SUPPLEMENTAL MATERIAL

Supplemental file 1:

Supplemental Methods:

Quantitation of Free Fatty Acids:

Free fatty acids were measured in mouse ventricular tissues using Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry (UPLC-MSMS). **Materials.** All free fatty acid standards were purchased from Nu-Chek Prep Inc. (Elysian, MN, USA). Deuterated palmitic acid (C16-D31 fatty acid) was purchased from C/D/N Isotopes Inc. (Quebec, Canada). All the solvents were LC-MS grade and purchased from Fisher Scientific (Pittsburgh, PA, USA) or Sigma-Aldrich Corp. (St. Louis, MO). **Sample preparation.** Samples were extracted with a liquid-liquid (chloroform:methanol) extraction as described before [1]. Briefly, 3 ml of chloroform:methanol (2:1 v/v) containing 1 nmol deuterated palmitic acid as internal standard were added to 0.5 ml of tissue homogenate disrupted in water. The mixture was vortexed again and centrifuged at 3,000 g for 10 min. The lower organic phase was transferred to a clean glass tube. Two ml of chloroform was added to the aqueous phase, vortexed and centrifuged. The lower organic phases were pooled and evaporated under nitrogen. Lipids extracts were reconstituted in 50 µl of methanol:acetonitrile (1:1 v:v) and transferred to LC/MS vial. **UPLC/MS.** LC-MS analysis was carried out on a Waters Xevo TQ MS integrated with an Acquity UPLC system (Waters, Milford, MA, USA). The system was controlled by MassLynx software 4. 1. Five microliters of the sample were loaded onto a Waters Acquity UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 µm) maintained at 40°C. The flow rate was 300 µl/min. The initial conditions were 15% phase A (H_2O containing 10 mM ammonium acetate) and 85% phase B (methanol containing 10 mM ammonium acetate). Phase B was increased linearly to 100% over 8 min, held for 2min, brought back to initial conditions and reconditioned for 2 minutes. Negative ESI-MS mass spectrometry with selected ion recording (SIR) mode was performed using the following parameters: capillary voltage -3.8 kV, source temperature 150 °C, desolvation temperature 500 °C,

desolvation gas flow 1000 L/hr. Concentrations of free fatty acids in the serum were quantitated by comparing integrated peak areas for those of each species against those of known amounts of purified standards. The range of LOQ of is 0.2-200 uM. Intra- and inter-assay precision are both below 8%.

Supplemental movie legend: example of VF in WT heart after 4 weeks of high saturated fat diet.

Supplemental Tables

Supplemental Table 1 Mouse body weights

	WT chow	WT HUFD	WT HSFD	NOX2KO HSFD
Initial Weight	26.0 ± 0.5	25.2 ± 0.3	25.7 ± 0.8	26.6 ± 0.8
Final Weight	29.3 ± 0.7	28.5 ± 0.3	29.6 ± 0.9	29.8 ± 1.1
mean change in weight	3.3	3.2	3.8	3.4

N= 8 each group

Supplemental Table 2. Echocardiogram measurements from WT HSFD and control (WT on regular chow) mice

