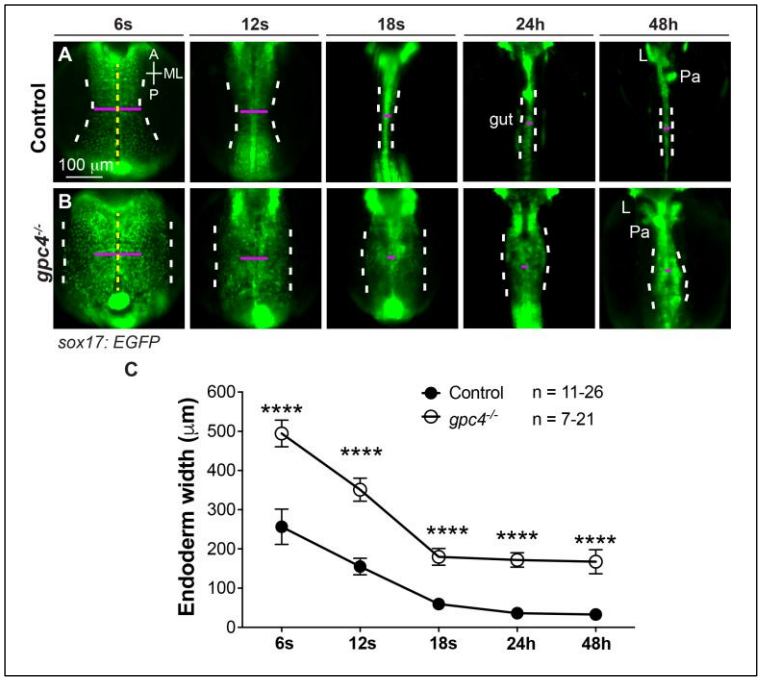


Fig. S1. Gpc4 is required for endoderm morphogenesis during segmentation.
(A-B) Epifluorescence imaging on endoderm in the posterior region of the indicated embryos at different stages. Dashed white lines, endoderm boundary; Magenta lines (equal length in embryos at the same stage), endoderm width; Yellow dashed lines, midline; L, liver; Pa, pancreas; A, anterior; P, posterior; ML, mediolateral. (C) Average endoderm width of embryos shown in A, B. The number of embryos analyzed is indicated. **** P <0.0001, Unpaired two-tailed student's t-test.

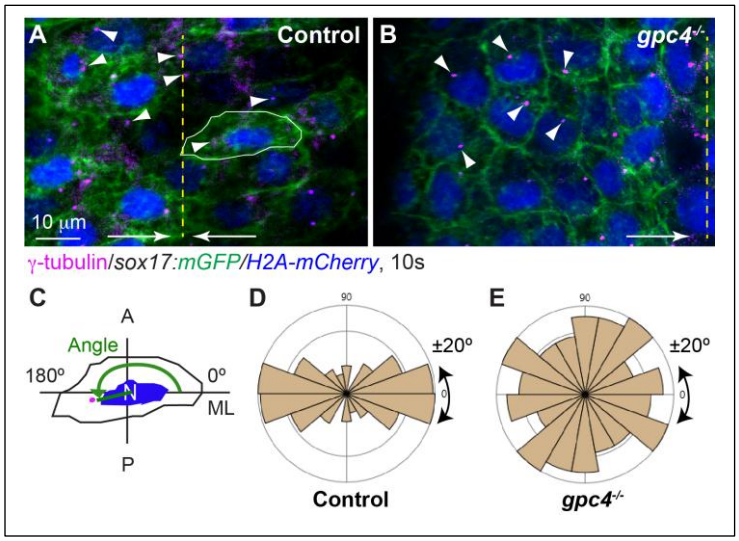


Fig. S2. Positioning of MTOCs in endodermal cells is impaired in *gpc4*$^{-/-}$ embryos.
(A,B) Confocal images (Z-projection) of endodermal cells showing expression of γ-tubulin, which labels microtubule organizing centers (MTOCs, arrowheads, detected by immunostaining) of endodermal cells. Yellow dashed lines, midline; arrows, the directions of endodermal convergence. (C) Schematic depicting how MTOC position is measured relative to the nucleus (N) and the ML axis. The angle measured is indicated by the green line. (D,E) Rose diagrams showing the distribution of the MTOC angle (each bin, 20º) in control embryos (79 cells, 6 embryos) and *gpc4*$^{-/-}$ embryos (90 cells, 5 embryos).

