EMBO Molecular Medicine

Long non-coding RNA-MALAT1 regulates retinal neurodegeneration through CREB signaling

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 15 October 2015

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the Reviewers whom we asked to evaluate your manuscript. We are sorry that it has taken longer than usual to get back to you on your manuscript.

As you will see the issues raised are many and fundamental. Although I will not dwell into much detail, I would like to highlight the main points.

Reviewers 1 points to an important caveat in your studies. Specifically, s/he notes that RGC-5 cells cannot be considered an appropriate retinal ganglion cell model; your conclusions are therefore confounded, and potentially compromised, by this issue. The reviewer also lists other items of importance that would need to be fully addressed to appreciate the validity of your conclusions.

Reviewer 2 is especially critical of the quality, analysis and presentation of the human samples (as is Reviewer 1). S/he also notes that many conclusions are not fully supported by the data with many missing controls and numerous other important issues. Also apparent are the mistakes in the figures, improper callouts of figures, and other serious errors. Finally, Reviewer 2 (and Reviewer 1) also notes, and we agree, that the quality of writing and English usages is far too low and that this impinges on readability and comprehension.

Reviewer 3 is less reserved but notes that immunostaining alone is not sufficient to support protein data and calls for additional experimental proof (e.g. western blotting) in that respect; s/he would also like to see stronger evidence for the CREB implication for MALAT1 function.

In conclusion, while publication of the paper cannot be considered at this stage, given the potential interest of your findings and after further discussion with my colleagues and reviewer cross-commenting, we have decided to give you the opportunity to address the above issues. We agreed however, that all the concerns must be addressed fully and to the satisfaction of the reviewers. This includes repeating the relevant experimentation on an appropriate cell line, much tighter experimentation to support the case for the role of MALAT1 and extensive re-writing and editing of the revised manuscript. While Reviewer 1 especially suggests removing altogether the human data, I would ask you instead to make an effort to add more data and considerably improve description, analysis and description of confounders to improve significance for the study. The overall aim is to significantly upgrade the clinical relevance and usefulness of the dataset, which of course is of paramount importance for our title.

I understand that if you do not have the required data available at least in part, to address all the above, this might entail a significant amount of time, additional work and experimentation and might be technically challenging, I would therefore understand if you chose to rather seek publication elsewhere at this stage. Should you do so, we would welcome a message to this effect.

However, should you decide to revise for EMBO Molecular Medicine, it is important that you be fully aware that it is EMBO Molecular Medicine policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version.

As you know, EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. However, I do ask you to get in touch with us after three months if you have not completed your revision, to update us on the status. Please also contact us as soon as possible if similar work is published elsewhere.

Finally, please note that EMBO Molecular Medicine now requires a complete author checklist (http://embomolmed.embopress.org/authorguide#editorial3) to be submitted with all revised manuscripts. Provision of the author checklist is mandatory at revision stage; The checklist is designed to enhance and standardize reporting of key information in research papers and to support reanalysis and repetition of experiments by the community. The list covers key information for figure panels and captions and focuses on statistics, the reporting of reagents, animal models and human subject-derived data, as well as guidance to optimise data accessibility. Additional information on manuscript preparation is available below.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System):

One of the cell lines used in this study cannot be used when making conclusions related to retinal ganglion cells. This will need to be addressed in a revised version of the manuscript.

While this is an interesting study, there are major weaknesses especially in relation to the use of RGC-5 cells as a cell line representative of retinal ganglion cells. These cells were reported to be of mouse origin and not rat and do not express markers of retinal ganglion cells. Indeed, it has been shown that the cells are likely a cell line called 661w cells that were contaminated by the lab that generated them. In general, the ophthalmology community has now been advised not to use them.

That said, the data are interesting but confounded by the use of these cells to make conclusions related to retinal ganglion cell biology.

Referee #1 (Remarks):

This manuscript details the role of MALAT1 (metastasis associated lung adenocarcinoma transcript 1), a noncoding nuclear-enriched component in mouse models of retinal degeneration. The authors

also use cell lines (retinal ganglion and muller cells) to come to conclusions related to the mechanism of involvment of MALAT1 in neurodegeneration.

Overall, the manuscript could do with proof reading throughout for English grammar corrections. The authors need to define exactly what MALAT1 is, as a reader unfamiliar with this gene will not realise the significance of the findings.

Figure 1
The use of RGC5 cells should be discouraged in ophthalmology research. These cells are a contaminated cell line and do not represent ganglion cells, (See Krishnamoorthy RR et al., IOVS, 2013). In addition, details need to be included on the Muller cells used in the study as it's not clear where they were sourced.

I don't know what NS stands for in the 3rd and 4th panel of Fig1D.

Images in Fig.1 E need to be lower magnification and RPE cells should be confluent for immunocytochemistry as their gene expression can change depending on confluency. It is celar from the images that they are sub-confluent. Again, RGC5 cells are now redundant for vision research and should not be included.

Figure 3
Please label all abbreviations in the figure legends as it is difficult to have to constantly check the main text to ascertain what they refer to.

Figure 4
Again, need clarity on the Muller cells being used and what exactly their source is. Contrary to what the authors state, the data in this figure do not show an alteration in Muller cell function but simply show changes in Muller cell viability following suppression of MALAT1.

Figure 5
These are not retinal ganglion cells that are being transfected in Fig 5A. In fact these cells are not even the same species as they are listed. They are listed as rat cells but are actually mouse. Their use should be discontinued as a ganglion cell line. For example, a recent editorial in Experimental Eye Research states: " Any conclusions made in the published papers using these cells (RGC-5) that relate to retinal ganglion cell-specific responses must be reconsidered. (See, http://www.sciencedirect.com/science/article/pii/S0014483513002418). Therefore, the data in the entirety of Fig 5 cannot robustly be related to retinal ganglion cells.

Figure 7
I'm unsure if the nerve fibre layer is being measured in the OCT images outlined in Fig 7B. It appears to include large swathes of GCL and inner plexiform layer in addition to the inner nuclear layer. This needs clarification and much higher magnification on the area in question. All abbreviations need to be clarified in the figure legend.

Figure 8
The analysis of human samples seems redundant to the entire study and the significance is weak overall. I

Referee #2 (Comments on Novelty/Model System):

Technical Quality:
The role of MALAT1 in mouse and rat models of trauma and diabetic retinopathy were well-presented, with a large variety of experimental techniques and statistical significance. However, the clinical analysis for the role of MALAT1 is limited and incomplete. VEPs were given only as traces, even though the methods state that the authors ran 100 traces per animal. Statistics and quantification are needed for this as well as the immunohistochemistry and electron microscopy images. For the Alzheimer's, tumor, and glaucoma patient samples, the controls are unlisted and the fact that these are all aging diseases are not mentioned. The authors should be careful if they did not

include age-matched samples, and should provide that information to the reader as it may have affected the results. In addition, other confounders such as gender are ignored.

