nature research

Peer Review Information

Journal: Nature Genetics
Manuscript Title: Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retroelement co-option
Corresponding author name(s): Dr. George Kassiotis

Editorial Notes:

NA

Reviewer Comments & Decisions:

Decision Letter, initial version:

Date: 14th Aug 20 07:50:16
Last Sent: 14th Aug 20 07:50:16
Triggered By: Catherine Potenski
From: Catherine.Potenski@us.nature.com
To: george.kassiotis@crick.ac.uk
Subject: Decision on Nature Genetics submission NG-LE55459
Message: 14th Aug 2020

Dear Dr Kassiotis,

Your Letter, "Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retrovirus co-option" has now been seen by 3 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Genetics, 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.

As you will see, Reviewer #1 asks for further validation experiments and thinks that it is very important to show how the smaller isoform potentially affects SARS-CoV-2 entry/replication. We would like to see you respond to this point, but we would be open to you presenting the findings with more caveats in the absence of the ability to perform more experiments.
Reviewer #2 is supportive, but raises question about the evolutionary conservation of

the LTR and asks for some further functional experiments to determine the LTR's behavior as a promoter. We think that these are reasonable requests.
Reviewer #3 has similar points to Reviewer #2; we think that you should definitely refine the evolutionary analysis and fully address the concern about the novel isoform originating from LTR16A1 elements.

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. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable 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:

*1) 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.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Letter format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

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 four to eight weeks. If you cannot send it within this time, please let us know.

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

Nature Genetics 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.

All the best,

Catherine

--

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Referee expertise:

Referee #1: immunology, genetics

Referee #2: immunology, IFN signaling

Referee #3: genetics, gene regulation, immunology

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
Authors demonstrate largely using already published datasets that ACE2 is not IFN-I inducible. They demonstrate that that a different isoform which encodes parts of ACE2 is. In the last figures they perform their own wet experiments to show that while LTR16A1-ACE2 can be detected at mRNA level they cannot detect it at the protein level, presumably due to low stability. Whilst this is timely, it is relatively simple, and requires validations.

1) Authors should express ACE2 and LTR16A1-ACE2 to demonstrate that one can and the other cannot allow for SARS-CoV2 entry/replication of live virus in a cell lines. This is very important as apart from stating that ACE2 is not IFN-I inducible, no function is

ascribed to LTR16A1-ACE2. At the very least authors should prove that this IFN-I inducible LTR16A1-ACE2 cannot aid in SARS-CoV-2 entry and replication (or that perhaps it can restrict it).

Reviewer #2:
Remarks to the Author:
The authors describe identification of a novel transcript expressed from the ACE2 locus that is IFN inducible. They show that expression of this transcript explains previous reports that ACE2 is an ISG by examining numerous databases. They also show that this transcript begins from an endogenous retroviral LTR element, which likely explains the differential regulation of the novel transcript with respect to full-length ACE2. They further analyze the tissue-specific distribution of the two transcripts coming from the ACE2 locus. Interestingly, they failed to detect a translation product from the novel transcript and conclude, based on these experimental data and on structure-based predictions, that it is unlikely to encode a stable protein.

These are important results, since they correct a previously published supposition that ACE2 itself was induced by IFN, which could complicate the use of IFN as an anti-COVID antiviral.

Sequence comparison data suggest that the LTR potentially responsible for expression of the novel transcript exists in all mammalian species, although it is highly diverged in mice. This observation raises a question concerning whether this phenomenon is primate-specific, or simply lacking in the mouse. It would be helpful if the authors could directly address this point.

It would also be helpful if the authors addressed whether the LTR is truly acting as the promoter for the novel transcript. Direct experimental evidence for the 5' end of the transcript (e.g., 5' RACE or 5' CAP capture) could demonstrate the lack of initiation further upstream. Experiments to demonstrate that the DNA sequence of the LTR encodes IFN-inducible regulatory activity could also address this point.

