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Pathogenic fungi neutralize plant-derived ROS via Srpk1 deacetylation

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(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.)

Thank you for submitting your manuscript to The EMBO Journal. We have now received comments from three reviewers, which are included below for your information.

As you will see from the reports, all reviewers find the study interesting, but they also indicate a number of controls and alternative experimental approaches, as well as a better depiction of microscopic images that would be needed to sufficiently strengthen the study and its conclusions, and state that a better description of the applied experimental approaches is needed. In particular, the reviewers point out the need better quantification of H2O2 levels during the infection (reviewer #1) and for alternative methods to verify identified Srpk1 interactors (reviewer #3) and Srpk1 phosphorylation and acetylation status (reviewer #2).

Based on the overall interest expressed in the referee reports, I would like to invite you to address the comments of all reviewers in a revised version of the manuscript. I think it would be helpful to discuss the revision in more detail via email or phone/videoconferencing - please let me know which option you prefer. I should also 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.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, please contact me as soon as possible upon publication of any related work to discuss the appropriate course of action. Should you foresee a problem in meeting this three-month deadline, please let us know in advance to arrange an extension.

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. Please also see the attached instructions for further guidelines on preparation of the revised manuscript.

Please feel free to contact me if have any further questions regarding the revision. Thank you for the opportunity to consider your work for publication, and I look forward to receiving the revised manuscript.

Referee #1:

Zhang et all present a quite comprehensive story (Srpk1 deacetylation initiates ROS detoxification in plant fungal infection) about how post-translational modifications of a conserved fungal protein counteract plant defense mechanisms, thereby enabling pathogen infection. They present important findings which will be interesting for the broad readership of EMBO and worth publishing. The manuscript is well-written and the results are well-presented.
However, before publishing, some issues need to be addressed to consolidate the underlying mechanisms. It was shown previously, that different H2O2 concentrations/ homeostasis determine the virulence of pathogenic fungi. It is important to determine the H2O2 concentration in the different Fol and Botrytis strains before plant infection and also after plant infection. The disruption and mutations of Srpk1 might also change the in situ H2O2 concentration and virulence of the different Fol and Botrytis strains. Furthermore, the experimental background of the various experiments is pretty often imprecisely and superficially described which should be revised.

In detail:

138: Suppl Fig. 1e-f. Explain more in detail the presented experiments...give more information about used samples and the tested combinations ...one sentence that summarises the findings from a Co-IP and Pull-down experiment is too superficial...

142: use "sequestering" instead of "distribution"

144: Fig 1 a: How was the quantitative H2O2 measurement performed? Did you directly infect the tomato roots, or did you infect the tomato leaves and subsequently measure the ROS accumulation in the roots?...briefly describe that in the results section.

The Fol-wildtype strain control is missing. FolSrpk1-GFP is a potential overexpression line. It would be important to compare H2O2 release/production after FolSrpk1-GFP and Fol wildtype strain infection.

Fig1 a: Please perform 3,3'-Diaminobenzidine (DAB)-staining to visualize H2O2 accumulation in tomato plants after mock and infection.

Fig 1 b: Please provide the robust statistical analysis (significance test) proofing the accumulation in the nucleus at 3dpi. Shouldn't there be a significant drop of FolSrpk1 in the cytoplasm at 3dpi? ...please comment on that point.

Fig 1 c,e: How many biological replicates have been performed...?
181: rephrase: it is likely that the modification

Fig 2 h: How many biological replicates have been performed...? The band at 2 and 3 dpi looks weird... any explanation for that phenomenon

Fig3 b How many hours after treatment?

Fig3c What is AcCoA doing in this experiment?...there is no explanation for that

225-226: describe in detail "leading to altered distribution of this protein in the cell (Fig. 3e)"...what and how is it altered?...be more precise!!!

Fig 3d-e: How many biological replicates were performed?

244: "but few alternative splicing events were observed". --> Why is that important...Please comment!

285-286: "Given the fact that FolSrpk1 nuclear translocation increases the expression level of antioxidant enzymes"...you cannot claim that because is not backed up by your findings....after Fol infection, only the expression level of antioxidant enzymes increases, but you haven't shown in detail that the specific translocation of FolSrpk1 changes the expression level of antioxidant enzymes ....Please correct that!

Fig 5a: Please perform DAB-stainings of these strains on PDA media to analyze whether the disruption of Srpk1 and its modifications affects the Fol in situ H_2O_2 accumulation !!!

Fig 5C: Please perform 3,3'-Diaminobenzidine (DAB)-staining to visualize H_2O_2 accumulation in Tomato plants after infection of these different Fol strains.

Fig 5d-e: "Quantification of the disease indexes of the indicated strains" What does that mean, provide more details. How many biological replicates were performed?

Fig 6 e: These images are of very poor quality. Please provide high-resolution images of these lesions in which the hyphae can be clearly distinguished from the plant cells and vice versa. Subsequently, one should be able to make out whether Botrytis or plant cells are stained.

Fig1-4, 6 and Supplementary figure 1, 3, 4, 6: How often did you repeat the blots? How many biological replicates with similar results were performed?

Referee #2:

EMBO Review
Srpk1 deacetylation initiates ROS detoxification in plant fungal infection
By Ning Zhang et. al.

