SUPPLEMENTAL TEXT AND FIGURES

SUPPLEMENTAL FIGURES AND LEGENDS

Figure S1

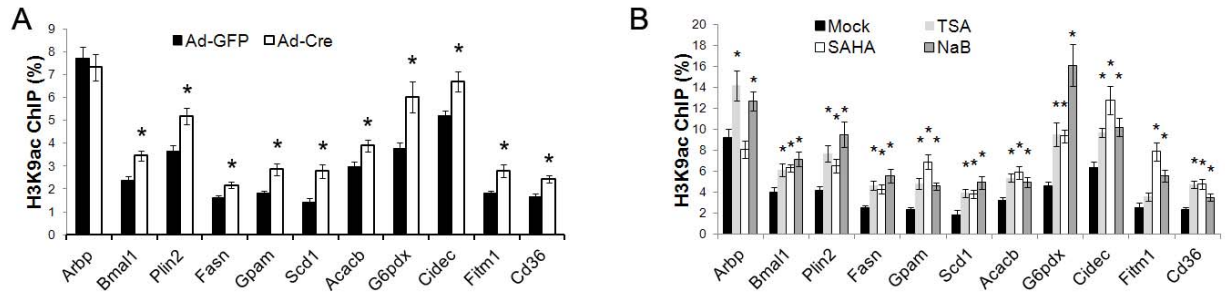

Figure S1, related to Figure 1. Histone acetylation near HDAC3 target genes promoters in primary hepatocytes. (A) ChIP-qPCR analysis of H3K9 acetylation using primers specific for the previously-determined HDAC3 sites near promoters of the target lipogenic gene in mouse primary hepatocytes 48 h after adenovirus infection. **(B)** ChIP-qPCR analysis was performed as above in primary hepatocytes treated with the indicated HDIs for 48 h. * indicates significant difference compared to control infection or treatment by student t test. Error bars, s.e.m from triplicate plates of cells.

Figure S2

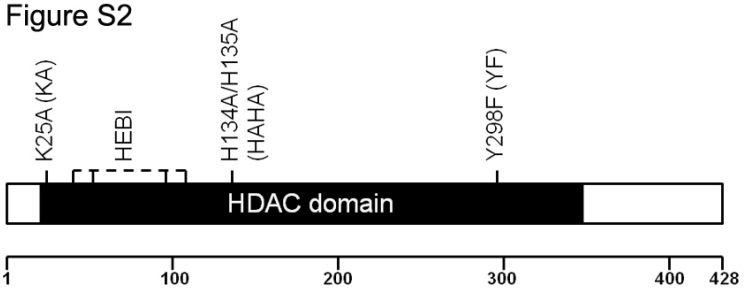

Figure S2, related to Figure 2. A diagram of HDAC3 protein showing domain architecture and mutations used in the study. The HDAC domain was shown in black. The scale bar shows numbers of amino acid residues.

