SUPPLEMENTARY MATERIAL

Expanded Methods

Transthoracic Echocardiography Mice were anesthetized with 1.5-2% isoflorane inhalation and put in supine position on a warming pad. The chest and lower neck area was gently shaved and washed with N/S to avoid any chemical burn injury. Transthoracic echocardiography was performed using the Vevo 2100 high-resolution echocardiography machine equipped with a 22-55 MHz transducer (VisualSonics, Toronto, ON, Canada). B-mode and color Doppler images were acquired of the ventricular chambers, aortic arch, carotid arteries, pulmonary artery, and left main coronary artery through right parasternal and left parasternal short axis window, respectively. All acquisitions were performed by the same experienced operator and all images were acquired at an average frame rate of 250 frame per second with 20% transmit power to avoid high power ultrasonic tissue injury. Standard LV dimensions including LVID, posterior and septal wall thickness were calculated by a digitally reconstructed anatomical M-mode view from the original short axis B-mode image. Pulmonary, coronary, aortic arch and carotid arteries flow was measured as VTIs multiplied by the heart rate and cross sectional area inferred from the luminal dimension derived from pulse wave Doppler and B-mode images respectively. We employed the VevoStrain software (VisualSonics, Toronto, ON, Canada) for functional data analysis. Three cardiac cycles were selected on a standard 2D B-Mode parasternal long axis image that showed clear endocardial and epicardial borders as well as both LV inflow and outflow. Semi-automatic tracing of endocardial and epicardial borders was performed and corrected if needed. Traced Images were then processed by speckle-tracking algorithm in a frame-by-frame manner. LV systolic and diastolic volumes, ejection fraction, and mass were recorded.

Due to lack of continuous flow Doppler capability on this echo machine, the pressure gradient over TAC site was calculated indirectly using the modified Bernoulli equation and the blood velocity before and at the TAC site measured by pulse wave Doppler with a wide sampling gate and using a 13-24 MHz transducer to resolve higher velocities.

qPCR Primer Sequences

β-MHC: 5'-AGGGCGACCTCAACGAGAT-3' and 5'-CAGCAGACTCTGGAGGCTCTT-3'
Acta: 5'-CCTGTATGCCAACAACGTCA-3' and 5'-CTCGTCGTACTCCTGCTTGG-3'
ANP: 5'-ATGGGCTCCTTCTCCATCA-3' and 5'-CCTGCTTCCTCAGTCTGCTC-3'
BNP: 5'-GGATCTCCTGAAGGTGCTGT-3' and 5'-TTCTTTTGTGAGGCCTTGGT-3'
Col1a: 5'- ACCTTCCTGCGCCTAATGTC-3' and 5'- AAGGGTGCTGTAGGTGAAGC-3'

MMP-9: 5′-AGAAGCAGTCTCTACGGCCG-3′ and 5′-TGATGGTCCCACTTGAGGCC-3′
TIMP1: 5’-AGTGATTTCCCCGCCAACTC and 5’-GGGCCATCATGGTATCTGCT-3’
Post:, 5’- AGGTGGCGATGGTCACTTATT-3’ and 5’- AACGGCCTTCTCTTGATCGT-3’
VE-Cad: 5’-AGACAAGGATGTGGTGCCAG-3’ and 5’- ACTGCCCATACTTGACCGTG-3’
VEGF: 5′-AGGAGAGCAGAAGTCCCATGA-3′ and 5′-CACAGGACGGCTTGAAGATGT-3′
VEGFr-2: 5’-TGGGCAGTCAAGTCCG AATC-3’ and 5’-GTTGGTGAGGATGACCGTGT-3’
SDF-1: 5’-ACCAGTCAGCCTGAG CTACC-3’ and 5′-CACTTTAATTTCGGGTCAATGC-3′
16s: 5’-GATTTGCTGGTGTG GATATT-3’and 5’- TCTTTGATCTCCTTCTTGGA-3’

