Supporting Materials

Table S1

Echocardiography following transverse aortic constriction, Males

	Surgery	Sham		TAC	
	Genotype	**WT**	**USP20-KO**	**WT**	**USP20-KO**
	Sample Size	**14**	**13**	**23**	**31**
Base	**Heart Rate (BPM)**	675 ± 38	741 ± 60	675 ± 64	739 ± 54
	IVSWth;d (mm)	1.14 ± 0.18	1.16 ± 0.14	1.05 ± 0.15	1.1 ± 0.15
	LVID;d (mm)	3.09 ± 0.38	3.12 ± 0.29	3.28 ± 0.37	3.25 ± 0.38
	LVID;s (mm)	1.2 ± 0.27	1.25 ± 0.3	1.42 ± 0.38	1.43 ± 0.46
	LVPWth;d (mm)	1.03 ± 0.15	1.02 ± 0.15	1 ± 0.13	1.01 ± 0.22
	EF (%)	90.71 ± 4.03	89.73 ± 4.95	87.48 ± 6	86.48 ± 7.87
	FS (%)	61.35 ± 6.23	60.1 ± 7.28	57.11 ± 7.53	56.72 ± 10.74
	LV Mass (mg)	99.11 ± 18.78	101.73 ± 19.23	98.52 ± 15.43	101.55 ± 16.11
2 weeks	**Heart Rate (BPM)**	691 ± 60	749 ± 61	662 ± 80	727 ± 48
	IVSWth;d (mm)	1.08 ± 0.19	1.11 ± 0.2	1.26 ± 0.22 ***§**	1.33 ± 0.19 ***§**
	LVID;d (mm)	3.1 ± 0.32	3.23 ± 0.36	3.19 ± 0.49	3.49 ± 0.59 *
	LVID;s (mm)	1.34 ± 0.25	1.48 ± 0.41	1.56 ± 0.64	2.19 ± 0.91 *#
	LVPWth;d (mm)	1.16 ± 0.22	1.03 ± 0.19	1.34 ± 0.37 *	1.3 ± 0.28 ***§**
	EF (%)	87.74 ± 4.39	85.39 ± 7.22	80.84 ± 15.84	67.39 ± 20.6 *#
	FS (%)	56.86 ± 5.95	54.61 ± 8.91	51.69 ± 15.46	39.53 ± 16.49 *#
	LV Mass (mg)	104.83 ± 21.16	102.34 ± 12.66	136.61 ± 29.15 ***§**	159.88 ± 41.03 ***§**
4 weeks	**Heart Rate (BPM)**	676 ± 62	736 ± 47	681 ± 64	706 ± 84
	IVSWth;d (mm)	1.12 ± 0.18	1.07 ± 0.13	1.42 ± 0.24 ***§$**	1.38 ± 0.21 ***§**
	LVID;d (mm)	3.19 ± 0.33	3.31 ± 0.39	3.21 ± 0.74	3.49 ± 0.76 *
	LVID;s (mm)	1.35 ± 0.35	1.57 ± 0.37	1.73 ± 0.98	2.22 ± 1.05*#
	LVPWth;d (mm)	1.14 ± 0.29	1.18 ± 0.2	1.46 ± 0.39 ***§**	1.37 ± 0.28 *
	EF (%)	88.04 ± 5.39	84.54 ± 6.02	76.84 ± 20.72 *	64.69 ± 25.71 *#
	FS (%)	57.99 ± 8.48	53.24 ± 7.21	48.84 ± 18.46 *	37.9 ± 19.87 *#
	LV Mass (mg)	110.20 ± 25.42	115.07 ± 23.46	163.15 ± 45.13 ***$§**	173.24 ± 52.69 ***$§**

# $p < 0.05$, versus all other samples at the time point of measurement
* $p < 0.05$, versus respective Base
$ $p < 0.05$, 4 weeks versus respective 2 weeks
§ $p < 0.05$, versus Sham
Values are expressed as mean ± SD. BPM, beats per minute; LV, left ventricle; s, systole; d, diastole; IVSWth, intra-ventricular septum wall thickness; LVID, left ventricle internal dimension; LVPWth, left ventricular posterior wall thickness; EF, ejection fraction; FS, fractional shortening of LV; Statistical significance was determined by two-way RM-ANOVA and Holm-Šídák's multiple comparisons test.

