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Supplementary Materials for

Mitochondrial ACSS1-K635 acetylation knock-in mice exhibit altered metabolism, cell senescence, and nonalcoholic fatty liver disease

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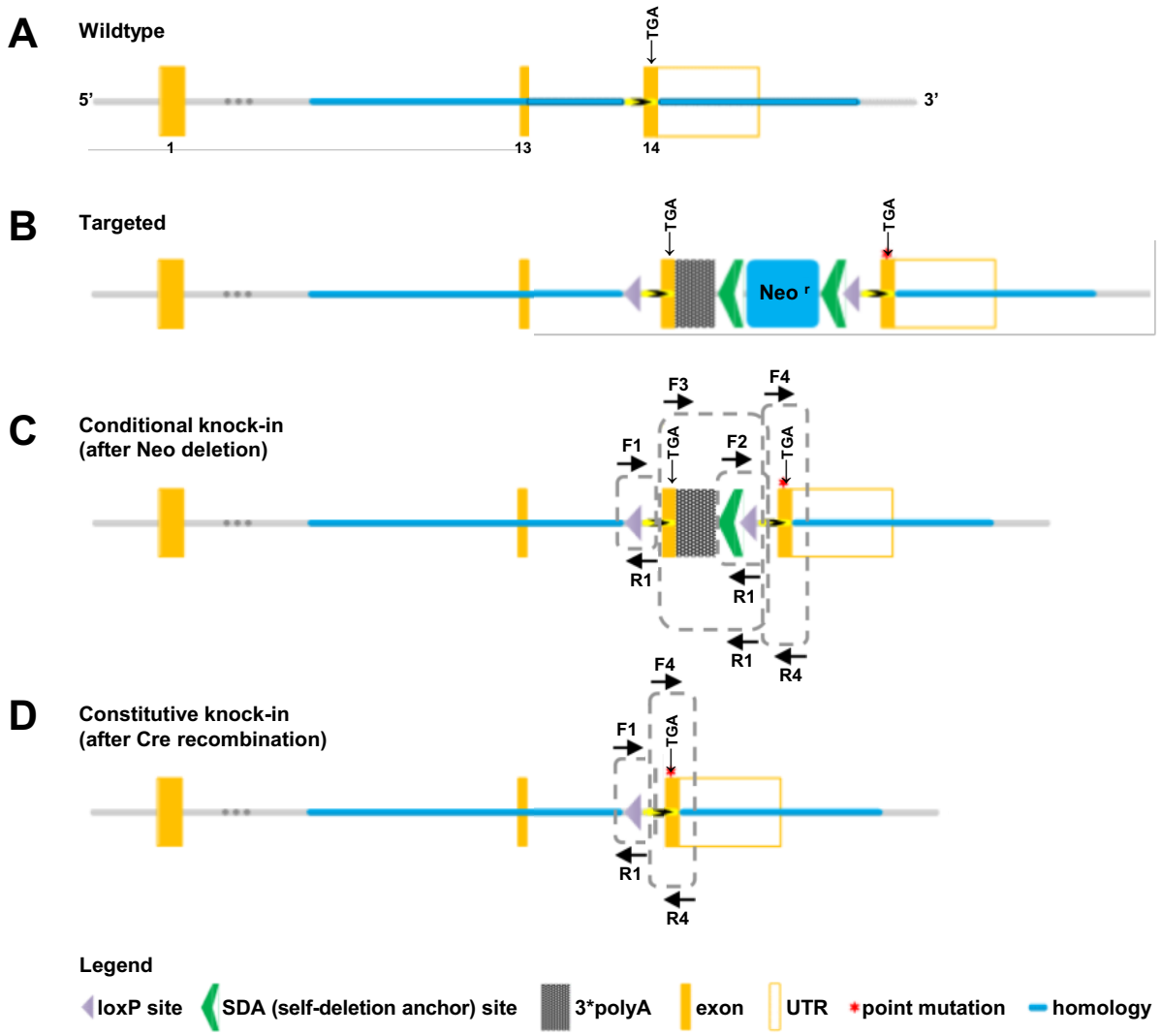

Primers for loxP PCR: **loxP-F (F1):** 5'-TTTTCCCCAACTAAGCCCGAG-3'
loxP-R (R1): 5'-TCCCCCTGCAAACTGAGCCTGAAG-3'

Primers for neo deletion PCR: **Neo-del-F (F2):** 5'-CGCAGATCTGCAAGCTAATTCCCAC-3'
loxP-R (R1): 5'-TCCCCCTGCAAACTGAGCCTGAAG-3'

Primers for KI PCR: **KI-F (F3):** 5'-GGCAAAGTTGCGTTGTAGTCCTCTG-3'
loxP-R (R1): 5'-TCCCCCTGCAAACTGAGCCTGAAG-3'

Primers for sequencing PCR: **Seq-F (F4):** 5'-GATGTCAGCTTCAGGCTCAGTTTG-3'
Seq-R (R4): 5'-GCACACCTCTCAGTTGGTAGCAGC-3'

Fig. S1. Schema for generation of the *Acss1*^{K635Q} mouse model. (A) Lysine 635 (the targeted allele or TGA) is located in the 14th and final exon of *Acss1*. **(B)** Mouse genomic fragments containing exon 14 with the *Acss1* point mutation (AAA > CAA), homology arms, and a conditional knockout region were amplified and then assembled into a targeting vector with recombination sites and selection markers. This targeting construct was linearized and used to transfect C57BL/6 embryonic stem (ES) cells, which were then selected and amplified. Correctly targeted ES cells were injected into albino C57BL/6 embryos and founders initially identified by coat color. **(C)** The presence of the knock-in and loxP sites and deletion of the neomycin resistance cassette were confirmed by PCR in founder conditional *Acss1* knock-in mice (see Figure S2). **(D)** Crossing homozygous conditional *Acss1* knock-in mice with the Sox2Cre transgenic line induced recombination of the conditional alle in the germline, replacing the endogenous exon 14 with the knock-in, and generating full-body constitutive *Acss1*^{K635Q} mice.

