JCB

Centriole-independent mitotic spindle assembly relies on the PCNT-CDK5RAP2 pericentriolar matrix

Sadanori Watanabe, Franz Meitinger, Andrew Shiau, Karen Oegema, and Arshad Desai

Corresponding Author(s): Arshad Desai, University of California, San Diego and Karen Oegema, University of California, San Diego

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July 16, 2020

Re: JCB manuscript #202006010

Dr. Arshad Desai
UC San Diego & Ludwig Cancer Research
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La Jolla, CA 92093

Dear Arshad,

Thank you for submitting your manuscript entitled "The PCNT-CDK5RAP2 matrix and centrioles can independently support spindle assembly in human cells". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that the reviewers are very positive about the quality and high interest of your study for the readership of JCB. Most of their constructive comments can be addressed through text changes and clarifications, as well as quantifications of your data (rev 1 p3, rev 2p3). With regards to the additional suggested experiments, we agree these would further strengthen your study and would welcome data to address these points. However, as they do not seem essential to your main conclusions, you may instead clarify these points for example with explicit statements regarding limitations in your text.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations

are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Tarun Kapoor, PhD
Monitoring Editor

Andrea L. Marat, PhD
Senior Scientific Editor

Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In their work, "The PCNT-CDK5RAP2 matrix and centrioles can independently support spindle assembly in human cells," the authors Watanabe, Meitinger, and colleagues, investigate the centriole-independent role of the pericentriolar material (PCM) in spindle assembly. This is a question of great interest to the field, as the independent roles of the centrioles and the PCM in spindle assembly have not yet been clearly delineated in human cells. Prior work has shown that there are indeed independent contributions by acentriolar bodies in mouse oocytes (Clift and Schuh, 2015, Nat Comm), but this work extends into the mitotic process of human cells.
The central approach in this work is to use the recently established PLK4 inhibitor, centrinone (by this group in Wong et al, 2015), to deplete centrioles in human cells. This allows the authors to investigate the centriole-independent contribution of PCM components to bipolar spindle assembly. They observe the formation of acentriolar poles that contain PCM components, and seem to be necessary for bipolar spindle assembly in one-centrosome cells. The authors probe the specific contribution of PCM components like pericentrin (PCNT) and CDK5RAP2 at these acentriolar poles via CRISPR/Cas9-mediated knockouts in HeLa and RPE-1 cell lines, as well as via siRNA mediated knockdown. They find that mitotic PCNT-CDK5RAP2-based PCM is dispensable for the assembly

of functional spindles due to the redundant, parallel function of centriole-dependent MT nucleation. However, centrinone treatment and depletion of these PCM components reveals that in cells lacking this centriole-dependent pathway, acentriolar PCM poles are crucial for bipolar spindle assembly.
Utilizing mutants of CDK5RAP2, the authors also find evidence that these acentriolar poles form via a similar mechanism used in centrosome maturation. They find that the well-characterized domains of CDK5RAP2, CM1 and CM2, are required for recruitment of γ-tubulin (and thus efficient MT nucleation) to acentriolar poles, as well as for the formation of the PCM foci, respectively. They also find that PLK1, a key regulator of centrosome assembly, is required for the formation of acentriolar PCM foci.
Finally, the authors conclude their study by attempting to replicate their HeLa and RPE-1 cell-based findings in the cancer cell lines DLD1 and U2OS. Ultimately, they find that these cancer cell lines do not form acentriolar PCM poles and do not require them to complete mitosis, even under centrinone treatment. This suggests that while PCM foci are required in some cell types, these findings are not generalizable to all human cells. Rather, this suggests that the acentriolar PCM pathway is one of likely several mechanisms that can compensate for centriole loss.

The manuscript is well-written and easy to follow. The work is of high quality and makes use of well-established techniques to generate new insights into the centriole-independent role of the PCM in spindle assembly. This work would be of great interest to readers of the Journal of Cell Biology.

Major Issues:
1) No major issues in this work.

Minor Issues:

2) The authors have made it clear in their introduction (page 5, bottom 6 lines) and discussion (page 18) that the acentriolar PCM poles are necessary for spindle assembly in the RPE-1 and HeLa cell lines, but not in DLD1 and U2OS. This same distinction should be made clear in the abstract.

3) Quantification of siRNA knockdowns:Western blots of all the PCM components targeted for knockdown or knockout were displayed. These should be quantified in figure 2F, 6B, and 6F. Besides helping understand the figures, it would allow to assess whether the knockdown of one component is affecting another relative to control.

Reviewer #2 (Comments to the Authors (Required)):

In this manuscript Watanabe, Meitinger et al., analyse the contributions of centrioles and the pericentriolar matrix (PCM) to spindle assembly in several cell types (mainly focusing on untransformed RPE-1 cells). In these cells, they confirm previous suggestions that depleting or knocking-out the PCM matrix proteins PCNT and/or CDK5RAP2 can have a surprisingly mild spindle phenotype, and that this is because the centrioles can still organise relatively normal spindle assembly without these proteins. They go on to confirm that cells depleted of centrioles can also organise relatively normal spindle assembly, but now show that this is because the matrix proteins PCNT and CDK5RAP2 help to organise acentriolar MTOCs (aMTOCs) that can organise MTs and help guide spindle assembly. In the absence of both pathways, spindle assembly is dramatically perturbed. The authors perform a comprehensive analysis of the requirements for aMTOC

assembly, and show that these are largely very similar to the requirements for centrosome matrix assembly. The centriole and matrix pathways they characterise in RPE1 cells also cooperate in spindle assembly in HeLa cells but, surprisingly, not in DLD1 or U2OS cells, which seem to use other mechanisms to support spindle assembly in the absence of both centrioles and the matrix proteins.

This is a really beautiful piece of work. The data is well presented and convincing, and these findings will be of great interest to a broad range of cell biologists as well as to those in the cancer field trying to understand how centrosomes and MTs can be exploited as therapeutic targets. I strongly support publication in JCB although I have a small number of points, detailed below, that the authors might like to consider prior to publication.

