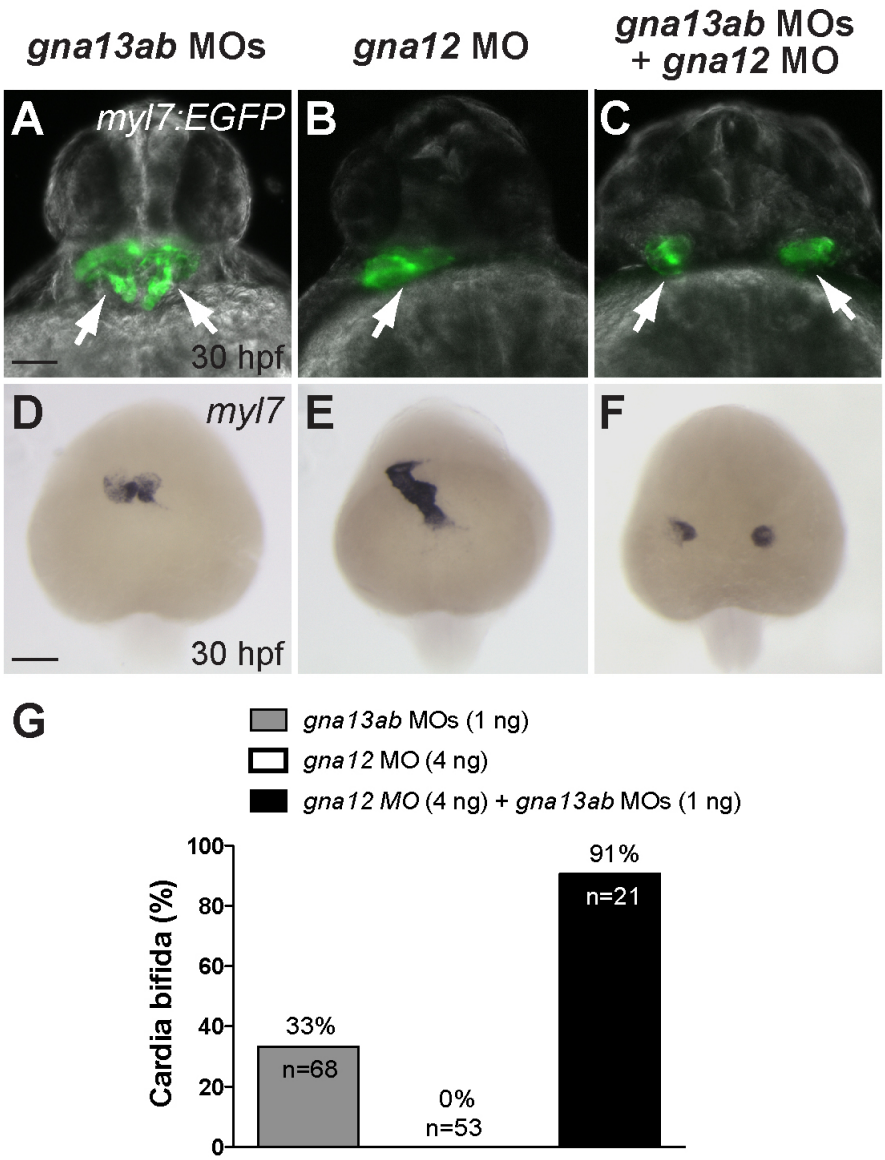


Fig. S1. Gα_{12} and Gα_{13} have partially redundant roles in myocardial migration. *Tg*(*myl7:EGFP*) embryos injected with *gnb13ab* MOs (1 ng each) and *gnb12* MO (4 ng), alone or in combination, at 30 hpf. (**A-C**) Overlay of epifluorescence and bright-field images. Ventral view; white arrows indicate hearts. (**D-F**) *myl7* expression, as detected by in situ hybridization. Dorsoanterior view with anterior upwards. (**G**) Frequencies of indicated embryos that exhibit cardia bifida at 35 hpf. Scale bars: 100 μm.

