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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	LEICA software LAS X was used for image acquisition.
Data analysis	For graphical statistics and statistical tests (t-test), GraphPad Prism 7.0a was used. To visualize the genes expression patterns in each embryo section, we wrote a program for visualization in MATLAB (version: 2015a). To analyze the genes expression dynamics during development (E5.25, E5.5, E5.75), we normalized the three sequencing batches to the same standard using ComBat DEGs analysis between epiblast and extra-embryonic ectoderm was performed using RankProd RNA raw reads were mapped to mm10 version of mouse genome using Tophat2 v2.0.4. We calculated fragment per kilobase per million (FPKM) as expression level using Cufflinks v2.0.2 with default parameters. Fiji image processing software was used for image processing and analysis. 3D reconstructions of cells were carried out on 3D Slicer software 4.0.0-1.

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 upon request. 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

RNA–seq data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE110808. Source data for Fig. 1b, 2c,2e,2g,2i,2j,2l,3c,3h and Supplementary Fig. 3c,3d have been provided as Supplementary Table 2. Source data for Fig.1g,h; Fig 3e,f; Fig. 4d,f; Fig. 5c,d and Fig. S3c are provided as supplementary movies. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-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)
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. Figure legends indicate the number of independent experiments performed in each analysis.
Randomization	Samples (mouse embryos) were allocated randomly into experimental groups
Blinding	The investigators were not blinded to group allocation. This study focuses on the identification of morphogenetic events driving tissue remodelling during mouse development and specifically during pro-amniotic cavity formation. Therefore the experiments were descriptive in their nature.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	Primary Antibodies used in whole mount immunofluorescence: ZO-1 (1:200; Thermofisher Scientific, 33-9100, Lot:SH255451) E-cadherin (1:300; Thermofisher Scientific, 13-1900, Lot:74960345A)

aPKC (1:100; Santa Cruz Biotechnologies, sc-216, discontinued)
Oct4(1:400; Santa Cruz Biotechnologies, sc-5279 Lot:E1017)
GM130 (1:200; BD, 610822, Clone 35/GM130)
Laminin (1:400;Sigma, L9393, Lot: 046M4837V)
Cdx2 (1:200; Biogenex, MU392A-UC)
β1-integrin (1:50; Ha2/5; BD,561796, Lot: 5203501)
active β1-integrin (1:50; 9EG7; BD, 553715, 5323932)
Ap2γ (1:300; Santa CruzBiotechnologies, sc-8977, discontinued)
Eomes (1:400; Abcam, ab23345, Lot:GR305614-2)
Elf5 (1:400; Santa Cruz Biotechnologies, sc-9645, Lot:B0216)
Podocalyxin (1:300; R&D systems, MAB1556, Clone:192703, Lot:1PF0317051)
Pard6b (SantaCruz Biotechnologies, sc-67393, Lot: C2014)
pMLC (1:100; Cell Signalling Technologies, 3671P, Lot3)
Collagen IV (1:100; Millipore, AB769, Lot: 2818807)
HSPG2 (1:100; Millipore, MAB1948P, Clone:A726, Lot:2890988)
Rab11a (1:100; Cell Signalling Technologies, 2413S, Lot:1)
Anxa2 (1:700; Abcam, ab41803, Lot: GR307369-2)

Secondary antibodies used
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)

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.
Primary Antibodies used in whole mount immunofluorescence:
ZO-1: displayed tight junction localisation as described before (Nature 552, 239-243).
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)
aPKC: displayed apical localisation as described before (Cell. 2014 Feb 27; 156(5): 1032–1044.)
Oct4: it specifically stained the epiblast at all stages tested, as expected (Science 356, doi:10.1126/science.aal1810).
GM130: it correctly displayed the punctate Golgi morphology (Nature 552, 239-243).
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)
Cdx2: it correctly stained only the extra-embryonic ectoderm cells in post-implantation embryos
β1-integrin: showed ECM enriched localisation
active β1-integrin: showed ECM enriched localisation
Ap2γ: it correctly stained the ExE lineage in postimplantation embryos and PGC after gastrulation as reported and expected (Science 356, doi:10.1126/science.aal1810).
Eomes: it correctly stained Exe and embryonic VE in post-implantation embryos (Science 356, doi:10.1126/science.aal1810).
Elf5: It correctly stained only the trophectoderm lineage
Podocalyxin; it correctly stained the lumen (Nature 552, 239-243)
Pard6b: showed apical localisation as described before (Cell. 2014 Feb 27; 156(5): 1032–1044.)
pMLC: signal is lost upon rock inhibition
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
Rab11a. showed staining of exocytotic vesicles as expected ((Nature 552, 239-243).
Anxa2: displayed extra-embryonic ectoderm specific localisation in agreement with our sequencing data and showed membrane localisation as described previously (PLoS One. 2015; 10(10): e0139506)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mouse trophoblast stem cells: wild-type TSCs (a gift from Jenny Nichols, University of Cambridge, UK)
Authentication	Plates were inspected for morphological evidence of differentiation (presence of trophoblast giant cells in TSC cultures). Additionally, the properties of Trophoblast Stem Cells were confirmed by RT-PCR for transcription factors necessary for TSCs pluripotency maintenance such as CDX2 and immunofluorescence for CDX2 and EOMES.
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	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, Lifeact-GFP and mTmG (both males and females). Females for natural matings were used at 3 +- 1 month of age.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.