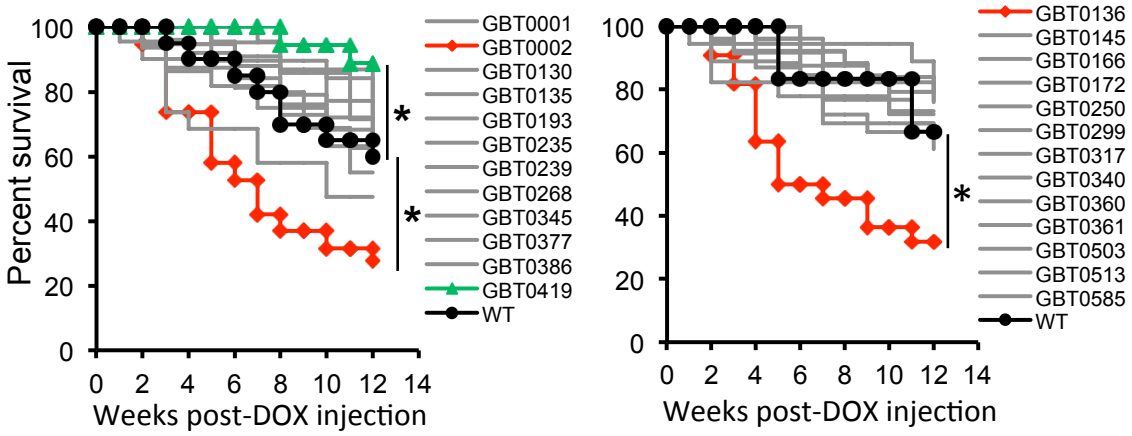
Supplemental Table

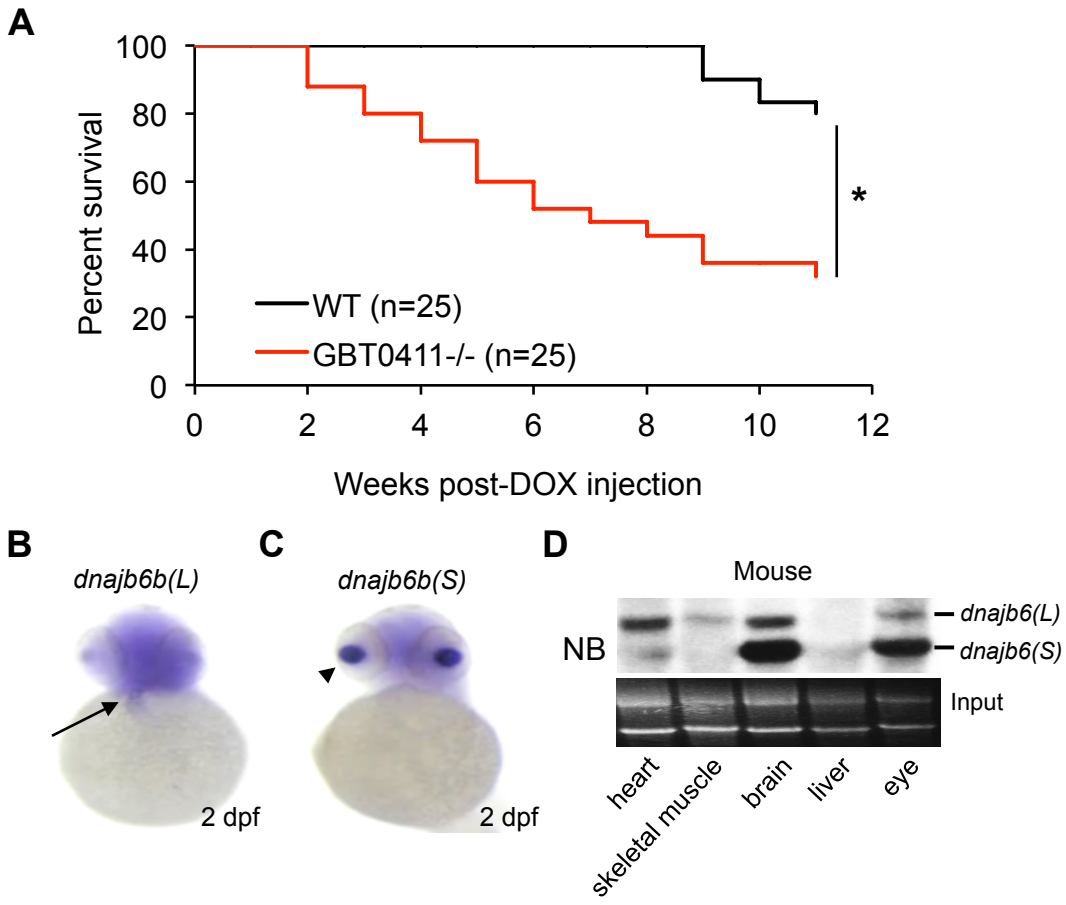
Screening method	Total GBT lines screened	Total ZIC mutants	GBT number of ZIC mutants
Screening for mutants with positive mRFP signal detected in embryonic hearts	405	27	0001*, 0002*, 0025, 0080, 0103, 0135, 0136, 0145, 0166, 0193, 0235, 0268, 0270, 0345, 0360, 0361, 0364, 0377, 0410, 0411, 0412, 0415, 0416, 0419, 0422, 0424, and 0425
Screening for mutants with positive mRFP signal detected in adult hearts	204	17	0130, 0159, 0172, 0239, 0249, 0250, 0298, 0299, 0317, 0340, 0386,, 0389, 0402, 0503, 0513, 0585, and 0589
Total	609	44	