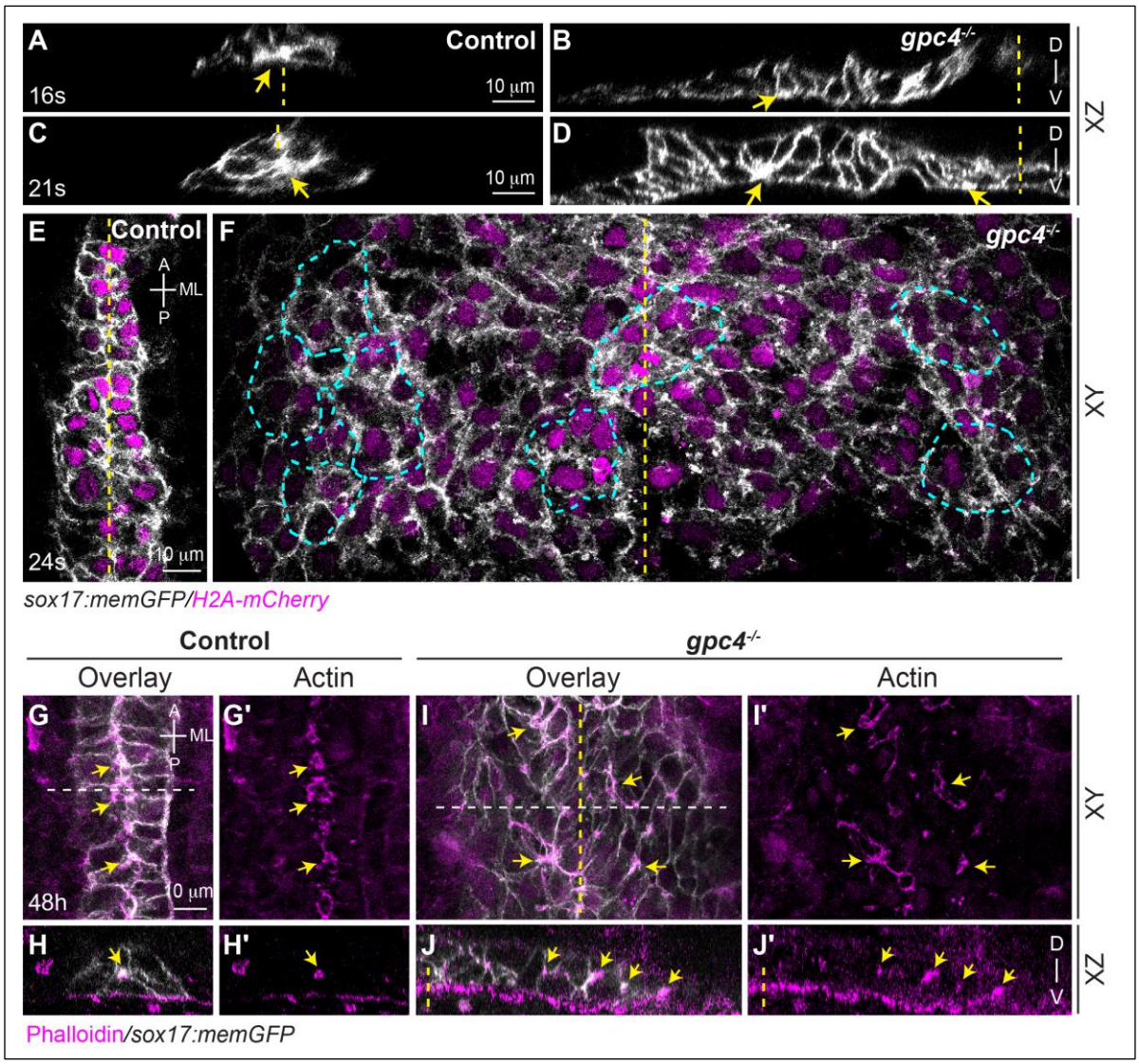


Fig. S3. Endodermal cells change shape during late segmentation.

(A-D) Confocal images (XZ view) of gut-endoderm in the indicated embryos. Only the left side of the endoderm is shown in B, D. Yellow arrows, apical constriction sites. (E-F) Z-projection confocal images (XY view) of the endoderm in the indicated embryos. Cyan dashed lines outline rosettes. (G-J′) Confocal images of gut endoderm in the indicated embryos, showing expression of Actin (detected by Phalloidin staining). (G-I′) Z projection images (XY view). White dashed lines: the region that the XZ cross section was taken. (H-J′) XZ view. Only the right side of the endoderm is shown in J-J′. Yellow arrows: actin enriched sites. Yellow dashed line, midline; D, dorsal; V, ventral; A, anterior; P, posterior; ML, mediolateral.

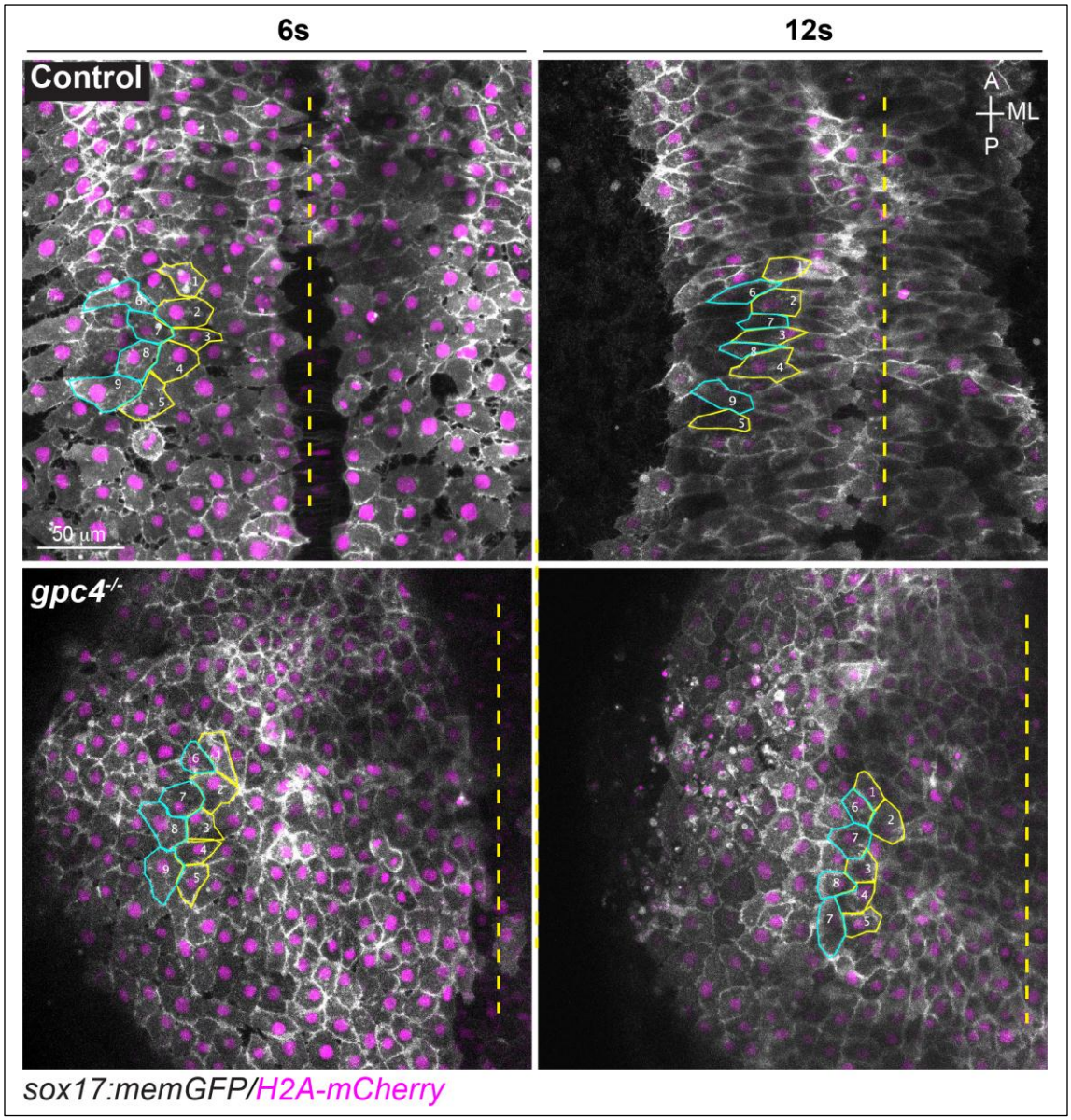


Fig. S4. Gpc4 is required for efficient endoderm convergence.

