Glucose Metabolism Drives Histone Acetylation Landscape Transitions that Dictate Muscle Stem Cell Function

Graphical Abstract

Highlights

- Histone acetylation levels change with muscle stem cell states during regeneration
- Mitochondrial glucose utilization determines overall histone acetylation levels
- PDH activity controls histone acetylation and myogenic differentiation potential
- PDK2 and PDK4 are required for skeletal muscle regeneration in vivo

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In Brief

Yucel et al. identify a link between stem cells’ metabolism and their fate and function. Mitochondrial glucose utilization determines remodeling of the histone acetylation landscape of muscle stem cells during tissue regeneration. Pyruvate dehydrogenase (PDH) is a pivotal control point for this and determines the differentiation potential of myogenic progenitors.
Glucose Metabolism Drives Histone Acetylation Landscape Transitions that Dictate Muscle Stem Cell Function

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SUMMARY

The impact of glucose metabolism on muscle regeneration remains unresolved. We identify glucose metabolism as a crucial driver of histone acetylation and myogenic cell fate. We use single-cell mass cytometry (CyTOF) and flow cytometry to characterize the histone acetylation and metabolic states of quiescent, activated, and differentiating muscle stem cells (MuSCs). We find glucose is dispensable for mitochondrial respiration in proliferating MuSCs, so that glucose becomes available for maintaining high histone acetylation via acetyl-CoA. Conversely, quiescent and differentiating MuSCs increase glucose utilization for respiration and have consequently reduced acetylation. Pyruvate dehydrogenase (PDH) activity serves as a rheostat for histone acetylation and must be controlled for muscle regeneration. Increased PDH activity in proliferation increases histone acetylation and chromatin accessibility at genes that must be silenced for differentiation to proceed, and thus promotes self-renewal. These results highlight metabolism as a determinant of MuSC histone acetylation, fate, and function during muscle regeneration.

INTRODUCTION

Skeletal muscle is a central metabolic organ. It comprises ~40% of body mass in mammals, 50%–75% of all protein, accounts for ~30% of the resting metabolic rate, and serves as the primary site (~80%) of post-prandial glucose uptake (Rasmussen and Phillips, 2003; Zurlo et al., 1990). Despite the role of muscle in glucose homeostasis, little is known about the impact of glucose metabolism on muscle regeneration.

Muscle stem cells (MuSCs), or satellite cells, are critical for the regeneration of skeletal muscle, and for maintaining muscle mass in adult tissues (Brack et al., 2005; Collins et al., 2005; Fry et al., 2015; Lepper et al., 2011; Sambasivan et al., 2011; Tanaka et al., 2009). In homeostasis, MuSCs are largely quiescent and characterized by the expression of the transcription factor PAX7 (Seale et al., 2000; von Maltzahn et al., 2013). Upon injury, MuSCs are activated, and transiently expand as proliferating MuSCs (or myoblasts), which express the transcription factor MYOD1 (Cooper et al., 1999; Megeney et al., 1996). As regeneration completes, MuSCs exit cell cycle to become differentiating progenitors characterized by the transcription factor Myogenin (MYOG) (Wright et al., 1989). Differentiating progenitors fuse to form new myofibers that express mature myosin heavy-chain isoforms (Silberstein et al., 1986). Intrinsic and extrinsic regulators of myogenesis during adult muscle regeneration have been identified, including biophysical, extracellular matrix, and molecular signaling factors. However, our understanding of the metabolic cues that regulate MuSC function during regeneration remains limited (Cerletti et al., 2012; Paia et al., 2018; Ryall et al., 2015; Tang and Rando, 2014). Here, we identify glucose metabolism as a crucial mediator of epigenetic landscape during MuSC fate transitions.

Epigenetic landscape, or the pattern of histone modifications that dictates DNA accessibility, is a determinant of cell identity. Of these modifications, histone lysine acetylation marks open chromatin and active gene expression. It is dynamically regulated by histone acetyl-transferases (HATs) and histone deacetylases (HDACs), as well as availability of acetyl-CoA, the eponymous substrate for HATs (Choudhary et al., 2014; Mousaieff et al., 2015; Wellen et al., 2009). Glucose has been shown to be a major source for histone acetylation (Evertts et al., 2013; Wellen et al., 2009), via the pyruvate dehydrogenase complex (PDH) in the mitochondria, which converts pyruvate to acetyl-CoA. Acetyl-CoA is then converted to citrate by citrate synthase (CS), which can be used in the tricarboxylic acid (TCA) cycle. Unused citrate can be transported out of the mitochondria and diffuse into the nucleus, where it is cleaved by ATP-citrate lyase (ACLY) to reform acetyl-CoA, which is used to acetylate histones. Consequently, glucose metabolism via PDH and ACLY is a regulator of the histone acetylation state.

Here, we use mass cytometry (CyTOF) (Bendall et al., 2011; Porpiglia et al., 2017) and flow cytometry to identify distinct...
Figure 1. Histone Acetylation Dynamics during Regeneration Revealed by CyTOF

(A) Visualization of t-distributed stochastic neighbor embedding (viSNE) plot of uninjured and injured (D6 after notexin injection) muscle. Sca1/CD31/CD11b/CD45^a7-integrin^ cells were clustered based on PAX7, MYOD1, and MYOG (Myogenin). viSNE plots of combined biological replicates (n = 3) for each time point shown. Color scale shows CD34, PAX7, MYOD1, or MYOG normalized expression. Significance determined using one-way ANOVA with Tukey’s test for multiple comparisons across samples (injured and uninjured), and repeated-measures ANOVA was used for comparisons between Activated or Self-Renewing Stem and Differentiated subsets within biological samples. Subsets outlined are as follows: (a) quiescent PAX7^+CD34^+MYOD1^+MYOG^ MuSCs (D0), gray; (b) self-renewing PAX7^+CD34^-MYOD1^-MYOG^- MuSCs (D6), black; (c) activated PAX7^+MYOD1^+MuSCs (D6), blue; and (d) differentiating PAX7^-MYOD1^-MYOG^- progenitors (D6 injured), red. (B) Expression of PAX7, CD34, MYOD1, and MYOG in subsets in (A).

(C) IdU incorporation subsets in (A).

(D) Histone acetylation normalized for total histone H3 content in subsets outlined in (A).

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histone acetylation states characteristic of quiescence, proliferation, and differentiation of MuSCs. We characterize distinct histone acetylation states in conjunction with metabolic signatures that mark these MuSC states. We show that low utilization of glucose for mitochondrial respiration in proliferating MuSCs leads to increased available acetyl-CoA and, in turn, elevated histone acetylation. In this state, PDH acts as a metabolic rheostat for glucose-derived histone acetylation. During differentiation, mitochondrial remodeling and increased reliance on glucose to support respiration restricts glucose-derived histone acetylation, resulting in a decreased histone acetylation that precedes MYOG. Increasing PDH activity results in elevation of histone acetylation and reduced differentiation capacity both in vitro and in vivo. Finally, we use chromatin immunoprecipitation (ChIP) and assay for transposable accessible chromatin (ATAC) followed by sequencing to characterize the effects of glucose-derived acetyl-CoA on the epigenetic landscape. We find that glucose-derived histone acetylation regulates chromatin accessibility at genes that must be turned off for differentiation. Thus, we identify metabolic regulation of histone acetylation state as a crucial component of myogenesis that acts in coordination with the canonical myogenic program.

**RESULTS**

**Single-Cell Analysis Reveals Distinct Histone Acetylation States during Regeneration**

We sought to identify global changes in histone acetylation in MuSCs during regeneration. To understand cellular heterogeneity, we analyzed histone acetylation state in single cells by CyTOF. CyTOF enables simultaneous analysis of MuSCs and progenitor cells during muscle regeneration (Porpiglia et al., 2017) concurrent with a range of well-studied histone acetylation marks (H3K9, H3K18, H4K16, and H2BK5) (Feller et al., 2015; Young et al., 2009). Tibialis anterior (TA) and gastrocnemius (GAS) muscles were collected in uninjured animals or 6 days following injury by notexin injection (Figures 1A–1D). MuSCs and progenitors were gated for absence of blood and endothelial cell lineage markers (Lin−; Sca1, CD11b, CD45, CD31) and the presence of α7-integrin* (Figure S1A), as well as expression of the quiescence marker CD34, and transcription factors Pax7, Myod1, or Myog. Myogenic cells were classified as (1) quiescent Pax7+/CD34−/MYOD1−, (2) self-renewing Pax7+/CD34+/MYOD1−, (3) activated Pax7+/CD34+/MYOD1+, (4) differentiating Pax7+/MYOD1−/MYOG+ progenitors from injured samples (Figures 1A, 1B, and S1B). Self-renewing Pax7+/MYOD1−/MYOG+ MuSCs have been shown to repopulate the stem cell pool, while committed activated MuSCs that express Myod1 go on to differentiate (Shea et al., 2010). All injured populations had ~15%–25% iododeoxyuridine (IdU) incorporation, while quiescent MuSCs were IdU negative, suggesting that self-renewing Pax7+/CD34+/MYOD1−/MYOG+ MuSCs are distinct from quiescent MuSCs in resting muscle (Figure 1C).