Reviewer #3:
Remarks to the Author:
Three manuscripts were posted on BioRxiv reporting the identification of a novel alternative gene product of ACE2. ACE2 gene encodes Angiotensin-converting enzyme 2, which is what SARS-CoV-2 uses as an entry point into cells. Previous reports suggest that ACE2 can be induced by IFN. Thus, the discovery of the novel ACE2 form that responses to IFN is highly significant, and any functional understanding of this isoform may have immediate clinical amplification. The paper under consideration is the 2nd of the three:

https://www.biorxiv.org/content/10.1101/2020.07.19.210955v1
https://www.biorxiv.org/content/10.1101/2020.07.24.219139v1
https://www.biorxiv.org/content/10.1101/2020.07.31.230870v1

Ng et al described a tissue-specific novel isoform of ACE2 derived from an LTR16A1

retroelement that is transcriptionally responsive to interferon induction in the select cell lines. The authors used a large set of publicly available RNA-seq data to establish the tissue specific expression pattern and expression ratio between ACE2 and the novel isoform. They also used cell line and SARS-CoV-2 infected patient data to establish that the novel ACE2 isoform responses to IFN. They examined squamous cell carcinoma cell lines SCC-4 and SCC-25, tried ectopic expressing the novel ACE2 isoform, mutated putative ubiquitination sites, but could not detect a protein product or enzymatic activity. Thus, they conclude that this novel isoform is not stable.

The discovery of the novel ACE2 isoform is highly significant especially in the current covid-19 pandemics. Thus, I strongly support that the result be published as soon as possible. However, the current study did not provide insight on any potential function of the novel ACE2 isoform, which dampens my enthusiasm. Other than the discovery of the novel isoform, most conclusions were somewhat rushed and lacked rigorous support. I'll elaborate a few points.

1) The authors claimed that the novel isoform is originated from an LTR16A1 elements. However, examining Fig S1 reveals that most of the FANTOM5 CAGE data support that the transcription start site (TSS) is in the neighboring MIRb element. In fact the LTR16A1 is antisense to the transcription of novel ACE2. Thus, I think that the evidence that the TSS is from an LTR16A1 is weak. I looked into this a bit further and I believe that both TSS and TATA box are in MIRb, whereas the start codon ATG is in LTR16A1. The authors should perform more rigorous analyses to determine how the transcript is initiated. For example, 5' RACE; analysis of distribution of TFBS in and around LTR16A1 and MIRb; promoter reporter assay; CRISPR deletion; among other standard assays that help define alternative TSSs.

2) Related to 1), the authors performed phylogenetic analysis on LTR16A1, but the analysis can become irrelevant if LTR16A1 is not the TSS or does not provide critical regulatory elements for the novel ACE2. The analysis itself also lacks stringency. For example, sequence identity between human and mouse LTR16A1 was determined to be 14.6%. You can't count truncation as mismatch. This is a cardinal sin in molecular evolution analysis.

3) Most of the evidence came from analysis of full-length ACE2 and LTR16A1-ACE from RNA-seq data (Figs. 2-4). Since the two transcripts share more than half of gene body, it is critical to show how the authors distinguish or assign reads to which isoform of ACE2. Did the authors use special software or algorithm to calculate TPM of each isoform? For example, from Fig 1A, LUSC has 37 reads supporting full-length isoform and 94 reads supporting short isoform. Is this how TPM was calculated? This detail should be included.

4) The authors should re-examine the ability to detect a protein product in the context of the other bioRxiv reports that appeared around the same time. The authors should state the lot number of the antibody, justify the choice of cell lines and cell types in which they search for a protein product, and perform experiments including ribo-profiling to determine if this is translational regulation or post-translational. The current data is not sufficient to draw conclusion on protein stability.

Author Rebuttal to Initial comments

We thank all three Reviewers for their astute and constructive comments. Below is a list of the changes we have made in response to these comments.

Reviewers' Comments:

Reviewer #1:

1) Authors should express ACE2 and LTR16A1-ACE2 to demonstrate that one can and the other cannot allow for SARS-CoV2 entry/replication of live virus in a cell lines. This is very important as apart from stating that ACE2 is not IFN-I inducible, no function is ascribed to LTR16A1-ACE2. At the very least authors should prove that this IFN-I inducible LTR16A1-ACE2 cannot aid in SARS-CoV-2 entry and replication (or that perhaps it can restrict it).

