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An alternatively transcribed TAZ variant negatively regulates JAK-STAT signaling

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14 November 2018

Thank you for the submission of your research manuscript to EMBO reports. We have now received 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, all referees think the manuscript is of high interest, but requires a major revision to allow publication in EMBO reports. All three referees have a number of concerns and/or suggestions to improve the manuscript, which we ask you to address in a revised manuscript. As the reports are below, I will not detail them here. However, we consider it essential that the physiological relevance of the findings is significantly strengthens (as also indicated by referee #3). In particular, we would require further evidence that cTAZ is expressed and can be detected at the protein level in vivo, an not only in cultured cells (e.g. in virus-infected tissues of animals - see comment by referee #3 - or in human samples).

Given the constructive referee comments, I would like to invite you to revise your manuscript with the understanding that all referee concerns must be addressed in the revised manuscript and/or 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 or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

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

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5

images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature.

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Please also format the references according to EMBO reports style. See:
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Regarding data quantification and statistics, can you please specify, where applicable, the number "n" for how many independent experiments (biological replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values in the respective figure legends. Please provide statistical testing where applicable. See:
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We now strongly encourage the publication of original source data with the aim of making primary data more accessible and transparent to the reader. The source data will be published in a separate source data file online along with the accepted manuscript and will be linked to the relevant figure. If you would like to use this opportunity, please submit the source data (for example scans of entire gels or blots, data points of graphs in an excel sheet, additional images, etc.) of your key experiments together with the revised manuscript. Please include size markers for scans of entire gels, label the scans with figure and panel number, and send one PDF file per figure.

Please also note that we now mandate that all corresponding authors list an ORCID digital identifier that is linked to his/her EMBO reports account!

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

REFEREE REPORTS

Referee #1:

The manuscript entitled "An alternatively transcribed TAZ variant negatively regulates JAK-STAT signaling" describes the novel role of a short TAZ isoform, cTAZ. TAZ is characterized to be a transcriptional co-activator in the Hippo signaling pathway; nevertheless, this short TAZ isoform is not involved in Hippo signaling. Instead, Fang et al. show that cTAZ negatively regulates JAK/STAT signaling in cultured cells. By employing extensive biochemistry, the authors demonstrate that cTAZ inhibits STAT1/2 nuclear localization presumably via forming a complex with STAT1/2. Additionally, cTAZ expression is controlled by type I interferon signaling, and STAT1 is shown to be a direct regulator of cTAZ expression. Thus, the authors propose that cTAZ functions as a negative feedback regulator of JAK/STAT signaling to fine-tune cellular antiviral response.

Overall, the findings, expanding the function of TAZ beyond the canonical role in the Hippo signaling pathway, are quite novel and of broad interest to the readers in the field of immunology, cancer biology, and/or cell signaling. Although lack of in vivo studies prompts follow-up investigations, the authors have thoroughly addressed their claims by performing experiments in multiple ways. The manuscript should be appropriate for publication if the following comments are addressed.

Major comments:

1.Presumably, full-length TAZ can also interact with STATs as the STAT-interacting domain is present in TAZ. Additionally, proximity labeling with TAZ and YAP identified STAT1. Did the authors test whether TAZ and YAP can also interact with STATs and inhibit their function? If they act redundantly, how would the authors interpret the cTAZ knockout phenotypes?
2.A set of genes is differentially expressed by loss and gain of cTAZ. The authors nicely validated the RNA-seq results by performing qRT-PCR. However, it is not certain whether the changes in mRNA levels can be reflected to the changes in protein levels. For instance, STAT1 mRNA levels are increased by cTAZ loss (Fig. 2C). However, the STAT1 protein levels don't appear to be increased in cTAZ knockout cells (Fig EV5A).

Minor comments:

1.The authors should provide more detailed information on the antibodies used in the study.
2.How RNA-seq data were analyzed to identify differentially expressed genes and enriched gene ontologies is not described.
3.There is no information on the error bars.
4.TBK1 needs to be defined.
5.Figure 6 is not mentioned in the main text.
6.A few typos: page 12, 'proof' → 'prove'; Fig EV6E legend, 'delated' → 'deleted'; Fig EV6G legend, 'protentiates' → 'potentiates'.

Referee #2:

The authors very originally find a novel and previously uncharacterized isoform of TAZ (cTAZ). It is transcribed by an alternative promoter, it is expressed in several cell lines and it lacks the N-terminal portion of the longer and more characterized isoform. Structurally, this isoform lacks the domains required for the interaction with either LATS1/2 or with TEAD. Functionally the canonical Hippo pathway doesn't modulate the cTAZ protein. It does not modulate oncogenic TEAD target gens, nor it modulates cell proliferation or EMT, differently from YAP and full-length TAZ. Indeed, the authors show for cTAZ a peculiar function in the inhibition of the antiviral JAK/STAT mediated response to IFNs, eventually inhibiting the expression of ISGs normally induced by IFNs. Mechanistically, the authors show that cTAZ binds to STAT1 and inhibits STAT1/2 dimerization and its interaction with importin5, thus reducing its nuclear translocation. Moreover, the authors show that viral infection induces the expression of cTAZ transcript and protein as a negative feedback loop. In the discussion section, the authors provide a fascinating evolutionary explanation for the lack of any oncogenic function of cTAZ. If viral infection would modulate a protein involved in tumorigenesis, an infected cell would then transform in an oncogenic fashion, and this would not be evolutionarily selected. Instead, it is likely that the evolution may have selected a TAZ isoform that has been exclusively specialized in the negative modulation of the antiviral response as a control mechanism necessary to avoid aberrant antiviral response that needs to be finely tuned. The work is novel and original and a lot of experiments have been well performed. However, the authors should address some points in order to render the work suitable for publication in EMBO REPORTS.

1. It is no clear how do the author construct a plasmid with a C-terminal HA-tagged YAP/TAZ. What does it mean? How is it possible to co-express YAP and TAZ in the same construct? Explain better than in the presented form.

2. Describe better figure 1F-H. It is not clear from the figure which are the primers targeting the novel 5'UTR (highlight it graphically). Moreover, is the aminoacidic sequence 145-400 encoded by the PCR product shown in figure 1G? Also, specify better that the only difference between the canonical exon in the aminoacidic sequence 145-400 and the alternative exon is in two aminoacids, and that this difference requires the novel identified 5'UTR. From the text, it is no clear.

3. It is also not clear how do the authors conclude that cTAZ is not produced by alternative splicing after the comparison of TAZ and cTAZ levels from RNA-seq and GTEx database analysis, or after comparison of the two protein isoform in different cell lines. These analyses only suggest that there is no correlation

between the two isoforms. They do not exclude the possibility of an alternative splicing.

4. In figure EV2C,D, it is not clear how do the authors discriminate between total or phosphorylated TAZ. From figure EV2C and D it is not possible to appreciate a reduction of total TAZ or an increase of phosphorylated TAZ upon serum starvation. It seems that total TAZ is increased, conversely to total YAP that is decreased, upon serum starvation. The authors should explain better this result. Also, specify in the text what are the residues that are phosphorylated in YAP and TAZ, respectively, upon serum starvation (there is more than one residue) and their position in the different long and short TAZ isoforms.

5. Figure EV2E does not suggest that cTAZ is not regulated by LATS1/2 or upstream signals. It only suggests tat it is more stable than full-length TAZ. Indeed, from that figure, YAP is more stable than both TAZ and cTAZ, but the previous figures show that instead YAP is regulated both by serum starvation and by LATS1/2 phosphorylation. The authors should clarify this point.

6. In figure EV3B, the authors show that the luciferase activity of 8XGTIIC reporter is not increased by cTAZ overexpression. However, by western blot analysis, it seems that Cyr61 protein expression is instead increased in the same conditions. Moreover, the authors should show a longer exposure of TAZ blot in hEK293t cells to show also endogenous full-length TAZ in control cells and cTAZ-transfected cells.

7. It was not possible to read legends relative to figures EV

8. In figure 2A ad EV4A it is not clear how do the authors knockdown specifically cTAZ without knocking down TAZ full length. Moreover, the authors should add as an important control cells knocked down for or overexpressing full lenght TAZ and analyze the genes that are commonly deregulated upon full length TAZ and cTAZ interference or overexpression and those that are specifically deregulated upon cTAZ interference or overexpression.

9. Figure EV4 A and B are not clear. In Figure EV4A, it seems that RKO cells are interfered for endogenous cTAZ, and then they are transfected for overexpression of exogenous full-length TAZ. How can the authors state that the analyzed genes are regulated by cTAZ and not by lull length TAZ? Control suggested in comment 8 could clarify this.

10. Add raw data (list of single genes, and genes in the indicated categories) about the analysis shown in figure 2A and 2B

11. Figure 2D and figure EV4C are not clear at all. It is not clear the rationale, what is ISRE? what is constitutively active RIG-I?

12. Page 10. Specify (at least in the discussion section) that in the work of Wang et al., both transcriptionally active or inactive YAP isoforms regulate the immune response in their experimental settings.

13. Figure EV4C,D and 2G are not clear at all. Explain better in the text and use more clear legends in the figure. It is difficult to appreciate what the authors are showing in the figure and its meaning. Moreover, JAK/STAT signaling is described very superficially in the paragraph without an appropriated introduction. Spend more words on it. Also the sentence "Taken together, these results suggest that cTAZ can regulate the robustness of JAK/STAT signaling directly" is not enough supported in my opinion, it can be mentioned only after in the manuscript.

