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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 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	MetaMorph Advanced 7.8.1.0, AxioVision 4.8
Data analysis	ImageJ 1.47t

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 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

Data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding authors upon 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	All experiments were conducted with at least two independent experiments and multiple biological replicates. Sample sizes were determined based on our previous experience and similar studies of other groups. Sample sizes were determined as sufficient since they led to similar results.
Data exclusions	Due to intrinsic inhomogeneity of Geltrex, some Geltrex continued to contract during experiments. Such experiments were excluded from data analysis. In rare cases, after initial cell seeding, concave Geltrex pockets between neighboring supporting posts could be damaged. Cell clusters formed within such damaged gel pockets were excluded from data analysis. No similar platform has been previously reported, thus the criteria were established specifically for this platform.
Replication	Reported results were repeated and confirmed for at least two independent experiments. Key experimental findings were reliably reproduced by two investigators involved in this work.
Randomization	Samples were randomly allocated to control and different experimental groups (see Methods). However, no particular randomization method was used in this work.
Blinding	Investigators were not blinded to group allocation, as no animal/human studies were conducted in this manuscript.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Only certified and company-validated antibodies were used in this work:

Primary antibodies:
EZRIN Sigma-Aldrich Cat# E8897, RRID:AB_476955 Mouse 1:2000
OCT4 Santa Cruz Biotechnology Cat# sc-5279, RRID:AB_628051 Mouse 1:200
OCT4 Cell Signaling Technology Cat# 2750, RRID:AB_823583 Rabbit 1:500
NANOG Cell Signaling Technology Cat# 4903, RRID:AB_10559205 Rabbit 1:500
SOX2 Stemgent Cat# 09-0024, RRID:AB_2195775 Rabbit 1:500
TFAP2A Santa Cruz Biotechnology Cat# sc-12726, RRID:AB_667767 Mouse 1:100
TFAP2C Santa Cruz Biotechnology Cat# sc-12762, RRID:AB_667770 Mouse 1:100
BRACHYURY Thermo Fisher Scientific Cat# PA5-46984, RRID:AB_2610378 Goat 1:100
SOX17 R and D Systems Cat# AF1924, RRID:AB_355060 Goat 1:500
CDX2 BioGenex Cat# AM392, RRID:AB_2650531 Mouse 1:300
FOXA2 Cell Signaling Technology Cat# 8186, RRID:AB_10891055 Rabbit 1:300
EOMES Abcam Cat# ab23345, RRID:AB_778267 Rabbit 1:200

Secondary antibodies:
Donkey anti-Rabbit 546 Thermo Fisher Scientific Cat# A10040, RRID:AB_2534016 1:500

Donkey anti-Mouse 488 Thermo Fisher Scientific Cat# A-21202, RRID:AB_141607 1:500
Donkey anti-Goat 647 Thermo Fisher Scientific Cat# A-21447, RRID:AB_2535864 1:500

The antibody information (including species, application, and catalog number) has been provided in Supplementary Information.

Validation

All antibodies have been validated by the companies from which they were purchased. The subcellular localization of all the proteins analyzed in this work is consistent with previous published literatures. This information was used to further validate the specificity. Details about validation statements of the manufacturer, relevant citations and antibody profiles can be found on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The following cell lines were used in this work:
H9 hESC line (WA09, WiCell; NIH registration number: 0062); H1 hESC line (WA01, WiCell; NIH registration number: 0043); A hiPSC line (1196a) originally reported in Villa-Diaz, L. G. et al. Nat. Biotechnol. 28, 581 (2010).

Authentication

All hPSC lines have been authenticated by the original sources and also authenticated in-house by immunostaining for pluripotency markers and successful differentiation to the three germ layer cells.

Mycoplasma contamination

All cell lines used in this work have been tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines listed by ICLAC were used in this work.