The manuscript describes experiments that are aimed at identifying the mechanism by which pathogens are able to counteract the effect of production of reactive oxygen species (ROS), that are deployed by the plant immune system. Pathogens contend with ROS release by a variety of mechanisms, including the production of antioxidant proteins and enzymes. However, the mechanism of such counteracting processes by pathogens remain largely unknown. The authors propose that deacetylation of the Fusarium oxysporum f. sp. lycopersici kinase Srpk1 is involved in counteracting ROS release during plant infection. They provide evidence that the deacetylation controls the disassociation of Srpk1 from a cytoplasmic protein, Aha1, which enables nuclear translocation of the kinase. Nuclear localisation of the kinase is then proposed to act upon Sr1 which is able to enhance transcription of different antioxidant enzymes. These enzymes are responsible for the removal of ROS thereby facilitating successful infection. This is an interesting paper which proposes a novel counteracting process for ROS removal. However, I have some concerns about the manner in which the work has been carried out which should be addressed by the authors.
1. The paper relies predominantly on western blot analysis using a variety of antibodies. Whilst in their own right these data are convincing, I am worried that this is the only line of evidence that was produced in support of any of the conclusions made. The phosphorylation status Srpk1 for instance and its acetylation status are based primarily on the use of antibodies. Biochemical analysis in particular, phosphoproteomic analysis would add much to this study and provide much more convincing evidence overall for the conclusions being made. In particular, the use of the anti-K304ac antibody is the only evidence shown regarding acetylation of Srpk1. For such an important major conclusion associated with the acetylation status of this kinase there really should be at least another form of evidence in support of such an assertion.
2. For the western blots shown in Fig. 1 there are loading controls missing for panels f and g. This is important because it is still not clear that the proteins are evenly loaded in spite of the way in which the blots are being presented. I think that if the original Coomassie blue stained images could be provided (or shown in supplement) this would also helps to support the conclusions made for all of the figures because loading controls are sometimes, but not always, used for many of the subsequent panels.
3. In Fig. 1c the images showing potential nucleal localisation of Srpk1 and the effect of putative deacetylation really require larger images. This is not clear from these images without extensive magnification. Arrows could also be added to highlight the nuclei more clearly.
4. In Fig. 3 the putative acetylation at K304 of Srpk1 is analysed purely on the basis of the use of the anti-K304ac antibody. I strongly believe that a second line of evidence is necessary for such an important proposition.

5. In Fig. 4 the role of K304 acetylation is tested on the transcription of antioxidant enzymes, this analysis would again benefit from enlarging panel d to show the phenotypic effect much more clearly. The data shown in panels e and f would be better presented as box and whisker plots with the original data points presented rather than bar charts. the spread of the data is not shown accurately in the current presentation and we cannot evalute variation between replicates.
6. Fig. 5 panel d is again too small to show the phenotypic effect on plants and plant disease effectively and panels e and f should show the original data points and be presented as box and whisker plots. The same is also true of Fig. 5b.
7. In Fig. 6 there is no loading control in panels a and d. Scale bars are needed for panel e and the data shown as bar charts in panels e and f would be better presented with the original data points and replications of the experiment shown.
8. In Fig. 7 the proposed model is still based entirely on a single form of evidence. The hyperphosphorylation of Sr1, for instance, is a speculation with no evidence in support.
9. Supplementary Fig. 3 showing the validation of the K304ac antibody should be shown as a panel in one of the main figures since it is the only evidence shown in support of the specificity of the antibody.
In summary, this is an interesting paper that provides evidence for a new and potentially novel mechanism of ROS removal by pathogens during infection as such it has considerable merit. The experiments carried out appear to have been performed carefully and the data presentation is of a high standard overall in spite of some of the concerns raised above. The most significant limitation in this study, however, is the absence of other forms of independent analysis to support the main conclusions regarding deacetylation of phosphorylation status. If these can be added and further evidence provided, or if the authors are able to highlight more clearly the current limitations of the study and modify their conclusions accordingly, then this would improve the manuscript very considerably.

Referee #3:

In this work, Zhang et al explore one essential process in the plant-pathogen interaction which is the capacity of the pathogen to reduce the level of ROS to infect the host. Using diverse and complementary methodologies, they show fascinating and very promising results suggesting that the acetylation level of Serine/arginine protein kinases determines their cellular localization. Once these proteins translocate to the nucleus, it activates a downstream cascade leading to the expression of antioxidant enzymes that actively participate in fungal virulence. The work needs to indicate replicates, controls, and statistics, and a few more experiments are required to sustain the exciting conclusions.

Specific major concerns essential to be addressed to support the conclusions
- FolSrpk1 localization is a very relevant piece of information because it determines the activity of the protein. Unfortunately, the images have an extremely low magnification and resolution. Please, improve them. Regarding the associated WB (1Sc), please, include the controls of total protein loaded for each line. I also recommend to move Fig S1B and C to Main figure.
- Microscopy images: not enough magnification and resolution. Replicates and quantifications missing: quantify distribution of the protein in nucleus/total in microscopy images. Include at least 3 replicates, with at least 3 hyphae/replicate and at least 3 cells/hyphae.
- Missing the loading controls in most WB. Missing information of replicates in all WB.
- Table S1 is incomplete. It has to include all replicates (4 is the minimum) and statistics of both FolSrpk1-GFP and the negative control GFP. Additional information like LFQ intensities are highly recommended.
- The potential FolSrpk1 interactors identified by co-IP MS/MS have to be confirmed with other methods. Co-IP is the exact same method but followed by visualization via WB (which, by the way, misses the negative control free-GFP). The interactions assays of heterologous expressed proteins (wrongly named "pull-down") are far to be ideal because the proteins are not in native conditions. I suggest Y2H and/or FRT-FLIM (or BiFC, at least).
- Some relevant conclusions are based on data obtained at certain dpi, but the biological meaning of these time points is missing; i.e., where is the fungus inside the root?
- Line 300: "ΔFolSrpk1 only grew slightly slower than the WT strain". The speed of infection was not quantified! This is an over conclusion. Indeed, the images shown in Fig 5a In Fig 5a show a clear reduction of ΔFolsrpk1 growth compared to WT in PDA (without H2O2). This suggest that this mutant is affected in growth, which disagrees with the statement mentioned in line 300. Please, quantify growth and growth speed because differences in growth speed can be determinant for Fo infection.
- Line 300-301: "FolSrpk1 acetylation or not had no obvious effect on its growth". This doesn't read correct based on Fig 5a, where FolSrpk1K304R shows a different color than WT in PDA (its color suggests melanin accumulation-stress). Please, quantify and discuss.
- Include mycelial growth on PDA plates of mutants used in Fig4c-f (images and quantifications), as done in Fig 5a.
- Include mycelial growth on PDA plates of BcSrpk1 mutants to evaluate if they're impaired in growth outside the plant.

- Methods:
o Line 433: RP27 is the promoter, right? Include "p" at the end: "RP27p". Importantly, what is this promoter? In Results, the authors indicate that they generated the FolSrpk1-GFP under its native promoter.
o Indicate the number of replicates for all experiments.
o Negative control of the IP-MS/MS? In the methods it reads "GFP", driven by which promoter? Please, also include in Table S1 (as requested above).