14. In figure EV5A, the author show a longer exposure blot showing that upon cTAZ interference, the abundance of full length TAZ is not affected. Moreover, from the blot, it appears that IFN treatment increases the expression of cTAZ in RKO cells (in fact later the authors show that viral infection induces cTAZ transcript and protein). Please, discuss on it.

15. Page 11, line 1 contains an imprecision: T705 of STAT1 is not the phosphorylation of other STAT proteins it is the phosphorylation of another residue of STAT1. Authors should correct this sentence.

16. In the material and methods section, the authors need to describe how the proximity-dependent biotin identification has been performed.

17. Figure EV5E: be sure that residues 137-317 of STAT1 are not required for cTAZ-STAT1 interaction. Again, in these experiments, analyze the interaction between full length TAZ and STAT1 as a control.
18. In each panel of figure EV5, specify which protein is truncated of the indicated aminoacids (STAT1 or cTAZ) in order to avoid confusion

19. In the material and methods section, describe how cTAZ-/- cells are obtained in RKO cells and in other cell lines in Figure 3C.

20. For each immunoprecipitation experiment, specify better whether the authors are analyzing endogenous or overexpressed proteins

21. In Figure 3C it is not clear whether in the blot the authors are showing TAZ full-length or cTAZ.

22. Figure 3E is not described correctly: in cTAZ-/_ cells, nuclear STAT1/2 is increased, not decreased

23. In figure 3F, quantify the nuclear STAT1 and describe in the material and methods section how quantification has been obtained

24. In figure 4A, indicate how many events have been used for the quantification of viral infection in the immunofluorescence. Same comment for figure 4A and the other immunofluorescence shown in the manuscript.

25. In figure 5F it is difficult to discriminate the enrichment of STAT1 on cTAZ promoter. Please provide a better figure

26. In the discussion section, the authors state that cTAZ does not modulate the production of IFNs. In order to support this notion, the authors should show the production of IFNs in cells were cTAZ levels are upregulated or downregulated.

27. In order to translate the molecular findings of this work into clinics, would it be possible to analyze the expression of cTAZ transcript in a published dataset of patients affected by specific viral infection and correlate the expression of cTAZ with their prognosis? Moreover, the authors show that in vitro cTAZ is not responsible of the oncogenic proliferative or metastatic phenotype, and it is not regulated by canonical Hippo pathway, nor it interacts with TEAD in the regulation of oncogenic targets, differently to YAP and full length TAZ. Would it be possible also to analyze the expression of cTAZ transcript in public available dataset of cancer patients in order to exclude an oncogenic role of cTAZ?

Referee #3:

The manuscript investigates the role of a short form (cTAZ) of the Hippo pathway protein TAZ. The novelty of the manuscript lies in showing that this short form does not act in the context of Hippo signaling as might be expected, but rather as a negative regulator of interferon (IFN)-induced Jak-Stat signaling. Specifically the authors provide data suggesting that cTAZ interferes with the association of STAT1 and STAT2 and, as a consequence, with STAT1 binding to Importin-a5 which is needed for nuclear translocation. The authors propose a negative feedback loop based on cTAZ expression and action in response to IFN signaling.

General comment: Collectively the data in the paper support the notion that cTAZ exerts an inhibitory activity on IFN-induced Jak-Stat signal transduction. They do not support the concept of a negative feed-back loop given that the induction of cTAZ mRNA by IFN is 1.5-fold under optimal conditions (figure 5, the model in figure 6 lacks the IRF9 subunit). The relationship of the cTAZ promoter analyzed in figure 5 to the activating histone marks in figure 1F remains unclear. The position of the critical binding sites appears inconsistent with the position of the histone marks relative to the alternative exon containing the translation start.

The biological relevance of the study is limited by the fact that much of the data is generated with vastly overexpressed proteins in 293 cells. Moreover, the authors show that 70% of the investigated cell lines

don't express cTAZ. Does this mean their response to IFN is stronger? Can these cells be induced to express cTAZ or does a cTAZ knockout affect their response to IFN? Do they contain the activating histone marks reported in figure 1F? Is cTAZ found expressed in virus-infected tissues in animals? Some of these questions could easily be addressed.

The manuscript contains some inconsistencies:
- the Irf 7 gene, a well-established and highly induced ISGF3 target is hardly affected by the cTAZ knockout.
- the sentence 'about 50% Stat1 protein was localized ...' (bottom of page 11) suggests that less ISGF3 translocated to the nucleus in cTAZ-/- cells. This is inconsistent with the main hypothesis of the paper.
- the w-blot in figure 3B shows Flag-Stat1 in the cell lysate. However, no Flag-Stat1 was precipitated with anti-Flag and no Flag-Stat1 was transfected according to the figure labeling. How is this explained?

Technical comments:
1. Fig. EV2E: the difference in stability between TAZ and cTAZ is marginal. Cells with-or without starvation should be compared.
2. Fig. EV3B: upregulation of the reporter by wt TAZ is barely 2-fold, questioning biological relevance.
3. Fig. 3: cell fractionation studies would be a more convincing way of quantifying the nuclear translocation of STATs.
4. Why were the antiviral assays shown in figure 4A performed with overexpressed Stat1/2? Why does cTAZ expression not reduce the activity of endogenous Stat1/2 (compare panels 2 and 4)?

Length:
- the part dealing with IFN-inducibility of cTAZ should be ommitted because the data is unconvincing. Similarly, the EVFs contain data that are redundant with the original figures and should be deleted from the manuscript.

1st Revision - authors' response 14 February 2019

Response to reviewers' comments
Fang et al., 2019
*Response in **blue**;
*all updates in figures were summarized in a table attached at the end of this letter

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Referee #1:

The manuscript entitled "An alternatively transcribed TAZ variant negatively regulates JAK-STAT signaling" describes the novel role of a short TAZ isoform, cTAZ. TAZ is characterized to be a transcriptional co-activator in the Hippo signaling pathway; nevertheless, this short TAZ isoform is not involved in Hippo signaling. Instead, Fang et al. show that cTAZ negatively regulates JAK/STAT signaling in cultured cells. By employing extensive biochemistry, the authors demonstrate that cTAZ inhibits STAT1/2 nuclear localization presumably via forming a complex with STAT1/2. Additionally, cTAZ expression is controlled by type I interferon signaling, and STAT1 is shown to be a direct regulator of cTAZ expression. Thus, the authors propose that cTAZ functions as a negative feedback regulator of JAK/STAT signaling to fine-tune cellular antiviral response.

Overall, the findings, expanding the function of TAZ beyond the canonical role in the Hippo signaling pathway, are quite novel and of broad interest to the readers in the field of immunology, cancer biology, and/or cell signaling. Although lack of in vivo studies prompts follow-up investigations, the authors have thoroughly addressed their claims by performing experiments in multiple ways. The manuscript should be appropriate for publication if the following comments are addressed.

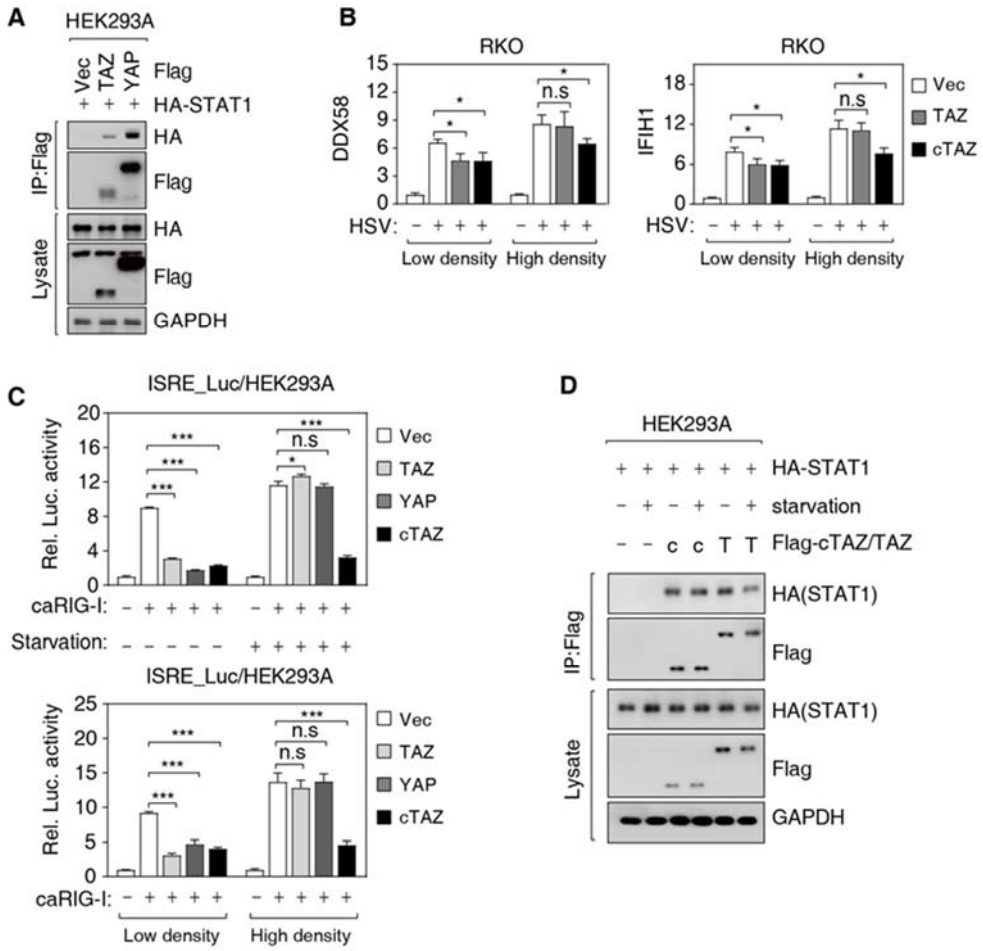
We thank this reviewer for recognizing the novelty and broad interest of our study, and for her/his valuable comments. In this revision, we have addressed all concerns from this reviewer, and we have also showed that cTAZ was present *in vivo* in mouse and human lymph nodes and mouse thymus (new Figure EV1C and D).

