SUPPLEMENTAL MATERIAL

Supplemental methods

cAMP Assay: The amount of cAMP in mouse hearts was measured with a cyclic AMP EIA kit (ewEast Biosciences) according to the instruction from the manufacturer. Briefly, harvested mouse ventricular tissue was first frozen and ground to a fine powder in liquid nitrogen. Once the liquid nitrogen evaporated, the tissue powder was weighed, homogenized and diluted in 0.1M HCl and subjected to cAMP detection.

Mouse cardiomyocyte isolation and treatments: To analyze STAT3 activation in adult cardiomyocytes by βAR agonists, freshly isolated adult single cardiomyocyes were incubated with dobutamine (1μM) or isoproterenol (1μM) for the indicated period of time before fixing by using 1% paraformaldehyde for 10 minutes at room temperature. Immunofluorescence staining was performed using standard procedures.[1] Neonatal mouse cardiomyocytes (NMCs) were isolated as previously described.[2] To assay STAT3 activation by βAR agonists, cultured NMCs were switched to low serum medium for 24 hours before incubating with dobutamine or isoproterenol for the indicated period of time. To block EGFR and Src kinase activity, NMCs were pre-incubated with AG1478 (1 μM, EGFR inhibitor) and Src kinase inhibitor I (1μM) for 5 minutes before addition of dobutamine. To block βARs, NMCs were pre-incubated with Proranolol (25 μM), Carvedilol (10μM), Metoprolol (20μM), Bisoprolol (10μM), and ICI-181,551 (1μM) for 1hr before addition of isoproterenol (1μM).

Histology: Hearts were harvested, fixed, embedded and sectioned using standard techniques. Minimal cardiomyocyte fiber diameter was calculated as described.[3] At least 400 randomly selected cardiomyocytes from each animal were analyzed. TUNEL analyses were performed on

adjacent sections using the ApopTag Apoptosis Detection kit according to the manufacturer's procedures (Chemicon International, Billerica, MA). Sirius red-Fast green staining was performed and quantitated on sections post-fixed in Bouin's solution as described previously.[3] EBD (Evans blues dye) injection was performed as previously described. Four transverse sections from each heart, sampled from the midpoint between the apex and base, were analyzed. The following primary antibodies were used in the IHC and IF assay: anti-STAT3 (Cell Signaling, 9139), anti-pSTAT3 (Tyr705) (Cell Signaling, 9415), anti-cleaved Caspase3 (Cell Signaling, 9661) and anti-C5b9 (abcam, ab55811).

Western blot: Mouse heart tissue and cultured NMCs were homogenized and lysed in Tumor Lysis buffer (50 mM Tris, PH8.0, 150mM NaCl, 5mM EDTA, 1% NP-40) containing EDTA-free cOmplete Protease Inhibitor Cocktail (Roche). Protein concentration of the supernatant was determined by the BAC protein assay (Bio-Rad). The following primary antibodies were diluted in blocking buffer (5%BSA in TBST buffer): anti-STAT3 (Cell Signaling, 9139), anti-pSTAT3 (Tyr705) (Cell Signaling, 9415), anti-ERK1/2 (Cell Signaling, 9102), anti-pERK1/2 (Cell Signaling, 9100), anti-P38 (Cell Signaling, 9212), anti-pP38 (Thr180/Tyr182) (Cell Signaling, 9216), anti-JNK (Cell Signaling, 9252), anti-pJNK (Thr183/Tyr185) (Cell Signaling, 4668), anti-Smad2 (Cell Signaling, 3103), anti-pSmad2(Ser465/467) (Cell Signaling, 3101), anti-AKT (Cell Signaling, 9272), anti-pAKT(ser473) (Cell Signaling, 9271), anti-Troponin I (Cell Signaling, 4002), anti-p-troponin I (Cell Signaling, 4004), anti-GAPDH (Santa Cruz, sc-25778), anti-α-tubulin (Sigma, T5168), anti-Pka ca(Cell Signaling, 4782), anti-Ca_v3.2 (alomone labs, ACC-025), anti-Cacnα1c (abcam, ab84814), Serca2 (Cell Signaling, 4388).

Luciferase Assay: Mouse Adrb1 gene promoter region fragments from nt-2099 to nt2 and nt-1301 to nt2 were suncloned and inserted into KpnI/HindIII site of pGL3Basic plasmid

(Promega). For promoter reporter assay, *Adrb1* promoter-luciferase reporter constructs were transfected alone or with Stat3 expression plasmid into P19 cells. Luciferase activity was assayed 48 hours after transfection using the Dual- luciferase Report Assay System (Promega).

***Isolated heart perfusion system (Langendorff)*:** Briefly, hearts were rapidly excised via a median sternotomy and placed in a 4°C KH solution. The aorta was rapidly cannulated and the heart was perfused in the isovolumetric Langendorff mode (70 mmHg). A water-filled latex balloon was passed into the left ventricle. End diastolic pressure was adjusted to a level between 8 and 15 mmHg. Based on the coronary flow rate, the dobutamine infusion rate and volume were calculated and preset to ensure that 1μg/g bodyweight of dobutamine was infused into isolated mouse hearts during the 20 minutes of infusion time widow. Coronary flow rate was monitored after dobutamine infusion. The dobutamine doses were chosen based on the literature.[5] The left ventricular developed pressure (LVDP) and the maximum positive and negative values of the first derivative of left ventricular pressure (±dP/d*t*) were continuously recorded using a PowerLab 8 preamplifier/digitizer (AD Instruments, Milford, MA). For ouabain treatment, isolated mouse hearts from *Stat3cKO* and their control littermates were transferred to a Langendorff prep. After equilibration with baseline recording of cardiac function, ouabain was infused at a concentration of 500nM for 20 minutes as previously reported. [6] Isolated mouse hearts were paced at 3 Hz (2-millisecond duration, 4 V) with bipolar electrodes attached to the left ventricle using a Grass SD9 stimulator, and the pacing was maintained throughout experiments.

ChIP assay: ChIP assay was performed as previously described. [7] In brief, adult hearts were minced before fixing with 1% PFA. Cells were lysed by cell lysis buffer (10 mM HEPES, pH 7.5, 85 mM KCl, 0.5% NP-40, protease inhibitor (78410, Pierce)). Nuclei were isolated by

disruption using a B dounce, and washed with SDS lysis buffer (1% SDS, 10 mM EDTA and 50 mM Tris, pH 8.1). Chromatin was sonicated to generate average fragment sizes of 100–300 base pairs (bp), and immunoprecipitated using anti-STAT3 antibody.

Statistical Analysis for calcium properties: As the experiments analyzing calcium properties (Ca^{2+} transient amplitude (F/F_0) and activation kinetics (T_{peak}), SR Ca^{2+} content and the frequency of Ca^{2+} sparks) used multiple cells per animal, the experiment results were analyzed using a mixed-effect model with a "proc mixed" in the statistical software SAS 9.4. The effect of the dobutamine treatment, the effect of STAT3 deletion, and the interaction effect of the dobutamine treatment and STAT3 deletion were fitted as fixed effects in the mixed model. The variation from each individual mouse was fitted as random effect. The effect of the dobutamine treatment on cells from Control mice, the effect of the dobutamine treatment on cells from *Stat3cKO* mice, the difference between the treated cells from WT mice and treated cells from *Stat3cKO* mice and their p-values were obtained from SAS output (See attachment).

The mixed model for data fitting is as following:

$$\boldsymbol{Y} = \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \boldsymbol{Z} \times \boldsymbol{u} + \boldsymbol{\epsilon}$$

Y is the result.

β_1: the expected result for cardiomyocte from control mice without dobutamine treatment.

β_2: the expected result for cardiomyocte from control mice with dobutamine treatment.

β_3: the expected result for cardiomyocte from *Stat3cKO* mice without dobutamine treatment.

β_4: the expected result for cardiomyocte from *Stat3cKO* mice with dobutamine treatment.

$X_1 = 1_{\{\text{Cardiomyocyte from control mice without dobutamine}\}}$

$X_2 = 1_{\{\text{ Cardiomyocyte from control mice with dobutamine}\}}$

$X_3 = 1_{\{\text{ Cardiomyocyte from } \textit{Stat3cKO} \text{ mice without dobutamine}\}}$

$X_4 = 1_{\{\text{ Cardiomyocyte from } \textit{Stat3cKO} \text{ mice with dobutamine}\}}$

$\boldsymbol{\mu}$: the random effect of each individual mouse, we assume that it follows normal distribution: $N(0, \sigma^2_{\text{random effect}})$.