Major Points:

1. I was surprised that the authors did not try to address whether Cep192 is essential for the formation of the aMTOCs described here. In the discussion they highlight this as an area for further study but I wonder why they did not attempt to look at this. If there is a reason why this cannot be done it should be explained here, as I'm sure I won't be the only reader puzzled by this. This point is particularly interesting in light of the finding that Spd-2/Cep192 is not required for the assembly of similar aMTOCs in flies, but Asl/Cep152 is required, even though it is very difficult to detect any Asl in these structures (Baumbach et al., 2015).

2. The authors are usually careful and accurate in their choice of words, but to me the phrasing used throughout the manuscript will give most readers the impression that PCNT and CDK5RAP2 are the only components of the PCM matrix, and that in the absence of these proteins the centrioles do not assemble any PCM matrix. I am not convinced that the data (presented here or elsewhere) is strong enough to support this conclusion. I'm sure that the authors will be aware that studies of these proteins in flies have come to a slightly different conclusion, whereby Spd-2/Cep192 can assemble a weak PCM matrix around the centriole on its own (i.e. in the absence of Cnn) and this residual matrix can still recruit and organise some residual PCM and MTs (e.g. Conduit et al., eLife, 2014; Alvarez-Rodrigo et al., eLife, 2020). This weak Spd-2 matrix normally recruits Cnn, which then forms its own matrix around the centriole that strengthens the Spd-2 matrix. Is it not possible that something similar is going on here (i.e. a weaker Cep192 matrix is responsible for the "centriole-anchored mechanism...that supports spindle assembly when PCM matrix proteins are absent")?
The authors do discuss the possibility that the residual PCM organised by the centrioles in the absence of PCNT/CDK5RAP2 may be dependent on Cep192 but, related to point #1 above, is there a reason why they don't test this? Importantly, do they have any evidence that the residual Cep192 that may be contributing to the centriole function in the absence of PCNT and CDK5RAP2 is not forming some sort of PCM-matrix around the centriole? Perhaps they could test this by examining whether Cep192 (and the other PCM components recruited to centrioles in PCNT/CDK5RAP2 depleted/knock-out cells) are really restricted to the centriole, and don't expand outwards to form any matrix around it?

I do understand that the centriole/PCM-matrix distinction here is a useful construction that makes it easy to explain the logic of the paper. But, if the authors feel they cannot rule out that Cep192 might form some sort of matrix around the centriole without PCNT/CDK5RAP2, I think they should discuss this possibility and perhaps tone down a few of the phrases that imply that these depleted cells lack any PCM matrix (see, for example, minor point #3, below).

3. I assume the authors are aware of a recent publication from the Kitagawa lab characterising the

acentriolar spindle poles in HeLa cells depleted of centrosomes by centrinone (Chenin et al., EMBO J., 2020). These authors concluded that these acentriolar spindles lacked PCNT, CDK5RAP2 and Cep192 at >90% of their spindle poles. In the current paper the authors show that this is very different in RPE-1 cells, and imply that it is also very different when they do the experiment in HeLa cells (Figure 7A)-although, surprisingly, they don't show any quantification of this phenotype in HeLa cells. This data really should be quantified and, if there is a discrepancy with the Chenin et al. paper, this should be discussed.

Minor Points:

1. CNN should really be Cnn to conform to the usual Drosophila nomenclature.

2. The authors conclude from their analysis of DLD1 and U2OS cells that different human cell types might use different mechanisms of spindle assembly. This is of course correct, but it seems to me equally possible that these highly abnormal cancer cells might simply have a different combination of dysregulated pathways that allow them to overcome the usual requirement for centrioles and/or PCM proteins that might be present in all/most human cells. This is a subtle but important distinction that the authors should mention.

3. At the start of the Results section the authors state that "In human cells, the PCM matrix is assembled from PCNT and CDK5RAP2". They don't quote a reference and, to my knowledge, there is not sufficient data from human cells to back such a strong statement. I would advise toning down, or at least reference the data on which it is based so readers can make up their own mind.

Reviewer #3 (Comments to the Authors (Required)):

In their elegant paper, Watanabe and colleagues study basic principles of mitotic spindles, and in particular, the respective contribution made by centrioles and the pericentriolar matrix (PCM) to mitosis. They utilise a small molecule inhibitor of centrosome duplication, centrinone, developed by the same group, to dissect the roles of the large PCM scaffolding proteins, CDK5RAP2, Pericentrin and CEP192 in this process. The key discovery of this work is the profound synthetic lethality that exists between centrinone-induced centriole loss and deletion (and depletion) of CDK5RAP2 or PCNT in USP28-deficient RPE1 cells. The authors present ample evidence to explain the source of this synthetic lethality and describe molecular mechanisms and interactions responsible for acentriolar PCM recruitment/assembly. A central role for CEP192 in the process is revealed with live cell analysis showing recruitment of CEP192 foci to acentriolar spindles and their subsequent coalescing at spindle poles. With careful quantitative analysis of live cell data, the authors demonstrate that PLK1 activity, microtubules, pericentrin and the conserved gamma-tubulin- and pericentrin-interacting domains of CDK5RAP2 are essential in recruiting/organising a CEP192-dependent acentriolar PCM. In addition, it is shown that cells entering mitosis with a single centrosome rely on the same CDK5RAP2-pericentrin-dependent pathway to form/stabilise the acentriolar pole.
Given that the centriole wall is capable of CEP192 recruitment, this mechanism is particularly attractive to explain how CDK5RAP2-pericentrin and centrioles operate parallel and largely redundant pathways to organise/stabilise spindle poles. It is intriguing that at least some cancer cell lines are able to bypass both pathways to build functional spindles and require no spindle pole-located CEP192.

This is a clearly written, well-executed and carefully controlled study where conclusions are

adequately supported by the data. It assigns new roles to centrosome sub-compartments and reveals intriguing redundancies between these. In my view this work is well-suited for JCB's readership and I therefore recommend it for publication pending response to the minor points listed below.

