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UV-B photoreceptor UVR8 interacts with MYB73/MYB77 to regulate auxin responses and lateral root development

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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14th May 2019

Thank you for submitting your manuscript for consideration by the EMBO Journal. I sincerely apologise for the unusual delay in the assessment of your work due to belated submission of referee reports. We have now received the full set of reviewers' reports on your manuscript, which are included below for your information.

As you will see from the comments, the reviewers appreciate the work and the topic. However, they also raise a number of concerns that need to be addressed before they can support publication here. Based on the overall interest expressed in the reports, I would like to invite you to submit a revised version of your manuscript in which you address the comments of all reviewers.

REFEREE REPORTS:

Referee #1:

The manuscript investigates the effect of UV-B light on plant root development, a topic that has received little attention to date. Whilst the authors demonstrate a clear effect of UV-B on root growth and development when plants are grown in agar, I am left wondering what was the relevance of this signal to roots growing in soil? The authors flag in the introduction the concept that light may be 'channelled' down to the roots, but has this been shown for UV-B? As a plant stress signal, UV-B may instead impact aerial plant growth and/or production of key hormones like auxin in shoots that are mobilised to roots to promote root branching.

The authors initially demonstrate UV-B negatively impacts Arabidopsis root growth and branching. NOTE: I was puzzled why they buried this important result in the Supplementary Figures?. The authors focus on the impact of UV-B on lateral root length, yet there also appears to be a clear effect of UV-B on primary root growth (see Fig S1A). Given this focus, it is not clear whether they checked if the number and density of LR primordia are also affected? This is important as these represent parameters dependent on the auxin response machinery which the authors show is

impacted by UV-B using the DR5 reporter and arf7, arf19 mutants.

How UV-B impacts auxin response is addressed in data presented in Fig. 1. The authors initially demonstrate that seedlings exposed to UV-B and white light (versus just white light) exhibited reduced inhibition to the auxin IAA supplemented in growth media. NOTE: can the authors rule out that reduced inhibition of root growth in all lines shown in Fig. 1A (when grown in white light + UV-B) is not due to UV-B accelerating the breakdown of this labile form of auxin?
This bioassay revealed UV-B modulates seedling root auxin responses via the UVR8 receptor.
NOTE: this first results section was particularly difficult to read, jumping between different bioassays ({plus minus}auxin, {plus minus}direct light), organs (root versus hypocotyl) and chemical treatments, making it hard for readers to follow the logic thread. I recommend a complete re-write!

How UVR8 blocks auxin response and inhibition of lateral root length was examined using elegant transgenic tools featuring steroid inducible constitutively active and inactive UV-B photo-receptor forms. This revealed that monomeric nuclear localised UVR8 is a key signalling component repressing auxin response. Next, using grafting of WT and uvr8 scions and roots, the authors demonstrated UVR8 must act directly in roots to mediate the effect of UV-B (despite roots not being directly exposed to UV-B - see Fig S3E - a result which I found quite puzzling to explain unless invoking a mechanism such as UV-B channelling from shoots to roots....)

The authors note the overlap between genes whose expression was altered following auxin and UV-B treatments. To uncover the connection(s) between UVR8 and auxin response machinery, a Y2H screen was performed, identifying MYB73/77. Intriguingly, MYB73 interacted with UVR8 in a UV-B dependent manner in both yeast and plant cells using several in vivo and in vitro methods. Given the dependence of UV-B for this interaction, I was surprised to read in the methods that UV-B treatment was not employed during the initial Y2H screen. Can the authors please clarify?
Additional analysis revealed a preference for the N terminus of MYB73/77 interacting with the N and C termini of the monomeric form of UVR8.
However, the mechanism for how UV-B promotes this interaction remains unclear.

Rather than address the latter question, the authors focused on characterising the genetic relationship between UVR8 and MYB73/77 using double and triple mutant combinations. They concluded that the latter transcription factors acted 'downstream' of UVR8 but, given they directly interact, this conclusion is debatable as they act at a common step of the UV signalling cascade, co-regulating targets like auxin responsive genes. Indeed, the last results section reports UVR8 functions by blocking MYB73/77 DNA binding activity.

In summary, this represents a promising manuscript, but several key questions remain to be addressed as detailed above.

Referee #2:

Yang et al. study how the UV-B photoreceptor UVR8 inhibits lateral root growth in Arabidopsis. They identified the MYB73 and MYB77 transcription factors as novel UVR8 interacting proteins. UV-B-dependent interaction of UVR8 with MYB73 and MYB77 interferes with their capacity to interact with target DNA, thus inhibiting their activity. As MYB73 and MYB77 affect lateral root growth, UVR8 is able to negatively regulate lateral root growth. The authors provide evidence, including grafting assays, that the function of UVR8 in regulating root development is tissue-autonomous. Although the physiological relevance of lateral root growth inhibition by UV-B stays unfortunately rather unclear, interesting findings are provided towards further understanding on how UVR8 may regulate gene expression and, in particular, represses auxin responses.

1.) Lines 226: "... expression was up-regulated in uvr8 mutant compared with WT but downregulated in rup1 rup2 mutants with UV-B treatment in both seedlings (Fig 4E) and roots (Fig 4G)" - this is a rather confusing way of describing what happens. Indeed, UV-B results in repression of the tested target genes in WT and stronger repression in rup1 rup2. However, uvr8 simply does not show repression of the target genes in response to UV-B, they are not "up-regulated" compared

to WT, but they are simply not repressed under UV-B. Please rephrase.
I am also confused by what is actually shown in the figures 4D&E as "relative expression". Fig. 4D: It seems that relative expression is shown for WT = 1 for IAA19, IAA5, SAUR63 and GH3.2, but then WT expression is different than 1 for SAUR20 and SAUR23 in the same graph. In Fig. 4E, GH3.2 WT is different than 1, but in this case SAUR20 is put to 1. Please correct and clarify.

2.) Line 159-161: The authors performed a careful control supporting that UV-B does not simply affect the stability of the exogenously applied NAA itself, at least under the conditions used. However, as is presently written, it will likely not be clear for the readers why this control has been performed. The authors should mention the potential and documented problem of photodestruction of auxins by UV-B to make clear why the effect of UV-B on NAA stability was tested.
Also, line 160: "... did not affect NAA stability,..." instead of "... did not affect the degradation of NAA,...".

3.) Fig 2A-C legend: "uvr8 mutant is hypersensitive to auxin, and rup1 rup2 mutant is insensitive to auxin" - this is misleading. What is shown is that UV-B inhibits the response of Arabidopsis seedlings to exogenously added NAA (please make clear in the Figure title as well that NAA is added). The data show that this is UVR8 dependent (i.e. the response to NAA is not repressed in uvr8, but this does not mean that uvr8 is hypersensitive to NAA). Please check wording thoughout the text. Such misleading statements make it sometimes difficult to read the manuscript and follow the argumentation.

4.) Figure 7F-H: It is not clear how the ChIP data are presented here. The y-axis and figure legend say "IP/Input" ratios. But then in the graphs, for example, F&H, all negative WT controls are = 1 (?). This cannot be true: as much IP signal as input signal in the WT negative control? This should be the background control. Also it should not be the same for all tested promoters. It seems to me that only Figure 7E shows "IP/Input" data as suggested by the y-axes, but not Figures 7F-I. Same problem also for Figures EV8E and G.

5.) Line 185: "... GR-UVR8R338A (Qian et al., 2016) (the constitutively monomeric UVR8 mutant form that is active regardless of their subcellular localization and UV-B light conditions)..." - although UVR8R338A is constitutively monomeric, it is not constitutively active (see e.g. data in the cited Qian et al., 2016). Also, the UV-B induced activity of the GR-UVR8 fusions requires Dex treatment (i.e. not independent of subcellular localization). Please correct.

6.) Lines 348-350: "Shoot and root need to coordinate so that a plant could grow well, when the growth of shoot is repressed by UV-B, the growth of root is also inhibited, so as to save the energy." - it is not clear to me why plants need to save energy under conditions where they are exposed to sunlight (high UV-B would also mean high PAR).

Minor points:

1.) Line 37: I assume it should mean "... demonstrates that UV-B antagonistically regulates auxin-induced gene expression", or similar.
2.) Line 83: "In those two stories, ..." - not clear what is meant by two stories, please rephrase.
3.) Line 236: BiLC should be "Bimolecular Luminescence Complementation" (not Bimolecular Fluorescence Complementation)
4.) Lines 216-217: "... repressed by UV-B in a UVR8-dependent manner and the repression was abolished in uvr8 mutant..." - seems to state 2-times the same.
5.) Figure 8: not clear what the smileys should indicate. It may be better to remove/reduce the arrows between "auxin response" and the seedling on the "+ UV-B" side.

