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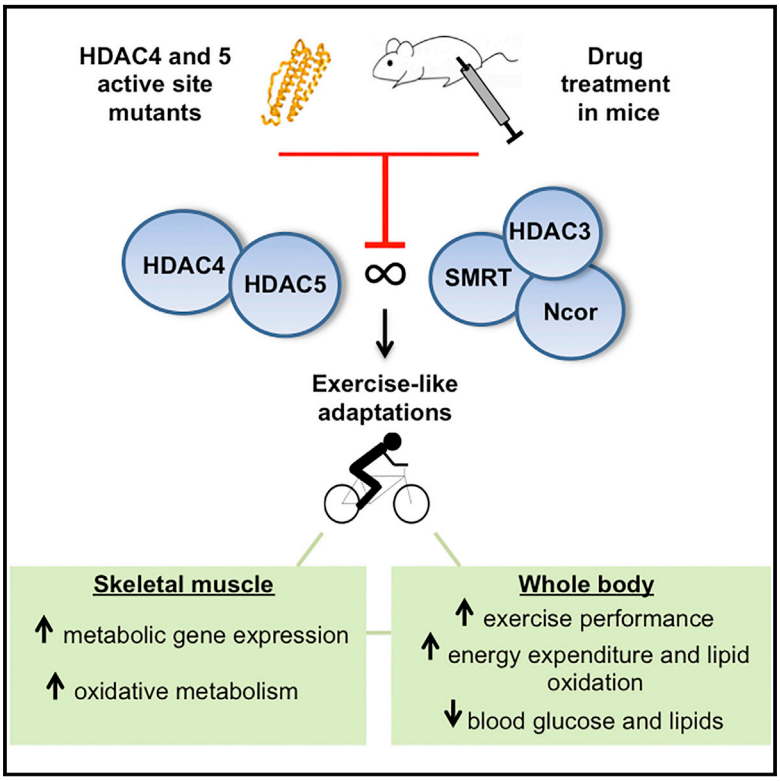
Disruption of the Class IIa HDAC Corepressor Complex Increases Energy Expenditure and Lipid Oxidation

Graphical Abstract



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In Brief

Physical inactivity is a major cause of chronic diseases. Drugs that mimic aspects of exercise could therefore reduce chronic disease burden. Gaur et al. identify and validate the class IIa histone deacetylases (HDACs) as drug targets to replicate aspects of the exercise adaptive response.

Highlights

- HDAC4/5 active-site mutants increase exercise-responsive genes and metabolism
- Scriptaid is a compound with similar phenotypic effects in vitro
- Acute Scriptaid administration in mice increases exercise-responsive genes
- Chronic Scriptaid administration reduces blood glucose and lipids in mice

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Disruption of the Class IIa HDAC Corepressor Complex Increases Energy Expenditure and Lipid Oxidation

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SUMMARY

Drugs that recapitulate aspects of the exercise adaptive response have the potential to provide better treatment for diseases associated with physical inactivity. We previously observed reduced skeletal muscle class IIa HDAC (histone deacetylase) transcriptional repressive activity during exercise. Here, we find that exercise-like adaptations are induced by skeletal muscle expression of class IIa HDAC mutants that cannot form a corepressor complex. Adaptations include increased metabolic gene expression, mitochondrial capacity, and lipid oxidation. An existing HDAC inhibitor, Scriptaid, had similar phenotypic effects through disruption of the class IIa HDAC corepressor complex. Acute Scriptaid administration to mice increased the expression of metabolic genes, which required an intact class IIa HDAC corepressor complex. Chronic Scriptaid administration increased exercise capacity, whole-body energy expenditure and lipid oxidation, and reduced fasting blood lipids and glucose. Therefore, compounds that disrupt class IIa HDAC function could be used to enhance metabolic health in chronic diseases driven by physical inactivity.

INTRODUCTION

Physical inactivity, which is related to energy imbalance and a loss of functional capacity (Booth et al., 2012), is recognized as a major cause of chronic disease and mortality (Mokdad et al., 2004). However, many patients and at-risk individuals do not, or cannot, engage in physical activity. Identification of the mechanisms mediating exercise adaptations could provide new molecular targets for therapeutic intervention.

Adaptations to exercise involve repeated transient changes in gene expression that alter protein levels and, ultimately, phenotype (Egan and Zierath, 2013). A number of studies implicate the class IIa histone deacetylase (HDAC) myocyte enhancer factor 2 (MEF2) axis in this process. The class IIa HDACs, which include isoforms 4, 5, 7, and 9, repress gene expression by interacting with the MEF2 transcription factors (McKinsey et al., 2001). Although the catalytic domain of the class IIa HDACs is structurally similar to the highly active class I HDACs, they have low intrinsic HDAC activity against acetyl-lysine substrates due to a single amino acid substitution within their active site (Lahm et al., 2007), and their repressive activity requires a corepressor complex that contains SMRT/N-Cor and HDAC3 (Fischle et al., 2002; Kao et al., 2000), a class I HDAC. We have found that this class IIa HDAC corepressor complex is disrupted by exercise in human skeletal muscle through phosphorylation-dependent nuclear export of HDAC4 and HDAC5 (McGee et al., 2009), which reduced their association with MEF2 (McGee and Hargreaves, 2004), increased MEF2 DNA binding (McGee

