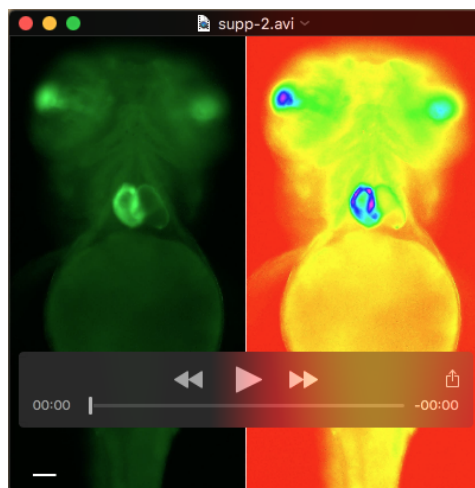
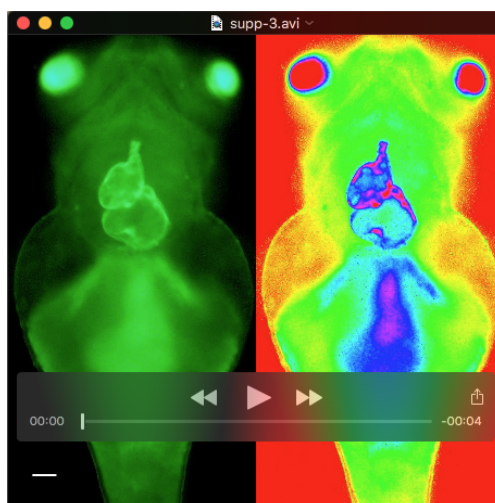


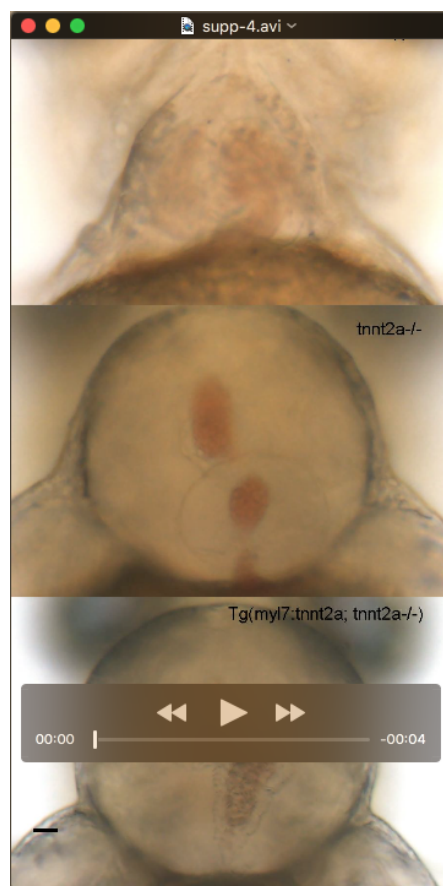
## Supplemental information



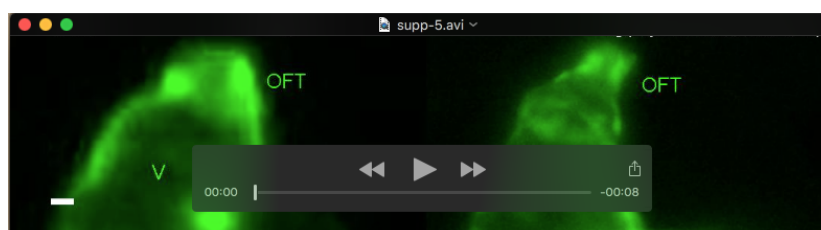
**Movie 1.** Time-elapse video and pseudo-colors of a 3 dpf *Tg(bactin2:GCamp6s); tnnt2a<sup>+/+</sup>* larva from the ventral view.



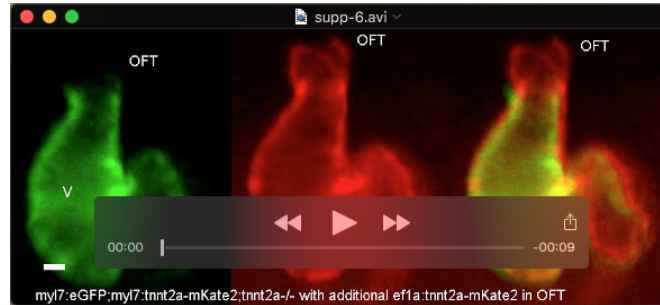
**Movie 2.** Time-elapse video and pseudo-colors of a 3 dpf *Tg(bactin2:GCamp6s); tnnt2a<sup>-/-</sup>* larva from the ventral view.



