Journal of Cell Science

MKLP2 functions in early mitosis to ensure proper chromosome congression

Morgan S. Schrock, Luke Scarberry, Benjamin R. Stromberg, Claire Sears, Adrian E. Torres, David Tallman, Lucas Krupinski, Arnab Chakravarti and Matthew K. Summers
DOI: 10.1242/jcs.259560

Editor: David Glover

Review timeline
Original submission: 10 November 2021
Editorial decision: 14 January 2022
First revision received: 3 May 2022
Accepted: 23 May 2022

Original submission

First decision letter

MS ID#: JOCES/2021/259560

MS TITLE: MKLP2 functions in early mitosis to ensure proper chromosome congression

AUTHORS: Morgan S Schrock, Luke Scarberry, Benjamin R Stromberg, Claire Sears, Adrian E Torres, David Tallman, Lucas Krupinski, Arnab Chakravarti, and Matthew K. Summers
ARTICLE TYPE: Short Report

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

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

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to

all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

Most of the prior studies of MKLP2 have focused on its function in cytokinesis. This makes sense because MKLP2 interacts with the CPC, localizing it to the midzone. This study is significant because of the finding that MKLP2 is required during metaphase for chromosome congression and alignment. These results are very exciting, revealing an previously unappreciated function for MKLP2. The authors use multiple approaches to deplete MKLP2 activity, including drug inhibition and siRNAs. They also observe similar defects with MKLP2 mutants. My main concern with this work is in the presentation of the results. In the text and Figures, there is often confusing language and lack of specifics, which make the manuscript unnecessarily challenging to read. These concerns are listed below.

Comments for the author

1) On using drugs and siRNA to inhibit or deplete MKLP2. On page 3, the Results begin with the results from siRNA treatment. This is abruptly followed by MKLP2i[1] and MKLP2[2], although he word “drug” is not mentioned until the end of the paragraph. A better transition from siRNA to drug treatment is needed with specific naming of each inhibitor. #1 = paprotrain, which is well known and should be specifically mentioned and referenced in the Results the first time the #1 nomenclature is used. Inhibitors 2 and 3 are referred to as “compounds” in the resources table and there is no reference for them. What are these?
2) The possibility is raised that the reason metaphase defects were not observed in previous drug studies is that they are observed with higher concentrations of inhibitor (eg. the metaphase functions require less MKLP2). It is not clear why these defects would not have been observed previously using siRNA treatment.
3) Pg 4, line 3 and beyond: The authors should use a better term than “pseudometaphase”. I don’t understand how this term accurately reflects a defective metaphase because of a congression/ alignment defect. Why not use standard terms like a congression or alignment defect?
4) While the incorporation of mutants is nice (pg 4), the authors should indicate whether these were resistant to the siRNA. I am guessing they are, otherwise, not sure how the experiment works.
5) Figure 2 or pg 4: While the methods indicate how “centromere distribution ratio” is calculated, a sentence or two defining this term in the results or figure legend would be helpful.
6) Pg 5 top: the authors conclude that the unstable KT-MT attachments are due to impairment of the error correction mechanism. This wording seems awkward. A defect in error correction, which leads to misaligned chromosomes, could lead to unstable attachments. That is, the errors are detected, leading to destabilization and metaphase arrest, but not corrected.
7) Pg 6: The authors connect the MKLP2 defect to AurkA activity, however, the authors should consider the possibility that this is indirect, or other factors could also be involved. They show that phospho-AurKB and AurkA are elevated, but for unknown reasons focus on AurkA. The experiment measures the effect of AurkA inhibition on cell cycle arrest. Thus, AurkA could be responsible for the arrest phenotype but not involved in error correction. No experiment conclusively rules out AurkB. The authors acknowledge this, but should also make it clear they have not directly linked AurkA to the error correction defect (although there is evidence for this in the literature). They do not show if AurkA is responsible for the increased pHEC1, which could depend on AurkB.
8) Figure 4 and chromosome instability. The FISH data is nice, showing MKLP2 associated aneuploidy. The results from the evaluation of cancer cell lines is not surprising. I would argue that the emphasis on cancer cells is misleading. Any cell experiencing CIN would then be extra sensitive to MKLP2 loss. This is confirmatory of other results in this paper.
9) The last paragraph in the conclusion should mention other possibilities in addition to AurkA, given the lack of data regarding the AurkB. Given the known interactions of MKLP2, a possible role for MKLP2-CPC interactions in metaphase should be discussed. Could the role of MKLP2 is bundling anti-parallel microtubules and its location in the center of the spindle be important for the observed metaphase defects.

