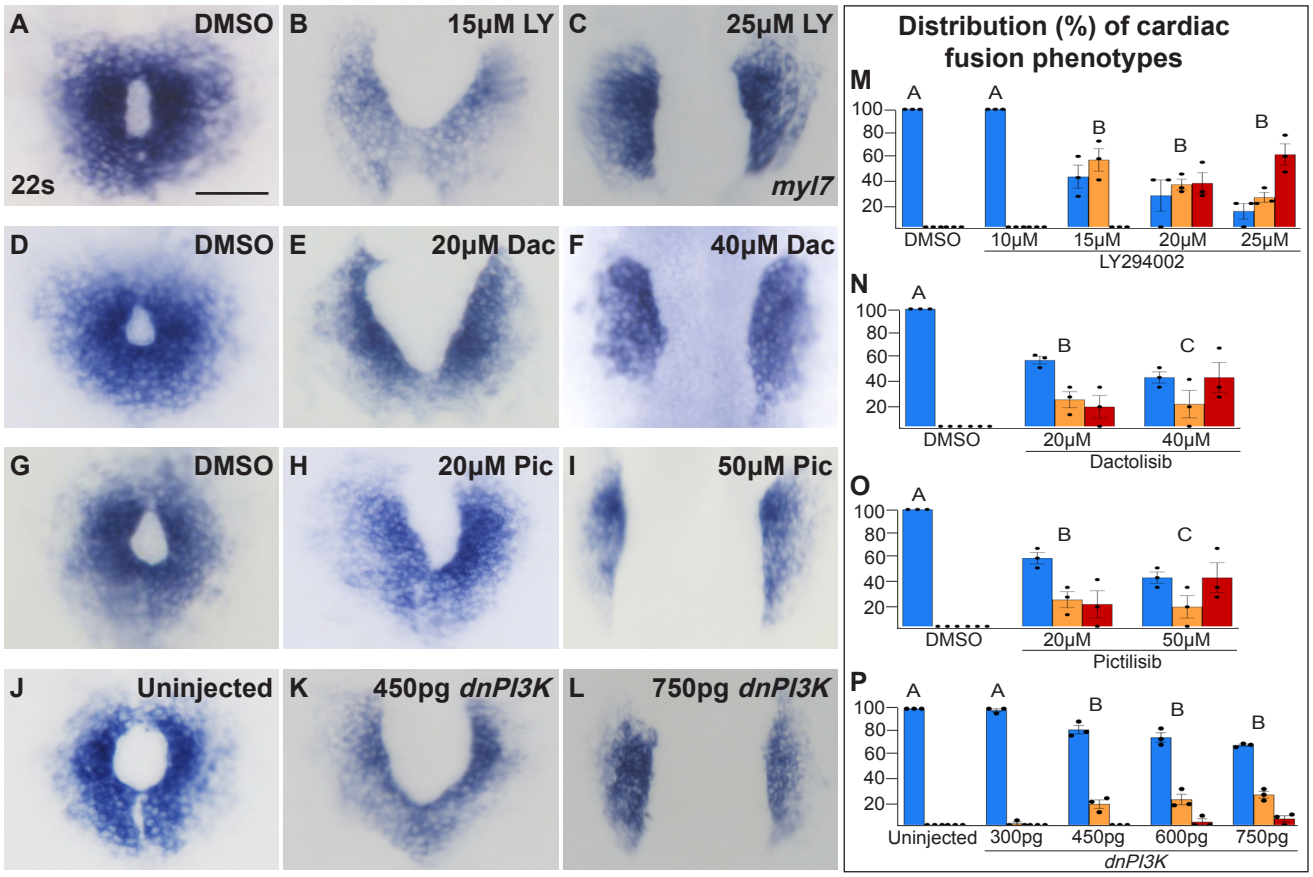
Supplemental Table S1. Primers for genotyping and cloning.

