EMBO reports

UBAP2L-dependent coupling of PLK1 localization and stability during mitosis

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Dear Izabela,

Thank you for the submission of your research manuscript to EMBO reports. It has now been seen by three experts in the field, whose detailed reports I have already forwarded to you (they are appended again below). I would also like to thank you for your pre-decision point-by-point response to their comments, and for our discussion yesterday.

The referees acknowledge that your manuscript provides novel and interesting insights into regulation of the key mitotic factor PLK1 by the ubiquitin-binding protein UBAP2L. However, they also raise several concerns, which should be addressed for further consideration of your manuscript. In particular, we think that their criticism that the available data support general control of PLK1 levels by UBAP2L through ubiquitin-mediated degradation rather than through specific removal from kinetochores is important and should be addressed experimentally and by textual revision of your manuscript if necessary. In addition, we agree with referee #3 that regulation of other mitotic factors by UBAP2L cannot be conclusively ruled out based on the presented data, and while an exhaustive investigation of this possibility will not be required, we would ask you to interpret the results and discuss all possibilities accurately in your revised manuscript. Furthermore, all referees provide a number of additional suggestions for the improvement of the study and the manuscript, which should be addressed.

Given these constructive comments and your point-by-point response outlining your proposed revision plan, we would like to invite you to revise your manuscript along the lines you suggested and with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript. If you have any questions or comments, we can also discuss the revisions in a video chat, if you like.

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we usually recommend a revision within 3 months (February 8th). Please discuss with me the revision progress ahead of this time if you require more time to complete the revisions.

*****
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See also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

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The following points must be specified in each figure legend:

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- If the data are obtained from n {less than or equal to} 2, use scatter plots showing the individual data points.

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Ioannis

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

This manuscript describes Ubiquitin-Binding Protein 2-Like (UBAP2L) protein as a Polo-like kinase 1 (PLK1) modulator. UBAP2L regulates PLK1 protein level through mediating degradation, and UBAP2L appears to be important to remove PLK1 from kinetochores before anaphase. During interphase, loss of UBAP2L causes markedly elevated PLK1 that persists at centromeres throughout interphase, instead of only during G2 (with UBAP2L present). A number of experiments address mechanisms and importantly, UBAP2L knockdown has effects on mitotic aberrations that are largely rescued with inhibition of Plk1, demonstrating a functional role of the excess residual activity in the absence of UBAP2L.
Overall, this is an important addition to literature. The paper builds on an important discovery of a new ubiquitin-binding protein and its regulation of Plk1. Careful experimental models with both knockout and knockdown experiments are used. Live-cell imaging corroborates many of the fixed imaging experiments and the different stages of the cell cycle and mitosis are carefully parsed out. The manuscript is written well. I found the rescue experiment at the end of the paper compelling. There are some concerns about the manuscript as noted below that could be addressed in revision.
Major:
1. Figure 1 experiments should be expanded and corroborated by siRNA in figure 1 (siRNA is in the final figure with different penetrance). The KO will have long-term UBAP2L loss, which may have secondary and downstream consequences of prior mitoses. The text says that there were no abnormal prior mitoses occur because only some, but not nuclei are misshapen-this claim is not warranted as some nuclei do have irregular shape and because abnormal mitoses need not always lead to observably misshapen nuclei. Of note, all displayed nuclei in Figure 1A UBAP2L KO cases appear larger than control and one has a tripolar division. These findings suggest polyploid cells with supernumerary centrosomes. To rectify this, the data should be corroborated with siRNA, and the cells in the KO case should be assessed for polyploidy and centrosome amplification.
2. The data shown do not support KT localization of UBAP2L. This is addressed in Figures 6-7. Only one close-up image of a KT is shown for each condition and the displayed localizations are distinct-in 6A UBAP2L is inner centromere (with BI); in 6B there are outer KT signals (outside CREST); in 7A there is diffuse signal spanning the entire KT region. The lack of colocalization is particularly evident in Figure 7C where most KTs clearly lack a UBAP2L signal and the chunky signals are much larger than the CREST foci in the flag-UBAP2L-FL case; further, in the Flag-UBAP2L-CT case span the entire cell not even located within the chromosome regions. Quantification of UBAP2L/CREST are insufficient to support colocalization as these only relate varying levels of UBAP2L in the regions of the chromosomes under distinct conditions but do not compare levels at versus away from KTs.
3. Timely removal of PLK1 from KT is claimed, but the data better supports regulatory control of PLK1 protein levels through ubiquitin-mediated degradation than specific removal from KTs. Prior studies with FRAP experiments have shown rapid turnover at KT with T1/2 of 12 s, so changing the dynamics of removal would have to be remarkable to remove protein [Kishi, Kazuhiro, et al. "Functional dynamics of Polo-like kinase 1 at the centrosome." Molecular and cellular biology 29.11 (2009): 3134-3150.]. Further evidence of this is seen with the increase in midbody Plk1 with siUBAP2L (Figures 8A and 8E for instance). Thus, data

in the manuscript support Plk1 degradation rather than removal from KT; if removal from the KT is claimed, measurement of protein dynamics at the KT would be important.
a. Line 351 "licensing the kinetochore removal of PLK1 prior to anaphase." As noted above, the data do not clearly show kinetochore removal, much less licensing for a separate removal process. This should be restated.
4. In Figure 2C, G only a small fraction of cells expresses PLK1. In Figures D and H, there are measurable levels of PLK1 in all cells. Is this because the cells that lack PLK1 expression are excluded and only the PLK1 expressing cells are shown in D/H?
5. Lines 263-5 describe that as early as prometaphase there are increased levels of Plk1 as well as cytoplasmic aggregates with UBAP2L depletion as shown in Figure 8A. However, 8B quantification does not show this-at 0h and 1h30, all cells express Plk1. What is the reason for the discrepancy? Should Plk1 level be quantified in a different way to support the claim?
6. Why does Plk1 coIP with UBAP2L in Figure 9B but fails to do so in Figure 9C?
Minor:
1. Please clarify the time between siRNA transfection to experiments in the methods and/or legends throughout but especially in Fig. 9.
2. Some cells may have both misaligned chromosomes or bridges; others may have multipolar divisions without misaligned chromosomes or bridges. Therefore, in Figure 1, consider quantifying % normal versus abnormal divisions encompassing all defects-with this quantification, cells that have multiple defects should be counted only once.
3. Figure 1F-G: Why are two timepoints used? This isn't mentioned in the text and the legend doesn't clarify the rationale (or state that Time490 means, apparently 490 minutes from prometaphase). Micronuclei didn't go up in 1F over time (i.e. after mitosis), but this is perhaps because there was already a large number of surviving micronucleated cells in the population from prior abnormal mitoses?
4. Do both UBAP2L KO clones show increased Plk1 protein levels? Figure 2E should include both.
5. Claims about effects of UBAP2L on Plk1 activity are not well supported. Intracellular activity is typically measured in a biochemical assay via IP-kinase. T210 phosphorylation in Figure 2J is only an approximate surrogate (since additional levels of activity control are at play; e.g. protein level and PBD binding). The BubR1 levels in Figure 2J helps, but this is only a single blot, and is not quantified; further the activity would be measured as the intensity of the upper (phosphorylated) versus lower (unophosphorylated) BUBR1 band, not the total levels. These are inadequate to infer activity.
6. Line 320: as above, pT210 isn't a measure of activity, but just one mechanism by which activity is controlled-at best it could be called a surrogate of activity.
7. Line 351 "the regulatory effect of UBAP2L towards PLK1 is specific." A survey of AurA, AurB, Cyclin B1, Plk2/3/4 isn't sufficient to assert this is specific. Only a handful of proteins have been excluded at a specific cell cycle state. This claim should be moderated here and in the abstract (lines 26-27).
8. Line 433 "UBAP2L directly interacts with PLK1, CUL3, and KLHL22...(Fig 9A)" This shows a coIP which doesn't address directness of the interaction.
9. Figure 6A,D and 7A,C: please include scale bars for insets and, if possible, adjust levels so they are not blown out.
10. Figure 7C: Please change label to UBAP2L to FLAG if the flag antibody is used; if not, clarify genetic background of cells (is this UBAP2L KO?)

