EMBO reports

A dual-function SNF2 protein drives chromatid resolution and nascent transcripts removal in mitosis

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1st Editorial Decision **23rd Dec 2022**

Dear Raquel,

Thank you for submitting your research manuscript for consideration by EMBO reports. It has now been seen by three experts in the field, and we have received the full set of their reports that is appended below.

As you will see, the referees acknowledge that the study uses elegant approaches to address a fundamental question that has been debated for a long time, and that the findings are interesting. However, they also identify limitations and raise a number of concerns, which should be addressed during revision. Several of their comments relate to the clarity of the presentation and suggest that the study should be better placed into context, and the relevant literature should be discussed more thoroughly. The referees also provide in their well-informed reports detailed suggestions for the improvement of the study and the manuscript.

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

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we usually recommend a revision within 3 months (March 22nd). Please discuss with me the revision progress ahead of this time if you require more time to complete the revisions.

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- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) Before submitting your revision, primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>).

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below (see also < https://www.embopress.org/page/journal/14693178/authorguide#dataavailability>). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

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8) We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the new policy (<https://www.embopress.org/competing-interests>) and update your competing interests statement if necessary. Please name this section 'Disclosure and competing interests statement' and place it after the Acknowledgements section.

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The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,
- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,
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- If the data are obtained from n {less than or equal to} 2, use scatter plots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

See also the guidelines for figure legend preparation:
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- Please also include scale bars in all microscopy images.

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directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

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Best regards,

Ioannis

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

The means by which transcriptional diminution in mitosis is linked to the cell cycle has been debated for years. Previous studies indicated a role for condensing-I mediated compaction as driving mitotic transcription diminution, but here the authors have used live-cell imaging and a genetic screen to show that the SNF2 helicase-like protein Lds1 in Drosophila, an ortholog of the human TTF2, plays an important role along with aiding DNA decatenation. Specifically, the authors claim that LDS promotes transcription termination during mitosis and resolves sister chromatin exchange. While the fundamentals of the paper are sound and the paper is generally suitable for EMBO, substantial work needs to be done on a revision, as detailed below. Note that many of the comments relate to clarity of the presentation.

1. In the paper, the authors talk about "transcription termination" , "abortion of ongoing transcription", "switch off of transcription" but these are diverse aspect of transcription process. "Transcription termination" it is when currently transcribed RNA is cleaved, polyadenylated and released from chromatin and that RNA pol is evicted from DNA; whereas the "switch off of transcription" it is when transcription is repressed and promote silenced. Moreover experiments proposed by authors this manuscript do not allow to study "termination of transcription" since they monitor nascent RNA and RNA steady state. The text needs to be edited extensively to be clear and specific about what they can conclude from their experiments.

2. To understand LDS function in transcription termination during mitosis, the author used MS2/MCP system in Drosophila embryos. The system monitors ongoing transcription thanks to the presence of M2 loop in 5'end-transcript of interest, therefore this approach allows to visualize the ON-OFF stage of a gene or nascent transcription, but not "transcript release" as proposed

by the authors (page 8 line 23). To prove that the authors observed a transcription release phenomenon, they have to determine whether transcription is initiated or not at promoter and that transcript is dissociated from chromatin (e.g by combining DNA- and RNA-FISH or other approaches). If it is not the case, the authors could not claim that LSD protein is involved in transcript detachment from chromatin during mitosis (as page 10 line 6).
3. The authors said "We used nanos Gal4 (nos-Gal4) driver to induce Lds depletion in the germline, which, similar to what we observed in the wing, leads to severe mitotic defects in these early embryonic divisions (Fig S1), that can be rescued by ectopic addition of Lds (Fig S2A)." Here, they proposed to compare Figure S1 and S2 but it is difficult to compare them (same for figure 1 and 3) since histograms do not contain the same category numbers. Could the authors update these figures to facilitate comparison, comprehension and conclusion of their results?

4.Could the authors add supplementary figures showing LDS mRNA depletion/rescue efficiency? (Related to Figure 3).

5. Page 11 line 9 : "We microinjected embryos with 1 mg/ml alpha-amanitin in early interphase, which is sufficient to abolish transcription, as evidenced by the absence of MS2-labelled reporter RNAs in the subsequent interphase (Fig S4)". Figure S4 DOES NOT show what the authors claimed here. Could the authors, please, add a figure showing RNApol II degradation and stop a loss of global and specific under alpha-amanitin. Morover in figure S4, add control conditions for A and B panels to allow comparison of data in WT and RNAi treated cells. Could the authors also quantify these results in order to allow conclusions from this figure?

6. Page 14 line 2 : "Moreover, we could not detect any delay in the kinetics of Rad21-EGFP removal at the metaphase to anaphase transition (Fig 4B)" Please add corresponding quantification analysis.

7. In Figure 5, control conditions (Luciferase RNAi) are missing therefore the readers cannot conclude anything for this Figure. Thus authors should add control condition to figure 5A and B and/or rephrase their conclusion page 14 line 13 and 18. Same for Figure 5C and line 22.

8. Page 15 line 10 the authors said "Notably, the rise in Lds-GFP does not follow the same kinetics as Top2. It increases faster than Top2 itself, reaching a steady-state on equimolar concentrations at later time points" suggesting that LDS binds/acts/is recruited before Top2, but the authors concluded page 15 line 12 that "Lds is sensitive to Top2 inhibition and is either co-enriched on mitotic chromosomes via direct binding to Top2 and/or is recognising topological changes on DNA induced upon Top2 inhibition". The authors never show a direct interaction between LDS and TOP2 allowing LDS trapping on mitotic chromatin, so they should rephrase their conclusion. To validate or not interaction between TOP2 and LDS, could the authors do a Co-IP experiment.

9.Page 15 line 2 : "Importantly, we observed that treatment of ICRF-193 increased the half-time of Top2 recovery significantly (t1/2= 4.43 {plus minus} 1.51 min, p<0.0001 One-way ANOVA), evidencing the differential mode of binding of this molecule to chromatin in the presence of the inhibitor"; This sentence is in opposition with results of Figure 6E, where TOP2 residence time is higher under DMSO treatment than ICRF-193.

10. Figure 2C: change color range, it is difficult to distinguish each categories. (Same for Figure S1B)

11. Figure S1: "Lagging segregation defect" category is missing in A and B panels, thus the sentence page 6 "Our results confirm that Lds is required for mitotic fidelity in developing wing epithelia, similar to what was reported for embryonic syncytial divisions" is not totally true, could you correct panels or rephrase, please. Moreover for easier comparison between mitosis occurring in wing disc and in syncytial embryos, add examples of wing disc mitotic defects.

12. Page 10 line 31: "Altogether, these results suggest that loop extrusion mediated by condensin I complexes does not contribute to the eviction of RNA PolII from chromatin during mitosis, and support the hypothesis that timely termination of transcription during mitosis relies on specific mechanisms, driven by the helicase-like protein Lds" : The authors should mitigate their conclusion since they never shown/observed directly RNApol II on mitotic chromatin.

Editorial comments:
1.Please add line numbers to your manuscript.

