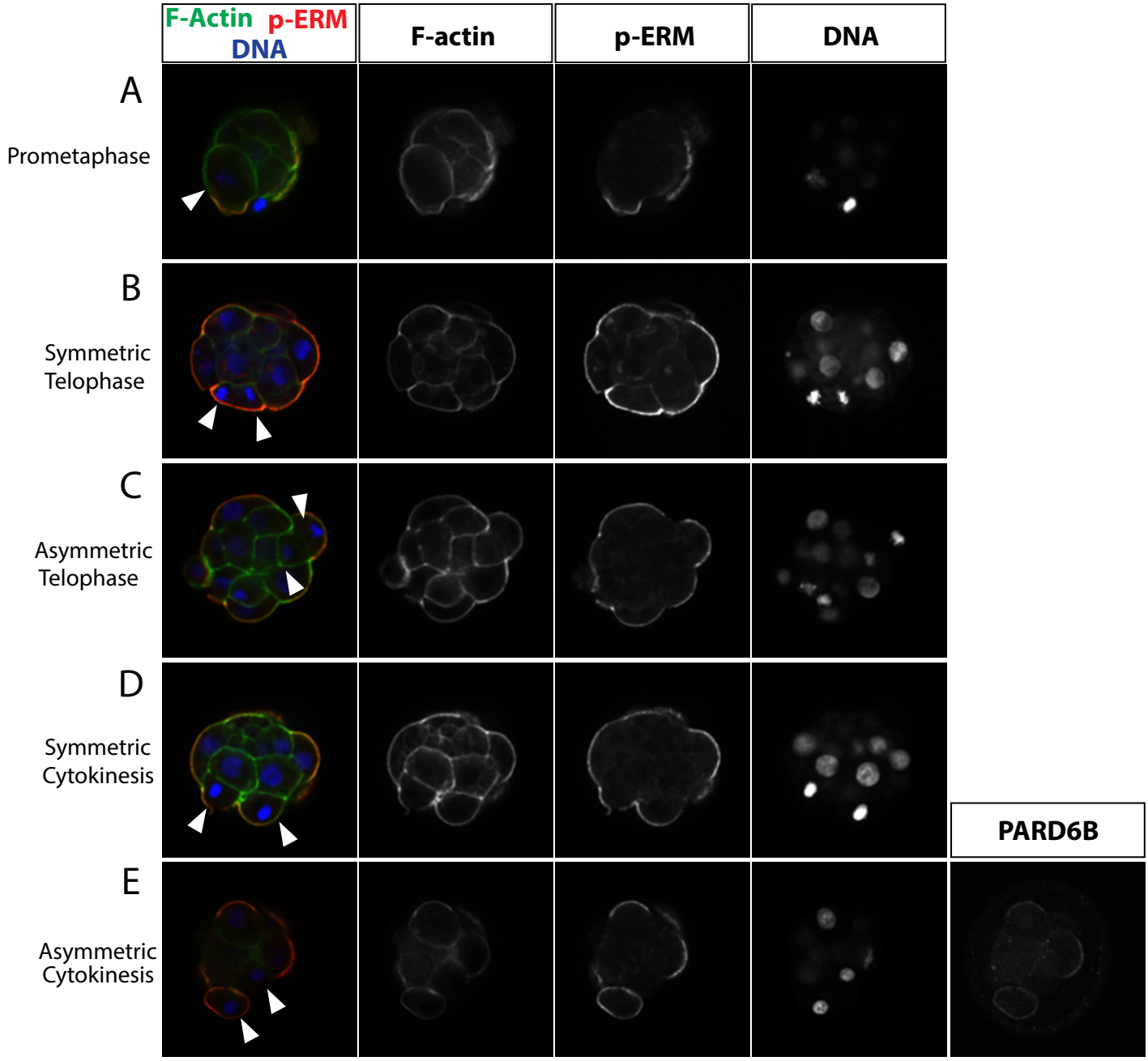
