PCMD-1 bridges the centrioles and the pericentriolar material scaffold in *C. elegans*

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AUTHORS: Lisa Stenzel, Judith Mehler, Alina Schreiner, Sim Uestuener, Elisa Zuccoli, Esther Zanin, and Tamara Mikeladze-Dvali

II have now received reviews of your manuscript from 3 experts. The reviewers' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, all 3 reviewers express interest in your study but also express concerns with the current version of the analysis and interpretation. They offer numerous excellent suggestions to address those concerns and improve your study and manuscript. All three reviewers request additional experiments, analysis and careful rewording of the manuscript. Reviewer 1 requests demonstration of a clear link between PCMD-1 and the PCM. Reviewer 2 requests additional experiments, including discussions on the role of the coiled coil domain in addition to the analysis on the role of this domain in the SPD-5, PLK-1 and SAS-4 are in any manner relevant to the interaction with PCMD-1. Such revisions would also likely address Reviewer 3's concern that the final conclusions are to suggestions/speculations at this time. While Reviewer 3 lays out a quick fix and a long fix, in terms of interest to the readership of Development, an analysis of the bridging function of PCMD-1 would be of interest (the long fix). All three reviewers provide excellent detailed suggestions on additional experiments, analysis and discussions on improving the study.

I invite you to consider the reviewers' suggestions and submit a revised manuscript that addresses their concerns. Your revised manuscript would be re-reviewed, and acceptance would depend on your satisfactorily addressing the reviewers' concerns. In your revised manuscript, please clearly HIGHLIGHT all changes made in the revised version. You should avoid using 'Tracked Changes' in

Word files as these are lost in PDF conversion. I also request a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of the reviewers' criticisms or suggestions, please explain why.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

To function as a microtubule organizing center, the centrosome must be assembled properly so that the pericentriolar material (PCM) organizes around the centrally positioned pair of centrioles. How the PCM is linked to centrioles is not well understood. The manuscript from Stenzel and colleagues focuses on the C. elegans protein PCMD-1 which is a component of both the centrioles and PCM and is required for incorporation of the major PCM component SPD-5. Here the authors show that PCMD-1 is recruited to centrosomes by the centriole protein SAS-7. However, using a yeast two-hybrid assay they do not detect an interaction between PCMD-1 and SAS-7. Rather, they find that PCMD-1 interacts with SAS-4, itself SAS-5 and the kinase PLK-1. They convincingly show that ectopically localized PCMD-1 is sufficient to recruit SPD-5 and PLK-1, and go on to map regions of PCMD-1 that may mediate its localization to centrioles.

The study provides some nice insights into the molecular activity of PCMD-1, but comes up well short of demonstrating that PCMD-1 serves as a link between the centrioles and PCM. This is largely a consequence of not clearly demonstrating how PCMD-1 attaches to centrioles. However, there are also a host of other problems with the manuscript. Some claims are not supported by the data (for instance the importance of the coiled-coil domain in localizing PCMD-1), some experimental results need further confirmation (two hybrid), some results are not quantitated, and some of the experiments do not address meaningful questions (the dependence of PCMD-1 localization on SAS-4), and the fate of sperm-derived PCMD-1). Further, there is a certain vagueness to the writing that often makes it is very difficult to clearly understand what question a particular experiment is designed to understand or how the authors are interpreting a particular result. Finally, there are a number of cases where the authors report an unexpected or unintuitive result without providing an explanation. This leaves the reader wondering if the authors even recognized the incongruency of these results.

This study does have the potential to answer an important question in centrosome biology and if successful would be of great interest to the readers of Development. While extensive changes are needed to achieve a publication-quality study, it seems all such improvements could be made in a reasonable amount of time.

Comments for the author

Major issues:
1. Figure 1B. GFP::SAS-7 signal should be quantified in wild-type vs pcmd-1 mutant. Likewise, the data in either Figure 1C or Figure S1A should be quantitated.

2. Figure 2A. The two-hybrid data appear to have yielded some important information regarding the molecular function of PCMD-1, but as this assay is notorious for yielding false positives it is imperative that the most important interactions (PCMD-1/SAS-4, PCMD-1/SPD-5, PCMD-1/PLK-1) be confirmed. This could involve simply reversing the bait and prey plasmids and redoing the two hybrid or using a different assay such as a pull down.

3. Figure 2C. I am not sure what question this experiment is designed to address. Are the authors trying to say that this experiment supports a role for the physical interaction between SAS-

4 and PCMD-1 in PCMD-1 recruitment or that at some level (direct or indirectly) SAS-4 is required for PCMD-1 localization to the centriole? If it's the former, then this experiment does not address that question in any meaningful way and if it's the latter, then the result is neither surprising nor interesting. The partial RNAi experiment also does not help in distinguishing between a direct or indirect role for SAS-4. Put another way, you would likely get this same result using RNAi against most other centriole factors (ZYG-1, SAS-5, or SAS-6).

In place of this experiment the authors should use their two-hybrid assay to map the PCMD-1-binding site in SAS-4 (or vice versa, the SAS-4 binding site in PCMD-1) and then use CRISPR/Cas9 to mutate it. By showing that the mutant SAS-4 localizes to centrioles but fails to recruit PCMD-1, they would be able to offer strong proof of their central argument that PCMD-1 is physically linking the centriole and PCM. An experiment like this one is vital to demonstrate a molecular link between the centriole and PCM.

4. Figure 3 B-D. Really nice assay! Regarding the question of whether the GFP::SPD-5 pool localized to the membrane represents an interphase core fraction or an expanded mitotic pool, most of the cells in the embryos shown in this figure look like they are in interphase and thus would be expected to have only the core fraction. Thus, I wondered if the failure to observe expanded PCM is due to most or all cells being in interphase or if the membrane localized GFP::SPD-5 lacks something critical for mitotic expansion. Could the authors do live time-lapse imaging of the embryos to see if the membrane-bound GFP::SPD-5 expands when cells enter mitosis? They could monitor GFP::SPD-5 levels at centrosomes in these same cells to determine when they enter mitosis. This seems like a simple experiment that might yield insightful results no matter what the answer is.

5. Figure S3A&B. Do the authors know if GFP::PCMD-1 is stably associated with embryonic centrosomes or if it exchanges with the cytoplasmic pool.? Based on the data in Figure S3, they conclude that sperm derived PCMD-1 turns over after fertilization. If PCMD-1 rapidly exchanges with the cytoplasmic pool, this result is expected and this experiment doesn't say much. If it does not usually exchange, then the removal of paternal PCMD-1 is likely a regulated developmental event with possible biological significance. Also, how many embryos were scored in these experiments?

6. Figure 4G. SPD-5 centrosome levels should be measured in control and pcmd-1(Δcc) embryos.

7. The authors seem to be overstating the importance of the coiled-coil domain in loading PCMD-1 onto centrosomes. It's true as shown in figure 4C and D, that a full-length version of PCMD lacking the coiled-coil localizes poorly to centrosomes. However, the data in Figure 5F shows that the C2 fragment that also lacks the coiled-coil localizes just fine. Thus, the data appear contradictory and leave a less than clear picture of the role of the coiled-coil in localizing PCMD-1. Also, some of the results in this section are counter intuitive and the authors again fail to offer any explanation why. For instance, why does fragment C localize to centrioles better than the ΔCC construct even though it lacks more of the PCMD-1 sequence? Similarly, why does the sub-fragment C2 localize to centrosomes better than the larger fragment C1 that contains the entire C2 sequence?

Minor issues:
1. The authors state that in multicellular embryos, GFP::PCMD-1 was detected at centrosomes. They do not specifically state that they are talking about sas-7(or452) embryos, but if they are, why do they think that GFP::PCMD-1 is absent at centrosomes in one cell mutant embryos but present at centrosomes in multicellular embryos? The authors should at least speculate why this is so.

2. Figure 1A. I am not sure what the relationship is between the schematic of the four cell embryo and the images of PCMD-1 and tagRFP::SAS-7. Are the fluorescent images showing centrosomes from the EMS cell of a four-cell embryo? This is not explained in the figure legend.

3. Page 3 first paragraph. The authors should point out that the pcmd-1(t3421) mutation is a likely null allele due to an early stop codon. Not immediately aware of the nature of the mutation, I wondered if the mutant protein might maintain some functions (such as recruiting SAS-7) while lacking other functions (recruiting PCM). I had to go back to the Erpf et al. paper to find out what the nature of the allele is and thus if the data presented in Figure 1C was definitive. Of course it is, but you had to know the allele was a protein null. You can save readers the trouble of researching the allele by describing it here.

4. Figure 3B: The authors show that GFP::PCMD-1 is not recruited to the membrane by PH::mKate2::PCMD-1. This is an unexpected result given the two-hybrid results and the authors offer no explanation that may account for this. This is just one of a few places in the manuscript where an unexpected result is observed and there is no attempt to provide a possible explanation.

5. Figure 3D. The authors should explain to the reader why sas-5(RNAi) does not target the gfp::sas-5 transgenes. Many readers may be unfamiliar with the use of recoded transgenes. Related to this, how do the authors control for the efficiency of sas-5(RNAi) in these strains?

6. I don't understand the purpose of Table 1. All of the data contained in this table is already reported in Figures 4, 5 and S4. It should be removed.

7. Discussion. The authors offer an explanation for why membrane tethered PCMD-1 fails to recruit centriole proteins SAS-4 and SAS-7 by stating that they are stably incorporated into the centriole. This assumes that there is very low to no cytoplasmic SAS-4 or SAS-7, which I don't think is true. It thus seems likely that another explanation is possible. For instance, post-translation modifications might control these interactions.

8. I think a model to account for the various protein interaction and localization dependence data is that PCMD-1 localizes to centrioles via two distinct but essential interactions. One is a direct interaction with SAS-4 and the second is a SAS-7-dependent interaction (that may or may not involve direct binding between SAS-7 and PCMD-1. If either interaction is disrupted, PCMD-1 fails to localize.

Typos and very minor issues:
1. Why is RFP at times referred to as RFP and others as tagRFP?
2. Page 4, line 10: centriole duplication fails "in" sas-4…
3. Page 4, 6th line of next section: "whether PCMD-1 is capable "of recruiting"
4. Page 6, line 15: "deleted the sequence from E86 "to" F118.
5. Page 6, line 26: "in" 3 out of 18 centrosomes
6. Page 6, line 16: "in the context of" should be rephrased
7. Page 7, line 16: "even had" a negative effect on the viability

Reviewer 2

Advance summary and potential significance to field

Stenzel et al present results indicating that the C. elegans centriolar coiled-coil protein PCMD-1 bridges the centrioles to the pericentriolar matrix (PCM). They show that the centriolar protein SAS-7 is required for PCMD-1 to localize to centrioles (but not vice versa), that the C-terminus of PCMD-1 is required for its centriolar localization, and that PCMD-1 both binds to (in yeast two-hybrid assays) and can recruit to an ectopic location the PCM proteins PLK-1 and SPD-5. Moreover, PCMD-1 loads onto centrioles prior to SPD-5 during the first mitotic division of the zygote. The authors also show that the coiled-coil domain of PCMD-1 is required for PCMD-1 to bind itself in yeast two hybrid and that a strain expressing an edited PCMD-1 that lacks the coiled coil domain is viable but has reduced levels of both the edited PCMD-1 and SPD-5, with a disorganized PCM. The authors have provided an extensive analysis of PCMD-1 function significantly advances our understanding of the link between centrioles and PCM assembly and maturation. Given the importance of centrosome assembly and maturation for cell division and development, these results will be of substantial interesting to a wide audience of cell and developmental biologists. However,

before the manuscript can be considered suitable for publication, the authors first need to address the following concerns.