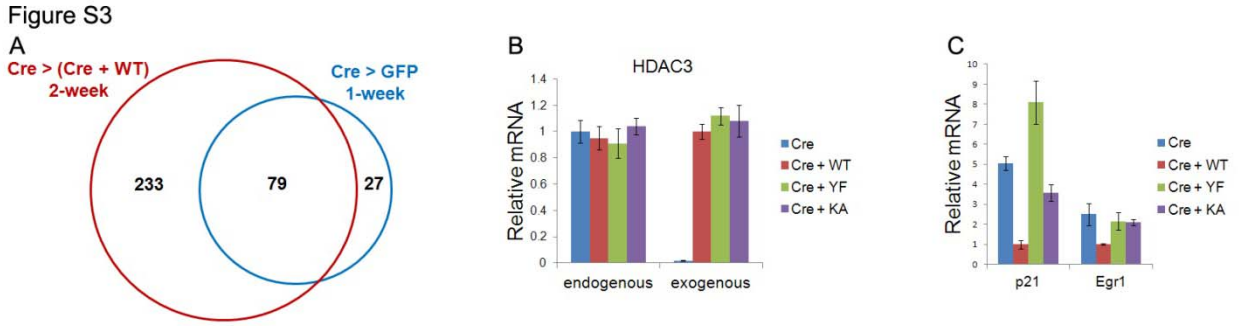

Figure S3, related to Figure 3. Transcriptional profiling in HDAC3 rescued livers. **(A)** Microarray analysis of livers from HDAC3$^{f/f}$ mice injected with AAV-Cre or AAV-Cre plus AAV-HDAC3 (WT) at 2-weeks post-injection. Shown in red are genes significantly upregulated in the Cre group ($q < 0.05$, fold-change ≥ 1.4) (GSE49386). Shown in blue are genes using the same cutoff from a previous microarray experiment with AAV-GFP or AAV-Cre at 1-week post-injection (Feng et al. 2011). The longer time since knockout is the likely cause of more upregulated genes in the 2-weeks array. **(B)** RT-qPCR analysis of HDAC3 in livers from the HDAC3 rescue experiment as shown in Figure 3. Primers specific for either the endogenous HDAC3 (spanning the floxed region) or the exogenous Flag-tagged human HDAC3 were used. n = 4. **(C)** RT-qPCR analysis of additional genes from livers as above. p21 (WAF1): also known as cyclin-dependent kinase inhibitor 1. Egr1: Early growth response protein 1n = 4. All error bars, s.e.m.

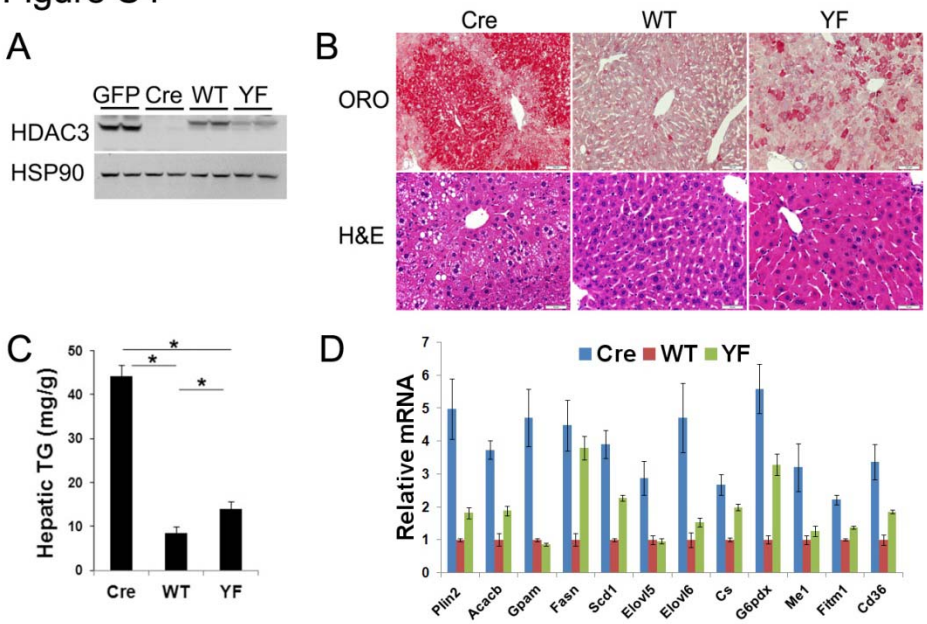

Figure S4, related to Figure 3. Increasing the virus dosage does not overcome the sub-physiological expression of YF. HDAC3$^{f/f}$ mice were injected with AAV-Cre along with 10-fold higher dosage of AAV-HDAC3 YF than the normal dosage used for AAV-HDAC3 WT. **(A)** Immunoblot analysis of livers. **(B)** ORO and H&E staining of the livers. **(C)** Liver triglyceride (TG) measurement, n = 4. **(D)** RT-qPCR analysis of livers, n = 4. Error bars, s.e.m.