Specific points:
1. To ensure that RPE1 and HeLa cells are not outliers, it would be important to demonstrate synthetic lethality between CDK5RAP2 depletion and centrinone treatment in at least one other untransformed cell line (i.e. MCF10A, hTERT-BJ1, Huvec etc).
2. I noticed that centrinone incubation times vary between cell lines. In particular, HeLa and DLD1 are treated only for 30+2 hours prior to live imaging, as opposed to 3-4 days in RPE1 cells. I would expect the majority of HeLa and DPD1 cells to contain at least one centriole at the beginning of filming. It would therefore be useful to demonstrate the penetrance of centriole depletion in each cell line at relevant timepoints. For instance, it is vital to prove that loss of CEP192/CDK5RAP2/PCNT signal from spindle poles in centrinone-treated DLD1 cells indeed reflects loss of centrioles from spindle poles rather than potentially independent effects of PLK4 inhibition. Even if DLD1 cells do not depend on centrioles and PCM to build a spindle, it is surprising that their acentriolar spindle poles do not recruit any PCM components at all. If mitosis is prolonged in these cells, do these components accumulate over time?
2. Fig. 2B: It would be useful to show centrosome content of these surviving cells with a centriolar marker as well. Also, the cells seem rather large; are they senescent?
3. Page 11: second paragraph, first line, correct arounds to around
4. Page 13: second paragraph, second line, correct to '' for the formation of PCM foci''
5. In Fig 2E: is the difference between mitotic durations of DMSO and Centrinone-treated Control cells not significant? It is unclear what ns refers to.

RESPONSE TO REVIEWERS **Watanabe, Meitinger et al. (JCB #202006010)**

Reviewer #1: "In their work, "The PCNT-CDK5RAP2 matrix and centrioles can independently support spindle assembly in human cells," the authors Watanabe, Meitinger, and colleagues, investigate the centriole-independent role of the pericentriolar material (PCM) in spindle assembly. This is a question of great interest to the field, as the independent roles of the centrioles and the PCM in spindle assembly have not yet been clearly delineated in human cells. Prior work has shown that there are indeed independent contributions by acentriolar bodies in mouse oocytes (Clift and Schuh, 2015, Nat Comm), but this work extends into the mitotic process of human cells. The central approach in this work is to use the recently established PLK4 inhibitor, centrinone (by this group in Wong et al, 2015), to deplete centrioles in human cells. This allows the authors to investigate the centriole-independent contribution of PCM components to bipolar spindle assembly. They observe the formation of acentriolar poles that contain PCM components, and seem to be necessary for bipolar spindle assembly in one-centrosome cells. The authors probe the specific contribution of PCM components like pericentrin (PCNT) and CDK5RAP2 at these acentriolar poles via CRISPR/Cas9-mediated knockouts in HeLa and RPE-1 cell lines, as well as via siRNA mediated knockdown. They find that mitotic PCNT-CDK5RAP2-based PCM is dispensable for the assembly of functional spindles due to the redundant, parallel function of centriole-dependent MT nucleation. However, centrinone treatment and depletion of these PCM components reveals that in cells lacking this centriole-dependent pathway, acentriolar PCM poles are crucial for bipolar spindle assembly.
Utilizing mutants of CDK5RAP2, the authors also find evidence that these acentriolar poles form via a similar mechanism used in centrosome maturation. They find that the well-characterized domains of CDK5RAP2, CM1 and CM2, are required for recruitment of γ-tubulin (and thus efficient MT nucleation) to acentriolar poles, as well as for the formation of the PCM foci, respectively. They also find that PLK1, a key regulator of centrosome assembly, is required for the formation of acentriolar PCM foci.
Finally, the authors conclude their study by attempting to replicate their HeLa and RPE-1 cell-based findings in the cancer cell lines DLD1 and U2OS. Ultimately, they find that these cancer cell lines do not form acentriolar PCM poles and do not require them to complete mitosis, even under centrinone treatment. This suggests that while PCM foci are required in some cell types, these findings are not generalizable to all human cells. Rather, this suggests that the acentriolar PCM pathway is one of likely several mechanisms that can compensate for centriole loss.

The manuscript is well-written and easy to follow. The work is of high quality and makes use of well-established techniques to generate new insights into the centriole-independent role of the PCM in spindle assembly. This work would be of great interest to readers of the Journal of Cell Biology.
Major Issues: 1) No major issues in this work."

We thank the reviewer for this positive evaluation of our work.

Reviewer #1 (minor point 1): "Minor Issues: 2) The authors have made it clear in their introduction (page 5, bottom 6 lines) and discussion (page 18) that the acentriolar PCM poles are necessary for spindle assembly in the RPE-1 and HeLa cell lines, but not in DLD1 and U2OS. This same distinction should be made clear in the abstract."

We have rewritten the abstract (reproduced below) to address this point.

> "Centrosomes, composed of centrioles that recruit a pericentriolar material (PCM) matrix assembled from PCNT and CDK5RAP2, catalyze mitotic spindle assembly. Here, we inhibit centriole formation and/or remove PCNT–CDK5RAP2 in RPE1 cells to address their relative contributions to spindle formation. While CDK5RAP2 and PCNT are

dispensable for spindle formation, they become essential when centrioles are absent. Acentriolar spindle assembly is accompanied by the formation of foci containing PCNT and CDK5RAP2 via a microtubule and Polo-like kinase 1-dependent process. Foci formation and spindle assembly require PCNT-CDK5RAP2-dependent matrix assembly and the ability of CDK5RAP2 to recruit γ-tubulin complexes. Thus, the PCM matrix can self-organize independently of centrioles to generate microtubules for spindle assembly; conversely, an alternative centriole-anchored mechanism supports spindle assembly when the PCM matrix is absent. Extension to three cancer cell lines revealed similar results in HeLa cells, whereas DLD1 and U2OS cells could assemble spindles in the absence of centrioles and PCNT-CDK5RAP2, suggesting cell type variation in spindle assembly mechanisms."

Reviewer #1: "3) Quantification of siRNA knockdowns: Western blots of all the PCM components targeted for knockdown or knockout were displayed. These should be quantified in figure 2F, 6B, and 6F. Besides helping understand the figures, it would allow to assess whether the knockdown of one component is affecting another relative to control."

We have added the measured band intensity values below the indicated blots in revised Figures 2 & 6. We do not see significant effect of depletion of PCNT on CDK5RAP2 levels or vice versa.

