JCB

Cell division requires RNA eviction from condensing chromosomes

Judith Sharp, Carlos Perea-Resa, Wei Wang, and Michael Blower

Corresponding Author(s): Michael Blower, Massachusetts General Hospital

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DOI: https://doi.org/10.1083/jcb.201910148

November 21, 2019

Re: JCB manuscript #201910148

Dr. Michael D Blower
Massachusetts General Hospital
Department of Molecular Biology
185 Cambridge St, CPZN-7250
Boston, MA 02114

Dear Dr. Blower,

Thank you for submitting your manuscript entitled "Prophase removal of chromosome-associated RNAs facilitates anaphase chromosome segregation". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. Thank you for your patience with the peer review process. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that the reviewers found your results implicating Aurora B in the removal of SAF-A:RNA complexes from prophase chromatin via direct phosphorylation of SAF-A and showing this is important for normal chromosome segregation interesting and the work well done. They provided succinct and constructive comments that we editorially feel are valid and important to address in revision. Rev#1 suggested deeper investigations of how the retention of SAF-A-RNA on chromatin leads to chromosome segregation errors: by identifying the source of the error (point #1) and assessing potential phenotypes for kinetochore-microtubule attachment and spindle organization (#3). The ref suggested resolving a conflict with previously published work showing chromosome segregation defects upon SAF-A depletion (#2). Rev#2 asked whether retention of SAF-A only, or nuclear RNA only, is what leads to the chromosome segregation defects (#1) and suggested ruling out a potential artifact (#2) through new live imaging analyses. These lines of investigations would in our view deepen the phenotypic analyses and the understanding of the role of the Aurora B-SAF-A:RNA removal circuit in cell division and should be addressed rigorously for publication. Please let us know if you anticipate any issues addressing these remarks or have any questions. We would be happy to discuss the revisions further as needed.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:

Text limits: Character count for an Article is < 40,000, not including spaces. Count includes title page, abstract, introduction, results, discussion, acknowledgments, and figure legends. Count does not include materials and methods, references, tables, or supplemental legends.

Figures: Articles may have up to 10 main text figures. Figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, http://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior

to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Articles may have up to 5 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

The typical timeframe for revisions is three months; if submitted within this timeframe, novelty will not be reassessed at the final decision. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to the Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Arshad Desai, PhD
Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In interphase cells, chromatin is decondensed and transcriptionally active. When cells enter mitosis, chromatin is condensed into sister chromatids, and gene transcription is attenuated. It is not clear how the nuclear and chromatin-associated RNAs were regulated after chromosome condensation and nuclear envelope breakdown during mitosis. In this paper, the authors investigated the molecular mechanisms that regulate nuclear RNA localization during mitosis. They found that SAF-A (hnRNP-U) tethers a large set of nuclear RNAs to chromatin during interphase through its ability to bind both DNA and RNA. The SAF-A-RNA complexes can be removed from mitotic chromosomes in an Aurora B-dependent manner. Aurora-B phosphorylates SAF-A at two sites in the its DNA-binding domain to release SAF-A-RNA complexes from chromatin during mitosis. Expression of nonphosphorylatable SAF-A leads to RNA retention on mitotic chromosomes and caused anaphase chromosome segregation defects.

Overall, this study presents several interesting findings, and the authors provide convincing

evidence on the mitotic regulation of SAF-A. On the other hand, the mitotic function of this elaborate mechanism of RNA removal is underexplored. The mitotic defects caused by nonphosphorylatable SAF-A are not well-characterized. It is unclear why retention of SAF-A-RNA on chromatin leads to chromosome segregation errors. This major deficiency needs to be corrected prior to publication. Specifically, the following points need to be addressed.

Major points
(1) The authors should further explore the potential causes of chromosome segregation defects in SAF-A-S14A S26A-GFP expressing cells. For example, the authors can examine the mitotic phenotypes of SAF-A-S14A S26A-GFP expressing cells using time-lapse microscopy. They can use markers of chromatin or DNA to monitor chromosome segregation in these cells. This experiment will help to pinpoint the source of the anaphase errors.

