Dear Dr. Ferguson,

Two reviewers who are both experts in the field have seen your manuscript and I have now received their reports. As you will see from their comments, I am afraid that your study does not provide sufficiently new information to reward publication in this journal. This criticism is serious enough to preclude publication.

Under these circumstances, I am really sorry to let you know you that your paper cannot be accepted for publication in Molecular Biology of the Cell. I am sure that you will be disappointed but I hope that you will find the reviewers' comments helpful when preparing the manuscript for resubmission elsewhere.

Thank you for the opportunity to examine this work. We hope that as your studies progress you will consider submitting future manuscripts to Molecular Biology of the Cell (MBoC).

If you have any questions regarding the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

Sincerely,
Jean Gruenberg
Monitoring Editor
Molecular Biology of the Cell

Reviewer #1 (Remarks to the Author):

This manuscript demonstrates the suitability of iPSC-derived, NGN2-induced human neurons (i3neurons) as a model system to investigate lysosomal transport in neurons, which could be particularly useful for the study of neurological disorders characterized by impaired lysosome transport/function. The authors characterize axonal lysosome transport, cathepsin content and pH using imaging-based techniques. Furthermore, the authors use the i3neuron system to demonstrate partially redundant roles for JIP3 and JIP4 in preventing lysosome accumulation in the axon and in the formation of axonal swellings, phenotypes that are associated with abnormal processing of the amyloid precursor protein. LAMP1 distribution and dynamics in i3neurons were already characterized to some extent by Boecker et al. (Traffic, 2019). The current study builds on the Traffic study by qualitatively assessing the presence of lysosomal cathepsins and pH in axonal lysosomes. However, the study provides little, if any, new information relative to what was already shown for lysosomes in other systems or other organelles in i3neurons. In addition, the experiments are minimally controlled and quantified. Finally, the manuscript is riddled with mistakes and inconsistencies.

Specific points:

1) Fig. 1A seems unnecessary. This panel could be moved to Supplemental Materials or deleted. Scale bars are missing. In the legend to this figure, the A label is missing.
2) Figs. 1B,C are redundant. The authors conclude that lysosomes are more concentrated in the soma than the neurites. However, the neurites are thinner than the soma, so it's not clear that there are actual differences in concentration; some quantification is needed. In this figure, it would have also been informative to label for both MAP2 and tau to compare the presence of lysosomes in dendrites and axons.
3) In the third panel of Fig. 1D, there is an extra asterisk.
4) In Fig. 1E, the insets are not properly aligned.
5) The study would benefit from the use of additional methodologies to characterize the presence of degradative lysosomes in the axon, for example, by using fluorogenic lysosomal enzyme substrates as previously done by Farfel-Becker et al. (Cell Rep., 2019).
6) The statement “the LAMP1-positive organelles localized in the soma of i3neurons were more enriched in multiple cathepsins (luminal proteases) compared to those localized in axons and dendrites (Fig. 1D-E)” on page 9 should be supported with more than just the micrographs shown in the figure, perhaps with some kind of quantification of the images (or, as suggested above, additional experiments using fluorogenic substrates). The images, particularly Fig. 1D, are not very convincing; the axonal lysosomes in this figure appear to contain cathepsin L. Although the staining for cathepsin L in the axon is faint, so is the staining for this enzyme in the soma and dendrites. I would think that the use of the word “multiple” requires the analysis of more than two lysosomal enzymes.
7) In Fig. 2A (extracts from differentiated i3neurons), endogenous LAMP1 runs at 120kDa. In Fig. 3B, endogenous LAMP1 in the same cells runs around 80kDa. The authors should explain or correct this discrepancy.
8) Legend to Fig. 2B: “LAMP1-GFP subcellular distribution aligns well with endogenous LAMP1 in i3Neurons (10 days of differentiation). The words “aligns well” suggests that co-localization with endogenous LAMP1 was conducted somehow, but that’s not in the image. To be informative, this experiment also requires markers for the axon and dendrites.

9) Fig. 3B lacks a loading control.

10) The legend to Fig. 4B could use some more explanation. Quantified from what? Blots like that in Fig. 4A? It also says n=3, but there are 4 points in each bar.

