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Supplemental Information

Chromatin Succinylation Correlates with Active Gene Expression and Is Perturbed by Defective TCA Cycle Metabolism

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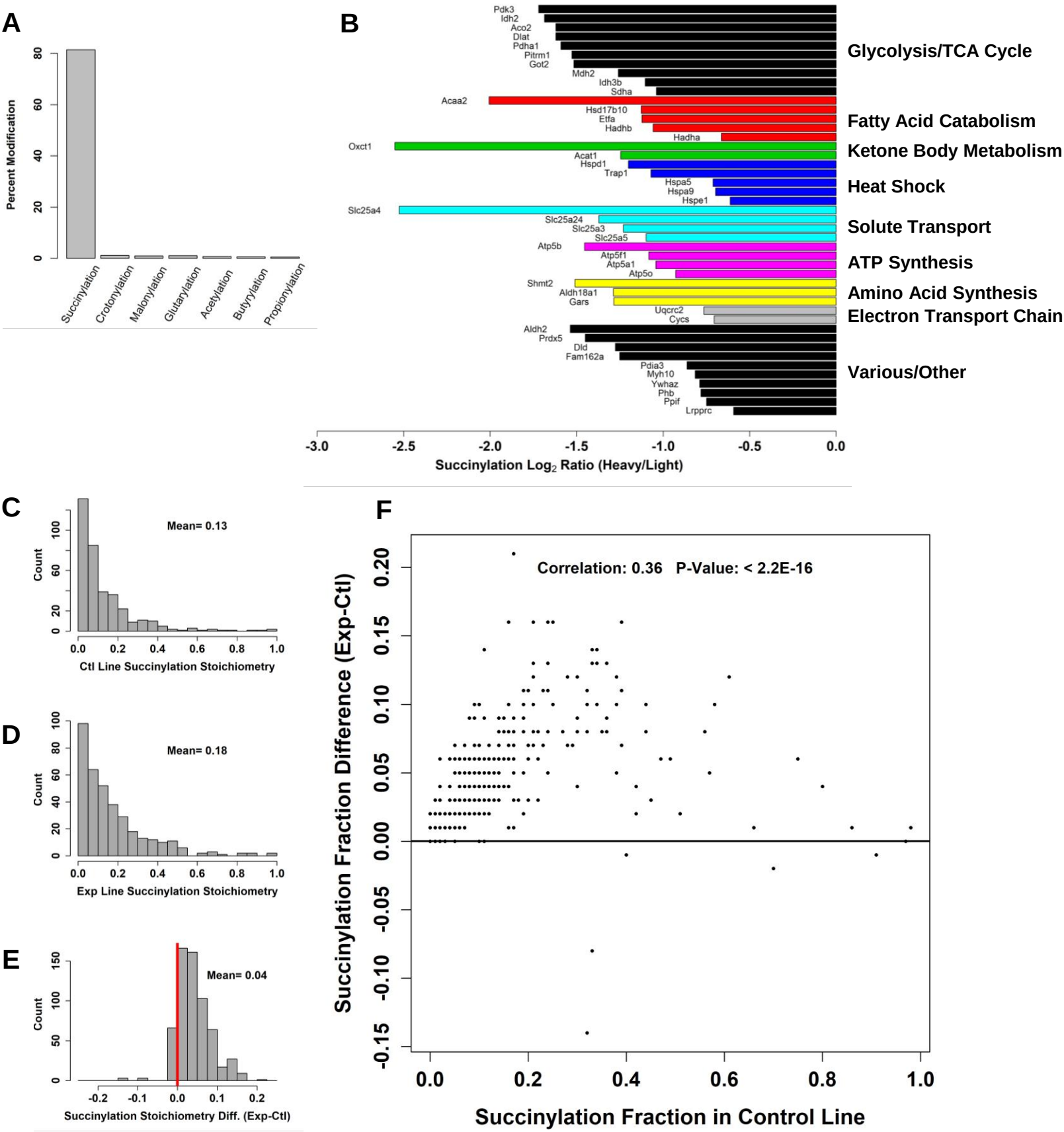

Figure S1. Related to Figure 1. Additional analysis of protein succinylation changes. A) Analysis of acylation modification prevalence in proteomic dataset following PTM-401 antibody pull-down. B) Relative succinylation changes for mitochondrial proteins identified as being differentially-succinylated in the context of SDHC loss, grouped according to functional role, supportive of Figure 1. C) Histogram showing distribution of succinylation stoichiometries in control cell line data set (N=364 succinylated sites quantified). D) Histogram showing distribution of succinylation stoichiometries in experimental cell line data set (N=364 succinylated sites quantified). E) Difference between succinylation stoichiometries identified in experimental and control cell line datasets. F) Analysis of correlation between succinylation stoichiometry difference (Exp-Ctl) and succinylation stoichiometry in control cell line.

