Supplemental Materials

Materials and Methods

Human Myocardial Samples

Myocardial samples were obtained from failing hearts from patients undergoing heart transplantation or mechanical circulatory support device implantation at the Virginia Commonwealth University as part of the Division of Cardiology Tissue Biobank under a long-standing protocol approved by the Internal Review Board (IRB #HM11452). Patients signed written consent for use of their explanted hearts for research purposes. Non-failing donor hearts were defined by no major cardiac history and a left ventricular echocardiography-based fractional shortening ≥ 25%. Non-failing left ventricular samples were obtained from organ donors whose hearts could not be placed for transplantation due to size, ABO mismatch, or other factors. Family members of organ donors signed written consent for research use of explanted cardiac tissue obtained by the local organ procurement agency. Immediately after explantation, left ventricular free wall that are remote from infarcted segments were immersed in liquid nitrogen, transported to the laboratory, and stored at -80 degree Celsius.

Experimental Animals

3-MST KO mice were generated by breeding 3-MST$^{flox+/flox+}$ mice to global Cre expressing mice as previously described[1]. Due to the well-established cardiovascular protective effects of estrogen seen in young-adult female mice[2], only male 3-MST KO and age matched C57BL/6J (Jackson Laboratories, Bar Harbor, Maine) wild-type control (9 to 11 weeks of age) were used in the studies described in the current manuscript. Studies using ovariectomized or postmenopausal female mice will be performed in the future to fully investigate the effects of 3-MST in cardiovascular system for both sexes. Minimal group sizes of n=7 to 10 for physiological experiments were determined using a power and sample analysis with the significance level at 5% and power at 80%. All experimental protocols were approved by the Institute for Animal Care and Use Committee at LSU Health Sciences Center-New Orleans and conformed to the Guide for the Care and Use of Laboratory Animals. All experimental animals received humane care in accordance with National Society of Medical Research and National Institutes of Health tenets.

In experiments involving treatments, animals were randomized through Research Randomizer (randomizer.org) prior to initiating treatment. No animals were excluded during the study, however; presented data may have different numbers of animals per endpoint due to procedural complications, limited sample collection (i.e., plasma volume), or lack of participation in involuntary treadmill running.

BCKD Kinase Inhibitor Treatment

3,6-dichlorobenzo1[b]thiophene-2-carboxylaic acid (BT2) was purchased from Sigma-Adrich (Burlington, MA) and administered at the dose of 40 mg/kg/day as previously described[3, 4].

Hydrogen Sulfide Donor Treatment

JK-1 was synthesized and the optimal dose were determined as previously reported[5, 6]. Mice were administered with either Vehicle or JK-1 at the dose of 200 mg/kg/day as previously described[6].

Transverse Aortic Constriction (TAC) Protocol

Cardiac hypertrophy and heart failure was induced via transverse aortic constriction (TAC) as previously described[6]. The animals were observed for 12 weeks following TAC surgery. The experimental protocol is described in ***Figure 2***.

Echocardiography

Echocardiography was performed at baseline and every 3 weeks during the 12-week protocol using a Vevo-2100 ultrasound system (Visual Sonics, Toronto, Canada) as previously described[6]. During the procedure, the mice were anesthetized with 3 to 4 % and maintained at 0.5 to 1.5% isoflurane during the acquisition of echocardiography images. LV chamber diameters at end diastole (LVEDD) and LV ejection fraction (LVEF) were measured using series of 5 M-mode images across the long-axis view. All images were taken within a heart rate range of 450 to 500 bpm.

Left Ventricular (LV) Hemodynamics Assessment

Invasive hemodynamic measurement was performed at the 12-week endpoint using a 1.2F Transonic high-fidelity pressure catheter with a solid-state sensor mounted at the tip (Transonic, NY, USA). The pressure catheters were fully calibrated using electronic two-point calibration. The mice were anesthetized with 3 to 4% isoflurane and maintained at 1 to 1.5% during the procedure, then the pressure catheter was inserted into the right common carotid and advanced into the LV lumen. LV end-diastolic pressure and relaxation constant Tau were presented.

Transmission Electron Microscopy

The morphology and structure of the myocardial mitochondria harvested from wild-type and 3-MST KO mice at 12 weeks post TAC were evaluated using transmission electron microscopy as previously described[7].

Mitochondria Isolation and Respiration Assays

Hearts were extracted from mice following cervical dislocation at 12 weeks post TAC and mitochondria were isolated using a commercially available kit (MITOISO1, Sigma-Aldrich, Burlington, MA) following manufacture's instruction. Then the mitochondrial specific H_2S production were also measured by combined gas chromatography - sulfur chemiluminescence method as described below with addition of 3-mercaptopyruvate as substrate. The respiration capacity of the isolated mitochondria was measured using a Clark-type oxygen electrode (Hansatech Instruments, Amesbury, MA) with the presence of pyruvate or palmitoyl-L-Carnitine, as previously described[8].

ATP Synthesis Efficiency

Aliquots of respiration reaction buffer were taken from the Clark-type oxygen electrode chamber over a 1-minute period after the addition of ADP. Then ATP concentration in these aliquots were quantified using an ATP determination kit (A-22066; Molecular Probes, Eugene, OR). Then the ATP concentration were normalized to the oxygen consumption rate and ATP synthesis efficiency is presented as ATP/Oxygen ratio.

Circulating B-type Natriuretic Peptide (BNP) Quantification

Circulating BNP levels were measured at 12 weeks post TAC using an enzyme-linked immunoassay. (EK-011-23, Phoenix Pharmaceuticals, Burlingame, CA).

H_2S and Sulfane Sulfur Measurement

H_2S and sulfane sulfur levels in the myocardial samples from patients and experimental animals were measured by combined gas chromatography – sulfur chemiluminescence methods (Agilent, Santa Clara, CA) as previously described[9-11].

Vascular Reactivity Studies

At 12 weeks following TAC, isolated thoracic aorta segments were harvested and vascular reactivity to acetylcholine (ACh) and sodium nitroprusside (SNP) were determined as previously described[6]. Briefly, aortic rings (2-3 mm) were equilibrated in oxygenated Krebs-Henseleit solution at 0.5 g tension for 30 min. Rings were pre-contracted with phenylephrine (1 μM) then challenged with increasing concentrations of acetylcholine (10^{-9} to 10^{-5} M) to assess endothelial-dependent vasorelaxation, or sodium nitroprusside ($10^{-10.5}$ to 10^{-7} M) to measure endothelial-independent vasorelaxation. Data are reported as percent of relaxation from maximum contraction to phenylephrine.

Western Blot Analysis

Human heart samples were homogenized, sonicated, and centrifuged at 10,000x g for 15 minutes at 4°C in RIPA buffer; a protease and phosphatase inhibitor cocktail were added accordingly (Halt, ThermoScientific). Total proteins (100μg) from each sample were separated by SDS-PAGE on 10% acrylamide gels, transferred onto a nitrocellulose membrane, and blocked with 5% nonfat dry milk in Tris-buffered saline. Membranes were incubated overnight with antibodies for CSE (1:1000; Santa Cruz Biotechnology SC-100583), MST (1:1000; Santa Cruz Biotechnology sc-86170), CBS (1:1000; Santa Cruz Biotechnology sc-67154) and GAPDH (1:5000 Cell Signaling). Membranes were then incubated with secondary antibodies 1:5000 in 5% dried milk TBS-T for 1.5 hours (Anti-mouse IgG (#NA931V) and Anti-Rabbit IgG (#NA934V)) with horseradish peroxidase-conjugated (GE healthcare, UK). Then the membranes were developed with enhanced chemiluminescence and acquired on Blue Ultra

Autorad Film (GeneMate). Images were electronically scanned, and protein levels were quantified using densitometry.

In mouse studies, whole cell fractions were obtained from mouse hearts. Protein concentrations were measured with the DC protein assay (Bio-Rad Laboratories, Hercules, CA). Equal amounts of protein were loaded into lanes of CriterionTGX (Tris-Glycine eXtended) Stain-Free PAGE gels (BioRad). The gels were electrophoresed and activated using a ChemiDoc MP Visualization System (BioRad) to ensure correct loading. The protein was then transferred to a polyvinylidene difluoride membrane. The membranes were then imaged using a ChemiDoc MP Visualization System to obtain an assessment of proper transfer and to obtain total protein loads. The membranes were then blocked and probed with primary antibodies overnight at 4°C. Immunoblots were next processed with 1:5000 Anti-Rabit-HRP secondary antibodies (7074S, Cell Signaling) for 1 hour at room temperature. Immunoblots were then probed with a Super Signal West Dura kit (Thermo Fisher Scientific) to visualize signal, followed by visualization using a ChemiDoc MP Visualization System (BioRad). Data were analyzed using Image Lab (BioRad). The total protein images were used as loading controls. For each protein of interest, the portion of the protein load image corresponding to the molecular weight of the protein of interest was used as the loading control. Antibodies used are as followed:

p-BCKDH - Cell Signaling 40368 - 1:2000

t-BCKDH - Cell Signaling 90198 - 1:2000

BCKDK - Novus Biologicals NBP2-15553 - 1:2000

PP2Cm - custom antibody as previously described[8] - 1:2000

Exercise Capacity Assessment

Treadmill exercise performance was assessed using a rodent specific treadmill (IITC, Woodland Hills, California) at 12 weeks post TAC as previously described[12]. Briefly, following treadmill acclimation, mice were subjected to a 30-degree inclined treadmill- running exercise protocol with speed progressively increased from 10 to 18 m/min. Exercise ended at the maximal exhaustion, defined as inability to maintain running speed despite 5 consecutive seconds contact with the electrical grid. Duration of the running session was recorded.

