SUPPLEMENTAL MATERIAL

Supplemental Methods

Mouse study

C57BL/6 mice of both genders were used for this study. Genotype- and sex-matched mice were randomly assigned to experimental groups mitigating cage effect. Researchers were blinded to the genotype of the animals until the end of the analysis. Animal Research was performed according to animal welfare regulations of the Indiana University's Institutional IACUC Committee. All procedures were conducted to conform with NIH guidelines (Guide for the care and use of laboratory animals). Carvedilol (Carv; Sigma-Aldrich) was dissolved in DMSO and micro-osmotic pumps (Alzet model 2001; DURECT Corporation) were then used to deliver Carv at the rate of 19 mg/kg/day for 3 or 7 days. In control mice, 10% DMSO administrated as a vehicle. Left ventricular (LV) tissues were then snap-frozen in the liquid N_2 for mRNA and protein analyses as described previously [11, 64].

Mouse model of myocardial infarction

Eight-sixteen week old mice were subjected to MI as previously published [65, 66]. Briefly, mice were anesthetized using 1-4% inhalant isoflurane and placed on a heating pad. Mice were intubated and ventilated with oxygen using a PhysioSuite MouseVent™ ventilator (Kent Scientific). The left anterior descending (LAD) coronary artery was visualized under a stereoscope and ligated by using an 8-0 nylon suture. Regional ischemia was confirmed by visual inspection for discoloration of the occluded distal myocardium. Sham-operated mice underwent the same procedure without LAD occlusion. One dose of buprenorphine SR Lab (0.05mg/kg; ZooPharm) was given subcutaneously immediately before the surgery. We used responses to toe/skin pinch and heart rate for optimal anesthesia and appropriate post-operative monitoring plan.

Transthoracic high-resolution echocardiography

LV performance was assessed by two-dimensional echocardiography using a Visual Sonics Vevo 2100 Ultrasound at baseline (pre-surgery) and post-MI (1, 2 and 4 weeks) as previously used [65, 66]. M-mode tracings were used to measure anterior and posterior wall thicknesses at end-diastole and end-systole. Parameters including LV internal diameter (LVID) in either diastole (LVIDd) or systole (LVIDs), end-diastolic volume (EDV), and end-systolic volume (ESV) were obtained. A single operator blinded to mouse genotypes performed echocardiography and data analysis. Fractional shortening (FS) was calculated according to the following formula: FS (%) = [(LVIDd-LVIDs)/LVIDd] X 100. Ejection fraction (EF) was calculated by: EF (%) = [(EDV-ESV)/EDV] X 100.

Histopathological and immunohistochemical analyses

Morphometric analyses of heart size and weights were conducted as we published [12, 65, 66]. Histopathological analyses of heart tissues such as fibrosis via Masson's trichrome staining were performed as described [67, 68]. For gross histological assessment, heart sections were stained with hematoxylin and eosin (H&E). Cardiac sections were also stained for TUNEL to assess apoptosis using *In Situ* Cell Death Detection Kit (Sigma-Aldrich).

Fractionation of different cardiac cell types isolated from mouse hearts

The thorax of WT mice was opened and the aorta was cannulated. The fractionation of highly purified cardiac cell populations was performed and the purity of isolated cell types was validated using QRT-PCR analyses of cell type-specific markers (i.e., *Tnnt2*: cardiac 6 muscle troponin T [for cardiomyocytes (CMs)], *Ptprc*: protein tyrosine phosphatase receptor type C [for cardiac inflammatory cells (CIs)], *Pecam1*: platelet 7 endothelial cell adhesion molecule-1 [for cardiac endothelial cells (CEs)], and *Ddr2*: discoidin domain receptor 2 [for cardiac fibroblasts (CFs)], as we published [12]. Briefly, freshly harvested mouse hearts were dissociated into single-

cell suspensions or thorough homogenates using a gentleMACS™ Dissociator (Miltenyi Biotec) according to the manufacturer's instructions (Miltenyi Biotec). The dissociated cells were then processed for cell fractionation. An incubation step with CD31 antibodies coupled to microbeads was first performed and subjected to magnetic affinity cell sorting according to the manufacturer's recommendations (Mouse CD31 microbead endothelial isolation kit, Miltenyi Biotec). An additional incubation step with CD31-deficient fraction and CD45 antibodies coupled to microbeads was performed and subjected to magnetic affinity cell sorting according to the manufacturer's recommendations (Mouse CD45 microbead leukocyte isolation kit, Miltenyi Biotec). CMs and CFs were then separated by a sedimentation step as previously described [69].

Transcriptome analysis

Total RNAs from 12 independent mouse left ventricles (3 sham WT, 3 sham MIAT KO, 3 MI WT and 3 MI MIAT KO samples) were prepared as described [70]. RNA quantity and quality were assessed by the Synergy LX FA Multi-Mode Microplate Reader (BioTek Instruments). The DNA microarray experiments were performed using the Mouse Microarray v3.0 (8 x 60K, Arraystar Inc) designed for the global profiling of 24,881 protein-coding transcripts as described [70-74]. Fifteen positive probes for housekeeping genes and 20 negative probes were also included in the array as hybridization quality controls.

Labeling and array hybridization

RNA labeling and array hybridization were conducted according to the One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology) as described [70, 75]. The Quick Amp Labeling Kit (Agilent Technologies) was used for sample labeling, and hybridization was conducted in the SureHyb Hybridization Chamber (Agilent Technologies). In brief, mRNAs were purified by using mRNA-ONLY™ Eukaryotic mRNA Isolation Kit (Epicentre Biotechnologies). Each sample was then transcribed into fluorescent cRNAs by using Flash RNA

Labeling Kit (Arraystar Inc). Next, the labeled cRNAs were purified by RNeasy Mini Kit (Qiagen). Each labeled cRNA was then fragmented, and the mixture was heated at 60°C for 30 minutes. Lastly, labeled cRNAs were loaded into gasket slides and assembled to microarray slides. The slides were then incubated for 17 hours at 65°C in a hybridization oven (Agilent Technologies) and scanned by using the DNA Microarray Scanner G2505C (Agilent Technologies).

Analysis of microarray dataset

The Feature Extraction software version 11.0.1.1 (Agilent Technologies) was used to analyze array images as published [70]. We performed more robust quantile normalization across all 12 samples than normalizing with one/some control probes (*i.e.,* the entire intensity distributions were normalized across the arrays, not just some reference points). The subsequent data processing was then conducted using the GeneSpring GX version 12.1 software package (Agilent Technologies). We then chose mRNAs, in which at least 6 out of 12 samples exhibited flags in Present or Marginal, for further data analyses. We identified differentially expressed (DE) mRNAs with statistical significance through Volcano Plot filtering between groups. We performed the pathway analysis for DE genes to identify the biological pathway by utilizing the GeneSpring Software GX version 12.1 (Agilent Technologies) and the latest Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg) database. We used Fisher's exact test to calculate the *P* values of the Pathway ID and converted to enrichment score by negative log10 transformation.

Western blotting and antibodies

LVs were washed once with PBS and solubilized in Nonidet P-40 lysis buffer as previously described [65]. Lysate samples were resolved by SDS-PAGE and transferred to PVDF membrane (Bio-Rad) for immunoblotting. HOXA4 (sc-515418, mouse, Santa Cruz) and GAPDH (sc-47724, mouse, Santa Cruz) primary antibodies were purchased and used at dilutions of 1:1,000 each.

Detection was carried out using ECL (Amersham Biosciences).

Primary adult human cardiac fibroblast culture and transfection

The primary adult human cardiac fibroblasts (HCFs) were purchased from PromoCell (C12375) and maintained according to the company's recommendation. The primary HCFs, which were isolated from the ventricles of the adult heart, display normal fibroblastic characteristics and cellular markers (*i.e.*, CD90-positive, smooth muscle α-actin-negative and slow muscle myosin-negative). We used multiple batches of HCFs with passage number of 3-5 to further ensure the specificity of used cells and normal growth pattern. The adult primary HCFs were cultured in PromoCell Fibroblast Growth Medium 3 (C-23025), which contains basic fibroblast growth factor (bFGF: 1ng/ml), recombinant human insulin (5ug/ml) and 10% fetal calf serum (FCS: 0.1ml/ml) in the supplement. HCFs were transfected with an ON-TARGETplus Non-targeting Negative Control siRNA (Dharmacon, cat#: D-001810-10-05) or siRNAs targeting human *HOXA4* (Dharmacon, cat#: 3201) with Lipofectamine™ 3000 reagent (Invitrogen) as previously described [12, 64]. To inhibit miR-150 expression in HCFs, we also transfected Ambion Anti-miR™ microRNA inhibitors (Life Technologies) specific to miR-150 (MH10070) and a miR inhibitor negative control (4464076) using Lipofectamine™ 3000 reagent (Invitrogen) as described previously [12]. Transfected HCFs were then processed for quantitative real-time RT-PCR, BrdU proliferation, and wound migration assays as mentioned below. For Carv stimulation experiments, HCFs were stimulated with Carv (1 μM; Sigma-Aldrich) in serum-free media for 4 hours as described previously [76], and were then processed for hypoxia/reoxygenation as below.

An *in vitro* model of hypoxia/reoxygenation

HCFs treated with Carv were incubated in an ischemia buffer that contained 118 mM NaCl, 24 mM NaH_2CO_3, 1 mM $NaHPO_4$, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 20 mM sodium lactate, 16 mM KCl, and 10 mM 2-deoxyglucose (pH 6.2). HCFs were then placed in the hypoxic chamber (5%

CO_2, 0.1% O_2) for 3 hours followed by 18 hours of reperfusion-mimicking conditions (*i.e.*, replacing the ischemic buffer with normal cell medium under normoxic conditions) as described [4, 12]. Based on our initial optimization experiments in HCFs with different time points (2-3 hours of hypoxia and 4-18 hours of reoxygenation, we chose 3 hours of hypoxia and 18 hours of reoxygenation when maximum increases in the expression of pro-fibrotic *POSTN* and *COL3A* were achieved, as compared to normoxia. HCFs were then processed for quantitative real-time RT-PCR as mentioned below.

Quantitative real-time RT-PCR

Total RNAs from HCFs and mouse hearts on infarct area were prepared using Trizol Reagent (Invitrogen) and treated with RNase-free DNase I as described [77, 78]. To measure mature miR-150, the TaqMan MicroRNA Reverse Transcription Kit (Life Technologies) was used to generate cDNAs. We used the miR-150 Taqman probe (000473; Life Technologies) to measure the evolutionarily conserved mature miR-150 by Real-Time RT-PCR. U6 snRNA probe, 001973 (Life Technologies) is used for an endogenous control. cDNAs for genes were generated using SuperScript III reverse transcriptase (Invitrogen) and random hexamers. Expression of genes or lncRNAs was detected using Taqman expression assays for mouse (*Nppa*, Mm01255747_g1; *Nppb,* Mm01255770_g1; *Ctgf*, Mm01192933_g1; *Tnf-α*, Mm00443258_m1; *Bak1*, Mm00432045_m1; *Egr2*, Mm00456650_m1; *P2x7r*, Mm00440578_m1; *Hoxa4*, Mm01335255_g1; *Fmo2*, Mm00490159_m1; *Lrrn4*, Mm00724014_m1; *Marveld3,* Mm01242978_m1; *Fat4,* Mm01291141_m1; *MIAT*, Mm01196418_g1 and *Gapdh*, Mm99999915_g1 for an endogenous control), and human (*HOXA4*, Hs01573270_m1; *FMO2*, Hs01025544_m1; *LRRN4*, Hs00379905_m1; *MIAT*, Hs00402814_m1 and *GAPDH*, Hs02786624_g1 for an endogenous control). Quantitative real-time PCR reactions were analyzed using a QuantStudio 3 Detection System (Life Technologies) as described previously [78]. Expression compared to endogenous controls was calculated using $2^{-\Delta\Delta Ct}$, and expression

levels were normalized to control.