Table S2

Echocardiography following transverse aortic constriction, Females

	Condition	Sham		TAC	
	Genotype	**WT**	**USP20-KO**	**WT**	**USP20-KO**
	Sample Size	**5**	**11**	**15**	**5**
Base	**Heart Rate (BPM)**	617 ± 98	741 ± 65	624 ± 79	625 ± 87
	IVSWth;d (mm)	0.98 ± 0.18	0.94 ± 0.13	0.91 ± 0.16	0.85 ± 0.07
	LVID;d (mm)	3.24 ± 0.25	3.1 ± 0.31	3.24 ± 0.23	3.03 ± 0.2
	LVID;s (mm)	1.52 ± 0.23	1.4 ± 0.3	1.27 ± 0.26	1.17 ± 0.31
	LVPWth;d (mm)	0.99 ± 0.1	0.98 ± 0.17	0.92 ± 0.13	0.92 ± 0.1
	EF (%)	84.78 ± 5.25	86.4 ± 4.98	90.31 ± 3.63	90.63 ± 5.02
	FS (%)	53.19 ± 6.58	55.13 ± 6.21	60.85 ± 6.18	61.6 ± 8.45
	LV Mass (mg)	91.05 ± 6.5	81.47 ± 10.57	81.86 ± 10.66	70.63 ± 8.3
2 weeks	**Heart Rate (BPM)**	643 ± 49	747 ± 64	610 ± 82	610 ± 99 **§**
	IVSWth;d (mm)	0.87 ± 0.14	0.99 ± 0.14	1.17 ± 0.2 ***§**	1.06 ± 0.23 *
	LVID;d (mm)	3.24 ± 0.35	3.19 ± 0.23	3.22 ± 0.53	3.47 ± 0.25
	LVID;s (mm)	1.47 ± 0.3	1.44 ± 0.34	1.65 ± 0.63 *	2.27 ± 0.66 ***§**
	LVPWth;d (mm)	0.91 ± 0.09	0.92 ± 0.12	1.28 ± 0.25 *	1.29 ± 0.17
	EF (%)	86.05 ± 4.89	86.02 ± 6.32	80.61 ± 11.84 *	62.87 ± 21.44 ***#**
	FS (%)	54.75 ± 6.25	55.27 ± 8.62	50.1 ± 12.16 *	35.12 ± 15.1 ***#**
	LV Mass (mg)	78.58 ± 9.69	85.44 ± 13.14	125.05 ± 18.77***§**	125.78 ± 37.33 ***§**
4 weeks	**Heart Rate (BPM)**	672 ± 73	710 ± 54	639 ± 78	529 ± 188 **§**
	IVSWth;d (mm)	1 ± 0.12	1 ± 0.12	1.27 ± 0.21 ***§$**	1.06 ± 0.08 *
	LVID;d (mm)	3.19 ± 0.34	3.16 ± 0.37	3.16 ± 0.64	3.76 ± 0.65 *
	LVID;s (mm)	1.37 ± 0.36	1.44 ± 0.26	1.67 ± 0.77 *	2.4 ± 1.1 ***#**
	LVPWth;d (mm)	1.02 ± 0.16	0.96 ± 0.25	1.4 ± 0.36 ***#§**	1.06 ± 0.13
	EF (%)	87.98 ± 5.14	85.57 ± 6.3	77.35 ± 14.64 *	63.72 ± 26.9 ***§**
	FS (%)	57.61 ± 7.85	54.26 ± 6.92	49.25 ± 18.97 *	37.12 ± 19 ***§**
	LV Mass (mg)	92.25 ± 12.38	88.07 ± 21.88	140.65 ± 31.48 ***$§**	128.39 ± 37.23 ***§**

# $p < 0.05$, versus all other samples at the time point of measurement
* $p < 0.05$, versus respective Base
$ $p < 0.05$, 4 weeks versus respective 2 weeks
§ $p < 0.05$, versus Sham
Values are expressed as mean ± SD. BPM, beats per minute; LV, left ventricle; s, systole; d, diastole; IVSWth, intra-ventricular septum wall thickness; LVID, left ventricle internal dimension; LVPWth, left ventricular posterior wall thickness; EF, ejection fraction; FS, fractional shortening of LV; Statistical significance was determined by two-way RM-ANOVA and Holm-Šídák's multiple comparisons test.

TABLE S3

βAR expression determined by radioligand binding

Genotype	Treatment, N	Total βAR Femto moles/mg membrane protein ± SD	β_1AR Femto moles/mg membrane protein ± SD	β_2AR Femto moles/mg membrane protein ± SD
WT	Sham, N=3	40.47 ±1.50	29.33 ±2.54	11.20±3.34
WT	TAC, N=2	26.90±4.67	18.01±2.40	8.9±2.26
USP20-KO	Sham, N=5	34.86 ±4.90	22.74 ±5.02	12.14±2.51
USP20-KO	TAC, N=3	22.30±2.12	16.67±3.53	5.6±1.7

