EMBO reports

Armitage determines Piwi-piRISC processing from precursor formation and quality control to inter-organelle translocation

Haruna Yamashiro, Mayu Negishi, Tatsuki Kinoshita, Hirotsugu Ishizu, Hitoshi Ohtani, and Mikiko C. Siomi

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Editorial Decision:	13 November 2019
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Editor: Esther Schnapp

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 5 August 2019

Thank you for the submission of your manuscript to EMBO reports. We have now received the enclosed referee comments.

As you will see, both referees acknowledge that the findings are interesting. They further raises several points to strengthen the study, which should all be addressed.

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 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 this further. You can either publish the study as a short report or as a full article. For short reports, the revised manuscript should not exceed 29,000 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.

Please correct the references to the numbered EMBO reports style that is also part of EndNote.

Regarding data quantification, please specify the number "n" for how many independent

experiments were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. This information must be provided in the figure legends. Please also include scale bars in all microscopy images.

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) 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>.

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.

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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 study biochemically dissects a critical segment of piRNA biogenesis pathway. This involves movement of the pre-piRNA intermediate complexes from processing bodies called Yb bodies to the mitochondrial surface where a key piRNA processing endonuclease is present. It examines the role of the RNA helicase Armitage in this movement to reveal novel insights. This will be valuable for the piRNA community.

Minor comments:
It is a very odd term to use: "inward RNA". Can the authors change it to pre-piRNA 3' extension? Or something similar.
Some of the instances in the text.
Piwi-pre-piRISC through inward RNA
Piwi-pre-piRISC via RNA inward (i.e., piRNA precursor)
Armi-Piwi-pre-piRISC association through inward piRNA precursor

A cartoon summarizing the different mutations and the delta34 deletion used for Armi, and other mutants of Piwi would be useful to the reader.

Referee #2:

This manuscript from Yamashiro et al dissects the role of Armitage, an RNA helicase, in the transit of the Piwi-pre-piRISC from the yb bodies to mitochrondria the site for piRNA biogenesis. The experiments are performed on Drosophila Ovarian somatic cells. Most of the experiments were performed in Zuc KD background to better understand the hierarchical regulation and recruitment of all these cytoplasmic factors known to be required in the somatic piRNA pathway. The authors confirmed previous results from Munafo et al, showing that Gasz located to the mitochondria is involved in the Zuc-dependent piRNA maturation. Furthermore, the authors showed an accumulation of Armi in the mitochiondria upon Zuc depletion in a Gasz-dependent manner suggesting that Armi shuttle between the Yb bodies and the mitochondria. As it has been shown by several previous studies, the authors revealed an interdependence of Armi and Piwi in the assembly of the Piwi-pre-piRISC.
Finally, they showed that the RNA binding activity of Armi is not required for Armi localization and retention in the yb bodies and they propose that this activity is necessary for the departure of the Piwi-pre-piRISC from the Yb bodies to the mitochondria.

While the in-depth characterization of each step between the yb bodies and the mitochondria in which piRNA biogenesis is initiated and completed is an important question in piRNA biology, the authors have to improve and simplify the manuscript.
Due to a multitude of KD combinations used in this study the manuscript is not easy to read and at the end it is difficult to draw a clear conclusion on such multilayered elaborate regulation.
This is an interesting paper that is suitable for publication in EMBO reports, as long as the following major and minor criticisms can be addressed.
Major concerns:

- Overall, it is not precised how many experiments were performed to draw each conclusion. In addition, it should be added larger immunostaining views with several cells on the same picture where similar phenotypes could be visualized. The legend of the figure should not be only a

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conclusion of the figure but should also include the type of experiment presented and all the details necessary to fully understand the experiment and the results. All the KD (endogenous gene KD by RNAi) and rescued experiment should be precise in the main text as well as in the figure legends.

- Previous studies showed that Gasz loss perturbs mitochondrial morphology, and Zuc depletion results in mitochondrial clustering which to my surprise was not discussed in the present manuscript. The authors should discuss their works.