Bromodeoxyuridine (BrdU) proliferation assay

Cell proliferation was detected *in situ* using BrdU as described [79]. In brief, HCFs were labeled with BrdU for 16 hours, and then fixed with ethanol and immunostained for BrdU incorporation using the BrdU-Labeling and Detection kit II (Sigma-Aldrich) according to manufacturer's recommendations. The total number of nuclei was determined by manual counting of DAPI-stained nuclei in 6 random fields per coverslip (original magnification, X20). All BrdU-positive nuclei were counted in each coverslip. Digital photographs of fluorescence were acquired with a Keyence microscope (BZ-X810) and processed with Adobe Photoshop.

Wound migration assay

Cell migration was detected as previously described [79]. In brief, $1X10^4$ HCFs were plated onto each well of a 2-well Culture-Insert 35 mm μ-Dishe (81176, Ibidi, Fitchburg, WI). Once the cells were confluent, the silicone insert on each dish was removed to reveal a defined cell-free gap. The medium was replaced, and images were taken at 0 and 24 hours. Subsequently, the distance between cell fronts was quantified in three wells of each group using Image J software. Initial open (cell-free) areas (0 hour) were measured to serve as the total open area, and the percentage of open area after 24 hours was calculated to determine the migratory potential of cells.

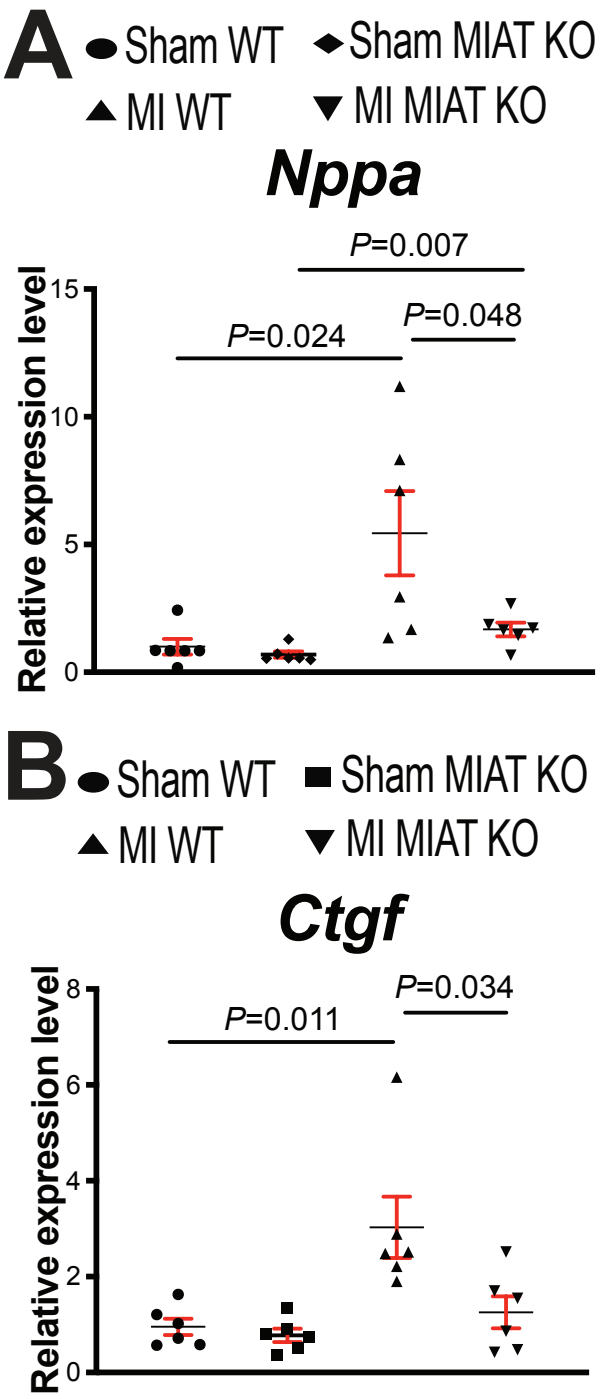
Luciferase reporter-based miR-150 targeting assay

The *Hoxa4* 3'-untranslated region (3'-UTR) with the miR-150 binding site was cloned into the pmirGLO Dual-Luciferase miR Target Expression Vector (E1130, Promega). The following oligonucleotide pairs were designed, annealed, and ligated into the pmirGLO Vector to generate WT and mutant luciferase constructs: WT forward primer, 5'-AAACTAGCGGCCGCTAGT

CTGGGGCACAGCGTTG***GGGAG***AT-3', WT reverse primer, 5'-CTAGAT***CTCCC***CAACGCTGTGCCCCAGACTAGCGGCCGCTAGTTT-3', mutant forward primer, 5'-AAACTAGCGGCCGCTAGTCTGGGGCACAGCGTTG***TAAG***AT-3', and mutant reverse primer, 5'-CTAGAT***CTTA***CAACGCTGTGCCCCAGACTAGCGGCCGCTAGTTT-3'.

Inserts and insertion sites were confirmed by ~140bp insert when digested with NotI due to NotI sites in the vector and oligonucleotides, which is followed by sequencing for all plasmids. H9c2 cells were transfected in 96-well plates with 0.1 μg of one of the luciferase plasmids along with either 50nM of miR mimic control or miR-150 mimic (MC10070; Life Technologies). At 72 hours after transfection, freshly collected cells were used for luciferase activities in Firefly and Renilla buffers measured by Dual-Glo Luciferase Assay System (E2920, Promega) with the Synergy LX FA Multi-Mode Microplate Reader (BioTek Instruments) as described [12, 65].