2.There are several mistakes concerning citation order of figures in the text.
-"In both wing discs and syncytial embryos, we observed that Lds is excluded from chromatin during interphase but gains access to mitotic chromatin around the time of nuclear envelope breakdown (NEBD) (Fig 2C,D)."
-Should be figure 2D,E?. Then figure S3 is cited before S2.
-Page 10 : "We used nanos Gal4 (nos-Gal4) driver to induce Lds depletion in the germline, which, similar to what we observed in the wing, leads to severe mitotic defects in these early embryonic divisions (Fig S1), that can be rescued by ectopic addition of Lds (Fig S2A)." : Figure S2A correspond to a experimental scheme and not to histograms.
- This delay is partially restored upon ectopic addition of mRNA coding for an RNAi-resistant form of lds (Fig S2B) : Fig S2B shows frequency of events whereas Fig S2C shows a length of time.
-"Next, we used the same approach to inhibit transcription in lds RNAi embryos. This analysis revealed that in the presence of

transcription (control H2O injection), lds RNAi embryos present ~43 % of abnormal anaphase figures. Upon alpha-amanitin injection, the frequency of these defects is not significantly reduced (41%). These findings imply that the presence of trapped nascent mRNAs on mitotic chromatin is not the primary source of defects that compromise mitotic fidelity in Lds-depleted embryos." No figures cited in this paragraph...
-We focused on the fact that the most prominent phenotype was a high frequency of chromatin bridges to investigate whether Lds contributes to the resolution of sister chromatids. Once again, No figures cited in this paragraph...
-Figure 6A is never citated in the text
-In the last paragraph page 15 and the first page 16 : Again, no figure is cited in the text.

3. Images from figure 2D are blurred.

4. "Sister chromatid exchange" instead of "sister chromatid"?

Referee #2:

Summary.
During mitosis chromatin dramatically reorganizes and condenses in preparation for segregation to daughter cells. During prophase the majority of cohesin complexes are removed from chromatin and replaced by condensin complexes, which triggers chromosome condensation. At the same time the vast majority of nuclear transcription is halted through a series of mechanisms that prevent new transcription initiation and remove elongating RNA Polymerases and nascent transcripts from chromatin. However, it is clear that specialized mechanisms control various aspects of transcription termination at various locations on the chromosomes and we do not have a complete understanding of mitotic transcriptional inhibition. Carmo et al. approach this problem using a combination of Drosophila genetics and live cell imaging. They find that Lodestar (Lds), which is a homolog of mammalian Transcription Termination Factor 2 (TTF2), genetically interacts with the cohesin and condensin complexes as well as with Topoisomerase 2. They use a very elegant live cell imaging assay to show that Lds mutants fail to properly terminate transcription during mitosis in syncytial Drosophila embryos. Additionally, Lds mutant embryos have dramatic mitotic defects likely caused with a failure to resolve catenated chromosomes. Interestingly, Lds localization is not affected by loss of condensin and that Lds mutants do not affect condensin or Top2 localization. Importantly, they show that loss of condensin or Top2 do not affect mitotic transcription termination. They conclude that Lds plays two independent roles during mitosis: 1. Transcription termination and 2. Interaction with the chromosome decatenation machinery. The strengths of this study are: the overall high quality of the data presented, clear demonstration that condensin and Top2 are not required for transcription termination, and elegant use of live cell imaging to investigate the function of Lds. The weaknesses are: a failure to fully investigate the genetic interactions of the cohesin pathway and Lds, and a failure to effectively put their results in the proper context of previous work on mammalian TTF2. Overall, this is strong study that will be an important addition to our understanding of mitotic transcriptional inhibition with appropriate revisions.

Major points:
1. The manuscript begins with the observation that Lds mutants suppress the phenotype of san mutants that exhibit weakened sister chromatid cohesion. The authors then show that Lds mutants do not dramatically effect cohesin localization (see also below). However, recent work (PMID: 32035037) showed that a failure to remove cohesin from chromosome arms during mitosis leads to a failure to properly remove elongating RNAPII from chromatin. Additionally, previous work on human TTF2 showed that TTF2 RNAi leads to a retention of elongating RNAPII on mitotic chromosomes (15125840). To fully integrate their results with the existing literature the authors should examine Lds localization in WAPL and Rad21 mutant cells/embryos. This would help to illuminate the mechanism of genetic interaction and understand if cohesin removal and Lds are working in parallel to terminate transcript during mitosis.
2. In Figure 4 the authors show that Lds RNAi does not dramatically change the localization of Rad21-GFP. This experiment would be much more convincing if the authors provided quantitation of Rad21-GFP levels during embryonic mitosis from many nuclei from several embryos. The current data may miss a small change in Rad21 levels or a slight delay in Rad21 movement during mitosis.
3. In the introduction the authors provide a nice summary of the literature related to mitotic transcription inhibition and the phenotypes of Lds mutants in Drosophila. However, I believe that they need to provide a more complete description of work performed on human TTF2. Jiang et al (15125840) showed that TTF2 is exclusively cytoplasmic during interphase and enters the nucleus during mitosis. Additionally, they show that TTF2 RNAi leads to a retention of elongating RNAPII on mitotic chromosomes, arguing that TTF2 is an important part of MTI. These key findings are replicated in the current work using in vivo imaging in a whole organism, which is a clear improvement. However, the authors need to carefully acknowledge this previous work in the introduction of their manuscript.

Minor Points:
1. On p15. The authors state that Lds-GFP "increases faster than Top2 itself, reaching a steady state on equimolar concentrations at later time points." What is Lds equimolar with? How were the relative molar concentrations of each protein determined? I find this sentence overstated and confusing.

2. Although it is probably not necessary for this manuscript it would be very interesting to determine if Lds mutants also result in transcript retention on mitotic chromosomes in wing discs and if transcription inhibition represses mitotic defects observed in Lds mutants.
3. It would be helpful if the authors could provide some experimental data to show the effectivity of the various RNAi treatments that they use throughout the manuscript. It is not possible to know if they are examining complete loss of function or hypomorphic mutant conditions.

Referee #3:

General comments
In this manuscript, Carmo and colleagues uncover a novel-dual role for Lds, the Drosophila ortholog of TTF2, in the dissociation of nascent RNAs from condensing chromatin and, in collaboration with Top2, in the decatenation of sister chromatid intertwines early in mitosis. The authors also claim that Lds role is key for appropriate chromosome segregation during mitosis.

The authors focus on Lds after its identification as a clear suppressor of cohesion defects. Using a combination of molecular and cellular approaches in adult and embryo tissues, Coma and colleagues study the Lds function during mitosis. Mitotic association of Lds with chromatin together with the fact that Lds knockdown cause a plethora of mitotic defects, evidence the fundamental role of this factor on chromosome segregation. Coma and colleagues identify a dual role of Lsd: Lsd promotes the chromatin eviction of nascent RNAs and participate in DNA decatenates resolution early in mitosis. Unfortunately, data presented here evidence no connection between both roles; the functional implication of Lsd role on nascent RNA eviction is missing; the connection of Lsd with DNA bridges is observational and lacks of a functional mechanism behind. In overall, I consider this study very interesting although lacks of important pieces that might be challenging to resolve under the frame of the quick review process for EMBO reports.

