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Nuclear Receptor Corepressors are Required for the Histone Deacetylase Activity of HDAC3 In Vivo

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Abstract

Histone deacetylase 3 (HDAC3) is an epigenome-modifying enzyme that is required for normal mouse development and tissue-specific functions. *In vitro*, HDAC3 protein itself has minimal enzyme activity, but gains its histone deacetylation function from stable association with the conserved deacetylase activation domain (DAD) contained in nuclear receptor corepressors NCOR1 and SMRT. Here we show that HDAC3 enzyme activity is undetectable in mice bearing point mutations in the DAD of both NCOR1 and SMRT (NS-DADm), despite normal levels of HDAC3 protein. Local histone acetylation is increased, and genomic HDAC3 recruitment is reduced though not abrogated. Remarkably, the NS-DADm mice are born and live to adulthood, whereas genetic deletion of HDAC3 is embryonic lethal. These findings demonstrate that nuclear receptor corepressors are required for HDAC3 enzyme activity *in vivo*, and suggest that a deacetylase-independent function of HDAC3 may be required for life.

INTRODUCTION

Acetylation of lysine residues in histone tails activates gene transcription by several mechanisms, including promotion of an open chromatin structure as well as attracting positively acting transcription factors to the genome[1–5]. Histone deacetylases (HDACs) catalyze the reversal of this process, promoting a closed state of chromatin and thus contributing to repression of gene expression[1,6–8]. HDACs are widely expressed in

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These have been requested and will be added when available.

AUTHOR CONTRIBUTIONS

S-H.Y. and M.A.L. conceived of the hypothesis and designed the experiments. S-H.Y., Z.S., and M.B. performed the experiments. H-W.L. and K-J.W. analyzed bioinformatics data. S-H.Y., Z.S., and M.A.L. analyzed and interpreted the data. S-H.Y. and M.A.L. wrote the manuscript.

metazoans, and are categorized as classical HDACs 1–11 as well as NAD-dependent HDACs known as sirtuins[9]. HDACs regulate many different biological processes including embryo development[10], cell cycle regulation[11–13], cell proliferation[12], cancer progression[14,15], lipid metabolism[16,17], circadian rhythm[16], macrophage alternative activation[18], etc.

HDAC3 is a Class I HDAC that has been suggested to interact with many protein partners. Unlike other HDACs, HDAC3 is present in specific complexes containing nuclear receptor corepressors NCOR1[19–22] and NCOR2 (also known as Silencing Mediator of Retinoid and Thyroid receptors, or SMRT)[19,21–24], and regulates many biological processes in a variety of tissues, as demonstrated by shRNA[17,25] and knockout (KO)[26–28]. For example, deletion of HDAC3 alters lipid metabolism in liver and heart[17,25, 10, 41], the polarization state of macrophages[18], and the cell cycle progression of fibroblasts[28].

HDAC3 interacts with NCOR1 and SMRT via a conserved repression domain containing a SANT (SWI3, ADA2, NCoR, TFIIB) motif which is similar to that found in other transcriptional regulators[29,30]. This SANT motif together with a unique N-terminal helical extension[31] is required to activate the catalytic function of the HDAC3 enzyme[20,32], and is thus referred to as the Deacetylase Activating Domain (DAD). NCOR1 and SMRT activate HDAC3 both *in vitro*[24,33] and *in vivo*[34,35] via the DAD. The structure of HDAC3 bound to the SMRT DAD was recently solved, revealing the details of the atomic interactions as well as the involvement of an inositol tetraphosphate (IP4) molecule in stabilizing the complex[36]. Of particular note, a tyrosine residue at position 470 (Y470) of SMRT, corresponding to Y478 of NCOR1 and previously shown to be required for interaction with HDAC3[31,33], makes a critical contact with the IP4[36]. The mutation of Y478 of NCOR1 to alanine (Y478A) abrogates repression by thyroid hormone receptor *in vivo*[34,35,37], and alters circadian and metabolic physiology in living mice[34].

While HDAC3 has been reported to contribute to many biological processes, the relation of these functions to NCOR1 and SMRT has been unclear. Indeed, although NCOR1 and SMRT are competent to activate the latent deacetylase activity of HDAC3, and other SANT domains tested to date have lacked this property[33], the question of whether interaction with NCOR1 or SMRT is required for HDAC3 activity *in vivo* remained open. To address this important question, we have generated mice homozygous for the Y470A mutation in SMRT (S-DADm) and crossed these with mice homozygous for the Y478A mutation in NCOR1 (N-DADm) to generate mice lacking functional DADs in both NCOR1 and SMRT (NS-DADm). Using this mouse model, we report here for the first time that the enzyme activity of endogenous HDAC3 indeed requires the DAD of either the NCOR1 or SMRT.

RESULTS

NS-DADm mice are born and live to adulthood

To determine whether NCOR1 and SMRT are required for HDAC3 enzyme activity *in vivo*, we generated C57BL/6 mice bearing mutations in the DAD of both alleles of *Ncor1* and *Ncor2*. In each case, the mutant protein contains a single tyrosine to alanine substitution in the DAD (Y478A in NCOR1, Y470A in SMRT) that prevents interaction with and

activation of HDAC3[33]. The *Ncor1* DAD mutant C57BL/6 mice (N-DADm) have been described previously[34], and the *Ncor2* DAD mutant mice (S-DADm) were generated in the C57BL/6 strain background using a similar strategy (Supplementary Figure 1). Mutants heterozygous of N-DADm and S-DADm were mated to generate mice that are heterozygous for both mutant alleles, and those mice were mated with each other to obtain male and female double homozygous mutant (referred to as NS-DADm) mice. Since this breeding strategy produces only 1 NS-DADm and 1 wild type (WT) of the same gender for every 32 pups, larger numbers of each genotype were generated by crossing WT and NS-DADm with each other. Interestingly, while loss of NCOR1, NCOR2, or HDAC3 is embryonically lethal[26,28,38,39], the NS-DADm mice exhibited no detectable embryonic lethality and lived to adulthood (Table 1).

