Direct and indirect regulation of Pom1 cell size pathway by the protein phosphatase 2C Ptc1

Veneta Gerganova, Payal Bhatia, Vincent Vincenzetti, and Sophie Martin

Corresponding author(s): Sophie Martin, University of Lausanne

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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.)

RE: Manuscript #E20-08-0508
TITLE: "Direct and indirect regulation of Pom1 cell size pathway by the protein phosphatase 2C Ptc1"

Dear Sophie:

I have received your manuscript and reviews. The work is very appropriate for MBoC. The manuscript is well written, and the data are generally of high quality. Your proposed revisions are reasonable to address the reviewer's comments. I hope you can try to do these revisions and submit a revised manuscript in the near future. I anticipate that I will able to evaluate these revisions without going back to the reviewers.

My additional suggestions (optional):
In the abstract, could you emphasize more that Ptc1 affects cell size (not just that it is part of a Pom1 pathway). I think that this will help the general readership interested in cell size control. I would also consider changing the "but" in this sentence: "We show that Ptc1 directly binds Pom1 and de-phosphorylates it in vitro. Further Ptc1 may regulate Pom1 localization indirectly in vivo in low glucose conditions by... " (The "but" seems to negate your in vitro results).
Suggestion of an alternate title: Protein phosphatase 2C Ptc1 regulates cell size through regulation of Pom1. (again, to emphasize the cell size regulation).

Sincerely,

Fred Chang

Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Martin,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

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To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
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UNIL | Université de Lausanne
Département de microbiologie fondamentale
bâtiment Biophore bureau 2310
CH-1015 Lausanne

Lausanne, 22nd December 2020

Dear Fred,

We are happy to submit the attached manuscript "*Direct and indirect regulation of Pom1 cell size pathway by the protein phosphatase 2C Ptc1*" for evaluation for publication in *MBoC*.

We have performed all the experiments we had proposed in our revision plan and have revised the manuscript accordingly. Detailed responses to the reviewers' comments are appended below. Briefly, we performed additional imaging using Airyscan to get better resolution images for critical strains, performed image quantifications, provided other examples of western blots as proposed, and obtained epistasis analysis showing that Ptc1's function in cell size control can occur from the cytosol.

I apologize for the delay in sending this revised version, which I hope will be appropriate for acceptance.

With best wishes,

Sophie Martin

Faculté de biologie et de médecine
Département de microbiologie fondamentale

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We thank the reviewers for their careful reading of our work and their constructive comments. Please find below our detailed responses.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

This manuscript by Gerganova et al. identifies the protein phosphatase 2C Ptc1 as a new regulator of the Pom1-Cdr-Wee1 cell cycle pathway in fission yeast. They find that Ptc1 is a dosage-dependent regulator of cell size, these effects are dependent on the kinase Pom1. The authors show that Ptc1 localizes to cell tips, and this localization depends at least in part on two other proteins: Skb5 and Mod5. The authors perform biochemical assays to show that Ptc1 can dephosphorylate Pom1 in vitro, consistent with a model where Ptc1 acts directly on Pom1 to regulate cell cycle signalling in cells. Interestingly, the authors also test the role of Ptc1-Pom1 regulation in low glucose conditions. The same lab previously showed a role for microtubules in regulating Pom1 and cell size under low glucose conditions. Here, they show that Ptc1 contributes to regulation of microtubules under these conditions, leading to indirect control over Pom1.

This paper provides a number of new insights that will contribute to this field, in particular the identification of a new component for this pathway. There are two weaknesses that limit the impact of the work. First, the cell size defect of ptc1 mutant cells is very small, leading to questions about its significance in the pathway. Second, it remains unclear how Ptc1 fits mechanistically in the pathway. The authors provide a nice description of this open question in the discussion section, but it is still not clear to me if Ptc1 acts directly on Pom1 in cells based on the data in this paper. Overall, the paper is a step forward for the field with additional questions to be answered in the future. I do have several concerns regarding technical aspects of experiments in the paper, and these concerns should be addressed to strengthen the paper.
We agree that the effect of *ptc1* is small and have been careful to not over-interpret any of the data.

