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SHORT REPORT

Figures and figure supplements

The Apelin receptor enhances
Nodal/TGFβ signaling to ensure proper cardiac development

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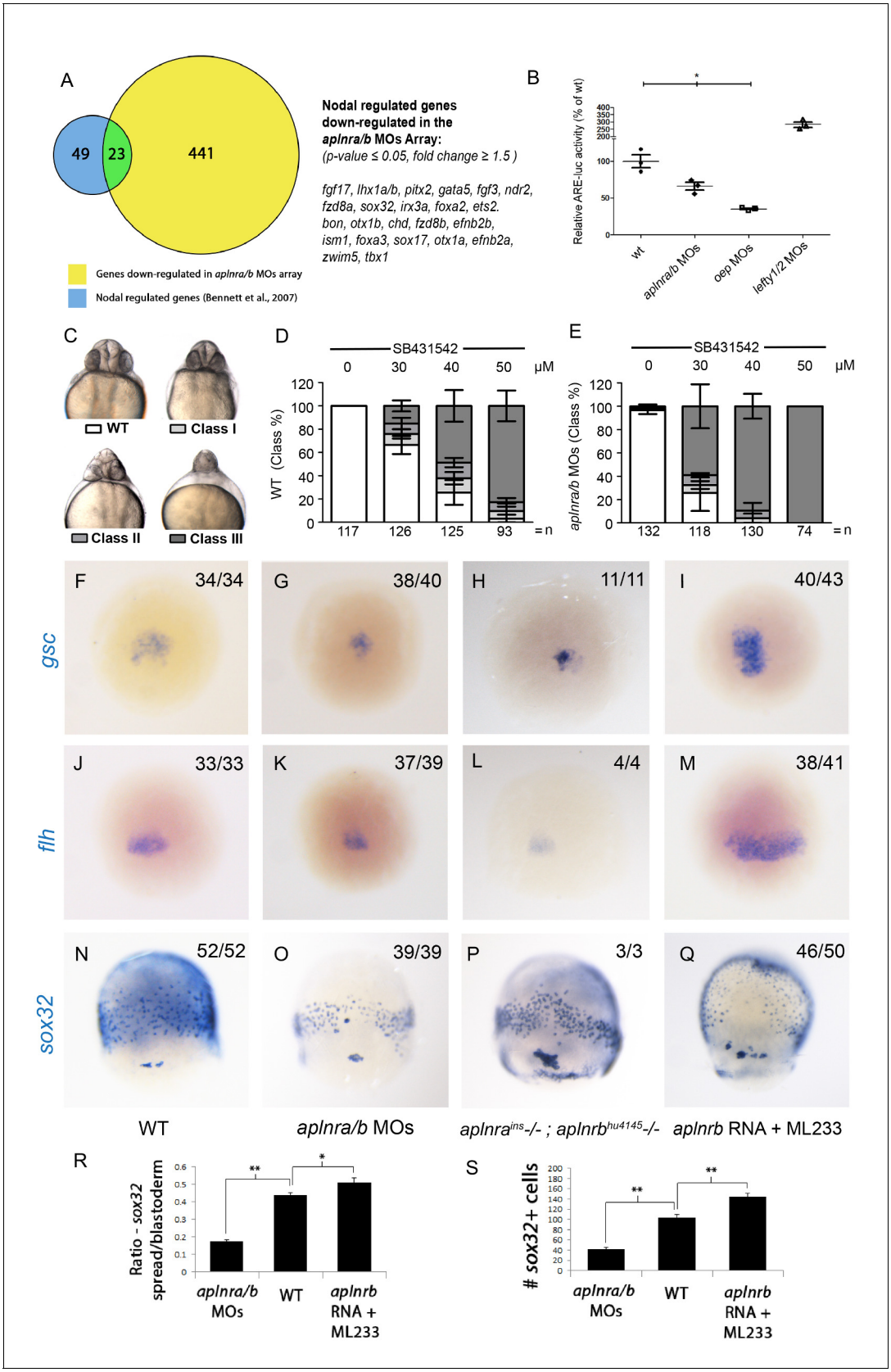

Figure 2. Aplnr deficient embryos exhibit a reduction in Nodal signaling. (**A**) List and Venn diagram of 23 Nodal target genes found to be down-regulated in a microarray of *aplnra/b* morphant embryos compared to WT at 50% epiboly (5.25 hpf). (**B**) Relative luciferase activity regulated by the

