EMBO reports

PTP-MEG2 regulates quantal size and fusion pore opening through two distinct structural bases and substrates

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1st Editorial Decision 19th Jan 2021

Dear Prof. Sun

Thank you for the transfer of your manuscript to EMBO Reports and thank you for your patience while we have editorially reviewed your manuscript.

Your revised manuscript had been reviewed by former referee 1 for our sister journal The EMBO Journal. The referee carefully analyzed the revised version and concluded that you have addressed most of the technical concerns and expanded your earlier data, yet the mechanistic insight was still limited. Since EMBO Reports does not necessarily require a full mechanistic understanding, we would like to offer publication of your dataset in EMBO Reports.

Please address all remaining concerns from referee 1 and please also provide a point-by-point response to these.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

1) Please provide a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

2) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). This information is still missing for Dr. Sun, Dr. Yu and Dr. Cui. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>)

3) Please list all funding information in the relevant fields in our online submission system.

4) Please provide the figures as individual production quality figure files (.eps, .tif, .jpeg, one file per figure)

5) Supplementary Information
- Please call the file "Appendix" and add a table of content including page numbers on the first or second page. Figures are called "Appendix Figure Sx", tables are called "Appendix Table Sx".

- Supplemental Table 2 is rather long and complex. To enhance its usefulness I suggest to remove it from the Appendix and to upload it either as Table EV1 or as Dataset EV1 in the form of an .xls or .xlsx file. The legend for the table can be given either in the first line of the Excel file or in a separate tab.

6) Materials and Methods:
- Please combine "Materials" and "Experimental model and subject detail under one header called "Materials and Methods"

- Key resources and other tables in the methods section: Please either rename these tables as Table 2 etc, or uploaded them separately as EV tables. Please note that we cannot typeset color in

tables.

- I noticed two paragraphs with the header "GST-pull down" in the methods section. Can these be combined or discriminated?

- Please include a statement on mouse husbandry and the ethics committee that approved the experiments using mice.

8) Additional Information: Please change to "Conflict of Interest"

9) Data availability section: Please add a link that resolves to the datasets deposited in PDB.

10) Please remove the list of abbreviations from the manuscript text and specify the abbreviations where needed in the text or figure legends instead.

11) We routinely inspect all images and figure panels before publication. This analysis indicated inconsistencies in the Western blots shown in Figure S13A. To avoid any ambiguities, we kindly ask you to provide the unmodified source data used to generate these panels.

12) I attach to this email a related manuscript file with comments by our data editors. Please address all comments and upload a revised file with tracked changes with your final manuscript submission. I have also taken the liberty to make some changes to the Abstract.

13) I have also looked at the Supplementary figures/Appendix legends and noticed several points that need your attention. Please find these below my signature.

14) Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-600 pixels large (width x height) in .png format. You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

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

Yours sincerely,

Martina Rembold, PhD
Senior Editor
EMBO reports

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Appendix Figure legends:

- S1A: please define the nature of the bars and error bars
- S1D-E: please define the meaning of EPI and NE, define the number of experiments (technical, biological/independent), the meaning of bars and error bars.
- S1H: please define bars and error bars and the number of experiments (and the nature of the

replicates, i.e., biological or technical)
- S1I-M: please define bars and error bars and the number of independent experiments that gave rise to the measurements of 72 chromaffin cells
I suggest summarizing the information that applies to all graphs under a header "Data information". E.g. Data information: the * in (A) indicates protein expression level....

- S2: please add information on the nature of the bars and error bars and the nature of the replicates (technical or biological) to D, E, F, H, I, J.
Again, I suggest using the header "Data information" to summarize the information on the statistical analysis.
If 'N' always refers to independent replicates, this can also be summarized in the final "Data information" paragraph, e.g. by saying that the N is indicated in the graphs and refers to independent experiments in all panels. This applies to all figures.

- S3, S6, S7, S8, S9, S10, S11, S12, S15, S16: please add information on the nature of the bars and error bars and on the nature of the replicates.

- S6D, S10B: please add a scale bar

- Figure S10C and D do not show histograms but bar graphs and the description is not entirely clear. Can you please update it?

- Figure S15: I suggest to add further references to the panels in the legend to orient the reader, e.g. "... to examine the binding of different PTP-MEG2 mutants to endogenous MUNC18-1 (C, E) and DYNAMIN2 (D, F) ....

Comments from referee 1.

In the revised version, Xu and colleagues have addressed most of the previous technical concerns and significantly expanded their study by analyzing the PTP-MEG2 - Dynamin2 interaction, using functional and biochemical assays. Importantly, in addition to the PTP-MEG2 mutagenesis, phosphomimetic mutants of Dynamin Y125 reveal effects on GTP hydrolysis, endocytosis and on fusion pore dynamics. The underlying mechanisms of fusion pore regulation still remain unclear and the phosphorylation/dephosphorylation of Munc18-1 and of Dynamin2 could also represent up-/downstream events, regulating aspects of the fusion machinery assembly, vesicle size and thus indirectly affecting fusion pore dynamics. (For example, the Munc18-1 Y145 phosphorylation/dephosphorylation seems to regulate the number of syntaxin1 binding-competent Munc18-1 molecules, thereby controlling SNARE complex assembly. See also Supplemental Fig. 11, comparing the effects of endogenous and overexpressed Munc18-1 wt and Y145E on PSF amplitude and charge.) The authors mention some of these aspects in the text and in the discussion. Thus, overall, the paper primarily provides novel insights into PEP-MEG2 - substrate interactions and their effects on exo-/ endocytosis, but it does not profoundly forward our molecular understanding of the fusion pore, which will require further mechanistic and structural analyses in the near future.

