THE EMBO JOURNAL

SIRT7 activates quiescent hair follicle stem cells to ensure hair growth in mice

Guo Li, Xiaolong Tang, Shuping Zhang, Meiling Jin, Ming Wang, Zhili Deng, Zuojun Liu, Minxian Qian, Wei Shi, Zimei Wang, Hongfu Xie, Ji Li, and Baohua Liu
DOI: embj.2019104365

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Review Timeline:

Submission Date:	27th Dec 19
Editorial Decision:	29th Jan 20
Revision Received:	10th May 20
Editorial Decision:	9th Jun 20
Revision Received:	11th Jun 20
Accepted:	14th Jun 20

Editor: Daniel Klimmeck

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 29th Jan 2020

Dear Dr Liu,

Thank you again for your interest and for the submission of your manuscript (EMBOJ-2019-104365) to The EMBO Journal. Thank you also for your patience with my response, which got delayed due to detailed discussions in the team. Your study has been sent to three referees, and we have received reports from all of them, which I enclose below.

The referees acknowledge the potential interest and novelty of your work, although they also express major concerns. In particular, referee #1 raises hesitations regarding the lack of support for a cell autonomous mechanism (pt.2) and asks you to rule out potential off-target effects of SIRT7. Referee #2 points to issues related to data inconsistencies and requests additional in vitro assays to support your proposed model (Ref#2, pts 3-5,7). Referee #3 agrees in that the interpretation is at this point not convincing in his-her view and states that more experiments are needed to discriminate SIRT7 dependence of germ versus stem cells and effects on timing versus HFSC numbers. In addition, the referees raise a number of issues related to related to missing controls, data quantification and illustration that would need to be conclusively addressed to achieve the level of robustness and clarity needed for The EMBO Journal.

I judge the comments of the referees to be generally reasonable and given their overall interest, we are in principle happy to invite you to revise your manuscript experimentally to address the referees' comments.
I need to stress though that we do need strong support from the referees on a revised version of the study in order to move on to publication of the work and as to the open outcome of the revisional work.

Please feel free to contact me if you have any questions or need further input on the referee comments.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Kind regards,

Daniel Klimmeck

Daniel Klimmeck, PhD
Editor
The EMBO Journal

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Instructions for preparing your revised manuscript:

Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
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Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition).

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability" section (placed after Materials & Method) that follows the model below (see also https://www.embopress.org/page/journal/14602075/authorguide#availabilityofpublishedmaterial). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

Our journal also encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at https://www.embopress.org/page/journal/14602075/authorguide#referencesformat

IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (http://emboj.embopress.org/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 28th Apr 2020.

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Referee #1:

In Li, et al, the authors sought to uncover mechanistic insight into how HFSCs become quiescent and fail to re-activate with age. They demonstrated that the loss of SIRT7 activity is at least partially responsible for the maintenance and activation of HF cycling, specifically through the regulation of NFATc1. The authors demonstrate that loss of SIRT7 deacetylation activity results in reduced degradation of NFATc1 by PA28γ, promoting its transcriptional activity in the nucleus and stabilizing the cells in the telogen phase. Through genetic models and cell work, they establish the relationship between SIRT7 and NFATc1. They also show similar observations in aged animals, suggesting a model by which age-related decline of SIRT7 produces quiescent HFSCs via stabilized NFATc1 signaling.
Overall, the authors have produced an excellent manuscript that tells a very compelling narrative of SIRT7's role in hair follicle aging. For the most part, their techniques and approach were quite thorough and their controls were appropriate for the bulk of their experiments. Figure 4, in particular, is an eloquent series of experiments that provide an incredible persuasive series of data. This care and focus is present throughout the manuscript.

However, there are a few issues that I believe the authors should address before final publication. The major issue is confirming that the effects are truly cell autonomous. Point #2 addresses possible solutions the authors can use to address this. These issues should not be difficult to accomplish in a timely manner and will help to strengthen and polish the final manuscript. This work is definitely thorough and insightful to SIRT7 biology and its role in stem cell biology and aging.

1.) In Figure 1C, the authors do not indicate if these stainings are from shaved or unshaved animals. Also, Figure 1M, 2H: How are these graphs generated? I.e. where does the data come from, or is it an abstraction as a schematic overview to illustrate the different cyclin patterns between the Sirt7 conditions? This should be described more extensively in the Figure legends or Methods, respectively.
2.) In regards to the epidermal stem cell-specific KO of SIRT7, the authors need to provide genotyping or qRT-PCR data for other tissues to rule out off-target contributions of SIRT7 activity. In general, this is the biggest concern for the validity of the findings, as the cell-autonomy of these findings is based off of these experiments.

Minor Issues
-Figure 3G-H: Why are Id3 and S11a each inversely regulated with respect to the NFAT target genes? Please provide some hypothesis or explanation to acknowledge this.

-Please quantify the Westerns for 3A. In general, any blot that is not showing a binary result should be quantified. Also, please indicate the n-value for the replicates in these works.

-Figure 5C and 5H should be quantified and the replicates indicated.

-Overall, please use different line styles to indicate different strains.

-Several figures throughout the manuscript need quantification. Fig 6A-B, Fig 7B and 7D.

-In discussion "Thus, it is plausible to speculate that to initiate a new HF cycle, Sirt7-mediated Nfatc1 degradation by PA28γ might be a better choice than ubiquitination relied, as this degradation pathway consumes less energy for quiescent HFSCs" is not supported in regards to the energy consumption. I suggest removing the part about energy consumption unless the authors want to include evidence.

-Figure 5 D-E: Looks like the Sirt7 H187Y mutant is less effective than wt, albeit the NFAT1c levels at 3h seem to be less those with EV. Authors should provide significance tests for Figure 5E and discuss the result.

-Figure 5K: The authors state: "Moreover, overexpression of HA-SIRT7, but not the H187Y mutant, promoted NFATc1 binding to PA28γ (Figure 5K)." In fact there is NFAT1c binding with the mutant Sirt7, although with lower yield in the IP. Authors should provide explanation and soften their statement.

-Page 17: "As such, Cyclosporin A (CsA), a potent calcineurin inhibitor, suppresses NFATc1 nuclear accumulation and renders NFATc1 activity (Hogan et al., 2003)." Statement is somewhat confusing by the use of the word "renders". Please rephrase for clarity.

Referee #2:

In this manuscript, Li et al. document a role for the NAD-dependent deacetylase Sirtuin 7 (Sirt7) protein in hair follicle stem cell (HFSC) activation, and hair follicle (HF) growth, using mouse genetic models. The authors turned to cell culture systems to show that Sirt7 interacts with Nfatc1, leading to Nfatc1 deacetylation, and nuclear degradation. Interestingly, the in vivo CSA treatment of Sirt7-/- mice, rescues HF growth compared to controls. Moreover, the authors established a correlation between Sirt7expression and skin ageing.
Overall, the findings in the manuscript are interesting. However, in its present form, the authors conclusions are not yet rigorously addressed to provide a conceptual advance to the field.

Specific comments:

1. Changes in CD34+ HFSC numbers. The authors show that the loss of Sirt7 does not affect the number of CD34+ HFSC, is this experiment conducted during the first HF cycle (Figure S2C)? Is the number of CD34+ HFSC still maintained during the second HF cycle in the Sirt7-/- mice?

2. Figure 1B. The authors indicate that the relative percentage of HF recovery after shaving is almost full by day P120 in control mice, while Sirt7-/- KO mice only showed 10% of relative hair recovery. However, the histogram does not represent that conclusion and indicates that KO mice exhibited a 100% recovery by day P80. Overall, although the data show a delay, the interpretation is not persuasive.

3. Figure 1C. The H&E images do not seem to be representative. Considering the results shown in Figure 1B, by day P90, approximately 85% of KO mice recovered HFs. Also, a better characterization of HF anagen stages and HF growth is needed. How does the wave of anagen entry look like in the Sirt7-/- KO mice that recovered their coat? Does it occur in a synchronized manner across the skin?

4. Figure 1H. In the same lines of ideas than point number 2. The authors indicate that the relative percentage of HF recovery after shaving is almost full by day P90 in Sirt7-TG mice. At the same time, controls only showed 10% of relative hair recovery, but the histogram does not represent that conclusion.

5. Figure 1F. The immunofluorescence analyses are not quantitative. The authors should also show that there are no quantitative changes in the number of CD34+ alpha-6 integrin+ HFSC by FACS upon the ablation of Sirt7 in the conditional Sirt7fl/fl; K15Cre model. The authors should also show if there any delays in the first HF cycle of the Sirt7fl/fl; K15Cre model. Showing these data will be helpful to support the conclusion that the loss of Sirt7 in HFSC does not affect HF morphogenesis.

6. Figure 2D, is the recovered HF growth in the Sirt7fl/fl; K15Cre mouse model synchronized?

7. Loss of Sirt7 and Nfatc1 in HFSC quiescence and activation. The authors isolated HFSC from Sirt7-/- mice, Sirt7-TG mice, and their control counterparts to conduct mechanistic analyses. However, the authors need to validate if there are any differences in the activation or quiescence of the HFSC cultures, to use them as a bonafide cell culture system to compare it with their in vivo findings. Are there any differences in the proliferation properties between the different cell types? Changes in colony formation? If so, is this due to quiescence, apoptosis, or differentiation?

8. The authors conducted a screen using isolated HFSC culture cells to test for concrete protein expression differences between Sirt7-/- mice, Sirt7-TG mice, and their control counterparts. They found an opposite correlation between the expression of Sirt7 and Nfatc1. Also, Sirt7 knockdown cells exhibited higher levels of Nfatc1, while overexpression of NFatc1 leads to NFatc1 reductions. However, in Figure S3, the skin of Sirt7-/- mice at day P100 shows no expression of Nfatc1 in the bulge. How do the authors reconcile the differences?