TABLE S4

WT versus USP20-KO differentially expressed proteins after 4 week TAC

Description	Gene Symbol (Mouse)	Gene Symbol (Human)	Log2 WT:KO
Collagen alpha-1(VIII) chain	Col8a1	COL8A1	1.2320
Tenascin	Tnc	TNC	1.0146
Myosin-7	Myh7	MYH7	0.9928
Angiopoietin-related protein 7	Angptl7	ANGPTL7	0.9710
Periostin	Postn	POSTN	0.9331
Latent-transforming growth factor beta-binding protein 2	Ltbp2	LTBP2	0.9324
Collagen alpha-1(XII) chain	Col12a1	COL12A1	0.8671
Thrombospondin-4	Thbs4	THBS4	0.8647
Myosin-1	Myh1	MYH1	0.8008
Adipocyte enhancer-binding protein 1	Aebp1	AEBP1	0.7926
Fibrillin-2	Fbn2	FBN2	0.7357
Asporin	Aspn	ASPN	0.7236
Cartilage oligomeric matrix protein	Comp	COMP	0.6905
Microfibrillar-associated protein 5	Mfap5	MFAP5	0.6795
Actin, alpha skeletal muscle	Acta1	ACTA1	0.6580
Microfibrillar-associated protein 2	Mfap2	MFAP2	0.6385
Fibrillin-1	Fbn1	FBN1	0.6326
Thrombospondin-3	Thbs3	THBS3	0.6313
Receptor-interacting serine/threonine-protein kinase 1	Ripk1	RIPK1	0.5959
Fibromodulin	Fmod	FMOD	0.5905
Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	Enpp1	ENPP1	0.5835
Nuclear receptor coactivator 3	Ncoa3	NCOA3	0.5519
Argininosuccinate synthase	Ass1	ASS1	0.5499
Retinol-binding protein 1	Rbp1	RBP1	0.5439
Collagen alpha-1(III) chain	Col3a1	COL3A1	0.5355
Collagen alpha-2(I) chain	Col1a2	COL1A2	0.5258
Double-stranded RNA-specific adenosine deaminase	Adar	ADAR	0.4997
Collagen alpha-1(I) chain	Col1a1	COL1A1	0.4961
Prolargin	Prelp	PRELP	0.4595
Reticulocalbin-3	Rcn3	RCN3	0.4534
Phosphorylated adapter RNA export protein	Phax	PHAX	0.4521
oluble scavenger receptor cysteine-rich domain-containing protein SSC5D	Ssc5d	SSC5D	0.4493
Lysyl oxidase homolog 1	Loxl1	LOXL1	0.4482
Fibulin-2	Fbln2	FBLN2	0.4412
Craniofacial development protein 1	Cfdp1	CFDP1	0.4385
Sterol O-acyltransferase 1	Soat1	SOAT1	0.4264
Tyrosine-protein phosphatase non-receptor type substrate 1	Sirpa	SIRPA	0.4173
Dihydropyrimidinase-related protein 1	Crmp1	CRMP1	0.4152
Progranulin	Grn	GRN	0.4093
Glutathione peroxidase 3	Gpx3	GPX3	0.4082
EGF-containing fibulin-like extracellular matrix protein 2	Efemp2	EFEMP2	0.3958
E3 ubiquitin-protein ligase MYCBP2	Mycbp2	MYCBP2	0.3942
HSPB1-associated protein 1	Hspbap1	HSPBAP1	0.3928
Podoplanin	Pdpn	PDPN	0.3821
Inactive phospholipase C-like protein 1	Plcl1	PLCL1	0.3819
Inter-alpha-trypsin inhibitor heavy chain H5	Itih5	ITIH5	0.3809
Biglycan	Bgn	BGN	0.3805
Fumarylacetoacetase	Fah	FAH	-0.3812
Cytochrome c oxidase assembly protein COX18, mitochondrial	Cox18	COX18	-0.3990
Mitochondrial 10-formyltetrahydrofolate dehydrogenase	Aldh1l2	ALDH1L2	-0.4256
Glycophorin-A	Gypa	GYPA	-0.4266
Bisphosphoglycerate mutase	Bpgm	BPGM	-0.4273
Large neutral amino acids transporter small subunit 3	Slc43a1	SLC43A1	-0.4318
Band 3 anion transport protein	Slc4a1	SLC4A1	-0.4353
Sorting nexin-21	Snx21	SNX21	-0.4805
Blood group Rh(D) polypeptide	Rhd	RHD	-0.4904
Carbonic anhydrase 1	Ca1	CA1	-0.5078
Alanine aminotransferase 2	Gpt2	GPT2	-0.5374
CD5 antigen-like	Cd5l	CD5L	-0.6530
Neurogranin	Nrgn	NRGN	-1.2303
Ubiquitin carboxyl-terminal hydrolase 33	Usp33	USP33	-1.3813

