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Cryo-EM analysis provides new mechanistic insight into ATP binding to Ca2+-ATPase SERCA2b

Yuxia Zhang, Satoshi Watanabe, Akihisa Tsutsumi, Hiroshi Kadokura, Masahide Kikkawa and Kenji Inaba
DOI: 10.15252/embj.2021108482

Corresponding author: Kenji Inaba (kenji.inaba.a1@tohoku.ac.jp)

Review Timeline:

Submission Date:	14th Apr 21
Editorial Decision:	21st May 21
Revision Received:	15th Jun 21
Editorial Decision:	24th Jul 21
Revision Received:	2nd Aug 21
Accepted:	4th Aug 21

Editors: Elisabetta Argenzio / Daniel Klimmeck

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 21st May 2021

Dear Dr. Inaba,

Thank you for submitting your manuscript entitled "Cryo-EM analysis provides new mechanistic insight into ATP binding to Ca2+-ATPase SERCA2b" [EMBOJ-2021-108482] to The EMBO Journal. Your study has now been assessed by two reviewers, whose reports are enclosed below.

As you can see, the referees concur with us on the potential interest of your findings. However, they also raise several critical points that need to be addressed before they can support publication here. In particular the referees ask you to better investigate the role of Ca2+ binding in the conformational transition of SERCA2b.

Given the overall interest of your study, I am pleased to invite submission of a manuscript revised as indicated in the reports attached herein. I would like to point it out that addressing all referees' points in a conclusive manner, as well as a strong support from the reviewers, would be essential for publication in The EMBO Journal.

I should also add that it is our policy to allow only a single round of major revision. Therefore, acceptance of your manuscript will depend on the completeness of your responses in this revised version.

We generally grant three months as standard revision time. As we are aware that many laboratories cannot function at full capacity owing to the COVID-19 pandemic, we may relax this deadline. Also, we have decided to apply our 'scooping protection policy' to the time span required for you to fully revise your manuscript and address the experimental issues highlighted herein. Nevertheless, please inform us as soon as a paper with related content is published elsewhere.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File and will therefore be made available online. For more details on our Transparent Editorial Process, please visit our website: http://emboj.embopress.org/about#Transparent_Process.

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I thank you again for the opportunity to consider this work for publication and look forward to your revision.

Yours sincerely,

Elisabetta Argenzio, PhD

Editor
The EMBO Journal

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Referee #1:

Zhang, Watanabe, Tsutsumi, Kadokura, Kikkawa and Inaba present a study of the Ca2+ bound state of SERCA2b studied by cryo-EM. By classification they find structures of an open E1-2Ca2+ state as also previously reported from crystal structures of SERCA1a 1SU4 (Toyoshima et al. 2000) and 2C9M (Jensen et al. 2006), but they find it to be inherently flexible and the map converges at 12 Å resolution. They place stronger emphasis on more compact states that are more akin to the SERCA E1-2Ca2+-AMPPCP and E1-2Ca2+-ADP-AlF4- forms that represent phosphorylating states (Mizutani 2004, Sørensen 2004), but in this study obtained in the absence of ATP analogs. In further support of the study, they also present Cys-bridge engineered constructs to show that the compact state indeed forms in an active sample (concluded by the indication of S-S- bridge formation between residue positions that are close in the compact state, and which can be unlocked for full activity by reduction). Hence, they conclude that they have revealed a compact functional state associated with Ca2+ binding prior to ATP binding, which is different from the open E1-2Ca2+ state
The technical quality of the work is good and cites also many earlier contributions from SERCA1a crystal structures (although it would seem fair to add also contributions of the Toyoshima lab a bit more frequently)

The question of the structural transitions in the SERCA cycle have long been debated and are of key interest to understanding kinetics and regulation of the transport activity and how it is affected by e.g. post-translational modifications and mutations. The study presents very valuable information for that discussion. However, the authors should discuss their findings in a broader context.

