Suppression of myeloid YAP antagonizes adverse cardiac remodeling during pressure overload stress

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Supplemental Materials:

4. Supplemental Materials and Methods

4.1 Mice. Conditional *Yap1* allele floxed mice(1) were crossed with myeloid (LysM) Cre recombinase transgenic mice (2) to generate conditional YAP deleted mice. Wild-type mice (stock #000664) and LysM-Cre mice (stock #004781) were purchased from The Jackson Laboratories. All mice were C57BL/6J background. Eight- to ten-week-old mice were used for these studies. Male and female age and gender matched littermate controls ($Yap^{F/F}$) were used for all experiments. All mouse studies were performed blinded to genotype. Mice were housed in a temperature-controlled environment with 12-hour light/dark cycles where they received food and water *ad libitum*. All protocols concerning the use of animals were approved by the Institutional Animal Care and Use Committee at Rutgers, The State University of New Jersey.

4.2 Transverse aortic constriction. The method for imposing pressure overload in mice has been described previously(3). Briefly, mice were subcutaneously injected with a small volume of bupivacaine, anesthetized with pentobarbital (60 mg/kg, i.p.), and mechanically ventilated. The left chest was opened at the second intercostal space. Aortic constriction was achieved by ligation of the transverse thoracic aorta between the innominate artery and left common carotid artery with a 27-gauge needle using a 7-0 braided polyester suture. Sham operation was performed without constricting the aorta. Mice with a pressure gradient < 50 mmHg after TAC surgery were excluded from the study.

4.3 Echocardiography. Mice were anesthetized using isoflurane (3% induction, 1-2% maintenance), and echocardiography was performed as described previously (4), using a 30-MHz linear ultrasound transducer. Two-dimensional guided M-mode measurements of LV internal diameter were obtained from at least three beats and then averaged. LV end-diastolic dimension (LVIDd) was measured at the time of the apparent maximal LV diastolic dimension, and LV end-systolic dimension (LVIDs) was measured at the time of the most anterior systolic excursion of the posterior wall.

4.4 Histology. Heart specimens were fixed with formalin, embedded in paraffin, and sectioned at 6 μm thickness. Interstitial fibrosis was evaluated by picrosirius red staining as described(5). Briefly, the

percentage of heart tissue area positive for picrosirius red was determined using multiple images that were obtained throughout each LV heart section, and the average area of fibrosis was calculated. Myocyte cross-sectional area was measured from images captured from wheat germ agglutinin (WGA)-stained sections as described previously(6). To evaluate apoptosis, DNA fragmentation was detected in situ using TUNEL as described previously(5).

4.5 Real-time qPCR. RNA was isolated, cDNA was generated, and quantitative real-time PCR performed as described previously(7). The primers used are listed in **Supplemental Table 3**. Targets were normalized to internal control (*18s*), and relative quantitation was determined using the comparative $\Delta\Delta C_T$ method.

4.6 Caspase-1 assay. The activity of caspase-1 was measured using a commercially available kit according to the manufacturer's instructions (Sigma, #218790).

4.7 Western blotting. For immunoblot analysis, protein was separated by SDS-PAGE and blotted as described previously(8). Densitometry was performed using ImageJ software. The antibodies used were NLRP3 (1:1000, 15101, Cell Signaling), Tubulin (1:1000, T6199, Sigma), YAP (1:1000, 14074, Cell Signaling), p-YAP (1:1000, 13008, Cell Signaling), VEGFA (1:1000, ab46154, Abcam), TAZ (1:1000, 8418, Cell Signaling), p62/SQSTM1 (1:1000, H00008878-M01, Abnova), LC3B (1:1000, NB100-2220, Novus Biologicals), PINK1 (1:1000, BC100-494, Novus Biologicals), Parkin (1:1000, 2132, Cell Signaling), TFEB (1:1000, A303-673A, Bethyl Labs), FoxO3a (1:1000, 12829, Cell Signaling), and Gasdermin D (1:1000, 93709, Cell Signaling).

4.8 Luciferase reporter assay. RAW264.7 cells were transfected using Lipofectamine LTX with PLUS reagent (Thermo Cat# 15228100) according to the manufacturer's instructions. A TEAD luciferase reporter gene (8xGTIIC-luciferase; Addgene plasmid #34615) was used to assess YAP-TEAD activity. For Nlrp3 and Il-1β promoter studies, designated sequences located proximal to the start of exon 1 in the mouse *Nlrp3* gene or the *Il-1b* gene were subcloned into the pGL3 Basic luciferase reporter plasmid (Promega). HEK293 cells were transfected using Lipofectamine2000 reagent (Invitrogen) according to

the manufacturer's instructions. Following transfection and treatments, cells were lysed with Passive Lysis Buffer (Promega), and luciferase activity was measured using the luciferase assay system (Promega) with an OPTOCOMP I luminometer (MGM instruments). The luminescence was normalized by protein content.

4.9 ELISA. IL-1β and IL-18 protein were measured using commercially available kits according to the manufacturer's instructions (Invitrogen #BMS6002, #BMS618-3). Mouse VEGF protein was measured according to manufacturer's instructions (R&D Systems, #MMV00).

4.10 PLGA nanoparticles. Verteporfin- or DMSO-loaded PLGA nanoparticles were synthesized using a single emulsion process. An oil phase containing dichloromethane with 50 mg/ml PLGA and 10 mg/ml verteporfin was first prepared. The emulsion was formed by adding 500 uL of the polymer/drug solution to 5 mL of water followed by sonication at 70% power pulsed (2 s on/1 s off) for 2 min on a Fisher Scientific 150E Sonic Dismembrator. The resulting emulsion was placed in a fume hood under gentle stirring for 3 h to evaporate the solvent. The particles were then collected by centrifugation at 80,000*g* in a Beckman Coulter Optima L-90K Ultracentrifuge and resuspended in 10% sucrose for lyophilization. Particle size, polydispersity (PDI), and surface zeta potential were characterized using DLS. Drug loading yield and release rate of replicate samples ($n = 3$) were quantified by high performance liquid chromatography (HPLC). Drug release was determined by dialyzing 500 µl of particle solution at a concentration of 2.67 mg/ml in PBS using 3.5 K MWCO Slide-A-Lyzers (Thermo Scientific). We prepared nanoparticles with an average diameter of 120 nm with a drug loading yield of at least 2%(wt). PLGA nanoparticles were reconstituted in sterile PBS immediately prior to use and administered to mice via retro-orbital injection (5 mg/kg nanoparticles encapsulating 2.5 µg of verteporfin).

4.11 Immunofluorescence. Mouse hearts were fixed in formalin or cryopreserved and sectioned at 6 µm thickness. Formalin fixed tissue sections were subjected to deparaffinization and antigen unmasking using citrate buffer and washed with PBS containing 0.3% Triton X-100. Samples were blocked with 5% BSA and incubated with primary antibody overnight and with Alexa Fluor 488- and

Alexa Fluor 568-conjugated secondary antibodies (Molecular Probes) at room temperature the following day. Primary antibodies used were mouse monoclonal anti-αSMA antibody (clone 1A4, Sigma), rabbit polyclonal anti-Troponin-I antibody (Santa Cruz, sc-15368), rat monoclonal anti-CD68 antibody (clone FA-11, Biolegend), rabbit monoclonal anti-CCR2 antibody (Abcam, ab273050), Alexa Fluor 488-conjugated rat monoclonal anti-Ly6G antibody (clone 1A8, Biolegend), mouse monoclonal anti-Troponin-T antibody (clone 13-11, Invitrogen), rhodamine-conjugated wheat germ agglutinin (Vector Labs, RL-1022), and FITC-conjugated isolectin B4 (Vector Labs, FL-1201). Nuclei were stained with DAPI. Imaging was performed using a Nikon fluorescence microscope.

