## SUPPLEMENTAL MATERIAL

### Supplemental Methods

### Mutant Mouse Lines

*mAKAP$^{fl}$ Allele:* The *mAKAP$^{fl}$* mouse was generated by the University of Cincinnati Gene Targeted Mouse Service. Briefly, a targeting vector containing negative (tk) and positive (neo) selectable markers was designed to conditionally delete Exon 9 of the *mAKAP (AKAP6)* gene. Targeted C57BL/6 ES cell clones were confirmed by Southern blot using probes "a" and '"b". The targeting vector introduced additional Xmn I and Nco I sites into the *mAKAP* gene. After breeding of targeted mice, the neo cassette was deleted by mating to a FLP recombinase transgenic mouse (B6;SJL-Tg(ACTFLPe)9205Dym/J, Jackson Laboratory). Mouse genotyping was performed by PCR with primers 44 and 45 and genomic DNA obtained by tail biopsy.

*Cre recombinase mice:* B6.C-Tg(CMV-cre)1Cng/j were obtained from the Jackson Laboratory to generate the constitutive, global mAKAP knock-out. After germline deletion was achieved, only mice lacking the Tg(CMV-cre) transgene were bred. B6;129-Nkx2-5tm1(cre)Rjs/H were obtained from the Mary Lyon Centre at MRC Harwell (www.har.mrc.ac.uk) to provide cardiac lineage knock-out.[1] Conditional cardiac myocyte-specific knock-out was obtained by mating *mAKAP$^{fl}$* and Tg(Myh6-cre/Esr1*)1Jmk/J mice (The Jackson Laboratory). [2] Starting at 8 weeks of age, tamoxifen-containing chow (125 mg/kg chow to provide ~20 mg/kg body weight, Harlan Teklad) was provided for one week. Mice were not used for experiments until 10 weeks of age, by which time they would have been fed normal chow for one week. Control cohorts included both MCM+Tam mice that control for effects due to LoxP insertion into the *mAKAP* gene and *mAKAP$^{fl/fl}$*;MCM mice that control for effects due to Tam administration.

*Reporter Mice:* NFAT-luciferase reporter mice (B6;FVB-Tg(Myh6/NFAT-luc)1Jmol/J) were obtained from Dr. Jeffrey Molkentin (Children's Hospital Medical Center, Cincinnati, OH).[3]

### Transverse Aortic Constriction

10 week old mice were subjected to TAC to induce pressure overload. All tools were sterilized with a Germinator 500 Dry Sterilizer and Betadine Solution (10% povidone-iodine topical solution). Anesthesia was induced with 5% isoflurane and maintained with 2% isoflurane and 100% oxygen at a flow rate of 1.5 L/min using a SurgiVet flow regulator via nose cone. Loss of consciousness was verified by toe pinch. Mouse fur over the left chest and sternum was removed with a calcium hydroxide lotion (e.g. Nair), and the surgical site was sterilized with betadine. The skin was incised exposing the pectoralis muscle and the second left intercostal space. The pectoralis muscle and the second rib were blunt dissected and retracted revealing the thymus within the mediastinum. The lobes of the thymus were retracted to reveal the transverse aortic arch as well as the right innominate and left common carotid arteries. Blunt dissection though the connective tissue between these 2 arteries and under the aorta allowed for the passage of a 6-0 silk using a modified ligation aid (Fine Science Tools 18062-12). A 27 gauge needle was placed on top of the aorta, and the 6-0 silk was tied around the needle. The needle was removed, leaving a constricted aorta. The chest was closed in 2 layers with 5-0 Polysorb Suture. Isoflurane administration was terminated, and the mice were maintained on 100% oxygen by nose cone until conscious. Immediately post-operatively, buprenorphrine (0.05-0.1 mg/kg s.c.) was administered and then q12 h prn. The mice were allowed to recover under a heat lamp until alert and active.

Sham-operated mice that experienced all but the placement of the aortic ligature served as controls. The <10% of the mice that did not survive the first week post-operative were excluded from study. For long-term TAC survival studies, mice in extremis were euthanized and counted as mortality events, i.e., mice that exhibited minimal movement, a moribund respiratory pattern and/or severe tachypnea, acute severe edema, or severe weight loss (20% of pre-surgical body weight).

**Chronic Isoproterenol Infusion**

Alzet 2002 osmotic pumps (Durect) were sterilely loaded with 200 μL saline or saline and isoproterenol to deliver 60 mg/kg/day for 14 days. 10-week old mice were anaesthetized using 5% isoflurane for induction and then 2% isoflurane and 100% oxygen at a flow rate of 1.5 L/min using a SurgiVet flow regulator via nose cone. Pumps were sterilely inserted subcutaneously into the shaved back through an intra-scapulae transverse incision. Wounds were closed with surgical staples and covered with betadine solution. Mice were housed separately after surgery. Immediately post-operatively, buprenorphrine (0.05-0.1 mg/kg s.c.) was administered and then q12 h prn.

**Chronic Exercise – Swimming**

10-week old Tam-treated mice were forced to swim in water tanks every day for 5 weeks. The swimming tank measured >225 $cm^2$ with a depth of 15 cm and a water temperature of 30-32°C. Mice were continuously observed to prevent drowning. The first day of training consisted of 2 10-min sessions separated by at least 4 h. Sessions were increased by 10 min each day until 90-min sessions were reached. Additional cohorts were housed normally without exercise to serve as a "resting" control group. Food and water were provided ad libitum throughout the month period for all mice.

**Echocardiography**

Mice minimally anesthetized with 1-4% isoflurane were studied using a Vevo 770®, High-Resolution Imaging System (VisualSonics). The pressure gradient following TAC was calculated from the pulse wave Doppler velocity at the point of ligation as follows: $P = 4v^2$; P = the induced pressure gradient (in mmHg) and $v$ = the velocity across the constriction (in m/s).[4] Calculated parameters were as follows: FS, fractional shortening = (LVID;d – LVID;s)/(LVID;d); EF, ejection fraction = (LVV;d – LVV;s)/(LVV;d), in which LVV (in μL) = $LVID^3 * 7/(2.4 + LVID)$ for either systole or diastole; LV Mass (in mg) = $0.84((LVID;d + LVPW;d + LVAW;d)^3 – LVID;d^3)$.

**Histochemistry**

Heart tissue was fixed in 3.7% formaldehyde in PBS. De-paraffinized, 5 μm tissue sections were stained using Alexa Fluor 555 Wheat Germ Agglutinin conjugate (Invitrogen), Picrosirius Red Stain Kit (Polysciences), and the *In Situ* Cell Death Detection Kit, TMR red (Roche) as recommended by the manufacturers. The cross-section area of >150 myocytes in >3 distinct regions of the left ventricle was measured for each mouse using images acquired by fluorescence microscopy of the wheat germ agglutinin-stained sections. Collagen content was assayed using the Picrosirius Red stained sections and linearly-polarized light microscopy for >3 40x magnification images per left ventricle. Montage images in Fig. 3H were assembled using Photoshop and ~20 images per heart. TUNEL staining was analyzed by fluorescence microscopy using 100x magnification images of the left ventricle. Morphometrics, collagen content, and nuclei counts were measured using IPLab microscope software (BD Biosciences).

**Adult Myocyte Isolation by Langendorff Perfusion**

Mice were anesthetized using Ketamine (80-100 mg/kg) and Xylazine (5-10 mg/kg) IP followed by 200 U heparin IP and cardiac excision. The heart was placed immediately in perfusion buffer (in mmol/L, NaCl 120, KCl 5.4, $Na_2HPO_4 \cdot 7H_2O$ 1.2, $NaHCO_3$ 20, $MgCl_2.6H_2O$ 1.6, Taurine 5, Glucose 5.6) equilibrated with 95% $O_2$ and 5% $CO_2$. The heart was attached via the aorta to the condenser outlet of a Harvard Langendorff apparatus. $Ca^{2+}$-free perfusion lasted for 5 minutes with a constant rate at 2.2 mL/min at 37$^{o}$C. The heart was digested by continuous perfusion with 25 mL buffer containing 25 mg type II collagenase (Worthington, 315 U/mg) and 1.3 mg protease (Sigma type XIV). After removal of the atria, the ventricles were then immersed in 5 mL of the same enzyme solution for dissociation by cutting into small pieces and by passing through a large bore pipette. The cell slurry was filtered through a 150 - 200 μm nylon mesh and the myocytes relaxed by incubation for 10 minutes in perfusion buffer containing 10 mM KCl. The cells were fixed in suspension in perfusion buffer containing 3.7% formaldehyde, before morphometric analysis by light microscopy. The maximum dimensions perpendicular (width) and parallel (length) to the myofibrils were measured for >100 freshly dissociated myocytes per

heart. The mean width and length of the myocytes for each individual mouse were included as single points in the cohort datasets, such that the data presented are the average of the means for the individual mice. Adult rat myocytes were similarly isolated for immunocytochemistry.

**Gene Expression**

Total RNA was quantified with a Nanodrop 8000 Spectrophotometer (Thermo Scientific) and quality controlled using with a Bioanalyzer 2100 and the RNA 6000 Nano kit (Agilent). The NanoString assay is based on direct, multiplexed measurement of gene expression without amplification, utilizing fluorescent molecular barcodes and single molecule imaging to identify and count multiple transcripts in a single reaction. After addition of a standard set of positive and negative control RNAs, 100 ng total RNA were hybridized in solution to a target-specific codeset overnight at 65˚C. The codeset contained dual, adjacently placed 50 bp oligonucleotide probes against a preselected panel of genes, one set of probes fluorescently bar-coded and the other biotinylated. The hybridization reactions were loaded onto the NanoString Prep station which removes excess oligonucleotides and binds the hybridized mRNA to the Streptavidin-coated cartridge surface. The cartridges were loaded onto the NanoString Digital Analyzer, and >1150 fields of view were fluorescently scanned to count only those individual mRNAs bound to both a biotinylated and fluorescently bar-coded probe. Datasets for each RNA sample were normalized to the positive controls and background-subtracted using the negative controls. Probe sequences are available upon request.

**NFAT Reporter Assay**

Luciferase enzymatic activity in tissue extracts was measured using the Luciferase Assay System (Promega E1501) according to the manufacturer's instructions. Apex heart tissue (20-30 mg) was homogenized using a Polytron and the kit buffer. Protein concentration was determined by Bradford assay. Luciferase activity was measured using a Centro $XS^3$ LB 960 luminometer (Berthold).

**Immunoprecipitation and Western Blotting**

Whole mouse and rat heart homogenates were lysed in buffer (20 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl, 5 mmol/L EDTA, 0.5% Triton, 50 mmol/L NaF, 1 mmol/L sodium orthovanadate, 1 mmol/L DTT, and protease inhibitors). Whole ventricular extracts containing 60 µg protein were analyzed by SDS-PAGE. Equal loading was confirmed by Ponceau S staining of the blots after transfer. Western blots were developed using horseradish peroxidase-conjugated, donkey secondary antibodies, Supersignal West Chemiluminescent Substrates (Thermo Scientific), and a Fujifilm LAS-3000 imaging system.

COS-7 cells were transfected with polyethylenimine "Max" (Polysciences) as previously described.[5] pCMH-mAKAPβ encoding myc-mAKAPβ was as previously described.[6] Expression plasmids for Flag-tagged HDAC4 WT and Ser-246/467/632-Ala and HA-tagged PDK1 were acquired from Addgene (catalog numbers 13821, 30486 and 10808) and were as previously described.[7, 8] The COS-7 cells were treated with 1 µmol/L okadaic acid and 1 µg/mL cyclosporine A for 40 minutes and 200 nmol/L phorbol myristate acetate for 20 minutes to maximally activate PKD1 before co-immunoprecipitation.

For immunoprecipitation, extracts were clarified by centrifugation at 1,000 *x g* for 15 minutes at 4°C and immunoprecipitated using either 10 µg purified antibody or 1-5 µL whole serum and 20 µL Protein G Sepharose (Millipore, Fastflow) for 3 hours to overnight at 4°C. The beads were washed 3-5 times with lysis buffer, and the immunoprecipitated proteins were eluted with 1x Laemmli buffer for western blotting by SDS-PAGE.

**Immunochemistry**

Cultured neonatal mouse or adult rat cardiomyocytes on plastic coverslips and frozen heart sections were fixed in 3.7% formaldehyde in PBS, permeabilized with 0.3% Triton X-100 in PBS, and blocked with PBS containing 0.2% BSA and 1% horse serum for 1 hour. The slides were then sequentially incubated for 1 hour with primary and Alexa fluorescent dye-conjugated specific-secondary antibodies (Invitrogen, 1:1000) diluted in blocking buffer. The slides were washed three times with blocking buffer. 1 µg/mL Hoechst 33258 was included in the last wash

stop to label nuclei. Slides were sealed in SlowFade Gold antifade buffer (Invitrogen) and imaged using either a LEICA DMI6000 wide-field fluorescence microscope or a Zeiss LSM710 spectral confocal microscope.

**Reagents**

Commercial antibodies are listed in Supplemental Table X. Oligonucleotides used for genomic PCR are listed in Supplemental Table XI.