First of all, it is important to note that ATP binds to SERCA throughout the functional cycle, with micromolar affinity and lower - SERCA will effectively be saturated by ATP at millimolar concentrations in the cell. The transition between compact states is expected to happen between E2-ATP states and then ATP-bound E1 states - an ATP-unbound state binding Ca2+ first would seem unlikely - or could particular conditions favor that, e.g. by ADP levels increasing? It would seem interesting to study this a bit further, both by the S-S formation probing the compact state and cryo-EM samples. I leave it to the authors to consider if they will ad experimental work here, but I woudl recommend it.
This also brings the discussion into important considerations of open states and if they may relate to the paused, incative states observed for the related H+-ATPase AHA2 (Veshaguri et al. & Stamou, 2016 Science), ie. bridging single-molecule observations to the structural states and bulk kinetics that are also being approached here. It would greatly improve the paper to consider these questions, and this discussion seems critical for a proper context of their findings on distributions betweeen compact and open E1 states

Referee #2:

This is an interesting and worthwhile contribution to our understanding of the calcium pump SERCA. Overall, most of the conclusions are valuable while also carefully stated to avoid over-reach. I have a few questions for the authors that I would like them to consider.

The conclusion that the cytosolic headpiece cluster of SERCA2b becomes even more compact upon ATP binding seems very reasonable. However, I would like to know more about the role of Ca2+ binding on the conformational transition. In my view, the compact state requires both bound ATP and the two ions. The, autophosphrylation is favored. One may think that the binding of any

ligands to a flexible protein may possibly induce a transition toward a given conformation that is otherwise not accessible. A slightly different view is that the conformation may have always been accessible all along, but the binding of the ligands serves to reduce the fluctuations around this conformational state, thereby increasing the rate of autophosphrylation. The main conclusion of the authors is that "the closed form with less mobile cytosolic domains can allow an ATP molecule to appropriately enter the ATP-binding cavity and drive ATP hydrolysis (Fig. 6b, right)." Nonetheless the authors are prudent to acknowledge that "we cannot exclude the possibility that SERCA2b in the E1·2Ca2+ state is in equilibrium between the open and closed forms and that the open form binds ATP and subsequently adopts the E1·2Ca2+-ATP state, the present study demonstrated that the closed form can do so even without returning to the open form via large cytosolic domain movements." My impression is that this conclusion is valid, but based on the implicit assumption that the Ca2+ ions are in their binding site all along? Without the bound Ca2+, is there existence of this closed ATP-free conformation. See for example the discussion in (Das et al, JMB 2017).

MINOR POINTS:

1. The idea that the cytoplasmic domains may undergo large conformational variations has been known for a while. In particular, the large difference between the Xray structures 1SU4 and 3W5A should be acknowledged and discussed.

2. Several computational studies seem to be of high relevance and should be incorporated in the discussion.

L.M. Espinoza-Fonseca, D.D. Thomas, Atomic-level characterization of the activation mechanism of SERCA by calcium, PLoS One 6 (2011), e26936. PMID: 22046418

Das A, Rui H, Nakamoto R, Roux B. Conformational Transitions and Alternating-Access Mechanism in the Sarcoplasmic Reticulum Calcium Pump. J Mol Biol. 2017 Mar 10;429(5):647-666. doi: 10.1016/j.jmb.2017.01.007. Epub 2017 Jan 16. PMID: 28093226 Free PMC article.

Thirman J, Rui H, Roux B. Elusive Intermediate State Key in the Conversion of ATP Hydrolysis into Useful Work Driving the Ca2+ Pump SERCA. J Phys Chem B. 2021 Mar 25;125(11):2921-2928. doi: 10.1021/acs.jpcb.1c00558. Epub 2021 Mar 15. PMID: 33720716

Referee #1:

Zhang, Watanabe, Tsutsumi, Kadokura, Kikkawa and Inaba present a study of the Ca^{2+} bound state of SERCA2b studied by cryo-EM. By classification they find structures of an open E1-2Ca^{2+} state as also previously reported from crystal structures of SERCA1a 1SU4 (Toyoshima et al. 2000) and 2C9M (Jensen et al. 2006), but they find it to be inherently flexible and the map converges at 12 Å resolution. They place stronger emphasis on more compact states that are more akin to the SERCA E1-2Ca^{2+}-AMPPCP and E1-2Ca^{2+}-ADP-AlF_4^- forms that represent phosphorylating states (Mizutani 2004, Sørensen 2004), but in this study obtained in the absence of ATP analogs. In further support of the study, they also present Cys-bridge engineered constructs to show that the compact state indeed forms in an active sample (concluded by the indication of S-S-bridge formation between residue positions that are close in the compact state, and which can be unlocked for full activity by reduction). Hence, they conclude that they have revealed a compact functional state associated with Ca^{2+} binding prior to ATP binding, which is different from the open E1-2Ca^{2+} state.