Major points:
1) Related to Figure 3. In this figure, authors attend to investigate the role of Lds in transcription termination following mitotic entry. Authors monitor the chromatin association of a reporter transcript (hb) fused to MS2 loops as a tool to evaluate nascent transcription and termination. Based on the retention of nascent hb-MS2 transcripts on mitotic chromatin under Lds depletion, authors claim a role for Lds on the eviction of nascent RNAs from condensing chromatin and, as a consequence, on transcription termination. Although results are encouraging, the study of a single transcript behavior weak this part of the study. I suggest to develop an approach to widely evaluate the effect on nascent transcription and Pol II. As an alternative, I would include the study of several other zygotic transcripts and include proofs that MS2 fusion does not impact the mRNA levels of the target gene. Importantly, authors did not mention or discuss the results from a previous article describing the role of Aurora B and SAFA in the general eviction of RNAs from condensing chromatin (Sharp et al., 2020 PMID: 33053167). This reference should be properly evaluated in a revised version of the manuscript.
In addition, the evidence demonstrating the chromatin association of hb-transcripts is not quite solid. Although co-localization with chromatin is a good indicator, I would suggest definitive proofs of a direct association between nascent transcript and the hb locus by performing DNA-FISH or a similar alternative.
Authors evaluate whether the chromosome segregation errors identified under Lds depletion are linked to defects on the dissociation of RNA from chromatin. Data indicate no connection as further demonstrated by the results in Figure 7, where authors find retention of nascent RNAs independently of Top2 inhibition. Thus, the functional implication of the Lds role on transcription termination remains unresolved and weakens the general interest of the article. More importantly, the presumable link between chromatin remodeling and transcription termination executed by Lsd remains undemonstrated.

2) Related to Figure5 and 6. Due to the high rate of chromosome bridges shown by Lds-depleted cells, the authors investigate the potential participation of Lds on the resolution of sister chromatids. Results demonstrate the localization of Lds protein at chromosome bridges. Moreover, Barren and Top2 localize at those areas under Lds depletion. However, whether Barren/Top2 accumulation at chromosome bridges is higher under Lsd absence is not explored.
Although the inhibition of Top2 activity cause a significant increase on the levels of chromatin-associated Lsd, FRAP data show that the resilience time is unaffected. Although data is compelling, the mix of results from studies looking at chromosome bridges and whole metaphase chromosomes makes the interpretation of the results difficult.
My overall feeling about this part of the article is that the connection between Lsd and DNA decatenation is there, how Lsd interplay with Top2 is not understood and might weaken the quality and interest of the story.

Minor points:
1) Authors widely use the RNA interference system (RNAi) to knockdown several genes of interest. Although I consider this a very appropriate approach, I highly recommend measuring and showing the effect on the levels of the target proteins. In this direction, I suggest testing at least the efficiency of RNAi against Lds in flies expressing Lds-GFP (Fig2D-E).

2) Related to Figure 2C. The authors show in this panel the quantification of mitotic defects associated with Lds knockdown. I suggest including the % for each category in order to improve clarity. I would also include the quantification of control flies to

determine whether the low percentage of normal mitosis (around 60%) is a characteristic of the cell niche used or if is caused by the RNAi transfection protocol.

3) Related to Figure 2D-E. The authors elegantly show here chromatin association of Lds specifically in mitosis by endogenously expressing a C-terminal fusion to GFP. I consider this is a very clear and relevant result but suggest to include an orthogonal method. Moreover, proof about the functionality of the fusion protein is missing. By performing IF including an anti-Lps antibody, if available, authors will show the behavior of a WT version of Lds.

We would like to thank all the reviewers for their constructive criticism that help us produce a much-improved version of the manuscript.

Referee #1:

The means by which transcriptional diminution in mitosis is linked to the cell cycle has been debated for years. Previous studies indicated a role for condensing-I mediated compaction as driving mitotic transcription diminution, but here the authors have used live-cell imaging and a genetic screen to show that the SNF2 helicase-like protein Lds1 in Drosophila, an ortholog of the human TTF2, plays an important role along with aiding DNA decatenation. Specifically, the authors claim that LDS promotes transcription termination during mitosis and resolves sister chromatin exchange. While the fundamentals of the paper are sound and the paper is generally suitable for EMBO, substantial work needs to be done on a revision, as detailed below. Note that many of the comments relate to clarity of the presentation.

1. In the paper, the authors talk about "transcription termination" , "abortion of ongoing transcription", "switch off of transcription" but these are diverse aspect of transcription process. "Transcription termination" it is when currently transcribed RNA is cleaved, polyadenylated and released from chromatin and that RNA pol is evicted from DNA; whereas the "switch off of transcription" it is when transcription is repressed and promote silenced. Moreover experiments proposed by authors this manuscript do not allow to study "termination of transcription" since they monitor nascent RNA and RNA steady state. The text needs to be edited extensively to be clear and specific about what they can conclude from their experiments.

We thank the reviewer for the valid point raised. We have rephrased the entire manuscript (including the title) to refer to the phenomenon we observed as a defect in “transcript release” from mitotic chromatin and left the possible interpretations of what that means in terms of regulation of the transcription cycle to the discussion. Corresponding changes are highlighted in grey throughout the manuscript.

2. To understand LDS function in transcription termination during mitosis, the author used MS2/MCP system in Drosophila embryos. The system monitors ongoing transcription thanks to the presence of M2 loop in 5'end-transcript of interest, therefore this approach allows to visualize the ON-OFF stage of a gene or nascent transcription, but not "transcript release" as proposed by the authors (page 8 line 23). To prove that the authors observed a transcription release phenomenon, they have to determine whether transcription is initiated or not at promoter and that transcript is dissociated from chromatin (e.g by combining DNA- and RNA-FISH or other approaches). If it is not the case, the authors could not claim that LSD protein is involved in transcript detachment from chromatin during mitosis (as page 10 line 6).

We have experimentally addressed this issue by several means:

a. We now provide a detailed characterization of how nascent mRNA signals disappear from chromatin during mitosis in control and lds-depleted embryos (Fig 3C). This analysis revealed that in most nuclei of control embryos (~60%), a signal is detected detaching from chromatin following NEBD. Considering that

single mRNA molecules would not be distinguishable from the cytoplasm background, these findings support that multiple mRNAs are being detected travelling away from chromosomes. These findings support that, in these embryos, the entry in mitosis triggers bulk transcript release. Importantly, we find that in Lds-depleted embryos, the frequency of detected “bulk” mRNA release is significantly reduced (frequency < 15%, see updated Fig. 3C).

b. We included quantitative analysis of the kinetics of mRNA levels decay on mitotic chromatin, relative to mitotic progression (Figure EV3C). This analysis reveals that the onset of decay is similar between lds-RNAi and controls, which supports that early mitotic events occur at a normal pace. However, the rate of transcript decay from chromatin is much reduced upon lds RNAi. This kinetic behaviour, although not a direct proof, is more consistent with a defect in transcripts removal.

c. We have included new data supporting that retained transcripts are located at the transcription site (See Fig. EV3D). Using anaphase-staged embryos, we have measured the location of the hb-MS2-lacz genomic loci using DNA FISH against MS2. Similar measurements revealed that the position of the MCP signal (ie, nascent mRNAs) is very similar to the one of the genomic loci. Moreover, analysis of the location of another reporter more distally placed (sna-MS2-Lacz-PP7) appears on anaphase chromatin in a region consistent with the respective genomic locus. We were unable to complement these observations by co-labelling DNA/RNA, as suggested, as our attempts to optimize DNA/RNA FISH in early embryos led to a very reduced relevant sample size (ie, embryos at the right mitotic stage with both probes efficiently incorporated), even when starting with large embryo collections (>300 embryos). We observed the expected result (co-localization) in a couple of embryos (n=2). But as the number of replicates did not meet publication standards, we opted not to include these experiments in the manuscript. Nevertheless, we trust the separate measurements of the signal positioning in anaphase strongly support our conclusion that retained RNAs are located at the transcription site.

