EMBO reports

Rapid endotheliitis and vascular damage characterize SARS-CoV-2 infection in a human lung-chip model

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 16th Mar 2021

Dear Dr. Thacker,

Thank you for the submission of your manuscript, the referee reports and your point-by-point response from your previous submission (to a journal outside EMBO press) to our editorial offices. I read your manuscript, went through the other files, and discussed your manuscript with my colleagues. We have also contacted an expert advisor, who examined your manuscript, the referee reports and the point-by-point response and stated that paper should be published in EMBO reports, if you revise the manuscript as indicated in your revision plan.

Moreover, the advisor has an important point that needs to be addressed in the revised version of your manuscript:

'The main question is whether there is infection of endothelial cells. Infection could be just entry of viral particles with minimal production of new viral RNA or proteins. In the p-b-p-response the authors state : "Viral genomes and viral proteins can be found in both cell types; however, we did not see instances of productive infection in endothelial cells." In the manuscript it is state: "Both genomic and antisense RNA are detected in endothelial cells at 1dpi (Fig. 5B), indicating that intracellular viral replication can also occur in these cells." These statements are not in line. Thus, the evidence of infection of endothelial cells needs to be clearly depicted and controls of the stainings shown in figure 5 are needed when referring to endothelial cells.'

Thus, please clarify/show in your revised manuscript if/that endothelial cells are indeed infected.

When submitting your revised manuscript, please also carefully review the instructions that follow below.

PLEASE NOTE THAT upon resubmission revised manuscripts are subjected to an initial quality control prior to exposition to re-review. Upon failure in the initial quality control, the manuscripts are sent back to the authors, which may lead to delays. Frequent reasons for such a failure are the lack of the data availability section (please see below) and the presence of statistics based on n=2 (the authors are then asked to present scatter plots or provide more data points).

When submitting your revised manuscript, we will require:

1) a .docx formatted version of the final manuscript text (including legends for main figures, EV figures and tables), but without the figures included. Please make sure that changes are highlighted to be clearly visible. Figure legends should be compiled at the end of the manuscript text.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure), of main figures and EV figures. Please upload these as separate, individual files upon re-submission.

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 file labeled Appendix. The Appendix should have page numbers and needs to include a table of content on the first page (with page numbers) and legends for all content.

Please follow the nomenclature Appendix Figure Sx, Appendix Table Sx etc. throughout the text, and also label the figures and tables according to this nomenclature.

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See also our guide for figure preparation:
http://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf

3) a .docx formatted letter INCLUDING your detailed point-by-point response (former revision plan) and a response to the comment of the advisor. 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.

4) a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14693178/authorguide). Please insert page numbers in the checklist to indicate where the requested information can be found in the manuscript. The completed author checklist will also be part of the RPF.

Please also follow our guidelines for the use of living organisms, and the respective reporting guidelines: http://www.embopress.org/page/journal/14693178/authorguide#livingorganisms

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

See also: http://embor.embopress.org/authorguide#datadeposition

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Methods) that follows the model below. This is now mandatory (like the COI statement). Please note that the Data Availability Section is restricted to new primary data that are part of this study.

Data availability

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

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

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

Moreover, I have these editorial requests:

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

7) Our journal 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: http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

8) 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, and also add a paragraph detailing this to the methods section. See:
http://www.embopress.org/page/journal/14693178/authorguide#statisticalanalysis

9) Please also note our new reference format:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

10) Please add up to 5 key words to the title page (below the abstract), and a conflict-of-interest statement and a paragraph detailing the author contributions to the manuscript text (next to the acknowledgements).

11) Please order the manuscript sections like this:
Title page - Abstract - Introduction - Results - Discussion - Materials and Methods -Data availability section - Acknowledgements - Author contributions - Conflict of interest statement - References - Figure legends - Expanded View Figure legends.

12) For microscopic images, please add scale bars of similar style and thickness to all the microscopic images, using clearly visible black or white bars (depending on the background). Please place these in the lower right corner of the images. Please do not write on or near the bars in the image but define the size in the respective figure legend.

In addition 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.

Finally, 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 the ORCID ID to the account in our manuscript tracking system in our Author guidelines: http://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

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.

Kind regards,

Achim Breiling
Editor
EMBO Reports

Dear Dr Breiling,

Please find below a summary of the changes made to the manuscript in this final round as well as a point-by-point response to the concerns raised by the advisor.