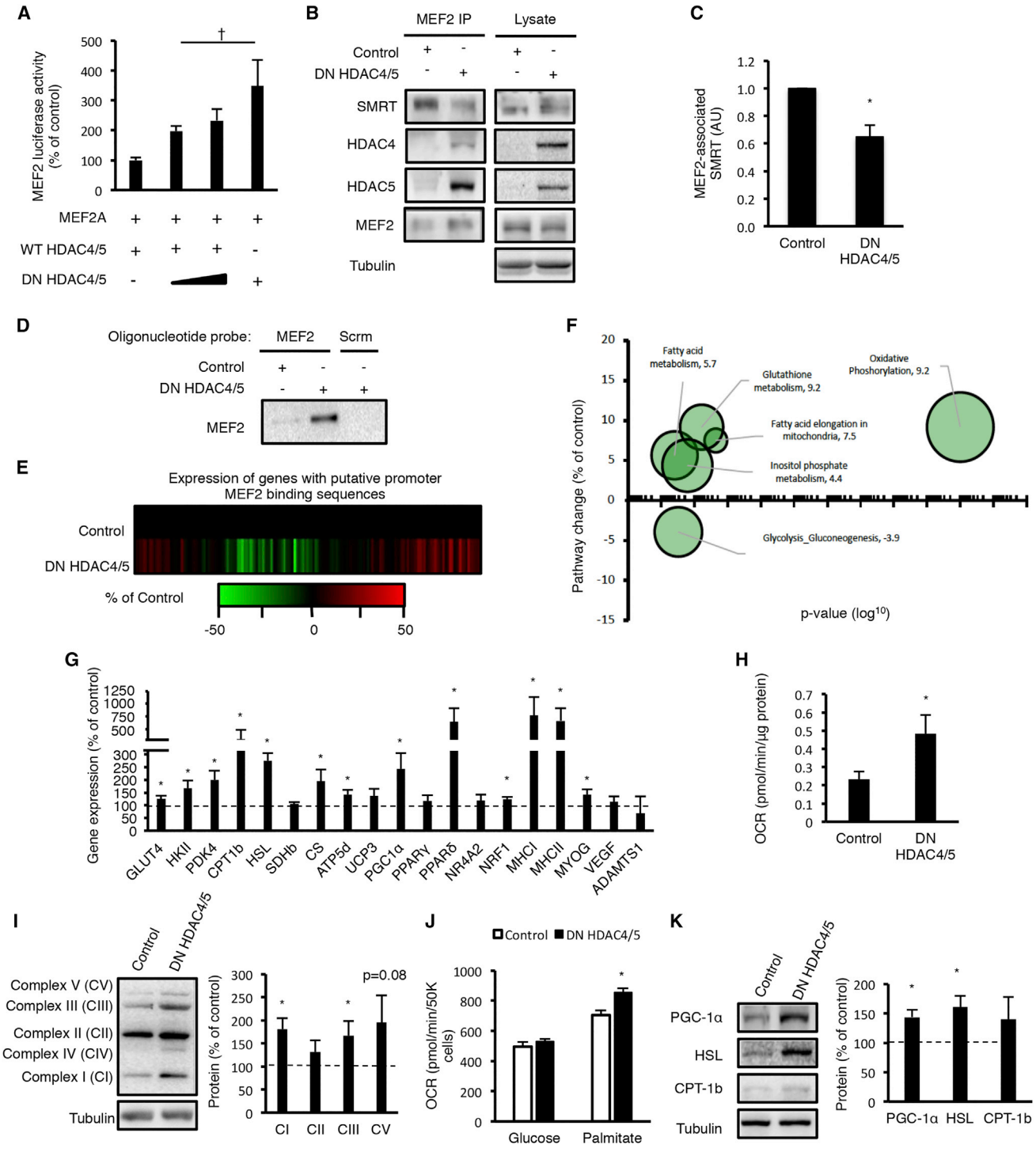

Figure 1. Active-Site Mutants DN HDAC4 and 5 Increase MEF2 Transcriptional Activity, the Expression of Exercise-Responsive Genes, and Oxidative Metabolism

(A) MEF2 luciferase reporter assays with various combinations of MEF2A, HDAC4, and HDAC5, with increasing amounts of DN HDAC4 and 5 (n = 3 biological replicates per group).

(B) Coimmunoprecipitation of the class IIa HDAC corepressor complex from extensor digitorum longus (EDL) muscles administered with empty rAAV6 (Control) or rAAV6 DN HDAC4 and rAAV6 DN HDAC5 via intramuscular injection.

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et al., 2006), and increased expression of MEF2-dependent genes (McGee and Hargreaves, 2004). However, the role of MEF2 in muscle post-development is unclear. Similarly, the gene networks that the class IIa HDACs regulate in adult muscle are unknown. Further understanding of this signaling axis could reveal the class IIa HDACs as targets for pharmacological induction of exercise adaptions.

Most HDAC inhibitors lack isoform selectivity and generally consist of a chelating moiety that interacts with the active-site zinc atom, a narrow linker region that spans the catalytic groove and a capping group (Sternson et al., 2001). It has emerged that some HDAC inhibitors can disrupt the class IIa HDAC corepressor complex by changing the conformation of the class IIa HDAC active site, which mediates physical association with N-Cor/SMRT (Hudson et al., 2015; Lahm et al., 2007). Therefore, HDAC inhibitors that were thought to only inhibit class I HDAC catalytic activity may have efficacy against class IIa HDAC transcriptional repression. Here, we found that expression of class IIa HDAC active-site mutants that disrupt their corepressor complex in skeletal muscle induced metabolic adaptations and identified a HDAC inhibitor, Scriptaid, which had similar phenotypic effects. Moreover, chronic administration of Scriptaid to mice induced skeletal muscle and systemic metabolic adaptive responses.