Reviewer #2: "In this manuscript Watanabe, Meitinger et al., analyse the contributions of centrioles and the pericentriolar matrix (PCM) to spindle assembly in several cell types (mainly focusing on untransformed RPE-1 cells). In these cells, they confirm previous suggestions that depleting or knocking-out the PCM matrix proteins PCNT and/or CDK5RAP2 can have a surprisingly mild spindle phenotype, and that this is because the centrioles can still organise relatively normal spindle assembly without these proteins. They go on to confirm that cells depleted of centrioles can also organise relatively normal spindle assembly, but now show that this is because the matrix proteins PCNT and CDK5RAP2 help to organise acentriolar MTOCs (aMTOCs) that can organise MTs and help guide spindle assembly. In the absence of both pathways, spindle assembly is dramatically perturbed. The authors perform a comprehensive analysis of the requirements for aMTOC assembly, and show that these are largely very similar to the requirements for centrosome matrix assembly. The centriole and matrix pathways they characterise in RPE1 cells also cooperate in spindle assembly in HeLa cells but, surprisingly, not in DLD1 or U2OS cells, which seem to use other mechanisms to support spindle assembly in the absence of both centrioles and the matrix proteins.

This is a really beautiful piece of work. The data is well presented and convincing, and these findings will be of great interest to a broad range of cell biologists as well as to those in the cancer field trying to understand how centrosomes and MTs can be exploited as therapeutic targets. I strongly support publication in JCB although I have a small number of points, detailed below, that the authors might like to consider prior to publication."

We thank the reviewer for this positive evaluation of our work.

Reviewer #2 (major point 1): "**Major Points:** 1. I was surprised that the authors did not try to address whether Cep192 is essential for the formation of the aMTOCs described here. In the discussion they highlight this as an area for further study but I wonder why they did not attempt to look at this. If there is a reason why this cannot be done it should be explained here, as I'm sure I won't be the only reader puzzled by this. This point is particularly interesting in light of the finding that Spd-2/Cep192 is not required for the assembly of similar aMTOCs in flies, but Asl/Cep152 is required, even though it is very difficult to detect any Asl in these structures (Baumbach et al., 2015)."

To address this point in the revision, we employed an inducible *CEP192* knockout RPE1 cell line that we recently generated (*iCEP192 KO*; Meitinger et al. 2020; PMID: 32908304). The gene encoding the mitotic duration sensor component USP28 was also knocked out to enable centrosome depletion without the complication of G1 cell cycle arrest due to p53 stabilization. In the experiment, cells were treated with centrinone for 4 days to deplete centrioles and then for 24 hours with doxycycline to induce *CEP192* KO before processing for immunofluorescence (see experimental schematic in **new Fig. S3B**). In centrinone-treated cells lacking centrioles, cells remain in a prometaphase-like state for an extended period of time (typically 45-70 minutes) while the spindle and PCM-containing foci form. Thus, in control centrinone-treated cells, only ~10% of mitotic cells are in a metaphase-like state with aligned chromosomes. In centrinone-treated cells in which CEP192 was inducibly knocked out (*iCEP192 KO*), the percentage of mitotic cells in a metaphase-like state was dramatically decreased (**new Fig. S3C**), suggesting failure of spindle assembly. As so few centrinone-treated *iCEP192 KO* cells were able to assemble a spindle and achieve a metaphase-like state, we compared CDK5RAP2-PCNT foci formation in cells that were in a prometaphase-like configuration. In contrast to control mitotic centrinone-treated cells with assembled spindles and chromosomes in a metaphase-like configuration, where CDK5RAP2-PCNT foci are observed in close to 100% of cells (**Fig. S5A**), clear foci are observed in ~30% of control mitotic centrinone-treated cells with chromosomes in a prometaphase-like configuration (**new Fig. S3B,C**). In mitotic centrinone-treated *iCEP192 KO* cells, no CDK5RAP2-PCNT foci were detected in cells with chromosomes in a prometaphase-like configuration (**new Fig. S3B,C**). These results strongly suggest that CEP192 is required to form the acentriolar PCM matrix foci. How CEP192 acts to stimulate PCM foci formation, and which of its previously defined functions including recruitment of PLK1, Aurora A and γ-tubulin (PMID: 22595525; PMID: 26012549; PMID: 25042804; PMID: 22895009; PMID: 17980596) are required will take future work to clarify.

Reviewer #2 (major point 2): "**Major Points:** "2. The authors are usually careful and accurate in their choice of words, but to me the phrasing used throughout the manuscript will give most readers the impression that PCNT and CDK5RAP2 are the only components of the PCM matrix, and that in the absence of these proteins the centrioles do not assemble any PCM matrix. I am not convinced that the data (presented here or elsewhere) is strong enough to support this conclusion. I'm sure that the authors will be aware that studies of these proteins in flies have come to a slightly different conclusion, whereby Spd-2/Cep192 can assemble a weak PCM matrix around the centriole on its own (i.e. in the absence of Cnn) and this residual matrix can still recruit and organise some residual PCM and MTs (e.g. Conduit et al., eLife, 2014; Alvarez-Rodrigo et al., eLife, 2020). This weak Spd-2 matrix normally recruits Cnn, which then forms its own matrix around the centriole that strengthens the Spd-2 matrix. Is it not possible that something similar is going on here (i.e. a weaker Cep192 matrix is responsible for the "centriole-anchored mechanism...that supports spindle assembly when PCM matrix proteins are absent")?"

"I do understand that the centriole/PCM-matrix distinction here is a useful construction that makes it easy to explain the logic of the paper. But, if the authors feel they cannot rule out that Cep192 might form some sort of matrix around the centriole without PCNT/CDK5RAP2, I think they should discuss this possibility and perhaps tone down a few of the phrases that imply that these depleted cells lack any PCM matrix (see, for example, minor point #3, below)."

We agree with the Reviewer's assertion that Spd-2/CEP192 or a Spd-2/CEP192 matrix associated with centrioles may be responsible for the centriole-anchored mechanism that supports spindle assembly. This is our favorite model and one that we mention in the discussion. We did not intend to imply that cells without PCNT and/or CDK5RAP2 lack any PCM. Throughout the text we use the term "PCM matrix" to refer to the outer mitotic PCNT and CDK5RAP2-dependent PCM matrix layer defined by 3D-SIM microscopy (Lawo et al., 2012). By contrast, we

use the broader term “pericentriolar material” to refer to the larger collection of all of the components that associate with centrioles. To make this distinction clear we have re-written the first paragraph of the introduction (reproduced below) and have checked all of the other instances where we use the term “PCM matrix” to ensure that we use the term consistently.

