SUPPLEMENTAL MATERIAL

Methods

Animals

Generation of MFAP4 knockout mice and C57BL6/N control (WT) mice was previously described (Drs. Schlosser and Sorensen) [13,14,17]. Male and female mice, 10 to 24 weeks old, were used in this study.

Cardiac injury was induced by transverse aortic constriction (TAC) to produce pressure overload as described previously [43]. In brief, the transverse aortic arch was visualized through a median sternotomy, and a 7-0 silk ligature was tied around the aorta using a 27-gauge wire to obtain a defined degree of constriction between the right brachiocephalic and left common carotid arteries. Antibody treatments were performed in 10-week-old wild type animals starting at 48 hours post-TAC through intraperitoneal injections. Neutralizing MFAP4 (house-made HG-HYB 7-14) and isotype-matched control IgG (Santa Cruz Biotechnology SC-2025) were administered at a dose of 100 μg/kg every 9 days. Infusion of angiotensin II (1.5 μg/kg of body weight/min, dissolved in sterile saline) (Sigma-Aldrich A9525) or vehicle (sterile saline) was performed with implantation of Alzet minipumps for 1 week (Durect Inc 1007D). Age-matched WT and MFAP4 KO mice were randomly assigned to TAC, sham, vehicle, and AngII groups prior to any procedure. No animals were excluded from this study.

Phenylephrine (PE) dissolved in sterile saline was injected intraperitoneally (IP) at 15 mg/kg of body weight, in 100 μl total volume (Sigma-Aldrich 1533002). After 0, 5, or 15 minutes, animals were placed into an isoflurane chamber containing 3% isoflurane for 2 minutes, then moved to a nose cone under 3% isoflurane for 1 minute. Adequate anesthesia was confirmed using bilateral toe pinch. The heart was removed and placed in liquid nitrogen at exactly 70 seconds following transfer to the nose cone.

Echocardiographic measurements were taken using a Vevo3100 Visual Sonics (Visual Sonics) system and MS-550 transducer. The mice were lightly anesthetized (1.5% isoflurane) and the left ventricular ejection fraction was determined in the M-mode using the parasternal short-axis view at the level of the papillary muscles. Ejection fraction was calculated from the average of at least three consecutive cardiac cycles using the VevoLAB program. All experiments involving animals were approved by the Institutional Animal Care and Use Committee at The Ohio State University.

Primary Cell Isolation and Culture

Rat primary aortic endothelial cells (ECs) were purchased from Cell Biologics (RA-6052). Cells were cultured at 37°C with 5% CO_2 in Complete Rat Endothelial Cell Medium containing growth factor supplements and 2% fetal bovine serum (FBS) (Cell Biologics M1266, Gibco 26140079). Trypsin-EDTA 0.25% (Gibco 25200056) was used for cell detachment. Cells used for experiments were at passages 5-9.

Mouse aorta/smooth muscle cells (MOVAS, SMCs) were purchased from ATCC (CRL-2797). Cells were cultured at 37°C with 5% CO_2 in Dulbecco's Modified Eagle Medium with 10% bovine growth serum and 1% penicillin-streptomycin mix (Gibco 11995073, Gibco 16170078, Gibco 15140163). Trypsin-EDTA 0.25% (Gibco 2520056) was used for cell detachment. Cells used for experiments were at passages 2-4.

For neonatal rat ventricular cardiomyocytes isolations, hearts were incubated with trypsin at 4°C overnight, followed by trypsin inhibitor and collagenase incubation at 37°C for 1 hour (Worthington Biochemical LS003703, LS003587, LK003240). Hearts were mechanically dissociated and incubated for an additional 15 minutes, followed by filtration through a 70 μm cell strainer (Corning 431751) and three rounds of sedimentation and resuspension in media supplemented with bovine growth serum. The supernatant from the resuspensions was collected for cardiac fibroblast culture (below). Cardiomyocytes were plated on 0.1% gelatin-coated dishes in the presence of 15% bovine growth serum and 1% penicillin-streptomycin mix (Gibco 16170078, Gibco 15140163). The following day, cells were washed twice with sterile PBS and cultured in M199 media without serum plus 1% penicillin-streptomycin mix or media derived from transfected ECs as described below (Corning 10-060-CV). Each preparation of neonatal ventricular cardiomyocytes consisted of cells derived by independent rat litters and each litter consisted of 10-14 pups. Cells derived from each rat were independently prepared and a minimum of three litters was used to complete each experiment. Neonatal rat cardiac fibroblasts from neonatal rat cardiomyocyte isolations were allowed to adhere to uncoated 10 cm dishes and cultured in Dulbecco's Modified Eagle Medium with 10% bovine growth serum and 1% penicillin-streptomycin mix (Gibco 11995073).

Adult mouse cardiomyocytes were isolated from WT animals as follows. Mice were deeply anesthetized with 5% isoflurane and 1 L/min O_2, the heart was rapidly excised, and the aorta was cannulated using a 22g cannula on a constant-flow Langendorff perfusion apparatus. Hearts were perfused for 2 minutes with Tyrode's solution (10 mM glucose, 5 mM HEPES, 5.4 mM KCl, 1.2 mM

$MgCl_2$, 150 mM NaCl, 2 mM sodium pyruvate, pH 7.4) and then digested for 10 minutes with Tyrode's solution containing .25 mg/mL Liberase DH (Roche, 5401054001). The ventricles were then minced in the digestion solution, filtered, and the resulting cardiomyocytes were equilibrated and sedimented in Tyrode's solution containing bovine serum albumin (BSA) and 1 mM $CaCl_2$ at room temperature.

Cell Treatments

For experiments, ECs were cultured in 6-well plates in Endothelial Cell Culture Basal Medium (Cell Biologics M1266b) with no serum and transfected with 3 μg of either rat MFAP4 or pEGFP control using Lipofectamine 3000 Reagent (Invitrogen L3000015). Cells were cultured for 48 hours to allow for protein production. Media was then collected from ECs and diluted 1:1 with M199 media without serum for cardiomyocyte treatments.

Hypertrophy was induced by culturing cardiomyocytes in the presence of 100 μM phenylephrine diluted in sterile saline (Sigma-Aldrich P6126). Cells were harvested 48 hours following treatments for either RNA isolation (described below) or immunofluorescence imaging. For immunofluorescence, cardiomyocytes were washed twice with sterile PBS and fixed for 20 minutes at room temp in 2% paraformaldehyde diluted in sterile PBS. All cultured cross-sectional cell area data and associated mRNA analyses are representative of at least two independent experiments.

When indicated, rat endothelial cells, neonatal rat cardiomyocytes, mouse smooth muscle cells, and neonatal rat cardiac fibroblasts were treated with 10 ng/ml of recombinant porcine TGFβ1 (R&D Systems 101-B1-010) for 48 hours. Recombinant MFAP4 was produced in mammalian cells as follows: *MFAP4* was transiently expressed in ExpiCHO-S cells. ExpiCHO-S cells (lot nr.1882582) were cultured in ExpiCHO Expression Medium in a shaker incubator set at 125 rpm, 37°C and 8.0% CO_2 until reaching a cell density of 6 x 10^6 cells/ml. The day before transfection, ExpiCHO-S cells were diluted to 3.5 x 10^6 per ml in ExpiCHO expression medium. Tranfection was performed using ExpiFectamine according to the manufacturer's protocol. Before transfection the cells were diluted to 6 x 10^6 cells/ml in ExpiCHO expression medium and then transfected using a total of 0.8 μg plasmid/ml of ExpiCHO-S cells. One day post transfection ExpiCHO feed and ExpiCHO enhancer were added according to the manufacturer's protocol and the cells were transferred to a shaker incubator set at 125 rpm, 32°C and 5.0% CO_2. Five days post transfection, ExpiCHO feed was added according to the manufacturer's protocol and day 12 post transfection the culture supernatants was harvested by centrifugation at 10.000 g for 30 min. Cells and reagents were from Thermo Fisher Scientific. For recombinant MFAP4

treatments, cardiomyocytes were treated with either 20 ng/ml bovine serum albumin (BSA) or 20 ng/ml recombinant human MFAP4 for 48 hours. For integrin inhibition treatments, cardiomyocytes were treated with cyclic RGD peptide in equimolar amounts to rMFAP4 treatment (for a final concentration of 0.2 μg/mL) (Sigma-Aldrich SCP-0111) and 1 μM FAK inhibitor PF-573228 (Sigma-Aldrich PZ0117) for 48 hours.

