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Peer Review Report for 10.1105/tpc.16.00313

The Arabidopsis Malectin-Like/LRR-RLK IOS1 Is Critical for BAK1-Dependent and BAK1-Independent Pattern-Triggered Immunity

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TPC2016-00313-RA	Submission received:	Apr. 22, 2016
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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2016-00049-RA 1st Editorial decision – *declined* Jan. 27, 2016

Your submission has been evaluated by members of the editorial board. They identified a number of issues that they would like to see resolved in order to consider having the manuscript formally entered into the review process. Therefore, we are returning the manuscript to you so that you can address the issues before we could re-evaluate a revised submission to determine whether it is then suitable for review. Please address the following points:

[Provided below along with author responses]

TPC2016-00313-RA Submission received Apr. 22, 2016

Editor comments on previously declined manuscript and author responses:

We would like to resubmit our IOS1 manuscript that was retracted in 2015 (Plant Cell. 2015 27(5):1563. Retraction. The Arabidopsis Malectin-Like Leucine-Rich Repeat Receptor-Like Kinase IOS1 Associates with the Pattern Recognition Receptors FLS2 and EFR and Is Critical for Priming of Pattern-Triggered Immunity. Plant Cell 26: 3201-3219). We have already sent a version where all the experiments performed by Ching-Wei Chen, the former first author that manipulated the data, were repeated. However, you asked us to complement further the manuscript. Thank you for your recommendations to make our manuscript of better quality. We have addressed most of your remarks.

Point 1. Figure 1: We don't understand what is shown in B. Please be explicit, how many plants were sampled for each bar in the graph. You say 3 technical replicates in the legend. Does this mean that you ground up the sample and plated it three times? (Elsewhere in the manuscript you also use the term technical replicates, please define it each time.) This should not be used as the method for plotting this type of experiment. We expect to see leaves from independent plants, is this what n=3 means? You should show just the biological replicates within one experiment. Technical replicates should not be used here. Please use ANOVA to analyze these growth experiments where you have multiple comparisons. Please use a consistent description of the data analysis. In C the description is different from B; is it really done differently? Unless you are pooling experiments, please use SD.

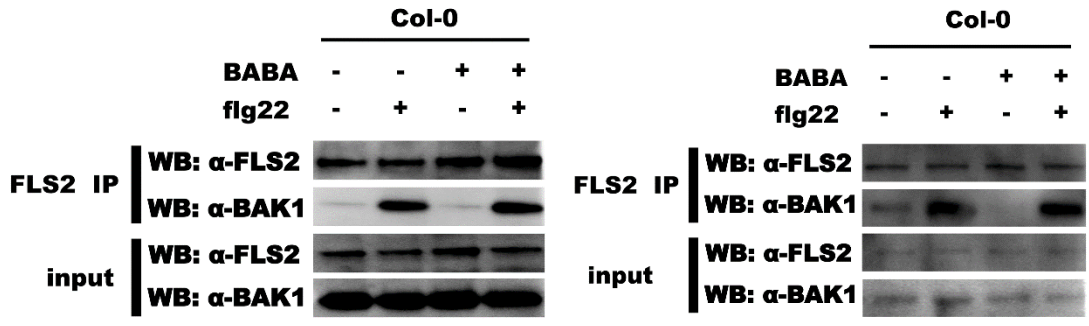
RESPONSE: First and as a general remark, we were asked to show all new replicates in the supplemental figures.

As you mentioned in point 5, there are too many supplemental figures and providing the other replicate(s) in the supplemental figures only for the new experiments would introduce inconsistency with figures not repeated (that have pooled replicates)(typical example is Figure 1B and 1C), as well as creating confusion for the readers. We thus agree with your remark and now, in this new version, show the pooled data in all main figures instead of having the second independent experiment in the supplemental (for graphs). As we cannot pool immunoblots, we only show one immunoblot in the main figure, and remove the second one from the supplemental (you have now seen the second repeat anyway). This will largely simplify the supplemental data section.

