THE
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JOURNAL

Phosphoproteomics reveals conserved exercise-stimulated signaling and AMPK regulation of store-operated calcium entry

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Transaction Report:

(Note: An earlier version of this manuscript was assessed by another journal and was then transferred to The EMBO Journal. As the original review of the manuscript was performed outside of The EMBO Journal's transparent review process policy, no Peer Review Process information is available for this article.)

1st Editorial Decision 3rd Jun 2019

Thank you again for your interest and submitting your manuscript for consideration by the EMBO Journal. I carefully assessed the manuscript together with your response to the referees from a previous venue and have also discussed this in the team here. I am happy to say that we find the context timely and the results presented of interest to the journal. We would thus have this work evaluated by an arbitrating expert, also to judge the additional data added during revision. Please note that this advisor is importantly not to raise new concerns but only to judge quality and advance provided. This process should allow us to draw a definitive conclusion in about ten days to two weeks.

There is one point of notice, which is the drosophila fatigue experiment mentioned, which in our view complements the other data with respect to the physiological relevance of the AMPK-p-STIM1 axis.

Before sharing your study with the external expert, we would thus ask you to add these data back to the manuscript. This should also be useful to better understand the arguments made earlier.

I would thus like to ask you to submit an amended version of the manuscript using the link enclosed below.

1st Revision - authors' response 4th Jun 2019

Thank you for considering our manuscript entitled “Phosphoproteomics reveals conserved nodes across exercise models and feedback regulation of store-operated calcium entry via AMPK” for publication at EMBO Journal and we are very happy that you consider our results timely and of interest.

Here we are resubmitting a new version of the manuscript which includes the Drosophila fatigue data which was referred to in the Reviewer comments we provided with our original submission, as you have requested. These data are shown in Figures 6 and S6 and described in the manuscript text. Figure 6A-C showing fly fatigue data was included in our previous submission to [another journal].

We then removed these data because at the time we lacked verification that the STIM1 overexpression levels were similar between the lines in Fig. 6C. We have recently obtained new data demonstrating equivalent expression (Figure S6A-B), giving us confidence in the results from 6C.

2nd Editorial Decision 14th Jun 2019

Thank you again for the submission of your manuscript (EMBOJ-2019-102578) to The EMBO Journal. We have carefully assessed your manuscript and the point-by-point response provided to the referee concerns that were raised during review at a different journal. In addition, and as mentioned before, we decided to involve an arbitrating expert to evaluate the revised version of your work, with respect to technical robustness, conceptual advance and overall suitability of your work for publication in The EMBO Journal.

As you will see from the report provided below, the arbitrating advisor states the interest and value of your work and is supportive of publication at The EMBO Journal.

Based on the overall positive expert's view together with our own assessment, we decided to proceed with publication of your work at The EMBO Journal pending the following minor issue related to the advisor's input is addressed:

• Please revise your manuscript regarding the arbitrator's point regarding the reduced Ca2+ flux and p-STIM1's roles in Ca2+ signaling, and relativise your statements introducing caveats where appropriate.

REFEREE REPORTS:

Arbitrating advisor's comments:

'These are excellent, highly informative studies reporting on skeletal muscle phosphoproteins regulated in response to exercise. The information in the manuscript should be a useful source of proteins phosphorylated in response to cell stress and not only in response to damaged caused by intense exercise and muscle fatigue.

Among the phosphoproteins changes in response to three models of muscle exercise, a prominent change was noted with the AMPK pathway and various Ca2+ signaling proteins. The authors then focused on the role of the AMPK in phosphorylating STIM1, a key protein regulating receptor-stimulated Ca2+ influx. STIM1 activates the store-operated Ca2+ channel Orai1. Importantly, excessive Ca2+ influx through Orai1 channels is associated with numerous Ca2+ toxicities in many inflammatory and cell stress associated diseases, including muscle fatigue and damage. The authors identified the STIM1 residues phosphorylated by AMPK, which impair STIM1 conformational change required for activation of Orai1 by STIM1 and thus restricts Ca2+ influx to reduce cell damage. The findings also reflect on a quite controversial issue in the field, the role of STIM1 phosphorylation on its function. The present study provides clear evidence for regulation of STIM1 function by phosphorylation, which sharply contrast with several prior studies, one of which is quite

recent (PMID: 19805124, 31064875). In my opinion, the authors fully addressed all concerns expressed by the reviewers.

