Supplemental Data

Hydrogen Sulfide Regulates Cardiac Mitochondrial Biogenesis via the Activation of AMPK

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Supplemental Data

Supplemental Table 1. Patient Characteristics

Group	Number	Age	% Female
Non-Failing	6	53.5±3.8	66% (4/6)
Heart Failure	10	56.0±2.6	40% (4/10)

Supplemental Table 2. Antibody List

Antibody	Concentration	Catalog #	Company
PGC1α	1:5,000	Ab54481	abcam
Fibrillarin	1:5,000	2639 S	Cell Signaling
p-AMPK	1.1,000	2535 S	Cell Signaling
t-AMPK	1:1,000	2532 S	Cell Signaling
Sirt1	1:1,000	2028 S	Cell Signaling
Phosphoserine	1:1,000	Ab1603	abcam
Acetyllysine	1:1,000	9441 S	Cell Signaling
p-LKB1	1:1,000	3482S	Cell Signaling
t-LKB1	1:3,000	3047	Cell Signaling
PP2Aa	1:1,000	KIT 9780T	Cell Signaling
PP2Ab	1:1,000	KIT 9780T	Cell Signaling
PP2Ac	1:1,000	KIT 9780T	Cell Signaling
Opa1	1:1,000	SC30573	SANTACRUZ
Mfn1	1:1,000	SC166644	SANTACRUZ
Mfn2	1:1,000	SC 50331	SANTACRUZ
Fis1	1:5,000	SC 98900	SANTACRUZ
p-eNOS	1:1,000	PAS-14957	THERMO
t-eNOS	1:1,000	32027 S	Cell Signaling
Biotin	1:10,000	5597 S	Cell Signaling

Supplemental Figure Legends

Supplemental Figure 1. (A) Levels of free H_2S and (B) sulfane sulfur from the hearts of Wild-Type, cystathionase-γ-lyase transgenic (CSE Tg^+), and CSE deficient (CSE KO) mice. (A) Levels of free H2S and (B) sulfane sulfur from the hearts of mice administered standard diet (Chow) or diet supplemented with SG-1002 (SG-1002; 20mg/kg/day) for 4 weeks. Values are means ±SEM. Number in bars represent sample sizes. *p<0.05 and ***p<0.001 vs. Wild-type of Chow.

Supplemental Figure 2. (A) Representative immunoblots and analysis of (B) Opa1, (C) Mfn1, (D) Mfn2, and (E) Fis1 from the hearts of Wild-Type, cystathionase-γ-lyase transgenic (CSE Tg^+), and CSE deficient (CSE KO) mice. (A) Representative immunoblots and analysis of (B) Opa1, (C) Mfn1, (D) Mfn2, and (E) Fis1 from the hearts of mice administered standard diet (Chow) or diet supplemented with SG-1002 (SG-1002; 20mg/kg/day) for 4 weeks. Values are means ±SEM.

Supplemental Figure 3. (A-B) Representative immunoblots and analysis of phosphorylated eNOS and total eNOS. Cardiac levels of (C) nitrite and (D) nitrosothiols (RXNO). (E) Ratio of mitochondrial DNA to nuclear DNA, and (F) Citrate synthase activity in samples collected from hearts of Wild-type (WT) and eNOS deficient (eNOS KO) mice administered standard diet (Chow) or diet supplemented with SG-1002 (SG; 20mg/kg/day) for 4 weeks. Values are means ±SEM. *p<0.05, **p<0.01, and ***p<0.001 vs. WT Chow.

