JCB

WDR62 localizes katanin at spindle poles to ensure synchronous chromosome segregation

Amanda Guerreiro, Filipe De Sousa, Nicolas Liaudet, Daria Ivanova, Anja Eskat, and Patrick Meraldi

Corresponding Author(s): Patrick Meraldi, University of Geneva

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September 26, 2020

Re: JCB manuscript #202007171

Prof. Patrick Meraldi
University of Geneva
Cell physiology and metabolism department
Centre Medical Universitaire Rue Michel Servet 1
Geneva 1211
Switzerland

Dear Patrick,

Thank you for submitting your manuscript "WDR62 localizes katanin at spindle poles to ensure synchronous chromosome segregation" to the Journal of Cell Biology. Three expert reviewers evaluated your manuscript as well as the related manuscript from Huang et al. Based on the reviewer feedback, we think the work needs revision that will involve additional experimentation as well as clarification/alteration of interpretations. In our evaluation, the reviewer feedback was on point and constructive, and we believe addressing their points will significantly improve the manuscript. To help guide your revision, we have highlighted key issues below. Please note that the revision will need to be accompanied by a detailed response to the reviewer feedback.

Specific points:
1) Microtubule flux verus minus-end depolymerization clarification (Rev 1).
2) Describe effect of WDR62 inhibition on astral microtubules (Rev 1 & Rev 3).
3) Need for rescue to confirm phenotypes (Rev 2) - we believe this is important to include in the revision given the confusing history of work on WDR62.
4) Cleaning up analysis of centriole duplication (Rev 2).
5) Distinguishing effects of WDR62 on MCAK versus katanin and adjusting conclusions based on the results; in particular, addressing if katanin inhibition affects MCAK (Rev 2 and Rev 3).
6) Better analysis of the spindle phenotypes; related to this, reviewers were concerned about differences between your and the Huang et al. manuscript and felt it would be beneficial to the field if these differences were resolved. We understand that such resolution may not always be possible but any efforts towards resolving differences would be beneficial in the longer term.

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,

https://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,

Arshad Desai, Ph.D.
Monitoring Editor
Journal of Cell Biology

Dan Simon, Ph.D.
Scientific Editor
Journal of Cell Biology

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

The work by Guerreiro et al., investigates the role of WDR62, a protein whose gene is often mutated in microcephaly, in mitotic spindle dynamics and chromosome segregation. The authors convincingly show that WDR62 localizes to spindle microtubule minus-ends, but not centrosomes, independently of ASPM, another protein implicated in microcephaly. Mechanistically, the authors

show that WDR62 is required to recruit the microtubule-severing enzyme Katanin to spindle microtubule minus-ends. Functional studies in cells revealed that both WDR62 and Katanin are required for microtubule minus-end depolymerization, which is essential to regulate normal spindle length and architecture (long microtubules buckle without WDR62 or Katanin). Surprisingly, both WDR62 and Katanin promote spindle microtubule turnover, without significantly affecting kinetochore microtubule-plus-end dynamics, as indirectly inferred by kinetochore tracking in living cells. In addition, loss of WDR62, but not Katanin, resulted in wider metaphase plates and asynchronous chromosome segregation in anaphase, without any evident aneuploidy. The authors propose a model in which asynchronous chromosome segregation in anaphase might have implications for the genesis of microcephaly by affecting chromosome positioning in the subsequent interphase nuclei. Overall, the study is technically well performed and well controlled and offers a fundamental cell biological explanation that might be of clinical relevance to microcephaly-associated disorders, and thus of interest to a wide audience.

Major issues:
1- There are a few inconsistencies in the data or its interpretation that are worth clarifying. For instance, the authors confuse the rate of microtubule minus-end depolymerization with the rate of poleward flux. This is not uncommon and must be clarified. Flux is the poleward motion of an entire microtubule relative to a fixed reference point within the spindle (e.g. the metaphase plate). What the authors have measured was the velocity of a fluorescent mark within a half-spindle relative to the corresponding pole - i.e. they measured the velocity of microtubule minus-end depolymerization, but then refer to these different phenomena interchangeably throughout the manuscript. However, as it is evident from the examples shown in figure 4G, the photoactivated mark in either perturbation is still moving at apparently normal velocity relative to the spindle equator (i.e. they flux), as defined from SiR-DNA. If spindle length is kept constant, the two velocities should in principle match, but it seems that over time (due to incorporation of new tubulin at microtubule plus-ends and microtubule poleward transport) spindle microtubules grow more than usual and buckle, because they cannot undergo minus-end depolymerization in response to poleward flux. Indeed, these two phenomena were able to be experimentally separated, as spindle microtubules continued to flux, without minus-end depolymerization after laser-induced generation of new microtubule minus-ends (Matos et al., JCB, 2009; similar to what Katanin does in the spindle). Also during spindle compression experiments, it was shown that the resulting increase in spindle length was due to cessation of microtubule minus-end depolymerization at the poles, without compromising poleward flux velocities (Dumont and Mitchison, Curr Biol, 2009). I suggest that the authors revise their nomenclature to reflect these clarifications. Alternatively, the authors could measure true poleward flux velocities in the different conditions, taking chromosomes at the equator as reference point. A possible feedback between minus-end depolymerization and the rate of flux (or plus-end polymerization) cannot be excluded. It would also help to characterize how spindles in WDR62 depleted cells become buckled and distorced, e.g. by live-cell imaging from the onset of mitosis.

2- A related point is that the authors are reporting "flux" velocities of aprox. 1 micrometer/min for human cells, which is 2x higher than reference values for flux abundant in the literature. This is another reason to revise their methodology to determine flux or, instead, explain the observed differences.

3- If the effects caused by WDR62 depletion essentially reflect a regulatory role over Katanin's severing activity at spindle poles, it is difficult to reconcile how only WDR62 depletion causes wide metaphase plates that move asynchronously in anaphase, based on the proposed model. Previous work in Drosophila cells suggested that Katanin plays a role in microtubule plus-end

depolymerization specifically during anaphase (Zhang, Sharp, JCB, 2007). Could it be that WDR62 or Katanin play pole-independent roles? For instance, how does the double WDR62+Katanin depletion compares to individual phenotypes? Which one stands out? Or how does a partial Katanin depletion (by RNAi) compare with WDR62 depletion that causes 70% reduction of Katanin at spindle poles? Could the authors track and quantify a direct MT plus-end polymerization marker, such as EB1, in both Katanin and WDR62 depleted cells?