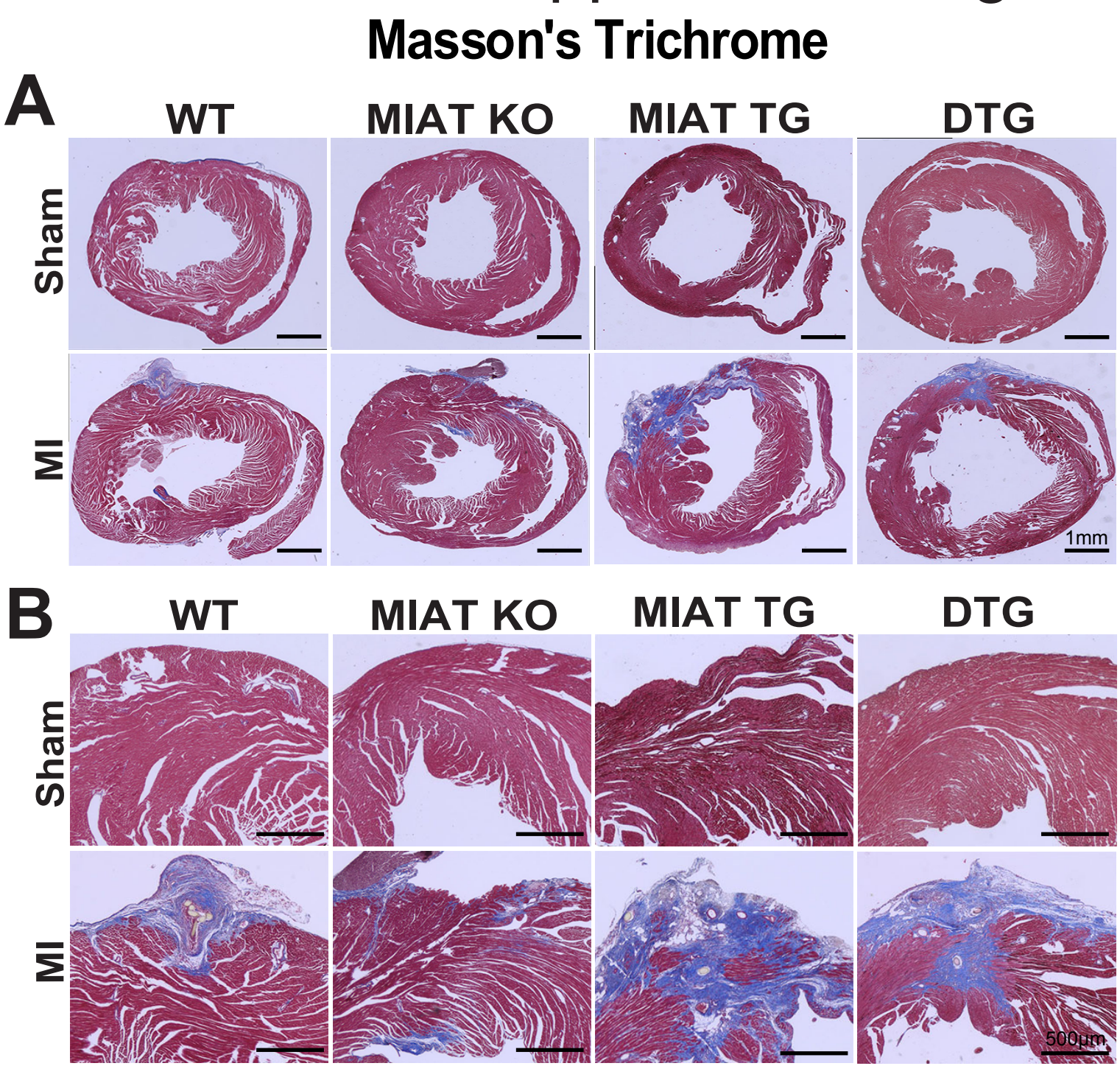
Supplemental Figure I



Supplemental Figure Legends

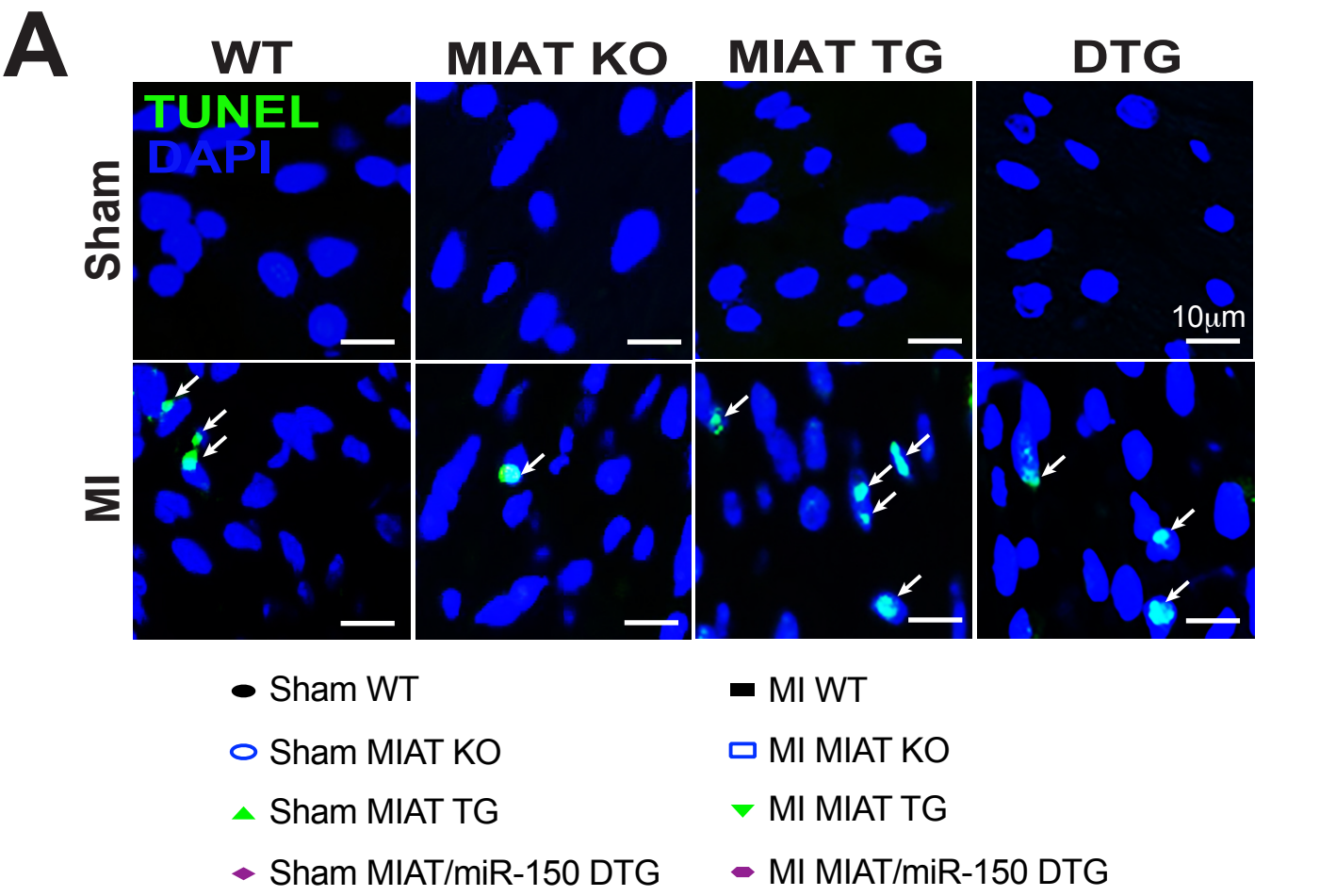
Supplemental Figure I. MIAT knockout (KO) hearts post-myocardial infarction (MI) display the reduced expression of fatal natriuretic peptide A (*Nppa*) and fibrotic connective tissue growth factor (*Ctgf*). Real-Time Quantitative Reverse Transcription (QRT)-PCR analyses of *Nppa* (**A**) and *Ctgf* (**B**) in left ventricles (LVs) from MIAT KO relative to wild-type (WT) controls at post-MI 4 weeks. Data are shown as fold induction of gene expression normalized to glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). Two-way ANOVA with Tukey multiple comparison test.

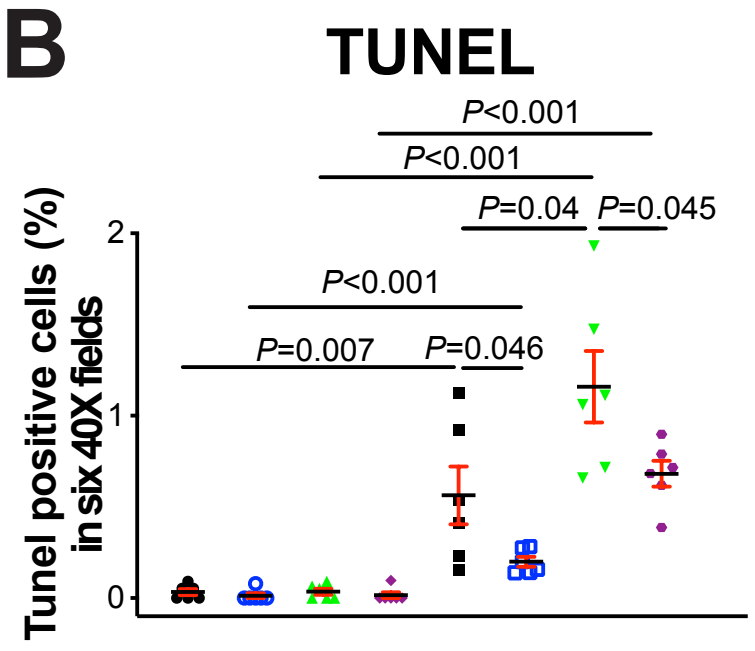
Supplemental Figure II

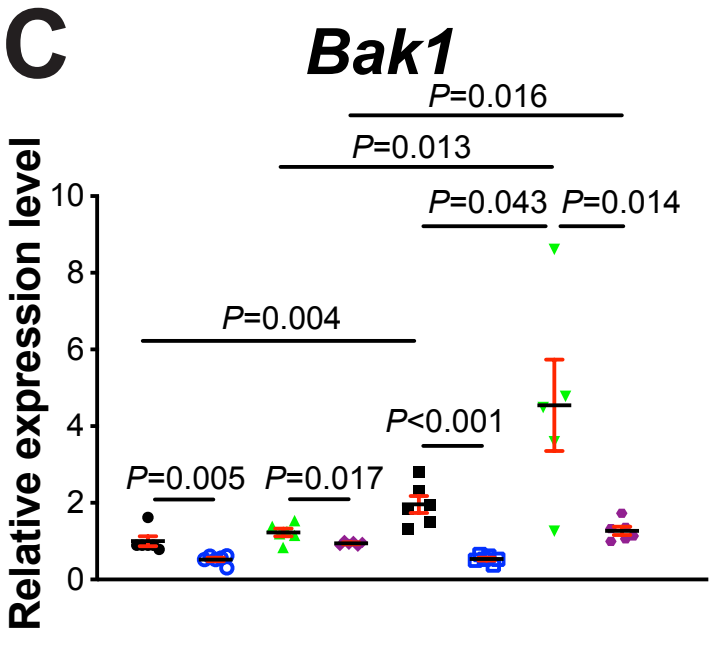


Supplemental Figure II. MIAT deletion decreases, while MIAT overexpression increases cardiac fibrosis post-MI in part by repressing miR-150. A-B, Representative Masson's trichrome images in heart sections in 8 experimental groups at 4 weeks post-MI. Fibrosis histology images from whole heart longitudinal sections (**A**: Scale bars: 1mm) and their zooming images on peri-ischemic border area (**B**: Scale bars: 500μm).

Supplemental Figure III

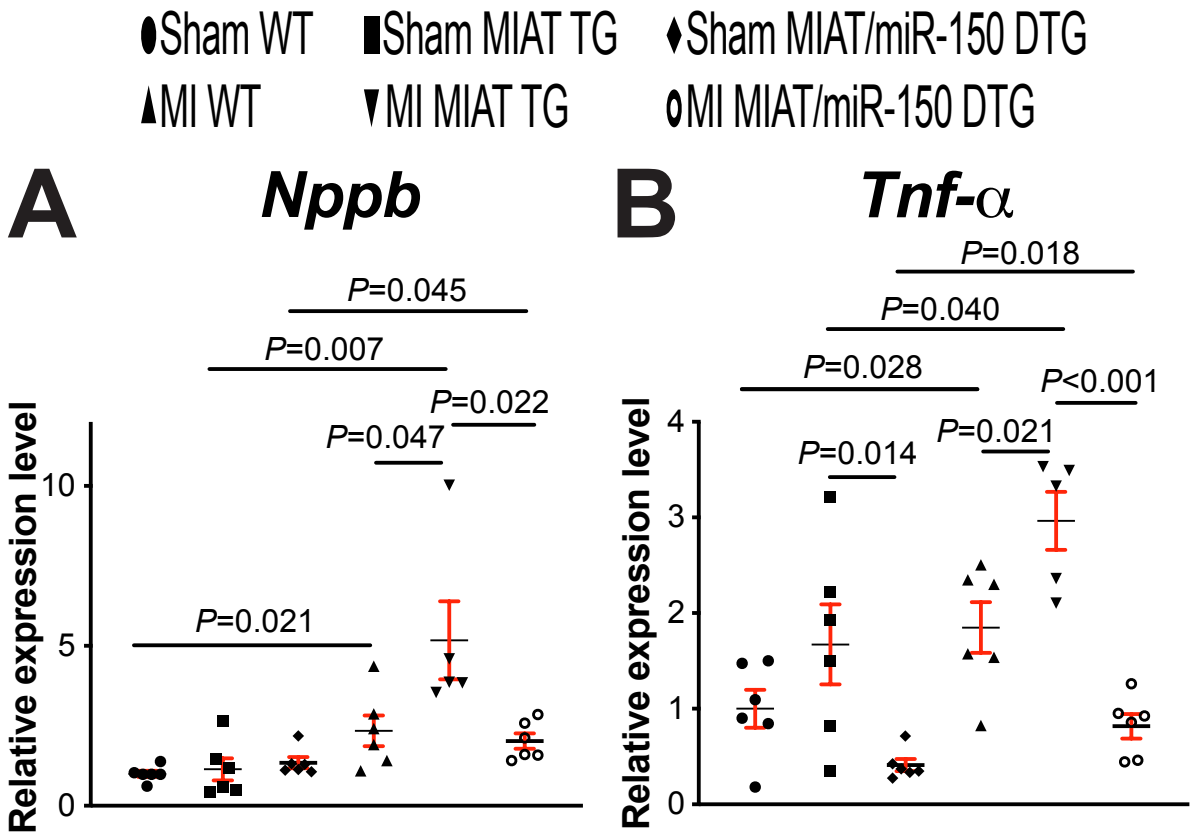






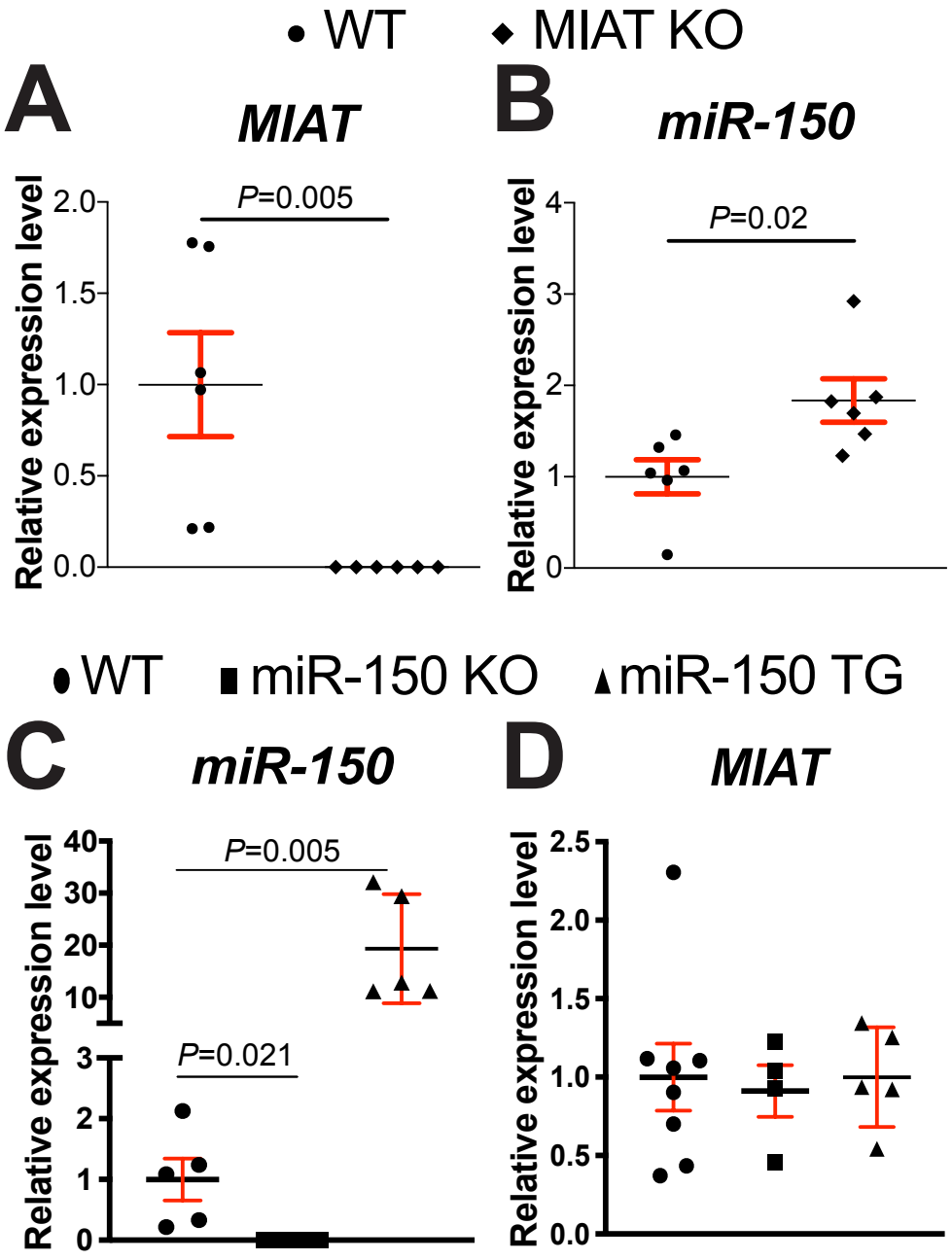
Supplemental Figure III. MIAT deletion represses, while MIAT overexpression induces cardiac apoptosis post-MI in part by repressing miR-150. A-B, Representative terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) images in heart sections of peri-ischemic border area from 8 experimental groups at post-MI 4 weeks (**A**) and quantification of apoptosis in six 40X fields (**B**). Scale bars: 10μm. **C**, QRT-PCR analysis of pro-apoptotic BCL2 Antagonist/Killer 1 (*Bak1*) expression in LVs from MIAT KO, TG and DTG compared to WT controls at 4 weeks post-MI. Data are shown as fold induction of *Bak1* expression normalized to *Gapdh*. N=6 per group. Two-way ANOVA with Tukey multiple comparison test.

