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# Chromosome misalignments induce spindle-positioning defects

Mihoko Tame, Jonne Raaijmakers, Pavel Afanasyev, Rene Medema

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**Review timeline:**

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| Revision received: | 08 December 2015 |
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Editor: Barbara Pauly

## 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 10 September 2015

Thank you for the submission of your research manuscript to our journal. I have taken over its handling as my colleague Barbara is currently not in the office. We have now received the full set of referee reports that is copied below.

As you will see, all referees acknowledge that the findings are interesting and should be published. However, both referees 1 and 2 point out that discrepancies between your findings and published data should be discussed and possible explanations provided. Referee 3 further notes that Plk1 substrates should be identified in the given context. While from their cross-comments it becomes clear that referee 2 does not think that substrate identification is necessary, referee 1 agrees that a role of NDR downstream of Plk1 should be investigated, and we therefore think that this should be done. Referee 3 also indicates that it should be examined whether chromosome misalignment can alter the cell division plane. The referees further pinpoint missing controls and clarifications that need to be added.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns 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.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient

 

for the revisions so that we can discuss the revisions further. Given the current format I suggest that we publish your study as a short report. For short reports, the revised manuscript should not exceed 35,000 characters (including spaces and references) and 5 main plus 5 expanded view figures. Commonly used materials and methods can further be moved to the supplementary information, however, please note that materials and methods essential for the understanding of the experiments described in the main text must remain in the main manuscript file.

Regarding data quantification, can you please specify the number "n" for how many experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends? This information is currently incomplete and must be provided in the figure legends. Please also include scale bars in all microscopy images.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The mitotic spindle ensures proper chromosome alignment and controls cleavage plate. Proper positioning of the spindle is important for normal cell division and development. Spindle positioning in many cell types is regulated by LGN-dynein-dependent cortical pulling forces exerted on astral microtubules. However, the molecular mechanisms regulating the localization of cortical dynein remain elusive. A previous study suggested that the chromatin-factor Ran-GTP gradient and spindle-pole-localized Plk1 negatively regulate the localization of cortical dynein to define spindle position (Kiyomitsu.T & Cheeseman IM, NCB, 2012). In the current study, however, the authors found a correlation between chromosome misalignments and spindle-positioning defects, and show a novel pathway by which KT-localized Plk1 on misaligned chromosomes controls spindle positioning by displacing cortical LGN. In general, the experiments were well designed and the results are compelling. I have some minor comments listed below, which should be clarified before this manuscript is accepted.

Major comments:
1. In sharp contrast to the previous study (Kiyomitsu.T & Cheeseman IM, NCB, 2012), the authors now show that Ran-GTP is not required for LGN delocalization (Fig S2), and that spindle-pole localized Plk1 restricts cortical LGN localization (Fig S3). Therefore, they should offer some explanation for these discrepancies, and/or repeat the same experiments.

2. Fig 3A: The authors performed siPBIP1 to deplete KT-enriched Plk1. Although they show the reduction of Plk1 at kinetochores, they should quantify the Plk1signal at centrosomes as a control.

Minor comments:
1. In Fig1, the authors show the results of measuring the spindle angle in each cell. However, the method of measurement is difficult to understand. They should provide a representative picture or schematic diagram that shows the method for measuring the spindle angle.

2. Fig 2B, Fig 3B, Fig S2 D: It would be better to distinguish the count numbers between the GFP-LGN positive and negative groups.

3. Fig S3 C right: Does the Y axis of the graph indicate different cells?

Referee #2:

Tame and coworkers have investigated the impact of chromosome alignment on spindle positioning. They report that depletion of Spindly, CLIP-170 and CENP-E all result in both impaired chromosome alignment and spindle misorientiation. Importantly, the recruitment of dynein to the cortex does not seem to be affected, raising the question of how misaligned chromosomes might affect spindle positioning. Previously, it had been reported, by Kiyomitsu and Cheeseman (ref 13),

 

that misaligned chromosomes disrupt an interaction of dynein/dynactin with LGN, a cortical docking factor, and that this effect was triggered by Ran-GTP. However, results reported in this new study from the Medema lab do not support this claim. These authors in fact found that i) LGN localization is locally reduced by the proximity of misaligned chromosomes and that this local restriction of LGN localization cannot be attributed to Ran. Instead, they identify kinetochore-associated Plk1 as a likely regulator of LGN localization. In support of this conclusion, they show that interference with KT-localization of Plk1 (via PBIP1 siRNA) rescues cortical LGN in proximity to misaligned chromosomes. Thus, they conclude that KT-associated Plk1 is responsible for displacing LGN from cortical sites in proximity to chromosome misalignment, thereby providing a causal link between chromosome misalignment and spindle orientation defects.

