EMBO reports

Planar cell polarity gene *Fuz* triggers apoptosis in neurodegenerative disease models

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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 19 December 2017

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees consider the findings of potential interest but they also note that further work is required to substantiate them. Furthermore, overstatements should be avoided, as indicated by referee 2. In particular, it will be important to further evaluate the threshold at which Fuz protein levels become toxic (ref 1, point 1) and to analyze the effect of core PCP genes and the role of Dvl in Fuz-mediated neuronal toxicity (ref 1, point 2).

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional

Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

Regarding data quantification, can you please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends? This information is currently incomplete and must be provided in the figure legends. Please also include scale bars in all microscopy images.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure or per figure panel.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
(Please see also our figure guidelines on the technical requirements for figure in EMBO press: http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf)
- a separate PDF file of any Supplementary information (in its final format)
- all corresponding authors are required to provide an ORCID ID for their name. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (http://embor.embopress.org/authorguide).

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS
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Referee #1:

The study by Chen et al showed that Fuz, a conserved PCP pathway effector, is highly expressed in the brain, but its overexpression can also promote Dvl punctae formation and induce apoptosis through the Dvl/Tiam/Rac1/MEKK/JNK/Caspase pathway in multiple assay systems. Moreover, in

both cell and animal models as well as human samples, Fuz showed elevated expression in multiple neurodegenerative diseases including polyQ, AD and PD, while its depletion suppressed the toxicity of polyQ proteins. The authors further provided evidence that polyQ-mediated up-regulation of Fuz is at epigenetic level through transcriptional repressor YY1 by sequestering YY1 into protein aggregates, thereby relieving the YY1-mediated hypermethylation of its promoter region. Lastly, the up-regulation of Fuz protein in several protein misfolding diseases might be explained by the observed hypomethylation of multiple Fuz promoter regions, mostly through YY1 except for that by aSynuclein. Together, the authors suggested that the transcriptional depression might induce Fuz-mediated pro-apoptotic pathway in multiple neurodegeneration diseases and contribute to their pathogenesis.

Overall this study has many interesting observations, the findings that Fuz is up-regulated in multiple brain diseases, mostly by epigenetically through YY1, and can promote cell death are novel and could potentially be valuable to the neurodegenerative field.

One main weak point is that as the study covered too many angles, it is a bit too spread out and not able to provide a deeper, more clear mechanistic picture of the pathways/regulation, either on the role of PCP or on YY1. Addressing some of the questions should strengthen the work and its significance.

Main concerns:

1. The level of Fuz that can cause toxicity: it was shown that endogenous Fuz is already highly expressed in the brain (Fig 1A). This raises the question on the threshold of the Fuz level when it become toxic: in Fuz overexpression studies, what are its expression levels as compared to endogenous Fuz? At what level will it become toxic to neurons? Is the overexpression effect too artificial?
Similarly, what are the levels of Fuz protein in polyQ and other neurodegenerative diseases? Are they significantly higher enough to cause toxicity? Table 1S only compiles the data on the levels of Fuz transcripts from different studies, not protein levels. It is not sure if Fuz levels in these setting are sufficient to cause toxicity.

2. The role of the general PCP pathway in Fuz-mediated apoptosis: considering Fuz is a conserved effector of PCP pathway, will manipulation of PCP pathway in general, such as modulating Dvl and Flamingo, affect the same apoptosis pathway, or is it just a function unique to Fuz? The functional significance of this important and well-studied pathway in Fuz-mediated apoptosis should be investigated in more details. The study touched on Dvl and the role of Fuz in affecting Dvl punctae formation, although this physical association of Dvl in Fuz-mediated neuronal toxicity has not been followed up further.
3. Similarly for YY1. The observation that YY1 is sequestered into aggregates in polyQ diseases and affect Fuz expression in several brain diseases is also interesting. This could suggest that YY1 is innately more sensitive to protein misfolding stress.
-What is the structural basis in YY1 protein for such sequestration in aggregates? Is there a polyQ or prion-like protein stretch in YY1? Will manipulation of such domain affect YY1 sequestration and Fuz expression?
- Given its central role in regulating Fuz expression, does YY1 itself affect neurodegeneration? This can be tested easily in the models used in the study.
-Is YY1 also being sequestered into plaques/aggregated in Tau and Abeta expressing cells, given its effect in regulating Fuz in these settings?