We apologize for the misuse of the term “technical replicates”. For us a “biological replicate” was an independent experiment with each plant representing a technical repeat. To summarize, for each CFU independent experiment, we took 9 leaf discs from 3 plants (3 leaf discs per plants pooled, n = 3). We corrected the legends of CFU figures to consider each plant as a biological replicate and wrote consistent cfu legends between different figures. We believe that the use of T-test is appropriate as we only compare the mutant or over-expression lines to the respective wild-type control (no additional chemical treatment involved here, so no multiple comparisons). We now provide a consistent description of the data analysis in the Figure legends.

Point 2. Figures with immunoblots: some figures seem to have very high contrast (eg figure 5, sup Fig 20). Please remake these figure without doing any adjustments of the contrast.

RESPONSE: When the immunoblot signal is strong, the image natively demonstrates a high contrast as acquired directly from the imaging system. Anyhow, we provided the original figures for Figure 5A and 5B, and Supplemental Figure 20 (now Supplemental figure 14) was performed again twice (IP with FLS2 instead of BAK1 as it was working better with this combination). See the 2 replicates below:



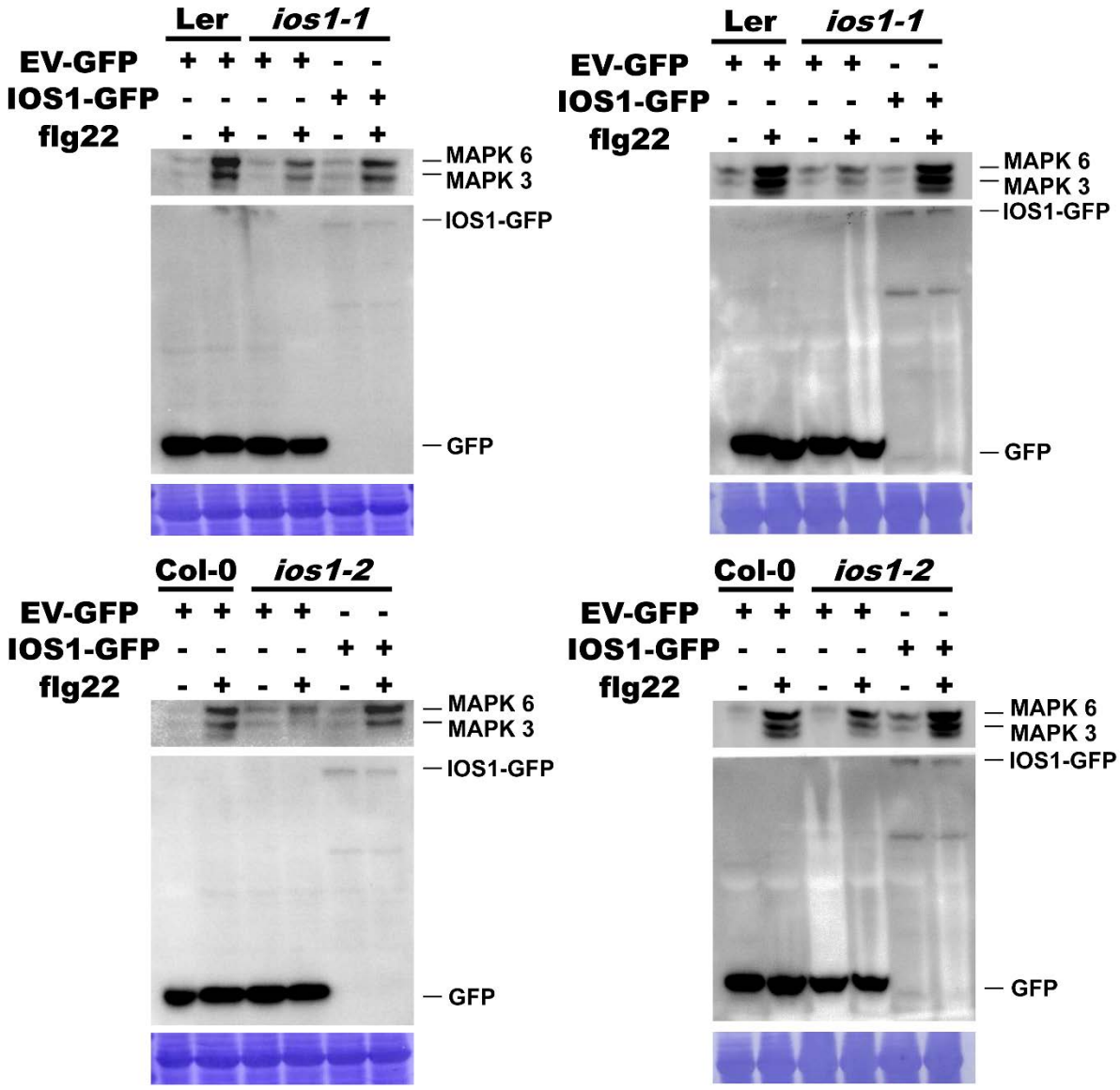
Supplemental Figure 14. BABA does not regulate ligand-induced FLS2-BAK1 association.