Major comments:

1.Presumably, full-length TAZ can also interact with STATs as the STAT-interacting domain is present in TAZ. Additionally, proximity labeling with TAZ and YAP identified STAT1. Did the authors test whether TAZ and YAP can also interact with STATs and inhibit their function? If they act redundantly, how would the authors interpret the cTAZ knockout phenotypes?

To test a potential link between full-length YAP/TAZ and STAT1, we performed co-immunoprecipitation (Co-IP), mRNA expression of interferon-stimulated genes (ISGs), and interferon-sensitive response element (ISRE) promoter luciferase assays under various conditions in the presence of full-length YAP/TAZ (New Appendix Fig S3, also

see below). Similar to BioID experiments, full-length YAP and TAZ could interact with STAT1 in a Co-IP assay (panel A below).



As we shown in the manuscript (new Figures EV2), the major difference between cTAZ and full-length YAP and TAZ was their response to Hippo pathway kinases and upstream signals. We therefore tested the effect of cTAZ and full-length YAP/TAZ on the expression of ISGs and ISRE promoter activities under different cell densities and serum concentrations (two robust upstream signals of the Hippo pathway[1, 2]. Interestingly, under low cell density or serum rich conditions, the effect of cTAZ and full-length YAP/TAZ on the expression of ISGs and ISRE promoter activity was indistinguishable; however, under high cell density and serum starvation conditions, cTAZ remained effective whereas full-length YAP/TAZ was unable to repress ISG expression or ISRE promoter activity (panels B and C above). The effect of full-length YAP towards ISG expression in a context-dependent manner was consistent with a previous report [3]. These results suggested that, compare to full-length YAP/TAZ, cTAZ might regulate antiviral response under diverse physiological and/or pathological conditions.

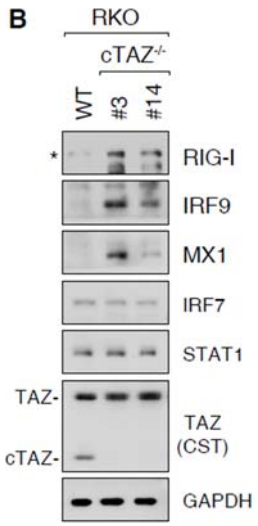
Our results suggest that full-length YAP/TAZ and cTAZ are not fully redundant. However, the exact mechanisms underlying the differences between cTAZ and full-

length YAP/TAZ are not fully understood. We showed that the interaction between STAT1 with full-length TAZ, but not cTAZ, was sensitive to serum starvation (Panel D above). The phosphorylation of full-length TAZ by Hippo pathway kinases may interfere with its interaction with STAT1. Therefore, the effect of cTAZ in antiviral response remains unchanged upon Hippo pathway activation.

Cells in animal tissues are normally densely packed, and the availability of serum factors is limited. High cell density or serum starvation are more similar to physiological conditions. Hence, our new results underscore a general role of cTAZ under physiological settings, and this further increases the novelty and relevance of our study. We have discussed the differences between full-length YAP/TAZ and cTAZ in the revised manuscript.

2.A set of genes is differentially expressed by loss and gain of cTAZ. The authors nicely validated the RNA-seq results by performing qRT-PCR. However, it is not certain whether the changes in mRNA levels can be reflected to the changes in protein levels. For instance, STAT1 mRNA levels are increased by cTAZ loss (Fig. 2C). However, the STAT1 protein levels don't appear to be increased in cTAZ knockout cells (Fig EV5A).

To analyze protein levels of ISGs, we obtained additional antibodies and performed immunoblotting assays. The protein level of RIG-I, IRF9, and MX1 was significantly increased in cTAZ knockout cells, whereas the expression of STAT1 and IRF7 was not changed (new Figure EV4B; also see below). The differences between mRNA and protein levels might be caused by two reasons: 1) The increase of STAT1 and IRF7 mRNA levels in cTAZ knockout cells was milder than that of RIG-I (DDX58), IRF9, and MX1 genes; 2) The basal protein level of STAT1 and IRF7 was higher, and the increase of mRNA expression had less impact on their protein level.



Minor comments:

1.The authors should provide more detailed information on the antibodies used in the study.

The source (Vendors and Catalog numbers) and dilution factors used for all antibodies were described in this revision (Appendix Table S6).

2.How RNA-seq data were analyzed to identify differentially expressed genes and enriched gene ontologies is not described.

We added details about RNA-seq data analysis in the revised Methods (revised manuscript Page 24), RNA-seq data was also shown in Appendix Table S3.

3.There is no information on the error bars.

We described error bars in Methods (revised manuscript Page 26) and Figure Legends when needed.

4.TBK1 needs to be defined.

TBK1 is defined as TANK Binding Kinase 1 in this revised manuscript (revised manuscript Page 5).

5.Figure 6 is not mentioned in the main text.

We cited this Figure (new Figure 5G) in this revision (revised manuscript Page 18).

6.A few typos: page 12, 'proof' → 'prove'; Fig EV6E legend, 'delated' → 'deleted'; Fig EV6G legend, 'protentiates' → 'potentiates'.

We corrected these typos in this revision. We would like to thank for this anonymous reviewer again for all these valuable comments.

Referee #2:

The authors very originally find a novel and previously uncharacterized isoform of TAZ (cTAZ). It is transcribed by an alternative promoter, it is expressed in several cell lines and it lacks the N-terminal portion of the longer and more characterized isoform. Structurally, this isoform lacks the domains required for the interaction with either LATS1/2 or with TEAD. Functionally the canonical Hippo pathway doesn't modulate the cTAZ protein. It does not modulate oncogenic TEAD target gens, nor it modulates cell proliferation or EMT, differently from YAP and full-length TAZ. Indeed, the authors show for cTAZ a peculiar function in the inhibition of the antiviral JAK/STAT mediated response to IFNs, eventually inhibiting the expression of ISGs normally induced by IFNs. Mechanistically, the authors show that cTAZ binds to STAT1 and inhibits STAT1/2 dimerization and its interaction with importin5, thus reducing its nuclear translocation. Moreover, the authors show that viral infection induces the expression of cTAZ transcript and protein as a negative feedback loop. In the discussion section, the authors provide a fascinating evolutionary explanation for the lack of any oncogenic function of cTAZ. If viral infection would modulate a protein involved in tumorigenesis, an infected cell would then transform in an oncogenic fashion, and this would not be evolutionarily selected. Instead, it is likely that the evolution may have selected a TAZ isoform that has been exclusively specialized in the negative modulation of the antiviral response as a control mechanism necessary to avoid aberrant antiviral response that needs to be finely tuned. The work is novel and original and a lot of experiments have been well performed. However, the authors should address some points in order to render the work suitable for publication in EMBO REPORTS.

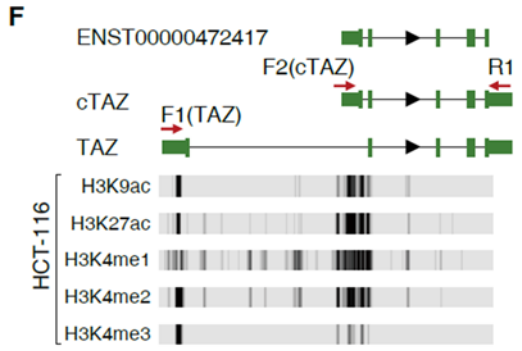
We thank this reviewer for recognizing the originality and novelty our study, and for her/his valuable comments. In this revision, we have addressed all concerns from this reviewer.

1. It is no clear how do the author construct a plasmid with a C-terminal HA-tagged YAP/TAZ. What does it mean? How is it possible to co-express YAP and TAZ in the same construct? Explain better than in the presented form.

We rephrased this sentence to “C-terminal HA-tagged YAP or TAZ”, and labeled these two constructs separately as YAP or TAZ (HA-tagged) (revised manuscript Page 7, new Fig 1E).

2. Describe better figure 1F-H. It is not clear from the figure which are the primers targeting the novel 5'UTR (highlight it graphically). Moreover, is the aminoacidic sequence 145-400 encoded by the PCR product shown in figure 1G? Also, specify better that the only difference between the canonical exon in the aminoacidic sequence 145-400 and the alternative exon is in two aminoacids, and that this difference requires the novel identified 5'UTR. From the text, it is no clear.

More details PCR primers were added in this revision. In new Figure 1F (also see below), the forward primers (F1 and F2) distinguishing TAZ and cTAZ were relabeled as F1(TAZ) and F2 (cTAZ) respectively. We also added sentences to show that the difference between TAZ (aa145-400) and cTAZ is the extreme N-terminal two amino acids, which is from an alternative exon and 5'UTR (revised manuscript Page 8).



3. It is also not clear how do the authors conclude that cTAZ is not produced by alternative splicing after the comparison of TAZ and cTAZ levels from RNA-seq and GTEx database analysis, or after comparison of the two protein isoform in different cell lines. These analyses only suggest that there is no correlation between the two isoforms. They do not exclude the possibility of an alternative splicing.