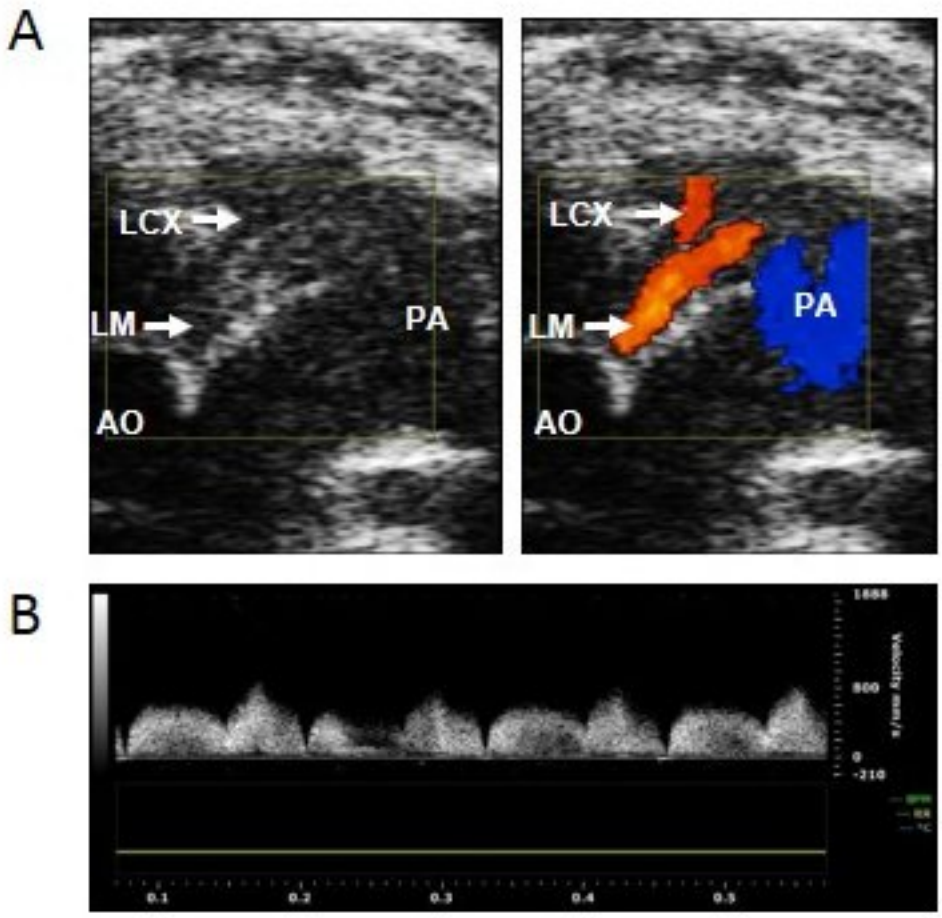
Supplementary Table 1 Animal numbers for specific experiments not otherwise detailed in the primary manuscript.

	WT Sham	WT TAC	KO Sham	KO TAC
Apoptosis (TUNEL, Bcl2/Bax)	5	5	5	5
Coronary Flow	6	5	4	7
Vascular qPCR	5	5	5	5
Vascular Density	6	5	4	7
Diff Vascular Density	3	3	3	5
HW/BW	6	9	5	7
Lung Dry/Wet	6	9	5	9
Hypertrophy qPCR	5	5	5	5
HIF Westerns	3	5	3	5
Fibrosis qPCR	5	7	5	7
Fibrosis Digital	4	7	5	6
CDA	5	5	4	6
CDA (Cmpiled CDAs)	9960	13875	7831	12846