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High-resolution Metabolomics (HRM) Sample Preparation

Metabolites were extracted from heart tissue by homogenizing the samples in ice cold acetonitrile (10:1 µL to mg sample wet weight) using a handheld pestle followed by vortexing and incubation on ice for 30 minutes. Samples were centrifuged at 14,000g for 10 minutes at 4°C to pellet proteins. The supernatants were transferred to autosampler vials and analyzed immediately on a Thermo Scientific Fusion HILIC ESI+/C18 ESI- (85-1,275 m/z) as previously described[13].

HRM Instrumental Analysis

Untargeted high-resolution mass spectrometric profiling was performed using a Dionex Ultimate 3000 ultra-high-performance liquid chromatography (UHPLC) system coupled to a Thermo Scientific Fusion. On the Fusion and LC column switching method using a reversed phase C18 column (Higgins Targa C18 2.1 x 50 mm, 3 µM particle size) and a hydrophilic interaction chromatography (HILIC) column (Waters XBridge BEH Amide XP HILIC column 2,1 x 50 mm, 2.6 µM particle size) were used for the analytical separation of extracts before high-resolution mass spectrometry analysis with HILIC/electrospray ionization plus (ESI^+) and C18/ESI^- at 120,000-resolution while of ions between 85-1,*275 m/z*[14].

HRM Data Processing and Statistics

Mass spectral files in .raw format were converted to .cdf files using xCalibur file converter software (Thermo Fisher, Waltham, MA) and were extracted using apLCMS and xMSanalyzer to generate feature tables which contain mass spectral features defined by mass-tocharge-ratio (m/z), retention time, and ion abundance[15, 16]. Data were then log2 transformed and quantile normalized after filtering. Statistical analysis of m/z features (hereafter termed metabolites) was performed using the *limma* function in xmsPANDA, an R-package for statistical analysis of metabolomics data[17-19]. Benjamini-Hochberg false discovery method was used for multiple hypothesis testing correction.

HRM Feature Selection, Metabolite Identification and Quantification

Metabolites with an unadjusted p value less than 0.05 were annotated using xmsAnnotator, a platform which assigns confidence scores for peak annotations. The metabolites were then referenced against an in-house reference library established with authentic chemical standards and matched within 5 ppm of the confirmed mass and within tens of the confirmed retention time[13, 20]. When no standard was available, MS isotopic ratios and MS/MS spectra (ion-trap-based collision-induced dissociation or higher energy collisional dissociation fragmentation as noted) were referenced against online spectral libraries mzCloud and METLIN. Standards were purchased when commercially available or synthesized as descried below. Reference standardization was used for quantification of identified metabolites[13]. Quality control samples consisting of pooled plasma were analyzed at the beginning, middle, and end of each analytical run (every twenty samples) to assess chromatographic peak quality (± 8s) and mass accuracy (observed mass within 5 ppm of expected mass)[13]. Data extraction quality control was performed to extract peaks that were reproducibly detected (technical replicate Pearson's R > 0.7). Metabolic set enrichment analysis on annotated metabolites with an adjusted p value less than 0.1 was performed using MetaboAnalyst 5.0. Individual log2 transformed values for annotated metabolites with unadjusted p values less than 0.05 are listed in ***Tables S1-2***. Mean log2 transformed values and p values for all significantly different metabolites are listed in ***Tables S3-5***.

Targeted Metabolomic analysis of BCAA

Metabolites were extracted from heart tissue by homogenizing at 4°C in 1:1 acetonitrile:methanol (10:1 µL to mg sample wet weight) using a bead homogenizer. Samples were centrifuged at 1811 RCF for 10 minutes at 4°C to pellet proteins. The supernatants were then mixed with 1:1 acetonitrile:methanol

(4:1 v/v) and incubated for 20 minutes at 4°C. The mixtures were then centrifuged at 1811 RCF for 10 minutes at 4°C and passed through a 0.22mm filter. The supernatants were transferred to autosampler vials and analyzed on a Sciex Q-TRAP 5500 paired with an ExionLC AD UPLC system (Sciex, Farmington, CT). Peaks were quantified with SCIEX OS-MQ software (1.6.1.29803). BCAA concentrations were calculated as nmoles per gram sample wet weight using authentic standards for leucine, isoleucine, and valine.

Statistical Analysis

Data was analyzed in a blinded manner to the genetic background of the animals in each study until all measurements were complete. All data except those generated in metabolomic analysis were analyzed using Prism 8 (GraphPad Software Inc). Normal distribution was tested by the D'Agostino & Pearson test when $n>6$ per group. For 2-group comparison, we used a student unpaired 2-tailed *t* test, or a Mann-Whitney test when the data did not follow a normal distribution or $n \leq 6$ per group. For comparison across multiple groups, data were analyzed with 1-way ANOVA analysis followed by a Bonferroni multiple comparison test, or Kruskal-Wallis test when the data did not follow a normal distribution or $n \leq 6$ per group. For comparison across different timepoints or treatment concentrations, data were analyzed using ordinary 2-way ANOVA analysis followed by a Bonferroni multiple comparison test. A p-value of <0.05 was considered statistically significant. Prior to conducting statistical analysis, an outlier test was performed using “ROUT” method developed by Prism 6 to identify and remove any outlier in the data set.

Table S1: Annotated Metabolites in WT mice.