Referee #2:

In Guerber et al. the authors study the role of the protein UBAP2L in mitosis and particularly in PLK1 localization and function. The authors convincingly show that UBAP2L regulates PLK1 amounts and clearance from kinetochores and propose that the UBAPL2-dependent PLK1 removal from kinetochores is coupled to degradation of the kinase.

The paper is clear while experiments are well performed and controlled. The results are solid and interesting, albeit missing the key mechanistic insight regarding PLK1 stabilization. A key point is whether the authors think that UBAP2L acts specifically at kinetochores or whether it generally controls PLK1 turnover. Following considerations may help clarify some issues and strengthen the manuscript.

Major
1. The authors show that PLK1 localizes to puncta in UBAP2L KO cells. The authors focus on kinetochores, but it is not clear, whether the majority of PLK1 puncta are on kinetochores, and what is the percentage of PLK1 puncta that do not colocalize with kinetochore structures. Indeed, it seems that PLK1 persists also on other spindle structures, like the midzone (as pointed out also by the authors) but possibly also at the spindle poles (see Fig. 8E, siUBAP2L, 60 min) and as cytoplasmic puncta in monastrol-treated cells. Since localization of UBAP2L is not specific for kinetochores, UBAP2L may be a general factor that controls PLK1 turnover rather than specifically removing PLK1 from KTs, as it is mostly suggested by the authors (or both). The authors could clarify and discuss this possibility. They could also use the PLK1-GFP-expressing line to assess the extent of PLK1 localization to other structures.

2. The authors use siG3BP to exclude that PLK1 regulation by UBAP2L is linked to stress signaling. An additional control would be to test PLK1 localization upon proteotoxic stress. How does PLK1 localize in MG132-treated cells? Moreover, is appearance of cytoplasmic PLK1 aggregates specific for monastrol-treated cells and does it depend on SAC activation (which could be

considered as a type of stress as well)?

3. Along the same lines, is the activity of PLK1 required for its localization in puncta?

4. The amount of GFP-PLK1 in Fig 9c input and its amount in the IP fraction is not elevated in the UBAP2L KO cells, but rather lower. Why? This influences the amount of conjugated Ub and the conclusion of the experiment.

Minor

The designation siNT (non-targeting) may cause confusion with the NT (N-terminus of UBAP2L.

Referee #3:

In their manuscript 'UBAP2L-dependent coupling of PLK1 localization and stability during mitosis', Guerber and co-workers study the role of UBAP2L in mitosis. In summary, they show that:
- UBAP2L inactivation leads to widespread mitotic defects in multiple cell lines
- Inactivation of UBAP2L leads to higher levels of Plk1.
- The C-terminal domain of UBAP2L regulates Plk1 levels, independently of stress granules
- UBA2L is described to localize at kinetochores.
- Plk1 levels are elevated at multiple sites during mitosis
- Plk1 inhibition in UBAP2L-depleted cells rescues mitotic defects to some extent.
Some remaining questions are: why is Plk1 not degraded by the APC/C-Cdh1 in G1? Does UBAP2L work epistatically with APC/C? Also, I am not very convinced by the PLK1 assessment. Many of the analysis use a thresholded microscopy analysis (eg Figure 4B), which emphasizes an effect that is much more complex and less pronounced when analyzed at the single cell level (eg Figure 4C). It is clear that UBAP2L-depletion gives mitotic defects, but how this is coordinated at kinetochores through Plk1 modulation remains insufficiently clear.

Comments:
-overall, the manuscript could be improved by textual editing. There are many grammatical errors.
-Abstract: it is claimed in the abstract that 'UBAP2L regulates Plk1 and no other factors'. That is a bold claim that cannot be made on the presented data. Aurora A and B, and Cyclin B are tested, and there is some rescue, but other targets may exist. Actually, BubR1 levels are up too (fig 2J). and AurA/B and Cyclin B are up too (not statistically significant but with good effect size, so just a matter of n). Also, the RGG domain is suggested in the text to regulate other mitotic factors.
-Graphical abstract: it remains unclear what the yellow and pink dots in the right-hand panels represent.
-Figure 1A: the nuclear size appears to be very different from the control cell and the KO cells. Is the scale bar the same? If yes, it could be that the KO have already undergone a defective round of mitosis, and will suffer from additional, indirect effects
-it remains incompletely clear what the mitotic phenotype is: chromosome alignment due to altered MT-KT interactions? Cohesin/condensin defects leading to anaphase bridges? Bipolar spindle formation.
-Figure 2A/E: these WBs and the corresponding conclusions would need a cell cycle analysis to confirm that there are not cell cycle changes.
-Figure 2B/F: are the nuclear Plk1 spots co-localizing with centromeres?
-Figure 2C/D and 2G/H: i do not understand how these plots are connected? Panels C/G appears to represent data from panel D/H with an artificial threshold. I would remove panel C/G. same goes for suppl. 1C/D/E. here single cell data should be included.
-Suppl. Figure S3A: the CHX experiment is relevant, but requires quantification over multiple experiments.
- does expression of FL or C-terminal UBAP2L also rescue mitotic defects? These data are missing.
- Figure 4D: a plk1 blot is missing.

Figure 5/6: I am not convinced by the UBAP2L/CREST co-localization. Actually, it appears that UBAP2L localizes outside kinetochores in untreated cells, and stronger localization next to kinetochores upon Plk1 inactivation (Fig6D).
Figure 8: I see much more Plk1 at kinetochores, but also at other structures, including outside the chromosome areas (centrosomes/stress bodies? and also non-crest structures at chromosome areas). To what extent are these pools of Plk1 also regulated by UBAP2L? Also, the conclusion is that UBAP2L removes Plk1 from kinetochores. This I support, but also Plk1 levels at centrosomes remain elevated, and a large fraction of Plk1 does move from kinetochores to the midzone/midbody, where it accumulate. The conclusion (and mechanism) is broader than the authors claim.
-Figure 5B/C/D: cell cycle synchronization is done using severe chemical perturbations: HU, Cdk1i. It would be better to follow thym/thym-block-release cells through the cell cycle at multiple time points to investigate Plk1 levels. In the current analysis many other pathways are perturbed (DDR signaling, Cdk1 effects).
-Figure 9: I find this experiment rather opportunistic. Plk1 inhibition has been described to lead to many defects, including defects beyond kinetochore effects. what does this concentration of Plk1 do on control cells (this important control is missing)? Is this amount enough to induce previously described effects in control cells?

