EMBO reports

Concerted regulation of mitochondrial and nuclear non-coding RNAs by a dual-targeted RNase Z

Stefan J. Siira, Giulia Rossetti, Tara R. Richman, Kara Perks, Judith A. Ermer, Irina Kuznetsova, Laetitia Hughes, Anne-Marie J. Shearwood, Helena M. Viola, Livia C. Hool, Oliver Rackham and Aleksandra Filipovska

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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.)

1st Editorial Decision 8 May 2018

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are potentially interesting. However, all referees also point out several technical concerns and have a number of suggestions for how the study should be strengthened, and I think that all of them should be addressed except for point 2 from referee 1, which is certainly interesting but beyond the scope of the current study.

Please also provide a better description of the RNA seq experiments as outlined by referee 2 and 3, including a better digestion of the results but also a more detailed description of the experimental details even if this description has been deposited alongside the data in GEO. Please also provide access to the data set deposited in GEO upon submission of the revised manuscript.

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

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Please contact us if a 3-months time frame is not sufficient for the revisions so that we can discuss the revisions further.

Supplementary/additional data: The Expanded View format, which will be displayed in the main

HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

The Supplemental tables represent rather complex datasets. I therefore suggest to submit them as Datasets with the legend in the first row of the excel file.

Regarding data quantification, please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. 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.

We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

When submitting your revised manuscript, we will require:

- a complete author checklist, which you can download from our author guidelines (http://embor.embopress.org/authorguide#revision). Please insert page numbers in the checklist to indicate where the requested information can be found.
- a letter detailing your responses to the referee comments in Word format (.doc)
- a Microsoft Word file (.doc) of the revised manuscript text
- editable TIFF or EPS-formatted figure files in high resolution
(In order to avoid delays later in the publication process, please check our figure guidelines before preparing the figures for your manuscript:
http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf)
- a separate PDF file of any Supplementary information (in its final format)
- all corresponding authors are required to provide an ORCID ID for their name. Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (http://embor.embopress.org/authorguide).

- Moreover, we kindly ask you to include a formal "Data and software availability" section (after Materials & Methods) that follows the example below:

Data and software availability
The datasets produced in this study are available in the following databases:
- RNA-Seq data: Gene Expression Omnibus GSE46843
(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)

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 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 version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

This manuscript by the Filipovska's group extends our knowledge on the role of the RNase Z-type ELAC2 on co-regulating mitochondrial and nuclear non-coding RNAs to facilitate protein synthesis. The study is performed in vivo using conditional mouse models that add biomedical significance to the study. The study is performed with great accuracy and it is sound technically and conceptually. I have two requests however to improve the manuscript:
1- In page 8 it is stated that because the levels of mitochondrial proteases where increased in the ELAC KO mice, this is the initial response to OXPHOS dysfunction. However, OXPHOS dysfunction is a too general term. In this case, perhaps is more accurate to talk about unbalanced mitochondrial protein synthesis, which is actually the cause of the OXPHOS dysfunction in the ELAC2 KO mice. There are several examples in the literature in this line that should be mentioned.
2- Mitoribosome assembly on unprocessed rRNAs is intriguing. Fig 4 presents very interesting information regarding the accumulation of processed and unprocessed rRNAs and accumulation of ribosome subunit subcomplexes. It will be very interesting to use a mass spec approach to identify all the mitoribosome proteins that accumulate in these subcomplexes and compare the pattern with the recently published human mitoribosome assembly pathway.

Referee #2:

Mutations in the RNA processing enzyme ELAC2 have been identified to cause severe mitochondrial disease involving cardiomyopathy by disruption of mitochondrial tRNA cleavage. This enzyme is produced in two variants, one present in mitochondria and one in the nucleus. In this study, the authors generated conditional k.o. mutants that lacked ELAC2 in heart and skeletal muscle cells and analyzed the consequences on a physiological and molecular level in detail. In particular they showed that ELAC2 is critical for the processing of mitochondrial and of nuclear encoded tRNAs. As a consequence, loss of ELAC2 leads to severe problems in mitochondrial (and nuclear) gene expression. This is a technically sound, very data-rich study. The results are well controlled and described in depth. This study clearly goes beyond the expected observation that a highly conserved RNAs present in the nucleus and the mitochondria is critical for gene expression. Some minor points still should be addressed.
Specific points:
1. I missed a quantitative measurement of the levels of mitochondrial (and nuclear) DNA levels in the cre tissues. This is absolutely essential as lower levels of RNA could be caused a rather general mtDNA depletion.
2. For many readers the information in the supplements will be particularly interesting. In particular the data obtained by the RNAseq experiments might be of interest. However, these data are currently very difficult to interpret as their description is very scarce. The authors should make an effort to digest them as good as possible in order to make their use by others easily possible.
3. The cre mutant shows only very low levels of mt ribosomes, in particular of assembled 55S ribosomes. Nevertheless, the levels of most mitochondrial translation products are only very moderately reduced. This should be explained.
4. Fig. 5B suggests that some mature tRNAs are increased in the cre tissues. This is surprising and needs an explanation. Which tRNAs are these?
5. Is it possible that the effects on the mitochondrial RNA levels are partially caused by alterations of nuclear ELAC2? Even if this is difficult to exclude this completely, this should be discussed, as well as why Nature chose to use the same enzyme for RNA processing in mitochondria and nuclei,

whereas for almost all other processes, mitochondria-specific isoforms are present.

Referee #3:

The manuscript by Siira et al present molecular analysis of the consequences of KO of ELAC2 gene in mice. ELAC2 encode tRNA processine RNase Z which localize both into the nucleus and mitochondria. In general this paper confirms previously suggested factions of ELAC2 using biologically relevant model. Furthermore, the authors analyzed the effect of KO on small RNA species like tRNA derived fragments. Although the level of novelty is limited, comprehensive analysis presented herein are interesting for a relatively broad group of scientist.
There are however several issues which need to be resolved before the manuscript will be suitable for publication.