Figure S5

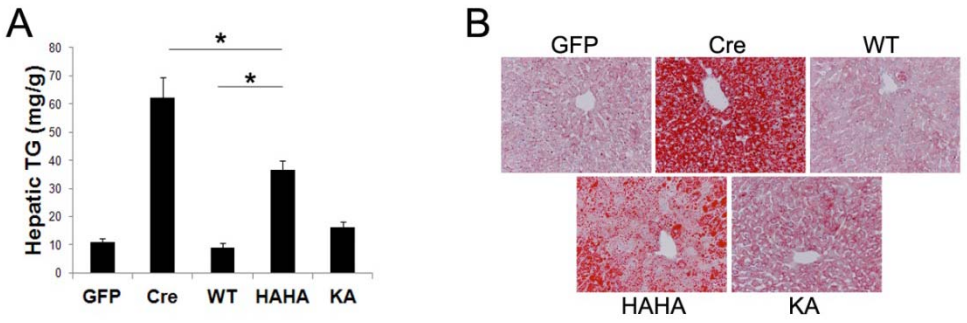

Figure S5, related to Figure 4. Additional metabolic characterization of HDAC3 rescued livers. (A) Liver triglyceride (TG) measurement, n = 4. **(B)** ORO staining of the livers. * indicates significant difference between two groups by student t test. Error bars, s.e.m.

Figure S6

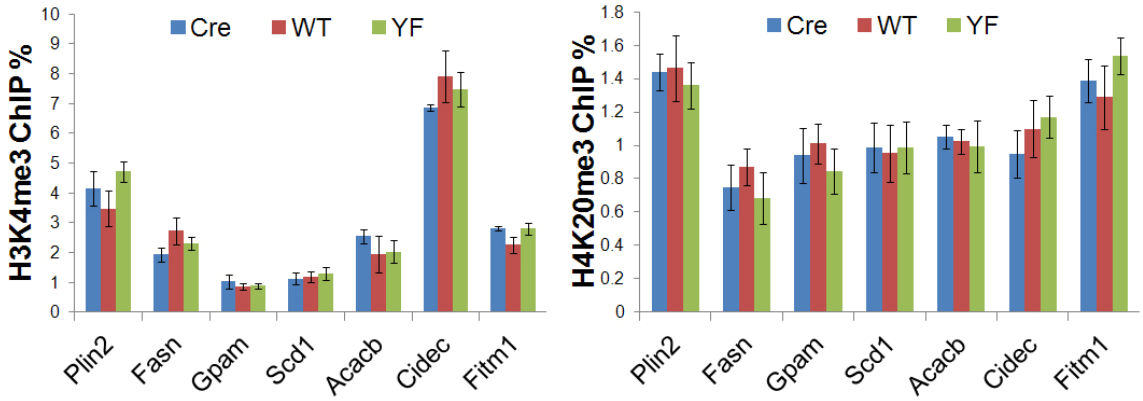

Figure S6, related to Figure 5. Histone methylation does not change in livers upon HDAC3 depletion. ChIP was performed on livers from the HDAC3 rescue experiment with antibodies specific for H3K4 trimethylation and H4K20 trimethylation. qPCR analysis was performed with primers for HDAC3 binding sites near the indicated genes. n = 4. Error bars, s.e.m.

