EMBO reports

Intellectual disability associated gene ftsj1 is responsible for 2'-O-methylation of specific tRNAs

Jing Li, Yan-Nan Wang, Beisi Xu, Ya-Ping Liu, Mi Zhou, Tao Long, Hao Li, Han Dong, Yan Nie, Peng CHEN, En-Duo Wang, and Ru-Juan Liu
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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 4th Mar 2020

Dear Prof. Liu,

Thank you for your patience while your manuscript was peer-reviewed at EMBO reports. I am sorry for the delay in getting back to you, I was traveling until yesterday. We have now received all referee reports pasted below, as well as cross-comments.

As you will see, while referee 2 is more critical, both referees 1 and 3 find your data interesting and support the publication of your study here.

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 do address referee 2's concerns to the best of your abilities, and discuss and place your data in context in an honest manner.

Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of major revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

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.

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.

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

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

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

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

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

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

Some of your Appendix tables are very long and should be called "Dataset" instead, please see below.

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

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

5) a complete author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

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

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

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

9) Our journal also encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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

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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.

Kind regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

Li et al. reports a Ftsj1-mediated tRNA methylation showing Wdr6 and Ftsj1 can mediate 2'-O-methylation at tRNA C32 and G34 (Cm32 and Gm34). Figure 6 is an example of comprehensive measurements of Nm32 and Nm34 methylation fractions for nearly all tRNA anticodon loops. The work can be accepted after some minor revisions.

In Figure 3F, why are there three peaks of red color? Are these m7G, m1G and m2G? If so, please mark these clearly in the figure to avoid confusion.

In Figure 3G, there are two peaks with red color, and one of them was annotated to o2yW by Li et al. Please provide explanations on the other peak there, is this an isoform of o2yW or some impurity?

For all data shown in Figure 3D-3H, add the corresponding bar charts together with statistics (like in Figure 6) to show the methylation levels of Cm/A, Gm/A, m1G/A and o2yW/A for all cellular treatments (WT, ftsj1 KO1, ftsj1 KO2, ...) with normalization using standard curve by mass spec.

A nice figure of qRT-PCR or western blotting to show Ftsj1 (and Wdr6) knockout efficiency needs to be added.

In Figure 5B and 5C, the color might be inverted? Also, Figure EV5.

In Figure 3F and 3G, there seems to be a balance at G37. G37 can be modified as either m1G37 or o2yW, and the authors demonstrated the 2'-O methylation could affect this balance. Ftsj1 KO showed a dramatic decrease of both Cm32 and Gm34, according to Fig. 3D and 3E. In this Ftsj1-depleted case, the data revealed an increase of the m1G level but decreased o2yW. Some explanations would be nice.

However, Wdr6 KO shows a decrease only for Gm34, according to Fig. 3E. the effects of 2'-O methylation seem to be minor compared to above. Why?

Procedures in materials and methods need to have more details.

Referee #2:

In their manuscript Li and coworkers characterize the intellectual disability associated gene ftsj1, which is responsible for 2-o-methylation of specific tRNA. Ftsj1 is the homologue of Trm7 in yeast, which was described by Pintard et al. in 2002 showing that it methylates 2-o-ribose of nucleotides in positions 32 and 34. This work has been extended by the Phizicky team in several papers showing the role of Trm7 in yeast but also that ftsj1 in humans is required for 2-o-methylation.
Li and coworkers purify WDR6 as an interactor of FTSJ1 using a chemical warhead and characterize it in tRNA modification field. They show which human tRNA are modified by FTSJ1 and WDR6 and show that the modifications are placed in a hierarchical order. In the end they use a reporter assay to suggest that translation efficiency of UUU codons is reduced in ftsj1 knockout cells. When analyzing genes that have a high bias for UUU codons they find that 40% of those are related to brain function.
The manuscript contains a series of solid experiments demonstrating the biochemical function of FTSJ1 and WDR6, in vitro and in a knock out cell line. They show the interaction by CoIP, chemical fishing and map a region that is necessary for the interaction. By in vitro assays and different substrates they show the hierarchy of adding modifications to tRNA substrates and how the Ftsj1 and/or WDR6 bind tRNA or SAM. Towards the end the manuscript the work becomes more speculative. Using a reporter assay they suggest that TTT codons are translated less efficiency than TTC codons and try to connect this to the use of TTT and TTC in mRNA expressed in the brain.
Even though the authors claim novelty for their findings, many of these findings have been described in yeast and were predicted. For example (Crecy-Lagard et al. 2019) lists WDR6 and FTSJ1 for 2-o-methylation. The Phizicky team has shown the function in vivo. What remains new is the biochemical demonstration that the yeast findings are correct for the human homologue. But this cannot claim novelty in a broader sense. The paper is therefore not for a broad audience and

even the modification field will only see this as a confirmation of what has already been expected. I recommend publication in a more specialized journal like e.g. the RNA journal but not EMBO reports.