## Supplemental Tables

### Supplemental Table I: M-mode Echocardiographic Data – Two Week Pressure Overload

| | | Sham-Operated | | | Transverse Aortic Constriction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM + Tam | *mAKAP*$^{fl/fl}$;MCM | mAKAP CKO | MCM + Tam | | *mAKAP*$^{fl/fl}$;MCM | | mAKAP CKO | |
| *n* | | 10 | 7 | 10 | 23 | | 14 | | 21 | |
| PG | mm Hg | | | | 53 ± 2 | *** | 52 ± 1 | *** | 52 ± 1 | ** ‡‡‡ ††† |
| LVPW;d | mm | 0.60 ± 0.02 | 0.55 ± 0.02 | 0.59 ± 0.02 | 0.89 ± 0.03 | *** | 0.85 ± 0.03 | ** | 0.70 ± 0.02 | * ††† |
| LVPW;s | mm | 0.91 ± 0.04 | 0.81 ± 0.04 | 0.85 ± 0.04 | 1.17 ± 0.04 | *** | 1.06 ± 0.04 | *** | 0.97 ± 0.03 | ** † |
| LVAW;d | mm | 0.65 ± 0.03 | 0.59 ± 0.02 | 0.65 ± 0.02 | 0.90 ± 0.03 | *** | 0.88 ± 0.03 | *** | 0.82 ± 0.03 | ** |
| LVAW;s | mm | 0.96 ± 0.03 | 0.87 ± 0.03 † | 0.92 ± 0.03 | 1.24 ± 0.04 | ** | 1.24 ± 0.06 | | 1.15 ± 0.04 | † |
| LVID;d | mm | 4.36 ± 0.08 | 4.17 ± 0.13 | 4.02 ± 0.11 † | 4.00 ± 0.07 | | 4.20 ± 0.10 | | 4.24 ± 0.07 | † |
| LVID;s | mm | 3.30 ± 0.09 | 3.24 ± 0.14 | 3.06 ± 0.17 | 3.04 ± 0.10 | | 3.27 ± 0.12 | | 3.32 ± 0.09 | |
| FS | % | 24.3 ± 1.4 | 22.4 ± 1.6 | 24.2 ± 2.4 | 24.4 ± 1.5 | | 22.5 ± 1.4 | | 21.9 ± 1.2 | |
| EF | % | 48.3 ± 2.2 | 45.2 ± 2.7 | 47.9 ± 3.9 | 48.3 ± 2.5 | | 45.2 ± 2.3 | | 44.1 ± 2.0 | |
| LV Mass | mg | 80 ± 6 | 66 ± 5 | 69 ± 4 | 110 ± 4 | *** | 114 ± 7 | *** | 98 ± 5 | ** |
| Body Weight | g | 21.7 ± 0.8 | 21.7 ± 1.4 | 20.8 ± 0.9 | 22.4 ± 0.5 | | 22.6 ± 0.5 | | 22.5 ± 0.6 | |
| Heart Rate | BPM | 467 ± 5 | 465 ± 13 | 470 ± 11 | 486 ± 4 | * | 486 ± 8 | | 490 ± 6 | |

LVPW, Left ventricular posterior wall thickness; LVAW, left ventricular anterior wall thickness; LVID, Left ventricular internal diameter; d, diastole; s, systole; FS, fractional shortening; EF, ejection fraction; LV mass, calculated left ventricular mass. * *p*-values vs. sham-operated for same genotype; † *p*-values vs. MCM+Tam; ‡ *p*-values vs. mAKAP$^{fl/fl}$;MCM. All data are mean ± sem.

### Supplemental Table II: Gravimetric Data – Two Week Pressure Overload

| | | Sham-Operated | | | Transverse Aortic Constriction | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM + Tam | *mAKAP*$^{fl/fl}$;MCM | mAKAP CKO | MCM + Tam | | *mAKAP*$^{fl/fl}$;MCM | | mAKAP CKO | |
| *n* | | 42 | 12 | 34 | 31 | | 18 | | 34 | |
| Biventricular Weight | mg | 90 ± 2 | 90 ± 4 | 89 ± 2 | 144 ± 5 | *** | 124 ± 5 | *** † | 113 ± 3 | *** ††† |
| Tibial Length | mm | 16.6 ± 0.1 | 16.5 ± 0.2 | 16.4 ± 0.1 | 16.7 ± 0.2 | | 16.6 ± 0.2 | | 16.7 ± 0.1 | * |
| Body Weight | g | 21.6 ± 0.4 | 21.3 ± 0.9 | 21.2 ± 0.4 | 22.0 ± 0.6 | | 21.6 ± 0.4 | | 21.9 ± 0.4 | |
| BiVW/BW | mg/g | 4.19 ± 0.05 | 4.24 ± 0.05 | 4.21 ± 0.04 | 6.59 ± 0.19 | *** | 5.76 ± 0.16 | *** † | 5.17 ± 0.11 | *** ††† ‡‡ |

BiVW/BW, biventricular weight/body weight. * *p*-values vs. sham-operated for same genotype; † *p*-values vs. MCM+Tam; ‡ *p*-values vs. mAKAP$^{fl/fl}$;MCM. All data are mean ± sem.

## Supplemental Table III: M-mode Echocardiographic Data – Chronic Isoproterenol Infusion

| | | Saline Infusion | | | Isoproterenol Infusion | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM + Tam | *mAKAP*$^{fl/fl}$;MCM | mAKAP CKO | MCM + Tam | | *mAKAP*$^{fl/fl}$;MCM | | mAKAP CKO | | | | |
| *n* | | 8 | 7 | 8 | 8 | | 5 | | 13 | | | | |
| LVPW;d | mm | 0.61 ± 0.02 | 0.59 ± 0.01 | 0.64 ± 0.02 | 0.86 ± 0.02 | *** | 0.90 ± 0.05 | *** | 0.73 ± 0.02 | * | ‡‡ | ††† | |
| LVPW;s | mm | 0.87 ± 0.03 | 0.84 ± 0.01 | 0.92 ± 0.05 | 1.19 ± 0.06 | *** | 1.22 ± 0.12 | ** | 1.13 ± 0.06 | * | | | |
| LVAW;d | mm | 0.66 ± 0.03 | 0.71 ± 0.01 | 0.68 ± 0.02 | 0.85 ± 0.05 | ** | 0.92 ± 0.07 | * | 0.76 ± 0.03 | | ‡ | | |
| LVAW;s | mm | 0.98 ± 0.04 | 0.98 ± 0.04 | 0.97 ± 0.03 | 1.31 ± 0.05 | *** | 1.35 ± 0.11 | ** | 1.17 ± 0.06 | * | | | |
| LVID;d | mm | 4.27 ± 0.12 | 4.26 ± 0.14 | 4.42 ± 0.12 | 4.36 ± 0.11 | | 4.46 ± 0.08 | | 4.59 ± 0.08 | | | | |
| LVID;s | mm | 3.26 ± 0.12 | 3.34 ± 0.15 | 3.38 ± 0.19 | 3.10 ± 0.13 | | 3.18 ± 0.18 | | 3.31 ± 0.11 | | | | |
| FS | % | 23.8 ± 1.1 | 21.7 ± 0.9 | 23.8 ± 2.3 | 29.1 ± 1.9 | * | 28.6 ± 3.4 | * | 27.8 ± 1.5 | | | | |
| EF | % | 47.6 ± 1.8 | 44.1 ± 1.7 | 47.2 ± 3.8 | 55.8 ± 2.8 | * | 54.6 ± 5.2 | | 53.6 ± 2.4 | | | | |
| LV Mass | mg | 79 ± 6 | 80 ± 5 | 87 ± 6 | 119 ± 8 | ** | 134 ± 11 | ** | 108 ± 6 | * | ‡ | | |
| Body Weight | g | 23.7 ± 1.0 | 22.9 ± 1.2 | 25.0 ± 1.1 | 24.9 ± 1.0 | | 25.8 ± 0.9 | | 25.7 ± 0.6 | | | | |
| Heart Rate | BPM | 489 ± 13 | 482 ± 13 | 497 ± 10 | 605 ± 3 | *** | 610 ± 4 | *** | 607 ± 2 | *** | | | |

LVPW, Left ventricular posterior wall thickness; LVAW, left ventricular anterior wall thickness; LVID, Left ventricular internal diameter; d, diastole; s, systole; FS, fractional shortening; EF, ejection fraction; LV mass, calculated left ventricular mass. * *p*-values vs. saline-infused for same genotype; † *p*-values vs. MCM+Tam; ‡ *p*-values vs. mAKAP$^{fl/fl}$;MCM. All data are mean ± sem.

## Supplemental Table IV: Gravimetric Data – Chronic Isoproterenol Infusion

| | | Saline Infusion | | | Isoproterenol Infusion | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MCM + Tam | *mAKAP*$^{fl/fl}$;MCM | mAKAP CKO | MCM + Tam | | *mAKAP*$^{fl/fl}$;MCM | | mAKAP CKO | | | | |
| *n* | | 14 | 8 | 11 | 14 | | 6 | | 15 | | | | |
| Biventricular Weight | mg | 96 ± 5 | 95 ± 7 | 102 ± 5 | 136 ± 4 | *** | 143 ± 7 | ** | 124 ± 4 | ** | † | ‡ | |
| Tibial Length | mm | 16.7 ± 0.2 | 17.0 ± 0.3 | 17.0 ± 0.2 | 16.7 ± 0.1 | | 17.0 ± 0.3 | | 16.8 ± 0.2 | | | | |
| Body Weight | g | 21.7 ± 0.6 | 22.2 ± 1.2 | 22.7 ± 0.7 | 24.4 ± 0.4 | ** | 25.3 ± 0.7 | | 24.6 ± 0.7 | | | | |
| BiVW/BW | mg/g | 4.48 ± 0.14 | 4.27 ± 0.13 | 4.51 ± 0.12 | 5.61 ± 0.18 | *** | 5.66 ± 0.20 | *** | 5.07 ± 0.08 | *** | † | ‡‡ | |

BiVW/BW, biventricular weight/body weight. * *p*-values vs. saline-infused for same genotype; † *p*-values vs. MCM+Tam; ‡ *p*-values vs. mAKAP$^{fl/fl}$;MCM. All data are mean ± sem.

## Supplemental Table V: M-mode Echocardiographic Data – Swimming

| | | Resting | | | Swimming | | |
|---|---|---|---|---|---|---|---|
| | | Control | mAKAP CKO | | Control | mAKAP CKO | |
| *n* | | 7 | 7 | | 7 | 7 | |
| LVPW;d | mm | 0.61 ± 0.02 | 0.54 ± 0.02 | † | 0.64 ± 0.03 | 0.57 ± 0.02 | |
| LVPW;s | mm | 0.81 ± 0.03 | 0.76 ± 0.02 | | 0.89 ± 0.05 | 0.76 ± 0.04 | |
| LVAW;d | mm | 0.67 ± 0.01 | 0.61 ± 0.02 | † | 0.69 ± 0.02 | 0.64 ± 0.01 | † |
| LVAW;s | mm | 0.91 ± 0.02 | 0.91 ± 0.03 | | 0.95 ± 0.04 | 0.92 ± 0.03 | |
| LVID;d | mm | 4.15 ± 0.10 | 4.11 ± 0.05 | | 4.09 ± 0.15 | 4.17 ± 0.12 | |
| LVID;s | mm | 3.29 ± 0.06 | 3.19 ± 0.09 | | 3.15 ± 0.15 | 3.19 ± 0.16 | |
| FS | % | 20.7 ± 1.2 | 22.5 ± 1.5 | | 23.1 ± 1.1 | 23.9 ± 1.8 | |
| EF | % | 42.5 ± 2.0 | 45.5 ± 2.4 | | 46.7 ± 1.9 | 47.7 ± 3.0 | |
| LV Mass | mg | 74 ± 3 | 65 ± 3 | † | 77 ± 8 | 70 ± 4 | |
| Body Weight | g | 22.9 ± 0.9 | 22.1 ± 0.8 | | 21.9 ± 1.0 | 21.4 ± 0.7 | |
| Heart Rate | BPM | 490 ± 14 | 488 ± 8 | | 447 ± 9 * | 433 ± 7 | *** |

Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. LVPW, Left ventricular posterior wall thickness; LVAW, left ventricular anterior wall thickness; LVID, Left ventricular internal diameter; d, diastole; s, systole; FS, fractional shortening; EF, ejection fraction; LV mass, calculated left ventricular mass. * *p*-values vs. resting mice for same genotype; † *p*-values vs. control. All data are mean ± sem.

## Supplemental Table VI: Gravimetric Data – Swimming

| | | Resting | | Swimming | |
|---|---|---|---|---|---|
| | | Control | mAKAP CKO | Control | mAKAP CKO |
| *n* | | 7 | 7 | 7 | 7 |
| Biventricular Weight | mg | 90 ± 3 | 86 ± 3 | 97 ± 7 | 88 ± 2 |
| Tibial Length | mm | 17.3 ± 0.3 | 17.1 ± 0.3 | 17.2 ± 0.3 | 17.3 ± 0.3 |
| Body Weight | g | 22.3 ± 0.8 | 21.7 ± 0.8 | 21.2 ± 1.0 | 21.0 ± 0.7 |
| Biventricular Weight/Body Weight | mg/g | 4.03 ± 0.06 | 3.97 ± 0.06 | 4.54 ± 0.15 * | 4.19 ± 0.05 * † |

Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. * *p*-values vs. resting mice for same genotype; † *p*-values vs. control. All data are mean ± sem.

