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Sequestration of LINE-1 in cytosolic aggregates by MOV10 restricts retrotransposition

Rajika Arora, Maxime Bodak, Laura Penoulty, Cindy Hackman, and Constance Ciaudo
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(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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision **21st Feb 2022**

Dear Constance,

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. We have received the comments from 2 referees but unfortunately the third referee got sick and seems to be unable to send her/his report, despite promising to do so. I will therefore make a decision based on the two reports we have. I will forward you the third report as well, should we receive it in the near future.

As you will see from the comments below, the referees acknowledge that the findings are interesting and they only raise minor concerns.

I would thus like to invite you to revise your manuscript with the understanding that the referee concerns must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major 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.

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 recommend a revision within 3 months (24th May 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 27,000 characters (including spaces but excluding materials & methods and references) and 5 main plus 5 expanded view figures. The results and discussion sections must further be combined, which will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. For a normal article there are no length limitations, but it should have more than 5 main figures and the results and discussion sections must be separate. In both cases, the entire materials and methods must be included in the main manuscript file.

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1) A data availability section providing access to data deposited in public databases is missing. If you have not deposited any data, please add a sentence to the data availability section that explains that.
2) Your manuscript contains statistics and error bars based on n=2. Please use scatter blots in these cases. No statistics should be calculated if n=2.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure). See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- 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.

4) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

5) a complete author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also

reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines
<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>

7) Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). 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 & Method (see also
https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study. * Note - All links should resolve to a page where the data can be accessed. *
If your study has not produced novel datasets, please mention this fact in the Data Availability Section.

8) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at
<https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

9) Our journal also encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should 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

10) Regarding data quantification (see Figure Legends:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat)

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,

- the nature of the bars and error bars (s.d., s.e.m.),

- If the data are obtained from n {less than or equal to} 2, use scatter blots 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.

- Please also include scale bars in all microscopy images.

11) The journal requires a statement specifying whether or not authors have competing interests (defined as all potential or actual interests that could be perceived to influence the presentation or interpretation of an article). In case of competing interests, this must be specified in your disclosure statement. Further information: https://www.embopress.org/competing-interests

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

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File (RPF) 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 are able to 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."

I look forward to seeing a revised form of your manuscript when it is ready. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The manuscript by Arora et al. entitled "Sequestration of LINE-1 in novel cytosolic bodies by MOV10 restricts retrotransposition" deciphers the molecular mechanism of an intriguing observation that was previously reported in 2017 by the same laboratory. Indeed, after knocking out Dicer (Dicer_KO), a protein involved in the biogenesis of micro- and siRNAs, in mouse embryonic stem cells (mESCs), they had observed an increase in L1 mRNA levels that only correlated with a marginal increase in retrotransposition.
Here, the authors show that, after the knockout, in this peculiar type of cell line, of either Dicer or Drosha, another protein involved in the biogenesis of microRNAs, there is also an increase of a L1 inhibitor, MOV10, at both the RNA and protein levels. Moreover, the MOV10 protein accumulates in cytoplasmic aggregates where it co-localises with L1-RNPs (containing both the L1 RNA and the L1 ORF1 protein).
In the next part of the work, the authors conclusively show that a two-to-three-fold forced endogenous upregulation of active L1 elements in wild-type mESCs is sufficient to upregulate L1 retrotransposition but not to create L1 RNP cytoplasmic aggregates, nor to increase MOV10 expression.
In fact, they show that the low level of MOV10 expression of these cells is caused by several miRNAs (miR-138-5p, miR-30-5p, miR-16-5p and miR-153-5p). My concern here is the high concentration of mimics that the authors have used to do the different experiments. Indeed, it is now more and more documented that an over-concentration of mimics during the transfection could lead to misinterpretations of the results. See Jin et al., 2015 (doi: 10.3389/fgene.2015.00340. eCollection 2015) and Mockly et al (bioRxiv 2021.02.11.430795; doi: https://doi.org/10.1101/2021.02.11.430795). Before concluding that these microRNAs have a role in fine-tuning MOV10 expression, the authors should try to reproduce their data using more physiological concentrations of mimics (1nM instead of 20nM or 50nM for the different experiments presented).
Moreover, in Figure 4C&D, the authors only performed the mimics experiments with Drosha_KO cells. I suggest the same experiments to be done with Dicer_KO cells, as well.
In a last series of experiments, they show that MOV10 over-expression is able to prevent L1 transposition in mESCs by sequestering L1 RNPs in cytoplasmic aggregates. On one hand, ectopic expression of MOV10 in mESCs upregulating L1 is sufficient to induce L1 RNP aggregate formation and to decrease L1 retrotransposition. On the other hand, downregulation of MOV10 by mimics can restore retrotransposition (what about the "aggregates" phenotype ?) in Drosha_KO mESCs.

