EMBO reports

Impaired activation of Transposable Elements in SARS-CoV-2 infection

Matan Sorek, Eran Meshorer, and Sharon Schlesinger
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1st Editorial Decision **25th Apr 2022**

Dear Dr. Schlesinger,

Thank you for the submission of your commentary to EMBO reports. We have now received the full set of referee reports that is pasted below.

As you will see, the referees acknowledge that the topic of the commentary is interesting. However, they also raise several concerns that would need to be addressed before we can consider the commentary for publication here. The referees point out that the current analyses are insufficient to draw clear conclusions, and that it remains unclear what the causal relations between TEs and IFN gene induction are. I would like to know whether you think that you can address all concerns. In this case, and only with the support from the referees, we can offer to publish your commentary.

If you like, we can also discuss the revisions in a video chat. Just let me know and we can set an appointment.

These are the format requirements for our commentaries:

- max ~5000 words
- max 30 references
- max 3-4 figures
- if you want to keep all figures, we suggest posting all to BioRxiv and referencing the preprint in the commentary
- A reasonable amount of suppl. materials is ok if it is important to make a definitive claim

Please submit with your revised commentary:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables).

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

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

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

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files.

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

5) a complete author checklist, which you can download from our author guidelines . Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

6) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines

7) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

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

Further instructions are available at https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

9) Regarding data quantification (see Figure Legends:
https://www.embopress.org/page/journal/14693178/authorguide#figureformat)

The following points must be specified in each figure legend:

- the name of the statistical test used to generate error bars and P values,

- the number (n) of independent experiments (please specify technical or biological replicates) underlying each data point,

- the nature of the bars and error bars (s.d., s.e.m.),

- If the data are obtained from n {less than or equal to} 2, use scatter blots showing the individual data points.

Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.

- Please also include scale bars in all microscopy images.

10) The journal requires a statement specifying whether or not authors have competing interests (defined as all potential or actual interests that could be perceived to influence the presentation or interpretation of an article). In case of competing interests, this must be specified in your disclosure statement. Further information: https://www.embopress.org/competing-interests

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I look forward to seeing a revised form of your manuscript when it is ready. Please use this link to submit your revision:
https://embor.msubmit.net/cgi-bin/main.plex

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

This paper, submitted as a commentary, reports a re-analysis of earlier data for transposable element (TE) expression after infection by SARS-CoV-2. They note that the virus causes only weak induction of TEs compared to other viruses. They cite several papers showing that many other viruses cause robust TE induction and also IFN induction, and suggest that TEs may form PAMPs that trigger IFN induction. The authors suggest that the weak TE induction by SARS-CoV-2 may contribute to the weak IFN induction by the virus.
The primary data analyzed here include data in papers by Blanco-Melo (2020) and Huang (2020), and more.

The authors sought to find correlates of the fold TE induction upon infection. Unsurprisingly, they see that higher viral load gives higher TE induction. Also (perhaps) unsurprisingly, high basal TE expression gives lower fold TE induction (as if there is a ceiling, a maximum level, allowed). Primary cells, and ageing cells, have higher basal levels, and so have lower fold induction. The authors argue this could explain weak innate immune responses of those cells.

There is a slightly odd flow to the paper. The main argument being made is that many viruses induce TEs first, and this then promotes IFN production (like Schmidt 2019). On p.7 they note that JAK inhibitors can allow TE induction and block subsequent IFN induction, which is consistent with the idea. But then they raise the idea that the...."IFN response upregulate TE expression", which is likely true, but this is a new causal pathway not really brought up yet.
I think the story might be clearer if it was started, very early, with the idea that both TEs and IFN are induced by many viruses,

and that we are not sure which causes which, and that the authors will end up suggesting that TEs often come first and IFN second - but that there is also feedback, with IFN causing more TE expression. The sentence at the top of p. 8 summarizes it well, and maybe this could be said in the introduction.

The paper then shows that genes near TEs are co-activated with TEs, and that IFN-related gene near TEs even more so. However, this pattern was less true for infection by SARS-CoV-2 than influenza. Later they suggest that the TE effect on IFN-related genes may be in trans rather than in cis - I think the opinion of the authors on this point is not made totally clear.

Next, the few TEs that are activated by SARS-CoV-2 were analyzed. The authors note that these were the TEs that already had activated histone marks, and low levels of a silencing mark, before infection. (This seems reasonable, as if they are already poised for induction.) Curiously, LINES that were activated by SARS infection had a combination of a silencing mark and an activating mark.

Finally, they show that genes induced along with TEs by SARS-CoV-2 were enriched for chromatin- and histone-related genes (including SETD2, and other interesting genes).

The summary of the paper near the end is good, but the last two sentences seem unnecessary and distracting - I would stop right before them.

All told, this is an interesting paper proposing interesting ideas about responses to virus infection. A little re-writing to clarify the basic idea up front, and some introductory sentences as we go into each new point, might help the flow. Also, perhaps some discussion as to why or how SARS-CoV-2 is different from most viruses could be included, though this is really a big area and it could only be mentioned briefly here.

Referee #2:

SARS-CoV-2 infection leads to a wide range of outcomes, from asymptomatic infection to death without treatment. While some antivirals now have joined immune modulators, and vaccines have helped to greatly reduce the burden on severe infection, emergence of new variants and uncertainty about future pathogenicity make the study of the pathogenesis of SARS-CoV-2 of continued importance. Within current studies of immunopathogenesis, how transposable elements (TEs) contribute to different inflammatory outcomes is an interesting area of study. TEs could be sensed by the innate immune response, or stimulate adaptive immunity, or enhance immune genes.

Major comments

There have already been several studies published on TE expression following SARS-CoV-2 infection Ferrarini et al, 2021; Marston et al, 2021; Hale 2021; Kitsou et al. 2021; Tovo et al 2021; Balestrieri et al.), and also with other respiratory viruses, including IAV. This paper repeats some of the previous studies and uses published datasets but comes up with different results. For figure 1, in which the authors claim that IAV caused a global increase in TE subfamilies expression, but SARS-CoV-2 did not, the data sets were from different studies, and cannot be directly compared. Some important methodological details are missing and controls lacking. The relationship between viral load in cells in vitro and TE expression, and TE expression in PBMC from patients with COVID-19, has been shown by others, from these and other data sets.