We identified distinct histone acetylation states in MuSCs in uninjured and injured tissue. Activated Pax7+/MYOD1−/MYOG+ MuSCs, from injured samples, had elevated acetylation on H3K9, H4K16, and H2BK5 compared to uninjured quiescent Pax7+/CD34−/MYOD1− MuSCs (Figures 1C and 1D). In contrast, Pax7+/MYOD1−/MYOG+ differentiating progenitors had reduced acetylation on all marks (H3K9, H3K18, H4K16, and H2BK5) compared to Pax7+/MYOD1−/MYOG+ activated MuSCs. These histone acetylation patterns were also observed by flow cytometric analysis, using CD34 and Myog as markers to identify quiescent, activated, and differentiating subsets (Figures 1E, 1F, S1C, and S1D). Qualitatively, acetylation changes were apparent by immunofluorescence of single quiescent (Pax7+/MYOD1−), activated (Pax7+/MYOD1+) MuSCs, and differentiating progenitors (Pax7+/MYOD1+ or MYOD1+/MYOG+) on freshly isolated and cultured myofibers (Figures 1G and S2A–S2C). Notably, by immunofluorescence, histone acetylation was barely perceptible in quiescent MuSCs, except for H3K18. This increased baseline could explain why for H3K18 acetylation is not further increased with activation (Figure S2B). These data demonstrate that changes in histone acetylation state accompany transitions in myogenic stem cell states in vitro and in vivo.

**General Reduction in Histone Acetylation during Early Myogenic Differentiation**

We were struck by the reduction in histone acetylation in Myog+ progenitors. This decrease in histone acetylation agrees with previous chromatin immunoprecipitation sequencing (ChiP-seq) studies, which have shown loss of acetylation on histones H3K9, H3K18, H4K12, and H3K27 in fully differentiated myotubes (Asp et al., 2011; Blum and Dynlacht, 2013). However, studies have also shown that histone acetylation of myogenic genes increases during differentiation (Francetic et al., 2012; Hamed et al., 2013; Iezzi et al., 2002, 2004). Therefore, we sought to reconcile and characterize the interplay between these two phenomena.

To eliminate confounding effects of serum withdrawal, we analyzed primary myoblasts, i.e., MuSCs isolated by fluorescence-activated cell sorting (FACS), cultured to confluency in high serum growth media (GM), which induces differentiation in a subset of cells. Using flow cytometry, we compared histone acetylation in Myog− versus Myog+ myoblasts in these heterogenous cultures (Figures 2A and S3A). We also assayed histone acetylation in undifferentiated, sub-confluent myoblasts in GM versus confluent myoblasts after induction of differentiation for 24 h in low serum differentiation media (DM). After 24 h in DM, 30%–60% of myoblasts were positive for Myog+ (Figures S3B
Figure 2. Histone Acetylation Is Reduced in MYOG+ Progenitors, and upon Induction of Myogenic Differentiation by Serum Removal, but Spares the Myogenic Program

A) Representative histogram (MYOG) and contour plot (H3K18ac versus MYOG) of confluent, heterogeneously differentiated myoblasts in GM (~15%–30% MYOG+). MYOG+ and MYOG gates are in red and black.

B) Normalized histone acetylation or total histone H3 intensity for MYOG versus MYOG+ myoblasts within same samples. n = 3–4 biological replicates are shown. Student’s paired t test was used for comparisons.

C) Histone acetylation in sub-confluent myoblasts in GM versus 24 h in DM. The percentage of MYOG+ cells in GM and DM was <5% and 40%–60%, respectively (Figures S3B and S3C). n = 3–4 biological replicates. Significance determined using one-way ANOVA with Tukey’s test for multiple comparisons across samples (GM and DM), and Student’s paired t test was used for comparisons between MYOG and MYOG+ cells within biological samples.

D) Histone acetylation in GM and 24-h DM myoblasts by LC-MS/MS. Lysine acetylation calculated as a fraction relative to each histone peptide (relative abundance). Histone peptides analyzed from the following, left to right: H3 9–17; H3 18–26; H4 4–17; and H2A 1–11. n = 3 biological replicates were run on a mass spectrometer three independent times. For statistical comparisons, two-way ANOVA was used with matching for experimental runs.

E) Enrichment of H3K9/14 acetylation across the genome for a 2-kbp window around transcriptional start sites (TSSs). ChIP-sequencing and gene expression data used from the Mouse ENCODE Consortium (GSE36023).
and S3C). Histone acetylation marks were examined by flow cytometry in MYOG+ and MYOG− cells (Figure 2B). In agreement with analyses of regenerating muscle, we observed reduced acetylation of all marks in MYOG+ compared to MYOG− cells. Similarly, myoblasts differentiated in DM for 24 h had reduced histone acetylation compared to those in GM. Notably, after 24 h in DM, both MYOG+ and MYOG− myoblasts had reduced histone acetylation compared to cells in GM (Figure 2C). These data demonstrate that loss of histone acetylation is associated with early myogenic differentiation and precedes MYOG expression.

In order to quantitatively measure histone acetylation without antibody bias, we performed liquid chromatography mass spectrometry (LC-MS/MS) to detect histone acetylation in subconfluent myoblasts in GM versus early differentiating myoblasts in DM (24 h). Acetylation was detected as relative abundance, or the fraction of each histone peptide that contained one or more acetylated lysine residues. Acetylation was quantified for peptides corresponding to histone 3 lysines 9 and 14 (H3K9/14), histone 3 lysines 18 and 23 (H3K18/23), histone 4 lysines 5, 8, 12, and 16 (H4K5/8/12/16), and histone 2 variant A.1 lysine 5 and 9 (H2A.1K5/9), respectively. Notably, acetylation of histone 2B, as well as histone H3 lysine 27 and lysine 56, was not detected at a reliable rate by this assay and was not included in subsequent analysis. We observed a decrease in mono acetylation at H3K9/14 (~27.5%), H3K18/23 (~12.8%), and H4K5/8/12/16 (~5%; p = 0.0659) (Figure 2D). In addition, we identified a decrease in the acetylation of H2A.1K5 (~37%) and the di- and tri-acetylated state of H4K5/8/12/16 (~12.6% and ~10.4%, respectively). In agreement with our antibody-based findings by CyTOF and flow cytometry, these results confirmed that histone acetylation loss at these lysine residues is occurring independent of histone tail cleavage or changes in histone variants.

Site-Specific Histone Acetylation Changes
Histone acetylation is generally considered a mark of open chromatin that occurs at the enhancers or transcriptional start sites (TSSs) of transcriptionally active or poised genes (Taylor et al., 2013; Wang et al., 2008). We sought to reconcile the global loss of histone acetylation with the known increase in histone acetylation at the promoters of myogenic genes during differentiation. To this end, we analyzed available ChIP-sequencing data of H3K9/14 in C2C12 myoblasts and 24 h after induction of differentiation in DM (ENCODE, GSE36023) and H3K9, H4K12, and H3K27 acetylation in C2C12 myoblasts versus myotubes (differentiated 7 days) (Asp et al., 2011; Blum and Dynlacht, 2013). Analysis of ENCODE data showed a global decrease in acetylation occurs at 24 h of differentiation in agreement with our findings (Figure 2E). In all data, this decrease occurs not only on genes that decrease expression, as expected, but also on average over genes that do not change, or those that increase expression. Analysis of these ChIP-sequencing data showed that, in total, 8,963 genes have associated ChIP peaks with decreased acetylation on one or more marks following differentiation. Genes with decreased histone acetylation on any one mark were enriched (1.3- to 1.94-fold) for chromatin remodeling, cell cycle, and metabolic processes. In contrast, increased acetylation was much more restricted. Only 1,987 genes had increased acetylation on one or more mark, and an even fewer 105 genes had associated peaks that showed increased acetylation on three or more marks (Figure 2F). Genes associated with peaks that increased histone acetylation were strongly enriched (up to 14-fold) for muscle specification and development, including myogenic differentiation factors Myog, Mef2c, and various Myh genes. We confirmed these results by ChIP-qPCR of H3K9/14 for a subset of genes in primary myoblasts 24 h after induction of differentiation (Figure S3D). Although on average histone acetylation was reduced, gain or loss of histone acetylation was concordant with changes in mRNA expression for a number of genes (Figure S3E). Overall, these data show that the histone acetylation landscape becomes specified early in differentiation (Cao et al., 2006, 2010; Dilworth et al., 2004; Puri et al., 1997b).