(2) Based on a report by Nozawa et al, 2017, SAF-A depletion itself can cause chromosome segregation defects in RPE1 cells. In contrast, the current study did not find chromosome segregation defects in cells depleted of SAF-A by auxin-induced degradation. This discrepancy needs to be resolved or at least discussed.

(3) SAF-A was reported to regulate kinetochore-microtubule attachment and spindle organization during mitosis. The authors should examine whether cells depleted of SAF-A indeed exhibit these defects and whether these defects underlie the phenotypes of SAF-A-S14A S26A-GFP cells.

Minor points
(1) On p8, "all RNA is labeled with BrdU..." should be "all RNA is labeled with BrU...".
(2) The last paragraph on mitotic bookmarking is too speculative. The current study does not shed light on mitotic bookmarking.

Reviewer #2 (Comments to the Authors (Required)):

Review of "Prophase removal of chromosome-associated RNAs facilitates anaphase chromosome segregation" by Sharp JA et al. in consideration at Journal of Cell Biology

Summary:

In this manuscript, the authors addressed the mechanisms of dynamic localization of nuclear RNAs during mitosis. Previous studies have shown that SAF-A tethers nuclear RNA to chromatin via DNA-binding and RNA-binding domains. Using a combination of imaging, biochemistry and molecular biology, the authors determine that 1) SAF-A:RNA complexes become excluded from mitotic chromosomes during mitosis, starting as early as prophase stage; 2) this change in localization is due to the kinase activity of Aurora B at specific residues of SAF-A; 3) the Aurora B-mediated phosphorylation of SAF-A reduces its affinity specifically to DNA; and 4) deregulation of this SAF-A:RNA dynamic localization leads to defects in chromosome segregation during anaphase. These findings lead the authors to conclude that removal of nuclear RNAs from chromatin during mitosis is essential for proper chromosome segregation.

The main strength of this manuscript is the multiple orthogonal approaches that provide convergent data to support the main findings. In particular, the combination of biochemical in vitro reconstitution experiments with molecular perturbations in cells shows that the main findings are

robust. Overall, this is a strong manuscript that could be ready for publication given some attention to the following concerns.

Major concerns:

1. While it is clear based on the data provided that retention of SAF-A:RNA complex on mitotic chromosomes leads to chromosome segregation defects, it is unclear whether retention of SAF-A only, or nuclear RNA only is sufficient to induce the phenotype. Given that the authors' main conclusion (and title) is that removal of nuclear RNA from chromosomes "facilitates anaphase chromosome segregation," it is important to distinguish between the two potential mechanisms. The authors can address this by mutating the RNA-binding motif of SAF-A, and testing for segregation defects.
2. Previous studies have identified a formaldehyde-based artifact that has been shown to exclude DNA-binding proteins from mitotic chromosomes in mitosis (Festuccia et al. 2019 Genome Research; Teves et al 2016 eLife; Pallier et al 2003 MBoC). To fully exclude the possibility this formaldehyde-based artifact is skewing the authors results, this reviewer suggests performing live imaging of SAF-A-GFP (or the mCherry knock-in) as a time course through mitosis.

Minor concerns:
1. Is SAF-A the primary (only) method for tethering nuclear RNAs to chromatin? If not, perhaps include this background info in the introduction.
2. On page 14 line 19, there is an error in the SAP mutation annotation.

Overall, this study presents several interesting findings, and the authors provide convincing evidence on the mitotic regulation of SAF-A. On the other hand, the mitotic function of this elaborate mechanism of RNA removal is underexplored. The mitotic defects caused by nonphosphorylatable SAF-A are not well-characterized. It is unclear why retention of SAF-A-RNA on chromatin leads to chromosome segregation errors. This major deficiency needs to be corrected prior to publication. Specifically, the following points need to be addressed.