I did identify an issue that is not fully addressed in the study, which is the prominently reduced Ca2+ release in response to SERCA pump inhibition by thapsigargin in cells expressing the phosphomimetic STIM1 mutants. This would suggest additional role of STIM1 phosphorylation on ER Ca2+ storage in addition to regulation of Ca2+ influx. However, I do consider this sufficient of an issue to precludes publication of the manuscript in the EMBO Journal and thus support the publication of the manuscript.'

2nd Revision - authors' response 27th Jun 2019

We are delighted that you have accepted our manuscript “Phosphoproteomics reveals conserved nodes and regulation of store-operated calcium entry by AMPK” for publication at EMBO Journal. Thank you for your support of our manuscript and guidance through this process.

In this final version we have addressed the following comment from the external arbitrator:

I did identify an issue that is not fully addressed in the study, which is the prominently reduced Ca2+ release in response to SERCA pump inhibition by thapsigargin in cells expressing the phosphomimetic STIM1 mutants. This would suggest additional role ofSTIM1 phosphorylation on ER Ca2+ storage in addition to regulation of Ca2+ influx. However, I do consider this sufficient of an issue to precludes publication of the manuscript in the EMBO Journal and thus support the publication of the manuscript.

To address this, we have added the following statement to the results section (highlighted in yellow in the manuscript):

These stores appear to be severely depleted in cells expressing either the L215S or S257E mutant, consistent with impaired STIM1 function and a reduced ability to refill SR Ca2+. We note that this is not observed with STIM1 KD alone. This suggests that the residual STIM1 activity in the KD cells is sufficient for the maintenance of SR Ca2+ stores, and depletion with the mutants occurs as a result of the mutants exerting a dominant negative effect. Taken together, these data show that phosphorylation of STIM1 at S257 causes a decrease in STIM1 SOCE activity.

We also believe we have addressed each of the formatting changes requested by the editorial team. Please let us know if any additional changes are required or if we can help any further.

3rd Editorial Decision 1st Jul 2019

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript 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.

THE EMBO JOURNAL

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

Corresponding Author Name: David E. James
Manusript Number: EMBOJ-2019-102578R

Reporting Checklist For Life Sciences Articles

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 (see link list at top right).

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 only for independent experiments and sample sizes where the application of statistical tests is warranted (error bars should not be shown for technical replicates)
- when n is small (n < 5), the individual data points from each experiment should be plotted alongside an error bar.
- 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 (see link list at top right).

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 $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

USEFUL LINKS FOR COMPLETING THIS FORM

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http://www.selectagents.gov/	List of Select Agents

B- Statistics and general methods

Please fill out these boxes ↓

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We performed power analyses using effect sizes estimated from previous experiments using similar methods, and specifying an alpha of .05 and power of
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	From our experience, the rodent exercise models used here produce large effect sizes (>2.5) and small deviation (<.3). Therefore, we chose sample sizes based on
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Inclusion criteria for animals was pre-established as 1) the animal must be healthy by all observable indications before undergoing the experimental
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.	Mice from the same cage were randomized across treatment groups to minimize cage bias. Mice within a cage were allocated to rest or running groups by the
For animal studies, include a statement about randomization even if no randomization was used.	Animals were randomized to treatments groups.
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.	Microscopy and subsequent image analysis was performed in a blinded manner.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Due to the nature of the rest vs exercise protocols, it was not possible for researchers to be blinded to the treatment.
5. For every figure, are statistical tests justified as appropriate?	Yes. When multiple groups are compared, a multiple comparison post-hoc test is applied.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes. Data normality distribution was tested using the D'Agostino-Pearson test.
Is there an estimate of variation within each group of data?	Yes.
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).	STIM1 total: https://www.cellsignal.com/products/primary-antibodies/stim1-antibody/4916?_=1560902011907&Ntt=stim1&tahead=true , ACC total: https://www.cellsignal.com/products/primary-antibodies/acetyl-coa-carboxylase-
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Lb rat myoblasts and HEK cells were originially obtained from ATCC. MEFs were obtained from mouse embryos then immortalized as described in the Methods

* 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.	Male Wistar rats were purchased from Animal Resources Centre (Murdoch, Australia) and were used for in situ contraction studies at 3 months of age.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal husbandry and experimentation was conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes
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 link list at top right). See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Relevant aspects of the animals used in this study are reported in compliance with ARRIVE guidelines.

E- Human Subjects

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
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
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' (see link list at top right).	N/A
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' (see link list at top right).	N/A

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition' (see link list at top right). Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD010452.
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).	Data not deposited as described above are provided as Appendix Tables.

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).	N/A
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section: Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	N/A
22. 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.	N/A

G- Dual use research of concern

23. 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.