- In a WT context, Gasz is located in the mitochondria fraction whereas Armi and Piwi are mainly found in the cyto fraction. It is really clear that in Zuc KD condition there is an enrichment of Armi in the mito fraction. In the experiment presented Fig 1C It is necessary to present (as presented in fig 1B) the cytoplasmic fraction to see whether there is in parallel a depletion of these proteins in the cytoplasmic part. In addition, how the authors explain that in Zuc KD, in the total fraction, the signal for HSP60 as well as Armi is less intense than the mitochondrial fraction. It seems that in addition to be mislocalized to the mitochondria in Zuc KD, Armi is more expressed as seen with WB on the mitochondrial fraction and immunostaining? The accumulation of Armi on the mitochondria is clear in Zuc KD but less for Piwi. In the Ev1B, in Zuc KD, Piwi seems to be localized more in the Yb body than on the mitochondria and in fig 1C the increase of Piwi in the mito fraction is not really clear. Could the author comment and adjust their statements in the text and in the title of figure EV1?

- The authors show that Gasz interact with Armi and Piwi on the mitochondria (Fig2A) in a control condition. Several questions can then be raised: Why any coimmunostaining are detected in OSC cells between Armi and Mitochondria in WT context? The figure 2A and 1A show an Armi detection which is very different, could the authors discuss this point? The figure 2B represent the mito fraction? How the authors explain that almost no coIP of Armi and flag-Gasz is found in the control of the figure 2B compared to the result found in figure 2A? Could the authors really conclude of an increase of Armi in a flag-Gasz complex in Zuc KD?

- Figure 2G: the author claimed that under Zuch and Piwi KD, Armi no longer stably associated with Gasz. Since the effect is very weak, could it be quantified? Could the experiment be done the other way around, a flag-Gasz IP to see whether Armi can be coIP.

- Figure 3C it is clearly showed that the PAZmut binds the precursor whereas the MIDmt not. However how the authors explain the results with the PIWI WT knowing that the experiment is performed in a Zuc KD context, why idefix piRNA are detected? A detailed legend is required to fully understand the figure.

- What is the consequence of the mutation N756A on the Idefix piRNA biogenesis and on the piRNA precursor accumulation?

- To explain the Armi localization to Yb bodies induced by Zuc + Piwi loss the authors proposed two scenarios. Based on the experiments presented in figure EV2, why the authors support the first scenario? The authors clearly showed that Armi and Gasz interact together, so how could the author explain that in Piwi + Zuc depleted cells, Armi is no longer associated with Gasz (fig 2G) ?

- How could the authors explain how the 2D6E11 antibody recognize only the long isoform?

- How could the author explain the discrepancy between the Protein-protein interaction assays and the IP experiments between Armi delta 34 and the Gasz proteins? For the Armi delta 34 the flag is fused to the Nterm or Cterm? it is not clear. The conclusion of this part is not clear because two experiments are going to the conclusion that the Nterminus of Armi is required for the interaction with Gasz and another shows that this domain is not required?

Minor Points:
- Page 3: In the introduction the beginning of the second paragraph is not well referenced. The author should add several references concerning the OSC and flamenco, such as Prud'homme et al Genetics 1995 - Zanni et al, PNAS 2013 - Niki el al, PNAS 2006 - Goriaux et al EMBO reports 2014...

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- Page 6 : In the result part the authors should precise in the main text what are the 4 yb-body components tested.
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- Figure EV2: OSC should be removed from the title of the figure.
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- Figure 3B : the immunostaining of N756A-Armi-F is not really clear, the authors should provide a better picture.
- The figure EV4A: why the beta-tubulin control for the WB with 2F8A9 is missing?

1st Revision - authors' response 14 October 2019

Referee #1:

This study biochemically dissects a critical segment of piRNA biogenesis pathway. This involves movement of the pre-piRNA intermediate complexes from processing bodies called Yb bodies to the mitochondrial surface where a key piRNA processing endonuclease is present. It examines the role of the RNA helicase Armitage in this movement to reveal novel insights. This will be valuable for the piRNA community.
We thank the reviewer for this positive comment.