**Supplemental Table VII: Gravimetric Data – Long Term Pressure Overload**

| | | Sham-Operated | | Long Term - TAC | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | mAKAP CKO | Control | | mAKAP CKO | | |
| *n* | | 24 | 30 | 17 | | 28 | | |
| Biventricular Weight | mg | 109 ± 4 | 111 ± 3 | 164 ± 7 | *** | 144 ± 4 | *** | † |
| Left Atrial Weight | mg | 3.1 ± 0.2 | 3.1 ± 0.3 | 5.9 ± 0.6 | *** | 4.2 ± 0.3 | * | † |
| Wet Lung Weight | mg | 144 ± 3 | 145 ± 2 | 184 ± 14 | ** | 152 ± 3 | * | † |
| Tibial length | mm | 18.0 ± 0.3 | 18.2 ± 0.2 | 17.4 ± 0.2 | | 18.1 ± 0.2 | | † |
| Body weight | g | 26.7 ± 0.9 | 27.6 ± 0.7 | 26.2 ± 0.9 | | 28.3 ± 0.7 | | |
| Biventricular Weight/Body Weight | mg/g | 4.09 ± 0.10 | 4.00 ± 0.06 | 6.29 ± 0.25 | *** | 5.15 ± 0.16 | *** | ††† |
| Left Atrial Weight/Body Weight | mg/g | 0.11 ± 0.01 | 0.11 ± 0.01 | 0.23 ± 0.02 | *** | 0.15 ± 0.01 | ** | †† |
| Wet Lung Weight/Body Weight | mg/g | 5.5 ± 0.1 | 5.3 ± 0.1 | 7.1 ± 0.5 | ** | 5.4 ± 0.1 | | †† |

Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. All mice were 16 weeks post-operative. * *p*-values vs. sham-operated mice for same genotype; † *p*-values vs. control. All data are mean ± sem.

**Supplemental Table VIII: M-mode Echocardiographic Data – Long Term Pressure Overload**

| | | Sham-Operated | | | Long Term - TAC | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | mAKAP CKO | | Control | | mAKAP CKO | | |
| 4 Weeks Post-Operative | | | | | | | | | |
| *n* | | 8 | 8 | | 21 | | 19 | | |
| PG | mm Hg | | | | 55 ± 1 | *** | 54 ± 1 | ** | ††† |
| LVPW;d | mm | 0.63 ± 0.01 | 0.61 ± 0.02 | | 0.86 ± 0.02 | *** | 0.73 ± 0.02 | * | † |
| LVPW;s | mm | 0.86 ± 0.04 | 0.83 ± 0.03 | | 1.06 ± 0.02 | ** | 0.98 ± 0.03 | | † |
| LVAW;d | mm | 0.69 ± 0.03 | 0.73 ± 0.03 | | 0.89 ± 0.03 | ** | 0.79 ± 0.02 | | |
| LVAW;s | mm | 0.99 ± 0.05 | 1.05 ± 0.05 | | 1.19 ± 0.03 | | 1.14 ± 0.03 | | |
| LVID;d | mm | 4.18 ± 0.11 | 4.36 ± 0.10 | | 4.32 ± 0.08 | | 4.33 ± 0.08 | | |
| LVID;s | mm | 3.29 ± 0.12 | 3.33 ± 0.15 | | 3.49 ± 0.10 | | 3.36 ± 0.10 | | |
| FS | % | 21.2 ± 2.1 | 23.9 ± 2.1 | | 19.4 ± 1.1 | | 22.7 ± 1.3 | | |
| EF | % | 43.0 ± 3.7 | 47.4 ± 3.5 | | 39.9 ± 1.9 | | 45.4 ± 2.2 | | |
| LV Mass | mg | 79 ± 5 | 87 ± 6 | | 121 ± 6 | *** | 101 ± 5 | | † |
| Body Weight | g | 23.8 ± 1.1 | 24.4 ± 1.2 | | 23.7 ± 0.4 | | 24.0 ± 0.7 | | |
| Heart Rate | BPM | 482 ± 10 | 479 ± 16 | | 493 ± 5 | | 489 ± 8 | | |
| 8 Weeks Post-Operative | | | | | | | | | |
| *n* | | 8 | 8 | | 19 | | 24 | | |
| PG | mm Hg | | | | 59 ± 1 | | 56 ± 1 | | |
| LVPW;d | mm | 0.61 ± 0.01 | 0.56 ± 0.01 | † | 0.95 ± 0.03 | *** | 0.78 ± 0.02 | *** | ††† |
| LVPW;s | mm | 0.87 ± 0.04 | 0.84 ± 0.05 | | 1.10 ± 0.04 | ** | 1.00 ± 0.02 | ** | † |
| LVAW;d | mm | 0.65 ± 0.02 | 0.71 ± 0.03 | | 0.98 ± 0.02 | *** | 0.84 ± 0.02 | ** | ††† |
| LVAW;s | mm | 0.94 ± 0.06 | 1.00 ± 0.04 | | 1.31 ± 0.03 | *** | 1.17 ± 0.03 | * | †† |
| LVID;d | mm | 4.13 ± 0.08 | 4.26 ± 0.14 | | 4.38 ± 0.11 | | 4.32 ± 0.07 | | |
| LVID;s | mm | 3.15 ± 0.11 | 3.24 ± 0.16 | | 3.58 ± 0.15 | | 3.44 ± 0.09 | | |
| FS | % | 23.7 ± 2.0 | 24.0 ± 1.9 | | 18.9 ± 1.7 | | 20.4 ± 1.1 | | |
| EF | % | 47.3 ± 3.3 | 47.8 ± 3.3 | | 38.4 ± 3.1 | | 41.5 ± 2.0 | | |
| LV Mass | mg | 73 ± 3 | 79 ± 8 | | 143 ± 8 | *** | 109 ± 4 | ** | ††† |
| Body Weight | g | 23.6 ± 1.0 | 25.7 ± 1.5 | | 25.0 ± 0.5 | | 25.3 ± 0.7 | | |
| Heart Rate | BPM | 495 ± 11 | 505 ± 13 | | 499 ± 8 | | 508 ± 8 | | |
| 12 Weeks Post-Operative | | | | | | | | | |
| *n* | | 8 | 8 | | 10 | | 13 | | |
| PG | mm Hg | | | | 58 ± 3 | | 62 ± 1 | | |
| LVPW;d | mm | 0.67 ± 0.02 | 0.62 ± 0.02 | | 0.99 ± 0.05 | *** | 0.83 ± 0.02 | *** | †† |
| LVPW;s | mm | 0.91 ± 0.03 | 0.91 ± 0.01 | | 1.19 ± 0.06 | ** | 1.08 ± 0.03 | ** | |
| LVAW;d | mm | 0.77 ± 0.04 | 0.73 ± 0.03 | | 0.97 ± 0.03 | ** | 0.88 ± 0.03 | ** | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| LVAW;s | mm | 1.07 ± 0.07 | 1.02 ± 0.03 | 1.29 ± 0.05 | * | 1.22 ± 0.05 | * | |
| LVID;d | mm | 4.24 ± 0.20 | 4.32 ± 0.20 | 4.29 ± 0.15 | | 4.32 ± 0.06 | | |
| LVID;s | mm | 3.24 ± 0.24 | 3.25 ± 0.23 | 3.43 ± 0.18 | | 3.40 ± 0.11 | | |
| FS | % | 24.2 ± 2.1 | 25.4 ± 1.9 | 20.5 ± 2.0 | | 21.5 ± 1.4 | | |
| EF | % | 48.1 ± 3.5 | 50.1 ± 3.3 | 41.6 ± 3.5 | | 43.3 ± 2.5 | | |
| LV Mass | mg | 93 ± 12 | 87 ± 9 | 139 ± 9 | ** | 118 ± 4 | ** | † |
| Body Weight | g | 26.5 ± 1.9 | 25.8 ± 1.6 | 25.3 ± 1.1 | | 26.2 ± 0.8 | | |
| Heart Rate | BPM | 492 ± 14 | 493 ± 6 | 503 ± 13 | | 496 ± 9 | | |
| | | | | | | | | |
| 16 Weeks Post-Operative | | | | | | | | |
| *n* | | 8 | 8 | 14 | | 19 | | |
| PG | mm Hg | | | 60 ± 2 | | 63 ± 1 | | |
| LVPW;d | mm | 0.65 ± 0.02 | 0.61 ± 0.01 | 1.04 ± 0.04 | *** | 0.86 ± 0.02 | *** | ††† |
| LVPW;s | mm | 0.89 ± 0.03 | 0.84 ± 0.03 | 1.18 ± 0.03 | *** | 1.05 ± 0.04 | ** | † |
| LVAW;d | mm | 0.67 ± 0.02 | 0.64 ± 0.02 | 1.03 ± 0.04 | *** | 0.90 ± 0.03 | *** | † |
| LVAW;s | mm | 0.95 ± 0.05 | 0.92 ± 0.04 | 1.30 ± 0.04 | *** | 1.24 ± 0.04 | *** | |
| LVID;d | mm | 4.26 ± 0.12 | 4.41 ± 0.11 | 4.33 ± 0.09 | | 4.46 ± 0.09 | | |
| LVID;s | mm | 3.34 ± 0.08 | 3.50 ± 0.11 | 3.55 ± 0.10 | | 3.56 ± 0.11 | | |
| FS | % | 21.6 ± 1.3 | 20.7 ± 1.4 | 18.1 ± 1.0 | * | 20.9 ± 1.0 | | |
| EF | % | 43.8 ± 2.2 | 42.1 ± 2.5 | 37.6 ± 1.8 | * | 42.5 ± 1.8 | | |
| LV Mass | mg | 82 ± 5 | 82 ± 6 | 155 ± 11 | *** | 128 ± 5 | *** | † |
| Body Weight | g | 27.7 ± 1.7 | 26.9 ± 1.5 | 26.4 ± 0.8 | | 28.4 ± 0.9 | | |
| Heart Rate | BPM | 500 ± 6 | 501 ± 5 | 489 ± 13 | | 499 ± 6 | | |

Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. LVPW, Left ventricular posterior wall thickness; LVAW, left ventricular anterior wall thickness; LVID, Left ventricular internal diameter; d, diastole; s, systole; FS, fractional shortening; EF, ejection fraction; LV mass, calculated left ventricular mass. * *p*-values vs. sham-operated mice for same genotype; † *p*-values vs. control. All data are mean ± sem. No mice were studied that were deemed to be in extremis and potentially not able to survive echocardiography.

