

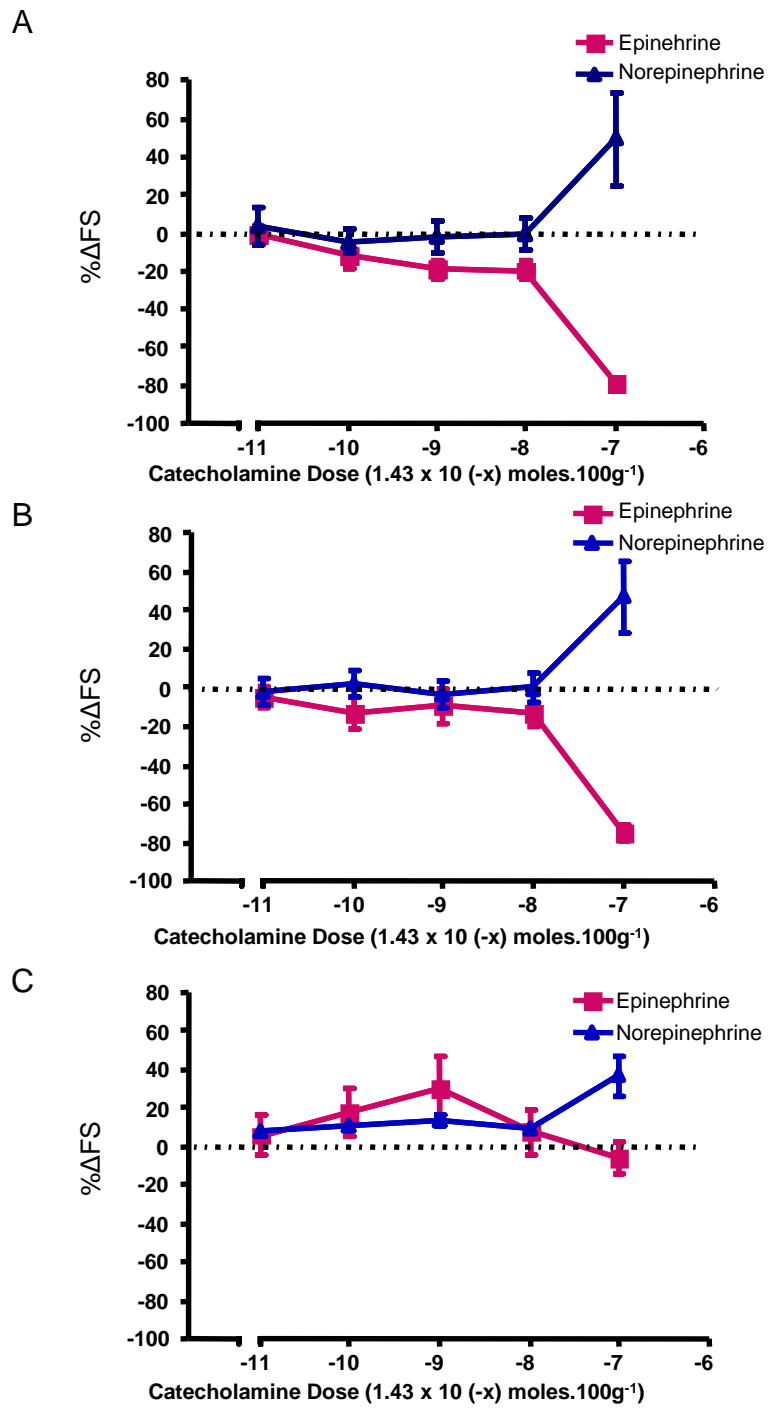
High levels of circulating epinephrine trigger apical cardiodepression in a β_2 -adrenoceptor/Gi-dependent manner: a new model of Takotsubo Cardiomyopathy

SUPPLEMENTAL MATERIAL

Cardiomyocyte contractility studies

Cardiomyocyte contraction experiments were performed using video edge tracking with the IonOptix system. Ca^{2+} concentrations were adjusted to give contraction amplitude of 50% to 75% of maximum to increase accuracy of contraction measurements (6-8mmol/L in human, 2mmol/L in rat). Experiments were carried out at 32°C with field stimulation at 0.2 Hz in human, while rat cardiomyocytes were paced at 0.5 Hz at 37°C. Cardiomyocyte contraction was expressed as the percentage shortening of cell length upon field stimulation. Cardiomyocytes used to determine the functional parameters were rod shaped without spontaneous contractions. β_2 AR-specific contractile responses were measured in rat cardiomyocytes using isoproterenol (1 $\mu\text{mol/L}$) plus the β_1 AR selective antagonist CGP20712A (300nmol/L).^{1,2} Calcium studies were performed with 4mmol/L Ca^{2+} +/- epinephrine pretreatment. For β -blocker experiments, the stably contracting human failing cardiomyocytes were subjected to blockers for 5-15 min, by which time a maximum decrease in contraction was observed. Only cardiomyocytes showing full reversal of the negative inotropic effect on washout of the β -blockers were used.

