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Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging

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(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. The original formatting of letters and referee reports may not be reflected in this compilation.)

Editor: Bernd Pulverer

1st Editorial Decision 08 November 2013

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below. I apologize for the slow review process - in this case exacerbated by problems with recruiting a high level panel of referees in a timely manner, followed by delayed reports.

You will see that all three referees are in principle positively disposed about the study, although a number of important and realistic experimental revisions are suggested, in particular by referees 2 and 3. I point out the most critical issues below, although we expect a response to all points by additions and revisions where appropriate.

referee 1 points out that both the Sirt1/Sirt2 mechanism of action and the mechanism downstream of NAMPT in NSPC self renewal remain unexplained. While we would encourage additional experimental data in this area, this is not a requirement for resubmission.
Referee 1 does request a repeat of the Sirt1/2 experiment with specific inhibitors; it would be useful to add this data, but again this is not a precondition for resubmission.
Apart from multiple useful recommendations about improvement to the text, the referee also points out that other markers should be considered, namely PdgPra, Sox10, NKx22.

referee 2 points out that neurogenesis and proliferation in the NMPT 'rescue' experiment should be measured directly.
The referee suggests to potentially remove the oligodendrocyte data, but we would prefer retention of this data.

The referee points out that the related literature needs to be discussed better

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referee 3 also takes issue with the in vivo data:
S/he request to show which cell populations are missing NAD exactly and also in which cells is NAMPT exactly deleted in the iNSPC-nampt-KO mice.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
http://www.nature.com/emboj/about/process.html

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.

I should also note that referee 3 commented that s/he agrees with referee 2's points to further the understanding of Nampt in ageing based on assessing neurogenesis and proliferation in the NMN treated mice. We appreciate that this may be a considerable experimental effort and we are prepared to discuss your plans for these experiments upfront if you prefer.

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

REFEREE REPORTS

Referee 1

This study addresses the mechanisms underlying the decrease of number and functions of neural stem/progenitor cells (NSPCs) residing in the mouse hippocampus during aging.
The rationale of this investigation is multi-faceted:
i) the number of proliferating NSPC in the subgranular zone (SGZ) of the dentate gyrus is known to decline dramatically during the first months of life until being absent by 24 months of age;
ii) earlier studies by several groups, and particularly by the Imai's group, demonstrated that in multiple peripheral tissues and organs aging is accompanied by significantly decreased intracellular levels of NAD+; this feature impacts on NAD+-driven cellular and organismal functions, including longevity, in several organisms;
iii) in mammals, the rate-limiting enzyme in NAD+ biosynthesis from its precursor nicotinamide is nicotinamide phosphoribosyltransferase (Nampt) which is strongly expressed in the hippocampus. Nampt catalyzes the formation of nicotinamide mononucleotide (NMN). Interestingly, in cells and tissues where Nampt activity is inhibited by pharmacological compounds (e.g., FK866), NMN is able to rescue the consequent drop of intracellular NAD+ and to counteract impairment of NAD+-mediated cell functions;
iv) in mice, Yoshino and Imai (Cell Metabolism (2011) 14:528-536) recently demonstrated that in multiple peripheral tissues aging is characterized by a significant decline of intracellular NAD+, and that this fact is causally related to a parallel decline of Nampt activity.
Based on these data, the study aimed at elucidating the role played by Nampt in the functional deficiencies of NSPCs, related to aging, that take place in the mouse hippocampus. To address such a complex issue, several converging approaches were designed and followed: the most remarkable

one was the combination of experimental strategies to explore, both in vivo and in vitro, whether and how NSPCs-specific deletion of Nampt affects the levels of intracellular NAD+ and accordingly the decision of NSPCs of self-renewal vs differentiation to specific cell lineages, notably to oligodendrocytes. Altogether, this major aim seems to have being achieved and the results reported in the manuscript demonstrate that Nampt deficiency in adult NSPCs is a valuable model system for investigating the decline of NSPCs functional properties during aging.
Salient and remarkable outcomes of the present study are the following:
a) Aging is accompanied by a decrease of Nampt-mediated NAD+ biosynthesis in murine hippocampus, where this decrease occurs mostly in the NSPCs of the SGZ of the dentate gyrus.
b) Genetic deletion of Nampt in NSPCs impairs NSPC proliferation and self-renewal in vivo.
c) Inhibition by FK866 of Nampt in neurospheres from the hippocampi (as an in vitro NSC culture model) decreases NAD+ biosynthesis in a NMN-rescuable way. This effect is paralleled by a decrease of the NSPC pool and proliferation.
d) Genetic ablation of Nampt in neurospheres results in a 73% decrease of intracellular NAD+, that is rescued by NMN. Concomitantly, Nampt- cells show a considerably decreased number and size, indicating reduced proliferation, compared with Nampt+ cells.
e) Genetic ablation of Nampt impairs the formation of Oligodendrocyte Precursor Cells (OPC) in vitro. This fact was related to a negative role, downstream of Nampt deficiency, mediated by Sirtuin1 and Sirtuin2 on oligodendrogenesis. However, the mechanisms of Sirt1 and Sirt2 are as yet elusive.
f) Genetic ablation of Nampt in adult NSPCs impairs NSPC self-renewal and differentiation toward oligodendrocytes in response to a challenge in vivo represented by the cuprizone model of demyelination and remyelination.
These findings are very interesting and novel. In addition, their biological significance is outstanding, because they suggest a mechanism-based possibility of increasing NAD+ biosynthesis and of upgrading cell functions in NSPCs. This may open new ways of counteracting age-related brain decline or of repairing brain damage. The experiments are of high scientific and technical quality, as expected by a group able to build up appropriate animal models and to investigate them deeply, using elegant and sound technologies.
Nevertheless, it is felt that the manuscript requires extensive revision with respect to two main weaknesses: i) lack of clarity, especially for a non-specialized audience; ii) related to this, attempts at interpreting the mechanisms of all experimental results, some of which remain in fact unexplained, with consequent overburden of the text.

Major criticisms:
1) The field of the study is related to cell biology of stem cells, specifically of NSPCs. A number of acronyms may be unfamiliar to several readers, especially as concerns markers of specific cells subpopulations and of functions thereof. As a suggestion, I believe that the Materials and Methods section should contain a dedicated paragraph indicating all markers analyzed and stating their cell and functional specificities. The addition of flow sheets of processes of proliferation/differentiation, similar to that reported in Fig. 6A, would help avoid confusion. An example of this is the failure to indicate the cell type specificity of the markers in the legend to Supplementary Figure 1. Another example is observed at p. 8, where the reader is presented with Type 1 and Type 2 NSPC markers which might be unknown to non-experts in the field. Again, at p. 9, the identity and the meaning of Ki67 and of doublecortin should be defined.
2) Please clarify the interpretation (p. 10, lines 10, 11) of quiescence of NSPCs at the stage of Type 2a cells. The results described at lines 6-8 do not lead to the obvious conclusion of this quiescence and more details are required. Similarly, at p. 11 (top part), the conclusion, even if presented as a possibility, of "preservation of Type 1 NSC population by preventing terminal fate decision" should be defined and supported more clearly.
3) P. 12, bottom. Description of Figure 4E should include not only the down-regulation of Cyclins E and A, but also a comment on the decrease in E2F1 expression which occurs following Nampt ablation. Otherwise, the finding appears abruptly in the Discussion (p. 22-23) and has no obvious mechanistic correlation with the stalling of NSPCs at G0/G1.
4) The experiments illustrated in Figure 6 and in Supplementary Figure 5 clearly demonstrated that genetic ablation of Nampt in neurospheres impairs formation of oligodendrocyte precursor cells (OPC) in vitro. However, they also yielded unexpected results as concerns the role of Sirt1 and Sirt2 in mediating oligodendrocyte differentiation from NSPCs. Indeed, while ablation of either single sirtuin failed to affect oligodendrogenesis (except for a partial reduction of O4+ immature oligodendrocytes in the Sirt1- neurospheres), pharmacological inhibition of Sirt1 or of Sirt2

completely suppressed oligodendrocyte formation (O+ cells, Figure 6D). To complicate the picture further, Sirt1/Sirt2 double-knockout neurospheres (Sirt1/2 DKO) were unable to form oligodendrocyte lineage cells, which led the Authors to postulate redundant roles of the two sirtuins in the regulation of oligodendrogenesis.
Concerning these experiments, I would suggest the following changes:
4a) Perform other experiments with addition of both inhibitors of Sirt1(EX527) and of Sirt2(AGK2), to compare the effects of their combination on oligodendrocyte formation with those produced in Sirt1/Sirt2 DKO neurospheres. These experiments might indicate differential roles of Sirt1/Sirt2 proteins, other from those of Sirt1/Sirt2 enzymatic activities.
4b) Check whether the marker "Olig1" displayed on the x axis of both Panel F and G of Figure 6 is correct or stands instead for "Olig2", as displayed in Panel A and elsewhere in the manuscript. In any case, please comment adequately in the text (at p. 17, bottom) what this specific result means, in the general context of the other markers, i.e. PdgPrα, Sox10, NKx22, when either Nampt or both Sirt1 and 2 are genetically ablated.
4c) Provide more details in the Discussion (p. 23, lines 6-14) on the possible links between Cyclin A, Cyclin E and p21. An alternative possibility might be to delete this part of the Discussion, which is inconclusive as it is.
4d) The part of the Discussion (p. 24, lines 10-19, and p. 25, lines 1-5) trying to explain the mechanisms accounting for "redundancy", i.e. reciprocal compensation between Sirt1 and Sirt2, in promoting NSPCs oligodendrocyte lineage fate decisions, is quite difficult to follow. The reported literature findings and the claim of their context dependency increase the degree of confusion and should be omitted for the sake of clarity. On the other hand, also the mechanisms downstream of Nampt that underlie NSPCs self-renewal and proliferation remain unexplained and require further investigations in the future.
4e) Please modify the general scheme depicted in Figure 8, downstream of Sirt1-Sirt2, and the legend to this Figure. By the way, what do the green and red rectangles below Sirt1 and Sirt2 represent?
5) The part of Results describing the experiments with the cuprizone model of demyelination as an insult in vivo is exceedingly complex and difficult to catch. First, the related reference (Skripuletz et al. 2011) reported in the Materials and Methods section, should be repeated under Results. Second, the experiments shown in Supplementary Figure S6C-G should be described in more detail and with better clarity, in order to provide a more convincing interpretation of the results (p. 19, lines 1-6). Finally, this part of the Results should give more focus on the reasons for comparing the data between the SGZ (Supplementary Figure S6) and the corpus callosum (Figure 7)
6) The Discussion is too long. In addition to the changes suggested above, I beg to strongly recommend that the part at p. 25, lines 9-19, and at p. 26, lines 1-12, be drastically shortened. The reason is that the reader may be distracted from the main achievements of this timely and interesting manuscript, as a result of the wealth of literature data which do not help identify the many mechanisms which they relate to.