	Name	Sequence (5'-3')
Primers to screen for the *Tg(myl7:dnPI3K)* transgene in F1 embryos	dnPI3K_F1	GCGGGAAGAGGACATTGACT
	dnPI3K_R1	GCGGGAAGAGGACATTGACT
Primers to clone lck-emGFP into the middle-entry vector of the tol2 gateway system	Hifi_lck_1F	CAGTCGACTGGATCCGGTACAGATCCGCTAGCCACCATG
	Hifi_lck_1R	CAGTCGACTGGATCCGGTACAGATCCGCTAGCCACCATG
	Hifi_emgfp_2F	GGTCGCCACCGTGTCCAAGGGCGAGGAG
	Hifi_emgfp_2R	GGTCGCCACCGTGTCCAAGGGCGAGGAG

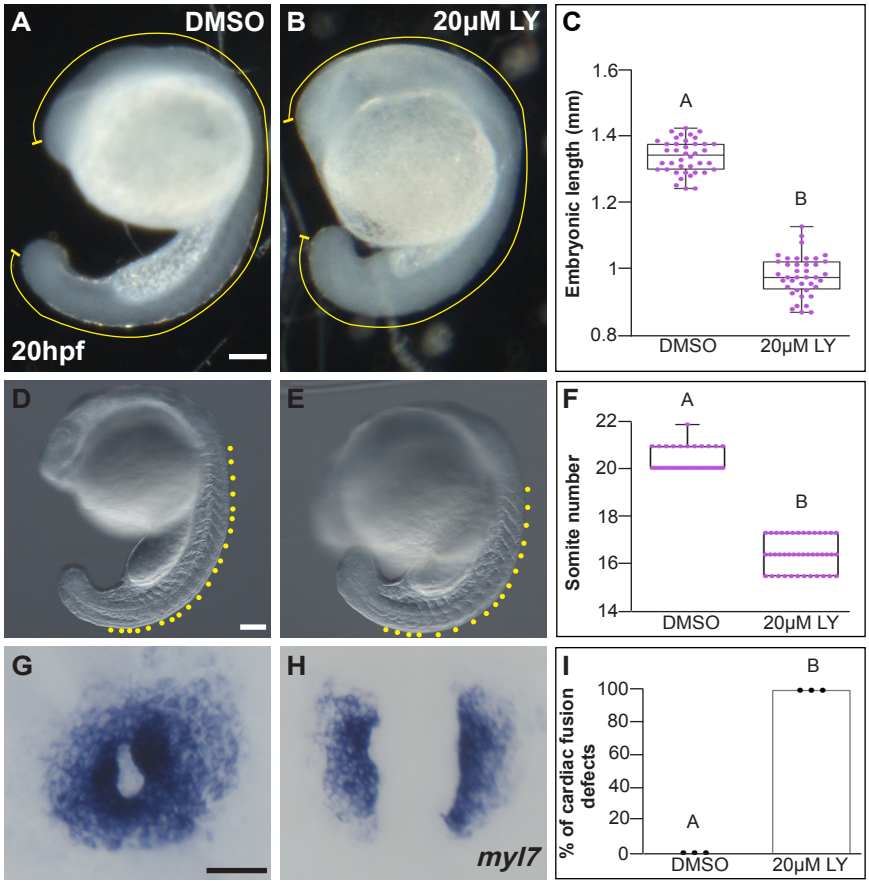
Source data: Excel file organized by figure containing data from which graphs and charts were derived including complete p-values, primer sequences and uncropped immunoblots.