11) While the presence of large amounts of lysosomes in axonal swellings in the JIP3 KO and particularly in the JIP3/JIP4 dKO already suggests these adaptors promote axonal lysosome transport, kymographs or some quantification of axonal lysosome transport would strengthen the data.

12) Fig. 2E is incorrectly called out in the text as Fig. 3E.

13) Fig. 3E,F: it would be helpful if there was a “fill” fluorescence, or an outline of the actual neurite.

14) Neurons in Fig. 3E,F neurons have fewer days of differentiation than those in 3G,H. They are not comparable.

15) Fig. 4B,C. I would recommend changing the color of the bars and points so that individual points are more visible - perhaps grey points on top of the black and red bars?

16) A reference is needed for the statement “Our previous studies had demonstrated that accumulation of axonal lysosomes in JIP3 KO mouse neurons was accompanied by increased levels of BACE1 (β-site APP cleaving enzyme), the protease that initiates amyloidogenic processing of APP.”

17) Fig. 5C-E should include staining for cathepsins and LysoTracker to better characterize the accumulated organelles.

18) Introduction: “we used of human iPSC-derived neurons”. Remove “of”

19) Inconsistency throughout the manuscript with the terms iPSC and i3neurons (all of the following are used: iPSC, iPSC, i3Neurons, iNeurons, i3Neurons, i3Neurons, i3 Neurons, i3 Neurons, and on page 11 it is misspelled as i3NNeurons)

20) (Figure 5C-E) in the last paragraph of results should be (Figure 5C-E)

21) Discussion on page 15 “a partial reduction JIP3 expression” should be “a partial reduction in JIP3 expression”

22) In the methods, the sentence “HEK 293 FT cells were transfected with the newly made LAMP1-GFP lentiviral vector, containing along with the psPAX2 (Addgene #12260) and PCMV (Addgene #8454) packaging plasmids” should not have the word “containing”.

23) Catalog numbers are missing for many reagents.

24) What company is the poly-ornithine from?

25) In methods, “incubated with 50nM Lysotracker DeepRed for 3 minutes and then and then rinsed gently twice”, remove “and then”.

26) Correct all other typos not mentioned here.

Reviewer #2 (Remarks to the Author):

Gowrishankar and co-workers present in their manuscript the use of differentiated human neurons to model lysosomal transport defects with a potential relevance to neurological and neurodegenerative diseases. Hereto they start from the established WTC-11 inducible iPSC cell line that permits an efficient differentiation into long-term cortical neurons and characterize these neurons with respect to lysosomal distribution and mobility in neurites. KO of JIP3 in these neurons result in accumulation of lysosomes along neurites, upregulation of APP processing with a resulting increased production of toxic Abeta peptides, confirming and supporting their previous findings in the more classical primary neuronal models derived from rodents and in vivo transgene models. Additional KO of JIP4 further aggravates the phenotype underscoring a redundant function in neuronal lysosomal transport regulation. Besides this, the major value of this work is for sure to validate the use of human neurons as a preferred system to model disease, particularly given the fact that disease-associated risk factors and their underlying molecular/functional networks are not necessarily fully preserved in rodent models. Moreover, knowledge acquired in these new models will provide a more reliable physiological context prior to translating mechanistic insights subsequently in appropriate in vivo models. To make it a solid model, I feel that the authors should however characterize these neurons and in particular the lysosomal aspects with more scrutiny. For instance, a drawback is that they do not provide evidence that these neurons actually polarize allowing to distinguish axons from neurons (whereas they define the lysosomal swellings in JIP3KO neurons as being ‘axonal’). In figure 1 they only show colocalizations with MAP2 (panels B-C) or with tau (D-E) but the stainings look very similar. From fig2B, they conclude that Lamp1-GFP lysosomes are more abundant in some/dendrites than axons, but this is just a single GFP-fluorescence image. This should be documented with more details for instance showing triple labeling that would allow to quantitate lysosomal abundance in dendrites vs axons. Also, some labeling for bona fide synaptic markers should be include to underscore a full maturation of neurons and the formation of functional networks and synapses. With the current data, including live imaging in fig 2, it is not possible to conclude that the authors are looking at axonal transport of lysosomes. Some additional TEM of ‘axonal’ swellings, kymographs of lysotracker in JIP3KO, Bace1 immunostaining etc could further document the parallel observations made in rodent neurons and in vivo and underscore the eventual dystrophic nature of these neurites. If promoted as a valuable human cell model, it is important to scrutinize the model on as many aspects as possible. Likewise, the authors demonstrate increased production of Abeta42, but they only measure this form and not others (like 40) and only intracellular. Is the increase of Abeta42 resulting from a decrease in Abeta40 (and thus shifting processivity of g-secretase, rather than merely increasing overall abeta production) and how does this relate to the secreted pool of Abeta. This is an important aspect not addressed in previous models related to JIP3 and could highlight the contribution of stalled lysosomes.
to more specific alterations in g-secretase processivity. After all, the increase in BACE1 does not define that but merely provides more substrate for subsequent g-sec processing.
Response to Reviewers

