# Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1

Abrar Aljiboury, Amra Mujcic, Erin Curtis, Thomas Cammerino, Denise Magny, Yiling Lan, Michael Bates, Judy Freshour, Yasir Ahmed-Braimeh, and Heidi Hehnly

*Corresponding author(s): Heidi Hehnly, Syracuse University*

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## Transaction Report:

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RE: Manuscript #E22-01-0015
TITLE: Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1

Dear Dr. Hehnly,

Thank you for sending your manuscript "Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1" to MBoC.

Now your manuscript has been evaluated two experts in the field. They raise important points that should be addressed for this manuscript acceptable in MBoC. In general, I would like you to address concerns/comments regarding the rigor of the paper (they point out issues that do not make sense), although you may not have to address all 'additional investigation' suggestions. If you choose not to address certain comments because you believe they are beyond the scope of the current paper, please clearly explain why you believe it is beyond the scope.

If you could address their comments thoroughly, I would like to invite you to submit the revised version.

Thank you for sending your work to MBoC.

Best wishes,
Yukiko

Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Hehnly,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments 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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Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office

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Reviewer #1 (Remarks to the Author):

The manuscript by Aljiboury et al. entitled "Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1" examines how failure to localize PLK1 to the centrosome in the absence of cenexin impacts PCM structure during metaphase. This manuscript shows that depletion of cenexin prevents PLK1, PCNT, and γ-Tubulin from localizing properly to the centrosome. It also suggests that mislocalization of PLK1 causes changes of PCM protein phosphorylation. Finally, this manuscript presents the phylogeny of cenexin and suggests that human cenexin functions in vivo in zebra fish embryos much like it does in vitro in HeLa cells. The majority of the experiments presented are done well with good controls and quantified appropriately. The addition of an in vivo model is also very interesting and add value to this study.

While the science is well done, this manuscript does not answer their overarching question, which is "How does cenexin depletion affect centrosome maturation in different model systems?" (p4, ln4-7). Every piece of data presented considers the centrosomes during metaphase, which is after centrosomes mature in G2 phase. As a result, this manuscript fails to present much novel data. Most of the conclusions reached with this data are the same as those reached in Soung et al. (2006) and Soung et al. (2009), both of which are cited within the manuscript. From these two previous papers, we know that cenexin contributes to the centrosomal localization of PLK1, PCNT, and γ-Tubulin and that the cenexin-PLK1 interaction through cenexin pS796 as well as PLK1 activity are required for the localization of these three proteins. The only novel findings given in this new manuscript are that Cep215 also fragments upon cenexin depletion (though not all of the data in the paper supports this claim) and the phylogeny of cenexin as well as the ability of human cenexin to rescue cenexin depletion in zebra fish embryos. Overall, I recommend this manuscript be accepted for publication after major revisions.

Major Comments

1. Figure 1E shows that Cep215 is fragmented upon depletion of cenexin in HeLa cells. However, figure 2E and 2G show that Cep215 is not fragmented upon depletion of cenexin. This discrepancy must be addressed, especially as the fragmentation of Cep215 is one of the more novel contributions in this study.
2. shRNA knockdown of cenexin is verified using fluorescence intensity as well as the claim that centrosomal protein levels are not changing (figure S1A and S1E). This should be done by Western blot.
3. Figures 2E, 3A, 3D, 4D, 4E, and 4F should all include another feature to show that what the authors are visualizing is actually a centrosome. Generally, this would not be necessary with centrosomal proteins, but the authors are looking at a phenotype where the proteins they stain for are not properly localized to the centrosome. If these are all mitotic cells, simply staining for α-Tubulin would reveal the spindle and confirm that these are actually centrosomes. Because of the multiple α-Tubulin aggregates, Figure 2E needs a centriole marker, like centrin, to show where the centrosome is. Currently the conclusion the authors draw from these data are questionable because they cannot demonstrate that the activity is happening at the centrosome.
4. The core question the authors attempt to answer in Figure 2 is whether cenexin loss causes centrosome disfunction (p6, ln19-20). Figure 2B shows a functional bipolar spindle, which suggests no, cenexin depletion does not interrupt centrosome function. This is surprising as Soung et al. (2006) and Soung et al. (2009) showed changes in spindle shape as one of the phenotypes of cenexin loss during mitosis. This discrepancy needs to be addressed. In addition, the authors should justify more clearly why they included the nocodazole treatment in this test.
5. From the FRAP experiment in Figure 3H, the authors conclude that, without cenexin, PLK1 is better able to phosphorylate PCM proteins and recruit them to the centrosome, which conflicts with the previously observed PCM fragmentation. This is similar to the previous conclusion from the nocodazole treatment in Figures 2B and 2E that the advent of more PCNT foci in cenexin-depleted cells results in a more PCM dense spindle because those PCNT aggregates are transported to the centrosome. This begs the question, why does cenexin depletion sometimes prevent PCNT from localizing to the centrosome and sometimes does not? This discrepancy needs to be addressed.
6. As mentioned above, this paper does not present much new data. Apart from the data on Cep215 and the data found in Figure 4, every piece of data either corroborates or contradicts data shown in Soung et al. (2006) and Soung et al. (2009), which show similar experiments in HeLa cells -- the same model that the authors primarily use. This is not mentioned in the text, and it should be. I suggest either citing the original work or presenting new data that furthers our understanding of how the interaction between cenexin and PLK1 contribute to PCM organization.