Minor criticisms:
1) The legend to Figure 1A should report a brief comment on the different pathways of NAD+ utilization, notably on sirtuins.
2) At p. 3, penultimate line, should read "...and they are almost completely absent...".
3) At p. 4, line 3, "that" should be deleted.
4) At p. 7, line 6, better: To assess in vivo colocalization...
5) At p. 9, lines 7 and 9, respectively, appropriate references to Ki67 and doublecortin would be helpful.
6) P. 10, lines 2-3 from bottom. The importance of this statement suggests it may be repeated and emphasized somewhere in the Discussion.
7) P. 11, lines 6-9. This statement is somewhat elusive and should be amplified and explained.
8) Panel A of Figure 6 should be supplemented with a time-scale, like for Panel B of Supplementary Figure S5.
9) In the References section, titles of papers should be made homogeneous (full vs abbreviated versions).

Referee 2

In this manuscript the authors found that Nampt expression levels are decreased in the SGZ of the hippocampus, leading to subsequently decreased levels of NAD levels in the hippocampus with aging. They go on to show that neural stem/progenitor cell (NSPC)-selective deletion of Nampt in vivo led to decreased NSPC proliferation and self renewal, and in vitro to an increase in quiescence cells as well as a decrease in NAD levels. They also show that deletion of Nampt reduced oligodendrogenesis in NSPCs both in vitro and in a demyelination model in vivo, and that this effect on oligodendrogenesis may be mediated through Sirt1 and Sirt2.
In summary this is an interesting study that presents novel data and will be of interest to the field. The manuscript is well written and the experimental design is straightforward. The effects of Nampt deletion on NSPC behavior in the adult hippocampus are convincing. Given recent data indicating an important role for cell metabolism to regulate proliferative activity and differentiation of somatic stem cells this paper will add to this emerging field.

However, the study has several shortcomings in its present form that should be addressed:
MAJOR CONCERNS
- A major concern is claimed connection with aging. The authors merely show correlative data for aging that they can mimic in some respects with Nampt knockout. However, the only true test they do for Nampt's role in aging is the 300mg/kg treatment of NMN (to increase NAD+ levels) for 12 months, where they report an increase in radial glial nestin+ cells that represents their rescue. They do not show, however, an effect on proliferation, and they do not report what happens to neurogenesis levels. As the typical NSPC aging effects in the hippocampus are a decrease in proliferation and a decrease in neurogenesis, it would be important to show that and they do not. Thus, the authors need to expand this part of their analyses and add measures of "net neurogenesis", e.g., at least using doublecortin numbers as a read out.
- The oligodendrocyte part of the manuscript is somewhat disconnected to the aging story (and not fully convincing; e.g., the origin of the cells in the subcallosal region from the SGZ of the DG is questionable; others, e.g., Kronenberg et al 2007 Brain Struct Funct). This is overall interesting data but they do substantially increase the key finding of the manuscript. (The authors may even consider taking out this part).
- The role of Nampt for oligodendrocyte differentiation (and the - previously described roles for Sirts in oligo differentiation by Prozorovoski and colleagues, as cited by the authors) is extensively discussed, as is the potential cellular mechanisms associated with aging. However, their discussion regarding the role that regulation of metabolism plays for somatic stem cell behavior and more specifically how NAD+ levels affect this is not "complete". The authors should discuss their findings in light of recent studies that identified a role for metabolism in somatic (and NSPC) stem cell control (e.g., Stoll et al 2011 Stem Cells; Ito et al 2012 Nature med; Knobloch et al 2013 Nature and others). This part of the discussion should be improved.

ADDITIONAL CONCERNS
Pg. 6 - "quantifying Nampt immunoreactivity in the SGZ of the DG..."
Pg. 6 - "almost all NeuN+ granule neurons expressed Nampt..." and pg. 7 "many intensely Nampt immunoreactive cells... did not express NeuN." These descriptive words seem to be in conflict, and this should really be expressed quantitatively. In the figure S1, not all NeuN cells have Nampt, and the image you are showing clearly does not look like "almost all." Also, your example picture where the cell doesn't have NeuN does not look like its Nampt is any brighter than the others. Again, this all needs to be quantified.
Fig. S1 - Olig2 and Ki67 example, some have Nampt, some don't. This really doesn't tell much and requires quantification.
In Fig. 2D, it may be more informative to do co-stainings that may label that types of cells that are expressing Nampt (For example Ming and Song's 2011 review) instead of single staining.
In Fig. 2E, I don't feel it is necessary to have the repeat lanes of neurospheres and adult HCs twice. It just makes it confusing.
In Fig. 3E legend, you say "per unit area of the dentate gyrus," yet in the text you say SGZ. Please be specific.
Pg. 8 - "Having seen specific enrichment of Nampt expression in NSPCs..." You also said before that it was in most NeuN cells - thus it is really not specific.
Pg. 8 - "Immunohistochemistry... undetectable Cre activity..." You did not show immunohistochemistry in this paper, only PCR. I don't think it is necessary, but it should be labeled (data not shown), or not put in at all.

In Supp. Fig. S2A - The labels are confusing - On top of the images should be like B - 28d post TAM, and on left , KO and Control as below in B.
In Supp. Fig. S2C - is one of the Tamoxifen mice not induced? If so, what does this mean?
In Sup. Fig. legend D-E - please identify in which mice you looked at the NestinGFP-positive cells.
In Fig. 3 B, C, D, E - please change y axis to say per SGZ are instead of DG area and keep this consistent also in 3I, S2F, and S2H.
Fig. 3I - please add more labels to the x axis of this graph instead of just 100 and 300.
Pg. 9 - "Incorporation of BrdU and the population of Ki67+ proliferating... were also significantly decreased." Ki67 was not significant.
In Fig. 4C - This example DMSO + NMN looks like there are less neurospheres than DMSO alone, while the numbers in B suggest that it should have the same number as in DMSO alone.
Pg. 10 - "reduced levels of colocalization of GFP with radial..."
In Supp. Fig. S4F - Why does one blot show less dividing cells? (no PCNA and Ki67)
Pg. 11 - "Having shown that NAD+ levels and Nampt expression in the SGZ..." The NAD+ data are not specific to the SGZ.
In Supp. Fig. S5H, I - It would be nice to have an overview picture of Sirt2 expression in the DG (costained with an NSPC marker) to determine a percentage of the cells that are Sirt2 positive, as opposed to just a zoomed image.
In Fig. 5C - please change y axis to "Neurosphere Diameter (AU)"
In Fig. 5E - There are only 2 neurospheres per well? You say you plate 100 cells/ml - but how many ml/well? What size is the well? Better to describe how many cells you plate/well and then report in the graph how many neurospheres you measure per well.
Fig. 6D, why did Olig2 numbers increase with the inhibitor of Sirt2? There is no discussion of this in the text.
Pg. 23 - "E2F1 expression that we observed upon Nampt ablation may explain this phenomenon." Where is this study done? Is there a citation or a figure?
Pg. 31 - "(FGF, 10ng/ml, R&D, 233-fb)"
Pg. 32 - "All tissue sections were (please remove were) and cells were incubated..."
In Methods section, the abbreviation 10m, for example, does not appear to represent 10 minutes, but rather looks like 10 meters. Please use min for the abbreviation for minutes instead of m.
Instead of "Blow up"' in Sup. Fig. legend 1B - please use zoom.

Referee 3

In this paper, Stein and Imai describe that Nampt and NAD+ play an important role in maintaining neural stem/progenitor cells and regulating their differentiation in the adult mouse brain. They showed that the deletion of Nampt in Nestin-CreERT2 expressing cells reduced the number of proliferating cells and Dcx positive newborn neurons in the hippocampal DG. Suppression of cell cycle in neurosphere-forming cells (neural stem/progenitor cells) by the inactivation of Nampt was confirmed in vitro by using the Nampt inhibitor FK866 or by deleting Nampt conditionally with Ade-Cre infection, which was rescued by the addition of NMN, a Nampt product. The levels of NAD+ and Nampt expression decline with age and, most strikingly, a long term (12 mo) administration of NMN suppressed the reduction of radial Nestin+ cells (neural stem cells) in the DG, although it did not affect the number of Ki67+ proliferating cells in the DG. The authors also showed that oligodendrocyte differentiation was impaired by deleting Nampt or the NAD+ targets Sirt1 and Sirt2 in neurosphere-forming cells. Nampt deletion also suppressed the emergence of newborn oligodendrocytes in the corpus callosum in response to a demyelination and remyelination treatment.

These findings on the functions of Nampt in adult NSCs are novel and of great interest to the researchers in the field. However, although the in vitro studies were performed well, some critical information is missing in the in vivo studies. Since adult neural stem cells tend to lose their characteristics by being cultured in vitro (e.g. when they are cultured as neurospheres in the presence of growth factors), the authors should provide more basic information in vivo as detailed below.

