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MARCO+ lymphatic endothelial cells sequester arthritogenic alphaviruses to limit viremia and viral dissemination

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1st Editorial Decision 2nd Aug 2021

Dear Tem,

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

As you can see, the referees find the analysis interesting but also find that we need some data to support the key conclusions and that LECs in lymph nodes limits viral spread. Should you be able to address the raised concerns then I would like to invite you to submit a revised version.

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

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.

I have attached a document with helpful tips on how to prepare the revised version. Please pay attention to the parts on the Data Availability Section and figure legends.

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

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

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Referee #2:

In this study Carpentier et al. have identified a new role for MARCO + lymphatic endothelial cells

(LECs) in lymph nodes (LNs) in limiting viral spread of CHIKV from tissues into the bloodstream and to distant body sites. MARCO has emerged as a novel marker of medullary LN LECs, but its function role in LECs has not been studied to date. At the same time, there are only a few studies that have thus far implicated LECs in direct host-pathogen interactions. The new findings of this study, demonstrating an active role of LN LECs in preventing viral dissemination, therefore are very timely and conceptually of great interest.

For their work, the authors have primarily performed in vivo infection experiments in MARCO-deficient mice, mice lacking LN (LTa-/-) or specific macrophages (Clec4F-DTR, CD169-DTR), combined with clodronate depletion experiment. Additional support for the involvement of MARCO+ LN LECs came from experiments performed with fluorescent CHIKV (combined with confocal microscopy of dLNs) as well as single-cell RNA-sequencing to demonstrate the presence of viral RNA in LN LEC subsets.

The experiments arriving at the conclusion that MARCO+ LN LECs are responsible for viral sequestration in draining LNs, seem generally well performed and in most cases sufficiently controlled. However, in its present form, the study is still lacking sufficient functional insights into how LECs mediate this protection ("sequestration"). Particularly, further evidence for the uptake of virus into LECs and the fate of infected LECs would be needed.

Major

"LN macrophages are not required for CHIKV accumulation the dLN or for limiting viral dissemination": In the depletion experiment in Fig. 5a, DT-mediated depletion in macrophages is only confirmed by one IFC image, showing the disappearance of all CD169+ cells (Fig. 5A). To back this finding, it would be important to show a FACS-based quantification of MARCO+ macrophage depletion in CD169-DTR mice.

Fig. 6: Uptake of CHIKV by LECS: Although the authors to do not formally claim in the experiments performed with CHIKC-mCherry that viral uptake into occurs, this is implicitly assumed by the subsequent experiments detecting CHIKV RNA in LECs (Fig. 7). Considering that the main finding of this study is that MARCO+ LECs "sequester" virus in LNs, it would be relevant to generate further evidence and mechanistic evidence for the suggested viral uptake. Fig. 6D: high magnification images of mCHerry, LYVE-1 and MARCO: please provide orthogonal views to address uptake of mCherry by LECs. In addition, please perform FACS experiments to quantify uptake efficiency of LN LECs (MARCO+ and MARCO-) as well as of Macrophages (MARCO+ / MARCO-).

On the same theme: to mechanistically strengthen the findings of MARCO-dependent interactions between LEC and CHIKV, the authors should perform in vitro assays with cultured LECs transduced (or not) with MARCO, to evaluate viral binding / uptake and possibly LEC survival. Performing such experiments with human endothelial cells would also help to address the translational relevance of these findings (i.e. whether mouse-specific or possibly also relevant for the human situation).

Minor
1) Introduction: Experiments in this study are mainly performed with CHIKV (some cases also with

ONNV and RRV, two other forms of arthritogenic alphaviruses). By contrast, most conclusions in the manuscript result sections refer broadly to alphaviruses, and the manuscript title even mentions arboviruses in general. The authors should either restrict their claims to the viruses analyzed (i.e. adapt the title and conclusions) or test MARCO binding more broadly for more alphaviruses / arboviruses.

2) The first paragraph of the introduction, although informative, doesn't add essential information for the aim of the paper. At the same time, the concept of LECS (definition, location) is not mentioned until the end of the introduction, and an introduction to the emerging role of LEC-pathogen interactions in host defense is missing.

3) Fig. Legends: It is typically not clear what is meant with e.g. "N=7-12, 3 experiments": pooled data from 3 experiments (total 7-12) or one representative out of 3 similar?

4) Data representation: please follow the same pattern of depicting your data in different Figures / Figure panels (symbol or color used of dots)

5) Why did the authors use 4 week-old mice (with a more immature immune system)? Have they tested that similar findings can be made in older mice?

6) Fig 2A: Why pool experiments in which mice are injected differently (i.v, i.p)?

7) Fig 2B: Abbreviations (e.g. PLL) should be better explained in the text and figure legend

8) Fig 2D: There is no legend/tags to explain the difference of the upper and the lower row of histology pictures, making it difficult to follow.

9) Fig 3: Could be moved to the supplementary

10) Fig.4: Statistical significance is confusing. Define better the bars on graphs to declare statistical significance or not

11) Size of the scale bars in images of 5A and Fig. 6 are not mentioned in the image/legend.