> “Centrosomes are the primary microtubule organizing centers in metazoan cells. Centrosomes consist of a centriolar core that organizes a layered proteinaceous structure, called the pericentriolar material (PCM; (Mennella et al., 2014)). During mitotic entry, centrosomes increase in size to help meet the increased demand for microtubule generation for spindle assembly (Palazzo et al., 2000). This increase in size is due to expansion of an outer PCM matrix layer whose assembly requires the large coiled-coil proteins pericentrin/PCNT (pericentrin-like-protein/PLP in *Drosophila*) and CDK5RAP2/CEP215 (centrosomin/Cnn in *Drosophila*, and SPD-5 in *C. elegans*) (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2014; Mennella et al., 2012; Woodruff et al., 2014). For convenience, we refer to this PCNT/CDK5RAP2-based matrix layer as the “PCM matrix”; noting that there are other PCM proteins, including CEP192 and NEDD1, that remain in a more centriole-proximal toroid, when the outer PCM matrix layer is absent (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012)…”

Reviewer #2 (major point 3): “The authors do discuss the possibility that the residual PCM organised by the centrioles in the absence of PCNT/CDK5RAP2 may be dependent on Cep192 but, related to point #1 above, is there a reason why they don't test this? Importantly, do they have any evidence that the residual Cep192 that may be contributing to the centriole function in the absence of PCNT and CDK5RAP2 is not forming some sort of PCM-matrix around the centriole? Perhaps they could test this by examining whether Cep192 (and the other PCM components recruited to centrioles in PCNT/CDK5RAP2 depleted/knock-out cells) are really restricted to the centriole, and don't expand outwards to form any matrix around it?”

CEP192 is a complex scaffold that interacts with PLK1, PLK4, Aurora A, γ-tubulin complexes, CEP295, and TRIM37 (PMID: 22595525; PMID: 26012549; PMID: 25042804; PMID: 22895009; PMID: 17980596; PMID: 27562453; PMID: 27562453). CEP192 is thought to localize to both the PCNT/CDK5RAP2-based matrix and to centrioles independently of PCNT-CDK5RAP2 and is important for spindle assembly in both the presence and absence of centrioles (e.g. **Fig. 2F**; PMID: 17980596; PMID: 18207742 PMID: 22595525; PMID: 32908304). The multifunctionality of CEP192 combined with the complexity of its centrosomal recruitment makes it difficult to remove CEP192 and draw clear conclusions as to whether it is specifically loss of centriole-anchored CEP192 that is responsible for failure of spindle assembly. With respect to the Reviewer’s specific question of whether CEP192 can itself polymerize to form a matrix, or whether, for example, CEP192 bound to CEP295 might constitute a centriole-anchored microtubule-generating matrix will require a greater understanding of how CEP192 interacts with its binding partners and the ability to generate mechanistically defined separation-of-function mutants. We think this level of analysis is beyond the scope of the current manuscript.

Reviewer #2 (major point 4): “3. I assume the authors are aware of a recent publication from the Kitagawa lab characterising the acentriolar spindle poles in HeLa cells depleted of centrosomes by centrinone (Chenin et al., EMBO J., 2020). These authors concluded that these acentriolar spindles lacked PCNT, CDK5RAP2 and Cep192 at >90% of their spindle poles. In the current paper the authors show that this is very different in RPE-1 cells, and imply that it is also very different when they do the experiment in HeLa cells (Figure 7A)-although, surprisingly, they don't show any quantification of this phenotype in HeLa cells. This data really should be quantified and, if there is a discrepancy with the Chenin et al. paper, this should be discussed.”

We now present a quantification of the immunofluorescence analysis of foci formation in HeLa, DLD1 and U2OS cells (**Fig. S5A,B**). We consistently observe foci containing CEP192, CDK5RAP2 and PCNT at spindle poles in HeLa and RPE1 cells (detected at 1 or both poles in 80-100% of cells). We do not understand why Chenin et al. (2020) did not detect PCM foci at the poles of their acentriolar HeLa cells. It is possible that the difference lies in antibodies used – at least for CEP192 we have found that our in-house antibody is significantly superior to commercial ones. Another possibility is that there are differences between the HeLa subclones that we employ versus those used by the Kitagawa group. To confirm the identity of our HeLa cell line, we submitted it to the ATCC for STR profiling and found that it is a perfect match for the CCL-2 HeLa cell line from the ATCC (*see attached report from ATCC*). As foci detection can be somewhat subjective, in our view, the critical distinction is the functional one – we see strong synthetic lethality between centrinone and CDK5RAP2-PCNT knockouts in our HeLa cell lines, a point that was not investigated in the prior work. We now point out the discrepancy with localization analysis in the prior report in the revision.

Reviewer #2 (minor point 1): "1. CNN should really be Cnn to conform to the usual Drosophila nomenclature."
We have changed CNN to Cnn throughout the text.

Reviewer #2 (minor point 2): "2. The authors conclude from their analysis of DLD1 and U2OS cells that different human cell types might use different mechanisms of spindle assembly. This is of course correct, but it seems to me equally possible that these highly abnormal cancer cells might simply have a different combination of dysregulated pathways that allow them to overcome the usual requirement for centrioles and/or PCM proteins that might be present in all/most human cells. This is a subtle but important distinction that the authors should mention."

Yes, whether these differences in the spindle assembly mechanisms reflect cell type of origin versus cancer-triggered dysregulation is a very interesting question, which we now mention in the discussion.

Reviewer #2 (minor point 3): "3. At the start of the Results section the authors state that "In human cells, the PCM matrix is assembled from PCNT and CDK5RAP2". They don't quote a reference and, to my knowledge, there is not sufficient data from human cells to back such a strong statement. I would advise toning down, or at least reference the data on which it is based so readers can make up their own mind."

