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Supplementary Table 1: Characteristics of individuals from whom human heart samples were obtained.

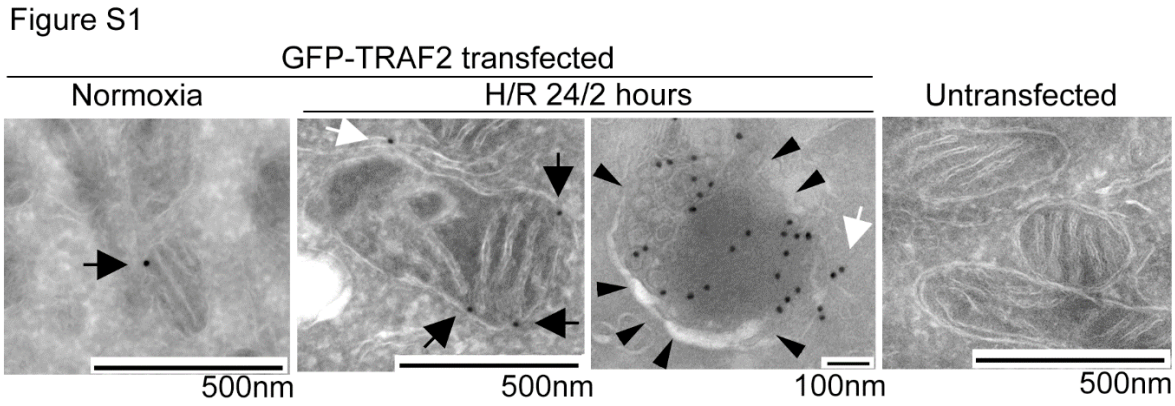
Individual Characteristics	Ischemic Cardiomyopathy (N=10)	Non-failing Donor (N=10)	Statistical Comparison
Male Gender (N)	10	8	P=ns
Age (years)	62.2 +/- 0.5	57.0 +/- 0.6	P=0.030
BMI (kg/m^2)	28.4 +/- 0.4	27.7 +/- 1.0	P=ns
LV Mass Index (g/kg)	178.9 +/-6.1	102.6 +/- 1.7	P<0.001
LVEDD (cm)	6.9 +/- 0.1	3.9 +/- 0.05	P<0.001
LVEF (%)	19.8 +/- 0.9	63.5 +/- 0.3	P<0.001
History of Diabetes (N)	0	0	P=ns
History of HTN (N)	10	3	P=0.003
History of ACE-I/ARB use (N)	8	0	P<0.001
History of Beta-blocker use (N)	9	0	P<0.001

All data shown are Mean±SEM. P values reported are by t-test. Categorical variables were analyzed by Fisher's exact test; 'ns' = not significant for $P \geq 0.05$.

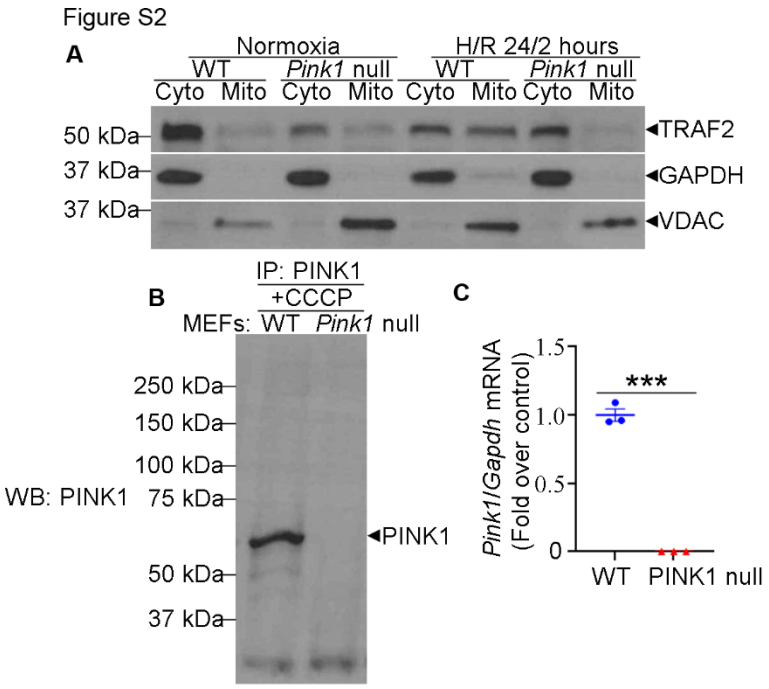
Supplementary Table 2: Mouse Primer sequences employed for qPCR analysis of the indicated genes.

Target gene	Forward Primer sequence (5'–3')	Reverse Primer sequence (5'–3')
GAPDH	ACTCCCACTCTTCCACCTTC	TCTTGCTCAGTGTCCTTGC
Acta1	ACCATCGGCAATGAGCGTTTCC	GCTGTTGTAGGTGGTCTCATGG
Myh7	GCTGGAAGATGAGTGCTCAGAG	TCCAAACCAGCCATCTCCTCTG
Myh6	GCTGGAAGATGAGTGCTCAGAG	CCAGCCATCTCCTCTGTTAGGT
Tlr9	CAAGAACCTGGTGTCACTGC	TGCGATTGTCTGACAAGTCC
Atp2a2	GTGAAGTGCCATCAGTATGACGG	GTGAGAGCAGTCTCGGTAGCTT
Nppa	TACAGTGCGGTGTCCAACACAG	TGCTTCCTCAGTCTGCTCACTC
Nppb	TCCTAGCCAGTCTCCAGAGCAA	GGTCCTTCAAGAGCTGTCTCTG
Map1lc3b	CGTCCTGGACAAGACCAAGT	ATTGCTGTCCCGAATGTCTC
Sqstm1	GCTGCCCTATACCCACATCT	CGCCTTCATCCGAGAAAC
Becn1	AATCTAAGGAGTTGCCGTTATAC	CCAGTGTCTTCAATCTTGCC