Minor Comments

1. The visual in Figure 1H is a great way to summarize your findings. Please detail whether or not it is an average of many different images of cenexin-depleted metaphase cells and what software or algorithm the authors used to caluculate that average and then to graph it out.
2. Add methodology for obtaining mitotic cells. The paper only gives specifics for how cells were synchronized for expansion microscopy.
3. FRAP experiment in Figure 3I should also include Control + BI2536. This could help explain why inhibition of PLK1 in cenexin-depleted cells does not rescue the FRAP phenotype as well as it does the other phenotypes shown in Figure 4.
4. p13, ln7-8 argues that the experiments done in Figures 4D-4H show that cenexin's role as seen in HeLa cells is conserved in vivo in zebrafish. Because the rescue is done with human cenexin, these experiments say little about how the zebrafish cenexin

homologue functions in relation to centrosomes. This is handled better throughout the rest of the manuscript.

Reviewer #2 (Remarks to the Author):

See file

We have commented to Reviewer 1 and 2 below in blue font. All changes made in the manuscript are also highlighted with blue font and denoted within the rebuttal. Based on the Reviewers points we have added 18 new/updated panels (Figures S1A, S1G-J, 2E-G, S2A, 3A, 3F-G, S3A-D, S3H, S4C) and one additional video. We argue that these additions have significantly strengthened our manuscript and we thank the Reviewers for their suggestions and critiques.

**Reviewer #1**

*"The manuscript by Aljiboury et al. entitled "Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1" examines how failure to localize PLK1 to the centrosome in the absence of cenexin impacts PCM structure during metaphase. This manuscript shows that depletion of cenexin prevents PLK1, PCNT, and γ-Tubulin from localizing properly to the centrosome. It also suggests that mislocalization of PLK1 causes changes of PCM protein phosphorylation. Finally, this manuscript presents the phylogeny of cenexin and suggests that human cenexin functions in vivo in zebra fish embryos much like it does in vitro in HeLa cells. The majority of the experiments presented are done well with good controls and quantified appropriately. The addition of an in vivo model is also very interesting and add value to this study.*

*While the science is well done, this manuscript does not answer their overarching question, which is "How does cenexin depletion affect centrosome maturation in different model systems?" (p4, ln4-7). Every piece of data presented considers the centrosomes during metaphase, which is after centrosomes mature in G2 phase. As a result, this manuscript fails to present much novel data. Most of the conclusions reached with this data are the same as those reached in Soung et al. (2006) and Soung et al. (2009), both of which are cited within the manuscript. From these two previous papers, we know that cenexin contributes to the centrosomal localization of PLK1, PCNT, and γ-Tubulin and that the cenexin-PLK1 interaction through cenexin pS796 as well as PLK1 activity are required for the localization of these three proteins. The only novel findings given in this new manuscript are that Cep215 also fragments upon cenexin depletion (though not all of the data in the paper supports this claim) and the phylogeny of cenexin as well as the ability of human cenexin to rescue cenexin depletion in zebra fish embryos. Overall, I recommend this manuscript be accepted for publication after major revisions."*

We thank the reviewer for their suggestions and for recommending our manuscript for publication. We have detailed below all the points we addressed in response to the reviewer's suggestions including additional experiments that could be performed.

