EMBO *reports*

Myogenin promoter-associated lncRNA Myoparr is essential for myogenic differentiation

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Submission date:	25 November 2018
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Transaction Report: Following peer-review at *The EMBO Journal* this manuscript was revised and transferred to *EMBO reports*

1st Editorial Decision 3 December 2018

Thank you for the transfer of your revised manuscript to EMBO reports. I have gone through your point by point response and the manuscript text now, and I am happy to tell you that we can in principle accept it. Only a few minor changes are still necessary.

Please add the statistical tests used to calculate the p-values to Figs 2,3,5,6,7,8, EV2,EV3,EV4 and App figs S2 - S5.

The legends for Fig 4 and 5 state n=2, in which case no error bars can be shown. You can show instead the single data points of both experiments along with their mean.

Please upload all main figures as individual files.

Please add page numbers to the Appendix and its table of content. App Fig S5C has an empty box. Please show the actual picture taken at the microscope instead.

Please move the Accession numbers from the Appendix file to the methods in the main manuscript file.

FIGURE CALLOUTS: Fig 2H is called out before 2G, please correct.
The panels of Appendix Fig S1A and S1B are not called out, please add.
The Appendix Table S2-5 are not called out, please add.

I would like to suggest some changes to the title and abstract. Please let me know if you agree with:

Myogenin promoter-associated lncRNA Myoparr is essential for myogenic differentiation

Promoter-associated long non-coding RNAs (lncRNAs) regulate the expression of adjacent genes; however, precise roles of these lncRNAs in skeletal muscle remain largely unknown. Here, we characterize a promoter-associated lncRNA, Myoparr, in myogenic differentiation and muscle

disorders. Myoparr is expressed from the promoter region of the mouse and human myogenin gene, one of the key myogenic transcription factors. We show that Myoparr is essential both for the specification of myoblasts by activating neighboring myogenin expression and for myoblast cell cycle withdrawal by activating myogenic microRNA expression. Mechanistically, Myoparr interacts with Ddx17, a transcriptional coactivator of MyoD, and regulates the association between Ddx17 and the histone acetyltransferase PCAF. Myoparr also promotes skeletal muscle atrophy caused by denervation, and knockdown of Myoparr rescues muscle wasting in mice. Our findings demonstrate that Myoparr is a novel key regulator of muscle development, and suggest that Myoparr is a potential therapeutic target for neurogenic atrophy in humans.

I would also like to suggest that you have the entire manuscript text corrected by a native speaker.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File (RPF) to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

I look forward to seeing a final manuscript as soon as possible. Please let me know if you have any questions.

Responses to senior Editor's comments are as follows:

Thank you for submitting your revised manuscript for consideration by The EMBO Journal and my apologies for the extended duration of the re-review period. Your study has now been seen by two of the original referees and their comments are shown below. As you will see, while the referees appreciate the work you have done to improve the manuscript I am afraid they find that several key points related to the mechanism at play remain unclear.

More specifically, ref #1 points to technical problems with the ChIRP data that was added in the revised manuscript that leave the whole experiment inconclusive. In addition, ref #2 finds that the DDX17-dependent trans-acting role for Myoparr is not conclusively supported by the present data and that another model may be needed to explain the effects observed. I would like to add that this concern also reflects our reading of your point-by-point response and quantification experiments. With only 5 copies of Myoparr per cell available to bind the likely thousands of DDX17 molecules it is conceptually hard to envision a model where direct RNA binding on each RNA helicase would give the widespread effects that you see in the present study.

Given the EMBO J focus on mechanistic understanding - in particular in the absence of broader functional/physiological evidence such as KO experiments - and in light of the referee concerns from both this and the previous round I am afraid we have to conclude that the revised manuscript does not offer the level of conclusiveness that we have to require for papers published in The EMBO Journal. We have therefore decided not to pursue publication here.

However, given the quality of the data related to the cis-acting effects on myogenin - and the general interest in the findings from the referees - I have taken the liberty to also mention your study to my colleague Esther Schnapp at our sister journal EMBO Reports. Since EMBO Reports focuses on novelty and functional insights more than detailed mechanism Esther has offered to publish your manuscript in EMBO Reports following relatively minor revision. ***This would involve toning down the conclusions for the trans-acting effect to better fit the current data and to remove the ChIRP-data that ref #1 found to be inconclusive. In addition, we would encourage you to discuss the question of stoichiometry between Myoparr and myogenin as well as Myoparr and DDX17 when***

it comes to the regulatory effects seen on distal loci.

