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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.

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	Lightcycler 480, ScanMaker i800Plus, Amersham Imager 600, Summit Software version 5.2,
Data analysis	GraphPad Prism 7, FlowJo V10, Fiji (NIH)

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

Previously published RNA–seq data that were re-analysed here are available with accession codes GSM2573084. All raw data associated with Figures are listed in Source data (Statistical source data). All the raw images for the western blots can be found in Source data (Unprocessed western blots and gels). The sequences of all vectors are provided in Supplementary Sequences.

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 chosen based on the results of previous works (PMID: 27214048, 25730490) in this field that used similar sample sizes to generate reproducible results.
Data exclusions	No data were excluded.
Replication	Details about the replication are stated in the figure legends and all attempts at replication were successful.
Randomization	Randomization was used in all experiments.
Blinding	Cells were not treated blindly due to the evident phenotypes. In addition, based on previous studies in this field, these assays do not require blinding. Thus, blinding was not used.

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	Immunofluorescence staining- Primary antibody used for staining was listing as follow: rabbit polyclonal Nanog antibody (1:100, #ab80892, Abcam), Tubb3 (1:500, MMS-435P, Covance) and mCherry (1:1000, M11217, Invitrogen). Secondary antibody included donkey anti rabbit-488 (1:1000, 711-545-152, Jackson Immunoresearch), donkey anti mus-647 (1:1000, 715-175-150, Jackson Immunoresearch) and donkey anti Rat-561 (1:1000, 712-165-153, Jackson Immunoresearch). Western blots-primary antibody: beta Actin,1:5000, 66009-1-Ig, Proteintech; Nanog, 1:1000, GT3312, Genetex).
Validation	All the antibodies are commercially available and have been validated by the producers, as well as previous literatures that are listed on the website of the manufacturers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	293T, N2a and mESC Cells were purchased from the Cell bank of Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences.
Authentication	Cell lines were not authenticated externally, since they were purchased from the commercial/original sources.
Mycoplasma contamination	Cells used in this study were free of mycoplasma contamination using PCR assays.

Commonly misidentified lines (See ICLAC register)	None of the cell lines used was listed in the database of ICLAC.

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	Cells were digested by trypsin (0.05%)
Instrument	MoFlo XDP (Beckman)
Software	Summit Software version 5.2, FlowJo X.
Cell population abundance	Cell population abundance was influenced by the size of the plasmids.
Gating strategy	Positive and negative boundaries were determined by control cells that were not transfected with any plasmids. For transient transfection, top 20% mCherry positive cells were used to quantify the mean fluorescence intensity.

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