As we highlight in our response to major point 2 above, in this manuscript we use the term "PCM matrix" to refer to the mitotic PCNT and CDK5RAP2-dependent PCM matrix layer defined by super-resolution microscopy (Lawo et al., 2012), and not the entirety of the pericentriolar material. This point is now clarified in the first paragraph of the introduction. We have also altered the wording of the sentence in question to read: "In human cells, the mitotic increase in centrosome size is due to expansion of a PCNT and CDK5RAP2-dependent layer of the PCM (Lawo et al., 2012), which we will refer to as the "PCM matrix".

With respect to the question of whether there is experimental support for the idea that PCNT and CDK5RAP2 are indeed the physical constituents of the PCM matrix layer that is lost when these components are knocked out or depleted, we have followed the strategy suggested by the Reviewer. We now outline the data in support of this idea in the second paragraph of the introduction (*reproduced below*).

> "Within the centrosome, PCNT/PLP is thought to link the PCM matrix layer to the centriole and ensure its proper organization. In humans and *Drosophila*, PCNT/PLP associates with the outer centriole wall via its C-terminal PACT domain and, in interphase

centrosomes, is oriented with its N-terminal domain facing outwards (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012). CDK5RAP2/Cnn is thought to assemble on this PCNT foundation, as loss of PCNT leads to a significant reduction in centrosomal CDK5RAP2/Cnn (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012). The mitotic expansion of the PCM matrix is controlled by the kinase PLK1 (Cabral et al., 2019; Conduit et al., 2014; Dobbelaere et al., 2008; Haren et al., 2009; Lane and Nigg, 1996; Lee and Rhee, 2011; Woodruff et al., 2015). Structural work on the *Drosophila* proteins has suggested that PCM matrix expansion is driven by a phosphorylation-regulated self-interaction between Cnn molecules (Feng et al., 2017) in which the CM2 motif at the Cnn C-terminus interacts with an internal Cnn leucine zipper (Citron et al., 2018; Feng et al., 2017). The Cnn CM2 motif also interacts with an internal region in PLP, and it has been suggested that this interaction may help tether the Cnn-based matrix to the centriole (Citron et al., 2018). Although similar structural work has not yet been done on the human proteins, the similar dependence on PCNT for assembly of a CDK5RAP2/PCNT matrix layer (Fu and Glover, 2012; Lawo et al., 2012; Mennella et al., 2012), the dramatic expansion of the matrix layer when either PCNT or CDK5RAP2 is overexpressed (Lawo et al., 2012), and experiments showing that the CM2 domain of CDK5RAP2 is important for its ability to accumulate around centrioles and to interact with PCNT (Kim and Rhee, 2014; Wang et al., 2010) suggest that that the human PCM matrix may assemble in a similar CM2-dependent fashion."

Reviewer #3: "In their elegant paper, Watanabe and colleagues study basic principles of mitotic spindles, and in particular, the respective contribution made by centrioles and the pericentriolar matrix (PCM) to mitosis. They utilise a small molecule inhibitor of centrosome duplication, centrinone, developed by the same group, to dissect the roles of the large PCM scaffolding proteins, CDK5RAP2, Pericentrin and CEP192 in this process. The key discovery of this work is the profound synthetic lethality that exists between centrinone-induced centriole loss and deletion (and depletion) of CDK5RAP2 or PCNT in USP28-deficient RPE1 cells. The authors present ample evidence to explain the source of this synthetic lethality and describe molecular mechanisms and interactions responsible for acentriolar PCM recruitment/assembly. A central role for CEP192 in the process is revealed with live cell analysis showing recruitment of CEP192 foci to acentriolar spindles and their subsequent coalescing at spindle poles. With careful quantitative analysis of live cell data, the authors demonstrate that PLK1 activity, microtubules, pericentrin and the conserved gamma-tubulin- and pericentrin-interacting domains of CDK5RAP2 are essential in recruiting/organising a CEP192-dependent acentriolar PCM. In addition, it is shown that cells entering mitosis with a single centrosome rely on the same CDK5RAP2-pericentrin-dependent pathway to form/stabilise the acentriolar pole.
Given that the centriole wall is capable of CEP192 recruitment, this mechanism is particularly attractive to explain how CDK5RAP2-pericentrin and centrioles operate parallel and largely redundant pathways to organise/stabilise spindle poles. It is intriguing that at least some cancer cell lines are able to bypass both pathways to build functional spindles and require no spindle pole-located CEP192.

This is a clearly written, well-executed and carefully controlled study where conclusions are adequately supported by the data. It assigns new roles to centrosome sub-compartments and reveals intriguing redundancies between these. In my view this work is well-suited for JCB's readership and I therefore recommend it for publication pending response to the minor points listed below.

We thank the reviewer for this positive evaluation of our work.

Reviewer #3 (point 1): "Specific points: 1. To ensure that RPE1 and HeLa cells are not outliers, it would be important to demonstrate synthetic lethality between CDK5RAP2 depletion and centrinone treatment in at least one other untransformed cell line (i.e. MCF10A, hTERT-BJ1, Huvec etc)."

We agree with the reviewer that analysis in more cell lines would be interesting but having characterized 4 cell lines, we believe extending the analysis to an additional untransformed cell line is unlikely to significantly amend the presented conclusions. Analysis in untransformed lines is challenging because of the irreversible senescence that occurs due to extended mitosis following centrosome loss (PMID: 25931445; PMID: 27432897). This is why all our RPE1 analysis was conducted in the *USP28*Δ background; USP28 is a critical component of the mitotic timer pathway that senses extended mitosis following centriole loss and triggers p53-dependent senescence (PMID: 27432897; PMID: 27432896; PMID: 27371829). We do not have other untransformed lines lacking USP28 and the amount of engineering required to first knockout USP28 and characterize clones in order to identify ones that lack the mitotic timer, followed by engineering knockouts of CDK5RAP2 and PCNT in that clonal background and characterizing them, is beyond the scope of a revision timeframe.

Reviewer #3 (point 2a): "2. I noticed that centrinone incubation times vary between cell lines. In particular, HeLa and DLD1 are treated only for 30+2 hours prior to live imaging, as opposed to 3-4 days in RPE1 cells. I would expect the majority of HeLa and DPD1 cells to contain at least one centriole at the beginning of filming. It would therefore be useful to demonstrate the penetrance of centriole depletion in each cell line at relevant timepoints.
For instance, it is vital to prove that loss of CEP192/CDK5RAP2/PCNT signal from spindle poles in centrinone-treated DLD1 cells indeed reflects loss of centrioles from spindle poles rather than potentially independent effects of PLK4 inhibition."

