nature research

Peer Review Information

Journal: Nature Immunology
Manuscript Title: SARS-CoV-2 infection of hACE2 transgenic mice causes severe lung inflammation and impaired function
Corresponding author name(s): Michael S. Diamond

Reviewer Comments & Decisions:

Decision Letter, initial version:

Subject: Decision on Nature Immunology submission NI-A30343A

Message: 24th Jul 2020

Dear Dr Diamond,

Your Article, "SARS-CoV-2 infection in the lungs of human ACE2 transgenic mice causes severe inflammation, immune cell infiltration, and compromised respiratory function" has now been seen by 2 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are [very] interested in the possibility of publishing your study in Nature Immunology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file in Microsoft Word format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/ni/authors/index.html. Refer also to any guidelines provided in

this letter.

* Please include a revised version of any required reporting checklist. It will be available to referees to aid in their evaluation of the manuscript goes back for peer review. They are available here:

Reporting summary:
https://www.nature.com/documents/nr-reporting-summary.pdf

When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please use the link below to submit your revised manuscript and related files:
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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within two weeks. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Immunology or published elsewhere.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Immunology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Jamie D.K. Wilson, D.Phil
Chief Editor
Nature Immunology
212 726 9207
j.wilson@us.nature.com

Referee expertise:

Referee #1:

Referee #2:

Referee #3:

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
This manuscript by Winkler et al. adds to the growing number of animal models to study COVID19. The authors specifically evaluate SARS-CoV-2 infected heterozygous mice transgenic for human ACE2 under the cytokeratin -18 promoter to drive expression in epithelial cells. The study is comprehensive in demonstrating high viral titers in lung tissues and viral spread, but lower infection in other organs coincident with levels of hACE2 expression. Lethality appears to be associated with lung pathology and decline in pulmonary function, with brain infection in a very limited number of animals. Moreover, inflammatory responses are demonstrated longitudinally by complementary assessment of leukocyte infiltration/composition, cytokine profiling, and RNAseq analysis.
Overall, results are comprehensive and well presented using multiple approaches, re viral titers and in situ viral RNA detection, histological and flow cytometric analysis, as well as physiological functions to characterize pathogenesis. As in other models, some features clearly reflect some characteristics of severe COVID19 in humans. Several other murine models of SARS CoV-2 infection have recently been published using hACE2 expressing mice, e.g. HFH4-hACE2 (Jiang RD et. al, 2020), CRISPR/Cas9 KI-hACE2 (Sun SH et al, 2020) and Ad5-hACE2 transduced mice (Sun J et al. 2020; Hassan et al 2020). This does not distract from the findings as all models show distinct severity of pathology, multi organ involvement, as well as mortality and thus provide distinct tools to assess therapeutics and vaccine outcome. The present studies are unique in providing physiological assessment of lung function as well as blood derived clinical chemistry and hematological parameters.
Results are well documented and conclusions supported by numerous technical approaches and careful evaluation. Statistics are used appropriately and conclusions are justified.

The manuscript would benefit from addressing items below.

Concerns/Suggested improvements:
1. Is there evidence for gender differences?
2. Fig.1 /ex Fig 1 show no statistics for titers/RNA. Are there no statistically significant differences across timepoints?
3. Brain histology: The text line 102 indicates sparing of olfactory bulb (OB) infection. Do the authors have data on d2 infected mice for nasal epithelium and
OB? Can earlier infection of the OB be excluded? Along similar lines, do brain tissues from mice without evidence of direct CNS infection show microglia/astrocyte activation (e.g. by Iba-1, GFAP morphology).
4. Do mice show T cell lymphopenia as in severe COVID19 patients?

Minor:
1. Lines 63/64. The statement that other models do not cause mortality should be modified as the Jiang RD report (HFH4hACE2 mice) does demonstrate a mixed survival phenotype of some mice.
2. Lines 184/185. The text indicates an increase in certain lymphocyte populations yet no statistical differences are noted in Fig. 4a?
3. Line 221-226/279…. As IFNg is elevated at day 7 (ex Fig 5), why is IFNg excluded as explaining distinct early and late ISG signatures?
4. The recent Sun …Zhao publication (Cell 182, 2020) should be included and discussed.

