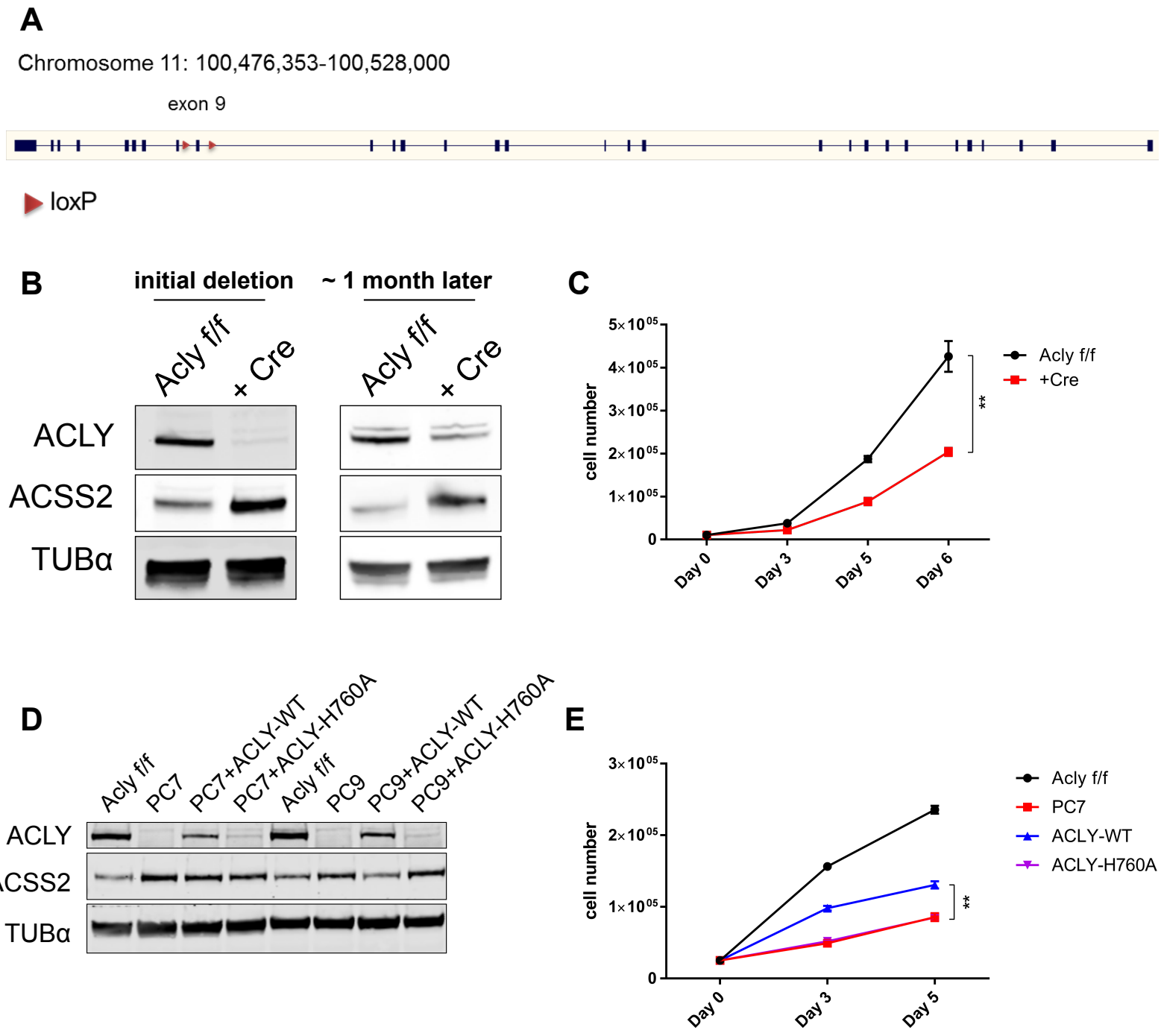

Figure S1. Deletion of *Acly* from mouse embryonic fibroblasts, related to Fig. 1.

A) Diagram of Acly locus in *Acly*$^{f/f}$ mice, loxP sites flanking exon 9 are depicted.

B) Western blot of ACLY and ACSS2 expression in *Acly*$^{f/f}$ MEFs, +/- Cre treatment at the time of initial deletion and one month later.

C) Proliferation curve of *Acly*$^{f/f}$ MEFs with or without Cre treatment over 6 days, mean +/- SEM of triplicate wells.

D) Western blot of ACLY and ACSS2 expression in *Acly*$^{f/f}$ MEFs and PC7 and PC9 knockout lines that have been reconstituted with wild type ACLY (+ACLY-WT) or catalytically dead ACLY (+ACLY-H760A).

E) Proliferation curve of *Acly*$^{f/f}$ MEF and PC7 lines compared to PC7 with reconstituted ACLY-WT or ACLY-H760A over 5 days, mean +/- SEM of triplicate wells, statistical significance compared to PC7.

For all panels: **, $p<0.01$.

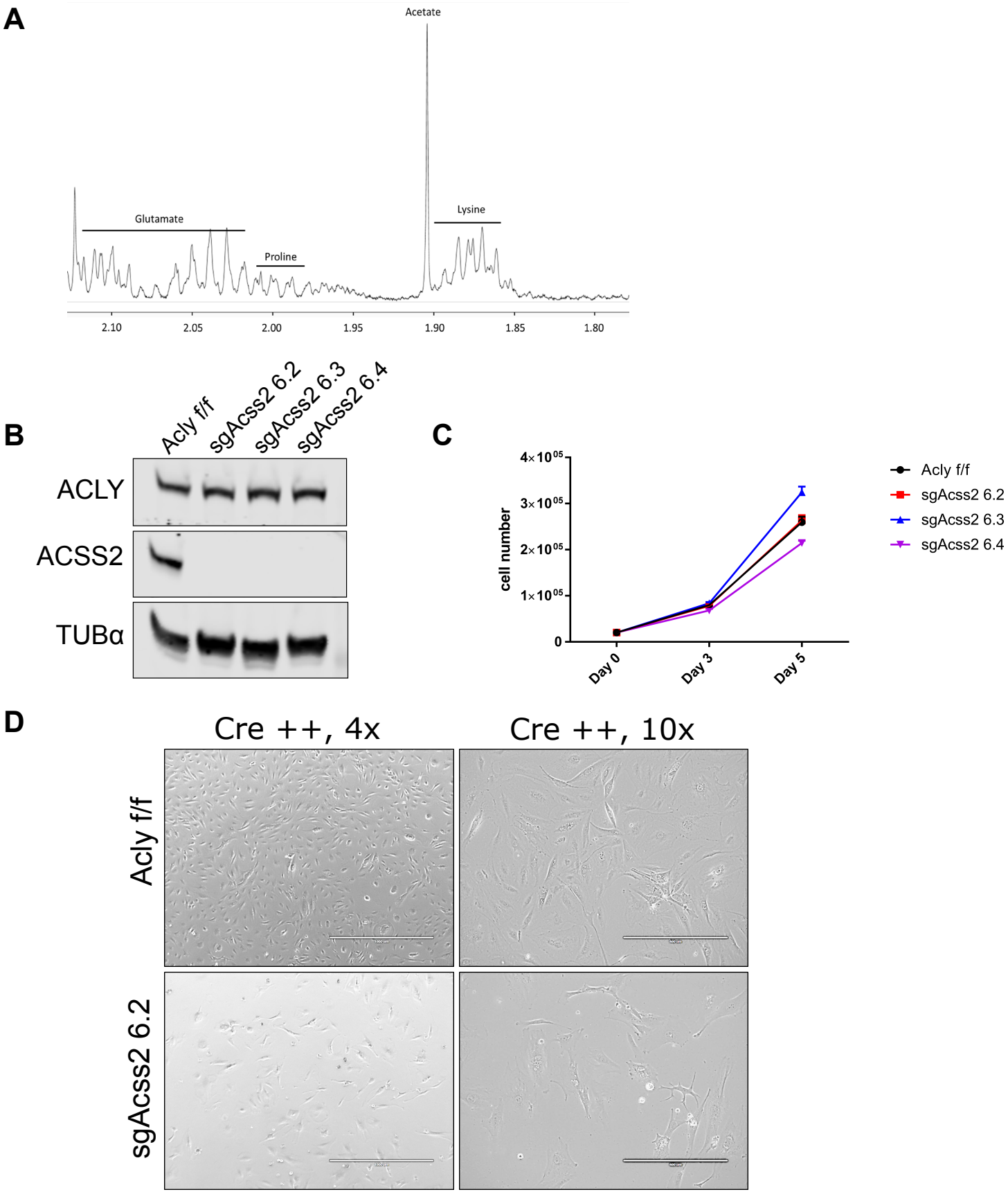

Figure S2. Acetate sustains viability in the absence of ACLY, related to Fig. 2.

