SWR1 Chromatin Remodeling Complex Prevents Mitotic Slippage during Spindle Position Checkpoint Arrest

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RE: Manuscript #E20-03-0179
TITLE: "SWR1 Chromatin Remodeling Complex Prevents Mitotic Slippage during Spindle Position Checkpoint Arrest"

Monitoring Editor (Remarks to Author):

Dear Gislene,
We have now received two reviews of your manuscript. I am pleased to inform you that they both found the findings to be of interest and worthy of publication. Both reviewers have several constructive comments. Reviewer 2 had many so I'll try to indicate the ones I think would be driving concerns for the paper. The comments in points 2 and 3 about a potential role of SWR1 in nucleolar function and Cdc14 partial release are very relevant. Considering you've done this (page 14, unpublished observation), I agree with the reviewer that a careful quantification of the dynamics would be helpful for the study.
While Reviewer 2 points 1 and 6 are important, they might be beyond the scope of this manuscript. However, please make sure you address the comment about the statistical analyses.
I am sure you will be able to address these and the additional comments and I look forward to seeing a revised manuscript.
Thank you for submitting this study to the Molecular Biology of the Cell.
Best,
Kerry

Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Pereira,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
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Reviewer #1 (Remarks to the Author):

See attached.

Reviewer #2 (Remarks to the Author):

In their manuscript, Caydasi et al. describe a genetic screen for new components of the spindle position checkpoint (SPOC), a surveillance mechanism that controls the correct orientation of the spindle during mitosis in budding yeast, and further characterize the participation in this checkpoint of one of the identified factors. Specifically, the authors reveal the chromatin-remodeling complex SWR1 as a novel SPOC component that is necessary to maintain the anaphase arrest induced by this surveillance mechanism when it needs to be prolonged for an extended period of time. Caydasi et al. propose that slippage from the SPOC-dependent anaphase arrest in cells lacking SWR1 requires the activity of the Cdc14-early anaphase release (FEAR) pathway and additional factors such as the SAGA complex or the mitotic CDK inhibitor Sic1. The conclusions of the manuscript could be of potential interest, but the lack of mechanistic insight is a significant deficit of this study. Hence, considering this lack of molecular understanding of the role of SWR1 in the functionality of the SPOC, the manuscript would require further experimental support to at least more strongly reinforce its main conclusions before granting publication in Molecular Biology of the Cell. Specifically, my main concerns about the data are the following:

1.- It is not evident why the authors decided to focus on the swr1Δ mutant when cells lacking other components of the SWR1 complex, such as the yaf9Δ mutant, seem to have a stronger SPOC deficiency phenotype. In fact, repeating some of the key experiments (SAC proficiency, slippage from SPOC arrest, etc.) using the yaf9Δ mutant would help to strengthen the authors' conclusions.

2.- Components of the SWR1 complex have been shown to be important for the proper structure and function of the nucleolus (e.g., Kitamura et al. 2015; DOI:10.1016/j.bbrc.2015.07.005). Hence, mitotic slippage imposed by SWR1 deletion could be due to premature Cdc14 release as a consequence of nucleolar defects, thereby explaining its dependence on the liberation of the phosphatase from this compartment. Did the authors check whether nucleolar morphology and, more specifically, whether condensation of the nucleolus is somehow affected by SWR1 deletion?

3.- Related to the previous point, and although the authors indicate that Cdc14 partial release did not occur prematurely in cells lacking SWR1 (page 14, unpublished observation), a precise estimation of the dynamics of nucleolar liberation of the phosphatase is not shown. This quantification is important in order to reinforce the conclusions of the manuscript. In this sense, and regarding the analysis of Cdc14 release by fluorescence time-lapse microscopy in Figure 3B, a more precise indication of how liberation of the phosphatase was evaluated (partial vs. full release, etc.) should additionally be provided. It is remarkable that the extent of Cdc14 release is already close to 40% in SWR1 cells with mispositioned spindles.