The technical quality of the work is good and cites also many earlier contributions from SERCA1a crystal structures (although it would seem fair to add also contributions of the Toyoshima lab a bit more frequently).

Response: We are grateful to Reviewer 1 for this positive overall evaluation and all the constructive and useful comments. We sincerely considered and addressed each of them as described below. As pointed out by this reviewer, we carefully reexamined previous works on structures and mechanisms of SERCA achieved by other groups including Toyoshima lab in order to fairly evaluate their contributions and thoroughly cite references in the text.

The question of the structural transitions in the SERCA cycle have long been debated and are of key interest to understanding kinetics and regulation of the transport activity and how it is affected by e.g. post-translational modifications and mutations. The study presents very valuable information for that discussion. However, the authors should discuss their findings in a broader context.

Response: It's our great pleasure that the present study received such a high evaluation from this reviewer. We totally agree that the paper could be more valuable and receive more attention by discussing our findings in a broader context. With this purpose, we especially rewrote the Discussion part by further linking the present findings to structural and mechanistic insights into SERCA and other P-ATPases so far gained and considering the physiological function and situation of SERCA2b in cells. For more details, please see below.

Comment 1: First of all, it is important to note that ATP binds to SERCA throughout the functional cycle, with micromolar affinity and lower - SERCA will effectively be

saturated by ATP at millimolar concentrations in the cell. The transition between compact states is expected to happen between E2-ATP states and then ATP-bound E1 states - an ATP-unbound state binding Ca^{2+} first would seem unlikely - or could particular conditions favor that, e.g. by ADP levels increasing? It would seem interesting to study this a bit further, both by the S-S formation probing the compact state and cryo-EM samples. I leave it to the authors to consider if they will add experimental work here, but I would recommend it.

Response: We thank the reviewer for this essential comment. We basically agree that the E2-ATP state can predominantly be formed before the ATP-bound E1 state under normal physiological conditions containing millimolar levels of ATP. Meanwhile, we believe that it is important to state the physiological meaning of the ATP-unbound E1 state, of which cryo-EM structure is first reported in this paper. Thus, we note that SERCA2b and IP3 receptor, a primary actor in Ca^{2+} release from the ER lumen, are enriched in the mitochondria-associated ER-membrane (MAM), where concentrations of Ca^{2+} and ATP/ADP are likely to fluctuate largely due to the chemical crosstalk between the ER and the mitochondria. Although we understand that this idea needs to be further examined experimentally, we added one Discussion paragraph with citation of related papers to expand our discussion from a physiological viewpoint (lines 334-349 on page 12).

To gain insight into the compactness of the cytosolic domain arrangement in the E2 states, we performed the additional experiment probing the S-S formation under the Ca^{2+}-free condition, as shown in new supplemental Fig. EV6b. Consequently, we found that without Ca^{2+}, the S-S bridge was formed only slightly between the A and N domains in either the presence or absence of AMPPCP, suggesting that Ca^{2+} binding has greater effect on tight interaction between the A and N domains in SERCA than ATP binding. We inserted a paragraph describing this important data in the Result section (lines 240-262 on pages 9 and 10).

We agree that cryo-EM analyses of SERCA2b in the E2 and E2-ATP states will provide further detailed information on structures in Ca^{2+}-free forms. We therefore plan to carry out the cryo-EM measurements using the E2-state samples soon, and wish to report the structures by making a new interesting story in another paper.

Comment 2: This also brings the discussion into important considerations of open states and if they may relate to the paused, inactive states observed for the related H^+-ATPase AHA2 (Veshaguri et al. & Stamou, 2016 Science), i.e. bridging single-molecule observations to the structural states and bulk kinetics that are also being approached here. It would greatly improve the paper to consider these questions, and this discussion seems critical for a proper context of their findings on distributions between compact and open E1 states.

Response: We again thank the reviewer for this noticeable information. Indeed, the ‘open’ conformations of Ca^{2+}-bound SERCA shown by the present cryo-EM analysis as well as the

previous crystallographic studies are reminiscent of the paused, inactive state observed for another P-type ATPase AHA2. To further discuss the functional significance of the open E1 state and make the present findings more conceptual and hence fascinating to broad readers, we inserted the following sentences into the Discussion section with citation of the suggested paper (lines 309-316 on page 11).