A) NMR spectrum of undiluted calf serum.

B) Western blot verification of *Acss2* knockout using CRISPR-Cas9 gene editing in *Acly*$^{f/f}$ MEFs.

C) Proliferation curve over 5 days of three ACSS2-deficient clonal cell lines as compared to *Acly*$^{f/f}$ MEFs, mean +/- SEM of triplicate wells.

D) Representative images of *Acly*$^{f/f}$ MEFs and sgAcss2 6.2 *Acly*$^{f/f}$ MEFs, treated twice with adenoviral Cre-recombinase at 4x zoom (left panels, bar represents 1000 μm) and 10x zoom (right panels, bar represents 400 μm).

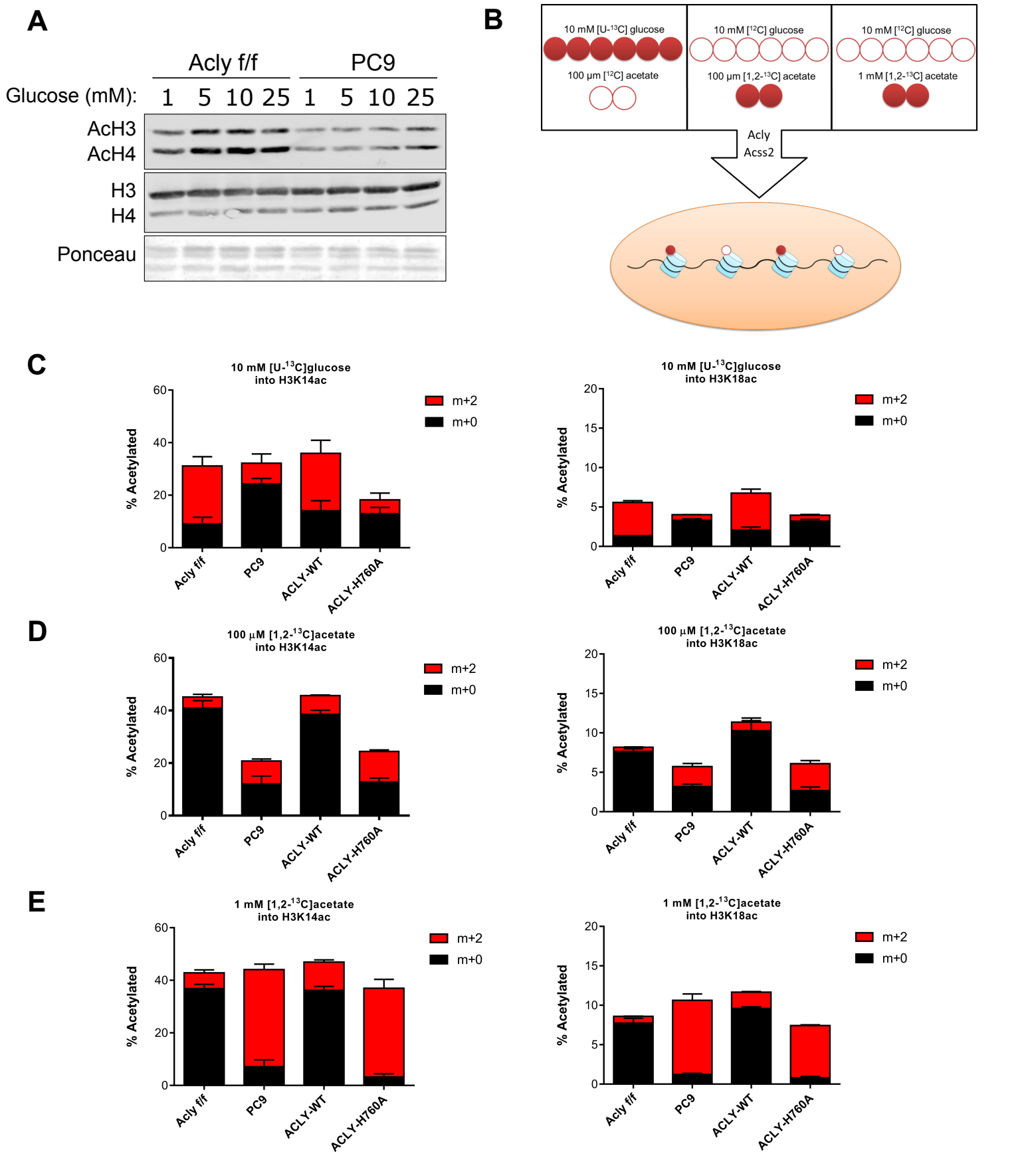

Figure S3. ACLY is required for sustaining histone acetylation levels, related to Fig. 4.

A) Histone acetylation in acid-extracted histones from *Acly*$^{f/f}$ and PC9 MEFs cultured for 24 hours in glucose-free DMEM supplemented with 10% dFBS and the indicated glucose concentrations.

B) Experimental design of heavy isotope labeling of histone acetylation using 10 mM [U-^{13}C]glucose with 100 µM unlabeled acetate present (left), 100 µM [1,2-^{13}C]acetate with 10 mM unlabeled glucose present (center), and 1 mM [1,2-^{13}C]acetate with 10 mM unlabeled glucose present (right).

C-E) Percent of total acetylation of H3K14 (left) and H3K18 (right) from labeled (red) and unlabeled (black) sources after labeling with 10 mM [U-^{13}C]glucose (C), 100 µM [1,2-^{13}C]acetate (D), or 1 mM [1,2-^{13} C]acetate (E), mean +/- SEM of triplicate samples.

