

Supplemental Information for “Inhibition of NADPH oxidase 2 (NOX2) prevents sepsis-induced cardiomyopathy by improving calcium handling and mitochondrial function ”

Table 1: Echocardiographic data from NOX2 inhibitor experiment

	LVIDd (mm)		LVIDs (mm)		FS, %	
	mean	SEM	mean	SEM	mean	SEM
control	2.77	0.13	1.80	0.19	35.10	4.54
LPS	3.08	0.28	2.62*	0.21	14.67*	2.10
LPS+apo	2.86	0.28	2.00	0.22	30.52	4.45
apo	2.97	0.08	1.93	0.18	35.06	4.17

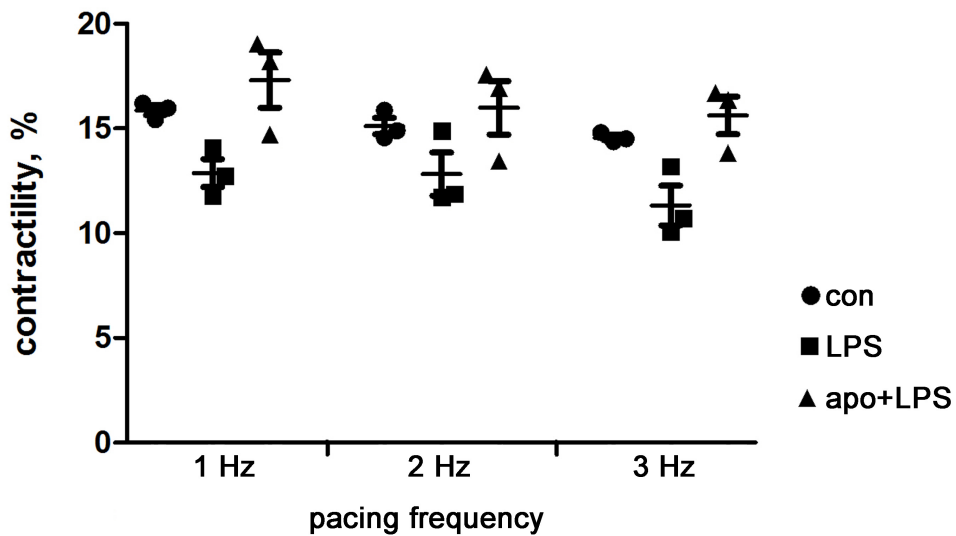
Table 2: Echocardiographic data from PKC inhibitor experiment

	LVIDd (mm)		LVIDs (mm)		FS, %	
	mean	SEM	mean	SEM	mean	SEM
control	3.30	0.06	1.98	0.16	39.89	5.00
LPS	3.30	0.05	2.71*	0.14	17.87*	3.26
LPS+PKC inhib	3.38	0.20	2.31	0.35	32.71	7.34

LVID = left ventricular internal diameter, diastolic and systolic, FS = fractional shortening, LVM = left ventricular mass, * indicates significantly different from control by post-hoc test.

