EMBO reports

The structure of fly Teneurin-m reveals an asymmetric self-assembly that allows expansion into zippers

Jingxian Li, Sumit Bandekar, and Demet Arac-Ozkan
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Editor: Ioannis Papaioannou

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.)

1st Editorial Decision **20th Feb 2023**

Dear Dr. Arac-Ozkan,

Thank you for the submission of your research manuscript to EMBO reports and for your patience during peer review. Your manuscript has now been seen by three experts in the field, and we have received the full set of their reports, which are included below.

As you will see, all referees acknowledge that the findings are interesting and the study comprehensive and well-performed. Referees #2 and #3 have a few suggestions for further strengthening of the work, as detailed in their reports. In addition, Ref. #3 points out that some minor edits and proofreading are necessary.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed 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. If you have any questions or comments, we can also discuss the revisions in a video chat, if you like.

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 usually recommend a revision within 3 months (May 19th). Please discuss with me the revision progress ahead of this time if you require more time to complete the revisions.

*****
IMPORTANT NOTE:
We perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:

1) If a data availability section providing access to data deposited in public databases is missing.

2) If your manuscript contains statistics and error bars based on n=2. Please use scatter plots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.
*****

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 unless you opt out of this (please see below for further information).

4) a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). 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 (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>)

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: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- 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) Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>).
Specifically, we would kindly ask you to provide public access to the following datasets/data:
- Structural data

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below (see also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>):

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

*** Note - The Data Availability Section is restricted to new primary data that are part of this study. ***

8) We request authors to consider both actual and perceived competing interests. Please review the new policy (<https://www.embopress.org/competing-interests>) and update your competing interests statement if necessary. Please name this section 'Disclosure and competing interests statement' and place it after the Acknowledgements section.

9) 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} 2, use scatter plots 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

10) We now request the publication of original source data with the aim of making primary data more accessible and transparent to the reader. 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.

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 <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

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<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>.

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

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Yours sincerely,

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

Very nice and interesting paper that is thoughtful, well-written and technically sound.

Referee #2:

This manuscript reports the structure of a large portion of the ectodomain of fly Tenurin-m and an accompanying biochemical analysis of its self-association. The interpretation of the structural data is generally thoughtful and comprehensive. The authors also extend their analysis to propose a structural basis for formation of teneurin nanoclusters at the cell surface based on their crystal-packing interface.

Major comments:

1) The authors show that their crystallization construct is capable of self-association based on size exclusion elution profiles and mass photometry. The data do not argue for the formation of a discrete dimer. Rather, the mass photometry analysis as a function of concentration argues that it self-associates both into dimers and additional higher order species as the concentration of protein is increased, a self-assembly process that is also promoted by changing ionic strength. These data, and the accompanying analysis of the oligomerization of mutations that disrupt the screw-axis interface, are fully consistent with the models of self-association proposed in Figures 3, 7, and the supplementary materials. Because there is no evidence for a discrete dimer (and the asymmetric interface promotes polymerization rather than dimerization), I would encourage the authors to rephrase their text using the term oligomerization, rather than dimerization.

2) The analysis in the current manuscript is limited to structural work and self-association studies of purified protein, and as a result, the models for oligomerization and self-assembly remain speculative. While not essential for publication, the addition of an experiment testing whether mutation of the same residues in a cellular context disrupts oligomer (nanocluster) formation in cells would make the paper stronger.

Minor comment:

The authors should label Y1217 in Fig. 5 to help interpret the mutational studies.

Referee #3:

The manuscript entitled 'The structure of fly Teneurin-m reveals an asymmetric homodimer that allows expansion into zipper' by Li et al. describes the structure of the major fly Teneurin (Ten-n). The authors observe that Ten-m can form homophilic dimers in solution in a concentration dependent manner. A putative dimerization interface identified in one of the crystallographic contact regions is consistent with protein-protein interaction properties and sequence conservation. This interface was validated using site directed mutagenesis and SEC chromatography methods. The authors further showed that an alternate arthropod splice variant does not disrupt this dimerization interface. Interestingly, the vertebrate splice sites on the beta-propeller are incompatible with this dimer interface and the author propose that vertebrates may have evolved this feature to regulate homophilic interactions in higher organisms.