Summary of changes in the final revised manuscript

Textual edits

We wonder if the use of the phrase 'epithelial cells' may be a cause of some confusion in the minds of the reader/referees whether we refer to upper airway epithelial cells or alveolar epithelial cells. We have therefore edited the manuscript thoroughly to use the acronym 'AT' to refer to the alveolar epithelial cells in the Main Text, Figures, and Figure Legends to avoid confusion with other studies that report on airway epithelial cells. Wherever relevant, we have replaced plots with $\log_{10}$ Fold Change to those with $\log_2$ Fold Change to allow our results to be compared to other studies in the literature. We have also implemented the following changes:

Additional Data

Figure 1J: the sample size for data from 3 dpi is now n=3

1. Figure EV1: new qRT-PCR data regarding the changes in expression of *FGF2*, *VEGFA*, and *VEGFR2* which are related to neuropilin-1 signalling and are important genes for vascular function. The data shows that the loss of *NRP1* expression coincides with a drop in *VEGFR2* expression which would alter endothelial cell function.
2. Appendix Fig S4: we have included additional examples of infected epithelial cells at 2 dpi with high levels of spike protein in new panels C-E.
3. New Appendix Fig. S6: We have provided the negative controls requested by Advisor 2.
4. Figure 2C: the X-axis labels in the panel should read 'N E N E' and were inadvertently swapped to 'N N E E'. This may have contributed to some confusion in interpreting our results, which we apologise for and will correct.
5. Appendix Fig. S3 C, D: the figure has been updated to increase sample size at 3 dpi to n=3.
6. Fig. 4M, N: the figure will be updated to increase sample size at 3 dpi to n=3.

Updates to the Discussion section

An overall summary of the broad changes to the discussion section is as follows:

1. An updated reference to published literature on lung-chip models for SARS-CoV-2 with a brief discussion of the merits of our system.
2. Updated references to published literature on animal models of SAR-CoV-2 and the correlation between their findings and the findings of this manuscript.
3. Updated references to autopsy reports of endothelial cell infection and its role in thrombosis and the relevance of these observations for our manuscript.
4. Updated references to reports by other groups on the inability to establish SARS-CoV-2 infections in endothelial cell monocultures.
5. Updated references and discussion of the results for Tocilizumab administration in clinical trials.
6. A discussion on the strengths and weaknesses of the model. Here we have briefly discussed the cell types used and compare the viral kinetics in our model versus those reported in A549-ACE2 cell lines as well as type II AT organoids. We have also described how the complexity of the model can be increased in a modular manner e.g., to mimic and probe coagulation *in situ*.

Point-by-point response

1. **Concerns regarding viral infection and replication**

The main question is whether there is infection of endothelial cells. Infection could be just entry of viral particles with minimal production of new viral RNA or proteins. In the p-b-p-response the authors state: "Viral genomes and viral proteins can be found in both cell types; however, we did not see instances of productive infection in endothelial cells." In the manuscript it is state: "Both genomic and antisense RNA are detected in endothelial cells at 1dpi (Fig. 5B), indicating that intracellular viral replication can also occur in these cells." These statements are not in line. Thus, the evidence of infection of endothelial cells needs to be clearly depicted and controls of the stainings shown in figure 5 are needed when referring to endothelial cells.

We apologise for the discrepancy between a previous version of the point-by-point response and the Main Text. We do detect instances of antisense RNA that would suggest limited replication of viral genomes does occur in endothelial cells. However, the full viral replication cycle does not appear to take place as we do not detect the release of infectious virions – this is what we refer as a lack of productive infection. In the revised manuscript, we have added the words 'limited' in the sentence quoted by the Advisor. Additionally, in a new Appendix Fig. S6 we provide negative controls for the RNAscope data that is shown in Fig. 5.

2. **Concerns regarding viral infection and replication**

"Overall, the manuscript mainly supports the earlier observations. My main concern is the quality of the virological assessments. I don't think there is convincing data to support infection of the endothelial cells and replication in the lung epithelial cells seems also very limited."

