JCB

TRIM37 prevents formation of condensate-organized ectopic spindle poles to ensure mitotic fidelity

Franz Meitinger, Dong Kong, Midori Ohta, Arshad Desai, Karen Oegema, and Jadranka Loncarek

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Review Timeline:

Submission Date:	2020-10-31
Editorial Decision:	2020-12-07
Revision Received:	2021-02-25
Editorial Decision:	2021-03-23
Revision Received:	2021-04-04

Monitoring Editor: Daniela Cimini

Scientific Editor: Melina Casadio

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

DOI: https://doi.org/10.1083/jcb.202010180

December 7, 2020

Re: JCB manuscript #202010180

Dr. Karen F Oegema
University of California, San Diego
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La Jolla, CA 92093

Dear Karen, Dear Jadranka,

Thank you for submitting your manuscript entitled "TRIM37 ensures bipolar mitotic division by suppressing formation of centrobin-PLK4 assemblies". The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

The reviewers found that documenting the impact of TRIM37 loss in cells that contain centrosomes is interesting. One reviewer felt that the results remained quite observational and suggested expanding the scope of the characterizations somewhat, and all reviewers made suggestions to clarify the phenotypes and their descriptions. From their comments, we encourage you to prioritize the following points:

1. All reviewers made suggestions to clarify the text, more consistently include statistical analyses and reproducibility information, make it clearer in the text what results are new and how they compare to published work. These are important suggestions to address in full.

2. The reviewers suggested deepening the spindle phenotype characterizations. We agree with them and, as suggested by two of the reviewers, a MT regrowth assay would represent an important functional analysis. The reviewers suggested better characterization of the multipolarity phenotype. You used live-cell imaging of cells with fluorescently tagged chromosomes. Reviewer #3 (#5) pointed out that looking at the DNA alone is not informative enough. These live-cell data could be complemented by fixed-cell data from cells immunostained for microtubules and centrosome markers (e.g., centrobin and centrin) combined with DNA staining to visualize the chromosomes. This would allow evaluation of multipolarity at different mitotic stages and provide further support to the transient multipolarity idea (this would be evident if the rates of multipolarity were high in prometaphase, but low in anaphase). Moreover, it would allow you to determine where the centrobin condensates localize in multipolar vs. bipolar spindles.

3. Rev#1 suggested extending the scope of the analyses. From their points, we do not feel that analyses of ciliation are needed or within the scope. The kinase studies may also be effort-intensive, and it is not clear yet how far one would need to look to find a hit, and we would not require such studies for publication. However, expanding the aneuploidy studies would increase the mechanistic understanding and disease relevance (see next point).

4. We agree with the reviewers that the link to Mulibrey nanism is exciting and opens up new lines

of investigation. However, like the referees state, the connection is still largely speculative. To support this connection, we would not require experiments in patient material unless cell lines are readily available. However, we think you should address the issue of chromosome mis-segregation and aneuploidy in the context of the current study. Specifically, quantifying the rates of lagging chromosomes in bipolar anaphase cells will be informative. This would provide support to the idea that the transient multipolarity causes chromosome missegregation. Furthermore, we feel you should perform chromosome counting at the cell cycle following the time point at which you observe formation of the Plk4-centrobin condensates and the transient multipolarity. These two data sets would provide information on whether chromosome missegregation occurs (lagging chromosomes) and whether the aneuploid cells that emerge from these abnormal mitoses persist in the population (cells with abnormal chromosome counts).

5. Please also address the remaining specific points from the reviewers to the best of your ability.

Please let us know if you have any questions or anticipate any issues addressing these points. We would be happy to discuss 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, https://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.

As you may know, the typical timeframe for revisions is three to four months. However, we at JCB realize that the implementation of social distancing and shelter in place measures that limit spread of COVID-19 also pose challenges to scientific researchers. Lab closures especially are preventing scientists from conducting experiments to further their research. Therefore, JCB has waived the revision time limit. We recommend that you reach out to the editors once your lab has reopened to decide on an appropriate time frame for resubmission. 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 Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

Daniela Cimini, PhD
Monitoring Editor, Journal of Cell Biology

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

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

In this work Meitinger and colleagues address the consequences of loss of the TRIM37 ubiquitin ligase. Effects have been recently described in cells without centrosomes, gaining widespread interest; here authors focus on the situation in the presence of centrosomes, with the aim of identifying phenotypes which may underlie the human disorder mulibery nanism, caused by TRIM37 mutations.
Authors find that Plk4 condensates, already described as a consequence of TRIM37 loss, contain the centrosomal protein centrobin and indeed depend on centrobin for their formation. They provide an extensive and excellent characterization of the structure of these condensates, by sophisticated imaging methodologies. They also show that TRIM37 regulates centrobin stability by mediating its ubiquitination, although molecular details are not investigated. Further, they build on previously published observations that these assemblies acquire microtubule nucleating ability in mitosis and that TRIM37 depleted cells undergo transient multipolarity, to show that condensates associate with additional poles in multipolar mitoses and most importantly that suppressing their formation restores bipolarity of cell division. Finally, they show that centrobin is not localized at ectopic Plk4 foci described in centrinone-treated TRIM37-null cells and consequently that centrobin loss does not influence the effects of TRIM37 loss on acentrosomal mitosis (previously shown to be dependent on these foci).
Overall, the paper provides novel information about centrosome regulation by TRIM37 and how its alteration contributes to pathologies, a topic that is acquiring increasing interest in the centrosomal field. Methodological approaches and cellular systems are advanced and well designed. Still, I find that assaying the functional properties of described structures and their consequences on mitosis and chromosome segregation has not been brought to the required depth of analysis and would require further investigation prior to publication.

There are a number of issues, mentioned in the Discussion session, that could be addressed with relatively straightforward experiments, to widen the significance of the work and improve its value.

1. Cilium formation. This is definitely something that is worth addressing, since centrobin is

important for cilium formation and cilia dysfunctions are pathologically relevant. Cilium formation can be easily triggered by serum starvation in the RPE1 cells used in this work, thus it seems that the authors have all required tools to ask whether TRIM37 loss influences (or not) cilium formation through regulation of centrobin and formation of centrobin-Plk4 condensates.
2. Interphase-to-Mitosis changes in Plk4-centrobin condensates. Phosphorylation is a widespread mechanism of centrosome mitotic regulation and authors mention in the discussion section that centrobin is phosphorylated by Plk1. Since specific kinase inhibitors are available it would be easily feasible to test the effects of distinct mitotic kinases on the microtubule organizing activity and centrosomal components recruitment, acquired by the condensates in mitosis.
3. Chromosome mis-segregation and aneuploidy. This is proposed as the mechanism that would contribute to the Mulibrey pathology, induced by transient spindle multipolarity. It is not clear why, despite having performed experiments that can apparently allow detection of chromosome mis-segregation events, their occurrence is not scored. Also, aneuploidy may be directly investigated by micronuclei scoring and FISH analysis.

Major specific points related to results that are shown are:

1. Throughout the paper a more detailed statistical analysis would be required, especially standard deviations and significances should be shown.

2. Condensates generate from the centrosome during interphase, but not in the cell which inherited the condensate from the previous cell cycle. Authors propose to investigate this issue, but it remains very open. Is centrobin the limiting factor for lack of new condensate formation in condensate-inheriting cells? Is condensate inheritance at mitotic exit, and/or formation in the following cycle, related to centriole age? Which is the model that authors propose for generation being "one per cell"?

3. Related to the previous point, given the observation that TRIM37 loss increases centrobin levels, can authors increase centrobin by an independent approach and assess whether this is sufficient to generate some of the reported phenotypes and structures?