4- Overall spindle architecture after perturbation of WDR62 or Katanin is difficult to assess with SiR-tubulin, which labels only stable microtubules. What is the impact of WDR62 or Katanin depletions on astral microtubules and could this explain phenotypic differences?

5- I find it somewhat speculative (I mean, beyond what would be reasonably acceptable for a model), that the proposed model of chromosome positioning in the subsequent interphase nuclei is not directly supported by data. This becomes particularly relevant given the absence of a clear chromosome segregatio phenotype in the WDR62 KO. Could the authors provide some measure of interphase nuclei architecture and/or chromosome distribution (FISH/Chromosome painting?) after WDR62 depletion?

Minor issues:
1- Introduction, page 3: "Kinetochore-fibers also constantly incorporate new tubulin dimers". This is not correct. As shown by Cameron, Salmon and co-workers (Cameron et al., JCB, 2006), when vertebrate kinetochores undergo poleward motion during metaphase oscillations, there is actually depolymerization at kinetochore microtubule plus-ends (i.e. pac-man activity), while the corresponding microtubules continue to undergo poleward flux (see also major point 1).

2- Results, page 10: "leads to buckling microtubules that generate an outward pushing force on the mitotic spindle". It would make more sense that microtubule buckling was instead generated by outward pushing forces (i.e. poleward flux) on the mitotic spindle.

3- Subsection title, page 11: "END" after "plus" is missing from "kinetochore-microtubule plus dynamics".

4- Results, page 12: the argument that WDR62 depletion allowed a more rigorous test of the role of flux in chromosome segregation because it only affected microtubule minus-end depolymerization is not a strong one, for the reasons explained in major point 1.

5- There are a few typos throughout the text that should be fixed.

Helder Maiato

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

The article by Guerrero and colleagues analysis the cellular consequences of WDR62 depletion knock out. WDR62 is microtubule (MT) binding protein with debatable functions in terms of the mitotic spindle. Unfortunately, many past studies have generated conflicting data that do not allow for an overall and comprehensive understanding of its function. This is rather important since mutations in the WDR62 gene are associated with MCPH, a neuro-developmental disorder characterised by a reduction in brain size at birth, where body size remains largely unaltered. Any

quality study addressing the functions of WD62 and the consequences of removing WDR62 is quiet important for the field and it seems to me that this study fall exactly in this category. The authors analysed either in RPE1 or in HeLa cells the consequences of WDR62 removal. They provide sound evidence that spindles that lack WDR62 are stabilized, form abnormal shapes, which might impact chromosome segregation errors. They also describe an important function of WDR62 in the recruitment of Katanin, a MT severing enzyme.
Overall the study is quite interesting. I am very positive about it and recommend its publication after revision. In particular I think the chromosome segregation errors associated with WDR62 loss deserve more attention and I have a few comments related with controls and with the accompanying paper.

1) This is just related with the format. In certain figures, we can see right away which cell type and depletion/knock-out method was used. In others, no. Can the authors just specify in each panel and graph which cell line /method they use? This will facilitate comprehension.

2) Are the siRNA and the KO rescued, in terms of phenotype? This is essential. IT will be also important to understand why the astral MT phenotype and number of centrosomes described in the Huang paper is different from what is described here.

3) In my opinion the results shown in Fig 2F are not given enough importance and well discussed in the text. If I understand correctly, these were obtained in HeLa cells. Can the authors repeat it using the KO RPE-1? According to the authors, these results show that WDR62 is not required for centriole duplication, which is consistent with certain past studies, but not with others. Strengthening this point will be really important for the field and to clarify the specificity of WDR62 in mitotic spindle biology independently of centrosomes.

4) In terms of spindle MT stability after WDR62 KO/depletion. I am slightly confused. The authors find that MCAK levels are marginally decreased, while Katanin levels are highly decreased, yes? They decrease Katanin levels in a series of experiences but these do not impact certain parameters such as velocity of chromosome movement or metaphase plate width, while WDR62 depletion does. So does this mean that the behaviours described here are not dependent at all on MT stabilization? I though this was confusing and maybe deserves clearer description and explanation?

5) The PA-GFP data is difficult to analyse. I am sure the authors are right, but the pictures of the spindle are difficult to interpret, most likely because the fluorescence is spreading to the poles of the spindle. In the Huang paper this is more easy to ascertain. Can they mark, with an arrow the position of the PA line in each panel? Maybe also showing these panels in black and white will be better? Or include movies.

6) The wavy spindles, typical of WDR62 depletion, I think they deserve more attention. Can the authors film these cells with sir-tubulin or any other MT marker that allows to characterise when this wavy behaviour is established? I think this will be quite important. Also, how do the authors explain the bridges?

7) Are the spindles that are wavy, the ones where chromosomes mis-segregate? If the authors want to defend that the defect in chromosome segregation comes from their broad alignment at the metaphase, they need to show it. And ideally to rescue it. Can this be rescued by MT destabilization (even if only partially) or even better increased minus-end depolymerisation? I think this is essential to prove the model.

8) This is only a minor point. The authors almost apologize by not seeing defects in spindle orientation. They should not. Also they might want to refer to the study from Insolera where removal of centrosomes from neuronal progenitors in mice did not result in spindle positioning defects.

9) On the causes of MCPH, the authors mentioned the Gogendeau and Marthiens papers as describing premature differentiation or apoptosis of neuronal progenitors. They are right, but in any case this was in response to aneuploidy generation, which I think goes exactly with what they are describing here. Maybe they should mention it?

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

The authors study the role of WDR62 for katanin recruitment to spindle poles and for the generation of microtubule flux in mitosis, using Hela and RPE1 cells. They find that WDR62 localizes to microtubules in the spindle pole region, and not to centrosomes, that it contributes to recruit katanin (and MCAK) to spindle poles, that it promotes microtubule flux and regular chromosome segregation in anaphase. The experiments are carefully performed and well documented. Several discrepancies reported in the literature are clarified and some interesting conceptual conclusions are drawn, the most interesting perhaps being a proposed mechanical uncoupling between microtubule plus end dynamics at kinetochores and microtubule minus end dynamics at spindle poles.

