EMBO reports

LINC00839 promotes colorectal cancer progression by recruiting RUVBL1/Tip60 complexes to activate NRF1

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Dear Prof. Zhou,

Thank you for the submission of your research manuscript to EMBO reports. We have now received the reports from the three referees that were asked to evaluate your study, which can be found at the end of this email.

As you will see, the referees think that these findings are of interest. However, they have several comments, concerns and suggestions, indicating that a major revision of the manuscript is necessary to allow publication of the study in EMBO reports. As the reports are below, and all their points need to be addressed experimentally as indicated in the reports, I will not detail them here.

Given the constructive referee comments, we would like to invite you to revise your manuscript with the understanding that all referee concerns must be fully addressed in the revised manuscript and in a detailed point-by-point response. Acceptance of your 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 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. Please contact me to discuss the revision should you need additional time.

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See also the guidelines for figure legend preparation:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat

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http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

5) that primary datasets produced in this study (e.g. RNA-seq, ChIP-seq, structural and array data) are deposited in an appropriate public database. If no primary datasets have been deposited, please also state this a dedicated section (e.g. 'No primary datasets have been generated and deposited'), see below.

See also: http://embor.embopress.org/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 & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

The datasets produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

Moreover, I have these editorial requests:

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

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11) Please order the manuscript sections like this:
Title page - Abstract - Introduction - Results - Discussion - Materials and Methods - DAS - Acknowledgements (including funding information) - Author contributions - Conflict of interest - References - Figure legends - Expanded View Figure legends

Finally, please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript. Please ask your co-corresponding author Philippe Plattet to do that. Please find instructions on how to link the ORCID ID to the account in our manuscript tracking system in our author guidelines:
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I look forward to seeing a revised version of your manuscript when it is ready. Please use this link to submit your revision:
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Please let me know if you have questions or comments regarding the revision.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

Referee #1:

In this work, Liu et al described a novel function of the lncRNA LINC00839 in colorectal cancer (CRC). They showed that LINC00839 expression positively correlates with CRC metastasis and low survival probability. Overexpression and KD experiments in colon cancer cells showed that LINC00839 promotes cell proliferation, migration, and invasion in vitro. They also provided in vivo data showing that LINC00839 accelerates CRC cell proliferation and metastasis. Further, the data indicated that LINC00839 can accelerate the EMT of CRC cells. Using RNA pulldown and RIP assays they showed that LINC00839 interacts with RUVBL1, a factor known to interact with the histone acetyltransferase Tip60. They also showed that the overexpression of LINC00839 enhances oxidative phosphorylation and that glucose metabolism provides a substrate for oxidative phosphorylation but not for glycolysis. RNAseq analyses revealed that many genes implicated in mitochondrial activities were affected upon overexpression of LINC00839. In particularly, one of the upregulated genes is NRF1, a key mediator of genes involved in oxidative phosphorylation and mitochondrial biogenesis. The data showed that expression levels of LINC00839 positively correlate with the occupancy of RUVBL1, Tip60, H4K5 and H4K8 at the NRF1 promoter. The data also showed that increased expression of NRF1 promotes oxidative phosphorylation and mitochondrial biogenesis in CRC cells and then promotes the EMT and tumor progression in CRC.

This is an interesting and original work and the authors provided many in vitro and in vivo data for the role of LINC00839 in CRC. However, I think that the mechanisms of LINC00839-mediated NRF1 regulation are not yet clear and are mainly based on correlations.

Lane 343. The author stated that "LINC00839 can serve as a scaffold to recruit RUVBL1 to the Tip60 complex". However, this was not proven. The authors should perform immunoprecipitation experiments in cells overexpressing wt and mutant LINC00839-Δ5 as well as its KD and determine whether TIP60 and Ruvb1 association is affected.

They should also determine whether the interaction of LINC00839 with RUVBL1/Tip60 affects the acetylase activity (i.e. acetylase activity in cells expressing LINC00839-Δ5 mutant). Similarly, they should determine whether the interaction of LINC00839 with RUVBL1/Tip60 is required for their association with NRF1 promoter sequences (RUVBL1/Tip60 ChIP in cells expressing LINC00839-Δ5 mutant). Finally, they should determine whether LINC00839 associates with NRF1 promoter as depicted in the model of Fig. 6F (i.e. ChIRP).

Minor points:

P3. Lane 62
"More than 70% of genes in the human genome are transcribed into noncoding RNAs". This statement is not correct. 70% of the human genome is transcribed but only 2% corresponds to genes encoding protein whereas the rest represent ncRNAs.

The authors should include the information on which chromosome the LINC00839 gene is located. Indeed, they are proposing a mechanism of action in trans and this should be clarified in the text

S2C. Colony assay upon LINC00839 overexpression. Please provide the quantification of the colonies as done for the KD experiments in Fig. S3E
S2DF. Transwell migration assay and matrigel invasion assay upon LINC00839 overexpression. Please provide the quantification as done for the KD experiments in Fig. S3
S2E & S3G. Wound-healing assay. The quality of images should be improved since it is very hard to visualize the cells.

S4A. Cells overexpressing LINC00839 exhibited EMT-like cell morphology. The quality of images should be improved since it is very hard to visualize the cells.
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There is no description of the RNaseq. How many genes are affected? How many genes are upregulated and downregulated upon overexpression of LINC00839? A list of regulated genes (log2FC and P values) should be provided in an excel file as Supplementary Table. Further the RNAseq data were not deposited in a data repository such as GEO.

Lanes 306, 337, 628. The authors often describe the results of the RIP as a"direct" interaction of LINC00839 with RUVBL1 or Tip60. This is not correct since the RIP was performed with a cell lysate and it cannot be excluded that the interaction occurs

through another factor in the complex.

Fig. 4H. The western blot clearly shows increased levels of TFAM, MT-ND5, MT-CYB, and MT-CO1 in cells overexpressing LINC00839. However, the effect in cells with LINC00839-KD is less evident. These results are not at all described and the authors should provide a comment on these data.

Lane 392. "LINC00839 can recruit the RUVBL1/Tip60 complex and increase its acetylase activity". This is not recruitment. The authors should replace "recruits" with "binds" or "associates". Further, there are no data showing that LINC00839 acts as scaffold for the formation of RUVBL1/Tip60 complex (see my major point above). The author should also clarify if they found Tip60 in the mass-spec.

The UCSC Genome Browser view of Fig. 5A does not clearly show the NRF1 gene. To which cells do the ChIPseq track correspond? Further, the track shows only peaks but not an enrichment of H4K5ac, H4K8ac and H4k12ac at the NRF1 gene. Maybe the authors should zoom out a bit to allow visual comparisons with neighboring sequences not enriched for these modifications. Why were the other modified histones excluded from the analysis?

Lane 407. "We divided the promoter into ten fragments". Please included the size of the promoter.

Fig. 5B. Why the P1-P10 fragments are represented with two different colors /blue and black)?

Lane 408. "NRF1 promoter DNA can bind only with H4K5ac and H4K8ac antibodies". The sentence is quite strange. Please modify with "the NRF1 promoter was significantly enriched in H4K5ac and H4K8ac whereas H4K12ac did not show any evident enrichment". The H4K12ac ChIP data should be shown. Wisely, EMBO Rep does not accept "data not shown".

Fig. 5I-L. The results using cells expressing LINC00839-Δ5 mutant are not described and I think they should since they are important.

The LINC00839-KD experiments have all been performed with the same shRNA sequences. However, in KD experiments is very important to assess the specificity of the effects that is usually demonstrated using another shRNA. The authors should provide some experiments using another shRNA.