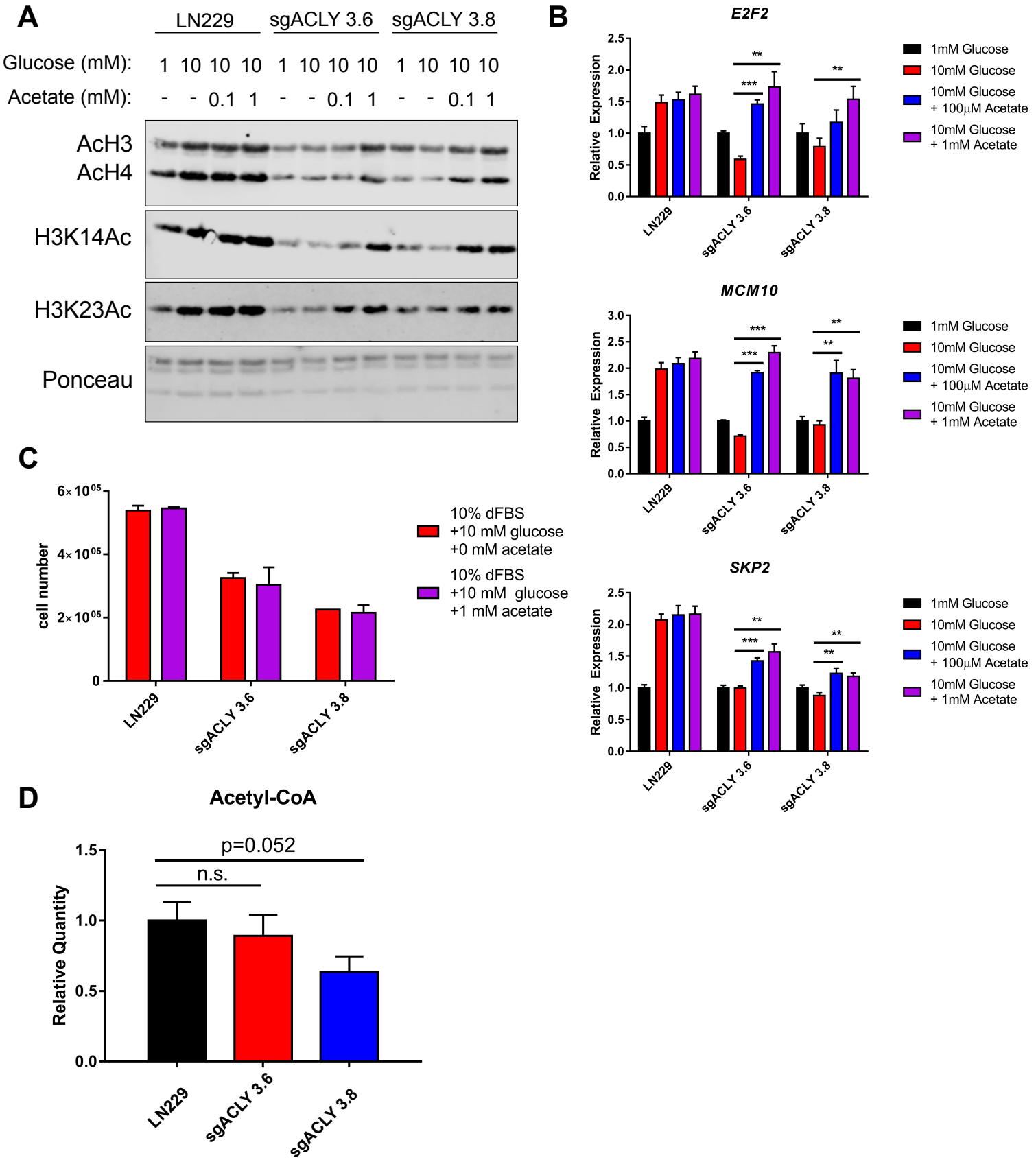

Figure S4. Acetate regulates histone acetylation and gene expression in ACLY-deficient glioblastoma cells, related to Fig. 4.

A) Histone acetylation in acid-extracted histones from LN229 parental and ACLY KO clones cultured for 24 hours in glucose-free RPMI + 10% dFBS + 1 or 10 mM glucose + 2 mM glutamine + 0, 0.1, or 1 mM acetate.

B) Relative expression of *E2F2*, *MCM10*, and *SKP2* in LN229 parental and ACLY KO clones after 24 hours cultured in the same conditions as in panel A.

C) Cell number after 48 hours of culture in indicated conditions.

D) Relative whole cell acetyl-CoA levels in LN229 parental and ACLY KO clones cultured in glucose-free RPMI + 10% dFBS + 10 mM glucose + 100 μM acetate + 2 mM glutamine for 6 hours, normalized to cellular volume, mean +/- SEM of triplicate samples.

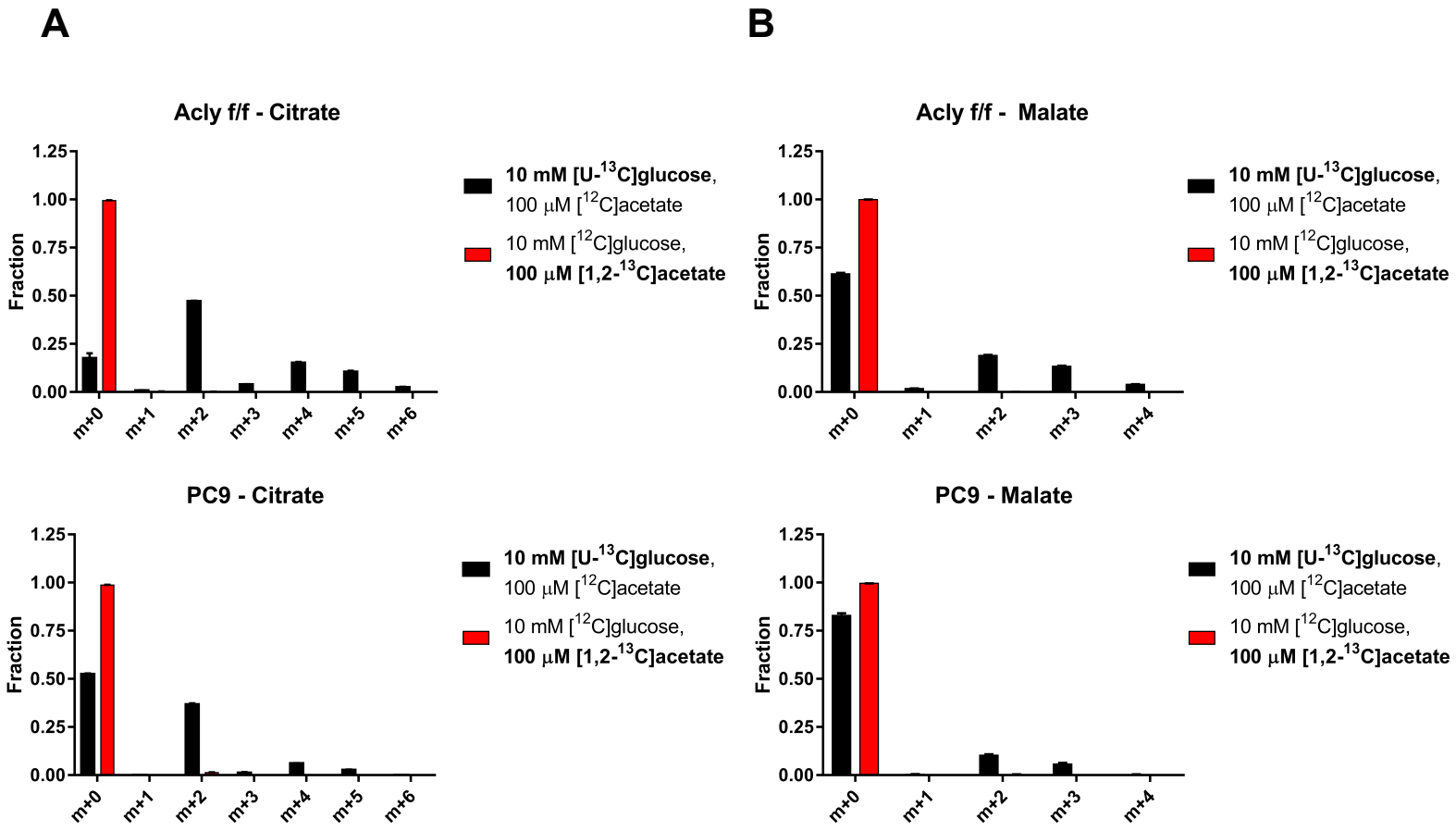

Figure S5. Acetate contributes minimally to mitochondrial metabolism in the absence of ACLY, related to Figure 5.
A,B) Isotopologue distribution of citrate (A) and malate (B) upon 24 hours labeling in 10 mM [U-^{13}C]glucose or 100 μM [1,2-^{13}C]acetate in *Acly$^{f/f}$* (top) and PC9 (bottom) MEFs, mean +/- SEM of triplicate samples.

