Supplemental Material

Methods

Experimental animals and *in vivo* treatment. The animal care and experimental protocols followed US National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committees (IACUC) of the University of California at Davis, the University of Utah and the Carver College of Medicine of the University of Iowa. C57BL/6 mice were purchased from Charles River. Six-week-old male WT, β_2AR global knockout (β_2KO) and β-arrestin2 global knockout (β-arr2 KO) mice randomly assigned to two groups fed ad libitum with either a low-fat diet or a matched high-fat diet (Research Diets Inc.) for six months (n=26). The low fat diet was D12450J (3.85 kcal/g; 10% of calories from fat, 20% of calories from protein and 70% of calories from carbohydrate) and the high fat diet (HFD) was D12492 (5.24 kcal/g; 60% of calories from fat, 20% of calories from protein and 20% of calories from carbohydrate). Blood glucose levels were measured after a fast of 6 hours. Cardiac function was assessed before and after 24 wk. HFD or chow diet by echocardiography under isoflurane anesthesia. Mice were subjected to intraperitoneal glucose tolerance testing (IPGTT) following each echocardiography study. For the treatment experiments, after 14 weeks of HFD feeding, WT mice (n=45) were randomly assigned to three groups according to the cardiac function. Each group were treated by daily oral gavage with vehicle (5% dimethyl sulfoxide (DMSO) in water), paroxetine (2.5 mg/kg in 5% DMSO, Ark Pharm, Libertyville, IL) or carvedilol (2.5mg/kg in 5% DMSO, Cayman Chemical, Ann Arbor, MI) for 4 wk. Metabolic parameters were monitored during the treatment period, including weekly body weight and food consumption and monthly fasting blood glucose levels. Intraperitoneal glucose tolerance tests (IPGTT) and cardiac function were determined prior to and after the respective diets and treatments. In a separate series of experiments, mice with cardiomyocyte-restricted deletion of insulin receptors (CIRKO) were fed a normal chow diet or a high fat diet for 36 weeks. In this study the HFD comprised 45% of calories from fat and 17% of calories from sucrose and the control diet comprised 10% of calories from fat and 7% of calories from sucrose. At the end of the experiment, the animals were euthanized; blood samples were collected; heart, fat, liver and muscles were weighed and stored at 80°C for further experimental analysis.

Human Atrial Appendage Samples. Right atrial appendage samples were obtained from diabetic and non-diabetic humans at the time of coronary artery bypass surgery from subjects described in a prior study [1].

Echocardiography. Echocardiography was performed using a Vevo 2100 imaging system from VisualSonics (Toronto, ON, Canada) with a 22-55 MHz MS550D transducer. Mice were anaesthetized with isoflurane (2% for induction and 1% for maintenance) supplemented with 100% O_2. Body temperature, respiratory rate, and ECG were constantly monitored. To minimize variation of the data, the heart rate was maintained at 450-550 beats per minute at the time when cardiac function was assessed. Cardiac function was recorded at baseline and after administration of the βAR agonist isoproterenol (ISO, 0.2 mg/kg, i.p.). Systolic function parameters including ejection

fraction (EF) and fractional shortening (FS) were measured in the two-dimensional parasternal short-axis imaging plane of M-mode tracings close to the papillary muscle level. Tissue Doppler imaging mode and pulsed-wave (PW) Doppler mode were applied to measure diastolic function as described [2, 3].

Intraperitoneal glucose tolerance test. Glucose tolerance tests were performed as described previously [4]. Briefly, mice in awake were fasted for 6h and weighed, and received an intraperitoneal injection of a glucose solution (1 mg/g i.p.). Blood samples were taken from a tail nick and glucose levels were measured using a Bayer Breeze2 standard glucometer 0, 15, 30, 60 and 120 min post injection (Bayer, Pittsburgh, PA). Area under the curve (AUC) was determined to quantify glucose intolerance.

Histology. Mice were euthanized and hearts were perfused with 4% paraformaldehyde and fixed in paraformaldehyde for 24h. Fixed hearts were then washed and stored in 70% ethanol at 4°C. Hearts were dehydrated and cleared on an Autotechnicon Mono Tissue Processor (Technicon Corporation, Tarrytown, NY), embedded in paraffin using HistoEmbedder (Leica Biosystems, Richmond, IL), and sectioned into five-micron slices using Leica RM2255 Microtomes. Tissue sections were stained with hematoxylin and eosin to examine heart morphology, and Masson's Trichrome (Sigma-Aldrich, St. Louis, MO) staining was used to quantify myocardial fibrosis. The staining was performed according to the manufacturer's protocol. For the semi quantitative analysis of collagen expression, the blue-stained areas in the sections were measured using the Image J program (NIH, Bethesda, MD, USA) by a researcher blinded to the samples. Apoptosis detection was done in fixed slices of hearts using the Promega DeadEnd™ Fluorometric TUNEL system according to manufacturer's instructions. Briefly, the slices were then permeabilized with 20 ug/mL Proteinase K and labeled with fluorescein-12-dUTP[(a)] using recombinant terminal deoxynucleotidyl transferase (rTdT). Vectashield Hard Set mounting medium with DAPI (Vector Labs) was used as the counter stain. Negative control samples were exposed to the same protocol, but lacked the rTdT enzyme treatment. Positive control samples were additionally exposed to 7.5 units/mL of DNase I (Promega) for 10 minutes at room temperature after permeabilization. The quantification was performed in six regions of each heart.

Blood insulin concentration. Plasma insulin levels were detected with an ALPCO mouse ultrasensitive insulin ELISA kit (Salem, NH) according to the manufacturer's instructions.

Cyclic AMP detection. Cyclic AMP levels in heart tissues were measured with the cAMP-Glo™ Assay kit (Promega, Madison, WI) following the manufacturer's instructions. 20 mg of heart tissue was homogenized in 500 µl chilled lysis buffer (25 mM HEPES, 0.15% Triton X-100, 150 mM NaCl, 0.5 mM IBMX, 100 µM Ro20-1724) using Lysing Matrix D Tubes (MP Biomedicals, Santa Ana, CA) using the FastPrep instrument (MP Biomedicals, Santa Ana, CA) for 40 seconds twice. The tubes were placed on ice for 5 minutes between each run. The lysates were sonicated on ice for three seconds, three times. The homogenate was centrifuged at 14,000 rpm for 15 minutes at 4 °C to eliminate tissue debris. Samples were heated for 10 minutes at 70 °C and were then

chilled on ice for 5 minutes. The samples were centrifuged to remove any precipitated proteins at 14,000 rpm at 4°C for 10 minutes. The supernatants were decanted and kept on ice and protein concentrations were measured using the Pierce BCA Protein Assay Kit (Life Technologies, Benicia, CA). The supernatants were diluted according to the protein concentration to 40 µl with lysis buffer and warmed to room temperature after which they were added into the appropriate wells of a white 96 well plate, one well was left with just lysis buffer as control. A series dilution of standard cAMP was used for the standard curve. Ten microliters of cAMP Detection Solution was added to all wells, and the plate was mixed by shaking for 2 minutes and incubated at room temperature for 20 minutes. Fifty micrograms of room temperature Kinase-Glo Reagent was added to all reactions, and the plate was mixed by shaking for 2 minutes and incubated at room temperature for 10 minutes. Relative luminescence was read on a SpectraMax M5 Multi-Mode Microplate Reader (Molecular Devices, Silicon Valley, CA) and ΔRLU was calculated by subtracting each value for each sample from that obtained with lysis buffer only. The cAMP levels are presented as ng/ug total protein.

