**Supplementary Movie Legends:**

**Movie S1**. 4-D reconstructed video of control embryonic zebrafish heart at 5 dpf. Video represents a loop of one cardiac cycle of a beating heart from a *Tg(fli1a:GFP)* zebrafish embryo. Scale bar, 50 μm.

**Movie S2**. 4-D reconstructed video of BDM-treated embryonic zebrafish heart at 5 dpf. Video represents a loop of one cardiac cycle of a beating heart from a *Tg(fli1a:GFP)* zebrafish embryo that was treated with BDM (10 mM) starting at 24 hpf. Scale bar, 50 μm.

**Movie S3**. 4-D reconstructed video of isoproterenol-treated embryonic zebrafish heart at 5 dpf. Video represents a loop of one cardiac cycle of a beating heart from a *Tg(fli1a:GFP)* zebrafish embryo that was treated with isoproterenol (100 μM) starting at 24 hpf. Scale bar, 50 μm.

**Movie S4**. 4-D reconstructed video of the heart of *NICD* mRNA-injected embryo at 5 dpf. Video represents a loop of one cardiac cycle of a beating heart from a *Tg(fli1a:GFP)* zebrafish embryo that was micro-injected with *NICD* mRNA at the one-to-two cell stage. Scale bar, 50 μm.