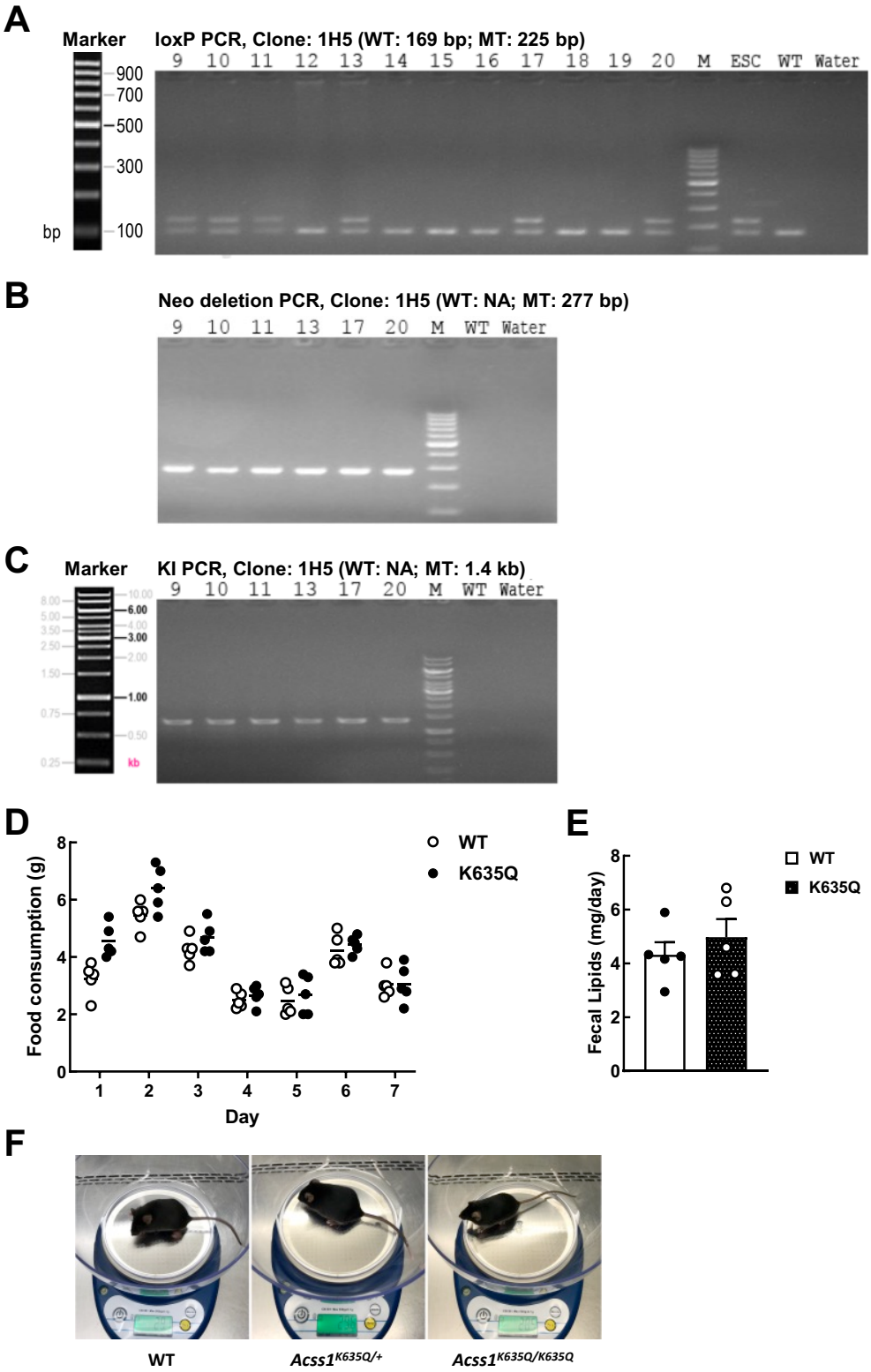

Fig. S2. Validation and characterization of the *Acss1*^{K635Q} mouse model. (A-C) Correctly targeted C57BL/6 embryonic stem cell clones were injected into C57BL/6 albino embryos, which were then implanted into CD-1 pseudo-pregnant females. Founder *Acss1*^{K635Q} conditional knock-in mice were identified by coat color and subsequent genotyping of offspring to confirm **(A)** presence of the loxP sites, **(B)** deletion of the neomycin resistance cassette, and **(C)** presence of the knock-in. **(D)** Body weight and **(E)** fecal lipids were measured in male wild-type and *Acss1*$^{K635Q/K635Q}$ mice over seven days ($N = 5$ mice per group). **(F)** Images of male *Acss1*$^{+/+}$, *Acss1*$^{K635Q/+}$, and *Acss1*$^{K635Q/K635Q}$ mice, weighing 29.1, 29.6, and 20.5 grams, respectively.

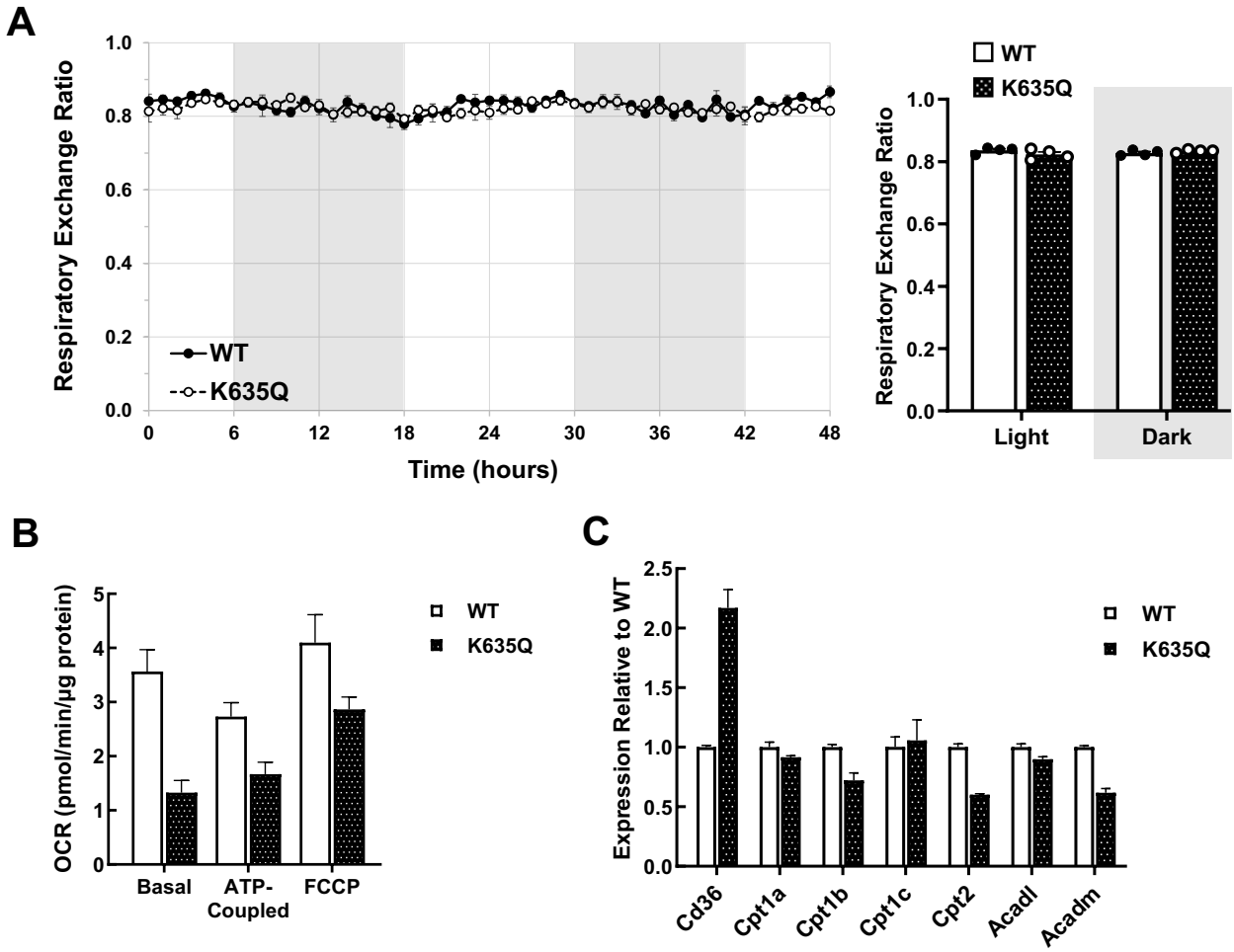

Fig. S3. Acss1-K636Q Alters Metabolism. (A) Respiratory exchange ratio (RER) in individually housed wild-type and *Acss1*$^{K635Q/K635Q}$ mice following a 3-week high-fat diet (HFD) (N = 4 mice per group). **(B)** Basal, ATP-coupled, and FCCP-uncoupled oxygen consumption rates (OCR) in wild-type and *Acss1*$^{K635Q/K635Q}$ primary hepatocytes with 0.5 mM glucose (N = 1 mice per group, 8 technical replicates each). **(C)** mRNA levels in *Acss1*$^{K635Q/K635Q}$ hepatocytes relative to wild-type days (N = 1 mouse per group, 4 technical replicates).

