

PRMT5 links lipid metabolism to contractile function of skeletal muscles

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DOI: [10.15252/embr.202357306](https://doi.org/10.15252/embr.202357306)

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Review Timeline:

Transfer from Review Commons:	6th Apr 23
Editorial Decision:	6th May 23
Revision Received:	30th May 23
Accepted:	5th Jun 23

Editor: Esther Schnapp

Review
COMMONS

Transaction Report: This manuscript was transferred to EMBO reports following peer review at Review Commons.

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Review #1

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The Kim et al. paper titled "PRMT5 links lipid metabolism to contractile function of skeletal muscles" reports how the arginine methyltransferase PRMT5 affects lipid metabolism in myofibers by stabilizing the mSREBP1 protein and repressing the expression of the PNPLA2 gene. The genetic deletion of PRMT5 in muscle results in the loss of lipid droplets in myofibers and a loss in muscle strength. Additionally, there is a change in muscle fiber types, moving from an oxidative state to a more glycolytic one. While the authors present compelling data on PRMT5's role in muscle metabolism, there are some concerns on the mouse model used and the sequencing data.

****Major concerns:****

1. The mouse model used in this study is PRMT5^{fl/fl}, Myl1^{cre} in order to genetically delete PRMT5 in skeletal muscle. While there is no issue with the KO mice, the WT mice are PRMT5^{fl/fl}, Myl1⁺ which is not an acceptable control. It is known that Cre itself can have a phenotype, and additionally Myl1 is very highly expressed. Thereby, there is a large amount of Cre in the KO mice, but none in the WT which may contribute to the differences seen between WT and KO mice. The appropriate WT control is PRMT5^{+/+} Myl1^{cre} and the experiments would need to be repeated using this mouse genotype as the WT.
2. In the scRNA-Seq the authors claim that PRMT5 is not expressed in quiescent muscle stem cells. However, the data set that is used only has approximately 250 muscle stem cells, which would not provide much coverage. It would be necessary to validate this claim by using other data sets, such as Tabula Muris or publicly available bulk RNA-Seq.
3. The ChIP-Seq data shown was performed on 3T3-L1 cells and is not appropriate for a muscle paper. The ChIP-Seq must be performed on muscle cells in order to confirm their conclusion.
4. The authors claim that loss of PRMT5 leads to a gradual loss of muscle fiber size but has no effect on myogenesis. The evidence to support that claim is shallow, being based solely on CSA and total number of myofibers, along with a loss of lean body mass. To confirm this statement, it would be best to quantify the CSA and # of myofibers in EDL and TA at P7 and P21. Further, a regeneration assay would also demonstrate if myogenesis is compromised or not.
5. The data presented shows that there is fiber type switch from oxidative to glycolytic, along with a decrease in muscle strength in the PRMT5 KO mice. This seems counterintuitive to what is known in the field as glycolytic fibers are viewed as being capable of generating more force than oxidative, while having less endurance. The authors should clarify this point and elaborate more on their conclusion that the loss of strength is due to an altered metabolism.

****Minor concerns:****

1. The term myocyte is not the most accurate word for describing skeletal muscle.

Myofibers would be best for fully differentiated muscle, myotubes for in vitro differentiation and myocytes should be reserved for differentiated cells that have not fused yet (1-2 nuclei/cell). I would recommend changing all mentions of myocyte to myofiber and changing "myocyte specific" with regards to the mouse models as "skeletal muscle specific"

2. Figure 1 Panel F and G would be clearer if they were labelled as 2 months old mice

3. Figure 2 Panel E, G and H the control line appears to be missing error bars

4. Figure 2 legend should specify that these mice are 2 months old for the sake of clarity

5. Figure 3 Panel G and H are not informative and are misleading. The supplemental panels show that when normalized to body mass there is no difference in O₂ consumption or CO₂ production. These should be replaced with the supplemental panels

6. Figure 4 Panel E and F, are these separate experiments? The values do not match between the 2 panels. If these are separate it should be made more clear.

- a. Error bars appear to be missing in Panel E

- b. As this is an experiment where the state of the cell is incredibly important (metabolism of myoblast is much different to myotube), the authors must demonstrate that there is no defect in in vitro differentiation by showing the fusion index assay for these cells and a representative image of the myotubes.

- c. The authors should mention in the legend that these are 3 DM myotubes

7. Figure 5 Panel G, a qPCR to confirm the O/E of the PRMT5 and SREBP1a in the test samples would be necessary.

- a. The double O/E cells are marked as significantly different, but it is unclear to which group they are significantly different to.

