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Supplemental information

A hydraulic feedback loop between mesendoderm cell migration and interstitial fluid relocalization promotes embryonic axis formation in zebrafish

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Supplementary Figure 1

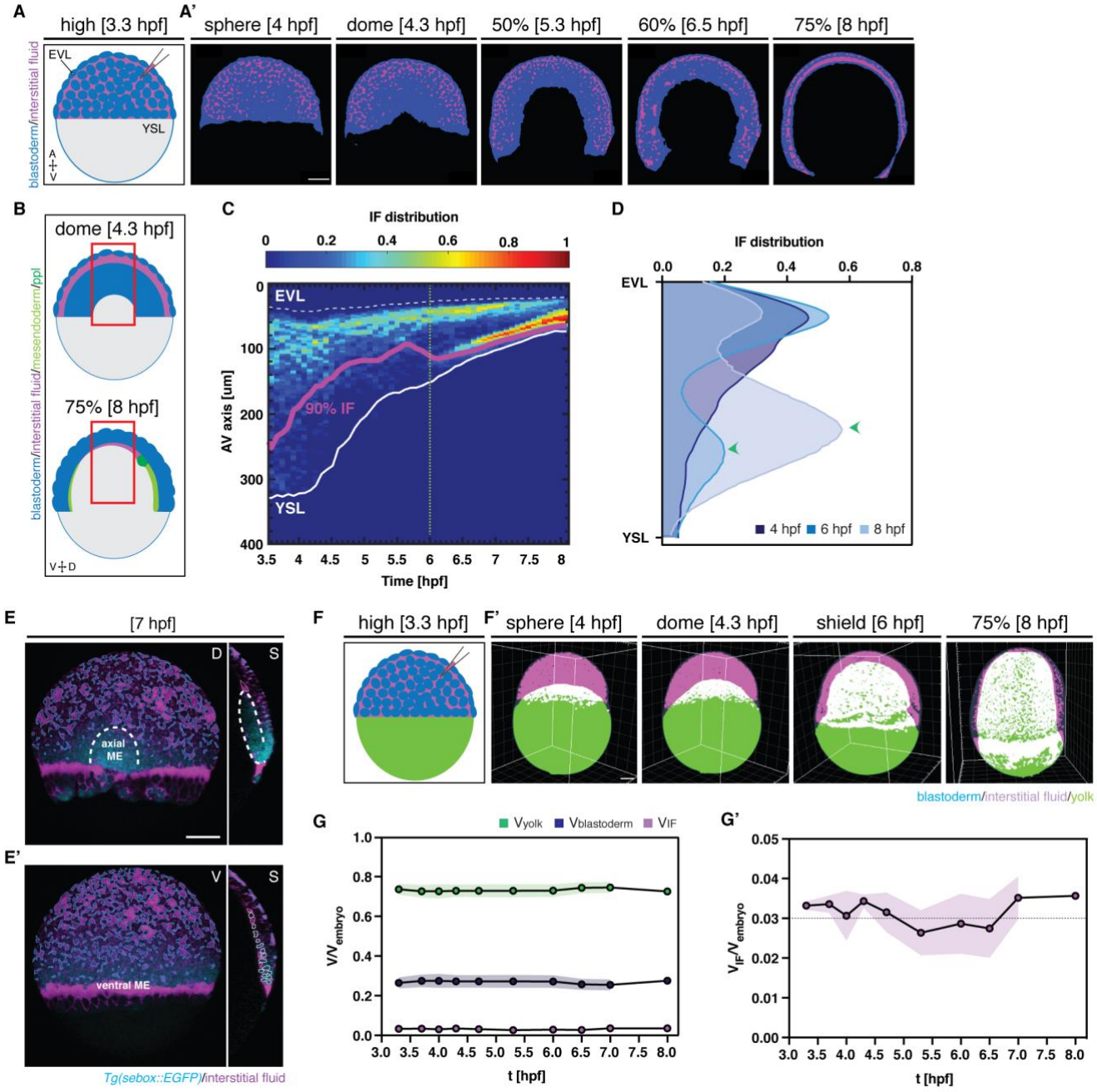

Figure S1. Interstitial fluid undergoes coordinated relocalisation during gastrulation - Related to Figure 1. (**A**) Schematic illustrating interstitial fluid (IF) labeling by injection of fluorescent dextran (purple) at 3.3 hours post-fertilization (hpf); AV, animal-vegetal axis; EVL, enveloping layer; YSL, yolk syncytial layer. (**A'**) Blastoderm and IF binary masks from 4 to 8 hpf; scale bar, 100 µm. (**B**) Schematic illustrating coordinated IF reorganization at 4.3 and 8 hpf; rectangle demarcates region of analysis in (**C,D**); DV, dorsoventral axis. (**C**) Representative kymograph of IF distribution along the AV axis of the blastoderm, with the position of EVL at the blastoderm surface and YSL at the blastoderm inside marked by dashed white and full white lines, respectively, as a function of developmental time; IF distribution is color-coded; the 90% IF line (magenta) marks the area along the AV axis of the blastoderm (from the line to the EVL on the blastoderm surface) containing 90% of the IF at a given time point; the vertical line at 6 hpf (dashed, green) marks the onset of