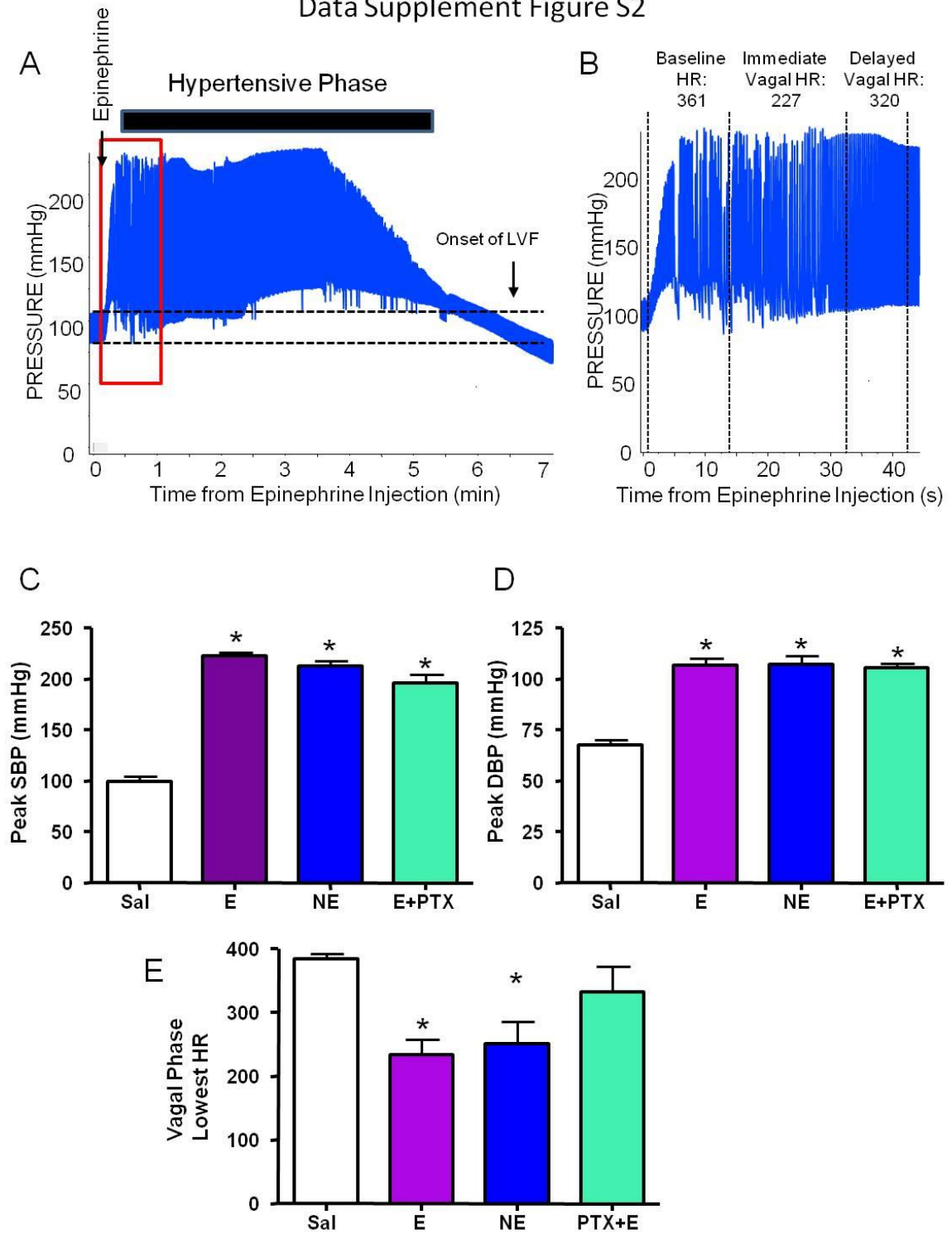
Data Supplement Figure S1



Supplement Figure S1

Inotropic effect of incremental increases in concentration of epinephrine (i.v.) and norepinephrine (i.v.) at the apex (a), MLV (b) and base (c). Values are expressed as the mean percentage change in baseline (untreated) left ventricular fractional shortening (FS) \pm SEM, measured for each concentration at 5 minutes post-catecholamine injection. N=5 (epinephrine), n=4 (norepinephrine).

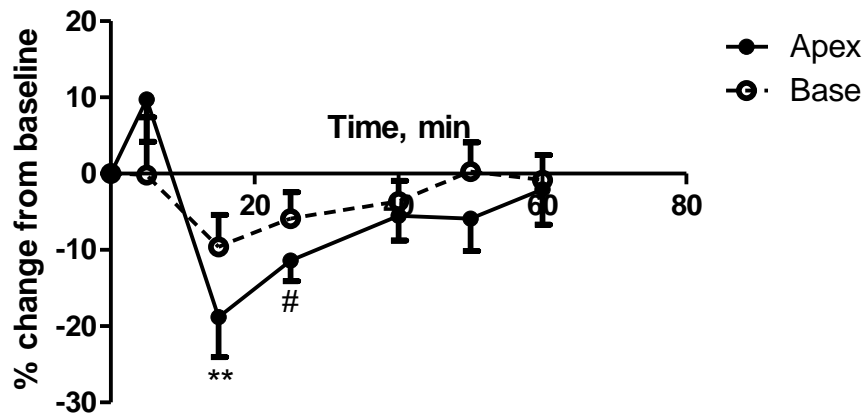
Data Supplement Figure S2



Supplement Figure S2

Haemodynamic arterial responses in in vivo Takotsubo cardiomyopathy model.