1. The Authors state that they performed RNA sequencing by Illumina Tru-Seq RNA-seq, small RNA-seq and miRNA referring to their previous work (Rackham 2016) without providing experimental details. Unfortunately, in the indicated paper the Authors used the Illumina Tru-Seq protocol and PARE approach but not small RNA-seq. Thus, it is not clear what is the difference between small RNA-seq and miRNA-seq. Are they equivalent to PARE protocol? Unfortunately, lack of this information essentially precludes critical evaluation of the significant part of the manuscript. Any steps of RNA size fraction (prior cDNA library preparation) or cDNA fractionation should be described. For the Illumina Tru-Seq protocol it should be stated whether the library was prepared in the strand-specific manner. Probably these important details are described along data deposited to GEO. However, the data has not been released and security token is absent in the section "Accession number".
2. Have the Authors prepared cDNA libraries in a way which includes steps essential for quantitative analysis of tRNA (e.g. PMID: 26214130)? If not it should be commented in the manuscript.
3. tRNA Phe and tRNA Val do not contribute to RNA19 (see PMID: 1732728). For the sake of clarity RNA19 should be labeled in Fig. 2A
4. In Fig2.A (hybridization detecting mt-Tl1) two processing intermediates are indicated. Inactivation of ELAC2 has opposite effect on their steady-state levels. Can the Authors confirm composition of indicated intermediates? Hybridization with other probes should help. Size marker would be welcome.
5. Does inactivation of ELAC2 affect processing of mitochondrial non-canonical processing sites (like ATP8-6, ND5-cytB). Actually, Fig3A suggests some changes in these sites upon ELAC2 inactivation.
6. Fig. 2C. It is not clear what are 5' aligned reads. Does the graph show mean or median value? We would suggest to mark a region on the figure which indicates uncleaved mt-tRNAs. The average length of mature mt-tRNAs should be included in the figure legend.
7. Fig. 3A. Description in the text suggests that the figure shows results of sequencing of libraries obtained using the Illumina Tru-Seq protocol (analysis of reads that span processed regions) , however, the figure legend suggests PARE results (3' end abundance). Please clarify.
8. Fig. 3A. Antisense Co1 (and Co3 to some extent) and mRNA ND3 seem to be upregulated but no other mtRNAs. Can the Authors confirm effect of ELAC2 KO on the levels of these transcripts by northern hybridization? Can you speculate why they are, if so, upregulated whereas others mtRNA are downregulated?
9. Fig. 3A. The figure is without scale. Thus, it is difficult to assess extent of changes since only log FC are shown. The Authors should also proved the coverages at least in the supplement.
10. The order of mt-tRNA processing events was described previously by others (PMID: 21593607) and further investigated using biochemical approach by Reinhard et al., (PMID: 29040705). These studies should be cited/discussed accordingly in the manuscript.
11. Fig. 4B. The Authors stated that mtDNA- and nuclear-encoded polypeptide components of OXPHOS are decreased. However, since a loading control they use (SDHB) is also a component of OXPHOS the result should be confirmed with a protein which is not a component of OXPHOS.
12. Page 8. " These findings suggest that the initial stress response to OXPHOS is....not increased transcriptional regulation of the mitochondrial genome". The Authors did not examined an effect of ELAC2 KO on mtDNA transcription. They tested the level of POLRMT but this is not sufficient to support the conclusion. Relevant data should be added or the sentence rephrased.
13. Page 8. "... we resolved the mitoribosomal subunits and assembled ribosomes..." This may imply

that mitoribosomes were isolated before sucrose gradient. I think that mitochondrial protein extracts were resolved and position of the mitoribosomal subunits and assembled ribosomes was examined by western blot. The same applies to description of cytoplasmic ribosomes (Page 12).

14. Fig. 4E, F. While the shift of large and small subunit proteins into less dense sucrose fraction can be noticed we don't see changes which would support impairments of monosome formation. Describe it better or draw this conclusion after Fig.4F. Actually, the data are not relevant for the main conclusions of the paper can be removed.
15. Fig. 5B shows the same data as Fig 6C and 6E. This should be described in the corresponding figure legends.
16. Fig. 5B, 6C, 6E. What are gray dots? It seems that there are some which do not represent any of the following RNAs: mature tRNA, precursor tRNA, snRNA, snoRNA, miRNA. There is a transcript (upper left corner) significantly depleted in KO mice which does not belong to any of mentioned RNA species. Can you label it? Does it downregulation have any functional implications?
17. Fig. 5F. Nuclear precursors are not detected. If their level is too low in order to be detected by hybridization please comment accordingly in the manuscript. If the resolution of the gel was not sufficient to distinguish between mature and precursor tRNA repeat the analysis. What is the percentage of the gel? Describe it in the manuscript.
18. Page 9. "Interestingly, these rRNAs were redistributed in the Elac2 knockout mice...". In fact 12S rRNA which seems to correspond to small subunit was not redistributed. Rephrase the sentence to avoid confusion.
19. FigS2. Include in the figure legend information that porin is a loading control.
20. Page 11. "Finally, the unique changes in the Elac2 knockout mice for molecular function largely overlapped with the molecular function changes in the Mrpp3 knockout....". I understand what the Authors intent to express, however, I would recommend rephrasing this sentence. The unique changes which overlap with other changes can be confusing.
21. Fig. 5. Panels appear in the main text (page 12) in the incorrect order (5D precedes 5C).
22. Page 12. "... and dramatic decrease in their mature levels (Supplementary Fig. S6A)". I guess there should be Fig. 5E, 5F instead of Supplementary Fig. S6A. tRNALys(CTT) is not shown on Fig. 5F, although, this is suggested in the text (page 12).
23. Page 12. The authors conclude that ELAC2 is required for 3'end processing of most tRNAs in the nucleus. Can you give an estimate (number out of number) based on RNAseq.
24. Page 14. Transition from tRF to miRNA is unclear for me. Please include more background/rephrase the paragraph.
25. Page 15. "... ~110 nt downstrem of the tRNA 3' end (Fig. 7D)". There should be Fig. 7E.
26. Fig 7E. Re-order sub-panels according to the order by which they appear in the main text. For example tRNAAsnGTT-3-1 should be on top. Indicate taRNA on genome browser images. "Precursor tRNA....." is misleading (for example the length of the pre-tRNAAsnGTT-3-1 is ~200 nt in the main text but is much shorter on the figure).
27. The Authors analysed nuclear encoded tRF. What happens with mitochondrial short RNAs which originate from mitochondrial tRNAs (PMID: 21854988).
28. Page 17. "Furthermore our findings indicate that ELAC2 plays a role in snoRNA...". In my opinion presented results suggest that ELAC2 may play indirect role in snoRNA-mediated RNA modification in the nucleolus.
29. Page 20. Immunoblotting - include antibodies cat. no.

1st Revision - authors' response 14 May 2018

Reviewer #1:

This manuscript by the Filipovska's group extends our knowledge on the role of the RNase Z-type ELAC2 on co-regulating mitochondrial and nuclear non-coding RNAs to facilitate protein synthesis. The study is performed in vivo using conditional mouse models that add biomedical significance to the study. The study is performed with great accuracy and it is sound technically and conceptually. I have two requests however to improve the manuscript:

We thank this reviewer for their positive comments and suggestions to improve this study.

