

Loss of CTLH component MAEA impairs DNA repair and replication and leads to developmental delay

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Editor: Zeljko Durdevic

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.)

20th Jun 2025

Dear Dr. Carnie,

Thank you for the submission of your manuscript to EMBO Molecular Medicine, and please accept my apologies for the unusual delay in getting back to you. We have now received feedback from two of the three reviewers who agreed to evaluate your manuscript. As the referee #2 will unfortunately not be able to return his/her report in a timely manner, we prefer to make a decision now in order to avoid further delay in the process. Should referee #2 provide a report, we will send it to you, with the understanding that we will not ask for an additional revision.

As you will see from their reports pasted below, both referees recognize potential interest of the manuscript but also raise important concerns that should be addressed in a major revision. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

Further consideration of a revision that addresses reviewers' concerns in full will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

We would welcome the submission of a revised version within three months for further consideration. Please let us know if you require longer to complete the revision.

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

When submitting your revised manuscript, please carefully review the instructions that follow below. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

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- 2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': (<https://www.embopress.org/page/journal/17574684/authorguide#figureformat>).
- 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 (<https://www.embopress.org/page/journal/17574684/authorguide#submissionofrevisions>). 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.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see <https://www.embopress.org/page/journal/17574684/authorguide#dataavailability>).

In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.). See also 'Figure Legend' guidelines: <https://www.embopress.org/page/journal/17574684/authorguide#figureformat>

8) At EMBO Press we ask authors to provide source data for the main manuscript figures. You will receive a separate email with instructions for providing source data with your revised manuscript, including how to upload and organize the files.

9) 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 .

10) 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.

- 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.

See detailed instructions here:

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This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.

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Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high.

15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines (<https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods>)

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

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

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

Medical impact is medium, because the authors study a very rare disorder.

Referee #1 (Remarks for Author):

This manuscript describes patients with mutations in the MAEA gene. The features of these patients are somewhat consistent with DNA replication stress. In parallel experiments, the MAEA gene is shown to have a role in the cellular response to DNA replication stress. Overall, this is a solid study that merits publication without revisions, except for addressing points 1 and 6.

Specific Points:

1. Fig. 1 shows the results of a CRISPR-Cas9 screen that identified genes encoding subunits of the CTLH complex, as genes whose loss leads to sensitivity to camptothecin. The results are clear. My only point is that Supplemental Table 3 does not show the results of all the genes that were targeted by the CRISPR-Cas9 knockout library. Can the authors provide all the results?
2. Fig. 2 identifies mutations associated with neurodevelopmental defects in humans in MAEA, a subunit of the CTLH complex. The functional defect of these mutations is then confirmed by expressing the mutant proteins in cells with knockout of the endogenous gene and testing for sensitivity to camptothecin. These results are clear.
3. Fig. 3 shows that loss of MAEA impairs RAD51 loading, but not end-resection. These results are also clear.
4. Fig. 4 shows that MAEA knockout cells are sensitive to agents that cause DNA replication stress, specifically hydroxyurea (HU), aphidicolin and an ATR inhibitor (ATRi). These results are also clear.
5. Fig. 5 shows that untreated MAEA knockout cells have decreased fork speed and increased fork stalling. After treatment with HU they have increased fork degradation, as well as decreased fork restart after HU wash-out. Similar results were obtained using fibroblasts from patients with MAEA mutations.
6. Table 1 shows pictures of patients with mutations in the MAEA gene. I presume that the authors have permission to use photographs of their patients. Yet, very soon photographs will be sufficient to identify individuals by AI using data from social media platforms. Should the pictures be shown? What do they add? Maybe the facial features should be cited, instead?

Referee #3 (Remarks for Author):

This study investigates the role of ubiquitin E3 ligases in the DNA damage response (DDR). Using a CRISPR-Cas9 knockout screen targeting E3 ligases, the authors identify the CTLH E3 ligase complex-focusing particularly on its subunit MAEA-as essential for handling single-ended DNA double-strand breaks (seDSBs). Furthermore, patient data reveal that mutations in MAEA are associated with neurodevelopmental disorders. Functional analyses indicate that MAEA deficiency impairs

homologous recombination (HR) and replication fork restart, likely by disrupting RAD51 loading at DNA damage sites. The study establishes a link between this defect, genomic instability, and developmental abnormalities.