4.- Deletion of SWR1 is shown to bypass a cdc15-1 arrest at the restrictive temperature (Figure 3C). However, in the experiment, cdc15-1 cells did not seem to maintain a stable anaphase block. The authors should check whether cells deleted for SWR1 or YAF9 could hold a more stable and prolonged anaphase arrest using a stronger conditional allele (e.g., cdc15-as1). In this sense, it is worth noting that, despite being able of maintaining a SAC-induced arrest, results from Figure 2A seem to indicate that the cells deleted for SWR1 already started degrading Pds1-3HA 135 min after the initial G1 release (compare with time point 90 min in the bub2 mutant), suggesting that they might probably not be able to maintain a stable arrest for a longer period of time in this case either.

5.- The possible contribution of the roles of the SWR1 complex in heterochromatin anti-silencing and DNA damage response to the inhibition of mitotic exit was evaluated by assessing the growth of the cells after KIN4 overexpression. This analysis, however, is sometimes complicated due to the different growth rate of the strains. In this way, and although deletion of SIR2 did not reverse the growth phenotype of cells lacking Swr1 and overexpressing Kin4, it did on the other hand significantly improve growth of the cells deleted for SWR1 when the SPOC kinase was normally expressed. A different approach, such as the determination of the SPOC deficiency index, would more strongly support the authors' conclusions.

6.- The authors finally propose that mitotic slippage of SPOC in cells lacking SWR1 could be due to changes in gene expression. It is therefore surprising, giving the numerous genome-wide expression analyses available, that at least an exploratory analysis of possible genes under the transcriptional control of Swr1 was not carried out to find changes in mRNA levels of known genes that could explain the observed phenotype. Along the same lines, did the authors check whether any of the factors found to be required for mitotic slippage in the absence of SWR1 is encoded by a gene that is transcriptionally regulated by this chromatin remodeling complex?

Finally, some minor points are:

1.- There is a general lack of statistical analyses.

2.- It would facilitate to evaluate the results from Figure 2F if images from wild type and swr1Δ cells were also displayed.

3.- The fact that deletion of SWR1 did not rescue the growth defect of MEN mutants (Figure S2A) does not necessarily mean that it does not bypass the MEN. This could be also due to cell lethality as a result of an untimely or unregulated mitotic exit giving rise to unviable cells.

4.- Similarly to what previously indicated for Cdc14 early release, activation of the cell wall integrity pathway in cells lacking or not SWR1 after overexpression of Kin4 is claimed to be not detected based on unpublished observations. The authors should include these evidences or, alternatively, remove this statement.

Point-to-point letter to reviewers

We would like to thank both reviewers for the constructive comments on our MS.

Reviewer 1

The spindle position checkpoint (SPOC) is the surveillance mechanism that delays mitotic exit and cytokinesis when the mitotic spindle is mispositioned in the budding yeast *S. cerevisiae.* The target of SPOC is the mitotic exit network (MEN), a kinase cascade that ultimately leads to activation of the Cdc14 phosphatase, which in turn promotes mitotic exit by reverting mitotic CDK-dependent phosphorylations.

This nice paper reports on the identification and initial characterization of novel factors involved in SPOC. In particular, it focuses on the SWR1-C chromatin remodeling complex, several subunits of which were identified through a genome-wide screen for non-essential genes that relieve the cell cycle arrest caused by overexpression of Kin4, a known SPOC kinase. The SWR1-C is shown to clearly contribute to a robust SPOC response and, although the underlying molecular mechanism remains to be defined, the data suggest that it might do so through its well-characterized transcriptional function, i.e. replacing histone H2A in nucleosome by its H2AZ variant.

The experiments are carefully designed and the paper is well written. In addition, the datasets with the outcome of the genetic screens will be a precious and inspiring source of data for people working in the field. I therefore fully support its publication in MBoC and only have some minor comments listed below:

It is quite puzzling that *KIN4* popped out of the screen for gene deletions that rescue the sick phenotype due to *KIN4* overexpression. What is the authors' explanation for this observation? What are the levels of overexpressed Kin4 relative to the endogenous protein?

In Gal1-inducing conditions, Kin4 expression levels were indeed lower in *GAL1-KIN4/kin4*Δ in comparison to *GAL1-KIN4/KIN4* strains (Supplementary table 2). Therefore, we believe that reduced expression levels were the reason for the rescue. To make it clear, we now included more detailed description of our screen in the result section (Page 6 and Figure 1A). We emphasized that the query strain had *GAL1-KIN4* in the *URA3* locus in addition to the endogenous *KIN4* (Figure 1A and the materials and methods). We also emphasize on Kin4 overexpression levels in the text (Page 6).