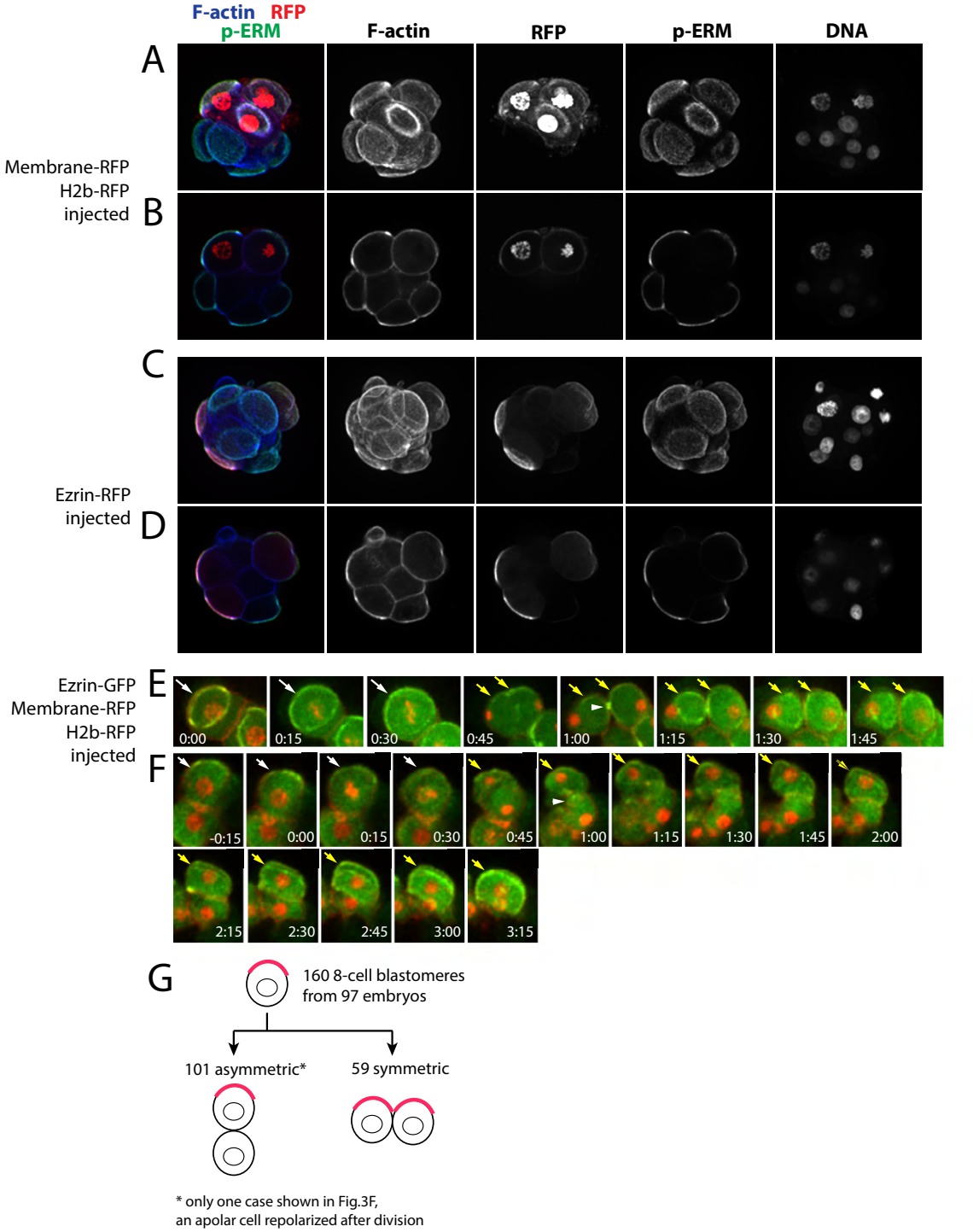