8. Figure 7 Panel B, body weight would be more informative in g rather than percentage.

9. It would be interesting to test whether the KO of Pnpla2 also rescues the fiber type switch.

2. Significance:

Significance (Required)

Overall the manuscript provides insight into the role of PRMT5 in regulating the metabolism of skeletal muscle. While the paper provides some interesting data, it is severely hampered by the improper WT controls lacking the cre alleles. In order for the data to be reliable, all of the WT samples will need to be replaced with PRMT5^{+/+} Myl1 cre mice. There are other papers looking at the role of PRMT5 in skeletal muscle, however these are more focused on muscle stem cells than the myofibers. Therefore this paper does fill in a gap with regards to the metabolic role of PRMT5 and how this can affect skeletal muscle function. This paper would most likely be of interest to a specialized audience, mostly those in the skeletal muscle field.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

More than 6 months

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No

Review #2

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

In the manuscript "PRMT5 links lipid metabolism to contractile function of skeletal muscles", Kim et al., describe the role for the arginine methyl transferase PRMT5 in maintaining skeletal muscle homeostasis. The authors showed that myocyte-specific deletion of PRMT5 results in the loss of muscle mass, reduced motor-performance, a fiber type switch from oxidative to glycolytic fibers along with reduced lipid content in the myofibers. The authors reasoned that absence of PRMT5 results in reduced methylation and stability of mSREBP1 and an increase in the expression of adipose triglyceride lipase (ATGL).

****Major Comments****

1. Experiments looking at the interaction between PRMT5 and mSREBP1a using overexpressed proteins are used to conclude that PRMT5 methylates SREBP1a. While the results are consistent with these conclusions, the finding remains correlative. Methylation assays using purified PRMT5 and SREBP1a would be required to make a definite conclusion that PRMT5 methylates SREBP1a. These should be performed. In the absence of such data, the authors would need to adjust their conclusions to say that in the presence of PRMT5, SREBP1a becomes methylated, and that it remains to be determined if this is directly mediated by PRMT5.

2. Stability studies in figure 5E have been performed in HEK293 cells using over-

expressed proteins. While these results show that PRMT5 protects SREBP1a from degradation, the significance of this in muscle is less clear. Western blots of these proteins in C2C12 cells show that the over expression of PRMT5 does not stabilize SREBP1a (Flag) despite the appearance of increased methylation. To solidify the concept that PRMT5-dependent methylation of SREBP1 leads to its stabilization, cycloheximide experiments should be performed in myotubes generated in culture from the PRMT5mko mice. The stability of the endogenous SREBP1a gene could be monitored using antibodies.

3. The measurement of methylation levels of SREBP1a are complicated by the fact that the protein levels are destabilized in the absence of PRMT5. The authors should use a PRMT5 inhibitor experiments in complement to these overexpression studies to measure the relative methylation of mSREBP1a (SMY10/mSREBP1).

****Minor Comments****

4. In figure 1H, there are more nuclei surrounding the myofibers. The authors should document the number of PAX7 cells per myofiber in the Control and Prmt5MKO mouse strains as it helps understand the additive effect of change in PAX7 cells during muscle atrophy.

5. In figure 5, the legend title mentioned ATGL. However the stability or methylation results of ATGL are not presented anywhere in the manuscript. Only in figure 6 did the authors show the differential expression of ATGL in the presence and absence of PRMT5. This title for Figure 5 should corrected.

6. For better representation, the authors should consider moving the western blot panel (S3A) and lipid droplet staining data (S3E) to main figures and some of the data on force generation and body weights to supplementary.

2. Significance:

Significance (Required)

This is an interesting manuscript that provides novel insight into the role for PRMT5 in muscle homeostasis. While previous studies have looked at the PRMT5 from a transcriptional standpoint during muscle differentiation, this work shows a role for PRMT5 in controlling the metabolic state of myofibers through transcriptional and potentially non-transcriptional mechanisms. Identification of the transcriptional regulation of Pnpla2 gene by PRMT5 is confirmed by mouse rescue experiments with the double KO mice. However the role for non-transcriptional control of SREBP1a stability through methylation by PRMT5 is not as clearly established. To strengthen this aspect of the manuscript, additional experiments are needed.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

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Yes

Review #3

1. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

The lipid droplets represent an energy store and central hub of lipid metabolism in cells. In the skeletal muscle, abundant lipid droplets are present in myofibers (especially in oxidative Type 1 and IIA myofibers) and thought to play a role in supplying energy through fatty acid oxidation (FAO) to power muscle contraction. Increased abundance of lipid droplets in myofibers is associated with poor muscle function and insulin resistance in patients of type 2 diabetes. Paradoxically, myofibers of trained athletes also contain higher than normal levels of lipid droplets but with better contractile function and insulin sensitivity. The molecular mechanism underlying this "Athlete's Paradox" has been unclear, due to the lack of understanding of what controls biogenesis and metabolism of lipid droplets in the myofiber.

