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A Fully Functional ROP Fluorescent Fusion Protein Reveals Roles for this GTPase in Subcellular and Tissue-level Patterning

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Review timeline:

TPC2020-00440-RA	Submission received:	June 8, 2020
	1st Decision:	July 14, 2020 *revision requested*
TPC2020-00440-BR1	1st Revision received:	Aug. 10, 2020
	2nd Decision:	Aug. 20, 2020 *accept with minor revision*
TPC2020-00440-BR2	2nd Revision received:	Aug. 25, 2020
	3rd Decision:	Aug. 31, 2020 *acceptance pending, sent to science editor*
	Final acceptance:	Sept. 7, 2020
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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.)

TPC2020-00440-RA 1st Editorial decision – *revision requested* July 14, 2020

The reviewers appreciated the technical strengths of this work and agreed that the functional ROP probe is a valuable tool. However, reviewers felt that the technical advance was not accompanied by significant new mechanistic work that advances our understanding of ROP function. For example, the tight association of ROPs with tip growth and cell polarization is already established, the phenotypic analysis did not provide much new insights, and ROP localization has previously been shown to be unaffected by LatB and oryzalin treatments. Similarly, while the polar localization of ROP4 after cytokinesis was noted to be interesting, its functional significance was not studied. The reviewers have provided several useful suggestions to strengthen this work. Please note that points 2 and 3 by Reviewer #1 can be addressed in the Discussion and do not have to be addressed experimentally.

Given the technical emphasis of this work, we suggest that you consider submitting the revised manuscript as a Breakthrough Report. However, you are welcome to re-submit as a Research Article if you prefer.

RESPONSE: As suggested, we have substantially modified the manuscript and resubmitted it as a Breakthrough Report. We thank the reviewers and editors for careful reading of the manuscript and for their constructive suggestions. We believe the revised manuscript better emphasizes the significance of studying localization of a functional ROP fusion in protonemata, which in contrast to root hairs and pollen tubes, is a developing tissue with undifferentiated apical stem cells exhibiting polarized growth. We view that this study is an important technical advance and describes exciting insights into ROP function in tissue development.

Here we briefly list the changes that we made to the figures, which was accompanied by significant reorganization of the text:

Figure 1 – we combined all the growth assay data, RNAi and stable knockouts (formerly part of Figure 2), demonstrating that the sandwich tagged ROP4 protein is functional.

Figure 2 – we quantitatively compared the localization of the N-terminal and sandwich tagged fusion at the cell apex in response to the reviews, demonstrating that ROP4-swmNG exhibits a steeper gradient and covers a smaller region of the cell tip. Data from this figure had formerly been in Figure 3 and Figure S1. The quantification is new

analysis.

Figure 3 – we added new data demonstrating the behavior of ROP4-swmNG at the cell apex for a cell that grew, paused, and then re-initiated growth (Figure 3A). This was combined with long-term oryzalin data showing ROP4-swmNG localization during growth initiation at ectopic sites as well as localization during frequent changes in growth direction (formerly Figure 5). We also included short-term responses to latrunculin B and oryzalin (formerly Figure 4) demonstrating that ROP localization depends on the cytoskeleton in recently divided apical cells.

Figure 4 – this was formerly Figure 2 in the original manuscript. We felt that the manuscript read better describing the null mutants closer to the localization data that demonstrates ROP is recruited to the cell cortex at the onset of mitosis in the apical cell and then remains at the future branching site many hours before a new branch emerges.

Figure 5 – this was formerly Figure 6.

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

[Reviewer comments shown below along with author responses]

TPC2020-00440-BR1 1st Revision received Aug. 10, 2020

Reviewer comments and **author responses**:

Reviewer #1:

This manuscript by Cheng and colleague describes roles of ROP GTPases using the newly designed functional ROP probe in the moss *Physcomitrella patens*. Based on the knowledge from yeasts, the authors produced genome-edited lines in which a fluorescent protein was inserted within the coding region of ROP4 GTPase (ROP4-swmNG). Using their unique RNAi technique, the authors show that the ROP4-swmNG was functional while N-terminally tagged ROP4 was not. The authors further demonstrated this by producing *ROP1/2/3* knockout lines. The authors analyzed the localization pattern and mobility of the functional ROP4 in protonemata. The authors found that the localization of ROP4 is basically independent of the cytoskeleton and well coupled with the polar cell growth. Interestingly, ROP4 marked not only growing tip but also sidewalls prior to cell division and showed polar localization after cytokinesis. This study potentially shows technical advance for ROP research in plants and provides new insights into ROP function in plants. However, considering that the tight association of ROPs with tip growth and cell polarization is already well known, I think further analysis is needed to reveal the exact roles of ROP GTPases.

