EMBO reports

PARK15/FBXO7 is dispensable for PINK1/Parkin-dependent mitophagy in iNeuron and HeLa cell systems

Felix Kraus, Ellen Goodall, Ian Smith, Yizhi Jiang, Julia Paoli, Frank Adolf, Jiuchun Zhang, Joao Paulo, Brenda Schulman, and J. Wade Harper
DOI: 10.15252/embr.202256399

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Review Timeline:

Submission Date:	1st Nov 22
Editorial Decision:	23rd Dec 22
Revision Received:	5th Apr 23
Editorial Decision:	9th May 23
Revision Received:	16th May 23
Accepted:	1st Jun 23

Editor: Martina Rembold

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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

The corresponding authors of Burchell VS *et al* (DOI: 10.1038/nn.3489) chose not to comment.

1st Editorial Decision 23rd Dec 2022

Dear Wade,

Thank you for the submission of your research manuscript to our journal. I apologize for the delay in its handling, but we have only recently received the third referee report and I have also discussed some of the points further with the referees and the editorial team. You find all three reports copied below my signature.

As you will see, all three referees acknowledge the quality of the data and consider the data overall convincing. However, they also raise a few points that would further strengthen the conclusion that FBXO7 does not play a general role in PINK1/Parkin-dependent mitophagy. Upon further discussion, we think that it would strengthen the data to test other stress and mitophagy-inducing conditions such as a stimulus that does not involve depolarization, as suggested by referee 2 and also referee 3. The results of the CCCP treatment or the results from HEK293 cells should be reported in case these experiments were performed already. Otherwise, these points should be discussed in the manuscript. It will not be necessary to repeat the experiments in dopaminergic neurons. While we all agree that this is a good suggestion, the analysis of dopaminergic neurons and ultimately the cause for the observed genetic linkage between PARK15/FBXO7 and PD, might be a task for future studies. Please address this concern in the discussion.

I am also happy to discuss these points and the revision further via e-mail or a video call, if you wish.

Taken together, 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.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (March 23). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).
Please download our Figure Preparation Guidelines (figure preparation pdf) from our Author Guidelines pages https://www.embopress.org/page/journal/14693178/authorguide for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines
()

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here:

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Please note that a Data Availability section at the end of Materials and Methods is now mandatory. See also <
https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>).

8) At EMBO Press we ask authors to provide source data for the main figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

Additional information on source data and instruction on how to label the files are available .

9) The journal requires a statement specifying whether or not authors have competing interests (defined as all potential or actual interests that could be perceived to influence the presentation or interpretation of an article). In case of competing interests, this must be specified in your disclosure statement. Further information: https://www.embopress.org/competing-interests

10) Figure legends and data quantification:
The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
- the nature of the bars and error bars (s.d., s.e.m.)

- If the data are obtained from n {less than or equal to} 5, show the individual data points in addition to the SD or SEM.
- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat

- Please also include scale bars in all microscopy images.

11) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

12) 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."

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

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

You can use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Kind regards,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

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Referee #1:

Kraus et al. undertook a systematic analysis to address the role of PARK15/FBXO7 in PINK1/Parkin-dependent mitophagy using HeLa cells and the i3Neuron model systems. Previous literature had linked an early onset form of parkinsonian-pyramidal syndrome showing a phenotype very similar to PRKN gene associated parkinsonism (Davison et al..,1954) with autosomal recessive genetic mutations in the risk gene PARK15/FBXO7 (Di Fonzo et al., 2009; Houlden & Singleton, 2012; Paisan-Ruiz et al. 2010). Subsequently, molecular studies have implicated the FBXO7 gene product, a subunit of the SCF (SKP1/cullin-1/F-box protein) E3 ubiquitin ligase complex, in the positive regulation of PINK1/Parkin-dependent mitophagy (Burchell et al. 2013) for its direct interaction with Parkin as assessed in immunoprecipitation experiments of overexpressed proteins in U2OS and HEX293T cells. They showed that FBXO7 promotes Parkin localization to depolarized mitochondria upon CCCP treatment and that overexpression of FBXO7 rescues Parkin mutant D. melanogaster flies. They also showed that FBXO7 directly interacts with PINK1, leading to PINK1 stabilization. A following study exploring anew its role in neurons functionally linked FBXO7 to the assembly of the proteasome (Vingill et al., 2016) and confirmed previous reports of its interaction with the SCF subunit SKP1 and the proteasomal regulator PI31, reporting in addition its interaction with the proteasomal subunit PSMA2 for the first time.