Teneurins play an essential role in developmental processes and synapse formation. While remarkable progress has been made to elucidate their structure and function there is still some unresolved questions, such how homophilic interacts are mediated at the molecular level. In this manuscript the authors have identified a new Ten-m asymmetric dimerization interface in flies that is different from that observed in mammalian Ten-4, which is largely symmetric (Meijer et al., 2022). The dimer organization of Ten-m is asymmetric and consistent with larger assemblies on the cell surface that resemble protocadherins. The results are new and open up the possibility to design further experiments to better understand the role of this interface during nervous system development in flies. It's uncertain how the importance of this interface will translate to vertebrate species given the low sequence conservation in this region between species. However, as the authors point out it is interesting that vertebrate Teneurins have acquired alternate slice sites at this interface, suggesting they have acquired this feature during evolution in more complex organisms. Further studies are required but this work provides a good basis to design new experiments to test this hypothesis.

The manuscript is concise and well written, although there are some edits and proof reading needed because errors appear to have occurred during refence insertion. The biochemical and X-ray crystallographic studies are of a high quality and well described. The results clearly add to the current knowledge of Teneurins and are of sufficient quality for publication in EMBO reports. However, I believe one or two further experiments could enhance the impact of this article (see below). I would therefore welcome the authors to comment on the two proposals below but am otherwise happy to support publication provided the PDB is deposited, I didn't see any submission code in the manuscript. I would also like to see the final PDB validation report even if the metrics reported in the manuscript look good.

1. While the dimer interface proposed has been validated using mutagenesis combined with SEC I was wondering did the authors ever considered trying small angle X-ray scattering? This would validate the overall shape of the at low resolution in solution? These experiments are relatively simple and would confirm the dimer orientation (or larger oligomers) proposed. If sample amounts are a problem then perhaps a low resolution Cryo-EM (or negative stain), without a 3D reconstruction, would be sufficient for such a characterisation.
2. Can the authors confirm that their dimer interaction is valid in relation to full length fly Ten-m. For example a cell aggregation assay could confirm if the 'trans' interaction occurs, or is it predominantly a 'cis' interaction.

Manuscript EMBOR-2022-56728-T: Response to Reviewer Comments

We thank the reviewers for their time and effort in considering our manuscript. Below we provide responses to the reviewers, and we plan to make any additional data available including the PDB validation report as requested by reviewer #3. Our responses are in bold.

Referee #1:

Very nice and interesting paper that is thoughtful, well-written and technically sound.

We are pleased that Reviewer 1 found our work thoughtful, well-written, and technically sound. We thank the reviewer for their time and effort in reading and critically evaluating our manuscript.

Referee #2:

This manuscript reports the structure of a large portion of the ectodomain of fly Tenurin-m and an accompanying biochemical analysis of its self-association. The interpretation of the structural data is generally thoughtful and comprehensive. The authors also extend their analysis to propose a structural basis for formation of teneurin nanoclusters at the cell surface based on their crystal-packing interface.

We thank this reviewer for their kind comments including their assessment that our paper is thoughtful and comprehensive. We respond to detailed comments below.

Major comments:

1. The authors show that their crystallization construct is capable of self-association based on size exclusion elution profiles and mass photometry. The data do not argue for the formation of a discrete dimer. Rather, the mass photometry analysis as a function of concentration argues that it self-associates both into dimers and additional higher order species as the concentration of protein is increased, a self-assembly process that is also promoted by changing ionic strength. These data, and the accompanying analysis of the oligomerization of mutations that disrupt the screw-axis interface, are fully consistent with the models of self-association proposed in Figures 3, 7, and the supplementary materials. Because there is no evidence for a discrete dimer (and the asymmetric interface promotes polymerization rather than dimerization), I would encourage the authors to rephrase their text using the term oligomerization, rather than dimerization.

