Sedletcaia, A. and Evans, T. (2011). Heart Chamber Size in Zebrafish is Regulated Redundantly by Duplicated Tbx2 Genes. Dev. Dyn. 240: 1548-1557.

Supplemental Information

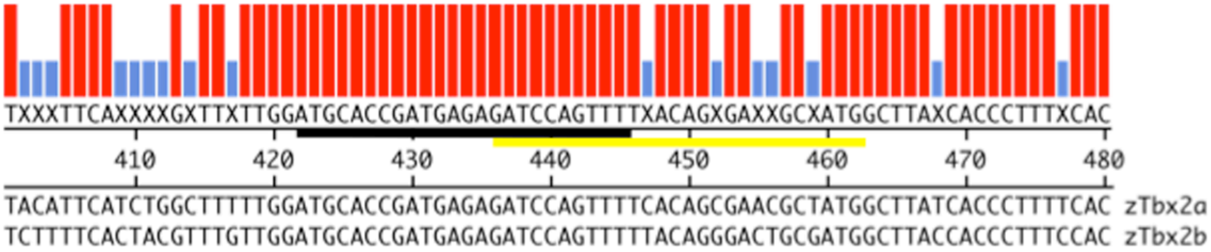

Fig. S1. Design of a single morpholino to inhibit expression of both *tbx2 genes*. Shown is the DNA sequence alignment of *tbx2a* and *tbx2b* around the region of the initiation ATG (located at 430, in this arbitrary numbering scheme). The sequence chosen for targeting by MO2ab is indicated by the black line, and is 100% conserved for both genes. The MO2a target site is represented by the yellow bar, with significant mis-matches to the *tbx2b* gene.

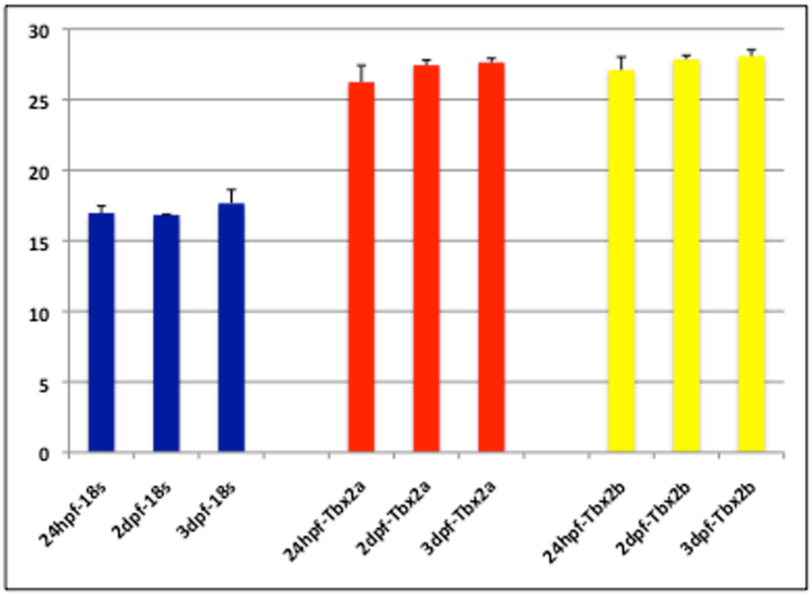

Fig. S2. Both Tbx2 genes are expressed by 24 hpf. Shown are the results of qPCR assays from three independent experiments. Plotted on the Y axis are the median Cp values for 18S rRNA, *tbx2a*, or *tbx2b* transcripts at 24 hpf, 1 dpf, or 3 dpf. We consistently detect transcripts for each *tbx2* gene around 27 cycles, even at 24 hpf. As discussed in the text, we also cloned out both cDNAs from purified 24 hpf cardiomyocytes and quantified levels readily from 24 hpf cardiomyocytes by RNA-sequencing (not shown).