Western blotting. Left ventricular extracts were prepared as previously described [5] in lysis buffer (25mM Tris HCl PH 7.6, 150 mM NaCl, 1% IGEPAL, 1% Sodium deoxycholate, 0.1% SDS, 1mM EDTA) with protease and phosphatase inhibitors (Na_3F 100mM, Na_2VO_4 1mM, glycerol 1mM, NaP_2O_7 2.5 mM, leupeptin 10 µg/ml, PMSF 1mM, aprotinin 10 µg/ml), and protein concentration was measured by BCA assay (Pierce, Rockford, IL). Lysates (50 µg total protein) were resolved by SDS-PAGE. The extracts were then transferred onto a PVDF membrane (Merck Millipore, Billerica, MA) and incubated with the primary antibody followed by IRDye 680CW or 800CW secondary antibodies and imaged with an Odyssey scanner (LI-COR Biosciences Lincoln, NE). The primary antibodies used for Western blotting were against following proteins: β_1AR (V-19), β_2AR (M-20), GRK2 (C-15), PDE2A (H-300), PDE5A (H-120), $G\alpha_i$ (C-10), insulin receptor-beta (C-19), IRS2 (M-19), and βarr2 (H-9) from SCBT, Santa Cruz, CA, and phospho-Akt (Ser473, #4051), AKT (#9272), phospho-MAP kinase (T202/T204, #9106), MAP kinase (p44/42, #91202), phospho-troponin (Ser23/24, #4004), troponin (#4002) from Cell Signaling, Danvers, MA, PDE3A (1098-1115, a gift from Vincent Manganiello, NIH), PDE4A (a gift from Marco Conti, University of California at San Francisco), PDE4D (ab14613, Abcam, Cambridge, MA), Serca (2A7-A1, Thermo, Rockford, IL), phospho-phospholamban (Ser16, Badrilla, London, UK), phospholamban (MA3-922, Affinity Bioreagent, Golden, CO), IRS1 (#06-248, Millipore, Billerica, MA), gamma-tubulin (T6557, Sigma-Aldrich, St Louis, MO). Signal intensity was quantitated by Image Studio software version 2.1 (LI-COR Biosciences, Lincoln, NE).

Quantitative RT/PCR. Fifty micrograms of finely minced heart tissue and 1 ml of ice-cold TRI Reagent (Sigma-Aldrich, St Louis, MO) was added to the Matrix Lysing D tube (MPbio, Santa Ana, CA). The tubes were secured in the Fast Prep machine (MPbio, Santa Ana, CA) and homogenized on speed 4.0 for 30 seconds. To ensure complete dissociation of nucleoprotein complexes, samples were allowed to stand for 5 minutes at room temperature. The TRI Reagent and sample were decanted into a 1 ml polypropylene tube after which 0.1 ml of 1-bromo-3-chloropropane was added. The sample was covered tightly, and vigorously shaken for 15 seconds, and the samples allowed to stand for

15 minutes at room temperature. The resulting mixture was then centrifuged at 12,000 × g for 15 minutes at 4 °C. The aqueous phase was then transferred to a fresh tube after which 0.5 ml of 2-propanol was added and the sample mixed. The sample was allowed to stand for 10 minutes at room temperature. Samples were centrifuged at 12,000 × g for 10 minutes at 4 °C. The RNA precipitate formed a pellet on the side and bottom of the tube. The supernatant was removed and the RNA pellet washed by adding 1 ml of 75% ethanol. The samples were vortexed and centrifuged at 7,500 × g for 5 minutes at 4 °C, after which the RNA pellet was dried for 10 minutes by air-drying. 40 µl of RNase-free water was added to dissolve the RNA pellet. All the reagents for mRNA isolation were from Sigma-Aldrich (St. Louis, MO). Messenger RNA was purified by utilizing a RNA Clean and Concentrator kit (Zymo Research, Irvine, CA) according to the manufacturer's instructions and quantified with a Synergy 2 Multi-Mode Reader (Bio-Tek, Winooski, Vermont). A260/280 reading was also performed to evaluate the purity of RNA extractions. One microgram RNA template was reverse-transcribed into cDNA by the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster, CA) using Applied Biosystems Veriti 96 Well Thermal Cycler. The sequence-specific primers were reconstituted and diluted to 100 nM in nuclease free water and stored at -20 °C until experimental use. All primer sequences are listed in Table 4. SYBR Advantage qPCR Premix kit (Clontech Laboratories, Inc. Mountain View, CA) was applied to measure mRNA expression according to kit directions using the ViiA™ 7 Real-Time PCR System (Life Technologies). The relative expression level of specific mRNA was determined by the comparative cycle threshold (CT) method ($2^{-\Delta\Delta CT}$), normalized to the endogenous control gene GAPDH. Each RNA sample was assayed in triplicate. The primers used in real-time PCR are listed below.

Gene	Primer	Sequences
α-MHC	F	5'-CAACGCCAAGTGTTCCTC-3'
	R	5'-AGCTCTGACTGCGACTCCTC-3'
β-MHC	F	5'-ATGTGCCGGACCTTGGAAG-3'
	R	5'-CCTCGGGTTAGCTGAGAGATCA-3'
ANP	F	5'-TCGTCTTGGCCTTTTGGCT-3'
	R	5'-TCCAGGTGGTCTAGCAGGTTCT-3'
BNP	F	5'-CTCCTGAAGGTGCTGTCC-3'
	R	5'-GCCATTTCCTCCGACTTT-3'
Col1a1	F	5'-TAGGCCATTGTGTATGCAGC-3'
	R	5'-ACATGTTCAGCTTTGTGGACC-3'
TGFβ1	F	5'-CGCCATCTATGAGAAAACC-3'
	R	5'-GTAACGCCAGGAATTGT-3'
PDE2A	F	5'-GGTGGCCTCGAAATCTGTGCTGG-3'
	R	5'-GCATGCGCTGATAGTCCTTCCG-3'
PDE3A	F	5'-CGACTCCGATTCTGACAGTG-3'
	R	5'-ATATTCCCAGACAGGCATCC-3'
PDE4A	F	5'-CTTCTGCGAGACCTGCTCCA-3'
	R	5'-GAGTTCCCGGTTCAGCATCC-3'
PDE4B	F	5'-AATGTGGCTGGGTACTCACA-3'
	R	5'-AAGGTGTCAGATGAGATTTTAAACG-3'

PDE4D	F	5’-ACCGCCAGTGGACGGACCGGA-3’
	R	5’-CATGCCACGCTCCCGCTCTCGG-3’
PDE5A	F	5’-AAATCAATTCAGTTTTGAAGATCC-3’
	R	5’-TGTTGAATAGGCCAGGGTTT-3’
GAPDH	F	5’-CATGGCCTTCCGTGTTCCTA-3’
	R	5’-CCTGCTTCACCACCTTCTTGAT-3’

Primary adult cardiomyocyte isolation and culture. The isolation of adult cardiomyocytes was carried out as described previously [5]. In brief, mice were anaesthetized with isoflurane (2%), and hearts were removed and placed in cannulation buffer (NaCl 120mM, KCl 5.4 mM, NaH_2PO_4 1.2 mM, $NaHCO_3$ 20 mM, $MgSO_4$ 1.2 mM, Glucose 5.6 mM, 2,3-Butanedione monoxime 10mM, Taurine 20mM, PH7.33. The solution was bubbled with 95% O_2 and 5% CO_2 for 10min, then filtered through a 0.22 micron filter). The heart was gently cannulated via the aorta using a small cannula attached to a 1 cc syringe and filled with cannulation buffer. After perfusing with the cannulation buffer for 4 minutes (3 ml per minute) and when there was about 10 ml buffer remaining in reservoir, 5 ml collagenase solution (containing 2.5 mg type II collagenase (Worthington Biochemical, Lakewood, NJ), 0.5 mg type XIV protease (Sigma-Aldrich) and 0.1 % BSA, filtered) was added into buffer for pre-digestion. Then the perfusion was continued with 20 ml of a fresh collagenase solution (containing 10 mg type II collagenase, 2 mg type XIV protease, 50 μM $CaCl_2$, 0.1 % BSA, filtered), which was recirculated to maintain perfusion for approximately 12-16 minutes until the heart became soft. The heart was cut below the atria into a dish containing 5 ml of collagenase solution with calcium, cut 5-10 times and transferred into a 15 ml tube. The supernatant was transferred into a new tube with 5 ml stopping buffer (12.5 μM $CaCl_2$ and 0.5 ml FBS), and centrifuged at 500 rpm for 1 minute, and repeated up to 2 times to ensure digestion. The cell pellet was resuspended and recovered in gradient calcium buffer to a physiological concentration (1mM). For myocyte cultures, the cell number was counted and 50,000 /ml cell was plated on natural mouse laminin (Life Technologies, Grand Island, NY) coated dishes with M1018 media (10.7 g Sigma M1018 Minimum Essential Medium Eagle, 0.35 g NaCHO3, 1% Penicillin-Streptomycin, PH 7.4, filtered).

