Supplementary Table 1. Quantitative PCR primer sequences

Gene symbol	Sequences (5' to 3')	
	Forward	Reverse
Mouse/Human 18s	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA
Mouse 36b4	GTTCTTGCCCATCAGCACC	AGATGCAGCAGATCCGCAT
Mouse Acox	GCTAGCAGCCTTACAGGGTG	TCAGAGGTCAAGGCCACTCTG
Mouse Cpt1a	GGTTTGACAAGTCCATCACCT	CCTTTACAGTGTCCATCCTCTG
Mouse Fasn	CTGACTCGGCTACTGACACG	TGAGCTGGGTTAGGGTAGGA
Mouse G6pc	GTCTGGATTCTACCTGCTAC	AAAGACTTCTTGTGTGTCTGTC
Mouse Gcgr	ACGGTACAGCCAGAAGATTG	TCTACCAGCAACCAGCAATAG
Mouse Kctd17	GAGCTCACACAGATGGTATCC	TGGTCCTCACTCCCATAGTT
Mouse Pck1	CCTGGAAGAACAAGGAGTGG	AGGGTCAATAATGGGGCACT
Mouse Phlpp1	AGGGTCCCGGAGACGATAAG	AGGGCGGAGATGTCTTTTGC
Mouse Phlpp2	GCCACAATCTTCTTACAGAGGTC	TCGAGGGGAATGTGCTCCA
Mouse Ppara	CCTACGCTTGGGGATGAAGAG	GACTGCCGTTGTCTGTCACTGT
Mouse Srebp1c	GAAGCTGTCGGGGTAGCGTCT	CTCTCAGGAGAGTTGGCACCTG
Human ACTB	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
Human DSCAML1	CATCGGCTATCCCTACTACTC	TTGAGGGTCCCATTCTCAAAC
Human GLIS3	CAACCAGATCAGTCCTAGCTTAC	CCAAAGACTCACGCGAAATAAG
Human JADE1	CGGGAGCAGGATGTCTTATTT	GGTCACCATGTAAGTGAGGTT
Human KCTD17	AGAGAAGGACTACACGGTCA	CCATCAGACATGGTGGAGAC
Human PHLPP2	CATACAGGAGGAGGCTACAAATC	GGTCTTTCCCTTGCGTACAT

Supplementary Table 2. Demographic, clinical and histological features in a cross-sectional liver biopsy cohort

	Liver biopsy cohort (n=158)
Age, years	45±10
Sex, female	95 (60)
BMI, Kg/m^2	37.8±8.5
Type 2 Diabetes, yes	41 (26)
ALT, IU/l	28 [18-157]
AST, IU/l	22 [17-33]
PNPLA3, 148M/M	25 (16)
Histological steatosis grade	
0	17 (11)
1	50 (32)
2	49 (31)
3	42 (27)
Definite NASH, yes	31 (19)

Data represent means of ± SD, median [interquartile range], or number (%).

Supplementary Table 3. Predictors of hepatic *KCTD17* expression in the cross-sectional liver biopsy cohort

	Univariate analysis			**Multivariate analysis**		
	Beta	SE	*P* value	Beta	SE	*P* value
Age, years	+0.03	0.01	0.001	+0.02	0.01	0.012
Sex, Female	-0.24	0.09	0.008	-0.07	0.10	0.46
BMI, Kg/m^2	-0.03	0.01	0.011	-0.02	0.01	0.061
Type 2 diabetes, yes	+0.33	0.10	0.001	+0.12	0.11	0.25
PNPLA3 148M, alleles	-0.08	0.13	0.55	-0.19	0.12	0.13
Steatosis grade	+0.37	0.09	<0.0001	+0.28	0.11	0.008
Histological activity (Necroinflammation + ballooning)	+0.31	0.09	0.0008	+0.02	0.11	0.87

SE: standard error. Comparisons were made by fitting data to generalized linear models, unadjusted (univariate analyses), or considering as independent variables: age, sex, BMI, type 2 diabetes, *PNPLA3* I148M alleles, histological steatosis grade, and activity. Hepatic *KCTD17* mRNA levels were normalized for β-actin and log transformed before analyses to ensure a normal distribution.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. *Phlpp2* expression is inversely correlated with lipogenic gene expression

(*A*) Gene set enrichment analysis (GSEA) using all hepatic transcriptomes (GSE60149) of BXD lines, highlighting the strains showing lowest (blue) or highest (red) *Phlpp2* expression. (*B*) Normalized enrichment score of GSEA indicating gene sets that show negative correlation with hepatic *Phlpp2* expression.

Supplementary Figure 2. Generation of conditional PHLPP2 knockout mice

(*A*) Targeting strategy to generate *PHLPP2*$^{flox/flox}$ allele, and (*B*) genotyping analysis of successfully targeted allele. (*C*) Western blots from liver, epididymal (eWAT) or inguinal white adipose tissue (iWAT), brown adipose tissue (BAT), and whole pancreas in *PHLPP2* floxed mice transduced with AAV8-TBG-GFP or AAV8-TBG-Cre. (*D*) Western blots in primary hepatocytes isolated from *PHLPP2*$^{flox/flox}$ mice, transduced with Ad-GFP or Ad-Cre.