Comments for the author

Major concerns
1. The authors present an impressive structure-function analysis of PCMD-1. It seems surprising the coiled-coil deletion is homozygous viable with very little embryonic lethality. Consistent with this, the C-terminal construct, which lacks the coiled-coil domain, almost completely rescues the loss of function t3421 allele. This indicates that most of the essential functions of PCMD-1 do not require the coiled-coil domain. The authors do not provide any discussion of this curious pair of observations, but rather emphasize how the coiled-coil domain is important for PMCD-1/PMCD-1 interactions. Apparently the PMCD-1 interaction with itself is of minor importance. The authors should provide some discussion of these observations and speculate on what parts of the proteins confer essential functions with regard to SPD-5 and PLK-1 recruitment. Some insight could be provided if the authors would report whether the two-hybrid interactions of PMCD-1 with SPD-5 and PLK-1 and SAS-4 required the coiled-coil domain of PMCD-1 (they only show that PMCD-1 requires this domain to interact with itself). Also see minor comment #4.

2. The authors need to address some quantification issues in their figures. They frequently note the number of embryos examined in almost all of the figure legends. However, they only show single representative embryos in Figures 1 and 2. Without some quantification of what the authors saw, these numbers don't mean much. In Figure 3C and 3E, how did the authors score whether or not GFP signal was present at the membrane or not? Did they apply some sort of threshold? This should be explained. Also the authors should note whether the images of membrane localization are single focal planes or projections. Can the authors explain why the levels of mKate::PCMD-1 at the membrane after heat shock vary considerably depending on which GFP background is being assessed?

Minor concerns
1. These authors have shown in their 2019 Current Biology manuscript that while SPD-2 largely depends on PMCD-1 for localization to centrosomes, PCMD-1 does not require SPD-2. The authors never mention in this manuscript that PCMD-1 does not require SPD-2 for its localization to the centrosome, even though they include SPD-2 in their two-hybrid assays. To provide a more complete overview of this network, it would help for the authors to note the lack of PMCD-1 dependence on SPD-2 for centriole localization. It might also be worth noting that SAS-7 is required for both PCMD-1 and SPD-2 to localize to centrioles, but neither PCMD-1 or SPD-2 are required for SAS-7 to localize to centrioles. In their 2019 Current Biology paper, the authors also assessed SAS-4 localization in pcmd-1(t3421) mutants. The authors should refer to these various results, presumably in the Discussion, to provide a more complete overview of these dependencies and dynamics.

2. In Figure 1A, the authors examine the partial co-localization of SAS-7 and PCMD-1. The text at the bottom of page 2 and top of page 3 describing this is somewhat confusing. It would help if the authors would make it clear in the text that PCMD-1 is present at more equal levels at the mother and daughter centrioles, while SAS-7 is more asymmetric. They refer to this asymmetry being consistent with a previously documented role for SAS-7 in daughter centrioles becoming competent to duplicate—the daughter-to-mother transition. Do the authors think SAS-7 is present at lower levels at the mother or the daughter centriole?

3. In Figure 1, the authors show some PCMD-1 localization to astral microtubules and kinetochores; the authors might want to comment on whether they think this has any function. Is it possible that loss of these pools contribute to lethality, given the surprising lack of lethality for the coiled-coil deletion, or does the rescue of most lethality by the C-terminal fragment argue against such a possibility?

4. The live imaging data in S1A seems worthy of being included as part of Figure 1.

5. The authors note (and show in S1C) that GFP::PCMD-1 signal is detected at much higher levels in later stage sas-7(or452) mutant embryos. The authors might want to speculate briefly about why there is such a substantial difference in early vs late levels.

6. On page 4, the authors state that "…PCMD-1 and SAS-7 were only detected at the mother centrioles (Figure 2C)." This implies that they could have been detected somewhere else (such as at daughter centrioles). But presumably in strong sas-4 knockdowns there are only mother and no daughter centrioles? If so, perhaps the authors could change the wording to say that PCMD-1 and SAS-7 were both detected at the mother centrioles, with daughter centrioles absent due to loss of SAS-4.

7. Might it be possible that the SAS-4 that is present in sas-7(-) mutants is present with a different conformation that explains the absence of PCMD-1, with the absent conformation serving as the bridge between SAS-7 and PCMD-1?

8. In Figure S3A, the authors fail to see sperm-derived GFP::PCMD-1 in mitotic embryos, after pronuclei have met. The authors do see oocyte-derived GFP::PCMD-1 at that time, and thus conclude that there is turnover. Can the authors detect sperm-derived PCMD-1 earlier, in association with the sperm pronucleus during meiosis I or II or shortly thereafter?

9. The authors further look at when oocyte-derived GFP::PMCD-1 loads onto centrioles, but they do not explicitly state how they staged the fixed oocytes to be during meiosis I, during meiosis II or after meiosis II. Presumably the staging was done based on the status of chromosome segregation and extrusion of polar bodies. This should be stated somewhere.

10. In Figure 4G, the authors state "Images of SPD-5 areas of centrosomes 1 and 3." What does this refer to?

11. In the text on page 7, lines 7 and 8 of the new section, the authors might insert "RNAi-resistant" to say "…to test the functionality of different RNAi-resistant truncations…" to be more explicit as to how the gene replacement strategy works, for readers who may not be familiar with it.

Some minor language edits:
1. Last sentence in the Introduction: maybe change "delocalize" to "recruit"? I understand de-localize refers to competing with the centrosome but that is not clear at this point and is a bit confusing without more information. I would think recruit serves fine here and might avoid some confusion.

2. page 4, line 10: change to "Centriole duplication fails IN SAS-4 depleted embryos." [Add "in"].

3. page 4, line 15: drop the comma after "Our results suggest that,…"

4. Page 6, line 11 of the new section: Change to "…from E86 TO AND including F118…" [Add "to and"]

5. Page 7, line 11 of the new section: Change to "…could RESCUE embryonic lethality of…" [Replace "restore" with "rescue".] This reads awkwardly as it first seems that "restore" refers to embryonic lethality not viability.

Reviewer 3

Advance summary and potential significance to field

The manuscript by Stenzel et al investigates the role of the centrosomal protein PCMD-1 in recruiting pericentriolar material (PCM) to the centrosome.
Using a combination of genetics and localization studies, they suggest that PCMD-1 is anchored at the centriole and acts as a scaffolding-protein for SPD-5, one of the main PCM proteins in C. elegans. As my comment indicate below the author convincingly show that Sas-7 functions upstream

of PCMD-1, which acts upstream of SPD-5. However, the authors overinterpret their results to state that PCMD-1 functions as a direct scaffold to link the centriole to the PCM. I do not believe the model is supported by the data; I outline my specific comments below.

Comments for the author

Specific Comments
Figure 1. The role of Sas-7 in recruiting PCM-1 Overall, the data in this figure nicely show that PCMD-1 co-localizes with Sas-7. However, the imaging does not allow for precise localization at the centriole wall. In the future, this will be required in order to classify PCMD-1 as a centriole to PCM linker.
1a. The authors state – "We observed that one PCMD-1 focus had a higher accumulation of the SAS-7 signal, while the second one colocalized to a lesser extent with SAS-7". The authors should quantify the difference between the two centrioles as it is not clear in the images that a mother vs the daughter has higher levels. Alternatively, remove the statement of a difference.
1b. Figure S1A shows some localization of PCMD-1 in sas-7 mutant. The Authors should move this to the main figure as it is important information the fixed analysis in 1C is not the full story. Also, the authors state "negligible GFP signal remained at the centrosome, reflecting the hypomorphic nature of the sas-7(or452) allele". It is not clear when a protein level is considered negligible. Quantification of levels or removing the word negligible is required. Overall, the conclusion should include that some PCMD-1 could be recruited independently of Sas-7
1c. The Ns for some of these experiments seem very low. Looking at 6 centrosomes suggests they analyzed 3 embryos, likely from one experiment. Is there a technical reason for the low Ns?

Figure 2. Interactions data Overall, the Y2H experiments are extremely over interpreted. Negative results are interpreted as no interaction, which is not necessarily the case. Furthermore, a positive interaction is also over interrupted as evidence that the interaction occurs in the cell, even at the centrosome.

False positives are common in Y2H analysis, making the follow-up experiments presented (cortical co-localization) important. The authors state that "In summary, PCMD-1 binds to the centriolar protein SAS-4, the PCM protein SPD-5, the mitotic kinase PLK-1, and to itself. Therefore, we propose that PCMD-1 physically bridges the centrioles and the PCM scaffold," – this is pure speculation and should be specifically phrased as a hypothesis at this point.

Figure 2. Sas4 knockdown

The conclusion that PCMD-1 requires Sas-4 for recruitment is not supported.

It is more likely that loss of Sas-4 results in absence of the daughter centriole, which would result in loss of Sas-7, PCMD-1, and likely anything else that would appear on a centriole. A direct Sas-4-PCMD-1 interaction is also not supported by their own data showing a lack of recruitment of Sas4 to sites of PH::mKate2::PCMD-1 in figure 3, and the lack of PCMD-1 localization to Sas-4 dots in Figure 1C bottom panel. There is also no indication that a nascent centriole is or is not present in figure 2C, and there is no measure of knockdown level in the partial vs the full RNAi.
Taken all of the Sas-4 data together, Sas-4 has not been functionally linked to PCMD-1 in this study

Figure 3. In vivo interaction assay

The major concern with this assay is interpreting something as sufficient.

This assay is similar to a co-IP, thus there is a difference between the following two statement, the first of which is what the authors use, but should avoid
a. "PCMD-1 is sufficient to recruit SPD-5 and PLK-1 to an ectopic location"
b. Positioning PCMD-1 at an ectopic site is sufficient to recruit SPD-5 and PLK-1 to that ectopic location.

Statement (a) suggests a direct protein-protein interaction between PCMD-1 and Spd-5 or Plk-1, while statement (b) does not infer direct binding. The authors should rephrase this entire section of the paper to better reflect the fact that indirect binding can account for the re-localization to the

membrane. This includes the statements that “These findings suggest that PCMD-1 would also be sufficient to recruit the PCM scaffold to the centrosome in the C. elegans one-cell embryo,” and “thus, PCMD-1 can independently recruit PLK-1 and SPD-5.” These results have not been shown. Showing sufficiency is really hard, the authors should remove the terms sufficiency and independently and simply state that PCMD-1 can act upstream of Spd-5 recruitment.
The proposal that “PCMD-1 physically bridges the centrioles and the PCM scaffold” to this point has not been show. The summary thus far is that PCMD-1 localization requires Sas-7, but Sas-7 might not be the direct anchor, or the only anchor. The role of Sas-4 in unknow. PCMD-1 acts upstream of Spd-5.

