Figure S1

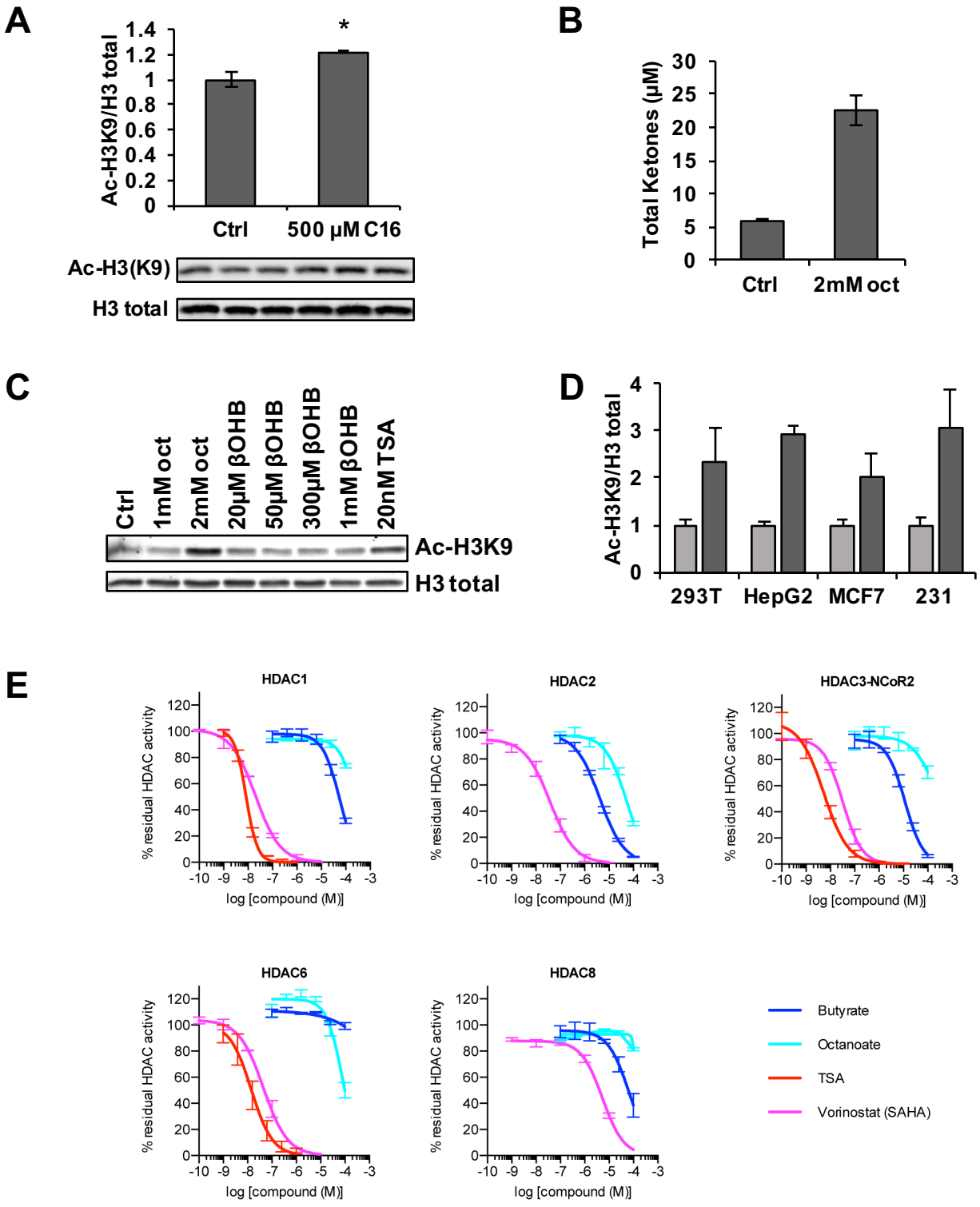

Figure S1, related to figure 1. Octanoate induced histone acetylation is not due to ketone body production. (A) Histone acetylation western blot and quantification of AML12 cells treated with 500 µM palmitate in the absence of glucose for 24 hours. Graph shows fold change compared to control, +/- SD. (B) Absolute levels of total ketone bodies produced from 2 mM octanoate after 72 hours treatment in AML12 cells, +/- SD. (C) Histone acetylation measured by western blot of acetylated histone H3K9 after AML12 cells were treated with octanoate or exogenous βOHB for 72 hours at doses equivalent to that produced by octanoate or several fold higher. (D) Quantification of histone H3K9 acetylation western blot from Fig 1G of multiple, non-ketogenic cell lines treated for 24 hours with 2 mM octanoate or vehicle control. Graph shows fold-change over compared control, +/- SD. Data are representative of experiments repeated at least twice. (E) Percent residual HDAC activity of HDACs 1,2,3,6, or 8 for dose curves of octanoate compared to the well-known HDAC inhibitors inhibitors butyrate, Trichostatin A (TSA) and suberanilohydroxamic acid (SAHA).