Minor issues:
1. Page 3: The following statement should be modulated, as it is not clear if neurodegeneration is really an apoptosis process or totally distinct. "Apoptosis has been reported as an essential executor for neuronal cell death in polyQ diseases (12, 13). Besides, the caspase cascade, a crucial mediator of apoptotic induction, is known to play key roles in polyQ neurodegeneration (14, 15)."
2. Fig S1B & C: will Fuz depletion also affect Dvl punctae formation?
3. YY1 binding site in Fuz promoters: are the YY1 site and CpG island also conserved in fly genome, considering that Fuz is also up-regulated in fly models of polyQ diseases?
4. Specific effect of YY1. Besides YY1, are there any other recognizable binding sites for other transcription factors in the polyQ-responsive promoter region of Fuz gene? Do they also get

sequested into aggregates and do they also potentially play any role in Fuz expression and toxicity in disease setting? This might help address how specific is YY1 in regulating Fuz expression.
5. Page 10, Fig 4A and 4B: "When compared with age-matched controls, all patientbrain samples exhibited reduced YY1 protein levels (Fig. 4 A and B)." This statement does not match well with the data in Figure 4A, as several controls also have quite low YY1 expression.

Referee #2:

Building previous data supporting a role for Fuz in regulating cellular proliferation versus apoptosis, this study presents data supporting the concept that Fuz is upregulated in neurodegenerative diseases, notably models of SCA3, via alterations in its promoter region methylation by a reduction in YY1. They further provide data implicating this pathway more broadly in PD, tauopathies, and AD. Overall this study presents data in support of an intriguing hypothesis. However, there are some issues which if addressed would enhance the impact of this work.

1. Throughout the manuscript the authors indicate that Fuz pathway has a major/critical role in regulating apoptosis in neurodegeneration. Yet in several instances the data indicate that Fuz is at best one of many regulators of cell death, e.g Figure 2A where a complete loss of Fuz reduced cell death by about 30%. A prudent course would be for the authors to present a more balanced view of the role of Fuz.

2. On page 11 based on data generated using Q81-EGFP-myc the authors conclude that Fuz has a general role in polyQ disorders. Their rational this is the case since this construct devoid of "disease protein-specific sequences, i.e. polyQ diseases are due to expression of a toxic polyQ peptide. This is a very controversial point and by no means universally accepted by the field. One could also argue that these data indicate that Fuz has a role in highly toxic situations and not is disease relevant conditions.

3. At several key places quantitative data are lacking. Most notably, to this reviewer, is Figure 1n-p.

1st Revision - authors' response 19 March 2018

Referee #1:

Overall this study has many interesting observations, the findings that Fuz is up-regulated in multiple brain diseases, mostly by epigenetically through YY1, and can promote cell death are novel and could potentially be valuable to the neurodegenerative field.
We thank the referee for his/her praise of our work.

One main weak point is that as the study covered too many angles, it is a bit too spread out and not able to provide a deeper, more clear mechanistic picture of the pathways/regulation, either on the role of PCP or on YY1. Addressing some of the questions should strengthen the work and its significance.
Based on the comments and suggestions received, we have performed additional experiments in an attempt to provide a deeper and clear mechanistic understanding on the role of PCP proteins in apoptosis, as well as the role of YY1 in multiple disease models of neurodegeneration.

Main concerns:

1. The level of Fuz that can cause toxicity: it was shown that endogenous Fuz is already highly expressed in the brain (Fig 1A). This raises the question on the threshold of the Fuz level when it become toxic: in Fuz overexpression studies, what are its expression levels as compared to endogenous Fuz? At what level will it become toxic to neurons? Is the overexpression effect too artificial?
Similarly, what are the levels of Fuz protein in polyQ and other neurodegenerative diseases? Are they significantly higher enough to cause toxicity?