**Supplemental Table IX: Gene Expression - Two Week Pressure Overload**

| Gene | Protein | Sham | | TAC | |
|---|---|---|---|---|---|
| | | Control | mAKAP CKO | Control | mAKAP CKO |
| **Stress Markers** | | | | | |
| *Nppa* | Atrial Natriuretic Factor | 1.00 ± 0.36 | 1.13 ± 0.32 | 8.38 ± 1.80 * | 2.57 ± 0.87 † |
| *Nppb* | Brain Natriuretic Factor | 1.00 ± 0.05 | 0.77 ± 0.07 | 3.69 ± 0.53 * | 2.55 ± 0.68 |
| **Sarcomeric Proteins** | | | | | |
| *Acta1* | Skeletal Muscle α-Actin | 1.00 ± 0.21 | 0.62 ± 0.15 | 20.04 ± 2.97 ** | 4.08 ± 1.83 † |
| *Actc1* | Cardiac Muscle α-Actin | 1.00 ± 0.11 | 1.28 ± 0.17 | 1.56 ± 0.12 * | 1.11 ± 0.11 † |
| *Myh6* | Cardiac Muscle α-Myosin Heavy Chain | 1.00 ± 0.08 | 1.17 ± 0.22 | 1.05 ± 0.11 | 0.80 ± 0.07 |
| *Myh7* | Cardiac Muscle β-Myosin Heavy Chain | 1.00 ± 0.44 | 1.84 ± 0.46 | 3.83 ± 0.47 * | 2.11 ± 0.61 |
| *Tnni3* | Cardiac Muscle Troponin I | 1.00 ± 0.07 | 1.09 ± 0.09 | 0.83 ± 0.06 | 0.74 ± 0.04 |
| *Tnnt2* | Cardiac Muscle Troponin T | 1.00 ± 0.17 | 1.33 ± 0.09 | 1.27 ± 0.05 | 1.00 ± 0.07 † |
| *Capg* | Gelsolin-Like Capping Protein | 1.00 ± 0.09 | 1.05 ± 0.18 | 2.41 ± 0.30 * | 1.16 ± 0.25 † |
| **Calcium Cycling** | | | | | |
| *Atp2a2* | Sarco/Endoplasmic Reticulum $Ca^{2+}$-ATPase 2 | 1.00 ± 0.16 | 1.29 ± 0.13 | 1.10 ± 0.07 | 0.82 ± 0.03 † |
| *Cacna1c* | L-Type Channel Channel Subunit α1c | 1.00 ± 0.20 | 1.23 ± 0.23 | 1.34 ± 0.21 | 0.85 ± 0.02 |
| *Pln* | Phospholamban | 1.00 ± 0.47 | 1.47 ± 0.32 | 1.65 ± 0.08 | 1.27 ± 0.11 † |
| **Interstitial Fibrosis** | | | | | |
| *Col1a1* | Collagen Type I α1 | 1.00 ± 0.13 | 0.93 ± 0.18 | 4.53 ± 0.73 * | 1.43 ± 0.38 † |
| *Col1a2* | Collagen Type I α2 | 1.00 ± 0.14 | 1.05 ± 0.13 | 3.73 ± 0.55 * | 1.31 ± 0.37 † |
| *Col3a1* | Collagen Type III α1 | 1.00 ± 0.09 | 0.96 ± 0.17 | 4.97 ± 0.55 ** | 1.77 ± 0.54 † |
| *Col5a1* | Collagen Type V α1 | 1.00 ± 0.05 | 1.02 ± 0.28 | 3.62 ± 0.76 * | 1.26 ± 0.22 † |
| *Col6a1* | Collagen Type VI α1 | 1.00 ± 0.10 | 1.03 ± 0.14 | 2.61 ± 0.37 * | 1.23 ± 0.22 † |
| *Col8a1* | Collagen Type VIII α1 | 1.00 ± 0.27 | 1.33 ± 0.31 | 12.53 ± 2.67 * | 3.18 ± 1.17 † |
| *Tgfb1* | Transforming Growth Factor β1 | 1.00 ± 0.05 | 0.93 ± 0.11 | 1.74 ± 0.21 * | 1.02 ± 0.19 |
| *Tgfb2* | Transforming Growth Factor β2 | 1.00 ± 0.02 | 1.62 ± 0.67 | 5.84 ± 1.41 * | 2.02 ± 0.52 |
| *Postn* | Periostin | 1.00 ± 0.25 | 1.03 ± 0.28 | 12.38 ± 2.55 * | 2.96 ± 1.27 † |
| *Eln* | Elastin | 1.00 ± 0.06 | 1.30 ± 0.10 | 4.92 ± 1.12 * | 2.27 ± 0.50 |
| *Fbn1* | Fibrillin-1 | 1.00 ± 0.08 | 0.83 ± 0.26 | 4.03 ± 1.41 | 1.60 ± 0.38 |
| *Fgl2* | Fibrinogen-Like Protein 2 | 1.00 ± 0.60 | 1.58 ± 0.55 | 3.49 ± 0.53 * | 1.79 ± 0.26 † |
| *Fn1* | Fibronectin 1 | 1.00 ± 0.14 | 1.00 ± 0.21 | 3.57 ± 0.63 * | 1.33 ± 0.30 † |
| *Mfap5* | Microfibrillar Associated Protein 5 | 1.00 ± 0.24 | 0.98 ± 0.00 | 5.08 ± 0.50 ** | 1.59 ± 0.27 †† |
| *Mmp2* | Matrix Metallopeptidase 2 | 1.00 ± 0.09 | 1.00 ± 0.07 | 2.46 ± 0.22 ** | 1.08 ± 0.19 † |

| | | | | | |
|---|---|---|---|---|---|
| *Pcolce* | Procollagen C-Endopeptidase Enhancer | 1.00 ± 0.08 | 1.10 ± 0.14 | 2.68 ± 0.42 * | 1.23 ± 0.19 † |
| *Rtn4* | Reticulon 4 (A.K.A Nogo) | 1.00 ± 0.22 | 1.26 ± 0.13 | 3.06 ± 0.42 * | 1.43 ± 0.13 † |
| *Srf* | Serum Response Factor | 1.00 ± 0.14 | 1.21 ± 0.07 | 1.54 ± 0.18 | 0.95 ± 0.16 |
| **Signal Transduction** | | | | | |
| *Adra1a* | α1-Adrenergic Receptor | 1.00 ± 0.28 | 1.54 ± 0.20 | 0.81 ± 0.15 | 0.91 ± 0.01 |
| *Adrb1* | β1-Adrenergic Receptor | 1.00 ± 0.19 | 1.39 ± 0.43 | 1.68 ± 0.23 | 1.02 ± 0.03 |
| *Adrb2* | β2-Adrenergic Receptor | 1.00 ± 0.32 | 1.09 ± 0.09 | 1.89 ± 0.20 | 1.29 ± 0.24 |
| *Dusp4* | Dual Specificity Protein Phosphatase 4 | 1.00 ± 0.51 | 0.78 ± 0.10 | 1.08 ± 0.03 | 0.71 ± 0.17 |
| *Mapk1* | Extracellular Signal-Regulated Kinase 2 | 1.00 ± 0.19 | 1.27 ± 0.09 | 1.43 ± 0.04 | 1.03 ± 0.09 † |
| *Mapk3* | Extracellular Signal-Regulated Kinase 1 | 1.00 ± 0.06 | 0.99 ± 0.12 | 1.43 ± 0.19 | 0.94 ± 0.12 |
| *Mapk7* | Extracellular Signal-Regulated Kinase 5 | 1.00 ± 0.09 | 1.20 ± 0.29 | 1.99 ± 0.30 * | 1.11 ± 0.19 |
| *Rps6ka1* | Ribosomal S6 Kinase 1 | 1.00 ± 0.16 | 0.99 ± 0.17 | 1.43 ± 0.31 | 1.03 ± 0.19 |
| *Rps6ka3* | Ribosomal S6 Kinase 2 | 1.00 ± 0.39 | 1.35 ± 0.25 | 2.43 ± 0.14 * | 1.61 ± 0.22 † |
| *Fhl1* | Four And A Half LIM Domains Protein 1 | 1.00 ± 0.16 | 1.19 ± 0.14 | 4.43 ± 1.25 * | 1.43 ± 0.22 |
| *Rcan1* | Regulator Of Calcineurin 1 | 1.00 ± 0.14 | 1.07 ± 0.04 | 2.46 ± 0.36 * | 1.15 ± 0.14 † |

Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. All mice were 2 weeks post-operative. Left ventricular total RNA was assayed by NanoString technology for the indicated mRNAs. All data (mean ± s.e.m.) are fold-expression normalized to the mean for the control, sham-operated cohort. $n$ = 3 for all cohorts. Friedman Test across all four cohorts was significant ($p < 0.0001$). In pairwise comparisons using Dunn's Multiple Comparison Test, the following were significant: control TAC vs. any of the other three cohorts ($p < 0.001$); control Sham vs. mAKAP CKO TAC ($p < 0.05$); Sham vs. TAC for mAKAP CKO mice and control vs. mAKAP CKO for Sham mice were not significant. By Student's $t$-test for individual genes: * $p$-values vs. Sham control mice; $^{\dagger}p$-values vs. control TAC cohort.

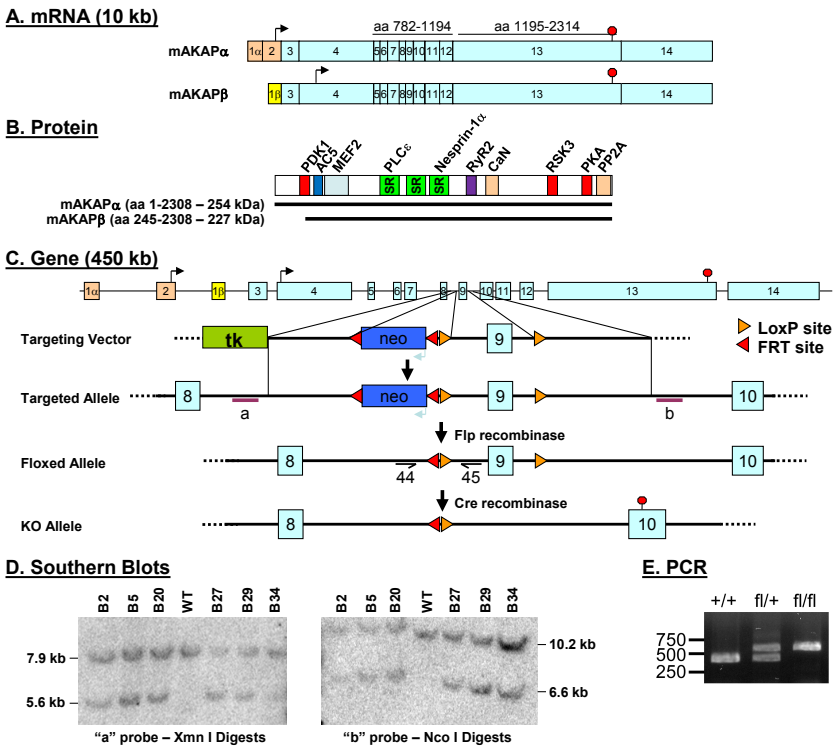
**Supplemental Table X: Antibodies used in this project**

| *Antigen* | *Species* | *Source* |
|---|---|---|
| mAKAP 245-340 | Rabbit FL100 | custom[9] |
| mAKAP 1406-2314 | Rabbit VO54 | custom[10] |
| mAKAP 1286-1831 | Rabbit OR010 | custom[11] |
| α-actinin | mouse monoclonal EA-53 | Sigma-Aldrich |
| ERK1/2 | Rabbit | Cell Signaling Technology 9102 |
| p44/p42 ERK Thr-202/Tyr-204 | Rabbit | Cell Signaling Technology 9101 |
| GSK-3β | Rabbit monoclonal 27C10 | Cell Signaling Technology 9315 |
| p-GSK-3β Ser-9 | Rabbit monoclonal 5B3 | Cell Signaling Technology 9323 |
| ERK5 | Rabbit | Cell Signaling Technology 3372 |
| p-ERK5 Tyr-218/Tyr-220 | Rabbit | Invitrogen 44-612G |
| RSK3 (detects all RSK family members)[9] | Mouse 1F6 | Abnova monoclonal H00006196-M01 |
| p-RSK3 Ser-218 (detects all RSK family members)[9] | Rabbit | R&D Systems AF893 |
| PKD/PKCμ | Rabbit | Cell Signaling Technology 2052 |
| p-PKD/PKCμ Ser-744/Ser-748 | Rabbit | Cell Signaling Technology 2054 |
| p-PKD/PKCμ Ser-916 | Rabbit | Cell Signaling Technology 2051 |
| MEF2D | Rabbit | Novus Biologicals NBP1-80567 |
| MEF2D | Mouse | BD Biosciences 610775 |
| NFATc2 | Rabbit monoclonal D43B1 | Cell Signaling Technology 5861 |
| HDAC4 | Rabbit | Cell Signaling Technology 7628 |
| HDAC4 | Mouse | Santa Cruz sc-46672 |
| HDAC5 | Rabbit | Cell Signaling Technology 2082 |
| HDAC5 | Mouse | Santa Cruz sc-133225 |
| p-HDAC4(Ser246)/HDAC5(Ser259)/HDAC7(Ser155) | Rabbit monoclonal D27B5 | Cell Signaling Technology 3443 |
| p-HDAC4(Ser632)/HDAC5(Ser498)/HDAC7(Ser486) -detects p-HDAC4 Ser467/632 and p-HDAC5 Ser 498/661. | Rabbit | Cell Signaling Technology 3424 |
| HIF-1α | Rabbit | Santa Cruz sc-10790 |

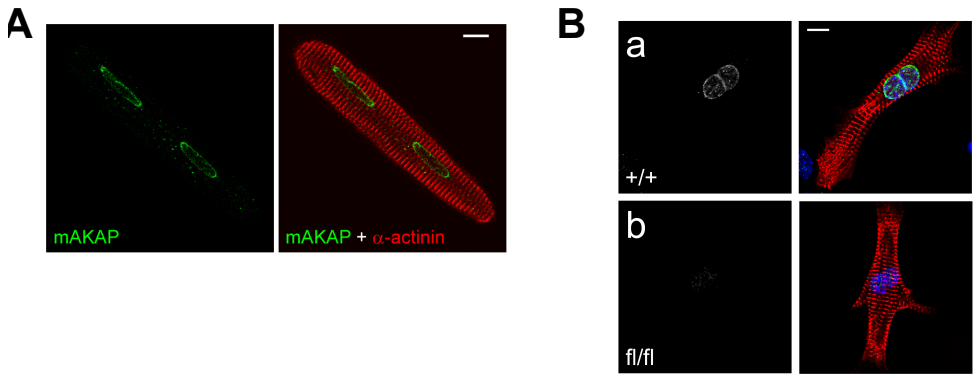
**Supplemental Table XI: Oligonucleotides used in this project for mouse genotyping**

| *Name* | *Sequence* |
|---|---|
| For mAKAP Floxed allele: | |
| VS1444 (“44”) | GCAGAGATAGGCAGGTAGACAG |
| VS1445 (“45”) | CACTTCCCAGGTTTGGAAAGC |
| VS369 (neo cassette) | CTGCCATAGCCACTCGAGAAG |
| VS1497 | ACCACTTCCCAACTCACGTAAC |
| VS1498 | TGTTATTTGGCAACGTGACTCC |
| | |
| For MCM transgene: | |
| HGH down | CGT CCT CCT GCT GGT ATA G |
| HGH up | GTC TGA CTA GGT GTC CTT CT |
| | |
| For NFAT-luc reporter: | |
| Luc down | AGATGAGATGTGACGAACGTG |
| Luc up | ATCCGCTGGAAGATGGAACCG |