I commend the authors for their comprehensive screening approach and for successfully integrating both clinical and mechanistic insights. However, several aspects require revision or clarification:

Cell viability should be assessed in MAEA knockout and hypomorphic cell lines, as well as in other gene knockout models, without camptothecin treatment. This will clarify whether MAEA loss alone affects cell viability in Figures 1D, 1E, and 1F.

The authors should provide a more detailed rationale for highlighting MAEA specifically over other CTLH complex components, such as WDR26, RMND5A, GID8, and RANBP9, within the manuscript.

To further support the link between MAEA and neurodevelopmental defects, the authors should validate their findings in primary murine neurons or in SH-SY5Y cells as an in vitro model system for the results shown in Figures 1D-F, 2C-F, 3D and 3G, and 4A-C.

In Figure 3, it is important to clarify whether MAEA knockout affects the expression levels of DNA repair-associated proteins regulated by RAD51, such as DNA2.

In Supplementary Figure 2B, it would be helpful to extend the immunoblot detecting MAEA to include the molecular weight of eGFP-MAEA. Similarly, the blot detecting eGFP should be shown to the size of eGFP-MAEA to confirm expression.

Providing clear explanations and additional data addressing these concerns will significantly strengthen the manuscript and further highlight the impact of the study's findings.

******* Reviewer's comments *********Referee #1 (Comments on Novelty/Model System for Author):**

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[We thank the Reviewer for their highly supportive feedback on our manuscript and are pleased to offer specific responses to their comments below.](#)

Specific Points:

1. Fig. 1 shows the results of a CRISPR-Cas9 screen that identified genes encoding subunits of the CTLH complex, as genes whose loss leads to sensitivity to camptothecin. The results are clear. My only point is that Supplemental Table 3 does not show the results of all the genes that were targeted by the CRISPR-Cas9 knockout library. Can the authors provide all the results?

[We thank the reviewer for highlighting this omission at our end; we have provided the full set of NormZ scores across the sgRNA library \(**Dataset EV1**\).](#)

2. Fig. 2 identifies mutations associated with neurodevelopmental defects in humans in MAEA, a subunit of the CTLH complex. The functional defect of these mutations is then confirmed by expressing the mutant proteins in cells with knockout of the endogenous gene and testing for sensitivity to camptothecin. These results are clear.

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[All such informed consent has already been obtained. However, the reviewer makes an important point about the future implications of AI. As such, we have removed the photographs from the clinical table and have added a comment to the Data Availability](#)

statement (lines 469-473 in the revised manuscript) stating that these are available from the corresponding authors upon reasonable request.

Referee #3 (Remarks for Author):

This study investigates the role of ubiquitin E3 ligases in the DNA damage response (DDR). Using a CRISPR-Cas9 knockout screen targeting E3 ligases, the authors identify the CTLH E3 ligase complex-focusing particularly on its subunit MAEA-as essential for handling single-ended DNA double-strand breaks (seDSBs). Furthermore, patient data reveal that mutations in MAEA are associated with neurodevelopmental disorders. Functional analyses indicate that MAEA deficiency impairs homologous recombination (HR) and replication fork restart, likely by disrupting RAD51 loading at DNA damage sites. The study establishes a link between this defect, genomic instability, and developmental abnormalities.

I commend the authors for their comprehensive screening approach and for successfully integrating both clinical and mechanistic insights. However, several aspects require revision or clarification:

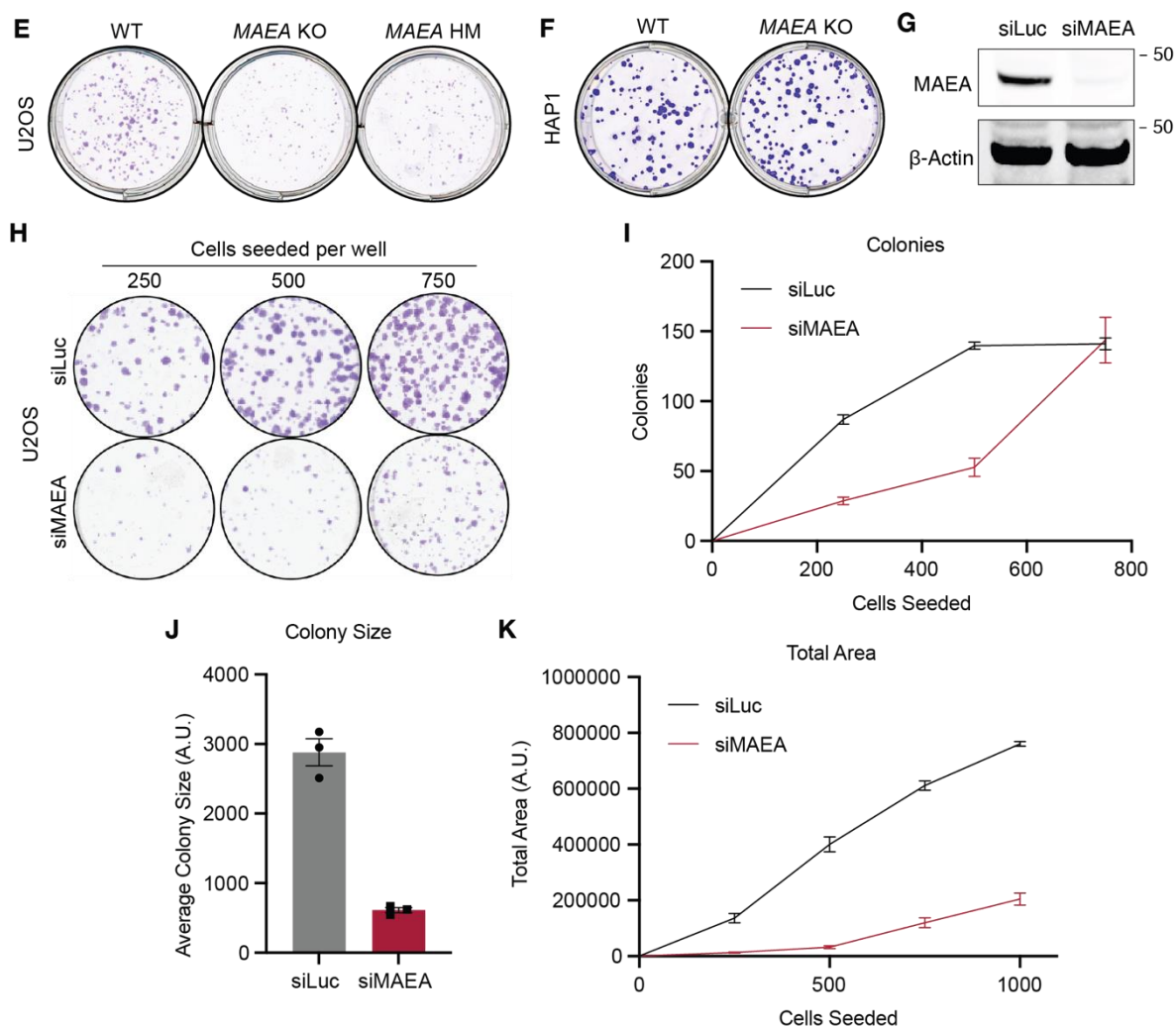
We thank the reviewer for their encouraging feedback on our manuscript, which has helped to enhance our study and expand the applicability of its findings. We offer our point-by-point responses to individual comments below:

Cell viability should be assessed in MAEA knockout and hypomorphic cell lines, as well as in other gene knockout models, without camptothecin treatment. This will clarify whether MAEA loss alone affects cell viability in Figures 1D, 1E, and 1F.

We have added representative images of untreated cells from clonogenic assays in MAEA-deficient U2OS and HAP1 cells, in which we observed that, in comparison to WT counterparts, MAEA knockout compromises colony formation and cell growth in U2OS cells but not in HAP1 cells (new Fig. EV1E,F and below). This could be consistent with the DNA replication defects we have observed in MAEA-deficient cells. Given that stable KO cell lines might have adapted somewhat to MAEA loss, we further tested clonogenicity of U2OS cells upon transient siRNA-mediated MAEA depletion and also observed reduced colony size and plating efficiency (new Fig. EV1G-K and below). These data are discussed in lines 119-123 in the revised manuscript.