Supplementary Figure 3. Prolonged Akt phosphorylation in *L-PHLPP2* mice leads to fatty liver

(*A*) Body weight, (*B*) normalized epidydimal (eWAT) or inguinal (iWAT) white adipose tissue weight, (*C*) liver cholesterol or (*D-F*) plasma cholesterol, non-esterified fatty acid (NEFA) and triglyceride in chow-fed Cre- or *L-PHLPP2* mice (n=9-10/group). (*G*) Western blots from primary hepatocytes isolated from *PHLPP2*$^{flox/flox}$ mice, transduced with Ad-GFP or Ad-Cre with or without 10 nM insulin for 6 h. (*H*) Blood glucose, (*I*) insulin, (*J*) intraperitoneal glucose tolerance test (GTT) and (*K*) intraperitoneal pyruvate tolerance test (PTT) in Cre- and *L-PHLPP2* mice. (*L*) Glucose released by primary hepatocytes isolated from *PHLPP2*$^{flox/flox}$ mice, then transduced with Ad-GFP or Ad-Cre prior to incubation with dexamethasone (Dex), forskolin (Fsk) and/or insulin for 4 h. (*M*) Liver expression of key regulators of fatty acid oxidation in chow-fed Cre- or

L-PHLPP2 mice (n-9-10/group). *P < .05 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m.

Supplementary Figure 4. HFD-fed *L-PHLPP2* mice show normal glucose/lipid homeostasis

(*A*) Experimental outline, (*B*) western blot, (*C*) liver weight and (*D*) triglyceride, (*E*) body weight, (*F*) blood glucose, (*G*) eWAT weight, (*H*) liver protein, (*I*) plasma triglyceride, (*J*) cholesterol and (*K*) NEFA levels, and (*L*) liver mRNA in Cre- or *L-PHLPP2* mice fed HFD for 16 weeks. (n=9-10/group). ***P < .001 as compared to Cre- mice by two-way ANOVA. All data are shown as the means ± s.e.m.

Supplementary Figure 5. *PHLPP2* KO cells have prolonged insulin-mediated lipogenic gene expression

(*A* and *B*) Generation of *PHLPP2 KO* cells using CRISPR/Cas9 by transducing HepG2 hepatoma cells with lentivirus expressing three different single guide RNA (sgRNA). (*C*) qPCR analysis of predicted potential off-target genes for sgRNAs. (*D*) Western blots from control or *PHLPP2* KO cells “pulsed” with 10 nM insulin for 30 min, then “chased” for 1-3 hours in insulin-free media. (*E*) qPCR from control and *PHLPP2* KO cells after insulin exposure for 6 h. *P < .05 and **P < .01 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m.

Supplementary Figure 6. Glucagon/PKA-induced phosphorylation of PHLPP2 at Ser1119 and Ser1210

(*A-C*) Western blots from primary hepatocytes incubated with insulin or the indicated kinase inhibitors (*A*), or transfected with PHLPP2-WT, S1119A, or S1210A (*B*), or S1119A/S1210A (2A) mutant with or without forskolin and the PKA inhibitor H-89 (*C*), prior to Phos-tag western blot.

(*D, E*) Validation of novel PHLPP2 Ser 1119- or 1210-specific antibodies in hepatocytes transfected with empty vector (Ctrl), PHLPP2-WT, S1119A, S1210A or -2A with or without forskolin. (*F*) Western blots from primary hepatocytes incubated in serum-free medium, then treated with forskolin, serum, insulin, amino acid deprivation (-AA) for 1 h or amino acid deprivation for 50 min followed by amino acid and serum for 10 min (AA + serum). (*G*) CoIP of WT but not 2A with phospho-PKA substrate antibody in response to glucagon or forskolin in primary hepatocytes.

Supplementary Figure 7. Glucagon/PKA-induced phosphorylation of PHLPP2 at Ser1119 and Ser1210 in fasted mice

(*A*) Western blots of liver lysate and (*B*) quantification of p-PHLPP2/total PHLPP2 and PHLPP2/β-actin. *$P < .05$ and **$P < .01$ as compared to fasted mice by two-way ANOVA. All data are shown as the means ± s.e.m.

Supplementary Figure 8. Similar expression of WT and 2A in obese mice

(*A*) Hepatic *Phlpp2* expression, (*B*) body weight, (*C*) normalized eWAT weight, (*D*) blood glucose, and (*E*) insulin levels in C57BL/6 WT mice fed HFD for 15 weeks, then transduced with Ad-GFP, Ad-PHLPP2 (WT), or Ad-PHLPP2 (2A) prior to sacrifice at a total of 16 weeks HFD feeding (n=6-7/group). ***$P < .001$ as compared to Ad-GFP-transduced mice by two-way ANOVA. All data are shown as the means ± s.e.m.

