# *Trp53* ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing

Alisa K. White, Marybeth Baumgartner, Madisen F. Lee, Kyle D. Drake, Gabriela S. Aquino and Rahul N. Kanadia
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**Original submission**

First decision letter

MS ID#: DEVELOP/2021/199591

MS TITLE: *Trp53* ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing

AUTHORS: Alisa K White, Marybeth Baumgartner, Madisen F Lee, Kyle D Drake, Gabriela S Aquino, and Rahul Kanadia

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing

how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

*Advance summary and potential significance to field*

In this manuscript, White and colleagues have used advanced mouse genetics to study epistatic interactions between the minor splicesome snRNA, Rnu11 and Trp53 (P53). Their previous work demonstrated the necessity of Rnu11 in radial glial cell (RGC) maintenance and ablation in the developing cortex led to defects in cell cycle and severe microcephaly at birth. In that study, they also observed enrichment for P53 apoptotic signalling pathway components, suggesting that RGC loss may be occurring in a P53 dependent manner.

Based on this work, their initial hypothesis was that the severe microcephaly observed when Rnu11 was ablated in the cortex was primarily due to defects in cell cycle, leading eventually to P53 mediated apoptosis. Here the authors used an Emx1-cre driver to induce ablation of Rnu11 in RGC from as early as E9. In parallel, they ablated Trp53 and observed that the microcephaly phenotype was partially rescued.

Perhaps surprising about this study is that even though some evidence suggests cell death is acting through the P53 signaling pathway, ablating Trp53 does not fully rescue microcephaly. The authors went on to show that removing Trp53 in Rnu11 depleted RGCs resulted in fewer cells undergoing apoptosis (E14.5). Transcriptional analysis of control, cKO and double KO (dKO) corticies revealed that the dKOs were transcriptionally more similar to the control than they were to the cKO. However, the minor intron splicing defects observed in Rnu11 cKOs is not rescued in the dKO. The minor intron splicing defects enriched for genes involved with cell cycle. This led the authors to test if the cKO and dKO mice displayed any defects in cell cycle length. Notably, cell cycle length was increased in the dKO. Finally, the authors observe that cell death in the cortex is blocked when Trp53 is ablated. However, what was possibly surprising was that DNA damage was delayed in the dKO.

*Comments for the author*

Major Points:
1. What is currently missing from this manuscript is a clear developmental timecourse of the phenotype in the cKO relative to the dKO. Currently, the representative images in many figures show opposing findings. For example the E14.5 dKO in Fig 2A shows a strong microcephaly, however E14.5 dKO in Fig 4A and Fig 5a does not show any sign of microcephaly. Then in Fig S3 there is again strong microcephaly at E15.5 in the dKO.

2. The findings regarding the microcephaly in Figure 1 are not clear. In Fig 1C' the weight of the dKO is significantly less than the cKO. However, both the neocortical thickness (Fig 1D') and the neocortical length (Fig 1D'') are significantly increased. These quantifications also indicate no significant difference in thickness compared to control corticies suggesting that the microcephaly is rescued when P53 is KO in combination with Rnu11. However, the relative cortical thickness in the representative images (Fig 1D) look visibly different. It is not clear what is contributing to these variances.

3. Microcephalies can also arise from smaller cell bodies and overall compaction of the neuropil. Since cell death is reduced in the dKO (ie. E14.5), are either of these factors contributing to the phenotype observed?

4. It should be noted that Emx1-cre expression also occurs in progenitors that later form the hippocampus and some interneurons of the olfactory bulb.
While the hippocampus appears to be entirely absent in the Rnu11 cKO mice this study would benefit from a careful characterization of how a loss of Rnu11 and Trp53 affects both progenitors and excitatory neurons of the hippocampus. By providing a more thorough analysis of other regions

derived from Emx1 positive progenitors, this may give greater insight into the cell type specific requirements of Rnu11 in development.

5. The way the graphs in figure 4b are currently presented is challenging to interpret. To assist the reader, it is suggested to present this in an optimized manner.