Supplementary Figures

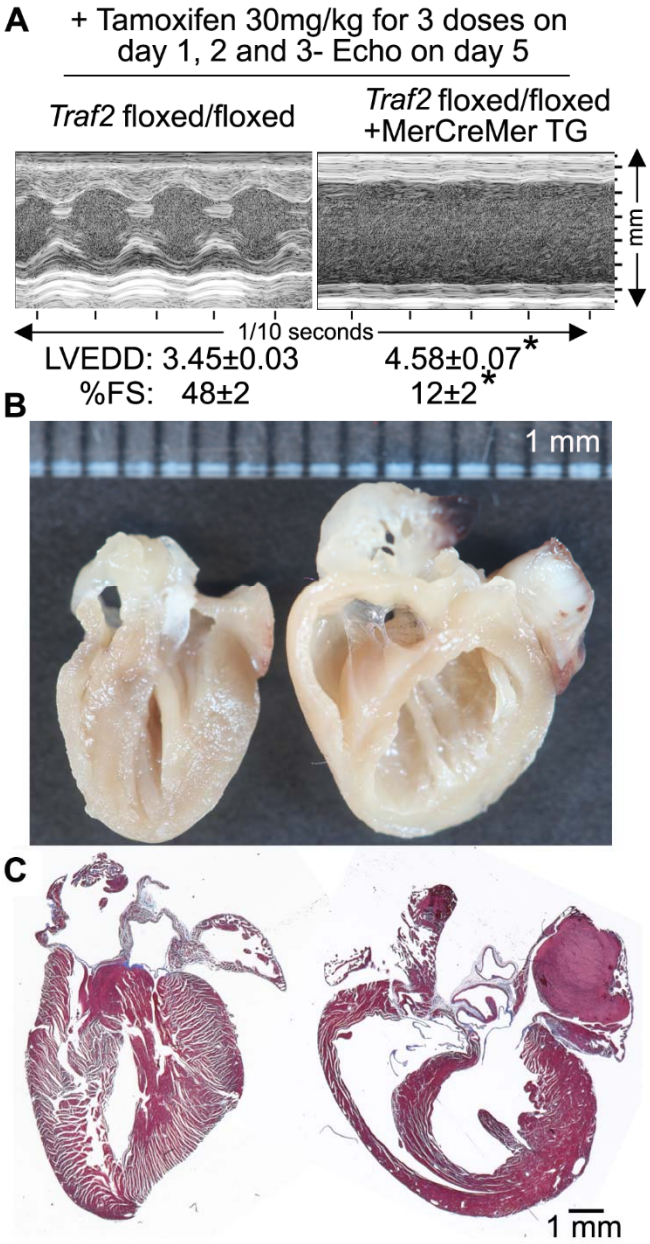


Supplementary Figure S1. TRAF2 localizes to the mitochondria and mitochondria-associated membranes in unstressed and hypoxia-reoxygenation stressed HEK293 cells. Representative transmission electron microscopic images in HEK293 cells transduced with GFP-tagged TRAF2 and subjected to immuno-gold detection for GFP under normoxic conditions or after hypoxia-re-oxygenation (H/R; 24 hours hypoxia followed by 2 hours re-oxygenation) injury. Image from similarly treated un-transfected cells is shown as control. Black arrows point to TRAF2 on mitochondria, white arrows indicate TRAF2 on MAM. Black arrowheads demonstrate TRAF2 localized to a mitochondria within an autophagosome.



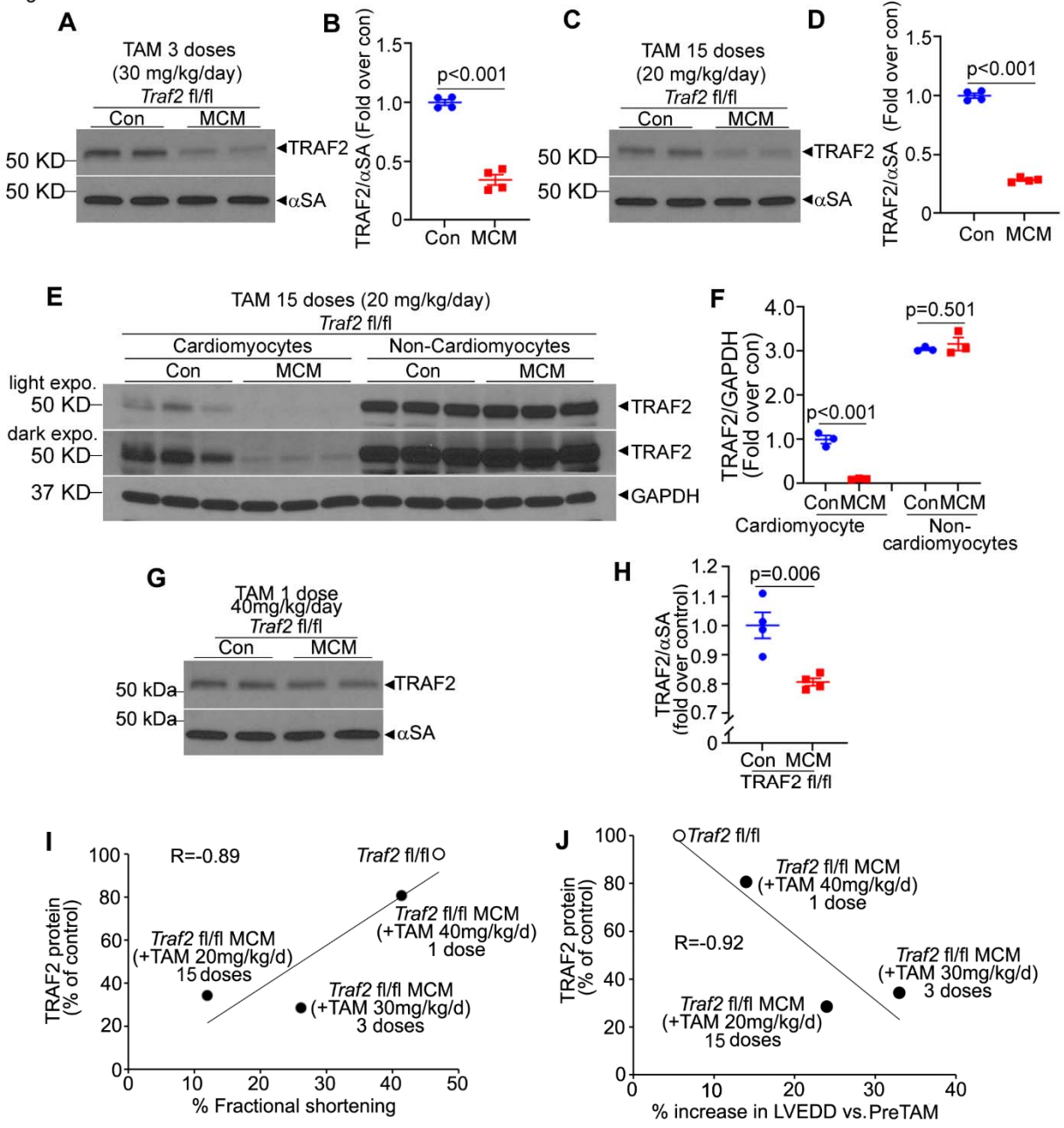
Supplementary Figure S2: PINK1 deficiency does not prevent TRAF2 localization to mitochondria in normoxic culture conditions, but abrogates it with hypoxia-reoxygenation injury. A. *Pink1* null and wild-type MEFs were subjected to hypoxia-re-oxygenation injury (H/R 24/2 hours) or nomoxia as control followed by biochemical fractionation into mitochondria-enriched (mito, detected by VDAC co-segregation) or cytosolic (cyto, detected with GAPDH expression) fractions. Representative immunoblot depicting detection of TRAF2 in mitochondrial fraction of both groups in normoxic culture conditions, but abrogation of increased TRAF2 abundance in the mitochondrial fraction in WT MEFs by *Pink1* ablation followed hypoxia-reoxygenation injury. **B**. Immunoblot depicting PINK1 in *Pink1* null and wild-type MEFs treated with carbonyl cyanide m-chlorophenyl hydrazone (CCCP, an ionophore that provokes loss of mitochondrial membrane potential and targets mitochondria for autophagic degradation; at 20µM for 24 hours) to stabilize PINK1. Cellular extracts were subjected to immuno-precipitation with anti-PINK1 antibody (MRC PPU products and reagents, S774C (DU17570)) and detection with an alternate PINK1 antibody (S086D (DU34559)) as shown in B. **C**. Detection of *Pink1* transcripts by quantitative PCR analyses in *Pink1* null and wild type MEFs. '***' indicates $P<0.001$ by t-test.