In this manuscript, Kuang and colleague provide compelling evidence to support a key role of PRMT5 in maintaining lipid droplets in the myofiber. They generated a conditional knockout mouse model to disrupt the Prmt5 gene in the myofibers. This leads to an astonishing depletion of lipid droplets in myofibers. The Prmt5 null myofibers also exhibited poor contractile function and classical signs of atrophy. To determine if the depletion of lipid droplets drives muscle atrophy or if muscle atrophy drives depletion of lipid droplets, the authors introduced a secondary lesion (ATGL-KO) in the Prmt5 null myofibers that would preserve the lipid droplets by preventing ATGL-mediated lipolysis. This restored lipid droplet content and largely rescued muscle contractile function, demonstrating that depletion of lipid droplets drives muscle atrophy and impairs muscle contractile function. The authors also performed a series of

biochemical and molecular biology assays to show that PRMT5 stabilizes SREBP1a through dimethylation, enhancing the activity of this master transcriptional regulator of de novo lipogenesis. PRMT5 also methylates H4R3, and the methylated H4R3 represses the transcription of Pnpla2 (ATGL - which controls lipolysis), therefore inhibiting degradation of lipid droplets. These results together illustrate the dual role of PRMT5 in promoting lipid biogenesis and inhibiting lipid droplet degradation.

2. Significance:

Significance (Required)

Collectively, this study identifies PRMT5 as a key regulator of lipid metabolism in the muscle and establishes a causal relationship between lipid droplet and muscle contractile function, and points to scarce lipid droplets as a driver of muscle atrophy. PRMT5 has previously been reported to regulate early myogenesis but its role in post-fusion myofibers has never been reported, therefore the conceptual novelty of this study is high.

There are a couple of minor points authors should consider:

1. While muscle-specific Prmt5-KO mice show reduced muscle mass, it is not clear whether this is a developmental effect or muscle atrophy. Authors should measure a few markers of muscle atrophy such as Atrogin1 and MuRF1 and overall levels of ubiquitination. Alternatively, authors can also subject the mice to conditions of muscle atrophy such as starvation and measure how various markers of atrophy are affected.
2. Please show representative EcoMRI images for body composition analysis.

3. How much time do you estimate the authors will need to complete the suggested revisions:

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Less than 1 month

4. *Review Commons* values the work of reviewers and encourages them to get credit for their work. Select 'Yes' below to register your reviewing activity at [Web of Science Reviewer Recognition Service](#) (formerly Publons); note that the content of your review will not be visible on Web of Science.

Web of Science Reviewer Recognition

No

Full Revision



Manuscript number: RC-2022-01749R

Corresponding author(s): Shihuan, Kuang

1. General Statements [optional]

Dear Dr. Monaco,

Thank you for the feedback on our manuscript entitled, "PRMT5 links to lipid metabolism to contractile function of skeletal muscles". I would like to also thank you for the detailed guidance on next steps provided via Zoom meeting.

The authors appreciate the time and effort that editor and reviewers have dedicated to providing constructive and insightful comments to improve our manuscript. We are happy to revise our manuscript for consideration by *Embo Journal*.

We have performed key additional experiments to address the comments raised by the reviewers. As you can see, we have attached a point-by-point response to the questions of the reviewers. Specific changes made to the figures (and associated text) are:

- Updated Figure 1F, G
- Figure 3G,H is replaced with Figure S3B,C
- Figure 5G is updated to show significant changes more specifically
- Figure 7D,I are moved from Supplementary data (reviewer suggested)
- Supplementary Fig.S1F is a new panel
- Supplementary Fig. S2 (A-G) is a brand new figure
- Supplementary Fig. S4A is a new panel
- Supplementary Fig. S5 (A-D) is a brand new figure
- Supplementary Fig. S6D-F are new panels
- Supplementary Fig. S6G,H are moved from main figures (reviewer suggested)
- Table 1 is a new table.

With these changes, we believe that we have address all comments raised by the three reviewers. We would also like to re-emphasize that the demonstration of PRMT5's role in linking lipid metabolism to muscle contractile function represents a conceptual advance that merits consideration by *Embo Journal*. No other molecules have been identified to exert this function in muscle. The ability to rescue PRMT5-loss-of-function by ATGL KO provide compelling in vivo evidence for the proposed role of PRMT5. Reviewer 1 may have underestimated this significance that is clearly highlighted by Reviewer 3.

Sincerely,

Shihuan Kuang, skuang@purdue.edu

2. Point-by-point description of the revisions

This section is mandatory. Please insert a point-by-point reply describing the revisions that were already carried out and included in the transferred manuscript.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

The Kim et al. paper titled "PRMT5 links lipid metabolism to contractile function of skeletal muscles" reports how the arginine methyltransferase PRMT5 affects lipid metabolism in myofibers by stabilizing the mSREBP1 protein and repressing the expression of the PNPLA2 gene. The genetic deletion of PRMT5 in muscle results in the loss of lipid droplets in myofibers and a loss in muscle strength. Additionally, there is a change in muscle fiber types, moving from an oxidative state to a more glycolytic one. While the authors present compelling data on PRMT5's role in muscle metabolism, there are some concerns on the mouse model used and the sequencing data.