Reviewer 1:

We appreciate that the reviewer has carefully read our manuscript and has taken the time to offer detailed feedback. In this revised manuscript, we have made multiple revisions based on their suggestions. However, we also wish to emphasize that the major scientific contributions of our study pertain to the JIP3/4-dependent regulation of lysosome axonal transport and its impact on APP processing. In order to perform and interpret such experiments, we also had to carry out some characterization of the iPSC-derived neurons as this is still a relatively new model system. We firmly believe that sharing the results of these characterization efforts will help others in this field and are synergistic with other recent studies (such as Boecker et al, Traffic 2019) which have performed similar but distinct characterization of the endolysosomal pathway in i3Neurons.

1) Fig. 1A seems unnecessary. This panel could be moved to Supplemental Materials or deleted. Scale bars are missing. In the legend to this figure,
   a. We feel that this is a helpful visualization of the iPSC to i3Neuron differentiation that does not detract from the rest of our study. We have performed the necessary corrections to the labeling.
2) Figs. 1B,C are redundant. The authors conclude that lysosomes are more concentrated in the soma than the neurites.
   We have moved panel B to Figure S1A; this stitched image highlights the concentration of lysosomes in soma across a wide field of neurons. We feel strongly that this image will help interested readers to better appreciate the distribution of lysosomes in these neurons and thus present it in the supplemental material. However, the neurites are thinner than the soma, so it's not clear that there are actual differences in concentration; some quantification is needed.
   We have now quantified the relative enrichment of lysosomal proteins in soma versus neurites (Figure 1E).
3) In this figure, it would have also been informative to label for both MAP2 and tau to compare the presence of lysosomes in dendrites and axons.
   We are limited by species specificity to label LAMP1, MAP2 and Tau together in the same image. We have independently co-labelled LAMP1 with each of these (Figures 1B, D), as well as with TRIM46, an axon initial segment (AIS) marker (Figure S1D).
4) In the third panel of Fig. 1D, there is an extra asterisk.
   a. We have corrected this.
5) In Fig. 1E, the insets are not properly aligned.
   a. Insets are now aligned.
6) The study would benefit from the use of additional methodologies to characterize the presence of degradative lysosomes in the axon, for example, by using fluorogenic lysosomal enzyme substrates as previously done by Farfel-Becker et al. (Cell Rep., 2019).
   We have used the DQ-BSA assay of lysosomal protease activity in i3Neurons and have confirmed that the signal is most enriched in lysosomes of the neuronal cell bodies (new Figure S1E).
6) The statement “the LAMP1-positive organelles localized in the soma of i3neurons were more enriched in multiple cathepsins (luminal proteases) compared to those localized in axons and dendrites (Fig. 1D-E)” on page 9 should be supported with more than just the micrographs shown in the figure, perhaps with some kind of quantification of the images (or, as suggested above, additional experiments using fluorogenic substrates). The images, particularly Fig. 1D, are not very convincing; the axonal lysosomes in this figure appear to contain cathepsin L. Although the staining for cathepsin L in the axon is faint, so is the staining for this enzyme in the soma and dendrites.
   We have added quantification to show relative deficiency of cathepsins in lysosomes in neurites compared to those in soma (Figure 1E). This is also highlighted in the insets (Figure 1C, D).