Supplemental Figure IV



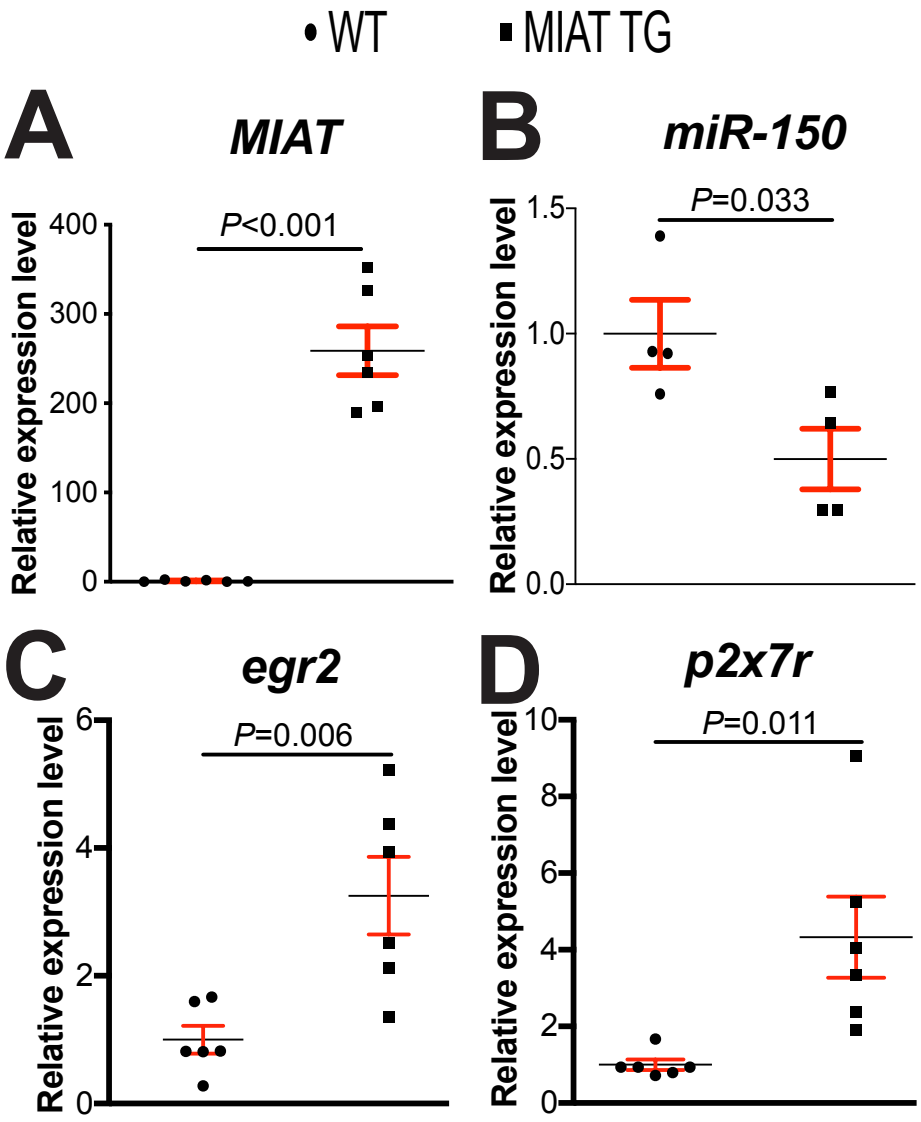
Supplemental Figure IV. MIAT transgenic (TG) MI hearts display the increased expression of fatal natriuretic peptide B (*Nppb*) and inflammatory tumor necrosis factor α (*Tnf-a*), which are reversed in part by miR-150 overexpression. QRT-PCR analyses of *Nppb* (**A**) and *Tnf-a* (**B**) in LVs from MIAT TG and MIAT/miR-150 double TG (DTG) relative to WT controls at post-MI 4 weeks. Data are shown as fold induction of gene expression normalized to *Gapdh*. Two-way ANOVA with Tukey multiple comparison test.

Supplemental Figure V



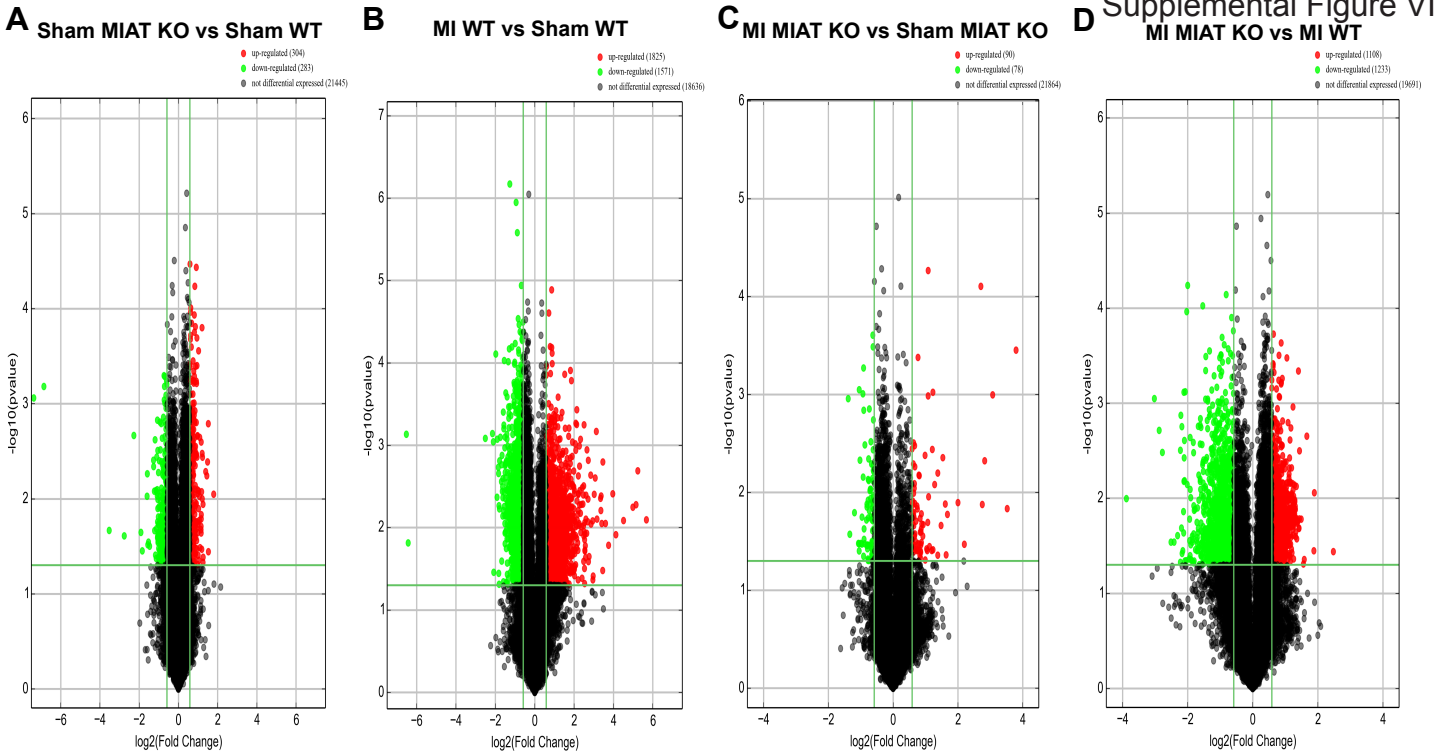
Supplemental Figure V. MIAT downregulates miR-150 in the heart, while miR-150 does not repress MIAT. A-B, The expression of MIAT (**A**) and miR-150 (**B**) in LVs from adult WT and MIAT KO mice. Unpaired 2-tailed t-test. **C-D**, The expression of miR-150 (**C**) and MIAT (**D**) in LVs from adult WT, miR-150 KO and miR-150 TG mice. One-way ANOVA with Tukey multiple comparison test.

Supplemental Figure VI



Supplemental Figure VI. MIAT overexpression downregulates miR-150 in the heart, thereby increasing two known direct target genes of miR-150. A-B, The expression of MIAT (**A**) and miR-150 (**B**) in LVs from adult WT and MIAT TG mice. **C-D**, The expression of early growth response 2 (*Egr2*: **C**) and purinergic P2X7 ionotropic receptor (*p2x7r*: **D**) in LVs from adult WT and MIAT TG mice. Unpaired 2-tailed t-test.