39	368.27942	27.1	HMDB13331	3 5-Tetradecadiencarnitine	0.50692186	22.01	22.92	22.29	22.57	24.40	21.53	21.62	22.01	21.01	22.17
40	372.3106	23.2	HMDB05066	Tetradecanoylcarnitine	0.163772375	23.47	24.32	23.70	23.98	24.40	22.87	22.58	22.96	22.04	23.55
41	246.17028	33.9	HMDB00378	2-Methylbutyroylcarnitine	0.218779619	21.42	21.99	21.30	21.61	22.82	21.05	20.62	20.20	20.05	21.09
42	246.17028	33.9	HMDB00688	Isovalerylcarnitine	0.218779619	21.42	21.99	21.30	21.61	22.82	21.05	20.62	20.20	20.05	21.09
43	246.17028	33.9	HMDB13128	Valerylcarnitine	0.218779619	21.42	21.99	21.30	21.61	22.82	21.05	20.62	20.20	20.05	21.09
44	246.17028	33.9	HMDB41993	Pivaloylcarnitine	0.218779619	21.42	21.99	21.30	21.61	22.82	21.05	20.62	20.20	20.05	21.09
45	256.10561	53	HMDB00855	Nicotinamide riboside	0.613630033	17.60	18.33	18.42	18.47	18.45	17.56	18.04	17.90	15.28	15.92
46	428.37312	25.5	HMDB00848	Stearoylcarnitine	0.229343411	22.85	24.25	23.31	23.31	23.76	22.76	22.23	22.26	20.97	22.53
47	178.08625	50.7	HMDB12490	12-Dehydrosalsolinol	0.069607536	21.65	21.71	21.76	21.88	21.17	20.51	20.94	19.98	20.16	19.46
48	240.10172	46.7	HMDB33519	N-Ornithyl-L-taurine	0.104767364	22.05	22.91	22.47	23.23	23.29	21.68	21.00	21.55	20.31	21.69
49	276.18051	35.8	HMDB13131	Hydroxyhexanoycarnitine	0.163772375	21.87	23.03	21.93	23.34	23.68	21.70	20.73	21.14	20.84	21.64
50	260.18558	34.1	HMDB00756	L-Hexanoylcarnitine	0.070313003	22.54	23.64	22.57	23.16	24.20	21.71	21.32	21.91	20.97	21.67
51	456.40461	25.1	HMDB06460	Arachidyl carnitine	0.044523452	18.95	20.29	19.03	19.24	19.88	17.42	17.03	17.99	18.01	17.71
52	232.15447	35.6	HMDB00736	Isobutyryl-L-carnitine	0.070030482	24.12	25.21	23.84	24.96	26.50	22.47	22.33	22.74	22.64	23.03
53	232.15447	35.6	HMDB02013	Butyrylcarnitine	0.070030482	24.12	25.21	23.84	24.96	26.50	22.47	22.33	22.74	22.64	23.03
54	248.14943	42.4	HMDB13127	Hydroxybutyrylcarnitine	0.04535137	25.88	27.59	25.62	27.14	27.91	23.66	22.26	24.54	23.30	24.22
55	105.00162	18.5	HMDB31520	2-Mercaptopropanoic acid	0.176993485	13.66	15.58	14.76	15.26	13.98	19.19	17.63	17.21	14.60	19.94
56	143.03506	22.1	HMDB31257	Dimethyl fumarate	0.022622051	17.95	17.17	17.51	18.62	17.75	19.31	20.12	20.59	20.68	20.64
57	131.03501	21	HMDB01844	Methylsuccinic acid	0.143400106	18.77	18.73	18.37	18.98	18.65	22.29	20.63	19.74	19.30	22.38
58	131.03501	21	HMDB06855	(S)-2-Acetolactate	0.143400106	18.77	18.73	18.37	18.98	18.65	22.29	20.63	19.74	19.30	22.38
59	159.03005	17.5	HMDB00225	Oxoadipic acid	0.098759863	14.99	15.98	16.67	13.99	15.36	17.70	16.68	17.68	17.11	18.53
60	179.05623	22.6	HMDB00122	D-Glucose	0.032938971	20.51	19.54	19.71	20.85	19.91	21.39	22.16	22.06	22.75	22.68
61	401.29136	254.7	HMDB29887	Sorbitan palmitate	0.052649966	21.38	21.07	20.69	21.60	20.49	23.64	22.05	22.97	22.42	23.09
62	153.01938	19.9	HMDB01856	Protocatechuic acid	0.280057722	15.56	15.74	15.52	15.31	16.10	15.41	16.45	18.48	18.17	17.88
63	153.01938	19.9	HMDB13677	35-Dihydroxybenzoic acid	0.280057722	15.56	15.74	15.52	15.31	16.10	15.41	16.45	18.48	18.17	17.88
64	299.22311	235.4	HMDB11531	MG(0:014:1(9Z)0:0)	0.079883616	19.55	20.09	19.59	20.75	19.54	21.62	20.68	21.34	21.24	22.19
65	178.05115	20	HMDB00714	Hippuric acid	0.065723444	19.02	20.47	20.01	20.30	20.29	21.12	22.04	21.35	21.39	21.67
66	178.05115	20	HMDB00992	3-Succinoylpyridine	0.065723444	19.02	20.47	20.01	20.30	20.29	21.12	22.04	21.35	21.39	21.67
67	253.21774	237.4	HMDB03229	Palmitoleic acid	0.097943032	25.07	26.06	25.38	26.02	25.26	26.77	26.08	27.15	27.22	27.73
68	277.21772	223.7	HMDB01388	Alpha-Linolenic acid	0.285120969	25.32	26.28	26.05	26.25	26.53	26.88	26.24	28.38	27.12	27.83
69	267.23333	256.1	HMDB40888	24-Heptadecanedione	0.143400106	21.26	22.24	21.65	22.36	21.63	23.26	22.20	23.22	22.79	23.57
70	396.27634	237	HMDB13628	PGF2a ethanolamide	0.516652173	16.66	15.71	16.00	17.18	14.93	17.12	16.26	16.99	17.80	17.56
71	187.00726	20.5	HMDB11635	p-Cresol sulfate	0.439759635	20.43	20.85	20.49	20.37	20.70	20.18	21.29	22.38	22.04	21.81
72	289.21764	222.6	HMDB00031	Androsterone	0.541513018	15.90	16.93	16.24	15.87	16.51	16.92	16.43	17.12	16.75	17.42
73	228.08159	21.8	HMDB03045	Ergothioneine	0.55778774	17.11	17.33	17.02	17.18	16.81	17.18	17.50	17.61	17.57	17.72
74	215.16539	80.4	HMDB10728	(R)-3-Hydroxydodecanoic acid	0.502984064	18.66	19.81	19.04	19.10	19.40	18.45	18.47	19.02	18.61	18.45
75	117.05571	20.3	HMDB00754	3-Hydroxyisovaleric acid	0.477511773	19.79	20.72	20.46	20.70	20.68	19.79	19.89	19.75	19.59	20.31
76	193.03528	143.8	HMDB00127	D-Glucuronic acid	0.447385823	20.49	21.28	21.42	21.53	20.85	20.34	20.46	20.80	20.55	20.34
77	184.09812	19.9	HMDB06348	Pseudoecgonine	0.187946883	16.59	16.63	16.74	16.50	16.51	15.67	16.10	15.88	16.26	15.96
78	215.16556	37.7	HMDB02059	12-Hydroxydodecanoic acid	0.076085695	20.28	20.94	20.56	20.41	20.67	19.47	19.76	19.82	19.72	19.17
79	225.18607	178.6	HMDB02000	Myristoleic acid	0.098759863	21.46	22.75	22.07	21.62	22.24	20.51	20.67	21.13	20.85	21.14
80	295.22819	158.4	HMDB04701	910-Epoxyoctadecenoic acid	0.155048129	22.50	23.36	22.99	23.14	23.17	21.70	21.04	22.96	21.59	21.71
81	103.04007	19.6	HMDB00011	(R)-3-Hydroxybutyric acid	0.05702144	22.16	23.06	22.53	22.59	22.68	21.09	20.16	21.73	20.88	21.42
82	345.20754	82.7	HMDB00015	Cortexolone	0.452078716	13.28	16.37	14.49	14.97	14.99	14.01	13.63	11.55	12.55	14.25
83	187.1342	22.5	HMDB10725	(R)-3-Hydroxydecanoic acid	0.032938971	19.33	20.33	19.50	19.37	19.87	17.78	18.29	17.96	18.31	17.54
84	159.10273	21.4	HMDB01954	3-Hydroxyoctanoic acid	0.035212065	18.51	19.73	18.37	18.10	18.75	16.87	17.10	16.64	17.15	16.20
85	117.01946	18.9	HMDB00254	Succinic acid	0.222183641	23.87	24.49	24.33	24.54	25.05	23.50	23.15	22.78	19.84	22.97

Table S2: Annotated Metabolites in 3-MST KO mice.

number	mz	time	chemical_ID	Name	adjusted.P.value	X3mstKO_sham1_001					X3mstKO_tac1_001				
1	340.10265	39.2	HMDB10362	6-Hydroxy-5-methoxyindole glucuronide	0.141499146	7.54	12.57	6.55	6.55	6.55	17.33	6.55	13.91	12.35	14.23
2	342.13938	96.9	HMDB06591	Lactosamine	0.051901094	11.39	11.39	11.39	11.39	12.39	15.65	18.65	20.43	13.46	14.33
3	289.08606	84.1	HMDB31870	gamma-Glutamyl-S-(1-propenyl)cysteine sulfoxide	0.101584475	10.80	15.37	10.80	11.80	13.38	19.80	14.00	13.71	18.00	18.03
4	123.59425	84	HMDB28964	Lysyl-Valine	0.125529361	15.47	18.70	15.48	16.03	17.67	22.74	17.09	17.75	20.48	20.41
5	234.1081	67	HMDB29037	Serinyl-Glutamine	0.063827932	18.54	19.89	19.20	19.61	19.82	22.39	20.47	23.90	20.97	21.10
6	100.02158	65.7	HMDB05843	Allyl isothiocyanate	0.011092958	14.51	15.49	14.47	14.80	15.21	17.49	16.41	16.65	16.27	17.05
7	100.02158	65.7	HMDB33116	4-Methylthiazole	0.011092958	14.51	15.49	14.47	14.80	15.21	17.49	16.41	16.65	16.27	17.05
8	808.51303	51.9	HMDB12425	PS(20:3(8Z11Z14Z)18:3(9Z12Z15Z))	0.136425217	12.78	14.60	12.78	12.78	12.78	17.58	14.81	14.21	14.47	13.78
9	216.06316	96.6	HMDB00114	Glycerylphosphorylethanolamine	0.160603393	17.85	20.21	17.93	18.47	19.57	22.80	19.53	18.81	20.73	21.05
10	703.57506	36.6	HMDB13464	SM(d18:016:1(9Z))	0.144236335	15.81	16.39	14.29	17.43	16.78	19.50	16.90	17.01	17.37	18.27
11	146.08118	73.2	HMDB12151	2-Keto-6-aminocaproate	0.016528718	18.23	18.50	18.52	17.75	18.81	20.66	19.77	19.70	19.45	20.24
12	244.11138	68.7	HMDB01458	Biotin amide	0.009193018	17.95	18.24	17.72	17.95	18.26	20.05	19.25	19.08	19.34	19.88
13	175.07127	62.3	HMDB00854	Formiminoglutamic acid	0.125529361	15.13	17.34	15.97	15.56	16.46	18.42	17.11	16.21	17.52	18.66
14	175.07127	62.3	HMDB06028	N-Acetylasparagine	0.125529361	15.13	17.34	15.97	15.56	16.46	18.42	17.11	16.21	17.52	18.66
15	150.05835	47.6	HMDB00696	L-Methionine	0.064013297	23.96	24.58	23.66	23.40	23.84	25.99	24.89	24.28	25.18	25.66
16	104.05291	46.7	HMDB33875	23-Dihydrothiophene	0.064251874	20.96	21.55	20.56	20.35	20.78	22.70	21.86	21.21	22.08	22.59
17	104.05291	46.7	HMDB33922	Divinyl sulfide	0.064251874	20.96	21.55	20.56	20.35	20.78	22.70	21.86	21.21	22.08	22.59
18	132.10194	43.9	HMDB00687	L-Leucine	0.022135178	26.07	26.50	26.03	25.97	26.36	27.90	27.23	26.80	27.36	27.71
19	238.04469	96.4	HMDB59660	sn-glycero-3-Phosphoethanolamine	0.161231062	16.35	18.16	16.19	16.71	17.78	19.32	17.79	17.11	18.12	18.87
20	120.06557	67.9	HMDB00167	L-Threonine	0.118104285	22.56	23.94	23.03	23.20	23.73	25.38	24.03	23.41	24.54	24.99
21	522.35573	26.6	HMDB02815	LysoPC(18:1(9Z))	0.123236824	21.62	21.88	21.21	21.42	21.72	24.11	22.02	21.77	22.65	22.97
22	182.0811	51.1	HMDB00158	L-Tyrosine	0.100983924	21.46	22.52	21.58	21.29	21.79	23.86	22.39	22.11	22.83	23.10
23	774.60091	50.5	HMDB09255	PE(20:1(11Z)18:0)	0.174486393	13.30	15.33	14.69	14.71	15.82	15.88	15.56	15.83	15.21	16.09
24	800.61607	52.8	HMDB09008	PE(18:022:2(13Z16Z))	0.105506897	17.80	17.41	18.09	17.64	18.21	19.16	18.64	17.89	18.58	19.30
25	306.08068	53.4	HMDB03333	8-Hydroxy-deoxyguanosine	0.039950186	18.35	18.78	18.32	18.17	18.91	19.74	19.16	19.22	19.27	19.53
26	166.08628	41.1	HMDB00159	L-Phenylalanine	0.113789959	25.20	25.77	25.26	25.12	25.50	26.91	25.68	25.64	26.17	26.40
27	118.0863	54.5	HMDB00043	Betaine	0.059747568	26.00	25.70	26.33	25.91	26.09	26.97	26.99	26.47	26.39	27.05
28	118.0863	54.5	HMDB00883	L-Valine	0.059747568	26.00	25.70	26.33	25.91	26.09	26.97	26.99	26.47	26.39	27.05
29	118.0863	54.5	HMDB01382	Vaporole	0.059747568	26.00	25.70	26.33	25.91	26.09	26.97	26.99	26.47	26.39	27.05
30	792.55425	39.3	HMDB09653	PE(22:5(7Z10Z13Z16Z19Z)18:1(9Z))	0.080195108	21.95	22.24	22.03	21.92	22.00	23.27	22.39	22.30	22.84	23.17
31	116.07066	54	HMDB00162	L-Proline	0.134225666	25.14	24.91	24.87	24.54	24.74	25.72	26.28	25.15	24.83	25.87
32	175.10772	65.4	HMDB28854	Glycyl-Valine	0.161159566	19.16	20.16	19.11	19.21	19.62	20.85	20.06	19.63	19.72	20.52
33	766.5746	34.7	HMDB08488	PC(20:4(8Z11Z14Z17Z)P-16:0)	0.125529361	21.46	21.18	21.33	21.22	21.19	21.08	20.83	20.78	20.92	20.91
34	142.08627	48.5	HMDB29427	L-Hypoglycin A	0.15315661	20.46	20.92	20.76	20.78	20.85	20.09	20.62	20.33	20.34	20.47
35	820.62089	48.5	HMDB08686	PC(22:5(4Z7Z10Z13Z16Z)P-18:0)	0.155409923	18.60	18.54	19.03	18.29	18.45	17.72	17.98	17.98	18.35	18.46
36	141.06587	289.3	HMDB02820	Methylimidazoleacetic acid	0.125529361	20.34	20.66	20.09	20.33	20.61	19.60	20.06	19.79	20.17	19.97
37	177.10226	32.1	HMDB00259	Serotonin	0.16716594	21.07	20.29	21.09	21.06	20.81	19.40	20.75	20.56	20.21	20.24
38	193.09717	36.6	HMDB01004	Oxoamide	0.134654206	20.11	19.72	20.24	20.18	20.15	18.61	19.94	19.87	19.09	19.40
39	244.0926	54.6	HMDB00089	Cytidine	0.125136689	20.79	21.51	21.13	21.02	21.42	21.11	19.78	20.38	20.75	19.69
40	113.03462	40.4	HMDB00300	Uracil	0.106741808	20.84	20.96	21.04	20.89	21.14	20.35	20.16	20.93	19.96	19.30