I would think that the use of the word "multiple" requires the analysis of more than two lysosomal enzymes.
We have added Cathepsin B (Figure S1C); we also changed the statement in the text in order to name the specific cathepsins that were examined.

7) In Fig. 2A (extracts from differentiated i3neurons), endogenous LAMP1 runs at 120kDa. In Fig. 3B, endogenous LAMP1 in the same cells runs around 80kDa. The authors should explain or correct this discrepancy. We have examined the images and have made the required correction.

8) Legend to Fig. 2B: "LAMP1-GFP subcellular distribution aligns well with endogenous LAMP1 in i3Neurons (10 days of differentiation). The words "aligns well" suggests that co-localization with endogenous LAMP1 was conducted somehow, but that's not in the image. To be informative, this experiment also requires markers for the axon and dendrites.
We have added results showing LAMP1-GFP and endogenous LAMP1 along with Tau (Figure S2B). We have updated the description of this data to now more generally state that the distribution of the endogenous and LAMP1 and the LAMP1-GFP are "similar".

9) Fig. 3B lacks a loading control.
As Fig. 3 B communicates a change in LAMP1 electrophoretic mobility and not abundance, a loading control would not meaningfully affect the interpretation.

10) The legend to Fig. 4B could use some more explanation. Quantified from what? Blots like that in Fig. 4A? It also says n=3, but there are 4 points in each bar.
The legend has been updated to reflect that we performed 4 independent biological replicates (lysates from 4 independent differentiation).

11) While the presence of large amounts of lysosomes in axonal swellings in the JIP3 KO and particularly in the JIP3/JIP4 dKOs already suggests these adaptors promote axonal lysosome transport, kymographs or some quantification of axonal lysosome transport would strengthen the data.
We have quantified the extent of LAMP1 accumulation in the single and double KO i3Neurons showing a significant enhancement of phenotype upon additional loss of JIP4 (Figure 5I).

12) Fig. 2E is incorrectly called out in the text as Fig. 3E.
This has now been fixed.

13) Fig. 3E,F: it would be helpful if there was a "fill" fluorescence, or an outline of the actual neurite.
Images showing co-staining of LAMP1 and Tau, where Tau outlines/ "fills" the neurites, have been added to this figure (Figure 3 G, H).

14) Neurons in Fig. 3E,F neurons have fewer days of differentiation than those in 3G,H. They are not comparable.
The important comparison for lysotracker labeling is between Figure 3 E and F (Control and JIP3 KO). However, we have added i3Neurons of the same age stained for LAMP1 and Tau in Figure 3 G, H. The original G and H (LAMP1 immunofluorescence in Control and JIP3 KO at a later age) has now been moved to Figure S3C.

15) Fig. 4B, C. I would recommend changing the color of the bars and points so that individual points are more visible - perhaps grey points on top of the black and red bars?
We agree that a better contrast would help visualize the points and have changed this as suggested.

16) A reference is needed for the statement "Our previous studies had demonstrated that accumulation of axonal lysosomes in JIP3 KO mouse neurons was accompanied by increased levels of BACE1 (β-site APP cleaving enzyme), the protease that initiates amyloidogenic processing of APP."
This reference has now been added.
17) **Fig. 5C-E should include staining for cathepsins and LysoTracker to better characterize the accumulated organelles.** We have added the requested lysotracker data (Figure 5 F-H) Given that we established the relative absence of cathepsins in axon (Figure 1 C-E), staining for cathepsins would not add anything meaningful to this figure.

18) **Introduction: “we used of human iPSC-derived neurons”. Remove “of”** We have corrected this.

19) **Inconsistency throughout the manuscript with the terms iPSC and i3neurons (all of the following are used: IPSC, iPSC, i3neurons, iNeurons, i3Neurons, i3 Neurons, i3 Neurons, and on page 11 it is misspelled as i3Nneurons)**

   We have made revisions to uniformly use the terms iPSC and i3Neurons.

20) **(Figure 5 C-E) in the last paragraph of results should be (Figure 5C-E).**

   Correction made.

21) **Discussion on page 15 “a partial reduction JIP3 expression” should be “a partial reduction in JIP3 expression”**

   Correction made.

