

Partially inserted nascent chain unzips the lateral gate of the Sec translocon

Lukas Kater, Benedikt Frieg, Otto Berninghausen, Holger Gohlke, Roland Beckmann, Alexej Kedrov

Review timeline:

Submission date:	29 March 2019
Editorial Decision:	9 May 2019
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Editorial Decision:	4 July 2019
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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision

29 March 2019

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

As you will see, the referees acknowledge the interest of your findings and support publication in EMBO reports after relatively minor revisions.

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

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

I include below some important points to consider before submitting your revised manuscript:

Please list the accession numbers and database for the cryo-EM and molecular model in a formal "Data Availability" section (placed after Materials & Method) that follows the model below (see also <http://embor.embopress.org/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

Our journal also 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 < <http://embor.embopress.org/authorguide#referencesformat>>.

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. 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. Please also include scale bars in all microscopy images.

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

When submitting your revised manuscript, we will require:

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

our Author guidelines (<http://embor.embopress.org/authorguide>).

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

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

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

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

REFeree REPORTS

Referee #1:

In their manuscript Kater et al. explore the structure of an early intermediate of a ribosome-nascent chain complex in contact with the SecYEG translocon. By using Cryo-EM, MD simulations and biochemical assays, they show that a short nascent chain induces a partial opening of SecY's lateral gate and that SecE unexpectedly appears to modulate lateral gate movements. The authors further suggest that the nascent chain remains in an unfolded or loosely folded state until it is exposed to the lipid environment.

Despite a wealth of structural and biochemical data, our understanding on how the SecYEG translocon mediates membrane protein insertion is still enigmatic and the structural characterization of an early insertion intermediate is therefore an important step forward in understanding this mechanism. The manuscript is well written and the data in general support the conclusions. Still, I have a few issues the authors should address for improving their study.

Major issues:

1. The authors suggest that the nascent chain achieves proper folding only when reaching the lipids. This conclusion is based on cross-linking experiments, which show that three consecutive residues (FtsQ40-FtsQ42) can be cross-linked to a single SecY residue. In my opinion, these observations are not sufficient to conclude that the nascent chain is indeed unfolded/loosely folded. Helix formation has been shown to occur already within the ribosomal tunnel and the cross-link pattern could also be explained by different orientations of a folded helix within the lateral gate. Thus the authors should tone down their statement or back it up by further experiments, e.g. FRET analyses.
2. Based on the CBB-stained gel in Fig. 1C, it appears that SecE/G are sub-stoichiometric to SecY. Although it is known that SecG is not easily stained by CBB, at least SecE should be detectable. Could the authors use a different staining method or immune detection for demonstrating that they have indeed primarily reconstituted SecYEG into their nanodiscs?

Minor issues:

1. Page 1: to me the description of SecE sounds as if SecE would have four transmembrane domains, which is not the case. The authors should rephrase this part.
2. The authors might want to refer to data by Beck et al. (2000) and Houben et al (2004). In both studies RNC cross-links to SecE have been observed, which was difficult to reconcile with the structure of the SecYEG complex. The current structure might provide an explanation for these contacts.
3. Fig. 1: The MW of SecY seems to be rather high; it usually migrates at approx. 35 kDa, but here it migrates at approx. 42 kDa. Is there any special reason?
4. In general, the manuscript would be much easier to comprehend, if the TMH and loops in the individual figures would be labeled; e.g. Fig. 3, Fig. 4G
5. The occasional SecY dimers seem to be rather pronounced in the absence of ribosomes/RNCs and

- are much weaker in their presence. Does this suggest that ribosomes/RNCs dissociate SecY dimer?
3. Add MW marker to Fig. 5E
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 5. Fig. S1: include length of the different parts of the RNC; typo: 'hemagglutinin'
 6. Fig. S4: typo 'quiescent'
 7. Fig. S5 needs labeling, define color code
 8. Fig. S10E is not described in the legend; define *
 9. Reference 51 is incomplete

Referee #2:

Membrane insertion of proteins is co-translationally achieved by the Sec translocon complex and the ribosome-nascent chain complex (RNC). Although many biochemical and biophysical analyses have been performed, the structural changes that SecYEG (bacterial Sec translocon) undergoes during this process are still not known. From the point of view of basic research, it is very important to obtain a structural snapshot of SecYEG. In this study, the most accurate structure of the SecYEG-nanodisc-RNC complex obtained thus far has been shown. SecYEG in the lipid bilayer of the nanodisc represents its pre-open form, possibly induced by the binding of the RNC complex. The EM structure shows separate densities of SecYEG TMHs, which is the most important and reliable point of this study. The conformational changes in SecYEG were supported by means of molecular dynamics simulation using the new structural model of SecYEG without the RNC complex. Importantly, I would like to point out that the reliability of the MD simulation would be low because of the moderate resolution model of SecYEG. However, performing the MD simulation as a successive analysis was a nice approach in this paper. Although it was very difficult to clearly identify the position of the substrate protein on the SecYEG from the presented EM data, the authors elucidated the positions of the substrate protein at the crevasse of SecYEG by performing site-directed crosslink analysis. As these data present key information for understanding membrane protein insertion, I strongly recommend publishing this paper in EMBO reports. To make your article easier to understand, I recommend that you address the following points:

A cited paper (Tanaka et al., 2015) in the text has presented the crystal structure (5CH4) of SecYEG with a peptide in its crevasse. In your paper, a comparison with this structure may be interesting. I suggest that the authors include and discuss this comparison.

In Fig. 1A, the SecYEG structure is from *Thermus thermophilus*, and not *Thermatoga maritima*. Please make this change.

In Fig. 1A, please label the sides, "in" and "out".

In Fig. 2B, please include the scale bar.

In Fig. 3D, please label the numbers of TMHs; this will help the readers easily understand this figure.

In Fig. 5B, the additional map does not seem to be clear. Please modify it for clarity.

In Fig. S4, please illustrate the membrane planes, and label the sides "in" and "out". Expanding the figure size would be better because the figures are a little complicated to comprehend.

In Fig. 10B, please indicate the precise mutated positions.

For Fig. 10E, the legend is missing. Please include this.

Because PE is one of the components of bacterial membrane, the EM map of SecYEG-nanodisc using POPG/POPE/POPC in Fig. S11 conveys important information. Please include the FCS graph in Fig. S11. In Fig. S11, the SecYEG-nanodisc (POPG/POPC) structure should be included to compare the structures. A superimposed figure may be apt to depict similar structures. Additionally, I recommend including detailed comments about the structure in the text.

Referee #1:

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Authors: Thank you for your positive comments!

Still, I have a few issues the authors should address for improving their study. Major issues:

Reviewer 1, Comment 1: *The authors suggest that the nascent chain achieves proper folding only when reaching the lipids. This conclusion is based on cross-linking experiments, which show that three consecutive residues (FtsQ40-FtsQ42) can be cross-linked to a single SecY residue. In my opinion, these observations are not sufficient to conclude that the nascent chain is indeed unfolded/loosely folded. Helix formation has been shown to occur already within the ribosomal tunnel and the cross-link pattern could also be explained by different orientations of a folded helix within the lateral gate. Thus the authors should tone down their statement or back it up by further experiments, e.g. FRET analyses.*

Authors: Thank you for the suggestion! We based our model of the partially unfolded nascent chain on the combination of cross-linking results, absence of helix-like densities in the cryo-EM map, and also geometrical considerations, which largely exclude the fully folded nascent chain forming an insertion hairpin within the translocon due to the insufficient length of the polypeptide chain. However, we agree that those considerations are rather indirect and alternative interpretations of the experimental results are possible. Thus, we have modified the abstract, discussion and the legend of Fig. 5F to describe a flexible state of the partially inserted nascent chain.

Reviewer 1, Comment 2: *Based on the CBB-stained gel in Fig. 1C, it appears that SecE/G are substoichiometric to SecY. Although it is known that SecG is not easily stained by CBB, at least SecE should be detectable. Could the authors use a different staining method or immune detection for demonstrating that they have indeed primarily reconstituted SecYEG into their nanodiscs?*

Authors: A new SDS-PAGE of the SecYEG-ND preparation after size exclusion chromatography have been included in the revised manuscript that validates the presence of SecE at stoichiometric amounts to SecY. In agreement, the presented cryo-EM map reveals SecY and SecE densities at comparable signal levels, thus verifying the stable inter-subunit assembly upon the translocon reconstitution into nanodiscs.

Minor issues:

Reviewer 1, Comment 3: *Page 1: to me the description of SecE sounds as if SecE would have four transmembrane domains, which is not the case. The authors should rephrase this part.*

Authors: The text has been modified for clarity.