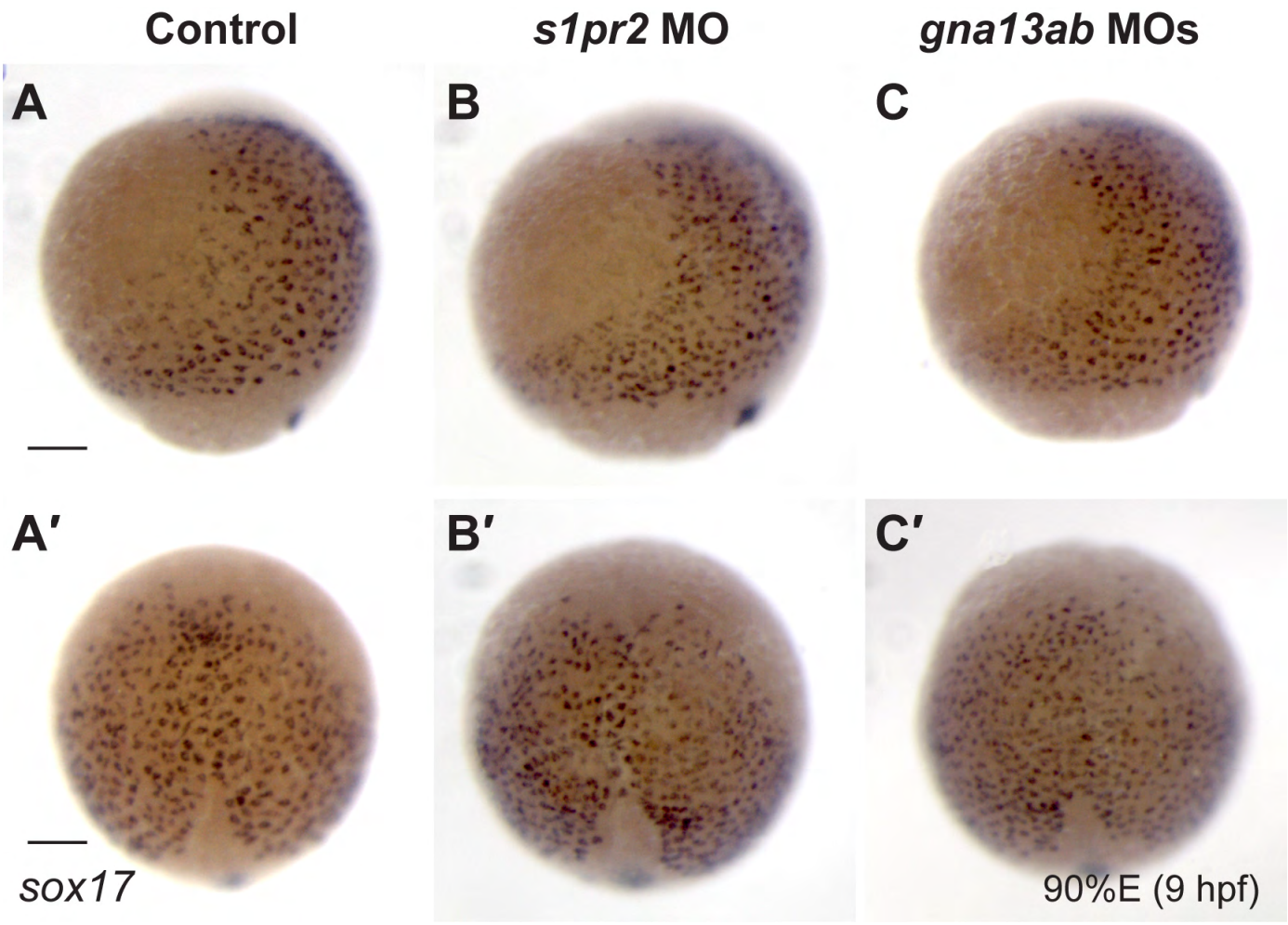


Fig. S2. The migration and differentiation of endodermal cells are normal at gastrula stage, in embryos defective for S1pr2/Gα_{13} signaling. *sox17* expression was examined by in situ hybridization in control (*n*=28), *s1pr2/mil* MO- (*n*=28) or *gnb13ab* MOs-injected (*n*=29) embryos, at 90% epiboly (9 hpf). (**A-C**) Dorsal view, anterior is upwards. (**A′-C′**) Lateral view, dorsal is towards the right. The distribution of *sox17*-expressing endodermal cells appears to be normal in *s1pr2* or *gnb13ab* morphants. Scale bars: 100 μm.