- Discussion: rephrase some conclusions to align them with the presented data. Please, differentiate more clearly between conclusions (from shown data and published works) and hypothesis (not proved). I would also appreciate more speculation about certain conclusions and hypothesis.
o Line 341: "(4) secreted detoxifying enzymes remove plant produced H2O2 to promote fungal infection". This is only proved indirectly: secreted proteins in PDB media and lower virulence of the corresponding mutants. Please, test the secretion of these proteins in planta or rephrase.
o Lines 342-343: "inactivation of FolSrpk1 or dys-deacetylation of K304 intervenes in ROS detoxification". No data are presented regarding actual ROS detoxification but ROS concentration.
o Lines 359-360: "Srpk1 might be regulated by distinct mechanisms in different eukaryotes." Can you please hypothesize about why this could be the case?
o Line 368: "role of ROS in plant defense". ROS can also reduce fungal reduced virulence. Both options are possible and none has been checked in this work (activation of plant defence and/or reduction of fungal virulence pathways).
o Lines 380-386: "We show here that the expression... and other phytopathogenic fungi". This section is redundant as was already mentioned at the beginning of the discussion. Please, substitute by more conclusions and hypothesis that put this work in context with the current literature and suggest paths to follow with the aim of understanding the mechanism of this pathway in more detail.

- Title: it is not clear who detoxifies the ROS (plant or fungus). Based on the data, the ROS detoxification is initiated by ROS perception.

- Some sentences are incorrect, please, recheck the English.

Minor concerns that should be addressed
- Introduction and discussion: some states are imprecise and citations are missing:
o line 52 "ROS burst kills the cells". Please, explain, this is too colloquial.
o lines 65-66. Include the concrete function of SR proteins and why they need to be hyperphosphorylated to play it. Do it here or in line 74, before mentioning FgSrk1.
o Line 71: "Akt". Give the complete name of the protein and briefly explain its function.
o Line 94: "CATs and PODs". Give the complete names of the proteins, briefly explain their function. And include citations.
o Line 361: "a hemibiotrophic pathogen, beginning its infection cycle as a biotroph but later changing to a necrotroph." Please, cite some published works to support this statement.
o Line 373: "these deleterious molecules are like a barrier". This is too not precise enough, please explain.

- Results: add some information to clarify the methodology, so the reader doesn't have to constantly check the methods:
o Lines 118-119: indicate that FolSrpk1p::FolSrpk1-GFP was generated by homologous recombination (so, it is in the folsrpk1 mutant background).
o Line 121: indicate how these hyphae were grown (media, not in planta).
o Line 132: indicate which negative control was included in the IP-MS/MS
o Line 136: add information about the line used to localize FolAha1 (promoter, fluorescent protein, homologous recombination?)
o Line 140: "inactivation of FolAha1". This is not correct, the generated mutant contains a deletion of FolAha1, "inactivation" implies that the protein is present but inactive. Please, rewrite.
o ΔFolsrpk1 (ex. fig 5) or ΔFolSrpk1 (ex. line 300). Please, choose one and be consistent

Any additional non-essential suggestions for improving the study (which will be at the author's/editor's discretion)
- Recommendation for future assays: free GFP is not the correct negative control or the IP. Please, consider using another cytosolic protein fused to GFP

Referee #1:

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However, before publishing, some issues need to be addressed to consolidate the underlying mechanisms. It was shown previously, that different H2O2 concentrations/ homeostasis determine the virulence of pathogenic fungi. It is important to determine the H2O2 concentration in the different Fol and Botrytis strains before plant infection and also after plant infection. The disruption and mutations of Srpk1 might also change the in situ H2O2 concentration and virulence of the different Fol and Botrytis strains. Furthermore, the experimental background of the various experiments is pretty often imprecisely and superficially described which should be revised.
Response: Thank you very much for the very constructive suggestions! Although scientists often perform DAB staining with rice sheath to detect H_2O_2 accumulation, we could not establish this system with tomato leaves and roots to see the hyphae and plant cells clearly at the same time after every efforts. Given this situation, we only determined the H_2O_2 concentration in different *Fol* and *Botrytis cinerea* strains before (on PDA plates) (Appendix Figure S8) but not after plant infection, and discussed this possibility in the Discussion section (lines 419-425). We have also added more experimental information to the revised manuscript.

In detail:

138: Suppl Fig. 1e-f. Explain more in detail the presented experiments...give more information about used samples and the tested combinations ...one sentence that summarises the findings from a Co-IP and Pull-down experiment is too superficial...
Response: As requested, we have explained the experiments in more detail. Please note that according to the suggestions of the Reviewer #3, we have replaced pull-down with yeast two hybrid (Y2H) (Fig EV1C) and bimolecular fluorescence complementation (BiFC) (Fig EV1D) analyses in the revised manuscript (lines 138-145).

142: use "sequestering" instead of "distribution"
Response: Change has been made (line 149).

144: Fig 1 a: How was the quantitative H2O2 measurement performed? Did you directly infect the tomato roots, or did you infect the tomato leaves and subsequently measure the ROS accumulation in the roots?...briefly describe that in the results section.
Response: *Fol* is a soil-borne fungus, so we directly infected the tomato roots and

then measured the ROS accumulation in the roots. We have added these information to the revised manuscript (lines 153-156).

The Fol-wildtype strain control is missing. FolSrpk1-GFP is a potential overexpression line. It would be important to compare H2O2 release/production after FolSrpk1-GFP and Fol wildtype strain infection.

Response: We are sorry for the misunderstanding. In fact, FolSrpk1-GFP was expressed under the control of its native promoter (the FolSrpk1p::FolSrpk1-GFP strain was generated by homologous recombination). We have changed the text to make it more clear (lines 118-119). As suggested, we have also determined H_2O_2 accumulation after infection by the WT strain with the currently used method and found there was no significant difference between the WT and FolSrpk1p::FolSrpk1-GFP strains (Fig 1D, lines 153-158).

Fig1 a: Please perform 3,3'-Diaminobenzidine (DAB)-staining to visualize H2O2 accumulation in tomato plants after mock and infection.