We concur with the reviewer that the lack of correlation between TAZ and cTAZ expression in diverse cell lines does not exclude the possibility of an alternative splicing. The presence of a unique 5'UTR and promoter-like features (Appendix Fig S1) strongly supports that cTAZ is alternatively transcribed, rather than a splicing variant.

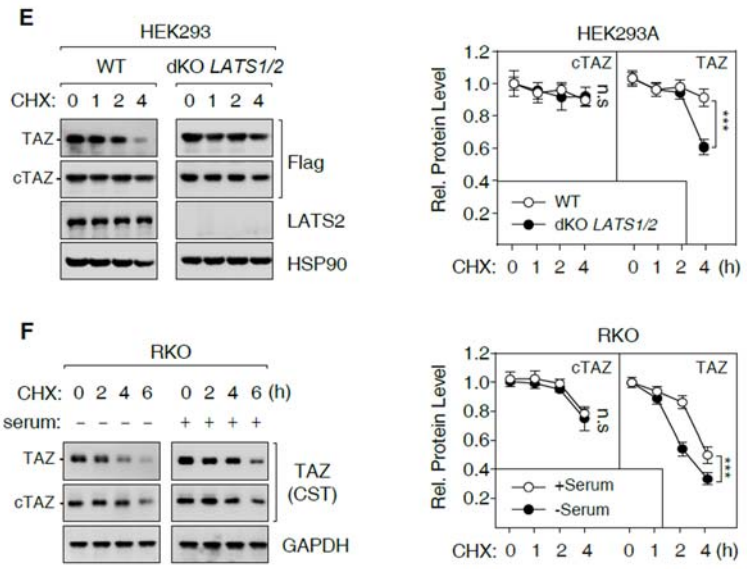
4. In figure EV2C,D, it is not clear how do the authors discriminate between total or phosphorylated TAZ. From figure EV2C and D it is not possible to appreciate a reduction of total TAZ or an increase of phosphorylated TAZ upon serum starvation. It seems that total TAZ is increased, conversely to total YAP that is decreased, upon serum starvation. The authors should explain better this result. Also, specify in the text what are the residues that are phosphorylated in YAP and TAZ, respectively, upon serum starvation (there is more than one residue) and their position in the different long and short TAZ isoforms.

We have specified TAZ phosphorylation sites in new Figure EV2A. When Hippo pathway kinases are activated, TAZ is phosphorylated and prone to ubiquitination and degradation. On SDS-PAGE, the migration of phosphorylated TAZ is retarded and the corresponding band appeared as a smear. Serum **addition** and serum **starvation** were experimental conditions used in old Figure EV2C and D respectively, which might have caused some confusion. To clearly show that TAZ rather than cTAZ is phosphorylated under serum starvation conditions, we performed SDS-PAGE in the presence of Phos-tag (which can dramatically separate phosphorylated and unphosphorylated proteins in

a gel), and the new results are incorporated into new Figure EV2D.

5. Figure EV2E does not suggest that cTAZ is not regulated by LATS1/2 or upstream signals. It only suggests tat it is more stable than full-length TAZ. Indeed, from that figure, YAP is more stable than both TAZ and cTAZ, but the previous figures show that instead YAP is regulated both by serum starvation and by LATS1/2 phosphorylation. The authors should clarify this point.

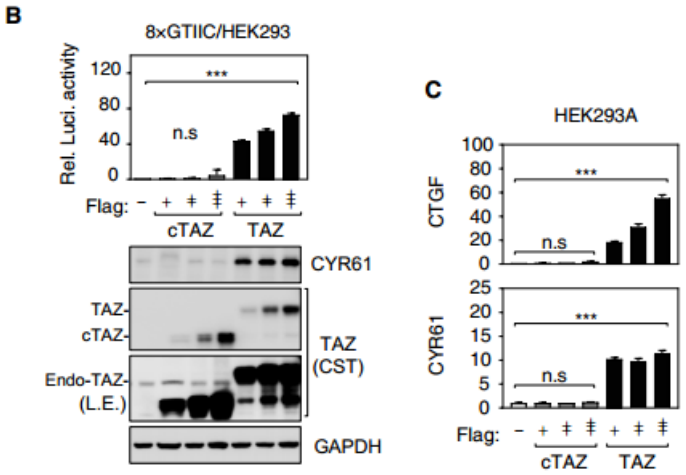
The lack of interaction between LATS1/2 and cTAZ (new Figure EV2B) suggests that cTAZ is not regulated by LATS1/2. We agree that data in old Figure EV2E is only about the protein stability of YAP, TAZ, and cTAZ. In this revision, we carefully examined the protein stability of TAZ and cTAZ in wild type or LATS1/2 double knockout cells, or under different serum concentrations, and clearly cTAZ was more stable than TAZ (new Figure EV2E and F, also see below). Protein stability of both YAP and TAZ is regulated by LATS1/2. However it has been reported previously that TAZ is more unstable, due to an additional phosphodegron at its N-terminal (Huang et al., 2012). To keep our work focused, we did not compare YAP protein stability in this revised manuscript.



6. In figure EV3B, the authors show that the luciferase activity of 8XGTIIC reporter is not increased by cTAZ overexpression. However, by western blot analysis, it seems that Cyr61 protein expression is instead increased in the same conditions. Moreover, the authors should show a longer exposure of TAZ blot in hEK293t cells to show also endogenous full-length TAZ in control cells and cTAZ-transfected cells.

The effect of TAZ expression on GTIIC promoter activity was mild (old Figure EV3B), we optimized this assay by increasing the amount of transfected GTIIC-Luc reporter (10 ng vs 100 ng per well in old and current experiment respectively), and the reporter was induced by TAZ for more than 40 folds. On the other hand, cTAZ expression failed

to change the GTIIC promoter activity significantly (new Figure EV3B, also see below). The ectopic expression of cTAZ had no effect on mRNA levels of *CTGF* and *CYR61*, two well-known YAP/TAZ target genes, whereas TAZ induced *CTGF* and *CYR61*expression for more than 10 folds (new Figure EV3C, also see below). We also analyzed endogenous TAZ protein level using immunoblotting, which was much lower than ectopic TAZ or cTAZ, and was detected under a long exposure (L.E.).

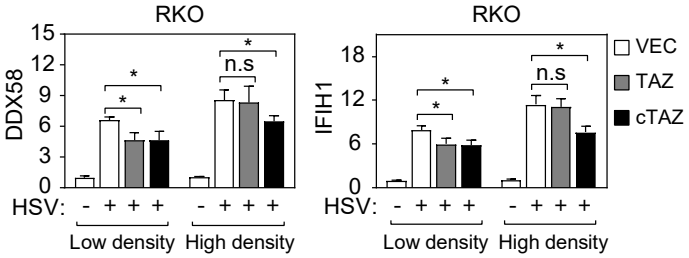


7. It was not possible to read legends relative to figures EV

We have presented more details in concise way for EV figure legends.

8. In figure 2A ad EV4A it is not clear how do the authors knockdown specifically cTAZ without knocking down TAZ full length. Moreover, the authors should add as an important control cells knocked down for or overexpressing full lenght TAZ and analyze the genes that are commonly deregulated upon full length TAZ and cTAZ interference or overexpression and those that are specifically deregulated upon cTAZ interference or overexpression.

We knocked out cTAZ using a CRISPR/cas9 guide targeting the 5'UTR and the alternative exon which are unique to cTAZ (new Figure EV1F and Appendix Fig S1), and this guide RNA does not target full-length TAZ. When cells were cultured at serum-rich or low-density conditions, both full-length TAZ and cTAZ similarly inhibited ISG expression. However, under serum starvation or high-density conditions, only cTAZ repressed ISG expression. These data suggest that full-length TAZ and cTAZ play non-redundant functions in ISG expression in a context-dependent manner (new Appendix Fig S3B and C, also see below).



9. Figure EV4 A and B are not clear. In Figure EV4A, it seems that RKO cells are interfered for endogenous cTAZ, and then they are transfected for overexpression of exogenous full-length TAZ. How can the authors state that the analyzed genes are regulated by cTAZ and not by lull length TAZ? Control suggested in comment 8 could clarify this.

To test if genes differentially expressed in cTAZ knockout cells could be rescued by re-introducing cTAZ, we expressed GFP-tagged full-length cTAZ (rather than full-length TAZ) in cTAZ KO cells, and RNA-seq experiments were performed for wild type, cTAZ KO, and cTAZ put-back cells. Coincidently, the molecular weight of GFP-tagged cTAZ was similar to endogenous full-length TAZ. We relabeled the figure to avoid confusion (new Figure EV 4A).

10. Add raw data (list of single genes, and genes in the indicated categories) about the analysis shown in figure 2A and 2B

A list of genes regulated by cTAZ and gene sets in each GO were provided in new Appendix Table S3.

11. Figure 2D and figure EV4C are not clear at all. It is not clear the rationale, what is ISRE? what is constitutively active RIG-I?

ISRE is interferon-sensitive response element. Constitutively active RIG-I (caRIG-I) is a plasmid express an active form of retinoic acid-inducible gene I (RIG-I, encoded by *DDX58* gene). We defined these two terms in the revised manuscript, and added references for caRIG-I (revised manuscript page 11). When overexpressed, caRIG-I can induce interferon expression, thus mimic IFN treatment.

12. Page 10. Specify (at least in the discussion section) that in the work of Wang et al., both transcriptionally active or inactive YAP isoforms regulate the immune response in their experimental settings.