In summary, it seems that depending on the cell line, MAEA loss alone can compromise cell viability. This is consistent with our difficulties in conducting viability assays upon MAEA depletion in SH-SY5Y cells (expanded upon below), and data from Depmap showing that approximately 23% of cell lines are dependent on MAEA.

Fig. EV1



New Fig. EV1. (E-F) Representative images of untreated WT and MAEA-deficient U2OS (E) and HAP1 (F) cells. (G) Immunoblot for MAEA in U2OS following siRNA transfection. (H-I) Representative images (H) and colony counts (I) of U2OS cells with or without siRNA-mediated MAEA depletion. (J) Average colony size of siRNA transfected U2OS cells at 500 cells per well. (K) Total area coverage of U2OS colonies following siRNA transfection. (I-K) Bars represent the mean $\pm$ SEM.

The authors should provide a more detailed rationale for highlighting MAEA specifically over other CTLH complex components, such as WDR26, RMND5A, GID8, and RANBP9, within the manuscript.

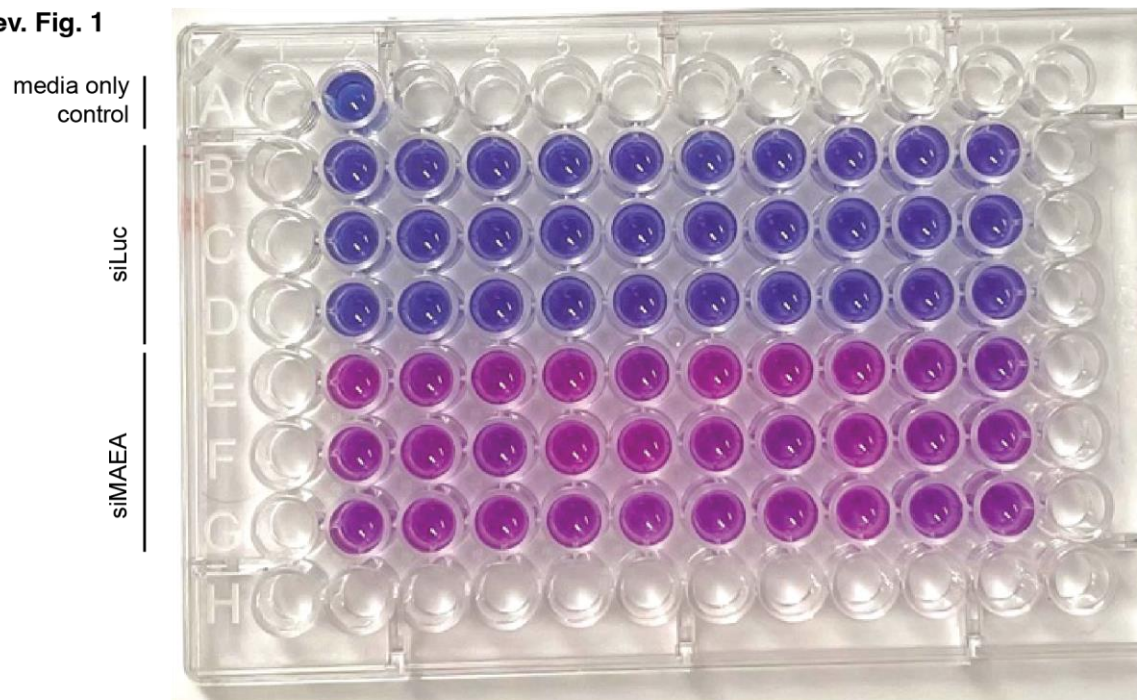
MAEA is the CTLH component known to possess a functional RING domain that is required for the CTLH to ubiquitylate its target proteins. Given that phenotypes arising from MAEA loss likely reflect dysregulation of ubiquitylation substrates of the CTLH complex, we focussed on MAEA as the functional 'core' of the complex. We have made this reasoning clearer in the text (see lines 102-103 in the revised manuscript). In addition, our identification of a new human disease associated with pathogenic variants in MAEA provided an ideal opportunity to study the clinical implications of the results of our CRISPR screen.