Co-immunoprecipitation analysis was performed with 5-week-old Arabidopsis pretreated (+) or not (-) **with 225 µM** BABA 2 days before treatment (+) or no treatment (-) with 100 nM flg22 for 10 min. Total proteins (input) were

subjected to immunoprecipitation (IP) with anti-FLS2 antibodies and IgG beads followed by immunoblot analysis using anti-FLS2 and anti-BAK1 antibodies.

Point 3. We would like to see evidence that the GFP fusion of IOS1 does not affect its biological function (i.e. does it complement the mutants? we don't consider dimerization enough proof that the protein is biologically functional).

RESPONSE: We tested complementation of ios1-1 and ios1-2 mutant protoplasts with the IOS1-GFP construct through the analysis of MPK3 and MPK6 phosphorylation after flg22 treatment. Expression of the IOS1-GFP construct did complement the defective phosphorylation of MPK3 and MPK6 in both ios1 mutants. Complementation data were added as Supplemental Figure 11. Both replicates are shown below.



Supplemental Figure 11. Complementation of defective MAPK activation in ios1-1 and ios1-2 mutants by IOS1-GFP.

Ler-0 and ios1-1 or Col-0 and ios1-2 Arabidopsis protoplasts expressing IOS1-GFP or the Empty Vector (EV)-GFP were treated with (+) or without (-) 100 nM flg22 for 5 min. Immunoblot analysis using phospho-p44/42 MAP kinase antibody is shown in top panel. Lines indicate the positions of MPK3 and MPK6. Anti-GFP antibody detects IOS1-GFP and EV-GFP (middle panel). Coomassie Brilliant Blue-staining is used to estimate equal loading in each lane (bottom panel).

Point 4. Please shorten the title to be more concise, we suggest: The Arabidopsis Malectin-like/LRR Receptor-like Kinase IOS1 is Critical for Pattern-Triggered Immunity.

RESPONSE: We propose a concise title: The Arabidopsis Malectin-like/LRR-RLK IOS1 is Critical for BAK1-Dependent and BAK1-Independent Pattern-Triggered Immunity.

Point 5. Please reduce the number of supplemental figures or provide in your cover letter explicit justification for each figure and why it is necessary for the manuscript. For example are there cases where you have replicated the experiment and you can pool the data? Consider whether all data in the supplement is really necessary to make your main point. Data that is integral to the main arguments of the paper should be in the main figures.

RESPONSE: Please see the first, general remark in point 1.

Point 6. There is concern about whether the data in Supplemental Fig.5 is reliable. The leaf samples shown and the lesion size measuring presented do not exactly convey the same message. Therefore, the board would like to see a second hemibiotrophic fungus tested (Erysiphe cruciferarum), which the Keller lab has used in their publication.

RESPONSE: We agree that the lesion sizes shown on pictures are not exactly matching the average results shown in the graph. We thus performed the Botrytis experiments twice again, and could reconfirm the data. Botrytis cinerea is not a hemibiotrophic pathogen, but a necrotrophic fungal pathogen. To further test the possible role of IOS1 against necrotrophic fungal pathogens we used Alternaria brassicicola that is commonly used as a pathogen involved in chitin recognition/signaling (Miya et al. 2007). Powdery mildews such as Erysiphe cruciferarum were already tested by the Keller laboratory. We therefore added more discussion about their results in the discussion section (see point 7).

Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., and Shibuya, N. (2007). CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in Arabidopsis. Proc. Natl. Acad. Sci. USA 104: 19613-19618.

Point 7. Please be sure to include a discussion of all published work relevant to your manuscript There seem to be at least one recent publications about ios1 mutants that are not discussed.

RESPONSE: We could only find one new article about IOS1 (from Keller lab, Hok et al., 2014). We already discussed about this article in the first part of the discussion section. Importantly, we now have added a discussion about why the role of IOS1 is not clear during Arabidopsis resistance to fungal pathogens that produce chitin in the chitin/CERK1 section of the discussion:

"By contrast, the role of IOS1 in Arabidopsis resistance against necrotrophic fungi such as B. cinerea and A. brassicicola was rather weak. In addition, the ios1-1 mutant is more resistant to the biotrophic fungus Erysiphe cruciferarum (Hok et al., 2014). These observations suggest that IOS1 role in the Arabidopsis resistance against pathogens producing the MAMP chitin is not critical. As suggested for oomycete pathogens (Hok et al., 2011), fungal pathogens may produce effectors that target IOS1 and the absence of IOS1 may result in WT or enhanced Arabidopsis resistance levels even though IOS1 is critical for a full chitin-mediated defense response."

Hok, S., Allasia, V., Andrio, E., Naessens, E., Ribes, E., Panabières, F., Attard, A., Ris, N., Clément, M., Barlet, X., Marco, Y., Grill, E., Eichmann, R., Weis, C., Hückelhoven, R., Ammon, A., Ludwig-Müller, J., Voll, L.M., and Keller, H. (2014) The receptor kinase IMPAIRED OOMYCETE SUSCEPTIBILITY1 attenuates abscisic acid responses in Arabidopsis. Plant Physiol. 166: 1506-1518.

TPC2016-00313-RA 1st Editorial decision – *revision requested* May 21, 2016

You will see from the reviewer comments there are a number of issues which you will need to address before we can reach a final decision. We are not asking you for additional experiments, but you will need to change the writing to accommodate the criticism raised by the two reviewers. In particular, this concerns the plasma membrane localization of IOS1 where the conclusions should be softened based on the criticism raised by reviewer #2. In addition, the issue that you used different concentrations of the MAMP in different experiments should be explained by saying that typical concentrations were used for the different assays but dose-dependent effects cannot be fully

excluded. To deal with the criticism of reviewer #2 with respect to the protoplast system and over-expression we consider it appropriate if you soften your conclusions somewhat and point out possible caveats. With respect to the MAMP response you should stress that there are differences at the single time point studied and you are not providing a kinetic analysis which could have revealed a faster or slower response. In addition, there are a large number of minor points which have been raised by the two referees and which you need to address. In general, both reviewers and all of us appreciate how you have handled the case of data manipulation. However, one reviewer noted that the retracted manuscript is still in your research gate profile, and we advise you to remove it.

---------- Reviewer comments:

Reviewer #1 (Comments for the Author):

This resubmitted work reports a malectin-like/leucine-rich repeat receptor-like kinase, IOS1, as a new partner protein of the pattern recognition receptors (PRRs), FLS2, EFR and CERK1, as well as the PRR-associated BAK1 adaptor/co-receptor protein. Interactions between IOS1 and the PRRs are independent of BAK1 and are ligand independent. Plants lines with IOS1 expression are altered in resistance to several pathogens. IOS1 is a component of the beta-aminobutyric acid (BABA)-mediated priming response.

While still lacking mechanistic insights on how it functions, it is an important contribution in identifying components that may reveal how BABA boosts defense response -possibly already at the PRR protein complex level. The work further shows the complexity of receptor components needed for modulating and/or transmitting signal perception of MAMPs.

I applaud the authors for their honesty and manner of handling the unfortunate situation with the data manipulation incident. On the whole, the core findings have not been compromised by this.