Referee #3:

This interesting article examines the control of root development by UV light, reporting that UV-B light impaired formation of lateral roots. The study identifies an important role for the transcription factor UVR8 in controlling this process. They also provide evidence that the effect of UV-B light is to reduce the positive effect of auxin, while revealing large groups of transcripts that show opposite

regulation by these two stimuli. They report that UVR8 interacts with two MYB transcription factors and show that this protein complex is needed for the developmental effects of UV-B light.
Major concerns:
This article uses appropriate methods and reveals an important role of UV light in controlling root development. There are a number of aspects of the work that need substantial edits to make the data set and the writing clearer. In particular, the case for the effect of UV-B in antagonizing auxin response is impaired as they don't ever provide a side by side comparison of growth with and without auxin and UV-B in the same figure. To show that UV-B modulates the auxin response (or vice versa) the effect of both treatments needs to be evident. So, they need to rearrange their data to make this point more clearly. Second the writing, particularly of the discussion, needs substantial editorial input. The communication is not clear nor is there an adequate synthesis of the science and integration into the framework of light and auxin effects on root development.
Detailed major and minor concerns:
1. The authors use the synthetic auxin NAA through out this work. They need to discuss why they use this instead of endogenous auxin, IAA. It is the case that the photostability of this synthetic auxin is higher than of IAA, which suggests it may be a better choice, but that needs to be explained.
2. The writing is not clear in many places, especially the discussion. Here are a few examples of places where the writing needs improvement. There are many more not listed here:
a. Line 83-the authors refer to two publications as "two stories".
b. Line 89 and 91 the same sentences is repeated twice with another sentence in between.
c. In many sections the text needs to be divided into additional paragraphs. One example is line 135.
3. The authors say in line 123 that UVB affects hypocotyl length, but they don't show that data or cite a publication to this effect. One or the other is needed.
4. The authors say that DR5-GUS tells one about "auxin activity" (line 145). They should be more precise here and other places indicating that this reporter tells about auxin induced gene expression
5. The DR5-GUS data in Figure 1 suggests that there is a difference in response to UV-B light. Yet, they show the root tip. As most of the paper focuses on the effect of this light treatment on lateral root development, they really should show auxin-responsive gene expression changes in lateral root primordia.
6. They discuss a yeast two hybrid screen to identify interactors with UVR8, but they don't report evidence from this screen. A complete summary of what they found and why they selected these two MYBs for further analysis would be a helpful addition.
7. The experiments examining the effects of UV-B light on root development of shaded roots in grafted seedlings is an excellent addition.
8. In many of the figures throughout the paper the colors of the bars with the two treatments are both very dark, making them impossible to resolve in black and white printouts.
9. Figure 1D reports the number of lateral roots at multiple stages. This is just reported for UV-B treated roots but needs to include data without UV-B as well.
10. Figure 2 is the first of multiple figures where the effect of UV-B is shown in the presence of auxin. The authors need to have comparison images and data for UV-B in the absence of auxin, so that the interactions of UV-B and auxin can be clarified.
11. The labels of the panels in Figure 3-C are not obvious. If they could add a functional label: inactive dimer and constitutively active monomer, that would make the images and the quantified data in B and C more intuitive.
12. The grafting experiment in Figure 3D, which is very important. The surprising thing is that the length of lateral roots not the number is quantified.
13. Figure 4- Panel A has data published by others and that should be clarified in the figure legend. Panel B needs error bars, Panel C, needs to make it clear that this was time after NAA addition in the x axis label. For panels D-G, each graph has a different y-axis maximum, making the comparison between graphs difficult. Those should be regraphed with consistent axes. Also, a more correct Y-axis label is relative transcript abundance (not relative expression), as qRT-PCR reports the steady state levels of transcripts, not their expression level.
14. Figure 5-These blots are important but are the least clean data in the manuscript. The authors do not fully discuss the very weak signaling in the UVR8-IP in figure A and B and the very messy bands in the same IPs in panels D-F. This experiment needs to be completed to make a more convincing data set.
15. Figure 6 needs the no auxin controls added and to have the data in panels D and E reported with similar y axis labels as in 12.
16. Figure 7. This is one of the clearest and well done EMSAs that I have ever seen. This is very nice data. I would suggest rather than using probe #, to just note the name of the gene from which

the promoter fragment is derived.
17. References. Some of these are not really the optimal reference. For example, Cheng et 2008 (line 92) is not an appropriate broad reference for auxin signaling as it just reports auxin effects in flower development, which is minimally studied. There are many great reviews available to provide a framework of auxin signaling.
18. The authors should provide a supplemental excel file of all the genes that they identified in their comparison of published microarray data.

1st Revision - authors' response 5th Jul 2019

Point by point response to Reviewer comments:

Referee #1:
The manuscript investigates the effect of UV-B light on plant root development, a topic that has received little attention to date. Whilst the authors demonstrate a clear effect of UV-B on root growth and development when plants are grown in agar, I am left wondering what was the relevance of this signal to roots growing in soil?

We checked the root phenotype in agar and also in soil. Line 135 "WT, uvr8 and rup1 rup2 ***grown in soil*** *were also treated with or without UV-B, UV-B treatment also inhibited the elongation of lateral roots, and the lateral root length of WT was longer than rup1rup2 but shorter than uvr8 only with UV-B treatment" (Fig EV1A).*

The authors flag in the introduction the concept that light may be 'channelled' down to the roots, but has this been shown for UV-B? As a plant stress signal, UV-B may instead impact aerial plant growth and/or production of key hormones like auxin in shoots that are mobilised to roots to promote root branching.

We appreciate the reviewer's suggestion. Broad band UV-B (280-315 nm) containing low-wavelength and high-energetic UV-B light is more likely to cause stress than narrow band UV-B (311-313 nm). The narrow band UV-B we used is mainly a signal to the plant. It has been reported that UV-B might regulate auxin levels or transport. Our reciprocal grafting experiments showed that the root UVR8 is responsible for regulating the lateral roots growth and auxin responses in roots. UV-B could regulate root development via regulating auxin biosynthesis, auxin distribution and also auxin signaling.

The authors initially demonstrate UV-B negatively impacts Arabidopsis root growth and branching. NOTE: I was puzzled why they buried this important result in the Supplementary Figures?. The authors focus on the impact of UV-B on lateral root length, yet there also appears to be a clear effect of UV-B on primary root growth (see Fig S1A). Given this focus, it is not clear whether they checked if the number and density of LR primordia are also affected? This is important as these represent parameters dependent on the auxin response machinery which the authors show is impacted by UV-B using the DR5 reporter and arf7, arf19 mutants.

We appreciate greatly this excellent point raised by the reviewer! We checked the impacts of UV-B on root growth and branching and did quantifications, those data are in Figure 1. Yes, UVR8 interacts with MYB73/MYB77 to affect the auxin responses, not only the lateral root length but also the primary root growth is affected by UV-B and UVR8. We checked the number of LR primordia in response

to UV-B treatment as reviewer suggested (Fig 1D). We think that UV-B affects the length of primary root via multiple pathways, including auxin biosynthesis, auxin distribution and also auxin signaling. MYB77 was reported to play critical role in lateral root growth, and we found that UVR8 physically interacted with MYB73/MYB77, so we focus on the growth of lateral roots.

How UV-B impacts auxin response is addressed in data presented in Fig. 1. The authors initially demonstrate that seedlings exposed to UV-B and white light (versus just white light) exhibited reduced inhibition to the auxin IAA supplemented in growth media. NOTE: can the authors rule out that reduced inhibition of root growth in all lines shown in Fig. 1A (when grown in white light + UV-B) is not due to UV-B accelerating the breakdown of this labile form of auxin? This bioassay revealed UV-B modulates seedling root auxin responses via the UVR8 receptor.
NOTE: this first results section was particularly difficult to read, jumping between different bioassays ({plus minus}auxin, {plus minus}direct light), organs (root versus hypocotyl) and chemical treatments, making it hard for readers to follow the logic thread. I recommend a complete re-write!
We appreciate greatly this excellent point raised by the reviewer! Later we also realized that UV-B might accelerating the breakdown of IAA, so we did check the effect of NAA. We also did experiments to check the effects of UV-B on the stability of IAA and NAA: IAA plates and NAA plates were pre-irradiated by UV-B for 7 days first, then they were used to conducted the root experiments. IAA plates exposed to UV-B have a reduced effect on root growth than plates exposed to white light without UV-B (Appendix Fig S1 D). NAA plates exposed to UV-B have the same effect on root growth as plates exposed to white light without UV-B (Appendix Fig S1 D). These results indicate that UV-B does not affect NAA stability significantly but does affect IAA stability. We used mainly NAA to do our experiments. The first part was re-wrote as suggested.