Figure S3

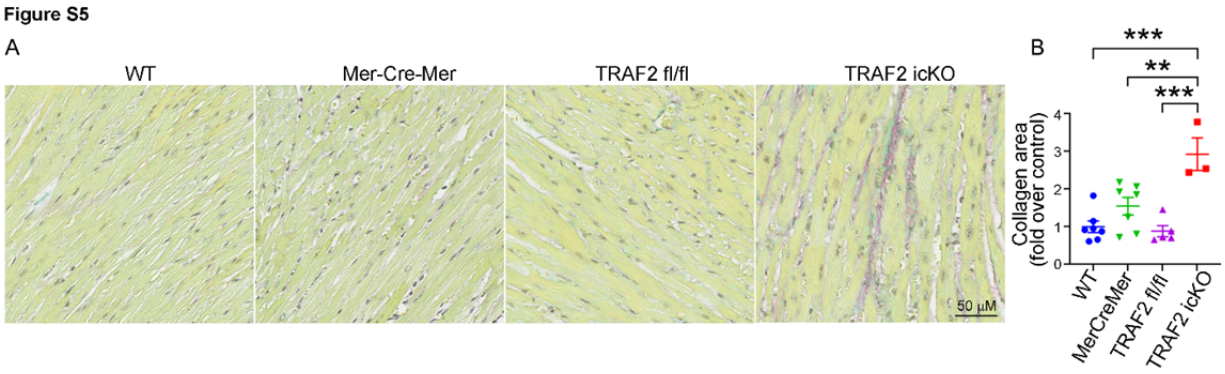


Supplementary Figure S3: Inducible cardiac myocyte TRAF2 ablation in young adult mice results in cardiomyopathy. A. Representative 2D-directed M mode echocardiographic images from *Traf2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only as control, followed treatment with three doses of tamoxifen i.p. as indicated. Left ventricular end-diastolic diameter (LVEDD) and endocardial fraction shortening (%FS) are reported below the images. N=3/group. * indicates P<0.05 by t-test vs. *Traf2* floxed controls. **B, C**. Representative photograph (B) and Trichrome stained coronal sections (C) from the hearts as in A.