Page 8: The sentence "Thus, mitotic slippage imposed by *SWR1* deletion occurs through Cdc14 release" should be rephrased. The related data show a correlation and not a cause-effect relationship.

We now changed the text to clarify our conclusion as:

"Thus, Cdc14 full release coincides with mitotic slippage of SWR1 deleted cells. This data indicates that the MEN becomes activated in cells with misaligned spindles in the absence of SWR1-C."

Fig. 3B shows that a high fraction (□40%) of *SWR1 kar9*□ _cells and almost 100% of *swr1*□ _*kar9*□ _cells undergo Cdc14 nucleolar release and Myo1 contraction. These results do not quite match the results in Fig. 3A showing that most *SWR1 kar9*□ cells and a fraction of *swr1*□ *kar9*□ cells remain arrested in mitosis for a long time. Could it be that Cdc14-GFP is hypermorphic? If yes, this should be noted.

The higher percentage of Cdc14-GFP release is most likely a consequence of Myo1 tagging because this effect was only observed in strains carrying Cdc14-GFP Myo1-3mCherry. However, this did not affect our conclusion because Cdc14-GFP release was higher in *swr1*Δ compared to *SWR1* cells, yet both cell types expressed Myo1-3mCherry.
To avoid confusion, we now mention the observed effect of Myo1 tagging upon Cdc14-GFP release in the legend of Figure 5B.

In the legend of Fig. 3D the temperature at which plates were incubated should be indicated.

The temperature was 30°C. This data indicated that *SWR1* deletion requires a fully functional MEN for promoting growth of *KIN4*-overexpressing cells. However, for simplicity, we are not showing this data in the current version of our manuscript.

Page 11: "In concordance with previous publications (Falk *et al.*, 2016; Caydasi *et al.*, 2017), deletion of FEAR network components *SPO12* and *SLK19* rescued SPOC deficiency of *kin4Δ* cells". The first evidence that FEAR inactivation rescues the SPOC defects of *kin4Δ* cells was reported in Scarfone et al., 2015 (*PloS Gen.* 11:e1004938), which should be quoted here.

Reference has been added.

In the Discussion the authors argue that SWR1-C does not impinge directly on FEAR or MEN pathways, but to my opinion the data gathered so far do not rule out this possibility. Even if Cdc14 nucleolar release does not occur prematurely in *swr1*□ cells during an unperturbed cell cycle (data not shown), SWR1-C could restrain FEAR or MEN activity only under specific circumstances, also depending on its own regulation.

We now changed the discussion to avoid confusion.

I found several typos in the Materials and Methods section.
Typos have been corrected.

Gal1 promoter should be written *GAL1* throughout the text and figures/tables

We now indicated the Gal1 promoter as *GAL1* through the text in the figures/figure legends and tables.

Reviewer #2 (Remarks to the Author):

In their manuscript, Caydasi et al. describe a genetic screen for new components of the spindle position checkpoint (SPOC), a surveillance mechanism that controls the correct orientation of the spindle during mitosis in budding yeast, and further characterize the participation in this checkpoint of one of the identified factors. Specifically, the authors reveal the chromatin-remodeling complex SWR1 as a novel SPOC component that is necessary to maintain the anaphase arrest induced by this surveillance mechanism when it needs to be prolonged for an extended period of time. Caydasi et al. propose that slippage from the SPOC-dependent anaphase arrest in cells lacking SWR1 requires the activity of the Cdc14-early anaphase release (FEAR) pathway and additional factors such as the SAGA complex or the mitotic CDK inhibitor Sic1. The conclusions of the manuscript could be of potential interest, but the lack of mechanistic insight is a significant deficit of this study. Hence, considering this lack of molecular understanding of the role of SWR1 in the functionality of the SPOC, the manuscript would require further experimental support to at least more strongly reinforce its main conclusions before granting publication in Molecular Biology of the Cell. Specifically, my main concerns about the data are the following:

1.- It is not evident why the authors decided to focus on the swr1Δ mutant when cells lacking other components of the SWR1 complex, such as the yaf9Δ mutant, seem to have a stronger SPOC deficiency phenotype. In fact, repeating some of the key experiments (SAC proficiency, slippage from SPOC arrest, etc.) using the yaf9Δ mutant would help to strengthen the authors' conclusions.