-figure 9: how do the authors explain that UBAP2L-depletion in combination with Plk1 inhibtion performs better than control cells (Fig 9F)?

Point by point response to reviewers

Referee #1

This manuscript describes Ubiquitin-Binding Protein 2-Like (UBAP2L) protein as a Polo-like kinase 1 (PLK1) modulator. UBAP2L regulates PLK1 protein level through mediating degradation, and UBAP2L appears to be important to remove PLK1 from kinetochores before anaphase. During interphase, loss of UBAP2L causes markedly elevated PLK1 that persists at centromeres throughout interphase, instead of only during G2 (with UBAP2L present). A number of experiments address mechanisms and importantly, UBAP2L knockdown has effects on mitotic aberrations that are largely rescued with inhibition of Plk1, demonstrating a functional role of the excess residual activity in the absence of UBAP2L.
Overall, this is an important addition to literature. The paper builds on an important discovery of a new ubiquitin-binding protein and its regulation of Plk1. Careful experimental models with both knockout and knockdown experiments are used. Live-cell imaging corroborates many of the fixed imaging experiments and the different stages of the cell cycle and mitosis are carefully parsed out. The manuscript is written well. I found the rescue experiment at the end of the paper compelling. There are some concerns about the manuscript as noted below that could be addressed in revision.

We thank the reviewer for the positive evaluation of our manuscript and appreciation of the importance of the presented findings and the quality of the experimental work. We are also grateful for helpful suggestions that significantly improved the study.

Major:

1. Figure 1 experiments should be expanded and corroborated by siRNA in figure 1 (siRNA is in the final figure with different penetrance). The KO will have long-term UBAP2L loss, which may have secondary and downstream consequences of prior mitoses. The text says that there were no abnormal prior mitoses occur because only some, but not nuclei are misshapen-this claim is not warranted as some nuclei do have irregular shape and because abnormal mitoses need not always lead to observably misshapen nuclei. Of note, all displayed nuclei in Figure 1A UBAP2L KO cases appear larger than control and one has a tripolar division. These findings suggest polyploid cells with supernumerary centrosomes. To rectify this, the data should be corroborated with siRNA, and the cells in the KO case should be assessed for polyploidy and centrosome amplification.

We agree with the reviewer that on the basis of our previously shown experiments, we cannot formally exclude that the phenotypes observed upon UBAP2L loss are the secondary consequences of defects in a previous mitosis. We also apologize for the confusion regarding the nuclear size in UBAP2L KO cells, as the scale bar used in the old Figure 1A was incorrect and the example showing tripolar division was not representative. In our new Figure 1A, we corrected the scale bars and removed the example of tripolar division. In addition, we repeated the live video experiment presented in Figure 1A using UBAP2L siRNA treatment to compare the severity of

mitotic phenotypes with those observed in UBAP2L KO cells. These new results are presented in Figures 1H-M and are described in the text (lines 120-122). Furthermore, we performed additional experiments to characterize in UBAP2L-depleted cells a) the number of centrosomes, b) the polarity of the mitotic spindle, and c) the nuclear size (also as per Reviewer 3 comments). Our new results have now excluded the concerns about centrosome amplification upon UBAP2L depletion and are presented in Figures 1G, 1N and 2A-H and in the text (lines 127-146).

2. The data shown do not support KT localization of UBAP2L. This is addressed in Figures 6-7. Only one close-up image of a KT is shown for each condition and the displayed localizations are distinct-in 6A UBAP2L is inner centromere (with BI); in 6B there are outer KT signals (outside CREST); in 7A there is diffuse signal spanning the entire KT region. The lack of colocalization is particularly evident in Figure 7C where most KTs clearly lack a UBAP2L signal and the chunky signals are much larger than the CREST foci in the flag-UBAP2L-FL case; further, in the Flag-UBAP2L-CT case span the entire cell not even located within the chromosome regions. Quantification of UBAP2L/CREST are insufficient to support colocalization as these only relate varying levels of UBAP2L in the regions of the chromosomes under distinct conditions but do not compare levels at versus away from KTs.

We agree with this criticism. Analysis of the kinetochore localization of UBAP2L is challenging because this protein is mainly found in the cytoplasm and tends to form aggregate-like structures. To overcome this issue and accurately characterize the subcellular localization pattern of UBAP2L during mitotic progression, we applied immunofluorescence protocols designed to extract cytoplasmic protein pools. We qualitatively assessed the localization of UBAP2L within different mitotic structures using markers for: the inner centromere (INCENP), outer kinetochore (BubR1), spindle microtubules (α-tubulin), centrosomes (γ-tubulin) and midbody (PRC1). Based on our new experiments, we conclude that the fraction of UBAP2L localized on chromosomes/kinetochores occurs rather randomly, because large UBAP2L foci overlap with few chromosome/kinetochore subregions. On the other hand, our new protocol demonstrated that UBAP2L tends to be enriched at the mitotic spindle and centrosomes throughout mitosis and at midbody during cytokinesis. In view of these new results, we therefore decided to remove the old Figures 6 and 7 from the manuscript and to refrain from any claims about the kinetochore localization of UBAP2L throughout the text. The new results are presented in Figure EV2A-E and described in the text (lines 147-161). Accordingly, and as explained below, we focused our manuscript on the more general role of UBAP2L in the regulation of stability of PLK1 at different subcellular locations.

3. Timely removal of PLK1 from KT is claimed, but the data better supports regulatory control of PLK1 protein levels through ubiquitin-mediated degradation than specific removal from KTs. Prior studies with FRAP experiments have shown rapid turnover at KT with T1/2 of 12 s, so changing the dynamics of removal would have to be remarkable to remove protein [Kishi, Kazuhiro, et al. "Functional dynamics of Polo-like kinase 1 at the centrosome." Molecular and cellular biology 29.11 (2009): 3134-3150.]. Further evidence of this is seen with the increase in midbody Plk1 with siUBAP2L (Figures 8A and 8E for instance). Thus, data in the manuscript

support Plk1 degradation rather than removal from KT; if removal from the KT is claimed, measurement of protein dynamics at the KT would be important.

We agree with the reviewer that the model of UBAP2L-dependent PLK1 kinetochore removal was not fully supported by the data presented, an opinion shared by all 3 reviewers. Therefore, we quantitatively assessed the localization of PLK1 upon UBAP2L depletion within different mitotic structures using markers for: the inner centromere (INCENP), outer kinetochore (BubR1), spindle microtubules (α-tubulin), centrosomes (pericentrin) and midbody (PRC1). Our new data indicate that all PLK1 pools are significantly increased in the absence of UBAP2L, suggesting that UBAP2L does not act specifically at kinetochores but rather controls ubiquitin-mediated PLK1 turnover in a global manner throughout mitosis. We have tuned down the claims about the regulation of the kinetochore removal of PLK1 by UBAP2L throughout the manuscript and focused our conclusions more on the regulation of PLK1 stability. The new results are presented in Figures 8A-G and EV5F-G and described in the text (lines 302-315).

a. Line 351 "licensing the kinetochore removal of PLK1 prior to anaphase." As noted above, the data do not clearly show kinetochore removal, much less licensing for a separate removal process. This should be restated.

This statement has been rephrased as suggested by reviewer.