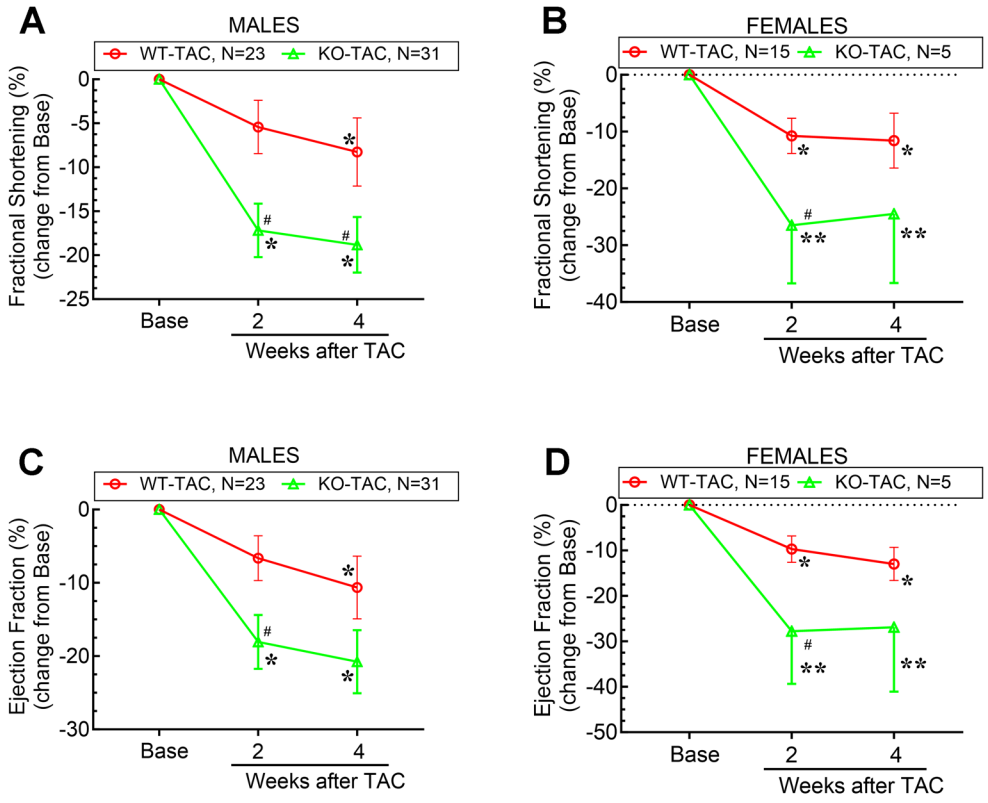

Figure S1. TAC-induced changes in %FS and ejection fraction in WT and USP20-KO. The line graphs summarize TAC-induced change in %FS in males (A) and females (B) ejection fraction in males (C) and females (D) as compared to respective base. Values are expressed as means ± SD from at least 5 mice per group. Statistical significance was determined by two-way RM-ANOVA followed by Holm-Šídák's multiple comparisons test. * $p < 0.05$ compared to respective Base; # $p< 0.05$ time point compared to all other groups.