4.12 Isolation of adult mouse cells. Mouse hearts were excised and placed in heparinized saline. After removal of epicardial fatty tissue and the aorta, the hearts were finely minced into ~1-2 mm pieces and blood removed by repeated washing in saline. The tissue was then digested with collagenase (1 mg/mL; Worthington), trypsin (0.1%; Gibco, Invitrogen), and DNAse (10 μg/mL; 5 Prime) in 10 mL RPMI media (Gibco) for 45 minutes at 37°C with occasional shaking (15 min each, total 45 min). Cells were pelleted at 350*g* for 5 minutes at 4°C, washed with PBS containing 1mM EDTA (to avoid cell aggregation), and filtered through a 100 μm cell strainer. Spleens were removed, triturated in HBSS (Mediatech, Inc.) at 4°C, and filtered through nylon mesh 40 μm cell strainer (BD Biosciences) with the end of a 3-ml syringe plunger. The cell suspension was centrifuged at 300g for 10 min at 4°C. Red blood cells were lysed with ACK lysis buffer, and the splenocytes were washed with HBSS. Blood was collected by retro-orbital bleeding using heparinized hematocrit capillary tubes containing 0.5M EDTA. Red blood cells were lysed with ACK lysis buffer, and the remaining cells were washed with HBSS. Cells from all tissues were resuspended in MACS buffer prior to FACS analysis.

4.13 Flow cytometry. Staining of single cell suspensions was performed at 4°C protected from light for 30 min and washed 2x in MACS buffer prior to fixation in PBS containing 2% paraformaldehyde solution for 10 min at room temperature. A portion of the heart samples was used for internal staining. Samples were permeabilized for intracellular staining by adding ice cold 90% methanol dropwise with

low vortexing. The samples were incubated on ice for 30 min protected from light and washed in MACS buffer. The pellets were then resuspended in MACS containing antibodies recognizing intracellular markers and incubated for 1-2 hours at room temperature protected from light. The samples were then washed 2x in MACS buffer and resuspended in MACS for flow cytometry. Antibodies used for flow cytometry were CD45-PE (clone 30-F11, 1:1000, 103106, BioLegend), CD11b-BUV395 (clone M1/70, 1:1000, 565976, BD Biosciences), F4/80-BV510 (clone T45-2342, 1:100, 743280, BD Biosciences), Ly6G-BV711 (clone 1A8, 1:100, 563979, BD Biosciences), CD3-FITC (clone 17A2, 1:200, 11-0032-82, Invitrogen), Cardiac Troponin T-PE (1:50, 130-120-545, Miltenyi), and Viability Dye eFluor-780 (1:100, 65-0865-14, Invitrogen). For gene expression experiments, RNA was isolated immediately following cell collection using the RNeasy Plus Micro kit according to the manufacturer's instructions (Qiagen).

4.14 CD11b cell isolation. Single cell suspensions were prepared from mouse hearts as described above and resuspended in MACS buffer. Viable cells were separated using the CD11b Microbeads UltraPure protocol (Miltenyi, 130-126-725). Briefly, the single cell suspension was incubated with CD11b Microbeads, washed, and passed through MS columns (130-042-201) using a MACS Separator. The columns were washed 3x with MACS buffer, removed, and flushed with MACS buffer to collect the enriched cell fraction.

4.15 Cell culture and reagents. The RAW264.7 cell line was purchased from ATCC (TIB-71) and maintained according to established protocols(9). RAW264.7 cells were transfected with eGFP (Addgene #13031) or YAPS127A (Addgene #27370). For bone marrow derived macrophage (BMDM) experiments, bone marrow cells were isolated from adult $Yap^{F/F}$;LysM-Cre and control $Yap^{F/F}$ mice(10). Cells were cultured in complete RPMI medium supplemented with 20ng/mL recombinant M-CSF (PeproTech) for 5-7 days. Cells were serum starved 24 hours prior to stimulation. Mouse recombinant IL-4 was purchased from R&D Systems. Lipopolysaccharide (LPS) and verteporfin were purchased from Sigma.

4.16 Chromatin immunoprecipitation (ChIP) assay. ChIP assays were performed using the SimpleChIP Plus Enzymatic Chromatin IP Kit (Cell Signaling) according to the manufacturer's

instructions. Briefly, RAW264.7 cells were transduced with 3x FLAG-YAP expression construct or control GFP for 24 hours and then cells were cross-linked using 1% formaldehyde for 10 minutes. Cells were washed with 1x PBS and glycine was added to stop the cross-linking reaction. Cells were then scraped, nuclei were isolated and lysed, and sheared chromatin was isolated after sonication. Immunoprecipitation reactions were carried out using chromatin extracts and anti-YAP (CST #14074) or control rabbit IgG (CST #2729) antibodies overnight at 4°C. The corrected genomic fragments were validated with quantitative PCR with the following primers 5'-ATGCAAGGGCCAGTGTAGGTCA and 5'-GGATCTGAGTTCAGTTCCCAGTCT. The primer sequences used for ChIP are as follows: Nlrp3 P1: 5'-GAGAGTCTTGCATATTTTTGCTATTAACTGC and 5'-CCAAAAAGCAACACAGCAAGAGGC; Nlrp3 P2: 5'-CCTTGGTGCTCTGGGGTTATTATAG and 5'-CACAGCCTTCACTGTTTCTCAACCT; Il-1b P1: 5'-TCCAAGCAAGAAGAGCTCCCCT and 5'-TGCACAGTGCAAAGGTGGTGAAC; Il-1b P2: 5'-TGTGAAAGGGCCACTTGACTCCAA and 5'-GAGGGGCGTCCTTCATATGTGTTT.

4.17 Statistical analysis. All data are reported as mean ± standard error of the mean (SEM). *P* values were determined by unpaired, two-tailed Student *t*-test to evaluate the difference in means between two groups. Evaluation between three or more groups was done using ordinary one-way analysis of variance (ANOVA). Post-hoc multiple pairwise comparisons were performed using Tukey's test. The normality of continuous variables was determined using the Shapiro-Wilk test. Statistical analyses were performed using Graph Pad Prism 9. A P value < 0.05 was considered statistically significant.

Supplemental References

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Supplemental Table S1. Echocardiographic analysis of mice after 4 weeks pressure overload.

Parameter	$Yap^{F/F}$ Sham	$Yap^{F/F}$;$LysM^{Cre}$ Sham	$Yap^{F/F}$ TAC	$Yap^{F/F}$;$LysM^{Cre}$ TAC
n	8	9	14	10
LVIDd (mm)	3.31±0.05	3.34±0.08	3.74±0.10*	3.85±0.18#
LVIDs (mm)	1.97±0.08	1.99±0.09	2.75±0.12*	2.55±0.16#
IVSd (mm)	0.79±0.03	0.75±0.03	1.22±0.03*	1.05±0.05#@
PWTd (mm)	0.81±0.03	0.76±0.02	0.99±0.04*	0.91±0.02#
LVEF(%)	78.3±2.4	78.3±1.8	59.7±3.5*	70.8±2.7@

Data are presented as mean ± S.E.M. *P<0.05 versus $Yap^{F/F}$ Sham. #P<0.05 versus $Yap^{F/F}$;$LysM^{Cre}$ Sham. @P<0.05 versus $Yap^{F/F}$ TAC. LVIDd (left ventricular end diastolic dimension), LVIDs (left ventricular end systolic dimension), IVSd (diastolic septal wall thickness), PWTd (diastolic posterior wall thickness), LVEF(%) (left ventricular ejection fraction).