1- In page 8 it is stated that because the levels of mitochondrial proteases where increased in the ELAC KO mice, this is the initial response to OXPHOS dysfunction. However, OXPHOS dysfunction is a too general term. In this case, perhaps is more accurate to talk about unbalanced mitochondrial protein synthesis, which is actually the cause of the OXPHOS dysfunction in the ELAC2 KO mice. There are several examples in the literature in this line that should be mentioned.

We agree with this reviewer and we have made this clarification to indicate that the increase in mitochondrial proteases is a consequence of unbalanced mitochondrial protein synthesis.

2- Mitoribosome assembly on unprocessed rRNAs is intriguing. Fig 4 presents very interesting information regarding the accumulation of processed and unprocessed rRNAs and accumulation of ribosome subunit subcomplexes. It will be very interesting to use a mass spec approach to identify all the mitoribosome proteins that accumulate in these subcomplexes and compare the pattern with the recently published human mitoribosome assembly pathway.

We agree that this would be a very interesting set of experiments to undertake and this is something we could pursue in the future, as we have previously (Rackham et al 2016), however this is beyond the scope of the current study.

Reviewer #2:

Mutations in the RNA processing enzyme ELAC2 have been identified to cause severe mitochondrial disease involving cardiomyopathy by disruption of mitochondrial tRNA cleavage. This enzyme is produced in two variants, one present in mitochondria and one in the nucleus. In this study, the authors generated conditional k.o. mutants that lacked ELAC2 in heart and skeletal muscle cells and analyzed the consequences on a physiological and molecular level in detail. In particular they showed that ELAC2 is critical for the processing of mitochondrial and of nuclear encoded tRNAs. As a consequence, loss of ELAC2 leads to severe problems in mitochondrial (and nuclear) gene expression. This is a technically sound, very data-rich study. The results are well controlled and described in depth. This study clearly goes beyond the expected observation that a highly conserved RNAase present in the nucleus and the mitochondria is critical for gene expression. Some minor points still should be addressed.

We thank this reviewer for their comments and insight and we address their suggestions below.

Specific points:
1. I missed a quantitative measurement of the levels of mitochondrial (and nuclear) DNA levels in the cre tissues. This is absolutely essential as lower levels of RNA could be caused a rather general mtDNA depletion.

In our revised manuscript, we include a quantitative measurement of both mitochondrial and nuclear DNA levels in the control and knockout mice and show that their levels are not affected significantly (Figure EV1D). This indicates that the defects are a result of impaired 3′ tRNA processing as a consequence of ELAC2 loss, not mtDNA depletion.

2. For many readers the information in the supplements will be particularly interesting. In particular the data obtained by the RNAseq experiments might be of interest. However, these data are currently very difficult to interpret as their description is very scarce. The authors should make an effort to digest them as good as possible in order to make their use by others easily possible.

We provide additional explanation the about the gene ontologies that were obtained from the differential analyses of the RNA-Seq data shown in the revised Appendix Supplementary Figure legends. We have clarified the purpose of the three different types of RNA-Seq libraries used in this study in the revised manuscript: on page 6 we describe the use of RNA-Seq to analyze mitochondrial RNA processing, on page 9 we describe that we used RNA-Seq to analyze differential expression as a consequence of ELAC2 loss, and on page 11 we describe the use of small RNA-Seq for capturing tRNAs and non-coding RNAs such as *Malat* and miRNA-Seq to analyze miRNAs and small RNAs such as tRNA fragments. We provide these extra details and information on the library

sizes of each dataset in the Methods section (page 20) and all the details on each of the RNA-Seq analyses are provided on pages 21-23, as the short format of EMBO Reports precludes us from including this extensive detail in the main text.

3. The cre mutant shows only very low levels of mt ribosomes, in particular of assembled 55S ribosomes. Nevertheless, the levels of most mitochondrial translation products are only very moderately reduced. This should be explained.

We have carried out many translation experiments and we consistently observe a significant decrease of mitochondrial protein synthesis (quantification of our translation experiments shows 71±2% decrease in protein synthesis in the knockout samples compared to controls) that is consistent with a reduction of the assembled ribosomes in the *L/L, cre* or knockout samples (Figure 4E, bottom four blots), compared to the *L/L* or control samples (Figure 4E, top four blots) and ~70% reduction of 55S ribosomes determined by qRT-PCR following sucrose gradient resolution (Figure 4F). Importantly, impaired tRNA processing and consequent loss of tRNAs also contribute to the reduction in mitochondrial protein synthesis.

4. Fig. 5B suggests that some mature tRNAs are increased in the cre tissues. This is surprising and needs an explanation. Which tRNAs are these?

The increased mature tRNA sequences are $tRNA^{Ala(TGC)}$, $tRNA^{Ala(AGC)}$ and $tRNA^{Gly(CCC)}$, and we have labelled these in Figure 5B in the revised manuscript. Interestingly, the $tRNA^{Ala(TGC)}$ and $tRNA^{Ala(AGC)}$ contained reads with non-templated CCA additions to the 3′ end, such that they must have been correctly cleaved at their 3′ ends. This suggests that an alternative 3′ tRNA processing activity exists in mammalian nuclei. This is also supported by our observation that abundant tRF-3s, which possess 3′-CCAs acquired after 3′ end processing, were found in the *Elac2* knockout mice.

5. Is it possible that the effects on the mitochondrial RNA levels are partially caused by alterations of nuclear ELAC2? Even if this is difficult to exclude this completely, this should be discussed, as well as why Nature chose to use the same enzyme for RNA processing in mitochondria and nuclei, whereas for almost all other processes, mitochondria-specific isoforms are present.

We agree with this reviewer that it is possible that the defects in nuclear ELAC2 and consequent decreased cytoplasmic protein synthesis could have downstream effects on mitochondrial protein synthesis. Since most mitochondrial proteins, and in particular the mitochondrial gene expression machinery proteins, are translated on cytoplasmic ribosomes the defects in nuclear ELAC2 would impact on mitochondrial function. However, the primary defects in mitochondrial RNAs are predominantly the result of loss of the mitochondrial ELAC2, since we observe dramatic defects in mitochondrial RNA processing and translation at a point when levels of nuclear-encoded mitochondrial RNA-binding proteins, ribosomal proteins, TFAM and POLRMT have yet to change (see Figure EV2). Nevertheless, the consequences of nuclear ELAC2 loss compound the mitochondrial defect, as this reviewer suggests, such that the mice die by 4 weeks.