Yaf9 is also a component of NuA4 acetyltransferase complex. Therefore, we focused on *swr1*Δ cells to be able to conclude on the function of SWR1-C only. For clarity, we now added our reasoning to focus our analysis on Swr1 in page 7.

2.- Components of the SWR1 complex have been shown to be important for the proper structure and function of the nucleolus (e.g., Kitamura et al. 2015; DOI:10.1016/j.bbrc.2015.07.005). Hence, mitotic slippage imposed by SWR1 deletion could be due to premature Cdc14 release as a consequence of nucleolar defects, thereby explaining its dependence on the liberation of the phosphatase from this compartment. Did the authors

check whether nucleolar morphology and, more specifically, whether condensation of the nucleolus is somehow affected by SWR1 deletion?

Thanks for pointing this out. We were not aware that Kitamura et al showed changes in nucleolar morphology upon ARP6 depletion in mammalian cells. Although we could not detect major differences in nucleolar morphology in yeast when comparing wild type and *swr1*Δ cells (figure below), we agree that slight changes in nucleolar morphology could influence the timing of Cdc14 release. For this reason, we decided to analyze Cdc14 premature release in more detail. For this, we quantified the timing of Cdc14-GFP release by FEAR (in the absence of MEN) and also analyzed Sli15 that associates with spindles in dependency of Cdc14 released by FEAR (Pereira&Schiebel, 2003). We see no premature release of Cdc14 in *swr1*Δ cells compared to wild type cells (Figure 6) .

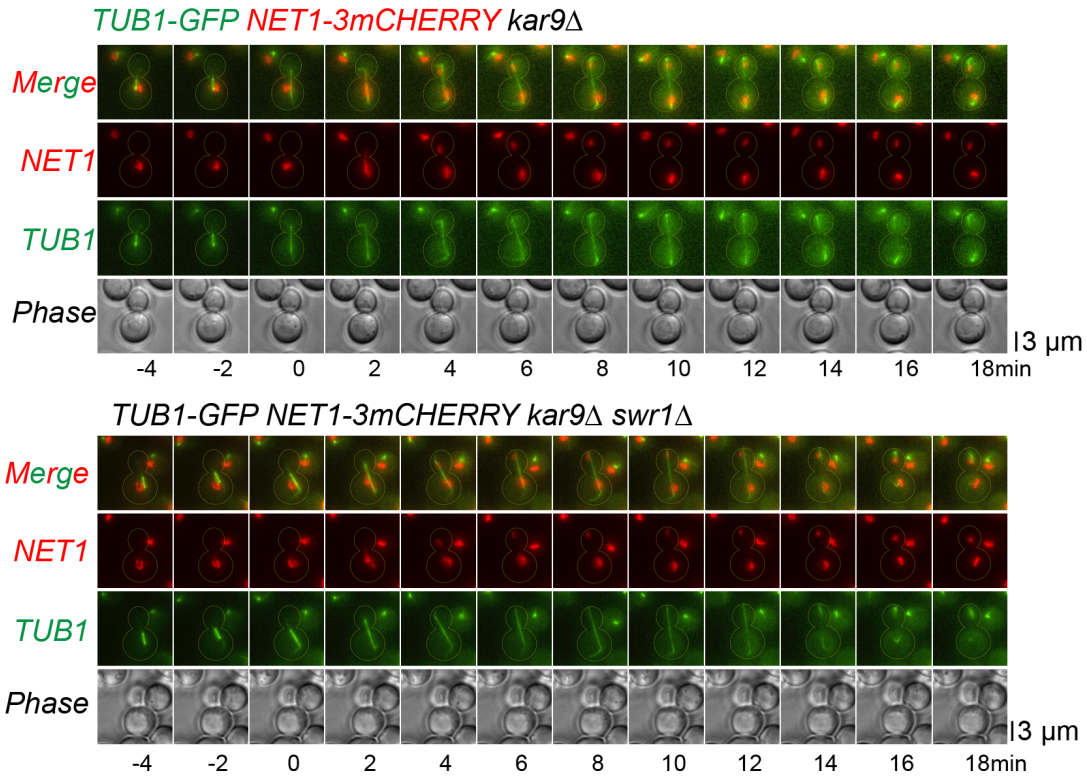

Figure shows still images of time lapse microscopy for the indicated strains. T=0 indicates the onset of anaphase.