The manuscript is well written and the authors present large amounts of data that were obtained by Immunostaining, RT-qPCR, Western-blotting, Northern blotting which required very good skills in the manipulation of mESCs. The experiments mostly support the conclusions drawn and I think that these results merit publication in EMBO Reports.

There are a few minor points:
The authors conclude that the observed aggregates are "novel cytosolic bodies", for me, the term "novel" may seem a bit excessive. So, the term "Novel" could be removed from the title.
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Sequestration of LINE-1 in novel cytosolic bodies by MOV10 restricts retrotransposition

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This work is based on previous work from the authors showing that DICER plays a role in regulating L1 elements in mouse embryonic stem cells (mESCs). In DICER knockout (KO) mESCs, there is an accumulation of L1 RNA, yet this is not sufficient to

induce active L1 retrotransposition. Here, the authors make the observation that in DICER KO mESCs, there is also a marked overexpression of MOV10, an RNA helicase, which has been implicated in inhibiting L1 retrotransposition in previous reports. The authors then employ DICER KO cells to investigate this mechanism further. They demonstrate that MOV10 forms aggregates as cytoplasmic foci together with L1 RNPs as measured by detection of MOV10, L1 ORF1 protein and L1 RNA using RNA FISH. MOV10 plays a role in restricting L1 mobility: Its targeted depletion in DICER KO mESCs rescues retrotransposition of a L1 reporter and MOV10 ectopic expression limits active retrotransposition of a L1 reporter transfected into mESCs, in which endogenous L1s are activated using CRISPRa. Finally, the authors uncover that DICER regulates MOV10 posttranscriptionally through miRNAs, which target the MOV10 3'UTR and which are upregulated in DICER KO mESCs. In general, the results are complete and clearly presented and the conclusions justified. I only have minor comments.

Minor comments

1.Figure 5: Can you induce L1 RNP aggregates and block retrotransposition simply by overexpressing the L1 retrotransposition reporter as a source of L1 RNA and ORF1p +/- MOV10 overexpression, i.e. without also needing to activate endogenous L1s using CRISPRa? CRISPRa of endogenous L1s may contribute off target effects by affecting expression of host genes that impact on L1 mobility. Can you verify that your CRISPRa sgRNAs don't affect expression of host genes?

2.Line 93: The authors state that DICER regulates the RNA helicase MOV10 through miRNAs and therefore, loss of DICER causes an increase in expression of MOV10. However, it is not clear from the introduction if/why there is also an increase in L1 RNA expression upon DICER KO. Please clarify this, linking to previous published work from the authors.

3.Line 110: The median number of L1 RNP foci per cell is given. Please clarify if this is indeed per cell or the median number of foci per field imaged within a cell.

4.Figure 1b: Can the authors comment on why only a proportion of DICER ko cells have L1 RNP foci/large foci (for example, line 112: in only '30-35% of Dicer_KO clones, L1 RNP were observed to co-localize in larger foci'). Are these different clones or different cells from a DICER KO bulk population? Can the authors comment on what are the differences between different DICER KO clones/cells making their response so varied?

5.Line 112: Some DICER KO clones harbour larger L1 RNP foci. The authors then state that 'These observations led us to hypothesize that sequestration of L1 RNP in the cytoplasm of Dicer_KO mESCs is preventing L1 retrotransposition'. Please explain how the hypothesis was developed more clearly as it could be assumed that more L1 RNPs are indicative of increased retrotransposition in the DICER KO cells. Citing references linking L1 RNP aggregates to negative regulation of retrotransposition would help.

6.Line 191: 'To parse out' please rephrase for clarity.