Reviewer #2:
Remarks to the Author:
Manuscript Nr: NI-A30343A
Winkler et al., "SARS-CoV-2 infection in the lungs of human ACE2 transgenic mice causes severe inflammation, immune cell infiltration, and compromised respiratory function"

The authors report yet another human ACE2 expressing mouse that can be infected with SARS-CoV-2. They argue that their animal model is unique due to the pathology that is observed, while infection induced disease was mild in other SARS-CoV-2 expressing mice, ferrets, hamsters and monkeys. In addition to virus replication in the lung, they observe SARS-CoV-2 in heart, brain and intestine in a subset of animals. Furthermore, they observe extensive inflammatory immune cell infiltrates in infected lungs and cytokine production with some similarities to previously published findings ins patients. They conclude that their animal model might be well suited to study treatments and the immunopathology of COVID-19.

Although potentially interesting the similarities with the human disease are not sufficiently explored to conclude that the presented animal model is more suitable to study COVID-19.

Major comments:
1. The choice of K18 as the promotor for ACE2 expression is not justified? A comparison between human ACE2 expression in different organs as well as cell types including leucocytes, and K18 expression should be provided.
2. The authors speculate that the increased severity of SARS-CoV-2 infection in K18-hACE2 mice is mainly due to immune pathology. However, no evidence for this, except for the kinetics of immune cell infiltration and cytokine production, is provided. A treatment that addresses immune pathology in humans and demonstrates some efficacy in patients,

like IL-6R blockade, should be tested to provide evidence for this hypothesis.
3. Lymphopenia has been observed in severe COVID-19 cases. The authors should report if they see any similarities in the blood leucocyte composition between SARS-CoV-2 infected K19-hACE2 mice and patients with severe disease.

Minor comments:
1. Since the K18-hACE2 transgenic integration in the used mouse background is not primarily in the X chromosome no gender effects can be investigated. This should be acknowledged.

In summary, the reported animal model is for sure an additional preclinical platform to investigate and treat SARS-CoV-2 infection, but its superiority to other already published models, is mainly based on more infected animals succumbing to infection but not in a comorbidity dependent fashion as in human patients. Therefore, further evidence for the faithful recapitulation of the human disease should be provided.

Author Rebuttal to Initial comments

Reviewers' Comments:

Reviewer #1:

This manuscript by Winkler et al. adds to the growing number of animal models to study COVID19. The authors specifically evaluate SARS-CoV-2 infected heterozygous mice transgenic for human ACE2 under the cytokeratin -18 promoter to drive expression in epithelial cells. The study is comprehensive in demonstrating high viral titers in lung tissues and viral spread, but lower infection in other organs coincident with levels of hACE2 expression. Lethality appears to be associated with lung pathology and decline in pulmonary function, with brain infection in a very limited number of animals. Moreover, inflammatory responses are demonstrated longitudinally by complementary assessment of leukocyte infiltration/composition, cytokine profiling, and RNAseq analysis.

We appreciate the concise summary.

Overall, results are comprehensive and well-presented using multiple approaches, re viral titers and in situ viral RNA detection, histological and flow cytometric analysis, as well as physiological functions to characterize pathogenesis. As in other models, some features clearly reflect some characteristics of severe COVID19 in humans. Several other murine models of SARS CoV-2 infection have recently been published using hACE2 expressing mice, e.g. HFH4-hACE2 (Jiang RD et. al, 2020), CRISPR/Cas9 KI-hACE2 (Sun SH et al, 2020) and Ad5-hACE2 transduced mice

(Sun J et al. 2020; Hassan et al 2020). This does not distract from the findings as all models show distinct severity of pathology, multi organ involvement, as well as mortality and thus provide distinct tools to assess therapeutics and vaccine outcome. The present studies are unique in providing physiological assessment of lung function as well as blood derived clinical chemistry and hematological parameters.

We greatly appreciate this supportive comment.

Results are well documented and conclusions supported by numerous technical approaches and careful evaluation. Statistics are used appropriately and conclusions are justified.

We again appreciate this comment.

The manuscript would benefit from addressing items below.

Concerns/Suggested improvements:

1. Is there evidence for gender differences?

We analyzed for this and found no sex-based differences in infection or disease. Unlike in humans, ACE2 is not expressed from the X chromosome in this model. This has been clarified in the Results (p. 5) and Discussion (p.15).

2. Fig.1 /ex Fig 1 show no statistics for titers/RNA. Are there no statistically significant differences across timepoints?

Indeed, we did observe significant differences in the lung. We have modified the Figure accordingly.

3. Brain histology: The text line 102 indicates sparing of olfactory bulb (OB) infection. Do the authors have data on d2 infected mice for nasal epithelium and OB? Can earlier infection of the OB be excluded? Along similar lines, do brain tissues from mice

without evidence of direct CNS infection show microglia/astrocyte activation (e.g. by Iba-1, GFAP morphology).