HDAC3 is enzymatically inactive in NS-DADm mice

We next studied the expression and function of HDAC3 in the tissues of mice bearing the DAD mutations in NCOR1 and SMRT. Gene expression of *Hdac3* is normal in the livers of NS-DADm mice (Figure 1a). Similarly, levels of hepatic HDAC3 protein were indistinguishable from those of WT mice (Figure 1b). Since expression of HDAC3, NCOR1, and SMRT was not significantly altered by the presence of the DAD mutations (Supplementary Figures 2a and 2b), we were able to test the hypothesis that endogenous HDAC3 requires NCOR1 or SMRT for its activity *in vivo*. Remarkably, whereas HDAC3 activity was readily measured in immunoprecipitates from WT liver, it was undetectable in liver from the NS-DADm mice (Figure 2a). Importantly, this was not due to inefficient immunoprecipitation relative to WT (Figure 2b). Similar loss of HDAC3 enzyme activity was observed in heart (Figure 2c) and skeletal muscle (Figure 2d). Moreover, no HDAC3 enzyme activity was detectable in embryos harvested on day 12.5 (Figure 2e), demonstrating the importance of the NCOR1 and SMRT DADs in all tissues, and that no other factor substitutes prenatally as an HDAC3 activator. Due to the background of the HDAC enzyme assay, it is possible that a small amount of residual activity exists. However these data prove that the NCoR and SMRT are required for the vast majority of the HDAC3 enzymatic activity in the tissues examined. Thus, the nuclear receptor corepressors are required for HDAC3 enzyme *in vivo*.

HDAC3 genome binding is reduced in the NS-DADm mice

Since HDAC3 is thought to be recruited to the genome by NCOR1 and SMRT, we hypothesized that this would be reduced in the NS-DADm mice. To test this, we located and quantitated the recruitment of HDAC3 to mouse liver using chromatin immunoprecipitation with HDAC3-specific antibody followed by massively parallel DNA sequencing (ChIP-seq). At 5 PM, when genomic recruitment is maximal in mouse liver[16], we detected HDAC3 at 5799 sites in WT mice, the majority of which were distant from transcription start sites or present in introns (Supplementary Figure 3), consistent with prior findings[40]. By contrast, using the same stringent peak calling criteria, only 600 HDAC3 binding regions were detected in the NS-DADm liver, the majority of which overlapped with WT binding (Supplementary Figure 4). It should be noted that HDAC3 binding remained detectable at most sites. The strength of binding in the NS-DADm liver decreased ~62.4% on average (Figure 3a), and individual HDAC3 binding sites reflect this decrease (Figure 3b). The

reduction of HDAC3 recruitment in the NS-DADm liver was validated at 10 sites by ChIP-qPCR (Figure 3c). The partial genomic interaction of HDAC3 is likely due to another region of NCOR1 or SMRT[33,41], or to direct interaction between HDAC3 and other transcription factors, neither of which would activate the HDAC3 enzyme. Nevertheless, these data demonstrate that the NCOR1 and SMRT DADs are critical for normal genomic recruitment of HDAC3 recruitment *in vivo*.

Local histone acetylation is increased in NS-DADm liver

Deletion of HDAC3 has been shown to result in increased local histone acetylation in liver[16] and macrophages[18], which is consistent with its *in vitro* histone deacetylase activity. We next tested whether the loss of HDAC3 activity alter local histone acetylation *in vivo.* Indeed, histone H3 lysine 9 acetylation (H3K9Ac), an activating mark[42], was locally increased at the 10 sites where loss of HDAC3 recruitment was validated by ChIP-qPCR, but not at control sites in the *Arbp* and *Ins* genes where HDAC3 is not bound (Figure 4a). Similar results were obtained after analysis of H3K27Ac, another activating mark (Figure 4b). Of note, the degree of elevated acetylation is comparable to that in the HDAC3 KO liver (Supplementary Figure 5a). Loss of HDAC3-DAD interaction did not alter the genomic recruitment of NCOR1 (Figure 4c) or SMRT (Supplementary Figure 5b), nor did the complete absence of HDAC3 affect the genomic recruitment NCOR1 or SMRT (Supplementary Figures 5c and 5d). Thus, the loss of HDAC3-DAD interaction leads to increased local nucleosomal histone acetylation in NS-DADm mice, despite normal binding of NCOR1 and SMRT at these genomic locations.

NS-DADm mice do not phenocopy mice lacking HDAC3 protein

The viability of the NS-DADm, which carry the mutant alleles in every cell, was quite different from the embryonic lethality of mice with germ line deletion of HDAC3[38,39], despite the fact that HDAC3 activity was undetectable in embryos from NS-DADm mice (Figure 2e). Since a major difference between these two genetic models is that HDAC3 protein is absent in the knockout but fully present in the NS-DADm mice, this result suggested that embryonic lethality of loss of HDAC3 is due to a deacetylase activity-independent function.

We also compared the livers of NS-DADm mice with those lacking HDAC3 in liver. Deletion of hepatic HDAC3 (“Liver HDAC3 KO”) was accomplished by injecting 10 week old male C57BL/6 mice bearing floxed *Hdac3* alleles (HDAC3$^{f/f}$) with an adeno-associated virus[43] in which the thyroxine-binding globulin (*Tbg*) promoter drives the liver-specific expression of either Cre recombinase or green fluorescent protein (GFP) as control. As previously described, the loss of HDAC3 in liver dramatically increased hepatic triglyceride levels[10,16,17]. Both mouse models exhibit undetectable hepatic HDAC3 activity (Figure 5a) and increased local histone acetylation at the lost binding sites[16] (Figures 3 and 4), as well as increased liver triglycerides. However, the ~2-fold increase of triglycerides activity in the NS-DADm livers was considerably more modest than the dramatic 5- to 10-fold increase observed in the mice lacking HDAC3 protein in liver (Figures 5b and 5c). This suggests that the continued presence of the inactive HDAC3 protein in the NS-DADm mice serves a function that normally contributes to the overall biological activity of HDAC3. Indeed,

whereas hepatic cholesterol levels increased in the liver HDAC3 KO**,** no significant change in cholesterol accumulation was observed in the NS-DADm mice (Figures 5d and 5e), suggesting that the cholesterol accumulation in the absence of HDAC3, which has been also observed by others[10], involves a deacetylation-independent function of HDAC3.