Figures 4 and 5
The goal of these two figures is to determine the role of the CC domain in PCMD-1 and define centrosome localization and protein-protein interaction sequences. The conclusions that can be made are:
Figure 4 – CC is necessary for centrosome recruitment in the contexts of full length PCMD-1 Figure 4 – CC is required for self-interaction.
Figure 5 – CC is not necessary for centrosome localization in the context of the c-terminal truncation Figure 5 and S4 - CC is not sufficient within construct (N) for centrosome localization

Taken all of these data together, it is clear that the role mechanism of PCMD-1 function is much more complicated than the presented model of being anchored on one end to the centriole, dimerizing at the CC, and linking to other end to SPD5. This model is reminiscent of the current view of PNCT/PLP, but lacks evidence. To propose a linker function between the centriole and PCM, one would have to:
1) Show detailed super resolution localization pf PCMD-1 not only to show it’s localization near the centriole wall, but also to show an extended molecule as depicted in their model.
2) Show direct binding between the proteins suggested in the model, better map the binding sites (does Spd-5 bind the delta CC for example), show a disruption of localization of PCM in targeted mutants that do not impact centrosome localization of PCMD-1, and show disruption of PCMD-1 localization without impacting centriole formation. One might even have to go one step further to rescue function by bypassing the normal interaction site and artificially linking the two proteins.

These are not easy experiments, but experiments along these lines are required to make the conclusions presented here. So my recommendation is one of two things:
1) Quick fix – Address some of the low Ns mentioned above and extensively changing the language in the paper to simply reflect that Sas-7 is upstream of PCMD-1 which is upstream of SPD-5. Removing all mention of sufficiency and direct linkage/bridging centriole with PCM from the title, abstract and results. Also removing the experiments on Sas-4 as there are alternate explanations for this. Finally, restrict the hypothesis that PCMD-1 is a bridge to the discussion.
2) Long fix – Use these results as preliminary data to begin testing the direct bridging function of PCMD-1.

First revision

Author response to reviewers' comments

Point-by-point response to Reviewers comments:

We thank the Reviewers for critically evaluating our manuscript and for giving us constructive inputs and suggestions. The manuscript has substantially improved by addressing the raised issues.
In the new version of the manuscript, we have added the following key data:

1. Using different assays, we confirmed all positive interactions between PCMD-1 and its interaction partners. Specifically, we reversed the baits/prey plasmids and show interaction for SAS-4/PCMD-1 and PLK-1/PCMD-1. Since SPD-5 bait autoactivated in the reversed Y2H assay, we turned to alternative approaches. We expressed the C. elegans SPD-5 and PCMD-1 in HEK295T

cells and successfully co-immunoprecipitated EGFP::PCMD-1 with mCherry::SPD- 5::6xHIS. Together this data strengthens our model that PCMD-1 is a link between the centriole and the PCM.

2. We added two C-terminal deletion constructs to the structure function analysis of PCMD-1. These constructs revealed a second centrosome localization region in the N-terminal region. These C-terminal deletion constructs localizes less efficiently to the centrosome, but can recruit SPD-5. Additionally, it allowed us to determine a region necessary for cilia localization.

3. Using the Y2H assay we narrowed down the PCMD-1/SAS-4 interaction to the C-terminal PCMD-1(C2) part.

4. By performing live-cell imaging of RFP::SPD-5 in the background of the constructs targeting the centrosome, we revealed that the PCMD-1(C2) construct missing the N-terminus of PCMD- 1 fails to recruit SPD-5 to the centrosome, splitting centrosome localization from the function. Our findings suggest that the N-terminal part of PCMD-1 is needed for the recruitment of the PCM scaffold.

Reviewer 1 Comments for the Author:
Major issues:

1. Figure 1B. GFP::SAS-7 signal should be quantified in wild-type vs pcmd-1 mutant. Likewise, the data in either Figure 1C or Figure S1A should be quantitated.

We added these quantifications to Figure 1C.

2. Figure 2A. The two-hybrid data appear to have yielded some important information regarding the molecular function of PCMD-1, but as this assay is notorious for yielding false positives it is imperative that the most important interactions (PCMD-1/SAS-4, PCMD-1/SPD- 5, PCMD-1/PLK-1) be confirmed. This could involve simply reversing the bait and prey plasmids and redoing the two hybrid or using a different assay such as a pull down.

We thank the Reviewer for raising this issue. We confirmed the interactions between SAS- 4/PCMD-1 and PLK-1/PCMD-1 by reversing the bait and the prey in the Y2H assay (Figure 2C). Unfortunately, the SPD-5 bait showed autoactivation. For the SPD-5/PCMD-1 interaction we first attempted co-immunoprecipitation from worm lysates using the CRISPR tagged RFP::SPD- 5 and GFP::PCMD-1. This approach failed due to low abundance of the proteins. Therefore, we turned to a heterologous system and expressed EGFP::PCMD-1 and mCherry::SPD-5::6xHIS in HEK295T cells. Using this system we could successfully co-IP EGFP::PCMD-1 with mCherry::SPD-5::6xHIS using a RFP-trap (Figure 2D).

3. Figure 2C. I am not sure what question this experiment is designed to address. Are the authors trying to say that this experiment supports a role for the physical interaction between SAS-4 and PCMD-1 in PCMD-1 recruitment or that at some level (direct or indirectly) SAS-4 is required for PCMD-1 localization to the centriole? If it's the former, then this experiment does not address that question in any meaningful way and if it's the latter, then the result is neither surprising nor interesting. The partial RNAi experiment also does not help in distinguishing between a direct or indirect role for SAS-4. Put another way, you would likely get this same result using RNAi against most other centriole factors (ZYG-1, SAS5, or SAS-6).

The aim of this experiment is to place SAS-7 and PCMD-1 into a genetic pathway with respect to SAS-4. When sas-4 is downregulated by RNAi the central tube of the centriole still forms and SAS-5 and SAS-6 still localize to the nascent centriole (Pelletier et al.2006). In contrast in ZYG-1, SAS-5, or SAS-6 RNAi the central tube is absent (Pelletier et al.2006). The purpose of this experiment is to test whether SAS-4 is genetically upstream of SAS-7 and PCMD-1, i.e. whether SAS-7 and/or PCMD-1 would still localize to the nascent centriole. Our results indicate that this is not the case. We are aware that based on our data a direct interaction of PCMD-1 with SAS-4 cannot be proposed. Furthermore SAS-4 is most likely not the only anchor of PCMD- 1 at the centriole. We recognize that the wording in the text lacked clarity and adjusted it accordingly (page 3-4, line

135-136, 146-147). The data was moved to Figure S2.

In place of this experiment the authors should use their two-hybrid assay to map the PCMD- 1-binding site in SAS-4 (or vice versa, the SAS-4 binding site in PCMD-1) and then use CRISPR/Cas9 to mutate it. By showing that the mutant SAS-4 localizes to centrioles but fails to recruit PCMD-1, they would be able to offer strong proof of their central argument that PCMD-1 is physically linking the centriole and PCM. An experiment like this one is vital to demonstrate a molecular link between the centriole and PCM.

As suggested by the Reviewer, we performed the Y2H assay with the C2 part of PCMD-1 and demonstrate that this part interacts with SAS-4 (Figure 5D). This interaction was confirmed by switching the bait and the prey. We also include live-cell imaging data using RFP::SPD-5 in the background GFP::PCMD-1(C2) and show that PCMD-1 localization to the centrosome is not affected, but SPD-5 recruitment fails (Figure 5B, S6). The combination of these experiment demonstrates that the PCMD-1(C2) is sufficient to localize the protein to the centrosome, interacts with SAS-4, but is insufficient to recruit SPD-5. In support of this, we also performed the Y2H between the PCMD-1(C2) bait and SPD-5 prey, which showed a highly reduced interaction (Figure for Reviewers 1A). Since the SPD-5 bait autoactivated, we could not confirm this data in the opposite setting and therefore, did not include it in the manuscript. If the reviewers find this data a valuable addendum, we would be happy to include it in the manuscript.

As an alternative approach we created transgenic worms in which PH::mkate2::SAS-4 is tethered to the plasma membrane. PH::mkate2::SAS-4 localized to the membrane upon heat shock, however very weakly (Figure for Reviewers 1B). We could not translocate PCMD-1 to the membrane in this setting. Interestingly, we never observed PH::mkate2::SAS-4 at centrioles, which is often the case for PH::mkate2::PCMD-1. To test whether this construct is really functional, it would require rescue experiments with new worm strains without the PH- domain. Due to time limitations, we decided to consider the results inconclusive and do not include them in the manuscript.

4. Figure 3 B-D. Really nice assay! Regarding the question of whether the GFP::SPD-5 pool localized to the membrane represents an interphase core fraction or an expanded mitotic pool, most of the cells in the embryos shown in this figure look like they are in interphase and thus would be expected to have only the core fraction. Thus, I wondered if the failure to observe expanded PCM is due to most or all cells being in interphase or if the membrane localized GFP::SPD-5 lacks something critical for mitotic expansion. Could the authors do live time-lapse imaging of the embryos to see if the membrane-bound GFP::SPD-5 expands when cells enter mitosis? They could monitor GFP::SPD-5 levels at centrosomes in these same cells to determine when they enter mitosis. This seems like a simple experiment that might yield insightful results no matter what the answer is.

In the translocation assay the heat shock promoter works only in multicellular embryos and the expression levels vary greatly from cell to cell. In addition, we are using the pcmd-1(t3421) mutant background where cell divisions are abnormal and cell fates are unspecified. In these settings, we are unable to reliably determine when a cell would divide. To perform live-cell imaging we would have to rely on a random event. Occasionally, we find dividing cells where some GFP::SPD-5 is actually at the membrane. The image in the GFP::SPD-5 panel Figure 3B was on purpose chosen to show a cell that to our best knowledge undergoes mitosis. Our assumption is based on the round shape of the cell and the arrangement of the centrosomes. In this sample the membrane localization in the 'mitotic' cell and the neighboring interphase cells appears similar. In the Figure for the Reviewer, we added another example, where the shape of the cell is round and centrosomes appear to be at the spindle poles (Figure Reviewers C). In both cases (single z-slices), some GFP::SPD-5 is located at the membrane. We do not see a major difference in the membrane localization of SPD-5 in these cells compared to other cells. However, due to the differences in PCMD-1 expression levels in different cells, a quantitative comparison of SPD-5 membrane localization would be very difficult. We adjusted the wording in the manuscript (page 5, line 189).

5. Figure S3A&B. Do the authors know if GFP::PCMD-1 is stably associated with embryonic centrosomes or if it exchanges with the cytoplasmic pool.? Based on the data in Figure S3, they

conclude that sperm derived PCMD-1 turns over after fertilization. If PCMD-1 rapidly exchanges with the cytoplasmic pool, this result is expected and this experiment doesn't say much. If it does not usually exchange, then the removal of paternal PCMD-1 is likely a regulated developmental event with possible biological significance. Also, how many embryos were scored in these experiments?

We observe the loss of the sperm-derived GFP::PCMD-1 signal very early, during female meiosis. We now include an image of an earlier embryo in Figure S4. Recently Garbrecht et al. 2021 published a FRAP experiments, where GFP::PCMD-1 is reported to be stably incorporated in prometaphase-stage embryos. Therefore, unlike SAS-4, which is stably incorporated and maintained at sperm-derived centrioles, the dynamics of PCMD-1 seems to change from fertilization to mitosis. The numbers of embryos analyzed (n=11) are mentioned in the figure legend S4A.