The Reviewer raises an interesting question. (a) Unfortunately, we did not analyze infection in nasal washes or turbinates. Although we agree we cannot exclude that the olfactory bulb was infected at all, we can certainly say that if it were infected, the level is much lower than the remainder of the brain (excluding the cerebellum). We have modified the text accordingly (p.6). (b) We have re-examined the brains for pathological changes in the brains of the mice that were negative by viral RNA ISH. This revealed no significant histopathological changes in brains of mice that were negative for viral RNA by ISH.

4. Do mice show T cell lymphopenia as in severe COVID19 patients?

Yes, we have analyzed data blood leukocyte data after infection. Indeed, as in humans, we see lower levels of T cells, B cells, and monocytes. This data is now included in ***Fig 4e-f****.*

Minor:
1. Lines 63/64. The statement that other models do not cause mortality should be modified as the Jiang RD report (HFH4hACE2 mice) does demonstrate a mixed survival phenotype of some mice.

The reviewer is correct, the Introduction has been modified to correct this.

2. Lines 184/185. The text indicates an increase in certain lymphocyte populations yet no statistical differences are noted in Fig. 4a?

Although CD45^{+} numbers are increased in SARS-CoV-2 infected mice at 2 and 4 dpi, these differences did not attain statistically significance. We have clarified this point in the text.

3. Line 221-226/279…. As IFNg is elevated at day 7 (ex Fig 5), why is IFNg excluded as explaining distinct early and late ISG signatures?

This is an interesting point raised by the reviewer. Although IFNg normally induces a distinct transcriptional response, a portion of the ISGs expressed at later time points also can be upregulated in response to IFNg signaling such as Irf1, Irf5, and Irf8. However, other genes induced in this late ISG signature such as IFITM1, SAMHD1, and Oas1 are classically thought to be the result of type I or III IFN signaling. This raises the possibility of IFNg in addition to type I/III IFNs contributing to the late ISG responses. Accordingly, we have added this possibility to the text (p.10).

4. The recent Sun …Zhao publication (Cell 182, 2020) should be included and discussed.

We agree and have added this to the Discussion as suggested.

Reviewer #2:

Winkler et al., "SARS-CoV-2 infection in the lungs of human ACE2 transgenic mice causes severe inflammation, immune cell infiltration, and compromised respiratory function"

The authors report yet another human ACE2 expressing mouse that can be infected with SARS-CoV-2. They argue that their animal model is unique due to the pathology that is observed, while infection induced disease was mild in other SARS-CoV-2 expressing mice, ferrets, hamsters and monkeys. In addition to virus replication in the lung, they observe SARS-CoV-2 in heart, brain and intestine in a subset of animals. Furthermore, they observe extensive inflammatory immune cell infiltrates in infected lungs and cytokine production with some similarities to previously published findings ins patients. They conclude that their animal model might be well suited to study treatments and the immunopathology of COVID-19.

Although potentially interesting the similarities with the human disease are not sufficiently explored to conclude that the presented animal model is more suitable to study COVID-19.

We appreciate the Reviewer's viewpoint but nonetheless feel strongly that this K18-hACE2 transgenic mouse and our analysis has several unique aspects.

(a) We characterized SARS-CoV-2 infection in a longitudinal manner and show peak lung infection within 2 to 4 days of intranasal inoculation, with persistence for approximately one week,

SARS-CoV-2 infection of pneumocytes progresses to an interstitial pneumonia characterized by marked inflammatory cell infiltrate and collapse of alveoli. This was associated with clinical morbidity as judged by substantive weight loss and ultimately lethality.

(b) We correlated the immunopathology observed in this model with changes in pulmonary physiology. We performed invasive mechanical ventilation and showed that infection and immunopathology are associated with extensive changes to the biomechanical properties of the lung parenchyma. These measurements are similar to the lung pathophysiology observed in severely ill COVID-19 patients.

(c) We demonstrated substantial infiltration of monocytes, neutrophils, and activated T cells in the lung and BAL coinciding with induction of pro-inflammatory cytokines. Theses inflammatory changes occur later in the course of SARS CoV-2 infection after the peak of viral replication. Moreover, we observed lymphopenia, which also is described in human patients.

(d) We performed longitudinal RNA sequencing analysis and showed distinct transcriptional signatures associated with early and late immune responses. A better understanding of these pathways could facilitate in the selection and development of immunotherapies for the treatment of severe SARS CoV-2 infection in humans.

Major comments:

1. The choice of K18 as the promotor for ACE2 expression is not justified? A comparison between human ACE2 expression in different organs as well as cell types including leucocytes, and K18 expression should be provided.