Major concerns:

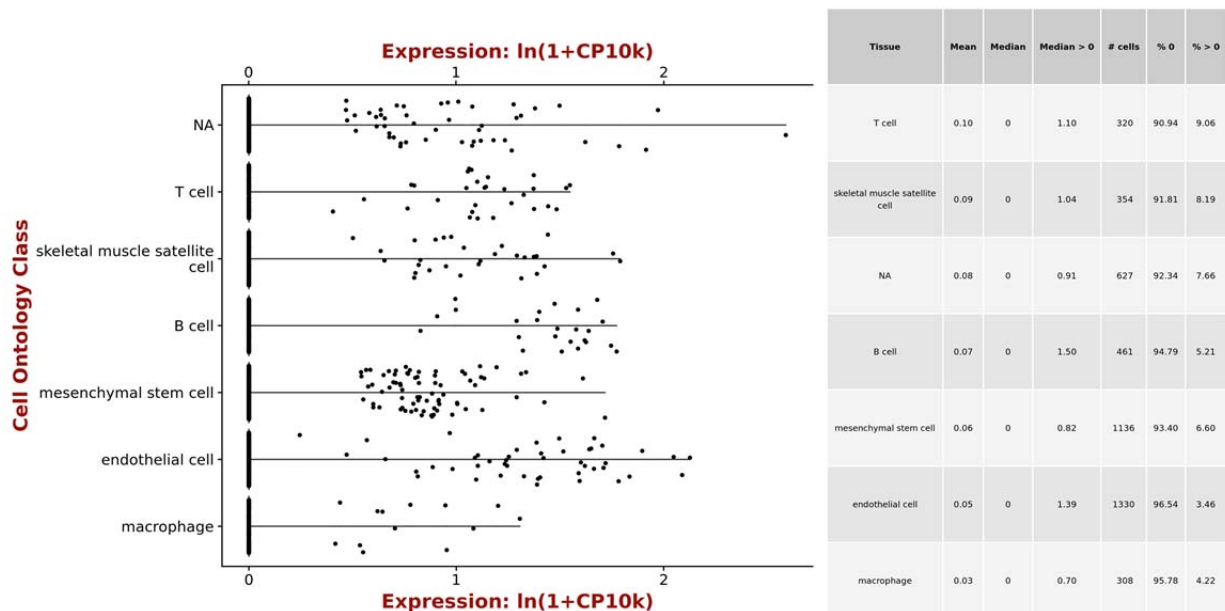
1. The mouse model used in this study is PRMT5^{fl/fl}, Myl1^{cre} in order to genetically delete PRMT5 in skeletal muscle. While there is no issue with the KO mice, the WT mice are PRMT5^{fl/fl}, Myl1⁺ which is not an acceptable control. It is known that Cre itself can have a phenotype, and additionally Myl1 is very highly expressed. Thereby, there is a large amount of Cre in the KO mice, but none in the WT which may contribute to the differences seen between WT and KO mice. The appropriate WT control is PRMT5^{+/+} Myl1^{cre} and the experiments would need to be repeated using this mouse genotype as the WT.

- We appreciated the comment and have analyzed many mice from the various control groups (WT, Myl1^{Cre}, Prmt5^{fl/fl}, and Myl1^{Cre}/Prmt5^{fl/+}). Long story short, we have maintained and used Myl1^{Cre} for multiple projects and in several previous publications (PMID: 25794679; PMID: 27644105), and never observed a phenotype of the Myl1^{Cre} mice. In the current study during the development of the Myl1^{Cre}-Prmt5^{fl/fl} mouse model, we had to first breed Myl1^{Cre} with Prmt5^{fl/fl} to generate the Myl1^{Cre}/Prmt5^{fl/+} mice, and then breed Myl1^{Cre}/Prmt5^{fl/+} with Prmt5^{fl/fl} mice to generate the Myl1^{Cre}/Prmt5^{fl/fl} mice. During the breeding we had generated many Myl1^{Cre}/Prmt5^{fl/+} mice (at least 10). We only observed phenotypes in the Myl1^{Cre}/Prmt5^{fl/fl} mice but not in the Myl1^{Cre}/Prmt5^{fl/+} (heterozygous KO) mice. In line with our observation, no phenotypes were described in the original report on the generation of the Myl1^{Cre} mice by Steve J. Burden and his colleagues ([Bothe, Genesis, 2000](#), PMID: 10686620). Also, in consistent to our choice of the floxed mice as control, [Pereira et al \(EMBO Molecular Medicine, 2020, PMC7005622\)](#) used the *Ndufs*^{fl/+}/Myl1^{Cre/-} or *Ndufs*^{fl/fl}/Myl1^{Cre/-} as control for their *Ndufs*^{fl/fl}/Myl1^{Cre+/-} KO mice.
- Given the situation, we trust that the reviewer will agree that the Prmt5^{fl/fl} mice are appropriate controls and repeating all the experiments with a new control model will not only require years of work but also violate IACUC's and NIH's 3R policy in reducing unnecessary use of animals. We added a sentence to state that the Prmt5^{fl/fl} are phenotypically identical to Myl1^{Cre}/Prmt5^{fl/+} mice in the revised manuscript.

