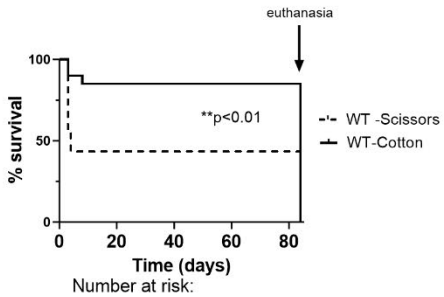
Supplemental Figure 1.

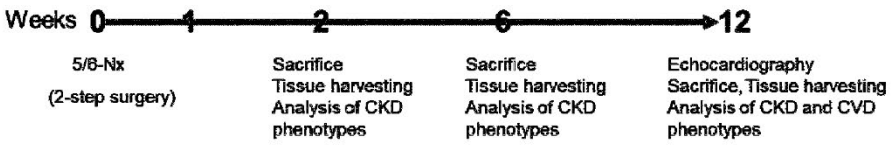
A



Number at risk:

Days	WT -Scissors	WT-Cotton
0.000	23	20
2.000		
3.000	23	20
4.000	13	
8.000		18
9.000		
78.000		
84.000	10	17

B



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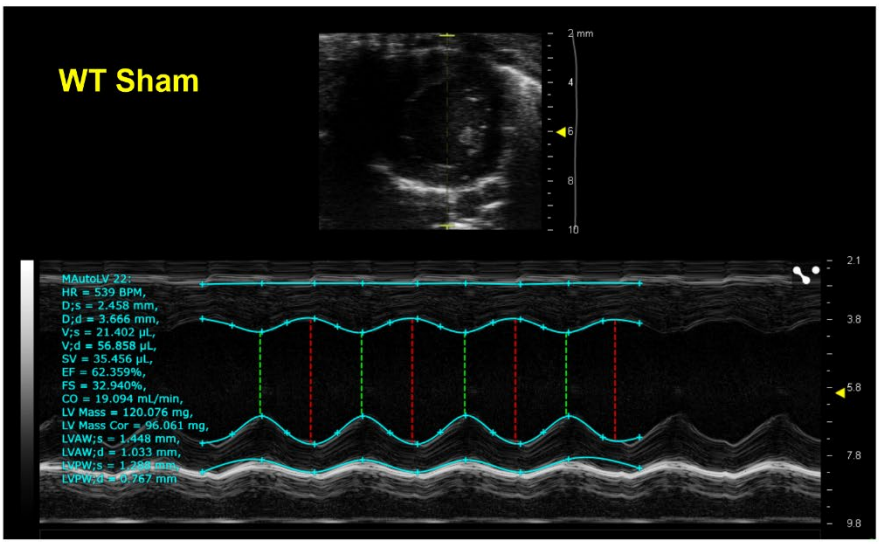