Response: Thank you very much for the good suggestion! Unfortunately, we failed to establish the DAB staining system with tomato roots to see the hyphae and plant cells clearly at the same time after every efforts. In addition, the quantification method employed in this research is sensitive to the detection of 1 umol/L H_2O_2 and is widely used by plant pathologists (please see the following representative publications).

(1) Liu C, Ou S, Mao B, et al. (2018) Early selection of bZIP73 facilitated adaptation of japonica rice to cold climates. Nat Commun 9: 3302
(2) Wang J, Nan N, Shi L, et al. (2020) Arabidopsis BRCA1 represses RRTF1-mediated ROS production and ROS-responsive gene expression under dehydration stress. New Phytol 228: 1591-1610
(3) Wei G, Lai Y, Wang G, et al. (2017) Insect pathogenic fungus interacts with the gut microbiota to accelerate mosquito mortality. Proc Natl Acad Sci U S A 114: 5994-5999
(4) Yin Y, Wu S, Chui C, et al. (2018) The MAPK kinase BcMkk1 suppresses oxalic acid biosynthesis via impeding phosphorylation of BcRim15 by BcSch9 in Botrytis cinerea. PLoS Pathog 14: e1007285
(5) Zhang H, Liu Y, Wen F, et al. (2014) A novel rice C2H2-type zinc finger protein, ZFP36, is a key player involved in abscisic acid-induced antioxidant defence and oxidative stress tolerance in rice. J Exp Bot 65: 5795-809

Fig 1 b: Please provide the robust statistical analysis (significance test) proofing the accumulation in the nucleus at 3dpi. Shouldn't there be a significant drop of FolSrpk1 in the cytoplasm at 3dpi? ...please comment on that point.

Response: As suggested, we performed statistical analysis by one-way ANOVA and the results showed that the amount of FolSrpk1 indeed significantly increased in the nucleus and decreased in the cytoplasm at 3 dpi (Appendix Fig S3A).

Fig 1 c,e: How many biological replicates have been performed...?
Response: At least three biological replicates have been performed. We have added these information to the revised manuscript (lines 899-900).

181: rephrase: it is likely that the modification
Response: Change has been made (line 192).

Fig 2 h: How many biological replicates have been performed...? The band at 2 and 3 dpi looks weird... any explanation for that phenomenon
Response: At least three biological replicates have been performed. We have added these information to the revised manuscript (lines 928-929). We guess it was due to short-exposure of the gel and have shown a new one (Fig 2J in the revised manuscript). We have also quantified the intensity of the bands and conducted statistical analysis by one-way ANOVA (Appendix Fig S3B).

Fig3 b How many hours after treatment?
Response: It is 2 hours after H_2O_2 treatment and this information has been added (lines 224 and 937).

Fig3c What is AcCoA doing in this experiment?...there is no explanation for that
Response: Acetyl-CoA (AcCoA) is the acetyl group donor. We have added this information to the revised manuscript (lines 229-230).

225-226: describe in detail "leading to altered distribution of this protein in the cell (Fig. 3e)"...what and how is it altered?...be more precise!!!
Response: We have rewritten the sentence to make it more clear (lines 237-238).

Fig 3d-e: How many biological replicates were performed?
Response: Three biological replicates have been performed. We have added these information to the revised manuscript (lines 959-960).

244: "but few alternative splicing events were observed". --> Why is that important...Please comment!
Response: Although Srpk1 plays critical roles in pre-mRNA alternative splicing in other organisms, no such events were observed in this analysis. We have added these information to the revised manuscript (lines 256-258).

285-286: "Given the fact that FolSrpk1 nuclear translocation increases the expression level of antioxidant enzymes"...you cannot claim that because is not backed up by your findings....after Fol infection, only the expression level of antioxidant enzymes increases, but you haven't shown in detail that the specific translocation of FolSrpk1

changes the expression level of antioxidant enzymes ....Please correct that!

Response: We have changed the statement to "To test the role of FolSrpk1 deacetylation in ROS scavenging, the WT and the *FolSrpk1* mutant strains were grown on PDA plates containing 10 mM H_2O_2". (lines 303-304).

Fig 5a: Please perform DAB-stainings of these strains on PDA media to analyze whether the disruption of Srpk1 and its modifications affects the Fol in situ H2O2 accumulation !!!

Response: Thank you very much for the good suggestion! As suggested, we performed DAB staining of these strains grown on PDA media and found that FolSrpk1 mutation had little effect on *in situ* H_2O_2 accumulation (Appendix Fig S8A, lines 421-423).

Fig 5C: Please perform 3,3'-Diaminobenzidine (DAB)-staining to visualize H2O2 accumulation in Tomato plants after infection of these different Fol strains.

Response: Thank you very much for the good suggestion! Unfortunately, we failed to establish the DAB staining system with tomato roots to see the hyphae and plant cells clearly at the same time after every efforts. Therefore, we stated in the Discussion that "it will be of considerable interest to determine the H_2O_2 concentration in different *Fol* strains after plant infection by establishing a detection system in future studies" (lines 423-425). In addition, as mentioned above, the quantification method employed in this research is sensitive to the detection of 1 umol/L H_2O_2 and is widely used by plant pathologists.

Fig 5d-e: "Quantification of the disease indexes of the indicated strains" □What does that mean, provide more details. How many biological replicates were performed?

Response: This means **"**quantification of the disease indexes of the indicated strains in D (four biological replicates; n= 24)" (lines 995-996). The detailed method was described in "Materials and methods" (lines 659-670).

Fig 6 e: These images are of very poor quality. Please provide high-resolution images of these lesions in which the hyphae can be clearly distinguished from the plant cells and vice versa. Subsequently, one should be able to make out whether Botrytis or plant cells are stained.

Response: Thank you very much for the good suggestion! Unfortunately, we failed to establish the DAB staining system with tomato leaves to see the hyphae and plant cells clearly at the same time after every efforts. Therefore, we enlarged photos in panel E to clearly show changed H_2O_2 accumulation in tomato leaves after infection by different *Botrytis* strains. We also performed DAB staining of these strains grown on PDA media and found that BcSrpk1 mutation had little effect on *in situ* H_2O_2 accumulation (Appendix Fig S8B, lines 421-423). Moreover, we stated in the Discussion that "it will be of considerable interest to determine the H_2O_2 concentration in different *B. cinerea* strains after plant infection by establishing a detection system in future studies" (lines 423-425).