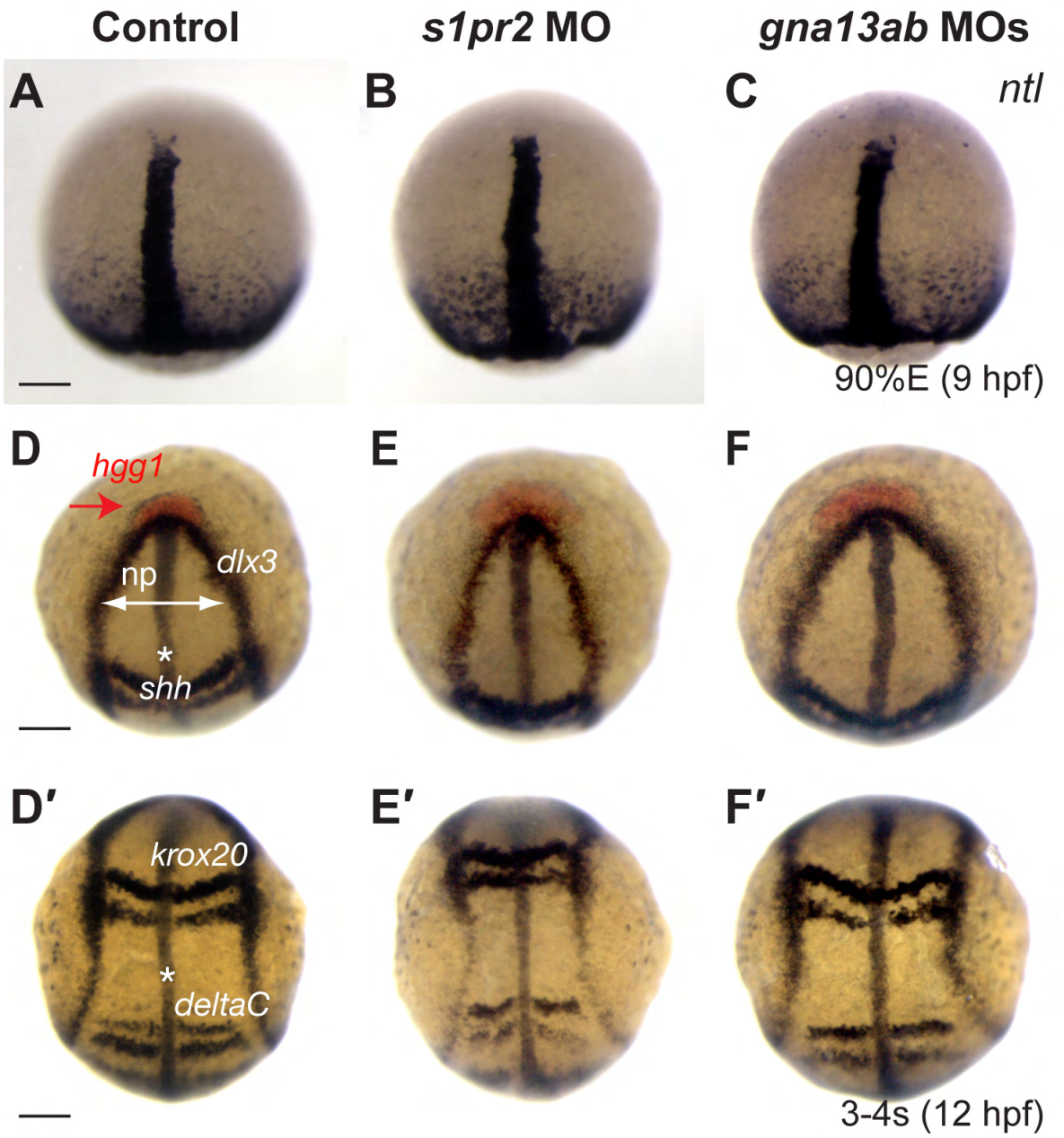


Fig. S3. Convergent and extension movements of the mesoderm and ectoderm are not affected by S1pr2/Ga_{13} signaling during zebrafish gastrulation. (**A-C**) Expression of *ntl*, as detected by in situ hybridization, at 90% epiboly (9 hpf). Dorsal view. (**D-F′**) Expression of *hgg1* (marks prechordal plate, red), *dlx3* (neural plate boundary), *krox20* (rhombomeres 3 and 5), *shh* (midline) and *deltaC* (somites) detected by in situ hybridization, at the 3- to 4-somite stage (12 hpf). (D-F) Dorsoanterior view; (D′-F′) dorsal view; *, notochord; white line with double arrows indicate neural plate (np). Scale bars: 100 μm.