41	127.0866	36.8	HMDB01169	4-Aminophenol	0.118359645	21.77	21.44	21.91	21.84	21.77	19.94	21.48	21.42	20.71	20.98
42	155.11791	32	HMDB04989	m-Tyramine	0.067374876	24.46	23.96	24.33	24.19	24.13	22.70	23.78	23.62	23.08	23.60
43	274.20118	35.3	HMDB13238	Heptanoylcarnitine	0.112202542	24.28	24.13	24.33	24.05	24.13	22.15	23.93	23.46	23.52	23.39
44	161.10733	32.3	HMDB00303	Tryptamine	0.119573825	21.72	20.81	21.73	21.47	21.30	19.40	21.28	20.70	20.26	20.50
45	246.17028	33.9	HMDB00378	2-Methylbutyroylcarnitine	0.098307214	21.55	21.98	22.19	21.45	21.72	21.87	20.39	20.02	20.67	20.67
46	246.17028	33.9	HMDB00688	Isovalerylcarnitine	0.098307214	21.55	21.98	22.19	21.45	21.72	21.87	20.39	20.02	20.67	20.67
47	246.17028	33.9	HMDB13128	Valerylcarnitine	0.098307214	21.55	21.98	22.19	21.45	21.72	21.87	20.39	20.02	20.67	20.67
48	246.17028	33.9	HMDB41993	Pivaloylcarnitine	0.098307214	21.55	21.98	22.19	21.45	21.72	21.87	20.39	20.02	20.67	20.67
49	276.18051	35.8	HMDB13131	Hydroxyhexanoycarnitine	0.043665838	22.27	22.94	22.91	22.83	22.77	22.13	20.68	20.61	21.66	21.77
50	456.40461	25.1	HMDB06460	Arachidyl carnitine	0.135164834	19.07	19.62	19.12	18.89	19.22	19.47	15.02	17.95	17.34	17.43
51	240.10172	46.7	HMDB33519	N-Ornithyl-L-taurine	0.0035634	23.32	23.62	23.32	23.17	23.46	21.89	21.03	21.22	21.77	21.48
52	260.18558	34.1	HMDB00705	Hexanoylcarnitine	0.051033775	23.40	23.49	23.42	23.49	23.17	22.93	19.48	21.20	21.61	21.25
53	260.18558	34.1	HMDB00756	L-Hexanoylcarnitine	0.051033775	23.40	23.49	23.42	23.49	23.17	22.93	19.48	21.20	21.61	21.25
54	248.14943	42.4	HMDB13127	Hydroxybutyrylcarnitine	0.003875555	27.27	27.33	27.15	27.18	27.11	24.68	24.52	22.71	24.28	24.61
55	132.03023	21.5	HMDB00191	L-Aspartic acid	0.07261269	18.43	20.69	18.54	19.13	20.11	25.04	20.87	19.92	22.80	22.90
56	226.99684	20.7	HMDB00094	Citric acid	0.005623905	17.25	17.62	16.62	15.96	16.95	19.71	18.32	19.50	18.76	19.35
57	885.54997	253.4	HMDB09842	PI(18:1(9Z)20:3(5Z8Z11Z))	0.177940975	17.70	19.96	18.74	18.23	19.12	23.37	19.03	18.77	21.21	20.70
58	885.54997	253.4	HMDB09894	PI(20:4(5Z8Z11Z14Z)18:0)	0.177940975	17.70	19.96	18.74	18.23	19.12	23.37	19.03	18.77	21.21	20.70
59	195.05127	20.9	HMDB00565	Galactonic acid	0.083073623	16.70	17.39	16.82	16.52	17.08	20.67	17.71	17.29	18.37	19.62
60	188.98665	20	HMDB59724	Pyrocatechol sulfate	0.00471047	17.07	16.27	16.76	16.63	16.70	18.72	19.21	18.15	17.76	18.53
61	209.03039	99.2	HMDB00663	Glucaric acid	0.086528238	14.25	14.40	15.32	12.62	14.20	17.23	15.52	15.68	14.71	16.05
62	174.04099	17.8	HMDB00812	N-Acetyl-L-aspartic acid	0.166026464	17.46	18.43	17.57	17.82	18.16	22.18	17.95	18.63	19.17	19.40
63	830.69924	39.3	HMDB13441	PC(o-20:020:0)	0.010070726	14.83	15.25	14.64	14.82	15.75	17.00	16.09	16.10	16.98	16.79
64	183.00673	21.1	HMDB00606	D-2-Hydroxyglutaric acid	0.077235609	19.71	20.10	19.82	19.75	20.27	22.81	20.87	20.89	20.19	22.04
65	181.0717	21.7	HMDB00247	Sorbitol	0.056139341	17.08	16.32	16.17	16.75	15.47	17.02	17.25	17.92	18.82	17.84
66	178.05115	20	HMDB00714	Hippuric acid	0.003263799	20.07	20.55	20.37	20.37	20.61	22.15	21.71	21.97	21.45	21.62
67	452.27895	207.3	HMDB11473	LysoPE(0:016:0)	0.162807081	18.95	21.79	19.29	20.88	20.68	21.72	21.45	21.41	22.20	20.93
68	147.02993	21.4	HMDB00426	Citramalic acid	0.072947809	20.03	20.74	20.14	20.40	20.58	22.89	21.21	20.90	21.04	21.87
69	129.01935	18.6	HMDB00634	Citraconic acid	0.023795461	23.54	24.42	23.74	24.11	24.21	25.61	25.07	24.68	24.77	25.30
70	767.48746	225.8	HMDB10652	PG(18:2(9Z12Z)18:3(9Z12Z15Z))	0.126897252	17.53	18.24	17.30	17.74	17.88	19.35	19.56	17.31	18.62	19.21
71	817.50284	228.8	HMDB10673	PG(18:3(6Z9Z12Z)22:5(7Z10Z13Z16Z19Z))	0.122103896	19.78	21.08	20.24	20.19	20.71	22.60	21.25	21.20	20.82	21.04
72	228.08159	21.8	HMDB03045	Ergothioneine	0.010609023	17.19	17.13	16.97	17.06	17.12	17.74	18.40	18.11	17.72	18.36
73	423.27592	233.8	HMDB00311	3b4b7a12a-Tetrahydroxy-5b-cholanoic acid	0.052809896	22.07	21.78	21.80	21.70	22.54	23.43	22.75	22.24	23.09	23.11
74	480.31002	215.3	HMDB10381	LysoPC(15:0)	0.091309314	20.86	22.11	21.30	21.77	22.18	23.33	22.21	22.20	22.56	22.62
75	151.06134	21.3	HMDB01851	L-Arabitol	0.126987939	17.13	16.66	16.69	16.76	16.78	18.52	17.19	16.72	18.09	17.68
76	165.04057	21.1	HMDB60256	L-Xylonate	0.148443348	18.70	18.99	18.62	18.83	19.10	20.74	19.45	19.37	18.91	19.54
77	173.00928	23.2	HMDB00072	cis-Aconitic acid	0.087226459	19.23	18.81	19.13	18.85	18.87	20.59	19.39	19.66	19.67	19.35
78	107.05022	20.1	HMDB02048	m-Cresol	0.074040616	16.87	16.45	16.27	16.68	16.51	17.93	17.52	17.03	16.83	17.21
79	187.00726	20.5	HMDB11635	p-Cresol sulfate	0.062992239	20.84	20.54	20.25	20.50	20.71	21.84	21.24	21.03	21.04	21.17
80	130.08728	24.2	HMDB00172	L-Isoleucine	0.070894308	21.51	22.37	21.85	22.02	21.98	22.46	23.04	22.46	22.53	22.54
81	204.06696	19.2	HMDB00671	Indolelactic acid	0.157698264	16.31	16.68	15.79	15.67	16.35	17.34	16.80	16.29	16.55	16.94
82	217.11977	24.8	HMDB29042	Serinyl-Isoleucine	0.1796041	19.75	19.90	20.04	20.07	20.05	19.34	19.62	19.89	19.32	19.76
83	285.22277	240.2	HMDB06221	13-cis Retinol	0.176090038	17.17	17.60	17.16	17.29	17.32	17.14	16.86	17.20	16.67	16.74
84	152.00247	17.6	HMDB00996	3-Sulfinoalanine	0.138418028	23.44	23.46	23.56	23.71	23.54	22.95	23.47	23.33	22.83	22.99
85	142.08726	280.4	HMDB04827	Proline betaine	0.089989787	14.66	14.74	15.00	15.10	14.82	14.38	14.40	14.50	14.31	14.52
86	182.0131	17.2	HMDB02205	L-Homocysteic acid	0.171996992	17.33	17.48	17.54	17.54	17.51	17.12	17.46	17.07	16.39	17.06
87	206.08267	19.9	HMDB00512	N-Acetyl-L-phenylalanine	0.160428582	21.14	21.14	21.32	21.37	21.26	20.18	21.08	21.17	20.40	20.85
88	172.09805	19.2	HMDB02087	Isovalerylsarcosine	0.127485365	22.66	22.63	22.83	22.92	22.80	21.63	22.60	22.61	21.62	22.27
89	158.08238	19	HMDB29230	4-Hydroxystachydrine	0.157510246	21.21	21.10	21.35	21.44	21.33	20.05	21.23	21.18	20.10	20.73
90	188.07172	33.7	HMDB02302	3-Indolepropionic acid	0.184113977	17.17	17.14	17.47	17.55	17.49	15.68	17.48	16.93	16.60	16.81
91	103.00366	21.9	HMDB00691	Malonic acid	0.082152336	17.45	16.49	17.17	17.38	16.44	16.27	16.60	16.08	16.01	16.07
92	235.07581	23.6	HMDB29045	Serinyl-Methionine	0.117500612	17.30	17.51	18.00	18.31	17.80	16.16	17.69	17.38	16.01	16.83
93	396.3124	251.4	HMDB13207	9-Hexadecenoylcarnitine	0.125236396	18.93	17.94	18.69	18.52	19.14	19.09	17.32	17.20	17.16	17.10
94	269.21259	159.4	HMDB30973	9-Oxohexadecanoic acid	0.071785454	21.73	22.63	21.70	22.04	22.19	21.43	21.82	20.30	20.91	20.14
95	215.16539	80.4	HMDB02059	12-Hydroxydodecanoic acid	0.006296605	19.17	19.58	19.31	19.71	19.77	18.39	17.96	18.55	18.61	18.04
96	297.24386	184.5	HMDB30980	10-Oxooctadecanoic acid	0.01187075	24.20	25.24	24.36	24.86	24.65	23.86	23.24	23.37	23.56	23.11
97	166.01796	18	HMDB06462	Homocysteinesulfinic acid	0.12070305	26.17	25.85	26.20	26.39	26.11	23.11	25.99	25.78	24.24	24.95
98	103.04007	19.6	HMDB00336	(R)-3-Hydroxyisobutyric acid	0.003633243	22.11	22.35	22.35	22.36	22.65	21.07	21.52	20.67	20.97	20.90
99	351.25461	175.4	HMDB11540	MG(0:018:3(9Z12Z15Z)0:0)	0.065942735	19.55	18.60	19.63	19.32	18.97	16.45	18.80	18.62	17.32	17.23
100	249.05552	17.3	HMDB01049	Gamma-Glutamylcysteine	0.095337632	17.81	18.04	17.89	18.57	17.96	14.69	17.74	17.44	15.89	16.78
101	117.01946	18.9	HMDB00254	Succinic acid	0.021905145	24.15	24.79	24.36	24.58	24.26	24.05	22.50	21.60	22.73	22.42
102	159.10273	21.4	HMDB02264	(R)-2-Hydroxycaprylic acid	0.019645152	18.47	18.67	18.53	18.77	18.67	16.18	16.58	18.02	15.95	17.52
103	191.04968	18.8	HMDB28768	Cysteinyl-Alanine	0.138800384	18.40	16.09	17.89	18.38	17.16	13.78	18.18	15.26	14.78	16.10
104	295.22819	158.4	HMDB04702	1213-EpOME	0.101010227	23.55	24.37	23.54	23.06	23.54	24.06	19.09	21.36	21.32	21.58
105	243.1968	168.5	HMDB02261	2-Hydroxymyristic acid	0.058439422	20.29	20.91	20.42	20.57	20.92	20.23	15.38	17.92	18.85	17.79