$\boldsymbol{\varepsilon}$: the error term, we assume that it follows normal distribution $N(0, \sigma^2_{\text{error}})$, and $\text{cov}(\boldsymbol{\mu}, \boldsymbol{\varepsilon}) = \boldsymbol{0}$

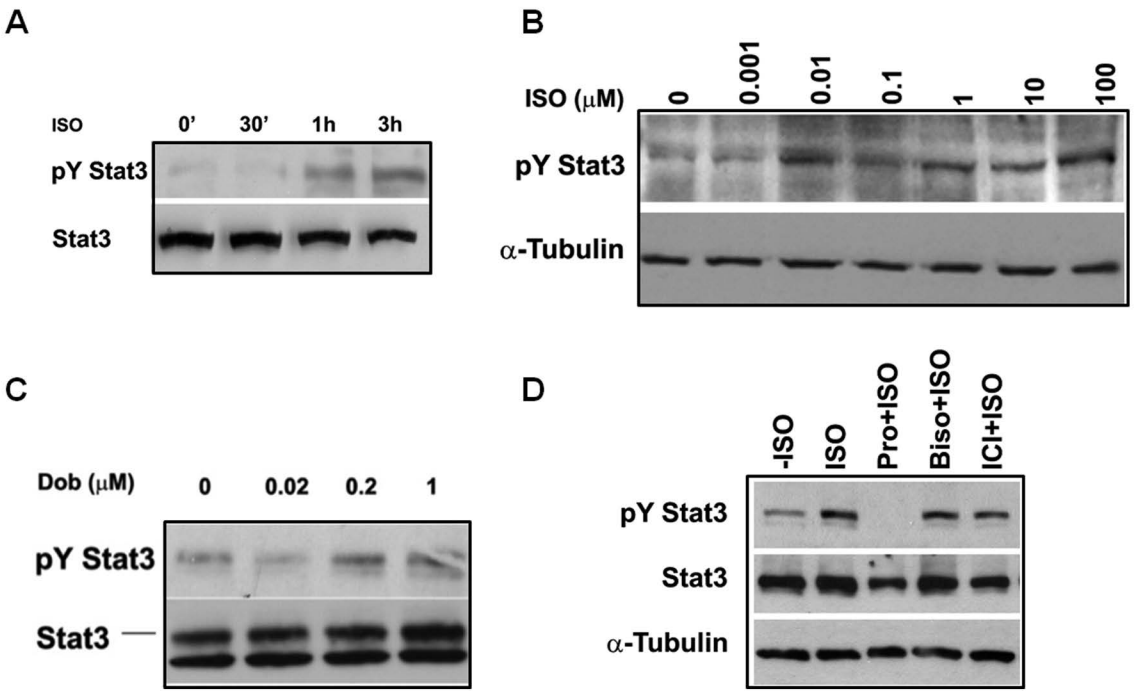
References

1. Li D, Liu Y, Maruyama M, Zhu W, Chen H, Zhang W, Reuter S, Lin SF, Haneline LS, Field LJ, Chen PS and Shou W. Restrictive loss of plakoglobin in cardiomyocytes leads to arrhythmogenic cardiomyopathy. *Human molecular genetics*. 2011;20:4582-96.
2. Jacoby JJ, Kalinowski A, Liu MG, Zhang SS, Gao Q, Chai GX, Ji L, Iwamoto Y, Li E, Schneider M, Russell KS and Fu XY. Cardiomyocyte-restricted knockout of STAT3 results in higher sensitivity to inflammation, cardiac fibrosis, and heart failure with advanced age. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100:12929-34.
3. Zhu W, Shou W, Payne RM, Caldwell R and Field LJ. A mouse model for juvenile doxorubicin-induced cardiac dysfunction. *Pediatric research*. 2008;64:488-94.
4. Zhou YY, Wang SQ, Zhu WZ, Chruscinski A, Kobilka BK, Ziman B, Wang S, Lakatta EG, Cheng H and Xiao RP. Culture and adenoviral infection of adult mouse cardiac myocytes: methods for cellular genetic physiology. *American journal of physiology Heart and circulatory physiology*. 2000;279:H429-36.
5. Sunagawa R, Nagamine F, Murakami K, Mimura G and Sakanashi M. Effects of dobutamine and acetylcholine on perfused hearts isolated from streptozocin-induced diabetic rats. *Arzneimittel-Forschung*. 1989;39:470-4.
6. Morgan EE, Li Z, Stebal C, Belliard A, Tennyson G, Salari B, Garlid KD and Pierre SV. Preconditioning by subinotropic doses of ouabain in the Langendorff perfused rabbit heart. *Journal of cardiovascular pharmacology*. 2010;55:234-9.
7. Hang CT, Yang J, Han P, Cheng HL, Shang C, Ashley E, Zhou B and Chang CP. Chromatin regulation by Brg1 underlies heart muscle development and disease. *Nature*. 2010;466:62-7.

Table 1: List of the genes in β-adrenergic signaling pathway that with promoter regions occupied by Stat3 in ES cells (from previous publications).

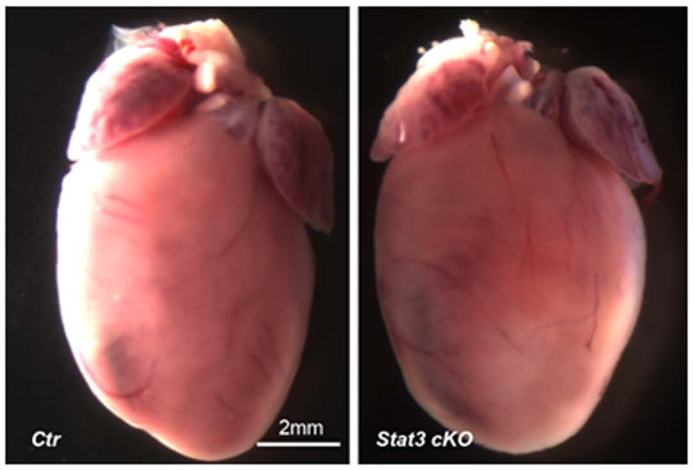
Accession	Gene symbol	Chromosome	Start binding Position	End binding position	TSS	Relative Position to TSS	P-Value	Ref
NM_007420	Adrb2	CHR18	62304610	62304866	62305328	-462		Cell, 2008, 133:1106-1107
NM_020263	Cacna2d2	CHR9	107379490	107379506	107257712	-121778		Cell, 2008, 133:1106-1107
AF247141	Cacna2d2	CHR9	107233237	107234054	107155501	-7736	1.32E-10	Plos Genet, 2008, 3(12); 19079581
NM_023116	CACNB2	CHR2	14611796	14612395	14553726	-58070	2.90E-08	Plos Genet, 2008, 3(12); 19079581
NM_009722	ATP2A2	CHR5	122710571	122710626	122762512	-51886		Cell, 2008, 133:1106-1107
NM_023868	RyR2	CHR13	11962332	11962346	12160998	70090		Cell, 2008, 133:1106-1107
NM_023868	RyR2	CHR13	12231488	12231988	12160998	70990		Plos Genet, 2008, 3(12); 19079581