The relationship between IFN expression and TE expression during SARS-CoV-2 has also already been published. The authors conclude that: "SARS-CoV-2 infection has a relatively weak effect on TE expression in primary lung epithelia", yet do not mention the Kitsou et al. paper in which there was marked upregulation of TEs in BALF and in epithelial cell lines, which is in line with the other papers published in this area.

Overall, the data in this paper does not support the authors' hypothesis that SARS-CoV-2 infection fails to upregulate the TEs coopted for immune activation.

Minor comments: the fast moving field of COVID-19 research is such that some of the references listed are now well outdated. There is a reference to "the understanding of the kinetics of IFN response in mild and severe COVID-19 patients is lacking (Park & Iwasaki, 2020)", but hundreds, of papers have been published since then addressing this topic - for example,
The cGAS-STING pathway drives type I IFN immunopathology in COVID-19 [https://www.nature.com/articles/s41586-022-04421-w} - one of many.

The authors should reference all published work on TEs and SARS-CoV-2 infection.

Referee #3:

Impaired activation of Transposable Elements in SARS-CoV-2 infection

Sorek, Meshorer and Schlesinger

In this study, the authors propose that SARS-CoV-2 infection is not accompanied by an immediate robust upregulation of transposable element transcription and that this is unusual in comparison to other viral infections. The authors also note that an upregulation of TEs is linked to an IFN response. It has not been demonstrated, of note, that an upregulation of TEs is necessary for an ensuing IFN response during infection, in this study or others. The authors show that IFN-beta treatment of primary lung cells induces upregulation of TEs, perhaps due to some of them acting as enhancers that are responsive to type I IFN signaling. This explanation is supported by the observation that a higher viral load induces a stronger TE response (through more induction of TE enhancers). However, the authors note that in A549 cells treated with a JAK1/2 inhibitor of IFN signaling, there is still upregulation of TEs, despite no induction of interferon-stimulated genes (ISGs). This suggests that TE-derived nucleic acids could have a role in inducing type I IFNs. In SARS-CoV-2 infected cells, the authors propose that there's delayed TE activation and that the spectrum of TEs induced is different, including less ISG-associated enhancer-TEs. Examination of epigenetic marks at upregulated TEs showed that these elements are naturally enriched for active marks even before infection, except for LINEs, which exhibited a bivalent signature of H3K9me3 and H3K36. This study, although only correlative, is interesting and timely, but there are some issues with the data as it stands, as detailed below. Apart from that, the text needs to be reworked to clarify that it is not known that TE nucleic acid sensing is required for the induction of type I IFN. This is an assumption that the manuscript is built on. In fact, while this is an intriguing hypothesis, it is not clear why you would require TE dsRNA sensing to initiate IFN responses in the presence of an RNA virus, which itself makes plenty of dsRNA through its own replication.

Major comments

1.It looks like the data in Figure 1a is based on only one replicate per sample. From this, it is not possible to say that there is a muted upregulation of TEs in response to SARS-CoV-2. In the NHBE cells, it could just be that the viral load of SARS-CoV-2 was low in that sample and if it were higher, the TE response would be higher. In Figure 1bc, it appears that the TE response tracks with the magnitude of viral infection rather than SARS-CoV-2 inducing a different response. This is in dispute with the title of the manuscript which states 'Impaired activation of Transposable Elements in SARS-CoV-2 infection'. More datasets and replicates should be included to ascertain if there is a difference in the TE response to SARS-CoV-2. It may be that side-by-side serial dilutions of SARS-CoV-2 vs. Flu virus on lung cells would show that both exhibit upregulation of the same TEs in a dose-dependent manner.

Minor comments

1.• 'Basal TE expression and viral load explain immune responses' in the key points is a bit vague and could be left out.

2.• Distinct chromatin and enhancer binding factors occupancy on TEs induced by SARS-CoV-2' in the key points should be modified as we don't know if these TFs are induced by SARS - just a correlation.

3.The abstract begins with two statements that are introduced as if they are set in stone facts upon which the rest of the study is based: 'Transposable element (TE) transcription is induced in response to viral infections. TE induction triggers a robust and durable interferon (IFN) response, providing a host defense mechanism'. Since these are not proven facts, these sentences should be re-worded.

4.In the abstract, the statement: This work provides a
functional explanation for SARS-CoV-2's success in its fight against the host immune system' should also be reworded because it is a correlation made analyzing public data rather than a functional explanation.

5.In the introduction, the authors build a case for TEs being linked to activation of IFN responses. I think that a bit more explanation would be useful there. For example, the statement: 'Lately, it was found that TEs induction stimulate antiviral response, via both cis and trans mechanisms' should be better explained.

6.TE induction upon infection is higher in cell lines compared to primary cells. If TEs are important for IFN responses in vivo, they should be observed in primary cells. Please comment.

7.There are many overstatements in the manuscript, which should be toned down to reflect the data. For example: These, and other 14,32 results, indicate that while IFNβ treatment activate TEs expression, TEs upregulation is an early response to viral infection, which precede the IFN response and induce it in a positive feedback loop 14.

8.The author focus on TEs activated in the presence of SARS-CoV2 infection of lung cell lines and find that they are enriched in active chromatin marks or bivalent H3K9me3 plus H3K36me3 (LINEs). Since these TEs are not associated with IFN induction and not ISG enhancers, they may represent transcriptional noise. It would be more interesting to focus on the TEs upregulated

upon influenza infection so see what is different about those (IFN-associated TEs), if anything in terms of epigenetic marks.

9.Its intriguing that SETD2, a H3K36 lysine trimethylase positively correlates with the TE response and is in the interactome of SARS-CoV-2 and not in the interactomes of other coronaviruses. This could be included in the manuscripts key points.

10.For analyses of ChIP-seq data, only uniquely-mapping reads were included. It should be mentioned that while this allowed the authors to assess the epigenetic signatures present at mappable TEs, it may be that there is missing data on highly repetitive TEs, which may be relevant to regulation of IFN responses.

11.In Figure 1b, PMBC should be corrected to PBMC.

12.Its not clear how IFN-related genes were selected. This is a vague term. Are these Interferon stimulated genes? How are they defined?

13.Figure 2G: This would be more informative if there was a comparison with another set of genes. For example, you could compare % IFN-stimulated genes nearby an up-TE vs. % IFN-stimulated genes not nearby an up-TE.

14.Figure 3: It's not clear if there are replicates here as there are no error bars. H3K9me3 should be assessed alone as its associated with TEs.

