Article

Inhibiting histone acetyltransferase activity rescues differentiation of emerin-null myogenic progenitors

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Abstract: Emery-Dreifuss Muscular Dystrophy (EDMD) is a disease characterized by skeletal muscle wasting, contractures of the major tendons, and cardiac conduction defects. Compromised skeletal muscle regeneration is predicted to result from impaired muscle stem cell differentiation. Mutations in the gene encoding emerin cause EDMD. We previously showed emerin-null myogenic progenitors fail to properly exit the cell cycle, delay myoblast commitment and form less myotubes. Treatments with theophylline, a HDAC3 activator, rescued myotube formation in differentiating emerin-null myogenic progenitors. This suggested emerin activation of HDAC3 activity to reduce H4K5 acetylation is important for myogenic differentiation. Pharmacological inhibitors of histone acetyltransferases (HATs) targeting acetylated H4K5 were used to test if the increased acetylated H4K5 was responsible for inhibiting emerin-null myogenic differentiation. Nu9056 and L002 were added to differentiating wildtype and emerin-null myogenic progenitors and differentiation was assessed. HAT inhibition rescued emerin-null myogenic progenitor differentiation. L002 also rescued myoblast commitment. Increased concentrations of L002 inhibit p300 and GCN5/pCAF, suggesting H3K9, H3K18 or H3K27 acetylation dynamics are important for myoblast commitment and are regulated by emerin. In contrast to treatment with these HAT inhibitors, emerin-null myogenic progenitors treated with SRT1720, which targets SIRT1, a NAD^+-dependent deacetylase, showed no significant change in myotube formation. Thus, we conclude emerin regulation of HDAC3 activity to affect H4K5 acetylation dynamics is important for myogenic differentiation.

Keywords: Cell signaling, Emerin, Emery-Dreifuss Muscular Dystrophy, Myogenic differentiation

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1. Introduction

The nuclear envelope is composed of two lipid bilayers, the outer nuclear membrane, which is contiguous with the endoplasmic reticulum, and the inner nuclear membrane [1]. Although the outer and inner nuclear membranes arise from a common membrane, they are functionally distinct membranes containing proteins localizing specifically to either the outer or the inner nuclear membrane. Underlying the inner nuclear membrane is a network of Type V intermediate filament proteins named lamins that provide nuclear rigidity and elasticity [2]. The inner nuclear membrane contains a large number of integral inner nuclear membrane proteins [3], many of which show cell-type-specific expression [4-11]. Inner nuclear membrane proteins function in diverse roles, including nuclear structure, genomic organization, chromatin architecture, gene expression, cell cycle regulation, and cytoskeletal organization [1, 12]. The nuclear lamins and its associated inner nuclear membrane proteins define the nuclear lamina.

Emerin is a lamin-binding, integral inner nuclear membrane protein. Mutations in the gene encoding emerin cause X-linked Emery-Dreifuss muscular dystrophy (EDMD1), an inherited disorder causing progressive skeletal muscle wasting, irregular heart rhythms, and contractures of major tendons [13-16]. Evidence suggests the skeletal muscle wasting seen in EDMD is caused by impaired differentiation of skeletal muscle stem cells and inefficient skeletal muscle regeneration. For example, skeletal muscle necrosis and increased skeletal muscle fiber permeability are rarely seen in EDMD patients [17]. Further supporting this hypothesis, emerin-null mice exhibit delayed skeletal muscle regeneration and repair, motor coordination defects, and mild atrioventricular conduction defects [18, 19]. Skeletal muscle from EDMD1 and EDMD2 patients and emerin-null mice showed altered expression of muscle regeneration pathway components [18, 20]. Emerin-null myogenic progenitors and emerin-downregulated C2C12 myoblasts exhibit impaired differentiation and myotube formation [21-23] due to aberrant temporal activation of myogenic differentiation genes [24] and disruption of key signaling pathways [25], suggesting defective muscle regeneration contributes to the EDMD skeletal muscle phenotype [18, 21, 22]. The coordinated temporal expression of MyoD, Myf5, Pax3 and Pax7 was also disrupted in emerin-null myogenic progenitors [26] due to the inability of the genome to properly reorganize during differentiation [18, 20, 25]. Emerin binds directly to HDAC3 and activates its deacetylase activity [27], which is required for proper dynamic reorganization of MyoD, Myf5, Pax3 and Pax7. The failure of the genome to properly reorganize during emerin-null myogenic differentiation supports the hypothesis that emerin-null myogenic progenitors fail to undergo the transcriptional reprogramming required for myogenic differentiation. It further suggests the regulation of HDAC3 activity by emerin is critical for transcriptional reprogramming during myogenic differentiation.

Whether H4K5 acetylation dynamics were important for myogenic differentiation was tested using histone acetyltransferase (HAT) inhibitors targeting HATs mediating H4K5 acetylation (e.g., Tip60/KAT5). HAT inhibition rescued emerin-null myogenic differentiation, showing increased H4K5 acetylation contributes to the impaired differentiation of emerin-null myogenic progenitors.