RESULTS

Active-Site Mutant HDAC4 and HDAC5 Increase MEF2 Transcriptional Activity, the Expression of Metabolic Genes, and Oxidative Metabolism

Mutation of key residues within the active site of class IIa HDACs disrupts the class IIa HDAC corepressor complex in vitro (Fischle et al., 2002; Hudson et al., 2015); however, the effect on MEF2 activity is unknown. In a MEF2 reporter assay that was sensitive to HDAC repression (Figures S1A and S1B), expression of HDAC4 and HDAC5 active-site mutants (D840N HDAC4 and D870N HDAC5 in human; D832N HDAC4 and D861N HDAC5 in mouse) increased MEF2-dependent transcription (Figure 1A), which required mutation of both HDAC4 and HDAC5 (data not shown). This shows that the D832N HDAC4 and D861N HDAC5 mutants (referred to hereinafter as DN HDAC4 and 5) act in a dominant-negative manner on MEF2-dependent transcription. To determine whether these mutants dissociated the class IIa corepressor complex in vivo, rAAV6 vectors expressing DN HDAC4 and 5 were administered to one hindlimb of C57BL6 mice via multiple intramuscular injections. The contralateral hindlimb musculature was administered with empty rAAV6 vector as a control (Figure 1B). Class IIa HDAC transcriptional repression is mediated, in part, through their association with SMRT (Hudson et al., 2015). Coimmunoprecipitation showed that MEF2-associated SMRT was reduced in extensor digitorum longus (EDL) muscles expressing DN HDAC4 and 5 (Figures 1B and 1C). To examine the capacity for MEF2 to bind DNA, a biotinylated oligonucleotide pull-down assay with probes containing either a MEF2 consensus binding sequence or a scrambled sequence was validated (Figure S1C). Oligonucleotide binding by MEF2 was increased by DN HDAC4 and 5 expression (Figure 1D). To determine the transcriptional response to DN HDAC4 and 5 expression, microarray analyses were performed. The expression of ~60% of genes with a putative MEF2 binding sequence (CTAAAAATAG) in their promoter region was increased (Figure 1E), and 1,412 genes, primarily involved in metabolic processes (Figure S1D), were differentially expressed by DN HDAC4 and 5 expression. Gene set enrichment analysis (GSEA) of metabolic pathways using the entire microarray dataset revealed that oxidative phosphorylation, fatty acid metabolism, glutathione metabolism, inositol phosphate metabolism, and fatty acid elongation pathways were significantly increased by DN HDAC4 and 5 expression (Figure 1F). This was further examined by qRT-PCR. Exercise-responsive genes involved in glucose (*HKII* and *GLUT4*), lipid (*PDK4*, *CPT-1b*, and *HSL*), mitochondrial (*CS* and *ATP5b*), and transcriptional (*PGC-1α* and *PPARδ*) regulation of metabolism were increased by DN HDAC4 and 5 expression, in addition to genes involved in muscle fiber type determination (*MHCI* and *II*, *MYOG*; Figure 1G). To test whether oxidative capacity was enhanced, respiration was assessed in the tibialis anterior (TA) muscle of mice administered rAAV6 vectors expressing DN HDAC4 and 5 or control rAAV6. State 3 respiration was higher in muscles expressing DN HDAC4 and 5 (Figure 1H), and components of mitochondrial oxidative phosphorylation complexes were increased (Figure 1I; complex IV subunits could not be reliably detected in these samples and were not quantified). To dissect oxidative metabolism further, DN HDAC4 and 5 were expressed in C2C12 myotubes (Figure S1F), which increased basal oxygen consumption rate (OCR; Figure S1F). Analysis of mitochondrial function revealed that this was due to an increase in ATP turnover and not due to alterations in uncoupled respiration (Figure S1G), indicating an increase in cell-autonomous energy expenditure through processes that consume ATP. Analysis of basal glucose and lipid oxidation showed that DN HDAC4 and 5 expression increased oxidation of the fatty acid palmitate but not glucose (Figure 1J). The expression of DN HDAC4 and 5 in the EDL also increased the protein levels of PGC-1α and HSL (Figure 1K), key regulatory

(C–F) MEF2-associated SMRT (C); MEF2 binding to an oligonucleotide probe with a consensus MEF2 binding site or a Scrambled (Scrm) sequence (D); Expression of genes with putative MEF2 promoter sequences (E); and bubble-plot representation of GSEA of metabolic pathways significantly altered by DN HDAC4 and 5 expression relative to control (empty rAAV6) in EDL muscle (F) (n = 5 per group).

(G–I) Expression profiles of exercise-responsive genes (G); oxygen consumption rate (OCR) measured under state 3 respiration conditions in tibialis anterior (TA) muscle (H); and (I) oxidative phosphorylation complex subunit protein in EDL muscle in mice administered empty rAAV6 (Control) or rAAV6 DN HDAC4 and rAAV6 DN HDAC5 (n = 10 per group).

(J) Substrate oxidation in C2C12 myotubes administered empty rAAV6 (Control) or rAAV6 DN HDAC4 and rAAV6 DN HDAC5 (n = 5 biological replicates per group).

(K) PGC-1α, HSL, and CPT-1b protein following DN HDAC4 and HDAC5 expression relative to control in EDL muscle (n = 6 per group).

Data are means ± SEM. †Versus cells expressing MEF2A and wild-type (WT) HDAC4 and HDAC5. *Versus control group. #Significantly enriched in dataset.

proteins involved in skeletal muscle lipid metabolism. Together, these data show that expression of class IIa HDAC active-site mutants that disrupt their corepressor complex enhanced the expression of metabolic genes and increased cell-autonomous energy expenditure that was supported by the oxidation of fatty acids.