Consistent with the physiological data, comparisons of transcriptomes revealed that the loss of HDAC3 in liver had more dramatic effects on gene expression than loss of HDAC3-DAD interaction in NS-DADm mice (Figure 6a; the complete list of significantly changed genes is available at GEO). In keeping with previous results[17], pathway analysis highlighted the upregulation of many genes associated with lipid metabolism in the livers lacking HDAC3 protein (Figure 6b), many of which could be confirmed by RT-qPCR (Figure 6c). By contrast, abrogation of HDAC3 enzyme activity but not the HDAC3 protein in NS-DADm liver caused upregulation of fewer genes involved in lipid metabolism (Figures 6b and 6d). These results reinforce the conclusion that while the NCOR1 and SMRT DADs control HDAC3 activity and local histone deacetylation, these effects contribute relatively modestly to the total effects of HDAC3 protein on hepatic gene expression and the physiology of liver lipid metabolism.

DISCUSSION

We have introduced point mutations into both alleles of NCOR1 and SMRT that specifically abolish their ability to activate HDAC3. Using this unique mouse model we show that the DADs of nuclear receptor corepressors NCOR1 and SMRT are required for HDAC3 enzyme activity *in vivo*. This finding is of great importance as HDAC3 has been shown to play pivotal roles in transcriptional regulation[1,6,7], cell cycle progression[11–13], inflammation[18], developmental events[10], and metabolism[16,17]. Our data clearly show that NCOR1 or SMRT is required for nearly all HDAC3 enzyme activity *in vivo*.

HDAC3 clearly functions as an epigenomic modifier in the liver[16], and indeed histone acetylation was increased in the NS-DADm liver at genomic locations where HDAC3 normally bind. This is most likely explained by the loss of HDAC3 catalytic activity. In addition, HDAC3 occupancy on the genome was significantly reduced in the NS-DADm, demonstrating the importance of the NCOR1 and SMRT DADs in recruiting HDAC3 to the genome, and providing a second mechanism for increased histone acetylation at site of endogenous HDAC3 recruitment in WT mice. Histone acetylation and deacetylation alters chromosome accessibility and affect functions of transcription factors (TFs) acting at the genome[2]. Many inhibitors of the enzyme activity of class I HDACs are being developed to treat diseases including several types of cancer[44,45]. Moreover, approximately 10% of currently prescribed drugs directly target TFs[46], including tamoxifen for breast cancer and bicalutamide for prostate cancer, which target nuclear receptors by regulating their interaction with HDAC3[47,48]. Therefore our findings towards understanding basic HDAC biology have important therapeutic implications.

The NS-DADm mice exhibited mild hepatic steatosis, and molecular analysis revealed reduced or absent HDAC3 binding and increased local histone acetylation at upregulated lipid metabolic genes. Therefore these effects are likely due to the absence of HDAC3-

dependent histone deacetylation. Nevertheless, mice lacking HDAC3 in liver manifest much more severe hepatic steatosis[10,16,17] along with disrupted cholesterol homeostasis[10,17], whereas NS-DADm mice exhibited no detectable alteration in hepatic cholesterol. Furthermore, while absence of cardiac HDAC3 causes lethality or diet-induced heart failure depending on when the HDAC3 is deleted[17,39], NS-DADm mice have normal hearts and are able to tolerate a high fat diet (data not shown). Thus, while our studies show for the first time that the nuclear receptor corepressors are required for the deacetylase activity of HDAC3 *in vivo*, they also suggest a DAD-independent or deacetylase-independent role of HDAC3. In addition to their roles in adult tissues, germ line deletion of NCOR1[26], SMRT[38], or HDAC3[10,39] all cause embryonic lethality. The viability of the N-DADm mice demonstrated that the DAD is not required for the essential functions of NCOR1[34] during embryonic development. The viability of the S-DADm mice makes this point about SMRT, which has a variety of functions besides HDAC3 activation[49–52]. The phenotype of the S-DADm mice will be further characterized. More remarkably, however, although HDAC3 enzyme activity is diminished to an almost undetectable level in embryos of NS-DADm, the mice are viable and live to adulthood. They also suggest that HDAC3 has a DAD-independent or deacetylase-independent function that is required for life.

The DAD-independent functions of HDAC3 are unlikely to be the regulation of the genomic recruitment of NCOR1 and SMRT, since the genomic occupancy of these corepressors was maintained in the livers of NS-DADm as well as in mice lacking hepatic HDAC3. Also, while it is possible that HDAC3 has nuclear receptor corepressor-independent ability to deacetylate non-histone substrates[53–58], this is unlikely because the substrate used in the HDAC3 assay is a short peptide rather than a full-length histone protein. One caveat is that, due to the relatively high background of the HDAC activity assay, it is also possible that there is a small amount of residual HDAC3 deacetylase activity that is not detectable over background but may contribute to the modest phenotype of NS-DADm mice relative to mice lacking HDAC3 protein. It should also be noted that the genome-wide localization of HDAC3 was reduced but not abrogated in NS-DADm mice, potentially due to the second region in NCOR1 and SMRT that interacts with HDAC3 but does not activate the enzyme[33]. Nonetheless, the present study in NS-DADm mice demonstrates for the first time that corepressors NCOR1 and SMRT are required for endogenous HDAC3 activity. It also raises the possibility that the HDAC3 protein is critical for embryonic development as well as adult physiology through non-enzymatic mechanisms.

ONLINE METHODS

Mice

NS-DADm were generated from crossing N-DADm and S-DADm and the generation of mice lacking HDAC3 in liver by injection of AAV-TBG-Cre into HDAC3$^{f/f}$ mice has been previously described[16,17]. Mice were housed under a 12 h light and 12 h dark cycle (lights on at 7 a.m. and lights off at 7 p.m.). We used adult male mice at the age of 3–7 months in all experiments, except where otherwise indicated. We harvested tissues at 5 p.m. without restricting the mice to food or water, unless otherwise indicated. All the animal care and use procedures followed the guidelines of the Institutional Animal Care and Use Committee of

the University of Pennsylvania in accordance with the guidelines of the US National Institutes of Health.

Antibodies and reagents

HDAC3 antibodies for ChIP-seq, immunoprecipitation, and western blot were purchased from Abcam (ab7030) and Millipore (05-813, clone 3G6). Acetylated H3K9 and H3K27 antibodies were purchased from Millipore (07-352) and Abcam (ab4729), respectively. IgG was purchased from Sigma (I8140).