***Table S3:* Metabolites That Are Common to Wildtype and 3-MST KO Mice following TAC.**

Changes in only 4 metabolites (1 up and 3 down) were found common to both WT and 3-MST KO hearts.

Metabolite	Identifier (HMDB)	WT Sham	WT TAC	Adjusted P value	3-MST KO Sham	3-MST KO TAC	Adjusted P Value
L-Hexanoylcarnitine	HMDB00756	23.22 ± 0.32	21.52 ± 0.17	0.070	23.39 ± 0.06	21.29 ± 0.55	0.051
Hydroxybutyrylcarnitine	HMDB13127	26.83 ± 0.46	23.60 ± 0.40	0.045	27.21 ± 0.04	24.16 ± 0.37	0.003
Hippuric acid	HMDB00714	20.02 ± 0.26	21.51 ± 0.16	0.065	20.39 ± 0.09	21.78 ± 0.12	0.003
12-Hydroxydodecanoic acid	HMDB02059	20.57 ± 0.11	19.59 ± 0.12	0.076	19.51 ± 0.12	18.31 ± 0.13	0.006

Table S4: **Metabolites That Are Unique to Wildtype Mice following TAC.**

In WT hearts subjected to 12 weeks of TAC a total of 17 metabolites (9 up and 8 down) were significantly changed compared to Sham-TAC WT.

Metabolite	Identifier (HMDB)	Sham	TAC	Adjusted P value
Stearoyllactic acid	HMDB33372	16.52 ± 0.08	18.18 ± 0.36	0.0839
MG(0:018:2(9Z12Z)0:0)	HMDB11538	18.26 ± 0.12	19.65 ± 0.25	0.0700
12-Dehydrosalsolinol	HMDB12490	21.63 ± 0.12	20.21 ± 0.25	0.0696
Arachidyl carnitine	HMDB06460	19.48 ± 0.26	17.63 ± 0.18	0.0445
Isobutyryl-L-carnitine	HMDB00736	24.93 ± 0.47	22.64 ± 0.12	0.0700
Dimethyl fumarate	HMDB31257	17.80 ± 0.24	20.27 ± 0.26	0.0226
Oxoadipic acid	HMDB00225	15.40 ± 0.45	17.54 ± 0.31	0.0988
D-Glucose	HMDB00122	20.10 ± 0.25	22.21 ± 0.25	0.0329
Sorbitan palmitate	HMDB29887	21.05 ± 0.21	22.83 ± 0.28	0.0526
MG(0:014:1(9Z)0:0)	HMDB11531	19.90 ± 0.24	21.41 ± 0.25	0.0799
3-Succinoylpyridine	HMDB00992	20.02 ± 0.26	21.51 ± 0.16	0.0657
Palmitoleic acid	HMDB03229	25.56 ± 0.20	26.99 ± 0.27	0.0979
Myristoleic acid	HMDB02000	22.03 ± 0.23	20.86 ± 0.12	0.0988
(R)-3-Hydroxybutyric acid	HMDB00011	22.60 ± 0.14	21.06 ± 0.27	0.0570
(R)-3-Hydroxydecanoic acid	HMDB10725	19.68 ± 0.19	17.98 ± 0.15	0.0329
3-Hydroxyoctanoic acid	HMDB01954	18.69 ± 0.28	16.79 ± 0.17	0.0352

***Table S5:* Metabolites That Are Unique to 3-MST KO Mice following TAC.**

12 weeks of TAC significantly changed 45 metabolites (30 up and 15 down) in the hearts of 3-MST KO mice.