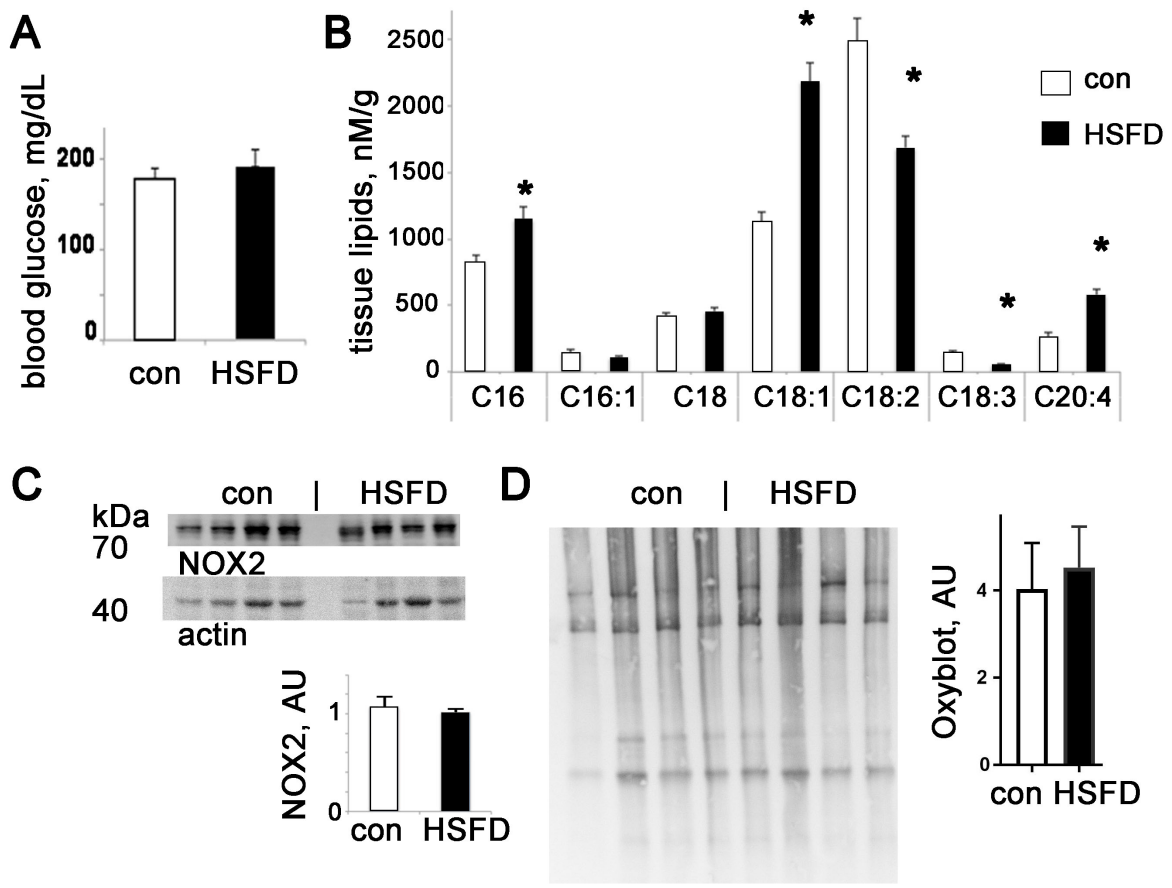
		HR/min	FS(%)	EF(%)	EDV (ml)	ESV (m)
control	mean	471.10	24.76	48.98	70.02	36.40
	SEM	7.26	2.68	4.40	2.92	4.34
HSFD	mean	482.38	22.53	45.59	74.28	40.23
	SEM	7.15	1.35	2.30	2.48	1.66
t-test		0.29	0.45	0.49	0.28	0.40

FS = fractional shortening, EF = ejection fraction, EDV = end diastolic volume, ESV = end systolic volume

N= 7 for control, N=8 HSFD

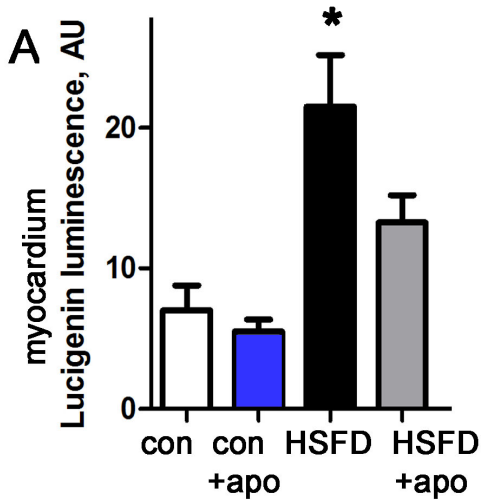
Supplemental Figures

Sup Figure 1:



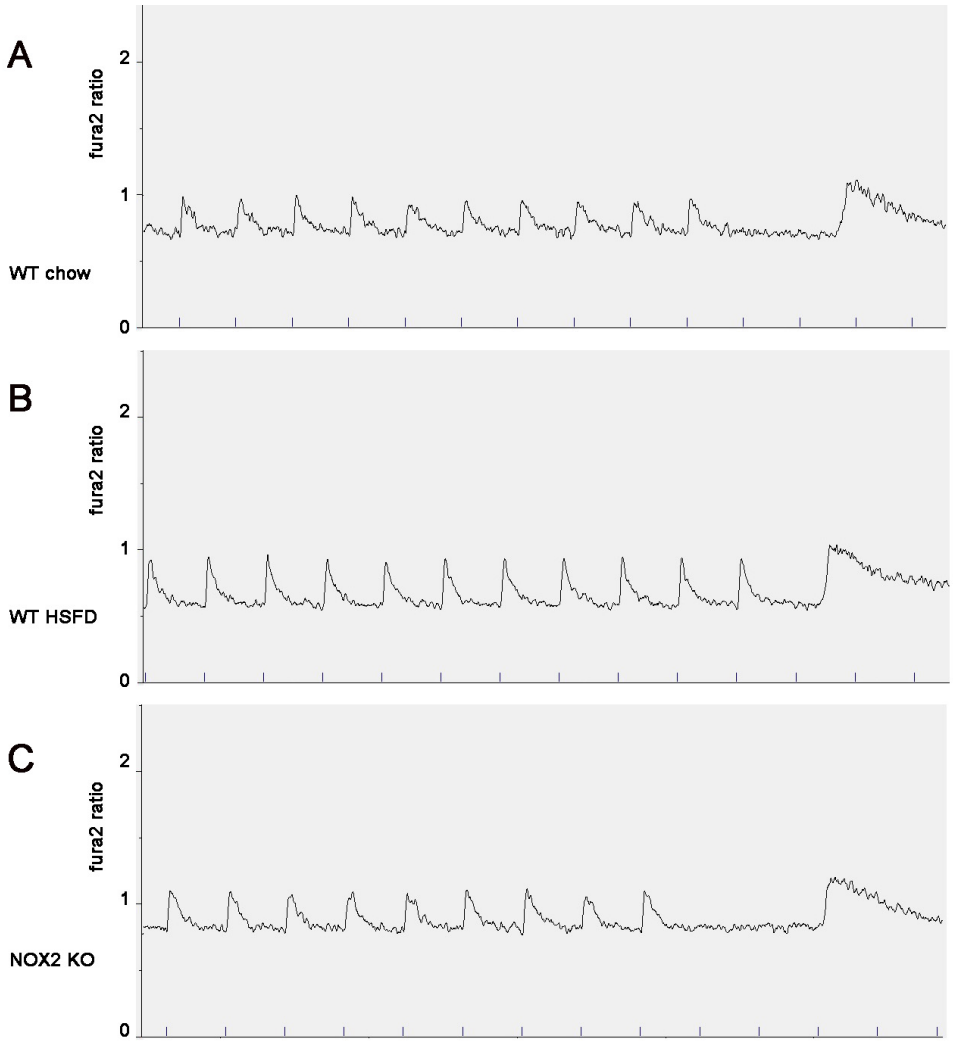
A. Graph of blood glucose after 4 weeks of diet.
B. Graph of subset of lipidomic analysis from left ventricular (LV) tissue samples after 4 weeks of diet.
C. NOX2 western blot from mouse ventricular tissue and graph of band quantification.
D. Blot of carbonylation of proteins from LV tissue samples and graph showing quantification in arbitrary units= AU.
In all panels error bars are SEM, * indicates $p<0.05$ compared to control.

Sup Figure 2:



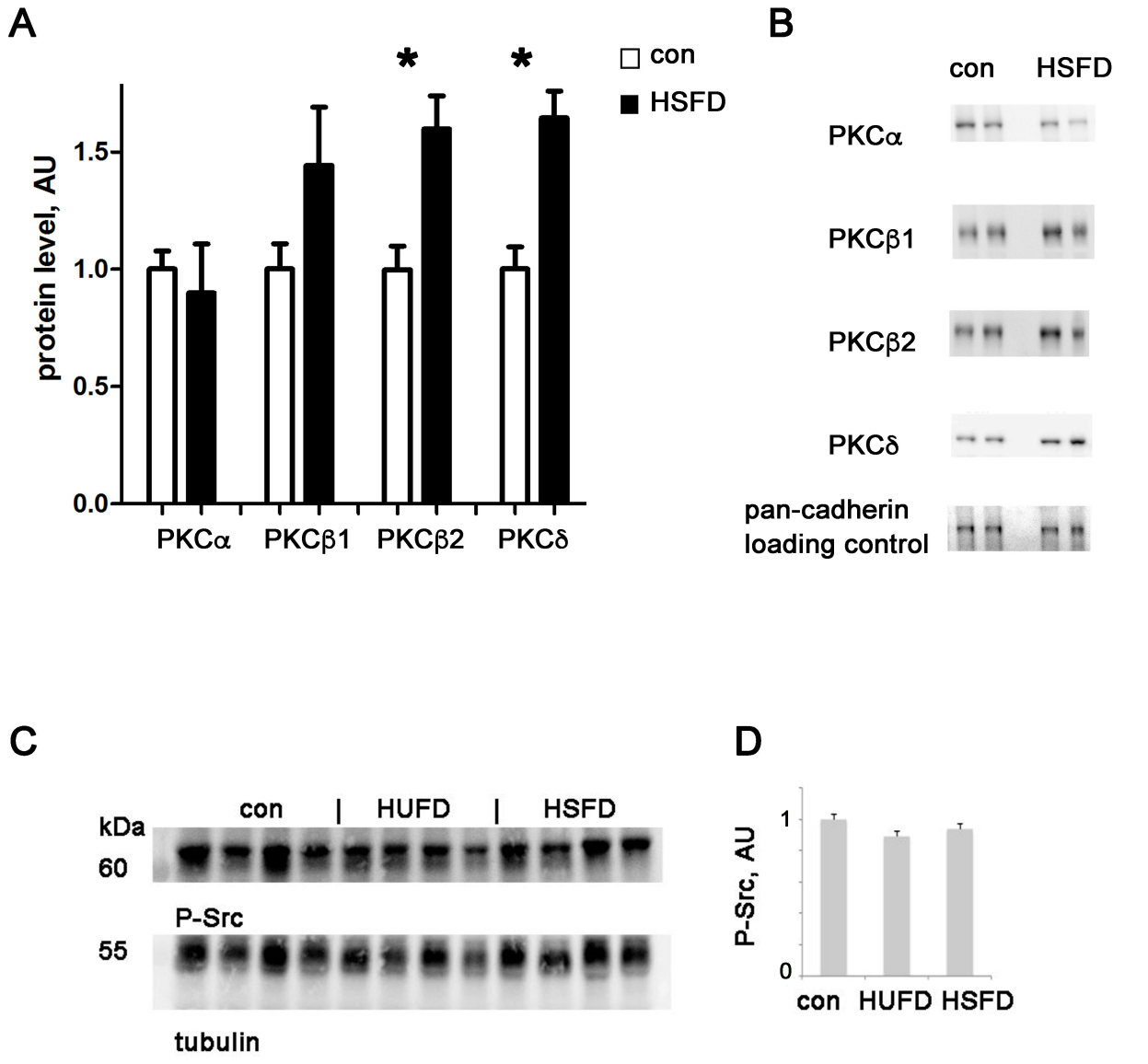
Supplemental Figure 2: High saturated fat diet activates NOX
High fat saturated fat diet increases NOX2 activity in cardiac ventricular tissue which is partially prevented by the NOX inhibitor apocynin.

Sup Figure 3:



Supplemental figure 3: Representative images of paced calcium transients and caffeine-evoked SR calcium load. A. WT chow **B.** WT HSFD **C.** NOX2 KO chow

