Supplementary Material

Figure S1

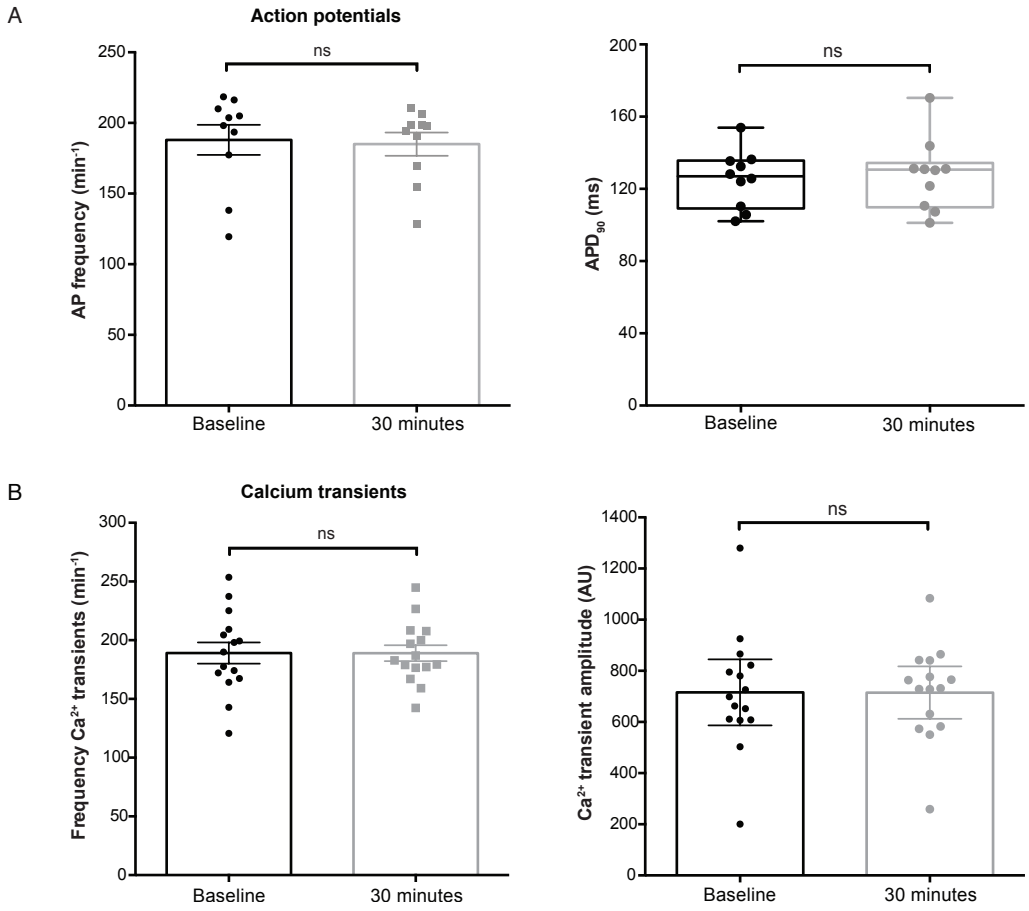

Figure S1. No effects of placebo treatment on cardiac electrophysiology and Ca^{2+} dynamics in embryonic zebrafish. Ventricular signals of 3 dpf chimeric VSFP-butterfly CY and GCaMP6f fish were recorded at baseline and after 30 min incubation in normal E3-Tricaine solution without added drugs, at 28 °C. **(A)** Bar graphs of AP-frequency, and boxplots of APD_{90} demonstrate no significant effect on both parameters in VSFP-butterfly CY fish, after 30 min incubation (mean ± SEM, n=10). **(B)** Bar graphs on frequency of Ca^{2+} transients, and Ca^{2+} transient amplitude demonstrates no significant effect on both parameters in GCaMP6f fish, after 30 min incubation (mean ± SEM, n=15). AU: arbitrary units.