mesendoderm internalization at the germ ring margin. (**D**) Average IF distribution within the blastoderm along the AV axis at 4, 6 and 8 hpf (N=8); arrowheads (green) indicate the emergence of a second peak of IF accumulation at the YSL-deep cell (DC) boundary. (**E,E'**) Maximum intensity projections (dorsal, D (**E**) and ventral, V (**E'**) views) and single cross-sections (sagittal views, S) of *sebox::EGFP* transgenic embryos expressing EGFP within the pan-mesendoderm (ME) and injected with fluorescent dextran to mark IF; dashed white lines outline the axial ME (**E**) and ventral ME (**E'**); dashed blue lines outline the IF at the EVL-DC boundary; dashed pink lines outline the IF at the YSL-DC boundary (sagittal view, S); scale bar, 100 µm. (**F**) Schematic illustrating the blastoderm, yolk cell and IF labeling for selective plane illumination microscopy (SPIM). (**F'**) 3D reconstitution images of dual-illumination, multi-view SPIM from 4 to 8 hpf; scale bar, 100 µm. (**G**) Quantification of yolk cell (green), blastoderm (blue), and IF volume (magenta) normalised to the total embryo volume from 3 to 8 hpf; mean ± SEM. (**G'**) Quantification of the normalised IF volume from 3 to 8 hpf; N=4; mean ± SEM. N, number of independent embryo replicates.