Scriptaid Increases MEF2 Transcriptional Activity, Metabolic Gene Expression, and Oxidative Metabolism In Vitro

We sought to identify an HDAC inhibitor that could induce similar phenotypic adaptations. We reasoned that these effects could be obtained through three different mechanisms: (1) inhibition of HDAC3, which is part of the class IIa HDAC corepressor complex; (2) direct inhibition of the class IIa HDACs; and/or (3) class IIa HDAC corepressor complex disruption. Three compounds—TSA (trichostatin A), Scriptaid, and MC1568—with different physicochemical properties were examined. TSA has high selectivity against class I HDACs (Bradner et al., 2010), while MC1568 is reportedly a class IIa-specific inhibitor (Mai et al., 2005). In contrast, Scriptaid shows weak inhibitory activity against class I HDACs (Bradner et al., 2010) but has a large capping region, the size of which has been linked to the capacity to disrupt the class IIa HDAC corepressor complex (Hudson et al., 2015). When administered to C2C12 myotubes, TSA and Scriptaid at 0.1 and 1 μM increased histone 3 lysine 9 acetylation (H3K9ac; Figure 2A). As some HDAC inhibitors have reported context-dependent cytotoxicity (Zhang et al., 2013), lactate dehydrogenase (LDH) release from myotubes in response to a therapeutically relevant dose (1 μM) of TSA, Scriptaid, and MC1568 was assessed. TSA increased LDH release, indicating reduced cell viability (Figure 2B), and was excluded from further analyses. Time-dependent expression profiling of candidate exercise-responsive metabolic genes in myotubes treated with Scriptaid or MC1568 for 60 min revealed that Scriptaid increased the expression of genes involved in lipid metabolism, mitochondrial biogenesis, and mitochondrial function (Figure 2C). Unexpectedly, MC1568 reduced the expression of all genes examined (Figure 2C). In addition to our recent structural reassignment of this compound (Fleming et al., 2014), we also found that MC1568 did not elicit inhibitory activity toward HDAC4 and HDAC5 against the class IIa HDAC-specific trifluoroacetyl-lysine substrate in cell-free assays (Figure S2A). Therefore, MC1568 was excluded from further analyses. Scriptaid increased MEF2-dependent transcriptional activity (Figure 2D) and basal OCR (Figure 2E). This was due to accelerated ATP turnover, and maximal respiratory capacity was also increased (Figure 2F). Scriptaid also increased palmitate oxidation (Figure 2G). These data show that Scriptaid enhanced metabolic gene expression, energy expenditure, and lipid oxidation and was selected for further mechanism of action and in vivo efficacy studies.

Scriptaid Alters Class IIa HDAC/Corepressor Interactions

Scriptaid action on class IIa HDAC function was assessed. To our knowledge, no studies have examined the inhibitory activity of Scriptaid against the class IIa HDACs for the non-physiological synthetic trifluoroacetyl-lysine substrate. In cell-free assays with recombinant HDAC isoforms, Scriptaid had only very weak inhibitory activity against HDAC3 (IC_{50} [half maximal inhibitory concentration], 0.5 μM; Figure S3), HDAC4, and HDAC5 (IC_{50} 20 μM and 0.5 μM, respectively; Figure S2B). In addition to the fact that no physiological trifluroacetyl-lysine substrates have been identified, this suggests that the action of Scriptaid on metabolic adaptation in muscle cells occurs independently of direct inhibition of class I and IIa HDACs. Some HDAC inhibitors can impair class IIa HDAC corepressor associations, which appear dependent on the compound's capping group (Hudson et al., 2015). As Scriptaid possesses a larger capping region than most hydroxamate-zinc-chelating HDAC inhibitors, we hypothesized that Scriptaid impairs class IIa HDAC corepressor interactions. Indeed, in high-throughput proteomics analyses, Scriptaid has been observed to dissociate a number of coregulator complex proteins from HDAC enzymes (Bantscheff et al., 2011). Docking of Scripatid to HDAC4 revealed a binding pose consistent with that of HDAC inhibitors (Figure 3A). The hydroxamic acid exhibits a bidentate interaction with the active-site zinc, while the aliphatic chain occupies the cleft. The large tricyclic capping region is exposed outside of the active site in close proximity to F812 and F871, which comprise the binding tunnel. This optimal binding orientation is consistent with reports that Scriptaid has a higher binding affinity (~4- to 5-fold) with the class IIa HDACs, compared with other HDAC classes (Bantscheff et al., 2011). To determine whether Scriptaid disrupts the class IIa corepressor complex, HDAC4 interaction with the corepressor SMRT was assessed by anisotropy assays using a fluorescence-labeled peptide corresponding to amino acids 1,450–1,469 of SMRT, which contains a class IIa HDAC-interacting domain (Hudson et al., 2015). Scriptaid reduced the maximal interaction polarization between wild-type (WT) HDAC4 and the SMRT peptide (Figure 3B), suggesting that Scriptaid binding influences the mobility of the fluorophore on the SMRT peptide, implying proximity of binding. Furthermore, Scriptaid reduced the binding affinity (higher dissociation constant, K_D) between the SMRT peptide and a gain-of-function (GOF) HDAC4 mutant (H976Y) that enhances its association with SMRT (Figure 3B). This was further analyzed in vivo, where Scriptaid was acutely administered to mice via intraperitoneal (i.p.) injection. A time and dose-response analysis of tissue H3K9ac was used to estimate Scriptaid pharmacokinetics. Scriptaid at 1 mg/kg was sufficient to increase H3K9ac in the EDL (Figure 3C), with less obvious alterations in H3K9ac in the pancreas and brain (Figure S3A). Modest increases in H3K9ac were also observed in the heart, while H3K9ac appeared to be reduced in the liver (Figure S3A). Scriptaid at 3 and 10 mg/kg body weight did not appear to have additional effects on H3K9ac in skeletal muscle (data not shown). Therefore, Scriptaid at 1 mg/kg body weight, which is an acceptable effective therapeutic dose, was used for all future experiments. Scriptaid reduced MEF2-associated SMRT in EDL muscle 60 min after its administration (Figure 3D), while MEF2 DNA binding capacity was increased (Figure 3E). Scriptaid had no effect on the phosphorylation of the class IIa HDACs (Figure S3B), which regulates class IIa HDAC corepressor interactions and the nuclear export of class IIa HDACs. Together, these data suggest that Scriptaid disrupts the class IIa HDAC corepressor complex.