Figure S2

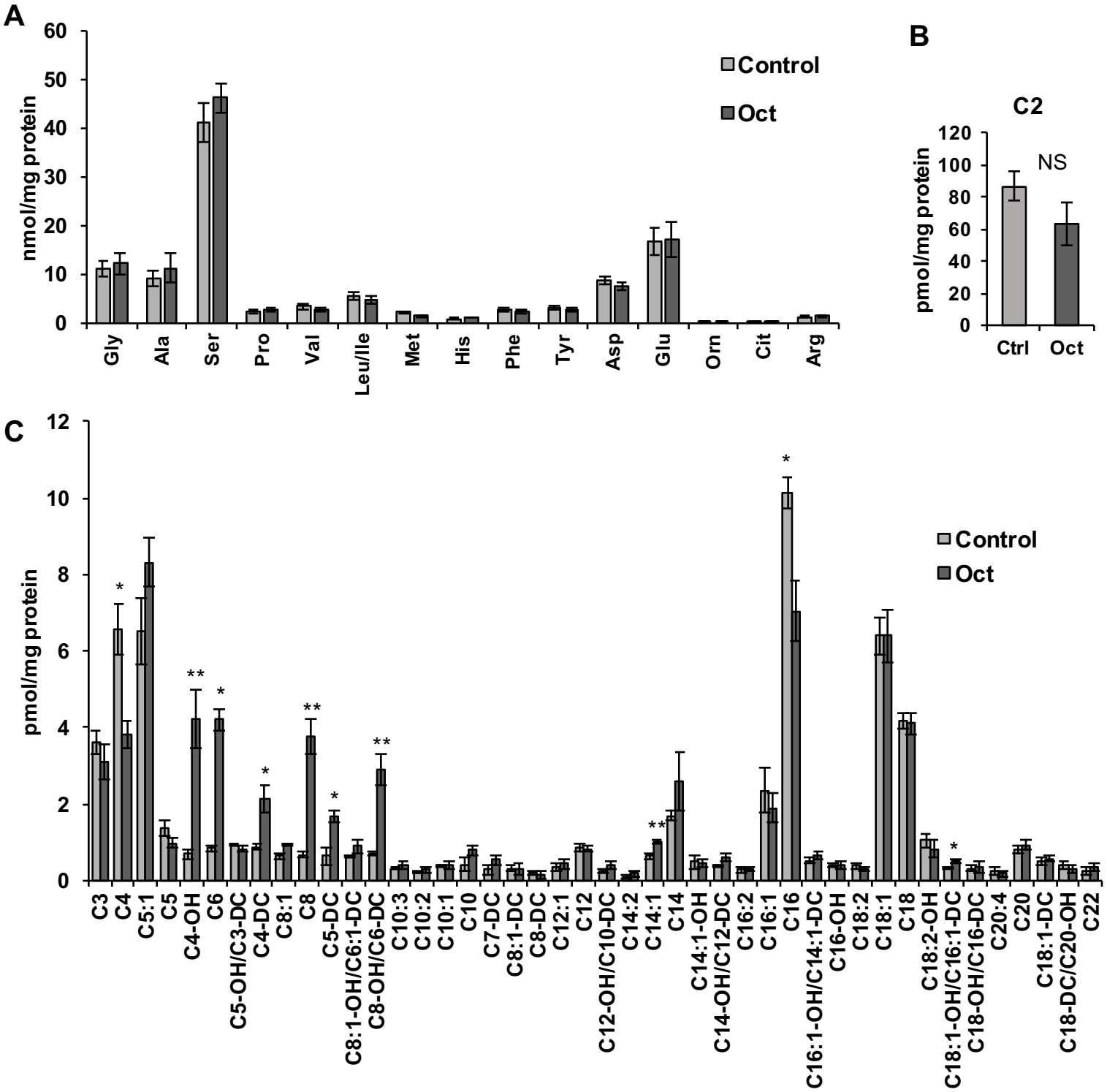

Figure S2, related to figure 3. Amino acid and acylcarnitine profile in octanoate treated AML12 cells. AML12 cells treated with 2 mM octanoate or vehicle in complete DMEM + 10% FBS in quadruplicate for 24 hours prior to metabolite extraction for organic acid analysis via tandem mass spectrometry (as in Figure 3A) of amino acids (A), acetylcarnitine (B) and acylcarnitines (C). Data are the average of quadruplicate samples +/- SEM. *p<0.05, **p<0.01 (Students t-test).

Figure S3

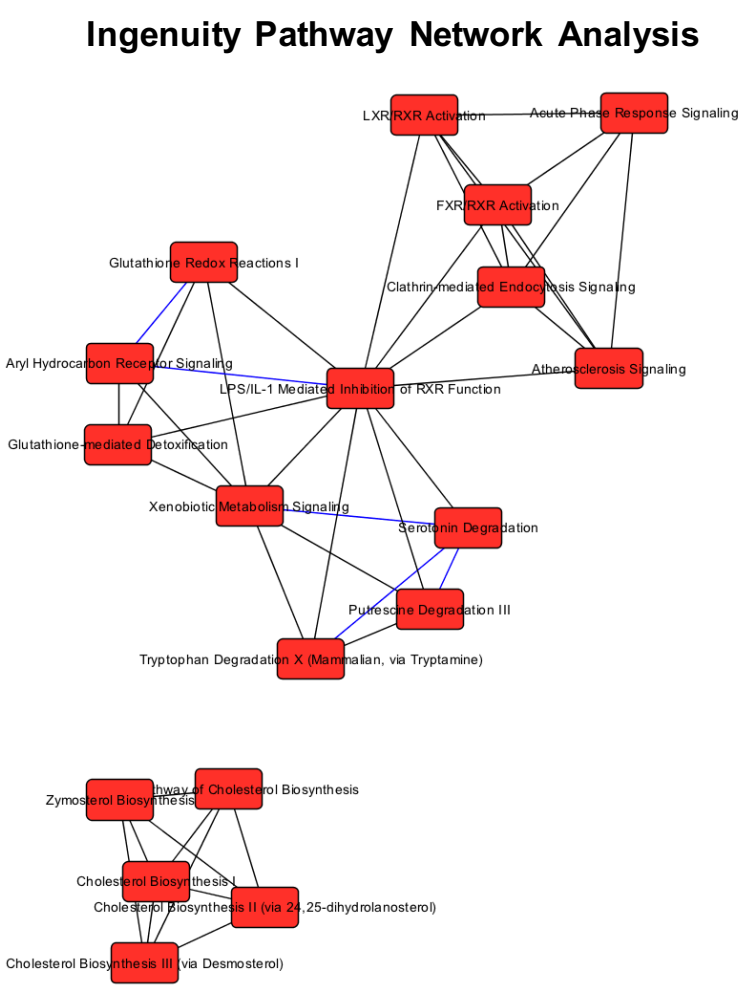

Fig S3, related to figure 4. IPA pathway analysis of activated pathways after octanoate treatment. Pathway network analysis of gene microarray from Fig 4 using Ingenuity Pathway Analysis (IPA) program with analyses criteria set at $\log_2$ fold change cutoff of 0.5 and p-value cutoff of 0.05.