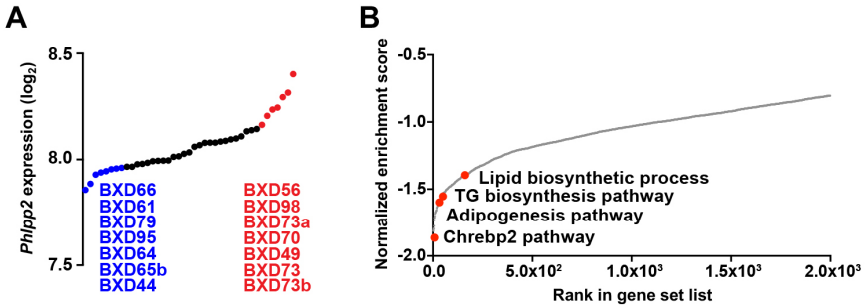
Supplementary Figure 9. Knockdown of KCTD17 prevents hepatic steatosis

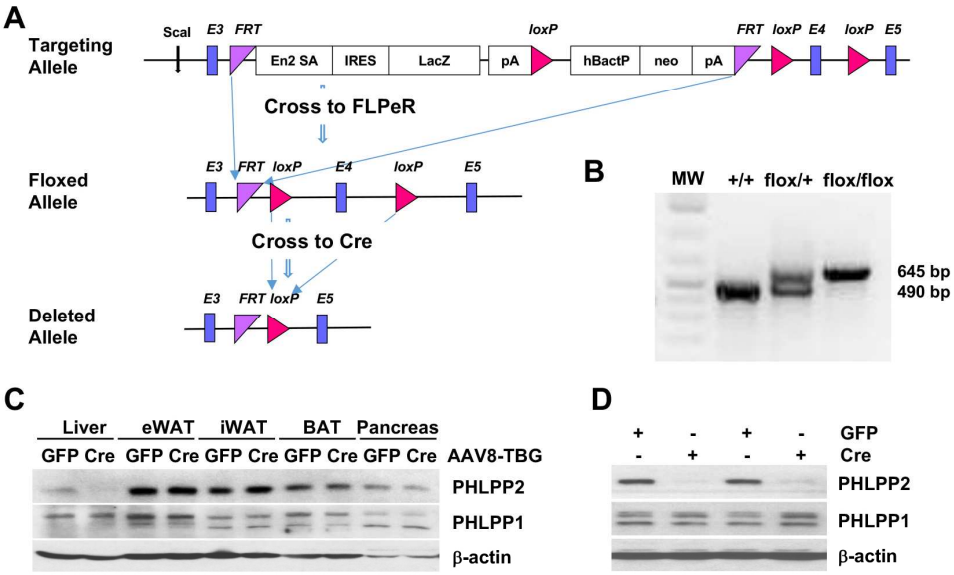
(*A*) Western blots and (*B*) qPCR from Hepa1c1c7 cells transduced with Ad-shControl or Ad-shKctd17. (*C*) Lipogenic gene expression in primary hepatocytes transduced with Ad-shControl or Ad-shKctd17 after insulin exposure for 6 h (n=4 biologic replicates). (*D*) qPCR, (*E*) body weight and (*F*) normalized eWAT weight in C57BL/6 WT mice fed HFD for 14 weeks, then

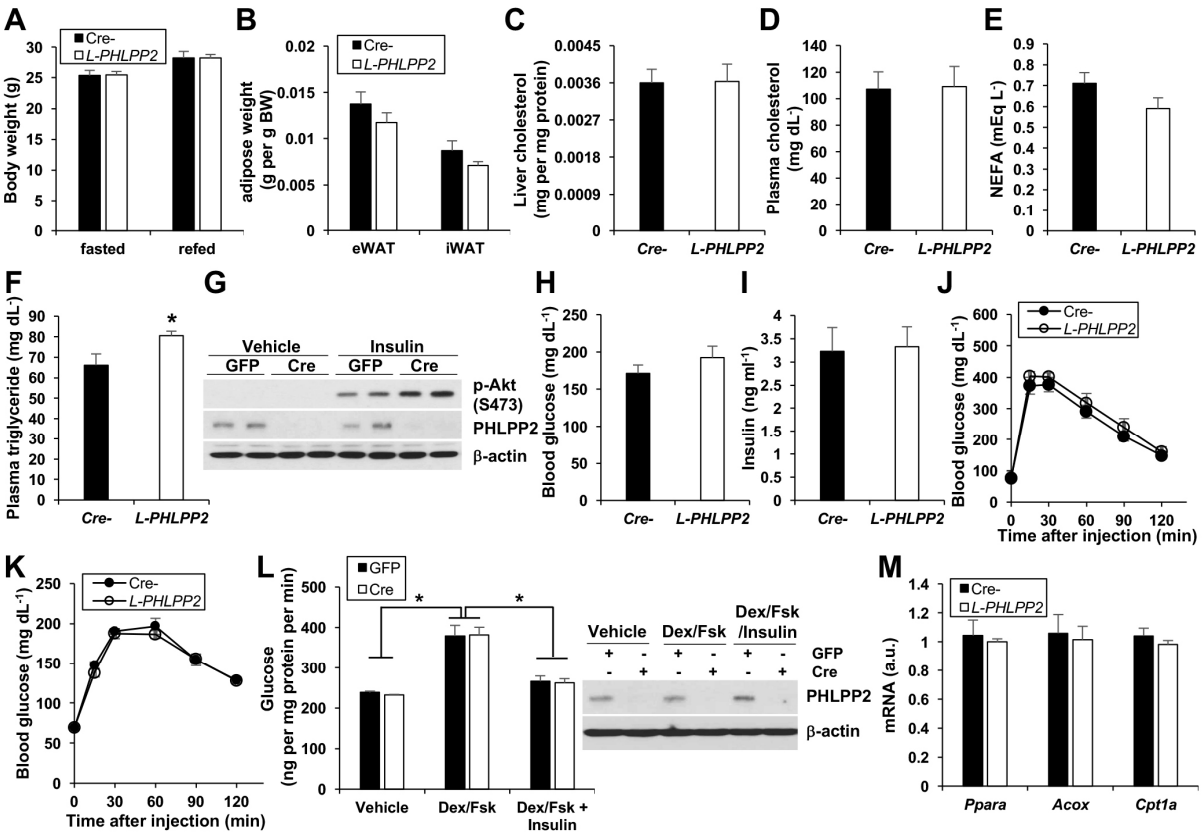
transduced with Ad-shControl or Ad-shKctd17 prior to sacrifice at a total of 16 weeks HFD feeding (n=6-7/group). *P < .05, **P < .01, and ***P < .001 as compared to the indicated control by two-way ANOVA. All data are shown as the means ± s.e.m.