6. Figure 4G. SPD-5 centrosome levels should be measured in control and pcmd-1(Δcc) embryos.

We added this data by quantifying live-cell imaging data of RFP::SPD-5 (Figure S5A, S5B).

7. The authors seem to be overstating the importance of the coiled-coil domain in loading PCMD-1 onto centrosomes. It's true as shown in figure 4C and D, that a full-length version of PCMD lacking the coiled-coil localizes poorly to centrosomes. However, the data in Figure 5F shows that the C2 fragment that also lacks the coiled-coil localizes just fine. Thus, the data appear contradictory and leave a less than clear picture of the role of the coiled-coil in localizing PCMD-1. Also, some of the results in this section are counter intuitive and the authors again fail to offer any explanation why. For instance, why does fragment C localize to centrioles better than the ΔCC construct even though it lacks more of the PCMD-1 sequence? Similarly, why does the sub-fragment C2 localize to centrosomes better than the larger fragment C1 that contains the entire C2 sequence?

The coiled-coil domain was deleted in the context of an endogenously CRISPR tagged protein. Instead, the single copy replacement system is using regulatory regions of the mai-2 gene. All constructs is under this promoter are expressed at higher levels than the endogenously tagged protein (unpublished observation). Therefore, it is hard to make a direct comparison. In accordance with the CRISPR deletion, the GFP::PCMD-1(C) under the mai-2 promoter also shows a tendency towards lesser centrosomal levels than the full-length protein (Figure 5C).

By 'C1' the Reviewer probably means the PCMD-1(C) fragment, which includes PCMD-1(C1) and PCMD-1(C2). The GFP::PCMD-1(C2) centrosomal levels are not significantly lower than GFP::PCMD-1 (mean 0.86+/-0.53 s.e.m. vs. 1), while GFP::PCMD-1(C) is slightly lower than GFP::PCMD-1(mean 0.73+/-0.08 s.e.m. vs. 1). In the context of the truncated constructs PCMD-1(C1) could negatively regulate PCMD-1(C2). However, the differences are minor and therefore, we do not include them in the manuscript.

Minor issues:

1. The authors state that in multicellular embryos, GFP::PCMD-1 was detected at centrosomes. They do not specifically state that they are talking about sas-7(or452) embryos, but if they are, why do they think that GFP::PCMD-1 is absent at centrosomes in one cell mutant embryos but present at centrosomes in multicellular embryos? The authors should at least speculate why this is so.

We propose that in older embryos a redundant mechanism recruits PCMD-1. This is the case in cilia, where SAS-7 is not present (unpublished observation by our group) and a positive feedforward loop between SPD-5 and PCMD-1 maintains these proteins at the ciliary base.

2. Figure 1A. I am not sure what the relationship is between the schematic of the four cell embryo and the images of PCMD-1 and tagRFP::SAS-7. Are the fluorescent images showing centrosomes from the EMS cell of a four-cell embryo? This is not explained in the figure legend.

The fluorescent images indeed show centrosomes from the EMS cell of a four-cell embryo. We

adjusted the image and clarified the figure legend 1A.

3. Page 3 first paragraph. The authors should point out that the pcmd-1(t3421) mutation is a likely null allele due to an early stop codon. Not immediately aware of the nature of the mutation, I wondered if the mutant protein might maintain some functions (such as recruiting SAS-7) while lacking other functions (recruiting PCM). I had to go back to the Erpf et al. paper to find out what the nature of the allele is and thus if the data presented in Figure 1C was definitive. Of course it is, but you had to know the allele was a protein null. You can save readers the trouble of researching the allele by describing it here.

The nature of the allele with the stop codon before the coiled-coil domain was added to the text to increase readability for the readers.

4. Figure 3B: The authors show that GFP::PCMD-1 is not recruited to the membrane by PH::mKate2::PCMD-1. This is an unexpected result given the two-hybrid results and the authors offer no explanation that may account for this. This is just one of a few places in the manuscript where an unexpected result is observed and there is no attempt to provide a possible explanation.

Protein interactions detected by the Y2H are not necessarily recapitulated by the translocation assay, this can have multiple reasons. As the Reviewer mentions below, interaction at the centrosome can be influenced by different posttranslational modifications or co-factors, which are absent in the cytoplasm and at the membrane. We suspect that PCMD-1 is strongly tethered to the centrosome through other interactions and therefore cannot be translocated. This and other explanations are discussed in the text (page 9).

5. Figure 3D. The authors should explain to the reader why sas-5(RNAi) does not target the gfp::sas-5 transgenes. Many readers may be unfamiliar with the use of recoded transgenes. Related to this, how do the authors control for the efficiency of sas-5(RNAi) in these strains?

By 'sas-5' the Reviewer most likely means spd-5. We added the clarification to the text (page 4, lane 185).

6. I don't understand the purpose of Table 1. All of the data contained in this table is already reported in Figures 4, 5 and S4. It should be removed.

We agree with the Reviewer and removed the table.

7. Discussion. The authors offer an explanation for why membrane tethered PCMD-1 fails to recruit centriole proteins SAS-4 and SAS-7 by stating that they are stably incorporated into the centriole. This assumes that there is very low to no cytoplasmic SAS-4 or SAS-7, which I don't think is true. It thus seems likely that another explanation is possible. For instance, post- translation modifications might control these interactions.

We thank the Reviewer for this comment. To test whether increasing the cytoplasmic pool of SAS-4 facilitates targeting to the membrane we included another dataset. For this we use the GFP::SAS-4(ΔTCP) strain in the translocation assay. Since the TCP domain is necessary for the interaction of SAS-4 with SAS-5, GFP::SAS-4(ΔTCP) represents the PCM or 'liberated' pool of SAS-4. PCMD-1 could not translocate GFP::SAS-4(ΔTCP) to the membrane (Figure S3C). This confirms the suspicion of the Reviewer and indeed suggest that either a posttranslational modification is needed or that the interaction requires a local environment close to centrioles.

8. I think a model to account for the various protein interaction and localization dependence data is that PCMD-1 localizes to centrioles via two distinct but essential interactions. One is a direct interaction with SAS-4 and the second is a SAS-7-dependent interaction (that may or may not involve direct binding between SAS-7 and PCMD-1. If either interaction is disrupted, PCMD-1 fails to localize.

The data acquired with PCMD-1(C2) strengthens our hypothesis that SAS-4 has a role in PCMD- 1 recruitment. Although the Y2H data does not provide information about where and when the two proteins would interact, we now suggest in the model that SAS-7 and SAS-4 act in parallel. The

wording in the model was adjusted accordingly.

Typos and very minor issues:

Minor issues have been addressed in the text.

1.Why is RFP at times referred to as RFP and others as tagRFP?
2.Page 4, line 10: centriole duplication fails “in” sas-4…
3.Page 4, 6th line of next section: “whether PCMD-1 is capable “of recruiting”
4.Page 6, line 15: “deleted the sequence from E86 “to” F118.
5. Page 6, line 26: “in” 3 out of 18 centrosomes
6. Page 6, line 16: “in the context of” should be rephrased
7.Page 7, line 16: “even had” a negative effect on the viability

Reviewer 2 Comments for the Author:
Major concerns

1. The authors present an impressive structure-function analysis of PCMD-1. It seems surprising the coiled-coil deletion is homozygous viable with very little embryonic lethality. Consistent with this, the C-terminal construct, which lacks the coiled-coil domain, almost completely rescues the loss of function t3421 allele. This indicates that most of the essential functions of PCMD-1 do not require the coiled-coil domain. The authors do not provide any discussion of this curious pair of observations, but rather emphasize how the coiled-coil domain is important for PMCD-1/PMCD-1 interactions. Apparently the PMCD-1 interaction with itself is of minor importance. The authors should provide some discussion of these observations and speculate on what parts of the proteins confer essential functions with regard to SPD-5 and PLK-1 recruitment. Some insight could be provided if the authors would report whether the two-hybrid interactions of PMCD-1 with SPD-5 and PLK-1 and SAS-4 required the coiled-coil domain of PMCD-1 (they only show that PMCD-1 requires this domain to interact with itself). Also see minor comment #4.

We attempted the proposed experiments in the Y2H setting by using a PCMD-1(ΔCC) bait and test for interaction with PCMD-1, PLK-1, SAS-4 and SPD-5 (Figure for Reviewers D). The PCMD- 1(ΔCC) bait still showed interaction with the PCMD-1 prey, while the PCMD-1(ΔCC) bait did not interact with the PCMD-1(ΔCC) prey (Reviewer Figure E, Figure 4F). For publication, we included the more stringent dataset with PCMD-1(ΔCC) bait.
Unfortunately, in this case of PCMD-1(ΔCC)/PLK-1, PCMD-1(ΔCC)/SAS-4, the Y2H results were inconclusive since interaction was only seen with PCMD-1(ΔCC) as prey, but not as bait. Due to autoactivation of the SPD-5 bait, we could not confirm the PCMD-1(ΔCC)/SPD-5 interaction. Therefore, we did not to include this data in the manuscript.
Our functional data using RFP::SPD-5 suggests that the N-terminal part together with PCMD-1(C1) are sufficient for SPD-5 recruitment, while the PCMD-1(C2) is not, narrowing down the region of SPD-5 interaction.

2. The authors need to address some quantification issues in their figures. They frequently note the number of embryos examined in almost all of the figure legends. However, they only show single representative embryos in Figures 1 and 2. Without some quantification of what the authors saw, these numbers don’t mean much. In Figure 3C and 3E, how did the authors score whether or not GFP signal was present at the membrane or not? Did they apply some sort of threshold? This should be explained. Also, the authors should note whether the images of membrane localization are single focal planes or projections. Can the authors explain why the levels of mKate::PCMD-1 at the membrane after heat shock vary considerably depending on which GFP background is being assessed?

We thank the Reviewer for pointing out these omissions:
For Figure 1, we have now included the quantifications of the centrosomal GFP::SAS-7 and GFP::PCMD-1 levels.
In Figure 3 all images show single Z-planes. Our scoring criteria for a positive embryo were either a minimum of two cells in case of a weak signal or one cell with a strong signal surrounding the whole circumference of this cell. This information was added to the methods. To our best

knowledge we did not see a difference in PH::mkate2::PCMD-1 recruitment between different GFP backgrounds. However, since a heat-shock promoter is used, PH::mkate2::PCMD-1 expression levels vary between embryos within an experiment, which might be reflected in the figures.

Minor concerns

1. These authors have shown in their 2019 Current Biology manuscript that while SPD-2 largely depends on PMCD-1 for localization to centrosomes, PCMD-1 does not require SPD-2. The authors never mention in this manuscript that PCMD-1 does not require SPD-2 for its localization to the centrosome, even though they include SPD-2 in their two-hybrid assays. To provide a more complete overview of this network, it would help for the authors to note the lack of PMCD-1 dependence on SPD-2 for centriole localization. It might also be worth noting that SAS-7 is required for both PCMD-1 and SPD-2 to localize to centrioles, but neither PCMD- 1 or SPD-2 are required for SAS-7 to localize to centrioles. In their 2019 Current Biology paper, the authors also assessed SAS-4 localization in pcmd-1(t3421) mutants. The authors should refer to these various results, presumably in the Discussion, to provide a more complete overview of these dependencies and dynamics.