12) Fig. 7: The authors show that in addition to MARCO+ LECs also a population of "undefined LECs" harbor high levels of CHIKV RNA (fig. 7G). Do these cells express the previously described receptor for entry, Mxra8? - It would be nice to compare the results of the FACS-based viral uptake (suggested as major point 2) with the sequencing results.

Referee #3:

Summary

In this paper, the authors provide novel insights into the role of MARCO in modulating the systemic

dissemination of CHIKV from the inoculation site to the blood. This is an important human pathogen, for which there is little published work defining roles for host immune responses that limit development and establishment of viremia. In this study they provide convincing and interesting novel data defining roles of key cell types in the draining lymph node. However, the data defining roles for liver phagocytes is more additive and represents only an incremental increase in our understanding by which blood virus is cleared.

Major concerns

This is a well-designed study that uses appropriate experimental tools and models to define a role for MARCO in limiting dissemination of virus in the draining lymph node and maintenance of viremia. Although there was no single major issue, the incremental/additive data that continues from their previously published work defining a role for liver phagocytes in CHIKV clearance from blood seems unnecessary.

Minor concerns and detailed comments

Figure 1 demonstrates how the absence of MARCO substantially increases host susceptibility to CHIKV and other similar alphaviruses. This is shown to be due to changes in systemic susceptibility, as initial replication of virus at inoculation site is mostly similar in both WT and KO mice. Virus is shown to spread more robustly in KO mice to distal tissues and thereby induce disease in a bilateral fashion.
Figure 1A,B. It is not clear why WT mice injected with WT virus demonstrate no change in weight and only a minor increase in disease score. Possible reasons for this should be clarified in the text e.g. Justification of CHIKV strain used would help here.
Figure 1D. The rate of clearance of CHIKV from circulation appears to be similar in WT and KO mice. The total length of time for which mice are viremic was increased in KO mice - however this most likely reflects the order of magnitude higher virus titre at 24 hours. i.e. one follows the other. The reference to this result in the main text (lines 129-133) should be clarified to avoid confusion.
Figure EV1B- It is not clear why virus levels are significantly increased in the proximal ipsilateral tissue in KO mice at day 7 (which is key timepoint, as this is when disease score peaks). This suggests an inherent advantage to virus replication at the local site of inoculation. The authors should explain why these data do not fit their overall narrative and change their conclusions accordingly.

Figure 2 adds to previously published work defining a role for liver-resident phagocytes in clearance of CHIKV from the blood, using genetic diphtheria toxin induced models that more specifically depletes CD169+ cells and also KC, leaving over cell types untouched. It is not clear in the text why this approach was used and what substantive information this adds to the existing published work. While the data support a role for KC in CHIKV, they also suggest an important role for other non-KC CD169 positive cells, which is not clear in their summarising statement for this section.

Figure 3. The use of i.v. CLL may also disrupt e.g. subcapsular populations of macrophages in the LN, which are known to modulate lymph borne virus dissemination from cutaneous sites (although LN macrophages are shown to be less important in later figures, they likely still have a role). The authors should ideally demonstrate or reference appropriate work that show lymph node CD169 macrophages are not disrupted by i.v. CLL treatment, or discuss appropriately.

Figure 4A - defines the relative contribution of lymph node and liver resident phagocytic cells, showing that LN phagocytes are not responsible for clearing the majority of circulating virus.

However roles for eg spleen cells are not examined.
Figure 4B. The introductory statement for justifying experiments in Figure 4B (lines 218-220) is confusing and should be clarified. The decrease in LN CHIKV RNA is fairly small compared to the large increase in serum virus. This discrepancy is not well explained and the relative role of LN MARCO+ve cells in preventing CHIV dissemination (with these data suggesting a minor role) should be clarified accordingly.

Figure 5 -7. contains the most interesting data and defines a novel role for MARCO+ LECs in modulating CHIKV dissemination. Figure 6 also suggests that CD169+ve macrophage have a minor or no role in sequestering lymph-borne virus, in which they show that the CD11b+ve macrophages are mostly negative for CHIKV. The discrepancies with other published work for other lymph borne viruses (eg VSV, Junt et al 2007) should be more carefully discussed and done so in more detail. Overall, the studies reported here are well done.
Figure 7 - nicely defines the LEC populations that have CHIKV RNA, using appropriate cell lineage markers to define the cell types involved.

Discussion summaries the work well.

RESPONSE TO REVIEWER COMMENTS

Note, all line numbers indicated in our responses below refer to the "MARKED" word file provided in the revised submission materials.

REVIEWER 2

In this study Carpentier et al. have identified a new role for MARCO + lymphatic endothelial cells (LECs) in lymph nodes (LNs) in limiting viral spread of CHIKV from tissues into the bloodstream and to distant body sites. MARCO has emerged as a novel marker of medullary LN LECs, but its function role in LECs has not been studied to date. At the same time, there are only a few studies that have thus far implicated LECs in direct host-pathogen interactions. The new findings of this study, demonstrating an active role of LN LECs in preventing viral dissemination, therefore are very timely and conceptually of great interest.

For their work, the authors have primarily performed in vivo infection experiments in MARCO-deficient mice, mice lacking LN (LTa-/-) or specific macrophages (Clec4F-DTR, CD169-DTR), combined with clodronate depletion experiment. Additional support for the involvement of MARCO+ LN LECs came from experiments performed with fluorescent CHIKV (combined with confocal microscopy of dLNs) as well as single-cell RNA-sequencing to demonstrate the presence of viral RNA in LN LEC subsets.