Minor points
1. In figure 2C,D it is unclear if or how many of the DEGs are shared or different in the control vs. cKO and the control vs dKO graphs. Presenting this data instead as a heat map or providing an excel table with such comparisons would be beneficial for the reader.

2. Please reword the following sentence:
“Here we report that blocking P53 mediated cell death failed to prevent microcephaly in the dKO mice. This finding suggests that microcephaly in minor spliceosome diseases is primarily driven by cell cycle defects, which were detected earlier and more severe in the dKO compared to the Rnu11 cKO.”

3. Please provide a citation for the previously published strategy referenced in this sentence:
“Utilizing a previously published, minor intron-focused alternative splicing-specific MSI calculation strategy, we identified 76, 179, and 215 AS events in the control, Rnu11 cKO, and dKO, respectively.”

Reviewer 2

*Advance summary and potential significance to field*

In this manuscript "Trp53 ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing", the authors examined the effect of Trp53 ablation in the Rnu11 cKO mice and found that there was no microcephaly rescue. They concluded that microcephaly in Rnu11 cKO is primarily driven by cell cycle defects. Overall, the manuscript investigates the molecular mechanisms of microcephaly, which is an important and relevant theme in the field of developmental neurobiology. However, there are some major issues that should be addressed. The main phenotype (cortical thickness as a readout of microcephaly) is not consistent amongst the figures. A thorough revision should be undertaken by the authors.

*Comments for the author*

Major comments:
1) The cortical wall thickness representative pictures showed in Figure2A for all the groups are strikingly different than the other examples chosen in the rest of the manuscript, such as Figure 4, Figure 5 and Figure 6 comparing the same age, E14.5, sagittal sections. In contrast, Figure S3 (E15.5) displays a similar phenotype than Figura 2A (E14.5). Authors should explain those differences and change the examples to depict the phenotype accordingly. It is not clear whether the embryonic cortical areas depicted in Figures 2, 4, 5, 6 and S3 are from the same rostro-caudal levels.

2) The number of animals used to reach the manuscript conclusions is not clearly shown. It should be stated in all Figure legends.

Minor comments:
1) The abstract should be revised for clarity purposes. The cell cycle defects cited in the abstract could be more detailed.

2) Authors should improve Figure 4 and Figure 6 alignment, to allow the cortical thickness comparisons.

3) In Figure 4, the E14. 5 dKo example seems to be upside down, with the Aurora B labelling instead of in the apical side, displayed in the basal side.

4) In Figure 4 or 5, the quantification of the PAX6+ number of cells in a correspondent cortical area in the different groups could contribute to the understanding of the phenotype.

5) Correct the misspelling of Martynoga in page 10 and page 12.

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**First revision**

Author response to reviewers' comments

**Reviewer 1**
Major:

1. "What is currently missing from this manuscript is a clear developmental timecourse of the phenotype in the cKO relative to the dKO. Currently, the representative images in many figures show opposing findings. For example, the E14.5 dKO in Fig 2A shows a strong microcephaly, however E14.5 dKO in Fig 4A and Fig 5a does not show any sign of microcephaly. Then in Fig S3, there is again strong microcephaly at E15.5 in the dKO."

   *Response:* We would like to thank the reviewer for pointing out these deficits in our first submission. To address the first point of the manuscript lacking a clear developmental time course, we have now included representative images of the whole brain, starting at E12.5, E14.5, E16.5, E18.5, and P0 (Figure S1). We would like to apologize for the images we used to represent the dKO which appeared to show opposing findings. However. this was not the case and we have remedied this issue by harvested new dorsal telencephalons and performed the IF for p53. Here we have provided a new representative image for the control, *Rnu11* cKO, and dKO for Figure 2A (IF for p53 at E14.5), which accurately reflect the cortical morphology shown in Figures 4A and 5A. Finally, Figure S3 (now Figure S6) shows strong microcephaly in the both the *Rnu11* cKO and dKO at E15.5 (TUNEL, CC3, yH2AX). One possibility for the identified variability by Reviewer 1 is that for E15.5 we analyzed cortices in the coronal plane, whereas previous analyses of the developing cortex were conducted in the sagittal plane. Our developmental progression analysis of microcephaly in the new Figure S1, show that microcephaly in both the *Rnu11* cKO and dKO follow a similar time course of appearance, with the first appearance of microcephaly at E14.5, continuing progression to P0, which is in concurrence with the observable microcephaly at E15.5 show in the coronal sections of Figure S6. We have kept Figure S6 in the coronal plane, however, have clearly stated in methods and the figure legend that this analysis was performed on sections collected in the coronal plane.

