SUPPLEMENTARY INFORMATION

Dynamics of anterior-posterior axis formation in the developing mouse embryo

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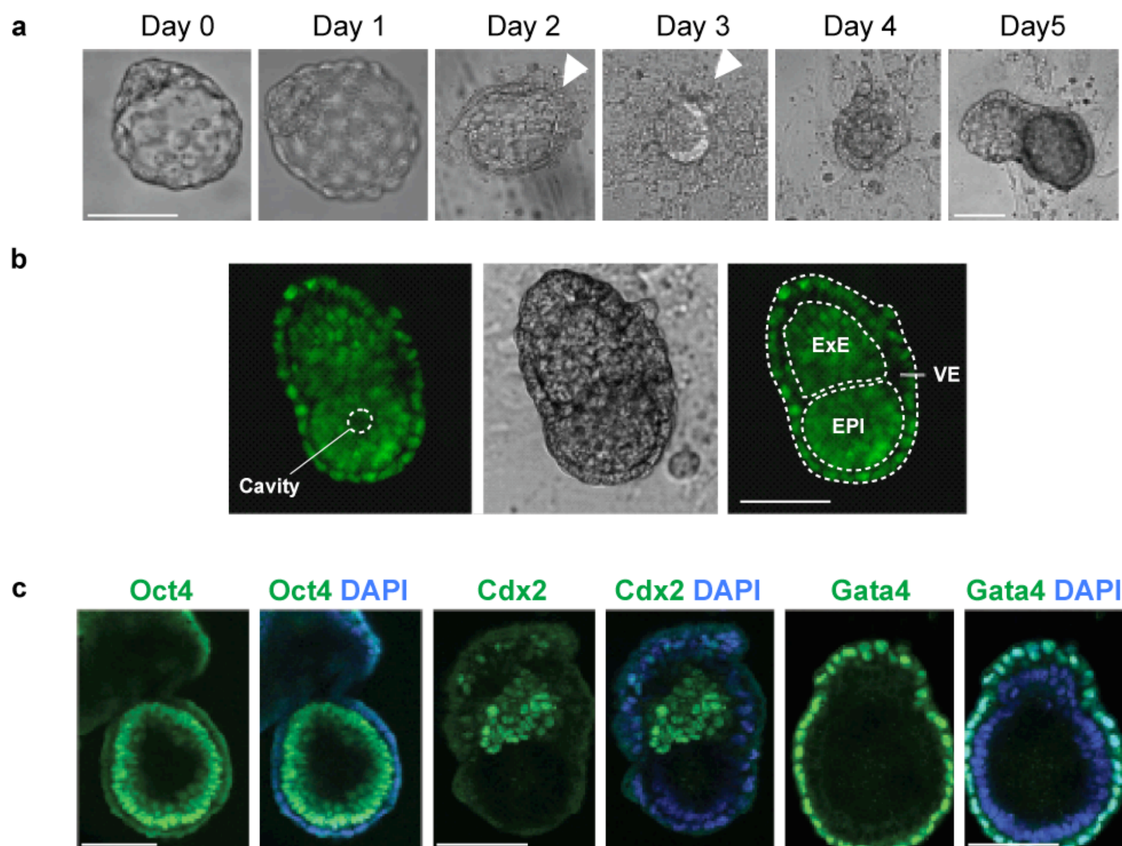