The experiments arriving at the conclusion that MARCO+ LN LECs are responsible for viral sequestration in draining LNs, seem generally well performed and in most cases sufficiently controlled. However, in its present form, the study is still lacking sufficient functional insights into how LECs mediate this protection ("sequestration"). Particularly, further evidence for the uptake of virus into LECs and the fate of infected LECs would be needed.

MAJOR

1. "LN macrophages are not required for CHIKV accumulation the dLN or for limiting viral dissemination": In the depletion experiment in Fig. 5a, DT-mediated depletion in macrophages is only confirmed by one IFC image, showing the disappearance of all CD169+ cells (Fig. 5A). To back this finding, it would be important to show a FACS-based quantification of MARCO+ macrophage depletion in CD169-DTR mice.

*The image provided in Fig 5A is representative of three biological replicates. This has been clarified in the figure legend (**see lines 1139-1140**). Moreover, CD169-DTR mice have been extensively characterized by numerous research laboratories. Collectively, the data shows that the administration of DT (at the dose and route used in this study) efficiently depletes CD169$^+$ macrophages in the sinuses of lymph nodes (1-3), which includes the medullary sinus macrophages that express MARCO.*

*To further address this reviewer concern, we now provide FACS-based quantification of lymph node macrophage depletion in CD169-DTR mice (**see new Appendix Figure S1**). As medullary sinus macrophages express MARCO, and to avoid the reviewer's concerns stated above regarding CD169, we focused these analyses on F4/80$^+$CD11b$^+$CD11c$^-$ cells (note, medullary sinus macrophages are F4/80$^+$ whereas subcapsular sinus macrophages are F4/80$^-$; both populations have low to undetectable levels of CD11c)(4). These analyses show a nearly 10-fold reduction in F4/80$^+$CD11b$^+$CD11c$^-$ cells in CD169-DTR mice treated with DT. Given that lymph nodes also contain populations of F4/80$^+$CD169$^-$ macrophages (4), these data further support that the MARCO$^+$CD169$^+$ medullary sinus macrophages are efficiently depleted from the draining lymph node of DT-treated CD169-DTR mice.*

2. Fig. 6: Uptake of CHIKV by LECS: Although the authors to do not formally claim in the experiments performed with CHIKC-mCherry that viral uptake into occurs, this is implicitly assumed by the subsequent experiments detecting CHIKV RNA in LECs (Fig. 7). Considering that the main finding of this study is that MARCO+ LECs "sequester" virus in LNs, it would be relevant to generate further evidence and mechanistic evidence for the suggested viral uptake. Fig. 6D: high magnification images of mCHerry, LYVE-1 and MARCO: please provide orthogonal views to address uptake of mCherry by LECs. In addition, please perform FACS

experiments to quantify uptake efficiency of LN LECs (MARCO+ and MARCO-) as well as of Macrophages (MARCO+ / MARCO).

*We appreciate this reviewer concern. To address this, we have performed new staining and analysis of LN sections. As shown in new **Movie EV1**, internalized CHIKV-associated antigen (mCherry) can be detected in Lyve1^{+}MARCO^{+} cells. These data further support the idea that MARCO^{+} LECs uptake CHIKV particles.*

Regarding FACS-based analysis, in our experience FACS-based approaches cannot be used to address this question. At a maximum, stromal cells represent ~1-2% of total lymph node cells (5). Lymphatic endothelial cells (LECs) are only a fraction of all lymph node stromal cells (~15%)(5), and LECs can be divided into numerous subsets (Fig 7 and (6)). Thus, identifying virus associated with such rare cell populations by flow cytometry is extremely challenging.

Finally, it should be noted that in addition to microscopy, we performed scRNAseq analysis to address this question. These data demonstrate that specific subpopulations of LECs harbor CHIKV RNA (Fig 7), further suggesting virus uptake by these cells.

3. On the same theme: to mechanistically strengthen the findings of MARCO-dependent interactions between LEC and CHIKV, the authors should perform in vitro assays with cultured LECs transduced (or not) with MARCO, to evaluate viral binding / uptake and possibly LEC survival. Performing such experiments with human endothelial cells would also help to address the translational relevance of these findings (i.e. whether mouse-specific or possibly also relevant for the human situation).

We agree with the reviewer that these data would be of interest. These experiments require careful selection and evaluation of endothelial cell type (endothelial cells derived from different tissue sources are highly diverse), cloning of murine MARCO (e.g., C57BL/6 allele) and distinct alleles of human MARCO (human MARCO is polymorphic), confirmation of stable cell surface MARCO expression, confirmation of appropriate MARCO function by binding and internalization assays using established MARCO ligands, and the development of appropriate positive (e.g., cells ectopically expressing Mxra8) and negative controls for viral internalization. Given the abundant new information reported in the current study, we consider these additional studies to be beyond the scope of this report.