We thank the reviewer for this insightful comment. We agree with the overall thought process that the dimer is an 'intermediate' species on the path towards formation of the Ten-m oligomer, depending on protein concentration and ionic strength. We chose to use the term dimer throughout our manuscript since it is the predominant species we observe in SEC at high concentrations (when compared to protein standards run on the same column). We include a revised Figure 1 including an overlay of our experimental chromatograms with gel filtration standards. We have revised the text to refer to the dimer as the 'dimer interface' instead. We agree with the reviewer that the dimer interface is simply a building block to make large Ten-m assemblies, and we

have no evidence that the dimeric species is of particular biological relevance compared to a trimer, tetramer, and so on. We have revised the text to indicate this.

2. The analysis in the current manuscript is limited to structural work and self-association studies of purified protein, and as a result, the models for oligomerization and self-assembly remain speculative. While not essential for publication, the addition of an experiment testing whether mutation of the same residues in a cellular context disrupts oligomer (nanocluster) formation in cells would make the paper stronger.

The authors agree that disrupting nanoclusters would be very informative to dissect the biological relevance of our Ten-m dimer interface. Unfortunately, observing teneurin nanoclusters requires advanced super resolution microscopy techniques that we do not currently have in our lab. We are interested in performing these experiments in collaboration, but it will take time to get started on these, and we hope the reviewers understand that we feel those experiments are out of the scope of this manuscript. We plan to conduct these studies and publish them in following manuscripts.

Minor comment:

The authors should label Y1217 in Fig. 5 to help interpret the mutational studies.

We agree with this thoughtful suggestion and we have updated Figure 5, panel A to include Y1217.

Referee #3:

The manuscript entitled 'The structure of fly Teneurin-m reveals an asymmetric homodimer that allows expansion into zipper' by Li et al. describes the structure of the major fly Teneurin (Ten-n). The authors observe that Ten-m can form homophilic dimers in solution in a concentration dependent manner. A putative dimerization interface identified in one of the crystallographic contact regions is consistent with protein-protein interaction properties and sequence conservation. This interface was validated using site directed mutagenesis and SEC chromatography methods. The authors further showed that an alternate arthropod splice variant does not disrupt this dimerization interface. Interestingly, the vertebrate splice sites on the beta-propeller are incompatible with this dimer interface and the author propose that vertebrates may have evolved this feature to regulate homophilic interactions in higher organisms.

Teneurins play an essential role in developmental processes and synapse formation. While remarkable progress has been made to elucidate their structure and function there is still some unresolved questions, such how homophilic interacts are mediated at the molecular level. In this manuscript the authors have identified a new Ten-m asymmetric dimerization interface in flies that is different from that observed in mammalian Ten-4, which is largely symmetric (Meijer et al., 2022). The dimer organization of Ten-m is asymmetric and consistent with larger assemblies on the cell surface that resemble protocadherins. The results are new and open up the possibility to design further experiments to better understand the role of this interface during nervous system development in flies. It's uncertain how the importance of this interface will translate to vertebrate species given the low sequence

conservation in this region between species. However, as the authors point out it is interesting that vertebrate Teneurins have acquired alternate slice sites at this interface, suggesting they have acquired this feature during evolution in more complex organisms. Further studies are required but this work provides a good basis to design new experiments to test this hypothesis.

The manuscript is concise and well written, although there are some edits and proof reading needed because errors appear to have occurred during refence insertion.

We thank the reviewer for this fine attention to detail. We have corrected these errors.

The biochemical and X-ray crystallographic studies are of a high quality and well described. The results clearly add to the current knowledge of Teneurins and are of sufficient quality for publication in EMBO reports.

We thank this reviewer for their comments including that the work is well-written, that the X-ray crystallography and biochemistry are high quality and well described, and that our results add to the knowledge of Teneurins.

However, I believe one or two further experiments could enhance the impact of this article (see below). I would therefore welcome the authors to comment on the two proposals below but am otherwise happy to support publication provided the PDB is deposited, …

We also thank the reviewer for suggesting the below experiments which were already in our mind, but not asking us to perform them for publication. We respond to detailed comments below.