Snapshots from confocal movies (movie #1 and #3) of the endoderm at the beginning and end of timepoints (6s and 12s) in control and *gpc4*$^{-/-}$ embryos. Selected cells are marked (the same cells are labeled with the same number) to show their relative positions at two different timepoints. Only the left side of the endoderm is shown in *gpc4*$^{-/-}$embryos. Yellow dashed line, midline; A, anterior; P, posterior; ML, mediolateral.

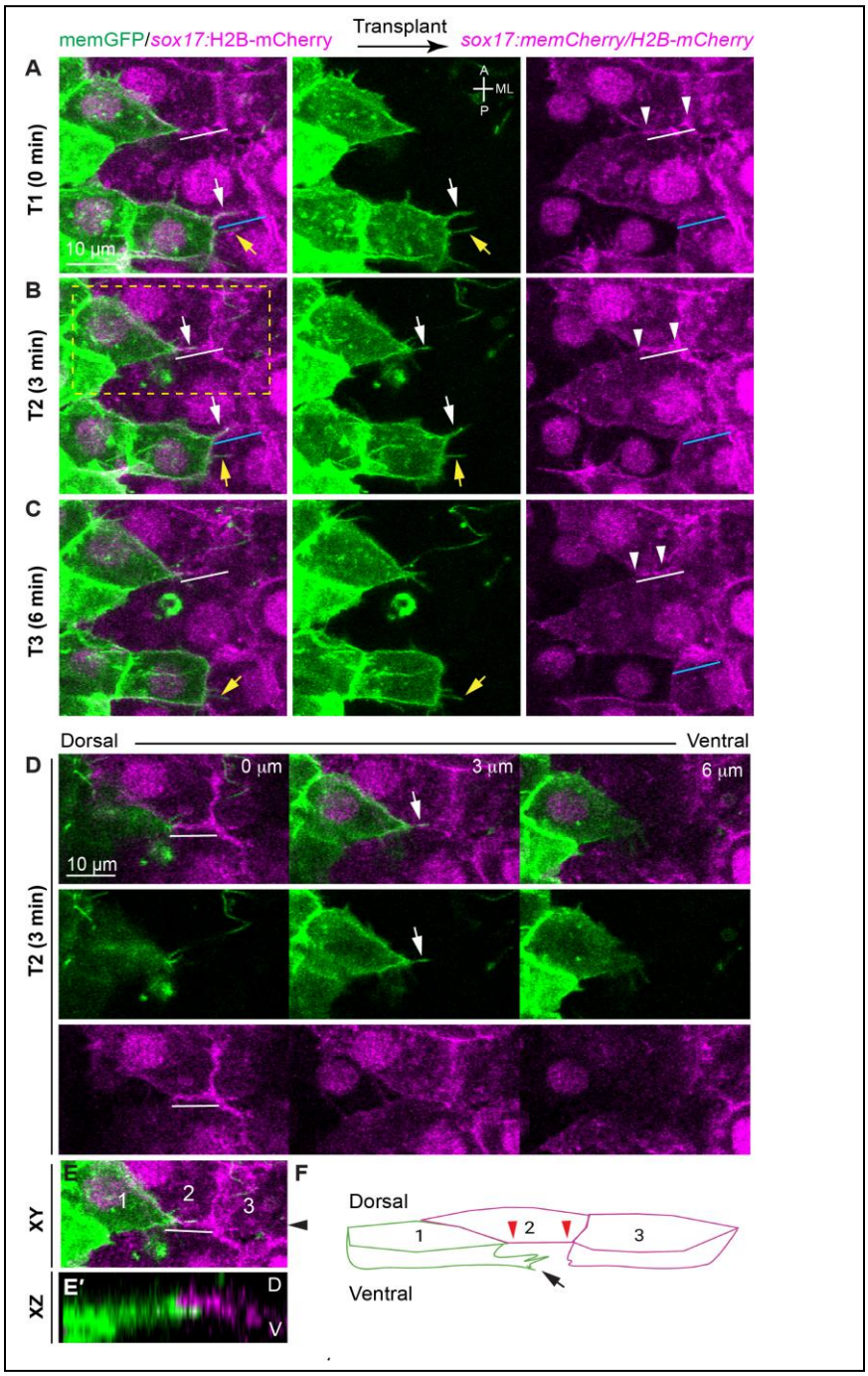


Fig. S5. **Cellular behaviors of endodermal cells during C&E.** Donor cells from *Tg(sox17:H2A-Cherry)* embryos injected with *memGFP* and *sox32* RNAs were transplanted into *Tg(sox17: memCherry/H2A-Cherry)* host embryos. Time-lapse experiments were performed on the host embryos at 6s, at which the endoderm contained memGFP-expressing donor endoderm cells. (A-C) Snapshots of Z-projected

confocal images at different time-points showing the protrusions extended from memGFP-expressing donor endodermal cells and the junctional changes in neighboring memCherry-expressing host endodermal cells. (D) Montage of Z-planes from the dorsal to ventral side of endodermal cells shown in the yellow dashed rectangle box in B. (E) Z-projected confocal image showing a donor cell protrusion into the ML junction of the neighboring endodermal cells, shown in the yellow dashed rectangle box in B. (E′) XZ view of image taken from E in the area indicated by the arrowhead. (F) Diagram depicting a cell protrusion and the shortening ML junction in the neighboring endodermal cells. White arrows, cellular protrusions extended toward the mediolateral junctions of neighboring cells; yellow arrows, cellular protrusions extended toward the neighboring cells; white and cyan lines, two different mediolateral junctions (equal length at the different timepoints); arrowheads, the ends of junctions. A, anterior; P, posterior; ML, mediolateral.