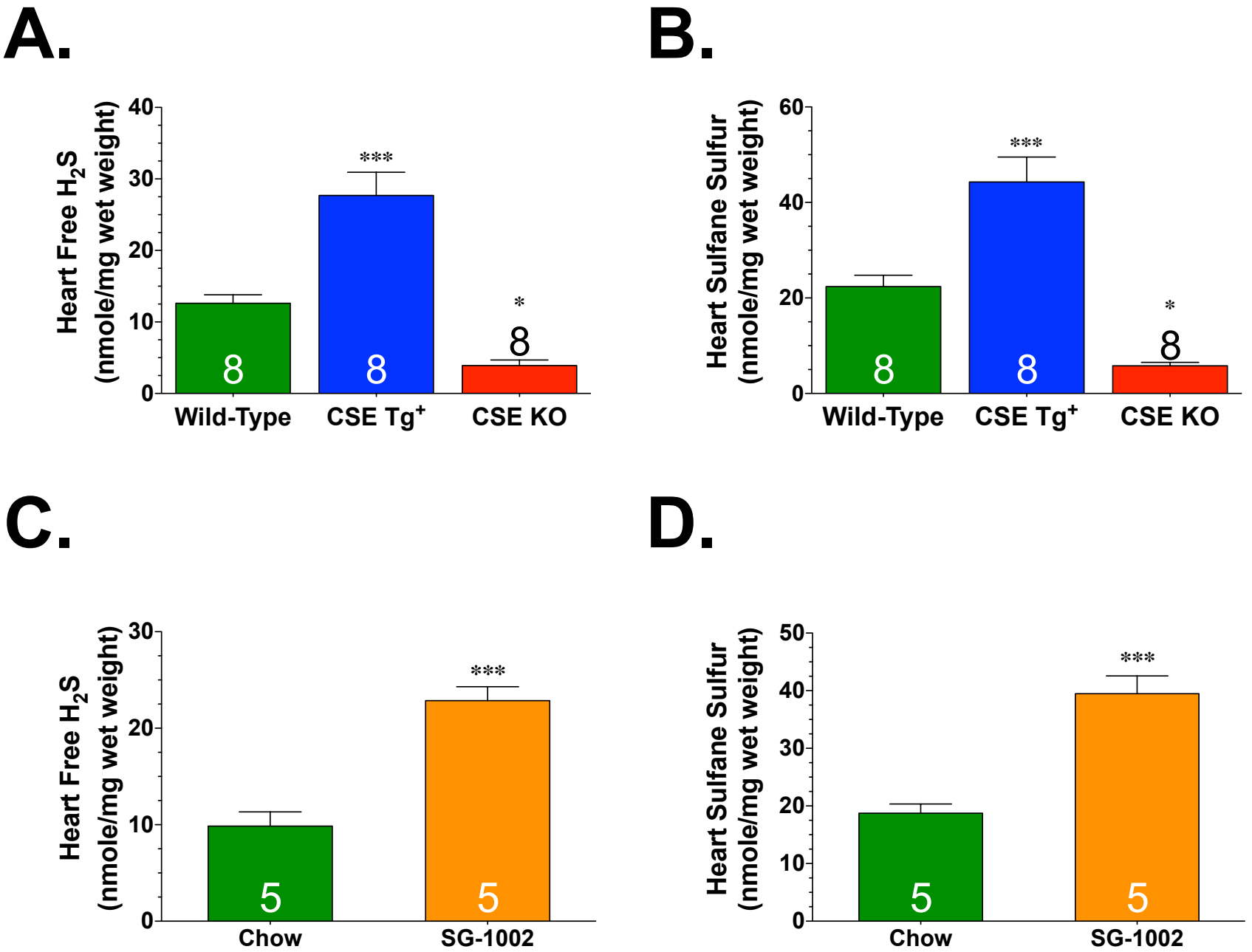
Supplemental Figure 4. (A) Representative immunoblots and (B) analysis from the modified biotin switch assay examining the global protein sulfhydration status from the hearts of mice administered standard diet (Chow) or diet supplemented with SG-1002 (SG-1002; 20mg/kg/day) for 4 weeks. (C) Representative immunoblots and (D) analysis from the tag switch method examining the sulfhydration status PP2Aa. (E) Representative immunoblots and (F) analysis from the tag switch method examining the sulfhydration status PP2Ab. (G) Representative immunoblots and (H) analysis from the tag switch method examining the sulfhydration status PP2Ac. Values are means ±SEM. *p<0.05 vs. WT Chow.