**Supplementary Materials:**

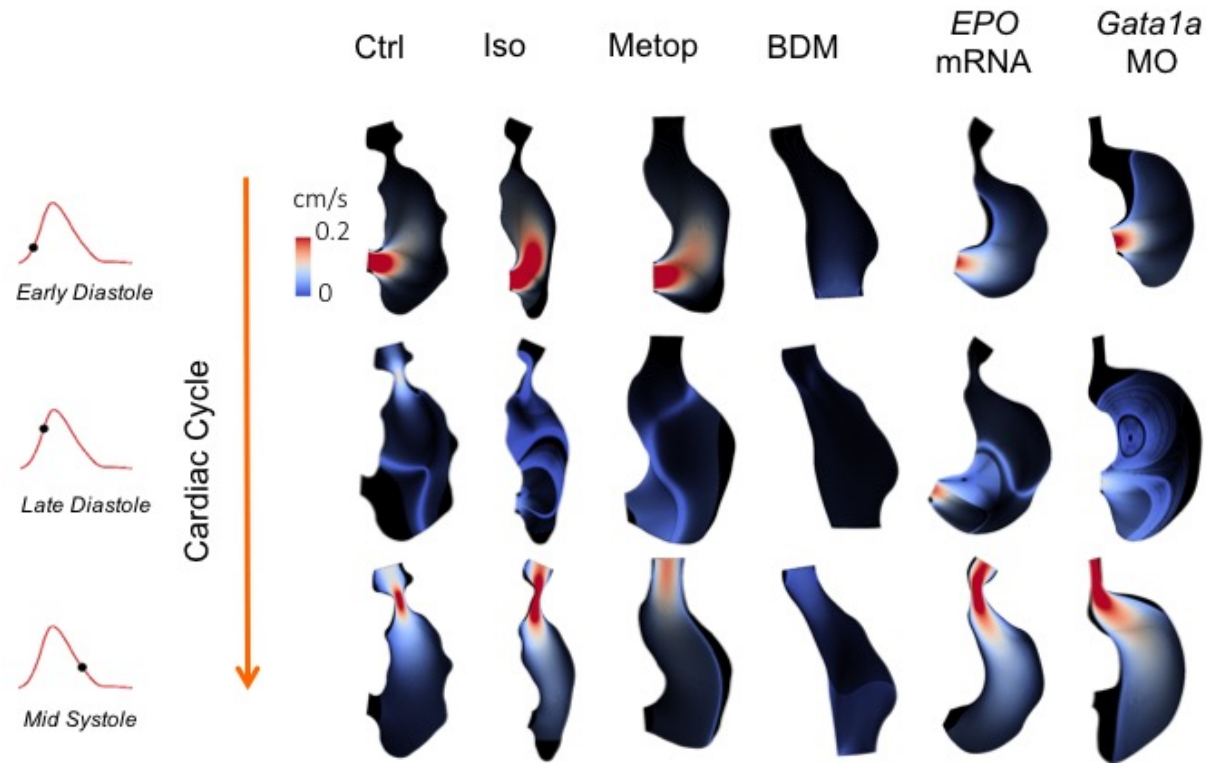


**Figure S1:** Velocity streamlines within the embryonic zebrafish ventricle. Moving-domain CFD was used to analyze images obtained from the light-sheet fluorescence microscopic imaging system and to model velocity streamlines within the *Tg(fli1a:GFP)* zebrafish ventricle in Early Diastole (top row), Late Diastole (middle row), and Mid Systole (bottom row) for control, isoproterenol, metoprolol, and BDM-treated embryos, as well as *EPO* mRNA and *Gata1a* MO micro-injected embryos, at 56 hpf. The outflow tract (OFT) is depicted by the black arrowhead, and the direction of blood flow is depicted by the red arrows. The figures shown are from representative samples from each group, with n=5 per group.

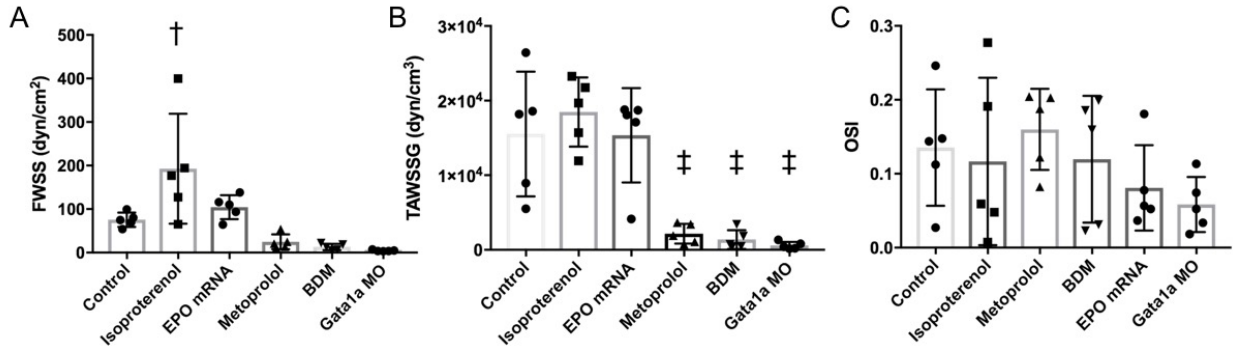


**Figure S2:** Moving-domain 2-D CFD assessment of wall shear stress properties within the developing OFT. Comparisons of the **(A)** FWSS, **(B)** TAWSSG, and **(C)** OSI between the treatment groups (n = 5 per group). Data are represented as means ± SD; * $p<0.05$, †$p<0.01$, ‡$p<0.001$, one-way ANOVA with Dunnett's multiple comparisons test.