However, I do have 2 minor points:

1. I wonder why the authors used different MAMP concentrations for the various PTI assays (10nM for ROS; 1μM for callose, 100nM or sometimes 50 nM for MAPK activation, or 0.75μM for BIK1 phosphorylation)? This complicates interpretation and comparison. In lines478-482, they discussed the altered MPK3/6 activities in the ios1 or IOS1-OE plants with respect to a BIK1-independent signaling cascade. Ranf et al 2014 (DOI: 10.1186/s12870-014-0374-4) also showed no dependence on BIK1 or PBL1 for the flg22-induced MAPK activation in a Col-0 background. However, they also showed that in the C24 background of their mutant, there was an effect for several pbl1 mutant alleles - and importantly, a dose-dependent effect on MAPK activation. That is my concern here that in this manuscript, various concentrations of MAMPs were used throughout the manuscript. Some justification should be made for this decision to use different concentrations.

2. In Fig. 7E, it is not immediately apparent that the IOS1-OE in the bak1-5 and bik1 backgrounds were actually independent transformation events. It should be mentioned and stated if independent transgenic lines had in fact been investigated.

Reviewer #2 (Comments for the Author):

Importance of findings:

The ms "The Arabidopsis Malectin-Like/LRR-RLK IOS1 is Critical for BAK1-Dependent and BAK1-Independent Pattern-Triggered Immunity" contains interesting data on the unexpected contribution of IOS1 to PTI and PRR complex formation in Arabidopsis. It suggests that IOS1 is constitutively associated with PRRs and BAK1 and involved in ligand-activated PRR complex formation, BAK1-dependent callose deposition, and BABA induced defense priming.

Quality of experiments:

Data appear largely sound but at several points additional controls and more accurate quantification is required. A lot of the data stem from transient over-expression systems in protoplast. These systems and particular the BIFC assay used are prone to artefacts. I therefore suggest additional controls (see below).

Points In favor:

Experimental setup and data appear generally sound and reliable. Although the manuscript does not provide a deep mechanistic understanding of how IOS1 acts at the biochemical level, genetics and protein interaction studies suggest that IOS1 functions in BAK1-dependent and BAK1-independent defense responses but not in BIK1-mediated responses. This is surprising because IOS1 was originally described as a susceptibility factor in interaction with filamentous biotrophs.

Points detracting:

Literature data on IOS1 have been incompletely discussed and partially interpreted in a way I cannot not really follow.

The discussion is partly redundant with the introduction and rather lists literature data without distinct discussion of possible mechanisms of IOS1 function.

Author actions necessary for acceptance:

1) Plasma membrane localization of IOS1 is insufficiently supported. The pattern of IOS1 is very uneven for a PM protein. Cytoplasms is largely out of focus and no z-stacks are provided. Please provide better pictures with co-expression of cytoplasmic red fluorescing protein to contrast this. Co-expression of a red ER-marker is further recommended.

2) All BIFC data stem from overexpression in protoplasts. I do not generally doubt all BIFC data but more proper controls should be provided. At the moment I am not fully convinced that IOS1 does not non-specifically interact with many types of RLKs. Since malectin proteins act in ER quality control and chaperoning of N-glycosylated proteins in the ER, it is well possible that IOS1 is a chaperoning RLK that interacts with many kinds of glycosylated RLKs. This could actually explain the pleiotropic phenotypes in other types of biotic stress responses.

3) BIFC is prone to artefacts if no point mutants of the interaction surface are taken as negative controls. If this is not available, FRET assays or at least a non-specific LRR-RLKs (instead of FLS2 and EFR) should be integrated as negative controls. The BIFC signals should be also normalized and quantified. Pictures are not satisfying in front of the general sensitivity of this methods for false positive signals.

4) The same negative controls should be used for Co-IP in this case.

5) Lines 264 ff. This is important because it provide protein interaction evidence without overexpressing EFR as far as I understand. However, I got confused here: Is this published data (Roux et al. 2011) to which you refer or new data. If it was published before, please move this into the discussion. If it is new data, please describe the experiment in more detail by mentioning how many proteins in total were Co-IPed specifically with EFR.

6) The MPK assays show only one point in time. It is impossible to conclude from this about a general increase of reduction of MPK response; it could also be a faster or slower response. Please provide kinetics (5, 10, 20 min).

7) Please adequately discuss IOS1 literature: The introduction reads as if nothing is known about the function of IOS1 in plant-microbe interactions. This is inadequate.