In our revised manuscript, we have characterized the infection of alveolar epithelial and lung microvascular endothelial cells in five different ways:

1. Determination of viral RNA in the effluent from the vascular channel and in an apical wash via a detection kit optimised for SARS-CoV-2 RNA detection (Fig. 2, Appendix Fig. S3)
2. Determination of the number of infectious virions released on the apical side via measurement of plaque forming units (Fig. 2)
3. Quantification of the intracellular viral load in the epithelial and endothelial layer of infected lung-chips via two independent qRT-PCR routes (Fig. 2, Appendix Fig. S3)

4. RNAscope measurements for viral genomic RNA and antisense RNA in cell within the epithelial and endothelial layer (Fig. 5, EV5, Appendix Fig. S7) which included a demonstration of heavily infected epithelial cells at 1 dpi (Fig. EV4).
5. Immunostaining for the spike protein using two different antibodies (Fig. 3 and Fig. 4 respectively) and a further comparison between infected epithelial cells in monoculture and on-chip at 1 and 2 dpi (Appendix Fig. S4).

   All these independent lines of evidence point to the same conclusions: there is a limited amplification of virions in the epithelial layer at an early stage of infection which then diminishes over time. Viral genomes and viral proteins can be found in both cell types; however, we did not see instances of productive infection in endothelial cells because no infectious virions were recovered from the effluent in the vascular channel. We have clarified this point in the abstract itself at **Lines 18-22**:

However, viral RNA and proteins are rapidly detected in the underlying endothelial cells, which are otherwise refractory to infection via the apical route in monocultures. Although endothelial infection on-chip is unproductive, it leads to the formation of endothelial cell clusters with low CD31 expression, a progressive loss of barrier integrity, and a pro-coagulatory microenvironment.

How do these results compare to in vitro and in vivo observations?

1.1 Epithelial cells

These kinetics are very different from infection of primary bronchial epithelial cells (https://www.biorxiv.org/content/10.1101/2020.04.27.062315v2) or the upper airway cell line Calu-3 which has been characterized by a number of groups (e.g., Blanco-Melo *et al.* PMID: 32416070). In contrast, there are no good cell alveolar epithelial cell lines that are permissible for SARS-CoV-2 infection. Many studies, including Blanco-Melo *et al.*, report on replication in alveolar epithelial cells use the type II-like A549 cell line transfected with the ACE2 protein, which makes these cells permissive to viral replication but is unphysiological because ACE2 expression is heterogenous but low in the alveolar space (Hikmet *et al.*, PMID: 32715618). The lung-chip model we present uses primary alveolar epithelial cells (ATs) obtained from a donor via a reputable supplier. ATs rapidly lose their in vivo differentiation into Type I and Type II, and so it is possible that the cells that we use have a lower proportion of Type II ATs compared to *in vivo*. However, we have made every effort to mitigate this by

seeding ATs obtained directly from the supplier on-chip and follow a protocol to enhance type II gene expression. There have been only a handful of papers that report on the kinetics of viral replication in primary ATs *in vitro*, a vast majority of these use cells from Type II AT organoids (e.g., Hekman *et al.* PMID: 33259812, Salahudeen *et al.* PMID: 33238290) wherein this cell population is enriched to an unphysiological level. It is therefore not surprising that these studies report a higher level of viral replication than we observe. These studies also use a high multiplicity of infection (MOI) to ensure high viral loads, whereas our aim was to mimic in vivo infection, where high MOI is unlikely given the small size of aerosols that can be delivered to the alveoli. In that context, it also worth noting that AT1 cells cover 95% of the surface area of the lungs and are in closest contact to the lung endothelium. Clearly, the accurate recreation of the correct type II to type I ratio in *in vitro* systems remains a challenge that needs to be overcome and is a work in progress. We have discussed these points in the Main Text at **Lines 421-446** as follows

The alveolar space has a strikingly different physiology from that of the upper airway. ACE2 is the canonical entry receptor used by SARS-CoV-2 yet is expressed in only a small fraction of alveolar cells, predominantly type II ATs. It has been speculated that high levels of ACE2 expression may be necessary for export of mature virions (Klein et al., 2020). Consequently, because most virology assays measure viral titre, studies of infection of alveolar epithelial cells have either used cell lines such as A549 transfected with ACE2 or utilized type II AT alveolar organoids that does not capture the functional diversity of the lung(Hekman et al., 2020). As a consequence, the infection of type I ATs, the role of alternate receptors such as NRP1 that is more abundant in the lower airways and the effects of uptake via NRP1 vs. ACE2 on the viral life cycle is relatively unknown.