Immunoprecipitation and Western blotting

Liver tissue was homogenized in modified radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitors. Lysates were incubated on ice for 1 hr and clarified by centrifugation. Supernatants were precleared and incubated with HDAC3 antibody at 4°C overnight followed by 1hr incubation with Protein A agarose beads at 4°C. Immunoprecipitates were washed 5X with modified RIPA, eluted by boiling in SDS-loading buffer and subject to immunoblot analysis.

For the western blot of the total lysates, tissues were lysed in modified RIPA buffer supplemented with protease inhibitors, and the samples were resolved by Tris-glycine SDS-PAGE, then transferred to polyvinylidene fluoride (PVDF) membranes and blotted with the indicated antibodies. All antibodies were used at 1:1,000 to 1:5,000 dilutions.

HDAC activity assay

Immunoprecipitation with HDAC3 antibody and control IgG were performed as described above. Immunoprecipitates were washed 2X with modified RIPA buffer and 2X with 1X PBS followed by HDAC assay according to the manufacture's instruction (Active Motif, 56200)

ChIP-seq

ChIP was performed independently on liver samples from 4 or 5 different mice. Detailed procedures were previously described[16,18]. High throughput sequencing was done by the Functional Genomics Core (J. Schug and K. Kaestner) of the Penn Diabetes Research Center using the Illumina Genome Analyzer IIx, and sequence reads were mapped to the mm8 mouse genome using ELAND pipeline. In each ChIP-seq sample, all the duplicate reads were removed except for one for each genomic position.

Computational analysis for HDAC3 peaks

Peak calling was carried out using HOMER[59] with a default option (FDR=0.001) on HDAC3 WT and NS-DADm samples against the matching input sample, and then 1 RPM cutoff was applied. In case of wild-type samples, two replicates were pooled into one before the peak calling, so the maximum tag counts per position were set to be two (-tbp 2). HDAC3 binding sites were annotated with the following priority. 1) pTSS (proximal promoter, from −1 kb to 100 b around TSS), 2) TTS (from −100 b to 1 kb around TTS), 3) Exon, 4) Intron, 5) dTSS (distal promoter, from −10 kb to +1 kb around TSS) and 6)

Intergenic (everything else). The heatmaps of normalized HDAC3 binding profiles surrounding peak centers were generated using heatmap.2 function in R package.

Quantitative PCR

Quantitative PCR was performed with Power SYBR Green PCR Mastermix and the PRISM 7500 instrument (Applied Biosystems), and analyses were performed by the standard curve method. Primer sequences provided in Supplementary Table 1.

Tissue TG and cholesterol

Liver samples were homogenized in tissue lysis buffer (140mM NaCl, 50mM Tris and 1% Triton-X, pH8.0). TG and cholesterol concentration in the lysates were then quantified using LiquiColor Triglyceride Procedure No. 2100 (Stanbio) and Cholesterol LiquiColor Test (Stanbio), respectively.

Microarray

Total RNA was extracted from liver using RNeasy tissue Mini kit (QIAGEN) according the manufacturer's instructions. Preparation of RNA for hybridization to Affymetrix MoGene 1.0 ST (Affymetrix, Santa Clara, CA) and scanning of the arrays were performed by the University of Pennsylvania Microarray Facility (http://www.bioinformatics.upenn.edu/index.html) according to the manufacturer's instructions. Robust Multiarray Averaging (RMA) signal extraction, normalization, and filtering were performed by the Microarray Facility's bioinformatics group (http://core.pcbi.upenn.edu/) using Partek Genomics Suite (Partek, St. Louis, MO).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Choudhary C, et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. Science. 2009; 325:834–40. [PubMed: 19608861]
2. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997; 389:349–52. [PubMed: 9311776]
3. Hebbes TR, Thorne AW, Crane-Robinson C. A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J. 1988; 7:1395–402. [PubMed: 3409869]
4. Lee DY, Hayes JJ, Pruss D, Wolffe AP. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. Cell. 1993; 72:73–84. [PubMed: 8422685]
5. Struhl K. Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. 1998; 12:599–606. [PubMed: 9499396]

6. Xu L, Glass CK, Rosenfeld MG. Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev. 1999; 9:140–7. [PubMed: 10322133]
7. Hassig CA, Schreiber SL. Nuclear histone acetylases and deacetylases and transcriptional regulation: HATs off to HDACs. Curr Opin Chem Biol. 1997; 1:300–8. [PubMed: 9667866]
8. de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): characterization of the classical HDAC family. Biochem J. 2003; 370:737–49. [PubMed: 12429021]
9. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet. 2009; 10:32–42. [PubMed: 19065135]
10. Knutson SK, et al. Liver-specific deletion of histone deacetylase 3 disrupts metabolic transcriptional networks. EMBO J. 2008; 27:1017–28. [PubMed: 18354499]
11. Brehm A, et al. Retinoblastoma protein recruits histone deacetylase to repress transcription. Nature. 1998; 391:597–601. [PubMed: 9468139]
12. Ferreira R, Magnaghi-Jaulin L, Robin P, Harel-Bellan A, Trouche D. The three members of the pocket proteins family share the ability to repress E2F activity through recruitment of a histone deacetylase. Proc Natl Acad Sci U S A. 1998; 95:10493–8. [PubMed: 9724731]
13. Luo RX, Postigo AA, Dean DC. Rb interacts with histone deacetylase to repress transcription. Cell. 1998; 92:463–73. [PubMed: 9491888]
14. Fraga MF, et al. Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet. 2005; 37:391–400. [PubMed: 15765097]
15. Yeung F, et al. Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. EMBO J. 2004; 23:2369–80. [PubMed: 15152190]
16. Feng D, et al. A circadian rhythm orchestrated by histone deacetylase 3 controls hepatic lipid metabolism. Science. 2011; 331:1315–9. [PubMed: 21393543]
17. Sun Z, et al. Hepatic Hdac3 promotes gluconeogenesis by repressing lipid synthesis and sequestration. Nat Med. 2012; 18:934–42. [PubMed: 22561686]
18. Mullican SE, et al. Histone deacetylase 3 is an epigenomic brake in macrophage alternative activation. Genes Dev. 2011; 25:2480–8. [PubMed: 22156208]
19. Fischle W, et al. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell. 2002; 9:45–57. [PubMed: 11804585]
20. Zhang J, Kalkum M, Chait BT, Roeder RG. The N-CoR-HDAC3 nuclear receptor corepressor complex inhibits the JNK pathway through the integral subunit GPS2. Mol Cell. 2002; 9:611–23. [PubMed: 11931768]
21. Yoon HG, et al. Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1. EMBO J. 2003; 22:1336–46. [PubMed: 12628926]
22. Li J, et al. Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. EMBO J. 2000; 19:4342–50. [PubMed: 10944117]
23. Guenther MG, et al. A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness. Genes Dev. 2000; 14:1048–57. [PubMed: 10809664]
24. Guenther MG, Yu J, Kao GD, Yen TJ, Lazar MA. Assembly of the SMRT-histone deacetylase 3 repression complex requires the TCP-1 ring complex. Genes Dev. 2002; 16:3130–5. [PubMed: 12502735]
25. Sun Z, et al. Diet-induced lethality due to deletion of the Hdac3 gene in heart and skeletal muscle. J Biol Chem. 2011; 286:33301–9. [PubMed: 21808063]
26. Jepsen K, et al. Combinatorial roles of the nuclear receptor corepressor in transcription and development. Cell. 2000; 102:753–63. [PubMed: 11030619]
27. Jepsen K, et al. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature. 2007; 450:415–9. [PubMed: 17928865]
28. Bhaskara S, et al. Deletion of histone deacetylase 3 reveals critical roles in S phase progression and DNA damage control. Mol Cell. 2008; 30:61–72. [PubMed: 18406327]
29. Perissi V, et al. Molecular determinants of nuclear receptor-corepressor interaction. Genes Dev. 1999; 13:3198–208. [PubMed: 10617569]