It is interesting that during evolution there are duplications of genes which diverge to become mitochondria- and nuclear-specific proteins, as well as single genes encoding dually targeted proteins, such is the case for ELAC2. The mechanisms behind this in evolution are intriguing but not clear, nevertheless it exemplifies that fact that through evolution it has not been necessary to have a mitochondria-specific gene for ELAC2. It is possible that the unique and compact organization of the mitochondrial genome in animals has necessitated the evolution of a distinct mitochondrial RNase P enzyme that can cope with the complexity of the polycistronic mitochondrial transcripts and extensive 5′ leaders, as 5′ tRNA processing precedes 3′ tRNA processing. While ELAC2 performs a similar function by cleaving the 3′ tRNA ends of already 5′-processed transcripts that are likely structurally similar to those in the nucleus. We have included this point in the discussion as suggested on page 15.

Reviewer #3:

The manuscript by Siira et al present molecular analysis of the consequences of KO of ELAC2 gene in mice. ELAC2 encode tRNA processine RNase Z which localize both into the nucleus and

mitochondria. In general this paper confirms previously suggested factions of ELAC2 using biologically relevant model. Furthermore, the authors analyzed the effect of KO on small RNA species like tRNA derived fragments. Although the level of novelty is limited, comprehensive analysis presented herein are interesting for a relatively broad group of scientist.
There are however several issues which need to be resolved before the manuscript will be suitable for publication.

We thank this reviewer for their comments and suggestions and we address all their points below. In addition, we believe that the findings in this manuscript provide many novel insights beyond what was previously known in the literature. Critically, the role of ELAC2 in nuclear tRNA processing and gene expression has never been studied in mammals before. We observed that ELAC2 is required for 3′ tRNA processing in both the nucleus and mitochondria, that its loss causes a dramatic imbalance in miRNAs and snoRNAs encoded by the nucleus, we confirm the predicted order of RNA processing in mitochondria, chart the transcriptome-wide response to a combined nuclear-mitochondrial RNA processing dysfunction for the first time, and discover a new class of small RNAs.

1. The Authors state that they performed RNA sequencing by Illumina Tru-Seq RNA-seq, small RNA-seq and miRNA referring to their previous work (Rackham 2016) without providing experimental details. Unfortunately, in the indicated paper the Authors used the Illumina Tru-Seq protocol and PARE approach but not small RNA-seq. Thus, it is not clear what is the difference between small RNA-seq and miRNA-seq. Are they equivalent to PARE protocol? Unfortunately, lack of this information essentially precludes critical evaluation of the significant part of the manuscript. Any steps of RNA size fraction (prior cDNA library preparation) or cDNA fractionation should be described. For the Illumina Tru-Seq protocol it should be stated whether the library was prepared in the strand-specific manner. Probably these important details are described along data deposited to GEO. However, the data has not been released and security token is absent in the section "Accession number".

Because of the number of different experiments performed and the brief format of EMBO Reports we were unable to include as much detail in the original submission. These details were included in the deposited data to GEO but also we have included this information in the Methods of the revised manuscript. We also describe the reasons why we chose to use three different types of libraries in the results section as described in the response to point 2 from Reviewer 2. We have provided a security token for the GEO submission (To review GEO accession GSE111228:
Go to https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111228
Enter token upmrieimdnchrir into the box). Briefly, PARE allows information on the 5′-OH ends of RNAs to be quantified in great depth that can directly analyse changes at the 5′ ends of RNAs. On the other hand, small RNA-Seq and miRNA-Seq are not equivalent to PARE. These methods capture the entire length of these small RNAs, enabling the identity of the 5′ and 3′ ends, as well as their overall sizes, to be determined. As ELAC2 cleaves at the 3′ ends of RNAs we decided that this approach would be more informative for this knockout model and used small RNA sequencing to capture tRNAs and miRNA-Seq to capture miRNAs and small RNAs such as tRFs. We have explained this in the results section and included the information in the methods section.

2. Have the Authors prepared cDNA libraries in a way which includes steps essential for quantitative analysis of tRNA (e.g. PMID: 26214130)? If not it should be commented in the manuscript.

The quoted paper on DM-tRNA-seq from Zheng *et al.* and the co-published paper on ARM-seq from Cozen *et al.* provide an approach for more efficient tRNA sequencing via demethylation of RNA prior to sequencing. This approach can increase the number of tRNA reads from small RNA libraries (but only a little more than 2x), however it is no more quantitative than small RNA-seq (see both Zheng *et al.* and Cozen *et al.*). In our experiments for this work, we have found that it is more effective to sequence small RNA-seq libraries to high depth without prior manipulation of the RNA samples. This also enables matched RNA samples to be used for small RNA-seq, miRNA-seq and standard RNA-seq in parallel, which enables reliable comparisons across different libraries.

3. tRNA Phe and tRNA Val do not contribute to RNA19 (see PMID: 1732728). For the sake of clarity RNA19 should be labeled in Fig. 2A.

We have corrected the statement in the results section so it is clear that we are not referring to tRNAPhe and tRNAVal as part of RNA19. The original labels referred to unprocessed polycistronic transcripts to indicate that loss of ELAC2 leads to accumulation of unprocessed intermediates. As suggested by this reviewer we have labelled RNA19 in Figure 2A of the revised manuscript for clarity.

4. In Fig2.A (hybridization detecting mt-Tl1) two processing intermediates are indicated. Inactivation of ELAC2 has opposite effect on their steady-state levels. Can the Authors confirm composition of indicated intermediates? Hybridization with other probes should help. Size marker would be welcome.

The tRNAL1 probe typically detects both the higher polycistronic transcript that includes the 12S and 16S rRNA because the lower unprocessed transcript is the RNA19 that is also detected by the mt-Nd1 probe. Unfortunately, it is difficult to ascertain the exact composition of the higher band because but is rarely detected using the remaining probes. We have shown previously, and in this work now, that 5′-tRNA processing precedes 3′-tRNA processing, therefore it is not surprizing that the higher polycistronic transcript is lowered since it would have been processed by the RNase P, while RNA19 is increased in the *Elac2* mice as a result of impaired 3′-tRNA processing. In addition, there is much more mature tRNA in the control mice compared to the almost absent tRNAs in the knockout mice indicating that proportionally the higher polycistronic RNA is also enriched and unprocessed in the knockout mice but appears less because there is overall less mitochondrial RNA in the knockout mice.

