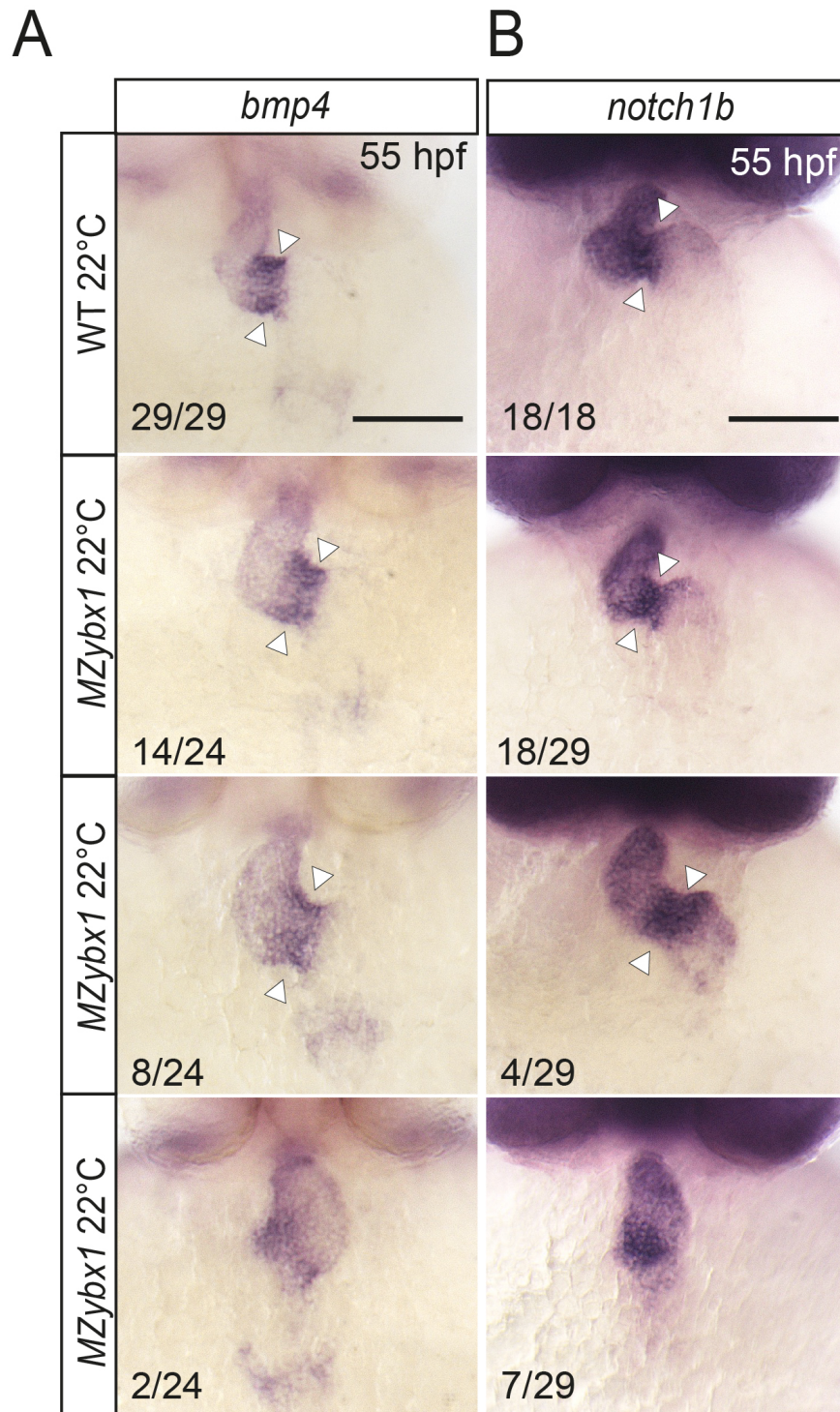


**Fig. S1. Total Spaw-GFP fusion protein fluorescence levels are similar in wild type and ybx1 mutant embryos.**

Quantification of fluorescence levels across the entire blastoderm at 1K, sphere and 30% epiboly; Spaw-GFP signal was normalised to mCherry mRNA reporter control.