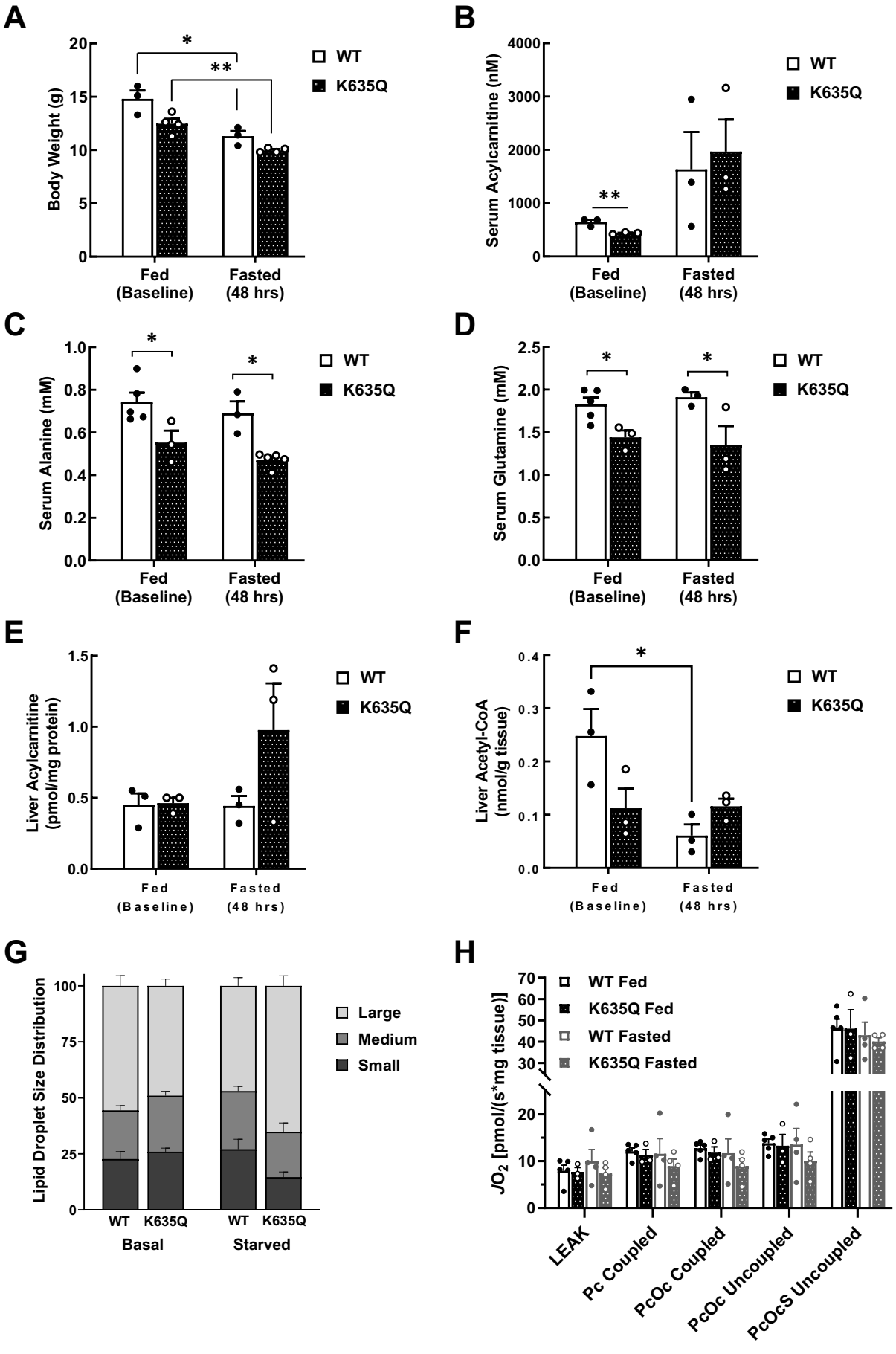
A
Body Weight (g)
20
15
10
5
0
*
**
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
B
Serum Acylcarnitine (nM)
4000
3000
2000
1000
0
**
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
C
Serum Alanine (mM)
1.0
0.8
0.6
0.4
0.2
0.0
*
*
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
D
Serum Glutamine (mM)
2.5
2.0
1.5
1.0
0.5
0.0
*
*
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
E
Liver Acylcarnitine (pmol/mg protein)
1.5
1.0
0.5
0.0
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
F
Liver Acetyl-CoA (nmol/g tissue)
0.4
0.3
0.2
0.1
0.0
*
WT
K635Q
Fed (Baseline)
Fasted (48 hrs)
G
Lipid Droplet Size Distribution
100
75
50
25
0
Large
Medium
Small
WT
K635Q
WT
K635Q
Basal
Starved
H
JO2 [pmol/(s*mg tissue)]
70
60
50
40
30
20
10
0
WT Fed
K635Q Fed
WT Fasted
K635Q Fasted
LEAK
Pc Coupled
PcOc Coupled
PcOc Uncoupled
PcOcS Uncoupled

Fig. S4. Metabolic dysregulation in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A) Body weight of wild-type (WT) and *Acss1*$^{K635Q/K635Q}$ (K635Q) mice at baseline and after a 48-hour fast. **(B-F)** Serum levels of **(B)** acylcarnitine, **(C)** alanine, and **(D)** glutamine and liver levels of **(E)** acylcarnitine and **(F)** acetyl-CoA in WT and K635Q mice at baseline and after a 48-hour fast. **(G)** Percentage of lipid droplets per cell scored as small (0.3-0.8 μm^2), medium (0.8-1.5 μm^2), or large (>1.5 μm^2) in WT and K635Q hepatocytes in basal conditions and after 1 hour starvation. **(H)** Oxygen flux (JO_2) per mass, measured in pmol oxygen per second per mg of liver tissue, stimulated by palmitoyl-carnitine but in the absence of ADP (LEAK respiration); palmitoyl-carnitine (Pc Coupled); palmitoyl-carnitine and octanoyl-carnitine (PcOc Coupled); palmitoyl-carnitine and octanoyl-carnitine with carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) (PcOc Uncoupled); and palmitoyl-carnitine, octanoyl-carnitine, and succinate with FCCP (PcOcS Uncoupled). Data represented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ calculated by two-tailed unpaired Student's *t*-test **(A-G)** or 2-way ANOVA **(H)**.

