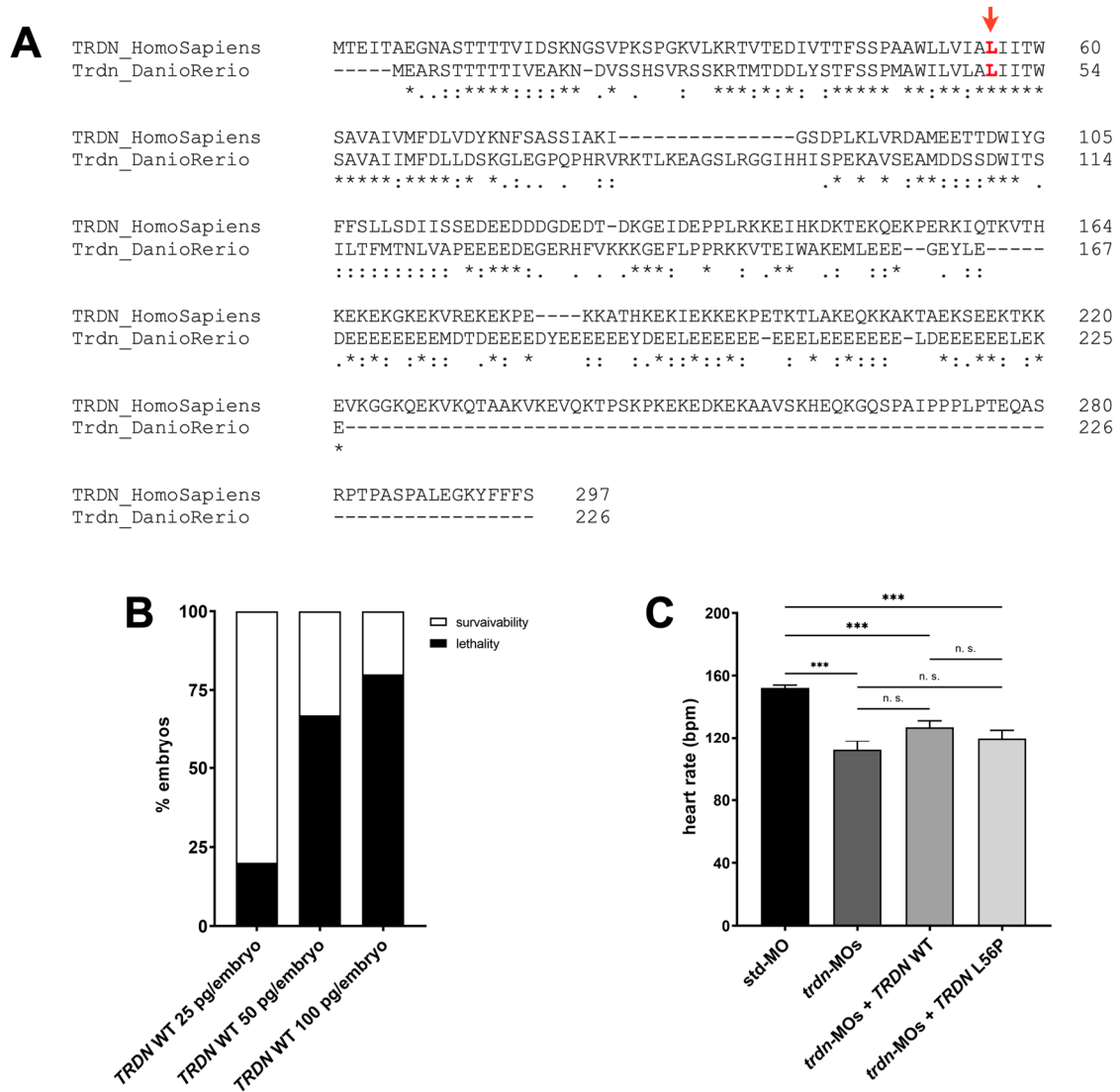
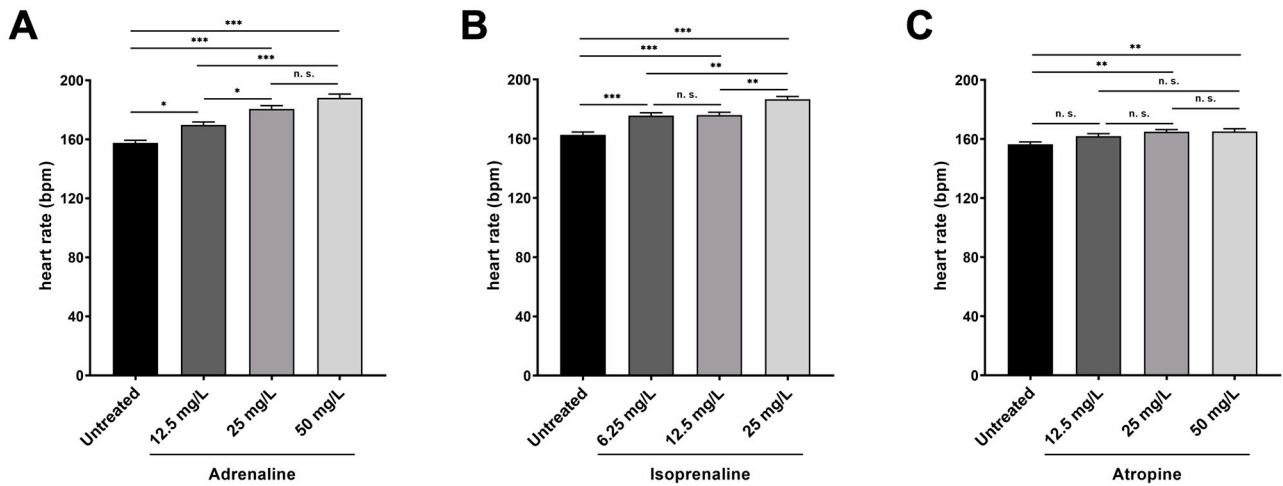