Novelty:
CREB and PP2 were already published to be involved with MALAT1 lncRNA. Therefore, the only novel finding is the roles that MALAT1 has in many neurodegenerative diseases. Furthermore, the analysis is still limited on the exact mechanism that ties together each of the studied disorders.

Medical Impact:
The fact that the authors show a role for MALAT1 lncRNA in a variety of complex human diseases increases its likelihood to be a target for therapeutic drugs and clinical trials.

Adequacy of Model System:
There are a variety of model systems used in this manuscript, including rat and mouse models of trauma (ONT) and diabetic retinopathy. Human cell lines and human patient samples were also analyzed. Based on the wide range of diseases and analysis, the evidence provided by the authors shows that MALAT1 lncRNA is involved in each disease model, but further data is needed for each disease to determine the full mechanism of action for MALAT1.

Referee #2 (Remarks):

Long noncoding RNA-MALAT1 regulates retinal neurodegeneration through CREB signaling

Long noncoding RNAs (lncRNAs) are key players in many biological processes and diseases. Previously, the authors have shown that lnc-RNA MALAT1 plays a role in vascular dysfunctions. Here, the authors investigate the role of MALAT1 after optic nerve transections (ONT) and diabetic retinopathy (DR), likely through CREB phosphorylation. The authors also make the observation that MALAT1 has a role not only in retinal degenerative diseases, such as DR and glaucoma, but also neurodegenerative diseases, such as Alzheimer's disease. The overall conclusion that MALAT1 may be a potential therapeutic target for neurodegenerative diseases is confirmed by the findings of this study, although the direct evidence for MALAT1's role in neurodegeneration is limited and incomplete.

Major Revisions:
1. The paper has many grammatical errors and spelling errors, both within the written manuscript and in the figures. It is extensive, and makes many portions of the manuscript difficult to read. Please correct.

2. The conclusions made by the authors of this manuscript tend to be ambiguous or overstated. Most of the discussion section is a repetition of the introduction and the results, with very little discussion included. The discussion section should be re-written, with a focus on the meaning behind the results, the role of CREB phosphorylation in MALAT1 functions for neurodegenerative disease, as well as potential confounders (other signaling pathways, other cell targets of MALAT1 besides Muller glial cells and RGCs, etc.). For instance, the photoreceptor cells were affected after MALAT1 knockdown in DR models, but not in the ONT model. What are the authors' ideas on why this is the case?

3. It cannot be said that all neurotrophic factors have a partial reduction after MALAT1 knockdown. Some had significant reductions, while others remained similar to the controls. In addition, the results are not the same between the ONT response and the DR response to MALAT1 knockdown, even though the authors claim that it is. Different factors were reduced, and this should be discussed.

4. Controls for some of the figures are listed as WT, even though there are no WT controls in the figures. Please change this to either DR or ONT as listed in the graphs for the figures. Or add in WT controls and perform statistical analyses for those as the main control groups.

5. Figure S13 is a duplication of S14. S13 based on the figure legend is missing entirely. Please provide and remove duplicated blots.

6. Is there any significance for Figure 5G (and corresponding supplementary figure)? This should be discussed in the discussion section.

7. Figure 6 results section mentions MALAT1 in a CREB immunoprecipitate, as well co-localization of MALAT1 and CREB in RGCs using FISH. There are no references to Figure 6 or any other figure, and no data appears to match these statements. The authors should provide this data or remove the statements from the manuscript.

8. The mechanism in Figure 6G is not mentioned in the results. If the authors would like to use this mechanism, it should be its own figure discussed in the discussion section. Please move accordingly.

9. Has the role of PP2A been examined for the RGCs, and not just the Muller glial cells? How about the effect of PP1, as the authors mention it in their discussion section?

10. Figure 7A needs to have the wave labels moved so that they can be read. The scrambled siRNA also looks similar to the VEP result for the MALAT1 siRNA. Since the authors ran 100 traces per animal, they should provide a quantification of the wave values. Please add this quantification and statistical analysis to show the true effect of MALAT1 knockdown.

11. Figure 7C-D should be quantified. Statistical analysis is needed.

12. Patient samples are not well described in the methods section. As these are neurodegenerative diseases, they are affected by aging. Were the samples taken from age-matched patients? What were the respective controls? Listing the age range is necessary in these cases. Also, potential gender bias could play a role. This can be discussed in the discussion section with other confounders.

Minor Revisions:
1. Acronyms for experimental methods, gene names, etc. need to be written out completely the first time that they appear in the manuscript. Then they can be shortened for the remainder of the manuscript. Please correct.

2. For figures using glutamate excitotoxicity and stress responses, the time between ONT and the treatment groups is not listed, only that the ONT treated groups were kept for 48 hours prior to testing. Please include the time between optic nerve transections and stress/drug treatments.

3. The injection procedures are not included in the methods. In addition, the route of injection is not always listed in the figures. In some, intravitreal injections are mentioned, but in others, it merely says retinal delivery. Is this a subretinal injection? Intravitreal? Please be specific.

4. For the injections, the authors state that the siRNA or treatment is delivered for the amount of time listed in the graphs. Is this a single injection? A weekly injection? At zero weeks, are the rats/mice uninjected? Please be clear.

5. The contrast for the immunofluorescence images in each of the figures is very high. Please reduce.

6. The figures list * and # as potential significance, but the p-values and comparison groups for these are only listed in one figure legend. Please include for all relevant figures.

7. Figure legends write out the acronym RGC, but it is not in a single figure of this manuscript. Please remove.

8. Scale bars are listed for all but one figure. However, in many figures the retinal histology images appear at the same magnification but the scale bars in the image are different sizes. Please make sure that the scale bar values are correct for all images.

9. Figure 1D qPCR has black outlines for parts of the graph, and not for others. Please correct.

10. In Figure 3 and S5, the BDNF treatment groups are either missing or not labeled. Please correct.

11. Figure 4E is missing labels. As are other immunofluorescent panels in multiple figures. Please provide information on what is stained for all figures.

12. Figure 5C is incorrectly organized, panels are not in the appropriate locations. Please correct.

13. In Figure 6D the y-axis is cut off and should be corrected.

14. Stating that glaucoma is an ocular Alzheimer's disease is debatable, the reasoning for looking into glaucoma patients should be restated.

15. Citations should not be listed in methods section titles. Please move to the paragraph below.

16. Sample sizes are missing for many graphs that contain error bars and statistical analyses. Please list the N for these in the figure legends.

17. There are multiple occasions when the authors state that an experimental method was performed "as shown." There are no references, no video files, and therefore they need to provide the experimental methods or a reference for these techniques.

18. S12 figure legend does not mention BDNF or GDNF treatment groups, n, or p-values. Please write a complete figure legend.

Technical Quality:
The role of MALAT1 in mouse and rat models of trauma and diabetic retinopathy were well-presented, with a large variety of experimental techniques and statistical significance. However, the clinical analysis for the role of MALAT1 is limited and incomplete. VEPs were given only as traces, even though the methods state that the authors ran 100 traces per animal. Statistics and quantification are needed for this as well as the immunohistochemistry and electron microscopy images. For the Alzheimer's, tumor, and glaucoma patient samples, the controls are unlisted and the fact that these are all aging diseases are not mentioned. The authors should be careful if they did not include age-matched samples, and should provide that information to the reader as it may have affected the results. In addition, other confounders such as gender are ignored.