Collectively, these new results substantiate our initial conclusion that Lds triggers the release of nascent mRNAs, which is consistent with its previous transcription termination activity detected in vitro. We have updated the description of the text to justify the rationale for our interpretation, considering the points raised above. We further highlight that our findings do not exclude that the retained transcripts may result from other perturbations in the transcription cycle (e.g. failures to inhibit transcription initiation), which are technically very difficult to evaluate in this experimental system (see line 165).

3. The authors said "We used nanos Gal4 (nos-Gal4) driver to induce Lds depletion in the germline, which, similar to what we observed in the wing, leads to severe mitotic defects in these early embryonic divisions ±(Fig S1), that can be rescued by ectopic addition of Lds (Fig S2A)." Here, they proposed to compare Figure S1 and S2 but it is difficult to compare them (same for figure 1 and 3) since histograms do not contain the

same category numbers. Could the authors update these figures to facilitate comparison, comprehension and conclusion of their results?

We apologize for the inconsistencies presented. The differences in scoring categories were caused by a variable approach to the minor percentage of cells in which the segregation defects could not be properly identified (originally scored as “others” in original figure S1 and Fig. 3) and the use of different transgenes across the tissues (e.g. use of centromere marker in the wing, Fig. 2).

To address this point and a similar raised below (#11), and in the interest of consistency between the different tissues/figures, we have now re-analysed all the data sets to present a uniformized scoring. In particular:

a. We have excluded from the analysis all images where segregation defects could not be properly assigned and refer to that selection in M&M. This led to minor changes in the overall frequencies as this percentage was <4%.
b. We have re-analysed all the segregation defects in the wing (Fig 2C), taking into account solely the DNA channel (excluding the centromere marker) to monitor DNA bridges. As such, the frequency of lagging chromosomes is now not included. This analysis does not reflect a decrease in the overall percentage of segregation errors presented, as most lagging chromosomes also display significant DNA bridges.

4.Could the authors add supplementary figures showing LDS mRNA depletion/rescue efficiency? (Related to Figure 3).

We have included new data demonstrating that RNAi is highly efficient in early embryos and wing discs. Due to the failure in producing a reliable anti-Lds antibody, we used strains carrying the GFP-tagged transgene for this analysis and probed with anti-GFP antibody or GFP fluorescence (Figure EV1 A, B).

As such, we were unable to use the same approach to estimate the efficiency of mRNA rescue (for which we used an untagged construct). To circumvent this limitation, we have estimated the efficiency of rescue by measuring the viability of embryos upon injection of mRNA, which was the same approach used in our rescue experiments (Fig. EV2 F,G) . This analysis revealed that while embryos derived from lds RNAi female are not viable, consistent with prior reports of its associated female sterility, embryos from lds-RNAi females that were injected with RNAi-resistant mRNA were able to hatch. The rescue observed is partial (10 % vs 54% in control conditions), which is consistent with the partial rescue observed for the segregation defects and transcripts eviction. The inability to obtain a full rescue is not unexpected considering the assay employed, which relies on the injection of mRNA that needs to be translated and folded in a timely manner (within ~1 hour).

5. Page 11 line 9 : "We microinjected embryos with 1 mg/ml alpha-amanitin in early interphase, which is sufficient to abolish transcription, as evidenced by the absence of MS2-labelled reporter RNAs in the subsequent interphase (Fig S4)". Figure S4 DOES NOT show what the authors claimed here. Could the authors, please, add a figure showing RNApol II degradation and stop a loss of global and specific under alpha-amanitin. Morover in figure S4, add control conditions for A and B panels to allow

comparison of data in WT and RNAi treated cells. Could the authors also quantify these results in order to allow conclusions from this figure?

As suggested, we have included a detailed quantitative analysis of the transcription levels upon alpha-amanitin injection that confirm that, at least for the loci measured, transcription is efficiently abolished (see updated Fig EV4). Unfortunately, measuring RNApol II degradation or analysis of global transcription is technically incompatible with the acute approach employed in these experiments.

Therefore, we have rephrased this sentence to refer to “impaired” transcription rather than “abolished” transcription as we cannot formally exclude that transcription may still occur at other genomic regions (line 202).

6. Page 14 line 2 : "Moreover, we could not detect any delay in the kinetics of Rad21-EGFP removal at the metaphase to anaphase transition (Fig 4B)" Please add corresponding quantification analysis.

We have now added quantifications of both the levels and kinetics of chromatin removal (see updated Fig 5C,D). These new results confirm our prior claims that the removal of Rad21-EGFP is unperturbed upon Lds depletion.

7. In Figure 5, control conditions (Luciferase RNAi) are missing therefore the readers cannot conclude anything for this Figure. Thus authors should add control condition to figure 5A and B and/or rephrase their conclusion page 14 line 13 and 18. Same for Figure 5C and line 22.

We have substantially altered this figure to include direct comparison with the corresponding control conditions (luciferase RNAi) and quantified both the levels in metaphase as well as the frequency of bridges with and without the specific protein enriched at the bridge. These new results confirm our previous interpretation that the levels of these proteins remain unaltered and that most bridges observed contain Top2/Barr/Lds. The new results can be seen in the updated Figure 6.

8. Page 15 line 10 the authors said "Notably, the rise in Lds-GFP does not follow the same kinetics as Top2. It increases faster than Top2 itself, reaching a steady-state on equimolar concentrations at later time points" suggesting that LDS binds/acts/is recruited before Top2, but the authors concluded page 15 line 12 that "Lds is sensitive to Top2 inhibition and is either co-enriched on mitotic chromosomes via direct binding to Top2 and/or is recognising topological changes on DNA induced upon Top2 inhibition". The authors never show a direct interaction between LDS and TOP2 allowing LDS trapping on mitotic chromatin, so they should rephrase their conclusion. To validate or not interaction between TOP2 and LDS, could the authors do a Co-IP experiment.

We apologize for the confusion as we did not wish to conclude a direct interaction between Top2 and Lds. In fact, as we discuss in the subsequent sentences, our results

on Lds turnover upon ICRF treatment (which remains highly mobile) suggest this is not the case. If Lds would be stably bound to Top2 it would become less mobile upon ICRF treatment, following the decrease in Top2 turnover. As such, we have changed the description of this part of the results to avoid an unsubstantiated inference of a potential interaction between these two proteins.