Fluorescence resonance energy transfer (FRET) measurements. Adult rat cardiomyocytes were cultured on glass coverslips and infected with regular ICUE3, an Epac based cAMP biosensor for 36 h. Coverslips with living myocytes were treated with insulin 100 nM, overnight in media and then maintained in PBS w/o calcium (KCl 2.68 mM, KH_2PO_4 1.47 mM, NaCl 136.89 mM, Na_2HPO_4 8.1 mM) for FRET recording as described previously [6]. Images were acquired using a Leica DMI3000 B inverted fluorescence microscope (Leica Biosystems, Buffalo Grove, IL) with a 40×/1.3 numerical aperture oil-immersion objective lens and a charge-coupled device camera controlled by Metafluor software (Molecular Devices, Sunnyvale, CA). FRET was recorded by exciting the donor fluorophore at 430-455 nm and measuring emission fluorescence with two filters (475DF40 for cyan and 535DF25 for yellow). Images were subjected to background subtraction, and were acquired every 20 seconds with exposure time of 200

Ms. The donor/acceptor FRET ratio was calculated and normalized to the ratio value of baseline. The binding of cAMP to ICUE3 decreased CFP–YFP FRET efficiency.

Adult cardiomyocyte contractility and calcium transient. Freshly isolated adult cardiomyocytes were loaded with Fluo-4 AM (5 μM; Molecular Probes, Grand Island, NY,) for 30 min. Cells were then placed in the middle of a glass-bottomed dish with 3 ml beating buffer (NaCl 120 mM, KCl 5.4 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, HEPES 20 mM, Glucose 5.5 mM, $CaCl_2$ 1mM, PH 7.1, filtered) and settled for 5 minutes. Platinum electrodes were placed near the cell and paced at 1 Hz with voltage of 30 V using the SD9 stimulator (Grass Technology, Warwick, RI) as described [7]. The calcium transient and contractile events of myocytes were recorded on an inverted microscope (Zeiss AX10, Dublin, CA) at 20× magnification enabling observation of 5-10 rod-shaped cardiomyocytes with clear striations per field of view using the Metamorph program (Molecular Devices, Sunnyvale, CA). The following settings were applied: time interval 1 minute, duration 10 minutes, 200 frames per movie, exposure time 25 ms, movie length 5 seconds. Drugs (Rolipram 100nM, ISO 100 nM) were added into the dish after two movies were acquired. The percentage of myocyte fractional shortening (FS) was calculated as (maximal cell length – minimal cell length)/ maximal cell length × 100%. The analysis was performed by Metamorph. The calcium transient were recorded with excitation at 488 nm and emission collected at >505 nm. Analysis was made using a homemade routine in interactive data language (ITT) as previously described [8].

Statistical analysis. All data are expressed as mean ± SEM. All statistical analysis was performed using GraphPad Prism 6 software (La Jolla, CA). The sample size for each group is shown in the figure legends or tables. The *in vitro* studies were done with at least three sets of independent experiments. Differences between two groups were evaluated by 2 -tailed Student's *t*-test; comparisons of multiple groups were performed using either one-way or two-way ANOVA followed by post hoc Tukey's test. $P < 0.05$ was defined as statistically significant.

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Supplementary Figure Legends for:

Inhibiting insulin-mediated β_2AR activation prevents diabetes-associated heart failure.

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Supplementary Figure 1. Characterization of metabolic profiles in HFD mice. a) Plasma glucose and insulin levels were measured in HFD- and NC-fed mice after a 6-hour fast (n=12). b) After 6 months of HFD GTT was performed on HFD- and NC-fed mice (n=12). Time course of plasma glucose levels after IP glucose injection (1g/kg, left); the area under the curve (AUC) was plotted (right). $^{\#}p < 0.05$ and $^{\#}p < 0.001$ by student *t*-test between paired groups. c) 6 months of HFD decreases protein levels of IRβ and IRS2, but increases phosphorylation of S6K1 (T389) and GSK3 (S9) in mouse hearts (n=12). ** $p < 0.01$ and *** $p < 0.001$ by student *t*-test between paired groups. d) Insulin (100 nM) induces activation of ERK (T202/Y204) and Akt (S473) in myocytes isolated from NC and HFD hearts (N = 3). * $p < 0.05$ and ** $p < 0.01$ by one-way ANOVA followed by post hoc Tukey's test.

Supplementary Figure 2. Cardiac tissue characterization of HFD-fed wildtype mice. a) Heart sections from HFD- and NC-fed mice were stained with hematoxylin and eosin to examine heart morphology, Masson's Trichrome to examine fibrosis, and TUNEL staining to examine apoptosis, respectively (n=4). Wall thickness of left ventricle, fibrosis, and apoptosis were quantified and plotted. b) mRNA levels were measured from

heart lysates in HFD- and NC-fed mice (n=12). $^{\#}p< 0.05$, $^{\#\#}$ $p < 0.01$, and $^{\#\#\#}$ $p < 0.001$ by student *t*-test between paired groups.

Supplementary Figure 3. Characterization of the insulin-induced transactivation of β_2AR-β-arrestin2-ERK pathway in cardiac myocytes. a) Insulin (100 nM, 5 minutes) promotes formation of a β_2AR-β-arrestin2-ERK complex in AVMs (N=3). * $p < 0.05$ by student *t*-test between paired groups. b) Insulin-induced ERK activation and PDE4D expression in AVMs from WT and β_1KO mice (n=3). * $p < 0.05$ by student *t*-test between paired groups. c) Insulin (100nM)-induced ERK activation (30 minutes) and PDE4D expression (12hrs) in mouse AVMs is blocked by a GRK2 inhibitor paroxetine (Parox, 100μM), but not by fluoxetine (Fluox 100μM, n=5). * $p< 0.05$ by one-way ANOVA followed by post hoc Tukey's test. d) Insulin (100nM)-induced ERK activation (30 minutes) and PDE4D expression (12 hours) in mouse AVMs is attenuated by β-blocker carvedilol (100 nM) and timolol (1μM). * $p< 0.05$ by one-way ANOVA followed by post hoc Tukey's test.

Supplementary Figure 4. Characterization of metabolic profiles in HFD-fed β_2AR-KO and β-arrestin2-KO mice. a and b) Plasma glucose and insulin levels were measured in HFD- and NC-fed mice after a 6-hour fast (n=12). c) After 6 months of HFD, GTT was performed on HFD and NC-fed mice (n=12). Time course of plasma glucose levels after IP of glucose (1g/kg, left); the area under the curve (AUC) was plotted (right). $^{*}p< 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by student *t*-test between paired groups.

Supplementary Figure 5. Cardiac tissue characterization of HFD-fed β_2AR-KO and β-arrestin2-KO mice. a and b) Heart sections from HFD- and NC-fed mice were stained with hematoxylin and eosin to examine heart morphology, Masson's Trichrome to examine fibrosis, and TUNEL staining to examine apoptosis, respectively (n=12). Wall thickness of left ventricle, fibrosis, and apoptosis were quantified and plotted. c and d) mRNA levels were measured from heart lysates in HFD- and NC-fed mice. $^{\#}p< 0.05$ and $^{\#\#}p < 0.01$ by student *t*-test between paired groups. $^{*}p< 0.05$, ** $p < 0.01$, and *** $p < 0.001$ by one-way ANOVA followed by post hoc Tukey's test.