Overall, I find this study very interesting. Prior to publication, I suggest that the authors address the following points:

Criticism:

1. In my opinion, the authors have a tendency to overemphasize the "novelty" of the link they have uncovered. For example, in the last sentence of the abstract, they refer to chromosome alignment and spindle orientation defects a "previously unlinked phenomena", giving the impression that this connection has not previously been explored. (Similar claims to novelty are made in several other places throughout the manuscript). Yet, Kiyomitsu and Cheeseman (ref 13) had previously investigated the connection between chromosome proximity to cortex and dynein localization, and much of the present paper is actually devoted to casting doubt on their conclusions (which was centered on Ran disrupting dynein-LGN interaction). I find the present data convincing, both those arguing against the role of Ran, as well as those implicating KT-associated Plk1 in the process. I wonder whether the authors can offer an explanation for why their findings on Ran and LGN differ so drastically from those reported in ref. 13?

2. p.5. last sentence of first paragraph: "Therefore, we conclude that misaligned chromosomes are the common cause..." In my view this is an exaggerated statement. The connection is plausible (tempting at the level of a speculation or a hypothesis), but not definitive. What the authors report, at this stage of the study, is primarily a CORRELATION, suggesting a common cause, but by no means proving it.

3. p. 5/6. Second paragraph. I wonder why the authors all of a sudden adopt noscapine treatment to cause chromosome misalignment - why not stick to (e.g.) Spindly depletion? Considering that the last paragraph of the introduction had whetted the reader's appetite for a study on "spindle-positioning defects seen after Spindly depletion", it comes as a bit of a surprise that many of the subsequent experiments actually rely on use of noscapine (and later on the Eg5 inhibitor STLC).

4. As confirmed in this study, spindly depletion is known to cause extensive spindle rotation (e.g. ref. 22). I wonder whether the authors have considered a possible impact of spindle rotation on some of their results? Could some of the "switching" they observe actually reflect spindle rotation? (Did they somehow control for this?)

5. Related to Fig. S3D, they state (page 7) that LGN switching and spindle movement are "abolished" after STLC and BI2356 treatment. "Reduced" would seem more appropriate. Regarding the line graphs shown in Figures S3C and D, I presume that each line represents the behaviour of one single cell? In any case, it would be helpful to have this better explained in the corresponding legend.

6. Figure 2: some of these IF images look strange: why does GPF-LGN staining appear as double lines in these images?

7. Figure 3A and B: considering that siPBIP1 only depletes Plk1 from about 35% of KTs (3A, right panel), I find the effect on cortical LGN surprisingly large (3B, right panel). What was actually counted in these histograms - only cells with Plk1 depleted from KT or all cells? (In the latter case, some 65% of all cells should still have Plk1 on KTs and for this majority of cells LGN localization should be indistinguishable from the siMOCK control).

 

Referee #3:

Review of 'Chromosome misalignments induce spindle-positioning defects' by Tame et al.

This manuscript uncovers a link between misaligned chromosomes and mitotic spindle alignment.

The key observations of this study are the following:
1. LGN oscillates on the cortex similarly to dynein
2. LGN is removed from cortex by kinetochore-associated Plk1
3. Reduction of kinetochore-bound Plk1 restores cortical LGN near misaligned chromosomes
4. Suppression of Plk1 activity reduces spindle orientation defects in response to misaligned chromosomes

This is a tidy, well-executed study with good quality data. The correlation between misaligned chromosomes and spindle orientation is an interesting and novel finding.

The manuscript places Plk1 upstream of LGN and shows that the kinase negatively regulates LGN localization to the cortex. Although this data contrasts with a report by the Cheeseman group (Nature Cell Bio, 2012), a recent study by Yan et al in Scientific Reports supports a similar role for Plk1. In addition, Yan et al identifies NDR kinase as a target of Plk1 in controlling LGN localisation; briefly, suppression of NDR kinase activity by Plk1 appears to control NuMA/ LGN accumulation in the cortex.
One criticism I have is that the manuscript by Tame at al provides no molecular insights as to how Plk1 might control LGN localisation. Is LGN a substrate of Plk1? If not, is it possible that loss of LGN from the cortex near misaligned chromosomes involved suppression of NDR by Plk1? The authors should at least try to address the latter.