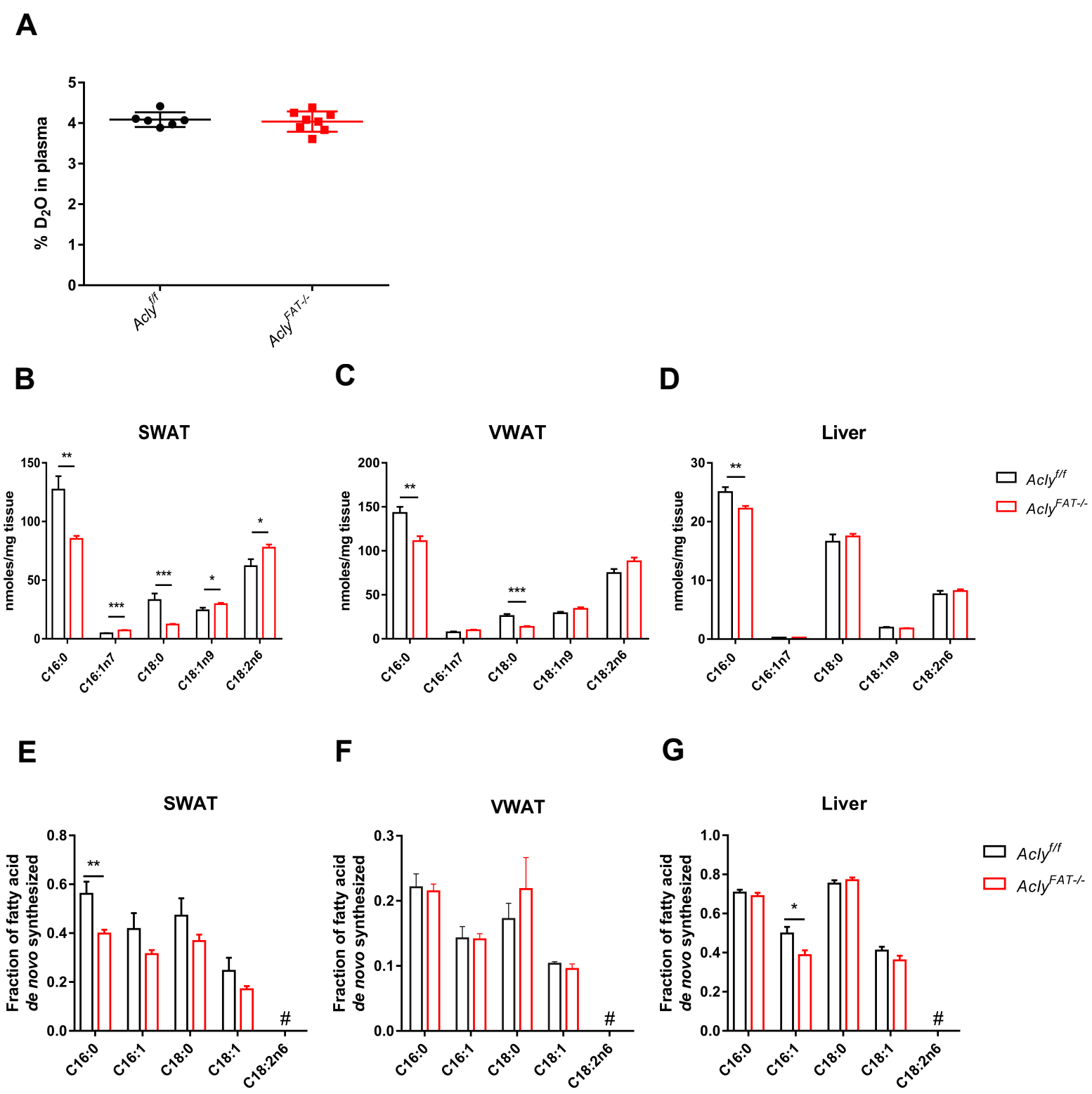

Figure S6. Tissue fatty acid levels and enrichment after D_2O labeling of *Acly*$^{f/f}$ and *Acly*$^{FAT-/-}$ mice, related to Fig. 7.

A) Plasma D_2O enrichment.

B-D) Abundance of fatty acids in SWAT (B), VWAT (C), and liver (D).

E-G) Fractions of fatty acids synthesized *de novo* present in SWAT (E), VWAT (F), and liver (G).

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Genotyping

Tail-snips from mice were placed in digestion buffer (10% SDS, 5M NaCl, EDTA, Tris, H_2O, proteinase K) for two hours while shaking at 56°C. Genomic DNA was isolated and then used for genotyping using the following primer sets:
Cre-Fw: TGCCACGACCAAGTGACAGC; Cre-Rv: CCAGGTTACGGATATAGTTCATG
tm1c (floxed allele)-Fw:AAGGCGCATAACGATACCAC; tm1c-Rv:CCGCCTACTGCGACTATAGAGA
Acly wild-type allele: WT-Fw:TGCAATGCTGCCTCCAATGAT; WT-Rv:GGAGCCAGAGGAGAAAAAGGC

Generation of *Acly*$^{f/f}$ MEFs

For mouse embryonic fibroblast (MEF) generation, two homozygous fertile females were placed on a dedicated mating cage with a homozygous fertile male. On day 15.5, pregnant females were sacrificed and the fetuses were surgically removed and placed in a 10-cm dish, washed two timed with PBS. Head and liver were removed from each fetus, the remaining part was trimmed, pooled in a 50-mL tube and washed again with PBS. Tissue remnants were digested with 5mL of Trypsin 0.25% at room temperature for 30 minutes. The digestion was stopped with DMEM+10% CS. Cells were pelleted and washed again with DMEM+10% CS. Finally cells were seeded in a 25-cm flask and cultured in DMEM+10% CS + 0.1 mM β-mercaptoethanol. Cells were immortalized by serial passaging (plated at 1:3 dilution and passaged at confluency) and began recovering from proliferation crisis after 13 (line 1) and 20 (line 2) passages.

Acly deletion and reconstitution in MEFs

For acute analysis, *Acly*$^{f/f}$ MEFs (line 2) were infected with adenoviral Cre recombinase (University of Pennsylvania Vector Core). For generation of stable lines PC7, PC8, and PC9, retroviral transduction of *Acly*$^{f/f}$ MEFs (line 2) with Cre recombinase was conducted as follows: A retroviral vector containing Cre recombinase (pBabe-puro-Cre, gift of L. Busino, University of Pennsylvania) was used to produce retrovirus in Phoenix E cells. MEFs were transduced with retrovirus and selected with 3 µg/mL of puromycin for 48 hours until mock infected MEFs displayed no viable cells. Following selection, single cell clonal populations were generated by plating cells in a limiting dilution. Deletion of *Acly* was confirmed by Western blot.
For reconstitution experiments, wild-type ACLY or catalytically inactive (H760A) ACLY were cloned into pBabe-hygro retroviral vector. Retrovirus was produced in Phoenix E cells. PC7 and PC9 cells were transduced with retrovirus and selected with hygromycin (400 µg/mL) for 48 hours until mock infected MEFs displayed no viable cells. Reconstitution was confirmed by immunoblotting for ACLY expression.

CRISPR-Cas9 genetic editing

Guide RNA sequences were generated using a CRISPR design tool (www.crispr.mit.edu). The guide sequences used are as follows: mAcss2 (GCTGCACCGGCGTTCTGTGG), hACLY (GACCAGCTGATCAAACGTCG). Guides were cloned into the LentiCRISPRv2 plasmid (Sanjana et al., 2014) followed by lentiviral production in HEK-293T cells. Cells were infected and selected with puromycin until a separate mock-infected plate displayed complete cell death. Single-cell clonal expansion of the selected population was done to ensure complete loss of the target gene. Loss of target gene was determined by immunoblotting for the target protein.