3.- Related to the previous point, and although the authors indicate that Cdc14 partial release did not occur prematurely in cells lacking SWR1 (page 14, unpublished observation), a precise estimation of the dynamics of nucleolar liberation of the phosphatase is not shown. This quantification is important in order to reinforce the conclusions of the manuscript.

We now show quantifications for Cdc14 FEAR release (Figure 6). We see no significant difference for the percentage of cells with FEAR-released Cdc14 in *swr1*Δ compared to wild type cells.

In this sense, and regarding the analysis of Cdc14 release by fluorescence time-lapse microscopy in Figure 3B, a more precise indication of how liberation of the phosphatase was evaluated (partial vs. full release, etc.) should additionally be provided.

In this figure (now Figure 5B) we quantified Cdc14 full release and Myo1 contraction. We now indicated this clearly in the figure and the figure legend (Figure 5B).

It is remarkable that the extent of Cdc14 release is already close to 40% in SWR1 cells with mispositioned spindles.

The high percentage (40%) of Cdc14 release in *SWR1* cells was only observed in Myo1-3mCherry tagged cells (see also answer to reviewer 1). We don't know the reason for this effect, but we think that it might be related to changes in binding dynamics of bud neck components that could influence mitotic exit or cytokinesis.

4.- Deletion of SWR1 is shown to bypass a cdc15-1 arrest at the restrictive temperature (Figure 3C). However, in the experiment, cdc15-1 cells did not seem to maintain a stable anaphase block. The authors should check whether cells deleted for SWR1 or YAF9 could hold a more stable and prolonged anaphase arrest using a stronger conditional allele (e.g., cdc15-as1).

Sorry for the confusion. *SWR1* deletion does not bypass Cdc15-1 ts or Cdc15-as cells (Figure 3C and Figure S3B).

In this sense, it is worth noting that, despite being able of maintaining a SAC-induced arrest, results from Figure 2A seem to indicate that the cells deleted for SWR1 already started degrading Pds1-3HA 135 min after the initial G1 release (compare with time point 90 min in the bub2 mutant), suggesting that they might probably not be able to maintain a stable arrest for a longer period of time in this case either.

Regarding SAC-arrest in *swr1*Δ cells, we don't see Pds1 premature degradation in *swr1*Δ cells (Figure 3). Pds1 behaves the same in WT, *kin4*Δ and *swr1*Δ cells. Pds1 behaviour is clearly different in *bub2*Δ cells, which are SAC deficient. We now also show Tubulin, Clb2 and Sic1 controls of the same experiment to have an additional level of analysis of SAC integrity. The decrease in Clb2 and increase in Sic1 between 75-90 min is only seen in *bub2*Δ but not in other cell types.

5.- The possible contribution of the roles of the SWR1 complex in heterochromatin anti-silencing and DNA damage response to the inhibition of mitotic exit was evaluated by assessing the growth of the cells after KIN4 overexpression. This analysis, however, is sometimes complicated due to the different growth rate of the strains. In this way, and although deletion of SIR2 did not reverse the growth phenotype of cells lacking Swr1 and overexpressing Kin4, it did on the other hand significantly improve growth of the cells deleted for SWR1 when the SPOC kinase was normally expressed. A different approach, such as the determination of the SPOC deficiency index, would more strongly support the authors' conclusions.

Thanks for the suggestion. We now checked the influence of deleting Yku80 and Sir2 on SPOC using *kar9*Δ cells. To our surprise, the SPOC deficiency of *swr1*Δ *kar9*Δ cells was reduced upon *SIR2* deletion. We now show this data in Figure 7B and discuss the differences observed between *GAL1-KIN4* and *kar9*Δ cells in the text.