Statistical analyses are not described in the corresponding Figure legends.

The acetylase activity assay is not described in material and method section

Please correct the text for the consent of publication.

Referee #2:

In this manuscript, Liu et al demonstrate the importance of lncRNA LINC00839 in colorectal cancer progression. They claim that LINC00839 regulates mitochondrial metabolism by modulating NRF1 levels via RUVBL1/Tip60-mediated acetylation of NRF1 promoter region. Despite the resonably strong data in favor of the claims, there are a few issues that need to be addressed:
1. Knockdown of RUVBL1 or treatment with MG149 is expected to affect general acetylation levels of histones in a global manner (which is also evident from their western blots). The authors do not sufficiently discuss how they can pin this effect on NRF1 alone.
2. There are controls missing for siRNA knockdowns. Fig 5J, how much knockdown of RUVBL1 achieved? Likewise Fig 6C how much knockdown of NRF1?
3. In Fig 6B, si-RUVBL1 hardly has any knockdown effect. Difficult to interpret the results.
4. Overall, the language in the manuscript is a bit difficult to understand.

Referee #3:

General comments:
In this study, Liu and colleagues report that LINC00839 interacts with the RUVBL1/Tip60 complex and enhances the NRF1 gene expression, resulting in activation of mitochondrial metabolism and biogenesis, which promotes CRC proliferation and metastasis.

The work is comprehensive, and includes many kinds of experiment, from patient tissue analyses, metastasis assays both in vitro and in vivo, RNA-pull down assays, epigenome analyses with ChIP, measurement of mitochondrial metabolism and biogenesis, and so on. Major concern is that the work lacks preciseness and critical experimental data. In addition, it is largely

unclear whether involvement of the LINC00839 is specific and significant to the RUVBL1/Tip60/NRF1 axis and CRC, because LINC00839 has been already reported in many cancers.

Major points:
1. The authors claim that mechanistic investigations revealed that LINC00839 can promote the acetylation of histones H4K5 and H4K8 at the promoter of NRF1 after recruiting the RUVBL1/Tip60 complex, in abstract for example. However, the direct binding of LINC00839 to the NRF1 promoter is not shown. RNA-pull down experiment to identify the genome binding sites, such as ChIRP-qPCR should be performed. In addition, ChIP-Seq of histone acetylation with the LINC00839 knockdown is also appreciated. These data also clarify how LINC00839 can function in trans, as LINC00839 and Tip60 are coded on different chromosomes: chromosomes 10 and 7, respectively.

2. The presence of the tertiary complex of RUVBL1/Tip60/LINC00839 should be rigorously validated. Interaction between LINC00839 and Tip60 is shown in Fig. 3H by the RNA pull down experiment followed by immunoblotting. On the other hand, Tip60 was not detected by the LC-MS/MS analysis. Interaction between Tip60 and RUVBL1 is not shown as well. For RNA-pulldown experiment in Fig. 3H, dot blot should be done to confirm the appropriate RNA pull down.

3. The authors claimed that cell proliferation, migration, and invasion abilities were enhanced in the cell lines stably expressing LINC00839 (Fig. 2A-E and Supplementary Fig. S2C-F). Also, they showed knockdown of LINC00839 by shRNA reduced cell proliferation, migration, and invasion (Supplementary Fig. S3C-H). Finally, they concluded that LINC00839 can promote cell proliferation, migration, and invasion in vitro. However, the interpretation should be more careful. Because LINC00839 affect cell proliferation, the results of migration and invasion assay should be normalized by the cell proliferation rate.

4. Fig. 5 is poorly organized. the authors should add ENCODE ID and describe how those particular data are appropriate for this study, including verification of right cell types with LINC00839 expression. It is unclear where the NRF1 promoter, TSS, and P1-P10 sites are located in Fig. 5A.

5. The authors claimed that LINC00839 is upregulated in CRC by showing "relative expression of LINC00839" in Fig. 1B and C, which is not convincing. The authors should clarify what was set to 1 and what was internal control.

6. The authors used the software I-TASSER to make the model of RUVBL1- LINC00839 complex in Fig. 3D, but the I-TASSER would provide only protein structure. The authors should describe how to make the model containing RNA. Also, they should describe accuracy of the model. Can the model explain the specific interaction between RUVBL1 and LINC00839?

7. The authors reported that MG149, a Tip60-selective inhibitor, repressed NRF1 expression level. It was observed in Fig. 5J, but not in Fig. S7G. Thus, the result is not reproducible.

8. The authors reported that the NRF1 knockdown in LINC00839-expressing cells inhibited mitochondrial biogenesis and OXPHOS (Supplementary Fig. S7C-F), but the immunoblotting indicated that almost all amount of NRF1still remained in the siNRF1 treated samples (Fig. S7C). Thus, the result does not support their conclusion. The authors also performed siNRF1 treatment in Fig. 6C, but they did not show the NRF1 level, and the knockdown is not confirmed. Thus, relationship between NRF1 and EMT markers are unclear. The authors also used siNRF1 for in vivo experiments but there is no description about the experimental design and no confirmation about the knockdown efficiency in vivo (Fig. 6 E-I). Taken together, roles of NRF1 in CRC is unclear.

9. The manuscript is poorly organized throughout. Not only there are typos, graphs are frequently mislabeled as describe below, and discussion sections appeared twice: one is shorter and the other is longer version !

Minor points:
1. In Fig. 3C, the loading control is not equal, unlike the author's claim. Quantification of the signals would be helpful.
2. In Fig. 3B and G, the label "expression" is inappropriate for the RIP assay. Also, authors should analyze other non-relevant RNAs to show the specificity.
3. In Figs. S2E and S3G, the labels of 0H and 24 H may be opposite.
4. In Fig. 4G, the label "Absorbance" is inappropriate for representing the NAD/NADH ratios.
5. In the manuscript, line 409 to 411, the authors wrote "only H4K5ac and H4K8ac can bind to NRF1 gene promoter DNA, especially in the P2 (-324~-174 bp) and P3 (-474~-325 bp) regions", but this pattern is not observed in the vector control. The authors should mention that they compared vector control and LINC00839 over expression.
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Point-by-point response to comments of the Reviewers

Referee #1:

In this work, Liu et al described a novel function of the lncRNA LINC00839 in colorectal cancer (CRC). They showed that LINC00839 expression positively correlates with CRC metastasis and low survival probability. Overexpression and KD experiments in colon cancer cells showed that LINC00839 promotes cell proliferation, migration, and invasion in vitro. They also provided in vivo data showing that LINC00839 accelerates CRC cell proliferation and metastasis. Further, the data indicated that LINC00839 can accelerate the EMT of CRC cells. Using RNA pulldown and RIP assays they showed that LINC00839 interacts with RUVB1, a factor known to interact with the histone acetyltransferase Tip60. They also showed that the overexpression of LINC00839 enhances oxidative phosphorylation and that glucose metabolism

provides a substrate for oxidative phosphorylation but not for glycolysis. RNAseq analyses revealed that many genes implicated in mitochondrial activities were affected upon overexpression of LINC00839. In particularly, one of the upregulated genes is NRF1, a key mediator of genes involved in oxidative phosphorylation and mitochondrial biogenesis. The data showed that expression levels of LINC00839 positively correlate with the occupancy of RUVB1, Tip60, H4K5 and H4K8 at the NRF1 promoter. The data also showed that increased expression of NRF1 promotes oxidative phosphorylation and mitochondrial biogenesis in CRC cells and then promotes the EMT and tumor progression in CRC.

This is an interesting and original work and the authors provided many in vitro and in vivo data for the role of LINC00839 in CRC. However, I think that the mechanisms of LINC00839-mediated NRF1 regulation are not yet clear and are mainly based on correlations.