A. Representative example of aortic pressure trace recorded before and after intravenous epinephrine bolus, demonstrating the rapid hypertensive response lasting several minutes, followed by a secondary hypotension as left ventricular impairment develops. Horizontal broken lines mark baseline systolic and diastolic blood pressure levels for reference. The tracing in the red box is expanded in **(B)** to show the immediate changes in blood pressure and heart rate during the first 40 seconds post epinephrine bolus. Maximum systolic **(C)** and diastolic **(D)** blood pressure response in cases treated with epinephrine (E), norepinephrine (NE), PTX-pretreated rats receiving epinephrine (E+PTX) or saline control. **E.** Minimum heart rate during bradycardic reflex phase. * $p < 0.05$ vs saline controls, one-way ANOVA. N=5-6 per study arm.

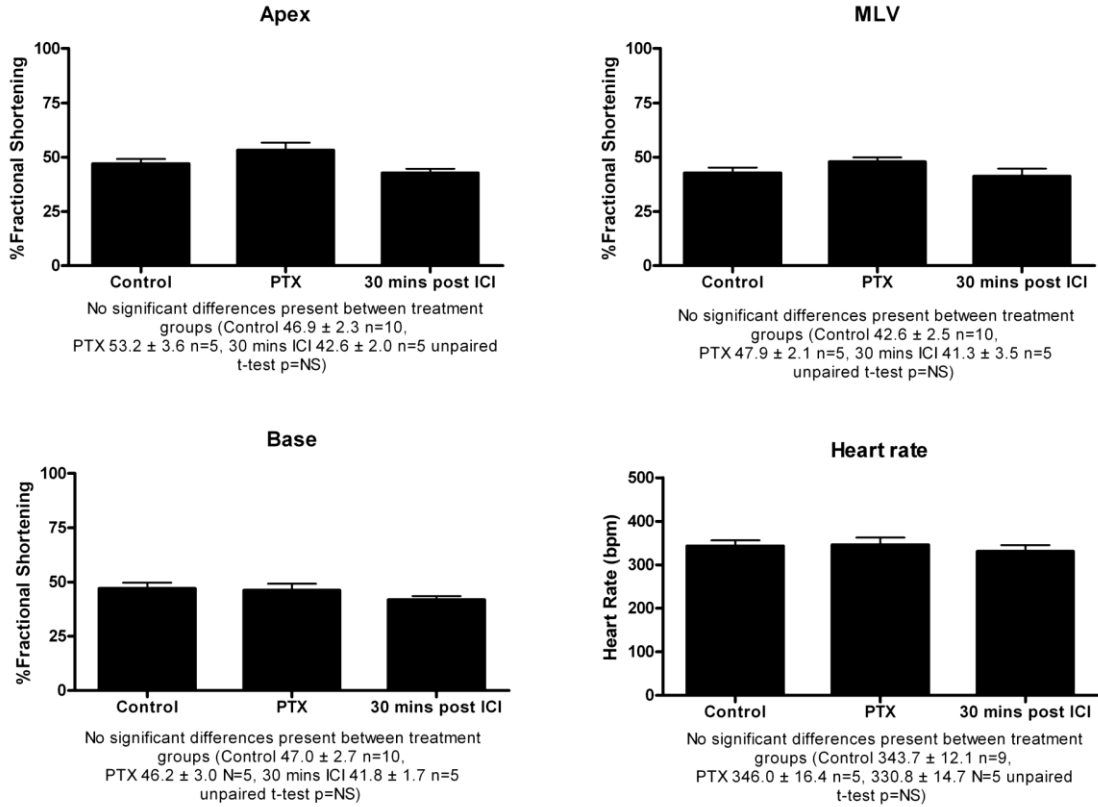


Supplement Figure S3

Time-dependent changes in ejection fraction measured in cross-sectional area from cardiac MRI-derived transverse apical (solid lines) and basal (broken lines) left ventricular slices after intravenous epinephrine (4.28×10^{-8} moles. $100g^{-1}$) # $P < 0.02$, ** $P < 0.01$ compared to baseline, $n=7$.