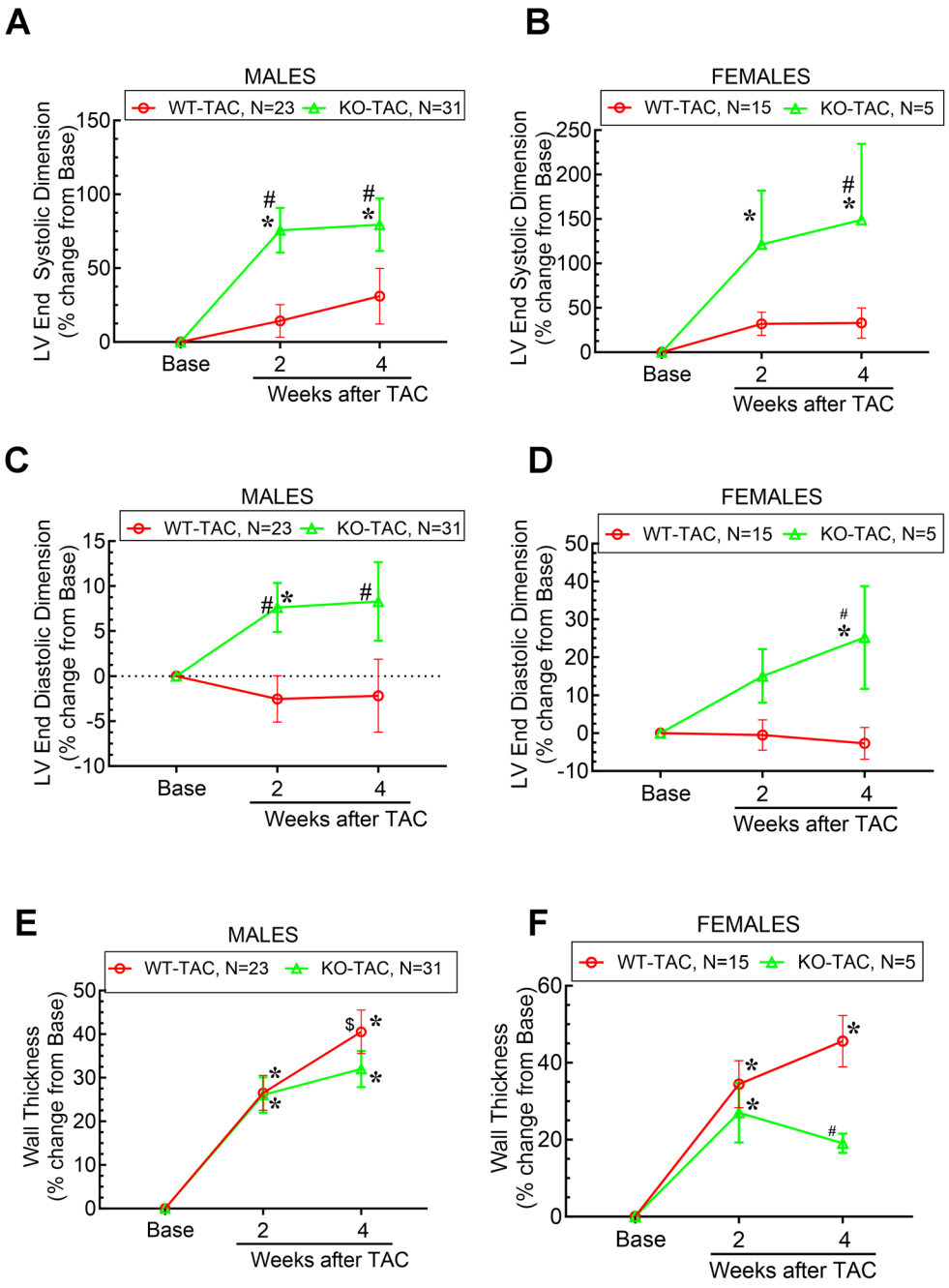

Figure S2. Analysis of changes in LV dimensions and wall thickness in WT and USP20-KO after 4-week TAC. Measurements were obtained by serial echocardiography, and summarized ± SD for all experimental mice for TAC-induced change in the following parameters: (1) LV end systolic dimensions as compared to respective base in males (A) and females (B). (2) LV end diastolic dimensions as compared to respective base in males (C) and females (D). (3) Wall thickness (LV posterior wall width + interventricular septum width) as compared to respective base in males (E) and females (F). # $p < 0.05$, versus all other samples at the time point of measurement, * $p < 0.05$, versus respective Base, $ $p < 0.05$, 4 weeks versus respective 2 weeks; two-way RM-ANOVA followed by Holm-Šídák's multiple comparisons test.

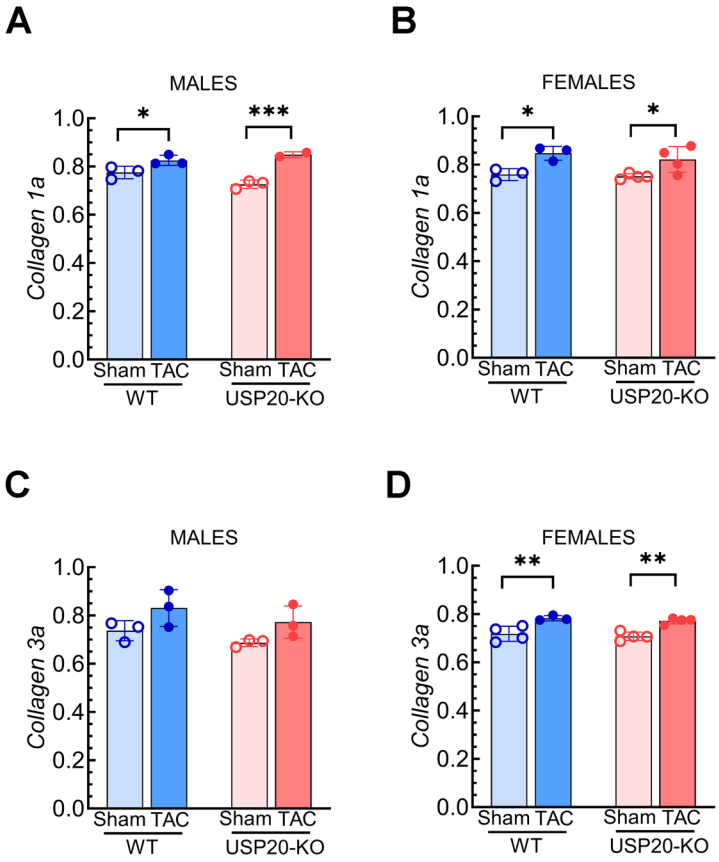

Figure S3. Collagen expression in WT and USP20-KO LV. A, & B) Collagen1a (Col1a) and C & D) Collagen3a (Col3a) mRNA normalized to Gapdh in WT and USP20-KO LV tissues at 1 week after TAC. Error bars indicate average ± SD, n = 3-4; * $p < 0.05$, ** $p<0.01$, ***$p<0.001$ as indicated, two-way ANOVA followed by Holm-Šídák's multiple comparisons test.

