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Lamin B1 decline underlies age-related loss of adult hippocampal neurogenesis

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1st Editorial Decision 9th Jun 2020

Thank you for transferring your manuscript from Review Commons to The EMBO Journal. I have now read your manuscript, the reviewer comments and your revision proposal.

I note that all reviewers acknowledge the interest of the findings, but also indicate a number of concerns regarding the depth of the analysis and the mechanistic understanding of the role of Lamin B1 in regulation of adult neurogenesis, most of which you are prepared to address during a major revision of the manuscript.

We thus would like to invite you to revise your manuscript for The EMBO Journal reports with the understanding that the referee concerns must be addressed as indicated in your revision plan. Acceptance of your manuscript will depend on a positive assessment by the original reviewers in the second round of review. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. I would be happy to discuss the revision in more detail via email or phone/videoconferencing.

Review #1

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

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Bedrosian and colleagues demonstrate a role for Laminin B1 in regulating neurogenesis in the adult mouse hippocampus and through aging, using in vivo and in vitro knockout/knockdown studies, coupled with transcriptomic analyses. Specifically, they find that Laminin B1 is highly expressed by ANSPCs and neuroblasts. KO in vitro results in increased neuronal differentiation and reduced proliferation. KO in vivo results in a transient increase in neuroblast production that is later lost due to an exhaustion of ANSPCs. They found that Laminin B1 expression decreases with age and postulate that Laminin B1 has a role in the maintenance of NSPCs in young mice which is later lost in older mice. While these final data are mostly correlative, they conclude decreased expression of Laminin B1 contributes to age-related decline in neurogenesis. In general the study is well performed but there are a number of areas that should be strengthened. **Major** 1) The expression of Laminin B1 decreases with age - and the assumption is that its function decreases too. This could be tested directly by acutely deleting Laminin B1 in young and old mice. If the model is true, then the phenotype in young mice should be larger than in the older cohort (each age would need its own age-matched wt controls). 2) The authors postulate Laminin B1 might have a role in neuroblast survival - could they measure the branching of neuroblasts to determine whether there is any gross phenotype to support this claim. If they are dying it's likely to be aberrant. 3) The de-repression of BMP4 and the general phenotype of the mice would suggest a role for the loss of quiescence of stem cells (radial ANSPCs). Could the authors assess Ki67 or Mcm2 staining in the radial ANSPCs. **Minor** 4) The choice to focus on anxiety like behavior and only to mention in passing that the mice did not have a memory defect in the Y-maze seems odd. The authors might consider discussing what was expected to happen here. 5) Phrasing: "Age-related anxiety" might be more conservatively described as reduced exploratory behavior in the Open Field test. It doesn't sound right to describe 11-month old wild-type mice as having anxiety because they spent less time in the center of a box.

3. Significance:

Significance (Required)

It is interesting that this nuclear intermediate filament protein has a very specific expression pattern in the dentate gyrus, and the role of Laminin As in progeria make the link to aging interesting too.

Review #2

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

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 3 and 6 months

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

Summary: Bedrosian et al. describe how age-related loss in LaminB1 in the hippocampus is related to decreased neurogenesis and anxiety behavior during aging. Given the intrinsic association of the nuclear lamina with heterochromatin, the authors present bioinformatic data supporting the notion that LaminB1 decay de-represses pro-differentiation genes. In KO mice, after an initial phase (3 weeks) in which neurogenesis is increased at the expense of NPC, follows exhaustion (>2 months) that precludes further neurogenesis. Not surprisingly, this premature loss in neurogenesis is associated with phenotypes associated with decreased neurogenesis, such as anxiety. **Major comments:** 1) I think that a major aspect missing is distinguishing effects in quiescent vs active NSC. This is important for many reasons. To start with, as the authors state, lamins are remarkably long-lived, hence, quiescent stem cells would be minimally affected, if any, in the conditional KO, which might result in confounding assessments when pulling active and quiescent together. On a more conceptual ground, quiescence is critical for stem cell maintenance and their age-related decline as 2 major aspects on which this study focuses. Combining some BrdU label retention at smart times before/after tamoxifen could address this and provide valuable insights. 2) Along the same lines, also addressable with smart S phase labeling, I notice that several quantifications (e.g. Fig 4) have very high variance (and some seem contradicting some statements, see below). Birthdating neurons would increase the accuracy of these data. An important control of these mice, provided in S1C, entirely lacks statistical assessment. Also concerning controls, it is a pity that the specificity of the shRNAs is mentioned as "data not shown". 3) I feel that too strong statements are made from rather correlative and indirect data e.g. when authors compare their transcriptome with other people' DamID data. These are correlative and indirect. Certain other statements are certainly off the mark e.g. "we provide in vivo functional evidence, supported by histological, genetic and behavioral profiling, that high levels of lamin B1 in ANSPCs

safeguard against a premature reduction in adult hippocampal neurogenesis and age-related anxiety-like behavior." Again, this is an indirect assumption derived from converse, loss of function, lamin KO experiment. Showing "safeguard" would require overexpression of lamin, that is certainly not provided here. **Minor comments:** 1.For Fig 4 I, authors suggest the production of newborn neurons started to decline at that time. I disagree; the quantification indicates the levels in the KO animals go back to the control levels. Some of the claims derived from this interpretation should be corrected. 2.In Fig 5H, it is not clear how did the authors defined this neuronal population. Do they refer to mature neurons? Did they use any specific marker? Without the aid of a neuron-specific marker (eg. NeuN) the cells could also be astrocytes (as the authors also show a major increase in GFAP and S100Beta after LaminB1 reduction, figs. 7 B, G). 3.It remains open whether LMNB1 overexpression can increase the stem/progenitor cell population? Perhaps rejuvenating the hippocampus? Particularly to support some authors' claims, eg "maintaining high levels of lamin B1 in ANSPCs is key for the long-term maintenance of adult neurogenesis." 4.Other inconsistencies arise from the literature. Differently to what is pointed in this manuscript Mahajani et al. (2017) described that upon Lamin B1 knockdown rather differentiate into GFAP+ astrocytes at the expense of the neuronal lineage. Contrarily, Lamin B1 overexpression leads to an increase in the neuron population. Authors should discuss these differencies. 5.The Discussion section focuses excessively on a possible neuronal cell death hypothesis to explain neuronal loss upon Lamin B1 KO. This is intriguing because their data already supports neuron loss due to progenitor exhaustion. Additionally, they did not test any apoptosis nor senescence marker. 6.sometimes the axes in the graphs are not clearly readable, eg. Fig. 5G, 6C-D

3. Significance:

Significance (Required)