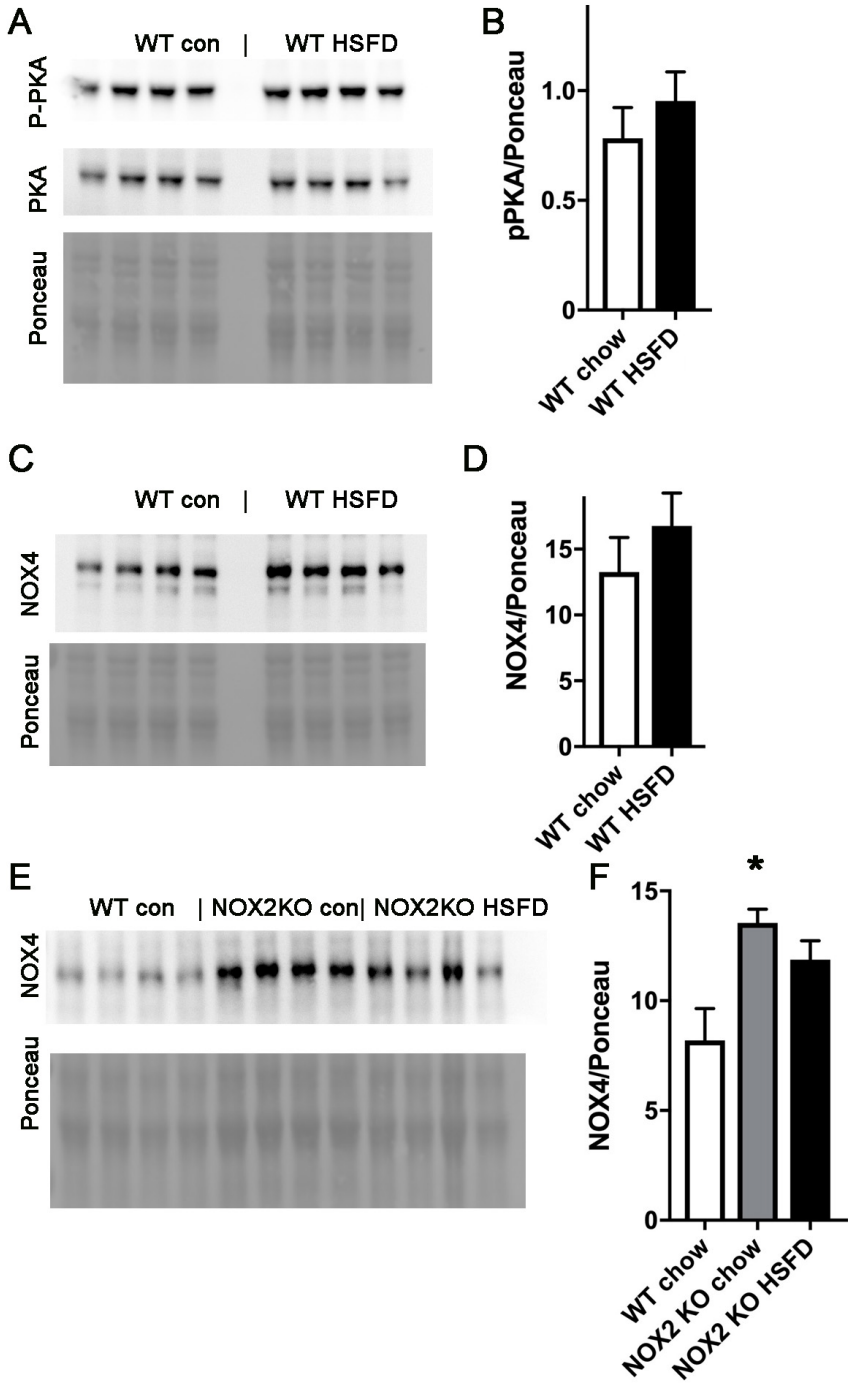
Sup Figure 4:



Supplemental figure 4: PKC and Src western blots from heart samples

A. Western blots of membrane fractions from mouse ventricular tissue for PKC alpha, beta, and delta. Pan-cadherin is a loading control for membrane fraction.
B. Representative images of PKC immunoblots.
C. Western blots of phosphorylated Src, with loading control.
D. Graphs of western blot, in arbitrary units. There are no significant differences by ANOVA.
n=4 animals for each group, error bars are SEM, * indicates $p<0.05$

Sup Figure 5:



Supplemental figure 5: PKA and NOX4 western blots from heart samples

A. Western blots of phosphorylated PKA (Thr197), total PKA, and Ponceau (loading control).
B. Graph of western blot, in arbitrary units. There is no significant difference by t-test.
C. Western blot of NOX4 and Ponceau (loading control).
D. Graph of western blot, in arbitrary units. There is no significant difference by t-test.
E. Western blot of NOX4 and Ponceau (loading control).
F. Graph of western blot, in arbitrary units. * indicates sig different from WT control**.**

Supplemental References

1. Clugston RD, Jiang H, Lee MX, Piantedosi R, Yuen JJ, Ramakrishnan R, Lewis MJ, Gottesman ME, Huang LS, Goldberg IJ, Berk PD, Blaner WS. Altered hepatic lipid metabolism in C57BL/6 mice fed alcohol: a targeted lipidomic and gene expression study. J Lipid Res Nov 2011;52:2021-2031.