Some feedback to the authors should they decide to submit the work to a different journal:

A main problem is the statement on translation efficiency. The authors use a reporter assay to reach their conclusion. Depending on the expression levels of the reporter the outcome will not reflect the natural situation in cells. Also 6x codons in a row are rarely found in nature. It is conceivable that the reporter does not show what happens in cells. Recent papers have used RiboSeq to show how translation efficiency is changed by RNA modifications (some of these are cited in the manuscript). tRNA overexpression is an alternative method to demonstrate the role of specific codons.

Some of the probes used for tRNA fishing match 100% zo long noncoding RNA.

The authors cannot claim that specific nucleotides are altered because they digest the tRNA to nucleotides.

Fig 3G: the authors write: "The o2yW levels moderately decreased in WDR6 cells". I do not see this change. Do the authors state this, because they want to claim that situation in humans is similar to yeast? They do not need this link in particular since it is not supported by their data.

Referee #3:

Although 2'-O-methylation has been studied in detail in yeast, to date nobdy has been able to reconstitute the activity of the equivalent enzyme (FTSJ1) in humans. Importantly, mutations in FTSJ1 lead to disease states and as such a better understanding of the workings of this enzyme is truly needed. In the present manuscript, Li et al. show for the firs time the in vitro reconstitution of the activity and discovered the importance of WDR6 as a partner. Interestingly, although FTSJ1 can methylate positions 32 and 34 of a number of tRNAs, WDR6 is only required for position 34, for example in tRNAPhe. Overall this is nice article and provides an extensive and detailed study on FTSJ1 and WDR6, which carefully shows their substrate specificity. I only have two comments:

1. The growth curves in figure 7 are lacking error bars. This needs to be done.
2. Throughout the paper the authors mention "biotin labeled DNA". Well the DNA is not really labeled. It should re-written as "biotinylated DNA" instead.

Otherwise, an excellent piece of work.

Dr. Esther Schnapp May 4th, 2020

Senior Editor of

EMBO Reports

Re: EMBOR-2020-50095-T (Intellectual disability associated gene *ftsj1* is responsible for 2'-O-methylation of specific tRNAs)

Dear Dr. Schnapp,

Thank you for your E. mail and for the constructive comments from the reviewers. We have revised the manuscript according to all the points raised by the reviewers. Changes have been marked in red in order to facilitate manuscript reviewing. Please read the response to the comments of reviewers following this letter.

We hope that we answered all questions and requirements from the reviewers and that this revised version of our paper is now suitable for publication in *EMBO Reports*.

Yours sincerely,

Ru-Juan Liu
Principle investigator
Lab of tRNA modifications
School of Life Science and Technology (SLST)
ShanghaiTech University

En-Duo Wang
Professor of Biochemistry and Molecular Biology
Institute of Biochemistry and Cell Biology
Shanghai Institutes for Biological Sciences
The Chinese Academy of Sciences

Response to Referee 1:

Li et al. reports a Ftsj1-mediated tRNA methylation showing Wdr6 and Ftsj1 can mediate 2'-O-methylation at tRNA C32 and G34 (Cm32 and Gm34). Figure 6 is an example of comprehensive measurements of Nm32 and Nm34 methylation fractions for nearly all tRNA anticodon loops. The work can be accepted after some minor revisions.

Response: Thanks for the nice comments.

In Figure 3F, why are three peaks of red color? Are these m7G, m1G and m2G? If so, please mark these clearly in the figure to avoid confusion.

Response: Thanks for your suggestions. The three peaks of red color from left to

right in Figure 3F were m^7G, m^1G and m^2G, respectively, and we have marked them in the current version.

In Figure 3G, there are two peaks with red color, and one of them was annotated to o2yW by Li et al. Please provide explanations on the other peak there, is this an isoform of o2yW or some impurity?