The concerns of this reviewer relate mostly to the logic underlying some conclusions given the presented experimental data and concern the presentation of some results/conclusions

1. Lines 126/132: wording: Is it justified to conclude that WDR62 localizes to microtubule "minus ends". This may be misunderstood. Do the authors really visualise minus ends and then observe co-localization? How close to minus ends does WDR62 localize? The data cannot answer this. It might be more appropriate to claim localization to microtubules in the spindle pole region (in contrast to centrosomes).

2. The authors construct knockout cells. It would be appropriate to demonstrate at the genomic and protein level that the knockout was successful for both alleles (and add the data to a Suppl. Fig.)

3. Do WDR62 knockout Hela cells show normal cell division times/propagate normally? Is their phenotype rather a mild one?

4. The authors re-investigate several previous results and thereby clean up the literature which is commendable. They refrain however from checking if the reported phenotype of a WDR62 depletion on astral microtubules is confirmed. What do they observe in their data? An effect on astral microtubules would be expected to be indirect given the reported localization of WDR62.

5. WDR62 depletion leads to 70% less katanin and 37% less MCAK at spindle poles. Which of the two reductions is more important for the effect on microtubule stability/dynamics?

6. Expression of exogenous WDR62 rescues the localisation defect (line 181). Does it also recue the

effect on microtubule dynamics? Can both katanin and MCAK also rescue the effect on dynamics/stability? Which one is more potent? Or do they maybe even need each other?

7. ASPM depletion leads to a 48% increase of WDR62 localization to poles. This seems to contradict the conclusion that they are "recruited independently". Instead, they seem to compete. Katanin depletion causes a 52% reduction of WDR62 and vice versa, they seem to cooperate. What happens to ASPM when katanin is depleted? What happens to MCAK when katanis is depleted? This seems to be important to know when assigning functions to particular proteins based on phenotypes caused by depletions, particularly in a situation where complex dependencies appear to exist.

8. Why is NUMA considered as marker for the density of minus ends? And implicitly being assumed to localize independent of WDR62 and katanin? A different logic compared to the other experiments is applied here. What if localization of NUMA to microtubules depends on WDR62? Why can this be excluded?

9. Quite interestingly, the authors conclude that poles and kinetochores are mechanically uncoupled. But can they really conclude this based on their observations? The main argument is that when flux is slowed down, then kinetochore dynamics are unaffected. But some other spindle features change (e.g. bendy microtubules). Could these features cause a feedback, based on mechanical coupling and lead to seemingly independent kinetochore and pole dynamics?

10. Points for the discussion:
a. The authors tend to conclude that the control of flux is functionally important for chromosome segregation, but they also conclude that kinetochore fiber stability is affected by WDR62. Could this be functionally more important than flux speed?
b. Why does WDR62 depletion lead to wider metaphase plates, but katanin depletion does not? Is this due to MCAK co-depletion?
c. What drives the remaining flux when WDR62 or katanin are depleted?

Minor points:
11. Method: how were photoactivated microtubules tracked for flux speed determination?
12. Statistics: no errors reported in Fig. 3I.
13. Space bar in Fig. 5a is missing
14. Language: Line 155: 'slow-kinetic' microtubules: what is meant here?

Point-by-point rebuttal letter:

First, we would like to thank all three reviewers for their appreciation of our work and their constructive comments. Here is our response to all their specific points:

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

The work by Guerreiro et al., investigates the role of WDR62, a protein whose gene is often mutated in microcephaly, in mitotic spindle dynamics and chromosome segregation. The authors convincingly show that WDR62 localizes to spindle microtubule minus-ends, but not centrosomes, independently of ASPM, another protein implicated in microcephaly. Mechanistically, the authors show that WDR62 is required to recruit the microtubule-severing enzyme Katanin to spindle microtubule minus-ends. Functional studies in cells revealed that both WDR62 and Katanin are required for microtubule minus-end depolymerization, which is essential to regulate normal spindle length and architecture (long microtubules buckle without WDR62 or Katanin). Surprisingly, both WDR62 and Katanin promote spindle microtubule turnover, without significantly affecting kinetochore microtubule-plus-end dynamics, as indirectly inferred by kinetochore tracking in living cells. In addition, loss of WDR62, but not Katanin, resulted in wider metaphase plates and asynchronous chromosome segregation in anaphase, without any evident aneuploidy. The authors propose a model in which asynchronous chromosome segregation in anaphase might have implications for the genesis of microcephaly by affecting chromosome positioning in the subsequent interphase nuclei. Overall, the study is technically well performed and well controlled and offers a fundamental cell biological explanation that might be of clinical relevance to microcephaly-associated disorders, and thus of interest to a wide audience.

Major issues:
1- There are a few inconsistencies in the data or its interpretation that are worth clarifying. For instance, the authors confuse the rate of microtubule minus-end depolymerization with the rate of poleward flux. This is not uncommon and must be clarified. Flux is the poleward motion of an entire microtubule relative to a fixed reference point within the spindle (e.g. the metaphase plate). What the authors have measured was the velocity of a fluorescent mark within a half-spindle relative to the corresponding pole - i.e. they measured the velocity of microtubule minus-end depolymerization, but then refer to these different phenomena interchangeably throughout the manuscript. However, as it is evident from the examples shown in figure 4G, the photoactivated mark in either perturbation is still moving at apparently normal velocity relative to the spindle equator (i.e. they flux), as defined from SiR-DNA. If spindle length is kept constant, the two velocities should in principle match, but it seems that over time (due to incorporation of new tubulin at microtubule plus-ends and microtubule poleward transport) spindle microtubules grow more than usual and buckle, because they cannot undergo minus-end depolymerization in response to poleward flux. Indeed, these two phenomena were able to be experimentally separated, as spindle microtubules continued to flux, without minus-end depolymerization after laser-induced generation of new microtubule minus-ends (Matos et al., JCB, 2009; similar to what Katanin does in the spindle). Also during spindle compression experiments, it was shown that the resulting increase in spindle length was due to cessation of microtubule minus-end depolymerization at the poles, without compromising poleward flux velocities (Dumont and Mitchison, Curr Biol, 2009). I suggest that the authors revise their nomenclature to reflect these clarifications. Alternatively, the authors could measure true poleward flux velocities in the different conditions, taking chromosomes at the equator as reference point. A possible feedback between minus-end depolymerization and the rate of flux (or plus-end polymerization) cannot be excluded. It would also

help to characterize how spindles in WDR62 depleted cells become buckled and distorced, e.g. by live-cell imaging from the onset of mitosis.