Figure S2

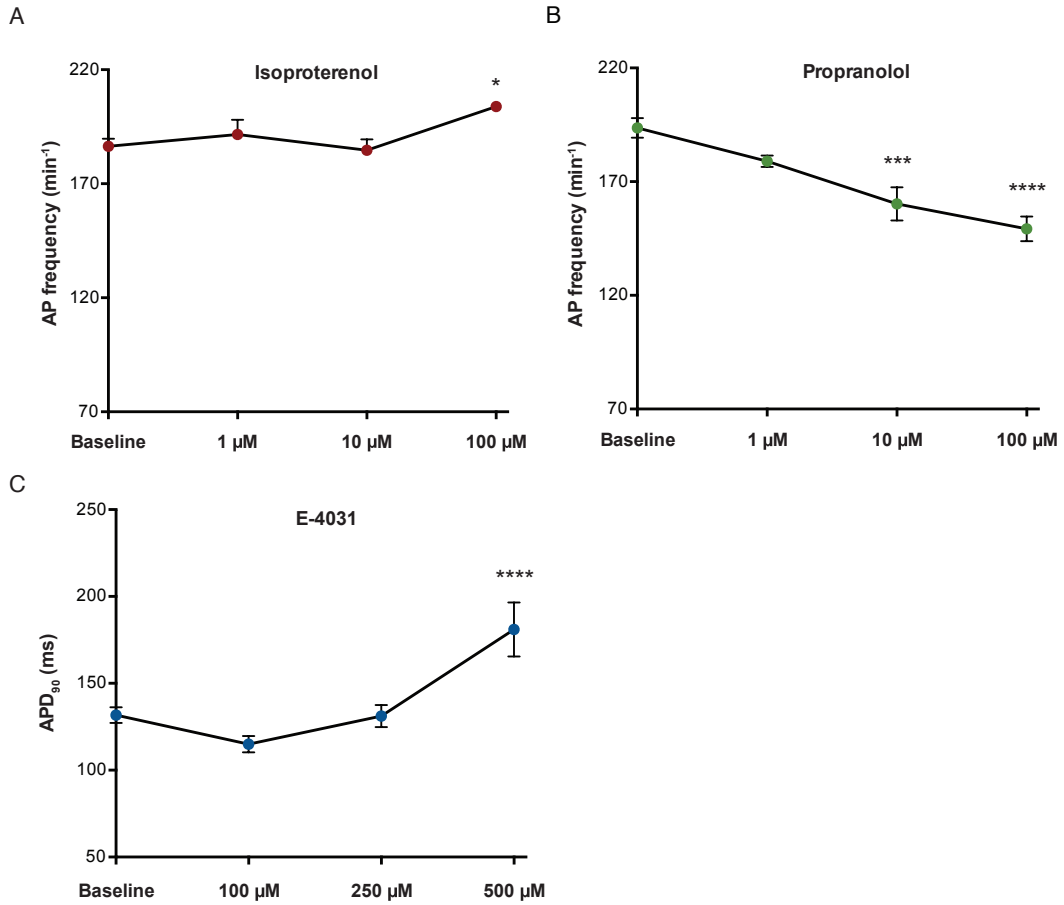

Figure S2. Dose-response effects of isoproterenol, propranolol and E-4031 in VSFP-butterfly CY fish. The effect of different concentrations (1-100 µM) isoproterenol and propranolol on AP frequency. **(A)** Showing a significant increase in AP frequency after treatment with 100 µM isoproterenol (mean ± SEM, $p<0.05$, one-way ANOVA, baseline: n=40, 1 µM: n=9, 10 µM: n=16, 100 µM: n=15). **(B)** Gradual decrease of AP frequency with increasing propranolol concentrations (mean ± SEM, ***$p\leq0.001$, ****$p\leq0.0001$, one-way ANOVA, baseline: n=39, 1 µM: n=11, 10 µM: n=12, 100 µM: n=16). **(C)** The effect of different concentrations (100-500 µM) E-4031 on ventricular APD_{90} showing a significant increase in APD_{90} after treatment with 500 µM (mean ± SEM, $p\leq0.0001$, one-way ANOVA, baseline: n=30, 100 µM: n=9, 250 µM: n=11, 500 µM: n=10). Baseline measurements of the different concentration groups were pooled into one group.