Response: Thanks for the instructive comments. The formation of eukaryotic o2yW is complicated, and many intermediate products will be formed. However, the complete formation process of o2yW from m^1G37 remains unclear. When the MS system monitored the Q1/Q3=541.2/409.0 of nucleosides which were digested from $tRNA^{Phe}$(GAA) that was isolated from cells, we observed two peaks of this ion. We marked the o2yW peak in Figure 3G according to the retention time of standard product. Considering the change of the other peak was similar to that of o2yW, we speculated this peak was generated by an intermediate product of o2yW with natural isotope labelled. We added this explanation in Figure 3 legends (**Line 37-39 on Page 24**).

For all data shown in Figure 3D-3H, add the corresponding bar charts together with statistics (like in Figure 6) to show the methylation levels of Cm/A, Gm/A, m1G/A and o2yW/A for all cellular treatments (WT, ftsj1 KO1, ftsj1 KO2, ...) with normalization using standard curve by mass spec.

Response: Thanks. Figure 3D-3H are the representative images of Cm, Gm, m^1G, o2yW and m^5C levels of $tRNA^{Phe}$(GAA) isolated from WT, *ftsj1* KO and *wdr6* KO cells. The corresponding bar charts together with statistics were shown in Figure EV3. We added this explanation in **Line 16-19 on Page 5**.

A nice figure of qRT-PCR or western blotting to show Ftsj1 (and Wdr6) knockout efficiency needs to be added.

Response: Thanks. We have added the western blotting to show *ftsj1* knockout efficiency and qRT-PCR to show *wdr6* knockout efficiency in Appendix Figure S2, and the description in **Line 12 on Page 5**.

In Figure 5B and 5C, the color might be inverted? Also, Figure EV5.

Response: Thanks. We have inverted the color in Figure 5B, 5C and EV5.

In Figure 3F and 3G, there seems to be a balance at G37. G37 can be modified as either m1G37 or o2yW, and the authors demonstrated the 2'-O methylation could affect this balance. Ftsj1 KO showed a dramatic decrease of both Cm32 and Gm34, according to Fig. 3D and 3E. In this Ftsj1-depleted case, the data revealed an increase of the m1G level but decreased o2yW. Some explanations would be nice.

Response: Thanks for the constructive comments. These results suggested that there is a balance at G37, which can be modified to m^1G or further modified to o2yW. In WT cells, m^1G37 is undetectable, suggesting that all the m^1G37 is hyper modified to

o2yW37; in *ftsj1* KO cells, the formation of o2yW37 is hindered, and G37 is mainly modified to m^1G37. We have added this discussion in **Line 32-35 on Page 5**.

However, Wdr6 KO shows a decrease only for Gm34, according to Fig. 3E. the effects of 2'-O methylation seem to be minor compared to above. Why?
Response: Thanks. FTSJ1 is responsible for Nm formation on different tRNA substrates at positions 32 and 34. However, as the MTase catalytic core, FTSJ1 needs auxiliary protein to recognize tRNA substrates. In the current study, we have identified that FTSJ1 interacts with WDR6 to 2'-O-methylate position 34. So, *wdr6* KO will not affect the level of 2'-O methylation at position 32. The protein that helps FTSJ1 targeting to position 32 of tRNA still needs further investigation and verification.

Procedures in materials and methods need to have more details.
Response: Thanks. We have added more details and marked as red in Materials and Methods section from **Page 13 to 17**.

Response to Referee 2:

In their manuscript Li and coworkers characterize the intellectual disability associated gene ftsj1, which is responsible for 2-o-methylation of specific tRNA. Ftsj1 is the homologue of Trm7 in yeast, which was described by Pintard et al. in 2002 showing that it methylates 2-o-ribose of nucleotides in positions 32 and 34. This work has been extended by the Phizicky team in several papers showing the role of Trm7 in yeast but also that ftsj1 in humans is required for 2-o-methylation.
Response: Thanks. Pintard et al showed that yeast Trm7 could independently catalyze 2'-O-methylation at positions 32 and 34 of $tRNA^{Phe}$ *in vitro*. Phizicky team demonstrated that yeast Trm7 requires Trm732 for Cm32 formation and Trm734 for Nm34 formation *in vivo*, respectively (Guy et al, 2012). $tRNA^{Phe}$ from *ftsj1* mutations or knockout cells lacks Cm32 and Gm34 (Guy et al, 2015), suggesting that FTSJ1 is a putative tRNA 32 and 34 2'-O-methyltransfearse. However, unlike Trm7, standalone FTSJ1 could not perform the 2'-O-methylation on tRNA substrates. Moreover, the reconstitution of the enzymatic activity of FTSJ1 *in vitro* has no success for years (personal communication), which hinders the study of the working mechanism and pathogenic mechanism of FTSJ1.