5. Does inactivation of ELAC2 affect processing of mitochondrial non-canonical processing sites (like ATP8-6, ND5-cytB). Actually, Fig3A suggests some changes in these sites upon ELAC2 inactivation.

Loss of ELAC2 does not affect processing of the non-canonical sites, as was also shown in cells previously (Lopez Sanchez *et al.* 2011 and Brzezniak *et al.* 2011). The decreased levels of these transcripts are a consequence of overall reduction of mitochondrial RNA levels and there is no enrichment of the *mt-Atp8/6-Co3* transcript in the absence of ELAC2 (as indicated in Figures 2A and 3A) to suggest that this site is cleaved by ELAC2.

6. Fig. 2C. It is not clear what are 5' aligned reads. Does the graph show mean or median value? We would suggest to mark a region on the figure which indicates uncleaved mt-tRNAs. The average length of mature mt-tRNAs should be included in the figure legend.

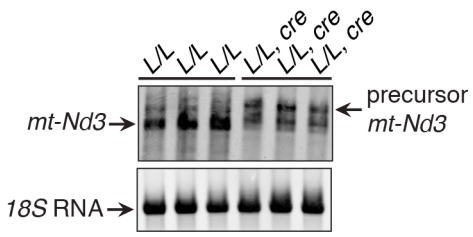
5' aligned reads are those that were collected based on their 5' ends aligning exactly to the canonical 5' ends of the mt-tRNA loci, the graph in this figure shows the mean reads per million (RPM). The suggestion to add sizes for mt-tRNAs is a good one, we have marked regions on the figure to indicate the uncleaved tRNAs and also the regions showing the average length of mature tRNAs (and also included this in the figure legend).

7. Fig. 3A. Description in the text suggests that the figure shows results of sequencing of libraries obtained using the Illumina Tru-Seq protocol (analysis of reads that span processed regions), however, the figure legend suggests PARE results (3' end abundance). Please clarify.

We have clarified this in the figure legend as suggested.

8. Fig. 3A. Antisense Co1 (and Co3 to some extent) and mRNA ND3 seem to be upregulated but no other mtRNAs. Can the Authors confirm effect of ELAC2 KO on the levels of these transcripts by northern hybridization? Can you speculate why they are, if so, upregulated whereas others mtRNA are downregulated?

The *mt-Nd3* RNA is not increased as shown below from a northern blot, the precursor transcript containing the *mt-Nd3* is enriched, and the combination of these reads is what is summarised in Figure 3A. The antisense regions such as those complementary to *mt-Co1* and *mt-Co3* are in very low abundance and the change is minimal but these are too low to detect by northern blotting in the mouse.



9. Fig. 3A. The figure is without scale. Thus, it is difficult to assess extent of changes since only log FC are shown. The Authors should also proved the coverages at least in the supplement.

We have included a scale in the figure legend for clarity. The coverages are included in the GEO submission and we have now added them in raw and normalized counts to Extended View Dataset 1.

10. The order of mt-tRNA processing events was described previously by others (PMID: 21593607) and further investigated using biochemical approach by Reinhard et al., (PMID: 29040705). These studies should be cited/discussed accordingly in the manuscript.

The study by Brzezniak *et al.* 2011 was already cited and pointed out in three different places in the manuscript and we have included the Reinhard *et al.* 2017 reference in the revised version.

11. Fig. 4B. The Authors stated that mtDNA- and nuclear-encoded polypeptide components of OXPHOS are decreased. However, since a loading control they use (SDHB) is also a component of OXPHOS the result should be confirmed with a protein which is not a component of OXPHOS.

We have included a porin loading control in the revised version.

12. Page 8. " These findings suggest that the initial stress response to OXPHOS is....not increased transcriptional regulation of the mitochondrial genome". The Authors did not examined an effect of ELAC2 KO on mtDNA transcription. They tested the level of POLRMT but this is not sufficient to support the conclusion. Relevant data should be added or the sentence rephrased.

We have re-phrased the sentence for clarity.

13. Page 8. "... we resolved the mitoribosomal subunits and assembled ribosomes..." This may imply that mitoribosomes were isolated before sucrose gradient. I think that mitochondrial protein extracts were resolved and position of the mitoribosomal subunits and assembled ribosomes was examined by western blot. The same applies to description of cytoplasmic ribosomes (Page 12).

We have clarified this on both pages.

14. Fig. 4E, F. While the shift of large and small subunit proteins into less dense sucrose fraction can be noticed we don't see changes which would support impairments of monosome formation. Describe it better or draw this conclusion after Fig.4F. Actually, the data are not relevant for the main conclusions of the paper can be removed.

Figures 4E and 4F show reduction in the monosome, so we have corrected the phrasing to indicate that there is less of the 55S monosome as opposed to impaired formation. We have emphasized this after Figure 4F as suggested. Both sets of data shown in the Figures are key to the work described in the manuscript, illustrating the effect of defective 3'-tRNA processing on ribosome assembly and translation, so we have not removed these data from the manuscript.

15. Fig. 5B shows the same data as Fig 6C and 6E. This should be described in the corresponding figure legends.

Each figure highlights the changes in different classes of small RNAs within the context of all detected small RNAs – it would be too difficult to see the changes clearly in a single panel. We have

clarified the relevance of each figure by providing the relevant information in the corresponding figure legend.

16. Fig. 5B, 6C, 6E. What are gray dots? It seems that there are some which do not represent any of the following RNAs: mature tRNA, precursor tRNA, snRNA, snoRNA, miRNA. There is a transcript (upper left corner) significantly depleted in KO mice which does not belong to any of mentioned RNA species. Can you label it? Does it downregulation have any functional implications?

The grey dots represent classes of RNA types other than the ones highlighted in colour, which may include any of the following: mature tRNAs, precursor tRNAs, mitochondrial tRNAs, miRNAs, miscRNAs, 5S rRNAs, scRNAs, scaRNAs, snoRNAs, snRNAs or sRNAs. The group of high-significance grey dots on the left side of the graph are mitochondrial tRNAs, and the grey dot in the upper left corner that is most significantly depleted is the mitochondrial $tRNA^{Asp}$.

17. Fig. 5F. Nuclear precursors are not detected. If their level is too low in order to be detected by hybridization please comment accordingly in the manuscript. If the resolution of the gel was not sufficient to distinguish between mature and precursor tRNA repeat the analysis. What is the percentage of the gel? Describe it in the manuscript.

