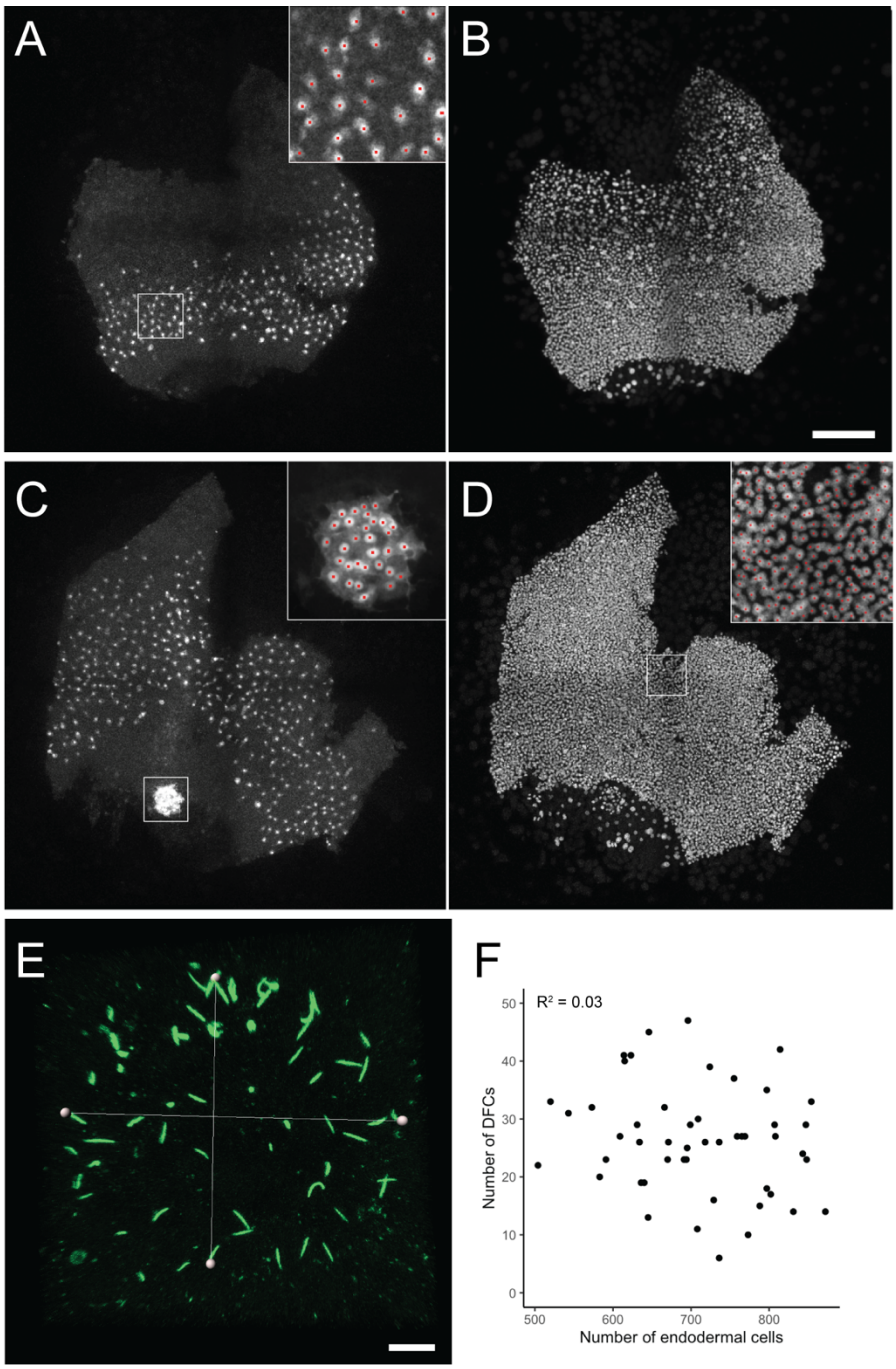

Figure S1. Quantification of endodermal cells, DFCs, total cell number and KV cilia number on flat mount preparations. (A-D) Confocal z-projections of a Tg[*sox17*:GFP] flat-mounted embryo at 75% epiboly stage. (A-B) dorsal side; (C-D) ventral side; (A-C) signal from an anti-GFP immunodetection coupled with a secondary antibody labeled with Alexa-568; (B-D) signal from nuclei staining with Hoechst. (A-C) Magnified regions show examples of cell quantification (red dots) for DFCs (taken separately, see Materials and Methods),