Figure S7

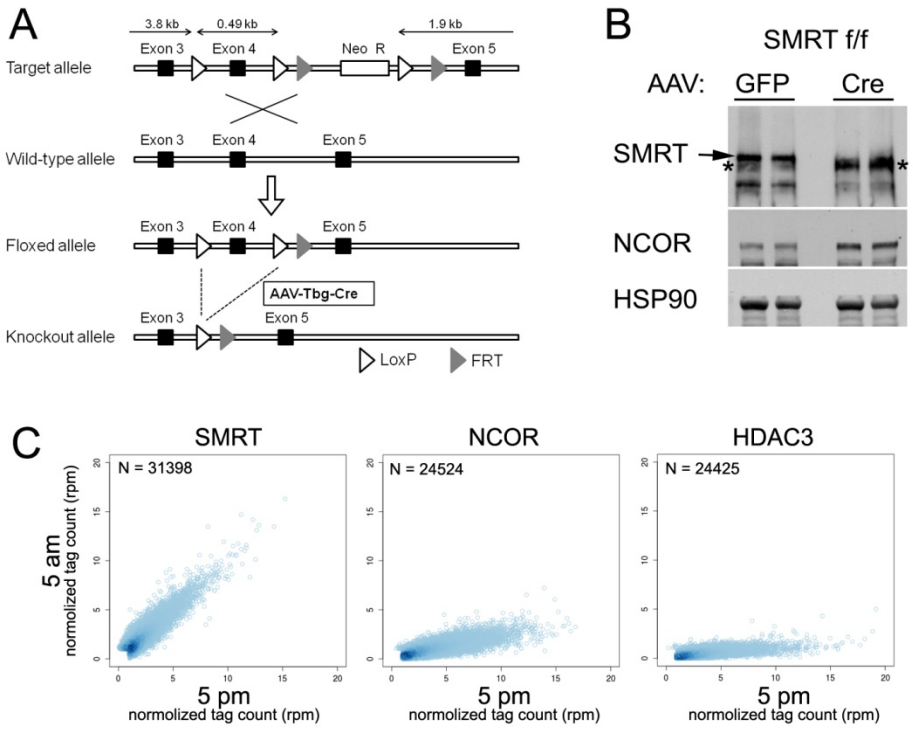

Figure S7, related to Figure 7. Characterization of SMRT in mouse liver. (A) Targeting strategy of SMRT$^{f/f}$ mice. To generate the SMRT conditional knockout, the targeting vector was constructed as follows. Three fragments of 3.8, 0.49, and 1.9 kb (respectively, the 5′, floxed, and 3′ arms) were amplified by PCR using 129S2/SvPas DNA as template and sequentially subcloned into an MCI proprietary vector. The linearized construct was electroporated into 129S2/SvPas mouse embryonic stem (ES) cells. After selection, 2 targeted clones were identified by PCR using external primers and further confirmed by Southern blot with 5′ and 3′ external probes. One targeted ES clone (#K175-78) was injected into C57BL/6J blastocysts, and derived male chimaeras were mated to female C57BL/6J mice that express the Flp recombinase under the control of the ubiquitous cytomegalovirus promoter. Offspring that contain the mutated allele, in which the selection marker was excised, and that lost the Flp transgene were selected. **(B)** Depletion of SMRT proteins in liver. Immunoblot analysis of livers from SMRT$^{f/f}$ mice injected with AAV. Arrow indicates SMRT proteins. * indicates nonspecific signals. **(C)** Genome-wide occupancy of SMRT in liver is not circadian. Comparison of SMRT ChIP-seq data in mouse livers harvested at 5 am versus 5 pm (GSE 51045). To draw a scatter plot, all the peaks from 5 am and 5 pm were pooled. Peaks with distance in-between the centers shorter than 200 bp were merged into one peak, from which total number of peaks (N) was calculated. Previously published NCOR and HDAC3 ChIP-seq data (Feng et al. 2011) was analyzed and plotted in the same way as controls.