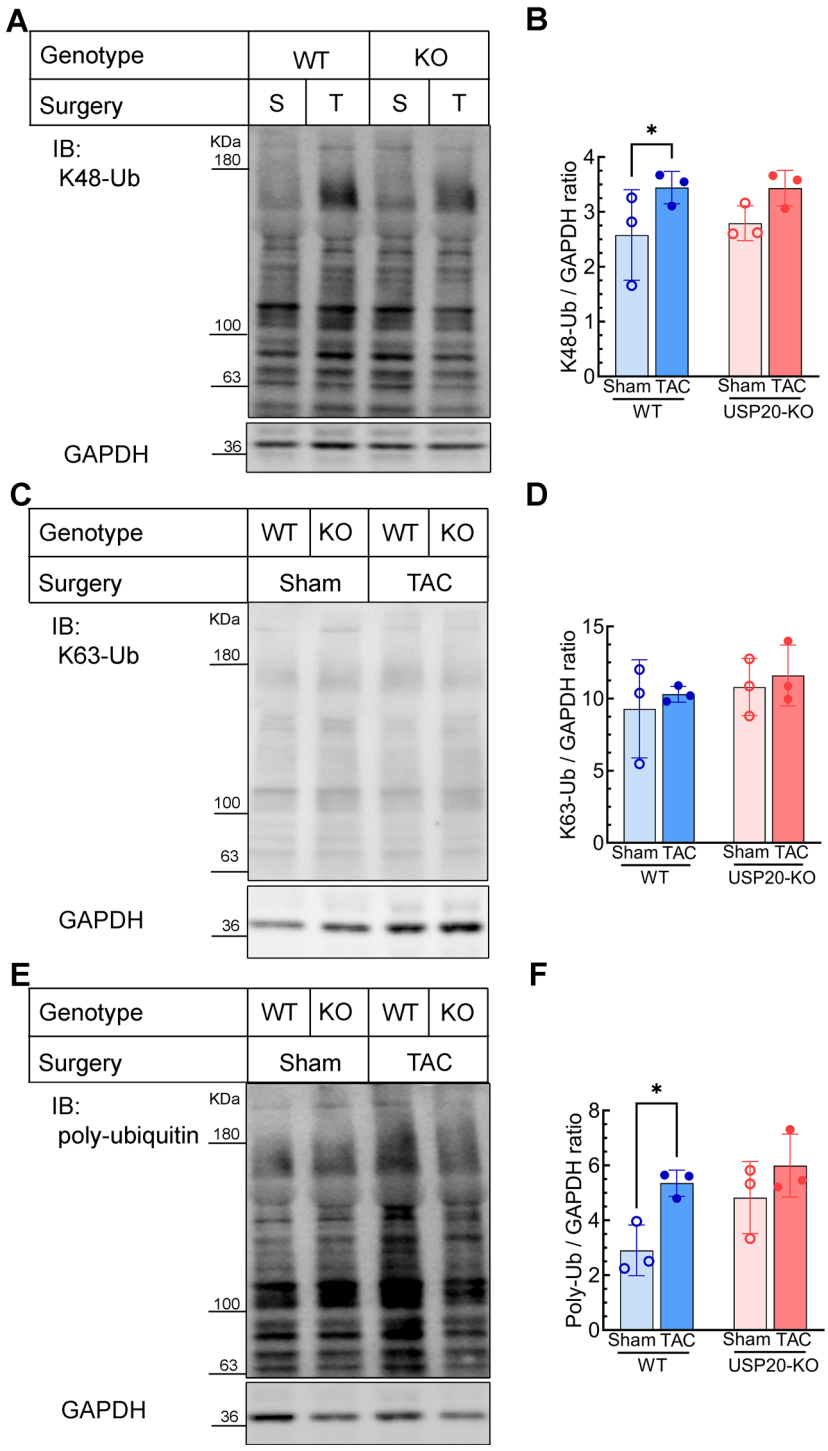

Figure S4. Assessment of ubiquitination levels in LV extracts of WT and USP20-KO mice. Solubilized LV extracts from Sham and TAC cohorts were analyzed by western blot for lysine-48 (A), lysine-63 (C), and total (E) polyubiquitins by using lysine-48-HRP, Apu3-K63, and FK1-Ub antibodies. The blots were reprobed for GAPDH. The graphs shown in B, D, and F summarize the quantitation of ubiquitin signals from each lane normalized to respective GAPDH. * $p<0.05$, as indicated, two-way ANOVA followed by Holm-Šídák's multiple comparisons test.

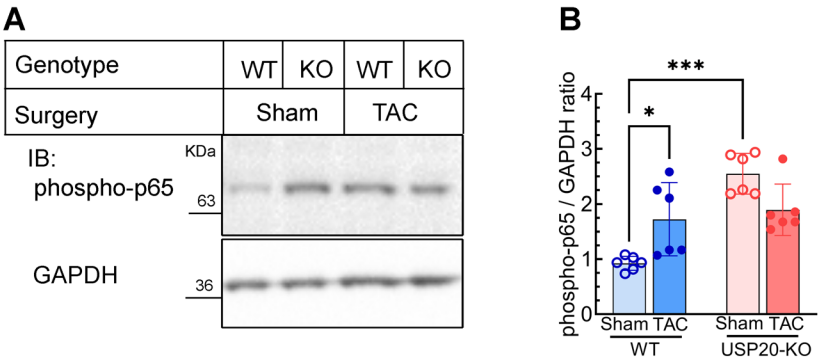

Figure S5. NFκB activity in USP20-KO and WT LVs. A) Representative immunoblots for NFκB activation determined by phosphorylation of p65 subunit (Serine 536) and GAPDH levels detected in LV extracts from WT and USP20-KO 4 weeks post Sham and TAC surgeries. B) Quantitation of phopho-p65 normalized to respective GAPDH bands obtained from 6 (WT) and 6 (USP20-KO) male LVs. *p<0.05 , ** p<0.01 as indicated, two-way ANOVA followed by Holm-Šídák's multiple comparisons test.