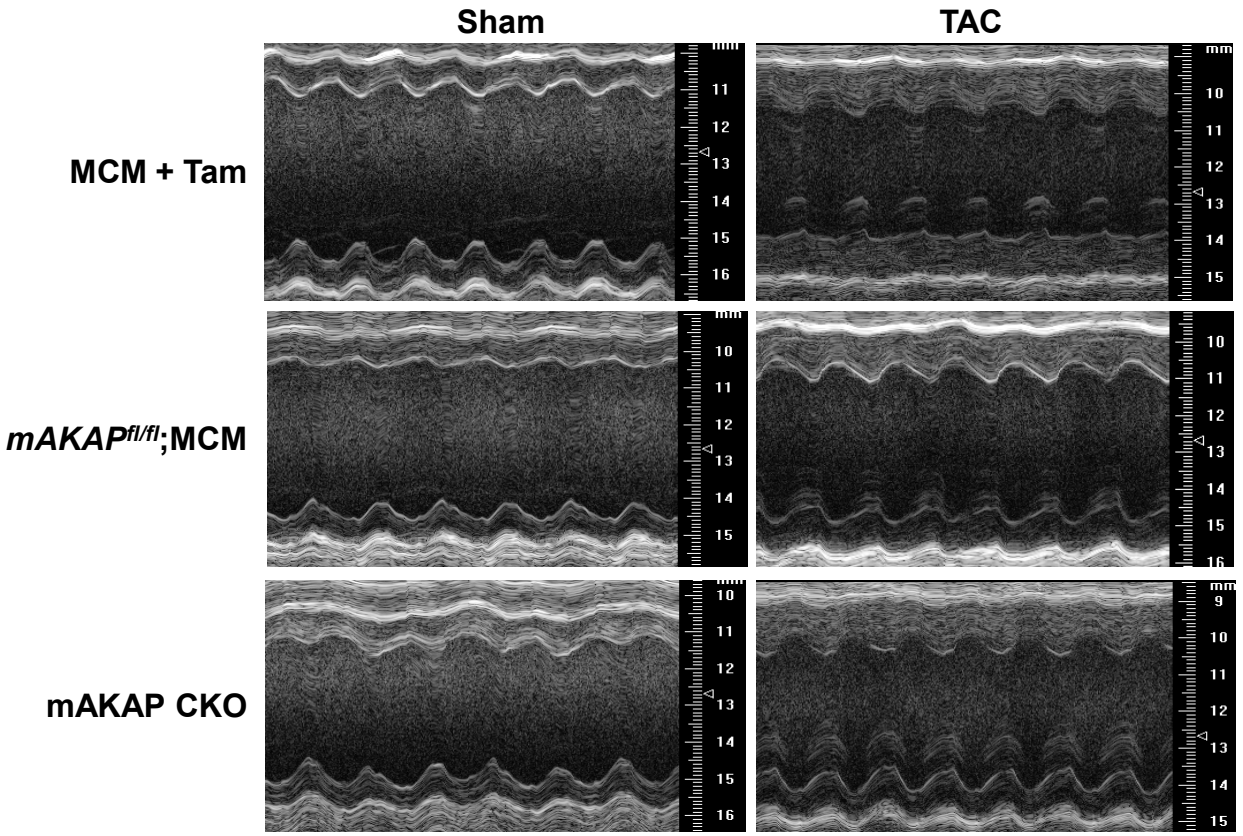
# Supplemental Figures



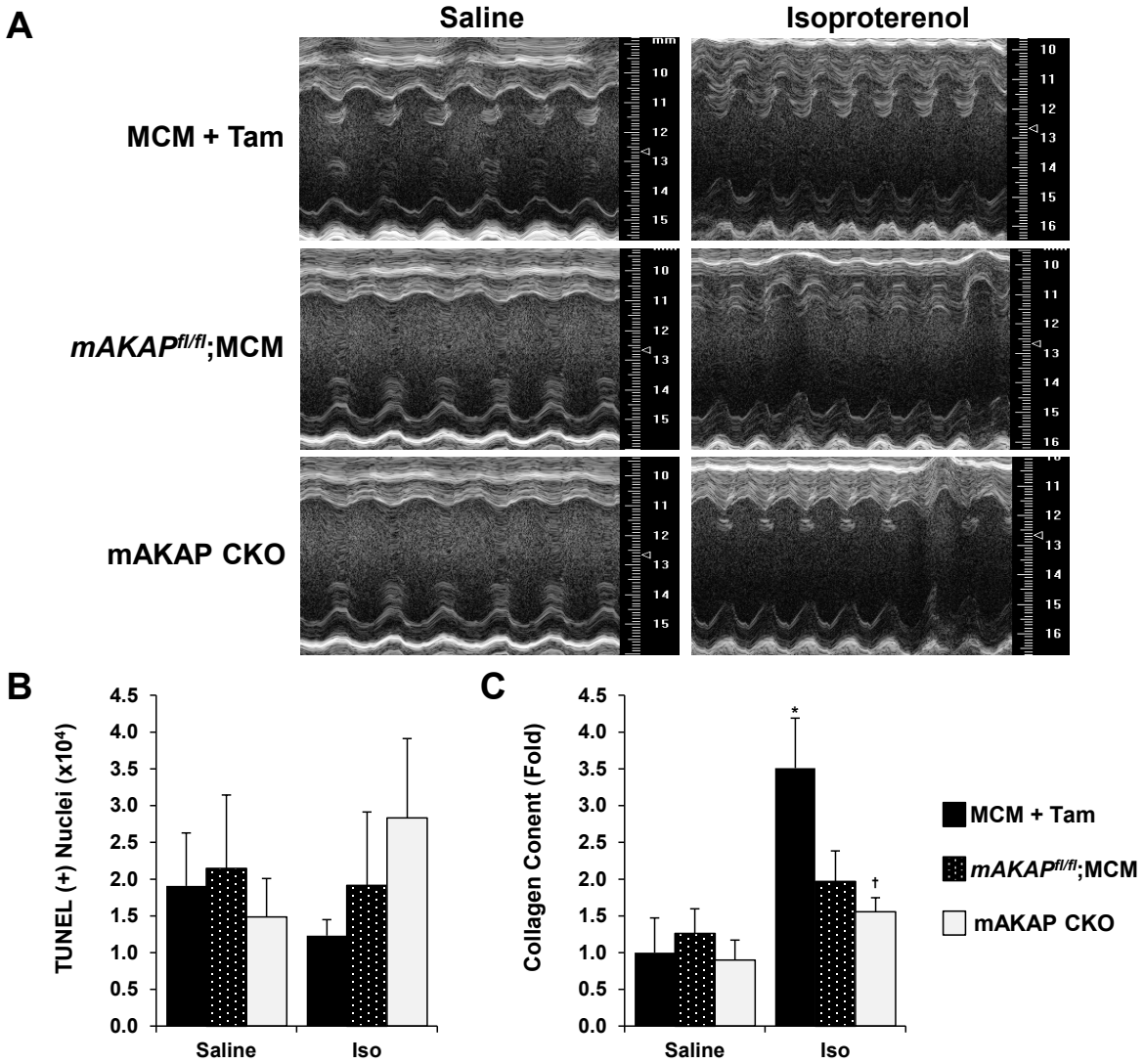

Supplemental Figure I. Generation of mAKAP conditional knock-out mouse. (A) mAKAP is expressed as one of two alternatively-spliced forms: mAKAPα in neurons, mAKAPβ in cardiac and skeletal muscle myocytes.[12] Exons are numbered. Alternatively spliced exons are in beige and yellow. Arrow and red circles indicate translation start and stop codons, respectively. (B) mAKAPβ is identical to mAKAPα 245-2308. Sites for proteins whose binding sites have been mapped are shown.[6, 9-17] AC5, adenylyl cyclase 5; CaN, calcineurin; MEF2, myocyte-enhancer factor-2; PDK1, phosphoinositide-dependent kinase 1; PKA, protein kinase A; PLCε. phospholipase Cε; PP2A, protein phosphatase 2A; RSK3, type 3 p90 ribosomal S6 kinase; RyR2, ryanodine receptor; SR, spectrin-like repeat domains. (C) Gene structure and design of floxed allele. Neomycin resistance (neo) and thymidine kinase (tk) were used for positive and negative selection during homologous recombination. FRT sites are recombination sites for Flp recombinase. LoxP sites are recombination sites for cre recombinase. Deletion of exon 9 results in a frame-shift and premature termination of translation within exon 10. (D) Southern blots probes with the fragments "a" and "b" showing that six ES cell lines were targeted. (E) Tail genomic DNA from a wildtype, heterozygous and homozygous mAKAP$^{fl}$ mouse analyzed by PCR using primers 44 and 45. mAKAPβ knock-out in Tam-treated *mAKAP*$^{fl/fl}$;MCM mice was verified by western blotting as shown in Figure 4A. No residual small mAKAPβ fragments or other proteins were detected using antibodies to multiple mAKAP domains (FL100, OR010, and VO54) in these mice (Figures 1 and 4 and Supplemental Figure II and data not shown).



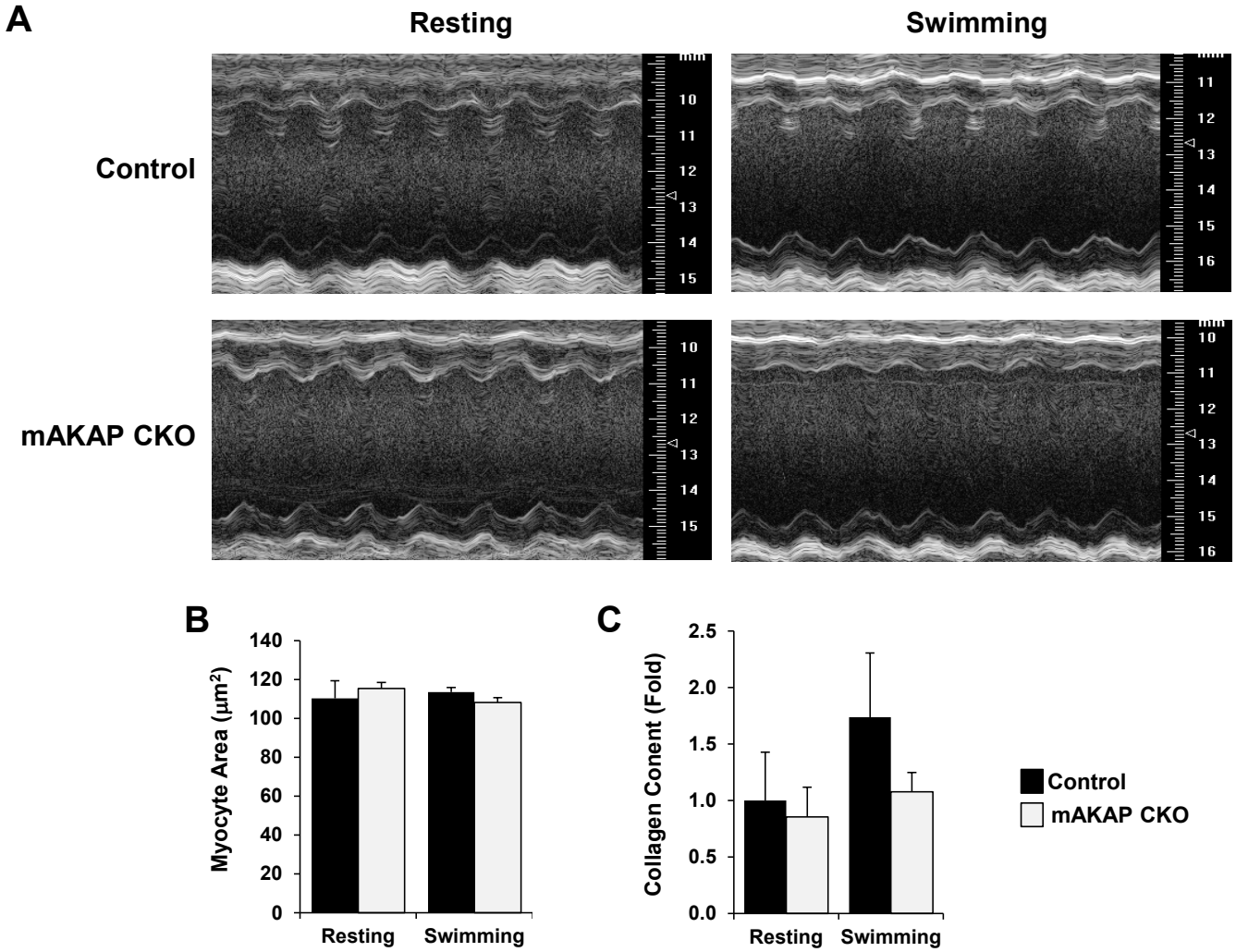

Supplemental Figure II. mAKAPβ is located at the nuclear envelope in mouse myocytes. (A) Adult rat ventricular myocyte stained with mAKAP OR010 (green) and α-actinin (red) antibodies. (B) Ventricular myocytes were isolated from *mAKAP*$^{+/+}$ (a) and mAKAP$^{fl/fl}$ (b) neonatal mice and infected with adenovirus that constitutively express cre recombinase. After 9 days, the myocytes were stained with mAKAP OR010 (green) and α-actinin (red) antibodies and Hoechst nuclear stain (blue). The mAKAP channel is reproduced in grayscale to the left of each composite, confocal fluorescent image. No significant mAKAP staining was detectable for α-actinin-positive, infected *mAKAP* $^{fl/fl}$ myocytes. Cultures were obtained from 4 mice of each genotype. All images were acquired with a confocal microscope. Bar = 10 μm for both panels. We have previously shown that mAKAPβ is targeted to the nuclear envelope by direct binding to the integral membrane protein nesprin-1α.[11]