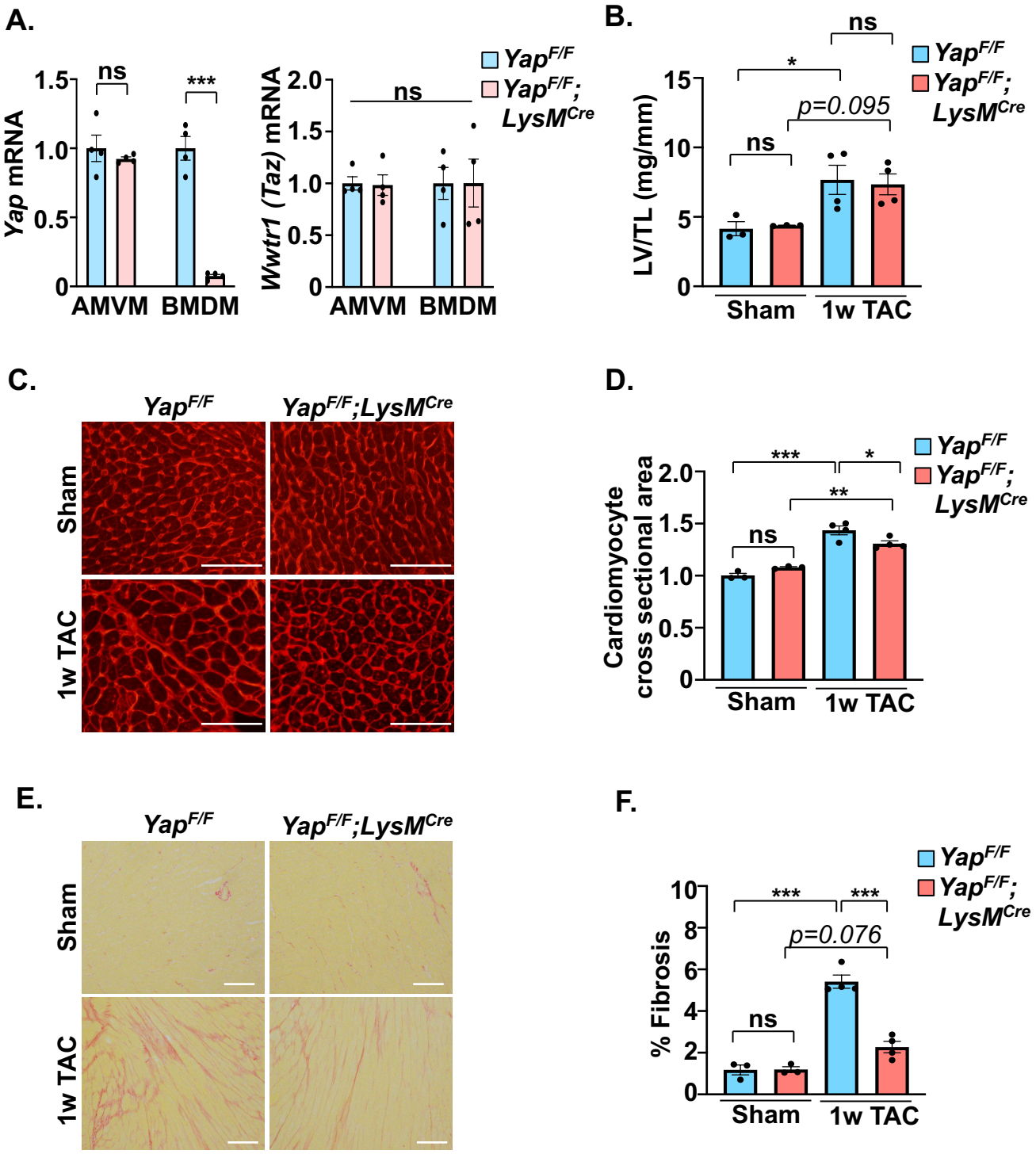
Supplemental Table S2. Echocardiographic analysis of mice after 2 weeks pressure overload with nanoparticle administration.

Parameter	NP-DMSO Baseline	NP-VP Baseline	NP-DMSO TAC	NP-VP TAC
n	4	3	4	3
LVIDd (mm)	3.52±0.12	3.71±0.25	3.62±0.19	3.61±0.06
LVIDs (mm)	2.37±0.09	2.49±0.16	2.55±0.19	2.44±0.13
IVSd (mm)	0.77±0.04	0.78±0.05	0.95±0.04*	0.88±0.02
PWTd (mm)	1.01±0.02	0.92±0.05	0.94±0.05	0.95±0.05
LVEF(%)	69.5±2.2	69.6±2.5	65.0±3.1	68.9±3.6

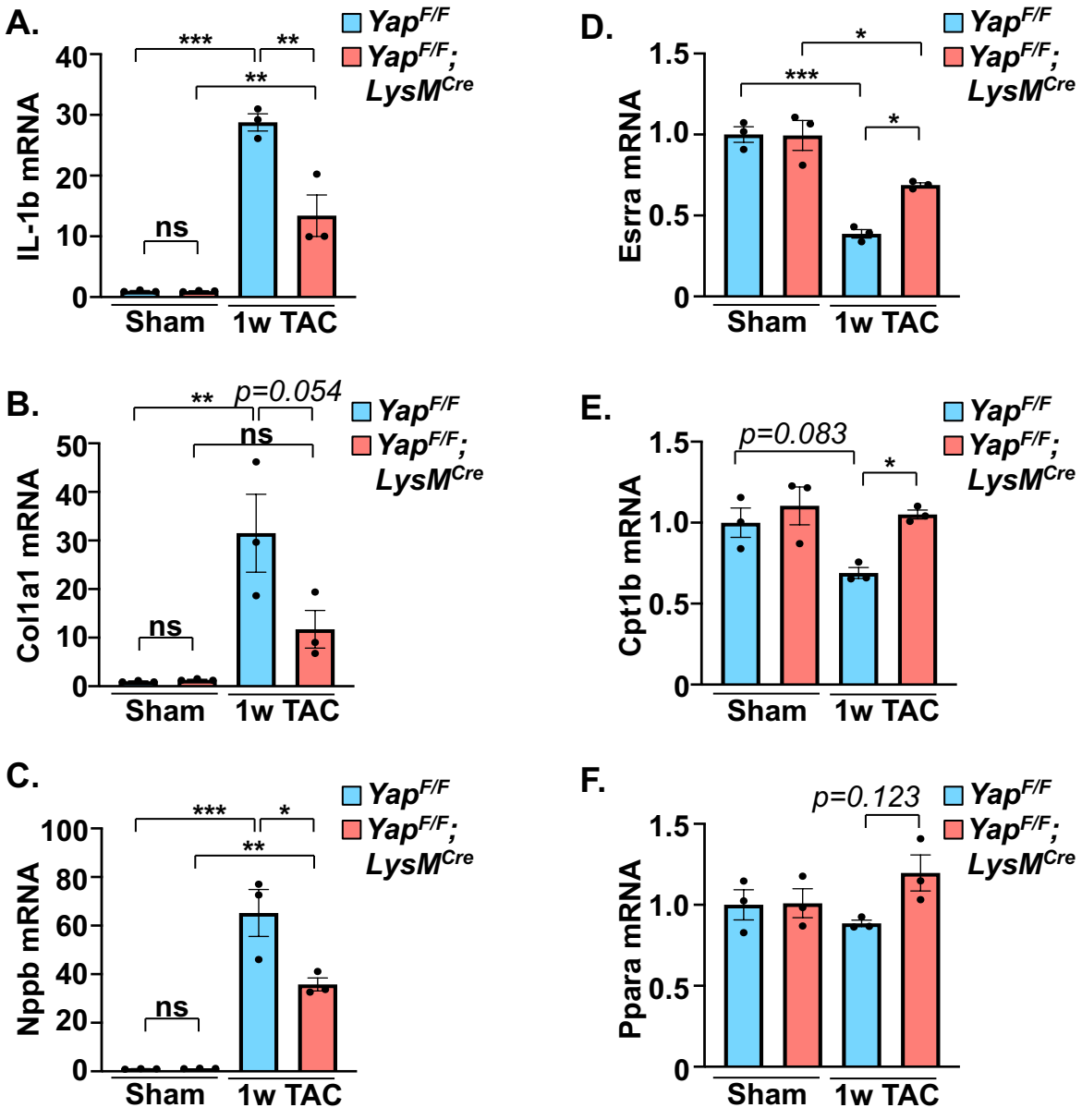
Data are presented as mean ± S.E.M. *$P<0.05$ versus NP-DMSO Baseline. LVIDd (left ventricular end diastolic dimension), LVIDs (left ventricular end systolic dimension), IVSd (diastolic septal wall thickness), PWTd (diastolic posterior wall thickness), LVEF(%) (left ventricular ejection fraction).

Table S3. Primer sequences used for quantitative qRT-PCR.