9. The authors nicely show that Sirt7 controls Nfatc1 deacetylation and stability through direct protein-protein interactions via specific domains. What is the biological role of precluding the Sirt7/Nfatc1 interaction in the quiescence/proliferation properties of the cultured cells?

10. In Fig 6, the authors show that treating Sirt7-/- deficient skin with CSA rescues HF growth when compared to the vehicle-treated region of the skin. It is a bit puzzling that the rescue is limited to the treated area without generating a HF growing wave throughout the skin.

11. Sirt7 and HF aging. Aging is a multifactorial process, and the authors show that full-body Sirt7-TG mice are in better shape than controls. Using hair plucking experiments, the authors evaluated the dynamics of HF regrowth and concluded that the Sirt7-Nfatc1 axis regulates it. At what time point was the expression of Sirt7 induced with Dox? The authors should induce the expression of Sirt7 in already aged mice, before conducting the experiments, to substantiate their claims.

Minor points:

1. Sirt1, Sirt6, and Sirt7 have aging phenotypes. It will be useful for the flow of the manuscript, to add a short sentence indicating the rationale to study Sirt7 in HF growth, as a representative member of the family.

2. Figure 1B, Figure 1H, Figure 2D. The plot of the graphs is confusing. Since the variable is the % Relative hair recovery, this should be changed to the Y-axis.

3. Figure 1D and 1G. Ki67 activation is shown at P90 in control HF in Figure 1D, and in P85 in Figure 1G. The areas shown do not represent the HF germ, but the HF matrix.

4. Figure 1C, Figure 1I, Figure 2E. Indicate the mouse skin age in the figure panels and legends, since it differs from the time points shown in the accompanying mouse images.

5. Figure S2D, in the manuscript text, the age of the mice is described as P72. The images show HF at P74.

Referee #3:

The manuscript by Guo et al. investigates the role of Sirt7 during the hair cycle. They show that Sirt7 is expressed in the hair follicle and that deletion of Sirt7 delays the transition from telogen to anagen, while the overexpression leads to an early anagen onset. Sirt7 expression is reduced in hair follicles of aged mice and aged Sirt7-/- mice present hair loss, which can be ameliorated by overexpression of Sirt7. Mechanistically, they uncover that Sirt7 deacetylates Nfatc1, a transcriptional regulator mediating hair follicle stem cell quiescence. Deacetylation of Nfatc1 leads to PA28y-mediated proteasomal degradation of Nfatc1 and stem cell activation.

While the findings are overall interesting, I do not share some of the interpretation and have a nuber off important questions that need to be addressed before publication. Furthermore, there are several experimental details missing that need to be added.

Figure 1:

The authors interpret their results as the direct effect of Sirt7 manipulation on hair follicle stem cells. However, Sirt7 is expressed in other hair follicle cells as well and the presented effects on proliferation look rather hair germ specific. The used marker P-cadherin is a hair germ marker. For bulge cells CD34 should be used. The second picture from the left in Fig. 1D (Sirt7+/+, P90) shows the matrix of a growing hair follicle. Proliferating cells in the matrix are classified as transient amplifying cells, which are distinct from hair follicle stem cells (HFSC) in the bulge and ORS. To study bulge HFSC activation the authors should focus on early anagen (anagen I and II). The best is to use EdU to show/quantify stem cell proliferation.

Important questions regarding HFSC phenotype when deleting/overexpressing Sirt7:

Are less/more HFSCs become activated in anagen or is only the timing different?

If fewer HFSCs activated in the knock out - what is the functional consequence of this when it comes to hair follicle growth?

Growth-mediating stem cell activation is synchronized between interfollicular epidermis (IFE), sebaceous gland (SG) and hair follicle (HF) (Reichenbach B et al., 2018, EMBO J). It would be important to know if Sirt7 is expressed in the IFE and SG basal layer?

Is basal layer proliferation in the IFE and SG affected in the Sirt7 knock out?

What is the promoter driving Sirt7 overexpression? Only in bulge/HG or in whole mouse? Please state the experimental details - important for interpretation.

In the text it is stated: "By P120, Sirt7+/+ mice recovered an almost full hair coat, while the Sirt7-/- mice showed only around 10% recovery (Figures 1A and 1B)."

However, the graph in Fig. 1B shows 100% hair recovery for Sirt7-/- mice at 75 days post shaving, which corresponds to P120. Please correct/explain!

The graph in Fig. 1H has a similar mismatch with the text as I stated regarding Fig. 1B above.

How is telogen duration measured in Fig. 1F?

Figure 2:

The upregulation of Sirt7 during telogen-anagen transition is insufficiently supported by the presented IHC images. To support this claim, the authors should purify CD34+ bulge cells and perform qPCR for Sirt7. It very much looks like as HF surrounding adipocytes express Sirt7 as well. Adipocyte lineage cells are important determinants of the hair cycle and control stem cell activation (Festa E et al., Cell. 2011). It is important to know if adipogenesis is altered in the Sirt7 knock out mice, as this alone could change the anagen onset.

As for IFE and SG, mentioned above, it is critical to evaluate the entire expression pattern of Sirt7 in the skin.

In text and figure a mifepristone K15-Cre mouse line is stated. Is the mouse model K15-CrePGR - please clarify with reference and mention the line in the methods. Please state how much/many injections of mifepristone are used.

In case of the K15-CrePGR line, it is not entire specific to HFSCs.

It looks like Sirt7 is predominantly expressed in the HG and expression of Sirt7 seem to correlate with Ki67/HG activation but not with bulge stem cells.

Figure 3:

Why do the authors use so many different cell types/lines for different experiments and without validation of the results in the other lines? It would be much better to use only HFSCs! Or at least epidermal keratinocytes.

Please address functional effects on Sirt7 knock out/over expression on HFSCs in vitro - colony formation, long term passaging, transcriptomics.

Figure 3G and 3H are not the same panel of genes - why not?? How are Nfact1 target genes defined?

Figure 4:

There are several sirtuins expressed in HFSC (1,2,6,7) - what happens to them when Sirt7 is lost/over expressed - is there compensation or redundancy? How was assessed that the NAM (sirtuin) inhibitor is active?

Figure 5:

The authors show direct interaction of Nfat/Sirt7 and Nfat/Pa28g. Wouldn't it make sense to probe for Sirt7/Pa28 or is Sirt7 and Nfat binding to Pa28 mutually exclusive or sequential?

Where on Nfat is Sirt7 deacetylating - where on Nfat is Pa28 binding? Are these events structurally linked?

Is the dynamics between Nfat phosphorylation and acetylation relevant?

Where is the calcineurin phosphorylation site?

Figure 6:

I do not think it is surprising that CSA works on Sirt7 knock out animals.

Would it not be more interesting to over express Nfat on Sirt7-TG background? One would expect that this has no effect on hair cycling, if Nfat is indeed deacetylated and downregulated?

Figure 7:

What is the phenotype of the aged Sirt7 knock out animals - are there fewer hair shafts/follicle?

Are there fewer HFSCs in aged knock out animals? Is the HG smaller?

Spelling and grammar should be looked over. Please use precise terms. The methods section lacks many details. Experiments need more detailed description.

Response to Editor's comments

The referees acknowledge the potential interest and novelty of your work, although they also express major concerns.
In particular, referee #1 raises hesitations regarding the lack of support for a cell autonomous mechanism (pt.2) and asks you to rule out potential off-target effects of SIRT7.
Referee #2 points to issues related to data inconsistencies and requests additional in vitro assays to support your proposed model (Ref#2, pts 3-5,7).
Referee #3 agrees in that the interpretation is at this point not convincing in his-her view and states that more experiments are needed to discriminate SIRT7 dependence of germ versus stem cells and effects on timing versus HFSC numbers. In addition, the referees raise a number of issues related to missing controls, data quantification and illustration that would need to be conclusively addressed to achieve the level of robustness and clarity needed for The EMBO Journal.
I judge the comments of the referees to be generally reasonable and given their overall interest, we are in principle happy to invite you to revise your manuscript experimentally to address the referees' comments.

Response

On behalf of my co-authors, I would like to thank you for your time in assessing our manuscript. We greatly appreciate the revision opportunity. We have addressed all the concerns raised by three referees, which we believe have helped us to greatly improve our manuscript. Our point-by-point responses are provided in this document.

Response to referees' comments

Referee #1:

In Li, et al, the authors sought to uncover mechanistic insight into how HFSCs become quiescent and fail to re-activate with age. They demonstrated that the loss of SIRT7 activity is at least partially responsible for the maintenance and activation of HF cycling, specifically through the regulation of NFATc1. The authors demonstrate that loss of SIRT7 deacetylation activity results in reduced degradation of NFATc1 by PA28γ, promoting its transcriptional activity in the nucleus and stabilizing the cells in the telogen phase. Through genetic models and cell work, they establish the relationship between SIRT7 and NFATc1. They also show similar observations in aged animals, suggesting a model by which age-related decline of SIRT7 produces quiescent HFSCs via stabilized NFATc1 signaling.

Overall, the authors have produced an excellent manuscript that tells a very compelling narrative of SIRT7's role in hair follicle aging. For the most part, their techniques and approach were quite thorough and their controls were appropriate for the bulk of their experiments. Figure 4, in particular, is an eloquent series of experiments that provide an incredible persuasive series of data. This care and focus is present throughout the manuscript.

Response #1-1

We appreciate the encouraging comments from this referee.

However, there are a few issues that I believe the authors should address before final publication. The major issue is confirming that the effects are truly cell autonomous. Point #2 addresses possible solutions the authors can use to address this. These issues should not be difficult to accomplish in a timely manner and will help to strengthen and polish the final manuscript. This work is definitely thorough and insightful to SIRT7 biology and its role in stem cell biology and aging.