Supplemental Figure 1

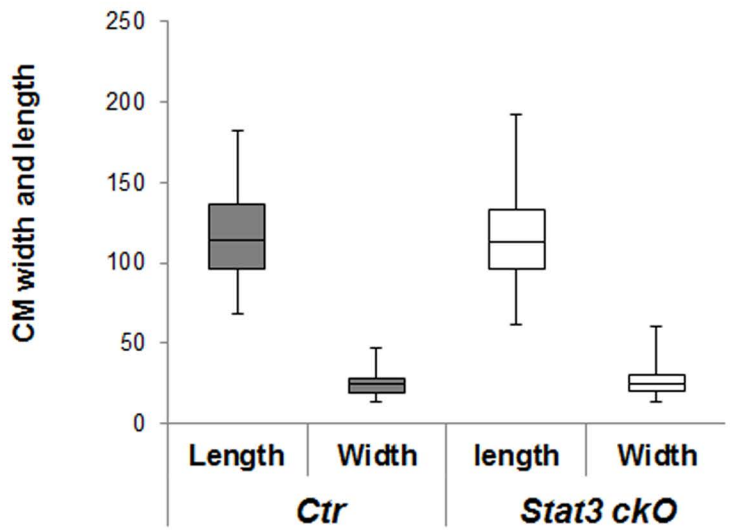


Supplemental Figure 2

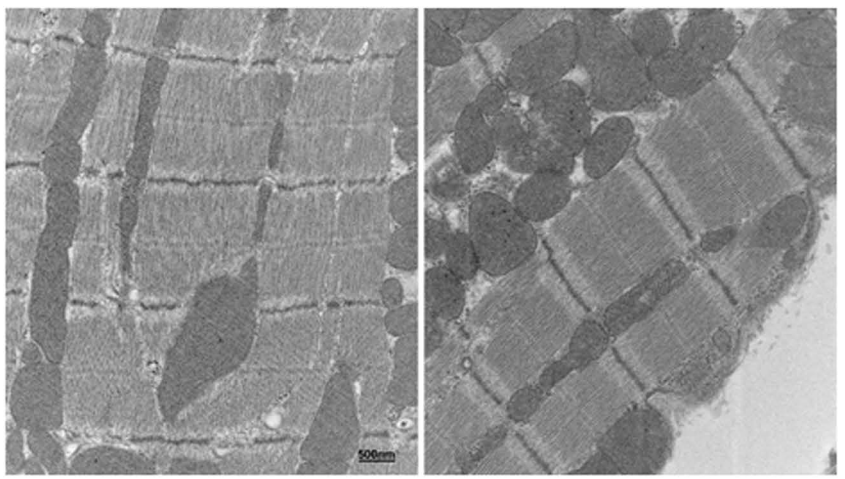
A



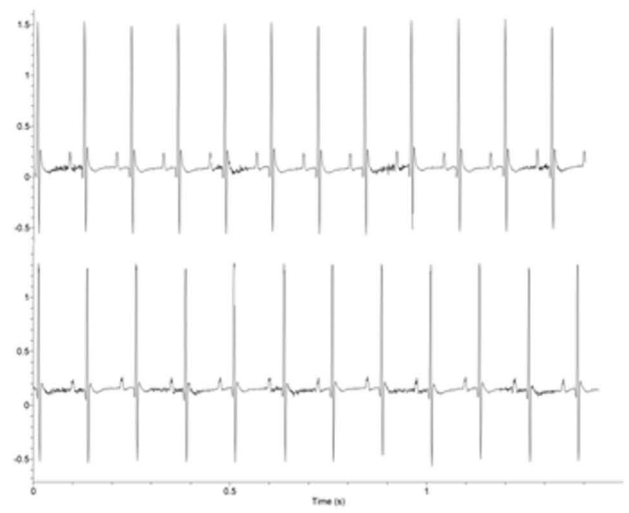
B



C



D

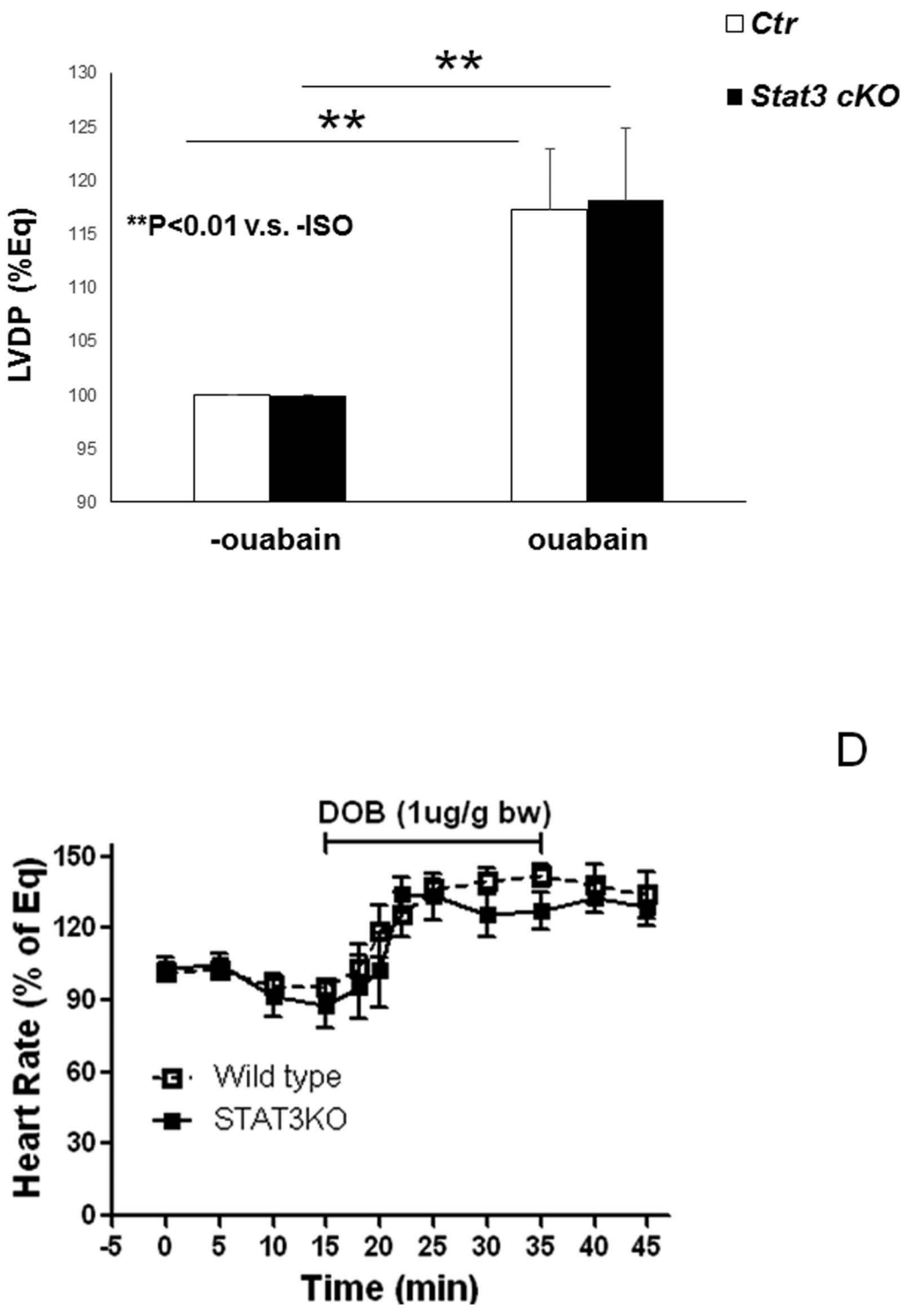


E

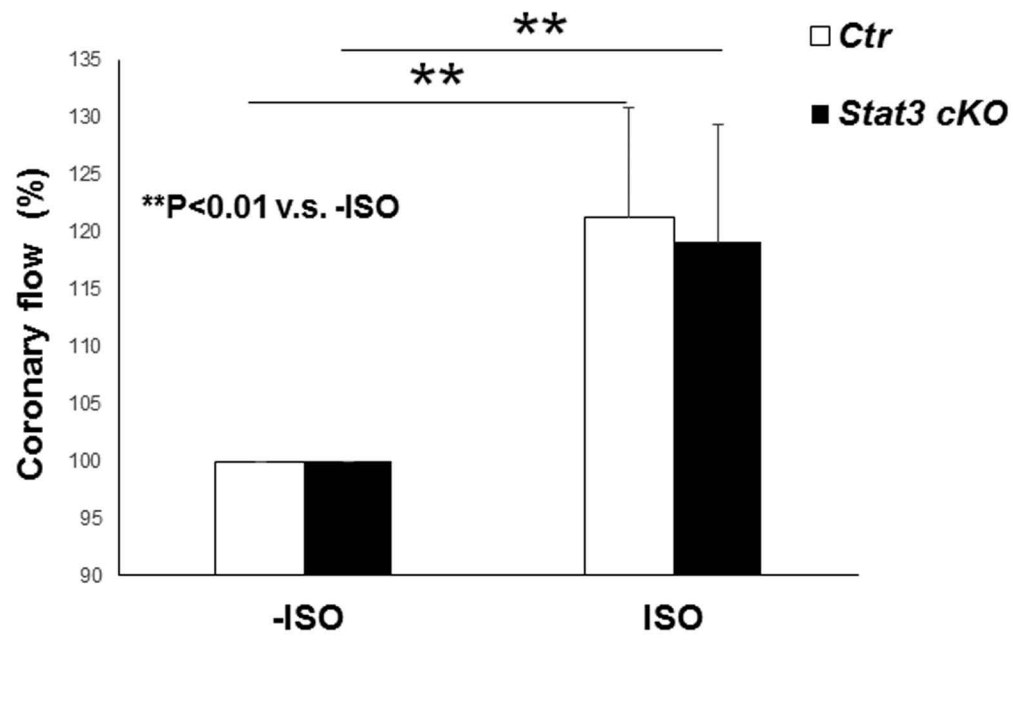
	H.R (BPM)	PP Interval (ms)	P wave (ms)	P.Q. Interval (ms)	QRS Interval (ms)
Ctr (n=6)	489.8±47.78	128±14.1	11.22±1.68	35.69±4.04	12.62 ±0.96
Stat3 cKO (n=6)	452.8±27.16	133±8.1	11.54±0.98	34.85±1.48	14.34±1.65*

