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Differential control of Eg5 dependent centrosome separation by Plk1 and Cdk1

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Transaction Report:

(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 06 September 2010

Thank you for submitting your manuscript on Plk1 and Cdk1 roles in Eg5-dependent centrosome separation for our consideration. I have solicited the input of three expert reviewers, whose evaluations (attached below) we have now received. I am afraid to say that taken together, these reports do currently not offer sufficiently strong support for publication in The EMBO Journal. On one hand, all referees clearly appreciate the importance and interest of the topic, and also acknowledge the technical quality of the data and the overall presentation of the study. At the same time, they however point out that the study remains at this stage mostly descriptive and, with regard to understanding of the different centrosome separation mechanisms, still somewhat too preliminary for publication in a broad general journal. As you will see, they raise a number of overlapping issues and question marks regarding the exact roles of each of the main CDKs and Plk1 (summarized most explicitly in the report of referee 2). Given the extent of these criticisms and the large number of open questions to be understood, I am afraid I have to concur with the opinion most clearly stated in referee 3's comments, that the study is currently not a good candidate for publication in The EMBO Journal. I therefore unfortunately do not see myself in the position to invite a regular revision of your present manuscript, as we can only afford to do this for studies that are met with enthusiasm from at least a majority of referees already upon initial review, and in cases where the absolutely required revision work appears to be manageable within a reasonable time frame.

Should you however feel confident that you may be able to further elucidate the underlying mechanisms along the lines suggested in detail especially by referees 1 and 2, then I would in light of the overall interest of the topic nevertheless be open to look at an extended new version of this study. Such an improved manuscript would however have to be treated as a new submission rather

than a revision (also with respect to the literature at the time of resubmission) and only sent back to our referees if we thought that the main issues had been largely answered and the insight into a majority of the open questions sufficiently advanced.

I am sorry we cannot be more positive on this occasion, but would in any case like to thank you for the opportunity to consider this manuscript, and I hope that you will find our referees' comments helpful.

With best regards,

Editor
The EMBO Journal

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REFEREE REPORTS

Referee #1 (Remarks to the Author):

Review Report Smith et al. EMBO J

This paper studies the role of Plk1, CDK1 and Eg5 in centrosome separation in prophase. In general this study is of high technical quality, the experiments are well controlled and the conclusions are supported by the data. The only drawback is that this study is rather descriptive, so there is limited mechanistic insight into how Plk1 and CDK1 might act to promote centrosome separation. Nonetheless, I think this study does provide several important novel insights into the process of centrosome separation in prophase, which has remained a rather mysterious process and, if the points mentioned below are adequately addressed, I feel it is in principle suited for publication in EMBO Journal.

Specific points:

One of the main points of the manuscript is that CDK1 is not essential for prophase centrosome separation and the authors mention that this is probably because CDK2 can compensate to some extent for CDK1 function, at least in Eg5 phosphorylation. However, it is not completely clear to what extent CDK1 and CDK2 have overlapping functions in prophase centrosome separation:

Is Eg5 still phosphorylated in a CDK2 RNAi/inhibition alone?

Does prophase centrosome separation still occur in CDK2 RNAi/inhibition and CDK2 RNAi/inhibition + RO (or roscovitine treated)? The authors refer to the Hochegger et al., 2007 JCB paper, in which it should show that combined inhibition of CDK1/2 blocks centrosome separation, however in this paper the authors show that inhibition of CDK1/2 results in defects in centrosome duplication, I could not find any experiments in the 2007 manuscript in which CDK1/2 were inhibited after duplication to look at centrosome separation in G2/prophase.

When CDK1 is inhibited and cells are arrested in G2/prophase with separated centrosomes, do centrosomes than collapse upon CDK2 inhibition?

Is centrosome separation also blocked by combined inhibition of CDK2 and Plk1? What happens to centrosome separation after Plk1 inhibition alone?

Also, it would be nice to see Eg5 localization on prophase asters in CDK1 inhibition, CDK2 inhibition and double inhibition, as lack of phosphorylation of T927 should decrease Eg5's binding to microtubules.