I am sorry that we cannot be more positive for The EMBO Journal on this occasion but I hope that you will be interested in transferring your manuscript to EMBO Reports for publication there. You can do so using the link provided below. Please also feel free to contact Esther directly (cc'ed here) for specific questions about the requirements for acceptance of the manuscript in EMBO Reports.

[Our Reply]

We appreciate the Editor's comments and suggestions. We discussed among the authors and agreed to tone down the conclusions for the trans-acting effect of *Myoparr* and to remove the ChIRP data about miRNAs loci presented in the previous Fig7C. We revised the words and sentences in Title, Abstract, Introduction, Results, Discussion, and Figure Legends to tone down our conclusions. Detail revised points are shown as follows. We have also discussed the mechanism how *Myoparr* regulates the expression of downstream genes at distal loci (See pages 19-20, lines 450-469).

The revised sentence in Title is from "*Coordination of cis and trans roles of Myoparr with Ddx17 is essential for myogenic differentiation*" to "*Myoparr* is essential for myogenic differentiation through the binding with coactivator Ddx17 protein".

The revised sentences in Abstract are,
1. from "*We showed that Myoparr is essential both for specification of myoblasts into differentiation lineage by activating myogenin expression in cis and for myoblast cell cycle withdrawal by activating myogenic microRNA expression in trans*." to "We showed that *Myoparr* is essential both for specification of myoblasts into differentiation lineage by activating neighboring *myogenin* expression and for myoblast cell cycle withdrawal by activating myogenic microRNA expression."
2. from "*Thus, our findings demonstrate that Myoparr is a novel key regulator of muscle development by controlling target gene expression in both a cis and trans manner, and suggest that Myoparr is a potential therapeutic target for neurogenic atrophy in humans*." to "Thus, our findings demonstrate that *Myoparr* is a novel key regulator of muscle development, and suggest that *Myoparr* is a potential therapeutic target for neurogenic atrophy in humans."

The revised sentences in Introductionis are,
1. from "*However, molecular characterization of these lncRNAs and their trans-acting functions in cell proliferation, differentiation, and diseases still remain unexplored*." to "However, molecular characterization of these lncRNAs in cell proliferation, differentiation, and diseases still remains unexplored."
2. from "*In addition, we found that Myoparr bound with Ddx17, a transcriptional coactivator of MyoD, and promoted the protein-protein interaction between Ddx17 and histone acetyltransferase PCAF, indicating a dual function of Myoparr through the binding with transcriptional activator during myogenesis*." to "In addition, we found that *Myoparr* bound with Ddx17, a transcriptional coactivator of MyoD, and promoted the protein-protein interaction between Ddx17 and histone acetyltransferase PCAF, indicating that *Myoparr* functions through the binding with transcriptional activator during myogenesis."

The revised sentences in Results are,
1. from "*From these results, we concluded that Myoparr is essential for specification of myoblasts to differentiation lineage through Myoparr (upstream) to myogenin (downstream) pathway, and that Myoparr also likely acts in trans on the cell cycle and cell division pathway as a myogenin-independent transcriptional regulator*." to "From these results, we concluded that *Myoparr* is essential for specification of myoblasts to differentiation lineage through *Myoparr* (upstream) to *myogenin* (downstream) pathway and that *Myoparr* also likely regulates the cell cycle and cell division pathway as a *myogenin*-independent manner."
2. from "*Thus, these results suggested that Myoparr may activate target gene expression by promoting the formation of Ddx17-PCAF complex on target locus in differentiating myoblasts*." to "Thus, these results suggested that *Myoparr* activates *myogenin* expression by promoting the formation of the Ddx17-PCAF complex on *myogenin* locus in differentiating myoblasts."
3. from "*To determine whether Ddx17 mediates the trans role of Myoparr in cell cycle and cell division pathway, we compared the genes regulated by Ddx17 KD and Myoparr KD by RNA-seq*."