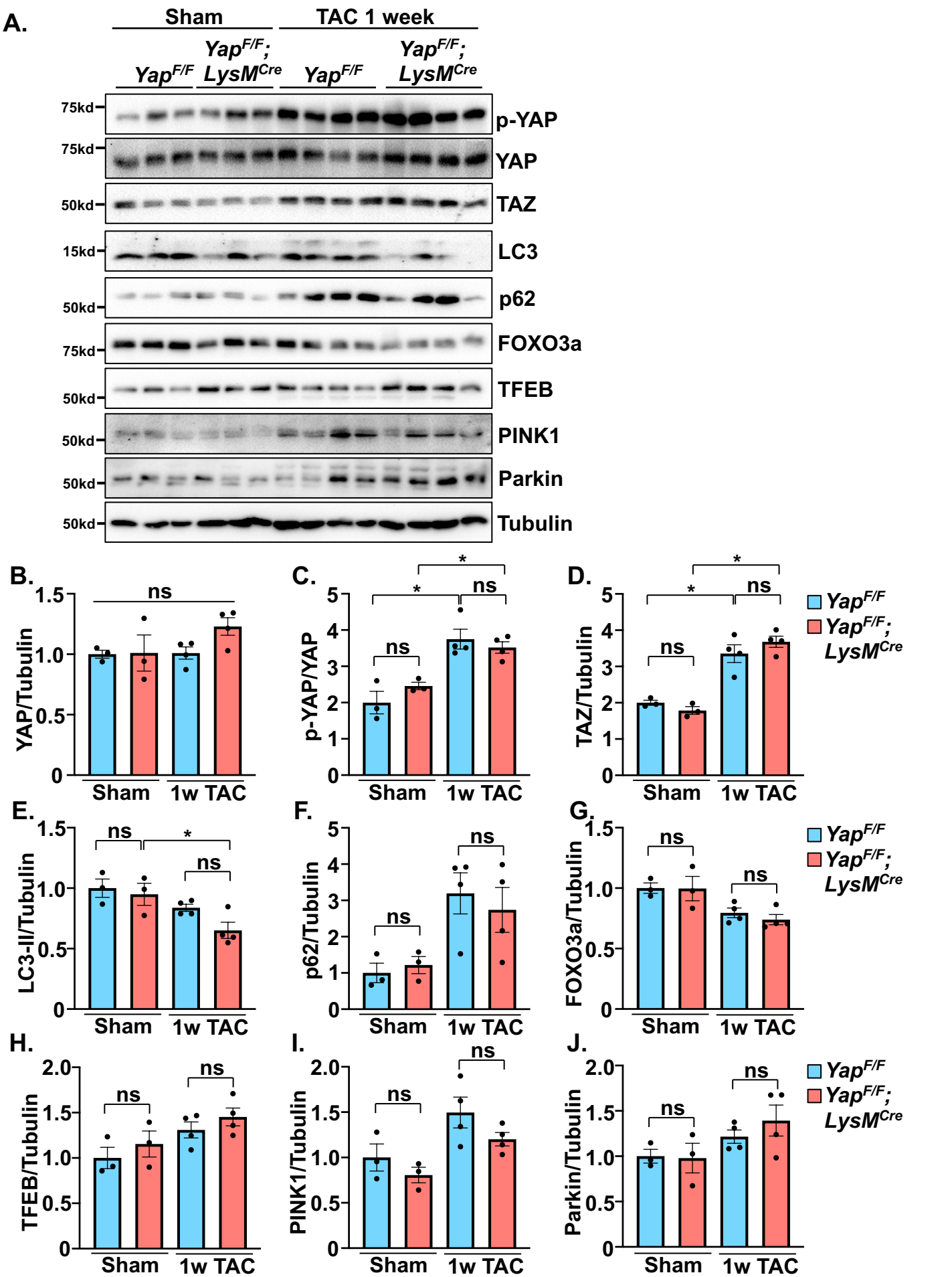
Gene	Forward (5'-3')	Reverse (5'-3')
Arg1	AGGCCCTGCAGCACTGAGGAA	GCCAGGTCCCCGTGGTCTCTCA
Ccl2	CATCCACGTGTTGGCTCA	AACTACAGCTTCTTTGGGACA
Ccl5	GCTGCTTTGCCTACCTCTCC	TCGAGTGACAAACACGACTG
Ccr2	ATCCACGGCATACTATCAACATC	TCGTAGTCATACGGTGTGGTG
Ccr7	TGTACGAGTCGGTGTGCTTC	GGTAGGTATCCGTCATGGTCTTG
Col1a1	GCTCCTCTTAGGGGCCACT	CCACGTCTCACCATTGGGG
Col3a1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Ctgf	CAAAGCAGCTGCAAATACCA	GGCCAAATGTGTCTTCCAGT
Cxcl2	CTTTCCAGGTCAGTTAGCCTT	CAGAAGTCATAGCCACTCTCAAG
Cxcr2	ATGCCCTCTATTCTGCCAGAT	GTGCTCCGGTTGTATAAGATGAC
Cyr61	CAAGAAATGCAGCAAGACCA	GGAACCGCATCTTCACAGTT
Il-1b	GACCTGTTCTTTGAAGTTGACG	CTCTTGTTGATGTGCTGCTG
Il-6	AGCCAGAGTCCTTCAGAGA	TCCTTAGCCACTCCTTCTGT
Il-10	CTCCTAGAGCTGCGGACTGCCTTCA	CTGGGGCATCACTTCTACCAGGTAAAA
Il-18	GACTCTTGCGTCAACTTCAAGG	CAGGCTGTCTTTTGTCAACGA
Itgal	CCAGACTTTTGCTACTGGGAC	GCTTGTTCGGCAGTGATAGAG
Itgam	ATGGACGCTGATGGCAATACC	TCCCCATTCACGTCTCCCA
Itgb2	CAGGAATGCACCAAGTACAAAGT	CCTGGTCCAGTGAAGTTCAGC
Nppa	ATACAGTGCGGTGTCCAACA	CGAGAGCACCTCCATCTCTC
Nppb	GGAAATGGCTCAGAGACAGC	CGATCCGGTCTATCTTCTGC
Nlrp3	ATGCTGCTTCGACATCTCCT	AACCAATGCGAGATCCTGAC
Nos2	GAGCGAGGAGCAGGTGGAAGACTA	GCGCTGCCCTTTTTTGCCCCATAG
Postn	CACGGCATGGTTATTCCTTCA	TCAGGACACGGTCAATGACAT
Relm	TGCCAATCCAGCTAACTATCC	GAGGCCCATCTGTTCATAGTC
Tnf	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Vegfa	GCACATAGAGAGAATGAGCTTCC	CTCCGCTCTGAACAAGGCT
Wwtr1	GAAGGTGATGAATCAGCCTCTG	GTTCTGAGTCGGGTGGTTCTG
Yap1	CCCGACTCCTTCTTCAAGC	CTCGAACATGCTGTGGAGTC
Ym1	GAAGCCCTCCTAAGGACAAAC	GCAGCCTTGGAATGTCTTTCT
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG



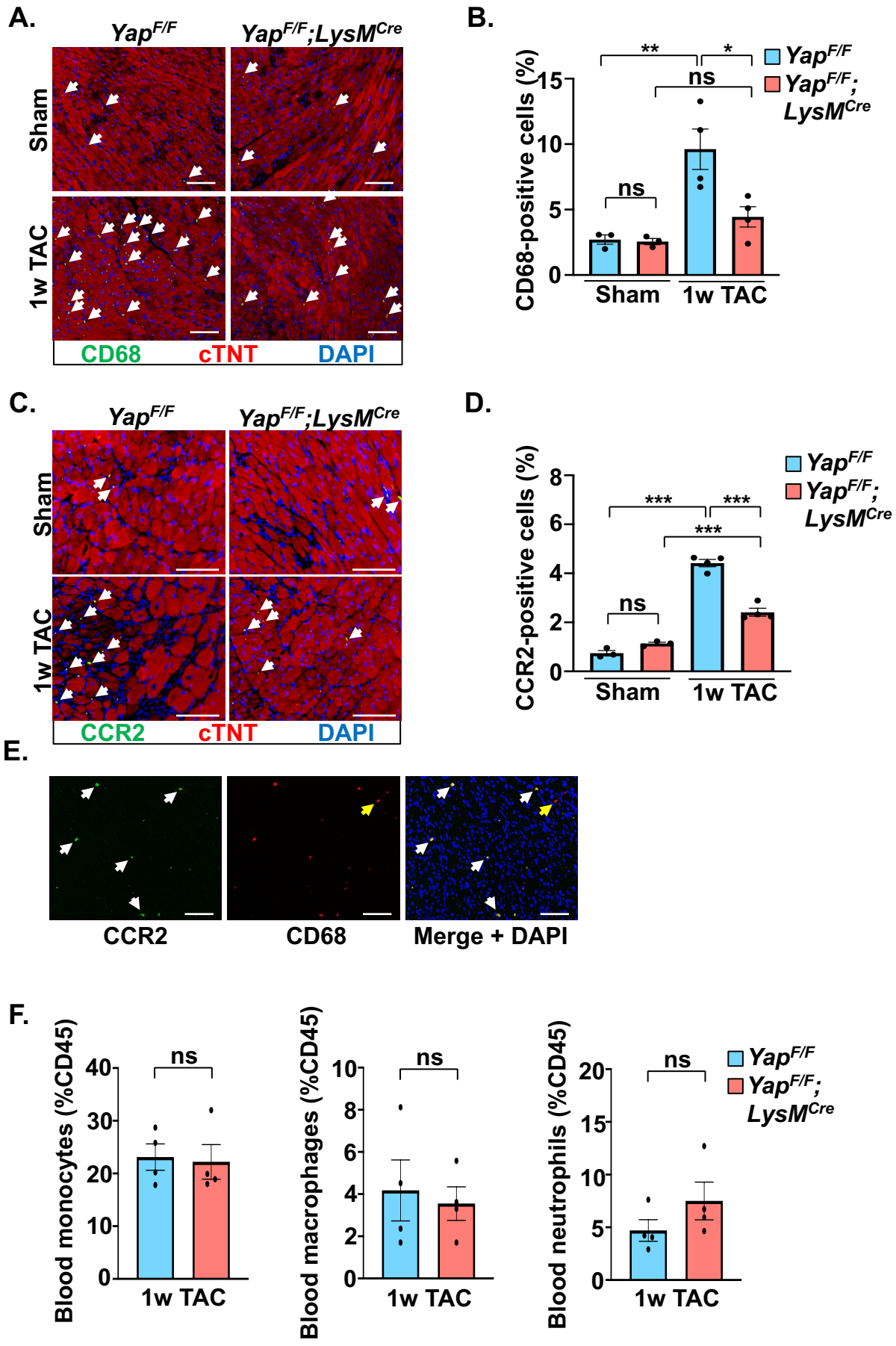
Supplemental Figure 1. Characterization of the cardiac phenotype after 1 week TAC. (A) *Yap* and *Wwtr1* (Taz) expression in isolated adult mouse ventricular cardiomyocytes (AMVM) and bone marrow-derived macrophages (BMDM) from control and myeloid YAP deficient mice. (B-D) Postmortem analysis of LV mass and wheat germ agglutinin (WGA) staining to determine cardiac hypertrophy in control and myeloid YAP deficient mice after 1 week TAC. (E-F) Collagen deposition was determined by PSR staining in control and myeloid YAP deficient LV tissue after 1 week TAC. Scale bar, 100 μm. N = 3-4 mice/group. All data are presented as mean ± SEM. *P* values were determined by unpaired, two-tailed Student *t*-test, or ordinary one-way ANOVA with multiple comparison. **P*<0.05. ***P*<0.01. ****P*<0.001, ns = not significant.



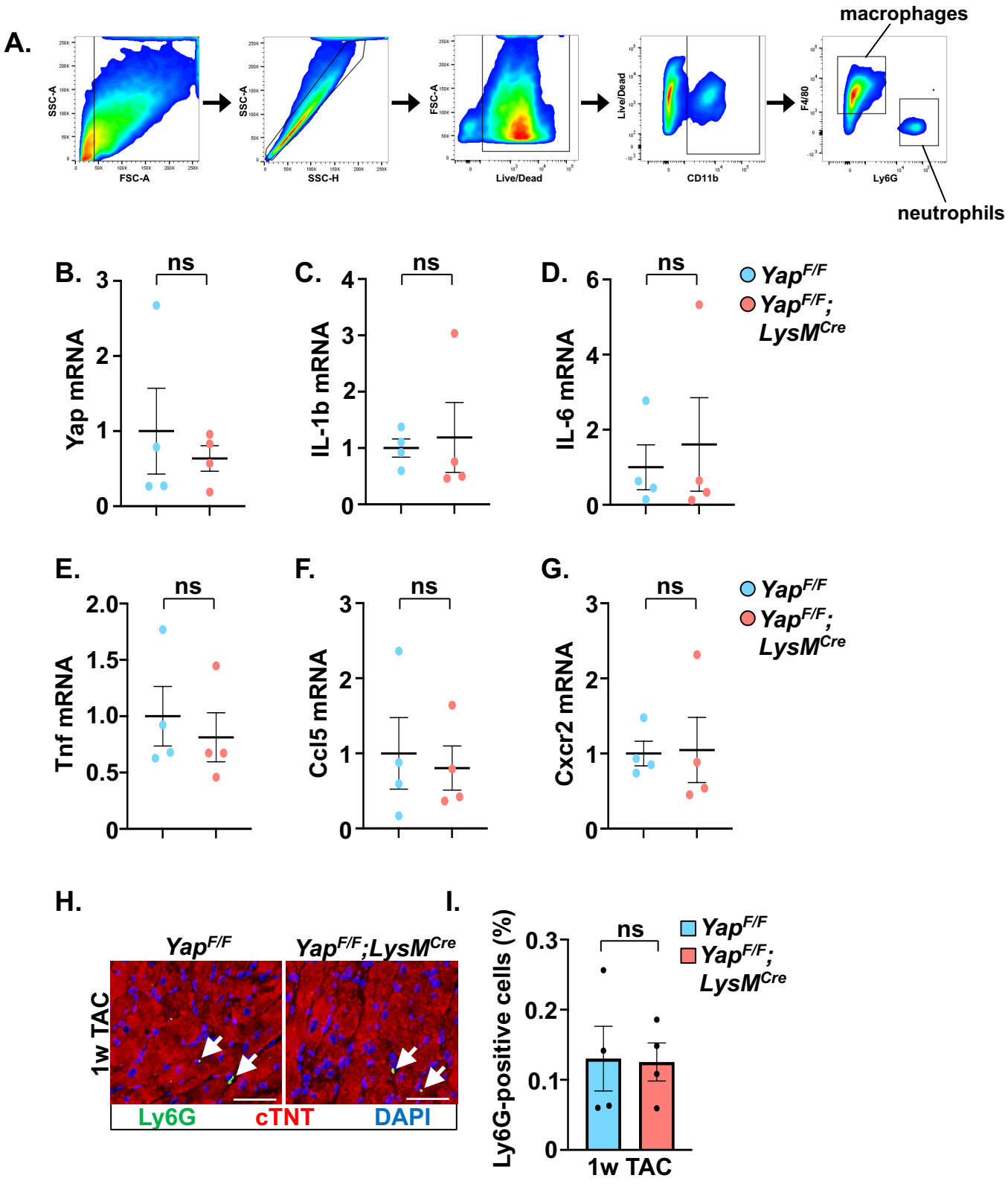
Supplemental Figure 2. Gene expression in control and myeloid YAP deficient hearts after 1 week TAC. (A-F) Quantitative PCR using RNA isolated from LV tissue measured gene expression in control and myeloid YAP deficient mice after 1 week TAC. N = 3 mice/group. All data are presented as mean ± SEM. *P* values were determined by ordinary one-way ANOVA with multiple comparison. **P*<0.05. ***P*<0.01. ****P*<0.001, ns = not significant.


