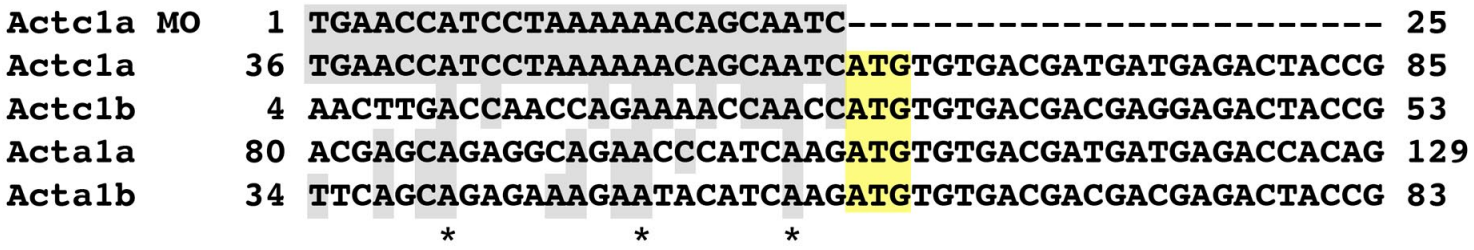
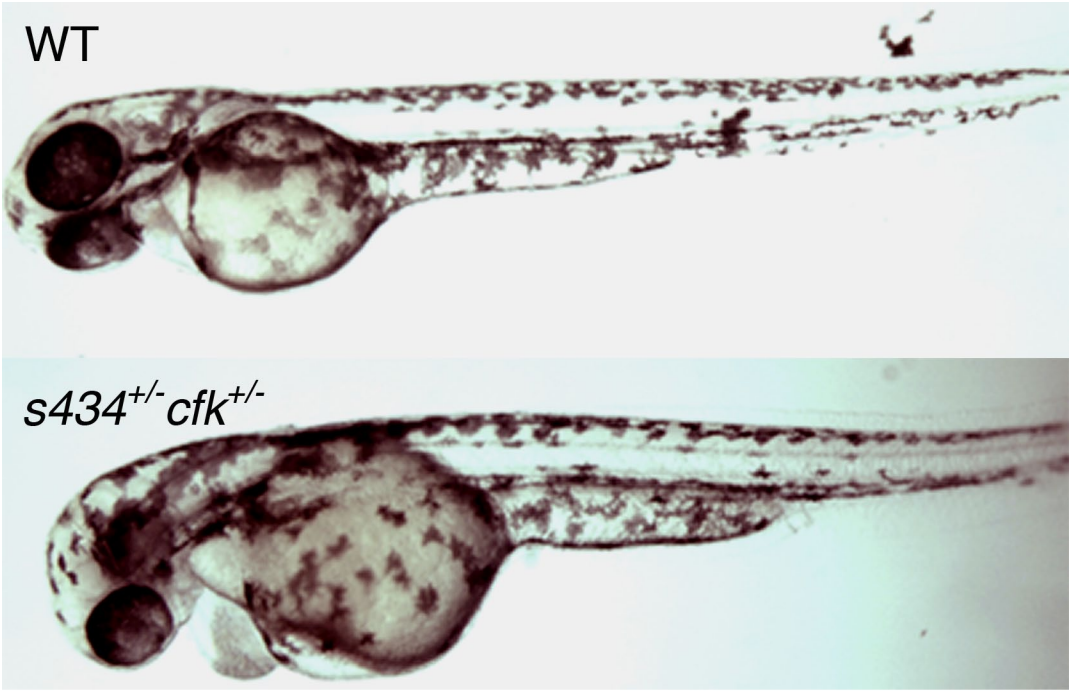