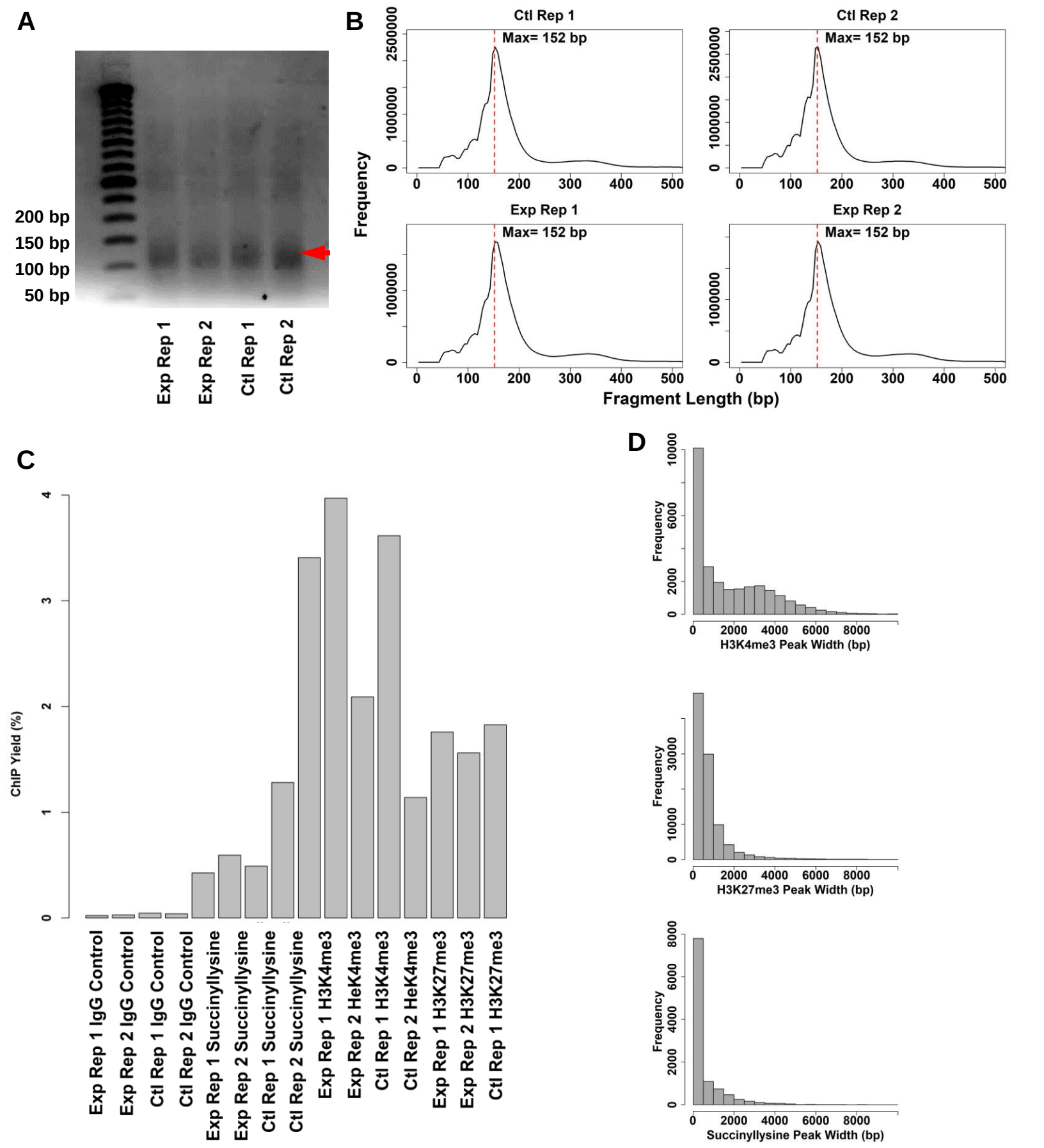

Figure S2. Related to Figure 2. Supplemental analysis of ChIP-seq experiment technical performance. A) Input chromatin size distribution assessed by gel electrophoresis (2% agarose gel; visualized with ethidium bromide) following cell lysis, MNase digestion, and sonication. Red arrow indicates position of highest band intensity. B) Paired end mapped fragment length quantified from ChIP-seq experiments. C) Quantified ChIP yields from pull-downs with anti-H3K4me3, anti-H3k27me3, and anti-succinyllysine antibodies. D) Widths of ChIP-seq peaks called via the MACS peak calling algorithm for H3K4me3, H3k27me3, and succinyllysine.