Human Heart Samples

All human heart tissue research was approved by The Ohio State University Institutional Review Board in compliance with all relevant ethical regulations. Informed consent for tissue collection was obtained from transplant patients and families of donors. Human heart tissues used in this study were de-identified and labeled with 6-digit random reference codes. Failing samples are obtained from patients with left ventricular hypertrophy and ischemic and non-ischemic heart failure, and nonfailing samples are from healthy donors without history of heart failure but with possible comorbidities including hypertension, diabetes, and modifying risk factors (including history of smoking and chronic alcohol consumption/abuse). Samples were obtained from The Ohio State University Cardiac Research Tissue Program or LifeLine of Ohio Organ Procurement Organization.

Western Blotting

Western blotting was performed from whole heart lysate, neonatal rat cardiomyocyte whole cell lysate, or rat endothelial whole cell lysate using standard procedures. Lysates were prepared by homogenizing cells or heart tissue from WT and MFAP4 KO mice in RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 1% w/v IGEPAL, 0.5% DOC, and 0.1% SDS) with protease and phosphatase inhibitors (Roche 11873580001, Calbiochem 524624, Calbiochem 524625). Protein concentration was determined using Bradford assay (Bio-Rad 5000006) and 10-20 μg protein was loaded onto 10% acrylamide gels (Bio-Rad 1610156, Bio-Rad 5671035). Membranes were transferred using the Bio-Rad Semi-Dry Turbo Transfer System (Bio-Rad 1704150), stained with Ponceau red, blocked for 1 hour in either 5% milk in TBS-T or 5% bovine serum albumin (BSA) in TBS-T, and then probed overnight (Fisher Scientific BP1600-100). Antibodies used were Histidine 1:1000 (Santa Cruz sc-804), phospho-p44/42 MAPK (Thr202/Tyr204) 1:2000 (Cell Signaling 9101), p44/42 MAPK 1:1000 (Cell Signaling 9102), phospho-FAK 1:2000 (Tyr397) (Cell Signaling 8556), FAK 1:1000 (Cell Signaling 3285), phoshpo-Akt 1:1000 (Ser473) (Cell Signaling 4060), Akt (pan) 1:1000 (Cell Signaling 4691), and GAPDH 1:5000 (Fitzgerald

Industries 10-1500). Peroxidase AffiniPure goat anti-rabbit IgG 1:10,000 (Jackson Immunoresearch 111-035-144), and Peroxidase AffiniPure goat anti-mouse IgG 1:10,000 (Jackson Immunoresearch 115-035-003). Densitometry of protein bands was quantified using NIH ImageJ software.

Histology and Immunostaining

Hearts were harvested from mice and fixed in either 1% paraformaldehyde/PBS for 1 hour at 25°C followed by 30% sucrose/PBS overnight at 4°C (for cryosections), or 10% neutral-buffered formalin overnight at 25°C followed by 70% ethanol (for paraffin-embedded sections). Hearts were then processed and paraffin embedded (for paraffin-embedded sections) or OCT-embedded on a frozen methylbutane layer. Both cryosections and paraffin-embedded sections were cut at a thickness of 5 μm.

Picrosirius red staining was performed from histological sections generated from paraffin-embedded hearts following deparaffinization and hydration in xylenes, ethanol, and water (NovaUltra IW-3012). Percent fibrosis was quantified using NIH ImageJ software. Apoptotic cell staining was performed from histological sections generated from paraffin-embedded hearts using the cardioTACS In Situ Apoptosis Detection Kit (R&D Systems, 4827-30-K). For quantifications of percent apoptotic nuclei, 3 20X images of left ventricular tissue were counted per mouse, and 3 mice were counted per condition. Numbers of apoptotic nuclei were quantified in relation to total nuclei. Immunostaining was performed on cryosections and fixed cells using antibodies to α-actinin 1:100 (Sigma-Aldrich A7811), FITC-conjugated MFAP4 1:100 (Schlosser and Sorensen laboratories), CD45 1:50 (BD Biosciences 550539), Alexa Fluor anti-mouse IgG 488 1:500 (Invitrogen A-11094), Alexa Fluor anti-rat IgG 488 1:500 (Invitrogen A-21208), and detection of the cell membrane was performed using rhodamine-labeled wheat germ agglutinin (Vector Labs RL-1022). Cryosections were permeabilized using blocking buffer containing 5% bovine serum albumin and 0.01% TritonX-100. Slides were mounted in VectaShield HardSet Antifading Mounting Medium with DAPI (Vector Labs H-1500) and imaged on an Evos FL Auto 2 microscope (Thermo Fischer) or Zeiss 780 confocal microscope. Secondary-only controls (using Alexa-Fluor anti-mouse IgG 488) were performed for staining of MFAP4. The cross-sectional area of cells was measured using NIH ImageJ Software.

mRNA Expression Analysis

RNA was extracted from whole heart tissue and cell cultures using Trizol (Invitrogen 15596026) and reverse transcription was performed using the High Capacity cDNA Reverse Transcription kit

(Applied Biosystems 4368814). Selected genes were analyzed by real-time polymerase chain reaction using SYBR green (Bio-Rad 1725272). In brief, the thermocycler conditions are as follows: polymerase activation and denaturation 30 seconds at 95°C; denaturation for 15 seconds at 95°C; annealing and extension 30 seconds at 60°C; 40 cycles; melt curve analysis from 65-95°C at 2 seconds / increment (Bio-Rad CFX96). mRNA expression was calculated using the ΔΔCt method. Quantified mRNA expression was normalized to Rpl7 (ribosomal protein L7) and expressed relative to controls. Primer sequences used for gene expression analysis are listed in the table below:

Human Primers		
Target	**5' sequence**	**3' sequence**
MFAP4	CGAGTGGACTTGGAGGACTT	CCGGTCGAAGGTAGAGAACT
RPL7	ATGCGCCAATTCCTCTTT	CAGCTCTGCGAAATTCCTTC
Rat Primers		
Target	**5' sequence**	**3' sequence**
Mfap1	GCTGAACTTCGGGCAAATGG	TGGTTTTATTGAAATGGTCCTCG
Mfap2	GGTCAACAAGGAAATATGTGTCC	TGGCTATCACACCACACTTG
Mfap3	GAGCTTTGAACTCTCGGCAG	CACGGTCATCAAAGGCTACG
Mfap4	TGCGGAGGATGGCTATA	TTGGCATAGGAGAGGTGGGA
Mfap5	ATGAGATCTGTTCCCGGCTT	TTTCACAGGGAGGAAGTCGG
Rpl7	AAAAGAAGGTTGCCGCTG	TAGAAGTTGCCAGCTTTCC
Nppa	CACAGATCTGATGGATTTCAAGA	CCTCATCTTCTACCGGCATC
Nppb	GTCAGTCGCTTGGGCTGT	CAGAGCTGGGGAAAGAAG
Myh6	GGAGGTGGAGAAGCTGGAA	ATCTTGCCCTCCTCATGCT
Myh7	CACCAACAACCCCTACGATT	AGCACATCAAAGGCGCTATC
Acta1	TGAAGCCTCACTTCCTACCC	CGTCACACATGGTGTCTAGTTTC
Mouse Primers		
Target	**5' sequence**	**3' sequence**
Mfap1	TGACTGAGGAAGAAAGGCGG	TGGTTTTGTTGAAATGGTCCTCA
Mfap2	CGCCTCTACTCCATCCACAA	TGCGGACACATATTTCCTTGT
Mfap3	TCTCAGCAGGTTCCTACTCAG	CACGGTCATCAAAGGCTACG
Mfap4	CTTCTGCGACATGACAACTGA	GCCAAAGCCCAGCTTGTA
Mfap5	TCAATAATGAGATCTGTTCCCGA	TCGGGTCTCTGCAAATTCATATT
Rpl7	TGGAACCATGGAGGCTGT	CACAGCGGGAACCTTTTTC
Nppa	GCTCCTTCTCCATCACCCTG	TACCGGCATCTTCTCCTCCA
Nppb	TCCTAGCCAGTCTCCAGAGC	CCGGTCTATCTTGTGCCCAA
Myh6	ATGGGCTGGCTGGAAAAGAA	TCTTCTTGCCTCCTTTGCCT

Myh7	CCTGCGGAAGTCTGAGAAGG	CTCGGGACACGATCTTGGC
Acta1	GTATGGAGTCTGCGGGGATC	TCCACACTGAGTACTTGCGC
Dapk1	ACGTGGACGACTACTACGACAC	ATTTCTTCACAACGGCGAAC
Bnip3	CTGGGTAGAACTGCACTTCAG	GGAGCTACTTCGTCCAGATTCAT
TP53	CCCCTGTCATCTTTTGTCCCT	AGCTGGCAGAATAGCTTATTGAG
Bnip3L	CTGGAGCACGTTCCTTCCTC	ACAGTGCGAACTGCCTCTTG
Cxcl10	GCTGCCGTCATTTTCTGC	TCTCACTGGCCCGTCATC
IL1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL-6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
Tnfa	TCACTGGAGCCTCGAATGTC	GTGAGGAAGGCTGTGCATTG
Atg5	TGTGCTTCGAGATGTGTGGTT	ACCAACGTCAAATAGCTGACTC
Becn1	ATGGAGGGGTCTAAGGCGTC	TGGGCTGTGGTAAGTAATGGA
Map1Lc3a	GACCGCTGTAAGGAGGTGC	CTTGACCAACTCGCTCATGTTA
Map1Lc3b	TTATAGAGCGATACAAGGGGGAG	CGCCGTCTGATTATCTTGATGAG
Sqstm1 (p62)	GAGGCACCCCGAAACATGG	ACTTATAGCGAGTTCCCACCA
TgfBr1	TCTGCATTGCACTTATGCTGA	AAAGGGCGATCTAGTGATGGA
TgfBr2	GACTGTCCACTTGCGACAAC	GGCAAACCGTCTCCAGAGTAA

Study Design

Surgical procedures, echocardiographic analysis, quantification of gene expression, histology, and fluorescence imaging were performed while experimenters were blinded to group assignment or genotype. Groups of mice within genotypes were assigned to surgical procedures and/or treatment groups with simple randomization using a random number generator. Sample size calculations were conducted to determine differences in cardiac function through unpaired, two-tailed t tests with an α of 0.05 and a power of 0.8 to detect an effect size of at least 0.5. No mice were excluded from the study. Representative images were obtained in a data-based method whereby the average for the group was taken into account and then an image selected from an individual or group of cells representing the average value.

Statistics

All results are presented as mean±SEM. Normality of data sets was determined using the Shapiro-Wilk test with a significance level (α) of 0.05. If data were normal, statistical analysis was performed with unpaired 2-tailed Student's t-test (for 2 groups), one-way ANOVA with Sidak's multiple

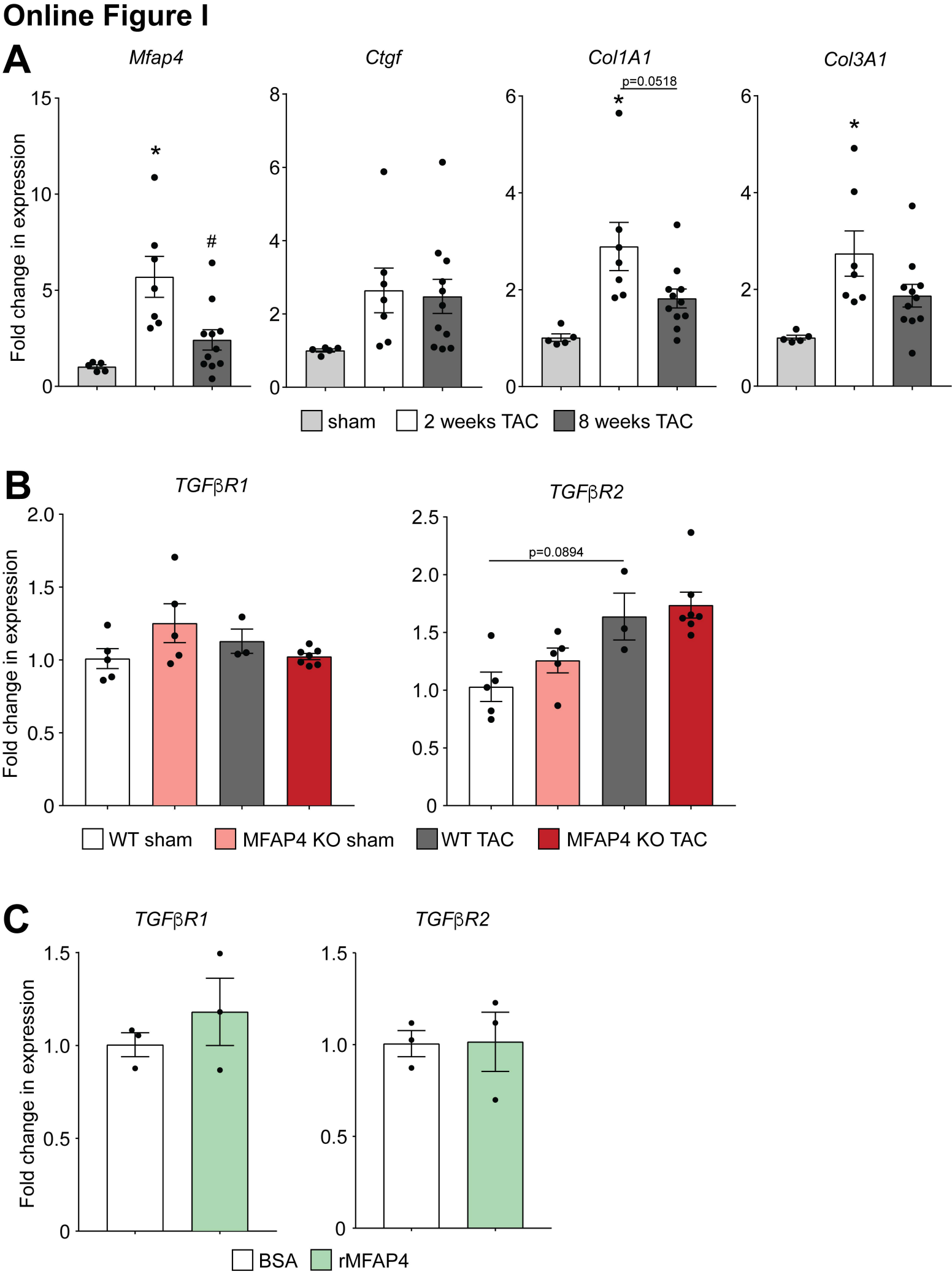
comparisons test (for groups of ≥3), or two-way ANOVA with Tukey's multiple comparison test, as noted in the figure legends. If data were not normal or sample size was small (n≤5), statistical analysis was performed with the two-tailed Mann-Whitney test (for 2 groups) or Kruskal-Wallis test with Dunn's multiple comparisons tests, as noted in the figure legends. *P* values <0.05 were considered significant. All exact *p* values are listed in the figure legends. All *p* values are adjusted for multiple comparisons as described above, and in all cases all pairwise comparisons were made. For groups of n≥100, the ROUT outlier test was performed with a Q value of 1%. All statistical analysis was performed using GraphPad Prism versions 8 and 9 (Graphpad).