Major points
(1) The authors should further explore the potential causes of chromosome segregation defects in SAF-A-S14A S26A-GFP expressing cells. For example, the authors can examine the mitotic phenotypes of SAF-A-S14A S26A-GFP expressing cells using time-lapse microscopy. They can use markers of chromatin or DNA to monitor chromosome segregation in these cells. This experiment will help to pinpoint the source of the anaphase errors.

We agree with the reviewer that the mitotic consequences of retention of SAF-A:RNP complexes on mitotic chromosomes were not explored in detail in our original submission. In our revision we have performed several experiments to explore the chromosome segregation defects in SAF-A$^{S14A\ S26A}$ cells. We performed the following experiments:

1. Live cell imaging of cells expressing SAF-A^{wt} or SAF-A$^{S14A\ S26A}$ and H2B-RFP. We found that SAF-A$^{S14A\ S26A}$ cells exhibit a significant prometaphase delay, often with defects in chromosome alignment (Figure 9A-E, Figure S2E). Interestingly, SAF-A$^{S14A\ S26A}$ cells showed defects in chromosome individualization immediately after nuclear envelope breakdown.
2. We performed a monastrol arrest and washout experiment to examine kinetochore microtubule error correction. We found that SAF-A$^{S14A\ S26A}$ cells exhibit higher rates of bipolar spindles with chromosome misalignment at early time points following monastrol washout (Figure S6A-C).
3. We examined chromosome alignment, kinetochore:microtubule attachment, and spindle length in cells arrested in metaphase by MG132. We found that SAF-A$^{S14A\ S26A}$ cells exhibited significantly shorter spindles and defects in chromosome alignment, but no major defects in kinetochore:microtubule attachment. These results are consistent with our live cell imaging approach and suggest that defects in factors that control chromosome alignment may underlie chromosome segregation errors in SAF-A$^{S14A\ S26A}$ cells (Figure 9-10).
4. We examined the localization of many different factors that control various aspects of mitosis in SAF-A^{wt} and SAF-A$^{S14\ S26A}$ cells. We examined proteins involved in: chromosome condensation (condensin I, condensin II, KIF4), kinetochore:microtubule attachment (Hec1, Aurora-B, autophosphorylated Aurora-B, CENP-E), chromosome alignment (KIF22, KIF4, CENP-E). We found a significant defect in CENP-E localization to the kinetochore in SAF-A$^{S14A\ S26A}$ cells and colocalization of SAF-A$^{S14A\ S26A}$ with KIF22, suggesting that defects in the localization or activity of these critical chromosome alignment factors underlie the mitotic defects in SAF-A$^{S14A\ S26A}$ cells (Figure 10).

Taken together we believe that these additional experiments provide new insight into the cause of chromosome segregation defects in cells that retain SAF-A:RNA complexes on mitotic chromosomes.

(2) Based on a report by Nozawa et al, 2017, SAF-A depletion itself can cause chromosome segregation defects in RPE-1 cells. In contrast, the current study did not find chromosome segregation defects in cells depleted of SAF-A by auxin-induced degradation. This discrepancy needs to be resolved or at least discussed.

We thank the reviewer for pointing out this discrepancy between our results and those published by Nozawa et al, 2017. To address this point we have taken several approaches. First, we created a SAF-A-AID cell line in the RPE-1 background. Treatment of this cell line with IAA induces complete SAF-A degradation in ~24 hours, similar to our DLD-1 SAF-A-AID cell line. We scored mitotic defects in RPE-1 cells lacking SAF-A within a single cell cycle after SAF-A depletion. Consistent with our results in DLD-1 cells, we do not detect any mitotic defects in SAF-A mutant cells in the RPE-1 background (Figure S4). We offer several suggestions for the discrepancies between our results and those of Nozawa et al.