Figure S4

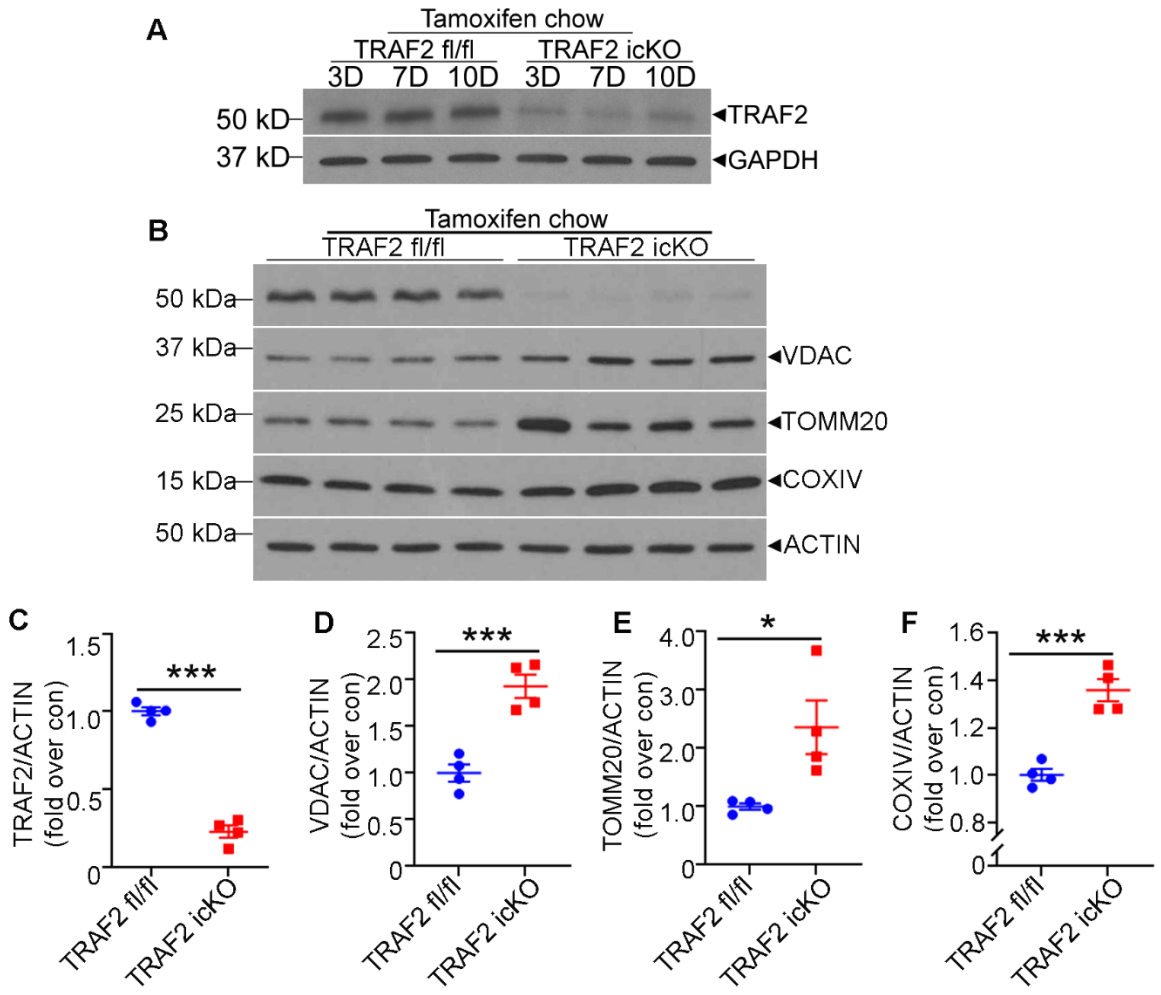

A
TAM 3 doses
(30 mg/kg/day)
Traf2 fl/fl
Con
MCM
50 KD
50 KD
TRAF2
αSA
B
TRAF2/αSA (Fold over con)
p<0.001
Con MCM
C
TAM 15 doses
(20 mg/kg/day)
Traf2 fl/fl
D
E
TAM 15 doses (20 mg/kg/day)
Traf2 fl/fl
Cardiomyocytes
Non-Cardiomyocytes
light expo.
dark expo.
37 KD
GAPDH
F
TRAF2/GAPDH (Fold over con)
p=0.501
Cardiomyocyte
Non-cardiomyocytes
G
TAM 1 dose
40mg/kg/day
50 kDa
H
TRAF2/αSA (fold over control)
p=0.006
TRAF2 fl/fl
I
R=-0.89
TRAF2 protein (% of control)
Traf2 fl/fl
Traf2 fl/fl MCM (+TAM 40mg/kg/d) 1 dose
Traf2 fl/fl MCM (+TAM 20mg/kg/d) 15 doses
Traf2 fl/fl MCM (+TAM 30mg/kg/d) 3 doses
% Fractional shortening
J
R=-0.92
% increase in LVEDD vs. PreTAM

Supplementary Figure S4: Tamoxifen treatment induces adult onset TRAF2 ablation selectively in cardiac myocytes. A-D. Representative immunoblots (A, C) demonstrating TRAF2 expression in *Traf2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only (as control) were treated with tamoxifen as indicated. αSARCOMERIC ACTIN (αSA) immunoblot is performed as loading control. Quantitation is for respective immunoblots is depicted in graphs to the right (B, D). '***' indicates P<0.001 by t-test. **E, F**. Immunoblot (E) and quantitative analyses (F) demonstrating TRAF2 expression in *Traf2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only (as control) 15 days after treatment with tamoxifen (20 mg/kg/d i.p. 5 days/week for 3 weeks), followed by enzymatic digestion to isolate cardiac myocytes from the non-myocyte cellular fraction. Two exposures (light, dark expo.) are shown. '***' indicates P<0.001 by t-test. No statistically significant differences were noted between the two groups for TRAF2 expression in non-cardiomyocytes by t-test. **G, H**. Representative immunoblot (G) demonstrating TRAF2 expression in *Traf2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only (as control), two weeks after treatment with a single dose of tamoxifen. αSA was detected as control (for A, C, G). '**' indicates P<0.01 by t-test. **I**. Correlation between mean reduction in TRAF2 protein levels and mean % fractional shortening by echocardiographic assessment in TRAF2-icKO mice generated with regimens depicted in A-F. Coefficient of correlation (R) is shown. **J**. Correlation between mean reduction in TRAF2 protein levels and change in left ventricular end-diastolic diameter (LVEDD) from baseline by echocardiographic assessment in TRAF2-icKO mice generated with regimens depicted in A-F. Coefficient of correlation (R) is shown.



