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A structural inventory of native ribosomal ABCE1-43S pre-initiation complexes

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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 6th May 2020

Thank you for submitting your manuscript reporting native ribosomal ABCE1-43S pre-initiation complex structures for consideration by The EMBO Journal. We have now received three referee reports on your study, which are included below for your information.

As you will see, the reviewers are overall positive and acknowledge the study's contribution to the field and its quality. Nonetheless they also raise some concerns that would need to be addressed in a revised manuscript. In particular referee #1's points 1, 5 and 6, and referee #3's last major concern should be addressed, by adding to the discussion and by providing further experimental data if needed. In addition, please also carefully respond to the other issues raised by the referees and revise the text and figures accordingly. When these concerns are resolved, we will be happy to consider this study further for publication, and I would therefore now like to invite you to prepare and submit a revised manuscript.

Please note that it is our policy to allow only a single round of major revision. We recognize that lab work worldwide is currently affected by the COVID-19/SARS-CoV-2 pandemic and can extend the revision time when needed. In addition, we have extended our 'scooping protection policy' to cover the period required for a full revision. However, it is nonetheless important to clarify any questions and concerns at this stage.

Please feel free to contact me should you have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication. I look forward to receiving your revised manuscript.

REFEREE REPORTS

Referee #1:

First off, I must apologize for the late review of this manuscript, as I have been flooded with such requests. This in no means reflects a negative opinion of this work; on the contrary, I found this a

careful analysis of both native yeast and human initiation complexes by the Beckmann group. This is a deep and dense manuscript that is rich in novel structural information regarding complexes directly isolated from cells after gradient analysis. Of course, linking this to the biochemistry and mechanism is challenging, but this manuscript is worthy of publication as it provides novel structural perspectives on the complex eukaryotic initiation process. There are several issues that should be rectified in a revised manuscript, outlined below.

1. I had some issues with their subunit splitting assay and analysis. They included Dom/Hbs in their "splitting factors" rather than eRF1/eRF3 for reasons that were unclear in the text. My guess is that they just see poor splitting rates with eRF1/3 - they are 10 fold slower than Dom/Hbs - but they are looking at canonical processes throughout the rest of the paper here (and eRF1 would be present for recycling/re-initiation, not Dom), so it reads pretty strangely to me. Plus, the effect of adding 3j is *really* small, and their signal/noise in that assay is not great. To rectify this,the authors could pull out these results (they can still cite the other paper), and scale back this specific claim in the discussion: "We could corroborate the finding that eIF3j assists in ABCE1-dependent splitting by in vitro dissociation assays and furthermore we established that eIF3j remains bound to the 40S together with ABCE1 after the splitting cycle."

2. The second intro paragraph does not make the point that ABCE1's FeSD seems to push on eRF1/pelota to elicit subunit splitting. This may be conceptually helpful for some readers, especially considering the FeSD is heavily discussed.
3. References are missing for eIF5B statement at the very end of third intro paragraph?
4. The fourth intro paragraph feels like a list of unknown structural details, maybe it would be better to edit this down and call out new findings throughout the text instead?
5. In processes such as recycling and reinitiation, splitting would presumably occur in the presence of eRF1, yet the authors performed splitting assays with Dom34 instead. Although Dom34 elicits faster subunit splitting in vitro, are these results meaningful in the context of recycling?
6. The effects of 3j on splitting with Dom/Hbs in 1C/S1D are quite subtle. Comparing +/- 3j, the addition of 3j not only increases the amount of subunits, but also increases the amount of 80S. The magnitude of the increase in 80S is substantial upon addition of 3j, and well outside what would be suggested by error bars for 80S. +3j/-SF and ++3j/-SF would also be good controls to include. The authors state "However, eIF3j alone did not exhibit any activity" so should include these results

Referee #2:

In this follow-up to their structural study of an in vitro reconstituted, archaeal post-splitting complex with ABCE1 bound (Heuer et al. 2020), Beckmann and co-workers set out to investigate the extent to which ABCE1 remains associated with initiation factor-bound 40S (43S-pre-initiation or 48S-initiation complexes). In order to do so, they obtained several cryo-EM structures of native small subunits from yeast and human cells, which show that ABCE1 stays bound to the SSU throughout 43S-PIC assembly and, to a lesser extent, in the 48S-IC. Moreover, the conformation of the ABCE1 NBDs differs from that observed in the complex reconstituted in vitro, seemingly as a result of an unknown molecule interacting with its nucleotide binding site, causing NBSI to remain in a semi-open conformation. Overall, this work sheds light on the various steps leading up to translation initiation in eukaryotes by providing a detailed series of cryo-EM snapshots, including a higher resolution view of human eIF3 interacting with the 43S PIC and of initiator tRNA interacting with eIF2 on the SSU.

