Manuscript EMBOR-2010-34567

# A member of the ETS family, EHF, and the ATPase RUVBL1 inhibit p53-mediated apoptosis

Kenzui Taniue, Takeaki Oda, Tomoatsu Hayashi, Masumi Okuno, Tetsu Akiyama

*Corresponding author: Tetsu Akiyama, The University of Tokyo, Inst. for Mol. and Cellular Biosciences*

**Review timeline:**

| | |
|---|---|
| Submission date: | 16 November 2010 |
| Editorial Decision: | 07 December 2010 |
| Revision received: | 09 March 2011 |
| Editorial Decision: | 28 March 2011 |
| Revision received: | 31 March 2011 |
| Editorial Decision: | 01 April 2011 |
| Accepted: | 01 April 2011 |

## 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 07 December 2010

Thank you for the submission of your research manuscript to our editorial office. We have now received the evaluation of three expert reviewers on your study.

As the reports are pasted below I would prefer not to repeat them here in detail, but to only summarize the main points raised by the referees. You will see that, while all referees agree on the potential interest of the findings, they also feel that in some instances additional work is needed.

All three referees feel that it should be shown that endogenous EHF activates RUVBL1 expression. Referee 1 remarks that the data on the effects of RUVBL1 knockdown on H2B monoubiquitination of p53, its target genes and control genes should be strengthened and referees 2 and 3 also make comments along those lines. Both referees 1 and 2 state that the changes in the expression of p53 and its target genes should be shown at the protein level. Finally, both referees 2 and 3 recommend performing the key experiments in the same cell lines, preferably in colon cancer cells.

Overall, given these evaluations, the reviewers constructive comments and the potential interest of the study, I would like to give you the opportunity to revise your manuscript, with the understanding that the main concerns of the referees (as outlined above and in their reports) must be addressed. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

 

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

I look forward to seeing a revised form of your manuscript when it is ready.

Yours sincerely

Editor
EMBO Reports

REFEREE REPORTS

Referee #1 (Remarks to the Author):

To the authors;
In this manuscript Taniue et al. demonstrate that a member of the ETS family, EHF, and the ATPase RUVBL1 inhibit p53-mediated apoptosis. This is a very interesting set of novel findings that add significantly to our understanding of the regulation of p53 function. However some of the data are rather preliminary and additional experiments are required to substantiate some of the author's claims.

Major concerns:
1) The direct effect of EHF on the RUVBL1 promoter is demonstrated only in settings in which EHF is overexpressed. Demonstration of binding of endogenous EHF to the RUVBL1 promoter (by ChIP analysis using untransfected cells) will strengthen the paper.
2) The increase in UbH2B on the p53 gene is not very convincing: A) The increase in UbH2B on the p53 gene upon knock down of RUVBL1 is not substantial. This should be discussed and explained by the authors. B) Why don't they see an increase in UbH2B on the PUMA and BAX genes after knock down of RUVBL1 while expression of these two genes is increased by this knock down of RUVBL1? C) The authors do not explain why RUVBL1 is detected bound to the GAPDH-TSS (Fig. 4C) while knock down of RUVBL1 does not lead to increased detection of UbH2B on the GAPDH gene (Fig. 4B).
3) The increase in levels of p53, Puma and Bax after knock down of RUVBL1 are presented only at the RNA level (Fig 3D). The authors should demonstrate also increase at the protein level.
4) The title suggests that the functional link that the authors have identified between EHF, RUVBL1 and p53 functions specifically in colon cancer cells. Since this is based on a couple of cell lines and, in addition, the data does not prove that this link is specific to colon cancer cells I think the title is a bit misleading and should be modified.

A more minor comment

1) On page 8 the authors state "a significant amount of RNF 20 was associated with the TSS region of p53...". However, the data presented in figure 4D shows a signal that is about 0.025% of the input. It is difficult to consider this very weak binding as an association of "a significant amount of RNF 20"

Referee #2 (Remarks to the Author):

In this manuscript, Taniue et al provide data to suggest that one of ETS family transcription factor, EHF, is required for the survival of p53-competent colon cancer cell lines by inducing the expression of Ruvbl1 ATPase, which in turn suppresses p53 expression by inhibiting RNF20-depenent ubiquitination of H2B. Although knockdown experiments have demonstrated that colon cancer cells are dependent on EHF and its immediate downstream target Ruvbl1 for suppression of p53-dependent apoptosis, data presented to support underlying mechanism of p53 repression by Ruvbl1 via RNF20 inhibition are not compelling. Among the different experiments that can be considered, authors should demonstrate that other components Paf1 complex are also recruited to p53 promoter.