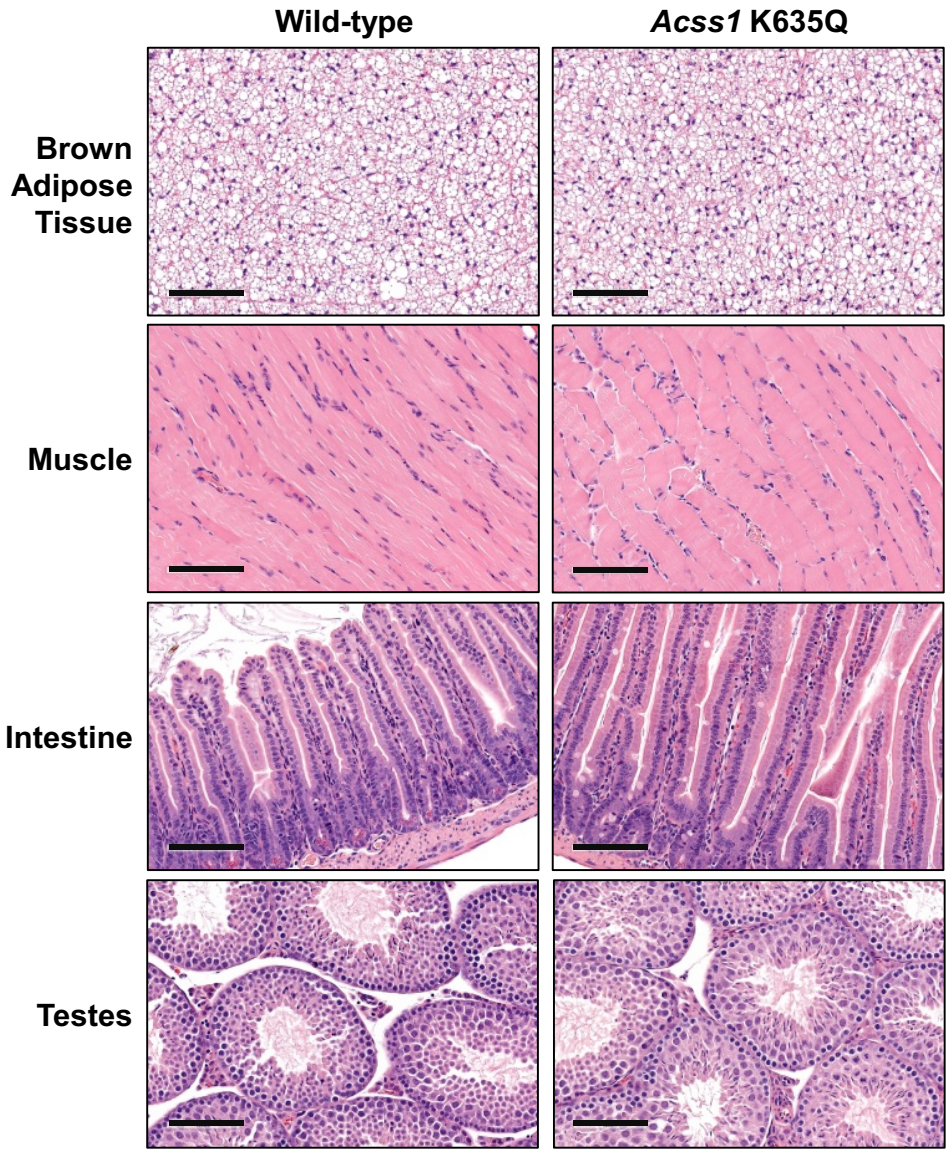

Fig. S5. Tissue histology with fasting. H&E staining of brown adipose tissue, muscle, intestine, and testes from wild-type and *Acss1*$^{K635Q/K635Q}$ mice after a 48-hour fast. Scale bar, 100 μm.


A
Hepatic Steatosis
WT
K635Q
MOGAT2
DDIT3
CD14
CIDEC
PDE4B
JUN
SOCS3
RORA
NOX4
IL1B
HMOX1
ACSL4
LIPE
NRIP1
CYP46A1
ADRB2
MYC
MAP3K14
ARNT
CBX2
IL1R1
DUSP1
UPP1
SUCNR1
GSTP1
E2F1
FBXO42
KLF2
TLR2
FABP4
PAQR3
CXCL10
MFSD2A
MAP3K5
LMNA
MDM2
MAPK13
EPAS1
LRP6
DDIT4
SLC2A1
TTC39B
CASP3
CD36
LPIN1
PPARGC1A
AGT
RALB
EIF6
RICTOR
IMPDH1
VSIG4
CAT
F8
METTL3
PDK2
IRF7
EHHADH
LZTFL1
SIRPA
AGK
GLDC
AGPAT2
ABCB11
CCR5
FGFR2
FANCC
GBP2
CAMKK2
STEAP2
BBS2
N6AMT1
NR1H3
PDE7B
PDE4C
IFIT3
TIMD4
CYP7A1
LEPR
PPARA
GPX7
CD48
LPL
B
NAFLD
WT
K635Q
DDIT3
FOS
JUN
SOCS3
NOX4
IL1B
LIPE
PPP2R5B
ATF6
SOCS7
GSK3B
TLR2
STAT3
TNFRSF1B
MAP3K5
ERN1
CASP3
CD36
FOXO3
PPP2R2A
LPIN1
PIK3C3
PPARA
C
Cellular Senescence
WT
K635Q
CDKN1A
GADD45A
EGR1
KLF6
SKIL
NOX4
JUNB
IL1B
RASSF1
HMOX1
SERPINE1
BMP4
CHD7
DUSP1
ATF6
TBPL1
E2F1
DYRK1A
CEBPB
PPARD
MNT
KLF4
CPEB1
USP28
UBC
KRAS
MAP3K5
BHLHE40
LMNA
RASSF5
SPRY4
ERCC5
IER2
ERN1
CD36
EIF4EBP1
ATXN1
MAP3K3
GABARAPL2
NUP62
AGT
TPR
RELA
LIN52
NCOA4
POLDIP2
CKS1B
SMARCAL1
CAT
DMPK
NUDT16L1
NUDT1
CDC14B
ZEB2
NBN
FGFR2
NCF1
LIN9
LIG1
TM4SF1
E2F6
BCL2L12
TERT
YEATS4
PATZ1
CUL7
D
Fatty Acid Metabolism
WT
K635Q
SLC10A2
CD14
SAA1
GDF15
EGR1
CHKA
MAP3K8
IL1B
ACOT2
LTC4S
PRNP
MAPKAPK2
ACSL4
LIPE
CYP46A1
AVPR1A
OSBPL2
MYC
MAP3K14
ARNT
ADA
SLC45A3
APOBEC1
PDK4
CS
AKAP13
SLC25A17
GSTP1
ABHD6
HMOX2
CEBPB
PPARD
TRIAP1
PIK3CB
TLR2
FABP4
PMP22
CYP1A2
ACSL3
MFSD2A
MAP3K5
SLCO2A1
NPC1
PLEKHA8
MDH2
SLC13A3
PCTP
SGMS1
ORAI1
TTC39B
GPC1
ABCA1
GM2A
CD36
KCNJ8
FOXO3
ABCA5
LPIN1
ANGPTL4
PPARGC1A
PLSCR1
AGT
EIF6
LIMK1
RELA
ECHDC3
SLC22A7
CBR4
APOF
ERLIN2
PPCS
STARD3
EHHADH
TSKU
ELOVL2
NCF1
CCL5
ABCD2
LEPR
FADD
SNCA
SIGIRR
DBP
2
1
0
-1
-2

Fig. S6. Altered gene expression in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A-D) Total RNAs were isolated from livers of wild-type and *Acss1*$^{K635Q/K635Q}$ mice after a 48-hour fast and sent for RNAseq ($N = 3$ mice per group). Sequence reads were mapped to the UCSC/mm9 mouse genome with HiSAT2 aligner and then quantified with StringTie. Differential expression analysis was done using the DESeq R package and the following significance criteria: FPKM > 1, adjusted P-value < 0.05, and fold change > 2. The differentially expressed genes were processed using Qiagen Ingenuity Pathway Analysis (IPA) software to determine enrichment and significance scores. Compared to fasted controls, the fasted *Acss1*$^{K635Q/K635Q}$ mice showed increased **(A)** hepatic steatosis, **(B)** non-alcoholic fatty liver disease (NALFD), and **(C)** cellular senescence, as well as **(D)** dysregulated fatty acid metabolism.