The role of the lamins family in premature aging is mainly described in peripheral tissues, but little is known about their influence in the brain. Given that in the CNS Lamin A is virtually absent, the study of Lamin B1 acquires notable importance. In this sense, the manuscript is relevant for the understanding of neurogenesis decline with aging, generally well written and executed

Review #3

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

Estimated time to Complete Revisions (Required)

(Decision Recommendation)

Between 1 and 3 months

2. Evidence, reproducibility and clarity:

Evidence, reproducibility and clarity (Required)

In their manuscript Bedrosian et al. addressed the role of LaminB1 in the control of hippocampal neurogenesis. They show that loss of LaminB1 function results in aberrant neurogenesis with a precocious decline in neuron production and aging-like phenotype. They show that the conditional LaminB1 knockout mice show an increased anxiety phenotype. They link this behavioral defect to a loss of newborn neurons in the dentate gyrus. This is an interesting manuscript and well presented. The experiments presented are convincing. The authors present gene expression data and make claims for a potential mechanism of LaminB1 action. However, none of these are tested experimentally. **Major concerns** Based on the behavioral changes, the authors should present more detailed analysis of recombination efficiency. What proportion of the newborn neurons are affected, how rapid is the loss of protein, what proportion of the stem cells rather than the IPCs are recombined? Does neurogenesis recover over time or is the production of neurons and stem cell numbers consistently reduced with age? The data are rather descriptive and there is no mechanism presented. It is unclear whether the authors favor the aberrant neurogenesis as the reason for the behavioral changes or the putative death of neurons. This needs to be clarified. The authors claim death of LaminB1 deficient neurons. They claim that LaminB1 plays a role in newborn neuron survival but do not formally show this. They either need to present data to show that the loss of neurons is a direct function of LaminB1 or a consequence of the initial increase in neuron production and then the block of stem/progenitor cell activity. If they wish to keep this statement they need to provide direct evidence of increased cell death related to LaminB1 function in neurons. The in vivo analysis of neurogenesis is rather rudimentary and does not really clarify the precise mode or cells that are affected. The analysis of the conditional knockout mice should be improved with a more detailed analysis of the neurogenic lineage at different time-points after knockout. The discussion of the mode of action including the upregulation of BMP4 following LaminB knockout is speculation. If the authors want to keep this conclusion, they would need to show validating data. The role of BMP in regulating DG quiescence in vivo is very controversial and recent evidence from the Guillemot lab indicates that BMP signaling may not be so important in regulation of quiescence in vivo. The authors should show whether the adult V-SVZ shows similar changes in neurogenesis following LaminB1 deletion. Presumably the conditional knockout approach also targets the lateral ventricle stem cells. **Minor concerns** The authors remain very close to their own story with their discussion. There is a whole wealth of data with putative mechanism of controlling DG stem cell ability that seem to have been omitted or neglected. A more balanced view would place their findings more in context of the current literature.

3. Significance:

Significance (Required)

The mechanisms controlling age-dependent decline in stem cell activity remain unclear. Understanding how and why stem cells enter a dormant state and fail to generate new

neurons in the adult brain is important as it provide novel targets for regenerative therapy and combating age-dependent loss of cognitive functions.

Response to reviewers “Lamin B1 decline underlies age-related loss of adult hippocampal neurogenesis”

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

Bedrosian and colleagues demonstrate a role for Laminin B1 in regulating neurogenesis in the adult mouse hippocampus and through aging, using in vivo and in vitro knockout/knockdown studies, coupled with transcriptomic analyses. Specifically, they find that Laminin B1 is highly expressed by ANSPCs and neuroblasts. KO in vitro results in increased neuronal differentiation and reduced proliferation. KO in vivo results in a transient increase in neuroblast production that is later lost due to an exhaustion of ANSPCs. They found that Laminin B1 expression decreases with age and postulate that Laminin B1 has a role in the maintenance of NSPCs in young mice which is later lost in older mice. While these final data are mostly correlative, they conclude decreased expression of Laminin B1 contributes to age-related decline in neurogenesis.

In general the study is well performed but there are a number of areas that should be strengthened.

We appreciate the reviewer’s constructive comments.

1) The expression of Laminin B1 decreases with age - and the assumption is that its function decreases too. This could be tested directly by acutely deleting Laminin B1 in young and old mice. If the model is true, then the phenotype in young mice should be larger than in the older cohort (each age would need its own age-matched wt controls).

This is an interesting point to test if the deletion of LaminB1 has a more pronounced effect in young versus aged mice. Unfortunately, due to COVID-19, we were asked to reduce cage numbers in the animal facility; therefore, we do not have aged cohorts to address this point in a reasonable timeframe. We have addressed the roles of lamin B1 in young mice in this manuscript. We discuss this point in the manuscript as shown below.

P22

“Since lamin B1 levels decrease in both RGL-ANSCs and ANPCs with age, it would be interesting to test whether lamin B1 deletion has a more pronounced effect in young mice versus aged mice that already have some natural degree of lamin B1 loss. Altogether, our data identify a novel cell-intrinsic mechanism underlying stem cell aging. It would also be intriguing to investigate what triggers lamin B1 levels in ANSPCs to become down-regulated during physiological aging. “

2) The authors postulate Laminin B1 might have a role in neuroblast survival - could they measure the branching of neuroblasts to determine whether there is any gross phenotype to support this claim. If they are dying it's likely to be aberrant.

We agree that it is an interesting point to assess whether LaminB1 KO induces morphological changes of immature neurons/neuroblasts. To address this point, we have conducted retroviral morphological tracing by injecting retrovirus expressing Cre under the control of a Sox2 promoter (pSox2-Cre) and analyzing the morphological development of adult-born neurons in LaminB1-cKO mice versus control mice 21 days after the retrovirus injection. We observed overgrowth of dendrites in KO cells compared with control cells (Fig R1-2). These data indicate that lamin B1 regulates not only the maintenance of ANSPCs but also the morphological development of neuroblasts/newborn neurons, which may be related to their survival. Indeed, we observed an increased number of active caspase-3 positive cells in lamin B1 cKO mice 2 months after TAM induction (Fig EV4 F-I). Alternatively, it is possible that this dendritic overgrowth is a consequence of earlier differentiation due to the reduction in lamin B1. Both possibilities are discussed in the manuscript. These data are added in the Fig. EV4 J-N in the revised manuscript.

Figure for reviewers removed

3) The de-repression of BMP4 and the general phenotype of the mice would suggest a role for the loss of quiescence of stem cells (radial ANSPCs). Could the authors assess Ki67 or Mcm2 staining in the radial ANSPCs.