"The coexistence of the open and closed cytosolic domain arrangements in SERCA is reminiscent of different functional states of P-type ATPase Arabidopsis thaliana isoform 2 (AHA2) revealed by the single-molecule observation using total internal reflection fluorescence (TIRF) microscopy (Veshaguri et al., 2016). Proton pumping by AHA2 is stochastically interrupted by long-lived inactive or leaky states, and the active and inactive states together define the bulk activity. In this analogy, the open conformation in the E1·2Ca^{2+} state of SERCA may serve as a regulatory state that can pause the proper ATP binding and the subsequent hydrolysis in the catalytic cycle."

Referee #2:

This is an interesting and worthwhile contribution to our understanding of the calcium pump SERCA. Overall, most of the conclusions are valuable while also carefully stated to avoid over-reach. I have a few questions for the authors that I would like them to consider.

Response: We are grateful to Reviewer 2 for this positive overall evaluation and all the constructive comments. We sincerely considered and addressed each of them as described below.

Comment 1: The conclusion that the cytosolic headpiece cluster of SERCA2b becomes even more compact upon ATP binding seems very reasonable. However, I would like to know more about the role of Ca^{2+} binding on the conformational transition. In my view, the compact state requires both bound ATP and the two ions. Then, autophosphorylation is favored. One may think that the binding of any ligands to a flexible protein may possibly induce a transition toward a given conformation that is otherwise not accessible. A slightly different view is that the conformation may have always been accessible all along, but the binding of the ligands serves to reduce the fluctuations around this conformational state, thereby increasing the rate of autophosphorylation. The main conclusion of the authors is that "the closed form with less mobile cytosolic domains can allow an ATP molecule to appropriately enter the ATP-binding cavity and drive ATP hydrolysis (Fig. 6b, right)." Nonetheless the authors are prudent to acknowledge that "we cannot exclude the possibility that SERCA2b in the E1·2Ca^{2+} state is in equilibrium between the open and closed forms and that the open form binds ATP and subsequently adopts the E1·2Ca^{2+}-ATP state, the present study demonstrated that the closed form can do so even without returning to the open

form via large cytosolic domain movements. " My impression is that this conclusion is valid, but based on the implicit assumption that the Ca^{2+} ions are in their binding site all along? Without the bound Ca^{2+}, is there existence of this closed ATP-free conformation. See for example the discussion in (Das et al, JMB 2017).

Response: We appreciate this essential comment. To address this reviewer's concern, we additionally performed the biochemical assay probing the disulfide between the A- and N-domains under the Ca^{2+}-free condition as well. We thus found that these two domains were bridged with much lower efficiency in the absence of Ca^{2+} than in the presence of Ca^{2+}. Thus, it is conceivable that the tight and stable interaction between the cytosolic domains is highly dependent on Ca^{2+}, in line with previous biophysical and computational works (Espinoza-Fonseca and Thomas, ***PLoS One*** 2011; Winters et al., Biochemistry 2008). Intriguingly, the interdomain disulfide bridge was only marginally enhanced by addition of ATP, suggesting that ATP binding has smaller effect on tight interaction between the A and N domains in SERCA than Ca^{2+} binding. The additional data is shown in new Fig. EV6b, and described in the main text of the revised manuscript (lines 240-262 on pages 9 and 10). Also, we additionally discuss the existence of another likely pathway that promotes ATP binding to Ca^{2+}-unbound E2 state in the Discussion section (lines 334-349 on page 12). Regarding these issues, please see also our response to the first comment of Reviewer 1.

MINOR POINTS:

1. The idea that the cytoplasmic domains may undergo large conformational variations has been known for a while. In particular, the large difference between the Xray structures 1SU4 and 3W5A should be acknowledged and discussed.

Response: As suggested, the large variations in structures of SERCA1a in the E1·2Ca^{2+} (1SU4) and E1·Mg^{2+} (3W5A) states have been additionally discussed in the revised manuscript with citation of the relevant papers (lines 292-298 on pages 10 and 11).

2. Several computational studies seem to be of high relevance and should be incorporated in the discussion.

L.M. Espinoza-Fonseca, D.D. Thomas, Atomic-level characterization of the activation mechanism of SERCA by calcium, PLoS One 6 (2011), e26936. PMID: 22046418

Das A, Rui H, Nakamoto R, Roux B. Conformational Transitions and Alternating-Access Mechanism in the Sarcoplasmic Reticulum Calcium Pump. J Mol Biol. 2017 Mar 10;429(5):647-666. doi: 10.1016/j.jmb.2017.01.007. Epub 2017 Jan 16. PMID: 28093226 Free PMC article.