Data Supplement Figure S4

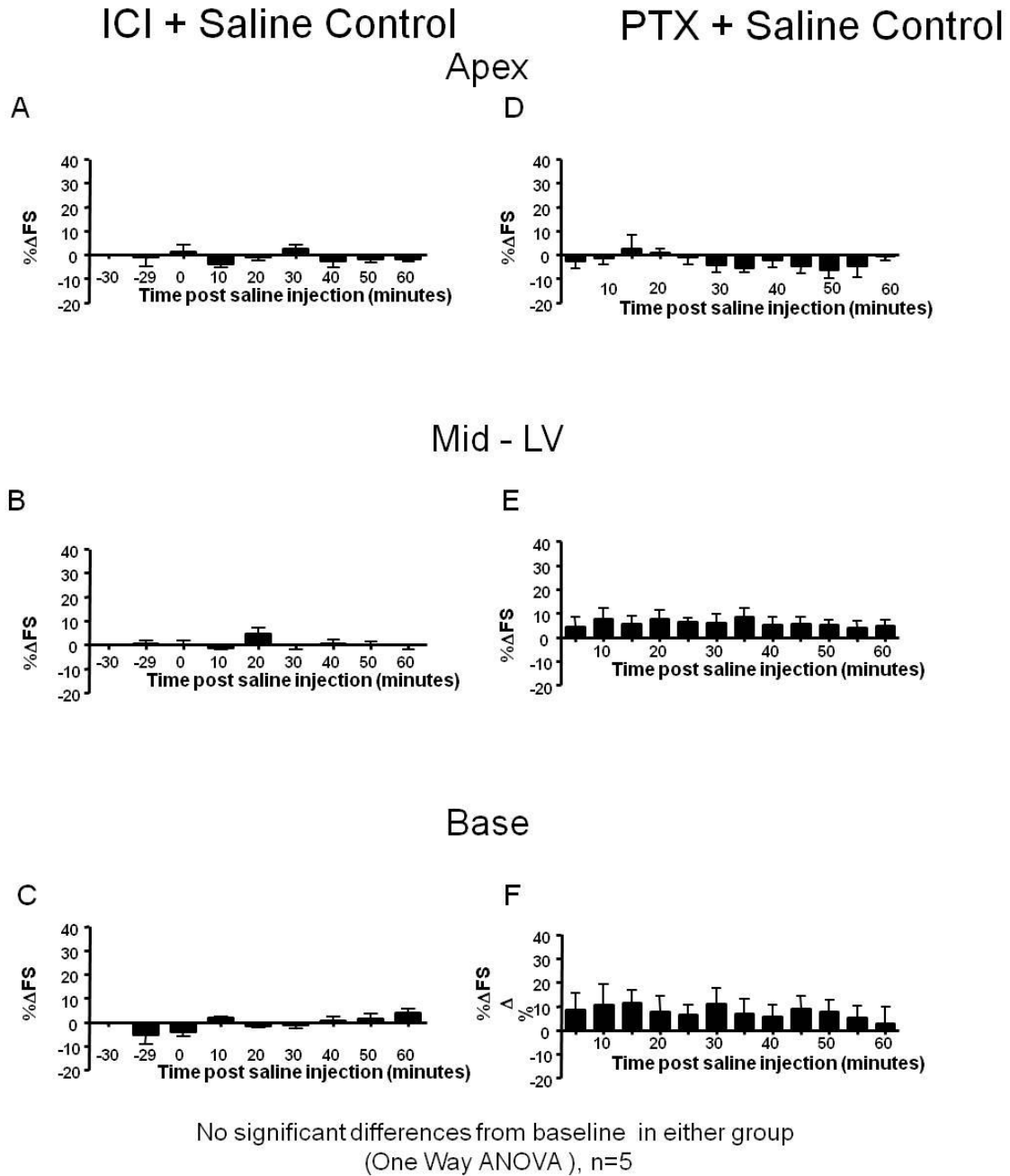
Baseline parameters for control groups



Supplement Figure S4

Baseline heart rate and regional ventricular fractional shortening from the apex, mid left ventricle (MLV) and basal left ventricle at baseline (pre-epinephrine) demonstrating no differences in animals pretreated with PTX or ICI 118,551 in vivo.