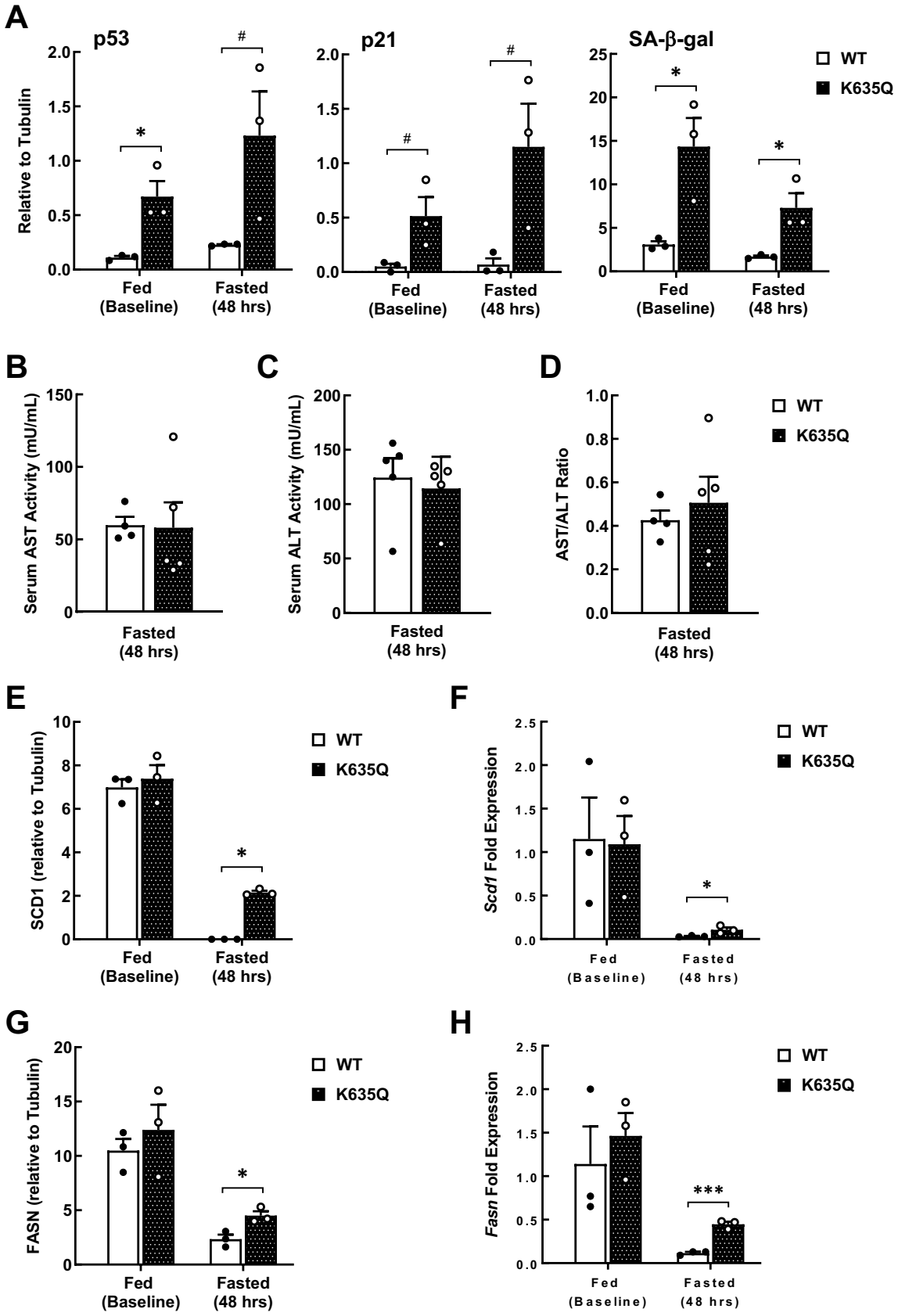

Fig. S7. Biomarkers of cell senescence, liver damage, and fatty acid synthesis in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A) Quantification of p53, p21, and SA-β-gal relative to Tubulin in immunoblots shown in main text Figure 5B. **(B-D)** Serum AST activity **(B)**, ALT activity **(C)**, and AST/ALT ratio

(D) in fasted wild-type and *Acss1*$^{K635Q/K635Q}$ mice. **(E)** Quantification of SCD1 band intensity relative to Tubulin in immunoblots shown in main text Fig. 6A. **(F)** Hepatic expression of *Scd1* as measured by qPCR in fed and fasted wild-type and *Acss1*$^{K635Q/K635Q}$ mice. **(G)** Quantification of FASN band intensity relative to Tubulin in immunoblots shown in main text Fig. 6C. **(H)** Hepatic expression of *Fasn* as measured by qPCR in fed and fasted wild-type and *Acss1*$^{K635Q/K635Q}$ mice. Data represented as mean ± SEM. $^{\#}P < 0.08$, $*P < 0.05$, $***P < 0.001$ calculated by two-tailed unpaired Student's *t*-test.

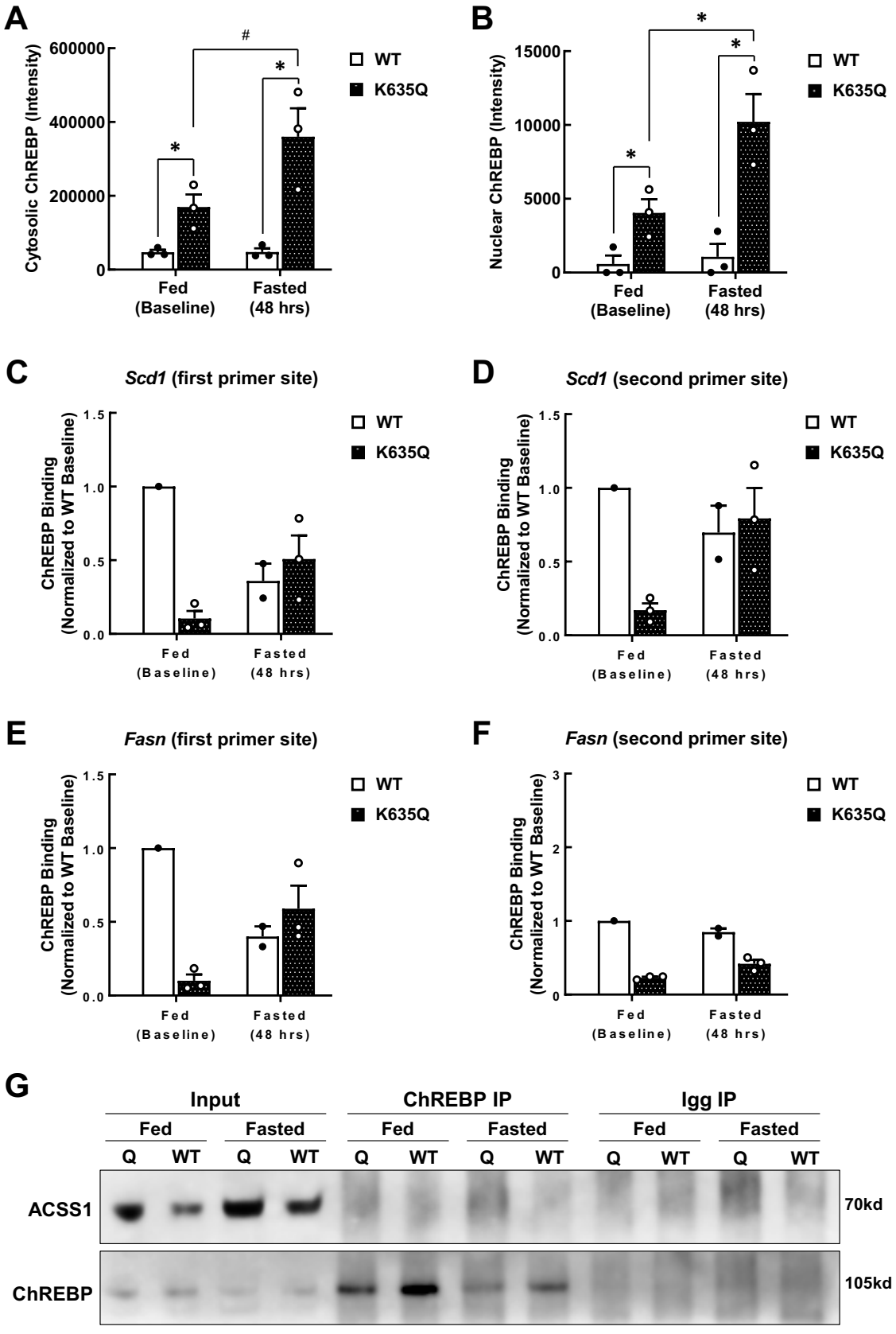

Fig. S8. ChREBP levels and binding. (A-B) Quantification of ChREBP band intensity in cytosolic **(A)** and nuclear **(B)** fractions of immunoblots shown in main text Figure 6E. Data represented as mean ± SEM. $^{\#}P < 0.08$, $^{*}P < 0.05$ calculated by two-tailed unpaired Student's *t*-test. **(C-F)** Binding of ChREBP to identified regions of *Scd1* **(C,D)** and *Fasn* **(E,F)**. Data normalized to wild-type baseline and shown as