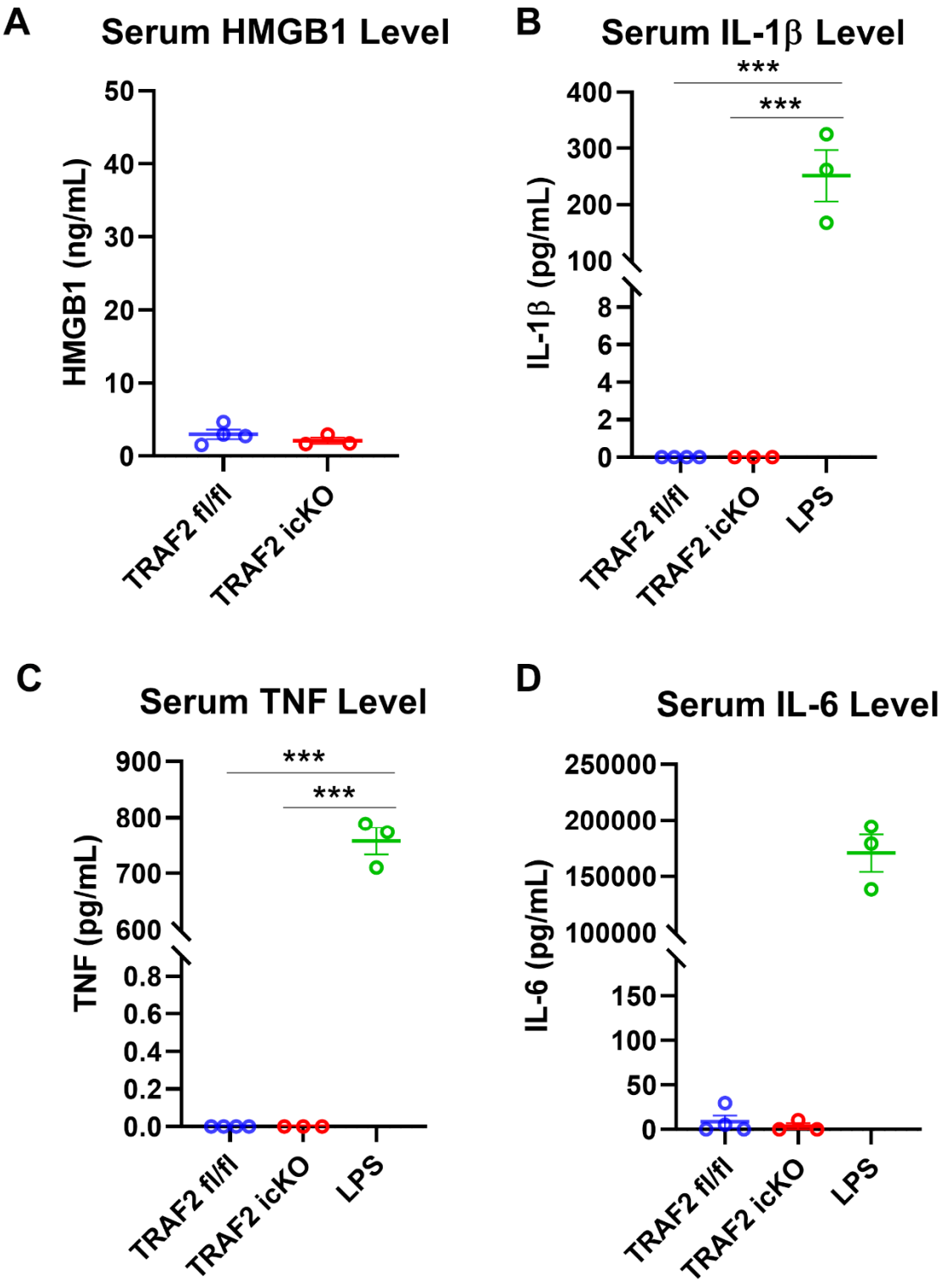
Supplementary Figure S5: Inducible cardiac myocyte TRAF2 ablation in young adult mice induces myocardial fibrosis. A. Representative myocardial sections stained with Picrosirius red from wild-type, TRAF2 floxed (*Traf2* fl/fl), MerCreMer (MCM) mice, and TRAF2 icKO mice (i.e. mice homozygous for *Traf2* floxed alleles carrying the MerCreMer transgene (*Traf2* fl/fl+MCM); 14 days after tamoxifen treatment (20 mg/kg/d, i.p. 5 days per week for 3 weeks). **B**. Quantitative assessment of fibrosis in mice as in A. '**' and '***' indicate P<0.01 and <0.001 by post-hoc test after one-way ANOVA.

Figure S6



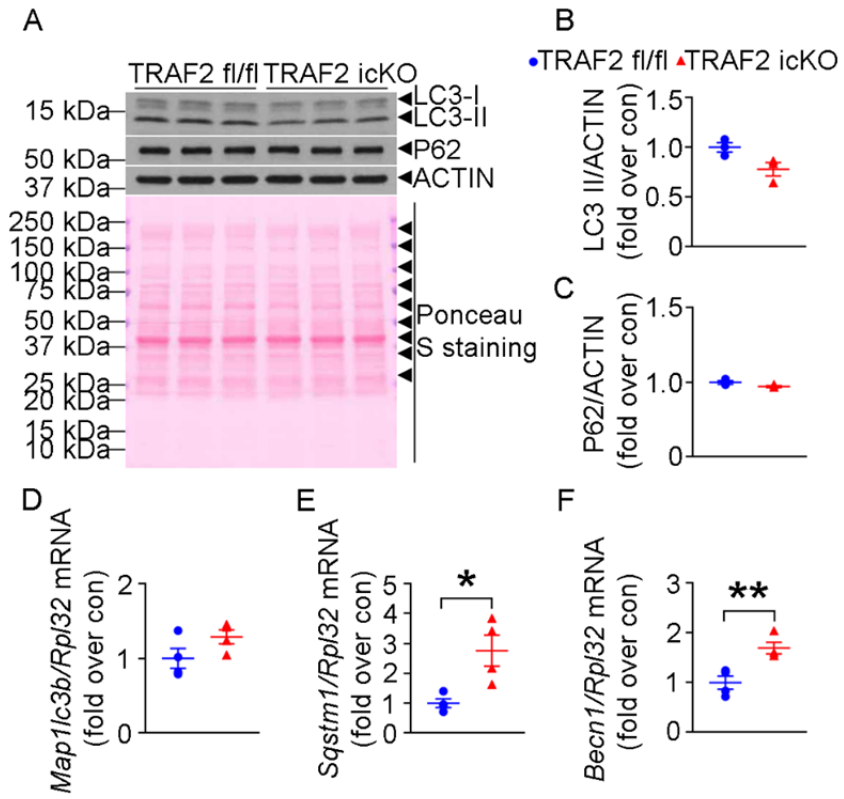
Supplementary Figure S6: Tamoxifen feeding induces adult onset TRAF2 ablation with accumulation of mitochondrial proteins. A. Immunoblot demonstrating TRAF2 expression in *Traf2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only (as control) two weeks after the mice were fed with tamoxifen chow for the indicated duration in days (D). **B-F**. Immunoblot (B) and quantitative analyses (C-F) demonstrating expression of TRAF2 (B, C), VDAC (B, D), TOMM20 (B, E) and COXIV (B, F) in *TRAF2* floxed mice carrying the *Myh6*-MerCreMer transgene or with *Traf2* floxed alleles only (as control) 2 weeks after the mice were fed with tamoxifen chow for 7 days**.** '*' and '***' indicate P<0.05 and <0.001 by t-test.