Point 1. The authors claim that ROP4swmNG is "fully" functional. I agree with the authors that, unlike GFP-ROP4, ROP4swmNG is functional. However, Fig1C shows slight but significant decrease in the area of ROP4-swmNG plants in the control condition. I wonder if ROP4swnNG may not be fully functional in terms of cell growth. To clarify the effect of swmNG, I suggest that the authors directly compare the phenotype between WT and WT/ROP4swmNG, and between delta *rop1/2/3* and delta *rop1/2/3/ROP4swmNG*, which are used in Fig 3.

RESPONSE: We thank the reviewer for the suggestion and have compared all three genotypes to WT in the revised Figure 1F and G.

Point 2. The Authors claim that delta *rop1/2/3/4* does not maintain cell polarity nor form gametophores. It is, however, still possible that the *rop* null plants actually form gametophore but it just looks like protonema due to a lack of cell polarity. The authors need to check the expression levels of gametophore-specific genes to demonstrate if they are indeed protonema.

RESPONSE: This is an interesting point. However, finding the conditions to optimally test this is challenging. The Δ*rop1/2/3/4* plants grow extremely slowly. Normally we would find gametophores in 3-4 week-old wild-type tissue. But if we do expression analysis in 3-4 week-old Δ*rop1/2/3/4* tissue, we would not be confident that this time point is developmentally equivalent. Thus, if we do not find expression of gametophore-specific genes, it may just be that we have not sampled at the appropriate time. It is unclear to us what the optimal time point would be for assaying gene expression. Given these difficulties and based on the editor's suggestion that we address this point in the Discussion, we have added the following sentences to the Discussion:

"Plants lacking all four *ROP* genes were characterized by a mass of loosely attached round cells with no obvious branching pattern. While △*rop1/2/3/4* plants did not develop any structures that resembled gametophores, it is possible that gametophore-specific gene expression still occurs. Nevertheless, even if appropriate gene expression were to occur in these plants, the lack of obvious morphological structures such as branching protonemata and gametophores demonstrate that ROP is essential for patterning tissues as well as single cells."

Point 3. In the Discussion, the authors mention that GFP-ROP had a larger fraction of cytosolic protein and a shallower tip gradient. This must be an important point to know why ROP4swmNG is functional but conventional GFP-ROP is not functional. So, I suggest that the authors demonstrate this experimentally. The gradient pattern and FRAP can be quantified to compare GFP-ROP4 and ROP-swmNG.

RESPONSE: Thank you for this very important comment. Since our paper has focused on the functionality of the sandwich tag, we agree with the reviewer that carefully documenting differences in localization between the N-terminal and sandwich tags should be included. As a result, we have reorganized the manuscript to reflect this. The new figure 1 compares growth of plants with the various tags demonstrating functionality. The new figure 2 quantitatively compares the apical localization of the N-terminal and sandwich fusions. While on average we did observe higher cytosolic fluorescence levels in the N-terminal tag as compared to the sandwich tag, the difference was not statistically significant for ROP4 tagged with a single fluorescent protein. Therefore, we removed that statement from the Discussion and instead focused on the robust difference between the apical tip gradient, quantifying both the steepness of the gradient as well as the width of the peak fluorescence normalized to the cell width. These data are now part of the new Figure 2.

Point 4. ROP pattern during cell division is very interesting but the exact role of ROP during cell division is still obscure. I suggest that the authors observe cell plates and cytoskeletons during cell division in the *rop* null plants to reveal how cytokinesis, division plane, and cell adhesion are affected at a subcellular level.