Online Table I: Echocardiographic parameters

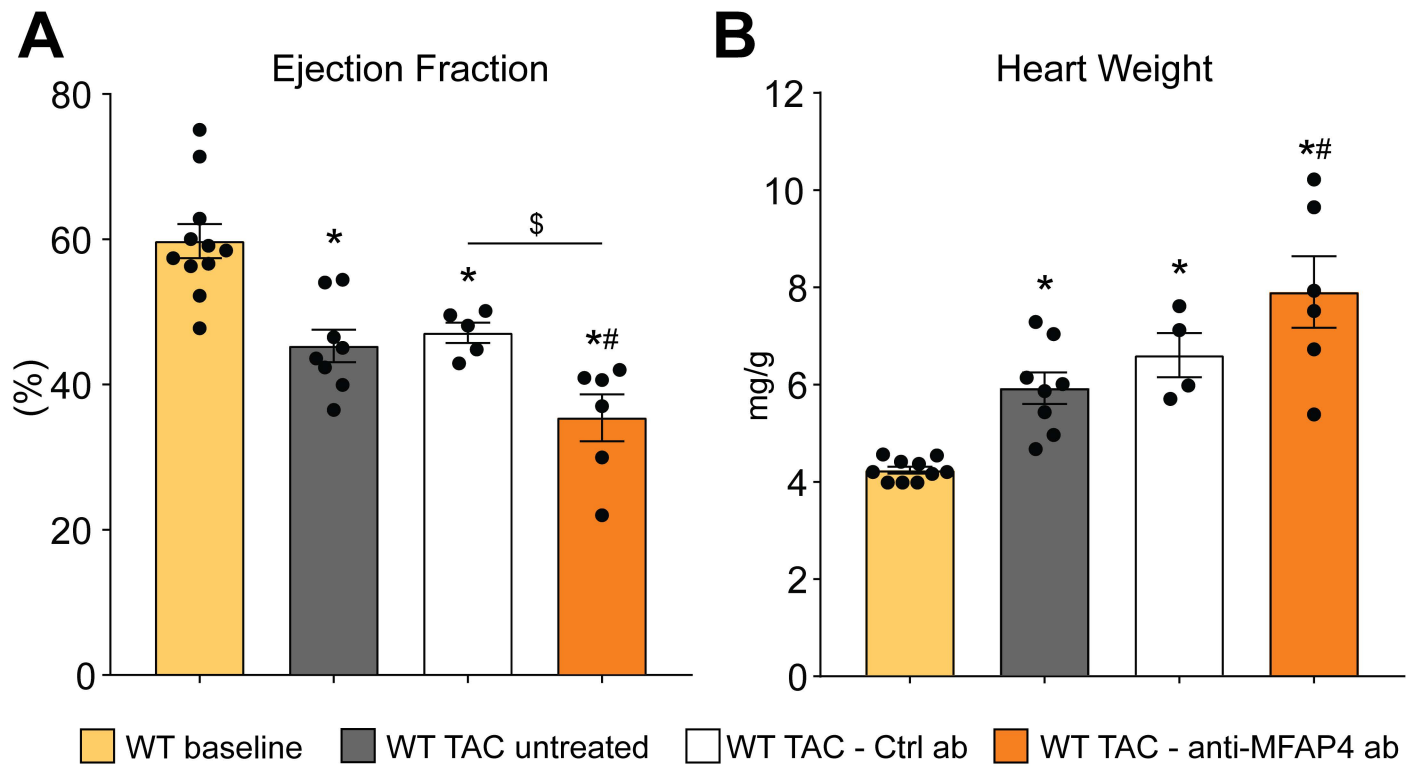
	2 week WT sham	2 week MFAP4 KO sham	2 week WT TAC	2 week MFAP4 KO TAC
IVS;d (mm)	.858±.066	.908±.041	1.104±.039	1.143±.077* p=0.0331
IVS;s (mm)	1.224±.026	1.316±.060	1.506±.064	1.484±.103
LVID;d (mm)	4.09±.164	4.046±.098	4.055±.093	4.436±.105
LVID;s (mm)	2.940±.181	2.895±.097	3.130±.112	3.637±.161
LVPW;d (mm)	.800±.014	.664±.015	.975±.053	.817±.043
LVPW;s (mm)	1.053±.060	.932±.057	1.184±.054	1.031±.062
EF (%)	54.922±3.426	55.447±1.581	46.397±2.161* p=0.0331	37.606±3.725* p=0.0002
FS (%)	28.314±2.121	28.501±.992	23.020±1.259	18.304±2.065* p=0.0132
	4 week WT sham	**4 week MFAP4 KO sham**	**4 week WT TAC**	**4 week MFAP4 KO TAC**
IVS;d (mm)	.829±.074	.824±.080	1.121±.024* p=0.0339	1.052±.032* p=0.0449
IVS;s (mm)	1.316±.124	1.233±.080	1.495±.008	1.306±.030
LVID;d (mm)	3.822±.088	3.749±.051	4.168±.037* p=0.0258	4.248±.138* p=0.0074
LVID;s (mm)	2.637±.060	2.637±.085	3.250±.021* p=0.0011	3.646±.137* p=0.000052
LVPW;d (mm)	.736±.076	.678±.014	.788±.021	.965±.050* p=0.0025; # p=0.0264
LVPW;s (mm)	.965±.026	.858±.056	.959±.057	1.083±.045* p=0.0405
EF (%)	59.077±2.601	57.490±2.088	40.628±4.416* p=0.0009	30.563±1.637* p=0.0000090; # p=0.0194
FS (%)	30.894±1.825	29.713±1.352	22.015±.865* p=0.0008	14.238±.832* p=0.000011; # p=0.0281
	WT vehicle	**MFAP4 KO vehicle**	**WT AngII**	**MFAP4 KO AngII**
IVS;d (mm)	.843±.035	.777±.041	1.021±.037* p=0.0098	1.043±.041* p=0.0002
IVS;s (mm)	1.231±.047	1.203±.032	1.393±.058	1.433±.036* p=0.0059
LVID;d (mm)	4.105±.135	4.158±.063	3.572±.190	3.679±.156
LVID;s (mm)	2.990±.084	3.000±.099	2.858±.236	2.831±.156
LVPW;d (mm)	.760±.086	.661±.049	1.056±.057* p=0.0289	.914±.022* p=0.0335
LVPW;s (mm)	1.004±.066	.905±.051	1.194±.111	1.127±.031
EF (%)	53.255±1.364	54.386±2.213	42.311±4.622	47.162±2.527
FS (%)	27.113±.900	27.925±1.452	20.448±2.524	23.176±1.431

Online Table I. Measurement of echocardiographic parameters are shown as mean ± standard error. IVS;d = Interventricular Septum Thickness; diastole. IVS;s = Interventricular Septum Thickness; systole. LVID;d = Left Ventricular Internal Dimension; diastole. LVID;s = Left Ventricular Internal Dimension; systole.
LVPW;d = Left Ventricular Posterior Wall thickness; diastole. LVPW;s = Left Ventricular Posterior Wall thickness; systole. EF = Ejection Fraction. FS = Fractional Shortening. Two-way ANOVA results: *$p \leq 0.05$ versus same genotype, different treatment; #$p \leq 0.05$ versus different genotype, same treatment. Exact p values are included in the table.

