JCB

GCN5 Maintains Muscle Integrity by Acetylating YY1 to Promote Dystrophin Expression

Gregory Addicks, Hongbo Zhang, Dongryeol Ryu, Goutham Vasam, Alex Green, Philip Marshall, Sonia Patel, Baeki Kang, Doyoun Kim, Elena Katsyuba, Evan Williams, Jean-Marc Renaud, Johan Auwerx, and Keir Menzies

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April 30, 2021

Re: JCB manuscript #202104022

Prof. Keir Menzies
University of Ottawa
451 Smyth Rd.
Ottawa, ON K1H 8M5
Canada

Dear Prof. Menzies,

Thank you for submitting your manuscript entitled "GCN5 Maintains Muscle Integrity by Acetylating YY1 to Promote Dystrophin Expression". Your manuscript has been reviewed by two experts in the field, whose comments are appended below. Although the reviewers express potential interest in this work, significant concerns unfortunately preclude publication of the current version of the manuscript in JCB. You will see that while both reviewers felt the paper made some strong points especially in the molecular analysis, they also felt that the analysis of the phenotype was not deep and complete enough. Editorially, we agree that this is a significant concern.

Based on these comments I am sorry to write that we cannot accept the paper for publication in the JCB in its current form. We are willing however to consider a revised manuscript that would need to address all the concerns expressed by each reviewer. Given the nature of the comments made by the reviewers I cannot insist enough on the necessity to address all their comments with significant new experimental data where requested. If you are willing to do so we would be happy to consider a revised manuscript that will be reviewed by the same two experts. Please bear in mind that the JCB allows only one round of extensive revision.

Please let us know if you are able to address the major issues outlined above and wish to submit a revised manuscript to JCB. Note that a substantial amount of additional experimental data likely would be needed to satisfactorily address the concerns of the reviewers. As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing measures that limit spread of COVID-19 also pose challenges to scientific researchers. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

If you choose to revise and resubmit your manuscript, please also attend to the following editorial points. Please direct any editorial questions to the journal office.

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Text limits: Character count is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Your manuscript may have up to 10 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, https://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Your manuscript may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

If you choose to resubmit, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

Regardless of how you choose to proceed, we hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised. You can contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for thinking of JCB as an appropriate place to publish your work. I wish you the best of luck with the revision if you decide to go that way.

Best regards,

Gerard Karsenty, MD, PhD
Monitoring Editor

Andrea L. Marat, PhD
Senior Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

The manuscript reports a potentially novel pathway for regulation of expression of the muscle protein dystrophin. The authors studied the effects of loss of function of GCN5 in skeletal muscle fibers. Through transcriptomic analyses expression of dystrophin and other DAPC members were significantly downregulated in GCN5skm-/- mice. The authors identified YY1 as a target of GCN5 acetylation, specifically two lysine residues located in the C-ter of YY1.
Overall, the study reports novel findings on regulation of muscle gene expression following acetylation (or loss of acetylation from GCN5). Interestingly, the authors report that expression of dystrophin and its associated protein complex might be a target of GCN5 regulation via YY1 modification. While these findings are novel and open the possibility of secondary regulation of dystrophin expression through epigenetic modifications, a number of revisions are suggested that can strengthen the overall message and conclusions of the manuscript.

