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Overexpression of MAPK Phosphatase-1 in obesity impairs PGC-1α-mediated regulation of myofiber type composition

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Figure S1. Growth curve of chow-fed C57BL6/J *mkp-1*$^{-/-}$ and C57BL6/J *mkp-1*$^{+/+}$ mice.mice.

(**A**) 129/J *mkp-1*$^{+/-}$ mice were backcrossed to wild type C57BL6/J mice for eight generations. At weaning, backcrossed *mkp-1*$^{+/+}$ (open circles) and *mkp-1*$^{-/-}$ (closed circles) mice were placed on a chow diet and weights were monitored weekly for 20 weeks. Data represent mean ± SEM (n= 3-16) for each time point.

Figure S2. Fatty acids do not induce cytotoxic effects in myoblasts and are increased in skeletal muscle after HFD.

(**A**) C2C12 myoblasts were starved overnight, and stimulated with 400 μM palmitate (C16:0), 100 μM palmitoleate (C16:1n7), a mixture of the two, 500 μM stearate (C18:0), or 100 μM oleate (C18:1n9) for 30 minutes. RNA was harvested, and MKP-1 mRNA levels were measured by RT-qPCR and normalized to 18S. Data represent the mean ± SEM (n=3-4, * ; P<0.05). (**B**) C2C12 myoblasts were starved overnight, and stimulated as in (**A**). Cells were stained with trypan blue and the percentage of trypan blue positive cells was assessed (n=5, * ; P<0.05). (**C**) Plantaris muscle was isolated from chow or

HFD-fed *mkp-1*$^{+/+}$ mice, lipid metabolites were extracted, and LC/MS/MS analysis was performed. Data represent the mean ± SEM of total acyl-CoA content as normalized to tissue weight (n=4-5, *; $P<0.05$) (**D**) Plantaris muscle was isolated and lipid metabolites were analyzed as in (**C**). Data represent the mean ± SEM of lipid metabolites as normalized to tissue weight (n=4-5, **; $P<0.005$).

Figure S3. Controls for skeletal muscle nuclear extracts and PGC-1α immunoblots. (**A**) Cytosolic and nuclear extracts were prepared from tibialis anterior muscle and immunoblotted for Na/K ATPase or Lamin-β1 as controls for the cytosolic and nuclear fractions, respectively. (**B**) Left panel: nuclear extracts, immunodepletions for pS265 or total PGC-1α, and positive control (C2C12 myoblasts transfected with PGC-1α and treated for 4 hours with LPS) were immunoblotted for pS265 PGC-1α. Right panel: nuclear extracts, pS265 and total PGC-1α immunodepletions, and positive control were immunoblotted for total PGC-1α. Endogenous PGC-1α can be seen in the dark exposures in nuclear extract and positive control lanes at ~115 kDa.

A.

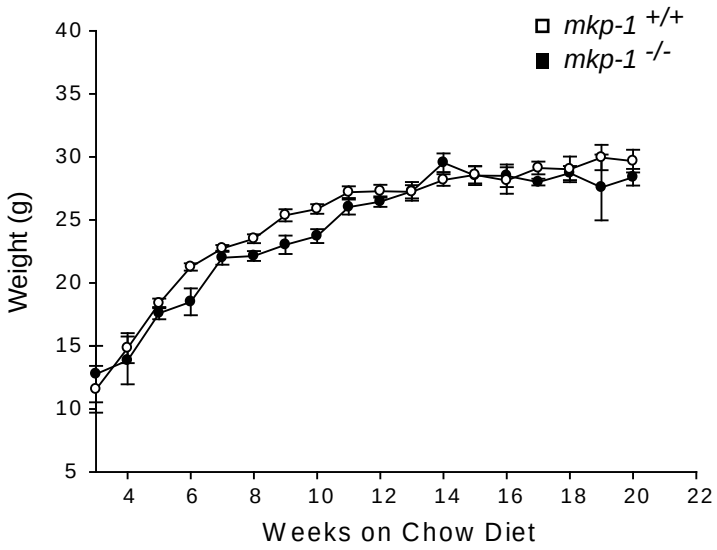

mkp-1 +/+
mkp-1 -/-
Weight (g)
40
35
30
25
20
15
10
5
4
6
8
10
12
14
16
18
20
22
Weeks on Chow Diet