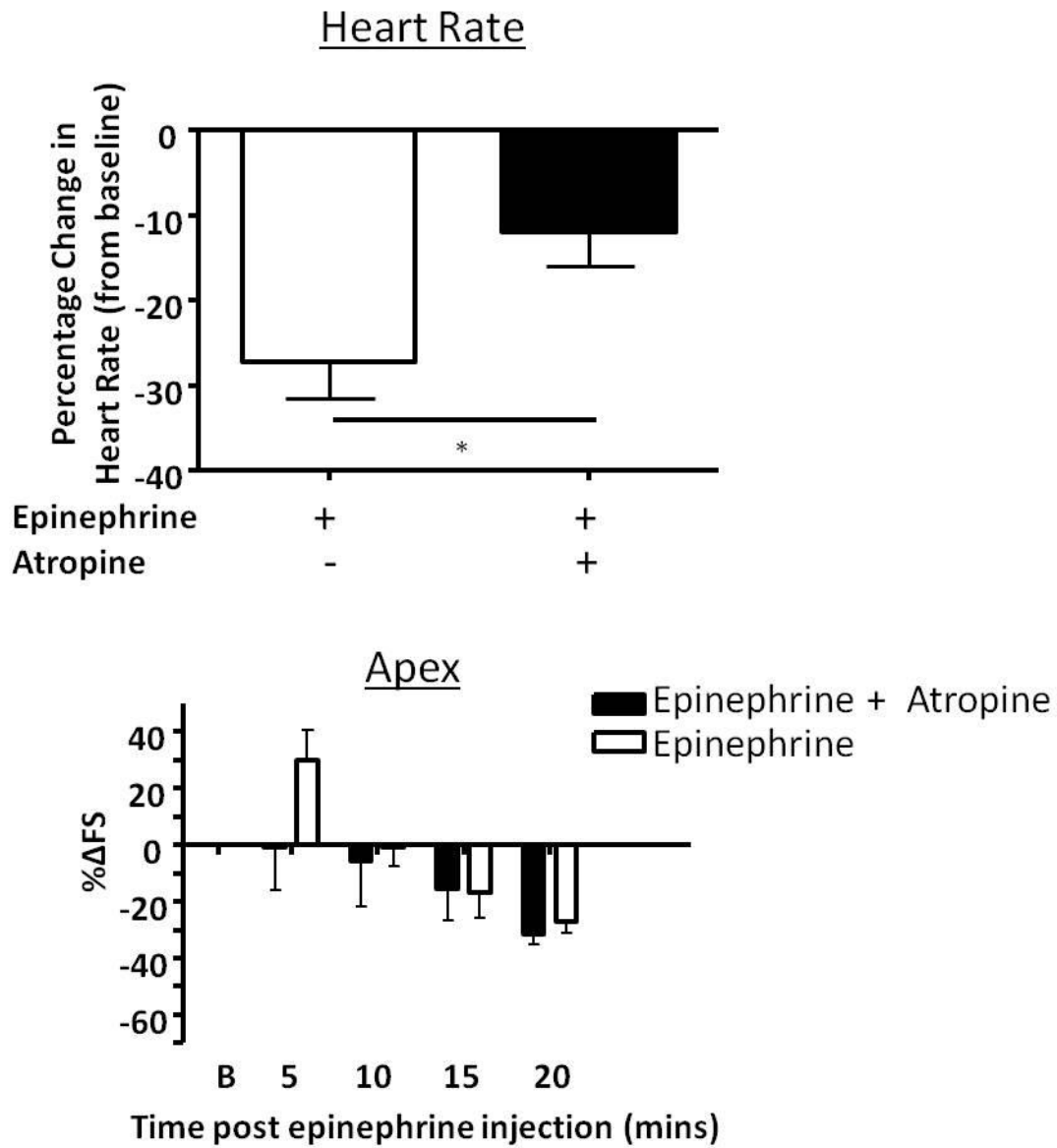
Data Supplement Figure S5



Supplement Figure S5

Time matched saline injected controls demonstrating no significant changes in regional ventricular fractional shortening from the apex, mid left ventricle (MLV) and basal left ventricle in cases pretreated in vivo with ICI 118,551 (left, A-C) or PTX (right, D-F).