Figure 2. In panel b, expression of dystrophin in WT animals appears to highlight the interstitial spaces between myofibers instead of the sarcolemma. It is not clear if the same primary antibody to dystrophin was used for western blot and IF analyses. If this is the case (mouse monoclonal primary), it is likely that some of the intermyofiber reactivity is due to binding of the secondary antibody to the extracellular matrix. It is recommended that a primary antibody raised in a different species is used to detect dystrophin (for example a rabbit anti-dystrophin). Additionally, a negative control staining should be included where the secondary antibody alone is applied to control and mutant tissue sections, to show no reactivity in the intermyofiber space. Microscopic images should be acquired at the same exposure for all samples.
For panels 2c and 2d, quantification of band intensities detected by western blot and statistical analyses should be performed to support that dystrophin protein is significantly decreased in mutant animals. There seems to be variability in expression in both WT and mutant animals that might not necessarily result in significantly different protein expression (as opposed to RNA expression data).
Confirmatory experiments on 'leakiness' of muscle fibers should be performed using an anti-IgM antibody.
Counting of centralized nuclei in 3 fields/section does not seem sufficiently comprehensive if low numbers of CLN myofibers are present, entire sections should be examined.
Please clarify in figure 2i what comparison is significant in WT vs mutant animals (what contraction number or overall contractions). Overall, mutant mice do not seem to show many deficiencies even when aged (Suppl. Fig. 2), therefore many of the noted transcriptional alterations might not result in muscle disease or significant weaknesses. Histological images of the diaphragm should be shown, as they might be able to exhibit some abnormalities, if indeed dystrophin reduction is more disruptive in this muscle.

Figure 4. Expression of dystroglycan or NOS should be validated at the protein levels, since special emphasis is given to these proteins in the manuscript (page 12).
In 4c, quantification of dystrophin reduction and whether it is statistically significant compared to baseline (no YY1 overexpression) should be provided.

Figure 5. In panel a, there is a bubble in one of the lanes where YY1ac-Lys is shown. A different blot should be provided. It also might be possible to quantify the amount of immunoprecipitated YY1ac-Lys using total (input YY1) as a denominator.

Reviewer #2 (Comments to the Authors (Required)):

The manuscript by Addicks et al. reports on a role for GCN5 in regulating expression of dystrophin and other dystrophin-associated protein complex factors in skeletal muscle. The authors show that GCN5 acetylates the transcriptional repressor YY1, thereby inhibiting its ability to bind DNA. YY1 represses dystrophin expression, so genetic removal of its inhibitor, GCN5, in skeletal muscle cells results in low levels of dystrophin. This in turn leads to signs of muscle damage in response to eccentric contraction. Meta-analysis of published transcriptome datasets reveals correlations between Gcn5/YY1 and dystrophin

expression in muscle atrophy and disease in mice and humans.

The findings reported in this manuscript are interesting and open a window on regulation of dystrophin expression. The molecular biology portions of the paper are strong, but the data characterizing the muscle phenotype need to be improved and broadened to be convincing. The lack of emphasis on this part of the study also makes the manuscript an awkward fit for JCB.

1. The data in Figure 2, which contains almost all the dystrophy-related phenotypic analysis of Gcn5(Skm-/-) mice, fall well below the standard set in the other figures, in breadth and quality. The myofibers in panel b appear mishappen, likely due to a sectioning artifact, resulting in thick, uneven staining for dystrophin, even in the control. In panel e, the duplicates for control and mutants are very different from each other. Is this meant to illustrate a spectrum of responses? For the Evans Blue dye uptake assay, in one control section the fibers all appear negative and in the other section the fibers all appear positive (if not quite as floridly as with the positive fibers in the mutant). Again, the sections do not appear to be of high quality. Micrographs of entire muscle sections, not just selected regions, would be helpful. Some basic information is also lacking: How many mice were studied in these experiments? How old were they? For these experiments (not just the metabolism studies), how long after the treadmill running were the mice analyzed? In panel h, again the sections are a problem, as individual myofibers are impossible to discern, so a reader is unable to identify the central nuclei (a similar problem exists for Figure S2).

I suspect that the authors' conclusions about the effects of Gcn5 loss on eccentric contraction injury are correct, but the figures do not make a compelling case for it.

2. The authors state that "GCN5 deficiency in skeletal muscle leads to a progressive muscle myopathy that is primarily identified by a susceptibility to eccentric muscle damage" and "...an overall phenotype that resembles muscular dystrophy". The phenotypic analysis does not support these conclusions. A time course of the phenotype in the absence and presence of injury is needed to establish a progressive nature and should include measurements of fiber size, fibrosis, etc. Additionally, the emergence of central nuclei, which is hard to visualize from the figures (see above), could be from accretion of muscle stem cell nuclei (as is true with Duchenne muscular dystrophy) or from nuclear movement from the periphery of the fiber (as is likely for centronuclear myopathies). Analysis of the muscle stem cell compartment is necessary to judge this. To call this a phenotype that resembles muscular dystrophy requires considerably more work.