Response:

Thanks for your positive comments and constructive suggestions. The reviewers have given us many suggestions and we have supplied some key experiments to confirm the interaction of LINC00839 and Ruvb1/Tip60 complex, as well as LINC00839 and NRF1 promoter. We think these data has strengthen our work greatly.

Lane 343. The author stated that "LINC00839 can serve as a scaffold to recruit Ruvb1 to the Tip60 complex". However, this was not proven. The authors should perform immunoprecipitation experiments in cells overexpressing wt and mutant LINC00839-Δ5 as well as its KD and determine whether Tip60 and

Ruvb1 association is affected.

Response:

We appreciate the reviewer for the very insightful comments and suggestions. Based on reviewer's comments, we performed further experiments to validate the conclusion. First, we confirmed the interaction of Ruvb1 and Tip60 in HCT116 and SW620 cells by Co-Immunoprecipitation (Co-IP) (Fig 3H). To further explore the role of LINC00839 in the interaction of Ruvb1 and Tip60 complex, we performed Co-IP in cells with LINC00839 overexpressed and nucleotides 1033-1290 of LINC00839 deleted (LINC-Δ5), as well as LINC00839 knockdown. Our findings showed that overexpression of LINC00839 promoted the binding of Ruvb1 and Tip60, while the LINC-Δ5 had little effect on the binding and LINC00839 KD restrained the binding compared with negative control (Fig 3I-J). These results indicates that Ruvb1 interacts with Tip60 in a LINC00839-dependent way.

The corresponding new data is described in the "Results" section (page 10, line 230-238) and the figures are shown in Fig 3H-J.

They should also determine whether the interaction of LINC00839 with RUVB1/Tip60 affects the acetylase activity (i.e. acetylase activity in cells expressing LINC00839-Δ5 mutant). Similarly, they should determine whether the interaction of LINC00839 with RUVB1/Tip60 is required for their association with NRF1 promoter sequences (RUVB1/Tip60 ChIP in cells expressing LINC00839-Δ5 mutant). Finally, they should determine whether LINC00839 associates with NRF1 promoter as depicted in the model of Fig. 6F (i.e. ChIRP).

Response:

We thank the reviewer for these important points and very constructive advice. First, we determined the acetylase activity in cells overexpressing LINC00839 and LINC-Δ5, as well as LINC00839 KD. The results showed that overexpression of LINC00839 increased the acetylase activity and the LINC-Δ5 do not have the similar effect, while knockdown of LINC00839 decreased the acetylase activity (Fig 3K-L). The corresponding data is described in the “Results” section (page 11, line 241-246).

Next, we determine whether the interaction of LINC00839 with Ruvb1/Tip60 is required for their association with NRF1 promoter. To explore the association, we performed ChIP-qPCR in cells with LINC-Δ5 expressed, MG149 treated, and RUVBL1 knocked down. The results showed that overexpression of LINC-Δ5 showed negligible impact on the acetylation of NRF1 promoter compared with negative control (Fig 4M-N). Further, treatment of MG149 and knockdown of Ruvb1 largely reversed the increase of the histone acetylation induced by LINC00839 (Fig 4M-N). Collectively, these data indicates that the interaction between LINC00839 and Ruvb1/Tip60 complex is essential for the acetylation of NRF1 promoter. The corresponding data is described in the “Results” section (page 15, line 327-337).

Furthermore, we also determined whether LINC00839 was associated with NRF1 promoter. CHIRP-qPCR was performed and showed significant enrichment of NRF1 promoter by the LINC00839 (Fig 4O). This indicates that LINC00839 binds with NRF1 promoter. The corresponding data is described in the “Results” section (page 15, line 337-343). Collectively, these data support

a model in which LINC00839, as a molecular scaffold of the Ruvb1/Tip60 complex, promotes histone acetylation of NRF1 promoter and facilitate its expression in CRC as depicted in Fig 5K.

Minor points:

P3. Lane 62

"More than 70% of genes in the human genome are transcribed into noncoding RNAs". This statement is not correct. 70% of the human genome is transcribed but only 2% corresponds to genes encoding protein whereas the rest represent ncRNAs.

Response:

Thanks for your correction. We have revised our statement. The corresponding amendment can be found on page 4, line 80.

The authors should include the information on which chromosome the LINC00839 gene is located. Indeed, they are proposing a mechanism of action in trans and this should be clarified in the text.

Response:

Many thanks for your kindly suggestion. The lncRNA LINC00839 is located on human chromosome 10q11.21, and the corresponding statement can be found on page 4, line 89. Based on the mechanism we revealed, we also discussed the in trans action of LINC00839 in the “Discussion” part (page 21, line 465).

S2C. Colony assay upon LINC00839 overexpression. Please provide the quantification of the colonies as done for the KD experiments in Fig. S3E

S2DF. Transwell migration assay and matrigel invasion assay upon LINC00839 overexpression. Please provide the quantification as done for the KD experiments in Fig. S3

Response:

Thanks for your reminding. Considering the chaotic composition of figure 2 and corresponding supplementary figures, we have reorganized the results. The colony assay and matrigel invasion assay were all quantified as specified in the “Materials and methods” part.

S2E & S3G. Wound-healing assay. The quality of images should be improved since it is very hard to visualize the cells.

Response:

Thanks for your comment. We have repeated the wound-healing assay using GFP-labeled cells. The migration of the cells was observed in the fluorescence microscope at 0 h and 48 h. The experiment method was described in the corresponding “Materials and methods” part. The revised figures were shown in Fig 2D and Fig EV1E.

S4A. Cells overexpressing LINC00839 exhibited EMT-like cell morphology.

The quality of images should be improved since it is very hard to visualize the cells.

Response:

Thanks for your comment. The images are now shown at a higher magnification (Fig EV1H).

S4G. The IF mages should be improved. It is very hard to visualize the red signal.

Response:

Thanks for your comment. We have repeated the IF experiments and the images are now shown at a higher magnification in the revised figure (Fig EV1J).

There is no description of the RNaseq. How many genes are affected? How many genes are upregulated and downregulated upon overexpression of LINC00839? A list of regulated genes (log2FC and P values) should be provided in an excel file as Supplementary Table. Further the RNAseq data were not deposited in a data repository such as GEO.

Response:

Thanks for your suggestions. we overexpressed LINC00839 in LoVo cells and identified the transcription profiles through RNA-seq. Remarkably, differentially

expressed genes (DEGs) analysis revealed that 38 genes were upregulated and 29 genes were downregulated in LINC00839 overexpressed cells (≥2 fold change, p value < 0.05). The DEGs list was provided in Appendix Table S3. The corresponding statement can be found on page 12, line 259-264. We also deposited our data in GEO dataset. The accession number for the RNA-sequencing data is GSE197706. This was stated in “Availability of data and materials” section (page 31, line 707).

Lanes 306, 337, 628. The authors often describe the results of the RIP as a "direct" interaction of LINC00839 with RUVB1 or Tip60. This is not correct since the RIP was performed with a cell lysate and it cannot be excluded that the interaction occurs through another factor in the complex.

Response:

Thanks for your correction. We have corrected our statement in the revised manuscript separately.

Fig. 4H. The western blot clearly shows increased levels of TFAM, MT-ND5, MT-CYB, and MT-CO1 in cells overexpressing LINC00839. However, the effect in cells with LINC00839-KD is less evident. These results are not at all described and the authors should provide a comment on these data.