Figure S3

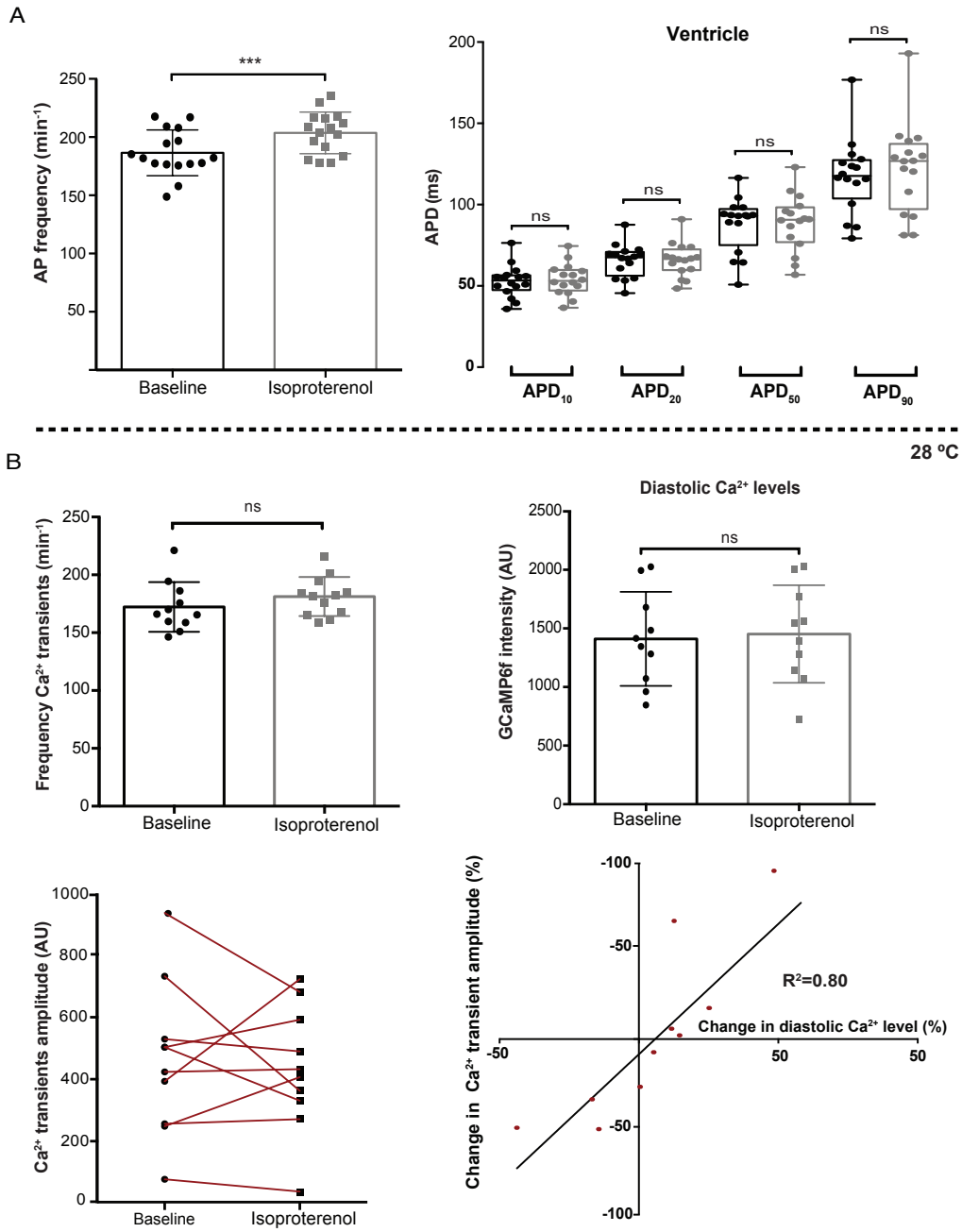

Figure S3. ***In vivo*** **effect of sympathetic stimulation on cardiac electrophysiology and Ca^{2+} dynamics at 28 °C.** 3 dpf VSFP-butterfly and GCaMP6f fish were treated for 30 min with β-adrenergic receptor agonist isoproterenol (100 µM), experiments were performed at 28 °C. **(A)** Bar graphs demonstrate a significant increase in AP frequency after isoproterenol treatment (mean ± SEM, $p \leq 0.001$, n=15). Boxplots show action potential duration (APD) parameters at baseline and after isoproterenol treatment. No significant changes were found in $APD_{10,20,50}$ and APD_{90} after treatment. **(B)** Effect of isoproterenol on Ca^{2+} dynamics at the physiological temperature of 28 °C, n=15. Bar graphs demonstrating no significant increase in the frequency of Ca^{2+} transients and no change in diastolic Ca^{2+} levels (mean ± SEM). The change in Ca^{2+} transient amplitude is plotted per individual fish, demonstrating no consistent positive response towards treatment. The correlation plot of the