We agree that it is important to assess the effects of lamin B1 KO on the proliferation of RGL-ANSCs. We have assessed the expression of Ki67 in RGL-ANSCs and found that Ki67-positive RGL-ANSCs were reduced 3 weeks, 2 months and 7 months after the administration of TAM in both RGL-ANSCs and NPCs (Fig R1-3), suggesting that lamin B1 is essential for the regulation of RGL-ANSCs.

Figure for reviewers removed

These data are added in Fig4 and Fig5 of the revised manuscript.

Minor

4) The choice to focus on anxiety like behavior and only to mention in passing that the mice did not have a memory defect in the Y-maze seems odd. The authors might consider discussing what was expected to happen here.

To test short-term memory, we also conducted a novel object recognition test, which did not show significant differences (Fig. R1-4). The results suggest that Lmnb1-cKO mice do not exhibit short-term memory deficits as far as we tested. We have added these data in Fig EV2 and discussed the possible function of LaminB1 in anxiety-like behavior as written below.

Figure for reviewers removed

P25

"The present data show that the reduction in lamin B1 in ANSPCs induces anxiety-like behavior. The enhanced anxiety was not due to decreased activity or decreased curiosity, as lamin B1 depletion did not modify total distance of exploration or novelty exploration in the novel-object recognition test. Intriguingly, a PCA analysis of behavioral data indicated that behavioral traits of lamin B1 cKO mice overlap better with old control mice than with young control mice. Thus, our data suggest lamin B1 is a possible link between brain aging and mood regulation, and lamin B1 cKO mice could be used as a model of cell-intrinsic neural stem aging and age-related mood dysregulation. One question that arises is how does lamin B1 depletion induce age-related anxiety-like behavior? One possibility is the lower survival of adult-born neurons. Our data indicated lamin B1 deficiency leads to lower survival rate, which is consistent with the survival rate of adult-born neurons in old mice (Kuipers et al., 2015). However, the total number of surviving neurons was only slightly lower 2 months after the induction of knockout (Fig EV4A). These observations raise several other possibilities to explain how lamin B1 deficiency contributes to age-related behavior. First, in addition to total numbers of adult-born neurons, the balance between immature adult-born neurons and mature adult-born neurons may be key. Lamin B1 cKO transiently increased neurogenesis but relatively reduced it from 3 weeks to 2 months after the induction of knockout. Therefore, the reduction in the rate of neurogenesis may underlie behavioral changes. The other possibility is change in functionality of adult-born neurons in laminB1 cKO neurons. In fact, we observed aberrant dendritic development in laminB1 cKO adult-born neurons. Mis-integration of adult-born neurons has been shown to cause anxiety-like behavior (Bergami, Rimondini et al., 2008). While a depletion of adult hippocampal neurogenesis could impair memory (Deng et al., 2009, Nakashiba et al., 2012, Saxe et al., 2006), lamin B1 cKO mice did not have deficits in short-term memory. Therefore, the effects of lamin B1 depletion may not be simply due to the depletion of adult-born neurons. Future investigation in this area is warranted."

5) Phrasing: "Age-related anxiety" might be more conservatively described as reduced

exploratory behavior in the Open Field test. It doesn't sound right to describe 11-month old wild-type mice as having anxiety because they spent less time in the center of a box.

We appreciate the reviewer's careful suggestion. However, thigmotaxis has been used as a standard measure of rodent anxiety like-response as described in "Seibenhener, M. L., & Wooten, M. C. (2015). Use of the open field maze to measure locomotor and anxiety-like behavior in mice. JoVE (Journal of Visualized Experiments), (96), e52434". or "Simon, P., Dupuis, R., & Costentin, J. (1994). Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behavioural brain research, 61(1), 59-64." or "David D. J. et al Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron, 4, 479-93, (2011). We have added these references to the revised manuscript. Our data indicated that total exploratory distance was not significantly changed by age or LaminB1-cKO, but the time spent in the center of the area was selectively reduced by age as well as cKO of LaminB1, which indicated the increased anxiety level. In addition, as an independent measurement, we conducted the novelty suppressed-feeding test, which supports the idea that the level of anxiety is increased in cKO mice as well as old mice. Therefore, our data suggest that those mice exhibited age-related anxiety.

Reviewer #1 (Significance (Required)):

It is interesting that this nuclear intermediate filament protein has a very specific expression pattern in the dentate gyrus, and the role of Laminin As in progeria make the link to aging interesting too.

We appreciate that the reviewer recognizes the significance and novelty of our manuscript.

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

Summary:

Bedrosian et al. describe how age-related loss in LaminB1 in the hippocampus is related to decreased neurogenesis and anxiety behavior during aging. Given the intrinsic association of the nuclear lamina with heterochromatin, the authors present bioinformatic data supporting the notion that LaminB1 decay de-represses pro-differentiation genes. In KO mice, after an initial phase (3 weeks) in which neurogenesis is increased at the expense of NPC, follows exhaustion (>2 months) that precludes further neurogenesis. Not surprisingly, this premature loss in neurogenesis is associated with phenotypes associated with decreased neurogenesis, such as anxiety.

Major comments:

1) I think that a major aspect missing is distinguishing effects in quiescent vs active NSC. This is important for many reasons. To start with, as the authors state, lamins are remarkably long-lived,

hence, quiescent stem cells would be minimally affected, if any, in the conditional KO, which might result in confounding assessments when pulling active and quiescent together. On a more conceptual ground, quiescence is critical for stem cell maintenance and their age-related decline as 2 major aspects on which this study focuses. Combining some BrdU label retention at smart times before/after tamoxifen could address this and provide valuable insights.

We appreciate the reviewer's thoughtful suggestions. We agree that it is important to segregate the effect of laminB1 KO between RGL-NSCs (quiescent) and non-RGL NPCs (active). To address this point, we independently counted the number of RGL-NSCs and non-RGL NPCs in our analyses (Fig4 and 5). To further deepen our understanding, we have conducted the following experiments.

1. To address which RGL-ANSC/ANPCs population are more prone to be affected in terms of the levels of lamin B1 during aging, we have segregated RGL-ANSCs and ANPCs in Figures 1 and 2 using pNestin-GFP transgenic mice. We found that non-RGL NPCs showed slightly higher lamin B1 expression than RGL-NSCs. During aging, the levels of laminB1 decreased both in RGL-ANSCs and ANPCs (Fig. R2-1 A-E), but the degree of reduction was larger in ANPCs. These data imply that both RGL-ANSCs and ANPCs could be affected by aging through the decline of lamin B1. These data are added to the Fig 1 and Fig 2 of the revised manuscript.