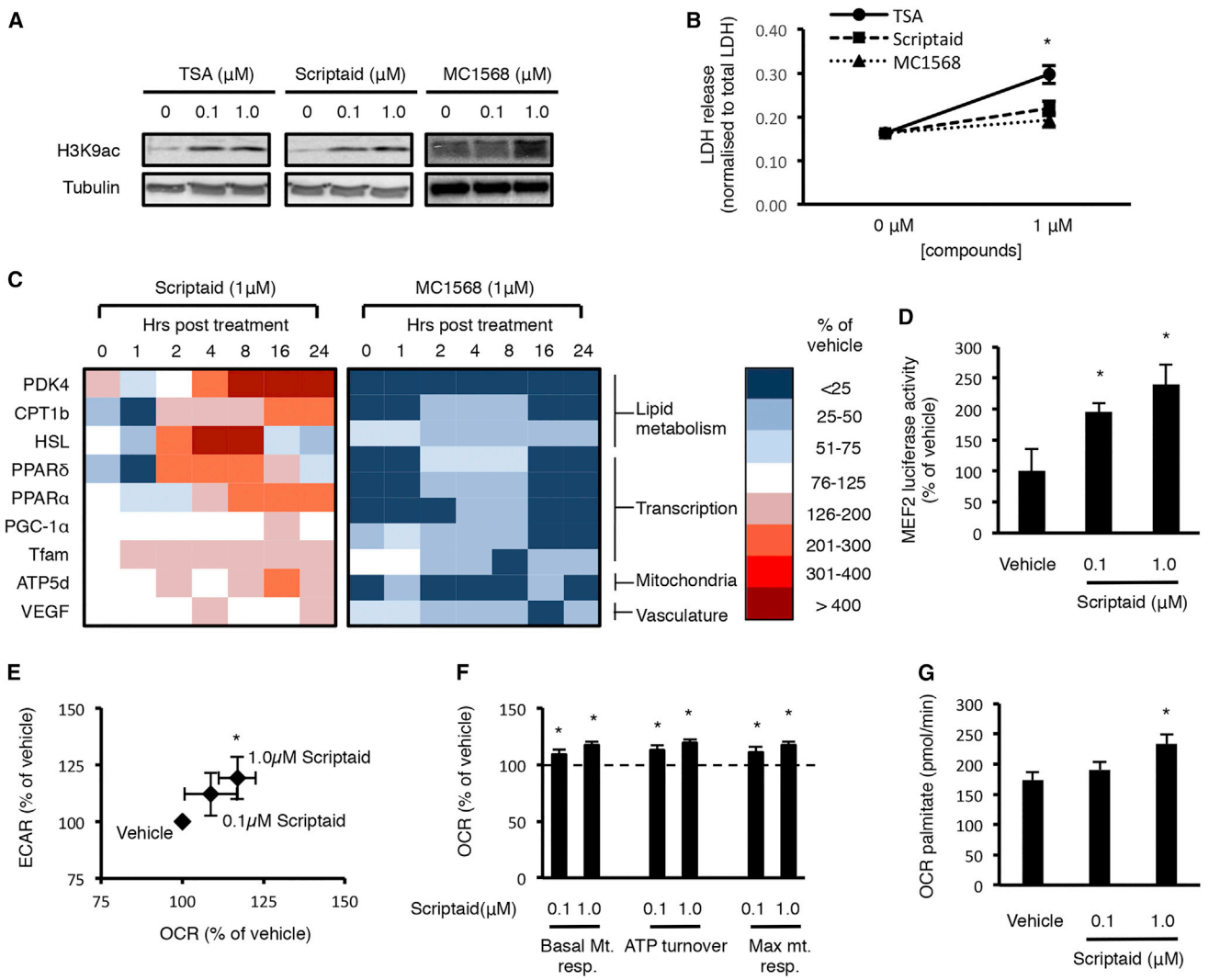

Figure 2. Pharmacological HDAC Inhibition Increases MEF2 Transcriptional Activity, Metabolic Gene Expression, and Oxidative Metabolism In Vitro

(A) C2C12 myotube histone 3 lysine 9 acetylation (H3K9ac) following 60-min exposure to vehicle (DMSO) or 0.1 μM or 1.0 μM TSA, Scriptaid, or MC1568.

(B) Lactate dehydrogenase (LDH) release from C2C12 myotubes treated with 1 μM TSA, Scriptaid, or MC1568 for 16 hr (n = 4 biological replicates per group).

(C) Time course of metabolic gene expression following 60-min exposure to 1.0 μM Scriptaid or MC1568 in C2C12 myotubes. Data normalized to vehicle-treated cells (n = 4 biological replicates per time point).

(D) MEF2 luciferase reporter assays following 60-min exposure to 0.1 and 1 μM Scriptaid (n = 3 biological replicates per group).

(E–G) Basal extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) (E); mitochondrial function indices (F); and palmitate oxidation in L6 myotubes treated for 2 consecutive days, 2× 1 hr a day, with 0.1 μM or 1.0 μM Scriptaid (G). Data were normalized to vehicle (n = 8 biological replicates per group). Mt. resp., mitochondrial respiration.

Data are mean ± SEM. *Versus vehicle group.