We have confirmed centrinone penetrance in all of the cell lines used here. Our standard protocol with centrinone is to treat cells for 3-4 cell cycles in order to generate a relatively uniform population of centriole-less cells; with any marker (CEP192, PCNT, CDK5RAP2), it is straightforward to discriminate between centriolar and acentriolar foci in mitotic cells – the centriolar foci are always brighter, singular and structurally well-organized.

With respect to the analysis of HeLa and DLD1 cell knockouts highlighted by the reviewer, the shorter timing of centrinone treatment was dictated by the outcomes. In this analysis, which was conducted in parallel to compare these two cell lines, we used a shorter incubation in centrinone because HeLa clones lacking PCNT or CDK5RAP2 show strong synthetic lethality with centrinone. Thus, in the live imaging experiments comparing HeLa and DLD1 knockouts, we are analyzing a significant number of 1-centrosome cells. Note that we showed earlier in the paper that for RPE1(*USP28*Δ) cells depleted of CDK5RAP2, both 0- and 1-centrosome cells fail mitosis (**Fig. 3E**). Thus, when there is a synthetic defect, it is manifest even when there is one centriolar pole. We have added mention of the likely presence of 1-centrosome in the Results section.

To address the second point raised by the reviewer, we conducted acute (<24h) centrinone treatment following RNAi of PCNT and CDK5RAP2 in RPE1(*USP28*Δ) cells. As centriole loss in centrinone requires cell division, such short term treatment allows us to distinguish between effects of PLK4 kinase activity inhibition and centriole loss. We do not observe any mitotic defects in cells first depleted of CDK5RAP2 or PCNT that undergo mitosis in a 14 hour interval after centrinone addition (**new Fig. S2B**). This data confirms that the reported effects of centrinone require centriole loss and not solely PLK4 inhibition.

Reviewer #3 (point 2b): "Even if DLD1 cells do not depend on centrioles and PCM to build a spindle, it is surprising that their acentriolar spindle poles do not recruit any PCM components at all. If mitosis is prolonged in these cells, do these components accumulate over time?"

In response to the reviewer's feedback and also the query from Reviewer 2, we have now more carefully assessed localization of PCM components in acentriolar mitotic HeLa, DLD1 and U2OS cells and report the analysis in **revised *Fig. 7A*** and **new *Fig. S4A***. We do observe accumulation of PCM components in a subset of DLD1 (and U2OS cells). Thus, the difference in PCM foci formation between RPE1/HeLa and DLD1/U2OS is one of degree and not absolute. We have observed γ-tubulin on the spindle in the doubly-inhibited DLD1 cells, but as there are many different mechanisms for γ-tubulin recruitment to the spindle, this does not lead to a clear conclusion. In our view, the critical data here is the functional difference in spindle assembly, mitotic progression and chromosome segregation, shown in **Figure 7**, which is emphasized in the conclusions.

Reviewer #3 (point 2c): "2. Fig. 2B: It would be useful to show centrosome content of these surviving cells with a centriolar marker as well. Also, the cells seem rather large; are they senescent?"

We suspect these cells have failed 1-centrosome division, as suggested by the analysis in **Fig. 3E**. This likely explains their larger size. As this is a minor lead-in point that is extended by the significant experimental effort that follows to establish the synthetic mitotic failure between centriole loss and PCNT-CDK5RAP2 inhibition, we have not conducted additional analysis of these cells.

Reviewer #3 (point 3): "3. Page 11: second paragraph, first line, correct arounds to around"

Thank you – this has been corrected.

Reviewer #3 (point 4): "4. Page 13: second paragraph, second line, correct to " for the formation of PCM foci"

Thank you – this has been corrected.

Reviewer #3 (point 5): "5. In Fig 2E: is the difference between mitotic durations of DMSO and Centrinone-treated Control cells not significant? It is unclear what ns refers to."

The comparisons were between the DMSO controls and DMSO *CDK5RAP2*Δ or DMSO *PCNT*Δ. These comparisons indicate that loss of CDK5RAP2 or PCNT does not significantly extend mitotic duration. We have redrawn the comparison lines for clarity.

We note that centrinone always significantly extends mitosis relative to DMSO-treated control cells. This point has been emphasized in prior work (e.g. Meitinger et al. 2016; 2020) and we have not drawn attention to it here in the graphs.

Cell Line Authentication Service

STR Profile Report

Sample Submitted By:	Ludwig Institute for Cancer Research Franz Meitinger
Email Address:	fmeitinger@ucsd.edu
ATCC Sales Order:	SO0665709
FTA Barcode:	STRB4124
Cell Line Designation:	HeLa
Date Sample Received:	Tuesday, September 08, 2020
Report Date:	Thursday, September 10, 2020

Methodology: Seventeen short tandem repeat (STR) loci plus the gender determining locus, Amelogenin, were amplified using the commercially available PowerPlex® 18D Kit from Promega. The cell line sample was processed using the ABI Prism® 3500xl Genetic Analyzer. Data were analyzed using GeneMapper® ID-X v1.2 software (Applied Biosystems). Appropriate positive and negative controls were run and confirmed for each sample submitted.

Data Interpretation: Cell lines were authenticated using Short Tandem Repeat (STR) analysis as described in 2012 in ANSI Standard (ASN-0002) Authentication of Human Cell Lines: Standardization of STR Profiling by the ATCC Standards Development Organization (SDO) and in Capes-Davis et al., Match criteria for human cell line authentication: Where do we draw the line? Int. J. Cancer. 2012 Nov 8. doi: 10.1002/ijc.27931

ATCC performs STR Profiling following ISO 9001:2008 and ISO/IEC 17025:2005 quality standards.

Technical questions?

ATCC Technical Support
(800) 638-6597 / +1 703-365-2700
tech@atcc.org

Ordering questions?