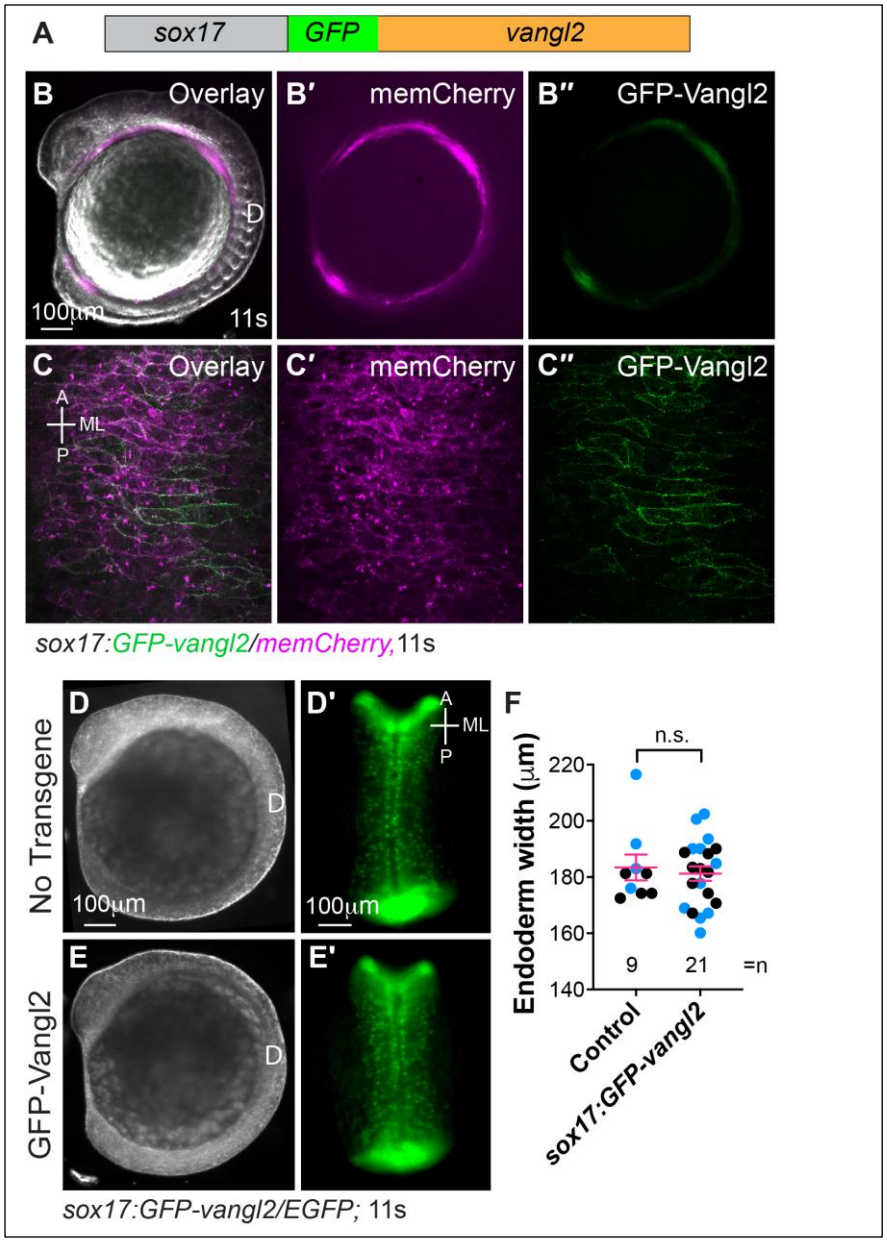


Fig. S6. Generation of the *Tg(sox17:GFP-vangl2)* line.

(A) Schematic of the zebrafish GFP-Vangl2 transgene under control by the endoderm-specific promoter *sox17* (gray box). (B-B′′) Lateral view of a live embryo expressing GFP-Vangl2 transgene; endoderm is labeled with memCherry. Overlay image of bright-field (B), epifluorescence of mCherry (B′) and GFP (B′′). (C-C′′) A confocal image (Z-projection) at 11s. Overlay (C) showing expression of mCherry (C′) and GFP-Vangl2 (C′′) on the plasma membranes of endodermal cells. (D-E′) Brightfield images (D, E, lateral view) and epifluorescence images of EGFP-labeled posterior endoderm (D′-E′, dorsal view) in the indicated embryos. (F) Average endoderm width in the indicated embryos from two independent experiments (represented by different color dots) with the number of embryos indicated. no significance or n.s., P >0.05; Unpaired two-tailed student's *t*-test. D, dorsal; A, anterior; P, posterior; ML, mediolateral.

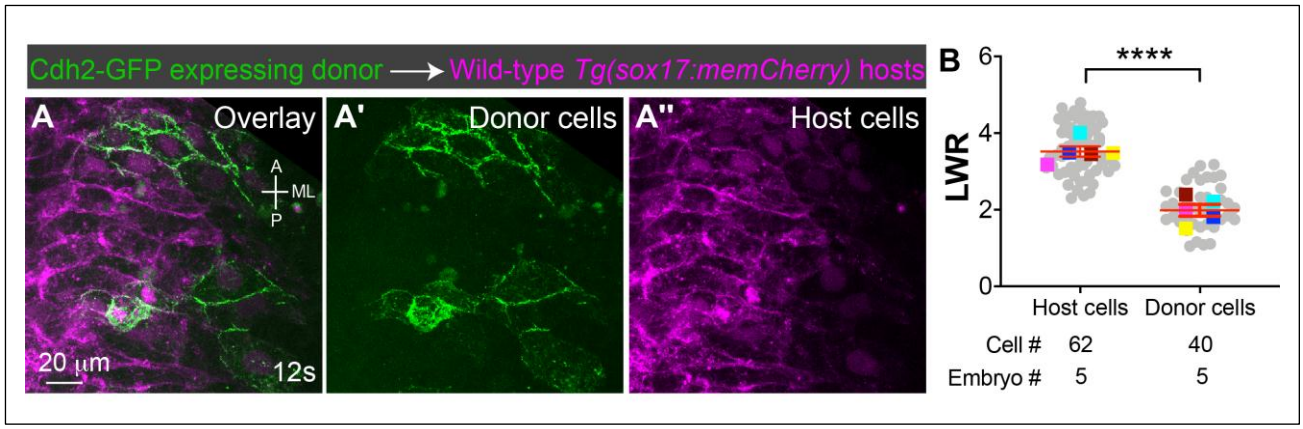


Fig. S7. Overexpression of Cdh2 in endodermal cells disrupts their cell polarity. (A-A'') Confocal images (Z-projections) showing Cdh2-overexpressing donor endodermal cells (green) in wild-type host endodermal cells. A, anterior; P, posterior; ML, mediolateral. (B) Average LWR of host and donor endodermal cells at 12s. Data from 5 chimeric embryos (represented by different color squares) and all cells (grey dots) are superimposed, with the number of cells and embryos indicated. ****, $P<0.0001$, Unpaired two-tailed student's *t*-test.