Supplemental Table 1: Summary of the Zebrafish Insertional Cardiac (ZIC) mutant collection.
∗,Gene-breaking transposon (GBT) lines based on R15 vector, other lines are based on GBT-RP2 vector (14,15). For more information about these GBT lines, see www.zfishbook.org.

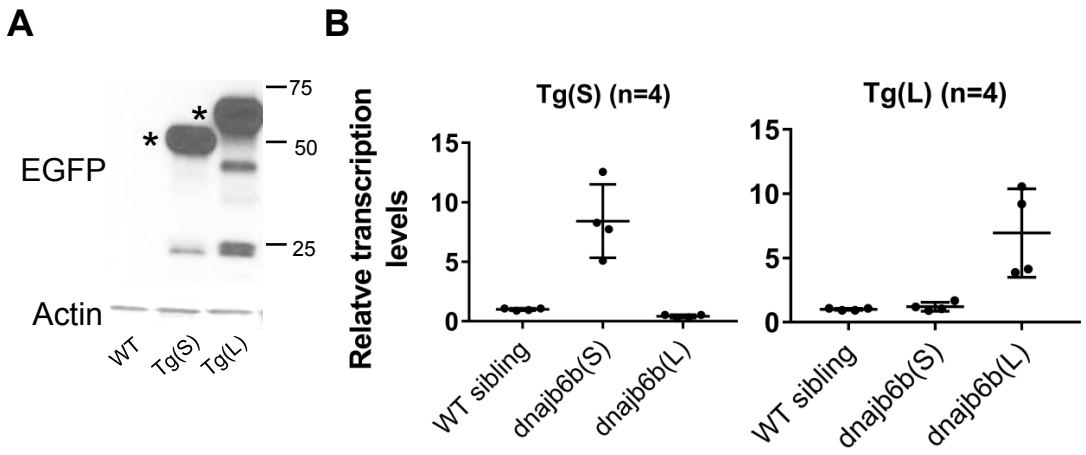
Supplemental Figures



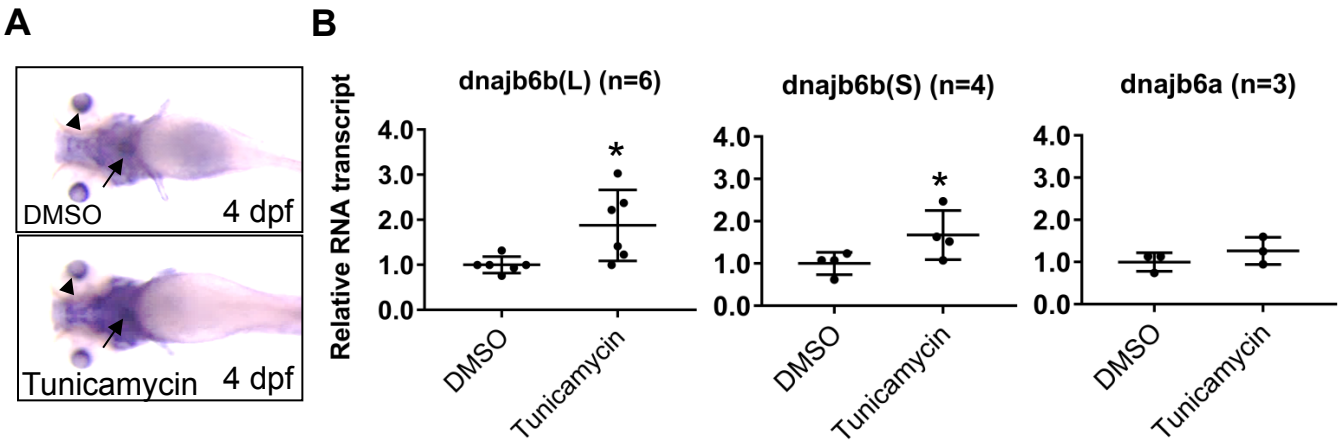
Supplemental Figure 1. A modifier screen of zebrafish insertional cardiac (ZIC) mutants identified 3 known cardiomyopathy-susceptibility genes. (**A**) Kaplan-Meier survival curves of adult wild type (WT) and gene-breaking transposon (GBT) heterozygous fish injected with a single bolus of 20 µg/gram body mass (gbm) doxorubicin (DOX). *GBT0002*/*sorbs2b* and *GBT0136*/*ano5a* were identified that accelerated DOX-induced fish death, while *GBT0419/rxraa* significantly reduced DOX-induced fish death (n=15-25). * *P*<.05 vs WT control, log-rank test.