We note that the precursor tRNAs are too low to detect by northern blotting using either polyacrylamide or 2% agarose gels which is why we used RNA-Seq, and the details of the gels were described in the methods of the original submission.

18. Page 9. "Interestingly, these rRNAs were redistributed in the Elac2 knockout mice...". In fact 12S rRNA which seems to correspond to small subunit was not redistributed. Rephrase the sentence to avoid confusion.

This is now rephrased.

19. FigS2. Include in the figure legend information that porin is a loading control.

We have included this information.

20. Page 11. "Finally, the unique changes in the Elac2 knockout mice for molecular function largely overlapped with the molecular function changes in the Mrpp3 knockout....". I understand what the Authors intent to express, however, I would recommend rephrasing this sentence. The unique changes which overlap with other changes can be confusing.

We agree and have rephrased this for clarity in the revised manuscript.

21. Fig. 5. Panels appear in the main text (page 12) in the incorrect order (5D precedes 5C).

We have corrected this in the revised manuscript.

22. Page 12. "... and dramatic decrease in their mature levels (Supplementary Fig. S6A)". I guess there should be Fig. 5E, 5F instead of Supplementary Fig. S6A. tRNALys(CTT) is not shown on Fig. 5F, although, this is suggested in the text (page 12).

We have clarified this in the revised version.

23. Page 12. The authors conclude that ELAC2 is required for 3'end processing of most tRNAs in the nucleus. Can you give an estimate (number out of number) based on RNAseq.

Based on the RNA-Seq analyses we estimate that ELAC2 is required for processing of ~80% of nuclear tRNAs.

24. Page 14. Transition from tRF to miRNA is unclear for me. Please include more background/rephrase the paragraph.

We have re-phrased the paragraph but unfortunately due to space limitations it is difficult to provide more background than what it already in that section. Briefly for this reviewer, some tRNA loci are known to produce precursors that can be processed by the canonical miRNA processing pathway producing normal ~21 nt miRNAs. The processing of miRNAs from these pre-tRNAs competes with the canonical tRNA processing (the folding of the 3' uncleaved pre-tRNAs is required and so cannot occur after ELAC2 cleavage), thus with the loss of ELAC2 a greater proportion of these pre-tRNAs are shunted towards the miRNA pathway.

25. Page 15. "... ~110 nt downstrem of the tRNA 3' end (Fig. 7D)". There should be Fig. 7E.

We have corrected this.

26. Fig 7E. Re-order sub-panels according to the order by which they appear in the main text. For example tRNAAsnGTT-3-1 should be on top. Indicate taRNA on genome browser images. "Precursor tRNA....." is misleading (for example the length of the pre-tRNAAsnGTT-3-1 is ~200 nt in the main text but is much shorter on the figure).

We have re-ordered them as suggested and labelled taRNA on the images. In the revised figure legend of this panel we indicate that the image shown summarises data from the small RNA library so that it is clear why the shorter fragments only are displayed.

27. The Authors analysed nuclear encoded tRF. What happens with mitochondrial short RNAs which originate from mitochondrial tRNAs (PMID: 21854988).

In the revised manuscript we have highlighted in Figure 2C that the mitochondrial short RNAs are decreased as a result of decreased mt-tRNA levels.

28. Page 17. "Furthermore our findings indicate that ELAC2 plays a role in snoRNA...". In my opinion presented results suggest that ELAC2 may play indirect role in snoRNA-mediated RNA modification in the nucleolus.

We agree with this reviewer and we do not claim that ELAC2 has a direct role in snoRNAs, which is why in the paragraph preceding this statement we describe how ELAC2 can influence snoRNA levels.

29. Page 20. Immunoblotting - include antibodies cat. no.

We have included the catalogue numbers of the antibodies used in the revised manuscript.

2nd Editorial Decision 14 June 2018

Thank you for the submission of your revised manuscript to EMBO reports. We have now received the full set of referee reports that is copied below.

As you will see, both referees are very positive about the study and request only minor changes to clarify text and figures and methods and one validation experiment. Regarding point 2 from referee #3: Please note that we have no word limit for the materials and methods section. We usually ask authors to include all methods in the main text but in case of methods that are of rather specialized interest, these can also be part of the Appendix.

From the editorial side, there are also a few things that we need before we can proceed with the official acceptance of your study.

- I noticed that the Author Contributions section is rather short. Could you please specify the individual contributions in a bit more detail?

- Please submit all EV figures as individual high-resolution files and add their figure legends to the main manuscript in a separate section "Expanded View figure legends" after the main figure

legends.

- Please upload the Data sets separately and zipped with their legends (as a plain text file)

- Please update the Figure callouts: currently there are references to Figs S4 and S5 in text, but these have become EV Figures and thus the callouts need to be corrected.

- Please provide the Accession number to the sequencing data in a dedicated "Data Availability" section at the end of Materials & Methods (suggested wording: "The [protein interaction | microarray | mass spectrometry] data from this publication have been deposited to the [name of the database] database [URL] and assigned the identifier [accession | permalink | hashtag])."

- The following information is missing in the figure legends:
- Fig. 1B: Please specify the number of mice analyzed, the nature of the error bars and the test used to calculate the p-values in the legend for Fig. 1B. Also specify the meaning of the '*'
- 1E: specify the scale bar in the figure legend
- 1F: define the meaning of ***
- 2B: nature of the error bars
- 4D: number of experiments
- EV1A: the scale bar in the image says "1 mm", the legend states "0.5 mm"
- EV1B, C: define the scale bar in the legend
- EV1D: define the nature of the bars and error bars

- During our routine figure check we noticed marbling in the blots in Figure 6D. It looks quite distinct to the usual plasticwrap effect. Could you please comment and explain?

- We also noticed that the Northern blots shown in Fig. 2A (right panel) and in Figure 5F (n-Tk[TTT]) look very similar with respect to their respective leftmost band, which also contains a white spot. There is not enough information in the text or figure legend to judge. Did you re-probe the same membrane? Please clarify and also provide the respective source data.

- Moreover, our routine text analysis tool revealed that the first paragraph of the results section is very similar to the description of Mrpp3 knockout mice in your recent Cell reports paper. I acknowledge that this is only a methodological description, but would nevertheless ask you to modify it a bit.
- Also a section on page 8 starting with "Therefore, to investigate the effects of impaired 3' tRNA processing on the assembly ..." is almost identical to a section in the above mentioned Cell reports paper (see attached screenshot of marked similarities). I suggest modifying the text further.

- Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (width x height). You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have questions or comments regarding the revision.