Reply: As a surrogate for viral entry, we have now used binding of recombinant S1 subunit of SARS-CoV-2 spike. The S1 subunit contains the receptor binding domain (RBD) and its binding to ACE2 is the first step in viral entry. While SARS-CoV-2 S1 bound ACE2-expressing cells, it did not bind LTR16A1-ACE2-expressing cells. These experiments were performed with expression of LTR16A1-ACE2 protein at levels detectable by Western blotting. Moreover, co-expression of the two isoforms in the same cells (even when the balance was shifted in favour of LTR16A1-ACE2) did not alter binding of SARS-CoV-2 S1 to ACE2. These results are shown in the new Figure 5g and argue that LTR16A1-ACE2 neither promotes nor hinders SARS-CoV-2 entry.

Reviewer #2:

1) Sequence comparison data suggest that the LTR potentially responsible for expression of the novel transcript exists in all mammalian species, although it is highly diverged in mice. This observation raises a question concerning whether this phenomenon is primate-specific, or simply lacking in the mouse. It would be helpful if the authors could directly address this point.

Reply: We have now performed RT-qPCR on a panel of primate and non-primate cell lines for *Ace2* and *LTR16A1-Ace2* using species-specific primers. For the *LTR16A1-Ace2* transcript in particular, primers were complementary to the respective *LTR16A1* and *Ace2* exon 10 sequences of each species (spanning the splice junction between the two). Full-length *Ace2* was expressed in all cell lines. *LTR16A1-Ace2* was detected in African green monkey CV-1 cells, but not Vero cells, consistent with the disrupted IFN pathway in Vero cells. Importantly, *LTR16A1-Ace2* was detected also in canine MDCK cells, but not in murine MCA-38 cells or leporine R9ab cells, which agrees with the phylogenetic analysis. Although there are species, such as mice and rabbits that seem to have lost the ability to produce the *LTR16A1-Ace2*

transcript, its presence in dog demonstrates that it is not primate-specific and may indeed exist in some other (but not all) mammalian species, as the Reviewer suggests. These results are shown in the new Extended data figure 3.

2) It would also be helpful if the authors addressed whether the LTR is truly acting as the promoter for the novel transcript. Direct experimental evidence for the 5' end of the transcript (e.g., 5' RACE or 5' CAP capture) could demonstrate the lack of initiation further upstream. Experiments to demonstrate that the DNA sequence of the LTR encodes IFN-inducible regulatory activity could also address this point.

Reply: We agree with the Reviewer and have now extended our analysis of the transcription start site(s) of the *LTR16A1-ACE2* transcript (please see also our response to Reviewer #3 point 1). The following changes were made:

a, we include a closer inspection of FANTOM5 CAGE data extending the analysis to the *MIRb* element immediately upstream of the *LTR16A1* element, both of which appear contain CAGE peaks. The distribution of peaks in the *MIRb* and *LTR16A1* elements also seems to depend on the cell type.

b, we looked for transcription factor binding sites and TATA-box in the *MIRb* and *LTR16A1* elements, particularly the IFN responsive ones. This analysis indicated the IRF-1 and IRF-2 sites and putative TATA-box reside in the *MIRb* element.

c, lastly, we performed 5'RACE PCR and sequencing in NHBE, SCC-4 and SCC-25 cells stimulated with IFNα, which uncovered transcription start sites in both the *MIRb* and *LTR16A1* elements, in agreement with CAGE data and also in a cell-type-dependent way.

These results suggest that the *MIRb* and *LTR16A1* elements act as a cryptic promoter for the *LTR16A1-ACE2* transcript and are shown in the new Extended data figures 1 and 2.