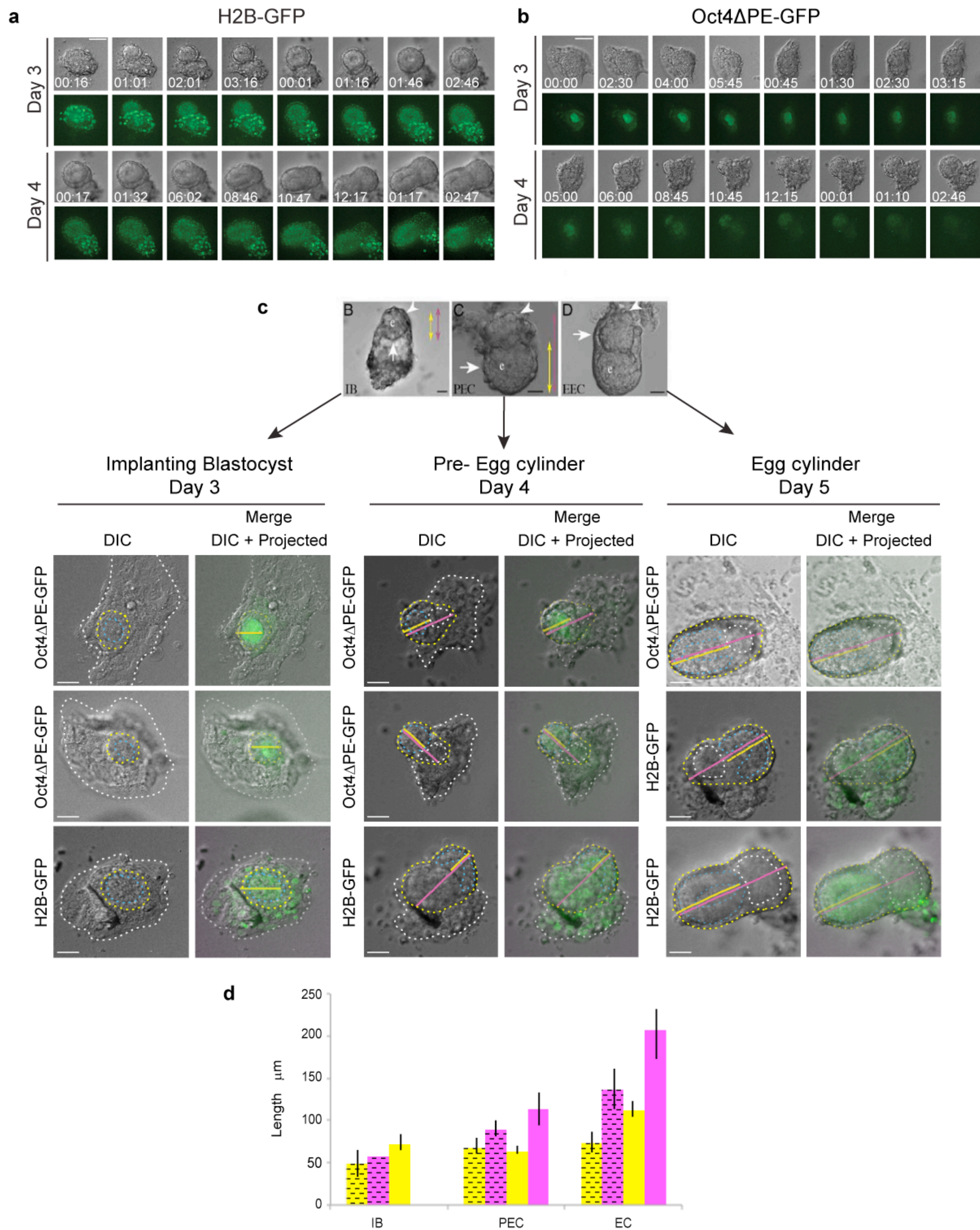
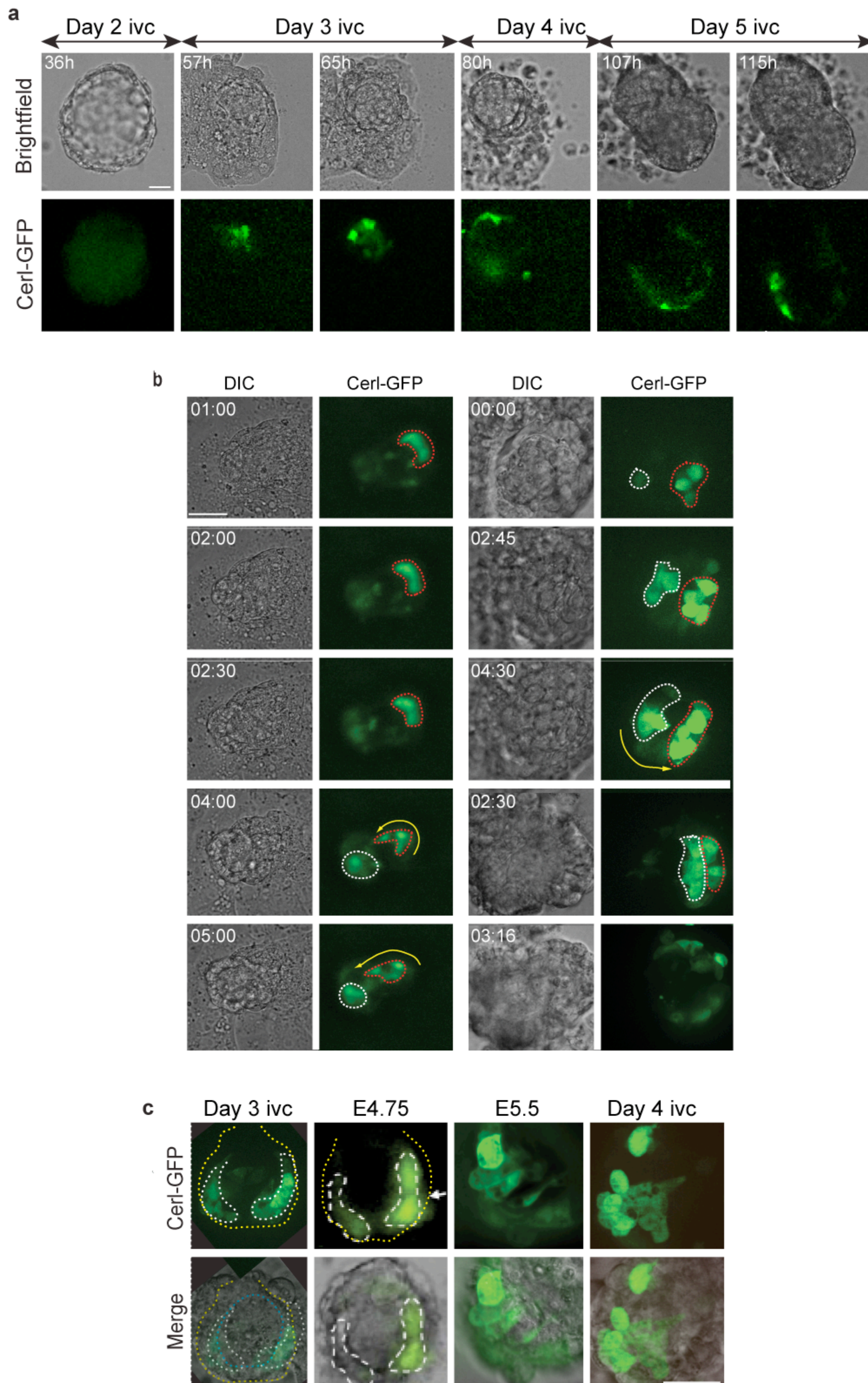