*Major Comments*

*1. "Figure 1E shows that Cep215 is fragmented upon depletion of cenexin in HeLa cells. However, figure 2E and 2G show that Cep215 is not fragmented upon depletion of cenexin. This discrepancy must be addressed, especially as the fragmentation of Cep215 is one of the more novel contributions in this study."*

We do agree that Cep215 fragmentation is one of the novel discoveries of this paper, along with Pericentrin. We observe fragmentation phenotypes (including that of Cep215 and Pericentrin) only during metaphase. The difference between Figure 1E from old 2E and old 2G is nocodazole treatment, where we find fragmentation of Cep215 and Pericentrin when microtubules are intact (Figure 1E), but when cells are treated with nocodazole that disassembles the microtubule-based spindle we find that Pericentrin is in acentrosomal fragments, but Cep215 is no longer at these fragments with Pericentrin. What this suggests to us is that when a spindle is fully intact Cep215 and Pericentrin may fragment similarly in a microtubule-dependent way, but when microtubules are dismantled Cep215 no longer stays in these acentrosomal fragments with pericentrin suggesting that the interaction between Cep215 and Pericentrin can no longer be maintained.

2. "*shRNA knockdown of cenexin is verified using fluorescence intensity as well as the claim that centrosomal protein levels are not changing (figure S1A and S1E). This should be done by Western blot."*

Our previous studies with these same stable cell lines have demonstrated a loss of cenexin mRNA in cenexin shRNA treated cells (Hung *et al.*, 2016; Colicino *et al.*, 2019), and as Reviewer 1 has stated we have confirmed depletion by immunofluorescence analysis (Figure S1A and S1B, similar to our studies in (Colicino *et al.*, 2019)). We struggle with performing successful Western blots toward cenexin with our current reagents and are limited to immunofluorescence and RT-PCR (Colicino *et al.*, 2019). We have performed Western blot analysis on Cep215, Pericentrin, Cep192 and γ-tubulin where we identified similar protein concentrations between control and cenexin depleted cells (Figure S1G).

3. *"Figures 2E, 3A, 3D, 4D, 4E, and 4F should all include another feature to show that what the authors are visualizing is actually a centrosome. Generally, this would not be necessary with centrosomal proteins, but the authors are looking at a phenotype where the proteins they stain for are not properly localized to the centrosome. If these are all mitotic cells, simply staining for α-Tubulin would reveal the spindle and confirm that these are actually centrosomes. Because of the multiple α-Tubulin aggregates, Figure 2E needs a centriole marker, like centrin, to show where the centrosome is. Currently the conclusion the authors draw from these data are questionable because they cannot demonstrate that the activity is happening at the centrosome."*

To address the reviewers concerns with including a centriole marker, centrin has been included as a centriole marker with α-tubulin in Figure 2B to demonstrate two centrioles with multiple α-tubulin acentrosomal asters. This is also performed in new Figure 2E. In addition, we have included a centriole marker (acetylated tubulin) in our expansion microscopy images now featured in Figure 3A and centrin as a centriole marker in all analysis for figure panels 3B and S3E-G featured in representative images S3B-D, and γ-tubulin in S3A. For the expansion microscopy image, we also include new volumetric analysis of the PCM in relation to the centriole as an additional video file (video 1).

4. "*The core question the authors attempt to answer in Figure 2 is whether cenexin loss causes centrosome disfunction (p6, ln19-20). Figure 2B shows a functional bipolar spindle, which suggests no, cenexin depletion does not interrupt centrosome function. This is surprising as Soung et al. (2006) and Soung et al. (2009) showed changes in spindle shape as one of the phenotypes of cenexin loss during mitosis. This discrepancy needs to be addressed. In addition, the authors should justify more clearly why they included the nocodazole treatment in this test."*

We thank the reviewer for their comment and have removed the language "disfunction". We now discuss how the centrosome microtubule nucleation capacity is upregulated with loss of cenexin. We would like to clarify that the spindles imaged in Figure 2B are a result of a nocodazole washout and not a spindle that is formed normally during the cell cycle. The nocodazole washout allows us to dismantle all the microtubules in the spindle and characterized how the spindle reforms from the mitotic centrosomes (Figure 2A). We did this experiment based on Soung et al.'s studies that found abnormal spindle morphologies with cenexin depletion (multipolar spindles similar to Figure 1G) present in ~20% of the population, suggesting that ~80% of the cells present with normal spindles. In our cell population studies, we see similar extreme multipolar phenotypes as Soung et al., with most of the population presenting with loss of PCM cohesion that we characterized as splayed and/or scattered PCM (Figure S1C). Based on this, we wanted to examine in cenexin-depleted cells if mitotic centrosomes that exhibit subtle PCM cohesion defects

behave similarly in their microtubule nucleation capacity compared to a control population. Thus, we performed a nocodazole washout experiment that is shown as a time-course in Figure 2B. We found that even when cenexin-depleted cells form multiple MT-asters at 5 min post nocodazole washout, they can form a normal shaped bipolar spindle by 20 min. However, during this time course the spindle was not formed in the same manner between control cells that mainly nucleated their MTs from the two centrin positive mitotic centrosomes compared to cenexin depleted cells that have robust MT nucleation events from both the centrin positive mitotic centrosomes and acentrosomal nucleation sites positive for pericentrin. We have clarified the text to reflect these ideas and better cite Soung et al.'s findings as our rationale in our summary discussion of Figure 1.