Response #1-2

We appreciate the constructive comment. Please refer to response #1-4 (Point #2).

1.) In Figure 1C, the authors do not indicate if these stainings are from shaved or unshaved animals. Also, Figure 1M, 2H: How are these graphs generated? I.e. where does the data come from, or is it an abstraction as a schematic overview to illustrate the different cyclin patterns between the Sirt7 conditions? This should be described more extensively in the Figure legends or Methods, respectively.

Response #1-3

We are sorry for the inadequate information provided for Figures 1 and 2 (revised Fig 1 and 2). In the revised Figure parts 1C, 1K and 2E, we performed a histological analysis of skin tissues in unshaved mice at the indicated times. To better demonstrate

the role of Sirt7 on hair cycle progression, we calculated the duration of each hair cycle phase and generated the schematic graphs (revised Fig 1M and 2I). To define the hair cycling phases, we considered two points: (1) that the skin pigment production in C57BL/6 mice positively correlates with hair follicle growth; we used this to assess the hair cycle phase post hair-shaving, e.g. from telogen to anagen, the dorsal skin color shifts from pink to black (dense pigmentation) (Muller-Rover et al., 2001; Stenn and Paus, 2001); (2) the first two postnatal hair cycles are relatively synchronous (Rishikaysh et al., 2014) and *Sirt7* knockout had a marginal effect on HF morphogenesis and the first hair cycle (Revised Appendix Fig S1D-E). We have included these details in the methods section of the revised manuscript.

2.) In regards to the epidermal stem cell-specific KO of SIRT7, the authors need to provide genotyping or qRT-PCR data for other tissues to rule out off-target contributions of SIRT7 activity. In general, this is the biggest concern for the validity of the findings, as the cell-autonomy of these findings is based off of these experiments.

Response #1-4

We appreciate this critical comment from the referee. *Sirt7* deletion in HFSCs was achieved by crossing *Sirt7*$^{f/f}$ mice to K15-Cre, which is preferentially expressed in the adult hair follicle bulge stem cells (Giroux et al., 2017; Lay et al., 2018; Liu et al., 2003). Considering the critical role for Sirt7 in multiple tissues/organs, we agree that evaluating the potential off-target effects elicited by K15-Cre is necessary. Indeed, Sirt7 was almost completely deleted in the bulge cells of *Sirt7*$^{f/f}$;K15-Cre mice but remained unaffected in epidermis cells, ascertaining the specificity of K15-Cre in HFs (revised Appendix Fig S2H). We also measured *Cre* and *Sirt7* expression in different organs/tissues by qPCR analysis. As shown, *Cre* expression was only observed in the skin of *Sirt7*$^{f/f}$;K15-Cre mice (revised Appendix Fig S2I), accompanied by an almost 50% reduction in *Sirt7* levels (skin contains multiple cell types including HSFCs). By contrast, *Cre* expression and *Sirt7* reduction were negligible in other organs/tissues, e.g. liver, lung, heart and skeletal muscle. Together, in our model, K15-Cre-mediated *Sirt7* deletion in bulge HFSCs had negligible off-target effects on other tissues/organs. We have included these new results in the revised manuscript.

Minor Issues
-Figure 3G-H: Why are Id3 and S11a each inversely regulated with respect to the NFAT target genes? Please provide some hypothesis or explanation to acknowledge this.

Response #1-5

In response to stimuli like a calcium signal, NFATc1 cooperates with transcriptional factors to regulate the expression of multiple genes (Mancini and Toker, 2009). However, under some circumstances, NFATc1 can suppress gene transcription. For

example, NFATc1 competes with p300 to interact with MyoD, thus repressing the transcription of downstream genes (Ehlers et al., 2014). Of note, *Id3* transcription is indeed dependent on p300 (San-Marina et al., 2008).

-Please quantify the Westerns for 3A. In general, any blot that is not showing a binary result should be quantified. Also, please indicate the n-value for the replicates in these works.
-Figure 5C and 5H should be quantified and the replicates indicated.
-Overall, please use different line styles to indicate different strains.
-Several figures throughout the manuscript need quantification. Fig 6A-B, Fig 7B and 7D.

Response #1-6

We thank the referee for the suggestion. We have quantified the western blot data in original Fig 3A (now revised Fig 3D and E) and Fig 5C and H (now revised Fig 5C, D and K), and also indicated n-value for the replicates in the revised manuscripts. The data in Fig 6A-B, Fig 7B and 7D were also quantified (see revised Fig 6A-D, Fig 7B-D and Fig 7F-G). As suggested, we have used different line styles to redraw the curves (revised Fig 5 and Fig 7) in the revised manuscript.

-In discussion "Thus, it is plausible to speculate that to initiate a new HF cycle, Sirt7-mediated Nfatc1 degradation by PA28γ might be a better choice than ubiquitination relied, as this degradation pathway consumes less energy for quiescent HFSCs" is not supported in regards to the energy consumption. I suggest removing the part about energy consumption unless the authors want to include evidence.

Response #1-7

We agree with the referee and have removed this sentence in the revised manuscript.

-Figure 5 D-E: Looks like the Sirt7 H187Y mutant is less effective than wt, albeit the NFAT1c levels at 3h seem to be less those with EV. Authors should provide significance tests for Figure 5E and discuss the result.

Response #1-8

We considered that this inconsistency was attributable to technical issues as the EV group was likely overexposed. We repeated this experiment and presented now a clearer blot in the revised Fig 5E-F.

-Figure 5K: The authors state: "Moreover, overexpression of HA-SIRT7, but not the H187Y mutant, promoted NFATc1 binding to PA28γ (Figure 5K)." In fact there is NFAT1c binding with the mutant Sirt7, although with lower yield in the IP. Authors should provide explanation and soften their statement.

Response #1-9

Thank you for this comment and we apologize for not being clear enough. In Figure 5K (revised Fig 5O), we didn't examine the interaction between NFATc1 and HA-SIRT7/H187Y, but rather showed that HA-SIRT7 promoted NFATc1 binding to PA28γ whereas SIRT7-H187Y enzyme dead mutant loses such ability.

-Page 17: "As such, Cyclosporin A (CsA), a potent calcineurin inhibitor, suppresses NFATc1 nuclear accumulation and renders NFATc1 activity (Hogan et al., 2003)." Statement is somewhat confusing by the use of the word "renders". Please rephrase for clarity.

Response #1-10

We are grateful for the suggestion. We have rephrased this sentence as "Cyclosporin A (CsA), a potent calcineurin inhibitor, suppresses this import and thus blocks NFATc1 nuclear activity" in the revised manuscript.

Referee #2:

In this manuscript, Li et al. document a role for the NAD-dependent deacetylase Sirtuin 7 (Sirt7) protein in hair follicle stem cell (HFSC) activation, and hair follicle (HF) growth, using mouse genetic models. The authors turned to cell culture systems to show that Sirt7 interacts with Nfatc1, leading to Nfatc1 deacetylation, and nuclear degradation. Interestingly, the in vivo CSA treatment of Sirt7-/- mice, rescues HF growth compared to controls. Moreover, the authors established a correlation between Sirt7expression and skin ageing.

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1. Changes in CD34+ HFSC numbers. The authors show that the loss of Sirt7 does not affect the number of CD34+ HFSC, is this experiment conducted during the first HF cycle (Figure S2C)? Is the number of CD34+ HFSC still maintained during the second HF cycle in the Sirt7-/- mice?

Response #2-1

Thank you for your question. As a *Sirt7* deficiency has a marginal effect on the first hair cycle and results in an extension of the second telogen, we analyzed CD34+ HFSCs population at the second telogen phase (revised Appendix Fig S2G). For clarity, we have incorporated the necessary information in the revised figure legend.

2. Figure 1B. The authors indicate that the relative percentage of HF recovery after shaving is almost full by day P120 in control mice, while Sirt7-/- KO mice only showed 10% of relative hair recovery. However, the histogram does not represent that conclusion and indicates that KO mice exhibited a 100% recovery by day P80. Overall, although the data show a delay, the interpretation is not persuasive.

Response #2-2

Thank you for these comments. In the current study, we quantified hair coat recovery based on skin pigmentation (Chai et al., 2019). Briefly, Mice were shaved at telogen. Then, the hair coat recovery rate (ranging from 0 to 100%) was calculated using the skin pigmentation levels and the hair shaft density. For example, 0 indicates no hair growth (no pigmentation); 50% indicates full-length hair growth on 50% of the dorsal skin area or pigmentation on 100% of the dorsal skin area without hair shafts yet; 70% indicates full-length hair growth on 70% of the dorsal skin or pigmentation on 100% of the dorsal skin with ~30-40% hair shafts; 100% indicates full-length hair growth on 100% of the dorsal skin area. We have included this description in the methods section of our revised manuscript.

As to the concerns in Figures 1B, 1H and 2D, we do apologize for making errors here which have led to confusion. The hair coats of *Sirt7*$^{+/+}$, *Sirt7*$^{-/-}$, *Sirt7*$^{f/f}$ and *Sirt7*$^{f/f}$;K15-Cre were clipped on P60; Sirt7-TG/WT mice were shaved at P45. The more accurate description should be "by P120, *Sirt7*$^{+/+}$ mice recovered an almost full hair coat, while the *Sirt7*$^{-/-}$ mice showed only ~25% recovery; by P100, *Sirt7*-TG mice had recovered an almost full hair coat, while their WT littermates recovered only 50%".