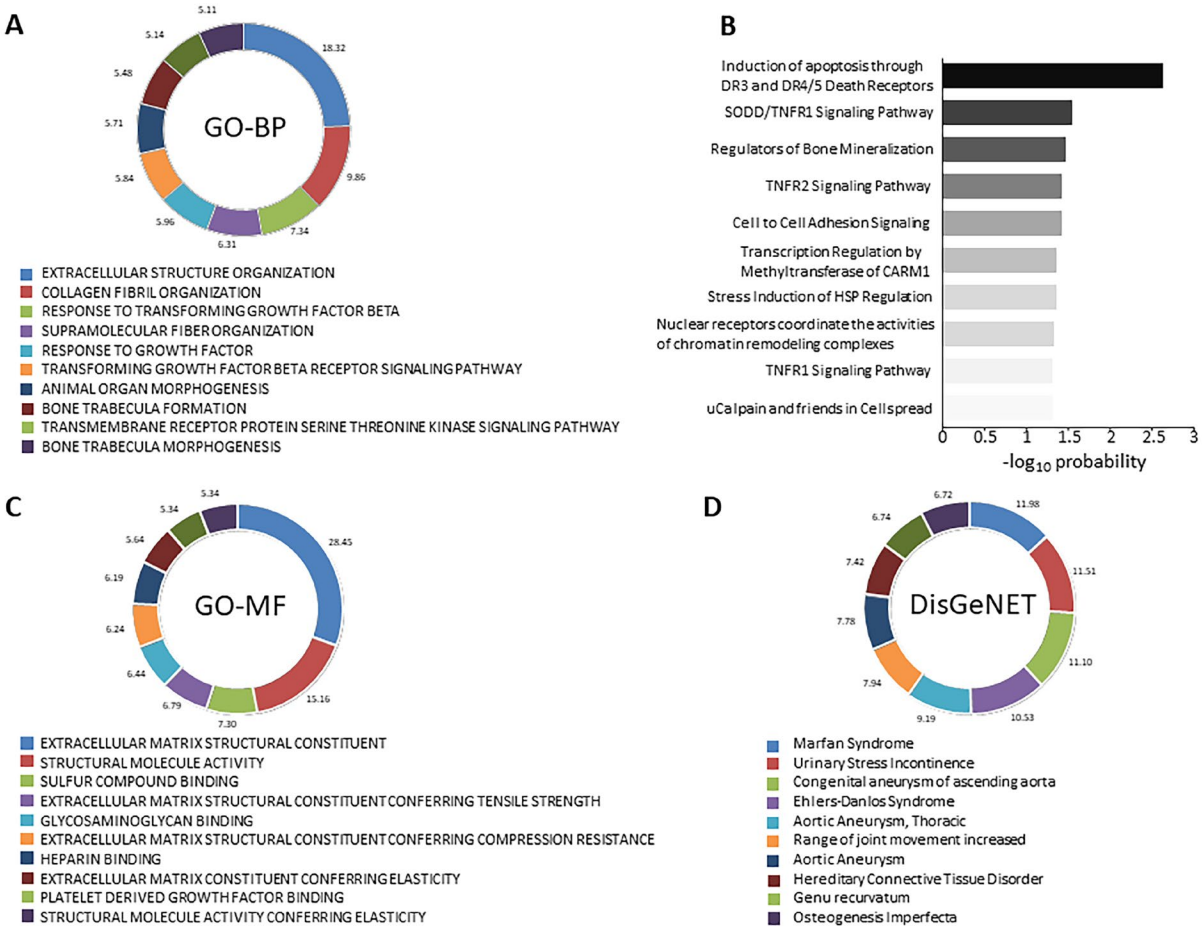

Figure S6. Comparison of differentially expressed proteins in USP20-KO and WT hearts by bioinformatics analyses. For the total significantly differentially expressed proteins between WT:KO, Gene Ontology (GO) term enrichment analysis was performed for biological processes (A) and molecular function (B). The top 10 most significantly enriched GO terms are depicted according to the negative $\log_{10}$ transform of the enrichment p value. (C) In addition, BioCarta pathway enrichment analysis was performed and again, the top 10 most highly enriched pathways are indicated. (D) For disease-based analysis the DisGeNET database was employed to investigate pathologies related to the input significant differential WT:KO USP20 dataset. The top 10 negative $\log_{10}$ transforms of the most significant enrichment p values are indicated in the diagram