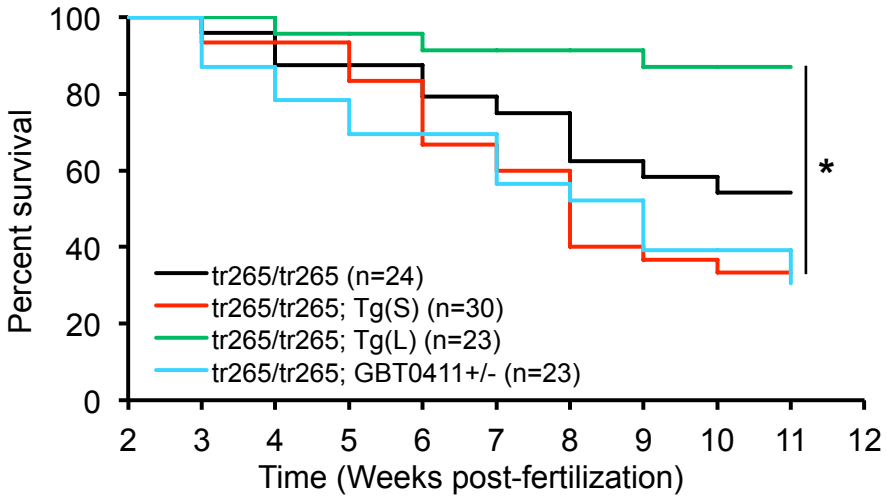
Supplemental Figure 2. Disruption of *dnajb6(L)*, the major isoform expressed in the heart, accelerated doxorubicin (DOX)-induced zebrafish death. (**A**) Kaplan-Meier survival curves of gene-breaking transposon (*GBT)0411-/-* and WT control fish injected with a single bolus of 20 µg/gbm DOX. * *P*<.05, log-rank test. (**B-C**) Whole mount In situ hybridization analysis of the *dnajb6b(L*) and *dnajb6b(S*) isoforms in zebrafish embryos at 2 days post-fertilization (dpf). Arrow indicates heart expression; arrowhead indicates lens expression. (**D**) Northern blot (NB) analysis of *dnajb6(S)* and *dnajb6(L)* isoforms in 3 months old WT C57BL/6 mouse tissues.



Supplemental Figure 3. Generation of cardiac- specific overexpression of *dnajb6b(S)* and *dnajb6b(L)* stable transgenic fish lines driven by the *cardiac myosin light chain-2* (*cmlc2)* enhancer. (**A**) Western blot analysis detected Dnajb6b(S)-EGFP and Dnajb6b(L)-EGFP fusion proteins from heart lysate of *Tg(cmlc2:dnajb6(S)-EGFP)* or *Tg(cmlc2:dnajb6(L)-EGFP*) transgenic fish. * indicate EGFP fusion proteins with expected size. (**B**) Quantitative reverse transcriptase polymerase chain reaction detected overexpression of *dnajb6b(S)* and *dnajb6b(L)* transcripts from corresponding transgenic fish hearts. Values are relative unit to WT sibling controls. Data represent mean ± SEM.



Supplemental Figure 4. *dnajb6b(L)* was activated transcriptionally upon tunicamycin treatment. (**A**) Whole-mount in situ hybridization analysis of *dnajb6b(S)* and *dnajb6b(L)* transcript levels in embryos with or without tunicamycin treatment at the concentration of 1 ?g/mL for 1 day from 3 to 4 days post-fertilization (dpf). Arrows indicate cardiac expression; arrowheads indicate lens expression. (**B**) Quantitative reverse transcriptase polymerase chain reaction analysis of the transcript levels of *dnajb6b(S)*, *dnajb6b(L)*, and *dnajb6a* in embryos at 4 dpf with or without tunicamycin at the concentration of 1 ?g/mL for 1 day from 3 to 4 dpf. *gapdh* was used as an internal input control. Values are relative unit to DMSO control. Data represent mean ± SEM. * $P<.05$, Student's *t* test.



Supplemental Figure 5. Overexpression of *dnajb6b(L)* in cardiomyocytes reduced the death rate in anemia-induced cardiomyopathy. Kaplan-Meier survival curves of *tr265/tr265* homozygous fish with or without double crossed with the cardiac-specific *Tg(cmlc2:dnajb6b(S)-EGFP)*, *Tg(cmlc2:dnajb6b(L)-EGFP),* or *GBT0411* heterozygous mutant. *tr265/tr265* is a zebraifsh homozygous mutant with a Band 3 mutation which results in anemia and cardiomyopathy due to disrupted erythrocyte formation (17). *tr265/tr265*; *Tg(cmlc2:dnajb6b(L)-EGFP)* versus *tr265/tr265* fish, * *P*<.05, log-rank test.