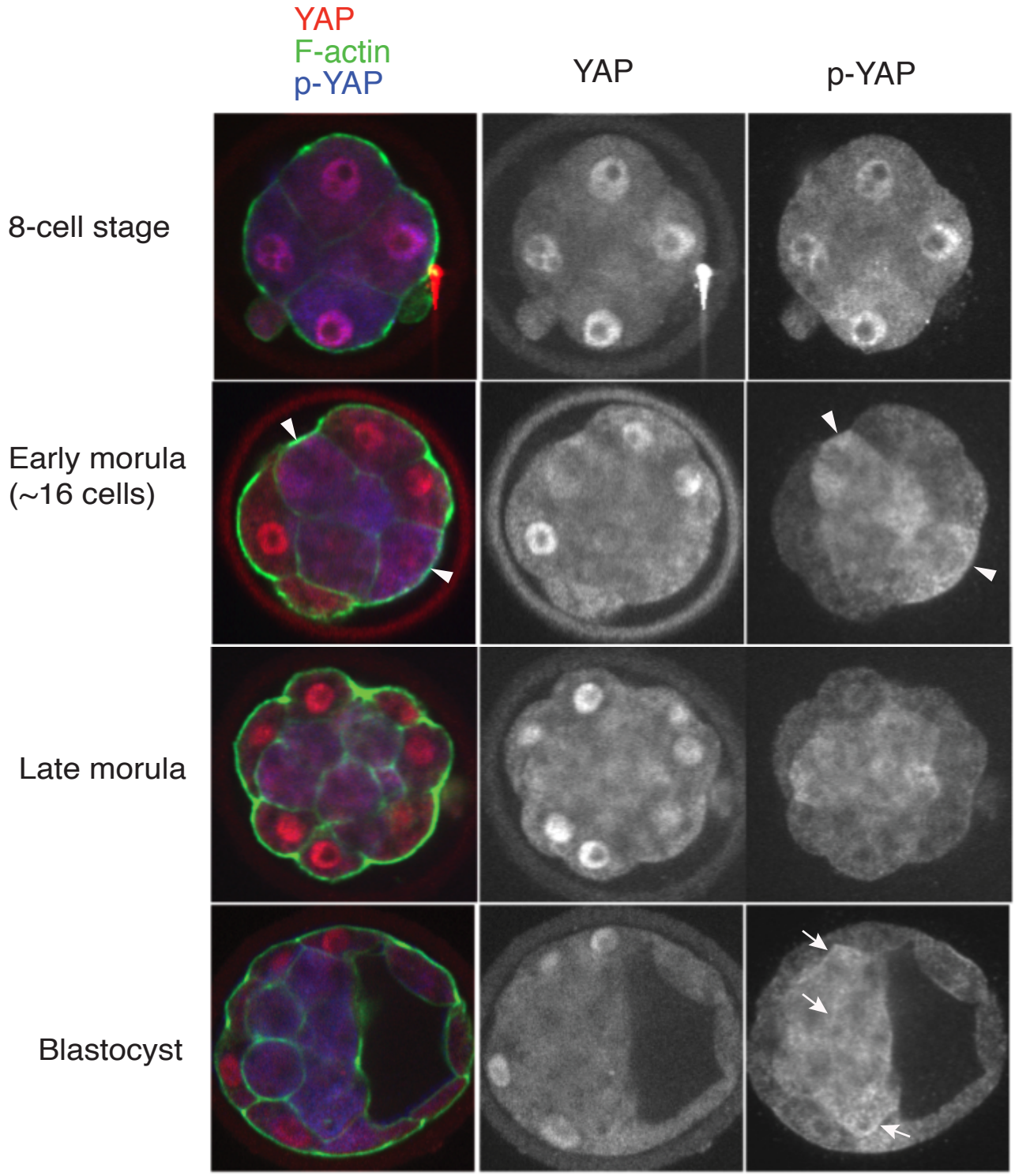


Supplementary Figure 1.