10) The authors should cite any other work in other organisms suggesting Kinesin 6s have metaphase functions. For example, the Drosophila orthologue of MKLP2 is Subito, which has been shown to have defects in spindle organization and chromosome alignment and bi-orientation during metaphase I of meiosis.
11) In all figures and legends, makes sure to label the colors.
12) Figure 1: The siRNAs are not indicated in panel A; they are in panel B. The authors should list what siRNAs 06 - 09 are. Panel D: what is the GFP panel? In A and F, what is the difference between metaphase and mitotic index? Why are the graphs different styles?
13) Figure 2: As mentioned above, the term "psuedometaphase" is problematic. For example, how is it different than "abnormal mitosis". In the figure legend, the second "D" should be "E)" and "E)" should be "F)".

Reviewer 2

Advance summary and potential significance to field

The manuscript proposed by Schrock et al., entitled "MKLP2 functions in early mitosis to ensure proper chromosome congression" provides the interesting observation that MKLP2 might be required earlier in mitosis than previously thought. Indeed, the kinesin MKLP2 was well known to transport Aurora B kinase from the kinetochore to the spindle midzone in anaphase, but no function had been documented during chromosome congression.
Here, the authors use a more potent MKLP2 inhibitor (MKLP2i3), to document this phenotype, that they can also observe, although to a much milder extent, by down-regulating MKLP2 with some siRNA.

Comments for the author

Although interesting, this manuscript could be dramatically improved in the following ways:
1- Figure 2 intend to show that MKLP2 deficiency leads to chromosome misalignment in prometaphase. For clarity, the Fig.2D should include the control with the siMKLP2 alone, without rescue by any of the MKLP2 isoforms.
2- Since Figure 2 was intended to prove that the effect scored after MKLP2i3 addition is not due to an off-target effect, it would be useful if the authors could show in a new Figure 2C (the present one is confusing, and could be removed) some kind of rescue by WT MKLP2 upon addition of very low doses of inhibitor (could 5 nM of inhibitor be, at least partially, rescued by WT MKLP2 over-expression? If so, please score for the effect of inhibition without rescue at the same dose).
3- In Figure 3L, scoring the effect of alisertib alone is missing to conclude. Also the statistics should compare +/- alisertib and not 12h versus 18 hours.
4- Figure 4 has several caveats. In figure A, the "modal deviation" is not easy to apprehend. Neither is understandable the "gene effect" of MKLP2 RNAi or CRISPR KO using "publically available data". "DepMap, Broad (2019)" is a bit short as a method description! The authors should better explain what they did with the publically available data, and how reliable it is. Alternatively, they could repeat the experiment themselves in a controlled way.
5- In figure 4, the authors provide a model stating that because Aurora A is more phosphorylated upon MKLP2 inhibition, more CENP-E remains phosphorylated, but they do not document this hypothesis. Could the authors try to prove their model using the reagents used in Kim et al., Cell 2010?
6- Alternatively, could the authors propose hypothesis to understand how MKLP2 depletion/inhibition could enhance Aurora A and Aurora B activities?

Reviewer 3

Advance summary and potential significance to field

The manuscript by Schrock and colleagues examines the hitherto almost entirely unreported role for the kinesin MKLP2 in mitotic chromosome congression. Towards this end the authors employ chemical genetic and siRNA perturbations in conjunction with quantitative live and fixed cell imaging. They report that MKLP2 is needed for the efficient congression of chromosomes to the

metaphase plate and timely anaphase onset. An examination of putative mechanisms identified Aurora A kinase as a major player. When MKLP2 function is perturbed beyond threshold amounts Aurora A levels are increased at the centrosomes (along with centromere associated Aurora B kinase). Further examination revealed that disruption of MKLP2 led to syntellic malorientations and a loss of k-fibre stability.
These data add to our knowledge of chromosome dynamics and genomic stability. They provide phenomenological as well as mechanistic insight. They are therefore of interest to the field and merit publication.

