**Prevotella timonensis** turns restrictive vaginal Langerhans cells into HIV-1 reservoirs for HIV-1 dissemination

Nienke van Teijlingen, Leanne Helgers, Ramin Sarrami-Forooshani, Esther Zijlstra-Willems, John van Hamme, Celia Segui-Perez, Marleen van Smoorenburg, Hanneke Borgdorff, Janneke van de Wijgert, Elisabeth van Leeuwen, Joris van der Post, Karin Strijbis, Carla Ribeiro, and Teunis Geijtenbeek

DOI: 10.15252/embj.2022110629

**Corresponding author(s):** Teunis Geijtenbeek (t.b.geijtenbeek@amsterdamumc.nl)

**Review Timeline:**
- Submission Date: 10th Jan 22
- Editorial Decision: 23rd Feb 22
- Revision Received: 7th Jun 22
- Editorial Decision: 28th Jun 22
- Revision Received: 8th Jul 22
- Accepted: 18th Jul 22

*Editor: Karin Dumstrei*

**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.)
Dear Theo,

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. A third referee had also agreed to review the manuscript for us, but I have not received the comments yet. I would like to give this referee until next week to submit the report. If we don't hear anything back by then, then we will go with the two reports on hand.

Given the positive feedback from the two referees I would like to ask you to start considering the revisions needed to address the raised concerns. Would also be good to discuss the revisions further either via email or a video call once we have sorted out the last report.

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: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Thank you for the opportunity to consider your work for publication.

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

I have attached a file with helpful tips on how to prepare the revised version.

Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (24th May 2022). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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

------------------------------------------------

Referee #1:

This is a study that investigates the functions of LANGERHANS CELLS in the context of MICROBIOTA. This is done in HUMANS, i.e., with human cells and human skin and vaginal epithelium models.

This work provides mechanistic insights into the clinically observed phenomenon, that women with vaginal dysbiosis, that is a disturbed composition of their vaginal microbiota, are at higher risk to acquire an HIV infection. The data

The authors find one culprit among the many bacteria species of the vagina, namely Prevotella timonensis. (They do discuss however, that there may be other, unstudied, perhaps unkown species that have similar effects.)

Langerhans cells that are treated with Prevotella and subsequently exposed to HIV
-- contain much more intracellular virus (compared to no pretreatment or to other bacterial species)
-- this is due to increased uptake, and not productive infection of Langerhans cells
-- interestingly, this uptake is independent of CD4, CCR5, Langerin/CD207
-- do not mature as indicated by lack of CD80, CD86 and CCR7 induction / increase
-- lose their capacity to degrade HIV intracellularly
-- transmit several HIV strains to a reporter cell line
-- transmit also founder HIV strains to the reporter cell line
1. Maturation. Data from Figure 3A-D. CD80/86 and CCR7 expression are compared side-by-side in enriched suspensions of Langerhans cells and of Langerhans cells migrated from epidermal explants. This is not a perfectly fair comparison. It would be more meaningful, or rather, more convincing to compare
(A) Langerhans cell suspensions untreated and treated with Prevotella and some / few other bacteria to directly test, whether this block in maturation, this failure to upregulate the co-stimulators is really Prevotella-specific.
(B) Conversely, epidermal/epithelial explants should be treated or not with Prevotella. Do the bacteria inhibit maturation of the migrated cell in this quasi-physiological model? (Does Prevotella perhaps inhibit migration??) Since the authors have previously shown that Langerhans cell maturation abrogates their protective function, these data are crucial and important.
(C) All maturation experiments were done with epidermal LC "only" but not with vaginal LC. The authors should specifically discuss and emphasize this point for the sake of clarity - or, if possible within a reasonable timeframe - add few confirmatory experiments with vaginal LC or vaginal explant.

2. In the Discussion there is this sentence: "P. 214 timonensis strongly enhanced HIV-1 uptake by mucosal vaginal LCs and induced HIV-1 transmission to target cells." In the wording of the Abstract, these data are not so stringently linked to vaginal LC. The reviewer recognizes that the authors have taken on this challenge successfully). The experiments dealing with TRANSMISSION to the target cell line U87.CD4.CCR5 were done with epidermal LC "only" but not with vaginal LC. The authors should specifically discuss and emphasize this point for the sake of clarity - or, if possible within a reasonable timeframe - add a small series of experiments like those in Figure 5A with "true" vaginal LC.

