

Life Sciences Reporting Summary

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▶ Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was determined based on our previous experience and the work of other groups using embryos and stem cells as experimental model systems.

2. Data exclusions

Describe any data exclusions.

Mouse epiblast samples for deep-sequencing analysis: those samples that did not pass the quality controls as well as those samples that showed contamination from primitive endoderm cells were excluded from the analysis (explained in the "Sample quality assessment" section of Methods and Extended Data Figure1).

Human embryos: only embryos that attached and survived in the in vitro culture were analyzed. For the analysis of amniotic cavity formation only embryos that preserved the epiblast lineage were included in the statistical analysis (explained in the "Human embryos" section of Methods).

3. Replication

Describe whether the experimental findings were reliably reproduced.

All the experimental data was replicated at least in two independent experiments, with the exception of the in vivo epiblast mRNA sequencing results (Fig. 1). These data were generated in a single experiment to avoid batch-effects (see also "Library preparation, RNA sequencing and mapping of reads" section of Methods). The experimental findings were reliably reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Samples were randomly allocated to control and experimental groups (Methods section).

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were not blinded to group allocation

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- | | |
|--------------------------|--|
| n/a | Confirmed |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The <u>exact sample size</u> (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A statement indicating how many times each experiment was replicated |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> A clear description of statistics including <u>central tendency</u> (e.g. median, mean) and <u>variation</u> (e.g. standard deviation, interquartile range) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Clearly defined error bars |

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

- Immunofluorescence and time-lapse images: analyzed with Fiji (version 1.0).
- Statistical analyses (excluding the sequencing data): analyzed with GraphPad Prism (version 6).
- Analysis of deep-sequencing data: analyzed with R

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

There are no restrictions on availability of unique materials

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The following antibodies were used:

Rabbit pAb anti-aPKC, sc-216, Santa Cruz Biotechnology (1:200)
Goat pAb anti-aPKC, sc-216-G, Santa Cruz Biotechnology (1:100)
Mouse mAb anti-aPKC, sc-17781, Santa Cruz Biotechnology (1:50)
Goat pAb anti-Brachyury, sc-17745, Santa Cruz Biotechnology (1:100)
Mouse anti- human CD130 Clone AM64, 555756 BD Biosciences (1:200)
Rabbit pAb anti-Cingulin, Home-made, kind gift of Rytis Prekeris lab (1:500)
Rat mAb anti-E-Cadherin, Clone ECCD-2, 13-1900, ThermoFisher Scientific (1:200)
Mouse mAb anti- γ -Tubulin, Clone GTU-88, T6557, Sigma (1:500)
Goat pAb anti-GATA6, AF1700, R&D Systems (1:200)
Rat mAb anti-GFP, Clone GF090R, Nacalai Tesque (1:500)
Chicken pAb anti-GFP, ab13970, Abcam (1:500)
Mouse mAb anti-GM130, Clone 35/GM130, 610822, BD Biosciences (1:250)
Rabbit pAb anti-KLF17, HPA024629, Sigma (1:200)
Rabbit pAb anti-Nanog, ab80892, Abcam (1:200)
Goat pAb anti-NANOG, AF1997, R&D Systems (1:200)
Mouse mAb anti-Oct-3/4, sc-5279, Santa Cruz Biotechnology (1:200)
Goat pAb anti-OTX2, AF1979, R&D Systems (1:200)
Rabbit pAb anti-Par3, 07-330, Merck Millipore (1:100)
Rabbit pAb anti-Par6, sc-67393, Santa Cruz Biotechnology (1:200)
Rabbit pAb anti-Phospho-Myosin Light Chain 2 (Thr18/Ser19), 3674, Cell Signaling Technology (1:200)
Rat mAb anti-Podocalyxin, Clone 192703, MAB1556, R&D Systems (1:500)
Mouse mAb anti-PODOCALYXIN, Clone 222328, MAB1658, R&D Systems (1:200)
Rabbit pAb anti-Rab11a, 2413, Cell Signaling Technology (1:100)
Rabbit pAb anti-Rab11Fip5, Home-made, kind gift of Rytis Prekeris lab (1:200)
Rabbit pAb anti-Sox2, ab59776, Abcam (1:100)
Mouse mAb anti-ZO1, Clone ZO1-1A12, 33-9100, ThermoFisher Scientific (1:100)
Alexa Fluor 488 Donkey anti-Goat, A-11055, ThermoFisher Scientific (1:500)
Alexa Fluor 568 Donkey anti-Goat, A-11057, ThermoFisher Scientific (1:500)
Alexa Fluor 647 Donkey anti-Goat, A21447, ThermoFisher Scientific (1:500)
Alexa Fluor 568 Donkey anti-Mouse, A10037, ThermoFisher Scientific (1:500)
Alexa Fluor 647 Donkey anti-Mouse, A31571, ThermoFisher Scientific (1:500)
Alexa Fluor 568 Donkey anti-Rabbit, A10042, ThermoFisher Scientific (1:500)
Alexa Fluor 647 Donkey anti-Rabbit, A-31573, ThermoFisher Scientific (1:500)
Alexa Fluor 488 Donkey anti-Rat, A-21208, ThermoFisher Scientific (1:500)
Alexa Fluor 594 Donkey anti-Rat, A21209, ThermoFisher Scientific (1:500)
Alexa Fluor 488 Phalloidin, A12379, ThermoFisher Scientific (1:500)
Alexa Fluor 647 Phalloidin, A22278, ThermoFisher Scientific (1:500)
This information is also included in Supplementary Table 2.