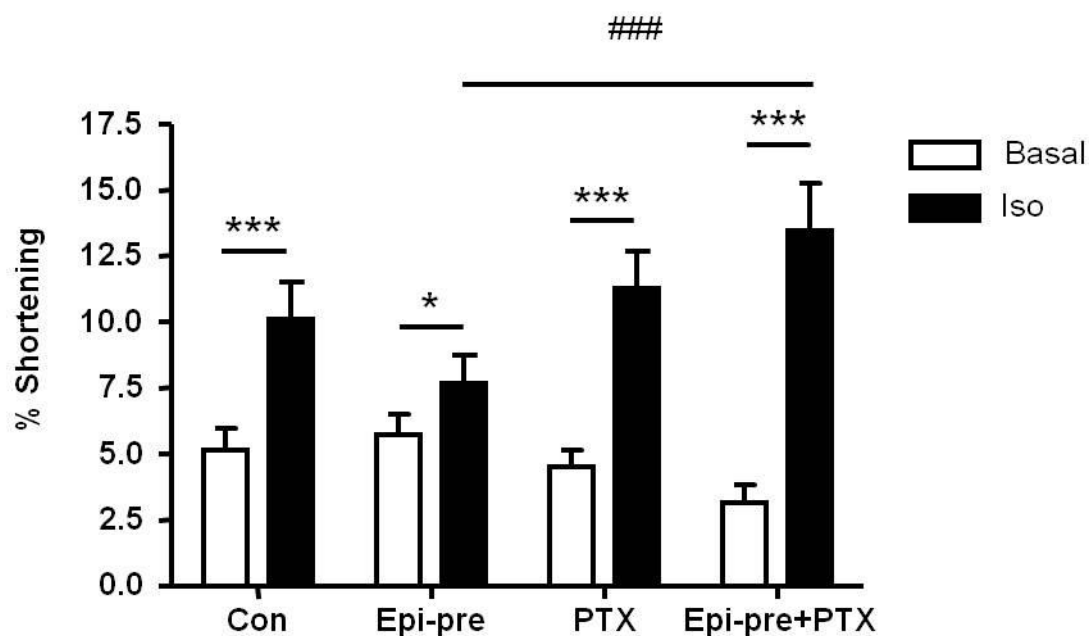
Data Supplement Figure S6



Supplement Figure S6

In vivo atropine pretreatment (10mg/kg, intravenous injection) reduced the reflex bradycardia following subsequent epinephrine injection (above), but failed to prevent epinephrine-induced apical hypokinesia (below). There was 100% mortality in the atropine-pretreated animals at 30 mins post epinephrine injection. * $p < 0.05$, unpaired t-test, $n = 5-6$ per study arm.

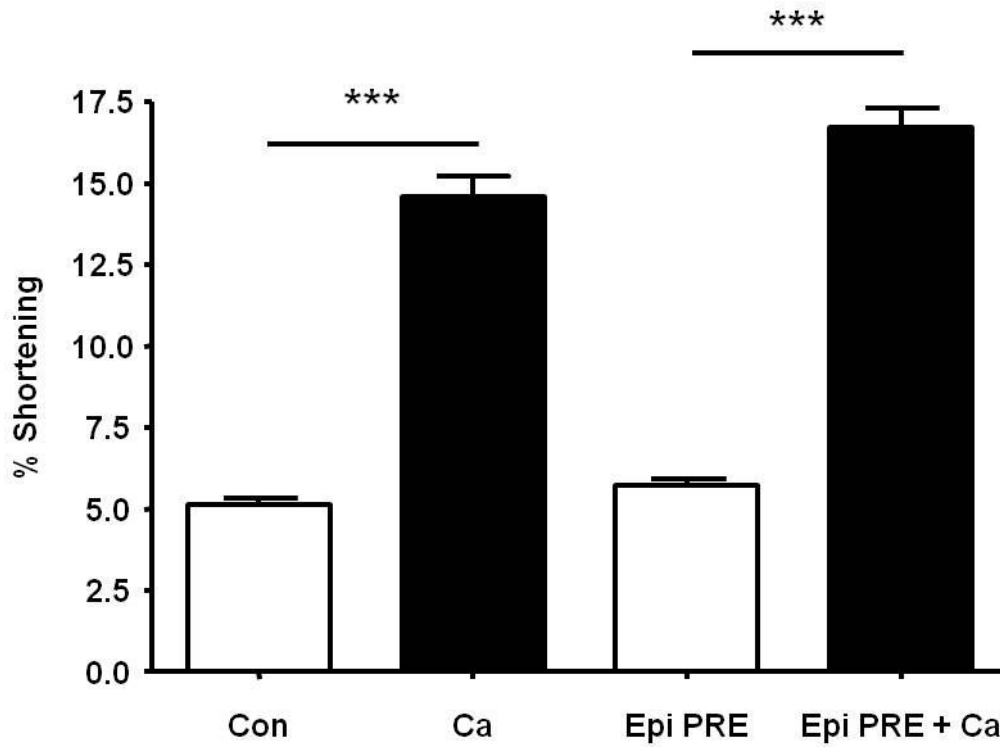
Data Supplement Figure S7



Supplement Figure S7

Epinephrine pretreatment reduced β_2 AR-dependent isoproterenol responses in a PTX-sensitive manner. Contraction amplitude (% shortening) of isolated rat ventricular myocytes in the presence and absence of $1\mu\text{M}$ isoproterenol plus 300nM CGP20712A (iso); untreated (Con, $n=15$), pre-treated for 20 min with $0.1\mu\text{M}$ epinephrine, 10 min wash (Epi-pre, $n=15$); preincubated with PTX (PTX, $n=6$); pretreated with PTX then exposed to epinephrine (Epi-pre PTX, $n=7$). ISO studies: * $p<0.05$, *** $p<0.001$, paired t-test. Epi-pre vs Epi-pre +PTX +/- ISO ### $p<0.0001$, unpaired t-test.