Supplemental Figure 5. (A) PP2A activity. (B) Representative immunoblots and (C) analysis of phosphorylated AMPK and total AMPK in H9c2 cells treated with 100 μM Na_2S. Cells were collected 10-60 minutes after treatment. (D) PP2A activity. (E) Representative immunoblots and (F) analysis of phosphorylated AMPK and total AMPK in H9c2 cells treated with 100 μM Na_2S for 3 consecutive days. Values are means ±SEM of three independent experiments of three biological replicates. *p<0.05, **p<0.01, and ***p<0.001 vs. Vehicle.

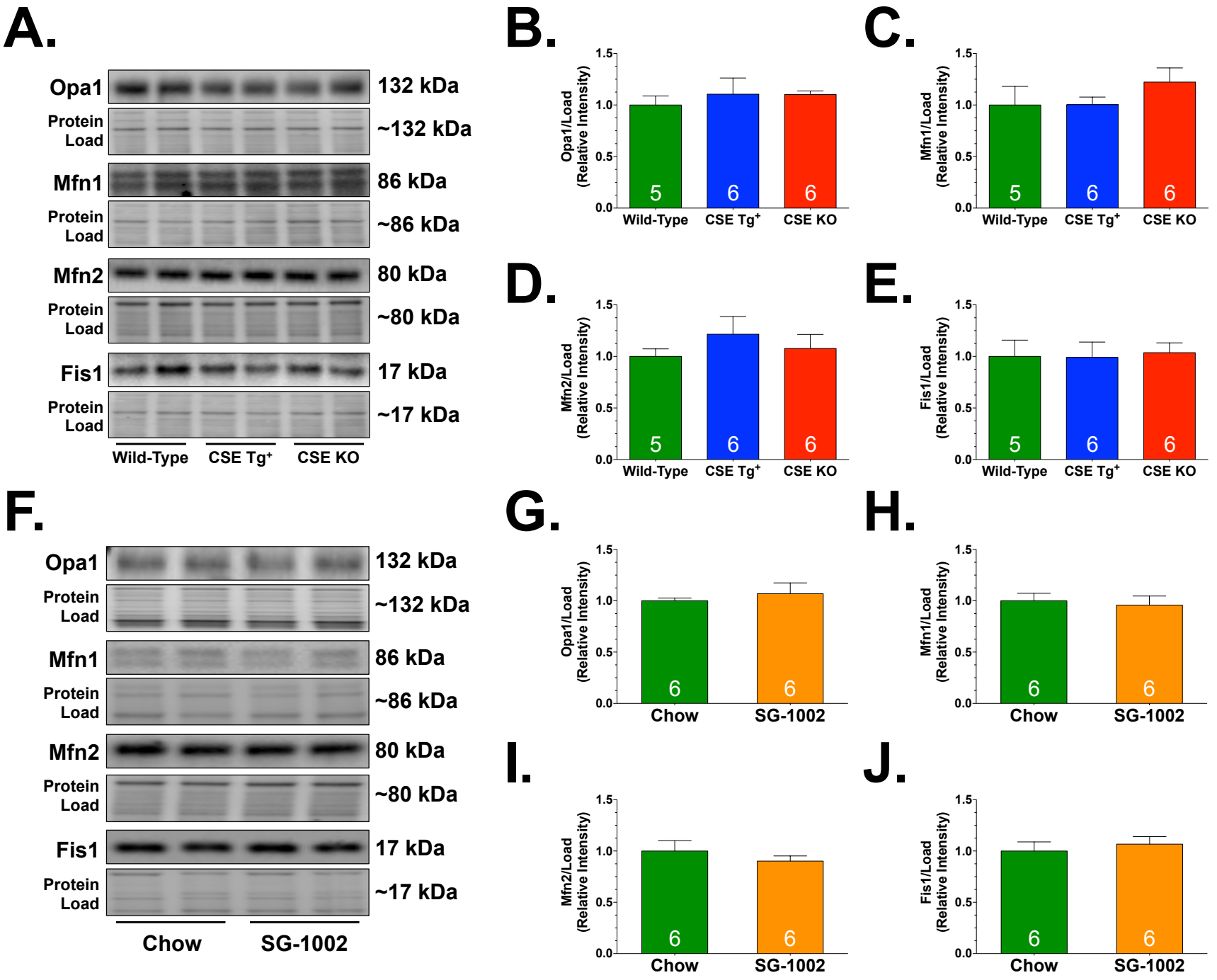
Supplemental Figure 6. (A) Representative immunoblots and (B) analysis from the modified biotin switch assay examining the global protein sulfhydration status from the hearts of mice subjected to 60 minute of myocardial ischemia and 2 weeks of reperfusion. Samples were collected from Sham operated mice and mice administered either a standard diet (I/R+Chow) or a diet supplemented with