The subcellular localization of all the proteins analyzed in this study has been previously reported. This was used to validate the specificity of the antibody.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

The following cell lines were used:

Mouse embryonic stem cells: E14 WT mESCs (kindly provided by Prof. Austin Smith, Stem Cell Institute, Cambridge, UK), 129 WT mESCs (kindly provided by Dr. Jacob Hanna, Weizmann Institute of Science, Israel), Δ PE-Oct3/4-GFP mESCs (kindly provided by Prof. Azim Surani, The Gurdon Institute, Cambridge, UK), Rex1::GFPd2 mESCs (kindly provided by Prof. Austin Smith, Stem Cell Institute, Cambridge, UK), Nanog-YFP mESCs, Dgcr8 KO mESCs (kindly provided by Dr. Jacob Hanna, Weizmann Institute of Science, Israel), Otx2 KO mESCs, and KH2 dox-inducible Nanog mESCs (kindly provided by Dr. Konrad Hochedlinger, Harvard Stem Cell Institute, Boston, US).

Feeder cells: CF-1 MEFs (GSC-6101G, Amsbio).

Human embryonic stem cells: WIBR3 Δ PE-OCT4-GFP dox-inducible NANOG KLF2 hESC line (kindly provided by Prof. Rudolf Jaenisch, MIT, Boston US).

b. Describe the method of cell line authentication used.

The self-renewal properties of WT ESCs were confirmed by RT-PCR and immunofluorescence (Extended Data Fig. 2 and 10), and chimera assays (Fig. 2h). KO mESCs were authenticated by PCR (primers provided in the Methods section) and immunofluorescence.

c. Report whether the cell lines were tested for mycoplasma contamination.

Cell lines were routinely tested for mycoplasma contamination by PCR

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

n/a

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Mice (*Mus musculus*) were used to obtain mouse embryos for this study. The following strains and genetically-modified models were used: F1 (C57Bl6xCBA), MF1, CD1, CAG-GFP and Nanog-YFP (both males and females). Superovulated females for chimera experiments were used at 6 +/- 1 week of age. Females for natural matings were used at 3 +/- 1 month of age.

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

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human research participants are couples eligible for IVF treatment. We expect embryo quality to vary from patient to patient. For this reason, for all the human embryo experiments presented in this study, human embryos coming from multiple couples were pooled together and randomly allocated to groups (control and experimental).