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Supplemental Information

Adipocyte ACLY Facilitates Dietary Carbohydrate Handling to Maintain Metabolic Homeostasis in Females

Sully Fernandez, John M. Viola, AnnMarie Torres, Martina Wallace, Sophie Trefely, Steven Zhao, Hayley C. Affronti, Jivani M. Gengatharan, David A. Guertin, Nathaniel W. Snyder, Christian M. Metallo, and Kathryn E. Wellen

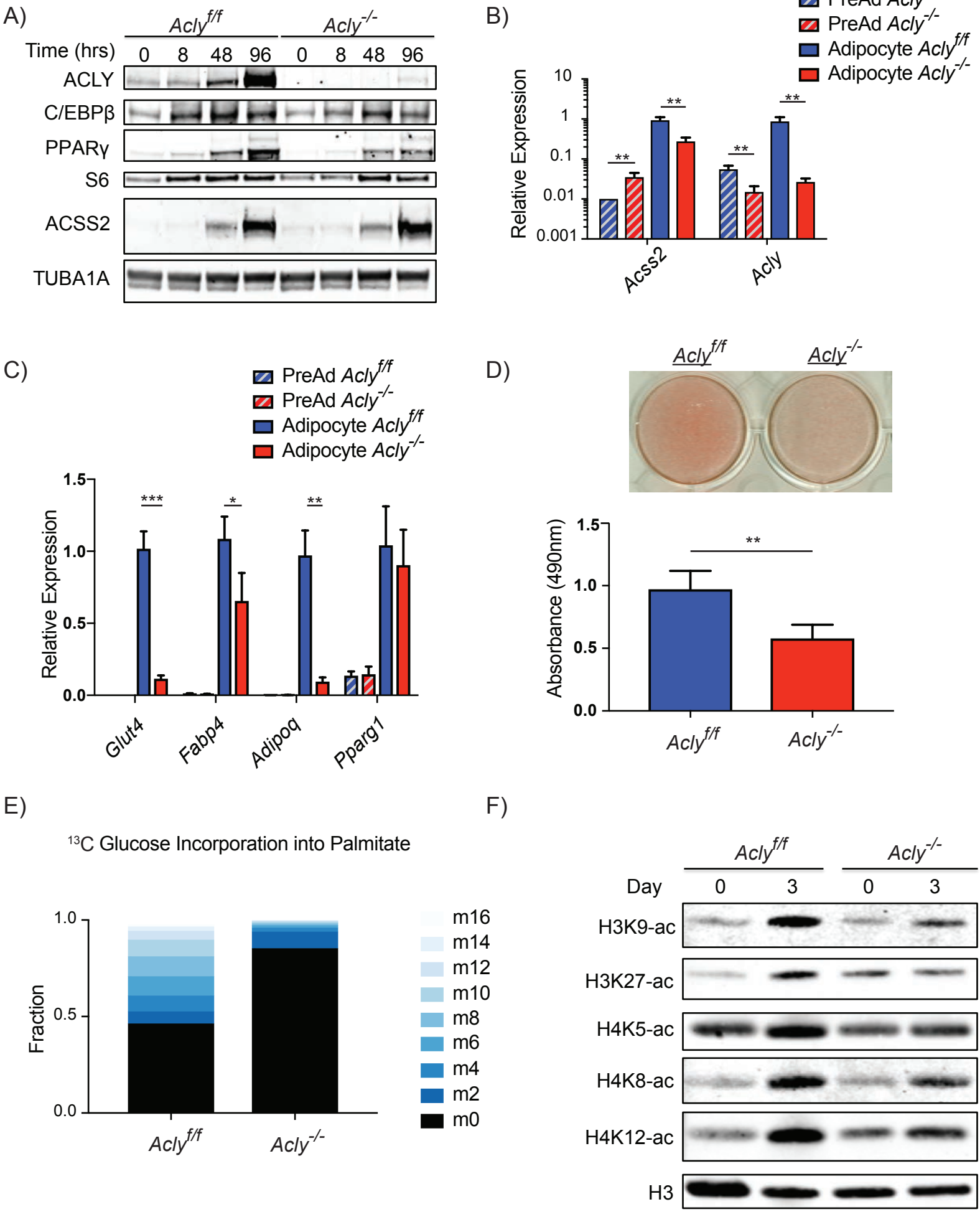
A)
Acly f/f
Acly -/-
Time (hrs)
0 8 48 96 0 8 48 96
ACLY
C/EBPβ
PPARγ
S6
ACSS2
TUBA1A
B)
PreAd Acly f/f
PreAd Acly -/-
Adipocyte Acly f/f
Adipocyte Acly -/-
Relative Expression
10
1
0.1
0.01
0.001
**
Acss2
Acly
C)
PreAd Acly f/f
PreAd Acly -/-
Adipocyte Acly f/f
Adipocyte Acly -/-
Relative Expression
1.5
1.0
0.5
0.0
***
*
**
Glut4
Fabp4
Adipoq
Pparg1
D)
Acly f/f
Acly -/-
Absorbance (490nm)
1.5
1.0
0.5
0.0
**
Acly f/f
Acly -/-
E)
13C Glucose Incorporation into Palmitate
Fraction
1.0
0.5
0.0
m16
m14
m12
m10
m8
m6
m4
m2
m0
Acly f/f
Acly -/-
F)
Acly f/f
Acly -/-
Day
0 3 0 3
H3K9-ac
H3K27-ac
H4K5-ac
H4K8-ac
H4K12-ac
H3

