Cell Reports, Volume *26*

Supplemental Information

Genetic Liver-Specific AMPK Activation Protects against Diet-Induced Obesity and NAFLD

Daniel Garcia, Kristina Hellberg, Amandine Chaix, Martina Wallace, Sébastien Herzig, Mehmet G. Badur, Terry Lin, Maxim N. Shokhirev, Antonio F.M. Pinto, Debbie S. Ross, Alan Saghatelian, Satchidananda Panda, Lukas E. Dow, Christian M. Metallo, and Reuben J. Shaw

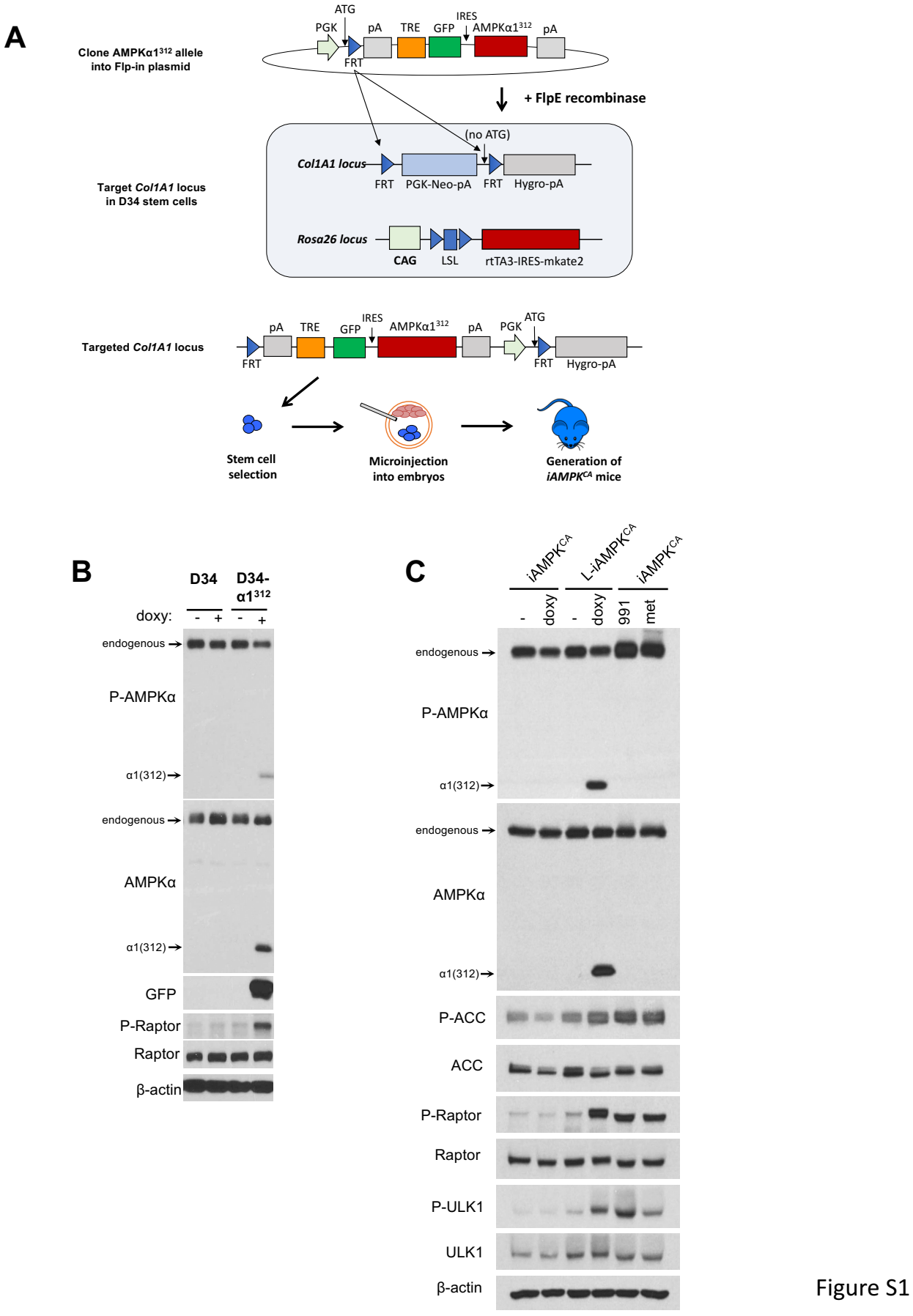

A
Clone AMPKα1312 allele into Flp-in plasmid
ATG
PGK
pA
TRE
GFP
IRES
AMPKα1312
pA
FRT
+ FlpE recombinase
(no ATG)
Target Col1A1 locus in D34 stem cells
Col1A1 locus
FRT
PGK-Neo-pA
FRT
Hygro-pA
Rosa26 locus
CAG
LSL
rtTA3-IRES-mkate2
Targeted Col1A1 locus
pA
TRE
GFP
IRES
AMPKα1312
pA
PGK
ATG
FRT
FRT
Hygro-pA
Stem cell selection
Microinjection into embryos
Generation of iAMPKCA mice
B
D34
D34-α1312
doxy:
- + - +
endogenous
P-AMPKα
α1(312)
endogenous
AMPKα
α1(312)
GFP
P-Raptor
Raptor
β-actin
C
iAMPKCA
L-iAMPKCA
iAMPKCA
- doxy - doxy 991 met
endogenous
P-AMPKα
α1(312)
endogenous
AMPKα
α1(312)
P-ACC
ACC
P-Raptor
Raptor
P-ULK1
ULK1
β-actin

Figure S1

Figure S1. Generation and validation of iAMPK^{CA} mice (related to Fig 1).

(A) Diagram detailing the cloning strategy for the generation of iAMPK^{CA} mice. FRT, Flp recombination target; pA, polyA signal; TRE, tet-response element; GFP, green-fluorescent protein; IRES, internal ribosome entry site; PGK, phosphoglycerate kinase promoter; Hygro^{R}, hygromycin resistance gene; CAG, CMV early enhancer chicken β-actin promoter; LSL, loxP-Stop (Neomycin-resistance gene)-loxP cassette; rtTA3, reverse tetracycline-controlled transactivator 3; mkate2, next-generation red-fluorescent protein.

(B) Western blot analysis of the expression of constitutively active AMPK and subsequent AMPK signaling activation in a correctly targeted D34 stem cell clone.

(C) Western blot analysis comparing AMPK activation in primary hepatocytes derived from iAMPK^{CA} and L-iAMPK^{CA} mice that were treated with or without doxycycline for 4 weeks relative to AMPK activation after one hour treatments of 991 (10 uM) or metformin (1 mM).