Suppl. Figure 1



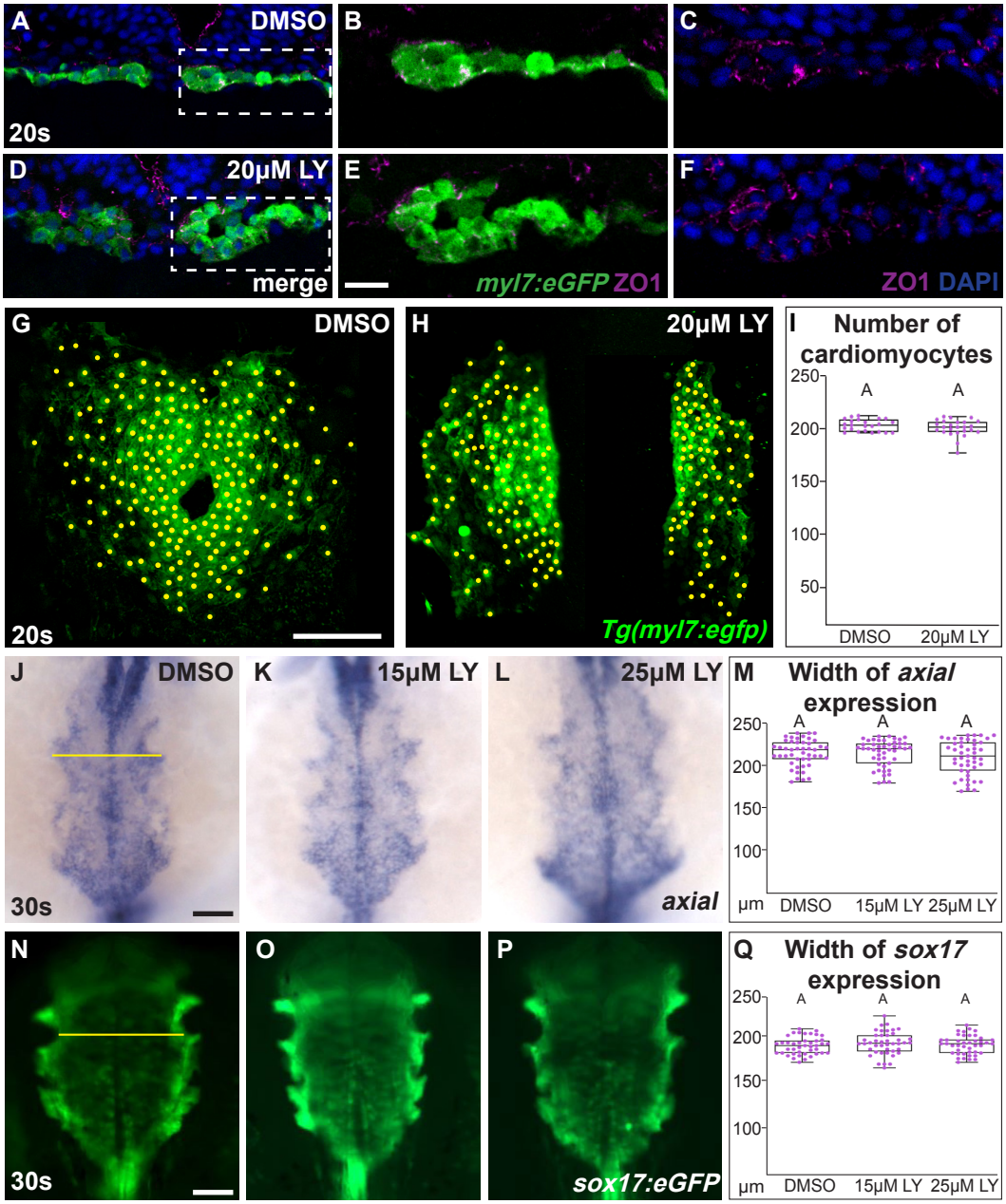
Supplemental Fig. 1: The penetrance and severity of cardiac fusion defects in PI3K-inhibited embryos is dose-dependent. A-L Dorsal views, anterior to the top, of the myocardium labeled with *myl7* at 22s. Incubation of embryos with LY (A-C), Dac (D-F), Pic (G-I) from bud stage to 22s or injection of embryos with *dnPI3K* mRNA (J-L) at the one-cell stage results in dose-dependent cardiac fusion defects at 22s. **M-P** Graphs depict the distribution of cardiac fusion defects in embryos treated with increasing concentrations of LY (M), Dac (N), Pic (O) or injected with increasing amounts of *dnPI3K* mRNA (P). Graphs reveal that both the percentage of embryos displaying cardiac fusion defects and the severity of those defects are dose-dependent. Total number of embryos analyzed (n) from > 3 treatments or injections at the indicated concentrations in (M-P): LY- 40, 40, 30, 31, 31; Dac: 38, 34, 39; Pic: 37, 39, 38, *dnPI3K* mRNA: 73, 52, 61, 57, 52, respectively. Dots indicate the percent of embryos displaying a specific phenotype per incubation. Blue - Cardiac ring/normal; Orange - fusion only at posterior end/mild, Red - cardia bifida/severe. Bar graphs, mean ± SEM. One-Way ANOVA comparing percent of cardiac fusion defects-letter change indicates $p < 0.05$. Scale = 60 μm. Data with full p-values included in the source file.