How UVR8 blocks auxin response and inhibition of lateral root length was examined using elegant transgenic tools featuring steroid inducible constitutively active and inactive UV-B photo-receptor forms. This revealed that monomeric nuclear localised UVR8 is a key signalling component repressing auxin response. Next, using grafting of WT and uvr8 scions and roots, the authors demonstrated UVR8 must act directly in roots to mediate the effect of UV-B (despite roots not being directly exposed to UV-B - see Fig S3E - a result which I found quite puzzling to explain unless invoking a mechanism such as UV-B channelling from shoots to roots....)
Those grafting experiments were repeated many times, and we got similar results. UV-B light might be conducted through the soil to root to activate UVR8, it is also possible that UV-B light is conducted through plant stem. We still do not know how could the root UVR8 get activated.

The authors note the overlap between genes whose expression was altered following auxin and UV-B treatments. To uncover the connection(s) between UVR8 and auxin response machinery, a Y2H screen was performed, identifying MYB73/77. Intriguingly, MYB73 interacted with UVR8 in a UV-B dependent manner in both yeast and plant cells using several in vivo and in vitro methods. Given the dependence of UV-B for this interaction, I was surprised to read in the

methods that UV-B treatment was not employed during the initial Y2H screen. Can the authors please clarify? Additional analysis revealed a preference for the N terminus of MYB73/77 interacting with the N and C termini of the monomeric form of UVR8.
However, the mechanism for how UV-B promotes this interaction remains unclear.

We did not write clearly in our previous version, the yeast two hybrid screening was done with and without UV-B treatment. We add in the method and also text that the UV-B treatment was employed in the yeast two hybrid screening (line 242 "we performed a yeast two-hybrid screen with a library of A. thaliana TF (transcription factor) ORFs (Castrillo, Turck et al., 2011) with and without UV-B to identify transcription factors that interact with UVR8 and are also involved in auxin responses", line 499 "Yeast cells were then grown on SD-Trp-Leu plates in dark and treated with or without UV-B light (2W/m^2) for 2 to 3 hours per day for 4 days. The interactions were tested by galactosidase assays.").

Rather than address the latter question, the authors focused on characterising the genetic relationship between UVR8 and MYB73/77 using double and triple mutant combinations. They concluded that the latter transcription factors acted 'downstream' of UVR8 but, given they directly interact, this conclusion is debatable as they act at a common step of the UV signalling cascade, co-regulating targets like auxin responsive genes. Indeed, the last results section reports UVR8 functions by blocking MYB73/77 DNA binding activity.
In summary, this represents a promising manuscript, but several key questions remain to be addressed as detailed above.

Thanks! We appreciate the reviewer's suggestions.

Referee #2:
Yang et al. study how the UV-B photoreceptor UVR8 inhibits lateral root growth in Arabidopsis. They identified the MYB73 and MYB77 transcription factors as novel UVR8 interacting proteins. UV-B-dependent interaction of UVR8 with MYB73 and MYB77 interferes with their capacity to interact with target DNA, thus inhibiting their activity. As MYB73 and MYB77 affect lateral root growth, UVR8 is able to negatively regulate lateral root growth. The authors provide evidence, including grafting assays, that the function of UVR8 in regulating root development is tissue-autonomous. Although the physiological relevance of lateral root growth inhibition by UV-B stays unfortunately rather unclear, interesting findings are provided towards further understanding on how UVR8 may regulate gene expression and, in particular, represses auxin responses.

1.) Lines 226: "... expression was up-regulated in uvr8 mutant compared with WT but downregulated in rup1 rup2 mutants with UV-B treatment in both seedlings (Fig 4E) and roots (Fig 4G)" - this is a rather confusing way of describing what happens. Indeed, UV-B results in repression of the tested target genes in WT and stronger repression in rup1 rup2. However, uvr8 simply does not show repression of the target genes in response to UV-B, they are not "up-regulated" compared to WT, but they are simply not repressed under UV-B. Please rephrase.

We appreciate the reviewer's suggestion. The sentence is rephrased as "Furthermore, the expression of these genes was similar in the seedlings (Fig 4D) and also roots (Fig 4F) of WT, uvr8 and rup1 rup2 mutants without UV-B treatment, while their expression was higher in uvr8 mutant compared with WT but

lower in rup1 rup2 mutants with UV-B treatment in both seedlings (Fig 4E) and roots (Fig 4G), because their expression was repressed in WT and stronger repressed in rup1 rup2 mutants but very weak repressed in uvr8 mutant by UV-B" (line 232).

I am also confused by what is actually shown in the figures 4D&E as "relative expression". Fig. 4D: It seems that relative expression is shown for WT = 1 for IAA19, IAA5, SAUR63 and GH3.2, but then WT expression is different than 1 for SAUR20 and SAUR23 in the same graph. In Fig. 4E, GH3.2 WT is different than 1, but in this case SAUR20 is put to 1. Please correct and clarify.

For all qPCR results, biological replicates of three experiments were used. For every single experiment, three technical repeats were used. The WT control was set to 1 for each single experiment, so that the results of three different experiments could be comparable to each other. We reanalyzed our data and all the WT control are set to 1 now.

2.) Line 159-161: The authors performed a careful control supporting that UV-B does not simply affect the stability of the exogenously applied NAA itself, at least under the conditions used. However, as is presently written, it will likely not be clear for the readers why this control has been performed. The authors should mention the potential and documented problem of photodestruction of auxins by UV-B to make clear why the effect of UV-B on NAA stability was tested.
Also, line 160: "... did not affect NAA stability,..." instead of "... did not affect the degradation of NAA,...".

We appreciate greatly this excellent point raised by the reviewer! We add the description of that "It has been reported that auxin homeostasis could be affected by UV-B via photo-oxidative damage, degradation, biosynthesis, and conjugation (Vanhaela et al 2016), so we first checked whether UV-B affected the stability of IAA and NAA. IAA plates and NAA plates were pre-irradiated by UV-B for 7 days first, then they were used to conduct the root experiments. IAA plates exposed to UV-B had a reduced effect on root growth than plates exposed to white light without UV-B (Appendix Fig S1 D). NAA plates exposed to UV-B have the same effect on root growth as plates exposed to white light without UV-B (Appendix Fig S1 D). These results indicate that UV-B does not affect NAA stability significantly, but does affect IAA stability (line 158). We used mainly NAA to do our experiments."

3.) Fig 2A-C legend: "uvr8 mutant is hypersensitive to auxin, and rup1 rup2 mutant is insensitive to auxin" - this is misleading. What is shown is that UV-B inhibits the response of Arabidopsis seedlings to exogenously added NAA (please make clear in the Figure title as well that NAA is added). The data show that this is UVR8 dependent (i.e. the response to NAA is not repressed in uvr8, but this does not mean that uvr8 is hypersensitive to NAA). Please check wording thoughout the text. Such misleading statements make it sometimes difficult to read the manuscript and follow the argumentation.

We appreciate the reviewer's suggestion. We changed the sentence to (line 744) "UV-B inhibits the response of Arabidopsis seedlings to exogenously added NAA in a uvr8 dependent manner, since the response to NAA was not repressed in uvr8 mutant." We checked through the manuscript and changed our descriptions.

4.) Figure 7F-H: It is not clear how the ChIP data are presented here. The y-axis and figure legend say "IP/Input" ratios. But then in the graphs, for example, F&H, all negative WT controls are = 1 (?). This cannot be true: as much IP signal as input signal in the WT negative control? This should be the background control. Also it should not be the same for all tested promoters. It seems to me that only Figure 7E shows "IP/Input" data as suggested by the y-axes, but not Figures 7F-I. Same problem also for Figures EV8E and G.

Our previous Figure 7E and I, Appendix Figure S8 H and I are the IP/Input ratios, while Figure 7F-H and Appendix Figure S8 E-G, the lowest IP/Input ratio of each primer was set to 1, and the other IP/Input ratios of the same primer was normalized to this lowest ratio. In our new Figure 7 and Appendix Figure S8, the IP/Input ratios are shown with SDs (n=3), without normalization.

5.) Line 185: "... GR-UVR8R338A (Qian et al., 2016) (the constitutively monomeric UVR8 mutant form that is active regardless of their subcellular localization and UV-B light conditions)..." - although UVR8R338A is constitutively monomeric, it is not constitutively active (see e.g. data in the cited Qian et al., 2016). Also, the UV-B induced activity of the GR-UVR8 fusions requires Dex treatment (i.e. not independent of subcellular localization). Please correct.

We appreciate that, it is corrected.