Supplemental Figure 3

A



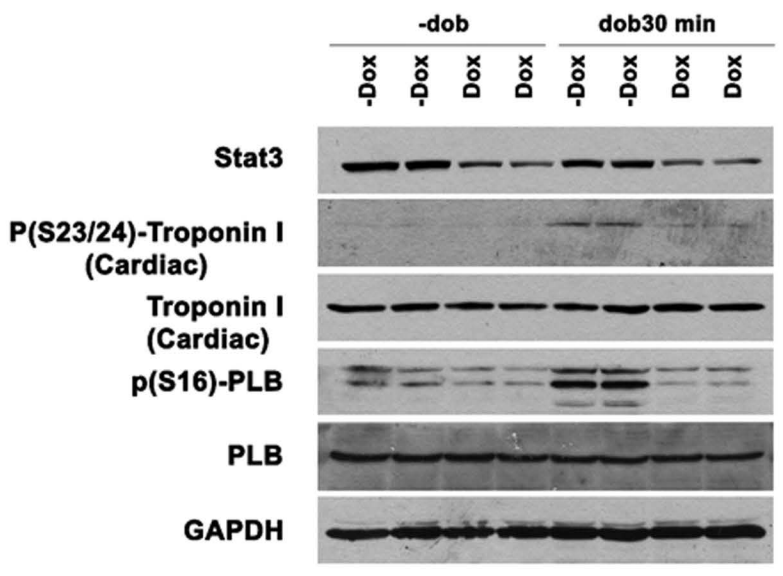
B



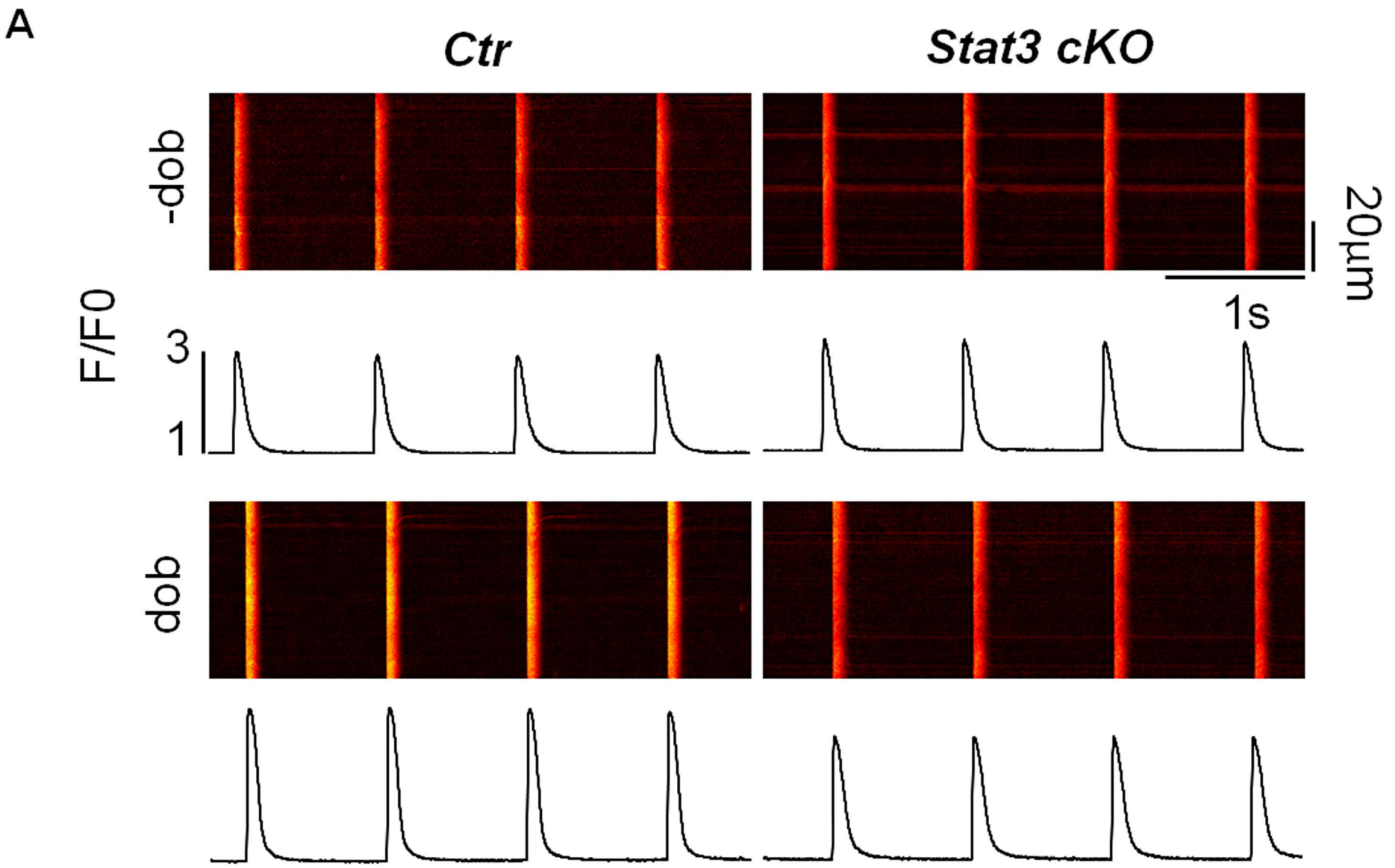
** P<0.01

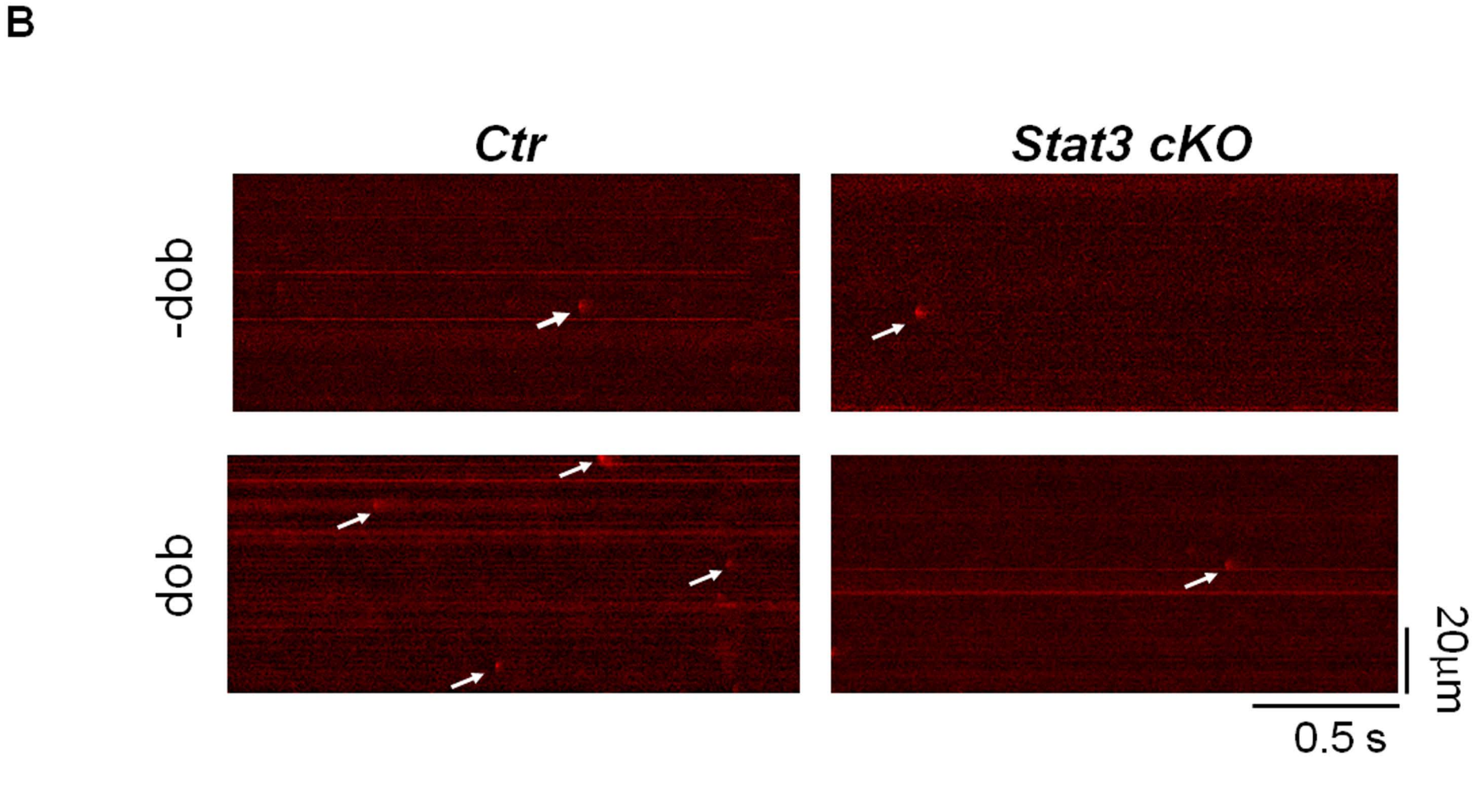
C

D

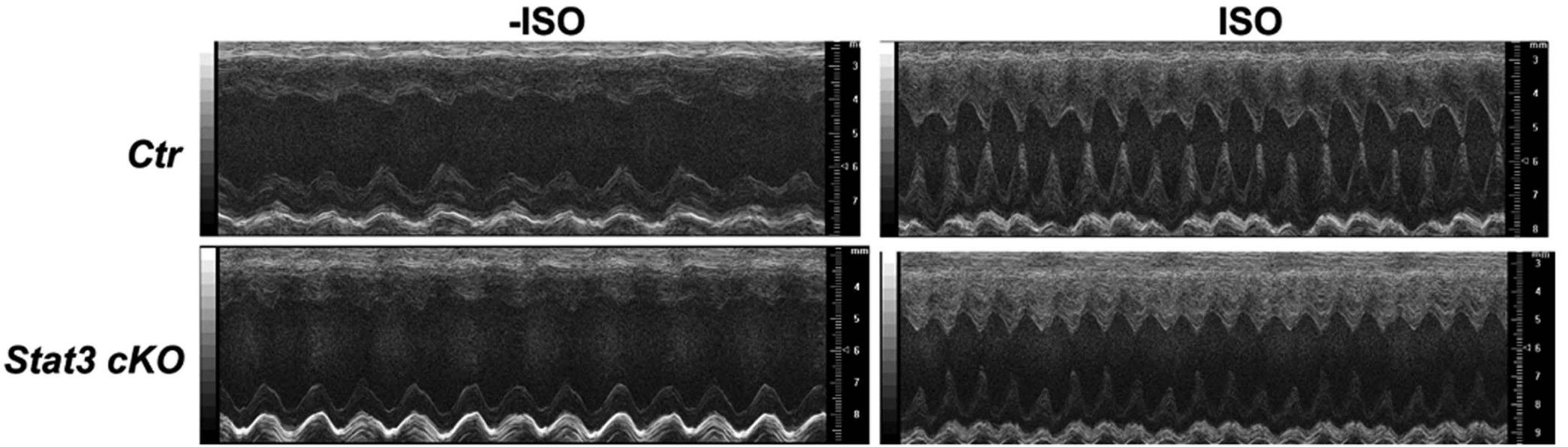


Supplemental Figure 4





Supplemental Figure 5

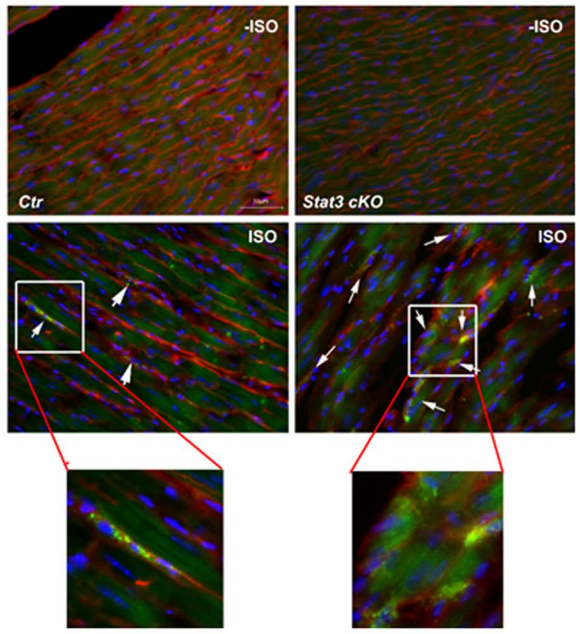