For the curves in the original Figures 1B, 1H and 2D, the value on Y-axis represents post-shaving days, e.g. 0 equals to postnatal day 60 in the original Fig. 1B. That said, y = 60 indicates postnatal day 120 (60+60). Nevertheless, we realized that those curves are somehow obscured. For clarity, we have replaced those curves (Fig 1B, 1H and 2D) with box-and-whisker plots in the revised Figures 1B, 1H and 2D, wherein the Y-axis now represents the postnatal day and the hair-shaving time is indicated by an arrow. We hope that this new layout is now clearer to interpret.

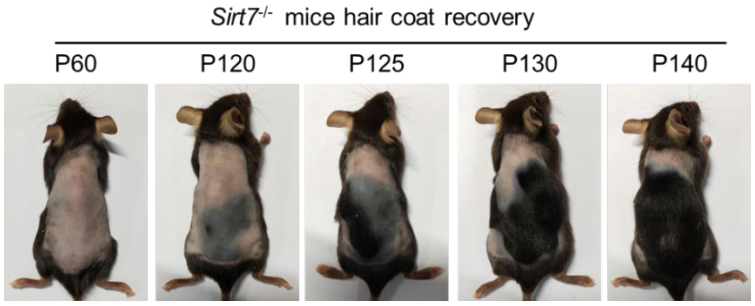
3. Figure 1C. The H&E images do not seem to be representative. Considering the results shown in Figure 1B, by day P90, approximately 85% of KO mice recovered HFs. Also, a better characterization of HF anagen stages and HF growth is needed. How does the wave of anagen entry look like in the Sirt7-/- KO mice that recovered their coat? Does it occur in a synchronized manner across the skin?

Response #2-3

We again apologize for this problem and similar issue as detailed in P#2; please refer to response #2-2.

On postnatal day 90, *Sirt7*$^{+/+}$ mice had nearly 25% hair coat recovery, indicating entry of anagen (revised Fig 1B). By contrast, *Sirt7*$^{-/-}$ mice had almost 25% recovery by postnatal day 120 (revised Fig 1B). Therefore, it is rational that for the *Sirt7*$^{+/+}$ mice, the hair follicle was in anagen at P90, whereas for KO mice the hair follicles mostly stayed in telogen. Indeed, when *Sirt7*$^{-/-}$ HFs prominently entered anagen at P130, *Sirt7*$^{+/+}$ mice had moved to the next telogen (revised Fig 1C, right).

The first two hair cycles were overall synchronous in *Sirt7*$^{-/-}$ mice. From the 3rd anagen phase, *Sirt7*$^{-/-}$ mice displayed asynchronous hair growth (rebuttal Fig 1). Indeed, hair cycle phases *per se* are no longer synchronous in mice starting from the 3rd hair cycle (Reichenbach et al., 2018). Moreover, *Sirt7* KO mice displayed minimal discrete domains of anagen-phase (black) interspersed with patches of telogen-phase (pink) in the 3rd hair cycle but rather formed growth waves throughout the skin (rebuttal Fig 1). Therefore, whether *Sirt7* KO mice have severe asynchronous hair growth needs to be evaluated over the next several hair cycles.



Rebuttal Fig 1. Post-shaving hair coat recovery in *Sirt7*$^{-/-}$ mice

4. Figure 1H. In the same lines of ideas than point number 2. The authors indicate that the relative percentage of HF recovery after shaving is almost full by day P90 in Sirt7-TG mice. At the same time, controls only showed 10% of relative hair recovery, but the histogram does not represent that conclusion.

Response #2-4

Please refer to our responses in #2-2 and #2-3.

5. Figure 1F. The immunofluorescence analyses are not quantitative.

Response #2-5

As suggested, we have now performed a statistical analysis of Fig 2F in revised Fig 2G-H and revised Appendix Fig S2K, showing the average percentage of Ki67 positive cells per HG (Pcad^{+} cells), the number of Ki67 positive cells per bulge (Cd34^{+}) and Cd34 positive cells per hair follicle.

The authors should also show that there are no quantitative changes in the number of CD34+ alpha-6 integrin+ HFSC by FACS upon the ablation of Sirt7 in the conditional Sirt7fl/fl; K15Cre model.

Response #2-6

As suggested, we have now analyzed the HFSC population via labelling Cd34/integrin alpha-6 at the second telogen phase in *Sirt7*$^{fl/fl}$;K15-Cre mice and littermate controls. The proportion of HFSCs was comparable between *Sirt7*$^{fl/fl}$;K15-Cre mice (14.6±0.6%) and controls (14.8±0.5%). We have incorporated these new data in the revised appendix Fig. S2J.

The authors should also show if there any delays in the first HF cycle of the Sirt7fl/fl; K15Cre model. Showing these data will be helpful to support the conclusion that the loss of Sirt7 in HFSC does not affect HF morphogenesis.

Response #2-7

Thank you for this suggestion. Keratin 15 promoter activity is not specific in skins of neonate mice. For example, it is also detectable in interfollicular epidermis cells. Keratin 15 becomes undetectable in the epidermis and prominent in HFSCs after postnatal day 21 (Liu et al., 2003). Thus, K15-Cre is particularly suitable for targeting adult HFSCs. In current study, to avoid nonspecific deletion at neonatal stages, we used an inducible K15-Cre (K15-CrePGR) mouse line and executed *Sirt7* deletion in adult mice at postnatal 42 (revised Fig 2I). As *Sirt7*$^{-/-}$ mice displayed similar skin morphology and a nearly identical 1st hair cycle compared to WT mice, we argue against the notion that *Sirt7* deletion dysregulates HF morphogenesis (Appendix Fig. S1D-E).

6. Figure 2D, is the recovered HF growth in the Sirt7fl/fl; K15Cre mouse model synchronized?

Response #2-8

Thank you for this comment. In revised Fig 2D, we found that all 6 *Sirt7*$^{fl/fl}$;K15-Cre mice entered the 3rd anagen on P100 and each individual mouse showed a similar hair recovery rate. *Sirt7*$^{fl/fl}$;K15-Cre mice showed a similar hair growth pattern as the *Sirt7*$^{-/-}$ mice, as mentioned in P#3 above.

7. Loss of Sirt7 and Nfatc1 in HFSC quiescence and activation. The authors isolated HFSC from Sirt7-/- mice, Sirt7-TG mice, and their control counterparts to conduct mechanistic analyses. However, the authors need to validate if there are any differences in the activation or quiescence of the HFSC cultures, to use them as a bonafide cell culture system to compare it with their in vivo findings. Are there any differences in the proliferation properties between the different cell types? Changes in colony formation? If so, is this due to quiescence, apoptosis, or differentiation?

Response #2-9

We appreciate the referee's insightful comments. We enriched bulge SCs that were Cd34^{+}/integrin-α6^{+} from telogen phase HFs in *Sirt7*$^{+/+}$, *Sirt7*$^{-/-}$ and *Sirt7*-TG mice. The cells were allowed to grow for 14 days, as previously described (Blanpain et al., 2004). As shown, *Sirt7* loss resulted in a decreased number of colonies. By contrast, *Sirt7* overexpression significantly boosted colony formation (revised Fig 3A-B). In addition, the *Sirt7*$^{-/-}$ colonies were a smaller size than WT; forced *Sirt7* overexpression largely promoted colony expansion (revised Fig 3C). Little difference in Annexin-V/PI signals was observed between WT and Sirt7 KO mice, ruling out a contribution of apoptosis to the observed differences (Appendix Fig S3H). Importantly, the stem cell marker Lgr5 was equivalent in both WT and Sirt7 KO colonies, arguing against altered cell differentiation (Appendix Fig S3I). Intriguingly, Cdk4, which is required for HFSC proliferation and cell cycle progression (Horsley et al., 2008), was significantly downregulated in *Sirt7* KO but elevated in Sirt7-TG

colonies, suggesting that Sirt7 has a critical role in promoting HFSC activation (Appendix Fig S3M). We have included these new results in the revised manuscript.

8. The authors conducted a screen using isolated HFSC culture cells to test for concrete protein expression differences between Sirt7-/- mice, Sirt7-TG mice, and their control counterparts. They found an opposite correlation between the expression of Sirt7 and Nfatc1. Also, Sirt7 knockdown cells exhibited higher levels of Nfatc1, while overexpression of NFatc1 (Sirt7) leads to NFatc1 reductions.
However, in Figure S3, the skin of Sirt7-/- mice at day P100 shows no expression of Nfatc1 in the bulge. How do the authors reconcile the differences?

Response #2-10

Nfatc1 is preferentially expressed in bulge HFSCs and maintains their quiescence (Keyes et al., 2013). An elegant work showed that Bmp signaling underlines *Nfatc1* transcription activation, which is essential to keep HFSCs quiescent in telogen (Horsley et al., 2008).

In our study, Sirt7 confers Nfatc1 destabilization to maintain normal HF cycling. *Sirt7*$^{-/-}$ HFs exhibited telogen-anagen delay, likely attributable to an Nfatc1 accumulation in bulge stem cells. Interestingly, as what we observed, *Sirt7* KO HFs eventually initiated anagen on P100 with attenuated Nfatc1 (revised Appendix Fig S3O). This finding suggests the existence of redundant mechanisms regulating Nfatc1. Indeed, Tgf-β signaling could antagonize the Bmp quiescence signal during the telogen-to-anagen shift (Oshimori and Fuchs, 2012). In turn, Bmp inhibition might attenuate *Nfatc1* transcription. Simultaneously, TGF-β leads to the activation of ERK1/2 and/or P38, which can directly phosphorylate NFATc1 and promote nuclear exportation (Alam et al., 2014; Ryu et al., 2006). Nonetheless, we reasoned that redundant mechanisms in hair follicle niches contribute to the Nfatc1 decrease, which eventually initiates a new hair cycle in *Sirt7* knockout HFs.

9. The authors nicely show that Sirt7 controls Nfatc1 deacetylation and stability through direct protein-protein interactions via specific domains. What is the biological role of precluding the Sirt7/Nfatc1 interaction in the quiescence/proliferation properties of the cultured cells?