Additional points
1. In lower right panel (DLD1 cell) of Fig 1, it is recommended to include experiments of other ETS factor knockdown such as ERF to serve as a positive control.
2. Authors are mentioning only Ruvbl1 among EHF target genes, which are selected from the microarray and promoter sequence analysis. It is desirable to describe the outcome of the analysis, such as the number of potential candidates.
3. In Fig 2, authors tried to confirm EHS-dependent Ruvbl1 expression by performing EHF overexpression experiments in HeLa cells. Are HeLa cells expressing EHF in low levels? What would be the result like if colon cancer cell lines were used?
4. In Fig 2D, ChIP experiment should be done to analyze the recruitment of the endogenous EHF to Ruvbl1 promoter in HCT116 cells instead of that of overexpressed EHF in HeLa cells.
5. In this report, the gene expression levels were checked only at the level of mRNA. It is required to confirm changes also at the protein levels, especially of Ruvbl1 and p53.
6. In Fig 3C, authors tried to rescue the effects of EHF knock down on apoptosis by exogenous Ruvbl1 overexpression. In this experiment, Ruvbl1 was forced to localize in nucleus by cloning the NLS in the Ruvbl1 expression vector. Is this Ruvbl1-NLS expression well reflecting the localization of endogenous Ruvbl1? Cellular distribution of endogenous Ruvbl1 should be shown.
7. In Fig 4, authors tried to figure out the underlying mechanism of Ruvbl1-dependent p53 repression by looking at the recruitment of Ruvbl1 and its possible inhibition target, RNF20 ubiquitin ligase. In Fig 4C, Ruvbl1 is well demonstrated to locate in TSS and TR of p53 gene and TSS of GAPDH. Upon Ruvbl1 depletion, RNF20 recruitments increased significantly only in TSS of p53 (Fig 4D). However, when looking at Ub-H2B, the immediate outcome of RNF20 action, the levels did not increase in this region (Figure 4B). Why?
8. Ruvbl1-dependent p53 repression (Fig 3D) and ChIP experiments (Fig 4) were done in RKO cells, though most of apoptosis analysis was performed in HCT116. It would be important to demonstrate that different aspects of the study are seen in the same cell line.
9. In terms of transcriptional repression, Ruvbl1/pontin has been documented as a transcriptional repressor of Myc, beta-catenin and NF-kB targets. It would make this study more complete if authors could look at the possibility that Ruvbl1-dependent p53 repression is mediated through interactions with these transcription factors.

Referee #3 (Remarks to the Author):

This interesting paper from the Akiyama lab defines a novel pathway through which colon tumor cells are able to evade p53 mediated apoptosis. The focus of the paper is on an Ets family protein known as EHF. They initially found enrichment of Ets binding sites in genes upregulated in colon cancer and then they systematically knocked down Ets family proteins in colon cancer cells finding that EHF loss appeared to specifically result in apoptosis of those lines with active p53. The next identified RUVBL1 as a potential target for EHF and showed that RUVBL1 expression is modulated by EHF through direct binding to EHF to specific consensus sites in the RUVBL1 promoter. They go on to demonstrate that RUVBL1 represses p53 expression, is associated with the p53 promoter, and that knockdown of RUVBL1 induces apoptosis predominantly in cells containing wild type p53. Moreover, ectopic expression of RUVBL1 partially suppresses apoptosis induced by EHF knockdown. Lastly, based on previous work of others showing that RUVBL1 controls histone H2B ubiquitylation, they show that in RUVBL1 knockdown cells, ubH2B levels in the transcribed

 

region of p53, but not other genes, increase most likely through increased recruitment of the RNF20 ligase.