15.Figure 4: Left: It's not clear what the purpose is of showing IFNE upregulated in viral infected cells. This is likely due to viral activation of innate immunity rather than through TEs and could be omitted. For the L1PA13 shown, the bivalent ChIP signal appears to relate to the chromatin region rather than being specific to the L1PA13. It would be useful to show at least 5 more examples in supplementary material so that we could see, for example, if epigenetic signatures are inherent to the repeat or to the surrounding chromatin region. This should be discussed.

16.Figure 5: Are there any replicates here and do phenotypic differences reflect differences in viral load? H3K9me3 should be assessed in uninfected cells and whether it changes upon infection, since it's associated with repressed repeats.

17.In the model, 'IFN' genes should be IFN-stimulated genes / IFN-response genes. It's not that clear what is happening from the model. Is H3K9me3 initially enriched on the TEs that increase in expression? If not, it's probably best to remove H3K9me3 from the diagram if it's not relevant to the model. What is proposed to be happening in the right-hand box in the presence of the virus? It's not clear from the diagram.

Referee #1:

This paper, submitted as a commentary, reports a re-analysis of earlier data for transposable element (TE) expression after infection by SARS-CoV-2. They note that the virus causes only weak induction of TEs compared to other viruses. They cite several papers showing that many other viruses cause robust TE induction and also IFN induction, and suggest that TEs may form PAMPs that trigger IFN induction. The authors suggest that the weak TE induction by SARS-CoV-2 may contribute to the weak IFN induction by the virus.
The primary data analyzed here include data in papers by Blanco-Melo (2020) and Huang (2020), and more.

The authors sought to find correlates of the fold TE induction upon infection. Unsurprisingly, they see that higher viral load gives higher TE induction. Also (perhaps) unsurprisingly, high basal TE expression gives lower fold TE induction (as if there is a ceiling, a maximum level, allowed). Primary cells, and ageing cells, have higher basal levels, and so have lower fold induction. The authors argue this could explain weak innate immune responses of those cells.

There is a slightly odd flow to the paper. The main argument being made is that many viruses induce TEs first, and this then promotes IFN production (like Schmidt 2019). On p.7 they note that JAK inhibitors can allow TE induction and block subsequent IFN induction, which is consistent with the idea. But then they raise the idea that the...."IFN response upregulate TE expression", which is likely true, but this is a new causal pathway not really brought up yet.
I think the story might be clearer if it was started, very early, with the idea that both TEs and IFN are induced by many viruses, and that we are not sure which causes which, and that the authors will end up suggesting that TEs often come first and IFN second - but that there is also feedback, with IFN causing more TE expression. The sentence at the top of p. 8 summarizes it well, and maybe this could be said in the introduction.

1. We thank the reviewer for this comment. As the reviewer suggested, we moved the message on IFN capable of inducing TE from p.8 to the introduction p. 4. We added the sentence: "These, and other (Badarinarayan & Sauter, 2021; Macchietto et al., 2020) results, indicate that while IFNβ treatment activates TEs expression (Attig et al, 2017; Hung et al, 2015), TEs upregulation is an early response to viral infection, which precede the IFN response and induce it in a positive feedback loop (Macchietto et al., 2020)."

The paper then shows that genes near TEs are co-activated with TEs, and that IFN-related gene near TEs even more so. However, this pattern was less true for infection by SARS-CoV-2 than influenza. Later they suggest that the TE effect on IFN-related genes

may be in trans rather than in cis - I think the opinion of the authors on this point is not made totally clear.

2. We thank the reviewer for this comment. We showed that during SARS-CoV-2 infection the capacity of TEs to upregulate nearby genes in *cis* is not affected. However, in practice, TEs do not induce the nearby IFN genes, but rather the mild IFN induction is the result of other processes. We clarified the text both in the Results section (p. 8) and in the Discussion (p. 13).

Next, the few TEs that are activated by SARS-CoV-2 were analyzed. The authors note that these were the TEs that already had activated histone marks, and low levels of a silencing mark, before infection. (This seems reasonable, as if they are already poised for induction.) Curiously, LINES that were activated by SARS infection had a combination of a silencing mark and an activating mark.

Finally, they show that genes induced along with TEs by SARS-CoV-2 were enriched for chromatin- and histone-related genes (including SETD2, and other interesting genes).

The summary of the paper near the end is good, but the last two sentences seem unnecessary and distracting - I would stop right before them.

3. We agree with the reviewer's comment and accordingly removed the last two sentences from the discussion.

All told, this is an interesting paper proposing interesting ideas about responses to virus infection. A little re-writing to clarify the basic idea up front, and some introductory sentences as we go into each new point, might help the flow. Also, perhaps some discussion as to why or how SARS-CoV-2 is different from most viruses could be included, though this is really a big area and it could only be mentioned briefly here.

4. We thank the reviewer for this suggestion. We now added a paragraph plainly explaining our hypothesis (page 5). We hope and trust the reviewer will find the revised and improved version suitable for publication.

Epithelial cells are the primary targets and first responders of both IAV and SARS-CoV-2 infections, which initiate the immediate immune response. Viral nucleic acids are recognized by the pattern recognition receptors and culminate in the production of ISGs and pro-inflammatory cytokines. Early type I interferon response is crucial for the host, and both IAV and SARS-CoV-2 produce proteins that interfere with interferon signaling. TEs that are up-regulated following IAV infection contribute to the induction of the antiviral responses (Shen et al, 2022).

Referee #2:

SARS-CoV-2 infection leads to a wide range of outcomes, from asymptomatic infection to death without treatment. While some antivirals now have joined immune modulators, and vaccines have helped to greatly reduce the burden on severe infection, emergence of new variants and uncertainty about future pathogenicity make the study of the pathogenesis of SARS-CoV-2 of continued importance. Within current studies of immunopathogenesis, how transposable elements (TEs) contribute to different inflammatory outcomes is an interesting area of study. TEs could be sensed by the innate immune response, or stimulate adaptive immunity, or enhance immune genes.

Major comments

There have already been several studies published on TE expression following SARS-CoV-2 infection Ferrarini et al, 2021; Marston et al, 2021; Hale 2021; Kitsou et al. 2021; Tovo et al 2021; Balestrieri et al.), and also with other respiratory viruses, including IAV. This paper repeats some of the previous studies and uses published datasets but comes up with different results.