Response #2-11

We appreciate this critical comment. We hypothesized that Sirt7 might interact with Nfatc1 as a deacetylation target. Indeed, in the revised version of our manuscript, we found that SIRT7 deacetylated NFATc1 at K612 (revised Fig 5 and Appendix Fig S4). We therefore generated cells overexpressing NFATc1 mutants, i.e. hypo-acetylation (K612R) and hyper-acetylation (K612Q) mimics and examined the single-cell colony formation efficiency (CFE). K612Q-expressing cells were difficult to form tight colonies and displayed a significant growth arrest compared to K612R-expressing

cells (revised Appendix Fig S5A-B). This result implicates the functional significance of a SIRT7/NFATc1 interaction in maintaining HFSC quiescence.

10. In Fig 6, the authors show that treating Sirt7-/- deficient skin with CSA rescues HF growth when compared to the vehicle-treated region of the skin. It is a bit puzzling that the rescue is limited to the treated area without generating a HF growing wave throughout the skin.

Response #2-12

We appreciate the point on the HF growing wave. We are also sorry for the misunderstanding owing to improper demonstration. We agree that hair follicles affect each other during anagen re-entry and thus form regenerative hair waves (Plikus and Chuong, 2008). Regenerative hair waves have limited domain expansion, i.e. growth waves stop at non-responsive regions and form boundaries. Multiple hair cycle domains are formed in adult mice and each domain has an intrinsic regeneration rhythm, i.e. an asynchronous hair cycle (Ge et al., 2020; Keyes et al., 2013; Plikus et al., 2008). A previous study showed that a CSA-treated region induced a new anagen phase, but the surrounding skin continued to stay at telogen with its own progression phase (Plikus et al., 2008). In revised Fig 6F, representative images show the recovered haircoat after CSA treatment, which was applied in the left part of the shaved skin. The dashed circle indicates the shaved area; the dotted line indicates the hair growth boundary.

11. Sirt7 and HF aging. Aging is a multifactorial process, and the authors show that full-body Sirt7-TG mice are in better shape than controls. Using hair plucking experiments, the authors evaluated the dynamics of HF regrowth and concluded that the Sirt7-Nfatc1 axis regulates it. At what time point was the expression of Sirt7 induced with Dox? The authors should induce the expression of Sirt7 in already aged mice, before conducting the experiments, to substantiate their claims.

Response #2-13

Thank you for these comments. In the current study, *Sirt7* expression was induced at age of 12 months when murine HFs start to decline (Goodier and Hordinsky, 2015; Matsumura et al., 2016). Laboratory mice suffer apparent hair thinning roughly 16 months after birth (Matsumura et al., 2016). Skin areas with great hair loss struggle to grow new hair even in the presence of growth inducers. We are afraid that it is not suitable to conduct Sirt7 rescue at an age after 16 months. Thus, we rather assessed the plucking-induced hair regeneration in mice aged 20-months-old. We found that aged Sirt7-TG mice (20 m) showed more active hair growth, underlining the essential roles of Sirt7 in maintaining HF growth in aged mice (revised Fig 7H-I).

Minor points:
1. Sirt1, Sirt6, and Sirt7 have aging phenotypes. It will be useful for the flow of the

manuscript, to add a short sentence indicating the rationale to study Sirt7 in HF growth, as a representative member of the family.

Response #2-14

Thank you; we have now stated the rationale in the revised manuscript.

2. Figure 1B, Figure 1H, Figure 2D. The plot of the graphs is confusing. Since the variable is the % Relative hair recovery, this should be changed to the Y-axis.

Response #2-15

We thank the referee for the suggestion. In those Figures, we intended to compare the time required for hair coat recovery. To avoid misunderstanding, we have replaced those curves with box-and-whisker plots in the revised manuscript.

3. Figure 1D and 1G. Ki67 activation is shown at P90 in control HF in Figure 1D, and in P85 in Figure 1G (Figure 2F). The areas shown do not represent the HF germ, but the HF matrix.

Response #2-16

We are grateful for the suggestion. The original Fig 1D has been replaced by another representative staining.

4. Figure 1C, Figure 1I, Figure 2E. Indicate the mouse skin age in the figure panels and legends, since it differs from the time points shown in the accompanying mouse images.

Response #2-17

We have now incorporated the relative information in the indicated Figures.

5. Figure S2D, in the manuscript text, the age of the mice is described as P72. The images show HF at P74.

Response #2-18

We appreciate the comment. We performed the staining at P74 in revised Appendix Fig S2H. We have amended the relevant text in the revised manuscript.

Referee #3:

The manuscript by Guo et al. investigates the role of Sirt7 during the hair cycle. They show that Sirt7 is expressed in the hair follicle and that deletion of Sirt7 delays the transition from telogen to anagen, while the overexpression leads to an early anagen onset. Sirt7 expression is reduced in hair follicles of aged mice and aged Sirt7-/- mice present hair loss, which can be ameliorated by overexpression of Sirt7. Mechanistically, they uncover that Sirt7 deacetylates Nfatc1, a transcriptional regulator mediating hair follicle stem cell quiescence. Deacetylation of Nfatc1 leads to PA28y-mediated proteasomal degradation of Nfatc1 and stem cell activation. While the findings are overall interesting, I do not share some of the interpretation and have a number of important questions that need to be addressed before publication. Furthermore, there are several experimental details missing that need to be added.

Figure 1:

The authors interpret their results as the direct effect of Sirt7 manipulation on hair follicle stem cells. However, Sirt7 is expressed in other hair follicle cells as well and the presented effects on proliferation look rather hair germ specific. The used marker P-cadherin is a hair germ marker. For bulge cells CD34 should be used. The second picture from the left in Fig. 1D (Sirt7+/+, P90) shows the matrix of a growing hair follicle. Proliferating cells in the matrix are classified as transient amplifying cells, which are distinct from hair follicle stem cells (HFSC) in the bulge and ORS. To study bulge HFSC activation the authors should focus on early anagen (anagen I and II). The best is to use EdU to show/quantify stem cell proliferation.

Response #3-1

We appreciate the referee's critical comment. In original Fig 1, we performed a phenotypical analysis to elicit the role of Sirt7 in adult hair follicle cycles, as stated that "*Sirt7* loss led to apparent delay of the 3rd hair cycle anagen entry". In Fig 1D and I, the anagen status was indicated by Ki67 staining of P-cadherin-labelled hair germ cells which are considered to immediately respond to anagen growth. We have replaced the image in original Fig 1D ($Sirt7^{+/+}$, P90) with a more representative one. As suggested, we also conducted a BrdU assay to assess whether *Sirt7* loss directly compromises HFSC activation. Please refer to our later response #3-2.

Important questions regarding HFSC phenotype when deleting/overexpressing Sirt7: Are less/more HFSCs become activated in anagen or is only the timing different? If fewer HFSCs activated in the knock out - what is the functional consequence of this when it comes to hair follicle growth?

Response #3-2

We thank the referee for raising these important questions. As $Sirt7^{f/f}$;K15-Cre HFs displayed a similar telogen-to-anagen delay as *Sirt7* KO mice (revised Fig 2), it

encouraged us to focus on HFSCs. To evaluate whether Sirt7 directly regulates HFSC activation, we herein conducted a BrdU pulse-chase experiment. *Sirt7*$^{+/+}$ and *Sirt7*$^{-/-}$ mice hair follicles at telogen were depilated to induce synchronous anagen entry. Depilation removes an inner bulge layer of BMP6 and FGF18-expressing cells that consequently poses strong activation of quiescent HFSCs (Hsu et al., 2011). As shown, on post-depilation day 2 and 4, much fewer *Sirt7*$^{-/-}$ bulge cells incorporated BrdU than WT cells (revised Appendix Fig S3A-B), which suggests that only few HFSCs are activated at early anagen. However, BrdU labelled *Sirt7*$^{-/-}$ bulge cells further increased in number, emphasizing that *Sirt7* deletion delays rather than blocks HFSC activation. Indeed, we have shown that *Sirt7*$^{-/-}$ mice can complete hair cycling and develop mature HFs (revised Figs 1A and 2F).

Growth-mediating stem cell activation is synchronized between interfollicular epidermis (IFE), sebaceous gland (SG) and hair follicle (HF) (Reichenbach B et al., 2018, EMBO J). It would be important to know if Sirt7 is expressed in the IFE and SG basal layer? Is basal layer proliferation in the IFE and SG affected in the Sirt7 knock out?

Response #3-3

We appreciate the insightful suggestion. As determined by IHC staining, at P56, P69, P74, (i.e. mid-telogen, late-telogen and anagen-I), Sirt7 levels were almost constant in IFE and largely comparable in SG (revised Appendix Fig S2B-C). To assess whether *Sirt7* loss affects basal layer cell proliferation, we performed Ki67 staining of *Sirt7*$^{+/+}$ and *Sirt7*$^{-/-}$ murine skin, at Pd0, Pd2d and Pd4d, respectively. A less obvious difference of Ki67 staining was observed at Pd0 between WT and *Sirt7* KO mice, suggesting that basal layer proliferation *per se* was negligibly affected (revised Appendix Fig S3C). While a similar pattern of Ki67 increase was observed from Pd2d to Pd4d in WT and *Sirt7*$^{-/-}$ mice, Ki67 signals were greater in WT epidermis cells (revised Appendix Fig S3D). Considering that the growth of IFE and SG is synchronized with hair cycle, the above observed difference is likely decided by the hair cycle delay in *Sirt7*$^{-/-}$ mice rather than the inhibition of cell proliferation.

What is the promoter driving Sirt7 overexpression? Only in bulge/HG or in whole mouse? Please state the experimental details - important for interpretation.