RESPONSE: While we agree that these are very interesting experiments to pursue, imaging in the ROP null plants is very challenging as they grow extremely slowly. Furthermore, it is difficult to draw definitive conclusions on possible effects to the cytoskeleton and subcellular processes in the context of a stable knockout with such a dramatic phenotype. It might be problematic to conclude that the effects are due directly to loss of ROP function rather than compensation or changes in cell geometry. Thus, we are currently in the planning stages to develop a system, where it would be possible to acutely remove the remaining ROP protein in the ROP4-swmNG line so that we can study the consequences to the cytoskeleton during division plane specification and cytokinesis as well as the effects on cell adhesion. This system will be able to identify the immediate consequences of the loss of ROP function. Unfortunately, setting up this system will take a significant amount of time and is beyond the scope of this study.

Point 5. Figure 4 needs control plants that were not treated with the inhibitors. I wonder if the susceptibility to oryzalin and latrunculin B depends on the growth rate of tip cells, rather than the time after cell division. Could the authors elaborate on this?

RESPONSE: We apologize for the omission, but we had already performed the control. We have modified the results to reflect this:

"First, we identified actively growing apical cells and then infused 0.5% DMSO and discovered that all actively growing cells retained ROP localization at the cell tip (N=34 cells). In contrast after infusion with latrunculin B, we observed that roughly 30-40% of cells lost the apical ROP gradient (Fig. 3D). Infusion with oryzalin, which disrupts microtubules, also resulted in a similar fraction of cells that lost the apical gradient."

If susceptibility to oryzalin and latrunculin B depended on growth rate, we would have expected to observe different growth rates for long and short cells. However, in our experience, an actively growing cell has the same growth rate throughout the whole cell cycle.

Point 6. Figure 5 and Figure 6 need quantification. In Figure 5, the direction of cell growth and the position of ROP relative to the apex can be plotted. This would visualize the spatial and temporal relationship between cell growth and ROP polarization. For Figure 6B, frequency of the protoplast expelling position can be counted. Ideally, ROP4-swmNG may be observed prior to cell wall digestion.

RESPONSE: We have now provided new data where an actively growing cell pauses and then resumes growth. This cell loses apical ROP localization, gains it to one side of the cell tip and then starts to grow towards the maximal ROP signal, shown in Figure 3A. Unfortunately, because these types of events are stochastic, it is difficult to get enough numbers to perform a quantitative analysis of this behavior. For that reason, we had initially performed the experiments with oryzalin since one can observe multiple pauses as well as initiation of new growth directions in a single cell. The quantification suggested by the reviewer is difficult in the oryzalin experiments because often it is not possible to morphologically identify the cell apex. For this reason, we instead tracked the trajectory of the ROP localization and overlaid that on the images so it would be possible to observe how the trajectory correlates with the ultimate growth of the cell. Video 2 is an optimal way to view the correlation between track and growth.

As suggested by the reviewer, we quantified the frequency with which protoplasts emerged from the wall adjacent to an existing cross wall. We have included the following sentences in the revised manuscript:

"Surprisingly, 47% of the time (N=15 cells) we found that protoplasts were expelled from subapical cells with no obvious branch protrusions (Fig. 5B black arrows, Video 11) at the site where we had observed ROP accumulation during branching. In contrast, 53% of the time, the protoplast emerged from the apical cell plate or a position on the side of the filament more basal to the expected future branching site, or did not emerge at all. Various factors could contribute to the inability of every protoplast to emerge from a future branch site. Positioning of the weak cell wall might be facing the cover slip or the top of the imaging chamber, not allowing the protoplast to escape. Or during normal protonemal development not all subapical cells are programmed to branch, thus weakening of the wall may not have occurred."

Point 7. Brief information on delta3'UTR lines is better to be shown in the legend of Figure 1. Also, it would be very helpful for the readers if the authors add some illustrations explaining the strategy of delta3'RNAi.

RESPONSE: We have modified the legend to Figure 1 by adding the following sentences:

"(A-B) Transient RNAi assay performed in a line containing a nuclear GFP:GUS reporter and a deletion of the *ROP4* 3'UTR, which renders the *ROP4* gene insensitive to the UTR RNAi construct as described in (Burkart et al., 2015). All RNAi constructs simultaneously silence nuclear GFP:GUS reporter and the target genes, with the control construct only silencing GFP:GUS."

Due to space constraints and the fact that this strategy was already published, we did not include an illustration of how it works but could do so if the reviewers/editors find that it is essential.