Novelty:
CREB and PP2 were already published to be involved with MALAT1 lncRNA. Therefore, the only novel finding is the roles that MALAT1 has in many neurodegenerative diseases. Furthermore, the analysis is still limited on the exact mechanism that ties together each of the studied disorders.

Medical Impact:
The fact that the authors show a role for MALAT1 lncRNA in a variety of complex human diseases increases its likelihood to be a target for therapeutic drugs and clinical trials.

Adequacy of Model System:
There are a variety of model systems used in this manuscript, including rat and mouse models of trauma (ONT) and diabetic retinopathy. Human cell lines and human patient samples were also analyzed. Based on the wide range of diseases and analysis, the evidence provided by the authors shows that MALAT1 lncRNA is involved in each disease model, but further data is needed for each disease to determine the full mechanism of action for MALAT1.

Referee #3 (Remarks):

In this manuscript, the authors have investigated the role of Long noncoding RNA-MALAT1 in neurodegeneration, Using rodent ONT models and RGC/Muller cell cultures. This is a well designed study with some interesting and potentially important findings.
The authors found that MALAT1 expression is up-regulated in ONT and diabetic retinas, and in cultured cells upon stress with (Hypoxia, HG, H2O2, Glutamate). Knockdown of MALAT1 affects RGC survival and Müller glial activation in animal retinas. In cell culture, Knockdown of MALAT1

affects cellular functions of Muller and RGC cells. With VEP, OCT and histological study on rodent eyes, they found that MALAT1 knockdown decreased p-wave amplitude, RNFL thickness and Induced more swellings / fragmentation/demyelination of RGC axons respectively. They also found MALAT1 regulates Müller and RGC function through CREB signaling. They concluded that Long noncoding RNA-MALAT1 regulates retinal neurodegeneration through CREB signaling. There are some concerns that it would be important to address:
1. In figures 2, 3, 4 5, all of the protein level data was from immunostaining evaluation. It would be much more convincing if confirming some of the key data (such as vimentin, GFAP...), using second method like western blotting analysis.
2. For MALAT1 and CREB signaling. It is important to see changed cell viability and cell proliferation under the condition of over-expressing MALAT1 plus inhibiting CREB signaling. Without those evidences, it would not be strong enough to conclude that "MALAT1 regulates Müller and RGC function through CREB signaling".

1st Revision - authors' response 24 November 2015

Referee #1 (Remarks):
This manuscript details the role of MALAT1 (metastasis associated lung adenocarcinoma transcript 1), a noncoding nuclear-enriched component in mouse models of retinal degeneration. The authors also use cell lines (retinal ganglion and muller cells) to come to conclusions related to the mechanism of involvement of MALAT1 in neurodegeneration. Overall, the manuscript could do with proof reading throughout for English grammar corrections. The authors need to define exactly what MALAT1 is, as a reader unfamiliar with this gene will not realize the significance of the findings.
Answer:
Thanks for your suggestion.
We have asked a company to improve the English expression. They claimed that this paper had been edited by a native speaker with science experience. Moreover, we have defined MALAT1 gene in the Introduction section in the revised manuscript.
The use of RGC5 cells should be discouraged in ophthalmology research. These cells are a contaminated cell line and do not represent ganglion cells, (See Krishnamoorthy RR et al., IOVS, 2013). In addition, details need to be included on the Muller cells used in the study as it's not clear where they were sourced.
Answer:
Thanks for your suggestions.
In the revised manuscript, we used the primary rat RGCs instead of RGC-5 cell line to investigate the role of MALAT1 in retinal ganglion cells. Müller cell line, rMC-1 line, was prepared from retinas of rats exposed to 2 weeks of constant light. It was immortalized by transfection with simian virus 40. It was obtained from Dr. V. R. Sarthy (Northwestern University, IL). rMC-1 cells were grown in 5 mM glucose-DMEM supplemented 10% FBS and 1% penicillin-streptomycin (P/S) at 37°C and 5% CO_2 in a humidified incubator.
I don't know what NS stands for in the 3rd and 4th panel of Fig1D.
Answer:
We are sorry for this incorrect abbreviation. NS should be NC (negative control, FISH conducted with MALAT1 sense probe).
Images in Fig.1 E need to be lower magnification and RPE cells should be confluent for immunocytochemistry as their gene expression can change depending on confluency. It is clear from the images that they are sub-confluent. Again, RGC5 cells are now redundant for vision research and should not be included.
Answer:
Thanks for your suggestion.
In the revised manuscript, we have provided the lower magnification for all cells. Moreover, we used the primary rat RGCs instead of RGC-5 to detect MALAT1 expression distribution.

Figure 3
Please label all abbreviations in the figure legends as it is difficult to have to constantly check the main text to ascertain what they refer to.
Answer:
Thanks for your suggestion.

We have labeled all abbreviations in the figure legends.

Figure 4

Again, need clarity on the Muller cells being used and what exactly their source is. Contrary to what the authors state, the data in this figure do not show an alteration in Muller cell function but simply show changes in Muller cell viability following suppression of MALAT1.

Answer:

Thanks for your question.

Müller cell line, rMC-1 line, was prepared from retinas of rats exposed to 2 weeks of constant light. It was immortalized by transfection with simian virus 40. It was from Dr. V. R. Sarthy (Northwestern University, IL).

Müller cells are usually activated against pathogenic stimuli. GFAP up-regulation is the most sensitive response to stress (Bringmann et al, 2009). In the revised manuscript, we also investigated the effect of MALAT1 knockdown on GFAP expression. We found that MALAT1 knockdown could significantly block GFAP up-regulation under high glucose and oxidative stress (Fig. S9), implying a role of MALAT1 in Müller cell gliosis in vitro.

Müller cell gliosis is usually associated with cell proliferation and up-regulation of the intermediated filaments, such as GFAP. Given the critical role of MALAT1 in Müller cell viability, cell proliferation, and GFAP regulation, we thus speculated that MALAT1 is involved in Müller cell gliosis.

Figure 5

These are not retinal ganglion cells that are being transfected in Fig 5A. In fact these cells are not even the same species as they are listed. They are listed as rat cells but are actually mouse. Their use should be discontinued as a ganglion cell line. For example, a recent editorial in Experimental Eye Research states: " Any conclusions made in the published papers using these cells (RGC-5) that relate to retinal ganglion cell-specific responses must be reconsidered. (See, http://www.sciencedirect.com/science/article/pii/S0014483513002418). Therefore, the data in the entirety of Fig 5 cannot robustly be related to retinal ganglion cells.

Answer:

Thanks for your suggestion.

We have deleted all previous results now, and re-conducted this section by using the primary rat RGCs instead of RGC-5 cell line to investigate the role of MALAT1 in ganglion cells.