Discussion of published literature about IOS1 is incomplete. Published data report on unchanged expression in pathogen interactions of FRK1, which is reported here to be differentially expressed. Callose deposition in response to fungi is unchanged in literature data but changes here in PAMP response. IOS1 influences ABA-triggered stomata closure but does not influence bacteria-triggered stomata closure. I guess this should be discussed somehow.

It is difficult to digest that a gene reported to be a susceptibility factor turns here into a key component of PTI. I do not doubt presented data but the discussion on this contradiction is neither satisfying nor conclusive. If IOS1 would be an effector target, this would still make the ios1 mutants more susceptible if IOS1 is primarily involved in PTI. Are you sure that Hok et al really discussed it that way? I did not read this out of their papers that way? They rather conclude that it is somehow used by the pathogen in supporting pathogenesis or in regulating metabolism required for biotrophy. They did not consider it as a regulator of immunity.

The function of IOS1 in ABA signaling is also neglected.

Reviewer comments on previous submission and author responses:

Many thanks for the positive feedback on our manuscript TPC2016-00313-RA entitled "The Arabidopsis Malectin-Like/LRR-RLK IOS1 is Critical for BAK1-Dependent and BAK1-Independent Pattern-Triggered Immunity", which we received on May 22, 2016. The reviewers have made helpful suggestions for improving the quality of our manuscript and we have addressed most of the reviewer's remarks.

Reviewer #1

Point 1. I wonder why the authors used different MAMP concentrations for the various PTI assays (10nM for ROS; 1μM for callose, 100nM or sometimes 50 nM for MAPK activation, or 0.75μM for BIK1 phosphorylation)? This complicates interpretation and comparison. In lines478-482, they discussed the altered MPK3/6 activities in the ios1 or IOS1-OE plants with respect to a BIK1-independent signaling cascade. Ranf et al 2014 (DOI: 10.1186/s12870-014-0374-4) also showed no dependence on BIK1 or PBL1 for the flg22-induced MAPK activation in a Col-0 background. However, they also showed that in the C24 background of their mutant, there was an effect for several pbl1 mutant alleles - and importantly, a dose-dependent effect on MAPK activation. That is my concern here that in this manuscript, various concentrations of MAMPs were used throughout the manuscript. Some justification should be made for this decision to use different concentrations.

RESPONSE: In general, different PTI outputs show different sensitivities to MAMPs. We tried to use the most relevant concentration for each PTI output, hence the use of different concentrations of MAMPs for each PTI assays. To soften our conclusion, we added the following sentence at the end of part 1 in the "Discussion" section: "Noteworthy, typical concentrations of MAMPs were used for each PTI assay resulting in the use of various concentrations of MAMPs in different experiments. We thus cannot fully exclude dose-dependent effects."

Point 2. In Fig. 7E, it is not immediately apparent that the IOS1-OE in the bak1-5 and bik1 backgrounds were actually independent transformation events. It should be mentioned and stated if independent transgenic lines had in fact been investigated.

RESPONSE: Since silencing of *IOS1* over-expression occurs frequently in subsequent generations, independent transformation of each mutants were used in Fig. 7E. To clarify this important point, we added this sentence in the legend of Fig. 7E: "For *IOS1*-OE lines in the *bak1-5* and *bik1* backgrounds, data represent 2 independent transformation events for each genotype."

Reviewer #2:

Point 1. Plasma membrane localization of IOS1 is insufficiently supported. The pattern of IOS1 is very uneven for a PM protein. Cytoplasms is largely out of focus and no z-stacks are provided. Please provide better pictures with co-expression of cytoplasmic red fluorescing protein to contrast this. Co-expression of a red ER-marker is further recommended.

RESPONSE: We provided a new picture of the PM localization with a more even pattern. It has to be noted that the GFP pattern follows exactly the blue pattern of the PM marker. We also soften our conclusions by adding "likely" in the title (line 204) and in the last sentence of the "Results" section mentioning IOS1 localization: "These data suggest that similarly to the PRRs FLS2 and EFR (Robatzek et al., 2006; Haweker et al., 2010), IOS1 is likely localized at the plasma membrane."