SUPPLEMENTAL TABLES

Table S1, related to Figures 1, 3, 4, 6, and 7. Primers used in ChIP-qPCR and RT-qPCR. Arbp: ribosomal protein, large, P0; also known as 36B4. Plin2: perilipin 2; also known as adipose differentiation related protein (Adrp). Fasn: fatty acid synthase. Gpam: glycerol-3-phosphate acyltransferase, mitochondrial. Scd1: stearoyl-Coenzyme A desaturase 1. Acacb: acetyl-CoA carboxylase beta; also known as ACC2. Elovl5: fatty acid elongase 5. Elovl6: fatty acid elongase 6. G6pdx: glucose-6-phosphate dehydrogenase X-linked. Cidec: cell death-inducing DFFA-like effector c; also known as fat specific protein 27 (Fsp27). Fitm1: fat storage-inducing transmembrane protein 1. Cd36: CD36 antigen, also known as fatty acid translocase. HDAC3 (endo): endogenous mouse HDAC3. HDAC3 (exo): exogenous human HDAC3. p57 (KIP2): cyclin-dependent kinase inhibitor 1C. Bmal1: aryl hydrocarbon receptor nuclear translocator-like, also known as Arntl. Me1: malic enzyme 1. Cs: citrate synthase. G0S2: G0/G1 switch 2. p21 (WAF1): also known as cyclin-dependent kinase inhibitor 1. Egr1: Early growth response protein 1. 18S: 18S ribosomal RNA.

ChIP-qPCR	Forward	Reverse
Arbp (36B4)	GAGGTGGCTTTGAACCAGAG	TCTTTGTCTCTGTCTCGGAAAA
Plin2	GGGTGACAGCAATAGGAAGG	CATGTTGGAGAGCCGTCATT
Fasn	CCAGCTTGCTCAAGACATCA	GGAAAGCAGCTCATGGAGTC
Gpam	CTATTGCCCAACCACACACA	ACGGTTGCCCAATGAGTTAT
Scd1	GCATTTCTCAGCCAGGAGTC	GGCACAGAGGAAGCTCAGTC
Acacb	TGGTGTGTGGCTTCTGTTTC	TGTTGCTGTCACAGGGTGTT
Elovl5	AGTGCCCTGGAGACACTGAC	CAAGGGTGAGAGGTGAAGGA
G6pdx	CAATGAGAACCCTGGCTTGTAGAC	GAGTCTGTACTGATTCCTCCCAGT
Cidec	TCAGGCAGCCAATAAAGTCC	AGCTAGCCCTTTCCCAGAAG
Fitm1	AAGGATCACAGGTGAAAGGTGGGA	ATTCCTGTGCCCAGCAACTCAA
Cd36	CCAGTTTGTCCTGGAAGCTC	GGGGTGATCAGTAACCCTCA
RT-qPCR	Forward	Reverse
HDAC3 (endo)	CCTGGAACAGGTGACATGTATGA	CGTAAGGGCACATTGAGACAATAG
HDAC3 (exo)	TCTGGCTTCTGCTATGTCAACG	CCCGGTCAGTGAGGTAGAAAG
p57 (KIP2)	CGAGGAGCAGGACGAGAATC	GAAGAAGTCGTTCGCATTGGC
Bmal1	TGACCCTCATGGAAGGTTAGAA	GGACATTGCATTGCATGTTGG
Plin2	AAGAGGCCAAACAAAAGAGCCAGGAGACCA	ACCCTGAATTTTCTGGTTGGCACTGTGCAT
Scd1	GCTCTACACCTGCCTCTTCG	GCCGTGCCTTGTAAGTTCTG
Gpam	CAACACCATCCCCGACATC	GTGACCTTCGATTATGCGATCA
Fitm1	CCTCTGCCTTACTGTACTTTGG	TAGCGAAGATCGTCCGAGAGT
G6pdx	AGACCTGCATGAGTCAGACG	TGGTTCGACAGTTGATTGGA
Me1	GGG ATT GCT CAC TTG GTT GT	GTT CAT GGG CAA ACA CCT CT
Cidec	GCATCATGGCTCACAGCTT	ATTGTGCCATCTTCCTCCAG
Cd36	GGAGCCATCTTTGAGCCTTCA	GAACCAAACTGAGGAATGGATCT
Acacb	GATGGAGCGCATACACTTGA	CCGAGTTTGTCACTCGGTTT
Fasn	TACAGGAGTTCTGGGCCAAC	GACCGCTTGGGTAATCCATA
Cs	TGACTGGCACCCAACATTTGA	CAGCTTGAGGCACAGCAGGTATAG
G0S2	AGTGCTGCCTCTCTTCCCAC	TTTCCATCTGAGCTCTGGGC
Elovl6	AATGGATGCAGGAAAACTGG	AACTTGGCTCGCTTGTTCAT
Elovl5	ATGGAACATTTCGATGCGTCA	GTCCCAGCCATACAATGAGTAAG
NCOR	AGATTTCAGCGAGTTGGTCAG	CCTCCATCAGCCCATTCATATT
SMRT	GTTCCATCATCCAGCCACA	AGCAGGGTATCGGGTAGTAG
p21 (CIP1)	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Egr1	TCGGCTCCTTTCCTCACTCA	CTCATAGGGTTGTTCGCTCGG
18S	AGTCCCTGCCCTTTGTACACA	CGATCCGAGGGCCTCACTA
36B4 (Arbp)	CTGGGACGATGAATGAGGAT	AGCAGCTGGCACCTAAACAG

