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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	LEICA software LAS X (v. 3.5.6.21594) was used for image acquisition.
Data analysis	The code used in this study are available at https://github.com/darogan/Kyprianou_Zernika-Goetz and https://doi.org/10.5281/zenodo.3610335 . . For graphical statistics and statistical tests (t-test), GraphPad Prism 7.0a was used. Fiji image processing software (v.1.52p) was used for image processing and analysis. Imaris 9.0 image processing software was used for image processing and analysis. Oriana Software (Version 4) was used for visualising circular statistics. Re-analysis of the GSE109071(from 10.1016/j.celrep.2019.02.031) dataset was performed using R (v3.4.4) and Seurat (v3.0.1). ChIP tracks for Smad2_3 from GSE70486(from 10.1016/j.stem.2016.10.002) in TDF (tiled data file) format were visualised in IGV (Integrative Genomics Viewer, 10.1038/nbt.1754).

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

Scripts and data for single cell RNA-seq and Chip-seq analysis are available from GSE109071,GSE70486 and https://github.com/darogan/Kyprianou_Zernika-Goetz. The source data used in all the presented graphs are provided as a Source Data files. Raw image files are available from the corresponding author upon 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 mouse embryos as experimental model systems. (Cell. 2014 Feb 27; 156(5): 1032–1044) (Nature Cell Biology volume 17, 113–122 (2015) (Nature Cell Biology volume 18, 1281–1291 (2016) (Nature Cell Biology volume 20, 1278–1289 (2018)
Data exclusions	No data were excluded from the analysis
Replication	In the study, all attempts at replication were successful. Each result described in the paper is based on at least two independent biological replicates but very often an experiment is based on more than two experiments.
Randomization	Samples (mouse embryos) were allocated randomly into experimental groups
Blinding	The investigators were not blinded to group allocation due to the nature of embryo recovery. Thus, it was not possible to blind the experimenters when i treatments were performed. When assessing treatment result on basement membrane appearance, it was either quantified or judged simply on presence or absence of perforations.

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	Primary Antibodies used in whole mount immunofluorescence: E-cadherin (1:300; Thermofisher Scientific, 13-1900; lot: TD2496212 and 74960345A) Oct4 (1:400; Santa Cruz Biotechnologies, sc-5279; lot: E1017 and E1818) Laminin (1:400; Sigma, L9393; lot: 046M4837V) Collagen IV (1:100; Millipore, AB769; lot: 2818807 and 3182110)

HSPG2 (1:100; Millipore, MAB1948P; lot: 2890988 and 3094623)
Otx2 (1:100; R&D Systems, AF1979; lot: KNO0615111 and KNO0616081)
Cerberus (1:500; R&D Systems, MAB1986; cl. 225807; lot: JUP031904A)
Foxa2 (1:200; Cell Signalling Technologies, D56D6; lot: 3)
T/Brachyury (R&D Systems, AF2085; lot: KQP0619031)
MMP14 (IF: 1:150; WB: 1:2000; Abcam, ab51074; cl. EP1264Y; lot: GR300562-16)
P-Histone H3 (S10) (1:300; Cell Signaling Technologies, 9701S; lot: 17)
Cleaved Caspase-3 (D175) (5A1E) (1:200; Cell Signaling Technologies, 9664S; lot: 21)
hAP-2gamma (1:200; R&D Systems, AF5059; lot: CBHR011904A and CBHR0219051)

Secondary antibodies used:
Alexa 488 donkey anti-mouse (1:500; Thermo Fisher Scientific, A21202; lot: 2018296)
Alexa 594 donkey anti-rat (1:500; Thermo Fisher Scientific , A21209; lot: 1870948)
Alexa 647 donkey anti-rabbit (1:500; Thermo Fisher Scientific, A31573; lot: 1903516)
Alexa 488 donkey anti-goat (1:500; Thermo Fisher Scientific, A11055; lot: 1942238)

Validation

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.
E-cadherin: it correctly stained the basolateral side of cells in the embryo as reported and as expected (Science 356, doi:10.1126/science.aal1810)
Oct4: it specifically stained the epiblast at all stages tested, as expected (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)
Collagen IV: it correctly stained the basement membrane between visceral endoderm and Exe or epiblast, as reported elsewhere and as expected (Dev Dyn 241, 270-283)
HSPG2: same as above
Otx2: it was correctly expressed in the epiblast and embryonic visceral endoderm of post-implantation embryos (eLife 2018;7:e32839)
Cerberus: correctly stained anterior visceral endoderm in post-implantation mouse embryos (Dev Bio 309, 97-112)
Foxa2: it was correctly expressed in the visceral endoderm and in the gastrulation stage mesoderm (Development 136, 1029-1038)
T/Brachyury: it correctly stained mesoderm at 6.5 and later as reported and expected (Dev Biol 288, 363-371)
c-Casp3: correctly stained apoptotic cells as reported before (Autophagy, doi.org/10.1080/15548627.2019.1630222)
MMP14: expected membrane staining in the epiblast cells where MMP14 is expressed according to single cell sequencing analysis and confirmed by immunofluorescence and western blot in wildtype and MMP14 knockout ES cell lines in this study.
AP2gamma: it correctly stained the ExE lineage in mouse postimplantation embryos as reported and expected (Genes Dev 29(23):2435-48.
Anti phospho histone 3 it correctly labelled mitotic cells, as expected (Science, 360(6384):99-102 2018)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Lifeact-GFP mESCs were derived from the Lifeact-GFP transgenic mouse line (Reidl, J. et al., 2010).
MMP14-KO mESCs were generated in this study in Lifeact-GFP mESCs.
E14 WT mESCs (kindly provided by Prof. Austin Smith, Stem Cell Institute, Cambridge, UK)
Mouse extraembryonic endoderm cells: EGFP XENs (a gift from Dr. Peter Rugg-Gunn Babraham Institute, Cambridge).

Authentication

Cells were maintained in conditions to preserve stem cell character and prevent differentiation. The self-renewal properties of WT ESCs were confirmed by immunofluorescence routinely and morphological characterstics. WT ESC colonies maintained a domed morphology and XEN cells showed a mixed morphology of fibroblast-like and rounded cells as expected.

Mycoplasma contamination

Cell lines were routinely tested for mycoplasma contamination by PCR and confirmed that they were 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

6-week old 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, Nodal fl/fl; RCre:T2, TTR:Cre; RhoA fl/fl and T:GFP (both males and females).

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight	All experiments involving mice have been regulated by the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 and additional ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). Experiments were authorised by the Home Office (Licence number: 70/8864).

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