9.Page 15 line 2 : "Importantly, we observed that treatment of ICRF-193 decreased the half-time of Top2 recovery significantly (t1/2= 4.43 {plus minus} 1.51 min, p<0.0001 One-way ANOVA), evidencing the differential mode of binding of this molecule to chromatin in the presence of the inhibitor"; This sentence is in opposition with results of Figure 6E, where TOP2 residence time is higher under DMSO treatment than ICRF-193.

We thank the reviewer for pointing this out. We of course meant “increased the half time of recovery” (ie, decreased turn-over). The sentence has been corrected accordingly (line 394).

10. Figure 2C: change color range, it is difficult to distinguish each categories. (Same for Figure S1B)

All graphs have been changed in colour scheme to facilitate their readability.

11. Figure S1: "Lagging segregation defect" category is missing in A and B panels, thus the sentence page 6 "Our results confirm that Lds is required for mitotic fidelity in developing wing epithelia, similar to what was reported for embryonic syncytial divisions" is not totally true, could you correct panels or rephrase, please. Moreover for easier comparison between mitosis occurring in wing disc and in syncytial embryos, add examples of wing disc mitotic defects.

As explained above (see reply to point #3) we have reanalysed the data to improve consistency between the different tissues and restricted our analysis to the evaluation of chromatin bridges. We have also included images in Fig 2C to depict examples of defects in the wing epithelia, to facilitate comparison with errors exemplified in the embryo (EV1).

12. Page 10 line 31: "Altogether, these results suggest that loop extrusion mediated by condensin I complexes does not contribute to the eviction of RNA PolII from chromatin during mitosis, and support the hypothesis that timely termination of transcription during mitosis relies on specific mechanisms, driven by the helicase-like protein Lds" : The authors should mitigate their conclusion since they never shown/observed directly RNApol II on mitotic chromatin.

We fully agree. We have rephrased this sentence to refer to the “removal of nascent mRNA transcripts” instead of RNApol II.

Editorial comments:

1.Please add line numbers to your manuscript.

Line numbers are now included in the revised version.

2.There are several mistakes concerning citation order of figures in the text.

-"In both wing discs and syncytial embryos, we observed that Lds is excluded from chromatin during interphase but gains access to mitotic chromatin around the time of nuclear envelope breakdown (NEBD) (Fig 2C,D)." -Should be figure 2D,E?.
Then figure S3 is cited before S2.
-Page 10 : "We used nanos Gal4 (nos-Gal4) driver to induce Lds depletion in the germline, which, similar to what we observed in the wing, leads to severe mitotic defects in these early embryonic divisions (Fig S1), that can be rescued by ectopic addition of Lds (Fig S2A)." : Figure S2A correspond to a experimental scheme and not to histograms.
- This delay is partially restored upon ectopic addition of mRNA coding for an RNAi-resistant form of lds (Fig S2B) : Fig S2B shows frequency of events whereas Fig S2C shows a length of time.
-"Next, we used the same approach to inhibit transcription in lds RNAi embryos. This analysis revealed that in the presence of transcription (control H2O injection), lds RNAi embryos present ~43 % of abnormal anaphase figures. Upon alpha-amanitin injection, the frequency of these defects is not significantly reduced (41%). These findings imply that the presence of trapped nascent mRNAs on mitotic chromatin is not the primary source of defects that compromise mitotic fidelity in Lds-depleted embryos." No figures cited in this paragraph...
-We focused on the fact that the most prominent phenotype was a high frequency of chromatin bridges to investigate whether Lds contributes to the resolution of sister chromatids. Once again, No figures cited in this paragraph...
-Figure 6A is never citated in the text
-In the last paragraph page 15 and the first page 16 : Again, no figure is cited in the text.

Reference to the figures has been thoroughly revised and updated according to the new figures' layout.

3. Images from figure 2D are blurred.

We have replaced this figure with another one with improved quality.

4. "Sister chromatid exchange" instead of "sister chromatid"?

We could not identify what this correction was referring to.

Referee #2:

Summary.
During mitosis chromatin dramatically reorganizes and condenses in preparation for segregation to daughter cells. During prophase the majority of cohesin complexes are removed from chromatin and replaced by condensin complexes, which triggers chromosome condensation. At the same time the vast majority of nuclear transcription is halted through a series of mechanisms that prevent new transcription initiation and remove elongating RNA Polymerases and nascent transcripts from chromatin. However, it is clear that specialized mechanisms control various aspects of transcription termination at various locations on the chromosomes and we do not have a complete understanding of mitotic transcriptional inhibition. Carmo et al. approach this problem using a combination of Drosophila genetics and live cell imaging. They find that Lodestar (Lds), which is a homolog of mammalian Transcription Termination Factor 2 (TTF2), genetically interacts with the cohesin and condensin complexes as well as with Topoisomerase 2. They use a very elegant live cell imaging assay to show that Lds mutants fail to properly terminate transcription during mitosis in syncytial Drosophila embryos. Additionally, Lds mutant embryos have dramatic mitotic defects likely caused with a failure to resolve catenated chromosomes. Interestingly, Lds localization is not affected by loss of condensin and that Lds mutants do not affect condensin or Top2 localization. Importantly, they show that loss of condensin or Top2 do not affect mitotic transcription termination. They conclude that Lds plays two independent roles during mitosis: 1. Transcription termination and 2. Interaction with the chromosome decatenation machinery. The strengths of this study are: the overall high quality of the data presented, clear demonstration that condensin and Top2 are not required for transcription termination, and elegant use of live cell imaging to investigate the function of Lds. The weaknesses are: a failure to fully investigate the genetic interactions of the cohesin pathway and Lds, and a failure to effectively put their results in the proper context of previous work on mammalian TTF2. Overall, this is strong study that will be an important addition to our understanding of mitotic transcriptional inhibition with appropriate revisions.

Major points:

1. The manuscript begins with the observation that Lds mutants suppress the phenotype of san mutants that exhibit weakened sister chromatid cohesion. The authors then show that Lds mutants do not dramatically effect cohesin localization (see also below). However, recent work (PMID: 32035037) showed that a failure to remove cohesin from chromosome arms during mitosis leads to a failure to properly remove elongating RNAPII from chromatin. Additionally, previous work on human TTF2 showed that TTF2 RNAi leads to a retention of elongating RNAPII on mitotic chromosomes (15125840). To fully integrate their results with the existing literature the authors should examine Lds localization in WAPL and Rad21 mutant cells/embryos. This would help to illuminate the mechanism of genetic interaction and understand if cohesin removal and Lds are working in parallel to terminate transcript during mitosis.

We have now included quantifications of Lds localization upon RNAi for WAPL and the cohesin subunit SA1. These experiments revealed that Lds levels remain unaltered in conditions where the cohesion cycle is perturbed. We thus trust that the Lds pathway in transcripts removal will likely work independently of cohesin.

2. In Figure 4 the authors show that Lds RNAi does not dramatically change the localization of Rad21-GFP. This experiment would be much more convincing if the authors provided quantitation of Rad21-GFP levels during embryonic mitosis from many nuclei from several embryos. The current data may miss a small change in Rad21 levels or a slight delay in Rad21 movement during mitosis.