1. Nozawa et al. show that the basal rate of chromosome missegregation in RPE-1 cells is ~23%, which is at least 10-fold higher than reported by our group and many other groups (e.g. PMID 31527146, 28539402). This suggests that the defects observed by Nozawa et al. may be related to basal defects in their RPE-1 isolate or culture conditions.
2. Additionally, the chromosome missegregation data in Nozawa et al. appear to come from a single experiment (Nozawa et al. 2017, Figure 7C), so it is difficult to know if this rate is reproducible.
3. We examined mitotic defects after 24 hours of SAF-A depletion while Nozawa et al. relied on slower protein depletion using RNAi (48h, or 2 cell cycles). The mitotic defects that they observed could be a result of indirect effects related to slow protein depletion or siRNA off-target effects. Nozawa et al. did not rescue SAF-A RNAi with a RNAi resistant wild-type SAF-A protein, so this explanation cannot be excluded.

SAF-A was reported to regulate kinetochore-microtubule attachment and spindle organization during mitosis. The authors should examine whether cells depleted of SAF-A indeed exhibit these defects and whether these defects underlie the phenotypes of SAF-A$^{S14A\ S26A}$-GFP cells.

To address this point we examined spindle assembly and kinetochore:microtubule attachment in SAF-A-depleted, SAF-A^{wt}, and SAF-A$^{S14A\ S26A}$ cells briefly arrested in metaphase with MG132. We found that spindles were completely normal in SAF-A^{wt} and SAF-A-depleted cells, consistent with a low rate of chromosome missegregation in these genotypes. SAF-A$^{S14A\ S26A}$ cells exhibited well-formed, but shorter spindles. However, we did not detect defects in kinetochore:microtubule attachment or in the localization of Hec1, which is a major factor controlling end-on kinetochore:microtubule attachment (Figure 9 and S4).

Minor points
(1) On p8, "all RNA is labeled with BrdU..." should be "all RNA is labeled with BrU...".

We have corrected this error.

(2) The last paragraph on mitotic bookmarking is too speculative. The current study does not shed light on mitotic bookmarking.

We have removed this paragraph.

Reviewer #2 (Comments to the Authors (Required)):

Review of "Prophase removal of chromosome-associated RNAs facilitates anaphase chromosome segregation" by Sharp JA et al. in consideration at Journal of Cell Biology

Summary:

In this manuscript, the authors addressed the mechanisms of dynamic localization of nuclear RNAs during mitosis. Previous studies have shown that SAF-A tethers nuclear RNA to chromatin via DNA-binding and RNA-binding domains. Using a combination of imaging, biochemistry and molecular biology, the authors determine that 1) SAF-A:RNA complexes become excluded from mitotic chromosomes during mitosis, starting as early as prophase stage; 2) this change in localization is due to the kinase activity of Aurora B at specific residues of SAF-A; 3) the Aurora B-mediated phosphorylation of SAF-A reduces its affinity specifically to DNA; and 4) deregulation of this SAF-A:RNA dynamic localization leads to defects in chromosome segregation during anaphase. These findings lead the authors to conclude that removal of nuclear RNAs from chromatin during mitosis is essential for proper chromosome segregation.

The main strength of this manuscript is the multiple orthogonal approaches that provide convergent data to support the main findings. In particular, the combination of biochemical in vitro reconstitution experiments with molecular perturbations in cells shows that the main findings are robust. Overall, this is a strong manuscript that could be ready for publication given some attention to the following concerns.

Major concerns:

1. While it is clear based on the data provided that retention of SAF-A:RNA complex on mitotic chromosomes leads to chromosome segregation defects, it is unclear whether retention of SAF-A only, or nuclear RNA only is sufficient to induce the phenotype. Given that the authors' main conclusion (and title) is that removal of nuclear RNA from chromosomes "facilitates anaphase chromosome segregation," it is important to distinguish between the two potential mechanisms. The authors can address this by mutating the RNA-binding motif of SAF-A, and testing for segregation defects.