Figure S7


A
Serum HMGB1 Level
HMGB1 (ng/mL)
50
40
30
20
10
0
TRAF2 fl/fl
TRAF2 icKO
B
Serum IL-1β Level
***
***
IL-1β (pg/mL)
400
300
200
100
8
6
4
2
0
TRAF2 fl/fl
TRAF2 icKO
LPS
C
Serum TNF Level
***
***
TNF (pg/mL)
900
800
700
600
0.8
0.6
0.4
0.2
0.0
TRAF2 fl/fl
TRAF2 icKO
LPS
D
Serum IL-6 Level
IL-6 (pg/mL)
250000
200000
150000
100000
150
100
50
0
TRAF2 fl/fl
TRAF2 icKO
LPS

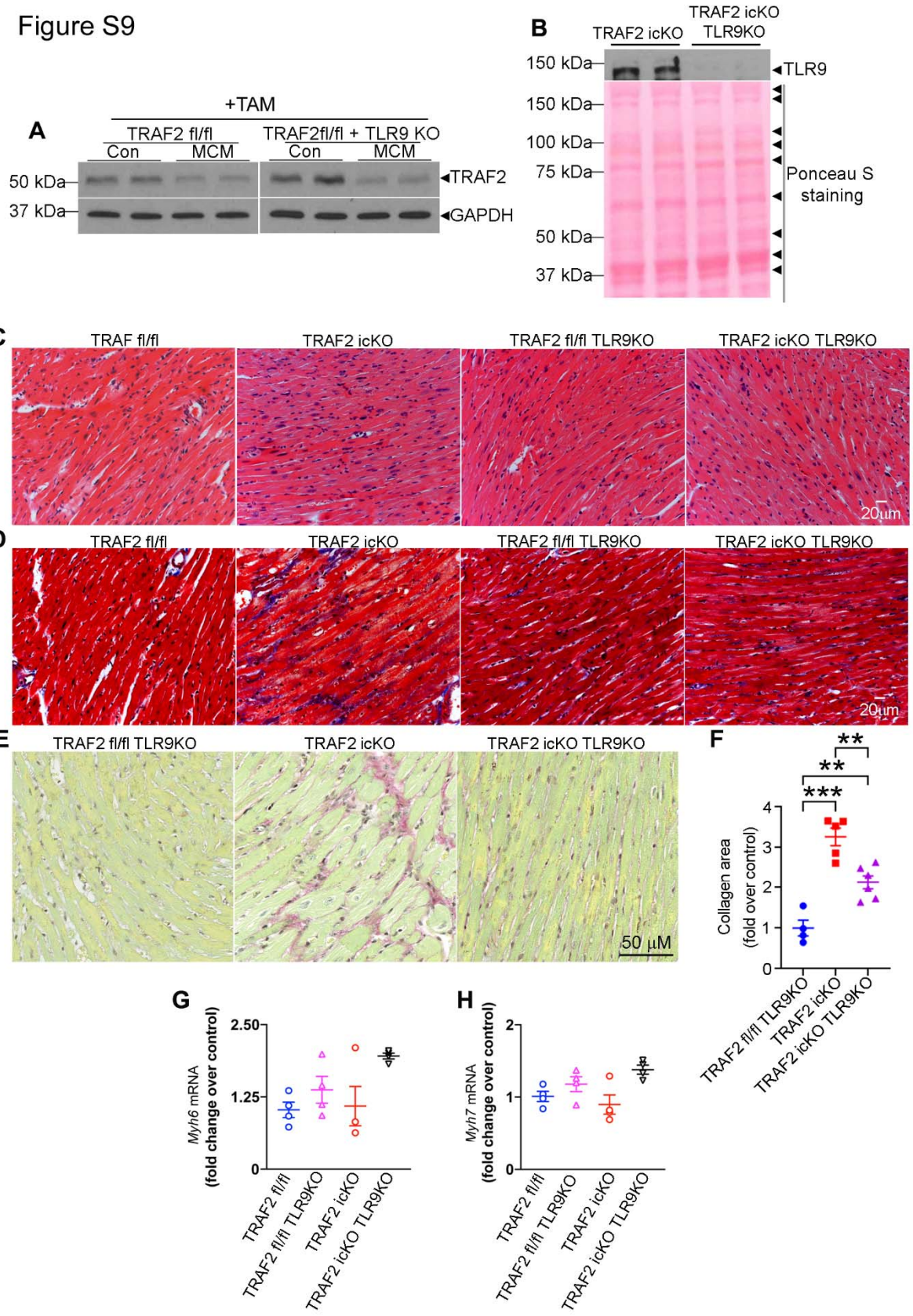
Supplementary Figure S7. Adult onset inducible TRAF2 ablation does not result in increased circulating markers of cellular necrosis or inflammation. A-D. Assessment of circulating levels of HMGB-1, IL1β, TNF and IL-6 in sera from mice modeled for inducible cardiac myocyte TRAF2 ablation (TRAF2-icKO) versus TRAF2 floxed mice as controls. Mice treated with a dose of lipopolysaccharide (LPS, 200μg) with sera harvested 90 minutes later were employed as an additional control. '***' indicates $P<0.001$ by post-hoc test after one-way ANOVA. No statistically significant differences were noted between the groups for HMGB-1 (A) by t-test or IL-6 (D) by Kruskal-Wallis test.