Thirman J, Rui H, Roux B. Elusive Intermediate State Key in the Conversion of ATP

Hydrolysis into Useful Work Driving the Ca2+ Pump SERCA. J Phys Chem B. 2021 Mar 25;125(11):2921-2928. doi: 10.1021/acs.jpcb.1c00558. Epub 2021 Mar 15. PMID: 33720716

Response: We greatly appreciate this useful information. We acknowledge that these computational studies provide important mechanistic insight into the ligand-induced conformational transitions in SERCA, hence are highly related to the present study. We therefore cited all of them at appropriate sites and expanded the discussion in the revised manuscript (e.g. lines 251-255 on page 9; 260-262 on page 10; lines 306-309 on page 11; lines 337-339 on page 12).

1st Revision - Editorial Decision 24th Jul 2021

Dear Dr. Inaba,

Thank you for submitting your revised study. Please accept my apologies for the delay in getting back with our decision. The manuscript has been sent back to the original referees and we have now obtained their reports, which are appended below for your information.

As you can see, the referees find that their criticisms have been adequately addressed and recommend the study for publication. However, referee #1 requests you to address a few technical points.

In addition, there are some editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

-> Provide 3-5 keywords for your study. These will be visible in the html version of the paper and on PubMed and will help increase the discoverability of your work.

-> Upload main and EV figures as single files (one file per figure).

-> Add callout for Fig 1A in the main manuscript text.

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-> In addition, I would encourage you to provide an image for the synopsis. This image should provide a rapid overview of the question addressed in the study but still needs to be kept fairly modest since the image size cannot exceed 550x400 pixels.

I thank you again for giving us the chance to consider your manuscript for publication in The EMBO Journal and look forward to your revision.

Kind regards,

Elisabetta

Elisabetta Argenzio, PhD
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Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
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IMPORTANT: When you send the revision we will require
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- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 22nd Oct 2021.

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Referee #1:

The authors present an important study that investigates a possible mechanism for ATP binding to

a compact, Ca2+ bound state of the SERCA2B cycle, superseding a seemingly inactive state with thte ATP binding N-domain detached from the core of the enzyme. In the revised version, they discuss possible relevance of this mechanism at for example mitochondrial contact sites, where local concentrations of Ca2+ and ADP may favor such a route rather than Ca2+ binding to a n . Furthermore, they discuss the relevance in relation to paused states observed from single-molecule activity studies of the related AHA2 H+-ATPase.
The authors have responded well to reviewer comments, and I have only a few technical comments.

line 446 - which slit width was used, 20 eV?
451 - which BIS scheme, 3x3?
452-454 - just state the total number of movies here
459 - how many classes? Which tau-fudge value was used?
459 - what is meant by "rapid data processing"? please describe the cryoSPARC data processing a bit further
465 - what exactly was masked and how? What were the extensions/softness in pixels of the mask? Was classification including local angle with mask?
479 - please describe real space refinement and model validation tools and criteria

The text in lines 335-340 and other places is a bit edgy and needs rephrasing, but it can be handled in copyediting

Referee #2:

I am satisfied with the revisions.

Referee #1:

The authors present an important study that investigates a possible mechanism for ATP binding to a compact, Ca2+ bound state of the SERCA2B cycle, superseding a seemingly inactive state with thte ATP binding N-domain detached from the core of the enzyme. In the revised version, they discuss possible relevance of this mechanism at for example mitochondrial contact sites, where local concentrations of Ca2+ and ADP may favor such a route rather than Ca2+ binding to a n . Furthermore, they discuss the relevance in relation to paused states observed from single-molecule activity studies of the related AHA2 H+-ATPase.
The authors have responded well to reviewer comments, and I have only a few technical comments.

Response: We are grateful to this referee for his/her careful review of our revised manuscript. It's our great pleasure to learn that Referee#1 acknowledged our proper responses to reviewer comments in the revised manuscript. Now, we have addressed the referee's technical comments as below, and added the requested information to the appropriate sites of the "Materials and Methods" section in the re-revised manuscript.

Comment 1: line 446 - which slit width was used, 20 eV?