Reviewer #3:

1) The authors claimed that the novel isoform is originated from an LTR16A1 elements. However, examining Fig S1 reveals that most of the FANTOM5 CAGE data support that the transcription start site (TSS) is in the neighboring MIRb element. In fact the LTR16A1 is antisense to the transcription of novel ACE2. Thus, I think that the evidence that the TSS is from an LTR16A1 is weak. I looked into this a bit further and I believe that both TSS and TATA box are in MIRb, whereas the start codon ATG is in LTR16A1. The authors should perform more rigorous analyses to determine how the transcript is initiated. For example, 5' RACE; analysis of distribution of TFBS in and around LTR16A1 and MIRb; promoter reporter assay; CRISPR deletion; among other standard assays that help define alternative TSSs.

Reply: We thank the Reviewer for astutely pointing this out. We had also noticed that CAGE peaks were distributed across the *MIRb* and *LTR16A1* elements and indeed our transcript assembly placed the putative TSS in the MIRb element, but we could not definitively rule out initiation also at the *LTR16A1*

element. As LTR promoters can be directional, the antisense orientation of the *LTR16A1* element should not preclude promoter activity. We have adopted the *LTR16A1-ACE2* term to describe the new transcript as splicing with the *LTR16A1* element led to its discovery and also the *LTR16A1* element encodes the first 10 amino acids of the protein. We have now looked into this point more carefully, as the Reviewer suggested (also in response to Reviewer #2, point 2), and provide evidence that the *MIRb* element is indeed the most frequent, but not exclusive TSS, in at least some cell types. The following changes were made:

a, we include a more detailed inspection of FANTOM5 CAGE data extending the analysis to the *MIRb* element immediately upstream of the *LTR16A1* element. Both these elements contain CAGE peaks, and indeed there are more peaks in *MIRb* than in *LTR16A1* in pooled data, but the distribution of the them also appears to depend on the cell type (in 4 distinct examples). On note, CAGE peaks reside almost exclusively in *MIRb* in bronchial epithelial cells, but spread to LTR16A1 in HEK293 cells.

b, we mapped transcription factor binding sites in the *MIRb* and *LTR16A1* elements and the IFN responsive IRF-1 and IRF-2 sites in the *MIRb* element, as well as a putative TATA box, immediately downstream of the IRF-1 binding site, as the Reviewer correctly identified.

c, lastly, we performed 5'RACE PCR and sequencing in NHBE, SCC-4 and SCC-25 cells stimulated with IFNα, which uncovered transcription start sites both in the *MIRb* and *LTR16A1* elements, in agreement with CAGE data. Again, the distribution of 5'RACE peaks dependent on the cell type.

Together, these results suggest that although the *MIRb* element is likely to be the dominant promoter, both the *MIRb* and *LTR16A1* elements contain transcription start sites, thus acting as a cryptic promoter for the *LTR16A1-ACE2* transcript. The new data are shown in the new Extended data figures 1 and 2.

2) Related to 1), the authors performed phylogenetic analysis on LTR16A1, but the analysis can become irrelevant if LTR16A1 is not the TSS or does not provide critical regulatory elements for the novel ACE2. The analysis itself also lacks stringency. For example, sequence identity between human and mouse LTR16A1 was determined to be 14.6%. You can't count truncation as mismatch. This is a cardinal sin in molecular evolution analysis.

Reply: We agree with the Reviewer and have now extended our phylogenetic analysis to cover both the *MIRb* and *LTR16A1* elements. We further modified our sequence identity analyses to exclude truncations and the new results are shown in the modified Figure 1b-c. Of note, the IRF-1 sites in *MIRb* is missing in the mouse.

3) Most of the evidence came from analysis of full-length ACE2 and LTR16A1-ACE from RNA-seq data (Figs. 2-4). Since the two transcripts share more than half of gene body, it is critical to show how the authors distinguish or assign reads to which isoform of ACE2. Did the authors use special software or algorithm to calculate TPM of each isoform? For example, from Fig 1A, LUSC has 37 reads supporting full-length isoform and 94 reads supporting short isoform. Is this how TPM was calculated? This detail should be included.