Supplementary Figure S1: Time–course of *in vitro* cultured blastocysts on standard plastic tissue culture dishes. (a) E3.5 blastocysts were seeded into culture in drops containing FCS (Day 0 of *in vitro* culture, scale bar represents 50 µm). After one day, blastocyst expansion was evident. Trophoblast outgrowths (white arrows) were observed after 2 days of culture indicating attachment to the substrate. At this point FCS was replaced by HCS for the remaining culture period. Development into an egg cylinder was observed between days 4 and 5 of culture. Scale bar represents 100 m. **(b)** Early egg cylinders recovered following *in vitro* culture of Histone H2B-GFP-expressing embryos. The epiblast (EPI), extra-embryonic ectoderm (ExE) and visceral endoderm (VE) domains, and the nascent pro-amniotic cavity are indicated. Scale bar represents 100µm. **(c)** Immunostaining of egg cylinders (carried out as described previously[1]) showing correct spatial expression of Oct4, Cdx2, and Gata4. Nuclei are counterstained with DAPI. Scale bars represent 100 µm.



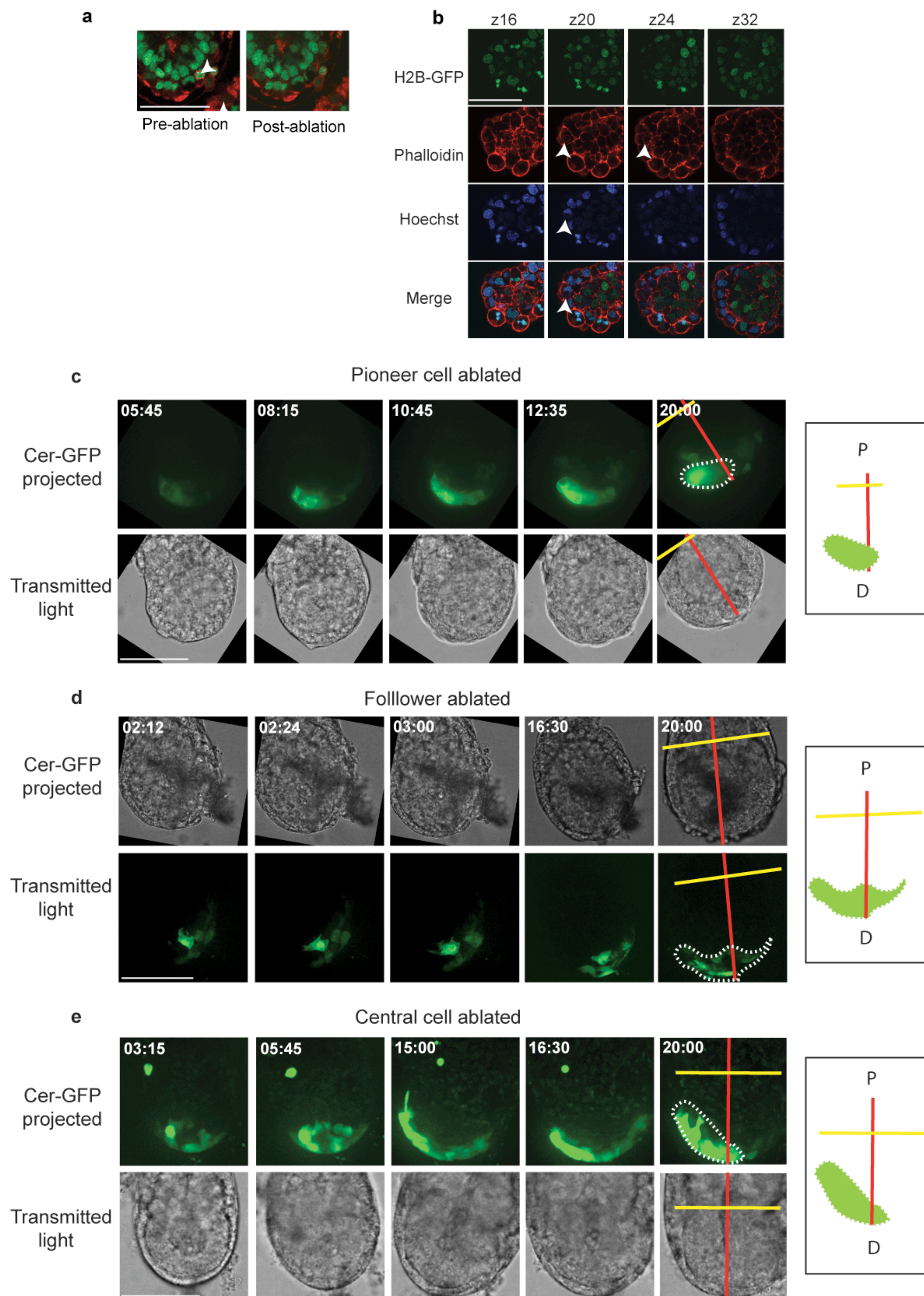
Supplementary Figure S2: Comparison of embryos cultured *in vitro* on collagen-coated hydrogels and embryos freshly collected from the uterus. **(a), (b)** DIC and projected GFP images at selected time-frames from a time-lapse series of *in vitro* development showing expression of Histone H2B-GFP **(a)** and Oct4ΔPE-GFP **(b)** transgenic embryos during *in vitro* culture. Indicated times at the bottom of each frame represent the time-frames from live-imaging. Scale bars represent 100 µm. **(c)**

Implanting blastocysts (IB), pre-egg cylinder (PEC) and egg cylinder (EC) stage embryos dissected from uteri[1] were compared to the corresponding developmental stages of *in vitro* cultured embryos (bottom panels). Three representative examples are given for each stage. Yellow solid lines represent the length of the embryonic part (Epi+VE), and pink solid lines the total length (embryonic + extra-embryonic). **(d)** Mean lengths of the embryonic part (yellow) and total egg cylinder (pink, embryonic + extraembryonic) of *in vitro* cultured embryos (filled columns) at the indicated stages (4 - 5 embryos measured in each case) alongside comparable measurements of counterparts developing *in vivo* (dashed columns[34]). Scale bars represent 50 μm.