Kraus et al. have clarified the role of PARK15/FBXO7 in PINK1/Parkin-dependent mitophagy as it employs state-of-the-art methods developed in the evolving field for the evaluation of ubiquitin-dependent mitochondrial turnover via the PINK1/Parkin axis. The authors employ CRISPR engineered FBXO7-/- HeLa and hES cell lines and compare these to their WT counterparts in fed conditions or upon induction of mitochondrial stress with the membrane potential uncouplers Antimycin A and Oligomycin. They monitor accumulation of phosphorylated ubiquitin at Ser65 (pUb) on Mitochondrial Outer Membrane (MOM) using Western Blotting analysis, map pUb localization to mitochondria using 3D super-resolution microscopy and measure mitophagic flux in iNeurons and HeLa cells. Consistently, throughout all these approaches, authors did not find differences between FBXO7-/- lines and controls thus arguing against a general role of PARK15/FBXO7 in PINK1/Parkin-dependent mitophagy. Last, using global proteomic analysis authors evaluated mitochondrial clearance in FBXO7-/- HeLa cells expressing Parkin and found no change when comparing the test and control lines.

This is a thorough and valuable of the role of PARK15/FBXO7 in the pathogenesis of parkinsonian-pyramidal syndrome. I support acceptance of this manuscript for publication in EMBO Reports with no major revisions. The following minor points should be addressed:

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- authors use both FBXO7/PARK15 and FBXO7/Park15 (Supplemental Figure 1). They should regularize the nomenclature;
- line 80, correct " F-box protein to binds to substrates "
- line 94, correct " FBOXO7 "
- line 210, correct " extendt "
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- line 237, add " Additionally, we have not been able to "
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- Figure 2 panel A and Supplemental Figure 2 panel A, yellow dotted boxes are absent in whole cell image for FBXO7-/- condition
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In this study, the authors closely examine the role of FBXO7 in PINK1 and Parkin mitophagy. FBXO7 is mutated in familial parkinsonism and in previous work was reported to play a role in driving PINK1 and Parkin mitophagy. Give its role in human disease, it is critically important that we have a clear understanding of how FBXO7 functions. Kraus et al show that FBXO7 does not play a role in PINK1 and Parkin mitophagy. The authors do so through very thorough and exhaustive analyses, using all major techniques available in the mitophagy field to very carefully examine any activity of FBXO7 in mitophagy. In addition, they examine HeLa based cell models expressing exogenous Parkin as well as neuronal models expressing endogenous Parkin, both of which lead to the same conlclusion that FBXO7 does not play a prominent role in PINK1 and Parkin mitophagy. The data are of very high quality, and are very clear and convincing. It is important for the field of mitophagy and for Parkinson's disease (PD) researchers to be aware of these findings, which will be of high and broad interest to the fields of mitophagy, PD, and ubiquitin signaling. I have only minor comments/suggestions.

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5. Figure 3F and G: Are the Y axes in these graphs correct; there is one mtDNA foci per cell in untreated conditions?