The lung-on-chip architecture is well-placed to mimic alveolar physiology. The endothelial layer is exposed to fluid flow and shear stress that is necessary for an accurate recreation of endothelial cell biology and is difficult to achieve in Transwell based systems. ATs can be maintained at an air-liquid interface which is necessary for surfactant production. Although Zhang et al. (Zhang et al., 2021) recently reported on a lung-on-chip platform to study SARS-CoV-2 infection of the alveolar space, the platform was not maintained at an air-liquid interface and used immortalized alveolar

epithelial cell lines and a lung microvascular endothelial cell line. In contrast, we populate our system with the relevant primary human cells that includes a mix of type I and type II ATs. We make every effort to minimize in vitro passages of ATs to avoid the loss of type II ATs, although it is likely that the cell population does not completely reflect the type II: type I AT ratio found in the lung. However, we report low ACE2 expression and high NRP1 expression, consistent with human alveolar physiology.

By and large, our observations agree with reports from *in vitro* infections of whole lung tissue and isolated AT cells (Hui *et al.*, PMID: 32386571). They are also in good agreement with the findings from (Hou *et al.*, PMID: 32526206) that isolated specific ATII and AT1 populations from the lungs of patients and demonstrated a clear 'replication gradient' in the lung when quantified with plaque forming unit assays. Furthermore, in many autopsy reports, infected AT cells are identified through RNA FISH measurements (as in Hou *et al.*) but culturable virus has rarely been extracted beyond day 8 or 9 of illness (e.g., La Scola *et al.* PMID: 32342252), although viral RNA can be obtained from the lung for many days subsequent. This correlates with the observations that viral load in the upper and lower respiratory tract peak in the first and second week of infection respectively (as reviewed in Cevik *et al.* PMID: 33521734 and other references contain therein)**.** Overall, the picture is one of rapid replication in the upper respiratory tract in early infection (and in many mild or asymptomatic cases the disease does not progress further) followed by infection of the lower respiratory tract (which leads to severe disease, but the patient is not infectious). Our observations, which directly model the lower respiratory tract infection, fit well with these clinical findings.

In the revised manuscript, we have mentioned these points at **Lines 453-460**:

In contrast to models of productive replication in epithelial cells, we observe slow intracellular viral replication in ATS without significant release of infectious virions, observations that are consistent with reports of observation of viral RNA in patients long after they cease to be infectious(Bussani *et al.*, 2020; Cevik *et al.*, 2020). Microscopy-based analyses also revealed that responses to infection are highly heterogenous; for example, the few foci of heavily infected cells might represent cells with constitutively higher levels of *ACE2* expression at the time of infection.

1.2 Endothelial cells

Our findings are all the more relevant when we consider endothelial cell infection. Although there is a clinical consensus that endothelialitis does occur in COVID-19, there conflicting reports on whether individual endothelial cells are infected and if infection itself is responsible for the inflammation (Basta, PMID: 33493794 for a summary) and compare the findings in Dorward *et al.* PMID: 33217246 vs. Busani *et al.* PMID**:** 33158808 as examples of opposing observations from autopsy reports**.** Given that endothelial cell monocultures *in vitro* do not get infected or show or show signs of inflammation, our results are an important confirmation that infection is indeed possible, although it is not productive and does not lead to amplification of virions as is likely to be the case as SARS-CoV-2 virus is rarely detected directly in the blood. We have discussed these points at Lines 483-497 in the Main Text as follows:

The lack of endothelial cell infection and inflammation in Zhang *et al.* (Zhang *et al.*, 2021)may be explained by the absence of air-liquid interface, which would alter cell-cell communication between the endothelial and epithelial cell layers, as well as the maintenance of flow in the epithelial channel which would wash away virions or inflammatory stimuli secreted by the epithelial cells. Indeed, Wang *et al.*(Wang *et al.*, 2020) reported that supernatants from infected alveolar epithelial cells were sufficient to induce changes in tight junction protein expression in endothelial cell monolayers, consistent with a role for cell-cell communication at the alveolar interface. Clinically, although endothelialitis is an accepted facet of COVID-19 pathophysiology, there are conflicting reports of the presence of viral antigens in endothelial cells in autopsy samples(Bussani *et al.*, 2020b; Basta, 2021; Dorward *et al.*, 2021). Thus, although the exact mechanisms of endothelial infection and damage remain to be elucidated, our results provide important verification that infection can occur and may well lead to the persistent infection and endothelial-cell specific cell damage observed in human patient samples.

To further convince both advisors, we have provided images from a negative control for the RNAscope in a new Appendix Fig. S6 and included additional examples of infected epithelial cells at 2 dpi with high levels of spike protein in new panels C-E in Appendix Fig S4.