3. A "subpoint" to the above mentioned issue. On most occasions the authors specify whether epidermal LC or vaginal LC were used - in the text - in the figure legends. Please double-check, whether this applies to really all occurrences throughout the manuscript and adapt accordingly if needed.

MINOR POINTS - minor concerns that should be addressed

4. In the METHODS, the abbreviation DMSZ should be defined as "German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig"

5. METHODS: ".....cells were stained in demi-water supplemented with 1.0 % BSA and 1x TBS for with PE-conjugated..... "demi-water" is jargon. Please use "demineralized water", which I guess, this is. What does "1xTBS" stand for? Means Tris-buffered saline. So, it is a "TBS supplemented with BSA", but not "demi-water supplemented with TBS". Correct?

6. Please make sure that "inoculation" is always used correctly. In Fig.5 it says "Ex vivo skin explants were stimulated O/N with L. crispatus (LC), M. elsdenii (ME), or P. timonensis (PT) and inoculated with HIV-1 (JRCSF)....." So, here HIV was indeed inoculated or injected. In the legend for Figure 4, however, it says "Immature vaginal LCs were stimulated O/N with P. timonensis (PT) followed by HIV-1 (SF162) inoculation." Here, in contrast, it was a cell suspension with which the virus was incubated or which was exposed to the virus (as the authors have worded it some other place).

7. Fig.1 and all other figures where this may apply to. In the y-axis it says "" HIV+ cells" - why not explicitly write "LC" rather than "cells"? Would be cleared.

8. "matured", not "maturated"

9. Please state explicitly, whether / that Langerin expression was always surface expression, not done on peremabilized cells.

10. Legend to Figure 5. May be helpful to modify/extend "....of the CD1a- fraction..." to "....of the CD1a- fraction, i.e., LC..."

11. few typos like Fischer for Fisher or Croning for Corning

Referee #2:

HIV transmission at mucosal surfaces is known to be affected by dysbiosis of the local microbiota but the underlying mechanisms are unclear. Van Teijlingen and colleagues focus on the impact of bacteria on the interaction of HIV-1 with Langerhans cells (LCs) isolated from epidermal or vaginal tissue. Screening a set of bacteria enriched at dysbiotic vaginal mucosa, the authors identify that specifically P. timonensis sensitizes LCs for enhanced uptake of HIV-1 particles into an intracellular compartment. This uptake does not result in production infection, fusion or degradation but internalized particles remain infectious over extended periods of time and can be transferred to new target cells. The study addresses a relevant topic
and describes an interesting new phenomenon in physiologically relevant primary cell systems. Experiments presented are well
designed and interpreted and the paper is well written. A few control experiments would be required to substantiate the
conclusions drawn from the data presented (points 1-3). The study would also benefit from providing some additional
mechanistic insight (point 4).
1) The authors use HIV-1 SF162 for most of the experiments on particle uptake (Figs 1-4) but this HIV-1 strain is not well
transmitted (Fig 6C). Transmission experiments then focus on JRCSF and CHO58. It would be important to gain some insight
into the reasons why transmission efficiencies differ between SF162 vs. JRCSF/CHO58. Comparing the compartments in which
these particles are internalized, including, as discussed by the authors, identifying the specific compartments, should be
informative.
2) Text and legend to Figure1 mention that epidermal LCs were exposed to L.crispatus and L.iners (LI) but panels A and C do
not show data for LI - please add the data or correct the text.
3) How do the amounts of bacteria used for experimental stimulation compare to those present on the mucosa in vivo? This
should be discussed and, if required, an experiment assessing the impact of physiological amounts of P. timonensis should be
added.
4) Initial experimental insight and a conceptual discussion regarding what distinguishes P. timonensis from the other bacteria
analyzed with respect to altering the interaction of LCs with HIV and by which signals P. timonensis reprograms HIV uptake by
LCs would significantly add to the study.
Response to the reviewers comments (EMBOJ-2022-110629)

Reviewer 1

This is a study that investigates the functions of LANGERHANS CELLS in the context of MICROBIOTA. This is done in HUMANS, i.e., with human cells and human skin and vaginal epithelium models. This work provides mechanistic insights into the clinically observed phenomenon, that women with vaginal dysbiosis, that is a disturbed composition of their vaginal microbiota, are at higher risk to acquire an HIV infection. The authors find one culprit among the many bacteria species of the vagina, namely *Prevotella timonensis*. (They do discuss however, that there may be other, unstudied, perhaps unknown species that have similar effects.)