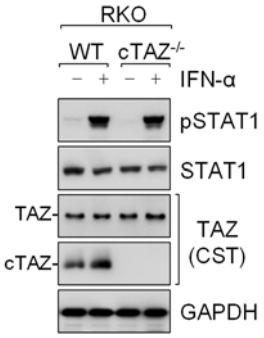
We have discussed this finding of Wang et al., in this revision (revised manuscript page 19).

13. Figure EV4C,D and 2G are not clear at all. Explain better in the text and use more clear legends in the figure. It is difficult to appreciate what the authors are showing in the figure and its meaning. Moreover, JAK/STAT signaling is described very superficially in the paragraph without an appropriated introduction. Spend more words on it. Also the sentence "Taken together, these results suggest that cTAZ can regulate the robustness of JAK/STAT signaling directly" is not enough supported in my opinion, it can be mentioned only after in the manuscript.

We have updated the corresponding text and figure legends for old Figures EV4C, D and 2G (new Figure EV4C, E, and 2E respectively). Background information about the JAK-STAT pathway was shown in Introduction (revised manuscript pages 4 and 5). We have added new Fig EV4G, and updated the sentence "cTAZ can regulate the robustness of JAK/STAT signaling directly" to "rather than modulating the production of IFNs, cTAZ can regulate JAK-STAT signaling directly" (revise manuscript page 12).

14. In figure EV5A, the author show a longer exposure blot showing that upon cTAZ interference, the abundance of full length TAZ is not affected. Moreover, from the blot, it appears that IFN treatment increases the expression of cTAZ in RKO cells (in fact later the authors show that viral infection induces cTAZ transcript and protein). Please, discuss on it.

Upon cTAZ knockout, the protein level of full-length TAZ was unchanged (new Figure EV5E; also see below). IFN treatment indeed increased the expression of cTAZ, and we have discussed this point in this revision (revised manuscript page 16).



15. Page 11, line 1 contains an imprecision: T705 of STAT1 is not the phosphorylation of other STAT proteins it is the phosphorylation of another residue of STAT1. Authors should correct this sentence.

We have made corrections (revised manuscript page 12) according to this comment.

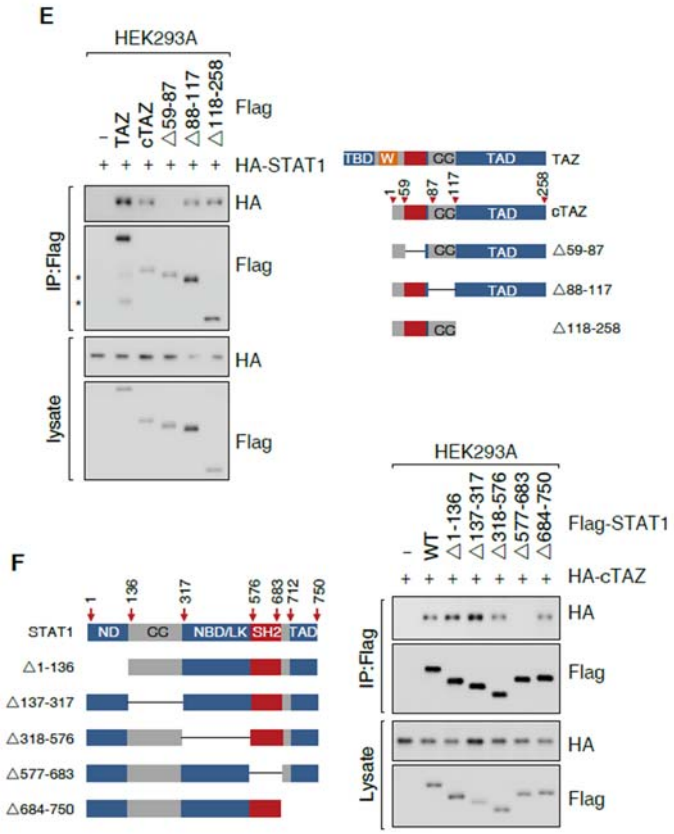
16. In the material and methods section, the authors need to describe how the proximity-dependent biotin identification has been performed.

We have described the BioID protocol in this revision (revised manuscript pages 24-25).

17. Figure EV5E: be sure that residues 137-317 of STAT1 are not required for cTAZ-STAT1 interaction. Again, in these experiments, analyze the interaction between full length TAZ and STAT1 as a control.

Co-IP experiments were repeated under conditions with normalized expression of Flag-tagged STAT1 or its deletion mutants. Residues 137-317 of STAT1 were not required

for cTAZ-STAT1 interaction (new Figure EV5F, also see below). We also included full-length TAZ in the Co-IP experiments, and full-length TAZ also interacted with STAT1 (new Figure EV5E, also see below), which was consistent to the BioID results.



18. In each panel of figure EV5, specify which protein is truncated of the indicated aminoacids (STAT1 or cTAZ) in order to avoid confusion

We have updated relevant Figures and labels in this revision (new Figures EV5E and F, also see response to #17).

19. In the material and methods section, describe how cTAZ-/- cells are obtained in RKO cells and in other cell lines in Figure 3C.

We have provided more details about cTAZ knockout and stable expression. We have also added a new Appendix Fig S1 to show the genomic structure of cTAZ, and updated (New Figure EV1F) to show the CRISPR/cas9 guide RNA positions.

20. For each immunoprecipitation experiment, specify better whether the authors are analyzing endogenous or overexpressed proteins

For IP experiments, ectopically expressed proteins were all labeled with corresponding tags, and when necessary, we indicated endogenous or overexpressed in figure legends.

21. In Figure 3C it is not clear whether in the blot the authors are showing TAZ full-length or cTAZ.

We have relabeled it to “TAZ (cTAZ)” in this revision (new Figure 3C).

22. Figure 3E is not described correctly: in cTAZ-/_ cells, nuclear STAT1/2 is increased, not decreased

We apologize for this mistake, and have made the correction in this revision (revised manuscript page 13).

23. In figure 3F, quantify the nuclear STAT1 and describe in the material and methods section how quantification has been obtained

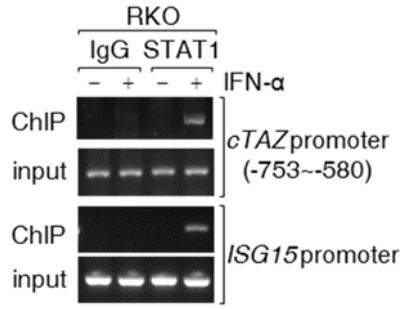
Based on the immunostaining pattern, we classified STAT1 subcellular localization into three categories: N>C: more nuclear; N=C: equal distribution; N<C: more cytosolic. One hundred cells were randomly selected and assessed. The results were shown in a bar-chart on the right. We have described this approach in revised figure legends (new Figure 3E).

24. In figure 4A, indicate how many events have been used for the quantification of viral infection in the immunofluorescence. Same comment for figure 4A and the other immunofluorescence shown in the manuscript.

Cells numbers were determined by staining nuclei using DAPI staining. About 300 cells were counted for each condition, and the ratio of GFP positive cells were determined. Method were briefly described in revised figure legends (new Figure4A).

25. In figure 5F it is difficult to discriminate the enrichment of STAT1 on cTAZ promoter. Please provide a better figure

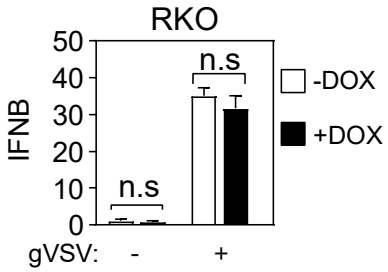
A better image was used in this revision (new Figure 5F; also see below).



26. In the discussion section, the authors state that cTAZ does not modulate the production of IFNs. In order to support this notion, the authors should show the

production of IFNs in cells were cTAZ levels are upregulated or downregulated.

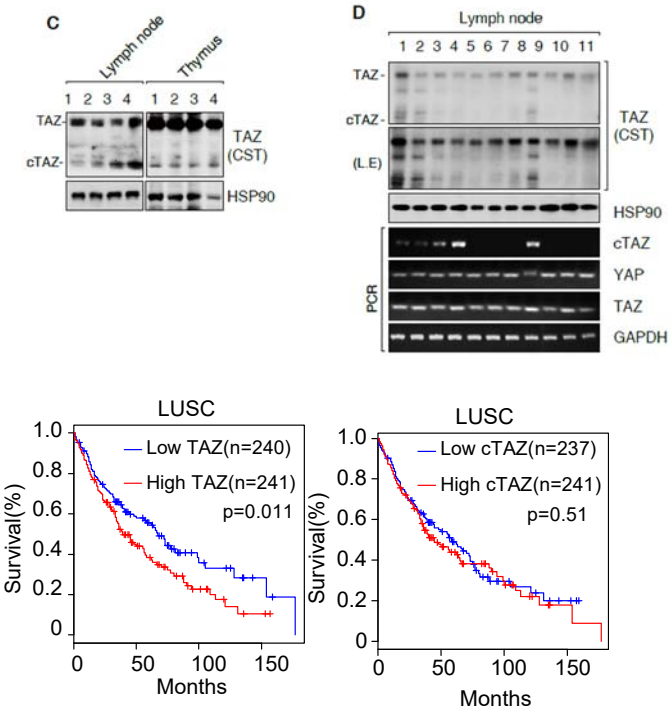
In cTAZ overexpressing cells, the mRNA level of IFNB was not different to control wild type cells, under either unstimulated or gVSV-treated condition (new Figure EV4G; also see below).