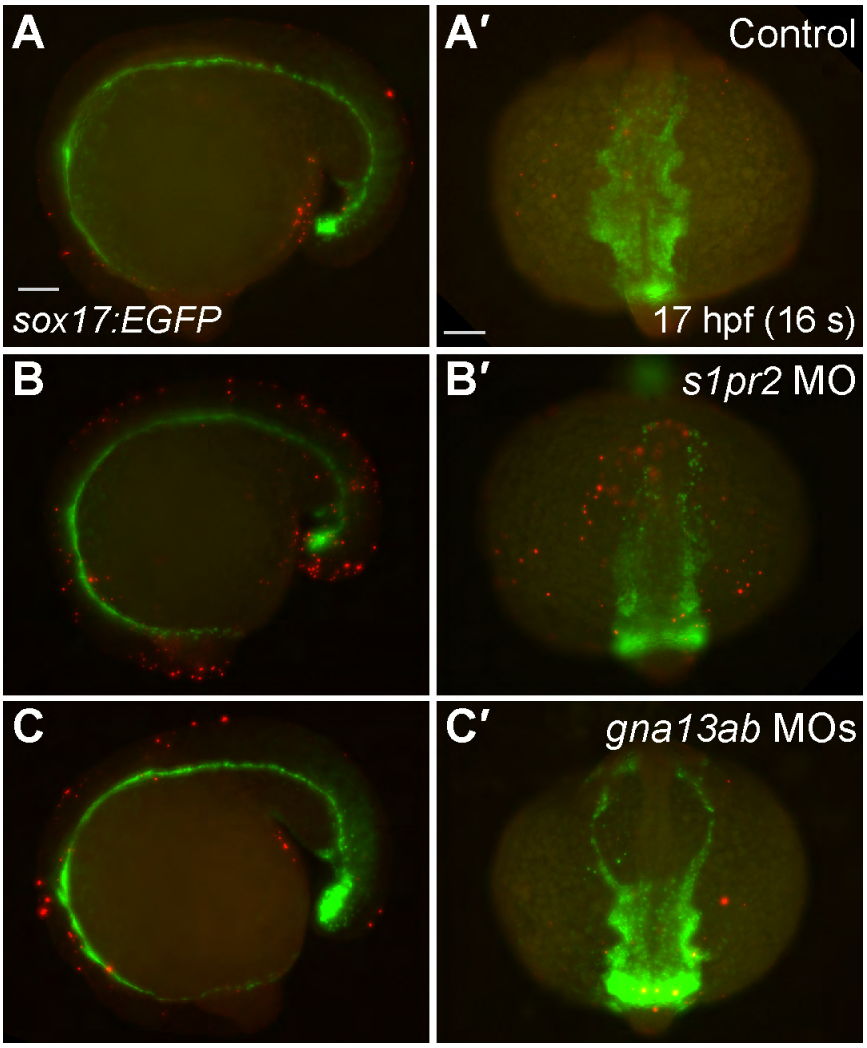


Fig. S4. Defects in S1pr2/Gα_{13} signaling do not induce apoptosis in the endoderm. Whole-mount TUNEL assay was performed in 17 hpf (16-somite stage) *Tg*(*sox17:EGFP*) control embryos (A-A′, *n*=15) and embryos injected with the *s1pr2/mil* MO (B-B′, *n*=15) or *gnb13ab* MOs (C-C′, *n*=15) using an apoptosis detection kit (ApopTag Red *in situ* apoptosis Detection Kit, Millipore) according to the manufacturer's instructions. Rhodamine-labeled apoptotic cells (red) are not detected in the EGFP-expressing endoderm. (A-C) Lateral view with anterior towards the left; (A′-C′) dorsoanterior view with anterior upwards. Scale bars: 100 μm.