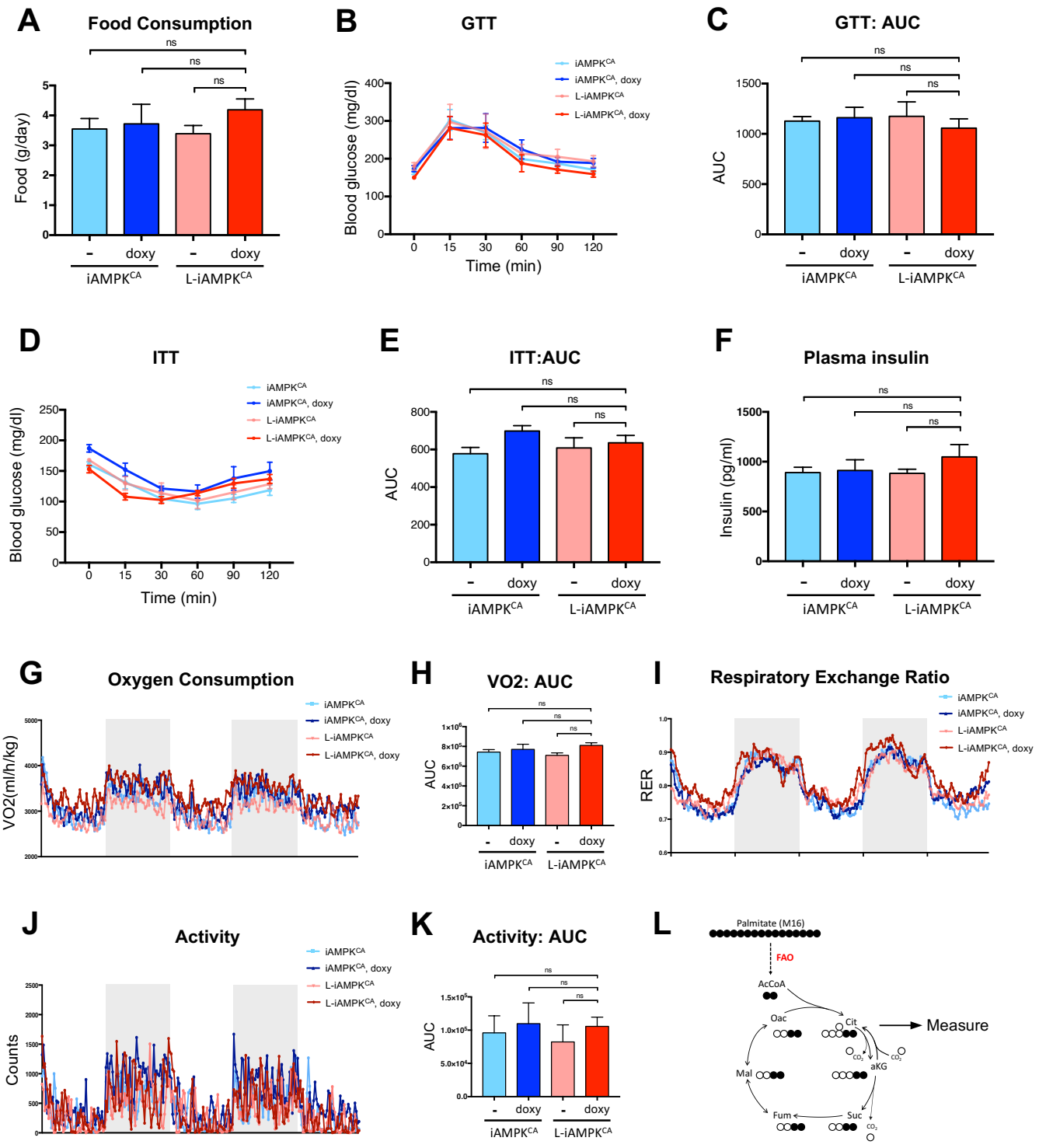

A
Food Consumption
Food (g/day)
ns
ns
ns
- doxy - doxy
iAMPKCA
L-iAMPKCA
B
GTT
Blood glucose (mg/dl)
Time (min)
iAMPKCA
iAMPKCA, doxy
L-iAMPKCA
L-iAMPKCA, doxy
C
GTT: AUC
AUC
D
ITT
E
ITT:AUC
F
Plasma insulin
Insulin (pg/ml)
G
Oxygen Consumption
VO2(ml/h/kg)
H
VO2: AUC
I
Respiratory Exchange Ratio
RER
J
Activity
Counts
K
Activity: AUC
L
Palmitate (M16)
FAO
AcCoA
Oac
Cit
Measure
aKG
Mal
Fum
Suc

Figure S2

Figure S2. AMPK activation reduces hepatic lipid levels (related to Fig 2).

(A) Food consumption rates in iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline.

(B) Glucose tolerance test (GTT) on iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. *n*=6 mice per condition.

(C) Quantification of the area under the curve in (B).

(D) Insulin tolerance test (ITT) on iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. *n*=6 mice per condition.

(E) Quantification of the area under the curve in (D).

(F) Fasting (6 hours) levels of plasma insulin in iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. *n*=5 mice per condition.

(G) Metabolic cage analysis of oxygen consumption (VO2) in iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. Shaded area (light gray) delineates night time (6:00 pm to 6:00 am). *n*=4 mice per condition.

(H) Quantification of the area under the curve in (G).

(I) Metabolic cage analysis of the respiratory exchange ratio (RER) in iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. Shaded area (light gray) delineates night time (6:00 pm to 6:00 am). *n*=4 mice per condition.

(J) Metabolic cage analysis of the activity of iAMPK^{CA} and L-iAMPK^{CA} mice fed chow with or without doxycycline for 4 weeks. Shaded area (light gray) delineates night time (6:00 pm to 6:00 am). *n*=4 mice per condition.

(K) Quantification of the area under the curve in (J).

