The transcription factor BES1 interacts with HSFA1 to promote heat stress resistance of plants

Pablo Albertos, Gönül Dündar, Philipp Schenk, Sergio Carrera, Philipp Cavelius, Tobias Sieberer, and Brigitte Poppenberger

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Editor: David del Alamo

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.)
Dear Prof. Poppenberger,

Thank you again for the submission of your manuscript entitled "The transcription factor BES1 cooperates with HSFA1 to promote the heat shock response of plants" and for your patience during the review process. Your study has been sent to three referees, and we have so far received reports from two of them, which I copy below. As both referees are convinced about the high interest, novelty and quality of your study, I would like to ask you to begin revising your manuscript according to the referees’ comments. Please note that this decision is made in the interest of time, and I will forward you the third report very likely including further requests, as soon as I receive it.

As you can see from their comments, all three referees are supportive of your work, but point out to several significant concerns that will require your attention before your manuscript can be published in The EMBO Journal. I will not repeat here the referee concerns, but in summary, in addition to other technical concerns, referee #1 believes that the single major caveat of the paper is the lack of BES1 loss-of-function analyses. Referee #3, in turn points out to some mechanistic and technical problems.

Based on the overall interest expressed in the reports, I would like to invite you to address the comments of all referees in a revised version of the manuscript. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but we are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic, so please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. Follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this "scooping protection policy" beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

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 available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Again, please contact me at any time during revision if you need any help or have further questions.

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

David

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David del Alamo, PhD.
Editor
The EMBO Journal

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.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised
6) We require a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) 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: http://bit.ly/EMBOPressFigurePreparationGuideline

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

8) 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.).

9) 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 .

10) 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.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called "Appendix", which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2” etc. See detailed instructions regarding expanded view here: .

- 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.

11) 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 .

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Link Not Available

Referee #1:
The manuscript of Albertos et al. reports that BES1, the transcription factor mediating the growth-promoting activity of BR, is involved in HS response by cooperating with HSFA1. More than 20 years ago, it had been demonstrated by Priti Krishna and coworkers that exogenous application of BR significantly enhances thermotolerance of plants, but how BR affects the HS response of plants remains an enigma. In this study, the authors provided several lines of evidence that BES1 is activated at high temperatures by dephosphorylation and directly regulates the transcription of HS response genes in cooperation with HSFA1. The findings explain the effect of exogenous BR on thermotolerance and point out a novel role of BES1 in HS response, which is quite exciting. However, the current work exclusively relied on bes1-D, a mutant allele encoding a constitutively active variant of BES1. The consequence of repressing BES1 has not been shown, which is the major weakness of the manuscript. It is essential to demonstrate to what extent HS response is affected when BES1 function is repressed or disrupted. Other outstanding issues are listed below:

1. L81-83: The authors said, “BES1 and BZR1 were previously shown to act upstream of HSFs and other heat-induced TFs in the control of the heat-responsive transcriptome (Divi et al., 2016; Lachowiec et al., 2013; Samakovli et al., 2014; Shigeta et al., 2015).” However, I could not find relevant data in the cited papers supporting such notion. The paper of Divi et al. did mention, “That some HSF genes are targets of BES1 remains a hypothesis to be tested in the future.” These citations are inaccurate, and correction is needed.

2. L186-188: The interaction between BES1 and HSFA1a detected by co-immunoprecipitation is not convincing. Additional evidence using other methods, such as BiFC, should be provided.

3. L333-340: The information about the heat stress assays is not comprehensive. Depending on the heating device used, the rate of temperature rise within a tissue culture vessel substantially varies. As discussed in the review paper of Yeh et al. (2012, Plant Sci. 195:10), temperature raises slower when heating is conducted in a growth chamber than in a water bath. The former device allows the seedlings to be acclimated before the air temperature inside the vessel reaches the setting. In the studies with a water bath, Arabidopsis seedlings could hardly tolerate an HS treatment at 44 degrees C for 45 min. However, most wild-type seedlings survived after incubation at 44 degrees C for up to 90 min in this study (Fig.1 F, top), probably because the basal heat tolerance assay was done in a growth chamber.