We agree with the reviewer that determining whether SAF-A alone or SAF-A:RNA complexes are the cause of mitotic defects is one of the most important questions raised by our study. We have addressed this point using a combination of *in vitro* assays to measure SAF-A:RNA

interactions and reconstitution of cell lines with RNA-binding mutants. We mapped the SAF-A RNA-binding domain to the central cluster of RGG repeats using *in vitro* EMSA assays (Figure 8 and S5). We then reconstituted our SAF-A AID cell lines with SAF-A with a complete RGG deletion (SAF-$A^{C\text{-}term\Delta}$) or SAF-A with a deletion of only the RGG residues required for RNA binding (SAF-$A^{RGG1\text{-}7\Delta}$). Interestingly, we found that mutations that block RNA binding also block chromatin binding in interphase and mitosis (Figure 8). Based on these results we conclude that RNA binding by SAF-A is required for chromatin interaction, which is an important conclusion that has not been reported. However, these results do not allow us to determine if SAF-A alone or SAF-A:RNA complexes are the cause of mitotic defects in SAF-$A^{S14A\ S26A}$ cells. We have modified the wording throughout the manuscript to reflect this uncertainty.

2. Previous studies have identified a formaldehyde-based artifact that has been shown to exclude DNA-binding proteins from mitotic chromosomes in mitosis (Festuccia et al. 2019 Genome Research; Teves et al 2016 eLife; Pallier et al 2003 MBoC). To fully exclude the possibility this formaldehyde-based artifact is skewing the authors results, this reviewer suggests performing live imaging of SAF-A-GFP (or the mCherry knock-in) as a time course through mitosis.

To address this issue we examined the mitotic localization of SAF-A-AID-mCherry knock-in, SAF-A^{wt}-GFP, and SAF-$A^{S14A\ S26A\text{-}GFP}$ cells using live cell imaging (Figure S2E). Our results are consistent with the fixed cell imaging presented in the initial version of the manuscript.

In addition, Figure 1B-C shows the coprecipitation of SAF-A with chromatin in interphase, but not mitosis. These IP experiments were performed in the absence of formaldehyde and provide additional evidence of cell cycle-dependent SAF-A chromatin interaction. We have added text to clarify that this experiment was performed without crosslinking, and corroborates SAF-A localization observed in live cells.

Minor concerns:
1. Is SAF-A the primary (only) method for tethering nuclear RNAs to chromatin? If not, perhaps include this background info in the introduction.

To our knowledge, there are no publications that have addressed the mechanisms of RNA tethering to chromosomes and release during mitosis. Based on our results we can conclude that SAF-A is a major mechanism of global DNA:RNA tethering, but cannot rule out additional pathways. We have added a sentence to the first paragraph of the Discussion to include this information.

2. On page 14 line 19, there is an error in the SAP mutation annotation.

We have fixed this mistake.

August 23, 2020

RE: JCB Manuscript #201910148R

Dr. Michael D Blower
Massachusetts General Hospital
Department of Molecular Biology
185 Cambridge St, CPZN-7250
Boston, MA 02114

Dear Dr. Blower,

Thank you for submitting your revised manuscript entitled "Prophase removal of chromosome-associated RNPs facilitates anaphase chromosome segregation". We have editorially assessed the revision. We greatly appreciated the changes made in revision to address the reviewers' points. We feel that this is a terrific revision and would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

1) JCB Articles are limited to 10 main and 5 supplementary figures. Each figure can span up to one entire page, with all panels fitting on the page. Could you please try to rearrange the data to meet this limit, perhaps rearranging the supplement to combine S3 and S5?

2) Titles, eTOC: Please consider the following revision suggestions aimed at increasing the accessibility of the work for a broad audience and non-experts.

