SUPPLEMENTARY MATERIAL

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Immunohistochemistry and electron microscopy

Whole-mount immunohistochemistry was performed essentially as described (Bessho et al., 2003). Embryos were incubated with anti-chicken PAPC (1:8,000), anti-mouse PAPC (1:8,000) and anti-CDH2 (Sigma; 1:1,000) at 4°C for two days. Secondary antibodies conjugated either with HRP or AlexaFluor (Molecular probes) were used at 1:1,000. For cryosections (12μm) immunolabeling, working dilution of the anti-chicken PAPC was 1:2,000, anti-CDH2 was 1:300, anti-Clathrin (Cell signaling) was 1:300, anti-ZO-1 (Zymed) was 1:50 and anti-GFP (Abcam) was 1:1,000. Antibodies were incubated at 4°C overnight. F-Actin was detected with fluorescent Phalloidin (Thermo Fisher Scientific) used at 1:300 and incubated at 4°C overnight. Samples were then analyzed by confocal microscopy with a Zeiss LSM5 Pascal, LSM780 or Leica SP2. For electron microscopy analysis, explants composed of the PSM, last formed somite and the associated neural tube were dissected and fixed for 1 hour at room temperature in 4% paraformaldehyde (PFA) with 2.5% glutaraldehyde in 0.1 M PBS. Following fixation, tissue was prepared for ultrathin (60 nm) frontal sections and stained for EM analysis. For immunolabelling, PSM was fixed for 1 hour at room temperature in 2% paraformaldehyde with 0.01% glutaraldehyde in 0.1 M PBS, 80 nm sections were incubated with anti-PAPC (1:200) and secondary antibody conjugated to gold beads (10 nm) at room temperature for 1 hour (Amersham, Piscataway, NJ). Sections were post-stained in uranyl acetate. Analysis was performed on a FEI microscope at 80 kV.

PAPC-CDH2 Colocalization measurement

Signal intensity and distribution for CDH2, PAPC and F-Actin (Phalloidin) on immunostained parasagittal chicken embryo sections were analyzed both at the tissue level (PSM areas) and subcellular level (cell-cell junctions).

PSM area level colocalization analysis was performed in Image J using the Coloc2 plugin. Each PSM subdomains was divided in 5 areas of 19µm^2 and used for subsequent quantification (n=3 embryos, 15 squares per subdomains). For each PSM area, the signal intensity and distribution for CDH2, PAPC and F-Actin stainings were compared 2 by 2 and a Pearson's coefficient was calculated (ranging from -1 to 1, with 1 corresponding to a total positive correlation). The analysis was done at a 200nm resolution.

Junction level co-localization analysis was performed in Image J. Along each cell-cell junction (over 3µm in length, n=20 junctions), the mean junctional signal intensity was collected using Plot profile function (line width 3 pixels) in Image J. Each signal was then cross-correlated 2 by 2 using IGORPro software (macro from (Munjal et al., 2015)) which generate a Pearson's coefficient for each pixel. A peak at 0 micron means that both signals are co-localized. Statistical significance was assessed using Kolmogorov-Smirnov test.

Phenotype quantifications

The distribution of electroporated cells was quantified on confocal images of parasagittal sections of the last three formed somites. Data were collected for at least six embryos per condition, with two to three sections per embryo analyzed. R-C distribution**:** distribution of electroporated cells between the rostral versus the caudal halves of newly formed somites. The mesenchymal index was defined as the ratio between the mesenchymal versus epithelial fraction of the cells by direct scoring of cells' location and morphology. Epithelial cells were defined as cells within the epithelial ring and exhibiting centripetal polarization based on F-Actin, CDH2 and fluorescent reporter expressions. Mesenchymal cells were defined as non polarized

cells within the somitocoele, the epithelial ring, and cells outside the ring structure when present. Statistical significance was assessed using Student's t-test.

For the cell-cell connectivity index, the anterior PSM subdomains S-I and S0 were first identified at low magnification by the overall tissue morphology and the presence of a forming acellular fissure. Then four high magnification 200µm^2 micrographs for each subdomains were acquired. Individual cell-cell contacts (number and length) were quantified using Image J. The tissue cell-cell connectivity index was defined as the

average length of cell-cell contact per cell, data are represented normalized to control S0 caudal domain value, fixed at 100. Electroporated embryos were processed in parallel. Two embryos per condition were analyzed. Statistical significance was assessed using Student's t-test.