7.LINE-1; full name is 'Long Interspersed Element-1s' rather than 'Long Interspersed Nucleotide Elements 1'.

8.Line 282: There is a mistake in the title of this results section. 'Restoring MOV10 expression in Drosha_KO mESCs leads to L1 retrotransposition' should be 'Reducing MOV10 expression in Drosha_KO mESCs rescues L1 retrotransposition'

9.Line 914: 'Mutant reporter plasmid'. Please specify what is mutated?

Responses to reviewers

We would like to thank the reviewers for their comments. We highlighted in red in the revised version of our manuscript all the changes performed.

Referee #1:

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In fact, they show that the low level of MOV10 expression of these cells is caused by several miRNAs (miR-138-5p, miR-30-5p, miR-16-5p and miR-153-5p). My concern here is the high concentration of mimics that the authors have used to do the different experiments. Indeed, it is now more and more documented that an over-concentration of mimics during the transfection could lead to misinterpretations of the results. See Jin et al., 2015 (doi: 10.3389/fgene.2015.00340. eCollection 2015) and Mockly et al (bioRxiv 2021.02.11.430795; doi: https://doi.org/10.1101/2021.02.11.430795). Before concluding that these microRNAs have a role in fine-tuning MOV10 expression, the authors should try to reproduce their data using more physiological concentrations of mimics (1nM instead of 20nM or 50nM for the different experiments presented).

We thank the reviewer for this suggestion and have now performed titration experiments using increasing concentrations of mimics (1, 2, 4, 5 and 20 nM). First, we assessed the impact of the different concentrations of mimics on the level of MOV10 protein by western blotting (Revised Figure 4C). We observed a stronger downregulation of MOV10 using 4 nM of mimics. Then, we reproduced the retrotransposition assay using again several concentrations of mimics and observed a higher retrotransposition rate using 4 nM (Revised Figure 4D), indicating indeed, as suggested by the reviewer, that high concentrations of mimics might have a stronger off target effect. Additionally, to further confirm the specificity and direct action of miRNA mediated post-transcriptional regulation of MOV10 using the luciferase assay, we mutated the MREs of miR16-5p and miR-153-3p in the 3'UTR sequence of *Mov10* (Fig EV3B). We find that the RENILLA Luciferase expression in the mutant plasmids is no longer sensitive to transfection with the corresponding mimic (Fig 4B), indicating a direct regulation by miRNAs.

Moreover, in Figure 4C&D, the authors only performed the mimics experiments with Drosha_KO cells. I suggest the same experiments to be done with Dicer_KO cells, as well.

We thank the reviewer for this suggestion, and indeed several tries for this experiment in *Dicer_KO* mESCs failed. As observed previously, the level of AGO2 protein in *Dicer*_KO cells is very low (Bodak *et al.,* 2017), which we believe might impair the loading of mimics. We now present in revised FigEV3C a western blotting comparing the levels of AGO2 in

Drosha and *Dicer*_KO mESCs, demonstrating the higher expression of AGO2 in *Drosha*_KO mESC line.

In a last series of experiments, they show that MOV10 over-expression is able to prevent L1 transposition in mESCs by sequestering L1 RNPs in cytoplasmic aggregates. On one hand, ectopic expression of MOV10 in mESCs upregulating L1 is sufficient to induce L1 RNP aggregate formation and to decrease L1 retrotransposition. On the other hand, downregulation of MOV10 by mimics can restore retrotransposition (what about the "aggregates" phenotype ?) in Drosha_KO mESCs.

We now assessed the presence of aggregates in *Drosha*_KO cells upon the transfection of mimics at 4 and 20 nM (Revised FigEV4B). Here, despite the downregulation of MOV10 at protein level, we are still observing the presence of aggregates in the cytoplasm of the cells. We previously showed (Fig2 and FigEV1A) that these aggregates do not undergo liquid phase separation and we believe that they are not dynamic and/or plastic structures but rather more solid aggregates, which once they are built, are very persistent as we never succeed to dissociate them despite using chemical and biological approaches (drugs, siRNA…). Additionally, from the Colony Forming Assay we know that retrotransposition is only observed in approximately 200 out of the 500,000 plated cells, leading us to speculate that the breakdown of the aggregates may be a too rare event to be visualized by IF.
We now discuss these points in our revised manuscript.