The structural data are of good quality, are well described and the assignment of the various densities observed is well supported by earlier studies and by the LC/MS data. As a result, I only have a few minor comments:

- In the discussion, the authors discuss a putative order of events during 43S PIC and 48S IC assembly. A schematic figure summarizing the discussion would be very helpful for the uninitiated reader.

- On p.7, it would be worth mentioning that complexes were affinity-purified directly from the lysate and not from the gradients. The way it is phrased at the moment is ambiguous.

- The order of the B and C panels in Fig. 5 should be inverted to match the order in which they are discussed in the text.

- Density for eIF3c could be shown in Fig. 6E. At the moment it is difficult to make out in panel A.

Referee #3:

In the manuscript entitled "Structural inventory of native ribosomal ABCE1-43S pre-initiation complexes" Beckmann and colleagues investigate the coupling of recycling and initiation steps of eukaryotic translation by cryo-EM. In particular they focus on the role played by the ATPase ABCE1 (Rli1 in yeast). While ABCE1 primarily functions as a recycling factor, the authors of this manuscript find it to remain associated in 43S and 48S stages of re-initiation. In addition, by sorting 43S-PIC particles and employing focused re-classification a near complete picture of the 43S stage of eukaryotic translation initiation could be discerned. As such this manuscript, represents a substantial advance in our understanding of this stage of translation initiation and expands the structural role played by ABCE1 in this process. While this justifies the publication of this manuscript, I feel some re-structuring of the manuscript is in order to extend its accessibility to a broader readership.

Major concerns:
- Most of the conclusions reached by the authors is based on the structural analysis of the human samples. However, they intermittantly refer to both the human and yeast samples within the manuscript. I presume the intention of the authors was to highlight that the observations they describe are validated by their simultaneous observation both in the human and yeast system. This is however confusing when reading the manuscript. Therefore, I would suggest to describe all structural findings based on the human samples and then summarise in one chapter the observation made in yeast, which justify the drawn conclusions.

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- regarding the hybrid state of ABCE1, the authors claim that the ominous extra density in Fig.3F is responsible for this. While this is plausible from their description, I feel the cannot exclude a contribution of eIF3j and/or eIF1. Since this is one of the central points of this manuscript, I feel there should be some experimental evidence to backup the authors´ claims. Considering that models are available Xl-MS should be able to reveal the identity of this factor?!

Minor concerns:
- Please add densities into the main manuscript figures, where interpretation of side chain interactions occur. This includes 5C, 5D, 6F, 7C and 7D.

Reviewers reports and response

Referee #1:

First off, I must apologize for the late review of this manuscript, as I have been flooded with such requests. This in no means reflects a negative opinion of this work; on the contrary, I found this a careful analysis of both native yeast and human initiation complexes by the Beckmann group. This is a deep and dense manuscript that is rich in novel structural information regarding complexes directly isolated from cells after gradient analysis. Of course, linking this to the biochemistry and mechanism is challenging, but this manuscript is worthy of publication as it provides novel structural perspectives on the complex eukaryotic initiation process. There are several issues that should be rectified in a revised manuscript, outlined below.

1. I had some issues with their subunit splitting assay and analysis. They included **Dom/Hbs in their "splitting factors" rather than eRF1/eRF3** for reasons that were unclear in the text. My guess is that they just see poor splitting rates with eRF1/3 - they are 10 fold slower than Dom/Hbs - but they are looking at canonical processes throughout the rest of the paper here (and eRF1 would be present for recycling/re-initiation, not Dom), so it reads pretty strangely to me. Plus, the effect of adding 3j is *really* small, and their signal/noise in that assay is not great. To rectify this, **the authors could pull out these results** (they can still cite the other paper), and **scale back this specific claim** in the discussion: "We could corroborate the finding that eIF3j assists in ABCE1-dependent splitting by in vitro dissociation assays and furthermore we established that eIF3j remains bound to the 40S together with ABCE1 after the splitting cycle.