SG-1002 (I/R+SG) starting 24 hours after reperfusion. (C) Representative immunoblots and (D) analysis from the modified biotin switch assay examining the sulfhydration status of PP2A subunits in hearts collected from Sham, I/R+Chow and I/R+SG mice. (E) PP2A activity in hearts collected from the experimental groups. (F) Representative immunoblots and (G) analysis of phosphorylated AMPK and total AMPK in hearts collected from the experimental groups. (H) Relative gene expression of tfam, atp5b, and cox1. Values are means ±SEM. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ vs. Sham.

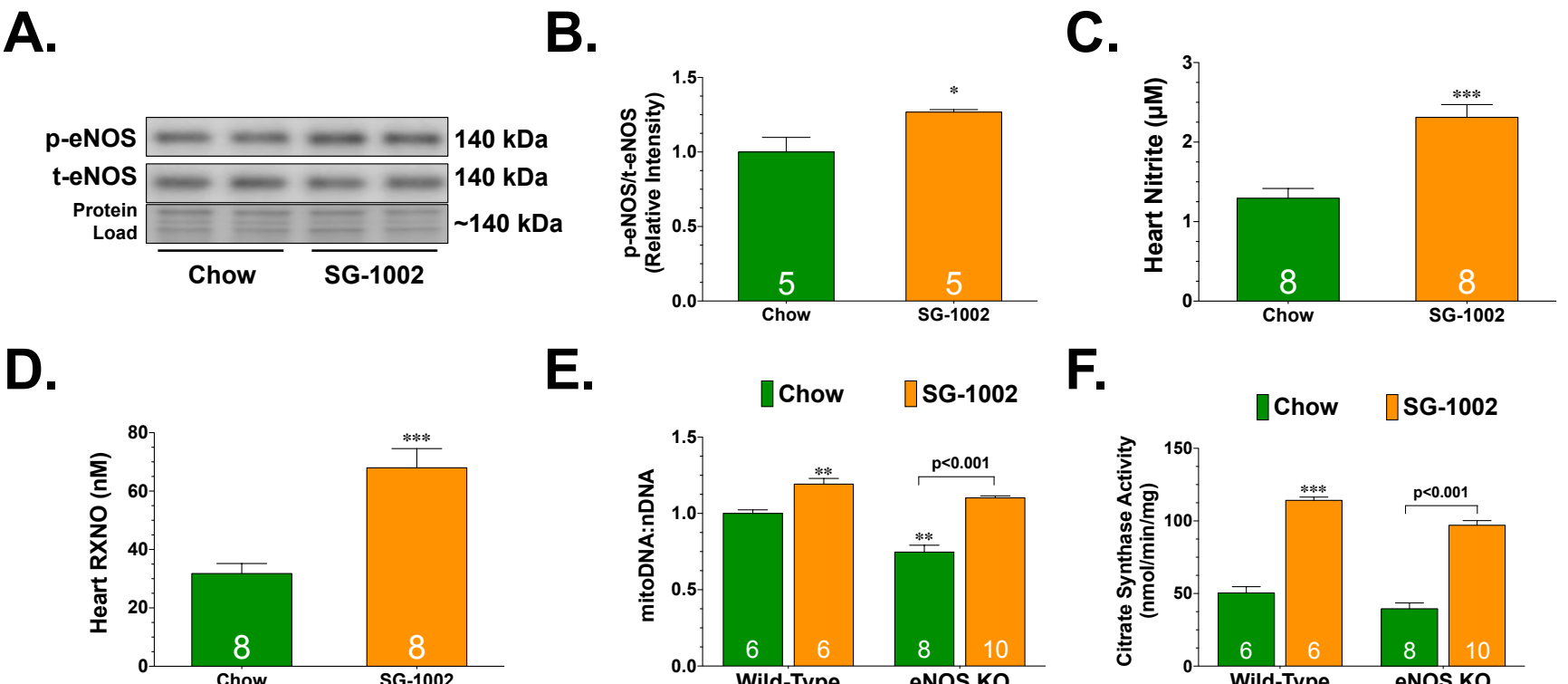