Figure S8



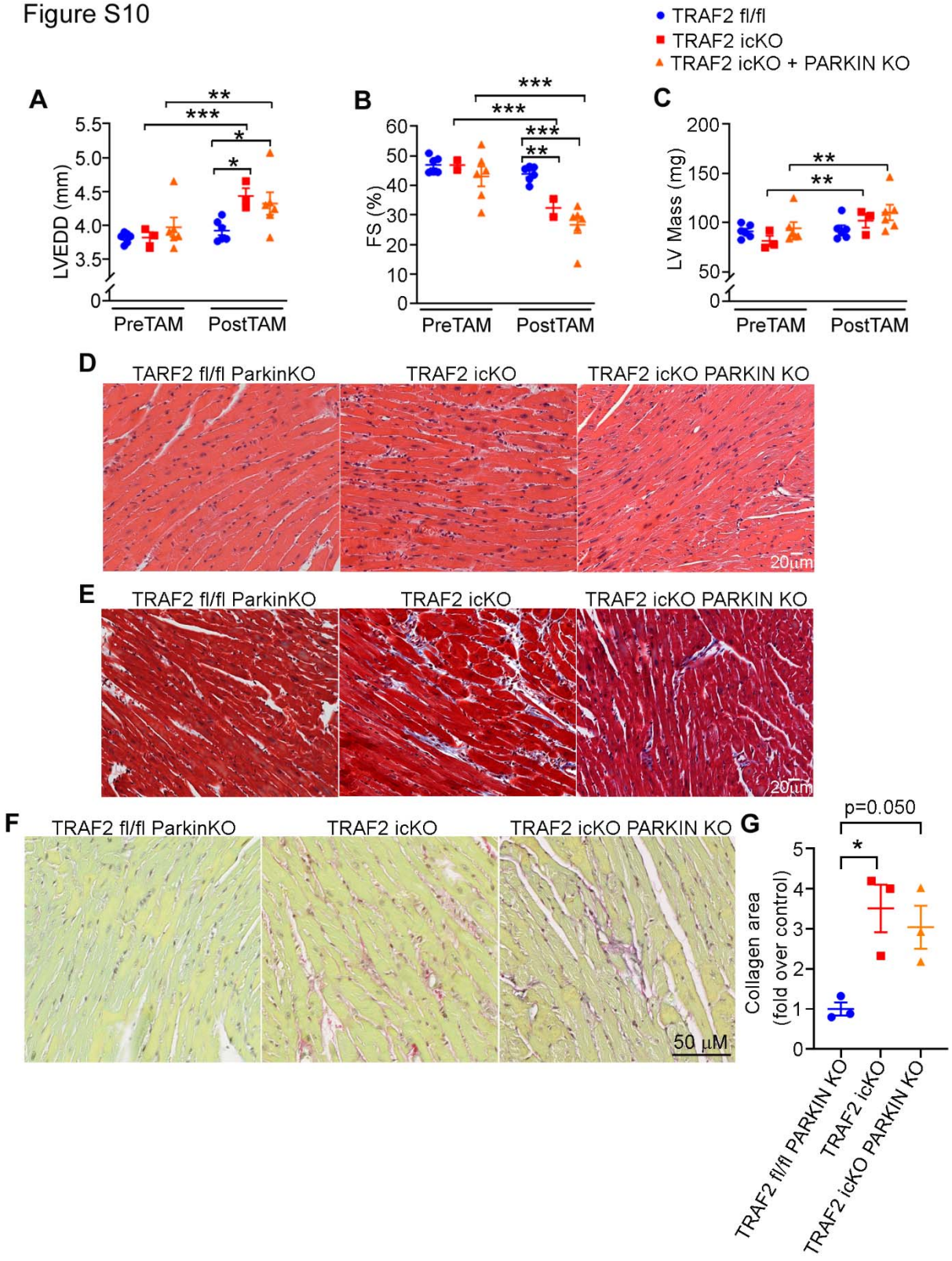
Supplementary Figure S8: Inducible cardiac myocyte TRAF2 ablation in young adult mice does not impair expression of autophagy pathway proteins. A. Representative immunoblot (B) showing expression of LC3 and p62 from cardiac extracts from TRAF2 floxed (*Traf2* fl/fl) mice, and mice homozygous for *Traf2* floxed alleles carrying the MerCreMer transgene (*Traf2* fl/fl+MCM); 14 days after tamoxifen treatment (20 mg/kg/d, i.p. 5 days per week for 3 weeks). **B, C**. Quantitative assessment of LC3-II and p62 in mice treated as in A. **D-F**. Expression of *Map1lc3b* (D), *Sqstm1* (E) and *Becn1* (F) transcripts in the myocardium from mice treated as in A. No statistically significant differences were noted between the groups in panels B-D by t-test. '*' and '**'indicate P<0.05 and <0.01, respectively, by t-test.

Figure S9


A
+TAM
TRAF2 fl/fl
TRAF2fl/fl + TLR9 KO
Con
MCM
Con
MCM
50 kDa
37 kDa
TRAF2
GAPDH
B
TRAF2 icKO
TRAF2 icKO TLR9KO
150 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
TLR9
Ponceau S staining
C
TRAF fl/fl
TRAF2 icKO
TRAF2 fl/fl TLR9KO
TRAF2 icKO TLR9KO
20μm
D
TRAF2 fl/fl
TRAF2 icKO
TRAF2 fl/fl TLR9KO
TRAF2 icKO TLR9KO
20μm
E
TRAF2 fl/fl TLR9KO
TRAF2 icKO
TRAF2 icKO TLR9KO
50 μM
F
Collagen area (fold over control)
TRAF2 fl/fl TLR9KO
TRAF2 icKO
TRAF2 icKO TLR9KO
G
Myh6 mRNA (fold change over control)
TRAF2 fl/fl
TRAF2 fl/fl TLR9KO
TRAF2 icKO
TRAF2 icKO TLR9KO
H
Myh7 mRNA (fold change over control)
TRAF2 fl/fl
TRAF2 fl/fl TLR9KO
TRAF2 icKO
TRAF2 icKO TLR9KO