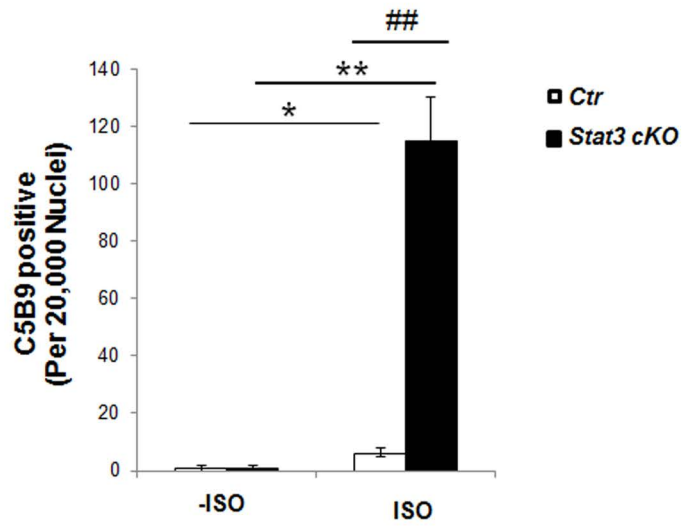
	-ISO				ISO			
	HR (BPM)	LVIDD (mm)	LVIDS(mm)	F.S. (%)	HR (BPM)	LVIDD (mm)	LVIDS (mm)	F.S. (%)
Control (n=10)	425±29	3.36±0.18	2.01±0.17	40.18±4.1	554±32	3.72±0.18	1.76±0.16	52.6±3.1
Stat3cKO (n=7)	414±39	3.28±0.21	1.95±0.19	40.54±3.7	538±48	3.54±0.14	1.8±0.11	47.7±2.5*