to "To determine whether Ddx17 mediates the role of *Myoparr* in cell cycle and cell division pathway, we compared the genes regulated by *Ddx17* KD and *Myoparr* KD by RNA-seq."
4. from "*As a result, Myoparr was required for the high level of Pol II occupancy at these promoters in differentiating C2C12 cells (Figure 7B). We also showed that endogenous Myoparr directly bound to these promoters by ChIRP assays (Figure 7C). These results indicate that Myoparr directly regulates the expression of these miRNAs in trans at transcriptional level. Regarding Ddx17 and PCAF, direct bindings of Ddx17 and PCAF to these promoters were observed by ChIP assays, compared with GAPDH locus as a negative control (Figure 7D-E).*" to "As a result, *Myoparr* was required for the high level of Pol II occupancy at these promoters in differentiating C2C12 cells (Figure 7B). Regarding Ddx17 and PCAF, direct bindings of Ddx17 and PCAF to these promoters were observed by ChIP assays, compared with *GAPDH* locus as a negative control (Figure 7C-D)."
5. from "*These results indicated that Myoparr acts in trans as a myogenin-independent transcriptional regulator with Ddx17 on myoblast cell cycle withdrawal by activating myogenic miRNA expression.*" to "These results indicated that *Myoparr* and Ddx17 are required for myoblast cell cycle withdrawal through the activation of myogenic miRNA expressions."

The revised sentences in Discussion are,
1. from "*Myoparr was required for Pol II recruitment to these promoters through the direct binding to chromatin. Consequently, Myoparr depletion prevented myoblast cell cycle arrest by increasing ERK1/2 activity and Cdc6 and Pola1 expression in a myogenin-independent manner. Taken together, our study showed a dual role of promoter-associated lncRNA Myoparr; one role is specification of myoblasts to differentiation lineage by activating neighboring myogenin expression in cis, and the other role is cell cycle withdrawal by activating the expression of myogenic regulatory miRNAs in trans.*" to "*Myoparr* was required for Pol II recruitment to these promoters. Consequently, *Myoparr* depletion prevented myoblast cell cycle arrest by increasing ERK1/2 activity and Cdc6 and *Pola1* expression. Taken together, our study showed the role of promoter-associated lncRNA *Myoparr*; one role is specification of myoblasts to differentiation lineage by activating neighboring *myogenin* expression, and the other role is myoblast cell cycle withdrawal by activating the expression of myogenic regulatory miRNAs in a *myogenin*-independent manner."
2. from "*The DEAD box protein Ddx17 plays key roles in transcription, miRNA processing, alternative splicing, and myogenic differentiation (Caretti et al, 2006; Fuller-Pace, 2013; Dardenne et al, 2014). In the present study, we discovered a new feature of Ddx17 as a Myoparr-interacting protein. Both Ddx17 and Myoparr were essential for myogenin expression. Deletion of the 341-nt DNA sequence, which corresponds to the Ddx17-binding region of Myoparr, from the myogenin upstream region largely reduced the promoter activity of myogenin. Moreover, comprehensive RNA-seq analyses revealed that Myoparr and Ddx17 regulated the expression of cell cycle-related genes in a myogenin-independent manner during C2C12 differentiation. Although not all the genes regulated by Myoparr KD completely overlapped with the genes regulated by Ddx17 KD, our findings indicated that the role of Myoparr in the activation of myogenin and myogenic miRNA expression is mediated by interaction with Ddx17 protein. In differentiating myoblasts, Ddx17 functions as a transcriptional coactivator of MyoD through association with histone acetyltransferases CBP, p300, and PCAF (Caretti et al, 2006). PCAF promotes transcriptional activities of Ddx17 and MyoD (Sartorelli et al, 1999; Dilworth et al, 2004; Shin & Janknecht, 2007) and is essential for myoblast cell cycle arrest (Puri et al, 1997). Although Myoparr was dispensable for the binding of Ddx17 and PCAF with target loci in our ChIP experiments, we showed that Myoparr is required to augment the interaction between Ddx17 and PCAF proteins. In addition, Myoparr was required for maximum Pol II recruitment to target promoters. Taken together, we proposed the model of cis and trans roles of Myoparr during myogenesis (Figure 9). In the absence of Myoparr, Ddx17 weakly interacts with PCAF. Thus, occupancies of both Ddx17 and PCAF on target loci (myogenin and miRNAs) are not sufficient for maximum Pol II recruitment to target promoters. In the presence of Myoparr, after binding to target loci in both a cis and trans manner, Myoparr interacts with Ddx17 and promotes the interaction between Ddx17 and PCAF on target loci. Thus, maximum Pol II recruitment to promoter regions of myogenin and miRNAs is achieved by Myoparr expression. Considering that genes regulated by both Myoparr and Ddx17 (e.g., myogenin, miR-133b/206, H19) are also regulated by MyoD (Rao et al, 2006; Borensztein et al, 2013), and the transactivation of MyoD is also required for its own transcription (Thayer et al, 1989), Myoparr may facilitate the transactivation of MyoD by strengthening the interaction between transcriptional coactivators Ddx17 and PCAF.*" to "The DEAD box protein Ddx17 plays key roles in transcription, miRNA processing, alternative splicing, and myogenic differentiation [22,23,32]. In the present study, we discovered a new feature of Ddx17 as a *Myoparr*-interacting protein. Both