Minor comments

It is a very odd term to use: "inward RNA". Can the authors change it to pre-piRNA 3' extension? Or something similar.
Some of the instances in the text.
Piwi-pre-piRISC through inward RNA
Piwi-pre-piRISC via RNA inward (i.e., piRNA precursor)
Armi-Piwi-pre-piRISC association through inward piRNA precursor
We changed “inward RNA” to “piRNA precursor” (pages 2, 12, 13, and 15) as suggested by the reviewer.

A cartoon summarizing the different mutations and the delta34 deletion used for Armi, and other mutants of Piwi would be useful to the reader.
A cartoon summary is provided as revised Fig 7.

Referee #2:

Major concerns:

Overall, it is not precised how many experiments were performed to draw each conclusion. In addition, it should be added larger immunostaining views with several cells on the same picture where similar phenotypes could be visualized.
All of the experiments were performed at least three times to draw each conclusion. This is now noted in the Materials and Methods section. Larger immunostaining views with several cells are provided for revised Figs 1C, 1D, 3A, 3B, EV1D (upper) and EV1E (left) as revised Figs EV1C (combined for 1C and D), EV3A (combined for 3A and B), EV1D (lower) and EV1E (right), respectively. In revised Figs 4B, 5B, 6B, EV4B (left), EV4C (left), EV5D (left), EV5E (left), and EV6D (left), we transfected OSCs with Piwi and Armi WT and mutant constructs upon RNAi treatments, and so it was very difficult to show other cells by showing larger immunostaining views. Therefore, we showed other sets of cells that show similar patterns to the original ones as revised EV4A, EV5C, EV6C, EV4B (right), EV4C (right), EV5D (right), EV5E (right), and EV6D (right), respectively. We hope that the amendments that we made satisfy his/her concerns.

The legend of the figure should not be only a conclusion of the figure but should also include the type of experiment presented and all the details necessary to fully understand the experiment and the results. All the KD (endogenous gene KD by RNAi) and rescued experiment should be precise in the main text as well as in the figure legends.

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The legends of the figures are modified in accordance with the reviewer's comments. It is noted that some of the type of experiments presented and the details appear in revised main text as well as in revised Materials and Methods section. We hope that the amendments that we made satisfy his/her concerns.

Previous studies showed that Gasz loss perturbs mitochondrial morphology, and Zuc depletion results in mitochondrial clustering which to my surprise was not discussed in the present manuscript. The authors should discuss their works.
We thank the reviewer for raising these important points. We added descriptions of changes in mitochondrial morphology in Zuc- and Gasz-depleted OSCs in the revised text (page 6 and 7, respectively). The sentences added are as follows: "*Zuc knockdown caused slightly mitochondrial clustering in OSCs (Figs 1C and EV1C), as has been reported previously* [23]*, although at present the effect of this phenomenon on piRNA biogenesis remains unclear*", and "*Previous studies showed that Gasz loss perturbs mitochondrial morphology in OSCs* [17,20]*, which was also observed but to a lesser extent in this study.*"

In a WT context, Gasz is located in the mitochondria fraction whereas Armi and Piwi are mainly found in the cyto fraction. It is really clear that in Zuc KD condition there is an enrichment of Armi in the mito fraction. In the experiment presented Fig 1C It is necessary to present (as presented in fig 1B) the cytoplasmic fraction to see whether there is in parallel a depletion of these proteins in the cytoplasmic part.
The data are now shown as revised Fig EV1B.

In addition, how the authors explain that in Zuc KD, in the total fraction, the signal for HSP60 as well as Armi is less intense than the mitochondrial fraction.
For "mito" blot, we used mitochondria isolated from five volumes of total lysates used in the "total" blot (Figs 1C and D). This was the explanation. We added this explanation in the revised Materials and Methods section (page 18).

It seems that in addition to be mislocalized to the mitochondria in Zuc KD, Armi is more expressed as seen with WB on the mitochondrial fraction and immunostaining?
The signals of HSP60 and Armi in "control" and "Zuc KD" lanes on "total" blot in Fig 1C clearly show that the abundance of Armi in the cells was hardly impacted by the loss of Zuc, although the immunostaining data "apparently" indicate higher expression of Armi upon Zuc loss. This may reflect that a large amount of Armi is concentrated into Yb bodes in control cells.
The abundance of Armi in the mitochondrial fraction became higher upon Zuc depletion. This was one of the points we would like to make in this report; namely, Armi aberrantly accumulates on the surface of mitochondria upon Zuc depletion, although the total expression level is hardly changed by the RNAi treatment.