* P<0.05

Supplemental Figure 6

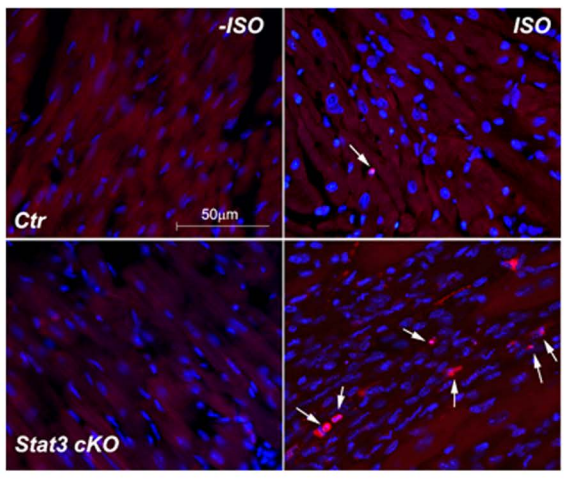
A

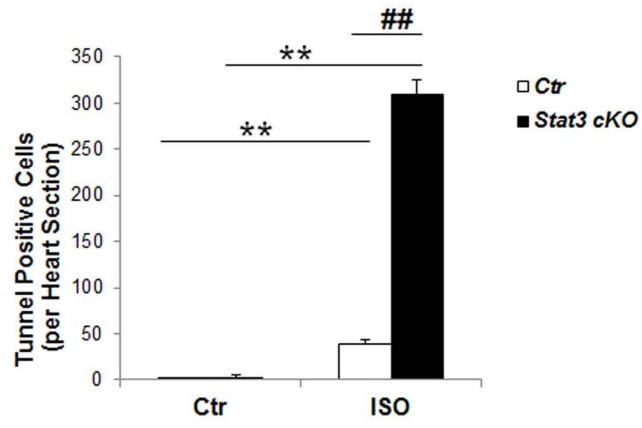




*P<0.05, **P<0.01 v.s. -ISO
##P<0.01 v.s. Ctr

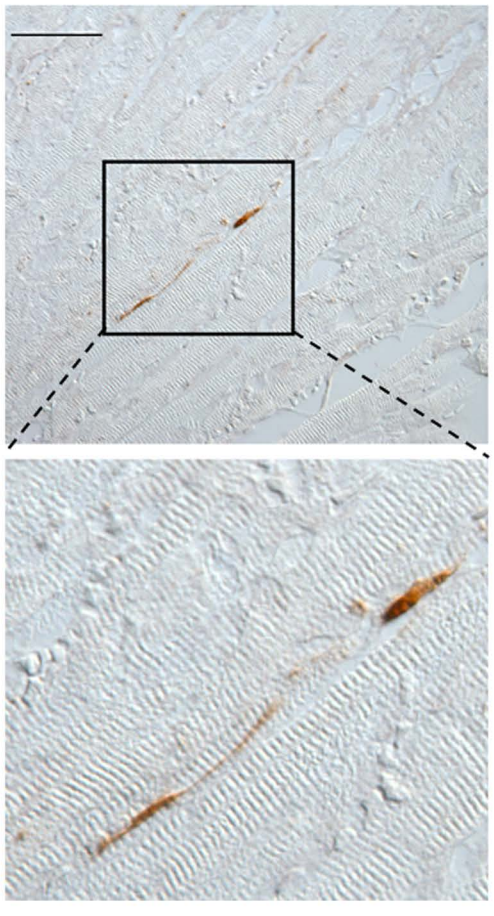
B

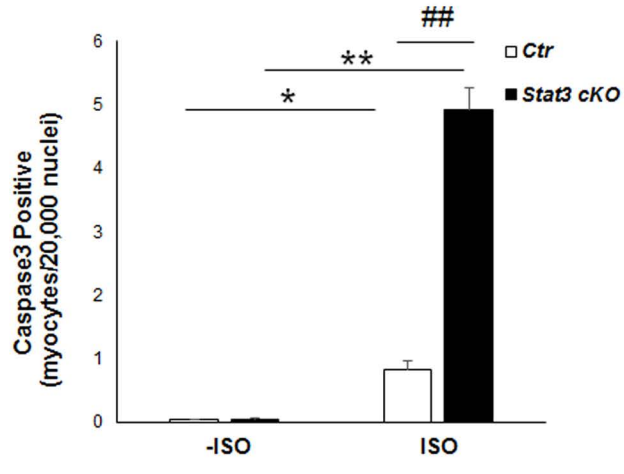




*P<0.05, **P<0.01 v.s. -ISO
##P<0.01 v.s. Ctr

C

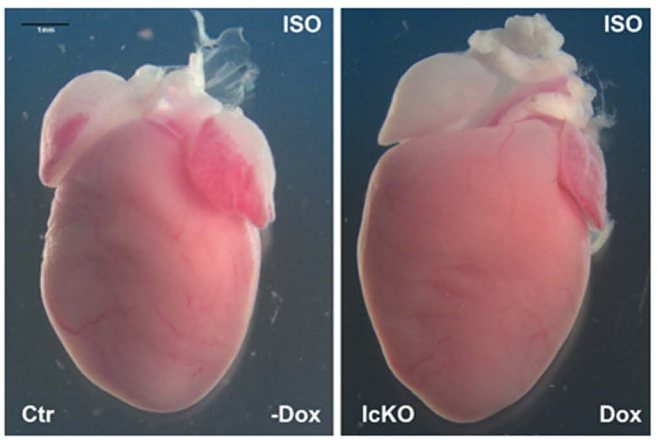


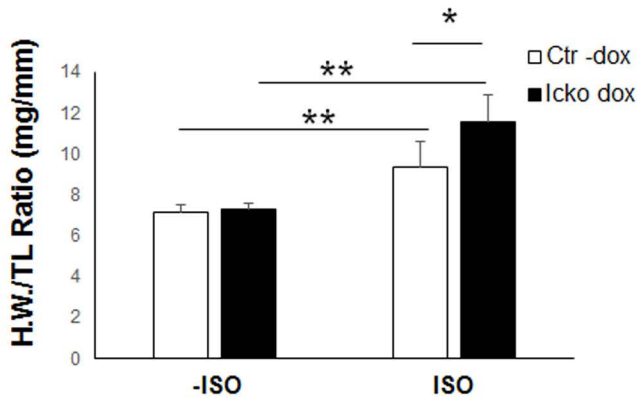


*P<0.05, **P<0.01 v.s. -ISO
##P<0.01 v.s. Ctr

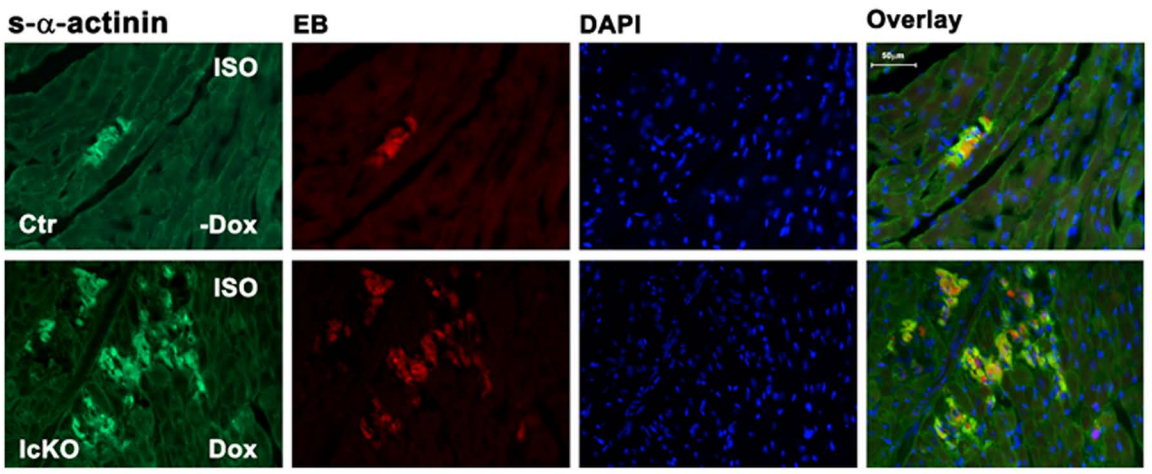
Supplemental Figure 7

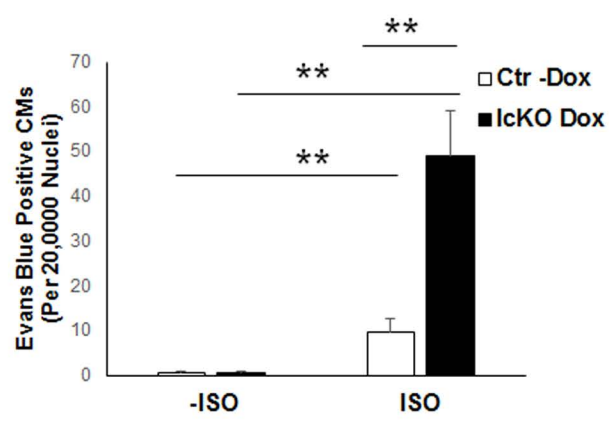
A



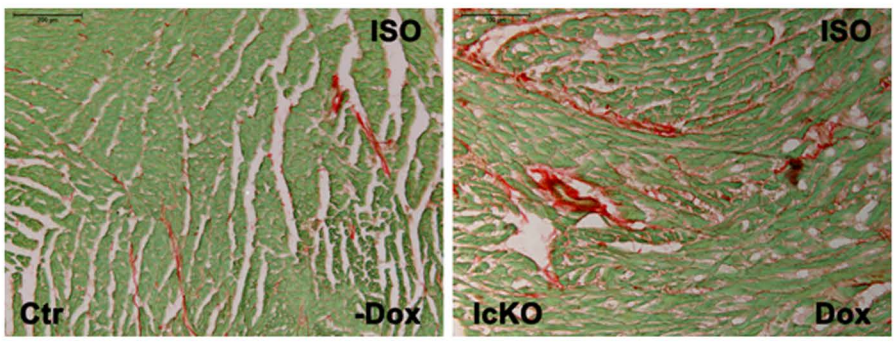


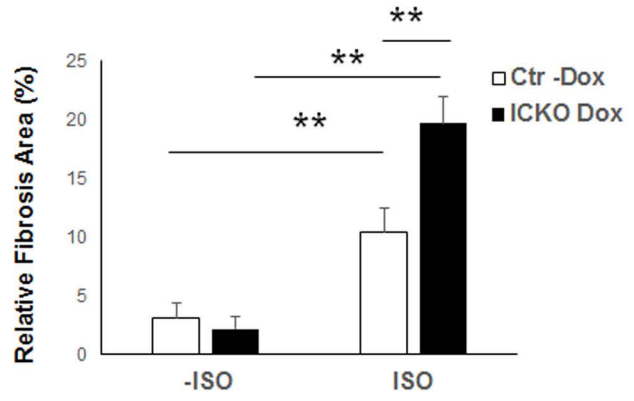
B



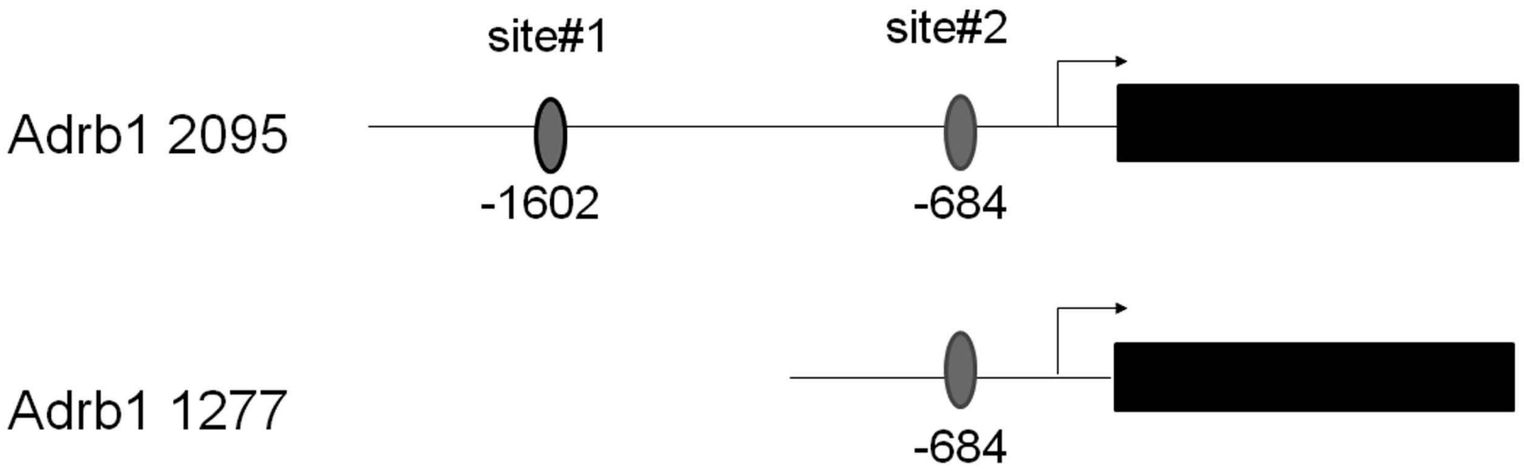


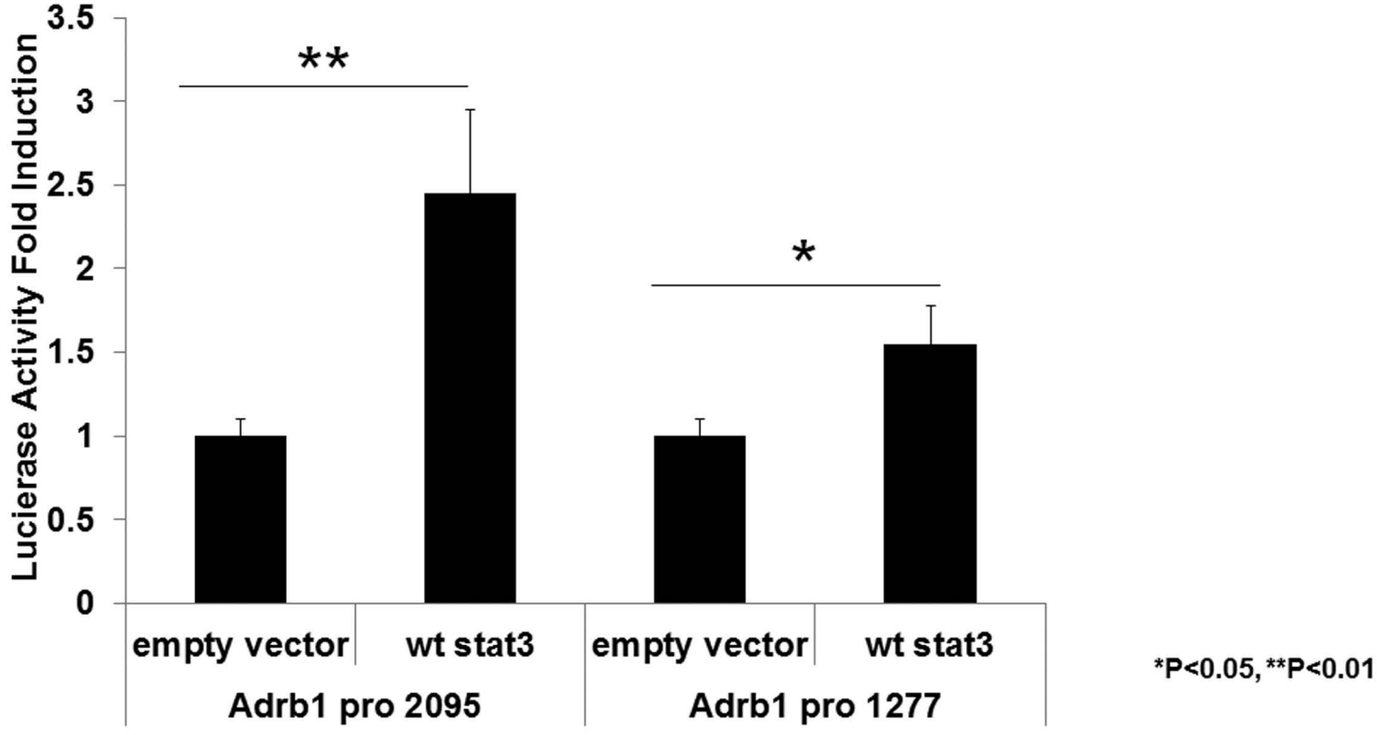
C



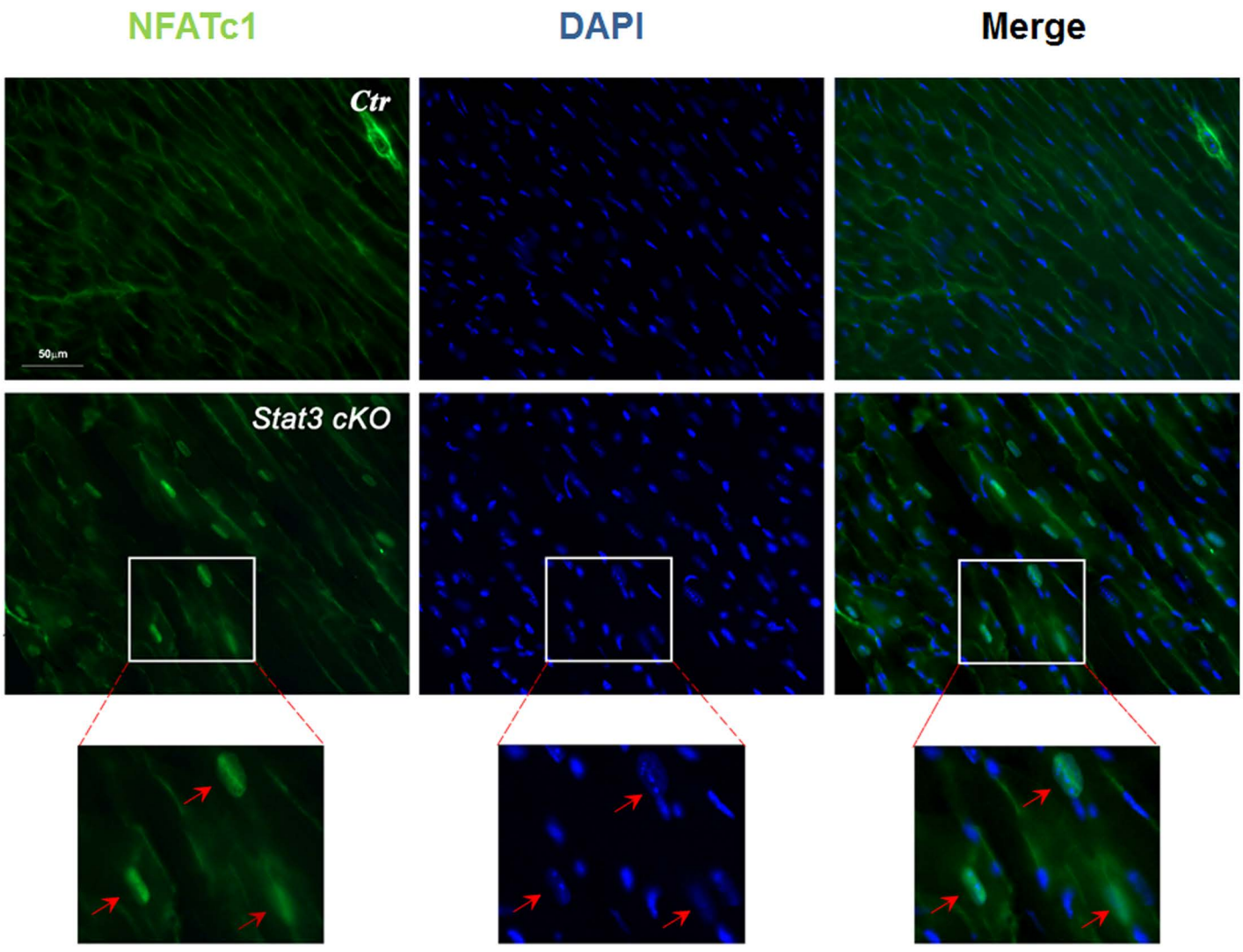


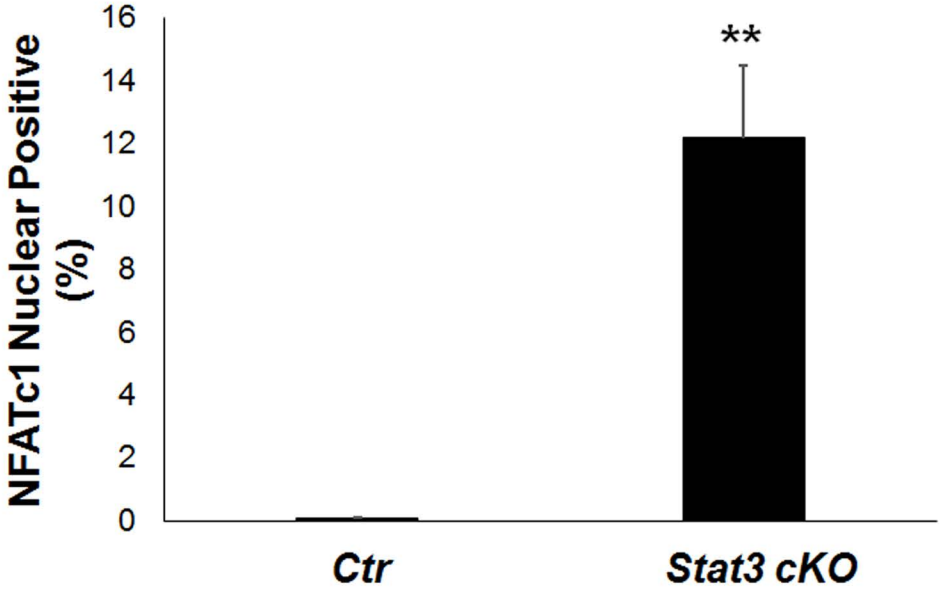
Supplemental Figure 8





Supplemental Figure 9





**P<0.01

Supplemental Figure Legend

Supplemental Figure 1: Activation of STAT3 by βAR agonist. **A**, Western blot analysis of pY-STAT3 in wild-type mouse ventricular tissue after the treatment with isoproterenol (ISO) for indicated times via intraperitoneal (ip) injection (1μg/g bodyweight). **B**, Western blot analysis of STAT3 activation in response to different concentrations (0.001 to 100 μM) of ISO for 30 minutes in mouse embryonic fibroblast (MEF) cells. **C**, Western blot analysis of STAT3 activation in neonatal mouse cardiomyocytes in response to different concentrations (0.02 to 1μM) of dobutamine stimulation. **D**, Western blot analysis of STAT3 activation in neonatal mouse cardiomyocytes in response to ISO after pre-incubation with Propranolol (Pro), Bisoprolol (Biso) or ICI-181,551 (ICI).

Supplemental Figure 2: Cardiomyocyte-restricted ablation of STAT3 does not have a noticeable effects on overall cardiac morphology (**A**), myocyte size (**B**), and myocyte ultrastructure (**C**) in 2-month old mice. The surface electrocardiogram (ECG) reveals largely normal electrophysiology in *Stat3cKO* mice (2-month old) (**D** and **E**).