(L) Diagram illustrating the trajectory of uniformly labeled palmitate into acetyl-CoA, which subsequently gets incorporated into citrate. Abbreviations: FAO, fatty acid oxidation; AcCoA, acetyl-CoA; Oac, oxaloacetate; Cit, citrate; aKG, alpha-ketoglutarate; Suc, succinate; Fum, fumarate; Mal, malate.

All values are expressed as means and error bars reflect SEM. Significance determined by ANOVA, ns= not significant.

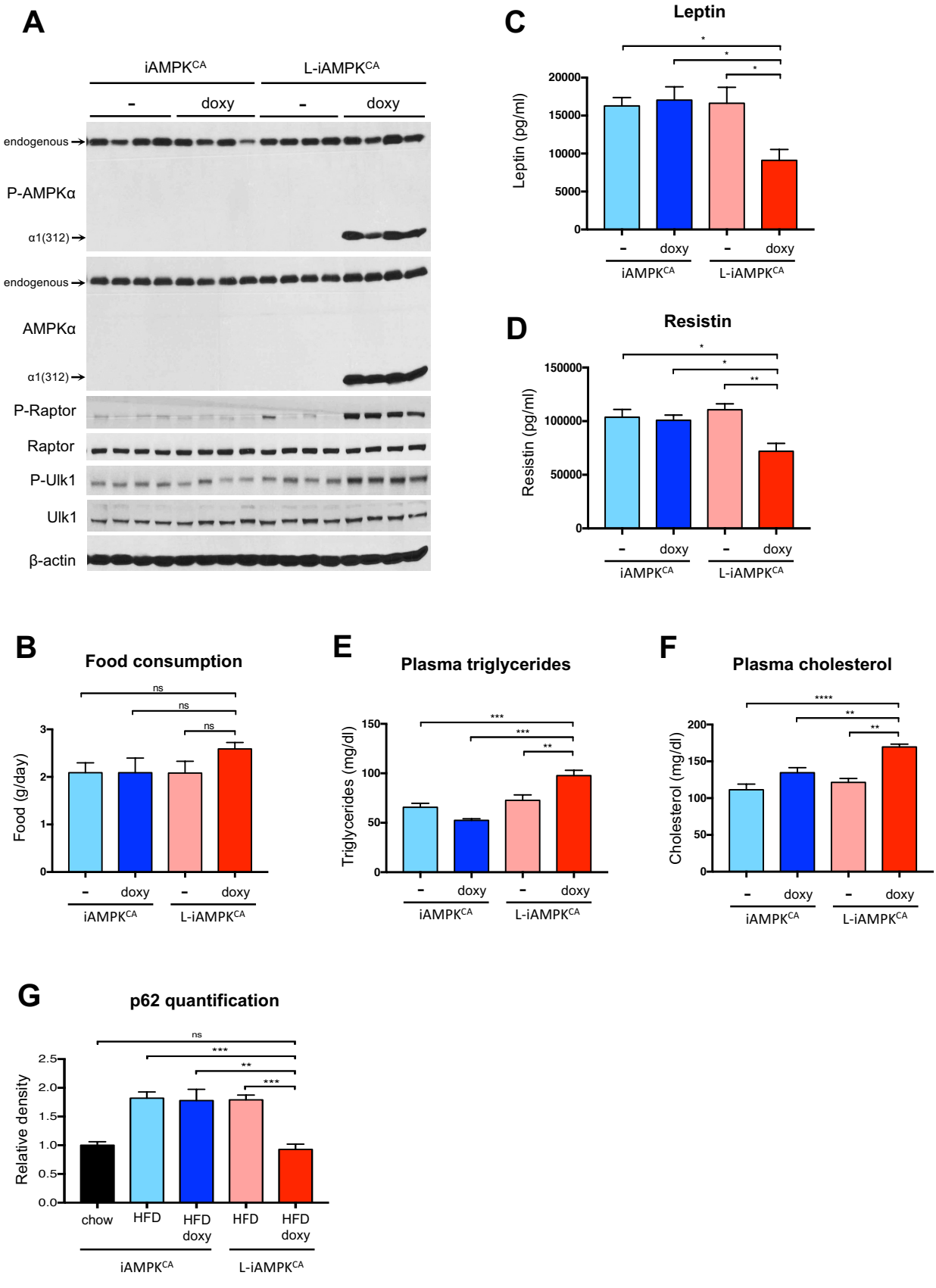

A
iAMPKCA
L-iAMPKCA
-
doxy
-
doxy
endogenous
P-AMPKα
α1(312)
endogenous
AMPKα
α1(312)
P-Raptor
Raptor
P-Ulk1
Ulk1
β-actin
C
Leptin
Leptin (pg/ml)
20000
15000
10000
5000
0
*
*
*
-
doxy
-
doxy
iAMPKCA
L-iAMPKCA
D
Resistin
Resistin (pg/ml)
150000
100000
50000
0
*
*
**
-
doxy
-
doxy
iAMPKCA
L-iAMPKCA
B
Food consumption
Food (g/day)
3
2
1
0
ns
ns
ns
-
doxy
-
doxy
iAMPKCA
L-iAMPKCA
E
Plasma triglycerides
Triglycerides (mg/dl)
150
100
50
0
***
***
**
-
doxy
-
doxy
iAMPKCA
L-iAMPKCA
F
Plasma cholesterol
Cholesterol (mg/dl)
200
150
100
50
0
****
**
**
-
doxy
-
doxy
iAMPKCA
L-iAMPKCA
G
p62 quantification