Response: During data collection, we employed a Gatan Quantum-LS Energy Filter (GIF) with a slit width of 25 eV. This information has been added to the revised text (lines 443-444).

Comment 2: 451 - which BIS scheme, 3x3?

Response: Yes, we performed data collection using a 3x3 BIS pattern. This information has been added to the revised text (lines 449-450).

Comment 3: 452-454 - just state the total number of movies here

Response: Yes, we added this information to the revised manuscript (line 450).

Comment 4: 459 - how many classes? Which tau-fudge value was used?

Response: For 2D classification, the particles were classified using the number of classes k=100 and a tau-fudge value of 2. Subsequently, 3D classification was performed using the number of classes k=4 and a tau-fudge value of 4. This information has been added to the revised text (lines 456-458).

Comment 5: 459 - what is meant by "rapid data processing"? please describe the cryoSPARC data processing a bit further

Response: To describe the cryoSPARC data processing, we rewrote this part as follows (lines

459-462).

Meanwhile, quick data assessment with 2D classification, ab-initio reconstruction, heterogenous refinement and non-uniform refinement by cryoSPARC v2.14 (Punjani et al., 2017) was performed to produce a 3D initial model, which was used as a reference for the 3D classification by RELION 3.1.

Comment 6: 465 - what exactly was masked and how? What were the extensions/softness in pixels of the mask? Was classification including local angle with mask?

Response: We thank the referee for this comment. Actually, we performed 3D classification without masking, and rephrased this part accordingly (line 466).

Comment 7: 479 - please describe real space refinement and model validation tools and criteria

Response: We thank the reviewer for this comment. As suggested, we additionally described our real space refinement procedure and a model validation tool with citation of the related papers in the re-revised text (lines 475-479).

Comment 8: The text in lines 335-340 and other places is a bit edgy and needs rephrasing, but it can be handled in copyediting.

Response: As suggested, we carefully reread the whole part of text and rephrased the indicated part in a clearer and less edgy way (lines 340, 344, and 348). I would appreciate copyeditor's careful check throughout the re-revised manuscript.

Referee #2:

I am satisfied with the revisions.

Response: We are grateful to this referee for his/her careful review of our revised manuscript. It's our great pleasure to learn that Referee#2 has been satisfied with our revisions.

2nd Revision - Editorial Decision 4th Aug 2021

Dear Dr Inaba,

Thank you for submitting the revised version of your manuscript.

Please note that I have taken on this process from my colleague Elisabetta Argenzio, as she has in the meantime left the office and transitioned into a different position.

We have now evaluated your amended manuscript in the editorial team and concluded that the remaining minor concerns have been sufficiently addressed.

Thus, I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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On a different note, I would like to alert you that EMBO Press is currently developing a new format for a video-synopsis of work published with us, which essentially is a short, author-generated film explaining the core findings in hand drawings, and, as we believe, can be very useful to increase visibility of the work. This has proven to offer a nice opportunity for exposure i.p. for the first author(s) of the study. Please see the following link for representative examples and their integration into the article web page:
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Please let me know, should you be interested to engage in commissioning a similar video synopsis for your work. According operation instructions are available and intuitive.

If you have any questions, please do not hesitate to call or email the Editorial Office.

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

Kind regards,

Daniel Klimmeck

Daniel Klimmeck, PhD
Senior Editor
The EMBO Journal
EMBO
Postfach 1022-40
Meyerhofstrasse 1
D-69117 Heidelberg
contact@embojournal.org
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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Kenji Inaba
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2021-108482

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.

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 sample size was chosen based on previous experience for each experiment to yield high power to detect specific effects. For cryo-EM single-particle analysis, we colleted 3531 micrographs and 2,497,830 particle images, which is above the standard sample size of this methodology. For ATPase activity and autophophorylation assays, we performed three independent experiments to calculate means and SD statistically.
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.	No.
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.	No.
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?	Yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. All statistical significance was calculated by one-way ANOVA followed by Tukey's test.
Is there an estimate of variation within each group of data?	Yes.

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Is the variance similar between the groups that are being statistically compared?	Yes.

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).	In this study, we did not use antibodies.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The source of a cell line used in this study is HEK293T (ATCC, CRL-3216) that was tested for mycoplasma contamination.

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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
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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	Yes, we have done.
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).	Yes, we have done.
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.
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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, our study could not fall under dual use research restrictions.