Reviewer 1, Comment 4: *The authors might want to refer to data by Beck et al. (2000) and Houben et al (2004). In both studies RNC cross-links to SecE have been observed, which was difficult to reconcile with the structure of the SecYEG complex. The current structure might provide an explanation for these contacts.*

Authors: Thank you for the suggestion! Indeed, co-translational cross-linking of both MtlA and the leader peptidase (Lep) to SecE have been previously described at different stages of insertion. The visualized proximity of SecE TMH 1 to the lateral gate may explain the crosslinks with the nascent chains being sufficiently long to be exposed beyond the lateral gate, as it is the case for Lep in Houben et al. (2004) *EMBO Reports*. However, clarifying the crosslinks between SecE and the nascent chain residues near the ribosomal exit tunnel as reported by Beck and co-workers is more challenging and would require structural analysis of the translocon engaged with the particular nascent chain (MtlA). The discussion and the reference to Houben et al. have been added to the manuscript (page 7).

In the revised manuscript we have also included a reference to a recent publication from Reid Gilmore's lab that describes the dynamics of the homologous Sec61 translocon and shows that the residues between the loop 6/7 and TMH 7 play a key role in opening of the lateral gate (Mandon et al. 2018 *J Biol Chem*) that is in a remarkable agreement with our results on the coupled dynamics of the ribosome-binding loop and the lateral gate.

Reviewer 1, Comment 5: *Fig. 1: The MW of SecY seems to be rather high; it usually migrates at approx. 35 kDa, but here it migrates at approx. 42 kDa. Is there any special reason?*

Authors: The increase in the apparent MW of SecY upto 40 kDa (Fig. 1C and EV5, panels C and D) is likely attributed to 28 amino acids extension at the N-terminal end: a deca-histidine tag followed by a flexible linker and the 3C protease cleavage site. The extension would not only increase the molecular weight of the protein by 3 kDa, but it also increases its polarity and interactions with SDS, thus affecting the migration within SDS-PAGE. The details on the used SecYEG variant have been added to the Methods section.

Reviewer 1, Comment 6: *In general, the manuscript would be much easier to comprehend, if the TMH and loops in the individual figures would be labeled; e.g. Fig. 3, Fig. 4G*

Authors: Following the advice (also Reviewer 2, Comment 5) labelling of the secondary structure elements was added to Figures 3 and 4.

Reviewer 1, Comment 7: *The occasional SecY dimers seem to be rather pronounced in the absence of ribosomes/RNCs and are much weaker in their presence. Does this suggest that ribosomes/RNCs dissociate SecY dimer?*

Authors: We agree with the Reviewer that there is a pronounced effect of RNCs on the appearance of SecY:SecY crosslinked adducts in Fig. 5D. Indeed, one hypothesis would be a dissociation of dimeric translocons, or a conformational change within the lateral gate that prevents SecY:SecY crosslinking. However, since the reconstituted SecYEG dimers represent a minute fraction of nanodiscs formed in excess of lipids and MSP (Taufik, Kedrov et al. 2013 *J Mol Biol*), and also a dual orientation of protomers within a disc is possible, explaining the role of the RNC binding on SecYEG quaternary dynamics based on the crosslinking results would be rather ambiguous.

Reviewer 1, Comment 8: *Add MW marker to Fig. 5E*

Authors: Position of the pre-stained 70 kDa MW marker was added to the image.

Reviewer 1, Comment 9: *In Fig. 5F, the early intermediate state depicts the nascent chain completely within the translocon, while Fig. S1 and the text leave the impression that the N-terminus of the RNC is already outside. This should be consistent.*

Authors: Thank you for the comment! As the structural and biochemical experiments suggest that the N-terminal end of the nascent chain leaves the translocon, the scheme in Fig. 5F has been modified. Additionally, the TMHs of the lateral gate have been indicated for clarity.

Reviewer 1, Comment 10: *Fig. S1: include length of the different parts of the RNC; typo: 'hemagglutinin'*

Authors: The lengths of each part has been added and the typo corrected. In the revised manuscript the figure is listed as the Expanded View Figure EV1.

Reviewer 1, Comment 11: *Fig. S4: typo 'quiescent'*

Authors: The typo has been corrected.

Reviewer 1, Comment 12: *Fig. S5 needs labeling, define color code*

Authors: Labeling and an explanation for the color code has been added. The rainbow color scale encodes α -distance between the two aligned models ranging from 0 Å (blue) to 3 Å or greater (red). In the revised manuscript the figure is listed as the Expanded View Figure EV3.