Supplementary Figure 2

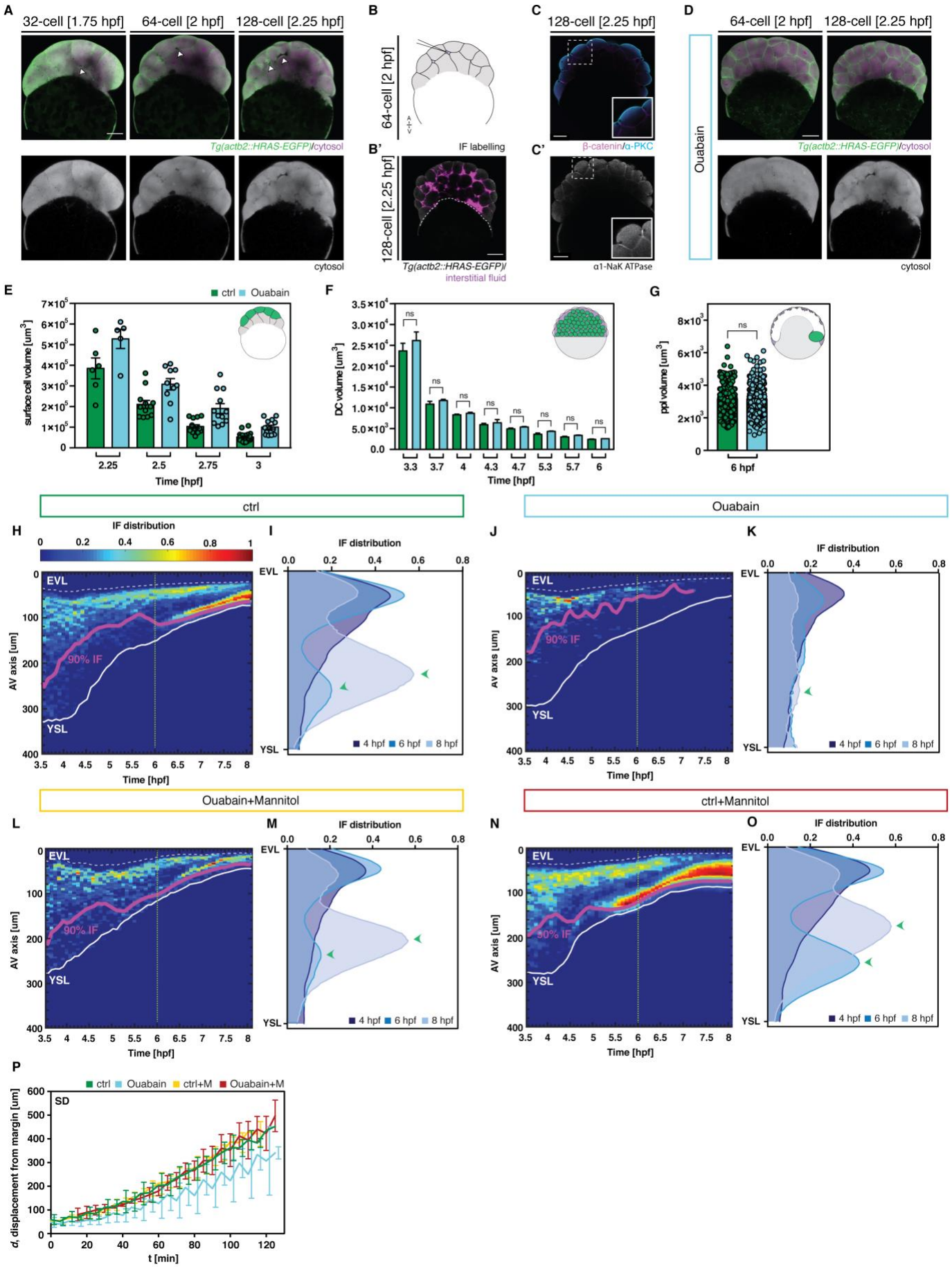

Figure S2. Interstitial fluid accumulation requires Na^+/K^+-ATPase activity - Related to Figure 1. (**A**) Representative confocal cross-section images of *actb2::HRAS-EGFP* transgenic embryos expressing EGFP to mark the plasma membrane and injected with fluorescent dextran to label the cytosol from 1.75 to 2.5 hours post fertilization (hpf); EGF and dextran labeling, upper row; dextran labeling only, lower row; arrowheads point at the first emerging interstitial spaces; scale bar, 100 µm. (**B**) Schematic illustrating interstitial fluid (IF) labeling by injection of fluorescent dextran at 2 hpf; AV, animal-vegetal axis. (**B'**) Representative confocal cross-section image of *actb2::HRAS-EGFP* transgenic embryos expressing EGFP to mark the plasma membrane and injected with fluorescent dextran to label the IF at 2.25 hpf; white dashed line outlines the blastoderm-yolk cell boundary; scale bar, 100 µm. (**C,C'**) Representative confocal cross-section images of ß-catenin and α-PKC (**C**), and α_1-Na^+/K^+-ATPase (**C'**) localisation detected by immunohistochemistry at 2.25 hpf; insets are high-magnification images of apical α-PKC and basolateral ß-catenin localisation (**C**), and basolateral α_1-Na^+/K^+-ATPase localisation (**C'**) in surface blastomeres; scale bar, 100 µm. (**D**) Confocal cross-section images of Ouabain-treated *actb2::HRAS-EGFP* transgenic embryos expressing EGFP to mark the plasma membrane and injected with fluorescent dextran to label the cytosol at 2 and 2.5 hpf when the first IF pockets emerge in control embryos; EGFP and dextran labeling, upper row; dextran labeling only, lower row; scale bar, 100 µm. (**E-G**) Surface cell (**E**; Control (N=1), Ouabain (N=2)), deep cell (**F**; DC; Control at 3.3 hpf (N=3), 3.7 hpf (N=3), 4 hpf (N=7), 4.3 hpf (N=6), 4.7 hpf (N=5), 5.3 hpf (N=2), 5.7 hpf (N=2), 6 hpf (N=2)) and *gsc*-expressing (prechordal plate, ppl) cell (**G**; Control (N=4), Ouabain (N=4)) volume quantification in control and Ouabain-treated embryos; individually plotted values represent volume quantifications of each individual cell; ns, not significant; mean ± SEM; Mann-Whitney test. (**H,J,L,N**) Representative kymographs of IF distribution (marked by fluorescent dextran injection) along the AV axis as a function of developmental time in control (**H;** from FigureS1; N=8), Ouabain-treated (**J**; N=8), Ouabain-treated and Mannitol-injected (**L**; N=6), and Mannitol-injected embryos (**N**; N=9). (**I,K,M,O**) Corresponding average IF distribution within the blastoderm along the AV axis at 4, 6 and 8 hpf; arrowheads (green) indicate the emergence of a second peak of IF accumulation at the yolk syncytial layer (YSL)-DC boundary. (**P**) Distance of the ppl leading edge to the germ ring margin from the onset of internalisation (6 hpf, corresponding to 0') for control (green, N=9), Ouabain-treated (blue, N=9), Ouabain-treated and Mannitol-injected (yellow, N=6), and Mannitol-injected embryos (red, N=4); mean ± SD. N, number of independent embryo replicates.