LCs that are treated with *P. timonensis* and subsequently exposed to HIV contain much more intracellular virus (compared to no pretreatment or to other bacterial species):

- this is due to increased uptake, and not productive infection of Langerhans cells
- interestingly, this uptake is independent of CD4, CCR5, Langerin/CD207
- do not mature as indicated by lack of CD80, CD86 and CCR7 induction / increase
- lose their capacity to degrade HIV intracellularly
- transmit several HIV strains to a reporter cell line
- transmit also founder HIV strains to the reporter cell line

1) Data from Figure 3A-D. CD80/86 and CCR7 expression are compared side-by-side in enriched suspensions of Langerhans cells and of Langerhans cells migrated from epidermal explants. This is not a perfectly fair comparison. It would be more meaningful, or rather, more convincing to compare

   (A) Langerhans cell suspensions untreated and treated with *Prevotella* and some / few other bacteria to directly test, whether this block in maturation, this failure to upregulate the co-stimulators is really *Prevotella*-specific.

As requested, we have conducted new experiments to determine maturation of epidermal isolated immature LCs in response to stimulation with different vaginal bacteria (*Lactobacillus crispatus*, *Megasphera esdenii* and *P. timonensis*) (Figure 3A-E). These data show that *L. crispatus* did not induce maturation of immature LCs, while *M. esdenii* and *P. timonensis* induced minor maturation of immature LCs as compared to mature LCs from the same donor that had migrated from the skin (Figure 3A-E). We have included these new data and discussed the results in the manuscript [136-148].

   (B) Conversely, epidermal/epithelial explants should be treated or not with *Prevotella*. Do the bacteria inhibit maturation of the migrated cell in this quasi-physiological model? (Does *Prevotella* perhaps inhibit migration??) Since the authors have previously shown that Langerhans cell maturation abrogates their protective function, these data are crucial and important.
We thank the reviewer for this comment. We have performed ex vivo experiments where epidermal explants were exposed to different vaginal microbiota and determined the absolute cell count and maturation of the migrated LC population [Figure 3F-H]. Migration of LCs from epidermal explants exposed to P. timonensis was slightly but not significantly reduced and LCs from P. timonensis-exposed explants did not induce higher levels of CD80 compared to untreated explants. M. eldenii decreased migration of LCs but induced some maturation as shown by increased CD80 and CD86 expression, whereas the positive control induced some migration as well as maturation [Figure 3F-H]. These data suggest that P. timonensis does not induce LC maturation. We have included our findings and discussion of these results in the manuscript [148-157].

(C) All maturation experiments were done with epidermal LC "only" but not with vaginal LC. The authors should specifically discuss and emphasize this point for the sake of clarity - or, if possible within a reasonable timeframe - add few confirmatory experiments with vaginal LC or vaginal explant.

As requested, we have performed maturation experiments with isolated immature vaginal LCs and we observed similar maturation responses as with epidermal LCs [Figure 3A-E]. L. crispatus did not induce maturation of immature LCs, while M. esdenii and P. timonensis induced minor maturation of immature LCs. We have included these novel data and discussed the results in the manuscript [136-148].

2) In the Discussion there is this sentence: "P. 214 timonensis strongly enhanced HIV-1 uptake by mucosal vaginal LCs and induced HIV-1 transmission to target cells." In the wording of the Abstract, these data are not so stringently linked to vaginal LC. The observations in this manuscript are very clear, and it is also legitimate to deduce functions for vaginal LC when shown in epidermal LC. (Understandably, working with human vaginal LC is logistically even more challenging than with human epidermal LC. The reviewer recognizes that the authors have taken on this challenge successfully!). The experiments dealing with TRANSMISSION to the target cell line U87.CD4.CCR5 were done with epidermal LC "only" but not with vaginal LC. The authors should specifically discuss and emphasize this point for the sake of clarity - or, if possible within a reasonable timeframe - add a small series of experiments like those in Figure 5A with "true" vaginal LC.

