsewhere (Nature 552, 239-243).
 E-cadherin: it correctly stained the basolateral side of cells in the embryo as reported and as expected (Nat Cell Biol 2000 Feb;2(2):70-5)
 Gata4: it correctly labelled the visceral endoderm in postimplantation embryos (eLife 2018;7:e32839) same as above (Science 356, doi:10.1126/science.aal1810)
 Laminin: it correctly stained the basement membrane between visceral endoderm and Exe or epiblast, as reported elsewhere and as expected (Dev Dyn 241, 270-283)
 Cleaved caspase-3: it correctly stained apoptosis cell (Science 356, doi:10.1126/science.aal1810)
 COX2: it localized in the luminal epithelium and underlying stromal cells solely at the sites of blastocyst attachment reaction. (Cell. doi.org/10.1016/S0092-8674(00)80402-X)
 PL1: it was correctly expressed in trophoblast giant cells (Placental lactogen I (P-17,sc34713) has been removed, but manufacturer recommend the sc-376436. Nature 557 doi: 10.1038/s41586-018-0051-0)
 CK: it was correctly expressed in the luminal epithelium as reported and expected (PNAS 114, doi: 10.1073/pnas.1616060114.)
 Cdx2: it was correctly expressed in post-implantation trophoblast cell (Nature 557 doi: 10.1038/s41586-018-0051-0).
 Cripto: it correctly stained the stem cells in the embryo as reported and as expected (Nat Commun. 10.1038/ncomms12589).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Mouse embryonic stem cells: G4 and G4-ACTB-dsRED-MST ESCs were obtained from Dr. Andras Nagy, Kristina Vintersten and Marina Gertsenstein's Laboratory in Mount Sinai Hospital & the Samuel Lunenfeld Research Institute.
 BVSC ESC line were obtained from Dr. Mitinori Saitou.
 Mouse trophoblast stem cell lines, extraembryonic endoderm stem cell lines , CF-1 MEF feeder cells, p53-/-, Lamc1-/- and Nodal-/- ES cells, UBC-EGFP TS cells were derived from our lab.

Authentication

p53-/-, Lamc1-/- and Nodal-/- mouse ES cells were authenticated by sequencing.
 Cells were maintained in conditions to preserve stem cell character and prevent differentiation. Plates were inspected for morphological evidence of differentiation and plates with differentiated cells were discarded. Furthermore, cell identities were confirmed routinely by immunofluorescence marker expressions.

Mycoplasma contamination

Cell lines were routinely tested for mycoplasma contamination by PCR.

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

The cells we used are not part of this database.

Palaeontology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

☐ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Animals and other organisms

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

Laboratory animals

CD1 and 129 mice were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. Actin-GFP mice were obtained from Shaorong Gao's Laboratory in Tongji University. All mice were maintained in specific pathogen-free (SPF) conditions with a 12-hour dark / 12-hour light cycle between 06:00 and 18:00 in a temperature controlled room (22 ± 2°C) with free access to

water and food.

Wild animals

No wild animals were used in the study.

Field-collected samples

No field-collected samples were used in the study.

Ethics oversight

All animal procedures and all of the mouse work were approved by the Animal Care and Use Committee of China Agriculture University (Permit Number: SKLAB-2016-01-04).

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

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission

Provide a list of all files available in the database submission.

Genome browser session

(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates

Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth

Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies

Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters

Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality

Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- ☐ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☐ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☐ All plots are contour plots with outliers or pseudocolor plots.
- ☐ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.

Instrument

Identify the instrument used for data collection, specifying make and model number.

Software

Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.

Cell population abundance

Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.

Gating strategy

Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.

- ☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

☐ Used

☐ Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ BothStatistic type for inference
(See [Eklund et al. 2016](#))

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involvement in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis