Circulation Research. 2024;134:00–00. DOI: 10.1161/CIRCRESAHA.123.323655

SUPPLEMENTAL METHODS AND MATERIALS

DETAILED MATERIALS AND METHODS:

Data, Materials, and Code Disclosure Statement

All data and materials have been made publicly available using the online *figshare* data repository and can be accessed at https://doi.org/10.6084/m9.figshare.c.7154392

Cell Culture

AC16 cells were obtained from Millipore Sigma Aldrich (32011203, Cat. #SCC109, Temecula, CA, USA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 IU/ml penicillin G, 100 μg/ml streptomycin (Invitrogen, Carlsbad, CA, USA), and 12.5% fetal bovine serum (FBS).

Cell Grouping

We grouped AC16 cells into two groups: Controls (AC16 cells without any transfections – Ctrls) and AKAP12-OX (AC16 cells stably transfected with human AKAP12 plasmid – Hygromycin selection). To be consistent, transient transfections of GloSensor and β2AR were always carried out for 48 hours.

Animal Studies

All animal studies have been approved by the Institutional Animal Care and Use Committee (IACUC; protocol #17-017) and the ethics committee at the University of Houston (UH; # UH-ACP-11-032). Animal care was provided for in AAALAC accredited animal barrier

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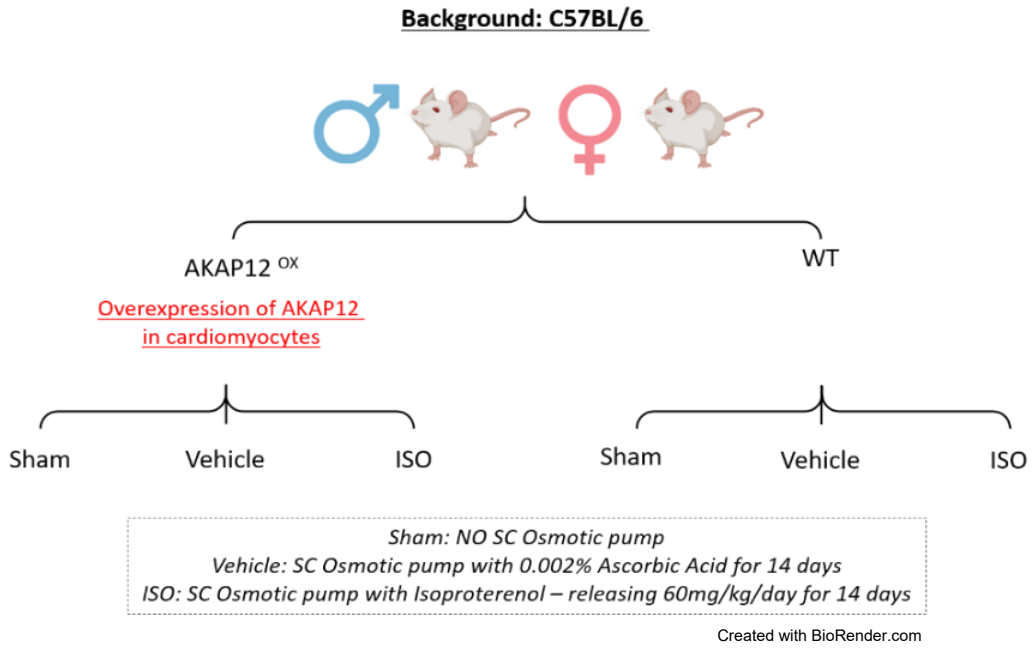
facilities at UH and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Generating Transgenic Mice

We generated mice with cardiac-specific AKAP12 overexpression (AKAP12^{OX}; using the mouse strain C57Bl6/J) which specifically overexpressed AKAP12 in their cardiomyocytes. The transgene consists of the well-documented αMHC promoter driving transcription of a mouse AKAP12 cDNA fused to a C-terminal Myc epitope tag, obtained from GeneCopoeia (OmicsLink expression clone EX-Mm12276-M09)[52]. The AKAP12-Myc fragment was PCR-amplified from EX-Mm12276-M09 with extended primers and inserted between the SalI and HindIII sites of the pJG/αMHC plasmid by recombination[53]. After BamHI excision, the fragment containing the αMHC-Gravin-Myc-bGH-polyA sequence was gel purified for pronuclear injection into mouse zygotes. Genotyping using primers specific for this transgene identified transgenic founders.

Animal Grouping



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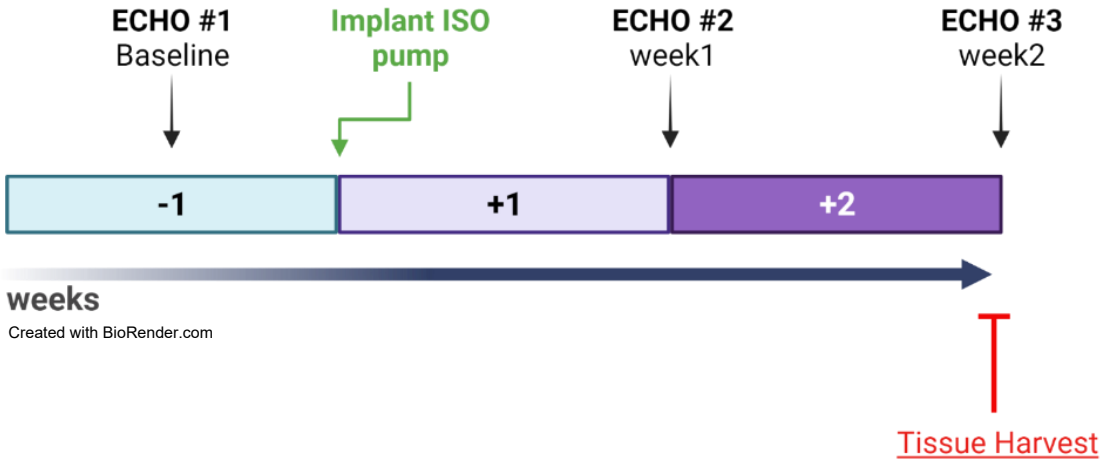
Subcutaneous Osmotic Pump Insertion



Eight to twelve week old mice were anesthetized using 3% Isoflurane before and during the procedure. A small incision in the area behind the neck was made followed by the insertion of Alzet Osmotic pumps (model 1002) with an infusion rate of 0.2 µL/hr. Isoproterenol (ISO) was dissolved in 0.002% Ascorbic Acid to prevent oxidation during pump priming. ISO dose was 60 mg/kg/day for 14 days. Pumps were primed at 37°C overnight before insertion. For the vehicle group, pumps were filled with 0.002% Ascorbic Acid alone and also primed overnight at 37°C.

Echocardiography Measurements

Baseline measurements by echocardiography were obtained at least one day prior to subcutaneous osmotic pump insertion. Each pump released 60 mg/kg/day of isoproterenol (ISO; a βAR agonist) or vehicle (0.002% Ascorbic Acid) for 14-days with a release rate of 0.2 µL/hr. Cardiac morphology and function were assessed by serial M-mode echocardiography with a VisualSonics Vevo 3100 High-Resolution In-Vivo Micro-Imaging System (VisualSonics In, Ontario, Canada) equipped with a 550X probe. Ventricular measurements in M-mode were taken at baseline, 7-days, and 14-days after ISO treatment with at least three readings per mouse. B-mode echocardiography was used for strain analysis. Mice that died during the 14-days study

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period or mice who survived but had a Heart Rate (HR) below 400 bpm or above 650 bpm in both baseline and treatment conditions were excluded from the analysis[54]. All measurements and analyses were performed by the same investigator.

Isolation of Adult Ventricular Mouse Cardiomyocytes for Measurement of Cell Shortening and Intracellular Calcium Levels Downstream β1AR and β2AR

Isolation of ventricular cardiomyocytes from the hearts of mice 8-12 weeks old; AKAP12^{OX}, WT was performed by a modified non-Langendorf approach[55], detailed protocol is listed at the end of the supplemental data. Following incremental calcium restoration, freshly isolated cardiomyocytes were resuspended in plating medium (M199 medium containing 5% FBS, 10mmol/L BDM and 100 U/ml penicillin G, and 100μg/ml streptomycin) and plated onto Geltrex-coated wells and allowed to adhere for 1 hour in the incubator at 37°C. Only rod-shaped cells that showed clear striations and no spontaneous contractions were used for measurements. Cardiomyocyte calcium and contractility measurements were collected using a Multi-Cell Lite® system (IonOptix LLC, Westwood, MA, USA) that allowed repeated measurements on the same cells with different treatments. Cells were paced at 1Hz; 20 Volts at 37°C, unless otherwise specified. Basal and post-treatment measurements were recorded for 10 sec. Data collected from the Multi-Cell Lite® system was analyzed using CytoSolver 3.0 automated analysis system (IonOptix LLC, Westwood, MA, USA).

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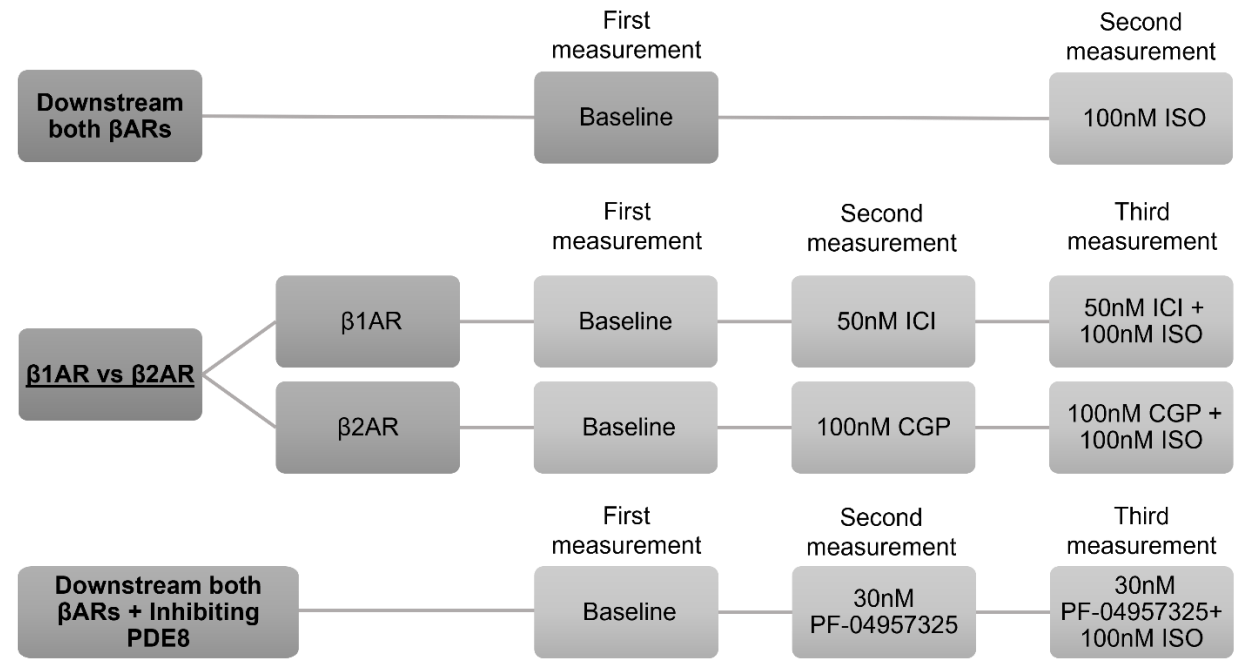

RNA Extraction, Real-Time qPCR, and RNA Sequencing

a. AC16 Cells

Total RNA was extracted from AC16 cells using PureLink® RNA Mini Kit according to the manufacturer's instructions. RNA Integrity and quality were assessed, and samples with 260/230 and/or 260/280 ratios < 2.0 were excluded. 1 µg of total RNA was used for cDNA synthesis using the SuperScript IV VILO (SSIV VILO) kit. For the 10 µL reaction, we combined 5 µL PowerUp™ SYBR™ Green with a 5 µL mix containing cDNA (5ng/reaction), 500nM forward and reverse primers diluted in water. We followed the manufacturer's recommendations for the thermal cycling conditions; UDG activation: 50° - 2 min, Dual-Lock DNA polymerase: 95°- 2 min, Denaturation: 95° - 15 sec, Anneal/Extend: 60° - 1 min, a total of 40 cycles. Real-time qPCR was carried out using the BioRad CFX-Maestro system (BioRad, Hercules, CA). Primer sequences are provided in **Table-1**. Expression data were normalized to mean GAPDH.

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b. Left-Ventricular Extracts (Mouse Tissue)

Total RNA was extracted from sections of mouse left ventricles using Qiagen – Rneasy Fibrous Tissue Mini Kit according to the manufacturer's instructions. RNA Integrity and quality were assessed, and samples with 260/230 and/or 260/280 ratios < 2.0 were excluded. 1 µg of total RNA was used for cDNA synthesis using the SuperScript IV VILO (SSIV VILO) kit. For the 10 µL reaction, we combined 5 µL PowerUp™ SYBR™ Green with a 5 µL mix containing cDNA (5ng/reaction), 500 nM forward and reverse primers diluted in water. We followed the manufacturer's recommendations for the thermal cycling conditions; UDG activation: 50° - 2 min, Dual-Lock DNA polymerase: 95°- 2 min, Denaturation: 95° - 15 sec, Anneal/Extend: 60° - 1 min, a total of 40 cycles. Real-time qPCR was carried out using the BioRad CFX-Maestro system (BioRad, Hercules, CA). Primer sequences are provided in **Table-1.** Expression data were normalized to RPL4 or HPRT1. Part of the samples was processed and analyzed by Novogene for RNA sequencing.

c. Left-Ventricular Extracts (Human Tissue)

Human left ventricular tissues were crushed using liquid nitrogen to make powdered samples. TRIzol was added to the powdered tissue for further homogenization and RNA extraction. Total RNA was isolated using a Direct-zol RNA MiniPrep kit (Zymo research 11-331) following the user's protocol. Dnase treatment step was excluded. Total RNA was eluted in nuclease free water and concentration was measured using a nanodrop. Then 500 ng of total RNA was used to construct cDNA using reverse-transcriptase (iScript™ Reverse Transcription mix, BIO-RAD #1708841).

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Protein Extraction

a. AC16 Cells

Whole-cell extracts were isolated using a mixture containing a final concentration of 1X RIPA Lysis Buffer supplemented with 1X Protease and Phosphatase Inhibitor Cocktail. After the cells were lysed, they were incubated for 30 mins on a tube rotator at 4°C. The cells were then centrifuged at 16000 Rcf for 20 mins at 4°C and the supernatant was collected. The concentration of the isolated proteins was determined using Pierce™ BCA Protein Assay Kit.

b. Left-Ventricular Extracts (Mouse Tissue)

Total protein from mouse left ventricular sections was isolated using a mixture containing a final concentration of 1X RIPA Lysis Buffer supplemented with 1X Protease and Phosphatase Inhibitor Cocktail. After the cells were lysed, they were then incubated for 120 mins on a tube rotator at 4°C. The concentration of the isolated proteins was determined using Pierce™ BCA Protein Assay Kit.

c. Left-Ventricular Extracts (Human Tissue)

Human left ventricular tissues were crushed using liquid nitrogen and further resuspended in freshly prepared lysis buffer (RIPA buffer with 1% CHAPS, phosphatase inhibitor – phos stop, protease inhibitor – complete mini protease inhibitor cocktail, 20 mM sodium fluoride (NaF), and 1 mM sodium orthovanadate (Na3VO4)). These homogenized samples in lysis buffer were sonicated 3 times for 1 sec each at 4°C followed by centrifugation at 18,000g for 20 mins to collect the supernatants as total lysates. The concentration of these lysates was measured using a nanodrop.

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Immunoblotting

6-10 µg of protein were separated on Stain Free Mini-PROTEAN® TGX™ Precast Gels for 40-45 mins at 180V. Afterward, the gels were imaged to assess protein integrity and concentration using the stain-free gel option on BioRad ChemiDoc MP Imaging System (BioRad, Hercules, CA). Electrophoretic transfer to 0.2 µm low fluorescence PVDF membranes was performed using the Trans-Blot® Turbo™ Transfer System (BioRad, Hercules, CA). For high molecular weight proteins (AKAP12), we used the high-molecular transfer option [10 mins] while for other proteins turbo option was used [7 mins]. Membranes were then blocked with 5% BSA in Tris Buffered Saline containing 0.1% Tween-20 (0.1% TBST) for 1 hour at room temperature. Subsequently, we incubated the membranes overnight at 4°C with primary antibodies diluted in 5% BSA in 0.1% TBST, unless otherwise stated. Prior to secondary antibodies incubation, membranes were washed 3 times with 0.1% TBST, each wash for 5 mins, shaking at room temperature. Secondary antibodies were freshly prepared at 1:1000, 5% BSA in 0.1% TBST, and membranes were incubated for 1 hour shaking at room temperature. Next, the membranes were washed 3 times with 0.1% TBST, each washed for 5 mins, shaking at room temperature. Total protein levels were visualized using the stain-free blot feature on BioRad ChemiDoc MP Imaging System [Auto optimal with membrane activation time 45 sec]. Finally, membranes were developed using the SuperSignal West Pico PLUS Chemiluminescent Substrate, by incubating it for 2 mins in the dark. BioRad ImageLab Software was used for the band intensity analysis and was normalized to total protein expression levels.

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Glosensor cAMP Assays

AC16 cells were seeded into 96-well microplates at a density of 2.0×10^4 cells/well. The next day, cells were transfected with 50 ng of β2AR plasmid and 50 ng of pGlo22F plasmid. Forty-eight hours post-transfection, cells were incubated in an equilibration medium (Optimem) with a 1% v/v Glosensor cAMP reagent. After 2 hours of incubation, baseline readings were performed at 37°C once every 1-2 minutes for 10 mins. Next, cells were treated with a vehicle or agonist (10 μM EPI or 25 μM FSK) in the presence or absence of one of the PDE inhibitors: Rolipram; selective PDE4 inhibitor, PF-04957325; selective PDE8 inhibitor, or IBMX; non-selective PDE inhibitor. All pretreatments with PDE inhibitors were 30 mins prior to measuring baseline values. Luminescence measurements were performed once every 1-2 minutes for an additional 60 mins using Synergy H1 Multi-Mode Microplate Reader (BioTek, Winooski, VT, USA).

Immunocytochemistry

Isolated cardiomyocytes were fixed in 4% formaldehyde for 20 min at room temperature and then washed with 1x PBS three times for 5 min each. Cells were overlaid with permeabilization reagent PBST (1XPBS + 0.2% Tween) for 30 min at 37 C then kept at room temperature for 10 min. Cells were blocked with blocking buffer (TNB) for 30 min at room temperature (Perkin Elmer, FP1020, Waltham, MA, USA) then they were incubated in primary antibodies at concentration 1:100 overnight at 4^{o}C (anti-AKAP12(Gravin), Sigma Aldrich #G3795, PDE8A, Protientech # 13956-1AP). The next day cells were washed with 1X PBS 3 times 5 min each then incubated with secondary antibodies at concentration 1:500 (antimouse Alexa Fluor 488 conjugate to detect AKAP12 and antirabbit Alexa Fluor 568 conjugate to detect PDE8A signal, life technologies # A11001, A10042) for one hour. Finally, cells were counterstained and then mounted using mounting media (Vectashield, H-1000-10, Burlingame, CA, USA). Confocal

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images were obtained using the Leica TCS SP8 confocal system. Colocalization analysis was performed using the ImageJ plugin; Colocalization Finder.

WGA Staining

Adult hearts were harvested and then fixed in 4% PFA overnight at 4°C. Samples were washed with 1XPBS 3 times for 10 mins each. Samples were embedded in OCT and then kept at -80°C overnight. Sectioning was done using cryostat at 10 μm thickness then kept at -80°C overnight before staining. The sections were washed with 1XPBS to remove OCT and then incubated for one hour at room temperature with WGA (wheat germ agglutinin) Alexa Fluor 488 conjugate. Molecular Probe, Life Technology, Cat.# W11261. The sections were then counterstained and mounted using mounting media (Vectashield, H-1000-10, Burlingame, CA, USA). ImageJ software was used to measure the cross-sectional area of cells.

Competitive ELISA cAMP Assay

Intracellular basal cAMP levels in isolated primary cardiomyocytes were assessed using the cAMP Assay Kit (Competitive ELISA, Colorimetric – ab290713) in technical duplicates. Isolated cardiomyocytes were promptly flash frozen before storage at -80°C immediately following isolation. To facilitate further analysis, the frozen tissue samples were ground into a fine powder under liquid nitrogen in a stainless-steel mortar. After evaporating the liquid nitrogen, the powdered samples were weighed in Eppendorf tubes and homogenized in 10 volumes of 0.1M HCl, with the volume determined by the sample weight (e.g., Sample 1 with a weight of 0.01758g was mixed with 0.1758 mL of 0.1M HCl). The homogenization step aimed to ensure uniformity across the samples. Subsequently, the homogenized samples underwent centrifugation at 12,000g, allowing debris to be pelted while collecting the supernatant for further analysis using the acetylation format protocol. Considering the possibility of low concentration due to the continued

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activity of endogenous phosphodiesterases during tissue digestion, the acetylation format was chosen for its enhanced sensitivity over the non-acetylation protocol. As per the protocol's requirements, the supernatant was diluted at a ratio of 1:32 in 0.1M HCl to boost assay sensitivity. This involved adding 10 μL of supernatant to 320 μL of 0.1M HCl, resulting in a total volume of 330 μL for each sample.

Statistical Analysis

Data were processed using Microsoft Excel (RRID: SCR_016137), Vevo LAB 5.6.1, Image Lab, IonWizard 7.7.1, CytoSolver 3.0, ImageJ, and GraphPad Prism 8.2.1. (RRID: SCR_002798). All values are reported as the Mean ± S.E.M. Numeric data were first analyzed for normality using the Shapiro-Wilk test. Data with parametric distribution were analyzed by unpaired two-tailed Student's T-test and 1-way ANOVA and Holm-Sidak or Tukey post hoc multiple comparisons test were used. When significant departures from normality were observed by the Shapiro-Wilk test, nonparametric tests were used. For echocardiograms, 2-way ANOVA and Sidak post hoc multiple comparisons test were used. P values of less than 0.05 were considered significant. Representative images and figures were chosen based on their proximity to the mean/average for each group.

Blinding

Mice genotypes were blinded during echocardiography acquisition and analysis. Human samples were blinded until both RT-qPCR and Western Blot analysis were completed.

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Table-1: Resources Table

Antibodies

Target Antigen	Vendor or Source	Catalog #	Working concentration
AKAP12 – C-terminal *Used for western blots*	Abcam Inc. Cambridge, UK	ab204559	1:1000
AKAP12 - Anti-Gravin Antibody, Mouse Monoclonal *Used for ICC*	Sigma-Aldrich	G3795	1:100
PDE8A Antibody Polyclonal *Used for western blots*	Thermo Fisher Scientific™, Waltham, MA, USA	PA5-88405	1:1000
PDE8A Polyclonal antibody *Used for ICC*	ProteinTech	13956-1-AP	1:100
WGA (wheat germ agglutinin) Alexa Fluor 488 conjugate *Used for ICC*	Thermo Fisher Scientific™, Waltham, MA, USA	W11261	100μg/ml
troponin I Rabbit Ab *Used for western blots*	Cell Signaling, Danvers, MA, USA	4002S	1:1000
p-troponin I(cardiac)(S23/24) Rabbit Ab *Used for western blots*	Cell Signaling, Danvers, MA, USA	4004S	1:1000
Anti-rabbit IgG, HRP-linked Antibody *Used for western blots*	Cell Signaling, Danvers, MA, USA	7074	1:1000
Anti-mouse IgG, HRP-linked Antibody *Used for western blots*	Cell Signaling, Danvers, MA, USA	7076	1:1000
Alexa Flour 488 goat anti-mouse *Used for ICC*	Invitrogen, life technologies	A11001	1:500
Alexa Flour 568 donkey anti-rabbit *Used for ICC*	Invitrogen, life technologies	A10042	1:500

DNA/cDNA Clones

Clone Name	Sequence	Source / Repository	Persistent ID / URL
Human AKAP12 (hAKAP12)	HG18215-UT	Sino Biologicals, Wayne, PA, USA	https://www.sinobiological.com/cdna-clone/human-akap12-hg18215-ut

Cultured Cells

Name	Vendor or Source	Persistent ID / URL
AC16 Human Cardiomyocyte Cell Line[56]	Millipore Sigma	https://www.sigmaaldrich.com/US/en/product/mm/scc109?gclid=CjwKCAiAqNSsBhAvEiwAn_tmxY5Ra9lYHp0xTVteqErJ2Ou1pAzyrv0AV1aVeEJzUsWowUB9IpMaQBoCHNMQAvD_BwE

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
RNAseq Data	data will be available using the online *figshare* data repository	
Raw data files and Prism analysis files organized by figure panels	data will be available using the online *figshare* data repository	

Other

Description	Source / Repository	Catalog Number
Reagents and Chemicals		
Dulbecco's modified Eagle's medium (DMEM) high glucose, pyruvate	Life Technologies, Grand Island, NY, USA	11-995-073
Fetal Bovine Serum (FBS)	Life Technologies, Grand Island, NY, USA	26-140-079
Antibiotic-Antimycotic (100X)	Life Technologies, Grand Island, NY, USA	15-240-062
DPBS, no calcium, no magnesium	Life Technologies, Grand Island, NY, USA	14-190-235
Opti-MEM™ I Reduced Serum Medium	Life Technologies, Grand Island, NY, USA	31-985-070
Bovine Serum Albumin	Sigma Aldrich, St. Louis, MO, USA	A7906-100G
10X RIPA Lysis Buffer	MilliporeSigma, Burlington, MA, USA	20-188
Paraformaldehyde Solution, 4% in PBS	Thermo Fisher Scientific™, Waltham, MA, USA	AAJ19943K2

Triton™ X-100	Sigma Aldrich, St. Louis, MO, USA	T8787
TWEEN® 20	Sigma Aldrich, St. Louis, MO, USA	P9416
ViaFect™ Transfection Reagent	Promega, Madison, WI, USA	E4982
Duolink® In Situ PLA® Probe Anti-Mouse MINUS	MilliporeSigma, Burlington, MA, USA	DUO92004
Duolink® In Situ PLA® Probe Anti-Rabbit PLUS	MilliporeSigma, Burlington, MA, USA	DUO92002
Duolink® In Situ Detection Reagents Red	MilliporeSigma, Burlington, MA, USA	DUO92008
PowerUp™ SYBR™ Green Master Mix	Thermo Fisher Scientific™, Waltham, MA, USA	A25776
Halt™ Protease and Phosphatase Inhibitor Single-Use Cocktail (100X)	Thermo Fisher Scientific™, Waltham, MA, USA	78442
SuperSignal West Pico PLUS Chemiluminescent Substrate	Thermo Fisher Scientific™, Waltham, MA, USA	34577

Kits		
Description	**Source / Repository**	**Catalog Number**
Trans-Blot Turbo RTA Mini 0.2 µm PVDF Transfer Kit	Bio Rad, Hercules, CA, USA	1704272
Stain Free Mini-PROTEAN® TGX™ Precast Gels	Bio Rad, Hercules, CA, USA	4568026
Pierce™ BCA Protein Assay Kit	Thermo Fisher Scientific™, Waltham, MA, USA	23225
PureLink® RNA Mini Kit	Life Technologies, Grand Island, NY, USA	12-183-020
SuperScript IV VILO Kit	Thermo Fisher Scientific™, Waltham, MA, USA	11-756-050
ELISA cAMP assay	Abcam	ab290713

Drugs		
Description	**Source / Repository**	**Catalog Number**
(-) – Epinephrine [RRID: SCR_003281]	Sigma Aldrich, St. Louis, MO, USA	E4250-10G

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Forskolin	Sigma Aldrich, St. Louis, MO, USA	F3917-10MG
PF-04597325	Thermo Fisher Scientific™, Waltham, MA, USA	50-202-9122-1MG
IBMX	Sigma Aldrich, St. Louis, MO, USA	I5879
Rolipram	Sigma Aldrich, St. Louis, MO, USA	R6520
PTX	Thermo Fisher Scientific™, Waltham, MA, USA	PHZ1174
ICI-118551	Sigma Aldrich, St. Louis, MO, USA	I127-25MG
CGP-20712A	Sigma Aldrich, St. Louis, MO, USA	C231-10MG

RTqPCR Primers – Human		
Name	**Forward Primer**	**Reverse Primer**
AKAP12	GCTGGACAGGAAACGGAGAA	CACTGCGGTTGACTCTGACT
PDE1A	AGGTCACTTCCAGCAAATTA	CCACATAGGAAGAAGTTTCG
PDE1C	AGATATTAGCCATCCAGCAA	CAACGGAGATGACAGAAT
PDE2A	CCTTCAACAAGCTAGAAGGA	CGGATCTCATAGCTCTCATC
PDE3A	CAACACTGTGTGTGTGTGTG	CAAGTGGTGCATAGCAGTAA
PDE4A	GCTGAAGACCTCATCGTAAC	ATTCTGTTTGTCCAGGAATG
PDE4C	AGAGTGGTACCAGAGCAAGA	TGGGAGCCACCTATAACTAA
PDE4D	CACCAAATGACCTTACCTGT	AGCTCCACTGTTACCTTTCA
PDE5A	GAAAAGGACTTTGCTGCTTA	TGATTTTGTTTGCATCATGT
PDE7A	GCAATATGAATTTGGCTTTC	GGAAAGAGCTGCAGTCTAAA
PDE7B	TCTTCAATACCCATGGACTC	ATCCTGTGTCATTTCCTTTG
PDE8A	ACCAATGTAATGGATTCTGC	TGAGTTACAAGCCCTGAGTT
PDE8B	AGAACAGGAGGAAAGAGTCC	CGTTTCCTGACAGTCTTCTC
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
HPRT1	CTTGAGCACACAGAGGGCTACA	CATTATGCTGAGGATTTGGAAAG
RTqPCR Primers – Mouse		
Name	**Forward Primer**	**Reverse Primer**
AKAP12	GTCCAAGAGGAAAGCCAGGATG	CTGTGGAACTGGCTGCCATTTC
PDE1A	TGACGTCACTCAAACTGTGCATT	CCATGGCTAAAATTTCCAGTTCA
PDE1C	GAAGACAGCCCTGCAGCAA	GTGTAACATGAGGGATAAGGCTTTC
PDE2A	ATGAGCTGCCACAGGAAGGA	ATTGCAACTCAGCCGCTTCT
PDE3A	TTCAGAATGGGACCACAAGAGA	TCACCCATCACAGCAATATCCA
PDE3B	GGGACTTGAAGCAGTGGTGTAAA	AGCACTGAAAGATCAACTCCATTTC
PDE4D	CCTAACTAATTCATGTATCCCCAGGTT	GGCCCCACTTGTTCACATCT
PDE5A	AATACCACCCCTGGAGCACC	TTCAAGGGCTCGCCAAAAGC
PDE8A	TCAGAGTGTGCAATGGCAAC	GTCCATCGAATGTTTCCTCC

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AC5	CACCGCCAATGCCATAGAC	CTTCAGCGCCACCTTGGT
AC6	CTTCATCTGTTTTATCCAGCTCCTT	CGGCATAAATCCCGAGTATCA
AC8	GGATCTACATCCATCGCTATGAGA	CGTGGAGAGGTTGGTAAATCCT
HPRT1	GCTTGCTGGTGAAAAGGACCTCTCGAAG	CCCTGAAGTACTCATTATAGTCAAGGGCAT
RPL4	GCCGCTGGTGGTTGAAGATAA	CGTCGGTTTCTCATTTTGCCC
Genotyping Primers – Mouse		
Name	**Forward Primer**	**Reverse Primer**
AKAP12 (OX)	ACTTGCGGTTTCTGATAGTTCTGA	CAGAGATGAGTTTCTGCTCGCTAG

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SUPPLEMENTAL FIGURES

Figure-S1:

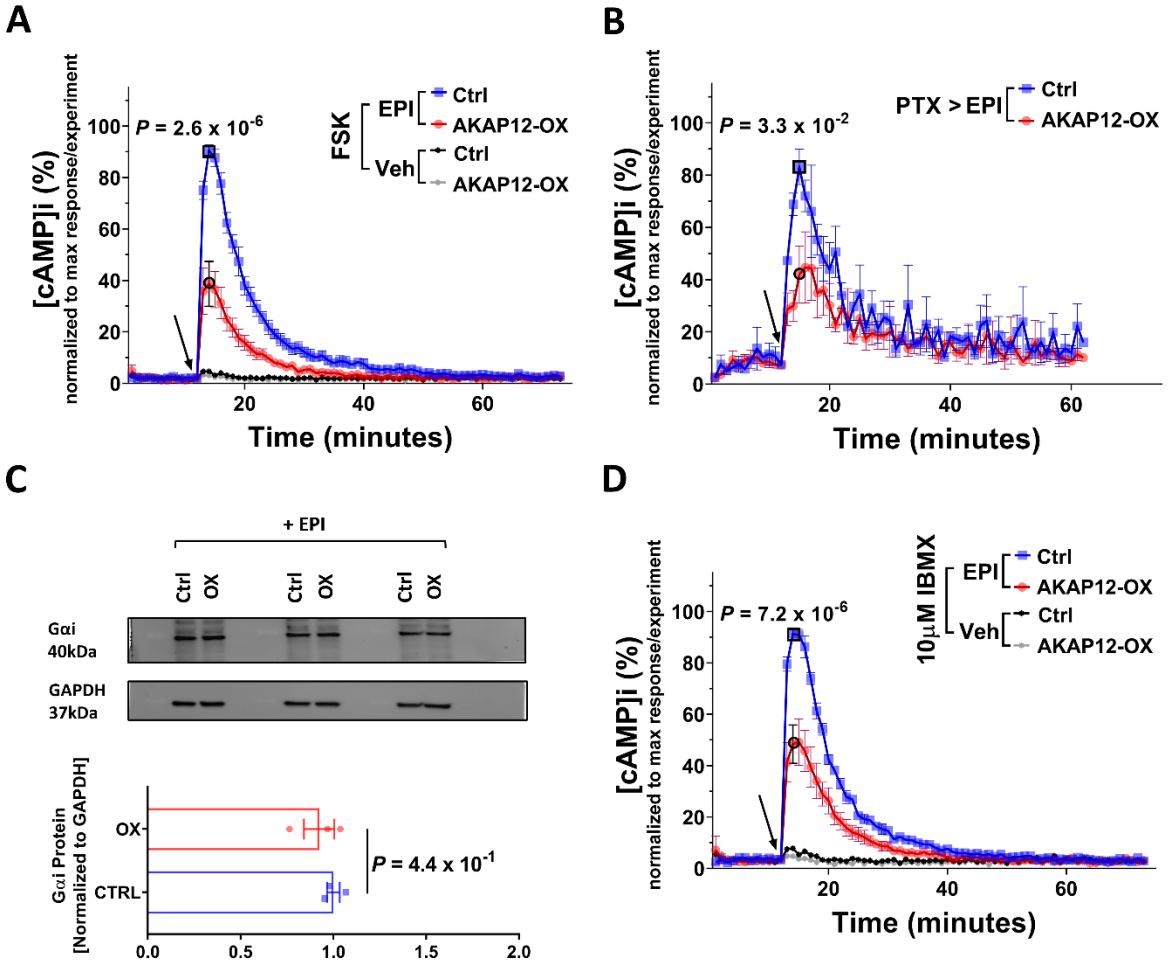

Figure-S1: Reduced intracellular cAMP levels in the AKAP12-OX group are not due to impaired Gαs pathway or higher Gαi pathway activity. A, Gαs pathway activity was assessed using Glosensor Luciferase cAMP assay in response to 25μM Forskolin (FSK), showing a significantly lower response in the AKAP12-OX group as compared to controls. **B,** Pre-treatment overnight with 50nM Pertussis Toxin (PTX); Gαi inhibitor, showed significantly lower intracellular cAMP levels in AKAP12-OX AC16 cells as compared to controls; cells were normalized to the vehicle. **C,** Western blots of Gαi protein expression level in AKAP12-OX AC16

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cells and controls treated with 10μM Epinephrine (EPI); non-selective βARs. **D,** Pre-treatment with 10μM IBMX for 30 mins showed significantly lower intracellular cAMP levels in AKAP12-OX AC16 cells as compared to controls. All data represented as average Mean±S.E.M. *panels A and D*; n=5 and *panel B* n=3. All experiments were performed as technical duplicates. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.05 (AKAP12-OX Veh in *panel D* had non-parametric distribution). Data were analyzed using two-way ANOVA followed by Sidak multiple comparisons post hoc test at the point of max response for *panels A and D*. Data in *panel B* was compared using unpaired 2-tailed Student *t*-test, at the point of max response. Data in *panel C* was compared using unpaired 2-tailed Student *t*-test. The point of max response has black borders. The arrow indicates the start of EPI, FSK, or vehicle (Optimem) addition. Veh: Vehicle, EPI: Epinephrine, FSK: Forskolin. AKAP12-OX is represented by the *red color*; Control is represented by the *blue color.*

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Figure-S2:

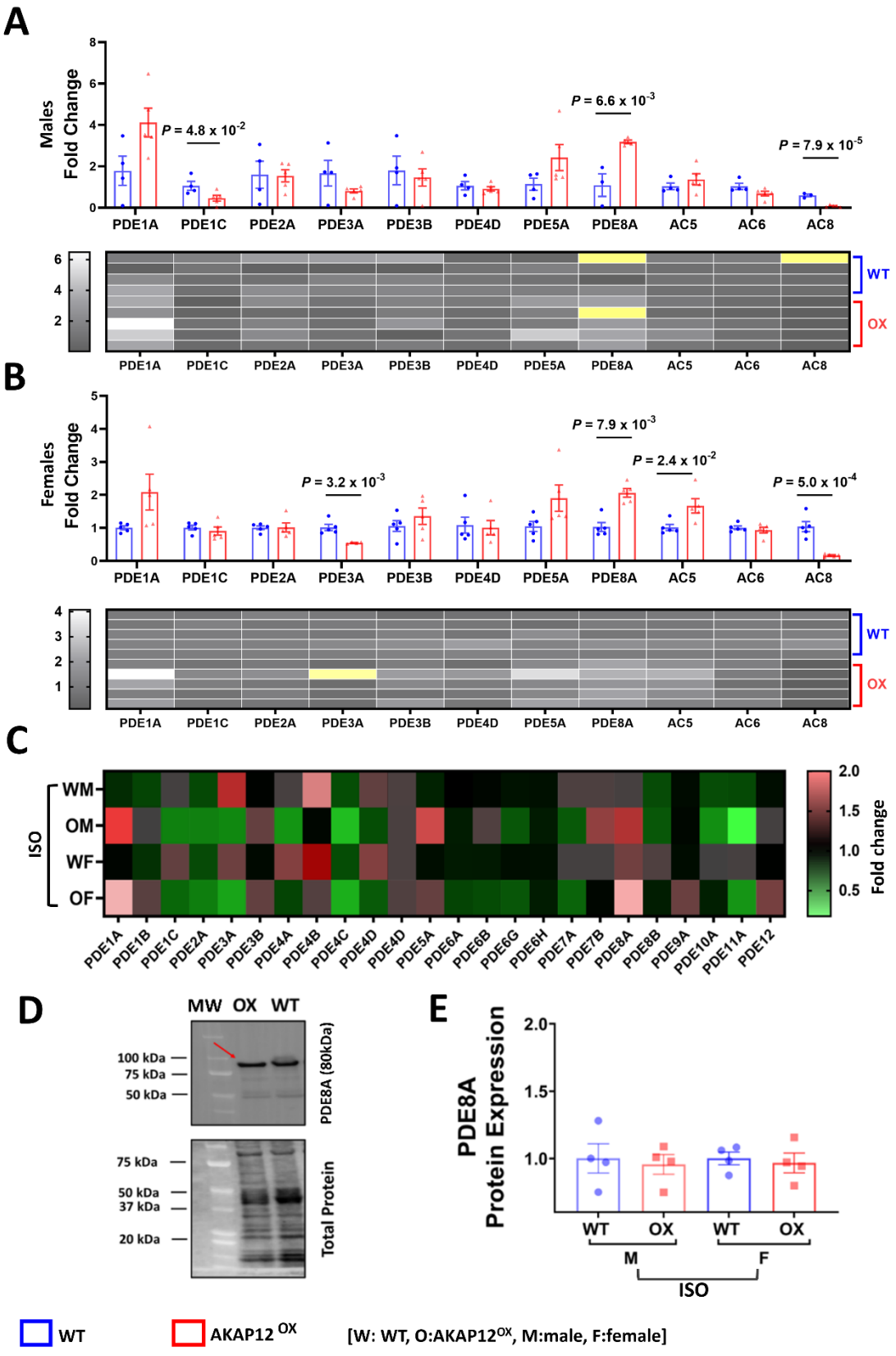

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Figure-S2: AKAP12^{OX} in mouse hearts upregulates PDE8A gene expression without affecting PDE8A protein levels. A, Quantification of gene expression levels from RTqPCR data of several PDEs and ACs in male AKAP12^{OX}; N=5 and WT; N=4 mice. **B,** Quantification of gene expression levels from RTqPCR data of several PDEs and ACs in female AKAP12^{OX} and WT mice, N=5 in each group. **C,** Heat map of PDE gene expression (average fold change), assessed in LV extracts from AKAP12^{OX} and WT male and female mice (8-12 weeks old) in the absence of ISO treatment (Sham) and after 14-days post-ISO treatment (ISO); N=3 in each group. **D,** Representative PDE8A western blot from LV extracts from WT and AKAP12^{OX} mice. **E,** Quantification of PDE8A protein levels; fold change, N=4 in each group. All data represented as average Mean±S.E.M. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.05 and were analyzed using unpaired 2-tailed Student *t*-test for *panels A and B*, except *panel A*; PDE8A group had nonparametric distribution, and data were compared with Mann-Whitney U test. In *panels A and B*, gene expression was normalized to RPL4 (yellow rectangles are excluded outliers), detected using the ROUT method; Q=2%. 2-way ANOVA followed by Tukey multiple comparisons post hoc test was used to compare data in *panel E*. AKAP12-OX is represented by the *red color*; Control is represented by the *blue color.*

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Figure-S3:

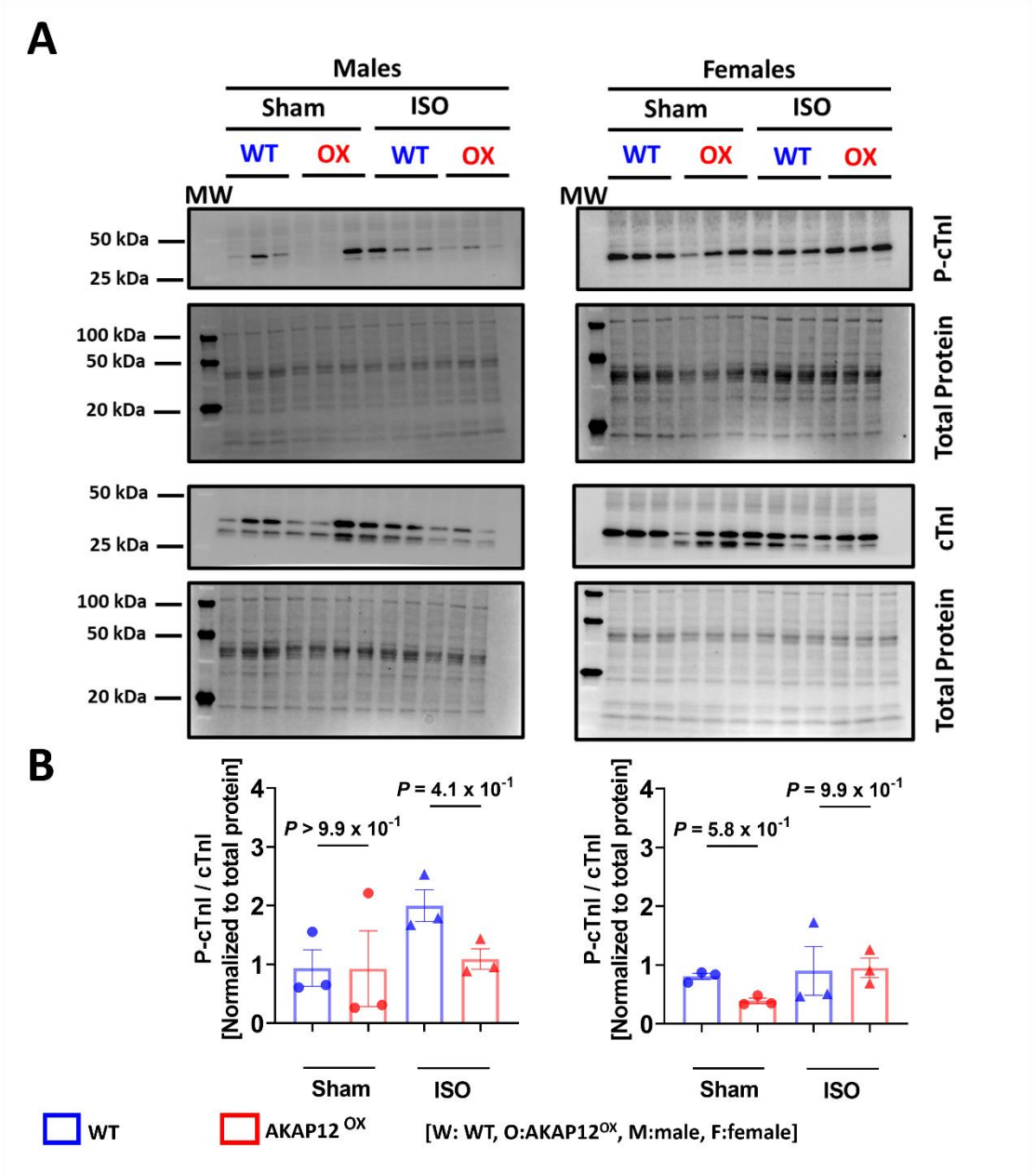

Figure-S3: Cardiac AKAP12^{OX} does not affect phosphorylation of cardiac troponin-I. A, Total cardiac troponin (cTnI) and Phospho cardiac troponin-I (P-cTnI) expression levels in left ventricular extracts from AKAP12^{OX} and WT male and female mice; sham (no ISO treatment) vs ISO (60mg/kg/day for 14 days). **B,** Quantification of P-cTnI represented as ratio P-cTnI/cTnI after normalizing each protein to total protein for each blot. All data represented as average Mean ±

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S.E.M. N=3 in each group. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.05 (AKAP12^{OX} male sham group had non-parametric distribution). Data were analyzed using two-way ANOVA followed by Tukey multiple comparisons post hoc test. AKAP12-OX is represented by the *red color*; Control is represented by the *blue color.*

Figure-S4:

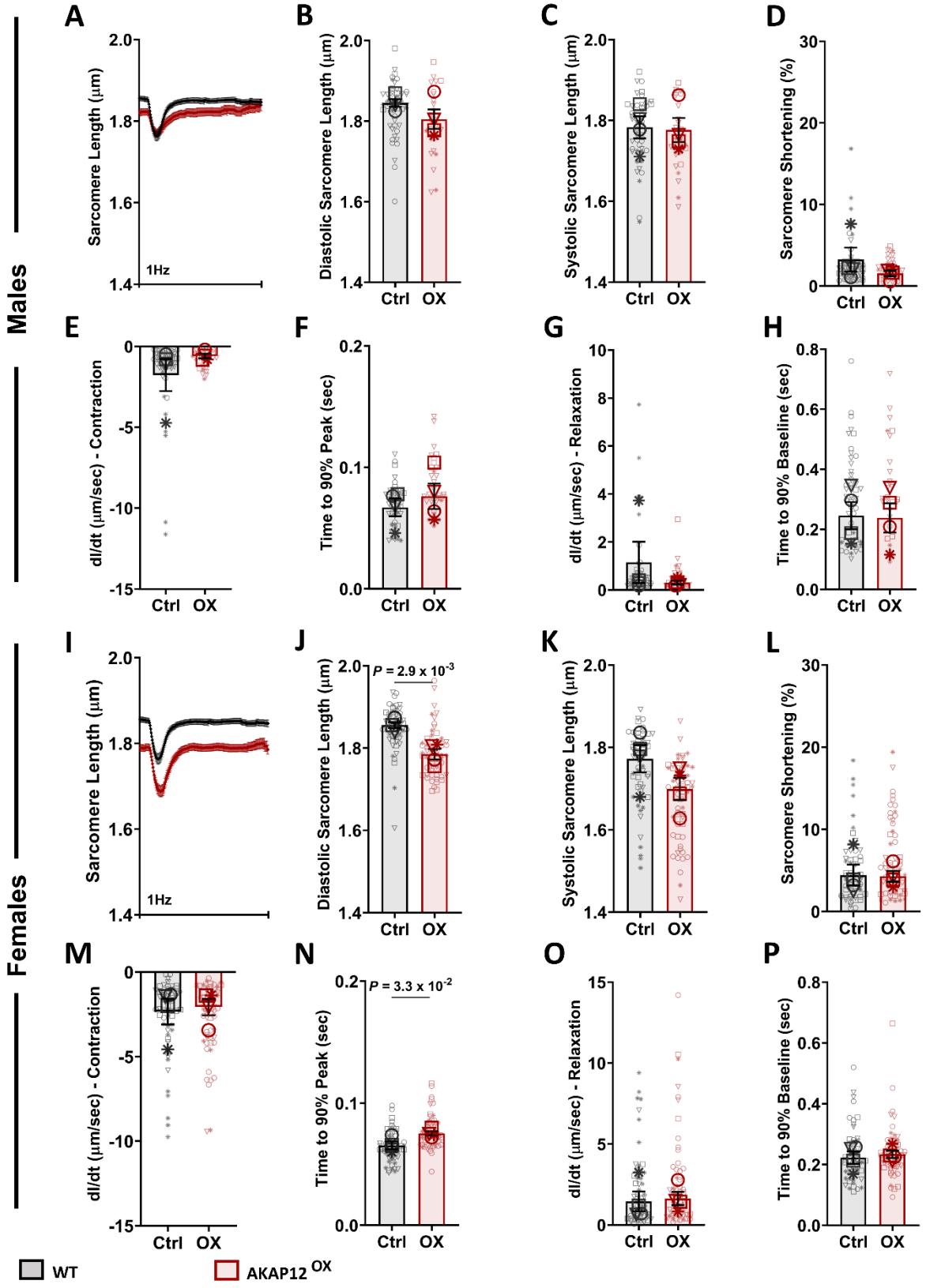
Males
Females
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Sarcomere Length (µm)
1Hz
Diastolic Sarcomere Length (µm)
Systolic Sarcomere Length (µm)
Sarcomere Shortening (%)
dl/dt (µm/sec) - Contraction
Time to 90% Peak (sec)
dl/dt (µm/sec) - Relaxation
Time to 90% Baseline (sec)
P = 2.9 x 10-3
P = 3.3 x 10-2
Ctrl
OX
WT
AKAP12 OX

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Figure-S4: Overexpressing AKAP12 in primary adult cardiomyocytes does not affect sarcomere shortening (%) at basal levels. A, Average tracings of sarcomere length from all primary adult cardiomyocytes isolated from male mice (8-12 weeks old) at basal levels, before being acutely treated with 100nM ISO. **B-H,** Quantification of contractility parameters among the $AKAP12^{OX}$ and WT males, n=50 in WT and n=31 in $AKAP12^{OX}$. Comparison of paced cells parameters measured includes (**B**) diastolic sarcomere length (μm), (**C**) systolic sarcomere length, (**D**) sarcomere shortening (%), (**E**) dl/dt contraction (μm/sec), (**F**) time to 90% peak (sec), (**G**) dl/dt relaxation (μm/sec), and (**H**) time to 90% baseline (sec). **I**, Average tracings of sarcomere length from all primary adult cardiomyocytes isolated from female mice (8-12 weeks old) at basal levels, before being acutely treated with 100nM ISO. **J-P,** Quantification of contractility parameters among the $AKAP12^{OX}$ and WT females, n=57 in WT and n=60-61 in $AKAP12^{OX}$. Comparison of paced cells parameters measured includes (**J**) diastolic sarcomere length (μm), (**K**) systolic sarcomere length, (**L**) sarcomere shortening (%), (**M**) dl/dt contraction (μm/sec), (**N**) time to 90% peak (sec), (**O**) dl/dt relaxation (μm/sec) and (**P**) time to 90% baseline (sec). All data represented as average Mean±S.E.M of *median values for each animal* except *panels A and I*, where the mean of individual cells was used to represent contraction trends. N=4 in each group. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.005 and were analyzed using an unpaired 2-tailed Student t-test. AKAP12-OX is represented by the *dark red color*; Control is represented by the *grey color.* ‡‡

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Figure-S5:

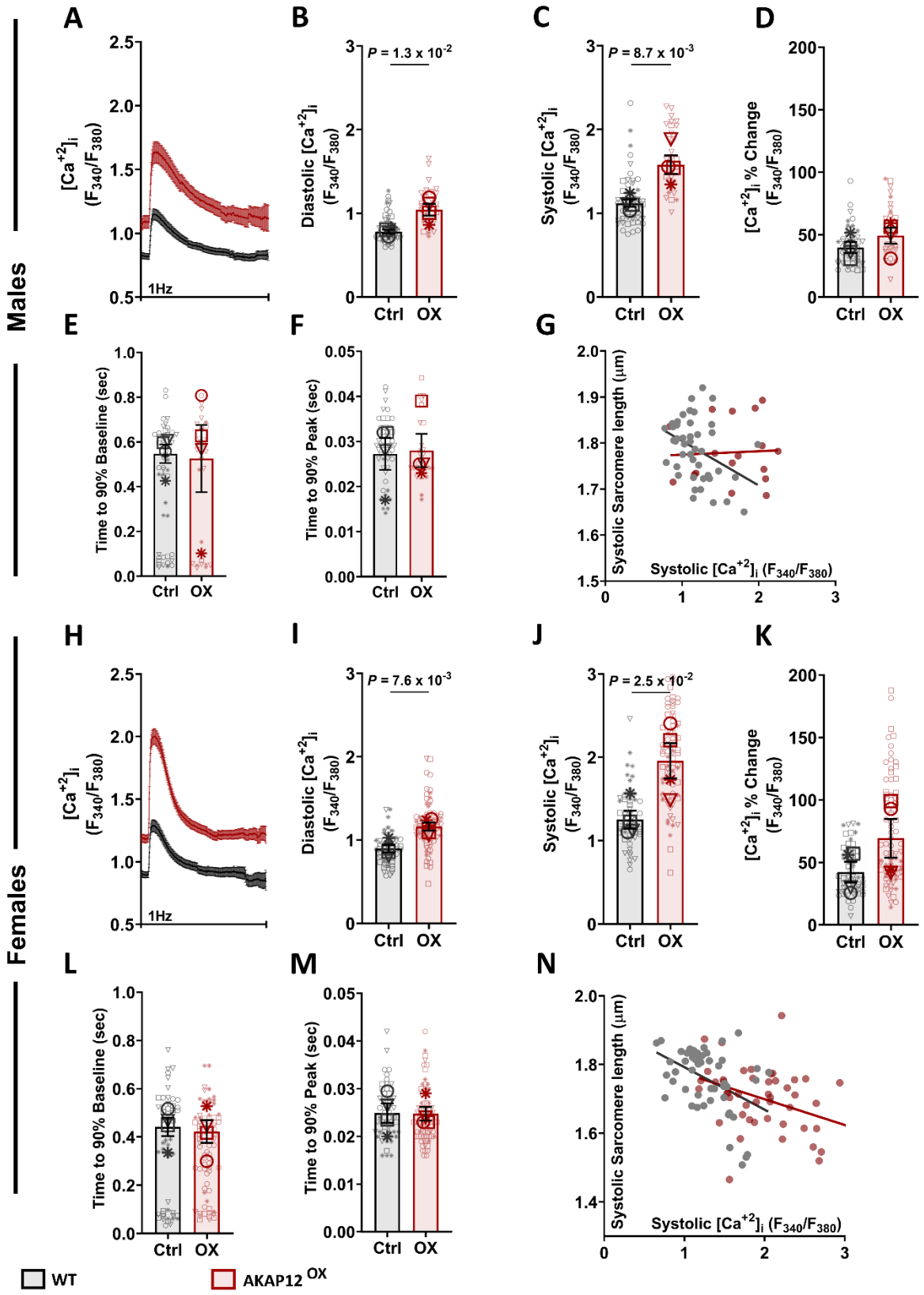

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Figure-S5: Overexpressing AKAP12 in primary adult cardiomyocytes significantly increases intracellular calcium at basal levels. A, Average tracings of $[Ca^{2+}]i$ represented by Fura-2 fluorescence ratio (340nm/380nm) from all primary adult cardiomyocytes isolated from male mice (8-12 weeks old) at basal levels, before being acutely treated with 100nM ISO. **B-F,** Quantification of $[Ca^{2+}]i$ and calcium kinetics among the $AKAP12^{OX}$ and WT males; n=51-52 in WT and n=30 in $AKAP12^{OX}$. Comparison of paced cells parameters measured includes (**B**) diastolic $[Ca^{2+}]i$ (F_{340}/F_{380}), (**C**) systolic $[Ca^{2+}]i$ (F_{340}/F_{380}), (**D**) $[Ca^{2+}]i$ change (%), (**E**) time to 90% baseline (sec), and (**F**) time to 90% peak (sec). **G,** Scatter plot of systolic $[Ca^{2+}]i$ (F_{340}/F_{380}) and systolic sarcomere length (μm). **H,** Average tracings of $[Ca^{2+}]i$ represented by Fura-2 fluorescence ratio (340nm/380nm) from all primary adult cardiomyocytes isolated from female mice (8-12 weeks old) at basal levels, before being acutely treated with 100nM ISO. **I-M,** Quantification of $[Ca^{2+}]i$ and calcium kinetics among the $AKAP12^{OX}$ and WT females; n=55-56 in WT and n=80-81 in $AKAP12^{OX}$. Comparison of paced cells parameters measured includes (**I**) diastolic $[Ca^{2+}]i$ (F_{340}/F_{380}), (**J**) systolic $[Ca^{2+}]i$ (F_{340}/F_{380}), (**K**) $[Ca^{2+}]i$ change (%), (**L**) time to 90% baseline (sec), and (**M**) time to 90% peak (sec). **N,** Scatter plot of systolic $[Ca^{2+}]i$ (F_{340}/F_{380}) and systolic sarcomere length (μm). All data represented as average Mean±S.E.M. of *median values for each animal* except *panels A and H* which represent Mean±S.E.M. of all cells. N=4 in each group. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.005 and were analyzed using an unpaired 2-tailed Student t-test. AKAP12-OX is represented by the *dark red color*; Control is represented by the *grey color.* ‡‡.

Figure-S6:

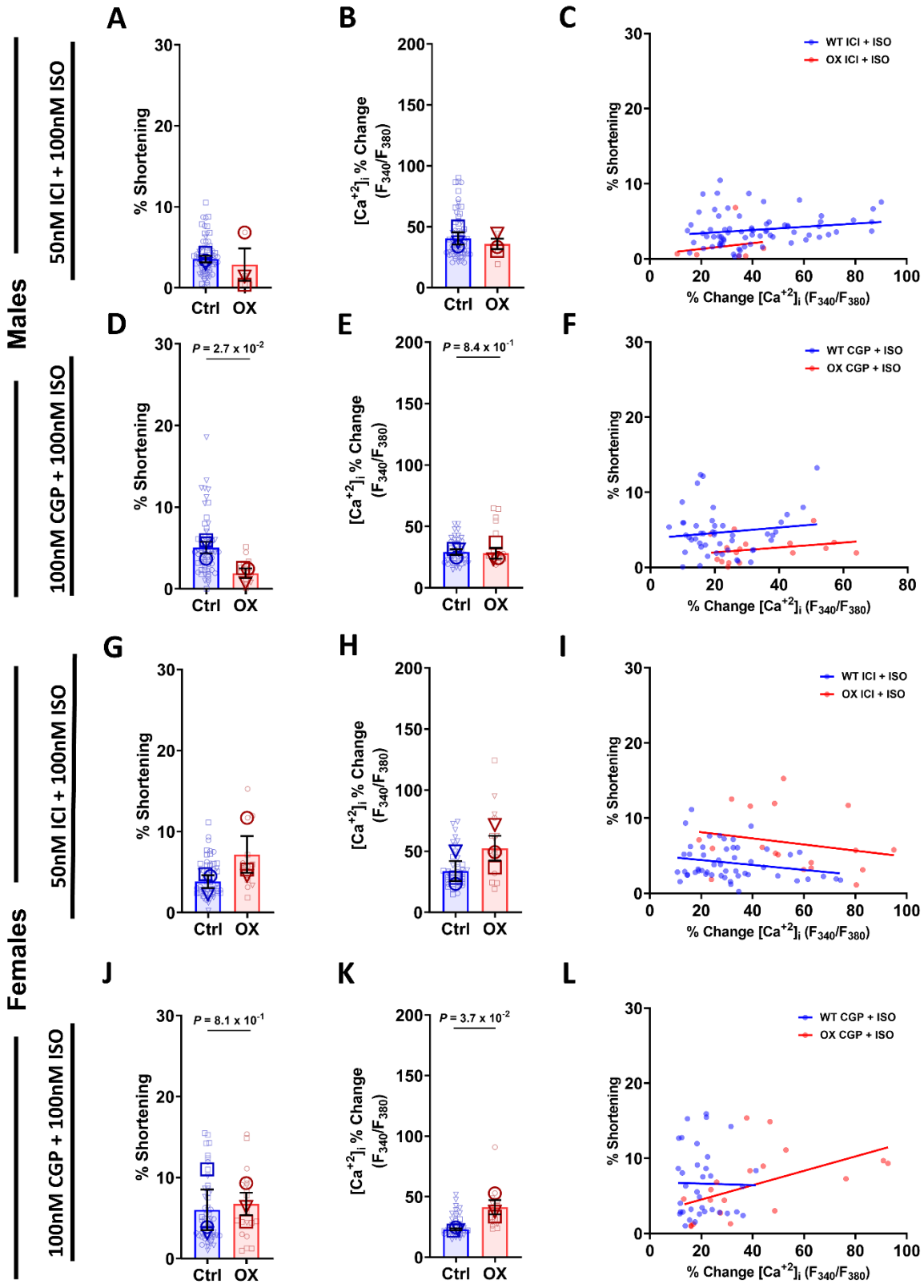
Males
Females
50nM ICI + 100nM ISO
100nM CGP + 100nM ISO
A
B
C
D
E
F
G
H
I
J
K
L
% Shortening
[Ca+2]i % Change (F340/F380)
% Change [Ca+2]i (F340/F380)
Ctrl
OX
P = 2.7 x 10-2
P = 8.4 x 10-1
P = 8.1 x 10-1
P = 3.7 x 10-2
WT ICI + ISO
OX ICI + ISO
WT CGP + ISO
OX CGP + ISO

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Figure-S6: Adult primary cardiomyocytes from AKAP12^{OX} mice have impaired contractility in response to higher intracellular calcium levels downstream β2AR. A-C, Quantification of selective β1AR stimulation (50nM ICI + 100nM ISO) on cardiomyocytes calcium and contractility and their scatter plot among the AKAP12^{OX} and WT males; n=63-73 in WT and n=6 in AKAP12^{OX}. **D-F,** Quantification of selective β2AR stimulation (100nM CGP + 100nM ISO) on cardiomyocytes calcium and contractility and their scatter plot among the AKAP12^{OX} and WT males; n=40-62 in WT and n=14-16 in AKAP12^{OX}. **G-I,** Quantification of selective β1AR stimulation (50nM ICI + 100nM ISO) on cardiomyocytes calcium and contractility and their scatter plot among the AKAP12^{OX} and WT females; n=40-55 in WT and n=15 in AKAP12^{OX}. **J-L,** Quantification of selective β2AR stimulation (100nM CGP + 100nM ISO) on cardiomyocytes calcium and contractility and their scatter plot among the AKAP12^{OX} and WT females; n=35-57 in WT and n=15-21 in AKAP12^{OX}. All data represented as average Mean±S.E.M *of median values per each animal*. N=3 in each group. Data were determined to have a parametric distribution by the Shapiro-Wilk test; α=0.005 and were analyzed using an unpaired 2-tailed Student *t*-test. AKAP12-OX is represented by the *red color*; Control is represented by the *blue color.*

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Figure-S7:

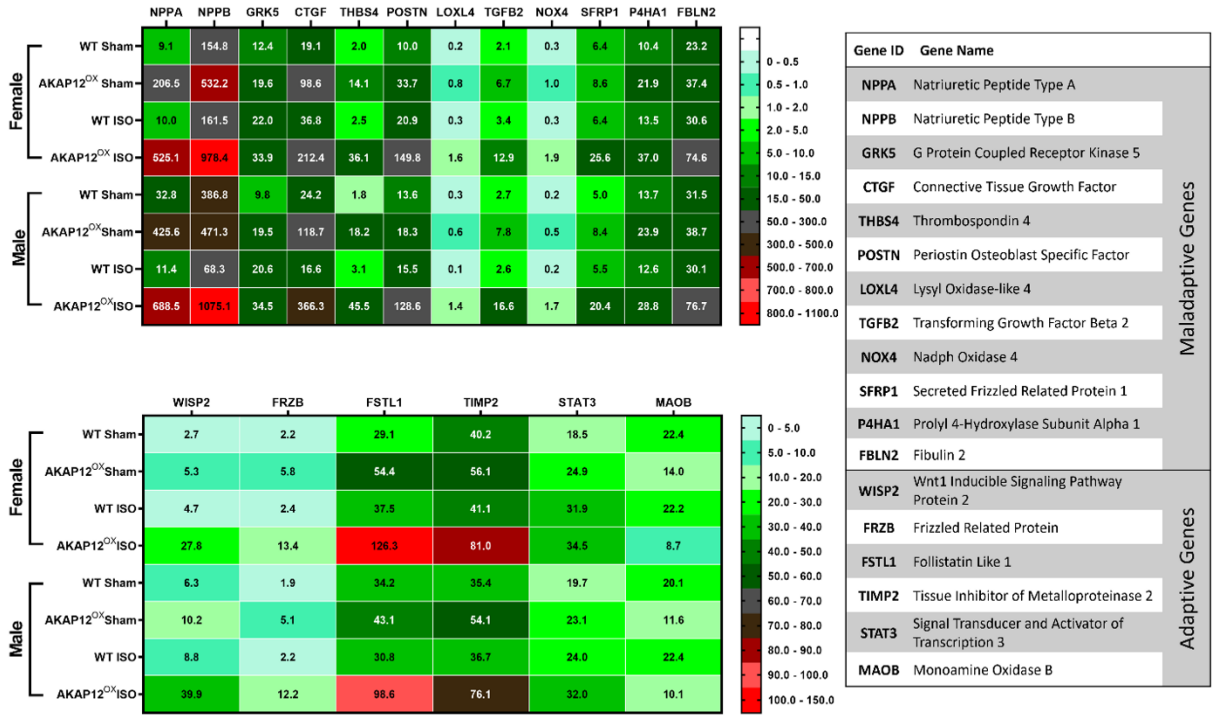

Figure-S7: Cardiomyocytes AKAP12^{OX} upregulates several maladaptive genes in the left ventricle post 14 days of ISO treatment. Heat map of maladaptive and adaptive gene expression (Average FPKM), assessed in LV extracts from AKAP12^{OX} and WT male and female mice (8-12 weeks old) in the absence of ISO treatment (Sham) and after 14-days post-ISO treatment (ISO); N=3 in each group.

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Figure-S8:

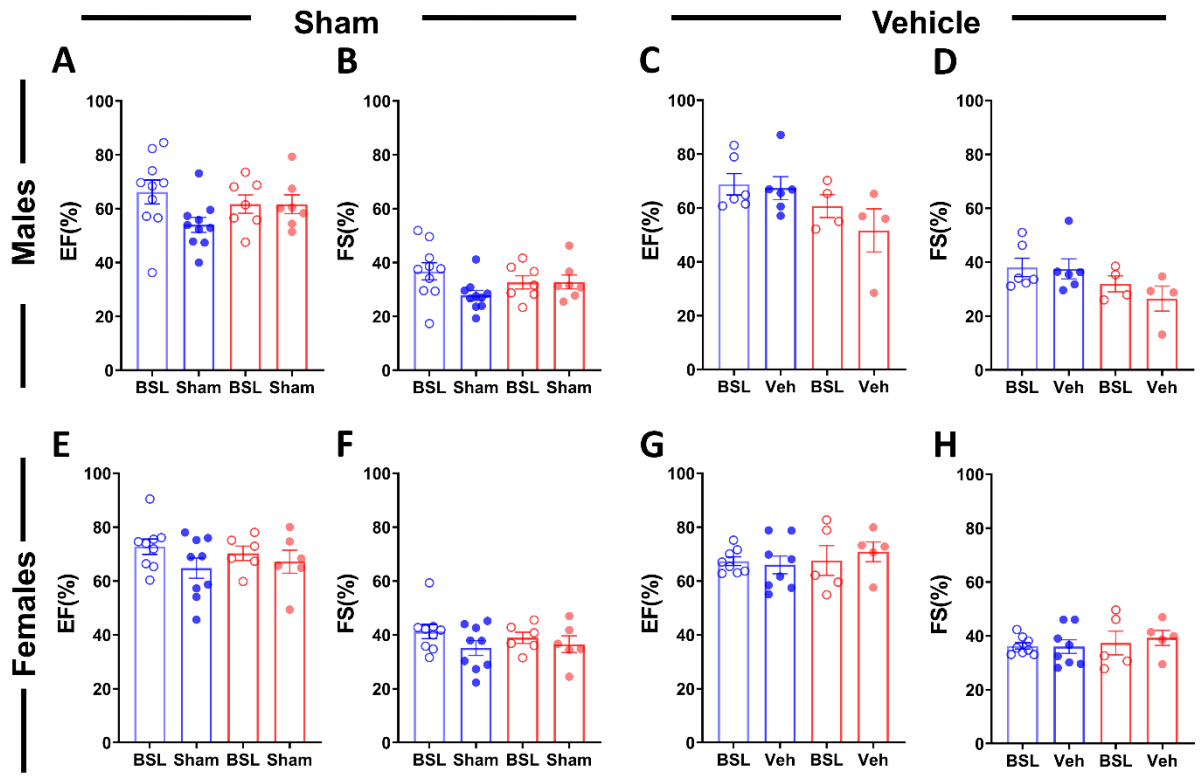

Figure-S8: Systolic function is not different between AKAP12^{OX} and WT mice sham groups or vehicle treated groups. Echocardiographic data showing systolic cardiac function parameters; EF% and FS% in sham groups (**A, B;** males and **E, F;** females) and vehicle treated groups (**C, D;** males and **G, H;** females). All data represented as average Mean±S.E.M.; N=>4 in each group for all panels. Statistical analysis was performed using 2-way ANOVA followed by Sidak multi comparisons post hoc test. *For clarity, this graph focused only on reporting significant p values of genotype effect; AKAP12^{OX} vs WT. Please refer to* ***Supplemental Raw Data D2 and D3*** *for detailed p-values and further group comparisons.*

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SUPPLEMENTAL TABLES

Table-S1: Echocardiographic Parameters of WT and AKAP12^{OX} Males and Females

Group/ Echo Parameter	WT M ISO N=9	AKAP12^{OX} M ISO N=6	P-value Males	WT F ISO N=8	AKAP12^{OX} F ISO N=9	P-value Females
IVS;d	1.23±0.15	1.42±0.11	$4.9x10^{-1}$	1.2±0.13	1.64±0.15	$5.6x10^{-2}$
IVS;s	1.70±0.15	1.75±0.12	$9.6x10^{-1}$	1.58±0.11	1.92±0.16	$1.3x10^{-1}$
HR	496.55±10.36	539.51±12.54	$1.0x10^{-1}$	487.33±12.14	493.96±16.74	$9.5x10^{-1}$
Diameter;s	2.39±0.21	3.16±0.21	$4.2x10^{-2}$	2.62±0.13	2.92±0.12	$2.9x10^{-1}$
Diameter;d	3.68±0.17	4.01±0.13	$3.3x10^{-1}$	3.75±0.11	3.71±0.09	$9.7x10^{-1}$
Volume;s	22.38±3.82	41.43±6.40	$1.4x10^{-2}$	25.8±2.99	33.65±3.34	$1.5x10^{-1}$
Volume;d	58.95±5.82	71.14±5.53	$2.8x10^{-1}$	60.52±4.31	59.13±3.35	$9.7x10^{-1}$
SV	36.57±2.29	29.7±3.45	$1.3x10^{-1}$	34.73±3.77	25.48±2.20	$5.5x10^{-2}$
EF%	65.22±4.07	42.97±5.78	$9.9x10^{-3}$	57.39±3.95	43.89±3.61	$3.5x10^{-2}$
FS%	36.21±3.40	21.38±3.53	$2.8x10^{-2}$	30.17±2.78	21.54±2.00	$5.1x10^{-2}$
CO	18.02±0.89	16±2.01	$4.4x10^{-1}$	16.7±1.49	12.80±1.48	$1.4x10^{-1}$
LVID;d	3.65±0.17	4.03±0.15	$2.5x10^{-1}$	3.73±0.12	3.60±0.09	$7.2x10^{-1}$
LVID;s	2.41±0.22	3.2±0.23	$4.2x10^{-2}$	2.65±0.12	2.88±0.11	$4.7x10^{-1}$
LVPW;d	1.04±0.13	1.10±0.08	$9.4x10^{-1}$	1.00±0.14	1.51±0.09	$1.7x10^{-3}$
LVPW;s	1.55±0.16	1.44±0.10	$8.6x10^{-1}$	1.46±0.17	1.75±0.10	$2.0x10^{-1}$
LV mass /BW	5.50±0.59‡	7.12±0.52	$8.0x10^{-2}$	6.40±0.77‡	10.51±1.09	$8.6x10^{-3}$

ISO; Isoproterenol, HR; heart rate, IVS; intraventricular septum, LVID; LV internal dimensions, LVPW; LV posterior wall, SV; stroke volume, EF; ejection fraction, FS; Fractional Shortening, CO; cardiac output, LV; LV, s; systole, d; diastole, M; male, F; female, WT; wild-type, OX; AKAP12^{OX}. All values are expressed as Mean ± S.E.M. Statistical analysis was performed using 2-way ANOVA followed by Sidak multiple comparison post hoc test. Please refer to the online figshare data repository ***Supplemental Raw Data D1*** *for detailed p-values and further group comparisons.* ‡; N=6.

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Table-S2: Echocardiographic Parameters of WT and AKAP12^{OX} Mice; Males-Sham

	WT BSL	***WT Sham***	***AKAP12^{OX} BSL***	***AKAP12^{OX} Sham***
Parameter	**N=10**	**N=10**	**N=7**	**N=7**
IVS;d	1.41±0.11	1.12±0.07*	1.44±0.13	1.47±0.07*
IVS;s	1.84±0.1	1.52±0.07	1.9±0.15	1.84±0.08
HR	485.68±16.39	509.14±15.74	507.18±20.05	462.77±29.85
Diameter;s	2.23±0.21	2.86±0.14	2.19±0.12	2.29±0.16
Diameter;d	3.45±0.18	3.94±0.12*	3.26±0.17	3.38±0.15*
Volume;s	19.43±4.43	32.41±3.6*	16.82±2.3	19.02±2.87*
Volume;d	51.48±6.41	68.46±4.87*	44.27±6.06	47.61±4.73*
SV	32.05±2.95	36.05±1.91	27.46±4.59	28.59±2.22
EF	66.21±4.43	54.01±2.77	61.69±3.43	61.64±3.51
FS	36.75±3.19	27.81±1.82	32.65±2.44	32.81±2.6
CO (LV Trace)	15.6±1.49	18.32±1.05*	13.79±2.29	12.92±0.75*
LVID;d	3.39±0.2	3.94±0.12*	3.23±0.17	3.31±0.16*
LVID;s	2.25±0.21	2.9±0.14	2.2±0.12	2.33±0.16
LVPW;d	1.19±0.11	0.86±0.08*	1.52±0.1	1.23±0.09*
LVPW;s	1.63±0.13	1.21±0.1	1.84±0.14	1.54±0.09
LV Mass (Corrected)	151.08±14.47	126.09±11.43	173.81±16.84	152.19±10.21