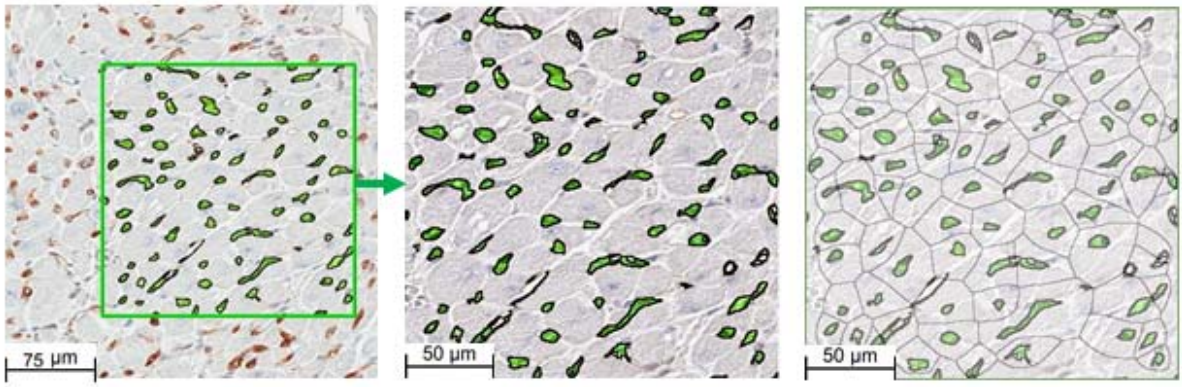
Supplementary Table 2 Complete serial echocardiographic data over the 6-week time course. P<0.05: *vs Baseline, †vs KO sham, ‡vs WT sham, §vs WT TAC (LVID, left ventricular internal dimensions; PW, posterior wall; FS, fractional shortening; EF, ejection fraction).

	Knock-Out	Wild Type	KO Sham	WT Sham
Baseline				
n=	8	7	5	6
LVIDs (mm)	2.84 ± 0.12	2.89 ± 0.08	2.77 ± 0.12	3.05 ± 0.16
LVIDd (mm)	3.98 ± 0.06	4.15 ± 0.10	3.94 ± 0.06	4.19 ± 0.14
PWs (mm)	1.09 ± 0.04	1.15 ± 0.05	1.10 ± 0.09	1.07 ± 0.05
PWd (mm)	0.74 ± 0.03	0.73 ± 0.03	0.77 ± 0.04	0.73 ± 0.05
FS (%)	30.85 ± 1.76	30.23 ± 1.21	30.38 ± 2.07	26.14 ± 1.79
LV Mass (mm)	81.25 ± 1.78	83.00 ± 2.19	75.600 ± 1.75	85.67 ± 4.47
EF (%)	47.25 ± 1.83	47.00 ± 1.45	47.40 ± 2.62	43.83 ± 2.41
1 Week Post TAC				
n=	8	7	5	6
LVIDs (mm)	3.15 ± 0.16	2.96 ± 0.22	2.87 ± 0.13	3.02 ± 0.06
LVIDd (mm)	4.06 ± 0.07	4.11 ± 0.15	4.02 ± 0.07	4.22 ± 0.07
PWs (mm)	1.30 ± 0.07	1.34 ± 0.09	1.16 ± 0.06	1.17 ± 0.05
PWd (mm)	1.01 ± 0.07 *	0.98 ± 0.04 *	0.83 ± 0.06	0.72 ± 0.03
FS (%)	22.45 ± 2.65 *	28.70 ± 2.44	28.59 ± 1.73	27.19 ± 0.88
LV Mass (mm)	100.88 ± 4.37 *	97.75 ± 6.1	81.00 ± 1.30	77.67 ± 5.74
EF (%)	34.50 ± 3.00 * †	41.57 ± 2.52	49.00 ± 0.95	46 ± 1.39
3 Week Post TAC				
n=	8	7	5	6
LVIDs (mm)	3.92 ± 0.23 * †	3.51 ± 0.31 *	2.77 ± 0.12	2.81 ± 0.15
LVIDd (mm)	4.50 ± 0.18 *	4.22 ± 0.28	3.95 ± 0.09	4.07 ± 0.10
PWs (mm)	1.18 ± 0.09	1.34 ± 0.06	1.10 ± 0.05	1.27 ± 0.08
PWd (mm)	1.04 ± 0.15 *	1.02 ± 0.07 *	0.79 ± 0.04	0.83 ± 0.07
FS (%)	16.52 ± 2.15 * †	24.61 ± 3.66	31.47 ± 2.36	31.34 ± 1.62
LV Mass (mm)	148.00 ± 8.85 * †	131.43 ± 12.92 * ‡	92.20 ± 7.28	88.33 ± 4.65
EF (%)	29.25 ± 2.53 * †	36.71 ± 3.60 *	50.80 ± 0.08	47.00 ± 2.16
4 Week Post TAC				
n=	8	7	5	6
LVIDs (mm)	4.15 ± 0.25 * †	3.54 ± 0.34 *	2.89 ± 0.07	2.98 ± 0.13
LVIDd (mm)	4.72 ± 0.17 * †	4.32 ± 0.26	4.03 ± 0.07	4.07 ± 0.12
PWs (mm)	1.13 ± 0.08	1.35 ± 0.10	1.15 ± 0.04	1.12 ± 0.02
PWd (mm)	0.96 ± 0.05	1.07 ± 0.06 * ‡	0.79 ± 0.02	0.76 ± 0.06
FS (%)	12.40 ± 2.01 * †	19.67 ± 3.17 *	28.10 ± 2.29	27.04 ± 1.50
LV Mass (mm)	153.50 ± 12.40 * †	145.86 ± 18.03 * ‡	92.40 ± 4.01	91.00 ± 8.33
EF (%)	23.63 ± 3.04 * † §	33.71 ± 3.08 * ‡	47.60 ± 1.29	47.50 ± 2.11
6 Week Post TAC				
n=	7	7	5	6
LVIDs (mm)	4.57 ± 0.29 * † §	3.78 ± 0.44 *	2.60 ± 0.12	3.00 ± 0.16
LVIDd (mm)	5.18 ± 0.23 * †	4.60 ± 0.36 *	3.90 ± 0.09	4.03 ± 0.16
PWs (mm)	1.12 ± 0.05	1.42 ± 0.12 ‡	1.18 ± 0.03	1.02 ± 0.08
PWd (mm)	0.91 ± 0.06	1.19 ± 0.11 * ‡	0.76 ± 0.04	0.68 ± 0.05
FS (%)	11.97 ± 2.48 * †	20.36 ± 3.80 *	31.85 ± 1.92	25.18 ± 1.86
LV Mass (mm)	170.86 ± 14.25 * †	157.71 ± 19.73 * ‡	92.20 ± 7.45	90.17 ± 4.28
EF (%)	20.57 ± 2.99 * † §	35.14 ± 4.79 * ‡	51.00 ± 1.55	46.67 ± 4.19