We have now added quantifications of both the levels and kinetics of chromatin removal (see updated Fig 5C,D). Although we cannot exclude that *slight changes* may take place that are undetected by our analysis, our results support that loss of Lds does not cause drastic changes in the dynamics of cohesin removal during mitosis.

3. In the introduction the authors provide a nice summary of the literature related to mitotic transcription inhibition and the phenotypes of Lds mutants in Drosophila. However, I believe that they need to provide a more complete description of work performed on human TTF2. Jiang et al (15125840) showed that TTF2 is exclusively cytoplasmic during interphase and enters the nucleus during mitosis. Additionally, they show that TTF2 RNAi leads to a retention of elongating RNAPII on mitotic chromosomes, arguing that TTF2 is an important part of MTI. These key findings are replicated in the current work using in vivo imaging in a whole organism, which is a clear improvement. However, the authors need to carefully acknowledge this previous work in the introduction of their manuscript.

We thank the reviewer for this comment as we had indeed made a mistake in the citation: Liu et al 1998 was cited instead of Jiang et al 2004 when referring to prior work on TTF2 in MTI. We have corrected the reference and extended the sentence to refer to the reported dynamic localization of TTF2 in human cells.

Minor Points:

1. On p15. The authors state that Lds-GFP "increases faster than Top2 itself, reaching a steady state on equimolar concentrations at later time points." What is Lds equimolar with? How were the relative molar concentrations of each protein determined? I find this sentence overstated and confusing.

We have removed the “equimolar concentrations” part of the sentence as we agree that our analysis is insufficient to determine the stoichiometry of Lds/Top2 on chromatin.

2. Although it is probably not necessary for this manuscript it would be very interesting to determine if Lds mutants also result in transcript retention on mitotic chromosomes in wing discs and if transcription inhibition represses mitotic defects observed in Lds mutants.

We agree that testing how the dual function of Lds varies across various Drosophila tissues, and how each function contributes to mitotic fidelity, will be a very interesting question for future studies. Establishing similar tools (e.g. MS2 system) to evaluate the role of Lds in wing discs is not compatible with the timely revision process required for this manuscript. Moreover, transcription inactivation experiments in a tissue that is activity relying on its transcriptional program is prone to highly pleiotropic effects. In this regard, the early embryo is quite a unique system as their transcriptional program is only required at later developmental stages.

3. It would be helpful if the authors could provide some experimental data to show the effectivity of the various RNAi treatments that they use throughout the manuscript. It is not possible to know if they are examining complete loss of function or hypomorphic mutant conditions.

We have included new data that support that our RNAi approach depletes the protein to undetectable levels in the two experimental systems used (wing and embryos):

1. In the wing-disc, we performed imaging of discs that revealed loss of Lds signal specifically in the RNAi-targeted area (pouch). - see new results in updated figure EV1A
2. In the embryo, we confirmed depletion levels using WB against GFP inn strains expressing lds-GFP (as attempts to produce an lds antibody failed) -see new results in updated figure EV1B

Referee #3:

General comments

In this manuscript, Carmo and colleagues uncover a novel-dual role for Lds, the Drosophila ortholog of TTF2, in the dissociation of nascent RNAs from condensing chromatin and, in collaboration with Top2, in the decatenation of sister chromatid intertwines early in mitosis. The authors also claim that Lds role is key for appropriate chromosome segregation during mitosis.

The authors focus on Lds after its identification as a clear suppressor of cohesion defects. Using a combination of molecular and cellular approaches in adult and embryo tissues, Coma and colleagues study the Lds function during mitosis. Mitotic association of Lds with chromatin together with the fact that Lds knockdown cause a plethora of mitotic defects, evidence the fundamental role of this factor on chromosome segregation. Coma and colleagues identify a dual role of Lsd: Lsd promotes the chromatin eviction of nascent RNAs and participate in DNA decatenates resolution early in mitosis. Unfortunately, data presented here evidence no connection between both roles; the functional implication of Lsd role on nascent RNA eviction is missing; the connection of Lsd with DNA bridges is observational and lacks of a functional mechanism behind. In overall, I consider this study very interesting although

lacks of important pieces that might be challenging to resolve under the frame of the quick review process for EMBO reports.

Major points:

1) Related to Figure 3. In this figure, authors attend to investigate the role of Lds in transcription termination following mitotic entry. Authors monitor the chromatin association of a reporter transcript (hb) fused to MS2 loops as a tool to evaluate nascent transcription and termination. Based on the retention of nascent hb-MS2 transcripts on mitotic chromatin under Lds depletion, authors claim a role for Lds on the eviction of nascent RNAs from condensing chromatin and, as a consequence, on transcription termination. Although results are encouraging, the study of a single transcript behavior weak this part of the study. I suggest to develop an approach to widely evaluate the effect on nascent transcription and Pol II. As an alternative, I would include the study of several other zygotic transcripts and include proofs that MS2 fusion does not impact the mRNA levels of the target gene.

To address this valid point, we have performed both experiments suggested by the reviewer.

1. We repeated the analysis on the timing of transcripts removal using two different reporters, each containing the promoter (and enhancers) of two other early zygotic genes. We find that while the timing of release differs among different constructs, they all show a significant delay in transcripts eviction upon Lds RNAi. (Figure EV3 E,F)
2. We have labelled recently transcribed RNAs using EUTP incorporation. These results also indicate that upon lds loss there is an increased retention of RNAs in the chromatin region (Figure EV3 G, H).

Both results are consistent with a wide role for Lds in removing transcripts (although we cannot formally exclude that some gene specificity may exist, undetected in our assays).

Importantly, authors did not mention or discuss the results from a previous article describing the role of Aurora B and SAFA in the general eviction of RNAs from condensing chromatin (Sharp et al., 2020 PMID: 33053167). This reference should be properly evaluated in a revised version of the manuscript.

We have now cited this important and relevant manuscript both in the introduction and discussion.

In addition, the evidence demonstrating the chromatin association of hb-transcripts is not quite solid. Although co-localization with chromatin is a good indicator, I would suggest definitive proofs of a direct association between nascent transcript and the hb locus by performing DNA-FISH or a similar alternative.

As explained in detail in response to reviewer #1 (see page 2, point 2a of this rebuttal letter), we now provide new evidence based on the localization of the retained transcripts in anaphase. We show that mRNAs retained (measured using MCP-EGFP in our live cell imaging) are found at the same distance within the segregating

chromatin mass as the genomic loci (measured by DNA FISH). Moreover, comparing the location of two reporters placed at different genomic regions demonstrate that the MCP signal is placed in accordance with the respective transcription site. The new results can be found in Figure EV3 D.

Due to technical difficulties, we have not been able to obtain co-labelling in the same embryo in reliable terms. We nevertheless trust that the indirect method used now addresses this important issue.

Authors evaluate whether the chromosome segregation errors identified under Lds depletion are linked to defects on the dissociation of RNA from chromatin. Data indicate no connection as further demonstrated by the results in Figure 7, where authors find retention of nascent RNAs independently of Top2 inhibition. Thus, the functional implication of the Lds role on transcription termination remains unresolved and weakens the general interest of the article. More importantly, the presumable link between chromatin remodeling and transcription termination executed by Lsd remains undemonstrated.

We agree that the mechanistic details of how lds drives the eviction of nascent transcripts remain to be established and will be the subject of future studies.