MINOR

1. Introduction: Experiments in this study are mainly performed with CHIKV (some cases also with ONNV and RRV, two other forms of arthritogenic alphaviruses). By contrast, most conclusions in the manuscript result sections refer broadly to alphaviruses, and the manuscript title even mentions arboviruses in general. The authors should either restrict their claims to the viruses analyzed (i.e. adapt the title and conclusions) or test MARCO binding more broadly for more alphaviruses / arboviruses.

We have modified the manuscript title and text as recommended.

2. The first paragraph of the introduction, although informative, doesn't add essential information for the aim of the paper. At the same time, the concept of LECS (definition, location) is not mentioned until the end of the introduction, and an introduction to the emerging role of LEC-pathogen interactions in host defense is missing.

The first paragraph of the Introduction provides context for readers that may not be as familiar with the public health concerns of arboviruses. Thus, we think it is important to include.

*As suggested, we have modified the Introduction section to include additional information about LECs and pathogen interactions (**see lines 113-116**).*

3. Fig. Legends: It is typically not clear what is meant with e.g. "N=7-12, 3 experiments": pooled data from 3 experiments (total 7-12) or one representative out of 3 similar?

We have modified each of the figure legends and Extended View figure legends for clarity.

4) Data representation: please follow the same pattern of depicting your data in different Figures / Figure panels (symbol or color used of dots)

With the exception of MARCO$^{-/-}$ mice and LTα$^{-/-}$ mice, the display of the data is consistent across figures. We have modified the color of the symbols in Fig 4A for LTα$^{-/-}$ mice to avoid this overlap.

5) Why did the authors use 4-week-old mice (with a more immature immune system)? Have they tested that similar findings can be made in older mice?

For many viruses, including alphaviruses, mice greater than 4-5 weeks in age develop less severe infection outcomes. Thus, we and many other groups use 4-week old mice for CHIKV pathogenesis studies (7-9).

6) Fig 2A: Why pool experiments in which mice are injected differently (i.v, i.p)?

Because the magnitude of the effect on viral clearance from the circulation in DT-treated Clec4f-DTR mice was less than observed in CLL-treated mice, we tested whether the inoculation route of DT would impact the results in Clec4f-DTR mice. As shown in Fig 2A, whether DT was inoculated i.v. or i.p. did not alter viral clearance.

7) Fig 2B: Abbreviations (e.g. PLL) should be better explained in the text and figure legend

*We have added clarification to the text (**see lines 175-176**) and the figure legends (**see lines 1061 and 1098**).*

8) Fig 2D: There is no legend/tags to explain the difference of the upper and the lower row of histology pictures, making it difficult to follow.

*For this figure, in order to convey to the readers the range of staining observed, we provided two representative images for each group. The legend has been modified for clarity (**see line 1085**).*

9) Fig 3: Could be moved to the supplementary

In our view, these are critical data that demonstrate the key differences observed in MARCO$^{-/-}$ mice and mice depleted of liver Kupffer cells. Thus, we have chosen to keep these data in the main figures.

10) Fig.4: Statistical significance is confusing. Define better the bars on graphs to declare statistical significance or not

We have modified the display of the statistical analysis in Fig 4.

11) Size of the scale bars in images of 5A and Fig. 6 are not mentioned in the image/legend.

We have added this information to the figure legends for Fig 5A and Fig 6.

12) Fig. 7: The authors show that in addition to MARCO+ LECs also a population of "undefined LECs" harbor high levels of CHIKV RNA (fig. 7G). Do these cells express the previously described receptor for entry, Mxra8? - It would be nice to compare the results of the FACS-based viral uptake (suggested as major point 2) with the sequencing results.

*We have performed a new analysis of the sequencing data (**see Appendix Figure S4**). This analysis revealed that the undefined LECs do not show expression of Mxra8. However, as originally discussed (**see lines 440-441**), these cells display reduced mouse gene expression levels.*

REVIEWER 3

In this paper, the authors provide novel insights into the role of MARCO in modulating the systemic dissemination of CHIKV from the inoculation site to the blood. This is an important human pathogen, for which

there is little published work defining roles for host immune responses that limit development and establishment of viremia. In this study they provide convincing and interesting novel data defining roles of key cell types in the draining lymph node. However, the data defining roles for liver phagocytes is more additive and represents only an incremental increase in our understanding by which blood virus is cleared.

MAJOR

This is a well-designed study that uses appropriate experimental tools and models to define a role for MARCO in limiting dissemination of virus in the draining lymph node and maintenance of viremia. Although there was no single major issue, the incremental/additive data that continues from their previously published work defining a role for liver phagocytes in CHIKV clearance from blood seems unnecessary.

We appreciate the positive comments from the reviewer. However, the new data using more specific depletion strategies demonstrates a key role for KCs in clearance of virus particles from the circulation and also a foundation for the identification of a second MARCO expression cell type (i.e., MARCO^{+} LECs) in the control of arthritogenic alphavirus dissemination.

Minor concerns and detailed comments

1. Figure 1A,B. It is not clear why WT mice injected with WT virus demonstrate no change in weight and only a minor increase in disease score. Possible reasons for this should be clarified in the text e.g. Justification of CHIKV strain used would help here.