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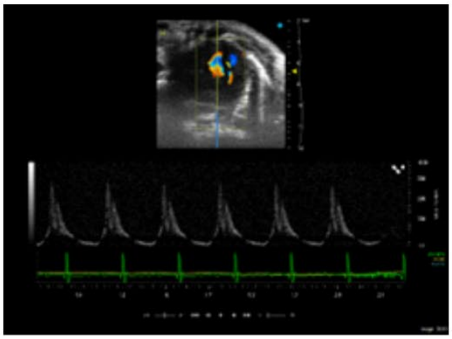
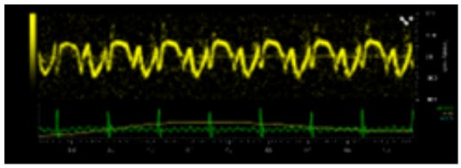
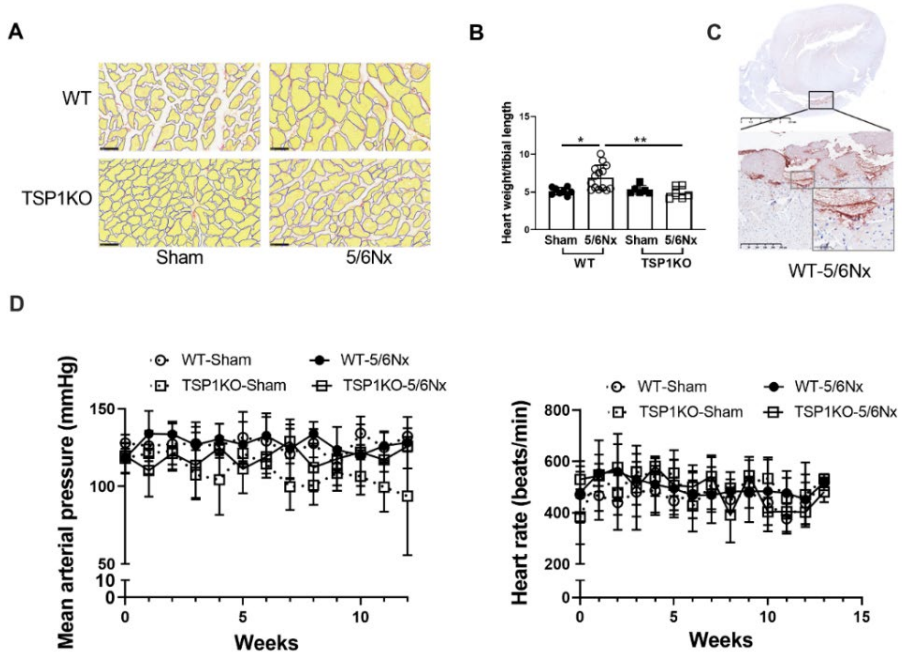


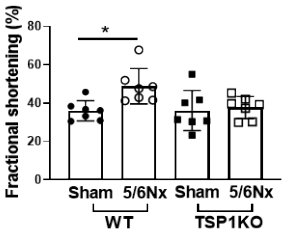
E

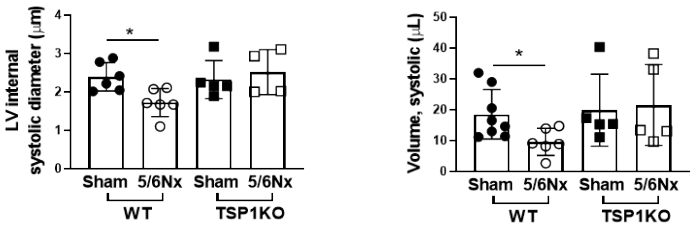


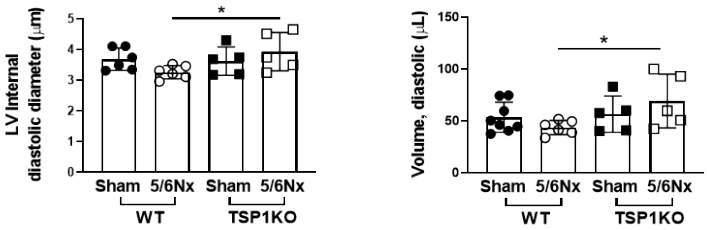
Supplemental Figure 1. Induction of CKD in a 5/6 nephrectomy (5/6Nx) mouse model. (A) Kaplan–Meier survival curves of wild-type (WT, C57BL/6) mice following different 5/6Nx techniques (n = 20-23). (B) Experimental scheme. B) 5/6 nephrectomy (5/6Nx) was performed by 2-step surgery. Evaluation of CKD was performed at 2, 6, and 12 weeks after second-step surgery. Evaluation of LV (dys)function was performed at week 12. (C) Echocardiography measurement in mice. A representative sample of image production and analysis pipeline. (D) Representative color Doppler pulse-wave image (PW) (D) and tissue doppler image (TDI) (E), both images are representing fromTSP1KO-Sham mice. "**$P < 0.01$ by Kaplan-Meier survival calculation" with "**$P < 0.01$ by log-rank test."