Data Supplement Figure S8

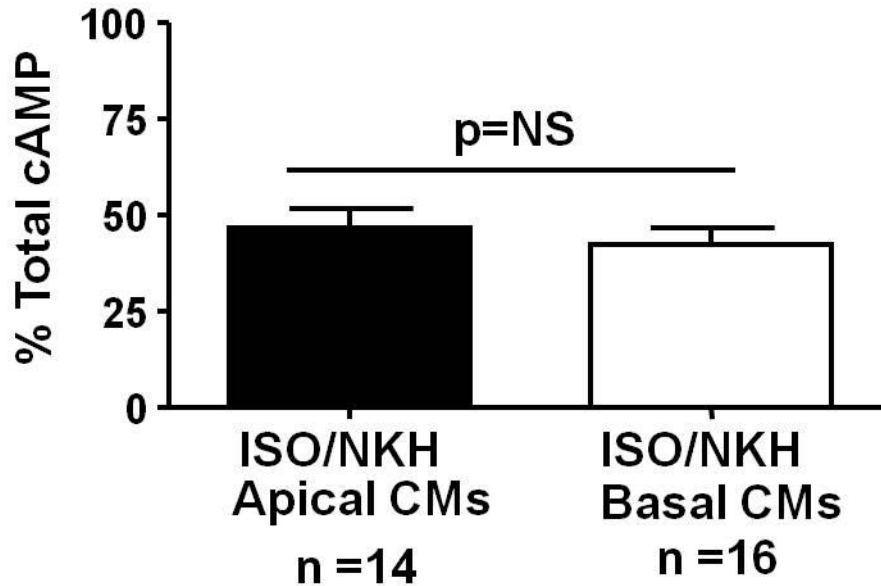
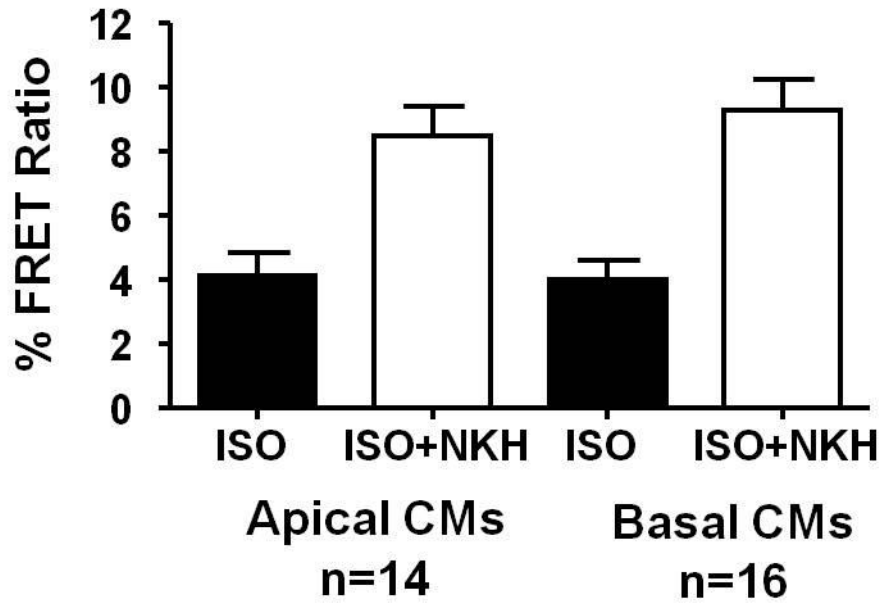


Supplement Figure S8

Calcium-dependent inotropy is preserved after epinephrine pretreatment.

Contraction amplitude (% shortening) of isolated rat ventricular myocytes in the presence (black bars) or absence (white bars) of $1\mu\text{M}$ isoproterenol plus 300nM CGP20712A (ISO); untreated (Con, $n=15$), treated for 20 min with $0.1\mu\text{M}$ epinephrine, 10 min wash (Epi-pre, $n=15$); 4mM calcium (Ca, $n=5$); 4mM calcium after epinephrine pretreatment (Epi-pre, Ca, $n=4$). *** $p<0.001$, paired t-test.

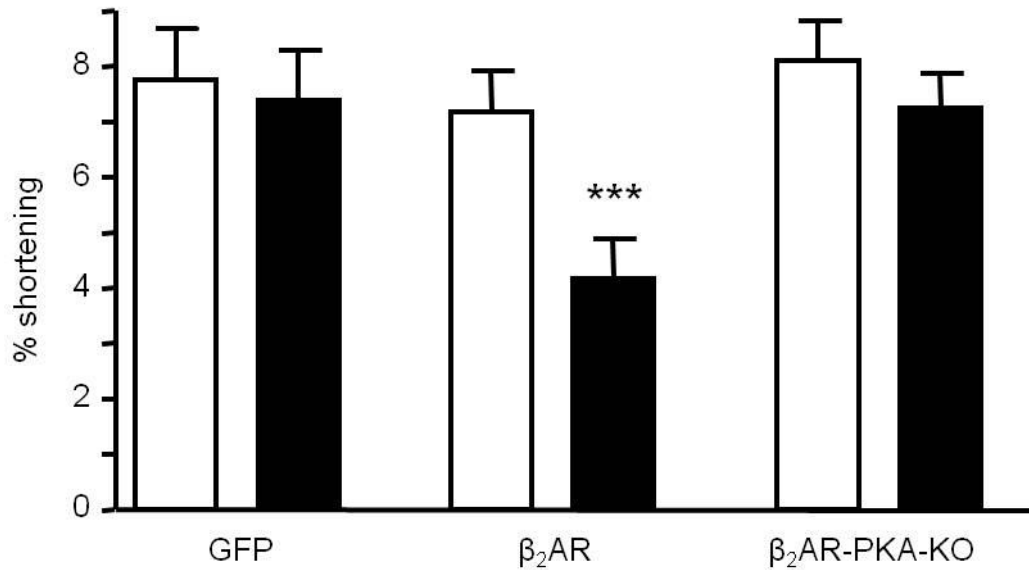
Data Supplement Figure S9



Supplement Figure S9

β_2 AR mediated cAMP response of apical and basal CMs (100nM Isoproterenol (ISO) + 100nM CGP20712A) was not significantly different in terms of raw FRET (above) or %total cAMP production (below) relative to NHK477 (a forskolin analog) (apical $46.7\% \pm 5.0$ n=14 vs. basal $42.4\% \pm 4.5$ n=16 p=NS: values are % maximal FRET response, P=NS unpaired t-test.)