Overall I found this paper to be of significant interest. It identifies and links together several new players in apoptotic pathways relevant to tumorigenesis. The experiments are well controlled and the results are generally compelling. The use of siRNAs to downregulate components of the pathway is particularly important in substantiating that EHF and RUVBL1 function at endogenous levels. While in principle it would be of interest to determine by microarray analysis what other genes are regulated by RUVBL1 the data provided here are sufficiently complete and worthwhile that I would recommend publication at this time without that additional data.

Some specific comments:

1. Fig. 1 - was the siERF tested in the DLD cells? If so the authors should mention whether it had an effect.
2. Why was the EHF ChIP on the RUVBL1 promoter carried out in HeLa cells rather than in colon cancer cells. I assume that anti-EHF is not available (is this true?)- otherwise it would have strengthened the results to have shown binding by endogenous EHF.
3. Fig. 3C - although the effect of siEHF could be overcome by increases RUVBL1 the effect was only partial. However only one RUVBL1 concentration was apparently examined. The authors should consider titrating the levels of RUVBL1.
4. Fig. 4 - was RUVBL1 binding tested in the Bax and Puma promoters? If RUVBL1 interacts with GAPDH promoter (4C) why aren't ubH2A levels affected in the siRNA knockdown (4B)?
5. The model shown in Supp. Fig. 4 should be included in the main text as it provides a good summary of the conclusions.
6. Minor point: on pg. 8 the authors write: "To prove this hypothesis...". I would recommend that they change the wording to " To test (or examine) this hypothesis...."

1st Revision - authors' response 09 March 2011

I am sending herewith our revised manuscript, "A member of the ETS family, EHF, and the ATPase RUVBL1 inhibit p53-mediated apoptosis". We have revised the text along the lines you indicated.

We believe that this study is of broad interest to a large readership of EMBO Reports. I would like to thank you and the referees for your helpful comments and revision. I hope that the revised manuscript is acceptable for publication in EMBO Reports.

Our replies to the refereeís comments are as follows:
Referee #1:

>Major concerns:
>1) The direct effect of EHF on the RUVBL1 promoter is demonstrated only in settings in which EHF is overexpressed. Demonstration of binding of endogenous EHF to the RUVBL1 promoter (by ChIP analysis using untransfected cells) will strengthen the paper.

We have performed a ChIP assay using anti-EHF antibody and confirmed that endogenous EHF binds directly to the TSS region of RUVBL1. The results are shown in Fig 2E and mentioned in page 6, lines 7-9 of the revised manuscript.

>2) The increase in UbH2B on the p53 gene is not very convincing:
>A) The increase in UbH2B on the p53 gene upon knock down of RUVBL1 is not substantial. This should be discussed and explained by the authors.

We have repeated the ChIP assay using anti-UbH2B antibody and obtained similar results. In addition, at the suggestion of reviewer 2, we have performed ChIP assays using anti-PAF antibody. We have found that knockdown of RUVBL1 leads to a significant decrease in PAF1 and H3K9

 

trimethylation associated with the p53 gene. We have also shown that knockdown of either PAF1 or CDC73, a component of the PAF1 complex, results in the upregulation of p53 expression. These results are consistent with a previous report showing that the PAF1 complex induces H3K9 trimethylation and thereby represses cyclin D1 transcription (Yang et al. Nuc. Acids Res., 38, 382-390, 2010). Thus, we speculate that the RUVBL1 represses p53 transcription by interfering with RNF20-mediated histone H2B monoubiquitination and promoting PAF1-mediated H3K9 trimethylation. These results are presented in Fig 4F-H and explained in page 8, line27 and page 9, line 1-12 of the revised manuscript.

>B) Why don't they see an increase in UbH2B on the PUMA and BAX genes after knock down of RUVBL1 while expression of these two genes is increased by this knock down of RUVBL1?

We have performed ChIP assays using anti-RUVBL1 antibody and have found that RUVBL1 is associated with the promoter regions of PUMA and BAX. However, ChIP assays with anti-RNF20 and anti-ubH2B antibodies have shown that RUVBL1 knockdown does not lead to increased detection of RNF 20 and ubH2B associated with the PUMA and BAX genes. Thus, RUVBL1-mediated inhibition of histone H2B monoubiquitination does not play a role in the repression of PUMA and BAX expression. The results are presented in Fig 4A-D and mentioned in page 8, lines 7-13 and 17-19 of the revised manuscript.