Supplemental Figure 2.



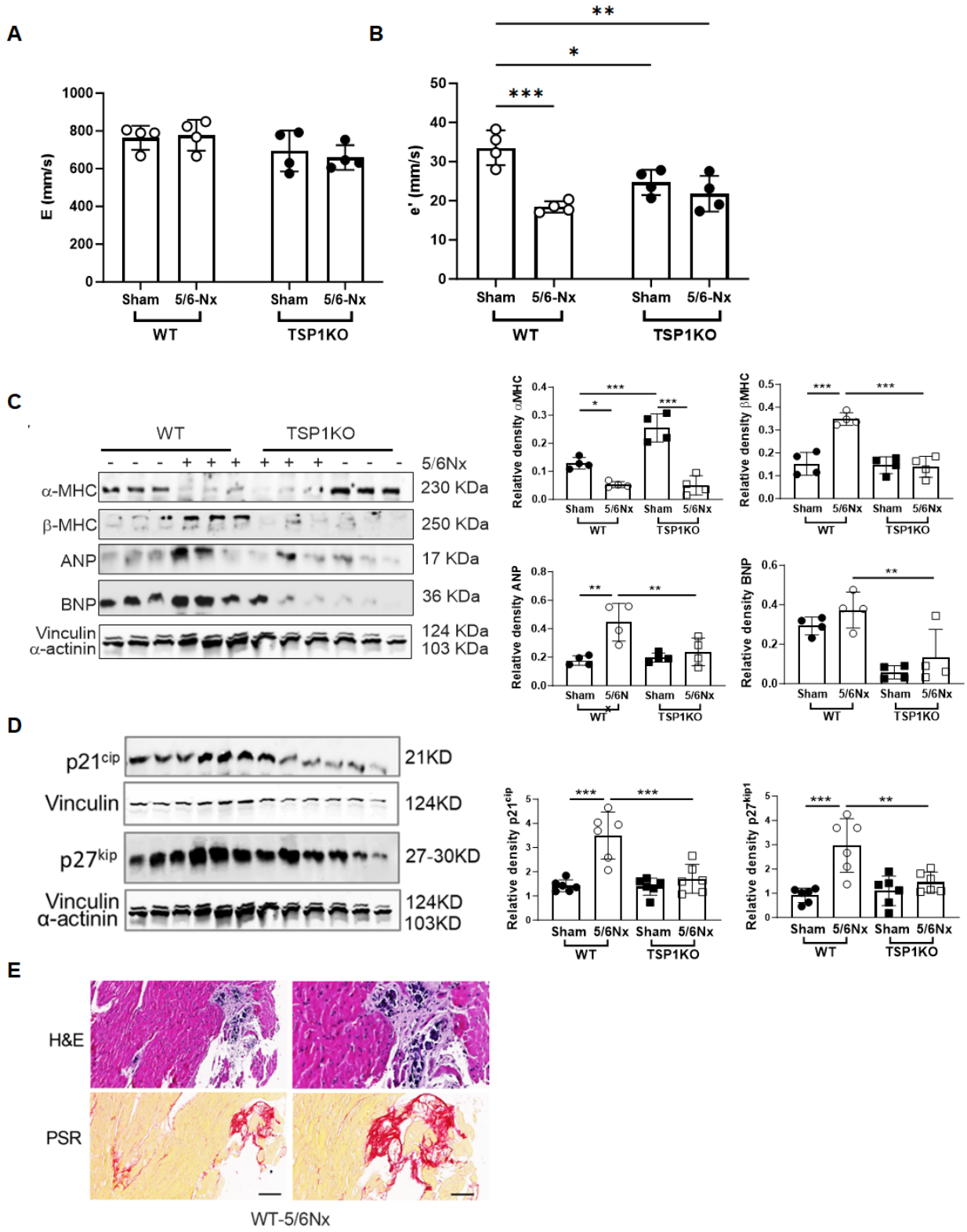






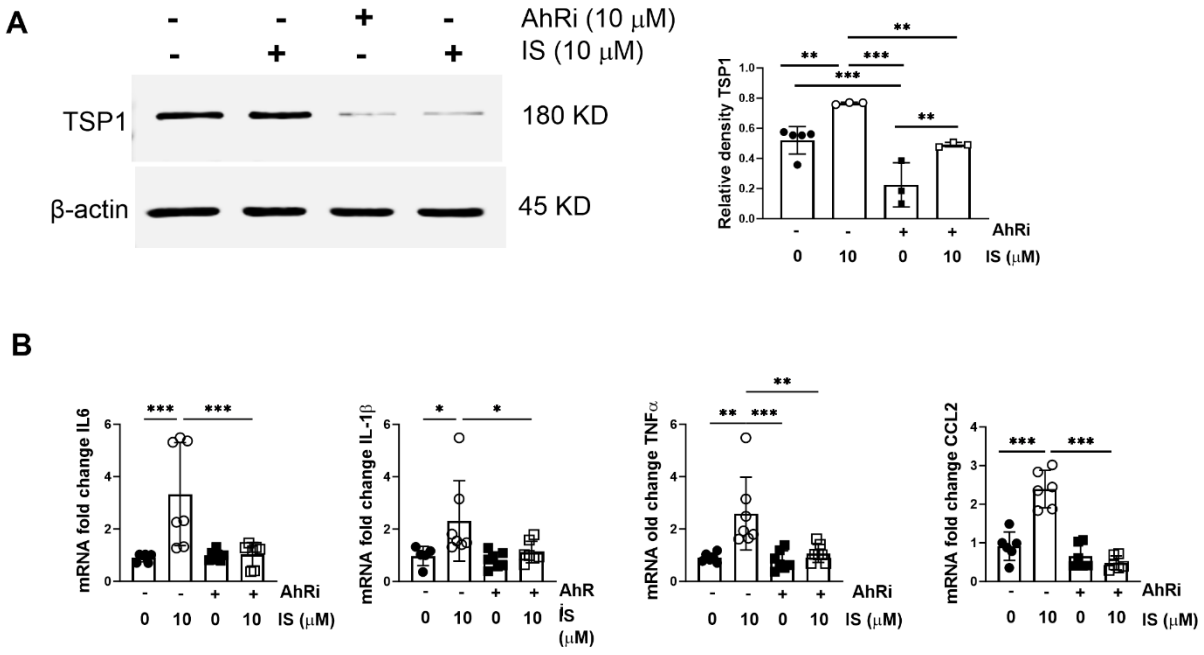
Supplemental Figure 2. 5/6 nephrectomy induced cardiovascular changes in mice. (A) Representative cross-sections of cardiomyocytes stained with picrosirius red (scale bar = 25 μm). (B) LV weight to tibial length (n = 6-14). (C) Perivascular expression of TSP1 in WT-5/6Nx heart (scale bars = 2.5 mm, 250 μm, and 50 μm). (D) Mean arterial pressure and heart rate using tail-cuff plethysmography over 12 weeks in WT or TSP1KO mice following 5/6Nx or sham operation (n = 20-23). M-mode echocardiography measurement of LV. (E) Percentage of fractional shortening (FS%) (n = 6), (F) LV systolic internal diameter (μm) and volume (μL) (n = 4-8), (G) LV diastolic internal diameter (μm) and volume (μL) (n = 5-8). All data are mean ± SD; **P* < 0.05; ***P* < 0.01 by 2-way ANOVA with Tukey's post-hoc test. Abbreviations: LV, Left ventricular; Nx, Nephrectomy; μL, microliter; μm, micrometer.

Supplementary Figure 3.