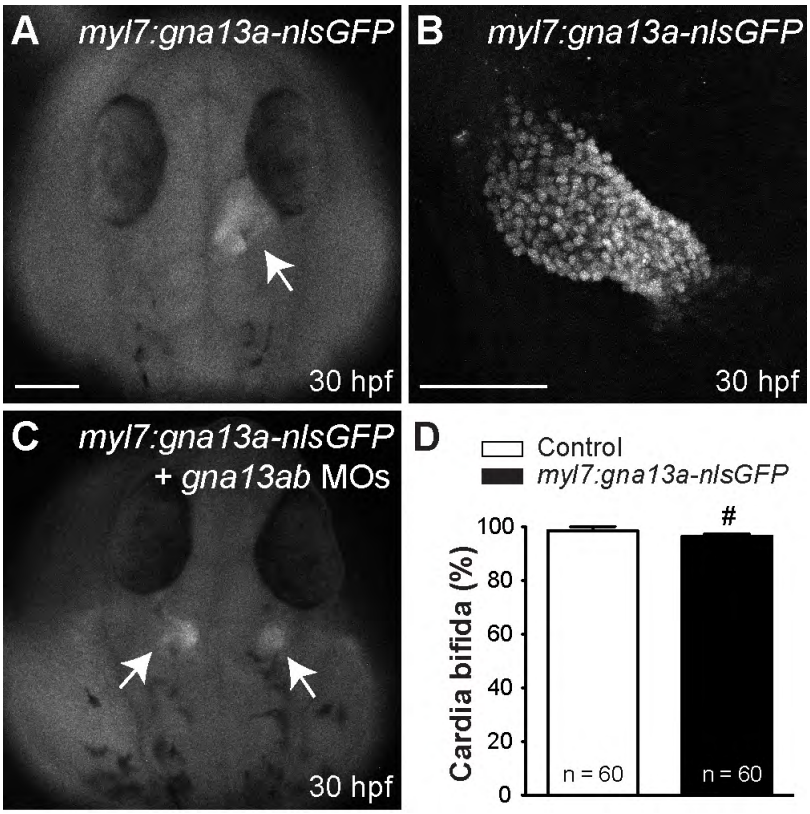


Fig. S5. Cardiac-specific expression of Gα_{13}a fails to rescue cardia bifida caused by global Gα_{13} inhibition. The transgene *myl7:gna13a-IRES-nlsGFP* was created using the Gateway system (Kwan et al., 2007; Villefranc et al., 2007), such that Gα_{13}a is expressed specifically in the myocardial cells under control of the *myl7* promoter (Huang et al., 2003). The expression of Gα_{13}a was monitored as nuclear GFP (nlsGFP) expression driven by an internal ribosomal entry site (IRES) (Kwan et al., 2007). The transgene plasmid DNA (50 pg) was co-injected with transposase RNA (30 pg) into the cytoplasm of wild-type embryos at the one-cell stage. A stable line expressing Gα_{13}a in the heart was identified. Injections of *gnb13ab* MOs in *Tg(myl7:gna13a-IRES-nlsGFP)* embryos still exhibited cardia bifida. (**A**) A representative epifluorescence image of 30 hpf-*Tg(myl7:gna13a-IRES-nlsGFP)* embryos, showing expression of nlsGFP in the heart (arrow). (**B**) A confocal Z-stack image showing that nlsGFP is expressed in all cardiomyocytes of *Tg(myl7:gna13a-IRES-nlsGFP)* embryos at 30 hpf. (**C**) A representative epifluorescence image of 30 hpf-*Tg(myl7:gna13a-IRES-nlsGFP)* embryos injected with *gna13ab* MOs showing cardia bifida (arrows). (**D**) Frequencies of cardia bifida in 30-hpf embryos. $^{\#}P>0.5$ versus control. Scale bar: 100 μm.