Fig1-4, 6 and Supplementary figure 1, 3, 4, 6: How often did you repeat the blots? How many biological replicates with similar results were performed?
Response: Each gel shown is a representative experiment carried out at least three times. We have added these information to the figure legend of the revised manuscript (lines 899-900, 928-929, 959-960, 969-970, 1023-1024, 1051-1052, 1059-1060, 1066, 1105-1106; the last two lines in page 2 of Appendix).

Referee #2:

EMBO Review
Srpk1 deacetylation initiates ROS detoxification in plant fungal infection
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The manuscript describes experiments that are aimed at identifying the mechanism by which pathogens are able to counteract the effect of production of reactive oxygen species (ROS), that are deployed by the plant immune system. Pathogens contend with ROS release by a variety of mechanisms, including the production of antioxidant proteins and enzymes. However, the mechanism of such counteracting processes by pathogens remain largely unknown. The authors propose that deacetylation of the Fusarium oxysporum f. sp. lycopersici kinase Srpk1 is involved in counteracting ROS release during plant infection. They provide evidence that the deacetylation controls the disassociation of Srpk1 from a cytoplasmic protein, Aha1, which enables nuclear translocation of the kinase. Nuclear localisation of the kinase is then proposed to act upon Sr1 which is able to enhance transcription of different antioxidant enzymes. These enzymes are responsible for the removal of ROS thereby facilitating successful infection. This is an interesting paper which proposes a novel counteracting process for ROS removal. However, I have some concerns about the manner in which the work has been carried out which should be addressed by the authors.

1. The paper relies predominantly on western blot analysis using a variety of antibodies. Whilst in their own right these data are convincing, I am worried that this is the only line of evidence that was produced in support of any of the conclusions made. The phosphorylation status Srpk1 (1) for instance and its acetylation status are based primarily on the use of antibodies. Biochemical analysis in particular, phosphoproteomic analysis would add much to this study and provide much more convincing evidence overall for the conclusions being made. In particular, the use of the anti-K304ac antibody (2) is the only evidence shown regarding acetylation of Srpk1. For such an important major conclusion associated with the acetylation status of this kinase there really should be at least another form of evidence in support of such an assertion.
Response: Our LC-MS/MS analysis identified K304 acetylation of FolSrpk1 (Supplementary Fig. 3c), and we then developed an antibody that specifically

recognized the WT but not the K304 mutant FolSrpk1 proteins (Supplementary Fig. 3d). Site-specific acetyl lysine antibodies are widely used in the study of acetylation status by scientists from diverse areas (please see the following representative publications). Based on these observations, we believe that the assertion of FolSrpk1 K304 acetylation is convincing. We have moved these two panels to the main figure (Fig 2A and B) in the revised manuscript to highlight the conclusion.
There is no phosphorylation status of FolSrpk1 in this research, and I guess that the reviewer is talking about FolSr1 phosphorylation. Accordingly, we carried out Western blot analysis using anti-Ser/Phosphoserine antibody to prove hyperphosphorylation of FolSr1 during plant infection (Fig EV5I). Given the lack of LC-MS/MS evidence of FolSr1 phosphorylation, we toned down the corresponding statement by adding the word "possibly" (lines 101, 297, 362 and 1035). We also clearly highlighted this limitation in the Discussion section with the statement "In addition, the conclusion of FolSr1 phosphorylation is based only on Western blot analysis, and further LC-MS/MS evidence will greatly strengthen the corresponding statement." (lines 425-427).

(1) Lin R, Tao R, Gao X, et al. (2013) Acetylation stabilizes ATP-citrate lyase to promote lipid biosynthesis and tumor growth. Mol Cell 51(4): 506-518
(2) North BJ, Rosenberg MA, Jeganathan KB, et al. (2014) SIRT2 induces the checkpoint kinase BubR1 to increase lifespan. EMBO J 33: 1438-53
(3) Choi SJ, Lee HC, Kim JH, et al. (2016) HDAC6 regulates cellular viral RNA sensing by deacetylation of RIG-I. EMBO J 35: 429-42
(4) He C, Danes JM, Hart PC, et al. (2019) SOD2 acetylation on lysine 68 promotes stem cell reprogramming in breast cancer. Proc Natl Acad Sci U S A 116(47): 23534-23541
(5) Garcia Morato J, Hans F, von Zweydorf F, et al. (2022) Sirtuin-1 sensitive lysine-136 acetylation drives phase separation and pathological aggregation of TDP-43. Nat Commun 13, 1223
(6) Saunders HA J, Johnson-Schlitz DM , Jenkins BV, et al. (2022) Acetylated α-tubulin K394 regulates microtubule stability to shape the growth of axon terminals. Currt Biol 32(3): 614-630

2. For the western blots shown in Fig. 1 there are loading controls missing for panels f and g. This is important because it is still not clear that the proteins are evenly loaded in spite of the way in which the blots are being presented. I think that if the original Coomassie blue stained images could be provided (or shown in supplement) this would also help to support the conclusions made for all of the figures because loading controls are sometimes, but not always, used for many of the subsequent panels.
Response: As suggested, we have added the original Coomassie brilliant blue (CBB) stained gels as loading controls to all Western blot analysis including these two panels (Fig EV2 in the revised manuscript)**.**

3. In Fig. 1c the images showing potential nucleal localisation of Srpk1 and the effect

of putative deacetylation really require larger images. This is not clear from these images without extensive magnification. Arrows could also be added to highlight the nuclei more clearly.

Response: As suggested, we have provided larger images that clearly show the distribution of the WT and mutant FolSrpk1 proteins. Arrows were also added to indicate the nucleus (Fig 2F in the revised manuscript).

4. In Fig. 3 the putative acetylation at K304 of Srpk1 is analysed purely on the basis of the use of the anti-K304ac antibody. I strongly believe that a second line of evidence is necessary for such an important proposition.