A.
Sham
TAC 1 week
Yap^F/F
Yap^F/F; LysM^Cre
Yap^F/F
Yap^F/F; LysM^Cre
75kd
p-YAP
75kd
YAP
50kd
TAZ
15kd
LC3
50kd
p62
75kd
FOXO3a
50kd
TFEB
50kd
PINK1
50kd
Parkin
50kd
Tubulin
B.
YAP/Tubulin
C.
p-YAP/YAP
D.
TAZ/Tubulin
E.
LC3-II/Tubulin
F.
p62/Tubulin
G.
FOXO3a/Tubulin
H.
TFEB/Tubulin
I.
PINK1/Tubulin
J.
Parkin/Tubulin
ns
*
Sham
1w TAC
Yap^F/F
Yap^F/F; LysM^Cre

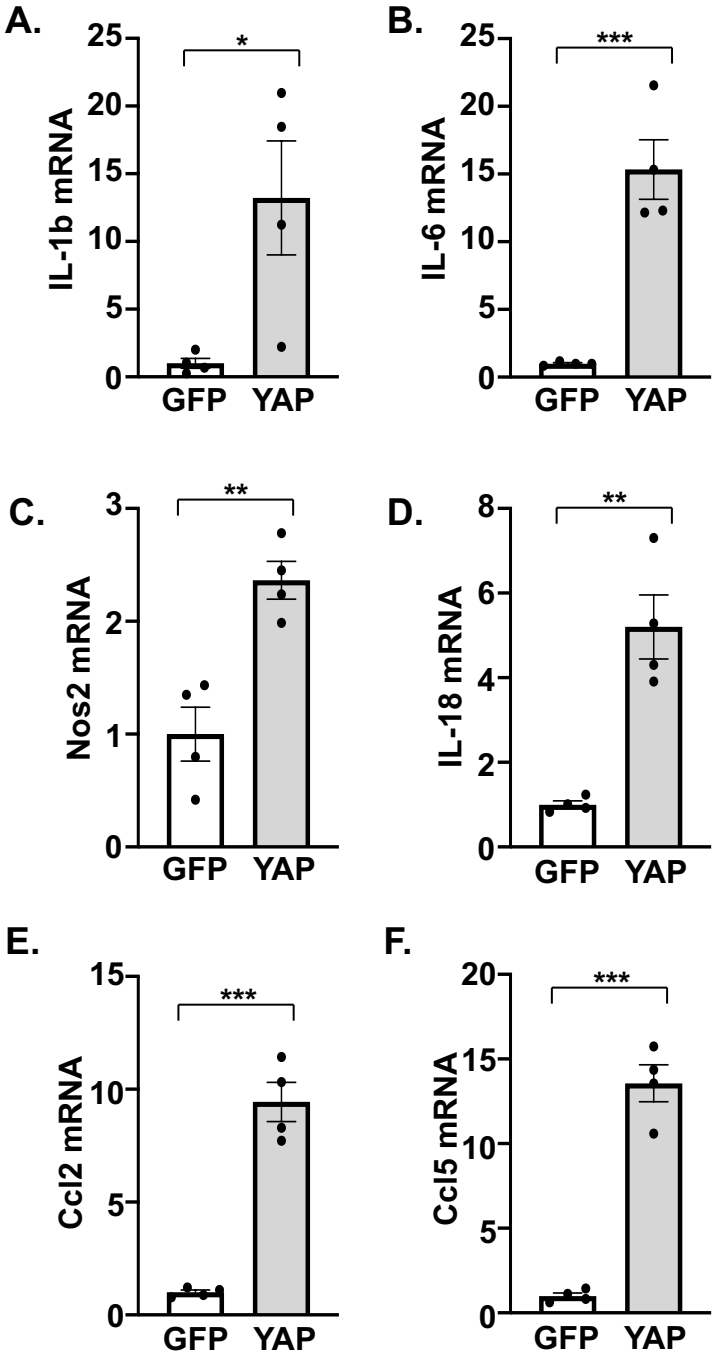
Supplemental Figure 3. Evaluation of Hippo-YAP pathway and autophagy/mitophagy-related proteins in control and myeloid YAP deficient hearts. (A) Representative blots from LV tissue of control and myeloid YAP deficient mice subjected to sham or 1-week post-TAC operation. (B-J) Quantification of western blots. N = 3-4 mice/group. All data are presented as mean ± SEM. *P* values were determined by ordinary one-way ANOVA with multiple comparison. **P*<0.05, ns = not significant.


A.
Yap F/F
Yap F/F;LysM Cre
Sham
1w TAC
CD68
cTNT
DAPI
B.
CD68-positive cells (%)
Yap F/F
Yap F/F; LysM Cre
**
*
ns
ns
Sham
1w TAC
C.
Yap F/F
Yap F/F;LysM Cre
Sham
1w TAC
CCR2
cTNT
DAPI
D.
CCR2-positive cells (%)
Yap F/F
Yap F/F; LysM Cre
***
***
***
ns
Sham
1w TAC
E.
CCR2
CD68
Merge + DAPI
F.
Blood monocytes (%CD45)
ns
1w TAC
Blood macrophages (%CD45)
ns
1w TAC
Blood neutrophils (%CD45)
ns
Yap F/F
Yap F/F; LysM Cre
1w TAC

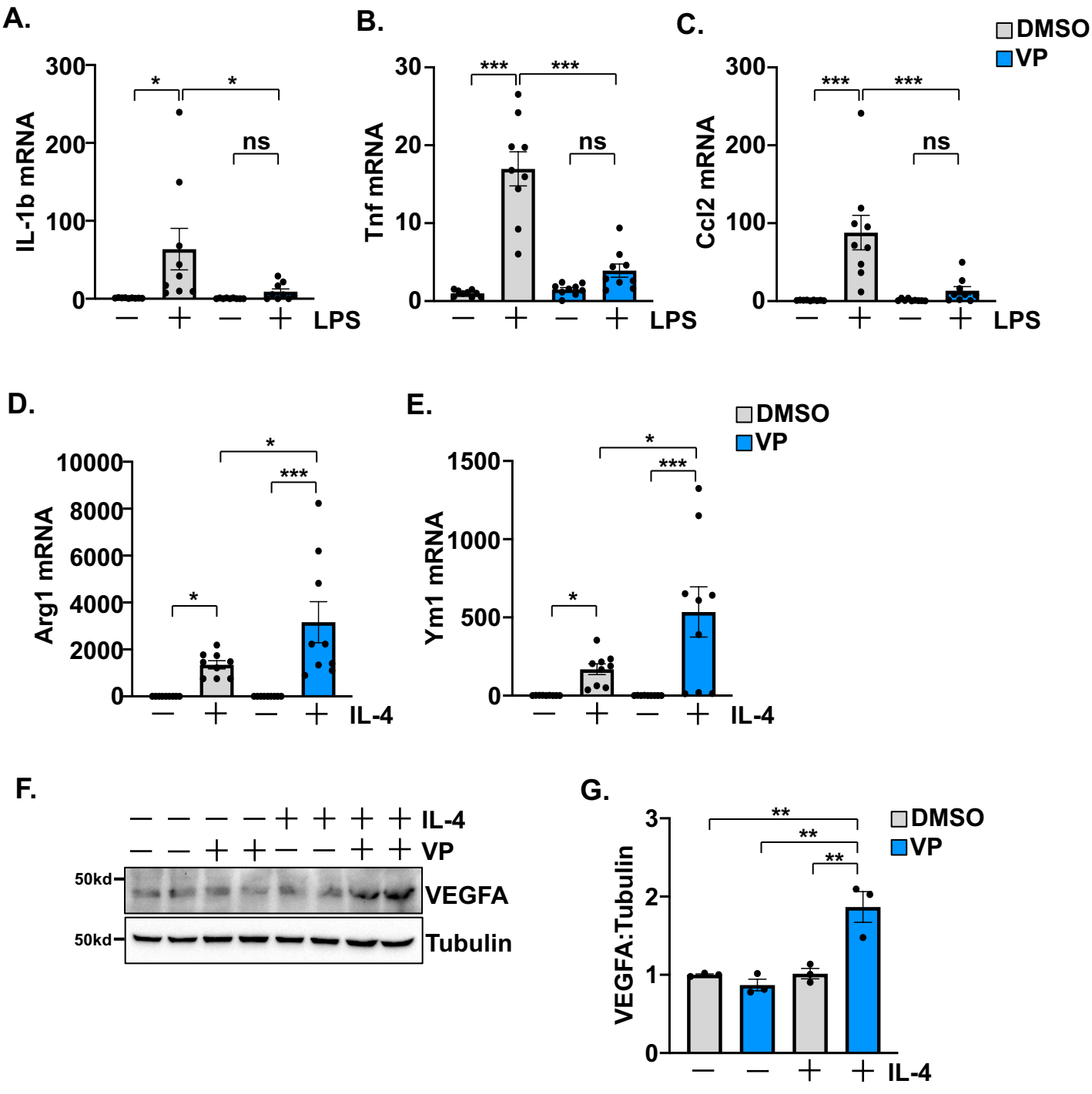
Supplemental Figure 4. Cardiac macrophage abundance in control and myeloid YAP deficient mice after 1 week TAC. (A-B) LV tissue was stained for CD68 (green), cardiac troponin T (red), and DAPI (blue). CD68-positive cells were determined. (C-D) LV tissue was stained for CCR2 (green), cardiac troponin T (red), and DAPI (blue). CCR2-positive cells were determined. (E) LV tissue was stained for CD68 (green) and counterstained for CCR2 (red) with DAPI (blue) merge. White arrows indicate CD68+CCR2+ double-positive cells. Yellow arrow indicates CD68+CCR2- cell. (F) Flow cytometry analysis of peripheral blood was performed to determine relative amounts of myeloid cells in control and YAP deficient mice after 1 week TAC. Monocytes, ($CD11b^{+}CD3^{-}Ly6G^{-}F4/80^{-}$); macrophages ($CD11b^{+}CD3^{-}Ly6G^{-}F4/80^{+}$); neutrophils ($CD11b^{+}CD3^{-}Ly6G^{+}F4/80^{-}$). Scale bar, 100 μm. N = 3-4 mice/group. All data are presented as mean ± SEM. *P* values were determined by unpaired, two-tailed Student *t*-test, or ordinary one-way ANOVA with multiple comparison. **P*<0.05. ***P*<0.01. ****P*<0.001, ns = not significant.