Figure S4

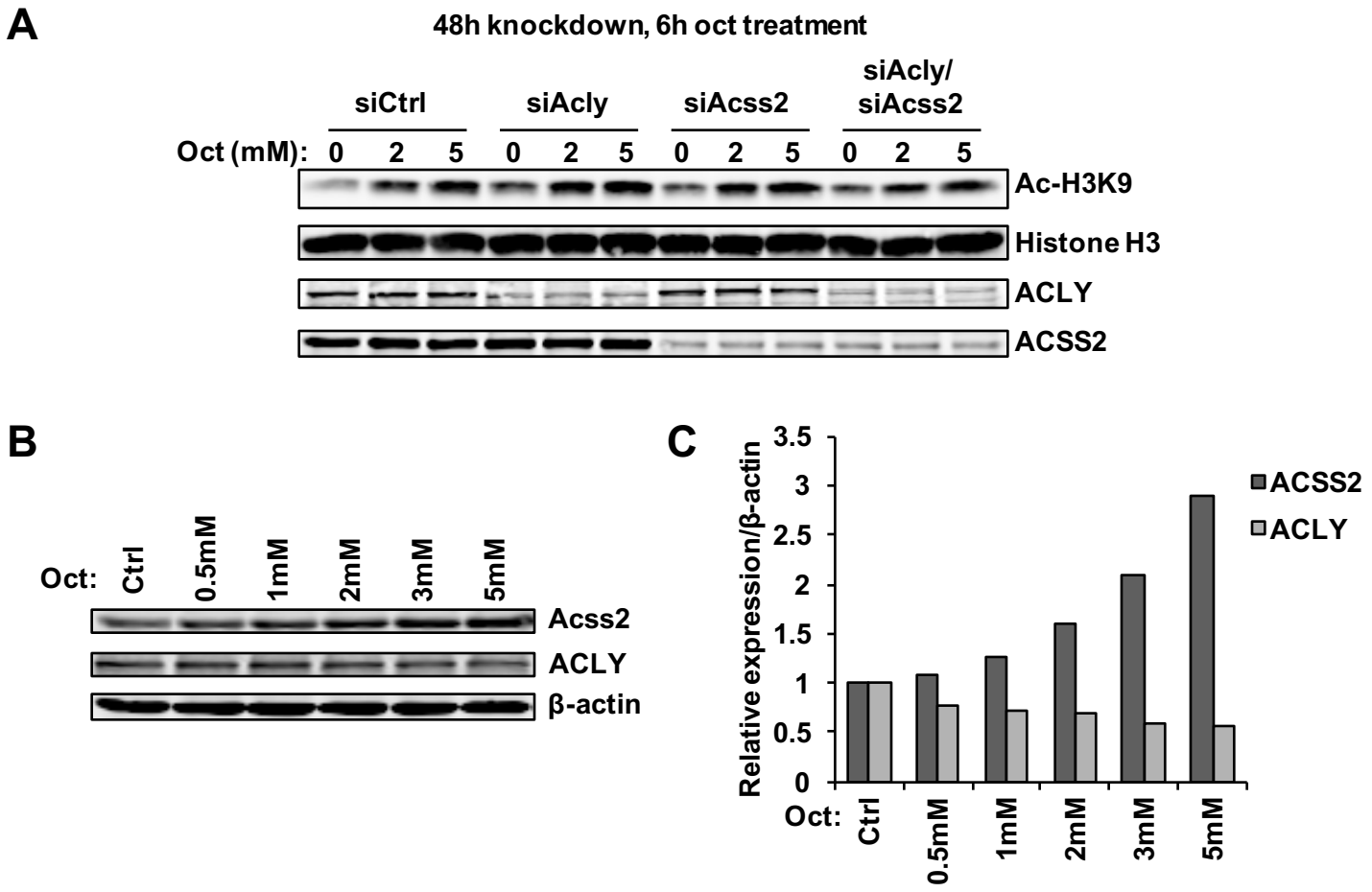

Figure S4. related to discussion. Testing the role of ACLY and ACSS2 in lipid-induced histone acetylation. (A) AML12 cells were treated with siRNAs to ATP-citrate lyase (*Acly*) and/or nucleocytosolic acetyl-CoA synthetase (*Acss2*). 48 hours was allowed for gene knockdown followed by 6-hour treatment with vehicle, 2 mM or 5 mM octanoate supplemented to complete DMEM + 10% FBS. Histone acetylation and protein knockdown were measured by western blot. Similar results were obtained after 24 hours treatment (data not shown). (B) Western blot of ACLY and ACSS2 in AML12 cells treated with increasing dose of octanoate for 24 hours in complete media. (C) Quantification of (B) showing increased ACSS2 expression with decreasing ACLY expression with increasing concentration of octanoate.

Table S1. TMT-based quantitation of PTM and protein abundance changes in octanoate-treated AML12 hepatocytes; related to Figure 1. Table containing analyzed proteomic data comparing the relative abundance of phosphopeptides, acetylpeptides, and protein abundance, displayed on the following four spreadsheet columns:

Tab 1) *Key*. Includes a detailed summary of the information fields included in the columns of the subsequent four tabs. The "comments" column indicates cases where certain rows or columns are hidden initially, but users can right click and select "unhide" to view the information.
Tab 2) *PTM-containing peptide quant*. Annotation and quantitative values of all acetylpeptides and phoshopeptides identified at 1% FDR.
Tab 3) *Protein quant*. Annotation and quantitative values of all "Master" proteins identified at 1% FDR.
Tab 4) *Protein group metadata*. Additional annotation, including summary of all sites on modification identified in this study, for all possible proteins to which the identified peptides map.

Table S2. ^{13}C-enrichment of acetylated histone peptides in [U-^{13}C]octanoate treated AML12 hepatocytes; related to Figure 2. Supplemental table containing analyzed ^{13}C-enrichment data for PBS and [U-^{13}C]octanoate-treated hepatocytes. File includes brief description of methods; validation of natural isotopic abundances in H3/H4 acetylpeptides; isotopic data (both uncorrected & corrected for natural isotopic abundances) of H3/H4 acetylpeptides for PBS/[U-^{13}C]octanoate-treated cells.

Table S3. Gene microarray of AML12 hepatocytes treated with 0 mM, 1 mM, 2mM, or 5mM octanoate for 24 hours; related to Figure 4. GEO accession number: GSE87156. Supplemental table containing analyzed genomic data containing ProbeID: Illumina Probe ID; GeneSymbol: Gene name associated with probe ID; Detected: Was the probe detected as present in at least 1 sample (detection p-value <= 0.05); LogFC: Log2 Fold-Change (Octanoate/Control); AveExpr: Average expression for probe across all samples; t: T statistic for difference between treatment and control; P.Value: P-Value; adj.P.Val: FDR corrected p-value.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Ketone body measurement

Total ketone bodies were measured in media collected from cells treated with octanoate. Media samples were then tested for total ketones using the Wako Diagnostics Total Ketone bodies kit and protocol (R1 cat# 415-73301 and R2 cat# 411-73401). Ketone levels were then normalized to total protein content determined by BCA assay or total RNA content.

qPCR primers used:

Hmgcs2 (For- CACATGAGACCCTCGATCTTTC, Rev-CACTGGCTTCTCTTTCGTGTAG),
BDH1 (For-CTTCCCCTTCTCCGAAGAGC, Rev-ATCTCATAGCGCAGGCAGTC),
BDH2 (For-TGCAGACTTCATCCAACAGG, Rev-ACCTTCCCGTCTTTTGTCTG) ,
Acaa2 (For-GACTTCTCTGCCACCGATTTA-, Rev-TTGCCCACGATGACACTATC),
CPT1a (For-CCAGGCTACAGTGGGACATT, Rev-GAACTTGCCCATGTCCTTGT),
Pdk4 (For-GAAGCTGATGACTGGTGTATCC, Rev-GACCCACTTTGATCCCGTAAA),
Mylcd (For-CCCTACCGGAGATGTTACTTC, Rev-AGGGCACTCCTTCACAATG),
CD36 (For-GAACCACTGCTTTCAAAAACTGG, Rev-TGCTGTTCTTTGCCACGTCA),
Acot1 (For-CGATGACCTCCCCAAGAACA, Rev-CCAAGTTCACCCCCTTTGGA),
Acaa1a (For-TCTCCAGGACGTGAGGCTAA, Rev-CTCAGAAATTGGGCGATGCG),
Acaa1b (For-GAGACTGCCTGATTCCTATGG, Rev-GCACAATCTCAGCATGGAAG),
Ech1 (For-CAACGAGCTGACCTTCTCTG, Rev-AGGGCTCTTGCTGGAAATATC),
Ephx1 (For-AGGAAGCAGGTGGAGATCCT, Rev-GGGAGGTTTCACGTGGATGA),
Aldh1a1 (For-GGCTCTTCACCTGGCATCTT, Rev-TTGATCCAGTGAAGGCCACC),
Ttr (For-AATCGTACTGGAAGACACTTGG, Rev-TGGTGCTGTAGGAGTATGGG),
Acly (For- CGGGAGGAAGCTGATGAATATG, Rev- GTCAAGGTAGTGCCCAATGAA),
Glut4 (For-CCAACAGCTCTCAGGCATCA, Rev- GAGACCAACGTGAAGACCGT),
HK2 (For- AGCCTCGGTTTCTCTATTTGG, Rev- GACGCATGTGGTAGAGATACTG),
Ldha (For-GCTCCCCAGAACAAGATTACAG, Rev-TCGCCCTTGAGTTTGTCTTC).

Metabolomic profiling
AML12 cells were plated at $2x10^6$ cells per 10 cm dish and allowed to seed overnight. The following day cells were treated with DMEM + 10% FBS containing either PBS or 2 mM octanoate for 24 hours. After 24 hours cells were washed with ice cold PBS, scraped from plates in ice cold PBS then spun down in a swinging bucked centrifuge at 500xg for 5 mins to pellet cells at 4°C. Cell pellets were then lysed in 300 µL 0.6% formic acid, 30 µL was then removed for protein quantification, followed by addition of 270 µL acetonitrile to give a final concentration of 0.3% formic acid and 50% acetonitrile. Amino acids, acylcarnitines and organic acids were analyzed using stable isotope dilution technique. Amino acids and acylcarnitine measurements were made by flow injection tandem mass spectrometry using sample preparation methods described previously (An et al., 2004; Ferrara et al., 2008). The data were acquired using a Waters TQD mass spectrometer equipped with Acquity™ UPLC system and controlled by MassLynx 4.1 operating system (Waters, Milford, MA). Organic acids were quantified using methods described previously (Jensen et al., 2006) employing Trace Ultra GC coupled to ISQ MS operating under Xcalibur 2.2 (Thermo Fisher Scientific, Austin, TX).

Isotope tracer experiments
For isotope tracing experiments, cells were seeded in 6-well dishes and harvested as described above. Metabolic steady-state was determined from cells collected at 2h, 6h, 12h and 24h post treatment. Cells were washed and scraped with PBS on ice. Pellets and collected media were stored at -80°C. Metabolites were measured intracellularly or from media as described below.

Gas chromatography/mass spectrometry
GC-MS analysis was performed on an Agilent 7890B GC system equipped with a HP-5MS capillary column (30 m, 0.25 mm i.d., 0.25 µm-phase thickness; Agilent J&W Scientific), connected to an Agilent 5977A Mass Spectrometer operating under ionization by electron impact (EI) at 70 eV. Helium flow was maintained at 1 mL/min. The source temperature was maintained at 230°C, the MS quad temperature at 150°C, the interface temperature at 280°C, and the inlet temperature at 250°C (intracellular metabolites, glucose) or 200°C (acetate). Mass spectra were recorded in selected ion monitoring (SIM) mode with 4 ms dwell time. Mass isotopomer distributions were obtained by integration of ion chromatograms (Antoniewicz Anal Chem 2007) and corrected for natural isotope abundances (Fernandez CA J Mass Spectrom 1996). ^{13}C-labeling of a metabolite was determined as previously described (Leighty and Antoniewicz, 2013). In brief, ^{13}C-labeling $= \frac{1}{n}\sum_{i=1}^{n} i(M+i)$, where n is the number of carbon atoms in a given MS fragment, and $M+i$ are the relative mass isotopomer abundances after correction for natural isotope abundances.

Intracellular metabolite derivatization and ^{13}C-assay
Harvested metabolite samples were allowed to thaw and 750 µL methanol was added to each sample. Samples were briefly vortexed before centrifugation at 14,000xg for 10 minutes. Protein-free supernatants were transferred and evaporated to dryness under N_2 gas-flow at 37°C using an evaporator. *Tert*-butyldimethylsilyl (TBDMS) derivatized metabolites were performed as described in (Ahn WS Metab Eng 2011) with slight modifications. Briefly, metabolites were resuspended in 25 µL of methoxylamine hydrochloride (2% (w/v) in pyridine) and incubated at 40°C for 90 minutes on a heating block. After brief centrifugation, 35 µL of MTBSTFA + 1% TBDMS was added and the samples were incubated at 60°C for 30 minutes. The derivatized samples were centrifuged for 5 minutes at 14,000xg and the supernatants were transferred to GC vials for GC-MS analysis. The injection volume was 1 µL and samples were injected in split or splitless mode depending on analyte of interest. GC oven temperature was held at 80°C for 2 minutes, increased to 280°C at 7°C/min, and held at 280°C for a total run time of 40 minutes. $^{13}C_2$-acetyl CoA was analyzed using a method published by Magnes et al. (Magnes et al., 2005), which relies on the extraction procedure described by Deutsch et al. (Deutsch et al., 1994). The acetyl CoA was further purified by solid phase extraction as described by Minkler et al. (Minkler et al., 2008). The acetyl CoA was measured by flow injection analysis using positive electrospray ionization on Xevo TQ-S, a triple quadrupole mass spectrometer (Waters, Milford, MA) employing methanol/water (80/20, v/v) containing 30 mM ammonium hydroxide as the mobile phase. Spectra were acquired in the multichannel acquisition mode monitoring the neutral loss of 507 amu.