For figure 1, in which the authors claim that IAV caused a global increase in TE subfamilies expression, but SARS-CoV-2 did not, the data sets were from different studies, and cannot be directly compared.

1. The datasets used in this figure are from the same study, i.e. Blanco-Melo et al., comparing NHBE cells following either SARS-CoV-2 or IAV infection.

Some important methodological details are missing and controls lacking. The relationship between viral load in cells in vitro and TE expression, and TE expression in PBMC from patients with COVID-19, has been shown by others, from these and other data sets.The relationship between IFN expression and TE expression during SARS-CoV-2 has also already been published. The authors conclude that: "SARS-CoV-2 infection has a relatively weak effect on TE expression in primary lung epithelia", yet do not mention the Kitsou et al. paper in which there was marked upregulation of TEs in BALF and in epithelial cell lines, which is in line with the other papers published in this area.Overall, the data in this paper does not support the authors' hypothesis that SARS-CoV-2 infection fails to upregulate the TEs coopted for immune activation.

2. We thank the reviewer for this comment. There are already several works that try to study the relations between viral load and TE response and between TE response and IFN response. Specifically, for the relevant papers that were mentioned by the reviewer:
a. Blasterieri et al. , 2021 (currently not cited): This paper shows that TEs are upregulated in SARS-CoV-2 leukocytes, and that TE expression is correlated with cytokines and with severity of the disease. This fits into our model, as we claim the TE is relatively lowly expressed in healthy subjects and when they are

upregulated in response to SARS-CoV-2 it's only in more advanced stages of the disease after the virus succeeded to evade the innate immune response, instead of during early stage.

b. Kitsou, 2021(currently not cited): This paper shows that there is upregulation of ERVs in BALF and not in PBMCs of COVID-19 patients, and an induction of some HERV families in senescence-induced HBECs compared to that in non-induced cells.

The HBEC model is not relevant for SARS-CoV-2 infection as it is a cancer senescence model. It is only relevant for the TE-IFN relation. The BALF and PBMC data are both taken from Xiong et al. (which we cite). We also analyzed the PBMC data in our paper, and obtained similar results to Kitsou et al., where there is no ERV upregulation following SARS-CoV-2 infection. For BALF, we find the data is problematic as Xinog et al. have generated only the covid patients data and not the control data, which they used from a completely different experiment, rendering this comparison flawed.

c. Tovo et al. (currently not cited): This paper used real-time PCR in order to evaluate the changes in expression of only a small number of genes and 3 HERV families. It claims that there is a correlation between TE and IFN, similar to our results. In addition, this paper claims that in mild cases of corona there is an upregulation of the HERV families while in severe cases- they are downregulated. These result are, in principle, in contrast to our results. However, when we examined the actual data in the paper, we found that the results have a very limited value:

1. They are based on only 3 HERV families, in contrast to full estimation of TEs as in our paper.

2. The actual results are very heterogeneous

3. For mild cases comparison: only 1/3 of the HERV families shows a trend of upregulation, where for the other two HERV families all of the effect is based on a few outliers (one of these results is also non-significant).

4. For severe cases: 2/3 are non-significant and 1/3 has a significant p-value that is larger than 0.04.

5. Finally, all of the results were not corrected for multiple comparisons, which would likely also turn most of the significant results to non-significant.

d. Ferrarini,2021 (currently cited): In this paper the authors analyze data from Blanco-Melo et al. and concentrate on TEs that are upregulated in A549 and Calu3 cells that are specific for the response to SARS-CoV-2 and not other viruses. Similar to our findings, there is an upregulation of TE in these cell lines. However, the authors did not analyze primary cells as we did, including the NHBE cells from

Blano-Mello paper, and therefore they did not recognize the lack of response to SARS-CoV-2 compared to other viruses.

e. Marston et al. (2021): In this paper, the authors analyze the data from Blanco-Melo et al., similar to our paper. Similar to our paper, the authors find a correlation between TE upregulation and immune related genes, and notice upregulation in TE in the cancerous cell lines in Blanco-Melo et al. in response to SARS-CoV-2 infection. The authors concentrate on shared TEs that are upregulated in response to SARS-CoV-2 infection in all cancerous cell lines and find a few HERV families that are consistent. They do not, however, analyze the TE response in NHBE cells, and they find downregulation in the PBMC in Xiong et al., in contrast to our results. We suspect that the differences arise as a result of the technical details of the analysis, as the authors of this paper did not remove TEs that are overlapping exons in the genome, therefore introducing a potential bias in the results.

In addition, the authors find the TE upregulation in A549 cells in response to SARS-CoV-2 in the largest response, compared with RSV, HPIV3 and IAV, which stands in contrast to our results. This comparison is problematic, as the number of replicates for SARS-CoV-2 (3 for mock and 3 for infected) is different from the rest of the viruses (2 for mock and 2 for infected). This results in worse p-values in general for the comparison of TE expression in the rest of the virus, and naturally mistakenly results in a smaller effect of TE upregulation in the rest of the viruses compared to SARS-CoV-2. For these reasons, in our paper we omitted this type of analysis.

In summary, most previous works show increased TE expression only in late or severe corona cases, when, according to our model, it is already after the SARS-CoV-2 virus has managed to escape detection by the innate immune sensing. The data we have examined represents the early stage of SARS-CoV-2 infection (24h post infection).

Following the reviewer's comment, in the new version of the manuscript we cited these papers:

In the introduction (page 5):

"Although some studies have addressed TEs expression following SARS-CoV-2 infection (Ferrarini et al, 2021; Marston et al, 2021), and detected minor TE expression changes in primary cells (Kitsou et al., Tovo et al.), none has considered the cell type and basal TE expression levels as important characteristics of their analysis.

And in the discussion (page 13):

"This is in line with previously published data that link TE expression to immune reaction (Chiappinelli et al., 2015; Roulois et al., 2015, kitsou et al. Tovo et al,

Martson et al), as well as studies that show suppression of dsRNA-activated early host responses following SARS-CoV-2 infection (Domizio et al., 2022; Neufeldt et al., 2022). In later stages of the disease and especially in severe cases, when the viral load significantly increases, TEs are eventually induced and generate the delayed IFN response (Blasterieri et al.). Our own analysis also shows a highly significant correlation between TE activation and the induction of IFN response.