Ddx17 and *Myoparr* were essential for *myogenin* expression. Deletion of the 341-nt DNA sequence, which corresponds to the Ddx17-binding region of *Myoparr*, from the *myogenin* upstream region largely reduced the promoter activity of *myogenin*. In differentiating myoblasts, Ddx17 functions as a transcriptional coactivator of MyoD through association with histone acetyltransferases; CBP, p300, and PCAF [22]. PCAF also promotes transcriptional activities of Ddx17 and MyoD [33-35]. Although *Myoparr* was dispensable for the binding of Ddx17 and PCAF with *myogenin* locus in our ChIP experiments, we showed that *Myoparr* is required to augment the interaction between Ddx17 and PCAF proteins. In addition, *Myoparr* was required for maximum Pol II recruitment to *myogenin* promoter. Taken together, we proposed the model of *Myoparr* function during myogenesis (Figure 9). In the absence of *Myoparr*, Ddx17 weakly interacts with PCAF. Thus, occupancies of both Ddx17 and PCAF on *myogenin* locus are not sufficient for maximum Pol II recruitment to *myogenin* promoter. In the presence of *Myoparr*, after binding to *myogenin* promoters, *Myoparr* interacts with Ddx17 and promotes the interaction between Ddx17 and PCAF. Thus, maximum Pol II recruitment to *myogenin* promoter is achieved by *Myoparr* expression."

3. from "*In conclusion, we identified a novel promoter-associated lncRNA, Myoparr, and revealed its dual role during myogenesis through the regulation of neighboring myogenin and distant myogenic regulatory miRNAs by promoting Ddx17-PCAF interaction. Thus, our findings revealed that the promoter-associated lncRNA does not merely regulate nearby genes but is also a trans-acting regulator of cell proliferation and differentiation by promoting protein-protein interaction between transcriptional activators. Besides transcriptional activator, we have identified hnRNPK and Tial1, key interactors of lncRNA regulating the p53 pathway (Huarte et al, 2010; Liu et al, 2016), as Myoparr-interacting proteins. Collectively, future studies using comprehensive analysis of Myoparr-interacting proteins will further define the transcriptional activator-independent role of promoter-associated lncRNA in skeletal muscle formation and disorders affecting muscles.*" to "In conclusion, we identified a novel promoter-associated lncRNA, *Myoparr*, and revealed its role during myogenesis through the regulation of neighboring *myogenin* expression by promoting the protein-protein interaction between transcriptional activators. In addition, *Myoparr* was also required for the expression of myogenic regulatory miRNAs in a *myogenin*-independent manner. Thus, our findings indicate that the promoter-associated lncRNA not merely regulates neighboring gene expression but also may affect gene expressions on distal loci in a neighboring gene independent manner. However, we did not exclude the possibility that *Myoparr* may function independently through the interaction of *myogenin* gene and Ddx17/PCAF proteins in myogenesis, because the genes affected by *Myoparr* KD didn't completely overlap with that by *Ddx17* or *myogenin* KD. Besides Ddx17, we have identified hnRNPK and Tial1, key interactors of lncRNA regulating the p53 pathway [44,45], as *Myoparr*-interacting proteins. Collectively, future studies using comprehensive analysis of *Myoparr*-interacting proteins will further define the other roles of *Myoparr* in skeletal muscle formation and disorders affecting muscles."