Response:

Thanks for your comment. We have repeated the experiments and qualified the expression levels of these proteins. The result showed that LINC00839

increased the expression of TFAM, MT-ND5, MT-CYB, and MT-CO1 in CRC cells (Fig. 4F). We also knocked down LINC00839 and the expression of TFAM, MT-ND5, MT-CYB, and MT-CO1 was decreased (Fig 4F). The expression of these proteins was quantified by the expression of β-actin.

Lane 392. "LINC00839 can recruit the Ruvb1/Tip60 complex and increase its acetylase activity". This is not recruitment. The authors should replace "recruits" with "binds" or "associates". Further, there are no data showing that LINC00839 acts as scaffold for the formation of RUVB1/Tip60 complex (see my major point above). The author should also clarify if they found Tip60 in the mass-spec.

Response:

Thanks for your suggestions. We have reconsidered our statement and revised the manuscript accordingly. In light of your constructive suggestion above, we also confirmed the interaction of Ruvb1 and Tip60 in HCT116 and SW620 cells by Co-Immunoprecipitation (Co-IP) (Fig 3H). To further explore the role of LINC00839 in the interaction of Ruvb1 and Tip60 complex, we performed Co-IP in cells with LINC00839 overexpressed and nucleotides 1033-1290 of LINC00839 deleted (LINC-Δ5), as well as LINC00839 KD. Our findings shows that LINC-Δ5 had little effect on the binding and LINC00839 KD restrained the binding compared with negative control (Fig 3I-J), indicating that Ruvb1 interacted with Tip60 in a LINC00839-depandent way.

As described in the manuscript (page 9, line 192), we performed RNA pull-down assay and observed an obvious enrichment at about 50 kDa (Fig

3A). The enrichment proteins were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Subsequently, we identify Ruvb1 and validated the binding of LINC00839 and Ruvb1 by RNA pull-down and RIP (Fig 3B-C). Considering Ruvb1 interacts Tip60 complex, we also confirmed that LINC00839 can bind to Tip60 by RNA pull-down and RIP (Fig EV2D and Fig 3F). As the molecular weight of Tip60 is 60 kDa, it cannot be found in the proteins we identified by LC-MS/MS. We further performed RNA pull-down assay and identified the enrichment of proteins at about 60 kDa by LC-MS/MS. Tip60 was included in the identified list (Appendix Table S2). Together, these data indicates that LINC00839 binds Ruvbl1/Tip60 complex in CRC cells.

The UCSC Genome Browser view of Fig. 5A does not clearly show the NRF1 gene. To which cells do the ChIP-seq track correspond? Further, the track shows only peaks but not an enrichment of H4K5ac, H4K8ac and H4k12ac at the NRF1 gene. Maybe the authors should zoom out a bit to allow visual comparisons with neighboring sequences not enriched for these modifications. Why were the other modified histones excluded from the analysis?

Response:

Thanks for your comment. It is reported that Tip60 complex can acetylate H2A lysine 5 (H2AK5), H3 lysine 14 (H3K14), H4 lysine 5 (H4K5), H4 lysine 8 (H4K8), and H4 lysine 12 (H4K12) at promoters. Thus, we analyzed the acetylation of these histones by the UCSC Genome Browser, which integrating the CHIP-seq data published online. Now, we focused on the NRF1 promoter before STT (from 129609720 to 129611720) and viewed the peak of these acetylated histones. As shown in Fig EV2F, the enrichment of H3K14ac, H4K5ac, H4K8ac, H4K12ac was widespread, especially at P2 (from -324 to

-174 bp) and P3 (from -474 to -325 bp) fragments, as predicted in different cells including H1 cell, trophoblast cell, H9 cell and the like.

Lane 407. "We divided the promoter into ten fragments". Please included the size of the promoter.

Response:

Thanks for your suggestion. We have included the size of the promoter in our statement (page 14, line 314) and the pattern of the ten fragments can be found in Fig EV2F.

Fig. 5B. Why the P1-P10 fragments are represented with two different colors /blue and black)?

Response:

Thanks for your comment. Fig 5B represent the schema of the 10 fragments we divided. There is no difference between the two colors. To better illustrate the schema, we revised the figure and showed it in Fig EV2F.

Lane 408. "NRF1 promoter DNA can bind only with H4K5ac and H4K8ac antibodies". The sentence is quite strange. Please modify with "the NRF1 promoter was significantly enriched in H4K5ac and H4K8ac whereas H4K12ac did not show any evident enrichment". The H4K12ac ChIP data should be shown. Wisely, EMBO Rep does not accept "data not shown".

Response:

Thanks for your correction. We changed the sentence into “Furthermore, the nucleosomes at the NRF1 gene promoter were acetylated as H4K5ac and H4K8ac instead of H4K12ac and H3K14ac (Fig 4K-L and Fig EV3H-I)” (page 14, line 319) and the ChIP-qPCR data of H4K12ac and H3K14ac were shown in Fig. EV3H-I.

Fig. 5I-L. The results using cells expressing LINC00839-Δ5 mutant are not described and I think they should since they are important.

Response:

Thanks for your comment. We have described the mutant LINC00839-Δ5 in the corresponding experiments and statements.

The LINC00839-KD experiments have all been performed with the same shRNA sequences. However, in KD experiments is very important to assess the specificity of the effects that is usually demonstrated using another shRNA. The authors should provide some experiments using another shRNA.

Response:

Thanks for your suggestion. Another shRNA is essential to excluded the off-target effect of a single shRNA. We designed another shRNA of LINC00839 with different targeting sequence. The knockdown of the shRNA was validated

by qPCR (FigEV1A). We next repeated CCK8, colony formation, wound healing, and matrigel invasion assays using the shRNAs and the results showed that knockdown of LINC00839 restrained the proliferation, migration, and invasion of CRC cells (Fig EV1B-F). The corresponding new data is described in the manuscript (page 7 lines 150-163) and the sequences of shRNA is listed in the Appendix Table S4.

Statistical analyses are not described in the corresponding Figure legends.

Response:

Thanks for your suggestion. We have stated the statistical analyses in detail in the corresponding figure legends.

The acetylase activity assay is not described in material and method section.

Response:

Thanks for your comments. We have added the detail of the acetylase activity assay in “Materials and methods” section (page 30).

Please correct the text for the consent of publication.

Response:

Thanks for your suggestion. we have revised the manuscript according to

reviewers' suggestion and strengthen our English language. In addition, the revised manuscript follows all points in the Author Guidelines of *EMBO reports.*

Referee #2:

In this manuscript, Liu et al demonstrate the importance of lncRNA LINC00839 in colorectal cancer progression. They claim that LINC00839 regulates mitochondrial metabolism by modulating NRF1 levels via RUVB1/Tip60-mediated acetylation of NRF1 promoter region. Despite the resonably strong data in favor of the claims, there are a few issues that need to be addressed:

1. Knockdown of RUVB1 or treatment with MG149 is expected to affect general acetylation levels of histones in a global manner (which is also evident from their western blots). The authors do not sufficiently discuss how they can pin this effect on NRF1 alone.

Response:

Thanks for your comment. The acetylase activity assays and western blots both indicated the altered acetylation levels of histones in a global manner. We also detected the acetylation levels of histones at NRF1 promoter by ChIP-qPCR. Our results showed that overexpression of LINC-Δ5, inhibiting the binding of LINC00839 and Ruvb1, showed negligible impact on the acetylation of NRF1 promoter compared with negative control (Fig 4M-N). Further, treatment of MG149 and knockdown of Ruvb1 largely reversed the increase of the histone acetylation induced by LINC00839 (Fig 4M-N). Taken together, these data indicated that LINC00839 increased the acetylase activity of

Ruvb1/Tip60 complex and acetylated the histone H4K5 and H4K8 at P2 and P3 sites of NRF1 promoter. Although the possibility that Ruvb1/Tip60 complex may affect the acetylation of histones at the other promoter cannot be excluded, it can indeed acetylate the histone at NRF1 promoter and promote its expression.