endodermal cells and total cell number, respectively. Scale bar = 200 μm. (E) Confocal z-projection of an 8-somite embryo's KV. The green signal shows individual cilia detected with anti-acetylated Tubulin (ac-Tub). The white lines indicate the two diameters used to calculate the area of the KV lumen as an ellipse. Scale bar = 10 μm. (F) Number of DFCs and endodermal cells detected per embryo, together with the coefficient of determination (R^2).

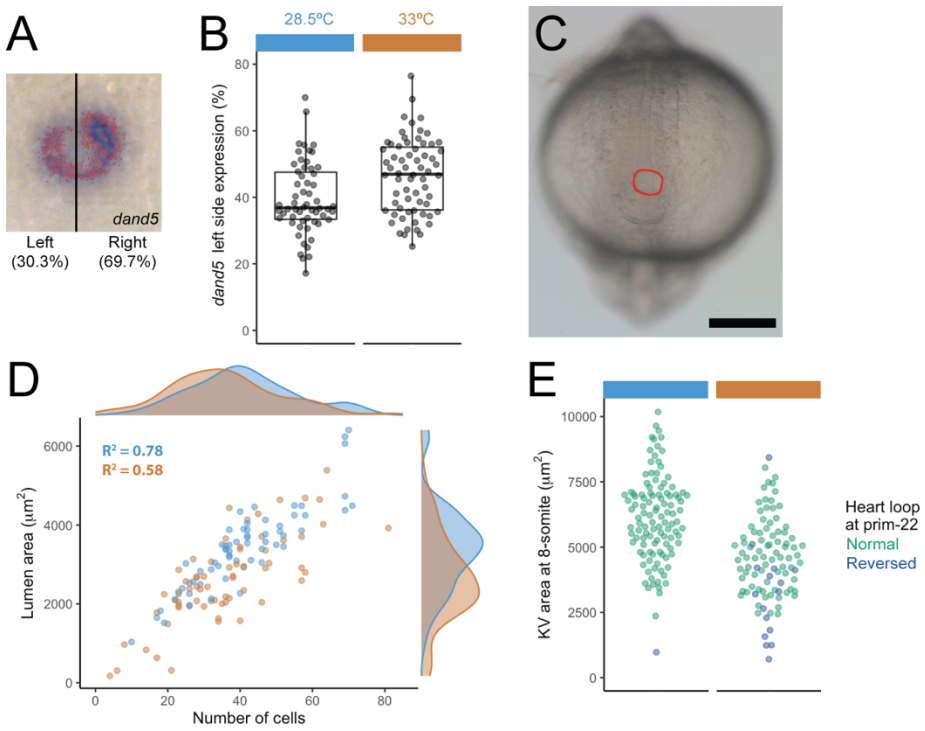

Figure S2. Incubation temperature affects early LR signaling and KV size. (A) *dand5* expression in the KV of a 10-somite embryo detected by *in situ* hybridization. Relative levels of *dand5* expression on the left and right side as determined after color thresholding in Fiji. (B) Column scatter plot of the *dand5* left side expression in embryos incubated at 28.5ºC (light blue) and 33ºC (orange), p = 0.017. (C) Photograph of the posterior part of an 8-somite stage embryo, showing the KV (red circle) below the notochord. Scale bar = 200 µm. (D) KV lumen area and cilia number in TL embryos incubated at 28.5ºC and 33ºC. The distributions for these two parameters are shown as marginal histograms at the top and right. Lumen area $p < 0.001$, cilia number p = 0.04. (E) Quantification of the KV area in live embryos at 8-somite stage together with the heart laterality phenotype observed on the same embryos at prim-22 , after incubation at 28.5ºC or 33ºC, $p < 0.001$.