We thank the reviewer for these in-depth comments. Indeed, if the spindle grows due to buckling microtubules during the photoactivation experiments, then minus-end depolymerization and poleward microtubule flux rates are not equivalent. However, we think that this condition is not present due to the following three reasons:

1. When re-analyzing pole-to-pole distance over the entire duration of our photoactivation experiment, we found no increase in spindle length, indicating that in metaphase, MG132-arrested cells have reached a steady-state in spindle length (new supplementary Fig. S3C).
2. When we re-analyzed flux rates using the metaphase plate position as a reference point we found the same results; a decrease in flux rates of about 50%, which is logic since the spindle length was constant during the time of the experiment.
3. Third, by analyzing our movies for buckling microtubules and correlating this behavior with possible lagging chromosomes in anaphase, we found that only half of the WDR62-depleted cells displayed buckling microtubules, yet flux rate is consistently reduced in all WDR62-depleted cells, indicating that microtubule buckling and reduced flux rate are independent (new supplementary Fig. S5D)

Given that spindle length is constant under those experimental conditions, we stand by our conclusion that WDR62 loss is associated with a 50% reduction in poleward microtubule flux rates, and we infer a corresponding 50% reduction in microtubule minus-end depolymerization. Nevertheless, we now explicitly state this reasoning in our manuscript to avoid any misunderstanding.

2- A related point is that the authors are reporting "flux" velocities of aprox. 1 micrometer/min for human cells, which is 2x higher than reference values for flux abundant in the literature. This is another reason to revise their methodology to determine flux or, instead, explain the observed differences.

Here, we report that our RPE1 PAGFP-tubulin cells have a flux velocity in the order of 0.9 micrometer/min. This is very similar to the flux rate observed by the McAinsh group with the exact same cell line in 2009 in our common publication in 2009 (0.85 micrometer/min; Toso et al., JCB, 2009), and in the same order of magnitude as the flux rate reported by the Tolic group for RPE1 cells (1.14 micrometer/min) in their Bioarchive manuscript (https://doi.org/10.1101/2020.12.30.424837). It is also in the same range as what we reported for HeLa cells (0.94 micrometer/min; Mchedlishvili et al., JCS, 2012), but faster than what has been reported for U2OS cells (median of 0.6 micrometer/min), which fits with our internal observations in the laboratory, as we also found that RPE1 cells flux consistently faster than U2OS cells. We therefore feel confident about our measurements, given that they were reproduced by other laboratories.

3- If the effects caused by WDR62 depletion essentially reflect a regulatory role over Katanin's severing activity at spindle poles, it is difficult to reconcile how only WDR62 depletion causes wide metaphase plates that move asynchronously in anaphase, based on the proposed model. Previous work in Drosophila cells suggested that Katanin plays a role in microtubule plus-end depolymerization specifically during anaphase (Zhang, Sharp, JCB, 2007). Could it be that WDR62 or Katanin play pole-independent roles? For instance, how does the double WDR62+Katanin depletion compares to individual phenotypes? Which one stands out? Or how does a partial Katanin depletion (by RNAi) compare with WDR62 depletion that causes 70% reduction of Katanin at spindle poles? Could the

authors track and quantify a direct MT plus-end polymerization marker, such as EB1, in both Katanin and WDR62 depleted cells?

A previous publication in drosophila cells had indeed found that Katanin's activity during mitosis was strictly restricted to anaphase. This is not what we found in human cells, independently of its association with WDR62. Indeed, our experiments indicate that Katanin depletion is associated with an increase in microtubule stability in metaphase, reduced poleward microtubule flux and slightly higher pole-to-pole distances. This indicates that in human cells Katanin has a pre-anaphase role. Based on its localization and the effects seen in WDR62-depleted cells, we conclude that most of these functions are controlled by a pole-based Katanin pool. Nevertheless, our experiments also indicate that Katanin has others, non-WDR62 related functions, that are most likely not linked to its pole-based pool. In particular we observe a reduced Anaphase A speed concomitant with a faster anaphase B speed. While the first aspect most likely reflects the known role of Katanin in the Pacman mechanism (Zhang et al., 2007), the second might reflect a role of Katanin at the midzone, two results which we know discuss explicitly.

4- Overall spindle architecture after perturbation of WDR62 or Katanin is difficult to assess with SiR-tubulin, which labels only stable microtubules. What is the impact of WDR62 or Katanin depletions on astral microtubules and could this explain phenotypic differences?

We thank the reviewer for this suggestion, and we have now quantified the density of astral microtubules in control, WDR62-depleted and Katanin-depleted cells. As shown in the new Figure 4A and B, both WDR62 and Katanin depletion led to a reduction in astral microtubule density. Since neither of the two reside at astral microtubules, as far as we can see, we suspect that this result is an indirect effect due to a higher proportion of tubulin present on spindle microtubules, a hypothesis we state in the revised discussion.

5- I find it somewhat speculative (I mean, beyond what would be reasonably acceptable for a model), that the proposed model of chromosome positioning in the subsequent interphase nuclei is not directly supported by data. This becomes particularly relevant given the absence of a clear chromosome segregatio phenotype in the WDR62 KO. Could the authors provide some measure of interphase nuclei architecture and/or chromosome distribution (FISH/Chromosome painting?) after WDR62 depletion?

We do appreciate the reviewer's concern and acknowledge that our model implied more than we can support with our current data. Unfortunately, testing whether WDR62 depletion leads to a statistically significant change in nuclear architecture at the single cell level is beyond the scope of a manuscript revision. Nevertheless, to emphasize that our speculation is just that, we now also propose another alternative, i.e. that the lagging chromosomes seen in WDR62-depleted cell could result in DNA damage due to an imperfect nuclear envelope reformation. We cite published reports supporting both propositions, and we emphasize in our discussion that they represent just that, propositions.

Minor issues:
1- Introduction, page 3: "Kinetochore-fibers also constantly incorporate new tubulin dimers". This is not correct. As shown by Cameron, Salmon and co-workers (Cameron et al., JCB, 2006), when vertebrate kinetochores undergo poleward motion during metaphase oscillations, there is actually depolymerization at kinetochore microtubule plus-ends (i.e. pac-man activity), while the corresponding microtubules continue to undergo poleward flux (see also major point 1).