Major Comments:

1.In figure 1B, the authors conclude that faint amounts of Ptc1 can be detected at the tips of skb5Δ cells, but I cannot see this result from the data provided. Further, this figure appears to be impacted by different contrasting between each panel, were the images processed and contrasted identically? As presented, it looks like Ptc1 protein levels might be different in the skb5 mutant versus the mod5 mutant.
We agree that the faint signal of Ptc1 present at the tips of some *skb5*Δ cells was indeed challenging to see. We have now repeated this imaging using Airyscan technology, which showed more clearly the remaining amounts of Ptc1 at the periphery of *skb5*Δ cells. This also revealed an interesting distribution that is more clustered than the smooth distribution in wildtype cells. All images were acquired with identical settings, but we increased contrast for the double mutant to ensure we did not miss some cortical localization. We have modified the text accordingly. We have also re-organized Fig 1 to pool together panels A and B, leading to renumbering of the other panels.

2.I have some concerns about the co-immunoprecipitation in Figure 1E. One problem is that HA-Mod5 is not seen in the second lane of the input blot. Its absence is a problem because this would be the negative control for HA-Mod5 interacting nonspecifically with the beads, but this

negative control is misleading if it is not present at the same level of other input samples. A second and smaller concern is that the Mod5-Ptc1 interaction appears weaker in the pom1 mutant, but the authors conclude that the interaction is independent of Pom1. I would recommend changing the interpretation to reflect this reduced level of coimmunoprecipitation if the reduction is reproducible.

Because HA-Mod5 is expressed from plasmids and induced from the *nmt* promoter, we found high variability in expression levels from experiment to experiment. We note that the first concern from the reviewer can be simply addressed by pointing out that the blots in the original Fig 1E have two lanes of negative control. While the first indeed has reduced HA-Mod5 levels, the second has equivalent levels to the first lane where Ptc1 is tagged with GFP.

However, in reviewing all the coIP data, we found that we were unable to locate the original blots that were used to construct Figures 1E and S2E. These experiments were performed 6 years ago, and we suspect that the original data was lost by failing to replace the films in their original location upon scanning for figure preparation at the time. The time elapsed since then is not an excuse, and we deeply apologize for this loss of data.

Because the original data is no longer available for these two blots, we have replaced them with one of two additional experiments for which we have the original data. In this repeat (now in Fig 1D), HA-Mod5 expression was done in both *mod5+* and *mod5Δ* cells, where it is more highly expressed. CoIP of HA-Mod5 is seen in both cases, though it is very weak in the *mod5+* background, perhaps because tagged Mod5 has slightly compromised function and is competed out by untagged Mod5.

Regarding the possible effect of Pom1 on the Ptc1-Mod5 interaction, we do not think that the interaction is reduced in the *pom1Δ* strain. If anything, the blot in the original Fig S2E showed a stronger interaction in *pom1Δ*. However, as these data have now been replaced with ones that do not include the *pom1Δ* strain, we have removed the comment on Pom1-independence from the text.

3.The authors have provided a nice analysis of factors impacting Ptc1 localization to cell tips, but then the relevance of this regulation to cell size was untested. If Ptc1 localization to cell tips is important for its regulation of the Pom1 pathway, then skb5 mutants and skb5 mod5 mutants should be elongated, and this phenotype would require ptc1 and pom1. It seems important to relate the localization to the proposed function in cell cycle regulation.

We agree with the reviewer that it is interesting to test this aspect, though one concern is that both Mod5 and Skb5 may affect cell size by additional ways (Mod5 is for instance well-known to affect Tea1 and Tea4, which will have knock-on consequences on Pom1). Nevertheless, to address these points, we measured the cell length at division of *skb5Δ mod5Δ* double and *skb5Δ mod5Δ ptc1Δ* triple mutants. We found that *skb5Δ mod5Δ* double mutants are slightly longer than wildtype cells, consistent with the idea that cytosolic Ptc1 may be less efficient in regulating the Pom1 pathway. However, we found that the *skb5Δ mod5Δ ptc1Δ* triple mutant was significantly longer than the double mutant. This indicates that, even from its cytosolic location, Ptc1 affects cell size. Thus, we conclude that Ptc1 localization at cell poles is not essential for its role in regulating the Pom1 pathway. We have added this information to the manuscript and Fig 2A.