Minor points:

1. Several of the western blots (Figures 2c, 2d, 5a) are somewhat unevenly loaded. These would benefit from quantification of the signals, relative to the loading controls.

2. Central nuclei are quantified in Figure 2 and S2 as centralized/total nuclei, a value that must be lower than 1. It appears that the control is set to 1 and that what is reported is a fold-increase value. The axis label should be corrected, but it would be valuable to report the percentage of centrally nucleated fibers for control and mutant mice.

Reply to reviewers

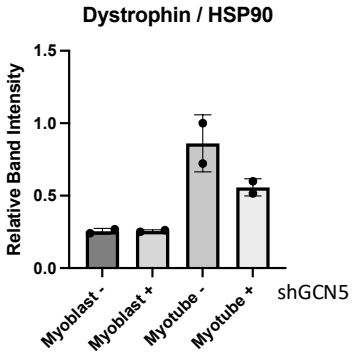
Reviewer 1:

Figure 2. In panel b, expression of dystrophin in WT animals appears to highlight the interstitial spaces between myofibers instead of the sarcolemma. It is not clear if the same primary antibody to dystrophin was used for western blot and IF analyses. If this is the case (mouse monoclonal primary), it is likely that some of the intermyofiber reactivity is due to binding of the secondary antibody to the extracellular matrix. It is recommended that a primary antibody raised in a different species is used to detect dystrophin (for example a rabbit anti-dystrophin). Additionally, a negative control staining should be included where the secondary antibody alone is applied to control and mutant tissue sections, to show no reactivity in the intermyofiber space. Microscopic images should be acquired at the same exposure for all samples.

We thank the reviewers for highlighting the need for improved immunohistochemical stains and proceeded with their suggested controls. Unfortunately, we suspect that some of the issues with staining arose from a low-quality freeze artifacts of the muscle tissue. As a result, we used a new cohort of mice to create a new set of tissues for isopentane/N2 freezing and sectioning. The resulting images included in Fig. 2b do not exhibit intermyofiber reactivity and include images for both rabbit anti-dystrophin/dapi and rabbit secondary/dapi controls. We have also updated the methods section to reflect the antibodies used (pg. 16 of supplementary). All our images are taken using the same exposure time.

For panels 2c and 2d, quantification of band intensities detected by western blot and statistical analyses should be performed to support that dystrophin protein is significantly decreased in mutant animals. There seems to be variability in expression in both WT and mutant animals that might not necessarily result in significantly different protein expression (as opposed to RNA expression data).

As suggested, we have analyzed dystrophin expression in GCN5$^{skm+/+}$ and GCN5$^{skm-/-}$ mice by quantifying western blot band intensities and normalizing to loading controls (i.e. HSP90) to demonstrate a significant change in dystrophin levels in Fig. 2c. Below we included the quantification of the western blot that exhibited myoblast differentiation (with and without shGCN5) (Fig. 2a), which shows a trend for reduced dystrophin expression in myotubes with (+) and without (-) shGCN5 treatment.



To further address the reviewer's comment, we have observed that there is some variability of dystrophin expression in both GCN5$^{skm+/+}$ muscle tissue and primary myotubes across mice. The variability in myoblast dystrophin expression is also observed in Fig 5c where 3 different control GCN5$^{skm+/+}$ animals were used to create 3 separate control myoblast cell lines which were then

differentiated. Specifically, myotubes differentiated from myoblasts isolated from animal #2 exhibit higher dystrophin expression than animal #1 and #3 in both control and YY1 OE experiments despite similar loading. Nonetheless, there is clear evidence that dystrophin expression is reduced via the GCN5/YY1 mechanism, as shown by Figures 1b,c; 2a,b,c,d; 5b,c,d; 7d, along with supporting evidence of a phenotype with similarities to Duchenne's muscular dystrophy.