We have integrated these points in the discussion (page 9).

2. In Figure 1A, the authors examine the partial co-localization of SAS-7 and PCMD-1. The text at the bottom of page 2 and top of page 3 describing this is somewhat confusing. It would help if the authors would make it clear in the text that PCMD-1 is present at more equal levels at the mother and daughter centrioles, while SAS-7 is more asymmetric. They refer to this asymmetry being consistent with a previously documented role for SAS-7 in daughter centrioles becoming competent to duplicate—the daughter-to-mother transition. Do the authors think SAS-7 is present at lower levels at the mother or the daughter centriole?

Indeed, we observed SAS-7 at higher levels at one of the two centrioles at multiple instances in the germline and in embryos. However, we do not have enough datapoints for a reliable quantitative readout at the same cell cycle stage in embryos. The experiment was performed at the Elyra 7 Lattice SIM Super-Resolution Microscope at the Zeiss headquarters. Due to COVID-19 restrictions we were not able to add more datapoints. Therefore, we removed this comment from the manuscript.

3. In Figure 1, the authors show some PCMD-1 localization to astral microtubules and kinetochores; the authors might want to comment on whether they think this has any function. Is it possible that loss of these pools contribute to lethality, given the surprising lack of lethality for the coiled-coil deletion, or does the rescue of most lethality by the C-terminal fragment argue against such a possibility?

While this is an absolutely valid possibility, we do not have enough data that would allow us to discriminate between a defect in PCM assembly and an independent disruption of astral or kinetochore microtubules in pcmd-1 mutants. So far our data is consistent with PCMD-1 rescuing lethality when RPF::SPD-5 is recruited and a failed rescue when RPF::SPD-5 is not efficiently recruited. In the coiled-coil deletion RPF::SPD-5 levels are not changed, only the integrity of the mitotic PCM scaffold is affected. Previous publications have shown that there is no major effect on embryo viability even when SPD-5 levels at the centrosome are very low, as in the GFP::SPD-5(2A) background (Woodruff et al. 2015), therefore it is not surprising that the deletion of the coiled-coil domain of PCMD-1 is viable.

4. The live imaging data in S1A seems worthy of being included as part of Figure 1.

This dataset was quantified moved for Figure 1.

5. The authors note (and show in S1C) that GFP::PCMD-1 signal is detected at much higher levels in later stage sas-7(or452) mutant embryos. The authors might want to speculate briefly about why there is such a substantial difference in early vs late levels.

We think that PCMD-1 can be recruited to the centrioles through multiple mechanisms. For

instance, at the ciliary base, where SPD-5 and PCMD-1 maintain each other through a positive feed forward loop, where SAS-4 and SASA-7 are not present (Garbrecht et al. 2021, unpublished observation by our group). We speculate that SAS-7 is absolutely necessary for the first cell divisions. In later divisions SPD-5, SAS-4 or another zygotically transcribed factor may work redundantly. We tried to clarify this in the manuscript (page 3, line 101).

6. On page 4, the authors state that "…PCMD-1 and SAS-7 were only detected at the mother centrioles (Figure 2C)." This implies that they could have been detected somewhere else (such as at daughter centrioles). But presumably in strong sas-4 knockdowns there are only mother and no daughter centrioles? If so, perhaps the authors could change the wording to say that PCMD-1 and SAS-7 were both detected at the mother centrioles, with daughter centrioles absent due to loss of SAS-4.

We adjusted the wording for this paragraph (page 3-4).

7. Might it be possible that the SAS-4 that is present in sas-7(-) mutants is present with a different conformation that explains the absence of PCMD-1, with the absent conformation serving as the bridge between SAS-7 and PCMD-1?

We thank the reviewers for this suggestion. This is a very plausible possibility and we added it to the Discussion (page 9, line 413).

8. In Figure S3A, the authors fail to see sperm-derived GFP::PCMD-1 in mitotic embryos, after pronuclei have met. The authors do see oocyte-derived GFP::PCMD-1 at that time, and thus conclude that there is turnover. Can the authors detect sperm-derived PCMD-1 earlier, in association with the sperm pronucleus during meiosis I or II or shortly thereafter?

We never detected a sperm-derived PCMD-1 signal after meiosis I (one polar body extruded). We added a picture of an earlier timepoint to the Figure S4A.

9. The authors further look at when oocyte-derived GFP::PMCD-1 loads onto centrioles, but they do not explicitly state how they staged the fixed oocytes to be during meiosis I, during meiosis II or after meiosis II. Presumably the staging was done based on the status of chromosome segregation and extrusion of polar bodies. This should be stated somewhere.

Meiotic stages of embryos in fixed samples were staged by the condensation state of the female DNA and the presence of the polar bodies. We incorporated this in the methods.

10. In Figure 4G, the authors state "Images of SPD-5 areas of centrosomes 1 and 3." What does this refer to?

We refer to the outline of the centrosome visualized by the overlayed mask to quantify its shape. We changed the wording in the corresponding figure legend.

11. In the text on page 7, lines 7 and 8 of the new section, the authors might insert "RNAi-resistant" to say "…to test the functionality of different RNAi-resistant truncations…" to be more explicit as to how the gene replacement strategy works, for readers who may not be familiar with it.

We inserted explanation in the current text (page 4, lane 185).

Some minor language edits:

These issues have been addressed in the current text.

1. Last sentence in the Introduction: maybe change "delocalize" to "recruit"? I understand de-localize refers to competing with the centrosome but that is not clear at this point and is a bit confusing without more information. I would think recruit serves fine here and might avoid some confusion.

2. page 4, line 10: change to “Centriole duplication fails IN SAS-4 depleted embryos.” [Add “in”].
3. page 4, line 15: drop the comma after “Our results suggest that,…”
4. Page 6, line 11 of the new section: Change to “…from E86 TO AND including F118…” [Add “to and”]
5. Page 7, line 11 of the new section: Change to “…could RESCUE embryonic lethality of…” [Replace “restore” with “rescue”.] This reads awkwardly as it first seems that “restore” refers to embryonic lethality, not viability.

Reviewer 3 Comments for the Author:
Specific Comments

Figure 1. The role of Sas-7 in recruiting PCM-1
Overall, the data in this figure nicely show that PCMD-1 co-localizes with Sas-7. However, the imaging does not allow for precise localization at the centriole wall. In the future, this will be required in order to classify PCMD-1 as a centriole to PCM linker.

1a. The authors state – “We observed that one PCMD-1 focus had a higher accumulation of the SAS-7 signal, while the second one colocalized to a lesser extent with SAS-7”. The authors should quantify the difference between the two centrioles as it is not clear in the images that a mother vs the daughter has higher levels. Alternatively, remove the statement of a difference.

We observed SAS-7 at higher levels at one of the two centrioles of multiple centrosomes in the germline and in the embryo. However, we do not have enough datapoints for a reliable quantitative readout at the same cell cycle stage in embryos. The experiment was performed at the Elyra 7 Lattice SIM Super-Resolution Microscope at the Zeiss headquarters. Due to COVID-19 restrictions we were not able to add more datapoints. Therefore, we removed this comment from the manuscript.

1b. Figure S1A shows some localization of PCMD-1 in sas-7 mutant. The Authors should move this to the main figure as it is important information, the fixed analysis in 1C is not the full story. Also, the authors state “negligible GFP signal remained at the centrosome, reflecting the hypomorphic nature of the sas-7(or452) allele”. It is not clear when a protein level is considered negligible. Quantification of levels or removing the word negligible is required. Overall, the conclusion should include that some PCMD-1 could be recruited independently of Sas-7

We quantified the datasets and moved the images to the main Figure 1.

1c. The Ns for some of these experiments seem very low. Looking at 6 centrosomes suggests they analyzed 3 embryos, likely from one experiment. Is there a technical reason for the low Ns?

The n=6 refers to the number of embryos that were collected in the course of multiple experiments. sas-7(or452) worms have a reduced brood size and therefore, finding embryos in immunostainings for a precise timepoint required multiple experiments. Nevertheless, we increased the embryo number to n=11 for this experiment.

Figure 2. Interactions data
Overall, the Y2H experiments are extremely over interpreted. Negative results are interpreted as no interaction, which is not necessarily the case. Furthermore, a positive interaction is also over interrupted as evidence that the interaction occurs in the cell, even at the centrosome. False positives are common in Y2H analysis, making the follow-up experiments presented (cortical co-localization) important. The authors state that “In summary, PCMD-1 binds to the centriolar protein SAS-4, the PCM protein SPD-5, the mitotic kinase PLK-1, and to itself.

Therefore, we propose that PCMD-1 physically bridges the centrioles and the PCM scaffold,”
– this is pure speculation and should be specifically phrased as a hypothesis at this point.

We thank the Reviewer for critically evaluating this data. We clearly state in the main text that the negative results only refer to this particular assay (page 9, line 406). We would like to highlight that our positive Y2H interactions only include interactions in which both growth and GFP

expression were observed. To support of our data, we now include experiments showing SAS-4 and PLK-1 baits interacting with PCMD-1 and a co-immunoprecipitation for the interaction of SPD-5 with PCMD-1. The SAS-4/PCMD-1 interaction is also seen for a shorter fragment of PCMD-1. Further, our translocation assay supports the interaction of PCMD-1 with PLK-1 and SPD-5. In addition, a recent preprint reports the PCMD-1/SPD-5 and PCMD- 1/PLK-1 interaction in an independent Y2H assay (https://www.biorxiv.org/content/10.1101/2021.05.20.444955v1). We are aware that in the absence of in vitro data with purified proteins these results do not infer direct protein-protein binding. However, the cumulative evidence and the localization dependencies strongly suggest that these proteins interact at the centrosome. We rephrased the text to make sure that the reader does not infer ***direct*** *protein-protein interaction, however we would still put forward a bridging model as a speculation.*

Figure 2. Sas4 knockdown
The conclusion that PCMD-1 requires Sas-4 for recruitment is not supported. It is more likely that loss of Sas-4 results in absence of the daughter centriole, which would result in loss of Sas-7, PCMD-1, and likely anything else that would appear on a centriole. A direct Sas-4- PCMD-1 interaction is also not supported by their own data showing a lack of recruitment of Sas4 to sites of PH::mKate2::PCMD-1 in figure 3, and the lack of PCMD-1 localization to Sas-4 dots in Figure 1C bottom panel. There is also no indication that a nascent centriole is or is not present in figure 2C, and there is no measure of knockdown level in the partial vs the full RNAi. Taken all of the Sas-4 data together, Sas-4 has not been functionally linked to PCMD-1 in this study.

As mentioned above in the comment to Reviewer #1, we disagree with the statement that in SAS-4 RNAi any centriolar protein would be missing. In SAS-4 RNAi embryos the central tube of the centriole is still formed (Pelletier et al. 2006). It is well established that SAS-5 and SAS- 6 still localize to the nascent centriole in this background (Delattre et al. 2004, Leidel et al. 2006) and were therefore genetically placed upstream of SAS-4. We have previously shown that SAS-4 still localizes to centrioles in pcmd-1(t3421) mutant embryos (Erpf et al. 2019), which already suggests that PCMD-1 is downstream of SAS-4, but PCMD-1 and SAS-4 could genetically act in parallel. The purpose of this experiment is to test whether SAS-4 is genetically upstream of SAS-7 and PCMD-1 i.e., whether SAS-7 and/or PCMD-1 would still localize to the nascent centriole. Our results indicate that this is not the case. While this might be an expected result, the conclusion cannot be made without showing the experiment. We do not infer any direct interaction of PCMD-1 with SAS-4, but simply place SAS-4 upstream of PCMD-1 and SAS-7. In cases when the SAS-4 RNAi results in a bipolar asymmetric spindle in the two-cell embryo, other groups (Kirkham et al. 2003) have already reported using TEM that a centriole with partial microtubules assembled around the central tube, is present. In our argumentation we rely on this analysis.