Endocytosis assays

Chicken embryos were electroporated with pCImG-PAPC-S or pCImG at stage 5HH then cultured on filter paper on agar/albumen plate (Chapman et al., 2001) for 24 hours. After 24 hours, embryos were treated for 20 min with a single 10µL drop of DMSO or of the clathrin-mediated endocytosis inhibitor Pitstop2 at 30 µM (Abcam, ab120687) deposited on the embryo's ventral side. Next, a sagittal slit was generated within the PSM using a tungsten needle and embryos were incubated with Dextran for 7 min at 37C (2µl of a 1mg/mL Dextran -PBS solution; 10,000MW, conjugated with Alexa Fluor 647; Molecular probes). Next, embryos were washed in cold PBS for 2 min at 4C and fixed in PFA 4% overnight for further immuno-staining processing. Dextran uptake by electroporated cells was measured as the intensity of the retained Dextran fluorescent signal after washes of the treated embryos. Number of embryos analyzed per conditions: pCImG/DMSO n=2, pCImG/Pitsop2 n=4, PAPC-S/DMSO n=7, PAPC-S/Pitstop2 n=6. For each embryos, electroporated PSM was subdivided in ~5 regions and corresponding Dextran signal intensity in GFP positive versus GFP negative cells was measured using Image J (>110 cells per conditions). The

corresponding CDH2 signal (detected with the anti-CDH2 antibody) and mGFP signal (detected with the anti-GFP antibody) intensities were also acquired. For Chlorpromazine treatment, bisected posterior embryo explants were cultured for 3-5 hours as described (Delfini et al., 2005), left side treated with DMSO (control) and right side with Chlorpromazine at 50 µM (Sigma). Three embryos per conditions were analyzed.

SUPPLEMENTARY FIGURES
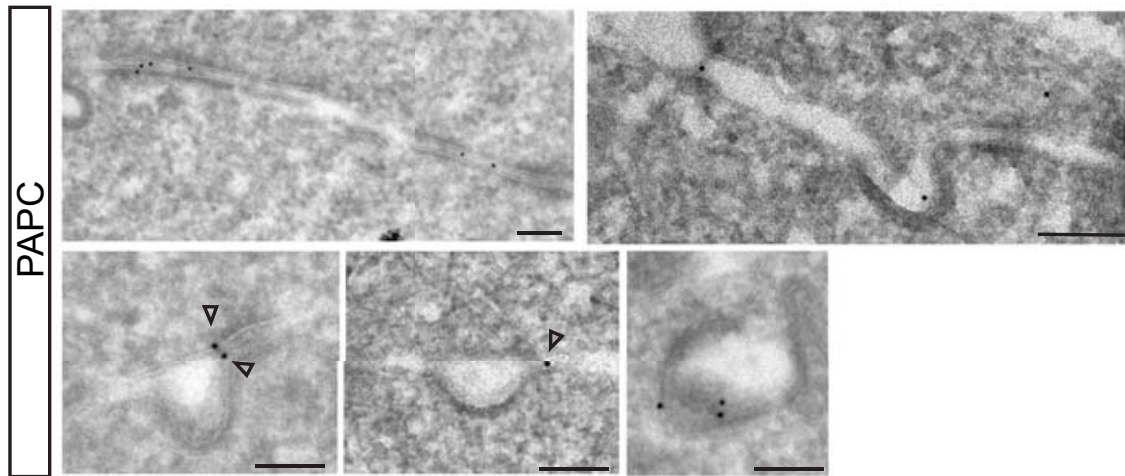
Figure S1: Immunogold localization of PAPC by electron microscopy of the PSM