To further support the link between MAEA and neurodevelopmental defects, the authors should validate their findings in primary murine neurons or in SH-SY5Y cells as an in vitro model system for the results shown in Figures 1D-F, 2C-F, 3D and 3G, and 4A-C.

As suggested, we have expanded our study to examine the effects of MAEA loss on neuronal cells. The neurodevelopmental features of DIADEM might suggest an effect of MAEA loss on pre-neuronal cells, since fully differentiated neurons are post-mitotic and will not undergo DNA replication or DNA repair by RAD51-dependent homologous recombination. With that in mind, we have conducted these experiments in SH-SY5Y cells rather than primary mouse neurons.

To perform **cell viability assays** (corresponding to Figures 1D-F, 2C-F, and 4A-C), we first tried CRISPR-mediated depletion of MAEA from SH-SY5Y cells. Unfortunately, we were unable to generate monoclonal *MAEA*^{-/-} SH-SY5Y cells, perhaps suggesting that MAEA loss compromises SH-SY5Y cells' fitness, in line with our findings detailed in response to the Reviewer's above question. Therefore, we next used CRISPR-Cas9 to deplete MAEA transiently in a polyclonal population, seeding clonogenic survival assays at short timepoints after targeting and selection with puromycin. However, the plating efficiency of these targeted cells was extremely poor and made clonogenic survival assays impossible. Finally, we used siRNA to deplete in SH-SY5Y cells and subjected the cells to alamarBlue viability assays. Unfortunately, MAEA depletion appeared to drastically alter the metabolism of SH-SY5Y cells, resulting in accelerated metabolism of the alamarBlue reagent and preventing this assay from being used (**see below**). This surprising finding might reflect the role of MAEA in regulating cellular metabolism (Yi et al, *Mol Cell* 2024, Pham et al *Cell Rep* 2025). Alternatively, it may be the case that this cell type cannot tolerate compromised RAD51 loading even in unperturbed conditions.

