EMBO Molecular Medicine

IL-10 producing type 2 innate lymphoid cells prolong islet allograft survival

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Transaction Report:

(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 8th Apr 2020

8th Apr 2020

Dear Prof. Cao,

Thank you for the submission of your manuscript to EMBO Molecular Medicine. We have now heard back from the two referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees acknowledge the interest of the study. However, they raise some concerns that should be addressed in a major revision of the present manuscript. Addressing the reviewers' concerns in full will be necessary for further considering the manuscript in our journal.

Acceptance of the manuscript will entail a second round of review. Please note that EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision.

We realize that the current situation is exceptional on the account of the COVID-19/SARS-CoV-2 pandemic. Therefore, please let us know if you need more than three months to revise the manuscript.

I look forward to receiving your revised manuscript.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

Overall this is an innovative paper which offers a novel way of prolonging the survival of transplanted islets. The experiments were performed carefully, however some additional data is required to be completely convincing:

1. IL-33 treatment was shown to have glucose-lowering effects on its own that are both insulin-dependent and insulin-independent (Molofsky et al., 2013; Dalmas et al., 2017). Considering that islet transplantation is performed after IL-33 treatment, authors should show the survival curve and glycemia of STZ-induced diabetic mice treated with IL-33 (but without islet graft). These data are required to control that prolonged islet allograft survival is not also due to a better glycemia status at the time of islet graft.
2. In Fig 1D, GTT was done only with 3-5 mice although 12 mice survived the 80 day time point (Fig 1 b). How were these few mice selected for the GTT?
3. The sustained ILC2s increase in the islets graft, long after exogenous IL-33 treatment, is of great interest. IL-33 have been shown to be locally produced in mouse islets, and especially in Balb/c mouse islets that are grafted here (Dalmas et al., 2017). Did the authors check for sustained increased expression of IL-33 (or any other ILC2-promoting factors) in the islet graft over time ? This could partly explain why ILC2s stay inside the graft so long.
4. Fig 6A shows in vitro the reduced production of IL10 upon knockdown CRISPR ko of IL10 in ILc210. Can this reduction only be detected in isolated cells in vitro or also in whole organs, does it affects IL10 plasma levels?
5. Figure 7 Do ILC2s from the local transplantation with the graft migrate in other organs? Does islet rejection occur because of the ILC2 death in the graft? Authors should show the staining of ILC2 in

islet grafts of islet transplant mice over time until rejection.
6. Please describe how the islets were isolated. Also, the number of islets isolated per mice seems unusually high, please control
7. PC61 (anti-CD25antibody) is not described in methods section, provider?
8. Mice: sex of mice is missing.
9. DEREG and IL-10-GFP transgenic mice should be better described, source, origin reference to production of mouse strains.
10. Description of islet transplantation is poor and also no reference to the details of the protocol is provided.
11. "Neutralizing anti-IL-10 antibodies (10 □g/ml Biolegend)", specify antibody used.
12. Provide rationale for IL-2/anti-IL2 mAB.
13. Description/Provider of ELISA assay for IL10 is missing
14. The description of the CRISPR-Cas9 transfection is very superficial.

Referee #2 (Remarks for Author):

In the manuscript entitled "IL-10 producing type 2 innate lymphoid cells induced by IL-33 prolong islet allograft survival," the authors present data that show that systemic IL-33 treatment of diabetic mice that receive islet allografts, prolongs the survival of the transplanted islets by increasing the levels of Tregs and especially IL10 secreting, ILC2 cells. The data are intriguing, but the manuscript would be strengthened with the follow data:
1) Whether, after systemic IL-33 treatment, ILC2 cells localize to other non-lymphoid organs (e.g., liver, heart, the kidney that did not receive islets) versus localizing to where the islet allografts are found and where an immune response in under way.
2) The relative number of infiltrating CD4+ versus CD8+ T cells in vehicle versus IL-33 treated animals to provide insight into the mechanism of rejection/acceptance in the experimental and control groups.
3) A control group in Fig 4 of islet + IL-33/PC61 without DT. Also, the authors show that Treg levels peak at day 7 in the kidney but ILC2 levels remain high up to day 30 in the kidney and up to day 80 within the islet allografts. Have the authors treated the recipients with PC61at day 30 and/or day 80? In addition, have they treated recipients with DT at those times? It could be that Tregs don't play as great a role in the later time points in IL-33 treated recipients and these additional studies could help explain the results in Figure 4D where 40% graft survival is observed after DT treatment.
4) In Figure 6F, the authors show suppressive activity by ILC210 cells in vitro. Have the authors performed similar experiment using ILC210 -IL10 cells? If so, are ILC210 -IL10 cells as suppressive and if not, does the addition of IL10 result in the recovery of their suppressive activity?
5) Regarding Figure 7, does injection of ILC210 -IL10 cells injected locally result in decreased survival of the islet allografts?