Comments for the author

As presented the manuscript is largely clearly written and the data solid. There are a few aspects that should be addressed prior to publication.
1) It is unclear how the metaphase index (Fig 1A) was determined. Was this a quantification of cells with non-polar placed chromosomes or chromosomes in a defined size region at the spindle equator?
2) in my version of the figures, the images of the chromosomes in Fig 2A have aberrations. This may be a reproduction error or an artefact of the z-series projection method employed.
3) It would be useful to include in the text the average and std error duration that all treatments spent in metaphase. This is important because the individual control cells shown in Fig 1D and 2A differ markedly in their timing of anaphase onset at approx. 35 and 75 min, respectively. This is a large variation if I am interpreting the images correctly.
4) An underlying assumption is that the polar proximal chromosomes are syntellically maloriented. Was this attachment defect ever directly imaged?
5) Figure 3E is not needed. The mechanism of polar malorientation correction by Aurora A can be elaborated upon in Fig 4E and more thoroughly referenced within the text.
6) Aurora A has been previously implicated in chromosome biorientation, chromosome congression and oscillations along with syntelly correction. This should be referenced given the predominant role that Aurora A is serving.
7) The MKLP2 rigor mutants are expressed in an siMKLP2 background. It is presumed that these constructs are siRNA tolerant through sequence differences. This should be stated if so.
7) The work relies heavily on quantitative imaging. Yet in many sections of the methods the details of the quantitation are not given. More detailed procedures should be provided.

First revision

Author response to reviewers' comments

First, we want to thank all the reviewers for providing feedback that we believe has greatly improved the manuscript. The quality of the comments and suggestions reflects the time and thoughtfulness each reviewer dedicated to our study and we greatly appreciate your efforts. Below we respond to each comment specifically and indicate where changes have been made in the figures or text (highlighted gray within the revised manuscript). We also want to let the reviewers know that due to requests from all the reviewers to include more discussion and include additional experiments that would put us outside the character limit for the 'Report' format, we decided to submit this resubmission as a 'Research Article' per the suggestion of the editor.

Reviewer 1 Comments for the author
1. On using drugs and siRNA to inhibit or deplete MKLP2. On page 3, the Results begin with the results from siRNA treatment. This is abruptly followed by MKLP2i[1] and MKLP2[2], although he word "drug" is not mentioned until the end of the paragraph. A better transition from siRNA to drug treatment is needed with specific naming of each inhibitor. #1 = paprotrain, which is well known and should be specifically mentioned and referenced in the Results the first time the #1 nomenclature is used. Inhibitors 2 and 3 are referred to as "compounds" in the resources table and there is no reference for them. What are these?

Our goal is to make our work as clear as possible, therefore in addition to the references to the patents and publications for each inhibitor and explanation of our coding system (eg. MKLP2i1, MKLP2i2, MKLP2i3) in the last paragraph of the Introduction, we have added additional explanations to the beginning of the Results section (Results, 1st paragraph) and further clarified the associated references in the Key Resources Table.

2. The possibility is raised that the reason metaphase defects were not observed in previous drug studies is that they are observed with higher concentrations of inhibitor (eg. the metaphase functions require less MKLP2). It is not clear why these defects would not have been observed previously using siRNA treatment.

Thank you for the opportunity to clarify our thoughts on this. In order to meet the word count limit for the 'Report' manuscript format, we were limited on how much we could discuss this particular point. However, after noting that all reviewers wanted more discussion regarding various points of our findings and consulting with the Editor, we decided to resubmit the manuscript as a Research Article and therefore, have the space to expand on this statement. We address this question in the first two paragraphs of the Discussion.

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3. Pg 4, line 3 and beyond: The authors should use a better term than "pseudometaphase". I don't understand how this term accurately reflects a defective metaphase because of a congression/ alignment defect. Why not use standard terms like a congression or alignment defect?

We want to be as clear as possible and this is a fair point, therefore we replaced the 'pseudometaphase' phrase with 'congression defect' throughout the manuscript and figures (highlighted throughout).