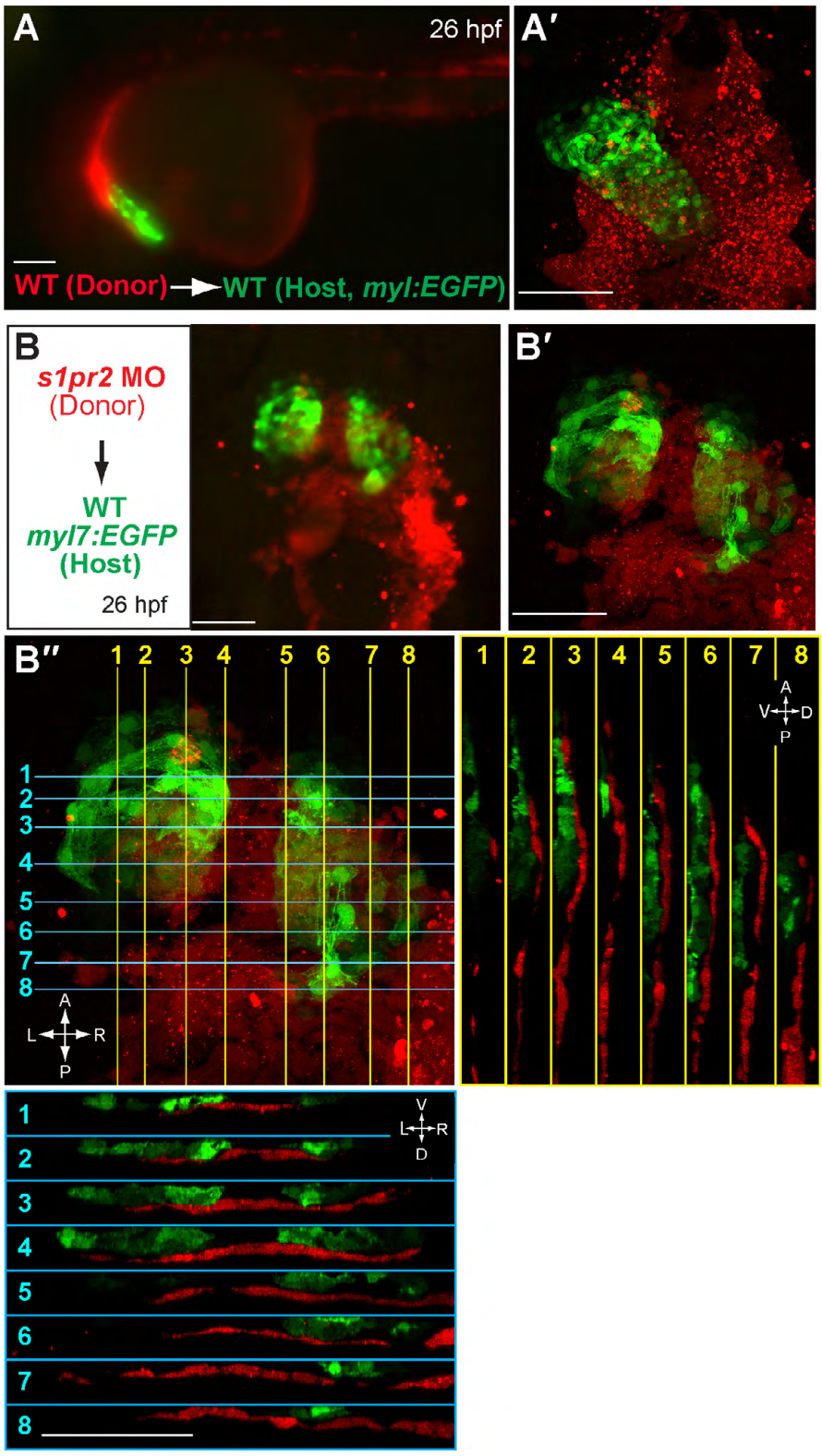
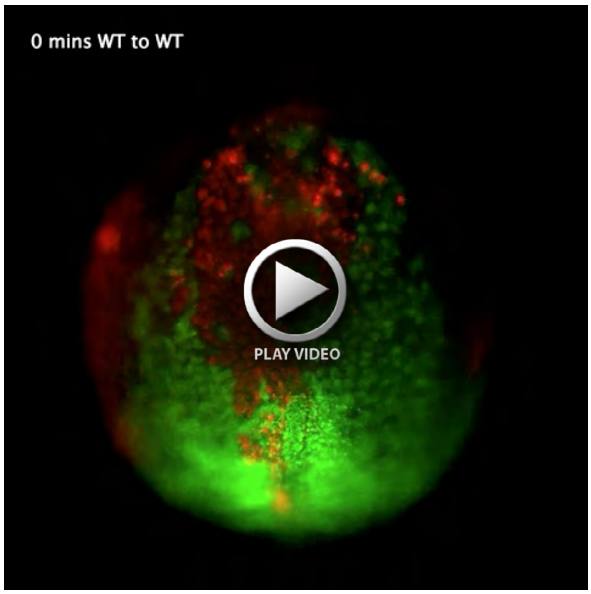
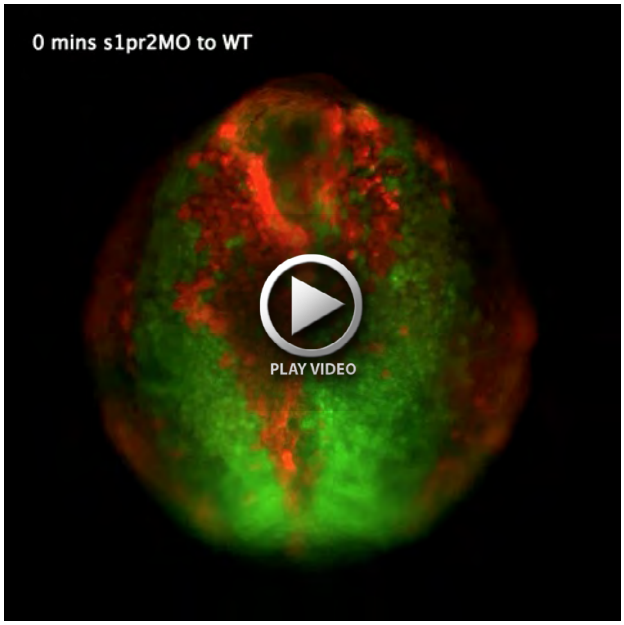