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Referee #3:

J. Wade Harper group presented a research article entitled 'PARK15/FBXO7 is dispensable for PINK1/1 Parkin mitophagy in iNeurons and HeLa cell systems'. This project aimed to investigate the roles of an ubiquitin ligase substrate receptor PARK15/FBXO7 in mitophagy; by the application of imaging, biochemistry, and mass spectrometry techniques in HeLa and/or iNeurons, they authors did not find any evidence of the essentiality of PARK15/FBXO7 in mitophagy. The data were in high quality, and were presented in a professional way, supporting its own conclusion: 'these data indicate that FBXO7 does not play a general role as a positive modulator of Parkin and PINK1-dependent mitophagy in HeLa and iNeuron systems. As the original reprot (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3827746/) was also well-done in its own systems (HEK293T cells and flies), addressing below questions are essential to consolidate the current statement.

Major concerns:
1. Cell types and mitophagy. There is a strong genetic association of FBOX7/PARK15 with Parkinson's Disease (Houlden & Singleton, 2012); in PD, it is the damage and loss of dopaminergic neurons but not other types of neurons (such as cortical neurons), highlighting a cell-type (not universal) impairment of the cell survival system. By presumption, there is a likelihood that PARK15/FBXO7 may play a role in eliminating damaged mitochondria in dopaminergic neurons, although not in cortical-like iNeurons. The authors are suggested to check roles of PARK15/FBXO7 in mitophagy in dopaminergic neurons.
2. Stress and mitophagy. As there are many mitophagy pathways, impairment of one mitophagy pathway may not lead to 'detectable impairment' of mitophagy due to the compensation by other mitophagy pathways; in this condition, some 'false negative data' may exisit. One example is that the PINK-/-;Parkin-/- mice only show Parkinson's-like phenotypes in high stress conditions (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7362342/). Whether this could be the same case in the settings of the

current paper? It would be nice if the authors to provide quantitative data on any changes of mitophagy in exogenous stress-exposed PARK15/FBXO7-/- cells (including iNeurons, HeLa cells, and a new dopaminergic neuronal line/neurons).
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4. In the Introduction section, a summary on the molecular mechanism of the PINK1-Parkin-dependent mitophagy was excellently presented. Adding a few more sentences on the roles of mitophagy maintenance in neuronal protection in common neurodegenerative diseases and the ageing brain are important, as this provides rationales of further deeper mechanistic studies of mitophagy in neuroprotection and health (PMID: 30742114; PMID: 31577933; PMID: 35134347).

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We thank the reviewer for their detailed summary of the paper and positive comments about the work.

1) the authors have chosen Antimycin A and Oligomycin to induce mitochondrial stress. Have they tried using CCCP (as in previous reports) and found a different outcome in any of the assays presented in the study?

We thank the reviewer for this suggestion. We have now performed several assays with CCCP in both HeLa and HEK293T cells with or without FBXO7, but again find no defects in any of the assays. This data is now included in Appendix Figure 1J, K.

2) the authors never mention the absolute amounts of Antimycin A and Oligomycin used for the treatments of both HeLa cells and i3Neurons. They only mention to have tested a 10-fold lower dose in respect to other treatments performed in the study (line 186);

The concentrations are provided in the text and figure legends. We used 5 μM Antimycin A and 10 μM oligomycin for standard assays and then 0.5 μM Antimycin A and 0.5 μM oligomycin for some experiments in iNeurons, as indicated. This is now mentioned in the methods section.

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We thank the reviewer for noting this. We have corrected the images.

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We have slightly rewritten the sentence to clarify and also added a reference (review article).

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We thank the authors for the suggestion. We have performed new experiments with WIPI, and again see no difference in recruitment to mitochondria in cells lacking FBXO7 (two independent clones). These data are now show in EV Figure 2J,K.

5. Figure 3F and G: Are the Y axes in these graphs correct; there is one mtDNA foci per cell in untreated conditions?

We thank the reviewer for spotting this mis-labelled axis. We added the "[normalized to fed]" to the Y-axis of both panels.

6. I view this as an optional comment, since the mitophagy data are overall very convincing. However, to cover all bases, the authors may wish to try a mitophagy stimulus that does not involve depolarisation, such as mitochondrial protein folding stress using G-TPP treatment. It would be more than sufficient to only conduct this experiment in the HeLa model lines.