Supplemental figures

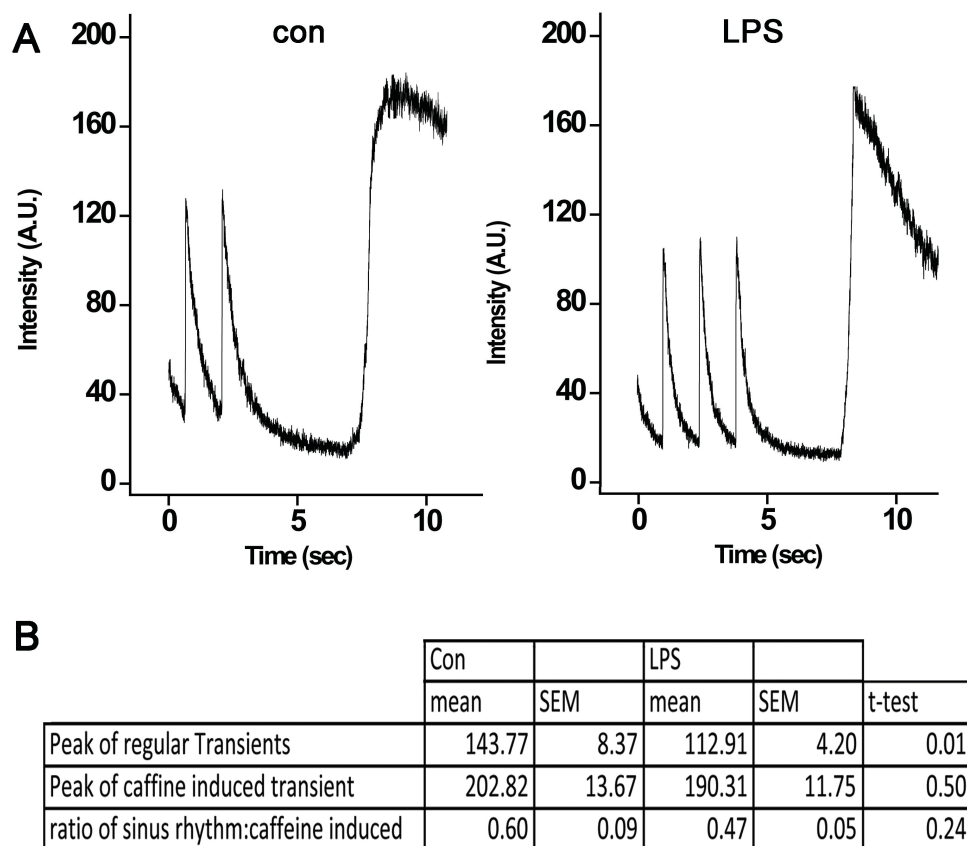
Supplemental figure 1:



Supplemental figure 1, Apocynin preserves contractility at multiple pacing frequencies:

Graph of isolated WT cardiomyocyte contractility paced at 1, 2, and 3 Hz, percent sarcomere shortening from baseline sarcomere length, for cardiomyocytes treated with LPS and/or apocynin, mean + SEM, n= 3 cardiomyocyte isolations from different hearts. At each frequency, the means are different by ANOVA.

Supplemental figure 2:

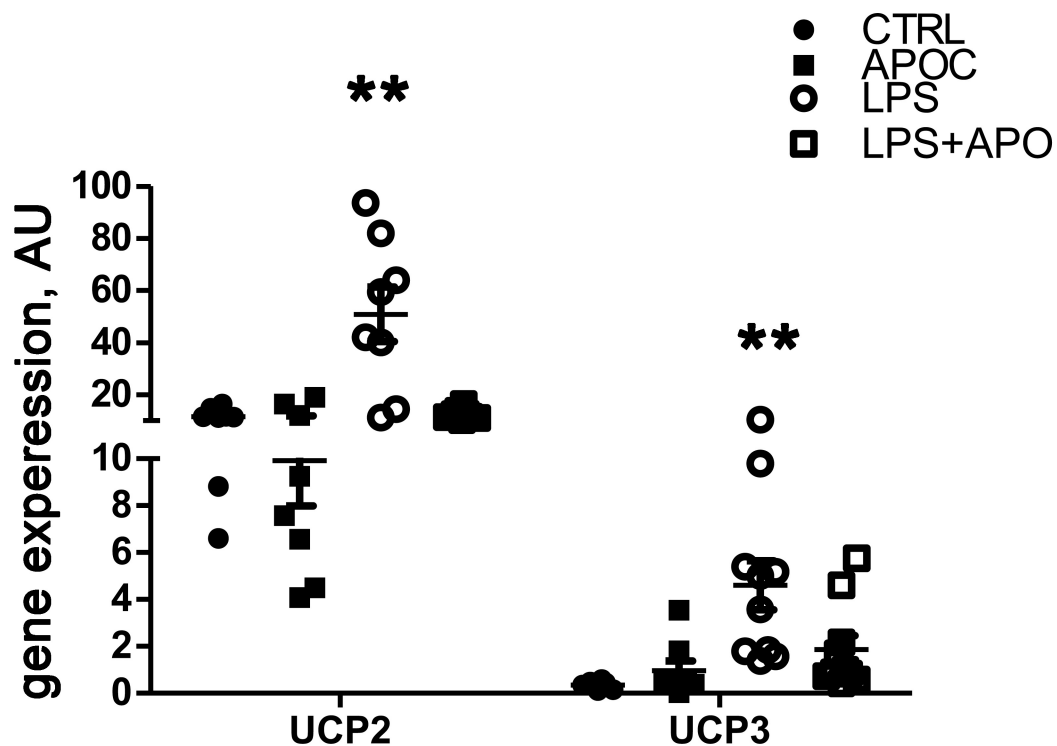


Supplemental figure 2: Sarcoplasmic reticulum calcium load

A. Examples of raw data from experiments using isolated WT cardiomyocytes with caffeine to determine SR calcium load.

B. Table quantifying results of caffeine experiments, n=6 each group from 2 separate cardiac isolations

Supplemental figure 3:

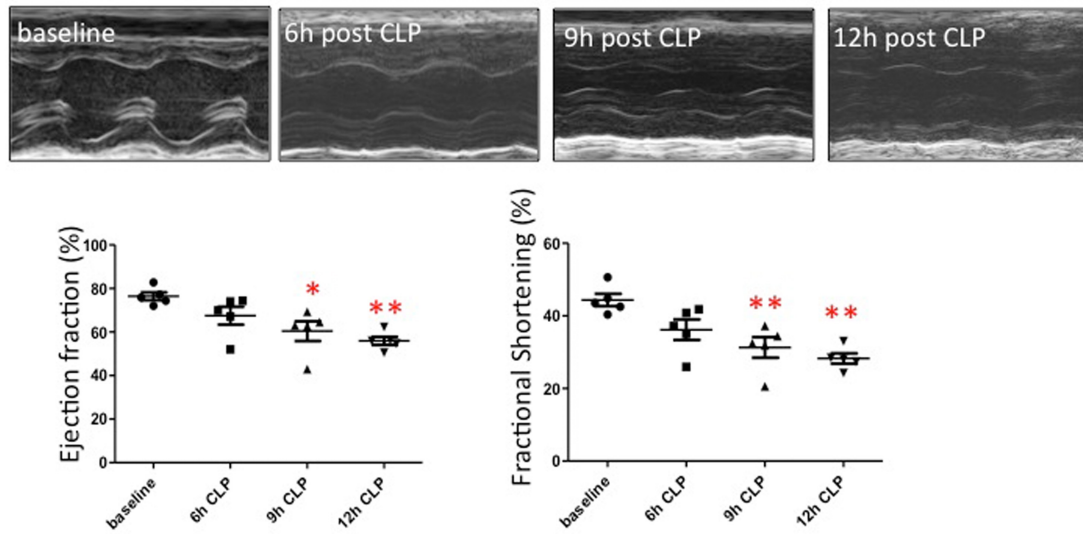


Supplemental figure 3: NOX inhibition prevents upregulation of UCP in the heart

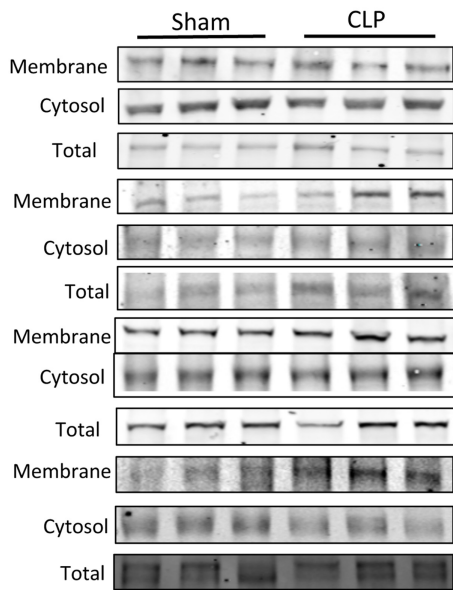
Graph of cardiac Ucp2 and Ucp3 mRNA expression. For each gene, the means are different by ANOVA, ** indicates $p < 0.01$ significant difference from control by post-hoc test.

Supplemental figure 4:

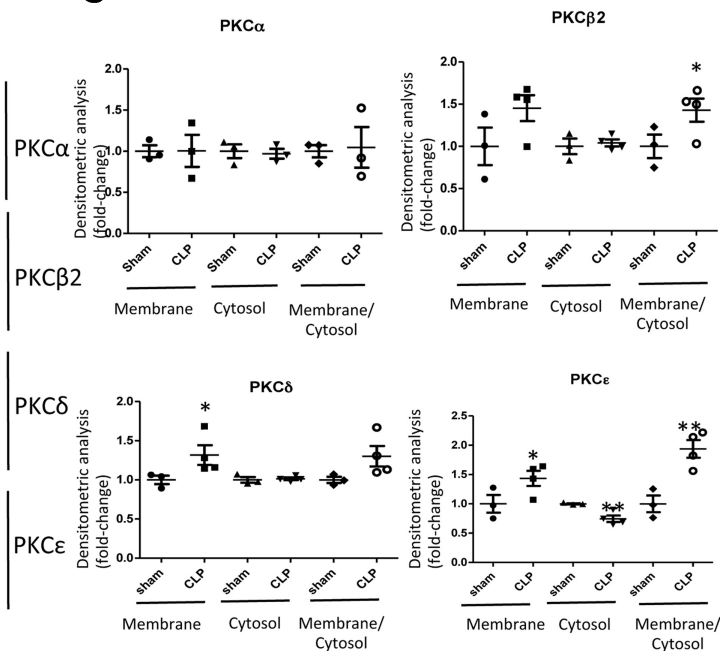
A



B



C



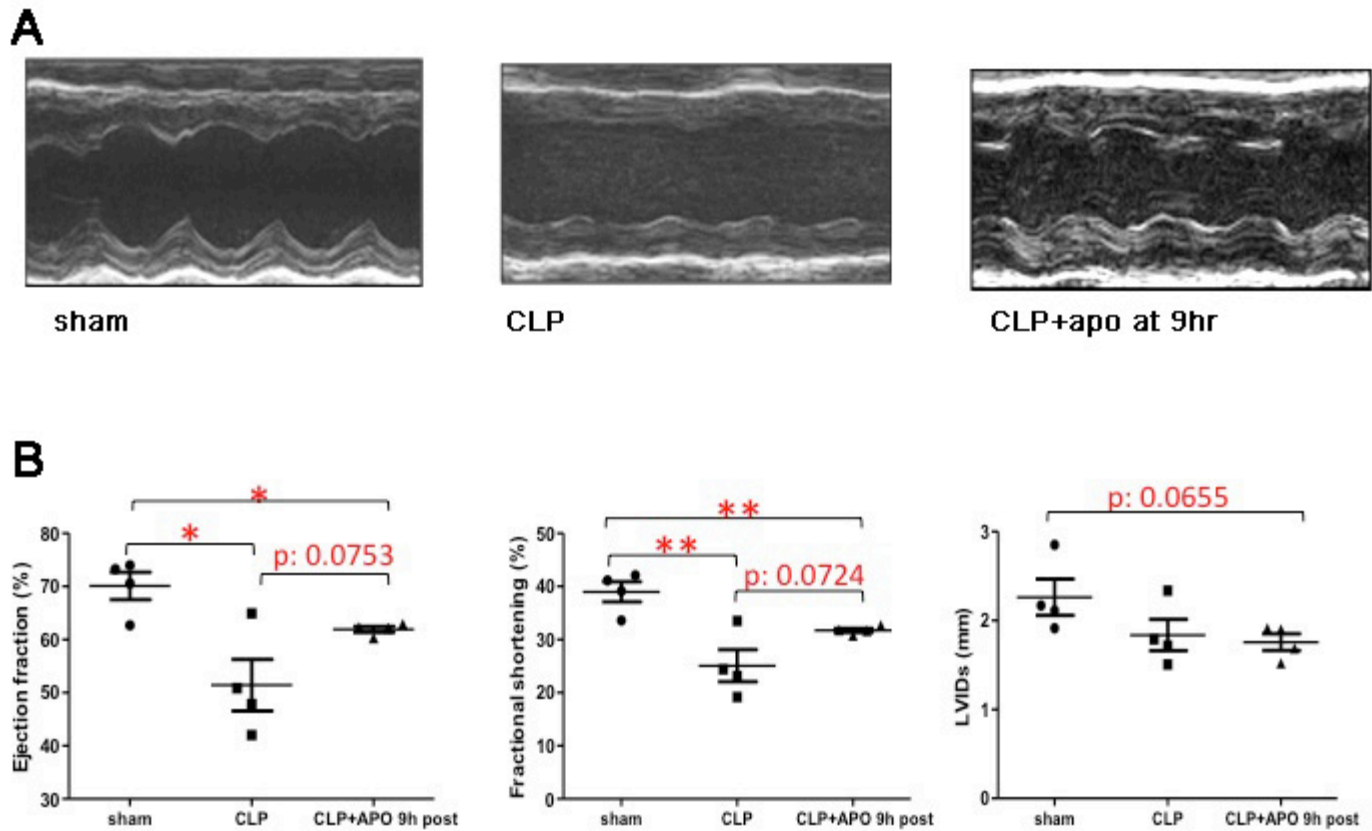
Supplemental figure 4: PKC isoforms are activated in the heart after cecal ligation and puncture (CLP)

A. Time-course of echocardiograms after CLP surgery. Cardiac dysfunction is statistically significant at 9hrs post CLP. N=5 mice, one way ANOVA analysis *p<0.05 vs baseline. **p<0.01 vs baseline. Data are presented as mean with SEM

B. PKC isoforms western blots, membrane and cytosolic fraction, from ventricular tissue of C57BL/6 mice undergoing sham surgery or CLP.