Supplementary Figure 3

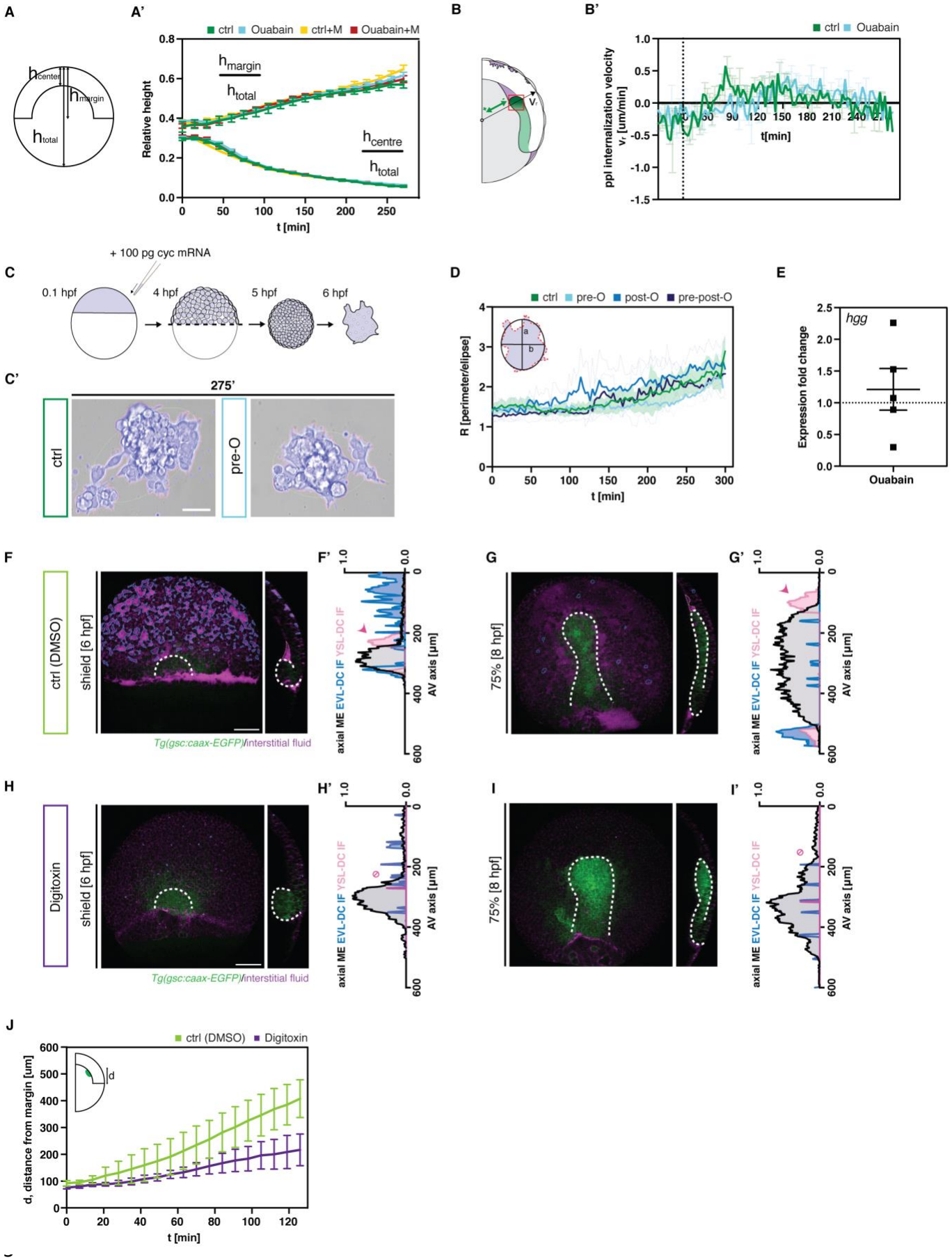

Figure S3. Na^+/K^+-ATPase activity non-cell-autonomously affects prechordal plate cell migration but is not required for cell-autonomous cell protrusion formation and fate specification - Related to Figures 1 and 2. (**A,A'**) Quantification schematic (**A**) and results (**A'**) of blastoderm thinning (h_{center}/h_{total}) and spreading (h_{margin}/h_{total}) in control (green; N=4), Ouabain-treated (blue; N=6), Ouabain-treated and Mannitol-injected (yellow; N=7), and Mannitol-injected embryos (red; N=6); mean ± SEM. (**B,B'**) Quantification schematic (**B**) and results (**B'**) of radial-directed velocities of individual ppl cells in control (green, N=5) and Ouabain-treated embryos (blue, N=4). (**C**) Schematic illustrating the *in vitro* cell spreading assay for ppl cells obtained from *gsc::caax-EGFP* transgenic embryos injected with *cyc* mRNA (100 pg); small cell clusters were seeded on fibronectin (FN) coated glass coverslips. (**C'**) Representative bright-field images of ppl cell clusters undergoing cell spreading *in vitro* explanted from control (green) and Ouabain-pre-treated (light blue) *gsc::caax-EGFP* transgenic embryos injected with *cyc* mRNA (100 pg) after 275' in culture; ppl cell cluster perimeter (red) was measured and an ellipse (green) was fitted to the analysed area (purple); scale bar, 50 µm. (**D**) Quantification (normalised cluster perimeter to fitted ellipse) of ppl cell cluster spreading explanted from control (green, N=9), Ouabain-pre-treated (embryos treated 1-3 hpf; light blue, N=5), Ouabain-post-treated (treated at 6 hpf by supplying 1 mM Ouabain to the cell culture medium; blue, N=3) and Ouabain-pre- and post-treated (embryos treated 1-3 hpf and addition of 1 mM Ouabain to the cell culture medium; dark blue, N=4) embryos; mean ± SEM. (**E**) Expression changes of *hatching