Media metabolite derivatization and GC-MS analysis
For measurement of $^{13}C_2$-acetate in the media, 50 µL of media was combined with 50 µL of 250 µM [D_3,$^{13}C_2$] acetate. Then, 20 µL of HCl was added to the samples, followed by 100 µL methyl propionate. Samples were vortexed for 1 minute before centrifugation for 10 minutes at 14,000 x g. For TBDMS-acetate, 20 µL MTBSTFA + 1% TBDMS was added to 50 µL of the top extract layer. Samples were incubated for 30 minutes at 60°C. After allowing samples to cool to room temperature, samples were centrifuged for 2 minutes at 14,000g before transfer to GC vials. The injection volume was 1 µL and samples were injected at split of 5. GC oven temperature was held at 80°C for 2 minutes, increased to 140°C at 5°C/min, and increased to 260°C at 20°C/min for a total run time of 20 minutes. Labeling of glucose in the medium was determined after aldonitrile propionate derivatization as described in (Antoniewicz et al., 2011).

Proteomic Materials
Protease (Complete mini EDTA-free) and phosphatase (PhosStop) inhibitor tablets (Roche). 6-plex Tandem Mass Tags (TMT) were purchased from Thermo Fisher Scientific. Sequencing Grade Modified Trypsin was purchased from Promega. Lysyl Endopeptidase (LysC) was purchased from Wako Chemicals. PTMScan Acetyl Lysine Motif IAP Beads and IAP Buffer were purchased from Cell Signaling Technology. tC18 SEP-PAK SPE columns were purchased from Waters. Ni-NTA Magnetic Agarose Beads were purchased from Qiagen.

Cell lysis and digestion for TMT proteomic experiment
AML12 cells were plated and seeded as above for metabolic profiling analysis. Cells were washed and scraped in PBS and pellets were frozen at -80°C. Cell pellets were re-suspended in 500 µL of ice-cold 8M Urea Lysis Buffer (8 M urea in 50 mM Tris, pH 8.0, 40 mM NaCl, 2 mM $MgCl_2$, 1x cOmplete mini EDTA-free protease inhibitor tablet, 10 mM Nicotinamide, 10 µM TSA) additionally supplemented with phosphatase inhibitors (1x PhosStop phosphatase inhibitor tablet 50 mM NaF and 10 mM Na pyrophosphate). The cells were disrupted by a combination of pipetting and vortexing, and subsequent sonication with a probe sonicator in three 5 second bursts (power setting of 3), incubating on ice in-between each burst. Samples were centrifuged at 10,000 x g for 10 min at 4°C and the supernatant was retained. Protein concentration was determined by BCA, and equal amount of protein (500 µg, adjusted to 2.5 mg/mL with Urea Lysis Buffer) from each sample was reduced with 5 mM DTT at 37°C for 30 min, cooled to RT, alkylated with 15 mM iodoacetamide for 30 min in the dark and unreacted iodoacetamide quenched by the addition of DTT up to 15 mM. Each sample was digested with 5 µg LysC (100:1 w/w, protein to enzyme) at 37°C for 4 hours. Following dilution to 1.5 M urea with 50 mM Tris (pH 8.0), 5 mM $CaCl_2$, the samples were digested with trypsin (50:1 w/w, protein:enzyme) overnight at 37°C. The samples were acidified to 0.5% TFA and centrifuged at 4000 x g for 10 min at 4°C to pellet insoluble material. The supernatant containing soluble peptides was desalted by solid phase extraction (SPE) with a Waters 50 mg tC18 SEP-PAK SPE column and eluted once with 500 µL 25% acetonitrile/0.1% TFA and twice with 500 µl 50% acetonitrile/0.1% TFA. The 1.5 ml eluate was frozen and dried in a speed vac. Each sample was re-suspended in 100 µL of 200 mM triethylammonium bicarbonate (TEAB), mixed with a unique 6-plex Tandem Mass Tag (TMT) reagent (0.8 mg re-suspended in 50 µL100% acetonitrile), and shaken for 4 hours at room temperature. After samples were quenched with 0.8 µL 50% hydroxylamine and shaken for 15 additional minutes at room temperature, all six samples were combined, frozen, and dried in a speed vac. The mixture was re-suspended in 1 mL 0.5% TFA and subjected to SPE again with a Waters 100 mg tC18 SEP-PAK SPE column as described above. The eluate was vortexed and split into one aliquot containing ~ 5% of the total peptide mixture (150 µg) and a second aliquot containing ~ 95% (2.85 mg). Both aliquots were frozen and dried in a speed vac. The 150 µg aliquot of the "input" material was saved at -80°C for quantification of unmodified peptides. The 2.85 mg aliquot was used for enrichment of post-translational modifications (PTMs) as described below.

Peptide labeling and PTM enrichment for TMT proteomic experiment
The 2.85 mg aliquot was resuspended in 1.4 mL 1X IAP Buffer and subjected to immunoprecipitation (IP) with PTMScan Acetyl Lysine Motif IAP Beads on a rotator overnight at 4°C. The next day, the antibody-peptide complexes were pelleted via centrifugation at 2000 x g for 30 seconds and the precipitate was washed 2 times in 1 mL of IP buffer and 3 times in 1 mL PBS (centrifuging each time). The flow-through