Figure S1

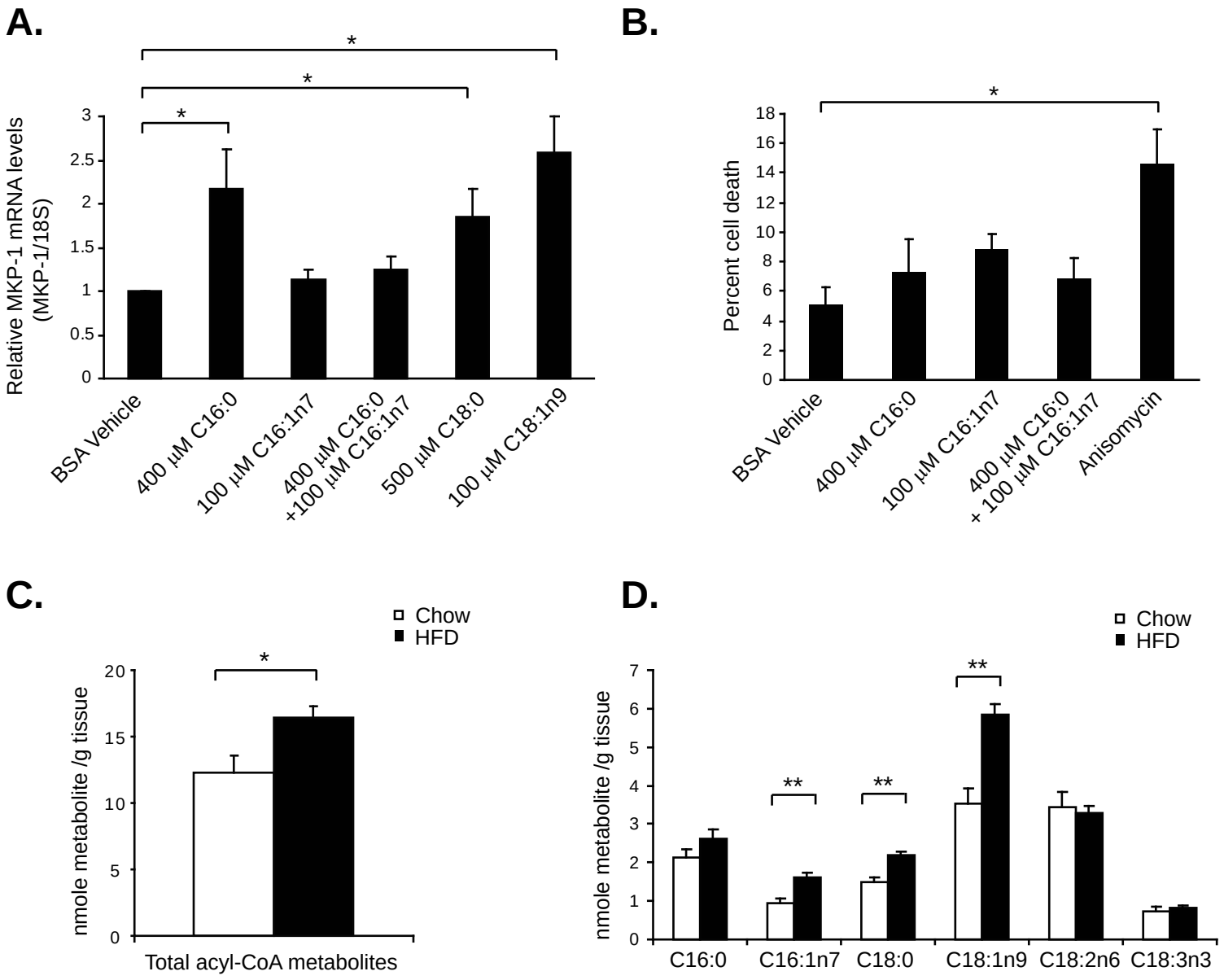
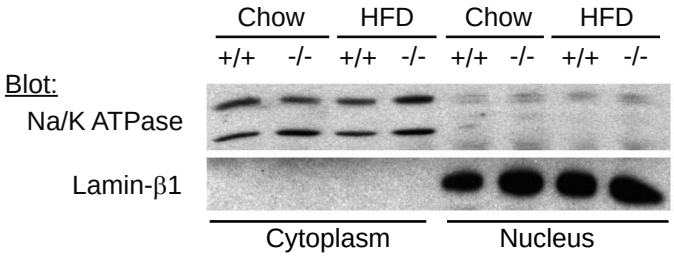