Activation of CDK1 in the presence of continued Plk1 inhibition results in rapid centrosome separation (Fig. 4). However, Eg5 is already maximally phosphorylated in CDK1 inhibited cells (at least on T927). So CDK1 must be doing something else to drive sudden centrosome separation. Could the function of CDK1 in this setup be simply to break centrosome cohesion, rather than activate outward pushing forces (which are already "on" due to CDK2-dependent phosphorylation of

Eg5)? In this context it is important to be able to distinguish between inhibition of centrosome separation due to defects in centrosome cohesion vs defects in pushing/pulling forces.
Perhaps the authors could analyze centrosome movement in C-NAP1 RNAi in the presence of the CDK/Plk1 inhibitors? And in a parallel approach look at C-NAP1 localization in the different conditions (which is displaced after loss of centrosome cohesion, Fry et al., 1998 JCB)?

Minor points:

In figure 3B is unclear to me what the difference is between the second and fourth condition

I don't think the authors of the Woodcock et al., 2009 paper suggest that Tiam1/Rac1 act through actin to regulated prophase centrosome separation, as mentioned in the discussion

STLC was first described in debonis et al., Mol cancer ther. 2004, not the 2006 paper cited here.

Referee #2 (Remarks to the Author):

Differential control of Eg5 dependent centrosome separation
by Plk1 and Cdk1 by Ewan Smith, Clare Vesely, Nadia HÈgarat, Isaac Roseboom, Hansjˆrg Streicher, Chris Larch, Kees Straatman, Toru Hirota, Ryoko Kuriyama and Helfrid Hochegger

The authors used a DT40 cell line expressing an allele sensitive CDK1 that can be inhibited by 1NMPP1. The manuscript then reports that centrosome separation can occur in the absence of CDK1 activity that was supposed to phosphorylate the kinesin EG5 on Thr927. The authors show that CDK2 replaces CDK1. They observed that separation is delayed in the absence of CDK1 and becomes dependent on PLK1 activity. This PLK1 dependent separation is also dependent on proteasome mediated proteolysis, while CDK1 dependent centrosome separation is not. In the absence of CDK1, they also found that inhibition of PLK1 in G2 triggers reversal of centrosome separation.

The manuscript is well written; the experiments have been well conducted and are well described. The authors identified different players and pathways involved in centrosome separation but overall I found the manuscript very descriptive. The authors do not really clarify the situation and leave the reader with too many questions.

To summarize:
Phosphorylation of EG5 on Thr927 is required for centrosome separation. The site is phosphorylated by CDK1 but when CDK1 is absent, CDK2 replaces CDK1
- DOES CDK2 PLAY ANY ROLE IN THE PRESENCE OF CDK1?

In the absence of CDK1 (that has multiple substrates) centrosome separation is delayed
- IF THR927 IS PHOSPHORYLATED, THEN WHY A DELAY IN CENTROSOME SEPARATION? ANOTHER NOT FULFILLED CDK1 FUNCTION?

In the absence of CDK1, PLK1 is required for centrosome separation (that appears to be much slower). This slow separation remains dependent on EG5
- WHAT IS THE FUNCTION OF PLK1? IT DOES NOT PHOSPHORYLATE EG5. WHAT DOES PLK1 PHOSPHORYLATE?
- WHAT IS THE KINETICS OF EG5 PHOSPHORYLATION ON THR927 WITH OR WITHOUT CDK1?
- DOES THE SPEED OF SEPARATION DEPEND ON THR927 PHOSPHORYLATION

PLK1 dependent separation requires proteasome dependent degradation
- OF WHAT PROTEIN(S)?
- THE REQUIREMENT OF PROTEASOME MEDIATED DEGRADATION IS PUZZLING. WHY WOULD DEGRADATION BE NEEDED ONLY IN THE ABSENCE OF CDK1. WOULD

THIS BE ONLY DUE TO THE FACT THAT CDK1 DOES INHIBIT PROTEOLYSIS?

Inhibition of PLK1 induces a reversal of centrosome separation in G2. The fact that PLK1 inhibition in a background of CDK1 depletion triggers reversion of centrosome separation seems to indicate that PLK1 is required to inhibit motor proteins that counteract EG5 (as suggested by the authors).
- WHAT ARE THOSE MOTOR PROTEINS?

The scheme in the last figure does not help

Referee #3 (Remarks to the Author):

In the submitted manuscript the authors report on a Cdk1-independent, Plk1- proteasome-dependent mechanism of centrosome separation in mammalian cells. In contrast to the Cdk1-driven centrosome separation, centrosomes separate slowly when Cdk1 is inactive. In the absence of Cdk1 activity, Eg5 is still phosphorylated at T927, probably by Cdk2. Inhibition of Plk1 does not abolish phosphorylation of T927.