Major points
1. Since the reduction of Nampt (and NAD+) in adult neural stem cells during aging is a central issue in this paper, they should show in which cell populations the expression of Nampt is reduced

with age in the hippocampal DG. They should determine the immunological signal intensity of Nampt in radial NSCs, TAPs, NBs, neurons and astrocytes and show their changes with age in Fig. 1.
2. Along the same line, the authors should determine in which cell populations Nampt is deleted in the iNSPC-Nampt-KO mice in Fig. 3 to support their main conclusion "Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging" (the title of this paper). Since Nestin-CreERT2 protein may stay in the progeny of neural stem cells (i.e. TAPs, NBs etc), the deletion of Nampt may take place in these cells as well. Therefore, the reduction of Nampt should be determined in NSCs and their progeny in iNSPC-Nampt-KO mice in Fig. 3.

Minor points
3. The % of cell type marker positive cells among GFP+ cells in iNSPC-GFP mice after tamoxifen injection should be examined in Supplementary Fig. 2.
4. Regarding Fig. 3H, the authors conclude that "differentiated cell lineage decisions were unaltered (by iNSPC-Nampt-KO) under basal conditions". However, this seems to be an overstatement since 72 h is too short to judge it.
5. Due to the rapid clearance of dead cells, the absence of caspase3 positive cells cannot rule out the occurrence of cell death. Therefore, "cell death does not appear to be a causative factor in these effects" and "loss of Nampt activity specifically caused a G1/S stall without causing appreciable cell death in NSPCs" may be overstatements.
6. The authors did not find a defect in oligodendrogenesis in the SGZ of iNSPC-Nampt-KO mice in vivo. If the authors would like to pursue basal oligodendrogenesis from adult NSCs in vivo, SVZ would be a better place to look at than SGZ.

1st Revision - authors' response 14 February 2014

To Referee 1

We are very glad to know that this referee considers our study “very interesting and novel” and its biological significance “outstanding”. We also appreciate many constructive suggestions from this referee to improve the clarity of our terminology and interpretation through the manuscript. We incorporated all suggestions and addressed all criticisms from this referee as described below:

General comments:
The manuscript requires extensive revision with respect to two main weaknesses:
i) lack of clarity, especially for a non-specialized audience;
ii) attempts at interpreting the mechanisms of all experimental results, some of which remain in fact unexplained, with consequent overburden of the text.

As described in detail below, we followed this referee’s constructive suggestions and made extensive revisions to our manuscript and figures. For example, we added flow sheets for proliferation/differentiation markers to Figures 3, 5, 7, and Supplementary Figure 1 and made all necessary textual changes. We believe that the revised manuscript is now significantly improved and less overbearingly interpretive.

Major criticisms:
1) The field of the study is related to cell biology of stem cells, specifically of NSPCs. A number of acronyms may be unfamiliar to several readers, especially as concerns markers of specific cells subpopulations and of functions thereof. As a suggestion, I believe that the Materials and Methods section should contain a dedicated paragraph indicating all markers analyzed and stating their cell and functional specificities.

In the Reagents section of Materials and Methods, we added the purpose and the cell type specificity of the antibodies used in this study.

The addition of flow sheets of processes of proliferation/differentiation, similar to that reported in Fig. 6A, would help avoid confusion. An example of this is the failure to indicate the cell type specificity of the markers in the legend to Supplementary Figure 1.

Following this great suggestion, we added flow sheets to Figures 3, 5, 7, and Supplementary Figure 1 (New Figures 3B, 5H, 7E, and S1D). We also added the cell type specificities to the Supplementary Figure 1 legend.

Another example is observed at p. 8, where the reader is presented with Type 1 and Type 2 NSPC markers which might be unknown to non-experts in the field.

We clarified the text by changing the sentence to, "To verify that the NestinGFP+ population consisted of NSPCs, we costained for the NSPC markers Sox2 and Gfap." (page 9, lines 5-6)

Again, at p. 9, the identity and the meaning of Ki67 and of doublecortin should be defined.

We clarified the text by changing the sentence to, "Indeed, incorporation of BrdU and the population of proliferating cells [Ki67+ (von Bohlen und Halbach, 2011)] were also decreased by 22% and 35%, respectively (Figure 3D-E). Consistent with this defect in the NSPC pool and proliferation, the pool of newborn neurons [doublecortin, Dcx+, (von Bohlen und Halbach, 2011)] was also significantly decreased by 26% in the DG (Figure 3F-G)." (page 9-10)

2*) Please clarify the interpretation (p. 10, lines 10, 11) of quiescence of NSPCs at the stage of Type 2a cells. The results described at lines 6-8 do not lead to the obvious conclusion of this quiescence and more details are required.*

We clarified the text by changing the sentence to, "The lack of increase in colocalization of BrdU with cell type specific markers may imply that a larger percentage of BrdU+ cells have failed to differentiate in iNSPC-Nampt-KO mice. iNSPC-Nampt-KO NSPCs could have stalled during differentiation after losing Nestin expression." (pages 10-11)

Similarly, at p. 11 (top part), the conclusion, even if presented as a possibility, of "preservation of Type 1 NSC population by preventing terminal fate decision" should be defined and supported more clearly.

We clarified the text by changing the sentence to, "The age-related depletion of the NSPC pool is thought to be caused by an increase in terminal fate decisions relative to self-renewal fate decisions (Encinas et al, 2011). Thus, it is possible that NMN administration maintains the NSPC pool by preventing the age-associated increase in terminal fate decisions." (pages 11-12)

3) P. 12, bottom. Description of Figure 4E should include not only the down-regulation of Cyclins E and A, but also a comment on the decrease in E2F1 expression which occurs following Nampt ablation. Otherwise, the finding appears abruptly in the Discussion (p. 22-23) and has no obvious mechanistic correlation with the stalling of NSPCs at G0/G1.

We revised the description of qRT-PCR results as follows: "Analysis of specific gene changes by qRT-PCR revealed that cyclins E and A, the two cyclins required for cellular progression from G1 to S, as well as their upstream transcriptional regulator E2F1 (Wong et al, 2011), were the primary cell cycle factors affected by this treatment (Figure 4E). These alterations in gene expression indicated that reducing Nampt activity stalls NSPCs at G0/G1. Supporting this notion, FACS analysis of neurospheres demonstrated that FK866 treatment increased the proportion of NSPCs in G0/G1 and decreased the proportion in S phase (Figure 4F)." (page 13)

4) The experiments illustrated in Figure 6 and in Supplementary Figure 5 clearly demonstrated that genetic ablation of Nampt in neurospheres impairs formation of oligodendrocyte precursor cells (OPC) in vitro. However, they also yielded unexpected results as concerns the role of Sirt1 and Sirt2 in mediating oligodendrocyte differentiation from NSPCs. Indeed, while ablation of either single sirtuin failed to affect oligodendrogenesis (except for a partial reduction of O4+ immature oligodendrocytes in the Sirt1- neurospheres), pharmacological inhibition of Sirt1 or of Sirt2 completely suppressed oligodendrocyte formation (O+ cells, Figure 6D). To complicate the picture further, Sirt1/Sirt2 double-knockout neurospheres (Sirt1/2 DKO) were unable to form oligodendrocyte lineage cells, which led the Authors to postulate redundant roles of the two sirtuins in the regulation of oligodendrogenesis. Concerning these experiments, I would suggest the following changes:

4a) Perform other experiments with addition of both inhibitors of Sirt1(EX527) and of Sirt2(AGK2), to compare the effects of their combination on oligodendrocyte formation with those produced in

Sirt1/Sirt2 DKO neurospheres. These experiments might indicate differential roles of Sirt1/Sirt2 proteins, other from those of Sirt1/Sirt2 enzymatic activities.

We appreciate the referee's insightful suggestion. However, we decided not to pursue this particular experiment because of the potential toxicity that this combinatorial approach would produce. Because we do not know whether and how these two drugs cross-interact in cells, it would be very difficult to interpret the results from this experiment. Therefore, we prefer to use genetic ablation, instead of inhibitors, whenever possible.

4b) Check whether the marker "Olig1" displayed on the x axis of both Panel F and G of Figure 6 is correct or stands instead for "Olig2", as displayed in Panel A and elsewhere in the manuscript. In any case, please comment adequately in the text (at p. 17, bottom) what this specific result means, in the general context of the other markers, i.e. PdgPrα, Sox10, NKx22, when either Nampt or both Sirt1 and 2 are genetically ablated.

For Figures 6F and G, Olig1 is the correct gene denoted. To further clarify the description of these results, we added the following text, "Olig1 expression showed no change or slight reduction by these genetic ablations, potentially due to its lesser expression in NSPCs relative to Olig2 (Ligon et al, 2007) and predominant roles in oligodendrocyte maturation and remyelination rather than specification (Arnett et al, 2004; Lu et al, 2002)." (page 18)

4c) Provide more details in the Discussion (p. 23, lines 6-14) on the possible links between Cyclin A, Cyclin E and p21. An alternative possibility might be to delete this part of the Discussion, which is inconclusive as it is.

We agree with this referee that it is important to add more detailed discussions on the connection between Cyclins A and E and p21. Therefore, we added the following text on page 25, "Because we also observed downregulation of E2F1 expression, which transcriptionally regulates Cyclin E, it is very likely that the downregulation of E2F1 contributes to the downregulation of Cyclin E. Interestingly, we also see upregulation of p21 upon loss of Nampt. p21 represses E2F activity and E2F activation is required to eliminate a p21-mediated block in cell cycle entry in late G1 (Polager & Ginsberg, 2009; Wong et al, 2011). Thus, the upregulation of p21 that we see upon loss of Nampt may also contribute to the downregulation of E2F/Cyclin E activity."