Analysis of *Acly*$^{FAT-/-}$ mice

From 4 weeks to 16 weeks of age, mixed background *Acly*$^{f/f}$ and *Acly*$^{FAT-/-}$ were fed normal chow and weighed weekly. At 16 weeks of age mice were sacrificed and white fat [visceral (epididymal) and subcutaneous (inguinal)] depots were harvested. Depots were dissected into thirds with a third of each being fixed in formalin for histological evaluation, a third being digested in Trizol for RNA expression analysis and the final third digested in protein lysis buffer for protein analysis. For analysis of histone acetylation and acetyl-CoA levels, a separate cohort of *Acly*$^{FAT-/-}$ (n=6) and WT (*Acly*$^{f/f}$; n=7) mice, females aged 10 to 11 weeks, backcrossed onto a C57Bl/6 background, were used. Mice were fasted for 6 hours, sacrificed, and liver, VWAT and SWAT were removed. Organs were split in half; half snap frozen for acyl CoA analysis and the other processed fresh for histone extraction, as described below. The University of Pennsylvania's Institutional Animal Care and Use Committee (IACUC) approved all animal experiments.

Immunoblotting

Protein was extracted from cells using NP-40 lysis buffer (150 mM NaCl, 1.0% NP-40, 50 mM Tris-HCl pH 8.0) with protease inhibitors (Roche). Mouse tissue was lysed in RIPA lysis buffer (1%NP-40, 0.5% Deoxycholate, 0.1% SDS, 150nM NaCl, 50mM Tris plus protease and phosphatase inhibitors). Fat was chopped with scissors on ice to fine pieces, followed by homogenization with TissueLyser (30 Hz for 20s x 2). Samples were chilled on ice for 30 min, spun down, and infranatant saved and then sonicated. Protein concentration was determined using the BCA protein assay (ThermoScientific). Proteins were separated by SDS-PAGE and

transferred to a nitrocellulose membrane (GE Health Sciences). Membranes were probed with the specified antibodies (see Antibodies and Reagents), and developed on a LI-COR Odyssey CLx scanner.

Antibodies and reagents
Antibodies used for Western blotting: ACLY (previously described (Wellen et al., 2009)), ACSS2 (Cell Signaling Technologies #3658S), Tubulin (Sigma T6199), FASN (Cell Signaling Technologies #3189S), Lamin A/C (Cell Signaling Technologies #2032S), Parp (Cell Signaling Technologies #9542S), Cleaved Parp (Cell Signaling Technologies #9544T), Cleaved Caspase 3 (Cell Signaling Technologies #9661S), Acetyl-H3 (Upstate 06-599), Acetyl-H4 (Millipore 06-866), H4K5Ac (Millipore 07-327), H3K14Ac (Cell Signaling #7627S), H3K18Ac (Cell Signaling 9675P), H3K23Ac (Cell Signaling 9674S), H3K27Ac (Abcam ab4729). Secondary antibodies were IRDye680RD Goat Anti-Mouse (LI-COR 926-68070) and IRDye800CW Goat Anti-Rabbit (LI-COR 926-32211). Reagents: ACLY inhibitor BMS-303141 (Tocris Bioscience)

Nuclear-cytoplasmic subcellular fractionation:
Fractionation was performed essentially as described (Wellen et al., 2009). Cells were harvested in cold Buffer A (10 mM HEPES pH 7.4, 10 mM KCl, 1.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM EGTA; Complete Mini (Roche) protease inhibitor (PIC) tablet and 0.1% NP-40 added fresh). Cells were lysed on ice for 15 minutes until the plasma membrane was broken (assessed by trypan blue staining). Cells were centrifuged at 1000 RCF for 5 min at 4ºC. Supernatant (cytosol) was transferred to a new microfuge tube and spun down again at high speed to clear debris. Pellet (nuclei) from initial spin was washed once with Buffer A without NP-40, then resuspended in equal volumes of cold Buffer B (10 mM HEPES pH 7.4, 0.42 M NaCl, 2.5% glycerol, 1.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM EGTA, 1 mM DTT; PIC added fresh). Samples were incubated on ice for 30 minutes with occasional vortexing, centrifuged 10 minutes at 15,000 RCF to clear debris, and the supernatant transferred to new tube (nuclei). Lamin A/C and FASN were used as nuclear and cytoplasmic markers, respectively.

Histone Acid Extraction for Immunoblotting
Acid extraction on isolated nuclei was performed as previously described (Lee et al., 2014). Histones for immunoblotting were extracted from nuclei by lysing cells with NIB-250 buffer (15 mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 250 mM sucrose, 1 mM DTT, 10 mM sodium butyrate, 0.1% NP-40, protease inhibitors) for 5 minutes on ice. Nuclei were pelleted by spinning lysate at 600 RCF for 5 minutes at 4^{o}C. Nuclei were washed with NIB-250 buffer without NP-40 twice. Histones were extracted from nuclei by resuspending the pellet in 0.4N H_2SO_4 and rotating overnight at 4^{o}C, insoluble nuclear debris was cleared by spinning at 11,000 RCF for 10 minutes at 4^{o}C. Histones were precipitated by adding 100% trichloroacetic acid (TCA) until final solution reached 20% TCA and allowed to precipitate overnight at 4^{o}C. Precipitated histones were spun down at 11,000 RCF for 10 minutes at 4^{o}C and washed with 1 mL acetone + 0.1% 12 N HCl followed by a wash of 1 mL acetone. Histone pellet was air dried at room temperature for at least 30 minutes and resuspended in glass distilled H_2O.

YSI metabolite analysis:
Culture medium (glucose-free DMEM (Gibco) supplemented with 10% dFBS (Gibson), 10 mM glucose, and 100 μM acetate) was collected from cells after culturing for 48 hours. Glucose, lactate, glutamine, and glutamate levels in culture medium were measured using a YSI 2950 Bioanalyzer. Because of differences in proliferation rate and cell volume between clones, measurements were normalized to cell volume (cell number X mean cell volume) area under the curve. Metabolite consumption was defined as $v = V(x_{medium\ control} - x_{final})/A$, where v is metabolite consumption/ production, V is medium volume, x is metabolite concentration, and A is total cell volume area under the curve. A was calculated as $N(T)d/\ln 2(1-2^{-T/d})$, where N(T) is the final cell count, d is doubling time, and T is time of experiment. Cell counts and volume measurements were taken on a Coulter Counter (Beckman Coulter), and final cell count N(T) was multiplied by mean cellular volume to obtain total cellular volume per sample. Doubling time was calculated as $d = (T)[\log(2)/\log(Q2/Q1)]$, where Q1 is starting cell number and Q2 is final cell number.

Quantitative RT-PCR
Cells were lysed using Trizol reagent (Ambion), and RNA was isolated as per Trizol extraction protocol. Adipose tissue were excised from animals and immediately frozen in liquid nitrogen, placed in Trizol, and lysed using a tissue homogenizer before RNA isolation as per Trizol extraction protocol. RNA was resuspended in DEPC H_2O and quantified on a Biotek Synergy HT Plate Reader. cDNA was generated from isolated RNA using High Capacity RNA-to-cDNA Kit (Applied Biosystems), and diluted 1:20 in nuclease free water for quantitative RT-PCR reactions (qRT-PCR). qRT-PCR was run using *Power* SYBR Green PCR Master Mix (Applied Biosystems) for 40 cycles at standard reaction speed on a ViiA 7 Real-Time PCR System (Applied Biosystems). Primer sequences listed in the table below.