Rev. Fig. 1



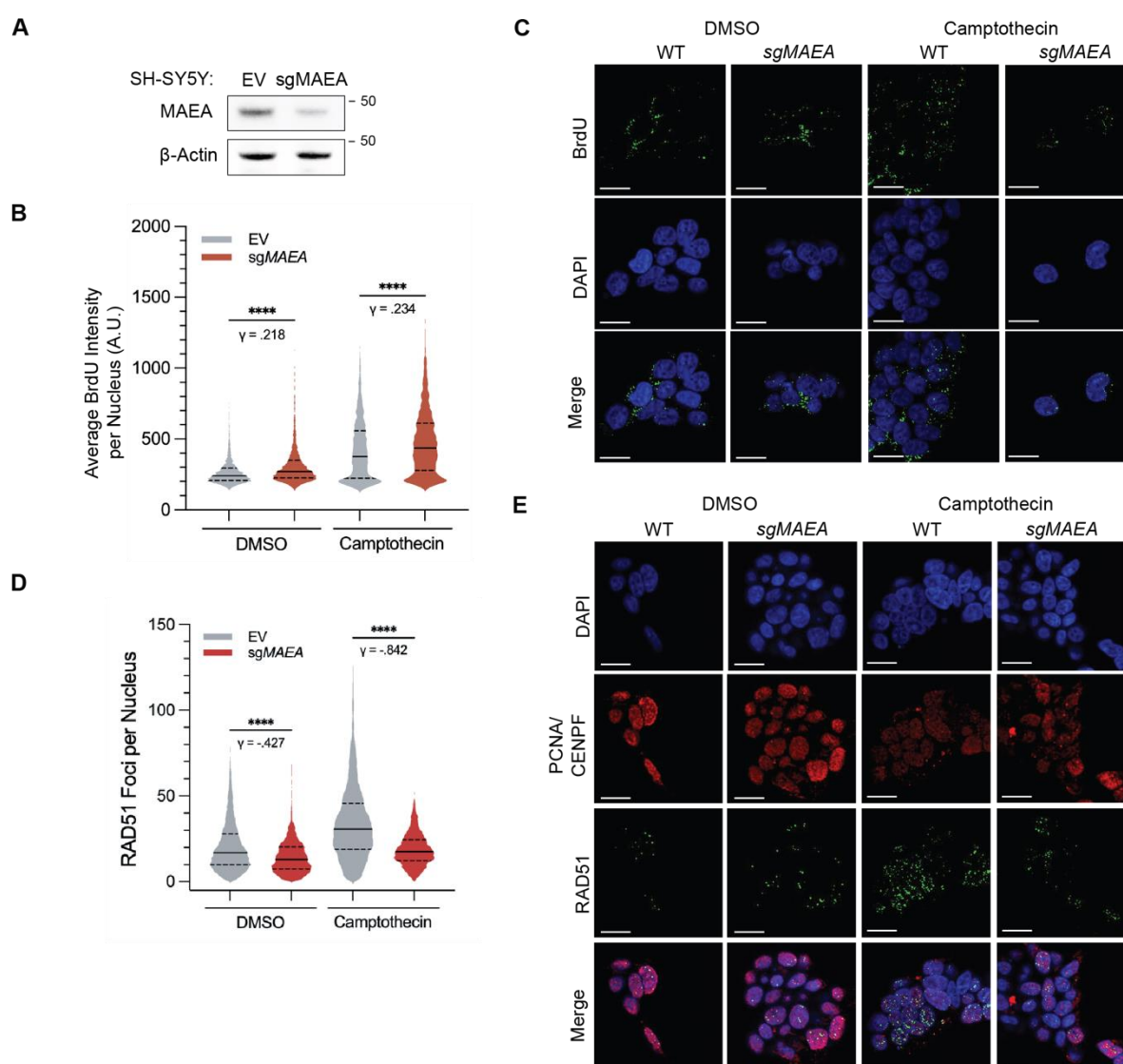
Rev. Fig. 1. AlamarBlue cell proliferation assay in SH-SY5Y cells with siLuc or siMAEA as indicated. MAEA-depleted cells metabolically reduced resazurin to fluorescent resorufin before control cells had performed the equivalent reaction to a sufficient extent to be distinguishable from the media only control.

However, using **immunofluorescence-based assays** (corresponding to Figures 3D,G), we were able to show that siRNA-mediated depletion of MAEA in SH-SY5Y cells recapitulated the cellular phenotypes previously observed in U2OS cells. Specifically, we observed that siMAEA in SH-SY5Y cells did not compromise DNA end resection following camptothecin treatment as measured using native BrdU staining for ssDNA, but caused a dramatic

reduction in RAD51 loading at seDSBs/damaged replication forks (**new Fig. EV4A-E** in the revised manuscript and below). These data are discussed in **lines 232-234** in the revised manuscript.

On balance, while we have not successfully recapitulated our cellular sensitivity phenotypes in SH-SY5Y cells due to technical or biological challenges, our finding that the key mechanistic basis of these phenotypes – impaired RAD51 loading – does recapitulate in these cells. This is strongly suggestive of a consistent source of genome instability across cell types. The observation that MAEA loss appears to have more severe consequences for cell fitness in SH-SY5Y cells could be consistent with the neurological basis of DIADEM. We will therefore pursue these ideas in follow-up studies and thank the reviewer for the suggestion that led us to these observations.

Fig. EV4



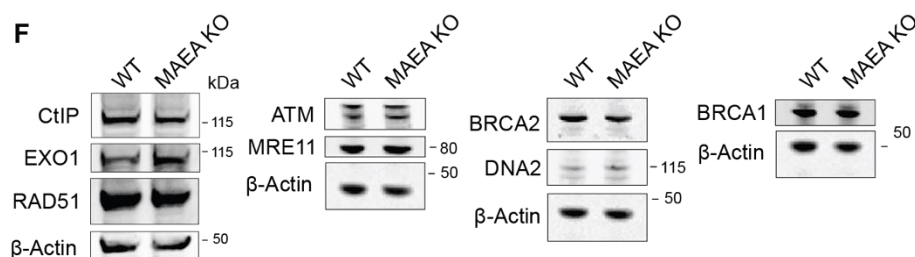
New Fig. EV4. (A) Immunoblot for MAEA depletion in polyclonal SH-SY5Y cells following CRISPR-Cas9 editing. (B) Quantification and (C) representative images of BrdU in SH-SY5Y cells treated with DMSO (1h) or camptothecin (1 μ M, 1 h). (D) Quantification and (E) representative images of RAD51 foci in S phase SH-SY5Y cells treated with DMSO or camptothecin. Bars in B and D represent median and interquartile range. P-values were generated by performing a two-tailed Kruskal-Wallis test. γ is a measure of effect size. Data