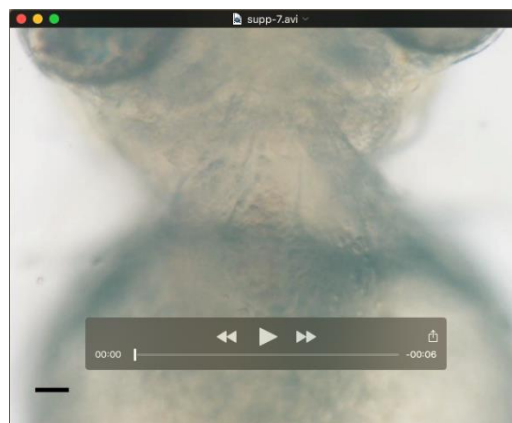
**Movie 3.** Bright field video of the heart regions of wildtype, *tnnt2a<sup>-/-</sup>*, and *Tg(myl7:tnnt2a); tnnt2a<sup>-/-</sup>* zebrafish at 3 dpf.



**Movie 4.** Time-elapse video of OFT behavior in wildtype and *Tg(myl7:tnnt2a); tnnt2a<sup>-/-</sup>* zebrafish at 3 dpf.



**Movie 5.** Time-elapse video of the heart region in a representative *Tg(myl7:tetOn; tre:tnnt2a-p2a-mKate2; myl7:eGFP); tnnt2a<sup>-/-</sup>* larvae carrying additional red fluorescence of mKate2 (and Tnnt2a) in the OFT (without overlapping green fluorescence).



**Movie 6.** Bright field video of a representative double-rescued *tnnt2a<sup>-/-</sup>* heart that was fully functional.

**Supplementary Table****Table S1. Fold changes of sarcomere and valve or OFT relative genes of wildtype and mutant zebrafish in RNA-seq**

	<i>tnnt2a</i> <sup>+/+</sup>	<i>tnnt2a</i> <sup>-/-</sup>	log <sub>2</sub> FoldChange ( <i>tnnt2a</i> <sup>-/-</sup> / <i>tnnt2a</i> <sup>+/+</sup> )	P value	FDR
<i>tnnt2a</i>	7.42	0.3	-4.6283848	3.44E <sup>-13</sup>	5.14E <sup>-12</sup>
<i>actc1a</i>	354.27	397.98	0.16784663	6.54E <sup>-06</sup>	4.06E <sup>-05</sup>
<i>myl7</i>	20.89	28.05	0.42518828	0.009689	0.0284507
<i>tnni1b</i>	6.91	10	0.53324238	0.342696	0.4976011
<i>tpm4a</i>	82.32	112.57	0.45150751	1.74E <sup>-13</sup>	2.66E <sup>-12</sup>
<i>tnnc1a</i>	10.89	9.5	-0.1970045	0.221544	0.3629792
<i>has2</i>	1.02	0.9	-0.1805722	0.704468	0.8029169
<i>tbx20</i>	2.24	2.38	0.08746284	0.807426	0.8733331
<i>gata4</i>	2.24	7.11	1.66635083	1.1E <sup>-05</sup>	6.75E <sup>-05</sup>
<i>gata5</i>	6.83	7.19	0.07410619	0.723448	0.8153896
<i>tbx2b</i>	27.77	23.48	-0.2420948	0.009189	0.0271485
<i>bmp2b</i>	12.42	9.23	-0.4282626	0.016952	0.045948

**Table S2. Fold changes of sarcomere and valve or OFT relative genes of wildtype and transgene rescue zebrafish in RNA-seq**