2. In the scRNA-Seq the authors claim that PRMT5 is not expressed in quiescent muscle stem cells. However, the data set that is used only has approximately 250 muscle stem cells, which would not provide much coverage. It would be necessary to validate this claim by using other data sets, such as Tabula Muris or publicly available bulk RNA-Seq.

- As suggested, we queried Tabula Muris on Prmt5 expression in skeletal muscles based on scRNA-seq. The results showed that Prmt5 is expressed at very low levels in various mononuclear cell populations in the skeletal muscle. Specifically, only 8% of satellite cells had detectable levels of Prmt5, while 92% satellite cells had no detectable levels of Prmt5 (Table 1). We included the results in the revised Supplemental Table S1.

Table 1. Prmt5 mRNA expression in various cell populations in skeletal muscle based on scRNA-seq. <https://tabula-muris.ds.czbiohub.org>



3. The ChIP-Seq data shown was performed on 3T3-L1 cells and is not appropriate for a muscle paper. The ChIP-Seq must be performed on muscle cells in order to confirm their conclusion.

- We trust that the reviewer understand that ChIP-seq only represents a discovery tool that needs to be experimentally validated. Although the ChIP-Seq data and identification of the PRMT5 binding peak at the *Pnpla2* gene was based on 3T3-L1 cells, we have validated enrichment of PRMT5 on the potential binding region through ChIP-qPCR experiments. Repeating the ChIP-seq on muscle cells will not add additional support to the conclusion.

4. The authors claim that loss of PRMT5 leads to a gradual loss of muscle fiber size but has no effect on myogenesis. The evidence to support that claim is shallow, being based solely on CSA and total number of myofibers, along with a loss of lean body mass. To confirm this statement, it would be best to quantify the CSA and # of myofibers in EDL and TA at P7 and P21. Further, a regeneration assay would also demonstrate if myogenesis is compromised or not.

- Thank you for this suggestion. As *Myf1^{Cre}* is only expressed in post-differentiation myocytes and myofibers (Bi et al, 2016, eLife, PMID: 27644105), we do not expect the *Myf1^{Cre}/Prmt5^{fl}* to impact muscle development. Nevertheless, we now provide data on analysis of muscles at P7 and P21, as well postnatal muscle regeneration. The results are included in Supplementary Fig S2.

5. The data presented shows that there is fiber type switch from oxidative to glycolytic, along with a decrease in muscle strength in the PRMT5 KO mice. This seems counterintuitive to what is known in the field as glycolytic fibers are viewed as being capable of generating more force than oxidative, while having less endurance. The authors should clarify this point and elaborate more on their conclusion that the loss of strength is due to an altered metabolism.

- We agree with the reviewer that an increased abundance of glycolytic myofibers should increase muscle force under normal conditions. However, the increased glycolytic fibers in the *Prmt5* mKO mice is associated with metabolic deficiencies. These fibers are atrophic (smaller than control fibers of the same myosin type), devoid of lipid droplets and had less force production. We added the following sentences to the discussion in this matter.

Minor concerns:

1. The term myocyte is not the most accurate word for describing skeletal muscle. **Myofibers** would be best for fully differentiated muscle, myotubes for in vitro differentiation and myocytes should be reserved for differentiated cells that have not fused yet (1-2 nuclei/cell). I would recommend changing all mentions of myocyte to myofiber and changing "myocyte specific" with regards to the mouse models as "skeletal muscle specific"

- Thank you for your suggestion. We updated the nomenclature accordingly.

2. Figure 1 Panel F and G would be clearer if they were labelled as 2 months old mice

We edited the Figure 1F, and 1G to include the mouse age.

3. Figure 2 Panel E, G and H the control line appears to be missing error bars

Error bars were in the original panels, but the variation was very small, making it hard to visualize the error bars.

4. Figure 2 legend should specify that these mice are 2 months old for the sake of clarity

We added mouse age to the Figure 2 legend.

5. Figure 3 Panel G and H are not informative and are misleading. The supplemental panels show that when normalized to body mass there is no difference in O₂ consumption or CO₂ production. These should be replaced with the supplemental panels

We reformatted the figure as suggested.

