THE EMBO JOURNAL

Opposing activities of IFITM proteins in SARS-CoV-2 infection

Guoli Shi, Adam Kenney, Elena Kudryashova, Ashley Zani, Lizhi Zhang, Kin Kui Lai, Luanne Hall-Stoodley, Richard Robinson, Dmitri Kudryashov, Alex Compton, and Jacob Yount
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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 24th Sep 2020

Dear Jacob,

Thank you for submitting your manuscript to The EMBO Journal. Your study has now been seen by two referees and their comments are provided below.

As you can see from the referees' comments, the manuscript received a bit of a mixed response. Referee #1 mentions that the findings are preliminary and also novelty issues. Regarding novelty, I see that the related manuscripts are posted on preprint servers, are not published yet and therefore doesn't count in our assessment of novelty so that issue is OK. However, the other points raised by referee #1 and #2 are relevant and would have to be addressed for consideration here.

Should you be willing to embark on significant revisions then I am open to consider a revised version. If you find yourself in apposition not to be able to do so then it would be in your best interest to seek publication elsewhere at this stage.

If you are able to address the concerns then it would be good to discuss the experiments and timeline further. Let me know when it is a good time for you.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
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We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

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- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 23rd Dec 2020.

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Referee #1:

Shi and colleagues have studied restriction of SARS-CoV2 by IFITM proteins. They show that IFITM 1 and 3 expression restricts infection and that specific IFITM3 mutants actually slightly enhance infectivity. They also show that TMPRSS2 expression reduces IFITM1/3 sensitivity but enhances infection increases in the presence of IFITM mutants. Overall, the data are quite preliminary and not very novel since a series of previous studies have described IFITM restriction of CoV2. There's also actually not a lot of data presented. This is an interesting start and its nicely written but I'm not sure what this study tells us and what insight it brings.

1. The effect sizes for CoV2 are pretty weak, particularly e.g. Fig 2D. Note the comparison with flu shows a complete block to infection in Fig2. Why is that? Are these real inhibition effects? I would

have liked to see some replication assays showing that IFITM is really a strong inhibitor. Also do replication assays show strong enhancement in the presence of the IFITM mutants. Its all a bit preliminary.

2. Graph labelling could be clearer. I'm not sure of the value of a control bar without errors, in each of the plots. The axis labels should be consistent, eg relative infection with 1 or 100, not a mixture of the 2.

3. Labeling the bar charts with the virus would improve clarity.

4. The facs analysis is a bit shaky in places. In Fig 2C the gate is too far to the left. It is clear that the effect size in Fig 3C with IFITM1 expression is very strong, ie a complete block to infection. But poor placing of the gate reads it as a 3 fold effect, which it clearly is not given the decent shift in the infected population. Here they're just counting the edge of the uninfected population. A proper look at all the Facs data is advised.

5. I don't like the fact that all the infection data are normalised. Have any titrations been done? Is the effect size MOI dependent?

Referee #2:

The manuscript submitted by Shi, et al. is an intriguing study that explores the role of the IFITM proteins in SARS-CoV-2 infection. The authors show that IFITM1, IFITM2 and IFITM3 restrict SARS-CoV-2. The experiments are well-designed and the use of both overexpression and deletion of IFITM proteins strengthens the claim of an important role for these innate immune proteins in viral infection. Overall, the data presented are strong and the authors make a compelling case for the function of IFITM3 that is distinct from other viral infections. The studies that show re-localization of IFITM3 results in enhancement, rather than the inhibition of infection are interesting. However, there are some points that can be addressed to strengthen the studies presented here.

1. There is less inhibition of infection with Caco2 cells which are infectable without overexpression of ACE2. Does this indicate an issue with ACE2 overexpression? What are the baseline levels of infection in each cell line?

2. Many of the experiments are performed in the context of ACE2 overexpression in cells that are not naturally susceptible to SARS-CoV-2 infection. These results should be verified in a more relevant context of infection, such as the Calu3 cell line used for the syncytia formation experiments.

3. The authors show that transfecting cells with IFITM3 can modestly inhibit syncytia formation when expressed in target (non-spike expressing cells) but that IFITM3 mutants enhanced syncytia formation in this context. Is this effect observed only when expressed in target cells? What is the result of co-expressing IFITM3 and mutants in SARS-CoV-2 spike-expressing cells? What is the effect of expressing IFITM1 and IFITM2?

4. The results showing that overexpression of TMPRSS2 decreases IFITM3-mediated SARS-CoV-2 inhibition are interesting. What is the result of overexpressing TMPRSS2 with IFITM1? Or with IFITM3 localization mutants?