change in diastolic Ca^{2+} level versus the change in Ca^{2+} transient amplitude after sympathetic stimulation presents a significant correlation, which is positive as well as negative ($p=0.0004$, $R^2=0.80$). AU: arbitrary units.

Figure S4

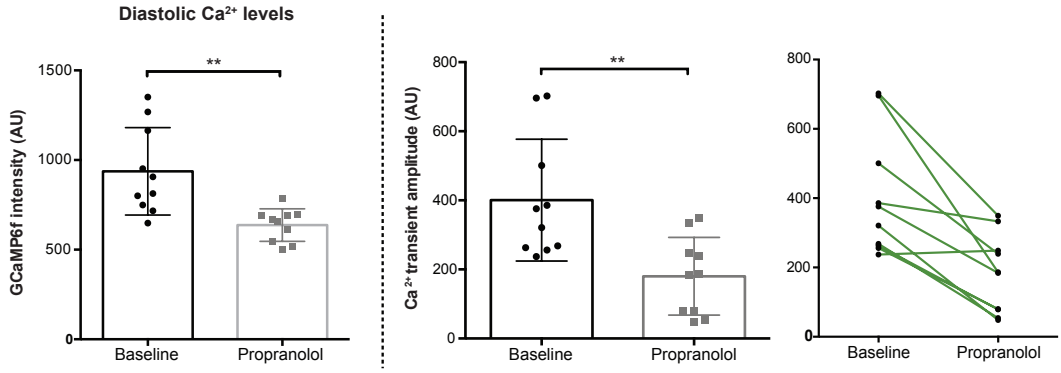

Figure S4. *In vivo* effect of sympathetic inhibition on Ca^{2+} dynamics in GCaMP6f fish. 3 dpf GCaMP6f fish were treated for 30 min with β-adrenergic receptor antagonist propranolol (100 μM), n=10. Bar graphs demonstrating a significant decrease in diastolic Ca^{2+} levels ($p \leq 0.01$) (left panel) and Ca^{2+} transient amplitude ($p \leq 0.01$) (middle panel) after treatment (mean ± SEM). Change in Ca^{2+} transient amplitude is plotted per individual fish (right panel), demonstrating a clear decrease in 9/10 fish after treatment (right panel). AU: arbitrary units.