We appreciate this reviewer question. Numerous studies from our group and other laboratories have demonstrated that infection of 4 week-old WT C57BL/6 mice with a variety of WT CHIKV strains does not result in altered weight gain or disease signs beyond swelling of the inoculated foot (9-11). Altered weight gain and other more severe disease signs can occur following WT CHIKV infection in younger mice or immunodeficient mice (12, 13). We have provided new references in the methods section to support the use of 4-week-old mice.

3. Figure 1D. The rate of clearance of CHIKV from circulation appears to be similar in WT and KO mice. The total length of time for which mice are viremic was increased in KO mice - however this most likely reflects the order of magnitude higher virus titre at 24 hours. i.e. one follows the other. The reference to this result in the main text (lines 129-133) should be clarified to avoid confusion.

*In the text, we state that the magnitude and duration of viremia are increased in MARCO$^{-/-}$ mice (**see lines 142-143**). As indicated by the reviewer, this statement is consistent with the data displayed in Fig 1D.*

4. Figure EV1B- It is not clear why virus levels are significantly increased in the proximal ipsilateral tissue in KO mice at day 7 (which is key timepoint, as this is when disease score peaks). This suggests an inherent advantage to virus replication at the local site of inoculation. The authors should explain why these data do not fit their overall narrative and change their conclusions accordingly.

We agree with the reviewer that this is an interesting observation. At this time, we cannot provide a molecular mechanism for this observation. Given that this effect is not observed until day 7 post-infection in MARCO$^{-/-}$ mice, it is possible that MARCO may regulate other aspects of the antiviral immune response. However, these data do not alter the conclusions that MARCO limits early viral dissemination and viremia.

5. Figure 2 adds to previously published work defining a role for liver-resident phagocytes in clearance of CHIKV from the blood, using genetic diphtheria toxin induced models that more specifically depletes CD169+ cells and also KC, leaving over cell types untouched. It is not clear in the text why this approach was used and what substantive information this adds to the existing published work. While the data support a role for KC in CHIKV, they also suggest an important role for other non-KC CD169 positive cells, which is not clear in their summarising statement for this section.

The use of CD169-DTR mice and Clec4f-DTR mice strengthens the conclusion that Kupffer cells are required for the clearance of CHIKV particles from the circulation.

6. Figure 3. The use of i.v. CLL may also disrupt e.g. subcapsular populations of macrophages in the LN, which are known to modulate lymph borne virus dissemination from cutaneous sites (although LN macrophages are shown to be less important in later figures, they likely still have a role). The authors should ideally demonstrate or reference appropriate work that show lymph node CD169 macrophages are not disrupted by i.v. CLL treatment, or discuss appropriately.

We appreciate this reviewer concern. To our knowledge, liposomes that are administered i.v. are rapidly internalized by blood-exposed phagocytes (14, 15). Thus, i.v. administered CLL does not deplete cells in the parenchyma of tissues or somehow traffic into the popliteal lymph node. For these reasons, injection of CLL in the footpad or calf is routinely used to deplete macrophages in the draining popliteal lymph node (16, 17). Moreover, as shown in Fig 3, i.v. administration of CLL had no impact on viral dissemination. Thus, if the LN macrophages were depleted (again, not known to occur), the data would suggest a limited role for these cells in the control of CHIKV dissemination.

7. Figure 4A - defines the relative contribution of lymph node and liver resident phagocytic cells, showing that LN phagocytes are not responsible for clearing the majority of circulating virus. However roles for eg spleen cells are not examined.

In prior studies, we found that viral particles were cleared efficiently from the circulation of mice in which the spleen had been surgically removed (18). In addition, we also found that virus accumulated in the liver and that this accumulation was diminished following clodronate-mediated depletion of blood-exposed phagocytes – which was not observed in the spleen (18).

8. Figure 4B. The introductory statement for justifying experiments in Figure 4B (lines 218-220) is confusing and should be clarified. The decrease in LN CHIKV RNA is fairly small compared to the large increase in serum virus. This discrepancy is not well explained and the relative role of LN MARCO+ve cells in preventing CHIV dissemination (with these data suggesting a minor role) should be clarified accordingly.

The findings presented in Fig 4A suggested that MARCO-expressing cells in the draining lymph node limit CHIKV dissemination. Because of this, as outlined in the manuscript, we designed experiments to specifically test the role of MARCO in accumulation of the virus in the LN and spread to the blood circulation. Finally, decrease in CHIKV RNA in the LN of MARCO-/- mice is nearly 10-fold, indicating that far less virus (i.e., ~10%) accumulates in the LN and the overwhelming majority (i.e., ~90%) spreads to the blood circulation.

9. Figure 5 -7. contains the most interesting data and defines a novel role for MARCO+ LECs in modulating CHIKV dissemination. Figure 6 also suggests that CD169+ve macrophage have a minor or no role in sequestering lymph-borne virus, in which they show that the CD11b+ve macrophages are mostly negative for CHIKV. The discrepancies with other published work for other lymph borne viruses (eg VSV, Junt et al 2007) should be more carefully discussed and done so in more detail. Overall, the studies reported here are well done.

*We appreciate this reviewer comment. We do address these issues at multiple points in the Discussion section including **lines 389-405** and **lines 420-429**. To further emphasize differences with other published studies, we modified the Discussion section (**lines 406-409**) and the Abstract (**lines 35-36**) to highlight the novelty of the findings.*

LITERATURE CITED

1. Asano K, Nabeyama A, Miyake Y, Qiu CH, Kurita A, Tomura M, Kanagawa O, Fujii S, Tanaka M. 2011. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. Immunity 34:85-95.