4. Formation of centrin foci is also described. I find slightly confusing that they are described separately from condensates description. Indeed, authors report that they are present in 96% condensates and that both condensates and foci are highly frequent phenotypes. Which is the % of cells displaying both? How are centrin foci distributed in mitoses? Do they also recruit centrosomal components? Authors describe those as independent phenotypes; still, centrin foci increase when centrobin condensates decrease: would this suggest that they are to some extent interdependent?

5. Characterization of multipolar mitoses/spindles should be improved. First, particularly in fixed samples, alpha-tubulin staining should be used, not only chromosome arrangement, to score multipolar spindles. Why is the wt not used in these assays, in parallel to the mutant, as in Figure 1? Are centrin foci not associated with spindle poles? Most importantly, microtubule regrowth assays should be performed, in mitosis and interphase, comparing centrin foci to centrobin-Plk4 condensates. This would clarify the mechanisms through which those distinct structures contribute to spindle assembly and MT organization.

6. I find it difficult in certain points to follow what was previously shown from what is the novel information, and sometimes I find indications that are apparently contradictory, e.g. that TRIM37-

depleted cells are delayed in mitosis (this study) while they are reported as normal mitosis length in Meitinger et al., 2020 or slightly delayed in Meitinger et al., 2016; or that upon centrinone treatment Plk4-centrobin condensates are still present while in Meitinger et al., 2020 large condensates are reported to leave place to smaller assemblies. A more thoroughly discussion of current data in respect to previously published ones would help the reader to better follow and appreciate the novelty of the work. This is reflected by the Model figure too, which is a comprehensive view of results in the field, but where it is not easy to focus on the novelty provided by the current study.

7. The link to the Mulibrey nanism disease is extremely interestingly. Significance would be obviously much improved by displaying evidence that such condensates can be observed in relevant samples (e.g., patients) or model systems. I realize that this kind of approach is likely not feasible in a revision phase. If so, connection to the disease -that is called in the abstract, paper, model figure- should be quite smoothened since it remains an intriguing hypothesis but -to my opinion- not supported by existing data.

Minor issues:
1. Authors should mention whether centrobin-ko cells are normal in terms of centrosomes, i.e. the loss of condensates is the only recorded effect. This may not be directly linked to the conclusions drawn in this manuscript but would help the reader to understand whether we are focusing on a specific feature within a more complex framework of defects or within an otherwise normal cell.
2. Please define ">2 centrioles + aberrant" in Fig 1.

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

Previous work, including work from these authors, has shown that the ubiquitin ligase TRIM37 localises to the centrosomes and its loss in cells lacking centrosomes improves mitotic spindle assembly by driving the formation of ectopic foci PLK4 foci that can recruit PCM proteins. Here, the authors extend their observations to somatic cells with normal centrosome number. They observe that loss of TRIM37 in normal cells leads the formation of ectopic PLK4-centrobin condensate, as previously shown. Using super resolution microscopy, the authors beautifully show that these condensates are highly structured. Interestingly, this condensate is unable to recruit PCM proteins in interphase, remaining inactive, but after mitotic entry it can indeed recruit other centrosomal proteins and acquire MTOC activity. This leads to increased functional MTOCs during mitosis and culminates with the formation of transient multipolar spindles. The authors propose that these results could help explaining the pathology of mulibrey nanism, a rare autosomal recessive disorder caused by loss-of-function mutations in the TRIM37 gene. In particular, they speculate that the intermediate multipolar phenotype could increase aneuploidy, explaining the increase propensity of patients with mulibrey nanism to cancer.

Overall this is a very nice story with high quality data that stems from the authors previous work. Experiments are well controlled and data is clear. While in my opinion this work should be considered positively, there are several issues that need to be addressed and suggestions to improve this work are highlighted below:

Main comments:

#1. From the way this manuscript is written it is not always easy to determine the novel aspects of this work. It is clear that a substantial number of observations have been published before in a

different context but the novel parts not always come through. I suggest perhaps using the supplementary figures to add some of the data that is less novel. This will also help the clarity of the main figures. Some text is quite repetitive, specially describing previous work, I think this could also improve readability.

#2. One of the novel aspects of this work is how these findings could relate to the pathology of mulibrey nanism. However, the authors only offer circumstantial evidence and comparisons with MVA syndrome to propose that aneuploidy that results from intermediary multipoolar spindles could explain some of the features of this disease, such as susceptibility to cancer. Could the authors quantify the levels of aneuploidy that results from TRIM37 KO? And are there cell lines available from patients with mulibrey nanism where aneuploidy could be quantified as well?

#3. In Figure 5C, the authors show that the presence of WT TRIM37 decreases centrobin protein levels. But this is assessed in different cell lines with clearly different levels of TRIM37 being expressed. Could the authors perform TRIM37 siRNA in cells expressing TRIM37 WT to determine that in the same cell line centrobin levels do indeed change in response to TRIM37?

#4. Similarly, to the comment above, in 5D-E could the pellet vs sup ratio of centrobin be the result of increased centrobin levels in Lig mut and TRIM37 KO cell lines? Is there a way to prevent centrobin degradation (e.g. proteasome inhibition) in cells expressing TRIM37 WT to determine if changes in centrobin levels could explain the pellet/ratio? As it is, I do not think there is strong evidence to suggest that WT TRIM37 increases centrobin solubility.

#5. In Figure 5F it is difficult to conclude that centrobin does not interact with WT TRIM37 due to the low levels of WT TRIM37, when compared to Lig mut.

#6. The authors show increased multipolar configuration upon loss of TRIM37, suggesting that the ectopic foci that forms can nucleate microtubules. Could the authors at least show microtubule staining in combination with centrobin to demonstrate that? Ideally microtubule re-growth assay should be use to show this.

#7. It is very interesting that only the condensates associated with multipolar spindle configurations have PCM components. Does this mean that not all condensates are able to recruit PCM? Is there any explanation for this? Or is the positioning of the condensates that determines PCM accumulation? If the authors look at earlier stages of mitosis after NEBD do they also observe differences in PCM accumulation? And are these ectopic condensates being inactivated as mitosis progresses? Resulting in bipolar divisions?

#8. Detailed statistical analyses and significance is missing in most graphs and should therefore be included.

Minor comments:

#9. Is there a reason why only 1 condensate forms in TRIM37 KD cells? Is it a matter of available centrobin/PLK4 levels? Did the authors test if OE PLK4 leads to increase foci in TRIM37 cells?

#10. Figure 1B. Images of control centrioles should be added for comparison.

#11. Page 13. "we analyzed interaction between centrobin and TRIM37 in the supernatant (Fig. 5B)." I do not think this is the correct description for this western blot that only shows crude

extracts.

#12. Figure 6B should be mentioned in the text before 6C

#13. Page 15. Fig. S5A should be Fig. S6A.

#14. Figure S5A looks different from 6C, where centrobin is visible in all poles. Or is this a matter of intensity levels?