2. "The findings regarding the microcephaly in Figure 1 are not clear. In Fig1C' the weight of the dKO is significantly less than the cKO. However, both the neocortical thickness (Fig 1D') and the neocortical length (Fig 1D'') are significantly increased. These quantifications also indicate no significant difference in thickness compared to control corticies, suggesting that the microcephaly is rescued when P53 is KO in combination with Rnu11. However, the relative cortical thickness in the representative images (Fig 1D) look visibly different. It is not clear what is contributing to these variances."

   *Response:* Again, we apologize for not presenting the data in manner that was clearly indicative of the phenotype. To better characterize the microcephaly phenotype at P0 we replaced our previous figure panel with new data that was quantified using a method presented by Ka, Moffat, & Kim, in a recent 2021 paper (https://doi.org/10.1007/s10571-021-01088-1). Here to characterize cortical parameters, we set-up matings to obtain fresh P0 brains for each genotype (control n=5, *Rnu11* cKO n=10, dKO n=5). After dissecting out the brains, we immediately imaged the brains from the top view and used the ImageJ to calculate hemi-cortical surface area, anterior-posterior length (AP length) and length measurements of the cortices of control, *Rnu11* cKO, and dKO. This analysis displayed that the microcephaly observed at P0 was comparable in the *Rnu11* cKO and dKO based on these cortical parameters.

3. “Microcephalies can also arise from smaller cell bodies and overall compaction of the neuropil. Since cell death is reduced in the dKO (ie.E14.5), are either of these factors contributing to the phenotype observed?”

   *Response:* We agree with the Reviewer in that it is possible that overall reductions in cell bodies or neuropil compaction, not just reductions in cell numbers could give rise to microcephaly in the dKO. To address this issue, we analyzed P0 Nissl-stained coronal sectioned of which we did not observe smaller cell bodies or neuropil compaction (lines 198-199). Moreover, we do begin to see cell death occurring in the dKO at E15.5. Thus, this cell death and cell cycle defect combined in the dKO most likely results in the microcephaly observed.
4. “It should be noted that Emx1-cre expression also occurs in progenitors that later form the hippocampus and some interneurons of the olfactory bulb. While the hippocampus appears to be entirely absent in the Rnu11 cKO mice, this study would benefit from a careful characterization of how a loss of Rnu11 and Trp53 affects both progenitors and excitatory neurons of the hippocampus. By providing a more thorough analysis of other regions derived from Emx1 positive progenitors, this may give greater insight into the cell type specific requirements of Rnu11 in development.”

   *Response*: We agree with the Reviewer’s suggestion regarding the lineage *Emx1*+ NPCs outside of the cortex. However, the original hypothesis we sought to test was the molecular underpinning of microcephaly in the *Rnu11* cKO cortex. To include a systematic analysis of the effect of *Rnu11* loss in Emx1+ progenitors on the hippocampus and olfactory bulb, we would have to initiate new crosses that would take well beyond a year to generate the animals needed. Moreover, data from this proposed experiment would not change the central finding of the current manuscript. Therefore, we believe that this specific experiment is beyond the scope of the current manuscript. To note we have now explicitly mentioned absence of the hippocampus in both the *Rnu11* cKO and dKO in the results (line 200) and have discussed (lines 453-463).

5. “The way the graph in Figure 4B was originally presented was challenging to interpret. It is suggested to present this in an optimized manner”.