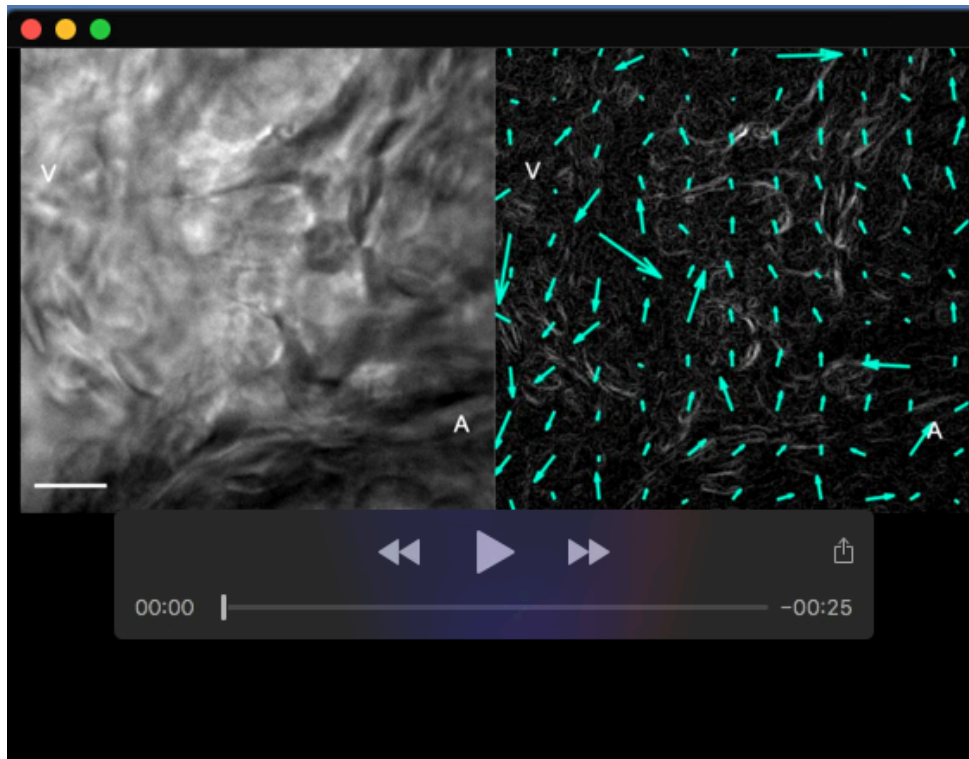
**Fig. S2. The heart appears enlarged in MZybx1 mutant embryos at 5 dpf**

- A) WISH showing *bmp4* expression in wild type and MZybx1 mutant embryos. White arrowheads point to the AV canal. Scale bar, 100  $\mu$ m.
- B) WISH with *notch1b* probe, showing expression in the AV canal at 55 hpf in wild type and MZybx1 mutant embryos. White arrowheads point to the AV canal, scale bar, 100  $\mu$ m.

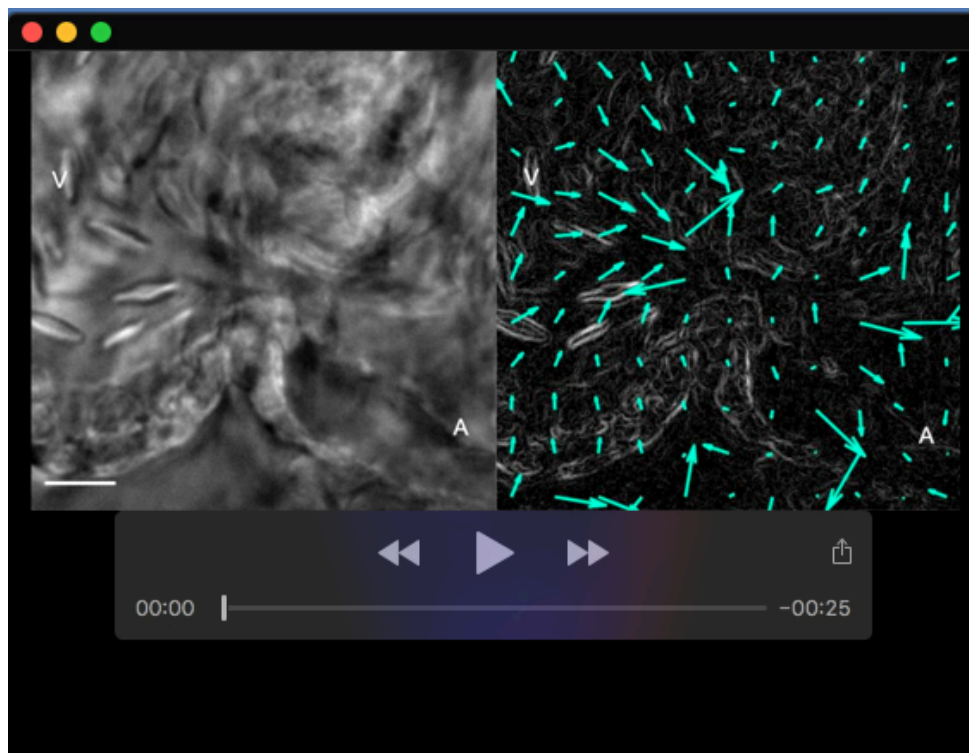
**Table S1.** List of primers and guide RNAs

Available for download at

<https://journals.biologists.com/dev/article-lookup/doi/10.1242/dev.201657#supplementary-data>



**Movie 1. Blood flow is altered in MZybx1 mutants compared to wild type embryos.** DIC movie of heart beating in a wild type embryo at 5 dpf (left); PIV analysis (right) shows direction of blood flow, cyan arrows indicate the red blood cells. Images captured at 13 frames per second (fps), V marks ventricle and A marks atrium; Scale bar, 20  $\mu$ m.



**Movie 2. Blood flow is altered in MZybx1 mutants compared to wild type embryos.** DIC movie of heart beating in an MZybx1sa42 mutant embryo at 5 dpf showing retrograde blood flow (left). PIV analysis (right) shows the movement of red blood cells. Images were captured at 13 fps; V marks ventricle and A shows atrium; Scale bar, 20  $\mu$ m