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Figure 2 continued

Activin response element (ARE) in WT, *aplnra/b* morpholino (MO), *oep* MO and *lefty1/2* MO injected embryos at 30% epiboly (4.7 hpf). Data are represented as means ± SEM. *p<0.05 unpaired two-tailed t-test. (**C–E**) Phenotypic characterization of WT (**D**) and *aplnra/b* morphant embryos (**E**) when treated with the indicated concentration of the Alk4/5/7 inhibitor SB431542 from the sphere stage (4 hpf) onwards. (**F–S**) Visualization of the expression of the canonical nodal target genes *gsc, flh* and *sox32* in WT (**F**,**J**,**N**), *aplnra/b* MOs injected (**G**,**K**,**O**), *aplnra*^{ins}*; aplnrb*^{hu4145} double mutant (**H**,**L**,**P**) and *aplnrb* RNA injected treated with the Aplnr agonist ML233 (**I**,**M**,**Q**) embryos at 8 hpf. Embryos are viewed from the dorsal side. Quantification of the number and spread of *sox32* expressing cells (R,S). Data are represented as means ± SEM. *p<0.05, **p<0.01 unpaired two-tailed t-test.

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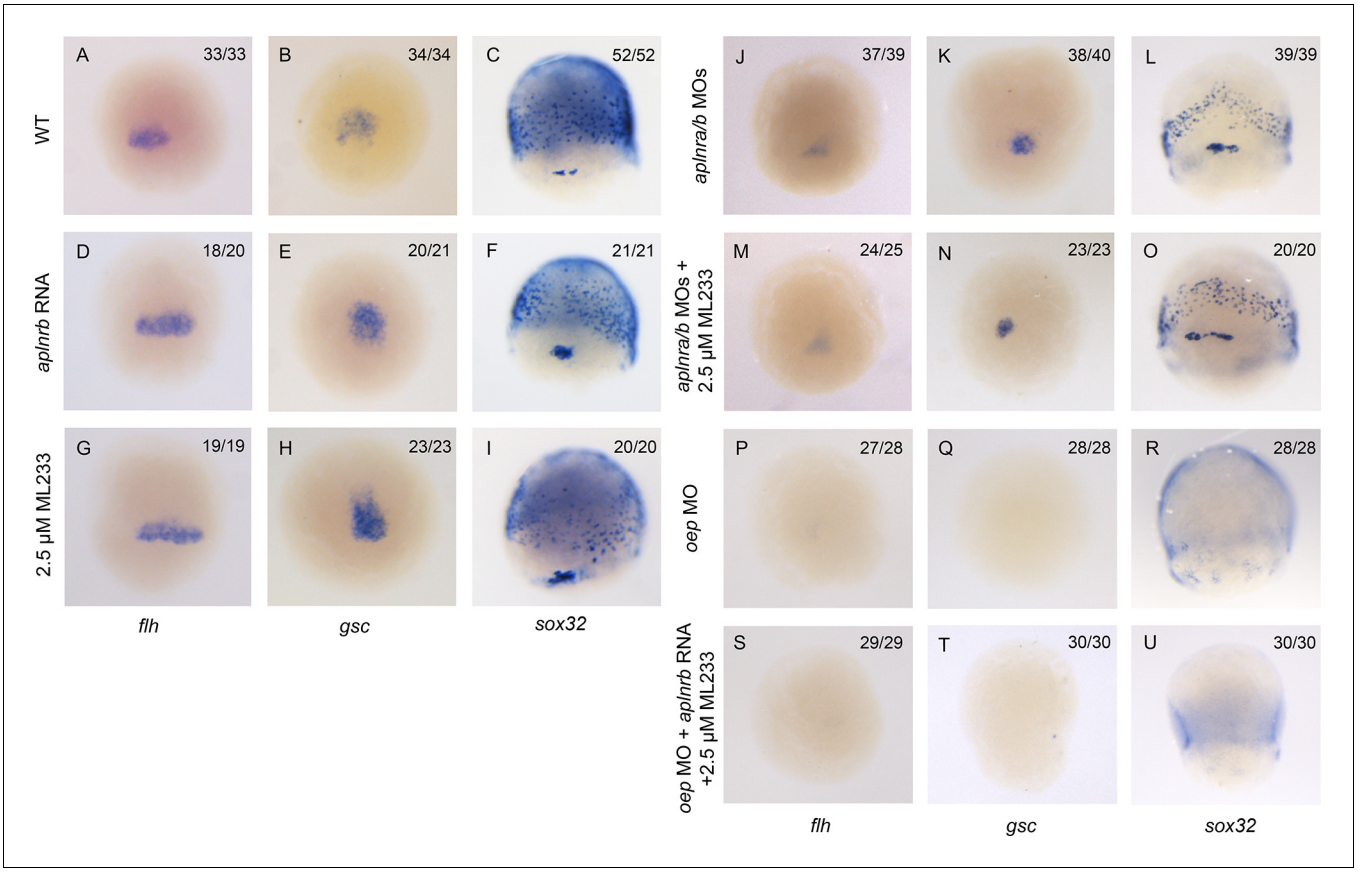