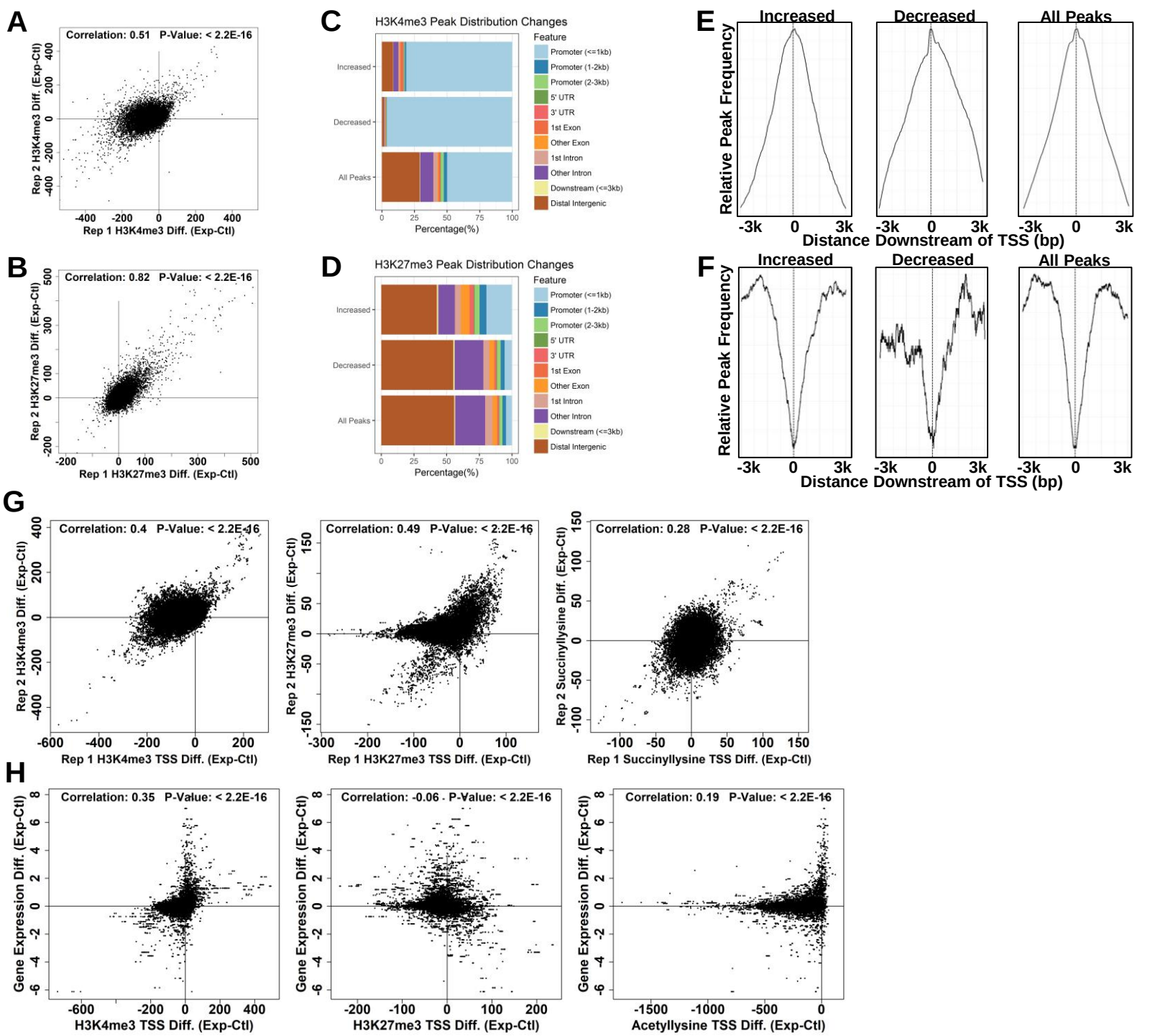

Figure S3. Related to Figure 4. Additional analysis of genome-wide H3K4me3 and H3K27me3 ChIP-peak intensity change patterns in response to SDHC loss and correlations with transcriptional effects. A) Correlation analysis of observed H3K4me3 peak integrated intensity differences (experimental minus control) between replicate experiments. B) Correlation analysis of observed H3K27me3 peak integrated intensity differences (experimental minus control) between replicate experiments. C) Analysis of genomic feature localization for H3K4me3 peaks showing dramatic integrated intensity differences resulting from SDHC loss. (0.05 and 0.95 quantiles, as shown in panel A). X-axis indicates percentage of identified peaks mapping to a given feature type. D) Analysis of genomic feature localization for H3K27me3 peaks showing dramatic integrated intensity differences resulting from SDHC loss. (0.05 and 0.95 quantiles, as shown in panel A). X-axis indicates percentage of identified peaks mapping to a given feature type. E) Average profile plot showing TSS relative position for H3K4me3 peaks showing dramatic integrated intensity differences resulting from SDHC loss. F) Average profile plot showing TSS relative position for H3K27me3 peaks showing dramatic integrated intensity differences resulting from SDHC loss. G) ChIP-seq difference correlations measured at TSS between biological replicate experiments, with each axis corresponding to differences between experimental and control lines. H) Correlations between changes in chromatin marks at TSS and changes in gene expression.