To investigate at which level Fuz protein becomes toxic, we overexpressed flag-Fuz in a dose dependent manner in rat primary cortical neurons to determine apoptosis induction using cleaved caspase-3 as readout. Caspase-3 cleavage was first observed in neurons transfected with 0.6 μg of *flag-Fuz*. Under this condition, the relative level of overexpressed flag-Fuz protein is approximately 2.5-fold higher than that of the endogenous Fuz protein (Fig 1A). Meanwhile, we observed a significant elevation of neuronal cell death in these neurons (Fig 1B). These results therefore suggest that a 2.5-fold increase of Fuz protein expression is sufficient to trigger apoptosis and cause cell death in neurons.

We also quantified the induced Fuz protein level in Fig 5A. When rat primary cortical neurons were treated with $A\beta_{1-42}$ peptide, or transfected with *Htttr-Q92*, *ATXN3tr-Q78*, *α-synuclein*, *Tau*, Fuz protein was induced at least 4-fold relative to controls (Fig 5A). It is worth noting that the induction level of endogenous Fuz protein in these disease models already exceeded the threshold of it to cause neuronal toxicity (~2.5 fold). This indicates that our Fuz overexpression condition (Fig 1A) is a biologically achievable condition.

2. Table 1S only compiles the data on the levels of Fuz transcripts from different studies, not protein levels. It is not sure if Fuz levels in these setting are sufficient to cause toxicity.
We agree with the referee's comments that Table 1S only compiles the induction level on *Fuz* transcripts in different neurodegenerative diseases. Table 1S as well as related discussion have been removed from the manuscript.

3. The role of the general PCP pathway in Fuz-mediated apoptosis: considering Fuz is a conserved effector of PCP pathway, will manipulation of PCP pathway in general, such as modulating Dvl and Flamingo, affect the same apoptosis pathway, or is it just a function unique to Fuz? The functional significance of this important and well-studied pathway in Fuz-mediated apoptosis should be investigated in more details.
We agree with the referee's comment, and have performed extra experiments to investigate the functional significance of the PCP pathway in Fuz-mediated apoptosis in more details. We knocked down the expression of *Dvl*, *Inturned*, *Fritz* and *Flamingo* in Fuz-overexpressing cells to investigate whether altering these other players in the PCP pathway would affect Fuz-mediated apoptosis. We found that *Dvl* knockdown suppressed Fuz-mediated caspase-3 cleavage (Fig 1K). By contrast, knockdown of *Inturned*, *Fritz* or *Flamingo* did not (Fig EV1I-K). In addition, we overexpressed Inturned, Fritz, Dvl and Flamingo in rat primary cortical neurons to examine whether these PCP pathway proteins would induce neuronal cell death. As shown in Fig EV1C, overexpression of Inturned, Fritz, Dvl and Flamingo did not induce neuronal cell death. The above results suggest that general manipulation of the PCP pathway does not affect the apoptosis pathway.

4. The study touched on Dvl and the role of Fuz in affecting Dvl punctae formation, although this physical association of Dvl in Fuz-mediated neuronal toxicity has not been followed up further.
Fuz was reported to interact with Dvl [1]. When we analyzed Dvl staining pattern in Fuz-expressing cells, we observed that Fuz protein colocalized with Dvl "punctae" (Fig EV1E), which is an indication of their interaction. In addition, we have performed a co-immunoprecipitation experiment and detected the physical interaction between Fuz and Dvl in our model (Fig EV1H).

5. What is the structural basis in YY1 protein for such sequestration in aggregates? Is there a polyQ or prion-like protein stretch in YY1? Will manipulation of such domain affect YY1 sequestration and Fuz expression?
We did not find a polyQ region in YY1 protein, neither did we detect a prion-like protein stretch in YY1 using the prediction software [2]. To further address this question, we made a series of YY1 deletion constructs (Fig 4E), and coexpressed these proteins in polyQ-expressing cells. We found that when the REcruitment of POlycomb (REPO) domain was removed, YY1 was no longer able to colocalize with polyQ protein aggregates (Fig 4F).

The REPO domain in YY1 is known for the recruitment of the Polycomb group proteins into a multimeric protein complex required for transcriptional silencing [3]. Thus, deleting the REPO domain is expected to disrupt YY1's function in suppressing *Fuz* expression. Indeed, we found that REPO-deleted YY1 ($YY1^{\Delta REPO}$) is less effective than full-length YY1 in suppressing the expanded polyQ-induced upregulation of Fuz (Fig EV4F).