Apical domains are maintained during 8-16 cell divisions.
A. Prometaphase. B. Telophase of symmetric division. C. Telophase of asymmetric division. D. Cytokinesis of symmetric division. E. Cytokinesis of asymmetric division. p-ERM and PARD6B localization at the apical domain are maintained through the mitotic phase.

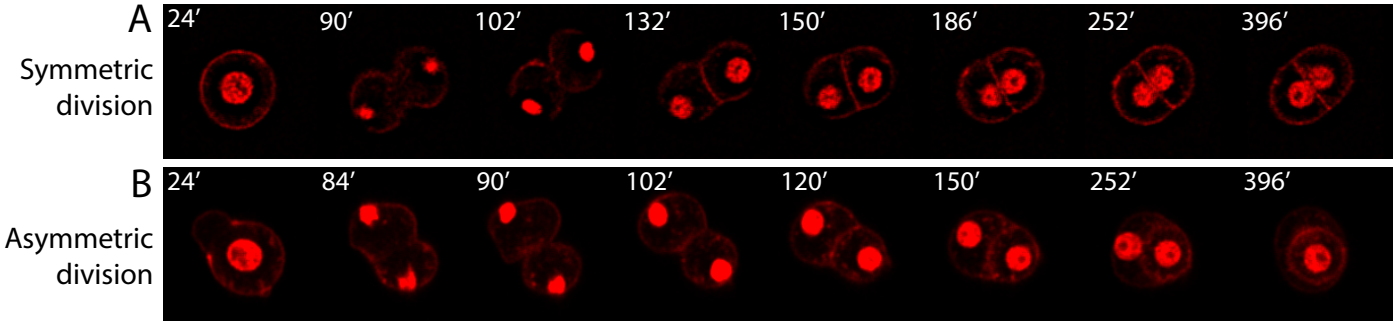


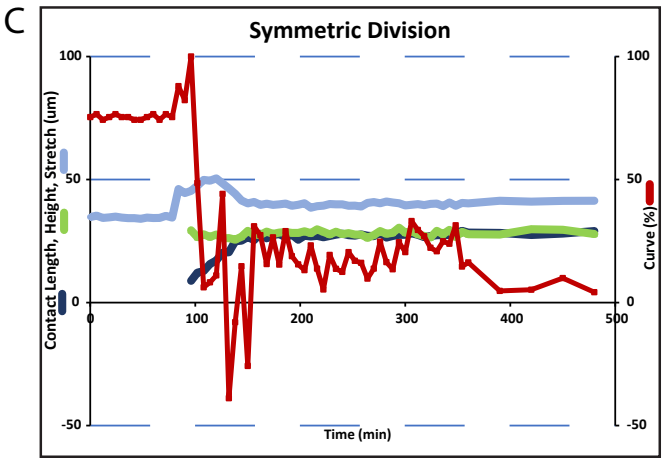
Supplementary Figure 2.
Fluorescent reporters, membrane-RFP and Ezrin GFP/RFP are enriched at the apical domain in progeny of mRNA injected blastomeres.
A and B. Membrane-RFP and histone H2b-RFP injected embryo. mRNAs of the reporters were injected into one of 2-cell blastomeres. A. Projected images. B. Optical section images. Membrane-RFP is enriched at the apical domain marked by F-actin and p-ERM staining. C and D. Ezrin-RFP injected embryo. C. Projected images. D. Optical section images. Ezrin-RFP enrichment co-localized with p-ERM staining. E and F. Time-lapse images of Ezrin-GFP, membrane-RFP and H2B-RFP injected embryos. Projected images. E. 8-cell blastomere dividing symmetrically. (t=0:00) One frame (15min) before entering mitosis. An apical domain is clearly visible (arrows). (t=0:30) Metaphase. (t=1:00) Cytokinesis. Contractile ring (arrowhead). (t=1:30) Two daughter polar 16-cells (yellow arrows). F. 8-cell blastomere dividing asymmetrically. (t=0:00) One frame before entering mitosis. Apical domain (arrows). (t=0:30) Prometaphase. (t=1:00) Cytokinesis. (t=1:30) One polar (yellow arrows) and one apolar daughter cell. (t=2:45) The apolar cell internalizes. Interestingly, Ezrin-GFP enrichment becomes less clear during cell division in live imaging, although it is still enriched in the fixed embryo shown in C and D. G. Schematic of division patterns of single 8-cell blastomeres. A total of 160 8-cell blastomeres from 97 embryos were observed. 101 blastomeres divided asymmetrically and 59 blastomeres divided symmetrically. Only one apolar cell repolarized after asymmetric division.