We have modified the sentence to "*At the same time microtubules within k-fibers undergo a conveyor-belt like movement called poleward microtubule flux that is driven by microtubule motors acting on kinetochore- and non-kinetochore microtubules, which over time results in new tubulin dimer incorporation at their plus-ends concomitant with tubulin dimer removal at minus-ends*" to better reflect the fact that k-fibers are not **constantly** incorporating new tubulin dimers.

2- Results, page 10: "leads to buckling microtubules that generate an outward pushing force on the mitotic spindle". It would make more sense that microtubule buckling was instead generated by outward pushing forces (i.e. poleward flux) on the mitotic spindle.

We now state: *"we often observed microtubule buckling in the bipolar spindle, implying a compression of microtubules as they push against the mitotic spindle (…). Overall, (…)but rather leads to buckling microtubules that arise due to the resistance of the mitotic spindle to outward pushing forces*", but emphasize that both formulations are the two faces of the same coin. By actio = reactio the buckling microtubules reflect a steady state of outward pushing forces and a resistance of the mitotic spindle to those same forces, resulting in microtubule compression.

3- Subsection title, page 11: "END" after "plus" is missing from "kinetochore-microtubule plus dynamics".

We have corrected and thank the reviewer

4- Results, page 12: the argument that WDR62 depletion allowed a more rigorous test of the role of flux in chromosome segregation because it only affected microtubule minus-end depolymerization is not a strong one, for the reasons explained in major point 1.

As stated under point 1, our results indicate that spindle length is constant during our flux measurements, and we therefore feel that our conclusions are supported by the data.

5- There are a few typos throughout the text that should be fixed.

We have tried our best to find and correct them.

Helder Maiato

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

The article by Guerrero and colleagues analysis the cellular consequences of WDR62 depletion knock out. WDR62 is microtubule (MT) binding protein with debatable functions in terms of the mitotic spindle. Unfortunately, many past studies have generated conflicting data that do not allow for an overall and comprehensive understanding of its function. This is rather important since mutations in the WDR62 gene are associated with MCPH, a neuro-developmental disorder characterised by a reduction in brain size at birth, where body size remains largely unaltered. Any quality study addressing the functions of WD62 and the consequences of removing WDR62 is quiet important for the field and it seems to me that this study fall exactly in this category. The authors analysed either in RPE1 or in HeLa cells the consequences of WDR62 removal. They provide sound evidence that spindles that lack WDR62 are stabilized, form abnormal shapes, which might impact chromosome segregation errors. They also describe an important function of WDR62 in the recruitment of Katanin, a MT severing enzyme.
Overall the study is quite interesting. I am very positive about it and recommend its publication after revision. In particular I think the chromosome segregation errors associated with WDR62 loss deserve more attention and I have a few comments related with controls and with the accompanying paper.

1) This is just related with the format. In certain figures, we can see right away which cell type and depletion/knock-out method was used. In others, no. Can the authors just specify in each panel and graph which cell line /method they use? This will facilitate comprehension.

We thank the reviewer for this comment and have now added the cell line name to all the relevant panels.

2) Are the siRNA and the KO rescued, in terms of phenotype? This is essential. IT will be also important to understand why the astral MT phenotype and number of centrosomes described in the Huang paper is different from what is described here.

As suggested by the reviewer we have now extended our rescue experiments. We now show in RPE1 cells, as we were previously showing in HeLa cells, that exogenous WDR62-eGFP expression rescues Katanin recruitment to spindle poles. These rescue experiments required the creation of a stable RPE1 WDR62-eGFP cell line, as transient transfection of a high expression plasmid was toxic to RPE1 cells. Moreover, we demonstrate that this construct also rescues the WDR62 depletion phenotype in terms of spindle microtubule stability (cold-stable assay). We were, however, not able to use this cell line to perform a rescue experiment in terms of lagging chromosomes during anaphase, as introduction of WDR62-eGFP led to lagging chromosomes in RPE1 cells in about 35% of the cells. To address the issue of potential off target effects in an alternative manner, we now analyzed the number of lagging chromosomes in two CRISPR/Cas9 WDR62 KO cell lines (the one from our laboratory and the one from Kai Jiang's laboratory). Our results, which are now based on a much larger sample size than in the original manuscript and a second KO cell line, confirm that WDR62 deletion leads to a significant 13% increase in the rate of cells with lagging chromosomes when compared to the respective parental Hela cell line. We conclude that WDR62 loss or depletion leads to lagging chromosomes after 3 different types of loss-of-function experiments: 1 siRNA depletion in RPE1 cell line, and 2 independent CRISPR/Cas9 KO cell lines, thus excluding a potential off target effect.

Second, as suggested by several reviewers we also quantified the density of astral microtubules and find consistent with the Huang manuscript that WDR62 leads to a mild, but reproducible reduction in the

density of astral microtubules. We also find that Katanin depletion results in a similar but slightly stronger reduction in the density of astral microtubules.
Finally, we also tested whether not only the long-term loss (KO) of WDR62 in Hela cells, but also the acute siRNA-mediated depletion of WDR62 in RPE1 cells would affect centrosome duplication. We found in both cases no changes in centrosome numbers, confirming that WDR62 has no major effect on centrosome duplication.

3) In my opinion the results shown in Fig 2F are not given enough importance and well discussed in the text. If I understand correctly, these were obtained in HeLa cells. Can the authors repeat it using the KO RPE-1? According to the authors, these results show that WDR62 is not required for centriole duplication, which is consistent with certain past studies, but not with others. Strengthening this point will be really important for the field and to clarify the specificity of WDR62 in mitotic spindle biology independently of centrosomes.

See our response under comment 2, where we confirm that WDR62 depletion in RPE1 has also no effect on centrosome duplication.

4) In terms of spindle MT stability after WDR62 KO/depletion. I am slightly confused. The authors find that MCAK levels are marginally decreased, while Katanin levels are highly decreased, yes? They decrease Katanin levels in a series of experiences but these do not impact certain parameters such as velocity of chromosome movement or metaphase plate width, while WDR62 depletion does. So does this mean that the behaviours described here are not dependent at all on MT stabilization? I though this was confusing and maybe deserves clearer description and explanation?

We thank the reviewer on both points. First, we re-analyzed the contribution of WDR62 depletion on the levels of MCAK at spindle poles. Our re-investigation led us to realize that the control siRNA that we originally used for the fixed cell imaging experiments had an off-target effect, resulting in increased MCAK levels at spindle poles when compared to un-transfected cells. We have now replaced this control siRNA with an alternative control siRNA set for all fixed cell experiments, verified that it does not change MCAK levels (and other proteins) in comparison to untransfected cells, and ultimately found that WDR62 does not affect the spindle pole bound pool of MCAK, making our analysis easier.