C. Densitometry analysis of PKC western blots, in relative units. Unpaired t-test *p<0.05, **p<0.01

Supplemental figure 5:

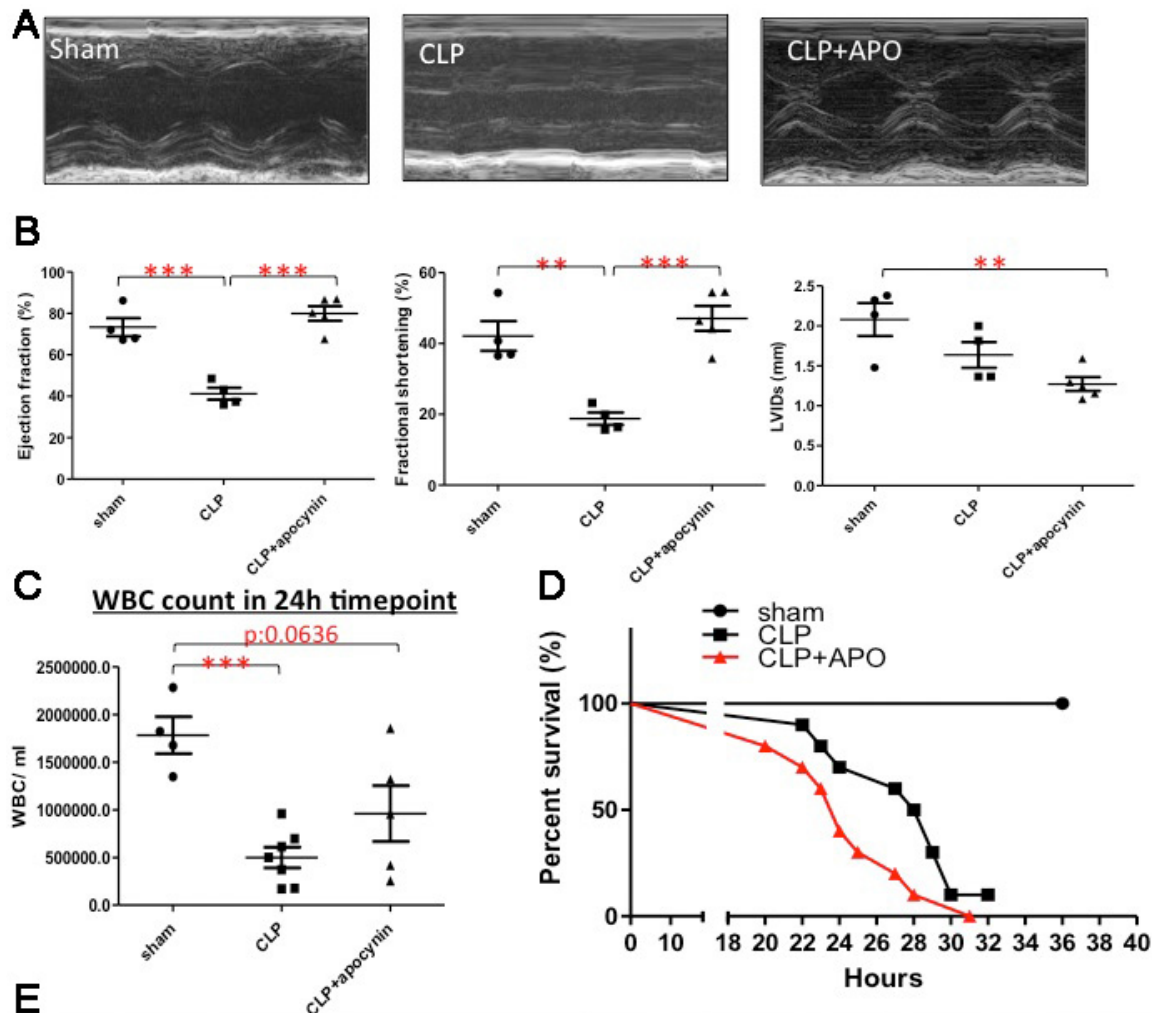


Supplemental figure 5: Apocynin given after the onset of cardiac dysfunction caused by cecal ligation and puncture (CLP)