30. Aasland R, Stewart AF, Gibson T. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIIB. Trends Biochem Sci. 1996; 21:87–8. [PubMed: 8882580]
31. Codina A, et al. Structural insights into the interaction and activation of histone deacetylase 3 by nuclear receptor corepressors. Proc Natl Acad Sci U S A. 2005; 102:6009–14. [PubMed: 15837933]
32. Hu X, Lazar MA. The CoRNR motif controls the recruitment of corepressors by nuclear hormone receptors. Nature. 1999; 402:93–6. [PubMed: 10573424]
33. Guenther MG, Barak O, Lazar MA. The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase 3. Mol Cell Biol. 2001; 21:6091–101. [PubMed: 11509652]
34. Alenghat T, et al. Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. Nature. 2008; 456:997–1000. [PubMed: 19037247]
35. You SH, Liao X, Weiss RE, Lazar MA. The interaction between nuclear receptor corepressor and histone deacetylase 3 regulates both positive and negative thyroid hormone action in vivo. Mol Endocrinol. 2010; 24:1359–67. [PubMed: 20427468]
36. Watson PJ, Fairall L, Santos GM, Schwabe JW. Structure of HDAC3 bound to co-repressor and inositol tetraphosphate. Nature. 2012; 481:335–40. [PubMed: 22230954]
37. Sinha RA, et al. Thyroid hormone stimulates hepatic lipid catabolism via activation of autophagy. J Clin Invest. 2012; 122:2428–38. [PubMed: 22684107]
38. Jepsen K, et al. SMRT-mediated repression of an H3K27 demethylase in progression from neural stem cell to neuron. Nature. 2007
39. Montgomery RL, et al. Maintenance of cardiac energy metabolism by histone deacetylase 3 in mice. J Clin Invest. 2008; 118:3588–97. [PubMed: 18830415]
40. Henck JW, Mattsson JL, Rezabek DH, Carlson CL, Rech RH. Developmental neurotoxicity of polybrominated biphenyls. Neurotoxicol Teratol. 1994; 16:391–9. [PubMed: 7968941]
41. Privalsky ML. The role of corepressors in transcriptional regulation by nuclear hormone receptors. Annu Rev Physiol. 2004; 66:315–60. [PubMed: 14977406]
42. Mann RK, Grunstein M. Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. EMBO J. 1992; 11:3297–306. [PubMed: 1505519]
43. Brownstein MJ, Palkovits M, Saavedra JM, Bassiri RM, Utiger RD. Thyrotropin-releasing hormone in specific nuclei of rat brain. Science. 1974; 185:267–269. [PubMed: 4209272]
44. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs. 2005; 14:1497–511.
45. Drummond DC, et al. Clinical development of histone deacetylase inhibitors as anticancer agents. Annu Rev Pharmacol Toxicol. 2005; 45:495–528. [PubMed: 15822187]
46. Gronemeyer H, Gustafsson JA, Laudet V. Principles for modulation of the nuclear receptor superfamily. Nat Rev Drug Discov. 2004; 3:950–64. [PubMed: 15520817]
47. Liu XF, Bagchi MK. Recruitment of distinct chromatin-modifying complexes by tamoxifen-complexed estrogen receptor at natural target gene promoters in vivo. J Biol Chem. 2004; 279:15050–8. [PubMed: 14722073]
48. Welsbie DS, et al. Histone deacetylases are required for androgen receptor function in hormone-sensitive and castrate-resistant prostate cancer. Cancer Res. 2009; 69:958–66. [PubMed: 19176386]
49. Hoberg JE, Yeung F, Mayo MW. SMRT derepression by the IkappaB kinase alpha: a prerequisite to NF-kappaB transcription and survival. Mol Cell. 2004; 16:245–55. [PubMed: 15494311]
50. Hong SH, Yang Z, Privalsky ML. Arsenic trioxide is a potent inhibitor of the interaction of SMRT corepressor with Its transcription factor partners, including the PML-retinoic acid receptor alpha oncoprotein found in human acute promyelocytic leukemia. Mol Cell Biol. 2001; 21:7172–82. [PubMed: 11585900]
51. Nofsinger RR, et al. SMRT repression of nuclear receptors controls the adipogenic set point and metabolic homeostasis. Proc Natl Acad Sci U S A. 2008; 105:20021–6. [PubMed: 19066220]

52. Varlakhanova N, Hahm JB, Privalsky ML. Regulation of SMRT corepressor dimerization and composition by MAP kinase phosphorylation. Mol Cell Endocrinol. 2011; 332:180–8. [PubMed: 20965228]