Minor comments: the fast moving field of COVID-19 research is such that some of the references listed are now well outdated. There is a reference to "the understanding of the kinetics of IFN response in mild and severe COVID-19 patients is lacking (Park & Iwasaki, 2020)", but hundreds, of papers have been published since then addressing this topic - for example,
The cGAS-STING pathway drives type I IFN immunopathology in COVID-19 [https://www.nature.com/articles/s41586-022-04421-w} - one of many. The authors should reference all published work on TEs and SARS-CoV-2 infection.

3. We agree with the reviewer's comment. We changed the sentence (together with the one before) and cited this paper and other relevant and recently published papers. The new version:

"This is partially achieved by viral proteins that antagonize various steps of dsRNA-activated early host responses (Domizio et al, 2022; Neufeldt et al, 2022). However, we are only beginning to understand the kinetics of IFN response in mild and severe COVID-19 patients (Paludan & Mogensen, 2022)"

Referee #3:

Impaired activation of Transposable Elements in SARS-CoV-2 infection

Sorek, Meshorer and Schlesinger

In this study, the authors propose that SARS-CoV-2 infection is not accompanied by an immediate robust upregulation of transposable element transcription and that this is unusual in comparison to other viral infections. The authors also note that an upregulation of TEs is linked to an IFN response. It has not been demonstrated, of note, that an upregulation of TEs is necessary for an ensuing IFN response during infection, in this study or others. The authors show that IFN-beta treatment of primary lung cells induces upregulation of TEs, perhaps due to some of them acting as enhancers that are responsive to type I IFN signaling. This explanation is supported by the observation that a higher viral load induces a stronger TE response (through more induction of TE enhancers). However, the authors note that in A549 cells treated with a JAK1/2 inhibitor of IFN signaling, there is still upregulation of TEs, despite no induction of interferon-stimulated genes (ISGs). This suggests that TE-derived nucleic acids could have a role

in inducing type I IFNs. In SARS-CoV-2 infected cells, the authors propose that there's delayed TE activation and that the spectrum of TEs induced is different, including less ISG-associated enhancer-TEs. Examination of epigenetic marks at upregulated TEs showed that these elements are naturally enriched for active marks even before infection, except for LINEs, which exhibited a bivalent signature of H3K9me3 and H3K36. This study, although only correlative, is interesting and timely, but there are some issues with the data as it stands, as detailed below. Apart from that, the text needs to be reworked to clarify that it is not known that TE nucleic acid sensing is required for the induction of type I IFN. This is an assumption that the manuscript is built on. In fact, while this is an intriguing hypothesis, it is not clear why you would require TE dsRNA sensing to initiate IFN responses in the presence of an RNA virus, which itself makes plenty of dsRNA through its own replication.

We thank the reviewer for appreciating the significance of the manuscript. In the introduction, we do not wish to claim that TE upregulation is necessary for IFN induction, but that it might precede and enhance the IFN response. To clarify this point, we added this sentence to the introduction: "These, and other (Badarinarayan & Sauter, 2021; Macchietto et al., 2020) results, indicate that while IFNβ treatment activates TEs expression (Attig et al, 2017; Hung et al, 2015), TEs upregulation is an early response to viral infection, which can precede the IFN response and induce it in a positive feedback loop (Macchietto et al., 2020)."

Major comments

1.It looks like the data in Figure 1a is based on only one replicate per sample. From this, it is not possible to say that there is a muted upregulation of TEs in response to SARS-CoV-2. In the NHBE cells, it could just be that the viral load of SARS-CoV-2 was low in that sample and if it were higher, the TE response would be higher. In Figure 1bc, it appears that the TE response tracks with the magnitude of viral infection rather than SARS-CoV-2 inducing a different response. This is in dispute with the title of the manuscript which states 'Impaired activation of Transposable Elements in SARS-CoV-2 infection'. More datasets and replicates should be included to ascertain if there is a difference in the TE response to SARS-CoV-2. It may be that side-by-side serial dilutions of SARS-CoV-2 vs. Flu virus on lung cells would show that both exhibit upregulation of the same TEs in a dose-dependent manner.

1. We thank the reviewer for this comment. Regarding the number of replicates per sample, for most samples the data reflects an average of 3 biological replicates. Following the reviewer's comment, we added these statistics to the legend of Figure 1. Regarding the statement on impaired response of TE in SARS-CoV-2 infection, while we think that it is easier to understand the data plotted in the current order, we agree with the reviewer that more clarification and

discussion is needed on this point. First, for NHBE cells while the viral load of SARS-CoV-2 and IAV is quantitatively different, there is a qualitative difference in the TE response, where SARS-CoV-2 does not induce any response and IAV does (Fig. 1A). In addition, for similar viral loads the TE response to IAV infection is larger. Therefore, following the reviewer's comment, we added the IAV data in Figure 1C to reflect this.