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Mice. All mice were housed under the 12 h light/ 12 h dark cycles (lights on at 7 a.m., off at 7 p.m.). Adult male mice between the ages of 2 - 7 months were used in all experiments. AAV2/8-Tbg-Cre was intravenously injected at 1.5×10^{11} GC per mouse to induce hepatocyte-specific gene knockout, using AAV2/8-Tbg-GFP as a negative control. Together with AAV2/8-Tbg-Cre, AAV2/8-Tbg-HDAC3 vectors containing mutations were injected at 4×10^{10} GC per mouse in all experiments except the one described in Figure S4. Within one experiment, each mouse received equal total dosages of AAV vectors through matching up with AAV2/8-Tbg empty vectors. All mice were euthanized by CO_2 inhalation followed by tissue harvest at 2 weeks after viral injection at 5 pm except indicated otherwise. All animal procedures followed the guidelines of the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Cell culture and DNA constructs. Primary hepatocytes were isolated from $HDAC3^{f/f}$ mice, plated in 12-well plates in DMEM medium containing 10% FBS, and cultured in Williams E medium containing Primary Hepatocyte Maintenance Supplements (Invitrogen CM4000). Recombinant adenoviral (Ad) vectors were obtained from Penn Vector core. 5×10^{8} GC of Ad-GFP or Ad-Cre was added into each well and incubated for 4 hrs. Cells were then switched to virus-free culturing medium for the indicated time duration. HDIs were used at 0.2 uM, 0.4 uM, 1 uM for TSA (Cell Signaling); 1 uM, 5 uM, 10 uM for SAHA (Cayman); and 1 mM, 2 mM, 5 mM for NaB (Sigma). HEK 293T cells were transfected with plasmids using Lipofectamine reagents (Invitrogen). Site-directed mutagenesis was performed using Stratagene kit.

Immunoprecipitation, immunoblot, and HDAC assay. Primary hepatocytes were either lyased directly in Laemmli sample buffer or acid extracted using 0.2 N HCl (Abcam histone extraction protocol), followed by SDS-PAGE for immunoblot. For immunoprecipitation, cells or tissues were lysed in lysis buffer (20 mM Tris pH7.5, 150 mM NaCl, 1% NP40) containing protease inhibitors, pre-cleared with protein A beads, and incubated with either Flag beads (Sigma) or anti-HDAC3 antibodies (Santa Cruz) with protein A beads. Immunoprecipitates were washed 3 times with 1% NP40 lysis buffer, unless indicated otherwise in figure legends. NCOR polyclonal rabbit antibodies were generated in lab. Other antibodies used in immunoblot were purchased from commercial resources: HDAC3 (Abcam), H3K9ac (Millipore), H3K27ac (Abcam), H3 (Abcam), GAL4 (Santa Cruz), Flag (Sigma), TBLR1 (IMGENEX), TCP-1a (Enzo), HSP90 (Cell Signaling), SMRT (Abcam), and Ran (BD). HDAC assay was conducted using a fluorescence kit (Active Motif) following manufacture's instruction.