We thank the reviewer for the comments and as requested we have now performed transmission experiments with vaginal LCs [Figure 5E,F]. Our data show that P. timonensis induced HIV-1 transmission by vaginal LCs similar as observed for epidermal LCs. We have included the new data (Figure 5 E,F) and discussed the results [201-206]. Additionally, we have clarified the use of vaginal or epidermal LCs throughout the manuscript and figures.

3. A "subpoint" to the above mentioned issue. On most occasions the authors specify whether epidermal LC or vaginal LC were used - in the text - in the figure legends. Please double-check, whether this applies to really all occurrences throughout the manuscript and adapt accordingly if needed.

We have clarified the use of vaginal or epidermal LCs throughout the manuscript
MINOR POINTS - minor concerns that should be addressed

4) METHODS: the abbreviation DMSZ should be defined as "German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig" – adjusted [371].

5) METHODS: ".....cells were stained in demi-water supplemented with 1.0 % BSA and 1x TBS for with PE-conjugated...... "demi-water" is jargon. Please use "demineralized water", which I guess, this is. What does "1xTBS" stand for? Means Tris-buffered saline. So, it is a "TBS supplemented with BSA", but not "demi-water supplemented with TBS". Correct? – adjusted [405-409].

6) Please make sure that "inoculation" is always used correctly. In Fig. 5 it says "ex vivo skin explants were stimulated O/N with L. crispatus (LC), M. elsdenii (ME), or P. timonensis (PT) and inoculated with HIV-1 (JRCSF)" So, here HIV was indeed inoculated or injected. In the legend for Figure 4, however, it says "Immature vaginal LCs were stimulated O/N with P. timonensis (PT) followed by HIV-1 (SF162) inoculation." Here, in contrast, it was a cell suspension with which the virus was incubated or which was exposed to the virus (as the authors have worded it some other place). – adjusted

7) FIGURE 1: In the y-axis it says "" HIV+ cells" - why not explicitly write "LC" rather than "cells"? Would be cleared. – adjusted

8) P635: "matured", not "maturated" – adjusted [632-650].

9) METHODS & FIGURE LEGENDS: Please state explicitly, whether / that langerin expression was always surface expression, not done on peremabilized cells. – adjusted [405-409; 637-640; 725-730].

10) FIGURE 5: May be helpful to modify/extend "....of the CD1a- fraction..." to "....of the CD1a- fraction, i.e., LC..." – adjusted [644; 646; 687; 691; 696].

11) METHODS: few typos like Fischer for Fisher or Croning for Corning – adjusted [346; 349; 362].

Reviewer 2

HIV transmission at mucosal surfaces is known to be affected by dysbiosis of the local microbiota but the underlying mechanisms are unclear. Van Teijlingen and colleagues focus on the impact of bacteria on the interaction of HIV-1 with LCs isolated from epidermal or vaginal tissue. Screening a set of bacteria enriched at dysbiotic vaginal mucosa, the authors identify that specifically P. timonensis sensitizes LCs for enhanced uptake of HIV-1 particles into an intracellular compartment. This uptake does not result in production infection, fusion or degradation but internalized particles remain infectious over extended
periods of time and can be transferred to new target cells. The study addresses a relevant topic and describes an interesting new phenomenon in physiologically relevant primary cell systems. Experiments presented are well designed and interpreted and the paper is well written. A few control experiments would be required to substantiate the conclusions drawn from the data presented (points 1-3). The study would also benefit from providing some additional mechanistic insight (point 4).

1) The authors use HIV-1 SF162 for most of the experiments on particle uptake (Figs 1-4) but this HIV-1 strain is not well transmitted (Fig 6C). Transmission experiments then focus on JRCSF and CH058. It would be important to gain some insight into the reasons why transmission efficiencies differ between SF162 vs. JRCSF/CH058. Comparing the compartments in which these particles are internalized, including, as discussed by the authors, identifying the specific compartments, should be informative.