and wash fractions were saved for phosphopeptide analysis (described below). Acetylpeptides were eluted in 55 µL of 0.1% TFA for 10 minutes followed by a quick wash in 50 µL of 0.1% TFA and the supernatants (2000 x g, 30 sec) from each elution were combined. The eluate was acidified to 0.5% TFA (and brought to a 1 mL volume), desalted on a 50 mg tC18 SEP-PAK SPE column and eluted as described above. The eluate was frozen on dry ice and dried in a speed vac. The flow-through and wash fractions from the acetyl IP described above were combined, acidified, and desalted using a 100 mg tC18 SEP-PAK SPE column. The elutate was dried in a speed vac and subjected to phosphopeptide enrichment via immobilized metal affinity chromatography (IMAC) using Ni-NTA Magnetic Agarose Beads as described previously (Cell Metab. 2012 Nov 7;16(5):672-83.), with slight modifications. Briefly, the beads were washed three times with water, incubated in 40 mM EDTA, pH 8.0 for 30 minutes while shaking, and subsequently washed with water three times. The beads were then incubated with 100 mM $FeCl_3$ for 30 minutes while shaking, and were washed four times with 80% acetonitrile/0.15% TFA. Samples were re-suspended in 1 ml 80% acetonitrile/0.15% TFA, added to the beads, and incubated for 30 minutes at room temperature while shaking. Samples were subsequently washed three times with 1 ml 80% acetonitrile/0.15% TFA and eluted for 1 minute by vortexing in 100 µl of 1:1 acetonitrile:0.7% NH_4OH in water. Eluted phosphopeptides were acidified immediately with 50 µl 4% formic acid, frozen and dried in a speed vac. The dried phosphopeptides were re-suspended in 12 µL 0.1% formic acid, placed in an auto-sampler vial, and frozen. All samples were submitted to the Duke University School of Medicine Proteomics Core facility for analysis by nLC-MS/MS described below. The input material described above (5% of the large-scale mixture, ~ 150 µg of TMT-labeled peptides) was re-suspended in 150 µL 100 mM Ammonium Formate, pH 10, 10% of which (15 uL,15 ug) was aliquoted into a separate tube and subjected to 5-step Stage-Tip High-pH reversed-phase fractionation. Each fraction, containing approximately 3 µg of material, aliquoted into glass vials, acidified to 1% formic acid, dried in a speedvac, and re-suspended in 15 µL.

Nano-LC-MS/MS for TMT proteomic experiment

All samples were subjected to *nano*LC-MS/MS analysis using a nano-Acquity UPLC system (Waters) coupled to a *Q Exactive Plus* Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fischer Scientific) *via* a nanoelectrospray ionization source. Prior to injection, the phosphopeptide sample was resuspended in 20 µL 1% TFA/2% acetonitrile supplemented with 10 mM citrate and the acetylpeptide sample was re-suspended in 12 µL 1% TFA/2% acetonitrile. Each of the PTM enriched samples were analyzed with technical replicate runs, with 4 µL of sample injected. The 5 input material fractions were analyzed in singlicate, with 3 uL injections. For each injection, the sample was first trapped on a Symmetry C18 20 mm × 180 µm trapping column (5 µl/min at 99.9/0.1 v/v water/acetonitrile), after which the analytical separation was performed over a 90 minute gradient (flow rate of 400 nanoliters/minute) of 3 to 30% acetonitrile using a 1.7 µm Acquity BEH130 C18 75 µm × 250 mm column (Waters Corp.), with a column temperature of 55ºC. MS^1 (precursor ions) was performed at 70,000 resolution, with an AGC target of $1x10^6$ ions and a maximum injection time of 60 ms. MS^2 spectra (product ions) were collected by data-dependent acquisition (DDA) of the top 20 most abundant precursor ions with a charge greater than 1 per MS1 scan, with dynamic exclusion enabled for a window of 30 seconds. Precursor ions were filtered with a 1.2 m/*z* isolation window and fragmented with a normalized collision energy of 30. MS2 scans were performed at 17,500 resolution, with an AGC target of $1x10^5$ ions and a maximum injection time of 60 ms.

***nano*LC-MS/MS Data Processing for TMT proteomic experiment**

Raw LC-MS/MS data data have been deposited to the ProteomeXchange Consortium via the PRIDE (Vizcaino et al., 2016) partner repository with the dataset identifier PXD005040. Raw LC-MS/MS data were processed in Proteome discoverer v2.1 with service pack 1 (PD2.1, SP1, Thermo Fisher Scientific), using both the Sequest HT and MS Amanda search engines. Data were searched against the UniProt mouse complete proteome database of reviewed (Swiss-Prot) and unreviewed (TrEMBL) proteins, which consisted of 50,189 sequences on the date of download (2/3/2016). Default search parameters included oxidation (15.995 Da on M) as a variable modification and carbamidomethyl (57.021 Da on C) and TMTplex (229.163 Da on peptide N-term and K). Phospho runs added phosphorylation (79.966 Da on S,T,Y) as a variable modification. Acetyl runs added acetylation (42.011 Da on K) and changed TMT to a variable modification on K (remaining fixed on peptide N-term). Data were searched with a 10 ppm precursor mass and 0.02 Da product ion tolerance. The maximum number of missed cleavages was set to a default value of 2 (but changed to 4 for acetyl runs) and enzyme specificity was trypsin (full). Considering

each data type (acetyl, phospho, input) separately, peptide spectral matches (PSMs) from each search algorithm were filtered to a 1% false discovery rate (FDR) using the Percolator node of PD2.1. For acetyl and phospho data, site localization probabilities were determined for all modifications using the ptmRS algorithm. PSMs were grouped to unique peptides while maintaining a 1% FDR at the peptide level and using a 95% sites localization threshold for PTMs. Peptides from all samples (phosho, acetyl, input) were grouped to proteins together using the rules of strict parsimony and proteins were filtered to 1% FDR using the Protein FDR Validator node of PD2.1. Reporter ion intensities for all PSMs having co-isolation interference below 0.3 (30% of the ion current in the isolation window) and average reporter S/N>10 for all reporter ions were summed together at the peptide group and protein level, but keeping quantification for each data type (phosho, acetyl, input) separate. Peptides shared between protein groups were excluded from protein quantitation calculations.

Statistical analysis for TMT proteomic experiment

Protein and peptide groups tabs in the PD2.1 results were exported as tab delimited .txt. files, opened in Microsoft EXCEL, and analyzed as described previously (Grimsrud et al., 2012). First, peptide group reporter intensities for each peptide group in the input material were summed together for each TMT channel, each channel's sum was divided by the average of all channels' sums, resulting in channel-specific loading control normalization factors to correct for any deviation from equal protein/peptide input into the six sample comparison. Reporter intensities for peptide groups from both the acetyl and phospho fractions, and for proteins from the input fraction were divided by the loading control normalization factors for each respective TMT channel. Analyzing the acetylpeptide, phosphopeptide, and protein datasets separately, all loading control-normalized TMT reporter intensities were converted to $\log_2$ space, and the average value from the six samples was subtracted from each sample-specific measurement to normalize the relative measurements to the mean. For the octanoate and vehicle comparison (n=3), condition average, standard deviation, p-value (p, two-tailed student's t-test, assuming equal variance), and adjustped p-value ($P_{adjusted}$, Benjamini Hochberg FDR correction) were calculated(Benjamini and Hochberg, 1995; Lesack and Naugler, 2011). For protein-level quantification, only Master Proteins–or the most statistically significant protein representing a group of parsimonious proteins containing common peptides--identified at 1% FDR were used for quantitative comparison. PTM level measurements (acetyl and phospho) were calculated both alone (referred to as ***abundance***) and with normalization to any change in the corresponding Master Protein (referred to as ***relative occupancy***), calculated by subtracting Log_2 Master Protein values from PTM-containing peptide quantitation values on a sample-specific basis.