	<i>tnnt2a</i> <sup>+/+</sup>	Tg( <i>myl7:tnnt2a</i> ); <i>tnnt2a</i> <sup>-/-</sup>	log <sub>2</sub> FoldChange (Tg; <i>tnnt2a</i> <sup>-/-</sup> / <i>tnnt2a</i> <sup>+/+</sup> )	P value	FDR
<i>tnnt2a</i>	7.42	5.25	-0.4991018	0.105368	0.2108288
<i>actc1a</i>	354.27	368.28	0.05595375	0.248902	0.40047213
<i>myl7</i>	20.89	21.1	0.01443051	0.980672	0.98679463
<i>tnni1b</i>	6.91	7.47	0.11242253	0.286924	0.44193824
<i>tpm4a</i>	82.32	112.5	0.45061011	7.54E <sup>-13</sup>	1.05E <sup>-11</sup>
<i>tnnc1a</i>	10.89	8.87	-0.2959979	0.468862	0.63137703
<i>has2</i>	1.02	0.51	-1	0.0677928	0.148043
<i>tbx20</i>	2.24	2.65	0.24249363	0.50083	0.65227157
<i>gata4</i>	2.24	7.69	1.77948487	2.03E <sup>-06</sup>	1.37E <sup>-05</sup>
<i>gata5</i>	6.83	9.1	0.41398097	0.029981	0.07629847
<i>tbx2b</i>	27.77	27.61	-0.0083363	0.869186	0.92671892
<i>bmp2b</i>	12.42	12.15	-0.0317089	0.803724	0.87933139

**Table S3.** Raw data of the RNA-seq in *tnnt2a*<sup>+/+</sup>; *tnnt2a*<sup>-/-</sup>; Tg(*myl7:tetOn;tre:tnnt2a-p2a-mKate2; myl7:eGFP*); *tnnt2a*<sup>+/+</sup>; Tg(*myl7:tetOn;tre:tnnt2a-p2a-mKate2; myl7:eGFP*); *tnnt2a*<sup>-/-</sup> at 3 dpf.

[Click here to Download Table S3](#)

**Table S4. Primers using in the construction of transgenic zebrafish**

Primers	Sequence
pPCRII_ <i>tmt2a</i> cDNA Forward	atgtcagacaacgaagaagt
pPCRII_ <i>tmt2a</i> cDNA Reverse	cttcctgaggcctcgcttggttct
pENTR-5'- <i>cmlc2</i> Forward	aaagcttaaatcagttgtgt
pENTR-5'- <i>cmlc2</i> Reverse	atggtcactgtctgctttg
pENTR-Tetone First Forward	ggggacaagttgtacaaaaagcaggctggaccatgtctagactggacaagagc
pENTR-Tetone First Reverse	tcttatcatgtctagatcttaccggggagcatgcaa
pENTR-Tetone Second Forward	gacatgctccccgggtaagatctagacatgataagatacatt
pENTR-Tetone Second Reverse	aaggcctcatcgataaactgtttattgcagctta
pENTR-Tetone Third Forward	agctgcaataaacaagttatcgatgaggcccttctgt
pENTR-Tetone Third Reverse	ggggaccactttgtacaagaaagctgggtgcccgatccaagtataaga

**Table S5. Real time RT-PCR primers list**

<b>Gene</b>	<b>Forward primers</b>	<b>Reverse primers</b>
<i>tnnt2a</i>	gcgacagaagcgtttggag*	ctgagagcagattcattggca
<i>actc1a</i>	tgatgagactaccgcactcg	gagcctcgtctcccacatag
<i>myl7</i>	gaaccgggatggagttatca	tgggtcattagcagcctctt
<i>tnni1b</i>	gctggcagataaggaagacg	tgaggggtaaattcaaacgc
<i>tpm4a</i>	tgacttctttggacgctgtg	gcggttctcgattacctca
<i>myh7</i>	atthtgggtgccagtggaaag	cctccctctgcttctgtttg
<i>tnnc1a</i>	gaaccctaccctgaagagc	ttttgtctccgtccctcatc
<i>mybpc3</i>	ccaaggaggactgtggacat	ccagatgaatcttgggagga
<i>has2</i>	aggtcatgtacaccgccttc	caagggtccgctgatacatt
<i>gata4</i>	gctcctctgaaggctcagtcg	cagaatcaggctgttccaca
<i>gata5</i>	cgacaacactgtggaggaga	gtagcgccagacactgttga
<i>gata6</i>	tatgtcacaccgcagctctc	agacatgcgcttctgaggtt
<i>tbx20</i>	gtttgccaaggttcagag	gctgagggagaggttgcag
<i>tbx2a</i>	gacgactcatcttgcgaaca	tctgcttcttttggagga
<i>tbx2b</i>	gcagagatgctggcattca	tcgtcttcttctccgcaat
<i>tbx1</i>	gtttgccaaggttcagag	ctggtgagtggtgcagaat
<i>bmp2b</i>	ccagcagagcaaacacgata	agccactcgtactggcatct
<i>notch1b</i>	cctggatgactgtcagagca	aagccattgactccatccac

