Supplementary Material

Figure S1

Figure S1. No effects of placebo treatment on cardiac electrophysiology and Ca\textsuperscript{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\textsubscript{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\textsuperscript{2+} transients, and Ca\textsuperscript{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≤0.001, ****p≤0.0001, 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 APD₉₀ showing a significant increase in APD₉₀ after treatment with 500 µM (mean ± SEM, p≤0.0001, 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. *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*≤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. *In vivo* effect of sympathetic inhibition on Ca\(^{2+}\) dynamics in GCaMP6f fish. 3 dpf GCaMP6f fish were treated for 30 min with \(\beta\)-adrenergic receptor antagonist propranolol (100 \(\mu\)M), \(n=10\). 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. Dose-response effect of nifedipine on ventricular Ca\textsuperscript{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\textsuperscript{2+} transient frequency (p≤0.01) and Ca\textsuperscript{2+} transient amplitude (p≤0.01) after 1 μM nifedipine treatment. These parameters recovered to baseline levels after washout (mean ± SEM, frequency p≤0.001, amplitude p≤0.01). (C) Bar graphs demonstrating a significant decrease in Ca\textsuperscript{2+} transient frequency (p≤0.001) and Ca\textsuperscript{2+} transient amplitude (p≤0.0001) after 10 μM nifedipine treatment. These parameters significantly recovered after washout (mean ± SEM, frequency p≤0.001, amplitude p≤0.0001). AU: arbitrary units.
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 significantly 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.