6. Given its central role in regulating Fuz expression, does YY1 itself affect neurodegeneration? This can be tested easily in the models used in the study.
The reduction of YY1 protein level was observed in the temporal neocortex and hippocampus regions from Alzheimer's patients, indicating a correlation between YY1 protein expression and neurodegeneration [4]. In addition, when YY1 was overexpressed, polyQ-induced caspase-3 cleavage was suppressed in rat primary cortical neurons (Fig EV3F).

7. Is YY1 also being sequestered into plaques/aggregated in Tau and Abeta expressing cells, given its effect in regulating Fuz in these settings?
We stained endogenous YY1 in $A\beta_{1-42}$ and Tau-expressing cells, and observed that YY1 was sequestered to $A\beta_{1-42}$ aggregates (Fig EV5K), whereas it was not recruited to Tau aggregates (Fig EV5L). When we overexpressed fluorescently-labelled YY1 deletion proteins (Fig 4E) in $A\beta_{1-42}$-treated cells, it was found that the N-terminal deleted $YY1^{155-414}$ protein did not colocalize with $A\beta_{1-42}$ aggregates (Fig EV5K). This suggests the N-terminal Acidic and His cluster regions of YY1 play some roles in its sequestration to $A\beta_{1-42}$ aggregates.

Minor issues:
1. Page 3: The following statement should be modulated, as it is not clear if neurodegeneration is really an apoptosis process or totally distinct. "Apoptosis has been reported as an essential executor for neuronal cell death in polyQ diseases (12, 13). Besides, the caspase cascade, a crucial mediator of apoptotic induction, is known to play key roles in polyQ neurodegeneration (14, 15)."
We thank the referee for pointing out this issue. On page 4, line 2, the statement has been revised as "In polyQ diseases, the misexpression of apoptotic gene triggers the induction of apoptosis, contributing to the pathology of the diseases. In particular, the caspase cascade is known to play a crucial role in polyQ-mediated apoptosis.".

2. Fig S1B & C: will Fuz depletion also affect Dvl punctae formation?
To investigate this, we stained Dvl in *Fuz* knockout cells (*Fuz*$^{-/-}$; Fig EV2A). Compared to the control parental HEK293 cells, the percentage of cells that displays Dvl "punctae" was significantly decreased (Fig EV1G). This demonstrates that *Fuz* depletion affects Dvl punctae formation.

3. YY1 binding site in Fuz promoters: are the YY1 site and CpG island also conserved in fly genome, considering that Fuz is also up-regulated in fly models of polyQ diseases?
A putative binding site of the *Drosophila* orthologue of YY1, Pleiohomeotic (4), could be identified in the *fuzzy* promoter region. However, we did not find putative CpG islands in the *fuzzy* promoter region.

4. Specific effect of YY1. Besides YY1, are there any other recognizable binding sites for other transcription factors in the polyQ-responsive promoter region of Fuz gene? Do they also get sequested into aggregates and do they also potentially play any role in Fuz expression and toxicity in disease setting? This might help address how specific is YY1 in regulating Fuz expression.
Putative binding sites of two additional conserved transcriptional factor were identified in the polyQ-responsive *Fuz* promoter region (Fig EV4A). These two neuronal transcriptional factors are nuclear receptor subfamily 3 group C member 1 (NR3C1; [5]) and Wilms tumor 1 (WT1; [6]). In contrast to YY1, we did not observe alteration of the soluble protein levels of NR3C1 and WT1 in polyQ-expressing cells (Fig EV4B), nor did we observe sequestration of these proteins to polyQ protein aggregates (Fig EV4C and D).