4.The coimmunoprecipitation result in Figure 2B is hard to interpret. There is much more Pom1-HA protein in the double-tagged strain, as compared to the single-tagged Pom1-HA strain (compare second and fourth lanes of the input). The increased concentration makes it impossible to compare this sample to the relevant controls, as it would drive interactions compared to the other samples. Also, what is the background band at the same size as Pom1-HA in the wt input sample?

We have done this experiment several times. In all cases, the Pom1-HA levels appear slightly higher in the double tagged strain in 2% glucose, suggesting possible stabilization of Pom1 when Ptc1 is tagged, though we have not probed this further. Please note however that this is not the case in 0.03% glucose (bottom blot). We have now replaced the blot with a second version of the experiment, where Pom1-

HA levels are more equal across lanes in the 2% glucose blot and there is no background band. In the 0.03% glucose blot, the Pom1-HA levels appear even lower in the double-tagged strain.

5. In Figure 2C, is there a negative control for the possibility that GST-Pom1 interacts with MBP? If I see it properly, the authors show that GST alone does not interact with MBP alone, but the more important control is testing GST-Pom1 with MBP alone.
We have performed the requested control, which is now presented in Fig 2C (right panel). In this experiment, GST or GST-Pom1 were bound to glutathione beads and binding of MBP or MBP-Ptc1 was assessed. The blot shows that MBP does not bind, whereas MBP-Ptc1 strongly binds GST-Pom1. Note that MBP-Ptc1 also shows some background binding to empty glutathione beads.

Minor comments:

1.In the introduction it should read: "...recent work proposed that this feature is cell surface area."
This is corrected in the text
2.Skb5 localization at cell tips: is it seen in all cells? This localization is so weak that it is hard to assess. It appears additional fluorescence (background mitochondria?) is so much stronger that it is near impossible to assess Skb5 localization in this strain.
Skb5 localization is indeed weak and there is consistently additional background fluorescence in this strain, for reasons we do not understand. In previous work, Skb5 had been shown to localize to cell poles upon overexpression when tagged with 3xGFP. We imaged the protein expressed from endogenous genomic locus. We have now improved the imaging by using Airyscan imaging, which helps distinguish the weak cortical localization of Skb5 from the background fluorescence.
3.It would be helpful to note in the legend for Figure 1E that the + indicates untagged wild type Ptc1.
We made the suggested change (now Fig 1D)
4.In figure S2E, I am not sure what AU and AUT refer to.
Sorry for this typo: this should have read as -T and +T, i.e. without or with thiamine, which serves to repress the *nmt* promoter. As stated above, we have now removed this blot.
5.Although it goes beyond the focus of the current paper, can the authors comment on whether division septa are commonly misplaced in ptc1Δ cells in 0.08% glucose?
We have quantified septum position in *ptc1*Δ cells, which is unchanged and presented in Fig S1B. Septa position in ptc1Δ grown in 0.08% glucose are also unchanged.
6.Can the authors comment on genetic interactions between ptc1 and dis2 mutants? Has past work shown if the double mutants are viable and have cell size defects?
Though it would be interesting to test, we have not examined possible genetic interaction between these two phosphatase-coding genes and are not aware of such information in previous literature.
7.Does Ptc1 over-expression, which makes cells smaller, affect Pom1 localization?
We have also not investigated this point.

Reviewer #1 (Significance (Required)):

The paper is significant in the addition of a new signaling component for a biologically important pathway. Further significance relates to the identity of this new component as a protein phosphatase, as the pathway has been primarily studied in the context of its kinase components. The work fits well in the known literature, and extends the basic knowledge of this field. It will be of interest to a cell biology community, particularly those studying cell cycle progression.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

This is an intereating study by Martin and colleagues on two different modes of regulation of the

polarity and division kinase Pom1 by phosphatase Ptc1. The work is very well performed and I only have minor comments.