Response: Our LC-MS/MS analysis identified K304 acetylation of FolSrpk1 (Supplementary Fig. 3c), and we then developed an antibody that specifically recognized the WT but not the K304 mutant FolSrpk1 proteins (Supplementary Fig. 3d). As mentioned above, site-specific acetyl lysine antibodies are widely used in the study of acetylation status by scientists from diverse areas. Based on these observations, we believe that the assertion of FolSrpk1 K304 acetylation is convincing. We have moved these two panels to the main figure (Fig 2A and B) in the revised manuscript to highlight the conclusion.

5. In Fig. 4 the role of K304 acetylation is tested on the transcription of antioxidant enzymes, this analysis would again benefit from enlarging panel d to show the phenotypic effect much more clearly. The data shown in panels e and f would be better presented as box and whisker plots with the original data points presented rather than bar charts. the spread of the data is not shown accurately in the current presentation and we cannot evalute variation between replicates.

Response: As suggested, we have enlarged panel d and replaced the column diagram with box and whisker plots in panels e and f (Fig 4D-F in the revised manuscript).

6. Fig. 5 panel d is again too small to show the phenotypic effect on plants and plant disease effectively and panels e and f should show the original data points and be presented as box and whisker plots. The same is also true of Fig. 5b.

Response: As suggested, we have enlarged panel d and replaced the column diagram with box and whisker plots in panels b, e and f (Fig 5B, D, E and F in the revised manuscript).

7. In Fig. 6 there is no loading control in panels a and d. Scale bars are needed for panel e and the data shown as bar charts in panels e and f would be better presented with the original data points and replications of the experiment shown.

Response: As suggested, we have added the original CBB stained gels as loading controls to panels a and d, and scale bar to panel e (Fig 6A, D and E in the revised manuscript)**.** We have also replaced the column diagram with box and whisker plots in panels f and g (Fig 6F and H in the revised manuscript).

8. In Fig. 7 the proposed model is still based entirely on a single form of evidence.

The hyperphosphorylation of Sr1, for instance, is a speculation with no evidence in support.

Response: We detected phosphorylation levels of FolSr1 during plant infection using anti-Ser/Phosphoserine antibody (Fig EV5I in the revised manuscript) and the results showed that FolSr1 was indeed hyperphosphorylated during the early infection stage (lines 295-296). Given the lack of LC-MS/MS evidence of FolSr1 phosphorylation, we toned down the corresponding statement by adding the word "possibly" (lines 101, 297, 362 and 1035). We also clearly highlighted this limitation in the Discussion section with the statement "In addition, the conclusion of FolSr1 phosphorylation is based only on Western blot analysis, and further LC-MS/MS evidence will greatly strengthen the corresponding statement." (lines 425-427).

9. Supplementary Fig. 3 showing the validation of the K304ac antibody should be shown as a panel in one of the main figures since it is the only evidence shown in support of the specificity of the antibody.

Response: As requested, we have moved panel d to the main figure (Fig 2B in the revised manuscript).

In summary, this is an interesting paper that provides evidence for a new and potentially novel mechanism of ROS removal by pathogens during infection as such it has considerable merit. The experiments carried out appear to have been performed carefully and the data presentation is of a high standard overall in spite of some of the concerns raised above. The most significant limitation in this study, however, is the absence of other forms of independent analysis to support the main conclusions regarding deacetylation of phosphorylation status. If these can be added and further evidence provided, or if the authors are able to highlight more clearly the current limitations of the study and modify their conclusions accordingly, then this would improve the manuscript very considerably.

Response: Thank you very much for the very constructive suggestions! As mentioned above, we kept the statement of K304 acetylation of FolSrpk1 and performed Western blot analysis to prove hyperphosphorylation of FolSr1 during the early infection stage (Fig EV5I in the revised manuscript). Given the lack of LC-MS/MS evidence of FolSr1 phosphorylation, we toned down the corresponding statement by adding the word "possibly" (lines 101, 297, 362 and 1035). We also clearly highlighted this limitation in the Discussion section with the statement "In addition, the conclusion of FolSr1 phosphorylation is based only on Western blot analysis, and further LC-MS/MS evidence will greatly strengthen the corresponding statement." (lines 425-427).

Referee #3:

In this work, Zhang et al explore one essential process in the plant-pathogen interaction which is the capacity of the pathogen to reduce the level of ROS to infect

the host. Using diverse and complementary methodologies, they show fascinating and very promising results suggesting that the acetylation level of Serine/arginine protein kinases determines their cellular localization. Once these proteins translocate to the nucleus, it activates a downstream cascade leading to the expression of antioxidant enzymes that actively participate in fungal virulence. The work needs to indicate replicates, controls, and statistics, and a few more experiments are required to sustain the exciting conclusions.

Specific major concerns essential to be addressed to support the conclusions

- FolSrpk1 localization is a very relevant piece of information because it determines the activity of the protein. Unfortunately, the images have an extremely low magnification and resolution. Please, improve them. Regarding the associated WB (1Sc), please, include the controls of total protein loaded for each line. I also recommend to move Fig S1B and C to Main figure.

Response: Thank you very much for the very constructive suggestions! As requested, we have provided larger images that clearly show the distribution of the WT and mutant FolSrpk1 proteins (Fig 1B, G and Fig 2F in the revised manuscript), and added the original Coomassie brilliant blue (CBB) stained gels as loading controls in Supplementary Fig 1c (Fig 1C in the revised manuscript). We have also move Fig S1B and C to the main figure (Fig 1B and C in the revised manuscript).

- Microscopy images: not enough magnification and resolution. Replicates and quantifications missing: quantify distribution of the protein in nucleus/total in microscopy images. Include at least 3 replicates, with at least 3 hyphae/replicate and at least 3 cells/hyphae.

Response: As requested, we have provided new images with enough magnification and resolution for all the microscopy analyses. We have also quantified the distribution of FolSrpk1 in the microscopy images (Fig 1G, H and Fig 2F, G in the revised manuscript) based on three independent replicates with three hyphae per replicate and three cells per hyphae (lines 890-895, 912-917).

- Missing the loading controls in most WB. Missing information of replicates in all WB.