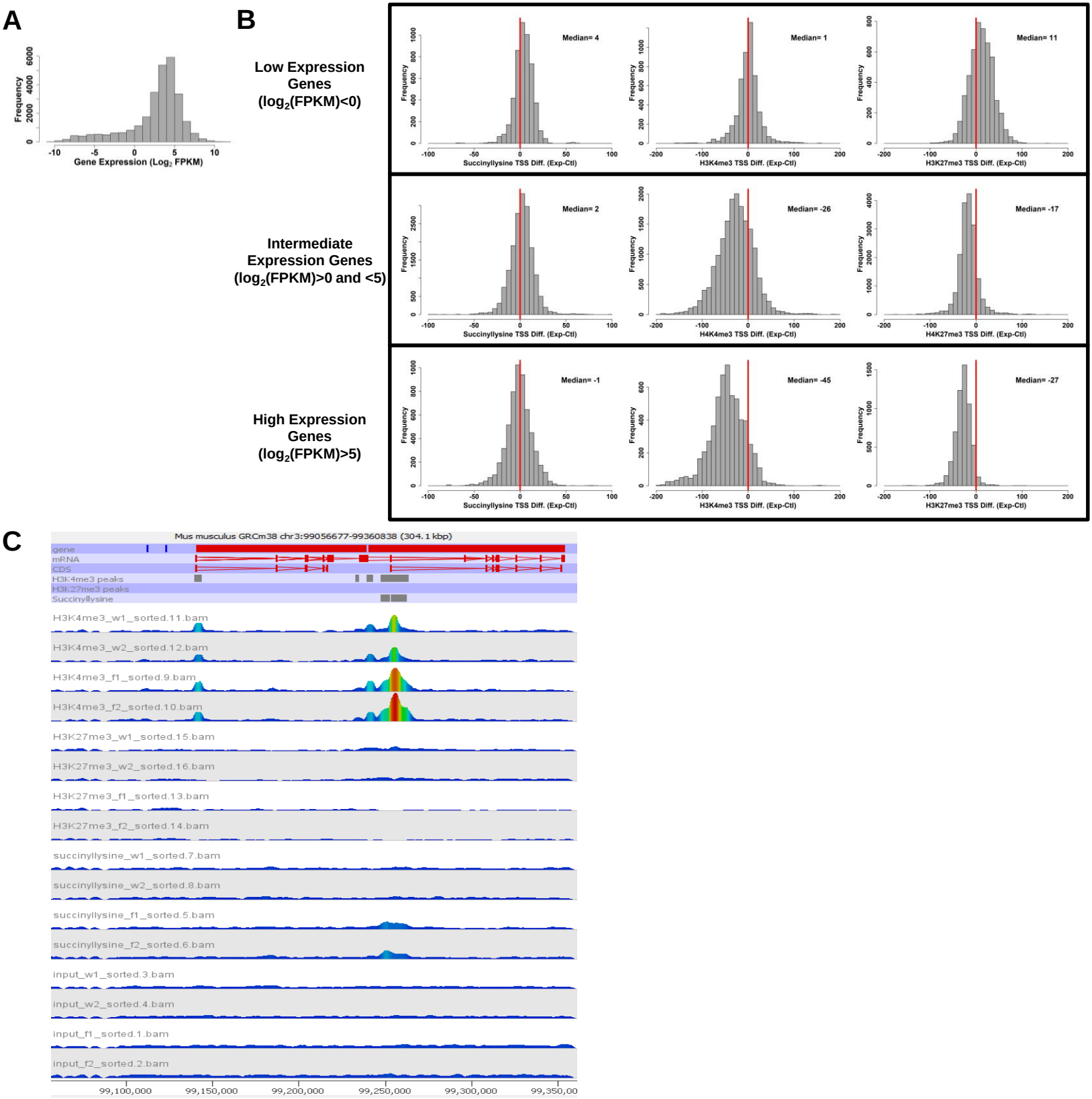

Figure S4. Related to Figure 4. Supplemental analysis of patterns of succinylation, H3K4me3, and H3K27me3 change at TSS in response to SDHC loss. A) Histogram showing distribution of gene expression values in control cell line. Gene expression is presented as $\log_2$-transformed FPKM values. B) Histograms showing distributions of succinyllysine, H3K4me3, and H3K27me3 changes (experimental minus control integrated intensities) measured at TSS, separated according to gene expression value in control cell line. Median values for distributions are indicated on plots. C) Representative genomic view of mouse chromosome 3 showing a succinyllysine peak dramatically affected by SDHC loss. Shown are genomic annotations for genes, mRNAs, CDS, identified ChIP-seq peaks, and wiggle plot representations of mapped reads from the various ChIP-seq experiments. Shown are two biological replicates for each experiment type.

Transparent Methods

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, L.J. Maher (maher@mayo.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Immortalized mouse embryonic fibroblast cell lines

The cell lines used in this study has been described previously (Smestad et al., 2017). Briefly, R26M2rtTA/+;TetOcre;Sdhcfl/fl (experimental) and R26M2rtTA/+;TetOcre;Sdhc fl/+ (control) mouse embryonic fibroblast lines (MEFs) were derived by crossing Sdhcfl/fl;R26M2rtTA/M2rtT mice with Sdhcfl/wt;TetOcre mice, harvesting and homogenizing day 13 embryos, and culturing in DMEM containing penicillin/streptomycin antibiotics (0.5 mg/mL), non-essential amino acids (100 micromolar each of glycine, alanine, asparagine, aspartic acid, glutamic acid, proline, and serine), sodium pyruvate (1 mM), and HEPES buffer (10 mM) at 21% O_2 and 5% CO_2. Cells were grown to superconfluence, split and transduced with SV-40 lentivirus, and then iteratively split at high dilution to yield purified populations of immortalized mouse embryonic fibroblasts (iMEFs). Sex of both experimental and control iMEF cell lines was determined to be female through analysis of sex chromosome aligned reads from ChIP-seq experiments. For routine cell culture subsequent to line derivation, cells were grown in DMEM containing 10% FBS, penicillin/streptomycin antibiotics (0.5 mg/mL each) at 21% O_2 and 5% CO_2.

METHOD DETAILS

TCA cycle metabolomics

For TCA cycle metabolomic studies, experimental (R26M2rtTA/+;TetOcre;Sdhcfl/fl) and control (R26M2rtTA/+;TetOcre;Sdhcfl/wt) iMEF cells were grown in DMEM containing 10% FBS, penicillin/streptomycin antibiotics (0.5 mg/mL each) at 21% O_2 and 5% CO_2. To induce *Sdhc* gene rearrangement, doxycycline hyclate (Sigma Aldrich cat# D9891-25G) was added to media to final concentration of 1 microgram/mL. Cells were grown for 16 d under these conditions, harvested by scraping, washed 3X with PBS, and then submitted to the Mayo Clinic Metabolomics Resource Core for targeted TCA cycle metabolomics studies. For both experimental and control cell lines, these experiments were performed in triplicate. Measured metabolite levels were normalized to total intracellular protein.

For succinyl-CoA measurements, cells were grown as describe above, and the same number of experimental and control cells harvested, washed 2X with PBS, homogenized in 10% trichloroacetic acid (TCA), and immobilized on a C18 SepPak cartridge pre-conditioned with methanol and water. Immobilized metabolites were washed 3X with 2 mL water and then eluted with 2X 0.75 mL methanol. Methanol eluents were diluted to 20% total volume and then lyophilized. Dried eluents were then reconstituted in 100 microliters 0.1M NaOH and CoA esters hydrolyzed by incubation at 40 degrees Celsius for 1 hour. Solutions were neutralized by the addition of 10 microliters of 1M HCl, and then the pH of the solutions stabilized by the addition of 20 microliters of 1 M tris-HCl (pH 7.5). Succinate concentrations in samples were then

quantified in triplicate using the BioVision succinate colorimetric assay kit (BioVision cat # K649).