4. In Figure 2C, G only a small fraction of cells expresses PLK1. In Figures D and H, there are measurable levels of PLK1 in all cells. Is this because the cells that lack PLK1 expression are excluded and only the PLK1 expressing cells are shown in D/H?

Old Figures 2C, G, (now 3C, 3G) show the analysis of the levels of PLK1 of cells in interphase which is low as expected. The PLK1 nuclear intensity shown in old Figures 2D, H (now 3D, 3H) was quantified only in the cells expressing PLK1 as predicted by reviewer. We provide a detailed description of these analyses in the revised Figure legends (lines 944-961).

5. Lines 263-5 describe that as early as prometaphase there are increased levels of Plk1 as well as cytoplasmic aggregates with UBAP2L depletion as shown in Figure 8A. However, 8B quantification does not show this-at 0h and 1h30, all cells express Plk1. What is the reason for the discrepancy? Should Plk1 level be quantified in a different way to support the claim?

We apologize for the confusion. In the revised Figure 7B we quantified PLK1 intensity rather than the percentage of cells expressing PLK1.

6. Why does Plk1 coIP with UBAP2L in Figure 9B but fails to do so in Figure 9C?

The IP in Figure 9C has been done under denaturing conditions, allowing for the detection of covalently attached PTMs but not protein-protein interaction. UBAP2L blot is presented here as an internal control in WT versus UBAP2L KO conditions.

Minor:

1. Please clarify the time between siRNA transfection to experiments in the methods and/or legends throughout but especially in Fig. 9.

We provide more details on the experimental design in the revised methods/legends as requested by reviewer.

2. Some cells may have both misaligned chromosomes or bridges; others may have multipolar divisions without misaligned chromosomes or bridges. Therefore, in Figure 1, consider quantifying % normal versus abnormal divisions encompassing all defects-with this quantification, cells that have multiple defects should be counted only once.

As suggested by the reviewer, we simplified the graphs which now show quantification of all mitotic defects including both misaligned chromosomes and DNA bridges. These graphs are presented in Figures 1D and 1K corresponding to the results in UBAP2L KO cells and in cells treated with specific UBAP2L siRNA, respectively.

3. Figure 1F-G: Why are two timepoints used? This isn't mentioned in the text and the legend doesn't clarify the rationale (or state that Time490 means, apparently 490 minutes from prometaphase). Micronuclei didn't go up in 1F over time (i.e. after mitosis), but this is perhaps because there was already a large number of surviving micronucleated cells in the population from prior abnormal mitoses?

We apologize for not including sufficient information. “Time490” indicates 490 min from the moment the filming was started (0 = before mitosis, 490 = end of live-video when most of the cells divided). To avoid confusion, we have replaced the graphs showing only the results obtained after mitosis in Figures 1E and 1F. Similar quantifications have been added for the complementary experiment in cells treated against UBAP2L siRNA and are presented in Figures 1L and 1M. Detailed description is provided in the revised Figure legends (lines 898-902 and 913-916). Moreover, as described in our response to reviewer’s major comment 1, we have now excluded that the phenotypes observed upon UBAP2L loss are the result of prior abnormal mitoses.

4. Do both UBAP2L KO clones show increased Plk1 protein levels? Figure 2E should include both.

Both UBAP2L KO clones show increased PLK1 protein levels. Representative WB of two UBAP2L KO clones is presented in Figure 3E.

5. Claims about effects of UBAP2L on Plk1 activity are not well supported. Intracellular activity is typically measured in a biochemical assay via IP-kinase. T210 phosphorylation in Figure 2J is only an approximate surrogate (since additional levels of activity control are at play; e.g. protein level and PBD binding). The BubR1 levels in Figure 2J helps, but this is only a single blot, and is not quantified; further the activity would be measured as the intensity of the upper

(phosphorylated) versus lower (unphosphorylated) BUBR1 band, not the total levels. These are inadequate to infer activity.

We thank the reviewer for this suggestion. We performed the quantification of the upper versus the lower BubR1 band from three individual experiments as suggested. This result is shown in Figure 3K and supports our observations on the effect of UBAP2L on Plk1 activity.

6. Line 320: as above, pT210 isn't a measure of activity, but just one mechanism by which activity is controlled-at best it could be called a surrogate of activity.

We modified the text accordingly (lines 188-190 and 345-346).

7. Line 351 "the regulatory effect of UBAP2L towards PLK1 is specific." A survey of AurA, AurB, Cyclin B1, Plk2/3/4 isn't sufficient to assert this is specific. Only a handful of proteins have been excluded at a specific cell cycle state. This claim should be moderated here and in the abstract (lines 26-27).

We tuned down the claims on the specific effects of UBAP2L on PLK1.

8. Line 433 "UBAP2L directly interacts with PLK1, CUL3, and KLHL22...(Fig 9A)" This shows a coIP which doesn't address directness of the interaction.

We modified the text accordingly (lines 456-458).

9. Figure 6A, D and 7A, C: please include scale bars for insets and, if possible, adjust levels so they are not blown out.

Please refer to our response to major comment 2.

10. Figure 7C: Please change label to UBAP2L to FLAG if the flag antibody is used; if not, clarify genetic background of cells (is this UBAP2L KO?)

Please refer to our response to major comment 2.

Referee #2

In Guerber et al. the authors study the role of the protein UBAP2L in mitosis and particularly in PLK1 localization and function. The authors convincingly show that UBAP2L regulates PLK1 amounts and clearance from kinetochores and propose that the UBAPL2-dependent PLK1 removal from kinetochores is coupled to degradation of the kinase.

The paper is clear while experiments are well performed and controlled. The results are solid and interesting, albeit missing the key mechanistic insight regarding PLK1 stabilization. A key point is whether the authors think that UBAP2L acts specifically at kinetochores or whether it generally controls PLK1 turnover. Following considerations may help clarify some issues and strengthen the manuscript.

We thank the reviewer for the positive evaluation of our manuscript and appreciation of the importance of the presented findings and the quality of the experimental work. We are also grateful for helpful suggestions that improved the study. We agree with the key point raised and shared by all 3 reviewers, and we have now tuned down the claims about the regulation of the kinetochore removal of PLK1 by UBAP2L throughout the manuscript focusing more our conclusions on the global PLK1 turnover model.

Major

1. The authors show that PLK1 localizes to puncta in UBAP2L KO cells. The authors focus on kinetochores, but it is not clear, whether the majority of PLK1 puncta are on kinetochores, and what is the percentage of PLK1 puncta that do not colocalize with kinetochore structures. Indeed, it seems that PLK1 persists also on other spindle structures, like the midzone (as pointed out also by the authors) but possibly also at the spindle poles (see Fig. 8E, siUBAP2L, 60 min) and as cytoplasmic puncta in monastrol-treated cells. Since localization of UBAP2L is not specific for kinetochores, UBAP2L may be a general factor that controls PLK1 turnover rather than specifically removing PLK1 from KTs, as it is mostly suggested by the authors (or both). The authors could clarify and discuss this possibility. They could also use the PLK1-GFP-expressing line to assess the extent of PLK1 localization to other structures.

We fully agree with this criticism that is shared by all 3 reviewers. Please refer to our detailed response to comment 3 of reviewer 1 and to the new Figures 8A-G and EV5F-G.