The accumulation of Armi on the mitochondria is clear in Zuc KD but less for Piwi. In the Ev1B, in Zuc KD, Piwi seems to be localized more in the Yb body than on the mitochondria and in fig 1C the increase of Piwi in the mito fraction is not really clear. Could the author comment and adjust their statements in the text and in the title of figure EV1?
The outcome that "*Piwi seems to be localized more in the Yb body*" agreed with our previous observation that the depletion of Zuc causes the aberrant accumulation of Piwi in OSCs (Saito et al. 2010). We stated this in the revised figure legend of Fig EV1D.
We originally noted in the original text (page 7), "*The mitochondrial localization of Piwi was unclear (Fig EV1B). This might be attributable to the reduced level of Piwi due to the loss of piRNA loading (Fig 1C)*".
The title of Figs 1 and EV1 was revised, which now reads "*Loss of Zuc and Gasz impacts the cellular localization of Armi and Piwi in OSCs.*"

The authors show that Gasz interact with Armi and Piwi on the mitochondria (Fig2A) in a control condition. Several questions can then be raised: Why any coimmunostaining are detected in OSC cells between Armi and Mitochondria in WT context?
Armi interacts with Gasz in the mitochondrial fraction and so Armi can be detected, although very minutely, on mitochondria in normal OSCs.

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The figure 2A and 1A show an Armi detection which is very different, could the authors discuss this point?
Unfortunately, we do not understand this question. Fig 1A contains northern blots, in which "*Armi detection*" was not performed.

The figure 2B represent the mito fraction? How the authors explain that almost no coIP of Armi and flag-Gasz is found in the control of the figure 2B compared to the result found in figure 2A? Could the authors really conclude of an increase of Armi in a flag-Gasz complex in Zuc KD?
Gasz is a mitochondrial protein anchored onto the outer membrane via its transmembrane region. When we used "whole-cell lysate" and "mitochondrial lysate" side-by-side in our preliminarily experiments, we realized that similar, comparative data indicating the presence of Armi and Piwi in the Gasz complex were obtained. Therefore, we employed "whole-cell lysate" in Fig 2B, which is now noted in the revised figure legend of Fig 2B. We also show the Armi data in Fig 2B with a longer exposure time as revised Fig EV2A, which clearly demonstrates the aberrant accumulation of Armi and Piwi upon Zuc depletion.

Figure 2G: the author claimed that under Zuch and Piwi KD, Armi no longer stably associated with Gasz. Since the effect is very weak, could it be quantified? Could the experiment be done the other way around, a flag-Gasz IP to see whether Armi can be coIP.
The quantified data now appear in revised Figs 3C and D and also their legends. We performed the experiment recommended by the reviewer: We immunopurified Flag-Gasz and probed the materials with anti-Armi and anti-Flag antibodies. The data now appear as revised Fig 3D.

Figure 3C it is clearly showed that the PAZmut binds the precursor whereas the MIDmt not. However how the authors explain the results with the PIWI WT knowing that the experiment is performed in a Zuc KD context, why idefix piRNA are detected? A detailed legend is required to fully understand the figure.
We depleted Zuc by RNAi and so residual Zuc may have remained in cells, where a tiny amount of piRNAs could be loaded onto Piwi. This is now noted in the revised figure legend of Fig 4A (original Fig 3C).

What is the consequence of the mutation N756A on the Idefix piRNA biogenesis and on the piRNA precursor accumulation?
We already showed in the original manuscript that the Armi N756A mutant produced only a trivial amount of *idefix*-piRNAs in OSCs (original Fig EV3B). We have not checked the impact on piRNA precursors experimentally, but Armi binding to Piwi requires piRNA precursor loading onto Piwi (original Fig 3E) and the Armi N756A mutant was able to bind with Piwi (original Fig 3A). These findings mean that piRNA precursors are still produced in OSCs where the Armi mutant was expressed alternative to Armi WT.