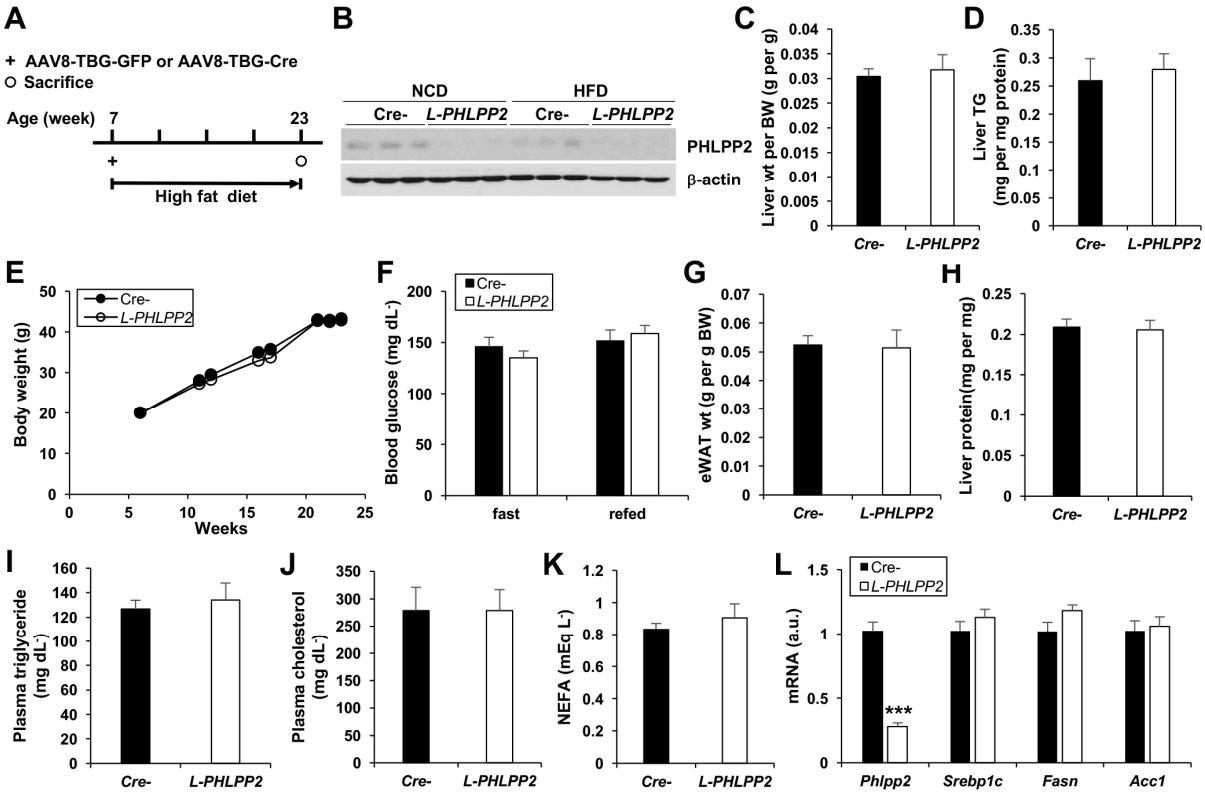
Supplementary Figure 10. *KCTD17* expression is correlated with multiple potentially pathogenic pathways in patients with NASH