To determine whether histone acetylation loss is directed by specific transcription factors, we performed promoter motif analysis. Remarkably, genes associated with decreased histone acetylation showed no significant motif enrichment (Figure 2G). The lack of enriched motifs diminishes the likelihood that a particular transcription or chromatin-modifying factor coordinates acetylation reduction. In contrast, promoter regions of genes associated with increased histone acetylation were highly enriched for MADS and E-Box motifs, which are bound by the MEF2 transcription factor family, and myogenic regulatory factors (MYF5, MYOD1, and MYOG), respectively (Bergstrom et al., 2002). This agrees with studies showing that MYOD1 associates with HATs p300 and CBP to activate transcription of myogenic targets (Polesskaya et al., 2001; Puri et al., 1997a). These data indicate that general reduction of histone acetylation occurs in conjunction with the gain of acetylation on myogenic genes. Given the lack of motif enrichment, we hypothesized that the histone acetylation reduction likely results from a broader change in cell state during differentiation.

Metabolic Alterations Characterize Distinct Myogenic Cell States
We postulated that changes in glucose metabolism could mediate the global reduction in histone acetylation during differentiation. Glucose-derived acetyl-CoA is known to be a source of histone acetylation, via PDH and ACLY (Evertts et al., 2013;
Wellen et al., 2009) (Figure 3A). Labeling experiments have shown that histone acetylation turnover is highly dynamic (Zheng et al., 2013); therefore, we hypothesized that decreased availability of acetyl-CoA could result in reduced histone acetylation over a large range of genes and marks.

To identify changes in glucose metabolism, we compared the transcriptional profile of quiescent (freshly isolated), proliferating (2-day cultured), and early differentiating myoblasts. Analysis was paired with published microarray datasets of myoblast differentiation (GSE24811) (Soleimani et al., 2012). Activated and proliferating MuSCs exhibited a >2.5-fold upregulation of genes in anaerobic ATP generation and in lactate production (Pgaml, Pgm1, Pklr, Ldha) (Figure 3B; Table S2). In early differentiation, however, this expression pattern reversed. The expression of the rate-limiting enzyme Pklm increased, while lower glycolysis genes decreased or stayed the same. In addition, expression of Pdha1 increased, as did the mitochondrial pyruvate transporters Mpc1 and Mpc2. These changes suggest reciprocal changes in glucose metabolism upon both MuSC activation and differentiation.

**Early Myogenic Differentiation Is Marked by Metabolic Dysfunction and Glucose Reliance**

We hypothesized that myoblasts increase glucose utilization for respiration during differentiation. We used Seahorse mitochondrial and glycoolytic stress tests to assay the metabolic profile in growing (in GM) or early differentiating myoblasts (after 24-h DM). To assay the contribution of glucose to respiration, mitochondrial stress tests were performed in glucose (25 mM) and glucose-free media conditions (Figures 3C–3F), as well as the presence of pyruvate, 5 mM glucose, and combinations of glucose and pyruvate (Figures S4A–S4D) to control for the different concentrations in GM and DM.

We observed that myoblasts in GM did not require glucose for basal oxygen consumption or ATP-linked respiration (Figures 3C, 3D, and S4A–S4D). In glucose-free conditions, however, cells lacked spare capacity. This suggests that while myoblasts are capable of utilizing glucose for mitochondrial respiration under metabolic stress (i.e., FCCP), non-glucose sources fuel basal respiration. Compared to myoblasts in GM, however, differentiating myoblasts had a striking loss of respiratory capacity (Figures 3C, 3D, and S4A–S4D). Loss of mitochondrial function is consistent with the mitochondrial remodeling during reported during early differentiation (Kim et al., 2013; Sin et al., 2016). Early differentiating myoblasts had reduced Mitotracker Red staining, indicative of reduced membrane potential, and increased expression of FIS1, a marker of mitofission (Figures S4E and S4F). Glycolysis and glycoalytic capacity were also reduced in differentiation (Figures 3E and 3F). Importantly, while glucose was not required for basal respiration or ATP production of proliferating myoblasts, both metrics were significantly reduced in early differentiating myoblasts without glucose. Spare capacity was almost completely absent in differentiating myoblasts, indicating that mitochondria are respiring at their maximum capacity.

PDH is a key regulator of mitochondrial utilization of glucose. Its activity is inhibited by pyruvate dehydrogenase kinases (PDKs) through reversible phosphorylation (Patel et al., 2014). Therefore, we sought to determine PDH activity at various stages of MuSC progression by assaying phosphorylation. Increased phosphorylated PDH (pPDH) was apparent in MuSCs on cultured single myofibers compared to freshly isolated fibers (Figures 3G and S4H). Using flow cytometry to measure pPDH and total PDH, we observed that pPDH ratio was increased ~5-fold in proliferating, cultured MuSCs compared to those freshly isolated (Figure 3H). Consistent with expression data and the increase in mitochondrial glucose utilization observed by Seahorse, early differentiating MYOG⁺ progenitors had reduced pPDH (Figure 3I). We validated this finding in regenerating muscles in vivo by CyTOF, which confirmed an increase in pPDH/PDH ratio specifically in PAX7⁺ MuSCs in injured tissue compared to uninjured, followed by a decrease in MYOG⁺ differentiating progenitors (Figure 3J). Together, these data confirm our hypothesis that altered mitochondrial glucose utilization occurs upon MuSC activation and differentiation.

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**Figure 3. Myogenic Fate Corresponds to Distinct Glycolytic States**

(A) Flux from glucose to histone acetylation.

(B) Expression of glycolytic genes in freshly isolated and 2-day hydrogel cultured MuSCs and primary myoblasts grown on collagen-coated plastic. Color scale shows Log2Fold compared to mean TPM (transcripts per million) of fresh MuSCs (left) or proliferating myoblasts (right). Genes in orange indicate ATP-generating phase/lactate-producing stage of glycolysis. Right, Log2Fold change shown for MuSC-derived myoblasts versus day 2 versus day 5 of induced differentiation of published microarray data (GSE24811; Soleimani et al., 2012). For statistical comparisons of all replicates of RNA-seq data, see Table S2.

(C–F) Bioenergetic profile of confluent primary myoblasts in growth media (GM) or after 24 h in differentiation media (DM). Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were measured using a Seahorse XF96 extracellular flux analyzer using the Mitochondrial Stress or Glycolytic Stress kits. n = 5–8 biological replicates. One-way ANOVA with Tukey’s test for multiple comparisons used.

(C) Oxygen consumption profile for GM (15% FBS) or 24-h DM (5% horse serum) myoblasts with or without 25 mM glucose.

(D) Basal respiration, ATP production, maximal respiration, and spare capacity for GM and DM = 25 mM glucose.

(E) Glycolytic stress profile for GM versus 24-h DM.

(F) Glycolytic rate, capacity, and reserve for GM and DM myoblasts.

(G) Immunostaining for pPDH, PAX7, and MYOD1 on isolated single fibers from soleus fixed immediately after isolation, or after 48 h in culture in GM.

(H) pPDH/PDH by flow cytometry for freshly isolated versus 48-h cultured MuSCs on hydrogels. n = 4 independent experiments. Student’s paired t test was used for statistical comparison.

(I) Quantification of pPDH/PDH for primary myoblasts in GM versus 24-h DM. n = 3 independent experiments. Student’s paired t test was used for statistical comparisons.

(J) pPDH/PDH of quiescent, self-renewing, and activated (MYOD⁺ and MYOD⁺) MuSCs, and MYOG⁺ differentiating progenitors CyTOF (as characterized in Figure 1). n = 3 biological replicates, one-way ANOVA with Tukey’s test for multiple comparisons used for comparisons against quiescent MuSCs, and repeated-measures ANOVA used to compared activated versus differentiated subsets within biological replicates.