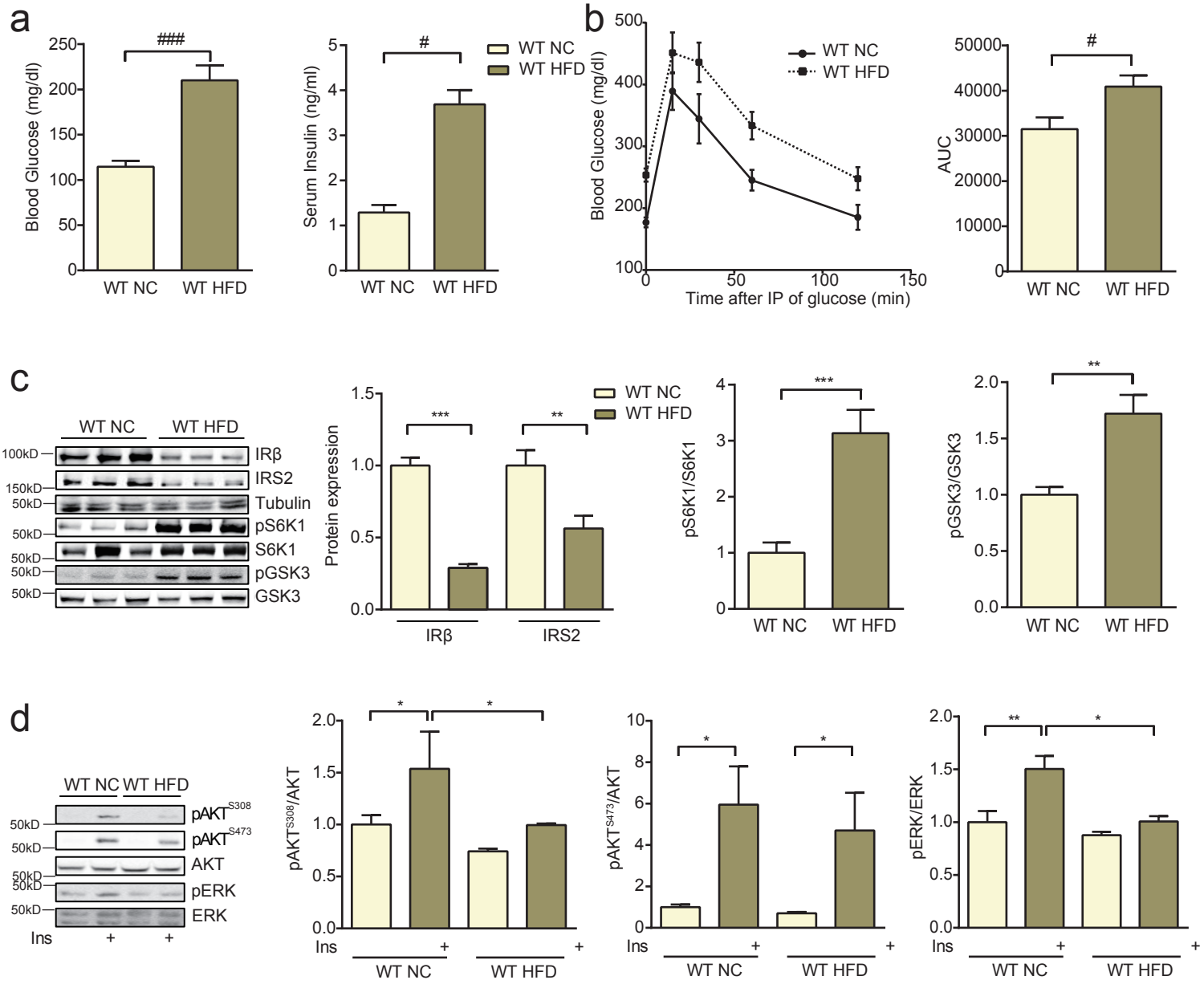
Supplementary Figure 6. Characterization of gene expression in HFD cardiac tissues. a) mRNA levels were measured from heart lysates in HFD- and NC-fed β_2AR-KO mice (n=12). b) mRNA levels were measured from heart lysates in HFD- and NC-fed β-arrestin2-KO mice (n=12). c) mRNA levels were measured from heart lysates in HFD- and NC-fed mice after drug therapies (n=12). *$p< 0.05$ and **$p < 0.01$ by student *t*-test between paired groups.

Supplementary Figure 7. Metabolic characterization of HFD-fed mice after treatment with paroxetine and carvedilol. a) After 4 months of HFD, mice were treated with vehicle, paroxetine (2.5 mg/kg body weight) or carvedilol (2.5 mg/kg body weight) for 4 weeks. Plasma glucose and insulin levels were measured in HFD- and NC-fed mice after a 6-hour fast (n=12). b). GTT was conducted on HFD and NC-fed mice after a 6-hour fast (n=12). Time course of plasma glucose levels after IP injection of glucose (1g/kg,

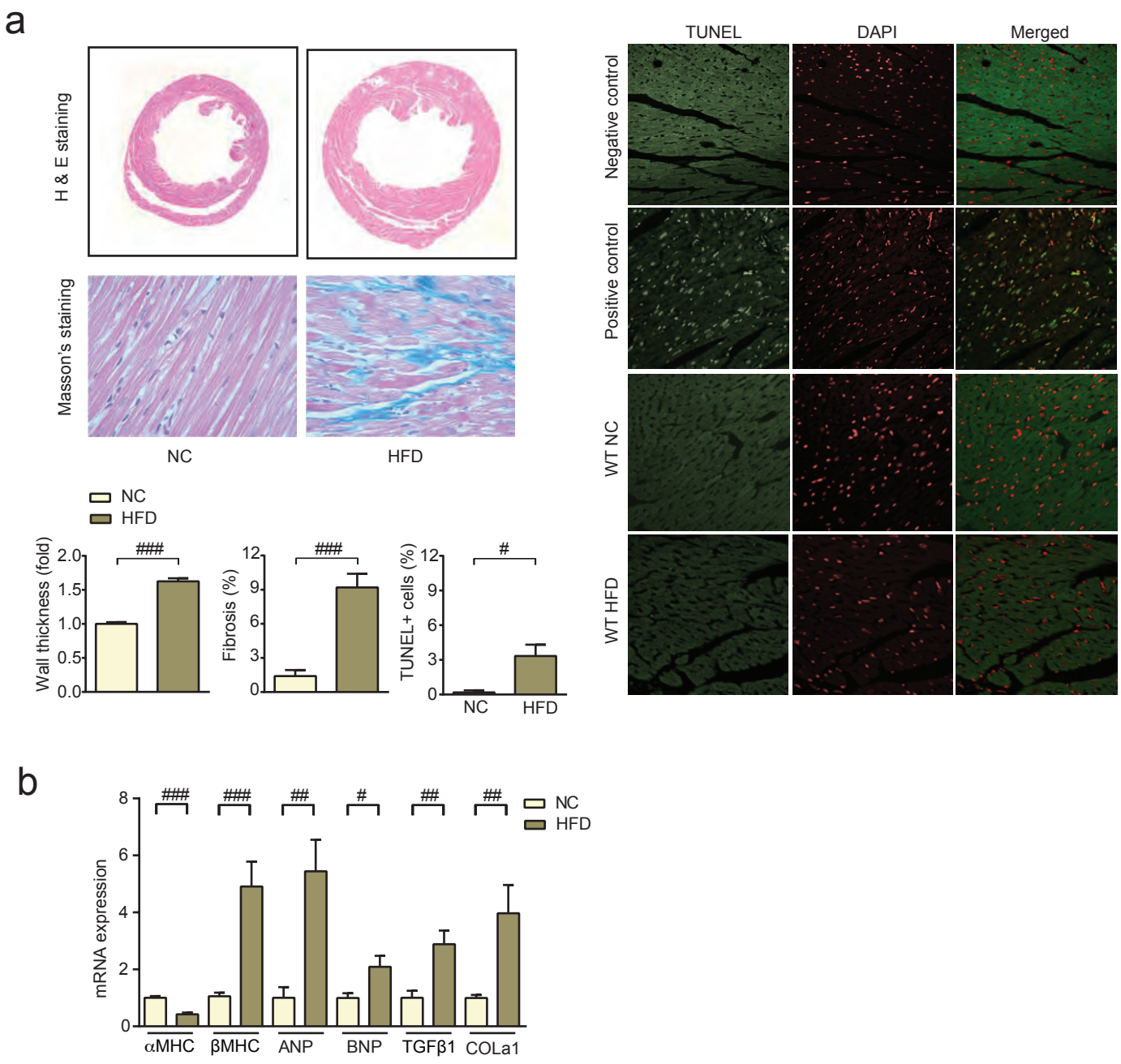
left); the area under curve (AUC) was plotted (right). *p< 0.05 and *** p < 0.001 by one-way ANOVA followed by post hoc Tukey's test.

