Supplemental Methods

Cardiac biochemical tissue measurements

Four different lysis buffer solutions were used for different biochemical assays and western blotting including (1) Lysis Buffer A (15mM Tris-HCl, 150 nmol/L NaCl, 1% Triton-X-100, 5mmol/L EDTA, 1mM $CaCl_2$, 1mM $MgCl–H_2O$, and 100ug/ml of Protease Inhibitor Cocktail [Sigma-Aldrich P8340; Saint Louis, MO]); (2) Lysis Buffer B (phosphate buffered saline containing 0.01% Triton X-100, 0.1% SDS, and 1mM Protease Inhibitor Cocktail); (3) Lysis Buffer C (phosphate buffered saline containing 0.1% Triton-X-100); & (4) Lysis Buffer D (phosphate buffered saline containing 2% SDS, 10mmol/L Tris-HCL, 10% [vol/vol] glycerol, and 1mM Protease Inhibitor Cocktail). Tissue lysates were centrifuged at 10,000g for 10 minutes at $4^{O}C$ and the supernatant was used for analysis. Protein concentration was determined using the Bradford method, and tissue lysates were stored at $-80^{O}C$. Cardiac NO substrate/production and nitrotyrosine were measured in LV tissue samples in Lysis Buffer A, and LV H_2O_2 was measured following homogenization in Lysis Buffer C.

Western Blotting. LV tissue samples resuspended in laemmli sample buffer (Bio-Rad Laboratories, Inc.; Hercules, CA) were separated by electrophoresis and transferred to nitrocellulose membranes (1h, 50V) that were subsequently blocked overnight at $4^{O}C$ (5%-nonfat dry milk in Tris-buffered saline and 0.1%-Tween-20). The membranes were then probed for proteins of interest (*see Supplemental Table 1*) and stained with ECL detection reagents (GE Healthcare; Pittsburgh PA) for development. All western blots were digitized and analyzed using Image J software. Protein abundance is expressed as relative units normalized to β-actin (1:1000-1:5000; Santa Cruz Biotechnology; Santa Cruz, CA) and a control sample loaded into each gel. No differences were observed between groups while conducting control analysis of β-actin ($p>0.05$), and exposure times fell within the linear portion of β-actin standard curves (band intensity vs. time).

Supplemental Figure Legends

Supplemental Figure 1

Representative color images of periodic acid schiff stained glomeruli with each grade of sclerosis including grade 0 (normal) (A), grade 1 (≦ 25% sclerotic area; minimal sclerosis) (B), grade 2 (25-50% sclerotic area; moderate sclerosis) (C), grade 3 (50-75% sclerotic area; moderate-to-severe sclerosis) (D), and grade 4 (≧75% sclerosis; severe sclerosis) (E). Images were taken at 200X magnification; ——— 100μm.