4d) The part of the Discussion (p. 24, lines 10-19, and p. 25, lines 1-5) trying to explain the mechanisms accounting for "redundancy", i.e. reciprocal compensation between Sirt1 and Sirt2, in promoting NSPCs oligodendrocyte lineage fate decisions, is quite difficult to follow.
The reported literature findings and the claim of their context dependency increase the degree of confusion and should be omitted for the sake of clarity. On the other hand, also the mechanisms downstream of Nampt that underlie NSPCs self-renewal and proliferation remain unexplained and require further investigations in the future.

We agree with the referee's criticism and therefore shortened this discussion as follows: "Previous work has generated contradictory conclusions in the function of Sirt1 in NSPC differentiation (Hisahara et al, 2008; Prozorovski et al, 2008; Rafalski et al, 2013; Saharan et al, 2013; Wang et al, 2011b; Zhang et al, 2011) and in the function of Sirt2 in oligodendrocyte differentiation (Ji et al, 2011; Li et al, 2007). Yet, these contradictory findings could be explained by compensatory activity between Sirt1 and Sirt2. Since Sirt1 is highly expressed in NSPCs (Hisahara et al, 2008; Prozorovski et al, 2008; Saharan et al, 2013) and Sirt2 is highly expressed in oligodendrocytes (Li et al, 2007; Tyler et al, 2011), it is possible that the activities of these two proteins trade off during oligodendrocyte maturation." (page 26)

As the referee points out, the mechanisms downstream of Nampt that underlie NSPCs self-renewal and proliferation remain unexplained. However, we feel that explaining these mechanisms is beyond the purview of this paper. Our future work will follow up on these issues.

4e) Please modify the general scheme depicted in Figure 8, downstream of Sirt1-Sirt2, and the legend to this Figure. By the way, what do the green and red rectangles below Sirt1 and Sirt2 represent?

We changed the green and red rectangles and revised the figure legend accordingly.

5) The part of Results describing the experiments with the cuprizone model of demyelination as an insult in vivo is exceedingly complex and difficult to catch. First, the related reference (Skripuletz et al. 2011) reported in the Materials and Methods section, should be repeated under Results.

We added the requested reference on page 19, line 19.

Second, the experiments shown in Supplementary Figure S6C-G should be described in more detail and with better clarity, in order to provide a more convincing interpretation of the results (p. 19, lines 1-6).

Following this suggestion, we rewrote this paragraph as follows:

"Cuprizone feeding did not alter the total number of NestinGFP+ cells present in the iNSPC-GFP DG (Supplementary Figure S6A-C), suggesting that NSPC proliferation was unaltered. On the other hand, cuprizone fed mice exhibited an increased percentage of NestinGFP+ cells that colocalized with the NSPC markers Nestin+ (from 13 to 35%) and Gfap+ (from 19 to 41%), suggesting that cuprizone treatment prevented SGZ NSPCs from terminally differentiating and instead resulted in their retention of NSPC characteristics, which could occur through increased self-renewal decisions and/or quiescence (Supplementary Figure S6D-E). To assess if the NestinGFP-marked NSPCs had differentiated into oligodendrocyte lineage cells in response to cuprizone, we next assessed colocalization between NestinGFP and oligodendrocyte specific markers, Sox10 and APC. However, the SGZ did not substantially produce oligodendrocytes even in response to demyelination (Supplementary Figure S6F-G). Thus, SGZ NSPCs do not appear to be the main mediators of short-term remyelination in the hippocampus." (page 20)

Finally, this part of the Results should give more focus on the reasons for comparing the data between the SGZ (Supplementary Figure S6) and the corpus callosum (Figure 7)

We added the following rationale to page 20, "While cuprizone treatment affects the hippocampus, its principle target of myelin injury is the corpus callosum (CC) (Doucette et al, 2010; Gudi et al, 2009; Norkute et al, 2009; Skripuletz et al, 2011). Under basal conditions and particularly during demyelination, NSPCs can proliferate to generate oligodendrocytes which integrate into the corpus callosum (Colak et al, 2008; Hack et al, 2005; Jablonska et al, 2010; Menn et al, 2006; Nait-Oumesmar et al, 1999; Picard-Riera et al, 2002; Soundarapandian et al, 2011). Thus, we assessed the fate decisions of migratory cells derived from the adult Nestin+ population in the subcallosal zone of the corpus callosum (Supplementary Figure S6A)." (page 20-21)

6) The Discussion is too long. In addition to the changes suggested above, I beg to strongly recommend that the part at p. 25, lines 9-19, and at p. 26, lines 1-12, be drastically shortened. The reason is that the reader may be distracted from the main achievements of this timely and interesting manuscript, as a result of the wealth of literature data which do not help identify the many mechanisms which they relate to.

We extensively reduced these sections of the discussion.

Minor criticisms:

1) The legend to Figure 1A should report a brief comment on the different pathways of NAD+ utilization, notably on sirtuins.

We added the requested text to the figure legend as follows, "While NAD^+ is commonly used in redox reactions, cells primarily require NAD^+ as a co-substrate for several families of enzymes, one of which is the sirtuin family of protein deacetylases. The sirtuin family includes Sirt1 and Sirt2, which cleave NAD^+ at its glycosidic bond, releasing ADP-ribose (Stein & Imai, 2012)."

2) At p. 3, penultimate line, should read "...and they are almost completely absent...".

We changed the text on the bottom of page 3 as follows, "and they are almost completely absent by 24 months of age."

3) At p. 4, line 3, "that" should be deleted.

We deleted "that" on the top of page 4.

4) At p. 7, line 6, better: To assess in vivo colocalization...

We added "*in vivo*" on page 7, line 6.

5) At p. 9, lines 7 and 9, respectively, appropriate references to Ki67 and doublecortin would be helpful.

We added the requested references on page 9.

6) P. 10, lines 2-3 from bottom. The importance of this statement suggests it may be repeated and emphasized somewhere in the Discussion.

We added the following text in the discussion on page 24, "Importantly for therapeutic purposes, this experiment and our finding that intraperitoneal injection of NMN substantially increases hippocampal NAD^+ levels within 15 minutes (Supplementary Figure S2G), strongly suggest that NMN can cross the blood-brain barrier."

7) P. 11, lines 6-9. This statement is somewhat elusive and should be amplified and explained.

We changed the text as follows, "The age-related depletion of the NSPC pool is thought to be caused by an increase in terminal fate decisions relative to self-renewal fate decisions (Encinas et al, 2011). Thus, it is possible that NMN administration maintains the NSPC pool by preventing the age-associated increase in terminal fate decisions." (pages 11-12)

8) Panel A of Figure 6 should be supplemented with a time-scale, like for Panel B of Supplementary Figure S5.

We added the requested the requested time-scale to Fig. 6A.

9) In the References section, titles of papers should be made homogeneous (full vs abbreviated versions).

We reformatted the references according to the format requested by EMBO Journal.

To Referee 2

We greatly appreciate very positive comments and many precise, constructive suggestions/criticisms from this referee. We addressed all criticisms from this referee as described below, which significantly improves our manuscript.

MAJOR CONCERNS

- A major concern is claimed connection with aging. The authors merely show correlative data for aging that they can mimic in some respects with Nampt knockout. However, the only true test they do for Nampt's role in aging is the 300mg/kg treatment of NMN (to increase NAD+ levels) for 12 months, where they report an increase in radial glial nestin+ cells that represents their rescue. They do not show, however, an effect on proliferation, and they do not report what happens to neurogenesis levels. As the typical NSPC aging effects in the hippocampus are a decrease in proliferation and a decrease in neurogenesis, it would be important to show that and they do not. Thus, the authors need to expand this part of their analyses and add measures of "net neurogenesis", e.g., at least using doublecortin numbers as a read out.

We appreciate this particular criticism from Referee 2. Whereas we know that it is very hard technically and practically to prove a strict cause-effect relationship for aging in general, we conducted the best possible experiment we could do in this study, namely, a year-long *in vivo* enhancement of NAD^+ by NMN administration. Therefore, we completely agree with this referee's opinion that we should show the number of Dcx+ cells in the SGZs of NMN-treated mice. We added the result to Supplementary Figure 2I and described it in the text as follows: "While not statistically significant, the population of newborn neurons (Dcx+) trended to increase (Supplementary Figure S2I)."

- The oligodendrocyte part of the manuscript is somewhat disconnected to the aging story (and not fully convincing; e.g., the origin of the cells in the subcallosal region from the SGZ of the DG is questionable; others, e.g., Kronenberg et al 2007 Brain Struct Funct). This is overall interesting data but they do substantially increase the key finding of the manuscript. (The authors may even consider taking out this part).

We have decided to retain the oligodendrocyte portion of the data for the following reasons. First, it has been reported that rates of remyelination decline with age due to slower recruitment of OPCs into areas of demyelination and slower differentiation of OPCs into remyelinating oligodendrocytes (Decker et al, 2002; Doucette et al, 2010; Sim et al, 2002). Therefore, our findings from the cuprizone experiment have a decent and important connection to age-associated pathophysiology, particularly in response to environmental insults. Second, we understand that there are groups that dispute the origin of the cells in the subcallosal zone. However, other studies have found that cells isolated from the subcallosal zone behave as neural stem cells *in vitro* and migrate into the corpus callosum to become oligodendrocytes *in vivo* (Seri et al, 2006). Moreover, SVZ NSPCs have been shown to be able to generate oligodendrocytes which integrate into the corpus callosum (Colak et al, 2008; Hack et al, 2005; Jablonska et al, 2010; Menn et al,

2006; Nait-Oumesmar et al, 1999; Picard-Riera et al, 2002; Soundarapandian et al, 2011). Our findings add new pieces of information to resolve this issue and also provide important insight into age-associated decline in insult-induced oligodendrogenesis *in vivo*.