Metabolite	Identifier (HMDB)	Sham	TAC	Adjusted P value
Lactosamine	HMDB06591	11.59 ± 0.20	16.50 ± 1.32	0.052
Serinyl-Glutamine	HMDB29037	19.41 ± 0.25	21.77 ± 0.62	0.064
4-Methylthiazole	HMDB33116	14.90 ± 0.20	16.77 ± 0.22	0.011
2-Keto-6-aminocaproate	HMDB12151	18.36 ± 0.18	19.96 ± 0.22	0.017
Biotin amide	HMDB01458	18.02 ± 0.10	19.52 ± 0.19	0.009
L-Methionine	HMDB00696	23.89 ± 0.20	25.20 ± 0.30	0.064
23-Dihydrothiophene	HMDB33875	20.84 ± 0.21	22.09 ± 0.27	0.064
8-Hydroxy-deoxyguanosine	HMDB03333	18.51 ± 0.14	19.38 ± 0.11	0.040
Betaine	HMDB00043	26.01 ± 0.10	26.77 ± 0.14	0.060
Vaporole	HMDB01382	26.01 ± 0.10	26.77 ± 0.14	0.060
PE(22:5(7Z10Z13Z16Z19Z)18:1(9Z))	HMDB09653	22.03 ± 0.06	22.79 ± 0.20	0.080
m-Tyramine	HMDB04989	24.21 ± 0.09	23.36 ± 0.20	0.067
Isovalerylcarnitine	HMDB00688	21.78 ± 0.14	20.72 ± 0.31	0.098
Hydroxyhexanoycarnitine	HMDB13131	22.74 ± 0.12	21.37 ± 0.31	0.044
N-Ornithyl-L-taurine	HMDB33519	23.38 ± 0.08	21.48 ± 0.16	0.004
Hexanoylcarnitine	HMDB00705	23.39 ± 0.06	21.29 ± 0.55	0.051
L-Aspartic acid	HMDB00191	19.38 ± 0.44	22.31 ± 0.89	0.073
Citric acid	HMDB00094	16.88 ± 0.28	19.13 ± 0.26	0.006
Galactonic acid	HMDB00565	16.90 ± 0.15	18.73 ± 0.62	0.083
Pyrocatechol sulfate	HMDB59724	16.69 ± 0.13	18.47 ± 0.25	0.005
Glucaric acid	HMDB00663	14.16 ± 0.43	15.84 ± 0.41	0.087
PC(o-20:020:0)	HMDB13441	15.06 ± 0.20	16.59 ± 0.21	0.010
D-2-Hydroxyglutaric acid	HMDB00606	19.93 ± 0.11	21.36 ± 0.47	0.077
Sorbitol	HMDB00247	16.36 ± 0.27	17.77 ± 0.31	0.056
Citramalic acid	HMDB00426	20.38 ± 0.13	21.58 ± 0.37	0.073
D-2-Hydroxyglutaric acid	HMDB00606	19.93 ± 0.11	21.36 ± 0.47	0.077
Citraconic acid	HMDB00634	24.00 ± 0.16	25.09 ± 0.17	0.024
Ergothioneine	HMDB03045	17.09 ± 0.04	18.07 ± 0.15	0.011
3b4b7a12a-Tetrahydroxy-5b-cholanoic acid	HMDB00311	21.98 ± 0.15	22.92 ± 0.20	0.053
LysoPC(15:0)	HMDB10381	21.64 ± 0.25	22.58 ± 0.21	0.091
L-Valine	HMDB00883	26.01 ± 0.10	26.77 ± 0.14	0.060
cis-Aconitic acid	HMDB00072	18.98 ± 0.08	19.73 ± 0.22	0.087
m-Cresol	HMDB02048	16.56 ± 0.10	17.30 ± 0.19	0.074
p-Cresol sulfate	HMDB11635	20.57 ± 0.10	21.26 ± 0.15	0.063
L-Isoleucine	HMDB00172	21.95 ± 0.14	22.61 ± 0.11	0.071
L-Leucine	HMDB00687	26.19 ± 0.10	27.40 ± 0.19	0.022
Proline betaine	HMDB04827	14.86 ± 0.08	14.42 ± 0.04	0.090
Malonic acid	HMDB00691	16.99 ± 0.22	16.21 ± 0.11	0.082
9-Oxohexadecanoic acid	HMDB30973	22.06 ± 0.17	20.92 ± 0.32	0.072
10-Oxooctadecanoic acid	HMDB30980	24.66 ± 0.18	23.43 ± 0.13	0.012

(R)-3-Hydroxyisobutyric acid	HMDB00336	22.36 ± 0.09	21.03 ± 0.14	0.004
MG(0:018:3(9Z12Z15Z)0:0)	HMDB11540	19.21 ± 0.19	17.68 ± 0.45	0.066
Gamma-Glutamylcysteine	HMDB01049	18.05 ± 0.13	16.51 ± 0.55	0.095
Succinic acid	HMDB00254	24.43 ± 0.11	22.66 ± 0.40	0.022
(R)-2-Hydroxycaprylic acid	HMDB02264	18.62 ± 0.05	16.85 ± 0.40	0.020
2-Hydroxymyristic acid	HMDB02261	20.62 ± 0.13	18.03 ± 0.79	0.058

Figure S1

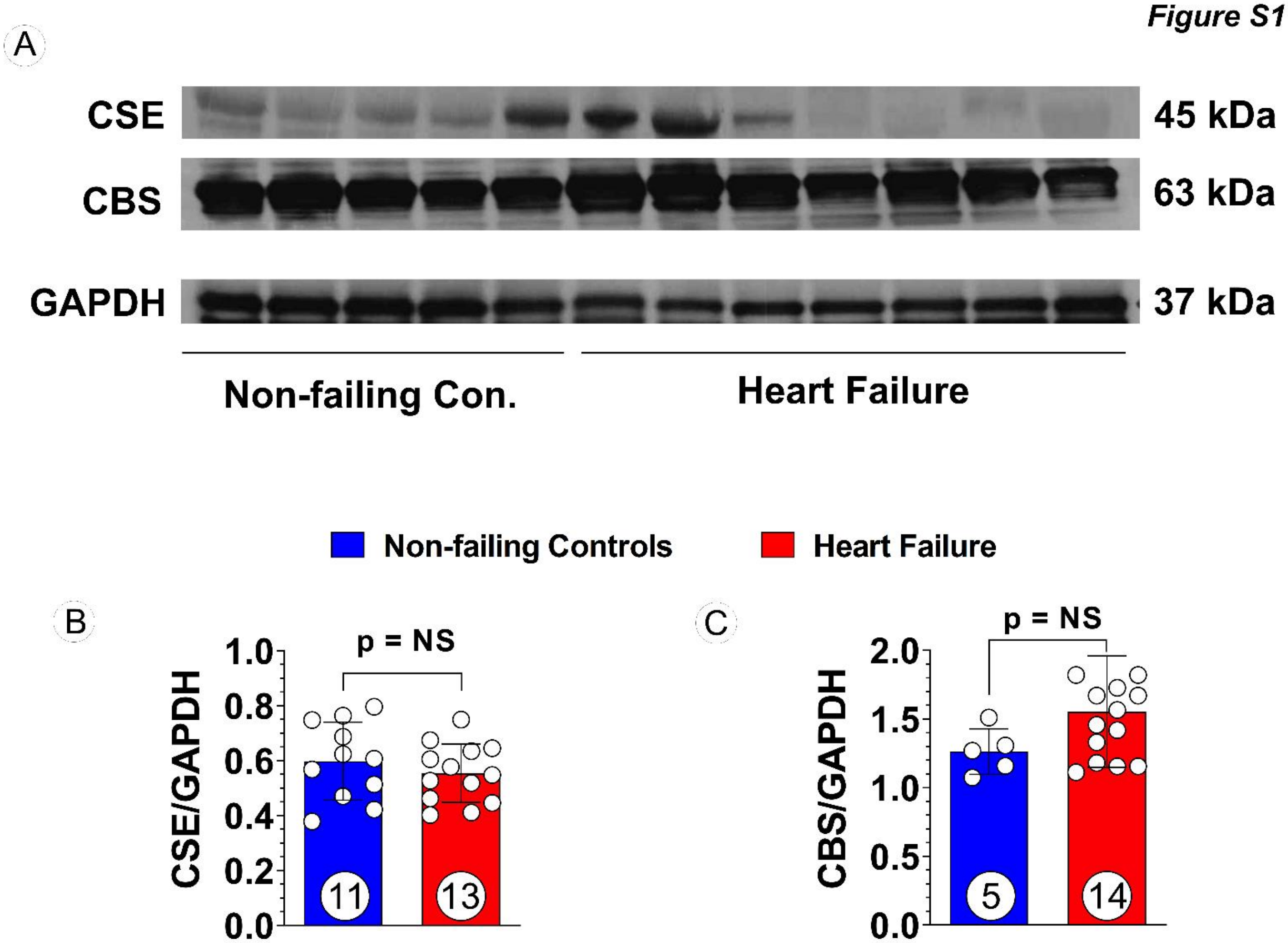

Figure S1: **Protein Expression of CSE and CBS in Human Non-failing Control Hearts and Failing Hearts.**

(A) Representative Western blots of human myocardial CSE and CBS. (***B***) and (***C***) Quantification of the Western blot images. Data were analyzed with student unpaired 2-tailed *t* test (***B***) or Mann-Whitney test (***C***) and presented as mean ± SD.