Li and coworkers purify WDR6 as an interactor of FTSJ1 using a chemical warhead and characterize it in tRNA modification field. They show which human tRNA are modified by FTSJ1 and WDR6 and show that the modifications are placed in a hierarchical order. In the end they use a reporter assay to suggest that translation efficiency of UUU codons is reduced in ftsj1 knockout cells. When analyzing genes that have a high bias for UUU codons they find that 40% of those are related to brain function.

The manuscript contains a series of solid experiments demonstrating the biochemical function of FTSJ1 and WDR6, in vitro and in a knock out cell line. They show the interaction by CoIP, chemical fishing and map a region that is necessary for the interaction. By in vitro assays and different substrates they show the hierarchy of adding modifications to tRNA substrates and how the Ftsj1 and/or WDR6 bind tRNA or SAM. Towards the end the manuscript the work becomes more speculative. Using a reporter assay they suggest that TTT codons are translated less efficiency than TTC codons and try to connect this to the use of TTT and TTC in mRNA expressed in the brain.

Response: Thanks for the nice comments.

Even though the authors claim novelty for their findings, many of these findings have been described in yeast and were predicted. For example (Crecy-Lagard et al. 2019) lists WDR6 and FTSJ1 for 2-o-methylation. The Phizicky team has shown the function in vivo. What remains new is the biochemical demonstration that the yeast findings are correct for the human homologue. But this cannot claim novelty in a broader sense. The paper is therefore not for a broad audience and even the modification field will only see this as a confirmation of what has already been expected. I recommend publication in a more specialized journal like e.g. the RNA journal but not EMBO reports.

Some feedback to the authors should they decide to submit the work to a different journal:

Response: Thanks. The review paper by Crecy-Lagard et al suggested FTSJ1 and WDR6 for 2'-O-methylation based on the former studies by Phizicky team and the sequence similarity between FTSJ1/WDR6 and Trm7/Trm734. In Phizicky's work, *ftsj1* could complement the growth defect of *S. cerevisiae* △Trm7, however, they also showed that co-expression of *wdr6* and *ftsj1* could not complement the growth defect of *S. cerevisiae* △Trm734△Trm7 (Guy & Phizicky, 2015), raising the question that whether WDR6 is the human functional equivalent of Trm734. Particularly, WDR6 and Trm734 only shares 20% identity and 37% similarity in primary sequence.

During the submission of our work, two Trm7 homologues were identified in *Drosophila*. One is responsible for Nm34 modification, and the other one is in charge of Nm32 modification (Nucleic Acids Res., 2020, 48(4): 2050-2072). These former findings suggest that the formation of tRNA 2'-O-methylation at positions 32 and 34 is complicated and distinct in different species. Therefore, we cannot simply draw conclusions from the results of yeast Trm7 to human FTSJ1.

We have been inspired by the nice works from Phizicky team and others, and have cited their papers in our manuscript. In the current study, we demonstrated that FTSJ1 directly binds to WDR6 and successfully reconstituted the 2'-O-methylation activity of FTSJ1-WDR6 complex *in vitro*. We also showed that this methylation by FTSJ1-WDR6 at position 34 requires m^1G37 as a prerequisite. Importantly, mutations in *ftsj1* lead to disease states, so a better understanding of the workings of this enzyme is truly needed. Our work, especially the enzymatic assay system for FTSJ1, would

largely benefit future study on the pathogenic mechanism of *ftsj1* mutations.