4. While the incorporation of mutants is nice (pg 4), the authors should indicate whether these were resistant to the siRNA. I am guessing they are, otherwise, not sure how the experiment works.

This is an important point brought up by Reviewer 3 as well. We apologize for having left out this key information. The siRNAs were purchased from Dharmacon and are directed toward MKLP2 3'UTR. We clarified this in the manuscript in the Results (2nd paragraph) and Methods sections.

5. Figure 2 or pg 4: While the methods indicate how "centromere distribution ratio" is calculated, a sentence or two defining this term in the results or figure legend would be helpful.

We thank the reviewer for this suggestion to improve clarity for the reader. In the revised text we include a description of how the ratio is calculated (Results, 2nd paragraph) in addition to a description in the Statistical section of the Methods.

6. Pg 5 top: the authors conclude that the unstable KT-MT attachments are due to impairment of the error correction mechanism. This wording seems awkward. A defect in error correction, which leads to misaligned chromosomes, could lead to unstable attachments. That is, the errors are detected, leading to destabilization and metaphase arrest, but not corrected.

We agree that your wording is easier to follow and have used it throughout the text.

7. Pg 6: The authors connect the MKLP2 defect to AurkA activity, however, the authors should consider the possibility that this is indirect, or other factors could also be involved. They show that phospho-AurKB and AurkA are elevated, but for unknown reasons focus on AurkA. The experiment measures the effect of AurkA inhibition on cell cycle arrest. Thus, AurkA could be responsible for the arrest phenotype but not involved in error correction. No experiment conclusively rules out AurkB. The authors acknowledge this, but should also make it clear they have not directly linked AurkA to the error correction defect (although there is evidence for this in the literature). They do not show if AurkA is responsible for the increased pHEC1, which could depend on AurkB.

We agree with all of the Reviewer's points and discuss this more extensively in the newly added Discussion. Also, to show that alisertib does not rescue cells from other antimitotic

drugs, we performed the alisertib experiment but with nocodazole and taxol. The arrest induced by these agents was not affected by alisertib (Suppl Figure 1B).

8. Figure 4 and chromosome instability. The FISH data is nice, showing MKLP2 associated aneuploidy. The results from the evaluation of cancer cell lines is not surprising. I would argue that the emphasis on cancer cells is misleading. Any cell experiencing CIN would then be extra sensitive to MKLP2 loss. This is confirmatory of other results in this paper.

Thank you for bringing up this point so that we could clarify it for the readers. It was not our intent to place an emphasis on cancer cells, but merely to utilize this large data set. We have attempted to improve the text describing this data (now Figure 5) in that area of the Results (paragraph 5).
To respond to your comments in detail here: We selected colorectal cancer cell lines because of the ability to test our hypothesis that MKLP2 is more essential in cells with CIN using the MSI / MSS dichotomy within that cancer type. We agree that given our finding that MKLP2 facilitates chromosome congression, one would expect the MSS cells with CIN to be more dependent on MKLP2, however, because this has not been shown previously and it reinforces our findings, we have chosen to include it in the manuscript. In addition, the data in Figure 5 indicates that not all MSS cells are more sensitive to MKLP2 loss than MSI cells. We hypothesize that the mechanism of CIN may play an important role-perhaps some mechanisms would not be further perturbed by loss of MKLP2. We are planning to examine this possibility in future work.

9. The last paragraph in the conclusion should mention other possibilities in addition to AurkA, given the lack of data regarding the AurkB. Given the known interactions of MKLP2, a possible role for MKLP2-CPC interactions in metaphase should be discussed. Could the role of MKLP2 is bundling anti-parallel microtubules and its location in the center of the spindle be important for the observed metaphase defects.

We thank the reviewer for this suggestion. We have incorporated a more in-depth discussion of MKLP2-CPC interactions within the newly added Discussion and have added discussion of the potential for MKLP2 to promote congression due to its ability to bundle microtubules.

10. The authors should cite any other work in other organisms suggesting Kinesin 6s have metaphase functions. For example, the Drosophila orthologue of MKLP2 is Subito, which has been shown to have defects in spindle organization and chromosome alignment and bi-orientation during metaphase I of meiosis.