represents three independent experiments. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. Scale bars are 10 μm . For **B-C**, the top and bottom 1% of nuclei according to BrdU intensity were removed from the analysis to remove artifacts.

In Figure 3, it is important to clarify whether MAEA knockout affects the expression levels of DNA repair-associated proteins regulated by RAD51, such as DNA2.

To assess this, we have as suggested tested the effects of MAEA on expression levels of relevant homologous recombination-related proteins in MAEA-deficient cells in addition to RAD51, which we reported was unchanged in MAEA^{-/-} cells in the original manuscript. We have now tested protein levels of the pro-resection factors CtIP, EXO1 and DNA2, as well as the major proteins known to promote RAD51 loading (BRCA1, BRCA2, PALB2 and RAD51 itself), and have not found any alterations in the expression level of any of these proteins - see below and **new Fig. EV3F** in the revised manuscript. We have described these findings in **lines 230-232** in the revised manuscript.

Figure EV3



New Fig. EV3. (F) Immunoblot for indicated proteins in U2OS WT or MAEA KO cells.

In Supplementary Figure 2B, it would be helpful to extend the immunoblot detecting MAEA to include the molecular weight of eGFP-MAEA. Similarly, the blot detecting eGFP should be shown to the size of eGFP-MAEA to confirm expression.

As requested, we have provided an updated image in **new Fig. EV2** that now shows the sizes of both MAEA and eGFP-MAEA together

Providing clear explanations and additional data addressing these concerns will significantly strengthen the manuscript and further highlight the impact of the study's findings.

11th Nov 2025

Dear Dr. Carnie,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Authors: E-mail correspondence to John Thomas and Guido Zagnoli Vieira could not be delivered. Please update their e-mail addresses and make sure to enter correct e-mail addresses for all authors in our submission system.

2) In the main manuscript file, please do the following:

- Please address all comments suggested by our data editors listed below:

- o Figure legends:

- 1. Please note that the exact p values are not provided in the legends of figures 3A, B, D, G; 4G, 5A, B, C, D, E, F; EV3 B, EV4 B, D.

- There is a callout for Supplemental Figure 3C, please update.

- Please remove Reagents and Tools Table from the manuscript file and uploaded it as a separate file. More information on how to adhere to this format as well as downloadable templates (.docx) for the Reagents and Tools Table can be found in our author guidelines: <https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods>

An example of a paper with Structured Methods can be found here:

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- Author contributions: Please remove it from the manuscript and specify author contributions in our submission system. CRediT has replaced the traditional author contributions section because it offers a systematic machine-readable author contributions format that allows for more effective research assessment. You are encouraged to use the free text boxes beneath each contributing author's name to add specific details on the author's contribution. More information is available in our guide to authors:

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- In the Figure EV3 legend please correct the reference to reused images Fig. 2C, E to Fig. 3C, E.

- Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.
- In data availability statement replace current text with the following sentence "This study includes no data deposited in external repositories."

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5) Funding: Please make sure that information about all sources of funding are complete in both our submission system and in the manuscript. Currently, RUK RadNET Cambridge C17918/A2887 is missing in our submission system. Please correct.

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9) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file).