2. Results

Histone acetyltransferase (HAT) inhibition rescues emerin-null myogenic differentiation

We previously showed emerin-null myogenic progenitors had impaired differentiation [23]. This impaired differentiation was rescued by activation of HDAC3. Histone acetyltransferase inhibitors (HATi) were used to independently test if the altered H4K5 acetylation dynamics was responsible for the impaired differentiation of emerin-null progenitors. HATi used for these studies were chosen because they preferentially inhibit acetylation of lysine residues targeted by HDAC3 (e.g., H4K5)[28]. Cell cycle withdrawal, myosin heavy chain (MyHC) expression and myotube formation were analyzed 36 hours post-differentiation induction within the same cell population during differentiation. Emerin-null and wildtype myogenic progenitors were treated with 0.5 μM L002 upon differentiation induction to test whether inhibition of H4K5 acetylation rescued myogenic differentiation of emerin-null progenitors, (Figure 1A). L002 was developed as a specific inhibitor of p300. However, in addition to inhibiting H3K18 and H3K27 acetylation, L002 also inhibited H4 acetylation in cells at low micromolar concentrations (0.3 μM)[29]. We confirmed L002 inhibited H4K5 acetylation at 0.5 μM L002 (Figure 2). L002-treated wildtype progenitors exited the cell cycle normally (Figure 1C′, J). 2.7% of emerin-null progenitors failed to exit the cell cycle after 36 hours, as expected

(Figure 1G, J). Emerin-null progenitors treated with L002 showed a trend toward reducing the number of emerin-null cells in the cell cycle (2.1%; p=0.06; Figure 1G′, J). L002 treatment significantly increased the percentage of differentiating emerin-null progenitors expressing MyHC (46%, Figure 1H, K; p=0.015). The number of MyHC-positive cells in L002-treated differentiating emerin-null progenitors is statistically similar to untreated wildtype progenitors (47.8% in wildtype, p=0.35; Figure 1D, H′, K), indicating rescue of myoblast commitment. L002 treatment increased myotube formation 1.8-fold in differentiating emerin-null progenitors (Figure 1 I, L) to completely rescued myotube formation to wildtype levels (p=0.97 for L002-treated emerin-null cells vs. wildtype cells; Figure 1E, I′, L).

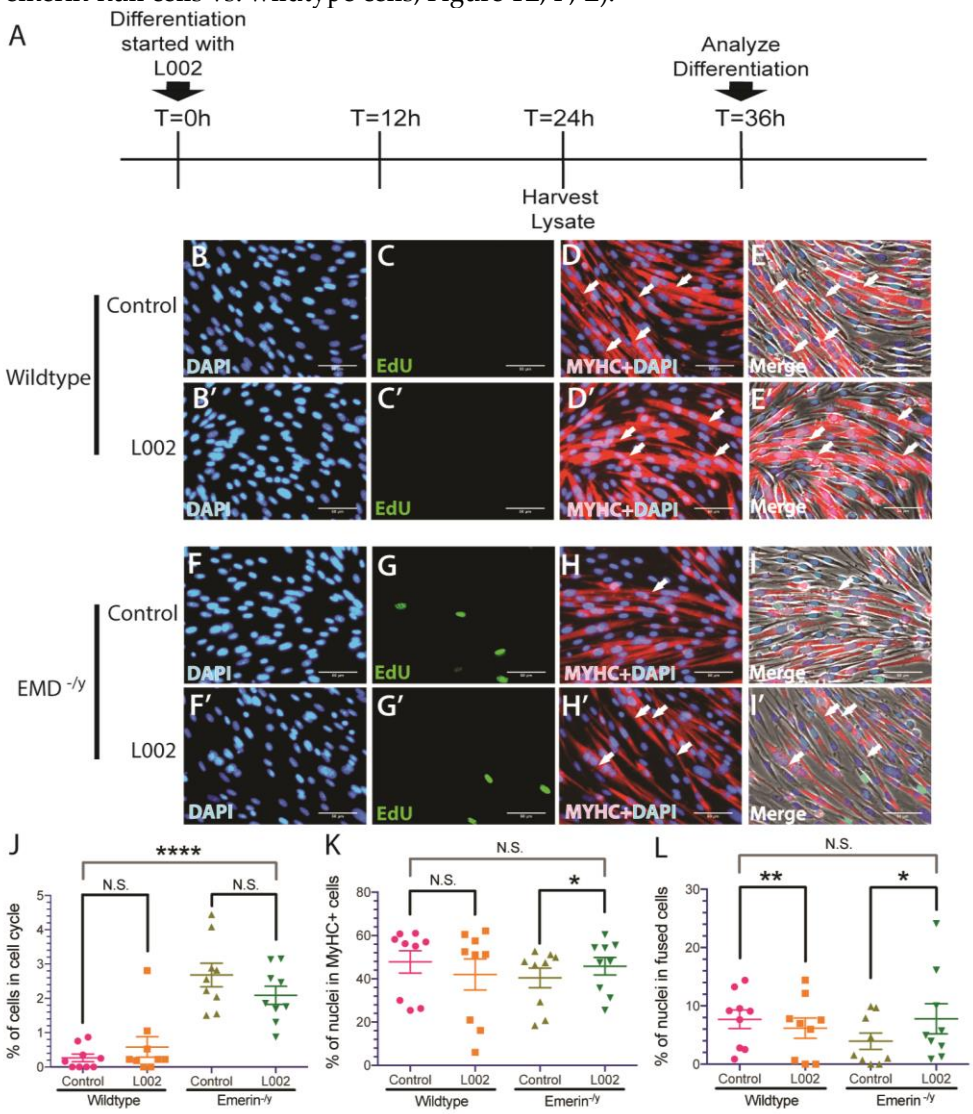
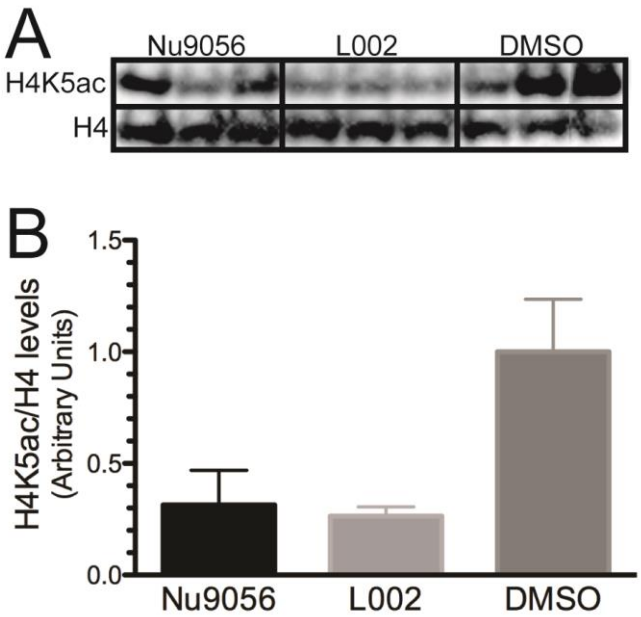