1.Is there a reason all of the experiments where done at 30C and not 24C?
There is no particular reason. 30°C is a very common culturing condition across yeast labs.

2.For Figure 1 b/c, It would be helpful if the authors could provide quantitative measurements of Ptc1-GFP cortical signals in the mutant strains such as Tea1, Tea4, skb5, and mod5. This is especially the case for the mod5/skb5 double mutant, as it appears there is some cortical Ptc1 signal there, although the authors suggest it is fully cytosolic.
We thank the reviewer for this suggestion. For *tea1*Δ and *tea4*Δ, where the Ptc1 cortical signal is decently distinguishable from cytosolic signal, we have now provided measurements of Ptc1-GFP along the cell cortex. These confirm the flatter distribution of Ptc1 in these mutants. For mod5Δ, skb5Δ and double mutant, we now provided improved imaging, using Airyscan technology, as stated in response to the first comment of reviewer 1. This also revealed a more particulate distribution of Ptc1 in the skb5Δ strain. We have not performed quantification in the double mutant, a this would be uninformative in quantifying mainly cytosolic signal.

3.In figure 1d, It would be interesting to know whether Mod5 also co-localises with Ptc1-GFP.
We unfortunately do not have either Mod5 or Ptc1 tagged in other colour than GFP, so cannot easily do this experiment. However, previous work had shown that Mod5 and Tea1 form complexes (Snaith et al, 2005) and that both Tea1 and Mod5 form aggregates at one pole of *tea4*Δ cells (Martin et al, Dev Cell 2005 – see Fig S3). Therefore, we think that the localization evidence we present, in addition to the coIP, is sufficient support for the notion that Ptc1 is recruited to Mod5.

4.In figure 2b, Mod5 co-immunoprecipitates with Ptc1 in the absence of Pom1, however it does seem to be reduced in the absence of Pom1 compared to the control. It would be helpful to clarify this. It would alsobe interesting to see whether there is change in amount of Mod5 co-immunoprecipiating in the absence of Skb5 i.e are they direct competitors for binding to Ptc1?
This comment is similar to comment 2 from reviewer 1. We do not think that the interaction between Ptc1 and Mod5 is reduced in the *pom1*Δ strain: the band was slightly weaker in the original Fig 1E but stronger in Fig S2E. However, we have now removed this data and any reference to it in the revised manuscript. We have not tested whether Mod5 interaction with Ptc1 is affected in *skb5*Δ.

5.For statistics done in figure 2 and 3, it would be helpful it they could provide a supplementary table with anova analysis to compare a few more of the strains rather than only doing t-tests on two strains at a time.
We are not sure we understand the point of this analysis. The meaningful comparisons are pairwise.

6.For figure 2a, authors state a N=300 cells. It's not clear whether this is total number of cells for all the strains or for each individual strain. In either case, this should be clarified.
We have clarified this in the figures. 300 septating cells per strain were measured, with a few exceptions.

Reviewer #2 (Significance (Required)):

The work is of broad interestt to the fields of cell biology and cell cycle. How cells modulate the size / volume in relation to polarity is of interest to all biologists.

The authors do an impressive job of linking the polarity and cell size fields.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

In this work, Gerganova et al. explore how the Pom1 intracellular gradient is regulated in fission yeast. They find that Ptc1, a phosphatase, plays a critical role in controlling the activity of Pom1, both through direct and indirect interactions. Consequently, Ptc1 plays a role in determining cell length in fission yeast cells. They utilize a range of genetic and drug perturbations to test the role of Ptc 1.

Overall, this is an interesting paper, which adds important knowledge about how subcellular gradients form and how they are regulated. The data presented - with some caveats highlighted below - is generally convincing. The paper is well written.

