Comments to the Authors

The authors addressed the molecular mechanism by which HDAC11 gene expression is upregulated in early muscle differentiation by comparing the chromatin states between proliferation and differentiation C2C12 cells at HDAC11 locus. The results showing the involvement of p300 and MyoD in the induction of HDAC11 expression at the onset of muscle differentiation, in normal and signal-enhanced (RXR) conditions, are clear and the figures are clear and well labelled and described. The paper structure and English language are correct.

However, there are several issues that need to be address and better discussed:

1- In the abstract, the authors refer to "*Through integrative omic analyses, we found that while some HDACs were differentially expressed, at the early stage of myoblast differentiation, the upregulation of Hdac11 gene expression was most pronounced and significant".*
However, this is not a novel finding because it was already published in 2020, by Núñez-Álvarez et al. These authors reported that HDAC11 was the HDAC member whose expression changes the most at the transition from proliferating myoblasts to early differentiating myocytes (day 1 of differentiation) in satellite cell-derived primary myoblast (Figure 1A and B). The authors also reported the HDAC11 upregulation in human primary myoblasts upon differentiation and in differentiated C2C12 (Figures S1 and 1C, respectively). They also showed a very fast induction, being detected as early as 12 hours post differentiation (Figure 1C).

   The abstract sentence should clarify that.

2- The introduction is fine, but in the last paragraph should include that it was previously published that HDAC11 was upregulated in C2C12 differentiating cells, as well as in human and mouse satellite cell-derived primary myoblast (Byun et al 2017 in Figures 1A and B; and Núñez-Álvarez et al.2020).

3- Regarding the methodology, the authors determine differential gene expression between proliferation and 24 hours of differentiation with a cut-off of $\geq \pm 1.5$ absolute fold change. The authors found 3.159 genes upregulated and 3.936 genes down regulated. However, they did not mention about which was the false discovery rate (FDR) used. FDR (the ratio of the number of false positive results to the number of total positive test results) allows to decide how many false positives they are willing to accept among all the results that can be called significant. It is important to mention which FDR has been used.

4- For MyoD and p300 ChIP, the authors do not indicate which is the control locus used and the corresponding primers in the Materials and Methods section.

5- In the discussion, the paragraph from lanes 238 to 243 should be rewritten, mentioning the two previous papers showing the up regulation of HDAC11 in early myoblast differentiation (Byun et al 2017; Núñez-Álvarez et al.2020). In addition, the authors should clarify that they compare mRNA levels, not protein levels, when they refer that HDAC11 rich the same level than HDAC1,2,3, and 7 upon differentiation.

6- In the discussion, the paragraph from lanes 244 to 251 is a bit confused. I think that the authors aim to comment about the fact that HDAC11 is upregulated by RXR signaling, but the RXR ChIP-Seq data do not exhibit specific RXR enrichment at the HDAC11 locus, suggesting an indirect effect. Please, revise this paragraph.

7- In the conclusion, the paragraph begins with "*Adult muscle regeneration is a multi-stage process…..*"

I would replace that for "*Myogenesis is a multi-stage process….*" , since the paper is not referring to muscle regeneration at any moment.

8- The last part of the conclusion should be modified, because is not focused on the paper results and because it is referred to the Núñez-Álvarez et al paper (reference 25) without being accurate.
"*In addition, Hdac11 ablation affects the expression of genes involved in cell cycle progression, which leads to persistent myoblast proliferation irrespective differentiation induction (25). Therefore, HDAC11 likely plays an important role in histone deacetylation at these gene promoters to mediate cell cycle arrest to permit myogenic activation at the onset of myoblast differentiation*".

However, in this paper the authors reported that HDAC11-deficient satellite cells showed sustained proliferation, consistent with the deregulated expression of cell cycle genes observed in the RNA-Seq data and the observed delay in cell cycle exit *in vitro* and *in vivo*. However, the level of H3 acetylation marks in the analyzed promoter regions of some cycle-related genes, such as Aurka, Aurkb, and Pcna, did not show differences in shRNA-HDAC11cells. That would suggest

that the upregulation of cell cycle-related genes is not mediated by increased H3 acetylation levels due to the reduction of HDAC11 histone deacetylase activity.

Minor comments to the authors

1- In lane 52, the word *enhances* I think it should be *enhancers*

2- In lane 202, when the authors refer to the HDAC11 upregulation as earlier as 12 hours of differentiation, I would also include Fig2A (Fig. 2A and 3A)

3- In Figure 3, it should be indicated DM or 24h of differentiation in the graphs corresponding to control and bexarotene treatment

4- In lane 206, please indicate in which conditions it was done the RXR ChIP-Seq. At 24 h of differentiation?

5- In figures 2A and 3A when it is indicated HDAC class, in HDAC11 class it is written Class V instead of Class IV (at least in my computer it is missed the number I)

6- In lane 218, it is written Figure 5D instead of Figure 3D

7- In Figures 2F and 3F better indicate in Y axis HDAC11 mRNA (fold change)

8- In lane 246, *we* should be in capital letter

9- In lane 267, it should be HDAC11 instead of HDAC1