Suppl. Figure 2



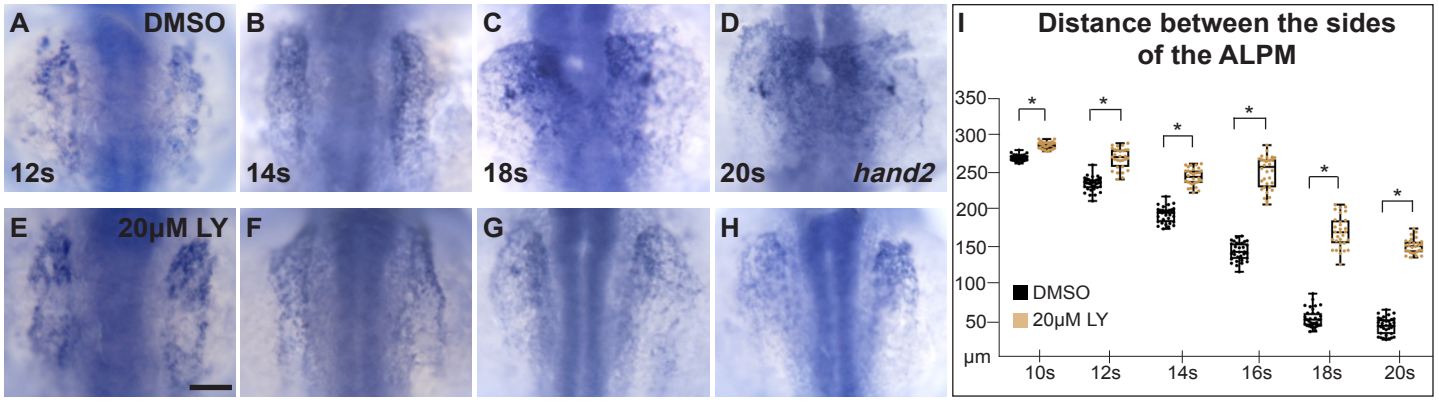
Supplemental Fig. 2: LY-incubation results in trunk extension and somite formation delays. A-B, D-E Lateral brightfield views of 20 hours post fertilization (hpf) embryos treated with DMSO (A, D) or 20µM LY (B, E) at bud stage. **C, F** Box-whisker plot depicting the average embryonic length (yellow curved line in A, B) or somite number (yellow dots in D, E) at 20 hpf. Total number of embryos (n) from >3 separate incubations = 40 (DMSO), 40 (20µM LY) for (C), and 39 (DMSO), 42 (20µM LY) for (F). Dots = measurements from individual embryos. Two sample t-test; p-value = 4.527×10^{-4} and 7.624×10^{-5}, respectively. **G-H** Dorsal views, anterior to the top, of the myocardium labeled with *myl7* at 20 hpf. Embryos treated with DMSO at bud stage show cardiac rings (G) whereas those treated with 20µM LY show cardia bifida at 20 hpf (H). **I** Graph depicts the average percentage of cardiac fusion defects in embryos treated with DMSO or 20µM LY. The total number of embryos examined over three separate incubations (n) = 45 (DMSO), 45 (20µM LY). Two sample t-test; p-value = 4.56×10^{-5}. Dots indicate the percent of embryos with cardiac fusion defects per incubation. Letter changes (C, F, I) indicate p-values < 0.05. Raw data and full p-values included in the source file.