Scriptaid Administration Induces Metabolic Adaptive Responses In Vivo

To examine the transcriptional response to Scriptaid and its requirement for an intact class IIa HDAC corepressor complex, one EDL muscle of C57BL6 mice was administered with rAAV6 vectors expressing DN HDAC4 and 5, while the contralateral EDL muscle was administered with empty rAAV6 vector (control) via intramuscular injection. Two weeks later, mice were administered vehicle (5% DMSO in 1× PBS) or Scriptaid, and skeletal muscles were collected 4 hr later. In control EDL muscles, Scriptaid increased the expression of ~70% of genes with a putative MEF2 binding sequence in their promoter region (Figure 4A) and altered the expression of 1410 genes involved in a variety of biological processes, including metabolism (Figure S3C). GSEA of metabolic pathways showed that, similar to DN HDAC4 and 5, Scriptaid significantly increased fatty acid metabolism and oxidative phosphorylation pathways (Figure 4B). Indeed, these two interventions showed remarkably similar metabolic pathway

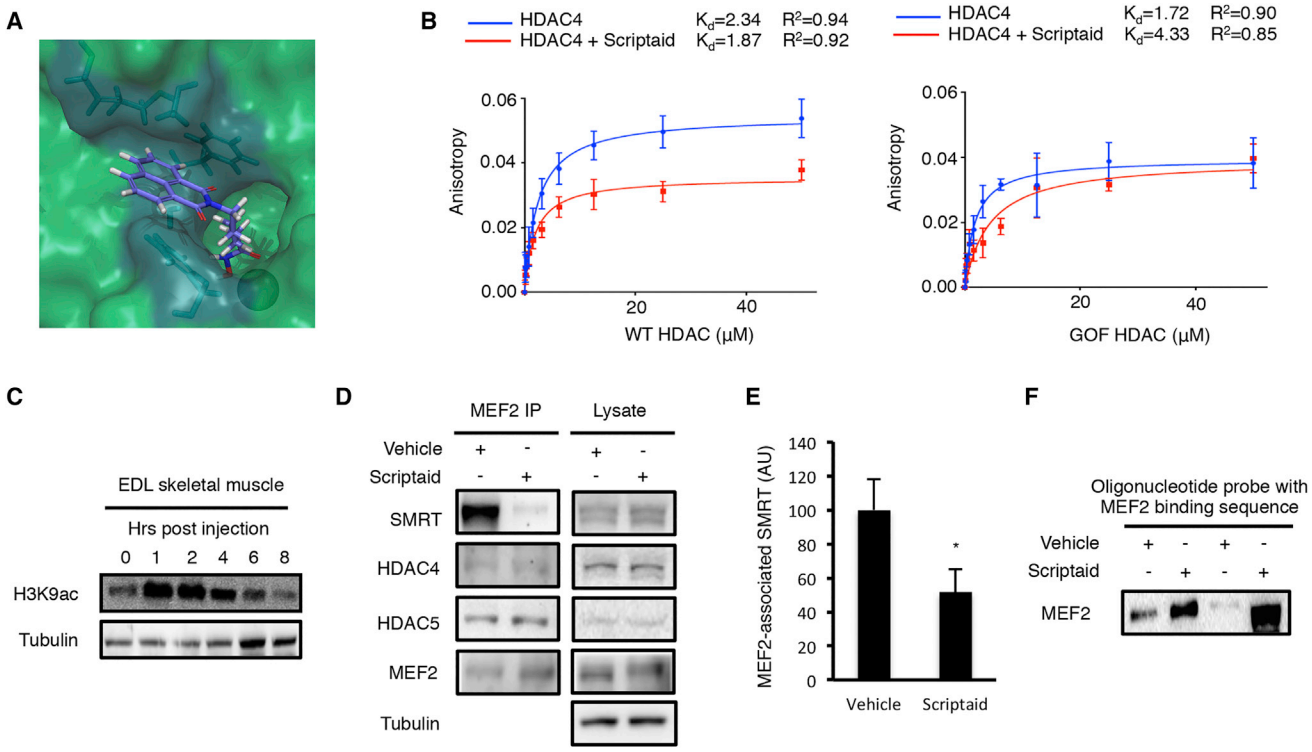

Figure 3. Scriptaid Disrupts the Class IIa HDAC Corepressor Complex

(A) Simulation of Scriptaid docking with the active site of HDAC4. Scriptaid coordinates with the active-site zinc (sphere) and its capping region interacts with F residues (teal) around the rim of the catalytic groove.

(B) Fluorescence anisotropy of WT and gain-of-function (GOF) mutant (H976Y) HDAC4 and a peptide corresponding to amino acids 1,450–1,469 of SMRT in the absence or presence of 2 M excess Scriptaid. Dissociation constants are given in micromolar.

(C) Histone 3 lysine 9 acetylation (H3K9ac) in the EDL muscle at 0, 1, 2, 4, 6, and 8 hr after a single administration of 1 mg/kg body weight of Scriptaid via intraperitoneal (i.p) injection.

(D–F) Coimmunoprecipitation of the class IIa HDAC corepressor complex (D); MEF2-associated SMRT (n = 8 per group) (E); and MEF2 DNA binding in EDL muscles of mice administered vehicle (5% DMSO in 1× PBS) or 1 mg/kg Scriptaid (F).

Data are mean ± SEM. *Versus vehicle group.

gene expression alterations (Figure S3D), suggesting that Scriptaid exerts many of its effects on skeletal muscle transcription through HDAC4 and 5. This was assessed directly by comparing the effects of Scriptaid on the expression of a selection of metabolic genes in control and EDL muscles expressing DN HDAC4 and 5. Scriptaid tended to increase all genes examined in control muscles and significantly increased *SDHb*, *ATP5d*, *CPT-1b*, and *PPARδ* (Figure 4C). In contrast, Scriptaid either did not alter or decreased these same genes in muscles in which DN HDAC4 and 5 were expressed (Figure 4C). Together, these data provide evidence that Scriptaid regulates a network of metabolic genes in vivo, which is dependent on normal class IIa HDAC function.