Response #3-4

Thanks for the comment and we apologize for not specifying these important details. *Sirt7* overexpression was manipulated by the rtTA promoter embedded in the tet-on system as previously described (Tang et al., 2017). Therefore, Sirt7 was non-selectively overexpressed in mouse tissues subjected into doxycycline feeding. Experimental details are provided in the methods section of the revised manuscript.

In the text it is stated: "By P120, Sirt7+/+ mice recovered an almost full hair coat, while the Sirt7-/- mice showed only around 10% recovery (Figures 1A and 1B)." However, the graph in Fig. 1B shows 100% hair recovery for Sirt7-/- mice at 75 days post shaving, which corresponds to P120. Please correct/explain!
The graph in Fig. 1H has a similar mismatch with the text as I stated regarding Fig. 1B above.

Response #3-5

Thank you for the comments and we apologize for our errors. Please refer to our earlier response #2-2. Briefly, the hair coats in *Sirt7*$^{+/+}$, *Sirt7*$^{-/-}$ and *Sirt7*$^{f/f}$, *Sirt7*$^{f/f}$;K15-Cre were clipped at P60, while that in *Sirt7*-TG and WT was clipped at P45. We have amended this in the revised manuscript, which should be "by P120, *Sirt7*$^{+/+}$ mice recovered an almost full hair coat, while the *Sirt7*$^{-/-}$ mice showed only ~25% recovery; by P90, *Sirt7*-TG mice had recovered an almost full hair coat, while their WT littermates recovered only up to 50% hair". The hair coat recovery curves seem somehow obscured; for clarity, we have replaced them with box-and-whisker plots (revised Fig 1B and H, Fig 2D).

How is telogen duration measured in Fig. 1F?

Response #3-6

Hair cycle analysis was based on pigmentation as pigment production is active during hair follicle growth, i.e. skin color is pink at telogen and becomes dense pigmentation once shifting to the anagen phase (Keyes et al., 2013; Reichenbach et al., 2018). In Fig 1F, to monitor the 2nd telogen duration, we clipped the dorsal hair when HFs of both genotypic mice were at telogen. Telogen ending was determined by the appearance of dense pigmentation. The time interval was defined as telogen duration.

Figure 2:
The upregulation of Sirt7 during telogen-anagen transition is insufficiently supported by the presented IHC images. To support this claim, the authors should purify CD34+ bulge cells and perform qPCR for Sirt7.

Response #3-7

As suggested, we have now purified bulge cells from the hair follicle by labeling CD34/integrin α6, at P56 and P74 respectively. As determined by qPCR analysis, the mRNA level of *Sirt7* in anagen HFSCs was ~2-fold greater than that in telogen cells (revised Appendix Fig S2E).

It very much looks like as HF surrounding adipocytes express Sirt7 as well. Adipocyte lineage cells are important determinants of the hair cycle and control stem cell activation (Festa E et al., Cell. 2011). It is important to know if adipogenesis is altered in the Sirt7 knock out mice, as this alone could change the anagen onset.

Response #3-8

We appreciate the insightful comment. Indeed, an elegant study revealed that dWAT (dermal white adipose tissue) constitutes to the HFSC niche and synergizes hair follicle cycling via PDGF signaling (Festa et al., 2011). Sirt7 is indeed essential for adipogenesis in subcutaneous and visceral white adipose tissue (Cioffi et al., 2015; Fang et al., 2017). Therefore, we agree that whether Sirt7 affects intradermal adipogenesis is important.

We found that *Sirt7* expression was rather constant from telogen to anagen in dWAT, suggesting that Sirt7 might not be directly associated to hair cycling-synchronizing adipogenesis (revised Fig Appendix S2D). To test this hypothesis, we compared intradermal adipogenesis in $Sirt7^{+/+}$ and $Sirt7^{-/-}$ mice during depilation-induced hair growth. As assessed by Perilipin staining of mature adipocyte, we found that *Sirt7* deletion had a negligible effect on intradermal adipogenesis, as we observed a less notable difference on adipocyte size and dWAT thickness during hair growth (revised Appendix Fig S3F-G). Moreover, $Sirt7^{f/f}$;K15-Cre mice showed a similar hair cycling phase as $Sirt7^{-/-}$ mice (revised Figs 1 and 2). Considering the specific *Sirt7* deletion in hair follicles, this finding argues against the potential effect of dWAT on hair cycling in *Sirt7* KO mice. Therefore, while Sirt7 has crucial role in subcutaneous and visceral white adipose tissues, whether it functions equivalently in dWAT needs to be further explored.

Importantly, hair-associated dWAT remodeling is uncoupled from other adipose tissues that are controlled by systemic metabolic demand. Instead, dWAT is coupled with hair follicle cycles by receiving mature HF signals, e.g. BMP and SHH (Guerrero-Juarez and Plikus, 2018). Meanwhile, as a response, plenty of cytokines and growth factors that accompany dWAT expansion and de-differentiation act on hair cycle progression (Festa et al., 2011; Guerrero-Juarez and Plikus, 2018; Kruglikov and Scherer, 2016). Altogether, our findings underline the effect of Sirt7 on HFSCs.

As for IFE and SG, mentioned above, it is critical to evaluate the entire expression pattern of Sirt7 in the skin.

Response #3-9

Please refer to response #3-3.

In text and figure a mifepristone K15-Cre mouse line is stated. Is the mouse model K15-CrePGR - please clarify with reference and mention the line in the methods. Please state how much/many injections of mifepristone are used. In case of the K15-CrePGR line, it is not entire specific to HFSCs.

Response #3-10

For the information of K15-CrePGR line and its specificity to HFSCs, please refer to our response #2-7. The mice were treated with mifepristone topically on the dorsal skin during telogen once a day for 7 days (12 mg in ethanol) as described (Lay et al., 2018; Morris et al., 2004). We have included the detailed information in the methods part of the revised manuscript.

It looks like Sirt7 is predominantly expressed in the HG and expression of Sirt7 seem to correlate with Ki67/HG activation but not with bulge stem cells.

Response #3-11

Thank you for the insightful comment. We agree that Sirt7 showed stronger expression in HG proliferative cells after anagen entry, e.g. at P74 and P85. However, it should not be ignored that the first wave of increase in Sirt7 expression appeared in bulge cells at P69 (late telogen) and the expression expanded to HG, suggesting that Sirt7 affects bulge stem cells prior to HG (revised Fig 2A, Appendix Fig S2A).

Depilation mostly removes an inner bulge layer cells that consequently activates outer bulge layer of HFSCs (Hsu et al., 2011). Interestingly, upon depilation, we observed a quick increase in Sirt7 expression in bulge stem cells at first (revised Fig 2B, Pd2h), confirming that Sirt7 initially acts on HFSCs. Importantly, at the refractory telogen phase (P42), forced ectopic Sirt7 expression in Sirt7-TG mice resulted in anagen growth at P60. More interestingly, $Ki67^{+}$ proliferative cells first appeared in the lower bulge/upper HG but not the lower HG. Later on at P65, the lower bulge had already accumulated proliferative cells, while the lower HG cells likely just started to increase (revised Fig 1J). Noting that both the lower bulge and upper HG are derived from bulge HFSCs, these results strongly suggest that precocious Sirt7-induced HFs growth corresponds to bulge HFSC activation. Additionally, K15-Cre-mediated *Sirt7* deletion led to similar HF cycling delay as *Sirt7* KO mice; *Sirt7* loss compromised HFSCs colony formation (Revised Figs 2D-I and 3A-C). These findings further support the roles of Sirt7 in HFSC activation. While it is not sufficient to rule out potential roles of Sirt7 in the HG, these results at least suggest a major function of Sirt7 in HFSCs.

Figure 3:
Why do the authors use so many different cell types/lines for different experiments and without validation of the results in the other lines? It would be much better to use only HFSCs! Or at least epidermal keratinocytes.

Response #3-12

Thank you for your query. To validate our findings, we repeated key experiments in human keratinocyte HaCaT cells, including endogenous Co-IP of SIRT7/NFATc1 (revised Fig 4G-H), endogenous NFATc1 (de)acetylation (revised Appendix Fig S4C), endogenous NFATc1 protein turnover rate (revised Appendix Fig S4G) and

endogenous Co-IP of NFATc1/PA28γ (revised Fig 5L, revised Appendix Fig S4I). These results were included in the revised manuscript.

Please address functional effects on Sirt7 knock out/over expression on HFSCs in vitro - colony formation, long term passaging, transcriptomics.

Response #3-13

As suggested, we enriched the bulge SCs that were $CD34^{+}$/integrin-$\alpha6^{+}$ from telogen phase HFs in *Sirt7*$^{+/+}$, *Sirt7*$^{-/-}$ and *Sirt7*-TG mice. The cells were allowed to grow for 14 days as described (Blanpain et al., 2004). *Sirt7* loss resulted in decreased number of colonies. By contrast, *Sirt7* overexpression significantly boosted colony formation (revised Fig 3A-B). The *Sirt7*$^{-/-}$ colonies showed smaller size than WT while forced *Sirt7* overexpression largely promoted colony expansion (revised Fig 3C).

We further performed four rounds of passaging with five colonies each time. Interestingly, for every passage, *Sirt7*$^{+/+}$, *Sirt7*$^{-/-}$ and *Sirt7*-TG colonies shared comparable colony formation efficiencies, suggesting equivalent self-renewal abilities. *Sirt7* KO colonies at different passages were still smaller (Fig Appendix S4J-L, passage 4). Together, these findings indicate that Sirt7 promotes HFSC proliferation, consistent with the inhibition effect of Nfatc1 on HFSC proliferation.