Figure 1. Inhibition of HAT activity with L002 treatment rescues myotube formation and myosin heavy chain expression in emerin-null myogenic progenitors. (A) Timelines showing the time point L002 was added and whole cell lysate collection for western blot analysis. Representative images at 40X magnification of vehicle-treated wildtype (B-E) or emerin-null (F-I) and L002-treated wildtype (B′-E′) or emerin-null (F′-I′) cells 36 h after initiating differentiation. Arrows mark myotubes (e.g., 4 myotubes in I′ vs 1 myotube in I). (J-L) Quantification of >500 nuclei for each experimental treatment (n=4) was carried out to determine the percentage of myogenic progenitors in the cell cycle (J), expressing MyHC (K) and formed tubes (L) 36 h after inducing differentiation.

112 Results are mean±s.d. of n=4; N.S., not significant; *, P<0.05; **, P<0.01; ****, P<0.0001 using paired,
113 two-tailed t-tests.



114

115 **Figure 2. H4K5 acetylation is decreased by treatment with L002 and Nu9056.** (A) Western blotting
116 of whole cell lysates treated with Nu9056 or L002 to analyze H4K5 acetylation during
117 differentiation of emerin-null progenitors. DMSO-only treatment was the control. Three biological
118 replicates are shown for each treatment. (B) Densitometry was performed and acetylated H4K5 in
119 each sample was normalized to total H4 protein in each sample. Levels of acetylated H4K5 for each
120 condition were normalized to DMSO-treated cells. Results are mean±s.d. of n=3 for each condition.

121 Wildtype and emerin-null myogenic progenitors were differentiated for 36 hours in the
122 presence of the HAT inhibitor Nu9056 to independently confirm HAT inhibition rescued emerin-
123 null myogenic differentiation. Nu9056 is a highly specific inhibitor of Tip60/KAT5 [30].
124 Tip60/KAT5 mediates the acetylation of H4K5, H4K8 and H4K12. Unlike L002, Nu9056 exhibits
125 greater specificity, as the IC_{50} of Nu9056 for p300 or pCAF/GCN5 is 20-40-fold higher than for
126 Tip60/KAT5. 0.5 μM Nu9056 in DMSO or DMSO alone were incubated with wildtype or emerin-
127 null myogenic progenitors upon differentiation induction (Figure 3A). Nu9056 treatment had no
128 effect on cell cycle withdrawal of wildtype or emerin-null myogenic progenitors, (Figure 3C, G,
129 J). Nu9056 treatment failed to rescue myoblast commitment, as the number of MyHC-expressing
130 cells was similar in Nu9056-treated (51.0%) and untreated emerin-null myogenic progenitors
131 (50.3%; Figure 3H, K). This suggests myoblast commitment results from inhibition of p300 or
132 pCAF/GCN5 activity with L002. Myotube formation in emerin-null progenitors was rescued by
133 Nu9056 treatment, as 15.1% of Nu9056-treated emerin-null progenitors fused to form myotubes,
134 compared to 10.8% of DMSO-treated emerin-null progenitors (Figure 3I, L). Myotube formation
135 in Nu9056-treated emerin-null progenitors was statistically similar to wildtype progenitors
136 (p=0.11).
137