We also agree that we did not sufficiently explain why WDR62 and Katanin depletion resemble each other for many mitotic parameters, but not for all. We now discuss these differences in detail in the discussion, as it points to other pools of Katanin acting at microtubule plus-end, consistent with the literature in the field (e.g. Zhang et al., JCB 2007)

5) The PA-GFP data is difficult to analyse. I am sure the authors are right, but the pictures of the spindle are difficult to interpret, most likely because the fluorescence is spreading to the poles of the spindle. In the Huang paper this is more easy to ascertain. Can they mark, with an arrow the position of the PA line in each panel? Maybe also showing these panels in black and white will be better? Or include movies.

We thank the reviewer for this comment, which we will use to lobby our bioimaging platform to upgrade our photo-activation confocal microscope. We tried our best, but we are aware that our images are not optimal. Nevertheless, to better illustrate our measurements we have, as suggested, put a mark both on the site of photoactivation (yellow dotted line) and the location of the fluxing signal (white arrow) in our images. Moreover, we included as suggested the movies as supplementary material.

6) The wavy spindles, typical of WDR62 depletion, I think they deserve more attention. Can the authors film these cells with sir-tubulin or any other MT marker that allows to characterise when this wavy behaviour is established? I think this will be quite important. Also, how do the authors explain the bridges?
7) Are the spindles that are wavy, the ones where chromosomes mis-segregate? If the authors want to defend that the defect in chromosome segregation comes from their broad alignment at the metaphase, they need to show it. And ideally to rescue it. Can this be rescued by MT destabilization (even if only partially) or even better increased minus-end depolymerisation? I think this is essential to prove the model.

Response to points 6 and 7: As suggested by the reviewer we have further characterized the buckling microtubules in relationship to chromosome mis-segregation by live cell imaging. This revealed that about 50% of the spindle contained buckling microtubules during metaphase, but that the microtubule buckling was not a predictor for lagging chromosomes, rather the opposite (new Supplementary Figure S5D). This suggests as we now state in the discussion, that buckling microtubules and lagging chromosomes in anaphase are not causally linked, but rather two independent consequences of the same original phenotype: a reduction in efficient microtubule minus-end depolymerization.

8) This is only a minor point. The authors almost apologize by not seeing defects in spindle orientation. They should not. Also they might want to refer to the study from Insolera where removal of centrosomes from neuronal progenitors in mice did not result in spindle positioning defects.

We thank the reviewer for suggesting this reference, which is indeed in line with our results.

9) On the causes of MCPH, the authors mentioned the Gogendeau and Marthiens papers as describing premature differentiation or apoptosis of neuronal progenitors. They are right, but in any case this was in response to aneuploidy generation, which I think goes exactly with what they are describing here. Maybe they should mention it?

We partially disagree, as our results indicate that WDR62 loss only very rarely leads to loss/gain of entire chromosomes, but rather leads to more subtle chromosome segregation defects, which we now state more explicitly in our discussion.

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

The authors study the role of WDR62 for katanin recruitment to spindle poles and for the generation of microtubule flux in mitosis, using Hela and RPE1 cells. They find that WDR62 localizes to microtubules in the spindle pole region, and not to centrosomes, that it contributes to recruit katanin (and MCAK) to spindle poles, that it promotes microtubule flux and regular chromosome segregation in anaphase. The experiments are carefully performed and well documented. Several discrepancies reported in the literature are clarified and some interesting conceptual conclusions are drawn, the most interesting perhaps being a proposed mechanical uncoupling between microtubule plus end dynamics at kinetochores and microtubule minus end dynamics at spindle poles.

The concerns of this reviewer relate mostly to the logic underlying some conclusions given the presented experimental data and concern the presentation of some results/conclusions

1. *Lines 126/132: wording: Is it justified to conclude that WDR62 localizes to microtubule "minus ends". This may be misunderstood. Do the authors really visualise minus ends and then observe co-localization? How close to minus ends does WDR62 localize? The data cannot answer this. It might be more appropriate to claim localization to microtubules in the spindle pole region (in contrast to centrosomes).*

We agree with reviewer 3 that our data are not sufficient to prove with 100% that WDR62 is precisely bound to microtubule minus-ends, as binding assays with recombinant protein and single microtubules would be necessary for such a statement. Nevertheless, we believe that our data in Figure 1C and D show that WDR62 accumulates on the terminal part of spindle pole microtubules, which is the localization that one would expect from a minus-end binding protein. We therefore state in the results that WDR62 localizes to the part of the spindle microtubules that terminate at spindle poles, in the vicinity or possibly at microtubule minus-ends, which we think reflects our results fairly.

2. *The authors construct knockout cells. It would be appropriate to demonstrate at the genomic and protein level that the knockout was successful for both alleles (and add the data to a Suppl. Fig.)*

We agree with the reviewer, but we emphasize that the information was already present in the original manuscript. Supplementary Figure 1A shows the genomic sequence of the WDR62 gene of the parental and KO cell line, indicating that CRISPR/Cas9 has led to a 11-nucleotide deletion on both alleles. This genomic information is complemented at the protein level by quantitative immunofluorescence (which we find to be more sensitive that immunoblotting), which indicates a complete absence of the WD62 protein.

3. *Do WDR62 knockout Hela cells show normal cell division times/propagate normally? Is their phenotype rather a mild one?*

WDR62 KO cells have near-normal mitotic timings as indicated in Supplementary Figure 5A and display no obvious cell proliferation defects. This is consistent with the CRISPR/Cas9 library dataset of the Broad Institute (https://depmap.org/portal/gene/WDR62?tab=overview), which indicates that loss of WDR62 in over 800 cell lines, leads only to a mild fitness decrease. This is also consistent with our extensive characterization of chromosome segregation efficacy, as we find a mild but consistent increase in the number of lagging chromosomes (New Supplementary Figures S5B and C).