mean ± SEM. **(G)** ChREBP was immunoprecipitated from liver lysate of fed and fasted wild-type and *Acss1*$^{K635Q/K635Q}$ mice and probed for ACSS1. Compare with Input (positive control) and Igg IP (negative control).

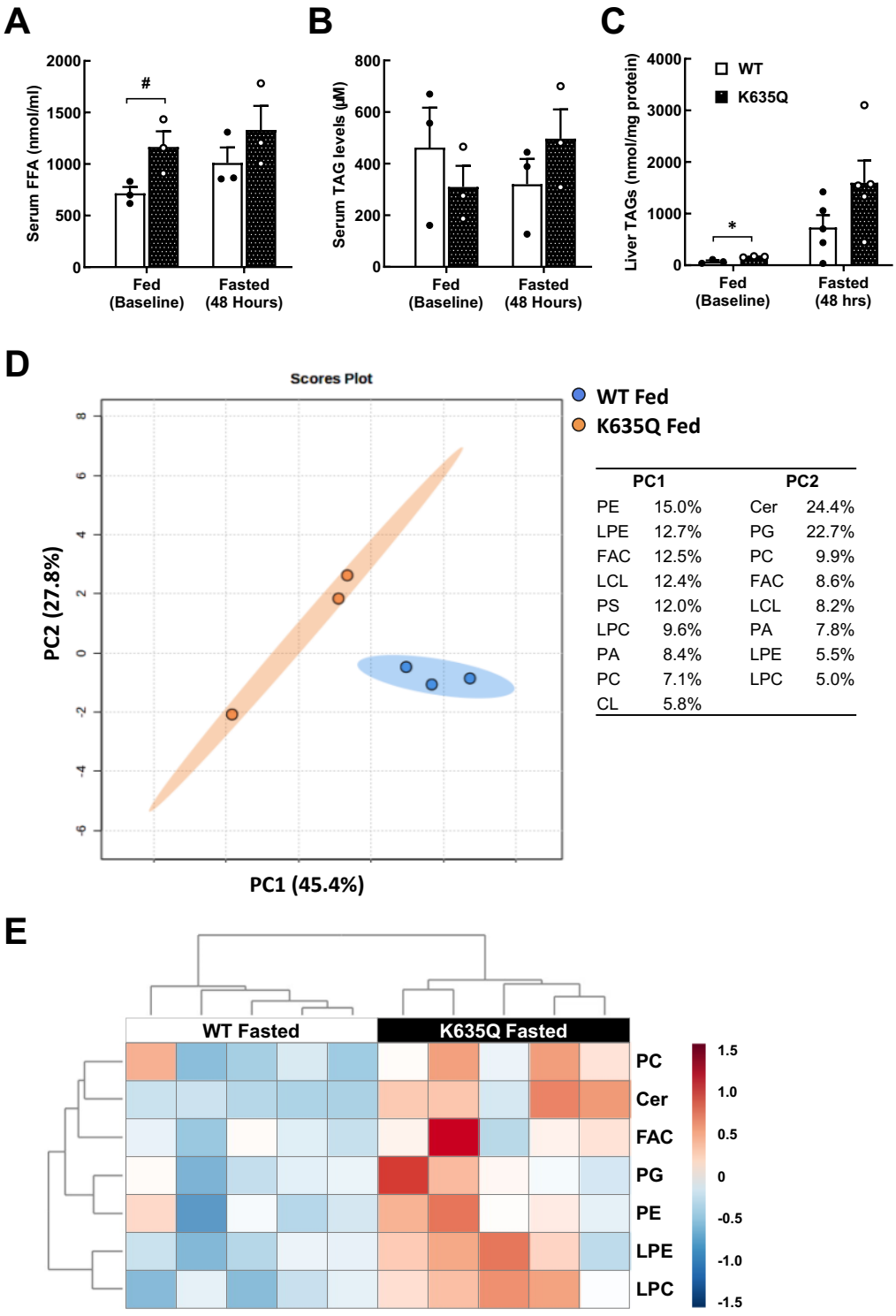

Fig. S9. Lipidomics in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A) Serum free fatty acid (FFA), **(B)** serum triacylglycerol (TAG), and **(C)** liver TAG levels in fed and fasted wild-type and *Acss1*$^{K635Q/K635Q}$ mice. Data represented as mean ± SEM. $^{\#}P < 0.08$, $^{*}P < 0.05$ calculated by two-tailed unpaired Student's *t*-test. **(D)** Principal component analysis of fed *Acss1*$^{K635Q/K635Q}$ mice vs. wild-type ($N = 3$ mice per group). **(E)** Top seven lipid classes by variance in fasted *Acss1*$^{K635Q/K635Q}$ vs. wild-type. Cer = ceramide, CL = cardiolipin, FAC = fatty acyl chains, LCL = lysocardiolipin, LPC = lysophosphatidylcholine, LPE = lysophosphatidylethanolamine, PA = phosphatidic acid, PC = phosphatidylcholine, PE = phosphatidylethanolamine, PG = phosphatidylglycerol, PS = phosphatidylserine