We apologize for the confusion. We have used different MOI for the transmission experiments with SF162/CH058 (Figure 6) and JRCSF (Figure 5). Infection and transmission experiments throughout this manuscript are performed with MOI of 0.5. We have now clarified this in the figure legends throughout the manuscript. However, in figure 6C the baseline transmission of T/F variant CH058 (MOI 0.5) already reached plateau-levels (>85% HIV-1+ U87.CD4.CCR5 cells), probably due to the immune evasive characteristics of T/F variants, which enables them to infect LCs more efficiently (Hertoghs et al, 2019). Hence, we used a lower MOI (0.25) for CH058 to distinguish between different conditions. To make a fair comparison between CH058 and SF162, MOI of SF162 had to be adjusted to 0.25 as well. Therefore, a direct comparison between SF162 and JRCSF cannot be made, as transmission experiments regarding JRCSF are performed using a higher MOI. Of note, we have now also performed transmission with vaginal LCs using SF162 (Figure 5E,F) and the transmission efficiency is similar as observed with JRCSF transmission by epidermal LCs (Figure 5C,D), suggesting that P. timonensis induces transmission of JRCSF and SF162 to a similar extent, whereas transmission by T/F CH058 is stronger probably due to the ability of T/Fs to escape from restriction in LCs as we have shown before (Hertoghs et al., 2019). We have now discussed this in the manuscript.

2) Text and legend to Figure1 mention that epidermal LCs were exposed to L. crispatus and L. iners but panels A and C do not show data for LI - please add the data or correct the text.

– we have corrected the text [600-613].
3) How do the amounts of bacteria used for experimental stimulation compare to those present on the mucosa in vivo? This should be discussed and, if required, an experiment assessing the impact of physiological amounts of *P. timonensis* should be added.

*As requested, we have now included a discussion about the amounts of bacteria present during bacterial vaginosis [266-274].*

4) Initial experimental insight and a conceptual discussion regarding what distinguishes *P. timonensis* from the other bacteria analyzed with respect to altering the interaction of LCs with HIV and by which signals *P. timonensis* reprograms HIV uptake by LCs would significantly add to the study.

*We agree with the reviewer that understanding of the mechanism induced by *P. timonensis* would be very interesting. We have now included data showing that, interestingly, *P. timonensis* induced increased fusion of HIV-1 but not viral integration nor replication [Figure 4D,E]. These data strongly suggest that *P. timonensis* does not abrogate the HIV-1 restriction pathway in LCs but enhances viral uptake and sequestration via another internalization pathway, leading up to increased HIV- transmission by LCs. We are currently investigating the routing of *P. timonensis*-induced HIV-1 uptake. We have included the novel data as well as discuss the potential mechanism [168-171; 289-298].*

References

Hertoghs N, Nijmeijer BM, van Teijlingen NH, Fenton-May AE, Kaptein TM, van Hamme JL, Kappes JC, Kootstra NA, Hahn BH, Borrow P et al (2019) Sexually transmitted founder HIV-1 viruses are relatively resistant to Langerhans cell-mediated restriction. *PLoS ONE* 14: e0226651-e0226651
Dear Theo,

Thank you for submitting your revised manuscript to The EMBO Journal. Your study has now been seen by the two referees. As you can see from the comments below, both referees appreciate the introduced changes. Referee #2 has some sensible text suggestions.

I am therefore very pleased to accept the MS for publication here. Before doing so there are just a few formatting issues to resolve:

You are missing 3-5 keywords

Regarding the 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: Data Availability: This study includes no data deposited in external repositories.

The conflict of interest statement needs to be updated to Disclosure & Competing Interests Statement - see also our guide to authors

Please move the reference section to below the Data Availability section and above the figure legends.

The Supplemental figures need to be re-labelled as EV figures 'Figure EV#'. Please also correct legends/callouts.

For the EV figure legends add the heading 'Expanded View Figure legends'. Remove the panel 'A' label from the figures as there is only one panel.

Our publisher has also done their pre-publication check on your manuscript. When you log into the manuscript submission system you will see the file "Data Edited Manuscript file". Please take a look at the word file and the comments regarding the figure legends and respond to the issues.

Please not that we don't encourage statistical analysis when N=2 (Figure 2A, 4F-G, 5F, 6B, S1) just show the data points.

Please submit a "clean" version of the MS file when you resubmit and include a point-by-point response as well.

That should be all - let me know if you have any further questions

Congratulations on a nice study!

Best Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Use the link below to submit your revision:
https://emboj.msubmit.net/cgi-bin/main.plex

------------------------------------------------

Referee #1:

The authors have responded in sufficient depth to my suggestions and concerns. In fact, they have performed additional sets of experiments in response to all of my respective request, even where I had left them the choice to "only" discuss the points.

For instance, Fig 3 now places the bacteria-induced upregulation of maturation markers in context to the well established "maximal maturation" of migrated Langerhans cells from skin explant.