G-TPP is an interesting molecule as it specifically inhibits mitochondrial HSP90 and leads to increased protein misfolding, which can clog the mitochondrial translocon and induce PINK1/Parkin activity. However, it is well known that the response of the Parkin/PINK1 pathway is significantly weaker with G-TPP and with depolarizing agents. We obtained commercial G-TPP and performed several assays to examine Parkin/PINK1 pathway activity. As expected, we do routinely detect pUb and Parkin recruitment using G-TPP in the HeLa and HEK293T systems. We found that pUb still accumulates and Parkin is still recruited to mitochondria in FBXO7-/- in both the HeLa and HEK293T system, indicating that FBXO7 is not required for the response. Interestingly, we routinely found that FBXO7 indeed had somewhat (~20-30%) elevated pUb signaling. We conclude that while FBXO7 is not required for the response to G-TPP, its absence may actually increase the cells response to mitochondrial protein misfolding (mtUPR). This data is now shown in EV Figure 4A-F.

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We are not exactly sure what type of exogenous stress beyond AO and CCCP the reviewer was referring to but to address this issue, we examined the mtUPR agent G-TPP in both HeLa and HEK293T cells with or without FBXO7. As indicated in the comments for reviewer 2, cells lacking FBXO7 still respond to mtUPR, and even have slightly higher levels of pUb and Parkin recruitment. These data indicate that FBXO7 is not required for cells to respond to matrix protein misfolding, and its absence may even amplify the cells response.

3. As the original reprot (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3827746/) was based on the data majorly generated in the HEK293T cells. Can the authors check changes of mitophagy in PARK15/FBXO7-/- HEK293 cells? Although the statement is excellently conditioned 'these data indicate that FBXO7 does not play a general role as a positive modulator of Parkin and PINK1-dependent mitophagy in HeLa and iNeuron systems', it is important to check the conclusion using the original system it was reported.

In order to address the reviewer's comments, we used CRISPR/Cas9 to create HEK293T cells lacking FBXO7. We obtained two independent clones that were validated as deletions using both sequencing of the mutant allele and immunoblotting of extracts (Appendix Figure 1). We then introduced GFP-Parkin into these cells as HEK293 cells have very low endogenous Parkin levels and therefore do not have a robust mitophagy activity in response to depolarization. We subsequently examined: 1) GFP-Parkin recruitment in response to depolarization, 2) pUb accumulation in response to depolarization, and 3) the response of cells to G-TPP (Appendix Figures 1 and 2, and EV Figure 4). In all cases, the FBXO7 null cells had activities that were similar to that seen with wild-type cells or somewhat elevated in the case of G-TPP, indicating that FBXO7 is not required for the Parkin/PINK1 in this cell line.

4. In the Introduction section, a summary on the molecular mechanism of the PINK1-Parkin-dependent mitophagy was excellently presented. Adding a few more sentences on the roles of mitophagy maintenance in neuronal protection in common neurodegenerative diseases and the ageing brain are important, as this provides rationales of further deeper mechanistic studies of mitophagy in neuroprotection and health (PMID: 30742114; PMID: 31577933; PMID: 35134347).

We appreciate the authors comments and have added a section on neuroprotection, including references.

1st Revision - Editorial Decision 9th May 2023

Dear Wade,

Thank you for the submission of your revised manuscript. As I informed you, we have meanwhile received the reports from the referees who were asked to assess it and both support publication without further revision.

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

- We usually publish manuscripts that contain up to 5 figures as "Report" with a combined Results and Discussion section and a character limit of approx. 27,000 characters (including spaces but excluding materials & methods and references). If however you prefer to have a separate discussion, which might be the better option in this specific case, then let me know and I keep the classification as 'Article'.

- Please update the 'Conflict of interest' paragraph to our new 'Disclosure and competing interests statement'. Please note that EMBO council members must disclose their relationship with EMBO in the author disclosure statement using the standard phrase "[Author] is an EMBO council member."
For more information see
https://www.embopress.org/page/journal/14693178/authorguide#conflictsofinterest

- Regarding the Author Contributions, we now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section, which therefore needs to be removed from the manuscript text. You can use the free text box in our system if you wish to provide more detailed descriptions. See also guide to authors https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines.