Point 2. All BIFC data stem from overexpression in protoplasts. I do not generally doubt all BIFC data but more proper controls should be provided. At the moment I am not fully convinced that IOS1 does not non-specifically interact with many types of RLKs. Since malectin proteins act in ER quality control and chaperoning of N-glycosylated proteins in the ER, it is well possible that IOS1 is a chaperoning RLK that interacts with many kinds of glycosylated RLKs. This could actually explain the pleiotropic phenotypes in other types of biotic stress responses.

RESPONSE: For BiFC, we first show that FLS2 and BAK1 do not interact before flg22 treatment (as expected) to show that the "no interaction" scenario can be observed with FLS2. In addition, we used the plasma membrane LTI6b that is commonly used for such studies to show that the "no interaction" scenario can also be observed with

IOS1. We also provided data of pull-down and Co-IP in protoplast for both FLS2 and EFR and in planta Co-IP with native FLS2 and BAK1 and proteomics analyses for EFR to support the association study and BiFC conclusions. Nevertheless, we added the sentence "Although we cannot exclude artifacts inherent to over-expression in protoplasts" at the concluding sentence of the BiFC "Result" section.

Point 3. BIFC is prone to artefacts if no point mutants of the interaction surface are taken as negative controls. If this is not available, FRET assays or at least a non-specific LRR-RLKs (instead of FLS2 and EFR) should be integrated as negative controls. The BIFC signals should be also normalized and quantified. Pictures are not satisfying in front of the general sensitivity of this methods for false positive signals.

RESPONSE: For controls, please look at our response at point 2. For quantifications, we did the BiFC assay many times (more than what mentioned in the manuscript) and, for what we concluded to be positive interactions, we always clearly see a signal (i.e. interactions) in most of the protoplasts. We thus believe that showing a representative picture is honest and sufficient.

Point 4. The same negative controls should be used for Co-IP in this case.

RESPONSE: For Co-IP in protoplasts and similarly to BiFC, we used LTI6b as a negative control. The protoplast Co-IP is supported by in planta Co-IP with native FLS2 and BAK1 minimizing possible artifacts related to over-expression. Also, IOS1 was identified by IP-MS using lines expressing EFR-GFP under its own promoter in a null *efr* mutant background.

Point 5. Lines 264 ff. This is important because it provide protein interaction evidence without overexpressing EFR as far as I understand. However, I got confused here: Is this published data (Roux et al. 2011) to which you refer or new data. If it was published before, please move this into the discussion. If it is new data, please describe the experiment in more detail by mentioning how many proteins in total were Co-IPed specifically with EFR.

RESPONSE: EFR IP was indeed performed using transgenics expressing constructs driven by the EFR native promoter. We agree that the way the EFR IP data are described is confusing. In fact, the EFR IP data were published before but only to mention SERKs (Roux et al. 2011) and RBOHD (Kadota et al., 2014) as EFR-associated proteins. In the current manuscript, we report that IOS1 is also in the list of candidate EFR-associated proteins identified by IP-MS. We thus removed the "Roux et al., 2011" citation at lines 266 and 439 as the text has nothing to do with SERKs. The fact that IOS1 was found in complex with EFR is new data (so no citations). The "Roux et al. 2011" and "Kadota et al., 2014" publications were instead cited in the "Intro" and "Discussion" sections, respectively. It is however difficult to mention how many proteins were Co-IPed with EFR as the list depends on whether antiGFP agarose or antiGFP magnetic beads were used and on the threshold used and the washing conditions. We thus prefer not to mention how many candidates were obtained. Importantly, IOS1 peptides were detected in both systems (antiGFP agarose and antiGFP magnetic beads).

References that mention the EFR IP experiment:

Kadota Y, Sklenar J, Derbyshire P, Stransfeld L, Asai S, Ntoukakis V, Jones JD, Shirasu K, Menke F, Jones A, Zipfel C. (2014). Direct regulation of the NADPH oxidase RBOHD by the PRR-associated kinase BIK1 during plant immunity. Mol Cell. 54: 43-55.

Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F.G., Tor, M., de Vries, S., and Zipfel, C. (2011). The Arabidopsis Leucine-Rich Repeat Receptor-Like Kinases BAK1/SERK3 and BKK1/SERK4 Are Required for Innate Immunity to Hemibiotrophic and Biotrophic Pathogens. Plant Cell 23: 2440-2455.

Point 6. The MPK assays show only one point in time. It is impossible to conclude from this about a general increase of reduction of MPK response; it could also be a faster or slower response. Please provide kinetics (5, 10, 20 min).

RESPONSE: We added this new sentence at the end of the "Result" section (line 202): "However, since we do not provide a kinetic analysis, we cannot exclude a slower or faster MPK response in *ios1* mutants or OE lines, respectively."

Point 7a. Please adequately discuss IOS1 literature: The introduction reads as if nothing is known about the function of IOS1 in plant-microbe interactions. This is inadequate.

RESPONSE: To mention already known IOS1 function in Arabidopsis immunity, the sentence: "IOS1 is known to contribute to disease caused by filamentous (hemi)biotrophs and to attenuate abscisic acid (ABA) responses in

Arabidopsis (Hok et al., 2011; 2014)." was added in the "Introduction" section.

Point 7b. Discussion of published literature about IOS1 is incomplete. Published data report on unchanged expression in pathogen interactions of FRK1, which is reported here to be differentially expressed. Callose deposition in response to fungi is unchanged in literature data but changes here in PAMP response. IOS1 influences ABA-triggered stomata closure but does not influence bacteria-triggered stomata closure. I guess this should be discussed somehow.

RESPONSE: We discussed the FRK1 expression and callose deposition aspects by adding the following sentence in the "Discussion" section (line 405): "By contrast, both FRK1 expression and callose deposition were not affected in the *ios1-1* mutant after inoculation with filamentous pathogens (Hok et al., 2014). These discrepancies may be explained by early (this work) versus late time point analyses (Hok et al., 2014)."

A short discussion was added to clarify the discrepancy between the ABA hypersensitivity and WT stomatal closure upon contact with bacteria in the *ios1-1* mutant: "Stomata of the *ios1-1* mutant are hyper-responsive to ABA (Hok et al., 2014), and ABA signaling is critical for stomatal immunity (Melotto et al., 2006; Desclos-Theveniau et al., 2012), but we observed WT stomatal closure in *ios1-1* after *Pst* DC3000 inoculation. As suggested by Hok et al. (2014), IOS1 may use different signalings for the activation of PTI in response to bacteria and in the down-regulation of ABA upon infection with filamentous pathogens."

Point 7c. It is difficult to digest that a gene reported to be a susceptibility factor turns here into a key component of PTI. I do not doubt presented data but the discussion on this contradiction is neither satisfying nor conclusive. If IOS1 would be an effector target, this would still make the ios1 mutants more susceptible if IOS1 is primarily involved in PTI. Are you sure that Hok et al really discussed it that way? I did not read this out of their papers that way? They rather conclude that it is somehow used by the pathogen in supporting pathogenesis or in regulating metabolism required for biotrophy. They did not consider it as a regulator of immunity.

RESPONSE: With the current limited mechanistic understanding of the function of IOS1 in Arabidopsis immunity, it is indeed difficult to imagine how IOS1 may act as a susceptibility factor for some type of pathogens and as a positive regulator during the PTI response for other pathogens. We thus prefer to limit our discussion on this matter to a minimum.

Point 7d. The function of IOS1 in ABA signaling is also neglected.

RESPONSE: Our story about the function of IOS1 in PTI does not involve ABA, but rather directly connect IOS1 to PRR complex formation at the plasma membrane. We thus did not discuss in details about the previously reported involvement of IOS1 in ABA signaling, as this most likely represent another function of IOS1. We have however added a discussion about the ABA hypersensitivity of *ios1-1* stomata (see point 7b).

TPC2016-00313-RAR1 2nd Editorial decision – *acceptance pending* June 8, 2016

We are pleased to inform you that your paper entitled "The Arabidopsis Malectin-Like/LRR-RLK IOS1 is Critical for BAK1-Dependent and BAK1-Independent Pattern-Triggered Immunity" has been accepted for publication in The Plant Cell, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership.

Final acceptance from Science Editor June 17, 2016