Subcellular localization of PAPC in anterior PSM cells by IEM. PAPC can be found (gold beads, black dots) specifically at cell-cell junctions and sites of membrane trafficking including clathrin - coated pits and endocytosis vesicles. Arrowheads mark PAPC specific distribution at the rim of the clathrin coated pits. Scale bar, 200nm.
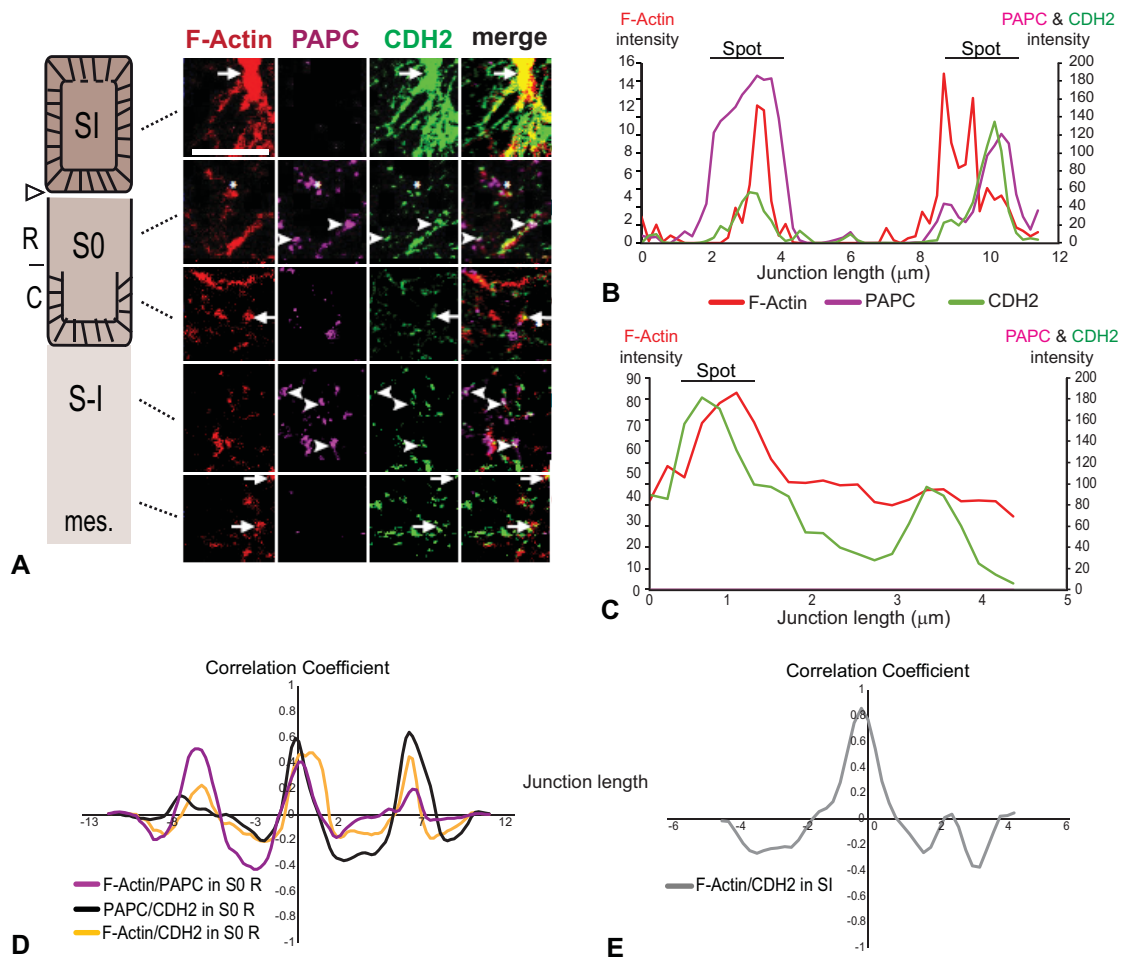
Figure S2: Tissular and junctional distribution of PAPC and CDH2 in the PSM

(A) (Left) Diagram of the PSM subdomains. S-I/0/I:somite -I/0/I; R: rostral half; C: caudal half; mes.: posterior PSM mesenchyme. (Right) Representative confocal sections for each subdomains as shown on the diagram (SI, top row; mes., bottom row), after co-staining for F-Actin (Phalloidin, red), PAPC (purple), CDH2 (green). Note that PAPC is detected only in PSM subdomains S-I and S0. Each image shows a representative image of the 19µm^2 areas used for the quantification shown in Fig. 3F. Arrows: F-Actin and CDH2 co-localization; Arrowheads: PAPC and CDH2 co-localization. Scale bar, 4µm.