qRT-PCR primer sequences

Gene	Primer Sequence
Acly (mouse) Forward	TTCGTCAAACAGCACTTCC
Acly (mouse) Reverse	ATTTGGCTTCTTGGAGGTG
Acss2 (mouse) Forward	GCTTCTTTCCCATTCTTCGGT
Acss2 (mouse) Reverse	CCCGGACTCATTCAGGATTG
Glut4 (mouse) Forward	GCCCGAAAGAGTCTAAAGC
Glut4 (mouse) Reverse	CTTCCGTTTCTCATCCTTCAG
FASN (mouse) Forward	ATTGGTGGTGTGGACATGGTC
FASN (mouse) Reverse	CCCAGCCTTCCATCTCCTG
FABP4 (mouse) Forward	ACAAAATGTGTGATGCCTTTGTGGGAAC
FABP4 (mouse) Reverse	TCCGACTGACTATTGTAGTGTTTGATGCAA
PPARg1 (mouse) Forward	TGAAAGAAGCGGTGAACCACTG
PPARg1 (mouse) Reverse	TGGCATCTCGTGTCAACCATG
PPARg2 (mouse) Forward	TGGCATCTCTGTGTCAACCATG
PPARg2 (mouse) Reverse	GCATGGTGCCTTCGCTGA
AdipoQ (mouse) Forward	GCACTGGCAAGTTCTACTGCAA
AdipoQ (mouse) Reverse	GTAGGTGAAGAGAACGGCCTTGT
18S (mouse) Forward	AAATCAGTTATGGTTCCTTTGGTC
18S (mouse) Reverse	GCTCTAGAATTACCACAGTTATCCAA
E2F2 (human) Forward	TTTACCTCCTGAGCGAGTCA
E2F2 (human) Reverse	AGCACGTTGGTGATGTCATAG
MCM10 (human) Forward	CGGAACAAACCTAGTGGGATAA
MCM10 (human) Reverse	AGAAGGCTTCCACACAGATG
SKP2 (human) Forward	GTGTACAGCACATGGACCTAT
SKP2 (human) Reverse	CCAGGCTTAGATTCTGCAACT

Gas Chromatography/Mass Spectrometry of Fatty Acid Methyl Esters (GC/MS-FAME)

To measure glucose incorporation into lipids, $2x10^5$ cells were plated and allowed to adhere overnight. Culture medium was changed the following day to DMEM without glucose (Gibco) supplemented with 10% dialyzed fetal bovine serum (dFBS) (Gibco 26400044, Lot. 1616514), 10 mM [U-^{13}C]glucose (Cambridge Isotope Laboratories), and 100 µM sodium acetate, and incubated for 48 hours. To measure acetate incorporation into lipids, DMEM without glucose was supplemented with 10% dFBS, 10 mM glucose, and 100 µM or 1 mM [1,2-^{13}C]acetate (Cambridge Isotope Laboratories). On day of harvest, cells were washed with 1x PBS, followed by 1x PBS + fatty acid free BSA before detachment with trypsin. Cells were spun down, and frozen at -80°C until day of extraction.

Fatty acids were extracted from cells by resuspending and sonicating cells in a mixture of methanol, distilled H_2O, and chloroform (2:1:2). Mixture was spun at 10,000 RCF for 10 minutes at 4°C to separate organic and aqueous phases. The organic phase was evaporated under nitrogen to obtain a dry lipid fraction for derivatization. Fatty acids were derivatized by adding 2 mL of IS solution (40 mL MeOH, 10 mL toluene, 5 mg butylated hydroxytoluene) and 2 µL of acetylchloride (Sigma) to the dried lipid fraction and heating at 95°C for 1 hour. Derivatized fatty acid methyl esters were then extracted by adding 5 mL of 6% potassium carbonate solution to separate hydrophobic and hydrophilic phases. The hydrophobic phase containing fatty acid methyl esters was analyzed by GC/MS on an Agilent GC/MS 7890A/5975A with a DB-5 column. Enrichment of ^{13}C into palmitate was determined using IsoCor (Millard et al., 2012).

Gas Chromatography/Mass Spectrometry of TCA cycle metabolites

Measurements of citrate and malate were conducted essentially as described (Shah et al., 2016). Briefly, $6x10^5$ cells (for 6 hour labeling) or $4x10^5$ cells (for 24 hour labeling) were plated and allowed to adhere overnight. Culture medium was changed the following day to DMEM without glucose (Gibco), supplemented with 10% dialyzed fetal bovine serum (dFBS) (Gibco 26400044, Lot. 1616514), 10 mM [U-^{13}C]glucose (Cambridge Isotope Laboratories), and 100 µM sodium acetate, and incubated for 6 or 24 hours. To measure acetate incorporation into TCA cycle metabolites, DMEM without glucose was supplemented with 10% dFBS, 10 mM glucose, and 100 µM [1,2-^{13}C]acetate (Cambridge Isotope Laboratories). At time of harvesting, media was removed from cells and cells were quickly scraped into 1 mL of cold methanol and collected into conical tubes. 0.3 mL of water was added to each sample, and samples were then sonicated for 60 seconds. Samples were then centrifuged for 15 minutes at 8,500 RPM at 4°C. Following centrifugation, supernatant was transferred to a 4 ml vial, and samples were heated under nitrogen to evaporate methanol.

For derivatization, pyridine and BSTFA-TCMS were added sequentially in a 1:1 ratio, and allowed to react at 54^{o}C for 30 minutes. Finally, samples were spun down for 10 minutes at 13,000 RPM at room temperature. Supernatants were transferred GC-MS vials with pulled glass inserts and were analyzed by GC/MS on an Agilent GC/MS 7890A/5975A with a DB-5 column. Enrichment of ^{13}C into TCA cycle intermediates was determined using IsoCor (Millard et al., 2012).

Mass Spectrometry Analysis of Histone Acetylation

To measure glucose incorporation into histone acetyl-groups, 10^5 cells were plated and allowed to adhere overnight. Culture medium was changed the following day to glucose-free DMEM (Gibco) supplemented with 10% dFBS (Gibson), 10 mM [U-^{13}C]glucose, and 100 μM acetate, and incubated for 24 hours. Measurement of acetate incorporation into histone acetyl-groups was done in identical conditions, but with 100 μM or 1mM [1,2-^{13}C]acetate and 10 mM glucose. Histones were acid extracted from cells using 0.4 N HCl. These samples were TCA precipitated, acetone washed, and prepared for mass spectrometry analysis as previously described (Kuo et al., 2014). A Waters (Milford, MA) Acquity H-class UPLC system coupled to a Thermo (Waltham, MA) TSQ Quantum Access triple-quadrupole (QqQ) mass spectrometer was used to quantify modified histones. Selected reaction monitoring was used to monitor the elution of the acetylated and propionylated tryptic peptides. Transitions were created to distinguish between normal and heavy (^{13}C) acetylation marks on the histone H3 tail: histone H3 lysine 9 (H3K9), H3K14, H3K18, and H3K23. The detailed transitions of H3 are reported in the Supplementary Information (Detection parameters of tryptic peptides from histone H3).

QqQ MS Data Analysis: Each acetylated and/or propionylated peak was identified by retention time and specific transitions. The resulting peak integration was conducted using Xcalibur software (version 2.1, Thermo). The fraction of a specific peptide (F_p) is calculated as $F_p = I_s / (\sum I_p)$, where I_s is the intensity of a specific peptide state and I_p is the intensity of any state of that peptide.