6.) Lines 348-350: "Shoot and root need to coordinate so that a plant could grow well, when the growth of shoot is repressed by UV-B, the growth of root is also inhibited, so as to save the energy." - it is not clear to me why plants need to save energy under conditions where they are exposed to sunlight (high UV-B would also mean high PAR).

Our point is that shoot and root need to coordinate. UV-B, blue, red and far red light all could inhibit growth though sun light is energy for photosynthesis. We change the sentence to "Shoot and root need to coordinate so that a plant could grow well, when the growth of shoot is repressed by UV-B, the growth of root is also inhibited" (line 363).

Plants grown on top of the mountain or in high altitude areas normally are smaller than plants grown at the foot of the mountain or low altitude areas, higher UV-B is one of the reasons. For those smaller plants, roots are also smaller.

Minor points:

1.) Line 37: I assume it should mean "... demonstrates that UV-B antagonistically regulates auxin-induced gene expression", or similar.

We appreciate that, it is corrected.

2.) Line 83: "In those two stories, ..." - not clear what is meant by two stories, please rephrase.

We appreciate that, it is changed.

3.) Line 236: BiLC should be "Bimolecular Luminescence Complementation" (not Bimolecular Fluorescence Complementation)

We appreciate that, it is corrected.

4.) Lines 216-217: "... repressed by UV-B in a UVR8-dependent manner and the repression was abolished in uvr8 mutant..." - seems to state 2-times the same.

We appreciate that, it is modified. We changed to "Many auxin responsive genes were repressed by UV-B and the repression was abolished in uvr8 mutant"(line 183).

5.) Figure 8: not clear what the smileys should indicate. It may be better to remove/reduce the arrows between "auxin response" and the seedling on the "+ UV-B" side.

We appreciate that, it is changed as suggested.

Referee #3:

This interesting article examines the control of root development by UV light, reporting that UV-B light impaired formation of lateral roots. The study identifies an important role for the transcription factor UVR8 in controlling this process. They also provide evidence that the effect of UV-B light is to reduce the positive effect of auxin, while revealing large groups of transcripts that show opposite regulation by these two stimuli. They report that UVR8 interacts with two MYB transcription factors and show that this protein complex is needed for the developmental effects of UV-B light.

Major concerns:

This article uses appropriate methods and reveals an important role of UV light in controlling root development. There are a number of aspects of the work that need substantial edits to make the data set and the writing clearer. In particular, the case for the effect of UV-B in antagonizing auxin response is impaired as they don't ever provide a side by side comparison of growth with and without auxin and UV-B in the same figure. To show that UV-B modulates the auxin response (or vice versa) the effect of both treatments needs to be evident. So, they need to rearrange their data to make this point more clearly. Second the writing, particularly of the discussion, needs substantial editorial input. The communication is not clear nor is there an adequate synthesis of the science and integration into the framework of light and auxin effects on root development.

We appreciate the reviewer's suggestion! New experiments are done so as to have comparison images and data for UV-B with and without auxin (Fig EV 2). The results indicate that supplement of low concentration of NAA (10 and 25 nM) promoted the growth of lateral roots while UV-B inhibited lateral root growth and development in a UVR8-dependent manner (Fig EV2A-C) (line 167). In Appendix Fig S2, we show that UV-B represses the auxin response to inhibit the hypocotyl elongation in a UVR8-dependent manner, comparison images and data for UV-B with and without auxin are also shown.

For the high concentration of NAA supplemented experiments in Fig 2 and Fig 6, seedlings of indicated genotypes were grown in LD on 1/2 MS plats without NAA supplemented for 5 days, then transferred to new plates containing 0.4 μM NAA then kept in continuous white light with or without UV-B for 7 days. We did not compare those high concentration of auxin supplemented experiments with those in the absence of auxin since they were done differently. The primary root length of plants grown directly in plates supplemented of high concentration of auxin is too

short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B.

Our discussion is modified as suggested, changes are highlighted.

Detailed major and minor concerns:

1. The authors use the synthetic auxin NAA through out this work. They need to discuss why they use this instead of endogenous auxin, IAA. It is the case that the photostability of this synthetic auxin is higher than of IAA, which suggests it may be a better choice, but that needs to be explained.

We really appreciate your suggestion! Both IAA and NAA were used. We did not put the IAA data in the manuscript because we found that UV-B accelerated the breakdown of IAA, while did not affect NAA. IAA plates and NAA plates were pre-irradiated by UV-B for 7 days first, then they were used to conduct the root experiments. IAA plates exposed to UV-B have a reduced effect on root growth than plates exposed to white light without UV-B□Appendix Fig S1 D□. NAA plates exposed to UV-B have the same effect on root growth as plates exposed to white light without UV-B (Appendix Fig S1 D). These results indicate that UV-B does not affect NAA stability significantly, but does affect IAA stability. We then used NAA in our experiments. We add our explanation (line 164).

2. The writing is not clear in many places, especially the discussion. Here are a few examples of places where the writing needs improvement. There are many more not listed here:

a. Line 83-the authors refer to two publications as "two stories".

We appreciate that, it is corrected.

b. Line 89 and 91 the same sentences is repeated twice with another sentence in between.

We appreciate that, it is changed.

c. In many sections the text needs to be divided into additional paragraphs. One example is line 135.

We appreciate that, it is corrected.

3. The authors say in line 123 that UVB affects hypocotyl length, but they don't show that data or cite a publication to this effect. One or the other is needed.

We appreciate that, references are added.

4. The authors say that DR5-GUS tells one about "auxin activity" (line 145). They should be more precise here and other places indicating that this reporter tells about auxin induced gene expression

We appreciate that, it is changed.

5. The DR5-GUS data in Figure 1 suggests that there is a difference in response to UV-B light. Yet, they show the root tip. As most of the paper focuses on the effect of this light treatment on lateral root development, they really should show auxin-responsive gene expression changes in lateral root primordia.

We appreciate the reviewer's suggestion! DR5p-GUS activity is also repressed by UV-B light in lateral root primordia, those data are added (Fig EV 1B) .

6. They discuss a yeast two hybrid screen to identify interactors with UVR8, but they don't report evidence from this screen. A complete summary of what they found and why they selected these two MYBs for further analysis would be a helpful addition.

We mentioned that "To figure out the mechanism by which UVR8 inhibits auxin responses, we performed a yeast two-hybrid screen with a library of A. thaliana TF (transcription factor) ORFs (Castrillo et al., 2011) with and without UV-B to identify transcription factors that ***interact with UVR8 and are also involved in auxin responses****. MYB73 was identified in this screen with UV-B treatment." MYB73 is the only transcription factor we get from the screening that interacts with UVR8 and also might be involved in auxin responses (its homologous protein MYB77 was reported to be involved in auxin responses and lateral root development). We also used yeast two hybrid to verify the interaction between UVR8 and MYB73 (Fig EV4A).*

7. The experiments examining the effects of UV-B light on root development of shaded roots in grafted seedlings is an excellent addition.

Thank you so much! We appreciate that.

8. In many of the figures throughout the paper the colors of the bars with the two treatments are both very dark, making them impossible to resolve in black and white printouts.

We appreciate the reviewer's suggestion. Bars with different treatments were made in blue and red.

9. Figure 1D reports the number of lateral roots at multiple stages. This is just reported for UV-B treated roots but needs to include data without UV-B as well.

The number of lateral roots were similar in WT, uvr8 and rup1 rup2 without UV-B treatment. The data is added as suggested (Fig 1D).

10. Figure 2 is the first of multiple figures where the effect of UV-B is shown in the presence of auxin. The authors need to have comparison images and data for UV-B in the absence of auxin, so that the interactions of UV-B and auxin can be clarified.

New experiments are done so as to have comparison images and data for UV-B with and without auxin (Fig EV 2). The results indicate that supplement of low concentration of NAA (10 and 25 nM) promoted the growth of lateral roots while UV-B inhibited lateral root growth and development in a UVR8-dependent manner (Fig EV2A-C) (line 166). In Appendix Fig S2, we show that UV-B represses the auxin response to inhibit the hypocotyl elongation in a UVR8-dependent manner, comparison images and data for UV-B with and without auxin are also shown.

For the high concentration of NAA supplemented experiments in Fig 2, seedlings of indicated genotypes were grown in LD on 1/2 MS plats without NAA supplemented for 5 days, then transferred to new plates containing 0.4 μM NAA then kept in continuous white light with or without UV-B for 7 days. We did not compare those high concentration of auxin supplemented experiments with those in

the absence of auxin since they were done differently. The primary root length of plants grown directly in plates supplemented of high concentration of auxin is too short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B.

11. The labels of the panels in Figure 3-C are not obvious. If they could add a functional label: inactive dimer and constitutively active monomer, that would make the images and the quantified data in B and C more intuitive.

Thanks! Those are added.