Figure 7

I'm unsure if the nerve fibre layer is being measured in the OCT images outlined in Fig 7B. It appears to include large swathes of GCL and inner plexiform layer in addition to the inner nuclear layer. This needs clarification and much higher magnification on the area in question. All abbreviations need to be clarified in the figure legend.

Answer:

Thanks for your suggestion.

We have re-conducted this experiment to determine the effect of MALAT1 knockdown on the change of retinal nerve fiber layer (RNFL) thickness using OCT. RNFL consists of the unmyelinated axons of retinal ganglion cells gathered into bundles lying just under the retinal surface. RNFL is damaged in many optic nerve diseases. Optical coherence tomography (OCT) measurement and quantitative analysis revealed that traumatic injury significantly decreased RNFL thickness compared with Wt group. MALAT1 knockdown further decreased RNFL thickness (Fig. 7B).

Figure 8

The analysis of human samples seems redundant to the entire study and the significance is weak overall.

Answer:

Thanks for your suggestion.

We have made an effort to add more data and improve description, analysis and description of clinical information. We hope that our finding has important clinical relevance and usefulness. We also hope taht the provided information could meet the requirement for publication. Please let us know if any further information required.

Referee #2 (Remarks):

Long noncoding RNAs (lncRNAs) are key players in many biological processes and diseases. Previously, the authors have shown that lnc-RNA MALAT1 plays a role in vascular dysfunctions.

Here, the authors investigate the role of MALAT1 after optic nerve transections (ONT) and diabetic retinopathy (DR), likely through CREB phosphorylation. The authors also make the observation that MALAT1 has a role not only in retinal degenerative diseases, such as DR and glaucoma, but also neurodegenerative diseases, such as Alzheimer's disease. The overall conclusion that MALAT1 may be a potential therapeutic target for neurodegenerative diseases is confirmed by the findings of this study, although the direct evidence for MALAT1's role in neurodegeneration is limited and incomplete.

Major Revisions:

1. The paper has many grammatical errors and spelling errors, both within the written manuscript and in the figures. It is extensive, and makes many portions of the manuscript difficult to read. Please correct.

Answer:

Thanks for your suggestion.

We have asked a company to improve the English expression of this paper. They claimed that this paper had been edited by a native speaker with science experience. We hope it could meet the requirement for publication now. We have tried our best to correct all grammatical errors and spelling errors.

2. The conclusions made by the authors of this manuscript tend to be ambiguous or overstated. Most of the discussion section is a repetition of the introduction and the results, with very little discussion included. The discussion section should be re-written, with a focus on the meaning behind the results, the role of CREB phosphorylation in MALAT1 functions for neurodegenerative disease, as well as potential confounders (other signaling pathways, other cell targets of MALAT1 besides Muller glial cells and RGCs, etc.). For instance, the photoreceptor cells were affected after MALAT1 knockdown in DR models, but not in the ONT model. What are the authors' ideas on why this is the case?

Answer:

Thanks for your suggestion. We have re-written the discussion section, with a focus on the meaning behind the results.

We hope it could meet the requirement for publication now.

3. It cannot be said that all neurotrophic factors have a partial reduction after MALAT1 knockdown. Some had significant reductions, while others remained similar to the controls. In addition, the results are not the same between the ONT response and the DR response to MALAT1 knockdown, even though the authors claim that it is. Different factors were reduced, and this should be discussed.

Answer:

We have re-analyzed the result, and corrected the descriptions in the revised manuscript as shown below:

Müller glia is the major glial component of the retina. Its activation protects the retina from a wide variety of pathological stimuli such trauma, ischemia, and degeneration via the release of neurotrophic factors. We revealed that MALAT1 knockdown reduced the expression of neurotrophic factors, including GDNF, NGF, NT-4, and BDNF in ONT retinas (Fig. 3A), and reduced the expression of neurotrophic factors, GDNF, NT-3, CNTF, and BDNF in diabetic retinas (Fig. S6).

We found that MALAT1 knockdown affected the expression of different neurotrophic factors between ONT retinas and diabetic retinas. Why this difference occurred has been discussed in the revised manuscript.

4. Controls for some of the figures are listed as WT, even though there are no WT controls in the figures. Please change this to either DR or ONT as listed in the graphs for the figures.

Answer:

We are sorry for the incorrect labeling.

In the revised manuscript, we have clearly mentioned the control group in each figure.

5. Figure S13 is a duplication of S14. S13 based on the figure legend is missing entirely. Please provide and remove duplicated blots.

Answer:

The previous S13 and S14 has been deleted

Now in Fig. S16,

Mass spectrometric analysis of CREB-interacting proteins affected by MALAT1 led us to focus on PP2A, a protein tyrosine phosphatase and a negative regulator of CREB signaling. Coimmunoprecipitation experiments showed that MALAT1 knockdown potentiated PP2A-CREB

interaction, whereas MALAT1 overexpression attenuated PP2A-CREB interaction in Müller cells (Fig. S16). CREB continuous activation usually attenuates via the dephosphorylation by phosphatases PP-1 and PP-2A. We also determined the effect of MALAT1 intervention on PP-1-CREB interaction (Fig. S16). The result showed that neither MALAT1 knockdown nor its overexpression affected PP-1-CREB interaction in Müller cells.

Moreover, we also found that MALAT1 intervention affects CREB-PP2A, but not CREB-PP-1 interaction in primary RGCs (Fig. S18).

We are sorry for the incorrect labeling in the previous manuscript.

6. Is there any significance for Figure 5G (and corresponding supplementary figure)? This should be discussed in the discussion section.

Answer:

We have discussed the result of Figure 5G in the discussion section now.

7. Figure 6 results section mentions MALAT1 in a CREB immunoprecipitate, as well co-localization of MALAT1 and CREB in RGCs using FISH. There are no references to Figure 6 or any other figure, and no data appears to match these statements. The authors should provide this data or remove the statements from the manuscript.

Answer:

We are sorry for our previous mistakes.

We also investigate whether MALAT1-mediated CREB signaling involved in regulating RGC function. RIP experiment showed that MALAT1 was abundantly existed in CREB-immunoprecipitate (Fig. S17A). FISH followed immunofluorescence revealed the co-localization between MALAT1 and CREB in primary RGCs (Fig. S17B). CREB knockdown impaired cell viability and proliferation in a manner similar to MALAT1 knockdown in RGCs, whereas CREB overexpression was able to rescue the effect of MALAT1 knockdown. MALAT1 overexpression-induced abnormal cell viability and hyper-proliferation was interrupted when CREB signaling was inhibited (Fig. S17C and S17D).

8. The mechanism in Figure 6G is not mentioned in the results. If the authors would like to use this mechanism, it should be its own figure discussed in the discussion section. Please move accordingly.

Answer:

Thanks for your suggestion.

We have deleted the previous figure 6G in the revised manuscript.

9. Has the role of PP2A been examined for the RGCs, and not just the Muller glial cells? How about the effect of PP1, as the authors mention it in their discussion section?