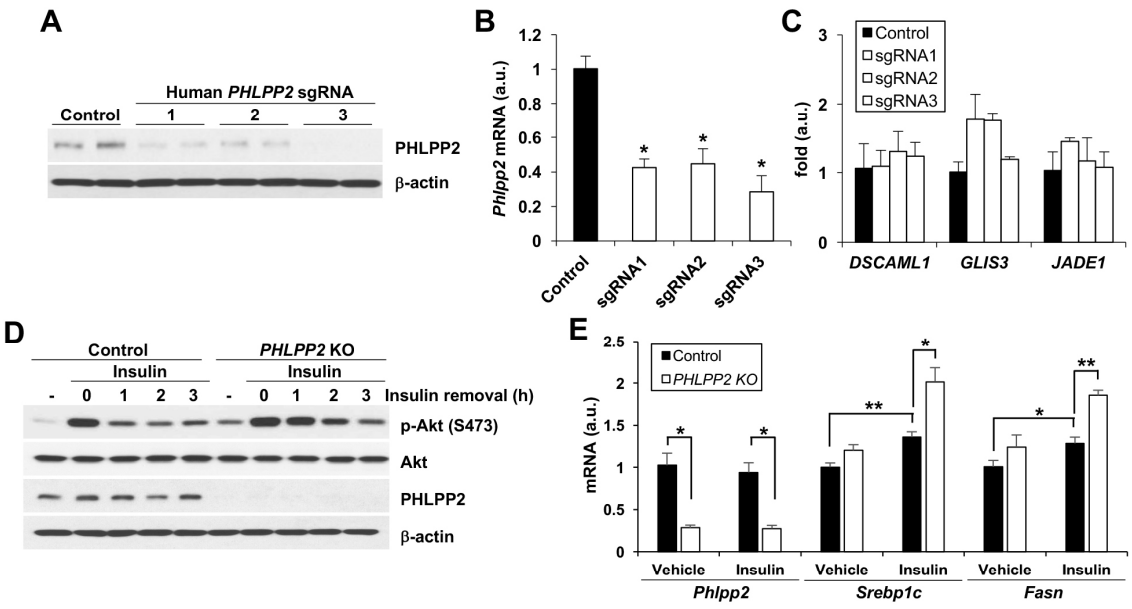
(*A*) Correlogram indicating Spearman's correlation between *KCTD17* and gene sets of indicated pathways from NASH subjects. (*B*) Interaction network showing *KCTD17* expression association with genes sets involved in DNL (light blue), fatty acid esterification (green), fatty acid oxidation (yellow), ER stress (purple), and inflammation (orange), with blue and red connecting lines indicating positive and negative correlations, respectively. Only correlations with Spearman's *Rho* > .5 or < -.5 are shown.

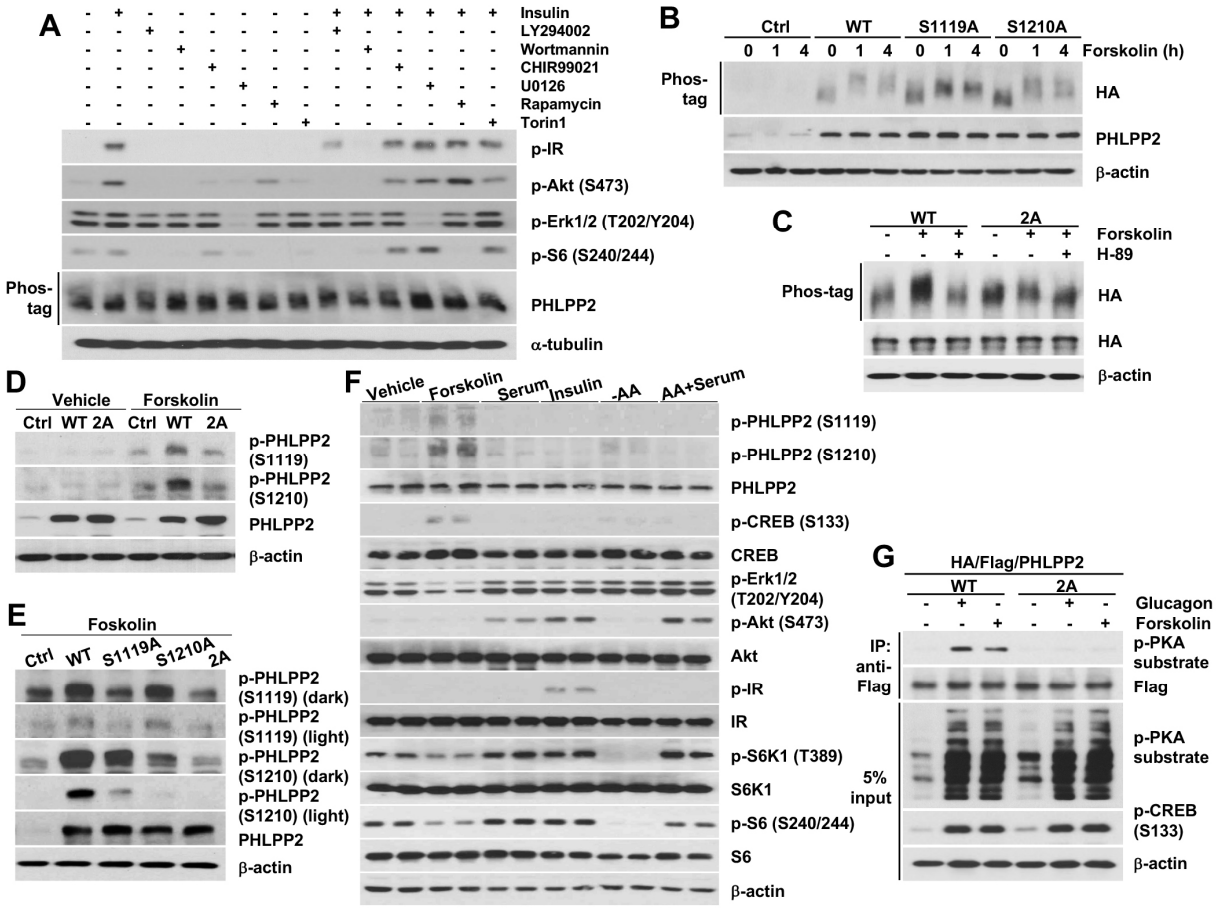

A
8.5
8.0
7.5
Phlpp2 expression (log2)
BXD66
BXD61
BXD79
BXD95
BXD64
BXD65b
BXD44
BXD56
BXD98
BXD73a
BXD70
BXD49
BXD73
BXD73b
B
-0.5
-1.0
-1.5
-2.0
Normalized enrichment score
Lipid biosynthetic process
TG biosynthesis pathway
Adipogenesis pathway
Chrebp2 pathway
0.0
5.0x10²
1.0x10³
1.5x10³
2.0x10³
Rank in gene set list