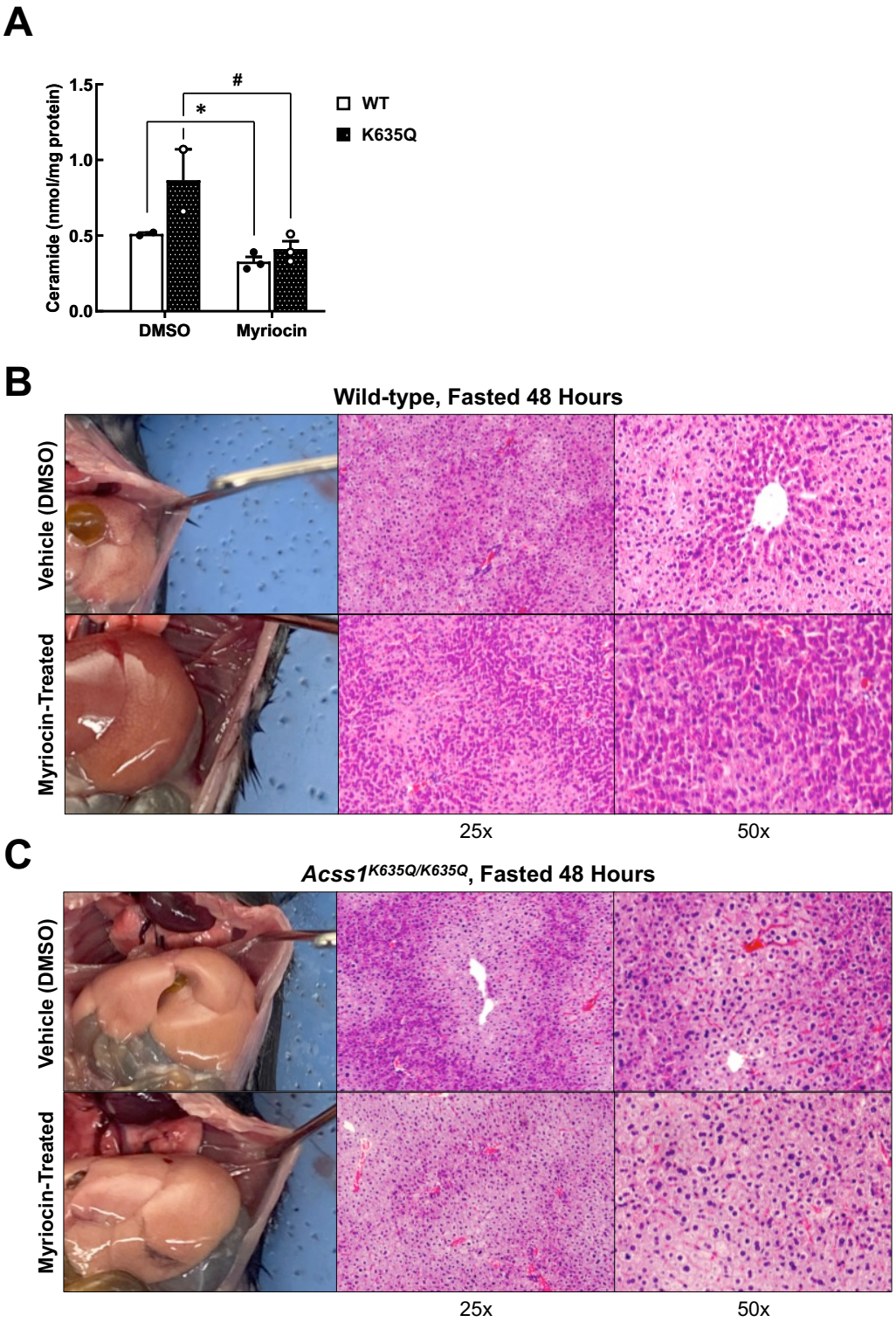

Fig. S10. Inhibition of ceramide synthesis does not prevent an NAFLD-like phenotype in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A) Ceramide levels in wild-type and *Acss1*$^{K635Q/K635Q}$ mice treated with myriocin vs. vehicle (DMSO) only. Data represented as mean ± SEM. $^{\#}P < 0.08$, $^{*}P < 0.05$ calculated by two-tailed unpaired Student's *t*-test. **(B-C)** Livers of **(B)** wild-type and **(C)** *Acss1*$^{K635Q/K635Q}$ mice treated with myriocin or vehicle only prior to a 48-hour fast.