Supplemental Figure 2

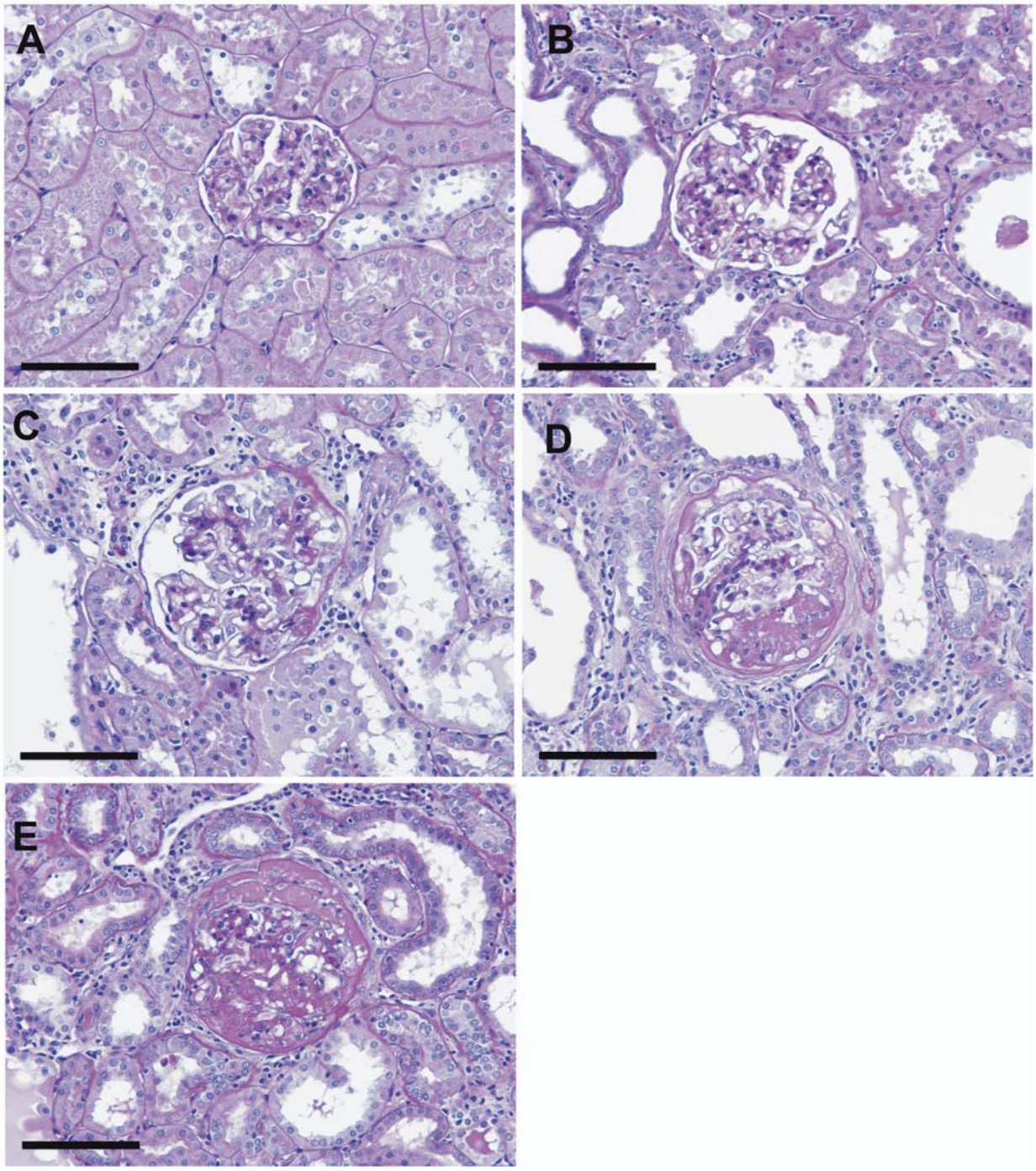
Heart rate **(A),** stroke volume **(B),** rate pressure product (HR X SP; **C**)**,** and stroke work (SP X SV; **D**) in sham (n = 8) and AI (n = 7) animals able to withstand the 15 min ischemic bout (i.e. achieve aortic overflow post-ischemia) during the course of the ischemia-reperfusion perturbation at 5, 10, and 15 min pre-ischemia as well as 20, 25, and 30 min post-ischemia. Heart rate and rate pressure product were not significantly reduced in AI relative to sham post-ischemia at any of the time points. In contrast, stroke volume and stroke work were impaired post-ischemia in AI. AI, 5/6 ablation-infarction animals, *$p<0.05$ vs. sham; $^{\#}p<0.05$ vs. 15 minute pre-ischemia time-point