Figure for reviewers removed

2. To investigate the effect of lamin B1cKO on RGL-ANSCs/ANPCs' proliferation, we assessed the number of Ki67+ cells in RGL-NSCs and NPCs (Fig. R2-1F). These data showed that laminB1 is essential for the regulation of both RGL-ANSC and NPCs. These data are added in the Figure 4 of the revised manuscript.

3. To address which cell types lost laminB1 protein and when they lost laminB1 proteins after TAM administration, we investigated laminB1 levels with cell type markers or a proliferation=
marker Ki67. We defined laminB1-deficient cells as having complete loss or more than 30%
reduction of lamin B1 immunofluorescent intensity compared to the average intensity of=
lamin B1 immunofluorescent signals in corresponding non-EYFP control cells (30% of the=
averaged signal intensity corresponds to the standard deviation of laminB1 signal intensity=
from corresponding control cells). The data suggest that lamin B1 proteins are reduced 10=
days after the induction of knockout in ANSPCs and neuroblasts. However, 3 weeks after the=
induction, although the fraction of lamin B-deficient cells was increased in neuroblasts, the=
fraction of laminB1-deficient cells was decreased from 10 days to 3 weeks in ANSPCs.=
These observations imply that laminB1-deficient ANPCs are not able to retain their state=
after laminB1 levels are downregulated. Consistent with this idea, our original observation=
indicated that the number of ANPCs was already reduced 3 weeks after the induction of=
lamin B1 knockout, and the number of neuroblasts was increased. In addition, with Ki67=
staining, we observed laminB1 deficiency in proliferating non-RGL-ANSCs but rarely=
observed proliferating RGL-ANSCs. These data imply that a reduction in lamin B1 requires=
the proliferation process to reduce lamin B1 protein levels once RGL-ANSCs are activated;=
activated ANSCs may differentiate instead of going back to a quiescent state (Fig. R2-2).

These data are added in Fig EV1.

Figure for reviewers removed

4. To address if cKO of LaminB1 affects the maintenance/return to quiescent NSCs, we utilized an established method for labeling slowly dividing NSCs. Briefly, after TAM treatment, BrdU is injected for 9 days to label active RGL-ANSCs, and the numbers of BrdU+GFAP+ RGL-ANSCs are analyzed three weeks after BrdU injection (FigR2-3). The data indicate that lamin B1 is important to return ANSCs to a quiescent state, and a knockout of lamin B1

inhibits the return of activated ANSCs into quiescent ANSCs. These data are added in Fig 4P in the revised manuscript.

Figure for reviewers removed

2) Along the same lines, also addressable with smart S phase labeling, I notice that several quantifications (e.g. Fig 4) have very high variance (and some seem contradicting some statements, see below). Birthdating neurons would increase the accuracy of these data. An important control of these mice, provided in S1C, entirely lacks statistical assessment. Also concerning controls, it is a pity that the specificity of the shRNAs is mentioned as "data not shown".

We really appreciate the reviewer's constructive comments. Regarding the reviewer's point, we have conducted BrdU birth-dating experiments to analyze new cell generation and survival (FigR 2-4). After the administration of Tamoxifen, BrdU was injected and samples were collected at different time points. Consistent with our original observation, we found an increased number of BrdU+ cells in Lmnb1 cKO right after the induction of knockout (Fig R2-4B, C). However, the number of BrdU+ cells was dramatically reduced in Lmnb1 cKO mice at a later time point. To estimate the survival ratio after cKO of laminB1, the total number of BrdU-positive cells at 2 months post injection of BrdU was divided by the average number of BrdU-positive cells at day 1 post injection of BrdU. The data indicate that survival rate was significantly lower in laminB1 cKO mice (FigR 2-4D).

In parallel, to measure the rate of new cell generation at different time points after the induction, BrdU was injected 3 weeks or 2 months after TAM administration, and the number of BrdU+ cells were measured. Consistent with our original observation, the levels of proliferation are higher in cKO mice 3 weeks after the induction of knockout, but the levels of neurogenesis are lower 2 months after the induction of cKO (Fig. R2-4G, H). These data are consistent with our original observation that laminB1 cKO transiently increases neurogenesis but eventually affects the maintenance of adult neurogenesis and survival. These data are added to Fig EV3 and Fig EV4.

Regarding the quantification of the original S1C, we added our assessment as shown in Fig R2-2.

Figure for reviewers removed

To further address which cell types lost LaminB1 protein and when they lost laminB1 proteins after TAM administration, we investigated LaminB1 expression with cell type markers at different time points as shown in Fig R2-2. Our data indicate that lamin B1 protein is preferentially lost/reduced in proliferating NPCs and neuroblasts. These data are added in Fig EV1.

We have shown that our shLmnb1 did not affect the levels of *LaminB2* (in original figure 6). We assume that the reviewer is referring to RNA-seq data 5.5 days after the knockout of LaminB1 in NPCs, where we referred to the specificity of LaminB1 manipulation as "data not shown". We have added the corresponding RNA-seq data as Table 2 to show the specificity of LaminB1 KO; the original data are updated in GEO: GSE156156.

..

3) I feel that too strong statements are made from rather correlative and indirect data e.g. when authors compare their transcriptome with other people' DamID data. These are correlative and indirect. Certain other statements are certainly off the mark e.g. "we provide in vivo functional evidence, supported by histological, genetic and behavioral profiling, that high levels of lamin B1 in ANSPCs safeguard against a premature reduction in adult hippocampal neurogenesis and age-related anxiety-like behavior." Again, this is an indirect assumption derived from converse, loss of function, lamin KO experiment. Showing "safeguard" would require overexpression of lamin, that is certainly not provided here.

We appreciate the reviewer's thoughtful suggestion. According to the reviewer's suggestion, in the revised manuscript, we have softened our statements which are supported by correlative or indirect evidence.