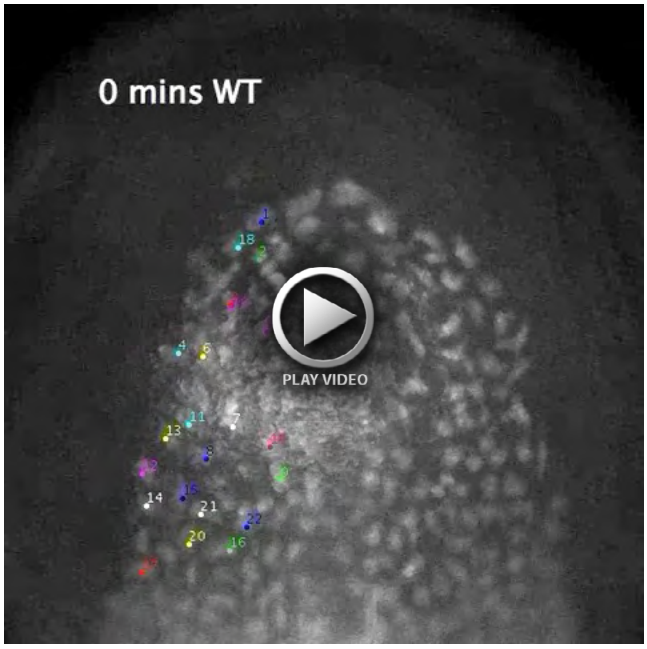
Fig. S6. Donor cells do not contribute to myocardium when transplanted into endoderm. Epifluorescence images (A,B) and confocal z-stack images (A′,B′) showing rhodamin-dextran labeled donor cells and GFP-labeled myocardium in host embryos at 26 hpf. (A-A′) Wild-type donor cells were transplanted into wild-type hosts in the Tg(myl7:EGFP) background. (B-B″) s1pr2 MO-injected donor cells were transplanted into wild-type hosts in the Tg(myl7:EGFP) background. (A) Lateral view; (A′ ′B,B″ ventral view; (B″) orthogonal view of B″ Blue lines indicate cross-section planes along the L/R axis of the embryos perpendicular to the midline; yellow lines indicate cross-section planes along the embryonic AP axis; blue and yellow lines are numbered to show the position of the corresponding orthogonal panel; the respective sections are shown within blue or yellow insets. A, anterior; P, posterior; L, left; R, right; D, dorsal, V, ventral. Scale bars: 100 μm.