Figure S1: Generation of a genetic model of *Acly* deficiency in adipocytes. Related to Figure 1. *Acly* was deleted from immortalized *Acly*$^{f/f}$ preadipocytes by adenoviral administration of Cre recombinase (denoted *Acly*$^{-/-}$ after deletion). *Acly*$^{f/f}$ and *Acly*$^{-/-}$ preadipocytes were induced to differentiate to adipocytes. A) Cells were analyzed by western blot at the indicated time points; B) mRNA expression of *Acly* and *Acss2*; C) mRNA expression of select genes upregulated during differentiation; D) Oil Red O staining and quantitation; E) GC-MS FAME analysis of ^{13}C-glucose incorporation into palmitate, mean of duplicates shown; F) western blots to evaluate histone acetylation in acid-extracted histones. In all panels, PreAd indicates Day 0 cells prior to induction, Adipocyte denotes cells 4 days after induction of differentiation. All bar graphs represent mean +/- SEM of triplicates; *, $p<0.05$; **, $p<0.01$, ***, $p<0.001$.

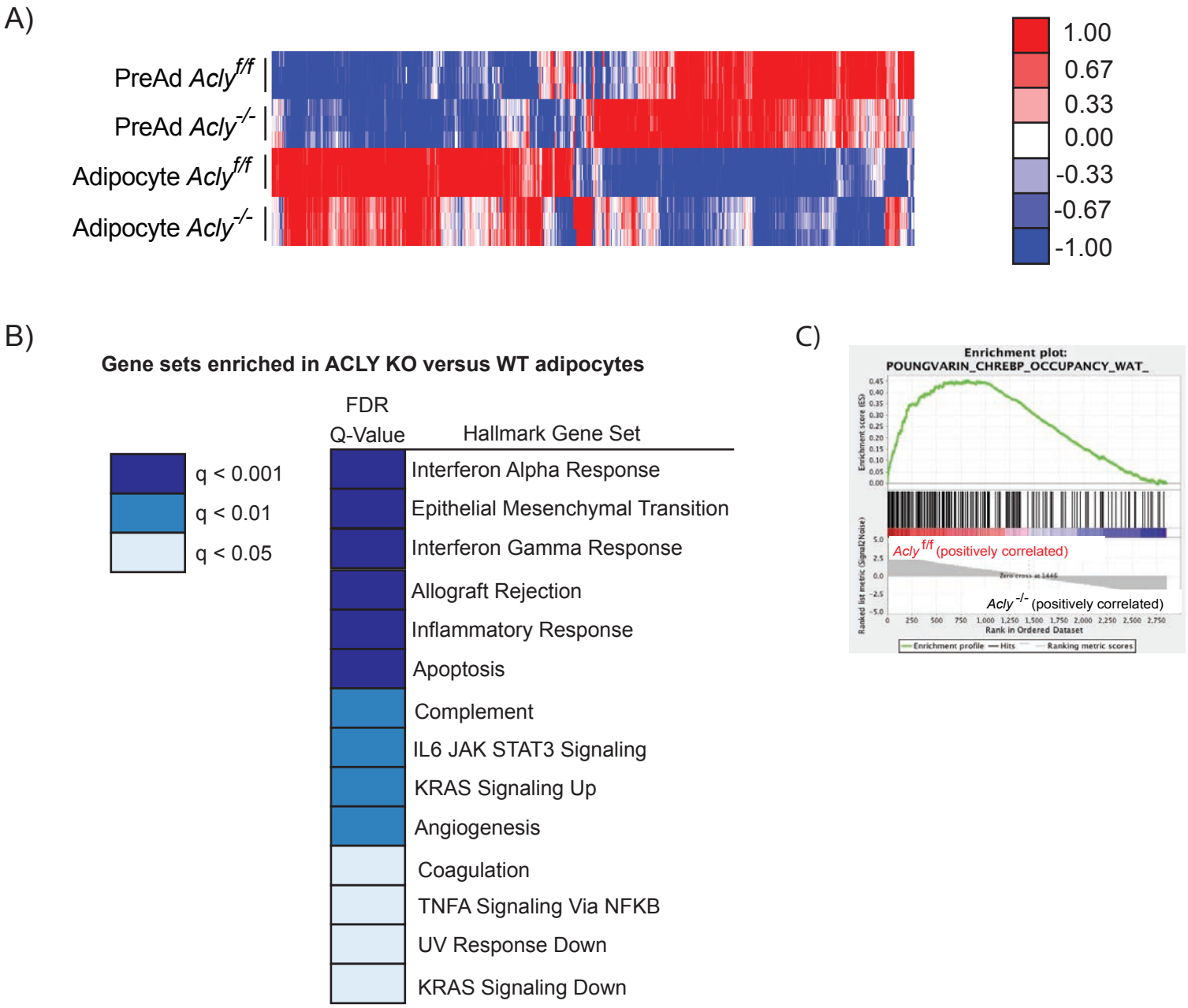

Figure S2. Inflammatory genes are upregulated in *Acly*$^{-/-}$ adipocytes. Related to Figure 1. A) Heat map clustering of RNA-seq data based on q<0.05. B) GSEA analysis of Hallmarks gene sets enriched in KO Ads versus WT Ads. C) GSEA analysis of top 1000 genes occupied by ChREBP in WAT, as defined by (Poungvarin et al., 2015), enriched in WT Ads versus KO Ads.