In Fig 5, new panels E and F impressively demonstrate the key effect of HIV transmission not "only" for epidermal Langerhans cells but also for the very relevant vaginal Langerhans cells - that are logistically a great challenge to work with.
Indeed, the manuscript has markedly gained in scientific “weight”.

I suggest to now ACCEPT this revised manuscript for publication in the EMBO Journal.

I HAVE NO MORE REMARKS FOR THE AUTHORS

Referee #2:

VAN TEIJLINGEN, HELGERS and colleagues present a revised version of their previous manuscript on the impact of mucosal microbiota and the interaction of Langerhans cells with HIV-1. The revised manuscript contains new experimental data as well as clarification that address my previous concerns and further improved this very interesting manuscript. I briefly comment on their replies below.

1) The authors clarify that they had to use different MOIs for different virus variants and convincingly explain the rationale for this in their rebuttal letter. In the revised manuscript, they now mention the MOIs used in the figure legend and materials and methods. In addition, it would be helpful for the reader to include the explanation from the rebuttal letter in the manuscript text.

2) Ok as corrected

3) Ok as corrected

4) The new data and explanation provide first mechanistic insight as requested. The authors may want to consider adding a schematic model on their current view of how P. timonensis affects HIV fusion/uptake efficiencies/pathways in LCs.
Dear Theo

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

Congratulations on a nice study!

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Your manuscript will be processed for publication in the journal by EMBO Press. Manuscripts in the PDF and electronic editions of The EMBO Journal will be copy edited, and you will be provided with page proofs prior to publication. Please note that supplementary information is not included in the proofs.

You will be contacted by Wiley Author Services to complete licensing and payment information. The required ‘Page Charges Authorization Form’ is available here: https://www.embopress.org/pb-assets/embo-site/tej_apc.pdf - please download and complete the form and return to embopressproduction@wiley.com

Should you be planning a Press Release on your article, please get in contact with embojournal@wiley.com as early as possible, in order to coordinate publication and release dates.

If you have any questions, please do not hesitate to call or email the Editorial Office. Thank you for your contribution to The EMBO Journal.

** Click here to be directed to your login page: https://emboj.msubmit.net
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.4122/osf.io/gmp4). Please follow the journal’s guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

### Abridged guidelines for 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.
   - Ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
   - Plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
   - If relevant, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
   - Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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 entities that are being measured.
   - An explicit mention of the biological and chemical entities 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 was independently replicated in the laboratory.
   - Definitions of statistical methods and measures:
     - Common tests, such as t-tests (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon or Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section.
     - 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.

### Materials

| Newly Created Materials | Information included in the manuscript? | In which section is the information available? |
|-------------------------|-----------------------------------------|---------------------------------------------|
| New materials and reagents need to be available; do any restrictions apply? | Yes | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Antibodies | Information included in the manuscript? | In which section is the information available? |
|------------|-----------------------------------------|---------------------------------------------|
| For antibodies provide the following information: Commercial antibodies: RRID (if possible) or supplier name, catalogue number and/or clone number. Non-commercial RRID or other. | Yes | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| DNA and RNA sequences | Information included in the manuscript? | In which section is the information available? |
|-----------------------|-----------------------------------------|---------------------------------------------|
| Short novel DNA or RNA including primers, probes: provide the sequences. | Not Applicable | |

| Cell materials | Information included in the manuscript? | In which section is the information available? |
|----------------|-----------------------------------------|---------------------------------------------|
| Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, and/or RRID. | Yes | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| Primary cultures: Provide species, strain, sex of origin, parent(s) modification status. | Yes | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| Report if the cell lines were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination. | Not Applicable | |

| Experimental animals | Information included in the manuscript? | In which section is the information available? |
|----------------------|-----------------------------------------|---------------------------------------------|
| Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, and/or RRID. | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
| Animal observed in or captured from the field: Provide species, sex, and age where possible. | Not Applicable | |
| Please detail housing and husbandry conditions. | Not Applicable | |

| Plants and microorganisms | Information included in the manuscript? | In which section is the information available? |
|---------------------------|-----------------------------------------|---------------------------------------------|
| Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). | Not Applicable | |
| Microbes: provide species and strain, unique accession number if available, and source. | Yes | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Human research participants | Information included in the manuscript? | In which section is the information available? |
|-----------------------------|-----------------------------------------|---------------------------------------------|
| If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants. | Not Applicable | (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |

| Core facilities | Information included in the manuscript? | In which section is the information available? |
|----------------|-----------------------------------------|---------------------------------------------|
| If your work benefited from core facilities, was their service mentioned in the acknowledgments section? | Yes | |