5. Page 10, Fig 4A and 4B: "When compared with age-matched controls, all patient brain samples exhibited reduced YY1 protein levels (Fig. 4 A and B)." This statement does not match well with the data in Figure 4A, as several controls also have quite low YY1 expression.
We apologize for an unclear description of the data in Fig 4A. Since different groups would have different levels of YY1 expression in their respective controls, the bands in Fig 4A were meant to be compared in a pair-wise manner only. The mean age of control and patient groups are 52.2 ± 11.1 and 51.8 ± 9.5 respectively (Appendix Table S2), and they are comparable. The average relative expression of YY1 protein is significantly reduced in the "SCA3" group when compared to the "control" group (Fig 4B). We now rephrased the statement to "When compared with age-matched control group, the SCA3 group exhibited reduced YY1 protein level (Fig 4A and B).".

Referee #2:

Building previous data supporting a role for Fuz in regulating cellular proliferation versus apoptosis, this study presents data supporting the concept that Fuz is upregulated in neurodegenerative diseases, notably models of SCA3, via alterations in its promoter region methylation by a reduction in YY1. They further provide data implicating this pathway more broadly in PD, tauopathies, and AD. Overall this study presents data in support of an intriguing hypothesis. However, there are some issues which if addressed would enhance the impact of this work.
We thank the referee for his/her comments and suggestions. We revised the manuscript to address the issues listed below, and hope these revisions would enhance the impact of this work.

1. Throughout the manuscript the authors indicate that Fuz pathway has a major/critical role in regulating apoptosis in neurodegeneration. Yet in several instances the data indicate that Fuz is at best one of many regulators of cell death, e.g Figure 2A where a complete loss of Fuz reduced cell death by about 30%. A prudent course would be for the authors to present a more balanced view of the role of Fuz.
In addition to the expanded polyQ protein, expanded CAG *polyQ* transcript also contributes to the polyQ pathology [7]. In cells transfected with *EGFP-CAG78*, a construct that only produces expanded polyQ transcript but not polyQ protein, induction of Fuz protein level was not detected (Fig EV4E). However, when cells were transfected with *ATXN3tr-CAA/G78*, a construct that possesses polyQ protein toxicity and exhibits diminished CAG RNA toxicity, Fuz induction was still observed (Fig EV4E). These results suggest Fuz induction is correlated with polyQ protein but not RNA toxicity. Therefore, a complete loss of *Fuz* only partially suppresses polyQ-mediated cell death may due to the inhibition of protein toxicity, while the RNA toxicity remains unaffected.

Meanwhile, we avoided mentioning the involvement of *Fuz* in AD, Tauopathy and PD, but used "$A\beta_{1-42}$, Tau and α-synuclein models" instead. We hope this modification could provide a more appropriate description of the involvement of *Fuz* in different experimental models.

2. On page 11 based on data generated using Q81-EGFP-myc the authors conclude that Fuz has a general role in polyQ disorders. Their rational this is the case since this construct devoid of "disease protein-specific sequences, i.e. polyQ diseases are due to expression of a toxic polyQ peptide. This is a very controversial point and by no means universally accepted by the field. One could also argue that these data indicate that Fuz has a role in highly toxic situations and not is disease relevant conditions.
We thank the referee for reminding us about this controversial point. The Q19-EGFP-myc/Q81-EGFP-myc data as well as related discussion have been removed.

3. At several key places quantitative data are lacking. Most notably, to this reviewer, is Figure 1n-p.
The quantification data are now added to the figures. The information regarding data quantification are also included in respective figure legends.

References

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anxiety. *Nat Genet* **23:** 99-103
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2nd Editorial Decision 25 April 2018

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, both referees are positive about the study and support publication in EMBO reports after minor revision. Please address the remaining concerns from referee 1 in the text and please also provide a point-by-point response or mark the changes in the text/discussion.

Browsing through the manuscript myself, I noticed a few editorial things that we need before we can proceed with the acceptance of your study.

- Callouts to figures: Appendix Table S3 is mentioned before Tables S1 and S2 in the text. It might be meaningful to reverse the order of the tables and rename Appendix Table S3 table to Appendix Table S1.

- Please provide a running title of max 40 characters (including spaces) on the first page of the manuscript.