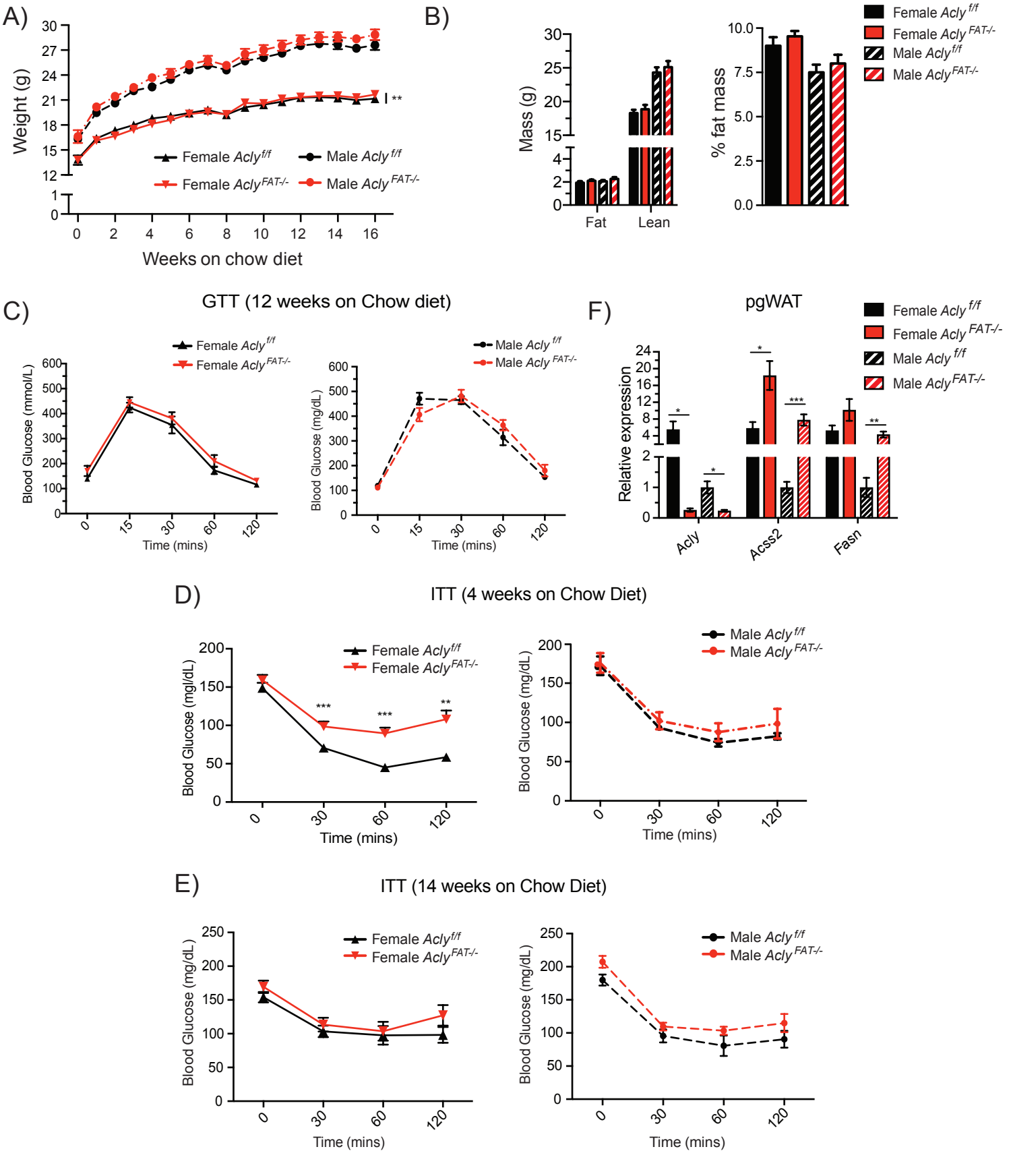
A)
Weight (g)
Weeks on chow diet
Female Acly f/f
Female Acly FAT-/-
Male Acly f/f
Male Acly FAT-/-
**
B)
Mass (g)
Fat
Lean
% fat mass
C)
GTT (12 weeks on Chow diet)
Blood Glucose (mmol/L)
Blood Glucose (mg/dL)
Time (mins)
F)
pgWAT
Relative expression
Acly
Acss2
Fasn
D)
ITT (4 weeks on Chow Diet)
Blood Glucose (mg/dL)
E)
ITT (14 weeks on Chow Diet)