gland* (*hgg*) gene, as a marker of ppl cell fate specification, in Ouabain-treated embryos normalised to the expression level of a housekeeping gene (*elongation factor 1a*). Fold change reflects the relative change of expression levels in Ouabain-treated compared to control embryos in qRT-PCR; dotted black line indicates 1-fold change in expression; N = 5; data are mean ± SEM; Mann-Whitney test; p=0,6825 (ns, not significant). (**F-I**) Maximum intensity projections (dorsal view, D) and single cross-sections (sagittal view, S) of *gsc::caax-EGFP* transgenic embryos expressing EGFP within the axial ME and injected with fluorescent dextran to mark IF in DMSO control (**F,G**) and Digitoxin-treated (**H,I**) embryos at 6 hpf (**F,H**) and 8 hpf (**G,I**); dashed white lines outline the prechordal plate (ppl) and, due to the leaky nature of this transgenic reporter line, also the posterior axial ME (pam); dashed blue lines outline the IF at the EVL-DC boundary; dashed pink lines outline the IF at the yolk syncytial layer (YSL)-deep cell (DC) boundary (sagittal view, S); scale bar, 100 µm. (**F',G',H',I'**) IF distribution profiles along the animal-vegetal (AV) axis relative to the position of ppl/pam, marked by *gsc::caax-EGFP* expression (black lines), in DMSO control (**F',G'**) and Digitoxin-treated (**H',I'**) embryos at 6 hpf (**F',H'**) and 8 hpf (**G',I'**); multi-color curves represent average values of N=3 independent embryo replicates of the position of ppl/pam (black lines) and IF distribution at the enveloping layer (EVL)-DC (blue lines) and YSL-DC (pink lines) boundarys; arrowheads (pink) indicate the IF accumulation ahead of the ppl. (**J**) Distance of the ppl leading edge to the germ margin from the onset of internalisation (6 hpf, corresponding to 0') for DMSO control (green, N=6) and Digitoxin-treated (purple, N=5) embryos; mean ± SD. N, number of independent embryo replicates.