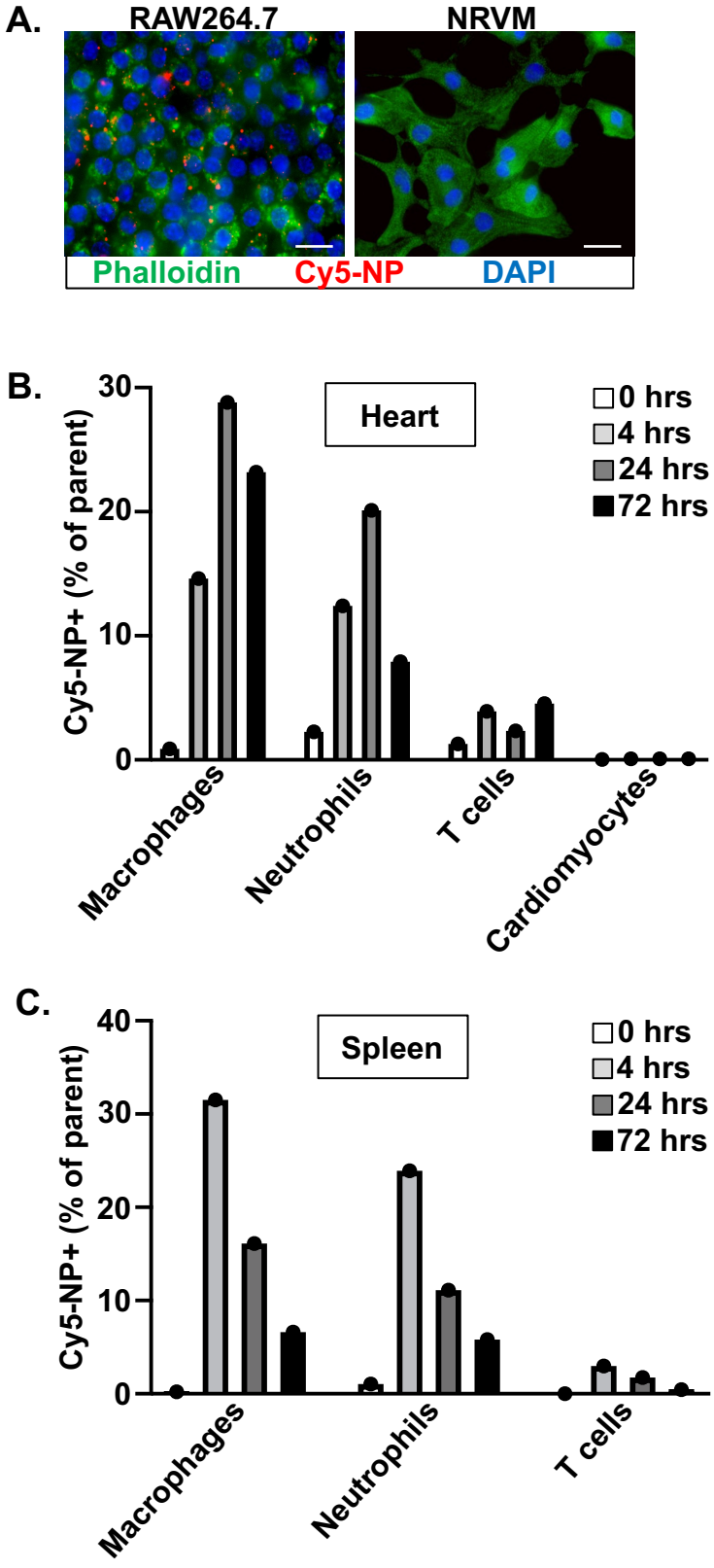
Supplemental Figure 5. Characterization of neutrophils from hearts of control and myeloid YAP deficient mice following PO stress. (A) Gating strategy for flow cytometry of cardiac myeloid cells. (B-G) Quantitative PCR using RNA isolated from myocardial flow-sorted neutrophils was performed to determine inflammatory and migration/chemotaxis-related gene expression in control and myeloid YAP deleted mice after 1-week TAC. (H-I) LV tissue was stained for Ly6G (green), cardiac troponin T (red), and DAPI (blue). Ly6G-positive cells (white arrows) were determined. N = 4 mice/group. All data are presented as mean ± SEM. *P* values were determined by unpaired, two-tailed Student *t*-test. ns = not significant.



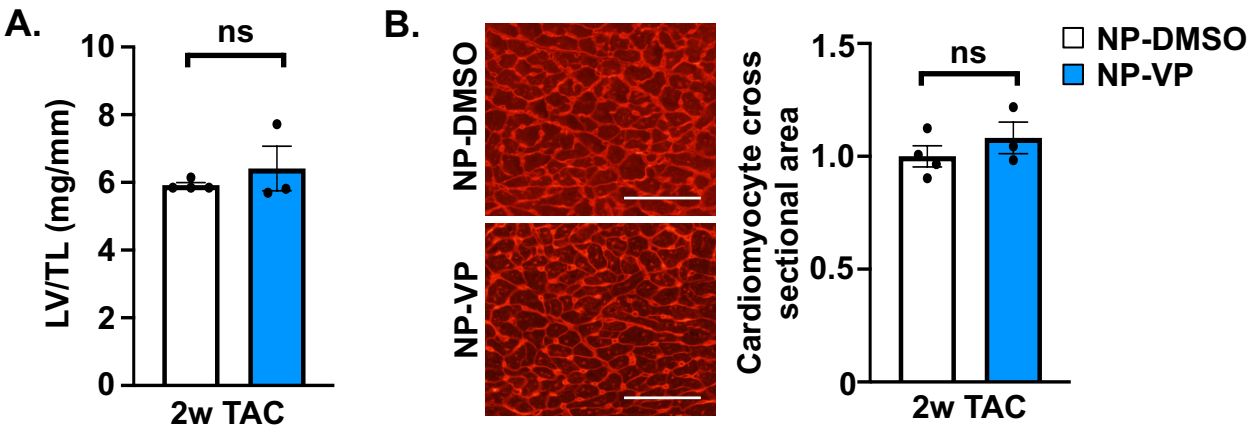
Supplemental Figure 6. Active YAP expression increased pro-inflammatory gene expression. (A-F) RAW264.7 cells were transfected with YAPS127A or GFP. Quantitative PCR determined levels of gene expression. N = 4 experimental replicates. All data are presented as mean ± SEM. *P* values were determined by unpaired, two-tailed Student *t*-test. **P*<0.05. ***P*<0.01. ****P*<0.001.