2. The authors use siG3BP to exclude that PLK1 regulation by UBAP2L is linked to stress signaling. An additional control would be to test PLK1 localization upon proteotoxic stress. How does PLK1 localize in MG132-treated cells? Moreover, is appearance of cytoplasmic PLK1 aggregates specific for monastrol-treated cells and does it depend on SAC activation (which could be considered as a type of stress as well)?

We thank the reviewer for these suggestions. To further investigate if UBAP2L-mediated PLK1 regulation during G1/S phase is linked to stress conditions, we have tested PLK1 localization in MG132-treated cells, as suggested by the reviewer. MG132 treatment alone leads to increased PLK1 levels in G1/S phase, although to a lesser extent than UBAP2L depletion. However, the effect of UBAP2L on PLK1 expression is not additive upon proteotoxic stress. These new results are presented in Figures 6C and 6D and described in the text (lines 269-276). Moreover, the appearance of cytoplasmic PLK1 aggregates during mitosis does not seem to be UBAP2L-dependent, neither is specific to monastrol treatment and/or stress induction. As shown in Figure EV5G, cytoplasmic PLK1 aggregates can be observed even under non-stress conditions in cells synchronized with double thymidine block and release (DTBR) treatment and their appearance is not UBAP2L-dependent. To avoid confusion and to simplify our conclusions, we edited the revised text and figures to not specifically focus on cytoplasmic PLK1 aggregates.

3. Along the same lines, is the activity of PLK1 required for its localization in puncta?

As stated in our response to the comment above, the appearance of cytoplasmic PLK1 aggregates (puncta) during mitosis is not UBAP2L-dependent and we apologize for the confusion arising from the description of our previous results. However, to address if PLK1 activity is required for its accumulation in puncta-like structures, we overexpressed GFP-PLK1 WT and the catalytically inactive mutant GFP-PLK1 K82R in HeLa WT and in UBAP2L KO cells synchronized in prometaphase-like stage by paclitaxel treatment. According to our results, the ability of PLK1 to form cytoplasmic aggregates is independent of both its own catalytic activity and of UBAP2L expression. Following the same rational as described in the previous point, we provide these results in our response letter and do not include them in the revised manuscript.

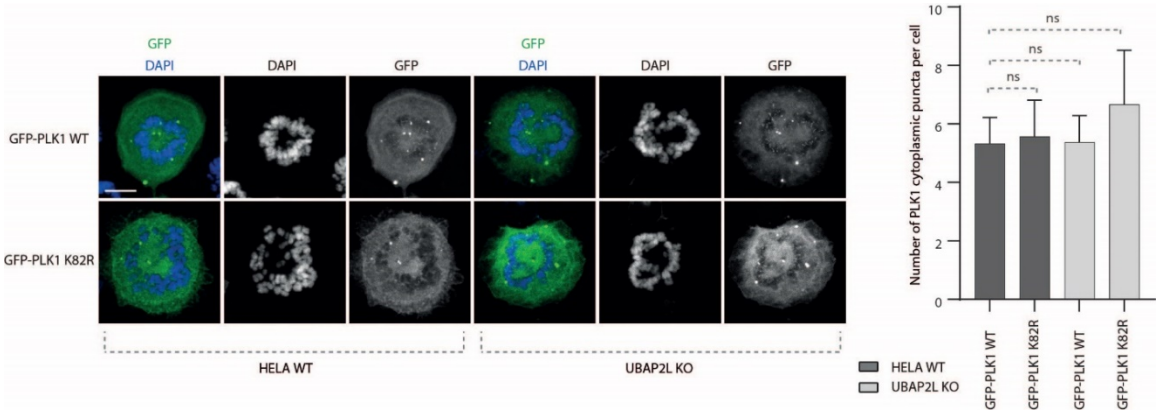

Figure: PLK1 catalytic activity is not required for its localization in cytoplasmic puncta.
(Left panel) Representative immunofluorescence (IF) pictures of HeLa WT or UBAP2L KO cells transfected with GFP-PLK1 WT or GFP-PLK1 catalytically inactive mutant K82R for 48h and synchronized in mitosis using 1µM Paclitaxel (taxol) for 16h. Scale bar, 5µm.
(Right panel) Quantification of the number of PLK1 cytoplasmic foci per cell. At least 50 cells were quantified per condition for each experiment. The graph represents the mean of three biological replicates ± SD (one-way ANOVA with Sidak's correction ns=non-significant).

4. The amount of GFP-PLK1 in Fig 9c input and its amount in the IP fraction is not elevated in the UBAP2L KO cells, but rather lower. Why? This influences the amount of conjugated Ub and the conclusion of the experiment.

We agree with the reviewer that the input levels of GFP-PLK1 are not elevated in UBAP2L KO cells, which is likely resulting from overexpression of the GFP-PLK1 form. This may perturb the typical regulation of PLK1 turnover by UBAP2L, which can be clearly observed on the endogenous PLK1 pools and on the endogenously tagged version of PLK1. We disagree that moderately lowered levels of GFP-PLK1 could explain the differences in Ub-conjugated forms of PLK1 in UBAP2L KO cells.

Minor

The designation siNT (non-targeting) may cause confusion with the NT (N-terminus of UBAP2L).

Thank you for this suggestion. We modified the text and designated “non-targeting” siRNAs as “control” siRNA (siCTL) in the entire revised manuscript.

Referee #3

In their manuscript 'UBAP2L-dependent coupling of PLK1 localization and stability during mitosis', Guerber and co-workers study the role of UBAP2L in mitosis. In summary, they show that:

- UBAP2L inactivation leads to widespread mitotic defects in multiple cell lines
- Inactivation of UBAP2L leads to higher levels of Plk1.
- The C-terminal domain of UBAP2L regulates Plk1 levels, independently of stress granules
- UBA2L is described to localize at kinetochores.
- Plk1 levels are elevated at multiple sites during mitosis
- Plk1 inhibition in UBAP2L-depleted cells rescues mitotic defects to some extent.

We thank the reviewer for helpful suggestions on the manuscript, which largely overlap with the comments of reviewers 1 and 2. We also appreciate that reviewer 3 recognized the importance of our findings on UBAP2L-dependent regulation of PLK1 during mitosis as stated above.

Some remaining questions are: why is Plk1 not degraded by the APC/C-Cdh1 in G1? Does UBAP2L work epistatically with APC/C?

We agree with the reviewer that this is an important remaining question. One possibility would be that the interaction of PLK1 with the APC/C co-activators CDH1 and CDC20 is affected in UBAP2L-deficient cells or that UBAP2L itself might interact with the APC/C complex during mitosis. Moreover, we cannot formally exclude that unknown E3-ubiquitin ligases, other than APC/C, contribute to the UBAP2L-dependent regulation of PLK1 turnover. Identification and characterization of such mechanisms constitutes an important future perspective of our study, which will require substantial amount of work and therefore falls beyond the scope of the current manuscript. Nevertheless, we carefully discuss this point in the revised version of the manuscript (lines 465-476).

Also, I am not very convinced by the PLK1 assessment. Many of the analysis use a thresholded microscopy analysis (eg Figure 4B), which emphasizes an effect that is much more complex and less pronounced when analyzed at the single cell level (eg Figure 4C). It is clear that UBAP2L-

depletion gives mitotic defects, but how this is coordinated at kinetochores through Plk1 modulation remains insufficiently clear.