Answer:

Thanks for your suggestion.

We have examined the role of PP2A in the RGCs. Coimmunoprecipitation experiments showed that MALAT1 knockdown potentiated PP2A-CREB interaction, whereas MALAT1 overexpression attenuated PP2A-CREB interaction in primary RGCs (Fig. S18).

CREB continuous activation usually attenuates via the dephosphorylation by phosphatases PP-1 and PP-2A. We also determined the effect of MALAT1 intervention on PP1-CREB interaction. The result showed that neither MALAT1 knockdown nor its overexpression affected PP-1-CREB interaction in Müller cells (Fig. S16). We also found that MALAT1 intervention did not affect CREB-PP-1 interaction in primary RGCs (Fig. S18).

10. Figure 7A needs to have the wave labels moved so that they can be read. The scrambled siRNA also looks similar to the VEP result for the MALAT1 siRNA. Since the authors ran 100 traces per animal, they should provide a quantification of the wave values. Please add this quantification and statistical analysis to show the true effect of MALAT1 knockdown.

Answer:

Thanks for your suggestion.

We have re-conducted this experiment to determine the effect of MALAT1 knockdown on visual function using visual evoked potentials (VEP). The representative VEP waveforms for each group were shown. Compared with ONT mice, MALAT1 knockdown could further increased the latency and reduced the amplitude of VEP. These VEP recordings indicated that MALAT1 knockdown could aggravate visual damage under traumatic condition (Fig. 7A). Moreover, we conducted the statistical analysis to show the true effect of MALAT1 knockdown (Fig. 7A).

11. Figure 7C-D should be quantified. Statistical analysis is needed.

Answer:

Thanks for your suggestion.

We have provided the statistical analysis for Figure 7C and 7D in the revised manuscript.

12. Patient samples are not well described in the methods section. As these are neurodegenerative diseases, they are affected by aging. Were the samples taken from age-matched patients? What were the respective controls? Listing the age range is necessary in these cases. Also, potential gender bias could play a role. This can be discussed in the discussion section with other confounders.
Answer:

Thanks for your suggestion.

We have provided more detailed information about patients' samples as shown in supplementary materials and Table S1, S2 and S3. We have paid great attention to age/ gender. MALAT1 expression was compared using the age-matched samples. We hope the provided information could meet the requirement for publication. Please let us know if any further information required. We also discussed this section in the discussion section.

Minor Revisions:
1. Acronyms for experimental methods, gene names, etc. need to be written out completely the first time that they appear in the manuscript. Then they can be shortened for the remainder of the manuscript. Please correct.
Answer:

Thanks for your suggestion.

We have corrected these errors.

2. For figures using glutamate excitotoxicity and stress responses, the time between ONT and the treatment groups is not listed, only that the ONT treated groups were kept for 48 hours prior to testing. Please include the time between optic nerve transections and stress/drug treatments.
Answer:

Thanks for your suggestion.

We have provided detailed information in the figure legend.

3. The injection procedures are not included in the methods. In addition, the route of injection is not always listed in the figures. In some, intravitreal injections are mentioned, but in others, it merely says retinal delivery. Is this a subretinal injection? Intravitreal? Please be specific.
Answer:

Thanks for your suggestion.

We have provided the specific injection procedures and methods in the revised manuscript.

4. For the injections, the authors state that the siRNA or treatment is delivered for the amount of time listed in the graphs. Is this a single injection? A weekly injection? At zero weeks, are the rats/mice uninjected? Please be clear.
Answer:

Thanks for your suggestion.

All injections were a weekly injection.

At zero weeks, the rats/mice are injected. We injected the rats/mice shRNA, and immediately extracted RNA from retinas. We aimed to determine whether injection operation affected MALAT1 expression. The result showed that only operation did not affect MALAT1 expression at zero weeks. We feel this result would create ambiguity. Thus, we deleted it in the revised manuscript.

5. The contrast for the immunofluorescence images in each of the figures is very high. Please reduce.
Answer:

Thanks for your suggestion.

We have reduced the contrast for the immunofluorescence images.

*6. The figures list * and # as potential significance, but the p-values and comparison groups for these are only listed in one figure legend. Please include for all relevant figures.*
Answer:

P-values and comparison groups have been clearly described now.

7. Figure legends write out the acronym RGC, but it is not in a single figure of this manuscript.
Answer:

We have written out the acronym RGC in the figure legend.

8. Scale bars are listed for all but one figure. However, in many figures the retinal histology images appear at the same magnification but the scale bars in the image are different sizes. Please make sure that the scale bar values are correct for all images.
Answer:

We are sorry for previous mistakes. We have carefully checked in the revised manuscript, and all scale bar value is correct now.

9. Figure 1D qPCR has black outlines for parts of the graph, and not for others. Please correct.
Answer:
We have corrected it.
10. In Figure 3 and S5, the BDNF treatment groups are either missing or not labeled. Please correct.
Answer:
The BDNF treatment group has been labeled figure 3 and S6 (Previous S5).
11. Figure 4E is missing labels. As are other immunofluorescent panels in multiple figures. Please provide information on what is stained for all figures.
Answer:
We have labeled Figure 4E.
We have provided information on what was stained for all figures.
12. Figure 5C is incorrectly organized, panels are not in the appropriate locations. Please correct.
Answer:
We have re-organized Figure 5 in the revised manuscript.
13. In Figure 6D the y-axis is cut off and should be corrected.
Answer:
We have corrected it.
14. Stating that glaucoma is an ocular Alzheimer's disease is debatable, the reasoning for looking into glaucoma patients should be restated.
Answer:
We have removed the debatable expression. In the revised manuscript, we have restated why we selected glaucoma patients for study. We mainly selected primary open-angle glaucoma patients due to it is a progressive optic neuropathy.
15. Citations should not be listed in methods section titles. Please move to the paragraph below.
Answer:
We have moved the citations to the paragraph below.
16. Sample sizes are missing for many graphs that contain error bars and statistical analyses. Please list the N for these in the figure legends.
Answer:
We have provided the missing N for relevant figure.
17. There are multiple occasions when the authors state that an experimental method was performed "as shown." There are no references, no video files, and therefore they need to provide the experimental methods or a reference for these techniques.
Answer:
We have corrected these descriptions, and provided the experimental methods in the revised manuscript.
18. S12 figure legend does not mention BDNF or GDNF treatment groups, n, or p-values.
Answer:
Now the figure is S15. We have mentioned BDNF or GDNF treatment groups, n, or p-values in the revised manuscript.