>C) The authors do not explain why RUVBL1 is detected bound to the GAPDH-TSS (Fig. 4C) while knock down of RUVBL1 does not lead to increased detection of UbH2B on the GAPDH gene (Fig. 4B).

ChIP assays with anti-RNF20 antibody have shown that knockdown of RUVBL1 does not induce the association of RNF 20 to the TSS region of GAPDH. Thus, UbH2B does not occur on GAPDH. The results are shown in Fig 4D and mentioned in page 8, lines 17-19 of the revised manusecipt.

>3) The increase in levels of p53, Puma and Bax after knock down of RUVBL1 are presented only at the RNA level (Fig 3D). The authors should demonstrate also increase at the protein level.

We have performed immunoblotting analysis and the results are presented in Fig 3E and mentioned in page 7, lines 17-19 of the revised manuscript

>4) The title suggests that the functional link that the authors have identified between EHF, RUVBL1 and p53 functions specifically in colon cancer cells. Since this is based on a couple of cell lines and, in addition, the data does not prove that this link is specific to colon cancer cells I think the title is a bit misleading and should be modified.

At the suggestion of the reviewer, we have modified the title.

A more minor comment

>1) On page 8 the authors state "a significant amount of RNF 20 was associated with the TSS region of p53...". However, the data presented in figure 4D shows a signal that is about 0.025% of the input. It is difficult to consider this very weak binding as an association of "a significant amount of RNF 20"

We have deleted “a significant amount of “.

Referee #2 (Remarks to the Author):

>In this manuscript, Taniue et al provide data to suggest that one of ETS family transcription factor, EHF, is required for the survival of p53-competent colon cancer cell lines by inducing the expression of Ruvbl1 ATPase, which in turn suppresses p53 expression by inhibiting RNF20-depenent ubiquitination of H2B. Although knockdown experiments have demonstrated that colon cancer cells are dependent on EHF and its immediate downstream target Ruvbl1 for suppression of p53-dependent apoptosis, data presented to support underlying mechanism of p53 repression by Ruvbl1 via RNF20 inhibition are not compelling. Among the different experiments that can be considered, authors should demonstrate that other components Paf1 complex are also recruited to

 

p53 promoter.

At the suggestion of the reviewer, we have performed ChIP assays using anti-PAF antibody. We have found that knockdown of RUVBL1 leads to a significant decrease in PAF1 and H3K9 trimethylation associated with the p53 gene. We have also shown that knockdown of either PAF1 or CDC73, a component of the PAF1 complex, results in the upregulation of p53 expression. These results are consistent with a previous report showing that the PAF1 complex induces H3K9 trimethylation and thereby represses cyclin D1 transcription (Yang et al. Nuc. Acids Res., 38, 382-390, 2010). Thus, we speculate that the RUVBL1 represses p53 transcription by interfering with RNF20-mediated histone H2B monoubiquitination and promoting PAF1-mediated H3K9 trimethylation. These results are presented in Fig 4F-H and explained in page 8, line27 and page 9, line 1-12 of the revised manuscript.

Additional points
>1. In lower right panel (DLD1 cell) of Fig 1, it is recommended to include experiments of other ETS factor knockdown such as ERF to serve as a positive control.

We have performed knockdown experiments with DLD-1 cells using the same set of ETS family genes that were used for HCT116(-/-) cells. The results are presented in Fig 1 (Lower right) of the revised manuscript.

>2. Authors are mentioning only Ruvbl1 among EHF target genes, which are selected from the microarray and promoter sequence analysis. It is desirable to describe the outcome of the analysis, such as the number of potential candidates.

We have presented the results of the analysis in Table S1 and described the number of potential candidate genes in page 5, lines 4-5 of the revised manuscript.

>3. In Fig 2, authors tried to confirm EHS-dependent Ruvbl1 expression by performing EHF overexpression experiments in HeLa cells. Are HeLa cells expressing EHF in low levels? What would be the result like if colon cancer cell lines were used?

The level of EHF expression in HeLa cells is low. This is mentioned in page 5, lines 16 of the revised manuscript. Overexpression of EHF resulted in a 1.5 fold increase in RUVBL1 mRNA expression in HCT116 cells.