Supplementary Figure 4

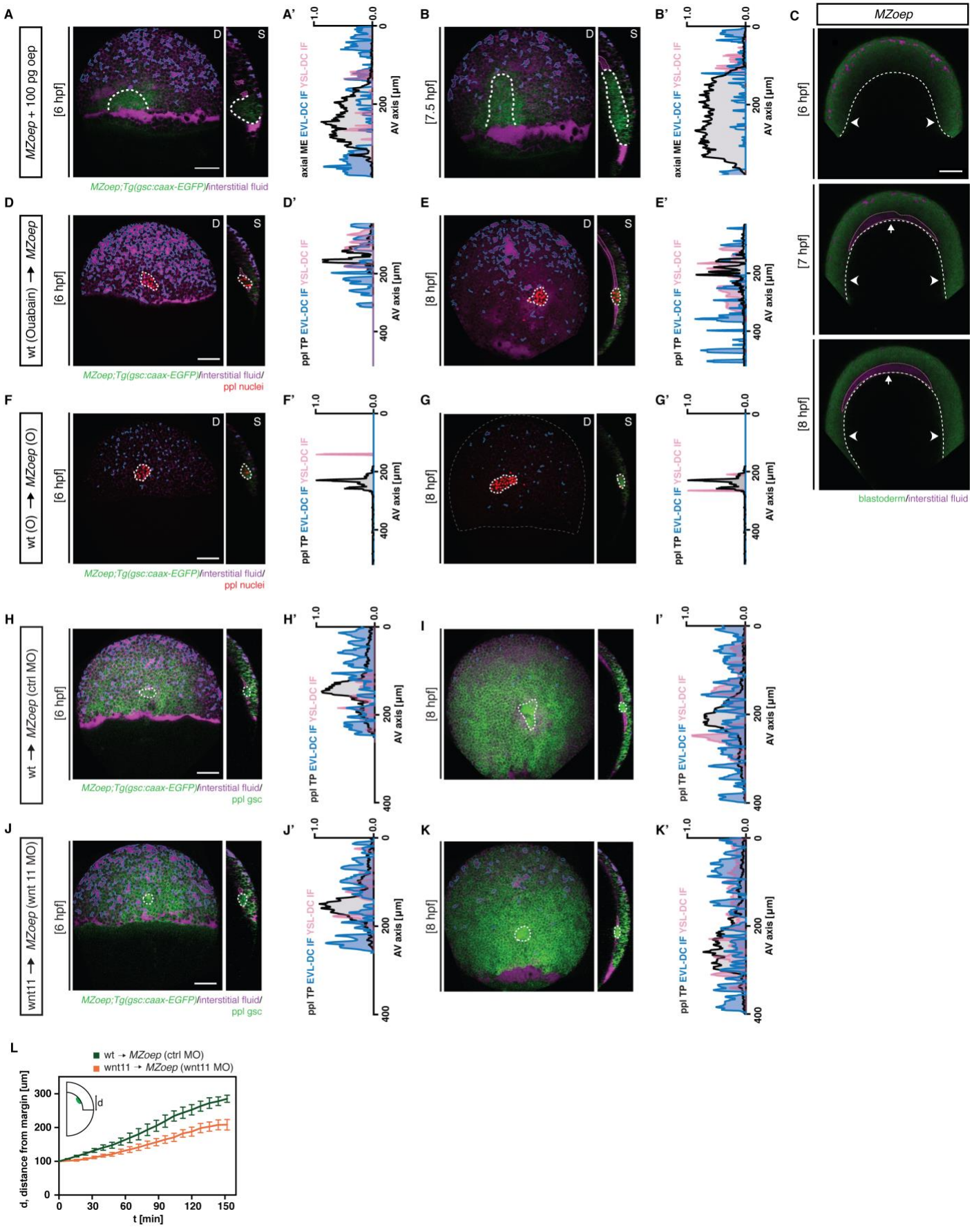

Figure S4. Prechordal plate migration and interstitial fluid relocalisation are interdependent - Related to Figures 3 and 4. (**A,B**) Maximum intensity projections (dorsal view, D) and single cross-sections (sagittal view, S) of *MZoep;gsc::caax-EGFP* mutant/transgenic embryos injected with *oep* mRNA (100 pg), expressing EGFP within the prechordal plate (ppl)/posterior axial mesendoderm (pam) at 6 (**A**) and 7.5 hours post fertilization (hpf) (**B**); for control embryos see Fig. 1B,C; dashed white lines outline the ppl/pam (dorsal view, D); dashed blue line outlines the interstitial fluid (IF, marked by fluorescent dextran injection) at the enveloping layer (EVL)-deep cell (DC) boundary; dashed pink line outlines the IF at the yolk syncytial layer (YSL)-DC boundary (sagittal view, S); scale bar, 100 µm. (**A',B'**) IF distribution profiles along the animal-vegetal (AV) axis relative to the position of ppl/pam, as indicated by *gsc::caax-EGFP* expression in *MZoep* mutant embryos injected with *oep* mRNA (100 pg) at 6 (**A'**) and 7.5 hpf (**B'**); multi-color curves represent average values of N=3 independent embryo replicates of the position of ppl/pam (black lines) and IF distribution at the EVL-DC (blue lines) and YSL-DC (pink lines) boundarys. (**C**) Fluorescence images of single cross-sections (sagittal view) of an *MZoep* mutant embryo injected with fluorescent dextran to label the cytosol (FITC) and IF (Alexa647) from 6 to 8 hpf; white dashed line outlines the boundary between the YSL and the blastoderm; IF accumulates at the YSL-DC boundary close to the animal pole (arrows, pink lines, 7-8 hpf), but remains absent from the YSL-DC boundary near the germ ring margin (arrowheads); scale bar, 100 µm. (**D-G**) Maximum intensity projections (dorsal view, D) and single cross-sections (sagittal view, S) of control (**D,E**) and Ouabain-treated (**F,G**) *MZoep;gsc::caax-EGFP* mutant/transgenic host embryos containing Ouabain-treated ppl transplants at 6 (**D,F**) and 8 hpf (**E,G**). (**H-K**) Maximum intensity projections (dorsal view, D) and single cross-sections (sagittal view, S) of control *morpholino*-injected (containing wild type ppl transplants) (**H,I**) and *wnt11f2 morpholino*-injected (containing *slb/wnt11f2* mutant ppl transplants) (**J,K**) *MZoep;gsc::caax-EGFP* mutant/transgenic host embryos; dashed white lines outline the ppl transplant (dorsal view, D) at 6 (**H,J**) and 8 hpf (**I,K**); dashed blue line outlines the IF at the EVL-DC boundary; dashed pink line outlines the IF at the YSL-DC boundary (sagittal view, S); scale bar, 100 µm. (**D'-K'**) IF distribution profiles along the AV axis relative to the position of the ppl transplant, as indicated by *gsc::caax-EGFP* expression, in control (containing Ouabain-treated ppl transplants) (**D',E'**), Ouabain-treated (containing Ouabain-treated ppl transplants) (**F',G'**), control *morpholino*-injected (containing wild type ppl transplants) (**H',I'**), and *wnt11f2 morpholino*-injected (containing *slb/wnt11f2* mutant ppl transplants) (**J',K'**) *MZoep;gsc::caax-EGFP* mutant/transgenic host embryos at 6 (**D',F',H',J')**) and 8 hpf (**E',G',I',K'**); multi-color curves represent average values of N=3 independent embryo replicates of the position of ppl/pam (black lines) and IF distribution at the EVL-DC (blue lines) and YSL-DC (pink lines) boundarys. (**L**) Distance of the ppl leading edge to the germ ring margin as a function of time from the onset of internalisation (6 hpf, corresponding to 0') for control *morpholino*-injected (containing wild type ppl transplants) (green, N=5) and *wnt11f2 morpholino*-injected (containing *slb/wnt11f2* mutant ppl transplants) (orange, N=7) *MZoep;gsc::caax-EGFP* mutant/transgenic host embryos; mean ± SD. N, number of independent embryo replicates.