As stated in the response to comments of reviewers 1 and 2, we rephrased and tuned down the claims about the regulation of the kinetochore removal of PLK1 by UBAP2L throughout the manuscript and focus our conclusions more on the global PLK1 turnover model. Our new detailed analysis on how UBAP2L regulates the stability and localization of PLK1 throughout mitosis presented in Figures 8A-G and EV5F-G, clearly shows a pronounced effect on PLK1 at different subcellular locations upon UBAP2L depletion.

Comments:

-overall, the manuscript could be improved by textual editing. There are many grammatical errors.

This opinion is not shared by reviewers 1 and 2. Nevertheless, we have carefully screened for and corrected any possible remaining typing and grammatical errors in the revised version of the manuscript.

-Abstract: it is claimed in the abstract that 'UBAP2L regulates Plk1 and no other factors'. That is a bold claim that cannot be made on the presented data. Aurora A and B, and Cyclin B are tested, and there is some rescue, but other targets may exist. Actually, BubR1 levels are up too (fig 2J). and AurA/B and Cyclin B are up too (not statistically significant but with good effect size, so just a matter of n). Also, the RGG domain is suggested in the text to regulate other mitotic factors.

We have carefully analyzed the levels of Aurora A/B and Cyclin B and no statistically significant differences could be observed in our hands. Moreover, as suggested by reviewer 1 in his/her minor comment 5, we quantified the upper (phosphorylated) versus the lower BubR1 (unphosphorylated) band. The results presented in Figure 3K clearly indicate that the total BubR1 levels are not affected by UBAP2L depletion. Nevertheless, we agree with the reviewer that we cannot exclude an effect of UBAP2L on additional mitotic factors that we have not tested in the current study. We therefore tuned down our statements about specific effects of UBAP2L on PLK1 and modified the abstract and the text accordingly.

-Graphical abstract: it remains unclear what the yellow and pink dots in the right-hand panels represent.

The graphical abstract has been designated more precisely and adjusted to emphasize the UBAP2L-mediated PLK1 global turnover (and not the kinetochore removal) model as discussed above.

-Figure 1A: the nuclear size appears to be very different from the control cell and the KO cells. Is the scale bar the same? If yes, it could be that the KO have already undergone a defective round of mitosis, and will suffer from additional, indirect effects

Please refer to our detailed response to major comment 1 of reviewer 1. We apologize for the nuclear size confusion, since the scale bar between WT and UBAP2L KO cells in Figure 1A was in fact different. The correct scale bars have now been added to Figure 1A. We also quantified the nuclear size of WT versus UBAP2L KO cells (Figure 1G) and of control (siCTL) versus UBAP2L siRNA-treated cells (Figure 1N), confirming that there is no statistically significant difference.

-it remains incompletely clear what the mitotic phenotype is: chromosome alignment due to altered MT-KT interactions? Cohesin/condensin defects leading to anaphase bridges? Bipolar spindle formation.

Maeda et al., demonstrated that UBAP2L depletion leads to defective KT-MT attachments such as increase in side-on attachments, decrease in end-on attachments and decreased K-fiber formation. We discuss these points throughout the manuscript. The possible effects on cohesin/condensin loading in UBAP2L-deficient cells could represent logical consequences of PLK1 turnover defects (Sumara *et al*, 2002, 2004). UBAP2L depletion does not affect the formation of a bipolar mitotic spindle as shown in Figures 2G, 2H.

-Figure 2A/E: these WBs and the corresponding conclusions would need a cell cycle analysis to confirm that there are not cell cycle changes.

We provide the blots for the missing cell cycle marker (cyclin E) as requested by the reviewer in the revised Figures 3A, 3E.

-Figure 2B/F: are the nuclear Plk1 spots co-localizing with centromeres?

We included representative images demonstrating that indeed PLK1 dots may co-localize with centromeres (CREST staining) in the nucleus. These results are presented in the revised Figures 3B, 3F and described in the text (lines 178-180).

-Figure 2C/D and 2G/H: i do not understand how these plots are connected? Panels C/G appears to represent data from panel D/H with an artificial threshold. I would remove panel C/G. same goes for suppl. 1C/D/E. here single cell data should be included.

Please refer to our response to major comment 4 of reviewer 1. Single cell data analysis for the results presented in the revised Figure EV3C-E would be rather confusing to the reader, because we did not detect a significant number of cells in interphase stage expressing AurA, AurB and CyclinB1, which would allow us to efficiently quantify the nuclear intensity of these proteins. Nevertheless, we present here as an example the single data analysis for AURA and AURB as requested by the reviewer.

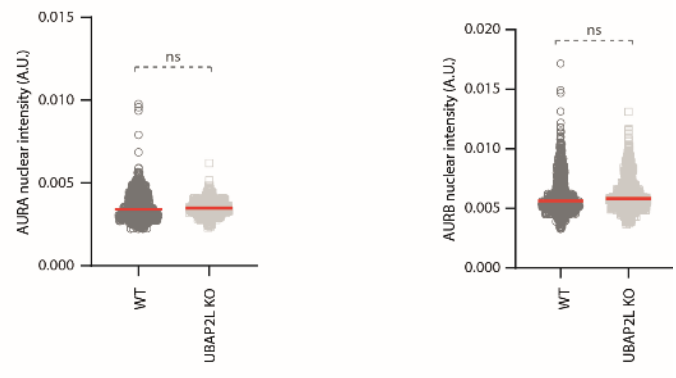

Figure: UBAP2L does not affect Aurora A and B nuclear intensity during interphase.
Quantification of AURA and AURB nuclear intensity (A.U.) in HeLa WT or UBAP2L KO cells synchronized in G1/S using Double Thymidine Block (DTB). At least 600 cells were quantified per condition for each replicate. Each dot of the graphs represents AURA or AURB nuclear intensity in a single nucleus where the signal was detectable. Interphasic cells which do not express AURA or AURB were excluded from the quantification. The measurements of three biological replicates are combined, red bars represent the mean (Mann-Whitney test, ns=non-significant).

-Suppl. Figure S3A: the CHX experiment is relevant, but requires quantification over multiple experiments.

All conclusions presented in our manuscript are derived from carefully repeated biological replicates. We feel that western blotting is not a precise quantitative method. Nevertheless, we present here the quantified PLK1 levels upon CHX treatment of the revised Figure 4A, as requested by the reviewer.

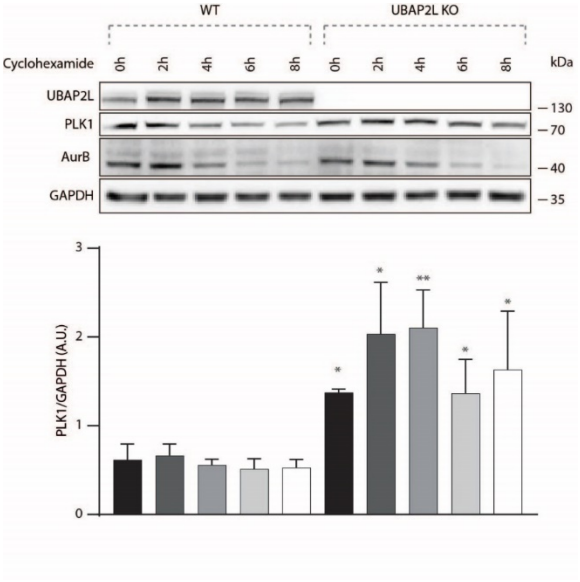

Figure: UBAP2L depletion promotes PLK1 protein stability.
(Upper panel) WB analysis of WT or UBAP2L KO HeLa lysates of G1/S synchronized (DTB) cells treated with 100µg/mL cycloheximide (CHX) for the indicated times. Proteins MW is indicated in kDa. WB is representative of three independent replicates.
(Lower panel) Quantification of the relative protein levels of PLK1. Graphs represent the average ratio of PLK1/GAPDH (A.U.) from three biological replicates ± SD (one-way ANOVA with Dunnett's correction *P<0,05, **P<0,01, ns=non-significant).