Figure 2—figure supplement 2. Aplnr activation enhances Nodal target gene expression. (**A–I**) Expression of the canonical Nodal target genes *gsc*, *flh* and *sox32* in WT (**A–C**), *aplnrb* RNA injected embryos (**D–F**) and embryos treated with the Aplnr agonist ML233 (**G–I**) at 8 hpf. Embryos are viewed from the dorsal side with anterior to the top. (**J–O**) Expression of the canonical Nodal target genes *gsc*, *flh* and *sox32* in *aplnra/b* morphant embryos with or without ML233 at 8 hpf. Embryos are viewed from the dorsal side with anterior to the top. (**P–U**) Expression of the canonical Nodal target genes *gsc*, *flh* and *sox32* in *oep* morphant embryos with or without the injection of *aplnrb* RNA and addition of ML233 at 8 hpf. Embryos are viewed from the dorsal side with anterior to the top.

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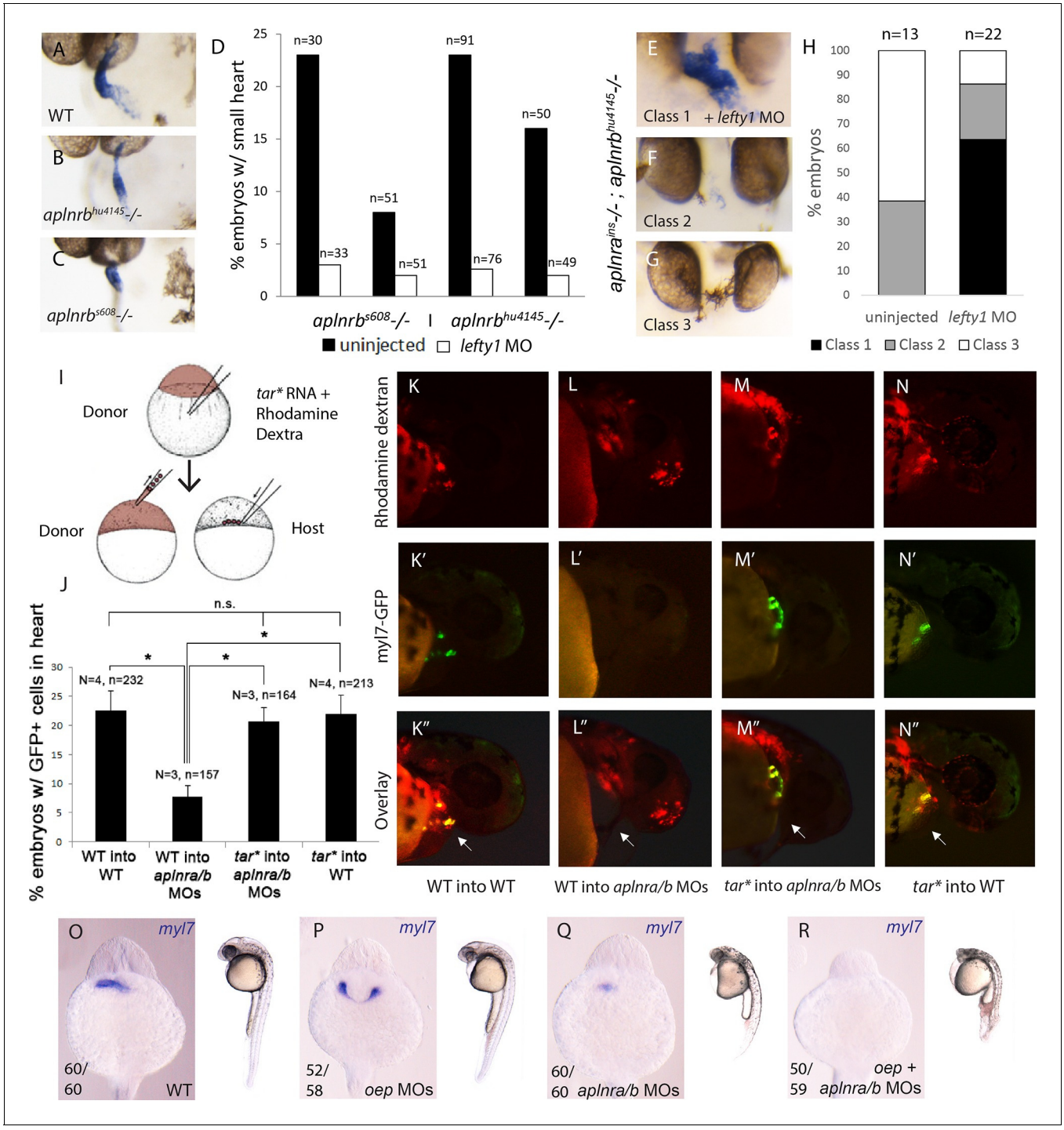