P21

"Here we provide *in vivo* evidence, supported by histological and behavioral profiling, that lamin B1 plays critical roles in the maintenance of adult hippocampal neurogenesis and in age-related anxiety-like behavior. Our correlative genomic data analyses also support the emerging notion

that cell type-specific nuclear architecture directed by nuclear structural proteins (e.g., nucleoporins or lamins) controls cell type-specific gene regulation and long-term maintenance of cell type-specific functions (Ibarra, Benner et al., 2016, Jacinto, Benner et al., 2015, Peric-Hupkes & van Steensel, 2010, Toda et al., 2017), such as continuous generation of new neurons from ANSPCs. "

To address if high levels of laminB1 inhibit differentiation of NPCs, we conducted LaminB1 overexpression experiments *in vitro*. Using retroviral vectors, we exogenously expressed LaminB1-IRES-GFP in cultured hippocampal NPCs and tested if high levels of LaminB1 inhibited differentiation. qRT-PCR analyses revealed that high levels of LaminB1 inhibit the expression of genes related to differentiation (Fig R2-5; A-D). These data suggest that high levels of LaminB1 inhibits differentiation of NPCs and support the idea that high levels of LaminB1 safeguard NPCs

To further address this point, we conducted LaminB1 overexpression using retroviral vectors *in vivo*. We injected RV harboring LaminB1-IRES-GFP in the SGZ and collected samples 7 days after the injection. We confirmed that LaminB1 is highly expressed *in vivo* (Fig R2-5F). The exogenous expression of laminB1 markedly reduced the fraction of DCX-positive neuroblasts compared to control whereas a subset of EGFP+ cells remained as Sox2+ cells. These data suggest that high levels of laminB1 contribute to maintaining NPCs to some extent. These data are added in Fig 7 in the revised manuscript.

Figure for reviewers removed

Minor comments:

1.For Fig 4 I, authors suggest the production of newborn neurons started to decline at that time. I disagree; the quantification indicates the levels in the KO animals go back to the control levels. Some of the claims derived from this interpretation should be corrected.

According to the reviewer's suggestion, we have revised our interpretation in the revised manuscript.

2.In Fig 5H, it is not clear how did the authors defined this neuronal population. Do they refer to mature neurons? Did they use any specific marker? Without the aid of a neuron-specific marker (eg. NeuN) the cells could also be astrocytes (as the authors also show a major increase in GFAP and S100Beta after LaminB1 reduction, figs. 7 B, G).

We defined the neuronal population using GFP-based neuronal morphology. As suggested by the reviewer, we re-assessed our observation using NeuN staining. The revised data are added in the Fig 5I and Fig Ev4A.

3.It remains open whether LMNB1 overexpression can increase the stem/progenitor cell population? Perhaps rejuvenating the hippocampus? Particularly to support some authors' claims, eg "maintaining high levels of lamin B1 in ANSPCs is key for the long-term maintenance of adult neurogenesis."

We appreciate the reviewer's thoughtful advice. To address this point, we have assessed LaminB1-overexpression *in vitro* and *in vivo* as shown in new Fig 7. Our data indicate that overexpression of LaminB1 inhibits neural differentiation of NPCs *in vitro* and *in vivo*, which support the idea that high levels of lamin B1 could contribute to retain ANPCs.

As the reviewer suggests, it would be very intriguing to test if LaminB1 expression can rejuvenate the hippocampal niche in future experiments. This point is now discussed in the discussion.

P28

"Interestingly, a recent study showed that an increase of only 100 newborn neurons could rejuvenate some hippocampus-dependent function in old mice (Berdugo-Vega, Arias-Gil et al., 2020). Since lamin B1 overexpression represses differentiation of ANPCs, it would be intriguing to test whether an exogenous expression of lamin B1 could retain more ANSPCs until older ages or even rejuvenate ANSPCs and hippocampal function by generating new neurons at older ages."

4.Other inconsistencies arise from the literature. Differently to what is pointed in this manuscript Mahajani et al. (2017) described that upon Lamin B1 knockdown rather differentiate into GFAP+ astrocytes at the expense of the neuronal lineage. Contrarily, Lamin B1 overexpression leads to an increase in the neuron population. Authors should discuss these differencies.

This is an interesting point raised by the reviewer. The inconsistency of laminB1 function between embryonic and adult neurogenesis may indicate the context-dependent roles of lamin B1. We discuss this point in the revised manuscript.

P26

"In contrast to the enhanced adult hippocampal neurogenesis observed with lamin B1 cKO, lamin B1 knockdown promotes astrogenesis in the developing cortex (Mahajani et al., 2017). This inconsistency suggests that lamin B1 may play distinct roles in a context-dependent manner. During corticogenesis, neurogenesis precedes astrogenesis and lamin B1 may regulate this transition from neurogenesis to astrogenesis (Namihira & Nakashima, 2013). Another factor contributing to this inconsistency could be environmental cues. The niche environment in the SGZ provides strong neurogenic cues such as Wnt signaling (Goncalves et al., 2016). Therefore, even though knockdown of lamin B1 in proliferating ANPCs *in vitro* induced the increased expression of both neuronal and glial markers, neurogenic cues in the SGZ may preferentially promote neurogenesis *in vivo*."

5.The Discussion section focuses excessively on a possible neuronal cell death hypothesis to explain neuronal loss upon Lamin B1 KO. This is intriguing because their data already supports neuron loss due to progenitor exhaustion. Additionally, they did not test any apoptosis nor senescence marker.

To address neuronal loss in laminB1 deficiency, we quantified the number of GFP+NeuN+ cells, which indicated that the total number of surviving adult-born neurons in cKO was significantly lower than control at 2 months and 6.5months after the induction of knockout (EV4A, Fig 5H). We also conducted BrdU retention experiments, and calculated the estimated survival rate by comparing the number of BrdU-positive cells right after the injection and 2 months after injections. We found that the survival rate in laminB1 cKO was significantly reduced (Fig. R2-3D), suggesting cells were lost, presumably through cell death.

To examine the mode of cell death, according to the reviewer's suggestion, we addressed this point using an apoptotic cell marker, active caspase3, at different time points, and we observed an increased number of active caspase3+ cells 2 months after TAM infusion (Fig EV4G-I).

P25

"The present data show that the reduction in lamin B1 in ANSPCs induces anxiety-like behavior. The enhanced anxiety was not due to decreased activity or decreased curiosity, as lamin B1 depletion did not modify total distance of exploration or novelty exploration in the novel-object recognition test. Intriguingly, a PCA analysis of behavioral data indicated that behavioral traits of lamin B1 cKO mice overlap better with old control mice than with young control mice. Thus, our data suggest lamin B1 is a possible link between brain aging and mood regulation, and lamin B1 cKO mice could be used as a model of cell-intrinsic neural stem aging and age-related mood dysregulation. One question that arises is how does lamin B1 depletion induce age-related anxiety-like behavior? One possibility is the lower survival of adult-born neurons. Our data indicated lamin B1 deficiency leads to a lower survival rate, which is consistent with the survival rate of adult-born neurons in old mice (Kuipers et al., 2015). However, the total number of surviving neurons was only slightly lower 2 months after the induction of knockout (Fig EV4A). These observations raise several other possibilities to explain how lamin B1 deficiency contributes to age-related behavior. First, in addition to total numbers of adult-born neurons, the balance between immature adult-born neurons and mature adult-born neurons may be key. Lamin B1 cKO transiently increased neurogenesis but relatively reduced it

from 3 weeks to 2 months after the induction of knockout. Therefore, the reduction of the rate of neurogenesis may underlie behavioral changes. The other possibility is a change in functionality of adult-born neurons in laminB1 cKO neurons. In fact, we observed aberrant dendritic development in laminB1 cKO adult-born neurons. Mis-integration of adult-born neurons has been shown to cause anxiety-like behavior (Bergami, Rimondini et al., 2008). While a depletion of adult hippocampal neurogenesis could impair memory (Deng et al., 2009, Nakashiba et al., 2012, Saxe et al., 2006), lamin B1 cKO mice did not affect short-term memory. Therefore, the effects of lamin B1 depletion may not be simply due to the depletion of adult-born neurons. Future investigation in this area is warranted."