Succinyllysine immunostaining

Following induction of SDHC loss, experimental and control cells were plated into 96 well plates at a density of 25,000 cells per well in 100 microliters of DMEM media containing 10% FBS, penicillin/streptomycin antibiotics (0.5 mg/mL each), 1 mM pyruvate, 1X MEM NEAA, and 10 mM HEPES buffer (pH7.2-7.5) at 21% O_2 and 5% CO_2. 6 hours after plating, media was aspirated and cells were washed with 100 microliters of PBS, fixed with 100 microliters of 3.7% formaldehyde in PBS for 20 minutes at room temperature, and permeabilized with 100 microliters of 0.1% Triton X-100 in PBS for 15 minutes at room temperature. Cells were then incubated with anti-succinyllysine (PTM Biolabs cat# PTM-401) primary antibody diluted 1:200 into PBS containing 10% FBS for 1 hour at room temperature with gentle shaking. Cells were then washed 5X with 100 microliters of PBS, and then incubated for 30 minutes at room temperature with goat anti-rabbit IgG (H+L) Alexa Fluor 488 (Invitrogen cat# A32731) diluted 1:500 into PBS containing 10% FBS. Cells were then washed 5X with 100 microliters PBS, and then counterstained with DAPI prior to imaging on a Zeiss LSM 780 confocal microscope. For each visual field, an autofocus routine was implemented to capture the plane with the highest DAPI staining intensity. Automated image analysis was then employed to quantify compartment-specific succinylation patterns, as previously described (Carpenter et al., 2006).

Gamma H2A.X phospho 139 immunostaining

Immunostaining for gamma H2A.X phospho 139 was performed using the same general protocol as described for succinyllysine immunostaining (see above), with a mouse monoclonal primary antibody against gamma H2A.X phospho 139 (Abcam cat# ab26350) used at 1:500 dilution, and a goat anti-mouse IgG (H+L) Alexa Fluor 594-conjugated secondary antibody (Invitrogen cat# A11032) used at 1:500 dilution.

Genotoxic drug studies

Cells from two stable SDH loss (experimental) and hemizygous control iMEF lines were plated into wells of a 96 well plate at concentration of 25,000 cells per 100 microliters of DMEM media (without phenol red) containing 10% FBS, penicillin/streptomycin antibiotics (0.5 mg/mL each), 1 mM pyruvate, 1X MEM NEAA, and 10 mM HEPES buffer (pH7.2-7.5) at 21% O_2 and 5% CO_2. Cells were allowed to attach to plate for 12 hours, and then 1 microliter of solutions containing genotoxic drugs (or vehicle control) were added to wells. Dilutions of etoposide were made into DMSO, with final concentrations of drugs ranging from 20 μM to 27 nM. Dilutions of gemcitabine were made into sterile water, with final concentrations ranging from 1 μM to 1 nM. After addition of drugs to cells, the plates were thoroughly mixed and incubated at 37 degrees with 21% O_2 and 5% CO_2 for 48 hours. 10 μL Alamar Blue (Thermo Fisher) cell viability reagent was added to each well. Plates were incubated for 36 h prior to taking absorbance measurements at 570 and 600 nm.

SILAC labeling

SILAC labeling of cells using heavy arginine and lysine was performed using with a commercially-available kit purchased through Thermo Fisher (cat # 89983 and cat #

88210). Reconstitution of arginine and lysine into media was performed according to product specifications. *Sdhc* fl/fl (experimental) cells were grown in "light" SILAC media and *Sdhc* fl/wt cells were grown in "heavy" media in T75 flasks at 37°C with 5% CO_2 and 21% O_2. For each cell line, labeling was performed in duplicate in separate flasks. On the same day as cells were started in SILAC media, doxycycline (2 micromolar) was added to the media to induce *Sdhc* gene rearrangement. Media was changed and cells passaged every 2-3 d to maintain between 20-90% confluence for a total of 16 d. On day 12 post induction with doxycycline, cells were expanded into T150 flasks. On day 16 post induction with doxycycline, media was aspirated, and adherent cells washed twice with 10 mL cold PBS. Cells were then harvested by scraping, transferred into 1.7 mL microcentrifuge tubes, subjected to centrifugation for 5 min at 500 X g, supernatant aspirated, and wet cell pellets flash frozen and stored in -80°.

Cell lysis and protein digestion

Following SILAC labeling, both experimental and control cells were homogenized in the urea lysis buffer (9M urea in PBS [pH 7.2] with 25 mM sodium butyrate, 50 mM nicotinamide and HALT protease inhibitor cocktail (Thermo Fisher)) followed by protein reduction and alkylation as previously described (Zhou et al., 2016). An equal amount of proteins from heavy and light labeled cells were mixed. The combined lysate was diluted six-fold in 1X PBS and proteins were digested with trypsin (1:50 enzyme to substrate ratio, w/w; Promega) at room temperature for overnight. A second tryptic digestion was performed (1:100 enzyme to substrate ratio, w/w) for 3 h to ensure complete digestion. Peptides were desalted with Sep-pack C18 cartridge (Waters) following the manufacturer’s instructions prior to enrichment.