For the mechanistic analysis, we performed a screening analysis of key pathways in HFSCs and found that Nfatc1 is a main causal factor, based on *in vitro* and *in vivo* evidence (revised Figs 3-5, Appendix Figs S3-4). Based on these results, we do not think that a transcriptomics analysis is necessary at this stage. Certainly, to obtain a deeper understanding of the potential signaling pathways manipulating Sirt7 expression in the telogen-to-anagen transition, a transcriptomics analysis would be helpful. We have included our new results in the revised manuscript.

Figure 3G and 3H are not the same panel of genes - why not?? How are Nfact1 target genes defined?

Response #3-14

We selected the target genes from published NFATc1 ChIP-seq data (Keyes et al., 2013). Due to technical issues or other reasons like feedback, we herein only presented readouts that reached statistical significance. Nevertheless, for consistency, we show the same panel of genes in the revised Fig 3K-L.

Figure 4:
There are several sirtuins expressed in HFSC (1,2,6,7) - what happens to them when Sirt7 is lost/over expressed - is there compensation or redundancy?

Response #3-15

In the current study, in terms of deacetylation, we considered whether fundamental issues associated with compensation or redundancy might occur if other sirtuins (Sirt1, 2, 6) share NFATc1 as a substrate. We thus checked the interaction between NFATc1 and SIRT1/2/6/7. SIRT7 had the strongest binding capacity (revised Appendix Fig S4F). While SIRT6 showed a weak interaction with NFATc1, it negligibly affected NFATc1 expression level, suggesting that it is probably not an NFATc1 deacetylase (revised Appendix Fig S4F, input NFATc1). Indeed, we did not observe significant changes in *Sirt1/2/6* in *Sirt7*$^{-/-}$ or *Sirt7*-TG HFSCs (revised Appendix Fig S5F-G). Altogether, we propose that with regards to NFATc1 deacetylation, any compensatory or redundancy effects elicited by these other sirtuins are likely minor.

How was assessed that the NAM (sirtuin) inhibitor is active?

Response #3-16

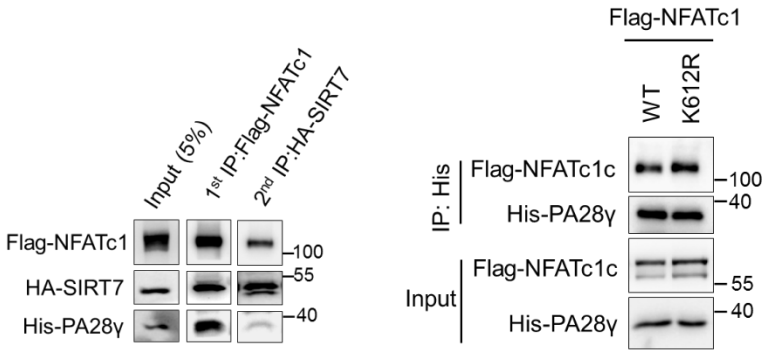
Here, we detected H3K9-acetylation levels to assess the effects of NAM (Kawahara et al., 2009). We have now included these results in the revised manuscript (revised Fig 4L, Appendix Fig S4E).

Figure 5:
The authors show direct interaction of Nfat/Sirt7 and Nfat/Pa28g. Wouldn't it make sense to probe for Sirt7/Pa28 or is Sirt7 and Nfat binding to Pa28 mutually exclusive or sequential?

Response #3-17

We have taken this suggestion to mean that there is a concern as to whether SIRT7/PA28γ/NFATc1 form complexes. To that end, we performed 2-step Co-IP. As shown in rebuttal Fig 3 (left), both SIRT7 and PA28γ were observed in the 1st Co-IP elution based on Flag-NFATc1, confirming an interaction between NFATc1 and SIRT7 and between NFATc1 and PA28γ.

Then, we used the Flag-NFATc1 elution for the 2nd Co-IP based on HA-SIRT7. While we still observed an interaction between SIRT7 and NFATc1, the PA28γ signal sharply declined to a negligible level compared to the 1st round Co-IP, suggesting that PA28γ is less likely to be involved in the NFATc1/SIRT7 complex. Moreover, with a comparable level of SIRT7, a hypo-acetylated K612R mutant showed stronger binding capacity to PA28γ (rebuttal Fig 3, right). Therefore, we conclude that SIRT7 firstly deacetylates NFATc1, which leads to PA28γ feasibly recognizing the K612-naked NFATc1. In other words, the interactions between NFATc1/SIRT7 and NFATc1/PA28γ are most likely sequential.



Rebuttal Fig 2. 2-step Co-IP based on Flag-NFATc1 and HA-SIRT7 (left); interaction of NFATc1 to PA28γ (Right)

Where on Nfat is Sirt7 deacetylating - where on Nfat is Pa28 binding? Are these events structurally linked?

Response #3-18

Thank you for your queries. In the revised manuscript, we show that SIRT7 deacetylates NFATc1 at K612 (revised Fig 4K-L, Appendix Fig S4D-E). We first screened for acetylated lysines on NFATc1 under NAM treatment by LC-MS/MS and identified a novel K612 site (revised Appendix Fig. S4D). We then validated that K612 was mainly deacetylated by sirtuins, as a NFATc1-K612R mutant failed to respond to NAM treatment (revised Appendix Fig. S4E). Using both *in vivo* and *in vitro* deacetylation assays, we confirmed that NFATc1 was directly deacetylated by SIRT7 at K612 (revised Figs 4K-L). The NFATc1-K612R mutant exhibited a faster protein turnover than WT (revised Fig 5I-J).

To explore the NFATc1 domains recognized by PA28γ, we performed a domain truncation analysis. As shown, the NFATc1 DNA binding domain (C terminal, also called DBD) strongly interacted with PA28γ (revised Appendix Fig S4J). Notably, K612 was embedded within this region. It is well described that PA28γ mainly locates in nucleus and forms a homoheptamer to activate 20S proteasome-mediated proteolysis (Mao et al., 2008; Realini et al., 1997). At present, the precise mechanism as to how PA28γ recognizes it substrates remains unclear. PA28γ elicits efficient proteolysis at basic residues (Li et al., 2001; Wilk et al., 2000). As mentioned above, PA28γ bound to the DBD domain in which K612 embedded. We speculate that K612 deacetylation by SIRT7 releases a free basic lysine which possibly helps properly expose the basic residue cluster (GKKM), and then activates PA28γ-mediated proteasome hydrolysis.

Is the dynamics between Nfat phosphorylation and acetylation relevant? Where is the calcineurin phosphorylation site?

Response #3-19

These are great questions. Under basal conditions, NFATc1 is hyper-phosphorylated and thus located in the cytoplasm. In response to calcium signaling, Nfatc1 is dephosphorylated by calcineurin, exposing the masked NLSs, and it subsequently translocates to the nucleus and then regulates gene expression (Clipstone and Crabtree, 1992; Flanagan et al., 1991; Gafter-Gvili et al., 2003). It is known that calcineurin dephosphorylates the SRR (serine-rich region), SP1, SP2 and SP3 motifs (SP, Serine-Proline rich) within the NFATc1 amino terminus (Beals et al., 1997a). Several studies disclosed that the PCAF/P300 acetylases are associated with NFATc1 nuclear import (Garcia-Rodriguez and Rao, 1998). Interestingly, RANKL stabilizes NFATc1 in an acetylation-dependent manner during osteoclast differentiation (Kim et al., 2011). Thus, we agree that it is interesting to explore the relevance of NFATc1 acetylation/phosphorylation dynamics.

For that purpose, we first evaluated whether NFATc1 phosphorylation and acetylation are mutually exclusive. GSK-3β phosphorylates nuclear NFATc1 at the SP motif (Beals et al., 1997b; Neal and Clipstone, 2001). As shown in revised Fig 4M, CA-GSK-3β (constitutively active form) significantly attenuated NFATc1 acetylation. By contrast, treatment with the GSK-3β inhibitor LiCl resulted in a robust increase in acetylation (revised Fig 4M). This finding suggests a mutual exclusion of NFATc1 acetylation and phosphorylation. To ascertain this, we generated CA-NFATc1 (CA), which mimics the constitutive de-phosphorylation status by mutating serines to alanines in the SRR/SP region (Neal and Clipstone, 2003). As expected, CA-NFATc1 showed hyper-acetylation compared to the WT form (revised Fig 4N). On the other hand, we found that an NFATc1-K612R mutant (hypo-acetylation) exhibited much higher phosphorylation levels than the K612Q (hyper-acetylation), ascertaining the intrinsic interplay between NFATc1 phosphorylation and acetylation (revised Fig 4O).

Figure 6:
I do not think it is surprising that CSA works on Sirt7 knock out animals. Would it not be more interesting to over express Nfat on Sirt7-TG background? One would expect that this has no effect on hair cycling, if Nfat is indeed deacetylated and downregulated?