4. *The authors re-investigate several previous results and thereby clean up the literature which is commendable. They refrain however from checking if the reported phenotype of a WDR62 depletion on astral microtubules is confirmed. What do they observe in their data? An effect on astral microtubules would be expected to be indirect given the reported localization of WDR62.*

As suggested by reviewer 2, we now also quantified the effects of WDR62- and Katanin-depletion on astral microtubules. Consistent with previous literature, we find that depletion of either gene leads to a reduction in the density of astral microtubules, particularly after the depletion of Katanin (see new Fig. 4A and B). Given the localization of WDR62, we agree that this can only be an indirect effect, and we now propose in the discussion that over-stabilization of kinetochore-microtubules in the mitotic spindle at constant tubulin concentrations might lead to a corresponding reduction in the density of astral microtubules. We also speculate as to why this partial reduction in astral microtubules does not lead to spindle orientation defects.

5. *WDR62 depletion leads to 70% less katanin and 37% less MCAK at spindle poles. Which of the two reductions is more important for the effect on microtubule stability/dynamics?*

As state under point 4 of reviewer 2, our revision work revealed that the relative MCAK reduction in WDR62-depleted cell, was due to an off-target effect of the control siRNA, which led to a MCAK increase at spindle poles. We have now repeated all the experiments with a novel control siRNA, which does not change MCAK levels, when compared to untransfected cells. This allows us to conclude that WDR62 depletion does not affect MCAK levels at spindle poles. We apologize for this confusion and thank the reviewer for having forced us to look at these experiments more carefully.

6. *Expression of exogenous WDR62 rescues the localisation defect (line 181). Does it also recue the effect on microtubule dynamics? Can both katanin and MCAK also rescue the effect on dynamics/stability? Which one is more potent? Or do they maybe even need each other?*

First, as we now show in our rescue experiments, exogenous WDR62 not only rescues Katanin recruitment, it also restores microtubule (in)stability in the mitotic spindle (new Figure 2D and E). Second, we attempted to restore Katanin localization in WDR62-depleted cells by introducing a PACT-tagged Katanin construct in cells by transient and stable transfection; this attempt, however, failed, as we found only few transfected, sick-looking interphase cells and not a single mitotic cell. We presume that exogenous Katanin recruitment at centrosome in interphase is toxic, due to an excessive microtubule severing activity, and that a more subtle approach, that goes beyond this revision will be necessary to address this particular point.

7. *ASPM depletion leads to a 48% increase of WDR62 localization to poles. This seems to contradict the conclusion that they are "recruited independently". Instead, they seem to compete. Katanin depletion causes a 52% reduction of WDR62 and vice versa, they seem to cooperate. What happens to ASPM when katanin is depleted? What happens to MCAK when katanis is depleted? This seems to be important to know when assigning functions to particular proteins based on phenotypes caused by depletions, particularly in a situation where complex dependencies appear to exist.*

We agree that our formulation was imprecise, and we now state that WDR62 and ASPM do each not require the presence of the other protein to localize to spindle poles, indicating that they recruit Katanin via separate pathways, which we think is more precise. With regard to ASPM levels in Katanin, Jiang et al. 2017, has previously shown that Katanin-depletion also reduced ASPM levels. Finally, since we found

that WDR62-depletion does not affect MCAK levels at spindle poles, we did not quantify its level in Katanin-depleted cells.

8. *Why is NUMA considered as marker for the density of minus ends? And implicitly being assumed to localize independent of WDR62 and katanin? A different logic compared to the other experiments is applied here. What if localization of NUMA to microtubules depends on WDR62? Why can this be excluded?*

First, as our current citation (Elting et al., 2014) shows, NuMA is recruited to newly created microtubule-ends within seconds of their creation, indicating that it is a valid microtubule minus-end marker. Nevertheless, it is true, that we cannot conclude that Katanin and WDR62 depletion do not have a direct, minor effect on NuMA recruitment. Our aim, which we now state more explicitly (lines 203-207), was to show that the mutual changes seen after WDR62 or Katanin-depletion go beyond the simple reduction in microtubule minus-ends, and are therefore specific.

9. *Quite interestingly, the authors conclude that poles and kinetochores are mechanically uncoupled. But can they really conclude this based on their observations? The main argument is that when flux is slowed down, then kinetochore dynamics are unaffected. But some other spindle features change (e.g. bendy microtubules). Could these features cause a feedback, based on mechanical coupling and lead to seemingly independent kinetochore and pole dynamics?*

We agree with the reviewer that our statement was too general, and we are now more specific, in stating that minus-end and plus-end dynamics can be experimentally uncoupled, i.e. that it is possible to change one without affecting the other. This is not always the case, and we and others have previously observed that changes at one end can influence the behavior of the other end of the mitotic spindle, and future work beyond the current manuscript will be necessary to understand, when (and when not) the two ends of the mitotic spindle can be experimentally coupled.

10. Points for the discussion:
a. *The authors tend to conclude that the control of flux is functionally important for chromosome segregation, but they also conclude that kinetochore fiber stability is affected by WDR62. Could this be functionally more important than flux speed?*

As we now state more explicitly, our results suggest that the change in microtubule stability observed after WDR62 depletion is not sufficient to induce lagging chromosomes, or only provides a very minor contribution, as microtubule stability mainly affects the correction of erroneous (merotelic) kinetochore-microtubule attachments. Such attachments are the main source of lagging chromosomes after a monastrol release, and the fact that WDR62 depletion has only a minor effect on the number of lagging chromosomes after a monastrol release, would suggest that changes in microtubule stability are not the main contributor for the lagging chromosomes in WDR62-depleted cells.

b. *Why does WDR62 depletion lead to wider metaphase plates, but katanin depletion does not? Is this due to MCAK co-depletion?*

As stated in point 5, our new results with a corrected control siRNA show that WDR62 depletion does not affect MCAK levels at spindle poles, reason we think that WDR62 depletion mainly acts via the removal of Katanin from spindle poles.

c. What drives the remaining flux when WDR62 or katanin are depleted?

As we now write more explicitly (line 228-231), flux is driven mostly by microtubule motors and plus-end polymerization; it does however necessitate minus-end depolymerization, as otherwise microtubules will pile up against spindle poles. As we now more explicitly state, loss of katanin at spindle poles or complete loss of katanin reduces flux speeds by 50%, as minus-end depolymerization is now most likely only driven by members of the kinesin-13 family, whose localization is not affected by WDR62 depletion. We hypothesize that the reduction of minus-end depolymerization creates a jam due to the persistent activity of microtubule motors and plus-end polymerization that results in microtubule buckling in about 50% of the cells, and more generally a slow-down of flux rates.