A main problem is the statement on translation efficiency. The authors use a reporter assay to reach their conclusion. Depending on the expression levels of the reporter the outcome will not reflect the natural situation in cells. Also 6x codons in a row are rarely found in nature. It is conceivable that the reporter does not show what happens in cells.
Recent papers have used RiboSeq to show how translation efficiency is changed by RNA modifications (some of these are cited in the manuscript). tRNA overexpression is an alternative method to demonstrate the role of specific codons.
Response: Thanks for the constructive comments. We agree with the reviewer that the 6×codons in a row are rarely found in nature, even though this method has been widely used for checking the translation efficiency of specific codons, such as in Cell, 2016, 167(3):816-828. To mimic the situation in nature, instead of using 6×codons, we constructed the *F-luc* gene with all the Phe codons using either TTT or TTC. It is noteworthy that the changes of the translation efficiency of *F-luc* with all TTT(Phe) or TTC(Phe) are consistent with that of 6×codons system (Figure 7J, K). We have added these results in **Line 10-12 and 17-19 on Page 9**.

We thank the reviewer's nice suggestion about using Riboseq or tRNA overexpression to investigate the role of specific codons. To investigate the role of $tRNA^{Phe}$(GAA) for FTSJ1, the WT and *ftsj1* KO cells were transfected with mature $tRNA^{Phe}$(GAA). Intriguingly, $tRNA^{Phe}$ (GAA) overexpression had no effect on the growth of WT HEK293T cells under normal culture condition (Fig 7C) or in the presence of paromomycin (Fig 7D); while $tRNA^{Phe}$(GAA) overexpression could significantly promote the growth of *ftsj1* KO cells under both conditions (Fig 7E, F). These results indicated that $tRNA^{Phe}$(GAA) serves as the main functional executor of FTSJ1. We have added these results in **Line 38-40 on Page 8 and Line 1-3 on Page 9**.

Some of the probes used for tRNA fishing match 100% zo long noncoding RNA.
Response: Thanks for the suggestions. The purity of fished tRNAs by biotinylated DNA probes were all detected by denatured electrophoresis (Figure EV5), and most of them show only one tRNA band. To avoid potential contamination from other RNAs, only those tRNAs with high purity were further subjected to UPLC-MS/MS analysis.

The authors cannot claim that specific nucleotides are altered because they digest the tRNA to nucleotides.
Response: Thanks for the instructive comments. We agree with the reviewer's point. We have re-written the description in **Line 8, 24-27 on Page 5**, and in **Line 20-22 on Page 8**.

Fig 3G: the authors write: "The o2yW levels moderately decreased in WDR6 cells". I

do not see this change. Do the authors state this, because they want to claim that situation in humans is similar to yeast? They do not need this link in particular since it is not supported by their data.
Response: Thanks. It is true that the o2yW level only decreased a little bit in *wdr6* KO2 cells, and showed no significant difference in *wdr6* KO1 cells compared with that of WT cells (Figure EV3). So, the hindered formation of o2yW in *ftsj1* KO cells may result from lacking both Cm32 and Gm34. The separate influence of Cm32 or Gm34 on the formation of o2yW still needs to be further explored. We removed the sentence "The o2yW levels moderately decreased in *wdr6* KO cells" in the current version.

Response to Referee 3:

Although 2'-O-methylation has been studied in detail in yeast, to date nobody has been able to reconstitute the activity of the equivalent enzyme (FTSJ1) in humans. Importantly, mutations in FTSJ1 lead to disease states and as such a better understanding of the workings of this enzyme is truly needed. In the present manuscript, Li et al. show for the first time the in vitro reconstitution of the activity and discovered the importance of WDR6 as a partner. Interestingly, although FTSJ1 can methylate positions 32 and 34 of a number of tRNAs, WDR6 is only required for position 34, for example in tRNAPhe. Overall this is nice article and provides an extensive and detailed study on FTSJ1 and WDR6, which carefully shows their substrate specificity. I only have two comments:

1. The growth curves in figure 7 are lacking error bars. This needs to be done.
Response: Thanks for the instructive comments. We have added the error bars in Figure 7.

2. Throughout the paper the authors mention "biotin labeled DNA". Well the DNA is not really labeled. It should re-written as "biotinylated DNA" instead.
Response: Thanks for the suggestions. We have re-written as "biotinylated DNA" in the present version.

Otherwise, an excellent piece of work.
Response: We thank the reviewer for the nice comments.

1st Revision - Editorial Decision 20th May 2020

Dear Prof. Liu

Thank you for the submission of your revised manuscript. I asked referee 1 to assess your reply to all referees, and I am happy to say that s/he supports the publication of your study now. We can therefore in principle accept your manuscript.