Relative density
2.5
2.0
1.5
1.0
0.5
0.0
ns
***
**
***
chow
HFD
HFD doxy
HFD
HFD doxy
iAMPKCA
L-iAMPKCA

Figure S3

Figure S3. AMPK activation in liver protects against diet-induced obesity (related to Fig3).

(A) Western blot analysis of AMPK activation in iAMPK^{CA} and L-iAMPK^{CA} mice fed HFD with or without doxycycline for 8 weeks. Each lane is a separate mouse.

(B) Food consumption rates in iAMPK^{CA} and L-iAMPK^{CA} mice fed HFD with or without doxycycline.

(C-D) Fasting plasma levels of adipokines leptin and resistin in iAMPK^{CA} and L-iAMPK^{CA} mice fed HFD with or without doxycycline for 8 weeks. n=6 mice per condition.

(E-F) Fasting plasma levels of triglycerides and cholesterol in iAMPK^{CA} and L-iAMPK^{CA} mice fed HFD with or without doxycycline for 8 weeks. n=6 mice per condition.

(G) Densitometry quantification of p62 levels on the western blot shown on Figure 3J.

All values are expressed as means and error bars reflect SEM. Significance determined by ANOVA, *= $p<0.05$, **= $p<0.01$, ***= $p<.001$, ****= $p<.0001$, ns= not significant.