12. The grafting experiment in Figure 3D, which is very important. The surprising thing is that the length of lateral roots not the number is quantified.

We appreciate greatly this excellent point raised by the reviewer! The density of lateral roots is added (Fig 3E).

13. Figure 4- Panel A has data published by others and that should be clarified in the figure legend. Panel B needs error bars, Panel C, needs to make it clear that this was time after NAA addition in the x axis label. For panels D-G, each graph has a different y-axis maximum, making the comparison between graphs difficult. Those should be regraphed with consistent axes. Also, a more correct Y-axis label is relative transcript abundance (not relative expression), as qRT-PCR reports the steady state levels of transcripts, not their expression level.

We did not explain it clearly, we re-analyzed previously reported microarray data-sets of genes affected by UV-B, UVR8 and auxin (ArrayExpress, E-MEXP-1957, E-GEOD-627), and found that genes affected by UV-B, UVR8 and auxin significantly overlap, Figure 4A was our new analysis. Panel B was also a re-analysis of those previously reported microarray data-sets. The x axis label of Fig 4C was time (hour of UV-B treatment). Different graphs in Figure 4D-G are not comparable. Those qPCR results are biological replicates of three experiments. For every single experiment, three technical repeats were used. The WT control was set to 1 for each single experiment, so that the results of three different experiments could be comparable to each other, and since the WT control was set to 1 in every graph, then different graphs are not comparable. Our purpose is to show that the expression of these genes was similar in the seedlings (Fig 4D) and also roots (Fig 4F) of WT, uvr8 and rup1 rup2 mutants without UV-B treatment, while their expression was higher in uvr8 mutant compared with WT but lower in rup1 rup2 mutants with UV-B treatment in both seedlings (Fig 4E) and roots (Fig 4G), because their expression was repressed in WT and stronger repressed in rup1 rup2 mutants but very weak repressed in uvr8 mutant by UV-B. Y-axis was changed to relative transcript abundance as suggested.

14. Figure 5-These blots are important but are the least clean data in the manuscript. The authors do not fully discuss the very weak signaling in the UVR8-IP in figure A and B and the very messy bands in the same IPs in panels D-F. This experiment needs to be completed to make a more convincing data set.

New experiments are done and those data are replaced by better data.

15. Figure 6 needs the no auxin controls added and to have the data in panels D and E reported with similar y axis labels as in 12.

For the high concentration of NAA supplemented experiments in Fig 6, it is the same as Fig 2, seedlings of indicated genotypes were grown in LD on 1/2 MS plats without NAA supplemented for 5 days, then transferred to new plates containing 0.4 μM NAA then kept in continuous white light with or without UV-B for 7days. We can't compare those high concentration of auxin supplemented experiments with those in the absence of auxin since they were done differently. The primary root length of plants grown directly in plates supplemented of high concentration of auxin is too short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B.

Different graphs in Figure 6D-E are not comparable (the same as Figure 4D-G). Those qPCR results are biological replicates of three experiments. For every single experiment, three technical repeats were used. The WT control was set to 1 for each single experiment, so that the results of three different experiments could be comparable to each other, and since the WT control was set to 1 in every graph, then different graphs are not comparable. Y-axis was changed to relative transcript abundance as suggested.

16. Figure 7. This is one of the clearest and well done EMSAs that I have ever seen. This is very nice data. I would suggest rather than using probe #, to just note the name of the gene from which the promoter fragment is derived.

We appreciate the reviewer's nice comments and suggestion. The labels are changed as suggested.

17. References. Some of these are not really the optimal reference. For example, Cheng et 2008 (line 92) is not an appropriate broad reference for auxin signaling as it just reports auxin effects in flower development, which is minimally studied. There are many great reviews available to provide a framework of auxin signaling.

We appreciate greatly this excellent point raised by the reviewer! New references are added (Leyser, 2018, Weigers & Wagner, 2016).

18. The authors should provide a supplemental excel file of all the genes that they identified in their comparison of published microarray data.

We appreciate greatly for this excellent suggestion. We provide the excel files as Appendix Tables.

2nd Editorial Decision 12th Sep 2019

Thank you for submitting a revised version of your manuscript. I apologise for the delay due to belated submission of referee reports. It has now been seen by two of the original referees, and their reports are included below for your information.

As you will see from the comments, while reviewer #2 finds that their concerns have been sufficiently addressed, reviewer #3 indicates several data presentation issues that should be further clarified before they can recommend publication. I have consulted with reviewer #2 regarding these points, who agreed that clarity and presentation of the data could be further improved, especially as indicated in point 1 (rearrangement of the data) and point 2 (please discuss the discrepancy in Fig

EV2 vs Fig EV5 and/or quantify the data in Fig EV5). Please also clarify the points 8 and 11 regarding statistical analysis and clarify the remaining issues at your discretion.

REFEREE REPORTS:

Referee #2:

This is a revised version. The authors have satisfactorily addressed my concerns.

Referee #3:

This revised article examines the control of root development by UV light, reporting that UV-B light impaired formation of lateral roots. The study identifies an important role for the transcription factor UVR8 in controlling this process through formation of complexes with MYB transcription factors. This article uses appropriate methods and reveals an important role of UV light in controlling root development. The revised manuscript is improved, yet there are still a number of points raised in the original reviews that are not fully addressed. In particular the organization of the figures and the text make the article difficult to read and follow the data, especially the organization of the figures that focus on the developmental interactions between auxin and UV-B light. The molecular and biochemical data is much clearer. The complicated and unclear presentation of the data on the interactions between auxin and UV light on root development mean that the framework to unify the molecular mechanisms is not optimal.
Major concerns:
There are a number of aspects of the work that need substantial edits to make the data set and the writing clearer.
1. The case for the effect of UV-B in antagonizing auxin response is not clear, as the authors do not provide a side by side comparison of growth with and without auxin and UV-B in the same figure in the primary article. To show that UV-B modulates the auxin response (or vice versa) the effect of both treatments needs to be evident. They need to rearrange their data to make this point more clearly in primary figures. The authors only partially address this request from the first review, by adding EV Figure 5, which is poorly described in the legend and does not appear to include UV-B treatment. indicating that this is difficult as there are large differences in roots with and without auxin. They could reduce the duration of the treatment with auxin to minimize these differences, to reduce this difference. Including this side by side comparison is always done in papers and is essential for this work.
2. Auxin increases the number and length of lateral roots, which has been shown in numerous reports, but in EV2 they do not report much of an increase in lateral roots or in length, so this added data is puzzling. This contrasts with EV5, where the standard increase in lateral roots in response to NAA is shown in images, but not quantified.
3. The authors added a figure with the goal of showing that IAA, but not NAA, is broken down by UV-B light in a new supplemental Figure 1. This is an important addition, but not yet clear. They need to show side by side the effect of IAA and NAA with and without UV-B on fresh plates, versus those treated with UV for extended periods of time. This figure also needs the 0 nM IAA in panel E and would be better if the time of treatment were shorter and the dose response curve in the presence and absence of UV-B.
4. They really need to rearrange Figure 1 and 2 so that they can show parallel images of roots or DR5-GUS staining with and without auxin in the same figure . For example, they can put all the DR5-GUS in one image and separate that from the root development. Figure 1A also has roots at too low a resolution, so it is not really possible to see the lateral roots. The rest of the manuscript shows images more clearly using fewer seedlings on a plate, and similar resolution images should be included here.
5. Some of the additions requested in the last review are in EV figures. The choices of the authors on where to put data in primary figures, EV figures, and supplemental figures needs to be adjusted. In a number of cases the data generated as a result of reviewer requests needs to be integrated into the primary figures. For example, the DR5-GUS images of lateral roots are far more pertinent than the leaf images and should be central to the manuscript and show up in the primary figures not EV. The leaf images should be supplemental and lateral root expression primary.

6. The writing still needs substantial improvement. There are cases where data is not discussed, where conclusions are not logical from the data and where the language needs improvement. Specific examples are listed below.
7. There is just too much data presented in a complex way, making it difficult for the reader to follow their results.
8. The statistical analysis of the bar graphs needs to be clarified. Significance is noted with both a "A" and "a or b". The legend indicates that A indicates significant differences, but all the bars marked with A do not look different. It is not clear what they are comparing as well with these statistics. As a result, I am now not convinced of which differences are meaningful on these graphs. They need a detailed section on statistics. They mention Tukey's test, which is usually performed as a post-hoc test after an ANOVA, but whether they did an ANOVA is not clear.
9. The reviewers asked the authors to better explain the idea of light being conducted from the shoots to roots. The authors did not adequately address this request in the text of the manuscript.
10. A fundamental point of confusion in their data that is not discussed is why rup1 rup2 has little DR5-GUS signal (in primary and lateral roots) but forms lateral roots and has auxin -induced lateral root formation.
11. Throughout the manuscript there are some issues with the bar graphs. For example, in Figure 1, 2, and 4, there is a capital A and small a and b used to denote statistical difference. Rather than red and blue they should be black and white (with an outline). Also, the multi panel bar graphs (like Figure 1 D) need statistics and to be organized in a way where the data is grouped so that the patterns and trends in the data are more evident.
12. The discussion is slightly, but not substantially improved. The reader needs synthesis of this extremely complicated data set as well as integration into the literature. The final paragraph is a very general summary but does not include sufficient synthesis of the complex dataset.