Reviewer #3 (Comments to the Authors (Required)):

The article by Meitinger and colleagues analyses the consequences of TRIM37 depletion in human RPE 1 cells. TRIM37 was initially identified by this group as being a suppressor of proliferation defects of cells treated with the PLK4 inhibitor. In a recent study the authors have shown that in cancers that that contain high levels of TRIM37, loss of centrosomes results in lethality. It has been shown that elevated TRIM37levels decreases the frequency of PCM foci, which are essential for timely acentrosomal spindle assembly. Here the authors focus their analysis on what happens in TRIM37depleted cells. Mulibrey nanism, a multi-syndrome rare disease is caused by mutations in TRIM37 and so is of interest from a cell biology point of view. The authors analysed TRIM37 depleted cells and found that in contrast to previous studies, centrosome number is not highly affected (see below). Using elegant CLEM and super resolution approaches, they also show that centriole structure is not affected. Interestingly, they report the formation of condensates that form unusual cellular structures. These condensates recruit centrobin and PLK4, but they are not centriole related. They then discuss the biogenesis of these structures and propose that they are formed from centrioles. They show that centrobin is essential to this process. Mechanistically, they show that lack of TRIM37 results in centrobin stabilization, leading to the assembly of condensates. They proposed that these can form ectopic poles and contribute to multipolar spindle assembly and chromosome mis-segregation. Finally, they distinguish between these centrobin condensates and the PCM foci that contribute to mitotic spindle assembly in the absence of centrosomes.
This study is interesting and reveals a novel function for TRIM37 in inhibiting the assembly of non-centriolar foci that can in principle generate chromosome mis-segregation. The CLEM data and super resolution analysis bring novelty to the field as this type of structure is quite unusual. I think this article will be of interest to JCB readers and should be considered for publication.
Points to be addressed:
1)The extra dost in acetylated tubulin shown in Figure 1D. Why are these not extra centrosomes? I can understand that the authors do not see overduplication. But could they represent centriolar mis-segregation. If at the end of mitosis a cell inherits 3 centrioles instead of two, they will duplicate to form six centrioles. It is a pity that the images provided in Fig 1D do not facilitate comprehension. Why using expansion microscopy, if it just looks like centrioles imaged with conventional microscopy?
2) The authors mention that loss of Trim 37 does not alter PLK4 protein levels. Where is this result shown? I find it difficult to interpret this statement in light of the findings that in TRIM37cells, PLK4 is found at the centrioles and on the condensate. This surely means that there is more protein, no? Or there is also a pool of PLK4 that it is not on centrioles in control cells. I thought cells normally had very low PLK4 levels.
3) Why is the USP28 mutant cell line used here as a control? I could not find this information. The authors have to justify the use of this cell line as control.

4) The genesis of condensates. The movies are great and provide a lot of information, however, it would be nice to see the whole cell so that the appearance of condensate de novo can be examined. Also, I could not find information about the number of cells analysed. If a daughter cell is born with condensate (like the example below in Fig 3F), does this mean that other condensates will not form? In the top cell, the authors point to structure that they call condensate based on what? This is just the same size of the neighboring centrosome. Does it go away, later on? I tried to examine the movies, but they were extremely slow and jumping most likely due to difficult internet connections during lockdown. I think the visualization of the whole cell will help to see the condensate behavior and biogenesis. A quantification of the frequency of these behaviors should also be included.

5) Figure 6E, the cell that divides in a multipolar manner in TRIM37 depleted cells has a much larger nucleus than the others. Is his always the case? For how long have these cells been depleted for TRIM37? Can it be an aneuploid or polyploid cell? I think to establish a correlation between the position of condensates and the fate of the cell in terms of cell division the authors need to analyse movies of cells expressing centrobin, a microtubule marker and DNA. Otherwise, the behavior of the DNA cannot be interpreted without the position and behavior of centrosomes and condensates.

6) The model is nice and it summarizes very well the different results. However, in my opinion it goes too far in stating that Mulibrey nanism results from merotelic attachments or tripolar divisions. I guess for such a statement the authors need to analyse patient derived fibroblasts or sequence these cells to detect aneuploidy. It is worth mentioning that TRIM37 was also described as a nuclear binding factor with roles in gene expression, so this type of function might also contribute to the disease.

RESPONSE TO REVIEWERS **Meitinger, Kong, Ohta et al. (JCB 202010180)**

We thank the reviewers for their feedback on our initial submission. We have addressed the Reviewer's comments by significantly extending the experimental analysis and restructuring the manuscript. In particular, we characterized spindle phenotypes in significant depth (***new Fig. 4, new Fig. 7 A,B, new Fig. S2B***), analyzed chromosome missegregation using 3 different assays (***new Fig. 5***), characterized a mulibrey nanism patient cell line (***new Fig. 1H & 4E***), and analyzed cilia formation (***new Fig. 1E***). We have also restructured the manuscript, most notably by placing greater emphasis on spindle phenotypes and their consequences on chromosome missegregation. Finally, we reworked the model (***Fig. 10***) to more accurately represent the findings on spindle defects and chromosome missegregation.

Reviewer #1: "In this work Meitinger and colleagues address the consequences of loss of the TRIM37 ubiquitin ligase. Effects have been recently described in cells without centrosomes, gaining widespread interest; here authors focus on the situation in the presence of centrosomes, with the aim of identifying phenotypes which may underlie the human disorder mulibery nanism, caused by TRIM37 mutations.
Authors find that Plk4 condensates, already described as a consequence of TRIM37 loss, contain the centrosomal protein centrobin and indeed depend on centrobin for their formation. They provide an extensive and excellent characterization of the structure of these condensates, by sophisticated imaging methodologies. They also show that TRIM37 regulates centrobin stability by mediating its ubiquitination, although molecular details are not investigated. Further, they build on previously published observations that these assemblies acquire microtubule nucleating ability in mitosis and that TRIM37 depleted cells undergo transient multipolarity, to show that condensates associate with additional poles in multipolar mitoses and most importantly that suppressing their formation restores bipolarity of cell division. Finally, they show that centrobin is not localized at ectopic Plk4 foci described in centrinone-treated TRIM37-null cells and consequently that centrobin loss does not influence the effects of TRIM37 loss on acentrosomal mitosis (previously shown to be dependent on these foci).
Overall, the paper provides novel information about centrosome regulation by TRIM37 and how its alteration contributes to pathologies, a topic that is acquiring increasing interest in the centrosomal field. Methodological approaches and cellular systems are advanced and well designed. Still, I find that assaying the functional properties of described structures and their consequences on mitosis and chromosome segregation has not been brought to the required depth of analysis and would require further investigation prior to publication.
There are a number of issues, mentioned in the Discussion session, that could be addressed with relatively straightforward experiments, to widen the significance of the work and improve its value."

Reviewer #1 (point 1): "1. Cilium formation. This is definitely something that is worth addressing, since centrobin is important for cilium formation and cilia dysfunctions are pathologically relevant. Cilium formation can be easily triggered by serum starvation in the RPE1 cells used in this work, thus it seems that the authors have all required tools to ask whether TRIM37 loss influences (or not) cilium formation through regulation of centrobin and formation of centrobin-Plk4 condensates."

Analyzing cilia formation is an important point with potential disease relevance. To address this point, we compared ciliogenesis in wild-type and *TRIM37*Δ RPE1 cells following serum starvation; the data are shown in ***new panels added as Fig 1E & Fig. S1A*.** The results suggest that cilium formation following serum starvation is normal in *TRIM37*Δ cells. We did observe a slight increase in both the frequency and length of cilia in *TRIM37*Δ RPE1 cells relative to the parental wild-type RPE1 cells; however, it is unclear whether these slight increases are due to loss of TRIM37 or represent clonal variation.

Reviewer #1 (point 2): "2. Interphase-to-Mitosis changes in Plk4-centrobin condensates. Phosphorylation is a widespread mechanism of centrosome mitotic regulation and authors mention in the discussion section that centrobin is phosphorylated by Plk1. Since specific kinase inhibitors are available it would be easily feasible to test the effects of distinct mitotic kinases on the microtubule organizing activity and centrosomal components recruitment, acquired by the condensates in mitosis."

To better understand the acquisition of microtubule nucleating capacity by the condensates, we have improved our analysis of the consequences on spindle assembly and also conducted microtubule regrowth experiments (***new panels in Fig. 4B-E; Fig. 4G; Fig. S2B***). Our data collectively show that 25-30% of the condensates acquire microtubule-nucleating activity during the transition into mitosis and generate ectopic spindle poles. We do not understand why only a subset of the condensates acquire nucleation activity – this is an important question for the future. As the reviewer suggests, it is likely that maturation of the condensates, like that of centrosomes, is regulated by PLK1. However, as PLK1 inhibition has strong effects on centrosome assembly and microtubule nucleation in general, we think that it will be challenging to inhibit PLK1 and interpret the effects on condensate maturation.