5. Human IFITM3-Y20A but not mouse IFITM3-Y20A increased infection compared to vector control cells. Do both mutants localize to the plasma membrane? This should be shown.

We thank the reviewers for taking the time to provide feedback on our work identifying the divergent activities of IFITMs on SARS-CoV-2 infection. Please find below in blue font our responses to the specific points raised by each reviewer. In sum, we have clarified several points of significance within the manuscript text and have made the following major changes:

1. We have provided a supplemental spreadsheet containing all non-normalized and normalized infection data that were utilized to generate each of the graphs in our manuscript.
2. We have added confocal imaging of mouse and human IFITM3 Y20A and L23Q mutants, demonstrating their localization at the cell periphery in comparison to intracellular punctate localization of WT IFITM3 (New Figures 3G and 4D).
3. New data have been added to Figure 7 to further address whether TMPRSS2 over-expression allows IFITM1 or IFITM3-Y20A to enhance infection. We conclude that IFITM1 is not able to enhance infection regardless of TMPRSS2 expression, and that TMPRSS2 does not provide statistically significant enhancement of infection beyond the enhancement already provided by IFITM3-Y20A.
4. We have added an entirely new set of experiments utilizing IFITM3 KO and IFITM locus-deleted MEFs to further confirm an overall restriction of SARS-CoV-2 infection by endogenous IFITMs. By stimulating these cells with type I IFN, we also demonstrate that IFITMs play a role in IFN-mediated inhibition of the virus.

We look forward to publishing this timely and important work with EMBO Journal.

Best regards,
Jacob Yount & Alex Compton

Referee #1:

Shi and colleagues have studied restriction of SARS-CoV2 by IFITM proteins. They show that IFITM 1 and 3 expression restricts infection and that specific IFITM3 mutants actually slightly enhance infectivity. They also show that TMPRSS2 expression reduces IFITM1/3 sensitivity but enhances infection increases in the presence of IFITM mutants. Overall, the data are quite preliminary and not very novel since a series of previous studies have described IFITM restriction of CoV2. There's also actually not a lot of data presented. This is an interesting start and its nicely written but I'm not sure what this study tells us and what insight it brings.

1. The effect sizes for CoV2 are pretty weak, particularly e.g. Fig 2D. Note the comparison with flu shows a complete block to infection in Fig2. Why is that? Are these real inhibition effects? I would have liked to see some replication assays showing that IFITM is really a strong inhibitor. Also do replication assays show strong enhancement in the presence of the IFITM mutants. Its all a bit preliminary.

The reviewer's comment noting the comparison with influenza highlights an aspect of our work that should have been better discussed, and that bolsters our conclusions. IFITM3 is primarily localized to endosomes and is thus able to very effectively inhibit infection by influenza virus, which enters cells entirely via endocytosis. As a contrast, we previously showed that metapneumovirus, which uses dual cell entry pathways (membrane fusion at either the plasma membrane or within endosomes), is restricted by IFITM3 only in its endocytic entry (McMichael,

J Infect Dis, 2018). Our results showing that SARS-CoV-2 is similarly partially inhibited by IFITM3 is consistent with the known ability of this virus to similarly utilize dual cell entry pathways. Our finding that IFITM3 at the plasma membrane enhances SARS-CoV-2 infection adds further unique complexity to our results. Taking all of this together, we would not expect full inhibition of SARS-CoV-2 as is seen for influenza virus. Overall, a partial inhibitory effect of WT IFITM3 is consistent with the known entry pathways of the virus and the opposing roles of IFITM3 that we report on here. **We now provide a more thorough discussion of these points.**

We have additionally added data in which IFITM3 KO MEFs and IFITM-locus deleted MEFs show increased infection with genuine SARS-CoV-2 as compared to WT cells (New Figure 5). These data further support one of our primary conclusions that IFITMs generally repress infection despite the ability of IFITM3 to enhance infection under certain circumstances.