We apologize for being unclear on why we used the given set of factors. We agree with the referee that the canonical termination factors eRF1 and eRF3 would in principle be more appropriate than the Dom34/Hbs1 system. We have nevertheless chosen the Dom34/Hbs1 system for several reasons. First, in order to use eRF1 and eRF3 for termination-coupled recycling, stop-codon containing pre-termination complexes need to be prepared in high enough yields from a yeast *in vitro* translation system. This is in principle possible using for example the stalling sequence encoded by the gp48 uORF2 of cytomegalovirus (coding for the “CMV-stalling” sequence) or by adding a release-deficient eRF1 GGQ-mutant. However, both approaches would have created rather complicated cases of pre-termination stalled substrates for recycling. Therefore, for practical reasons we chose the use the mechanistically equivalent Dom34/Hbs1-ABCE1 system, since this system works with vacant 80S ribosomes and is - as the referee pointed out - also more efficient. Moreover, as for canonical splitting using termination factors, splitting of vacant ribosomes with Dom34/Hbs1 and ABCE1 results in the same end product, namely 40S subunits to which ABCE1 may remain bound under certain conditions, e.g. using non-hydrolyzable nucleotide analoga. Because of this mechanistic equivalence and the fact that also these subunits eventually enter a new phase of initiation, we consider this system suitable to investigate the effect of Hcr1/eIF3j on ABCE1-mediated splitting.

We also agree that the eIF3j-effect is indeed weak but still significant (see point 6). It may indeed be possible, that this effect would be stronger using canonical termination factors, but for reasons discussed above we haven’t performed these assays with “real” termination complexes.

As requested, we actually confirmed the interaction between eIF3j and ABCE1 in native 40S initiation complexes by chemical crosslinking coupled to mass spectrometry (XL-MS) as well as with additional cryo-EM data of such a cross-linked 43S complex (see in detail in the response to reviewer 3). Since these data strengthen our *in vitro a*ssays, we kindly ask to not follow the reviewer's suggestion to scale back our claims and pull out these results. We rather added more controls and a clearer explanation of our system. For further justification please see also the response to point 6.

2. The second intro paragraph does not make the point that ABCE1's FeSD seems to push on eRF1/pelota to elicit subunit splitting. This may be conceptually helpful for some readers, especially considering the FeSD is heavily discussed.

As suggested, we inserted a sentence in the second intro paragraph (line 57): "According to current models, the conformational change occurring during site-occlusion would be transmitted via the FeSD of ABCE1 to the bound A site factor (eRF1 or Dom34), whereby the FeSD exerts a force on the A site factor which ultimately leads to ribosome splitting (Becker et al., 2012; Heuer et al., 2016; Nürenberg-Goloub *et al.*, 2020)."

3. References are missing for eIF5B statement at the very end of third intro paragraph?

We agree and added the appropriate references (Pestova *et al.*, 2000; Lee *et al.*, 2002; Acker *et al.*, 2006; Acker *et al.*,2009) in the third intro paragraph (line 84).

4. The fourth intro paragraph feels like a list of unknown structural details, maybe it would be better to edit this down and call out new findings throughout the text instead?

Here, we are not sure, if we understand what the reviewers refers to. In the fourth paragraph we are giving a general overview over the different subunits and modules of eIF3 that we refer to later, but we don't give any structural details here and all this information is commonly known for a long time in the translation field. We thus would ask to not change the text there.

5. In processes such as recycling and reinitiation, splitting would presumably occur in the presence of eRF1, yet the authors performed splitting assays with Dom34 instead. Although Dom34 elicits faster subunit splitting in vitro, are these results meaningful in the context of recycling?

As explained in point 1.) we used the Dom34/Hbs1 system primarily for technical reasons, because we can use vacant 80S ribosomes for splitting. We still think that this is a reasonable approximation to study the effects of eIF3j/Hcr1 in splitting because the mechanism of ABCE1 action in both processes (recycling after canonical termination and after ribosome rescue) is most likely highly similar. While we cannot rule out that eIF3j could have a more prominent effect on splitting after termination, our and other data show, that eIF3j rather affects/assists ABCE1 itself. Thus, we think, when observing a Hcr1 effect on Dom34/Hbs1-mediated splitting, this is also conferrable to canonical termination.