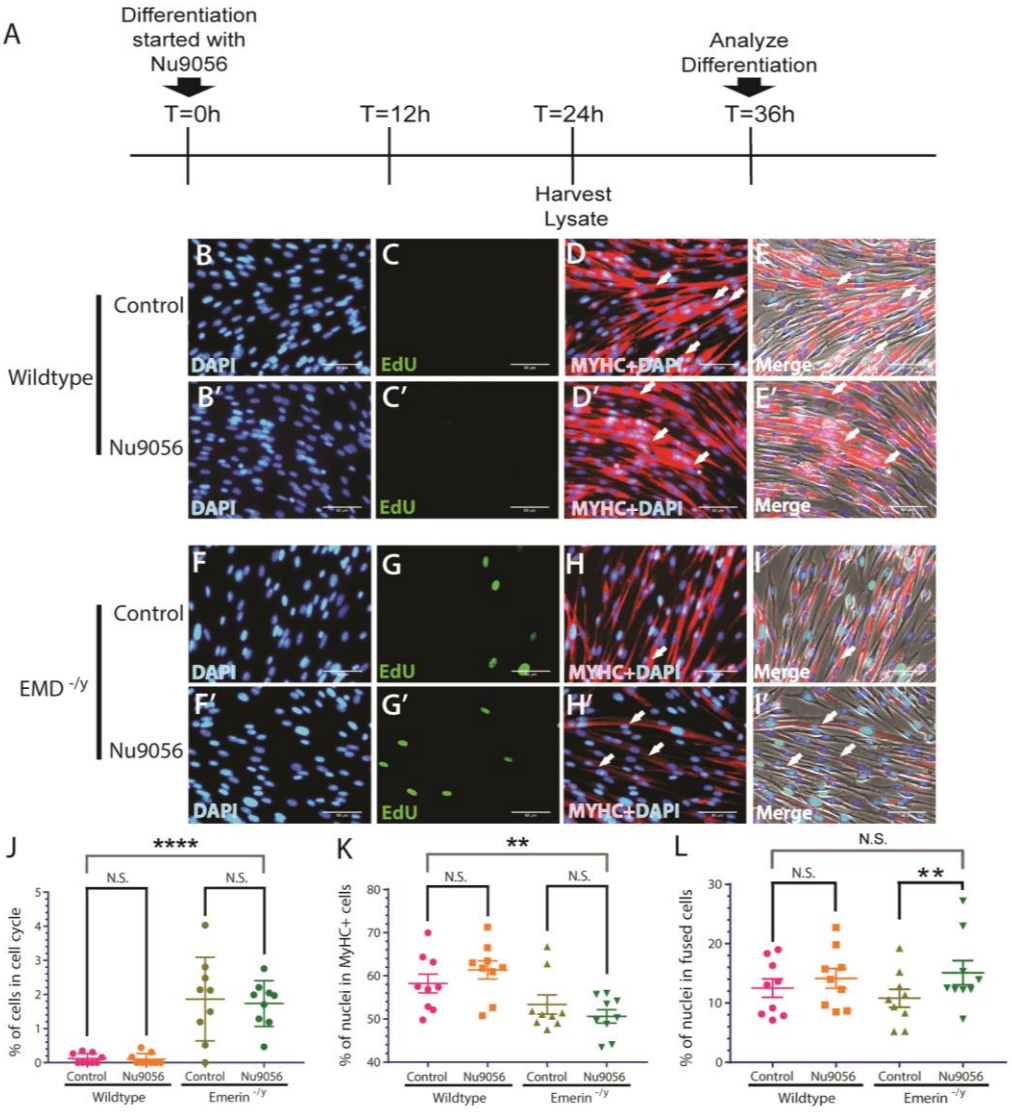
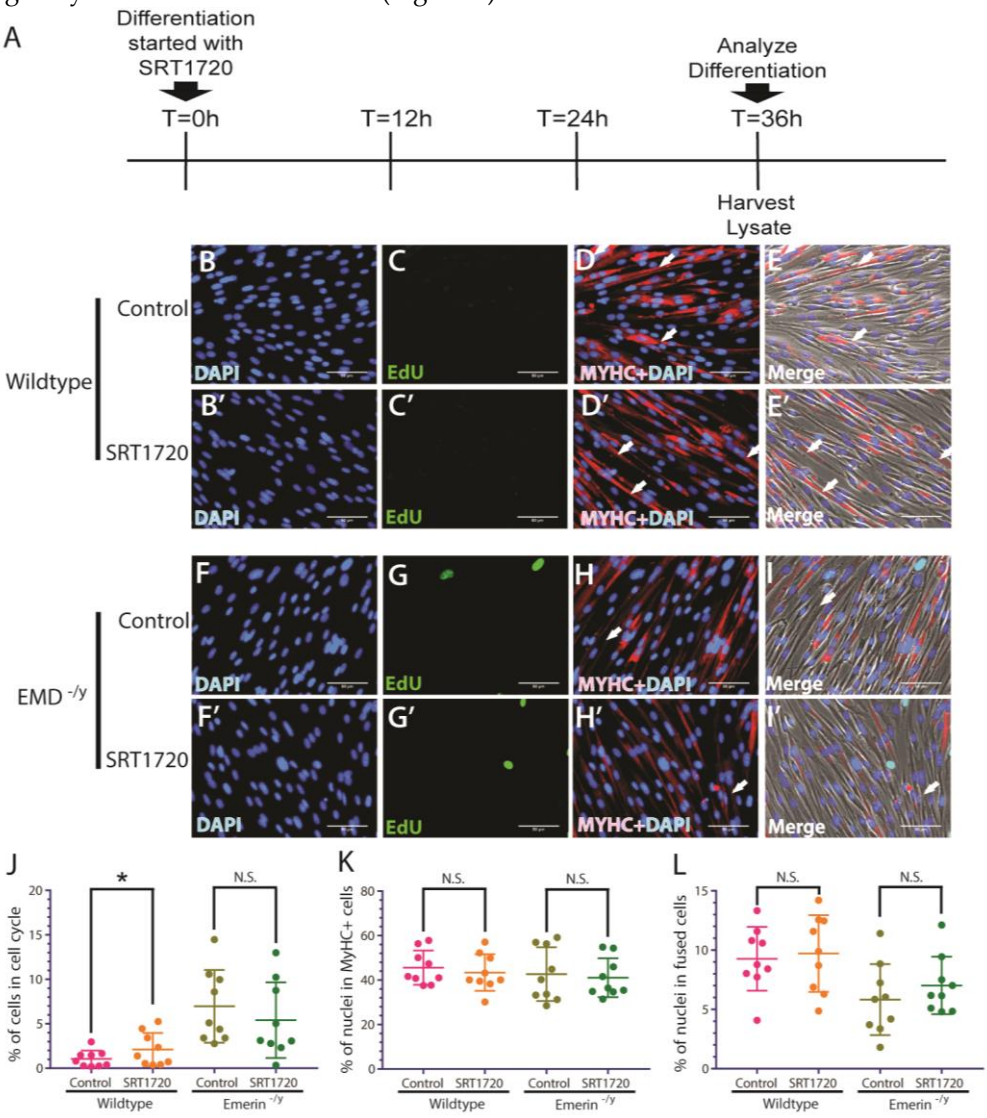