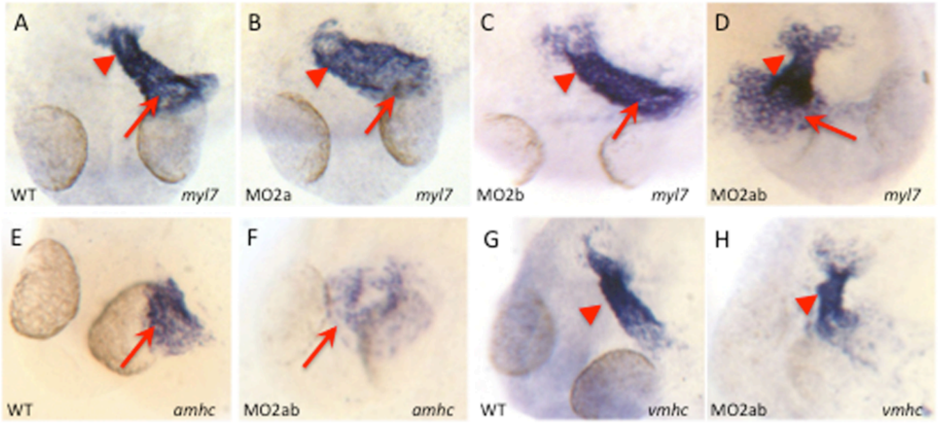

Fig. S3. Some morphological disruption can be noted in the tbx2ab double morphants at 24 hpf. Shown are representative embryos following *in situ* hybridization to detect transcript patterns for *myl7* in cardiomyocytes of wildtype (A), the *tbx2a* morphant (B), the *tbx2b* morphant (C), or the *tbx2ab* double morphant (D) at 24 hpf. Bottom panels compare *amhc* expression in the presumptive atrium of wildtype (E) or *tbx2ab* morphant embryos (F), and *vmhc* expression in presumptive ventricle of wildtype (G) or *tbx2ab* morphant embryos (H). Arrows mark the presumptive atrial domain and arrowheads mark the presumptive ventricular domain. Note particularly the expanded atrial domain marked by the pattern of markers in the double morphants. For each panel n is at least 10 embryos.

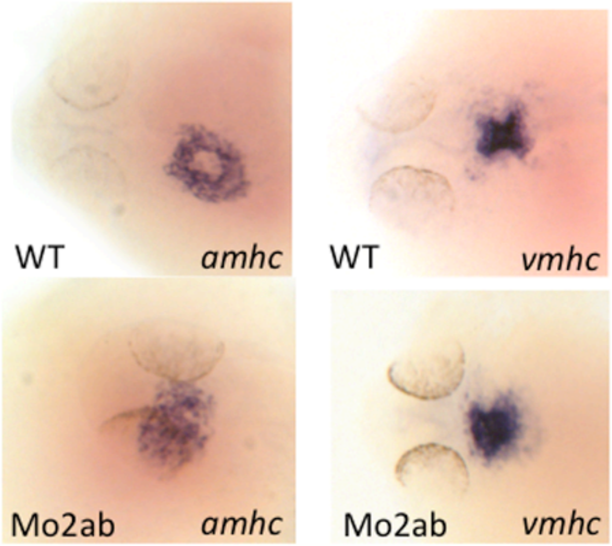

Fig. S4. Chamber-specific progenitors appear to be normal at the pre-heart tube stage in *tbx2ab* morphant embryos. Shown are representative embryos (n is at least 10 for each) following *in situ* hybridization to detect transcript patterns for atrial-specific *amhc* and ventricular-specific *vmhc* at 19.5 hpf cardiac cone stage, comparing wildtype (WT) and double morphant (Mo2ab) embryos, as indicated. It does not appear that progenitor domains are disturbed in the morphant embryos.

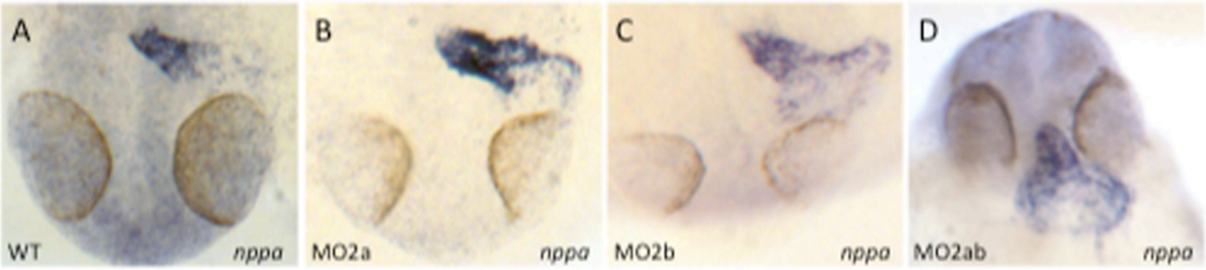

Fig. S5. Expression pattern for nppa transcripts levels are enhanced at 24 hpf in the tbx2a morphant. Shown are representative embryos following in situ hybridization analysis to detect the nppa transcript patterns. Compared to wildtype (A) the transcript levels appear enhanced in the tbx2a morphant (B) but not the tbx2b (C) or double morphant (D).