27. In order to translate the molecular findings of this work into clinics, would it be possible to analyze the expression of cTAZ transcript in a published dataset of patients affected by specific viral infection and correlate the expression of cTAZ with their prognosis? Moreover, the authors show that in vitro cTAZ is not responsible of the oncogenic proliferative or metastatic phenotype, and it is not regulated by canonical Hippo pathway, nor it interacts with TEAD in the regulation of oncogenic targets, differently to YAP and full length TAZ. Would it be possible also to analyze the expression of cTAZ transcript in public available dataset of cancer patients in order to exclude an oncogenic role of cTAZ?

We found cTAZ was expressed in mouse and human lymph nodes (new Figure EV1C and D, also see below), although the cell types in lymph nodes that harbored cTAZ signal were unclear. Most public datasets of patients affected by specific viral infection consisted of RNA expression data of macrophages or T cells, but the expression of cTAZ and full-length TAZ in these cells were extremely low, and cTAZ signals were usually filtered out in these datasets. Thus, based on public datasets, we were unable to assess the association between cTAZ expression and the prognosis of patients infected with virus. We agree with this reviewer that it is a great future direction to explore the relationship between cTAZ expression and prognosis.

Regarding the oncogenic role of TAZ or cTAZ, we analyzed the expression level of TAZ or cTAZ in lung squamous cell carcinoma (LUSC) (from TCGA). It was clear that high TAZ expression correlated with poor survival; on the other hand, cTAZ expression was not associated with survival. We included these new results in this revision (new Figure EV3G), and would like to thank this reviewer again for the insightful and constructive comments.


C
Lymph node
Thymus
1 2 3 4 1 2 3 4
TAZ-
cTAZ-
TAZ (CST)
HSP90
D
Lymph node
1 2 3 4 5 6 7 8 9 10 11
TAZ-
cTAZ-
(L.E)
TAZ (CST)
HSP90
cTAZ
YAP
TAZ
GAPDH
PCR

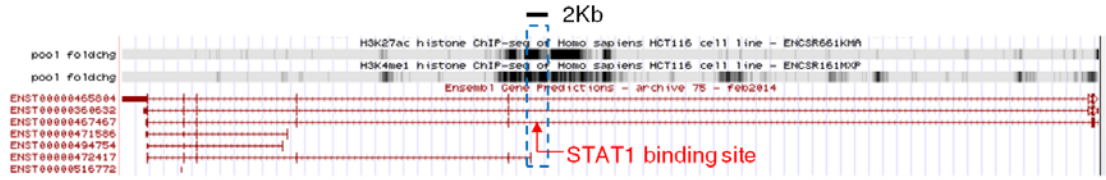
LUSC
Low TAZ(n=240)
High TAZ(n=241)
p=0.011
Survival(%)
Months
LUSC
Low cTAZ(n=237)
High cTAZ(n=241)
p=0.51
Survival(%)
Months

Referee #3:

The manuscript investigates the role of a short form (cTAZ) of the Hippo pathway protein TAZ. The novelty of the manuscript lies in showing that this short form does not act in the context of Hippo signaling as might be expected, but rather as a negative regulator of interferon (IFN)-induced Jak-Stat signaling. Specifically the authors provide data suggesting that cTAZ interferes with the association of STAT1 and STAT2 and, as a consequence, with STAT1 binding to Importin-a5 which is needed for nuclear translocation. The authors propose a negative feedback loop based on cTAZ expression and action in response to IFN signaling.

General comment: Collectively the data in the paper support the notion that cTAZ exerts an inhibitory activity on IFN-induced Jak-Stat signal transduction. They do not support the concept of a negative feed- loop given that the induction of cTAZ mRNA by IFN is 1.5-fold under optimal conditions (figure 5, the model in figure 6 lacks the IRF9 subunit). The relationship of the cTAZ promoter analyzed in figure 5 to the activating histone marks in figure 1F remains unclear. The position of the critical binding sites appears inconsistent with the position of the histone marks relative to the alternative exon containing the translation start.

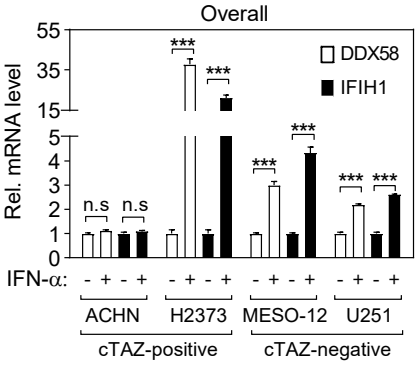
We thank this reviewer for the appreciation of the novelty of our study and for the valuable comments. We consistently observed 1.5-fold induction of cTAZ expression by IFN treatment. IRF9 was included in the working model in this revision. The position of STAT-binding sites and histone marks overlapped (see below). Our ChIP results indicated that upon IFN stimulation, STAT1 was recruited to *cTAZ* and *ISG15* promoters, whereas the H3K27ac status on these two promoters were largely unchanged. We think that epigenetic modifications (such as H3K27ac) may facilitate STAT1 recruitment upon IFN stimulation.



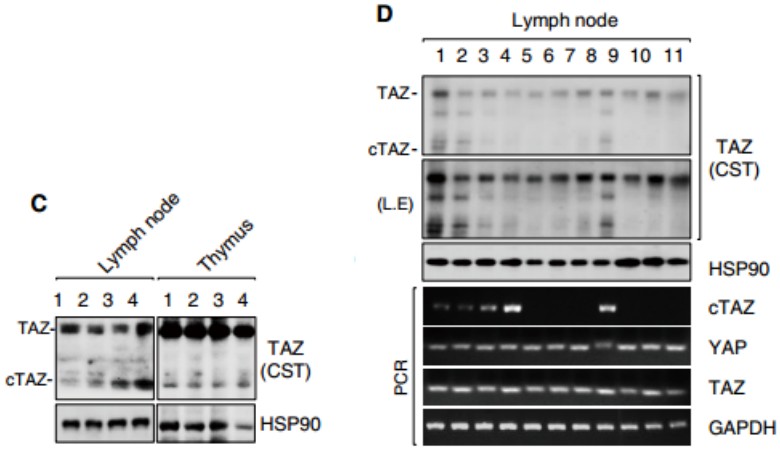
The biological relevance of the study is limited by the fact that much of the data is generated with vastly overexpressed proteins in 293 cells. the authors show that 70% of the investigated cell lines don't express cTAZ. Does this mean their response to IFN is stronger? Can these cells be induced to express cTAZ or does a cTAZ knockout affect their response to IFN? Do they contain the activating histone marks reported in figure 1F? Is cTAZ found expressed in virus-infected tissues in animals? Some of these questions could easily be addressed.

The expression level of cTAZ in different cell lines exhibited great variation. We treated cTAZ-negative or -positive cell lines with IFN, and the induction of ISG expression

appeared not associated with basal cTAZ levels (below). We reasoned that multiple factors, such as JAK expression, epigenetic modifications of ISG genes, as well as cTAZ, contribute to the expression of ISGs, rendering it difficult to observe a negative association between cTAZ and sensitivity to IFN. Even though the transfected cTAZ promoter in HEK293A cells was responsive to IFN treatment, we failed to induce endogenous cTAZ expression in this cell line. The epigenetic status of cTAZ promoters could control both basal expression of cTAZ and the responsiveness to IFN. We analyzed histone marks around cTAZ gene in different cell lines, and the active histone marks were significantly lower in MCF7 (new Figure EV1F) and HEK293 (not shown), two cell lines without cTAZ expression.



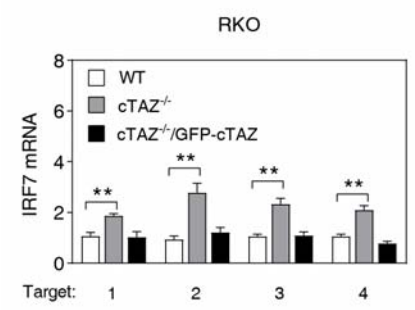
The expression of cTAZ RNA in most human tissues was low (Appendix Table S2). In mouse tissues, we overserved that cTAZ expression could be detected in lymph nodes and Thymus by immunoblotting (new Figure EV1C, also see below). We also analyzed cTAZ expression in anterior cervical lymph nodes from 11 thyroid cancer patients, and about half had cTAZ expression at mRNA and protein levels (new Figure EV1D, also see below). These new results suggested that cTAZ is expressed *in vivo*.



The manuscript contains some inconsistencies:
- the Irf 7 gene, a well-established and highly induced ISGF3 target is hardly affected by the cTAZ knockout.

IRF7 gene was upregulated in cTAZ KO cells (2.86 fold), as showed by RNA-seq (Appendix Table S3). We designed another three pairs of IRF7 PCR primers (#2-4, #1

was the old primer; see below), and the new result was consistent with the RNA-seq data, and new data generated from primer set 2 was incorporated into new Figure 2C.



- the sentence 'about 50% Stat1 protein was localized ...' (bottom of page 11) suggests that less ISGF3 translocated to the nucleus in cTAZ-/- cells. This is inconsistent with the main hypothesis of the paper.

The data were not accurately described in the previous version of the manuscript. We updated this sentence in this revision: In an IF assay, about 30% of WT cells and 50%-60% of cTAZ-/- cells respectively showed strong nuclear STAT1/2 localization following IFN-α stimulation (revised manuscript page 13).