Detection parameters of tryptic peptides from histone H3

	Precursor ion (m/z)	Product ions (m/z)	Collision energy (eV)	Retention time (min)
KhSTGGKhAPR	495.282	817.444	18	0.4
		730.412	18	
		572.343	18	
KhSTGGKaAPR	494.276	815.437	18	0.4
		728.405	18	
		570.336	18	
KhSTGGKpAPR	501.286	829.453	18	0.7
		742.421	18	
		584.351	18	
KaSTGGKhAPR	494.278	817.444	18	0.4
		730.412	18	
		572.343	18	
KaSTGGKaAPR	493.275	815.437	18	0.4
		728.405	18	
		570.336	18	
KaSTGGKpAPR	500.282	829.453	18	0.7
		742.421	18	
		584.351	18	
KpSTGGKhAPR	501.288	817.444	18	0.7
		730.412	18	
		572.343	18	
KpSTGGKaAPR	500.284	815.437	18	0.7

KpSTGGKpAPR	507.291	728.405	18	1.1
		570.336	18	
		829.453	18	
		742.421	18	
KhQLATKhAAR	537.826	584.351	18	2.7
		774.474	19	
		661.390	19	
KhQLATKaAAR	536.822	590.353	19	2.7
		772.468	19	
		659.384	19	
KhQLATKpAAR	543.830	588.346	19	3.5
		786.483	19	
		673.399	19	
KaQLATKhAAR	536.824	602.362	19	2.7
		774.474	19	
		661.390	19	
KaQLATKaAAR	535.819	590.353	19	2.7
		772.468	19	
		659.384	19	
KaQLATKpAAR	542.827	588.346	19	3.5
		786.483	19	
		673.399	19	
KpQLATKhAAR	543.832	602.362	19	3.6
		774.474	19	
		661.390	19	
KpQLATKaAAR	542.829	590.353	19	3.6
		772.468	19	
		659.384	19	
KpQLATKpAAR	549.835	588.346	19	4.4
		786.483	19	
		673.399	19	
		602.362	19	

*p= propionylated, a= acetylated, h= 13C acetylated

Determination of acetyl-CoA and ^{13}C incorporation into acyl-CoAs:

Internal standard generation:
[$^{13}C_3{}^{15}N_1$]-labeled acyl-CoA internal standard was generated by culturing *pan*6-deficient *Saccharomyces cerevisiae* with [$^{13}C_3{}^{15}N_1$]-pantothenate (Isosciences, King of Prussia, PA), as described previously (Snyder et al., 2015). A 500 ml culture at stationary phase was resuspended in 100 ml of 10% (w/v) trichloroacetic acid (Sigma-Aldrich, St. Louis, MO cat. #T6399). The cells were dismembranated in 10 ml aliquots by sonication (60 0.5 s pulses) with a probe tip sonicator (Thermo Scientific) and centrifuged at 3000 g for 10 mins at 4°C. The cleared supernatant was stored at -80°C.

Cell treatment and harvest:

[U-^{13}C]glucose and [U-^{13}C]acetate incorporation into acyl-CoA thioesters were analysed in cells incubated in the presence of 10 mM [U-^{13}C]glucose (Cambridge Isotope Laboratories CLM-1396-1) or 100 µM [U-^{13}C]acetate (Cambridge Isotope Laboratories CLM-440-1) for 6 hours. For relative acetyl-CoA determination cells were incubated in the same conditions in the absence of labeled substrate. Cells were removed from culture dish by scraping on ice and resuspended directly in the cell culture medium. Cell volume and concentration were determined by Coulter counter (Beckman-Coulter). An appropriate volume of each cell sample was pelleted by centrifugation (500 x g for 10 min at 4 °C) such that total cell volume in each cell pellet was equal.

Short chain acyl-CoA extraction:
Frozen tissue samples were cut to ~ 50 mg on a super chilled ceramic tile on dry ice. The weighed samples were added to 1 mL of thawed [$^{13}C_3{}^{15}N_1$]-labeled acyl-CoA internal standard in 1.5 mL Eppendorf tubes on ice. Cell pellets were resuspended in 1 ml 10% (w/v) trichloroacetic acid. For relative acyl-CoA quantitation, 100 µl of [$^{13}C_3{}^{15}N_1$]-labeled acyl-CoA internal standard was added to each sample. Internal standard was omitted for ^{13}C labeling experiments. Samples were homogenized and dismembranated by 60 (for tissues) or 20 (for cell pellets) 0.5 s pulses with a probe tip sonicator (Thermo Scientific). The homogenised samples were centrifuged at 13,000 × *g* for 10 min at 4 °C. Supernatants were purified by solid-phase extraction using Oasis HLB 1cc (30 mg) SPE columns (Waters). Columns were washed with 1 mL methanol, equilibrated with 1 mL water, loaded with supernatant, desalted with 1 mL water, and eluted with 1 mL methanol containing 25mM ammonium acetate. The purified extracts were evaporated to dryness under nitrogen then resuspended in 55 µl 5% (w/v) 5-sulfosalicylic acid in water.

Liquid chromatography:
Analytes were separated before introduction to the mass spectrometer using a reversed-phase Phenomenex HPLC Luna C18 column with 5 mM ammonium acetate in water as solvent A, 5 mM ammonium acetate in acetonitrile/water (95:5, v/v) as solvent B, and acetonitrile/water/formic acid (80:20:0.1, v/v/v) as solvent C. Gradient conditions were as follows: 2% B for 1.5 min, increased to 25% over 3.5 min, increased to 100% B in 0.5 min and held for 8.5 min, washed with 100% C for 5 min, before equilibration for 5 min. The flow rate was 200 µl/min. For determination of [^{13}C]acetate incorporation into acetyl-CoA in primary mature adipocytes an alternative LC method was used, as described (Guo et al., 2016).

Mass-spectrometry:
For relative quantitation of acetyl-CoA levels in cells, samples were analyzed using an API 4000 triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) in the positive ESI mode as described previously (Snyder et al., 2015). Acetyl-CoA was quantified by single reaction monitoring (SRM) of *m/z* 810.1/303.1 and the [$^{13}C_3{}^{15}N_1$]-labeled internal standard at *m/z* 814.1/307.1. Samples (10 µl) were injected using a Leap CTC autosampler (CTC Analytics, Switzerland) and data were analyzed with Analyst 1.4.1 software (Applied Biosystems).

For [U-^{13}C]glucose and [U-^{13}C]acetate labeling and mouse tissue experiments, samples were injected by an Ultimate 3000 autosampler and analytes were detected using a Thermo Q Exactive instrument in positive ESI mode as described elsewhere (Frey et al., 2016). Briefly, scan parameters were alternating full scan from 760 to 1800 *m/z* at 140,000 resolution and data-independent acquisition (DIA) looped three times with all fragment ions multiplexed at a normalized collision energy (NCE) of 20 at a resolution of 280,000. An isolation width of 7 *m/z* with an offset of 3 *m/z* was used to capture all relevant isotopologues for targeted acyl-CoA thioesters. Parent ion and product ion *m/z* transitions detected are indicated in the table below.

Species	Isotopologue	Parent *m/z*	Product *m/z*
Acetyl-CoA	M0	810.1331	303.1373
Acetyl-CoA	M1	811.1364	304.14066
Acetyl-CoA	M2	812.13976	305.14401
Acetyl-CoA	M3	814.14311	306.14737
Acetyl-CoA	M4	814.14647	307.15072
Acetyl-CoA	M5	815.14982	308.15408
Acetyl-CoA	$[^{13}C_3{}^{15}N_1]$-internal standard	814.1402	307.1444
Succinyl-CoA	M0	868.13853	361.14278
Succinyl-CoA	M1	869.14188	362.14614
Succinyl-CoA	M2	870.14524	363.14949
Succinyl-CoA	M3	871.14859	364.15285
Succinyl-CoA	M4	872.15195	365.1562
Succinyl-CoA	M5	873.1553	366.15956
Malonyl-CoA	M0	854.12288	347.12713
Malonyl-CoA	M1	855.12623	348.13049
Malonyl-CoA	M2	856.12959	349.13384
Malonyl-CoA	M3	857.13294	350.1372
Malonyl-CoA	M4	858.1363	351.14055
HMG-CoA	M0	912.16474	405.169
HMG-CoA	M1	913.1681	406.17235
HMG-CoA	M2	914.17145	407.17571
HMG-CoA	M3	915.17481	408.17906
HMG-CoA	M4	916.17816	409.18242
HMG-CoA	M5	917.18152	410.18577
HMG-CoA	M6	918.18487	411.18913
HMG-CoA	M7	919.18823	412.192482

For [^{13}C]acetate incorporation into acetyl-CoA in primary mature adipocytes, samples were injected by an Ultimate 3000 autosampler and analytes were detected using a Thermo Q Exactive HF instrument with HESI in negative mode. Instrument parameters were as follows, spray voltage 3000 V, capillary temperature 325 °C sheath gas 40 arbitrary units, auxillary gas 10 arbitrary units, spare gas 2 arbitrary units, S-lens RF level 55. Scan parameters were alternating full scan from 70 to 950 *m/z* at 120,000 resolution. Acetyl-CoA isotopologue ions were detected as listed in the table below.