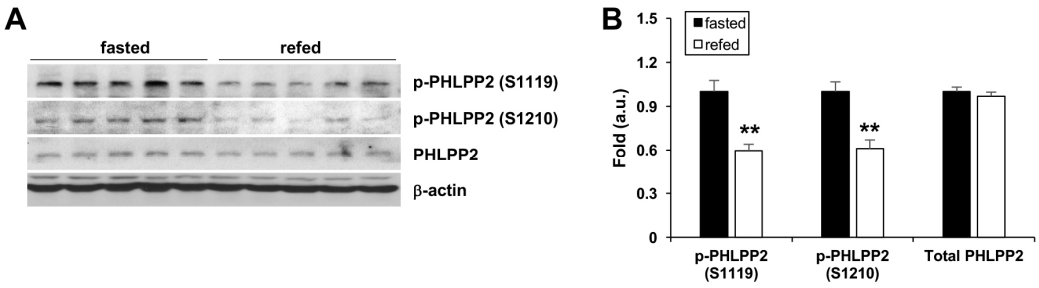

A
Targeting Allele
ScaI
E3
FRT
En2 SA
IRES
LacZ
pA
loxP
hBactP
neo
pA
FRT
loxP
E4
loxP
E5
Cross to FLPeR
Floxed Allele
E3
FRT
loxP
E4
loxP
E5
Cross to Cre
Deleted Allele
E3
FRT
loxP
E5
B
MW
+/+
flox/+
flox/flox
645 bp
490 bp
C
Liver
eWAT
iWAT
BAT
Pancreas
GFP Cre GFP Cre GFP Cre GFP Cre GFP Cre
AAV8-TBG
PHLPP2
PHLPP1
β-actin
D
+ - + -
- + - +
GFP
Cre
PHLPP2
PHLPP1
β-actin

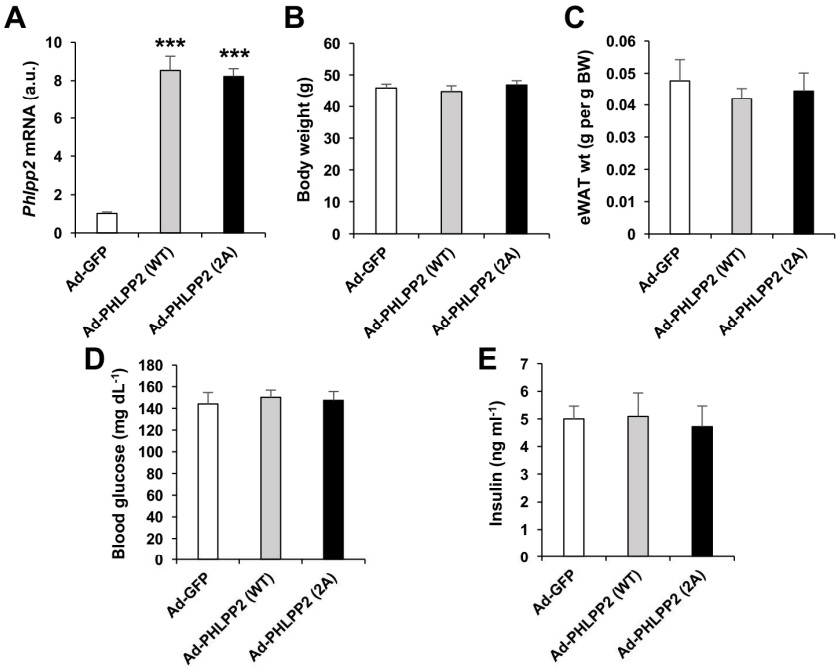

A
Cre-
L-PHLPP2
Body weight (g)
fasted
refed
B
adipose weight (g per g BW)
eWAT
iWAT
C
Liver cholesterol (mg per mg protein)
Cre-
L-PHLPP2
D
Plasma cholesterol (mg dL-1)
E
NEFA (mEq L-1)
F
Plasma triglyceride (mg dL-1)
G
Vehicle
Insulin
GFP
Cre
p-Akt (S473)
PHLPP2
β-actin
H
Blood glucose (mg dL-1)
I
Insulin (ng ml-1)
J
Time after injection (min)
K
L
GFP
Cre
Glucose (ng per mg protein per min)
Vehicle
Dex/Fsk
Dex/Fsk + Insulin
Dex/Fsk /Insulin
M
mRNA (a.u.)
Ppara
Acox
Cpt1a