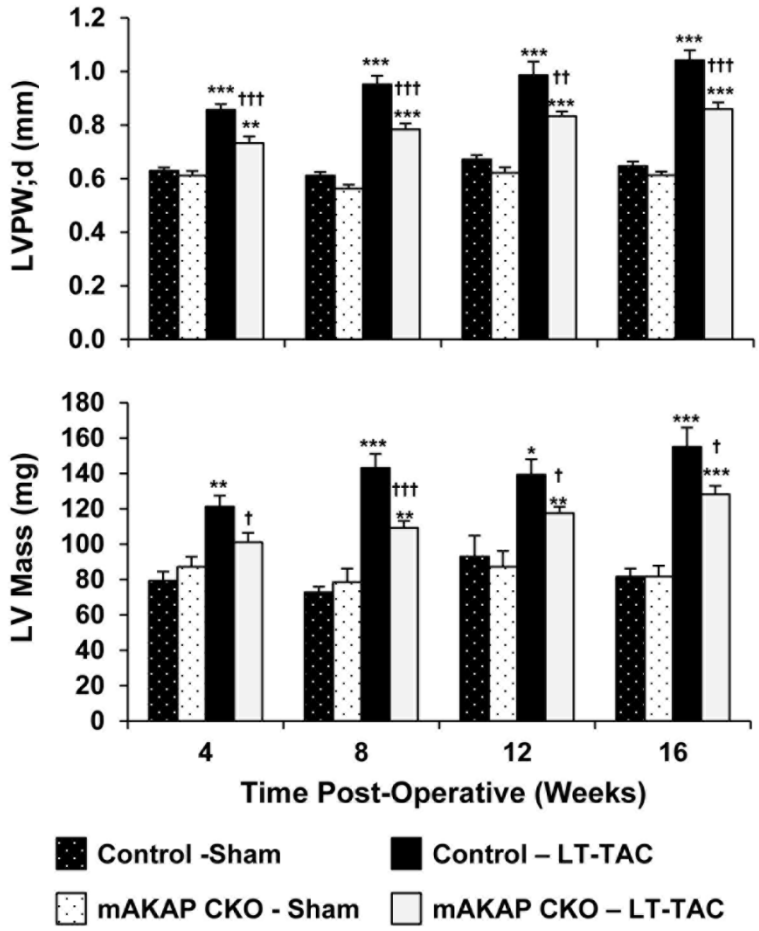

Supplemental Figure III. Representative M-mode echocardiography of 2-week TAC and sham-operated mice. Cf. Supplemental Table I.



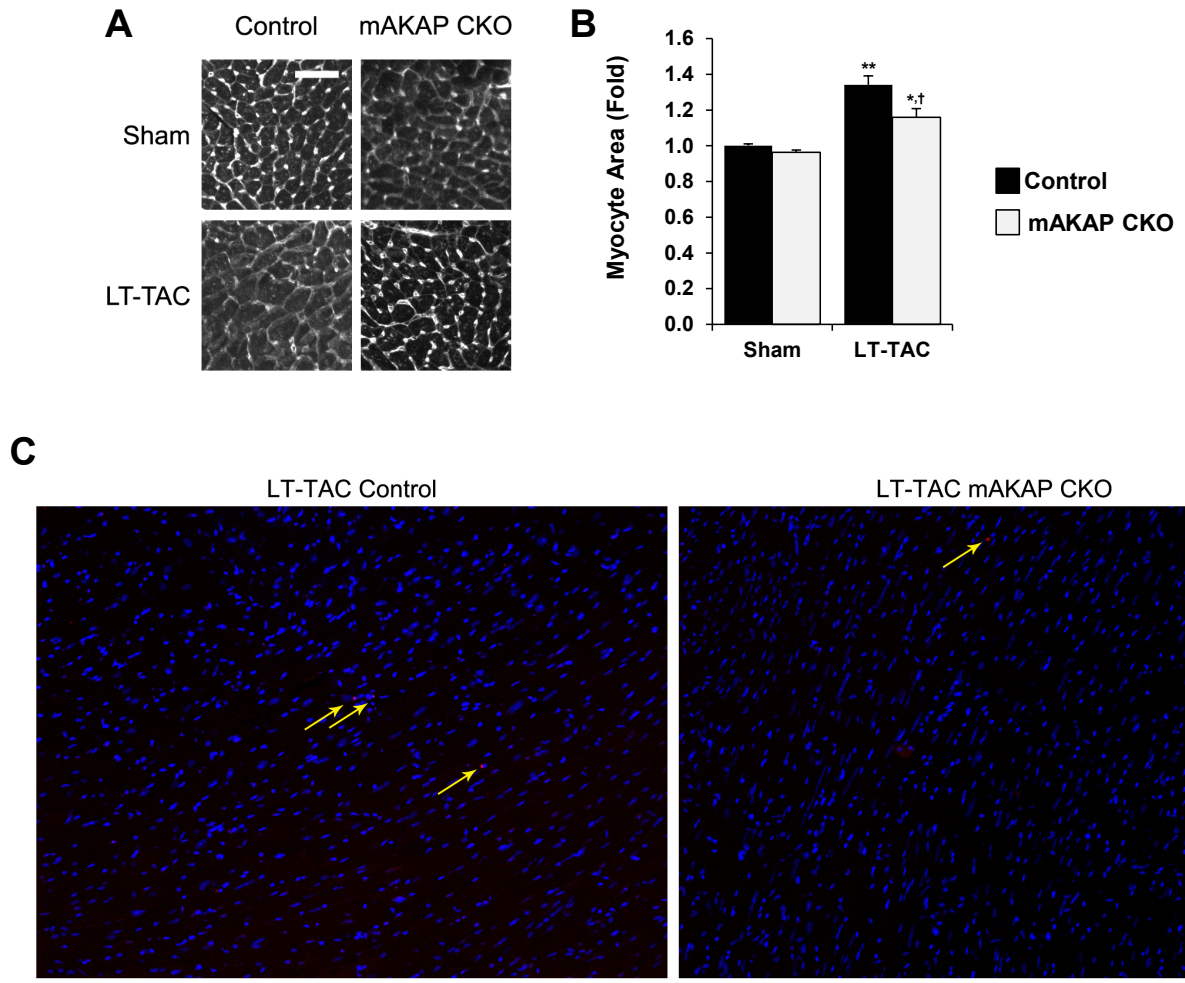

Supplemental Figure IV. Additional data for mice stressed by chronic catecholamine exposure. (A) Representative M-mode echocardiography of Iso and saline-infused mice. Cf. Supplemental Table III. (B) No significant cell death as measured by TUNEL assay was induced by Iso infusion for two weeks. *n*=4 (C) Collagen content was assayed using Picrosirius Red stained sections. Fibrosis was significantly induced by Iso only for the MCM+Tam cohort. * *p*-values vs. saline-infused mice of same genotype; † *p*-values vs. MCM+Tam. All data are mean ± sem. *n*=4-8.



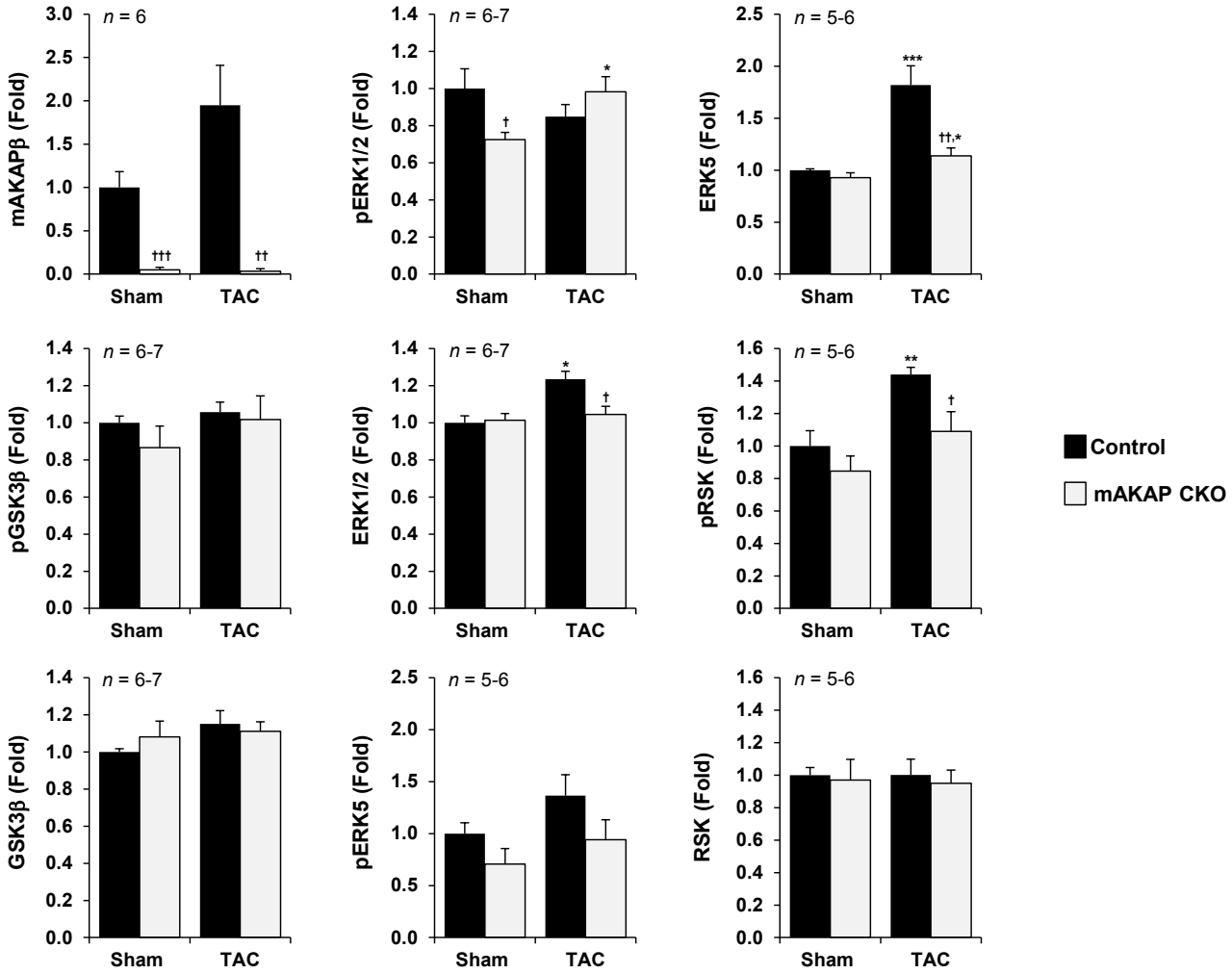

Supplemental Figure V. Additional data for mice conditioned by swimming. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. (A) Representative M-mode echocardiography for mice resting or swimming for five weeks. Cf. Supplemental Table V. (B) Myocyte cross-section area was measured by wheat-germ agglutinin-staining of heart sections. (C) Collagen content was assayed using Picrosirius Red stained sections. *n*=3-7. No significant myocyte hypertrophy or interstitial fibrosis was detected.