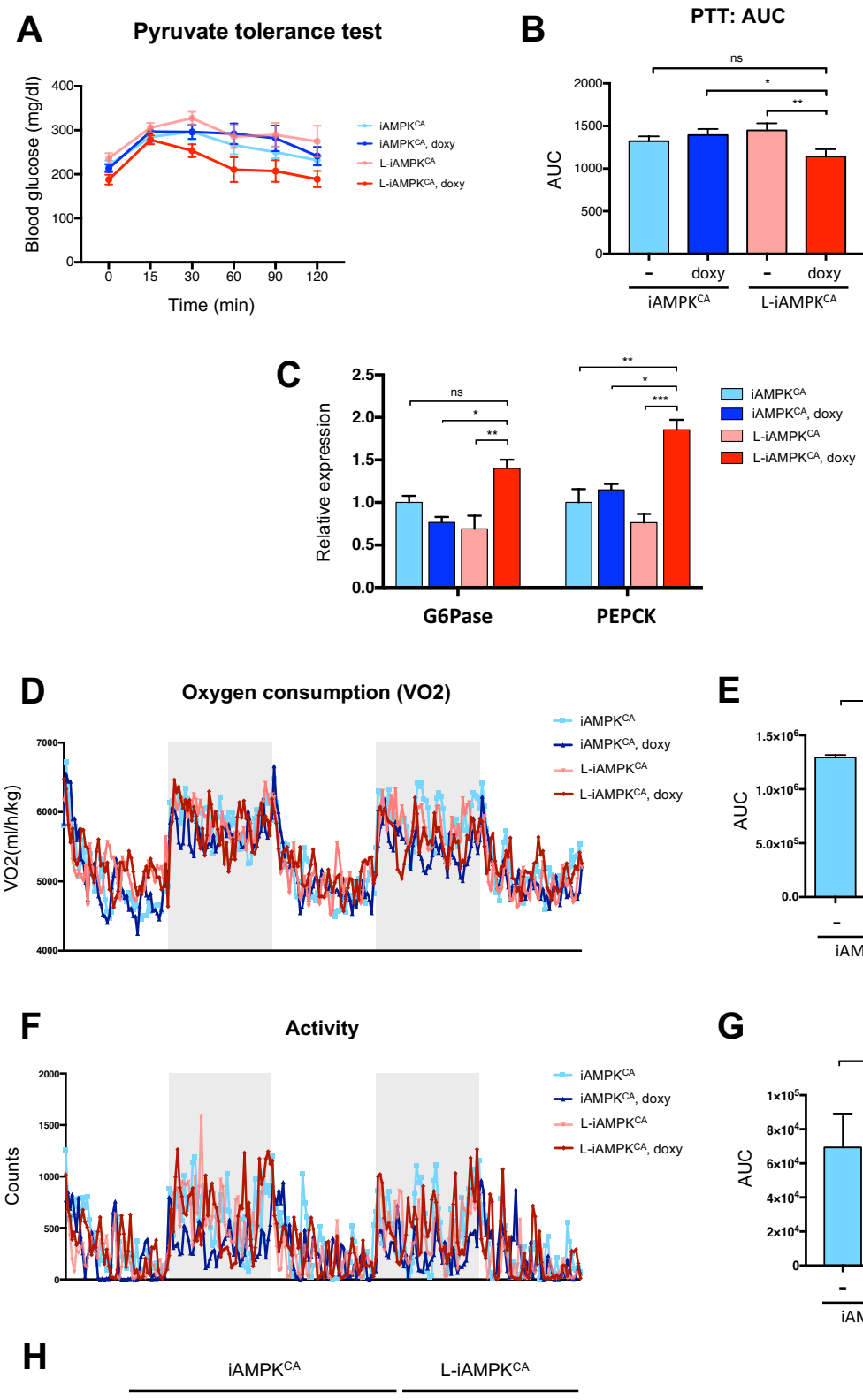

A
Pyruvate tolerance test
Blood glucose (mg/dl)
Time (min)
iAMPKCA
iAMPKCA, doxy
L-iAMPKCA
L-iAMPKCA, doxy
B
PTT: AUC
AUC
ns
*
**
doxy
iAMPKCA
L-iAMPKCA
C
Relative expression
G6Pase
PEPCK
***
D
Oxygen consumption (VO2)
VO2(ml/h/kg)
E
VO2: AUC
F
Activity
Counts
G
Activity: AUC
H
chow
HFD
HFD+doxy
PKCε
β-actin

Figure S4

Figure S4. AMPK activation in liver modestly improves glucose homeostasis in the context of diet-induced obesity (related to Fig 4).

(A) Pyruvate tolerance test (PTT) on iAMPK^{CA} and L-iAMPK^{CA} mice fed a HFD with or without doxycycline for 8 weeks. *n*=6 mice per condition.

(B) Quantification of the area under the curve in (A).

(C) Gene expression analysis (qPCR) of *G6pc* (G6Pase) and *Pck1* (PEPCK) in livers from iAMPK^{CA} and L-iAMPK^{CA} mice fed a HFD with or without doxycycline for 8 weeks. *n*=3 mice per condition.

(D) Metabolic cage analysis of oxygen consumption (VO2) in iAMPK^{CA} and L-iAMPK^{CA} mice fed a HFD with or without doxycycline for 8 weeks. Shaded area (light gray) delineates night time (6:00 pm to 6:00 am). *n*=4 mice per condition.

(E) Quantification of the area under the curve in (D).

(F) Metabolic cage analysis of the activity of iAMPK^{CA} and L-iAMPK^{CA} mice fed a HFD with or without doxycycline for 8 weeks. Shaded area (light gray) delineates night time (6:00 pm to 6:00 am). *n*=4 mice per condition.

(G) Quantification of the area under the curve in (F).

(H) Western blot analysis of total PKCε levels in livers from iAMPK^{CA} and L-iAMPK^{CA} mice fed either chow, or HFD with or without doxycycline for 8 weeks. Each lane is a separate mouse.