6.sometimes the axes in the graphs are not clearly readable, eg. Fig. 5G, 6C-D

We thank the reviewer for the careful suggestion. We corrected the data presentation to ensure all axes are readable.

Reviewer #2 (Significance (Required)):

The role of the lamins family in premature aging is mainly described in peripheral tissues, but little is known about their influence in the brain. Given that in the CNS Lamin A is virtually absent, the study of Lamin B1 acquires notable importance. In this sense, the manuscript is relevant for the understanding of neurogenesis decline with aging, generally well written and executed

We appreciate the reviewer's recognition of the importance of our work.

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

In their manuscript Bedrosian et al. addressed the role of LaminB1 in the control of hippocampal neurogenesis. They show that loss of LaminB1 function results in aberrant neurogenesis with a precocious decline in neuron production and aging-like phenotype. They show that the conditional LaminB1 knockout mice show an increased anxiety phenotype. They link this behavioral defect to a loss of newborn neurons in the dentate gyrus. This is an interesting manuscript and well presented. The experiments presented are convincing. The authors present gene expression data and make claims for a potential mechanism of LaminB1 action. However, none of these are tested experimentally.

We thank the reviewer for interest in our work and the quality of data we presented.

Major concerns

Based on the behavioral changes, the authors should present more detailed analysis of recombination efficiency. What proportion of the newborn neurons are affected, how rapid is the loss of protein, what proportion of the stem cells rather than the IPCs are recombined? Does

neurogenesis recover over time or is the production of neurons and stem cell numbers consistently reduced with age?

We appreciate the reviewer's suggestion that more detailed analysis of recombination efficiency would strengthen our data. We analyzed the efficiency of the recombination at 10 days and 3 weeks after KO with cell type markers or a proliferation marker. With this information, we investigated when and which cell types start to lose laminB1 proteins (FigR3-1). Our data indicate that lamin B1 proteins are depleted/reduced from 10 days after the induction of knockout, and lamin B1 loss preferentially occurs in proliferating cells. These data are added in Fig EV1.

Figure for reviewers removed

We also examined the proliferation of RGL-NSCs and NPCs using Ki67 and cell type-specific markers to check if the proliferation of stem cell pools recovers. Our data indicate that the proliferation of RGL-NSCs as well as NPCs was reduced in cKO from 3 weeks and 6 months after the induction of cKO (FigR3-2). In contrast, the proliferation of neuroblasts was transiently increased 3 weeks after TAM infusion (Fig3-2G), but it was reduced 2 months after TAM administration (Fig R3-2H). EYFP+Neuroblasts were completely depleted 6 months after TAM administration (Fig5F in the manuscript). These data suggest that, after the induction of lamin B1 knockout, neurogenesis is transiently increased due to the increase of neuroblasts. However, ANSPCs are depleted continuously, and as a consequence, neuroblasts are also depleted at a later time point.

Figure for reviewers removed

To confirm our observation, we measured new cell generation by BrdU assay 3 weeks after TAM administration and BrdU was injected. The density of BrdU+ cells was increased in lamin B1- cKO mice (FigR3-3A-C). However, when BrdU was injected 2 months after TAM administration, the density of BrdU+ cells was reduced in laminB1 cKO. As our original data for 6.5 months after TAM indicated, new cell generation was substantially depleted (Current Fig EV3G-I). These data are consistent with the idea that lamin B1 cKO transiently increases neurogenesis, but eventually depletes it.

Figure for reviewers removed

The data are rather descriptive and there is no mechanism presented. It is unclear whether the authors favor the aberrant neurogenesis as the reason for the behavioral changes or the putative death of neurons. This needs to be clarified.

It is challenging to discern which of these two closely related mechanisms contributes to behavioral effects. To begin to clarify whether aberrant neurogenesis and/or putative neuronal death causes behavioral changes, we asked whether the timing of neuronal loss and/or cell death correlates with the timing of differences in anxiety-like behavior.

First, we addressed the number of adult-born neurons by counting GFP+NeuN+ neurons at 2 and 6 months after TAM infusion. GFP+ NeuN+ neurons represent the accumulated total number of surviving neurons produced after the recombination induced by TAM. Since newborn neurons were generated in greater numbers in cKO at 3 weeks after TAM administration (Fig 4H, increased DCX+ neuroblasts/newborn neurons), the reduction of GFP+NeuN+ neurons indirectly indicates the loss of adult-born neurons. We observed fewer GFP+NeuN+ cells at 2 months and 6 months after TAM infusion, suggesting that the total number of adult-born neurons was reduced but not totally depleted when we conducted behavioral tests, which occurred at 2months after TAM infusion (FigR3-4A,B).

Second, using a BrdU retention assay, we checked the survival rate of newborn cells after TAM administration (Fig. R3-4C-F). Our data indicate that cKO of laminB1 transiently increases neurogenesis, but the number of BrdU-positive cells declines profoundly in cKO, suggesting that laminB1 cKO induced cell death. The survival rate of adult-born cells is significantly lower in lamin B1 cKO mice. These data indicate that significant cell death occurs between 3 weeks and 2 months after the induction of lamin B1 cKO, well in advance of our original behavioral testing.

Third, we directly addressed the death of cells using an apoptotic marker active-caspase-3 after TAM infusion. We observed an increased active-caspase-3+ cells at 2 months after the induction of KO. It is possible that ongoing neuronal loss, as well as continued aberrant neurogenesis, both contribute to behavioral changes.

Figure for reviewers removed

Taken together, our data indicate that lamin B1 loss-induced cell death begins to occur between 3 weeks and 2 months after TAM infusion, before our behavioral testing. These data support an idea that neuronal death may contribute to the behavioral changes. However, the total number of neurons at 2 months was only slightly lower in cKO (Fig EV4A). Thus, our data indicate that, in addition to the reduction of adult-born neurons, other possibilities such as the relative reduction of neurogenesis rate (Fig EV3A-D) may be contributing. These points are discussed in the revised manuscripts.