- The role of Nampt for oligodendrocyte differentiation (and the - previously described roles for Sirts in oligo differentiation by Prozorovoski and colleagues, as cited by the authors) is extensively discussed, as is the potential cellular mechanisms associated with aging.
However, their discussion regarding the role that regulation of metabolism plays for somatic stem cell behavior and more specifically how NAD+ levels affect this is not "complete". The authors should discuss their findings in light of recent studies that identified a role for metabolism in somatic (and NSPC) stem cell control (e.g., Stoll et al 2011 Stem Cells; Ito et al 2012 Nature med; Knobloch et al 2013 Nature and others). This part of the discussion should be improved.

We greatly appreciate the referee's constructive suggestion. We added a discussion on the connection between stem cell metabolism and cell fate control with suggested references (pages 27-28).

ADDITIONAL CONCERNS

Pg. 6 - "quantifying Nampt immunoreactivity in the SGZ of the DG..."

We have added "SGZ of the" on page 6 to the sentence, "Consistent with this finding, quantifying Nampt immunoreactivity in the SGZ of the DG by both a thresholded level of Nampt intensity as well as a count of the number of thresholded Nampt+ cells demonstrated that 18 month-old mice exhibit 52-66% of the Nampt immunoreactivity present in 6 month-old mice (Figure 1C-E)."

Pg. 6 - "almost all NeuN+ granule neurons expressed Nampt..." and pg. 7 "many intensely Nampt immunoreactive cells... did not express NeuN."
These descriptive words seem to be in conflict, and this should really be expressed quantitatively. In the figure S1, not all NeuN cells have Nampt, and the image you are showing clearly does not look like "almost all."
Also, your example picture where the cell doesn't have NeuN does not look like its Nampt is any brighter than the others. Again, this all needs to be quantified.

Following this referee's constructive suggestion, we added the quantification to Supplementary Figure S1A and revised the text for further clarification. Indeed, the discrepancy is due to the extent of colocalization between Nampt and NeuN+ in the granule layer vs. the SGZ of the DG. All observed NeuN+ cells in the granule layer expressed Nampt, whereas ~80% of NeuN+ cells expressed Nampt in the SGZ of 6 month-old mice. A similar result is also shown in Figure 2F. We have thus clarified the text as well on pages 6-7: "Consistent with this finding, immunohistochemistry for Nampt and cell type specific markers revealed almost all NeuN+ neurons in the granule layer of the DG expressed Nampt, while almost no S100b+ glial cells did (Supplementary Figure S1A-E). However, we also noticed that many intensely Nampt immunoreactive cells along the SGZ of the DG did not express NeuN (Supplementary Figure S1B,E)."

Fig. S1 - Olig2 and Ki67 example, some have Nampt, some don't. This really doesn't tell much and requires quantification.

We added the requested quantification to Supplementary figure 1G and revised the text as follows: "Additionally, Ki67+ and Olig2+ cells along the SGZ also expressed Nampt (Supplementary Figure S1G-I)."

In Fig. 2D, it may be more informative to do co-stainings that may label that types of cells that are expressing Nampt (For example Ming and Song's 2011 review) instead of single staining.

We have extensively looked through Ming and Song's 2011 review, and cannot find methodology that differs from what we used here. In Fig. 2D, we report co-staining between Nampt and the specified markers. In Ming and Song's 2011 review, they suggest using the markers that we used as well as Nestin, Mash1, Dcx. We cannot use these antibodies with our Nampt antibody due to similar species and/or duel need for signal amplification which results in crosstalk after sequential staining. Nonetheless, we added an assessment of Nampt/NeuN colocalization to Figure 2F and Supplementary Figure 1E. We also found that Nampt co-localizes with Calretinin, but did not add this result since it provides the same conclusion as the NeuN data.

In Fig. 2E, I don't feel it is necessary to have the repeat lanes of neurospheres and adult HCs twice. It just makes it confusing.

We removed the extra two lanes from Figuire 2E.

In Fig. 3E legend, you say "per unit area of the dentate gyrus," yet in the text you say SGZ. Please be specific.

We changed the text from “SGZ” to “DG” in the paragraph on page 9-10.

Pg. 8 - "Having seen specific enrichment of Nampt expression in NSPCs..." You also said before that it was in most NeuN cells - thus it is really not specific.

We removed the word “specific” and changed the phrase to “Having seen enrichment of Nampt in NSPCs,”.

Pg. 8 - "Immunohistochemistry... undetectable Cre activity..." You did not show immunohistochemistry in this paper, only PCR. I don't think it is necessary, but it should be labeled (data not shown), or not put in at all.

We mentioned “immunohistochemistry” based on the data shown in Supplementary Figure S2A and B. To ameliorate the ambiguity, we changed the text on page 8 from “Immunohistochemistry and recombination PCR for NestinGFP confirmed that there was undetectable Cre activity in vehicle injected mice (Supplementary Figure S2A-C)” to “Immunohistochemistry and recombination PCR for NestinGFP confirmed that there was undetectable recombination present in vehicle injected mice (Supplementary Figure S2A, C).” (page 9).

In Supp. Fig. S2A - The labels are confusing - On top of the images should be like B - 28d post TAM, and on left , KO and Control as below in B.

Supplementary Figures S2A and S2B actually used different mouse lines. Thus, the same labels cannot be used. Supplementary Figure S2A compares oil-injected and TAM-injected iNSPC-GFP mice, whereas Supplementary Figure S2B compares TAM-injected iNSPC-GFP mice (Control) and TAM-injected iNSPC-Nampt-KO mice. To ameliorate the ambiguity, we added the following text to the figure legend, “A) Control iNSPC-GFP mice were treated with oil or TAM to ensure that there was no leaky NestinGFP reporter expression. B) iNSPC-Nampt-KO or iNSPC-GFP mice were treated with TAM.”

In Supp. Fig. S2C - is one of the Tamoxifen mice not induced? If so, what does this mean?

In lane 5 of previous Supplementary Figure S2C, the recombination band is fainter than other recombination bands in lanes 1, 4, and 7, but still present. Nonetheless, this depiction did not represent the overall success of tamoxifen-induced recombination. We apologize this misrepresentation in this particular figure. We replaced this panel with another of increased sample size and more robustness.

In Sup. Fig. legend D-E - please identify in which mice you looked at the NestinGFP-positive cells.

We changed the figure legend to read “D) Quantification of the percentages of NestinGFP-positive cells that also express NSPC (Sox2: n=190 cells from 7 mice; Gfap: n=208 cells from 7 mice) or neuronal (Dcx, NeuN, n=473 cells from 7 mice) markers in 3 to 6 month old iNSPC-GFP mice 7 days post initial TAM injection. E) Quantification of the percentages of NestinGFP-positive cells that also express Nampt in iNSPC-Nampt-KO and iNSPC-GFP mice in the DG at the indicated days post initial TAM injection (n= more than 350 cells from 7 mice).”

In Fig. 3 B, C, D, E - please change y axis to say per SGZ are instead of DG area and keep this consistent also in 3I, S2F, and S2H.

In previous Figures 3B-E, I, and Supplementary Figure2 2F and H (new Figures 3C-F, J, and Supplementary Figures 2F and H), data were all normalized by DG area, not SGZ area. Thus, we changed all references to these figures to be consistent with this. The DG is easily recognizable by Dapi staining, whereas the SGZ is a more ambiguous 2-3 cell layer thick zone lying between the SGZ and the hilus. Thus, we chose to normalize by DG area, not SGZ area, because it was easier to be consistent between samples.

Fig. 3I - please add more labels to the x axis of this graph instead of just 100 and 300.

We revised these labels for previous Figure 3I (new Figure 3J) as requested. We also made similar changes for Supplementary Figures 2H and I.

Pg. 9 - "Incorporation of BrdU and the population of Ki67+ proliferating... were also significantly decreased." Ki67 was not significant.

We removed the word "significantly" and changed the sentence as follows: "Indeed, incorporation of BrdU and the population of proliferating cells [Ki67+ (von Bohlen und Halbach, 2011)] were also decreased by 22% and 35%, respectively (Figure 3D-E)." (pages 9-10)

In Fig. 4C - This example DMSO + NMN looks like there are less neurospheres than DMSO alone, while the numbers in B suggest that it should have the same number as in DMSO alone.

We appreciate this referee's keen eyes. We replaced the picture for DMSO + NMN in Figure 4C with the one that better represents the results in Figure 4B.

Pg. 10 - "reduced levels of colocalization of GFP with radial..."

We believe that with this comment, this referee is referring to the statement, "iNSPC-Nampt-KO mice displayed significantly reduced levels of colocalization with radial Nestin+ cells (Figure 3H)." This assessment was done with colocalization of Nestin and BrdU, and thus we changed the text as follows: "iNSPC-Nampt-KO mice displayed significantly reduced levels of colocalization of BrdU with radial Nestin+ cells (Figure 3I),".

In Supp. Fig. S4F - Why does one blot show less dividing cells? (no PCNA and Ki67)

We mistakenly omitted to mention in the figure legend that the second blot is after 2 days of differentiation. We apologize this mistake. We better annotated the figure and added to the figure legend, "Neurospheres were grown under proliferation conditions (left blot) or differentiated for 2 days (right blot)." (This is now Figure S4E.)

Pg. 11 - "Having shown that NAD+ levels and Nampt expression in the SGZ..." The NAD+ data are not specific to the SGZ.

We changed the text on page 12 to "Having shown that NAD^+ levels in the hippocampus and Nampt expression in the SGZ decreased with age,".

In Supp. Fig. S5H, I - It would be nice to have an overview picture of Sirt2 expression in the DG (costained with an NSPC marker) to determine a percentage of the cells that are Sirt2 positive, as opposed to just a zoomed image.