   *Response:* We agree with the reviewer that the graph that we presented could be difficult to interpret. Therefore, we have now simplified the presentation to the most salient phenotypes, including separated bar charts of only the % Mitotic RGCs (Pax6+) in prometaphase and telophase in the main body (Figure 4B). The full bar chart has been moved to supplement to show the % of all M-phase RGCs in the stacked bar chart orientation (Figure S4).

Minor points

1. “In figure 2C,D it is unclear if or how many of the DEGs are shared or different in the control vs. cKO and the control vs dKO graphs. Presenting this data instead as a heat map or providing an excel table with such comparisons would be beneficial for the reader.”

   *Response:* We agree with the Reviewer’s concern regarding the shared/unique DEGs presented in Figure 2. To address this point, we have now presented the gene expression data as an Upset plot (Figure 2C), moving the scatterplots of gene expression to supplement (Figure S2). Here we can more clearly plot the intersection of gene expression changes occurring the *Rnu11* cKO vs control, and how those bins of genes then responded in the dKO vs the control. Additionally, we can more clearly highlight how MIGs specifically are changing in the two comparisons.

2. “Please reword the following sentence: “Here we report that blocking P53 mediated cell death failed to prevent microcephaly in the dKO mice. This finding suggests that microcephaly in minor spliceosome diseases is primarily driven by cell cycle defects, which were detected earlier and more severe in the dKO compared to the Rnu11 cKO.””

   *Response:* We have reworded the identified statement to clarify the conclusion being made

to: “Here we report that blocking p53-mediated cell death still resulted in primary microcephaly in the dKO mice. This combined with the fact that cell cycle defects were detected earlier and were more severe in the dKO, compared to the *Rnu11* cKO, suggests that microcephaly observed in minor spliceosome disease is primarily driven by cell cycle defects” (lines 163-166).

3. Please provide a citation for the previously published strategy referenced in this sentence: “Utilizing a previously published, minor intron-focused alternative splicing- specific MSI calculation strategy, we identified 76, 179, and 215 AS events in the control, Rnu11 cKO, and dKO, respectively.”

   *Response:* We have now added a citation to this sentence (line 315).

Reviewer 2
Major comments:

1. “The cortical wall thickness representative pictures showed in Figure2A for all the groups are strikingly different than the other examples chosen in the rest of the manuscript, such as Figure 4, Figure 5 and Figure 6 comparing the same age, E14.5, sagittal sections. In contrast, Figure S3 (E15.5) displays a similar phenotype than Figure 2A (E14.5). Authors should explain those differences and change the examples to depict the phenotype accordingly. It is not clear whether the embryonic cortical areas depicted in Figures 2, 4, 5, 6 and S3 are from the same rostro-caudal levels.”

   *Response:* Both Reviewer 2 and Reviewer 1 shared similar concerns with the representative images used to display microcephaly in Figure 2A, as well as the developmental time course of microcephaly progression. Please see response to Reviewer 1’s concerns.

2. “The number of animals used to reach the manuscript conclusions is not clearly shown. It should be stated in all Figure legends.”

   *Response:* Apologies for not having n-value clearly state in the legend, as these numbers were a part of Table S3. However, we have now clearly stated the n-value in each of the figure legends.

Minor comments:

1. “The abstract should be revised for clarity purposes. The cell cycle defects cited in the abstract could be more detailed.”

   *Response:* We have re-written the abstract to more clearly reflect the cell cycle defects.

2. “Authors should improve Figure 4 and Figure 6 alignment, to allow the cortical thickness comparisons.”

   *Response:* The representative dorsal telencephalon images in Figures 4 and 6 have been realigned to address concerns of Reviewer 2 and allow for easier cortical thickness comparison.

3. “ In Figure 4, the E14. 5 dKO example seems to be upside down, with the Aurora B labelling instead of in the apical side, displayed in the basal side.