Species	Isotopologue	*m/z*
Acetyl-CoA	M0	808.1185
Acetyl-CoA	M1	809.12185
Acetyl-CoA	M2	810.12521
Acetyl-CoA	M3	811.12856
Acetyl-CoA	M4	812.13192

Data were processed in Xcalibur, TraceFinder (Thermo), and isotopic enrichment was calculated to compensate for the non-linearity of isotopic enrichment as outlined and applied previously (Fernandez et al., 1996; Worth et al., 2014). For acetyl-CoA determination

in mouse tissues, the parent ion peak for acetyl-CoA M0 and the [$^{13}C_3{}^{15}N_1$]-acetyl-CoA internal standard were integrated to determine relative abundance between samples.

***In vivo de novo* lipogenesis analysis**
Plasma D2O enrichment:

The ^2H labeling of water from samples or standards was determined via deuterium acetone exchange (McCabe et al., 2006) (Yang et al., 1998). 5 uls of sample or standard was reacted with 4 uls of 10N NaOH and 4 uls of a 5% (v/v) solution of acetone in acetonitrile for 24 hours. Acetone was extracted by the addition of 600 ul chloroform and 0.5 g Na2SO4 followed by vigorous mixing. 100 uls of the chloroform was then transferred to a GCMS vial. Acetone was measured using an agilent DB-35MS column (30 m 3 0.25mm i.d. 3 0.25 mm, Agilent J&W Scientific) installed in an Agilent 7890A gas chromatograph (GC) interfaced with an Agilent 5975C mass spectrometer (MS) with the following temperature program: 60 °C initial, increase by 20 °C/min to 100 °C, increase by 50 °C/min to 220 °C, and hold for 1 min. The split ratio was 40:1 with a helium flow of 1 ml/min. Acetone eluted at approximately 1.5min. The mass spectrometer was operated in the electron impact mode (70 eV). The mass ions 58 and 59 were integrated and the % M1 (m/z 59) calculated. Known standards were used to generate a standard curve and plasma % enrichment was determined from this. All samples were analyzed in triplicate.

Total fatty acids were extracted from tissues and plasma using a bligh and dyer based methanol/chloroform/water extraction with C16 D31 as an internal standard. Briefly, 500 uls MeOH, 500 uls CHCL3, 200 uls H2O and 10 uls 10 mM C16 D31/ 10 mgs tissue were added to weighed pre-ground tissue. This was vortexed for 10 minutes followed by centrifugation at 10,000 g for 5 minutes. The lower chloroform phase was dried and then derivitised to form fatty acid methyl esters via addition of 500 uls 2% H2SO4 and incubation at 50°C for 2 hours. FAMES were extracted via addition of 100 uls saturated salt solution and 500 uls hexane and these were analyzed using a Select FAME column (100m x 0.25mm i.d.) installed in an Aglient 7890A GC interfaced with an Agilent 5975C MS using the following temperature program: : 80 °C initial, increase by 20 °C/min to 170 °C, increase by 1 °C/min to 204 °C, then 20 °C/min to 250 °C and hold for 10 min.

Calculations:
The % mass isotopomer distributions of each fatty acid was determined and corrected for natural abundance using in-house algorithms adapted from Fernandez et al.(Fernandez et al., 1996). Calculation of the fraction of newly synthesized fatty acids (FNS) was based on the method described by Lee et al. (Lee et al., 2000), where FNS is described by the following equation:

FNS=ME/(n x p)

Where ME is the average number of deuterium atoms incorporated per molecule(ME =1 x m_1 + 2 x m_2 +3 x m_3 ...), p is the deuterium enrichment in water and n is the maximum number of hydrogen atoms from water incorporated per molecule. N was determined using the equation:

m2/m1 = (N-1) /2 x p/q

As described by Lee et al (Lee et al., 1994) where q is the fraction of hydrogen atoms and p + q = 1. The molar amount of newly synthesized fatty acids was determined by:

MNS = FNS x total fatty acid amount (nmoles/mg tissue).

Acetate measurements:
*Protein filtration from the samples***:** 200 ml of sample was filtered through 3 kDa cutoff nanosep centrifugation device (Pall Inc. Port Washington, NY), and recovered volume of the filtrate noted.

Sample preparation for NMR spectroscopy: 180 µl of filtrate was added to 20 µl of DSS (4,4-Dimethyl-4-silapentane-1-sulfonic acid, Cambridge Isotope Limited, Andover, MA) in D_2O to a final concentration of 0.16 mM.

Nuclear Magnetic Resonance (NMR) Spectroscopy: All NMR spectra were acquired in Bruker Avance III HD NMR spectrometer equipped with a triple resonance inverse (TXI) 3 mm probe (Bruker Biospin, Billerica, MA) and a Bruker Samplejet for sample handling. One-dimensional NMR spectra were acquired using the first transient of a 2 dimensional NOESY and generally of the form RD-90-t-90-t_m-90-ACQ (Beckonert et al., 2007). Where RD = relaxation delay, t = small time delay between pulses, t_m = mixing time

and ACQ = acquisition. The water signal was saturated using continuous irradiation during RD and t_m. The spectra were acquired using 76K data points and a 14 ppm spectral width over 384 scans with a 1 second interscan (relaxation) delay and 0.1 second mixing time. The FIDs were zero filled to 128K; 0.1 Hz of linear broadening was applied followed by Fourier transformation, baseline and phase correction using an automated program provided by Bruker Biospin.

Profiling of acetate signal from the NMR spectra: The acetate signal was quantitatively profiled from the spectra using Chenomx v 8.0 (Edmonton, Canada) (Weljie et al., 2006) by quantifying the acetate peak at 1.90 ppm (Supplementary Fig. 2A) relative to the DSS peak area. Proper care was taken to omit the effects of the overlapping signals (for example, lysine and arginine overlapping with the 1.90 ppm acetate peak) using the Chenomx targeted spectral fitting algorithm (Weljie et al., 2006).

Histology

For histology, subcutaneous and visceral white fat tissue was fixed in formalin overnight, deyhydrated and submitted to the AFCRI Histology Core for paraffin embedding, sectioning and hematoxylin and eosin staining.

Primary adipocyte ^{13}C-acetate uptake

Primary adipocyte isolation was conducted as previously described (Wellen et al., 2007), with minor modifications. Briefly, visceral white adipose tissues (VWAT) were removed from mice ages 12-16 weeks and weighed. Isolation buffer (1X Krebs-Ringer-Phosphate Buffer, 2% Hepes, 25 mg/mL BSA, 0.2 mM adenosine, 10 mM glucose, 100 µM [1,2-^{13}C]acetate, pH 7.5) and 1 mg/mL collagenase was prepared ahead of time and added to VWAT at 2 mL per gram of tissue while on ice. VWAT fat pads were chopped with scissors in the buffer for 5 minutes until no large chunks of tissue remained, and then incubated at 37^{o}C for 45 minutes while shaking to allow collagenase digestion to occur. Following collagenase digestion, tissue suspension was passed through a 100 µm mesh filter and allowed to sit at room temperature until primary adipocytes separated from infranatant. Infranatant was subsequently removed and remaining adipocytes were washed 3x in isolation buffer without collagenase. Following washes, primary adipocytes were re-suspended in 3x cell volume of isolation buffer containing 100 µM [1,2-^{13}C]acetate and incubated at 37^{o}C for 4 hours while shaking. Following incubation, suspension was allowed to sit at room temperature until primary adipocytes separated from infranatant. Infranatant was subsequently removed and the remaining primary adipocytes were re-suspended in ice cold 10% tricholoroacetic acid, and frozen at -80^{o}C until samples could be analyzed for acyl-CoA species by mass spectrometry as described above.

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