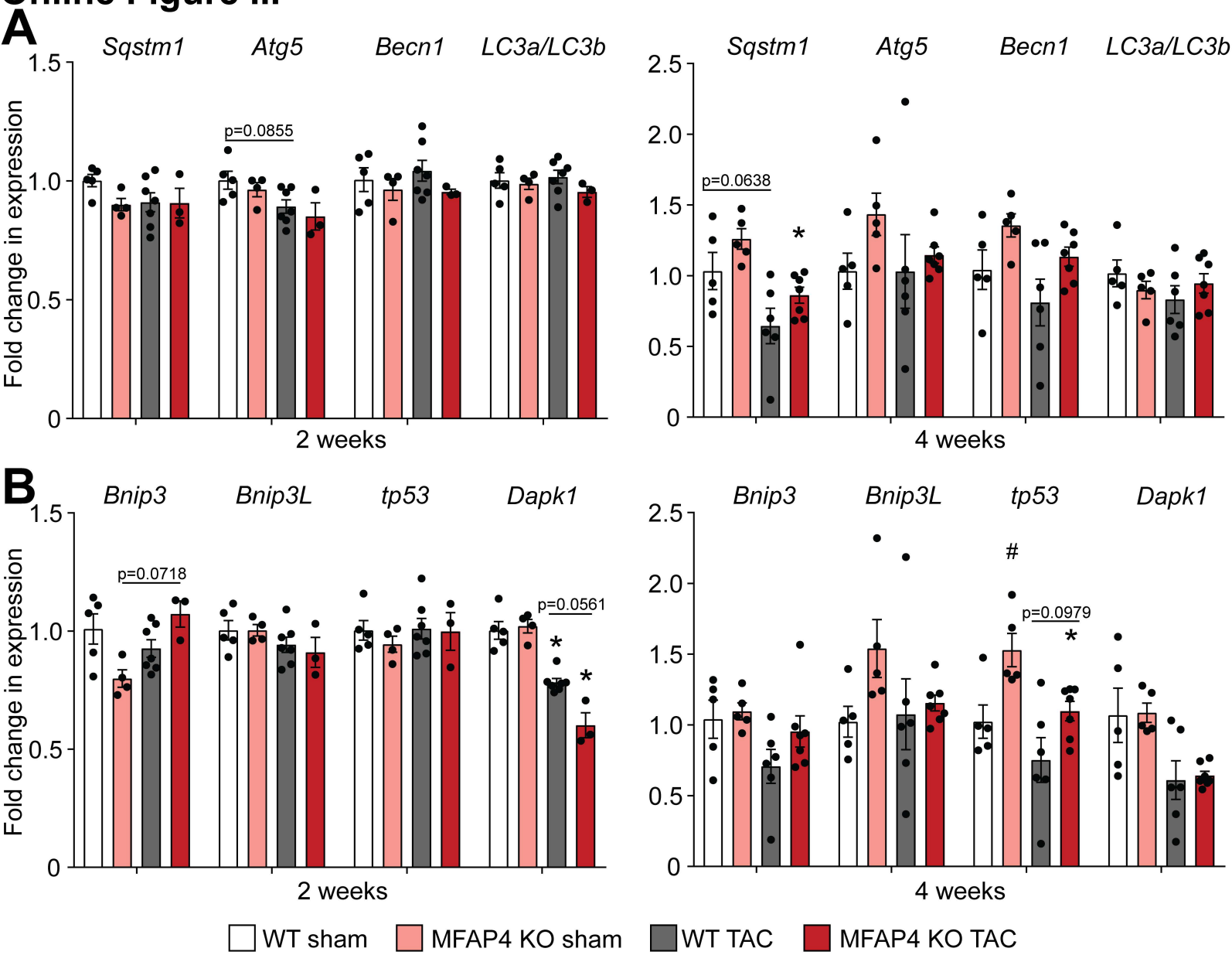
Online Figure I

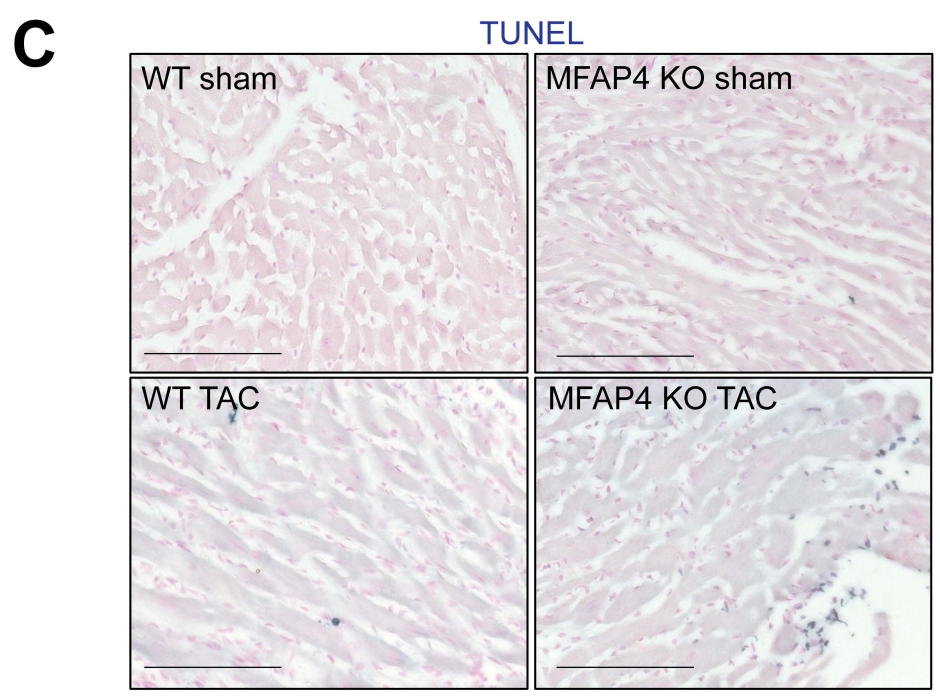


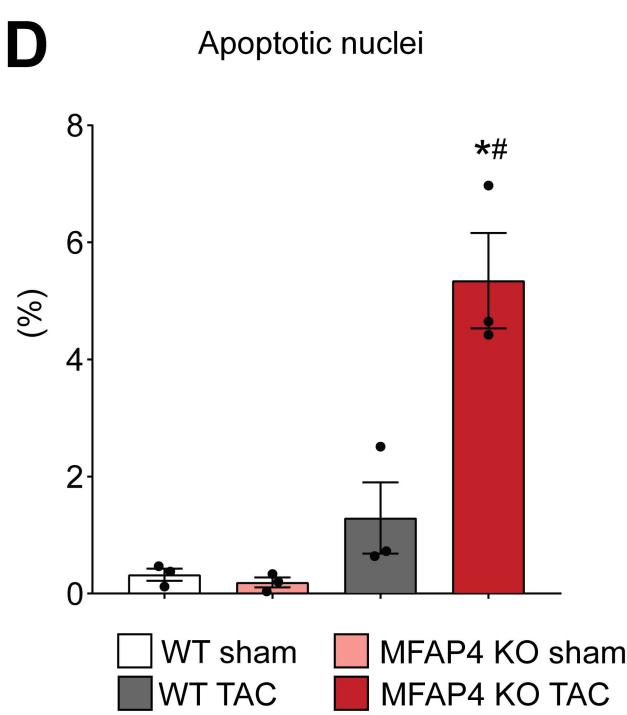
Online Figure II



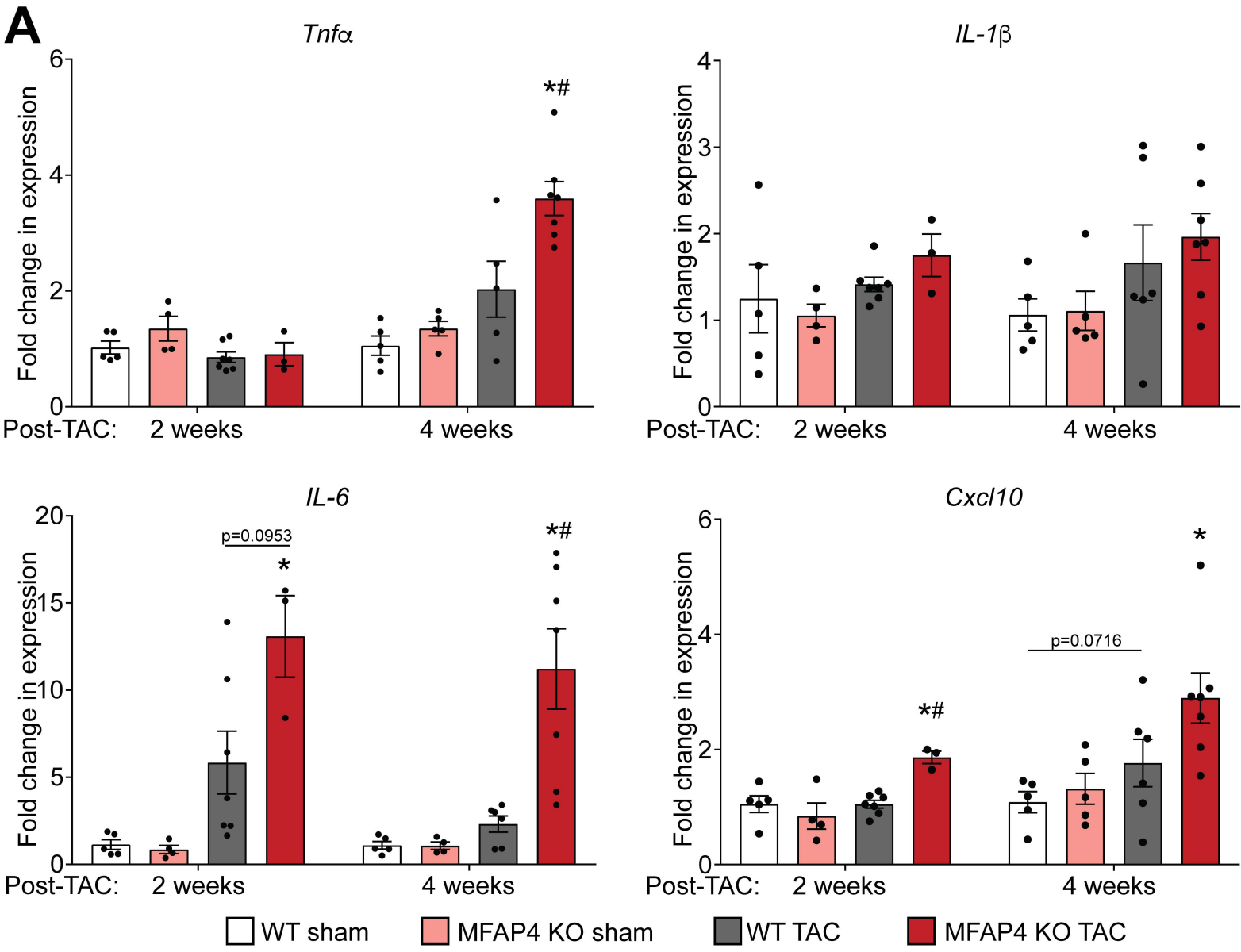
Online Figure III

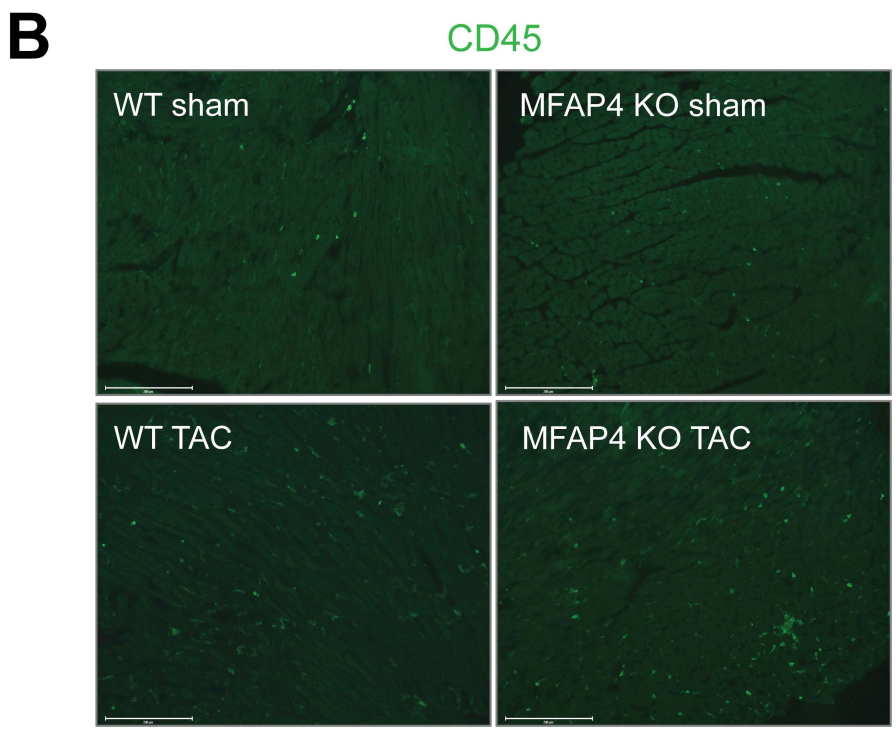


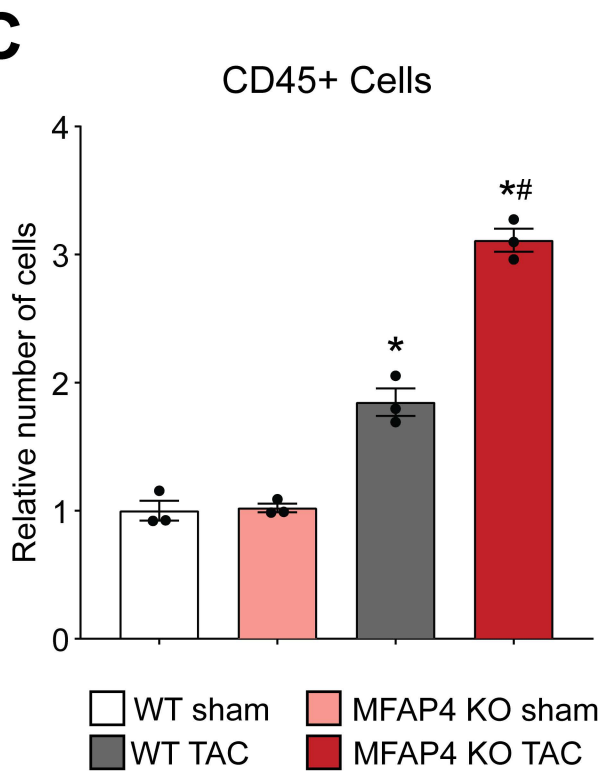




Online Figure IV

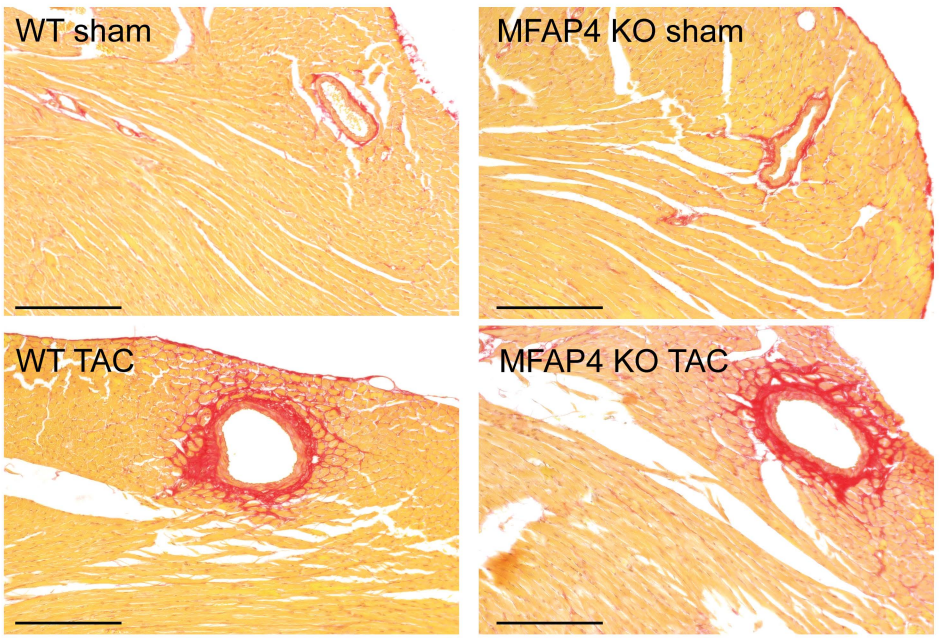


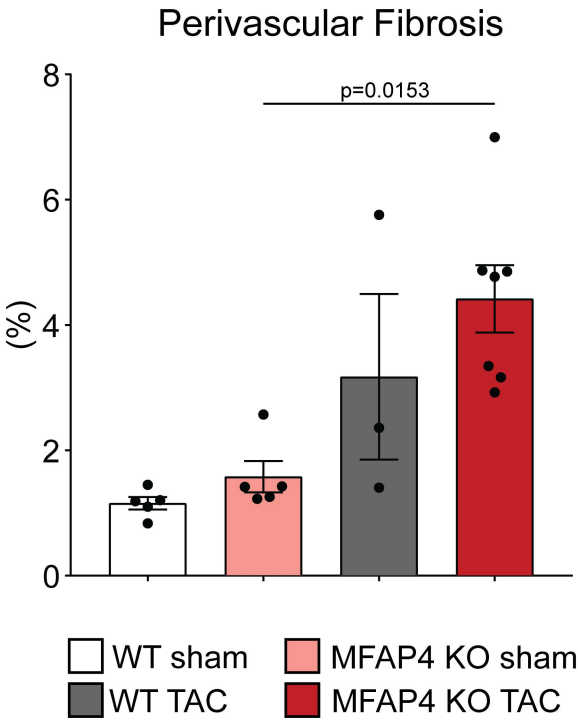




Online Figure V

A





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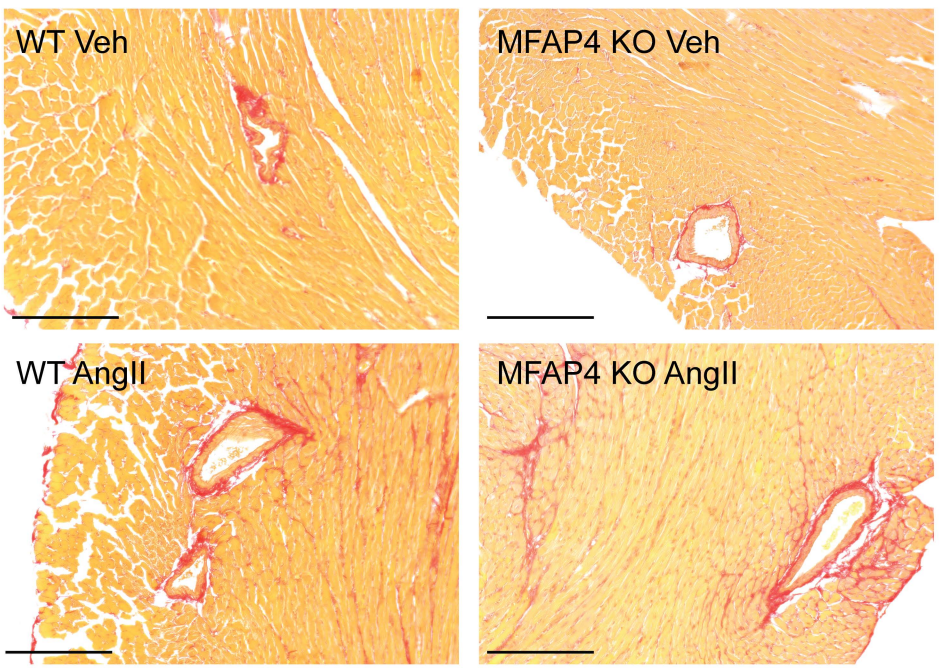

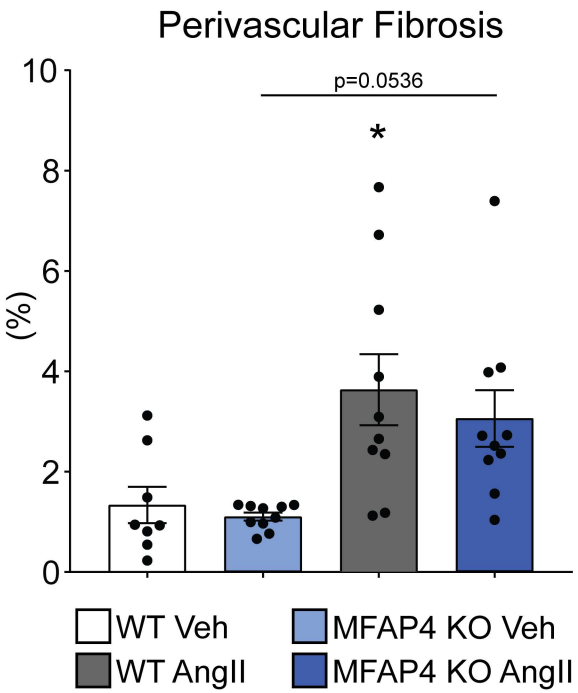