Major comments

- Though most of the conclusions are strongly supported by the data, the claim that "Ptc1 controls division timing" is not well-substantiated. On page 6 it is claimed that the reduction in cell length in ptc1 mutants is due to a timing defect. Yet, sizer effects may also be playing a role (indeed, seems more likely, given its effect on the spatial extent of pom1). There is no substantiation of this claim and the conclusions is based on a significant assumption. The results presented in the paper are generally very well supported, and so stretching them to make this claim about timing is unnecessary and distracting. I suggest the authors remove all claims that it is acting as timer (though of course it can be a discussion point at the end). If they choose to leave it in, more direct evidence is required to support this claim.
We agree with this comment. By using the phrase “division timing”, our intention was not to present Ptc1 as a timer. We have replaced these instances with “size at division” and modified the text to remove inference that Ptc1 acts as a timer.

- I have some concerns about how the gradients were measured. If I understand the Methods correctly, a sum projection was taken and then the intensity measured along the long axis of the cell. Yet, the effect of Ptc1 is being explored on the cell cortex. The intensity measurements should be taken along the gradient of Ptc1. The current used method will tend to distort the actual signal present at the cortex.
We apologize if our description for how these measurements were made was unclear. All measurements were done at the cortex of medial focal plane images by drawing a line from the cell tip towards the cell middle. The sum projection is a temporal projection of 5 consecutive medial plane images, which helps reduce noise. We have reworded the corresponding section in the methods to clarify.

- In a related point to the above, some data could do with improved quantification or analysis. Regarding Fig. 1B, it is claimed that there is faint Ptc1 signal at the tips. The highlighted signal in the zoomed region still looks like that signal could easily just be noise. How did the authors confirm this signal was real? The presented evidence is currently not sufficiently convincing. Similarly, in Fig. 1C we are told "Ptc1-GFP was spread more evenly around the cell cortex". Yet, from the data as presented, one could imagine that the signal is simply higher but still have a gradient. This is also relevant for Fig. 3C. Clearer analysis is needed to substantiate these claims.
We have now quantified the distribution of Ptc1 at the cortex in tea1Δ, tea4Δ, skb5Δ and mod5Δ, as also stated in response to reviewers 1 and 2. We have further re-imaged Ptc1 in skb5Δ and mod5Δ using Airyscan technology, which helped get better resolution images and revealed a more clustered distribution of Ptc1 in the skb5Δ strain. We have also measured cortical profiles in Fig 3C.

Minor comments

- Page 4: In reference to the ptc1 tagged at the endogenous locus, it is described as "largely functional".

This seems a somewhat odd terminology. The authors refer to cell length, but in what sense does this mean it is largely functional? A better description of the observed phenotypes should be given here.
We exchanged “largely” with “apparently” from the text. This stems from an abundance of caution, as one can never be completely sure that a tagged protein is fully functional.
- Page 5: Is "MAPKKK Mkh1" a typo?
This is not a typo. Mkh1 is a MAP kinase kinase kinase.
- Page 6: I find the use of the word "landmark" to describe the proteins Skb5 and Mod5 unclear. These are not landmarks - unless they have a highly localized spatial location.
We agree and have reworded the text accordingly.
- The p-values presented in Fig 2A are implausible. The Methods says that three replicates were done of the experiment with a certain number of cells in each replicate. What is the variability in the cell lengths between each replicate? This will provide a better estimate of the statistical certainty. For example, given WT = 13.7{plus minus}1.0 and ptc1Δ=14.4{plus minus}1.1, it is implausible to claim a p-value of 1.1e-13. The n is actually 3 (for the 3 replicates), not the 100s of separate cells.
We agree with the reviewer and apologize for having reported *p*-values across the hundreds of cells, rather than across individual experiments. We have now updated the table with p-values across experiments (N = 3 or 6, as indicated). Please note that the cell length values for the ptc1 overexpression (nmt1-ptc1, induced and non-induced in Fig 2A) changed slightly in comparison to the early version of the manuscript: we had to re-do these experiments in triplicate, as our early measurements had not been labelled per experiment. We also changed the p-values to those of comparison between experiments in Fig 3B.
- It would be helpful to add pointers to Fig. 2B and 2C. The figure panels are quite dense and some guidance to the reader would improve presentation.
We have added pointers as suggested.
- It is claimed that "Ptc1 was evenly spread around the cell cortex" in low glucose conditions (Fig. 4A). Yet, in Fig. S4B, there are clear peaks and troughs in many of the profiles shown. Overall, the gradient is definitely flatter than high glucose, but it really doesn't look "evenly spread". It looks like there are distinct clusters across the membrane, leading to localized high signal. I suggest changing the wording.
We agree with this comment and have changed the wording in the text.
- Fig. 4D. The images look very low quality, almost out-of-focus. These should be improved, as it is currently difficult to validate the claimed results for these panels.
This is not out of focus, but was acquired on an epifluorescence microscope, whereas other panels were acquired on a spinning disk confocal (which since nearly died). We apologize for the lower quality of these images. However, we feel that that take-home message that there are no microtubules in *ptc1*Δ in 0.08% glucose, whereas microtubules are present in similarly treated WT cells is well supported by these images.