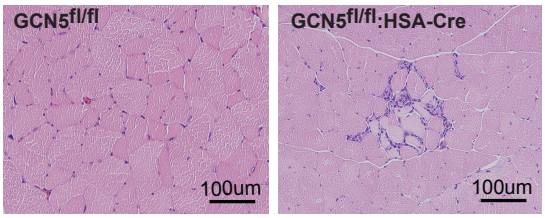
Confirmatory experiments on 'leakiness' of muscle fibers should be performed using an anti-IgM antibody.

We created a new cohort of 6-month-old animals for downhill running to improve the quality of our tissue sections. We confirmed again that moderate downhill running induced muscle damage in GCN5$^{skm-/-}$ mice as dictated by increased Evans Blue and anti-IgM staining. Fig 3a shows full images of the entire right quadriceps muscle for both the anti-IgM/dapi and anti-IgM/Evans blue/dapi conditions. This illustrates a strong consensus between the two techniques (IgM versus Evans blue) and confirms our "leakiness" phenotype. We thank the reviewers for this suggestion as these images now better reveal the phenotype of GCN5$^{skm-/-}$ mice.

Counting of centralized nuclei in 3 fields/section does not seem sufficiently comprehensive if low numbers of CLN myofibers are present, entire sections should be examined.

We counted approximately 900 nuclei per genotype (300 nuclei per animal) with up to 135 of those nuclei being centralized in the gastrocnemius of GCN5$^{skm-/-}$ mice, which was sufficient for our statistical analysis. In addition, we repeated this same assay in the quadriceps for these animals yielding similar results. We now show our results for both the gastrocnemius and quadriceps muscles in the main figures (Fig. 3e). To substantiate the damage found in aged GCN5$^{skm-/-}$ mice we also observed lesions in these mice that were not present in the wt mice but did not see consistent results across all GCN5$^{skm-/-}$ mice to include in our manuscript (see below).

Gastroc



Please clarify in figure 2i what comparison is significant in WT vs mutant animals (what contraction number or overall contractions).

Thank you for finding this oversight in our explanation of our statistics. Our analysis was a two-way ANOVA and upon revisiting the stats we observed that the level of significance for the main effect was understated and should have been described as $P < 0.0001$ (Fig. 3d). There was no effect observed for any individual time point when performing multiple comparisons. Although not included in the manuscript, unpaired t-tests show significance for contraction #s 4, 5, 6, 7, 9 and 10.

Overall, mutant mice do not seem to show many deficiencies even when aged (Suppl. Fig. 2), therefore many of the noted transcriptional alterations might not result in muscle disease or significant weaknesses.

Given that there is not a complete ablation of dystrophin expression in GCN5$^{skm-/-}$ mice, the expected phenotype deficiencies would be less severe than those in mdx mice (the knockout model of dystrophin), which are also not very drastic unless exposed to eccentric contractions. This has been known as a potential limitation of the mdx mouse model for muscular dystrophy, given that the phenotype of these mdx mice do not replicate the robust deterioration in muscle function in humans at young ages. Notably, we found reduced force production in GCN5$^{skm-/-}$ versus GCN5$^{skm+/+}$ diaphragms in response to *ex vivo* eccentric contraction (Fig. 3d) and more muscle damage with downhill running in GCN5$^{skm-/-}$ quadriceps versus control mice (Fig. 3a), as would be expected with reduced dystrophin expression. Nonetheless, the primary finding rests on the importance of identifying a novel signalling pathway that can regulate dystrophin expression via the GCN5-directed acetylation of YY1.
Our updated manuscript also now includes additional observations of diaphragm fibrosis (Fig. 2d) and abnormal sarcomere structures in EDL muscle of GCN5$^{skm-/-}$ mice (Fig. 2f). Ultimately, we feel that the findings presented in this manuscript could lead to potential pharmaceutical implications during aging or disease where enhanced dystrophin expression could have a beneficial impact on muscle integrity and health.