1st Revision - Editorial Decision 15th Apr 2021

Dear Dr. Thacker,

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the report from the advisor that was asked to re-evaluate your study, you will find below. As you will see, the advisor now fully supports the publication of your study in EMBO reports. The advisor has one further point I ask you to fix during a final reversion of the manuscript.

Further, I have these editorial requests I ask you to address:

- Please shorten the title of the manuscript to not more than 100 characters (including spaces).

- Per journal policy, we do not allow 'data not shown' (see pages 7 and 15 of your manuscript). All data referred to in the paper should be displayed in the main or Expanded View figures, or the Appendix. Thus, please add these data, or remove the statement, if these data are not essential. See:
http://www.embopress.org/page/journal/14693178/authorguide#unpublisheddata

- The callouts for the Appendix figures should be 'Appendix Figure Sx' throughout the text. Several callouts for these figures miss the word 'Appendix'. Please correct this.

- The callouts for the Appendix tables should be 'Appendix Table Sx' throughout the text. The callouts for these tables presently miss the word 'Appendix'. Please correct this.

- There seems to be no legend for panel 7H. Please check.

- Please call out the figure panels in a sequential manner (or change their position). Presently Fig. 4C+D are called out before Fig 1F, Fig. 3D is called out before 3A and Fig. EV2F is called out before EV2A.

- There is a callout to Fig EV5F, but there is no such panel. Please check.

- Please make sur that regarding data quantification and statistics, where applicable, the number "n" for how many independent experiments and the type or replicate (biological or technical replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is stated in the respective figure legends (of main, EV and Appendix figures). Please provide statistical testing where applicable (for main, EV and Appendix figures). Presently many diagrams have no (or only partially) statistics. Please add statistical testing to all diagrams with n>2. Please also indicate (with n.s.) if testing was performed, but the differences are not significant. It would also render the diagrams less crowded, if the significance would be marked in the diagrams with asterisks, and the p-values would be mentioned only in the legend.

- Please display the references according to our new reference format (et al only if there are more than 10 authors):
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text. Please provide your final manuscript file

with track changes, in order that we can see the modifications done.

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.

Kind regards,

Achim Breiling
Editor
EMBO Reports

Advisor:

In the revised version the authors have addressed basically most comments raised earlier by the other reviewers, but more importantly some additional statements have been added regarding the infection of endothelial cells by SARS-CoV-2 in their model system. In addition, control stainings have been added to further support the findings (Fig S6).

Especially the statement added to the abstract puts the findings made in the right perspective "However, viral RNA and proteins are rapidly detected in the underlying endothelial cells, which are otherwise refractory to infection via the apical route in monocultures. Although endothelial infection on-chip is unproductive, it leads to the formation of endothelial cell clusters with low CD31 expression, a progressive loss of barrier integrity, and a pro-coagulatory microenvironment." The revised discussion on their findings in relation to what is published also improved the manuscript substantially.

In the end, some of the observations made are maybe a bit surprising but may trigger other researchers to repeat such experiments. Therefore I think this is an important contribution.
The kinetics of infection of endothelial cells in the LoC and the fact that these are infected through release from the basolateral side of the epithelial cells poses some problems in verifying this technically. However, the fact that changes to the endothelial cells are not seen in monocultures is a strong point of the manuscript (infection and downstream effects on the cells).

One minor comment:
Please add in Fig 1 and FigS3 viral genomes/ml or PFU/ml on the y-axis.

Dear Dr Breiling,

Thank you very much for your Decision Letter. Please find below a summary of the changes made to the manuscript in this final round in response to the editorial requests as well as the final changes requested by the advisor. I hope with this final revision the manuscript will be entirely suitable for rapid publication at EMBO Reports and thank you again for your efforts in expediting the process.

- Please shorten the title of the manuscript to not more than 100 characters (including spaces).

AU Response: We have shortened the title as requested.

- Per journal policy, we do not allow 'data not shown' (see pages 7 and 15 of your manuscript). All data referred to in the paper should be displayed in the main or Expanded View figures, or the Appendix. Thus, please add these data, or remove the statement, if these data are not essential.

AU Response: We have removed the statement of 'data not shown' at both locations and explicitly written that no plaques were observed (at page 7) and IL-1B and IP-10 were not detected via ELISA (page 15).

- The callouts for the Appendix figures should be 'Appendix Figure Sx' throughout the text. Several callouts for these figures miss the word 'Appendix'. Please correct this.