The paper addresses an interesting finding and the presented data are of good quality. However, the physiological relevance and the mechanism of Plk1-dependent separation remains enigmatic. Given the plethora of Plk1 functions the discussion about balance of forces is too speculative. I recommend that the authors submit their manuscript at a more specialized journal. Alternatively, the authors should dissect the underlying mechanism. Which are the residues phosphorylated by Plk1 how does the phosphorylation event affect the enzymatic properties of Eg5 ?

Resubmission 03 February 2011

We believe we have addressed the specific comments of the reviewers and have added considerable mechanistic insight to our previous findings. The previous MS showed that Plk1 and Cdk1 trigger centrosome separation independently. Plk1 dependent separation is slow and staggering, while Cdk1 induced separation proceeds in a fast and linear movement. Both pathways depend on the motor protein Eg5. We found that Cdks already phosphorylated Eg5 at Thr927 in interphase, which could explain why centrosome separation could occur independently of Cdk1. These findings left a number of questions unanswered, as pointed out by the reviewers.

Firstly both reviewer 1 and 2 raised the question on which Cdk actually phosphorylates Eg5 in interphase. Using Cdk2 knock out DT40 cells we clarified this point and show that either Cdk1, or Cdk2 can execute this step, and only inactivation of both kinases results in the inhibition of this Thr927 phosphorylation. Secondly, we needed to further clarify the mechanism by which Plk1 triggers centrosome separation. We show now that Plk1 is involved in both displacement of C-Nap1 from the centrosome, and also plays a critical role in centrosome loading of Eg5 in parallel with Cdks. The third major question concerns the difference in the dynamics of Cdk1 versus Plk1 triggered separation. We pursued two possible hypotheses: Cdk1 could further modify Eg5 thereby enhancing its activity. We undertook a search for posttranslational changes in Eg5 purified from either G2 arrested or mitotic cells using Mass Spectrometry. However, we could only identify the Thr927 phosphorylation, which was detectable in both G2 and M-phase Eg5. This suggests that there is no major change in Eg5 itself, between G2 and M-phase cells and that the crucial regulatory step triggered by mitotic Cdk1 may involve another target. We then hypothesized that this regulatory mechanism may involve the forces that push centrosomes back together in interphase. If this were true, then it should be possible to speed up Plk1 dependent centrosome separation by simply removing these Eg5 opposing forces. We developed an assay to probe these forces in G2 phase and found that they may be identical to the forces that maintain centrosome positioning in interphase. These have been shown to involve long astral microtubules reaching to the cell cortex. Accordingly we found that disruption of the actin cytoskeleton and perturbation of microtubule

polymerization abrogates the Eg5 opposing movements of the centrosomes. Strikingly, we found that it was indeed sufficient to disrupt these forces to significantly speed up Plk1 dependent centrosome separation. This suggests that Eg5 is already primed for action by Plk1 and Cdk2 in interphase, but held in check by MTs that push on the centrosomes from the cell cortex. Cdk1 would then trigger the process simply by increasing dynamic instability of the long interphase microtubules. We confirmed that MT stability is dramatically decreased following Cdk1 activation as has been previously observed in many different models. We also show that artificial MT destabilization enhances Plk1 dependent centrosome separation, while MT stabilization slows down separation induced by Cdk1, providing further support for our model. In summary, we believe that this work constitutes an important advance in our understanding of the regulation of centrosome separation and that we have added significant new findings in this revised version. We have been looking at the concerted control of centrosome disjunction, separation and reversion of separation and uncovered several novel regulatory principles.

Below we give a point-by-point rebuttal to the reviewer's comments. We would be grateful if you could re-consider this revised study for publication in EMBOJ.

Reviewers
Referee #1 (Remarks to the Author):
One of the main points of the manuscript is that CDK1 is not essential for prophase centrosome separation and the authors mention that this is probably because CDK2 can compensate to some extent for CDK1 function, at least in Eg5 phosphorylation. However, it is not completely clear to what extent CDK1 and CDK2 have overlapping functions in prophase centrosome separation:
Is Eg5 still phosphorylated in a CDK2 RNAi/inhibition alone?

We have performed this experiment using Cdk2 knock out DT40 cells and show in Figure 5E that either Cdk1 or Cdk2 can phosphorylate Eg5 at Thr927, and that only inactivation of both results in a inhibition of this phosphorylation.