Enrichment of lysine succinylated peptides

Succinylated peptides were enriched following the procedure as previously described (Park et al., 2013). Briefly, peptides were incubated with the agarose beads conjugated with the pan-anti succinyllysine antibody (PTM Biolabs) at 4 °C overnight. Following incubation, the beads were washed with NETN buffer (50 mM Tris HCl [pH 8.0], 100 mM NaCl, 1 mM EDTA, 0.5% NP40) and ETN buffer (50 mM Tris HCl [pH 8.0], 100 mM NaCl, 1 mM EDTA) sequentially. Peptides were eluted with 0.1% TFA and dried in a Speed-Vac device (Thermo Fisher) prior to desalting and fractionation. A small aliquot of peptides retained prior to the enrichment was processed directly for SCX fractionation and LCMS as described below for total protein quantification.

SCX fractionation

Peptides were desalted and fractionated with SCX using Empore membranes (3M) using a previously described method (Rappsilber et al., 2007). Briefly, peptides were loaded onto a Stage-tip containing Empore Cation Exchange-SR membranes (3M). The peptides were washed with 0.1% formic acid and then sequentially eluted with buffers consisting of 20% acetonitrile (v/v), 0.1% formic acid (v/v), and NH_4OAc with respective concentrations of 50, 75, 125, 200, 300, 500 mM. Subsequently, peptides from each fraction were desalted with C18 Stage-tips and dried with the Speed-Vac (Thermo Fisher).

LC-MS/MS measurement

Peptide samples were resuspended with HPLC buffer A (0.1% formic acid in water, v/v) and loaded onto an C18 column packed in-house (15 cm × 75 µm, ReproSil-

Pur Basic C18, 2.5 µm, Dr. Maisch GmbH). The samples were separated and analyzed with a Proxeon Easy nLC 1000 Nano-UPLC system connected online to an Orbitrap Fusion mass spectrometer (Thermo Fisher). Peptides were eluted with a 56-min gradient of 5-30% of HPLC buffer B (0.1% formic acid in acetonitrile, v/v) at a flow rate of 200 nL/min. The Fusion Orbitrap was operated in a data-dependent mode, prioritizing the most intense precursor ions. Full mass spectra were acquired with a resolution of 60,000 at m/z 300-1500 and MS/MS spectra were acquired using collision dissociation (CID) with 35% collision energy for detection in the ion trap.

Cell fixation

Experimental and control cell lines were grown for 16 d post induction with doxycycline, as described for the TCA metabolomics experiments. Cells (~10E6 count) were harvested by trypsinization and fixed with formaldehyde (methanol-free, Pierce cat# 28906) at 1% final concentration at room temperature for 10 min with gentle agitation. The fixation reaction was quenched by the addition of 0.5 mL 2.5 M glycine per 10 mL total volume, and incubated with mixing at room temperature for 5 min. Cells were then pelleted by centrifugation (3000 X g for 5 min), washed with 3 mL of cold TBS (50 mM Tris-HCl pH7.4, 150 mM NaCl) and counted with a hemacytometer, subjected to centrifugation again (3000 X g for 5 min at 4°C), and then wet cell pellets were stored at -80°C until use in ChIP experiments.

Chromatin preparation

Cell pellets were resuspended in 3 mL cell lysis buffer (10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 0.5% IGEPAL) with gentle vortex agitation, and then incubated on ice for

15 min. Cells were pelleted by centrifugation (3000 X g for 5 min at 4°C) and the supernatant discarded. The pellet was resuspended in 450 microliters of MNase digestion buffer (20 mM Tris-HCl, pH 7.5, 15 mM NaCl, 60 mM KCl, 1 mM $CaCl_2$), subjected to centrifugation (12,000 rpm for 3 min), and supernatant discarded. 250 microliters of MNase digestion buffer was then added (with protease inhibitor cocktail; Thermo Fisher cat # 78430) to resuspend. MNase (NEB Cat# M0247S, 1 microliter) was then added and cell lysates were mixed by gently pipetting. Samples were then incubated at 37°C for 20 min on an orbital shaker to allow for digestion of chromatin into mono- and di-nucleosomes. 250 microliters 2X stop/ChIP/sonication buffer (100 mM Tris-HCl, pH 8.1, 20 mM EDTA, 200 mM NaCl, 2% Triton X-100, 0.2% Sodium deoxycholate) were added and samples were agitated by vortex mixing. Samples were then sonicated using a Diagenode Bioruptor Pico using 20 total cycles with 30 sec sonication followed by 30 sec rest in each cycle. Samples were then subjected to centrifugation (17,000 X g for 5 min at 4°C), and the supernatant transferred to a new tube. Qubit high-sensitivity assay (Thermo Fisher cat # Q32851) was used to estimate total amount of soluble chromatin. SDS was then added to samples to final concentration of 0.05% to reduce background in subsequent ChIP-experiments. The size of input chromatin fragments were profiled using agarose gel electrophoresis (2% gel).

Chromatin immunoprecipitation and DNA purification

For ChIP reaction, 7 micrograms of prepared chromatin was diluted to a final volume of 450 microliters using 1X ChIP buffer (50 mM Tris-HCl, pH 8.1, 10 mM EDTA, 100 mM NaCl, 1% Triton X-100, 0.1% Sodium deoxycholate) containing 0.05% SDS.