Reviewer #1 (point 3): "3. Chromosome mis-segregation and aneuploidy. This is proposed as the mechanism that would contribute to the Mulibrey pathology, induced by transient spindle multipolarity. It is not clear why, despite having performed experiments that can apparently allow detection of chromosome mis-segregation events, their occurrence is not scored. Also, aneuploidy may be directly investigated by micronuclei scoring and FISH analysis."

To address this point, we analyzed lagging chromosomes, multipolar segregation and micronuclei frequency, which are now included in ***new panels in Fig. 5A,B***. We additionally conducted FISH analysis of 2 chromosomes (Chr. 17 and Chr. 18) which was added as ***new panel in Fig. 5C***. The results of these approaches support the conclusion that loss of TRIM37 increases chromosome segregation errors, micronuclei formation and aneuploidy. In particular, *TRIM37*Δ cells exhibited ~6.5% multipolar segregation, which is never observed in wild-type RPE1 cells. They also exhibited elevation of lagging chromosomes (~3.2% in *TRIM37*Δ compared to 0.6% in controls) and substantial missegregation, even when spindles ultimately achieved a bipolar configuration.

Reviewer #1 (point 4): "Major specific points related to results that are shown are: 1. Throughout the paper a more detailed statistical analysis would be required, especially standard deviations and significances should be shown."

We sincerely apologize for the lack of some of this information in the first submission, which arose due to an error on our part. All information has now been added in the legends for the graphs. For significance, we use the standard labeling scheme with **** ($p<0.0001$), *** ($p<0.001$), ** ($p<0.01$) and n.s. (not significant) – this scheme is described in the final section of the Materials and Methods titled "Statistical Analysis". We note that for many of the graphs focused on condensates, we are comparing control RPE1 cells (either parental or *USP28*Δ) to *TRIM37*Δ RPE1 cells and we have never observed a single condensate in the control cell populations. In these cases, we report all of the data for condensates in *TRIM37*Δ cells but there is no means of comparison with the control RPE1 cells other than indicating that the control cells have no condensates.

Reviewer #1 (point 5): "2. Condensates generate from the centrosome during interphase, but not in the cell which inherited the condensate from the previous cell cycle. Authors propose to investigate this issue, but it remains very open. Is centrobin the limiting factor for lack of new condensate formation in condensate-

inheriting cells? Is condensate inheritance at mitotic exit, and/or formation in the following cycle, related to centriole age? Which is the model that authors propose for generation being “one per cell”? “

These are very interesting questions. One possibility as to why cells that inherit an existing condensate do not generate a second one is that new centrobin that is produced is preferentially incorporated into the existing condensate, preventing accumulation around the centriole to enable generation of a new condensate. Along these lines, it would be interesting to follow condensates over multiple cell cycles to see if they increase in size. We have not done more work on this topic for the revision since addressing these questions requires the development of new tools (such as an inducible *TRIM37* deletion, which we currently lack, and cell lines with endogenously-tagged centrobin that would allow photomarking of existing condensates).

Reviewer #1 (point 6): “3. Related to the previous point, given the observation that TRIM37 loss increases centrobin levels, can authors increase centrobin by an independent approach and assess whether this is sufficient to generate some of the reported phenotypes and structures?”

We agree that this is potentially interesting and prior overexpression of centrobin has been reported to lead to the formation of aggregates (PMID: 24700465); however, a detailed structural analysis of these was not conducted. Based on editorial feedback, we focused our revision on extending the analysis of spindle defects and chromosome missegregation and the suppression of these defects by knockout of centrobin.

Reviewer #1 (point 7): “4. Formation of centrin foci is also described. I find slightly confusing that they are described separately from condensates description. Indeed, authors report that they are present in 96% condensates and that both condensates and foci are highly frequent phenotypes. Which is the % of cells displaying both? How are centrin foci distributed in mitoses? Do they also recruit centrosomal components? Authors describe those as independent phenotypes; still, centrin foci increase when centrobin condensates decrease: would this suggest that they are to some extent interdependent?”

Preventing the formation of centrobin condensates by removing centrobin in *TRIM37*Δ cells does not have much impact on the centrin foci (a slight increase in the number of centrin foci was observed; **Fig. 6B**), suggesting that loss of TRIM37 causes centrin to form foci independently of the centrobin condensate. Centrobin condensates are typically larger than centrin foci and more than 20% of cells have 2 or more centrin foci. When a centrobin condensate and centrin foci are present in the same cell, a single small centrin focus is observed immediately adjacent to the larger centrobin condensate, often with one or more other centrin foci elsewhere in the cytoplasm. We do not know why centrin foci tend to associate with the centrobin condensate. Although centrin foci persist when centrobin is knocked out (**Fig. 6B,C**; **Fig. S4C**), removal of centrobin suppressed the spindle multipolarity phenotypes observed in *TRIM37*Δ cells (**Fig. 7A-D**), suggesting that centrin foci cannot nucleate microtubules to form functional poles. Due to the seeming lack of a functional impact on spindle assembly and chromosome segregation, we have chosen not to focus further on the centrin foci in this paper.

Reviewer #1 (point 8): “5. Characterization of multipolar mitoses/spindles should be improved. First, particularly in fixed samples, alpha-tubulin staining should be used, not only chromosome arrangement, to score multipolar spindles. Why is the wt not used in these assays, in parallel to the mutant, as in Figure 1? Are centrin foci not associated with spindle poles? Most importantly, microtubule regrowth assays should be performed, in mitosis and interphase, comparing centrin foci to centrobin-Plk4 condensates. This would clarify the mechanisms through which those distinct structures contribute to spindle assembly and MT organization.”

To address this point, we have followed these suggestions and significantly extended analysis of the spindle phenotypes using both fixed and live imaging. All of this data is shown in a **new Fig. 4 and new panels A&B in Fig. 7**. We note that we always analyze control cells in parallel and have never observed a condensate; thus, when the analysis is focused on the behavior of condensates we do not always show control images. The data clearly show condensates at the center of ectopic spindle poles in *TRIM37*Δ cells (**new panels in Fig. 4B-D**) and also in mulibrey nanism fibroblasts (**new panel in Fig. 4E**) . We additionally show 2-color live imaging of condensates, marked with ligase-mutant TRIM37-mNG, and microtubules (**new panels in Fig. 4G,H and Video 4**). Finally, we conducted regrowth assays which showed that a subset of the condensates nucleate microtubules beginning in prophase/prometaphase (**Fig. S2B**). All of this analysis strengthens the conclusion that ~25-30% of the condensates acquire microtubule-nucleating activity during mitotic entry to form ectopic spindle poles in *TRIM37*Δ cells.

We note that a key part of our manuscript is the inducible centrobin knockout, which eliminates the ectopic spindle poles in *TRIM37*Δ cells but does not affect centrin foci. We do not see proteins such as CEP192 localized at centrin foci in mitosis. Consequently, we have not expended further effort on the centrin foci.

Reviewer #1 (point 9): "6. I find it difficult in certain points to follow what was previously shown from what is the novel information, and sometimes I find indications that are apparently contradictory, e.g. that TRIM37-depleted cells are delayed in mitosis (this study) while they are reported as normal mitosis length in Meitinger et al., 2020 or slightly delayed in Meitinger et al., 2016; or that upon centrinone treatment Plk4-centrobin condensates are still present while in Meitinger et al., 2020 large condensates are reported to leave place to smaller assemblies. A more thoroughly discussion of current data in respect to previously published ones would help the reader to better follow and appreciate the novelty of the work. This is reflected by the Model figure too, which is a comprehensive view of results in the field, but where it is not easy to focus on the novelty provided by the current study."