Supplementary Figure 8. Cardiac tissue characterization of HFD-fed mice after treatment with paroxetine and carvedilol. a and b) Heart sections from HFD- and NC-fed mice were stained with hematoxylin and eosin to examine heart morphology and Masson's Trichrome to examine fibrosis, and TUNEL staining to examine apoptosis, respectively (n=4). Wall thickness of the left ventricle, fibrosis, and apoptosis were quantified and plotted. c) mRNA levels were measured from heart lysates in HFD- and NC-fed mice (n=12). * p< 0.05, ** p < 0.01, and *** p < 0.001 by one-way ANOVA followed by post hoc Tukey's test.

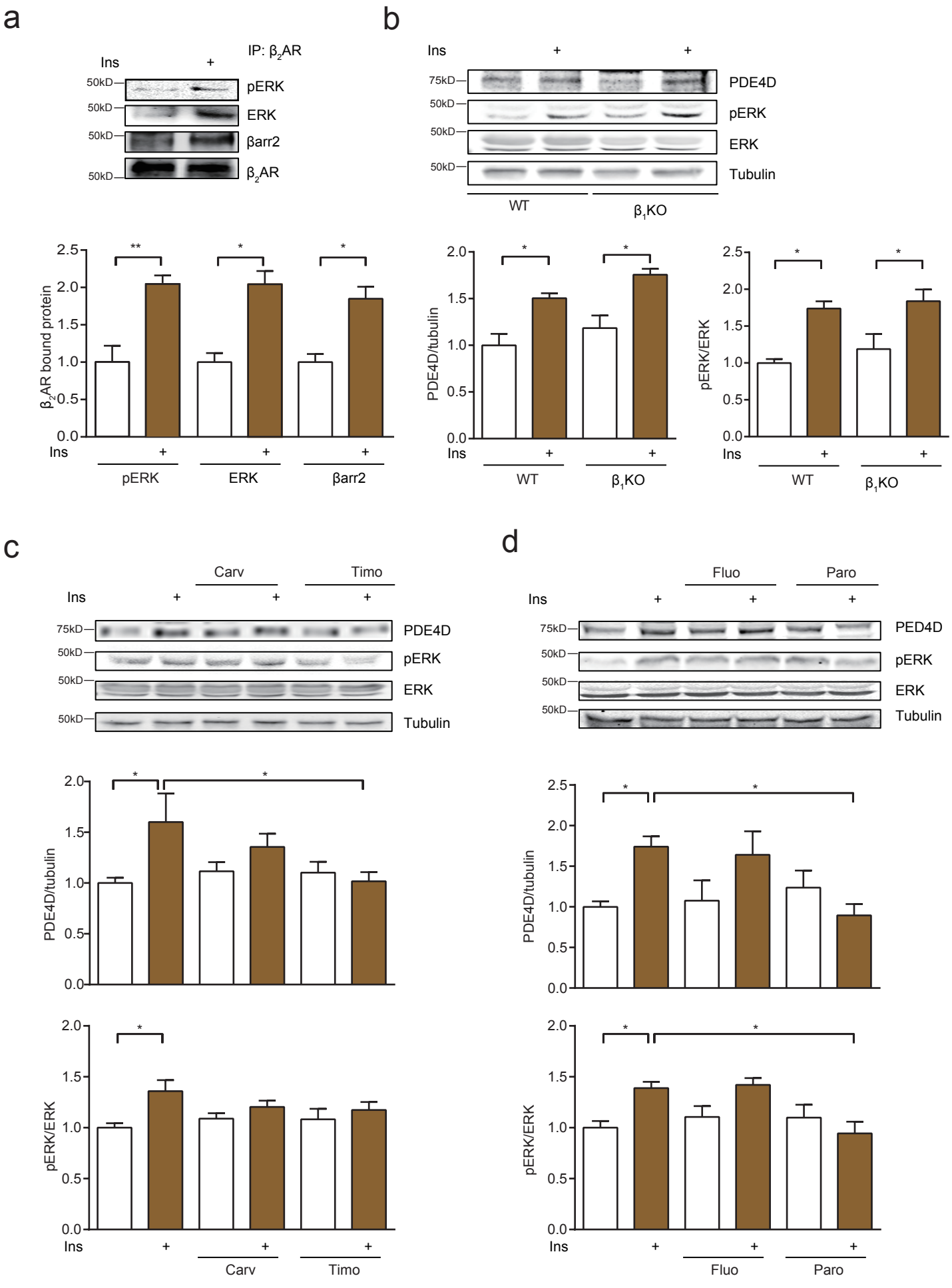
Supplemental Figure 1



Supplemental Figure 2



Supplementary Figure 3



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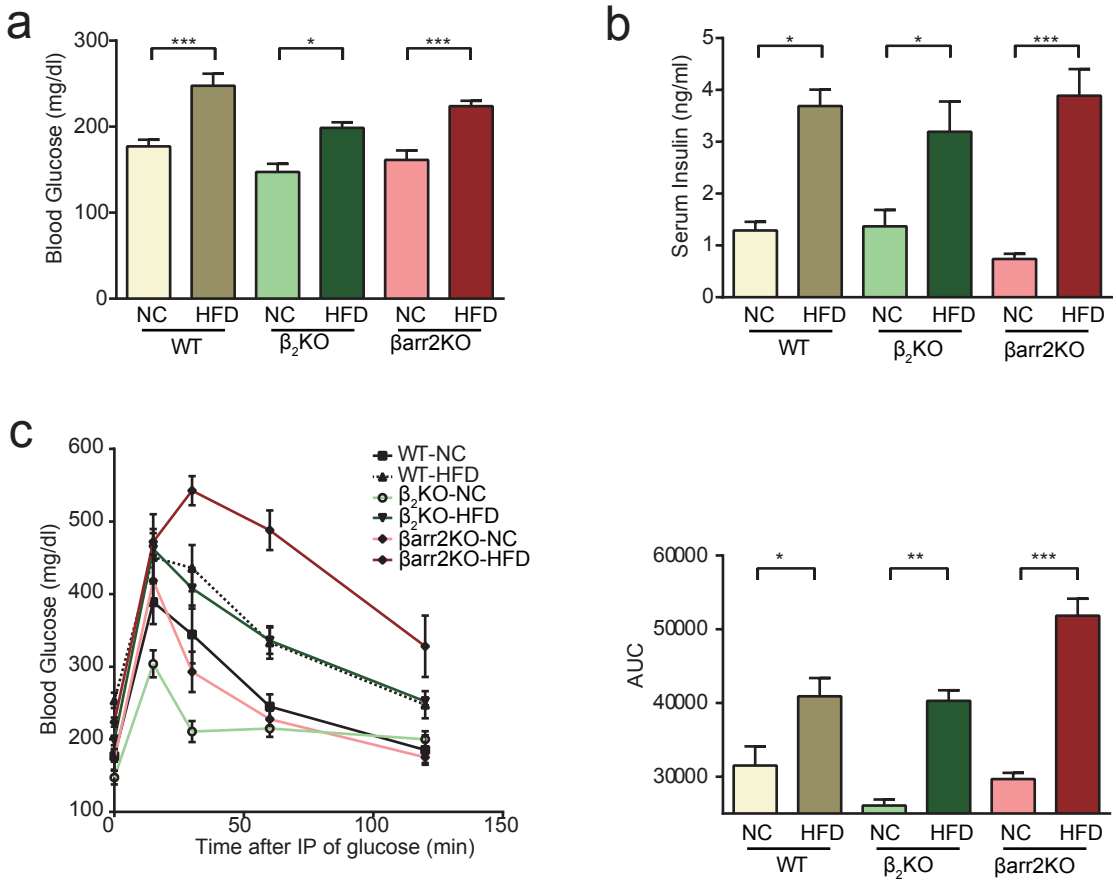