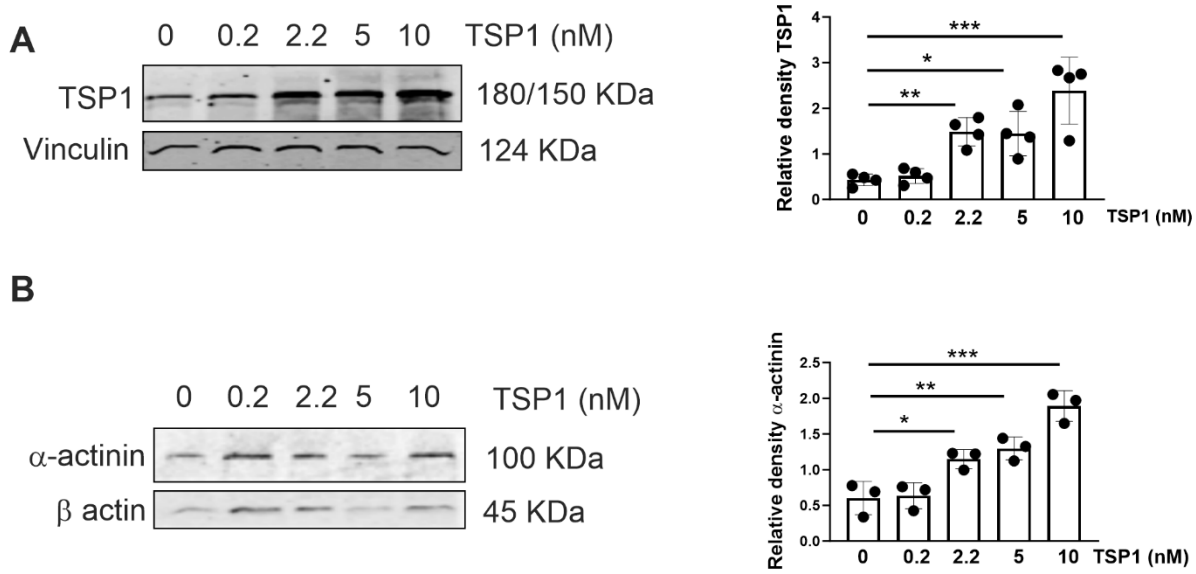
Supplemental Figure 3. CKD in mice promotes left ventricular hypertrophy (LVH). Four apical view echocardiography measurement demonstrating (A) early diastolic transmitral flow velocity and (B) peak early diastolic annular velocity. (C) Mice left ventricle (LV) homogenates were analyzed by Western blotting for expression of α-myosin heavy chain (MHC), β-MHC, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP). (D) Mice left ventricle (LV) homogenates were analyzed by Western blotting for expression of $p21^{cip}$ and $p27^{kip}$. Band density was normalized with vinculin and combined densitometries were shown (n = 4-6). Note that representative internal control vinculin was shown with α-actinin due to the close molecular weight. (E) Representative H&E and picrosirius red staining of left ventricle (LV) demonstrating heterotopic ossification only in WT-5/6Nx hearts (scale bars = 100 μm and 50 μm). Graphs are mean ± SD; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by 2-way ANOVA with Tukey's post-hoc test. Abbreviations: LV, Left ventricular.

Supplemental Figure 4.