Analysis of RNA-seq data and our experiments revealed that LINC00839 can promote OXPHOS and mitochondrial biogenesis. It is widely reported that NRF1 can regulate the expression of genes related to mitochondrial energy metabolism and biogenesis, and LINC00839 can also promote the expression of NRF1. Thus, we speculate that NRF1 may be a hub gene in LINC00839-induced OXPHOS and mitochondrial biogenesis. We also validated the essential role of NRF1 in this progress by knocking down NRF1. To illustrate the crucial function of the histone acetylation modification, we treated the cells with MG149 and the OXPHOS and mitochondrial biogenesis were inhibited. We also overexpressed NRF1 in the cells treated with MG149, the inhibited phenotype was reversed again. All these data demonstrated the importance of LINC00839-Ruvb1/Tip60-NRF1 signaling in OXPHOS and mitochondrial biogenesis of CRC.

It cannot be confirmed that Ruvb1/Tip60 complex can pin their effect on NRF1 alone, and it also seems impossible that Ruvb1/Tip60 complex affect NRF1 exclusively. But the mechanism that Ruvb1/Tip60 complex acetylates the histone at NRF1 promoter and promotes its expression indeed plays a key role in OXPHOS, mitochondrial biogenesis and the progression of CRC. In light of your constructive suggestion, we have discussed the question in the “Discussion" part of the revised manuscript (page 21, line 464-471).

2. There are controls missing for siRNA knockdowns. Fig 5J, how much knockdown of RUVB1 achieved? Likewise Fig 6C how much knockdown of NRF1?

Response:

Thanks for your comment. We have validated the knockdown efficiency of siRUVBL1 and siNRF1 before the experiment. The corresponding data were shown in Fig EV2E and Fig EV3J.

3. In Fig 6B, si-RUVB1 hardly has any knockdown effect. Difficult to interpret the results.

Response:

Thanks for your comment. We have added the negative control of siRUVB1 and repeated the experiments. The result showed that LINC-Δ5 showed impaired the EMT of CRC compared with wild-type, and treatment of MG149 and RUVBL1 knockdown reversed the EMT induced by LINC00839 (Fig EV4C).

4. Overall, the language in the manuscript is a bit difficult to understand.

Response:

Thanks for your comment. We have reorganized the data and revised the

statement literally. And the English language was also strengthened and polished.

Referee #3:

General comments:

In this study, Liu and colleagues report that LINC00839 interacts with the RUVB1/Tip60 complex and enhances the NRF1 gene expression, resulting in activation of mitochondrial metabolism and biogenesis, which promotes CRC proliferation and metastasis.

The work is comprehensive, and includes many kinds of experiment, from patient tissue analyses, metastasis assays both in vitro and in vivo, RNA-pull down assays, epigenome analyses with ChIP, measurement of mitochondrial metabolism and biogenesis, and so on. Major concern is that the work lacks preciseness and critical experimental data. In addition, it is largely unclear whether involvement of the LINC00839 is specific and significant to the RUVB1/Tip60/NRF1 axis and CRC, because LINC00839 has been already reported in many cancers.

Response:

Thanks for the positive feedback, as well as the constructive comment. In light of the reviewers' suggestion, we have suppled some key and critical experiments to strengthen our work. The specific and significant role of LINC00839 in Ruvb1/Tip60/NRF1 was illustrated by a series of experiments.

Overexpression of LINC00839 enhanced the association between Ruvb1 and Tip60, and knockdown showed weak association (Fig 3I-J). Overexpression of LINC00839 increased the acetylase activity of Ruvb1/Tip60 complex and Knockdown decreased the acetylase activity (Fig 3K). Besides, overexpression of LINC00839 increased the acetylation of histone at NRF1 promoter and the expression of NRF1, and knockdown decreased its expression (Fig. 4I-N). All of these data supported that LINC00839 is specific and significant to the Ruvb1/Tip60-NRF1 axis.

We have demonstrated LINC00839 is an important oncogenic lncRNA in CRC by loss- and gain-of-function studies in vitro and in vivo. But whether its function is specific in CRC is still unclear. Previous studies have reported that LINC00839 is aberrantly expressed in several cancers and functions as a miRNA sponge. Whether LINC00839 is involved in Ruvb1/Tip60-NRF1 axis and mitochondrial function in other cancers is unclear and still necessary for further studies.

Major points:

1. The authors claim that mechanistic investigations revealed that LINC00839 can promote the acetylation of histones H4K5 and H4K8 at the promoter of NRF1 after recruiting the RUVB1/Tip60 complex, in abstract for example. However, the direct binding of LINC00839 to the NRF1 promoter is not shown. RNA-pull down experiment to identify the genome binding sites, such as ChIRP-qPCR should be performed. In addition, ChIP-Seq of histone acetylation with the LINC00839 knockdown is also appreciated. These data also clarify how LINC00839 can function in trans, as LINC00839 and Tip60 are coded on different chromosomes: chromosomes 10 and 7, respectively.

Response:

Thanks for your insightful suggestions. In light of your comments, we determined whether LINC00839 was associated with NRF1 promoter. CHIRP-qPCR was performed and showed significant enrichment of NRF1 promoter by the LINC00839 (Fig 4O). This showed that LINC00839 binds with NRF1 promoter. These data support a model in which LINC00839, as a molecular scaffold of the Ruvb1/Tip60 complex, promotes histone acetylation of NRF1 promoter and facilitate its expression as depicted in Fig 5K. The corresponding data is described in the “Results” section (page 15, line 338).

For identification of acetylation of histones at NRF1 promoter, we performed the ChIP-qPCR in cells with LINC-Δ5 expressed, MG149 treated, and Ruvb1 knocked down. The results showed that overexpression of LINC-Δ5 showed negligible impact on the acetylation of NRF1 promoter compared with negative control (Fig 4M-N). The corresponding data is described in the “Results” section (page 15, line 327-337).

LINC00839 locates on human chromosome 10 and NRF1 locates on chromosome 7. LINC00839 can bind NRF1 promoter as detected by CHIRP-qPCR and upregulate expression of NRF1. These data can support the in trans function of LINC00839 and the function strategy was discussed in the “Discussion” part (page 21, line 464-471).

2. The presence of the tertiary complex of RUVB1/Tip60/LINC00839 should be rigorously validated. Interaction between LINC00839 and Tip60 is shown in Fig.

3H by the RNA pull down experiment followed by immunoblotting. On the other hand, Tip60 was not detected by the LC-MS/MS analysis. Interaction between Tip60 and RUVB1 is not shown as well. For RNA-pulldown experiment in Fig. 3H, dot blot should be done to confirm the appropriate RNA pull down.

Response:

We appreciate the reviewer for the very insightful comments and suggestions. Based on reviewer's comments, we performed further experiments to validate the conclusion. First, we confirmed the interaction of Ruvb1 and Tip60 in HCT116 and SW620 cells by Co-Immunoprecipitation (Co-IP) (Fig 3H). To further explore the role of LINC00839 in the interaction of Ruvb1 and Tip60 complex, we performed Co-IP in cells overexpressing wide-type LINC00839 and mutant LINC00839-Δ5, as well as LINC00839 KD. Our findings showed that mutation and knockdown of LINC00839 restrained the binding of Ruvb1 and Tip60 complex (Fig 3I-J), indicating that Ruvb1 interacted with Tip60 in a LINC00839-depandent way.