Data are represented as mean ± SEM (“p < 0.05, “p < 0.01, and ***p < 0.001). See also Figure S4 and Table S2.
Figure 4. Glucose Flux toward Histone Acetylation Decreases upon Myogenic Differentiation

(A) Scheme of $^{13}$C glucose labeling. Sub-confluent primary myoblasts in GM were switched to GM or GM + DCA (10 mM) or DM for 6 h. In parallel, 24-h differentiated, confluent myoblasts were switched to labeled DM for 6 h. $^{13}$C glucose was added to a final concentration of 25 mM. n = 3 biological replicates used for each condition.

(B) Quantification of $^{13}$C incorporation by LC-MS/MS as outlined in (A). Incorporation of labeled glucose quantified as fraction of labeled (+2-Da shift) over total acetylation (labeled + unlabeled) for each mark. Incorporation on singly acetylated lysines was analyzed: H3 K9–17; H3 K18–26; H4 K4–17; and H2A K4–11. n = 3 biological replicates were run on a mass spectrometer three independent times. For comparisons, two-way ANOVA was used with matching for each experimental run.

(C) H3K9, H3K18, H4K16, H2bK5, H3K27, and H3K56 acetylation of proliferating myoblasts after 24-h DCA, normalized for untreated controls. n = 3 biological replicates; statistics calculated by Student’s paired t test.

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Glucose Metabolism Regulates Histone Acetylation State and Myogenic Differentiation

We used $^{13}$C$_6$-D-glucose isotopic labeling to directly measure glucose-derived histone acetylation in proliferation versus differentiation. In addition, we assayed the role of PDH by treating proliferating myoblasts with the PDK inhibitor dichloroacetate (DCA), which increases PDH activity (Stacpoole et al., 1998). Incorporation of labeled acetyl-CoA into histones was quantified by LC-MS/MS following 6 h of incubation with $^{13}$C$_6$-D-glucose media (GM, GM+DCA, or DM). Labeling was quantified as the fraction of labeled acetyl-CoA incorporated. Acetylation of histones in GM and DM was significantly reduced compared to untreated controls, suggesting maintenance of the stem cell state (Figures S5D and S5E). In accordance with the reduction in spontaneous differentiation, treatment of myoblasts with DCA in GM alone was sufficient to reduce the percentage of myoblasts expressing MYOG after 24 h in DM (Figures 4D and 4E). In striking contrast, treatment of myoblasts with DCA in DM alone had no effect on differentiation, suggesting that DCA cannot reverse or prevent MYOG expression following commitment. Finally, at late differentiation (day 4), persistent DCA treatment (GM and DM) significantly reduced the percent of nuclei in MyHC⁺ cells (Figures S5F and S5G). These data show that increased PDH activity in myoblasts, which increases glucose metabolism, Treatment of proliferating myoblasts with FCCP, a mitochondrial uncoupler that stimulates oxygen consumption, also reduced histone acetylation (Figure S8H). These data show that glucose-derived acetyl-CoA is critical for maintaining histone acetylation

Glucose-Derived Histone Acetylation Is a Key Modifier of Myogenic Differentiation

To establish whether glucose-derived histone acetylation regulates MuSC function and myogenic differentiation, we assessed the differentiation capacity of myoblasts following DCA treatment. DCA-treated MuSCs cultured on hydrogels for 7 days retained PAX7 expression and gave rise to fewer MYOG⁺ cells compared to untreated controls, suggesting maintenance of the stem cell state (Figures S5D and S5E). In accordance with the reduction in spontaneous differentiation, treatment of myoblasts with DCA in GM alone was sufficient to reduce the percentage of myoblasts expressing MYOG after 24 h in DM (Figures 4D and 4E). In striking contrast, treatment of myoblasts with DCA in DM alone had no effect on differentiation, suggesting that DCA cannot reverse or prevent MYOG expression following commitment. Finally, at late differentiation (day 4), persistent DCA treatment (GM and DM) significantly reduced the percent of nuclei in MyHC⁺ cells (Figures S5F and S5G). These data show that increased PDH activity in myoblasts, which increases glucose metabolism, Treatment of proliferating myoblasts with FCCP, a mitochondrial uncoupler that stimulates oxygen consumption, also reduced histone acetylation (Figure S8H). These data show that glucose-derived acetyl-CoA is critical for maintaining histone acetylation

(D) Effect of DCA on differentiation. Cells were untreated (−/−), treated with DCA only in DM (−/+), treated in GM but not DM (+/−), or treated with DCA in both GM and DM (+/+/). Differentiation quantified as percentage positive for MYOG. n = 5–6 biological replicates. (E) Histogram of MYOG in myoblasts differentiated for 24 h following DCA treatment in GM, DM, or both (−/−, −/+), (+/−, +/+/). MYOG⁺ gate in green. (F) Contour plot of MYOG versus H3K9/14ac following 72-h treatment with scrambled siRNA or siAcly. MYOG⁺ gate outlined. (G) H3K9/14 histone acetylation following siAcly or SiScramble. DCA or vehicle added after 24-h knockdown and treatment continued for 48 h, for a total of 72-h siRNA treatment. n = 3. (H) MYOG⁺ quantification following siAcly or SiScramble, +/− DCA as described in (G). n = 3. (I) H3K9/14 acetylation following siCs or SiScramble, +/− DCA by flow cytometry. n = 3. (J) MYOG⁺ quantification following siCs or SiScramble, +/− DCA. n = 3. (K) H3K9/14 histone acetylation following DMSO (vehicle) or 20 µM Mdivi-1 treatment of myoblasts in GM or DM for 24 h. (L) Histogram, MYOG⁺ after 24-h treatment with DMSO or 20 µM Mdivi-1 in GM or DM. (M) MYOG⁺ quantification of myoblasts in GM or DM treated with DMSO or Mdivi-1 for 24 h. n = 3. Data are represented as mean ± SEM (p < 0.05, **p < 0.01, and ***p < 0.001). Unless otherwise indicated, significance was calculated by one-way ANOVA with Tukey’s test for multiple comparisons for n = 3 biological replicates. See also Figure S5.
Figure 5. Genomic Characterization of Glucose-Metabolism Mediated Histone Acetylation

(A) ChIP-seq of H3K9/14 acetylation of proliferating myoblasts in GM ± DCA for 24 h. Enrichment shown for a ±1-kb window around TSSs.

(B) Genomic tracks for representative housekeeping (left) and myogenic genes (right).

(C) ChIP-seq signal for peaks in control versus DCA treated myoblasts. The dotted line indicates slope of 1 (x = y).

(D) Gene set enrichment analysis of genes associated with increased ChIP-seq peak signal in DCA versus control myoblasts. Overlap with genes with gain or loss of at least one histone acetylation mark in myotube versus myoblasts (see Figure 2F) shown.

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levels and that overall level of histone acetylation modulates commitment toward differentiation.

Mitochondrial Remodeling Restricts Histone Acetylation during Differentiation

A transient phase of mitosis and mitophagy occurs at the early stages of myogenic differentiation (Sin et al., 2016). We hypothesized that this could contribute to the decrease in histone acetylation. To assay the role of mitochondrial remodeling in histone acetylation, myoblasts were treated with Mdivi-1, a Drp1 inhibitor that prevents mitochondrial fission, in GM or DM (Cassidy-Stone et al., 2008). Mdivi-1 had no effect on histone acetylation or percentage of cells expressing MYOG in GM. However, Mdivi-1 treatment in DM increased histone acetylation and reduced MYOG+ cells after 24 h differentiation (Figures 4K–4M). Intriguingly, histone acetylation in Mdivi-1-treated differentiating progenitors was increased compared to untreated myoblasts in GM. This is likely due to the increased PDH activity observed during early differentiation (Figures 3I and 3J). These findings suggest that mitochondrial remodeling during early differentiation limits glucose-derived histone acetylation, despite increased PDH activity. Overall, these findings confirm that glucose metabolism is a critical determinant of histone acetylation and differentiation capacity, and loss of histone acetylation is mediated by mitochondrial remodeling during early differentiation.

Increased Glucose-Derived Acetylation Reinforces the Epigenetic Landscape of Proliferating Myoblasts

To address how glucose metabolism modifies the epigenetic landscape, we assayed H3K9/14ac by ChIP-sequencing and chromatin accessibility by ATAC-seq following 24-h DCA treatment. On average, DCA-treated cells had significantly increased histone acetylation near TSSs across the genome (Figures 5A–5C), consistent with the observed increase in overall histone acetylation. Increased histone acetylation was primarily seen at promoters regions that already showed detectable H3K14ac (Figures 5B and 5C). Promoters with low signal in control samples, such as at myogenic differentiation genes in GM, had no change or reduced histone acetylation upon DCA treatment. This is consistent with increased substrate levels for existing HAT activity, which results in an overall increase in existing histone acetylation sites, rather than de novo deposition of acetylation marks by an increased activity of a specific transcription factor.