Detailed minor concerns:

1. Figure 2 is the first of multiple figures where the effect of UV-B is shown in the presence of auxin. The authors need to have comparison images and data for UV-B in the absence of auxin, so that the interactions of UV-B and auxin can be clarified, as requested previously and noted above.
2. The introduction discussed R2R-MYB transcriptional factors that control flavonol synthesis. This ties into their finding of MYBs interacting with UVR8, but there is literature on the role of MYB12 that functions to control flavonol synthesis in roots, which are not cited and more pertinent to this work.
3. Examples of writing issues:
a. Results first heading: roots should be singular
b. The word "dramatically" is used to describe results. They should report fold change, instead of using this descriptor.
c. Line 145The authors conclude that that UV light effects are acting through arf7/arf19, as these double mutants have no roots with or without UV. This conclusion is not logical, as if a plant cannot form lateral roots, even if there is a signal acting through a different pathway, it still won't form lateral roots.
d. Line 186: The phrase roots are mainly grown in soil is awkward.
e. Line 309: Should read: Next we examined the abundance of auxin-regulated transcripts and found that their levels descreated in myb73 myb77
f. Line 304 refers to the lateral root phenotype of uvr8, but whether they mean with or without auxin and the developmental differences need to be defined here
g. Discussion begins with the phrase "There are quite some reports..."
h. Line 362 negative should be negatively
i. Line 366 autonomic should be autonomous
j. The figure legends (especially the supplemental figures) have numerous grammatical errors or insufficient information on the experiments.

2nd Revision - authors' response 28th Sep 2019

Point-by-point response

Editor:

Clarity and presentation of the data could be further improved, especially as indicated in point 1 (rearrangement of the data) and point 2 (please discuss the discrepancy in Fig EV2 vs Fig EV5 and/or quantify the data in Fig EV5). Please also clarify the points 8 and 11 regarding statistical analysis and clarify the remaining issues at your discretion.

We appreciate your suggestions. We rearranged the data as suggested. We moved previous Fig EV2 into Fig 2A-B, seedlings were grown on 1/2 MS with the addition of a series of low concentration (0, 10 and 25 nM) of NAA in continuous white light condition and white plus UV-B condition for two weeks, so as to provide a side by side comparison of growth with and without auxin and UV-B in the same figure in the primary article. The DR5-GUS staining of previous Fig 2D and E were moved to Fig EV 2B and C, so the results of DR5-GUS are only shown in Fig 1E and F in the primary article. Appendix Fig 3A-B were moved to Appendix Fig 2A-B, so we explain this root phenotype before hypocotyl.

Previous Fig EV2 (current Fig 2A-B and Fig EV2), seedlings were grown on 1/2 MS with the addition of a series of low concentration (0, 10 and 25 nM) of NAA in continuous white light condition and white light plus UV-B condition for 2 weeks. For previous Fig EV5B (current Figure EV5C), seedlings of indicated genotypes were grown in LD for 5 days, then transferred to new plates containing 0.4 μM NAA and kept in UV-B for 7days. The data in those two figures were done differently, for Fig EV5, 5-day-old seedlings were transferred to new plats containing auxin, since primary root length of plants grown directly in plates supplemented of high concentration of auxin is too short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B. For Fig EV2, seedlings were not transferred.

The data in Fig EV5 is quantified and added as Fig EV5D.

Point 8 and 11 regarding statistical analysis are clarified as suggested. In our previous data, statistical analysis of data with and without UV-B treatment were done separately. We redid the statistical analysis of data with and without UV-B treatment together. Letters "a" to "d" indicate statistically significant differences for the indicated values, as determined by a one-way analysis of variance (ANOVA), followed by Tukey's least significant difference (LSD) test ($P < 0.05$). And those bar graphs are redone as suggested and they are black and white now.

There are also a few further editorial issues that I would like to ask you to address in the final version of the manuscript:

1. I would like to propose minor modifications in the abstract text (please see the file in the attachment). As indicated by the reviewers, the rest of the manuscript would also benefit from language editing, so I recommend to have the manuscript checked by a native speaker or a language editing service.

Your help and suggestions are highly appreciated! The manuscript is modified by a language editing service.

2. Please add scale bars in Fig EV4B,D

Scale bars are added, thanks!

3. Please remove the section "Figures and Figure Legends" from the main manuscript file (the separate figure legend sections should remain in the file)

The section “"Figures and Figure Legends" is removed.

4. Xuan Li is not mentioned in the "Author contributions" section
P.C and X.L performed the genomic expression analysis, we made mistake in X.L before (L.Z), it is corrected now, thanks a lot!

5. Please add the "Conflict of Interest" section
It is added, thanks!

6. Please remove the legends for Table EV1 and EV2 from the main manuscript text and add to the respective files in a separate tab
The legends for Table EV1 and EV2 are removed.

7. We generally encourage the publication of source data for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. We would need one file per figure (which can be a composite of source data from several panels) in jpg, gif or PDF format, uploaded as "Source data files". The gels should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files will be published online with the article as supplementary "Source Data". Please let me know if you have any questions about this policy.
We upload our source data for electrophoretic gels and blots as suggested.

8. Papers published in The EMBO Journal include a 'Synopsis' to further enhance discoverability. Synopses are displayed on the html version of the paper and are freely accessible to all readers. The synopsis includes a short introductory paragraph, as well as 2-5 one-sentence bullet points that summarise the paper. Please send us your suggestions for the synopsis text and a synopsis image (size max 550x400 pixels).
The synopsis is added, thanks!

Referee #3:

This revised article examines the control of root development by UV light, reporting that UV-B light impaired formation of lateral roots. The study identifies an important role for the transcription factor UVR8 in controlling this process through formation of complexes with MYB transcription factors. This article uses appropriate methods and reveals an important role of UV light in controlling root development. The revised manuscript is improved, yet there are still a number of points raised in the original reviews that are not fully addressed. In particular the organization of the figures and the text make the article difficult to read and follow the data, especially the organization of the figures that focus on the developmental interactions between auxin and UV-B light. The molecular and biochemical data is much clearer. The complicated and unclear presentation of the data on the interactions between auxin and UV light on root development mean that the framework to unify the molecular mechanisms is not optimal.
Major concerns:
There are a number of aspects of the work that need substantial edits to make the data set and the writing clearer.
1. The case for the effect of UV-B in antagonizing auxin response is not clear, as

the authors do not provide a side by side comparison of growth with and without auxin and UV-B in the same figure in the primary article. To show that UV-B modulates the auxin response (or vice versa) the effect of both treatments needs to be evident. They need to rearrange their data to make this point more clearly in primary figures. The authors only partially address this request from the first review, by adding EV Figure 5, which is poorly described in the legend and does not appear to include UV-B treatment. indicating that this is difficult as there are large differences in roots with and without auxin. They could reduce the duration of the treatment with auxin to minimize these differences, to reduce this difference. Including this side by side comparison is always done in papers and is essential for this work.

We moved previous Fig EV2 into Fig 2A-B to provide a side by side comparison of growth with and without auxin and UV-B in the same figure in the primary article. In this experiment, seedlings were grown on 1/2 MS with the addition of a series of low concentration (0, 10 and 25 nM) of NAA in continuous white light condition and white plus UV-B light condition for 2 weeks, so as to provide a side by side comparison of growth with and without auxin and UV-B in the same figure. We rearrange our data to make this point more clearly in primary figures.

In the previous revision, Fig EV2 (not Fig EV5) was added, and UV-B treatment was included. Supplement of 10 nM NAA increased the number and length of lateral roots under the white light condition, while supplement of 25 nM of NAA still increased the number of lateral roots but not the length of lateral roots, which is consistent with reported before (Shin et al., 2007), the growth of lateral root is very sensitive to auxin, high concentration of auxin repressed the growth of main roots and lateral roots, while promoted the generation of lateral roots. UV-B inhibited lateral root growth and development in a UVR8-dependent manner (Fig 2A and B, EV2A). In Appendix Fig S2C-H, we show that UV-B represses the auxin response to inhibit the hypocotyl elongation in a UVR8-dependent manner, comparison images and data for UV-B with and without auxin are also shown.