5. "*From the FRAP experiment in Figure 3H, the authors conclude that, without cenexin, PLK1 is better able to phosphorylate PCM proteins and recruit them to the centrosome, which conflicts with the previously observed PCM fragmentation. This is similar to the previous conclusion from the nocodazole treatment in Figures 2B and 2E that the advent of more PCNT foci in cenexin-depleted cells results in a more PCM dense spindle because those PCNT aggregates are transported to the centrosome. This begs the question, why does cenexin depletion sometimes prevent PCNT from localizing to the centrosome and sometimes does not? This discrepancy needs to be addressed."*

We apologize that Reviewer 1 took from our studies and text that we conclude that "without cenexin, PLK1 is better able to phosphorylate PCM proteins and recruit them to the centrosome". The first part of that sentence we do conclude that loss of cenexin causes over phosphorylation of PCM proteins by PLK1, however we do not think that this results in better recruitment to the centrosome. We think cenexin-loss doesn't affect PCM recruitment to the centrosome but may influence PCM cohesion. For instance, PCM proteins are packed tightly at the centrosome when PLK1 phosphorylation events are normal or low (control conditions or under conditions of BI treatment) that present experimentally as PCM proteins with lower mobility (Figure 3F, S3H) and less fragmentation (Figure 3D, *new* live Figure S1J), whereas with cenexin depletion there is an increase in phosphorylation events (Figure 3B) and an increase in fragmentation (Figure 3A, 3C-D, *new* live Figure S1J) that corresponds with an increase in mobility of PCM components (Figure 3F, S3H). This increase in mobility and fragmentation is alleviated when cells are treated with the PLK1 inhibitor, BI (Figure 3F, S3G). We also find no increase in the levels of Cep215 or Pericentrin recruitment to the centrosome (Figure S1F) but find an increase in centrosome area/fragmentation (Figure 3D, S3G) suggesting a decrease in packing of these components at the centrosome in cenexin depleted cells compared to control that can be rescued with PLK1 inhibition.

6. "*As mentioned above, this paper does not present much new data. Apart from the data on Cep215 and the data found in Figure 4, every piece of data either corroborates or contradicts data shown in Soung et al. (2006) and Soung et al. (2009), which show similar experiments in HeLa cells -- the same model that the authors primarily use. This is not mentioned in the text, and it should be. I suggest either citing the original work or presenting new data that furthers our understanding of how the interaction between cenexin and PLK1 contribute to PCM organization."*

We have addressed this concern by referencing Soung et al. where appropriate and clarified in the text where our studies confirm and add upon Soung et al's work as outlined in point 5 and point 4.

*Minor Comments*

*1. "The visual in Figure 1H is a great way to summarize your findings. Please detail whether or not*

*it is an average of many different images of cenexin-depleted metaphase cells and what software or algorithm the authors used to calculate that average and then to graph it out."*

We thank the reviewer for their comment and suggestion. The cartoon of the different centrosome proteins in Figure 1H reflect tracings of a single representative pole reflecting the average phenotype for each protein, whereas the numbers provided reflect the overall averages of the area of each centrosome protein from a representative experiment that was performed across n=3 experiments. We have clarified this in the text.

2. "*Add methodology for obtaining mitotic cells. The paper only gives specifics for how cells were synchronized for expansion microscopy."*

We have addressed this comment in the methods section highlighted in blue where we used ProTAME as our synchronization agent. All synchronization methods were consistent throughout the study.

3. "*FRAP experiment in Figure 3I should also include Control + BI2536. This could help explain why inhibition of PLK1 in cenexin-depleted cells does not rescue the FRAP phenotype as well as it does the other phenotypes shown in Figure 4."*

We thank the reviewer for their suggestion. We have included FRAP analysis on Control+BI2536 as the reviewer suggested; see Figure 3F and S3H.