2) Related to Figure5 and 6. Due to the high rate of chromosome bridges shown by Lds-depleted cells, the authors investigate the potential participation of Lds on the resolution of sister chromatids. Results demonstrate the localization of Lds protein at chromosome bridges. Moreover, Barren and Top2 localize at those areas under Lds depletion. However, whether Barren/Top2 accumulation at chromosome bridges is higher under Lsd absence is not explored.

We have reformulated this figure extensively to include quantifications on the frequency of bridges that are enriched for Barren/top2 levels. Our findings suggest that most of the bridges observed upon Lds depletion are enriched for top2 and Barre. Direct comparisons with controls are virtually impossible as the frequency of bridges in control embryos is reduced.

Although the inhibition of Top2 activity cause a significant increase on the levels of chromatin-associated Lsd, FRAP data show that the resilience time is unaffected. Although data is compelling, the mix of results from studies looking at chromosome bridges and whole metaphase chromosomes makes the interpretation of the results difficult.
My overall feeling about this part of the article is that the connection between Lsd and DNA decatenation is there, how Lsd interplay with Top2 is not understood and might weaken the quality and interest of the story.

We agree that our studies open several questions on the mechanistic details for the reported cooperation. These are beyond the scope of the present manuscript and will be the subject of future studies.

Minor points:

1) Authors widely use the RNA interference system (RNAi) to knockdown several genes of interest. Although I consider this a very appropriate approach, I highly recommend measuring and showing the effect on the levels of the target proteins. In this direction, I suggest testing at least the efficiency of RNAi against Lds in flies expressing Lds-GFP (Fig2D-E).

We have included detailed analysis on the efficiency of lds depletion in both wing discs and embryos. These analyses reveal that our approach is very efficient at depleting the targeted protein. The updated results can be found in Figure EV1 B and C.

2) Related to Figure 2C. The authors show in this panel the quantification of mitotic defects associated with Lds knockdown. I suggest including the % for each category in order to improve clarity. I would also include the quantification of control flies to determine whether the low percentage of normal mitosis (around 60%) is a characteristic of the cell niche used or if is caused by the RNAi transfection protocol.

The quantifications shown in this figure have been repeated and the scoring categories simplified (to increase consistency among the graphs in the manuscript, see reply to reviewer #1, page 3, point 3). In the updated graph we have also included the control strain without the RNAi, as requested (which in our experiments involved genetically encoding of hairpin RNA and not transfection). Mild bridges are indeed commonly observed in this tissue, in both control conditions. We have therefore re-labelled the major category to “no bridge” rather than “normal” as mild bridges should not be considered abnormal. We have also included a better description of each category in the figure legend stating that mild bridges are defined by fine bridges that are readily resolved whereas severe bridges are solely considered when thick DNA bridges remain unresolved for >4 min after anaphase onset.

3) Related to Figure 2D-E. The authors elegantly show here chromatin association of Lds specifically in mitosis by endogenously expressing a C-terminal fusion to GFP. I consider this is a very clear and relevant result but suggest to include an orthogonal method. Moreover, proof about the functionality of the fusion protein is missing. By performing IF including an anti-Lps antibody, if available, authors will show the behavior of a WT version of Lds.

We have attempted to produce an antibody against Lds to directly address this issue, however, all sera produced were unable to recognize Lds specifically by WB or IF. Hence, we were unable to confirm localization by other means. Nevertheless, to prove for full functionality of the established GFP-fusion, we performed fertility tests on the viable strains (note that absence of Lds is viable but females are sterile). The new results confirming full fertility of the established strain can be found in Figure EV1C. We trust these results support the full functionality of our tagged strains and support the reported localization is likely to reflect the one of the endogenous protein.

Dear Raquel,

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

As you will see, all referees find the revised manuscript significantly improved, they mention that all major concerns were successfully addressed, and they now recommend publication. Referees #2 and #3 request only few minor changes in the text (please see their comments below) that I need you to address in a revised version before I can accept the manuscript.

From the editorial side, there are also a few things that we need from you before acceptance of your article:

- Please provide no more than 5 keywords in your revised manuscript (you currently list 6).

- Please rename the heading of your conflict-of-interest statement to "DISCLOSURE AND COMPETING INTERESTS STATEMENT".

- Please note that no author contributions statement should be included in the revised manuscript. Instead, we now use CRediT to specify the contributions of each author in the journal submission system. Please use the free text box to provide more detailed descriptions. See also our guide to authors: <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>.

- Please make sure that the panels of Fig. 6 are called out in alphabetical order.

- We also noticed that figure callouts for Fig. EV3G-H, EV4A-D, and EV4A-C are missing, please make sure that all panels are called out in your revised manuscript.

- Please remove the synopsis text (short summary and bullet points) from the main manuscript file; instead, upload this information in a separate Word file.

- The synopsis image should be exactly 550 pixels wide and 300-600 pixels high (the height is variable). Could you please increase the font size to make sure that all text is easily readable at the final image size?

- During a standard image analysis we detected possible re-use of a nucleus in Fig. EV1D, and we would like to clarify this issue before acceptance of your paper. In particular, a single nucleus appears to be reused in two microscopy images of this panel (top left corner of the "telophase"-"no bridge" image and bottom right corner of the "telophase"-"mild bridges" image). We kindly request you to check the composition of these images and clarify the issue.

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

Please also note that as part of the EMBO publications' Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You can opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

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

We look forward to seeing a final version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best regards,

Ioannis

Ioannis Papaioannou, PhD
Editor
EMBO reports

Referee #1:

Apologies for the delay, this was a complicated manuscript to review with the many critiques and changes. Overall the authors have done a good job addressing the many initial comments. Particularly useful was the use of different reporters to confirm that Lds activity as described occurs on other genes. The paper has many creative assessments of transcription termination and sister chromatid cohesion control in mitosis. It is mainly a cell biological/genetic study. While it doesn't address how the two phenomena are linked by Lds, the study still adds substantial new information suitable for EMBO Reports. I recommend to accept.

Referee #2:

Summary
This revised manuscript describes two roles for the SNF2 helicase Lds during mitosis. First, Lds functions independently of cohesin to promote transcription termination and transcript release from mitotic chromatin. Second, Lds functions to promote sister chromatid decatenation or resolution. Overall, this is an interesting study that identifies a new protein that plays a role in mitotic transcriptional inhibition and chromosome decatenation. This works sets that stage for future work examining the mechanisms through which Lds accomplishes these tasks. In my previous review I raised several experimental and presentation points that I thought should be addressed prior to publication. The authors have substantially revised this manuscript and added several valuable new pieces of data and better integrated their work with the existing literature. There are a couple of minor issues that should be fixed prior to publication. I believe that this study is now a valuable addition to the literature.