We added an overview picture of Sirt2 expression in the DG costained with NestinGFP to Supplementary Figures 5E and F.

In Fig. 5C - please change y axis to "Neurosphere Diameter (AU)"

We changed the y axis as requested.

In Fig. 5E - There are only 2 neurospheres per well? You say you plate 100 cells/ml – but how many ml/well? What size is the well? Better to describe how many cells you plate/well and then report in the graph how many neurospheres you measure per well.

We added the requested information to the figure legend of Figure 5E as follows: "E) The number of neurospheres formed 7 days after plating dissociated cells at 100 cells/ml, 0.5 ml/well in 24-well plates (n=8 independent samples, 48-84 wells)." We also verified that the graph reports the number of neurospheres measured per well.

Fig. 6D, why did Olig2 numbers increase with the inhibitor of Sirt2? There is no discussion of this in the text.

We are also interested in this phenomenon. Sirt2 is expressed in oligodendrocytes (Harting & Knoll; Ji et al, 2011; Li et al, 2007; Southwood et al, 2007; Tyler et al, 2011), whereas Sirt1 is expressed in adult subventricular zone and hippocampal NSPCs (Hisahara et al, 2008; Iwahara et al, 2009; Prozorovski et al, 2008; Saharan et al, 2013). Thus, we predict that while Sirt1 and Sirt2 can function redundantly, Sirt1 may act before Sirt2 under normal conditions, due to downregulation of Sirt1 expression and upregulation of Sirt2 expression. If this is the case, loss of Sirt1 activity may stall cells as Nestin+, whereas loss of Sirt2 activity may stall cells as Olig2+. Nonetheless, this is still speculative, and unfortunately, we do not have enough space, due to character limitations, to fully discuss such a possibility. We feel that this interesting issue belongs to our future study.

Pg. 23 - "E2F1 expression that we observed upon Nampt ablation may explain this phenomenon." Where is this study done? Is there a citation or a figure?

We apologize that the statement might sound confusing. We changed this sentence as follows: "As E2F1-deficient mice have significantly reduced hippocampal NSPC death (Cooper-Kuhn et al, 2002), the decrease in E2F1 expression that we observed upon inhibition of Nampt may explain this phenomenon (Figure 4E)." (page 25)

Pg. 31 - "(FGF, 10ng/ml, R&D, 233-fb)"

We changed the text from "FGF (FGF, 10 ng/mL, R&D, 233-fb" to "fibroblast growth factor (FGF, 10 ng/mL, R&D Systems, 233-fb)." (page 33)

Pg. 32 - "All tissue sections were (please remove were) and cells were incubated..."
In Methods section, the abbreviation 10m, for example, does not appear to represent 10 minutes, but rather looks like 10 meters. Please use min for the abbreviation for minutes instead of m.

We changed all abbreviations of "m" to "min".

Instead of "Blow up"' in Sup. Fig. legend 1B - please use zoom.

We changed the text to "B) Zoom of boxed region shown in A)." (page 60)

To Referee 3

This referee considers our study "novel and of great interest to the researchers in the field". Nonetheless, this referee still recognizes several places to improve, particularly regarding *in vivo* analyses. We deeply appreciate all criticisms/suggestions from this referee. We addressed all of them and revised our manuscript and figures accordingly, as described below.

Major points
1. Since the reduction of Nampt (and NAD+) in adult neural stem cells during aging is a central issue in this paper, they should show in which cell populations the expression of Nampt is reduced with age in the hippocampal DG. They should determine the immunological signal intensity of Nampt in radial NSCs, TAPs, NBs, neurons and astrocytes and show their changes with age in Fig. 1.

We agree that this is a very important issue for our paper, although it is not easy to follow up such changes during aging. To address this issue, we decided to focus on Sox2+ NSPCs and NeuN+ neurons because 99% of Nampt+ cells are either Sox2+ or NeuN+. We compared percentages of the thresholded Nampt+ cells for colocalization with the neuronal marker NeuN and the NSPC marker Sox2 between 6 month-old and 18 month-old mice. Interestingly, we found that with age, the percentage of intensely Nampt immunoreactive cells that colocalized with NeuN increased slightly, whereas the percentage of intensely Nampt immunoreactive cells that colocalized with Sox2 decreased from 21% to 4%. Similarly, in the SGZ, the percentage of NeuN+ that expressed Nampt increased with age, while the percentage of Sox2+ cells that expressed Nampt decreased. These findings suggest that at least part of the decrease in Nampt expression in the SGZ with age is due to loss of Sox2+ NSPCs.

To show these important results, we added new Figure 2F and Supplementary Figures 1E-F. We also added a new paragraph that describes these results on page 8 in our revised manuscript.

2. The authors should determine in which cell populations Nampt is deleted in the iNSPC-Nampt-KO mice in Fig. 3 to support their main conclusion "Specific ablation of Nampt in adult neural stem cells recapitulates their functional defects during aging" (the title of this paper). Since Nestin-CreERT2 protein may stay in the progeny of neural stem cells (i.e. TAPs, NBs etc), the deletion of Nampt may take place in these cells as well. Therefore, the reduction of Nampt should be determined in NSCs and their progeny in iNSPC-Nampt-KO mice in Fig. 3.

We appreciate this insightful criticism from the referee. In Supplementary Figure S2D, we show that 61% of Sox2+ cells and 34% of radial Gfap+ cells coexpressed NestinGFP 7 days post tamoxifen, suggesting that the NestinGFP+ population consisted of NSPCs. In Supplementary Figure S2E, we also show that Nampt was deleted in the majority of cells marked by the NestinGFP reporter in iNSPC-Nampt KO mice. It is true that the Nestin-CreERT2 protein can stay in progeny of NSPCs. These cells were easily detectable by a 7-day labeling period. Seven days post injection of TAM, 95 ± 3% of NestinGFP+ cells were found in the SGZ, and 97 ± 1% of NestinGFP+ cells

had moderately intense GFP expression (n=473 cells from 7 mice). However, the remaining 3 ± 1% of NestinGFP+ cells had extremely strong GFP expression, were localized to the granule layer, and expressed NeuN. While this 3 ± 1% of NestinGFP+ cells is likely an artifact of the Nestin-CreERT2 protein staying in the progeny of NSPCs, this small population of cells is unlikely to affect any of the conclusions that we draw here.

Because of the character limitation for the main text, we were not able to add this argument to the main text. Therefore, we added the following sentence to the figure legend of Supplementary Figure 2D: "Separate from these SGZ-localized cell populations, 3 ± 1% of NestinGFP+ cells had extremely strong GFP expression, were localized to the granule layer, and expressed NeuN, likely due to residual CreERT2 protein left in the progeny of previously differentiated NSPCs."

Minor points

3. The % of cell type marker positive cells among GFP+ cells in iNSPC-GFP mice after tamoxifen injection should be examined in Supplementary Fig. 2.

We have re-analyzed the percentages of cell type marker positive cells among GFP+ cells in iNSPC-GFP mice and replaced this graph with the additional results of two other lineage markers (Dcx and NeuN) (new Supplementary Figure S2D).

4. Regarding Fig. 3H, the authors conclude that "differentiated cell lineage decisions were unaltered (by iNSPC-Nampt-KO) under basal conditions". However, this seems to be an overstatement since 72 h is too short to judge it.

We agree with this criticism and therefore changed the text as follows: "However, iNSPC-Nampt-KO mice exhibited normal levels of BrdU colocalization with neuronal (Dcx+), astrocytic (Gfap+) and oligodendrocytic (Olig2+) markers, indicating that alterations in differentiated cell lineage decisions were undetectable under basal conditions." (pages 10-11)

We chose the time point of 72h because at longer time points, astrocytes and oligodendrocytes will migrate away from the SGZ, thus making ambiguous whether the BrdU marked cells were generated from local OPCs and astrocytes or from SGZ NSPCs. We feel that coupling SGZ localization and BrdU+ cells provides a more accurate assessment of the actual production of SGZ NSPCs. Moreover, the 72h time point is reported to be sufficient for BrdU+ cells to express the markers we assessed (Encinas et al, 2011).

5. Due to the rapid clearance of dead cells, the absence of caspase3 positive cells cannot rule out the occurrence of cell death. Therefore, "cell death does not appear to be a causative factor in these effects" and "loss of Nampt activity specifically caused a G1/S stall without causing appreciable cell death in NSPCs" may be overstatements.

We changed the text from "suggesting that cell death does not appear to be a causative factor in these effects" to "providing evidence against a potential contribution of cell death to the observed effects." (page 10)

We also changed the text from "without causing appreciable cell death in NSPCs" to "without causing detectable cell death in NSPCs." (page 25)

6. The authors did not find a defect in oligodendrogenesis in the SGZ of iNSPC-Nampt-KO mice in vivo. If the authors would like to pursue basal oligodendrogenesis from adult NSCs in vivo, SVZ would be a better place to look at than SGZ.

We appreciate this suggestion from the referee. To assess basal oligodendrogenesis from adult NSPCs in the SVZ, we quantified the percentages of NestinGFP+ cells 7 days post injection that coexpressed the oligodendrocyte marker Olig2. We found that ~7% of NestinGFP+ cells coexpressed Olig2 in the control SVZ. Interestingly, the percentage of Olig2+ cells among NestinGFP+ cells in the SVZ significantly decreased in iNSPC-Nampt-KO mice, consistent with our *in vitro* findings and our assessment of oligodendrogenesis upon cuprizone treatment. We added these new results to Figure 7A.

2nd Editorial Decision 23 March 2014

Thank you very much for submitting your revised manuscript for further consideration by the EMBO Journal. It has now been seen by the three referees,§ whose comments are enclosed. I am

pleased to note that all three referees are in favour of publication, pending satisfactory minor revision.