Reply: Indeed, TPM calculations are based on uniquely-mapping, isoform-specific reads. Our preferred method for TPM calculations was described in a previous publication (ref. 18), but we have now briefly described it here, too. TPMs are calculated using Salmon v0.12.0 (ref. 35), which uses the abundance of reads unique to each isoform (not just the spliced reads) to build a probabilistic model, based on which it then assigns any reads that are common to both isoforms.

4) The authors should re-examine the ability to detect a protein product in the context of the other bioRxiv reports that appeared around the same time. The authors should state the lot number of the antibody, justify the choice of cell lines and cell types in which they search for a protein product, and perform experiments including ribo-profiling to determine if this is translational regulation or post-translational. The current data is not sufficient to draw conclusion on protein stability.

Reply: We agree with the Reviewer and have now re-assessed the potential of the *LTR16A1-ACE2* transcript to produce protein, in light also of the other two reports on detection of the truncated protein. We now provide further evidence that the protein can be made, but is unstable, consistent with all the observations.

a, we have repeated the overexpression experiments, considerably increasing the concentration of our pcDNA3.1-LTR16A1-ACE2-DYK-P2A-GFP plasmid in HEK293T cells and we have been able to detect a protein of expected size (52.7 kDa) using the anti-FLAG M2 antibody (Sigma), which is agreeing with other reports. However, the protein was only detected at RNA expression levels (determined by RT-qPCR in these transfected cells) that were one order of magnitude higher than those in IFN-stimulated NHBE or SCC-4/25 cells. In contrast, at physiological RNA expression levels, where full-length ACE2 is still readily available, we have not been able to detect the LTR16A1-ACE2 product, under the same conditions and using the same antibody. These experiments, shown in the revised Figure 5c, suggest that the LTR16A1-ACE2 product can indeed be made when overexpressed, but is far less stable than ACE2.

b, we should also note that the constructs used for these experiments have the ACE2 protein isoforms linked to GFP with a P2A peptide. The very low ratio of LTR16A1-ACE2 to GFP (translated from the same RNA molecule) to that of ACE2 to GFP, also suggest instability of the LTR16A1-ACE2 product. This effect would be at the post-translational level.

c, we additionally directly tested the stability of the LTR16A1-ACE2 protein product using a cycloheximide chase assay. While full-length ACE2 was stable for 4 hours following cycloheximide treatment, LTR16A1-ACE2 was rapidly degraded and was not detectable after 3 hours of cycloheximide treatment. These results are shown in the revised Figure 5e.

d, lastly, we have modified the discussion to indicate that the LTR16A1-ACE2 protein can indeed be found under certain in vitro conditions, in agreement with the other two reports, leaving open the possibility that it may also be found in vivo. Onabajo et al., also failed to detect the LTR16A1-ACE2 protein in cells that express the transcript naturally (or after viral infection), using the same polyclonal antibody (ab15348, Abcam) we have used on Western blotting, but did detect a Myc-DDK-tagged version upon overexpression in T24 cells, entirely consistent with our findings in HEK293T cells. Blume et

al., did report the presence of a slightly smaller (~50kDa) band in primary nasal epithelial cells using the same polyclonal antibody (ab15348, Abcam) and indeed this band is also reported by the suppliers of the antibody (Abcam) in human kidney lysate (https://www.abcam.com/ace2-antibody-ab15348.html#description_images_4). It is currently unclear if this band corresponds to the LTR16A1-ACE2 protein. We now also explain the choice of HEK293T cells for these experiments (negative for endogenous ACE2 that would confound detection of ACE2 produced by transfection).