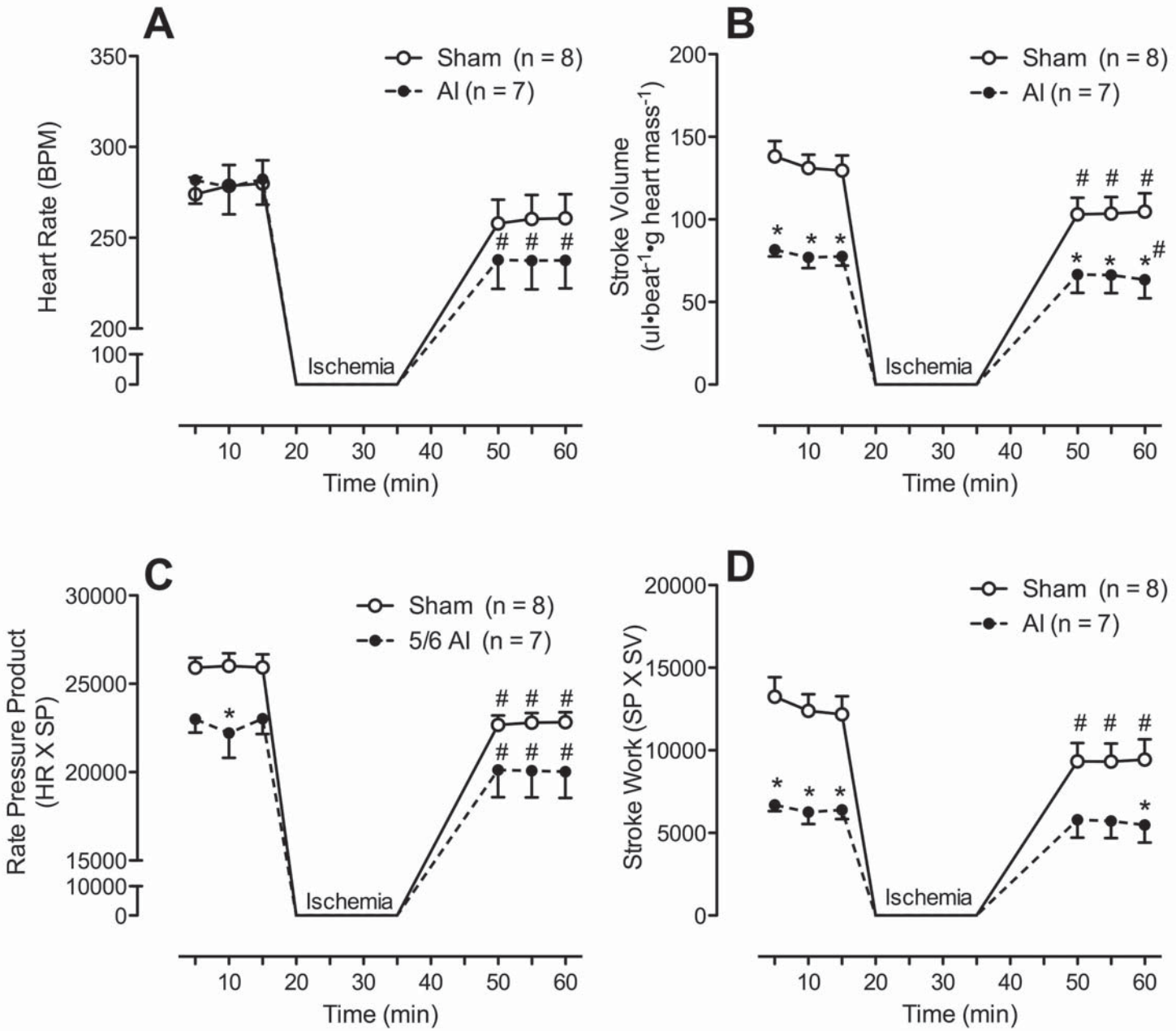
Tables

Table S1. Western blotting conditions and antibodies used.

Protein	Lysis Buffer	% Acrylamide gel	Electrophoresis conditions	Amt of Protein Loaded (μg)	Primary antibody/dilution	Secondary antibody/dilution
eNOS	B	10	100 V, 75 min	20	BD Pharmingen, 1:200	Rabbit anti-mouse (Abcam 6728); 1:2000
Nox-4	D	10	100 V, 60 min	50	Santa Cruz, 1:500	Donkey anti-goat (Santa Cruz Sc-2020); 1:2000
Nox-2	D	10	100 V, 60 min	50	Santa Cruz, 1:500	Rabbit anti-mouse (Abcam 6728); 1:2000
SOD-1	A	15	100 V, 60 min	10	Santa Cruz, 1:500	Rabbit anti-mouse (Abcam 6728); 1:2000
SOD-2	A	15	100 V, 60 min	10	Santa Cruz, 1:500	Rabbit anti-mouse (Abcam 6728); 1:2000
GPx-1/2	A	15	100 V, 60 min	10	Santa Cruz, 1:500	Rabbit anti-mouse (Abcam 6728); 1:2000
Catalase	A	15	100 V, 60 min	10	Santa Cruz, 1:1500	Rabbit anti-mouse (Abcam 6728); 1:2000

eNOS, endothelial nitric oxide synthase; NADPH-oxidase isoform-4, Nox-4; NADPH-oxidase isoform-2, Nox-2; superoxide dismutase-1, SOD-1, superoxide dismutase-2, SOD-2; glutathione peroxidase homologs 1/2, GPx-1/2


A
B
C
D
E


A
Sham (n = 8)
AI (n = 7)
Heart Rate (BPM)
350
300
250
200
100
0
Ischemia
10
20
30
40
50
60
Time (min)
B
Sham (n = 8)
AI (n = 7)
Stroke Volume
(ul•beat-1•g heart mass-1)
200
150
100
50
0
Ischemia
Time (min)
C
Sham (n = 8)
5/6 AI (n = 7)
Rate Pressure Product
(HR X SP)
30000
25000
20000
15000
10000
0
Ischemia
Time (min)
D
Sham (n = 8)
AI (n = 7)
Stroke Work (SP X SV)
20000
15000
10000
5000
0
Ischemia
Time (min)