The revised sentence in Figure Legends is from "*Figure 9. Proposed model of cis and trans roles of Myoparr during myogenesis. Ddx17 and PCAF bind to promoters of target genes, however, in the absence of Myoparr, the interaction between Ddx17 and PCAF is weak. Thus, occupancies of both Ddx17 and PCAF on target loci (myogenin and miRNAs) are not sufficient for maximum Pol II recruitment to target promoters. In the presence of Myoparr, Myoparr binds to target loci (myogenin and miRNAs) in both a cis and trans manner, and interacts with Ddx17 to promote the interaction between Ddx17 and PCAF. Enhanced Ddx17-PCAF interaction by Myoparr may be sufficient for maximum Pol II recruitment to target promoters. Thus, Myoparr activates the target gene expression.*" to "Figure 9. Proposed model of *Myoparr* function during myogenesis Although Ddx17 and PCAF bind to *myogenin* promoter, the interaction between Ddx17 and PCAF is weak in the absence of *Myoparr*. Thus, occupancies of both Ddx17 and PCAF on *myogenin* promoter are not sufficient for maximum Pol II recruitment to *myogenin* promoter. In the presence of *Myoparr*, after binding to *myogenin* promoter, *Myoparr* interacts with Ddx17 to promote the Ddx17-PCAF interaction. Enhanced Ddx17-PCAF interaction by *Myoparr* may be sufficient for maximum Pol II recruitment to *myogenin* promoter. Thus, *Myoparr* is required for specification of myoblast lineage into myogenic differentiation through *Myoparr* (upstream) to *myogenin* (downstream) pathway. In addition, *Myoparr* is involved in the regulation of myoblast cell cycle withdrawal by activating the expression of myogenic regulatory miRNAs in a *myogenin*-independent manner."

Responses to Referees' concerns are as follows:

Referee #1:

The reviewer appreciates the effort that the authors have made in responding to the concerns.
In particular, the ChIP experiments performed in the presence or absence of Myoparr have allowed the authors to consolidate their data and to appropriately refine the model.
Instead, the reviewer is still concerned about the data on the trans-acting activity of Myoparr. In particular the ChIRP experiments performed to examine the direct binding of Myoparr to cis (myogenin promoter) and trans (miRNAs) loci indicate a very different pull down efficiency of the odd and even oligos. However, while the data on the cis binding are statistically consistent, the same is not true for the trans loci.
In Fig7C, the authors show the binding in trans of Myoparr to miR-133b, miR-206 and H19 coding regions. From the quantification of the enrichments, the EVEN probes seem to pull down very little the miR-206 region in comparison to the ODD probes. The effect is even more extreme when analyzing H19, where only the ODD probes pull-down the associated DNA.
Although it is known that the presence of probe-specific noises (unique to each pool) may explain slight variations in the appearance of signals, big differences in the pull-down efficiencies should not be underestimated.
Therefore, it is difficult to accept the authors' conclusion on the trans effect of Myoparr unless more definitive proof is provided.

[Our reply]
We appreciate the referee's comments and evaluation of the ChIP experiments to refine the model. We feel sorry that we did not provide sufficient ChIRP data, particularly the data on the trans loci in Fig7C to satisfy the referee's concerns. We discussed among the authors and removed the ChIRP data about trans miRNAs loci in the previous Fig7C. We also toned down the conclusions for the trans-acting effect of *Myoparr* to better fit the current data and revised the sentences throughout the manuscript and Figure Legends. Detail revised points are shown above. We also discussed the potential mechanism how *Myoparr* regulates the expression of downstream genes at distal loci (See pages 19-20, lines 450-469).

Referee #3:

The revised version of the paper is improved and the reviewers have addressed most of my comments. However, I still have some concerns and considerations regarding the proposed molecular mechanism that I think at least deserve discussion.