Figure S2

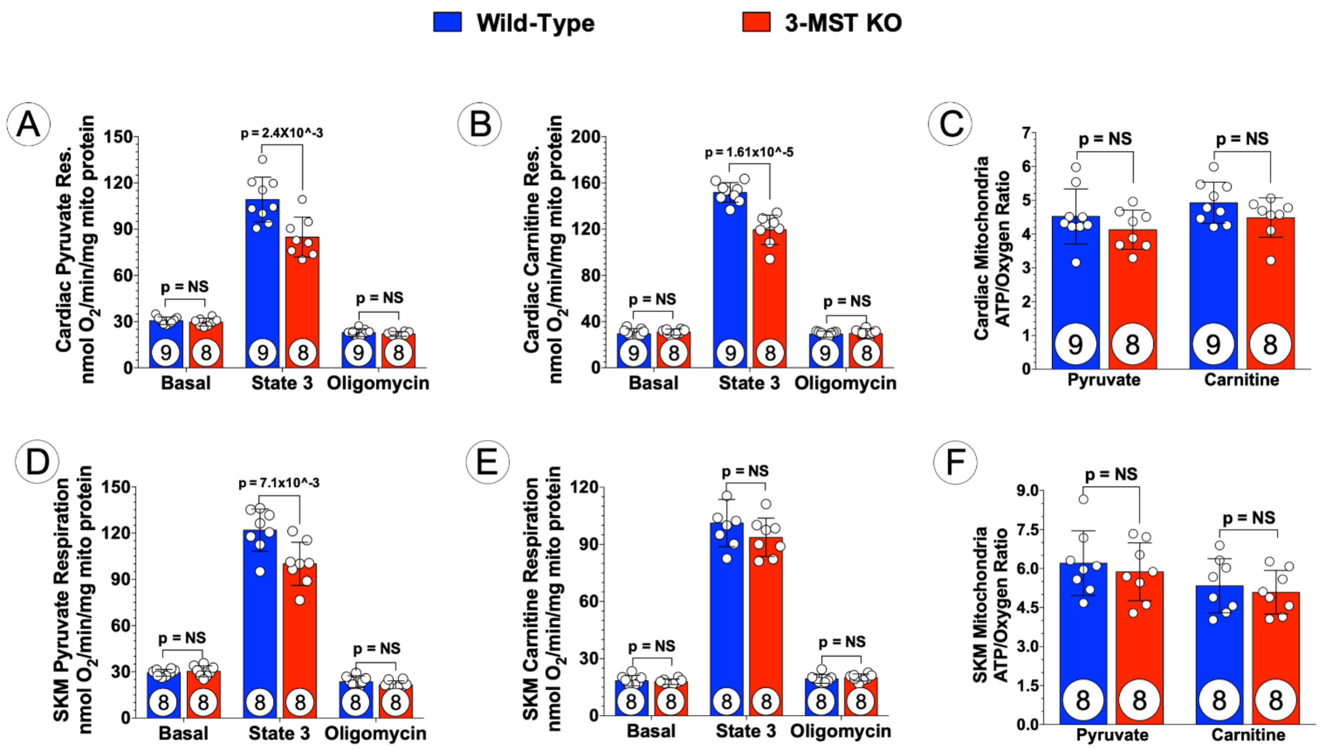

Figure S2: **Mitochondrial Respiration in Wildtype and 3-MST KO Mice under Baseline Condition.**

Respiration using ***(A)*** pyruvate or (***B***) carnitine as substrate and (***C***) ATP synthesis efficiency in mitochondria isolated from wildtype or 3-MST KO hearts at 10 weeks of age under baseline condition. Respiration using ***(D)*** pyruvate or (***E***) carnitine as substrate and ***(F)*** ATP synthesis efficiency in mitochondria isolated from wildtype or 3-MST KO skeletal muscle at 10 weeks of age under baseline condition. Circles inside bars indicates samples size. Data were analyzed with student unpaired 2-tailed *t* test and presented as mean ± SD.

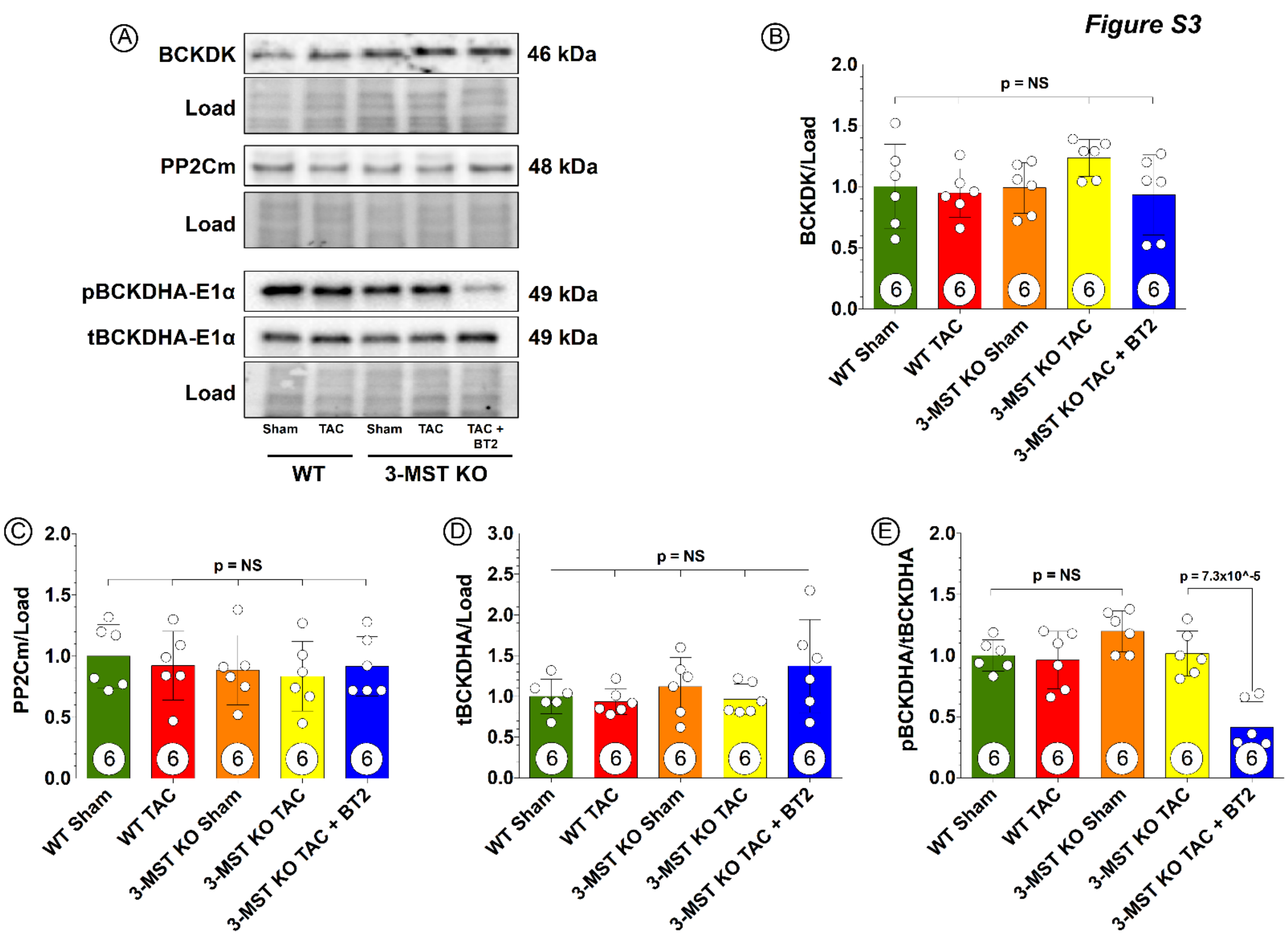

Figure S3: **Protein Expression of Key BCAA Catabolic Enzymes in Wildtype and 3-MST KO Mice.**

(A) Representative Western blots of BCKDK, PP2Cm, phosphorylated BCKD and total BCKD, quantified ***(B)*** BCKDK, ***(C)*** PP2Cm, ***(D)*** total BCKD, and ***(E)*** phosphorylated BCKD/total BCKD ratio. Circles inside bars indicates samples size. Data were analyzed with Kruskal-Wallis test and presented as mean ± SD.