This hypothesis was confirmed by Gene Ontology (GO) analysis of genes associated with histone acetylation changes. Promoter regions (±<2 kb from TSS) with >2-fold increase in histone acetylation following DCA were annotated to 5,136 genes. A comparison with myogenic differentiation showed that about one-half of these genes (2,787) lost histone acetylation on one or more marks (H3K9, H4K12, H4K16, and H3K27) in myotubes (Figure 5D). Genes that both lost histone acetylation in myotubes and gained histone acetylation with DCA were enriched for chromatin regulation, cell cycle, gene expression regulation, and muscle cell proliferation. There was little overlap (283 genes) between genes that gained histone acetylation in myotubes with those that gained histone acetylation with DCA. This is in contrast to the genes that gained histone acetylation during differentiation but did not respond to DCA, which are enriched for muscle-specific GO terms (Figure 5D).

In concordance with H3K9/14ac accumulation at the enhancers or promoters of poised or actively transcribed genes, we found increased expression in genes associated with increased histone acetylation at proximal promoters (±<2 kb from TSS), and to a lesser extent, distal enhancers (−100 kb) (Figure 5E). Overall, these data support that glucose metabolism is rate limiting for histone acetylation, and that PDH activity acts as a rheostat for acetylation of established enhancers and promoters.

We used ATAC-seq to assess chromatin accessibility changes upon DCA treatment. ATAC-seq signal correlates with accessible chromatin and is enriched in enhancers and promoter elements (Buenrostro et al., 2013). Consistent with the hypothesis that PDH-mediated histone acetylation occurs primarily on already acetylated, open chromatin, we observed increased H3K9/14ac ChIP signal at peaks closely associated with ATAC-seq peaks, which was also correlated with the number of associated ATAC-seq peaks (Figures 5F–5H). Furthermore, ATAC-seq signal was increased for a subset of peaks in DCA-treated (Figure 5I) and was associated with an increase in expression of genes with ATAC-seq peaks in their proximal promoters (±<2 kb from TSS) (Figure 5J). Motif analysis of regions that gained accessibility with DCA showed enrichment for consensus sequences of several transcription factors, in particular the core promoter element CCAAT (Figure 5K). This motif is

(E) Average change in expression in absolute transcripts per million (TPM) following DCA treatment. Change in expression of all genes was compared with those with increased ChIP-sequencing peaks following DCA treatment in proximal promoters (±2 kb) or distal enhancer (−100 kb). Statistical comparison was made versus average change in TPM over all genes (0).
(F) Comparison of ChIP-sequencing and ATAC-seq. Chromatin accessibility by ATAC-seq was assayed in myoblasts in GM ± DCA. Change in ChIP-sequencing signal shown as a function of distance to nearest ATAC-seq peak.
(G) Comparison of ChIP-sequencing and ATAC-seq. Chromatin accessibility by ATAC-seq was assayed in myoblasts in GM with or without DCA treatment. Change in ChIP-sequencing signal is shown as a function of number of associated ATAC-seq peaks.
(H) Genomic track of H3K9/14 ChIP-sequencing and ATAC-seq peaks for control and DCA-treated myoblasts.
(I) ATAC-seq signal of peaks in control versus DCA treated myoblasts. The dotted line shows slope of 1 (x = y).
(J) Change in expression in absolute transcripts per million (TPM) following DCA treatment. Expression of all genes was compared with those with increased ATAC-seq peaks following DCA treatment in proximal promoters (±2 kb) or distal enhancer (−100 kb). Statistical comparison made versus average change in TPM over all genes (0).
(K) HOMER motif enrichment analysis of genes that show increased accessibility by ATAC-seq following DCA treatment. Data are represented as mean ± SEM (*p < 0.05, **p < 0.01, and ***p < 0.001). Unless otherwise indicated, significance was calculated by one-way ANOVA with Tukey’s test for multiple comparisons. See also Table S3.
Figure 6. PDK dKO Mice Show Muscle Regeneration Defects and MuSC-Specific Histone Acetylation and Engraftment Capacity

(A) Real-time qPCR of Pdk1-4, Pdha1, and Pax7 expression in cultured MuSCs isolated from WT or dKO animals. n = 3 biological replicates.

(B) Immunohistochemistry for pPDH in WT and dKO animals at D0, or D6 and D12 after injury by notexin injection.

(C) Quantification of Pax7+ cells per area (1 mm²) at D0, D6, and D12. n = 4–6 animals per time point.

(D) Representative image of Pax7+ MuSCs in WT and dKO muscle at D12. DAPI, Laminin co-staining.

(E) H3K18 and H4K16 acetylation by flow cytometry for uninjured (D0) MuSCs (Lin⁻/α7-integrin⁺/CD34⁺/MYOG⁻). Histone acetylation normalized for uninjured WT controls in each experiment. n = 4–10 animals.

(F) H3K18 and H4K16 acetylation for injured (D6) MuSCs (Lin⁻/α7-integrin⁺/CD34⁺/MYOG⁻). Histone acetylation normalized for injured WT controls in each experiment. n = 3–12 animals.

(legend continued on next page)
bound by CCAAT-enhancer binding proteins (C/EBPs), which are known to recruit transcription factors as well as chromatin modifiers (Mink et al., 1997; Nerlov, 2007). C/EBPβ has been shown to have high expression in MuSCs and declines during early differentiation (Marchildon et al., 2012). Overexpression of C/EBPβ has been shown to inhibit myogenic differentiation, while loss of C/EBPβ leads to precocious differentiation (Lalatabbert et al., 2016). These data highlight the role of C/EBPs in preserving the stem/progenitor cell chromatin state. Moreover, they support our hypothesis that PDH activity is a rate-limiting step in histone acetylation and dictates the accessibility around myoblast genes. Accordingly, increasing PDH activity in proliferating myoblasts reinforces histone acetylation and chromatin accessible regions that are already present, delaying progression toward differentiation.

**Dysregulation of PDH In Vivo Results in Aberrant Histone Acetylation and Reduced Skeletal Muscle Regeneration**

To assess the role of PDH in muscle regeneration, we used the Pdk2/Pdk4 double-knockout (dKO) mouse model, which has increased acetyl-CoA due to constitutively active PDH (Jeoung et al., 2012; Rahimi et al., 2014). We confirmed the absence of Pdk2 and Pdk4, and the lack of compensation in proliferating MuSCs by expression of other PDK isoforms (Pdk1 and Pdk3) (Figure 6A), as well as the lack of PDH phosphorylation during regeneration (Figure 6B).

Regeneration and MuSC dynamics were assessed in uninjured TA muscle (D0) and 6 or 12 days (D6 and D12, respectively) postinjury by notexin injection. At D0, no difference in the number of Pax7+ MuSCs was observed between dKO and wild-type (WT) (Figure 6C). At D6, both genotypes exhibited a similar increase in numbers of Pax7+ MuSCs. No differences in KI67+ MuSCs, or Cleaved Caspase-3 were observed at D6, consistent with no differences in proliferation or cell death (Figures 5A and 5B). However, at D12, dKO animals exhibited a ~50% increase in the number of MuSCs (Figures 6C and 6D), suggesting that constitutive PDH activity could be enforcing self-renewal over differentiation, as we observed in vitro (Figures 4D and 4E). Flow cytometry of H3K18 and H4K16 acetylation showed that MuSCs in dKO animals had no difference in histone acetylation prior to injury (Figure 6E) but accumulated increased histone acetylation following injury (D6) (Figure 6F). Furthermore, we observed a decreased proportion of Myog+ myogenic progenitors in dKO mice at D6 (Figures 6G and 6H). Underscoring a differentiation defect, dKO muscles had reduced cross-sectional area at D12 postinjury (Figures 6I and 6J), despite increased numbers of MuSCs at this time point. Together, these results suggest that the MuSCs of Pdk2/4 dKO mice develop normally but are biased toward self-renewal and reduced differentiation capacity due to an aberrant accumulation of histone acetylation. To confirm the differentiation deficits we observed in dKO mice are MuSC-intrinsic, we assayed engraftment of WT or dKO MuSCs transplanted in Pdk2/4−/− recipient muscle using bioluminescence imaging (BLI) (Sacco et al., 2008). Isolated MuSCs were labeled with a luciferase-expressing lentivirus and transplanted into irradiated hind-limb muscles of immunodeficient NOD scid gamma (NSG) animals (Figure 6K). 28 days after transplantation, dKO MuSCs showed reduced bioluminescence signal, indicative of reduced differentiation and contribution of donor dKO MuSCs to myofibers (Figures 6J and 6K). This suggests that dKO MuSCs have a cell-autonomous reduction in differentiation capacity, which is attributable to the irreversible dysregulation of PDH activity. These data substantiate our findings that glucose metabolism regulates histone acetylation during proliferation and modulates myogenic differentiation both in vitro and in vivo.