Reviewer #2:

The submitted manuscript by Cheng et al. represents a significant technical advance by creating a new type of ROP-fusion protein that is based on previous work in yeast. This fusion protein is an internal tag and was previously shown to generate a fully functional Cdc42 in yeast. Cheng et al. take a similar approach here in moss and show that a similar tagging strategy generates a fully functional ROP. This is only possible in moss because of the clear redundancy of the ROP family and the presence of four orthologs that have all been knocked out by the Bezanilla group previously, providing a clean assay for functionality. This is important because the plant literature is riddled with data of tagged ROPs with no clear indication that the proteins are functional. This work provides a clearer path forward in terms of live cell imaging of ROPs. The authors go on to describe the localization patterns of ROP and observe interested localization at polar growth sites well in advance of a cell shape phenotype.

Point 1. The major weakness of this paper is that it just descriptive. There is no data on ROP activators or effectors that might lead to some mechanistic insight into ROP function. It is a purely technical advance.

RESPONSE: *P. patens* has 12 GEFs, 8 GAPs, and an unknown number of effectors. Thus, we think it is beyond the scope of this manuscript to narrow down the regulators mediating specific ROP activities. Furthermore, based on Bascom et al. 2018, we have evidence that many of these GEFs and GAPs function redundantly, so we would need to generate a significant number of higher order null mutants, which even in moss will take a substantial time investment. Therefore, based on the editor's advice and in response to the positive views of the importance of this technical advance, we have revised and resubmitted the manuscript as a Breakthrough Report.

Point 2. The authors should more completely acknowledge the use of the Cdc42 data that guided their work.

RESPONSE: We mentioned that our study was inspired by work in fission yeast twice in the manuscript. However, to make it more prominent, we now also include it in the abstract:

"Motivated by work in fission yeast (Bendezú et al., 2015), we generated a fluorescent fusion of the *P. patens* ROP4 protein by inserting mNeonGreen after Glycine 134."

Reviewer #3:

From a technical standpoint, this manuscript represents a significant effort, with carefully executed and sound results. The manuscript is well written. The breakthrough, demonstrated function of a ROP with a sandwich insertion, will provide valuable insight into this and other ROP proteins.

Point 1. The phenotypic analysis did not provide as much insight as expected, considering the substantial information already known about ROPs both in *P. patens* and other plants. In this paper, all *P. patens* ROPs were knocked-out (assuming that either deletion of 22 amino acids or a large insertion between two exons represents a null mutant of *rop4*), and, although the quadruple mutant lost all polarized growth, the quadruple mutant still grows vegetatively. This result demonstrates, in contrast to a negative result in a recent report by Yi and Goshima 2020, that a quadruple mutant can be generated.

RESPONSE: We have genotyped more Δ*rop1/2/3/4* isolates and have uncovered two more independent alleles with stop codons introduced in the protein as described on pages 13-14 of the revised text and shown in Figure S5. All four of these isolates have exactly the same phenotype, strongly suggesting that these four distinct alterations to the *ROP4* locus result in a null mutant. The ability to generate a plant lacking all ROP function is remarkable. Previous RNAi results (Burkart, et al. 2015) were transient and it was not clear that the proteins were completely eliminated. Yi and Goshima 2020 also used RNAi, which may still have residual protein. Yi and Goshima 2020 were able to generate Δ*rop1/2/3*+rop4 with a point mutation, which has a significantly different phenotype from our four isolates of Δ*rop1/2/3/4* – Δ*rop1/2/3*+rop4 point mutation generates small rod shaped cells that remain adhered to one and other, but importantly still branch and develop gametophores. Given that Δ*rop1/2/3/4* plants lack polarity at the cellular and tissue level has definitively demonstrated ROP's role in both cellular and tissue-level patterning. Even though this is an expected result, our data is the first to truly test it and to establish a plant that can grow indefinitely without any ROP proteins.

Point 2. The N-terminally tagged ROP4, with mEGFP or 3XGFP inserted right after the start codon at the ROP4 locus did not restore polarized growth in a line that silenced all ROPs with RNAi. Instead, mNeonGreen was inserted after G134, similar to fission yeast CDC42, again at the ROP4 locus, and grew as well as the parental line. Removal of the 3'UTR of ROP4, together with silencing the other ROPs, led to smaller plants, indicating that either ROP4 alone wasn't expressed enough (as suggested by the authors) or that ROP4 requires its 3'UTR for full function. Perhaps alternative possibilities could be discussed in the text?