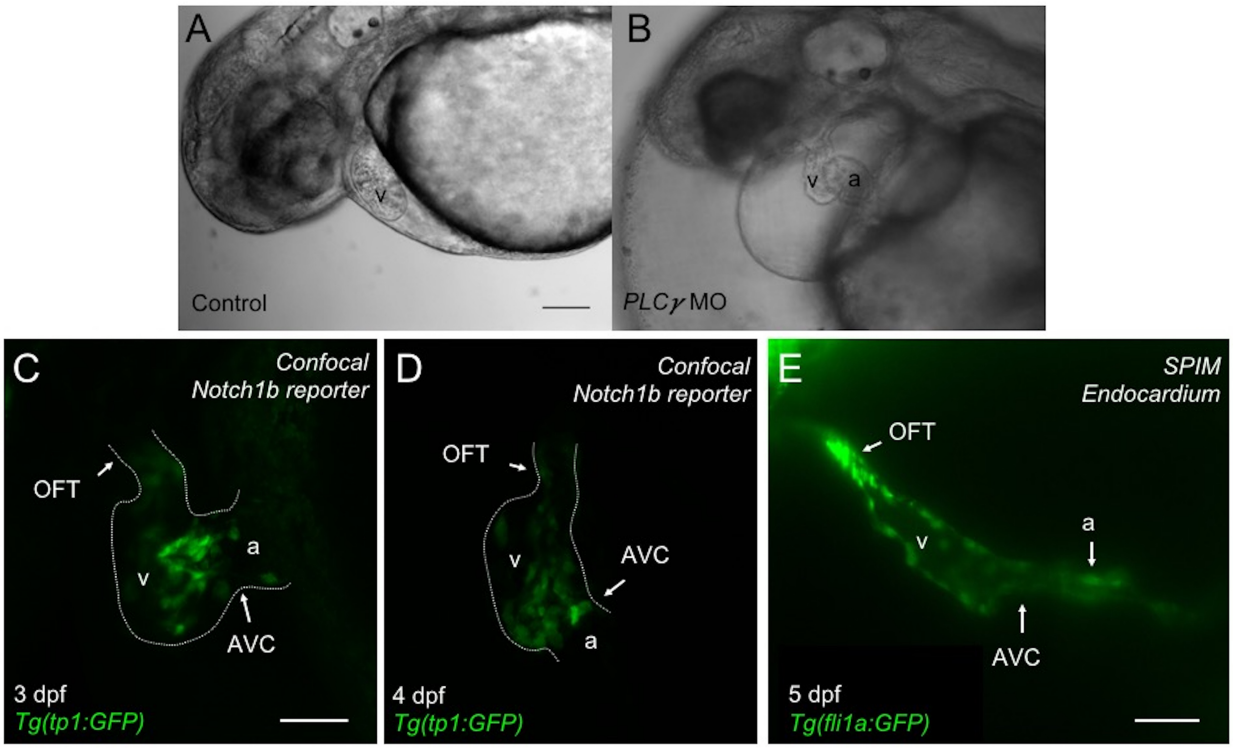


**Figure S3:** Microscopic images of zebrafish embryos showing inhibition of ventricular contractility with *PLCγ1* inhibition. Brightfield microscopic images (10X) at 48 hpf of **(A)** Control and **(B)** *PLCγ1* MO-injected embryos. Scale bar, 100 μm. **(C-D)** Confocal microscopy of *PLCγ1* MO-injected transgenic *Tg(tp1:GFP)* embryos at **(C)** 3 dpf and **(D)** 4 dpf, demonstrating minimal *Notch1b* activity in the ventricle (v) and outflow tract (OFT). The atrium (a) and atrioventricular canal (AVC) are shown. Scale bar, 50 μm. **(E)** SPIM image of a *PLCγ1* MO-injected transgenic *Tg(fli1a:GFP)* embryo, demonstrating the endocardium of the embryonic heart. Notably, no valves are seen in the OFT or AVC. Scale bar, 50 μm.