- Our data editors have already inspected the figure legends for accuracy and completeness. Please see their suggestions and open questions in the attached Word document (with track changes).
- I have also taken the liberty to suggest some changes to abstract and title in the attached document. I introduced some changes in the abstract to more clearly indicate the disease models used. Moreover, I think that the title introduces a very strong statement "...Fuz triggers apoptosis in neurodegenerative diseases". While Fuz clearly triggers apoptosis in cells and neurons when overexpressed this has not been shown in the context of a disease. I therefore suggest to change the title to "....Fuz triggers apoptosis in neurodegenerative disease models." Please let me know if you agree with this suggestion.

- I noticed that some of the figures are very crowded with small panels and text. As a consequence the data will be difficult to see in final print size. I therefore suggest to split the following figures: Fig. 1, Fig. 3, EV1, EV5.
The amount of main figures can be extended. You currently have only 6 figures. But please note that we can only accommodate up to five EV figures. If you end up having more supplemental figures you will have to include some of them in the Appendix.

- Please provide a scale bar for Fig. 2E, EV2E, and G.

- Please note that the author checklist will be published alongside the manuscript (as part of the review process file). I had some open questions regarding some points (please see attached file).

- Moreover, our routine image analysis showed that many Western blot panels have a rather high contrast modification, e.g., the panels in Fig. 1I, 1J, 1K, 3B, 3L, EV1D, EV1J, EV1L, EV2A, EV3F, EV5F etc. Please reduce the amount of contrast modification as much as possible and please provide the original, unmodified source data for all Western blot and DNA gel data (as one pdf per figure).

- It also appears that the beta-actin blot in Figure EV5E has been spliced. Please confirm that these are the correct control samples for the Fuz blot and please also indicate the splicing with a stippled line.

We look forward to seeing a final version of your manuscript as soon as possible. Please let me

know if you have questions or comments regarding the revision.

REFEREE REPORTS
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Referee #1:

The revision addressed most of my early questions. The potential mechanism of Fuz/YY1 mediated regulation of neuronal survival is better clarified. Overall, I am satisfied with the revision and support its acceptance for publication.

Minor points:
1. Page 4: "In particular, the caspase cascade is known to play a crucial role in polyQ-mediated apoptosis [16,17]."
This statement is not accurate. Although overexpression of polyQ might induce acute cell death, it is not clear if polyQ diseases are primarily due to apoptosis, as overall polyQ diseases (and other brain degenerative disorders) are slow progression and might be caused by mechanisms not related to apoptosis at all. Similar, apoptosis might not be the main cause for AD, PD and other neurodegenerative diseases. Apoptosis might contribute, but the over-emphasis on the link between apoptosis and these brain diseases might not reflect the overall thinking of the field.

2. The new data that except for Dvl and Fuz, other PCP pathway genes tested showed no effect suggest that the neurotoxic effect of Fuz/Dvl might be unique to these two proteins (likely through the JNK/Rac, as described), independent of the PCP pathway. This point should be discussed more clearly.

3. Levels of Fuz protein and its toxicity:
Fig 1A suggested that a 2.5x increase of Fuz levels in cultured neurons was sufficient to activate Caspase 3. However, the expression data in the brain (Fig EV1B) suggested that Fuz protein is already present at relatively high levels in brain neurons than other cells, suggesting that increased levels of Fuz might not be necessarily linked to neuronal toxicity, otherwise their expression should be down-regulated in the brain. Additional discussion should be included to clarify that high levels of Fuz might not necessarily correlated with neuronal death, but likely need additional insults from other sources to initiate the pathogenic process.

Referee #2:

In this revised version the authors have for the most part addressed issues raised regarding the format/wording. Importantly, they have nicely where appropriate added quantitation data to the figures

2nd Revision - authors' response 15 May 2018

Responses to the editor:

1. Callouts to figures: Appendix Table S3 is mentioned before Tables S1 and S2 in the text. It might be meaningful to reverse the order of the tables and rename Appendix Table S3 table to Appendix Table S1.
We have corrected the order of Appendix Tables.

2. Please provide a running title of max 40 characters (including spaces) on the first page of the manuscript.
A running title “Fuz cause apoptosis in SCA3 degeneration” is now provided on the first page of the manuscript.

3. Our data editors have already inspected the figure legends for accuracy and completeness. Please see their suggestions and open questions in the attached Word document (with track changes).
We have revised the figure legends according to data editors' suggestions and comments.