A.
Relative MKP-1 mRNA levels (MKP-1/18S)
BSA Vehicle
400 μM C16:0
100 μM C16:1n7
400 μM C16:0 +100 μM C16:1n7
500 μM C18:0
100 μM C18:1n9
B.
Percent cell death
BSA Vehicle
400 μM C16:0
100 μM C16:1n7
400 μM C16:0 + 100 μM C16:1n7
Anisomycin
C.
Chow
HFD
nmole metabolite /g tissue
Total acyl-CoA metabolites
D.
Chow
HFD
nmole metabolite /g tissue
C16:0
C16:1n7
C18:0
C18:1n9
C18:2n6
C18:3n3

Figure S2

A.


Chow
HFD
Chow
HFD
+/+ -/- +/+ -/- +/+ -/- +/+ -/-
Blot:
Na/K ATPase
Lamin-β1
Cytoplasm
Nucleus

B.

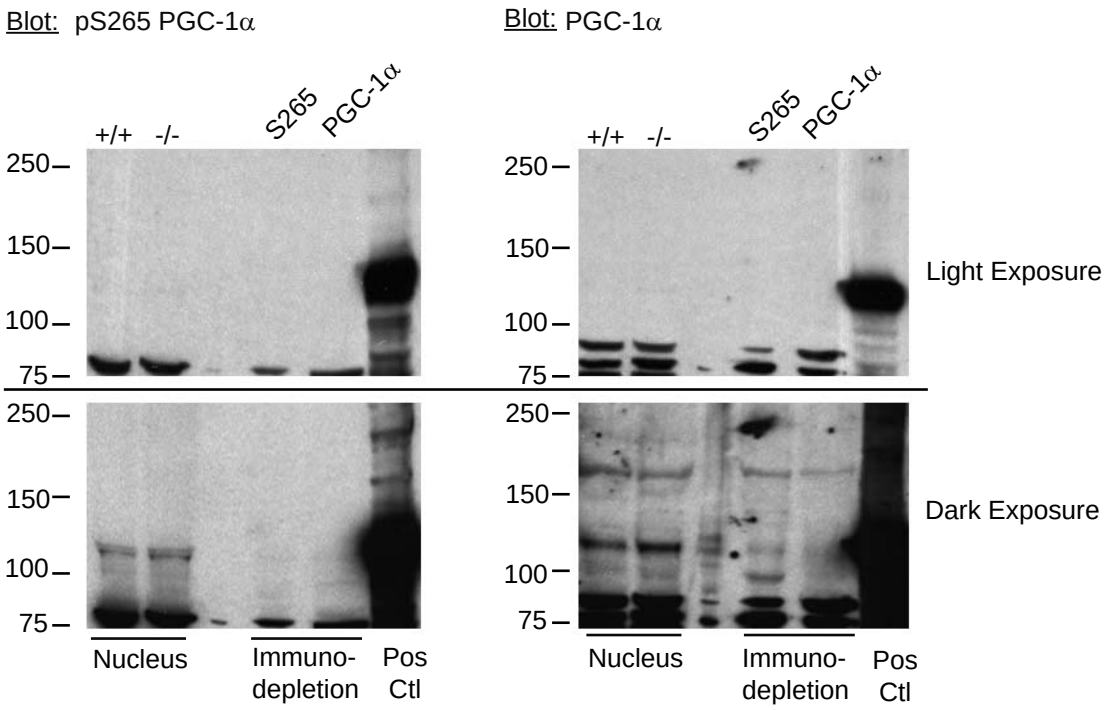

Blot: pS265 PGC-1α
Blot: PGC-1α
+/+ -/-
S265
PGC-1α
250
150
100
75
Light Exposure
Dark Exposure
Nucleus
Immuno-depletion
Pos Ctl

Figure S3