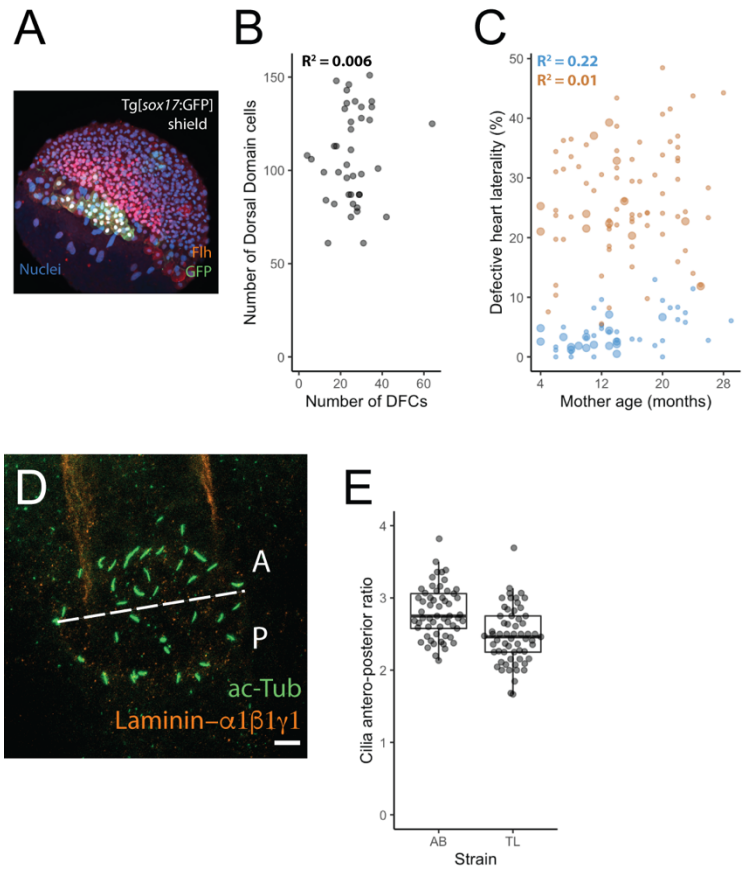

Figure S3. Origin of DFC number fluctuations. (A) Z-projection of a Tg[*sox17*:GFP] shield stage embryo (dorsal side) after nuclear staining (blue), anti-Flh (red), and anti-GFP immunolocalization (green). (B) Correlation between the numbers of DFCs and dorsal domain cells detected per embryo.. (C) Percentage of DHL versus the age of the mother used for each TL cross. (D) KV z-projection at 8-somite stage, cilia (ac-Tub) and notochord (Laminin-α1β1γ1) are shown; the latter is used as reference to set the KV anterior and posterior regions and estimate the anteroposterior ratio. Scale bar – 10µm. (E) Measurements of the anteroposterior ratio of cilia numbers in the KV of AB and TL 8-somite embryos; $p < 0.001$.