- does expression of FL or C-terminal UBAP2L also rescue mitotic defects? These data are missing.

We fully agree with the reviewer that these data were missing. We repeated rescue experiments in UBAP2L KO cells with the FL, N-terminal and C-terminal part of UBAP2L and quantified the mitotic defects. Re-expression of UBAP2L FL or the UBAP2L C-terminal fragment, but not the UBAP2L N-terminal protein part, partially rescued the mitotic defects in UBAP2L depleted cells, consistently with or data on cell proliferation (Figure 5E, 5F). Our new results are presented in Figures 5C-D and EV4C and described in the text (lines 235-240). In view of the partial rescue of mitotic defects by UBAP2L and UBAP2L-CT, we additionally discussed the possibility that regulation of mitosis by UBAP2L may not be restricted to the control of PLK1 stability (lines 410-416)

- Figure 4D: a plk1 blot is missing.

We added the missing PLK1 blot as requested in the revised Figure EV4D.

Figure 5/6: I am not convinced by the UBAP2L/CREST co-localization. Actually, it appears that UBAP2L localizes outside kinetochores in untreated cells, and stronger localization next to kinetochores upon Plk1 inactivation (Fig6D).

We fully agree with this criticism. Please refer to our detailed response to major comment 2 of reviewer 1 and to the revised Figure EV2A-E.

Figure 8: I see much more Plk1 at kinetochores, but also at other structures, including outside the chromosome areas (centrosomes/stress bodies? and also non-crest structures at chromosome areas). To what extent are these pools of Plk1 also regulated by UBAP2L? Also, the conclusion is that UBAP2L removes Plk1 from kinetochores. This I support, but also Plk1 levels at centrosomes remain elevated, and a large fraction of Plk1 does move from kinetochores to the midzone/midbody, where it accumulate. The conclusion (and mechanism) is broader than the authors claim.

We fully agree with the reviewer on his/her point raised regarding the broader regulation of PLK1 by UBAP2L, a criticism shared by all 3 reviewers. Please refer to our detailed response to major comment 3 of reviewer 1 and to the revised Figures 8A-G and EV5F-G.

-Figure 5B/C/D: cell cycle synchronization is done using severe chemical perturbations: HU, Cdk1i. It would be better to follow thym/thym-block-release cells through the cell cycle at multiple time points to investigate Plk1 levels. In the current analysis many other pathways are perturbed (DDR signaling, Cdk1 effects).

We agree with the reviewer that treatment with strong cell cycle inhibitors may perturb other pathways. However, as shown in Figures 1A-C and 1H-J in experiments performed using double thymidine protocol, UBAP2L KO cells and/or cells treated with siRNA against UBAP2L are characterized by delays in mitotic progression. Therefore, it would not be possible to compare reliably PLK1 levels throughout the cell cycle using this method, as cells would not be at the same cell cycle stage.

-Figure 9: I find this experiment rather opportunistic. Plk1 inhibition has been described to lead to many defects, including defects beyond kinetochore effects. what does this concentration of Plk1 do on control cells (this important control is missing)? Is this amount enough to induce previously described effects in control cells?

This criticism is not shared by reviewers 1 and 2 who appreciated that moderate inhibition of PLK1 by BI2536 restored proper chromosome segregation in UBAP2L deficient cells. We present here our results demonstrating that even at this low concentration of the PLK1 inhibitor (10 nM as compared to typical 100 nM widely used in the literature), control cells remain arrested in prometaphase and no effects on fidelity of chromosome segregation can be assessed. We describe in more details the experimental design and the underlying concept in the revised figure legend (lines 1083-1085).

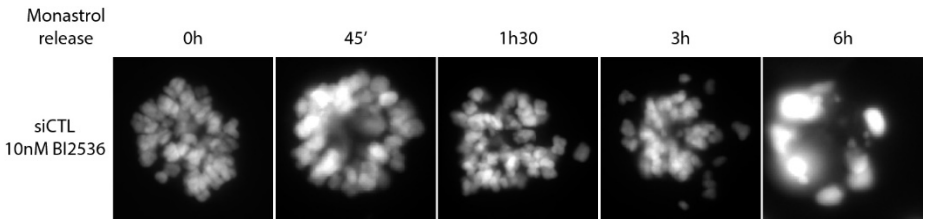

Figure: Effect of BI2536 on control HeLa cells in prometaphase.
DAPI staining of HeLa cells transfected with siCTL for 48h, synchronized in prometaphase with 1mM monastrol for 16h, treated with 10nM BI2536 for 45min and released from monastrol at the indicated time point. Cells remained blocked in prometaphase and died from prolonged arrest.

-figure 9: how do the authors explain that UBAP2L-depletion in combination with Plk1 inhibtion performs better than control cells (Fig 9F)?

This is only true for Figure 9F but not for Figures 9G and 9H. We believe that it will be very difficult to predict how a fine balance of localized PLK1 activity is changed in the course of these rescue experiments and therefore we would prefer to refrain from any speculation on this point.

References

Sumara I, Giménez-Abián JF, Gerlich D, Hirota T, Kraft C, de la Torre C, Ellenberg J & Peters J-M (2004) Roles of polo-like kinase 1 in the assembly of functional mitotic spindles. *Curr Biol* 14: 1712–1722

Sumara I, Vorlaufer E, Stukenberg PT, Kelm O, Redemann N, Nigg EA & Peters J-M (2002) The dissociation of cohesin from chromosomes in prophase is regulated by Polo-like kinase. *Mol Cell* 9: 515–525

Dear Izabela, dear Eva,

Thank you for the submission of your revised manuscript to EMBO reports and for your patience during peer review. We have now received the full set of reports from the three referees that were asked to re-evaluate your study. Please find their comments included below.

As you will see, all referees find the revised version significantly improved with all major criticisms successfully addressed, and referees #2 and #3 now recommend publication. Referee #1 is also positive but still has a number of minor concerns, which should be addressed before we can proceed to accept your manuscript. Please address all comments in a complete point-by-point response, and make sure that all changes are highlighted to be clearly visible in the revised manuscript file.

From the editorial side, there are also a few things that we need from you:

- Please provide up to 5 keywords in your revised manuscript.

- Please note that a data availability statement is mandatory. If your study does not include any datasets requiring deposition in public databases, please add the statement "This study includes no data deposited in external repositories." under the heading "Data availability" at the end of Materials and Methods.

- Please update your competing interests statement: the heading should be "Disclosure and competing interests statement" and the statement "The authors declare that they have no conflict of interest.", since you have no competing interests to declare.