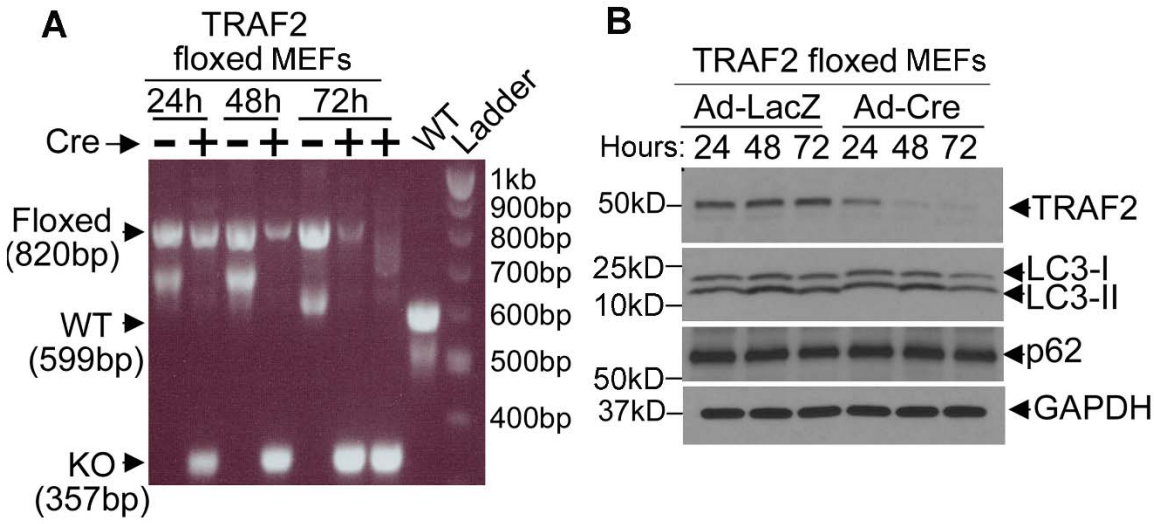
Supplementary Figure S9: TLR9 ablation prevents fibrosis in mice with inducible cardiac myocyte TRAF2 ablation in the short term. A. Representative immunoblot depicting TRAF2 expression in cardiac extracts from mice with adult-onset inducible TRAF2 ablation (TRAF2-icKO) and TRAF2 floxed controls, without and with concomitant germline TLR9 ablation, 2 weeks after TRAF2 ablation. **B.** Representative immunoblot depicting TLR9 expression in mice with adult-onset inducible TRAF2 ablation (TRAF2-icKO) with and without concomitant TLR9 ablation. **C, D**. Representative images with hematoxylin and eosin staining (C), and trichrome staining (D) to evaluate myocardial structure and fibrosis, respectively, in mice as treated in A. **E, F**. Representative Picrosirius red stained images (E) with quantitative assessment of fibrosis (F) in mice with adult-onset inducible TRAF2 ablation (TRAF2-icKO) without and with concomitant germline TLR9 ablation and TLR9 null controls, 2 weeks after TRAF2 ablation. "**" and '***' indicate $P<0.01$ and <0.001 by post-hoc test after one-way ANOVA. **G, H.** Expression of *Myh6* (E) and *Myh7* (F) transcripts in mice modeled as in A. No statistically significant differences were detected by one-way ANOVA.

Figure S10


TRAF2 fl/fl
TRAF2 icKO
TRAF2 icKO + PARKIN KO
A
LVEDD (mm)
PreTAM
PostTAM
B
FS (%)
C
LV Mass (mg)
D
TARF2 fl/fl ParkinKO
TRAF2 icKO
TRAF2 icKO PARKIN KO
20µm
E
TRAF2 fl/fl ParkinKO
TRAF2 icKO
TRAF2 icKO PARKIN KO
20µm
F
TRAF2 fl/fl ParkinKO
TRAF2 icKO
TRAF2 icKO PARKIN KO
50 µM
G
p=0.050
Collagen area (fold over control)
TRAF2 fl/fl PARKIN KO
TRAF2 icKO
TRAF2 icKO PARKIN KO

Supplementary Figure S10: Parkin ablation does not alter cardiac structure or function in mice with inducible cardiac myocyte TRAF2 ablation. A-C. 2D-directed M-mode echocardiography-derived left ventricular (LV) end-diastolic diameter (LVEDD, A), endocardial fractional shortening (%FS, B), left ventricular mass (LV mass, C) in *Traf2* floxed, *Traf2* floxed bearing MerCreMer transgene (TRAF2-icKO), and *Traf2* floxed bearing MerCreMer transgene in a *Park*2 null (KO) background (TRAF2-icKO-PARKIN KO) mice before (PreTAM) and 14 days after tamoxifen treatment for 1 week (postTAM). '*', '**' and '***' indicate $P<0.05$, <0.01 and <0.001, respectively, by post-hoc test after two-way ANOVA. No statistically significant differences were noted by post-hoc testing between TRAF2-icKO and TRAF2-icKO-PARKIN KO groups post-TAM treatment. **D, E**. Representative images with hematoxylin and eosin staining (D), and trichrome staining (E) to evaluate myocardial structure and fibrosis, respectively, in mice as treated in A. **F,G.** Representative Picrosirius red stained images (F) with quantitative assessment of fibrosis (G) in mice treated as in A. '*' indicating $P<0.05$, and P value ($=0.050$) shown are by post-hoc test after one-way ANOVA. No statistically significant differences were detected by post-hoc test between TRAF2-icKO and TRAF2-icKO-PARKIN-KO groups.

Figure S11



Supplementary Figure S11. TRAF2 ablation in murine embryonic fibroblasts does not alter macro-autophagy. A. Murine embryonic fibroblasts (MEFs) carrying *Traf2* floxed alleles were modeled for TRAF2 ablation with adenoviral Cre (or LacZ as control, each at MOI=100) treatment for 24, 48 and 72 hours, with assessment for ablation of *Traf2* gene by PCR analysis. **B**. MEFs treated as above were also subjected to immunoblotting for TRAF2, LC3, SQSTM1 (p62) and GAPDH proteins.