Minor points:
1. P5. Line 117 should read CRISPR instead of "CRISP".
2. P9 line 159. The authors state that "transcripts start to decay". This implies that the transcripts on chromatin are being degraded by a defined pathway, which is not addressed in any way in their work. I think that the authors mean to say that the fluorescent signal on chromatin decreases because the RNA is being displaced into the cytoplasm. They should clarify the wording.
3. P11 line 302. The half-time of TOP2 on chromatin increases in response to ICRF-193. This sentence still states that the half-time of TOP2 decreases. This was raised in the previous review and needs to be corrected.
4. Figure 7E-F. Please make the points and lines on these plots semi-transparent. In the current version one set of points and lines com

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Referee #3:

In this resubmission of the work entitled "A dual-function SNF2 protein drives chromatid resolution and nascent transcripts removal in mitosis" authors provide with an extensive reviewed version of the initial manuscript. Authors have addressed most of referee's comments and included most of the suggestions to improve the clarity and robustness of the report. In overall, I find this new version adequate for its publication in EMBO reports. I would just suggest a minor adjustment described below:

Minor points:

Line 343-351: authors state that previous literature have only uncovered "passive" mechanisms involved on MTI. I find this assumption inaccurate and unnecessary. As already included in the introduction, several mechanisms play an active role driving MTI as the inactivation of the general transcriptional machinery, the clearance of active elongating pol II, or the active removal of pol II driven by cohesin dissociation from chromatin.

All changes to the previously submitted version have been highlighted in yellow:

Editorial issues:

- Please provide no more than 5 keywords in your revised manuscript (you currently list 6).
This has been changed.

- Please rename the heading of your conflict-of-interest statement to "DISCLOSURE AND COMPETING INTERESTS STATEMENT".
This has been changed.

- Please note that no author contributions statement should be included in the revised manuscript. Instead, we now use CRediT to specify the contributions of each author in the journal submission system. Please use the free text box to provide more detailed descriptions. See also our guide to authors: <https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>;

No author contributions are listed in the manuscript.

- Please make sure that the panels of Fig. 6 are called out in alphabetical order.

To address this issue and maintain the order these figures are called in the text, we have changed the labels of the panels. Figure legends and all other references to this figure have been updated accordingly.

- We also noticed that figure callouts for Fig. EV3G-H, EV4A-D, and EV4A-C are missing, please make sure that all panels are called out in your revised manuscript.

EV3G-H was already called out in the previous version. Reference to EV4A-D has been included now in lines 203 and 477.

- Please remove the synopsis text (short summary and bullet points) from the main manuscript file; instead, upload this information in a separate Word file.

Synopsis text has been removed from the main manuscript and uploaded as a separate file.

- The synopsis image should be exactly 550 pixels wide and 300-600 pixels high (the height is variable). Could you please increase the font size to make sure that all text is easily readable at the final image size?

Font size in the synopsis image has been increased.

- During a standard image analysis we detected possible re-use of a nucleus in Fig. EV1D, and we would like to clarify this issue before acceptance of your paper. In particular, a single nucleus appears to be reused in two microscopy images of this panel (top left corner of the

"telophase"-"no bridge" image and bottom right corner of the "telophase"-"mild bridges" image). We kindly request you to check the composition of these images and clarify the issue.

As the figure legend states, the images are examples of segregation defects observed under the same experimental condition (Lds depletion). All images depicted were obtained from a single embryo where different types of segregation defects can be observed. The duplicated nuclei result from the top example shown being a neighboring nucleus of the mild bridge depicted. Hence, we see no issue in the point raised. In case you do not accept this duplication, please let us know and we will reformulate the figure.

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

I confirm all changes are appropriate and the comments raised have been addressed.

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I accept the publication of the Review process file.

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

A cover image has been submitted.

Referee #1:

Apologies for the delay, this was a complicated manuscript to review with the many critiques and changes. Overall the authors have done a good job addressing the many initial comments. Particularly useful was the use of different reporters to confirm that Lds activity as described occurs on other genes. The paper has many creative assessments of transcription termination and sister chromatid cohesion control in mitosis. It is mainly a cell biological/genetic study. While it doesn't address how the two phenomena are linked by Lds, the study still adds substantial new information suitable for EMBO Reports. I recommend to accept.

We thank the reviewer for the nice comments on our work.

Referee #2:

Summary

This revised manuscript describes two roles for the SNF2 helicase Lds during mitosis. First, Lds functions independently of cohesin to promote transcription termination and transcript release from mitotic chromatin. Second, Lds functions to promote sister chromatid decatenation or resolution. Overall, this is an interesting study that identifies a new protein that plays a role in mitotic transcriptional inhibition and chromosome decatenation. This works sets that stage for future work examining the mechanisms through which Lds accomplishes these tasks. In my previous review I raised several experimental and presentation points that I thought should be addressed prior to publication. The authors have substantially revised this manuscript and added several valuable new pieces of data and better integrated their work with the existing literature. There are a couple of minor issues that should be fixed prior to publication. I believe that this study is now a valuable addition to the literature.

We thank the reviewer for the nice comments on our work.

Minor points:
1. P5. Line 117 should read CRISPR instead of "CRISP".
Corrected (line 114).

2. P9 line 159. The authors state that "transcripts start to decay". This implies that the transcripts on chromatin are being degraded by a defined pathway, which is not addressed in any way in their work. I think that the authors mean to say that the fluorescent signal on chromatin decreases because the RNA is being displaced into the cytoplasm. They should clarify the wording.

We thank the reviewer for pointing the incorrect wording. We have now rephrased the sentence to *"the number of transcripts detected on chromatin, monitored by the fluorescence of MCP-EGFP signal, starts to decrease shortly before NEBD"* (line 155)

3. P11 line 302. The half-time of TOP2 on chromatin increases in response to ICRF-193. This sentence still states that the half-time of TOP2 decreases. This was raised in the previous review and needs to be corrected.
This has been corrected (line 300).

4. Figure 7E-F. Please make the points and lines on these plots semi-transparent. In the current version one set of points and lines com

As suggested, we have modified these graphs and reduced opacity of dots and lines. Additionally, we have changed the color scheme. We trust these changes improve readability of the graphs, particularly in areas of overlapping data points.

Referee #3:

In this resubmission of the work entitled "A dual-function SNF2 protein drives chromatid resolution and nascent transcripts removal in mitosis" authors provide with an extensive reviewed version of the initial manuscript. Authors have addressed most of referee's

comments and included most of the suggestions to improve the clarity and robustness of the report. In overall, I find this new version adequate for its publication in EMBO reports. I would just suggest a minor adjustment described below:

We thank the reviewer for the nice comments on our work.

Minor points:

Line 343-351: authors state that previous literature have only uncovered "passive" mechanisms involved on MTI. I find this assumption inaccurate and unnecessary. As already included in the introduction, several mechanisms play an active role driving MTI as the inactivation of the general transcriptional machinery, the clearance of active elongating pol II, or the active removal of pol II driven by cohesin dissociation from chromatin.

We have modified the sentence to include reference to other studies that also suggest that MTI is an active process. We nevertheless wish to keep emphasis on the idea that this process is driven by dedicated machinery (“active”) rather than an indirect consequence of entry into mitosis.

Dr. Raquel Oliveira
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IGC campus
Rua da Quinta Grande, 6
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Portugal

Dear Raquel,

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Reporting Checklist for Life Science Articles (updated January 2022)

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Reagents Availability

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Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Materials and Methods

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Design

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Include a statement about **blinding** even if no blinding was done.	Yes	Materials and Methods
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For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Materials and Methods

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In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Materials and Methods, Figure Legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure Legends

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