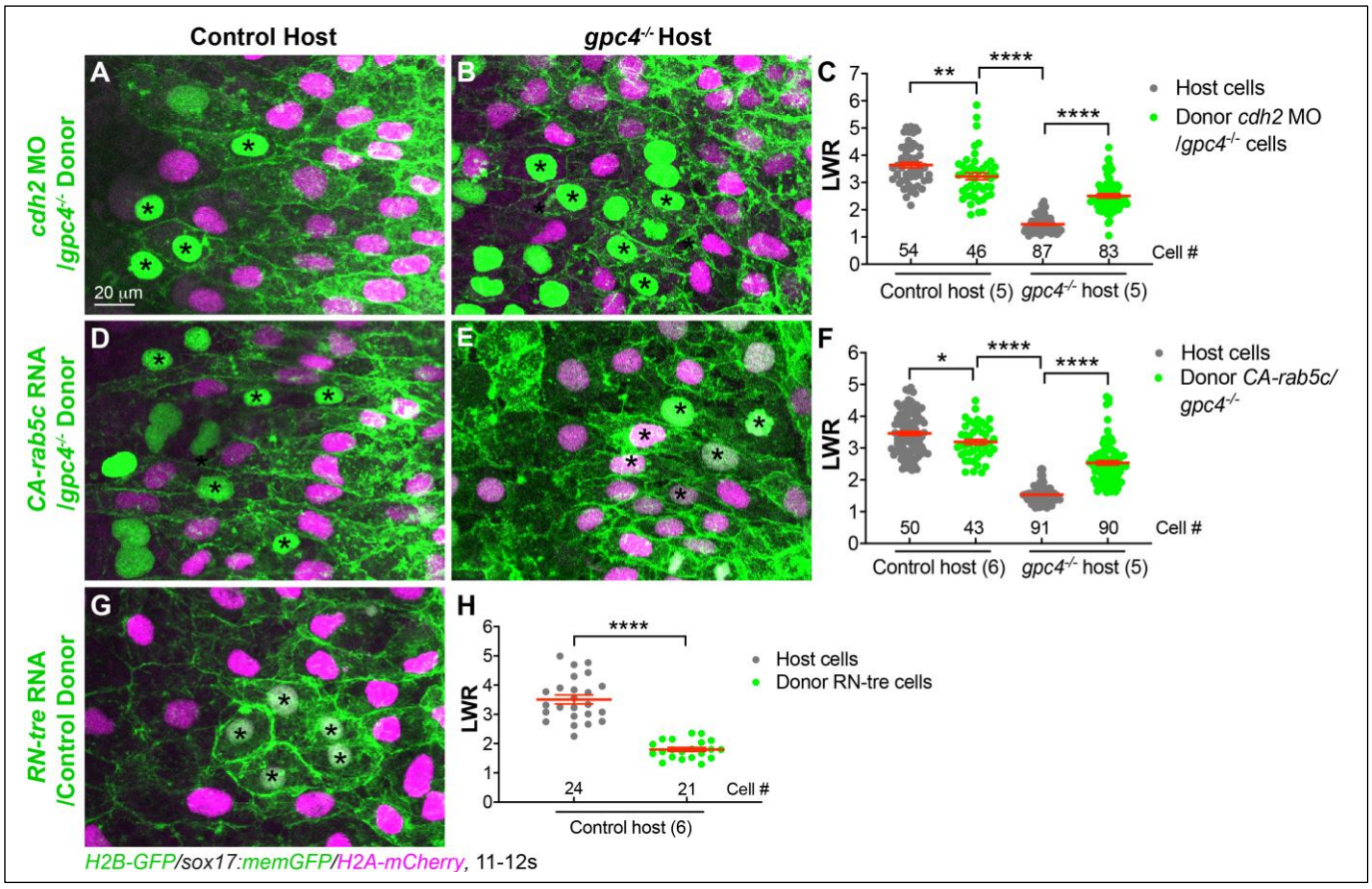


Fig. S8. Cell-autonomous functions of Gpc4, Rab5c and Cdh2 in regulating endodermal planar polarity. Transplantation was performed in the indicated embryos. (A,B,D,E,G) Confocal images (Z-projection) at XY view showing endodermal cells in the indicated host embryos transplanted with the indicated donor endoderm cells (whose nuclei are green, asterisk). (C,F,H) Graphs showing average LWR of host and donor endodermal cells in the indicated host embryos. All cells analyzed are plotted and the number of embryos and cells analyzed is shown. no significance or n.s., P >0.05; *, P <0.05; **, P <0.01; ****, P <0.0001; Unpaired two-tailed student's t-test.