All values are expressed as means and error bars reflect SEM. Significance determined by ANOVA, *= $p<0.05$, **= $p<0.01$, ***= $p<.001$, ns= not significant.

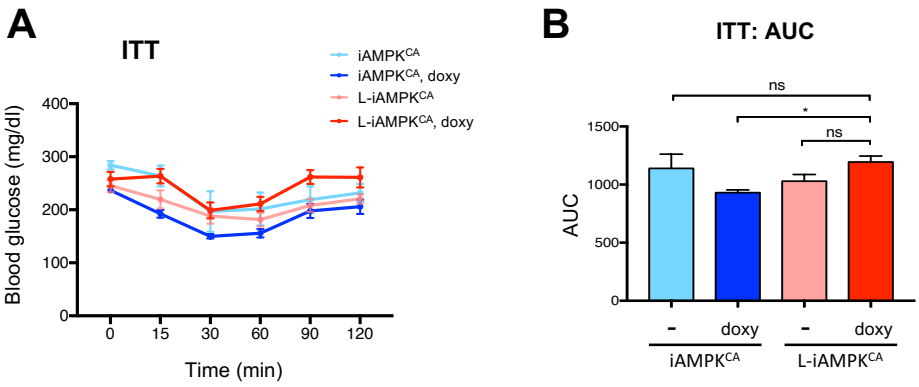

A
ITT
iAMPK^CA
iAMPK^CA, doxy
L-iAMPK^CA
L-iAMPK^CA, doxy
Blood glucose (mg/dl)
400
300
200
100
0
0
15
30
60
90
120
Time (min)
B
ITT: AUC
ns
*
ns
AUC
1500
1000
500
0
-
doxy
-
doxy
iAMPK^CA
L-iAMPK^CA

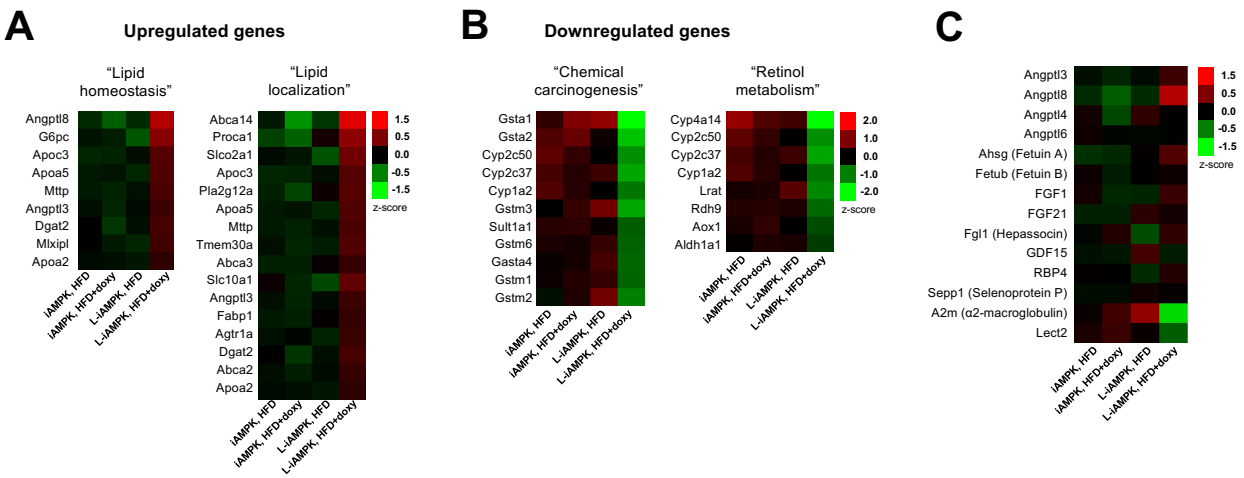
Figure S5

Figure S5. AMPK activation in liver inhibits progression of established diet-induced obesity (related to Figure 6).

(A) Insulin tolerance test (ITT) on iAMPK^{CA} and L-iAMPK^{CA} mice treated as in Figure 6A. *n*=7 mice per condition.

(B) Quantification of the area under the curve in (A).

All values are expressed as means and error bars reflect SEM. Significance determined by ANOVA, *= $p<0.05$, ns= not significant.