Thank you for this suggestion. We have included discussion regarding MKLP2 role in spindle organization and how that may play a role in promoting chromosome congression within the Discussion.

11. In all figures and legends, makes sure to label the colors.

After double checking figures and legends, we presume this comment is meant for Fig 1F in which the colors were meant to indicate different MKP2 inhibitors, but were not clearly coded in a legend. Since the MKLP2 inhibitors are clearly labeled along the x axis, we decided to separate inhibitors with a dashed line and keep the color a uniform gray. We also added labels for the chromosome probes and DAPI staining in Figure 4B.

12. Figure 1: The siRNAs are not indicated in panel A; they are in panel B. The authors should list what siRNAs 06 - 09 are. Panel D: what is the GFP panel? In A and F, what is the difference between metaphase and mitotic index? Why are the graphs different styles?

Thank you for bringing up these points of confusion. We changed Fig 1 caption to more clearly indicate that panel A corresponded to panel B. The siRNA 06-09 correspond to Dharmacon catalog numbers, which have now been added to the Key Resources table. We also altered the graph in panel F to match the graph in panel A (style and title of y axis) We apologize for the confusion that the aberrant labeling caused. The GFP-panels represent Histone H2B-GFP. We have added this to the figure legend and have replaced "GFP" with "H2B" in the figure.

13. Figure 2: As mentioned above, the term “psuedometaphase” is problematic. For example, how is it different than “abnormal mitosis”. In the figure legend, the second “D” should be “E)” and “E)” should be “F)”.

Thank you for the opportunity to clarify these points for the reader. As noted in response to comment 3, above, we have replaced ‘pseudometaphase’ with ‘congression defect’. We also corrected the mislabeled panels in Figure 2. The ‘abnormal mitosis’ is described in the text as “mitosis leading to 3 daughter cells or mitosis where cells exit mitosis with no apparent segregation of DNA” (Results, 1st paragraph) and we added a brief description in the figure legend as well.

Reviewer 2 Advance summary and potential significance to field
The manuscript proposed by Schrock et al., entitled “MKLP2 functions in early mitosis to ensure proper chromosome congression” provides the interesting observation that MKLP2 might be required earlier in mitosis than previously thought. Indeed, the kinesin MKLP2 was well known to transport Aurora B kinase from the kinetochore to the spindle midzone in anaphase, but no function had been documented during chromosome congression.
Here, the authors use a more potent MKLP2 inhibitor (MKLP2i3), to document this phenotype, that they can also observe, although to a much milder extent, by down-regulating MKLP2 with some siRNA.

Reviewer 2 Comments for the author
Although interesting, this manuscript could be dramatically improved in the following ways:
1. Figure 2 intend to show that MKLP2 deficiency leads to chromosome misalignment in prometaphase. For clarity, the Fig.2D should include the control with the siMKLP2 alone, without rescue by any of the MKLP2 isoforms.

Thank you for pointing this out. We have added data to this panel which is now Fig 2C that shows the mitotic outcomes of cells treated with siMKLP2 + empty vector. We apologize for leaving this out of the initial figure.

2. Since Figure 2 was intended to prove that the effect scored after MKLP2i3 addition is not due to an off-target effect, it would be useful if the authors could show in a new Figure 2C (the present one is confusing, and could be removed) some kind of rescue by WT MKLP2 upon addition of very low doses of inhibitor (could 5 nM of inhibitor be, at least partially, rescued by WT MKLP2 over-expression? If so, please score for the effect of inhibition without rescue at the same dose).

This was a nice suggestion. We did this experiment and as the reviewer suggested, incorporated it into Fig 1, Panel G. We also removed the original table depicting the types of cell fates under the ‘abnormal mitosis’ category.

3. In Figure 3L, scoring the effect of alisertib alone is missing to conclude. Also the statistics should compare +/- alisertib and not 12h versus 18 hours.

This is a good point. We thank the reviewer for this comment as we had missed inclusion of these data. We agree that the suggested changes better show the impact of alisertib, therefore we have updated this panel which is now Figure 4G to include these controls and to compare the data between MKLP2 treatment +/- alisertib.