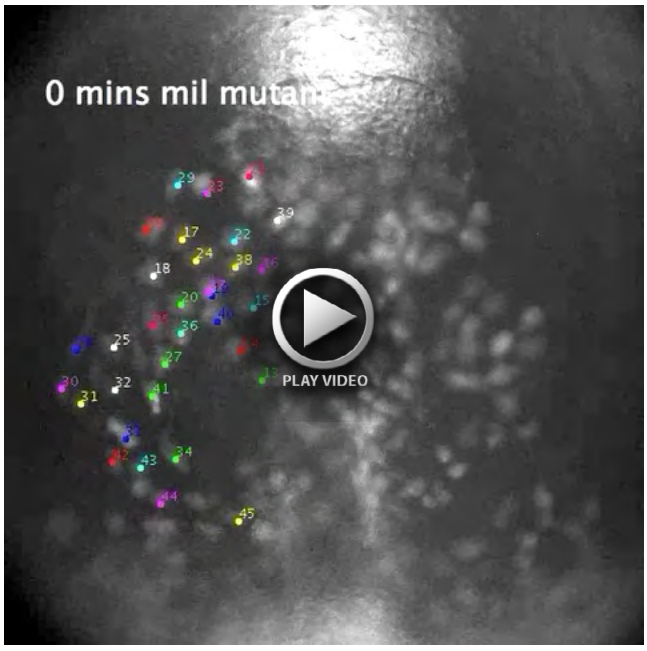
Movie 1. Convergent movement of wild-type donor cells in wild-type host embryo. Representative time-lapse movie of the anterior-region endoderm of a *Tg(sox17:EGFP)* embryo transplanted with sox32-expressing and rhodamine-labeled wild-type cells, from 10.5 hpf to 14 hpf (from 3 to 10 somites) at 25°C. Images were acquired at 5-minute intervals; the movie plays at 7 frames/second.



Movie 2. Convergent movement of *s1pr2*-deficient donor cells in wild-type host embryo. Representative time-lapse movie of the anterior-region endoderm of a *Tg(sox17:EGFP)* embryo transplanted with sox32-expressing, rhodamine-labeled *s1pr2* MO-injected cells from 10.5 hpf to 14 hpf (from 3 to 10 somites) at 25°C. Images were acquired at 5-minute intervals; the movie plays at 7 frames/second.



Movie 3. Cell movements in the anterior endoderm of wild-type embryo. Representative time-lapse movie of the most anterior region of the endoderm in a *Tg(sox17:EGFP)* embryo, from 12 hpf to 14.5 hpf (5 to 10 somites), at 25°C. Images were captured at 5-minute intervals, and the movie plays at 7 frames/second.



Movie 4. Cell movements in the anterior endoderm of a *mil*$^{93(-/-)}$ embryo. Representative time-lapse movie of the most anterior region of the endoderm in a *mil*$^{93(-/-)}$ embryo on a *Tg(sox17:EGFP)* background, from 12 hpf to 14.5 hpf (5 to 10 somites), at 25°C. Images were captured at 5-minute intervals, and the movie plays at 7 frames/second.

Table S1. Genetic interactions

Manipulation	Number of embryos	% Embryos	
		Cardia bifida	Tail blistering
gna13ab MOs (0.5 ng each)	45	6.7	20.0
s1pr2 MO (2.5 ng)	56	8.9	3.6
gna13ab MOs (0.5 ng each) + *s1pr2* MO (2.5 ng)	49	73.5	81.6
arhgef11RGS RNA (250 pg)	45	17.8	15.6
arhgef11RGS RNA (250 pg) + *s1pr2* MO (2.5 ng)	45	73.3	47.2

Embryos were injected with the indicated MOs or RNAs at the one-cell stage and raised at 32°C. The frequency of embryos exhibiting cardia bifida and tail blistering was assessed at 32 hpf and 48 hpf, respectively.