6. Figure 4 Panel E and F, are these separate experiments? The values do not match between the 2 panels. If these are separate it should be made more clear.

a. Error bars appear to be missing in Panel E

Error bars were included (as it was obvious after FCCP) but the error bars are small for the rest of the time points.

b. As this is an experiment where the state of the cell is incredibly important (metabolism of

myoblast is much different to myotube), the authors must demonstrate that there is no defect in in vitro differentiation by showing the fusion index assay for these cells and a representative image of the myotubes.

The differentiation of the KO cells was normal as *Myf1^{cre}* is only expressed after differentiation. we isolated myoblasts from WT and *Prmt5* MKO mice and differentiated for 3 days to stain MyoG and MF20 to measure fusion index. However, we did not see any change in fusion index from differentiated myotubes (Supplementary Fig. S4A)

c. The authors should mention in the legend that these are 3 DM myotubes

Figure legends are updated.

7. Figure 5 Panel G, a qPCR to confirm the O/E of the PRMT5 and SREBP1a in the test samples would be necessary.

We added the PRMT5 and SREBP1 O/E data (Supplementary Fig S5C, D).

a. The double O/E cells are marked as significantly different, but it is unclear to which group they are significantly different to.

We edited the figure to highlight the comparison.

8. Figure 7 Panel B, body weight would be more informative in g rather than percentage.

We reformatted the figure as suggested.

9. It would be interesting to test whether the KO of *Pnpla2* also rescues the fiber type switch.

We added Fiber type staining in dKO muscles. Based on quantification in Sol and EDL muscles, increased Type IIB (glycolytic myofibers) in *Prmt5*^{MKO} mice was significantly decreased in *Prmt5/Pnpla2*^{MKO} mice (Supplementary Fig S6D-F).

Reviewer #1 (Significance (Required)):

Overall the manuscript provides insight into the role of PRMT5 in regulating the metabolism of skeletal muscle. While the paper provides some interesting data, it is severely hampered by the improper WT controls lacking the cre alleles. In order for the data to be reliable, all of the WT samples will need to be replaced with PRMT5+/+ *Myf1 cre* mice. There are other papers looking at the role of PRMT5 in skeletal muscle, however these are more focused on muscle stem cells than the myofibers. Therefore this paper does fill in a gap with regards to the metabolic role of PRMT5 and how this can affect skeletal muscle function. This paper would most likely be of interest to a specialized audience, mostly those in the skeletal muscle field.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

In the manuscript "PRMT5 links lipid metabolism to contractile function of skeletal muscles", Kim et al., describe the role for the arginine methyl transferase PRMT5 in maintaining skeletal muscle homeostasis. The authors showed that myocyte-specific deletion of PRMT5 results in the loss of muscle mass, reduced motor-performance, a fiber type switch from oxidative to glycolytic fibers along with reduced lipid content in the myofibers. The authors reasoned that

absence of PRMT5 results in reduced methylation and stability of mSREBP1 and an increase in the expression of adipose triglyceride lipase (ATGL).

Major Comments

1. Experiments looking at the interaction between PRMT5 and mSREBP1a using overexpressed proteins are used to conclude that PRMT5 methylates SREBP1a. While the results are consistent with these conclusions, the finding remains correlative. Methylation assays using purified PRMT5 and SREBP1a would be required to make a definite conclusion that PRMT5 methylates SREBP1a. These should be performed. In the absence of such data, the authors would need to adjust their conclusions to say that in the presence of PRMT5, SREBP1a becomes methylated, and that it remains to be determined if this is directly mediated by PRMT5.

We thank the reviewer for this comment and wished we could perform in vitro methylation assay to address whether SREBP1a is directly methylated by PRMT5. However, our co-author (Dr. Changdeng Hu) who is an expert of PRMT5 biochemistry unfortunately died recently, hampering the validation of SREBP1 as PRMT5 substrate. We would also like to mention that several other studies have reported SREBP1 as substrate of PRMT5 in cancer cells (Liu et al, 2016, Cancer Research, <https://doi.org/10.1158/0008-5472.CAN-15-1766>). We cited and discussed the paper in the manuscript. Our new results using enzymatic inhibitor of PRMT5 (BLL3.3) further supports that PRMT5 mediates methylation of SREBP1 (Supplementary Fig. S5A).

2. Stability studies in figure 5E have been performed in HEK293 cells using over-expressed proteins. While these results show that PRMT5 protects SREBP1a from degradation, the significance of this in muscle is less clear. Western blots of these proteins in C2C12 cells show that the over expression of PRMT5 does not stabilize SREBP1a (Flag) despite the appearance of increased methylation. To solidify the concept that PRMT5-dependent methylation of SREBP1 leads to its stabilization, cycloheximide experiments should be performed in myotubes generated in culture from the PRMT5mko mice. The stability of the endogenous SREBP1a gene could be monitored using antibodies.