Regarding replication assays: IFITM3 is different from many classical interferon effectors in that it affects virus entry processes and not intracellular virus replication (Feeley, et al. *PLoS Pathogens*, 2011). Analysis of virus protein production early in infection as done in our flow cytometry assay provides a measure of whether or not virus was able to fuse with cellular membranes and begin to produce protein. This is thus a gold standard assay used for direct examination of IFITM activity in virus infections as demonstrated by the large number of high-impact papers that have utilized similar assays in studying IFITMs (Brass, *Cell*, 2009; Yount, *Nat Chem Biol*, 2010; Huang, *PLOS Pathogens*, 2011; Feeley, *PLOS Pathogens*; Everitt, et al. *Nature*, 2012; Lin, *Cell Rep*, 2013; Compton, *Cell Host Microbe*, 2015; Savidis, *Cell Rep*, 2016; Compton, *EMBO Rep*, 2016; Percher, *PNAS*, 2016; Chesarino, *EMBO Rep*, 2017; Huang, *PNAS*, 2017; Monel, *EMBO J*, 2017; McMichael, *JID*, 2018; Wu, *Cell*, 2018; Kenney, *PNAS*, 2019; Shi, *PNAS*, 2019; Ahi, *mBio*, 2020). **We have added a statement in the results section with an explanation of why early detection of virus protein is the standard assay for studying IFITM3 activity.**

2. Graph labelling could be clearer. I'm not sure of the value of a control bar without errors, in each of the plots. The axis labels should be consistent, eg relative infection with 1 or 100, not a mixture of the 2.

Control bars are based on a normalization to 100 so error bars are not shown for the controls. **We have, however, now provided a supplemental data sheet that contains all non-normalized and normalized percent infection data that were used in generating all graphs.**

We have made axis labels consistent throughout the manuscript as requested.

3. Labeling the bar charts with the virus would improve clarity.

We have added virus names to the y axes of the infection experiment graphs as requested.

4. The facs analysis is a bit shaky in places. In Fig 2C the gate is too far to the left. It is clear that the effect size in Fig 3C with IFITM1 expression is very strong, ie a complete block to infection. But poor placing of the gate reads it as a 3 fold effect, which it clearly is not given

the decent shift in the infected population. Here they're just counting the edge of the uninfected population. A proper look at all the Facs data is advised.

We respectfully disagree with these statements. The flow cytometry gates for infected cells were set based on lack of positive cells in non-infected samples. We also point out that altering the gates as suggested by the reviewer, may strengthen results concerning IFITM1, but would not affect our overall conclusions.

5. I don't like the fact that all the infection data are normalised. Have any titrations been done? Is the effect size MOI dependent?

The infection data is normalized because of day to day variation in the maximum percent infection observed in replicate experiments. We provided representative non-normalized flow cytometry plots for every normalized figure to show the general magnitudes of infections that we achieved, i.e., 7 – 20% maximal infection in different experiments. Importantly, despite slight variations in infections, the data trends for effects of IFITMs are consistent across experiments as shown by statistical significance observed in comparisons of the normalized data.

Regarding MOIs, effects of IFITMs on virus infections are generally saturable by increasing virus MOI. For our experiments we chose an MOI of 1, which was the highest virus dose allowed by the titer of our virus stock. An MOI of 1 resulted in reasonable, but not saturating, infection levels in HEK293T cells.

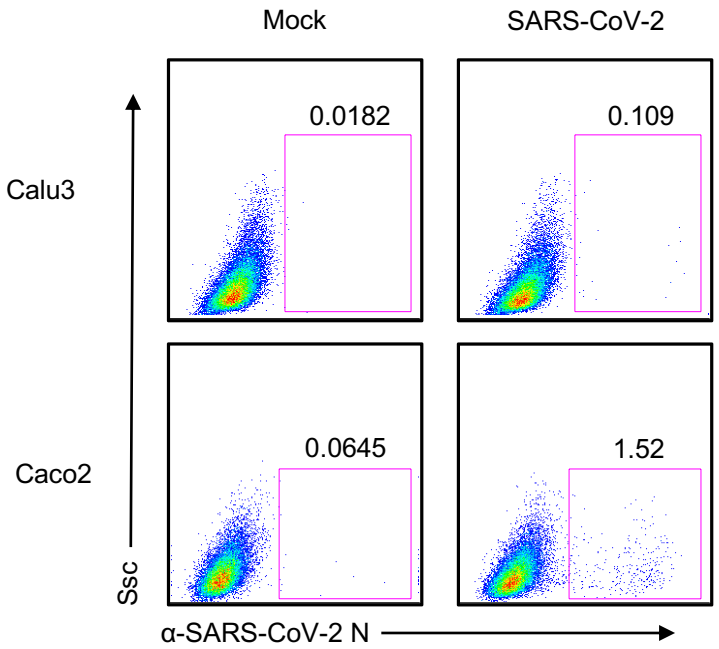
Referee #2:

The manuscript submitted by Shi, et al. is an intriguing study that explores the role of the IFITM proteins in SARS-CoV-2 infection. The authors show that IFITM1, IFITM2 and IFITM3 restrict SARS-CoV-2. The experiments are well-designed and the use of both overexpression and deletion of IFITM proteins strengthens the claim of an important role for these innate immune proteins in viral infection. Overall, the data presented are strong and the authors make a compelling case for the function of IFITM3 that is distinct from other viral infections. The studies that show re-localization of IFITM3 results in enhancement, rather than the inhibition of infection are interesting. However, there are some points that can be addressed to strengthen the studies presented here.