The results of our prior work and the current study are not contradictory – the major focus in the 2016 paper was comparison of mitotic duration in centrosome-less cells generated following centrinone treatment. Mitotic duration increases approximately 2-fold to ~70 minutes when centrosomes are removed, and this increase is significantly suppressed by *TRIM37* deletion. By contrast, the delay without centrinone treatment is quite subtle (~7 min on average) and in Meitinger et al. (2020), the mild mitotic delay of *TRIM37*Δ cells in mitosis was significant relative to control RPE1 cells (p=0.004), similar to what we report here in **Fig. 7D**. Neither prior paper addressed the reason for the mild mitotic delay or for the observed elevation of transient multipolarity and multipolar segregation (briefly mentioned in the 2016 paper). It is only with the identification of centrobin-scaffolded condensates, their ability to form ectopic spindle poles and the combination of *TRIM37*Δ with the inducible *CNTROB* knockout reported here that these phenotypes can be explained.

Following centrinone treatment, in Meitinger et al. (2020), the larger condensate could not be distinguished from the many smaller foci that formed, all of which labeled for PLK4 . Here, we were able to distinguish the large condensate by labeling for centrobin, which is not present in the other PLK4-labeled foci. To clarify this complexity, we have reworked **Figure 9** (*formerly Fig. 7*) and added a new panel **Fig. 9E** with images of *TRIM37*Δ cell spindles labeled for centrobin and PLK4, with and without inducible *CNTROB* knockout. We also include a schematic summary in **Fig. 9F**.

With respect to novel findings, we note that this manuscript is focused on:
1) The identification of centrobin as a core constituent of condensates observed in *TRIM37*Δ cells (novel to this work and while this paper was in revision independently reported by Balestra et al. 2021 (PMID: 33491649))
2) The description of highly ordered condensate morphology and birth at centrosomes in *TRIM37*Δ cells

3) The functional significance of the condensates in mitosis and their consequence on mitotic fidelity (with causality established using the inducible centrobin knockout)
4) The link to mulibrey nanism pathology
5) The direct regulation of centrobin by TRIM37
6) The independence of PLK4-scaffolded foci and the centrobin-scaffolded condensate revealed by centrinone treatment in conjunction with inducible *PLK4* and *CNTROB* knockouts

All of these points are novel to this work. We have significantly reworked the text, including title and abstract, to highlight these points. Broadly speaking, our prior studies on TRIM37 emphasized acentrosomal cells generated by centrinone treatment and the current effort is focused on TRIM37 loss-of-function in the presence of centrosomes, which is relevant to mulibrey nanism. We felt it important to include one experiment with centrinone to clarify the point that the reviewer highlighted (**Fig. 9**).

We have modified the model figure (**now Fig. 10**) to highlight the results of our analysis of centrosomal *TRIM37*Δ cells, which is the focus of this manuscript and relevant to defining the cellular-level origin of mulibrey nanism pathology.

Reviewer #1 (point 10): "7. The link to the Mulibrey nanism disease is extremely interestingly. Significance would be obviously much improved by displaying evidence that such condensates can be observed in relevant samples (e.g., patients) or model systems. I realize that this kind of approach is likely not feasible in a revision phase. If so, connection to the disease -that is called in the abstract, paper, model figure- should be quite smoothened since it remains an intriguing hypothesis but -to my opinion- not supported by existing data."

We obtained a patient-derived mulibrey nanism fibroblast cell line and show that there are similar centrobin condensates present and that these condensates generate ectopic spindle poles in mitosis. This data is shown in **new panels Fig. 1H and Fig. 4E** and strengthens the connection to mulibrey nanism.

Reviewer #1 (point 11): "Minor issues: 1. Authors should mention whether centrobin-ko cells are normal in terms of centrosomes, i.e. the loss of condensates is the only recorded effect. This may not be directly linked to the conclusions drawn in this manuscript but would help the reader to understand whether we are focusing on a specific feature within a more complex framework of defects or within an otherwise normal cell."

We have not observed any significant defects in centrosomes in the inducible knockout cells and have stated this in the revision. Note that the inducible knockout is analyzed 4 days after Cas9 induction; constitutive knockouts of centrobin were previously described and exhibited relatively normal proliferation but defects in ciliogenesis (PMID: 29440264).

Reviewer #1 (point 11): "2. Please define ">2 centrioles + aberrant" in Fig 1."

We have renamed this extremely rare category to ">2 (some partial")" as it reflects presence of extra structures labeled with acetylated tubulin and CEP290 that appear to be partially formed centrioles.

Reviewer #2: "Previous work, including work from these authors, has shown that the ubiquitin ligase TRIM37 localises to the centrosomes and its loss in cells lacking centrosomes improves mitotic spindle assembly by driving the formation of ectopic foci PLK4 foci that can recruit PCM proteins. Here, the authors extend their observations to somatic cells with normal centrosome number. They observe that loss of TRIM37 in normal cells leads the formation of ectopic PLK4-centrobin condensate, as previously shown. Using super resolution microscopy, the authors beautifully show that these condensates are highly structured. Interestingly, this condensate is unable to recruit PCM proteins in interphase, remaining inactive, but after mitotic entry it can indeed recruit other centrosomal proteins and acquire MTOC activity. This leads to increased functional MTOCs during mitosis and culminates with the formation of transient multipolar spindles. The authors propose that these results could help explaining the pathology of mulibrey nanism, a rare autosomal recessive disorder caused by loss-of-function mutations in the TRIM37 gene. In particular, they speculate that the intermediate multipolar phenotype could increase aneuploidy, explaining the increase propensity of patients with mulibrey nanism to cancer.

Overall this is a very nice story with high quality data that stems from the authors previous work. Experiments are well controlled and data is clear. While in my opinion this work should be considered positively, there are several issues that need to be addressed and suggestions to improve this work are highlighted below:"

Reviewer #2 (Point 1): "Main comments: #1. From the way this manuscript is written it is not always easy to determine the novel aspects of this work. It is clear that a substantial number of observations have been published before in a different context but the novel parts not always come through. I suggest perhaps using the supplementary figures to add some of the data that is less novel. This will also help the clarity of the main figures. Some text is quite repetitive, specially describing previous work, I think this could also improve readability."

We have significantly reworked the text and figures in response to this feedback. We now focus earlier in the manuscript on the ectopic spindle pole formed by the condensate and the consequences of its presence on chromosome segregation fidelity (**new Figures 4 & 5**). This structure motivates interest in the condensates by highlighting their functional impact. We have also streamlined the text to avoid repetition and keep emphasis on the advances presented here.

With respect to emphasizing novel findings, we note that this manuscript is focused on:
1) The identification of centrobin as a core constituent of condensates observed in *TRIM37*Δ cells (novel to this work and while this paper was in revision independently reported by Balestra et al. 2021 (PMID: 33491649))
2) The description of highly ordered condensate morphology and birth at centrosomes in *TRIM37*Δ cells
3) The functional significance of the condensates in mitosis and their consequence on mitotic fidelity (with causality established using the inducible centrobin knockout)
4) The link to mulibrey nanism pathology
5) The direct regulation of centrobin by TRIM37
6) The independence of PLK4-scaffolded foci and centrobin-scaffolded condensates revealed by centrinone treatment in conjunction with inducible *PLK4* and *CNTROB* knockouts

All of these points are novel to this work. We have significantly reworked the text, including title and abstract, to highlight these points. Broadly speaking, our prior studies on TRIM37 emphasized acentrosomal cells generated by centrinone treatment and the current effort is focused on TRIM37 loss-of-function in the presence of centrosomes, which is relevant to mulibrey nanism. We felt it important to include one experiment with centrinone to clarify the point that the reviewer highlights. We show that there are two types of assemblies following centrinone treatment of *TRIM37*Δ cells: the centrobin-scaffolded condensate and

PLK4-scaffolded foci, and that the latter are responsible for accelerating spindle assembly in acentrosomal cells generated by centrinone treatment.

We have also modified the model figure (**now Fig. 10**) to more accurately represent the outcomes observed in our analysis.