I would like to invite you to incorporate the revisions as appropriate to the text and the figures.
Referee 2 asks for a toning down of the ageing connection in the absence of a more definitive rescue experiment. Please revise to use language that cannot be misread as suggesting a firm link in both the abstract and title.
Referee 1 recommends dramatic shortening of the discussion on pages 24-25, as well as clarification of fig. 8. We would actually suggest to follow ref 2's advice here (last point) to add a table or figure to summarize these findings and to avoid undue speculation.
I will not list all the detailed recommendations made - if you disagree with any of them we are happy to discuss the matter before you resubmit your revision.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
http://emboj.embopress.org/about#Transparent_Process

We hope to receive a revision in about two weeks and assuming the revision addresses the referee recommendations where appropriate, we will proceed with rapid publication without additional review. 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.

I very much look forward to see you interesting work published in the EMBO Journal.

REFEREE REPORTS

Referee #1:

I have read with interest the revised version of the manuscript by L. Stein and S.I. Imai. I appreciate that the Authors have complied with the criticisms and questions raised to the original text. I keep believing that this is an outstanding piece of work shedding light on the still unidentified role of Nampt and of intracellular NAD in adult neural stem/progenitor cell (NSPC) proliferation, self-renewal and oligodendrogenesis in vitro and in vivo. A major outcome of the study is the demonstration that enhancing intracellular NAD+ levels via supplementation with NMN may become a preventive/therapeutic tool to counteract the age-related decline in NSPC functions.
I take note that my suggestions to perform additional experiments, especially on pharmacological inhibition of Sirt1 and Sirt2, have been considered not to be strictly required for resubmission. However, I believe that the complexity of the field and the wealth of experimental results would encourage a further effort to increase clarity in few parts of the manuscript, especially in the Discussion.
1) The links between Cyclin A, Cyclin E and p21 (p. 24, last 8 lines, and p. 25, lines 1-3) are still difficult to follow and I strongly suggest to delete this part to avoid confusion. The point is that the suggested correlations between the present findings and literature data seem to be somewhat unwarranted and inconclusive.
2) Same considerations apply to the lengthy part devoted to commenting on the still unknown factors downstream Sirt1/2 promoting oligodendrocyte fate decisions (p. 25, last line, to p. 26, 5 lines from bottom).For the sake of concision, this part should be drastically squeezed. A specific suggestion is to replace it with the last 5 lines of the legend to Figure 8, with few selected references.
3) Figure 8 itself keeps raising problems of interpretation in the part below Sirt1/Sirt2. Simply, I don't catch from this scheme (boxes, heavy/light lines) the opposite variations of Pdgfra, Sox10 and Nkx2.2 on one side and of p21 on the other. Instead, these variations, as induced by dissociated Nampt Ad-Cre and Sirt1/2 DKO neurospheres, are quite clear as shown in Figure 6F-G and by the relevant analysis of the experimental data at p. 18.

Minor remarks

1) I am satisfied by the introduction of a brief comment on sirtuins, specifically Sirt1 and Sirt2, in the legend to Figure 1A. However, I would leave out (lines 8-9 of the legend) "...releasing ADP-ribose", or replace it with "...releasing nicotinamide", as it is 2'-O-Acetyl-ADPribose, or 2'-O-Acyl-ADPribose, to be directly generated by sirtuins.
2) In the Supplementary Information, paragraph on Immunofluorescence, there is a typo in the 1st line, which should read "All tissue sections and cells were incubated in...".

Referee #2:

The authors show that Nampt and NAD levels in the SGZ are decreased during aging, and that Nampt ablation decreases NSPC proliferation, self-renewal and decreased oligodendrogenesis. Upon demyelination insult, oligodendrogenesis is also impaired in Nampt knockout, and that this effect may be mediated through Sirt1/Sirt2.
The authors have improved their manuscript with additional data as well as an expanded discussion. The main problem still remains that the "aging" aspect of the title is over-reaching and somewhat misleading and should be changed. The authors do discuss Nampt and NAD levels during aging and have one partial rescue experiment, however, the majority of the paper is not about aging. In fact, the key experiment to truly associate their findings to aging is the rescue experiment we had asked for in the previous round. However, NMN administration obviously failed to significantly enhance neurogenesis (see the authors' initial response to our first major concern). Thus, we feel that the title does not reflect the key findings presented here.

Additional comments:
- It is surprising that conditional deletion of Nampt induced by Tamoxifen leads to an immediate phenotype regarding the Nestin+ population (Fig 3H and Fig 3I). Is Nampt lost within minutes? (BrdU will be only available in the tissue for very few hours).
Furthermore, BrdU/GFAP positive cells early after BrdU injections are certainly not classical astrocytes but rather radial glia-like neural stem cells. Thus, the interpretation of their finding that the number of Nestin+ cells is decreased after Nampt deletion while not changing fate decisions is not convincing. In fact, virtually 100% of Nestin-expressing cells with a radial process are GFAP+ (see data from Enikolopov and Kempermann labs). This needs to be rediscussed.

- In Fig. 1, it would be more clear if you could draw a line (as in your other pictures) where the SGZ is.

- Pg. 7 - you say "significant population of NSPCs expressed Nampt (should be Figures 2A-D)", however the images are showing half of the cells are expressing Nampt, and the graph is significant to what? Please remove "significant."

- Pg. 7 - (NestinGFP, Figure 2C-D)

- Figure 2E - The western (perhaps due to the unequal loading), does not reflect the results shown in the quantification. In addition, the graph should be ordered in the same order as the blot so as not to create confusion.

- Pg. 8 and Figure 2 - you say that "at least part of the decrease in Nampt expression in the SGZ with age is due to the loss of Sox2+ NSPCs." You are showing that Sox2+ cells' expression of NAMPT is decreased, but that does not mean that there is a loss of Sox2+ NSPCs in your results. Please change this wording to reflect "the loss of expression from Sox2+ NSPCs."

- Figure 3J - The relevance of this partial rescue in terms of other rescue strategies (enriched environment, running, etc.) should be discussed.

- Pg. 9 - you should not state that Ki67 was "decreased" if that decrease does not reach significance. This could be misleading to the reader.

- Pg. 10 - It is not clear what you mean by maturation of newborn neurons in the text or the figure. Please clarify.

- Pg. 13 and Figure S3C - you say levels of "activated caspase 3 were only slightly increased," however, it goes from nothing to present in the FK866 condition, so better that it be quantified.

- Pg. 16 - It would be better to be more specific and say "knockdown of Nampt prevents the successful differentiation of oligodendrocytes from NSPCs, potentially due to quiescence as indicated by a retention of nestin staining."

- Supp. Fig. 4E - Why does NAMPT come up in the cre+ animals that are treated with Staurosporine?

- Supp. Fig. 4F - Please change the y axis so that the differences are clearer between conditions.

- Pg. 16 and Figure legend 6B - Figure legend says experiment was done at 2 days, but the text says 6-7 days. Please correct.

- Pg. 18 and Supp. Figure 5- How does the double knockout of Sirt1 and 2 affect proliferation?

- Pg. 20 - Figure S6 - It appears that SGC NSPCs do have increased levels APC and Sox10 in control animals, so the statement "Thus, SGZ NSPCs do not appear to be the main mediators of short-term remyelination in the hippocampus" should not really be used.

- Pg. 24 - The very long and detailed discussion on cyclins and Nampt would be better explained through use of a graph, schematic or table.

Referee #3:

The authors adequately addressed my comments.

2nd Revision - authors' response 27 March 2014

Referee #1:

1. *The links between Cyclin A, Cyclin E and p21 (p. 24, last 8 lines, and p. 25, lines 1-3) are still difficult to follow and I strongly suggest to delete this part to avoid confusion. The point is that the suggested correlations between the present findings and literature data seem to be somewhat unwarranted and inconclusive.*

   Following this suggestion, we removed this portion of the text from the Discussion section.

2. *Same considerations apply to the lengthy part devoted to commenting on the still unknown factors downstream Sirt1/2 promoting oligodendrocyte fate decisions (p. 25, last line, to p. 26, 5 lines from bottom). For the sake of concision, this part should be drastically squeezed. A specific suggestion is to replace it with the last 5 lines of the legend to Figure 8, with few selected references.*

   We followed this suggestion and shrunk our discussion to make it as concise as possible. Nonetheless, we prefer to keep this specific discussion because we feel that it is important to compare and contrast our results with other reports in the literature.

3. *Figure 8 itself keeps raising problems of interpretation in the part below Sirt1/Sirt2. Simply, I don't catch from this scheme (boxes, heavy/light lines) the opposite variations of Pdgfra, Sox10 and Nkx2.2 on one side and of p21 on the other. Instead, these variations, as induced by*

dissociated Nampt Ad-Cre and Sirt1/2 DKO neurospheres, are quite clear as shown in Figure 6F-G and by the relevant analysis of the experimental data at p. 18.

We apologize for the confusion. To make Figure 8 clearer, we have removed the boxes and heavy/light lines and replaced them with gene names with arrows of directionality. We also revised the figure legend. We hope that these changes ameliorate the problem.

Minor remarks

1. *I am satisfied by the introduction of a brief comment on sirtuins, specifically Sirt1 and Sirt2, in the legend to Figure 1A. However, I would leave out (lines 8-9 of the legend) "...releasing ADP-ribose", or replace it with "...releasing nicotinamide", as it is 2'-O-Acetyl-ADPribose, or 2'-O-Acyl-ADPribose, to be directly generated by sirtuins.*

   Following this suggestion, we replaced "ADP-ribose" with "nicotinamide" in the figure legend for Figure 1.

2. *In the Supplementary Information, paragraph on Immunofluorescence, there is a typo in the 1st line, which should read "All tissue sections and cells were incubated in...".*

   Thank you very much for finding this typo. We have made the correction.