Does prophase centrosome separation still occur in CDK2 RNAi/inhibition and CDK2 RNAi/inhibition + RO (or roscovitine treated)? The authors refer to the Hochegger et al., 2007 JCB paper, in which it should show that combined inhibition of CDK1/2 blocks centrosome separation, however in this paper the authors show that inhibition of CDK1/2 results in defects in centrosome duplication, I could not find any experiments in the 2007 manuscript in which CDK1/2 were inhibited after duplication to look at centrosome separation in G2/prophase.

We show in Figure S3F that centrosome separation is blocked only when both Cdk1 and Cdk2 is inactivated

When CDK1 is inhibited and cells are arrested in G2/prophase with separated centrosomes, do centrosomes than collapse upon CDK2 inhibition?

Yes we see a similar collapse, as shown in Figure S03F and Figure 6D

Is centrosome separation also blocked by combined inhibition of CDK2 and Plk1?
What happens to centrosome separation after Plk1 inhibition alone?

In the absence of Plk1 initial separation proceeds only with a slight delay. Following NEBD, gamma Tubulin is dispersed from the centrosomes and the spindles collapse (see Figure S2 and 3).

Also, it would be nice to see Eg5 localization on prophase asters in CDK1 inhibition, CDK2 inhibition and double inhibition, as lack of phosphorylation of T927 should decrease Eg5 s binding to microtubules.

We show in Figure 5F that both Cdk1/2 and Plk1 are required for Eg5 localization at the centrosome.
Activation of CDK1 in the presence of continued Plk1 inhibition results in rapid centrosome separation (Fig. 4). However, Eg5 is already maximally phosphorylated in CDK1 inhibited cells (at least on T927). So CDK1 must be doing something else to drive sudden centrosome separation. Could the function of CDK1 in this setup be simply to break centrosome cohesion, rather than activate outward pushing forces (which are already "on" due to CDK2-dependent phosphorylation of Eg5)? In this context it is important to be able to distinguish between inhibition of centrosome separation due to defects in centrosome cohesion vs defects in pushing/pulling forces.

We show that both timing of disjunction but also velocity of separation is slowed down in the absence of Cdk1 (See Figure 3). A crucial new finding in the revised version suggests that Cdk1 regulates the Eg5 opposing forces rather then Eg5 itself. Accordingly, we can speed up Plk1 dependent separation considerably by artificially blocking the Eg5 opposing force (See Figure 06 and 07).

Perhaps the authors could analyze centrosome movement in C-NAP1 RNAi in the presence of the CDK/Plk1 inhibitors? And in a parallel approach look at CNAP1 localization in the different conditions (which is displaced after loss of centrosome cohesion, Fry et al., 1998 JCB)?

We have addressed that this question and found the Plk1 acts both upstream of C-Nap1 displacement, but is also required for the actual separation process. Thus, C-Nap1 siRNA is not sufficient to overcome the block imposed by Plk1 inhibitors (see Figure 4).

In figure 3B is unclear to me what the difference is between the second and fourth condition

We have corrected the mistake

I don't think the authors of the Woodcock et al., 2009 paper suggest that Tiam1/Rac1 act through actin to regulated prophase centrosome separation, as mentioned in the discussion

Our own findings now provide evidence for an involvement of the actin cytoskeleton in opposing centrosome separation. We have amended the discussion accordingly.

STLC was first described in debonis et al., Mol cancer ther. 2004, not the 2006 paper cited here.
We have changed the citations as suggested.

Referee #2 (Remarks to the Author):
Phosphorylation of EG5 on Thr927 is required for centrosome separation. The site is phosphorylated by CDK1 but when CDK1 is absent, CDK2 replaces CDK1
- DOES CDK2 PLAY ANY ROLE IN THE PRESENCE OF CDK1?

We show in Figure 5D that either Cdk1 or Cdk2 are sufficient to allow Thr927 phosphorylation in interphase.

In the absence of CDK1 (that has multiple substrates) centrosome separation is delayed
- IF THR927 IS PHOSPHORYLATED, THEN WHY A DELAY IN CENTROSOME SEPARATION? ANOTHER NOT FULFILLED CDK1 FUNCTION?

We provide new evidence that this delay is due to the Eg5 opposing forces and not due to further activation of Eg5 by Cdk1. Accordingly, we find that inhibition these Eg5 opposing forces speeds up Plk1 dependent centrosome separation (Figure 6). Our experiments in Figure 7 suggest that Cdk1

controls these forces by enhancing the dynamic instability of MTs in mitosis. In the absence of CDK1, PLK1 is required for centrosome separation (that appears to be much slower). This slow separation remains dependent on EG5

- WHAT IS THE FUNCTION OF PLK1? IT DOES NOT PHOSPHORYLATE EG5. WHAT DOES PLK1 PHOSPHORYLATE?