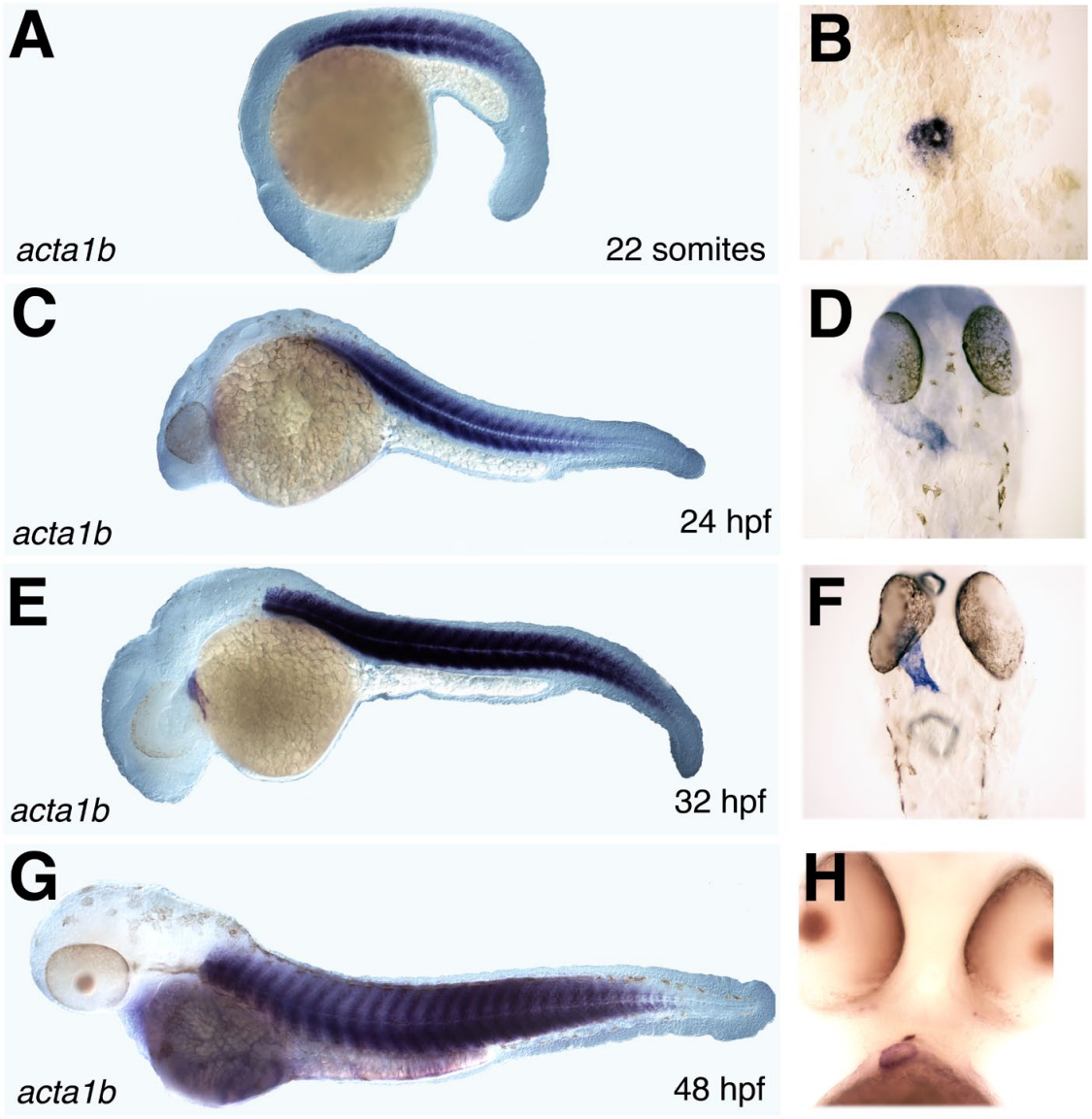