Further points:

1. The link between chromosome misalignment and spindle orientation is convincingly demonstrated. However, due to the spindle assembly checkpoint cells with chromosome misalignment do not initiate anaphase, and thus it is unclear if chromosome misalignment can indeed change the plane of cell division or rather its effects are only transient. The study would have more physiological relevance if the authors demonstrated that sustained chromosome misalignments can alter the cell division plane (e.g. using Cenp-E/Msp1 inhibitors as in Bennett et al., 2015 Oncotarget).

2. Another question is whether mitotic arrest (as a result of unaligned chromosomes or MG132 for instance) contributes to the observed random spindle orientation. MG132-treated cells could be included as controls. In Suppl Fig 2B mitosis seems long; cells are still in metaphase 72 minutes after NEB. Are these perhaps MG132-treated?

3. Title of Suppl Fig 2 is 'Ran does not displace cortical LGN near misaligned chromosomes'. The title would be clearer if it followed the main text, for example 'Ran depletion causes global reduction in cortical LGN recruitment'.

4. It would be useful to include a schematic of how spindle orientation is determined in Fig 1A.

1st Revision - authors' response 08 December 2015

Referee #1:

The mitotic spindle ensures proper chromosome alignment and controls cleavage plate. Proper positioning of the spindle is important for normal cell division and development. Spindle positioning in many cell types is regulated by LGN-dynein-dependent cortical pulling forces exerted on astral microtubules. However, the molecular mechanisms regulating the localization of cortical dynein remain elusive. A previous study suggested that the chromatin-factor Ran-GTP gradient and spindle-pole-localized Plk1 negatively regulate the localization of cortical dynein to define spindle position (Kiyomitsu.T & Cheeseman IM, NCB, 2012). In the current study, however, the authors found a

 

correlation between chromosome misalignments and spindle-positioning defects, and show a novel pathway by which KT-localized Plk1 on misaligned chromosomes controls spindle positioning by displacing cortical LGN. In general, the experiments were well designed and the results are compelling. I have some minor
comments listed below, which should be clarified before this manuscript is accepted.

Major comments:
1. In sharp contrast to the previous study (Kiyomitsu.T & Cheeseman IM, NCB, 2012), the authors now show that Ran-GTP is not required for LGN delocalization (Fig S2), and that spindle-pole localized Plk1 restricts cortical LGN localization (Fig S3). Therefore, they should offer some explanation for these discrepancies, and/or repeat the same experiments.

Indeed, our results on the regulation of cortical LGN recruitment by Ran-GTP are in contrast to previously published data (Kiyomitsu.T & Cheeseman IM, NCB, 2012). In order to rule out the possibility that the disparities are due to differences in our experimental setups, we have now included a third independent experiment in Fig EV3D to re-assess the function of Ran-GTP in cortical LGN recruitment. We have employed the exact same experimental setup as described in the paper of Kiyomitsu and Cheeseman by expressing the dominant-negative RanT24N mutant in GFP-LGN expressing cells. Consistent with our previous data obtained with the siRNA-mediated depletion of Ran (Fig EV3B) and the inhibition of the Ran-GTP by importazole treatment (Fig EV3C), we observed a decrease in the fraction of cells with proper cortical LGN enrichment in the presence of the dominant negative Ran mutant, thus pointing again towards a global positive role of Ran in cortical LGN recruitment.

The contrasting results might possibly be explained by the difference in the timing at which we conducted our analyses. We have employed live-cell imaging for most of our assays in order to be able to distinguish the early and late effects of Ran on cortical LGN. In our hands, Ran depletion or expression of a dominant negative RanT24N mutant leads to the loss of cortical LGN in the early phases of mitosis; however, we often observed slight appearance of LGN at cortical regions when cells were delayed in mitosis for long periods of time (indicated also in Fig EV3B lower panel), which might implicate the presence of two distinct pathways of cortical LGN recruitment depending on the mitotic phase potentially similar to what was shown before for the recruitment of NuMA in anaphase (Kotak et al. 2014 EMBO J). However, considering that we were interested in the function of chromosome-proximity dependent signaling on cortical LGN in the early phase of mitosis, we believe our exclusion of Ran as a possible candidate is justified.

2. Fig 3A: The authors performed siPBIP1 to deplete KT-enriched Plk1. Although they show the reduction of Plk1 at kinetochores, they should quantify the Plk1 signal at centrosomes as a control.