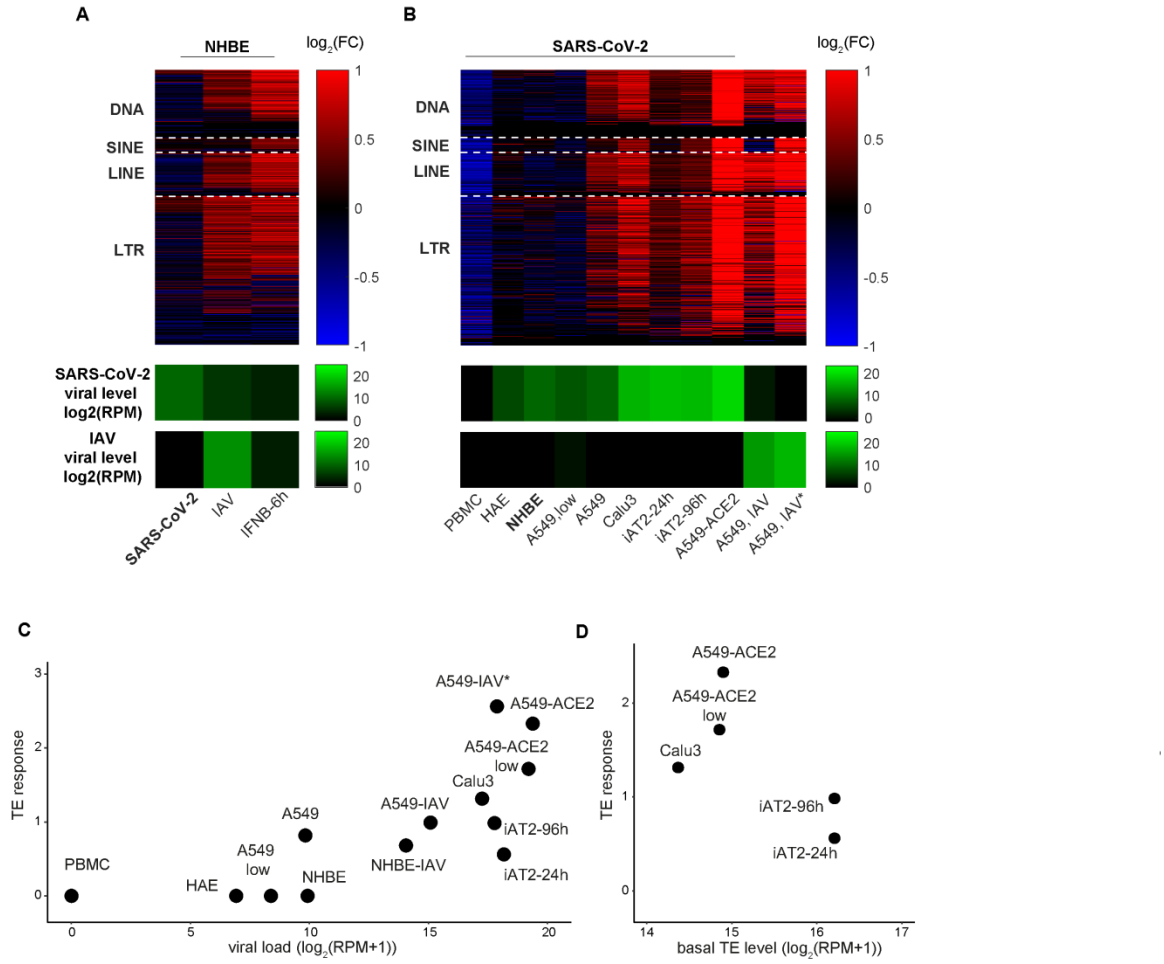


Figure 1. See changes in 1C.

Minor comments

1.• 'Basal TE expression and viral load explain immune responses' in the key points is a bit vague and could be left out.

1. Following the reviewer's comment, we removed it from the key points.

2.• Distinct chromatin and enhancer binding factors occupancy on TEs induced by

SARS-CoV-2' in the key points should be modified as we don't know if these TFs are induced by SARS - just a correlation.

2. We changed this point, according to the reviewer's suggestion, to "SARS-CoV-2 induces distinct chromatin and enhancer binding profile on TEs "

3.The abstract begins with two statements that are introduced as if they are set in stone facts upon which the rest of the study is based: 'Transposable element (TE) transcription is induced in response to viral infections. TE induction triggers a robust and durable interferon (IFN) response, providing a host defense mechanism'. Since these are not proven facts, these sentences should be re-worded.

3. We accept the reviewer's comment, and accordingly made the following change in the abstract:

'Transposable element (TE) transcription is induced in response to viral infections. TE induction triggers a robust and durable interferon (IFN) response, providing a host defense mechanism' -> 'Emerging evidence shows that Transposable element (TE) transcription is induced in response to viral infections. This TE induction is suggested to trigger a robust and durable interferon (IFN) response, providing a host defense mechanism'

4.In the abstract, the statement: This work provides a
functional explanation for SARS-CoV-2's success in its fight against the host immune system' should also be reworded because it is a correlation made analyzing public data rather than a functional explanation.

4. Following the reviewer's comment, we reworded the sentence:
"This work provides a possible explanation for SARS-CoV-2's success in its fight against the host immune system."

5.In the introduction, the authors build a case for TEs being linked to activation of IFN responses. I think that a bit more explanation would be useful there. For example, the statement: 'Lately, it was found that TEs induction stimulate antiviral response, via both cis and trans mechanisms' should be better explained.

5. We thank the reviewer for this comment. Accordingly, we rewrote this paragraph (starting with "Lately") to make the explanation clearer.

6.TE induction upon infection is higher in cell lines compared to primary cells. If TEs are important for IFN responses in vivo, they should be observed in primary cells. Please comment.

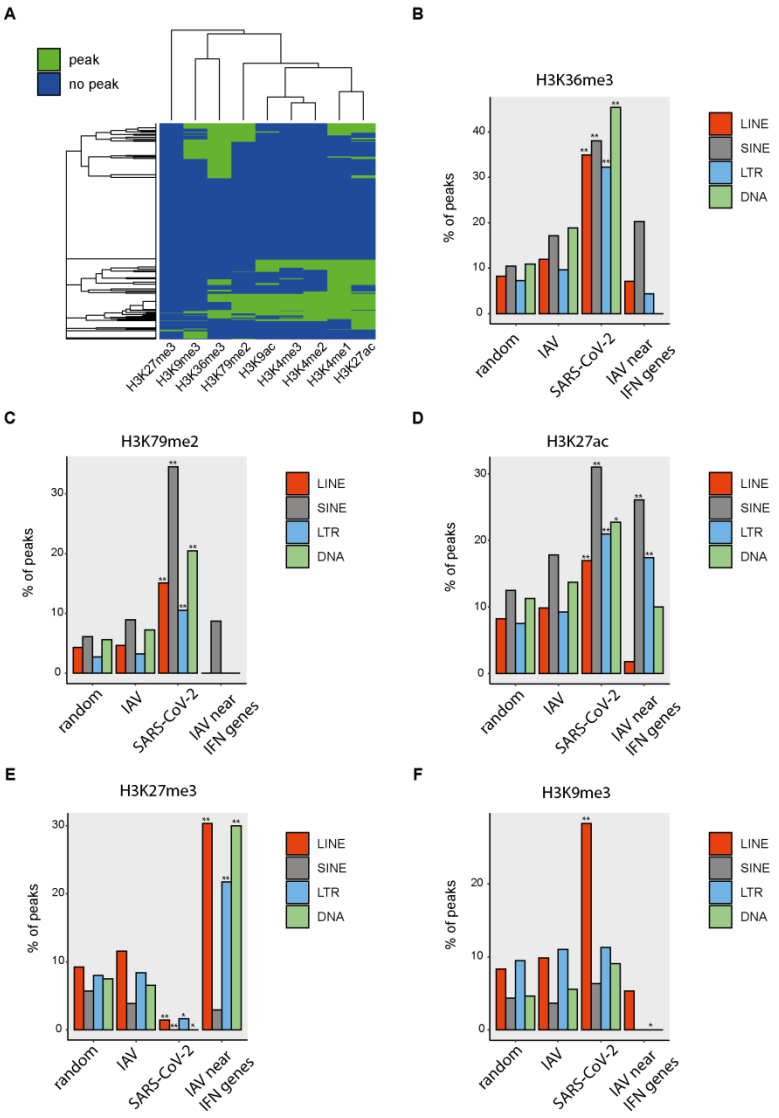
6. We do see a TE response in NHBE primary cells in response to IAV, and also in response to high levels of SARS-CoV-2 in later time points in iAT2 primary-like cells. Therefore, we can speculate that the primary epithelial cells can recognize familiar viruses, but might be slower to respond to unknown viruses like SARS-CoV-2. However, the TE response is lower compared to cell lines. This could be related to the basal TE level, which we found to be higher in uninfected primary cells. This in turn might elicit the TE activation less available for viral detection and therefore these cells produce slower innate response.