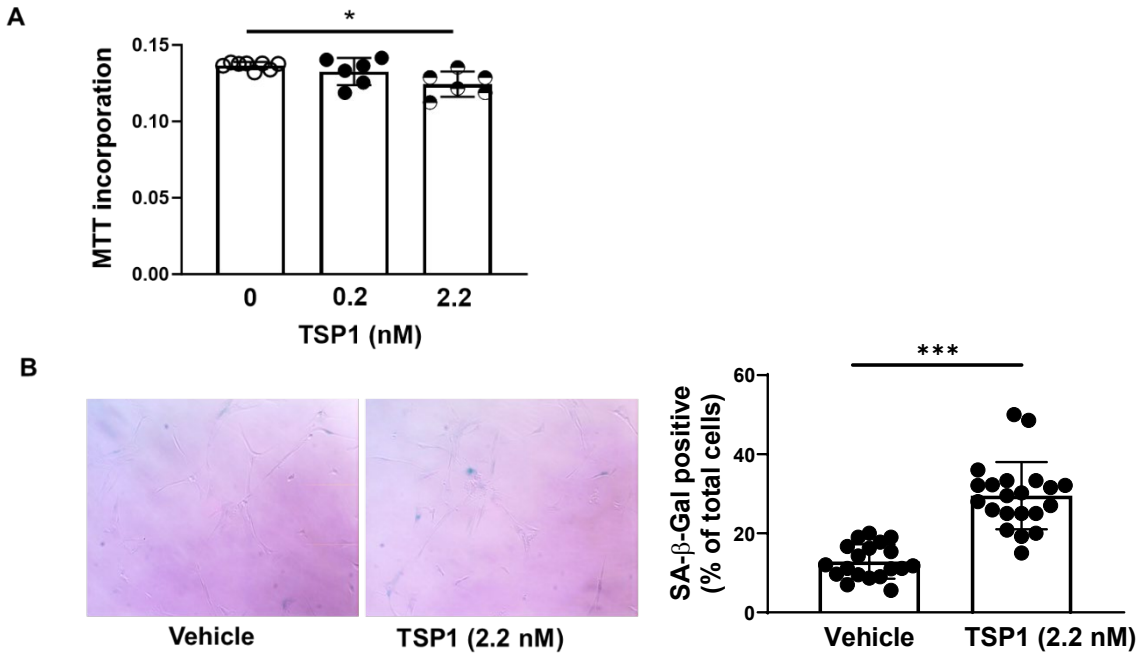
Supplemental Figure 4. Involvement of AhR in induction of TSP1 by indoxyl sulfate (IS). Human vascular smooth muscle cells were pretreated with AhR inhibitor (AhRi) (10 μM) for 2 h and then treated indoxyls sulfate for 24 h. Cell lysates were probed for (A) TSP1 or (B) mRNA expression of IL-6, IL-1, TNF, and CCL2 (n = 3-7, independent experiments, normalized to 18S). All data are mean ± SD. Representative Western blots and combined densitometry relative to β-actin are shown (n = 3). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ by 1-way ANOVA with Tukey's post-hoc test. Abbreviations: AhR, aryl hydrocarbon receptor; TSP1, thrombospondin 1; siRNA, small interfering ribonucleic acid.

Supplemental Figure 5.