Reviewer #2 (Point 2): "#2. One of the novel aspects of this work is how these findings could relate to the pathology of mulibrey nanism. However, the authors only offer circumstantial evidence and comparisons with MVA syndrome to propose that aneuploidy that results from intermediary multipoolar spindles could explain some of the features of this disease, such as susceptibility to cancer. Could the authors quantify the levels of aneuploidy that results from TRIM37 KO? And are there cell lines available from patients with mulibrey nanism where aneuploidy could be quantified as well?"

To quantify the effect of *TRIM37* deletion on chromosome segregation and aneuploidy, we have added a **new Fig. 5** which analyzes chromosome segregation defects based on DNA staining, micronuclei frequency, and chromosome-specific FISH. To establish a better connection to mulibrey nanism, we have analyzed a mulibrey nanism patient cell line to show that it has condensates that form ectopic spindle poles (**new panels added as Fig. 1H and Fig. 4E**). We had great difficulty obtaining and working with these primary cell lines – we finally obtained viable cells on a third attempt and were able to conduct limited fixed analysis to show presence of condensates and ectopic poles. However, these cells were not robust enough for us to conduct functional analysis of chromosome segregation. Nonetheless, the new data added to the revision greatly strengthen the proposal that the ectopic condensate pole-based elevation of chromosome missegregation contributes to the pathology of mulibrey nanism.

Reviewer #2 (Point 3): "#3. In Figure 5C, the authors show that the presence of WT TRIM37 decreases centrobin protein levels. But this is assessed in different cell lines with clearly different levels of TRIM37 being expressed. Could the authors perform TRIM37 siRNA in cells expressing TRIM37 WT to determine that in the same cell line centrobin levels do indeed change in response to TRIM37?"

Figure 8C (*formerly 5C*) is a co-expression assay conducted in Freestyle 293F cells and not in different cell lines. All of the analysis is conducted in parallel with one cell line and equivalent transfection mixes – the mixes are generated using empty vectors when one particular component is left out. In this type of analysis, TRIM37 WT expresses at a low level because TRIM37 activity reduces its own stability; such self-regulation is a common property of RING family ubiquitin ligases (PMID: 21372847). Thus, ligase-mutant TRIM37 is always expressed at a higher level than wild-type TRIM37.

In terms of regulation of centrobin levels in a normal cell line, we show this in **Fig. 8A**, comparing centrobin protein levels in RPE1 cells with and without TRIM37. We find that TRIM37 loss elevates centrobin levels ~3.5 fold. Notably, we had conducted RNA-Seq analysis of these same cell lines and there was no change in *CNTROB* mRNA levels; specifically, the *CNTROB* mRNA ratio between the *TRIM37*Δ and parental RPE1 cell lines was 0.95 (RNA-Seq data from Meitinger et al., 2020).

Reviewer #2 (Point 4): "#4. Similarly, to the comment above, in 5D-E could the pellet vs sup ratio of centrobin be the result of increased centrobin levels in Lig mut and TRIM37 KO cell lines? Is there a way to prevent centrobin degradation (e.g. proteasome inhibition) in cells expressing TRIM37 WT to determine if changes in centrobin levels could explain the pellet/ratio? As it is, I do not think there is strong evidence to suggest that WT TRIM37 increases centrobin solubility."

The data we present show that TRIM37 ligase activity modifies centrobin and regulates its levels and that ligase-mutant TRIM37 robustly associates with centrobin, We were careful to state that we are presenting "relative solubility" in the supernatant versus pellet analysis and do not state that wildtype TRIM37 increases centrobin solubility independently of effect on its levels. Most importantly, we have added text at the end of this Results section to indicate that there are two possibilities: 1) TRIM37-dependent ubiquitination solely regulates centrobin levels and higher centrobin levels in the absence of TRIM37 lead to condensate formation; 2) TRIM37-dependent ubiquitination prevents centrobin self-assembly into condensates and the formation of highly stable condensates in the absence of TRIM37 leads to an elevation in total centrobin levels. We hope to distinguish between these two models and address the detailed biochemical mechanism in future work.

Reviewer #2 (Point 5): "#5. In Figure 5F it is difficult to conclude that centrobin does not interact with WT TRIM37 due to the low levels of WT TRIM37, when compared to Lig mut."

We did not mean to imply that centrobin does not interact with WT TRIM37. As noted above, WT TRIM37 is expressed at a low level because the TRIM37 ligase activity controls its own stability, a feature commonly observed for RING family ubiquitin ligases (PMID: 21372847). In addition, WT TRIM37 triggers ubiquitination and degradation of centrobin. These factors make analysis of TRIM37 WT interaction with centrobin difficult to conduct as the levels of both proteins are very low. This is why we used ligase-mutant TRIM37 to conduct interaction analysis in both this effort and in our prior work. Based on the reviewer's comments, we additionally performed an experiment incorporating treatment with MG132 for 6 hours and were able to sufficiently stabilize centrobin to be able to immunoprecipitate it and observe its interaction with WT TRIM37 (**added as new panel in Fig. S5**).

Reviewer #2 (Point 6): "#6. The authors show increased multipolar configuration upon loss of TRIM37, suggesting that the ectopic foci that forms can nucleate microtubules. Could the authors at least show microtubule staining in combination with centrobin to demonstrate that? Ideally microtubule re-growth assay should be use to show this."

We have now performed immunofluorescence staining for centrobin and microtubules (**new panels added as Fig. 4B-E**) and have performed a live analysis of condensates and microtubules in a cell line expressing mNeonGreen-tagged Lig^{mut} TRIM37 along with an mRuby-tagged fusion with the microtubule binding domain of MAP4 (**new panels added as Fig. 4F-H**). We also performed a microtubule regrowth assay (**new Fig. S2B**). The results of all of these experiments support the conclusion that condensates in ~25% of *TRIM37*Δ cells act as mitotic MTOCs to form ectopic spindle poles.

Reviewer #2 (Point 7): "#7. It is very interesting that only the condensates associated with multipolar spindle configurations have PCM components. Does this mean that not all condensates are able to recruit PCM? Is there any explanation for this? Or is the positioning of the condensates that determines PCM accumulation? If the authors look at earlier stages of mitosis after NEBD do they also observe differences in PCM accumulation? And are these ectopic condensates being inactivated as mitosis progresses? Resulting in bipolar divisions?"

About 25% of condensates acquire microtubule nucleation-promoting PCM components during the transition from prophase to prometaphase-metaphase and become ectopic spindle poles, whereas the remaining condensates do not. We do not understand what parameters dictate whether a condensate will or will not become an ectopic MTOC in mitosis. In terms of inactivation, we have not observed condensates that become ectopic poles to lose activity. Rather, the ectopic poles cluster to make bipolar spindles or, in rarer cases, lead to multipolar segregation. This is particularly evident in live imaging analysis of

microtubules (see example in **Fig. 4G**; **Video 3**). In ~5% of mitotic *TRIM37*Δ cells, the clustering does not occur and multipolar segregation is observed, with the condensate organizing the non-centrosomal ectopic pole (**Fig. 4G,H; Fig. 5A,C**).

Reviewer #2 (Point 8): "#8. Detailed statistical analyses and significance is missing in most graphs and should therefore be included."

We sincerely apologize for the absence of this information in the first submission due to an error on our part. All statistical information has now been added. We note that for many of the graphs focused on condensates, we are comparing control RPE1 cells (either parental or *USP28*Δ) to *TRIM37*Δ RPE1 cells and we have never observed a single condensate in the control cell populations. In these cases, we report all of the data for condensates in *TRIM37*Δ cells but there is no meaningful way of conducting a comparison with the control RPE1 cells other than indicating that the control cells have no condensates.