The “basal heat stress resistance” mentioned in this study is more or less acquired resistance if this is the case.

4. Fig. 3E: The purpose of introducing BES1 effector into the bes1-D protoplasts is unclear. Because bes1-D is constitutively active, it makes no sense to supplement the cells with more BES1. It would be more meaningful if a construct were introduced to repress the expression of BES1.

5. The role of HSFA1 on repressing BES1's function in BR-controlled growth is quite preliminary, only based on the response of bes1-D x hsfA1qM shown in Fig. 4C, D. This assertion is not in agreement with the responses of hsfA1qM to epiBL and BRZ, which are similar to that of the two wild type lines.

Referee #3:

The authors examined the function of BES1, a transcription factors mediating Brassinosteroid (BR) signaling, in heat shock (HS) responses. They showed that BES1 accumulates as unphosphorylated form in response to HS (similar to BR treatment), which is independent of BRs and BR signaling through BR receptor BRI1 (Figs 1-2). They further showed that BES1 and HSFA1 can bind to HSP gene promoters and activate their expression (Fig 3). On the other hand, they provided genetic evidence showing that HSFA1 negatively regulates BES1 function in promoting BR-regulated plant growth (Fig 4). They conclude that BES1 integrates heat stress response and growth through its regulation by HS, cooperation with HSFA1 in activating HS response gene expression and repression by HSFA1 in BR-regulated growth. The studies revealed new function and regulation of BES1, a central regulator for plant growth and stress responses, in HS responses and are therefore of wide interests to the field. The genetic evidence is strong and some molecular mechanisms are presented. Further mechanistic studies can further strengthen the manuscript (1-4 major concerns, 5 minor concern):

1. An important question the authors should at least partially address is how does HS lead to accumulation of BES1 as unphosphorylated form. The authors should test BIN2 with loss-of-function or gain-of-function BIN2 mutants and/or inhibitors. It's possible that heat inhibits BIN2 and thus allow BES1 accumulation as unphosphorylated form and such regulation would not require BRs and BRI1.

2. The authors should test if addition of both BES1 and HSFA1a can further activate the HSP promoter-Luc reporters in Fig 3E.

3. The authors should try to improve the DNA binding experiments in Fig 3D. Alternatively/additionally, ChIP assay should be performed to confirm that both BES1 and HSFA1 can bind these promoters in vivo (only BES1 ChIP results are presented).
4. The author showed quite clearly that HSFA1 inhibit BR-regulated growth as the hsfA1qM showed increased BR-regulated growth (Fig 4). It would be helpful if the authors could examine the expression of several growth-related genes (e.g. SAUR-AC, XETs, expansins) in different mutants to provide some molecular evidence.

5. The authors showed that hsfA1qM can enhance bes1-D growth phenotype, suggesting that HSFA1 inhibits BES1-regulated plant growth. It would be helpful to examine if HSFA1 overexpression can suppress bes1-D phenotype.
Point-by-point response

Referee #1:

The manuscript of Albertos et al. reports that BES1, the transcription factor mediating the growth-promoting activity of BR, is involved in HS response by cooperating with HSFA1. More than 20 years ago, it had been demonstrated by Priti Krishna and coworkers that exogenous application of BR significantly enhances thermotolerance of plants, but how BR affects the HS response of plants remains an enigma. In this study, the authors provided several lines of evidence that BES1 is activated at high temperatures by dephosphorylation and directly regulates the transcription of HS response genes in cooperation with HSFA1. The findings explain the effect of exogenous BR on thermotolerance and point out a novel role of BES1 in HS response, which is quite exciting.

Thank you for the positive feedback and the helpful comments and suggestions!

However, the current work exclusively relied on bes1-D, a mutant allele encoding a constitutively active variant of BES1. The consequence of repressing BES1 has not been shown, which is the major weakness of the manuscript. It is essential to demonstrate to what extent HS response is affected when BES1 function is repressed or disrupted.