80% of genes altered by Ddx17 KD overlap with genes regulated by Myoparr KD. This suggests that Ddx17 function is mainly mediated through Myoparr. But taking into consideration that Ddx17 is an RNA helicase that has been involved in multiple processes (RNA splicing, translation regulation, ribosomal RNA processing...) it is difficult to think that the effects observed upon Ddx17 KD are exclusively due to the result of its interaction with Myoparr and PCAF. On the other hand, most of the genes affected by Myoparr KD don't overlap with Ddx17 or Myogenin KDs. Although the data show that there is a relationship between Ddx17, Myoparr and Myogenin, the proposed mechanism does not fully explain the data.

[Our reply]
We appreciate the referee's comments. We feel sorry that we did not provide sufficient description about the mechanism of *Myoparr* to completely satisfy the referee's concerns. We also think that the effects observed upon knockdown of Ddx17 are not exclusively due to the result of its interaction with *Myoparr* and PCAF. Although Ddx17 function is mainly mediated through *Myoparr* as commented by the referee, we did not exclude the possibility that Ddx17 may function independently through the interaction with *Myoparr* and PCAF. It is also of note that Ddx5, a highly similar homolog of Ddx17, is still expressed in our experimental condition. Thus, we would observe the Ddx17 function that could not be rescued with Ddx5 expression, that is the function mediated by *Myoparr* and PCAF. We also discussed what the genes affected by *Myoparr* KD didn't completely overlap with that by *Ddx17* or *myogenin* KD (See page 22, lines 503-510). We hope that the referee would kindly agree with our answer. In revised manuscript, we toned down the conclusions for the trans-acting effect of *Myoparr* to better fit the current data and discussed the mechanism how

Myoparr regulates the expression of downstream genes at distal loci (See pages 19-20, lines 450-469).

1st Revision - authors' response 17 December 2018

Thank you for the transfer of your revised manuscript to EMBO reports. I have gone through your point by point response and the manuscript text now, and I am happy to tell you that we can in principle accept it. Only a few minor changes are still necessary.

We are also grateful for your evaluation. We have responded to each of the comments in a point-by-point manner. In addition, we have provided a revised version of the manuscript with changes highlighted in red color. We hope that the changes incorporated into the revised manuscript satisfactorily address the concerns raised. Please see the point-by-point responses to your comments as follows.

Point-by-point responses to senior Editor's comments:

Please add the statistical tests used to calculate the p-values to Figs 2,3,5,6,7,8, EV2,EV3,EV4 and App figs S2 - S5.
[Our Reply]
We appreciate the Editor's comments. In the revision, we provided the description of the statistical tests we used in Figure legends of Figs 2, 3, 5, 6, 7, 8, EV2, EV3, EV4 and Appendix Figs S2 - S5.

The legends for Fig 4 and 5 state n=2, in which case no error bars can be shown. You can show instead the single data points of both experiments along with their mean.
[Our Reply]
We appreciate the Editor's comment. In revision, we replaced Fig 4E-H, Fig 5C, and Fig EV2C with new figures according to the comments. The similar data presentation is found in Fig 4 in EMBO reports (2018) 19: e46222.

Please upload all main figures as individual files.
[Our Reply]
We appreciate the comment. We uploaded the all main figures as individual files with sufficient resolution.

Please add page numbers to the Appendix and its table of content.
[Our Reply]
We appreciate the comment. We added the page numbers to the Appendix file. For readers to refer easily, we also changed the Appendix Figures and the Appendix Figure legends to be displayed on the same pages.

App Fig S5C has an empty box. Please show the actual picture taken at the microscope instead.
[Our Reply]
We appreciate the Editor's comment. We replaced the Appendix Fig S3C with the actual picture. We hope the editor kindly agree with us.

Please move the Accession numbers from the Appendix file to the methods in the main manuscript file.
[Our Reply]
We appreciate the Editor's comment. We moved Appendix Table S5 to the material and methods section (Pages 37-38, lines 880-889).

FIGURE CALLOUTS: Fig 2H is called out before 2G, please correct.
The panels of Appendix Fig S1A and S1B are not called out, please add.
The Appendix Table S2-5 are not called out, please add.
[Our Reply]

We appreciate the Editor's comments. We revised our manuscript. As described above, we moved Appendix Table S5 to the methods. Please see details as follows:
Fig 2G and H (Page 8, line 171).
Appendix Fig S1A and S1B (Page 6, line 136).
Appendix Table S2 (Page 24, line 560 and Page 28, line 658).
Appendix Table S3 (Page 25, line 591, Page 31, line 736, Page 34, line 790, Page 36, line 843, and Page 37, line 862).
Appendix Table S4 (Page 36, line 856).