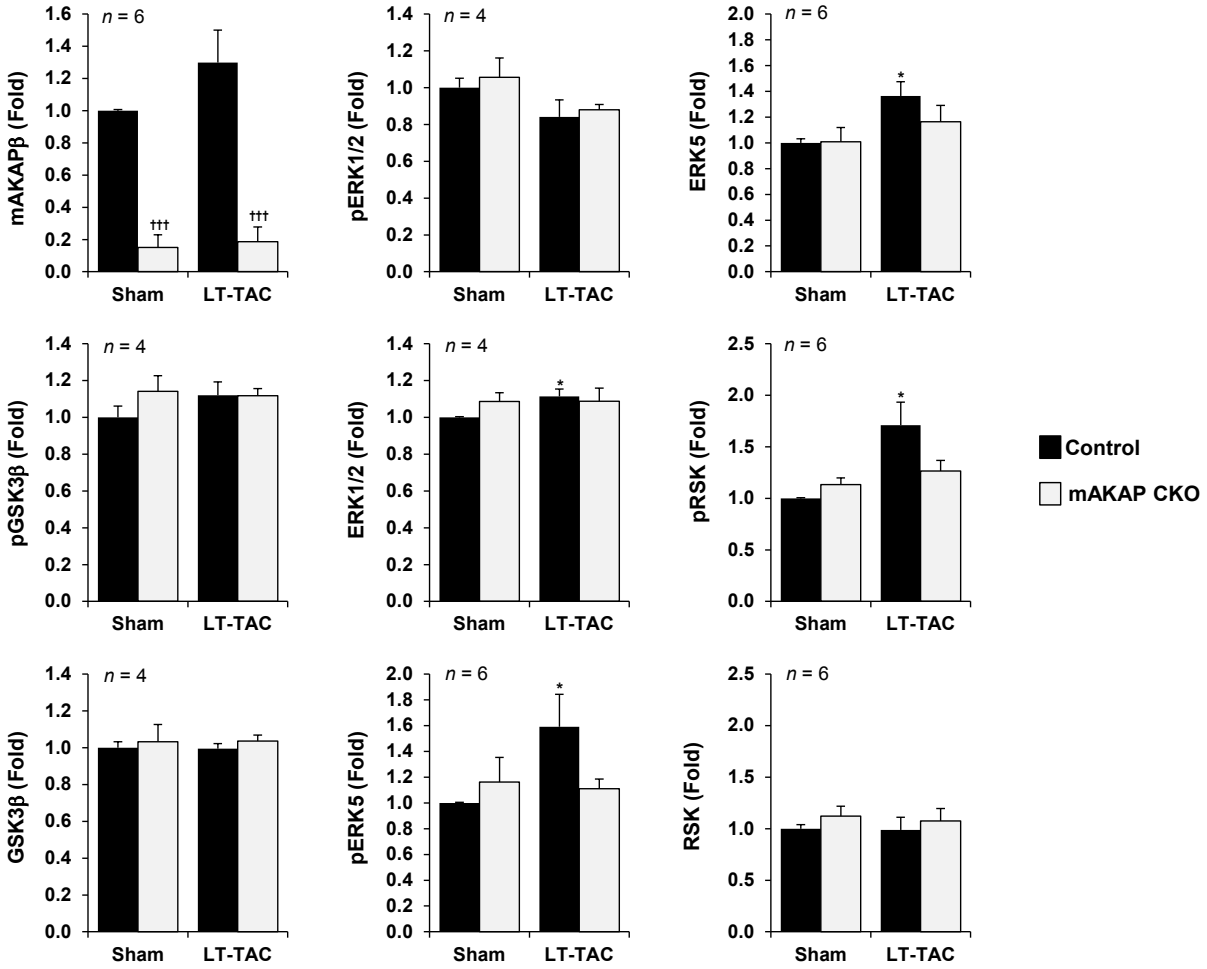

Supplemental Figure VI. Long-term TAC induces a progressive left ventricular hypertrophy attenuated by mAKAPβ knock-out. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. Left ventricular posterior wall thickness in diastole (LVPW;d) and calculated left ventricular mass (LV Mass) determined by M-mode echocardiography are shown. **p*-values vs. sham-operated mice of the same genotype; $^{\dagger}$ *p*-values vs. control cohort. Cf. Supplemental Table VIII for full echocardiographic data.



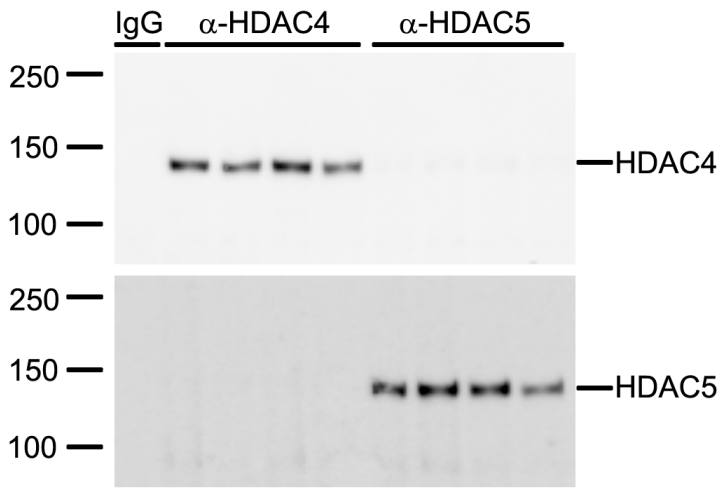

Supplemental Figure VII. Additional data for mice stressed by long-term pressure overload. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. (A) Wheat-germ agglutinin-stained heart sections. Bar = 50 μm. (B) Quantitation of wheat-germ agglutinin-stained heart sections for myocyte cross-section area. *n*=4-6. * *p*-values vs. sham-operated mice of same genotype; † *p*-values vs. control. All data are mean ± sem. (C) Representative TUNEL stained sections showing nuclei (blue) and TUNEL staining (red). Positive nuclei are indicated by the yellow arrows. Bar = 100 μm. Quantification is presented in Fig. 3F.



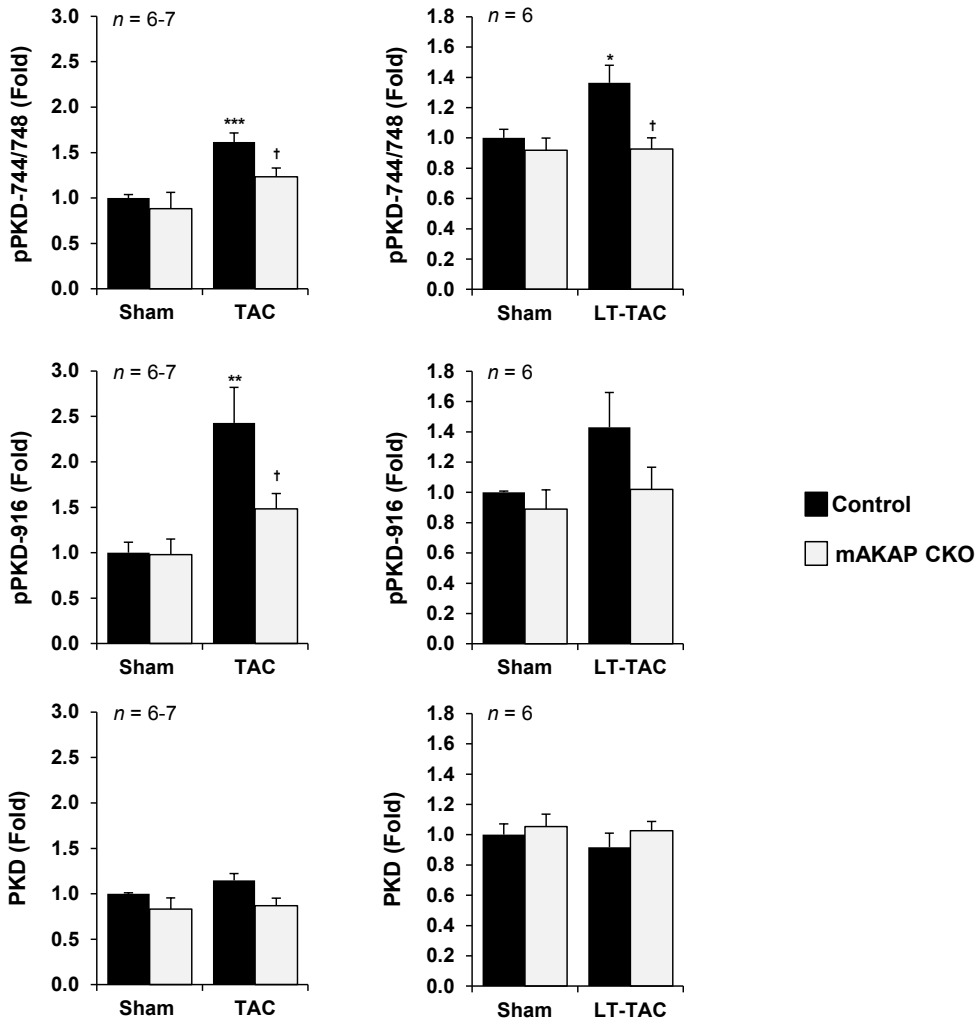

Supplemental Figure VIII. Quantification of western blots for Figure 4A. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. All mice were two-weeks post-TAC or sham operation. All data are mean fold + s.e.m. compared to the mean for control, sham-operated mice. Data regarding phospho-proteins are not normalized to the values for total protein and represent the total amount of phosphorylated species present in the extracts. * *p*-values vs. sham-operated mice of the same genotype; † *p*-values vs. control cohort.



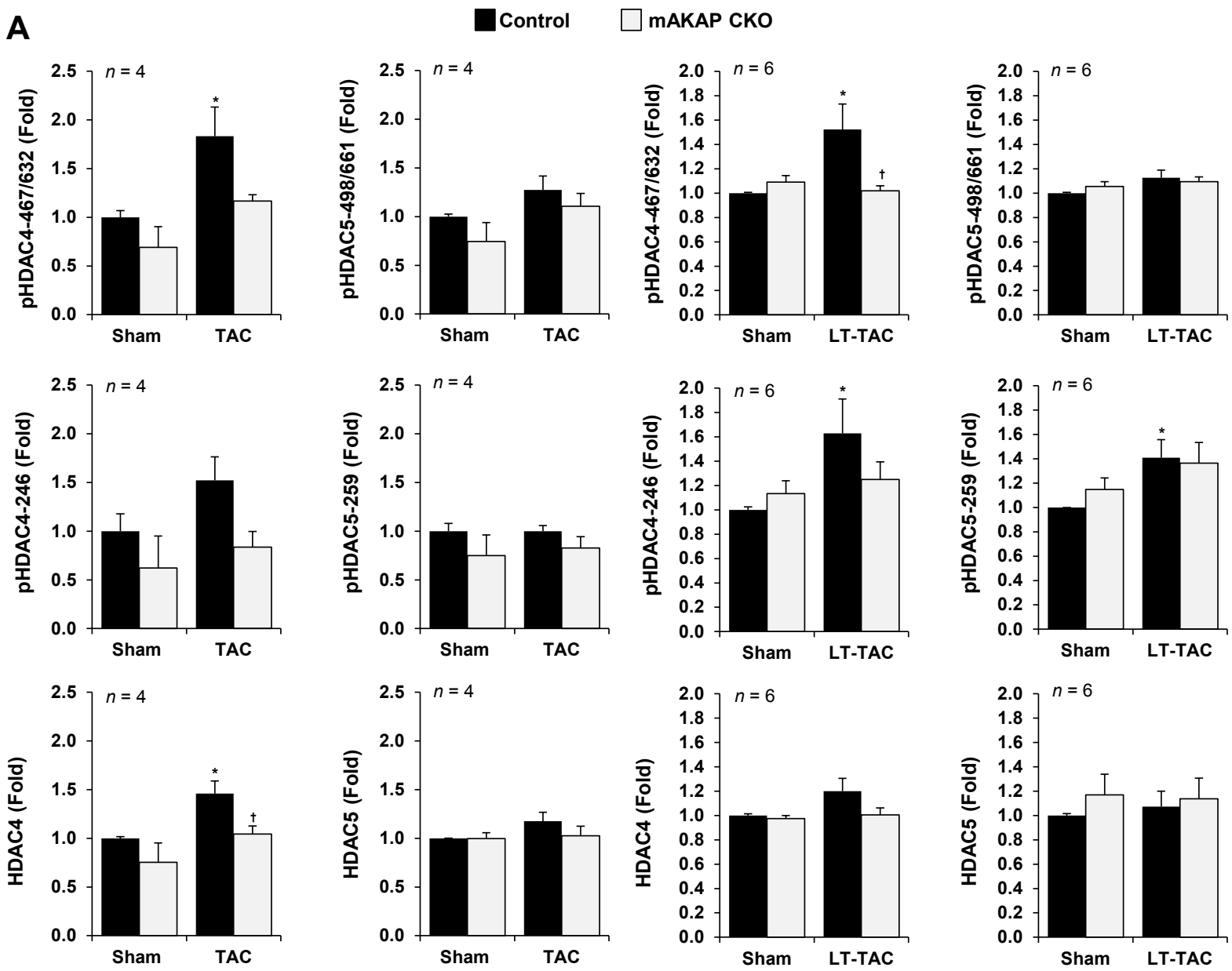

Supplemental Figure IX. Quantification of western blots for Figure 4B. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. All mice were 16-weeks post-TAC or sham operation. All data are mean fold + s.e.m. compared to the mean for control, sham-operated mice. Data regarding phospho-proteins are not normalized to the values for total protein and represent the total amount of phosphorylated species present in the extracts. * *p*-values vs. sham-operated mice of the same genotype; † *p*-values vs. control cohort.