Suppl. Figure 3



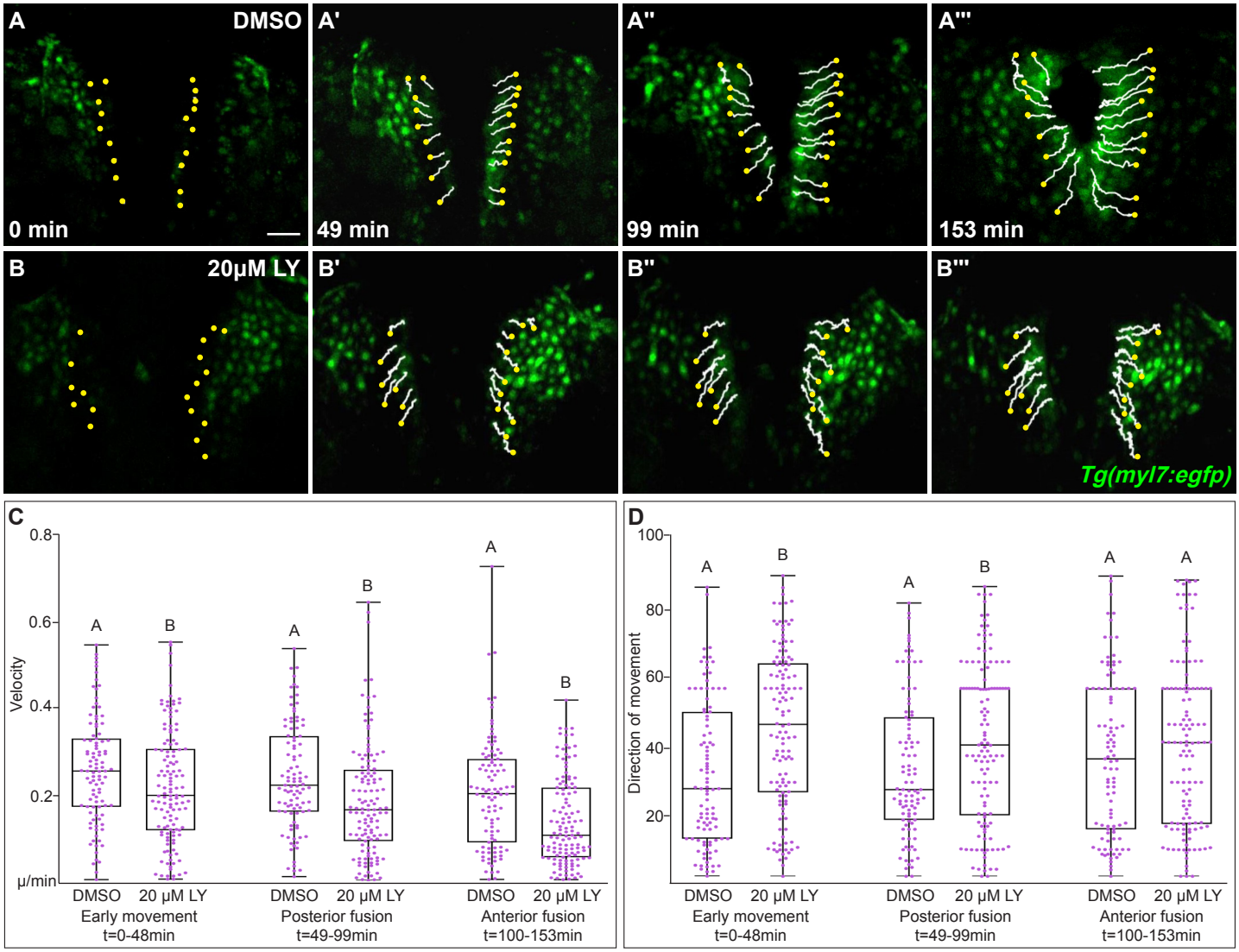
Supplemental Fig 3: The morphologies of the myocardium and anterior endoderm are not compromised in PI3K-inhibited embryos. A-F Representative transverse cryosections, dorsal to the top, compare the morphology of the myocardium, visualized with *Tg(myl7:eGFP)* (green), ZO1 (purple) and DAPI (blue) between DMSO- (A-C) and 20μM LY- (D-F) treated (bud-20s) embryos. Box (A, D) indicate region magnified in B, C, E, F. **G-I** Representative 3D confocal images of the myocardium at 20s, which were used to count myocardial cells in DMSO- (G) or 20μM LY - (H) treated embryos. Yellow dots indicate individual myocardial cells counted using ImageJ. Box-whisker plots depict the average number of myocardial cells. 21 (DMSO) and 25 (LY) embryos from 4 separate bud-20s incubations were analyzed (I). **J-Q** Dorsal views, anterior to the top, of the anterior endoderm labeled with *axial* (J-L) or the *Tg(sox17:eGFP)* transgene (N-P) at 30s. Embryos incubated with either DMSO (J, N) or 15μM LY (K, O) or 25μM LY (L, P) from the bud stage to 30s show no observable difference in the appearance or width of the anterior endoderm. Box-whisker plots of the average width of the anterior endoderm labeled with either *axial* (M) or *Tg(sox17:eGFP)* (Q). 47 (*axial)* and 42 (*Tg(sox17:egfp)*) embryos per inhibitor concentration from three separate incubations were analyzed. Yellow lines: width of the endodermal sheet. Purple dots (I, M, Q) indicate individual embryos. Letter differences indicate a p-value < 0.05 as tested by 1-way ANOVA. Scale bars, 10 (A-F), 42 (G-H), 60 (J-L), and 50 (N-P) μm. Raw data and full p-values included in the source file.