2. Tacconi C, Commerford CD, Dieterich LC, Schwager S, He Y, Ikenberg K, Friebel E, Becher B, Tugues S, Detmar M. 2021. CD169(+) lymph node macrophages have protective functions in mouse breast cancer metastasis. Cell Rep 35:108993.
3. Gray EE, Friend S, Suzuki K, Phan TG, Cyster JG. 2012. Subcapsular sinus macrophage fragmentation and CD169+ bleb acquisition by closely associated IL-17-committed innate-like lymphocytes. PLoS One 7:e38258.
4. Phan TG, Green JA, Gray EE, Xu Y, Cyster JG. 2009. Immune complex relay by subcapsular sinus macrophages and noncognate B cells drives antibody affinity maturation. Nat Immunol 10:786-93.
5. Tamburini BA, Burchill MA, Kedl RM. 2014. Antigen capture and archiving by lymphatic endothelial cells following vaccination or viral infection. Nat Commun 5:3989.
6. Jalkanen S, Salmi M. 2020. Lymphatic endothelial cells of the lymph node. Nat Rev Immunol 20:566-578.
7. Kim AS, Zimmerman O, Fox JM, Nelson CA, Basore K, Zhang R, Durnell L, Desai C, Bullock C, Deem SL, Oppenheimer J, Shapiro B, Wang T, Cherry S, Coyne CB, Handley SA, Landis MJ, Fremont DH, Diamond MS. 2020. An Evolutionary Insertion in the Mxra8 Receptor-Binding Site Confers Resistance to Alphavirus Infection and Pathogenesis. Cell Host Microbe 27:428-440 e9.
8. Jones JE, Long KM, Whitmore AC, Sanders W, Thurlow LR, Brown JA, Morrison CR, Vincent H, Peck KM, Browning C, Moorman N, Lim JK, Heise MT. 2017. Disruption of the Opal Stop Codon Attenuates Chikungunya Virus-Induced Arthritis and Pathology. mBio 8.
9. Chen W, Foo SS, Zaid A, Teng TS, Herrero LJ, Wolf S, Tharmarajah K, Vu LD, van Vreden C, Taylor A, Freitas JR, Li RW, Woodruff TM, Gordon R, Ojcius DM, Nakaya HI, Kanneganti TD, O'Neill LAJ, Robertson AAB, King NJ, Suhrbier A, Cooper MA, Ng LFP, Mahalingam S. 2017. Specific inhibition of NLRP3 in chikungunya disease reveals a role for inflammasomes in alphavirus-induced inflammation. Nat Microbiol 2:1435-1445.
10. Hawman DW, Carpentier KS, Fox JM, May NA, Sanders W, Montgomery SA, Moorman NJ, Diamond MS, Morrison TE. 2017. Mutations in the E2 Glycoprotein and the 3' Untranslated Region Enhance Chikungunya Virus Virulence in Mice. J Virol 91.
11. Miner JJ, Cook LE, Hong JP, Smith AM, Richner JM, Shimak RM, Young AR, Monte K, Poddar S, Crowe JE, Jr., Lenschow DJ, Diamond MS. 2017. Therapy with CTLA4-Ig and an antiviral monoclonal antibody controls chikungunya virus arthritis. Sci Transl Med 9.
12. Morrison TE, Oko L, Montgomery SA, Whitmore AC, Lotstein AR, Gunn BM, Elmore SA, Heise MT. 2011. A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. Am J Pathol 178:32-40.
13. Couderc T, Chretien F, Schilte C, Disson O, Brigitte M, Guivel-Benhassine F, Touret Y, Barau G, Cayet N, Schuffenecker I, Despres P, Arenzana-Seisdedos F, Michault A, Albert ML, Lecuit M. 2008. A mouse model for Chikungunya: young age and inefficient type-I interferon signaling are risk factors for severe disease. PLoS Pathog 4:e29.
14. van Rooijen N, Kors N, Kraal G. 1989. Macrophage subset repopulation in the spleen: differential kinetics after liposome-mediated elimination. J Leukoc Biol 45:97-104.
15. Van Rooijen N, Sanders A. 1994. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. J Immunol Methods 174:83-93.
16. Delemarre FG, Kors N, Kraal G, van Rooijen N. 1990. Repopulation of macrophages in popliteal lymph nodes of mice after liposome-mediated depletion. J Leukoc Biol 47:251-7.
17. Kastenmuller W, Torabi-Parizi P, Subramanian N, Lammermann T, Germain RN. 2012. A spatially-organized multicellular innate immune response in lymph nodes limits systemic pathogen spread. Cell 150:1235-48.
18. Carpentier KS, Davenport BJ, Haist KC, McCarthy MK, May NA, Robison A, Ruckert C, Ebel GD, Morrison TE. 2019. Discrete viral E2 lysine residues and scavenger receptor MARCO are required for clearance of circulating alphaviruses. Elife 8.

1st Revision - Editorial Decision 7th Sep 2021

Dear Tem,

Thank you for submitting your revised manuscript. Your revision has now been re-reviewed by the two referees and their comments are provided below.