800-638-6597 or 703-365-2700
Fax 703-365-2750
Email: sales@atcc.org

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRB4124

ATCC Sales Order: SO0665709

Test Results for Submitted Sample					ATCC Reference Database Profile			
Locus	**Query Profile: HeLa**				**Database Profile: HeLa; Cervical Adenocarcinoma; Human**			
D3S1358	15	18						
TH01	7				7			
D21S11	27	28						
D18S51	16							
Penta_E	7	16	17					
D5S818	11	12			11	12		
D13S317	12	13.3			12	13.3		
D7S820	8	12			8	12		
D16S539	9	10			9	10		
CSF1PO	9	10			9	10		
Penta_D	8	15						
Amelogenin	X				X			
vWA	16	18			16	18		
D8S1179	12	13						
TPOX	8	12			8	12		
FGA	21							
D19S433	13	14						
D2S1338	17							

Number of shared alleles between query sample and database profile:	16
Total number of alleles in the database profile:	16
Percent match between the submitted sample and the database profile:	100

The allele match algorithm compares the 8 core loci plus amelogenin only, even though alleles from all loci will be reported when available.

NOTE: *Loci highlighted in grey (8 core STR loci plus Amelogenin) can be made public to verify cell identity. In order to protect the identity of the donor,* ***please do not publish*** *the allele calls from all the STR loci tested.*
Electropherograms showing raw data are attached.

Explanation of Test Results
Cell lines with 80% match are considered to be related; i.e., derived from a common ancestry. Cell lines with between a 55% to 80% match require further profiling for authentication of relatedness.

☐ The submitted sample profile is human, but not a match for any profile in the ATCC STR database.

☒ The submitted profile is an exact match for the following ATCC human cell line(s) in the ATCC STR database (8 core loci plus Amelogenin): CCL-2

☐ The submitted profile is similar to the following ATCC human cell line(s):

☐ An STR profile could not be generated.

Additional Comments:

Submitted sample, STRB4124 (HeLa), is an exact match to CCL-2 (HeLa).

e-Signature, Technician:	snicholson 9/10/2020
e-Signature, Reviewer:	Bchase 9/10/2020

Cell Line Authentication Service
STR Profile Report

FTA Barcode: STRB4124

ATCC Sales Order: SO0665709

Addendum: Comparative Output from the ATCC STR Profile Database

% Match	ATCC® Cat. No.	Designation	D5S818	D13S317	D7S820	D16S539	vWA	TH01	AMEL	TPOX	CSF1PO
100	STRB4124	HeLa	11,12	12,13.3	8,12	9,10	16,18	7	X	8,12	9,10
100	CCL-2	HeLa; Cervical Adenocarcinoma; Human	11,12	12,13.3	8,12	9,10	16,18	7	X	8,12	9,10

Definitions of terms used in this report:

Peak Area Difference (PAD):
Refers to a heterozygous peak imbalance.
Two alleles at a single locus should amplify in a similar manner; and therefore produce peaks of similar height and area. Peaks which are above threshold (50 rfu) but are not of similar area, within 50% of each other, are referred to as a PAD. Due to their nature cell lines do not amplify in the same manner as a sample taken from a fresh buccal swab. PAD is far more common in cell line samples.

Stutter:
A stutter peak is a small peak which occurs immediately before the true peak. It is defined as being a single repeat unit smaller than the true peak. The stutter peak should be less than 15% of the true peak. The stutter is caused by the polymerase.

+4 Peak:
A +4 is similar to a stutter but occurs immediately after the true peak. A stutter peak should be less than 5% for a homozygous and 10% for a heterozygous.

Below Threshold Peak(s):
Cell lines can produce unusual profiles and occasionally a peak will amplify poorly and be below threshold. Where we find a below threshold peak which we believe is valid we indicate it as a below threshold peak. Our cell line analysis criteria, Homozygous and Heterozygous peaks must be equal to or above the set height threshold for it to be considered a true peak.

Ladder/ Off Ladder Peak(s):
The allelic ladder consists of most or all known alleles in the population and allows for precise assignment of alleles. Those which do not align are termed ‘off ladder.

Artifact:
A non-allelic product of the amplification process, an anomaly of the detection process, or a by-product of primer synthesis

Pull-up:
A term used to describe when signal from one dye color channel produces artificial peaks in another, usually adjacent, color.

Spike:
An extraneous peak resulting from dust, dried polymer, an air bubble, or an electrical surge.

Dye blob:
Free dye not coupled to primer that can be injected into the capillary (A known and documented dye blob is often found at the D3S1358 locus.)

September 29, 2020

RE: JCB Manuscript #202006010R

Dr. Arshad Desai
University of California, San Diego
9500 Gilman Dr
9500 Gilman Drive
La Jolla, CA 92093

Dear Arshad,

Thank you for submitting your revised manuscript entitled "The PCNT-CDK5RAP2 matrix and centrioles can independently support spindle assembly in human cells". We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

** In your original cover letter, you had discussed co-ordination with a related study also submitted to JCB. Therefore, please let us know how you would like to proceed, as it is your choice to publish immediately or wait for the possible publication of that paper.

1) Text limits: Character count for Articles is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

2) Figures limits: Articles may have up to 10 main text figures.

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (either in the figure legend itself or in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you

used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

5) Abstract and title: The abstract should be no longer than 160 words and should communicate the significance of the paper for a general audience. The title should be less than 100 characters including spaces. Make the title concise but accessible to a general readership.
While your current title will be appreciated by the specialists, we do not feel that it will be accessible to a broader cell biology audience. Therefore, we suggest the following alternative

Centriole-independent assembly of human mitotic spindles relies on pericentriolar material

6) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.

7) Please be sure to provide the sequences for all of your primers/oligos and RNAi constructs in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers (where appropriate) for all of your antibodies. Please also indicate the acquisition and quantification methods for immunoblotting/western blots.

8) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. Imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

9) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

10) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental display items (figures and tables).. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section.

11) eTOC summary: A ~40-50-word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person.

12) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the

following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

13) ORCID IDs: ORCID IDs are unique identifiers allowing researchers to create a record of their various scholarly contributions in a single place. At resubmission of your final files, please consider providing an ORCID ID for as many contributing authors as possible.

14) A separate author contribution section following the Acknowledgments. All authors should be mentioned and designated by their full names. We encourage use of the CRediT nomenclature.

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Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Tarun Kapoor, PhD

Monitoring Editor

Andrea L. Marat, PhD
Senior Scientific Editor

Journal of Cell Biology