P25

"The present data show that the reduction in lamin B1 in ANSPCs induces anxiety-like behavior. The enhanced anxiety was not due to decreased activity or decreaed curiosity, as lamin B1 depletion did not modify total distance of exploration or novelty exploration in the novel-object recognition test. Intriguingly, a PCA analysis of behavioral data indicated that behavioral traits of lamin B1 cKO mice overlap better with old control mice than with young control mice. Thus, our data suggest lamin B1 is a possible link between brain aging and mood regulation, and lamin B1 cKO mice could be used as a model of cell-intrinsic neural stem cell aging and age-related mood dysregulation. One question that arises is how does lamin B1 depletion induce age-related anxiety-like behavior? One possibility is the lower survival of adult-born neurons. Our data indicated that lamin B1 deficiency leads to a lower survival rate, which is consistent with the survival rate of adult-born neurons in old mice (Kuipers et al., 2015). However, the total number of surviving neurons was only slightly lower 2 months after the induction of knockout (Fig EV4A). These observations raise several other possibilities to explain how lamin B1 deficiency contributes to age-related behavior. First, in addition to total numbers of adult-born neurons, the balance between immature adult-born neurons and mature adult-born neurons may be key. Lamin B1 cKO transiently increased neurogenesis but relatively reduced it from 3 weeks to 2 months after the induction of knockout. Therefore, the reduction of the rate of neurogenesis may underlie behavioral changes. The other possibility is a change in functionality of adult-born neurons in laminB1 cKO neurons. In fact, we observed aberrant dendritic development in laminB1 cKO adult-born neurons. Mis-integration of adult-born neurons has been shown to cause anxiety-like behavior (Bergami, Rimondini et al., 2008). While a depletion of adult hippocampal neurogenesis could impair memory (Deng et al., 2009, Nakashiba et al., 2012, Saxe et al., 2006), lamin B1 cKO mice did not affect short-term memory. Therefore, the effects of lamin B1 depletion may not be simply due to the depletion of adult-born neurons. Future investigation in this area is warranted."

The authors claim death of LaminB1 deficient neurons. They claim that LaminB1 plays a role in newborn neuron survival but do not formally show this. They either need to present data to show that the loss of neurons is a direct function of LaminB1 or a consequence of the initial increase in neuron production and then the block of stem/progenitor cell activity. If they wish to keep this statement, they need to provide direct evidence of increased cell death related to LaminB1 function in neurons.

We appreciate the reviewer's question. A recent paper from the Young lab (PNAS, 2019, 12, 1691) clearly showed that laminB1 deficiency directly leads to neuronal cell death. Our data also support that laminB1 cKO leads to cell death (Fig R3-4). To assess when neurons die after the induction of cKO, we have investigated neuronal loss using three approaches. First, a BrdU

retention experiment indicates that cKO of laminB1 significantly reduces the survival rate 2 month after neurogenesis. Second, NeuN staining of EYFP+ cells shows that the number of mature neurons is also reduced in cKO 2 months after the induction of knockout. Finally, we used an apoptotic marker at different time points and observed an increased apoptosis 2 months after TAM infusion. Taken together, these data suggest that lamin B1 deficiency leads to cell death.

The in vivo analysis of neurogenesis is rather rudimentary and does not really clarify the precise mode or cells that are affected. The analysis of the conditional knockout mice should be improved with a more detailed analysis of the neurogenic lineage at different time-points after knockout.

We really appreciate the reviewer's constructive suggestion. We agree that further analysis is important to understand how LaminB1 regulates neurogenesis. As suggested by the reviewer, we addressed which proliferating cell types are affected using Ki67 staining combined with cell type markers. We have found that the proliferation of both RGL-ANSCs and NPCs are impaired 3 weeks after TAM administration. In contrast, the number of proliferating neuroblasts transiently increased, presumably due to the differentiation of NSPCs to neuroblasts, but eventually declined 2 months after TAM administration (Fig R3-2, R3-3). The data indicate that lamin B1-knockout transiently increases neurogenesis through the increase of neuroblasts but reduces ANPCs. Subsequently, the proliferation of RGL-ANSCs, ANPCs and neuroblasts was reduced, which eventually decreased neurogenesis at later time points. These data are added in Fig. 4 and Fig5 and Fig EV3.

Furthermore, we addressed whether lamin B1 is essential for the maintenance of RGL-ANSCs using a BrdU retention assay. After the induction of knockout, mice were injected with BrdU and sampled 3 weeks later (Fig R3-5). We found a reduction in BrdU+ RGL-ANSCs, indicating that the return of activated RGL-ANSCs to a quiescent state was reduced, which would underlie the gradual reduction of RGL-ANSCs in lamin B1-cKO. These data are added in Fig 4P in the revised manuscripts.

Figure for reviewers removed

The discussion of the mode of action including the upregulation of BMP4 following LaminB knockout is speculation. If the authors want to keep this conclusion, they would need to show validating data. The role of BMP in regulating DG quiescence in vivo is very controversial and recent evidence from the Guillemot lab indicates that BMP signaling may not be so important in regulation of quiescence in vivo.

We agree that the validation of BMP signaling downstream of LaminB1 is important and interesting. A recent paper from the Urban/Guillemot lab showed that BMP4-induced Id4 plays a critical role in the maintaining quiescent state of RGL-NSCs by inhibiting Ascl1. Other previous papers (Bonaguidi et al 2008, Mira et al., 2010, Martynoga et al 2013, Meyers et al 2016) also showed that BMP signaling is important in the maintenance of RGL-NSCs and aging of the niche environment. Consistent with these observations, our RNA-seq data indicated that not only BMP-4 (P = 4.21e-24, fold changes = 6.7) but also Id4 levels (P = 1.70e-23, fold changes = 2.5) are significantly upregulated after LaminB1 KO in NPCs (FigR3-6A, in EV5F). To validate these RNA-seq data, we first used immunohistochemistry to determine whether BMP4 or phospho-Smads, BMP-signaling mediators, are upregulated in the SGZ of LaminB1-cKO *in vivo*. Unfortunately, although we tested commonly used antibodies (Anti-BMP4, Millipore, MAB1049, pSmad1/5/9, CST, 13820), we were not able to detect reliable signals of BMP-4 and pSmads in the brain sections.