For low concentration of auxin, we can do a side by side comparison of growth with and without auxin and UV-B in the same figure. To further confirm our conclusion, we also used high concentration of auxin. For the high concentration of NAA supplemented experiments in Fig 2 and Fig 6, seedlings of indicated genotypes were grown in LD on 1/2 MS plats without NAA supplemented for 5 days, then transferred to new plates containing 0.4 μM NAA then kept in continuous white light with or without UV-B for 7 days. We did not compare those high concentration of auxin supplemented experiments with those in the absence of auxin since they were done differently. The primary root length of plants grown directly in plates supplemented of high concentration of auxin is too short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B.

2. Auxin increases the number and length of lateral roots, which has been shown in numerous reports, but in EV2 they do not report much of an increase in lateral roots or in length, so this added data is puzzling. This contrasts with EV5, where the standard increase in lateral roots in response to NAA is shown in images, but not quantified.

For previous Fig EV2 (current Fig 2A-B and Fig EV2), supplement of 10 nM NAA increased the number and length of lateral roots under the white light condition, while supplement of 25 nM of NAA still increased the number of lateral roots but not the length of lateral roots, which is consistent with reported before (Shin et al., 2007), the growth of lateral root is very sensitive to auxin, high concentration of auxin repressed the growth of main roots and lateral roots, while promoted the generation of lateral roots.

For low concentration of auxin, we can do a side by side comparison of growth with and without auxin and UV-B in the same figure. To further confirm our conclusion, we also used high concentration of auxin. For previous Fig EV5 B (current Fig EV5C), seedlings of indicated genotypes were grown in LD (16-h light/ 8-h dark) conditions on 1/2 MS plats without NAA supplemented for 5 days, then transplanted to new plates containing 0.4 μM NAA with UV-B treatment for 7 days. We did not compare those high concentration of auxin supplemented experiments with those in the absence of auxin since they were done differently. The primary root length of plants grown directly in plates supplemented of high concentration of auxin is too short, it is hard to analyze the lateral roots, so the plants have to be grown in normal conditions for several days, then transferred to plates with high concentration of auxin supplemented. The purpose of this experiment is to compare the auxin responses between white light and UV-B.

The data in Fig EV5B is quantified as suggested (Fig EV5 D).

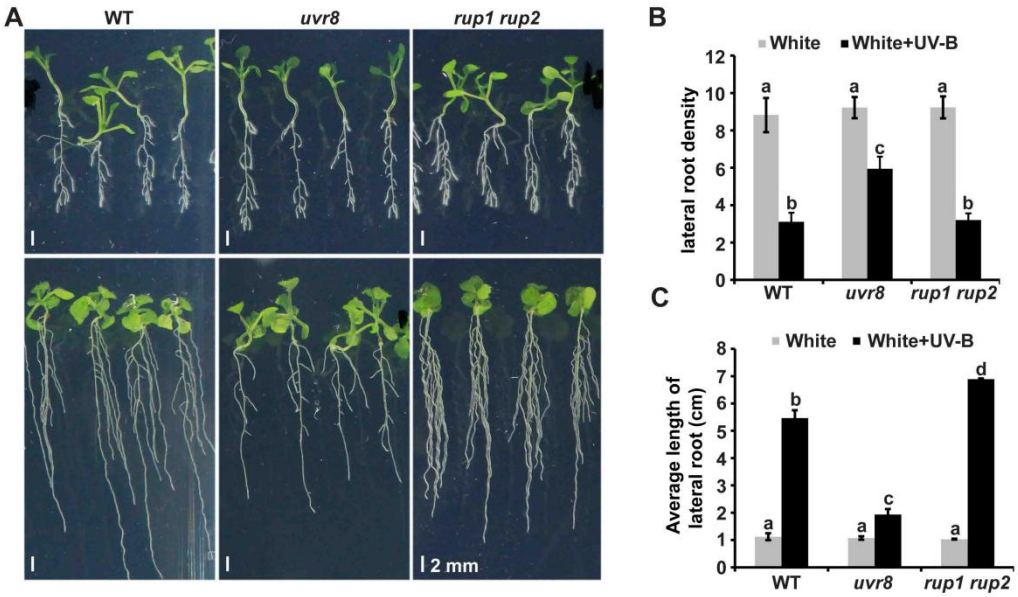
3. The authors added a figure with the goal of showing that IAA, but not NAA, is broken down by UV-B light in a new supplemental Figure 1. This is an important addition, but not yet clear. They need to show side by side the effect of IAA and NAA with and without UV-B on fresh plates, versus those treated with UV for extended periods of time. This figure also needs the 0 nM IAA in panel E and would be better if the time of treatment were shorter and the dose response curve in the presence and absence of UV-B.

It has been reported that auxin homeostasis could be affected by UV-B via photo-oxidative damage, degradation, biosynthesis, and conjugation (Vanhaelewyn, Prinsen et al., 2016). And we would like to check again in our experiments, the results of Appendix Fig 1D showed IAA but not NAA was affected by UV-B via photo-oxidative damage. For this reason, we used mainly NAA to do our experiments.

Fig S1 E would like to show that high concentration of auxin inhibits the lateral root growth. Seedlings of WT was grown in LD condition on 1/2 MS plates without NAA supplemented for 5 days, then transplanted to new medium with the addition of a series of concentrations of NAA and kept in continuous white condition for 7 days. This experiment tested the effects of high concentration of NAA on the growth and development of lateral root. Base on the result of this experiment, we choose 0.4 μM NAA to perform the followed experiments to check the lateral root growth in responses to high concentration of auxin with and without UV-B, so we didn't perform this experiment under UV-B.

We did high concentration of IAA supplemented experiments, seedlings of indicated genotypes were grown in LD on 1/2 MS plats without IAA supplemented for 5 days, then transferred to new plates containing 1 μM IAA then kept in continuous white light with or without UV-B for 7 days. Supplement of high concentration of IAA repressed the growth of main roots and lateral roots, while promoted the generation of lateral roots in WT without UV-B treatment. We found

that the repression of lateral root growth and the promotion of lateral root generation were suppressed with UV-B treatment. Furthermore, high concentration of IAA still inhibited the lateral root growth of uvr8 but did not inhibit lateral root growth of rup1rup2 with UV-B treatment (as shown here Fig A-C). Our results is similar with supplement of high concentration of NAA, but since it has been reported and we also found that IAA homeostasis could be affected by UV-B via photo-oxidative damage, degradation, biosynthesis, and conjugation (Vanhaelewyn, Prinsen et al., 2016), the results supplement of IAA is not solid enough so we used NAA.



(A-C) Seedlings of indicated genotypes were grown in LD (16-h light/ 8-h dark) conditions for 5 days, then transplanted to new plates containing 1 μM IAA and kept in continuous white light or white light plus UV-B for 7 days. Images are shown in (A); bars = 2 mm. The lateral root density (number of lateral roots/length of primary root) (B) and average length of lateral roots (C) of the indicated genotypes were measured. SDs (n > 8 independent seedlings) are indicated. Letters "a" to "d" indicate statistically significant differences for the indicated values, as determined by a one-way analysis of variance (ANOVA), followed by Tukey's least significant difference (LSD) test (P < 0.05).

4. They really need to rearrange Figure 1 and 2 so that they can show parallel images of roots or DR5-GUS staining with and without auxin in the same figure . For example, they can put all the DR5-GUS in one image and separate that from the root development. Figure 1A also has roots at too low a resolution, so it is not really possible to see the lateral roots. The rest of the manuscript shows images more clearly using fewer seedlings on a plate, and similar resolution images should be included here.

We appreciate greatly this excellent point raised by the reviewer! Figure 1 and Figure 2 are arranged as suggested. We moved previous Fig EV2 into Fig 2A-B, seedlings were grown on 1/2 MS with the addition of a series of low concentration (0, 10 and 25 nM) of NAA in continuous white light condition and white plus UV-B condition for two weeks, so as to provide a side by side comparison of growth with and without auxin and UV-B in the same figure in the primary article. The DR5-GUS staining of previous Fig 2D and E were moved to Fig EV 2B and C, so the

results of DR5-GUS are only shown in Fig 1E and F in the primary article. Appendix Fig 3A-B were moved to Appendix Fig 2A-B, so we explain this root phenotype before hypocotyl.

5. Some of the additions requested in the last review are in EV figures. The choices of the authors on where to put data in primary figures, EV figures, and supplemental figures needs to be adjusted. In a number of cases the data generated as a result of reviewer requests needs to be integrated into the primary figures. For example, the DR5-GUS images of lateral roots are far more pertinent than the leaf images and should be central to the manuscript and show up in the primary figures not EV. The leaf images should be supplemental and lateral root expression primary.