To address this concern, we assessed two bes1 knock-out alleles in the heat stress assays and found that they showed reduced resistance. The degree of phenotypes was correlated with the degree of BES1 activity changes. bes1-1, a weak knock-out allele, showed a weaker phenotype than bes1-2, which is a complete knock-out. bes1-D, which hyper-accumulates BES1, showed the clearest changes to wild-type. The results confirm results of a previous study (Setsungnern et al., 2020, Plant Sci. 296:110470), which had also found that a loss of BES1 function decreased heat stress tolerance and support the idea that BES1 acts as a positive regulator of heat stress resistance.

Other outstanding issues are listed below:
1. L81-83: The authors said, "BES1 and BZR1 were previously shown to act upstream of HSFs and other heat-induced TFs in the control of the heat-responsive transcriptome (Divi et al., 2016; Lachowiec et al., 2013; Samakovli et al., 2014; Shigeta et al., 2015)." However, I could not find relevant data in the cited papers supporting such notion. The paper of Divi et al. did mention, "That some HSF genes are targets of BES1 remains a hypothesis to be tested in the future." These citations are inaccurate, and correction is needed.

   Thank you for pointing this out! We have corrected this statement, to accurately cite what was shown in the work from the lab of Priti Krishna.

2. L186-188: The interaction between BES1 and HSFA1a detected by co-immunoprecipitation is not convincing. Additional evidence using other methods, such as BiFC, should be provided.

   Thank you for this suggestion! We carried out the BiFC assays, which showed that BES1 can directly interact with HSFA1a also in vivo.

3. L333-340: The information about the heat stress assays is not comprehensive. Depending on the heating device used, the rate of temperature rise within a tissue culture vessel substantially varies. As discussed in the review paper of Yeh et al. (2012, Plant Sci. 195:10), temperature raises slower when heating is conducted in a growth chamber than in a water bath. The former device allows the seedlings to be acclimated before the air temperature inside the vessel reaches the setting. In the studies with a water bath, Arabidopsis seedlings could hardly tolerate an HS treatment at 44 degrees C for 45 min. However, most wild-type seedlings survived after incubation at 45 degrees C for up to 90 min in this study (Fig.1 F, top), probably because the basal heat tolerance assay was done in a growth chamber.
The "basal heat stress resistance" mentioned in this study is more or less acquired resistance if this is the case.

Thank you for pointing this out! We have included a clearer description of the experimental set-up and cite the paper mentioned, to highlight, that in the conditions that we used for the basal resistance assays, the plants may actually be able to acquire some resistance.

4. Fig. 3E: The purpose of introducing BES1 effector into the bes1-D protoplasts is unclear. Because bes1-D is constitutively active, it makes no sense to supplement the cells with more BES1. It would be more meaningful if a construct were introduced to repress the expression of BES1.

We agree. We only included it for a comprehensive effector+mutant background combination and would like to keep it (as a control).

5. The role of HSFA1 on repressing BES1’s function in BR-controlled growth is quite preliminary, only based on the response of bes1-D x hsfA1qM shown in Fig. 4C, D. This assertion is not in agreement with the responses of hsfA1qM to epiBL and BRZ, which are similar to that of the two wild type lines.

We agree that the results on the ability of the hsfA1qM background to promote BR responses in bes1-D were preliminary, so we quantified the expression of BR-controlled genes. This showed that DWF4 and BR6ox2 were significantly more repressed and GA3ox1, IAA5 and SAUR-AC1 were significantly more induced in bes1-DxhsfA1qM than in bes1-D, supporting the idea that HSFA1s repress bes1-D effects. This of course, is not evidence for a physiological function of HSFA1s in repressing BR responses (in wild-type) and we discuss this more clearly now.

Referee #3:

The authors examined the function of BES1, a transcription factors mediating Brssinosteroid (BR) signaling, in heat shock (HS) responses. They showed that BES1 accumulates as unphosphorylated form in response to HS (similar to BR treatment), which is independent of BRs and BR signaling through BR receptor BRI1 (Figs 1-2). They further showed that BES1 and HSFA1 can bind to HSP gene promoters and activate their expression (Fig 3). On the other hand, they provided genetic evidence showing that HSFA1 negatively regulates BES1 function in promoting BR-regulated plant growth (Fig 4). They conclude that BES1 integrates heat stress response and growth through its regulation by HS, cooperation with HSFA1 in activating HS response gene expression and repression by HSFA1 in BR-regulated growth. The studies revealed new function and regulation of BES1, a central regulator for plant growth and stress responses, in HS responses and are therefore of wide interests to the field. The genetic evidence is strong and some molecular mechanisms are presented.