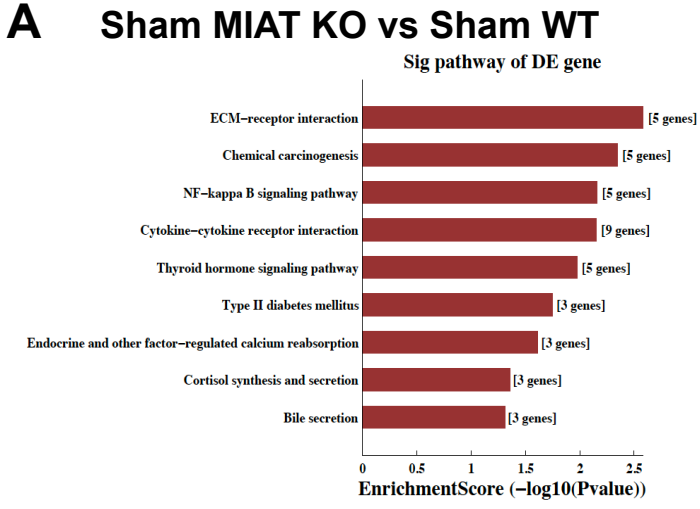
Supplemental Figure VII

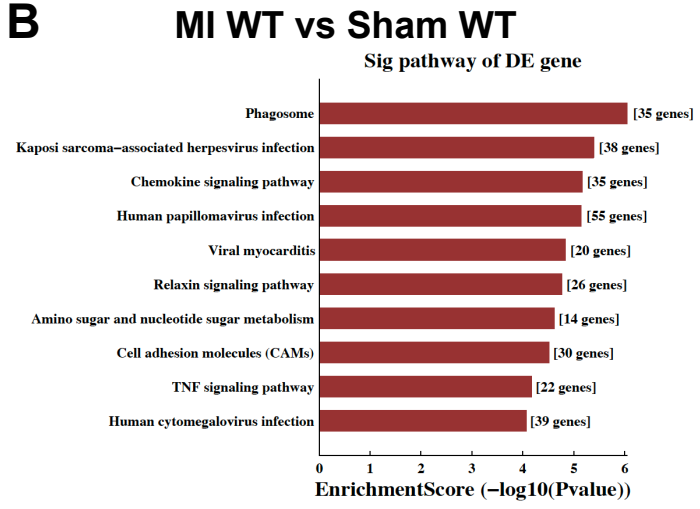


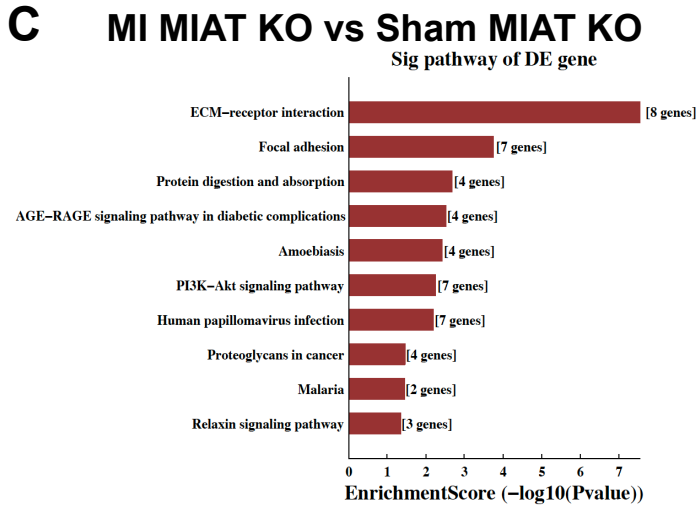
Supplemental Figure VII. Volcano plot analyses of differentially expressed cardiac genes to discover gene signatures regulated by MIAT. A-D, Volcano Plots are constructed using fold-change values and P values that allow visualization of the relationship between fold-change and statistical significance, taking both magnitudes of change and variability into consideration. The vertical lines correspond to 1.5-fold up and down, and the horizontal line represents a P value of 0.05. So, the red (up) or green (down) points in the plot represent the differentially expressed (DE) mRNAs with statistical significance. DE genes in sham MIAT KO compared to sham WT controls are shown in **A**, DE genes in MI WT compared to sham WT controls are shown in **B**, DE genes in MI MIAT KO compared to sham MIAT KO are shown in **C**, and DE genes in MI MIAT KO compared to MI WT at 4 weeks post-MI are shown in **D.**

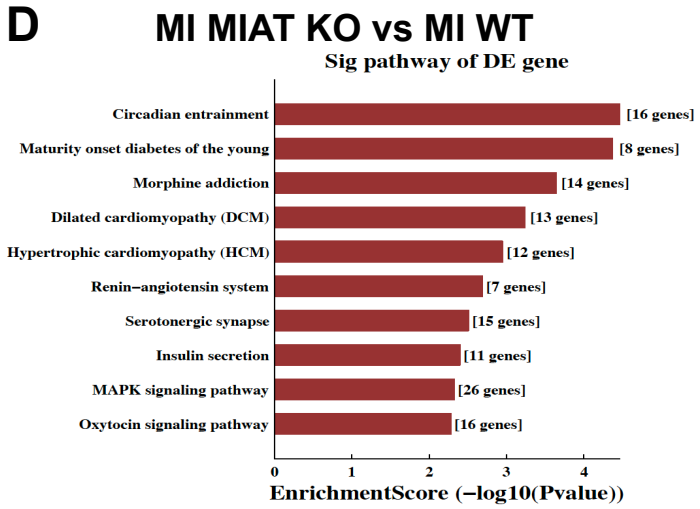
Supplemental Figure VIII

Upregulated Signaling Pathways





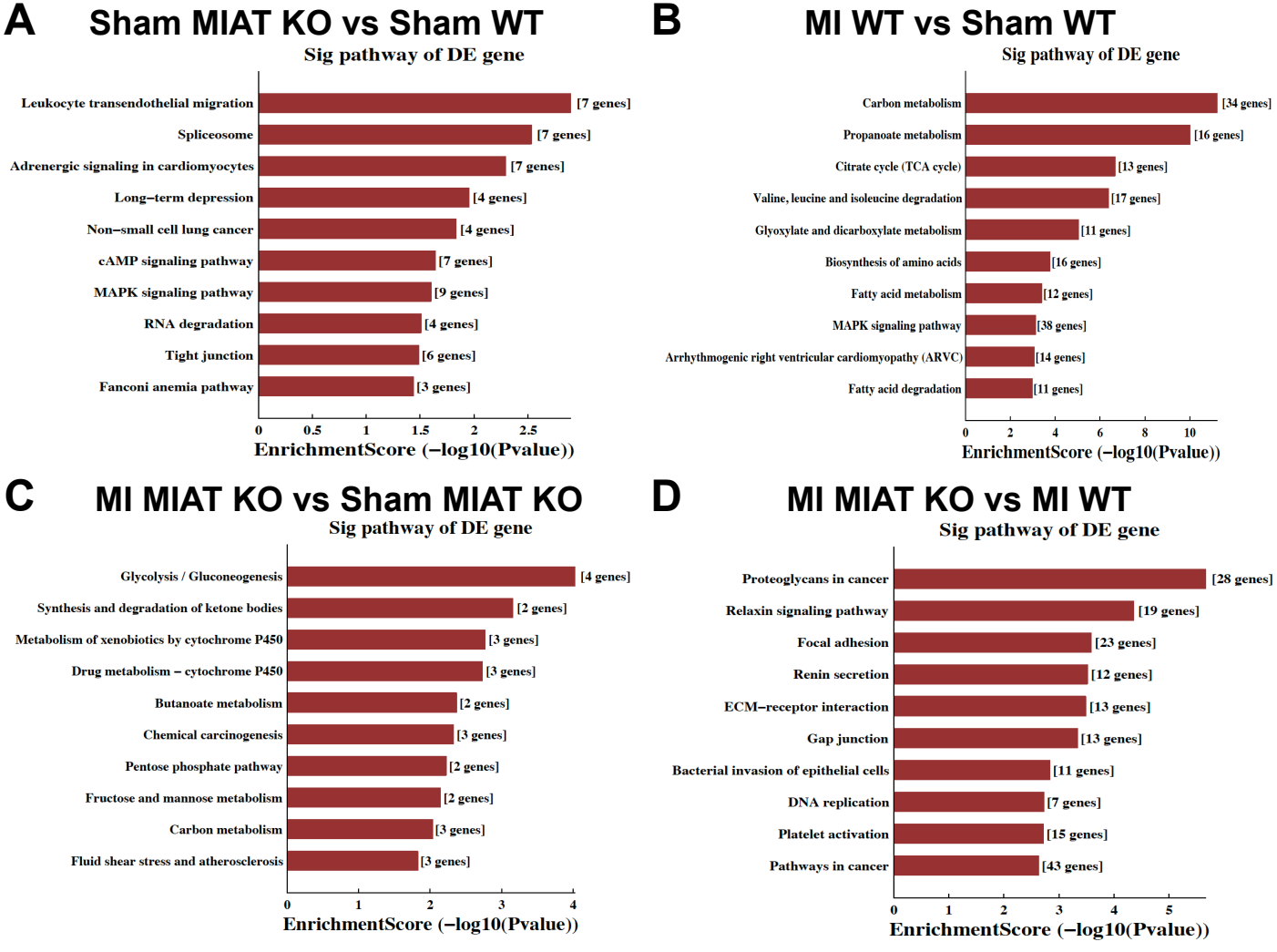




Supplemental Figure VIII. Signaling pathway analysis of differentially upregulated genes. The functional pathway analysis is performed by mapping differentially expressed (DE) genes to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Fisher's exact test is used. The *P* value indicates the significance of the pathway between groups (**A:** sham MIAT KO compared to sham WT control, **B:** MI WT compared to sham WT, **C:** MI MIAT KO compared to sham MIAT KO, and **D:** MI MIAT KO compared to MI WT). Enrichment Score is the value of the Pathway ID, which equals to -log10(Pvalue). The bar plots represent the top ten enrichment score values of the significant enrichment pathway. Among DE genes discovered in microarray analyses (Supplemental Table VI), upregulated genes are only shown.