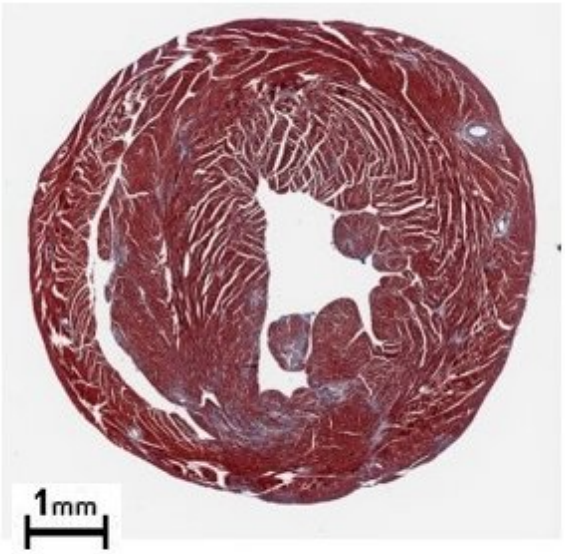
Supplementary Figure 1 Coronary artery flow measurements. (A) Color flow echocardiogram of left main coronary artery. (B) Velocity time index by Doppler at left main for calculating flow. (AO – aorta; LM – left main; LCX – left circumflex; PA – pulmonary artery)



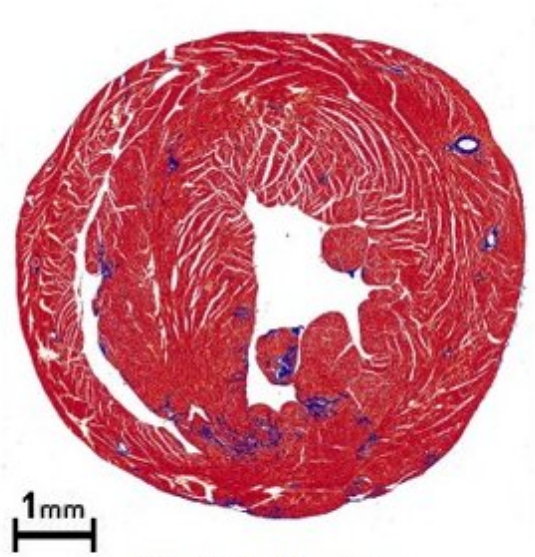
Supplementary Figure 2 Microvessel density digital analysis (green) and sites of capillary domain area measurement.