Thank you for the positive feedback and the helpful comments and suggestions!

Further mechanistic studies can further strengthen the manuscript (1-4 major concerns, 5 minor concern):

1. An important question the authors should at least partially address is how does HS lead to accumulation of BES1 as unphosphorylated form. The authors should test BIN2 with loss-of-function or gain-of-function BIN2 mutants and/or inhibitors. It’s possible that heat inhibits BIN2 and thus allow BES1 accumulation as unphosphorylated form and such regulation would not require BRs and BRI1.
We used the dominant bin2-1 mutant to address this point. The 35S:BES1-CFP reporter was introduced into bin2-1 by crossing, homozygous progeny was isolated, subjected to heat stress treatments and the BES1 phosphorylation state was analyzed. This showed that even in the bin2-1 background BES1 de-phosphorylation by heat was not compromised, indicating that it is not only a repression of BIN2 that is causal. We also performed heat stress phenotyping of bin2-1, which showed that the mutant does not exhibit an altered resistance as compared to wild-type supporting this idea.

To further investigate, how heat stress may de-phosphorylate BES1, we tested if ABA may be implicated, since it has been shown to inhibit BES1/BZR1 de-phosphorylation via the PP2C phosphatases ABI1 and ABI2. Treatment of wild-type with ABA, repressed BES1 de-phosphorylation by heat, whereas treatment with the ABA biosynthesis inhibitor Fluridone promoted it. In addition, in an abi1 higher order knock-out mutant (the triple mutant abi1-2 hab1-2 pp2ca-1 line) heat-induced BES1 de-phosphorylation was impaired and heat stress resistance was compromised, providing first evidence for their contribution. We included these results in Fig 2 and 3 and discuss them in detail.

2. The authors should test if addition of both BES1 and HSFA1a can further activate the HSP promoter-Luc reporters in Fig 3E.

We had tried these assays in the past, but had technical difficulties, since we didn’t obtain efficient expression of both effector constructs. This is why we changed the approach, and used genetic backgrounds in which either BES1 is hyperactive (bes1-D) or HSFA1 activity is lost (hsfA1qM), to test the impact of gain or loss of activity of one factor on the performance of the other.

3. The authors should try to improve the DNA binding experiments in Fig 3D. Alternatively/additionally, ChIP assay should be performed to confirm that both BES1 and HSFA1 can bind these promoters in vivo (only BES1 ChIP results are presented).

We agree that the EMSAs don’t look perfect, but there were problems with bad migration of these protein-DNA complexes into the gel, and we were unable to improve this. However, since efficient competition for binding occurred in the competitor experiments and since we don’t rely on the in vitro analysis only, we hope this is acceptable.

Unfortunately, we don’t have tagged HSFA1 over-expression lines to do ChIPs with HSFA1. However, the ability of HSFA1 to bind HSEs in HSP promoters is very well established. Moreover, in the LUC assays we show that the constitutive expression of the HSP70.4p-LUC and HSP90.1p-LUC constructs is strongly reduced in the hsfA1qM background as compared to wild-type and that HSFA1a (as an effector) can activate all promoters, evidence for its ability to directly bind them in vivo.

4. The author showed quite clearly that HSFA1 inhibit BR-regulated growth as the hsfA1qM showed increased BR-regulated growth (Fig 4). It would be helpful if the authors could examine the expression of several growth-related genes (e.g. SAUR-AC, XETs, expansins) in different mutants to provide some molecular evidence.