The translocation assay cannot be taken as a direct complement to the Y2H assay. Like SAS-4, PCMD-1 self-interaction cannot be mimicked by placing PCMD-1 at the membrane. There are several explanations for why this is the case. The proteins might require specific posttranslational modifications that only occur at the local environment at the centrosome or the interactions might need a co-factor, which is not present at the membrane. Finally, measuring precise RNAi knockdown levels by western blot in worms extract is only an approximation and does not provide information about the knockdown efficiency in the specific embryos analyzed. Therefore, we decided to group SAS-4 RNAi knock down based on the phenotype and RNAi strength (28h feeding for partial RNAi, 30h feeding for full RNAi).
We recognize that the wording in the text was not clear and adjusted it accordingly (page 3- 4, line 135-147).

Figure 3. In vivo interaction assay
The major concern with this assay is interpreting something as sufficient. This assay is similar to a co-IP, thus there is a difference between the following two statement, the first of which is what the authors use, but should avoid

a. "PCMD-1 is sufficient to recruit SPD-5 and PLK-1 to an ectopic location"
b. Positioning PCMD-1 at an ectopic site is sufficient to recruit SPD-5 and PLK-1 to that ectopic location.

Statement (a) suggests a direct protein-protein interaction between PCMD-1 and Spd-5 or Plk-1,

while statement (b) does not infer direct binding. The authors should rephrase this entire section of the paper to better reflect the fact that indirect binding can account for the re-localization to the membrane. This includes the statements that “These findings suggest that PCMD-1 would also be sufficient to recruit the PCM scaffold to the centrosome in the C. elegans one-cell embryo,” and “thus, PCMD-1 can independently recruit PLK-1 and SPD-5.” These results have not been shown. Showing sufficiency is really hard, the authors should remove the terms sufficiency and independently and simply state that PCMD-1 can act upstream of Spd-5 recruitment.

We thank the reviewer for pointing this out. By ‘independent’ we meant that PLK-1 recruitment is not dependent on SPD-5, we did not intend to exclude the possibilities that other proteins are bridging the interaction. We adjusted the wording accordingly (page 5, lane 192- 3).

The proposal that “PCMD-1 physically bridges the centrioles and the PCM scaffold” to this point has not been show. The summary thus far is that PCMD-1 localization requires Sas-7, but Sas-7 might not be the direct anchor, or the only anchor. The role of Sas-4 in unknow. PCMD-1 acts upstream of Spd-5.

We removed the notion of ‘physical’ bridge in the current text and model.

Figures 4 and 5

The goal of these two figures is to determine the role of the CC domain in PCMD-1 and define centrosome localization and protein-protein interaction sequences. The conclusions that can be made are:

Figure 4 – CC is necessary for centrosome recruitment in the contexts of full length PCMD-1
Figure 4 – CC is required for self-interaction.
Figure 5 – CC is not necessary for centrosome localization in the context of the c-terminal truncation
Figure 5 and S4 - CC is not sufficient within construct (N) for centrosome localization

Taken all of these data together, it is clear that the role mechanism of PCMD-1 function is much more complicated than the presented model of being anchored on one end to the centriole, dimerizing at the CC, and linking to other end to SPD5. This model is reminiscent of the current view of PNCT/PLP, but lacks evidence. To propose a linker function between the centriole and PCM, one would have to:

1) Show detailed super resolution localization pf PCMD-1 not only to show it’s localization near the centriole wall, but also to show an extended molecule as depicted in their model.

The experiment in Figure 1A was performed at the Elyra 7 Lattice SIM Super-Resolution Microscope at the Zeiss headquarters and is the highest resolution we could achieve for live- cell imaging. Due to COVID-19 restrictions we were not able to examine fixed material for a more detailed analysis. Even if we would have the opportunity, showing an extended molecule in a super-resolution setting will be challenging due to the small size of the C. elegans centrioles (around 150nm) and the length of PCMD-1 (630aa). We adjusted the orientation of the molecules in the schematics of the model.
However, we add to the model a picture, which indicates that the C-terminal part PCMD-1(C2) interacts with SAS-4, but is unable to recruit SPD-5.

2) Show direct binding between the proteins suggested in the model, better map the binding sites (does Spd-5 bind the delta CC for example), show a disruption of localization of PCM in targeted mutants that do not impact centrosome localization of PCMD-1, and show disruption of PCMD-1 localization without impacting centriole formation.

By including more C-terminal truncations of PCMD-1 in the structure function analysis, it becomes apparent that there is more than one region involved in targeting it to the centrosome. The PCMD-1(C2) part is sufficient for centrosomal targeting but cannot recruit RFP::SPD-5. This

strain represents a targeted mutant that does not disrupt centrosome localization but affects the PCM and indicates that SPD-5 is recruited by the more N-terminally located parts of the protein.

We used the Y2H assay to test the PCMD-1(ΔCC)/SPD-5 interaction (Reviewers Figure D). PCMD-1(ΔCC) bait did not interact with the SPD-5 prey, however, due to autoactivation of the SPD-5 bait we could not confirm this interaction in the reciprocal orientation and therefore did not include the data in the manuscript.

One might even have to go one step further to rescue function by bypassing the normal interaction site and artificially linking the two proteins.

We agree with the Reviewer that this would be an interesting experiment, however there are putative technical challenges. Since SPD-5 is an extremely dynamic protein (Woodruff et al. 2015, Wueseke et al. 2016), linking it to PCMD-1 might have a dominant-negative effect and thus, worms might not be viable. One could imagine targeting SPD-5 to the centrosome in the absence of PCMD-1, but due to the lack of a bona fide centriole targeting sequence, as the PACT domain, this experiment is currently not feasible.

These are not easy experiments, but experiments along these lines are required to make the conclusions presented here. So my recommendation is one of two things:

1) Quick fix – Address some of the low Ns mentioned above and extensively changing the language in the paper to simply reflect that Sas-7 is upstream of PCMD-1 which is upstream of SPD-5. Removing all mention of sufficiency and direct linkage/bridging centriole with PCM from the title, abstract and results. Also removing the experiments on Sas-4 as there are alternate explanations for this. Finally, restrict the hypothesis that PCMD-1 is a bridge to the discussion.

In the current version of the manuscript, we have addressed the above-mentioned issues and added a new data set in support of our hypothesis.

2) Long fix – Use these results as preliminary data to begin testing the direct bridging function of PCMD-1.

Second decision letter

MS ID#: DEVELOP/2020/198416

MS TITLE: PCMD-1 bridges the centrioles and the PCM scaffold in *C. elegans*

AUTHORS: Lisa Stenzel, Alina Schreiner, Elisa Zuccoli, Judith Mehler, Sim Ustuner, Esther Zanin, and Tamara Mikeladze-Dvali

I have now received all the referees reports on the above manuscript, and have reached a decision. The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. In particular, Reviewer 1 suggests either changing the text or providing additional experimental evidence to PCMD-1 functions as a centriole-PCM link rather than playing a role in PCM itself. Similarly the reviewer points out that at this time, the manuscript does directly provide evidence for SAS-4(TCPd) in the cytoplasm interact with PCMD-1. This is an intriguing point and should be clarified in the text. In addition, the reviewer provides recommendations for clarifying some of the analysis and presentation of data. Reviewer 3 recommends generating a model to summarize the discussion. This is a great idea, since it will help broader readers in the field. Addressing these recommendations would greatly help the field and the readers and I invite you to submit a revision. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so. Please highlight the changes in the field, but do not use track changes.

Reviewer 1

Advance summary and potential significance to field

To function as a microtubule organizing center, the centrosome must be assembled properly so that the pericentriolar material (PCM) organizes around the centrally positioned pair of centrioles. How the PCM is linked to centrioles is not well understood. The manuscript from Stenzel and colleagues implicates PCMD-1 in linking the centrioles and PCM. They show that PCMD-1 interacts with both centriole and pcm proteins and map some of the interaction domains. They carefully investigate how mutations in PCMD-1 that affect its interaction with itself and with SAS-4 affect PCMD-1 localization and PCM assembly. Finally, they uncover a domain that is important for targeting PCMD-1 to a distinct microtubule organizing center at the base of cilia.
This work represents a significant step forward in our understanding of centrosome biology and will be of great interest to those working in the field.

Comments for the author

The revised manuscript from Stenzel and colleagues is significantly improved compared to the original version. Most, but not all, of my initial concerns have been addressed. The study contains several important findings concerning the role of PCMD-1 in PCM assembly and will be of great interest to those in the field. In principle, I feel that manuscript is suitable for publication, but several outstanding issues need to be addressed beforehand.

1. The central point that the authors are trying to make is that PCMD-1 functions to link the centriole and PCM. The data presented in this study certainly indicate such a role for PCMD-1 as the authors have shown that PCMD-1 physically associates with the centriole protein SAS-4 and the PCM proteins SPD-5 and PLK-1. The phenotype of PCMD-1 mutants is also consistent with such a role. However, despite the authors' best attempts, definitive proof of such a role is lacking. They would need to show that detaching PCMD-1 from either the centriole or PCM would result in dissociation of the PCM and centriole. Such a result would clearly demonstrate that PCMD-1 is functioning as a centriole-PCM link rather than playing a role in PCM formation (like the centriole and PCM protein SPD-2). I should also point out that at best PCMD-1 is likely functioning as a link rather than as the link. What I mean by this is that PCMD-1 is largely dispensable for viability at low temperature indicating that at least some PCM is still associated with the centriole in the absence of PCMD-1. Thus, the authors should be very careful with their wording in order to avoid making it seem like their data demonstrates that PCMD-1 links the centriole and PCM. One place where this is a problem is the first sentence of the discussion. In my opinion they have not yet demonstrated the mechanism by which PCMD-1 anchors the PCM to the centriole.

2. Page 135 and Figure S2. Both reviewer 3 and myself took issue with the experiment investigating the dependance of PCMD-1 recruitment to nascent daughter centrioles on SAS-4. The problem here is that strong knockdown of SAS-4 blocks daughter centriole formation and thus there is no structure for PCMD-1 to localize to. Weak knockdown allows a partial structure to be built but this structure likely has SAS-4 and thus doesn't allow the authors to investigate how PCMD-1 recruitment is affected by the absence of SAS-4. As the authors point out in their rebuttal, prior studies have shown that SAS-5 and SAS-6 are able to localize and form a central tube in the absence of SAS-4. So, in theory, one could ask if PCMD-1 localized to this structure in sas-4(RNAi) embryos. The problem is that this structure is unstable and would probably not be present during the second cell cycle when the authors were scoring for the presence of PCMD-1 and SAS-7. Essentially, the experiment is designed wrong. If the authors want to properly address this issue, they should score for PCMD-1 (and SAS-7) recruitment during the first cell cycle, similar to the approach used by Dammerman et al 2008 JCB 180: 771-785. Scoring during the second cell cycle complicates analysis.