*4. "p13, ln7-8 argues that the experiments done in Figures 4D-4H show that cenexin's role as seen in HeLa cells is conserved in vivo in zebrafish. Because the rescue is done with human cenexin, these experiments say little about how the zebrafish cenexin homologue functions in relation to centrosomes. This is handled better throughout the rest of the manuscript."*

We thank the reviewer for the comment. Our data show that human cenexin localizes to centrosomes where it is expected and that it overlaps with γ-tubulin signal at the centrosome. Additionally, sequence alignment of human and zebrafish cenexin demonstrates the two sequences share 70% functional similarity (now highlighted in Figure 4A). These results coupled with evidence that the depletion of cenexin *in vivo* results in similar phenotypes where centrosome cohesion is disrupted strongly suggest that the function of zebrafish cenexin is conserved. We have updated the text to clarify this.

**Reviewer #2**

*"The manuscript by Aljiboury and colleagues investigates the role of the protein cenexin in organizing mitotic pericentriolar material (PCM). Cenenxin was identified as one of a few proteins that recruit Polo Like Kinase 1 (Plk1) to the centrosome. Plk1 recruitment to centrosomes is critical for phosphorylating PCM protein substrates, which then leads to centrosome maturation as cells enter mitosis. Thus, understanding how Plk1 is recruited to centrosomes is a major goal in the field. Very little work has been done on cenexin as it relates to Plk1 recruitment, thus the topic of this study is interesting and important.*

*The data in the manuscript are presented and quantified well, and the conclusions overall match the data without over interpretation. All the data in manuscript can be summarized by stating that loss of cenexin, or it simply its interaction with Plk1, leads to fragmentation, or loss of PCM cohesion. That conclusion is supported by in vitro and in vivo data presented in figures 1, 2, 3 and the bottom of 4. Authors also show that fragmentation following loss of cenexin relies of Plk1 activity. Thus, the concluding sentence in the abstract includes the new finding in this manuscript -*

*that cenexin is required for PCM integrity, but also includes what was already known about cenexin recruiting Plk1 for its role in phosphorylating PCM for proper PCM function.*

*Having highlighted the new finding, the manuscript does not show, or really suggest, how cenexin normally functions in wild-type cells to ensure proper cohesion. In fact, I am less clear on the role of cenexin as it relates to Plk1 recruitment and function during maturation. Cenexin does not seem to simply bring Plk1 to centrosomes. While the concluding sentences at the end of many paragraphs describe the phenotypes following loss of function and inhibition, they do not speculate on how cenextin normally functions to prevent these phenotypes. For example page-lines (6-14), (8-1), (8-17), (11-10). The page 11 line 10 summary is interesting, but how do the authors suggest cenexin-Plk1 complex prevents substrate phosphorylation which then prevents PCM mobility? How might this happen?"*

We thank the reviewer for their comments, especially the last enquiring about our potential model. We speculate that cenexin sequesters a population of PLK1 preventing it from over phosphorylating PCM proteins. If cenexin is absent, the cenexin-bound population of PLK1 is now free and available to phosphorylate PCM proteins. This may be why we don't see losses of PLK1 at the centrosome (Figure S3F) or significant increases in pPLK1(T210), but we do find a significant increase in pS/T substrates with cenexin-loss that is PLK1 dependent (Figure 3B-C). We hypothesize that this hyper phosphorylation of PCM proteins disrupts the cohesion of the PCM making the PCM more mobile as evidenced by our FRAP experiment (Figure 3F, S3H) and potentially allowing the PCM to be more competent for microtubule nucleation events (Figure 2). When cenexin is present, the cenexin-bound population of PLK1 is unavailable to PCM proteins resulting in normal PCM cohesion and microtubule nucleation. We have edited the manuscript to clarify these points.

*"Similarly, how do the author explain the massive increase in Plk1 activity without an increase in Plk1 levels and even a slight decrease in phosphor-Plk1. How might cenexin function to negatively regulate Polo kinase activity?"*

Like the discussion above, we propose that the amount of centrosome localized PLK1 and basal activity of PLK1 may not be drastically altered (Figure S3C-F), but that loss of cenexin may release a sub-population of PLK1 to act on substrates causing an increase in pS/T levels in a PLK1 dependent manner (Figure 3A-B). This discussion is more clearly outlined and discussed in the text. An ideal experiment to perform is to examine the mobility of PLK1 with loss of Cenexin, we are currently trying to make a zebrafish transgenic line with PLK1 fluorescently tagged at its endogenous locus where we can examine these ideas *in vivo*. These experiments are ongoing, and we hope to have a follow up paper further exploring these ideas with Cenexin and other potentially conserved scaffolds during zebrafish embryo development.