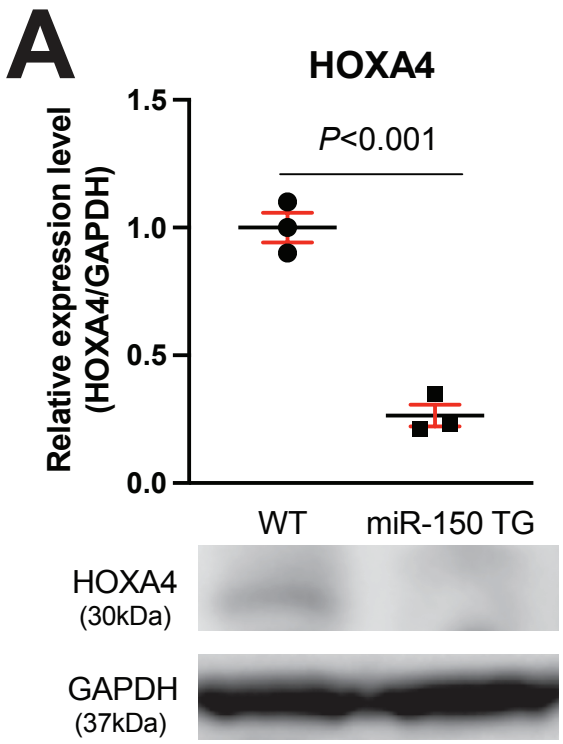
Supplemental Figure IX

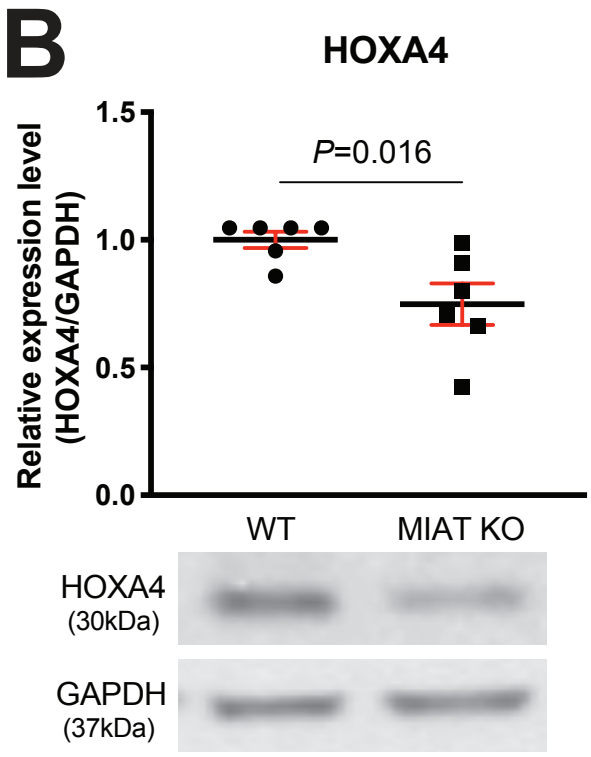
Downregulated Signaling Pathways

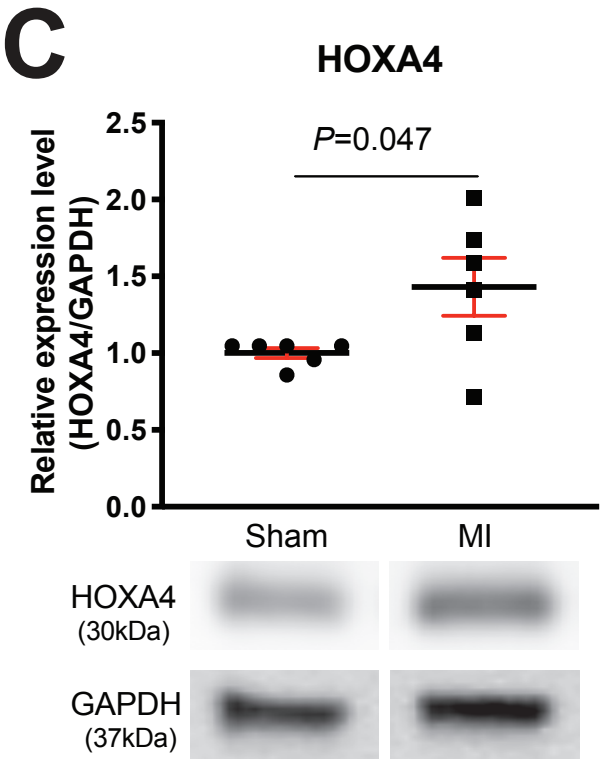


Supplemental Figure IX. Signaling pathway analysis of differentially downregulated genes. The functional pathway analysis is performed by mapping differentially expressed (DE) genes to KEGG pathways. Fisher's exact test is used. The *P* value indicates the significance of the pathway between groups (**A:** sham MIAT KO compared to sham WT control, **B:** MI WT compared to sham WT, **C:** MI MIAT KO compared to sham MIAT KO, and **D:** MI MIAT KO compared to MI WT). Enrichment Score is the value of the Pathway ID, which equals to -log10(Pvalue). The bar plots represent the top ten enrichment score values of the significant enrichment pathway. Among DE genes identified in microarray analyses (Supplemental Table VI), downregulated genes are only shown.

Supplemental Figure X



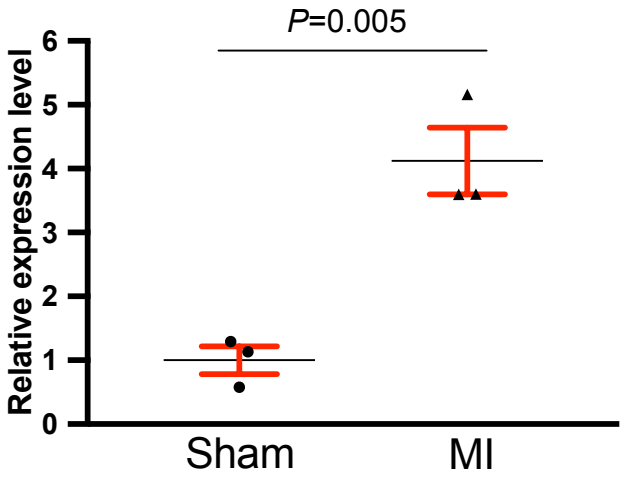




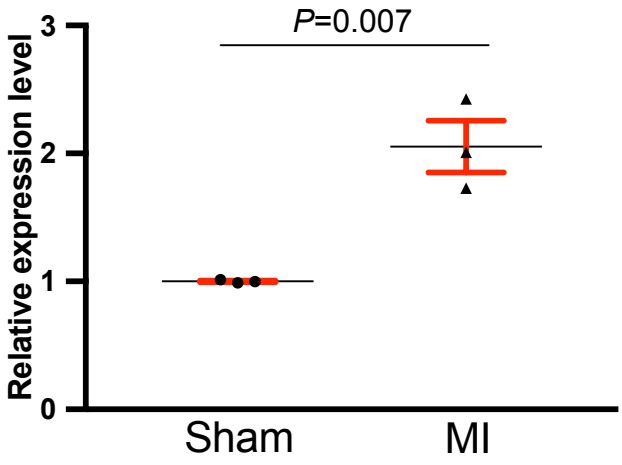
Supplemental Figure X. Homeobox a4 (HOXA4) is downregulated in miR-150 TG and MIAT KO hearts, but it is upregulated in post-MI hearts. HOXA4 protein levels were measured in left ventricle lysates from miR-150 TG (**A**) and MIAT KO mice (**B**) compared to WT, or from WT mice subjected to sham or MI for 4 weeks (**C**). Representative blot images in **C** were cropped from noncontiguous lanes in the same blot that was run on the same gel, and the images are separated by white spaces. N=3 (**A**) or N=6 (**B-C**). Unpaired 2-tailed t-test.

Supplemental Figure XI

A ***Hoxa4 at 3 days post-MI***

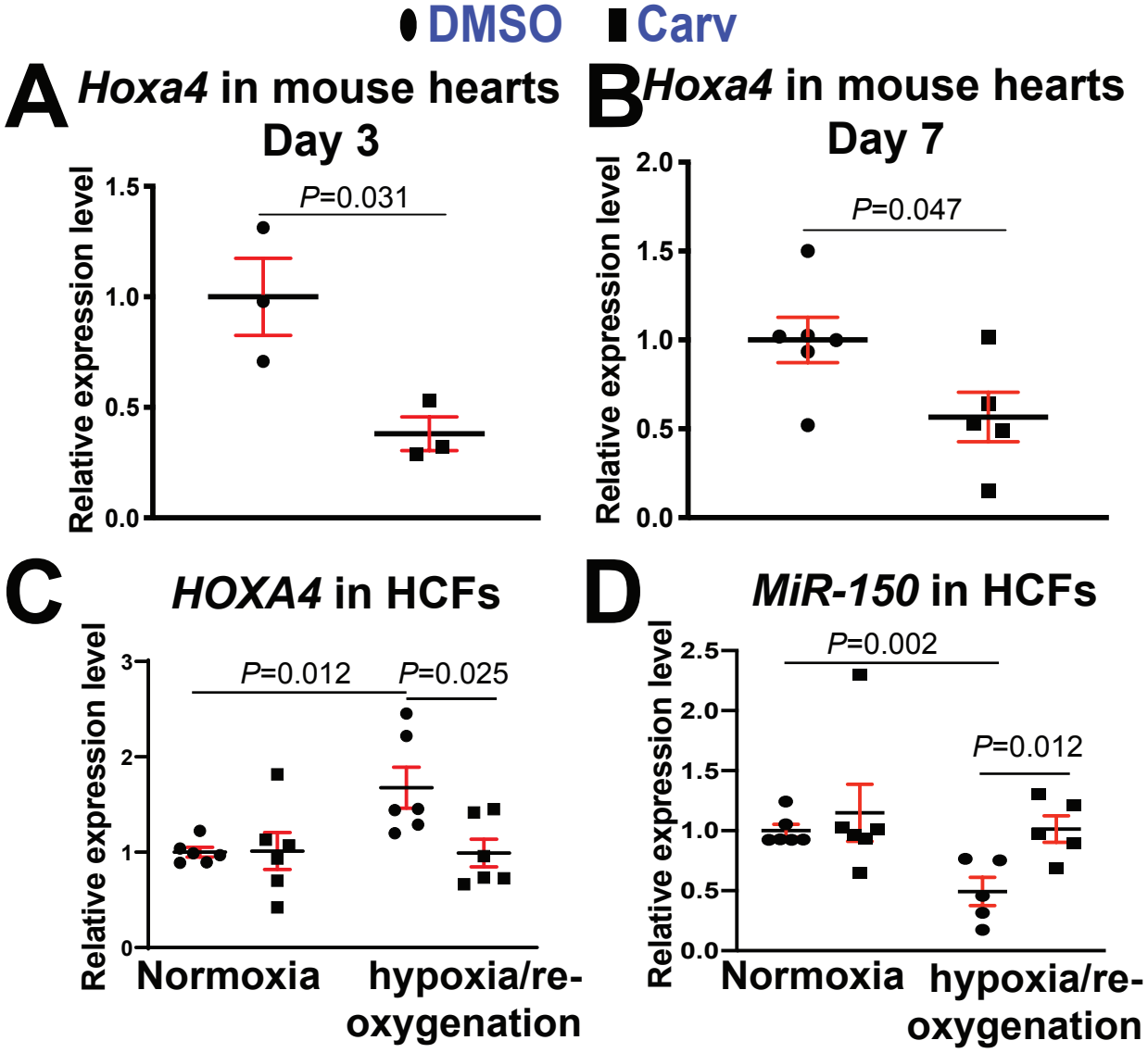


B ***Hoxa4 at 7 days post-MI***



Supplemental Figure XI. *Hoxa4* is upregulated in post-MI hearts. QRT-PCR analyses of *Hoxa4* in LVs from WT mice subjected to sham or MI for 3 days (**A**) and 7 days (**B**). Data are shown as fold induction of gene expression normalized to *Gapdh*. N=3 per group. Unpaired 2-tailed t-test.

Supplemental Figure XII



Supplemental Figure XII. *Hoxa4* is downregulated in mouse hearts and human cardiac fibroblasts by carvedilol. A-B, *Hoxa4* expression was detected by QRT-PCR in LVs from adult mouse hearts stimulated with carvedilol (Carv: 19 mg/kg per day) or vehicle for 3 (**A**) and 7 (**B**) days. Carv inhibits the expression of *Hoxa4* in mouse hearts. N=3-6. Data are shown as fold induction of *Hoxa4* expression normalized to *Gapdh*. Unpaired 2-tailed t-test. **C-D**, Human primary CFs (HCFs) were treated with 1 μM Carv for 4h and subjected to either normoxia (basal) or hypoxia/reoxygenation. QRT-PCR analyses for *HOXA4* (**C**) and miR-150 (**D**) were then performed. Carv inhibits the expression of *HOXA4* in HCFs subjected to hypoxia/reoxygenation, while activating miR-150. Moreover, *HOXA4* is upregulated in HCFs after hypoxia/reoxygenation, concurrent with downregulation of miR-150. Data are shown as fold induction of expression normalized to *GAPDH* (**C**) or U6 small nuclear RNA (*U6 SNRNA:* **D**). Two-way ANOVA with Tukey multiple comparison test.

Supplemental Table I. Demographic characteristics of human LV tissue samples

Etiology	Non-failing	HFrEF
Number	8	10
Age (years)	76 ± 4.65	47.60 ± 5.47
Sex male (%)	70	50
LVEF (%)	56 ± 0.06	22 ± 3.00

Supplemental Table II. Echocardiographic parameters in anesthetized WT, MIAT KO, MIAT TG or MIAT/miR-150 DTG mice before they were randomly assigned to 8 experimental groups.