The manuscript is well written and the authors present large amounts of data that were obtained by Immunostaining, RT-qPCR, Western-blotting, Northern blotting which required very good skills in the manipulation of mESCs. The experiments mostly support the conclusions drawn and I think that these results merit publication in EMBO Reports.

We thank the reviewer for noticing the quality of our work.

There are a few minor points:
The authors conclude that the observed aggregates are "novel cytosolic bodies", for me, the term "novel" may seem a bit excessive. So, the term "Novel" could be removed from the title.
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Line 925 : Hek293T have to be written properly.
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Figure S2 D: the detection of L1 by Northern in Cl1 is not so clear. Could the authors comments ?
We thank the reviewer for noticing the differences between the two $L1^{UP}$ clones. Indeed, the clone 1 has been generated using one sgRNA and the clone 2 using 2 sgRNAs targeting both the 3'UTR of the L1 Tf elements (as presented in Revised Fig EV2B). The impact on the upregulation of the endogenous L1 is lower in the clone 1 compared to the clone 2 as shown in the northern blot in FigEV2D. We now explain this discrepancy better in the text of our revised manuscript.

In figure S2, the CRISPRa targeting seems suggest that all the L1 family are upregulated. Indeed, the statistical unsignificant for Cl2 does not seem very relevant, there is only more variability in the experiments. The authors should rewrite this part.
As noticed by the reviewer, we indeed observed an upregulation by qRTPCR of all L1 family in our $L1^{UP}$ clones. We show the alignments to highlight the homology of the different sequences and the targeting sequences of the sgRNAs in revised FigEV2B and modified the text accordingly.

Referee #2:

Sequestration of LINE-1 in novel cytosolic bodies by MOV10 restricts retrotransposition

Arora et al.

This work is based on previous work from the authors showing that DICER plays a role in regulating L1 elements in mouse embryonic stem cells (mESCs). In DICER knockout (KO) mESCs, there is an accumulation of L1 RNA, yet this is not sufficient to induce active L1 retrotransposition. Here, the authors make the observation that in DICER KO mESCs, there is also a marked overexpression of MOV10, an RNA helicase, which has been implicated in inhibiting L1 retrotransposition in previous reports. The authors then employ DICER KO cells to investigate this mechanism further. They demonstrate that MOV10 forms aggregates as cytoplasmic foci together with L1 RNPs as measured by detection of MOV10, L1 ORF1 protein and L1 RNA using RNA FISH. MOV10 plays a role in restricting L1 mobility: Its targeted depletion in DICER KO mESCs rescues retrotransposition of a L1 reporter and MOV10 ectopic expression limits active retrotransposition of a L1 reporter transfected into mESCs, in which endogenous L1s are activated using CRISPRa. Finally, the authors uncover that DICER regulates MOV10 posttranscriptionally through miRNAs, which target the MOV10 3'UTR and which are upregulated in DICER KO mESCs. In general, the results are complete and clearly presented and the conclusions justified. I only have minor comments.

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We thank the reviewer for this suggestion and now performed the proposed experiments in WT (Revised Figure 6). Indeed, we are able to create the aggregates in WT mESCs by overexpression full L1 elements from a plasmid at the same time than MOV10 (Figure 6A). Interestingly, we were also able to create the aggregates by overexpressing only ORF1 protein and MOV10 (Figure 6B), indicating that MOV10 and ORF1p overexpression are sufficient to create the cytoplasmic aggregates in WT mESCs.

Can you verify that your CRISPRa sgRNAs don't affect expression of host genes?

We indeed observed an impact on host genes expression using CRISPRa against L1 Tf elements. We performed RNA seq in both $L1^{UP}$ clones, and while a few genes were found to be in common between the two clones we observed a variety of genes to be misregulated as compared to the control cell line (Figure 1). L1 expression has been linked to changes in transcriptome (Kashkush, Feldman and Levy, 2003; Belancio, Hedges and Deininger, 2008; Rangwala, Zhang and Kazazian, 2009; Chatterjee *et al.*, 2018; Ahn *et al.*, 2021), so we cannot rule out any off-target effects of sgRNAs or an impact from the L1 overexpression in $L1^{UP}$ clones (specifically since we do not know which L1s are targeted on the genome!). Nevertheless, we used these clones only to understand if the sole upregulation of L1 was sufficient to create the aggregates, which is not the case. The addition of the experiments proposed by the reviewer showing that we can also create the aggregates in WT cells by overexpression of L1 from a plasmid with MOV10 reinforce our preliminary results.