- the w-blot in figure 3B shows Flag-Stat1 in the cell lysate. However, no Flag-Stat1 was precipitated with anti-Flag and no Flag-Stat1 was transfected according to the figure labeling. How is this explained?

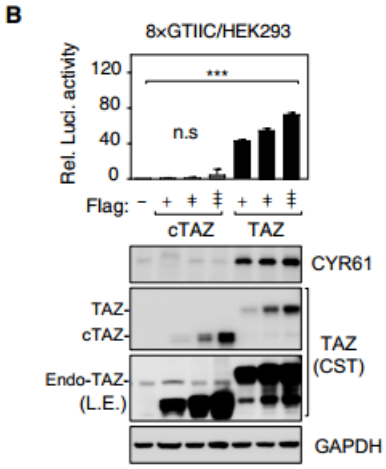
We apologize for the mistake. The labeling has been updated in this revision (new Figure 3B).

Technical comments:
1. Fig. EV2E: the difference in stability between TAZ and cTAZ is marginal. Cells with- or without starvation should be compared.

We assessed protein stability under serum rich and serum starvation conditions. TAZ protein was more unstable under serum starved condition, whereas cTAZ protein stability was not significantly affected by serum (new Figure EV2E, F).

2. Fig. EV3B: upregulation of the reporter by wt TAZ is barely 2-fold, questioning biological relevance.

The effect of TAZ expression on GTIIC promoter activity was mild (old Figure EV3B), we optimized this assay by increasing the amount of transfected GTIIC-Luc reporter (10 ng vs 100 ng per well in old and current experiment respectively), and the reporter was induced by TAZ for more than 40 folds. On the other hand, cTAZ expression failed to change the GTIIC promoter activity significantly (new Figure 3B, also see below).

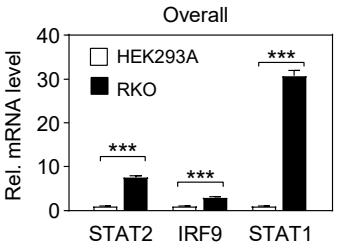


3. Fig. 3: cell fractionation studies would be a more convincing way of quantifying the nuclear translocation of STATs.

We have performed cell fractionation as suggested, the results were consistent with immunofluorescence staining (new Figure 3F).

4. Why were the antiviral assays shown in figure 4A performed with overexpressed Stat1/2? Why does cTAZ expression not reduce the activity of endogenous Stat1/2 (compare panels 2 and 4)?

HEK293A cells used in this assay expressed very low levels of STAT1/2 (see below), we thus overexpressed STAT1 in this antiviral assay, which was also why we did not see a significant reduction in the activity of endogenous STAT1. We also performed antiviral assay using IFN treatment (new Figure 4C).



Length:
- the part dealing with IFN-inducibility of cTAZ should be ommitted because the data is unconvincing. Similarly, the EVFs contain data that are redundant with the original figures and should be deleted from the manuscript.

The induction of cTAZ by IFN was not dramatic but firmly reproducible. Nevertheless, we are open to the suggestion of omitting this part. We have also removed some figures to Appendix to reduce the length of the manuscript, with all the changes summarized in the table below. We would like to thank this reviewer again for the constructive comments on our study.

Appendix Table: A summary of changes on figures in this revision.

Current Figures	Previous Figures	Remarks
Fig 1A	Fig 1A	
Fig 1B	Fig 1B	
Fig 1C	Fig 1C	
Fig 1D	Fig 1D	
Fig 1E	Fig 1E	
Fig 1F	Fig 1F	With data update
Fig 1G	Fig 1G	
Fig 1H	Fig 1H	
Fig 1I	Fig 1I	
Fig 2A	Fig 2A	
Fig 2B	Fig 2B	
Fig 2C	Fig 2C	With data update
Fig 2D	Fig 2D	With data update
Fig 2E	Fig 2G	
Fig 3A	Fig 3A	
Fig 3B	Fig 3B	With data update
Fig 3C	Fig 3C	
Fig 3D	Fig 3D	
Fig 3E	Fig 3E	With data update
Fig 3F	Fig EV5J	
Fig 3G	Fig 3F	With data update
Fig 4A	Fig 4A	
Fig 4B	Fig 4B	
Fig 4C	Fig 4C	
Fig 4D	Fig 4D	With data update
Fig 4E	Fig 4E	With data update
Fig 4F	Fig 4F	
Fig 4G	Fig 4G	
Fig 4H	Fig 4H	
Fig 4I	Fig 4I	
Fig 5A	Fig 5A	
Fig 5B	Fig 5B	
Fig 5C	Fig 5C	
Fig 5D	Fig 5D	
Fig 5E	Fig 5E	
Fig 5F	Fig 5F	With data update
Fig 5G	Fig 6	

Current Figures	Previous Figures	Remarks
Fig EV1A	Fig EV1A	
Fig EV1B	Fig EV1B	
Fig EV1C		New
Fig EV1D		New
Fig EV1E	Fig EV1C	
Fig EV1F	Fig EV1D	
Fig EV1G	Fig EV1E	
Fig EV1H	Fig EV1F	
Fig EV1I		New
Fig EV2A	Fig EV2A	
Fig EV2B	Fig EV2B	
Fig EV2C	Fig EV2C	
Fig EV2D	Fig EV2D	With data update
	~~Fig EV2E~~	Deleted
Fig EV2E		New
Fig EV2F		New
Fig EV3A	Fig EV3A	
Fig EV3B	Fig EV3B	With data update
Fig EV3C		New
Fig EV3D	Fig EV3C	
Fig EV3E	Fig EV3D	
Fig EV3F	Fig EV3E	
Fig EV3G		New
Fig EV4A	Fig EV4A	
	~~Fig EV4B~~	Deleted
Fig EV4B		New
Fig EV4C	Fig EV4C	With data update
Fig EV4D	Fig 2E	
Fig EV4E	Fig EV4D	
Fig EV4F	Fig 2F	
Fig EV4G		New
Fig EV5A	Fig EV5A	
Fig EV5B	Fig EV5B	
Fig EV5C		New
Fig EV5D	Fig EV5C	
Fig EV5E	Fig EV5D	With data update
Fig EV5F	Fig EV5E	With data update
	~~Fig EV5F~~	Deleted
Fig EV5G	Fig EV5G	
Fig EV5H	Fig EV5H	
Fig EV5I	Fig EV5I	With data update

Current Figures	Previous Figures	Remarks
Appendix Fig S2A	Fig EV6A	
Appendix Fig S2B	Fig EV6B	
Appendix Fig S2C	Fig EV6C	
Appendix Fig S2D	Fig EV6D	
Appendix Fig S2E	Fig EV6E	
Appendix Fig S2F	Fig EV6F	
Appendix Fig S2G	Fig EV6G	With data update
Appendix Fig S2H	Fig EV6H	
Appendix Fig S2I	Fig EV6I	
Appendix Fig S2J	Fig EV6J	
Appendix Fig S2K	Fig EV6K	
Appendix Fig S2L	Fig EV6L	
Appendix Fig S4A	Fig EV7A	
Appendix Fig S4B	Fig EV7B	
Appendix Fig S4C	Fig EV7C	
Appendix Fig S4D	Fig EV7D	
Appendix Fig S4E		New
Appendix Fig S3A		New
Appendix Fig S3B		New
Appendix Fig S3C		New
Appendix Fig S3D		New
Appendix Table S1	Expended Table 1	Rename
Appendix Table S2		New
Appendix Table S3		New
Appendix Table S4	Expended Table 3	Rename
Appendix Table S5	Expended Table 2	Rename
Appendix Table S6		New

1. Zhao B, Wei X, Li W, Udan RS, Yang Q, Kim J, Xie J, Ikenoue T, Yu J, Li L, *et al.* (2007) Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes Dev* **21**: 2747-61

2. Yu FX, Zhao B, Panupinthu N, Jewell JL, Lian I, Wang LH, Zhao J, Yuan H, Tumaneng K, Li H, *et al.* (2012) Regulation of the Hippo-YAP pathway by G-protein-coupled receptor signaling. *Cell* **150**: 780-91

3. Zhang Q, Meng F, Chen S, Plouffe SW, Wu S, Liu S, Li X, Zhou R, Wang J, Zhao B, *et al.* (2017) Hippo signalling governs cytosolic nucleic acid sensing through YAP/TAZ-mediated TBK1 blockade. *Nat Cell Biol* **19**: 362-374

2nd Editorial Decision 7 March 2019

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the three referees that were asked to re-evaluate your study (you will find below). As you will see, the referees now support the publication of your manuscript in EMBO reports. However, referees #2 and #3 have some further suggestions, we ask you to address in a final revised version of your manuscript. Please provide your final manuscript file with track changes, in order that we can see the modifications done.

Further, I have these editorial requests, which I ask you to also address in the final revised version of the manuscript:

- I seems the author list has increased, however the new authors have not been entered in our system. Please add all new authors upon re-submission of the final version in the online submission system.

- Please also check that in the author contributions all authors are mentioned. It seems that presently the author Zhaolan Hu is missing. Moreover, there are two authors JL and YZ, respectively, and it is unclear who contributed what. Please use abbreviations that distinguishes these authors. There is also an author YN mentioned, who is not found on the title page. Finally, we would ask you to consider the ICNJE authorship recommendations:
http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html

- Please add a running title (not more than 40 characters including spaces), and up to five key words to the title page.