Supplementary Figure 3 Example of whole heart digital image analysis for extracellular collagen content in a wildtype mouse following 6 weeks of pressure-overload. Microscopic view in primary manuscript Figure 4B.

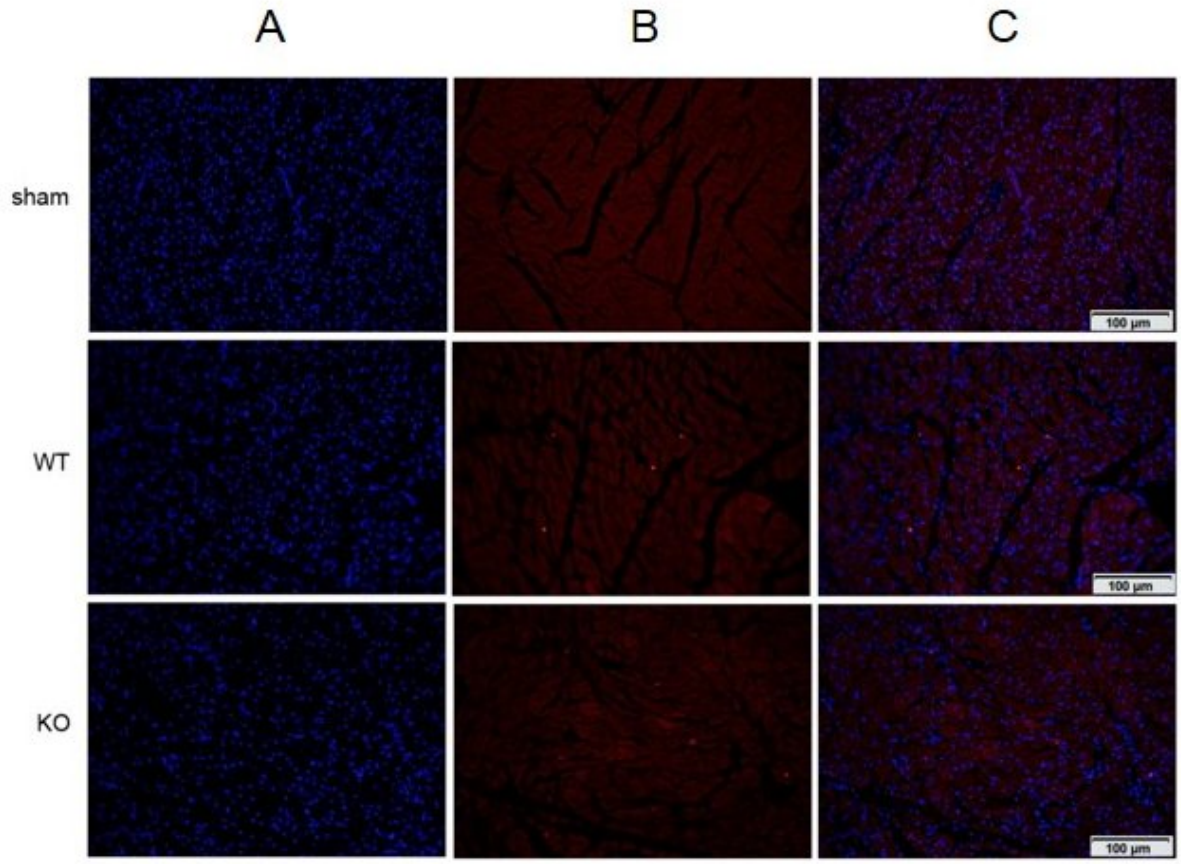


Before Digital analysis



After Digital Analysis

Supplementary Figure 4 TUNEL staining in respective animals 6 weeks after TAC. A- DAPI, nuclei; B – TUNEL; C- merged.



Supplementary Figure 5 Quantification of vascular density moving from the endocardium to epicardium (*p<0.05 vs sham KO).