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Corresponding author(s): Daniel J. Garry and Mary G. Garry

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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	The single-cell RNA-seq data of human pre-implantation embryonic development were obtained from Petropoulos et al. Cell 165, 1012–1026 (2016). The raw reads were mapped to the human genome (hg19) using TopHat (v2.0.13), and the raw read counts were obtained by HTSeq (v0.6.0) with default parameters.
Data analysis	To combine the human data with porcine data, we mapped the porcine genes to human orthologues using R's BiomaRt package, and only kept the porcine genes that can be uniquely mapped to the human orthologues. The scNCA package (https://github.com/gongx030/scNCA) was used to remove the batch effects of human and porcine cells. For statistical analyses, Prism 8 (v8.4.1, GraphPad Software Inc.) was used.

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

The single-cell RNA-seq data from wild-type porcine morula and blastocyst embryos have been deposited in the NCBI Sequence Read Archive (SRA) database, with project accession number PRJNA509275. All unique materials used are readily available from the authors or from commercial sources (see Supplementary Tables 1–3). Gene-edited primary cell lines are available yet limited in number because of supply constraints.

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 sizes were determined to achieve statistical significance wherever possible.
Data exclusions	No data were excluded.
Replication	All attempts at replication for standard assays (FACS, qPCR and immunohistochemistry) were successful. We observed that the production of null embryos was reliable and reproducible. SCNT, chimerism and complementation data reflect low efficiencies that are well established in the field.
Randomization	Random allocation (as was possible).
Blinding	Investigators were blinded whenever possible (for FACS analysis, manual cell counting, culture studies and qPCR, in particular). Embryos were processed, however, in the order that they were delivered to maximize preservation and to inform next steps, which limited investigator blinding in some instances.

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	Green Fluorescent Protein: Abcam Cat# ab150175 Chicken anti-GFP @ 1:500 secondary; AF488 Donkey anti-Chicken Jackson Immunoresearch Cat# 703-545-155 @ 1:400; cGFP Ab Origene Cat# TA15007 Chicken Polyclonal @ 1:300. Secondary: AF488 Donkey anti-Chicken Jackson Immunoresearch Cat# 703-545-155 @ 1:400; Desmin Ab Abcam Cat# ab15200 Rabbit Polyclonal @ 1:200. Secondary: AF555 Donkey anti-Mouse Life Technology Cat# A31570 @ 1:400. Sarcomeric α-actinin Ab: Abcam Cat# 9465 Mouse Monoclonal @ 1:250. Secondary: See Desmin. Transcription Factors: MYOD Ab Santa Cruz Cat# sc-304 Rabbit Polyclonal @ 1:150. Myogenin Ab Santa Cruz Cat# sc-576 Rabbit Polyclonal @ 1:150. MYF5 Ab Santa Cruz Cat# sc-302 Rabbit Polyclonal @ 1:150. Secondary:AF555 Donkey anti-Rabbit Life Technology Cat# A31572 @ 1:400. Pax7 Developmental Studies Hybridoma Bank Mouse Monoclonal @ 5µg/ml. Secondary:AF546 Goat anti-Mouse Life Technology Cat# A21123 @ 1:200. Laminin Abcam Cat# ab11576 Rat Monoclonal @ 1:250. Secondary:AF647 Donkey anti-Rat Abcam Cat# ab150151 @ 1:400. OCT4 Abcam Cat# ab18976 Rabbit Polyclonal @ 1:100. Secondary:AF594 Donkey anti-Rabbit Jackson Cat# 711-585-152 @ 1:400. SSEA4 Abcam Cat# ab16287 Mouse Monoclonal @ 1:150. Secondary: AF647 Donkey anti-Mouse Jackson Cat# 715-805-150 @1:400. NANOG Abcam Cat# ab21624 Rabbit Polyclonal @ 1:150. Secondary: AF647 Donkey anti-Rabbit Abcam Cat# ab150063 @1:400. SOX2 Santa Cruz Cat# sc17320 Goat Polyclonal @1:150. Secondary: AF647 Donkey anti-Goat Jackson cat# 705-605-147 @1:400. SSEA1 Abcam cat# ab16285 Mouse Monoclonal @1:150. Secondary: AF555 Donkey anti-Mouse Life Technologies cat# A31570 @1:400. beta III Tubulin Abcam cat# ab18207 Rabbit Polyclonal @1:150. Secondary: AF555 Donkey anti-Rabbit Life Technologies cat# A31572 @1:400. Mouse anti-HNA (Abcam Cat# ab191181 @1:250) with Donkey anti-Mouse AF647 (Jackson Immunoresearch Cat#715-805-150 @1:400); Rabbit anti-Nestin (Abcam Cat# ab105389 @1:200) with Donkey anti-rabbit AF594 (Jackson Immunoresearch Cat# 711-585-152 @1:400). Nuclei: Hoechst 33342 Dye 20mM Solution Pierce Cat# 62249 @ 1:1000 or DAPI Invitrogen Cat#D21490 @ 1:1000. Mounting Media: VectaShield (Vector Labs Cat#H-1000) or Immu-Mount (Shandon Cat.# 9990402).
Validation	Validation statements are provided on the manufacturer's website for each antibody used. All catalogue number, clone number,

dilution and supplier information are listed above. Appropriate positive and negative controls were used in each experiment to validate the manufacturer's statement for our system.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Porcine fetal fibroblasts were derived from E35 wild-type embryos. Wild-type fibroblasts were gene-edited using CRISPR/Cas9 to generate MYF5/MYOD/MYF6-null fibroblasts. Human iPS (RhiPSC11) cells were generated in our laboratory using human skin embryonic fibroblasts (8FW; GM00011). copGFP (cGFP) labeling and TP53 deletion were performed in our laboratory using the RhiPSC11 cells to generate cGFP+–TP53-null hiPSCs line (clone 1 and 16).
Authentication	All cell lines were authenticated using karyotypic analysis. Additionally, hiPSCs were authenticated using pluripotency assays and teratoma formation.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	Mixed strains of Landrace x Yorkshire X Duroc porcine gilts were used.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	IACUC (Institutional Animal Care and Use Committee) SCRO (Stem Cell Research Oversight)

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

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	Skeletal muscles were washed with PBS without Ca++/Mg++, then dissociated with collagenase type II (1mg/ml) /dispase (2mg/ml) (ThermoFisher Scientific) for 10 min, followed by addition of trypsin/EDTA (0.05%) for 5min at 37°C and FACS sorted for GFP. Propidium Iodide (Thermo Fisher, P3566) was used to exclude dead cells.
Instrument	FACSAria(TM) (BD)
Software	FlowJo Ver 10.4.2 (FloJo, LLC)
Cell population abundance	Total single cell events were acquired based on the forward scatter area (FSC-A) and side scatter area (SSC-A) profile (all cells), followed by forward scatter width (FSC-W) and forward scatter height (FSC-H). These gated populations and events were further acquired based on the Side Scatter width (SSC-W) and Side Scatter height (SSC-H) to obtain the single-cell populations. Live cells were gated based on the propidium iodide (PI) negative and PE channel (negative) plots. Single live cells were sorted using FITC-Channel vs FSC-H gating strategy, and cells were pooled into GFP- (FITC–) and GFP+ (FITC+) fractions for further analysis.
Gating strategy	FACS gating was based on endogenous GFP fluorescence and gated for GFP-negative and GFP-positive cell populations.

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