We have now replaced Fig 3A with a new Fig 3A including proper quantification of KT-localized Plk1 by measuring Plk1 intensity levels over the ACA staining. Furthermore, we have added an experiment with the quantification of Plk1 signal at centrosomes in the same figure. Using this quantification method, we confirm a significant reduction of KT-localized PLK1 upon depletion of PBIB1, with unaffected levels of centrosome-associated Plk1.

Minor comments:
1. In Fig1, the authors show the results of measuring the spindle angle in each cell. However, the method of measurement is difficult to understand. They should provide a representative picture or schematic diagram that shows the method for measuring the spindle angle.

We apologize for the confusion about the experimental set-up. We have now included in the new Fig 1A a schematic depicting the details of our analysis method for the micropatterning experiments.

2. Fig 2B, Fig 3B, Fig S2 D: It would be better to distinguish the count numbers between the GFP-LGN positive and negative groups.

We have now depicted in Figures 2B, C, 3B, EV3F and EV4A the total number of cells quantified as well as the absolute count numbers per category (LGN-positive and LGN-negative) for each experimental condition in the respective graphs.

 

3. Fig S3 C right: Does the Y axis of the graph indicate different cells?
We apologize for the confusion. Indeed, each bar in the graph indicated individual cells. Considering that reviewer #2 raised the same question under point 5, we have now simplified the graphs in Figs EV5C and D by depicting the mean+SD of spindle oscillations of all cells analyzed in each condition.

Referee #2:

Tame and coworkers have investigated the impact of chromosome alignment on spindle positioning. They report that depletion of Spindly, CLIP-170 and CENP-E all result in both impaired chromosome alignment and spindle misorientiation. Importantly, the recruitment of dynein to the cortex does not seem to be affected, raising the question of how misaligned chromosomes might affect spindle positioning. Previously, it had been reported, by Kiyomitsu and Cheeseman (ref 13), that misaligned chromosomes disrupt an interaction of dynein/dynactin with LGN, a cortical docking factor, and that this effect was triggered by Ran-GTP. However, results reported in this new study from the Medema lab do not support this claim. These authors in fact found that i) LGN localization is locally reduced by the proximity of misaligned chromosomes and that this local restriction of LGN localization cannot be attributed to Ran. Instead, they identify kinetochore-associated Plk1 as a likely regulator of LGN localization. In support of this conclusion, they show that interference with KT-localization of Plk1 (via PBIP1 siRNA) rescues cortical LGN in proximity to misaligned chromosomes. Thus, they conclude that KT-associated Plk1 is responsible for displacing LGN from cortical sites in proximity to chromosome misalignment, thereby providing a causal link between chromosome misalignment and spindle orientation defects.

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We agree that the discrepancy between our data and that of the paper of Kiyomitsu and Cheeseman should have been better addressed, and we have provided additional data in Fig EV3D and provide a possible explanation in the main text and under point 1 of reviewer #1.

Furthermore, we acknowledge that the previously published work demonstrated clearly an effect of chromosome proximity to cortical dynein/LGN localization. We therefore now changed some of our novelty claims accordingly. However, we feel our data brings novelty on several aspects. First, the mechanism described by Kiyomitsu and Cheeseman was found to be important for the establishment of polarized LGN-localization during an unperturbed mitosis to enable proper spindle centering. We believe our data showing the effect of a few misaligned chromosomes (without affecting bipolar spindle formation and/or microtubule dynamics), having such a dramatic effect on spindle positioning is in fact very novel. Second, our data describes a role for KT-associated Plk1 in negatively regulating LGN at the cell cortex, a mechanism which was previously not uncovered.

2. p.5. last sentence of first paragraph: "Therefore, we conclude that misaligned chromosomes are the common cause..." In my view this is an exaggerated statement. The connection is plausible (tempting at the level of a speculation or a hypothesis), but not definitive. What the authors report, at this stage of the study, is primarily a CORRELATION, suggesting a common cause, but by no

 

means proving it.

We agree that at this point of the manuscript our statement appeared as a premature conclusion. We have now removed the sentence on page 6.

3. p. 5/6. Second paragraph. I wonder why the authors all of a sudden adopt noscapine treatment to cause chromosome misalignment - why not stick to (e.g.) Spindly depletion? Considering that the last paragraph of the introduction had whetted the reader's appetite for a study on "spindle-positioning defects seen after Spindly depletion", it comes as a bit of a surprise that many of the subsequent experiments actually rely on use of noscapine (and later on the Eg5 inhibitor STLC).