Two micrograms of antibody (H3K4me3 antibody, Abcam cat # ab8580; H3K27me3 antibody, Abcam cat # ab6002; pan-succinyllysine antibody, PTM Biolabs cat # PTM-401; IgG isotype control antibody, Thermo Fisher cat # 02-6102) were then added and samples were rocked overnight at 4°C to allow for antibody binding to chromatin fragments. 0.07 micrograms of input chromatin for each preparation was saved. Following overnight antibody incubation, 30 microliters of pre-washed (300 microliters 1X ChIP buffer) protein G magnetic beads (Pierce Cat#88802) were added to each sample and rocked at 4°C to capture bound chromatin fragments. Samples were then subjected to centrifugation at 700 RPM for 5 min and beads magnetically trapped to facilitate removal of the supernatant. Beads were then washed 2X with 1 mL 1X ChIP buffer, 2X with 1 mL high salt buffer (1X ChIP buffer + 0.5M NaCl), 2X with 1 mL Tris/LiCl buffer (10 mM Tris-HCl, pH 8.0, 0.25 M LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA), and 1 mL TE buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA). Beads were resuspended in 50 microliters of 1X ChIP elution buffer (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, 150 mM NaCl, 5 mM DTT, 1% SDS) and incubated at 65°C for 15 min with vortexing performed every 3 min. The eluent was saved in a separate tube and the elution repeated a second time. Both eluents were pooled and incubated at 65°C overnight to reverse formaldehyde cross-links. At this point, the 1% input chromatin sample was diluted into 100 microliters of elution buffer and eluted overnight alongside the ChIP samples. The following day, samples were briefly centrifuged, 2 microliters of RNAse A (Thermo Fisher cat # EN0531) added, samples vortexed to mix, and incubated at 37°C for 1 h to degrade chromatin-associated RNA. 10 microliters of Proteinase K (Ambion cat # AM2546) was then added, samples

vortexed to mix, and then incubated at 37°C for 2 h to degrade chromatin proteins. DNA was purified using Qiagen MinElute columns. Briefly, 500 microliters of Qiagen buffer PB was added to samples, vortexing was performed to mix, samples were loaded onto Qiagen MinElute columns, samples subjected to centrifugation at 9000 RPM for 1 min, eluent discarded, columns washed with 650 microliters of wash buffer, spun to remove residual solvent, and purified DNA eluted with 16 microliters of Qiagen column purification buffer, and subjected to centrifugation at 15,000 RPM for 1 min. Purified DNA was used directly for ChIP-seq deep sequencing library preparation.

ChIP-seq library preparation

Concentration of purified DNA was determined using the Qubit high-sensitivity DNA assay (Thermo Fisher cat # Q32851). Deep sequencing library preparation was performed using 10 ng of purified DNA and the Rubicon Genomics ThruPLEX DNA-seq 48D kit as per the manufacturer's instructions. Libraries were then purified using AMPure XP beads (Beckman Coulter Cat# A63880). Briefly, 50 microliters of beads were combined with each sample and incubated at room temperature for 10 min. Using a magnet, the supernatant was discarded and the beads were washed 2X with 190 microliters of 80% ethanol. Beads were then air dried at 50°C using a heating block, and purified libraries eluted using 21.5 microliters of Qiagen buffer EB at room temperature for 5 min.

Deep sequencing

Deep sequencing was performed by the Mayo Clinic Medical Genome Core Facility using an Illumina HiSeq 4000 instrument, multiplexing 8 bar-coded libraries per lane. Sequencing was performed using 50 cycles and paired end indexed read type.

ChIP-Seq read alignment

FASTA sequences for assembled chromosomes of mouse reference genome GRCm38/mm10 were downloaded from NCBI. Individual chromosomes were concatenated into a single FASTA file, which was used to build Bowtie 2 (version 2.2.3) indices using the “bowtie2-build” function (Langmead and Salzberg, 2012). Paired end read alignments of ChIP-seq FASTQ reads to the indexed mouse reference genome were then performed in Bowtie 2 using default settings on the Mayo Clinic Research Computing Services high performance Beowulf-style Linux-based cluster. The resulting SAM files were converted to BAM format using the Samtools “view” function (Li et al., 2009). BAM files were then sorted by chromosome and region using the Samtools “sort” function, and BAM file indices were generated using the Samtools “index” function.

QUANTIFICATION AND STATISTICAL ANALYSIS

Raw proteomic data processing

MaxQuant (v 1.5.3.12) was used for identification and quantification from MS/MS spectra in the raw data (Cox and Mann, 2008). Lysine succinylation (+100.0160 Da), methionine oxidation and protein N-terminal acetylation were selected as variable modifications. Cysteine carbamidomethylation was selected as a fixed modification. Arginine 6 and Lysine 6 were selected as amino acid labels with three maximum-

labeled amino acids. Trypsin was selected as the proteolytic enzyme and a maximum of two missing cleavages was allowed. The ion mass tolerance for the precursor ion and fragment ion was 4.5 ppm and 0.5 Da respectively. The database search employed the Uniprot mouse database (released on 2013/09/27 and containing 43310 sequences). Peptides were filtered with a 1% False Discovery Rate (FDR) at the peptide, protein and modification site levels. A minimum Andromeda score of 40 was required for the identification of modified peptides. Stoichiometry determination of succinylation sites was achieved following a SILAC-based computational analysis as previously described (Chen, 2016; Olsen et al., 2010). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Vizcaino et al., 2016) partner repository with the dataset identifier PXD007874.

Proteomic data secondary analysis

Secondary analysis of quantified proteomic data was performed in R. SILAC ratios for biological replicate experiments were median-normalized. Analysis of Pearson correlations between replicate experiments or between protein abundance change and succinylation change was performed using the “cor.test” function in R, included in the stats package. Analysis of functional annotation enrichment for differentially succinylated peptides was performed by extracting the gene identifiers for peptides having succinylation $\log_2$(fold-change) differing by more than 0.2 from the corresponding peptide abundance $\log_2$(fold-change). The extracted list of gene identifiers was filtered to remove duplicate gene identifiers, and functional enrichment analysis of gene ontologies (molecular functions, biological processes, and cellular components) and KEGG pathways was performed using the DAVID functional

annotation tool (Huang da et al., 2009). For data display, a criteria was enforced such that all terms must have a p-value < 0.0001 for the enrichment analysis.

