Supplemental Materials

Methods:

Mitochondrial complex separation and visualization. Intact mitochondrial complexes were visualized using the Abcam MitoProfile® Total OXPHOS Blue Native (BN) WB Antibody Cocktail, according to the manufacturer's instructions. Briefly, samples were solubilized and centrifuged, and the protein concentration was determined in the supernatant. Coomassie blue was then added to the supernatant, and the samples were run on a 3.5-16% acrylamide gradient gel to separate on the first dimension (size). For the second dimension, the lanes of the gel were incubated in 1% mercaptoethanol and 1% SDS, then run on an SDS-PAGE gel and visualized using the Abcam antibody cocktail.

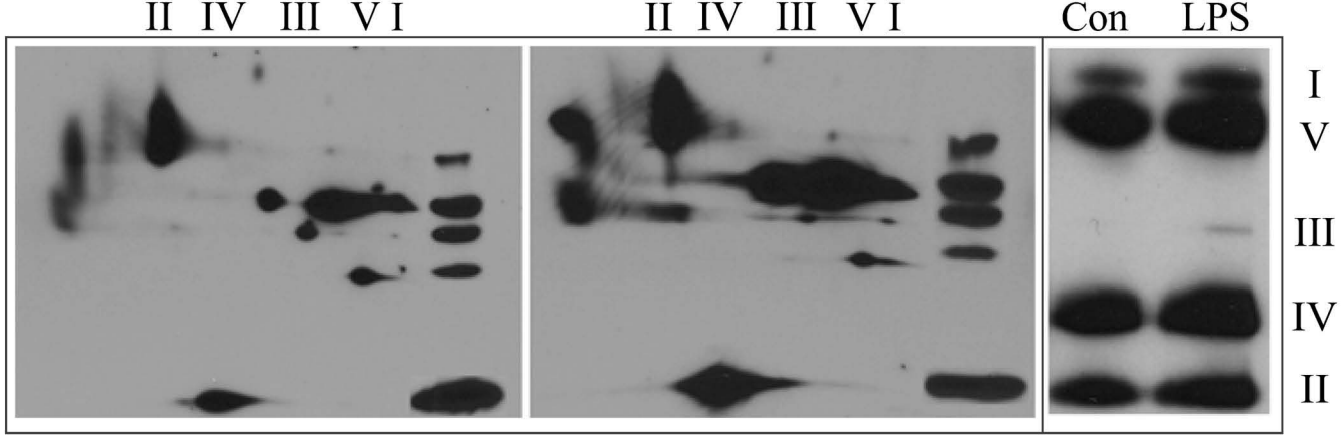
Figure legends:

Supplemental Figure 1. 2-D visualization of mitochondrial complex expression. Lysates from control (left) and LPS-treated (24 h, right) were stained with Coomassie blue and separated on an acrylamide gradient gel (right panel). The lanes were then excised, incubated in beta-mercaptoethanol and 1% SDS, run on an SDS-PAGE gel and then visualized using the Abcam antibody cocktail.

Supplemental Figure 2. LPS stimulates mitochondrial biogenesis *via* AMPK and Akt activation and requiring ROS. A-B. Both induction of TFAM mRNA and mtDNA content were significantly suppressed by inhibitors of TLR (polymixin B), ROS (NAC), AMPK (Compound C) and AKT (LY). Inhibitors to p38 (SB), MEK/ERK (U0126), CamKII (KN62) and NOS (L-NAME) did not affect LPS-induced TFAM mRNA or mtDNA levels. **C-D.** LPS exposure for 3 h

significantly increased the levels of p-AMPK (**C**) and p-AKT (**D**), which were attenuated by co-incubation with Compound C (**C**) and LY (**D**), respectively. **E**. LPS induced transient elevation of ROS in neurons. At the indicated time after LPS exposure, neurons were incubated with CM-H2DCFDA (10 μM), a specific sensor for H_2O_2, in the dark for 30 min. DCF fluorescence was measured under 485-nm excitation and 530-nm emission, and the data are presented as the fold increase of DCF fluorescence over vehicle controls. All quantitative data are mean±SE, from 3 independent experiments using cultures of different dams. *p<0.05, **p<0.01 compared to vehicle controls; #p<0.05, ##p<0.01 compared to LPS only.

Supplemental Figure 3. Inhibition of AMPK does not affect LPS-induced activation of AKT. LPS exposures (1μg/mL) for 3 or 6 hr lead to increases in AKT phosphorylation (p-AKT) in neurons. Co-incubation with the AMPK inhibitor Compound C failed to affect LPS-induced AKT phosphorylation. Data were from 3 independent experiments using cultures of different dams. n.s., not significant.