*"The conclusion in Figure 3 title is not supported by figure 3 data. The data show that cenexin is required for PCM cohesion and that Plk1 is required for PCM dispersion."*

We thank the reviewer for their comment and on their use of the word "cohesion", which we now have incorporated throughout the text. This much better discusses the findings we are trying to describe. We have also incorporated your point into the title for Figure 3, which encompasses the findings more accurately. Thank you again for this suggestion.

*"This reviewer can only reconcile all the data through two parallel pathways, one in which cenexin promotes Polo activity for PCM cohesion and another pathway that prevents Polo activity required for other roles that must be turned off in mitosis such as PCM dispersion (which cell might require in anaphase). The pathways might look like this:*

*Polo phosphorylates → Cenexin → anchors Polo →exclusively phosphorylates PCM component (Cep192, Cep215) →centrosome maturation → PCM cohesion*

*** In anchoring Polo for the above role, cenexin acts to prevent Polo function in other context as follow*
*Cenexin --| Polo → other functions including PCM dispersion → uncontrolled MT nucleation*
*I would urge the authors to think about the above model to see if it makes sense to them and if it explains all/most their data, and other published data such as Figure 6B from Soung 2009 that shows multipolar spindles that could be a result of loss of PCM cohesion. The manuscript would really benefit from a clear model or pathway that the reader can follow.*

*If the authors agree with these models or come up with any alternative model to explain their data, then they could really design experiments to test the model directly. As it stands, however, the manuscript lacks clear insight into cenexin function and simply reports a new phenotype."*

We thank the reviewer for their suggestion and making us commit to a model that provides a much clearer proposed function for cenexin. The model we support is like an outlined model above:

CDK1 phosphorylates cenexin → Cenexin binds and sequesters PLK1 --| unregulated PCM phosphorylation → loss of PCM cohesion and uncontrolled MT nucleation

Soung et al's studies demonstrated that cenexin is phosphorylated by CDK1 (Soung *et al.*, 2006, 2009) allowing for Cenexin to bind PLK1. Our findings suggest that this binding may sequester PLK1 preventing it from becoming activated and causing unregulated PCM phosphorylation that results in a loss of PCM cohesion and uncontrolled microtubule nucleation events. The studies included that support this model is an increase in PCM phosphorylation with cenexin-loss (Figure 3A-C), a loss of PCM cohesion (Figure 3A, 3C-D, S3B, S3G), and an increase in MT nucleation (Figure 2B and *new* Figure 2E-F) that are all rescued with BI treatment. We agree with the reviewer that the multipolar spindles found in Soung et al's studies are a likely result of a loss of PCM cohesion as we demonstrate in Figure 1G. One reason for this is we don't see any significant changes in centriole number between control and depleted conditions (Figure S1D) and find the PCM fragmentation phenotype to be robust (Figure 1G, S1C). We have included this in our discussion and incorporated a model to highlight these findings (Figure 3G).

*Other concerns*

*"If cenexin is only found on the mother centrosome, why do we see dispersion defect on both?"*

Cenexin is enriched at the mother centrosome (oldest) with decreasing amounts found at the daughter. The ratio of cenexin enrichment at mother over daughter centrosome during metaphase was nicely demonstrated in both Hung *et al* Figure 3B-D and Colicino *et al* Figure 3A-C. Colicino *et al* identified that there was 1.5 times more cenexin at the mother than the daughter. In this study, Figure 4E-F presents examples in zebrafish embryo dividing cells demonstrating cenexin's distribution between the two mitotic centrosomes.

*Where do the other non-centriole based cytoplasmic PCM blobs come from? Are they initially part of the mitosis centrosome? Are they present in interphase when Plk1 should be inactive.*
We don't see the fragmentation occurring in interphase (new Figure S1H-I). This suggests that at interphase the requirement of cenexin to anchor PLK1 may be less of a necessity than at later cell cycle stages when PLK1 activity starts to climb. We also think that the PCM is well constructed in prometaphase but then tends to lose cohesion and disperse which we have been able to visualize live (refer to new Figure S1J).