Reviewer #3 (Significance (Required)):

This work provides an important advance in understanding how subcellular gradients form. This work builds on recent literature, both looking at the role of pom1 and environment in cell length control. In particular, the direct and indirect modes of action by Ptc1 on Pom1, and the sensitivity to glucose levels are particularly interesting.

The issue of cell size control is a major challenge in cell biology. This work will likely be of general interest as it provides a new player in understanding the mechanisms of cell size control. It provides insights that give new directions for future work in other systems as well, increasing its potential impact.

Review expertise: quantitative biology, developmental biology, biophysics

Consultation comments:

I agree with the comments of referee 1. There is a lot of good potential here, but a lot of the data and some of the causal links should be strengthened to maximise impact of the work

RE: Manuscript #E20-08-0508R
TITLE: "Direct and indirect regulation of Pom1 cell size pathway by the protein phosphatase 2C Ptc1"

Dear Sophie,

I have reviewed your manuscript myself. The quality of the work is high, and the work is interesting. Your revisions have addressed the concerns of the reviewers. However, before I can accept this, I request that you address some extremely minor issues:

1. Fig 1A: can you state in legend that the contrast setting is different in the double mutant.

2. Fig 1D : can you add in the text to describe the data that the IP interaction is much more apparent in the mod5D background.

3. Discussion, first paragraph. "In agreement with previous data" Is there a reference to this?

4. I could not find Tables S1 S2 in the submission materials. Can you please submit them?

I am sorry about the delay.

Yours sincerely,
Fred Chang

Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Martin,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
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UNIL | Université de Lausanne
Département de microbiologie fondamentale
bâtiment Biophore bureau 2310
CH-1015 Lausanne

Lausanne, 10th January 2021

Dear Fred,

Thank you for reviewing our paper and your interest in it. Here are the few additional requested changes:

1. Fig 1A: can you state in legend that the contrast setting is different in the double mutant.
This is already stated as "Contrasting were increased post-acquisition on the *mod5Δskb5Δ* image".

2. Fig 1D can you add in the text to describe the data that the IP interaction is much more apparent in the mod5Δ background.
We added: "Note that co-immunoprcipitation of HA-Mod5 was more marked in absence of endogenous untagged Mod5".

3. Discussion, first paragraph. "In agreement with previous data" Is there a reference to this?
Sorry for the omission. The reference is Gaits et al, JBC 1997. It is now added.

4. I could not find Tables S1 S2 in the submission materials. Can you please submit them?
I am sorry if these were forgotten in the previous submission.

With best wishes,

Sophie

Faculté de biologie et de médecine
Département de microbiologie fondamentale

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RE: Manuscript #E20-08-0508RR
TITLE: "Direct and indirect regulation of Pom1 cell size pathway by the protein phosphatase 2C Ptc1"

Dear Sophie,

I am pleased to accept your manuscript for publication in Molecular Biology of the Cell. Congrats!

Sincerely,

Fred Chang
Monitoring Editor
Molecular Biology of the Cell

Dear Prof. Martin:

Congratulations on the acceptance of your manuscript.

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