I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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 - 4) a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word file).
 - 5) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting
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 - the results obtained and
 - their clinical impact.This may be edited to ensure that readers understand the significance and context of the research. Please refer to any of our published articles for an example.
 - 6) Author contributions: the contribution of every author must be detailed in our submission system.
 - 7) EMBO Molecular Medicine now requires a complete author checklist (<https://www.embopress.org/page/journal/17574684/authorguide>) to be submitted with all revised manuscripts. Please use the checklist as guideline for the sort of information we need WITHIN the manuscript. The checklist should only be filled with page numbers where the information can be found. This is particularly important for animal reporting, antibody dilutions (missing) and exact values and n that should be indicated instead of a range.
 - 8) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one sentence bullet points that summarise the paper. Please write the bullet points to summarise the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.
- You are also welcome to suggest a striking image or visual abstract to illustrate your article. If you do please provide a jpeg file 550 px-wide x 300-600px high.
- 9) A Conflict of Interest statement should be provided in the main text
 - 10) Please note that we now mandate that all corresponding authors list an ORCID digital identifier. This takes <90 seconds to complete. We encourage all authors to supply an ORCID identifier, which will be linked to their name for unambiguous name identification.

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Each figure should be given in a separate file and should have the following resolution:

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

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

The disease being studied is very rare. This limits medical impact. However, the molecular mechanisms being studied are quite important.

Referee #1 (Remarks for Author):

The authors have addressed all my comments in this revised version of the manuscript.

Referee #3 (Remarks for Author):

Successfully revised the manuscript by substantiating our findings with multiple supporting experiments in response to reviewer feedback.

Point-by-point reply and summary of changes

1) Authors: E-mail correspondence to John Thomas and Guido Zagnoli Vieira could not be delivered. Please update their e-mail addresses and make sure to enter correct e-mail addresses for all authors in our submission system. [Done](#)

2) In the main manuscript file, please do the following:

- Please address all comments suggested by our data editors listed below:

- o Figure legends:

1. Please note that the exact p values are not provided in the legends of figures 3A, B, D, G; 4G, 5A, B, C, D, E, F; EV3 B, EV4 B, D. [As suggested below, we have included exact values in Appendix Table S1 and added callouts to this table in the relevant legends.](#) Note that during this annotation, we noted a minor display error in Fig. 5D, in which two asterisks were missing from the WT vs E349K comparison. We have rectified this and uploaded a new PDF of this figure.

- There is a callout for Supplemental Figure 3C, please update. [Updated to Fig. EV3C-D](#)

- Please remove Reagents and Tools Table from the manuscript file and uploaded it as a separate file. More information on how to adhere to this format as well as downloadable templates (.docx) for the Reagents and Tools Table can be found in our author guidelines: <https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods>

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- In the Figure EV3 legend please correct the reference to reused images Fig. 2C, E to Fig. 3C, E.

[Done](#)

- Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.

Done – as suggested, we have included an Appendix Table S1 for conveying exact P-values and added callouts in the relevant legends.

- In data availability statement replace current text with the following sentence "This study includes no data deposited in external repositories." [Done](#)

3) Tables: Please upload EV Tables with their legends as separate excel files. [Done](#)

4) Source data: Please upload a completed source data checklist. [Done](#)

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9) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file). [Done](#)

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

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Successfully revised the manuscript by substantiating our findings with multiple supporting experiments in response to reviewer feedback.

We thank the reviewer for taking the time to review our manuscript and for their constructive feedback.

24th Nov 2025

Dear Dr. Carnie,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

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Yours sincerely,
Zeljko Durdevic

Zeljko Durdevic
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EMBO Molecular Medicine

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Manuscript Number: EMM-2025-21907-V2

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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

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
- 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

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 χ^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;
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Please complete ALL of the questions below.

Select "Not Applicable" only when the requested information is not relevant for your study.

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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	Reagents and Tools, Materials and Methods, Figures, Data Availability
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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	Reagents and Tools, Materials and Methods
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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Reagents and Tools, EV tables
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Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Reagents and Tools, Materials and Methods, EV Tables
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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.	Yes	EV Tables
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Include a statement about sample size estimate even if no statistical methods were used.	Yes	Figures, Materials and Methods
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Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not Applicable	
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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	Figures, Materials and Methods

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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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.	Yes	Materials and Methods
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.	Yes	Materials and Methods
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	
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The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

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

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	Data Availability, EV Dataset 1
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?	Yes	Reagents and Tools, Materials and Methods
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	