Title: Cell division requires RNA eviction from condensing chromosomes

Running title (50 characters max, including spaces): RNA eviction is needed for cell division fidelity

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to S2D.
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis. Please add molecular weight with unit labels on the following panels: 1B, S2C, S4C, S5B

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends.
Please indicate n/sample size/how many experiments the data are representative of: S6C

5) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.

- All cell lines, plasmids, etc. should be presented with a brief description of the basic genetic features **even if gifted from other investigators or described in other published work** OR database/catalog IDs should be provided (e.g., ATCC, Addgene, etc.) **even if gifted from other investigators or described in other published work**
- Please include sequences for all siRNA oligos if they were made available to you from the manufacturer, including for negative control siRNAs.
- Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature
d. imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

6) A summary paragraph of all supplemental material should appear at the end of the Materials and methods section.
- Please include one sentence briefly describing each supplemental item, including tables.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, http://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and video files: See our detailed guidelines for preparing your production-ready images, http://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

**The license to publish form must be signed before your manuscript can be sent to production. A

link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.**

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

Arshad Desai, PhD
Editor, Journal of Cell Biology

Melina Casadio, PhD
Senior Scientific Editor, Journal of Cell Biology

2nd Revision - Authors' Response to Reviewers: August 28, 2020

1) JCB Articles are limited to 10 main and 5 supplementary figures. Each figure can span up to one entire page, with all panels fitting on the page. Could you please try to rearrange the data to meet this limit, perhaps rearranging the supplement to combine S3 and S5?

We combined Figure S3 and S5 into Figure S4. S3 was eliminated. We have modified the text to reflect the new changes in Figure order.

2) Titles, eTOC: Please consider the following revision suggestions aimed at increasing the accessibility of the work for a broad audience and non-experts.

Title: Cell division requires RNA eviction from condensing chromosomes

Running title (50 characters max, including spaces): RNA eviction is needed for cell division fidelity

Thank you for the suggestions. We have changed both titles.
Title: Cell division requires RNA eviction from condensing chromosomes
Running title: RNA removal from chromosomes in mitosis

3) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications. Please add scale bars to S2D.

Scale bar was added to S2D and specified in the Figure legend.

Molecular weight or nucleic acid size markers must be included on all gel electrophoresis. Please add molecular weight with unit labels on the following panels: 1B, S2C, S4C, S5B

Molecular Weight added to 1B, S2C, S3C, S4C. (Figure numbers reflect new order.)

4) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends.
Please indicate n/sample size/how many experiments the data are representative of: S6C

Each figure legend with quantitative data specifies that error bars represent the standard deviation. We have included information for the number of independent data points (n), and specified the statistical test used. The Materials and methods describe statistical tests and experimental replicates (p. 56-57).

We have corrected the figure legend in Figure S5C to specify that two experiments were performed. (S5C is the new figure number).

5) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions in the text for readers who may not have access to referenced manuscripts.
- All cell lines, plasmids, etc. should be presented with a brief description of the basic genetic features **even if gifted from other investigators or described in other published work** OR database/catalog IDs should be provided (e.g., ATCC, Addgene, etc.) **even if gifted from other investigators or described in other published work**

We added catalog numbers for RPE-1, 293T, and DLD-1 cells.
Cell lines constructed in this study are described in detail on p. 57-60 of the Materials and Methods section.
Plasmids constructed in this study are described in detail on p. 48, 53, 54, 59, and 60.

- Please include sequences for all siRNA oligos if they were made available to you from the manufacturer, including for negative control siRNAs.

We have updated p. 49 to include siRNA sequences.

- Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
yes
b. Type, magnification, and numerical aperture of the objective lenses

yes
c. Temperature
yes
d. imaging medium
yes
e. Fluorochromes
yes
f. Camera make and model
yes
g. Acquisition software
yes
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).
yes

Details of Microscope image acquisition are described on p. 49-50.

6) A summary paragraph of all supplemental material should appear at the end of the Materials and methods section.
- Please include one sentence briefly describing each supplemental item, including tables.

added to p. 60-61.