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ZAK-beta is activated by cell compression and mediates contraction-induced MAPK signaling in muscle

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Transaction Report:

(Note: Please note that the manuscript was previously reviewed at another journal. As EMBO Press has a transfer agreement (including the identities of the referees) with that journal, revision was invited based on the reports from that previous external submission upon request by the authors.)

1st Editorial Decision **20th May 2022**

Dear Simon,

Thank you for transferring your manuscript together with referee reports from another journal to The EMBO Journal. Following our editorial assessment of the study and your responses to the previous reviews, we have concluded that the study will be of interest to our readership. I am therefore inviting you to submit the revised manuscript for publication in The EMBO Journal. Please check the manuscript for textual consistency in figure legends and figures as indicated in the final comments by reviewer #5. Further addition of experimental data is not needed. Please do not hesitate to contact me in order to discuss any specific points ahead of resubmission.

Detailed information on preparing, formatting and uploading a revised manuscript can be found below and in our Guide to Authors - adhering to these guidelines as closely as possible should greatly facilitate editorial processing at the resubmission stage.

Thank you again for the opportunity to consider this work for The EMBO Journal, and I look forward to receiving your revised manuscript!

Best regards,

Ieva

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When submitting your revised manuscript, please carefully review the instructions below and include the following items:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/Author Checklist%20-%20EMBO%20J-1561436015657.xlsx). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

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In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.
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7) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession

number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data can be provided as individual .xls or .csv files (including a tab describing the data). For 'blots' or microscopy, uncropped images should be submitted (using a zip archive or a single pdf per main figure if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

9) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

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- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

10) 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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11) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

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Point-by-point reply to the reviewers' comments

Reviewer #5:

The revised manuscript "ZAKbeta is activated by cellular compression and mediates contraction-induced MAP kinase signaling in skeletal muscle" by Nordgaard and colleagues has definitely improved a lot and the authors were able to address most of the criticism raised by the reviewers. Briefly, excluding the first two proteomics datasets (SILAC and TMT) from the manuscript was definitely the right decision. I can only congratulate the authors and the editorial board coming to this conclusion, as these two datasets did not add any additional value to the manuscript.

Our response:
We thank the reviewer for his/her positive attitude to our work, and we have done our utmost to rectify the last points of dissatisfaction.

We understand that it is also often difficult and cumbersome to generate many replicates for Omics-type of analyses. Also for similar scenarios a practical approach would be to condense the outcome of such "single shot" screens in a table and present the data as a source for generating hypotheses, which could be tested by orthogonal approaches in the same paper. We are aware of the pioneering work (done by the Olsen laboratory) in employing phosphatase treated phosphopeptide samples as a negative control for direct DIA PTM analyses and we were just wondering is the same approach was utilized in the manuscript of Nordgaard et al.

Our response:
Our use of phosphatase treated samples as controls was only done in the first paper when we described the methodology. We did not perform similar controls for the present dataset.

In general, data reduction has also led to a considerable gain in the readability/comprehension of the manuscript. We would also like to thank the authors for the additional "reviewer figures" that rectify initial concerns regarding the technical quality or reproducibility of western blot and IF data. In addition, we are glad to hear about the initial establishment of several CRISPR founder lines and we acknowledge the kind of rescue experiments carried out by in vivo electroporation of GFP-ZAKbeta. In summary, we feel that the manuscript in its current form is now suitable for publication in Nature communications.

Our response:
Again, we thank the reviewer for his/her positive remarks!

Nevertheless, we have still a few comments listed below.
The resulting re-structuring/re-distribution of different panels between the figures and supplementary figures has created some additional sort of confusion, which can easily be sorted out together with the editorial team. For example, main figure 3 is a mixture of data from experiments with "compression" or "stretching" in U2OS cells (panels 3a-3f) and experiments that are not addressing compression but that report results after applying osmotic shock in C2C12 cells (panels 3g-3j). Unfortunately, both have the running title "The C-terminal stress

fiber binding domain in ZAKbeta is a sensor of cell compression". Likewise, supplementary figure S3 contains experiments in C2C12 cells, including "stretching" (in panels a-c) as well as diverse data ranging from characterization of ZAK-/- mice to in-situ contraction studies (in the lower mouse hind-limb); common headline: "Cell stretch does not activate ZAKbeta". It would be probably a lot easier for the reader to follow the story if on one hand each figure and supplementary figure would have a common title/headline and on the other hand if each supplementary figure would only contain experiments that accompany a single main figure. As an example, the quantification of a western blot seen in main figure 4e is shown in supplementary figure S3 (j), while data that belonging to main figure 4d are depicted in supplementary figure S4.