*The K18-hACE2 transgenic mice were described previously in the context of the original SARS-CoV infection. Here, we showed that the tissues supporting SARS-CoV-2 infection in this model mirrored the pattern of hACE2 expression, with the highest receptor mRNA levels in the lungs, colon, kidney, and brain (**Extended Data Fig 1a**).*

2. The authors speculate that the increased severity of SARS-CoV-2 infection in K18-hACE2 mice is mainly due to immune pathology. However, no evidence for this, except for the kinetics of immune cell infiltration and cytokine production, is provided. A treatment that addresses immune pathology in humans and demonstrates some efficacy in patients, like IL-6R blockade, should be tested to provide evidence for this hypothesis.

We agree this is an important question. However, there is some element of "fishing" here since even in humans there is not good data to know which iintervention would work and whether IL-6R blockade is a therapeutic answer. Respectfully, we believe such experiments are beyond the scope of this paper, which establishes the model and characterizes the immune cell and inflammation phenotypes, and their associated consequences. That said, we are indeed initiating studies that will take several months with a large panel of immunomodulatory agents to address this question.

3. Lymphopenia has been observed in severe COVID-19 cases. The authors should report if they see any similarities in the blood leucocyte composition between SARS-CoV-2 infected K18-hACE2 mice and patients with severe disease.

*We agree with this comment and have added data showing lymphopenia after SARS-CoV-2 infection of K18-hACE2 mice (**Fig 4e-f**).*

Minor comments:

1. Since the K18-hACE2 transgenic integration in the used mouse background is not primarily in the X chromosome no gender effects can be investigated. This should be acknowledged.

We agree and have added a comment on this point (p.15).

In summary, the reported animal model is for sure an additional preclinical platform to investigate and treat SARS-CoV-2 infection, but its superiority to other already published models, is mainly based on more infected animals succumbing to infection but not in a comorbidity dependent fashion as in human patients. Therefore, further evidence for the faithful recapitulation of the human disease should be provided.

We have added the requested data and clarified the utility and advances of the model, as described above.

Decision Letter, first revision:

Subject: Nature Immunology - NI-A30343B pre-edit
Message: Our ref: NI-A30343B

29th Jul 2020

Dear Dr. Diamond,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Immunology manuscript, "SARS-CoV-2 infection in the lungs of human ACE2 transgenic mice causes severe inflammation, immune cell infiltration, and compromised respiratory function" (NI-A30343B). Please follow the instructions provided here and in the attached files, as the formal acceptance of your manuscript will be delayed if these issues are not addressed. Once accepted this paper will be fast-tracked for publication.

When you upload your final materials, please include a point-by-point response to the points below. We won't be able to proceed further without this detailed response.

General formatting:
1. Please include a separate "Data availability" subsection at the end of your Online Methods. This section should inform our readers about the availability of the data used to support the conclusions of your study and should include references to source data, accession codes to public repositories, URLs to data repository entries, dataset DOIs, and any other statement about data availability. We strongly encourage submission of source data (see below) for all your figures. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, these should be included in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see: http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf.

2. The title should provide a clear and compelling summary of the main findings in fewer than 100 characters including spaces and without punctuation.

3. Your abstract must be fewer than 150 words and should not include citations.

4. As a guideline, Articles allow up to 50 references in the main text. An additional 20 references can be included in the Online Methods. Only papers that have been published or accepted by a named publication or recognized preprint server should be in the numbered list. Published conference abstracts, numbered patents and research data sets that have been assigned a digital object identifier may be included in the reference list.

5. All references must be cited in numerical order. Place Methods-only references after the Methods section and continue the numbering of the main reference list (i.e., do not start at 1).

6. Genes must be clearly distinguished from gene products (e.g., "gene Abc encodes a kinase," not "gene Abc is a kinase"). For genes, provide database-approved official symbols (e.g., NCBI Gene, http://www.ncbi.nlm.nih.gov/gene) for the relevant species the first time each is mentioned; gene aliases may be used thereafter. Italicize gene symbols

and functionally defined locus symbols; do not use italics for proteins, noncoding gene products and spelled-out gene names.

Figures and Tables:

7. All figures and tables, including Extended Data, must be cited in the text in numerical order.

8. Figure legends should be concise. Begin with a brief title and then describe what is presented in the figure and detail all relevant statistical information, avoiding inappropriate methodological detail.