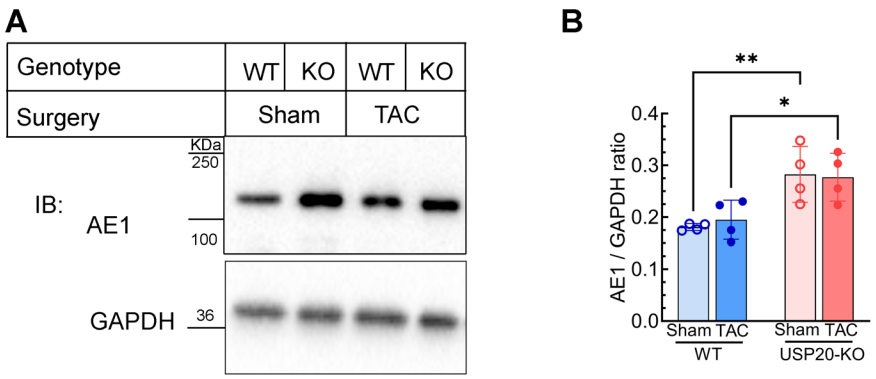

Figure S7. Expression levels of AE1 in mouse LV extracts. A) Representative immunoblots for AE1 and GAPDH detected in LV extracts from WT and USP20-KO 4 weeks post Sham and TAC surgeries. B) Quantitation of AE1 band in A obtained from 4 (WT) and 4 (USP20-KO) male LVs. *$p<0.05$ as indicated, two-way ANOVA followed by Holm-Šídák's multiple comparisons test.

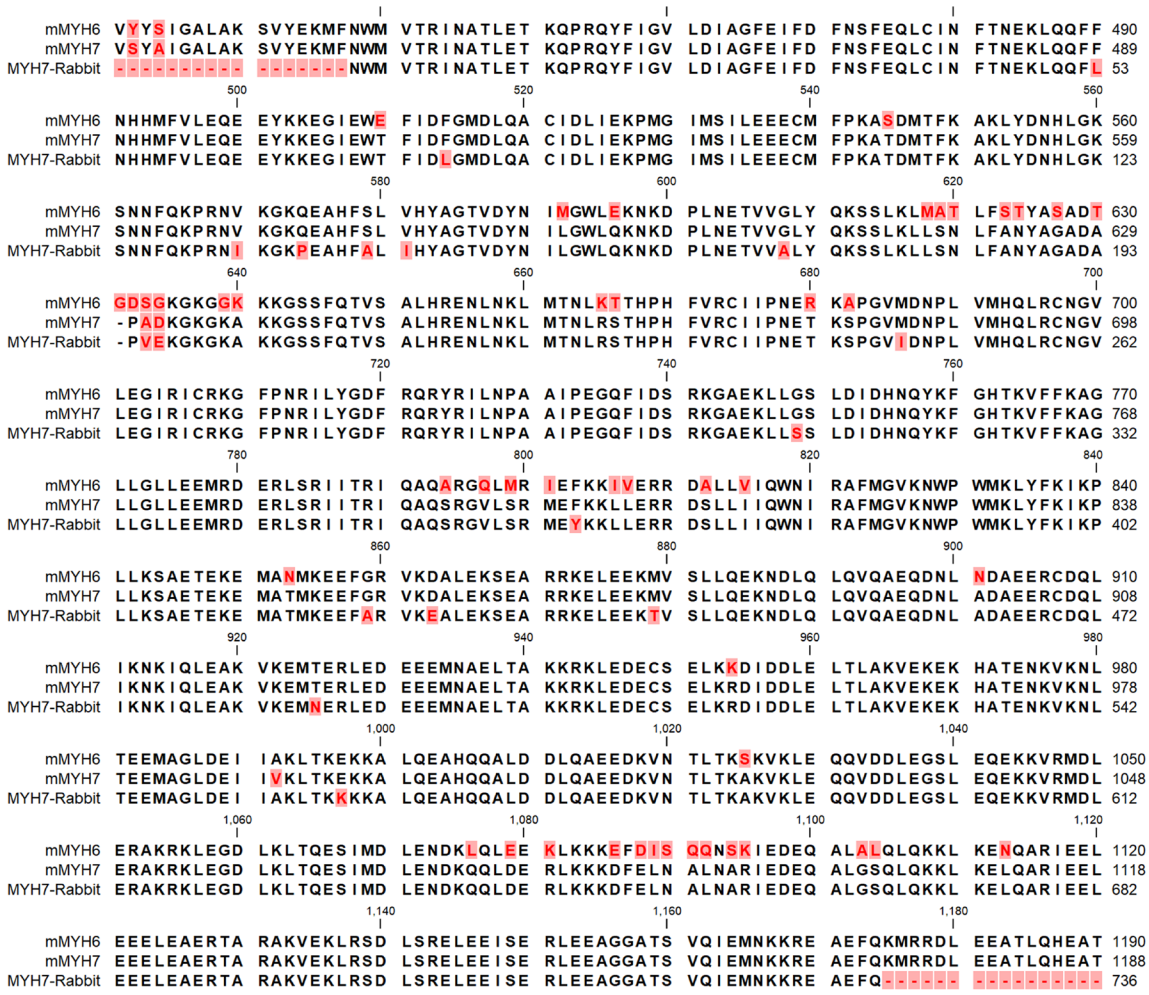

Figure S8: Amino acid sequence alignment comparing murine MYH6 and MYH7 with the MYH7 immunogen, rabbit MYH7. MYH7 rabbit protein sequence of the immunogen was aligned against mouse MYH6 and MYH7 using CLC Sequence Viewer multiple sequence alignment program. Unique residues are shown in red.