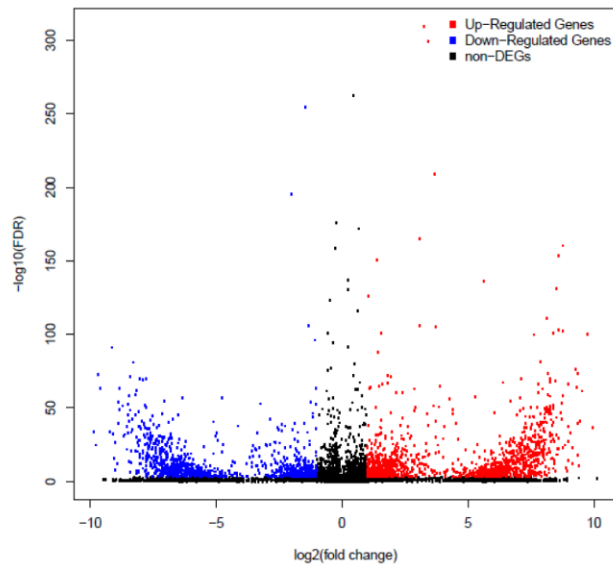
\*: *tnnt2a* qPCR forward primer spans the mutation site.

## Supplementary Figures

### Figure S1

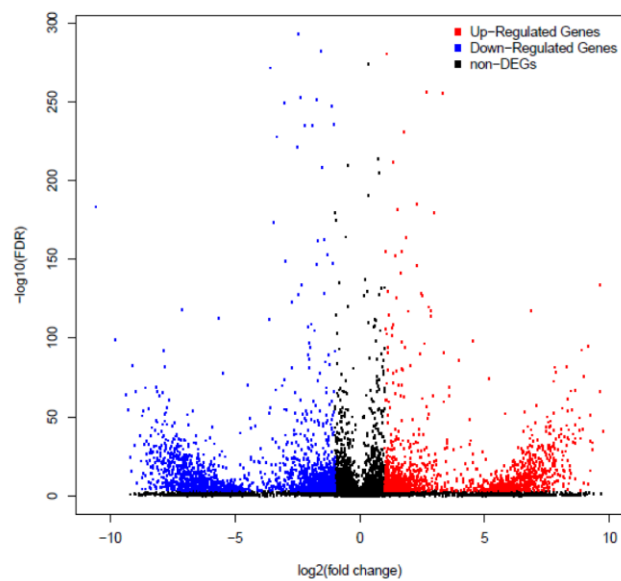
A

volcano plot of 3dpf *tnnt2a*<sup>-/-</sup> vs. Tg(*tnnt2a*);*tnnt2a*<sup>-/-</sup> RNA-seq



B

volcano plot of 3dpf *tnnt2a*<sup>+/-</sup> vs. *tnnt2a*<sup>-/-</sup> RNA-seq



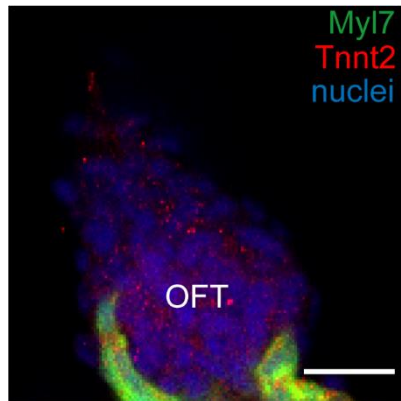
### Figure S1 Volcano plot of RNA-seq

A: Volcano plot of differential expressed genes in *tnnt2a*<sup>-/-</sup> vs Tg(*myl7:TetOn;tre:tnnt2a-mKate2;myl7:eGFP*);*tnnt2a*<sup>-/-</sup>

B: Volcano plot of differential expressed genes in *tnnt2a*<sup>+/-</sup> vs *tnnt2a*<sup>-/-</sup>



**Figure S2**



**Figure S2** Cytoplasm localization of Tnnt2 in OFT

Immunofluorescence of Tnnt2 and Myl7 staining in wildtype zebrafish heart outflow tract at 5 dpf: nuclei is stained by Hoechst 33342, OFT: outflow tract; bar: 25  $\mu$ m