Referee #3 (Remarks):
In this manuscript, the authors have investigated the role of Long noncoding RNA-MALAT1 in neurodegeneration, Using rodent ONT models and RGC/Muller cell cultures. This is a well designed study with some interesting and potentially important findings.
The authors found that MALAT1 expression is up-regulated in ONT and diabetic retinas, and in cultured cells upon stress with (Hypoxia, HG, H_2O_2, Glutamate). Knockdown of MALAT1 affects RGC survival and Muller glial activation in animal retinas. In cell culture, Knockdown of MALAT1 affects cellular functions of Muller and RGC cells. With VEP, OCT and histological study on rodent eyes, they found that MALAT1 knockdown decreased p-wave amplitude, RNFL thickness and induced more swellings / fragmentation/demyelination of RGC axons respectively. They also found MALAT1 regulates Muller and RGC function through CREB signaling. They concluded that Long noncoding RNA-MALAT1 regulates retinal neurodegeneration through CREB signaling. There are some concerns that it would be important to address:
1. In figures 2, 3, 4 5, all of the protein level data was from immunostaining evaluation. It would be much more convincing if confirming some of the key data (such as vimentin, GFAP...), using second

method like western blotting analysis.
Answer:

Thanks for your suggestion.

In the revised manuscript, we have used the western blots to verify that MALAT1 knockdown reduced vimentin, GFAP, NeuN and TUBB3 expression levels (Fig. S3). MALAT1 knockdown did not affect the expression of calbindin, Rhodopsin, PKCα, and calretinin (Fig. S3). Western blots also showed that MALAT1 knockdown affected the expression of progenitor markers such as nestin and vimentin (Fig. S7).

2. For MALAT1 and CREB signaling. It is important to see changed cell viability and cell proliferation under the condition of over-expressing MALAT1 plus inhibiting CREB signaling. Without those evidences, it would not be strong enough to conclude that "MALAT1 regulates Muller and RGC function through CREB signaling".
Answer:

Thanks for your suggestion.

In the revised manuscript, we showed that CREB knockdown impaired Müller cell viability and cell proliferation in a manner similar to MALAT1 knockdown, whereas CREB overexpression was able to rescue the effect of MALAT1 knockdown. MALAT1 overexpression-induced abnormal cell viability and hyper-proliferation was interrupted when CREB signaling was inhibited (Fig. 6E and 6F). In RGCs, we observed similar phenomena (Fig. S17C and S17D).

2nd Editorial Decision 18 December 2015

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now heard back from the three Reviewers whom we asked to re-evaluate your manuscript.

You will see that while the Reviewers are globally positive, Reviewers 2 and 3 especially, still have important reservations and comments, which we find all pertinent. One shared concern is that there is still a certain degree of inaccuracy in reporting of experimentation and English usage.

I look forward to seeing a revised form of your manuscript as soon as possible.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System):

While the authors have removed the RGC-5 data, the question still remains as to the specificity of the effects they see in their experiments.

Referee #1 (Remarks):

I am still not convinced by the clinical data and feel it should be removed from the study.

Referee #2 (Comments on Novelty/Model System):

Technical Quality:
The role of MALAT1 in mouse and rat models of trauma and diabetic retinopathy were well-presented, with a large variety of experimental techniques and statistical significance. The authors addressed most of my previous concerns for the clinical analysis for the role of MALAT1.

Novelty:
CREB and PP2 were already published to be involved with MALAT1 lncRNA. Therefore, the only novel finding is the roles that MALAT1 has in many neurodegenerative diseases. The authors enhanced their analysis for the interaction of MALAT1, CREB phosphorylation and PP2, although it is still limited on the exact mechanism that ties together each of the studied disorders.

Medical Impact:
The fact that the authors show a role for MALAT1 lncRNA in a variety of complex human diseases increases its likelihood to be a target for therapeutic drugs and clinical trials.

Adequacy of Model System:
There are a variety of model systems used in this manuscript, including rat and mouse models of trauma (ONT) and diabetic retinopathy. Human cell lines and human patient samples were also analyzed. Based on the wide range of diseases and analysis, the evidence provided by the authors shows that MALAT1 lncRNA is involved in each disease model, but further data is needed for each disease to determine the full mechanism of action for MALAT1.

Referee #2 (Remarks):

Long noncoding RNA-MALAT1 regulates retinal neurodegeneration through CREB signaling

Long noncoding RNAs (lncRNAs) are key players in many biological processes and diseases. Previously, the authors have shown that lnc-RNA MALAT1 plays a role in vascular dysfunctions. Here, the authors investigate the role of MALAT1 after optic nerve transections (ONT) and diabetic retinopathy (DR), likely through CREB phosphorylation. The authors also make the observation that MALAT1 has a role not only in retinal degenerative diseases, such as DR and glaucoma, but also neurodegenerative diseases, such as Alzheimer's disease. The overall conclusion that MALAT1 may be a potential therapeutic target for neurodegenerative diseases is confirmed by the findings of this study, although the direct evidence for MALAT1's role in neurodegeneration is limited.

Major Revisions:
The authors provided a greatly improved discussion section and addressed most of my previous concerns. There are still some points to consider:

1. The staining for calbindin and calretinin in supplementary figure 2 shows a significant decrease of both proteins when examined by immunofluorescence. However, supplementary figure 3 shows western blot results where there is no change in protein levels. Please explain.

2. Supplementary figure 5 repeats supplementary figure 2 and supplementary figure 3 for DR instead of ONT models. However, the authors only look at fluorescence and do not perform the western blot analysis. As there were differences in expression based on the analysis, a western blot should be run for the DR model system.

3. The authors corrected many of the grammatical and spelling errors in the manuscript, however many of them are still present and the English will need to be revised before publication.

Minor Revisions:

1. Figure 1D qPCR has black outlines for parts of the graph, and not for others. Please correct.

2. Figure 1 has a scale bar that is a different size than the others, with only one scale bar size noted in the figure legend. Please correct.

3. Multiple figures claim statistical significance with bar graphs that have overlapping error bars (e.g. Figure 2, supplementary figure 10, supplementary figure 13). These are questionable, especially since error bars represent SEM and not standard deviations. Can the authors explain this?

4. Immunofluorescent panels in multiple figures are missing staining information in the figure itself. Please provide information on what is stained for all figures. They also correspond to bar graphs but it is not clear without the legends which graph relates to which staining. Please organize as well as possible.

5. Figure 5E is incorrectly organized, panels are not in the appropriate locations. Please correct.

6. Figure 6 and supplementary figure 1 have symbols for p-values, but these are not listed in the figure legend. Please correct.

7. Figure 7A has a p-value listed in the figure legend that is not in the figure itself. Please correct.

8. Supplementary figure 14 has error bars with SEM shown above and below the mean, and some only above. Please make consistent with other figures/graphs.

Referee #3 (Remarks):

1.The authors have addressed all the issues raised from my previous comments.
2.The authors should carefully check their work for accuracy and consistency. For example, in revised Figure legend Fig. 6, listed as "MALAT1 regulates Müller and RGC cell function through CREB signaling", but the descriptions, from A to F, are all about Müller cells--not RGCs.

2nd Revision - authors' response 05 January 2016

***** Reviewer's comments *****

Referee #1
I am still not convinced by the clinical data and feel it should be removed from the study.
Answer:

Thanks for your suggestions.