Decision Letter, first revision:

Date: 21st Sep 20 12:29:49

Last Sent: 21st Sep 20 12:29:49

Triggered By: Catherine Potenski

From: Catherine.Potenski@us.nature.com

To: george.kassiotis@crick.ac.uk

CC: kevin.ng@crick.ac.uk,jan.attig@crick.ac.uk,William.Bolland@crick.ac.uk,George.Young@crick.ac.uk,jack.major@crick.ac.uk,antoni.wrobel@crick.ac.uk,steve.gamblin@crick.ac.uk,Andreas.Wack@crick.ac.uk

Subject: Decision on Nature Genetics submission NG-LE55459R

Message: Our ref: NG-LE55459R

21st Sep 2020

Dear Dr. Kassiotis,

Thank you for submitting your revised manuscript "Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retrovirus co-option" (NG-LE55459R). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we will be happy in principle to publish it in Nature Genetics, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

** Note that we will send you a checklist detailing these editorial and formatting requirements in about a week. Please do not finalize your revisions or upload the final materials until you receive this additional information.**

In recognition of the time and expertise our reviewers provide to Nature Genetics's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retrovirus co-option". For those reviewers who give their

assent, we will be publishing their names alongside the published article.

While we prepare these instructions, we encourage the Corresponding Author to begin to review and collect the following:

-- Confirmation from all authors that the manuscript correctly states their names, institutional affiliations, funding IDs, consortium membership and roles, author or collaborator status, and author contributions.

-- Declarations of any financial and non-financial competing interests from any author. For the sake of transparency and to help readers form their own judgment of potential bias, the Nature Research Journals require authors to declare any financial and non-financial competing interests in relation to the work described in the submitted manuscript. This declaration must be complete, including author initials, in the final manuscript text.

If you have any questions as you begin to prepare your submission please feel free to contact our Editorial offices at genetics@us.nature.com. We are happy to assist you.

Thank you again for your interest in Nature Genetics.

All the best,

Catherine

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Reviewer #1 (Remarks to the Author):

Unfortunately authors did not address my concerns. Binding assays in an overexpression system needs validation in orthogonal assays. Authors need to use live virus, as that is how SARS-CoV-2 entry should be assessed. Otherwise likelihood of reporting errors is high.

Thus authors should express ACE2 and LTR16A1-ACE2 to demonstrate that one can and the other cannot allow for SARS-CoV2 entry/replication of live virus in a cell line. This is very important as apart from stating that ACE2 is not IFN-I inducible, no function is ascribed to LTR16A1-ACE2. At the very least authors should prove that this IFN-I inducible LTR16A1-ACE2 cannot aid in SARS-CoV-2 entry and replication (or that perhaps it can restrict it).

Reviewer #2 (Remarks to the Author):

The authors have adequately addressed the issues raised during the primary review of this

work by providing key confirmatory data. The data are of interest, particularly in terms of settling a confounding issue of whether CoV-2 viral entry might be enhanced by IFN responses. The authors have dealt appropriately with the ambiguities that remain concerning the potential function (or lack thereof) of truncated ACE2.

I recommend acceptance.

[REDACTED]

Reviewer #3 (Remarks to the Author):

I am glad to see the authors were able to detect a protein product. I support publication of this significant and timely finding, even though there hasn't been any breakthrough in functionalizing this novel isoform. I would make one suggestion: please do not call this isoform LTR16A1-ACE2. It is clear that the promoter activity comes from MIRb. Data supporting LTR16A1 as TSS is very weak. LTR16A1-ACE2 ignores the major contribution of MIRb and will likely unnecessarily sensationalize the role of LTR retrotransposon in driving novel expression patterns.

ORCID

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Date: 21st Sep 20 16:11:00

Last Sent: 21st Sep 20 16:11:00
Triggered By: Catherine Potenski
From: Catherine.Potenski@us.nature.com
To: george.kassiotis@crick.ac.uk
Subject: Your manuscript, NG-A55459R
Message: Our ref: NG-A55459R

21st Sep 2020

Dear Dr. Kassiotis,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Genetics manuscript, "Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retrovirus co-option" (NG-A55459R). Please follow the instructions provided here and in the attached files.

When you upload your final materials, please:

A) Fill out and upload the attached ***Publishing Policy Worksheet For Authors***, which contains information on how to comply with our legal guidelines for publication and links to 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. We will not be able to proceed further without these files:
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g) Institutional Open Access Waiver (if applicable)
h) Inventory of Supplementary Information

B) Include a tracked-changes Word file of your revised article.