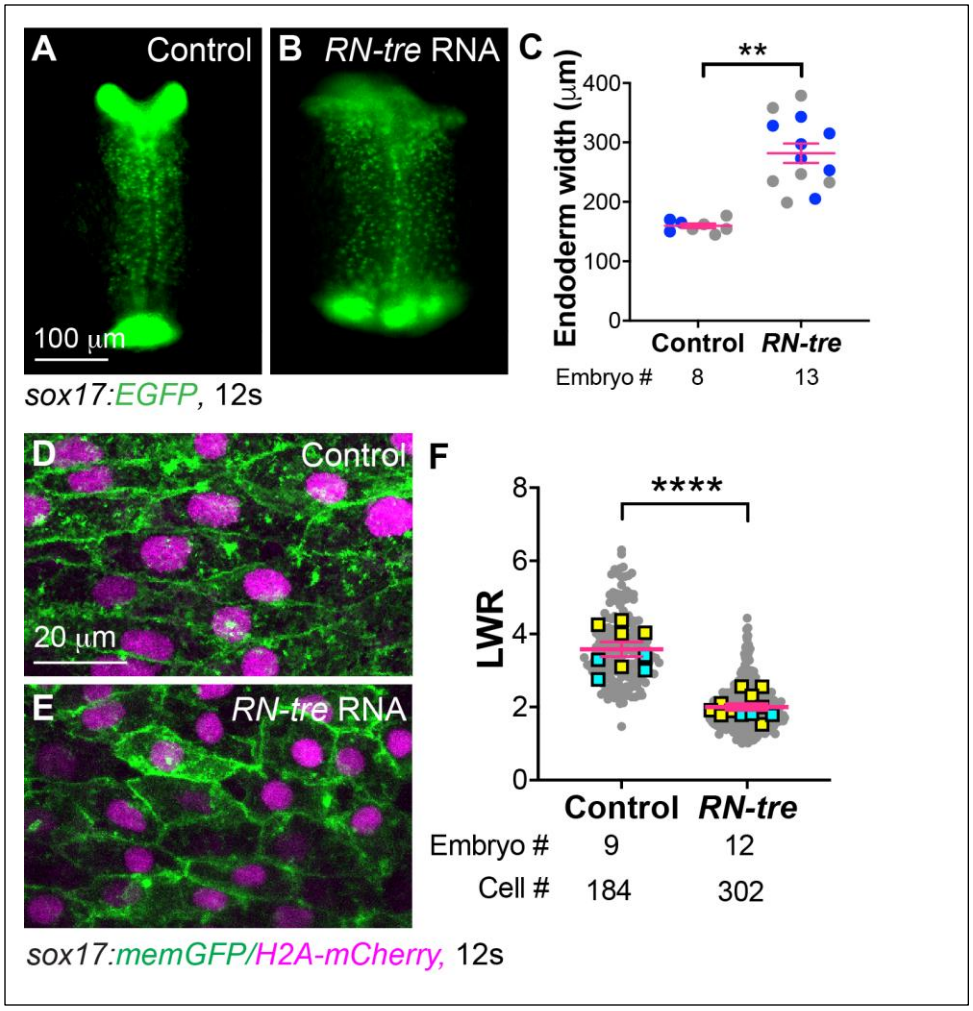
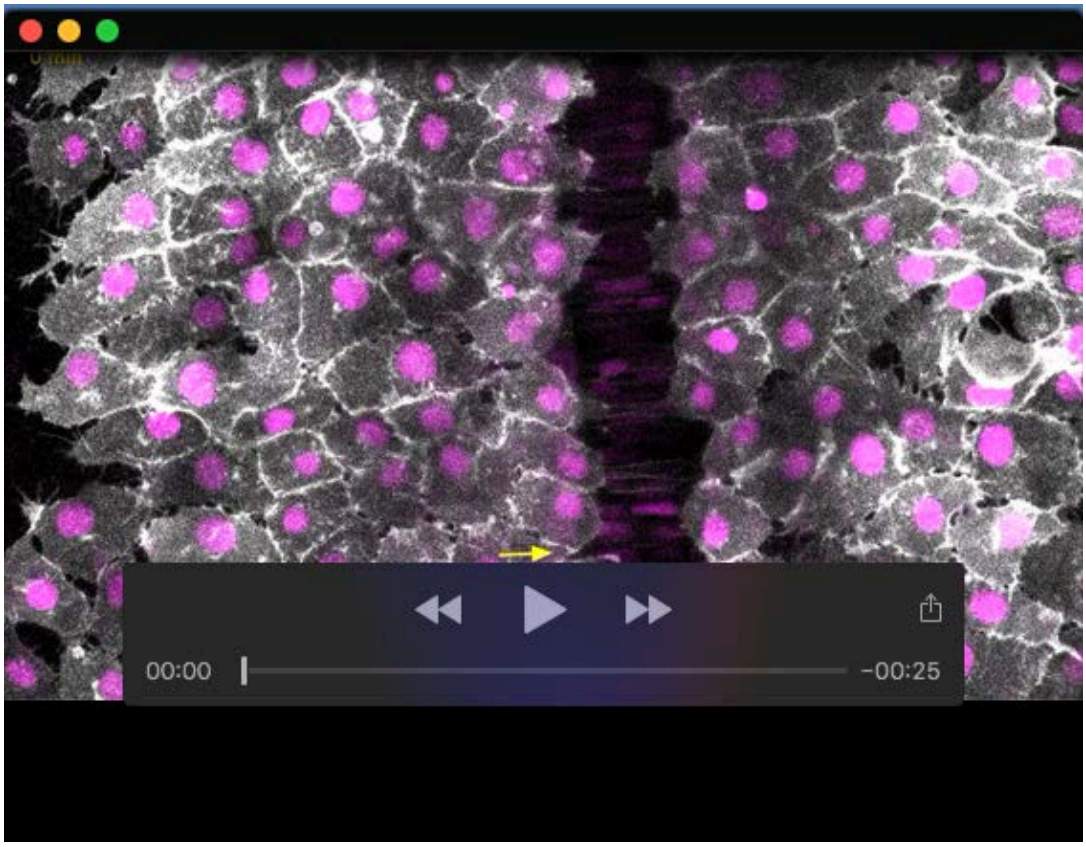
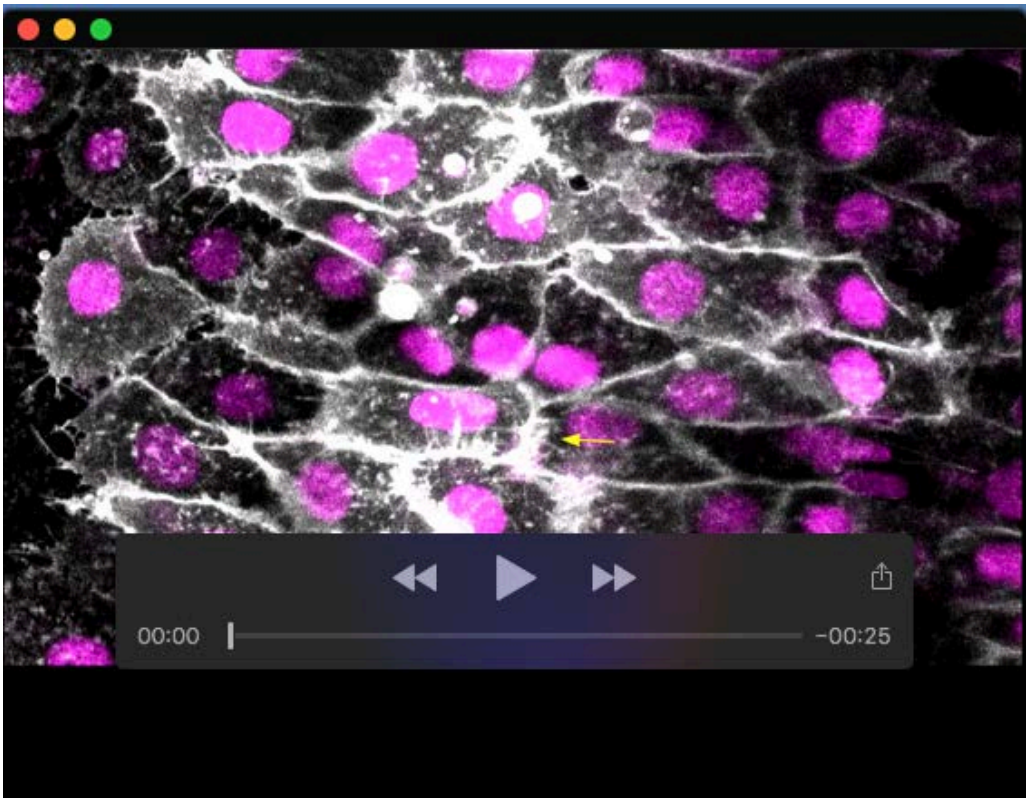


Fig. S9. Rab5 function is required for endodermal cell polarity and endoderm C&E. (A, B) Epifluorescence images of the posterior endoderm in uninjected or *RN-tre* mRNA injected embryos at the 12s. (C) Average posterior endoderm width in embryos shown in A, B. from two independent experiments (represented by different color dots) with the number of embryos and cells indicated. **, p<0.01, Student's *t*-test. (D, E) Confocal images (Z-projections) of XY view showing endodermal cells in the embryos. (F) Graph showing average LWR of endodermal cells in D, E. Data from all embryos (squares, different experiments are shown in different colors) and from all cells (grey circles) are superimposed, with number of cells and embryos indicated. ****, *P* <0.001, Unpaired two-tailed student's *t*-test.