53. Juan LJ, et al. Histone deacetylases specifically down-regulate p53-dependent gene activation. J Biol Chem. 2000; 275:20436–43. [PubMed: 10777477]

54. Luo J, Su F, Chen D, Shiloh A, Gu W. Deacetylation of p53 modulates its effect on cell growth and apoptosis. Nature. 2000; 408:377–81. [PubMed: 11099047]

55. Ashburner BP, Westerheide SD, Baldwin AS Jr. The p65 (RelA) subunit of NF-kappaB interacts with the histone deacetylase (HDAC) corepressors HDAC1 and HDAC2 to negatively regulate gene expression. Mol Cell Biol. 2001; 21:7065–77. [PubMed: 11564889]

56. Hubbert C, et al. HDAC6 is a microtubule-associated deacetylase. Nature. 2002; 417:455–8. [PubMed: 12024216]

57. Shimazu T, et al. Regulation of SV40 large T-antigen stability by reversible acetylation. Oncogene. 2006; 25:7391–400. [PubMed: 16767160]

58. Gregoire S, et al. Histone deacetylase 3 interacts with and deacetylates myocyte enhancer factor 2. Mol Cell Biol. 2007; 27:1280–95. [PubMed: 17158926]

59. Heinz S, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Mol Cell. 2010; 38:576–89. [PubMed: 20513432]

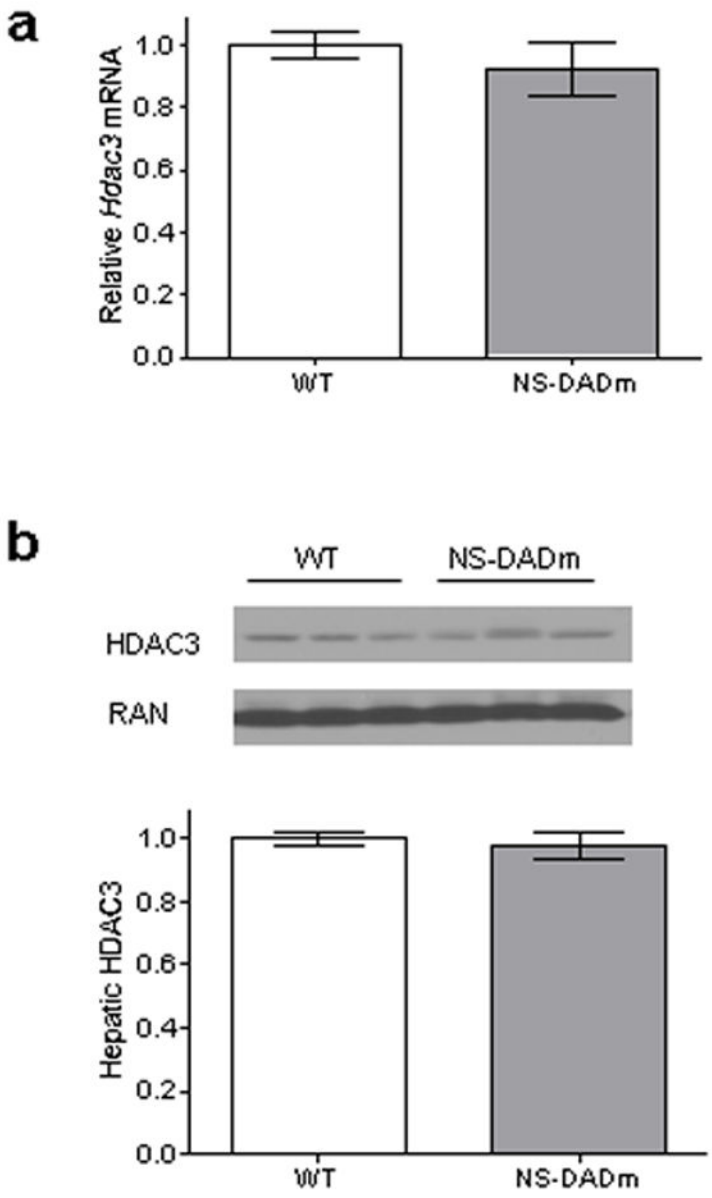

Figure 1. NS-DADm mice expressed normal levels of HDAC3

(a) qPCR analysis of *Hdac3* expression in wild type (WT) and NS-DADm liver. (b) Western blot analysis of hepatic HDAC3 was measured in WT and NS-DADm mice and its quantitation normalized by RAN expression. All error bars represent standard error of the mean (s.e.m.) by Student's two-tailed *t* test.