**DISCUSSION**

In this study, we elucidate the role of glucose metabolism as an epigenetic regulator of proliferating MuSCs, and a modulator of differentiation capacity. We characterize distinct, interrelated metabolic and histone acetylation states of MuSCs in uninjured and injured muscle tissues, as well as in differentiating progenitors. In particular, we identify PDH activity as a pivotal regulator of glucose-derived histone acetylation during MuSC expansion, which dictates the transition toward differentiation.

MuSCs in uninjured tissues exist in a quiescent cell state with low metabolic demand and few mitochondria (Muir et al., 1965; Pala et al., 2018). Nevertheless, quiescent MuSCs show signatures of glucose and fatty acid oxidation and low lactate-producing glycolysis (Ryll et al., 2015). This results in low levels of histone acetylation, due to utilization of acetyl-CoA, as well as high levels of NAD+ leading to active histone deacetylation by SIRT1 (Ryll et al., 2015). Upon activation, MuSCs undergo mitochondrial biogenesis, and increase lactate-producing glycolysis (Pala et al., 2018; Rodgers et al., 2014). This is marked by increased expression of lower-glycolysis genes (Figure 3B), diminished requirement for glucose to support respiration (Figures 3C and 3D), and increased PDH phosphorylation (Figures 3G, 3H, and 3J). In addition to the resulting decreased SIRT1 deacetylase activity (Ryll et al., 2015), these metabolic changes result in overall increased histone acetylation (Figure 4B). In this state, PDH activity is rate limiting for glucose-derived histone acetylation (Figures 4B, 4C, and 5A–5D).

(G) Histogram of Myog+ cells within Lin− α7-integrin+ population at D6 after injury.
(H) Myog+ quantification of cells as described in (G), n = 3.
(I) Histogram of fiber cross-sectional area in dKO and WT, D12 after injury.
(J) Median fiber cross-sectional area of WT and dKO at D6 postinjury, n = 3 animals.
(K) Transplant assay, MuSCs were isolated from WT or dKO donors, and transduced with a Luciferase reporter virus. After 24 h, 500 cells were transplanted into irradiated NSG recipient mice. MuSCs from WT and PDK mice were transplanted into contralateral legs to minimize biological variability.
(L) BLI signal for dKO and WT; images shown for same animal.
(M) BLI signal of engrafted MuSCs 28 days after transplantation, calculated as radiance (photons/second), n = 5.
Student’s unpaired t test was used for all statistical comparisons. Data are represented as mean ± SEM (**p < 0.01, and ***p < 0.001). See also Figure S6.
At the onset of differentiation, metabolic remodeling drives reduction in histone acetylation (Figures 1C–1G and 2A–2D). Mitophagy and mitochondrial fission decrease capacity of mitochondria, which in turn have increased reliance on glucose (Figures 3C–3F) (Sin et al., 2016). These metabolic changes reduce the contribution of glucose to histone acetylation (Figure 4B), resulting in a global reduction in histone acetylation across multiple marks during differentiation in vivo (Figure 1) and in vitro. Conversely, forced reduction of glucose-derived histone acetylation results in precocious induction of differentiation (Figure 4), which has been shown to be dependent on histone acetylation (Bracha et al., 2010). We find that histone acetylation is lost across the genome during differentiation; however, genes associated with differentiation such as MYOG increase histone acetylation. Thus, metabolic histone acetylation regulation acts in conjunction with regulation by histone modifiers and transcription factors.

It is important to note that metabolic modulation of histone acetylation is not equivalent to regulation by transcription factors, HDACs, or HATs. Our site-specific analyses suggest that, with substrate limitation, the availability of acetyl-CoA modulates histone acetylation at sites already targeted by HAT and HDACs. Sites not targeted by HATs have low acetylation turnover, or are maintained at a low acetylation state by HDACs, are less susceptible to substrate limitations. Conversely, HDAC inhibitors (HDA-Cis) will increase histone acetylation at genes targeted by HDACs. Even though both HDACi and DCA treatment result in increased histone acetylation, the affected sites are different. During proliferation, MYOD1 has been shown to act with HDACs to deacetylate myogenic differentiation genes (Lu et al., 2000; Puri et al., 2001). Thus, when proliferating myoblasts are treated with HDACis, histone acetylation increases on myogenic differentiation genes to promote differentiation (Iezzi et al., 2002, 2004). Treatment with DCA, on the other hand, does not affect differentiation genes (Figures 5A–5D). Instead, DCA treatment reinforces the overall epigenetic landscape of proliferating progenitors and stabilizes expression of stem cell genes (Figures 5D and 5E).

During differentiation, loss of histone acetylation around stem cell genes facilitates a change in cell identity, transitioning toward de novo acetylation around muscle-specific genes. Intriguingly, while loss of glucose-derived histone acetylation triggers differentiation, knockdown of the HAT CBP and p300 inhibits differentiation (Polesskaya et al., 2001; Puri et al., 1997a). This is because CBP/p300, also directed by MYOD1, is required to acetylate and activate transcription of myogenic differentiation genes. As such, HDACi treatment at the onset of differentiation by serum removal abolishes differentiation. Further supporting this idea, Acly knockdown in proliferation conditions induces differentiation (Bracha et al., 2010), while knockdown in differentiation conditions reduces muscle gene expression and myotube hypertrophy (Das et al., 2017). Recent insights from single-cell ATAC-seq agree with this idea (Pliner et al., 2018), wherein differentiation begins with closing of a large number of sites, followed by a subset of specific sites that gain acetylation and accessibility (of which 70% are bound by MYOD1) (Cao et al., 2010).

We thus propose two complimentary mechanisms of histone acetylation changes that coordinate during myogenic differentiation: (1) the active deacetylation and acetylation of myogenic genes by the interaction of MYOD1 with HDACs and HATs, and (2) regulation by shifts in metabolic state described here. Global histone acetylation loss, mediated by altered glucose metabolism, has also been observed in differentiating embryonic stem cells (Moussiaeff et al., 2015), but has not been previously described in the context of tissue-specific stem cells in regeneration. Our results raise the possibility that the metabolic regulation of histone acetylation on non-lineage specific genes may be a hallmark of tissue-specific stem cell specification.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2019.05.092.

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DECLARATIONS OF INTEREST

The authors declare no competing interests.

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STAR METHODS

KEY RESOURCES TABLE

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Chemicals, Peptides, and Recombinant Proteins

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### Experimental Models: Cell Lines

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### Experimental Models: Organisms/Strains

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| MOUSE: C57Bl6/J: PDK2/PDK4 double knockout | Robert Harris (Indiana University) | Jeoung et al., 2012 |
| MOUSE: NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup>/SzJ (NSG) | Jackson Laboratories | 005557 |

### Recombinant DNA

| Lentivirus Luc-IRES-GFP | N/A |

### Sequence-Based Reagents

| ChIP-qPCR Primer: Tmem8c-F | ATGGCCTCCCAT GTAGAAACG | N/A |
| ChIP-qPCR Primer: Tmem8c-R | TTGTTAGCCAAA CTGCTCGTG | N/A |
| ChIP-qPCR Primer: Myh3-F | CTCACCAAGGTCT GGTCGT | N/A |
| ChIP-qPCR Primer: Myh3-R | CAGCAGTTGTGG ACCTATGG | N/A |
| ChIP-qPCR Primer: Myogenin-F | AGCGCAAGGC TCCTCTCTT | N/A |
| ChIP-qPCR Primer: Myogenin-R | CTTAGCAGTGGACAGT | N/A |
| ChIP-qPCR Primer: Mef2a-F | TCTCTCGGCT TCTCCTCTT | N/A |
| ChIP-qPCR Primer: Mef2a-R | TGTCCATGACA AACCCTTCA | N/A |
| ChIP-qPCR Primer: Myod1-F | CGCCTGAGCA AAGTGAATG | N/A |
| ChIP-qPCR Primer: Myod1-R | CAGGATCTCC ACCTTGGGTA | N/A |
| ChIP-qPCR Primer: Myf5-F | AGGCCCACATG AGGCAGTG | N/A |
| ChIP-qPCR Primer: Myf5-R | CGGAGCACACA AAGCTGAG | N/A |
| ChIP-qPCR Primer: Pax7-F | CCTGGAGGGT GCCATCTCA | N/A |
| ChIP-qPCR Primer: Pax7-R | TTGTTAGCCAAA CTGCTCGTG | N/A |
| ChIP-qPCR Primer: Rpsa-F | TGGTTGGTGTTG GAGTGCTA | N/A |
| ChIP-qPCR Primer: Rpsa-R | GTGCAGGGTCAG TCAGTCTT | N/A |

(Continued on next page)
LEAD CONTACT AND MATERIALS AVAILABILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Helen Blau (hblau@stanford.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The Stanford University Administrative Panel on Laboratory Animal Care (APLAC) approved all animal protocols. We performed all experiments in compliance with the institutional guidelines of Stanford University. C57BL/6J mice were purchased from Jackson Laboratories and used at 8-16w. PDK2/PDK4 double knockout mice were provided by a generous gift from Robert Harris (Indiana University). All experiments were conducted using age and gender-matched controls, and littermates randomly assigned to experimental groups. Both male and female animals were used.