We show that Plk1 acts on C-NAP1 displacement (Figure 4) and also on Eg5 loading on the centrosome (Figure 04) in concert with Cdk activity.

- WHAT IS THE KINETICS OF EG5 PHOSPHORYLATION ON THR927 WITH OR WITHOUT CDK1?

Cdk1 activity is not essential for Thr927 phosphorylation but this step can also be performed by Cdk2. The levels of the phosphorylation are not changed with or without the Cdk1 activity when G2 and M-phase cells are compared in Figure 5.

DOES THE SPEED OF SEPARATION DEPEND ON THR927 PHOSPHORYLATION

New experiments in Figure 6 and 7 show that the increase in speed triggered by Cdk1 is not due to increased Eg5 phosphorylation, but on the inactivation of the Eg5 opposing forces. However, Thr927 has been shown to be essential for Eg5 interaction with MTs and localisation to the spindle and is likely to be essential for the process.

PLK1 dependent separation requires proteasome dependent degradation
- OF WHAT PROTEIN(S)?
- THE REQUIREMENT OF PROTEASOME MEDIATED DEGRADATION IS PUZZLING. WHY WOULD DEGRADATION BE NEEDED ONLY IN THE ABSENCE OF CDK1. WOULD THIS BE ONLY DUE TO THE FACT THAT CDK1 DOES INHIBIT PROTEOLYSIS?

Figure 04 now includes experiments implicating Plk1 in the displacement of CNap1 in the disjunction step. We have taken the proteasome data out of the revised version, because we do not have a mechanistic explanation for this phenomena, and the focus of the paper has shifted towards the balance of pushing and pulling forces on the centrosome.
Inhibition of PLK1 induces a reversal of centrosome separation in G2. The fact that PLK1 inhibition in a background of CDK1 depletion triggers reversion of centrosome separation seems to indicate that PLK1 is required to inhibit motor proteins that counteract EG5 (as suggested by the authors).

- WHAT ARE THOSE MOTOR PROTEINS?

We have further investigated the forces that oppose Eg5 in interphase. Our experiments in Figure 06 and 07 suggest that these forces depend on stable astral MTs and the actin cytoskeleton and may be identical to the forces described by Burakov et al. that push the centrosome in the cell centre. In current models this force could be provided by the dynactin/actin complex, by an unknown kinesin, or by MT polymerization pushing against the cortex and we discuss these possibilities in detail on page 17. We also provide evidence that this force may be the regulatory target to trigger fast separation in mitosis. This is a novel link and has major implications on the mechanism of centrosome separation.

The scheme in the last figure does not help

We have replaced the scheme in Figure 07.

Referee #3 (Remarks to the Author):
The paper addresses an interesting finding and the presented data are of good quality. However, the physiological relevance and the mechanism of Plk1- dependent separation remains enigmatic. Given the plethora of Plk1 functions the discussion about balance of forces is too speculative. I recommend that the authors submit their manuscript at a more specialized journal. Alternatively, the authors should dissect the underlying mechanism. Which are the residues phosphorylated by Plk1 how does the phosphorylation event affect the enzymatic properties of Eg5 ?

In this revision we have considerably advanced our mechanistic understanding of the balance of forces controlling centrosome separation. We show that Plk1 and interphase Cdk activity prime the centrosome for separation in G2 phase by controlling C-Nap1 displacement and Eg5 loading on the centrosomes, but that opposing forces, which require the actin cytoskeleton and stable interphase MTs, hinder separation. We show that under these circumstances it is sufficient to break these Eg5 opposing forces to speed up the separation and we provide evidence that this is a novel regulatory step that is controlled by mitotic Cdk1 through changes in MT dynamics. We believe that our study provides several important novel findings and significantly advances our understanding of centrosome separation.

We would like to thank all the reviewers for their constructive comments and suggestions. We believe that the revision and the additional experiments have significantly improved the quality of the MS and hope that we have satisfied all the requirements made by the reviewers.