RESPONSE: We thank the reviewer for pointing this out. It is possible that deletion of the 3'UTR has impaired expression. We have added the following sentences to the text:

"The fact that the sandwich tagged lines transformed with the control RNAi constructs were smaller than the untagged control parental line might reflect differences in expression and/or stability of the sandwich tagged fusion protein as a result of the deletion of the 3'UTR.

To control for possible expression effects resulting from deletion of the 3' UTR and to ensure that incomplete silencing of *ROP* did not account for the observed rescue of polarized growth, we isolated several lines where the *ROP4* locus with an intact 3' UTR was tagged appropriately with swmNG and also carried null mutations in *ROP1, 2* and *3*, indicating that ROP4-swmNG does not affect plant viability."

Point 3. So much work has been done to generate the lines, it is surprising that instead of measuring the growth of these polarized cells, circularity is used to describe how well polarization and the subsequent cell growth occurs. Time-lapse imaging may be used to directly measure the rate of growth (Figure 2).

RESPONSE: Because not all cells are actively growing, it can be difficult to assess growth rates without continually

imaging to ensure that the cell was growing the entire time during the imaging of that particular filament. This approach has very low throughput. In contrast, because protonemal tissue grows by tip growth, measuring plant area is a much more robust measure over large numbers of samples and provides significant statistical power. In the revised manuscript we have used solidity instead of circularity to describe the overall polarity of the plants. This measure has been used for all previous ROP studies form our lab (Burkart et al. 2015 and Bascom et al. 2018) and we wished to remain consistent with those studies.

Point 4. Figure 1: Please double check (and mention in the text if so) that the data are normally distributed before using ANOVA, instead of a non-parametric test. The variation seems larger in the ROP4-swmNG-84 sample.

RESPONSE: We have included in the legend to Figure 1 the following sentence:

"Based on the Kolmogorov-Smirnov Test, the data in (C, D, F, G) are normally distributed."

Point 5. Figure 2: These images might be used to assess growth. That ROP2 in the *rop1/3/4* triple mutant loses polarity in subapical cells but does not prevent gametophore generation is interesting, but not explored in sufficient depth. Which ROP or combination is required for polarized growth in branches? These lines have been generated in Burkart et al. 2015 and Yi and Goshima 2020.

RESPONSE: In Burkart et al. 2015, only single mutants were generated. These were all normal with respect to branching and all single mutants made gametophores. We generated Δ*rop1/2/3* in this study and Yi and Goshima 2020 generated Δ*rop2/3/4* (as well as Δ*rop1/2/3*). Both of these triple mutants make gametophores, and swelling of the subapical cell was not reported for any trip mutants in the Yi and Goshima 2020 study. Similar to Yi and Goshima 2020, we also did not observe swelling of the subapical cell in Δ*rop1/2/3*. Given that the ROP proteins in moss are nearly identical, we expect that the reason that Δ*rop1/3/4* exhibits such a unique phenotype is because *ROP2* has the lowest expression of all ROP genes in protonemata (Burkart et al. 2015). Importantly, Δ*rop1/3/4* loses polarity for branch formation, but not for gametophore formation. Thus, it is possible that either *ROP2* expression is upregulated during gametophore formation or that less protein overall is required during gametophore development. In the context of branching protonemata, Δ*rop1/3/4* does not have enough ROP protein to localize to the forming cell plate and the branch sites without losing polarized growth in the apical cell, leading to misplaced division planes most likely resulting from the inability to develop a branch.

Point 6. A fascinating feature of the rop quadruple mutant is the constriction during cell division. Is there a proposed mechanism?

RESPONSE: In fact, we do not think that there is a constriction during cell division. Rather, the cell divides normally, as we are able to observe the cell plate develop across the middle of the cell and it is mostly normal. However, due to lack of cell adhesion in the new cell plate, the cells become "unglued" and this then looks like constriction.

Point 7. Apical ROP localization in protonemal cells and to the cell plate is similar to other ROPs in other tip-growing cells, and to previously published work from Yi and Goshima in *P. patens* 2020.