Figure 3. Inhibition of HAT activity with Nu9056 treatment rescues myotube formation in emerin-null myogenic progenitors. (A) Timelines showing the time point Nu9056 was added and whole cell lysate collection for western blot analysis. Representative images at 40X magnification of vehicle-treated wildtype (B-E) or emerin-null (F-I) and Nu9056-treated wildtype (B′-E′) or emerin-null (F′-I′) cells 36 h after initiating differentiation. Arrows mark myotubes (e.g., 3 myotubes in I′ vs 1 myotube in I). (J-L) Quantification of >500 nuclei for each experimental treatment (n=3) was carried out to determine the percentage of myogenic progenitors in the cell cycle (J), expressing MyHC (K) and formed tubes (L) 36 h after inducing differentiation. Results are mean±s.d. of n=3; N.S., not significant; **, P<0.01; ****, P<0.0001 using paired, two-tailed t-tests.

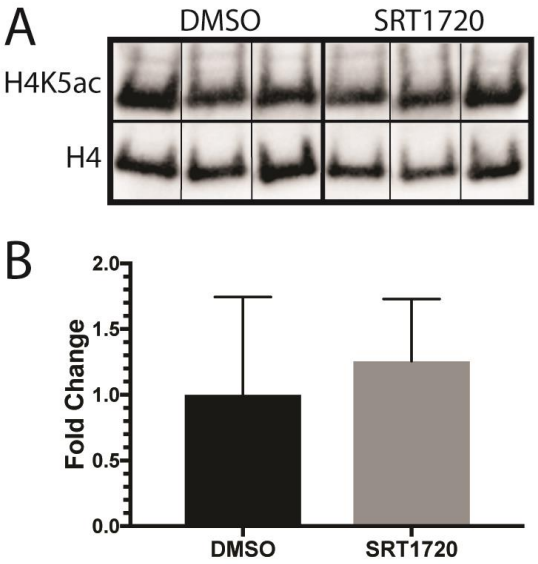
Western blotting using antibodies against H4 and H4 acetylated on lysine 5 (H4K5ac) was used to confirm L002 and Nu9056 inhibited H4K5 acetylation. We previously showed emerin-null cells increased H4K5ac by 1.9-fold [23]. Treatment of emerin-null progenitors with L002 during differentiation reduced H4K5ac 2.4-fold (Figure 2), comparable to H4K5ac levels in wildtype progenitors. Nu9056 treatment decreased H4K5ac 3.2-fold in emerin-null myogenic

progenitors (Figure 2). Decreased H4K5ac seen in emerin-null myogenic progenitors treated with Nu9056 is similar to the H4K5ac levels seen in differentiating wildtype progenitors.

A Sirtuin 1 (SIRT1) activator was used to confirm rescue of emerin-null progenitor differentiation was due to changes in acetylation of HDAC3 target residues (e.g., H4K5ac). Unlike HDAC3, SIRT1 is an NAD+-dependent protein deacetylase that was shown to deacetylate a number of protein targets within the cell, including p53 and PGC1◎ [31]. SIRT1 has not been shown to effect H4K5, H4K8 or H4K12 acetylation [32]. 1.5 μM SRT1720 was added to wildtype or emerin-null myogenic progenitors upon differentiation induction and differentiation was analyzed after 36 hours (Figure 4A). SRT1720 failed to rescue cell cycle withdrawal of differentiating emerin-null progenitors, as 7.0% of DMSO-treated and 5.4% of SRT1720-treated cells were cycling (Figure 4G, J; p=0.09). 41.1% of SRT1720-treated differentiating emerin-null progenitors expressed MyHC compared to 42.7% of DMSO-treated emerin-null progenitors (Figure 4H, K; p=0.49). SRT1720 treatment also failed to rescue myotube formation in emerin-null progenitors (Figure 4I, L; p=0.44). Western blotting confirmed the levels of H4K5ac were unchanged by treatment with SRT1720 (Figure 5).



170 **Figure 4. SIRT1 activation with SRT1720 treatment does not change cell cycle exit, myosin heavy**
171 **chain expression, or myotube formation in emerin-null myogenic progenitors.** (A) Timelines
172 showing the time point SRT1720 was added and whole cell lysate collection for western blot
173 analysis. Representative images at 40X magnification of vehicle-treated wildtype (B-E) or emerin-
174 null (F-I) and SRT1720-treated wildtype (B′-E′) or emerin-null (F′-I′) cells 36 h after initiating
175 differentiation. Arrows mark myotubes (e.g., 1 myotube in I′ and I). (J-L) Quantification of >500
176 nuclei for each experimental treatment (n=3) was carried out to determine the percentage of
177 myogenic progenitors in the cell cycle (J), expressing MyHC (K) and formed tubes (L) 36 h after
178 inducing differentiation. Results are mean±s.d. of n=3; N.S., not significant; *, P<0.05 using paired,
179 two-tailed t-tests.



180

181 **Figure 5. H4K5 acetylation remains unchanged SRT1720 treatment.** (A) Western blotting of whole
182 cell lysates treated with DMSO or SRT1720 to analyze changes in H4K5 acetylation. DMSO was the
183 treatment control. Each treatment was done in triplicate. (B) Quantification of western blots was
184 done by densitometry. H4K5 levels were normalized to H4 levels for each sample. SRT1720-treated
185 emerin-null cells were normalized to DMSO-treated emerin-null cells. Results are mean±s.d. of n=3
186 for each condition; N.S., not significant using paired, two-tailed t-tests.