Figure 3. Elevation of Nodal signaling in *aplnr* mutant/morphant embryos rescues cardiogenesis. (**AC**) *myl7* WISH showing a representative heart phenotype at 48 hpf in a WT embryo (**A**) and two different *aplnrb* mutant alleles; *hu4145* (**B**) and *s608/grinch* (**C**). Anterior is oriented towards the left. (**D**) Quantification of the number of embryos with a small heart at 48 hpf from individual clutches of embryos in which half were injected with *lefty1* MO. Clutches were obtained from crosses of two different *aplnrb* heterozygous mutants (*hu4145* and *s608/grinch* as indicated). (**E–H**) Classification of heart phenotype in *aplnra*^{ins}*; aplnrb*^{hu4145} double mutant embryos at 48 hpf when injected with *lefty1* MO as compared to un-injected embryos. Severity of cardiac phenotypes was scored based on *myl7* WISH (**H**). (**I**) Schematic displaying the transplantation of injected donor cells into the margin of host embryos. Contribution of transplanted cells to the heart is scored based on expression of the *myl7:EGFP* transgene in donor cells. (**J–N″**) Margin transplants of WT or *tar** (activated Nodal receptor) overexpressing *myl7:EGFP* cells into WT or *aplnra/b* morphant embryos at 48 hpf. Arrow indicates the heart. Embryos are displayed from a lateral view with the anterior of the embryo towards the right. Data are represented as means ± SEM. *p<0.05, n.s. = not significant, Tukey's Multiple Comparison test following significant (p<0.05) one way ANOVA. (**O–R**) Gross morphology and *myl7* expression

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at 24 hpf in WT (**O**), embryos injected with a sub-optimal dose of *oep* MOs (**P**), *aplnra/b* morphant embryos (**Q**) and *aplnra/b/oep* morphant embryos (**R**).