1. There is less inhibition of infection with Caco2 cells which are infectable without overexpression of ACE2. Does this indicate an issue with ACE2 overexpression? What are the baseline levels of infection in each cell line?

We have tried extensively over the past several months to achieve robust infections of Calu3 and Caco2 cells, which as the reviewer notes, endogenously express ACE2. Using an MOI of 1, which provides up to 20% infection of HEK293T-ACE2-GFP cells, we detected infection of Calu3 and Caco2 cells at a very low percentage within the cultures (shown below). We note that most published data with these lines do not measure percent infection, but rather show infection via qPCR, which is not informative as to the number of cells infected within a culture.

Using higher virus doses could possibly give higher infections, but this is not possible given our virus stock titer. We note, however, that we provide data in **Figure 1** with Caco2 cells in which concentrated Spike-pseudotyped virus was used to achieve a robust infection allowing us to measure effects of endogenous IFITMs in this relevant line.



2. Many of the experiments are performed in the context of ACE2 overexpression in cells that are not naturally susceptible to SARS-CoV-2 infection. These results should be verified in a more relevant context of infection, such as the Calu3 cell line used for the syncytia formation experiments.

As shown above, the low infection rates of Caco2 and Calu3 cells with authentic SARS-CoV-2 precludes us from confidently examining roles of IFITMs in these lines. Instead, HEK293T-ACE2-GFP cells have provided an ideal model for us to dissect the opposing roles of IFITMs because 1) they are robustly infected by SARS-CoV-2, 2) the virus can utilize both plasma membrane and endocytic entry pathways in this line, and 3) we can manipulate the virus entry pathway in these cells for mechanistic studies by overexpression of TMPRSS2 and IFITMs. The dual effects of IFITMs on the entry of specific coronaviruses has been controversial and confusing in the field, particularly in the context of SARS-CoV-2 infections. Our results offer a clearer mechanistic understanding of how IFITM3 uses an amphipathicity-based mechanism to inhibit virus entry at endosomes while also enhancing plasma membrane entry in an amphipathicity-independent manner.

As an additional test of effects of endogenous IFITM proteins, **we have now added data in which WT, IFITM3, and IFITM-locus deficient (IFITMdel) MEFs were transduced with hACE2 and infected with SARS-CoV-2 (New Figure 5)**. Compared to WT cells, we observed an increased infection in IFITMdel cells. Consistent with our Caco2 cell experiments in Figure 1, IFITM3 KO and broad IFITM deficiency both prevented type I IFN from fully inhibiting SARS-

CoV-2 infections, overall indicating that IFITMs are generally restrictive of infection and that they are among the critical IFN effectors that limit SARS-CoV-2 infections.

3. The authors show that transfecting cells with IFITM3 can modestly inhibit syncytia formation when expressed in target (non-spike expressing cells) but that IFITM3 mutants enhanced syncytia formation in this context. Is this effect observed only when expressed in target cells? What is the result of co-expressing IFITM3 and mutants in SARS-CoV-2 spike-expressing cells? What is the effect of expressing IFITM1 and IFITM2?

While these are interesting questions, we performed the syncytia assays specifically to have additional confirmation via a distinct assay that IFITM3 is able to enhance SARS-CoV-2 Spike-mediated fusion at the plasma membrane. Indeed, this assay confirmed this ability of IFITM3 when expressed in target cells. As for roles of other IFITMs and expression of IFITMs in Spike-expressing effector cells, we note that a full and comprehensive manuscript on these exact topics has been published as a preprint by the group of Dr. Olivier Schwartz (Pasteur Institute), demonstrating that an in-depth investigation of this topic could easily comprise a full manuscript and is outside the scope of our current study.

4. The results showing that overexpression of TMPRSS2 decreases IFITM3-mediated SARS-CoV-2 inhibition are interesting. What is the result of overexpressing TMPRSS2 with IFITM1? Or with IFITM3 localization mutants?