187 **3. Discussion**

188 The studies presented here used a cell-based system to follow differentiation, in which
189 myotubes are formed by myoblast-to-myoblast fusion or myoblast-to-myotube fusion. Upon
190 stimulation of myogenic progenitors to differentiate, transcriptional reprogramming is initiated,
191 leading to cell cycle exit. This reprogramming activates the myogenic differentiation program
192 and represses the proliferative program, thereby leading to myoblast commitment followed by
193 fusion to form myotubes. Transcriptional reprogramming is compromised in emerin-null
194 progenitors [33]. The failure of emerin-null progenitors to coordinate the temporal reorganization
195 of their genome during differentiation is predicted to cause this defective transcriptional
196 reprogramming.
197
198 Emerin binds directly to HDAC3, the catalytic component of the Nuclear Co-Repressor
199 (NCoR) complex [27, 34] and activates its activity. Emerin-binding recruits HDAC3 to the nuclear

envelope. The functional interaction between emerin and HDAC3 coordinates the spatiotemporal nuclear envelope localization of genomic regions containing important transcription factors that control the temporal expression of differentiation genes [26, 27]. Loss of emerin disrupts this genomic reorganization resulting in impaired myogenic differentiation. Activation of HDAC3 rescues emerin-null myotube formation [23, 26]. Nuclear envelope localization of HDAC3 is also important in cardiomyocyte differentiation [35]. In the absence of nuclear envelope-localized HDAC3, repressed genomic loci were aberrantly localized to the nuclear interior resulting in precocious differentiation. Thus, controlling HDAC3 nuclear envelope localization and activation is an important regulatory mechanism used to regulate differentiation.

The results presented here support the role of emerin in controlling histone acetylation dynamics by regulating HDAC3 activity. We predict loss of emerin disrupts these dynamics resulting in failure to coordinate the transcription activation and repression needed for transcriptional reprogramming at the onset of differentiation. This initial reprogramming event triggers the coordinated temporal expression of transcription factors required for later differentiation transitions. Emerin is proposed to play an important role in regulating myogenic differentiation by regulating chromatin dynamics, since treatment of emerin-null progenitors with small molecules that restore histone acetylation dynamics (e.g., HDAC3 activators, HAT inhibitors) rescue differentiation.

Using HATi specifically targeting acetylation of residues deacetylated by HDAC3, we found HAT inhibition rescued emerin-null differentiation. This recapitulated the rescue seen by treatment of emerin-null progenitors with an HDAC3 activator. Thus, H4K5 acetylation dynamics are predicted to be important for ensuring proper transcriptional reprogramming upon differentiation induction (Figure 6). Similar to HDAC3 activation, HDAC inhibition primarily affected later differentiation transitions [23], suggesting emerin regulation of HDAC3 activity controls the temporal expression of these later genes. This may result from failure to completely reprogram the transcriptome upon differentiation induction or by specifically regulating the latter steps of the gene expression program.

HAT inhibition and HDAC3 activation rescued the latter steps of emerin-null myogenic differentiation. This suggests emerin regulation of H4K5ac dynamics during transcriptional reprogramming may impair the complete reprograming during differentiation to effect genes that act at the latter stages of differentiation (e.g., myotube formation). Alternatively, emerin regulation of H4K5ac levels, and thus transcriptional activity, may specifically regulate genes important for cell fusion or myotube maturation. Consistent with these results, HDAC3 inhibition by RGFP966 blocked MyHC expression and fusion in both differentiating wildtype and emerin-null myogenic progenitors [23]. We propose H4K5 acetylation levels are tightly regulated and that increases or decreases in H4K5 acetylation levels impairs the transition from committed, differentiating myoblasts to myotubes by altering transcription reprogramming upon differentiation induction. These results support pharmacological targeting of H4K5 acetylation as a promising therapeutic approach for rescuing muscle regeneration in EDMD.

Interestingly, L002 rescued myoblast commitment, whereas Nu9056 failed to rescue myoblast commitment, as measured by MyHC expression. Myoblast commitment regulation is independent of H4K5ac dynamics. Rather, it is likely caused by L002 inhibiting p300- or pCAF/GCN5-mediated acetylation of H3K18 and H3K27 or H3K9, respectively. L002 was identified as a p300-specific inhibitor [29]. p300 specifically acetylates H3K18 and H3K27.

However, at submicromolar levels, L002 inhibits H4 acetylation and can inhibit H3K9ac. Thus, we predict the acetylation dynamics of H3K9ac, H3K18ac and H3K27ac are altered during emerin-null differentiation to inhibit myoblast commitment (Figure 6). Supporting this hypothesis, emerin-null myogenic progenitors have less H3K27me3 and H3K9me3 and more H3K4me3, indicative of more relaxed chromatin [27]. It will be important to elucidate how emerin regulates the dynamic epigenetic changes occurring during myogenic differentiation to control the transcriptional programs needed for passage through specific transition points.