Acid extraction of histones

Proteomics of acetylated histones using [U-^{13}C]octanoate. Cells were plated at $2x10^6$ cells per well in 10cm dishes, allowed seed overnight, then treated for 24h with complete DMEM + 10% FBS in the presence or absence of 2 mM [U-^{13}C]octanoate. Histones were extracted by following the Abcam histone extraction protocol for western blots. Briefly, cells were washed with ice cold PBS, scraped and pelleted. Pellets were then resuspended in 1 mL triton extraction buffer (TEB), which consisted of PBS containing 0.5% TritonX100, HALT protease inhibitor cocktail, 0.02%NaN_3 and 10 µM TSA. Cells were lysed in a 4°C room while rotating for 10 mins. Nuclei were then spun down by centrifugation at 6,500xg for 10 mins at 4°C. Supernatant was then discarded and nuclei were washed in 500 µL TEB buffer and centrifuged at 6,500xg for 10 mins at 4°C. Pellet was resuspended 200 µL in 0.2N HCL and rotated overnight at 4°C to acid extract histones. Samples were then centrifuged as before and supernatant containing histone proteins were frozen at -20°C.

***nano*LC-MS/MS** data **analysis of ^{13}C acetylation of histones**

Acid-extracted histones (~750 uL in 0.2N HCl) were supplemented with 75 uL M Tris pH8 to neutralize the pH, then spiked with enough 8M Urea Lysis Buffer (197.5 uL) to achieve a final concentration of 1.5 M urea. Samples were sonicated, reduced, alkylated, as described above. Trypsin was added at 100:1 (protein:enzyme) and samples were incubated overnight at 37°C and desalted by SPE, and dried down in 95% and 5% aliquots as described above. For each sample, the larger aliquot (95%) was subjected to acetyl IP and the eluate analyzed by LC-MS/MS as described above, but with 2 µL (of 12 µL) run in duplicate over a 60-minute gradient of 5-40%, and a DDA top10 method with an MS2 AGC target of $5x10^4$, an NCE of 27, and dynamic exclusion enabled for 20 seconds. Raw data was searched in PD2.1 with oxidation (M), acetylation (K), methylation (R), demethylation (R), and trimethylation (R) as variable modifications, full

tryptic cleavage and 7 missed cleavages (which was optimized by several rounds of searching). PTM localization, peptide and protein FDR were conducted as described above. The PD2.1.msf search results file and the FASTA database use for the search were uploaded to Skyline to analyze by MS1 filtering. To extract the M+0 through M+8 isotopologues for each peptide identified, the results were analyzed as if they were SILAC labeled, considering the M+0 through M+4 isotopologues as the isotope envelope for the "light" peptide (peptide identified by the PD2.1 search) and the M+4 through M+8 isotopologues as the "heavy" peptide (+4.01342 Da was added under the "Isotope Modifications" in the "Peptide Settings" menu to represent the addition of four ^{13}C isotopes). Extracted ion chromatograms were produced for all matching scans containing precursors ("p" ion types) of charge states 2 through 7, selecting the same resolution (70,000 at *m/z* 400) that the data was collected. Data was exported as MSstats Input and was analyzed in Excel. As a quality control, the overlapping M+4 peaks (from treating each peptide as if were SILAC labeled) were compared and were determined to have negligible differences. For histone acetylpeptide isoforms overlapping with the TMT experiment, peak areas were further analyzed in MATLAB for the ten acetylpeptides that increased to the greatest extent with octanoate treatment in the TMT dataset.

Radiolabeled oxidation assays

AML12 cells were plated at 80,000 cells per well in 24-well plates and treated the next day with 2 mM octanoate or PBS in complete media with 10% FBS. 24h later media was removed and replaced with media containing ^{14}C labeled substrate for 1.5 hours. Octanoate was not present during the oxidation period. After 1.5h the media was collected and added to a NaOH trap to capture CO_2. The trap was set up with medium collected from well placed at the bottom of a glass tube, which had a smaller tube elevated inside of it that contained NaOH. The trap was capped with a rubber septa and perchloric acid was added via syringe to the bottom of the trap to promote the release of CO_2. Traps were then placed on a shaker for 1h, after which the NaOH fraction was collected and added to scintillation vials and ^{14}C radioactivity was measured with a scintillation counter. ^{14}C DMP was normalized to protein content determined by BCA assay (Bicinchoninic acid) solution (Sigma catalogue # B9643) and Copper(II) Sulfate Solution (Sigma catalogue # 2284).

Gene Microarray

AML12 cells were plated at 350,000 cells per well in 6-well plates and allowed seed overnight. The following day cells were treated with PBS, 1 mM, 2 mM or 5 mM octanoate for 24h added to complete media with 10% FBS. After 24h media was removed, cells were washed with PBS, lysed with Bio-Rad Aurum RNA lysis buffer and frozen at -80°C. RNA was collected using the Bio-Rad Aurum mini kit as described above for qPCR. Illumina expression microarray data underwent a strict quality control evaluation using the *lumi* (Du et al., 2008) Bioconductor (Gentleman et al., 2004) package from the R statistical programming environment. Raw data underwent a variance stabilizing transformation followed by robust spline normalization to eliminate systematic differences across the arrays. A linear model with a moderated test statistic was used to identify genes that were differentially expressed between the drug treatment and control samples using the *limma* (Ritchie et al., 2015) package. The false discovery rate method was used to correct for multiple hypothesis testing. Gene set enrichment analysis (Mootha et al., 2003) was performed to identify differentially regulated pathways and gene ontology terms for each of the comparisons performed. Ingenuity Pathway Analysis (Qiagen) was used to identify possible transcription factor activation or inhibition based on the gene expression profile in octanoate-treated cells. Using the vehicle control group compared to the 2 mM group from normalized microarray data and a $\log_2$ cutoff fold change of 0.5 and a p-value cutoff of 0.05. Raw data were deposited to NCBI gene expression omnibus (GEO) under the accession GSE87156.