Figure 1

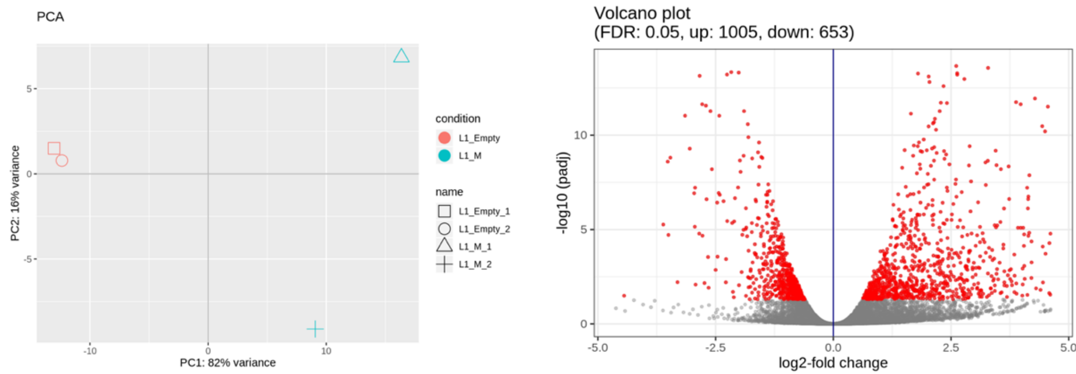

Figure 1: RNA-seq analysis was performed in L1^{UP} clones (L1_M_1 and L1_M_2) transfected with CRISPRa SgRNA and compared to control cell lines (L1_Empty_1 and L1_Empty_2) transfected with Empty SgRNAs. Principal component analysis (PCA) plot revealed a high variability in the data between the two L1 UP clones, this variability was not observed amongst the control cell lines. (B) Volcano Plot showing differentially expressed genes (red) in L1^{UP} clones as compared to the control cell line.

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We apologize here for the unclarity. Yes we showed previously that in *Dicer*_KO mESCs L1 are strongly upregulated at RNA levels (Bodak *et al*., 2017). We now clarify this point in our revised manuscript.

3.Line 110: The median number of L1 RNP foci per cell is given. Please clarify if this is indeed per cell or the median number of foci per field imaged within a cell.

Done (per cell).

4.Figure 1b: Can the authors comment on why only a proportion of DICER ko cells have L1 RNP foci/large foci (for example, line 112: in only '30-35% of Dicer_KO clones, L1 RNP were observed to co-localize in larger foci'). Are these different clones or different cells from a DICER KO bulk population? Can the authors comment on what are the differences between different DICER KO clones/cells making their response so varied?

The two *Dicer*_KO clones presented in this manuscript are coming from two different combination of sgRNAs (Bodak *et al*., 2017). We are observing different cells from these different clones. We believe that the variability observed comes from the cycle defects previously observed in *Dicer*_KO mESCs (Bodak *et al*., 2017) where around 30-35% of the cells are blocked in G1.

5.Line 112: Some DICER KO clones harbour larger L1 RNP foci. The authors then state that 'These observations led us to hypothesize that sequestration of L1 RNP in the cytoplasm of Dicer_KO mESCs is preventing L1 retrotransposition'. Please explain how the hypothesis was developed more clearly as it could be assumed that more L1 RNPs are indicative of increased retrotransposition in the DICER KO cells. Citing references linking L1 RNP aggregates to negative regulation of retrotransposition would help.

We thank the reviewer for this suggestion and have now added several references (Goodier *et al.*, 2007; Guo *et al.*, 2014)

6.Line 191: 'To parse out' please rephrase for clarity.
We have changed the text according to the reviewer's suggestion.

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9.Line 914: 'Mutant reporter plasmid'. Please specify what is mutated?
We have changed the text and added the relevant information in the M&M section.