>4. In Fig 2D, ChIP experiment should be done to analyze the recruitment of the endogenous EHF to Ruvbl1 promoter in HCT116 cells instead of that of overexpressed EHF in HeLa cells.

We have performed a ChIP assay using anti-EHF antibody and confirmed that endogenous EHF binds directly to the TSS region of RUVBL1. The results are shown in Fig 2E and mentioned in page 6, lines 7-9 of the revised manuscript.

>5. In this report, the gene expression levels were checked only at the level of mRNA. It is required to confirm changes also at the protein levels, especially of Ruvbl1 and p53.

We have performed immunoblotting analyses and the results are presented in Fig 2B, 2C and 3E and mentioned in page 7, lines 17-19 of the revised manuscript

>6. In Fig 3C, authors tried to rescue the effects of EHF knock down on apoptosis by exogenous Ruvbl1 overexpression. In this experiment, Ruvbl1 was forced to localize in nucleus by cloning the NLS in the Ruvbl1 expression vector. Is this Ruvbl1-NLS expression well reflecting the localization of endogenous Ruvbl1? Cellular distribution of endogenous Ruvbl1 should be shown.

Endogenous RUVBL1 is localized in the nucleus. This is shown in Fig S2 and mentioned in page 7, line 5-8 of the revised manuscript.

>7. In Fig 4, authors tried to figure out the underlying mechanism of Ruvbl1-dependent p53 repression by looking at the recruitment of Ruvbl1 and its possible inhibition target, RNF20 ubiquitin ligase. In Fig 4C, Ruvbl1 is well demonstrated to locate in TSS and TR of p53 gene and

 

TSS of GAPDH. Upon Ruvbl1 depletion, RNF20 recruitments increased significantly only in TSS of p53 (Fig 4D). However, when looking at Ub-H2B, the immediate outcome of RNF20 action, the levels did not increase in this region (Figure 4B). Why?

We have repeated the ChIP assays and found that RNF20 is also associated with TR of p53 although the amount of RNF20 associated with TSS is much larger. This result appears to be consistent with previous reports (Zhu et al. Mol. Cell, 20, 601-611, 2005; Shema et al. Genes Dev., 22, 2664-2676, 2008). In addition, at the suggestion of reviewer 2, we have performed ChIP assays using anti-PAF antibody. We have found that knockdown of RUVBL1 leads to a significant decrease in PAF1 and H3K9 trimethylation associated with the p53 gene. We have also shown that knockdown of either PAF1 or CDC73, a component of the PAF1 complex, results in the upregulation of p53 expression. These results are consistent with a previous report showing that the PAF1 complex induces H3K9 trimethylation and thereby represses cyclin D1 transcription (Yang et al. Nuc. Acids Res., 38, 382-390, 2010). Thus, we speculate that RUVBL1 represses p53 transcription by interfering with RNF20-mediated histone H2B monoubiquitination and promoting PAF1-mediated H3K9 trimethylation. These results are presented in Fig 4F-H and explained in page 8, line27 and page 9, line 1-12 of the revised manuscript.

8. Ruvbl1-dependent p53 repression (Fig 3D) and ChIP experiments (Fig 4) were done in RKO cells, though most of apoptosis analysis was performed in HCT116. It would be important to demonstrate that different aspects of the study are seen in the same cell line.

We have performed SubG1 assays with RKO cells. The results are presented in Fig 3B of the revised manuscript.

9. In terms of transcriptional repression, Ruvbl1/pontin has been documented as a transcriptional repressor of Myc, beta-catenin and NF-kB targets. It would make this study more complete if authors could look at the possibility that Ruvbl1-dependent p53 repression is mediated through interactions with these transcription factors.

We have found that knockdown of RUVBL1 along with Myc, beta-catenin or NF-kB does not result in an alteration in p53 expression. We have also found that knockdown of either Myc, beta-catenin or NF-kB does not lead to a change in p53 expression. These results are presented in Fig S6 and explained in page 9, lines 14-24 of the revised manuscript.