Reviewer 1, Comment 13: *Fig. S10E is not described in the legend; define **

Authors: The figure panel has been described. Thank you for the remark! In the revised manuscript the figure is listed as the Expanded View Figure EV5.

Reviewer 1, Comment 14: *Reference S1 is incomplete.*

Authors: The reference has been corrected.

Referee #2:

Membrane insertion of proteins is co-translationally achieved by the Sec translocon complex and the ribosome-nascent chain complex (RNC). Although many biochemical and biophysical analyses have been performed, the structural changes that SecYEG (bacterial Sec translocon) undergoes during this process are still not known. From the point of view of basic research, it is very important to obtain a structural snapshot of SecYEG. In this study, the most accurate structure of the SecYEG-nanodisc-RNC complex obtained thus far has been shown. SecYEG in the lipid bilayer of the nanodisc represents its pre-open form, possibly induced by the binding of the RNC complex. The EM structure shows separate densities of SecYEG TMHs, which is the most important and reliable point of this study. The conformational changes in SecYEG were supported by means of molecular dynamics simulation using the new structural model of SecYEG without the RNC complex. Importantly, I would like to point out that the reliability of the MD simulation would be low because of the moderate resolution model of SecYEG. However, performing the MD simulation as a successive analysis was a nice approach in this paper. Although it was very difficult to clearly identify the position of the substrate protein on the SecYEG from the presented EM data, the authors elucidated the positions of the substrate protein at the crevasse of SecYEG by performing site-directed crosslink analysis. As these data present key information for understanding membrane protein insertion, I strongly recommend publishing this paper in EMBO reports.

Authors: Thank you for the positive evaluation!

To make your article easier to understand, I recommend that you address the following points:

Reviewer 2, Comment 1: A cited paper (Tanaka et al., 2015) in the text has presented the crystal structure (5CH4) of SecYEG with a peptide in its crevasse. In your paper, a comparison with this structure may be interesting. I suggest that the authors include and discuss this comparison.

Authors: We apologize for leaving the mentioned structure out of the initial manuscript. Indeed, the structure 5CH4 may be interpreted as a very early intermediate in the lateral gate opening by a signal sequence, where the outward displacement of TMH 2b widens the lateral gate by ~3 Å relatively to the quiescent state (5AWW). Interestingly, our molecular model, as well as previous high-resolution structures describe inwards movement of TMH 2b upon the nascent chain insertion in the presence of a ribosome. Furthermore, no movement of TMH 7 is observed in 5CH4, that agrees with our findings that the dynamics of TMH 7 is mediated by the ribosome docking on the loop 6/7. The reference and a short discussion have been added to the manuscript (page 8).

Reviewer 2, Comment 2: In Fig. 1A, the SecYEG structure is from *Thermus thermophilus*, and not *Thermatoga maritima*. Please make this change.

Authors: Thank you for the remark! We apologize for the mistake.

Reviewer 2, Comment 3: In Fig. 1A, please label the sides, "in" and "out".

Authors: The membrane interfaces in Fig. 1A and also the Appendix Figure S2 (formerly S4) have been indicated as "cytoplasm" and "periplasm".

Reviewer 2, Comment 4: In Fig. 2B, please include the scale bar.

Authors: The scale bar (20 nm) has been added.

Reviewer 2, Comment 5: In Fig. 3D, please label the numbers of TMHs; this will help the readers easily understand this figure.

Authors: Following the Reviewer's advice, labelling of the secondary structure elements was added.

Reviewer 2, Comment 6: In Fig. 5B, the additional map does not seem to be clear. Please modify it for clarity.

Authors: We have modified the figure (transparency levels, the view angle) to improve the presentation quality.

Reviewer 2, Comment 7: In Fig. S4, please illustrate the membrane planes, and label the sides "in" and "out". Expanding the figure size would be better because the figures are a little complicated to comprehend.

Authors: The approximate membrane borders and sidedness have been indicated. We also optimised the figure assembly that, we hope, allow for better view of superimpositions. In the revised manuscript the figure is listed as the Appendix Figure S2.

Reviewer 2, Comment 8: In Fig. 10B, please indicate the precise mutated positions.

Authors: Cysteine positions within the lateral gate have been specified according to the Reviewer's suggestion.

Reviewer 2, Comment 9: For Fig. 10E, the legend is missing. Please include this.

Authors: The figure panel has been described. Thank you for the remark!