Only a few more minor changes will be required:

- please add up to 5 keywords with your manuscript

- in the author checklist, please answer the questions 1-4 in section B statistics

- please upload the EV figures as individual files

- please add a legend/title to the first tab of the excel file of Dataset EV1

- please upload the source data as one file per figure

- please increase the scale bar visibility in Fig 1A

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

I would like to suggest a few changes to the abstract that needs to be written in present tense. Please let me know if you agree with the following:

tRNA modifications at the anticodon loop are critical for accurate decoding. FTSJ1 was hypothesized to be a human tRNA 2'-O-methyltransferase. tRNAPhe(GAA) from intellectual disability patients with mutations in ftsj1 lacks 2'-O-methylation at C32 and G34 (Cm32 and Gm34). However, the catalytic activity, RNA substrates, and pathogenic mechanism of FTSJ1 remain unknown, owing, in part, to the difficulty in reconstituting enzymatic activity in vitro. Here, we identify an interacting protein of FTSJ1, WDR6. For the first time, we reconstitute the 2'-O-methylation activity of the FTSJ1-WDR6 complex in vitro, which occurs at position 34 of specific tRNAs with m1G37 as a prerequisite. We find that modifications at positions 32, 34, and 37 are interdependent and occur in a hierarchical order in vivo. We also show that the translation efficiency of the UUU codon, but not the UUC codon decoded by tRNAPhe(GAA), is reduced in ftsj1 knockout cells. Bioinformatics analysis reveal that almost 40% of the high TTT-biased genes are related to brain/nervous functions. Our data potentially enhance our understanding of the relationship between FTSJ1 and nervous system development.

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.

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have addressed my comments.

Dr. Esther Schnapp May 24th, 2020

Senior Editor of

EMBO Reports

Re: EMBOR-2020-50095V3 (Intellectual disability associated gene *ftsj1* is responsible for 2'-O-methylation of specific tRNAs)

Dear Dr. Schnapp,

Thanks for your decision, and we are very glad to publish our paper on *EMBO Reports*. We have revised the manuscript according to your last E. mail.

In the manuscript, we have added five keywords (On page 1) and addressed all comments raised by the data editors. We agreed with the abstract in present tense, and have revised in the current manuscript. We uploaded a synopsis together with the revised manuscript in the author system.

We have answered the questions 1-4 in section B statistics of author checklist; and uploaded the EV figures as individual files; we also added a title to the tab of the excel file of Dataset EV1; and uploaded the source data as one file per figure; and increased the scale bar visibility in Fig 1A.

We hope that this revised version of our paper is now suitable for publication in *EMBO Reports*.

Yours sincerely,

Ru-Juan Liu
Principle investigator
Lab of tRNA modifications
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2nd Revision - Editorial Decision 27th May 2020

Prof. Ru-Juan Liu
ShanghaiTech University
China

Dear Prof. Liu,

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

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

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, 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.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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."

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

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

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

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THINGS TO DO NOW:

You will receive proofs by e-mail approximately 2-3 weeks after all relevant files have been sent to our Production Office; you should return your corrections within 2 days of receiving the proofs.

Please inform us if there is likely to be any difficulty in reaching you at the above address at that

time. Failure to meet our deadlines may result in a delay of publication, or publication without your corrections.

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Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: En-Duo Wang and Ru-Juan Liu
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2020-50095-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 iustified
- ➔ 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 initially performed the pilot experiments to determine the sample size.
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?	For the nucleosides analysis by HPLC, peak with signal-to-noise ratio no <10 was considered as a detectable nucleoside.
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.	In cell proliferation experiments, equivalent WT or FTSJ1 KO cells calculated by cell counter were seeded into each well. In luciferase assay, we employed the pmirGlo mutated reporter normalized to the pmirGlo empty vector.
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.	In cellular experiments,control and experimental groups were cultured under the same conditions, and we checked daily the status of cells. For in vitro enzymatic assays and HPLC-MS performace, the samples were all done blinded.
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?	Yes, for every figure, statistical tests were justified.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	We used Graphpad Prism software to assess the normal distribution.
Is there an estimate of variation within each group of data?	Yes

USEFUL LINKS FOR COMPLETING THIS FORM

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

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-tumo

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/

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).	Yes, all the antibodies were profiled and have a reference to an antibody validation profile.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	HEK293T was purchased from ATCC and tested for mycoplasma contamination.

* 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