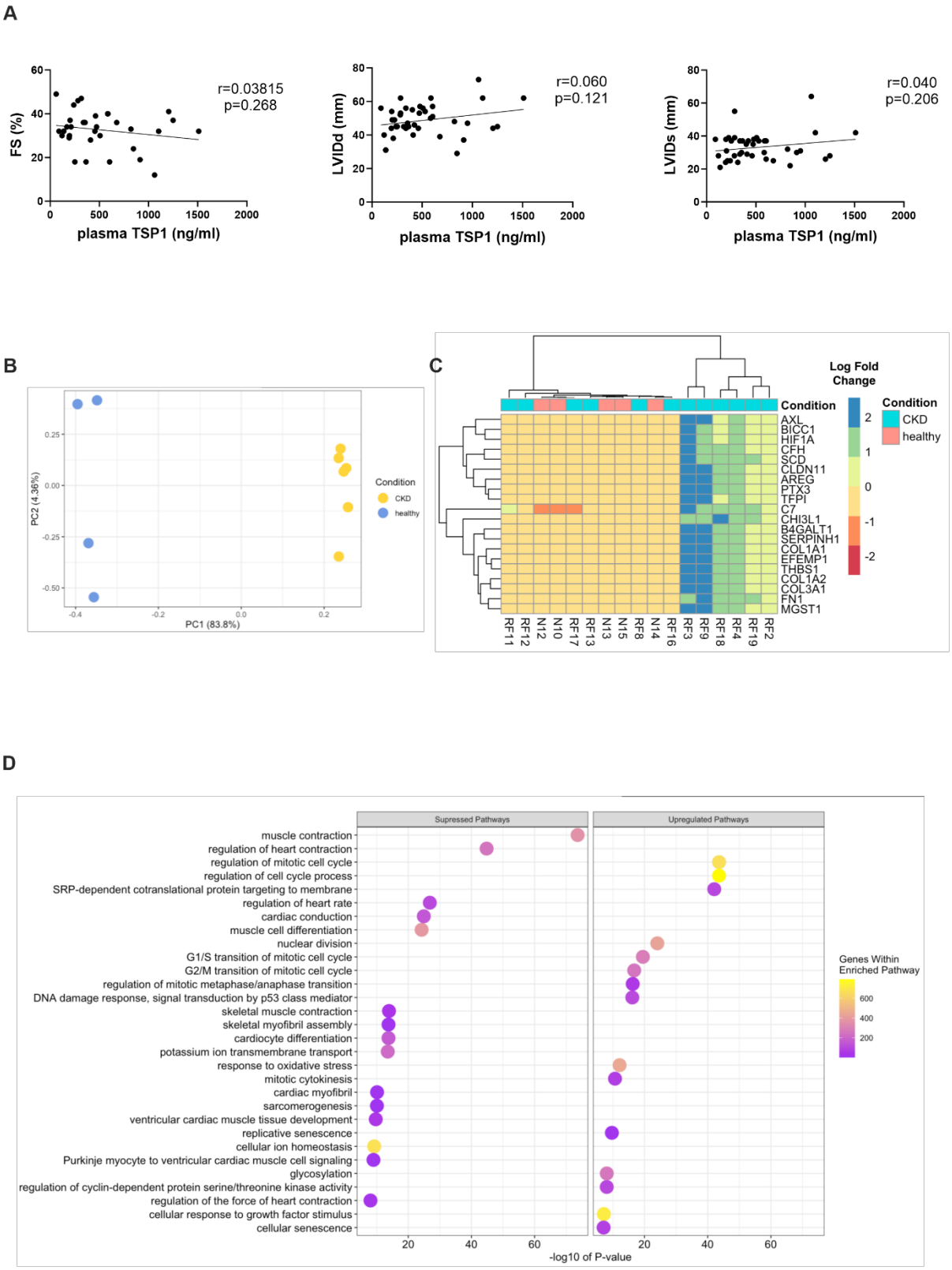
Supplemental Figure 5. TSP1 induces cardiomyocyte hypertrophy. (A) Human cardiomyocytes (HCM) were treated with increasing concentrations of TSP1 and probed for TSP1. (B) HCM were treated with increasing concentrations of TSP1 and probed for α-actinin. Band density was normalized vinculin (A) and β-actin (B). Representative Western blots and combined densitometry are shown (n = 3-4). All data are mean ± SD; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by 1-way ANOVA with Sidak's multiple comparisons test.

Supplemental Figure 6.



Supplementary Figure 6. Thrombospondin 1 (TSP1) reduced proliferation and increased senescence in cardiac fibroblasts. (A) Effects of TSP1 in MTT incorporation in human cardiac fibroblasts following incubation with TSP1 (0, 0.2, and 2.2 nM) (n = 6, independent experiments). (B) SA-β-gal staining following incubation with TSP1 (2.2 nM). Graph is from n = 4-10 observations (total = 41) from randomly chosen fields, from n = 3 independent experiments. Data are mean ± SD; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 by 1-way ANOVA with Sidak's multiple comparisons test (A) or Student's *t*-test (B). Abbreviations: MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide; SA-β-gal, senescence-associated β-galactosidase.

Supplemental Figure 7.