4. I have also taken the liberty to suggest some changes to abstract and title in the attached document. I introduced some changes in the abstract to more clearly indicate the disease models used. Moreover, I think that the title introduces a very strong statement "...Fuz triggers apoptosis in neurodegenerative diseases". While Fuz clearly triggers apoptosis in cells and neurons when overexpressed this has not been shown in the context of a disease. I therefore suggest to change the title to "....Fuz triggers apoptosis in neurodegenerative disease models." Please let me know if you agree with this suggestion.
Thank you for taking the time to revise the abstract for us. We have revised the title and abstract accordingly.

5. I noticed that some of the figures are very crowded with small panels and text. As a consequence the data will be difficult to see in final print size. I therefore suggest to split the following figures: Fig. 1, Fig. 3, EV1, EV5.
The amount of main figures can be extended. You currently have only 6 figures. But please note that we can only accommodate up to five EV figures. If you end up having more supplemental figures you will have to include some of them in the Appendix.
We have modified the figures accordingly.

6. Please provide a scale bar for Fig. 2E, EV2E, and G.
Scale bars are now included in these figures.

7. Please note that the author checklist will be published alongside the manuscript (as part of the review process file). I had some open questions regarding some points (please see attached file).
We have revised the author checklist accordingly.

8. Moreover, our routine image analysis showed that many Western blot panels have a rather high contrast modification, e.g., the panels in Fig. 1I, 1J, 1K, 3B, 3L, EV1D, EV1J, EV1L, EV2A, EV3F, EV5F etc. Please reduce the amount of contrast modification as much as possible and please provide the original, unmodified source data for all Western blot and DNA gel data (as one pdf per figure).
The Western blot panels are modified to reduce the amount of contrast as much as possible. The unmodified source data for all Western blots and DNA gels are now provided along with the figures.

9. It also appears that the beta-actin blot in Figure EV5E has been spliced. Please confirm that these are the correct control samples for the Fuz blot and please also indicate the splicing with a stippled line.
A correct beta-actin control blot is now provided in Figure EV5E.

Responses to the referee #1:

1. Page 4: "In particular, the caspase cascade is known to play a crucial role in polyQ-mediated apoptosis [16,17]."
This statement is not accurate. Although overexpression of polyQ might induce acute cell death, it is not clear if polyQ diseases are primarily due to apoptosis, as overall polyQ diseases (and other brain degenerative disorders) are slow progression and might be caused by mechanisms not related to apoptosis at all. Similar, apoptosis might not be the main cause for AD, PD and other neurodegenerative diseases. Apoptosis might contribute, but the over-emphasis on the link between apoptosis and these brain diseases might not reflect the overall thinking of the field.
We thank the referee for pointing out this issue. The statement has been revised as "In polyQ diseases, the misexpression of apoptotic gene triggers the induction of apoptosis, which may contribute to the pathogenesis of the diseases. In particular, the caspase cascade has been shown to be activated in polyQ-mediated apoptosis."

2. The new data that except for Dvl and Fuz, other PCP pathway genes tested showed no effect suggest that the neurotoxic effect of Fuz/Dvl might be unique to these two proteins (likely through

the JNK/Rac, as described), independent of the PCP pathway. This point should be discussed more clearly.
This point is now mentioned more clearly in the discussion section as "Interestingly, knockdown of other PCP genes, including *Inturned*, *Fritz* and *Flamingo*, did not affect Fuz-induced caspase-3 cleavage (Fig EV1I-K). This strongly suggests that Fuz-induced apoptosis is independent of the PCP signalling pathway, but may specifically involve the pro-apoptotic functions of Dvl. Since the regulation of apoptosis by Dvl involves the canonical Wnt signalling pathway, future investigations may examine the involvement of molecules such as GSK3β and β-catenin in Fuz-induced apoptosis."