Referee #2:

The main problem still remains that the "aging" aspect of the title is over-reaching and somewhat misleading and should be changed. The authors do discuss Nampt and NAD levels during aging and have one partial rescue experiment, however, the majority of the paper is not about aging. In fact, the key experiment to truly associate their findings to aging is the rescue experiment we had asked for in the previous round. However, NMN administration obviously failed to significantly enhance neurogenesis (see the authors' initial response to our first major concern). Thus, we feel that the title does not reflect the key findings presented here.

We toned down the language in the abstract by changing "implicating" to "giving rise to the possibility that". We also toned down the running title from "Nampt regulates neural stem cells during aging" to "Nampt regulates neural stem cell function". Nonetheless, we would prefer to keep the current title for the following reasons:

1) With our use of the word "recapitulates", our current title does not directly suggest the causality.
2) As Reviewer #1 pointed out, the results from our NMN administration experiment are a major outcome of this study, suggesting that the defect in NAMPT-mediated NAD^+ biosynthesis does exist in adult neural stem/progenitor cells during aging. Thus, the results we present in this paper will provide very important information regarding the age-associated functional defects in neural stem/progenitor cells.
3) The 12-month-long NMN administration we performed is as significant of an effort as possible towards a "definitive rescue experiment". In fact, it is hard to conceive of a cleaner "definitive rescue experiment".
4) In response to Reviewer #2's request in the first round of review, we successfully added the result of newborn neurons (Dcx+) in Supplementary Figure 2I. Although the difference did not reach statistical significance, the trend of increase in Dcx+ newborn neurons in NMN-treated mice provides important support to our notion. We do not think that it is appropriate to simply disregard this result as a "failure". We believe that it is important to report this trend as well.

Additional comments:

1. *It is surprising that conditional deletion of Nampt induced by Tamoxifen leads to an immediate phenotype regarding the Nestin+ population (Fig 3H and Fig 3I). Is Nampt lost within minutes? (BrdU will be only available in the tissue for very few hours).*

   Based on the data we present in Supplementary Figure 2E and Supplementary Figure 4A-D, we do not believe that Nampt is lost within minutes. Instead, Supplementary Figure 2E shows that 40% of Nampt protein is lost 3 days post deletion *in vivo*, and Supplementary Figures 4A-D show that 60% of Nampt mRNA and protein is lost 2 days post deletion, with concomitant effects on NAD^+ levels, *in vitro*. Thus, we believe that the majority of Nampt is lost within 48 to 72 hours. As such, this is the time course that we used for Figure

3H and 3I. Namely, BrdU was injected twice at an 8 hour interval and mice were sacrificed 72 hours later. BrdU is actually bioavailable for about 2 hours post injection (Taupin et al., 2007), thus, with 2 separate injections, this protocol labeled cells over a period of 4 hours, and assessed the deletion in this population of labeled cells, over 72 hours later. We believe that our protocol is sufficient to make the assessments and conclusions that we present here.

BrdU/GFAP positive cells early after BrdU injections are certainly not classical astrocytes but rather radial glia-like neural stem cells. Thus, the interpretation of their finding that the number of Nestin+ cells is decreased after Nampt deletion while not changing fate decisions is not convincing. In fact, virtually 100% of Nestin-expressing cells with a radial process are GFAP+ (see data from Enikolopov and Kempermann labs). This needs to be rediscussed.

Encinas et al., (2011) do show that the majority of BrdU+Gfap+ cells are also NestinGFP+ 3 days post BrdU injection. However, in our study, the percentages of BrdU+ cells that also express Dcx or Olig2 are unchanged. Thus, we feel that it is valid to conclude that fate decisions into these lineages are unchanged (Figure 3I). If we assume that all the BrdU+Gfap+ cells presented in Figure 3I are also Nestin+, as this referee cautioned, it would not change our conclusions as we show similar percentages of BrdU+Gfap+ and BrdU+Nestin+ cells in control mice, but a significant decrease in BrdU+Nestin+ cells in iNSPC-Nampt-KO mice. Thus, the NSPCs in iNSPC-Nampt-KO mice must have already abnormally lost Nestin expression but have retained Gfap expression on their way to quiescence. However, to account for this possibility, we have added that Gfap also markers NSPCs on page 11.

2. *In Fig. 1, it would be more clear if you could draw a line (as in your other pictures) where the SGZ is.*

   We have added lines denoting the SGZ in Figure 1.

3. *Pg. 7 - you say "significant population of NSPCs expressed Nampt (should be Figures 2A-D)", however the images are showing half of the cells are expressing Nampt, and the graph is significant to what? Please remove "significant."*

   We changed “a significant population of NSPCs” to “a majority of NSPCs” on page 7.

4. *Pg. 7 - (NestinGFP, Figure 2C-D)*

   We changed (NestinGFP, Figure 2C) to (NestinGFP, Figure 2C-D).

5. *Figure 2E - The western (perhaps due to the unequal loading), does not reflect the results shown in the quantification. In addition, the graph should be ordered in the same order as the blot so as not to create confusion.*

   Per Referee 2’s previous request of “In Fig. 2E, I don't feel it is necessary to have the repeat lanes of neurospheres and adult HCs twice. It just makes it confusing,” we removed those additional lanes. Therefore, we decided to keep the current panel but reordered the graph to match the blot as requested.

6. *Pg. 8 and Figure 2 - you say that "at least part of the decrease in Nampt expression in the SGZ with age is due to the loss of Sox2+ NSPCs." You are showing that Sox2+ cells' expression of NAMPT is decreased, but that does not mean that there is a loss of Sox2+ NSPCs in your results. Please change this wording to reflect "the loss of expression from Sox2+ NSPCs."*

   We changed “to loss of Sox2+ NSPCs” to “to loss of expression from Sox2+ NSPCs” on page 8.

7. *Figure 3J - The relevance of this partial rescue in terms of other rescue strategies (enriched environment, running, etc.) should be discussed.*

   We added the requested discussion to the Discussion section (page 24).

8. *Pg. 9 - you should not state that Ki67 was "decreased" if that decrease does not reach significance. This could be misleading to the reader.*

   To avoid such a possibility of misleading, we added the p values to this sentence: “decreased by 22% (p=0.019) and 35% (p=0.064), respectively” (now page 10).

9. *Pg. 10 - It is not clear what you mean by maturation of newborn neurons in the text or the figure. Please clarify.*

   We described the definition of immature and mature newborn neurons in the Supplementary Figure 2F figure legend: "Immature cells had no or horizontal projections. Mature cells had vertical projections spanning the granule cell layer." To clarify, we added the following clause to page 10, "as assessed by categorization of Dcx+ cells with no or horizontal projections as immature neurons and Dcx+ cells with vertical projections spanning the granule cell layer as mature neurons."

10. *Pg. 13 and Figure S3C - you say levels of "activated caspase 3 were only slightly increased," however, it goes from nothing to present in the FK866 condition, so better that it be quantified.*

    Since activated caspase 3 cells go from nothing to present in the FK866 condition, it is difficult to quantify and compare signal intensities without a reference value. Thus, we have instead changed our wording from "activated caspase 3 were only slightly increased" to "activated caspase 3 became detectable" (now page 12).

11. *Pg. 16 - It would be better to be more specific and say "knockdown of Nampt prevents the successful differentiation of oligodendrocytes from NSPCs, potentially due to quiescence as indicated by a retention of nestin staining."*

    We changed "NSPC characteristics" to "nestin staining" (page 16).

12. *Supp. Fig. 4E - Why does NAMPT come up in the cre+ animals that are treated with Staurosporine?*

    We apologize for the confusion. The cells treated with Staurosporine were $Nampt^{+/+}$ cells, rather than $Nampt^{flox/flox}$ cells to have positive controls for activated caspase 3. We added this detail to the Supplementary Figure 4E figure legend as well as to Supplementary Figure 4E.

13. *Supp. Fig. 4F - Please change the y axis so that the differences are clearer between conditions.*

    We changed the y axis on Supp. Fig. 4F from a maximum of 5% to a maximum of 2%.

14. *Pg. 16 and Figure legend 6B - Figure legend says experiment was done at 2 days, but the text says 6-7 days. Please correct.*

    Actually, both the figure legend and the text for Figure 6B say 6-7 days (previous text: "To assess oligodendrocyte formation, dissociated neurospheres were harvested after 6-7 days of differentiation (B). To assess OPC formation, dissociated neurospheres were examined after 2 days of differentiation (C)." However, to avoid confusion, we moved the figure letters before their respective sentences.

15. *Pg. 18 and Supp. Figure 5- How does the double knockout of Sirt1 and 2 affect proliferation?*

    We did not observe a difference in proliferation in the Sirt1/2 DKO cells.

16. *Pg. 20 - Figure S6 - It appears that SGC NSPCs do have increased levels APC and Sox10 in control animals, so the statement "Thus, SGZ NSPCs do not appear to be the main mediators of short-term remyelination in the hippocampus" should not really be used.*

    We removed this sentence from page 20.

17. *Pg. 24 - The very long and detailed discussion on cyclins and Nampt would be better explained through use of a graph, schematic or table.*

    Per Referee 1's suggestions, we dramatically shortened the discussion of cyclins and clarified Figure 8. Per Referee 2's suggestions, we summarized these findings in Figure 8.

18. *Pg. 7 - (NestinGFP, Figure 2C-D)*

    We changed (NestinGFP, Figure 2C) to (NestinGFP, Figure 2C-D).

19. *Figure 2E - The western (perhaps due to the unequal loading), does not reflect the results shown in the quantification. In addition, the graph should be ordered in the same order as the blot so as not to create confusion.*

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    We changed “to loss of Sox2+ NSPCs” to “to loss of expression from Sox2+ NSPCs” on page 8.

21. *Figure 3J - The relevance of this partial rescue in terms of other rescue strategies (enriched environment, running, etc.) should be discussed.*
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    We did not observe a difference in proliferation in the Sirt1/2 DKO cells.

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