Supplemental Figure 1. Actc1a MO does not target other actin muscle isoforms.
This alignment demonstrates that the translation-blocking MO designed against actc1a in the 5' UTR does not target other actin isoforms. The actc1a MO only shares 40% similarity with a copy of cardiac actin, actc1b, and only 32% and 36% with skeletal actins acta1a and acta1b, respectively. The start site of each actin isoform is highlighted in yellow.



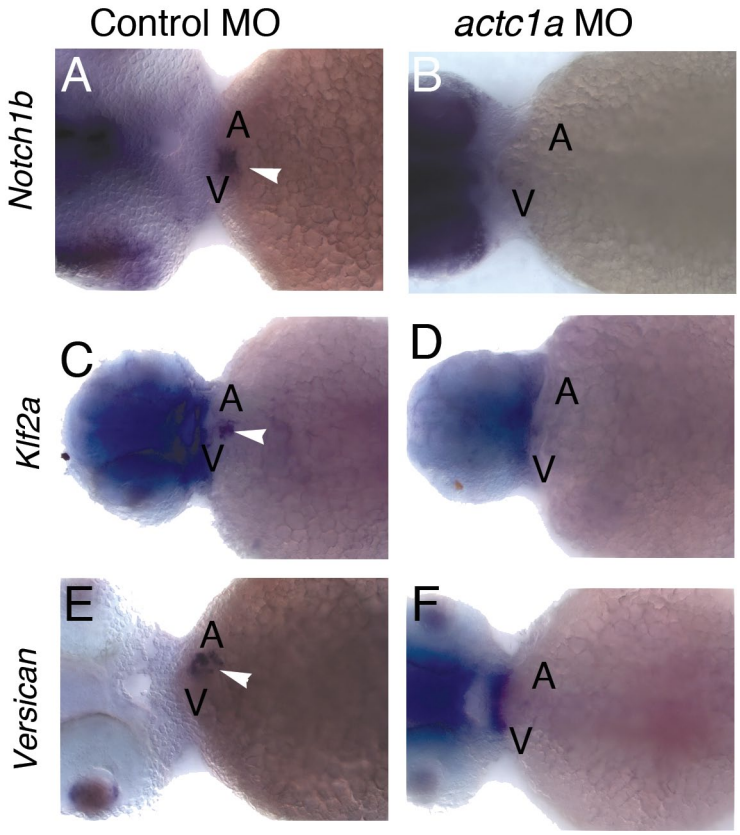
Supplementary Figure 2. The actc1as434 and cfk mutants fail to complement each other

cfk+/-, actc1a+/- double heterozygous embryos display a heart-specific phenotype including pericardial edema, similar to *actc1a*^{s434} mutants (bottom panel). Heterozygous carriers for the *actc1a*^{s434} and cfk mutation were bred and one quarter of the resulting progeny displayed the phenotype shown at 72 hpf.



Supplementary Figure 3. The expression domain of *cfk*, much like that of *actc1a*, is restricted to cardiac and somitic muscle.

(A-H) As analyzed by in situ hybridization, *cfk* transcript is expressed within the somitic muscle and in the heart tube at the 22-somites (A-B), 24hpf (C-D), 32hpf (E-F) and 48hpf stages (G-H). Expression of *cfk* does not appear to be as strong as actc1a expression at these stages in the heart, although cfk expression does become restricted to the ventricle at 48hpf, much like *actc1a* expression. (B,D,F,H) Ventral view of the heart field, anterior is toward the top.



Supplementary Figure 4. Actc1a MO Injected Morphant Embryos display improper AVC differentiation

(A, B) *notch1b* is localized to the AVC in the wild type embryos (A), however it appears absent in the morphants at 55 hpf (B). (C, D) *klf2a* expression is restricted to the AVC in the wild type embryos (C) at 55 hpf, however it is lost in the morphants (D). (E, F) *versican* transcript is also localized to the AVC in the wild type embryos (E) at 55 hpf, however it appears absent in the morphants (F).

SUPPLEMENTAL MOVIE LEGENDS

Supplementary Movies 1 and 2.

Movie 1 shows normal heart function of a wild type embryo at 48 hpf. Note the circulating blood cells throughout the head vasculature and body. Movie 2 shows the *s434* heart, which does not circulate blood and regurgitates blood from the ventricle to the atrium.

Supplementary Movies 3 and 4.

Movie 3 shows a high-speed movie of a wild type embryo at 48 hpf, demonstrating normal heart function and some regurgitant flow, which is quantified in Figure 10D as an RFF value. Movie 4 shows a mutant embryo at 48 hpf, which has a higher RFF value than the wild type, quantified in Figure 10D.

Supplementary Movie 5.

Movie 5 shows the swimming and locomotion activity of wild type (Wt) and s434 mutant (s434-/-) embryos at 72 hpf. When they are startled, either by shaking the dish or by touching with a glass pipette, wt embryos scatter quickly to the periphery of the petri dish. s434-/- mutant embryos also move away from the center of the dish when startled, however they do not swim as far as their wild type siblings. This is likely because mutant embryos have a severe edema, due to the lack of circulation.