Notably, the origin of the ABCE1-bound 43S/48S complexes found in our native pullouts is not clear and likely represents a mix. While assuming that these complexes originate mostly from recycling after canonical termination, an unknown portion will be the result of Dom34-dependent ribosome quality control pathways or of vacant ribosome splitting. For yeast, it was also reported that as a final step of

ribosome biogenesis a translation-like cycle occurs, that (Lebaron *et al.*, 2012; Strunk *et al.*, 2012) involves splitting of premature 80S ribosomes by Dom34-Hbs1-ABCE1.

6. The effects of 3j on splitting with Dom/Hbs in 1C/S1D are **quite subtle**. Comparing +/- 3j, the addition of 3j not only increases the amount of subunits, but also increases the amount of 80S. The magnitude of the increase in 80S is substantial upon addition of 3j, and well outside what would be suggested by error bars for 80S. **+3j/-SF and ++3j/-SF would also be good controls to include**. The authors state "However, eIF3j alone did not exhibit any activity" so should include these results

We can only partly agree with the referee at this point. It is correct that the amount of 80S slightly increases in the “+SF +eIF3j” condition, but when then comparing the error bars with the “+SF” condition (the control experiment without eIF3j), the increase in 80S is not “well outside what would be suggested by these error bars”, but still within 1.5 standard deviations. Please note, that this control experiment has an overall higher deviation, as also observable in the original triplicates of the gradients in Fig EV1D. While we agree that the addition of only 4-fold molar excess (indicated as “+” in the figures) is not very substantial, the effect upon addition of 20-fold molar excess (indicated as “++” in the figures) shows a significant effect on the splitting rates.
As suggested by the reviewer, we added the suggested control experiments ++3j -SF as well as a control with only Dom34-Hbs1 and only ABCE1 to Fig EV1E.

Referee #2:

In this follow-up to their structural study of an in vitro reconstituted, archaeal post-splitting complex with ABCE1 bound (Heuer et al. 2020), Beckmann and co-workers set out to investigate the extent to which ABCE1 remains associated with initiation factor-bound 40S (43S-pre-initiation or 48S-initiation complexes). In order to do so, they obtained several cryo-EM structures of native small subunits from yeast and human cells, which show that ABCE1 stays bound to the SSU throughout 43S-PIC assembly and, to a lesser extent, in the 48S-IC. Moreover, the conformation of the ABCE1 NBDs differs from that observed in the complex reconstituted in vitro, seemingly as a result of an unknown molecule interacting with its nucleotide binding site, causing NBSI to remain in a semi-open conformation. Overall, this work sheds light on the various steps leading up to translation initiation in eukaryotes by providing a detailed series of cryo-EM snapshots, including a higher resolution view of human eIF3 interacting with the 43S PIC and of initiator tRNA interacting with eIF2 on the SSU.

The structural data are of good quality, are well described and the assignment of the various densities observed is well supported by earlier studies and by the LC/MS data. As a result, I only have a few minor comments:

We are happy about the reviewer's overall very positive comments.

- In the discussion, the authors discuss a putative order of events during 43S PIC and 48S IC assembly. A schematic **figure summarizing the discussion** would be very helpful for the uninitiated reader.

As suggested by the reviewer we added a cartoon summarizing our findings in Fig 8.

- On p.7, it would be worth mentioning that complexes were affinity-purified directly from the lysate and not from the gradients. The way it is phrased at the moment is ambiguous.

The samples used for quantitative mass spectrometry (LC-MS/MS) were indeed affinity purified from the lysates and not from the gradient peaks. We corrected this in the revised manuscript.

- The order of the B and C panels in Fig. 5 should be inverted to match the order in which they are discussed in the text.

We agree that this should be consistent. While we kept the order of the two panels, we adjusted the text accordingly.

- Density for eIF3c could be shown in Fig. 6E. At the moment it is difficult to make out in panel A.

We added an additional panel into Fig EV6D to show the density for the N-terminus of eIF3c.