To assess whether chronic Scriptaid administration enhances functional capacity and metabolism in vivo, mice were treated with 1 mg/kg Scriptaid via daily i.p. injection for 4 weeks. Scriptaid treatment did not alter body weight (Figure S4A) or composition (Figures S4B and S4C) but did increase food intake (Figure S4D). Consistent with Scriptaid-mimicking aspects of the exercise-adaptive response, Scriptaid-treated mice had a greater time to fatigue than vehicle-treated mice in a treadmill-based incremental exercise test (Figure 4D). Consistent with in vitro observations, Scriptaid invoked a small but significant increase in whole-body energy expenditure (Figure 4E) and lipid oxidation (Figure 4F). There was no effect on carbohydrate oxidation (Figure 4G) or voluntary activity (Figure S4E). Respiration analyses were performed to determine the tissues contributing to these effects. Scriptaid administration increased ADP-stimulated respiration in EDL muscle (Figure 4H), which was associated with greater respiration linked to ATP production, but not uncoupled respiration (Figure 4I). Scriptaid administration had no effect on respiration in liver (Figure S4F) or white adipose tissue (Figure S4G), although a role for brown fat could not be excluded, which tended to be higher in Scriptaid-treated animals, but was not significantly different (Figure S4H). Together with enhanced exercise capacity, these data suggest that skeletal muscle is the major tissue for Scriptaid action. Scriptaid had limited effects on skeletal muscle expression of metabolic genes when assessed 24 hr after the last administration (Figure S4I), but it did increase PGC-1α, HSL (Figure S4J), and mitochondrial complex protein abundance (Figure 4J). Scriptaid reduced a number of plasma lipids, including specific species of dihydroceramides, ceramides, sphingomyelins, and

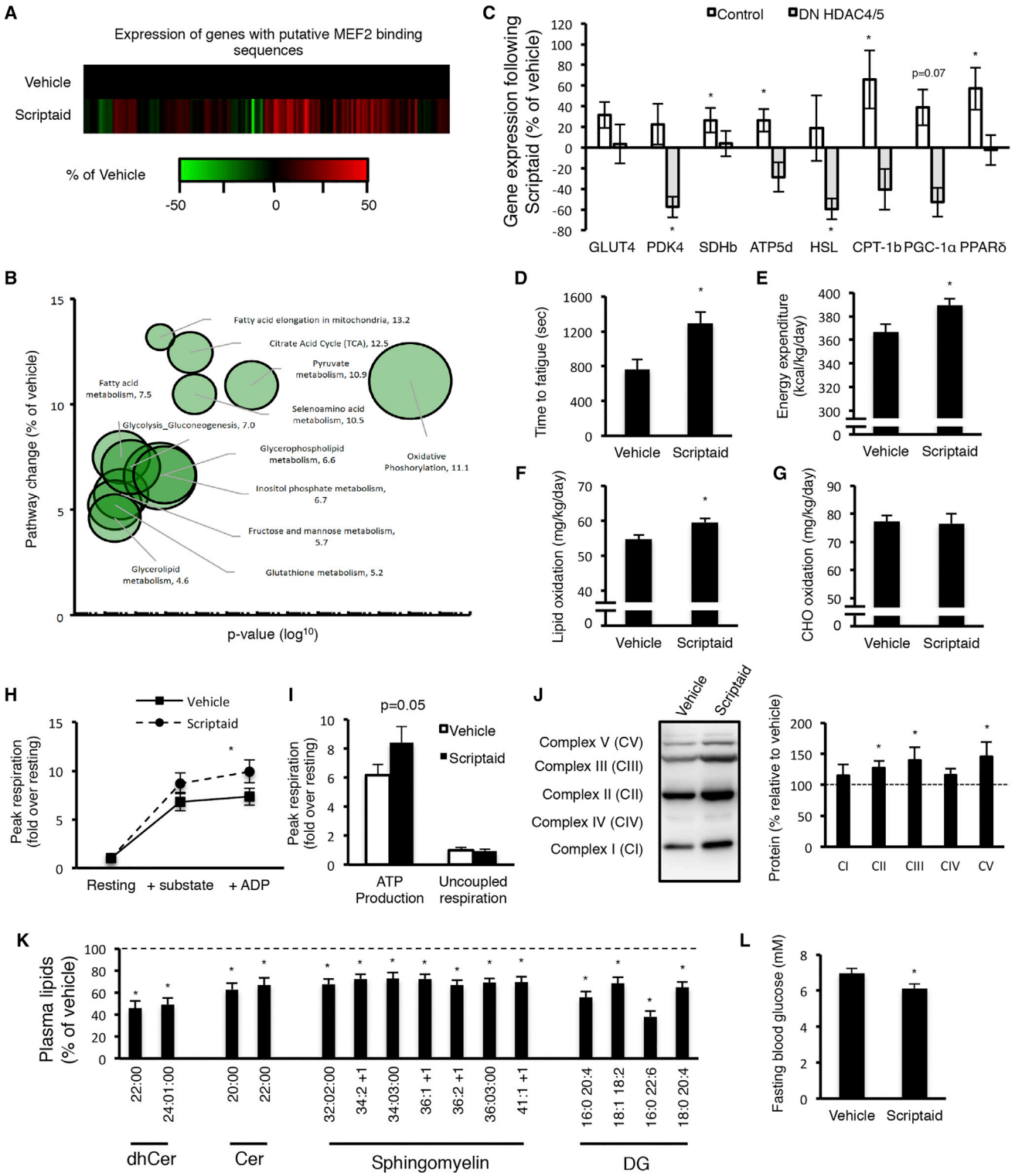

Figure 4. The Effects of Scriptaid In Vivo

(A–C) Expression of genes with putative MEF2 promoter sequences (A); bubble-plot representation of GSEA of significantly altered metabolic pathways (B); and expression of metabolic genes 4 hr after 1 mg/kg body weight Scriptaid administration in EDL muscles administered empty rAAV6 (Control) or rAAV6 DN HDAC4 and rAAV6 DN HDAC5 (n = 6 per group) (C).