Online Figure Legends

Online Figure I. A. qPCR analysis for the indicated extracellular matrix genes in sham (n=5) and post 2 (n=7) or 8 weeks (wks) (n=11) of TAC relative to the housekeeping gene *Rpl7*. (One-way ANOVA results for *Mfap4*: sham vs 2wk TAC *p=0.0020; 2wk TAC vs 8 wk TAC #p=0.0082. One-way ANOVA results for *Ctgf*: 2wk TAC vs 8wk TAC p=0.0518. One-way ANOVA results for *Col1A1*: sham vs 2 wk TAC *p=0.0038; 2wk vs 8wk TAC p=0.0518. One-way ANOVA results for *Col3A1*: sham vs 2 wk TAC *p=0.0087). **B-C.** qPCR analysis for transforming growth factor beta receptor (Tgfbr1 and Tgfbr2) gene expression relative to *Rpl7* in the indicated groups from total heart extracts (B) or isolated adult cardiomyocytes treated for 48 hours with either BSA control or recombinant MFAP4 (rMFAP) (C). (WT and KO sham n=5, WT TAC n=3, KO TAC n=7; BSA n=3, rMFAP4 n=3). (Two-way ANOVA results for *Tgfbr1* in (B): all conditions ns. Two-way ANOVA results for *Tgfbr2* in (B): WT sham vs TAC p=0.0894. Mann-Whitney test results for *Tgfbr1* in (C): ns. Mann-Whitney test results for *Tgfbr2* in (C): ns.).