***In vitro* histone deacetylase inhibition assays**

Assay materials: HDACs 1, 2, 8, and HDAC3-NCoR2 were purchased from BPS Bioscience (San Diego, CA 92121) and HDAC6 and trichostatin A (TSA) were purchased from Enzo Life Sciences (Postfach, Switzerland). Trypsin (10,000 units/mg, TPCK treated from bovine pancreas) was from Sigma Aldrich (Steinheim, Germany). The fatty acids were purchased from Sigma Aldrich and used without further purification. The assay buffer contained HEPES (50 mM), KCl (100 mM), tween-20 [0.001% (v/v)], tris(2-carboxyethyl)phosphine (TCEP, 200 μM), BSA (0.5 mg/mL), pH = 7.4. Protocols were adapted from Bradner *et al.* (Bradner et al., 2010) using Ac-Leu-Gly-Lys(Ac)-AMC as substrate for HDACs 1–3 and 6 and Ac-Leu-Gly-Lys(Tfa)-AMC as substrate for HDAC 8. Dose–response experiments with internal

controls were performed in black low-binding Corning half-area 96-well microtiter plates. The appropriate dilution of inhibitor (5 μL of 5 × the desired final concentration, prepared from 10 mM DMSO stock solutions with a maximum final DMSO concentration of 1%) was added to each well followed by substrate in HDAC assay buffer (10 μL). *Ac-Leu-Gly-Lys(Ac)-AMC* was used at a final concentration of 20 µM for HDACs 1, 2, 3, and 6. *Ac-Leu-Gly-Lys(Tfa)-AMC* was used at a final concentration of 200 µM for HDAC8. Finally, a freshly prepared solution of the appropriate HDAC (10 μL) was added and the plate (containing a final volume of 25 µL in each well) was incubated at 37 °C for 30 min. The final concentrations of enzyme were as follows: HDAC1: 3 ng/μL, HDAC2: 1.5 ng/μL, HDAC3: 0.7 ng/μL, HDAC6: 4 ng/μL, and HDAC8: 0.7 ng/μL. Then trypsin (25 μL, 0.4 mg/mL) was added and the assay development was allowed to proceed for 15 min at room temperature, before the plate was read using a Perkin-Elmer Enspire plate reader with excitation at 360 nm and detecting emission at 460 nm. The data were analyzed by non-linear regression using GraphPad Prism to afford IC_{50} values from the dose-response experiments, and K_i values were determined from the Cheng-Prusoff equation, $K_i = IC_{50}/(1+[S]/K_m)$, assuming standard fast-on–fast-off mechanism of inhibition, and using previously reported K_m values (Bradner et al., 2010). Each value is based on at least two individual experiments performed in duplicate.

SUPPLEMENTAL REFERENCES

An, J., Muoio, D.M., Shiota, M., Fujimoto, Y., Cline, G.W., Shulman, G.I., Koves, T.R., Stevens, R., Millington, D., and Newgard, C.B. (2004). Hepatic expression of malonyl-CoA decarboxylase reverses muscle, liver and whole-animal insulin resistance. Nature medicine *10*, 268-274.

Antoniewicz, M.R., Kelleher, J.K., and Stephanopoulos, G. (2011). Measuring deuterium enrichment of glucose hydrogen atoms by gas chromatography/mass spectrometry. Anal Chem *83*, 3211-3216.

Benjamini, Y., and Hochberg, Y. (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. J Roy Stat Soc B Met *57*, 289-300.

Bradner, J.E., West, N., Grachan, M.L., Greenberg, E.F., Haggarty, S.J., Warnow, T., and Mazitschek, R. (2010). Chemical phylogenetics of histone deacetylases. Nature chemical biology *6*, 238-243.

Deutsch, J., Grange, E., Rapoport, S.I., and Purdon, A.D. (1994). Isolation and quantitation of long-chain acyl-coenzyme A esters in brain tissue by solid-phase extraction. Analytical biochemistry *220*, 321-323.

Du, P., Kibbe, W.A., and Lin, S.M. (2008). lumi: a pipeline for processing Illumina microarray. Bioinformatics *24*, 1547-1548.

Ferrara, C.T., Wang, P., Neto, E.C., Stevens, R.D., Bain, J.R., Wenner, B.R., Ilkayeva, O.R., Keller, M.P., Blasiole, D.A., Kendziorski, C.*, et al.* (2008). Genetic networks of liver metabolism revealed by integration of metabolic and transcriptional profiling. PLoS genetics *4*, e1000034.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J.*, et al.* (2004). Bioconductor: open software development for computational biology and bioinformatics. Genome biology *5*, R80.

Grimsrud, P.A., Carson, J.J., Hebert, A.S., Hubler, S.L., Niemi, N.M., Bailey, D.J., Jochem, A., Stapleton, D.S., Keller, M.P., Westphall, M.S.*, et al.* (2012). A Quantitative Map of the Liver Mitochondrial Phosphoproteome Reveals Posttranslational Control of Ketogenesis. In Cell metabolism, pp. 672-683.

Jensen, M.V., Joseph, J.W., Ilkayeva, O., Burgess, S., Lu, D., Ronnebaum, S.M., Odegaard, M., Becker, T.C., Sherry, A.D., and Newgard, C.B. (2006). Compensatory responses to pyruvate carboxylase suppression in islet beta-cells. Preservation of glucose-stimulated insulin secretion. The Journal of biological chemistry *281*, 22342-22351.

Leighty, R.W., and Antoniewicz, M.R. (2013). COMPLETE-MFA: complementary parallel labeling experiments technique for metabolic flux analysis. Metabolic engineering *20*, 49-55.

Lesack, K., and Naugler, C. (2011). An open-source software program for performing Bonferroni and related corrections for multiple comparisons. Journal of pathology informatics *2*, 52.

Magnes, C., Sinner, F.M., Regittnig, W., and Pieber, T.R. (2005). LC/MS/MS method for quantitative determination of long-chain fatty acyl-CoAs. Analytical chemistry *77*, 2889-2894.

Minkler, P.E., Kerner, J., Ingalls, S.T., and Hoppel, C.L. (2008). Novel isolation procedure for short-, medium-, and long-chain acyl-coenzyme A esters from tissue. Analytical biochemistry *376*, 275-276.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstrale, M., Laurila, E.*, et al.* (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nature genetics *34*, 267-273.

Ritchie, M.E., Phipson, B., Wu, D., Hu, Y., Law, C.W., Shi, W., and Smyth, G.K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic acids research *43*, e47.

Vizcaino, J.A., Csordas, A., del-Toro, N., Dianes, J.A., Griss, J., Lavidas, I., Mayer, G., Perez-Riverol, Y., Reisinger, F., Ternent, T.*, et al.* (2016). 2016 update of the PRIDE database and its related tools. Nucleic acids research *44*, D447-456.