As you can see from their comments the referees appreciate the introduced changes and support publication here. Referee #2 has a few minor remaining concerns that should be easy enough to sort out in a final revision.

When you submit the revised version will you also address the following points:

"Declaration of Interests" should be re-labeled as Conflict of Interest.

Figs. EV2, EV4 & Appendix S1 panel callouts are missing.

There is a callout to Fig. S8, but no such figure.

The figure legends for the appendix figures should be removed from the main manuscript file.

The movie should be ZIPed with its legend. The legend should be removed from the manuscript file.

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.

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 upload the final version.

Congratulations on a nice study!

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 6th Dec 2021.

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

Referee #2:

The authors have performed several experiments and successfully revised the manuscript to address most of my comments.

In specific, they now provide a FACS-based quantification of LN macrophages, which demonstrates the effective depletion of CD169+ macrophages in CD169-DTR mice (response to major comment 1).

With regards to the uptake of CHIKV by LN LECs (response to major comment 2), I accept that a FACS-based analysis of uptake of fluorescent virus into LN LECs might be technically challenging, even though this has been performed and reported by several studies for s.c. injected fluorescent ovalbumin in the past (e.g. Tamburini et al., Nat. Comm. 2014; Vokali et al., Nat. Comm. 2020). Importantly, the authors now provide additional documentation of CHIKV uptake into LN LECs by showing a movie (EV1), in which internalized CHIKV (mCherry+) can be seen within Lyve1+MARCO+ cells in LN sections. Since a movie obviously is not quantitative, I would like to suggest that the authors either provide several movies (from several experiments) or at least write in the legends that this movie is representative for several experiments performed.

With regards to my third major comment (in vitro uptake studies with MARCO +/ - LECs) the authors have clearly explained the difficulty of such experiments and convinced me that these additional studies would go beyond the scope of their current study.

Also, my minor comments were largely addressed by the authors. As a general remark: it would have been helpful if the changes made to the manuscript had been indicated in some way in the text or if at least correct line numbers had been provided in the rebuttal.

The only reply I am not satisfied with is the one for minor comment 2: While I respect that the authors want to keep the text about public health concerns of arbovirus infections, I still strongly believe that a section / a few sentences on the emerging role of LEC-pathogen interactions (including references like e.g. PMID: 29343625; PMID: 24905362; PMID: 24905362; PMID: 15668734; doi: https://doi.org/10.1101/2021.08.26.457551 - or a dedicated review on this topic) is missing and would be relevant for the general readership of this study.

The authors wrote that this information has been added, but I cannot spot it, and the line numbers provided (lines 113-116) seem wrong.

Referee #3:

Concerns have been addressed in the authors rebuttal. In summary, defining a role for lymph node LECs in limiting viraemia is an important finding and should be published.

RESPONSE TO EDITOR and REVIEWER COMMENTS

EDITOR

1. "Declaration of Interests" should be re-labeled as Conflict of Interest.

This has been changed to Conflict of Interest.

2. Figs. EV2, EV4 & Appendix S1 panel callouts are missing.

Callouts to each of these panels are present in the manuscript file. A callout to Fig EV2 and Appendix Fig S1 can be found in the subsection entitled "LN Macrophages are not required for CHIKV accumulation in the dLN or for limiting viral dissemination." A callout to Fig EV4 can be found in the subsection entitled "MARCO$^+$ LECs harbor CHIKV RNA."

3. There is a callout to Fig. S8, but no such figure.

This has been removed.

4. The figure legends for the appendix figures should be removed from the main manuscript file.

The legends for the appendix figures have been removed from the main manuscript file.

5. The movie should be ZIPed with its legend. The legend should be removed from the manuscript file.

The legend for the movie has been removed from the main manuscript file. A ZIP file including the movie and the legend has been provided.

6. 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.

We have addressed all of the comments in the Data Edited Manuscript file.

7. 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.

Outlined below, we have provided a general summary statement and 3-5 bullet points that capture the key findings of the paper:

Lymphatic endothelial cells (LECs) in the draining lymph node and Kupffer cells (KCs) in liver sinusoids limit alphavirus dissemination to tissues and the development of viremia, which are essential for arbovirus pathogenicity and transmission, via the scavenger receptor MARCO.

1. *The draining lymph node (dLN) impedes viremia and viral dissemination via the scavenger receptor MARCO during arthritogenic alphavirus infections.*
2. *MARCO$^+$ lymphatic endothelial cells (LECs) in the dLN capture and internalize arthritogenic alphavirus particles to limit viral dissemination to the blood.*
3. *Kupffer cells in the liver capture circulating alphavirus particles that breach the lymph node barrier to access the bloodstream, providing a second line of MARCO-dependent defense.*
4. *MARCO$^{-/-}$ mice have an increased magnitude and duration of viremia, enhanced viral dissemination, and more severe disease outcomes.*

8. We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels**).**

A summary figure has been provided.

REVIEWER 2

Importantly, the authors now provide additional documentation of CHIKV uptake into LN LECs by showing a movie (EV1), in which internalized CHIKV (mCherry+) can be seen within Lyve1+MARCO+ cells in LN sections. Since a movie obviously is not quantitative, I would like to suggest that the authors either provide several movies (from several experiments) or at least write in the legends that this movie is representative for several experiments performed.