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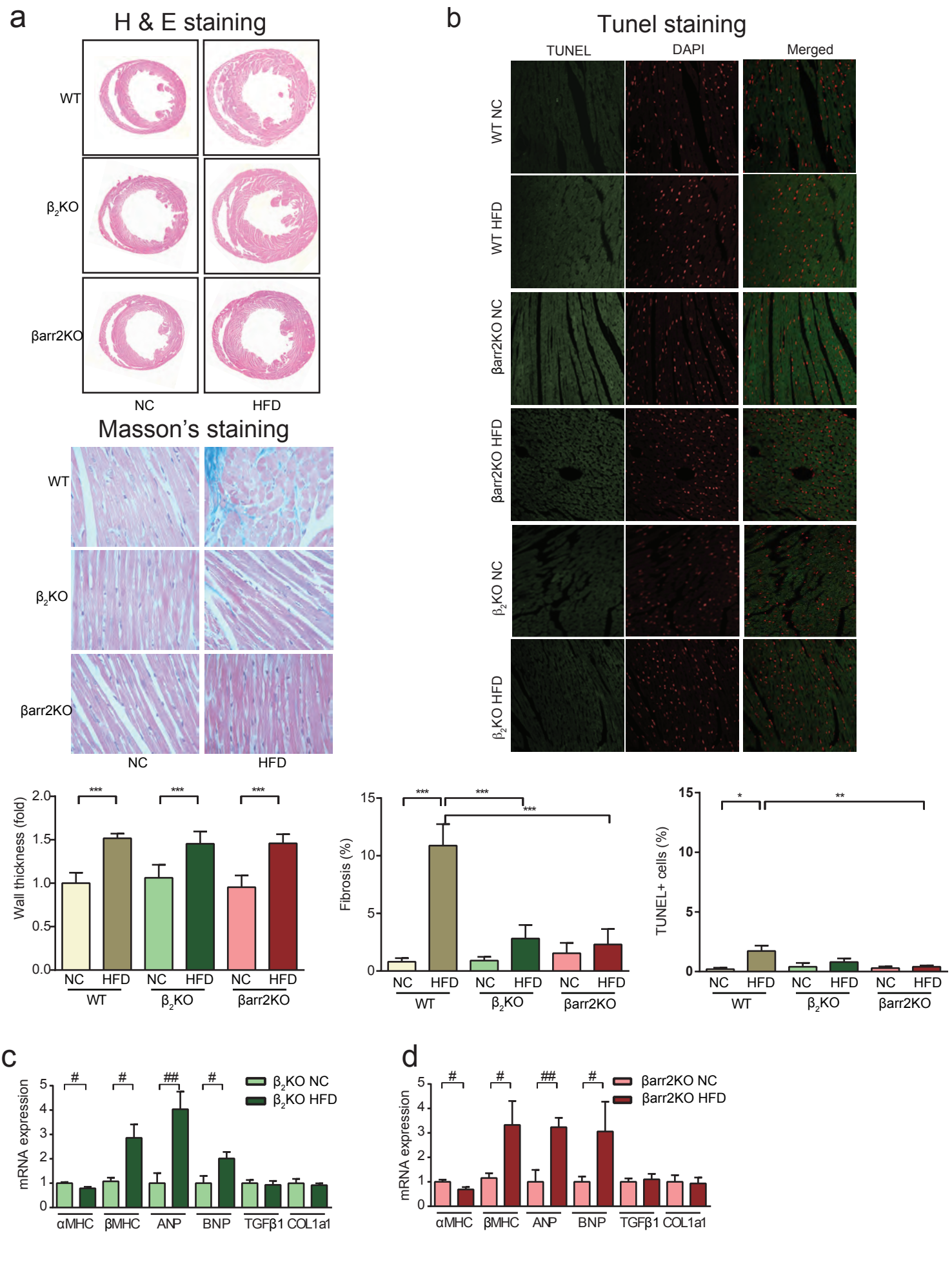
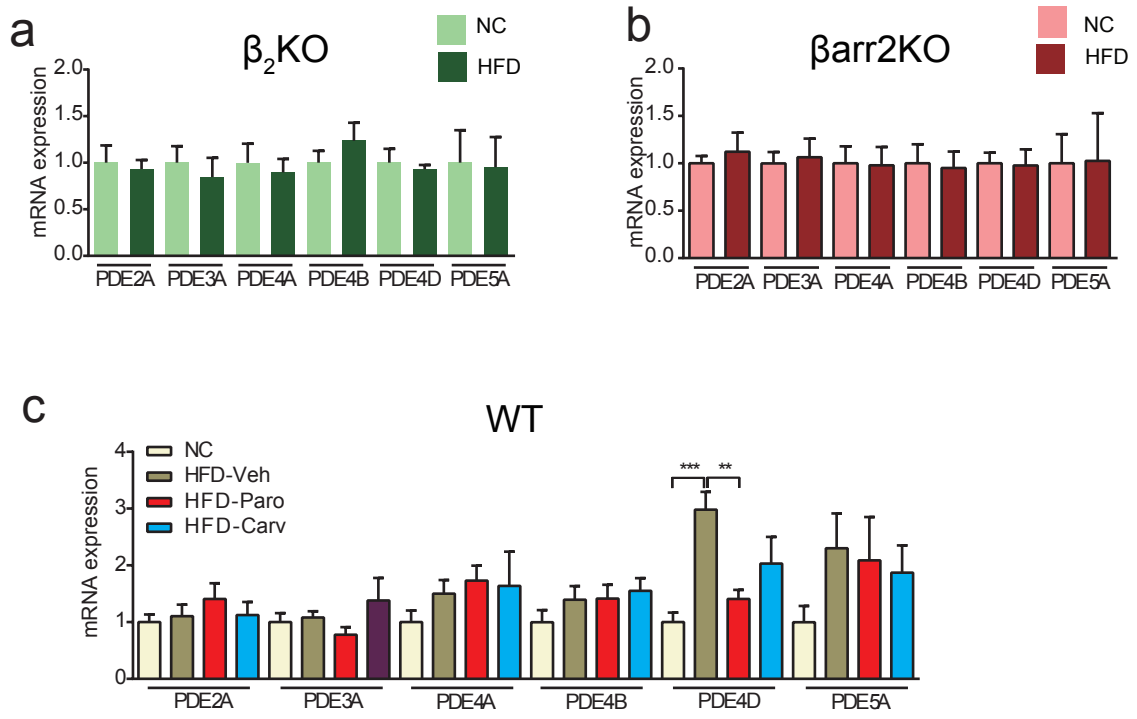
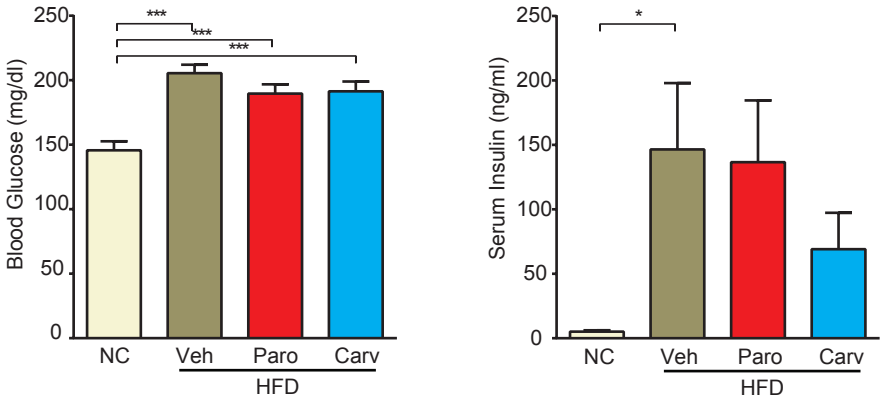