Online Figure II. A. Echocardiographic quantification of percentage ejection fraction in WT mice at baseline (n=10) and untreated WT mice (n=8), WT mice treated with normal mouse IgG (Ctrl) antibody (n=4), and WT mice treated with anti-MFAP4 neutralizing antibody (n=6) at 4 weeks following TAC. (Two-way ANOVA results: baseline to WT untreated TAC *p=0.0004; baseline to WT Ctrl ab TAC *p=0.0019; baseline to WT MFAP4 ab TAC *p=0.0000025; WT untreated TAC to WT MFAP4 ab TAC #p=0.0181; WT Ctrl ab TAC to WT MFAP4 ab TAC $p=0.0334). **B.** Heart weight to body weight ratio of WT mice at baseline and untreated WT mice, WT mice treated with normal mouse IgG (Ctrl) antibody, and WT mice treated with anti-MFAP4 neutralizing antibody at 4 weeks following TAC. (Two-way ANOVA results: WT baseline vs WT untreated TAC *p=0.0254; WT baseline vs WT Ctrl ab TAC *p=0.0069; WT baseline vs WT MFAP4 ab TAC *p=0.000052; WT untreated TAC vs WT MFAP4 ab TAC #p=0.0119).

Online Figure III. A. qPCR analysis for the indicated autophagy genes after 2 and 4 weeks (wks) of sham or TAC surgeries in WT and MFAP4 KO hearts. Gene expression is represented

relative to the housekeeping gene *Rpl7*. (WT sham 2 weeks n=5, KO sham 2 weeks n=4, WT TAC 2 weeks n=7, KO TAC 2 weeks n=3; WT sham 4 weeks n=5, KO sham 4 weeks n=5, WT TAC 4 weeks n=6, KO TAC 4 weeks n=7). (Two-way ANOVA results for *Sqstm1* at 2 weeks: all conditions ns. Two-way ANOVA results for *Atg5* at 2 weeks: WT sham vs TAC p=0.0855. Two-way ANOVA results for *Becn1* at 2 weeks: all conditions ns. Two-way ANOVA results for *LC3a/b* at 2 weeks: all conditions ns. Two-way ANOVA results for *Sqstm1*at 4 weeks: WT sham vs TAC p=0.0638; KO sham vs TAC *p=0.0399. Two-way ANOVA results for *Atg5* at 4 weeks: all conditions ns. Two-way ANOVA results for *Becn1* at 4 weeks: all conditions ns. One-way ANOVA results for *LC3a/b* at 4 weeks: all conditions ns.) **B.** qPCR analysis for the indicated cell death genes after 2 and 4 weeks (wks) of sham or TAC surgeries in WT and MFAP-KO hearts. Gene expression is represented relative to the housekeeping gene *Rpl7*. (Two-way ANOVA results for *Bnip3* at 2 weeks: KO sham vs TAC p=0.0718. Two-way ANOVA results for *Bnip3L* at 2 weeks: all conditions ns. Two-way ANOVA results for *tp53* at 2 weeks: all conditions ns. Two-way ANOVA results for *Dapk1* at 2 weeks: WT sham vs TAC *p=0.0128; KO sham vs TAC *p=0.0005; WT vs KO TAC p=0.0561. Two-way ANOVA results for *Bnip3* at 4 weeks: all conditions ns. Two-way ANOVA results for *Bnip3L* at 4 weeks: all conditions ns. Two-way ANOVA results for *tp53* at 4 weeks: WT vs KO sham #p=0.0185; KO sham vs TAC *p=0.0317; WT vs KO TAC p=0.0979. Two-way ANOVA results for *Dapk1* at 4 weeks: all conditions ns.) **C**. Representative images of apoptotic cell staining on cardiac sections from the indicated genotypes and treatments. Scale bar 200 um. **D**. Quantification of percentage apoptotic cells in cardiac sections from WT and MFAP4 KO post 2 weeks of sham or TAC surgeries (n=3). (Two-way ANOVA results: KO sham vs TAC *p=0.0021; WT vs KO TAC #p=0.0074).

Online Figure IV. A. qPCR analysis for the indicated inflammatory genes after 2 and 4 weeks (wks) of sham or TAC surgeries in WT and MFAP4 KO hearts. Gene expression is represented relative to the housekeeping gene *Rpl7*. (WT sham 2 weeks n=5, KO sham 2 weeks n=4, WT TAC 2 weeks n=7, KO TAC 2 weeks n=3; WT sham 4 weeks n=5, KO sham 4 weeks n=5, WT TAC 4 weeks n=6, KO TAC 4 weeks n=7). (Two-way ANOVA results of *Tnfa* at 2 weeks: all conditions ns. Two-way ANOVA results of *Tnfa* at 4 weeks: KO sham vs TAC *p=0.0034; WT vs KO TAC #p=0.0313. Two-way ANOVA results of *IL-1b* at 2 weeks: all conditions ns. Two-way ANOVA results of *IL-1b* at 4 weeks: all conditions ns. Two-way ANOVA results of *IL-6* at

2 weeks; KO sham vs TAC *p=0.0044; WT vs KO TAC p=0.0953. Two-way ANOVA results of *IL-6* at 4 weeks; KO sham vs TAC *p=0.0024; WT vs KO TAC #p=0.0022. Two-way ANOVA results of *Cxcl10* at 2 weeks: KO sham vs TAC *p=0.0058; WT vs KO TAC #p=0.0198. Two-way ANOVA results of *Cxcl10* at 4 weeks: KO sham vs TAC *p=0.0229; WT vs KO TAC p=0.0716.) **B.** Representative images of immunofluorescence staining for CD45+ cells in WT and MFAP4 KO animals following sham or 2 weeks of TAC surgery. Scale bar 200 um. **C**. Quantification of the number of CD45+ cells from staining of cardiac sections from the indicated genotypes and treatments (n=3). (Two-way ANOVA results: WT sham vs TAC *p=0.0006; KO sham vs TAC *p=0.000003; WT vs KO TAC #p=0.000063).

Online Figure V. A. Representative picrosirius red-stained images of perivascular fibrosis in WT and MFAP4 KO animals subjected to sham or TAC surgery at 4 weeks following surgery. Scale bar 200 um. **B**. Quantification of perivascular fibrosis from picrosirius red-stained cardiac sections from the indicated groups. (WT sham n=5, KO sham n=5, WT TAC n=3, KO TAC n=7). (Two-way ANOVA results: KO sham vs TAC p=0.0153). **C**. Representative picrosirius red-stained images of perivascular fibrosis in WT and MFAP4 KO animals subjected to 1 week of Vehicle or AngII infusion and associated quantification. Scale bar 200 um. **D**. Quantification of perivascular fibrosis from picrosirius red-stained cardiac sections from the indicated groups. (WT Veh n=8, KO Veh n=10, WT Ang n=10, KO Ang n=10). (Two-way ANOVA results: WT Veh vs AngII *p=0.0197; KO Veh vs AngII p=0.0536).