Please complete ALL of the questions below. Select “Not Applicable” only when the requested information is not relevant for your study.
| Study protocol | Information included in the manuscript? | In which section is the information available? |
|----------------|----------------------------------------|-----------------------------------------------|
| If study protocol has been pre-registered, provide DOI in the manuscript. For clinical trials, provide the trial registration number OR the DOI. | Not Applicable | (Protocol and Trust Tab: Materials and Methods, Figures, Data Availability Section) |
| Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable. | Not Applicable | (Protocol and Trust Tab: Materials and Methods, Figures, Data Availability Section) |

| Laboratory protocol | Information included in the manuscript? | In which section is the information available? |
|---------------------|----------------------------------------|-----------------------------------------------|
| Provide DOI or other data details if external detailed step-by-step protocols are available. | Not Applicable | (Protocol and Trust Tab: Materials and Methods, Figures, Data Availability Section) |

| Experimental study design and statistics | Information included in the manuscript? | In which section is the information available? |
|-----------------------------------------|----------------------------------------|-----------------------------------------------|
| Include a statement about sample size estimate even if no statistical methods were used. | Yes | Figure legends |
| Have any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g., randomization procedure)? If yes, have they been described? | Not Applicable | |
| Include a statement about blinding even if no blinding was done. | Not Applicable | |
| Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established? | Not Applicable | |
| If sample or data points were omitted from analysis, report if this was due to addition or intentional exclusion and provide justification. | Not Applicable | |
| For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared? | Yes | Methods, Figure legends |

| Sample definition and in-laboratory replication | Information included in the manuscript? | In which section is the information available? |
|-----------------------------------------------|----------------------------------------|-----------------------------------------------|
| In the figure legends: state number of times the experiment was replicated in laboratory. | Yes | Figure legends |
| In the figure legends: define whether data describes technical or biological replicates. | Yes | Figure legends |

| Ethics | Information included in the manuscript? | In which section is the information available? |
|--------|----------------------------------------|-----------------------------------------------|
| Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s)), provide reference number for approval. | Not Applicable | |
| Studies involving human participants: 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. | Not Applicable | |
| Studies involving human participants: For publication of patient photos, include a statement confirming that consent to publish was obtained. | Not Applicable | |
| Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s)), provide reference number for approval. Include a statement of compliance with ethical regulations. | Not Applicable | |
| Studies involving specimen and field samples: State if consent permits obtained, provide details of authority approving study; if none were required, explain why. | Yes | W15_069 # 15.0103, W13_046 # 13.17.2000 |

| Dual Use Research of Concern (DURC) | Information included in the manuscript? | In which section is the information available? |
|--------------------------------------|----------------------------------------|-----------------------------------------------|
| Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC https://www.selectagents.gov/index.html, Department of Health and Human Services Bioselect Information System). | Not Applicable | |
| If you used a select agent, is the security level of the lab appropriate and reported in the manuscript? | Not Applicable | |
| If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for regulatory approval provided in the manuscript? | Not Applicable | |

| Reporting | Information included in the manuscript? | In which section is the information available? |
|-----------|----------------------------------------|-----------------------------------------------|
| Adherence to community standards | Not Applicable | |
| For studies that are phase I and II randomized controlled trials, please refer to the CONSORT flow diagram (see link at top right) and submit the CONSORT checklist (see link at top right) with your submission. See author guidelines, under Reporting Guidelines. Please confirm you have submitted this list. | Not Applicable | |

| Data Availability | Information included in the manuscript? | In which section is the information available? |
|------------------|----------------------------------------|-----------------------------------------------|
| Have primary datasets been deposited according to the journal’s guidelines (see “Data Deposition” section) and the respective accession numbers provided in the Data Availability Section? | Not Applicable | |
| Have human clinical and genomic datasets deposited in a public-access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement? | Not Applicable | |
| Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided? | Not Applicable | |
| If publicly available data were reused, provide the respective data citations in the reference list. | Not Applicable | |