Figure S5

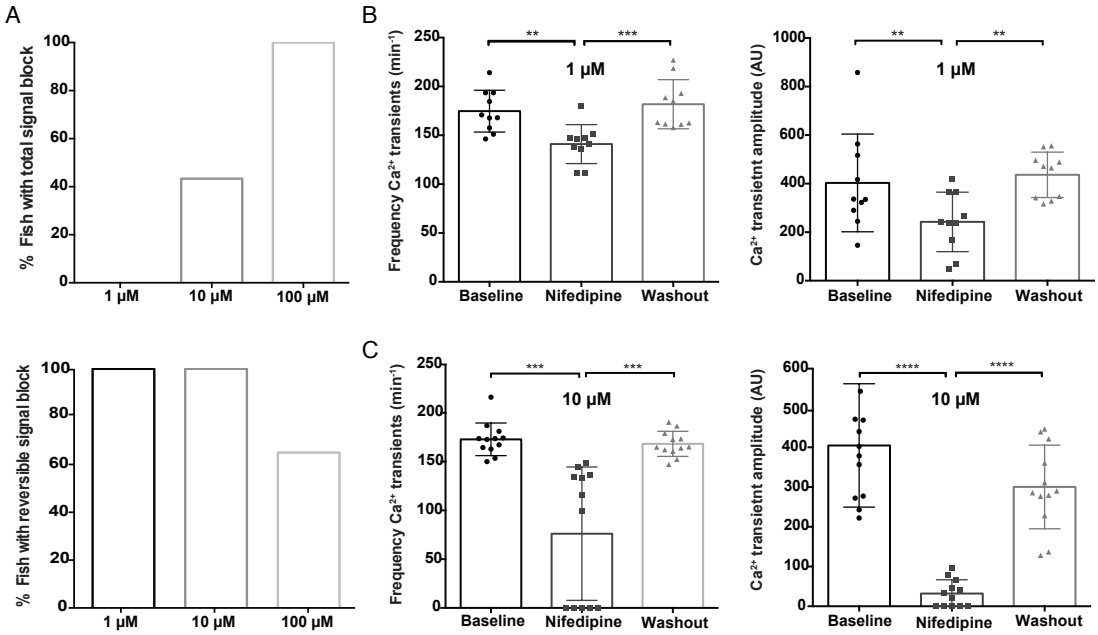

Figure S5. Dose-response effect of nifedipine on ventricular Ca^{2+} dynamics in GCaMP6f fish. 3 dpf GCaMP6f fish were treated for 30 min with 1, 10 and 100 µM nifedipine. **(A)** Bar graphs demonstrating the percentage of fish in which the ventricular GCaMP6f signal was blocked 100% after treatment (top panel) and the percentage of fish in which this block was reversible after washout in E3 medium for 180 min (bottom panel). Y-axis numbers are representable for the signal intensity across the entire heart tube. No total signal block was observed after 1 µM treatment, but there was a total block in 41.7% of fish when treated with 10 µM and total block in 100% of the fish when treated with 100 µM nifedipine. In addition, this total block was reversible in all fish after treatment with 10 µM and in 65% of fish treated with 100 µM nifedipine (data are presented as mean, 1 µM: n=10, 10 µM: n=12, 100 µM: n=19). **(B)** Bar graphs demonstrating a significant decrease in Ca^{2+} transient frequency ($p \leq 0.01$) and Ca^{2+} transient amplitude ($p \leq 0.01$) after 1 µM nifedipine treatment. These parameters recovered to baseline levels after washout (mean ± SEM, frequency $p \leq 0.001$, amplitude $p \leq 0.01$). **(C)** Bar graphs demonstrating a significant decrease in Ca^{2+} transient frequency ($p \leq 0.001$) and Ca^{2+} transient amplitude ($p \leq 0.0001$) after 10 µM nifedipine treatment. These parameters significantly recovered after washout (mean ± SEM, frequency $p \leq 0.001$, amplitude $p \leq 0.0001$). AU: arbitrary units.