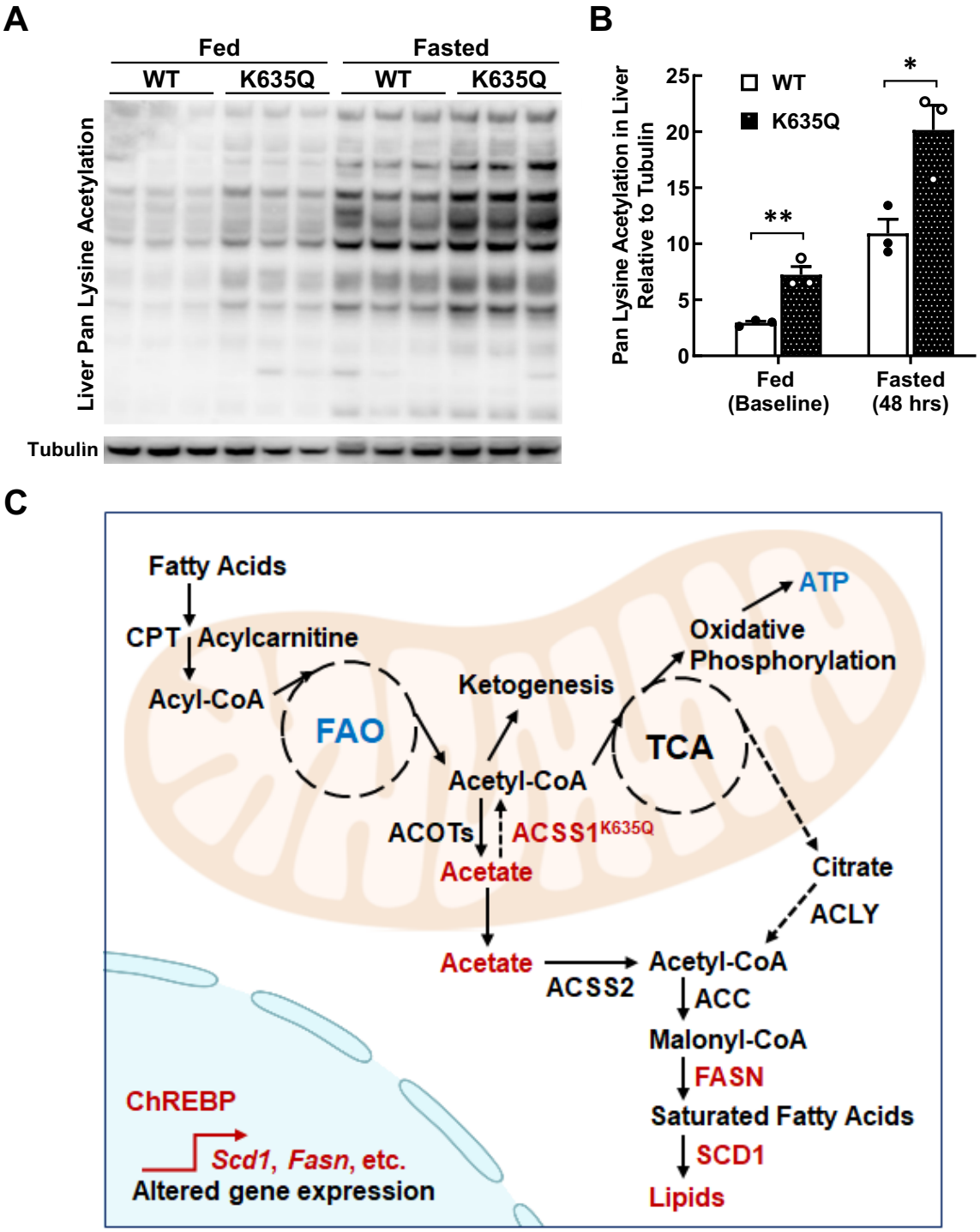

Fig. S11. Lysine acetylation and gene expression in fasted *Acss1*$^{K635Q/K635Q}$ mice. (A) Immunoblot showing liver pan-lysine acetylation in fed and fasted wild-type (WT) and *Acss1*$^{K635Q/K635Q}$ (K635Q) mice. **(B)** Quantification of Panel A. Data represented as mean ± SEM. *$P < 0.05$, **$P < 0.01$ calculated by two-tailed unpaired Student's *t*-test. **(C)** Schema representing *Acss1*$^{K635Q/K635Q}$ hepatocyte after 48-hour fast. Red text represents genes, metabolites, and other factors that are upregulated in the liver of fasted K635Q compared to fasted control. Blue text represents factors that are downregulated relative to fasted control. ACC = Acetyl-CoA Carboxylase, ACLY = ATP Citrate Lyase, ACOT = Acyl-CoA Thioesterase, ACSS = Acyl-CoA Synthetase Short Chain Family Member, ChREBP = Carbohydrate Response Element Binding Protein, CPT = Carnitine Palmitoyltransferase, FAO = Fatty Acid Oxidation, FASN = Fatty Acid Synthase, SCD = Stearoyl-CoA Desaturase, TCA = Tricarboxylic Acid Cycle.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Acetyllysine (1:1000 for IB)	PTMBIO	#PTM-105
Anti-ACSS1 (1:1000 for IB)	Proteintech	#17138-1-AP
Anti-ChREBP	Cell Signaling	#58069
Anti-ChREBP (1:250 for IB)	Santa Cruz	#sc-515922
Anti-FASN (1:1000 for IB)	Cell Signaling	#3180
Anti-GAPDH (1:1000 for IB)	Proteintech	#60004-1-Ig
Anti-MDM2 (SMP-14) (1:500 for IB)	Santa Cruz	#sc-965
Anti-p21 (SX118) (1:1000 for IB)	BD	#556430
Anti-p53 (1C12) (1:1000 for IB)	Cell Signaling	#2524S
Anti-SA-β-Galactosidase (4F4F4) (1:1000 for IB, 1:300 for IHC)	Proteintech	#66586-1-Ig
Anti-SCD1 (1:1000 for IB)	Cell Signaling	#2438
Anti-Tubulin (1:1000 for IB)	Cell Signaling	#5335
Anti-rabbit IgG, HRP linked Antibody (1:3000 for IB)	Cell Signaling	#7074
Critical Commercial Assays and Reagents		
Acetate Assay Kit	Abcam	#ab204719
Acetyl CoA PicoProbe Fluorometric Assay Kit	BioVision	#K317-100
Alanine Assay Kit	Abcam	#ab83394
ALT Assay Kit	BioVision	#K752-100
AST Assay Kit	Cayman Chemical	#701640
ATP Assay Kit	BioVision	#K354-100
BCA Protein Assay Kit	Pierce	#23225
Glutamine Assay Kit	BioAssay Systems	#EGLN-100
HCS LipidTOX^{TM} Green Neutral Lipid Stain	Invitrogen	#H34475
Ketone Body Assay Kit	Sigma-Aldrich	#MAK134
Lactate Assay Buffer	BioVision	#K607-100
Myriocin	Cayman Chemical	#63150
NucleoSpin RNA Kit	Macherey-Nagel	#740955
RIPA Buffer (10x)	Cell Signaling	#9806
RNeasy Mini Plus Kit	Qiagen	#74134
High-Capacity cDNA Reverse Transcription Kit	ABI	#4374966
Diets		
Envigo Teklad Rodent Diet traditional formula LM-485	Inotiv	#7012
Rodent Diet with 60% kcal fat (high-fat diet)	Research Diets, Inc.	#D12492i
Experimental Models		
Mouse: ACSS1^{K635Q}	This paper	N/A
Mouse: C57BL/6	Jackson Laboratory	#000664
Mouse: B6.Cg-Edil3$^{Tg(Sox2-cre)1Amc}$/J	Jackson Laboratory	#008454
Primers		
*Acss1*_F_seq_primer 5'-TTTTCCCCAACTAAGCCCGAG-3'	This paper	N/A
*Acss1*_R_seq_primer 5'-TCCCCCTGCAAACTGAGCCTGAAG-3'	This paper	N/A
*Acss1*_F_qPCR_primer 5'-ACCCTGATGCTGGTCGTTAC-3'	This paper	N/A
*Acss1*_R_qPCR_primer 5'-CGTGGTTGATAGGCTCTCCC-3'	This paper	N/A
*Acadl*_F_qPCR_primer 5'-TCTTTTCCTCGGAGCATGACA-3'	This paper	N/A
*Acadl*_R_qPCR_primer 5'-GACCTCTCTACTCACTTCTCCAG-3'	This paper	N/A
*Acadm*_F_qPCR_primer 5'-AACACAACACTCGAAAGCGG-3'	This paper	N/A

*Acadm*_R_qPCR_primer 5'-TTCTGCTGTTCCGTCAACTCA-3'	This paper	N/A
*Acsl1*_F_qPCR_primer 5'-TGCCAGAGCTGATTGACATTC-3'	This paper	N/A
*Acsl1*_R_qPCR_primer 5'-GGCATACCAGAAGGTGGTGAG-3'	This paper	N/A
*CD36*_F_qPCR_primer 5'-ATGGGCTGTGATCGGAACTG-3'	This paper	N/A
*CD36*_R_qPCR_primer 5'-TTTGCCACGTCATCTGGGTTT-3'	This paper	N/A
*Cpt1a*_F_qPCR_primer 5'-CTCCGCCTGAGCCATGAAG-3'	This paper	N/A
*Cpt1a*_R_qPCR_primer 5'-CACCAGTGATGATGCCATTCT-3'	This paper	N/A
*Cpt1b*_F_qPCR_primer 5'-GCACACCAGGCAGTAGCTTT-3'	This paper	N/A
*Cpt1b*_R_qPCR_primer 5'-CAGGAGTTGATTCCAGACAGGTA-3'	This paper	N/A
*Cpt1c*_F_qPCR_primer 5'-TCTTCACTGAGTTCCGATGGG-3'	This paper	N/A
*Cpt1c*_R_qPCR_primer 5'-ACGCCAGAGATGCCTTTTCC-3'	This paper	N/A
*Cpt2*_F_qPCR_primer 5'-CAGCACAGCATCGTACCCA-3'	This paper	N/A
*Cpt2*_R_qPCR_primer 5'-TCCCAATGCCGTTCTCAAAAT-3'	This paper	N/A
*Fasn*_F_qPCR_primer 5'-CTGACTCGGCTACTGACACG-3'	This paper	N/A
*Fasn*_R_qPCR_primer 5'-AATGGGGTGCACAAGGAACA-3'	This paper	N/A
*Gapdh*_F_qPCR_primer 5'-AGGTCGGTGTGAACGGATTTG-3'	This paper	N/A
*Gapdh*_R_qPCR_primer 5'-GGGGTCGTTGATGGCAACA-3'	This paper	N/A
*Scd1*_F_qPCR_primer 5'-CACCTGCCTCTTCGGGATTT-3'	This paper	N/A
*Scd1*_R_qPCR_primer 5'-CTTTGACAGCCGGGTGTTTG-3'	This paper	N/A
*Tubulin*_F_qPCR_primer 5'-GGCAGTGTTCGTAGACCTGGAA-3'	This paper	N/A
*Tubulin*_R_qPCR_primer 5'-CTCCTTGCCAATGGTGTAGTGG-3'	This paper	N/A
*Fasn*_ChREBP_F1_primer 5'-GCGCAGCCCCGACGCTCATT-3'	This paper	N/A
*Fasn*_ChREBP_R1_primer 5'-CGGCGCTATTTAAACCGCGG-3'	This paper	N/A
*Fasn*_ChREBP_F2_primer 5'-CCGACGCCCTCATCTCTGT-3'	This paper	N/A
*Fasn*_ChREBP_R2_primer 5'-GCCCTGGTGTCCTCCTCAGT-3'	This paper	N/A
*Scd1*_ChREBP_F1_primer 5'-GTGGTACCAAATTCCCATCG-3'	This paper	N/A
*Scd1*_ChREBP_R1_primer 5'-CGGTGTCCAGGAGTCAGAAT-3'	This paper	N/A
*Scd1*_ChREBP_F2_primer 5'-TGAGGTCACCGGGACTAACAGA-3'	This paper	N/A
*Scd1*_ChREBP_R2_primer 5'-CTCACGCTGGAGTCATGCAA-3'	This paper	N/A

Table S1. Key Resource Table.