References:
Ahn, A. *et al.* (2021) 'Transcriptional reprogramming and constitutive pd-l1 expression in melanoma are associated with dedifferentiation and activation of interferon and tumour necrosis factor signalling pathways', *Cancers*, 13(17), p. 4250. doi: 10.3390/CANCERS13174250/S1.

Belancio, V. P., Hedges, D. J. and Deininger, P. (2008) 'Mammalian non-LTR retrotransposons: For better or worse, in sickness and in health', *Genome Research*, 18(3), pp. 343–358. doi: 10.1101/GR.5558208.

Bodak, M. *et al.* (2017) 'Dicer, a new regulator of pluripotency exit and LINE-1 elements in mouse embryonic stem cells', *FEBS Open Bio*, 7(2), pp. 204–220. doi: 10.1002/2211-5463.12174.

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Goodier, J.L., L.E. Cheung, and H.H. Kazazian. 2012. MOV10 RNA Helicase Is a Potent Inhibitor of Retrotransposition in Cells. *PLoS Genetics*. 8. doi:10.1371/journal.pgen.1002941.

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1st Revision - Editorial Decision 20th Jun 2022

Dear Prof. Ciaudo,

Thank you for the submission of your revised manuscript. We have now received the enclosed reports from referees 1 and 2. Referee 2 still has a minor suggestion that I would like you to incorporate before we can proceed with the official acceptance of your manuscript.

A few editorial requests will also need to be addressed:

- Please reduce the number of keywords to 5.

- Please add a Data Availability Section (DAS) to the end of the methods. If you have not generated and deposited data in public databases please mention this fact in the DAS.

- Please correct the conflict of interest subheading to "Disclosure and Competing Interest Statement".

- Please correct the reference format to the EMBO reports (Harvard) style. Not more than 10 authors may be listed and the year needs to be in brackets.

- Please correct the callouts for the Appendix figure to "Appendix Fig S1". Please add page numbers to the Appendix table of content.

- The SOURCE DATA need to be uploaded as one file or folder per figure.

- The synopsis image looks good, but the text is not readable at the final size of 550x280 pixels. Can you please increase the text size and send us a new image? Also, please send us the short summary and bullet points as text file.

- Please remove the instructions from the template of the Reagents Table.

- Please add the heading 'Expanded View Figure Legends'.

- I attach to this email a related ms file with comments by our data editors. Please address all comments in the final ms.

I would like to suggest a few minor changes to the abstract, and made 2 comments below that need to be addressed. Please let me know whether you agree with the following:

LINE-1 (L1) retroelements have retained their ability to mobilize. Mechanisms regulating L1 mobility include DNA methylation in somatic cells and the piRNA pathway in the germline. During pre-implantation stages of mouse embryonic development, however, both pathways are inactivated leading to a window necessitating alternative means of L1 regulation. We previously reported an increase in L1 levels in Dicer_KO mouse embryonic stem cells (mESCs), which was accompanied by only a marginal increase in retrotransposition, suggesting additional mechanisms suppressing L1 mobility. Here, we demonstrate that L1 Ribonucleoprotein complexes (L1 RNP) accumulate as aggregates in Dicer_KO cytoplasms [of mESC? Please specify] along with the RNA helicase MOV10. The combined overexpression of L1 ORF1p and MOV10 is sufficient to create L1 RNP aggregates. In Dicer_KO mESCs, MOV10 is upregulated due to the loss of its direct regulation by miRNAs. The newly discovered post-transcriptional regulation of Mov10, and its role in preventing L1 retrotransposition by driving cytosolic aggregation provides alternative routes to explore for therapy [what kind of therapy? You do not mention any disease here. please explain].

I 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 wishes,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #2:

All of my concerns have now been addressed thank you. Note that in the new Figure 6A, labels are missing on the left side of

the IF images to enable the reader to know the difference between the upper and lower row of images. These should be added.

The authors have addressed all minor editorial requests.

2nd Revision - Editorial Decision 30th Jun 2022

Prof. Constance Ciaudo
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D-Biol
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Dear Prof. Ciaudo,

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

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

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Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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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.
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- an explicit mention of the biological and chemical entity(ies) that are being measured.
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- 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.).
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  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
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Please complete ALL of the questions below.
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