Suppl. Figure 4



Supplemental Fig. 4: Myocardial movement towards the midline is disrupted in PI3K-inhibited embryos throughout cardiac fusion. A-H Dorsal views, anterior to the top, of embryos displaying the expression of *hand2* in the anterior lateral plate mesoderm (ALPM) at (A, E) 12s, (B, F) 14s, (C, G) 18s and (D, H) 20s, treated with either DMSO (A-D) or 20μM LY (E-H) at bud stage. **I** Box-whisker plots depict the average distance between the sides of the ALPM. Although, *hand2* is properly expressed in LY-exposed embryos, ALPM convergence is affected as early as the 10s stage, with a dramatic difference in convergence starting at 12s. The total number of embryos analyzed (n), from 3 separate incubations at the noted stages (I) are: n = 34, 33, 31, 32, 34, 34 (DMSO); 32, 29, 30, 34, 31, 28 (20μM LY), respectively. Dots indicate the distance between ALPM sides per embryo. Student's t-test: asterisk indicates p values < 0.05. Scale bar, 100μm. Raw data and full p-values included in the source file.

Suppl. Figure 5



Supplemental Fig. 5. PI3K signaling directs myocardial movement during the early stages of cardiac fusion and regulates velocity throughout cardiac fusion. A-B Time-lapse confocal reconstructions from Figure 3 overlaid with cell movement tracks, starting at t = 0 (yellow dots). Scale bar, 60μm. **C, D** Box-whisker plots display the average velocity (C) and direction of movement (D) sub-divided at three distinct stages of cardiac fusion: early movement (0 - 48min), posterior fusion (49 - 99min) and anterior fusion (100 - 153min). The average velocity of myocardial cells in LY-treated embryos is consistently slower than the velocity of DMSO-treated embryos which is consistent throughout cardiac fusion (C). However, in LY-treated embryos myocardial cells display a more angular average direction of movement compared to DMSO-treated embryos during the early stages of cardiac fusion (Early movement, Posterior fusion), after which wild-type myocardial cell movement becomes more angular matching myocardial cell movements in LY-treated embryos. Two sample t-test, letter change indicates $p < 0.05$. Raw data and full p-values included in the source file.