Thank you for this suggestion! We did this experiment and found that there are no clear changes in BR marker genes in the hsfA1qM mutant. This is in line with its’ BR-response phenotypes, which are similar to wild-type Ws. In the bes1-DxhsfA1qM mutant however, several bes1-D-regulated genes were more strongly altered (including SAUR-AC1, GA3ox1, and IAA5), supporting the idea that HSFA1s repress bes1-D effects on a subset of BR responsive genes. While this is interesting, it’s not conclusive evidence for a physiological
function of HSFA1s in repressing BR responses (in wild-type) and we discuss this more clearly now.

5. The authors showed that hsfA1qM can enhance bes1-D growth phenotype, suggesting that HSFA1 inhibits BES1-regulated plant growth. It would be helpful to examine if HSFA1 overexpression can suppress bes1-D phenotype.

We agree that this line may be helpful, however we were unable to generate it in the time we were given for the revision. Also, it would not allow us to conclusively answer the question if HSFA1s can repress BES1 activity in BR responsive growth, which we plan to address in a follow up study.
Dear Prof. Poppenberger,

Thank you for the submission of your revised manuscript to The EMBO Journal and please accept my apologies for the delay in responding. The referees now consider that you have properly dealt with their concerns and your manuscript is almost ready for publication. There are however a few editorial points that will need to be addressed before it can be accepted:

- Referee #1 makes reference to a typo that should be corrected.
- The correct nomenclature for appendix elements is "Appendix Figure S1" or "Appendix Table S1". Please correct and make sure all callouts in the manuscript are modified accordingly. There is a callout in the text for Appendix Figure S4D and it should be S4C.
- Please provide the text part of the synopsis: a short 'blurb' text summarizing in two sentences the study (max. 250 characters). Add as well three to four 'bullet points' highlighting the main findings. Bullet points and standfirst text should be submitted as a separate manuscript file in LaTeX, RTF or MS Word format.
- Your synopsis image has been provided but it is not of the correct size. The image should be PNG or JPG format with pixel dimensions of 550 x 300-600 (width x height).

Please let me know if you have any further questions regarding any of these points. Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving the final version.

Yours sincerely,

David del Alamo
Editor
The EMBO Journal

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

Please click on the link below to submit the revision online:

Link Not Available

Referee #1:

The authors have addressed my questions satisfactorily in the revised manuscript. There is one mistake to be taken care of. In lines 37-375, HSFA3 should be corrected as HSF3.

Referee #3:

In this revision, the authors addressed my major concerns and provided reasonable explanations for others. Particularly, the finding that heat stress induced BES1 activation is at least partially mediated by ABA signaling pathway is interesting. The study establishes the functional mechanisms of BES1 in heat stress response and is of wide interest.
The authors performed the requested editorial changes.
Dear Prof. Poppenberger,

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

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: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

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If you have any questions, please do not hesitate to call or email the Editorial Office. Thank you for your contribution to The EMBO Journal.

Yours sincerely,

David del Alamo
Editor
The EMBO Journal
Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Authorship guidelines in preparing your manuscript consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal’s guidelines in preparing your manuscript.

B - Statistics and general methods

For animal studies, include a statement about randomization even if no randomization was used.

A. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?

B. What is the effect size of interest for this study?

C. What is the statistical power of the study to detect the effect size of interest?

D. What is the minimum effect size that is considered meaningful for this study?

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Z. What is the minimum effect size that is considered meaningful for this study?
In the variance similar between the groups that are being statistically compared? Yes. All the groups have a similar variation.

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).

8. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.

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.

9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.

10. We recommend consulting the ARRIVE guidelines (see link list at top right) (Furqan, B. et al, 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.

E- Human Subjects
11. Identify the committee(s) approving the study protocol.

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.

13. For publication of patient photos, include a statement confirming that consent to publish was obtained.

14. Report any restrictions on the availability (and/or on the use) of human data or samples.

15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.

16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under ‘Reporting Guidelines’. Please confirm you have submitted this list.

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.

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 GSE19682, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for ‘Data Deposition’.

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small-molecules
- Functional genomics data
- Antibodies
- Proteomics and molecular interactions
- Structural genomics data
- All other datasets

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 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 link list at top right or equivalent) where applicable.

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. It 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-centralized repositories such as dbGaP (see link list at top right) or EGA (see link list at top right).

21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (e.g., 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 EGA (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.

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 (APHS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.