RESPONSE: We agree with the reviewer that the apical localization has already been demonstrated. In fact, we showed apical localization in Burkart et al. 2015. However, we knew the tag wasn't functional, so we put these data in the supplement. With a functional tag in hand, it was important to analyze its behavior. Any function that was demonstrated by others could be due to clipping off the GFP, which was shown in a supplemental figure in Yi and Goshima 2020. In the revised manuscript in Figure 2, we now quantitatively compare the localizations of the N-terminal tagged and the sandwich tagged ROP fusion proteins.

Point 8. Figure 4: Molendijk et al. 2001 showed similar results in Arabidopsis root hairs, that ROP localization was unaffected by LatB or oryzalin treatment. The identification of conditions when ROP localization was altered in shorter apical cells needs further analysis.

RESPONSE: Root hairs and protonemata are very different cell types. Root hairs are fully differentiated and the apical cell of a protonemal filament is a stem cell. Thus, comparing protonemal apical cells to cell types such as dividing yeast cells may actually be more appropriate. Nevertheless, since the dynamics of apical ROP have not been investigated in protonemata, we thought this was an important experiment to perform. And we discovered that maintenance of apical localization may depend on the cytoskeleton in a cell cycle dependent manner. Future

studies with cell cycle markers and/or mutants in addition to ROP4-swmNG might help to focus how these processes are linked.

Point 9. Figure 5: ROP localization correlates with the direction of cell expansion. Time-lapse imaging was used to examine the correlation between growth direction and ROP localization. These results are similar to ROP localization during pollen tube growth, e.g. Luo et al. 2017 Nat Comm.

RESPONSE: Similar to root hairs, pollen tubes are terminally differentiated and quite different from protonemata. We wanted to take advantage of imaging a fully functional ROP fusion during growth changes in protonemata. It is exciting that there are such strong similarities between these divergent cell types, which we have noted in the revised Discussion with the following sentence:

"This behavior likely reflects a conserved function of ROP since it occurs in diverse tip growing cells, from the protonemal stem cell in mosses to fully differentiated pollen tubes in angiosperms (Luo et al., 2017)."

Point 10. Figure 6: ROP localization prior to polarized growth has been observed across multiple species and in many cell types. Cell wall softening has also been proposed e.g. Luo et al. 2017 Nat. Comm.

RESPONSE: With a functional fusion in hand, we needed to document how the fusion behaved under these circumstances. With regards to cell wall softening, we have added a sentence to the Discussion reflecting the findings in pollen tubes:

"Here, cell wall remodeling likely occurs as we discovered that the cell wall is weakest at these sites (Fig. 5B, video 11), suggesting that ROP is required to remodel the wall before tip growth can occur similar to what has been found at the tip of growing pollen tubes (Luo et al., 2017)."

TPC2020-00440-BR1 2nd Editorial decision – *accept with minor revision* Aug. 20, 2020

We have received reviews of your manuscript entitled "Fully functional ROP fluorescent fusion reveals roles in both subcellular and tissue level patterning." On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in The Plant Cell. This acceptance is contingent on revision based on the comments of our reviewers. In particular, please consider the following:

There are a couple of places where the text takes away from the focus on technical advancement. For this reason, we suggest that you either condense or delete the sections listed below.
1. Section on "ROP is essential for developmental patterning" on page 13. While these findings are interesting, they are not critical to appreciate the significance and application of the functional ROP fusion. Please also delete references to these findings in the next results section (e.g., line 387) and in the Discussion section.
2. Similarly, from a technical breakthrough standpoint, we feel that the second paragraph on page 17 (lines 446-466) and both paragraphs on page 18 could be condensed significantly or deleted.
3. Minor point. Line 321: should be "does not depend on microtubules..."
Trimming or removal of this material will also help to shorten the text for the Breakthrough Report format.

TPC2020-00440-BR2 2nd Revision received Aug. 25, 2020

RESPONSE: Taking the editors' suggestions, we condensed the section in the Results that described Figure 4 into one short paragraph (highlighted in the highlighted version of the manuscript) and removed Figure 4B into the supplemental material. Additionally, we deleted three paragraphs in the Discussion. We thank you for your helpful suggestions, as this has nicely focused the manuscript.

TPC2020-00440-BR2 3rd Editorial decision – *acceptance pending* Aug. 31, 2020

We are pleased to inform you that your paper entitled "Fully functional ROP fluorescent fusion reveals roles in both subcellular and tissue level patterning" 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	**Sept. 7, 2020**