… happy to support publication provided the PDB is deposited, I didn't see any submission code in the manuscript. I would also like to see the final PDB validation report even if the metrics reported in the manuscript look good.

We have included the PDB code in the manuscript and added the final PDB validation report as part of the files with the revised manuscript.

1. While the dimer interface proposed has been validated using mutagenesis combined with SEC, I was wondering did the authors ever considered trying small angle X-ray scattering? This would validate the overall shape of the at low resolution in solution? These experiments are relatively simple and would confirm the dimer orientation (or larger oligomers) proposed. If sample amounts are a problem, then perhaps a low resolution Cryo-EM (or negative stain), without a 3D reconstruction, would be sufficient for such a characterization.

We thank the reviewer for this suggestion and agree that SAXS and cryo-EM would be an interesting way to address this problem. In regards to this manuscript, we think the information SAXS provides will be largely redundant with the experiments we present in this manuscript. The shape of the dimer is provided by the packing in our crystal structure. We confirm that our crystal packing represents in-solution through our analytical size exclusion chromatography experiments. However, we are planning to

pursue cryoEM and SAXS in a detailed follow up manuscript to describe Ten-m oligomers in solution and in cells.

2. Can the authors confirm that their dimer interaction is valid in relation to full length fly Ten- m. For example, a cell aggregation assay could confirm if the 'trans' interaction occurs, or is it predominantly a 'cis' interaction.

Thank you for the kind suggestion. We recently optimized a cell aggregation assay using HEK293T cells in our lab and we are in the process of optimizing full-length Ten-m expression in mammalian cells. We plan to include this assay in a detailed follow up manuscript characterizing Ten-m oligomers and determining the functional effect of them using SAXS, super-resolution microscopy, and cryo-EM (or ET perhaps).

Dear Dr. Arac-Ozkan,

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the comments of the two referees that re-evaluated your study. Please find their comments included below.

Both referees are generally positive about the study and the manuscript, but there are two remaining issues that we need you to address before we can accept your manuscript for publication in EMBO reports:
1. Please make sure that the title, abstract, subheadings, and text (as well as the synopsis text and bullet points) are accurate and that all claims and conclusions throughout the manuscript are fully supported by the available data (taking on board the advice of referee #1). Please highlight all changes (to be clearly visible) in a revised version of the manuscript.
2. Thorough proof reading of the text is necessary before acceptance of the manuscript.

From the editorial side, there are also a few things that we need from you:

- Your revised title should be up to 100 characters (including spaces), and your revised abstract should not exceed 175 words. Please remember to update the title also on the first page of your Appendix.

- Please update your competing interests statement: the heading should be "Disclosure and competing interests statement" and the statement "The authors declare that they have no conflict of interest.", since you have no competing interests to declare.

- Figure callouts for Figure 2B&C, EV3D, EV4A&B, EV5A, Appendix Fig S1A&B are missing. Please make sure that all Figure panels are called out in your revised manuscript.

- Please include page numbers in the Table of Contents (first page) of your Appendix.

- Please note that the synopsis image should be exactly 550 pixels wide (the height is variable, between 300-600 pixels) and all text readable at the final size.

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- Please remove the numbered paragraph formatting from the Materials and Methods.

- Your manuscript has been inspected by our data editors for completeness and accuracy. Please see the required changes in the attached Word file and address them all in your revised manuscript (highlighted or with tracked changes).

Please also note that as part of the EMBO publications' 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.

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

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

Yours sincerely,

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #2:

Honestly, I am quite disappointed that the revision is virtually non-responsive to the comments of reviewers 2 and 3 other than acknowledgment in the response that the reviewers' points are sound. In particular, the title still reads "The structure of fly Teneurin-m reveals an asymmetric homodimer that allows expansion into zippers" even though the authors recognize in the written response to review that there is no evidence to support claims that there is a discrete dimer that has functional importance. It will take little effort to revise the text and title to replace the terms dimer and dimeric interface with the terms oligomer and self-association interface, which are more accurate descriptions of the findings.
For the title, I suggest: "The structure of fly Teneurin-m reveals an asymmetric self-association interface that allows expansion into zippers." The abstract, text, and subheadings in the manuscript should likewise be revised to use the appropriate terms for oligomers and self-association interfaces.