(B, C) Cell-cell junction co-localization analysis. Plots showing representative individual signal intensity and distribution of CDH2, PAPC and F-Actin (Phalloidin) on confocal sections along an individual cell-cell junction in the rostral half of Somite 0 (B) or Somite I (C). Note the absence of PAPC in the SI domain. These intensity profiles where used to cross-correlate the signal using IgorPro software as quantified in Fig. 3G (see Material and Methods for details).

(D-E) Cell-cell junction co-localization analysis. Plots showing representative individual cross-correlation signals obtained from the Somite 0 rostral half and Somite I (in D and E) respectively . The pearson coefficient is represented over the junction length indicating how much and where the signals are the most similar. A maximum at 0µm means that most of the signal in both channels co-localize, at a 200nm resolution.
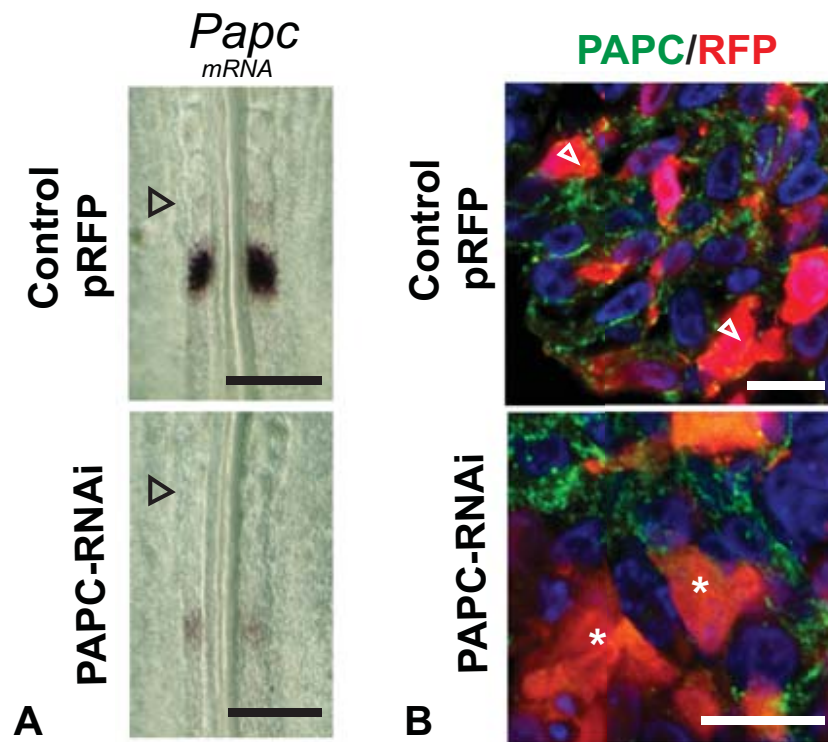
Figure S3: Validation of PAPC-Rnai contruct activity

(A) Expression of *PAPC* in pRFP control (upper panel) and PAPC-RNAi (lower panel) electroporated two-day-old chicken embryos. *PAPC* mRNA is detected by *in situ* hybridization (n=3 per condition). Arrowheads mark the last formed boundary. Dorsal view, anterior to the top. Scale bar, 200μm.