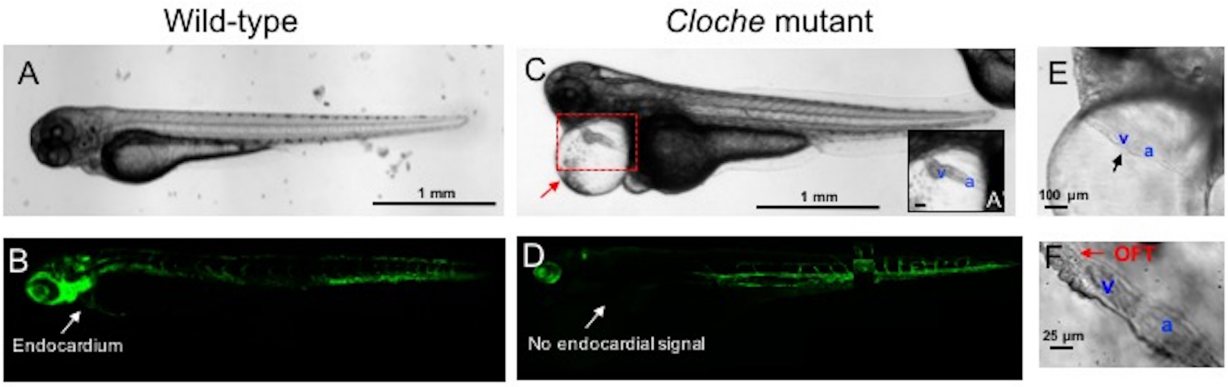


**Figure S4:** Depletion of endocardial lining in the *cloche* mutant results in the absence of valvular structure. Brightfield and fluorescence microscopic images (2X) at 3 dpf of embryonic wild-type **(A-B)** and *cloche* mutants **(C-D)** that were crossed with transgenic *Tg(fli1a:GFP)* the zebrafish line. *Cloche* mutants developed marked pre-cardiac edema (red arrow in **C**). The *fli1a* reporter is present in the vascular endothelium and endocardium, and it is notably absent in the *cloche* heart due to the absence of the endocardium. **(E)** Magnified (10X) image of the developing heart at 5 dpf, with visualization of the ventricle (v) and atrium (a) and absence of valvular structures. **(F)** Magnified (40X) brightfield image of the developing heart at 5 dpf, demonstrating an absence of cardiac looping, hypoplastic cardiac chambers, and an underdeveloped outflow tract (OFT). Scale bar, 25 µm.

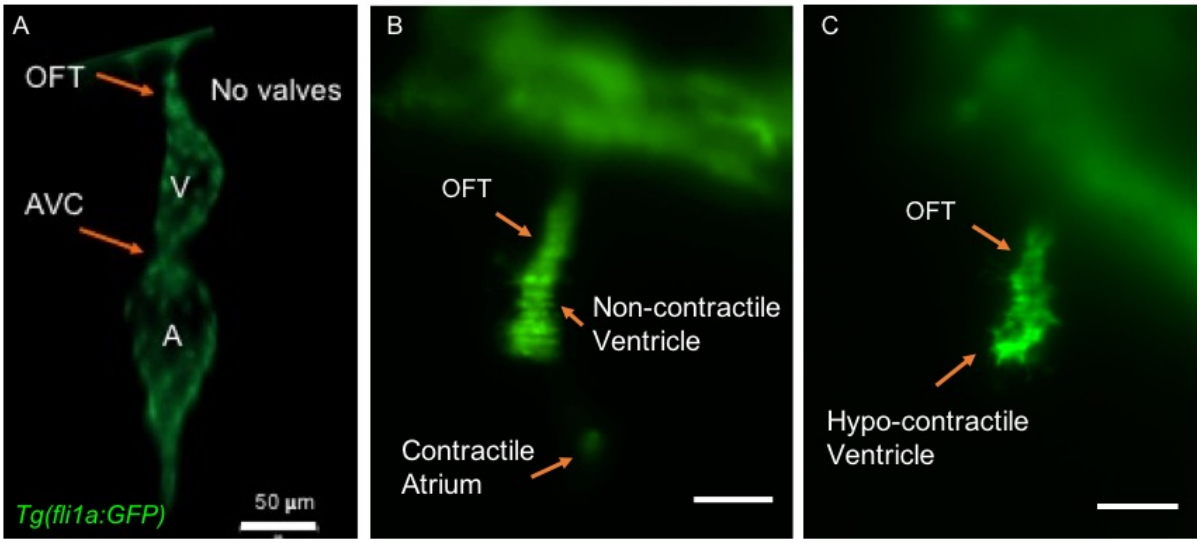


**Figure S5:** Activating Notch signaling is unable to rescue valve formation in non- and hypo-contractile conditions. **(A)** *Tnnt2a* MO and *NICD* mRNA: 3-D reconstruction of SPIM-acquired images of an embryonic zebrafish heart at 5 dpf, which was co-injected with *Tnnt2a* MO and *NICD* mRNA. Image demonstrates absence of cardiac looping, hypoplastic cardiac chambers (V, ventricle; A, atrium), underdeveloped outflow tract (OFT), and absence of cardiac valves. **(B)** *Plcγ1* MO and *NICD* mRNA. 2-D optical slice through the ventricle and OFT of embryo co-injected with *Plcγ1* MO and *NICD* mRNA. In the presence of a contractile atrium but non-contractile ventricle, no valve forms in the OFT. **(C)** BDM and *NICD* mRNA. 2-D optical slice through the ventricle and OFT of embryo injected with *NICD* mRNA and treated with BDM. In the presence of a hypo-contractile ventricle, no valve forms in the OFT. Images are representative images of n = 5 samples for each group. Scale bars, 50 µm.