Referee #1 (Remarks for Author):

Overall this is an innovative paper which offers a novel way of prolonging the survival of transplanted islets. The experiments were performed carefully, however some additional data is required to be completely convincing:

1. IL-33 treatment was shown to have glucose-lowering effects on its own that are both insulin-dependent and insulin-independent (Molofsky et al., 2013; Dalmas et al., 2017). Considering that islet transplantation is performed after IL-33 treatment, authors should show the survival curve and glycemia of STZ-induced diabetic mice treated with IL-33 (but without islet graft). These data are required to control that prolonged islet allograft survival is not also due to a better glycemia status at the time of islet graft.
Thanks for this valuable suggestion, following which we examined the survival rate and glycemia of STZ-induced diabetic mice treated with IL-33. The additional experiments showed that IL-33 treatment (starting from day 6 after STZ injection) in diabetic mice without islet transplantation improved the fasting and non-fasting glycemia at day 15 and 18 post-STZ injection, but not at other time points. IL-33 treatment did not enhance survival of STZ-induce diabetic mice within 30 days (new Figure S1). These results indicate that short-term IL-33 treatment only temporarily improved hyperglycaemia, which might contribute to prolonged islet allograft survival. However, we further demonstrated that Tregs and ILC2s played critical roles in IL-33-mediated islet graft protection (Figures 4, 6 and 7).

2. In Fig 1D, GTT was done only with 3-5 mice although 12 mice survived the 80 day time point (Fig 1 b). How were these few mice selected for the GTT?
We conducted two independent animal experiments in Figure 1, but only showed the data from one set of animal experiments in Figure1D and E. We now added all data in Figure 1.

3. The sustained ILC2s increase in the islets graft, long after exogenous IL-33 treatment, is of great interest. IL-33 have been shown to be locally produced in mouse islets, and especially in Balb/c mouse islets that are grafted here (Dalmas et al., 2017). Did the authors check for sustained increased expression of IL-33 (or any other ILC2-promoting factors) in the islet graft over time ? This could partly explain why ILC2s stay inside the graft so long.
According to the reviewer's suggestion, the expression of ILC2-promoting factors, including IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) were examined in islet graft tissue at day 7, 30 and 80 post-islet transplantation. We observed a consistent increase of IL-33, but not IL-25 or TSLP, in islet graft tissue of mice treated with IL-33, which could partly explain why ILC2s were found within the graft for so long. However, the reason for the consistent increase of IL-33 in islet graft needs future investigation.

4. Fig 6A shows in vitro the reduced production of IL10 upon knockdown CRISPR ko of IL10 in ILc210. Can this reduction only be detected in isolated cells in vitro or also in whole organs, does it affects IL10 plasma levels?
Additional experiments were performed to examine expression of IL-10 in islet graft and in plasma. The expression of IL-10 in islet graft tissue was significantly increased in mice transfused with $ILC2^{10}$, but reduced in mice transfused with IL-10-deleted $ILC2^{10}$ (Figure 6D). There was no significant change of IL-10 levels in plasma in mice transfused with $ILC2^{10}$, IL-10-deleted $ILC2^{10}$ or non-$ILC2^{10}$. There results indicate that transfused $ILC2^{10}$ only affect IL-10 expression in local islet graft tissue, but not systemic IL-10 levels in plasma.

5. Figure 7 Do ILC2s from the local transplantation with the graft migrate in other organs? Does islet rejection occur because of the ILC2 death in the graft? Authors should show the staining of ILC2 in islet grafts of islet transplant mice over time until rejection.
Thanks for another valuable suggestion. Additional experiments using CD45.2 and CD45.1 mice were performed to examine the number and phenotype of locally transferred $ILC2^{10}$ in islet graft over time (new Figure 7C-F). There was no difference in the number of CD45.2+ $ILC2^{10}$ in rejected islet graft and in accepted islet graft (survival for 80 days post-islet transplantation) (Figure 7D and E), suggesting that the occurrence of islet rejection is not because the number of ILC2 reduces in the graft over time. We did not detect CD45.2+ $ILC2^{10}$ in other organs, including kidney, liver and lung, by flow cytometry in recipient mice with local $ILC2^{10}$ transplantation. Furthermore, we found phenotypic changes of $ILC2^{10}$ in rejected islet graft, especially reduced expression of IL-10 (Figure 7F and Figure S5), which could possibly explain why locally transferred $ILC2^{10}$ did not lead to long-term islet graft survival in 5 out of 9 islet transplanted mice.