(B) Expression of PAPC protein (green) in the anterior, PAPC-positive, PSM, electroporated with the control vector pRFP or PAPC-RNAi construct (red). Nuclei are stained with Dapi (blue). Cells expressing control vector are dispersed and express PAPC (arrowheads), conversely, cells expressing PAPC-RNAi (asterisks) are clustered and lack PAPC expression relative to neighbours (n=3 per condition). Scale bar, 10μm.
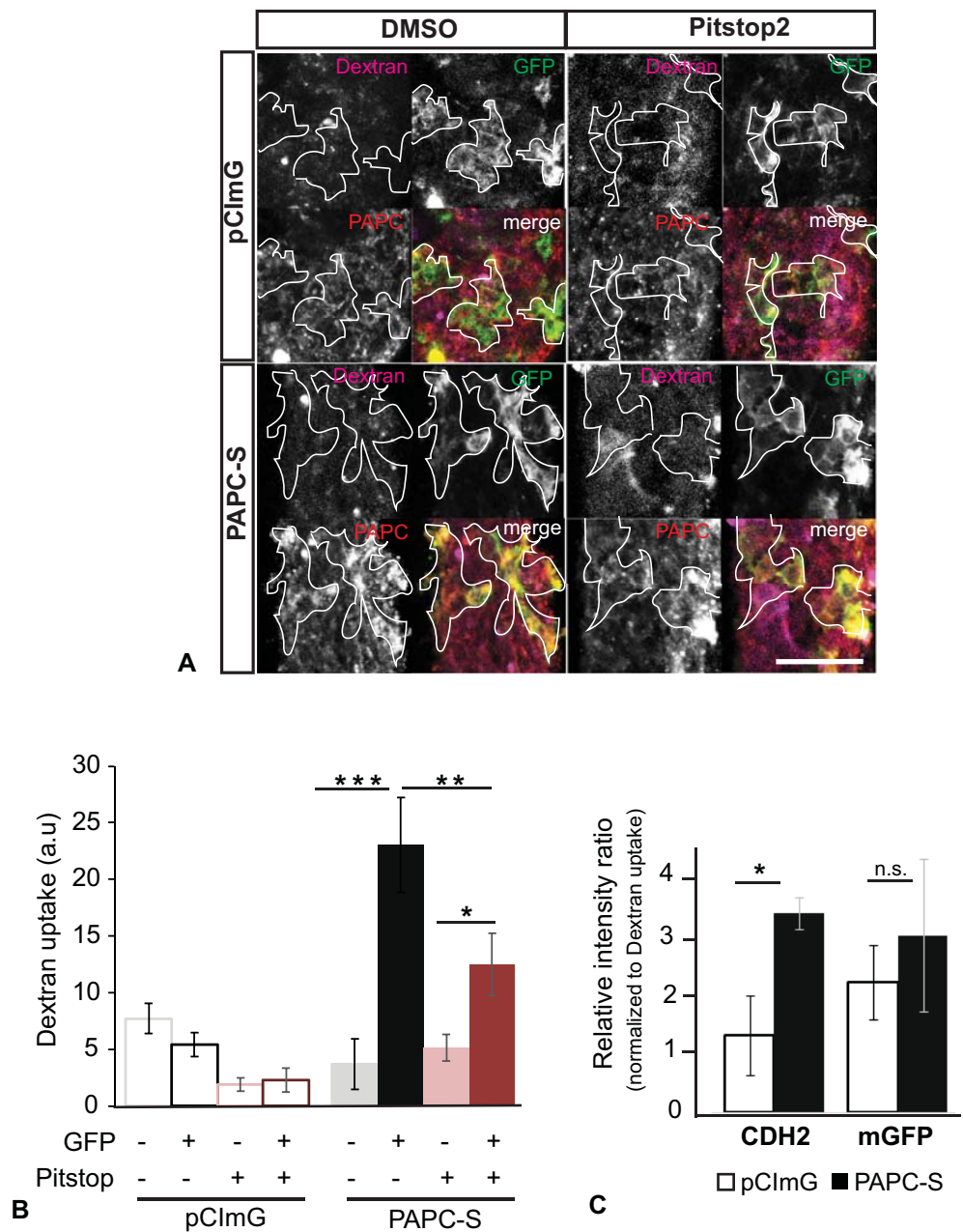
Figure S4: PAPC-dependent endocytosis and Dextran uptake assay

(A) Representative confocal images of the cellular localization of GFP (green), PAPC (magenta), and Dextran (red) in embryos electroporated with pCImG control vector or PAPC-S construct and subsequently treated with either DMSO (left) or the clathrin-mediated endocytosis inhibitor Pitstop2 (right). Regions of interest with electroporated GFP+ cells are delimited by a solid white line. Scale bar, 15μm.