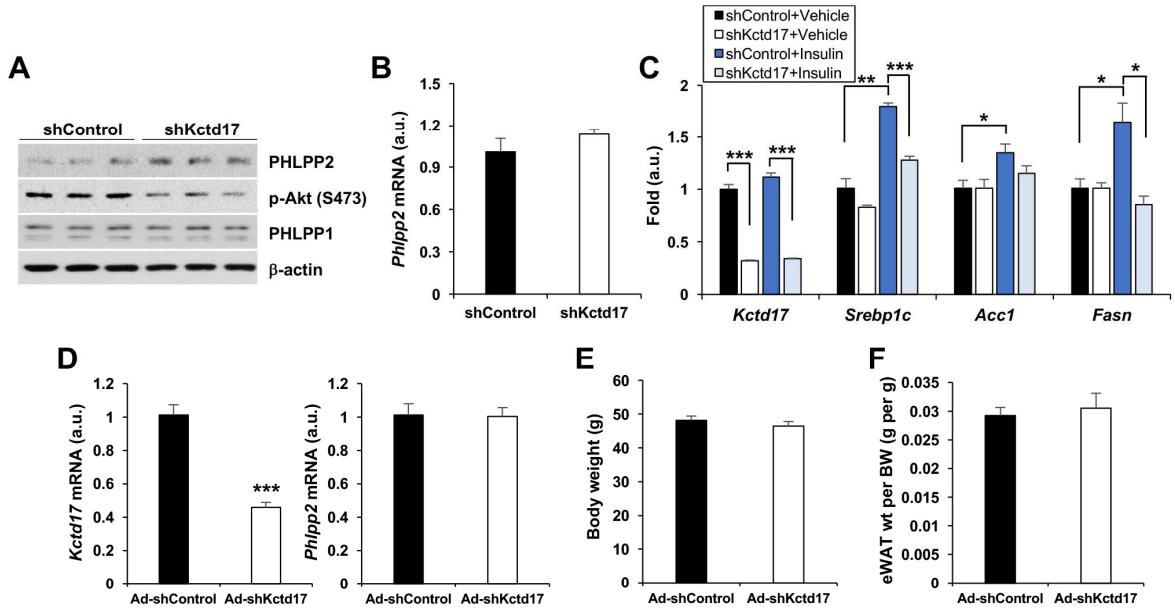

A
+ AAV8-TBG-GFP or AAV8-TBG-Cre
O Sacrifice
Age (week) 7
23
High fat diet
B
NCD
HFD
Cre-
L-PHLPP2
Cre-
L-PHLPP2
PHLPP2
β-actin
C
Liver wt per BW (g per g)
Cre-
L-PHLPP2
D
Liver TG (mg per mg protein)
Cre-
L-PHLPP2
E
Body weight (g)
Cre-
L-PHLPP2
Weeks
F
Blood glucose (mg dL-)
Cre-
L-PHLPP2
fast
refed
G
eWAT wt (g per g BW)
Cre-
L-PHLPP2
H
Liver protein(mg per mg)
Cre-
L-PHLPP2
I
Plasma triglyceride (mg dL-)
Cre-
L-PHLPP2
J
Plasma cholesterol (mg dL-)
Cre-
L-PHLPP2
K
NEFA (mEq L-)
Cre-
L-PHLPP2
L
Cre-
L-PHLPP2
mRNA (a.u.)
***
Phlpp2
Srebp1c
Fasn
Acc1


A
Human PHLPP2 sgRNA
Control
1
2
3
PHLPP2
β-actin
B
Phlpp2 mRNA (a.u.)
1.2
1
0.8
0.6
0.4
0.2
0
Control
sgRNA1
sgRNA2
sgRNA3
C
Control
sgRNA1
sgRNA2
sgRNA3
fold (a.u.)
3
2
1
0
DSCAML1
GLIS3
JADE1
D
Control
PHLPP2 KO
Insulin
Insulin
- 0 1 2 3 - 0 1 2 3 Insulin removal (h)
p-Akt (S473)
Akt
PHLPP2
β-actin
E
Control
PHLPP2 KO
mRNA (a.u.)
2.5
2
1.5
1
0.5
0
Vehicle
Insulin
Vehicle
Insulin
Vehicle
Insulin
Phlpp2
Srebp1c
Fasn


A
Insulin
LY294002
Wortmannin
CHIR99021
U0126
Rapamycin
Torin1
p-IR
p-Akt (S473)
p-Erk1/2 (T202/Y204)
p-S6 (S240/244)
Phos-tag
PHLPP2
α-tubulin
B
Ctrl
WT
S1119A
S1210A
0 1 4 0 1 4 0 1 4 0 1 4
Forskolin (h)
Phos-tag
HA
PHLPP2
β-actin
C
WT
2A
Forskolin
H-89
Phos-tag
HA
HA
β-actin
D
Vehicle
Forskolin
Ctrl WT 2A Ctrl WT 2A
p-PHLPP2 (S1119)
p-PHLPP2 (S1210)
PHLPP2
β-actin
E
Foskolin
Ctrl WT S1119A S1210A 2A
p-PHLPP2 (S1119) (dark)
p-PHLPP2 (S1119) (light)
p-PHLPP2 (S1210) (dark)
p-PHLPP2 (S1210) (light)
PHLPP2
β-actin
F
Vehicle
Forskolin
Serum
Insulin
-AA
AA+Serum
p-PHLPP2 (S1119)
p-PHLPP2 (S1210)
PHLPP2
p-CREB (S133)
CREB
p-Erk1/2 (T202/Y204)
p-Akt (S473)
Akt
p-IR
IR
p-S6K1 (T389)
S6K1
p-S6 (S240/244)
S6
β-actin
G
HA/Flag/PHLPP2
WT
2A
Glucagon
Forskolin
IP: anti-Flag
p-PKA substrate
Flag
5% input
p-PKA substrate
p-CREB (S133)
β-actin