II IV III V I
II IV III V I
Con LPS
I
V
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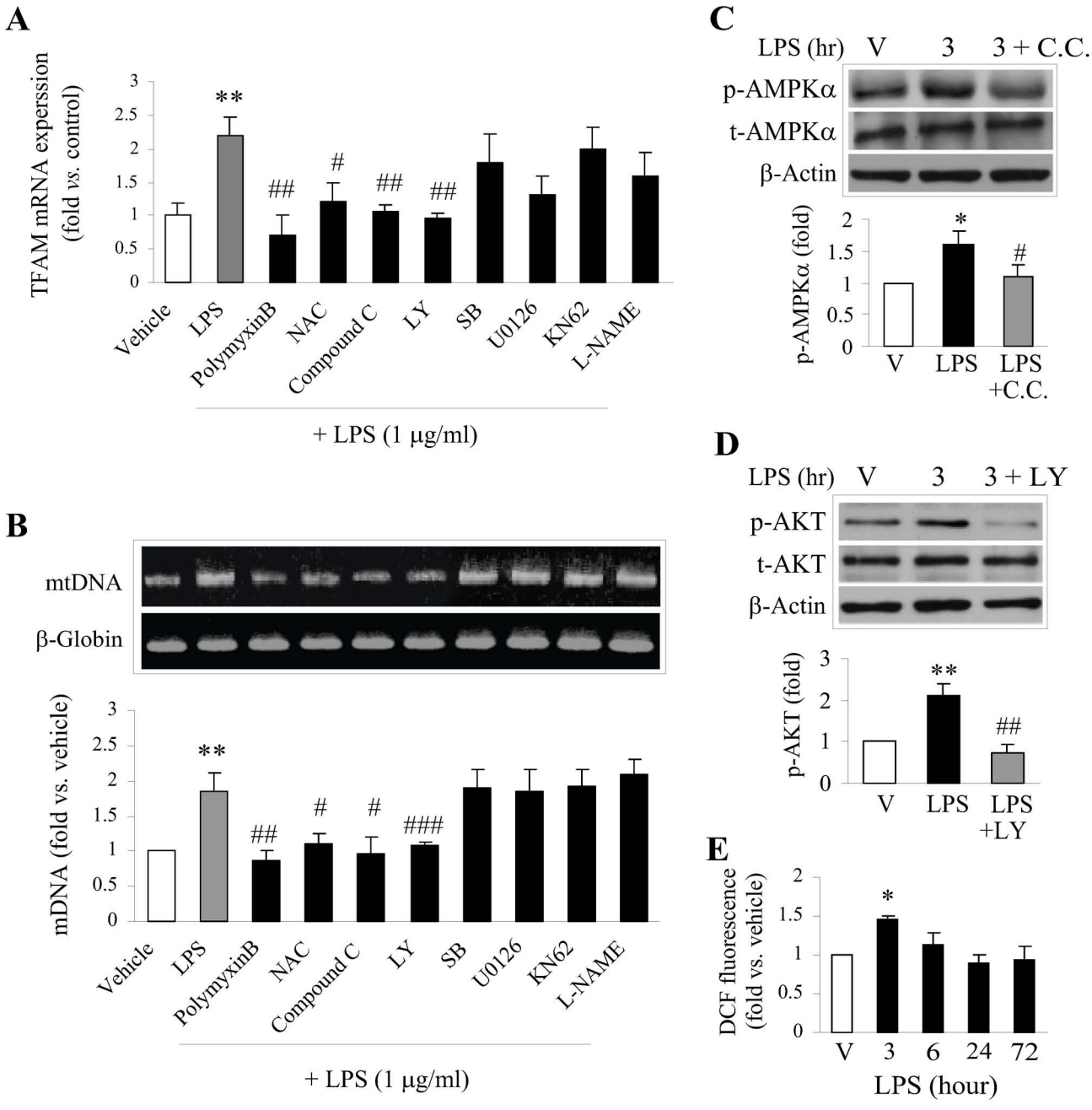
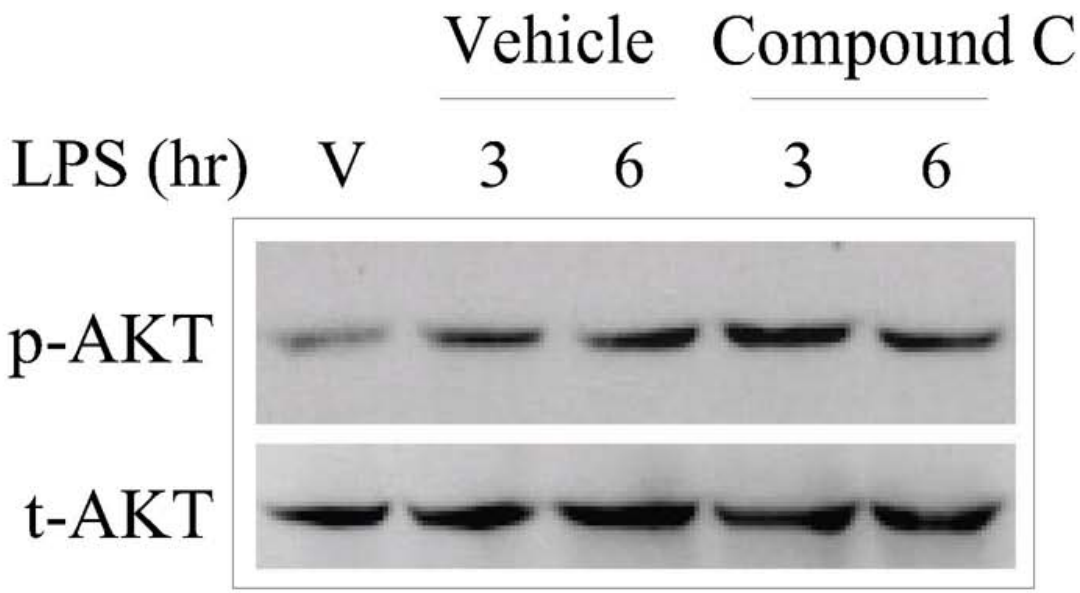
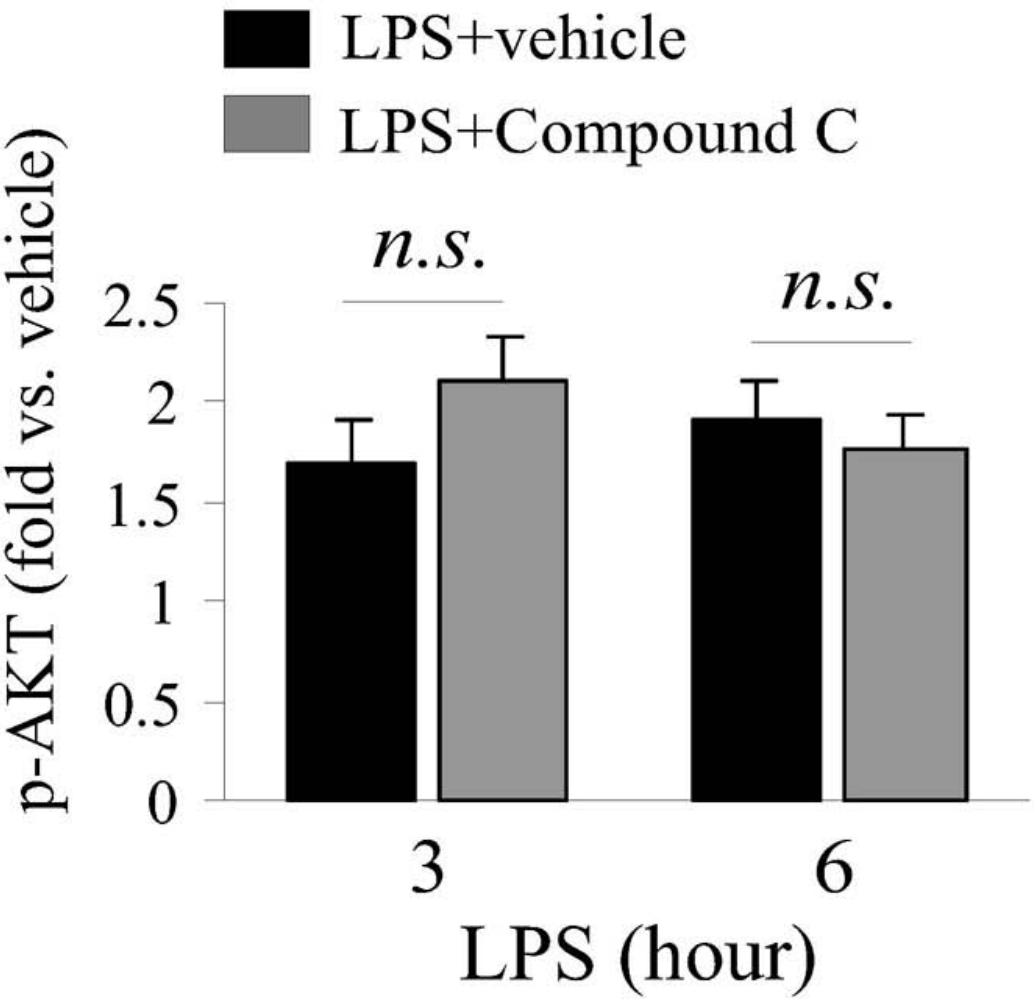
IV
II
Control
LPS


A
TFAM mRNA expression (fold vs. control)
Vehicle
LPS
PolymyxinB
NAC
Compound C
LY
SB
U0126
KN62
L-NAME
+ LPS (1 μg/ml)
B
mtDNA
β-Globin
mDNA (fold vs. vehicle)
+ LPS (1 μg/ml)
C
LPS (hr) V 3 3 + C.C.
p-AMPKα
t-AMPKα
β-Actin
p-AMPKα (fold)
V
LPS
LPS +C.C.
D
LPS (hr) V 3 3 + LY
p-AKT
t-AKT
β-Actin
p-AKT (fold)
V
LPS
LPS +LY
E
DCF fluorescence (fold vs. vehicle)
V 3 6 24 72
LPS (hour)


Vehicle
Compound C
LPS (hr)
V
3
6
3
6
p-AKT
t-AKT


LPS+vehicle
LPS+Compound C
n.s.
n.s.
p-AKT (fold vs. vehicle)
2.5
2
1.5
1
0.5
0
3
6
LPS (hour)