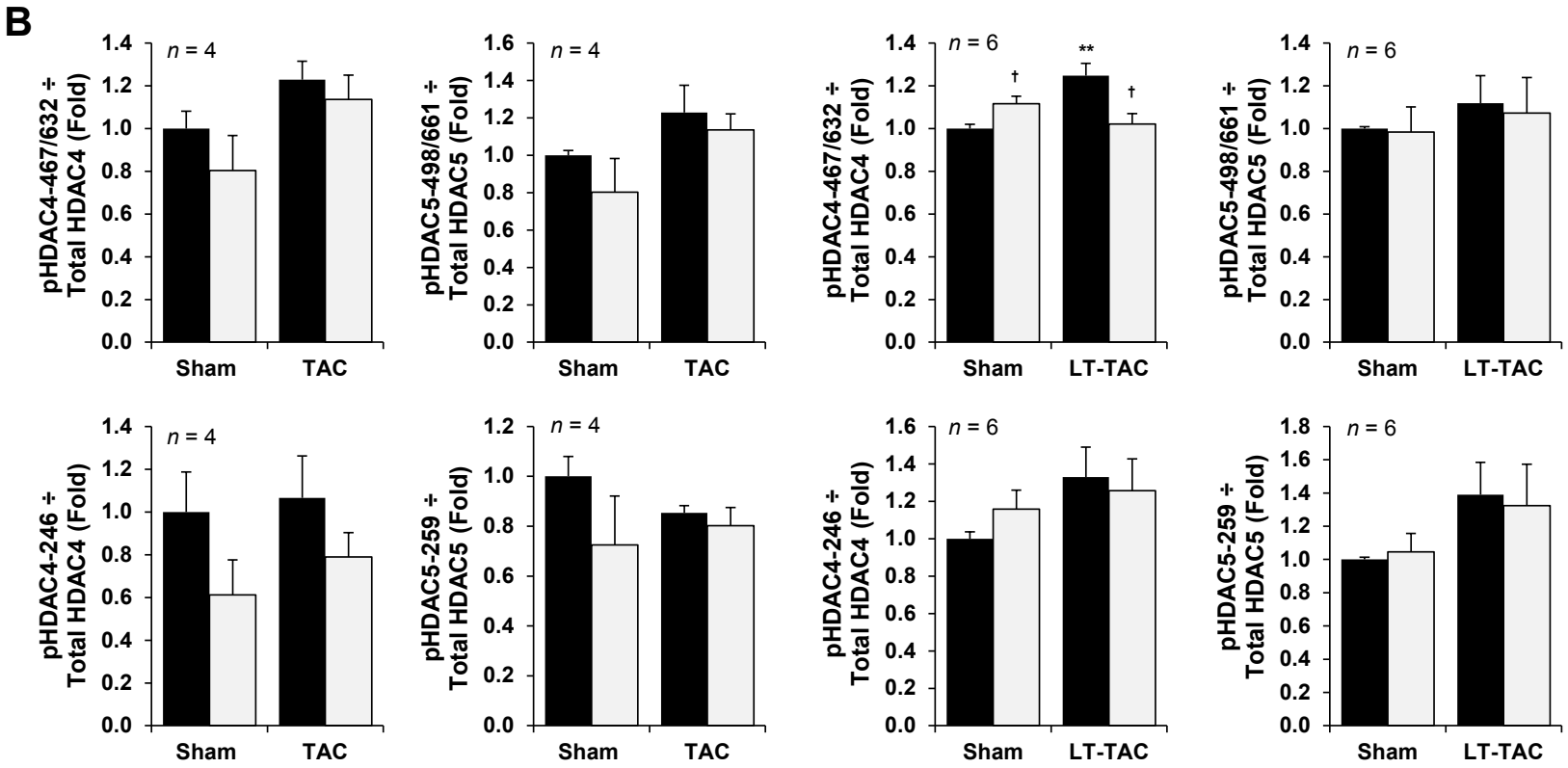



Supplemental Figure X. Specificity of immunoprecipitation-western blots for HDAC4 and HDAC5. Protein complexes in heart extracts were immunoprecipitated with either IgG, HDAC4, or HDAC5 antibodies, and proteins detected using HDAC4 and HDAC5 antibodies. Four independent immunoprecipitation reactions are shown.

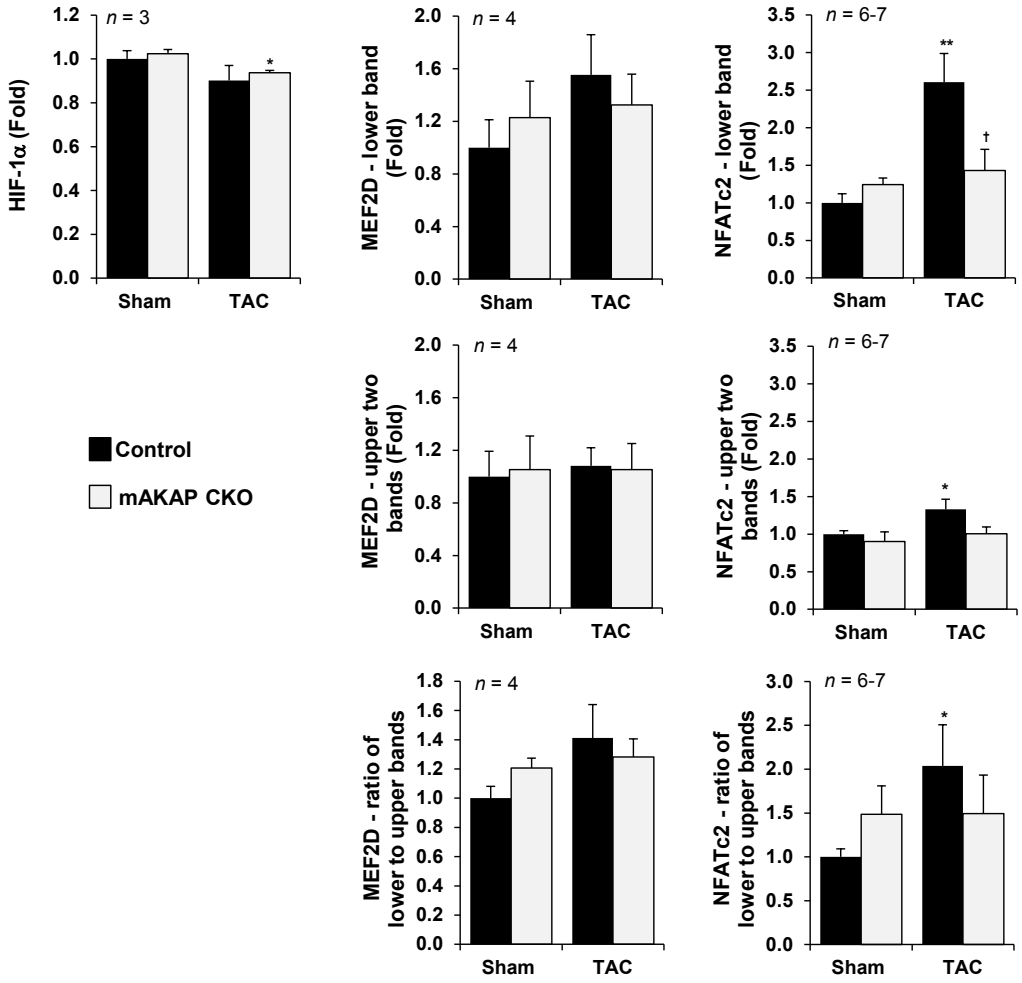



Supplemental Figure XI. Quantification of western blots for Figure 5E. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. Mice were 2 weeks (left graphs, TAC) or 16 weeks (right graphs, LT-TAC) post-TAC or sham operation. All data are mean fold + s.e.m. compared to the mean for control, sham-operated mice. Data regarding phospho-proteins are not normalized to the values for total protein and represent the total amount of phosphorylated species present in the extracts. * *p*-values vs. sham-operated mice of the same genotype; † *p*-values vs. control cohort.

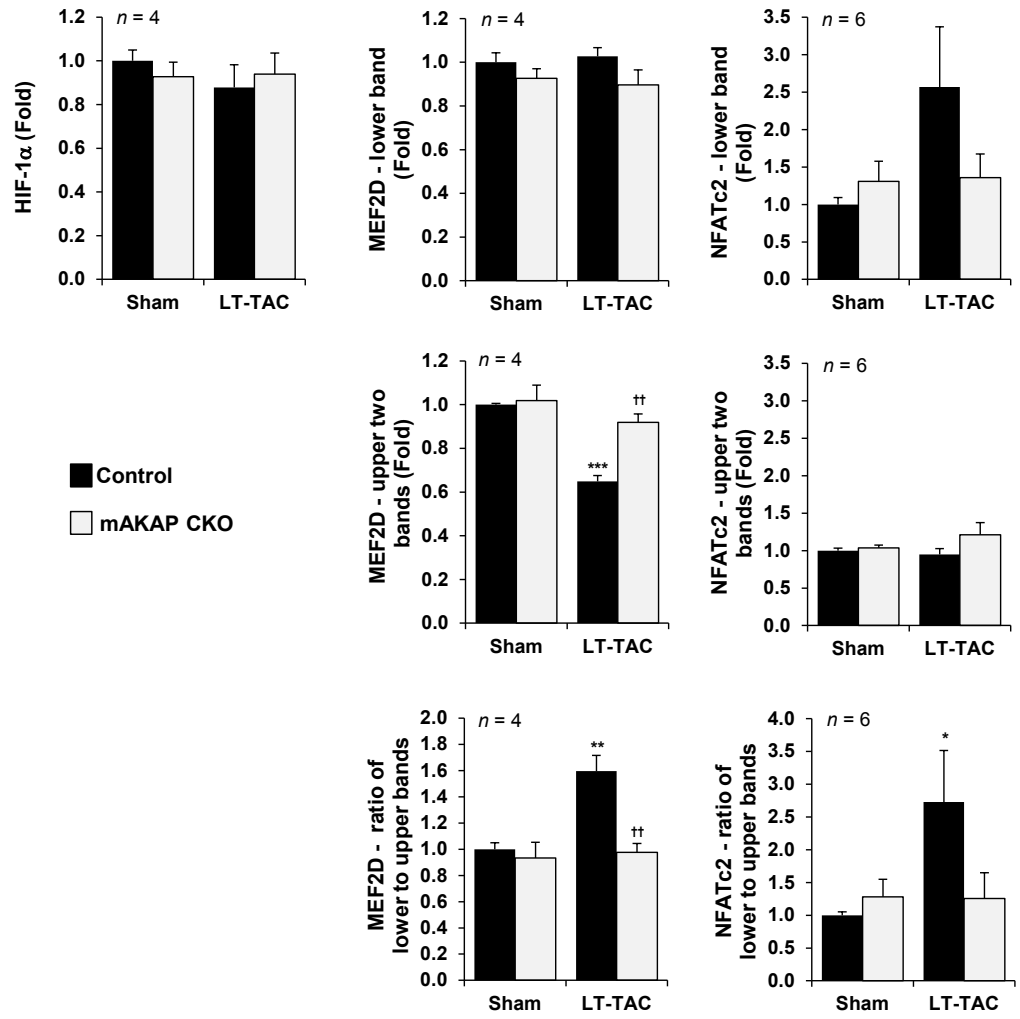

A
Control
mAKAP CKO
pHDAC4-467/632 (Fold)
pHDAC5-498/661 (Fold)
pHDAC4-467/632 (Fold)
pHDAC5-498/661 (Fold)
pHDAC4-246 (Fold)
pHDAC5-259 (Fold)
pHDAC4-246 (Fold)
pHDAC5-259 (Fold)
HDAC4 (Fold)
HDAC5 (Fold)
HDAC4 (Fold)
HDAC5 (Fold)
n = 4
n = 6
Sham
TAC
LT-TAC
*
†



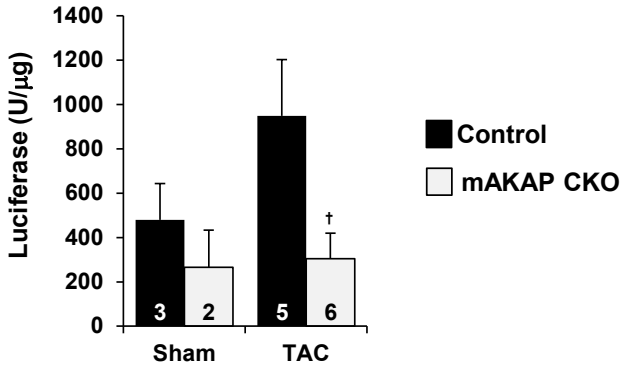

Supplemental Figure XII. Quantification of western blots for Figure 5F. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. Mice were 2 weeks (left graphs, TAC) or 16 weeks (right graphs, LT-TAC) post-TAC or sham operation. (A) All data are mean fold + s.e.m. compared to the mean for control, sham-operated mice. Data in these graphs regarding phospho-proteins are not normalized to the values for total protein and represent the total amount of phosphorylated species present in the extracts. (B) Phosphorylation of HDAC4 and HDAC5 after normalization to HDAC expression. * *p*-values vs. sham-operated mice of the same genotype; † *p*-values vs. control cohort.




Supplemental Figure XIII. Quantification of western blots for Figure 6A. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. Mice were 2 weeks post-TAC or sham operation. All data are mean fold + s.e.m. normalized to the mean for control, sham-operated mice. * *p*-values vs. sham-operated mice of the same genotype; † *p*-values vs. control cohort. For MEF2D and NFATc2, the lowest band was measured by densitometry separately from the upper bands.




Supplemental Figure XIV. Quantification of western blots for Figure 6B. Control mice were MCM + Tam, and mAKAP CKO were mAKAP$^{fl/fl}$;MCM + Tam. Mice were 16 weeks post-TAC or sham operation. All data are mean fold + s.e.m. normalized to the mean for control, sham-operated mice. * *p*-values vs. sham-operated mice of the same genotype; $^{\dagger}$ *p*-values vs. control cohort. For MEF2D and NFATc2, the lowest band was measured by densitometry separately from the upper bands.




Supplemental Figure XV. mAKAPβ knock-out inhibits the expression of a NFAT-luciferase reporter construct in mice following short term pressure overload. Additional mAKAP$^{fl/fl}$;MCM mice were bred to mice bearing a NFAT reporter transgene, in which luciferase activity is increased in the heart following the activation of NFATc transcription factors.[3] The cohorts studied were MCM; Tg(Myh6/NFAT-luc) + Tam (control), and mAKAP$^{fl/fl}$;MCM; Tg(Myh6/NFAT-luc) + Tam (mAKAP CKO). Mice were two-weeks post-TAC or sham operation. Data are presented as relative luciferase units per µg protein in total heart extracts. † *p*-value vs. control cohort. *n* as indicated on the bars.

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