Response: As requested, we have added the original CBB stained gels as loading controls for all the WB analyses (Fig 1C, E, I; Fig 2H; Fig 3B, E; Fig 4B; Fig 6A, D; Fig EV1F; Fig EV2; Appendix Fig S2 in the revised manuscript) and indicated the information of replicates in the legends (lines 899-900, 928-929, 959-960, 969-970, 1023-1024, 1051-1052, 1059-1060, 1066, 1105-1106; the last two lines in page 2 of Appendix).

- Table S1 is incomplete. It has to include all replicates (4 is the minimum) and statistics of both FolSrpk1-GFP and the negative control GFP. Additional information like LFQ intensities are highly recommended.

Response: As suggested, four replicates including the negative control GFP were

presented in Table S1. LFQ intensities and statistics analysis were also included.

- The potential FolSrpk1 interactors identified by co-IP MS/MS have to be confirmed with other methods. Co-IP is the exact same method but followed by visualization via WB (which, by the way, misses the negative control free-GFP). The interactions assays of heterologous expressed proteins (wrongly named "pull-down") are far to be ideal because the proteins are not in native conditions. I suggest Y2H and/or FRT-FLIM (or BiFC, at least).

Response: As suggested, we removed the pull-down assay and conducted Y2H and BiFC assays to confirm potential FolSrpk1 interactors (Fig 2D, E; Fig EV1C, D; Fig EV3E, F; Fig EV5E, F). We kept Co-IP analyses because this approach has also been used to show the interaction strength of different forms of FolSrpk1 with FolAha1 under normal or oxidative stress conditions. Please note that negative control free-GFP was included in the Co-IP analyses to show the association of FolSrpk1 with FolAha1, FolArd1, FolSir2 and FolSr1 (Fig EV1B; Fig EV3C and D; Fig EV5D).

- Some relevant conclusions are based on data obtained at certain dpi, but the biological meaning of these time points is missing; i.e., where is the fungus inside the root?

Response: We are very sorry for the unclearness. According to the previous publications, *Fol* spores germinate at the initial application sites at 12 hai, after which hyphae spread along the main root and mycelium formed, which densely coated the entire root at 2 dpi. At 3 dpi, hyphae crossed the epidermis, penetrated into the cortex and progressed through parenchymal cell borders reaching the xylem vessels at 7 dpi. To make it more clear, we changed the sentence to "……measured ROS accumulation in the roots during the *Fol* colonization stage" and cited the corresponding literatures in the revised manuscript (lines 154-156).

- Line 300: "ΔFolSrpk1 only grew slightly slower than the WT strain". The speed of infection was not quantified! This is an over conclusion. Indeed, the images shown in Fig 5a In Fig 5a show a clear reduction of ΔFolsrpk1 growth compared to WT in PDA (without H2O2). This suggest that this mutant is affected in growth, which disagrees with the statement mentioned in line 300. Please, quantify growth and growth speed because differences in growth speed can be determinant for Fo infection.

Response: We measured the colony diameter of these strains on PDA plates 12 h-72 h after cultivation and found that ΔFolSrpk1 grew slower than the WT strain (Appendix Fig S6A). Therefore, we have changed the statement to "Although ΔFolSrpk1 grew slower than the WT strain" (lines 315-316).

- Line 300-301: "FolSrpk1 acetylation or not had no obvious effect on its growth". This doesn't read correct based on Fig 5a, where FolSrpk1K304R shows a different color than WT in PDA (its color suggests melanin accumulation-stress). Please, quantify and discuss.

Response: We quantified melanin accumulation using a previously described method

and found that there was no difference among the tested strains (Appendix Fig S6B, lines 317-318). We guess it is due to the color of the background but not the mycelia and we have shown a new photo of FolSrpk1^{K304R} (Fig 5A).

- Include mycelial growth on PDA plates of mutants used in Fig4c-f (images and quantifications), as done in Fig 5a.

Response: As requested, we have shown the images and quantifications of these strains grown on PDA plates. The results indicate that deletion of these three genes has no obvious effect on *Fol* mycelial growth on PDA plates (Appendix Fig S5, lines 275-276).

- Include mycelial growth on PDA plates of BcSrpk1 mutants to evaluate if they're impaired in growth outside the plant.

Response: As requested, we have shown the images and quantifications of these strains grown on PDA plates, and found that although ΔBcSrpk1 grew slower than B05.10, K367 acetylation or not had little effect on its growth (Appendix Fig S7C, lines 343-344).

- Methods:

o Line 433: RP27 is the promoter, right? Include "p" at the end: "RP27p". Importantly, what is this promoter? In Results, the authors indicate that they generated the FolSrpk1-GFP under its native promoter.

Response: We have added “p” to the end of “RP27”, a constitutive promoter for over-expression. In fact, the overexpression strain RP27p::FolSrpk1-GFP was used for LC-MS/MS, and FolSrpk1-GFP driven by its native promoter (FolSrpk1p::FolSrpk1-GFP) was used for subcellular localization and phenotypic analyses. We have added these information to the revised manuscript (lines 118-119, 130-131, 464, 470, 479-485).

o Indicate the number of replicates for all experiments.

Response: We have indicated the number of replicates for all the experiments in the figure legends.

o Negative control of the IP-MS/MS? In the methods it reads "GFP", driven by which promoter? Please, also include in Table S1 (as requested above).

Response: The negative control was RP27p:: GFP. We have added this information to the revised manuscript (lines 132-133) and included it in Table EV1.