****: Significant difference between AKAP12^{OX} and WT Sham.*** *All values are expressed as Mean ± S.E.M. Statistical analysis was performed using 2-way ANOVA followed by Sidak multiple comparison post hoc test. For clarity, only significant values focused on AKAP12^{OX} and WT Sham at 14 days are shown in this table. Please refer to the online figshare data repository Supplemental Raw Data D2 for detailed p-values and further group comparisons.*

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Table-S3: Echocardiographic Parameters of WT and AKAP12^{OX} Mice; Females-Sham

	WT BSL	***WT Sham***	***AKAP12^{OX} BSL***	***AKAP12^{OX} Sham***
Parameter	**N=9**	**N=9**	**N=6**	**N=6**
IVS;d	1.31±0.13	1.41±0.12	1.2±0.14	1.66±0.12
IVS;s	1.62±0.11	1.79±0.14	1.66±0.14	1.99±0.13
HR	477.49±9.82	499.65±18.63	499.14±13.86	498.43±24.61
Diameter;s	1.76±0.14	2.15±0.13	1.93±0.15	1.91±0.19
Diameter;d	2.95±0.14	3.3±0.09	3.14±0.16	2.98±0.15
Volume;s	10.28±1.83	16.17±2.47	12.31±2.66	12.54±3.41
Volume;d	34.78±3.66	44.68±2.91	40.04±5.06	35.41±4.5
SV	24.5±1.96	28.51±1.8	27.73±2.69	22.87±1.16
EF	72.75±2.85	64.78±3.76	70.3±2.66	67.18±4.27
FS	41.25±2.63	35.15±2.74	38.93±2.07	36.54±3.1
CO (LV Trace)	11.8±1.1	14.1±0.73	13.73±1.23	11.3±0.41
LVID;d	2.84±0.16	3.27±0.09	3.17±0.15	2.96±0.11
LVID;s	1.79±0.15	2.16±0.12	1.95±0.16	1.96±0.21
LVPW;d	1.21±0.13	1.1±0.1	1.26±0.08	1.28±0.14
LVPW;s	1.55±0.14	1.49±0.15	1.7±0.06	1.66±0.16
LV Mass (Corrected)	112±11.8	134.31±10.67	123.84±8.96	152.39±15.34

****: Significant difference between WT and AKAP12^{OX} Sham.*** *All values are expressed as Mean ± S.E.M. Statistical analysis was performed using 2-way ANOVA followed by Sidak multiple comparison post hoc test. For clarity, only significant values focused on AKAP12^{OX} and WT Sham at 14 days are shown in this table. Please refer to the online figshare data repository Supplemental Raw Data D2 for detailed p-values and further group comparisons.*

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Table-S4: Echocardiographic Parameters of WT and AKAP12^{OX} Mice; Males-Vehicle

	WT BSL	***WT Veh***	***AKAP12^{OX} BSL***	***AKAP12^{OX} Veh***
Parameter	**N=6**	**N=6**	**N=4**	**N=4**
IVS;d	1.46±0.17	1.09±0.1	1.42±0.18	1.56±0.14
IVS;s	1.91±0.14	1.61±0.09	1.81±0.22	1.91±0.17
HR	486.84±17.1	507.62±20.87	477.77±26.18	521.23±6.54
Diameter;s	1.91±0.14	2.3±0.22	2.4±0.16	2.81±0.3
Diameter;d	3.08±0.14	3.63±0.18	3.51±0.18	3.79±0.19
Volume;s	12.13±2.27	19.78±3.6	21.1±3.18	31.67±8.32
Volume;d	38.11±4.11	56.77±5.87	52.65±6.26	62.56±6.84
SV	25.98±2.77	36.99±2.53	31.55±4.54	30.89±3.56
EF	68.79±3.98	67.33±4.26	60.69±4.26	51.65±8
FS	38.07±3.42	37.5±3.74	31.96±2.98	26.49±4.65
CO (LV Trace)	12.68±1.48	18.85±1.71	15.04±2.25	16.13±1.94
LVID;d	3.03±0.14	3.63±0.15	3.46±0.2	3.79±0.19
LVID;s	1.93±0.14	2.31±0.21	2.4±0.14	2.83±0.34
LVPW;d	1.28±0.19	0.91±0.14	1.51±0.08	1.39±0.11
LVPW;s	1.68±0.17	1.49±0.17	1.81±0.09	1.63±0.07
LV Mass (Corrected)	124.42±16.88‡	117.93±20.21‡*	186.62±22.92	211.45±12.24*

****: Significant difference between WT and AKAP12^{OX} groups post vehicle treatment, ‡ N=5.*** *All values are expressed as Mean ± S.E.M. Statistical analysis was performed using 2-way ANOVA followed by Sidak multiple comparison post hoc test. For clarity, only significant values focused on AKAP12^{OX} and WT Sham at 14 days are shown in this table. Please refer to the online figshare data repository Supplemental Raw Data D3 for detailed p-values and further group comparisons.*

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Table-S5: Echocardiographic Parameters of WT and AKAP12^{OX} Mice; Females-Vehicle

	WT BSL	***WT Veh***	***AKAP12^{OX} BSL***	***AKAP12^{OX} Veh***
Parameter	**N=8**	**N=8**	**N=5**	**N=5**
IVS;d	1.38±0.17	1.28±0.14	1.58±0.09	1.38±0.08
IVS;s	1.79±0.14	1.79±0.22	1.96±0.1	1.75±0.13
HR	485.56±9.31	511.11±17.35	510.59±11.15	501.82±18.51
Diameter;s	1.89±0.11	2.13±0.18	1.93±0.24	1.77±0.18
Diameter;d	2.96±0.13	3.29±0.17	3.02±0.2	2.9±0.2
Volume;s	11.6±1.59	16.59±3.1	13.02±3.44	10.12±2.63
Volume;d	34.69±3.5	45.16±5.21	36.73±5.34	33.54±5.34
SV	23.09±1.99	28.57±2.19	23.71±2.26	23.43±3.31
EF	67.36±1.6	66.01±3.31	67.65±5.53	70.85±3.65
FS	36.29±1.2	36.01±2.53	37.37±4.4	39.26±2.82
CO (LV Trace)	11.22±0.98	14.6±1.26	12.13±1.22	11.93±1.95
LVID;d	2.98±0.13	3.27±0.18	3±0.2	2.85±0.2
LVID;s	1.91±0.12	2.08±0.18	1.93±0.22	1.79±0.2
LVPW;d	1.17±0.12	1.06±0.14	1.43±0.18	1.2±0.17
LVPW;s	1.54±0.1	1.6±0.15	1.88±0.17	1.61±0.16
LV Mass (Corrected)	125.02±17.09	121.87±16.62	163.64±20.37	115.63±12.39

****: Significant difference between WT and AKAP12^{OX} groups post vehicle treatment.*** *All values are expressed as Mean ± S.E.M. Statistical analysis was performed using 2-way ANOVA followed by Sidak multiple comparison post hoc test. For clarity, only significant values focused on AKAP12^{OX} and WT Sham at 14 days are shown in this table. Please refer to the online figshare data repository Supplemental Raw Data D3 for detailed p-values and further group comparisons.*

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Table-S6: Human HF Patient Information

	S4	S5	S8	S9	S10	S13	S14	S15	S17
Age (Y)	28.1	42.3	71.4	28.2	43.6	44	18	53	51
Sex	M	M	M	F	M	M	M	F	F
Beta Blocker	Metoprolol	No	Metoprolol	Coreg	Coreg				
Diuretic	Bumex	Bumex	Lasix	No	Bumex				
ACE Inhibitor	No	No	No	Lisinopril	Lisinopril				
ARB	No	No	No	No	No				
CaC Blocker	No	No	No	No	No				
Statin	No	No	Yes	No	No				
Hypertension	No	No	Yes	No	No				
Prior MI	No	No	Yes	No	No				
Diabetes	No	No	Yes	No	No				
HF Type	Ischemic	Non-Ischemic	Non-Ischemic	Peripartum	Non-ischemic	Non-ischemic	Non-ischemic	Non-ischemic	Non-ischemic
LVEF (%)	30	<20	<20	<20	<20				

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Primary Cardiomyocytes Extraction Protocol – *Adjusted from Ackers Johnson Langendorff-free Protocol*[55]

Buffers used are the same as buffers used in the original publication[55], except for specific cases where 1 mM Blebbistatin was utilized in the buffers instead of BDM, these occurrences are noted as ‡‡ at the end of figure legends.

Pre-digestion Buffer Administration:

EDTA and Perfusion buffers used are identical to the original protocol[55]. Initially, administer 7 ml of the EDTA buffer into the right ventricle, ensuring that the entire volume is delivered within a one-minute timeframe. It is imperative to inflate the heart during the injection process, observing the onset of a subtle pallor. To achieve optimal results, position the syringe just 1-2 mm inside the heart. Subsequently, apply a clamp to the aorta and carefully excise the heart from its cavity by cutting above the clamp. Following this, place the excised heart into a dish containing 5 ml of EDTA buffer, ensuring complete coverage of the organ with the buffer solution. Proceeding to the left ventricle, inject 7 ml of EDTA buffer at the apex, employing a gradual administration over approximately 6 minutes. The heart should remain inflated throughout this procedure. Notably, the left ventricle may exhibit resistance to the syringe, necessitating unhurried insertion. In the subsequent step, transfer the heart into an alternative dish filled with 5 ml of perfusion buffer, ensuring consistent immersion in the buffers. Concluding this part, inject 3 ml of perfusion buffer into the left ventricle through the same opening, taking care to execute the process slowly over a 5-minute duration.

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Cardiac Digestion

Digestion Buffer:

Mix 50 ml Perfusion buffer with 225 μL of Liberase (5 mg/ml stock solution). Place the heart in a dish containing 5ml of digestion buffer. Subsequently, a controlled volume of 20-30 ml of digestion buffer is slowly injected into the heart through 2 or 3 10 ml injections, each administered over a 5–7-minute period per injection. Variability in required volume across mice necessitates vigilance to prevent over-digestion. Needles containing digestion buffer are to be kept on a heat pad. Following the removal of the clamp, the heart is sectioned using forceps, with minimal force applied. The designated heart chamber (left ventricle in this case) is transferred to another plate containing 3 ml of digestion buffer. Muscle separation is carried out until fragments are of a size suitable for passage through a 1ml pipette tip with a wide opening. Subsequent digestion of the heart gently for an additional 2-3 minutes using the pipette tip.

Stopping Digestion:

Buffer used: 10% FBS in perfusion buffer (kept at room temperature). Heart digestion is stopped by adding a volume of stop buffer to achieve a 1:1 ratio of digestion-buffer : stop-buffer in the plate. The additional pipetting step is performed for an extra 2 minutes.

Isolation of Healthy Rod-Shaped Cells:

70 μm cell strainer is placed on top of a sterile 50 ml tube, through which the entire cell volume is passed. While keeping the strainer in place, an additional stop buffer is introduced; volume added = total volume passed through the strainer. Distribute the resulting volume into two

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15 ml tubes and allow the cells to settle by gravity for 15-20 minutes, sometimes as brief as 10 minutes. Healthy cells should sediment more rapidly, with dead cells remaining at the top. Calcium reintroduction steps were identical to the original protocol[55]; however, all were performed at 37ºC rather than room temperature.