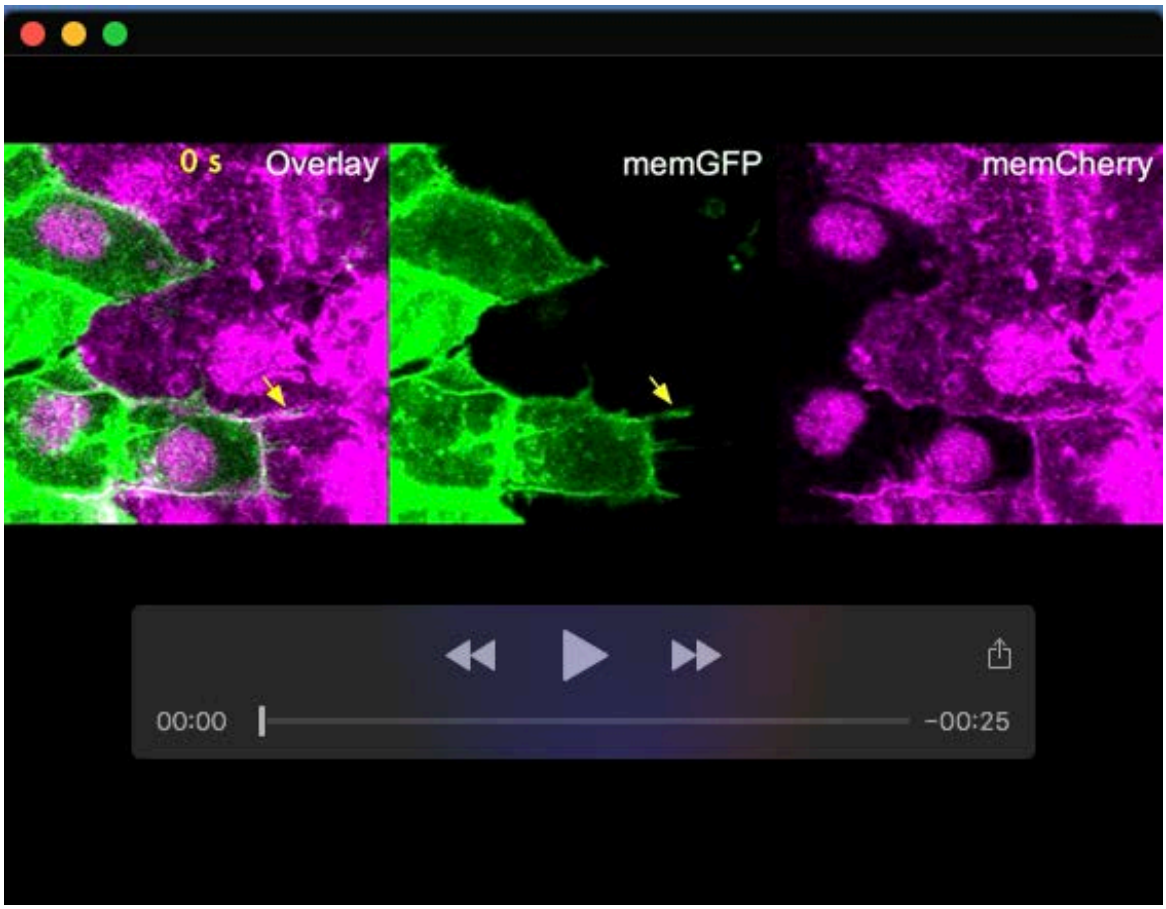
Movie 1. Endodermal cells display junctional changes and polarized cellular protrusions during endoderm C&E in control embryos at 7-12s.

Confocal time-lapse experiment was performed on *Tg(sox17:memGFP/H2A-mCherry)* embryos at 7-12s using Zeiss LSM880 confocal microscope with a LD C-Apo 20×/NA 0.8. Images were acquired at 5 min intervals and the movie plays at 4 frames/sec. Green lines: Type 3 junctions (T3), mediolaterally shrinking junctions; Red lines: Type 1 junctions (T1), anterior-posteriorly expanding junctions; Dashed yellow lines: rosettes; Yellow arrows: the leading edge of the endodermal cells that squeezed between neighboring cells; Green arrowheads: cellular protrusions in the leading edge of endoderm cells at the midline.