6. Please describe how the islets were isolated. Also, the number of islets isolated per mice seems unusually high, please control
We have added the protocol of islets isolation in the revised manuscript. Pancreatic islets were separated from the pancreata of donor (BALB/c) mice at a ratio of four pancreata per recipient. The “2000 IEQ/mouse” means “2000 IEQ per recipient mouse”. We have made correction in method section.

7. PC61 (anti-CD25antibody) is not described in methods section, provider?
We have added the source for anti-CD25 antibody, PC61.

8. Mice: sex of mice is missing.
Male mice were used in all animal experiments, which has been described on page 18 in the Methods section.

9. DEREG and IL-10-GFP transgenic mice should be better described, source, origin reference to production of mouse strains.
We have added the information for DEREG and IL-10-GFP transgenic mice.

10. Description of islet transplantation is poor and also no reference to the details of the protocol is provided.
The detailed protocol and reference have been added in the Methods section.

11. "Neutralizing anti-IL-10 antibodies (10 μg/ml Biolegend)", specify antibody used.
The clone number of anti-IL-10 antibody (JES5-16E3) has been added in the Methods section.

12. Provide rationale for IL-2/anti-IL2 mAB.
The rationale for using IL-2/anti-IL2 mAB is added in the revised manuscript (page 10).

13. Description/Provider of ELISA assay for IL10 is missing
The description and provider of ELISA assay have been added in the Methods section.

14. The description of the CRISPR-Cas9 transfection is very superficial.
A detailed description of the CRISPR-Cas9 transfection has been added in the Methods section.

Referee #2 (Remarks for Author):

In the manuscript entitled "IL-10 producing type 2 innate lymphoid cells induced by IL-33 prolong islet allograft survival," the authors present data that show that systemic IL-33 treatment of diabetic mice that receive islet allografts, prolongs the survival of the transplanted islets by increasing the levels of Tregs and especially IL10 secreting, ILC2 cells. The data are intriguing, but the manuscript would be strengthened with the follow data:

1) Whether, after systemic IL-33 treatment, ILC2 cells localize to other non-lymphoid organs (e.g., liver, heart, the kidney that did not receive islets) versus localizing to where the islet allografts are found and where an immune response in under way.
Thanks for your very helpful suggestions. We have previously shown that systemic IL-33 treatment induced ILC2 expansion in non-lymphoid organs, such as kidney, liver and lung.[1] Considering that islet transplantation is performed after IL-33 treatment, we proposed that ILC2s will migrate into islet graft after islet transplantation. We found that a greater amount of ILC2s were found in islet graft than in kidney and liver (Figure S3), which indicates that ILC2s tend to migrate to islet graft undergoing immune response.

2) The relative number of infiltrating CD4+ versus CD8+ T cells in vehicle versus IL-33 treated animals to provide insight into the mechanism of rejection/acceptance in the experimental and control groups.
Additional experiments were performed to examine the ratio of infiltrating CD4+ versus CD8+ T cells in mice treated with vehicle or IL-33. We observed a significant increase of ratio of CD4 T cells/CD8 T cells in islet graft of mice treated with IL-33 (Figure. S2), suggesting that IL-33 treatment may prevent islet graft rejection through modulating local CD4 and CD8 T cell responses.

3) A control group in Fig 4 of islet + IL-33/PC61 without DT. Also, the authors show that Treg levels peak at day 7 in the kidney but ILC2 levels remain high up to day 30 in the kidney and up to day 80 within the islet allografts. Have the authors treated the recipients with PC61at day 30 and/or day 80? In addition, have they treated recipients with DT at those times? It could be that Tregs don't play as great a role in the later time points in IL-33 treated recipients and these additional studies could help explain the results in Figure 4D where 40% graft survival is observed after DT treatment.
Following the reviewer's suggestion, a control group of islet + IL-33/PC61 without DT treatment was included in new Figure 4. Administration of anti-CD25 antibody (PC61) successfully depleted both Tregs and ILC2s in vivo as CD25 is highly expressed on both Tregs and ILC2s. Tregs and ILC2 depletion by PC61 completely abolished the protective effects of IL-33 on islet transplantation, indicating both Tregs and ILC2s play critical roles in IL-33-mediated islet graft protection. However, we have not treated the recipients with PC61 at day 30 and/or day 80 because the recipient mice that received PC61 treatment prior to islet transplantation rejected their graft within 30 days. We have not performed later stage depletion of Tregs (DT treatment at day 30 or 80) because IL-33 induced Tregs levels that peaked at day 7 and were down to normal at day 14 (Figure 2 and 3). Therefore, DT treatment at day 30 or 80 could not be used to demonstrate the protective effects of Tregs which were induced by IL-33. Additional ILC2 depletion in Treg-depleted DEREG mice completely abolished the protective effects of IL-33 on islet transplantation (Figure 4), suggesting that ILC2, but not Tregs, play an important role in the later time points in IL-33 treated recipients.