- Tables EV1-EV8 should be renamed to Dataset EV1-EV8 with the corresponding callouts in the manuscript text. The format is fine otherwise.

- I noticed that you supplied a list of all reagents and tools in Table EV9. We can typeset this table into the Materials and Methods section. In this case, please call it "Reagents and Tools table" and use the style we apply to these tables, at least use the column headers "Reagent/Resource", Reference or Source" and "Identifier or Catalog number". Please also see our guidelines on Structured Methods (https://www.embopress.org/page/journal/14693178/authorguide).

- We noticed that the small inserts in Figure 2A are reused in Figure EV1 B. Please clarify this in the legend of Figure EV1B. I also recommend to clearly state that the 1h AO images in Fig. 2A and EV1A are the same.

- The same applies to the reuse of the small inserts in EV Figure EV1A and EV1B. This should be clearly stated in the figure legend.

- We noticed that the quantification in Figure 3E is based on two experiments. In this case we ask you to only show the two data points instead of the mean and error bars and remove the statistical analysis.

- Please note that the order of the sections should be: abstract, introduction, results, discussion, materials & methods, data availability section, acknowledgments, disclosure statement and competing interests, references, main figure legends, expanded view figure legends

- I attach to this email a related manuscript file with comments by our data editors. Please address all comments and upload a revised file with tracked changes with your final manuscript submission.

- Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x300-600 pixels large (width x height) in PNG for JPG format. You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

I look forward to seeing a new revised version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Kind regards,

Martina

Martina Rembold, PhD

Senior Editor
EMBO reports

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Referee #2:

The authors have done an excellent job addressing the reviewer comments. Congratulations on a very important study.

Referee #3:

The authors did a good job in addressing all the major questions raised by this reviewer and the other one. The quality of the current paper is greatly improved.

The authors have addressed all minor editorial requests

2nd Revision - Editorial Decision 1st Jun 2023

Dr. J. Wade Harper
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Cell Biology
Harvard Medical School
Boston, orcid|||||| USA-Boston, MA 02115
United States

Dear Wade,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Best wishes,

Martina

Martina Rembold, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: J. Wade Harper
Journal Submitted to: EMBO Reports
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Reporting Checklist for Life Science Articles (updated January 2022)
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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 χ2 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.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	New materials and reagents includes CRISPR edited cell lines and there is no restrictions on their availability.

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	All antibody information can be found in Table EV 9, including RRID numbers where available.

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Sequences are provided in the Materials and Methods section.

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Information provided in Supplemental Table S9, including RRID numbers where available.
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	hESCs were karyotyped to confirm chromosomal integrity. Cells are tested for mycoplasma on a regular basis.

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Not Applicable	
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

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Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

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If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgments section.

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Yes	We use protocols.io for sharing of step-by-step protocols associated with this work. Link: https://www.protocols.io/view/kraus-et-al-2022-fbxo7-park15-kxygx99pwg8j/v2. Macros and pipelines are available on https://github.com/harperlaboratory/FBXO7.git

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	No sample-size calculation was performed. For proteomics experiments, we chose n=3 or n=4 given the limitation of the available TMT channels. For Flow-cytometry experiments, we analyzed >10,000 cells with triplicate experiments which showed consistent results through-out the replicates. Confocal microscopy experiments were done at least in triplicate and quantification was done.
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Image acquisition was automated as much as possible, allowing for random field of view selection by the NIS-software for imaging.
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	No data was excluded a priori
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Yes. All figures have statistical tests where appropriate and p values are provided in the legend.

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In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Stated in figure legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Stated in figure legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: 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.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
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Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
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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.	Not Applicable	

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Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Proteomics datasets have been deposited at ProteomeXchange Consortium by the PRIDE partner. The PRIDE project identification number is PXD037797.
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	