Histological images of the diaphragm should be shown, as they might be able to exhibit some abnormalities, if indeed dystrophin reduction is more disruptive in this muscle.

We agree with the above comment, specifically since we observed a reduction in *ex vivo* diaphragm force production with repeated eccentric contractions. We hence generated a new cohort of mice to obtain diaphragm tissues and now confirm that dystrophin expression is reduced in the diaphragm (Fig. 2d) leading to fibrosis (Fig. 2e) in GCN5$^{skm-/-}$ mice compared to otherwise healthy tissue of control mice. Not included in the manuscript, we also observed that some diaphragm fibres were stained with Evans blue in one of three GCN5$^{skm-/-}$ mice examined and in none of the control GCN5$^{skm+/+}$ animals (see below). We suspect that this phenotype could become significant if we were to have time to age a cohort of mice to 12 months or beyond.

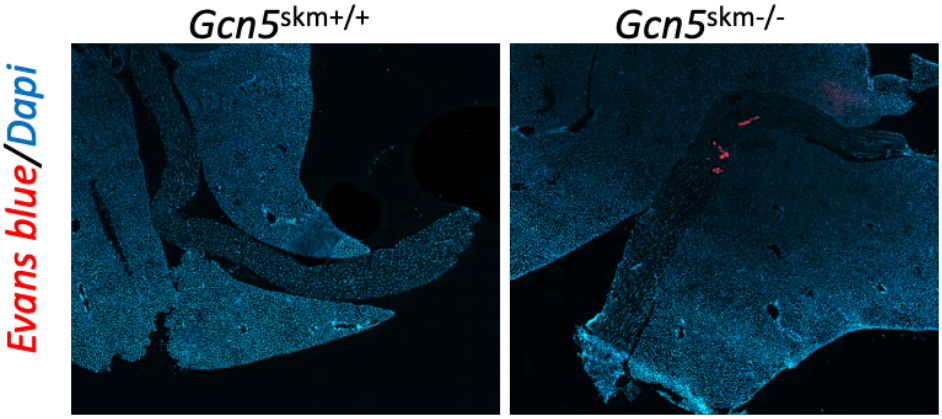


Figure 4. Expression of dystroglycan or NOS should be validated at the protein levels, since special emphasis is given to these proteins in the manuscript (page 12).

We noted dystroglycan binding and nitric oxide synthase regulator activity as downregulated pathways in our comparison of *Gcn5*$^{skm-/-}$ and muscle-specific *Yy1* KO biosets (pg. 12; Fig. 5a) and revealed how these were similar to pathways reduced in the *Gcn5*$^{skm-/-}$ versus *Gcn5*$^{skm+/+}$ bioset comparison (Fig. 1b). We therefore acknowledged the suggestion of the reviewers and examined the expression of α-DG and nNOS and found that there was a significant reduction in α-DG in *Gcn5*$^{skm-/-}$ animals with no change in nNOS (Fig. 2g).

In 4c, quantification of dystrophin reduction and whether it is statistically significant compared to baseline (no YY1 overexpression) should be provided.

We have quantified the reduction of dystrophin between control and YY1 OE and found that the reduction was significant (Fig. 5c).

Figure 5. In panel a, there is a bubble in one of the lanes where YY1ac-Lys is shown. A different blot should be provided. It also might be possible to quantify the amount of immunoprecipitated YY1ac-Lys using total (input YY1) as a denominator.

The IP for YY1 and following immunoblot for ac-Lys was very challenging to perform using mouse muscle tissue. This immunoblot is still our cleanest version following lengthy troubleshooting. Moreover, the data is already substantiated through molecular techniques used to identify GCN5-directed acetylation sites on YY1 as presented in Fig. 6c, S5, and Fig. 7a,c,d.

Reviewer 2.

1. The data in Figure 2, which contains almost all the dystrophy-related phenotypic analysis of Gcn5(Skm-/-) mice, fall well below the standard set in the other figures, in breadth and quality. The myofibers in panel b appear mishappen, likely due to a sectioning artifact, resulting in thick, uneven staining for dystrophin, even in the control.