AU Response: We apologise for the oversight and have corrected this throughout the manuscript.

- The callouts for the Appendix tables should be 'Appendix Table Sx' throughout the text. The callouts for these tables presently miss the word 'Appendix'. Please correct this.

AU Response: We apologise for the oversight and have corrected this throughout the manuscript.

- There seems to be no legend for panel 7H. Please check.

AU Response: We apologise for the oversight and have corrected this in the revised manuscript.

- Please call out the figure panels in a sequential manner (or change their position). Presently Fig. 4C+D are called out before Fig 1F, Fig. 3D is called out before 3A and Fig. EV2F is called out before EV2A.

AU Response: We have edited the revised manuscript to remove the reference to Fig. 4C+D along with Fig. 1F. We have also edited the text so that Fig. 3D is now not called out before Fig. 3A. The reference to Fig. EV2F should actually have been Appendix Fig. S2F, and this has been corrected which also solved the issue of the incorrect callout.

- There is a callout to Fig EV5F, but there is no such panel. Please check.

AU Response: The callout to the correct panel (Fig. EV2F) has now been inserted.

- Please make sur that regarding data quantification and statistics, where applicable, the number "n" for how many independent experiments and the type or replicate (biological or technical replicates) were performed, the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is stated in the respective figure legends (of main, EV and Appendix figures). Please provide statistical testing where applicable (for main, EV and Appendix figures). Presently many diagrams have no (or only partially) statistics. Please add statistical testing to all diagrams with n>2. Please also indicate (with n.s.) if testing was performed, but the differences are not significant. It would also render the diagrams less crowded, if the significance would be marked in the diagrams with asterisks, and the p-values would be mentioned only in the legend.

AU Response: As requested we have thoroughly edited the Figure Legends to include all the information requested and also to address the queries raised by the technical editors (inserted as responses to the comments). We have also provided statistical testing for the qRT-PCR measurements across all the Figures (main, EV, Appendix) and these are indicated with an asterisk. Differences that are not significant have been labelled 'ns'. We also noticed that we had omitted to convert the plots in Fig. 6 B and 6E to a $\log_2$ (Fold change) scale, as we had done for all other panels in the last round of revision. We have therefore corrected this omission. Where necessary, sentences in the main text have been slightly modified to highlight gene expression differences that are statistically significant to improve readability.

- Please display the references according to our new reference format (et al only if there are more than 10 authors):

AU Response: We have incorporated the new reference format as requested.

One minor comment:

Please add in Fig 1 and FigS3 viral genomes/ml or PFU/ml on the y-axis.

AU Response: We believe the advisor referred to Fig. 2 and Appendix Fig. S3, which we have duly modified to show viral genomes/ml and/or PFU/ml where appropriate.

2nd Revision - Editorial Decision 26th Apr 2021

Dr. Vivek Thacker
Ecole Polytechnique Federale de Lausanne
Switzerland

Dear Dr. Thacker,

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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Journal Submitted to: EMBO Reports
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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 $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	This is not relevant to the study
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No animals were used in this study
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	This is not relevant to the study
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For animal studies, include a statement about randomization even if no randomization was used.	No animals were used in this study
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.	This is not relevant to the study
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5. For every figure, are statistical tests justified as appropriate?	Yes, the statistical tests used to calculate the P values are mentioned in the figure legend of each figure.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The sample size was too small to assume normality, therefore we used the Kruskall-Wallis One-Way ANOVA test which does not assume a normal distribution.

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Is there an estimate of variation within each group of data?	No
Is the variance similar between the groups that are being statistically compared?	No

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibodies obtained from a commercial source are listed in Appendix Table S2 and include the catalogue number and clone number. Antibodies obtained from the group of Prof Carolyn Machamer (listed at page 29, line 734) are linked to a publication where they were validated
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	The source of the cells is mentioned in the Materials and Methods (Page 25, line 570) and were verified to be free of mycoplasma contamination by the supplier. Cells passaged in-house were tested and verified to be free of mycoplasma contamination.

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	No animals were used in this study
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	No animals were used in this study
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.	No animals were used in this study

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	This is not relevant to this study
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.	This is not relevant to this study
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	This is not relevant to this study
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	This is not relevant to this study
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	This is not relevant to this study
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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.	This is not relevant to this study

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	The required data availablity statement is in the manuscript at page 32, line 809
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G- Dual use research of concern

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