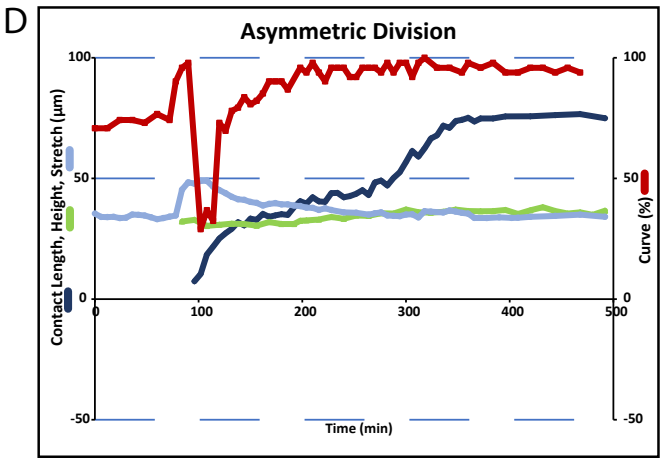


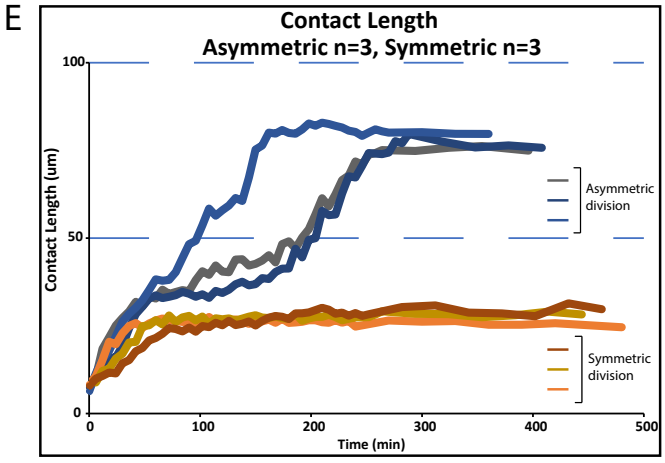
Supplementary Figure 3.
YAP and phosphorylated YAP (p-YAP) localization in preimplantation mouse embryos.

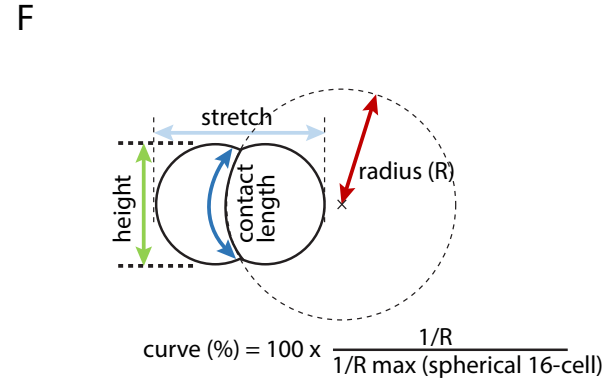
8-cell stage. YAP nuclear localization is observed in all blastomeres. At this stage, p-YAP is also localized in the nucleus.