We thank the reviewer for their comments related to the quality of our muscle sections. Given that this was likely due to freezing artifacts we used a new cohort of mice to create another set of tissues for isopentane/N2 freezing and sectioning. As a result, we replaced anti-dystrophin sections as seen in Fig. 2d. These images do not exhibit intermyofiber reactivity and include images for both rabbit anti-dystrophin/dapi and anti-rabbit secondary/dapi controls.

In panel e, the duplicates for control and mutants are very different from each other. Is this meant to illustrate a spectrum of responses? For the Evans Blue dye uptake assay, in one control section the fibers all appear negative and in the other section the fibers all appear positive (if not quite as floridly as with the positive fibers in the mutant). Again, the sections do not appear to be of high quality. Micrographs of entire muscle sections, not just selected regions, would be helpful. Some basic information is also lacking: How many mice were studied in these experiments? How old were they? For these experiments (not just the metabolism studies), how long after the treadmill running were the mice analyzed?

As mentioned in response to reviewer 1, we feel that we have now adequately addressed the common concerns with regards to our evidence for increased susceptibility to muscle damage in the *Gcn5*$^{skm-/-}$ animals. As explained above, we created a new cohort of animals for downhill running to improve the quality of our tissue sections for staining of muscle damage. This new experimental cohort confirmed our original data. Specifically, from this new cohort we have now included images of the entire right quadricep muscle for both the anti-IgM/dapi and anti-IgM/Evans blue/dapi conditions (Fig. 3a), which were obtained from another 3 mice/group that were injected with Evans blue 15 min after running and sacrificed 24hrs following the downhill run. We have now also added the important basic information that was missing in this figure legend (and other figure legends) and supplementary methods (supplementary pgs.14-15) and apologize for the previous oversight.

In panel h, again the sections are a problem, as individual myofibers are impossible to discern, so a reader is unable to identify the central nuclei (a similar problem exists for Figure S2).

We thank the reviewer for pointing out this issue with our identification of centralized nuclei in samples taken from 12-month-old animals. Given that we could not rapidly create a new cohort of 12-month aged animals to help clarify this data, we have improved the quality/size of the images in the figure and used arrowheads to help readers identify centralized nuclei (Fig. 3e). We also moved Figure S2 to the main Figure panels to help demonstrate the significantly different phenotype that we observed in both quadriceps and gastrocnemius muscles side by side. We feel that maintaining this observed phenotype of aged animals in this figure corroborates data exhibiting susceptibility to muscle damage in younger mice (Figs. 2e, f and 3a, b, d), along with evidence of increased muscle regeneration (Fig. 3c).

I suspect that the authors' conclusions about the effects of Gcn5 loss on eccentric contraction injury are correct, but the figures do not make a compelling case for it.

Given the substantial improvement in figures exhibiting both Evans blue permeated fibers and IgM staining in quadriceps following eccentric damage (Fig. 3a), combined with the increased creatine kinase release from *Gcn5*$^{skm-/-}$ muscle (Fig 3b) and markers of muscle regeneration (Fig. 3c) with eccentric exercise, we feel that we have solidified the clear difference in susceptibility to damage between *Gcn5*$^{skm-/-}$ and *Gcn5*$^{skm+/+}$ animals. In addition, our new observations of reduced dystrophin expression and increased fibrosis (Fig. 2d-e), a known end result of continual muscle damage in muscular dystrophy, in *Gcn5*$^{skm-/-}$ mouse diaphragms helps corroborate our existing data that showed the increased loss in diaphragm strip force during a series of eccentric contractions (Fig. 3d).

2. The authors state that "GCN5 deficiency in skeletal muscle leads to a progressive muscle myopathy that is primarily identified by a susceptibility to eccentric muscle damage" and "...an overall phenotype that resembles muscular dystrophy". The phenotypic analysis does not support these conclusions. A time course of the phenotype in the absence and presence of injury is needed to establish a progressive nature and should include measurements of fiber size, fibrosis, etc.