RT-qPCR, microarray, ChIP-qPCR, and ChIP-seq. Total RNA was extracted using TRIzol (Invitrogen) and RNeasy mini kit (Qiagen). RT-qPCR was performed with High Capacity RT kit, SYBR Master Mix, and the 7900HT instrument (ABI) using absolute quantification method with standard curves. 18S RNA or 36B4 (Arbp) was used as housekeeping controls. For microarray, RNA samples from different mice (n = 3-4) were individually processed and hybridized to the Mouse Gene array (Affymetrix). ChIP and

ChIP-seq were described previously (Feng et al., 2011). Briefly, livers were grounded in liquid N_2 and cross-linked in 1% Formaldehyde for 20 min. Whole cell extracts were sonicated followed by immunoprecipitation with antibodies for H3K9ac (Millipore), HDAC3, H3K4me3, H4K20me3, or SMRT (Abcam). All primers for qPCR analysis are summarized in Supplemental Table 1. For ChIP-seq, ChIP was performed independently on livers from difference mice (n = 3-4). The precipitated DNA were then pooled and amplified according to the guide of Illumina, followed by deep sequencing on Illumina Genome Analyzer IIx.

Computational analysis. Microarray data from $HDAC3^{f/f}$; AAV-Cre versus AAV-Cre + AAV-HDAC3-WT at 2-weeks post-injection (GSE 49386) and $NCOR^{f/f}$; AAV-Cre versus AAV-GFP (GSE 49387) were independently analyzed as described previously (Sun et al., 2011). Differential gene expression was determined using the Significance Analysis of Microarrays (SAM) algorithm with cut-offs of an FDR of 5%. The H3K9ac ChIP-seq samples in two rescue experiments (GSE 49365) were mapped to mouse genome (mm8) using Bowtie v0.12.8. SMRT ChIP-seq samples at 5 p.m. versus 5 a.m. (GSE 51045) were processed using ELAND pipeline. Peak calling was carried out by HOMER v4.2 (FDR 0.1%) using uniquely mapped reads. For generating heat maps of H3K9ac, total of 589 genes were first determined as significantly upregulated genes in $HDAC3^{f/f}$ Cre versus WT based on the microarray (GSE 49386) using fold-change >1.3 and $q<0.05$ as cut-offs. Total of 1301 HDAC3 binding peaks within 50kb from TSSs (transcription start site) upregulated genes were analyzed. ChIP-seq reads aligned to bins under each peak were calculated using Bedtools (v2.16.2). Gene ontology analysis of microarray results was performed using DAVID.

Histology and metabolites measurements. Liver tissues were fixed overnight in 4% paraformaldehyde and embedded either in paraffin for H&E staining or in OCT for cryosection followed by ORO staining. For measuring triglyceride, livers were lysed in lysis buffer (140 mM NaCl, 50 mM Tris and 1% Triton X-100, pH 8.0) followed by triglyceride assay using LiquiColor kit (Stanbio). For measuring glycogen, livers were homogenized in 0.5 N KOH. Glycogen was precipitated by ethanol and digested with 0.25 mg/ml amyloglycosidase (Sigma), followed by glucose assay using HK kit (Sigma).

SUPPLEMENTAL REFERENCES

Feng, D., Liu, T., Sun, Z., Bugge, A., Mullican, S.E., Alenghat, T., Liu, X.S., and Lazar, M.A. (2011). A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science *331*, 1315–1319.

Sun, Z., Singh, N., Mullican, S.E., Everett, L.J., Li, L., Yuan, L., Liu, X., Epstein, J.A., and Lazar, M.A. (2011). Diet-induced lethality due to deletion of the Hdac3 gene in heart and skeletal muscle. J. Biol. Chem. *286*, 33301–33309.