Referee #3:

The authors have addressed my critical comments in their rebuttal letter. I have also checked the PDB validation file included and the metrics conform to a macromolecule of this size and resolution. I therefore support publication in EMBL reports. However, the authors should carry out a thorough proof reading as many punctuations are misplaced (especially before/after) reference insertions.

Manuscript EMBOR-2022-56728V3: Response to Reviewer Comments

We thank the reviewers for their time and effort in considering our manuscript. Below we provide responses to the reviewers in bold text.

Reviewer #2:

Honestly, I am quite disappointed that the revision is virtually non-responsive to the comments of reviewers 2 and 3 other than acknowledgment in the response that the reviewers' points are sound. In particular, the title still reads "The structure of fly Teneurin-m reveals an asymmetric homodimer that allows expansion into zippers" even though the authors recognize in the written response to review that there is no evidence to support claims that there is a discrete dimer that has functional importance. It will take little effort to revise the text and title to replace the terms dimer and dimeric interface with the terms oligomer and self-association interface, which are more accurate descriptions of the findings.
For the title, I suggest: "The structure of fly Teneurin-m reveals an asymmetric self-association interface that allows expansion into zippers." The abstract, text, and subheadings in the manuscript should likewise be revised to use the appropriate terms for oligomers and self-association interfaces.

We apologize that we were not sufficiently clear in our previous response to reviewer 2. As stated in our previous response document, we agreed with the sentiment from the reviewers that the dimer is only one of several potential oligomeric states with biological relevance. However, the dimer is the minimum functional unit of larger oligomeric states so we did not feel we were being dishonest in the use of the word "dimer" to describe the self-association interface of Teneurin-m. That being said, we do agree with the reviewer in this case, and we have modified our manuscript using terms such as "homophilic interaction, self-association interface, self-assembly, and oligomer" in place of dimer whenever possible. We planned to use the reviewer's suggested title, however it is too long for the journal's restrictions, so we instead chose: "Structure of Teneurin-m reveals an asymmetric self-assembly that allows expansion into zippers".
We do distinctly see dimeric species in size exclusion and mass photometry experiments, so we believe that dimer is an accurate term to describe these results. We include "observed dimer" to delineate that this is merely what we observe and it may not have particular biological relevance.

Reviewer #3:

The authors have addressed my critical comments in their rebuttal letter. I have also checked the PDB validation file included and the metrics conform to a macromolecule of this size and resolution. I therefore support publication in EMBL reports. However, the authors should carry out a thorough proof reading as many punctuations are misplaced (especially before/after) reference insertions.

We thank reviewer 3 for their time and effort in reviewing the structural data associated with our manuscript. We have carried out careful proof-reading of our revised document and we have shifted all reference insertions before periods at the end of sentences as seen in published EMBO reports papers.

Dr. Demet Arac-Ozkan
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Biochemistry and Molecular Biology
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University of Chicago GCIS W210
Chicago, IL 60637
United States

Dear Dr. Arac-Ozkan,

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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If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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."

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Ioannis Papaioannou, PhD
Editor
EMBO reports

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EMBO Press Author Checklist

Corresponding Author Name: Demet Araç
Journal Submitted to: EMBO Reports
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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?	Not Applicable	

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	Not Applicable	

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	Materials and Methods

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	Materials and Methods
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.	Not Applicable	

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

Design

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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	

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Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
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?	Not Applicable	
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	
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?	Not Applicable	

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

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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.	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	
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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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Reporting

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.

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For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
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	

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	We have deposited the structural data in the Protein Data Bank and we provide the PDB validation report. Other data are provided in Source Data.
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	