RGC degeneration and reactive gliosis are two important features of retinal neurodegeneration. MALAT1 is dysregulated in the retinas of ONT and DR animals which are associated with retinal neurodegeneration. MALAT1 can regulate the function of RGCs and Müller cells in vivo and in vitro. Thus, it is not surprised that MALAT1 is involved in retinal neurodegeneration.

The eye is known as an extension of the brain. It displays many similarities to the brain in terms of anatomy, functionality, stress response, and immunology. Alzheimer's disease (AD) is a neurodegenerative disease characterized by neuropathological changes in the brain. MALAT1 levels in CSF are down-regulated in AD patients compared with the age-matched controls. Glioma is the most common and aggressive brain tumor with poor clinical outcome. MALAT1 levels are up-regulated in glioma tissues compared with peritumoral tissues. Glaucoma is characterized by progressive retinal ganglion cell death. Glaucomatous injury to retinal ganglion cells has also profound effects on target vision structures within the brain. MALAT1 dysregulation would affect RGC survival, and alter the development of glaucomatous neurodegeneration. Collectively, MALAT1 dysregulation is emerging as a common pathological feature in neurodegenerative and neuro-oncological disorders. MALAT1 intervention may become a potential target for treating these diseases. We hope this study could combine the clinical research and basic biology, and our finding is relevant to human disease. Thus, we still provide these data in the revised manuscript. Additional studies are required to verify the direct role of MALAT1 in AD, glioma , and glaucoma in the future studies.

Referee #2 (Remarks):
Major Revisions:
The authors provided a greatly improved discussion section and addressed most of my previous concerns. There are still some points to consider:
1. The staining for calbindin and calretinin in supplementary figure 2 shows a significant decrease of both proteins when examined by immunofluorescence. However, supplementary figure 3 shows western blot results where there is no change in protein levels. Please explain.
Answer:

Thanks for your suggestion.

Calretinin is expressed by ganglion cells and amacrine cells, whereas calbindin is mainly expressed by ganglion cells, amacrine and horizontal cells. Immunolabeling experiments showed

that calretinin-labeled cells in the GCL and calbindin-labeled cells in the GCL were affected by MALAT1 knockdown. We detected the number of Calretinin or calbindin-positive cells in different retinal layer. “The change of cell number” is not the same as “the change of protein expression levels”. Western blots provide the ability to detect specific protein expression levels from cells or tissues. Western blot signal was visualized by chemiluminescence (HRP-conjugated secondary antibody), whereas immunolabeling signal was visualized by fluorescence. Chemiluminescence is the production of visible light (luminescence) occurring as a result of a chemical reaction. Fluorescence occurs when light is absorbed from an external (excitation) source by a fluorescent molecule (fluorophore) and subsequently emitted. Chemiluminescence is typically about 2 orders of magnitude more sensitive than fluorescence. Although we did not detected the number change of calretinin-labeled cells in the INL, and the number change of calbindin-labeled horizontal and amacrine cells by fluorescence experiments, we cannot rule out the possibility that expression of calretinin-labeled cells in the INL, and the change of calbindin-labeled horizontal and amacrine cells was altered when detecting by chemiluminescence (western blots). Western blots detected the total amount of calretinin and calbindin levels in the retinas. However, they cannot differentiate calretinin and calbindin expression in different retinal cells. Fluorescence experiments can differentiate calretinin and calbindin expression in different retinal cells. Calretinin and calbindin may have opposite expression pattern in different retinal cells when detecting in high sensitivity of western blots, whereas the total amount of calretinin and calbindin may not change. In addition, calretinin or calbindin in the GCL only accounted for a small fraction of the total calretinin or calbindin expression in the retinas. If the expression of calretinin or calbindin in different retinal cells were calculated together, we may not detect the statistical difference of total calretinin or calbindin expression affected by MALAT1 knockdown.

ONT animal models only lasted for two weeks, while DR animal models last for about six months. We detected the expression change of total calbindin by western blots in diabetic retinas. We speculated that the change of total calretinin or calbindin expression may experience a very long time. Thus, we did not find the change of total calretinin or calbindin expression in ONT animal models due to a short time than DR animals.

2. Supplementary figure 5 repeats supplementary figure 2 and supplementary figure 3 for DR instead of ONT models. However, the authors only look at fluorescence and do not perform the western blot analysis. As there were differences in expression based on the analysis, a western blot should be run for the DR model system.

Answer:

Thanks for your suggestion.

We are sorry for not providing the data of western blots for diabetic retinas in the previously revised manuscript.

Retinal neurodegeneration is also implicated in the pathogenesis of diabetic retinopathy. We observed a similar scenario in diabetic rat retinas as shown in ONT retinas. Fluorescence experiments revealed that MALAT1 knockdown affected reactive gliosis and RGC survival, but had a minor effect on the number of horizontal cells, amacrine cells, photoreceptors, and bipolar cells. Western blots revealed that MALAT1 knockdown reduced vimentin, GFAP, NeuN and TUBB3 expression levels in the retinas of diabetic rats (Supplementary Fig S6).

3. The authors corrected many of the grammatical and spelling errors in the manuscript, however many of them are still present and the English will need to be revised before publication.

Answer:

Thanks for your suggestion.

We have asked another company which provides English Language Editing Services to revise this manuscript. They claimed that this paper had been edited by a native speaker with science experience. We also carefully checked, and corrected the grammatical and spelling errors. We hope it could meet the requirement for publication now.

Minor Revisions:

1. Figure 1D qPCR has black outlines for parts of the graph, and not for others. Please correct.

Answer:

Thanks for your suggestion.

We have corrected this error.

2. Figure 1 has a scale bar that is a different size than the others, with only one scale bar size noted in the figure legend. Please correct.

Answer:

Thanks for your suggestion.

We have corrected this error.

3. Multiple figures claim statistical significance with bar graphs that have overlapping error bars (e.g. Figure 2, supplementary figure 10, and supplementary figure 13). These are questionable, especially since error bars represent SEM and not standard deviations. Can the authors explain this?

Answer:

Thanks for your suggestion.

SD error bars quantify the scatter among the values. Looking at whether the error bars overlap lets you compare the difference between the mean with the amount of scatter within the groups. But the *t* test also takes into account sample size. If the samples were larger with the same means and same standard deviations, the *P* value would be much smaller. If the samples were smaller with the same means and same standard deviations, the *P* value would be larger. When the difference between two means is statistically significant ($P < 0.05$), the two SD error bars may or may not overlap. Likewise, when the difference between two means is not statistically significant ($P > 0.05$), the two SD error bars may or may not overlap. Thus, knowing whether SD error bars overlap or not does not let you conclude whether difference between the means is statistically significant or not.

SEM error bars quantify how precisely you know the mean, taking into account both the SD and sample size. Looking at whether the error bars overlap, therefore, lets you compare the difference between the mean with the precision of those means. By taking into account sample size and considering how far apart two error bars are, Cumming (2007) came up with some rules for deciding when a difference is significant or not. If two SEM error bars do overlap, and the sample sizes are equal or nearly equal, then you know that the P value is (much) greater than 0.05, so the difference is not statistically significant. The opposite rule does not apply. If two SEM error bars do not overlap, the *P* value could be less than 0.05, or it could be greater than 0.05. If the sample sizes are very different, this rule of thumb does not always work.