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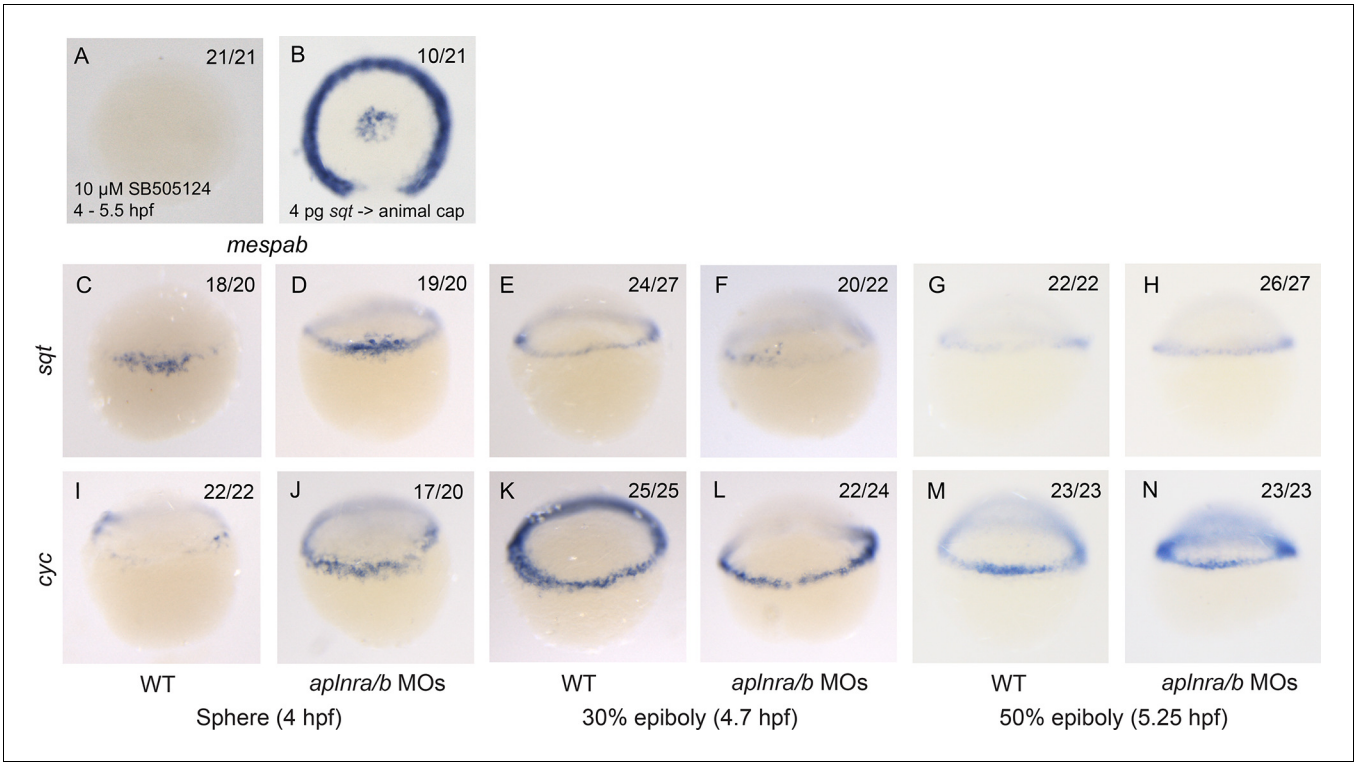

Figure 4—figure supplement 1. *mespaa* and *mespab* are Nodal target genes and Nodal ligand expression is not affected in *aplnra/b* morphant embryos. (**A**) Animal view of *mespab* expression at 50% epiboly (5.25 hpf) in embryos treated with 10 µM of SB505124 from 4–5.25 hpf. Animal cap view with dorsal to the bottom. (**B**) Animal view of *mespab* expression at 50% epiboly (5.25 hpf) in embryos in which cells expressing 4 pg of *sqt* RNA were transplanted into the animal cap. Animal cap view with dorsal to the bottom. (**C–N**) Lateral view of the Nodal ligands *sqt* (**C–H**) and *cyc* (**I–N**) expression at sphere (4 hpf), 30% epiboly (4.7 hpf) and 50% epiboly (5.25 hpf) in WT and *aplnra/b* morphant embryos.