- The author contributions statement should be removed from the manuscript file. Instead, we now use CRediT to specify the contributions of each author in the journal submission system. Please use the free text box to provide more detailed descriptions. See also guide to authors: <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>.

- We noticed that some sections of the author checklist have not been completed. Please provide an updated checklist with all sections completed.

- Please enter all relevant funding information in the online submission system when you submit your revised manuscript. The funding information in the system should match exactly the information provided in the Acknowledgments paragraph of the manuscript.

- Figure panels should be called out alphabetically. In the current version of your manuscript, Fig. 1G is called out after Fig. 1M. Please call them out in alphabetical order or revise your figure respectively if necessary.

- Please also note that there is a callout to Fig. EV2F, which does not exist.

- Your Figure legends have been inspected by our data editors for completeness and accuracy. Please see the required changes in the attached Word file and address all comments in your revised manuscript (with tracked changes).

- Each movie should be uploaded together with its legend in a single ZIP folder.

- Please make sure that all text in your synopsis image is easily readable.

- We noticed that more source data are provided than are ticked on the relevant checklist. Please update your source data checklist as necessary.

- Please also note that the manuscript sections are not in the correct order. Please follow the order: Title page, Abstract, Keywords, Introduction, Results, Discussion, Materials and Methods, Data availability, Acknowledgements, Disclosure and competing interests statement, References, Figure legends, Expanded View Figure legends.

- The movie legends should be removed from the manuscript file.

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We would also welcome the submission of cover suggestions or motifs to be used by our Graphics Illustrator in designing a cover.

We look forward to seeing a final version of your manuscript as soon as possible.

Best regards,

Ioannis

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

Authors focused more on the general effect of UBAP2L on PLK1 localization and stability this time.
UBAP2L downregulation leads to mitotic progression, as well as increased PLK1 level and activity. PLK1 degradation is attenuated in UBAP2L downregulated cells. UBAP2L downregulation caused PLK1 localization at centrosome, kinetochore and spindle, and this phenotype can be rescued by UBAP2L full length or C-terminus expression. UBAP2L interacts with CUL3 and mediates the interaction of PLK1 and CUL3. PLK1 inhibition can rescue the mitotic phenotypes caused by UBAP2L KD.
Minor Concerns:
1. Authors use nuclear size to assess ploidy status (Fig 1G). Is it possible to use flow sorting/centromere FISH to determine it?
2. Authors should make a note that PLK1 ectopically localized to centrosomes in UBAP2L downregulated cells in G1/S. (Fig 3B and 3F)
3. In Figure 3B-D, 3E-H, the text claimed that PLK1 was enriched at peri-centromere localization during interphase. Enlarged images for colocalization with CREST might be necessary.
4. CREST staining in Fig 3F seems not specific to centromeres.
5. The colony formation image in Fig 5E is not as representative compared to Fig 5F&G.
6. Fig 5F&G use different ways for comparison (compare with WT+Flag or UBAP2L KO+Flag).
7. In Figure 7 authors examine PLK1 dynamics after monastrol release. It is important to note if UBAP2L downregulated cells have any delay in mitotic progression.
8. Overall the control cells have relatively high mitotic error rate (20-40% in Fig 1D&E&K&L and in Fig 9F).

Referee #2:

In the revised version, the authors have adequately addressed the major criticisms and the main message of the paper is clear and interesting. For points that are less clear, the authors now refrain from over interpretations. Therefore, I think that the paper is suitable for publication in EMBO reports.

Referee #3:

The authors have addressed my comments appropriately, with a much better nuanced description of the data in the manuscript, and including experimental data to resolve questions. I support publication of the manuscript.

Point by point response to reviewers

Referee #1:

Authors focused more on the general effect of UBAP2L on PLK1 localization and stability this time.
UBAP2L downregulation leads to mitotic progression, as well as increased PLK1 level and activity. PLK1 degradation is attenuated in UBAP2L downregulated cells. UBAP2L downregulation caused PLK1 localization at centrosome, kinetochore and spindle, and this phenotype can be rescued by UBAP2L full length or C-terminus expression. UBAP2L interacts with CUL3 and mediates the interaction of PLK1 and CUL3. PLK1 inhibition can rescue the mitotic phenotypes caused by UBAP2L KD.

We thank the reviewer for the positive evaluation of our revised manuscript.

Minor Concerns:
1. Authors use nuclear size to assess ploidy status (Fig 1G). Is it possible to use flow sorting/centromere FISH to determine it?
We performed FACS analysis to assess the DNA content in WT versus UBAP2L KO cells. These results are included in the new Figure EV1D and demonstrate that UBAP2L KO cells do not display changes in ploidy status relative to WT cells.

2. Authors should make a note that PLK1 ectopically localized to centrosomes in UBAP2L downregulated cells in G1/S. (Fig 3B and 3F)
We modified the text accordingly (lines 195-196 and 198)

3. In Figure 3B-D, 3E-H, the text claimed that PLK1 was enriched at peri-centromere localization during interphase. Enlarged images for colocalization with CREST might be necessary.
We included the corresponding ROI images in the revised Figures 3B and 3F as requested.

4. CREST staining in Fig 3F seems not specific to centromeres.
We presented more representative images in Figure 3F showing specific CREST centromere signal.

5. The colony formation image in Fig 5E is not as representative compared to Fig 5F&G.
We presented more representative images in Figure 5E which correspond to the quantifications displayed in Figures 5F and 5G.

6. Fig 5F&G use different ways for comparison (compare with WT+Flag or UBAP2L KO+Flag).
The graphs in Figures 5F and G show comparison to WT+flag (G) and/or UBAP2L KO+Flag (F) as indicated by the corresponding brackets.

7. In Figure 7 authors examine PLK1 dynamics after monastrol release. It is important to note if UBAP2L downregulated cells have any delay in mitotic progression.
We thank the reviewer for this comment. It is true that according to our results presented in Figure 1, UBAP2L downregulated cells are characterized by delays in mitotic progression. Therefore, we cannot exclude the possibility that the enriched PLK1 protein levels after the monastrol release presented in Figure 7D can be partially attributed to the fact that cells are not at the same cell cycle stage. However, in the data presented in Figure 7A we compared PLK1 levels in cells of the same mitotic stage. To avoid confusion, we modified the text accordingly (lines 310-312).

8. Overall the control cells have relatively high mitotic error rate (20-40% in Fig 1D&E&K&L and in Fig 9F).
It is widely known that the genome of HeLa cells is error-prone, which can explain the relatively high rate of mitotic errors in the control conditions in particular in cells treated by repeated exposure to light (Fig.1 live video) or by chemicals (Fig 9E, F, DMSO).

Referee #2:

In the revised version, the authors have adequately addressed the major criticisms and the main message of the paper is clear and interesting. For points that are less clear, the authors now refrain from over interpretations. Therefore, I think that the paper is suitable for publication in EMBO reports.
We thank the reviewer for the positive evaluation of our revised manuscript and for supporting its publication.

Referee #3:

The authors have addressed my comments appropriately, with a much better nuanced description of the data in the manuscript, and including experimental data to resolve questions. I support publication of the manuscript.
We thank the reviewer for the positive evaluation of our revised manuscript and for supporting its publication.

Dr. Evanthia Pangou
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Illkirch, Grand Est 67400
France

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The data shown in figures should satisfy the following conditions:

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- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

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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.
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