(B) Quantification of Dextran uptake as a measure of endocytosis level. Signal intensity of fluorescent Dextran was measured in cells treated as described in (A). Dextran signal intensity in GFP+ and GFP- cells in pCImG or PAPC-S electroporated regions and subsequently treated with DMSO (control) or Pitstop2 (red). Number of embryos analyzed per condition: pCImG/DMSO n=2; pCImG/Pitsop2 n=4; PAPC-S/DMSO n=7; and PAPC-S/Pitstop2 n=6. Mean +/- s.d. t-test *$p<0.05$; ** $p<0.005$; ***$p<0.0005$ **(C)** Comparison of CDH2 and overall membrane trafficking (GFP) in cells electroporated with control pCImG or PAPC-S constructs. Signal intensities were normalized to Dextran signal as a proxy for endocytosis activity. Note selective CDH2 signal increased over mGFP in PAPC-S overexpressing cells (>110 cells per conditions). * $p<0.05$; n.s. $p>0.05$
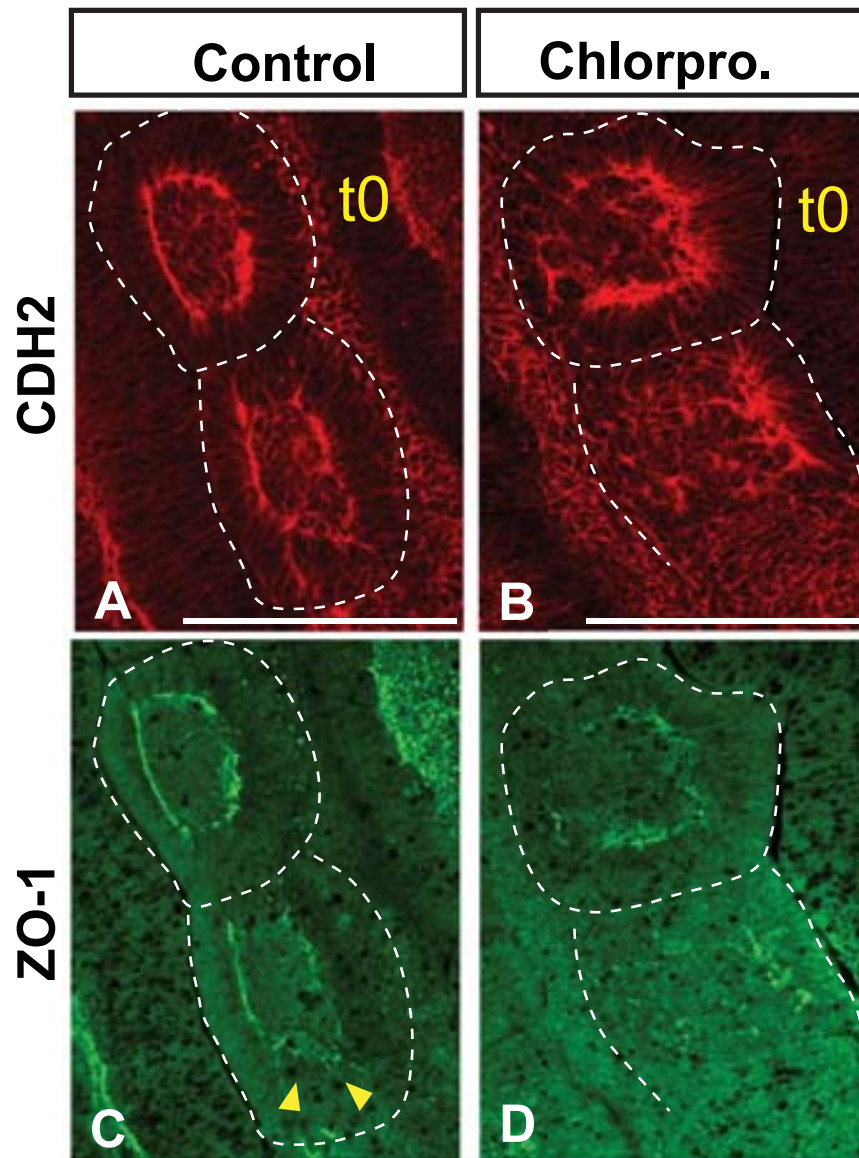
Figure S5: Inhibition of endocytosis blocks PSM segmentation

 (A-D) CDH2 and ZO-1 distribution by immunofluorescence in chicken PSM explants cultured 3 hours in the presence of DMSO (0.2%) (A,C) and Chlorpromazine (50µM) (B,D) (n=3 per condition). Dorsal view, anterior to the top. t0: forming somite at the time of treatment start. Somite limits are highlighted by dashed white lines. Yellow arrowheads show posterior epithelial wall assembly. Dorsal views, anterior to the top. Scale bar, 100µm.