Supplementary Figure S3: Dynamics of Cerl-GFP expression in *in vitro* cultured embryos. (a) Example of an E3.5 Cerl-GFP-expressing embryo cultured on collagen-coated polyacrylamide gel from blastocyst to egg cylinder stage. The selected time-

frames are taken from time-lapse movie 5. Total hours of culture are indicated above each frame. Scale bar represents 20 μm **(b)** Patterns of coalescence of domains of Cerl-GFP expression. Time-lapse observations from 2 different embryos showing how the two principal domains of Cerl-GFP expression coalesce at the distal tip. The first domain of Cerl-GFP expression to appear is encircled by a red dotted line and the second, by a white dotted line. The yellow arrowhead indicates the directional movement of the domains. Two different situations are shown: an original bright domain moves towards a dim domain, the intensity of which increases (left); a dim domain moves towards an original bright domain and becomes as bright as an original domain (right). Scale bar represents 50 μm**.** **(c)** Comparison of Cerl-GFP expression pattern in embryos developing *in vitro* and *in vivo*. On the left hand side, a selected time-frame from an embryo after 3 days of *in vitro* development (*ivc*) is compared to an E4.75 embryo developing *in utero* (E4.75[1]). On the right hand side, a selected time-frame of an egg cylinder formed *in vitro* (Day 4) is compared to a freshly collected E5.5 embryo. Scale bar represents 50 μm**.**



Supplementary Figure S4: Ablation of leading AVE cells blocks AVE migration and expansion. (a) Stills from live-imaging of an E5.5 H2B-GFP embryo, stained with FM4-64 to visualise membranes. The VE cell highlighted is laser ablated (circled in

the left frame). Following ablation, no changes are observed in the underlying epiblast. **(b)** Example of a fixed H2B-GFP embryo, stained with phalloidin immediately following ablation of the arrowed cell. Inspection of several optical (z) sections shows that the epiblast remains intact after ablation of a VE cell. **c)** Stills from live-imaging of an E5.5 Cerl-GFP embryo in which the leading AVE cell has been ablated, preventing AVE migration. Red and yellow bars highlight the ExE-Epiblast boundary, with the schematic representing the extent of AVE migration. **d)** Stills from live-imaging of an E5.5 Cer-GFP embryo where the cell following the leading AVE cell has been ablated. Deletion of this cell prevents migration of AVE but not its expansion. **e)** Control in which a central cell has been ablated. As a result, expansion and migration of AVE are observed. Scale bars represent 100 μm.

Supplementary Table S1: ***In vitro*** **egg cylinder formation in optically friendly plastic bottom and glass bottom culture dishes compared to conventional plastic tissue culture dishes.**

Dish	Day 2 (N) (attachment)	Day 2 (%) (attachment)	Day 5 (N) (Egg cylinder)	Day 5 (%) (Egg cylinder)
Nunc tissue culture	10	100	4	40
Mat Tek glass	2	20	1	50
Mat Tek collagen coated	3	30	1	33
WPI plastic fluorodish	3	30	0	0

For each condition, two culture drops were prepared in each case where 5 E3.5 blastocysts were seeded as described in the Materials and Methods. Embryo development was monitored on a daily basis. From Day 2 onwards the analysis refers to the attached embryos. Formation of egg cylinders only occurred in conventional plastic tissue culture dishes.

Supplementary Table S2: Embryo development on acrylamide matrices with different coatings.

Coating	N total	Attached (N)	Attachment (%)	Egg cylinder (N)	Egg cylinder (%)
Collagen	15	13	87	5	38
Laminin	15	2	13	0	0
Fibronectin	15	2	13	0	0
Combination	15	12	80	5	41

In vitro development of E3.5 blastocysts into egg cylinders was evaluated on a daily basis. The table summarises observations at Day 2 (attachment period) and Day 5 (end point, egg cylinder formation) of *in vitro* culture. Three different individual gels carrying 5 blastocysts each were prepared for each case. From Day 2 onwards the analysis refers to the attached embryos. Optimal outcome was achieved with collagen coating of the acrylamide support.

Supplementary Table S3: Embryo development on acrylamide matrices prepared on plates with glass bottom wells.

Trial	Seeded	Attachment	EC	%
1	8	8	2	25
2	10	10	1	10
3	5	5	2	40
4	11	11	2	18

In vitro development of E3.5 blastocyst into egg cylinders was evaluated on a daily basis. The table summarises observations at Day 2 (attachment period) and Day 5 (end point, egg cylinder (EC) formation) of *in vitro* culture. Four independent experiments are evaluated.

SUPPLEMENTARY REFERENCES

34. Perea-Gomez, A., *et al.* Regionalization of the mouse visceral endoderm as the blastocyst transforms into the egg cylinder. *BMC Dev Biol* **7**, 96 (2007).

35. Torres-Padilla, M.E., *et al.* The anterior visceral endoderm of the mouse embryo is established from both preimplantation precursor cells and by de novo gene expression after implantation. *Dev Biol* **309**, 97-112 (2007).