Early morula stage (~16 cells). Polar cells show YAP nuclear localization with low levels of p-YAP in the nucleus and cytoplasm, while apolar cells show higher cytoplasmic p-YAP. No YAP nuclear localization in apolar cells. At this stage, some apolar cells are at outer positions (white arrowheads).
Late morula stage (~32 cells). Outer polar cells show YAP nuclear localization with low levels of pYAP, while apolar cells show higher cytoplasmic p-YAP. By this stage, all apolar cells are located at inner positions with high p-YAP in the cytoplasm.
Blastocyst stage. Outer TE cells show YAP nuclear localization while ICM cells show high p-YAP in the cytoplasm. Enrichment of p-YAP at cell membrane/cortex is observed (white arrows).



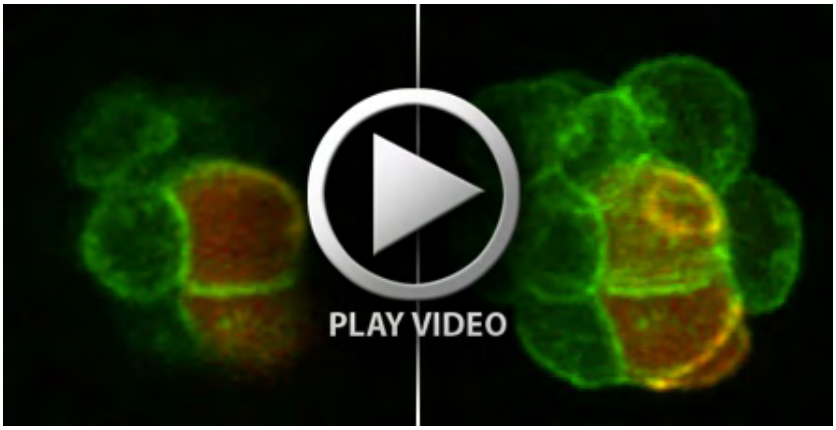








Supplementary figure 4.
Division dynamics in isolated 8-cell blastomeres injected with membrane-RFP and H2b-RFP mRNAs.
A, B. Time-lapse images of isolated 8-cell blastomeres dividing symmetrically (A) or asymmetrically (B). Images were taken every 3 minutes (min). Time= 0 is set at 30 min (10 frames) before nuclear envelope breakdown. A. After completion of cytokinesis (t=102 min), two polar sister cells quickly establish cell-cell contact and are opposed. B. After completion of cytokinesis (t=90 min), a polar (left) and an apolar (right) cell quickly establish cell-cell contact (by t=102 min). The cell-cell contact boundary curves toward the polar cell (t= 120 to 252 min). The polar cell starts enveloping the apolar cell (t= 253 to 396 min). C and D. Quantification of four parameters of membrane- and H2b-RFP mRNA-injected isolated 8-cell blastomere dividing symmetrically (C) or asymmetrically (D). E. Variability in timing of envelopment in polar/apolar 2/16 couplets. Basically, we observed the same cell dynamics observed in DIC images shown Figure 5. F. The parameters are: Height (µm, green), stretch (µm, light blue), contact length (µm, blue) and curve (%, red). Curve is indicated as % of the maximum (defined as 1/R of a circle drawn to fit an enveloped 16-cell blastomere).



Supplementary movie 1.
Time-lapse movie showing the internalization process of apolar outer cells. The embryo is labeled with GAP-43 GFP for cell shape visualization and Ezrin-RFP for apical domain visualization. The left movie shows optical section images at Z=2 and the right movie shows projected images generated from 9 optical sections. 15 min/frame.

Supplementary movie 2.
Time-lapse movie showing divisions of isolated 8-cell blastomeres and subsequent 16-cell couplet formation. The left movie shows a blastomere dividing symmetrically and the right shows one dividing asymmetrically. Three Z-section images were collected. The middle section is shown. 3 min/frame.