Figure S3. Adipocyte ACLY deficiency results in insulin resistance in young female chow-fed mice. Related to Figure 3. Upon weaning, male and female, *Acly*$^{f/f}$ and *Acly*$^{FAT-/-}$ mice were fed a standard chow diet and monitored for 16 weeks. A) Body weights measured weekly, analyzed by 2-way ANOVA; B) Body composition measured by MRI after 16 weeks on chow diet (age 20 weeks); C) Glucose tolerance tests in males (left panel) and females (right panel) after 12 weeks on diet; D) Insulin tolerance tests in males (left panel) and females (right panel) after 4 weeks on diet; E) Insulin tolerance tests in males (left panel) and females (right panel) after 14 weeks on diet. F) Gene expression in pgWAT. Error bars indicate mean +/- SEM for all panels. 2-tailed t-tests used for analysis, unless ANOVA is indicated for panel. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

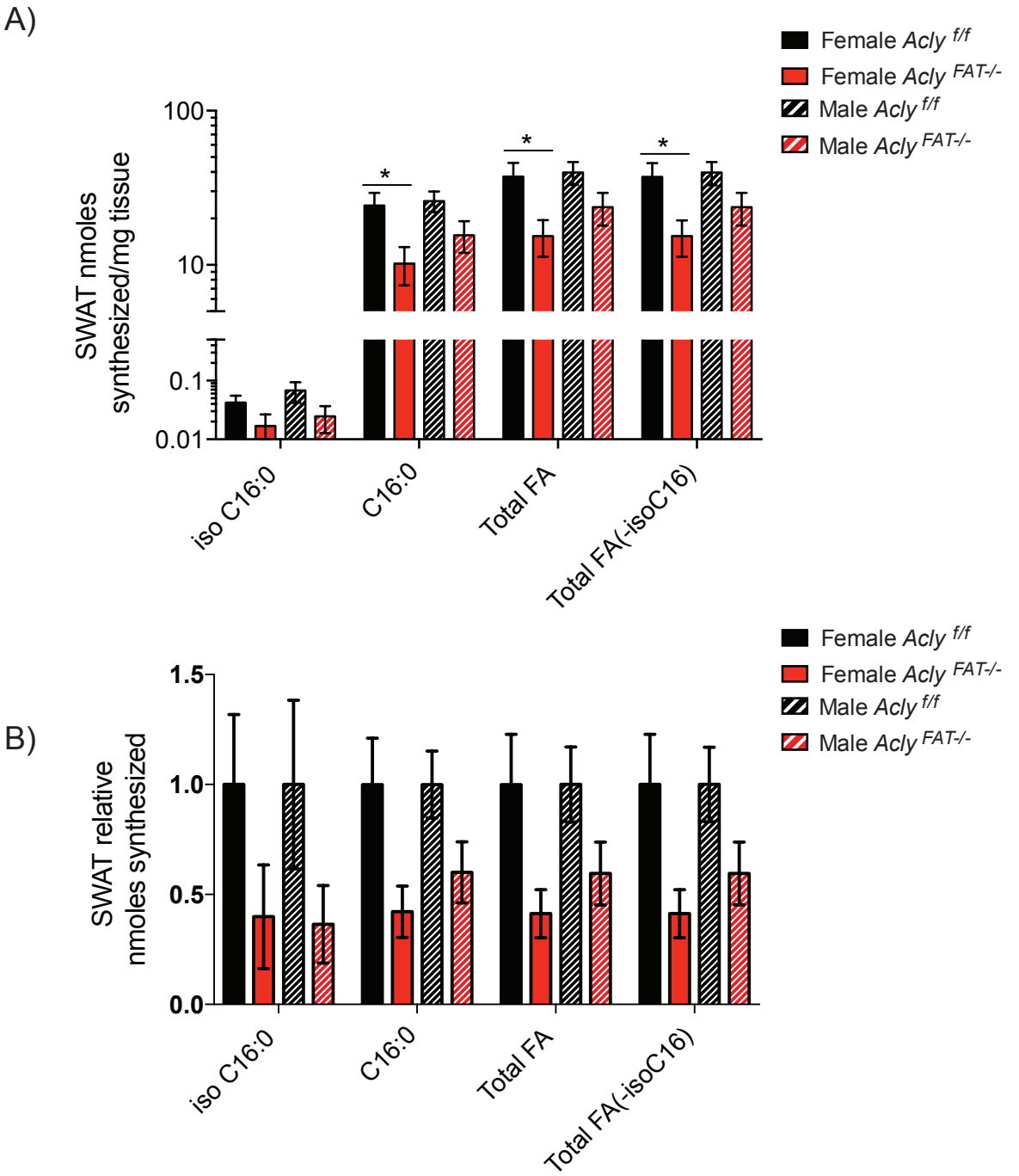

Figure S4. mmBCFA synthesis is comparably suppressed in the absence of ACLY in males and females. Related to Figure 4.
A, B) Absolute (A) and relative (B) nmoles of indicated fatty acids synthesized per mg tissue. For relative analysis, *Acly* $^{f/f}$ mice of each sex is set to 1. Error bars indicate mean +/- SEM. 2-tailed tests used for analysis, *, $p<0.05$.

GENE (SPECIES)	PRIMER	SEQUENCE (5'-3')	SOURCE	IDENTIFER
18S (mouse or human)	Forward	AAATCAGTTATGGTTCCTTTGGTC	Designed In House	N/A
18S (mouse or human)	Reverse	GCTCTAGAATTACCACAGTTATCCAA	Designed In House	N/A