Reviewer #2 (Point 9): "Minor comments: #9. Is there a reason why only 1 condensate forms in TRIM37 KD cells? Is it a matter of available centrobin/PLK4 levels? Did the authors test if OE PLK4 leads to increase foci in TRIM37 cells?"

This is a very interesting question. One speculation that we have as to why cells that inherit an existing condensate do not generate a second is that new centrobin that is produced is preferentially incorporated into the existing condensate, preventing its accumulation around the centriole to enable generation of a new condensate. We have not overexpressed PLK4 because it causes overduplication of centrioles but hope to investigate the reasons for the formation of one condensate in future work.

Reviewer #2 (Point 10): "#10. Figure 1B. Images of control centrioles should be added for comparison."

We have conducted significant expansion microscopy analysis of control RPE1 cells in parallel with *TRIM37*Δ cells and there is no difference in the G1, S, G2 and M centriole structures observed in *TRIM37*Δ cells versus control cells; the only difference is the minor percentage of ">2(separated)" and the even rarer ">2(some partial") centriole configurations observed in *TRIM37*Δ cells. To avoid redundancy in images given space limitations, we have shown example images from *TRIM37*Δ RPE1 cells in **Fig. 1B** but show quantification for all conditions, including parental RPE1 cells in **Fig. 1C & 1D**.

Reviewer #2 (Point 11): "#11. Page 13. "we analyzed interaction between centrobin and TRIM37 in the supernatant (Fig. 5B)." I do not think this is the correct description for this western blot that only shows crude extracts."

This text is correct - **Fig. 8B** (*formerly 5B*) is the schematic summarizing the different types of analysis conducted using co-expression in Freestyle 293F cells; it does not refer to a specific blot.

Reviewer #2 (Point 12): "#12. Figure 6B should be mentioned in the text before 6C"

This no longer applies as the figures were restructured.

Reviewer #2 (Point 13): "#13. Page 15. Fig. S5A should be Fig. S6A."

This no longer applies as the figures were restructured.

Reviewer #2 (Point 14): "#14. Figure S5A looks different from 6C, where centrobin is visible in all poles. Or is this a matter of intensity levels?**"**

Centrobin is detectable at centrosomes in *TRIM37*Δ cells although its levels are reduced to about 30% of that at centrosomes in control RPE1 cells (quantified in **Fig. 1G**). We have now added a rescaled inset in **Fig. 1G** to depict the effect of *TRIM37* deletion on the levels of centrosomal centrobin.

Reviewer #3: "The article by Meitinger and colleagues analyses the consequences of TRIM37 depletion in human RPE 1 cells. TRIM37 was initially identified by this group as being a suppressor of proliferation defects of cells treated with the PLK4 inhibitor. In a recent study the authors have shown that in cancers that that contain high levels of TRIM37, loss of centrosomes results in lethality. It has been shown that elevated TRIM37levels decreases the frequency of PCM foci, which are essential for timely acentrosomal spindle assembly. Here the authors focus their analysis on what happens in TRIM37depleted cells. Mulibrey nanism, a multi-syndrome rare disease is caused by mutations in TRIM37 and so is of interest from a cell biology point of view. The authors analysed TRIM37 depleted cells and found that in contrast to previous studies, centrosome number is not highly affected (see below). Using elegant CLEM and super resolution approaches, they also show that centriole structure is not affected. Interestingly, they report the formation of condensates that form unusual cellular structures. These condensates recruit centrobin and PLK4, but they are not centriole related. They then discuss the biogenesis of these structures and propose that they are formed from centrioles. They show that centrobin is essential to this process. Mechanistically, they show that lack of TRIM37 results in centrobin stabilization, leading to the assembly of condensates. They proposed that these can form ectopic poles and contribute to multipolar spindle assembly and chromosome mis-segregation. Finally, they distinguish between these centrobin condensates and the PCM foci that contribute to mitotic spindle assembly in the absence of centrosomes.
This study is interesting and reveals a novel function for TRIM37 in inhibiting the assembly of non-centriolar foci that can in principle generate chromosome mis-segregation. The CLEM data and super resolution analysis bring novelty to the field as this type of structure is quite unusual. I think this article will be of interest to JCB readers and should be considered for publication.

Reviewer #3 (Point 1): "Points to be addressed: 1)The extra do[ts] in acetylated tubulin shown in Figure 1D. Why are these not extra centrosomes? I can understand that the authors do not see overduplication. But could they represent centriolar mis-segregation. If at the end of mitosis a cell inherits 3 centrioles instead of two, they will duplicate to form six centrioles. It is a pity that the images provided in Fig 1D do not facilitate comprehension. Why using expansion microscopy, if it just looks like centrioles imaged with conventional microscopy?

We have restructured **Figure 1** and **Fig. 1B** now shows blown-up views of the centrioles obtained by expansion microscopy. To add in new data obtained during the revision and meet space limitations, we removed the former *Fig. 1D*. We note that there are 2 categories of abnormal centriole configurations in the clonal TRIM37Δ cell population: the rare ">2 separated" and the extremely rare ">2 (some partial)". As the Reviewer indicates, we have analyzed over 1000 S-phase *TRIM37*Δ cells by expansion microscopy and never observed overduplication around the mother centriole, excluding centriole overduplication as the cause of these cells with extra centrioles. In the Results section associated with Figure 4 ("**Condensates exhibit ectopic spindle pole activity during mitosis"**), we suggest that these small proportion of cells arise from the condensate "dominant" configuration (one cell inherits both centrosomes and the other cell inherits the condensate; **Fig. 4I & 4J**) and also potentially from cytokinesis failure following multipolar division. We note that the RPE1 cell line we use has a functional p53 pathway. Thus, we suggest that these low frequency errors occur constantly and defective cells are selected against, resulting in the persistence of a low frequency of abnormal centriole configurations in the clonal *TRIM37*Δ population.

Reviewer #3 (Point 2): "2) The authors mention that loss of Trim 37 does not alter PLK4 protein levels. Where is this result shown? I find it difficult to interpret this statement in light of the findings that in TRIM37cells, PLK4 is found at the centrioles and on the condensate. This surely means that there is more protein, no? Or there is also a pool of PLK4 that it is not on centrioles in control cells. I thought cells normally had very low PLK4 levels."

The lack of an effect of *TRIM37*Δ on PLK4 protein levels was published previously (*see Fig. 2B in PMID: 32908304*); the band was verified as being PLK4 using centrinone treatment (*see Extended Data Fig. 3B in PMID: 32908304*). Consistent with this observation, in co-expression analysis PLK4 levels were not affected by WT TRIM37 (*see Fig. 2J in PMID: 32908304*), even though PLK4 bound to and was ubiquitinated by TRIM37. By contrast, as we show here, centrobin levels are elevated in *TRIM37*Δ (**Fig. 8A**) and significantly reduced by co-expression of WT TRIM37 (**Fig. 8C**).

Reviewer #3 (Point 3): "3) Why is the USP28 mutant cell line used here as a control? I could not find this information. The authors have to justify the use of this cell line as control."

Thank you for this comment. We have added text justifying the use of this cell line in the first *Results* section. We wanted to analyze the consequences of inducibly removing centrobin-scaffolded condensates in centrosome-containing and centrosome-less *TRIM37*Δ cells. The *USP28*Δ cell line is a good control for such analysis because it has been similarly treated as the *TRIM37*Δ cell line and because loss of USP28 inactivates a sensor pathway that triggers p53-dependent arrest in response to extended mitosis (PMID: 27432897; PMID: 27432896; PMID: 27371829). Thus, we can analyze mitosis in both the presence and absence of centrosomes.