The following minor points should be addressed:

• Fig. 1A-D: Briefly mention how NE and EPI secretion was measured. Amperometry? (If appropriate, please include the method in the experimental section.)

Reply: We thank the referee for his/her helpful comments and suggestions. The NE and EPI secretion amount was measured with ELISA method. We have added corresponding descriptions in the figure legend.

• The authors may consider to improve in some figures (e.g. Fig. 2) the order/positioning/ numbering of the subfigures. (The readers may appreciate a more systematic approach going from left to right and/or top to bottom.)

Reply: We thank the referee for his/her helpful comments and suggestions. We organized the order and positioning of the subfigures according to referee's suggestion.

• Fig. 2F: Briefly mention in the figure legend the structural fragment highlighted in blue.

Reply: We thank the referee for his/her helpful comments and suggestions. The structural fragment highlighted in blue was "P loop" of MEG2, we have added corresponding description in the figure legend and added the illustration in the figure.

• Fig. 2D: Briefly explain the purpose of peroxide treatment.

Reply: We thank the referee for his/her helpful comments and suggestions. Hydrogen peroxide was added to inhibit protein tyrosine phosphatases (PTP) activity because it is permeable to cell and can oxidize the catalytic cysteine located in the active site of the PTP catalytic domain. This method was widely used to suppress PTP activity in cell experiments, thus elevating the overall protein tyrosine phosphorylation levels in cells [1, 2]. We have explained that in the manuscript.

• Comparing Supplemental Figure 9 with the corresponding previous Supplemental Figure 7-1, the quantitative values of the tyrosine phosphorylation (panel E, Munc18, VAMP7) have profoundly changed in the dephosphorylation assay using PTP-MEG2. Please provide an explanation.

Reply: We thank the referee for his/her helpful comments and suggestions. We have replicated several key experiments during the revision. We found that the student performed with wrong protocol for these indicated experiments, he did not added phosphatase inhibitor pervanadate in the lysis buffer in previous Supplemental Figure 7-1, which were important to suppress the residual phosphatase activity during the lysis procedure. We have reperformed corresponding experiments and included these new results in the revision.

• Supplemental Figs. 11-13: It is not necessary to start labeling figure panels with A, because subsequent labels such as B, ... are missing.

Reply: We thank the referee for his/her helpful comments and suggestions. We revised it accordingly.

• In the Methods section, the authors use centrifugation to isolated/purify proteins. To ensure reproducibility by other scientists please provide information about the employed rotors/ centrifuges.

Reply: We thank the referee for his/her helpful comments and suggestions. We have added the parameters for it.

Reference

1. Ostman A, Frijhoff J, Sandin A, Bohmer FD. Regulation of protein tyrosine phosphatases by reversible oxidation. Journal of biochemistry. 2011;150(4):345-56. Epub 2011/08/23.
2. Kappert K, Sparwel J, Sandin A, et al. Antioxidants relieve phosphatase inhibition and reduce PDGF signaling in cultured VSMCs and in restenosis. Arteriosclerosis, thrombosis, and vascular biology. 2006;26(12):2644-51. Epub 2006/09/23.

1st Revision - Editorial Decision 18th Feb 2021

Prof. JinPeng Sun
Shandong University
Biochemistry and Molecular Biology
Shandong University Medical School, 44 Wenhua Western Rd.
Jinan, Shandong 250012
China

Dear Jinpeng,

Thank you for sending the further revised files. I have uploaded all of them to our online submission system, except for Figure 8 itself, since it seemed unchanged.

I am now very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

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

Yours sincerely,

Martina Rembold, PhD
Senior Editor
EMBO reports

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Corresponding Author Name: JinPeng Sun
Journal Submitted to: EMBO reports
Manuscript Number: EMBOR-2020-52141-T

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 iustified
- 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?	All data were from at least 6 biological replicates.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	statement inlcuded. Thanks.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	female mice (6–8 weeks) were used for the isolation of primary chromaffin cells.
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.	N/A
For animal studies, include a statement about randomization even if no randomization was used.	N/A
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5. For every figure, are statistical tests justified as appropriate?	All stastitical tests are justified as appropriate
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The test assumptions were normal distribution, tested with SPSS.
Is there an estimate of variation within each group of data?	

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Is the variance similar between the groups that are being statistically compared?	N/A

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).	All the information of antibodies were detailed in Appendix Table.
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 cell line sources were reported

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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.	All the information of animals were reported in Materials and Methods.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The statement was provided.
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11. Identify the committee(s) approving the study protocol.	N/A
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.	N/A
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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	Data Availability section was provided.
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