METHOD DETAILS

Notexin-induced muscle injury
For muscle injury, we performed an intramuscular injection of 10 μL of notexin (10 μg ml−1; Latoxan) into the tibialis anterior or gastrocnemius muscle. For uninjured controls, we collected muscle from completely uninjured mice. Muscles were collected in uninjured mice, as well as day 6 after injury for flow cytometry and CyTOF analyses, and day 6 and day 12 for immunohistochemistry analysis.

Florescence activated cell sorting
Muscle stem cells were isolated using a modified version of the protocol previously described (Sacco et al., 2008). In brief, hind-limb muscles were isolated and dissociated using the gentleMACS Octo Dissociator with a modified manufacturer protocol.
injured C57/BL6 mice were obtained as described in Idu at 8hr and 30minutes before sample collection to label dividing cells. Whole muscle single-cell suspensions of uninjured and notexin-9-12wk old wild-type C57/Bl6 were injured 6 days before collection by notexin injection. Injured and uninjured mice were injected with Single Cell Mass Cytometry (CyTOF) the LSR-II.UV (Becton Dickinson) and analyzed by FlowJo. acetyl-Histone H3, and total histone H3 were used. DNA content was measured by DAPI staining or 7-AAD. Data were collected using mouse anti-Pax7. For acetylation analysis by flow, rabbit anti-H4K16ac, rabbit anti-H3K18ac, mouse anti-H3K9ac, rabbit anti-acetyl-Histone H3, and total histone H3 were used. DNA content was measured by DAPI staining or 7-AAD. Data were collected using the LSR-II.UV (Becton Dickinson) and analyzed by FlowJo.

**Flow Cytometric Analysis**

For analytical flow cytometry, cells or whole-muscle mixtures were fixed in 1.6% paraformaldehyde for 10min at room temperature (myoblasts, whole tissue digestes) or on ice (MuSCs), and permeabilized on ice with ice-cold MeOH. Cells were used after 10 minutes on ice, or after storage in 0°C for up to two weeks. After washing with PBS, cells were blocked using sterile filtered 0.5% bovine serum albumin (BSA) in PBS. Cells were then permeabilized with primary antibody for 1hr at room temperature at a density of 1 × 10⁶ cells/mL, washed 2X in PBS then stained in secondary for 20 minutes. Three washes were done before resuspension in DAPI/PBS and analyzed by LSR.II.UV. For myogenic staining, antibodies used were mouse anti-Myogenin, rabbit anti-Myogenin and mouse anti-Pax7. For acetylation analysis by flow, rabbit anti-H4K16ac, rabbit anti-H3K18ac, mouse anti-H3K9ac, rabbit anti-acetyl-Histone H3, and total histone H3 were used. DNA content was measured by DAPI staining or 7-AAD. Data were collected using the LSR-II.UV (Becton Dickinson) and analyzed by FlowJo.

**Single Cell Muscle Cytometry (CyTOF)**

9-12wk old wild-type C57/B6 were injured 6 days before collection by notexin injection. Injured and uninjured mice were injected with ldu at 8hr and 30minutes before sample collection to label dividing cells. Whole muscle single-cell suspensions of uninjured and notexin-injured C57/B6 mice were obtained as described in Fluorescence Activated Cell Sorting. Single-cell suspensions were passed twice through a 40uM filter to remove debris, and fixed using 1.6% paraformaldehyde. Cells were first labeled with metal-conjugated surface antibodies for 1hr at room temperature, and then permeabilized for 10 minutes on ice with 90% methanol. Permeabilized cells were labeled with metal-conjugated intracellular markers, washed and kept overnight at 4°C with iodium DNA intercalator. All antibodies were conjugated to isotopically pure lanthanide metal using the MaxPAR antibody conjugation kit according to the manufacturer’s recommended protocol. Labeled antibodies were stored at 4-C in PBS-based Antibody Stabilizer (Candor Bioscience). Samples were run on the CyTOF mass cytometer (DVS Sciences). Muscle antibody panel developed by Ermelinda Porpiglia and histone acetylation antibody panel developed by Sean Bendall and Michael Angelo. See Table S1 for antibodies and metal conjugation.

**RNA Sequencing**

α7-integrin*CD34* muscle stem cells were isolated as described above and processed either fresh or after 48hr of culture on 12kPa hydrogels. RNA was isolated using QIAGEN RNAEasy Micro kit from 5-10,000 cells, and cDNA generated and amplified using NuGEN Ovation RNA-Seq System v2 kit. Libraries were constructed from cDNA with the TruSEQ RNA Library Preparation Kit v2 (Illumina), and sequenced to 30-40x10⁶ 1x75bp reads per sample on a HISEQ 2500.

**Chromatin Immunoprecipitation**

~1 × 10⁷ primary myoblasts at 50%-75% confluence were cross-linked in 1% formaldehyde for 15 min, with constant agitation, and quenched with 150mM glycine for 5 min. Micrococcal nuclease (New England Biolabs, Inc.) was used to fragment chromatin to a range of 200-400 bp. Chromatin was incubated with H3ac antibody overnight at 4°C with constant agitation. Protein A Dynabeads was incubated with antibody-protein complexes for 2 hr at 4°C with constant agitation. After de-crosslinking, DNA was isolated using a MinElute column (QIAGEN). See Quantitative Real Time PCR for quantification of target genes.

**ATAC-Seq**

50,000 primary myoblasts were prepared according to Buenrostro et al. (2013) using the Nextera DNA Library Preparation Kit. In brief, cells were washed with PBS and lifted using 0.125% trypsin for 5 mins at 37°C then pelleted. The pellet were resuspended in ice cold lysis buffer (10 mM Tris–HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl2, 0.1% IGEPAL CA-630), pelleted and resuspended in Trn5 transposase according to manufacturer’s instructions. The reaction was incubated for 30 minutes at 37°C on a shaker. Transposed DNA was isolated using the QIAGEN MinElute Reaction Cleanup Kit. Libraries were amplified for a total of 8-10 PCR cycles (adjusted to 25% of saturation by qPCR), and sequenced in parallel to 97 x10⁶ and 91 x10⁶ 2x37bp reads for PBS and DCA-treated samples, respectively, on Illumina NextSeq High Output. After removal of mitochondrial DNA reads, 90.6 x10⁶ and 92.3 x10⁶ paired end reads with mapQ scores of 10+ were used in downstream analysis for PBS and DCA samples, respectively.

**Immunofluorescence and histology**

Muscle tissues were prepared for histology as described previously described (Sacco et al., 2008). For immunofluorescence, anti-Pax7, anti-laminin, anti-phosphoPDH (Ser293), anti-PDH E1 alpha subunit, anti-Ki67, anti-Cleaved Caspase-3 and mouse anti-Myosin Heavy Chain antibodies were used. All AlexaFluor secondary antibodies were used (1:500), and nuclei counterstained with DAPI.
Histones were extracted and derivatized similar to previously described methods (Lin and Garcia, 2012). Frozen cell pellets were resuspended in nuclear isolation buffer (NIB; 15 mM Tris HCl pH 7.5, 15 mM NaCl, 60 mM KCl, 5 mM MgCl₂, 1 mM CaCl₂, 250 mM sucrose supplemented with 1 mM DTT, 10 mM sodium butyrate, 500 μM AEBSF, and 5 mM microcystin) containing 0.2% NP-40 alternative and incubated on ice for 10 mins. Nuclei were pelleted by centrifugation at 500 x g for 5 mins at 4°C and washed twice in detergent-free NIB. Histones were extracted from nuclei with 0.4N H₂SO₄ for approximately 4 hr at 4°C with rotation. Histone extracts were cleared by centrifugation at 3400 x g for 5 mins at 4°C and the supernatants were precipitated by adding 1 volume of 100% trichloroacetic acid to 3 volumes of sample and incubating overnight at 4°C. Precipitated histones were pelleted at 3400 x g for 5 mins at 4°C and washed sequentially with 0.1% HCl in acetonitrile and then acetone alone. After air drying, histone pellets were resuspended in 100 mM ammonium bicarbonate. Approximately 8-10 μg of protein were subjected to derivatization, in which free lysine residues were propionylated by adding 1 volume of 25% propionic anhydride/75% 2-propanol to 2 volumes of sample and incubating for 15 mins at 37°C. Additional ammonium bicarbonate salt was added at the beginning of the reaction to prevent the pH from becoming too acidic. Samples were dried in a speed vac and then propionylated a second time. Propionylated histones were resuspended in 100 mM ammonium bicarbonate and digested with trypsin (1/20 ratio by mass) overnight at room temperature. Digested peptides were propionylated twice more as above and then desalted with C18 stage tips prior to mass spectrometry analysis.