3. Levels of Fuz protein and its toxicity:
Fig 1A suggested that a 2.5x increase of Fuz levels in cultured neurons was sufficient to activate Caspase 3. However, the expression data in the brain (Fig EV1B) suggested that Fuz protein is already present at relatively high levels in brain neurons than other cells, suggesting that increased levels of Fuz might not be necessarily linked to neuronal toxicity, otherwise their expression should be down-regulated in the brain. Additional discussion should be included to clarify that high levels of Fuz might not necessarily correlated with neuronal death, but likely need additional insults from other sources to initiate the pathogenic process.
The aim of the expression data in Fig. EV1A and EV1B was to demonstrate which tissue(s) and which part of the brain normally expresses *Fuz*. While the expression level in the normal brain (~100%) was much higher than that of other tissues such as the liver (~5%) and the spleen (~3%) (Fig. EV1A), the ~100% expression level in the brain does not cause detectable level of apoptosis. The data from Fig. 1A suggested that approximately 2.5-fold increase of Fuz levels would induce caspase-3 activation in neurons. Thus, it is expected that an at least 250% increase in expression level of *Fuz* would be observed in the disease brains. To demonstrate this, we have now included Fig. 2D, which showed that the average relative level of *Fuz* transcript in SCA3 patient brains were approximately 3-fold higher than that of normal human brains (Fig. 2D). This suggests that the expression level of *Fuz* in the normal human brain (~100%) is likely not high enough to induce detectable apoptosis, but could be sufficient to maintain other functions of *Fuz* in neural tissues, such as regulation of planar cell polarity.

3rd Editorial Decision 24 May 2018

Thank you for your patience while we have editorially assessed your revised manuscript. Thank you for incorporating all requested changes and for providing all relevant source data. I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- I noticed that the exposure levels for Fuz DNA gels are often quite different between the figure and the source data (e.g., in Fig. 2B, EV2A, EV2B, EV2C, EV5B, EV5C). In the source data file much more transcript can be seen in WT. I suggest to either modify the relevant figure panels accordingly or to supply source data files with a similar exposure time.

- Moreover, I noticed that the left YY1 blot in Figure 6A has a bright spot in the source data file, probably dust, which is not visible in the figure panel. Could you please revisit this panel and clarify?

- Also the panels in Figure 7D and EV3E (HpaII) do not match well with the supplied source data. It appears that these represent very different exposures or modifications of the contrast. Could you please also have a second look at these panels?

3rd Revision - authors' response 25 May 2018

The authors made the requested revisions.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Ho Yin Edwin CHAN
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2017-45409V2

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

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

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

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The statistical analyses were performed based on the experimental results obtained from at least three biological replicates. The actual number are indicated in the figure legends.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The statistical analyses were performed based on the experimental results obtained from three biological replicates.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded.
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.	We have used an unbiased approach when allocating animals or samples to treatment.
For animal studies, include a statement about randomization even if no randomization was used.	No randomization was used.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
5. For every figure, are statistical tests justified as appropriate?	Yes. The statistical tests used are stated in the figure legends.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, the data meet the assumptions of the tests.
Is there an estimate of variation within each group of data?	Yes.

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Is the variance similar between the groups that are being statistically compared?	Yes. The statistical tests used are stated in the figure legends.

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).	It is described in Materials and Methods section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	It is described in Materials and Methods section.

* 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.	It is described in Materials and Methods section. Mice were maintained in a C57BL/6 background strain. Mice were housed in cages with a maximum number of five animals and maintained in a standard 12-hour light/dark cycle with food and water ad libitum.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were conducted with the approval of the Animal Experimentation Ethics Committee of The Chinese University of Hong Kong.
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.	All animal experiments were conducted following the ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Human SCA3 tissues were obtained from the NIH Neurobiobank at the University of Maryland. Control brain tissues and one SCA3 case were obtained from the New York Brain Bank, Columbia University.
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 participants singed the informed consent and this study was performed in accordance with the Declaration of Helsinki.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	There are no patient photos in the manuscript.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	There are no restritions for human data reported in the manuscript.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	There are no clinical trials in this manuscript.
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.	There are no clinical trials in this manuscript.
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.	There are no tumor marker prognostic studies in the manuscript.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	In Materials and Methods section, Data Availability statement is included.
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).	We confirm to have consulted the data deposition recommendations.
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).	We confirm to have consulted the data deposition recommendations.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	We confirm to have consulted the data deposition recommendations.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	We did not use any of the listed agents and toxins in the present manuscript.