C) Include a point-by-point response to the points below:

We note that Reviewer #2 and Reviewer #3 are supportive of publication, while Reviewer #1 thinks that the viral entry data are weak and need validation. While we appreciate this view, we are mainly interested in this study for the discovery of this shorter, INF-inducible isoform of ACE2, and the finding that ACE2 is not induced by IFN/virus. The mechanistic details about what the shorter isoform does still need to be worked out.

We agree with Reviewer #3's comments that you should rename this isoform as not to give prominence to the LTR, and ask that you adjust the title and text accordingly. We note that another group has deposited this sequence in GenBank (MT505392)..

General formatting:

1. Article: Our standard word limit is 4,000 words for the Introduction, Results and Discussion. Your current manuscript is 3,725 words, which is fine. We have updated the article type from a "Letter" to an "Article", as this was formatted as the latter.
2. Please ensure that sections are in the following order within the same manuscript file: Title, Authors, Affiliations, Abstract, Introduction, Results (with subheadings), Discussion, Acknowledgements, Author Contributions, References for main text, Figure Legends for main text, Tables, Online Methods, Data Availability Statement, Code Availability statement (if applicable), Methods-only references.
3. Online Methods do not have a strict limit, but we suggest 3,000 words as a target. Your methods section is currently 1,600 words, which is fine. All primer sequences should be moved to a Supplementary Table.
4. For the title, we suggest "Tissue-specific and interferon-inducible expression of non-functional ACE2 transcripts".
5. Your abstract must be fewer than 150 words and should not include citations.
6. Please include an author contributions statement. Please ensure that there is an Author contribution for every main author listed. Initials must match the author name as written in the author list. Authors with the same initials must be distinguished in some way.
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15. All figures and tables, including Extended Data, must be cited in the text in numerical order.
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please note that in a few days we will send you detailed comments on your reproducibility checklist. You may have to modify some of the reporting in the manuscript at that time.

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2. 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.

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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.

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If you have any further questions, please feel free to contact me- I'd be more than happy to assist.

Thank you very much.

All the best,

Catherine

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Reviewer #1:
Remarks to the Author:
Unfortunately authors did not address my concerns. Binding assays in an overexpression system needs validation in orthogonal assays. Authors need to use live virus, as that is how SARS-CoV-2 entry should be assessed. Otherwise likelihood of reporting errors is high.

Thus authors should express ACE2 and LTR16A1-ACE2 to demonstrate that one can and the other cannot allow for SARS-CoV2 entry/replication of live virus in a cell line. This is very important as apart from stating that ACE2 is not IFN-I inducible, no function is ascribed to LTR16A1-ACE2. At the very least authors should prove that this IFN-I inducible LTR16A1-ACE2 cannot aid in SARS-CoV-2 entry and replication (or that perhaps it can restrict it).

Reviewer #2:
Remarks to the Author:
The authors have adequately addressed the issues raised during the primary review of this work by providing key confirmatory data. The data are of interest, particularly in terms of settling a confounding issue of whether CoV-2 viral entry might be enhanced by IFN responses. The authors have dealt appropriately with the ambiguities that remain concerning the potential function (or lack thereof) of truncated ACE2.

I recommend acceptance.

[REDACTED]

Reviewer #3:
Remarks to the Author:
I am glad to see the authors were able to detect a protein product. I support publication of this significant and timely finding, even though there hasn't been any breakthrough in functionalizing this novel isoform. I would make one suggestion: please do not call this isoform LTR16A1-ACE2. It is clear that the promoter activity comes from MIRb. Data supporting LTR16A1 as TSS is very weak. LTR16A1-ACE2 ignores the major contribution of MIRb and will likely unnecessarily sensationalize the role of LTR retrotransposon in driving novel expression patterns.

Author Rebuttal, first revision:

Reviewers' Comments:

Reviewer #1:

Unfortunately authors did not address my concerns. Binding assays in an overexpression system needs validation in orthogonal assays. Authors need to use live virus, as that is how SARS-CoV-2 entry should be assessed. Otherwise likelihood of reporting errors is high.