We performed that study as suggested. Consistent with the notion that PRMT5 stabilizes SREBP1, we found extremely low levels of SREBP1 proteins (nearly undetectable) in Prmt5 KO myotubes, in contrast to the robust expression of SREBP1 in WT myotubes (Supplementary Fig. S5B). This extremely low levels of SREBP1 precludes us from examining degradation after cycloheximide treatment

3. The measurement of methylation levels of SREBP1a are complicated by the fact that the protein levels are destabilized in the absence of PRMT5. The authors should use a PRMT5 inhibitor experiments in complement to these overexpression studies to measure the relative methylation of mSREBP1a (SMY10/mSREBP1).

Full Revision

We used PRMT5 inhibitor, BLL 3.3, to support that mSREBP1a methylation is mediated by PRMT5 (Supplementary Fig. S5A).

Minor Comments

4. In figure 1H, there are more nuclei surrounding the myofibers. The authors should document the number of PAX7 cells per myofiber in the Control and Prmt5MKO mouse strains as it helps understand the additive effect of change in PAX7 cells during muscle atrophy.

We quantified the number of Pax7⁺ cells in muscles and myofibers of WT and KO mice (Supplementary S2D,E).

5. In figure 5, the legend title mentioned ATGL. However the stability or methylation results of ATGL are not presented anywhere in the manuscript. Only in figure 6 did the authors show the differential expression of ATGL in the presence and absence of PRMT5. This title for Figure 5 should be corrected.

Thanks for clarifying. We fixed title of Figure 5 in the manuscript.

6. For better representation, the authors should consider moving the western blot panel (S3A) and lipid droplet staining data (S3E) to main figures and some of the data on force generation and body weights to supplementary.

We updated the figures as suggested.

Reviewer #2 (Significance (Required)):

This is an interesting manuscript that provides novel insight into the role for PRMT5 in muscle homeostasis. While previous studies have looked at the PRMT5 from a transcriptional standpoint during muscle differentiation, this work shows a role for PRMT5 in controlling the metabolic state of myofibers through transcriptional and potentially non-transcriptional mechanisms. Identification of the transcriptional regulation of Pnpla2 gene by PRMT5 is confirmed by mouse rescue experiments with the double KO mice. However the role for non-transcriptional control of SREBP1a stability through methylation by PRMT5 is not as clearly established. To strengthen this aspect of the manuscript, additional experiments are needed.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

The lipid droplets represent an energy store and central hub of lipid metabolism in cells. In the skeletal muscle, abundant lipid droplets are present in myofibers (especially in oxidative Type 1 and IIA myofibers) and thought to play a role in supplying energy through fatty acid oxidation (FAO) to power muscle contraction. Increased abundance of lipid droplets in myofibers is associated with poor muscle function and insulin resistance in patients of type 2 diabetes. Paradoxically, myofibers of trained athletes also contain higher than normal levels of lipid droplets but with better contractile function and insulin sensitivity. The molecular mechanism underlying this "Athlete's Paradox" has been unclear, due to the lack of understanding of what

controls biogenesis and metabolism of lipid droplets in the myofiber.

In this manuscript, Kuang and colleague provide compelling evidence to support a key role of PRMT5 in maintaining lipid droplets in the myofiber. They generated a conditional knockout mouse model to disrupt the *Prmt5* gene in the myofibers. This leads to an astonishing depletion of lipid droplets in myofibers. The *Prmt5* null myofibers also exhibited poor contractile function and classical signs of atrophy. To determine if the depletion of lipid droplets drives muscle atrophy or if muscle atrophy drives depletion of lipid droplets, the authors introduced a secondary lesion (ATGL-KO) in the *Prmt5* null myofibers that would preserve the lipid droplets by preventing ATGL-mediated lipolysis. This restored lipid droplet content and largely rescued muscle contractile function, demonstrating that depletion of lipid droplets drives muscle atrophy and impairs muscle contractile function. The authors also performed a series of biochemical and molecular biology assays to show that PRMT5 stabilizes SREBP1a through dimethylation, enhancing the activity of this master transcriptional regulator of de novo lipogenesis. PRMT5 also methylates H4R3, and the methylated H4R3 represses the transcription of *Pnpla2* (ATGL - which controls lipolysis), therefore inhibiting degradation of lipid droplets. These results together illustrate the dual role of PRMT5 in promoting lipid biogenesis and inhibiting lipid droplet degradation.

Reviewer #3 (Significance (Required)):

Collectively, this study identify PRMT5 as a key regulator of lipid metabolism in the muscle and establish a causal relationship between lipid droplet and muscle contractile function, and point to scarce lipid droplets as a driver of muscle atrophy. PRMT5 has previously been reported to regulate early myogenesis but its role in post-fusion myofibers has never been reported, therefore the conceptual novelty of this study is high.