Week 0	WT		MIAT KO		MIAT TG		MIAT/miR-150 DTG	
	Sham (n=15)	MI (n=17)	Sham (n=12)	MI (n=12)	Sham (n=16)	MI (n=17)	Sham (n=12)	MI (n=12)
CO (ml/min)	19.58 ± 0.74	20.64 ± 0.57	20.54 ± 0.73	20.71 ± 0.72	21.17 ± 0.93	20.01 ± 0.71	18.96 ± 1.14	18.88 ± 0.73
EF (%)	74.50 ± 0.90	72.92 ± 0.90	75.11 ± 0.73	74.63 ± 0.77	73.79 ± 0.94	74.34 ± 0.63	72.31 ± 0.82	73.72 ± 0.65
FS (%)	42.57 ± 0.82	41.15 ± 0.79	43.10 ± 0.66	42.73 ± 0.66	41.85 ± 0.81	41.95 ± 0.51	40.58 ± 0.64	41.74 ± 0.53
HR (bpm)	516 ± 11.69	508 ± 8.38	503 ± 10.22	524 ± 8.61	538 ± 13.51	512 ± 12.50	510 ± 16.12	520 ± 13.50
SV (μl)	37.97 ± 1.20	40.60 ± 0.89	41.05 ± 1.65	39.65 ± 1.47	39.50 ± 1.68	39.04 ± 1.05	36.95 ± 1.56	36.33 ± 1.20
Volume, diastole (μl)	51.58 ± 2.05	53.16 ± 1.27	54.88 ± 2.20	53.24 ± 2.14	52.48 ± 2.39	52.19 ± 1.47	51.38 ± 2.65	49.43 ± 1.94
Volume, systole (μl)	13.61 ± 0.92	12.56 ± 0.48	13.83 ± 0.78	13.60 ± 0.83	12.98 ± 0.84	13.15 ± 0.57	14.43 ± 1.13	13.10 ± 0.79
LVAW, diastole (mm)	0.77 ± 0.01	0.77 ± 0.02	0.80 ± 0.01	0.78 ± 0.02	0.74 ± 0.01	0.76 ± 0.01	0.75 ± 0.02	0.76 ± 0.01
LVAW, systole (mm)	1.04 ± 0.04	1.02 ± 0.03	1.13 ± 0.02	1.11 ± 0.02	0.98 ± 0.02	0.97 ± 0.03	1.04 ± 0.02	1.04 ± 0.01
LVID, diastole (mm)	3.45 ± 0.05	3.47 ± 0.04	3.57 ± 0.07	3.56 ± 0.06	3.39 ± 0.06	3.44 ± 0.04	3.50 ± 0.07	3.45 ± 0.06
LVID, systole (mm)	1.98 ± 0.04	2.04 ± 0.03	2.03 ± 0.04	2.04 ± 0.05	1.97 ± 0.04	2.00 ± 0.03	2.09 ± 0.06	2.01 ± 0.05
LVPW, diastole (mm)	0.81 ± 0.03	0.84 ± 0.02	0.80 ± 0.02	0.82 ± 0.03	0.82 ± 0.02	0.81 ± 0.02	0.77 ± 0.01	0.79 ± 0.02
LVPW, systole (mm)	1.04 ± 0.01	1.09 ± 0.02	1.08 ± 0.02	1.11 ± 0.03	1.08 ± 0.02	1.05 ± 0.01	1.09 ± 0.02	1.09 ± 0.02

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM.

Supplemental Table III. Echocardiographic parameters in anesthetized WT, MIAT KO, MIAT TG or MIAT/miR-150 DTG mice at 1 week after Sham or MI surgery.

1 week post-surgery	WT		MIAT KO		MIAT TG		MIAT/miR-150 DTG	
	Sham (n=15)	MI (n=15)	Sham (n=12)	MI (n=12)	Sham (n=16)	MI (n=15)	Sham (n=12)	MI (n=12)
CO (ml/min)	20.38 ± 0.68	18.15 ± 1.07	22.57 ± 0.79	17.77 ± 0.81***	20.10 ± 0.95	18.24 ± 1.20	21.18 ± 1.62	16.26 ± 0.82*
EF (%)	**72.94 ± 0.76**	**49.76 ± 1.22*** **	71.07 ± 0.53	53.43 ± 1.41***	**72.72 ± 0.65**	**44.78 ± 1.93***#**	**73.04 ± 1.13**	**50.16 ± 1.49***§**
FS (%)	**41.21 ± 0.63**	**24.90 ± 0.73*** **	39.67 ± 0.43	26.90 ± 0.99***	**40.96 ± 0.56**	**22.06 ± 1.07***#**	**41.27 ± 0.94**	**25.06 ± 0.90***§**
HR (bpm)	556 ± 10.58	532 ± 9.99	568 ± 11.92	563 ± 11.38	546 ± 10.45	531 ± 11.70	558 ± 16.97	526 ± 18.79
SV (µl)	36.80 ± 1.38	33.96 ± 1.38	39.73 ± 1.19	31.77 ± 1.74**	36.66 ± 1.38	34.33 ± 2.19	37.78 ± 2.38	30.88 ± 0.93*
Volume, diastole (µl)	**50.66 ± 2.21**	**66.04 ± 1.83*** **	55.97 ± 1.82	60.32 ± 3.30	**53.69 ± 2.42**	**83.64 ± 9.19***#**	**51.98 ± 3.66**	**61.89 ± 1.97*§**
Volume, systole (µl)	**13.86 ± 0.89**	**32.08 ± 1.60*** **	16.24 ± 0.70	28.54 ± 2.10***	**17.04 ± 1.78**	**49.31 ± 7.56***#**	**14.20 ± 1.48**	**31.02 ± 1.66***§**
LVAW, diastole (mm)	0.74 ± 0.02	0.69 ± 0.03	0.74 ± 0.02	0.73 ± 0.03	0.72 ± 0.02	0.63 ± 0.03*	0.77 ± 0.03	0.79 ± 0.03§§§
LVAW, systole (mm)	0.99 ± 0.04	0.91 ± 0.04	1.08 ± 0.04	0.98 ± 0.05	1.02 ± 0.03	0.81 ± 0.05**	1.10 ± 0.04	1.03 ± 0.02§§§
LVID, diastole (mm)	3.54 ± 0.06	3.95 ± 0.07***	3.64 ± 0.05	3.74 ± 0.09	**3.49 ± 0.06**	**4.20 ± 0.13*** **	**3.51 ± 0.10**	**3.79 ± 0.05*§**
LVID, systole (mm)	**2.08 ± 0.05**	**2.94 ± 0.08*** **	2.20 ± 0.04	2.74 ± 0.09***	**2.06 ± 0.05**	**3.28 ± 0.13***#**	**2.07 ± 0.08**	**2.85 ± 0.06***§**
LVPW, diastole (mm)	0.82 ± 0.02	0.81 ± 0.02	0.80 ± 0.03	0.80 ± 0.03	0.81 ± 0.02	0.76 ± 0.03	0.87 ± 0.05	0.84 ± 0.03
LVPW, systole (mm)	1.14 ± 0.03	1.07 ± 0.03	1.19 ± 0.04	1.04 ± 0.07	1.11 ± 0.02	1.00 ± 0.03**	1.18 ± 0.03	1.05 ± 0.04*

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM. All values are expressed as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. sham within same group. $^{\#}P<0.05$ vs. WT MI. $^{\S}P<0.05$ or $^{\S\S\S}P<0.001$ vs. MIAT TG MI. Only parameters, which were statistically significant between groups and sham vs. MI within same group, are highlighted with bold texts.

Supplemental Table IV. Echocardiographic parameters in anesthetized WT, MIAT KO, MIAT TG or MIAT/miR-150 DTG mice at 2 weeks after Sham or MI surgery.

2 weeks post-surgery	WT		MIAT KO		MIAT TG		MIAT/miR-150 DTG	
	Sham (n=15)	MI (n=15)	Sham (n=12)	MI (n=12)	Sham (n=16)	MI (n=15)	Sham (n=12)	MI (n=12)
CO (ml/min)	21.87 ± 0.91	20.55 ± 1.07	23.60 ± 1.07	20.18 ± 1.29	22.79 ± 0.98	19.84 ± 0.97*	24.27 ± 1.17	18.08 ± 1.31**
EF (%)	**72.77 ± 0.94**	**50.10 ± 1.09*****	**70.99 ± 0.94**	**53.71 ± 1.18*****#	**73.98 ± 0.84**	**39.94 ± 1.96*****###	**74.17 ± 0.95**	**48.52 ± 1.76*****§§
FS (%)	**41.15 ± 0.81**	**25.23 ± 0.66*****	**39.70 ± 0.76**	**27.35 ± 0.72*****#	**42.13 ± 0.71**	**19.50 ± 1.10*****###	**42.41 ± 0.79**	**24.17 ± 1.07*****§§
HR (bpm)	538 ± 9.52	523 ± 12.24	558 ± 10.29	553 ± 6.90	555 ± 12.36	528 ± 11.83	556 ± 18.83	550 ± 9.60
SV (µl)	40.66 ± 1.58	39.22 ± 1.76	42.29 ± 1.81	36.47 ± 2.21	41.00 ± 1.27	37.60 ± 1.68	43.66 ± 1.50	32.84 ± 2.22***
Volume, diastole (µl)	55.62 ± 2.16	77.04 ± 3.33***	59.63 ± 2.65	68.48 ± 4.60	59.15 ± 3.08	102.01 ± 11.77**	58.99 ± 2.18	67.71 ± 3.92§
Volume, systole (µl)	**14.96 ± 0.86**	**37.82 ± 1.82*****	17.34 ± 1.08	32.01 ± 2.68***	**18.16 ± 2.81**	**64.41 ± 10.87*****#	**15.33 ± 0.94**	**34.87 ± 2.29*****§
LVAW, diastole (mm)	0.76 ± 0.01	0.69 ± 0.02**	0.74 ± 0.02	0.66 ± 0.02**	0.75 ± 0.02	0.62 ± 0.03***	0.77 ± 0.03	0.77 ± 0.03§§
LVAW, systole (mm)	**1.12 ± 0.04**	**0.97 ± 0.04****	1.20 ± 0.04	0.88 ± 0.05***	**1.10 ± 0.04**	**0.76 ± 0.06*****##	**1.25 ± 0.06**	**0.99 ± 0.05****§§
LVID, diastole (mm)	**3.60 ± 0.06**	**4.15 ± 0.09*****	3.73 ± 0.07	3.94 ± 0.11	**3.56 ± 0.06**	**4.55 ± 0.10*****##	3.72 ± 0.06	3.93 ± 0.10§§§
LVID, systole (mm)	**2.11 ± 0.05**	**3.11 ± 0.07*****	2.25 ± 0.05	2.87 ± 0.10***	**2.06 ± 0.05**	**3.67 ± 0.12*****###	2.14 ± 0.05	**2.98 ± 0.08*****§§§
LVPW, diastole (mm)	0.88 ± 0.02	0.78 ± 0.03**	0.82 ± 0.01	0.77 ± 0.03	0.84 ± 0.02	0.76 ± 0.04	0.85 ± 0.03	0.79 ± 0.03
LVPW, systole (mm)	1.14 ± 0.03	0.97 ± 0.05**	1.21 ± 0.03	1.08 ± 0.05*	1.16 ± 0.03	0.98 ± 0.06**	1.27 ± 0.05	1.03 ± 0.05**

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM. All values are expressed as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. sham within same group. $^{\#}P<0.05$, $^{\#\#}P<0.01$ or $^{\#\#\#}P<0.001$ vs. WT MI. $^{\S}P<0.05$, $^{\S\S}P<0.01$ or $^{\S\S\S}P<0.001$ vs. MIAT TG MI. Only parameters, which were statistically significant between groups and sham vs. MI within same group, are highlighted with bold texts.