Supplemental Figure 1



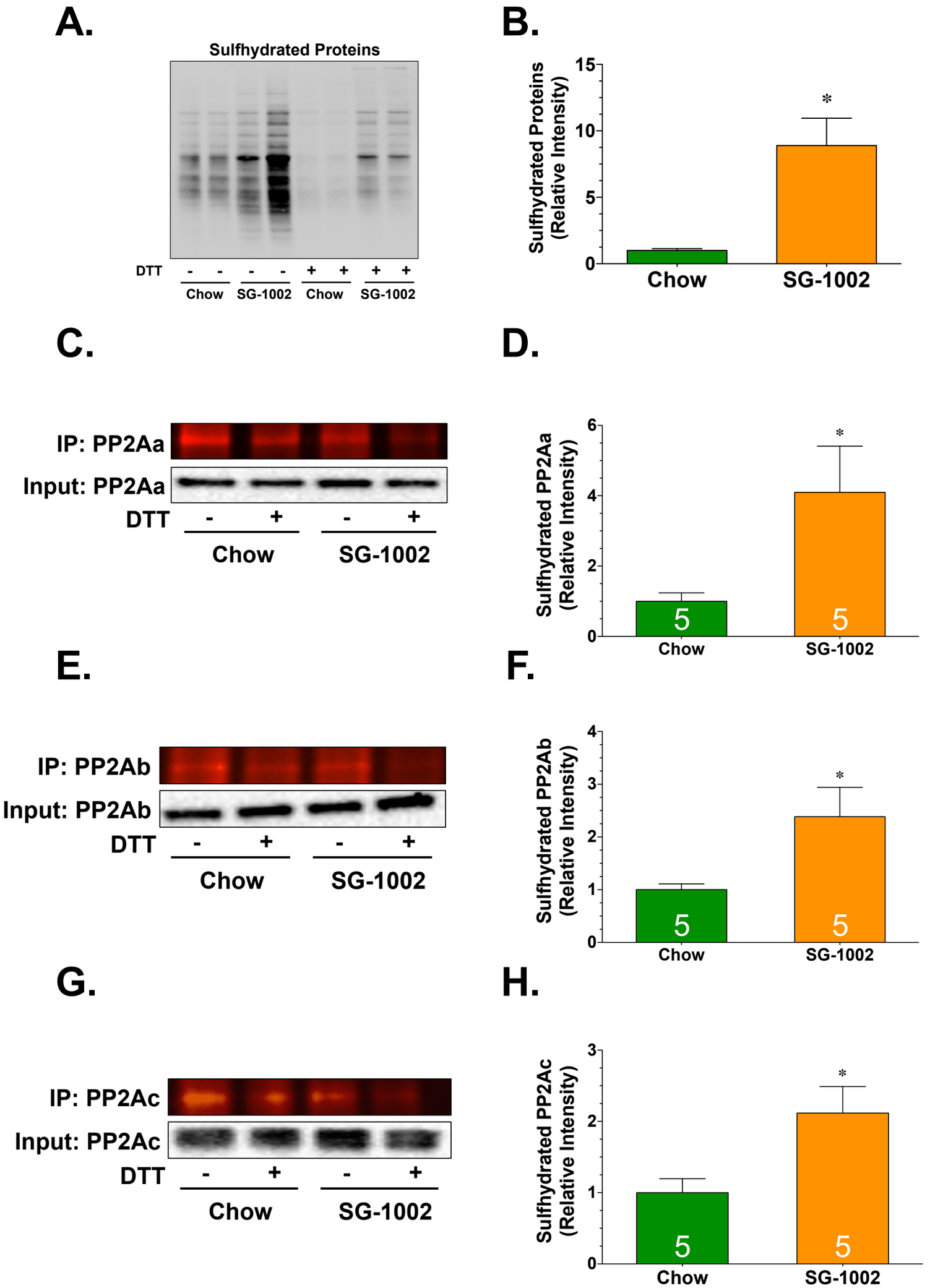
Supplemental Figure 2



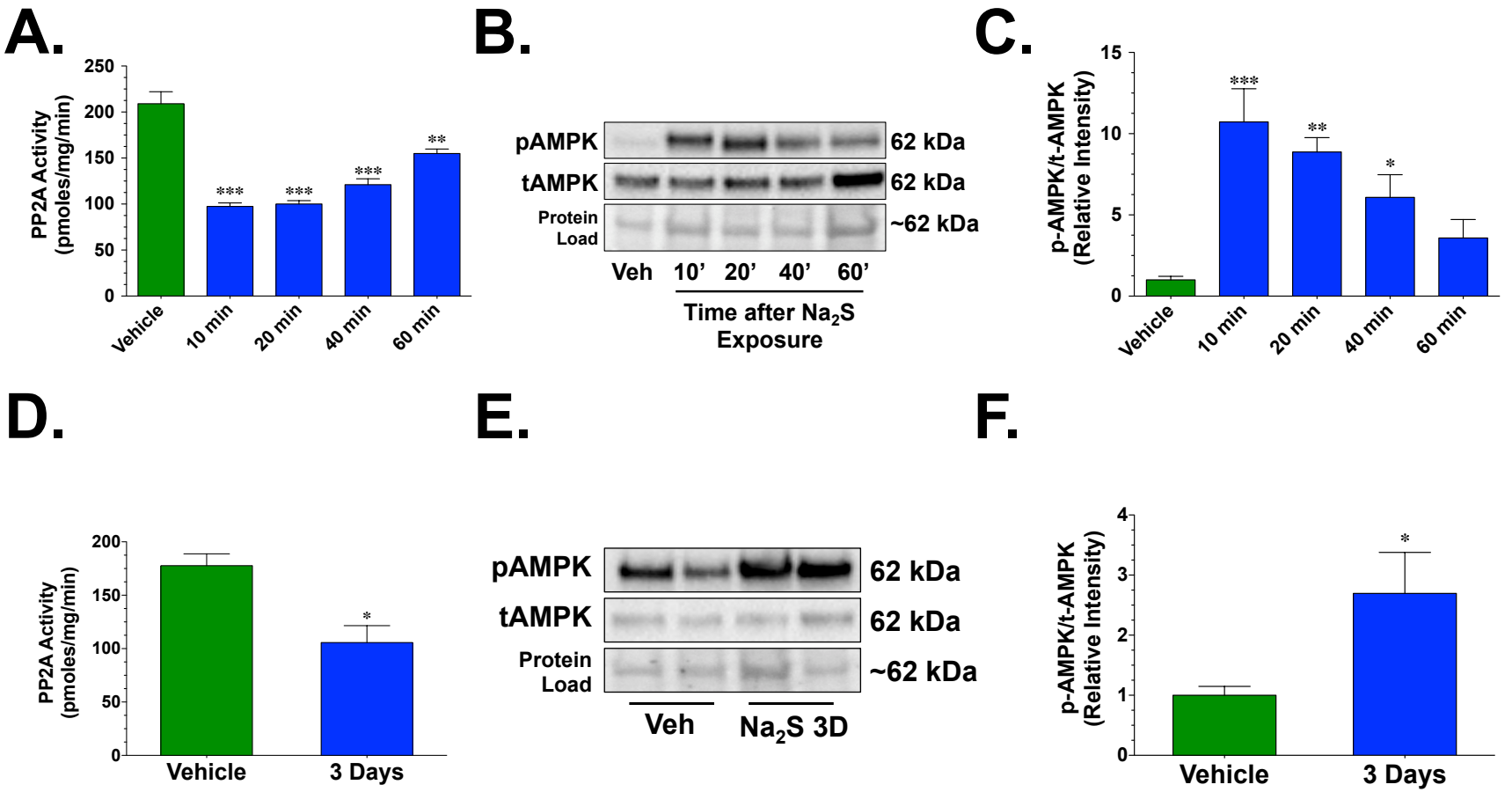
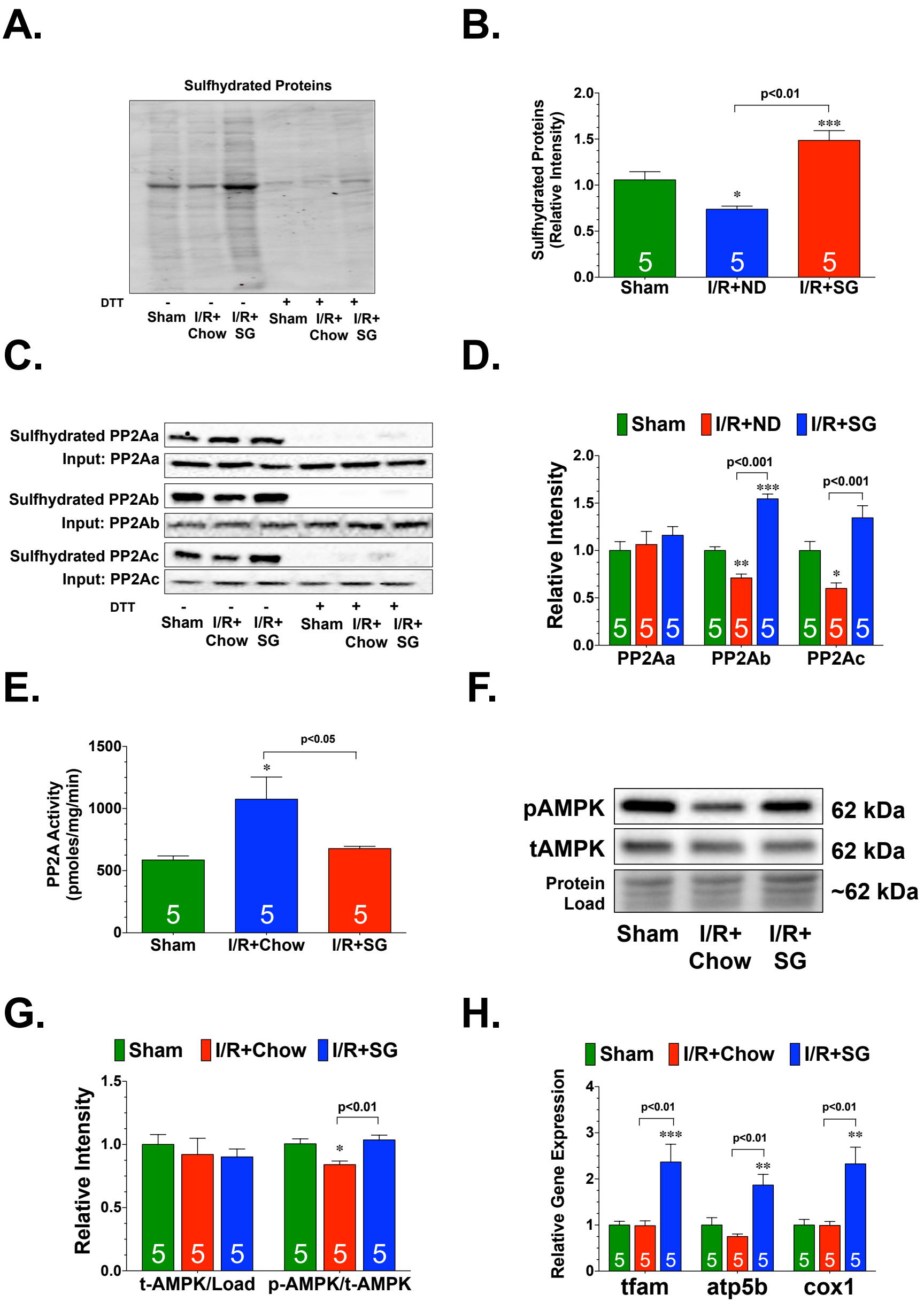
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6