Reviewer #3 (Point 4): "4) The genesis of condensates. The movies are great and provide a lot of information, however, it would be nice to see the whole cell so that the appearance of condensate de novo can be examined. Also, I could not find information about the number of cells analysed. If a daughter cell is born with condensate (like the example below in Fig 3F), does this mean that other condensates will not form? In the top cell, the authors point to structure that they call condensate based on what? This is just the same size of the neighboring centrosome. Does it go away, later on? I tried to examine the movies, but they were extremely slow and jumping most likely due to difficult internet connections during lockdown. I think the visualization of the whole cell will help to see the condensate behavior and biogenesis. A quantification of the frequency of these behaviors should also be included."

We reported quantification of all events we have observed in **Fig. 3E** (which additionally shows that condensate formation does not require PLK4 kinase activity). We imaged 24 untreated cells and 23 cells treated with centrinone. As RPE1 cells move rapidly and crawl over each other, we have to manually crop out the centrosome areas from selected frames and align then to generate movies (given the time scales involved, daughter cells frequently move into adjacent fields and this makes it difficult to show movies that feature both cells generated by division). We have thus not modified the movie showing condensate budding.

Reviewer #3 (Point 5): "5) Figure 6E, the cell that divides in a multipolar manner in TRIM37 depleted cells has a much larger nucleus than the others. Is his always the case? For how long have these cells been depleted for TRIM37? Can it be an aneuploid or polyploid cell? I think to establish a correlation between the position of condensates and the fate of the cell in terms of cell division the authors need to analyse movies of cells expressing centrobin, a microtubule marker and DNA. Otherwise, the behavior of the DNA cannot be interpreted without the position and behavior of centrosomes and condensates."

We have significantly extended analysis of spindles and of chromosome segregation defects in TRIM37-deficient cells: **new Figure 4**, **new Figure 5**, **new Figure 7A& B** and **new Fig. S2B**. We have also included a video showing condensate and microtubule behavior in live mitotic cells (**new Video 3**). Collectively these new data greatly strengthen the conclusion that centrobin-scaffolded condensates make ectopic spindle poles that elevate chromosome missegregation rates. With respect to the question of whether cells that divide in multipolar manner start off as aneuploid, our analysis suggests that the majority are not. Analysis of *TRIM37*Δ RPE1 cells with FISH probes for chromosomes 17 and 18 (**new Fig. 5C**) revealed a high frequency of multipolar divisions with the vast majority of these being divisions of cells with the expected diploid numbers of each of these chromosomes (out of 8 multipolar divisions captured for chromosome 17 none were aneuploid; out of 10 multipolar divisions captured for chromosome 18, 8 were diploid and 2 were triploid for chromosome 18).

Reviewer #3 (Point 6): "6) The model is nice and it summarizes very well the different results. However, in my opinion it goes too far in stating that Mulibrey nanism results from merotelic attachments or tripolar divisions. I guess for such a statement the authors need to analyse patient derived fibroblasts or sequence these cells to detect aneuploidy. It is worth mentioning that TRIM37 was also described as a nuclear binding factor with roles in gene expression, so this type of function might also contribute to the disease."

We have conducted the requested analysis and have added a **new Fig. 5** which analyzes chromosome segregation defects based on DNA staining, micronuclei frequency and chromosome-specific FISH. We have additionally analyzed a mulibrey nanism patient cell line to show that it has condensates that form ectopic spindle poles (**new Fig. 1H** and **new Fig. 4E**). We have had great difficulty obtaining and working with these primary cell lines – we finally obtained viable cells on a third attempt and were able to conduct limited fixed analysis to show presence of condensates and ectopic poles. Although these cells are not robust enough for us to conduct functional analysis of chromosome segregation, their similarity to the *TRIM37*Δ RPE1 cells strengthens the significance of the analysis conducted in the model cell line. These new data added to the revision greatly strengthen the proposal that the ectopic condensate pole-based elevation of chromosome missegregation contributes to the pathology of mulibrey nanism.

March 23, 2021

RE: JCB Manuscript #202010180R

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La Jolla, CA 92093

Dear Dr. Oegema,

Thank you for submitting your revised manuscript entitled "TRIM37 prevents formation of condensate-organized ectopic spindle poles to ensure mitotic fidelity". You will see that the reviewers praised the revision efforts and now recommend publication. We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below) and pending revisions to address Reviewer #1's concern with the statistical analyses, which we agree is important.

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

1) Figure formatting: Scale bars must be present on all microscopy images, including inset magnifications.
- Please add scale bars to 2A (first image), 2B (first image shown), 4B (magnifications), S1E (low magnification images), S1C (first image), S2B magnifications
Molecular weight or nucleic acid size markers must be included on all gel electrophoresis.
- Please add *unit labels* for figure 8 gels (all), S5 all gels

2) 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.

3) Tables should be provided as individual, separate, editable files (e.g., Word or Excel).

4) 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.
- For all cell lines, vectors, constructs/cDNAs, etc. - all genetic material: please include database / vendor ID (e.g., Addgene, ATCC, etc.) or if unavailable, please briefly describe their basic genetic features *even if described in other published work or gifted to you by other investigators*
- Please include **species** and source for all antibodies, including secondary, as well as catalog numbers/vendor identifiers if available.
- Sequences should be provided for all oligos: primers, si/shRNA, gRNAs, etc.

- 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.).

5) A summary paragraph of all supplemental material should appear at the end of the Materials and methods section.
- Please include one brief descriptive sentence per item (including supp figures, tables, movies).

6) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

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

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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 the Journal of Cell Biology.

Sincerely,

Daniela Cimini, PhD
Monitoring Editor, Journal of Cell Biology

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

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

In the revised version of the manuscript, Meitinger and colleagues have addressed most of raised issues, focusing on those indicated by Editors but also including additional ones, such as the impact of TRIM37 deficiency on cilia formation. I feel that now conclusions are better supported by data. In particular, results with patients-derived cell lines, a more detailed mitotic analysis and the inclusion of specific assays for chromosome mis-segregation together strengthen the link between TRIM37 depletion, aneuploidy and Mulibrey nanism features. I also appreciate text reorganization and schematics modifications in Figures. I therefore now recommend publication in The Journal of Cell Biology. The only thing I recommend before publication is that standard deviations from the biological replicates are shown when the % of phenotypes are indicated below IF panels and within histograms, in all figures throughout the paper. Although I understand that significance is not shown when the phenotype is totally absent in control cultures (point 4 of the rebuttal), I find unusual and incomplete not to show standard deviations. If data have been treated differently, so to justify lack of standard deviations, this should be made clear and the type of statistical analysis that has been applied to take into account the experimental variability should be described.

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

The authors have addressed my main concerns. The new additional data and text clarifications significantly improved this work.The links to mulibrey nanism are still supported by limited data, but new additional experiments help to support the authors hypothesis.

i have no additional comments and would support its publication at JCB.

Reviewer #3 (Comments to the Authors (Required)):

The revised version of this manuscript has improved substantially. I think it is ready to be published. The authors have addressed all my comments.
Juts a few typos- Fig 3B, and also the authors might want to decide to name SIM always and not some of the panels, while others have the full text.
While this version is much easier to follow than the previous one, I still feel that it is somehow difficult with figures very complex and full of data. Is it possible to transfer some of the data in supplementary data? Or increase the number of figures? Or try to simplify the text? Even if I am not sure this is feasible...
I just have one question that if the authors can clarify in manuscript this would be great. The authors use FISH to show that daughter nuclei have different chromosome contents (Fig 5C). I am assuming this is exactly this- not just neighbouring nuclei but rather nuclei at anaphase or telophase. How to explain to have two anaphase nuclei with 3 chromosome 18? Can it be that the mother cell had 6 chromomes 18? Was it polyploid? While I know that this is not possible to answer with FISH, if I analyse the size of these nuclei, they indeed seem larger than the other nuclei. Maybe bipolar aneuploid category can be illustrated just as with mis-segregation, type of cell? In the end a mis segregation even indeed results in aneuploidy, while the correct segregation of 3 chromosomes in two daughter cells is more difficult to explain, unless the mother cell was already aneuploid.