   *Response:* Reviewer 2 identified that the AuroraB staining in Figure 4A (dKO at E14.5) appeared to have the basal and apical edges flipped. Here the basal and apical edges were not flipped, however the fluorescent signal appears caught in ependymal/red blood cells, which would confuse readers. We appreciate the reviewers observation, and thus a new representative image has been used for this figure.

4. “In Figure 4 or 5, the quantification of the PAX6+ number of cells in a correspondent cortical

area in the different groups could contribute to the understanding of the phenotype".

*Response:* To address this concern, we have provided a bar graph of both the % of RGCs in S-phase and the raw number of RGCs across time, to supplement the cell cycle and S-phase length phenotypes in Figure S5.

5. "Correct the misspelling of Martynoga in page 10 and page 12."

*Response:* The spelling has now been corrected. Apologies for the mistake.

As detailed above, I hope that you agree we have made substantial progress in addressing most of the reviewer's comments. Accordingly, I hope you agree that the current manuscript is suitable for publication in Development.

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Second decision letter

MS ID#: DEVELOP/2021/199591

MS TITLE: *Trp53* ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing

AUTHORS: Alisa K White, Marybeth Baumgartner, Madisen F Lee, Kyle D Drake, Gabriela S Aquino, and Rahul Kanadia

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the remaining referees' comments can be satisfactorily addressed through text modification. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

*Advance summary and potential significance to field*

In this manuscript, White and colleagues have used advanced mouse genetics to study epistatic interactions between the minor splicesome snRNA, Rnu11 and Trp53 (P53). Their previous work demonstrated the necessity of Rnu11 in radial glial cell (RGC) maintenance and ablation in the developing cortex led to defects in cell cycle and severe microcephaly at birth. In that study, they also observed enrichment for P53 apoptotic signalling pathway components, suggesting that RGC loss may be occurring in a P53 dependent manner.

Based on this work, their initial hypothesis was that the severe microcephaly observed when Rnu11 was ablated in the cortex was primarily due to defects in cell cycle, leading eventually to P53 mediated apoptosis. Here the authors used an Emx1-cre driver to induce ablation of Rnu11 in RGC from as early as E9. In parallel, they ablated Trp53 and observed that the microcephaly phenotype was partially rescued.

Perhaps surprising about this study is that even though some evidence suggests cell death is acting through the P53 signaling pathway, ablating Trp53 does not fully rescue microcephaly. The authors went on to show that removing Trp53 in Rnu11 depleted RGCs resulted in fewer cells undergoing apoptosis (E14.5). Transcriptional analysis of control, cKO and double KO (dKO) corticies revealed that the dKOs were transcriptionally more similar to the control than they were to the cKO. However, the minor intron splicing defects observed in Rnu11 cKOs is not rescued in the dKO. The minor intron splicing defects enriched for genes involved with cell cycle. This led the authors to test if the cKO and dKO mice displayed any defects in cell cycle length. Notably, cell cycle length was increased in the dKO. Finally, the authors observe that cell death in the cortex is blocked when Trp53 is ablated. However, what was possibly surprising was that DNA damage was delayed in the dKO.

*Comments for the author*

The revised manuscript by White et al. mostly addresses the major points raised during the initial review. Below I elaborate on two of these points in greater detail.
The new Fig S1 addresses and clarifies my most pressing concern and now better demonstrates the progression of microcephaly formation. However, part of the revised text in the main manuscript is still unclear. Specifically line 196-198 in reference to Fig 1F. Is this statement a qualitative assessment of the provided nissl stains? It is not apparent from these images if the dKO truly displays less severe microcephaly. Combined with the timecourse provided in Fig S1, I see no quantitative evidence for this statement.
In my initial review I inquired if the authors had observed any phenotypes in the hippocampus or olfactory bulbs of Rnu11 cKO mice. Given how pronounced the phenotype is in both the cortex and the hippocampus, I find it interesting that olfactory bulb interneurons generally seem fine implying a certain degree of cell type specific requirements for the minor splicesome activity. While the authors are correct that the pool of progenitors that produce olfactory bulb interneurons is limited, a substantial fraction of interneurons located in the olfactory bulb granular layer are derived from the Emx1 lineage. Therefore the lack of any morphological difference here is quite an interesting finding, in my opinion.
Overall, the revised manuscript more clearly communicates the main message that a loss of Rnu11 results in microcephaly formation, mainly in a P53-independent manner. Instead, the minor splicesome appears to act on the mitotic progression of radial glial cells, resulting in severe impact on cell cycle progression.
Collectively, the revisions to the manuscript expand our understanding of the role of minor intron splicing in cortex development while opening new questions for follow-up studies.