7.There are many overstatements in the manuscript, which should be toned down to reflect the data. For example: These, and other 14,32 results, indicate that while IFNβ treatment activate TEs expression, TEs upregulation is an early response to viral infection, which precede the IFN response and induce it in a positive feedback loop 14.

7. Following the reviewer's comment, we moved this sentence to the Introduction and toned it down.

8.The author focus on TEs activated in the presence of SARS-CoV2 infection of lung cell lines and find that they are enriched in active chromatin marks or bivalent H3K9me3 plus H3K36me3 (LINEs). Since these TEs are not associated with IFN induction and not ISG enhancers, they may represent transcriptional noise. It would be more interesting to focus on the TEs upregulated upon influenza infection so see what is different about those (IFN-associated TEs), if anything in terms of epigenetic marks.

8. We thank the reviewer for this important comment. Following the reviewer's comment, we expanded our analysis of the epigenetic profile of the upregulated TEs, focusing on the TEs that are upregulated in response to SARS-CoV-2 and IAV and reside in the proximity of IFN response genes which are upregulated following viral infection. For SARS-CoV-2 this results in merely 3 upregulated TEs near upregulated IFN response genes, in line with our observation that the contribution of upregulated TEs to the *cis* activation of IFN genes is low in SARS-CoV-2 infection. In contrast, for IAV this results in ~180 upregulated TEs, for which we calculated the enrichment of different histone marks, which we added to Figure 3. This analysis revealed several differences from the epigenetic profile of the upregulated TEs in response to SARS-CoV-2. The most striking difference is in the H3K27me3 mark, where while it is depleted on the SARS-CoV-2 induced TEs, it is enriched on the TEs that are induced in response to IAV infection and are in the proximity of induced IFN response genes.



New Figure 3.

Following these results, we also updated the text (page 10) which corresponds to these results and our model (Figure 4D) to reflect the potential mechanisms between SARS-CoV-2 and IAV infections.

9.Its intriguing that SETD2, a H3K36 lysine trimethylase positively correlates with the TE response and is in the interactome of SARS-CoV-2 and not in the interactomes of other coronaviruses. This could be included in the manuscripts key points.

9. We agree with the reviewer that it is intriguing and we further emphasized this point in the revised manuscript, but since it is a correlation, we preferred not to include this in the key points.

10.For analyses of ChIP-seq data, only uniquely-mapping reads were included. It should be mentioned that while this allowed the authors to assess the epigenetic signatures present at mappable TEs, it may be that there is missing data on highly repetitive TEs, which may be relevant to regulation of IFN responses.

10. This comment is true not only for the ChIP-Seq analysis, but also for the RNA-Seq analysis at the level of single loci (in contrast to subfamilies). Therefore, these TEs would not only have a low signal of ChIP-Seq but also will not appear as an expressed (and as induced following infection). So these TEs are completely out of the game. This is mostly relevant for Figure 2G, where the % of upregulated IFN that are located near upregulated TE is a lower bound on the real number. For the ChIP-Seq analysis, there is no way to know how would this affect the results. Following the reviewer's comment, we added a sentence in the discussion:

"One caveat of our analysis is that as we analyze only uniquely mapped reads, we may potentially miss data on highly repetitive TEs which may be relevant to the regulation of the IFN response."

11.In Figure 1b, PMBC should be corrected to PBMC.

11. Corrected.

12.Its not clear how IFN-related genes were selected. This is a vague term. Are these Interferon stimulated genes? How are they defined?

12. This information is included in the Methods section : "In order to robustly calculate the IFN response, we used all genes associated with the following GO terms: GO_0035458, GO_0035457, GO 0035456, GO_0035455, GO_0034340, as well as genes associated with the following pathways in pathcards (https://pathcards.genecards.org/Pathway): Immune response IFN alpha/beta signaling super-pathway and pathways 2747, 2388, 213 (Table EV1)."

Following the reviewer's comment, we also added a sentence in the Results section briefly explaining that and referring to the Methods section: "we next tested the relationship between IFN genes (defined based on gene ontology, see Methods for details) and TEs expression during SARS-CoV-2 infection"

13.Figure 2G: This would be more informative if there was a comparison with another set of genes. For example, you could compare % IFN-stimulated genes nearby an up-TE vs. % IFN-stimulated genes not nearby an up-TE.

13. We thank the reviewer for the suggestion. We now try to clarify that the "% IFN-stimulated genes not nearby an up-TE" is just the complement (100%-X) of the "% IFN-stimulated genes nearby an up-TE". The other aspect, the TE effect on nearby genes, calculated by "% non-IFN stimulated genes *located* near upregulated TEs" vs % "non-IFN stimulated genes *not-located* near upregulated TEs". This is now shown in Figure 2F.

14.Figure 3: It's not clear if there are replicates here as there are no error bars. H3K9me3 should be assessed alone as its associated with TEs.

14. This data is not composed of replicates. For every ChIP-Seq we used the relevant processed file from ENCODE. If there were ChIP replicates in the original experiment we used the processed file that is based on all replicates (based on IDR method). Following the reviewer's comment, we added a sentence to clarify this in the Methods section.

For H3K9me3, we thank the reviewer for this comment as H3K9me3 was accidentally omitted from the results. We therefore added this histone mark as Figure 3F and moved the bivalent signature enrichment analysis to Figure EV2.