I would like to suggest some changes to the title and abstract. Please let me know if you agree with:
Myogenin promoter-associated lncRNA Myoparr is essential for myogenic differentiation,
Promoter-associated long non-coding RNAs (lncRNAs) regulate the expression of adjacent genes; however, precise roles of these lncRNAs in skeletal muscle remain largely unknown. Here, we characterize a promoter-associated lncRNA, Myoparr, in myogenic differentiation and muscle disorders. Myoparr is expressed from the promoter region of the mouse and human myogenin gene, one of the key myogenic transcription factors. We show that Myoparr is essential both for the specification of myoblasts by activating neighboring myogenin expression and for myoblast cell cycle withdrawal by activating myogenic microRNA expression. Mechanistically, Myoparr interacts with Ddx17, a transcriptional coactivator of MyoD, and regulates the association between Ddx17 and the histone acetyltransferase PCAF. Myoparr also promotes skeletal muscle atrophy caused by denervation, and knockdown of Myoparr rescues muscle wasting in mice. Our findings demonstrate that Myoparr is a novel key regulator of muscle development, and suggest that Myoparr is a potential therapeutic target for neurogenic atrophy in humans.
[Our Reply]
We appreciate the Editor's suggestion. The title and abstract that you suggested are essentially fine for us. We discussed among the authors and agreed to change the title and abstract.

I would also like to suggest that you have the entire manuscript text corrected by a native speaker.
[Our Reply]
We appreciate the Editor's advice. We subjected our manuscript to be checked by a native speaker. Please see the attached certificate.

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.
[Our Reply]
We appreciate the Editor's suggestion of a cover. We attached a Storyboard as a cover suggestion and motifs. Please see our attached file in detail.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File (RPF) to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.
You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."
[Our Reply]
We agree to publish the Review Process File (RPF) to accompany accepted manuscripts.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Kunihiro Tsuchida
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2018-47468

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	No statistical method was used to predetermine the sample size. We performed the several experimets for all results with duplicate or triplicate samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample size was estimated on prior information in the literature and/or previous experiments conducted in the lab.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No data were excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No steps taken to minimize the effects of subjective bias. No randomization procedures were used.
For animal studies, include a statement about randomization even if no randomization was used.	No randomization was used.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No blinding was applied.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was applied.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Nnormal distribution was assessed using F test.
Is there an estimate of variation within each group of data?	Yes

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

http://www.ebi.ac.uk/ega

http://biomodels.net/

http://biomodels.net/miriam/
http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes. When the variance is similar between the groups, statistical analyses were performed using Student's t-test. In cases of unequal variances, Welch's t-test was used.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Catalog numbers and clone numbers are provided in the Materials and Methods section for all antibodies used.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	C2C12 cells were purchased from Marinpharm. NIH3T3 and C3H10T1/2 cells were purchased from American Type Culture Collection (ATCC) prior to this study. Cells were tested for mycoplasma contamination using MycoAlert Mycoplasma Detection Kit (Lonza).

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	All mice (male, 8 weeks old, and Pregnant female) were C57BL/6J strain, purchased from the Japan SLC, and housed in a pathogen–free, environmental-controlled animal facility with a constant temperature (24°C) and a 12:12-h light-dark cycle.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal experiments were conducted under protocols approved by the Institutional Animal Care and Use Committee of Fujita Health University.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	The Ethical Review Board for Clinical Studies at Fujita Health University.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Informed consent was obtained for all subjects. Experiments were conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The raw data for each sample of the RNA-seq reported in this paper were deposited in the DDBJ Sequence Read Archive under the Accession No. DRA005527. The gene sequences reported in this paper were submitted to the DDBJ database (accession numbers; AB921551, AB921552, AB921553, AB921554, AB921555, AB921556, AB921557, LC366983, LC366984, LC388421, and LC388422).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The correspondence between the accession numbers and raw data is described in Appendix Table S2.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A