There are couple of minor points authors should consider:

1) While muscle-specific *Prmt5*-KO mice show reduced muscle mass, it is not clear whether this is a developmental effect or muscle atrophy. Authors should measure a few markers of muscle atrophy such as Atrogin1 and MuRF1 and overall levels of ubiquitination. Alternatively, authors can also subject the mice to conditions of muscle atrophy such as starvation and measure how various markers of atrophy are affected.

Thank you for this suggestion. As the *Myf1Cre* is only expressed in post-differentiation myocytes and myotubes/myofibers, we did see any developmental defects during postnatal myogenesis (Supplementary Fig S2A-C). We also checked Atrogin-1 and MuRF1 in WT and KO muscle tissues based on your advice, but we did not find any significant difference (Supplementary Fig S1F). These two genes are muscle-specific E3 ubiquitin ligases involved in protein degradation by the ubiquitin proteasome pathway. This finding is consistent with the idea that there are multiple mechanisms that can lead to muscle atrophy, and our study clearly elaborates that dysregulation of lipid catabolism by PRMT5 is the main pathway associated with muscle wasting.

Full Revision

2) Please show representative EcoMRI images for body composition analysis.

Thank you for your comments, but the EcoMRI equipment does not provide images of body composition. It only provides data of lean mass, fat mass and water in gram. We presented those data in the manuscript.

Dear Dr. Kuang,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from the referees and I am happy to say that both support its publication now. Only some editorial requests will need to be addressed before we can proceed with the official acceptance of your manuscript.

- Please correct the text of the "Data Availability Section" (DAS). This section should only list links to data generated in this study and deposited in public databases. If you have not generated such data in this study, please mention this fact in the DAS.
- Please correct the conflict of interest subheading to "Disclosure and Competing Interest Statement".
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- Fig 1L, S3A callouts are missing, please add.
- Please delete the figure legends from the figure files.
- Fig S4 only has one panel so doesn't need the 'A' panel label or callout.
- There are 6 Supplementary figures, which could be combined down to 5 EV figures. We cannot process more than 5 EV figures. EV figures and Appendix have replaced supplementary figures at EMBO press. You can find more info in our guide to authors online.
- The Supplementary table should be called Table EV1.
- I attach to this email a related ms file with comments by our data editors. Please address all comments in the final ms.

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I would like to suggest some minor changes to the abstract, that needs to be written in present tense. Please let me know whether you agree with the following:

Skeletal muscle plays a key role in systemic energy homeostasis besides its contractile function, but what links these functions is poorly defined. Protein Arginine Methyl Transferase 5 (PRMT5) is a well-known oncoprotein but also expressed in healthy tissues with unclear physiological functions. As adult muscles express high levels of Prmt5, we generated skeletal muscle-specific Prmt5 knockout (Prmt5MKO) mice. We observe reduced muscle mass, oxidative capacity, force production and exercise performance in Prmt5MKO mice. The motor deficiency is associated with scarce lipid droplets in myofibers due to defects in lipid biosynthesis and accelerated degradation. Specifically, Prmt5 deletion reduces dimethylation and stability of Sterol Regulatory Element-Binding Transcription Factor 1a (SREBP1a), a master regulator of de novo lipogenesis. Moreover, Prmt5MKO impairs the repressive H4R3 symmetric dimethylation at the Pnpla2 gene, elevating the level of its encoded protein ATGL, the rate-limiting enzyme catalyzing lipolysis. Accordingly, skeletal muscle-specific double knockout of Pnpla2 and Prmt5 normalizes muscle mass and function. Together, our findings delineate a physiological function of PRMT5 in linking lipid metabolism to contractile function of myofibers.

I look forward to seeing a final version of your manuscript as soon as possible. Please use this link to submit your revision:
<https://embor.msubmit.net/cgi-bin/main.plex>

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have adequately addressed all of my previous concerns.

Referee #2:

The authors have been very receptive to critiques and made appropriate changes to the manuscript. I have no further concerns with the manuscript.

The authors have addressed all minor editorial requests.

Dr. Shihuan Kuang
Purdue University
Animal Sciences and Center for Cancer Research
901 West State St
Smith Hall 174B
West Lafayette, IN 47907
United States

Dear Dr. Kuang,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

There are 2 remaining issues: Some of the text in the synopsis image is too small, especially the chromatin modifications. Can you please send us a new synopsis image with larger text? Also, not all the suggested changes were included in the abstract. Please let me know whether you agree with the changes I suggested, so that we can use this version of the abstract. Thank you.

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Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Best regards,
Esther

Esther Schnapp, PhD
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Reporting Checklist for Life Science Articles (updated January 2022)

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

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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.
- ☒ ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- ☒ plots 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. Any statistical test employed should be justified.
- ☒ Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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:
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 - are tests one-sided or two-sided?
 - are there adjustments for multiple comparisons?
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Include a statement about blinding even if no blinding was done.	Yes	Materials and Methods
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not Applicable	
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For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figures
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