We have chosen to employ noscapine for two reasons: 1. Noscapine treatment induces chromosome alignment defects in all cells, whereas siRNA-mediated depletion of proteins in general is not 100% penetrant. 2. The cell synchronization step using thymidine can be skipped, as we can control the timing of induction of chromosome misalignments. While the reason for adopting the noscapine treatment is purely technical, we do acknowledge that it was inconsistent with the preceding experiments. Therefore, we have now included a micropatterning experiment in the presence of noscapine in Fig 1F as a fourth method to induce chromosome misalignments and in line with the other methods, we confirm spindle orientation defects upon noscapine treatment.

Related to the employment of STLC in Fig EV5C/D, we would like to point out that this drug being a specific inhibitor of the kinesin Eg5 (S. DeBonis et al., Mol. Cancer Ther.,(2004)) causes the formation of monopolar spindles. The purpose in the experiment shown in Fig EV5C/D was to examine the effect of spindle pole-proximity on cortical LGN enrichment after Plk1 inhibition. As Plk1 inhibition preceding mitotic entry has been described previously to cause monopolar spindles (P. Lénárt et al., Curr. Biol., (2007)), we decided to use STLC to obtain a comparable spindle configuration in BI2536 treated and DMSO control conditions. We now explain the use of STLC for this specific inhibitor in the main text of the manuscript (page 8).

4. As confirmed in this study, spindly depletion is known to cause extensive spindle rotation (e.g. ref. 22). I wonder whether the authors have considered a possible impact of spindle rotation on some of their results? Could some of the "switching" they observe actually reflect spindle rotation? (Did they somehow control for this?)

This is an interesting point. Indeed, Spindly-depletion was previously linked to spindle rotation defects. In this manuscript, we link misaligned chromosome (as seen in Spindly-depleted cells) to locally perturbed LGN. A consequence of these perturbations is that the spindle re-positions, again followed by local LGN perturbations. These continuous perturbations, followed by spindle re-positioning lead to continuous LGN movements around the cortex as shown in Fig. 2A and video 4 following CENP-E inhibition, respectively, which is reduced upon depletion of Plk1 from KTs (Fig 3D). Thus, in fact, rather than the rotational defects impacting our results, we believe that our findings explain the rotation defects seen upon depletion of Spindly or other players involved in chromosome alignment.

5. Related to Fig. S3D, they state (page 7) that LGN switching and spindle movement are "abolished" after STLC and BI2356 treatment. "Reduced" would seem more appropriate. Regarding the line graphs shown in Figures S3C and D, I presume that each line represents the behaviour of one single cell? In any case, it would be helpful to have this better explained in the corresponding legend.

The sentence has been corrected according to the reviewers' suggestion on page 8. The latter point has been addressed under referee #1 minor point 3 and has been adjusted in Fig EV5C and D.

6. Figure 2: some of these IF images look strange: why does GPF-LGN staining appear as double lines in these images?

We recognize that LGN appears as double lines/rings in some of our example images. These double lines of GFP-LGN originated from including a broader range of z-stacks for generating maximum projection images as compared to example images shown in other figures. It has been now adjusted to a range of 10µm at the center of the cell as described in the live cell microscopy section of

 

materials & methods.

7. Figure 3A and B: considering that siPBIP1 only depletes Plk1 from about 35% of KTs (3A, right panel), I find the effect on cortical LGN surprisingly large (3B, right panel). What was actually counted in these histograms - only cells with Plk1 depleted from KT or all cells? (In the latter case, some 65% of all cells should still have Plk1 on KTs and for this majority of cells LGN localization should be indistinguishable from the siMOCK control).

We apologize for the confusion. In the previous version of the figure, we categorized only cells as Plk1-negative when all KTs were Plk1-negative. Cells with overall low signals of KT-localized Plk1 or cells with a few Plk1-positive KTs were categorized as positive, which lead to an underestimation of the phenotype. We have now quantified the absolute KT- and centrosome associated Plk1 levels based on immunofluorescence intensity values in Fig 3A, as was suggested by reviewer 1 under major comments point 2.

Referee #3:

Review of 'Chromosome misalignments induce spindle-positioning defects' by Tame et al.

This manuscript uncovers a link between misaligned chromosomes and mitotic spindle alignment.

The key observations of this study are the following:
1. LGN oscillates on the cortex similarly to dynein
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3. Reduction of kinetochore-bound Plk1 restores cortical LGN near misaligned chromosomes
4. Suppression of Plk1 activity reduces spindle orientation defects in response to misaligned chromosomes

This is a tidy, well-executed study with good quality data. The correlation between misaligned chromosomes and spindle orientation is an interesting and novel finding.