6.- The authors finally propose that mitotic slippage of SPOC in cells lacking SWR1 could be due to changes in gene expression. It is therefore surprising, giving the numerous genome-wide expression analyses available, that at least an exploratory analysis of possible genes under the transcriptional control of Swr1 was not carried out to find changes in mRNA levels of known genes that could explain the observed phenotype. Along the same lines, did the authors check whether any of the factors found to be required for mitotic slippage in the absence of SWR1 is encoded by a gene that is transcriptionally regulated by this chromatin remodeling complex?

We now show the microarray data in the manuscript (Figure S3 and Supplementary table 3), as a reference for genes under Swr1 control in late anaphase arrested cells. We see changes in transcription, yet we could not see enrichment of known mitotic exit related genes that could explain our phenotype. We have tested single component deletions of the top hits but they did not cause SPOC deficiency or rescued the SPOC deficiency phenotype of *swr1*Δ *kar9*Δ cells (data not shown). This is now discussed in pages 16-17 as:
“Owing to the role of SWR1-C in gene expression and the fact that the histone variant, Htz1, was also required for SPOC, it is tempting to speculate that SWR1-C dependent transcriptional regulation is important for prevention of mitotic slippage during SPOC arrest. Indeed, microarray analysis of SWR1-C dependent gene expression in a late anaphase arrest (cdc15-as) showed that transcriptional profiles of late anaphase-arrested cells differed in the presence and absence of SWR1-C. However, using this data we failed to identify individual genes or group of genes involved in the late anaphase arrest of cells with or without spindle misalignment. More needs to be done to understand the contribution of transcriptional regulation on control of mitotic exit."

Finally, some minor points are:

1.- There is a general lack of statistical analyses.

Statistical analyses are now shown for all relevant figures, tables and figure legends.

2.- It would facilitate to evaluate the results from Figure 2F if images from wild type and swr1Î cells were also displayed.

Bfa1 localization in wild type cells has been included in Figure 4E.

3.- The fact that deletion of SWR1 did not rescue the growth defect of MEN mutants (Figure S2A) does not necessarily mean that it does not bypass the MEN. This could be also due to cell lethality as a result of an untimely or unregulated mitotic exit giving rise to unviable cells.

In addition to drop test (Figure 3C), we analyzed cell cycle progression of *cdc15-1* and *cdc15-1 swr1*Δ cells in liquid medium using synchronized cultures (figure below). Both cell types arrest in late anaphase with fully elongated spindles, indicating that deletion of *SWR1* does not cause cell death. We also observed similar behaviour using cdc15-as allele (Figure S3B).

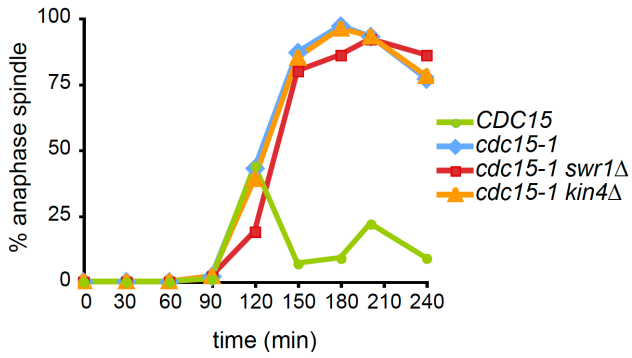

Figure shows cell cycle progression of indicated strains carrying Tub1-GFP. Cells were arrested in the G1-phase with alpha-factor (t=0) and released in medium lacking alpha-factor at 37°C to inactive the MEN kinase Cdc15-1. The percentage of cells with fully elongated spindles and separated DNA (anaphase cells) were counted over time.

4.- Similarly to what previously indicated for Cdc14 early release, activation of the cell wall integrity pathway in cells lacking or not SWR1 after overexpression of Kin4 is claimed to be not detected based on unpublished observations. The authors should include these evidences or, alternatively, remove this statement.

This data is now included in figure S5.

RE: Manuscript #E20-03-0179R
TITLE: "SWR1 Chromatin Remodeling Complex Prevents Mitotic Slippage during Spindle Position Checkpoint Arrest"

Dear Gislene,

Thank you for your thorough response to the reviewers comments. I am very pleased to inform you that your paper is now acceptable for publication in the Molecular Biology of the Cell. Congratulations to you and your co -authors.
Kerry

Kerry Bloom
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Pereira:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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