EMBO reports

Plk1 protects kinetochore-centromere architecture against microtubule pulling forces

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 22 July 2019

Thank you for submitting the revised version of your manuscript. It has now been seen by two of the original referees.

As you can see, both referees find that the study is significantly improved during revision and recommend publication. Before I can accept the manuscript, I need you to address the below minor/editorial points:

• Please address the remaining minor concerns of both referees textually.
•

REFEREE REPORTS

Referee #1:

The revised manuscript by Lera et al. investigates the role of PLK1 in protecting centromeric-kinetochore architecture during chromosome segregation. The authors have added multiple experiments to support the hypothesis that PLK1 is required to maintain CENPA and associated kinetochore components on chromosomes under tension. The addition of these experiments addresses several of the concerns identified in the review process. The added experiments include ensuring the loss of kinetochore protein signal is not the result of detection limitations via the addition of IF and FISH experiments on chromosome spreads. Additionally, the authors alleviate concerns of antibody detectability through the use of mCherry-CENPA expressing cell lines (response Fig. 1b-c). To address concerns for the role of PLK1 being distinct from its function in regulating kinetochore-microtubule attachments, the authors added analysis for attachment status in PLK1 inhibited cells (response Fig. 1g). The authors also provide evidence that PICH maintains an active role in this process through the addition of PICH knockdown experiments. These results, in addition to others not noted here, provide a more cohesive look at how PLK1 inhibition affects

kinetochore architecture. Therefore, we recommend this manuscript be accepted for publication in EMBO reports. However, the authors were not able to address the direct mechanism in which PLK1 functions to maintain centromeric or kinetochore architecture. While the data presented in the manuscript is important and sheds light on a novel role for PLK1, it is important the authors address this by specifically commenting on the possible direct or indirect roles in the discussion.

Referee #2:

In the current manuscript Lera and colleagues demonstrate that PLK1 activity is required for maintaining centromere and kinetochore integrity in face of microtubule pulling forces exerted along the inter kinetochore axis during mitosis. Loss of PLK1 activity resulted in chromosome mis-segregation after initial alignment concurrent with an asymmetric loss of the kinetochore from the misaligned chromosome. The experiments are generally well performed with proper controls and the authors do a great job in carefully characterizing the observed loss of centromere and kinetochore integrity upon PLK1 inhibition. However, the manuscript still raises several concerns, specifically regarding the 'interaction' between PLK1 (activity) and PICH in light of the observed Plk1 dependent centromere/kinetochore disruption.

Major points

The authors use FISH to show centromeres are stretched, coincident with disturbed/fragmented CENP-C signals. However, the figure is confusing. Figure 5C is labelled suggesting the left two stacked images are stained for DAPI and CENP-C in red. However, in the BI-2536 treated cell, the inset shows a green signal. Is this CENP-C? Then the right two stacked images show FISH using two different centromere probes, yet the figure legend only mentions Cen2. The images are very fuzzy/unclear, making it very hard to interpret what I'm looking at. The mere two lines of text describing this is of no help. This is in my opinion an important point the authors are making (the stretching/distortion of centromeric DNA) and could be expanded on in the text/discussion. Now, looking at the images, one could conclude based on the Cen1 signal in the DMSO control that they are also heavily distorted. Based on the quality of these images I find it difficult to draw any conclusions. The data should also be quantified as for CENP-C in Fig 5B. Finally, the signal of CENP-C in the overview of the spreads in Fig. 5A, C, J are very weak/unclear. This makes it difficult to get a good overview picture to compare the insets with.

The authors conclude in the discussion: "PICH recruitment is increased during metaphase and anaphase but this is impaired in PLK1as cells inhibited by 3-MB-PP1 (Fig. 6C)". However, no significant differences are observed. See, Fig 6C first three lanes. Instead, the expression of H2B or INCENP-PLK1 actually increases the number of segregated PICH (+) chromosomes, similar to inhibition of PLK1 using BI-2536 in Fig 5G. These data highlight inconsistencies between the use of BI-2536 in normal cells and 3-MB-PP1 in PLK1as cells regarding PICH localization, making it difficult to draw conclusions on PLK1 and PICH being involved in the same pathway. So, while the authors show that restoring PLK1 activity in PLK1as cells treated with 3-MB-PP1 rescues successful chromosome alignment (Fig 6B) and depletion of PICH rescues distorted CENP-C foci in BI-2536 treated RPE1 cells the relationship between PLK1 activity and PICH localization seems to be opposite in these two experiments. The authors provide little to no discussion on these important discrepancies. These data and this segment require much better explanation/discussion to somehow resolve this. Now the text is just confusing, raising more questions than actually answering.