The manuscript places Plk1 upstream of LGN and shows that the kinase negatively regulates LGN localization to the cortex. Although this data contrasts with a report by the Cheeseman group (Nature Cell Bio, 2012), a recent study by Yan et al in Scientific Reports supports a similar role for Plk1. In addition, Yan et al identifies NDR kinase as a target of Plk1 in controlling LGN localisation; briefly, suppression of NDR kinase activity by Plk1 appears to control NuMA/ LGN accumulation in the cortex.

One criticism I have is that the manuscript by Tame at al provides no molecular insights as to how Plk1 might control LGN localisation. Is LGN a substrate of Plk1? If not, is it possible that loss of LGN from the cortex near misaligned chromosomes involved suppression of NDR by Plk1? The authors should at least try to address the latter.

Thank you for the suggestion. Indeed, the centrosome- and KT- associated kinase NDR1 has been recently reported as a target of Plk1 involved in the regulation of cortical LGN/NuMA enrichment and spindle orientation. Considering that NDR1 has been described as a downstream effector of Plk1, we have now assessed the effect of Plk1 inhibition on cortical LGN enrichment after siRNA-mediated depletion of NDR1. As shown in the new Fig EV4, we still observed enrichment of cortical LGN near misaligned chromosomes in the absence of this factor when Plk1 activity is inhibited. This assay does not exclude NDR1 as a downstream target of Plk1; however, it strongly suggests the existence of (an)other direct or indirect target(s) of Plk1 required for the delocalization of cortical LGN in our system.

Further points:

1. The link between chromosome misalignment and spindle orientation is convincingly demonstrated. However, due to the spindle assembly checkpoint cells with chromosome misalignment do not initiate anaphase, and thus it is unclear if chromosome misalignment can indeed change the plane of cell division or rather its effects are only transient. The study would have

 

more physiological relevance if the authors demonstrated that sustained chromosome misalignments can alter the cell division plane (e.g. using Cenp-E/Msp1 inhibitors as in Bennett et al., 2015 Oncotarget).

We understand the concern of the cells not exiting mitosis in the case of induced chromosome misalignments as a result of spindle checkpoint activation. We therefore have now included a micropattern experiment in which we combine the Cenp-E- and Mps1 inhibitors to force cells out of mitosis in the presence of misaligned chromosomes in the new Figs EV2D-F. We find that cells do not re-orient the spindle into the correct position after initiating anaphase upon addition of the Mps1-inhibitor. We conclude that chromosome misalignments induce not only a transient effect on spindle orientation, but can indeed alter the cell division plane.

2. Another question is whether mitotic arrest (as a result of unaligned chromosomes or MG132 for instance) contributes to the observed random spindle orientation. MG132-treated cells could be included as controls. In Suppl Fig 2B mitosis seems long; cells are still in metaphase 72 minutes after NEB. Are these perhaps MG132-treated?

We have included new Figs EV2A-C in which we induce mitotic arrest without chromosome alignment defects by RNAi-mediated depletion of Cdc20, which similarly to MG132-treatment prevents the degradation of essential mitotic proteins such as Cyclin B1/securin, thereby preventing anaphase onset. We show that these cells are capable of properly positioning the mitotic spindle at time-points when chromosome misalignments prompted spindle-positioning defects (32 minutes after NEB). We therefore conclude that the spindle misorientation phenotype in the presence of misaligned chromosomes is not due to mitotic arrest. We now also explain this more clearly in the text.

In Fig S2B (now EV3B), we previously chose to display a control cell that took longer time in mitosis than average to be able to show the cortical switching of LGN. However, considering that a similar example is shown in Fig EV5A, we have now replaced the example cell in Fig EV3B with one that displayed normal mitotic timing.

3. Title of Suppl Fig 2 is 'Ran does not displace cortical LGN near misaligned chromosomes'. The title would be clearer if it followed the main text, for example 'Ran depletion causes global reduction in cortical LGN recruitment'.

Thank you for the suggestion. We have changed the title of the figure.

4. It would be useful to include a schematic of how spindle orientation is determined in Fig 1A.

Same point as reviewer #1 minor comments 1. We have included the schematic in Fig 1A.

2nd Editorial Decision 04 January 2016

Many thanks for your patience while we were waiting to hear back from the referees who were asked to assess the revised version of your manuscript titled 'Chromosome misalignments induce spindle-positioning defects'. I am happy to tell you that the two referees who saw the new version now fully support publication of the study in our journal.

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

 