Referee #3:

In the manuscript entitled "Structural inventory of native ribosomal ABCE1-43S pre-initiation complexes" Beckmann and colleagues investigate the coupling of recycling and initiation steps of eukaryotic translation by cryo-EM. In particular they focus on the role played by the ATPase ABCE1 (Rli1 in yeast). While ABCE1 primarily functions as a recycling factor, the authors of this manuscript find it to remain associated in 43S and 48S stages of re-initiation. In addition, by sorting 43S-PIC particles and employing focused re-classification a near complete picture of the 43S stage of eukaryotic translation initiation could be discerned. As such this manuscript, represents a substantial advance in our understanding of this stage of translation initiation and expands the structural role played by ABCE1 in this process. While this justifies the publication of this manuscript, I feel some re-structuring of the manuscript is in order to extend its accessibility to a broader readership.

Major concerns:
- Most of the conclusions reached by the authors is based on the structural analysis of the human samples. However, they intermittantly refer to both the human and yeast samples within the manuscript. I presume the intention of the authors was to highlight that the observations they describe are validated by their simultaneous observation both in the human and yeast system. This is however confusing when reading the manuscript. Therefore, I would suggest to describe all **structural findings based on the human samples and then summarise in one chapter the observation made in yeast, which justify the drawn conclusions**.

While we agree with the referee that it may be clearer to describe all findings with the human sample, we need to point out that our findings in yeast and human complexes are not always redundant, in a few cases rather complementary. For example, the conformation of eIF3j in ABCE1 containing classes differs between human and yeast, and in the yeast structures eIF1A is present while in the human structure it is absent. Moreover, that ABCE1-containing 48S complex was only found in yeast. For this reason, we decided to arrange the manuscript in a way, that yeast and human complexes from similar stages of initiation can be compared. We thus feel that restructuring the manuscript as suggested by the reviewer would not lead to an improved intelligibility. Nevertheless, we tried to simplify the manuscript wherever possible in order to make it less confusing.

- The authors describe that they were able to redefine existing models for the PCI-MPN core in particular and trace flexible parts of eIF3 in general. For the PCI-MPN core the claim to be able to correct register errors of former models. This is excellent and will be invaluable in future. However, in the main manuscript the authors only present C-alpha traces of the final model or side chains in selected areas. Densities are only presented in the Supplemental section. To substantiate the claims (in particular the correction of registry shifts) the **authors should provide figures along with densities in the main manuscript, where the density allows the reader to appreciate why registry shifts were corrected.**

As suggested, we added an Expanded View Figure showing model-to-map fits (including side chains) from PCI-MPN core regions (Fig EV5), as well as a new Appendix Figure S6, where the register shifts are shown. For the sake of clarity, however, we decided to not show this in the main figure. Notably in EMBO J, Expanded View figures are displayed together with the main figure online. This should give the reader an excellent possibility to retrace the claims made in the main figure.

- regarding the hybrid state of ABCE1, the authors claim that the ominous extra density in Fig.3F is

responsible for this. While this is plausible from their description, I feel the cannot exclude a contribution of eIF3j and/or eIF1. Since this is one of the central points of this manuscript, I feel there should be **some experimental evidence to backup the authors´ claims. Considering that models are available XI-MS should be able to reveal the identity of this factor**?!

We thank the referee for his request and we agree that the identification of the extra density at the ABCE1 nucleotide binding site would significantly strengthen the central claim of the manuscript. To that end, we prepared a similar native 40S initiation complex sample as used in the original manuscript: We noted that ABCE1 (Rli1) and eIF3j (Hcr1)-containing (pre)-initiation complexes are enriched in native pullouts using a TAP-tagged Nip1 (eIF3c) as a bait. We prepared native small 40S subunits from this sample and performed XL-MS in collaboration with the Herzog lab in the Gene Center Munich. In addition, we subjected this crosslinked sample to cryo-EM and single particle analysis.
Amongst a large number of crosslinks (43 inter-protein and 74 intra-protein crosslinks) largely confirming our previously presented structural data, XL-MS yielded two robust inter-protein crosslinks between a lysine in the eIF3j N-terminal domain and two lysines in the nucleotide-binding cleft of ABCE1-NBD1, unambiguously confirming our claim, that the N-terminus of eIF3j binds into the nucleotide binding cleft of ABCE1-NBD1. Further, with this new sample, we could improve the local resolution of the 40S-bound ABCE1-eIF3j cryo-EM map to around 3 Å. This allowed us to describe the structure of 40S-bound eIF3j for the first time in molecular detail. We now observe a direct density connection between the 6-helix bundle of the eIF3j dimer and the "extra density" in ABCE1. Unfortunately, the local resolution was still too low to build a molecular model for the eIF3j region (1-135) preceding the three C-terminal helices and thus for the peptide that accommodates within ABCE1. Yet, the position determined by the XL-MS experiment, 18 amino acids N-terminal of the eIF3j helix bundle (K118), would perfectly agree with extra density within ABCE1.
Taken together, we now can state with high certainty that the eIF3j N-terminal domain binds to the NBSI of ABCE1 and stabilizes this asymmetric ATPase in a novel hybrid conformation.