3. In several figures the authors use bar graphs to display their data (figure 1C and 1E, Figure 4 D and E Figure 5C), and in other places use scatter plots (for example Figure 4H). I strongly prefer the use of scatter plots as it allows the reader to more deeply examine the underlying data. I know that not all bar graphs can be converted to scatter plots but for those that can there really isn't any reason to avoid presenting the data in the most transparent way possible.

4. Line 88 “we crossed an in situ tagged gfp::pcmd-1 with sas-7(or452)..” This sentence is not clear and should be reworded. Also, what is the difference between in situ tagged and endogenously tagged?

5. Line 199. The authors describe using a mutant version of SAS-4 (TCPΔ) that is cytoplasmic to test for an interaction with PCMD-1 in the translocation assay. The thought here is that removing the TCP domain would liberate SAS-4 from centrioles and allow it to be recruited by membrane tethered PDMD-1.
This is a clever experimental design, but the results were negative leaving me with a question. Do the authors know if SAS-4(TCPΔ) interacts with PCMD-1? Without knowing this, the meaning of the experimental results is ambiguous. They should either test this construct for interaction with PCMD-1 or not include the experiment.

6. Line 273 and Figure 4F. Using a version of PCMD-1 with the coiled-coil domain deleted (ΔCC), the authors show that PCMD-1(ΔCC) will not self-interact in the two-hybrid assay. This is true, but inexplicably as shown in Figure 4F, PCMD-1(ΔCC) can interact with wild-type PCMD-1. Included in my comments on the first version of the manuscript I mentioned that in several instances the authors simply ignored unexpected results and did not attempt to provide possible explanations. It’s a bit frustrating to see this again. If the coiled-coil domain was the only self-interaction domain then deleting it in either the prey or bait should abolish the interaction, but that is not the case here. I suspect that there is a second weaker self-interaction domain. This domain might not drive self-interaction unless the protein dimerizes (which the wild-type prey can do in this experiment). Once dimerized, PCMD-1 could bind additional PCMD-1 monomers through this domain to form higher order oligomers. The authors need to address this finding in one way or another.

7. Line 320 Typo. I believe the authors meant to say SAS-4 prey.

8. Line 371 As shown by McNally et al. 2012, SPD-5 is recruited to the centrioles after meiosis. Not during meiosis.

Reviewer 2

Advance summary and potential significance to field

The authors provide important new insights into the early steps of centrosome maturation in C. elegans, and the link between centriole structure and the PCM during maturation. Their insights into the important and intriguing role(s) of PCMD-1 represent a significant contribution.

Comments for the author

The authors have thoroughly addressed all of the reviewers' comments and have added significant new data concerning the different protein/protein interactions and domain requirements. The authors also provide what to me are compelling arguments justifying their conclusions concerning the epistasis analysis as to whether SAS-4 functions upstream of PCMD-1 and SAS-7. As long as the manuscript clearly states their reasoning (and also clearly states their reasoning for why it is not surprising the the homozygous coiled-coil domain mutant is homozygous viable), the manuscript provide important insights into centrosome maturation in C. elegans and into the complex and intriguing functions of an important early player in this process (and of other players). Thus in my opinion, the manuscript will be of substantial interests to a broad audience of cell and developmental biologists and is worthy of publication in Development.

Reviewer 3

Advance summary and potential significance to field

I have now read the new version of the manuscript titled “PCMD-1 bridges the centrioles and the PCM scaffold in C. elegans” and the accompanying rebuttal letter. The manuscript is greatly improved and I thank the authors for considering the reviewers’ recommendations. The overall criticism in my initial review revolved around the definition of a protein bridge, and the distinction between a direct protein link and a genetic link. The authors have done a good job making this distinction clearer in the new version and carefully address this in the discussion section. The final paragraph in the discussion is very nicely worded and appreciated.

Comments for the author

I have one recommendation to help with summarizing the data and accompanying the discussion. I suggest adding a summary figure of the genetic pathway using gene names and arrows. Such as, two pathways feeding into PCM1 recruitment shown by arrows from Sas4 and Sas7. You might consider even two arrows feeding into Sas7 recruitment, one from Sas4 and one from ‘?’. Indicated on this genetic pathway could also be the direct protein-protein interactions such as the Sas4 to PCM1 arrow and PCM1-SPD5 arrow. Personally, I feel that this would really help the field place these proteins in a working model that is more helpful than the cartoon in 6B, although 6B is also useful and should remain.

Second revision

Author response to reviewers' comments

We thank the Reviewers for positively evaluating our manuscript. To address the last concerns of the Reviewers we integrated the following changes in the manuscript:

- *All changes in the main text are marked in blue*
- *Figure 6 now includes a genetic framework for the PCMD-1 interactions*
- *In Figure S6D the panel for pcmd-1(t3421) RFP::SPD-5; GFP::PCMD-1(C2) was exchanged for an embryo where the detachment of PCM from the centrioles is apparent*
- *Figures 1C, 1E, 4D, 4E, S6C, S6E, S5B now include dot plots*
- *The figure legends were adjusted accordingly*

Please find a point-by-point response below.

Reviewer 1 Advance Summary and Potential Significance to Field:
To function as a microtubule organizing center, the centrosome must be assembled properly so that the pericentriolar material (PCM) organizes around the centrally positioned pair of centrioles. How the PCM is linked to centrioles is not well understood. The manuscript from Stenzel and colleagues implicates PCMD-1 in linking the centrioles and PCM. They show that PCMD-1 interacts with both centriole and pcm proteins, and map some of the interaction domains. They carefully investigate how mutations in PCMD-1 that affect its interaction with itself and with SAS-4 affect PCMD-1 localization and PCM assembly. Finally, they uncover a domain that is important for targeting PCMD-1 to a distinct microtubule organizing center at the base of cilia. This work represents a significant step forward in our understanding of centrosome biology and will be of great interest to those working in the field.

Reviewer 1 Comments for the Author:
The revised manuscript from Stenzel and colleagues is significantly improved compared to the original version. Most, but not all, of my initial concerns have been addressed. The study contains several important findings concerning the role of PCMD-1 in PCM assembly and will be of great interest to those in the field. In principle, I feel that manuscript is suitable for publication, but several outstanding issues need to be addressed beforehand.

1. The central point that the authors are trying to make is that PCMD-1 functions to link the centriole and PCM. The data presented in this study certainly indicate such a role for PCMD- 1 as the authors have shown that PCMD-1 physically associates with the centriole protein SAS-4 and the PCM proteins SPD-5 and PLK-1. The phenotype of PCMD-1 mutants is also consistent with such a role. However, despite the authors' best attempts, definitive proof of such a role is lacking. They would need to show that detaching PCMD-1 from either the centriole or PCM would result in dissociation of the PCM and centriole. Such a result would clearly demonstrate that PCMD-1 is functioning as a centriole-PCM link rather than playing a role in PCM formation (like the centriole and PCM protein SPD-2). I should also point out that at best, PCMD-1 is likely functioning as a link rather than as the link. What I mean by this is that PCMD-1 is largely dispensable for viability at low temperature indicating that at least some PCM is still associated with the centriole in the absence of PCMD-1. Thus, the authors should be very careful with their wording in order to avoid making it seem like their data demonstrates that PCMD-1 links the centriole and PCM. One place where this is a problem is the first sentence of the discussion. In my opinion they have not yet demonstrated the mechanism by which PCMD-1 anchors the PCM to the centriole.

In some pcmd-1(t3421) mutant embryos the PCM scaffold forms, but is detached from the centrioles. This was already published in Erpf et al. (2019). The Figure 4E clearly shows that spherical structures of SPD-5 are located away from centrioles marked by IFA (please find a screenshot of Figure 4 at the end of the point-by-point response). A similar phenotype was observed during the structure-function analysis, for the construct comprising only the C2-terminal part of PCMD-1 (pcmd-1(t3421) RFP::SPD-5; GFP::PCMD-1(C2)). For a better illustration we replaced the panel in Figure S6D with an embryo where some RFP::SPD-5 scaffold forms, but it does not co-localize with centrioles. A corresponding note was added to the figure legend. For this reason, we argue that PCMD-1 plays a role in anchoring of the PCM to the centrioles rather than the formation of the PCM scaffold.
We are aware that PCMD-1 is not the sole and only anchor for the PCM and therefore, intentionally avoided using the term 'the linker' throughout the manuscript. The anchoring function of PCMD-1 might be cell cycle dependent. To clarify this point for the reader, we added SPD-2 to the genetic interaction framework and included an additional sentence in the discussion (Figure 6B and lines 485-488).

2. Page 135 and Figure S2. Both reviewer 3 and myself took issue with the experiment investigating the dependance of PCMD-1 recruitment to nascent daughter centrioles on SAS-4. The problem here is that strong knockdown of SAS-4 blocks daughter centriole formation and thus there is no structure for PCMD-1 to localize to. Weak knockdown allows a partial structure to be built but this structure likely has SAS-4 and thus doesn't allow the authors to investigate how PCMD-1 recruitment is affected by the absence of SAS-4. As the authors point out in their rebuttal, prior studies have shown that SAS-5 and SAS-6 are able to localize and form a central tube in the absence of SAS-4. So, in theory, one could ask if PCMD-1 localized to this structure in sas-4(RNAi) embryos. The problem is that this structure is unstable and would probably not be present during the second cell cycle when the authors were scoring for the presence of PCMD-1 and SAS-7. Essentially, the experiment is designed wrong. If the authors want to properly address this issue, they should score for PCMD-1 (and SAS-7) recruitment during the first cell cycle, similar to the approach used by Dammerman et al 2008 JCB 180: 771-785. Scoring during the second cell cycle complicates analysis.

There is no scientific evidence that the central tube is unstable and would not be present in the second cell cycle. Besides, we intentionally chose to include images of partial RNAi conditions where sufficient SAS-4 is present to form a structure stable enough to support a spindle pole in the second cell cycle (see Figure S2A). However, this same amount of SAS-4 is insufficient to recruit detectable signal of PCMD-1 and SAS-7 in more than 50% of spindle poles. This data makes us confident to claim that SAS-4 is genetically upstream of PCMD-1 and SAS-7. To clarify this point we adjusted the wording in the text (lanes 141-148).

3. In several figures the authors use bar graphs to display their data (figure 1C and 1E, Figure 4 D and E, Figure 5C), and in other places use scatter plots (for example Figure 4H). I strongly prefer the use of scatter plots as it allows the reader to more deeply examine the underlying data. I know that not all bar graphs can be converted to scatter plots but for those that can there really isn't any reason to avoid presenting the data in the most transparent way possible.

Dot plots were added for all the datapoints where intensities were scored (Figures 1C, 1E, 4D, 4E, S6C, S6E, S5B).

4. Line 88 “we crossed an in situ tagged gfp::pcmd-1 with sas-7(or452)..” This sentence is not clear and should be reworded. Also, what is the difference between in situ tagged and endogenously tagged?

‘in situ tagged’ is a commonly used term for the endogenous in locus tagging of a gene by CRISPR/Cas9. But if the Reviewer prefers, we do not mind using an alternative term (lanes 85 and 259).