Supplemental Figure 6



Supplemental Figure 7

a



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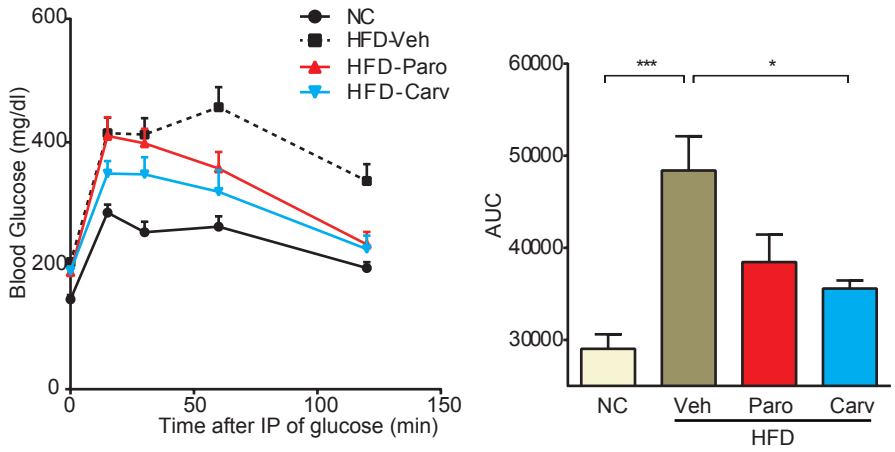
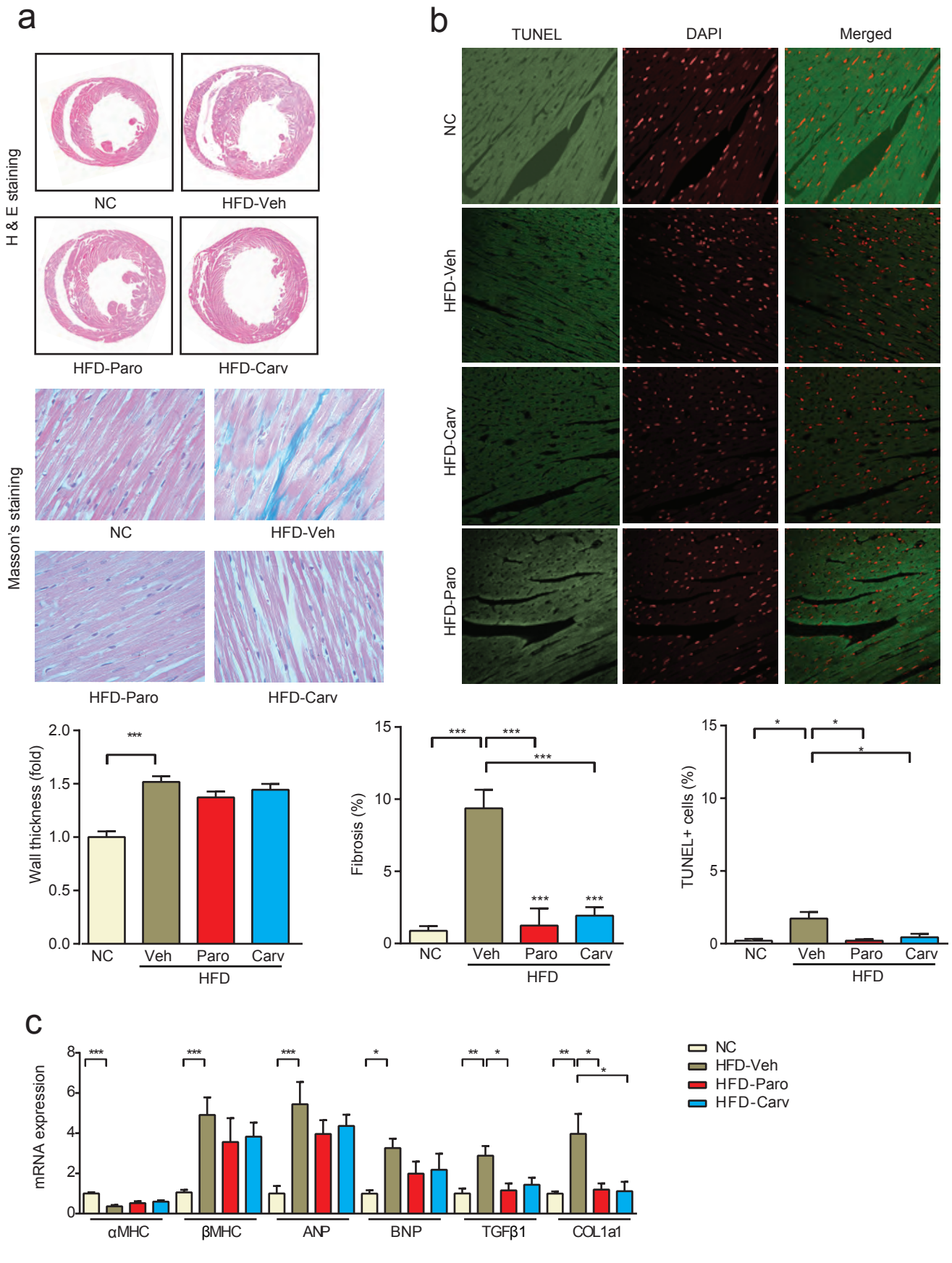


Supplemental Figure 8



Supplemental Table 1. Echo data of WT mice of 6 months' high fat feeding (mean±SEM)

	NC	HFD
HR	466.4±6.153	435.0±21.87
IVS d(mm)	0.6318±0.02536	0.6881±0.05523
IVS s(mm)	0.9639±0.03162	0.9404±0.08069
LVID; d(mm)	3.841±0.06359	4.054±0.1472
LVID; s(mm)	2.853±0.05864	3.281±0.1291$^{\#\#}$
LVPW; d(mm)	0.7191±0.03534	0.7854±0.03776
LVPW; s(mm)	0.9212±0.03767	0.9676±0.04710
EF %	52.16±0.7499	39.55±2.891$^{\#\#\#}$
FS %	26.25±0.4707	19.04±1.601$^{\#\#\#}$
LV Mass (mg)	88.24±4.952	109.4±12.21
Mass (corre)	70.59±3.962	87.52±9.770
LV Vol; d(uL)	63.75±2.514	72.99±6.123
LV Vol; s(uL)	31.13±1.568	44.05±4.177$^{\#\#}$
A'	-18.08±0.8494	-17.14±1.685
E'	-19.07±0.8388	-15.47±2.163$^{\#\#\#}$
ET	46.29±1.095	46.13±2.813
IVCT	17.69±1.079	21.08±2.331
IVRT	14.77±0.6579	24.57±1.099$^{\#\#\#}$
MPI[(IVRT+IVCT)/ET]	0.7084±0.04848	1.002±0.05992$^{\#\#}$
A'/E'	0.9564±0.04641	1.346±0.1302$^{\#\#}$
E'/A'	1.065±0.04972	0.7711±0.07352$^{\#\#}$
MV A(mm/s)	400.2±16.95	313.3±36.97$^{\#}$
MV E(mm/s)	751.8±44.31	381.5±31.04$^{\#\#\#}$
MV E/A	1.873±0.05027	1.275±0.06518$^{\#\#\#}$
E/E'	-40.17±3.531	-30.29±3.412
Vcf=FS/ET	0.5578±0.01776	0.4215±0.04441$^{\#\#}$

$^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ WT HFD *vs* WT NC group; IVS d: intact ventricular septum end diastole; IVS s: intact ventricular septum end systole; LVID d: Left ventricular internal diameter end diastole and end diastole; LVID s: Left ventricular internal diameter end diastole and end systole; LVPW d Left ventricular posterior wall end diastole; LVPW s Left ventricular posterior wall end systole; EF, ejection fraction; FS, fractional shortening; LV Mass, left ventricular mass; Mass (corre), corrected LV mass; LV Vol d, left ventricular volume end diastole; LV Vol s, left ventricular volume end systole; A', late diastolic mitral annular velocities; E', early diastolic mitral annular velocities; ET, ejection time; IVCT, isovolumic contraction time; IVRT, isovolumetric relaxation time; MPI, myocardial performance index; MV A, mitral valve A; MV E, mitral valve E; E/E', relationship between maximal values of passive mitral inflow (E) and lateral early diastolic mitral annular velocities (E'); Vcf, mean velocity of LV shortening through the minor axis.