REFEREE REPORTS

Referee #1:

The authors have responded to the previous criticism and therefore I recommned acceptance of the manuscript for publication.

Referee #3:

The Authors made little effort to improve the manuscript. In many cases, where we just asked to

improve the data presentation and clarity of the text, our remarks were not followed. Below we describe a few specific cases which need to be addressed:
1) We asked for presentation of normalized read coverages of RNA-seq data for the mitochondrial genome and instead we got just differentially expressed counts. We understand that the accumulation of unprocessed transcripts in mitochondria is better visible using log fold changes but readers should be able to appreciate the quality of the data as they are without performing bioinformatics analysis of they own.
2) We asked for a comment on the tRNA sequencing protocols. This is because it is well established that without demethylation and usage of specific reverse transcriptase a large faction of reads stops prematurely. Thus, in case of a paper focused on tRNA processing the reader should be informed about potential caveats of the approach used.
3) We asked for confirmation of the identity of the longer unprocessed transcript detected by the northern blot. Actually, we suspect that this is simple non-specific cross hybridization since, although it is longer than any other RNA species analyzed, it was not detected by other probes. Please clarify and conduct proper validation experiments!
These drawbacks notwithstanding, we are convinced that the paper present valuable data which shod be shared with the scientific community.

2nd Revision - authors' response 26 June 2018

Editorial and Reviewers' comments:

Editoral comments:

- *I noticed that the Author Contributions section is rather short. Could you please specify the individual contributions in a bit more detail?*

We have included more detail in this section.

- *Please submit all EV figures as individual high-resolution files and add their figure legends to the main manuscript in a separate section "Expanded View figure legends" after the main figure legends.*

We have submitted all Extended View figures as individual high-resolution eps files and added their figure legends to the main manuscript under the "Expanded View figure legends" that follows the main figure legends.

- *Please upload the Data sets separately and zipped with their legends (as a plain text file)*

We have uploaded the Data sets separately and zipped their legends in a plain text file.

- *Please update the Figure callouts: currently there are references to Figs S4 and S5 in text, but these have become EV Figures and thus the callouts need to be corrected.*

We have corrected the Figure callouts, we now refer to them as Appendix Fig S1-3.

- *Please provide the Accession number to the sequencing data in a dedicated "Data Availability" section at the end of Materials & Methods (suggested wording: "The [protein interaction | microarray | mass spectrometry] data from this publication have been deposited to the [name of the database] database [URL] and assigned the identifier [accession | permalink | hashtag])."*

We have provided this information at the end of the Materials & Methods section in a dedicated "Data Availability" section using the recommended wording.

- *The following information is missing in the figure legends:*
- *Fig. 1B: Please specify the number of mice analyzed, the nature of the error bars and the test used to calculate the p-values in the legend for Fig. 1B. Also specify the meaning of the '*'*

We have included this information.

- *1E: specify the scale bar in the figure legend*

We have included this information.

- *1F: define the meaning of ****

We have included this information.

- *2B: nature of the error bars*

We have included this information.

- *4D: number of experiments*

We have included this information.

- *EV1A: the scale bar in the image says "1 mm", the legend states "0.5 mm"*

We have corrected the figure legend.

- *EV1B, C: define the scale bar in the legend*

We have included this information.

- *EV1D: define the nature of the bars and error bars*

We have included this information.

- *During our routine figure check we noticed marbling in the blots in Figure 6D. It looks quite distinct to the usual plasticwrap effect. Could you please comment and explain?*

We use the Odyssey Infrared Imaging system for all our northern scans. We place our wet blot between two sheets of transparency and scan the blot within the transparency sheets. The swirls or marbling are from the wetness that is in contact with the transparency sheets that is detected by the Odyssey scanner that is very sensitive. This is particularly the case when the signal from the blots is not as strong as would be expected from very lowly abundant non-coding RNAs such as those shown in Figure 6D. This is not the case for very highly abundant tRNAs from either the nucleus or mitochondria. The original blots shown in Fig. 6D have been sent to the editor so they can see the entire blots.

- *We also noticed that the Northern blots shown in Fig. 2A (right panel) and in Figure 5F (n-Tk[TTT]) look very similar with respect to their respective leftmost band, which also contains a white spot. There is not enough information in the text or figure legend to judge. Did you re-probe the same membrane? Please clarify and also provide the respective source data.*

The membrane shown in Fig. 2A was stripped and re-probed for the nuclear tRNA lysine or n-Tk(TTT), hence the same white spot that has carried through from the fact that the same membrane was re-probed. The original blots shown in Fig. 2A and Fig. 5F have been sent to the editor so they can see this.

- *Moreover, our routine text analysis tool revealed that the first paragraph of the results section is very similar to the description of Mrpp3 knockout mice in your recent Cell reports paper. I acknowledge that this is only a methodological description, but would nevertheless ask you to modify it a bit.*

We have modified the text so that it is sufficiently different.

- Also a section on page 8 starting with "Therefore, to investigate the effects of impaired 3′ tRNA processing on the assembly ..." is almost identical to a section in the above mentioned Cell reports paper (see attached screenshot of marked similarities). I suggest modifying the text further.

We have modified the text further.

- Finally, EMBO reports papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (width x height). You can either show a model or key data in the synopsis image. Please note that the size is rather small and that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

We have prepared a summary statement of the findings and their significance, 3 bullet points highlighting the results and a synopsis image. We have uploaded this information on the website.

Reviewer #1:

The authors have responded to the previous criticism and therefore I recomnned acceptance of the manuscript for publication.

We thank this reviewer for their comments and to review this work.

Reviewer #3:

The Authors made little effort to improve the manuscript. In many cases, where we just asked to improve the data presentation and clarity of the text, our remarks were not followed. Below we describe a few specific cases which need to be addressed:
1) We asked for presentation of normalized read coverages of RNA-seq data for the mitochondrial genome and instead we got just differentially expressed counts. We understand that the accumulation of unprocessed transcripts in mitochondria is better visible using log fold changes but readers should be able to appreciate the quality of the data as they are without performing bioinformatics analysis of they own.

We had included the normalized read coverages of the RNA-Seq data in Dataset EV1 in our revision and in response to this comment from Reviewer 3, in addition to the differentially expressed counts. We are not certain why this reviewer could not see them, perhaps they hadn't scrolled further into the very large document. Nevertheless, these data are included now in a separate Dataset EV 4 to make it easier for readers to find these data.