Supplemental Figure 3: Evaluation of acute LV developed pressure (LVDP) in responses to ouabain stimulation (**A**), and the increase of coronary artery flow rate (B) and heart rate (C) in response to dobutamine in *Stat3cKO* and control hearts using a Langendorff perfusion system. N=6 in each group, ** $P<0.01$. **D**, Western blots show that the levels of phosphorylated troponin I and phospholamban (PLB) after 30 minutes of dobutamine stimulation are reduced in *Stat3icKO* (*Stat3$^{f/f}$;Tnnt2-rtTA;Tre-cre* with doxycycline administration) hearts when compared to control (*Stat3$^{f/f}$;Tnnt2-rtTA;Tre-cre* without doxycycline administration) hearts.

Supplemental Figure 4: Representative Ca^{2+} transient (**A**) and Ca^{2+} sparks (**B**) traces in control and *Stat3cKO* cardiomyocytes with or without dobutamine stimulation. Arrows denote the Ca^{2+} sparks.

Supplemental Figure 5: Echocardiographic analysis of cardiac function in control and *Stat3cKO* mice prior and post 7 days of ISO stimulation. Control mice, n = 10; *Stat3cKO* mice, n = 7. *P<0.05

Supplemental Figure 6: Chronic ISO stimulation induces excessive necrosis and apoptosis in *Stat3cKO* heart. **A**, Representative immunofluorescent staining of anti- C5b9 (green) and α-actinin (sarcomere) antibodies (red) microscopic images (400x) in control and *Stat3cKO* heart (left ventricle) after 7 days of ISO stimulation. Hoechest 33342 staining is in blue. Quantitation of C5b9 positive cardiomyocytes per 20,000 nuclei is shown in the right panel. **B,** Representative images of TUNEL staining (red) of control and *Stat3cKO* hearts with or without ISO administration (400x) (Left panel). Quantitation of TUNEL positive myocytes in control and *Stat3cKO* heart (N = 5/each group) (Right panel). **C**, Representative images of activated caspase-3 in cardiomyocytes from Stat3cKO heart after 7 days of ISO treatment. Quantitation of activated caspase-3 immune reactive cardiomyocytes in control and *Stat3cKO* after 7 days of ISO treatment (Right panel).