9. All relevant figures must have scale bars (rather than numerical descriptions of magnification).

10. All relevant figures must have defined error bars.

11. All bar graphs should be converted to a dot-plot format or to a box-and-whisker format to show data distribution. All box-plot elements (center line, limits, whiskers, points) should be defined.

12. When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Statistics and Reproducibility:

13. The Methods must include a statistics section where you describe the statistical tests used. For all statistics (including error bars), provide the EXACT n values used to calculate the statistics (reporting individual values rather than a range if n varied among experiments) AND define type of replicates (e.g., cell cultures, technical replicates). Please avoid use of the ambiguous term “biological replicates”; instead state what constituted the replicates (e.g., cell cultures, independent experiments, etc.). For all representative results, indicate number of times experiments were repeated, number of images collected, etc. Indicate statistical tests used, whether the test was one- or two-tailed, exact values for both significant and non-significant P values where relevant, F values and degrees of freedom for all ANOVAs and t-values and degrees of freedom for t-tests.

14. Reporting Guidelines– Attached you will find an annotated version of the

Reporting Summary you submitted, along with a Word document indicating revisions that need to be made in compliance with our reproducibility requirements. These documents detail any changes that will need to be made to the text, and particularly the main and supplementary figure legends, including (but not limited to) details regarding sample sizes, replication, scale and error bars, and statistics. Please use these documents as a guide when preparing your revision and submit an updated Reporting Summary with your revised manuscript. The Reporting Summary will be published as supplementary material when your manuscript is published.

Please provide an updated version of the Reporting Summary and Editorial Policy Checklist with your final files and include the following statement in the Methods section to indicate where this information can be found: "Further information on research design is available in the Nature Research Reporting Summary linked to this article."

The Reporting Summary and Editorial Policy Checklist can be found here:
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Note that these forms are smart "dynamic" PDFs which cannot be opened by most web browsers. Download them or right-click and choose "save as" in order to save them to your computer desktop and fill them in using Adobe Acrobat.

Supplementary Information:
All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

25 EXTENDED DATA: Extended Data are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data figures, and each must be referred to in the main text. Each Extended Data figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

26 SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

All Extended Data must be called you in your manuscript and cited as Extended Data 1, Extended Data 2, etc. Additional Supplementary Figures (if permitted) and other items are

not required to be called out in your manuscript text, but should be numerically numbered, starting at one, as Supplementary Figure 1, not SI1, etc.

27 SOURCE DATA: We encourage you to provide source data for your figures whenever possible. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Statistics source data should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (https://figshare.com/) or the Image Data Resource (https://idr.openmicroscopy.org).

Other
28 As mentioned in our previous letter, all corresponding authors on a manuscript should have an ORCID – please visit your account in our manuscript system to link your ORCID to your profile, or to create one if necessary. For more information please see our previous letter or visit www.springernature.com/orcid.

29 Nature Research journals encourage authors to share their step-by-step experimental protocols on a protocol sharing platform of their choice. Nature Research's Protocol Exchange is a free-to-use and open resource for protocols; protocols deposited in Protocol Exchange are citable and can be linked from the published article. More details can found at www.nature.com/protocolexchange/about.

30 TRANSPARENT PEER REVIEW
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In addition to addressing these points, please refer to the attached policy and rights worksheet, which contains information on how to comply with our legal guidelines for

publication and describes the files that you will need to upload prior to final acceptance. You must initial the relevant portions of this checklist, sign it and return it with your final files. I have also attached a formatting guide for you to consult as you prepare the revised manuscript. Careful attention to this guide will ensure that the production process for your paper is more efficient.

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We ask that you aim to return your revised paper within 7 days. If you have any further questions, please feel free to contact me.

Best regards,

Jamie D.K. Wilson, D.Phil
Chief Editor
Nature Immunology
212 726 9207
j.wilson@us.nature.com

Final Decision Letter:

In reply please quote: NI-A30343C

Dear Dr. Diamond,

I am delighted to accept your manuscript entitled "SARS-CoV-2 infection of hACE2 transgenic mice causes severe lung inflammation and impaired function" for publication in an upcoming issue of Nature Immunology.

The manuscript will now be copy-edited and prepared for the printer. Please check your calendar: if you will be unavailable to check the galley for some portion of the next month, we need the contact information of whom will be making corrections in your stead. When you receive your galleys, please examine them carefully to ensure that we have not inadvertently altered the sense of your text.

Acceptance is conditional on the data in the manuscript not being published elsewhere, or announced in the print or electronic media, until the embargo/publication date. These restrictions are not intended to deter you from presenting your data at academic meetings and conferences, but any enquiries from the media about papers not yet scheduled for publication should be referred to us.

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