Supplemental Table 2 Tissue weight of WT, β_2KO and βarr2KO mice of 6 months' high fat feeding (mean±SEM)

Groups	Body weight (g)	Brain weight (mg)	Heart weight (mg)	Liver weight (g)	Muscle weight (mg)	Fat weight (g)	Tibia length (cm)	Heart/Tibia (mg/cm)
WT NC	37.74±0.47	420.60±12.60	133.20±5.56	1.42±0.06	56.59±1.04	2.01±0.10	2.33±0.03	57.16±2.09
WT HFD	54.28±1.28***	415.10±8.60	154.80±4.22*	3.15±0.22***	60.29±1.79	4.04±0.19***	2.33±0.03	66.55±1.46**
β_2KO NC	34.69±0.87	423.80±4.94	135.50±2.88	1.27±0.08	53.22±0.93	1.68±0.15	2.32±0.02	58.34±1.28
β_2KO HFD	57.45±1.35$^{@@@}$	415.00±8.74	155.70±3.37$^{@@}$	4.03±0.14$^{@@@\$\$\$}$	56.47±1.09	4.18±0.22$^{@@@}$	2.31±0.02	67.77±1.67$^{@@@}$
βarr2KO NC	31.49±1.22$^{\Delta}$	450.80±6.06	143.80±3.52	1.02±0.05	55.16±0.73	0.82±0.18$^{\Delta\Delta}$	2.37±0.03	60.66±1.56
βarr2KO HFD	48.44±1.53$^{@@@\$}$	436.30±9.35	158.90±6.33	1.82±0.15$^{@\$\$\$}$	54.71±1.44	3.90±0.09$^{@@@}$	2.34±0.02	69.67±2.22$^{@}$

**P<0.05, **P<0.01, ***P<0.001* WT HFD *vs* WT NC group; $^{@}$*P<0.05,* $^{@@}$*P<0.01,* $^{@@@}$*P<0.001* β_2KO HFD *vs* β_2KO NC group or βarr2KO NC *vs* βarr2KO HFD; $^{\$}$*P<0.05,* $^{\$\$\$}$*P<0.001* β_2KO HFD or βarr2KO HFD *vs* WT HFD group, $^{\Delta}$*P<0.05,* $^{\Delta\Delta}$*P<0.01* βarr2KO NC *vs* WT NC group.

Supplemental Table 3. Echo data of Paroxetine treatment mice (mean±SEM)

	NC		HFD					
			Vehicle		Paroxetine		Carvedilol	
	16W	20W	16W	20W	16W	20W	16W	20W
HR	466.40±6.15	490.70±19.37	455.05±11.87	447.70±10.77	452.83±9.42	535.50±14.44$^{@@@}$	460.38±6.35	438.80±14.31
IVS d(mm)	0.63±0.03	0.71±0.03	0.71±0.02	0.81±0.05	0.69±0.02	0.79±0.03	0.69±0.03	0.74±0.03
IVS s(mm)	0.94±0.04	1.22±0.06	1.04±0.04	1.09±0.06	1.03±0.04	1.25±0.04	1.01±0.07	1.25±0.05
LVID; d(mm)	3.86±0.07	4.22±0.09	3.90±0.04	3.97±0.10	3.87±0.06	3.99±0.06	3.83±0.08	4.07±0.08
LVID; s(mm)	2.86±0.07	3.09±0.06	2.97±0.05	3.18±0.09	2.95±0.06	2.78±0.07$^{@@}$	2.88±0.09	2.89±0.07$^{@}$
LVPW; d(mm)	0.70±0.04	0.77±0.03	0.97±0.04***	0.88±0.03	0.95±0.03***	0.83±0.03	0.99±0.07***	0.78±0.02
LVPW; s(mm)	0.94±0.05	1.15±0.05	1.28±0.03***	1.04±0.03	1.19±0.02***	1.22±0.04$^{@}$	1.22±0.06***	1.22±0.04$^{@}$
EF %	51.77±1.67	54.27±1.50$^{@@@}$	47.23±1.52*	43.11±1.74	47.08±1.37*	58.04±1.86$^{@@@}$	47.10±1.47*	57.36±1.47$^{@@@}$
FS %	25.91±0.86	27.95±1.03$^{@@@}$	23.35±0.90*	21.03±0.98	23.85±0.77*	30.46±1.27$^{@@@}$	23.55±0.73*	29.93±1.00$^{@@@}$
LV Mass (mg)	87.25±4.16	115.60±3.54	121.9±3.06***	125.60±7.48	116.0±2.29***	120.40±6.33	118.2±2.25***	113.50±4.96
Mass (corre)	69.80±3.33	92.48±2.83	91.21±3.04***	100.50±5.99	94.12±1.79***	96.33±5.07	96.07±4.08***	90.81±3.97
LV Vol; d(uL)	64.48±2.56	80.12±4.10	66.09±1.64	69.91±4.16	64.57±2.74	69.94±2.29	67.40±1.54	73.59±3.55
LV Vol; s(uL)	31.20±1.98	37.92±1.92	34.55±1.27	41.15±2.86	33.43±1.96	29.51±1.84$^{@@}$	35.00±1.12	32.51±2.09$^{@}$
A'	-17.85±1.26	-19.34±1.24	-15.01±0.87	-18.03±1.37	-15.58±1.17	-20.21±1.85	-15.51±1.33	-17.05±1.11
E'	-18.72±0.66	-20.01±1.96$^{@}$	-12.74±0.87***	-13.33±0.76	-12.21±1.06***	-19.76±1.61$^{@}$	-12.10±1.03***	-17.96±1.59
ET	45.58±1.01	46.72±1.24	44.70±0.94	47.32±1.83	44.33±1.25	44.69±0.71	45.24±1.09	48.52±1.22
IVCT	17.20±0.95	19.78±1.22	21.20±0.94*	20.24±1.00	20.83±1.06*	18.76±0.80	22.04±1.28*	17.89±0.99
IVRT	14.75±0.69	17.64±1.27$^{@}$	24.01±1.25***	23.55±1.38	24.33±1.44***	18.41±1.18$^{@}$	25.46±2.01***	18.93±1.14$^{@}$
MPI[(IVRT+IVCT)/ET]	0.71±0.05	0.81±0.04	1.017±0.04***	0.94±0.05	1.064±0.05***	0.83±0.04	1.055±0.05***	0.77±0.04$^{@}$
A'/E'	0.95±0.04	1.02±0.06$^{@@}$	1.18±0.05*	1.41±0.05	1.28±0.08*	1.04±0.06$^{@@}$	1.28±0.07*	1.05±0.09$^{@@}$
E'/A'	1.05±0.05	1.03±0.06$^{@@}$	0.85±0.04*	0.72±0.02	0.78±0.07*	1.01±0.06$^{@@}$	0.78±0.06*	0.96±0.07$^{@}$
MV A(mm/s)	385.40±7.06	335.50±27.21	346.40±25.72	335.40±25.09	328.60±35.79	304.60±23.45	331.70±22.45	282.90±19.81
MV E(mm/s)	713.70±29.85	632.90±35.43$^{@@@}$	473.00±5.04***	432.40±36.81	450.00±40.51***	551.90±27.87$^{@}$	449.30±37.63***	527.90±25.56
MV E/A	1.851±0.06	1.96±0.09$^{@@@}$	1.37±0.02***	1.32±0.05	1.37±0.04***	1.90±0.08$^{@@@}$	1.35±0.04***	1.94±0.08$^{@@@}$
E/E'	-38.32±1.76	-35.55±3.27	-36.48±2.93	-33.05±2.81	-34.47±3.56	-30.76±2.79	-37.34±3.53	-32.41±2.80
Vcf=FS/ET	0.57±0.02	0.66±0.04$^{\#}$	0.53±0.01	0.47±0.03	0.54±0.02	0.71±0.03$^{@@@}$	0.52±0.02	0.70±0.05$^{@@}$

**P<0.05, **P<0.01, ***P<0.001* HFD, Paroxetine, Carvedilol 16W *vs* NC 16W; $^{@}$*P<0.05,* $^{@@}$*P<0.01,* $^{@@@}$*P<0.001* 20W *vs* 16W.