The authors conclude PICH plays a major role in the centromere/kinetochore instability upon PLK1 inhibition based on the depletion experiments rescuing the inhibition. However, they hardly go in to the potential mechanism regarding how PICH, which is part of a much larger functional complex with potentially multiple other helicases such as BLM, would cause the observed phenotype. While I appreciate that the entire mechanism cannot be solved here highlighting some potential mechanisms is important such as for example how PLK1 could control PICH recruitment (related to the opposite effects the authors see!). The authors do comment that PLK1 does not appear to control PICH ATPase activity but it is unclear if this is required for the observed phenotypes to begin with seeing as other helicases such as BLM are also recruited downstream of PICH. Do the authors think the activity of these helicases plays an active role in unravelling centromeres/kinetochores, in conjunction with the pulling forces exerted by the mitotic spindle? Furthermore, the discussion

mentions nothing regarding how PLK1 activity results in centromere/kinetochore disruption such as potential substrates. I understand that the authors are working on this behind the scenes and have ruled out several candidates but it at least deserves mention that this is something required to get a full understanding of the observed phenotype. I think these are key points missing in the discussion.

Figure 6B has stars above several bars indicating significance. But of what? What is being compared? For example, expression of WT PLK1 results in ****. Is that a significant increase in cells which successfully segregate their chromosomes compared to expression of the Flag control? And clone B2 expressing PLK1-CENP-B, has twice as many correctly segregating cells ({plus minus}60%) compared to control cells expressing Flag (30%), but the this is not significant? The way this is presented is unclear and does not allow the reader to draw any conclusions. Moreover, I have the impression the statistics are not done correctly. Despite being presented as a percentage the data is categorical in nature: a cell has successfully segregated its chromosomes: yes or no. This would preclude the use of ANOVA which requires the variable to be continuous, i.e. at the interval or ratio level. The authors should use contingency tables/chi square analysis to assess if there is an interaction between the different categories.

Minor points

Page 7, paragraph 2: "These findings are concordant with recent findings...."

Figure 6, the assay should be much better explained in the text, not only in the Fig. legend. Are the PLK1 chimeras expressed in the RPE1 PLK1as cells? Do the RPE1 PLK1as cells have one or two AS alleles? This is also related to Fig EV1a-c. I realize there is a reference noted that describes the cell line but the info is crucial to understanding the experiment and what is meant exactly by partial inhibition.

Fig 6C the dots are color coded for cells with normal chromosome segregation or the presence of lagging chromosomes, blue/purple and red respectively. Yet the Flag control are all a lighter shade of red and some other red dots are of a larger size? I couldn't find what that means, if anything.

Taken together, the manuscript, shows a novel phenotype of Plk1 at centromeres. I feel the manuscript has significantly improved, through the removal of several, too strong statements and the inclusion of the centromere FISH and PICH depletion studies. Still, these data or their explanation (see above) would require some extra work to warrant publication in EMBO reports.

1st Revision - authors' response 3 August 2019

As you will see, we have addressed each point as indicated by the very thoughtful referees. With these edits/clarifications, we believe the revised manuscript has improved markedly.

I would like to highlight one, we believe minor, suggestion of referee #2 that we did not act on in Major Points paragraph 4, second half of the paragraph: “Despite being presented as a percentage the data is categorical in nature: a cell has successfully segregated its chromosomes: yes or no. This would preclude the use of ANOVA which requires the variable to be continuous, i.e. at the interval or ratio level.” We want to clarify that we are measuring a percentage, and the distribution shown by the reported errors describe the repeatability of that measurement in biological replicates. By the central limit theorem, we expect the errors in the measurements of percentages can be reasonably assumed to be normally distributed, especially as none are close to 0% or 100%. Thus, we prefer to retain the current analysis of continuous data. Nevertheless, we appreciate the feedback from the referee who allowed us to reconsider this issue.

1st Editorial Decision 9 August 2019

Thank you for submitting your revised manuscript. I have now looked at everything and all looks fine. Therefore I am very pleased to accept your manuscript for publication in EMBO Reports.

EMBO PRESS

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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Mark Burkard
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-48711

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	Sample size was selected for cell biology experiments based on prior experience and biologically significant effect size. The sample sizes are indicated in each figure legend and the statement is included in the Methods Section, Immunofluorescence Microscopy, Analysis, last paragraph
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	Slide label concealment was used during microsocopy experiments. This statement is included in the Methods Section, Immunofluorescence Microscopy, Analysis, last paragraph.
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	No. We tested each data set for normality using Prism 8. Frequently, one or more samples failed to pass the normality test, so we used Mann-Whitney and Kruskal-Wallis tests.

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Is there an estimate of variation within each group of data?	No
Is the variance similar between the groups that are being statistically compared?	No

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	This table is available as Appendix Table S2
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	This information is included in the Methods Section, Cell Line Derivation and Culture Procedures, Cell Culture.

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11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
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20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
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G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No