2nd Editorial Decision 18 March 2011

Thanks for your patience during the re-review of your manuscript (previously EMBOJ-2010-75652) by the three original reviewers. We have now received all their reports (attached below), and I am happy to inform you that a majority of referees is now in favor of publication. Referee 3 is still not convinced by some of the data and conclusions, but following further consultations with one of the other referees (whom I asked to comment on the validity/significance of referee 3's remaining concerns), I do not think that these issues should further prevent publication in The EMBO Journal. We are therefore happy to accept the manuscript for publication!

Before we shall be able to proceed with formal acceptance, there are a number of minor presentational issues I need you to take care of:
- please provide individual files for the manuscript text (in a text file format) and for each of the main figures, as well as one single PDF combining all the 'printable' supplementary information
- accordingly, please remove supplementary information from the main text file
- include a brief 'author contribution' explanation at the end of the manuscript text (next to the 'acknowledgements' section)
- include a brief 'conflict of interest' declaration at the end of the manuscript text
- we will definitely require higher quality figure files - in the current version, conversion and compression artifacts compromise the quality, see e.g. the background shadows in Fig 1B's FACS profiles, or around the blot bands in Fig 5

I am thus returning the paper to you one more time, to allow you to upload the required files using the link below. Once we will have received and checked the revised/improved files, we should then be able to swiftly proceed with acceptance and production. Please not that given the extra input I received on the remaining referee criticisms, I will not require additional responses to those point from you anymore.

With best regards,
Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

The authors have addressed all my concerns. I think this is a very nice and technically high-quality study that will be of interested to both the cytoskeleton and cell cycle fields and I feel this paper is now suitable for publication in The EMBO Journal.

Referee #2 (Remarks to the Author):

This is a revised version of a previously submitted manuscript (sept 2010).

The authors observed that centrosome separation can occur in the absence of Cdk1, in this case inhibition of Plk1 blocked separation as well as inhibition of Eg5.
By comparing both Cdk1 and Plk1 inhibition, the authors observed different kinetics in centrosome separation, slower in the absence of Cdk1.
The effect of Plk1 on centrosome separation is reported to be due to C-Nap1 displacement from centrosomes but not only since depletion of C-Nap1 did not rescue Plk1 inhibition. The authors also report that both Cdk2 and Cdk1 phosphorylate Thr927 in interphase and that this phosphorylation is required to localize Eg5 at the centrosome only in the presence of Plk1. Then the authors analysed forces working in opposition of Eg5 during centrosome separation reversion. They report that inhibition of actin or MT polymerisation inhibits centrosome separation reversion.

The present manuscript has been greatly improved. The authors answered all my questions and performed requested experiments. This is now a very nice paper, and I don't have any further comments.

Referee #3 (Remarks to the Author):

In the submitted manuscript, Smith and colleagues describe a mechanism of centrosome separation in mammalian cells. Inhibition of Cdk1 results in untimed centrosome separation. Co-inhibition of Plk1 and/or Eg5 suppresses Cdk1-independent centrosome separation suggesting that an alternative, less efficient centrosome pathway exist that depends on Eg5 and Plk1. Depolymerization of actin promotes Plk1-mediated centrosome separation. The submitted manuscript describes an interesting finding. However, I still think that the paper is suitable for a more specialized journal. Figures 1-4 are convincing but do not provide much new data. Figures 5 -6 are less convincing.
In their response to reviewer #2, the authors answer the question ".....What is the function of Plk1?" with "… We show that Plk1 acts on C-NAP1 displacement...."
However, as shown in figure 4 depletion of C-Nap1 does not rescue centrosome separation in Plk1 inhibited cells. The data regarding Eg5 loading are not convincing. If Eg5 is phosphorylated despite Cdk1 inhibition (Fig. 5E) and no further Cdk sites are present in Eg5 this implies that the failure of Eg5 localization in Cdk1 Cdk2 inhibited cells is indirect. Are the cells in the same cell cycle state? Thus, neither C-Nap depletion is sufficient to restore centrosome separation in Plk1-inhibited cells nor are the data regarding Eg5 convincing. This is the weakest point of the paper and the postulated major novel finding.
Fig. 3A shows and it is stated in the text that the GFP-g-tubulin signal disappears after 20 min with Cdk1on/Plk1off. How can the authors quantify under these conditions centrosome separation up to 180min as shown in figure 2.
On p13 the authors argue that microtubule destabilization with low doses of nocodazole should speed up centrosome separation in G2 but not M. The actual concentration of nocodazole used in this experiment (20ng/ml = app 66nM) stabilizes microtubules. This effect of low doses of nocodazole is well described in the literature.