22) **In the methods, the sentence “HEK 293 FT cells were transfected with the newly made LAMP1-GFP lentiviral vector, containing along with the psPAX2 (Addgene #12260) and PCMV (Addgene #8454) packaging plasmids” should not have the word “containing”**

   Correction made.

23) **Catalog numbers are missing for many reagents.**

   The revised document contains catalog numbers for key reagents such as antibodies.

24) **What company is the poly-ornithine from?**

   This information has been added.

25) **In methods, “incubated with 50nM Lysotracker DeepRed for 3 minutes and then and then rinsed gently twice”, remove “and then”.**

   The requested correction has been made.

26) **Correct all other typos not mentioned here.**

   In the course of revising the manuscript, we have made such corrections.

**Reviewer 2:**

Gowrishankar and co-workers present in their manuscript the use of differentiated human neurons to model lysosomal transport defects with a potential relevance to neurological and neurodegenerative diseases. Here to they start from the established WTC-11 inducible iPS cell line that permits an efficient differentiation into long-term cortical neurons and characterize these neurons with respect to lysosomal distribution and mobility in neurites. KO of JIP3 in these neurons result in accumulation of lysosomes along neurites, upregulation of APP processing with a resulting increased production of toxic Abeta peptides, confirming and supporting their previous findings in the more classical primary neuronal models derived from rodents and in in vivo transgene models. Additional KO of JIP4 further aggravates the phenotype underscoring a redundant function in neuronal lysosomal transport regulation. Besides this, the major value of this work is for sure to validate the use of human neurons as a preferred system to model disease, particularly given the fact that disease-associated risk factors and their underlying molecular/functional networks are not necessarily fully preserved in rodent models. Moreover, knowledge acquired in these new models will provide a more reliable physiological context prior to translating mechanistic insights subsequently in appropriate in vivo models. To make it a solid model, I feel that the authors should however characterize these neurons and in particular the lysosomal aspects with more scrutiny. For instance, a drawback is that they do not provide evidence that these neurons actually polarize allowing to distinguish axons from neurons (whereas they define the lysosomal swellings in jIP3KO neurons as being ‘axonal’). In figure 1 they only show co-stainings with MAP2 (panels B-C) or with tau (D-E) but the stainings look very similar.
We thank the reviewer for their insightful comments and have made the following key revisions in response to their feedback:

a) We have added axon initial segment (AIS) staining (TRIM46 staining; Figure S 1D) to further characterize i3Neuron polarization.
Likewise, in our dendritic (MAP2) staining of Control i3Neurons, we can clearly identify LAMP1 vesicles in MAP2-negative neurites (Current Figure 1 B; see white arrows) which we interpret to be axons. Additionally, in JIP3 KO neurons, we show that lysosomes build up in MAP2B-negative neurites Figure S3A, B.

From fig2B, they conclude that Lamp1-GFP lysosomes are more abundant in some/dendrites than axons, but this is just a single GFP-fluorescence image. This should be documented with more details for instance showing triple labeling that would allow to quantitate lysosomal abundance in dendrites vs axons.
We have co-labelled for LAMP1-GFP and total LAMP1 and Tau (Figure S2B). We have also independently labeled LAMP1-GFP with MAP2B to show that LAMP1 accumulates in MAP2-negative axons in JIP3 KO i3Neurons (Figure S3A, B).

Also, some labeling for bona fide synaptic markers should be included to underscore a full maturation of neurons and the formation of functional networks and synapses.
Although questions of synaptic function are not directly related to our investigation of the endo-lysosomal pathway, we now show synapsin staining (Figure S2A) as a measure of synaptic vesicle accumulation within presynaptic terminals. We also note that staining for vGLUT1 to demonstrate glutamatergic synapses has previously been demonstrated in these i3Neurons (Wang et al, 2017).

With the current data, including live imaging in fig 2, it is not possible to conclude that the authors are looking at axonal transport of lysosomes.
For the live imaging studies, neurons were grown at low densities and axons were identified based on their length (tracing the longest neurite). Separate experiments, including staining for AIS markers, confirm the establishment of polarity and validity of this approach for axon identification.