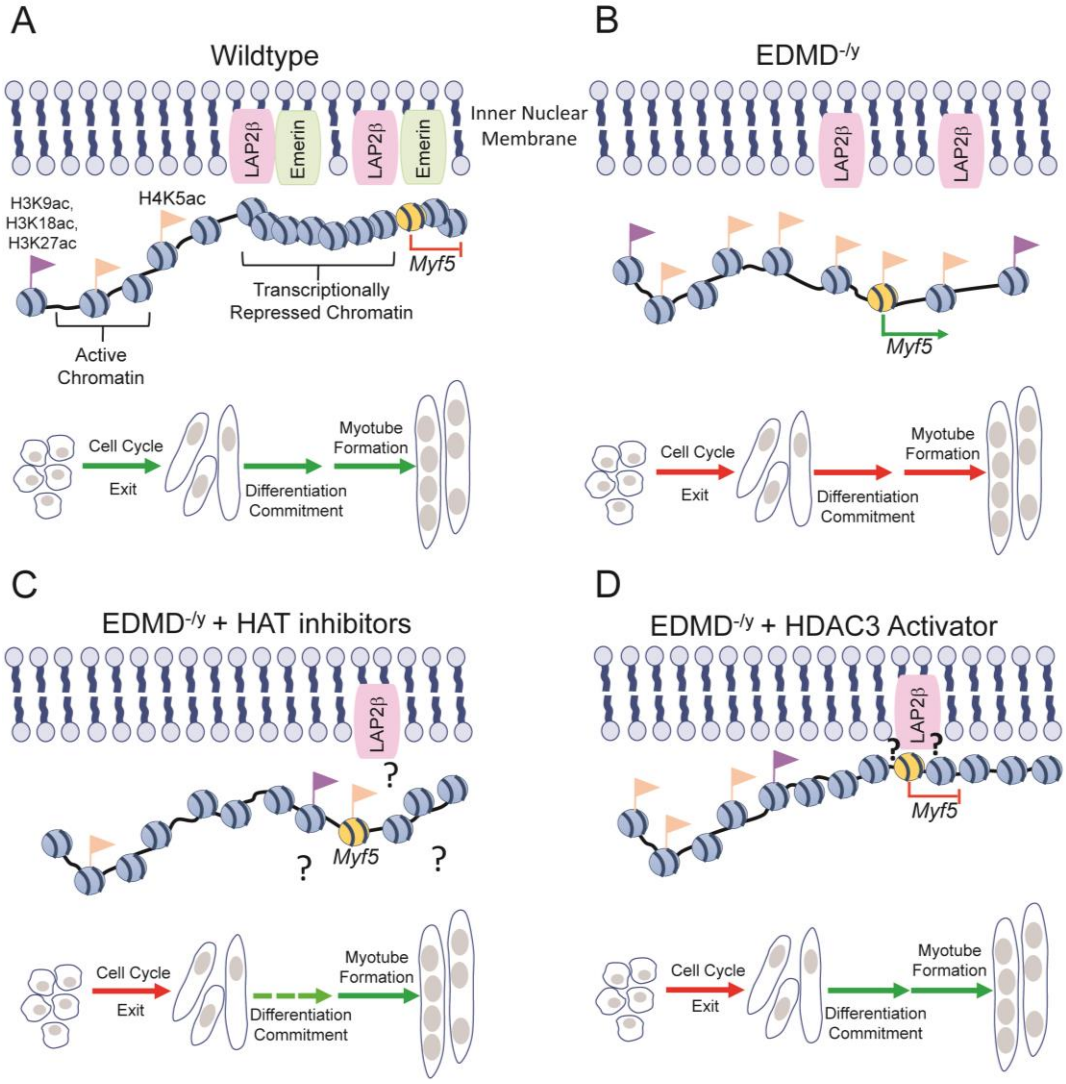


Figure 6. Effects of altered H4K5 acetylation dynamics on myogenic differentiation. (A) Wildtype myogenic differentiation. (B) Lack of emerin results in impaired differentiation with loss of Myf5 localization and increased H4K5 and H3 acetylation states. (C, D) Treatment with HAT inhibitors (C) and HDAC3 activators (D) restore the H4K5 and H4K5ac equilibrium and rescue myotube formation with no effect on cell cycle exit. (C) H3K9, H3K18, and H3K27 acetylation states are partially blocked by treatment with the HAT inhibitor L002 causing incomplete rescue of differentiation commitment. (D) Treatment with HDAC3 activators induces Myf5 nuclear envelope localization. Red arrows indicate impaired differentiation programming, solid dark green arrows indicate normal differentiation programming, and dashed light green arrow signifies partially impaired differentiation.