These experiments are included in Figure 7 of our manuscript, and we have added an additional experimental replicate. IFITM1 inhibition of infection was lost upon overexpression of TMPRSS2, but enhancement of infection was not observed. For IFITM3-Y20A, which enhances infection, statistical significance was not reached comparing infection with or without TMPRSS2 overexpression.

5. Human IFITM3-Y20A but not mouse IFITM3-Y20A increased infection compared to vector control cells. Do both mutants localize to the plasma membrane? This should be shown.

We note that both mouse and human IFITM3 have a conserved YxxΦ endocytosis motif involving Y20, **and now make this clear in the manuscript text**. Indeed, we previously showed that this motif regulates cellular localization of both mouse and human IFITM3 (Chesarino, *JBC*, 2014). For the current manuscript, **we have added confocal imaging which shows plasma membrane localization for Y20A mutants from both species.**

1st Revision - Editorial Decision 10th Nov 2020

Dear Jacob and Alex,

Thanks for sending me the revised manuscript. The study has now been seen by the original referees and their comments are provided below. As you can see from the comments the referees appreciate the introduced changes. I am therefore very happy to let you know that we will accept the manuscript for publication here.

Before sending you the formal acceptance letter there are just a few things to sort out.

- We need 3-5 keywords

- We also need a data availability section. This is the place to enter accession numbers etc. As far as I can see no data is generated that needs to be deposited in a database. If this is correct please state: This study includes no data deposited in external repositories. Please place it after the Materials and methods and before Acknowledgements

- Please also list author contributions

- The figure files need to uploaded as individual figures

- Please split the source data into 1 file per figure.

- I have asked our publisher to do their pre-publication checks on the paper. They will send me the file within the next few days. Please wait to upload the revised version until you have received their comments.

- We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels). You can also use something from the figures if that is easier.

That should be all - you can use the link below to submit the revised version.

Congratulations on a nice study.

With best wishes

Karin

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Instructions for preparing your revised manuscript:

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IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
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- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 8th Feb 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The reviewers have addressed my comments effectively. I'm right about the facs but its up to them how they present these data.The work is of good impact and will be of interest to a wide audience. The conclusions are justified.
I have no further concerns, suggestions or comments.

Referee #2:

In general, I believe this is an interesting and important study that is appropriate for the broad readership of this journal. The authors have addressed the majority of my points and have added sufficient new data and clarifications within the text to strengthen the manuscript. There is still an

outstanding concern of the primary use of ACE2 over-expression cell lines. However, the data support the overall conclusions.

2nd Revision - Editorial Decision 19th Nov 2020

Dear Jacob,

Thank you for submitting your revised manuscript to The EMBO Journal. I have now had a chance to take a look at everything and all looks good. I am therefore very pleased to accept the manuscript for publication here.

Congratulations on a nice study!

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
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Corresponding Author Name: Jacob S. Yount and Alex Compton
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020-106501R

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 iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	No pre-specified effect size was chosen. Experiments with three bilogical replicates were performed a minimum of two times to achieve a minimum of six data points for statistical comparison. Most experiments were performed at least 3 times providing nine data points.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	ANOVA tests were used to evaluate signifcant differences in mean values for >2 groups.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Data met the assumptions of the statiscal test employed (ANOVA): 1) The responses for each factor level have a normal population distribution. 2) These distributions have the same variance. 3) The data are independent.
Is there an estimate of variation within each group of data?	Yes

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http://biomodels.net/

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

Is the variance similar between the groups that are being statistically compared?	Yes

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).	Antibody catalog numbers are provided in the Materials and Methods section, but are listed again here - Anti-SARS-CoV-2 N (Sino Biological, 40143-MM08); Anti-IFITM2/3 (ProteinTech, 11714-1-AP); Anti-IFITM1 (Cell Signaling Technologies, 13126S); Anti-mouse-AlexaFluor-555 (ThermoFisher, A-21424); Anti-rabbit-AlexaFluor-647 (ThermoFisher, A-31573); Anti-rabbit-AlexaFluor-488 (ThermoFisher, A-21206); Anti-mouse-AlexaFluor-488 (A-11029).
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Antibody catalog numbers are provided in the Materials and Methods section. Calu3, Caco2, and HEK293T cells were purchased from ATCC. U2OS cells were verified recently by STR profiling. All cell lines were treated for two weeks with Mycoplasma Removal Agent (MP Biomedicals) as a precaution for two weeks when thawed. This information is provided in the Materials and Methods Section.

* 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.	NA
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
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.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
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.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	NA
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

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

G- Dual use research of concern

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