5. Line 199. The authors describe using a mutant version of SAS-4 (TCPll) that is cytoplasmic to test for an interaction with PCMD-1 in the translocation assay. The thought here is that removing the TCP domain would liberate SAS-4 from centrioles and allow it to be recruited by membrane tethered PDMD-1. This is a clever experimental design, but the results were negative leaving me with a question. Do the authors know if SAS-4(TCPll) interacts with PCMD-1? Without knowing this, the meaning of the experimental results is ambiguous. They should either test this construct for interaction with PCMD-1 or not include the experiment.

We are thanking the Reviewer for finding the design of the experiment ‘clever’. The TCP domain of SAS-4/CPAP was thoroughly mapped as an interaction domain with SAS-5/STIL in different species (Cottee et al. 2013). This makes it very unlikely that the same site would be the interaction site with PCMD-1. We agree with the Reviewer that we do not have a formal proof that the deletion of the site would not compromise the interaction with PCMD-1.
Therefore, as the editor suggested we included an paragraph in the text to clarify this caveat (lanes 208-201 and 398-406).

6. Line 273 and Figure 4F. Using a version of PCMD-1 with the coiled-coil domain deleted (llCC), the authors show that PCMD-1(llCC) will not self-interact in the two-hybrid assay. This is true, but inexplicably as shown in Figure 4F, PCMD-1(llCC) can interact with wild-type PCMD-1. Included in my comments on the first version of the manuscript I mentioned that in several instances the authors simply ignored unexpected results and did not attempt to provide possible explanations. It’s a bit frustrating to see this again. If the coiled-coil domain was the only self-interaction domain then deleting it in either the prey or bait should abolish the interaction, but that is not the case here. I suspect that there is a second weaker self- interaction domain. This domain might not drive self-interaction unless the protein dimerizes (which the wild-type prey can do in this experiment). Once dimerized, PCMD-1 could bind additional PCMD-1 monomers through this domain to form higher order oligomers. The authors need to address this finding in one way or another.

As the Reviewers previously suggested, we use stringent criteria for the Y2H interaction and only take in account data that was validated by both bait/prey combination. For the interaction of the full length and deltaCC constructs, interaction was found only in one of the two combinations. Since this data was not fitting our criteria, we chose to not include any speculations on it.

The argument of the Reviewer would be true if PCMD-1 would dimerize through the coiled- coil domain. An alternative explanation is that the coiled-coil domain interacts with a different region of PCMD-1. In this case deleting the region in one of the two interaction partners would still allow for binding through the undeleted coiled-coil domain of the second partner. When both domains are missing the interaction would be abolished. We now included a comment in the discussion (lanes 456-461).

7. Line 320 Typo. I believe the authors meant to say SAS-4 prey.

The typo was corrected accordingly (lane 320).

8. Line 371 As shown by McNally et al. 2012, SPD-5 is recruited to the centrioles after meiosis. Not

during meiosis.

We adjusted the wording accordingly (lane 370).

Reviewer 2 Advance Summary and Potential Significance to Field:
The authors provide important new insights into the early steps of centrosome maturation in C. elegans, and the link between centriole structure and the PCM during maturation.
Their insights into the important and intriguing role(s) of PCMD-1 represent a significant contribution.

Reviewer 2 Comments for the Author:
The authors have thoroughly addressed all of the reviewers' comments and have added significant new data concerning the different protein/protein interactions and domain requirements. The authors also provide what to me are compelling arguments justifying their conclusions concerning the epistasis analysis as to whether SAS-4 functions upstream of PCMD-1 and SAS-7. As long as the manuscript clearly states their reasoning (and also clearly states their reasoning for why it is not surprising the the homozygous coiled-coil domain mutant is homozygous viable), the manuscript provide important insights into centrosome maturation in C. elegans and into the complex and intriguing functions of an important early player in this process (and of other players). Thus in my opinion, the manuscript will be of substantial interests to a broad audience of cell and developmental biologists and is worthy of publication in Development.

We thank the Reviewer for positively evaluating the revised manuscript. The reasoning for the lack of lethality of the coiled-coil deletion was clarified in the first revised version of the manuscript (lanes 447-449). Now we added SPD-2 to the genetic interaction framework and included a comment to clarify that PCMD-1 is not the sole anchor for the PCM. This also means that redundant mechanisms can recruit sufficient SPD-5 when PCMD-1 levels are low at the centrosome (lanes 485-488).

Reviewer 3 Advance Summary and Potential Significance to Field:

I have now read the new version of the manuscript titled “PCMD-1 bridges the centrioles and the PCM scaffold in C. elegans” and the accompanying rebuttal letter. The manuscript is greatly improved and I thank the authors for considering the reviewers’ recommendations. The overall criticism in my initial review revolved around the definition of a protein bridge, and the distinction between a direct protein link and a genetic link. The authors have done a good job making this distinction clearer in the new version and carefully address this in the discussion section. The final paragraph in the discussion is very nicely worded and appreciated.

Reviewer 3 Comments for the Author:
I have one recommendation to help with summarizing the data and accompanying the discussion. I suggest adding a summary figure of the genetic pathway using gene names and arrows. Such as, two pathways feeding into PCM1 recruitment shown by arrows from Sas4 and Sas7. You might consider even two arrows feeding into Sas7 recruitment, one from Sas4 and one from ‘?’. Indicated on this genetic pathway could also be the direct protein-protein interactions such as the Sas4 to PCM1 arrow and PCM1-SPD5 arrow. Personally, I feel that this would really help the field place these proteins in a working model that is more helpful than the cartoon in 6B, although 6B is also useful and should remain.

We thank the Reviewer for positively evaluating the revised manuscript and for suggesting to include a genetic interaction scheme in the model. The proposed scheme was added to the Figure 6 and it indeed helps the reader to map our findings onto a genetic network. We also included SPD-2, to point out that we do not consider PCMD-1 to be the sole anchor of the PCM (lanes 485-488). However, we did not include the ‘?’ in the model, since the analysis of SAS-7 was not the primary scope of our manuscript.

Third decision letter

MS ID#: DEVELOP/2020/198416

MS TITLE: PCMD-1 bridges the centrioles and the PCM scaffold in *C. elegans*

AUTHORS: Lisa Stenzel, Alina Schreiner, Elisa Zuccoli, Sim Ustuner, Judith Mehler, Esther Zanin, and Tamara Mikeladze-Dvali

I have now received reviewer 1's report on the revised manuscript. As you will note from the review, the reviewer points out, accurately so, that there are published reports that in the absence of *sas-4*, the central tube is not stable and the structure likely collapses. The reviewer provides the associated published studies for reference. Because loss of *sas-4* likely results in loss of a stable central tube and its capacity to assemble the singlet microtubules, I agree with the reviewer that loss of a perceived association between *sas-4* and *sas-6* at the stage of embryogenesis which is assessed could be entirely due to loss of the structure at which they do associate, rather than because of loss of direct protein interaction between SAS-4 and SAS-6. I invite a final revision and recommend one of two possibilities (1) caveat the findings, add the associated publications and state that the loss of interaction between *sas-4* and *sas-6* could be due to loss of structure rather than loss of direct interaction (which cannot be assessed at the stage assayed anyways); (2) remove the experiment at this time from the manuscript.

Reviewer 1

Advance summary and potential significance to field

I have already stated my position in a prior review

Comments for the author

After reviewing the manuscript a third time, I have just one comment. I stand by my criticism of the experiment investigating the dependence of PCMD-1 recruitment on SAS-4. The author believes there is no scientific evidence that the central tube is unstable in the absence of SAS-4 but I say there is. To support my position, I quote from two papers:

Centriole assembly in Caenorhabditis elegans. Pelletier L, O'Toole E, Schwager A, Hyman AA, Müller-Reichert T.Nature. 2006 Nov 30;444(7119):619-23. doi: 10.1038/nature05318.
”Most interestingly, in sas-4(RNAi) embryos at PNA central tubes of daughter centrioles still formed (Fig. 4c). After PNR, daughter centriole central tubes were longer (Supplementary Movie ‘SAS4RNAi’ and Supplementary Table 1), suggesting that tube elongation can still occur in sas-4(RNAi) embryos. Daughter centriole central tubes failed to increase in width, and seemed defective in the assembly of both singlet microtubules and hook-like appendages (Supplementary Table 1 and data not shown). In older embryos daughter centriole central tubes were often difficult to discern (data not shown).”

So, central tubes form in the absence of SAS-4, but after pronuclear rotation (PNR) are “difficult to detect”. I interpret this to mean that the tubes were unstable. The authors appeared to feel the same way as indicated by the following statement from the same paper: “In sas-4(RNAi) embryos, central tube assembly is not perturbed although its stability and capacity to assemble singlet microtubules are compromised. “

A second paper shows that GFP-SAS-6 does not persist at centrioles in SAS-4 depleted embryos: SAS-6 defines a protein family required for centrosome duplication in C. elegans and in human cells.

Leidel S, Delattre M, Cerutti L, Baumer K, Gönczy P.Nat Cell Biol. 2005 Feb;7(2):115-25. doi: 10.1038/ncb1220.

“To independently assess whether GFP–SAS-6 is recruited to the mother centriole, we examined sas-4(RNAi) embryos, in which only mother centrioles are present because daughter centriole formation does not occur2. As shown in Fig. 5b, we found that GFP–SAS-6 is present at centrioles in such embryos.
Therefore, GFP–SAS-6 is recruited to the mother centriole or a closely associated structure.
Interestingly analysis of GFP–SAS-6 distribution throughout the cell cycle in sas-4(RNAi) embryos revealed that the protein is not present at centrioles between late prophase and early telophase (Fig. 5c, 5d). By contrast centriolar GFP–SAS-6 is present throughout the cell cycle in otherwise wild-type embryos (Fig. 4a, 5a).
Therefore, daughter centriole formation or sas-4 function is required for persistence of GFP–SAS-6 at centrioles.”
So, SAS-6, a component of the central tube can be recruited to centrioles in the absence of SAS-4 but does not persist and is not present in M phase.
If the author can provide evidence that the central tube is present in the two-cell embryos where SAS-4 is missing entirely, and PCMD does not localize then I will accept their findings. Otherwise, this experiment should be removed as it shows nothing.

Third revision

Author response to reviewers' comments

In response to the point raised by Reviewer #1 regarding the sas-4(RNAi) we would like to add the following: we agree with the reviewer that in full sas-4 RNAi conditions the central tube might not persist to a two-cell embryo. However, as we pointed out before, the main purpose of this experiment was to analyze two-cell embryos under a partial sas-4(RNAi) condition, where a weak/small spindle pole is still present. Similar two-cell partial sas-4(RNAi) embryos were examined by TEM in Kirkham et al. 2003 (doi.org/10.1016/S0092-8674(03)00117-X). This analysis shows a central tube-like structure at the small spindle pole (Figure 6D, panel b and especially e). Since PCMD-1 is not detectable at the small spindle poles under similar RNAi conditions, we argue that SAS-4 is genetically upstream of PCMD-1. Unfortunately, we do not have the capacity to repeat the TEM analysis performed by Kirkahm et al. on our embryos and therefore we remove the sas-4(RNAi) experiment from the manuscript, as the reviewer requested (lines 134-136).

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I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.