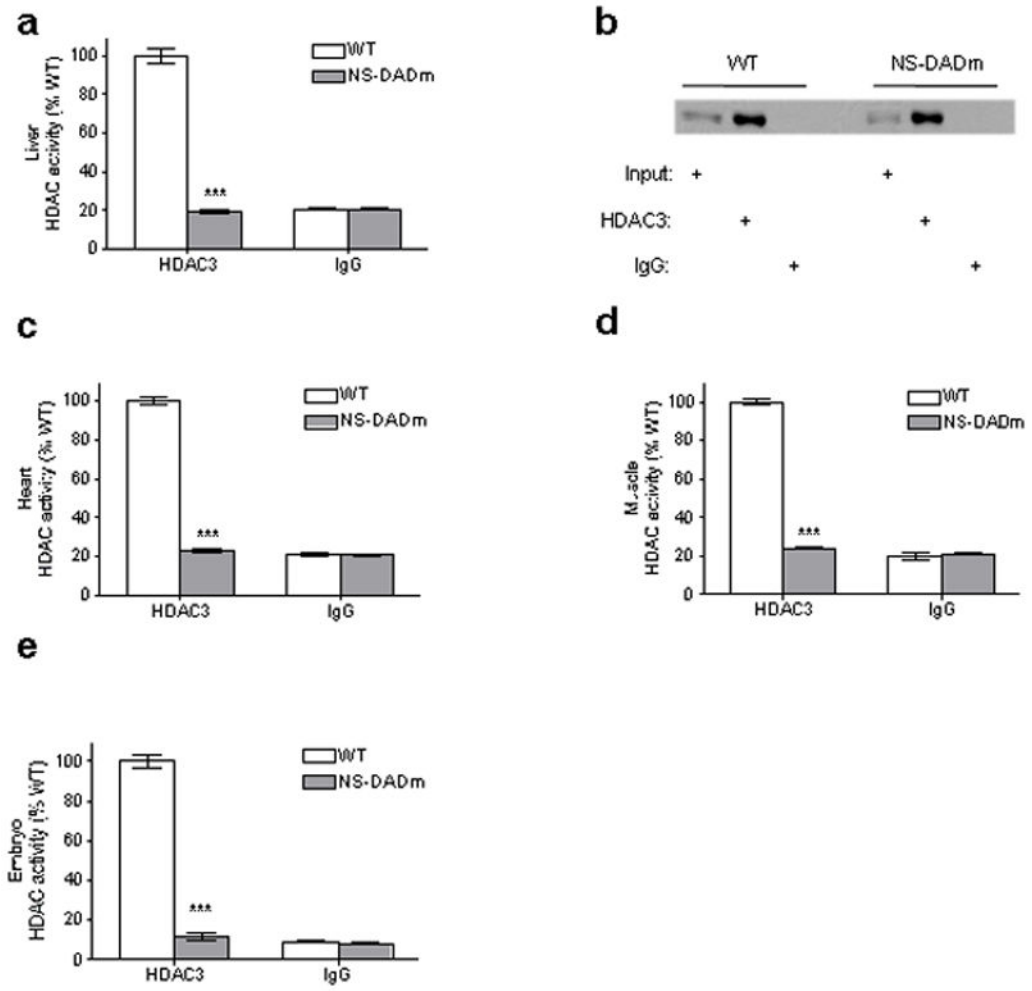

Figure 2. HDAC3 was enzymatically inactive in various tissues of NS-DADm mice

(a) HDAC activity was measured after immunoprecipitation with HDAC3 specific antibody or IgG in adult liver, heart (c), muscle (d), and embryo (E12.5) (e). (b) Western blot analysis of liver HDAC3 after immunoprecipitation with either HDAC3 or IgG. N=4, all error bars = s.e.m. ***$p < 0.001$ by Student's two-tailed *t* test.

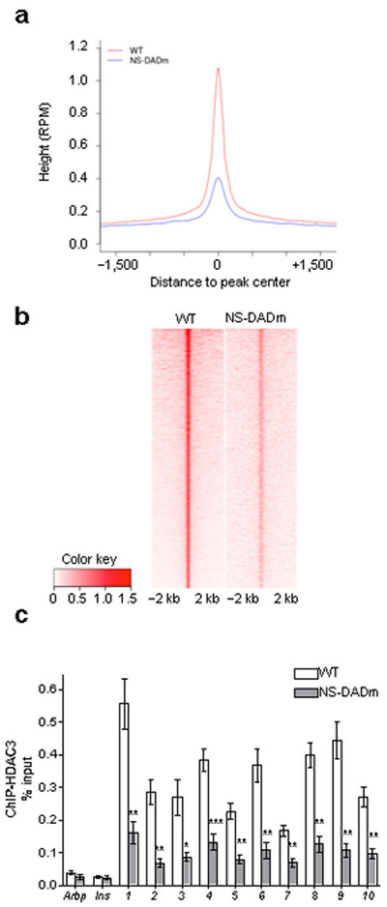

Figure 3. Genomic localization of HDAC3 was significantly reduced in NS-DADm mice

(a) Average HDAC3 signal from −1.5kb to +1.5kb surrounding center of all the HDAC3 binding sites. (b) Heat map of HDAC3 in WT (left) and NS-DADm (right) liver. Each row represents a single HDAC3 binding site that is continuous from WT to NS-DADm and sorted by the peak heights in WT. The color scale indicates the signal per million total reads. (c) ChIP-PCR analysis of HDAC3 recruitment to ten binding sites interrogated in WT and NS-DADm liver. PCR primers and genomic location provided in Supplementary Table 1. All error bars = s.e.m. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by Student's two-tailed t test.

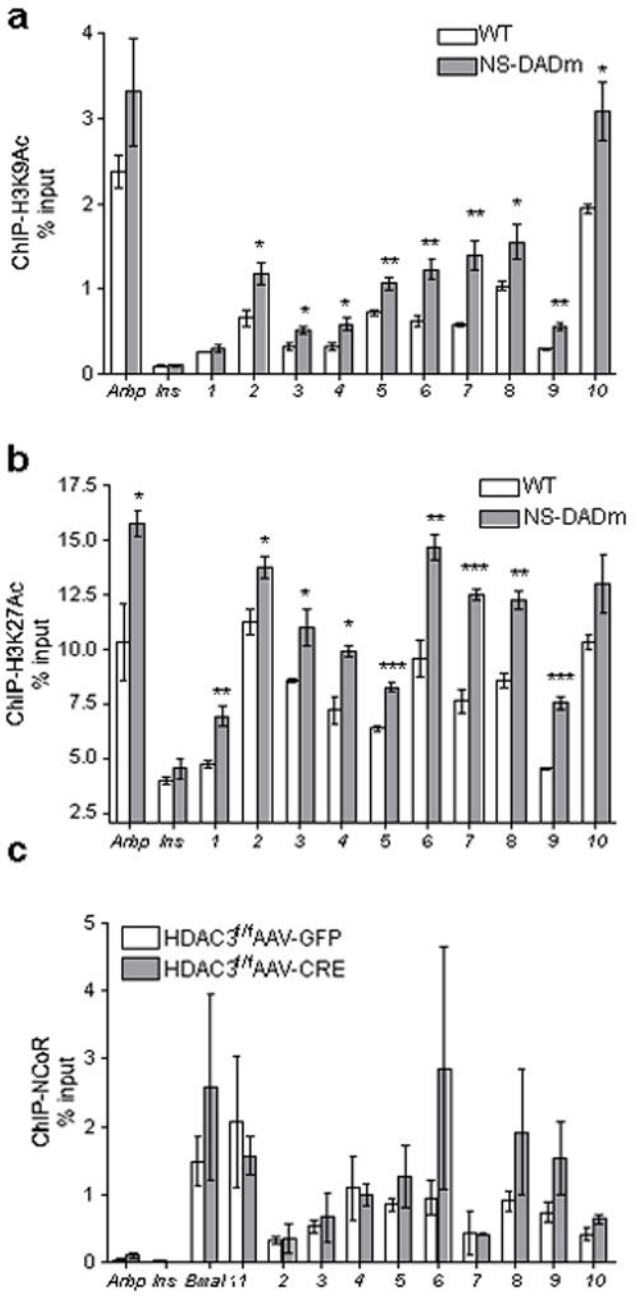

Figure 4. Local histone acetylation was increased in NS-DADm mice

ChIP-PCR analysis in WT and NS-DADm liver at sites shown in Figure 3c. (a) H3 lysine 9 acetylation. (H3K9Ac) (b) H3 lysine 27 acetylation (H3K27Ac). (c) NCOR1. N=4, All error bars = s.e.m. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by Student's two-tailed *t* test.