To explain the Armi localization to Yb bodies induced by Zuc + Piwi loss the authors proposed two scenarios. Based on the experiments presented in figure EV2, why the authors support the first scenario? The authors clearly showed that Armi and Gasz interact together, so how could the author explain that in Piwi + Zuc depleted cells, Armi is no longer associated with Gasz (fig 2G)?
Our *in vitro* assays clearly showed that Gasz and Armi have the intrinsic ability to interact with each other (original Fig EV2D). However, *in vivo* they failed to bind when Piwi was depleted in Zuc-lacking OSCs (original Fig 2G). In this context, Armi was detected at Yb bodies (original Fig 2G). Based on these observations, we support the first scenario. If Gasz and Armi fail to bind with each other in *in vitro* assays, we would support the second scenario.

How could the authors explain how the 2D6E11 antibody recognize only the long isoform?
2D6E11recognized only Armi WT (*i.e.,* longer isoform) but not the DN34 mutant lacking the N-terminal 34 residues (*i.e.,* shorter isoform) in western blotting (original Fig EV4B). On the other hand, 2F8A9 recognized both Armi WT and DN34 mutant. Based on these data, we conclude that 2D6E11 recognizes only the long isoform.

How could the author explain the discrepancy between the Protein-protein interaction assays and the IP experiments between Armi delta 34 and the Gasz proteins? For the Armi delta 34 the flag is fused to the Nterm or Cterm? it is not clear.

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In revised Figs 6A, B, C, EV5A, B, E, EV6C, and D, the DN34 mutant has a Flag peptide on the C-terminal end (Armi-F). In revised Fig EV6B, the DN34 mutant has a Flag peptide on the N-terminal end (F-Armi). To clarify this point, we added new sentences in the Materials and Methods section (page 18), "*Armi-Flag means that a Flag peptide was added to the C-terminal end of Armi. Flag-Armi means that a Flag peptide was added to the N-terminal end of Armi (Figs 6D and EV6B).*"

The conclusion of this part is not clear because two experiments are going to the conclusion that the Nterminus of Armi is required for the interaction with Gasz and another shows that this domain is not required?
Our conclusion was that the N-terminal end of Armi was dispensable for the interaction with Gasz but was necessary for Armi to depart from Yb bodies (page 14).

Minor Points:
Page 3: In the introduction the beginning of the second paragraph is not well referenced. The author should add several references concerning the OSC and flamenco, such as Prud'homme et al Genetics 1995 - Zanni et al, PNAS 2013 - Niki el al, PNAS 2006 - Goriaux et al EMBO reports 2014...
We thank the reviewer for raising this important point. We now added more references in the revised Introduction section. We hope that the amendment satisfies the reviewer's concern.

Page 6: In the result part the authors should precise in the main text what are the 4 yb-body components tested.
"*Four Yb body components*" are Armi, SoYb, Yb, and Vret, which are now indicated in the revised text (page 5).

Page 7: What does it mean reasonable? I found "reasonable" a bit non-specific.
The corresponding sentence was removed in the revision process.

Figure EV2: OSC should be removed from the title of the figure.
We removed "in OSCs" from the title of Figs 2 and EV2 accordingly.

Figure 3C: Zuc KD + Piwi KD??? Is not precise in the legend.
We depleted Zuc and Piwi by RNAi and then ectopically expressed Piwi WT, Piwi PAZmt, or Piwi MIDmt, all of which were RNAi-resistant. We revised the figure legend to clarify this in the revised text.

Figure 3B: the immunostaining of N756A-Armi-F is not really clear, the authors should provide a better picture.
Better pictures of immunostaining of N756A-Armi-F were provided as revised Fig 5B.

The figure EV4A: why the beta-tubulin control for the WB with 2F8A9 is missing?
In the figure, we used the same blot for 2F8A9 and 2D6E11. The signals of 2F8A9 were stripped away and then the blot was reprobed with 2D6E11. We clarified this in the revised figure legend.

2nd Editorial Decision 13 November 2019

Thank you for the submission of your revised manuscript. We have now received the enclosed report from the referee who was asked to assess it. Referee 2 still has a few minor suggestions that I would like you to incorporate before we can proceed with the official acceptance of your manuscript.