A. Representative echocardiograms after sham, CLP surgery, or CLP with apocynin given 9 hours after CLP surgery.

B. Graphs of echo parameters from experiments involving WT mice in the following groups: sham, CLP surgery, or CLP with apocynin given 9 hours after CLP surgery. N= 4 mice/group; for EF%; *p<0.05, **p<0.01.

Supplemental figure 6:



Supplemental figure 6: Apocynin effects 24 hours after cecal ligation and puncture (CLP)

A. Representative echocardiograms. Sham: n=4, CLP: n=4, CLP+apocynin: n=5

B. Graphs of echo parameters. The means are different by ANOVA; **p:<0.01 ***p:<0.001

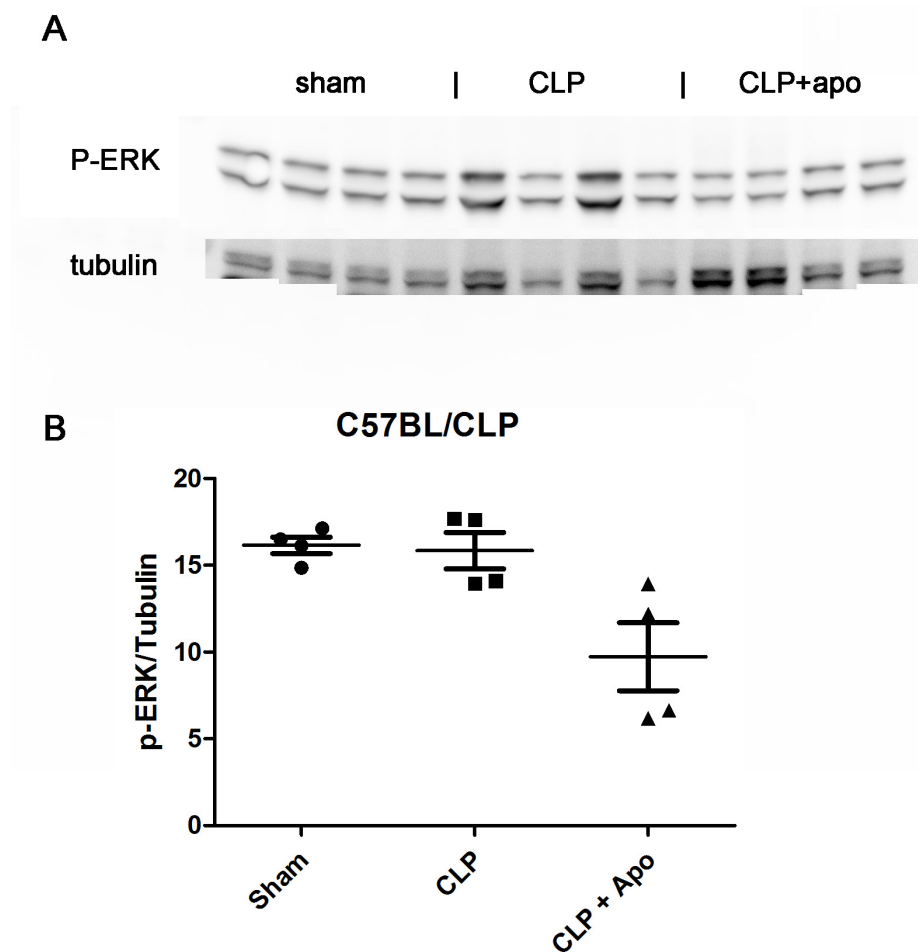
C. WBC count from CLP mice

D. Survival curves. N=4 mice in the sham group, N=10 mice in the CLP group, N=10 mice in the CLP+apocynin group.

The drug was given during the surgery and then again at 12 hrs. The sham and CLP mice received DMSO (vehicle control) at these time-points.

E. Table of echocardiogram measurements

Supplemental Figure 7:



Supplemental Figure 7: P-ERK western blot after CLP

A: Western blot of P-ERK and tubulin loading control from the same membrane.

B: Graph of quantification of P-ERK western blot adjusted for tubulin loading control.