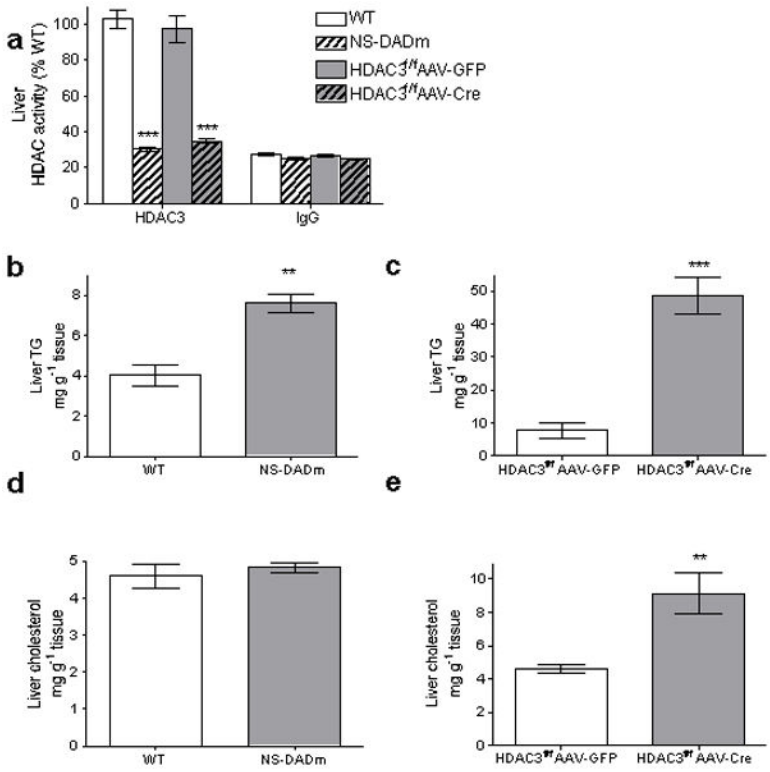

Figure 5. The liver phenotype of NS-DADm mice was more modest than mice lacking hepatic HDAC3

(a) HDAC activity was measured after immunoprecipitation with HDAC3 specific antibody or IgG in WT, NS-DADm, HDAC3$^{f/f}$ AAV-GFP and HDAC3$^{f/f}$ AAV-Cre liver. (b-c) Hepatic triglyceride (TG) and (d-e) liver cholesterol were measured in WT, NS-DADm, HDAC3$^{f/f}$ AAV-GFP and HDAC3$^{f/f}$ AAV-Cre mice. N=4–5, all error bars = s.e.m. $^{**}p < 0.01$ and $^{***}p < 0.001$ by Student's two-tailed *t* test.

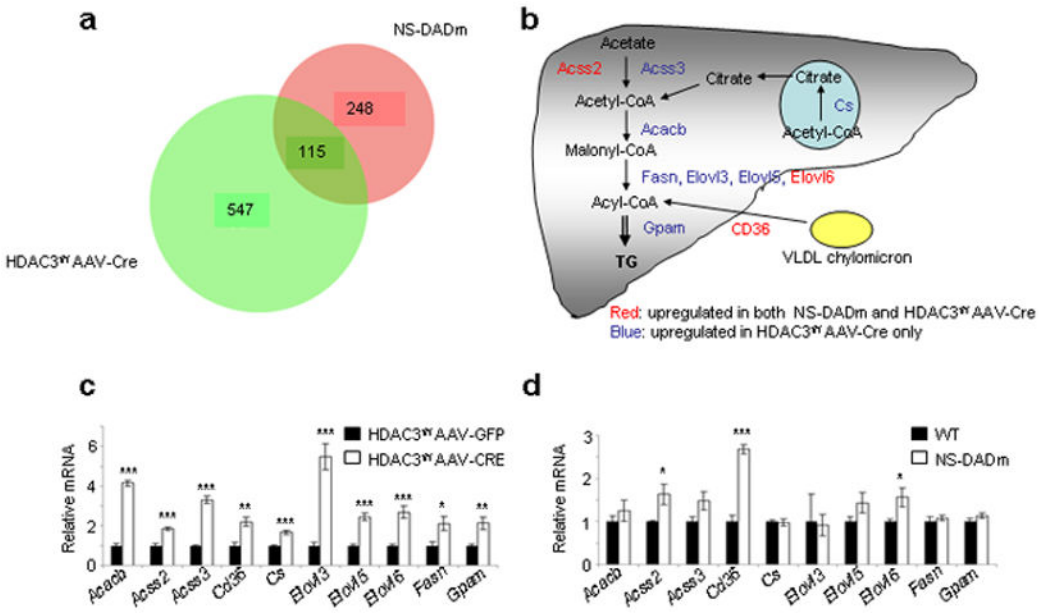

Figure 6. Gene expression changes in NS-DADm livers were mild compared to HDAC3 protein depletion

(a) Venn diagram demonstrating overlap of upregulated genes of NS-DADm versus HDAC3 liver-specific KO (HDAC3$^{f/f}$ AAV-Cre) from liver microarray. (b) Biochemical pathways of genes that are upregulated in both NS-DADm and HDAC3$^{f/f}$ AAV-Cre livers (red) and upregulated only in HDAC3$^{f/f}$ AAV-Cre liver (blue). (c-d) qPCR validation of results summarized in (b). N=4–5, all error bars = s.e.m. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ by Student's two-tailed *t* test.

Table 1

NS-DADm mice are viable without excess mortality.

Genotype	# of matings	# of pups born	Average pups/mating
WT	12	77	6.4
NS-DADm	13	87	6.7