Referee #3 (Remarks to the Author):

Some specific comments:

1. Fig. 1 - was the siERF tested in the DLD cells? If so the authors should mention whether it had an effect.

We have performed knockdown experiments with DLD-1 cells using the same set of ETS family genes that were used for HCT116(-/-) cells. The results are presented in Fig. 1 (Lower right) of the revised manuscript.

2. Why was the EHF ChIP on the RUVBL1 promoter carried out in HeLa cells rather than in colon cancer cells. I assume that anti-EHF is not available (is this true?)- otherwise it would have strengthened the results to have shown binding by endogenous EHF.

We have performed a ChIP assay using anti-EHF antibody and confirmed that endogenous EHF binds directly to the TSS region of RUVBL1. The results are shown in Fig. 2E and mentioned in page 6, lines 7-9 of the revised manuscript.

3. Fig. 3C - although the effect of siEHF could be overcome by increases RUVBL1 the effect was only partial. However only one RUVBL1 concentration was apparently examined. The authors should consider titrating the levels of RUVBL1.

We have titrated the level of RUVBL1 expression and the result is presented in Fig. 3C of the revised manuscript. However, the effect of RUVBL1 overexpression is still partial. Consistent with

 

this result, we have found that EHF also inhibits apoptotic cell death by another novel mechanism. We would like to deal with this mechanism in the next paper.

4. Fig. 4 - was RUVBL1 binding tested in the Bax and Puma promoters? If RUVBL1 interacts with GAPDH promoter (4C) why aren't ubH2A levels affected in the siRNA knockdown (4B)?

We have performed ChIP assays using anti-RUVBL1 antibody and have found that RUVBL1 is associated with the promoter regions of PUMA and BAX. However, ChIP assays with anti-RNF20 and anti-ubH2B antibodies have shown that RUVBL1 knockdown does not lead to increased detection of RNF 20 and ubH2B associated with the PUMA and BAX genes. Thus, RUVBL1-mediated inhibition of histone H2B monoubiquitination does not play a role in the repression of PUMA and BAX expression. The results are presented in Fig. 4A-D and mentioned in page 8, lines 7-13 and 17-19 of the revised manuscript.

5. The model shown in Supp. Fig. 4 should be included in the main text as it provides a good summary of the conclusions.

The model shown in Supp. Fig. 4 is presented in Fig. 4I of the revised manuscript.

6. Minor point: on pg. 8 the authors write: "To prove this hypothesis...". I would recommend that they change the wording to " To test (or examine) this hypothesis...."

We have corrected the wording to ìTo test this hypothesisî.

2nd Editorial Decision 28 March 2011

Thank you for the submission of your revised manuscript to our offices. We have now received the enclosed reports from the referees that were asked to assess it. I am happy to tell you that both referees now support publication of your study in EMBO reports.

Before we can proceed with the official acceptance of your manuscript I do have one minor thing that I would kindly ask you to address:

First, I have noticed that the manuscript contains only a very succinct materials and methods section. Basic materials and methods essential to the understanding of the experiments must be described in the main body of the manuscript and may not be presented as supplementary information. To enable you to incorporate an extended version of this section I can increase the character count to 27,500 (including spaces and references), which gives you about 2500 additional characters.

Thank you very much for your cooperation and I assure you that once these minor things have been addressed we will proceed with the acceptance and publication of your manuscript without further delay.

I look forward to seeing the final version of your manuscript as soon as possible.

Please do not hesitate to contact me if you require any further information.

Kind regards

Editor
EMBO Reports

REFEREE REPORTS

 

Referee #1 (Remarks to the Author):

In the revised version the authors have addressed most of the major concerns I raised regarding the original version. One concern that was not addressed is the issue of binding of ENDOGENOUS EHF to the RUVBL1 promoter. The authors added an experiment in which binding of transfected EHF was detected but they still do not show tat endogenous EHF behaves similarly. This issue was raised also by reviewer #3 (point #2 in his review). Nevertheless, I think this paper may be published in EMBO Reports.

Referee #2 (Remarks to the Author):

the authors have performed adequate revisions and the current manuscript is suitable for publication in ER

2nd Revision - authors' response 31 March 2011

The authors have adequately addressed all concerns.

3rd Editorial Decision 01 April 2011

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.

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.

Yours sincerely,

Editor
EMBO Reports

 