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diacylglycerides (Figure 4K), in addition to total ceramides and total dihydroceramides (Figures S4N and S4O). Scriptaid also reduced fasting blood glucose levels (Figure 4L) in the absence of changes in circulating insulin (Figure S4P). Importantly, there were no adverse effects on cardiac morphology in Scriptaid-treated mice (Figure S4Q). These data show that chronic Scriptaid administration induces metabolic adaptations in vivo, including enhanced whole-body energy expenditure and lipid oxidation and reduced plasma lipids and glucose.

DISCUSSION

This study has used previous observations on the molecular responses to exercise to validate the class IIa HDACs as effective targets to pharmacologically manipulate aspects of the exercise adaptive response. Disruption of the class IIa HDAC corepressor complex through genetic or pharmacological strategies increased the expression of exercise-responsive genes in skeletal muscle, enhanced exercise performance, increased whole-body energy expenditure and fatty acid oxidation, and reduced blood lipids and glucose. Although an increase in energy expenditure was observed in mice treated with Scriptaid, no alterations in body composition were observed, due to a small increase in food intake. Nonetheless, Scriptaid treatment reduced the levels of a number of plasma lipids that have been linked to obesity and type 2 diabetes (Meikle et al., 2013), cardiovascular diseases (Fernandez et al., 2013), and non-alcoholic fatty liver disease (Gorden et al., 2015). The broad-spectrum inhibitory profile of this compound toward a number of HDAC isoforms would likely preclude its use in chronic disease states. Nonetheless, these studies provide the impetus for the development of compounds to specifically target the class IIa HDACs and/or its corepressor complex in metabolic diseases.

In conclusion, this study has validated the class IIa HDACs as targets to pharmacologically enhance skeletal muscle metabolic adaptations, including enhanced functional capacity, energy expenditure, and lipid oxidation. This approach could be a useful prevention strategy to enhance metabolic health in chronic diseases.

EXPERIMENTAL PROCEDURES

Mouse HDAC4 and HDAC5 cDNA were sub-cloned into an AAV expression plasmid. AAV6 pseudotyped vectors containing the muscle-specific CK6 promoter (Gregorevic et al., 2004) were packaged, purified, and titered as previously described (Winbanks et al., 2013). MC1568 was synthesized using previously described procedures (Fleming et al., 2014). Scriptaid and TSA were purchased from Santa Cruz Biotechnology and Sigma-Aldrich, respectively. All animal experiments were approved by the Deakin University Animal Welfare Committee, which is subject to the Australian Code for the Responsible Conduct of Research. Male, 8-week-old C57BL6 mice were obtained from the Animal Resource Centre in Western Australia. For AAV experiments, mice were anesthetized with isoflurane before intramuscular injection of rAAV6 vectors expressing D832N HDAC4 and D861N HDAC5 into the anterior and posterior compartments of the hindlimb targeting the soleus, TA, and EDL muscles. Each 30-μl injection contained 2.5×10^{10} vector genomes of each mutant HDAC vector diluted in Hank's balanced salt solution (HBSS). Control injections of the contralateral limb included 5.0×10^{10} vector genomes of empty rAAV6 vector in 30 μl of HBSS. Scriptaid and vehicle (5% DMSO in 1× PBS) were administered via i.p. injection. For chronic drug administration studies, mice received single daily i.p. injections of Scriptaid (1 mg/kg body weight) or vehicle (5% DMSO in 1× PBS) in the afternoon (n = 10 per group) for 28 days until they were humanely killed. In the final 14 days of treatment, 24 hr of indirect calorimetry, an incremental exercise test, and body composition analysis by EchoMRI were performed. Mice were fasted overnight prior to cervical dislocation and tissue collection. Bioenergetic and mitochondrial function assays were performed as previously described (Martin et al., 2014; Selathurai et al., 2015). Detailed materials and methods for all other procedures can be found in the Supplemental Information. All data are expressed as means ± SEM. Data normality was assessed using SPSS statistical software. For normally distributed data, differences between groups were assessed with a t test or one-way ANOVA as appropriate, using Minitab statistical software. Specific differences between groups were identified using Tukey post hoc tests. For non-normally distributed data, we performed non-parametric tests using SPSS statistical software. Differences were considered statistically significant where $p < 0.05$.

ACCESSION NUMBERS

The accession number for the microarray dataset reported in this paper is GEO: GSE54642.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and four figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.08.005.

AUTHOR CONTRIBUTIONS

M.H. and S.L.M. conceived the research. V.G., T.C., K.R.W., M.H., and S.L.M. designed experiments. V.G., T.C., A.S., C.R.B., D.C.H., S.D.M., S.T.B., K.A.M., L.K.-B., T.D.A., M.W., L.S.P.W., D.C., and G.M.H. performed experiments and analyzed data. C.F., J.W.R.S., K.B., M.A.F., P.G., and F.M.P. provided technical expertise and reagents. V.G., T.C., and S.L.M. wrote the manuscript. All authors edited and approved the manuscript.

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(D–L) Incremental treadmill test time to fatigue (D); whole-body energy expenditure (E); lipid oxidation (F); carbohydrate (CHO) oxidation (G); substrate (malate, succinate glutamate) and ADP-stimulated respiration in EDL muscle (H); ATP-linked and uncoupled respiration in EDL muscle (I); oxidative phosphorylation complex subunit protein in EDL muscle (J); fasting (16 hr) plasma lipids (K); and fasting (16 hr) blood glucose in mice treated with 1 mg/kg body weight Scriptaid or vehicle (5% DMSO in 1× PBS) via daily i.p. injection (n = 9–10 per group) (L).
Data are means ± SEM. *Versus vehicle-treated group.

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