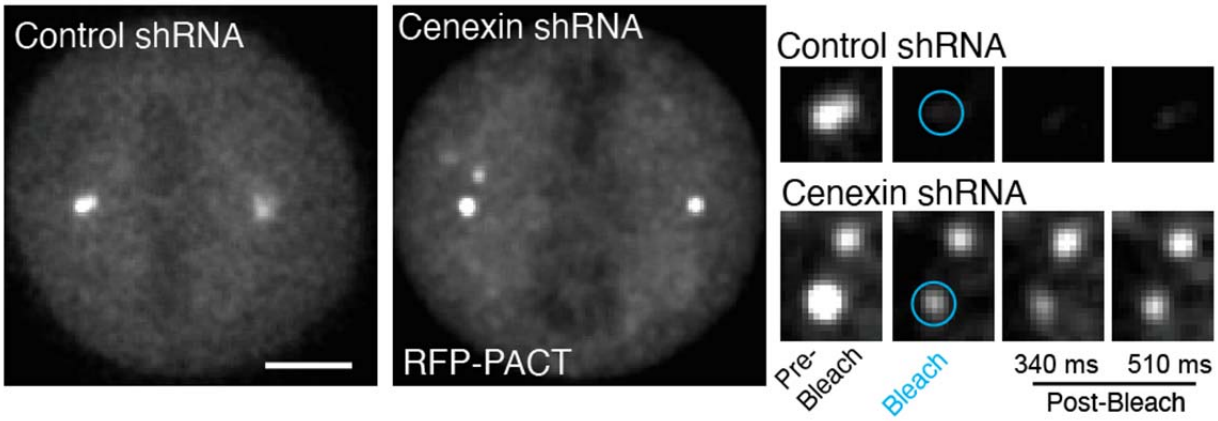
*"What is figure 2B telling us about cenextin function? Is cenextin normally required for tempering nucleation? If so, then is it only for a brief period of time? What does non-regrowth assay, steady state look like? Does this massive nucleation burst rely on Plk1?"*

To address your question if the massive nucleation burst from cenexin-depletion relies on PLK1, we treated cenexin depleted cells in our nocodazole washout experiments (Figure 2) with BI2536 to inhibit PLK1 activity (*new* Figure 2E-G) where we found that the MT nucleation burst does rely on PLK1. We propose cenexin functions to bind PLK1 during metaphase entry and sequester its ability to phosphorylate PCM components in an unregulated way (Figure 3). If a population of PLK1 is free to act on whatever it wants at the mitotic centrosome then PCM loses cohesion (Figure 1A, S1A, S1C) that is not associated with a change in centriole number (Figure S1D). The PCM fragmentation, and a loss of pericentrin-Cep215 co-localization, is associated with robust acentrosomal MT nucleation events (Figure 2B, D) that occurs in a PLK1 dependent manner (Figure 2E-G).

Cenexin has been linked to microtubule organization specifically during metaphase previously. Our previous studies demonstrated that cenexin depleted cells had astral microtubule defects (Hung *et al.*, 2016) and a separate study demonstrated that the central spindle of cenexin depleted cells had less stabilized microtubules (Gasic *et al.*, 2015). Ishikawa et al's studies concluded that there was no defect in MTOC activity in interphase cells between cenexin null MEFs (ODF2$^{-/-}$) and wild-type cells (Ishikawa *et al.*, 2005). Based on this previous work and the connection between the microtubule nucleation burst identified following nocodazole washout in Cenexin-depleted mitotic cells that is PLK1 dependent, we propose that cenexin may be tempering this burst as cells are going through prometaphase to metaphase. However, without performing the nocodazole washout it was difficult to identify any overt defects in MT organization beyond the astral MT-and central spindle defects reported in Hung *et al*. and Gasic *et al.*.

*"All images showing LUTs and combined colors should also be shown in greyscale. This includes Figure 1A, 2B, 3A and H.*
*Why?????"*

We find that Fire LUTs are useful when demonstrating changes in intensities of a single fluorophore between treatments or between locales, such that intense/concentrated signals are "hot" colors and dimmer signals are cooler colors that can correspond with a colorimetric scale as included in Figure 3E. We argue that with Figure 3E, using the LUT highlights the subtle changes occurring post Bleach (refer to black and white insert here versus FIRE LUT in Figure 3E). However, since this may be distracting or confusing, we have included greyscale images for updates (now included in Figure S1A, S1H, S1E, S1J, S2A, S3A-D, S4B).




*"Phylogenetic analysis is nice, but it does not help shed light on the role of cenexin in maintaining PCM cohesion. Figure 4B should be placed in supplement."*

We thank the reviewer for their comment. We argue that the phylogeny is an important part of the paper that we would like presented as part of a main figure. The phylogeny adds to the field as it contributes important information about what species may require PLK1 to be regulated by cenexin and how conserved that may be. Reviewer #1 supports this as they stated that the phylogeny is one of the novel findings presented in this paper.