Our response:
We have changed the headings of figures 3 and S3 to more accurately reflect the data depicted. Regarding linking main figures and supplemental figures, everything is presented in a chronological order. Figures S3 and S4 are highly packed as it is, and with EMBO Journal's policy of only allowing five "Expanded View" figures for typesetting, we did not find a good way to improve our layout further.

Please double-check whether the (supplementary) figure titles still fit the data presented in the figures and whether the figure legends are still correct or updated. For example, the authors have re-labeled some western blot pictures in which ZAKbeta was detected by the anti-ZAK antibody with ZAKbeta instead of ZAK, but the figure legends still say "analyzed by immunoblotting with the indicated antibody". So when reading the legend one could assume that an isoform-specific antibody was used. In addition, in blots with several ZAKbeta bands, just replacing ZAK (the antibody used) with ZAKbeta has not really helped clarifying what is shown. In figure 1g bands running at three different heights can be seen (which are presumably endogenous ZAKbeta, S-HA-ZAKbeta and an autophosphorylated form of S-HA-ZAKbeta). Now the figure is labeled with" ZAKbeta ~55kDa" but an arrowhead is missing. To be more reader-friendly, it would be best to have a tick mark on the right side of the blot at the position/height of the marker band labeled with "XY" kDa (as seen for example in Fig3i) as well as relevant information on which antibody was used (HA, ZAK or GFP). The same applies to figures 2h, 3c and 4j, in which full-length and truncated (1-132) versions of ZAKbeta constructs are discussed.

Our response:
We have changed the labelings of the relevant western blots to accurately indicate the antibody used (= the protein (isoform) being blotted for). It is now correct to use statements such as "analyzed by immunoblotting with the indicated antibodies" in the figure legends. We thank the reviewer for alerting us to this textual inconsistency. We did not find space in our tightly packed figures to insert molecular weight markers on the right side of all relevant blots, and instead do it on the left side (below the antibody info). In this way, we always indicate the size of the smallest band recognized by the total ZAK antibody. In addition, we indicate the molecular weights of the two ZAK isoforms in the schematic in Fig. 1c, alerting the reader to what the subsequent molecular weight indications imply. For fusion proteins such as HA- or GFP-tagged ZAK, we believe that the reader can easily infer the resulting reduced mobility in our gels.

Presumably, it would be more straightforward to replace "WT" by "FL" or "full-length" in figures 2h, 3c and S1i, because "wild-type" could be mistaken with the endogenous protein. Furthermore, please also pay attention to cross-references in the figure legends. As suggested before, you could indicate the sex of the mice studied in figure 5a and 5c on top of the images.

Our response:
We have chosen not to follow the advice of replacing "WT" with "FL", as we did not want to introduce further abbreviations. We have indicated the sex of mice in Fig. 5a,c as suggested.

Minor points:
In the "Methods" paragraph "Cell culture and reagents" please kindly remove the description of SILAC labeling since these data are not presented in the revised manuscript.
Figure 2: Please add "5 min" after "Sorbitol" in the labeling of the immunoblot.
Figure S4a appears a bit blurry. Maybe check the resolution of the picture.
Figure legend S4b: "Buttom" instead of Bottom, "cluster" should probably mean "clusters" (plural) here
Figure 5b and 5d: Please add "Soleus" on top of the bar charts
Figure S4h: Please add "TA" or "Tibialis anterior" on the top of the bar chart
Supplementary Table S2: We would like to thank the authors for compiling the legend of this table! Such a detailed description is immensely helpful when dealing with different kinds of proteomics data. Maybe consider to change "M2" (means doubly phosphorylated) for another abbreviation.
On a general note, please replace "mice" (plural) with mouse (singular) when you are referring to single animals.

Our response:
We have now corrected all of these things and checked the resolution of Fig. S4a

1st Revision - Editorial Decision **22nd Jun 2022**

Dear Simon,

Thank you for addressing the final editorial issues in the revised manuscript. I am now happy to inform you that your manuscript has been accepted for publication.

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here:
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Thank you again for this contribution to The EMBO Journal and congratulations on a successful publication!

Best regards,

Ieva

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