We have carefully considered the reviewer's opinion about our statements and agree with their suggestion for a time course. Given the time constraints for revisions, we were unable to complete a time series and therefore we have excluded the word "progressive" from our comments about the observed myopathy in *Gcn5*$^{skm-/-}$ mice. We have, however, demonstrated a preponderance of evidence supporting that the myopathy resembles features of muscular dystrophy.

Additionally, the emergence of central nuclei, which is hard to visualize from the figures (see above), could be from accretion of muscle stem cell nuclei (as is true with Duchenne muscular dystrophy) or from nuclear movement from the periphery of the fiber (as is likely for centronuclear myopathies). Analysis of the muscle stem cell compartment is necessary to judge this.

We do appreciate that the accretion of centralized nuclei recapitulates a phenotype found in Duchenne's muscular dystrophy that can be explained by the activation of muscle satellite cells. Beyond the repetitive damage that occurs in mouse models of Duchenne's muscular dystrophy due to the lack of muscle integrity, the satellite cell compartment is also now known to be affected by a lack of dystrophin during satellite cell self-renewal which leads to eventual stem cell exhaustion (PMID: 26569381). However, given that our mouse model is an HSA-cre-driven skeletal muscle-specific GCN5 LOF model, stem cells from this model would not lack GCN5 or dystrophin and so these mice would therefore not recapitulate the satellite cell-specific effects observed in mouse models that lack dystrophin systemically (i.e. mdx mice). Future work will examine the muscle satellite cell compartment using inducible PAX7-cre-driven satellite cell-specific GCN5 LOF mice.

To call this a phenotype that resembles muscular dystrophy requires considerably more work.

We have attenuated our concluding statements (pg. 19) that compare *Gcn5*$^{skm-/-}$ mice to muscular dystrophy to reflect the fact that *Gcn5*$^{skm-/-}$ mice are characterized by a reduction in YY1-directed dystrophin expression, which would not be as severe as the complete lack of dystrophin that is seen in mdx mice. Despite this, we have substantially improved our phenotyping of these mice (improved images representing eccentric damage to quadriceps (Fig. 3a), discovery of diaphragm fibrosis (Fig. 2e) and transmission electron microscopy of EDL muscle demonstrating abnormal sarcomeres (Fig. 2f)) and have already performed tests that are considered to be gold standard measurements for muscular dystrophy (*ex-vivo* and *in vivo* eccentric contractions (Fig. 2a-d)) along with a state of the art unbiased comparison of the *Gcn5*$^{skm-/-}$ transcriptome to all available GEO biosets (Fig. 4a,b), which identified human Duchenne's muscular dystrophy as one of top correlated datasets.

November 19, 2021

RE: JCB Manuscript #202104022R

Prof. Keir Menzies
University of Ottawa
451 Smyth Rd.
Ottawa, ON K1H 8M5
Canada

Dear Prof. Menzies:

Thank you for submitting your revised manuscript entitled "GCN5 Maintains Muscle Integrity by Acetylating YY1 to Promote Dystrophin Expression". I am happy to write that both reviewers were satisfied with the revisions to the paper and that your paper is now acceptable for publication in JCB pending final revisions necessary to meet our formatting guidelines (see details below). I command you for having addressed very extensively the comments of the reviewers and congratulate you on a very fine study.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

2) Figures limits: Articles may have up to 10 main text figures.

3) Figure formatting: * Scale bars must be present on all microscopy images, including inset magnifications. * Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (either in the figure legend itself or in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title: The abstract should be no longer than 160 words and should communicate the significance of the paper for a general audience. The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.

* While your current abstract will be appreciated by the specialists, we do not feel that it will be accessible to a broader cell biology audience. Therefore, please ensure your abstract include introductory sentences outlining the biological problem.

6) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.

7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies. Please also indicate the acquisition and quantification methods for immunoblotting/western blots.

8) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses

c. Temperature
d. Imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

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