ChIP-Seq peak calling and secondary analysis

Secondary analysis of aligned reads was performed in SeqMonk (https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/). Within SeqMonk, the MACS peak calling algorithm was implemented to detect ChIP-seq peaks (p-value cutoff: 1E-5; sonicated fragment size: ~200), comparing mapped read counts for the various ChIP experiments to the corresponding input control (Zhang et al., 2008). Read counts for the various ChIP experiments and input controls were quantified at the positions of ChIP-seq peaks or transcriptions start sites using the feature read count quantitation functionality in SeqMonk. Pearson and Spearman correlations between read counts quantified in the various ChIP experiments was performed in R using the "cor.test" function. For comparisons between signals measured in different types of ChIP experiments, or between ChIP and RNA-seq experiments, data was first scaled in the range of [0,1] using the 0.001 and 0.999 quantiles prior to visualization and analysis of correlation. Analysis of genomic localization of identified ChIP-seq peaks was performed in R using the ChIPseeker package available through the Bioconductor project (Yu et al., 2015). ChIPseeker was also used for generating aligned probe plots and probe trend plots for ChIP-seq peak localization near transcription start sites. For analysis of succinyllysine ChIP peak differential succinylation in the context of SDH loss, peaks were ranked according to observed differences in succinylation (normalized read counts) between experimental and control cell lines. The bottom 0.05 and top 0.95 quantiles of ChIP peaks were extracted, based upon succinylation differences between

experimental and control lines. These subsets of data were then analyzed using ChIPseeker to identify specific types of genomic features impacted by differential succinylation. For analysis of differential promoter succinylation in the context of SDHC loss, promoters genome-wide were ranked according to absolute difference between quantified ChIP-seq read counts in experimental and control samples, and the top 0.1 quantile of differentially succinylated promoters were extracted. The corresponding gene identifiers were extracted and used to perform term enrichment analysis using the DAVID functional annotation database (Huang da et al., 2009). Mean succinylation difference (experimental minus control) for differentially-succinylated genes was calculated in R.

Genotoxic drug study analysis

Amount of reduced Alamar Blue was calculated using the obtained absorbance measurements and the following equation,

$$AR_{570} = A_{570} - (A_{600} X R_o)$$

, where AR570 is the amount of reduced Alamar Blue, A570 and A600 are the absorbance measurements at 570 and 600 nm, respectively, and Ro (0.69) is the empirically-determined ratio of A570 and A600 absorbances for Alamar Blue in media with no cells. Percent difference in Alamer Blue reduction was then determined between drug-treated cells and vehicle-treated cells for each tested cell line.

DATA AND SOFTWARE AVAILABILITY

ChIP-seq data have been deposited in the NCBI GEO under ID code GSE104362. Proteomic data have been deposited in the PRIDE database under ID code PXD007874.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal IgG isotype control antibody	Thermo Fisher	Cat#02-6102; RRID:AB_2532938
Rabbit polyclonal anti-H3K4me3 IgG antibody	Abcam	Cat#ab8580; RRID:AB_306649
Mouse monoclonal anti-H3K27me3 IgG antibody	Abcam	Cat#ab6002; RRID:AB_305237
Rabbit polyclonal anti-pan-succinyllysine IgG antibody	PTM Biolabs	Cat#PTM-401; RRID:AB_2687628
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Doxycycline hyclate	Sigma Aldrich	Cat#D9891-25G
L-Arginine-HCl, 13C6 for SILAC	Thermo Fisher	Cat#88210
Critical Commercial Assays		
ThruPLEX DNA-seq 48D Kit	Rubicon Genomics	Cat#R400406
SILAC protein quantitation kit (LysC), DMEM	Thermo Fisher	Cat#A33969
Qubit dsDNA HS Assay Kit	Thermo Fisher	Cat#Q32851
Deposited Data		
Raw and analyzed ChIP-seq data	This paper	GEO: GSE104362
Raw and analyzed RNA-seq data	Smestad and Hamidi, 2017	GEO: GSE103662
Raw and analyzed proteomic data	This paper	PRIDE: PXD007874
Experimental Models: Cell Lines		
R26M2rtTA/+;TetOcre;Sdhcfl/fl immortalized mouse embryonic fibroblast cell line	Smestad and Hamidi, 2017	N/A
R26M2rtTA/+;TetOcre;Sdhcfl/wt immortalized mouse embryonic fibroblast cell line	Smestad and Hamidi, 2017	N/A

Experimental Models: Organisms/Strains		
Oligonucleotides		
Recombinant DNA		
Software and Algorithms		
Samtools	Li et al., 2009	http://samtools.sourceforge.net/
Bowtie2	Langmead and Salzberg, 2012	http://bowtie-bio.sourceforge.net/bowtie2/index.shtml
SeqMonk	Babraham Bioinformatics	https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/
MACS	Feng and Liu, 2013	http://liulab.dfci.harvard.edu/MACS/
ChIPseeker	Yu, Wang, and He, 2015	http://bioconductor.org/packages/release/bioc/html/ChIPseeker.html
MaxQuant	Cox and Mann, 2008	http://www.biochem.mpg.de/5111795/maxquant
Other		

Supplemental References

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