Those data are arranged as suggested (the DR5-GUS images of lateral root primordia are in Fig 1E now, and the leaf images are in Fig EV1B).

6. The writing still needs substantial improvement. There are cases where data is not discussed, where conclusions are not logical from the data and where the language needs improvement. Specific examples are listed below.

We appreciate the reviewer's suggestion. We modified our manuscript and it is also modified by a language editing service.

7. There is just too much data presented in a complex way, making it difficult for the reader to follow their results.

We rearranged our data as suggested. Appendix Fig 3A-B were moved to Appendix Fig 2A-B, so we explain this root phenotype before hypocotyl.

8. The statistical analysis of the bar graphs needs to be clarified. Significance is noted with both a "A" and "a or b". The legend indicates that A indicates significant differences, but all the bars marked with A do not look different. It is not clear what they are comparing as well with these statistics. As a result, I am now not convinced of which differences are meaningful on these graphs. They need a detailed section on statistics. They mention Tukey's test, which is usually performed as a post-hoc test after an ANOVA, but whether they did an ANOVA is not clear.

In our previous data, statistical analysis of data with and without UV-B treatment was did separately. We redid the statistical analysis of data with and without UV-B treatment together. Letters "a" to "d" indicate statistically significant differences for the indicated values, as determined by a one-way analysis of variance (ANOVA), followed by Tukey's least significant difference (LSD) test ($P < 0.05$).

9. The reviewers asked the authors to better explain the idea of light being conducted from the shoots to roots. The authors did not adequately address this request in the text of the manuscript.

UV-B light might be conducted through the soil to root to activate UVR8, it is also possible that UV-B light is conducted through plant stem. We have that in our discussion. (line 376)

10. A fundamental point of confusion in their data that is not discussed is why rup1

rup2 has little DR5-GUS signal (in primary and lateral roots) but forms lateral roots and has auxin -induced lateral root formation.

RUP1 and RUP2 physically interact with UVR8 to disrupt the UVR8-COP1 interaction and mediate UVR8 re-dimerization. RUP1 and RUP2 are suppressor of UVR8, UVR8 is more active in rup1 rup2 double mutant, so the decrease in GUS activity was more severe in rup1 rup2 mutant than in the WT after UV-B treatment since UV-B repressed auxin response via UVR8.

Low concentration of auxin induced lateral root growth and formation without UV-B treatment, while the effects were repressed by UV-B in a UVR8-dependent manner, and the repression of lateral root growth by UV-B was more sever in rup1 rup2 than in the WT after the UV-B treatment, since rup1 rup2 is hypersensitive to UV-B.

High concentration of auxin repressed the elongation of lateral roots and promoted the generation of lateral roots, while the effects were repressed by UV-B treatment in a UVR8-dependent manner, and the repression by UV-B treatment was more significant in rup1 rup2 than in the WT, since rup1 rup2 is hypersensitive to UV-B.

11. Throughout the manuscript there are some issues with the bar graphs. For example, in Figure 1, 2, and 4, there is a capital A and small a and b used to denote statistical difference. Rather than red and blue they should be black and white (with an outline). Also, the multi panel bar graphs (like Figure 1 D) need statistics and to be organized in a way where the data is grouped so that the patterns and trends in the data are more evident.

Those bar graphs are redone as suggested and they are black and white now. The multi panel bar graph of Fig 1D is reorganized as suggested, statistics are added.

12. The discussion is slightly, but not substantially improved. The reader needs synthesis of this extremely complicated data set as well as integration into the literature. The final paragraph is a very general summary but does not include sufficient synthesis of the complex dataset.

The discussion is modified as suggested.

Detailed minor concerns:

1. Figure 2 is the first of multiple figures where the effect of UV-B is shown in the presence of auxin. The authors need to have comparison images and data for UV-B in the absence of auxin, so that the interactions of UV-B and auxin can be clarified, as requested previously and noted above.

We appreciate this great suggestion, comparison data of with and without NAA is added in current Fig 2. Seedlings were grown on 1/2 MS with the addition of a series of low concentration (0, 10 and 25 nM) of NAA in continuous white light condition and white plus UV-B condition for two weeks.

2. The introduction discussed R2R-MYB transcriptional factors that control flavonol synthesis. This ties into their finding of MYBs interacting with UVR8, but there is literature on the role of MYB12 that functions to control flavonol synthesis in roots, which are not cited and more pertinent to this work.

We appreciate this great suggestion. This publication was cited in our previous version, and we add that "MYB12 controls flavonol biosynthesis mainly in the root, while MYB111 controls flavonol biosynthesis primarily in cotyledons" in the revision (line 97).

3. Examples of writing issues:
a. Results first heading: roots should be singular
Thanks! It is corrected and the manuscript is checked by a language editing service.

b. The word "dramatically" is used to describe results. They should report fold change, instead of using this descriptor.
Your suggestion is highly appreciated! We changed the sentence to "The density of lateral root in WT is dramatically (about 3.6 times) lower with UV-B treatment than without (Fig 1B)" (line 123).

c. Line 145The authors conclude that that UV light effects are acting through arf7/arf19, as these double mutants have no roots with or without UV. This conclusion is not logical, as if a plant cannot form lateral roots, even if there is a signal acting through a different pathway, it still won't form lateral roots.
As we mentioned that arf7 and arf19 single mutants were more sensitive to UV-B treatment. Our conclusion was based on the results of the single mutants. The phenotype of arf7 arf19 double mutant is too strong, simply had no lateral roots even without UV-B.
We change our sentence to "The arf7 and arf19 single mutants were more sensitive to UV-B treatment than the WT, exhibiting an even greater decrease than the WT in lateral root length with UV-B relative to white light (the lateral root length ratio of WT White+UV-B/White was 57.13%, that of arf7 was 0%, and that of arf19 was 25.24%), indicating that UV-B may regulate lateral root growth via inhibition of ARF7 and ARF19, arf7 arf19 double mutants had no lateral roots regardless of UV-B treatment (Appendix Fig S1A-C). " (Line 141)

d. Line 186: The phrase roots are mainly grown in soil is awkward.
We change the sentence to "In nature, roots are usually covered by soil"(line 177).

e. Line 309: Should read: Next we examined the abundance of auxin-regulated transcripts and found that their levels descreated in myb73 myb77
It is changed (line 319), thanks!

f. Line 304 refers to the lateral root phenotype of uvr8, but whether they mean with or without auxin and the developmental differences need to be defined here
We change the sentence to "however, the lateral root phenotype of uvr8 was partially suppressed in the myb73 myb77 uvr8 triple mutant both with (it showed less sensitive to high concentration of auxin than uvr8 under UV-B light) and without (it had less and shorter lateral root than uvr8 under UV-B light) the addition high concentration of auxin under UV-B light (Fig 6A–C, EV5B), even when the roots were covered (Appendix Fig S7A)" (line 311)

g. Discussion begins with the phrase "There are quite some reports..."

We change to "There are many reports…." (line 359)

h. Line 362 negative should be negatively
It is corrected, thanks! (line 372)

i. Line 366 autonomic should be autonomous
It is corrected, thanks! (line 375)

j. The figure legends (especially the supplemental figures) have numerous grammatical errors or insufficient information on the experiments.
The figure legends are modified, thanks a lot!

3rd Editorial Decision 17th Oct 2019

Thank you for implementing the requested changes in a revised version of your manuscript. Unfortunately there remain a few issues that still have to be ironed out before I can extend formal acceptance of the manuscript.

3rd Revision - authors' response 21st Oct 2019

The authors performed the requested editorial changes.

4th Editorial Decision 17th Oct 2019

Thank you for implementing the final changes in your manuscript. I am now pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal. Thank you for your contribution to our journal, and congratulations on successful publication!

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Hongtao Liu
Journal Submitted to: EMBO J
Manuscript Number: EMBOJ-2019-101928

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	There was not a pre-specified effect size.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Not relevant to this study.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples were excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No
For animal studies, include a statement about randomization even if no randomization was used.	Not relevant to this study.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	Not relevant to this study.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Yes, we confirmed that the data showed normal distribution.
Is there an estimate of variation within each group of data?	The variation has been shown by standard deviation (SD)
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

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).	Myc antibody (Millipore, 05-724), His antibody (MBL, D291-3), GST antibody (Abmart, M20007),UVR8 antibody was a polyclonal antibody made by Youke Company (Shanghai, China)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Not relevant

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Not relevant
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Not relevant
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Not relevant

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Not relevant
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.	Not relevant
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not relevant
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	Not relevant
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not relevant
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.	Not relevant
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Not relevant to this study.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Not relevant to this study.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Not relevant to this study.
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 (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Not relevant to this study.

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 (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No,our study does not fall under dual use research restrictions.