According to the above-mentioned descriptions, we speculated that the error bars overlapped may be the following reasons: (1) mistakenly used SD instead of SEM; (2) great variability in biology experiments. To solve these problems, we have carefully checked all bar graphs, and re-conducted the statistical analysis with the help of a statistician to guarantee the correctness of statistical analysis. As for some results which had overlapped error bars, we re-conducted some experiments, and conducted reduplicate experiments to increase the sample size from n=5 to n=8 for some data in Figure 2, supplementary figure 10, and supplementary figure 13.

It is generally known that the SEM quantifies how accurately you know the true mean of the population. The SEM gets smaller as your samples get larger. This makes sense, because the mean of a large sample is likely to be closer to the true population mean than is the mean of a small sample. However, it is difficult to unboundedly increase sample size of experimental groups due to the heavy workload and high cost. Thus, at the beginning of this study, we used more than one methods to detect cell function, such as MTT, Hoechst 33342, Calcein-AM/PI, JC-1 staining. Unavoidably, it may detect the statistical significance from the result of one method, but not in another method. When increasing the number of independent experiments, we felt we may get the objective and satisfactory answers. The error bars and other statistics is only a guide. We also need to use our biological understanding to appreciate the meaning of the numbers shown in any figure.

We are sorry for our previous errors. We ensured that all data were obtained from numerous cell and animal experiments. Thanks for your valuable knowledge of statistics.

4. Immunofluorescent panels in multiple figures are missing staining information in the figure itself. Please provide information on what is stained for all figures. They also correspond to bar graphs but it is not clear without the legends which graph relates to which staining. Please organize as well as possible.

Answer:

Thanks for your suggestion.

We have corrected these errors, and organized as well as possible.

5. Figure 5E is incorrectly organized, panels are not in the appropriate locations. Please correct.

Answer:

Thanks for your suggestion.

We have corrected this error.

6. Figure 6 and supplementary figure 1 have symbols for p-values, but these are not listed in the figure legend. Please correct.

Answer:

Thanks for your suggestion.

We have corrected these errors.

7. Figure 7A has a p-value listed in the figure legend that is not in the figure itself. Please correct.

Answer:

Thanks for your suggestion.

We have corrected this error.

8. Supplementary figure 14 has error bars with SEM shown above and below the mean, and some only above. Please make consistent with other figures/graphs.

Answer:

Thanks for your suggestion.

We have made the error bars consistent for all figures/graphs.

Referee #3 (Remarks):

1.The authors have addressed all the issues raised from my previous comments.

Answer:

No response.

2. The authors should carefully check their work for accuracy and consistency. For example, in revised Figure legend Fig. 6, listed as "MALAT1 regulates Muller and RGC cell function through CREB signaling", but the descriptions, from A to F, are all about Muller cells--not RGCs.

Answer:

Thanks for your suggestion.

In the revised manuscript, we carefully checked our work for accuracy and consistency. We hope that there are no errors existed now.

3rd Editorial Decision 27 January 2016

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine.

We have now received the enclosed report from the reviewer who was asked to re-assess it. As you will see that s/he is now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendment:

Please note that, as the reviewer mentions, your manuscript could still benefit from a careful revision of English usage in the manuscript.

***** Reviewer's comments *****

Referee #2 (Comments on Novelty/Model System):

Technical Quality:
The role of MALAT1 in mouse and rat models of trauma and diabetic retinopathy were well-presented, with a large variety of experimental techniques and statistical significance. The authors addressed most of my previous concerns for the clinical analysis for the role of MALAT1.

Novelty:
CREB and PP2 were already published to be involved with MALAT1 lncRNA. Therefore, the only novel finding is the roles that MALAT1 has in many neurodegenerative diseases. The authors enhanced their analysis for the interaction of MALAT1, CREB phosphorylation and PP2, although it is still limited on the exact mechanism that ties together each of the studied disorders.

Medical Impact:
The fact that the authors show a role for MALAT1 lncRNA in a variety of complex human diseases increases its likelihood to be a target for therapeutic drugs and clinical trials.

Adequacy of Model System:

There are a variety of model systems used in this manuscript, including rat and mouse models of trauma (ONT) and diabetic retinopathy. Human cell lines and human patient samples were also analyzed. Based on the wide range of diseases and analysis, the evidence provided by the authors shows that MALAT1 lncRNA is involved in each disease model, but further data is needed for each disease to determine the full mechanism of action for MALAT1.

Referee #2 (Remarks):

The authors addressed most of my previous concerns, although the clinical data is still limited and there are grammatical errors still present in the manuscript.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

Corresponding Author Name: Biao Yan, Qin Jiang
Journal Submitted to: EMOBO MOL MED
Manuscript Number: EMM-2015-05725

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

B- Statistics and general methods

	Please fill out these boxes ↓
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample sizes for all experiments were chosen based on previous experiences
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We have provided the sample size in animal studies.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Data were tested for normality by the D'Agostino–Pearson omnibus normality test and similar variance by F test.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Data entry and all analyses were performed in a randomization procedure.
For animal studies, include a statement about randomization even if no randomization was used.	Data were tested for normality by the D'Agostino-Pearson omnibus normality test and similar variance by F test.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	All analyses were performed in a blinded fashion.
4.b. For animal studies, include a statement about blinding even if no blinding was done	We conducted the animal studies in a blined fashion
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Comparison between any two groups was by two-tailed unpaired t test for normally distributed data or non-parametric Mann-Whitney test for non-normally distributed data. Multiple group comparison was done by one-way analysis of variance (ANOVA) for data with normal distribution. The Kruskal-Wallis test was used for data with non-normal distribution.
Is there an estimate of variation within each group of data?	Yes
Is the variance similar between the groups that are being statistically compared?	Yes

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C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We have provdied the detailed information of antibodies used in this study including the company and the dilution.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Müller cell line, rMC-1 line, was prepared from retinas of rats exposed to 2 weeks of constant light. It was immortalized by transfection with simian virus 40. It was from Dr. V. R. Sarthy .

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	we have provided all the required information.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All experimental animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approved by the Animal Care and Use Committee of Nanjing Medical University.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We have consulted the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	The clinical study was approved by the ethics committee of Nanjing Medical University (Nanjing, China). The surgical specimens were handled according to the Declaration of Helsinki.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	All patients were gave the informed consent before inclusion.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	No photo published
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	No further restrisions on the availability of human data or samples
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	No clinical trial invovled in this study.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	No phase II and III controlled trials is invovled in this study.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	The study is not a tumor marker prognostid study.

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	No deposited data in this study.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	All siRNA and shRNA sequences have been provided in the supplementary document.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	No restrictions on human clinical and genomics datasets.
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	No computational models exised in this study.

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	The study does not fall under dual used research restrictions.