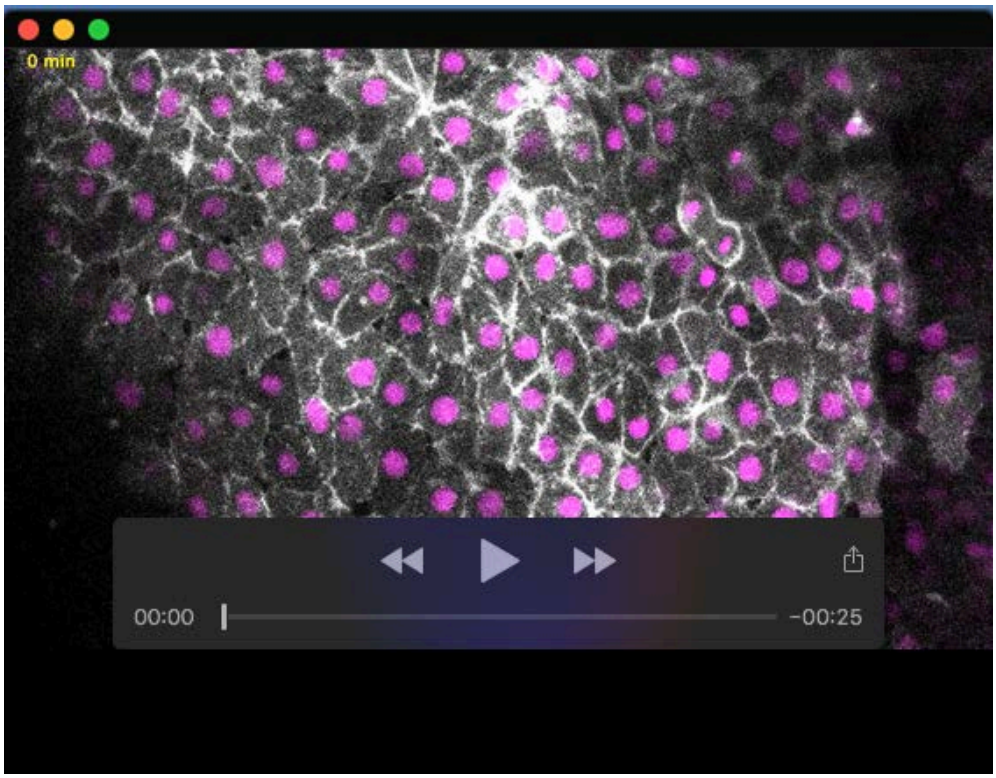


Movie 2. **Endodermal cells extended broad lamellipodia along ML axis in control embryos during 7-12s**.

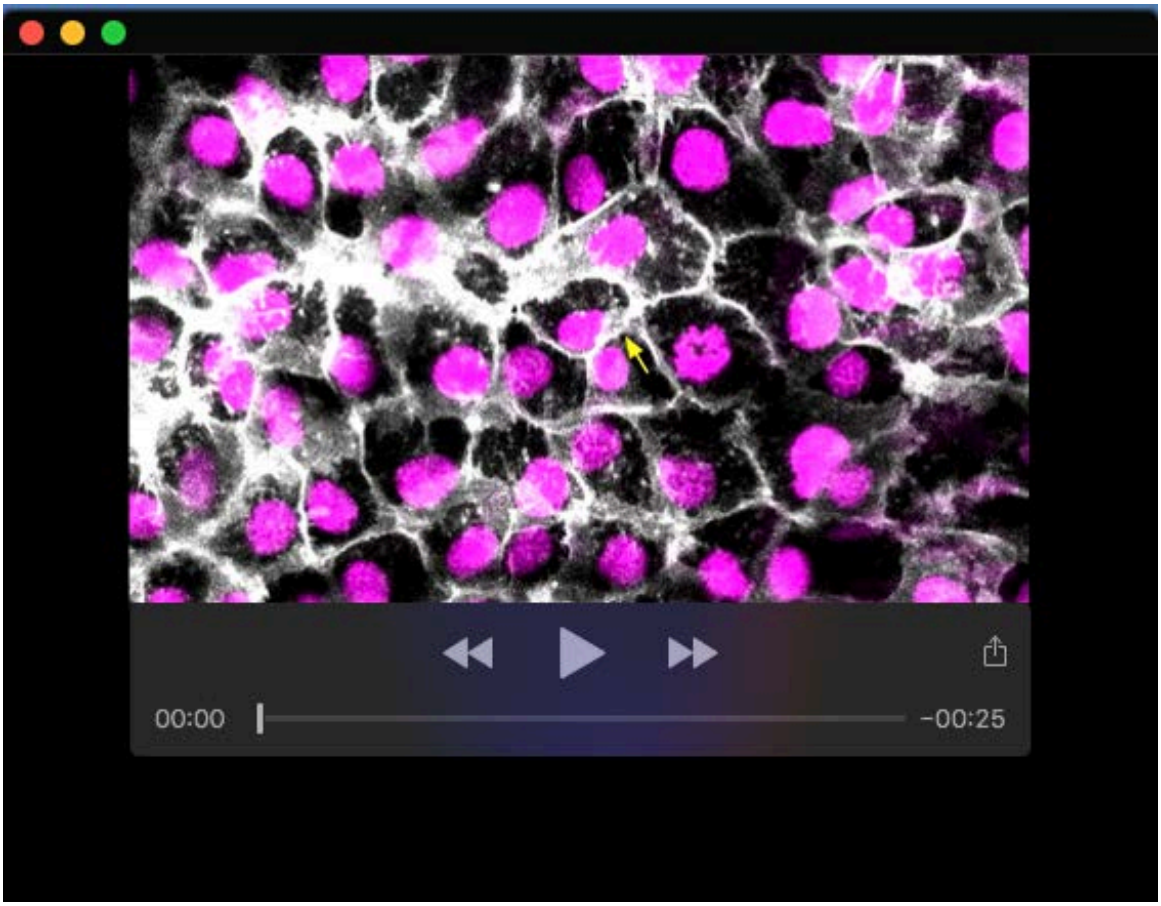
Confocal time-lapse experiment was performed on *Tg(sox17:memGFP)* embryos at 7-12s using Zeiss LSM880 confocal microscope with a LD C-Apo 40×/NA 1.1 water objective at 1.4 zoom. Images were acquired at 15 sec intervals and the movie plays at 4 frames/sec. Yellow arrows: lamellipodial like protrusions.



Movie 3. Junctional changes and polarized cellular protrusions occur in the same endodermal cells that are undergoing cell intercalations during 7-12s.
Donor cells from *Tg(sox17:H2A-Cherry)* embryos injected with *memGFP* and *sox32* RNAs were transplanted into *Tg(sox17: memCherry/H2A-Cherry)* host embryos. Time-lapse experiments were performed on the host embryos at 6s, in which the endoderm containing memGFP-expressing donor endoderm cells were imaged at 30s interval and movies play at 4 frames/sec. Yellow arrows: cells protrusions invading ML junctions that are shortening.



Movie 4. **Endodermal cells display junctional changes but not polarized cellular protrusions during endoderm C&E in *gpc4*$^{-/-}$ mutant embryos during 7-12s.** Confocal time-lapse experiment was performed on *gpc4*$^{-/-}$*/Tg(sox17:memGFP/H2A-mCherry)* embryos using the same settings with that in Movie 1 and the movie plays at 4 frames/sec. Labeling in this movie is the same as that of Movie 1.



Movie 5. **Endodermal cells extended lamellipodia in random directions in *gpc4*$^{-/-}$ embryos at 7-12s.**

Confocal time-lapse experiment was performed on *gpc4*$^{-/-}$*/Tg(sox17:memGFP)* embryos using the same settings with that in Movie 2 and the movie plays at 4 frames/sec. Labeling in this movie is the same as that of Movie 2.