Thus authors should express ACE2 and LTR16A1-ACE2 to demonstrate that one can and the other cannot allow for SARS-CoV2 entry/replication of live virus in a cell line. This is very important as apart from stating that ACE2 is not IFN-I inducible, no function is ascribed to LTR16A1-ACE2. At the very least authors should prove that this IFN-I inducible LTR16A1-ACE2 cannot aid in SARS-CoV-2 entry and replication (or that perhaps it can restrict it).

Reply: Whilst the use of live SARS-CoV-2 is possible at our institute, such experiments need to be fully justified against competing experiments with live SARS-CoV-2 due to limited capacity in the containment facilities. We cannot, at present, easily justify the suggested experiment using live virus for the following reasons:

a, The truncated ACE2 protein cannot be detected in cells expressing the physiological levels of mRNA even after IFN induction.

b, Vero cells, the only cell line consistently permissive to SARS-CoV-2 infection and replication, do not express the novel isoform, owing to defective IFN signalling.

c, Even under overexpression of truncated ACE2 protein, no binding of recombinant SARS-CoV-2 S1 subunit can be demonstrated.

With respect, the lack of any measureable binding to SARS-CoV-2 S1 when truncated ACE2 is overexpressed, does not really leave a lot of room for the possibility that binding will occur in the absence of overexpression, where truncated ACE2 is not even produced at detectable levels.

Our understanding of the experiment the Reviewer is suggesting does involves overexpression, but uses live virus as opposed to recombinant S1 as a surrogate for entry.

We discussed in the text that a potential functional role for the truncated ACE2 protein product in SARS-CoV-2 infection or, indeed, beyond SARS-CoV-2 infection is not completely ruled out. However, the focus of this study is the description of the new isoform and the contrasting effect of IFN or viral infection on the expression of the two isoforms.

Reviewer #2:

The authors have adequately addressed the issues raised during the primary review of this work by providing key confirmatory data. The data are of interest, particularly in terms of settling a confounding issue of whether CoV-2 viral entry might be enhanced by IFN responses. The authors have dealt appropriately with the ambiguities that remain concerning the potential function (or lack thereof) of truncated ACE2.
I recommend acceptance.

Reply: We thank the Reviewer for his recommendation.

Reviewer #3:
I am glad to see the authors were able to detect a protein product. I support publication of this significant and timely finding, even though there hasn't been any breakthrough in functionalizing this novel isoform. I would make one suggestion: please do not call this isoform LTR16A1-ACE2. It is clear that the promoter activity comes from MIRb. Data supporting LTR16A1 as TSS is very weak. LTR16A1-ACE2 ignores the major contribution of MIRb and will likely unnecessarily sensationalize the role of LTR retrotransposon in driving novel expression patterns.

Reply: We agree with the Reviewer's suggestion and have now renamed the new isoform as MIRb-ACE2 and made the corresponding changes to the text to avoid overemphasising the contribution of the LTR element LTR16A1 at the expense of the non-LTR element MIRb.

Final Decision Letter:

ate: 29th Sep 20 14:30:18
Last Sent: 29th Sep 20 14:30:18
Triggered By: Catherine Potenski
From: Catherine.Potenski@us.nature.com
To: george.kassiotis@crick.ac.uk
CC: rjsproduction@springernature.com
Subject: Decision on NG-A55459R1 Kassiotis
Message: In reply please quote: NG-A55459R1 Kassiotis

29th Sep 2020

Dear Dr. Kassiotis,

I am delighted to say that your manuscript "Tissue-specific and interferon-inducible expression of non-functional ACE2 through endogenous retroelement co-option" has been accepted for publication in an upcoming issue of Nature Genetics.

Prior to setting your manuscript, we may make minor changes to enhance the lucidity of the text and with reference to our house style. We therefore ask that you examine the proofs most carefully to ensure that we have not inadvertently altered the sense of your text in any way.

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Congratulations on the paper!

All the best,

Catherine

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