As described in the manuscript (page 9, line 192), we performed RNA pull-down assay and observed an obvious enrichment at about 50 kDa (Fig. 3A). The enrichment proteins were identified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Subsequently, we identify Ruvb1 and validated the binding of LINC00839 and Ruvb1 by RNA pull-down and RIP (Fig 3B-C). Considering Ruvb1 interacts Tip60 complex, we also confirmed that LINC00839 can bind to Tip60 by RNA pull-down and RIP (Fig EV2D and Fig 3F). As the molecular weight of Tip60 is 60 kDa, it cannot be found in the proteins we identified by LC-MS/MS. We further performed RNA pull-down assay and identified the enrichment of proteins at about 60 kDa by LC-MS/MS.

Tip60 was included in the identified list (Appendix Table S2). Together, these data indicates that LINC00839 binds Ruvbl1/Tip60 complex in CRC cells.

RNA pull-down and WB were performed to illustrate the binding of LINC00839 and Tip60 in Fig EV2D, and the dot blot has done to confirm the appropriate RNA pull down.

3. The authors claimed that cell proliferation, migration, and invasion abilities were enhanced in the cell lines stably expressing LINC00839 (Fig. 2A-E and Supplementary Fig. S2C-F). Also, they showed knockdown of LINC00839 by shRNA reduced cell proliferation, migration, and invasion (Supplementary Fig. S3C-H). Finally, they concluded that LINC00839 can promote cell proliferation, migration, and invasion in vitro. However, the interpretation should be more careful. Because LINC00839 affect cell proliferation, the results of migration and invasion assay should be normalized by the cell proliferation rate.

Response:

Thanks for your suggestion. In the migration and invasion assays, the cells were cultured in the serum-free media. Serum-free media or low serum medium containing less than 2% FBS can greatly decrease the effect of proliferation. This method is widely used in migration and invasion assays of cancer cells. We have described the detail in the “Materials and methods” section.

4. Fig. 5 is poorly organized. the authors should add ENCODE ID and describe how those particular data are appropriate for this study, including verification of

right cell types with LINC00839 expression. It is unclear where the NRF1 promoter, TSS, and P1-P10 sites are located in Fig. 5A.

Response:

Thanks for your suggestion. In light of the reviewer' suggestions, we have improved our conclusion by supplying some additional information. We have reorganized the data and the statement of the corresponding results. The ENCODE ID and the cell lines were listed in the revised figures. Although these online data were not performed in CRC cell lines, the predicted results were validated in CRC by CHIP-qPCR (Fig 4K-L and Fig EV3H-I). In the revised figure EV2F, the NRF1 promoter and P1-P10 are clearly showed now.

5. The authors claimed that LINC00839 is upregulated in CRC by showing "relative expression of LINC00839" in Fig. 1B and C, which is not convincing. The authors should clarify what was set to 1 and what was internal control.

Response:

Thanks for your comment. As we stated in the revised qPCR method, a kind of relative qualification method was used in analyzing qPCR data. We calculated the expression of the tumor and the normal tissue separately. The minimum CT value of normal group was set to 1. The internal control of both group is GAPDH and general expression level of the two group is comparable.

6. The authors used the software I-TASSER to make the model of RUVB1-LINC00839 complex in Fig. 3D, but the I-TASSER would provide only protein

structure. The authors should describe how to make the model containing RNA. Also, they should describe accuracy of the model. Can the model explain the specific interaction between RUVB1 and LINC00839?

Response:

We thank the reviewer for the point. We did not clearly clarify the molecular binding model. We have reorganized the data and restated the related data. As the RNA pull-down assays and subsequent WB confirmed the binding of LINC00839 and Ruvb1 (Fig 3B), we identified their specific binding sites. According to the predicted binding ability of different regions of LINC00839 with Ruvb1 (Fig EV2B), we constructed different fragments of LINC00839 and performed RNA pull-down experiment, and the RNA hairpin structures were mostly preserved in each fragment we designed. The results showed that only nucleotides 775-1548 of LINC00839 binds to Ruvb1 (Fig 3D). To further narrow down the specific binding sites, the nucleotides 775-1548 was further divided into three fragments. The RNA pull-down experiment indicated that nucleotides 1033-1290 of LINC00839 binds to Ruvb1 (Fig 3D). Importantly, LINC00839 with nucleotides 1033-1290 delated showed little binding ability to Ruvb1 (Fig 3E). These data indicated that nucleotides 1033-1290 of LINC00839 binds to RUVB1. I-TASSER software from Zhang's lab was used to predict the 3D structure of the Ruvb1 protein, and the C-score of the predicted model was −0.59, which signifies model accuracy with a high degree of confidence. Then, we further used RNA fold and structure software to predict the secondary structure of LINC00839.

Fig EV2C visualizes the structure of the LINC00839-Ruvb1 binding complex. The construction of the binding model was based on the experiments and

on-line prediction. The corresponding modification and statement are described on page 9, line 200-220.

7. The authors reported that MG149, a Tip60-selective inhibitor, repressed NRF1 expression level. It was observed in Fig. 5J, but not in Fig. S7G. Thus, the result is not reproducible.

Response:

Thanks for your comment. The NRF1 expression was repressed with MG149 treated. But it was not easily identified as the qualification was missing. We have repeated our experiment considering your comment, and the qualification was also performed to confirmed the changes. The result showed that NRF1 expression was repressed after the treatment of MG149 (Fig EV4K).

8. The authors reported that the NRF1 knockdown in LINC00839-expressing cells inhibited mitochondrial biogenesis and OXPHOS (Supplementary Fig. S7C-F), but the immunoblotting indicated that almost all amount of NRF1 still remained in the siNRF1 treated samples (Fig. S7C). Thus, the result does not support their conclusion. The authors also performed siNRF1 treatment in Fig. 6C, but they did not show the NRF1 level, and the knockdown is not confirmed. Thus, relationship between NRF1 and EMT markers are unclear. The authors also used siNRF1 for in vivo experiments but there is no description about the experimental design and no confirmation about the knockdown efficiency in vivo (Fig. 6 E-I). Taken together, roles of NRF1 in CRC is unclear.

Response:

Thanks for your comments. Although the NRF1 was still remained in the siNRF1 treated samples, it is knocked down significantly (the quantification data has not provided previously). The validation was performed before the experiments in vitro and in vivo. We have supplied the data in Fig EV3J and repeated the experiments. The results were consistent with previous studies.

Furthermore, we clarified the details about study design of in vivo experiments in the corresponding results section (page 17, lines 371-378) the knockdown efficiency was confirmed by WB before experiments. The experiment was performed using shRNA. The shRNA was constructed with the siRNA sequences validated before. Taken together, these results demonstrated that NRF1 plays a key role in mitochondrial biogenesis and OXPHOS, and CRC progression induced by LINC00839.

9. The manuscript is poorly organized throughout. Not only there are typos, graphs are frequently mislabeled as describe below, and discussion sections appeared twice: one is shorter and the other is longer version!

Response:

Thanks for your correction. In light of the reviewers' comments and suggestions, we have supplied and reorganized the manuscript thoroughly. All statement and graphs were checked carefully. We also revised the discussion section seriously. We think the modified version has improved greatly.

Minor points:

1. In Fig. 3C, the loading control is not equal, unlike the author's claim. Quantification of the signals would be helpful.

Response:

Thanks for your comment. We have quantified the strephtavidin-HRP signal. The quantification did not change the result and conclusion.

2. In Fig. 3B and G, the label "expression" is inappropriate for the RIP assay. Also, authors should analyze other non-relevant RNAs to show the specificity.