Minor points:
11. Method: how were photoactivated microtubules tracked for flux speed determination?

As we now specify in the material and methods:

"Half-spindles were photo-activated with a 500 ms 405 nm laser pulse at 30-80% intensity depending on the PA-GFP-α-tubulin expression levels, using a 1 pixel-thick and 100 pixel-long ROI stretched across the spindle. Single focal planes were imaged every 20 s for 4 min. Photo-activated kinetochore-microtubule bundles were tracked for 80 s. By computing manually the mean distance between the photo-activation mark on kinetochore-microtubule bundles and the corresponding spindle poles at different time points, we calculated the mean displacement of the photoactivation mark over time. Note that since spindle length did not change during the recording period, the rate of progression towards the spindle pole and the rate of displacement away from the metaphase plate is equivalent".

12. Statistics: no errors reported in Fig. 3I.

There is indeed no error bar, since this result is only based on one experiment. The goal of this experiment was to demonstrate that overall Katanin levels were not strongly reduced in WDR62-depleted cells, not to make a precise quantification. We now specify in the Figure legend that the graph is built on a N=1.

13. Space bar in Fig. 5a is missing

We thank the reviewer for pointing out this error.

14. Language: Line 155: 'slow-kinetic' microtubules: what is meant here?

We now specify that SiR-tubulin only binds to microtubules with a turnover rate of more than 10s

May 7, 2021

RE: JCB Manuscript #202007171R

Prof. Patrick Meraldi
University of Geneva
Cell physiology and metabolism department
Centre Medical Universitaire Rue Michel Servet 1
Geneva 1211
Switzerland

Dear Prof. Meraldi,

Thank you for submitting your revised manuscript entitled "WDR62 localizes katanin at spindle poles to ensure synchronous chromosome segregation". We would be happy to publish your paper in JCB pending final revisions necessary to address minor text revision requests and 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.

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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 (both in the figure legend itself and 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) 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 (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

6) 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.

7) 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.).

8) 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.

9) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures and 10 videos. 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.

10) 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. It should begin with "First author name(s) et al..." to match our preferred style.

11) 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."

12) A separate author contribution section is required following the Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames. We encourage use of the CRediT nomenclature (https://casrai.org/credit/).

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.

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

Sincerely,

Arshad Desai, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology

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

The authors have done a great job clarifying and revising all the issues raised by the reviewers and I can now recommend publication of this study in JCB.

Helder Maiato

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

The article by Guerreiro and colleagues has improved substantially. The authors deserve to be congratulated on their very nice study. I think the article should be accepted by publication by JCB. I just have two minor comments. Both can be addressed just with a few more explanations in the text.

1) Related to the sentence and figures present in the article- Fig 4H for example- "Moreover, during the recording of the spindle decay assay, we often observed microtubule buckling in the bipolar spindle, implying a compression of microtubules as they push against the mitotic spindle.
When I read this sentence I interpreted right away that buckling was referring to astral MT and its impaired interaction with the cortex. Like when astral MTs reach the cortex and then bent back towards the cytoplasm. This is often the case for buckling descriptions. I know the word bucking can have slightly different meanings but maybe the authors can use just twist or fold or distort? Might avoid confusion..

2) The second point relates with the characterization of bridges and lagging chromosomes. I agree that the pictures shown in Fig6C show on the left bridges and in the middle a lagging chromosome. However, the picture on the right does not show a micronucleus. It does show a lagging chromosome that most likely will end up outside the main nucleus, but still. The frequency of micronuclei has to be established much later after nuclear envelope reformation. Since these events are so rare, I will just add them to the overall category of lagging chromosomes. However the question is, how do the authors explain bridges if they are depleting a spindle associated factor? They do not mention it in the text. This deserves an explanation, in particular because it is in absent in Ctrls. It might be that the authors have no explanation, and I also can acknowledge that the frequency is very low, but still, should be mentioned.

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

The authors have nicely addressed all concerns of this reviewer.

Point-by-point rebuttal letter:

We were very happy to read that all three reviewers appreciated our revision and that they now support publication of this study in *Journal of Cell Biology.* Here is how we have addressed the two minor points of reviewer 2:

1) Related to the sentence and figures present in the article- Fig 4H for example- "Moreover, during the recording of the spindle decay assay, we often observed microtubule buckling in the bipolar spindle, implying a compression of microtubules as they push against the mitotic spindle. When I read this sentence I interpreted right away that buckling was referring to astral MT and its impaired interaction with the cortex. Like when astral MTs reach the cortex and then bent back towards the cytoplasm. This is often the case for buckling descriptions. I know the word bucking can have slightly different meanings but maybe the authors can use just twist or fold or distort? Might avoid confusion..

We thank reviewer 2 for pointing out this potential mis-understanding. We would like to stick with the notion of buckling microtubules, as this implies a compression, while twist, fold or distort is not linked to a compression. Nevertheless, to be more specific we now state:

"Moreover, during the recording of the spindle decay assay, we often observed buckling ***of spindle microtubules between the two poles****, implying a compression of microtubules as they push against the mitotic spindle (Fig. 4H; Tolić et al., 2019)"*

We hope that this should resolve any possible misunderstanding.

2) The second point relates with the characterization of bridges and lagging chromosomes. I agree that the pictures shown in Fig6C show on the left bridges and in the middle a lagging chromosome. However, the picture on the right does not show a micronucleus. It does show a lagging chromosome that most likely will end up outside the main nucleus, but still. The frequency of micronuclei has to be established much later after nuclear envelope reformation. Since these events are so rare, I will just add them to the overall category of lagging chromosomes. However the question is, how do the authors explain bridges if they are depleting a spindle associated factor? They do not mention it in the text. This deserves an explanation, in particular because it is in absent in Ctrls. It might be that the authors have no explanation, and I also can acknowledge that the frequency is very low, but still, should be mentioned.

We fully agree concerning the labelling of the picture in Fig. 6C. and have now changed the labelling to "lagging that will result in a micronucleus", which is a more precise description. With regard to the chromosome bridges, we are now explicit in the text and explain that in contrast to lagging chromosomes their incidence was not significantly changed by WDR62 depletion (RPE1) or deletion (HeLa) and we now state the p-values to bolster that argument. Finally, with regard to the last point, we are in agreement with the reviewer, we counted the lagging chromosomes resulting in micronuclei to the general population of lagging chromosomes, but only labelled them in addition in our graph to emphasize their rare incidence.