Supplemental Figure 7: **Chronic administration of isoproterenol (*ISO*) induces excessive hypertrophy, cardiomyocyte necrosis, and cardiac fibrosis in *Stat3icKO*. A,** Representative heart images of *Stat3icKO* mice (*Stat3$^{f/f}$;Tnnt2-rtTA;Tre-cre* mice with doxycycline administration) and control mice (*Stat3$^{f/f}$;Tnnt2-rtTA;Tre-cre* mice without doxycycline administration) in response to 7-day ISO perfusion. Right panel shows heart weight vs tibia

length ratios (N = 5/each group), *P<0.05, **P<0.01. **B**. Chronic ISO stimulation induces excessive cardiomyocyte necrosis in *Stat3icKO* hearts when compared to controls. Left panel shows representative images of Evans blue (in red) uptake in *Stat3icKO* and control hearts after 7-day ISO perfusion (400x), anti-Sarcomere-α-actinin, in green; DAPI, in blue. Right panel shows the quantitation of Evans blue positive myocytes in *Stat3icKO* and control hearts after 7-day ISO perfusion (N = 5/each group). **C,** Sirius red and fast green staining to demonstrate the collagen deposition in *Stat3icKO* and control hearts after 7-day ISO perfusion. Right panel shows the quantification of collagen deposition contents, *P<0.05, **P<0.01 (N = 5/each group).

Supplemental Figure 8: Overexpression of STAT3 enhances Adrb1 promoter activities. **A**, Diagram of Adrb1 promoter luciferase reporter constructs Adrb1 2099 and Adrb1 1301. **B**, The luciferase activities of Adrb1 2099 and Adrb1 1301 increased 2.5-fold and 1.5-fold with co-transcfection of STAT3 compared to control, respectively.

Supplemental Figure 9: Immunofluorescent staining of NFATC1 (in green) in the left ventricular regions of control and *Stat3cKO* hearts. DAPI is used for counterstaining of nucleus. Arrows indicate co-localization of NFATC1 and DAPI staining in nucleus in the Stat3cKO hearts.