Tnfa (mouse)	Forward	CCCTCACACTCAGATCATCTTCT	Designed In House	N/A
Tnfa (mouse)	Reverse	GCTACGACGTGGGCTACAG	Designed In House	N/A
Adipoq (mouse)	Forward	GCACTGGCAAGTTCTACTGCAA	Designed In House	N/A
Adipoq (mouse)	Reverse	GTAGGTGAAGAGAACGGCCTTGT	Harms Et Al, 2014	PMID: 24703692
GLUT4 (human)	Forward	ACCAGAGCAGCCATGGAG	Designed In House	N/A
GLUT4 (human)	Reverse	TTGATGCCTCCGTCCACGAT	Designed In House	N/A
CHREBPβ (human)	Forward	AGCGGATTCCAGGTGAGG	Herman et al, 2012	PMID: 22466288
CHREBPβ (human)	Reverse	TTGTTCAGGCGGATCTTGTC	Herman et al, 2012	PMID: 22466288
ACLY (human)	Forward	AAACTGTGGGTCCTTTACTCG	Designed In House	N/A
ACLY (human)	Reverse	GGATGACGATACAGCCCCTG	Designed In House	N/A
Acly (mouse)	Forward	TTCGTCAAACAGCACTTCC	Designed In House	N/A
Acly (mouse)	Reverse	ATTTGGCTTCTTGGAGGTG	Designed In House	N/A
Acss2 (mouse)	Forward	GCTTCTTTCCCATTCTTCGGT	Neess et al, 2011	PMID: 21106527
Acss2 (mouse)	Reverse	CCCGGACTCATTCAGGATTG	Neess et al, 2011	PMID: 21106527
Glut4 (mouse)	Forward	GCCCGAAAGAGTCTAAAGC	Designed In House	N/A
Glut4 (mouse)	Reverse	CTTCCGTTTCTCATCCTTCAG	Designed In House	N/A
Fapb4 (mouse)	Forward	ACAAAATGTGTGATGCCTTTGTGGGAAC	Designed In House	N/A
Fabp4 (mouse)	Reverse	TCCGACTGACTATTGTAGTGTTTGATGCAA	Designed In House	N/A
Pparg1 (mouse)	Forward	TGAAAGAAGCGGTGAACCACTG	Designed In House	N/A
Pparg1 (mouse)	Reverse	TGGCATCTCGTGTCAACCATG	Designed In House	N/A

Table S2: Primers for qPCR. Related to STAR Methods