4. Figure 4 has several caveats. In figure A, the “modal deviation” is not easy to apprehend. Neither is understandable the “gene effect” of MKLP2 RNAi or CRISPR KO using “publicly available data”. “DepMap, Broad (2019)” is a bit short as a method description! The authors should better explain what they did with the publicly available data, and how reliable it is. Alternatively, they could repeat the experiment themselves in a controlled way.

Thank you for highlighting these areas which needed clarification. Modal deviation is the standard way to report the degree of chromosome number change seen with the satellite enumeration probe experiment, as done in Godek et al. published in Cancer Discovery in 2016 by the Compton Lab, however we concede that we needed a better explanation. We attempted

to clarify within the text (Results, 6th paragraph). We have more clearly identified the data in Figure 4C (now Fig 5C) as coming from the Cancer Dependency Map and also attempted to clarify 'Gene Effect Score', which utilizes data from over 1000 cell lines to reflect the likelihood that a given gene is either non-essential or essential in a given cell line. We have also improved our explanation of our methods used in analyzing the DepMap data (Methods, highlighted).

5. In figure 4, the authors provide a model stating that because Aurora A is more phosphorylated upon MKLP2 inhibition, more CENP-E remains phosphorylated, but they do not document this hypothesis. Could the authors try to prove their model using the reagents used in Kim et al., Cell 2010?

This was a really good suggestion, but unfortunately, we weren't able to bring it to fruition. We reviewed the Kim et al. manuscript and found that the only reagent which would be useful for our studies was the pCENPE T422 antibody for detecting increased levels of pCENPE in the MKLP2 treated cells which we would anticipate being increased by MKLP2 inhibition. We reached out to the Cleveland Lab who was extremely responsive but no longer had that antibody in-hand. However, they did connect us to Dr. Benjamin Vitre who is located in France and had some of a similar antibody he was willing to share. We initiated the MTA to receive the antibody on January 26th (just 12 days after receiving the comments from the journal) but it is still pending approval from the granting institution and consequently we have not been able to assess pCENPE levels.

6. Alternatively, could the authors propose hypothesis to understand how MKLP2 depletion/inhibition could enhance Aurora A and Aurora B activities?

We agree with the reviewer that this discussion was lacking in the previous version of the manuscript. We have now included a Discussion section within the manuscript and have incorporated thoughts on potential mechanisms whereby Aurora kinase activities could be impacted.

Reviewer 3 Advance summary and potential significance to field
The manuscript by Schrock and colleagues examines the hitherto almost entirely unreported role for the kinesin MKLP2 in mitotic chromosome congression. Towards this end the authors employ chemical genetic and siRNA perturbations in conjunction with quantitative live and fixed cell imaging. They report that MKLP2 is needed for the efficient congression of chromosomes to the metaphase plate and timely anaphase onset. An examination of putative mechanisms identified Aurora A kinase as a major player. When MKLP2 function is perturbed beyond threshold amounts Aurora A levels are increased at the centrosomes (along with centromere associated Aurora B kinase). Further examination revealed that disruption of MKLP2 led to syntellic malorientations and a loss of k-fibre stability.
These data add to our knowledge of chromosome dynamics and genomic stability. They provide phenomenological as well as mechanistic insight. They are therefore of interest to the field and merit publication.

Reviewer 3 Comments for the author
As presented the manuscript is largely clearly written and the data solid. There are a few aspects that should be addressed prior to publication.

1. It is unclear how the metaphase index (Fig 1A) was determined. Was this a quantification of cells with non-polar placed chromosomes or chromosomes in a defined size region at the spindle equator?

We are glad Reviewer 3 brought up this point since we inadvertently had 'metaphase index' on the y axis instead of 'mitotic index'. As is standard, the mitotic index was determined by quantifying the percentage of cells in any of the phases (prophase through telophase) of mitosis. This was clarified within the Quantification and Analysis section of the Methods.

2. In my version of the figures, the images of the chromosomes in Fig 2A have aberrations. This may be a reproduction error or an artefact of the z-series projection method employed.

Thank you for pointing this out. We replaced the 'normal mitosis' image with a cell that better represents a normal mitosis and the timing of metaphase for the DMSO-treated cells.