266 **4. Materials and Methods**
267
268 *4.1. Pharmacological treatments*
269 A 1.0 mM stock solution of L002 in DMSO was added to a final concentration of 0.5 μM in
270 differentiation medium. A 1.0 mM stock solution of Nu9056 in DMSO was added to a final
271 concentration of 0.5 μM in differentiation medium. A 3.0 mM stock solution of SRT1720 in DMSO
272 was added to a final concentration of 1.5 μM in differentiation medium. Differentiation media
273 containing each inhibitor or DMSO was added to induce differentiation of wildtype or emerin-
274 null myogenic progenitors.
275
276 *4.2. Cell culture*
277 Cell culture of proliferation and differentiation of H2Ks were done as previously described
278 [23]. Briefly, for proliferation, wildtype and emerin-null H2K myogenic progenitors were seeded
279 onto tissue culture plates (Falcon cat no. 353046 and 3530003) and maintained at 33°C and 10%
280 CO_2 in proliferation medium (high glucose DMEM supplemented with 20% heat-inactivated fetal
281 bovine serum, 2% L-glutamine, 2% chick embryo extract, 1% penicillin/streptomycin, sodium
282 pyruvate, 20 units/ml γ-interferon, ThermoFisher Scientific). The plates were coated with 0.01%
283 gelatin (Sigma-Aldrich) prior to seeding.
284
285 Wildtype and emerin-null H2K myogenic progenitors were seeded onto 12 well tissue
286 culture plates coated with 0.01% gelatin (Sigma-Aldrich) for differentiation induction. Cells were
287 seeded at 23,500 cells/cm2 in proliferation media for 24h at 33°C and 10% CO_2. Differentiation
288 was stimulated by replacing the proliferation medium with differentiation medium (high glucose
289 DMEM with sodium pyruvate, 5% horse serum, 2% L-glutamine, ThermoFisher Scientific). The
290 cells were maintained at 37°C and 5% CO_2 throughout differentiation.
291
292 *4.3. EdU assays and immunofluorescence microscopy*
293 Cells were treated with 10 μM EdU (ThermoFisher Scientific) in DMSO 2h prior to fixing,
294 while incubating at 37°C and 5% CO_2. Cells were then fixed with 3.7% formaldehyde for 15 min
295 and washed three times with PBS. Fixed cells were then stored at 4°C with 0.1% sodium azide in
296 PBS. The cells were permeabilized with 0.5% triton X-100 in PBS for 20 minutes, washed twice
297 with 3% BSA in PBS for five minutes per wash and treated with the Click-IT EdU reaction
298 cocktail for 25 minutes. Cells were washed with PBS and blocked for 1 h at room temperature
299 with 3% BSA with 0.1% Triton X-100. MyHC antibodies (1:20, Santa Cruz Biotechnologies, H-300
300 for L002 and Nu506 experiments; 1:50, Santa Cruz Biotechnologies, B-5 for SRT1720 treatments)
301 were added and the cells were incubated at room temperature for 1 h. The cells were washed
302 with PBS three times and treated with Alexa Fluor 594 secondary antibodies (1:200, C10637;
303 A11032, ThermoFisher Scientific) at room temperature for 1 hour, washed with PBS and
304 incubated with DAPI for 5 minutes.
305
306 Images were taken using the EVOS-FL imaging system (ThermoFisher LifeSciences) for
307 experiments with L002. The remainder of the images were taken with the EVOS-FL Auto
308 (ThermoFisher LifeSciences). All images were obtained using a long working distance 40x
309 objective. At least three replicates, with each replicate containing three culture wells per group,
310 were done for each drug treatment. Images from five different sections from each well were
311 taken, with each section containing approximately 50-200 cells. The total number of cells
312 analyzed for each experiment ranged between 500-1500.
313

The cell counter plugin on ImageJ was used to count proliferating cells. The percent of cells still in the cycle was determined by dividing the number of EdU positive nuclei by the total number of nuclei. The DAPI and MyHC images were superimposed to calculate the percentage of cells expressing MyHC. Myotube formation was determined by superimposing the phase contrast image, which allowed for monitoring nuclei within a shared cytoplasm, with DAPI and MyHC images. Myotube formation, or the differentiation index, was determined by counting the number of nuclei in MyHC positive cells that contained three or more nuclei divided by the total number of nuclei in the field.

4.4. Western Blotting

Differentiated H2K cells were resuspended directly in sample buffer and 50,000 to 100,000 cell equivalents were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were blocked either at room temperature for 2 h or overnight at 4°C in 3% BSA in PBST (PBS with 0.1% Tween 20). Antibodies against H4 (1:50,000; Millipore, 05-858) and H4K5-ac (1:1,000; Millipore, 07-327), were then incubated either at room temperature for 2 h or overnight at 4°C. The membranes were washed three times in PBS and incubated with Goat Anti-Rabbit HRP secondary antibody (1:10,000; ThermoFisher Scientific) in PBST either at room temperature for 2 h or overnight at 4°C. The membranes were treated with ECL chemiluminescence detection reagent (GE healthcare, product # RPN2106V1 and RPN2106V20 and imaged using the Bio-Rad Chemidoc system (Bio-Rad Laboratories). Densitometry was done using ImageLab software (Bio-Rad Laboratories) as per the manufacturer's instructions.

Author contributions: Conceptualization, methodology, and validation: J.M.H.; Formal analysis: J.M.H. and K.A.B.; Investigation: K.A.B. and J.A.E.; Resources: J.M.H.; Data curation: J.M.H., K.A.B., and J.A.E.; Writing-original draft: J.M.H.; Writing- reviewing and editing: J.M.H., K.A.B., and J.A.E.; Visualization: K.A.B., J.A.E., and J.M.H.; Supervision: J.M.H.; Project administration: J.M.H., K.A.B., and J.A.E. Funding acquisition: J.M.H.

Acknowledgements: We thank the members of the Holaska laboratory for the many helpful discussions regarding these studies and preparation of this manuscript. This study was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number R15AR069935 (to J.M.H.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Conflicts of interests: The authors declare no competing or financial interests.

Abbreviations:

BAF: Barrier-to-Autointegration Factor
BSA: Bovine Serum Albumin
bHLH: Basic Helix-Loop-Helix
DAPI: 4',6-diamidino-2-phenylindole
DMEM: Dulbecco's Modified Eagle's medium
DMSO: Dimethyl Sulfoxide
EDMD: Emery-Dreifuss Muscular Dystrophy
EdU: 5-Ethynyl-2'-deoxyuridine
H4K5: Histone 4 lysine 5
H4K5ac: Histone 4 acetylated on lysine 5
HAT: Histone Acetyltransferase
HATi: Histone Acetyltransferase inhibitor
HDAC: Histone Deacetylase

HDACi: Histone Deacetylase Inhibitor
LAD: Lamina Associated Domain
LAP: Lamina Associated Polypeptide
NCoR: Nuclear Co-Repressor
MEF: Myocyte Enhancer Factor
MyHC: Myosin Heavy Chain
PBST: Phosphate Buffered Saline with Tween
SIRT1: Sirtuin 1

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