Reviewer 2

*Advance summary and potential significance to field*

Dear Editor,

In this manuscript "Trp53 ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing", the authors examined the effect of Trp53 ablation in the Rnu11 cKO mice and found that there was no microcephaly rescue. They concluded that microcephaly in Rnu11 cKO is primarily driven by cell cycle defects. Overall, the manuscript investigates the molecular mechanisms of microcephaly, which is an important and relevant theme in the field of developmental neurobiology. The authors have addressed the issues identified in the revision and the manuscript has improved significantly.

*Comments for the author*

The authors have addressed the issues identified in the revision and the manuscript has improved significantly.

**Second revision**

Author response to reviewers' comments

Dear Editor,
We would like to thank the reviewers for their comments on the resubmission of our manuscript by White et al., 2021 entitled, “Trp53 ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing.” Detailed below are the changes made to address Reviewer 1’s minor concerns regarding the manuscript. We believe these changes address their concerns and should make our manuscript suitable for publication in Development.

Reviewer 1
Minor :

1. “The new Fig S1 addresses and clarifies my most pressing concern and now better demonstrates the progression of microcephaly formation. However, part of the revised text in the main manuscript is still unclear. Specifically, line 196-198 in reference to Fig 1F. Is this statement a qualitative assessment of the provided nissl stains? It is not apparent from these images if the dKO truly displays less severe microcephaly. Combined with the timecourse provided in Fig S1, I see no quantitative evidence for this statement.”

   Response: We have removed the clause “albeit less severe in the dKO”, as the Reviewer correctly points out that the data presented was to how progression of microcephaly during embryonic development, and not the difference in progression in Rnu11 cko and dKO. The objective here was to address the Reviewer's previous comments that we show the in between embryonic timepoints prior to P0 where microcephaly was not rescued in the dKO. Now we believe that our conclusion for Figure 1F and Figure S1 more accurately reflect the observed phenotype, with no claims of significance.

2. “In my initial review I inquired if the authors had observed any phenotypes in the hippocampus or olfactory bulbs of Rnu11 cKO mice. Given how pronounced the phenotype is in both the cortex and the hippocampus, I find it interesting that olfactory bulb interneurons generally seem fine, implying a certain degree of cell type specific requirements for the minor spliceosome activity. While the authors are correct that the pool of progenitors that produce olfactory bulb interneurons is limited, a substantial fraction of interneurons located in the olfactory bulb granular layer are derived from the Emx1 lineage. Therefore the lack of any morphological difference here is quite an interesting finding, in my opinion.”

   Response: Again, we would like to thank the reviewer for drawing out attention to the interesting finding that impaired minor intron splicing does not appear to affect the progenitors of the olfactory bulb interneurons, whereas the progenitors of the cortex and hippocampus targeted by Emx1-cre have such pronounced phenotypes. We have added a subheading to the discussion to further highlight this point, as we also find the heterogeneity in the response to impaired minor intron splicing extremely fascinating. While we hope this current manuscript can draw attention to this progenitor cell heterogeneity, we still believe that exploration into these progenitors for this current paper would not change the main conclusions of the paper.

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Third decision letter

MS ID#: DEVELOP/2021/199591

MS TITLE: *Trp53* ablation fails to prevent microcephaly in mouse pallium with impaired minor intron splicing

AUTHORS: Alisa K White, Marybeth Baumgartner, Madisen F Lee, Kyle D Drake, Gabriela S Aquino, and Rahul Kanadia
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.