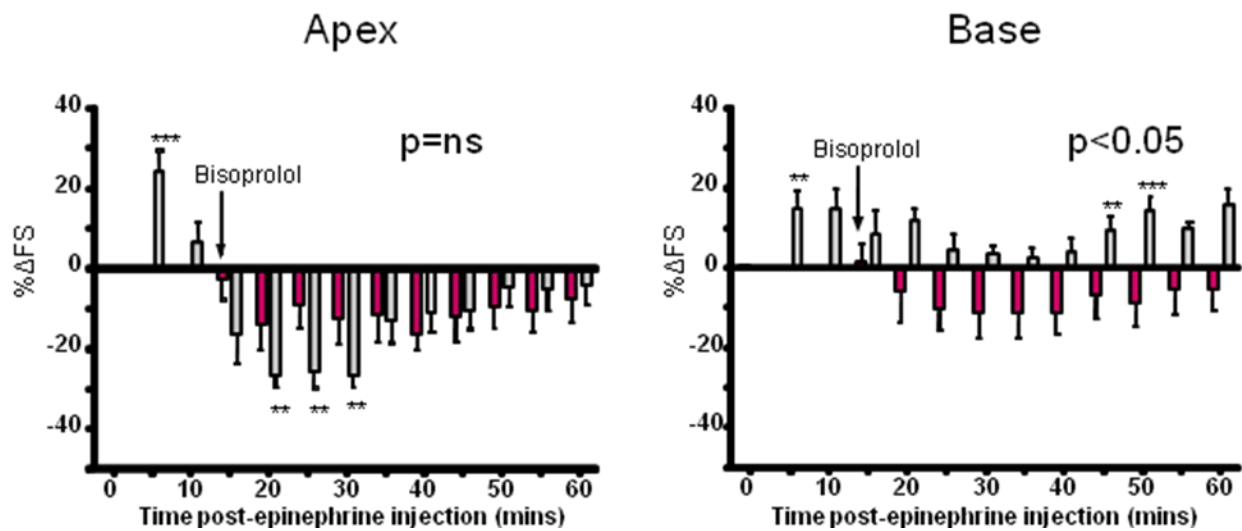
Data Supplement Figure S10



Supplement Figure S10

Negative inotropic effect of the β_2 AR-specific blocker ICI 118,551 (1 μ M) is induced in rat ventricular myocytes by overexpression of wild type β_2 AR but not β_2 AR with mutations at PKA phosphorylation sites 261, 262, 345, 346 S/A (β_2 AR-PKA-KO). Contraction amplitude in 2mM Ca^{2+} untreated (white bars) or in the presence of 1 μ M ICI 118,551 (black bar, 10 min) N=5 preparations, 9 myocytes per condition, ***P<0.001 vs respective control, paired t-test.

Data Supplement Figure S11



Supplement Figure S11

The β -blocker bisoprolol (4.28×10^{-12} moles. $100g^{-1}$ (i.v.)) did not alter epinephrine-mediated contractility of the apex but it did reduce the increased contractility of the base, in the *in vivo* rat model. Values are expressed as the mean percentage change in LV FS from baseline (untreated) levels \pm SEM at each 5 minute time point following intravenous injection of epinephrine. N=6 (epi), n=6 (epi+bisoprolol). (**p<0.01, ***p<0.001, vs baseline FS = 0). RM ANOVA epi vs. epi+bisoprolol: P=NS apex, P<0.05 base. Time: P<0.001 apex; P<0.05 base.

Supplementary Movie File S1

Representative *in vivo* cardiac cine-MRI of rat heart. Basal and apical short axis and 2 chamber long axis views were acquired prior to and at multiple time points after adrenaline infusion. Note the reduction in apical contractility at 15 and 30 mins.

1. Gong H, Sun H, Koch WJ, Rau T, Eschenhagen T, Ravens U, Heubach JF, Adamson DL, Harding SE. The specific β_2 AR blocker, ICI 118,551, actively decreases contraction through a Gi-coupled form of the β_2 AR in myocytes from failing human heart. *Circulation*. 2002;105:2497-503.
2. Gong H, Adamson DL, Ranu HK, Koch WJ, Heubach JF, Ravens U, Zolk O, Harding SE. The effect of Gi-protein inactivation on basal, β_1 - and β_2 AR-stimulated contraction of myocytes from transgenic mice overexpressing the β_2 -adrenoceptor. *Br J Pharmacol*. 2000;131:594-600.