In addition, the improved resolution allowed us to also identify and *de novo* build the ultimate C-terminus of eIF3j which locates in the mRNA entry channel (also confirmed by XL-MS). This finding explains previous experiments showing that mRNA- and eIF3j binding are antagonistic, which plays a role during mRNA recycling from the 40S as well as influencing/coordinating mRNA loading and even start-site selection during initiation.

Minor concerns:

- **Please add densities** into the main manuscript figures, where interpretation of side chain interactions occur. This includes 5C, 5D, 6F, 7C and 7D.

As mentioned above, for better clarity, we added model-to-map fits as new Expanded View Figures.

- For Figs 5C and 5D the model is show with cryo-EM density in Figs EV5B and C.
- For Fig 6F the model is show with cryo-EM density in Fig EV6B.
- For Figs 7C and 7D the model is show with cryo-EM density in Appendix Figure S9C.

2nd - Editorial Decision 10th Sep 2020

Thank you for submitting your revised manuscript, we have now received the reports from the three initial referees (see comments below). I am pleased to say that they overall find that their comments have been satisfactorily addressed and now support publication. Referee #3 raises a minor issue regarding how model quality is reported, which can be resolved in the final revised version. In addition, I would like to ask you to also address a number of editorial issues that are listed in detail below. Please make any changes to the manuscript text in the attached document only using the "track changes" option. Once these remaining issues are resolved, we will be happy to formally accept the manuscript for publication.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving your final revision. Please feel free to contact me if you have further questions regarding the revision or any of the specific points listed below.

REFEREE REPORTS

Referee #1:

The revised manuscript has addressed my minor concerns and is now acceptable for publication

Referee #2:

The authors have adequately addressed my concerns and I am fully satisfied with the quality of the manuscript and of the very interesting findings it reports. No additional revisions are needed at this point in my opinion.

Referee #3:

In the revised version of this manuscript Kratzat, Beckmann and colleagues have addressed all my concerns and, as I can see from the point-to-point response, the comments of the other referees. With this I congratulate the authors for a well executed study and recommend acceptance.

There are some minor comments remain which I encourage the authors to address in the final versions of this manuscript: In Appendix tables 1 and 2 the legends for model quality (such as correlation coefficients, rotamer outliers, etc) state percentage values. The authors however cite fractional values, both descriptors should be consistent:either state the values in percentage or remove percent from the legend.

2nd Authors' Response to Reviewers 21st Sep 2020

The Authors have made the requested editorial changes.

Accepted 29th Sep 2020

Thank you again for submitting the final revised version of your manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Prof. Dr. Roland Beckmann
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2020-105179

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 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.

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.

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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?	The main method used in this publicatiomn is cryo-EM followed by single particle analysis. Sample size (number of particles) was chosen to enable molecular interpretation of the obtained maps.
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

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).	Antibodies used: anti-Nog1: a rabbit polyclonal antibody (Saveanu et al., 2003), Peroxidase Anti-Peroxidase (PAP) complex antibody produced in rabbit (P1291 Sigma).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293 T-Rex Flp-In cells were purchased from Thermo Scientific and have not been further authenticated. All cell lines used tested negative for mycoplasma contamination.

* 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	Cryo-EM maps and resulting molecular models have been deposited in the EMDB and PDB databases and accession codes are listed in the "data availability" section as requested.
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).	Cryo-EM maps and resulting molecular models have been deposited in the EMDB and PDB databases and accession codes are listed in the "data availability" section as requested.
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.	NA