Figure S4

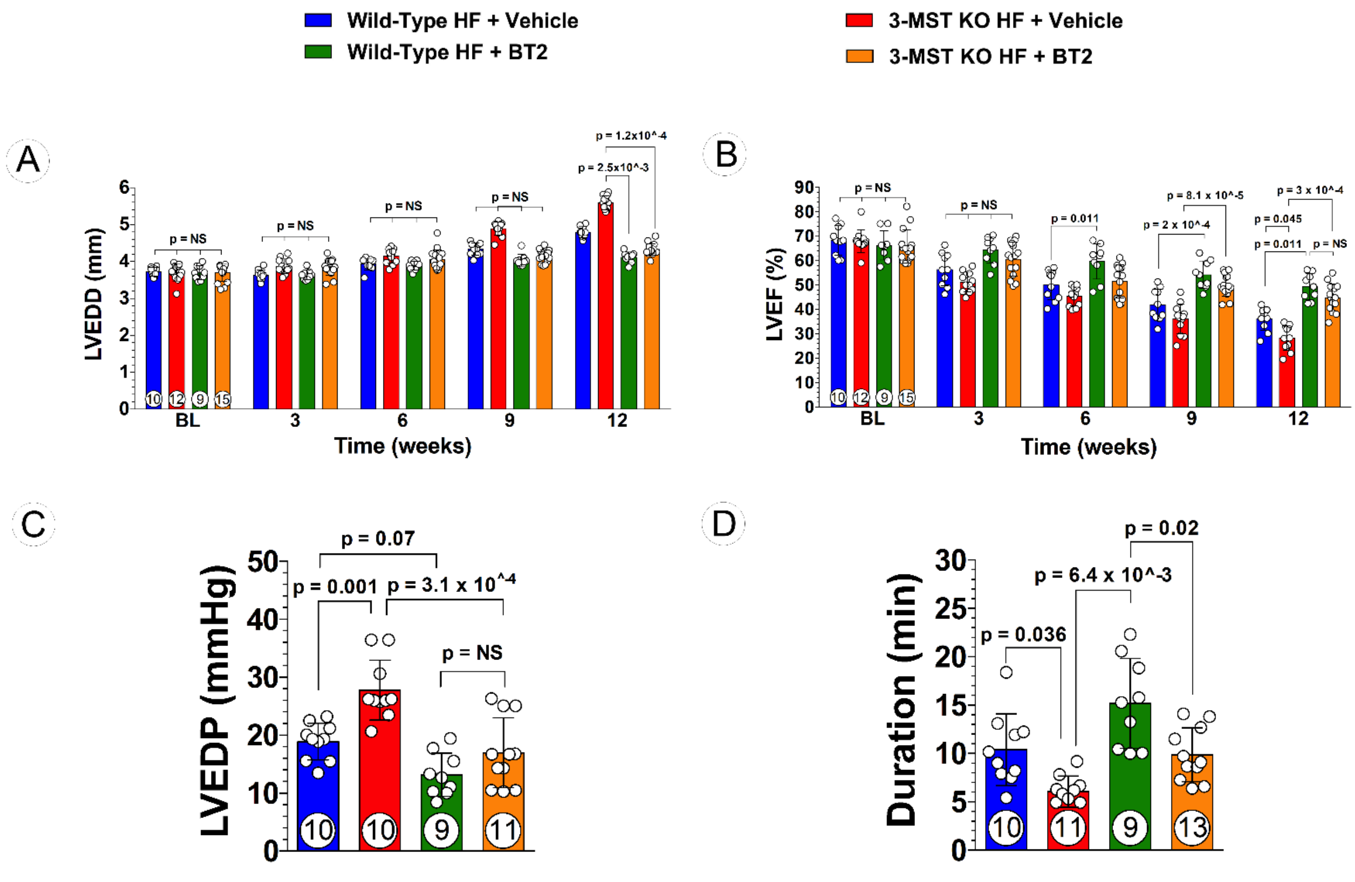

Figure S4: **Cardiac Structure, Function, LVEDP, and Exercise Capacity in Wildtype or 3-MST KO Mice Treated with Vehicle or BT2 following TAC.**

(A) LV end-diastolic diameter (LVEDD) and ***(B)*** LV ejection fraction (LVEF) throughout the 12 weeks study for 3-MST KO and wildtype (WT) control mice treated with either Vehicle or BT2. ***(C)*** LV end-diastolic pressure (LVEDP) and ***(D)*** treadmill running duration in 3-MST KO and WT control mice treated with either Vehicle or BT2 at 12 weeks post TAC. Circles inside bars indicates samples size. Data in (***A***) and (***B***) were analyzed with ordinary 2-way ANOVA; data in (***C***) and (***D***) were analyzed with ordinary 1-way ANOVA. All data are presented as mean ± SD.

Figure S5

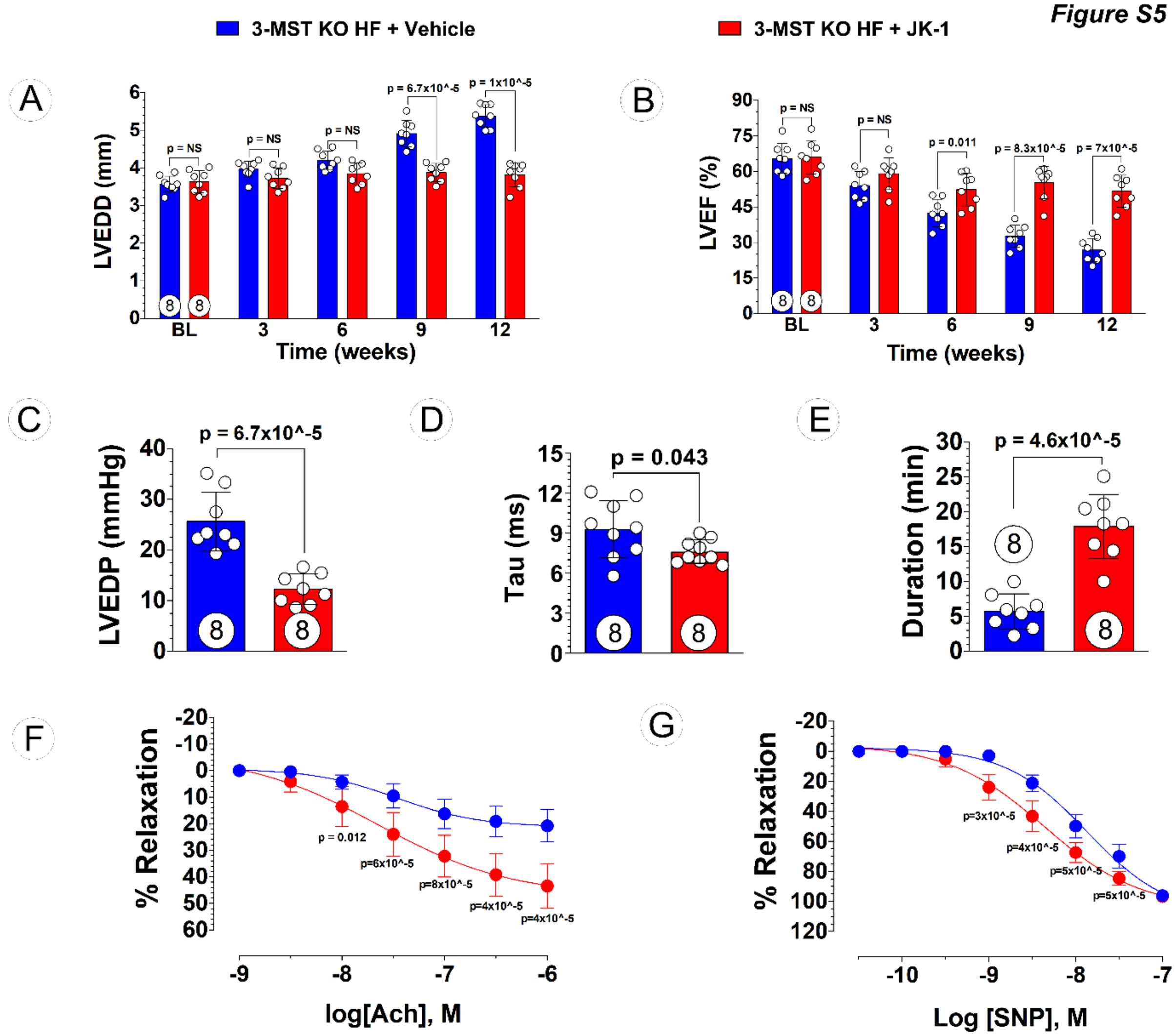

Figure S5: **Cardiac Structure, Function, LV hemodynamics, vascular reactivity, and Exercise Capacity in Wildtype or 3-MST KO Mice Treated with Vehicle or JK-1 following TAC.**

(A) LV end-diastolic diameter (LVEDD) and ***(B)*** LV ejection fraction (LVEF) throughout the 12 weeks study for 3-MST KO and wildtype (WT) control mice treated with either Vehicle or JK-1. ***(C)*** LV end-diastolic pressure (LVEDP), ***(D)*** relaxation constant Tau, ***(E)*** treadmill running duration, ***(F)*** aortic vascular reactivity to acetylcholine (Ach), and ***(G)*** aortic vascular reactivity to sodium nitroprusside (SNP) in 3-MST KO and WT control mice treated with either Vehicle or BT2 at 12 weeks post TAC. Circles inside bars indicates samples size. Data in (***A***), (***B***), (***F***), and (***G***) were analyzed with ordinary 2-way ANOVA; data in (***C***) to (***E***) were analyzed with student unpaired 2-tailed *t* test. All data are presented as mean ± SD.