**Figure S1.** Validation of *trdn*-MOs efficacy and dose-dependency assays. (A) Schematic representation of splice-MO validation by RT-PCR. To assess dose-dependent effect of splice-MO, RT-PCR was performed by using forward and reverse primers annealing to *trdn* exon 1 and exon 2, respectively, so that both exon 1 skipping and intron 1 (> 9000 bp length) retention determined no amplification. F and R arrows show the position of forward and reverse primers, respectively. E1, exon 1; E2, exon 2; I1, intron 1. (B) RT-PCR performed on RNA extracted from zebrafish embryos injected with std-MO or *trdn* splice-MO at different concentrations (0.3, 0.6, 1.2 pmol/embryo). Zebrafish  $\beta$ -actin was used as an internal control in each sample. (C) Western blot analysis of Trdn expression in zebrafish embryos injected with std-MO or *trdn* ATG-MO at different concentrations (0.3, 0.6, 1.2 pmol/embryo). Tubulin was used as internal control in each sample. (D-E) Quantification of embryos phenotypes following *trdn* splice-MO (D) and ATG-MO (E) injection. Values indicate the number of embryos for each phenotype.



**Figure S2.** Human *TRDN* injection in zebrafish embryos. **(A)** Human and zebrafish cardiac Triadin amino acid sequences alignment. Asterisks represent fully conserved residues; colons indicate positions with conservation between amino acid groups of similar properties; periods indicate positions with conservation between amino acid groups of weakly similar properties. Mutated amino acid in the mutant human Triadin (L56P) is indicated in red. **(B)** Quantification of embryos lethality following human *TRDN* WT mRNA injection at different doses. **(C)** Quantification of heartrate in embryos at 48 hpf injected with std-MO, *trdn*-MOs, *trdn*-MOs + *TRDN* WT and *trdn*-MOs + *TRDN* L56P. At least 30 embryos were analyzed for each group. Values are expressed as mean  $\pm$  SEM. Std-MO: mean = 152, IQR = 12; *trdn*-MOs: mean = 110, IQR = 44; *trdn*-MOs + *TRDN* WT: mean = 127, IQR = 20; *trdn*-MOs + *TRDN* L56P: mean = 120, IQR = 44. \*\*\* $p$  < 0.001, n.s. = not significant, Kruskal-Wallis followed by Dunn's multiple comparisons test.



**Figure S3.** Dose-effect test for adrenaline, isoprenaline and atropine. Heartbeat count in 3 dpf zebrafish embryos untreated or treated with increasing doses of adrenaline (**A**), isoprenaline (**B**) and atropine (**C**). At least 30 embryos were analyzed for each group. Values are expressed as mean  $\pm$  SEM. (**A**) Untreated: mean = 158, IQR = 12; 12.5 mg/L adrenaline: mean = 170, IQR = 20; 25 mg/L adrenaline: mean = 181, IQR = 20; 50 mg/L adrenaline: mean = 188, IQR = 13. \* $p < 0.05$ , \*\*\* $p < 0.001$ , n.s. = not significant, Kruskal-Wallis followed by Dunn's multiple comparisons test. (**B**) Untreated: mean = 163, IQR = 8; 6.25 mg/L isoprenaline: mean = 176, IQR = 13; 12.5 mg/L isoprenaline: mean = 176, IQR = 13; 25 mg/L isoprenaline: mean = 187, IQR = 16. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , n.s. = not significant, Kruskal-Wallis followed by Dunn's multiple comparisons test. (**C**) \*\* $p < 0.01$ , n.s. = not significant, one-way ANOVA followed by Tukey post-hoc correction.