Supplemental Figure 7. Differential gene expression and functional parameters in human hearts with CRS. (A) Linear regression analysis of plasma TSP1 levels and fractional shortening (FS%), LV internal diameter in diastole (LVIDd) or LV internal diameter in systole (LVIDs) in patients with CKD. (B) Principal component analysis and (C) heat map of differentially expressed genes in hearts from a subset of healthy or CRS patients. (D) Gene ontology (GO) analysis of differentially expressed genes in CRS-affected hearts. Abbreviations: TSP1, thrombospondin 1; LV, left ventricular; CKD, chronic kidney disease; CRS, cardiorenal syndrome.

Supplemental Table 1.

Study		WT mice		TSP1KO mice	
Method optimization (12 weeks)		**Sham**	**5/6Nx (Euthanized)**	**Sham**	**5/6Nx (Euthanized)**
	Scissors	**0**	**23 (13)**	**0**	**0**
	Cotton	**0**	**20 (3)**	**0**	**0**
2 weeks	**Cotton**	**6**	**8 (1)**	**6**	**8 (1)**
6 weeks	**Cotton**	**6**	**8 (1)**	**6**	**9 (1)**
12 weeks	**Cotton**	**8**	**10 (1)**	**8**	**12 (2)**
4 apical view imaging (12 weeks)	**Cotton**	**4**	**5 (1)**	**4**	**5 (1)**
Total		**24**	**74 (20)**	**24**	**34 (5)**

Supplemental Table 1. Number of mice. Euthanized mice are shown in parentheses for each experiment.

Supplemental Table 2.

Sample size	N = 44
eGFR (ml/min) (mean ± SD)	24.2 ± 20.9
Stage CKD: 1 2 3 4 5	 2 (4.5%) 3 (6.8%) 12 (27.3%) 9 (20.5%) 18 (including 16 on dialysis) (40.9%)
Age (years) (mean ± SD)	62.1 ± 14.8
Gender (%)	M = 27 (61.4%) F = 17 (38.6%)
Comorbidities [number (%)] HT IHD DM PVD Smoker	 32 (74%) 19 (44%) 28 (65%) 7 (16.3%) 3 (6.9%)
Plasma TSP1 (nmol/L) (mean ± SD)	515 ± 411.8 (range 59.8 – 2300)
Echocardiography [number (%)] EF (%) FS (%) LVMI LVIDs LVIDd	 55.2 ± 12.5 32.7 ± 9.7 119.7 ± 43.5 33.3 ± 9.8 48.1 ± 11.9

Supplemental Table 2. Demographic characteristics and comorbidities of patients with measured plasma TSP1 and echocardiography. Abbreviations: eGFR: estimated glomerular filtration rate, HT: hypertension, IHD: ischemic heart disease, DM: diabetes mellitus, PVD: peripheral vascular disease, EF: ejection fraction, FS: fractional shortening, LVMI: left ventricular mass index.

Supplemental Table 3.

Gene symbol	Gene name	Log(2) Fold change	P value
FN1	Fibronectin 1	4.607012	2.44E-04
COL1A2	Collagen type 1 alpha 2 chain	4.393553	2.90E-04
COL3A1	Collagen type 3 alpha 1 chain	3.854918	1.10E-03
EFEMP1	EGF containing fibulin extracellular matrix protein 1	4.376233	2.77E-04
COL1A1	Collagen type 1 alpha 1 chain	7.032401	3.47E-08
BICC1	BicC family RNA binding protein 1	4.246978	2.94E-04
PTX3	Pentraxin 3	6.932395	1.34E-07
CFH	Complement factor H	3.679006	1.74E-03
HIF1A	Hypoxia inducible factor 1 alpha	3.609801	1.83E-03
CLDN11	Claudin 11	6.651719	1.68E-08
THBS1	Thrombospondin-1 (TSP1)	3.712572	1.49E-03
TFP1	Inhibitor of carbonic anhydrase pseudogene	3.882673	6.63E-04

Supplemental Table 3. DEGs in hearts of healthy versus CKD patients.