Supplemental Figure 7. YAP inhibition attenuated pro-inflammatory gene expression and augmented expression of resolution genes. (A-C) RAW264.7 cells were treated with LPS (100 ng/mL) in the presence of verteporfin (1 μM) or DMSO control. Quantitative PCR determined levels of gene expression. (D-E) RAW264.7 cells were treated with IL-4 (20 ng/mL) in the presence of verteporfin (1 μM) or DMSO control. Quantitative PCR determined levels of gene expression. (F-G) Representative western blots with quantitation. N = 3 experimental replicates. All data are presented as mean ± SEM. *P* values were determined by ordinary one-way ANOVA with multiple comparison. **P*<0.05. ***P*<0.01. ****P*<0.001, ns = not significant.



Supplemental Figure 8. Nanoparticle characterization. (A) RAW264.7 cells or primary neonatal rat cardiomyocytes (NRVM) were incubated with Cy5-loaded nanoparticles (NP) for 1 hour. Cells were stained with FITC-conjugated phalloidin (green) and imaged (Cy5-NP, red; DAPI, blue). Scale bar, 50 μm. (B-C) Mice were administered Cy5-NP and sacrificed at indicated time points. Flow cytometry was performed to detect the presence of Cy5-NPs in cells isolated from heart (panel B) and spleen (panel C).



Supplemental Figure 9. Wild type mice were subjected to TAC surgery and administered control DMSO-loaded nanoparticles (NP-DMSO) or verteporfin-loaded nanoparticles (NP-VP) for 2 weeks. (A) Postmortem analysis of LV mass after TAC. (B) Individual cardiomyocyte size was measured using WGA staining after TAC. All data are presented as mean ± SEM. *P* values were determined by unpaired, two-tailed Student *t*-test. ns = not significant.

Figure 5A

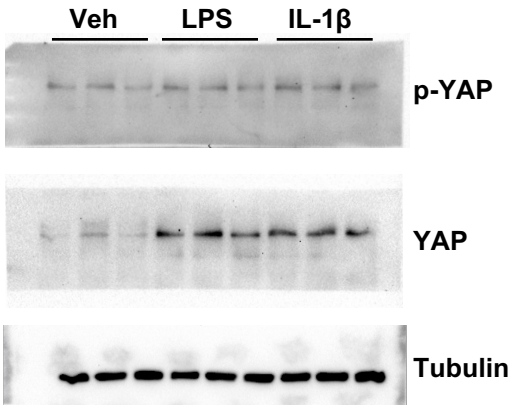
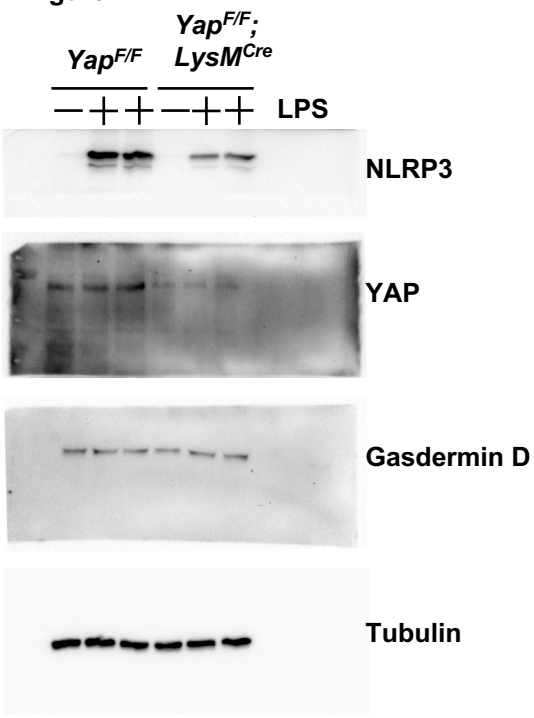
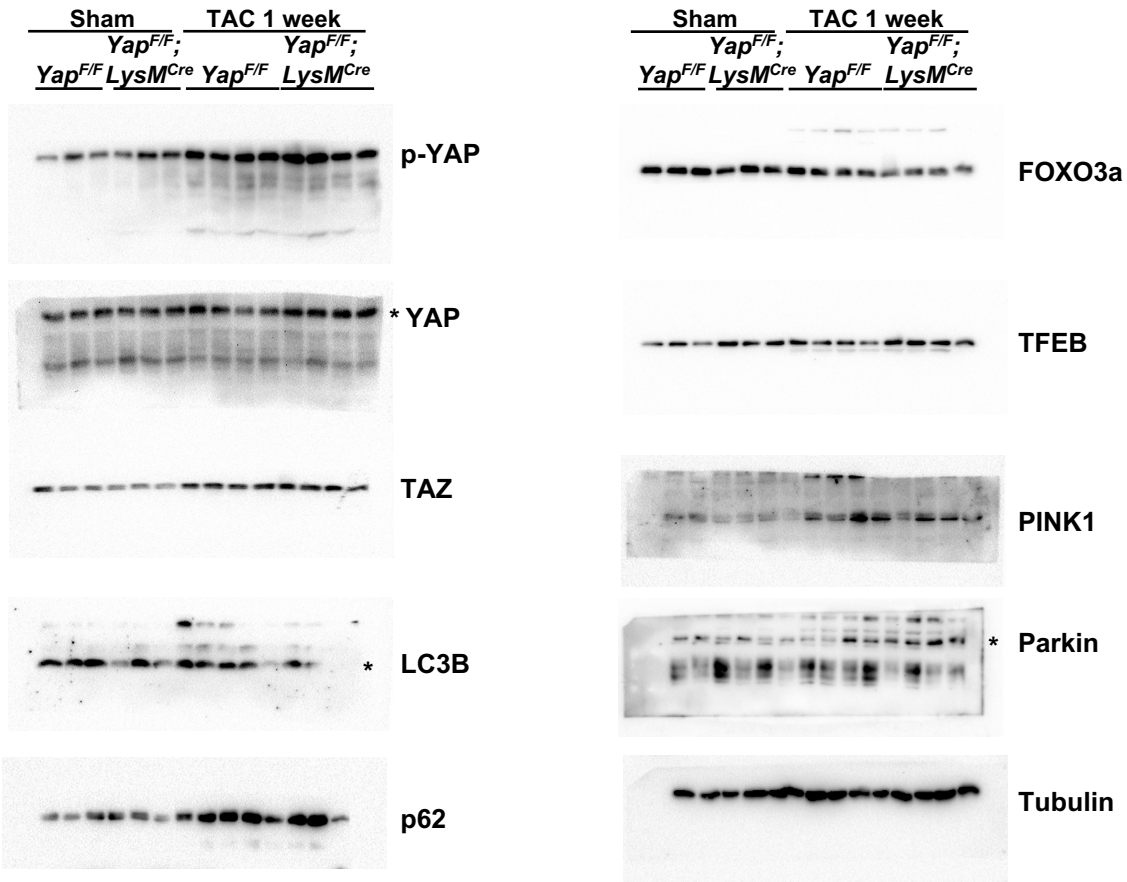


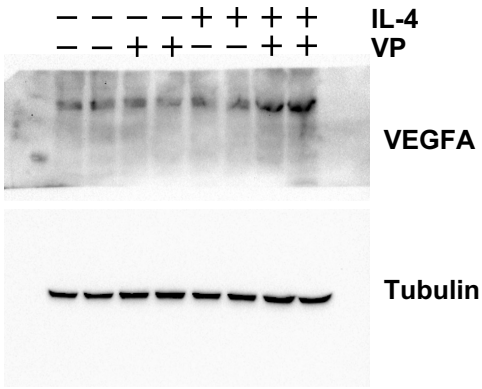
Figure 7B



Supplemental Figure 3A



Supplemental Figure 7F



Supplemental Figure 10. Original uncropped blots.