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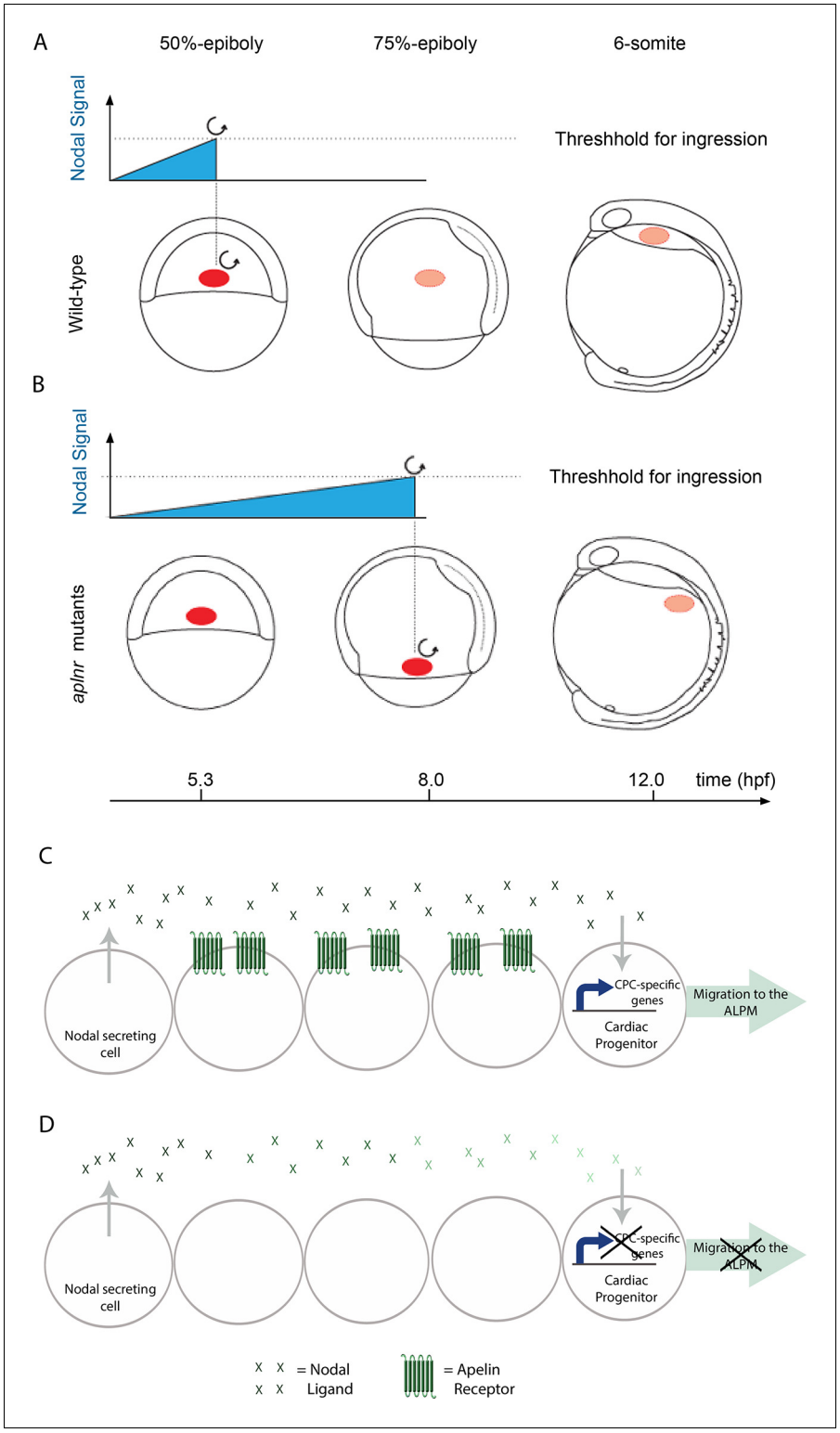

Figure 4—figure supplement 2. Aplnr is required to enhance Nodal signaling for proper cardiac development. (**A–B**) Model for the Nodal-mediated nature of the Aplnr phenotype. In WT embryos, Aplnr is required for the appropriate Nodal threshold to be reached in order to initiate the expression of the downstream transcriptional program to drive the ingression of cardiac progenitors and their migration towards the anterior of the embryo. Threshold refers to the integrated level of Nodal signaling that a cell is required to receive in order to activate this program. In *aplnr* mutant embryos it takes a longer period of time in order for this threshold to be reached and

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results in a delay in gene expression and ingression of the mesendoderm. As a result cardiac progenitors are unable to migrate all the way to the heart forming regions. Red circle denotes the location of cardiac progenitors in the embryo. (**C–D**) Model for the autonomy of Aplnr function in the context of regulating Nodal signaling. Aplnr appears to be required not in Nodal secreting cells or the cardiac progenitor cells receiving the signal, but instead in the surrounding environment. This role is important for the activation of the transcriptional program required for development of cardiac progenitor cells (CPCs). In the absence of Aplnr, the Nodal signal received by the cardiac progenitor is diminished (demonstrated by a reduction in the colour of the green Nodal ligands). This may reflect improper ligand processing or activity.

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