To circumvent this technical limitation, we used our *in vitro* system to check if lamin B1 deficiency increased BMP-4 expression and downstream signaling. First, we validated the increase of *BMP-4* and *Id4* expression by qRT-PCR in lamin B1 cKO NPC (Fig R3-6B, Fig EV5G). Subsequently, we examined if phospho-Smads expression or Id4 expression was upregulated in laminB1 cKO by immunostaining. We observed increased immunofluorescent signals of pSmad1/5/9 and Id4 in lamin B1 cKO NPCs. These observations support an idea that lamin B1 reduction/deficiency could activate BMP signaling (FigR3-6C-F). However, of course these data do not directly indicate if this phenomenon could happen *in vivo*. Therefore, we softened our conclusion in the revised manuscript.

To address whether high-laminB1 levels could repress the differentiation of ANPCs, we exogenously expressed lamin B1 in NPCs *in vitro* and *in vivo*. Our data indicated that upregulated genes in lamin B1 cKO NPCs such as *NeuroD1, Tubb3, Prox1* and *S100b* were significantly repressed by the exogenous expression of lamin B1 (New Fig 7A-D), suggesting that high lamin B1 levels inhibited upregulation of differentiation-related genes. Furthermore, exogenous expression of lamin B1 in ANPCs also repressed its differentiation into neuroblasts *in vivo* (Fig7E-H). Thus, our data indicate that higher levels of lamin B1 could repress differentiation of ANSPCs and possibly contribute to retain ANSPCs for the long term. These data are added in Fig7.

Figure for reviewers removed

The authors should show whether the adult V-SVZ shows similar changes in neurogenesis following LaminB1 deletion. Presumably the conditional knockout approach also targets the lateral ventricle stem cells.

This is an interesting point raised by the reviewer. However, the adult neurogenesis of V-SVZ is not the focus of this study, and we will leave this point for future experiments.

Minor concerns

The authors remain very close to their own story with their discussion. There is a whole wealth of data with putative mechanism of controlling DG stem cell ability that seem to have been omitted or neglected. A more balanced view would place their findings more in context of the current literature.

According to the reviewer's suggestion, we have incorporated more current literature in the discussion in the revised manuscript.

Reviewer #3 (Significance (Required)):

The mechanisms controlling age-dependent decline in stem cell activity remain unclear. Understanding how and why stem cells enter a dormant state and fail to generate new neurons in the adult brain is important as it provide novel targets for regenerative therapy and combating age-dependent loss of cognitive functions.

Our new data show how lamin B1 decline impairs the maintenance of adult hippocampal neurogenesis through the reduction of proliferating ANSPCs and the reduced return of activated RGL-ANSCs to a quiescent state. In addition, we show that the exogenous expression of lamin B1 could delay ANPC's differentiation, suggesting that lamin B1 acts as a safeguard of ANPCs at some extent. We believe that these additional data provide a novel insight into how lamin B1 decline underlies the age-dependent reduction of adult hippocampal neurogenesis, and they improved the quality of our manuscript.

1st Revision - Editorial Decision 16th Oct 2020

Thank you for submitting a revised version of your Review Commons manuscript. It has now been evaluated by two of the original referees, who find that their main concerns have been addressed and are now broadly in favour of publication of the manuscript. There now remain only a few editorial issues that have to be addressed before I can extend formal acceptance of the manuscript.

Referee #1:

The authors have done an excellent job at revising their manuscript with a large number of new experiments and extensive changes to the text, which altogether address most of my concerns and those of the other reviewers.

Remaining minor issues:

p. 6: the statement "the intermediate ANPCs exhibited greater laminB1 expression than RGL-ANSCs (Fig 1C)." is based on one image without quantification. This should be quantified or the conclusion tone down.

p. 7: the sentence "When we refer to this combined population, we use the term ANSPCs, in accordance with existing literature. However, where possible we have sought to segregate cells into RGL-ANSCs and ANPCs using additional features." should come earlier in the manuscript, as the terms ANSPCs, RGL-ANSCs and ANPCs have already been used in the introduction p. 5 and at the beginning of the Results p. 6.

Referee #2:

The authors have done an impressive revision work. All my points are thoroughly addressed. In fact, they have addressed them beyond what was strictly necessary and by multiple and complementary approaches. As one example, my suggestion to assess quiescent cells could have been fulfilled, technically-speaking, by the 4th set of experiments only (long-term BrdU retention, Fig. R2-3). Instead, the authors decided to (over- ?) kill my point adding also quantifications of radial morphology and Ki67 (Fig. R2-1 and R2-2). While I am personally not convinced that radial morphology is sufficient to identify quiescent NSC, the authors have also looked at this additional aspect allowing the readers to decide by themselves the significance of radial morphology with regard to stemness. Including many new experiments, and a substantial revision of the text, the study is now substantially expanded and thorough with a broader significance, novelty and interest.

2nd Authors' Response to Reviewers 23rd Oct 2020

The authors performed the requested changes.

2nd Revision - Editorial Decision 9th Nov 2020

Editor accepted the manuscript.

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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 χ2 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)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Cellular and histological analyses were performed at least in triplicates or more, as specificed in the text. For behavioral experiments, sample size was determined at least 9 animals per group (Effect size calculates as 1.5, alpha error + 0.05 and beta error = 0.8).
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	See above.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
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.	Most of the Experiments are genotype-dependent, so animals were distributed accordingly. All of them received the same treatment, independent of their genotype, to minimize bias. For behavior, the experimenter was blind throught the test and only notificed the genotype after data are collected.
For animal studies, include a statement about randomization even if no randomization was used.	Behavioral test are mostly automated and the experimenter and the analyzed person were independent. Mice of either genotype were couterbalanced that sent to behavioral platfrom with only with cage and ID numbers. At the end of behavioral tests and finalization of analyses, genetyped were matched to each recored data.
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.	Behavior experiments were blind as discribed above. In histology, all EYFP+ cells in the DG were assessed, and the investigators were blind to genotype.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Behavior experiments were blind as discribed above.
5. For every figure, are statistical tests justified as appropriate?	Performed statistical analyese are described in the method and figure legends
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Performed statistical analyese are described in the method and figure legends
Is there an estimate of variation within each group of data?	Variability was statistically assessed as indicated in the main text and methods

Is the variance similar between the groups that are being statistically compared?	Standard error or standard deviation was reported in all the figures.

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).	A table specifying catalog number, concentration and company of all the anibodies used in the study was provided as supplementary materials
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	n/a

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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.	Species, strain, gender, age and husbandry conditions of animals used in the study are included in the main text and the method.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Animal procedures were performed according to the official regulation and approved by IACUC of the salk institute or the Saxony local authority (TVA2/2019).
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E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
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.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
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15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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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	GSE156156
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).	N/A
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G- Dual use research of concern

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