We have indicated in the figure legend that the movie is representative of two independent experiments.

The only reply I am not satisfied with is the one for minor comment 2: While I respect that the authors want to keep the text about public health concerns of arbovirus infections, I still strongly believe that a section / a few sentences on the emerging role of LEC-pathogen interactions (including references like e.g. PMID: 29343625; PMID: 24905362; PMID: 24905362; PMID: 15668734; doi: https://doi.org/10.1101/2021.08.26.457551 - or a dedicated review on this topic) is missing and would be relevant for the general readership of this study.

The current Introduction section of the manuscript includes references to several reviews that discuss pathogen interactions with endothelial cells, including a comprehensive 2020 Nature Reviews Immunology article (Jalkanen and Salmi). Among the references recommended by the reviewers, one study (24905362), which the reviewer listed twice, is already cited in our manuscript three times and the major findings (i.e., that lymph node LECs can capture and archive antigen) are discussed in the Introduction, Results, and Discussion sections of the manuscript. Among the new suggestions provided by the reviewer, one study (PMID: 29343625) describes how skin infection with Staphylococcus aureus alters lymphatic vessel contractility and lymph flow, likely due to bacteria exotoxin-mediated death of lymphatic muscle cells. Another study (15668734) describes factors that influence lymphangiogenesis during inflammation of airways. Finally, a non-peer reviewed preprint reports that skin infection with vaccinia virus results in the tightening of interendothelial junctions via VEGFR2 signaling, which seems to limit viral dissemination to the draining lymph node and improve priming of virus-specific CD8+ T cell responses. While we agree with the reviewer that these are all interesting findings, they address rather distinct questions. In our view, incorporating all of these ideas into the Introduction section of this manuscript would be not only be challenging, but also would be confusing for the reader.

2nd Revision - Editorial Decision 10th Sep 2021

Dear Tem,

Thank you for submitting your revised manuscript to The EMBO Journal. I have now had a chance to look at the introduced changes 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
The EMBO Journal

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Corresponding Author Name: Thomas E. Morrison
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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 $\chi 2$ 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.

USEFUL LINKS FOR COMPLETING THIS FORM

http://www.antibodypedia.com
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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?	Sample sizes were determined based on datasets from similar prior studies.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	No statistical methods were used to predetermine sample size.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No samples or animals were excluded from the analysis.
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.	Animals were assigned randomly to experimental groups.
For animal studies, include a statement about randomization even if no randomization was used.	Mice were distributed randomly into groups containing approximately even division of sexes.
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.	Investigators were not blinded.
4.b. For animal studies, include a statement about blinding even if no blinding was done	No blinding was done.
5. For every figure, are statistical tests justified as appropriate?	Data (in some cases, log transformed) was analyzed by parametric or non-parametric analyses with corrections for multiple comparisons.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The data meets the assumption of the tests. Histograms used to visualize distribution.
Is there an estimate of variation within each group of data?	Sum of squares used in ANOVA tests.

Is the variance similar between the groups that are being statistically compared?	When relevant, F tests were performed to compare variances.

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).	BioLegend Antibodies: anti-CD45 clone 30-RF11, anti-PDPN clone 8.1.1., anti-CD31 clone 390, anti-CD169 clone 3D6.112, anti-CD11c clone BV510, anti-CD11b clone M1.70, anti-B220 clone RA3-6B2, anti-TCR-beta clone H57-597, anti-F4/80 clone BM8, anti NK1.1 clone PL136. Novus Antibodies: anti-MARCO clone 2359A, anti-mCherry polyclonal (catalog # NBP2-25157). Thermofisher Antibodies: anti-B220 clone RA3-6B2, anti-Lyve-1 clone ALY7. BioRad Antibodies: anti-MARCO clone ED31.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Vero cells (ATCC CCL81) and BHK-21 cells (ATCC CCL10) were obtained directly from the American Type Culture Collection. Cells were tested for mycoplasma contamination during the course of these studies and found to be negative.

* 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.	WT C57BL/6 and congenic Lymphotoxin alpha-/- mice were obtained from the Jackson Laboratory. Congenic CD169-DTR mice were provided by Jason Cyster (University of California San Francisco) and congenic MARCO-/- mice were provided by Dawn Bowdish (McMaster University). Clec4F-DTR+ C57BL/6 mice were provided by Martin Guilliams (Ghent University). Mice were housed and bred at the University of Colorado School of Medicine under specific pathogen-free conditions and were distributed randomly intp groups containing approximately even division of sexes for experiments. WT C57BL/6 male mice were purchased commercially and were age matched and distributed randomly across groups.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All of the animals were handled according to approved institutional animal care and use committee (IACUC) protocols (#00026) of the University of Colorado School of Medicine (Assurance Number A3269-01).
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.	All animal studies are adequately reported according to the 10 essential ARRIVE guidelines.

E- Human Subjects

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

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	Raw and processed data for this study have been deposited at NCBI GEO under accession GSE174667.
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).	A reproducible analysis pipeline is available at https://github.com/rnabioco/morrison-chikv.
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).	N/A
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.	N/A

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.	N/A