A few other changes are also required:

- Please upload Table EV1 as either word or excel file.

- Figs EV1 + EV4 run over 2 pages. All figures must fit on a single page. We allow a maximum of 5 EV figures, and exceptionally allow 6 EV figures. However, you cannot have more than 6 EV

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figures.

I would like to suggest a few minor changes to the abstract. Do you agree with the following:

Piwi and piRNAs form the piRNA-induced silencing complex (piRISC) to repress transposons. In the current model, Armitage (Armi) brings the Piwi–piRISC precursor (pre-piRISC) to mitochondria, where Zucchini-dependent piRISC maturation occurs. Here, we show that Armi is necessary for Piwi–pre-piRISC formation at Yb bodies and that Armi triggers the exit of Piwi–pre-piRISC from Yb bodies and the translocation to mitochondria. Piwi–pre-piRISC resists leaving Yb bodies until Armi binds Piwi–pre-piRISC through the piRNA precursors. Lack of the Armi N-terminus also blocks the Piwi–pre-piRISC exit from Yb bodies. Thus, Armi determines Piwi–piRISC processing, in a multilayered manner, from precursor formation and quality control to inter-organelle translocation for maturation.

EMBO press 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 (the height is variable). You can either show a model or key data in the synopsis image. Please note 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.

REFEREE REPORT

Referee #2:

In this revised manuscript the authors have addressed most of the criticisms I previously raised and the current version is much easier to follow thanks to all the schemes that have been added. Overall, the results are very interesting. My only concerns are:

Page 6: In the sentence," Gasz was detected " it should be precised "in the mitochondrial fraction"

As precised by the authors, in my first review I made a mistake when I wrote "The figure 2A and 1A show an Armi detection which is very different....". Of course, I wanted to compare Figure 2A and 2B (The control part)? How the authors explain these differences in Armi detection? Could it just be due to the fact that in A only the mito fraction is considered and in B it is the whole-cell Lysate?

2nd Revision - authors' response 16 November 2019

Referee #2:

In this revised manuscript the authors have addressed most of the criticisms I previously raised and the current version is much easier to follow thanks to all the schemes that have been added. Overall, the results are very interesting.
We thank the reviewer for this positive comment.

My only concerns are:
Page 6: In the sentence," Gasz was detected " it should be precised "in the mitochondrial fraction"
We added "in the mitochondrial fraction" in page 7 of re-revised manuscript.

As precised by the authors, in my first review I made a mistake when I wrote "The figure 2A and 1A show an Armi detection which is very different....". Of course, I wanted to compare Figure 2A and 2B (The control part)? How the authors explain these differences in Armi detection? Could it just be due to the fact that in A only the mito fraction is considered and in B it is the whole-cell Lysate?
Yes, it is due to the difference in lysate types. The data showed us that the level of Gasz□Armi complex in mitochondrial fraction is higher than that in whole cell lysate.

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Accepted 18 November 2019

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.

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Manuscript Number: EMBOR-2019-48769

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.

USEFUL LINKS FOR COMPLETING THIS FORM

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

http://grants.nih.gov/grants/olaw/olaw.htm
http://www.mrc.ac.uk/Ourresearch/Ethicsresearchguidance/Useofanimals/index.htm
http://ClinicalTrials.gov
http://www.consort-statement.org
http://www.consort-statement.org/checklists/view/32-consort/66-title

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

http://datadryad.org

http://figshare.com

http://www.ncbi.nlm.nih.gov/gap

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

http://biomodels.net/

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

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?	Sample size was chosen based on previous studies in the field. No statistical method was used to pre-determine the sample sizes.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
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.	No.
For animal studies, include a statement about randomization even if no randomization was used.	N/A
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.	No.
4.b. For animal studies, include a statement about blinding even if no blinding was done	N/A
5. For every figure, are statistical tests justified as appropriate?	N/A
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	N/A

Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

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).	Materials and Methods section, pages 19-20.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Materials and Methods section, page 16.

* 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.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
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.	N/A

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	N/A
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).	N/A
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