A
fasted
refed
p-PHLPP2 (S1119)
p-PHLPP2 (S1210)
PHLPP2
β-actin
B
fasted
refed
Fold (a.u.)
1.5
1.2
0.9
0.6
0.3
0
**
**
p-PHLPP2 (S1119)
p-PHLPP2 (S1210)
Total PHLPP2


A
Phlpp2 mRNA (a.u.)
10
8
6
4
2
0
***
***
Ad-GFP
Ad-PHLPP2 (WT)
Ad-PHLPP2 (2A)
B
Body weight (g)
60
50
40
30
20
10
0
Ad-GFP
Ad-PHLPP2 (WT)
Ad-PHLPP2 (2A)
C
eWAT wt (g per g BW)
0.06
0.05
0.04
0.03
0.02
0.01
0
Ad-GFP
Ad-PHLPP2 (WT)
Ad-PHLPP2 (2A)
D
Blood glucose (mg dL-1)
180
160
140
120
100
80
60
40
20
0
Ad-GFP
Ad-PHLPP2 (WT)
Ad-PHLPP2 (2A)
E
Insulin (ng ml-1)
7
6
5
4
3
2
1
0
Ad-GFP
Ad-PHLPP2 (WT)
Ad-PHLPP2 (2A)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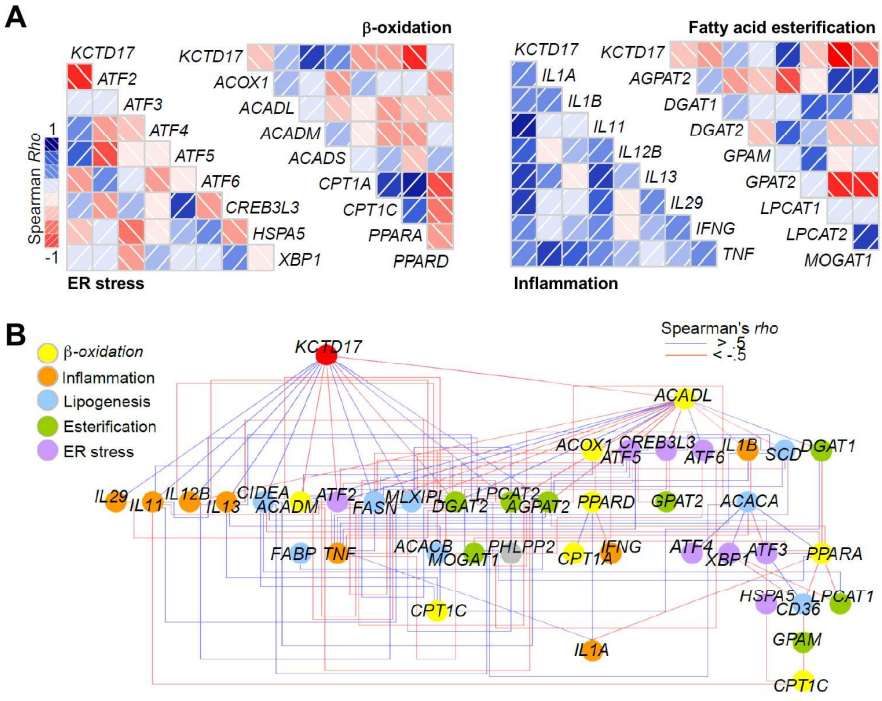
shControl
shKctd17
PHLPP2
p-Akt (S473)
PHLPP1
β-actin
B
Phlpp2 mRNA (a.u.)
shControl
shKctd17
C
shControl+Vehicle
shKctd17+Vehicle
shControl+Insulin
shKctd17+Insulin
Fold (a.u.)
Kctd17
Srebp1c
Acc1
Fasn
D
Kctd17 mRNA (a.u.)
Phlpp2 mRNA (a.u.)
Ad-shControl
Ad-shKctd17
E
Body weight (g)
F
eWAT wt per BW (g per g)


A
β-oxidation
Fatty acid esterification
KCTD17
ATF2
ATF3
ATF4
ATF5
ATF6
CREB3L3
HSPA5
XBP1
ACOX1
ACADL
ACADM
ACADS
CPT1A
CPT1C
PPARA
PPARD
IL1A
IL1B
IL11
IL12B
IL13
IL29
IFNG
TNF
AGPAT2
DGAT1
DGAT2
GPAM
GPAT2
LPCAT1
LPCAT2
MOGAT1
Spearman Rho
1
-1
ER stress
Inflammation
B
β-oxidation
Inflammation
Lipogenesis
Esterification
ER stress
Spearman's rho
> .5
< -.5
KCTD17
ACADL
ACOX1
CREB3L3
ATF5
ATF6
IL1B
SCD
DGAT1
IL29
IL11
IL12B
IL13
CIDEA
ACADM
ATF2
FASN
MLXIPL
DGAT2
LPCAT2
AGPAT2
PPARD
GPAT2
ACACA
FABP
TNF
ACACB
MOGAT1
PHLPP2
CPT1A
IFNG
ATF4
XBP1
ATF3
PPARA
HSPA5
CD36
LPCAT1
CPT1C
GPAM
IL1A
CPT1C