Histone Extraction and Derivatization
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Mass Spectrometry
Derivatized histone peptides were loaded into an Easy nLC 1000 (Thermo) liquid chromatography system fitted with 75 μm i.d. x 20 cm fused silica columns (Polymicro Tech), pulled and packed in house with ReproSil-Pur 120 C18-AQ (3 μm, Dr. Maisch GmbH), in line with an Orbitrap Elite or Velos (Thermo). Peptides were separated using a gradient of 2%–30% buffer B (0.1% formic acid in acetonitrile) in buffer A (0.1% formic acid in water) over 35 mins and 30%–98% over 20 mins. Full scan mass spectra from 290-1400 m/z were acquired at a resolution of 60,000 with automatic gain control set at 1E6. Tandem mass spectra were acquired by CID fragmentation at a normalized collision energy of 35 and maximum injection time of 150 ms with automatic gain control set at 5E4. MS data were searched and quantified with EpiProfile.

Oxygen Consumption and Extracellular Acidification analysis
Primary myoblasts were plated at a 1x10⁴ cells/well of a collagen coated 96-well Seahorse cell culture plate and grown for 48hrs ± DCA in standard myoblasts growth media (+ 1g/L glucose, 1mM Pyruvate). As a negative control, one row of the culture plate was left without only media and no cells as a negative control. One day before measurements, a Seahorse sensor cartridge was prepared according to manufacturer’s instructions, with overnight incubation at 37°C in a CO₂-free incubator. On the day of measurements, cells were washed twice and switched to un-buffered DMEM + Glutamine (Agilent) supplemented with 15% dialyzed fetal bovine serum (FBS) adjusted to pH of 7.4. For each condition (±DCA, pH 7.4), one row was supplemented with 1g/L glucose and 1mM pyruvate to assay respiration with available carbohydrate substrates, and one row left un-supplemented to assay non-carbohydrate respiration and lactate production. Cells were equilibrated at 37°C in a CO₂-free incubator for one hour. Inhibitors (Oligomycin, Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP), and Rotenone/Antimycin) were added each at a final concentration of 1 μM after injection to the appropriate port of the sensor cartridge. The cell culture plate and sensor cartridge were run on the Seahorse XF-96, and data analyzed by Excel.

Quantitative Real Time PCR
Total RNA was isolated using the RNAeasy Mini kit (QIAGEN). The RNA concentration was determined with a spectrophotometer, and up to 1ug was used to prepare cDNA (Ready-To-Go, T-Primed First-Strand Kit, Amersham Bioscience 27-9263-01). Inventoried TaqMan FAM probes (Applied Biosystems) were used for the relative quantification of the mRNA levels of Pdha1, Pdk1, Pdk2, Pdk3, Pdk4, Pax7 and Acly. For ChIP-qPCR, SYBR green was used with primers designed to span 80-100bp around H3ac ChIP-Seq peaks identified in C2C12 data (ENCODE/Caltech, GEO accession: GSE36023). For full primer sequences see Table S2.

Lentiviral Infection
Sorted MuSCs were plated on collagen coated 24-well plates for 24hrs and infected with a luc-IRES-GFP lentivirus for 24hrs. Efficiency of transduction was evaluated by GFP staining of excess cells after 6 days in culture.
**Bioluminescent Imaging**

Five hundred MuSCs transduced with luc-IRES-GFP virus for 24 hours were injected in a volume of 10μL into the tibialis anterior muscle of NSG recipient animals. Recipient animals were irradiated with 18Gy 48hrs prior to transplantation. Bioluminescent imaging was performed using a XENOGEN-200 system as previously described (Sacco et al., 2008). Briefly, we anesthetized mice using isoflurane and administered 100 μL D-luciferin (0.1 mmol kg⁻¹, reconstituted in PBS; Caliper LifeSciences) by intraperitoneal injection. We analyzed BLI using a 60 s exposure acquired at 12 min after luciferin injection. Digital images were recorded and analyzed using Living Image software (Caliper LifeSciences). We analyzed images with a consistent region-of-interest (ROI) placed over each hindlimb to calculate a bioluminescence signal. We used a bioluminescence signal value of 70,000 photons per second to define an engraftment threshold.

**Hydrogel fabrication**

We fabricated polyethylene glycol (PEG) hydrogels from PEG precursors, synthesized as described previously (Gilbert et al., 2010). Briefly, we formed hydrogels by mixing 10% (wt/vol) solutions of PEG-sulfhydryl (4-arm 10-kDa PEG-SH) and PEG-vinylsulfone (8-arm 10-kDa PEG-VS) precursor polymers diluted in water or triethanolamine, respectively. We functionalized hydrogel surfaces through covalent cross-linking reaction with PBS-dialyzed laminin protein (Roche). We fabricated soft 12-kPa (Young’s modulus) stiffness hydrogels to 1-mm thickness (Gilbert et al., 2010). We fabricated hydrogels to a 1-μm thickness directly onto tissue culture plastic dishes to achieve a rigid 1 × 106-kPa stiffness, as hydrogels of ≤ 1-μm thickness allow cells to sense the underlying substrate mechanical properties, resulting in an effective rigidity equivalent to tissue culture plastic (Engler et al., 2006; Gilbert et al., 2010), while providing equivalent material surface chemistry as thicker soft gels. This non-swelling gel chemistry results in equivalent laminin protein concentrations (estimated to be 7.5 ng cm⁻² in ref. 32) on both soft and rigid hydrogels. We cut and adhered all hydrogels to cover the surface area (2.0 cm²) of 24-well culture plates.

**Data Reporting**

Sample size was determined based on practical and experimental consideration, with no statistical methods used to predetermine sample size. For florescence data, investigators were blinded to allocation for image acquisition and analyses.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Statistical Analysis**

All values expressed as mean ± s.e.m. Differences with p value < 0.05 were considered significant (*p < 0.05, **p < 0.01, ***p < 0.001). Comparisons between two groups were performed using Student’s t test (two-tailed, paired and unpaired where indicated). For multiple comparisons, 1-Way or Repeated Measures ANOVA with Tukey’s Test or 2-way ANOVA with Bonferonni correction were conducted.

**Sequencing Analysis**

RNA, ChIP and ATAC-seq data were aligned against the *Mus musculus* mm10 genome using STAR (Dobin et al., 2013) or BWA (Li and Durbin, 2009). (for RNA and ChIP/ATAC, respectively). RSEM (Li and Dewey, 2011) was used for calling transcripts and calculating transcripts per million (TPM) values, as well as total counts. MACS2 (Zhang et al., 2008) was used for peak calling for ChIP and ATAC-seq data. ATAC-seq peaks were further refined as ± 75bp regions of peak summits identified by MACS2. Common satellite repeat regions were removed against the UCSC Repeatmasker track. A counts matrix containing the number of counts for each gene/peak and each sample was obtained. This matrix was analyzed by DESeq to calculate statistical analysis of significance (Anders and Huber, 2010) of genes between samples. Gene annotation for ChIP and ATAC peaks were performed using GREAT using regions ± 2 kb proximal and 100kb distal from the transcription start site (TSS) as well as curated regulatory domains. Motif analyses were performed using HOMER using either −300bp to +50bp proximal promoter regions of genes of interest or within peaks identified by ChIP or ATAC-seq.

**DATA AND CODE AVAILABILITY**

Sequencing (RNA, ChIP, ATAC) data have been submitted in MIME-compliant format to GEO, accession number super series GSE129280. Data available upon publication.