Some additional TEM of 'axonal' swellings, kymographs of lysotracker in JIP3KO, Bace1 immunostaining etc could further document the parallel observations made in rodent neurons and in vivo and underscore the eventual dystrophic nature of these neurites. If promoted as a valuable human cell model, it is important to scrutinize the model on as many aspects as possible.
While our BACE1 antibody did not work for immunofluorescence assays on these human neurons, we have additionally examined APP-BACE1 interactions using Split Venus fragments (as previously established by Das et al, 2016), where the reconstitution of Venus protein fluorescence depends on APP-BACE1 interaction/proximity. Our experiments examining APP-BACE1 proximity in Control and JIP3 KO neurons demonstrate that APP/BACE1 vesicles build up in JIP3 KO axons (Figure 4C).

Likewise, the authors demonstrate increased production of Abeta42, but they only measure this form and not others (like 40) and only intracellular. Is the increase of Abeta42 resulting from a decrease in Abeta40 (and thus shifting processivity of g-secretase, rather than merely increasing overall abeta production) and how does this relate to the secreted pool of Abeta. This is an important aspect not addressed in previous models related to JIP3 and could highlight the contribution of stalled lysosomes to more specific alterations in g-secretase processivity. After all, the increase in BACE1 does not define that but merely provides more substrate for subsequent g-secret processing.
We focused on measuring Abeta at its site of formation within the neurons. Our data indicates more Abeta 42 is made in JIP3 KO neurons and we now additionally show that APP and BACE1 positive vesicles build up in axonal swellings in these neurons (Figure 4C). This establishes this system as a useful human neuron culture model for future studies of APP processing (including examining different APP fragments as well as effect of different enzyme inhibitors).
Reference
1 Marwaha, R. & Sharma, M. DQ-Red BSA Trafficking Assay in Cultured Cells to Assess Cargo Delivery to Lysosomes. Bio Protoc 7, doi:10.21769/BioProtoc.2571 (2017).
Dear Dr. Ferguson,

Your revised manuscript has been seen by the same two reviewers, and I have now received their reports. I am very happy to let you know that both reviewers recommend publication. However, reviewer two has one minor editorial point that needs to be addressed before the paper can be formally accepted, since some changes may be needed in the text.

Looking forward to receiving the final corrected version of your manuscript,

Sincerely,
Jean Gruenberg
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Ferguson,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,
Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

The authors have performed additional experiments to address the comments of the reviewers. Although the study is still somewhat short on novelty, the characterization of lysosome transport in iPSC-derived neurons may be helpful to others working on the cell biology of neurons and neurological disease.

Reviewer #2 (Remarks to the Author):

The authors have revised their manuscript reporting on the impact of JIP3/JIP4 deficiencies on lysosomal transport. The effect of JIP3 LOF on accumulation of lysosomes in axons is documented in different model systems but here the authors succeed for the first time to recapitulate these findings in the more relevant context of differentiated human neurons. In particular they used the NGN2-induced direct and rapid differentiation resulting in so-called I3 neurons that, they show convincingly, provides a much improved model system for studying this and the increasing number of other genes recently identified in relation to neurodegeneration and neurodevelopmental problems. I've read in detail through the improved manuscript and notice that the authors have adequately and in detail addressed all my critiques, and added a significant number of new data panels to further support the validity of their neuronal model system. Overall and although it remains for some aspects confirmatory I can agree with the statement of the authors that it is important as well to have this done in a more optimized model system of differentiated neurons. Besides one small comment, I have no further restrictions with respect to this manuscript.

Comment: With respect to figure S3B, it is not clear to me whether this is a zoomed picture from the area indicated in panel A with yellow arrows. It doesn't seem so, but it should be clarified in the legends and, as well, these panels should be identified with the proper genotype or antibodies used because now there is besides a yellow arrow no indication what it represents.
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We thank the reviewer for pointing this out. We have added the labels to the figure and have updated the corresponding figure legend.
Dear Dr. Ferguson,

Thank you very much for this revised version of your manuscript. I am very happy to let you know that your manuscript has now been accepted for publication in Molecular Biology of the Cell.

Congratulations for a very nice piece of work!

Sincerely,
Jean Gruenberg
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Ferguson:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mbo/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,
Eric Baker
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