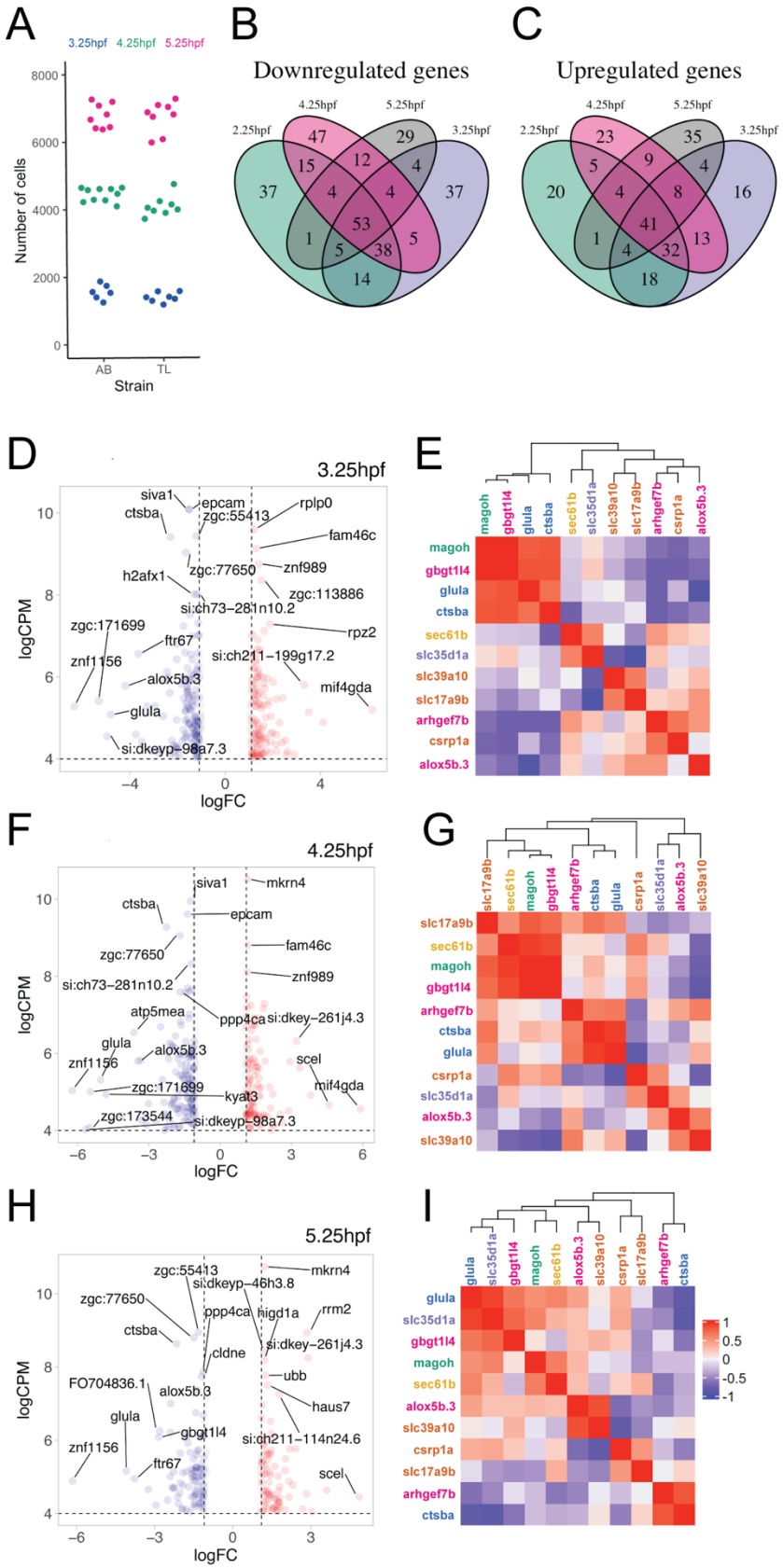

Figure S4. Transcriptomic comparison of TL vs AB embryos during early embryogenesis.

(A) Total cell number estimation with 2-photon microscopy for embryos from the same batch

used for transcriptomic analysis at the timepoint indicated on top. (B-C) Venn diagrams showing the intersection of the number of differentially expressed genes shared across different stages. (D, F, H) Volcano plot showing differentially expressed genes in TL compared to AB embryos at 3.25, 4.25 and 5.25hpf, respectively. The top 20 DE genes are shown. (E, G, I) Pairwise Pearson correlation between the downregulated genes at 3.25, 4.25 and 5.25hpf, respectively, for TL embryos. The gene names are color-coded by embryonic structure as in (3F).