Reviewer 2, Comment 10: Because PE is one of the components of bacterial membrane, the EM map of SecYEG-nanodisc using POPG/POPE/POPC in Fig. S11 conveys important information. Please include the FCS graph in Fig. S11. In Fig. S11, the SecYEG-nanodisc (POPG/POPC) structure should be included to compare the structures. A superimposed figure may be apt to depict similar structures. Additionally, I recommend including detailed comments about the structure in the text.

Authors: The presented cryo-EM map serves as a direct proof of the RNC:translocon complex assembly in presence of PE lipids, and an overlay of SecYEG-ND cryo-EM density map with the built molecular model has been included in the revised manuscript, as it is suggested by the Reviewer (the figure is currently listed as the Appendix Figure S6). Unfortunately, the cryo-EM reconstruction obtained in presence of POPE did not allow for detailed structural analysis due to the limited resolution (filtered at 9 Å). Upon single-particle analysis we have observed high heterogeneity within SecYEG-ND, that agrees well with previous structural and biochemical studies on PE-containing nanodiscs (Shenkarev *et al.* 2013, *BBA*; Henrich *et al.* 2017 *eLife*). One possible explanation is poor compatibility of PE lipids with planar bilayers, as it has been documented for DOPE, but may also account for POPE employed in our work. Furthermore, the relatively high transition temperature of POPE (25 °C vs. 3 °C for POPG and POPC) may cause separation of lipids based of physico-chemical properties under ambient temperature, thus adding to the complexity and the heterogeneity of the nanodisc preparation.

2nd Editorial Decision

5 June 2019

Thank you for your patience while we have editorially assessed your revised manuscript. I am now writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once a few minor issues/corrections have been addressed, as follows.

- Please provide a legend for movie EV1 as follows: the legend is a simple README.txt file. Then the legend and the movie are zipped together and the zip file is uploaded (i.e., one .zip file including legend and movie).

- Please provide a link to the database in the Data availability section. The description of the data(base) should follow this model:

- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

- Please provide up to five keywords on the first page of the manuscript.

- Figure callouts in the text: We noticed that the callouts to Appendix Figs S1 and S2 are missing the word 'Appendix'.

- In our routine analysis of all figures we noticed the following issues that need your attention:

- Fig 2A has a blank square in the top left corner. Please remove it.

- Fig 5C,D, and E appear overcontrasted. Please supply scans with less contrast modification, if available.

- Fig 4C and Appendix Fig S3B are identical. Please clearly indicate this in the figure legend. The same applies to Fig 4F and Appendix Fig S4B.

- Our data editors from Wiley already inspected the Figure legends for completeness and accuracy. Please see their suggested changes in the attached Word file. I have also taken the liberty to make a small change to the title. Could you please review it?

Once you have made these minor revisions, please use the following link to submit your corrected manuscript:

<https://embor.msubmit.net/cgi-bin/main.plex?el=A5Ij5GF3A1CqJZ6J1A9ftdsu50j9SHFRh8lAFkutt22QY>

If all remaining corrections have been attended to, you will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports.

2nd Revision - authors' response

10 July 2019

The authors performed all minor editorial changes.

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr. Alexej Kedrov, Prof. Dr. Roland Beckmann, Prof. Dr. Holger Gohlke

Journal Submitted to: EMBO Reports

Manuscript Number: EMBOR-2019-48191

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
 - common tests, such as t-test (please specify whether paired vs. unpaired), simple 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;
 - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	NA
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

<http://www.antibodypedia.com>
<http://1degreebio.org>
<http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo>

<http://grants.nih.gov/grants/olaw/olaw.htm>
<http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm>
<http://ClinicalTrials.gov>
<http://www.consort-statement.org>

<http://www.consort-statement.org/checklists/view/32-consort/66-title>

<http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun>

<http://datadryad.org>

<http://figshare.com>

<http://www.ncbi.nlm.nih.gov/gap>

<http://www.ebi.ac.uk/ega>

<http://biomodels.net/>

<http://biomodels.net/miriam/>

<http://jij.biochem.sun.ac.za>

http://oba.od.nih.gov/biosecurity/biosecurity_documents.html

<http://www.selectagents.gov/>

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	A reference for antibodies from Santa Cruz Biotechnology Inc. is included
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The Data Availability section (PDB, EMDB) is included in the manuscript
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	The source data from cryo-EM experiments is available upon request
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No
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