4) In Figure 6F, the authors show suppressive activity by ILC210 cells in vitro. Have the authors performed similar experiment using ILC210 -IL10 cells? If so, are ILC210 -IL10 cells as suppressive and if not, does the addition of IL10 result in the recovery of their suppressive activity?

Following the reviewer's suggestion, we performed similar experiments using IL-10-deleted $ILC2^{10}$, shown in Figure 6G. $ILC2^{10}$ effectively suppressed allogeneic splenocyte induced CD4 T cell proliferation in a dose dependent manner, and genetic ablation of IL-10 diminished the suppressive role of $ILC2^{10}$ on CD4 T cell proliferation (new Figure 6F and 6G), indicating that IL-10 is an important mediator in $ILC2^{10}$-mediated immune suppression. Regarding the suggestion of adding back IL-10 into cell culture system, this could not be used to demonstrate the recovery of suppressive effect of $ILC2^{10}$, as IL-10 alone has immunosuppressive effects.

5) Regarding Figure 7, does injection of ILC210 -IL10 cells injected locally result in decreased survival of the islet allografts?

Additional experiments showed that local transfer IL-10-deleted $ILC2^{10}$ failed to prolong islet graft survival in comparison with $ILC2^{10}$ (new Figure 7). These data further confirmed that $ILC2^{10}$ prolonged islet graft survival in an IL-10-dependent manner.

Reference

1. Cao Q, Wang Y, Niu Z, *et al.* Potentiating Tissue-Resident Type 2 Innate Lymphoid Cells by IL-33 to Prevent Renal Ischemia-Reperfusion Injury. *J Am Soc Nephrol* 2018; **29:** 961-976.

10th Sep 2020

Dear Prof. Cao,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed reports from the referees that were asked to re-assess it. As you will see the reviewers are now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

The authors made a great effort and addressed well the critics

Responses to your comments:

Referee #1 (Remarks for Author):
The authors made a great effort and addressed well the critics
We thank the reviewer’s generous comments.

2nd Revision - Editorial Decision 16th Sep 2020

The authors performed the requested changes.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: QI CAO
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2020-12305

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	The sample size was chosen based on our experience from previous studies. No statistical power analysis was conducted.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The sample size was chosen based on our experience from previous studies. No statistical power analysis was conducted.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	STZ-induced diabetic C57BL/6 mice with a blood glucose value >16 mmol/liter were selected as transplant recipients.
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.	The animals were randomized according to their body weight and blood glucose before starting therapy to avoid any bias.
For animal studies, include a statement about randomization even if no randomization was used.	The animals were randomized according to their body weight and blood glucose before starting therapy to avoid any bias.
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.	Randomization was used to allocate animals to the treatment groups and analysis was blinded.
4.b. For animal studies, include a statement about blinding even if no blinding was done	Researchers were blinding for measurement of blood glucose, IPGTT and islet graft sections.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Statistical tests included unpaired, two-tailed Student's t test using Welch's correction for unequal variances and one-way ANOVA with Tukey's multiple comparison test. Graft survival was analyzed using the Kaplan-Meier method, and survival curves were compared using the log-rank test. Statistical analyses were performed using Prism (Version 7, GraphPad). A P<0.05 was considered statistically significant.
Is there an estimate of variation within each group of data?	Yes, there is an estimate of variation shown as shown by error bar indicating the standard error mean for each group data. Whereever possible, we show individual data points in figures.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
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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).	All antibodies were validated and cross-referenced in the Materials and Method section.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* 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.	C57BL/6 (CD45.2+), congenic C57BL/6 (CD45.1+) C57BL/6 and BALB/c mice were purchased from the Animal Resources Centre (Perth, Australia) and Shanghai Laboratory Animal Center of Chinese Academy of Science. DEREG mice (Lahl, Loddenkemper et al., 2007, C57BL/6-Tg23.2Spar/Mmjax, JAX strain 32050) and IL-10-GFP mice (Kamanaka, Kim et al., 2006, B6.129S6-Il10tm1Flv/J, JAX strain 008379) were obtained from Jackson Lab and bred at the Department of Animal Care at Xinxiang Medical University (XMU) and Westmead Hospital Animal House. For all studies adult (8–10 weeks of age) male mice were used in accordance with the animal care and use protocols approved by Animal Ethics Committee of XMU or Western Sydney Local Health District (WSLHD).
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal procedures were approved by Animal Ethics Committee of XMU or Western Sydney Local Health District (WSLHD).
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.	We confirm compliance.

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.	
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	
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.	
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.	

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).	NA
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