Supplementary Figure 5

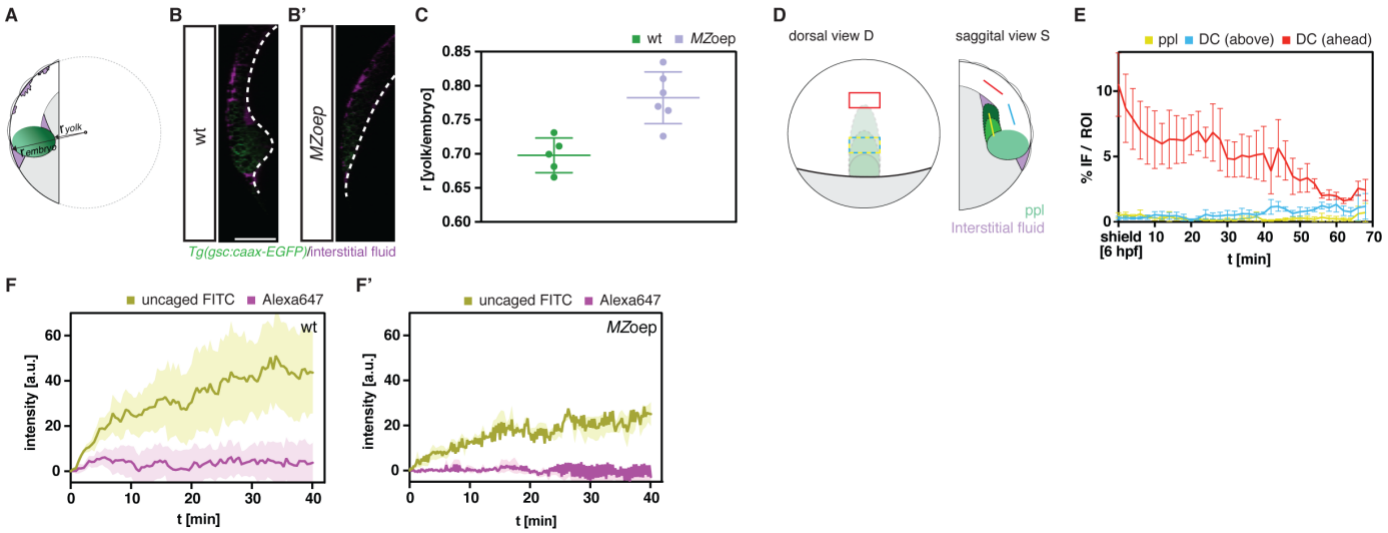

Figure S5. Yolk cell indentation, deep cell tissue packing, and interstitial fluid relocalization analysis - Related to Figure 5. (**A**) Schematic of yolk cell indentation analysis; r, radius (yolk, embryo). (**B,B'**) Single cross-sections (sagittal view) of wild type (wt) *gsc::caax-EGFP* (**B**) and *MZoep;gsc::caax-EGFP* mutant/transgenic embryos (**B'**) injected with fluorescent dextran to mark the interstitial fluid (IF) at 6 hpf; dashed white lines outline the yolk cell shape; scale bar, 100 µm. (**C**) Quantification of yolk cell indentation by normalising the yolk cell radius to the whole embryo radius in wt *gsc::caax-EGFP* (green, N=5) and *MZ*oep;*gsc::caax-EGFP* (lilac, N=6) mutant/transgenic embryos; mean ± SD. (**D**) Schematic of deep cell (DC) tissue packing analysis by measuring interstitial fluid fraction (% IF) within the tissue during prechordal plate (ppl) cell migration; dorsal, D and sagittal, S views. (**E**) Quantification of % IF as a function of developmental time in the deep cell tissue ahead (red) and above (blue) the ppl, and within the ppl itself (yellow); box size = 4000 µm^2; 0' corresponds to 6 hpf (shield stage); N=3; mean ± SEM. (**F,F'**) Quantification of uncaged FITC and Alexa647 intensities at the yolk syncytial layer (YSL)-deep cell (DC) boundary in wt (N=4) (**F**) and *MZoep* mutant (N=4) (**F'**) embryos as a function of time after the onset of internalization (6 hpf; 0'); mean ± SEM. N, number of independent embryo replicates.

Supplementary Figure 6

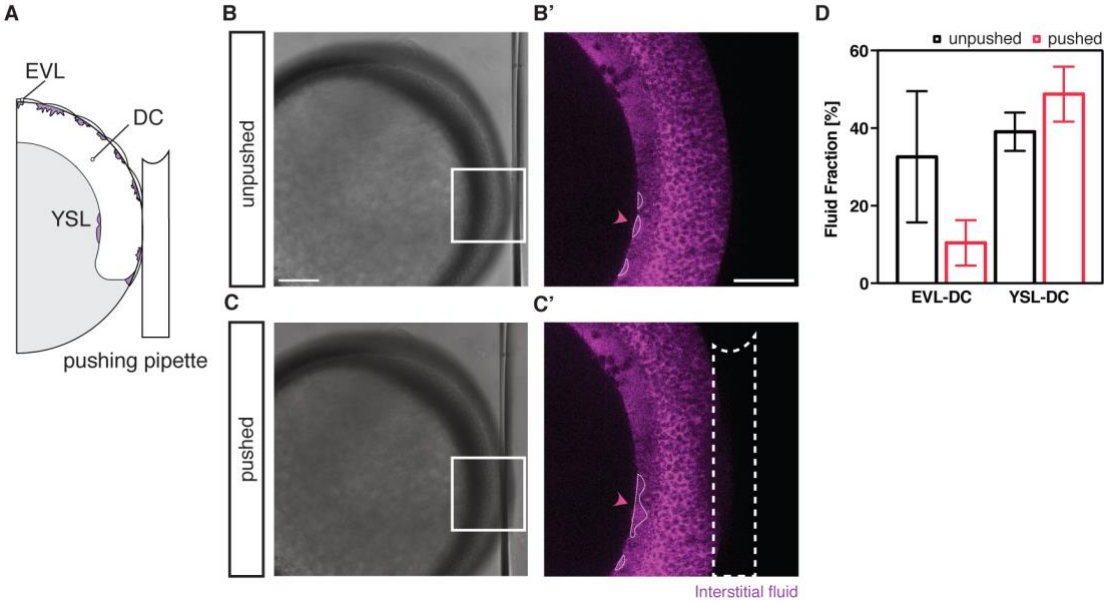

Figure S6. Mechanical pushing onto the EVL triggers interstitial fluid relocalisation - Related to Figure 6. (**A**) Schematic of interstitial fluid (IF) relocalisation from the enveloping layer (EVL)-deep cell (DC) boundary to the yolk syncytial layer (YSL)-DC boundary of an *MZoep* mutant embryo by a mechanical push onto the enveloping layer (EVL) with a glass micropipette. (**B-C'**) Brigthfield (**B,C**) and maximum intensity projection images of the deep cell layer injected with fluorescent dextran (purple) to mark IF (**B',C'**) showing the embryo before (**B,B'**) and after (**C,C'**) the micropipette push; white squares highlight the area of micropipette contact with the EVL; white lines and pink arrowheads outline IF accumulations at the YSL-DC boundary; dashed rectangle indicates the position of the micropipette; scale bar, 100 µm. (**D**) Quantification of IF fraction (%) at the EVL-deep cell and YSL-deep cell boundary in control and pushed embryos; N=3; mean ± SD. N, number of embryo replicates.