15.Figure 4: Left: It's not clear what the purpose is of showing IFNE upregulated in viral infected cells. This is likely due to viral activation of innate immunity rather than through TEs and could be omitted. For the L1PA13 shown, the bivalent ChIP signal appears to relate to the chromatin region rather than being specific to the L1PA13. It would be useful to show at least 5 more examples in supplementary material so that we could see, for example, if epigenetic signatures are inherent to the repeat or to the surrounding chromatin region. This should be discussed.

15. We agree with the reviewer that w/o long read sequencing or hiC we cannot prove that that IFNE is upregulated due to activation in *cis* from the adjacent L1PA13 repeat or as a result of enhancer activity making direct contact between these regions. However, because we see an enrichment of the bivalency mark on upregulated LINEs in response to SARS-CoV-2 infection, the data implies that this is a reasonable possibility. Figure S2 (now EV2) is intended to serve as one such example. Following the reviewer's comment and within the format limitations, we added another example of an upregulated TE in response to SARS-CoV-2 infection which is bivalently marked and is adjacent to an upregulated gene (Figure EV2C). In general, the bivalent mark is not specific to the repeat itself but is rather spread along the repeat and its flanking sequences.

16.Figure 5: Are there any replicates here and do phenotypic differences reflect differences in viral load? H3K9me3 should be assessed in uninfected cells and whether

it changes upon infection, since it's associated with repressed repeats.

16. We are unsure which figure the reviewer related to as there are only four figures. Regarding H3K9me3, the only ChIP-Seq data that is available is for uninfected cells. This data was accidentally omitted from the manuscript and we thank the reviewer for pointing our attention. The data can now be found in Figure 3F.

17.In the model, 'IFN' genes should be IFN-stimulated genes / IFN-response genes. It's not that clear what is happening from the model. Is H3K9me3 initially enriched on the TEs that increase in expression? If not, it's probably best to remove H3K9me3 from the diagram if it's not relevant to the model. What is proposed to be happening in the right-hand box in the presence of the virus? It's not clear from the diagram.

17. Following this comment and comment (8), we edited figure 4, to reflect the main point: in contrast to IAV infection where the upregulated TEs are enriched in the repressive H3K27me3 mark prior to infection and induce IFN response genes that are located in the proximity of these TEs, in SARS-CoV-2 the TEs that are upregulated mostly contain active histone marks and do not reside near IFN response genes. In addition, in the model figure we changed IFN to "IFN-response genes".

Dear Dr. Schlesinger,

Thank you for the submission of your revised commentary. We have now received the enclosed reports from the referees and both support its publication now. Referee 3 still has a minor suggestion that I would like you to incorporate before we can proceed with the official acceptance of your manuscript.

A few editorial requests will also need to be addressed:

- Please reduce the number of keywords to 5.

- Please correct the conflict of interest subheading to "Disclosure and Competing Interest Statement".

- Please callout all EV figure panels. There are callouts to Appendix Figs S14 and S25 which are incorrect.

- Tables EV2, EV3, EV4, EV5 (please remove colors) need to be named Dataset EV1, Dataset EV2, etc. Please also correct all callouts in the manuscript text.

- Please add the subheading 'Expanded View Figure Legends'.

- I attach to this email a related ms file with comments by our data editors. Please address these in the final manuscript file. If possible, please add the number "n" on which the statistical analyses are based in Fig 3B-F and Fig 4C.

EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is exactly 550 pixels wide and 200-600 pixels high (the height is variable). Your synopsis image is fine, but please send us the short summary and 2-3 bullet points along with your final manuscript.

I look forward to seeing a new revised version of your manuscript as soon as possible. Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Best regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

The authors have made revisions that address all of the suggestions made in the earlier review. The flow of the argument in the paper is much improved and is easy to follow.
I consider the paper suitable for publication.

Referee #3:

The authors have now answered all of my questions thank you. I think, though that a sentence should be added to the end of the discussion or elsewhere to explain that TE activation is not known to contribute to IFN responses (and subsequent adaptive immunity) in a viral infection. There are several studies linking TE induction to IFN responses (Chiappinelli et al. as cited and some more recent studies) but these studies are in the absence of a viral infection. Therefore future work will be needed to elucidate not only how TEs drive IFNs but also whether these mechanisms are fundamental to viral clearance.

Dear Esther,

Thank you very much for your email. We have added a sentence in the discussion as reviewer 3 suggested, removed 2 keywords, and added the text and bullet points for the synopsis (also attached here).

I have had trouble with adding the orchid number of Prof. Meshorer and Dr. Sorek, but those are the numbers: Meshorer: *0000-0003-4777-986X*
Sorek: *0000-0002-8233-4762*
if those could be added, it'll be nice

We thank you for your support and look forward to the EMBO Reports publication
All the best
Sharon

2nd Revision - Editorial Decision **21st Jul 2022**

Dear Dr. Schlesinger,

Thank you for submitting your revised manuscript files.

I am not sure whether you have addressed referee 3's last comment?

The current Datasets 6, 7 and 8 should remain EV tables, as they are not newly generated Datasets. Please correct this and all manuscript callouts in the text.

Callouts for Appendix Figs S1A-F, S2A-F, Figs EV2A-C and EV3A-D are still missing, please add. Callouts to Appendix Figs S14 and S25 are still incorrect, please correct.

When starting a new manuscript version, you should be able to bring forward old manuscript files, that can then be corrected. If you have any questions please feel free to contact us.

Thank you,

Esther Schnapp, PhD
Senior Editor
EMBO reports

The authors have addressed all minor editorial requests.

Dr. Sharon Schlesinger
The Hebrew University of Jerusalem
Israel

Dear Sharon,

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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Best,
Esther

Esther Schnapp, PhD
Senior 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.
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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.

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New materials and reagents need to be available; do any restrictions apply?	Yes	Materials and Methods

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	Not Applicable	

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Short novel DNA or RNA including primers, probes: provide the sequences.	Not Applicable	

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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
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Yes	Materials and Methods
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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Animal observed in or captured from the field: Provide species, sex, and age where possible.	Not Applicable	
Please detail **housing and husbandry conditions**.	Not Applicable	

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Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

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If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Not Applicable	

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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)
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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.	Not Applicable	
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.	Not Applicable	
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	Materials and Methods

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In the figure legends: define whether data describe **technical or biological replicates**.	Not Applicable	

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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.	Not Applicable	
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

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	
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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.

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State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
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.	Not Applicable	
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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?	Not Applicable	
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?	Not Applicable	
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?	Yes	Materials and Methods
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	Materials and Methods