## References

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Hung, H-F, Hehnly, H, and Doxsey, S (2016). The Mother Centriole Appendage Protein Cenexin Modulates Lumen Formation through Spindle Orientation. Curr Biol 26, 793–801.

Ishikawa, H, Kubo, A, Tsukita, S, and Tsukita, S (2005). Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. Nat Cell Biol 7, 517–524.

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Soung, NK, Park, JE, Yu, LR, Lee, KH, Lee, JM, Bang, JK, Veenstra, TD, Rhee, K, and Lee, KS (2009). Plk1-Dependent and -Independent Roles of an ODF2 Splice Variant, hCenexin1, at the Centrosome of Somatic Cells. Dev Cell 16, 539–550.

RE: Manuscript #E22-01-0015R
TITLE: "Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1"

Dear Dr. Hehnly,

Thank you for submitting your revised version of the manuscript 'Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1' to MBoC. Now your manuscript was reviewed by original reviewers, who now recommend publication upon minor textual revision.

Please revise your manuscript according to their suggestion, and I look forward to receiving the manuscript.

Best,
Yukiko

Yukiko Yamashita
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Hehnly,

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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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):
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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
mbc@ascb.org

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Reviewer #2 (Remarks to the Author):

I have now read the new manuscript and the rebuttal and I think overall the work has been improved enough to recommend publication. I offer the following final recommendations for the editor and authors to consider:

- The manuscript remains difficult to follow in terms of the precise model of cenexin function, even with the added functional pathway. Interestingly, however, the authors do provide a much clearer summary of their entire work in the rebuttal letter. This can be found in the middle of page 3 (reply to point 5 reviewer 1) and throughout page 5, 6, and 7. I strongly recommend adding a summary section at the end of this manuscript that synthesized the model and redirects the reader to the pertinent results. Otherwise, I suspect the sentiment of both reviewers in the first round of the lack of novelty and confusion of the cenexin's role will be share by all readers.

- Including Sas-4 and Ana1 as Polo centrosome recruiters (work from the Raff lab) in the intro.
- Page 8 line 3 - adding "number of" before a-tubulin
- Page 10 lines 14 and 15. It's not clear what "remains at the centrosome but is less-restrained" means. These terms seem to oppose one another. Consider rephrasing.
Page 13 line 13 - replace "centrosome area" with "cenexin area"

We have commented to Reviewer 2 below in blue font. All changes made in the manuscript are highlighted with blue font.

Reviewer #2 (Remarks to the Author):

I have now read the new manuscript and the rebuttal, and I think overall the work has been improved enough to recommend publication. I offer the following final recommendations for the editor and authors to consider:

We thank the reviewer for recommending our manuscript for publication.

- The manuscript remains difficult to follow in terms of the precise model of cenexin function, even with the added functional pathway. Interestingly, however, the authors do provide a much clearer summary of their entire work in the rebuttal letter. This can be found in the middle of page 3 (reply to point 5 reviewer 1) and throughout page 5, 6, and 7. I strongly recommend adding a summary section at the end of this manuscript that synthesized the model and redirects the reader to the pertinent results. Otherwise, I suspect the sentiment of both reviewers in the first round of the lack of novelty and confusion of the cenexin's role will be share by all readers.

We have added a summary paragraph that includes language highlighted in page 3, 5,6, and 7 of our previous response to reviewer comments file to highlight the novelty and potential mechanism cenexin may have in modulating PCM cohesion.

- Including Sas-4 and Ana1 as Polo centrosome recruiters (work from the Raff lab) in the intro.
We thank the reviewer for their suggestion. We have edited the manuscript introduction to incorporate references from the Raff lab representing Sas-4 and Ana1 as recruiters of PLK1.

- Page 8 line 3 - adding "number of" before a-tubulin
Here we are discussing the intensity of $\alpha$-tubulin rather than the number of clusters. We have edited the text to clarify that.

- Page 10 lines 14 and 15. It's not clear what "remains at the centrosome but is less-restrained" means. These terms seem to oppose one another. Consider rephrasing.
We thank the reviewer for their suggestion. We have edited the manuscript to clarify this statement.

-Page 13 line 13 - replace "centrosome area" with "cenexin area"
We thank the reviewer for their comment. We have edited the text to reflect this change.

RE: Manuscript #E22-01-0015RR
TITLE: "Pericentriolar matrix (PCM) integrity relies on cenexin and Polo-Like Kinase (PLK)1"

Dear Dr. Hehnly:

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

Sincerely,
Yukiko Yamashita
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Hehnly:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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