Response:

Thanks for your comment. We have replaced the label “expression” with “RNA enrichment (RIP/IgG)”. In the meantime, we also selected “U6” and “GAPDH” as negative control primers to demonstrated the specificity. The new data was stated in the “Results” part (page 9, line 202) and shown in Fig 3C.

3. In Figs. S2E and S3G, the labels of 0 H and 24 H may be opposite.

Response:

Thanks for your correction and sorry for our careless. We have corrected the corresponding labels.

4. In Fig. 4G, the label "Absorbance" is inappropriate for representing the

NAD/NADH ratios.

Response:

Thanks for your correction. We have calculated the NAD/NADH ratios according to the manual and replaced the label with “NAD/NADH ratios”.

5. In the manuscript, line 409 to 411, the authors wrote "only H4K5ac and H4K8ac can bind to NRF1 gene promoter DNA, especially in the P2 (-324~-174 bp) and P3 (-474~-325 bp) regions", but this pattern is not observed in the vector control. The authors should mention that they compared vector control and LINC00839 over expression.

Response:

Thanks for your suggestion. Our previous description was not serious and we have revised our statement. Ruvb1 and Tip60 were enriched around NRF1 promoter, especially P2 (-324~-174 bp) and P3 (-474~-325 bp), in cells overexpressing LINC00839 compared with control (Fig EV3F-G). The corresponding revision is on page 14, line 316.

6. In the manuscript, line 409 to 411, the authors wrote "only H4K5ac and H4K8ac can bind to NRF1 gene promoter DNA, especially in the P2 (-324~-174 bp) and P3 (-474~-325 bp) regions", but this pattern is not observed in the vector control. The authors should mention that they compared vector control and LINC00839 over expression. In addition, the description can be changed as "the nucleosomes at the NRF gene promoter is acetylated as

H4K5ac and H4K8ac".

Response:

Thanks for your suggestion. Our previous description was not serious and we have revised our statement as follows. Ruvb1 and Tip60 were enriched around NRF1 promoter, especially P2 (-324~-174 bp) and P3 (-474~-325 bp), in cells overexpressing LINC00839 compared with control (Fig. EV3F-G). Furthermore, the nucleosomes at the NRF1 gene promoter were acetylated as H4K5ac and H4K8ac instead of H4K12ac and H3K14ac (Fig 4K-L and Fig EV3H-I). The revised statement in on page 14, line 316-321.

Dear Prof. Zhou,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the referees that were asked to re-evaluate your study, you will find below. As you will see, referees #1 and #3 now fully support the publication of your study in EMBO reports. Referee #3 has a remaining concern I ask you to address in a final revised manuscript. Referee #2 now indicates that the manuscript is better suited for a more specialized journal. However, as this referee has not brought up this point in his/her previous assessment, and as no reasons for this view are mentioned, I decided to proceed with publication of the study.

Moreover, I have these editorial requests I also ask you to address:

- Please provide a more comprehensive title with not more than 100 characters (including spaces) describing what LINC00839 actually does.

- Please provide the abstract written in present tense.

- We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

- Please make sure that all figure panels are called out sequentially (as they show up in the figure) and separately. Please check or change the order of the panels in the figures. Presently, Fig. EV2F is called out after Fig EV3, and Fig EV3J is called out after Fig EV4. Please check.

- Please add scale bars of similar style and thickness to the microscopic images (main, EV and Appendix figures), using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend. Presently, the scale bars for many images are too thin or hard to see.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main, EV and Appendix figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.

- As the Western blots shown are significantly cropped, please provide the source data for the blots. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data for all the Western blots shown in the main and EV figures (scans of entire blots) together with the final revised manuscript. Please include size markers for the scans of entire blots, label the scans with figure and panel number, and send one PDF file per figure.

- Appendix Tables S2 and S3 are better displayed as datasets. Please upload the original excel files as Dataset EV1 and Dataset EV2. Please put a name and a legend for these on the first TAB of the excel file. In Table S2 part of the data or text is not legible (e.g. ####### in the last column) or partly cropped. Please make sure all is legible. Finally, please change the callouts for these tables to Dataset EV1 and Dataset EV2 in the manuscript text.

- Appendix Tables S1 and S4-S6 should be renumbered and compiled in an Appendix file. Please upload this Appendix as finally formatted pdf file with page numbers and a table of contents on the first page.

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four short bullet points highlighting the key findings of your study.
- a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

I look forward to seeing the final revised version of your manuscript when it is ready. Please let me know if you have questions

regarding the revision.

Best,

Achim Breiling
Senior Editor
EMBO Reports

Referee #1:

In this revised manuscript, Liu et al. have reasonably addressed all my previous comments. I therefore recommend publication of this manuscript.

Referee #2:

The authors seem to have addressed this reviewer's comments. However, this manuscript seems like it could be better suited for a more specialized journal since it may not be interesting for a general audience.

Referee #3:

The authors have addressed most of the points I raised. There is still one thing to be cleared. They need to provide quantification of NRF1 expression in Fig. EV4K and EV3J. In their responses, they claim as if they quantified this time. However, I could not find the actual values in those figures. It is especially not clear whether NRF1 is repressed with MG149, in LoVo cells (Fig. EV4K).

Point-by-point response to comments of Senior Editor and Reviewers

Senior Editor:

Please provide a more comprehensive title with not more than 100 characters (including spaces) describing what LINC00839 actually does.

Response:

Thanks for your suggestion. We have revised our title. The new title is "LINC00839 promotes colorectal cancer progression by recruiting RUVBL1/Tip60 complex to activate NRF1".

Please provide the abstract written in present tense.

Response:

Many thanks for your kindly suggestion. We have revised the abstract and the corresponding amendment can be found on page 3, line 44-59.

We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

Response:

Thanks for your reminding. We have reorganized the competing interests. The corresponding amendment can be found on page 36, line 737.

Please make sure that all figure panels are called out sequentially (as they show up in the figure) and separately. Please check or change the order of the panels in the figures. Presently, Fig. EV2F is called out after Fig EV3, and Fig EV3J is called out after Fig EV4. Please check.

Response:

Thanks for your correction. We have reorganized the Expanded View figures. Now all figure panels are called out sequentially.

Please add scale bars of similar style and thickness to the microscopic images (main, EV and Appendix figures), using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend. Presently, the scale bars for many images are too thin or hard to see.

Response:

Thanks for your reminding. We have added scale bars of similar style and thickness to the microscopic images.

Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main, EV and Appendix figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams. Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant.

Response:

Thanks for your suggestions. In light of your constructive suggestion, we have reconsidered our statement and revised the manuscript accordingly, and we have indicated this in the corresponding figure legends. The corresponding amendment can be found in the revised manuscript.

As the Western blots shown are significantly cropped, please provide the source data for the blots. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. Please submit the source data for all the Western blots shown in the main and EV figures (scans of entire blots) together with the final revised manuscript. Please include size markers for the scans of entire blots, label the scans with figure and panel number, and send one PDF file per figure.

Response:

Thanks for your comment. We have included all the source data of western blots in the main and EV figures and showed it in the PDF files.

Appendix Tables S2 and S3 are better displayed as datasets. Please upload the original excel files as Dataset EV1 and Dataset EV2. Please put a name and a legend for these on the first TAB of the excel file. In Table S2 part of the data or text is not legible (e.g. ####### in the last column) or partly cropped. Please make sure all is legible. Finally, please change the callouts for these tables to Dataset EV1 and Dataset EV2 in the manuscript text.