3. It would be useful to include in the text the average and std error duration that all treatments spent in metaphase. This is important because the individual control cells shown in Fig 1D and 2A differ markedly in their timing of anaphase onset at approx. 35 and 75 min, respectively. This is a large variation if I am interpreting the images correctly.

The incorporation of a more representative image for Figure 2A depicts a more average mitotic duration for HeLa cells. We agree that the duration of mitosis is important and therefore was the focus of Figure 1C where we quantified the length of mitosis for thousands of cells treated with various doses of MKLP2i2. In order to add to this data for MKLP2i3, we further characterized the cell fates of cells treated with MKP2i3 to include whether cells exited mitosis following the congression defect phenotype (Figure 2B-C).

4. An underlying assumption is that the polar proximal chromosomes are syntelically maloriented. Was this attachment defect ever directly imaged?

Thank you for this suggestion. Indeed, the original submission had not confirmed the chromosomes arrested at the poles were syntelically attached, however in this revision, we have added Figure 3E and 3F which confirm the absence of merotelic and amphitelic attachments and the prevalence of syntelic and monotelic KT-MT attachments in pole-proximal chromosomes.

5. Figure 3E is not needed. The mechanism of polar malorientation correction by Aurora A can be elaborated upon in Fig 4E and more thoroughly referenced within the text.

We have removed this panel from the figures. In reconsidering our data and the comments of the reviewers, we felt that although we had the alisertib rescue experiment (Figure 4G), we needed to include multiple possibilities for how MKLP2 inhibition could cause the congression defect, among which included AURKB involvement and the possibility of MKLP2 playing a role in tubulin bundling. We expand on these ideas within the Discussion.

6. Aurora A has been previously implicated in chromosome biorientation, chromosome congression and oscillations along with syntelly correction. This should be referenced given the predominant role that Aurora A is serving.

We thank the reviewer for this comment. We have added additional discussion and references of AURKA roles in the Discussion section.

7. The MKLP2 rigor mutants are expressed in an siMKLP2 background. It is presumed that these constructs are siRNA tolerant through sequence differences. This should be stated if so.

Thank you for pointing this out. We neglected to include this information and it was mentioned by Reviewer 1 as well. The siRNAs were purchased from Dharmacon and are directed toward MKLP2 3'UTR. We clarified this in the manuscript in the Results (2nd paragraph) and Methods sections.

8. The work relies heavily on quantitative imaging. Yet in many sections of the methods the details of the quantitation are not given. More detailed procedures should be provided.

Thank you for suggesting this. We want to be as transparent as possible. We added more detail regarding IF image processing and quantification, as well as tracking cells in the live imaging experiments to the 'Quantification and Statistical Analysis' section of the Methods.

Second decision letter

MS ID#: JOCES/2021/259560

MS TITLE: MKLP2 functions in early mitosis to ensure proper chromosome congression

AUTHORS: Morgan S Schrock, Luke Scarberry, Benjamin R Stromberg, Claire Sears, Adrian E Torres, David Tallman, Lucas Krupinski, Arnab Chakravarti, and Matthew K. Summers
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The significance of this paper is that MKLP2 is required during metaphase for chromosome congression and alignment. This is a previously unappreciated function for MKLP2. Almost all previous studies have focused on a role during anaphase and cytokinesis. The experiments are rigorous and come to these conclusions with a combination of inhibitors, siRNAs and MKLP2 mutants.

Comments for the author

The authors have done an excellent job responding to the previous reviews. I have no further comments.

Reviewer 2

Advance summary and potential significance to field

The manuscript proposed by Schrock et al., entitled “MKLP2 functions in early mitosis to ensure proper chromosome congression” provides the interesting observation that MKLP2 is required earlier in mitosis than previously thought. Indeed, the kinesin MKLP2 was well known to transport Aurora B kinase from the kinetochore to the spindle midzone in anaphase, but no function had been documented during chromosome congression.
Here, using a more potent MKLP2 inhibitor, they could show that MKLP2 facilitates error correction, thus preventing aneuploidy.

Comments for the author

The authors adressed all of my concerns and the format change was a wise choice to allow space for discussion.

Reviewer 3

Advance summary and potential significance to field

My concerns raised over the initial submission have been addressed. I recommend the manuscript for publication

Comments for the author

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