D

Motif	Motif name	q-value (Benjamini)	# targets with motifs
	ISRE(IRF)/ThioMac-LPS-Expression(GSE23622)	0	37
	IRF1(IRF)/PBMC-IRF1-ChIP-Seq(GSE43036)	0	44
	IRF2(IRF)/Erythroblas-IRF2-ChIP-Seq(GSE36985)	0	36
	PU.1:IRF8(ETS:IRF)/pDC-Irf8-ChIP-Seq(GSE66899)	0	43
	Atf3(bZIP)/GBM-ATF3-ChIP-Seq(GSE33912)	0	70
	Fra1(bZIP)/BT549-Fra1-ChIP-Seq(GSE46166)	0	60
	BATF(bZIP)/Th17-BATF-ChIP-Seq(GSE39756)	0.0001	67
	Fosl2(bZIP)/3T3L1-Fosl2-ChIP-Seq(GSE56872)	0.0003	43
	AP-1(bZIP)/ThioMac-PU.1-ChIP-Seq(GSE21512)	0.0005	71

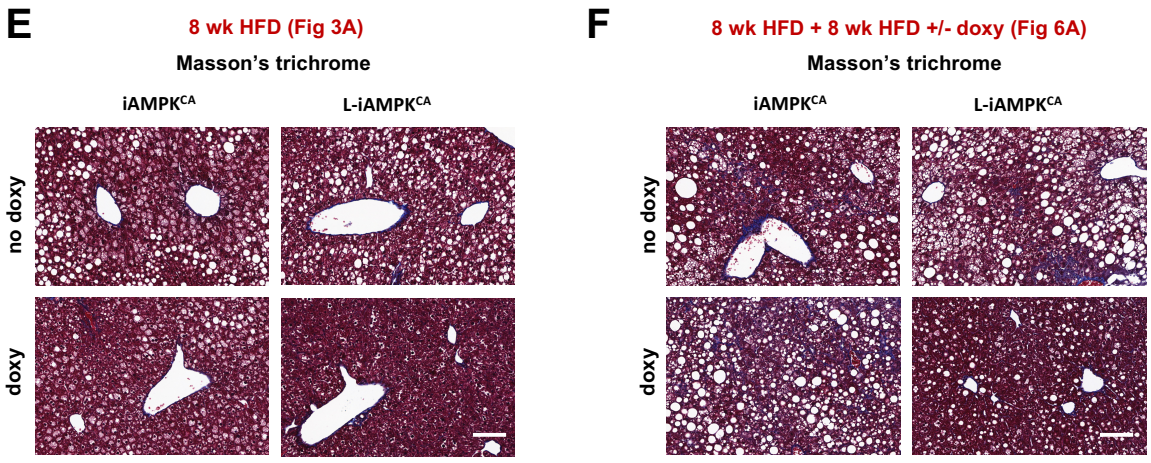


Figure S6

Figure S6. AMPK activation downregulates inflammation and fibrosis-related transcriptional programs (related to Figure 7).

(A) Heat maps showing upregulated genes in the terms “Lipid homeostasis” and “Lipid localization” from Metascape pathway analysis in Figure 7C.

(B) Heat maps showing downregulated genes in the terms “Chemical carcinogenesis” and “Retinol metabolism” from Metascape pathway analysis in Figure 7D.

(C) Heat map showing the expression profile of several known hepatokines in mice fed 8 weeks HFD, plus or minus doxycycline (from RNA seq data).

(D) Regulatory transcriptional motifs enriched in genes downregulated in doxycycline-treated L-iAMPK^{CA} mice versus doxycycline-treated iAMPK^{CA} mice (8 weeks HFD), identified using motif enrichment analysis.

(E) Representative images of Masson trichrome staining on livers from iAMPK^{CA} and L-iAMPK^{CA} mice fed 8 weeks HFD, plus or minus doxycycline (Protection study, Figure 3A). Collagen fibers are stained blue. Scale bar = 100 μM.

(F) Representative images of Masson trichrome staining on livers from iAMPK^{CA} and L-iAMPK^{CA} mice fed 8 weeks HFD and then an additional 8 weeks of HFD, plus or minus doxycycline (Intervention study, Figure 6A). Collagen fibers are stained blue. Scale bar = 100 μM.