- Discussion: rephrase some conclusions to align them with the presented data. Please, differentiate more clearly between conclusions (from shown data and published works) and hypothesis (not proved). I would also appreciate more speculation about certain conclusions and hypothesis.

o Line 341: "(4) secreted detoxifying enzymes remove plant produced H2O2 to promote fungal infection". This is only proved indirectly: secreted proteins in PDB

media and lower virulence of the corresponding mutants. Please, test the secretion of these proteins in planta or rephrase.
Response: We have changed the sentence to “the detoxifying enzymes remove plant-produced H_2O_2 to promote fungal infection” (lines 363-364).

o Lines 342-343: "inactivation of FolSrpk1 or dys-deacetylation of K304 intervenes in ROS detoxification". No data are presented regarding actual ROS detoxification but ROS concentration.
Response: We have changed the sentence to “deletion of *FolSrpk1* or dysdeacetylation of K304 leads to higher ROS accumulation in infected roots and impaired virulence of *Fol*” (lines 364-366).

o Lines 359-360: "Srpk1 might be regulated by distinct mechanisms in different eukaryotes." Can you please hypothesize about why this could be the case?
Response: We guess it is due to evolution and have changed the sentence to “Therefore, nuclear translocation of Srpk1 might be regulated by distinct mechanisms in evolutionary distant species” (lines 381-383).

o Line 368: "role of ROS in plant defense". ROS can also reduce fungal reduced virulence. Both options are possible and none has been checked in this work (activation of plant defence and/or reduction of fungal virulence pathways).
Response: Thank you very much for the good suggestion! We have changed the sentence to “……the role of ROS in the activation of plant defense and/or the attenuation of fungal virulence pathways of *Fol*” (lines 392-393).

o Lines 380-386: "We show here that the expression... and other phytopathogenic fungi". This section is redundant as was already mentioned at the beginning of the discussion. Please, substitute by more conclusions and hypothesis that put this work in context with the current literature and suggest paths to follow with the aim of understanding the mechanism of this pathway in more detail.
Response: As suggested, we have rewritten this part (405-414).

- Title: it is not clear who detoxifies the ROS (plant or fungus). Based on the data, the ROS detoxification is initiated by ROS perception.
Response: To make it more clear, we have changed the title to “Srpk1 deacetylation activates fungal detoxification of plant-derived ROS during infection”.

- Some sentences are incorrect, please, recheck the English.
Response: We have carefully checked the English and made all the necessary changes.

Minor concerns that should be addressed
- Introduction and discussion: some states are imprecise and citations are missing:
o line 52 "ROS burst kills the cells". Please, explain, this is too colloquial.

Response: We have changed the sentence to “ROS burst kills the cells mainly through damaging DNA, protein and cell membrane……” (lines 52-53).

o lines 65-66. Include the concrete function of SR proteins and why they need to be hyperphosphorylated to play it. Do it here or in line 74, before mentioning FgSrk1.
Response: Hyperphosphorylated SR proteins are recruited to the transcription and splicing machineries to regulate gene expression and pre-mRNA splicing. We have added these information to the revised manuscript (lines 79-81).

o Line 71: "Akt". Give the complete name of the protein and briefly explain its function.
Response: We searched literatures but could not find the complete name of Akt. Akt, commonly known as protein kinase B (PKB), is a serine/threonine protein kinase that plays key roles in regulating cell survival, insulin signaling, angiogenesis and tumor formation. We have added these information to the revised manuscript (lines 74-76).

o Line 94: "CATs and PODs". Give the complete names of the proteins, briefly explain their function. And include citations.
Response: Please note that the complete names of CATs and PODs and their functions have been presented in the first paragraph of this section with citations (lines 57-65).

o Line 361: "a hemibiotrophic pathogen, beginning its infection cycle as a biotroph but later changing to a necrotroph." Please, cite some published works to support this statement.
Response: Citations have been added (lines 385-386).

o Line 373: "these deleterious molecules are like a barrier". This is too not precise enough, please explain.
Response: The function of ROS has been comprehensively described in the Introduction (lines 50-57). We have changed the statement to “……indicate that essentially all plant pathogens need to overcome these deleterious molecules to accomplish their invasion” (lines 398-399).

- Results: add some information to clarify the methodology, so the reader doesn't have to constantly check the methods:
o Lines 118-119: indicate that FolSrpk1p::FolSrpk1-GFP was generated by homologous recombination (so, it is in the folsrpk1 mutant background).
Response: Change has been made accordingly (lines 118-119).

o Line 121: indicate how these hyphae were grown (media, not in planta).
Response: These hyphae were grown in YPD medium (line 121).

o Line 132: indicate which negative control was included in the IP-MS/MS

Response: The RP27p::GFP transformant served as the negative control in this analysis (lines 132-133).

o Line 136: add information about the line used to localize FolAha1 (promoter, fluorescent protein, homologous recombination?)

Response: FolAha1p::FolAha1-GFP strain generated by homologous recombination was used in this analysis (lines 136-137).

o Line 140: "inactivation of FolAha1". This is not correct, the generated mutant contains a deletion of FolAha1, "inactivation" implies that the protein is present but inactive. Please, rewrite.

Response: As suggested, “inactivation of FolAha1” has been changed to “deletion of *FolAha1*” (lines 147-148).

o ΔFolsrpk1 (ex. fig 5) or ΔFolSrpk1 (ex. line 300). Please, choose one and be consistent

Response: We used ΔFolSrpk1 throughout the manuscript.

Any additional non-essential suggestions for improving the study (which will be at the author's/editor's discretion)

- Recommendation for future assays: free GFP is not the correct negative control or the IP. Please, consider using another cytosolic protein fused to GFP

Response: Thank you very much for the constructive suggestions! We will include another cytosolic protein fused to GFP as the negative control in future studies.

Thank you for submitting a revised version of your manuscript. Your study has now been seen by two of the original referees, who find that most of their previous concerns have been addressed and now recommend publication of the manuscript. There remain only a few editorial points that have to be addressed before I can extend formal acceptance of the manuscript.

Please let me know if you have any questions regarding any of these points. You can use the link below to upload the revised files.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving the final version.

Referee #1:

Authors appropriately addressed all comments including additional experimental details and statistical analysis. Although authors could not measure/visualize H2O2 accumulation in plants after infection, I now understand technical difficulties in tomato plant. Overall conclusion would be acceptable without direct measurement of H2O2 accumulation in plants after infection.

Referee #2:

I am happy with the changes made by the authors. I am still a little concerned about some of the loading controls, as they do not all show even loading. However, the important thing is that this is now open to all readers to see and make their own evaluation. Transparency is the most important thing. My other concerns have mainly been addressed and I am grateful to the authors for their work in revising the paper. I support publication based on the revisions made.

The authors addressed the remaining editorial issues.

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

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The data shown in figures should satisfy the following conditions:
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- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
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- an explicit mention of the biological and chemical entity(ies) that are being measured.
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- 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.
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  - 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?
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  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	Data Availability
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	