Figure S6

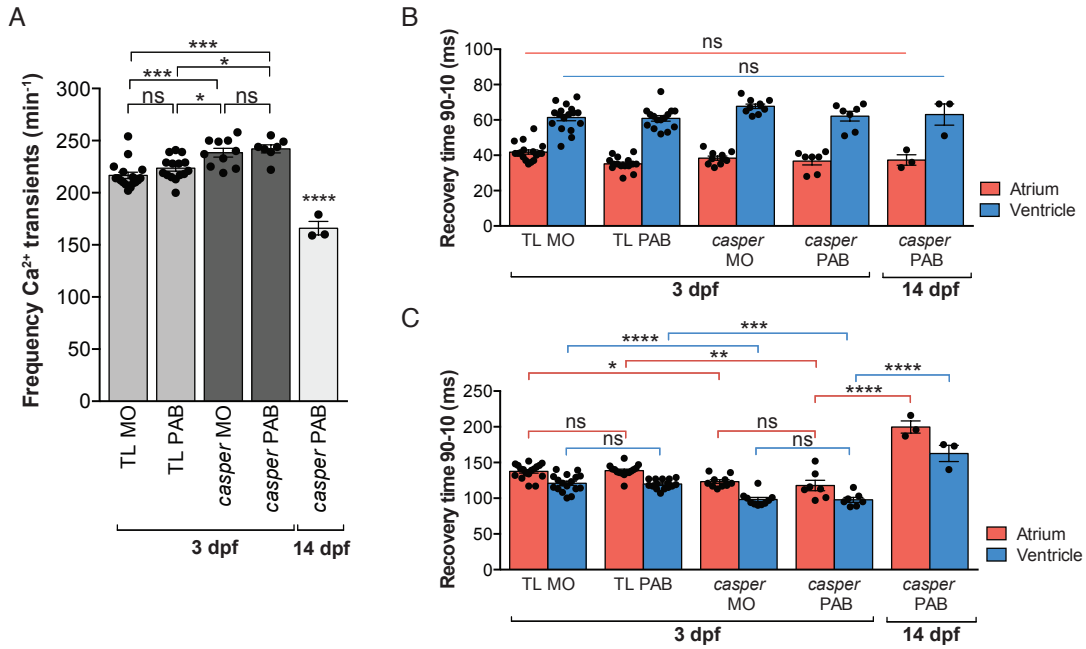

Figure S6. In vivo Ca^{2+} dynamics of embryonic zebrafish with different genetic backgrounds and different types of contraction block. Comparison in Ca^{2+} transient dynamics between different experimental groups (TL MO n=17, TL PAB n=15, *casper* MO n=10, *casper* PAB n=7, 14 dpf *casper* PAB n=3). **(A)** Bar graphs demonstrating no significant difference in Ca^{2+} transient frequency between MO and PAB treated fish within the TL or *casper* background. Embryonic *casper* fish have a higher Ca^{2+} transient frequency compared to embryonic TL fish. Ca^{2+} transient frequency is significant lower in 14 dpf juvenile fish compared to both 3 dpf *casper* and TL fish (mean ± SEM, *p≤0.05, ***p≤0.001, one-way ANOVA**)**. **(B)** Bar graphs demonstrating no significant differences in atrial and ventricular upstroke time between TL and *casper* fish, between 3 dpf and 14 dpf, nor between MO and PAB treated fish (mean ± SEM, one-way ANOVA). **(C)** Bar graphs demonstrating a significant difference in atrial and ventricular Ca^{2+} transient recovery time in 3 dpf TL versus 3 dpf *casper* fish, as well as a significant difference in atrial and ventricular Ca^{2+} transient recovery time of 3 dpf versus 14 dpf *casper* fish (mean ± SEM, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001, one-way ANOVA). No significant differences were observed between MO and PAB treated fish (mean ± SEM, one-way ANOVA). MO: morpholino; PAB: para-amino-blebbistatin; TL: Tupfel long fin.