Response:

Thanks for your suggestion. We have displayed the Appendix Tables S2 and S3 as Dataset EV1 and Dataset EV2 and change the callouts for these tables in the manuscript text. The corresponding amendment can be found on page11-12 and page14.

Appendix Tables S1 and S4-S6 should be renumbered and compiled in an Appendix file. Please upload this Appendix as finally formatted pdf file with page numbers and a table of contents on the first page.

Response:

Thanks for your suggestion. We have renumbered and compiled in an Appendix file and change the callouts for these tables in the manuscript text.

Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file with track changes, in order that we can see any modifications done.

Response:

Thanks for your reminding. We have revised the manuscript with track changes.

a short, two-sentence summary of the manuscript (not more than 35 words)

Response:

Many thanks for your kindly suggestion. We have added the summary of the manuscript in the Hightlights file.

two to four short bullet points highlighting the key findings of your study.

Response:

Many thanks for your kindly suggestion. We have added four short bullet points in the Hightlights file.

a schematic summary figure (in jpeg or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

Response:

Many thanks for your kindly suggestion. We have added a schematic summary figure of the manuscript.

Referee #1:

In this revised manuscript, Liu et al. have reasonably addressed all my previous comments. I therefore recommend publication of this manuscript.

Response:

Great thanks for your kind approval on my manuscript.

Referee #2:

The authors seem to have addressed this reviewer's comments. However, this manuscript seems like it could be better suited for a more specialized journal since it may not be interesting for a general audience.

Response:

Thanks for your comment.

Referee #3:

The authors have addressed most of the points I raised. There is still one thing to be cleared. They need to provide quantification of NRF1 expression in Fig. EV4K and EV3J. In their responses, they claim as if they quantified this time. However, I could not find the actual values in those figures. It is especially not clear whether NRF1 is repressed with MG149, in LoVo cells (Fig. EV4K).

Response:

Thanks for your insightful suggestions. We have revised our Expanded View figures according to reviewer's comments. The quantification data of knockdown NRF1 was shown in the Fig EV4A. And we have repeated our

experiment considering your comment, and the qualification was also performed to confirmed the changes. The result showed that NRF1 expression was repressed after the treatment of MG14 (Fig EV4G). The quantification data of NRF1 expression was repressed after the treatment of MG14 was also showed in Figure_EV4_Source_Data.

Dear Prof. Zhou

Thank you for the submission of your further revised manuscript to our editorial offices. I now went through the manuscript again and I think the remaining points of referee have been adequately addressed.

Before we can proceed with formal acceptance, I have these further editorial requests:

- I would suggest this slightly amended title:
LINC00839 promotes colorectal cancer progression by recruiting RUVBL1/Tip60 complexes to activate NRF1

- Could smaller manuscript files be provided? Some figure files (also some of the source data files) are exceedingly large and the merged manuscript file has now nearly 800 MB, which is difficult to handle. Please try to reduce the file sizes.

- Please have your final manuscript text file carefully proofread by a native speaker. There are several typos and grammatical errors that need to be fixed.

- Thanks for providing the Western blot source data. However, this needs to be better organised. Please group the blots according to the sub-panels in the figure (i.e. group those together that belong to one cell type - SW480 or LoVo - and label these accordingly) and in the order they show up in the figure. Presently this is not always the case, and it is hard to find out which SD blot belongs to which figure blot. Please also make sure that the boxes indicating the area in the final figure panel indeed match that area (see e.g. SD for Fig. 3), and that the source data blots are correctly labelled (presently SD for Fig. 4F is e.g. labelled 4I). Moreover, please show the blots in the figure in the same contrast and brightness as in the source data. Presently this is not the case, and it is often impossible to see if the source data indeed fits to the blot in the figure. Please carefully check that the right blots are shown in the source data. Finally, could also source data for the blots in Fig. 3B/D/E and EV2D be provided? Please upload all the source data files for one figure (main and EV figures) as separate files.

I look forward to seeing the final revised version of your manuscript when it is ready. Please let me know if you have questions regarding the revision.

Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best,

Achim Breiling
Senior Editor
EMBO Reports

Point-by-point response to comments of Senior Editor and Reviewers

Senior Editor:

Thank you for the submission of your further revised manuscript to our editorial offices. I now went through the manuscript again and I think the remaining points of referee have been adequately addressed.

Before we can proceed with formal acceptance, I have these further editorial requests

Response:

Great thanks for your kind approval on my manuscript.

I would suggest this slightly amended title:

LINC00839 promotes colorectal cancer progression by recruiting RUVBL1/Tip60 complexes to activate NRF1

Response:

Many thanks for your kindly suggestion and your constructive correction.We have revised the title.

Could smaller manuscript files be provided? Some figure files (also some of the source data files) are exceedingly large and the merged manuscript file has now nearly 800 MB, which is difficult to handle. Please try to reduce the file sizes.

Response:

Thanks for your reminding. We have smaller manuscript files and the merged manuscript file has now nearly 275 MB.

Please have your final manuscript text file carefully proofread by a native speaker. There are several typos and grammatical errors that need to be fixed.

Response:

Thanks for your reminding. In light of your constructive suggestion, the manuscript text file carefully proofread by AJE. And we believe that these revisions have substantially strengthened our paper.

Thanks for providing the Western blot source data. However, this needs to be better organised. Please group the blots according to the sub-panels in the figure (i.e. group those together that belong to one cell type - SW480 or LoVo - and label these accordingly) and in the order they show up in the figure.Presently this is not always the case, and it is hard to find out which SD blot belongs to which figure blot. Please also make sure that the boxes indicating the area in the final figure panel indeed match that area (see e.g. SD for Fig. 3), and that the source data blots are correctly labelled (presently SD for Fig. 4F is e.g. labelled 4I).

Response:

Thanks for your suggestions and your correction. We have reorganised the Western blot source data, and the blots according to the sub-panels in the figure have been grouped together that belong to the cell type. We also have revised and coreected the source data blots.

Moreover, please show the blots in the figure in the same contrast and brightness as in the source data. Presently this is not the case, and it is often impossible to see if the source data indeed fits to the blot in the figure. Please carefully check that the right blots are shown in the source data.

Response:

Thanks for your suggestions. We also have revised the source data blots and now the source data indeed fits to the blot in the figure,and the blots in the figure in the same contrast and brightness as in the source data.

Finally, could also source data for the blots in Fig. 3B/D/E and EV2D be provided? Please upload all the source data files for one figure (main and EV figures) as separate files.

Response:

Thanks for your comment. We have provided source data for the blots in Fig. 3B/D/E and EV2D, and all the source data of western blots in the main and EV figures and showed it in the PDF files.

Prof. Jun Zhou
Southern Medical University
1838 North Guangzhou Avenue
Guangdong 5010515
China

Dear Prof. Zhou,

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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Yours sincerely,

Achim Breiling
Editor
EMBO Reports

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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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 χ2 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.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Materials and methods,page 29

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and methods,page 26

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	Data Availability Section)

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	Materials and methods,page 24
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	Materials and methods,page 24

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	Materials and methods,page 28
Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Yes	Materials and methods,page 28

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Not Applicable	

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Materials and methods

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Yes	Materials and methods

Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	Yes	Materials and methods
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Yes	Materials and methods
Include a statement about **blinding** even if no blinding was done.	Yes	Materials and methods
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Materials and methods
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figure Legends

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Figure Legends
In the figure legends: define whether data describe **technical or biological replicates**.	Yes	Figure Legends

Ethics

Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving **human participants**: 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.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Yes	Materials and methods
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Yes	Materials and methods

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Yes	Materials and methods
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.	Yes	Materials and methods
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.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	**Data Availability Statement**
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Yes	Data Availability Statement
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	