Supplemental Table V. Echocardiographic parameters in anesthetized WT, MIAT KO, MIAT TG or MIAT/miR-150 DTG mice at 4 weeks after Sham or MI surgery.

4 weeks post-surgery	WT		MIAT KO		MIAT TG		MIAT/miR-150 DTG	
	Sham (n=15)	MI (n=15)	Sham (n=12)	MI (n=12)	Sham (n=16)	MI (n=15)	Sham (n=12)	MI (n=12)
CO (ml/min)	22.94 ± 0.85	19.46 ± 0.90**	22.04 ± 0.76	19.31 ± 0.97*	22.59 ± 0.72	19.25 ± 0.77**	22.48 ± 1.10	19.09 ± 1.17*
EF (%)	**73.22 ± 1.07**	**48.20 ± 1.48*** **	**73.49 ± 0.73**	**53.09 ± 1.50*** #**	**73.02 ± 0.85**	**40.80 ± 1.83*** ##**	**71.89 ± 0.95**	**48.78 ± 1.38*** §§**
FS (%)	**41.69 ± 0.89**	**24.07 ± 0.86*** **	**41.67 ± 0.60**	**26.95 ± 0.93*** #**	**41.45 ± 0.75**	**19.89 ± 1.01*** ##**	**40.42 ± 0.80**	**24.36 ± 0.82*** §§**
HR (bpm)	528 ± 10.23	522 ± 8.45	553 ± 9.91	548 ± 12.07	551 ± 6.77	541 ± 11.22	540 ± 17.40	526 ± 10.71
SV (µl)	43.36 ± 1.16	37.47 ± 1.93*	39.96 ± 1.54	35.18 ± 1.47*	41.06 ± 1.27	35.58 ± 1.23**	41.65 ± 1.46	36.22 ± 2.02*
Volume, diastole (µl)	**61.03 ± 1.94**	**75.36 ± 5.49***	55.36 ± 2.49	67.65 ± 3.75*	**59.03 ± 1.64**	**90.34 ± 3.88*** #**	**58.07 ± 2.23**	**74.22 ± 3.63** §§**
Volume, systole (µl)	**17.67 ± 0.98**	**38.55 ± 4.57*** **	15.32 ± 1.11	32.47 ± 2.67***	**17.97 ± 1.79**	**54.76 ± 3.63*** ##**	**16.42 ± 1.02**	**38.00 ± 2.09*** §§§**
LVAW, diastole (mm)	**0.75 ± 0.02**	**0.67 ± 0.02****	0.76 ± 0.02	0.70 ± 0.01**	**0.78 ± 0.02**	**0.59 ± 0.02*** ##**	0.79 ± 0.01	0.76 ± 0.03§§§
LVAW, systole (mm)	1.05 ± 0.03	0.93 ± 0.04*	1.12 ± 0.02	1.01 ± 0.04*	**1.08 ± 0.04**	**0.83 ± 0.04*** **	**1.15 ± 0.03**	**0.99 ± 0.03** §§**
LVID, diastole (mm)	**3.74 ± 0.06**	**4.12 ± 0.14***	3.54 ± 0.08	3.93 ± 0.09**	**3.70 ± 0.03**	**4.44 ± 0.08*** #**	**3.69 ± 0.06**	**4.09 ± 0.08*** §§**
LVID, systole (mm)	**2.18 ± 0.06**	**3.06 ± 0.16*** **	2.07 ± 0.06	2.88 ± 0.10***	**2.16 ± 0.04**	**3.57 ± 0.10*** #**	**2.20 ± 0.05**	**3.09 ± 0.07*** §§§**
LVPW, diastole (mm)	0.86 ± 0.03	0.77 ± 0.03*	0.84 ± 0.03	0.81 ± 0.02	0.86 ± 0.02	0.76 ± 0.03**	0.83 ± 0.03	0.81 ± 0.02
LVPW, systole (mm)	1.22 ± 0.05	0.97 ± 0.04***	1.16 ± 0.03	1.05 ± 0.05	1.11 ± 0.05	0.97 ± 0.03*	1.20 ± 0.05	1.01 ± 0.03**

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM. All values are expressed as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. sham within same group. $^{\#}P<0.05$ or $^{\#\#}P<0.01$ vs. WT MI. $^{\S\S}P<0.01$ or $^{\S\S\S}P<0.001$ vs. MIAT TG MI. Only parameters, which were statistically significant between groups and sham vs. MI within same group, are highlighted with bold texts.

Supplemental Table VI. Differentially expressed genes.

Please refer to the Supplemental Table VI. Differentially Expressed Genes.xls file

Supplemental Table VII. Echocardiographic parameters in anesthetized WT or *Hoxa4* KO mice before they were randomly assigned to 4 experimental groups.

Week 0	WT		*Hoxa4* KO	
	Sham (n=4)	MI (n=4)	Sham (n=4)	MI (n=4)
CO (ml/min)	18.94 ± 0.50	19.28 ± 0.62	17.88 ± 0.28	18.76 ± 0.77
EF (%)	68.97 ± 1.79	67.76 ± 0.52	68.51 ± 1.74	70.68 ± 1.21
FS (%)	37.92 ± 1.41	36.93 ± 0.39	37.55 ± 1.41	39.22 ± 1.03
HR (bpm)	524 ± 6.16	527 ± 12.82	502 ± 6.14	520 ± 12.88
SV (μl)	36.14 ± 0.85	36.64 ± 1.02	35.64 ± 0.87	36.10 ± 1.22
Volume, diastole (μl)	52.49 ± 1.53	53.85 ± 1.46	52.07 ± 1.22	51.05 ± 1.28
Volume, systole (μl)	16.35 ± 1.28	17.21 ± 0.50	16.44 ± 1.12	14.95 ± 0.63
LVAW, diastole (mm)	0.70 ± 0.04	0.66 ± 0.02	0.67 ± 0.06	0.64 ± 0.01
LVAW, systole (mm)	0.99 ± 0.03	0.93 ± 0.04	0.98 ± 0.02	0.99 ± 0.01
LVID, diastole (mm)	3.54 ± 0.04	3.56 ± 0.03	3.53 ± 0.03	3.50 ± 0.04
LVID, systole (mm)	2.20 ± 0.07	2.23 ± 0.01	2.21 ± 0.06	2.13 ± 0.04
LVPW, diastole (mm)	0.74 ± 0.03	0.75 ± 0.04	0.67 ± 0.03	0.67 ± 0.03
LVPW, systole (mm)	0.97 ± 0.03	0.96 ± 0.02	0.96 ± 0.04	0.96 ± 0.01

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM.

Supplemental Table VIII. Echocardiographic parameters in anesthetized WT or *Hoxa4* KO mice at 1 week after Sham or MI surgery.

1 week post-surgery	WT		*Hoxa4* KO	
	Sham (n=4)	MI (n=4)	Sham (n=4)	MI (n=4)
CO (ml/min)	**18.99 ± 0.79**	**13.86 ± 0.32****	**17.65 ± 0.13**	**16.26 ± 0.46**##**
EF (%)	**71.86 ± 0.73**	**44.73 ± 1.06*****	**69.99 ± 0.72**	**53.16 ± 0.78***##**
FS (%)	**40.17 ± 0.63**	**21.75 ± 0.61*****	**38.57 ± 0.59**	**26.80± 0.49***##**
HR (bpm)	542 ± 4.51	514 ± 9.65	517 ± 10.52	528 ± 11.80
SV (µl)	**35.01 ± 1.32**	**27.01 ± 0.48****	**34.16 ± 0.52**	**30.81 ± 0.59*##**
Volume, diastole (µl)	51.01 ± 1.05	60.43 ± 0.46***	48.80 ± 0.30	57.98 ± 1.16***
Volume, systole (µl)	**16.00 ± 0.54**	**33.42 ± 0.88*****	**14.64 ± 0.30**	**27.17 ± 0.84***##**
LVAW, diastole (mm)	0.68 ± 0.03	0.65 ± 0.02	0.69 ± 0.03	0.62 ± 0.02
LVAW, systole (mm)	1.02 ± 0.03	0.88 ± 0.03*	1.00 ± 0.01	1.00 ± 0.01#
LVID, diastole (mm)	3.50 ± 0.03	3.76 ± 0.01***	3.44 ± 0.01	3.70 ± 0.03***
LVID, systole (mm)	**2.10 ± 0.02**	**2.94 ± 0.03*****	**2.11 ± 0.02**	**2.71 ± 0.03***##**
LVPW, diastole (mm)	0.70 ± 0.02	0.74 ± 0.01	0.66 ± 0.02	0.67 ± 0.03
LVPW, systole (mm)	1.00 ± 0.02	0.97 ± 0.03	0.95 ± 0.02	0.92 ± 0.03

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ or $^{***}P<0.001$ vs. sham within same group. $^{\#}P<0.05$ or $^{\#\#}P<0.01$ vs. WT MI. Only parameters, which were statistically significant between groups and sham vs. MI within same group, are highlighted with bold texts.

Supplemental Table IX. Echocardiographic parameters in anesthetized WT or *Hoxa4* KO mice at 2 weeks after Sham or MI surgery.

2 weeks post-surgery	WT		*Hoxa4* KO	
	Sham (n=4)	MI (n=4)	Sham (n=4)	MI (n=4)
CO (ml/min)	19.51 ± 0.29	13.91 ± 0.37***	18.62 ± 0.24	15.19 ± 0.46**
EF (%)	**69.85 ± 2.20**	**43.83± 0.63*** **	**67.60 ± 0.62**	**49.92 ± 0.40***###**
FS (%)	**38.65 ± 1.76**	**21.22 ± 0.36*** **	**36.73 ± 0.49**	**24.79 ± 0.25***###**
HR (bpm)	546 ± 6.49	528 ± 13.03	541 ± 6.72	521 ± 11.94
SV (µl)	**35.79 ± 0.76**	**26.38 ± 0.20*** **	**34.44 ± 0.70**	**29.13 ± 0.33**###**
Volume, diastole (µl)	51.41 ± 1.76	60.23± 0.78**	50.93 ± 0.59	58.34 ± 0.33***
Volume, systole (µl)	**15.62 ± 1.58**	**33.85 ± 0.78*** **	**16.49 ± 0.17**	**29.21 ± 0.23***## **
LVAW, diastole (mm)	0.71 ± 0.02	0.64 ± 0.04	0.72 ± 0.03	0.64 ± 0.02
LVAW, systole (mm)	1.03 ± 0.03	0.75 ± 0.01***	1.01± 0.02	0.81 ± 0.03**
LVID, diastole (mm)	3.51 ± 0.05	3.76 ± 0.02**	3.50 ± 0.02	3.71 ± 0.01***
LVID, systole (mm)	**2.16 ± 0.09**	**2.96 ± 0.03*** **	**2.22 ± 0.01**	**2.79 ± 0.01***## **
LVPW, diastole (mm)	0.69 ± 0.02	0.73 ± 0.03	0.68 ± 0.02	0.66 ± 0.02
LVPW, systole (mm)	1.01 ± 0.02	0.98 ± 0.03	0.93 ± 0.03	0.90 ± 0.01

Abbreviations: MI = myocardial infarction, CO = cardiac output, EF = ejection fraction, FS = fractional shortening, HR = heart rate, SV = stroke volume, LVAW = left ventricular anterior wall thickness, LVID = left ventricular interior diameter, LVPW = left ventricular posterior wall thickness. All values are expressed as mean ± SEM. $^{**}P<0.01$ or $^{***}P<0.001$ vs. sham within same group. $^{\#\#}P<0.01$ or $^{\#\#\#}P<0.001$ vs. WT MI. Only parameters, which were statistically significant between groups and sham vs. MI within same group, are highlighted with bold texts.