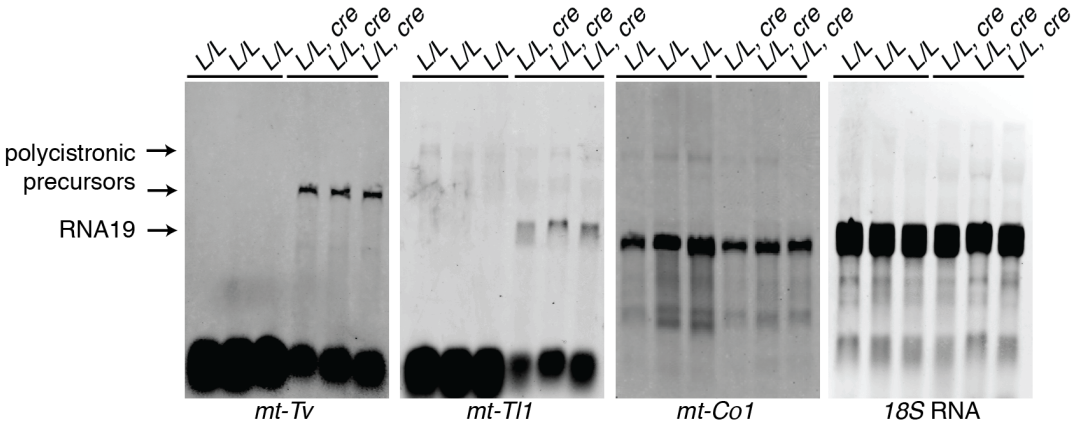
2) We asked for a comment on the tRNA sequencing protocols. This is because it is well established that without demethylation and usage of specific reverse transcriptase a large faction of reads stops prematurely. Thus, in case of a paper focused on tRNA processing the reader should be informed about potential caveats of the approach used.

We have included a comment on the tRNA sequencing protocol on pages 20-21.

3) We asked for confirmation of the identity of the longer unprocessed transcript detected by the northern blot. Actually, we suspect that this is simple non-specific cross hybridization since, although it is longer than any other RNA species analyzed, it was not detected by other probes. Please clarify and conduct proper validation experiments!
These drawbacks notwithstanding, we are convinced that the paper present valuable data which shod be shared with the scientific community.

We have carried out additional validation northern blotting and include this below showing the same unprocessed transcript identified in the original images of the manuscript. The precursor transcripts only accumulate in the *Elac2* knockout mice indicating that the detected bands are not non-specific and accumulate because they are not processed by ELAC2. We identify an unprocessed transcript (middle arrow) using the $tRNA^{Val}$ probe that according to its size suggests it contains the 12S rRNA,

16S rRNA, $tRNA^{Leu1}$ and *mt-Nd1* mRNA. We also observe this transcript using the $tRNA^{Leu1}$ probe (although it is less abundant than the RNA19 precursor). In addition, we also observe the RNA19 transcript (bottom arrow) that accumulates in the *Elac2* knockout mice containing only 16S rRNA, $tRNA^{Leu1}$ and *mt-Nd1* mRNA. Finally the $tRNA^{Leu1}$ probe also detects a larger unprocessed transcript (top arrow) that is reduced in the *Elac2* knockout mice when probed with the *mt-Co1* mRNA probe indicating that this precursor likely contains the 12S rRNA, 16S rRNA, $tRNA^{Leu1}$, *mt-Nd1* mRNA and *mt-Nd2* mRNA. We note two caveats to using northern blotting to identify precursor transcripts, the differences in hybridization of each probe that would detect each transcript at varied levels, hence the differences in intensities of the bands when using different probes. The blots have to be overexposed to detect the precursor transcripts that have variable stabilities since these are unstable and readily degraded if not modified by enzymes or bound by ribosomal or RNA-binding proteins or modified to stabilize them. To overcome these limitations we carried out three different types of RNA Sequencing that can effectively detect precursor transcripts because of the high depth coverage but also verify the identity of the precursors by aligning to the mitochondrial transcriptome. Therefore we have taken two different approaches to indicate that loss of ELAC2 impairs tRNA processing and leads to precursor accumulation determined by northern blotting and RNA-Seq, which identified the nature of the accumulated precursors.



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PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Aleksandra Filipovska
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2018-46198-T

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs 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 and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	We have not used a statistical method to predetermine size and the sample size was chosen based on our previous studies. We typically use a sample size of at least eight and we carry out multiple biologically indpependent experiments
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We have used at least eight biological replicates and at least three indpependent experiments.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	We have not excluded any samples from our study.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	We have used animals from different, age and sex matched litters to avoid any littermate bias.
For animal studies, include a statement about randomization even if no randomization was used.	We have used animals from different, age and sex matched litters to avoid any littermate bias.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	As described above we used animals from different parents, the isolation of the samples was carried out on separate occassions and animals were only chosen based on genotype and sex.
4.b. For animal studies, include a statement about blinding even if no blinding was done	We did not carry out any blinding studies because the animals needed to be identified by genotype before analyses could be carried out.
5. For every figure, are statistical tests justified as appropriate?	We have used Student's t tests for all our animal studies and the statistical analyses for the bioinformatic analyses are outlined in the methods section.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	All the data meet the assumptions of the tests based on a normally distributed population and for the bioinformatic data built-in statistacal analyses were used that were part of the software as described in the methods section of the manuscript.
Is there an estimate of variation within each group of data?	Yes

USEFUL LINKS FOR COMPLETING THIS FORM

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http://1degreebio.org
http://www.equator-network.org/reporting-guidelines/improving-bioscience-research-repo

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http://www.consort-statement.org
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http://www.equator-network.org/reporting-guidelines/reporting-recommendations-for-tun

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http://www.ebi.ac.uk/ega

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http://jjj.biochem.sun.ac.za
http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
http://www.selectagents.gov/

Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We have provided catalog numbers for all antibodies used.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Mus musculus, C57BL/6N background, male, 4-week old transgenic mice where Elac2 was knocked out or lox P sites flanked exon 8. Mice were housed in standard cages under 12 h light/dark schedule in controlled environmental conditons of 22 ˚C and 50% humidity and fed a normal chow diet. The animals were obtained from the European Mouse Mutant Archive (EMMA), Biomodels, Austria.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The study was performed in accordance with Principles of Laboratory Care (NHMRC Australian code for care and use of animals for scientific purposes 8th Edition 2013). The study was approved by the University of Western Australia Animal Ethics Committee.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm complience with these guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	We have provided a Data Availability section and have deposited our data in the Gene Expression Omnibus, as described in the manuscript.
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	We have included the supplementary data in the Extended View submission.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A