Response #3-20

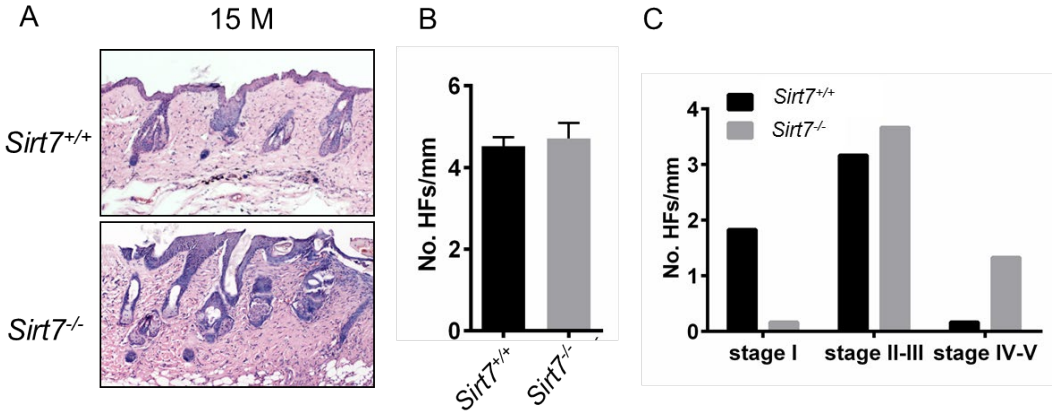
We agree with the referee that in principal, overexpressing NFATc1 in *Sirt7*-TG mice to examine hair cycling is interesting and may solidify our conclusions. However, to overexpress NFATc1, for the timing issue, we have no choice but to use a virus system to infect topical skin. It seems exceedingly difficult to choose a reasonable virus dosage, because a high dose might not only induce apoptosis and inflammation, but also bypass the effect of Sirt7 overexpression in *Sirt7*-TG mice. Conversely, a low dose might not be sufficient to distinguish the phenotypic difference between WT and *Sirt7*-TG mice. Moreover, Nfatc1 is specifically expressed in bulge stem cells, which discourages the possibility of cell-specific expression by virus at present. Nonspecific

Nfatc1 might impose strong cell cycle arrest in epidermal cells and/or T-cell dysfunction (Keyes et al., 2013), which might unexpectedly disrupt hair cycle progression. Indeed, we have tried using an Nfatc1-adenovirus (50 µl/injection, 1 × E10 PFU/ml) in pilot experiments. We noticed that the Nfatc1-virus elicited hair growth arrest even in *Sirt7*-TG mice. Thus, we suppose that while CSA treatment was not a precise rescue targeting Sirt7, it at least partially points out that Nfatc1 is crucial for Sirt7 loss-mediated HFSCs quiescence, in terms of NFATc1 stabilization.

Figure 7:
What is the phenotype of the aged Sirt7 knock out animals - are there fewer hair shafts/follicle? Are there fewer HFSCs in aged knock out animals? Is the HG smaller?

Response #3-21

In the current study, *Sirt7*$^{-/-}$ mice displayed diffuse patterns of hair thinning at 15 months-of-age. Histological analyses indicated no significant decrease in HFs in *Sirt7*$^{-/-}$ mice (Rebuttal Fig 3A-B). According to the shortening of the permanent portion (bulge/HG, shorted as PP) and retention of the DP, aged HFs are classified as stage I to V (Lei and Chuong, 2016; Matsumura et al., 2016). Interestingly, *Sirt7*$^{-/-}$ HFs already exhibited a slight increase in stage-IV/V HFs, indicating a loss of bulge and HGs (Rebuttal Fig 3C). Nevertheless, to further analyze age-related phenotypes, much older mice should be used in future studies.



Rebuttal Fig 3. Phenotypical analysis of HFs in 15 months old *Sirt7*$^{+/+}$ and *Sirt7*$^{-/-}$ mice.

Spelling and grammar should be looked over. Please use precise terms. The methods section lacks many details. Experiments need more detailed description.

Response #3-22

We are grateful for the helpful suggestions. We have carefully revised the manuscript and increased the level of detail provided, especially in the Methods section.

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1st Revision - Editorial Decision 9th Jun 2020

Dear Dr Liu,

Thank you for submitting your revised manuscript for consideration by The EMBO Journal. Your amended study was sent back to the three referees for re-evaluation, and we received re-comments from all of them, which I enclose below. As you will see the referees find that the concerns raised have been sufficiently addressed and are now broadly in favour of publication, pending minor revision.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending some minor issues, which need to be adjusted at re-submission.

Please consider the remaining point of referee #3 regarding consistent report of the ageing phenotypes observed and related claims by adding the rebuttal experiments to the main manuscript and introducing caveats where appropriate or revising the discussion.

In addition we need you to consider a number of minor points related to formatting and data representation, which are listed below.

Please contact me at any time if you need any help or have further questions.

Thank you for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to your final revision.

Again, please contact me at any time if you need any help or have further questions.

Kind regards,

Daniel Klimmeck

Daniel Klimmeck PhD
Editor
The EMBO Journal.

****

Formatting changes required for the revised version of the manuscript:

>> The appendix .pdf file requires a ToC on its first page; figure nomenclature needs to be changed to "Appendix Figure S1, S2". Please change callouts in the main text and legends accordingly.

>> Please consider additional changes and comments from our production team as indicated by attached .doc file and leave changes in track mode.

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Referee #1:

The authors addressed all of my concerns.

Referee #2:

In this revised version of the manuscript, the authors have incorporated additional experiments and clarified several aspects that improved their manuscript. In its present form, I found that it provides a

conceptual advance about the functional involvement of SIRT7 in the activation of HFSC, including mechanistic insight and a potential connection of its downregulation with hair loss and aging skin phenotypes.

Referee #3:

The revision greatly improved the manuscript.

The only remaining criticism I have is regarding the role of Sirt7 in aging related fur changes. The authors added "during aging" to the title and state that "Sirt7-/- mice exhibit prominent hair loss with aging." in the abstract. However, in the results they only state "severely diffuse patterns of hair thinning" and in the rebuttal letter Response #3-21 they provide data showing that the number of hair follicles per mm is not altered in aged Sirt7-/- compared to Sirt7+/+ mice. And state: "Histological analyses indicated no significant decrease in HFs in Sirt7-/- mice (Rebuttal Fig 3A-B)." Hair thinnening and hair loss are two different things. Having the same density of hair follicles indicates that there is no hair loss. Hair thinnening can be measured but has not been assessed by the authors.
In summary, there is inconsistency between the provided data and the statements regarding the aging phenotype that need to be corrected. The content of Rebuttal Fig. 3 should be included in the manuscript as it is important to understand the aging related phenotype.
In fact, the results on aged mice, provided in Fig. 7, match the results on younger mice in Fig. 1. However, they only show that Sirt7 is important for HFSC activation in young and old mice. This is also in line with Rebuttal Fig. 3C. The only data provided on the aging phenotype of Sirt7-/- mice is the picture of a single mouse back in Figure 7E. This is completely insufficient to justify the statements made in title and abstract.

I would suggest to either perform a thorough phenotype characterization of aged mice or to remove these claims.

Response to reviewers' comments

Referee #1:
The authors addressed all of my concerns.

Response:
We appreciate all the valuable comments and suggestions which helped improve our manuscript.

Referee #2:
In this revised version of the manuscript, the authors have incorporated additional experiments and clarified several aspects that improved their manuscript. In its present form, I found that it provides a conceptual advance about the functional involvement of SIRT7 in the activation of HFSC, including mechanistic insight and a potential connection of its downregulation with hair loss and aging skin phenotypes.

Response:
We appreciate the reviewer's positive comments on our revised manuscript.

Referee #3:
The revision greatly improved the manuscript.

Response:
Thank you for the encouraging comments on our revised version of manuscript.

The only remaining criticism I have is regarding the role of Sirt7 in aging related fur changes. The authors added "during aging" to the title and state that "Sirt7-/- mice exhibit prominent hair loss with aging." in the abstract. However, in the results they only state "severely diffuse patterns of hair thinning" and in the rebuttal letter Response #3-21 they provide data showing that the number of hair follicles per mm is not altered in aged Sirt7-/- compared to Sirt7+/+ mice. And state: "Histological analyses indicated no significant decrease in HFs in Sirt7-/- mice (Rebuttal Fig 3A-B)."
Hair thinning and hair loss are two different things. Having the same density of hair follicles indicates that there is no hair loss. Hair thinning can be measured but has not been assessed by the authors.
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7E. This is completely insufficient to justify the statements made in title and abstract. I would suggest to either perform a thorough phenotype characterization of aged mice or to remove these claims.

Response:
We appreciate the reviewer's points on aging-related hair thinning and hair loss. As suggested, we have removed these claims of aging-related hair loss and hair thinning from the title and the abstract. The title is amended as "SIRT7 evokes quiescent hair follicle stem cells and ensures hair growth in mice". We also made necessary changes (in track mode) in the revised manuscript.

2nd Revision - Editorial Decision 14th Jun 2020

Dear Dr Liu,

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript and concluded that the remaining minor concerns have been sufficiently addressed.

Thus, I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. I would thus like to ask for your consent on keeping the rebuttal figures included in this file.

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Kind regards,

Daniel Klimmeck

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Corresponding Author Name: Baohua Liu
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2019-104365

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 special stastatical methods were taken to evaluate sample size. Most western blot experiments were repeated for 3 times and quantificatied signals, or showed representative results. The other stastatic details were stated in figure legends and methods.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Sample sizes were not estimated by using statistical methods. Animal numbers were based on experience, e.g. for hair cycling analysis, 6 mice were used each time.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals were excluded from the analysis.
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
For animal studies, include a statement about randomization even if no randomization was used.	For hair cycle analysis, animals were collected randomly.
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
4.b. For animal studies, include a statement about blinding even if no blinding was done	Animal experiments were not done in blinded condition.
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.	Parametric statistical tests were use for normally distributed data and nonparametric tests were used in case of non normally distributed data.
Is there an estimate of variation within each group of data?	NO

USEFUL LINKS FOR COMPLETING THIS FORM

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

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-tumo

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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, the variance was anlyzed by Prism Software.

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).	They were reported in "Meaterials and methods" part in manuscript.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Our cells were purchased from ATCC. All cells were tested to confirm that they were not contaminated by mycoplasma (TaKaRa PCR Mycoplasma Detection Set, Japan).

* 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 were reported in "Materials and Methods" part in manuscript.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All the procedures were performed in accordance with instructions and permissions of the ethical committee on the Use of Live Animals in Teaching and Research of Shenzhen 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.	Current study applies to the ARRIVE guidelines for animal research.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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.	NA
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.	NA

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	NA
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).	NA
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).	NA
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.	NA

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.	NA