- We would like to publish the paper as Scientific Report. For a Scientific Report we require that results and discussion sections are combined in a single chapter called "Results & Discussion". Please do that for your manuscript. I think it would help this section if sub-headings were introduced. Please do that. For more details please refer to our guide to authors: http://embor.embopress.org/authorguide#manuscriptpreparation

- Please provide editable TIFF or EPS-formatted single figure files in high resolution for main figures and EV figures. Also check that figures conform to our guidelines. See here our guide for figure preparation:
http://www.embopress.org/sites/default/files/EMBOPress_Figure_Guidelines_061115.pdf

- Presently, the panel YAP/TAZ Lysate in figures 1D and EV1A looks identical, although this is supposed to come from different cell lines. Please check!

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

- Please deposit the RNA-seq. raw data at a public database and provide the accession number in the material & methods section (data deposition). See also:
http://embor.embopress.org/authorguide#datadeposition

- Appendix Tables S3 and S4 need to be datasets. Please name these files Dataset EV1 and Dataset EV2, add a legend/description on the first Tab of the excel sheet, and rename their call-outs in the manuscript file.

- Please add the Appendix Tables S1, S2, S5 and S6 directly to the Appendix, including legends. Name these Appendix Tables S1, S2, S3 and S4, and change their call-outs accordingly.

- Please add page numbers to the Appendix and provide the Appendix TOC (table of contents) with page numbers.

- Please fill in field D-10 in the author check list indicating that you comply with the ARRIVE guidelines. Please upload a version of the author checklist where this field is filed in. See also: http://embor.embopress.org/authorguide#livingorganisms

- 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 the modifications done.

When submitting your revised manuscript, we will require:
- a Microsoft Word file (.doc) of the final revised manuscript text (with track changes).
- a point-by-point response addressing the final requests by the referees.
- editable TIFF or EPS-formatted single figure files (main figures and EV figures) in high resolution.
- The revised Appendix.
- The requested source data.

Further, I would need from you:
- a short, two-sentence summary of the manuscript
- two to three 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.

REFEREE REPORTS

Referee #1:

The authors have thoroughly addressed the concerns. I recommend the manuscript to be accepted for publication.

Referee #2:

The authors addressed all the comments and the manuscript is now complete and suitable for publication. In light of the new version, however, just few points to be addressed before publication, are listed below:
1. New figures EV2B, EV3A, should be described better. Why is it possible to see two bands (TAZ and YAP) on lane 5, blot EV2B and lane 4 and 6, blot EV3A? Is YAP co-expressed with TAZ in those lanes? It is not clear.
2. Describe better figure EV3C. Are the authors analysing exogenous or the endogenous CTGF and CYR61 transcripts?
3. As already asked previously, in Page 11,explain better in the text why the authors use an ISRE reporter (Expand the acronymous "ISRE", describe better ISRE, RIG1, not only in the letter to the reviewer)
4. Introduce better the experiments in Figure EV4D-G, 2D-E. In particular, explain before describing the results why those experiments have been performed (i.e"in order to check whether cTAZ modulate the production of IFNs throug IRF, cTAZ or can regulate the robustness of JAK-STAT signaling directly...."
5. In figure 3A, discuss that the interaction between cTAZ and STAT1 is increased upon IFN treatment.
6. Figure S2A, correct SATA1/2 with STAT1/2. Moreover, in figure S2A it is not clear the rescue of viral infection in STAT1/2 + cTAZ cells. Provide a better quality figure

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Referee #3:

The authors have added a considerable amount of work to address the reviewers' concerns. By and large the responses to my critique is adequate. However, I still think that a 1.5-fold induction of cTAZ by IFN

doesn't justify the idea of a negative feed-back loop. The corresponding data should be omitted. With all the revisions the manuscript is now overly long (though not the authors' fault). The model in fig. 5G should be adjusted and not show cTAZ as a IFN-inducible gene.

2nd Revision - authors' response 18 March 2019

Referee #1:

The authors have thoroughly addressed the concerns. I recommend the manuscript to be accepted for publication.
We thank this reviewer again for the constructive comments.

Referee #2:

The authors addressed all the comments and the manuscript is now complete and suitable for publication. In light of the new version, however, just few points to be addressed before publication, are listed below:
We thank this reviewer for the constructive comments, and address all concerns below.

1. New figures EV2B, EV3A, should be described better. Why is it possible to see two bands (TAZ and YAP) on lane 5, blot EV2B and lane 4 and 6, blot EV3A? Is YAP co-expressed with TAZ in those lanes? It is not clear.
For Lane 5 of blot EV2B, the weak band (closed to TAZ molecular weight) is likely a degraded YAP fragment. For lanes 4 and 6 of blot EV3A, we only see one band for YAP (see lysate), for IP, these is an additional IgG band (close to TAZ), we emphasized this by adding an asterisk in the revised figure and description in revised legends.

2. Describe better figure EV3C. Are the authors analysing exogenous or the endogenous CTGF and CYR61 transcripts?
Endogenous CTGF and CYR61 were measured, which was clarified (page 38).

3. As already asked previously, in Page 11,explain better in the text why the authors use an ISRE reporter (Expand the acronymous "ISRE", describe better ISRE, RIG1, not only in the letter to the reviewer)
ISRE has been defined on page 4, caRIG-1 has been defined on page 11.

4. Introduce better the experiments in Figure EV4D-G, 2D-E. In particular, explain before describing the results why those experiments have been performed (i.e"in order to check whether cTAZ modulate the production of IFNs throug IRF, cTAZ or can regulate the robustness of JAK-STAT signaling directly...."
We appreciate this comment, and have explained the rational before presenting related results (page 11).

5. In figure 3A, discuss that the interaction between cTAZ and STAT1 is increased upon IFN treatment.
We discussed this point in this revision (page 13)

6. Figure S2A, correct SATA1/2 with STAT1/2. Moreover, in figure S2A it is not clear the rescue of viral infection in STAT1/2 + cTAZ cells. Provide a better quality figure
We made corrections, and provided a better image in this revision (new Figure S2A).

Referee #3:

The authors have added a considerable amount of work to address the reviewers' concerns. By and large the responses to my critique is adequate. However, I still think that a 1.5-fold induction of cTAZ by IFN doesn't justify the idea of a negative feed-back loop. The corresponding data should be omitted. With all the revisions the manuscript is now overly long (though not the authors' fault). The model in fig. 5G should be adjusted and not show cTAZ as a IFN-inducible gene.

We thank this reviewer for the constructive comments. In this revision, we removed the claim of "a negative feedback loop", instead we indicated that there is a trend of induction of cTAZ expression upon IFN treatment. We also made changes on the model (new Figure 5G), these changes included: 1) highlight the effect of cTAZ on STAT1/2 nuclear translocation and ISG expression; 2) cTAZ regulate STAT1/2 in a Hippo pathway independent manner, and 3) question marks and dotted line were introduced to show a relatively weak induction of cTAZ by JAK-STAT signaling.

3rd Editorial Decision 20 March 2019

Thank you for the submission of your revised manuscript to our editorial offices. I think that the remaining concerns of the referees have now been adequately expressed. Thank you also for providing the source data for the WB panels.

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

- Could you please provide the source data files with the blots ordered like they show up in the figure panel, and with a label identifying which protein is detected. This will help enormously to identify which blot belongs to which panel.

- Please upload the source data files as single files (not as part of the Appendix), one PDF file per figure (main and EV). It is fine to upload the source data for the Appendix as one file.

- We need separate files for the source data for main and EV figures, as they will be linked to these. Thus, please provide two files, one for Fig.1 and one for Fig. EV1, which contain all the relevant files. Currently, SD for panel EV1A is contained in the SD file for Fig. 1. There is no problem, if some blots show up twice.

- For several panels the source data shows significantly different contrast and/or band intensities. This is e.g. very apparent for Fig. 1B, panel YAP/TAZ (NCI-H28 - the upper band of the doublet at 40 kD); or panel 1D TAZ(CST) IP A: TAZ, the upper 60 kD band (here it does look, as the source data blot is not identical to the one shown in the figure); or the GAPDH loading control in Fig. 1I. Please go through the source data and ensure that the contrast and band intensities are not severely different from the published panel. Please show the data in the final panels as unmodified as possible.

- Finally, please remove any writing form the scale bars (e.g. 3E/G, EV5I), and indicate the size of the scale bars only in the figure legend.

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.

3rd Revision - authors' response 25 March 2019

The authors performed all minor editorial changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Fa-Xing Yu
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2018-47227V1

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple 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.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For cell based assays, we repeat at least three times.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	We used four mice/group in our study, we did not apply statistical testing.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	We randomly pick cells for analysing protein subcellular localization and GFP expression levels.
For animal studies, include a statement about randomization even if no randomization was used.	We used all four mice provided by a vendor, no randomization was used.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
5. For every figure, are statistical tests justified as appropriate?	NA
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

USEFUL LINKS FOR COMPLETING THIS FORM

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

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

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

http://datadryad.org

http://figshare.com

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

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

http://biomodels.net/

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

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Appendix tables 3-4 show details about all antibodies and oligos used in this study.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Cells used in this study were not authenticated, and were routnely tested mycoplasm free.

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	4 weeks old, male C57BL6 mice were used in this study, mice were housed under spf condition.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	The animal work in this study was approved by institutional (institutes of biomedical sciences) ethical committee.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We followed these guidelines in animal studies.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	Ethical Committee of Fudan University Shanghai Cancer Center (050432-4-1212B)
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	informed consent was obtained from all patients
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	RNA-seq data was deposited in GEO database (accession number: GSE128257).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

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