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Lymphatic vessels interact dynamically with the hair follicle stem cell niche during hair regeneration in vivo

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 12th Mar 2019

Thank you again for your interest and the submission of your manuscript (EMBOJ-2019-101688) to The EMBO Journal. Your manuscript has been sent to three referees for consideration, and we have received reports from all of them, which I enclose below.

As you will see, the referees acknowledge the potential high interest and novelty of your work, although they also express a number of issues that will have to be addressed before they can support publication of your manuscript in The EMBO Journal. Referee #1 states that the interplay between HF cycling, differentiation and lymph vessel dynamics should be explored by additional experiments. Referee #3 argues that the analysis of the genes differentially expressed in lymph vessel during telogen phases should be expanded to consolidate the results and concept proposed. In addition, the referees point to issues related to data representation, missing controls and methods annotation that would need to be conclusively addressed to achieve the level of robustness needed for The EMBO Journal.

I judge the comments of the referees to be generally reasonable and given their overall interest, we are in principle happy to invite you to revise your manuscript experimentally to address the referees' comments.

Please note that while per se well taken, the point raised by referees #2 and #3 on conservation of the findings in human skin is not at the core of the current study in our view, thus can be left for future work.

REFEREE REPORTS:

Referee #1:

Hair follicles (HFs) of the murine skin have previously been shown to be associated with and

remodel in concert with the blood vessels within the dermis. In this paper, the authors have described that lymphatic vessels (LVs), the complementary network to the cardiovascular system that circulates lymph, are also closely associated with hair follicles (HF) and may represent a niche for the stem cells of the HFs. This is a very novel idea and a valuable contribution to the field as this is a previously unexplored niche and relationship for HF regeneration. This study finds that in back skin, LVs can be found in a stereotypic organization with HFs whereby they are regularly found on the anterior side of HFs in patterns of triads. They are persistently found to be associated with the HFSC pool throughout the HF cycle and that induced growth of HFs was perturbed when LVs were ablated.

Major Concerns:

• The organization of LVs with triads of HFs is an interesting observation and suggests a tightly regulated organization that persists through HF cycling. However, the measurements of LV area in Figure EV2 and caliber in Figure 4 could be further expanded in light of the findings in Figure 5 that many pathways involving lymphatic remodeling are altered during HF growth phases. Do lymphatic endothelial cells undergo proliferation or apoptosis during phases of HF growth/regression similar to what has been observed for blood vessels?

• The finding that ablation of LVs abrogates the precocious entry of HFs into anagen is extremely compelling and suggests and important function of LVs for normal HF cycling (Figure 6). Does ablation of LVs affect the normal cycling of HFs in non-pharmacologically induced growth and regression? And furthermore does it affect the normal differentiation mechanisms that HF cells utilize during growth?

Minor Concerns:

• The abstract seems to focus on the relationship between LVs and HF stem cells but this might undercut the overall novelty of the paper as many of the results point towards LVs being important for HF development, cycling and organization as a whole, a process that could involve more than the outlined HFSCs. We suggest reframing the abstract to state the importance of LVs to the process of HF regeneration.

• The finding that specific ablation of Wntless if K15 cells results in a reorganization of associated LVs is a very interesting point, suggesting that the HFSCs could be responsible for a niche for LVs that direct their organization within the dermis (Figure 1). The authors should include in the figure legends what age these mice were.

• In Figure 2, the authors suggest that development of HFs from E17.5 onwards is coupled to recruitment of lymphatic capillaries. This is an interesting observation and makes one wonder what is the functional consequence of this early association of the HFs and LVs. We would suggest adding a section in the discussion to explore these possibilities.

Referee #2:

This manuscript carefully documents the structural and functional relationships between lymphatic vessels and hair follicles. The data are of high quality and the conclusions are well-justified and novel. The finding that depletion of lymphatic vessels can block the pharmacological induction of hair follicle growth is of particular interest. This study will likely form the foundation for future research into the role of lymphatics in the biology of the hair follicle. Specific issues that need to be addressed are as follows:

Major points

1. The manuscript documents the distribution of lymphatic vessels relative to hair follicles in the back skin of mice. It would enhance potential clinical relevance to test some human skin samples to see if this relative distribution is similar in the human setting.

Minor points

2. Figure 1B is labelled "P55" but the legend indicates the mice were P49. Were the mice in panel A P49 or P55? What does the labelling above the columns represent in panel F? What statistical test was used in F? Experimental Procedures refers to Student t test but surely multiple group testing was conducted.

3.What multiple group statistical testing was conducted for Figure 4B, Figure 6B and D and Figure

EV2B and C?

Referee #3:

Comments on "Dynamic interactions of lymphatic vessels at the hair follicle stem cell niche during hair regeneration". I read with interest this manuscript by Dr. Peña-Jimenez and colleagues.
In this work, the authors investigated the distribution of lymphatic vessels (LV) in the skin and their interaction with the hair follicle (HF) unit. First, the authors studied the distribution and the dynamic modifications of lymphangiogenesis occurring during the hair cycle and then they demonstrated the importance of LV during HF regeneration. The authors nicely showed in vivo the dynamic communication through lymphatic vascularization, which potentially facilitates the spreading of signals that mediate HF cycling. The authors then performed RNAseq on FACS isolated LV cells from two stages of HF cycling to identify genes that regulated LV remodeling during HF regeneration.
This is a novel and original study that demonstrates the important role of LV in controlling HF stem cell activation and cycling. The study is well executed and I have only few comments before the publication of this study.

Major comments:
1/ The authors investigated the transcriptome of LV cells by comparing two telogen phases. They found potentially interesting genes but the data analysis is relatively superficial and no specific molecular candidates were selected and validated (e.g by qPCR or immunostaining) or functionally tested (e.g to check the protein expression by staining or to perform in vitro experiments).
2/ It could be also interesting to reinforce the relevance of these findings on human skin (e.g to check the staining of some candidate genes on human skin).

Minor comments:
• There are some typing errors that need to be corrected
Back skin instead of Backskin
• In page 10, you should complete the sentence
... where 60% of the cells represent lymphatic endothelial cells.....
• Figure 1c: The line separating epidermis and dermis should be replaced correctly.

1st Revision - authors' response 12th Jul 2019

Response to the reviewers' comments

Referee #1

1. The organization of LVs with triads of HFs is an interesting observation and suggests a tightly regulated organization that persists through HF cycling. However, the measurements of LV area in Figure EV2 and caliber in Figure 4 could be further expanded in light of the findings in Figure 5 that many pathways involving lymphatic remodeling are altered during HF growth phases. Do lymphatic endothelial cells undergo proliferation or apoptosis during phases of HF growth/regression similar to what has been observed for blood vessels?

In the new Fig panels EV2D and EV2E, we now show the quantification of the number of proliferating lymphatic cells ($BrdU^+$, $LYVE1^+$) and apoptotic lymphatic cells (cleaved caspase 3^+, $LYVE1^+$) using double immunofluorescence analyses during different HF cycle stages (P5, P12, P16, P23, P35, P45, P49, P55, P70, P85). Except for the HF stages P5, P12, and P16, no changes in LV proliferation were observed (Fig EV2D), suggesting that LV were still growing and reorganizing to HF growing from morphogenesis. Also, no changes in LV cell death were observed during the HF cycle (Fig EV2E). These new data revealed that lymphatic cells do not undergo proliferation or

apoptosis during HF growth/regression phases.

2. The finding that ablation of LVs abrogates the precocious entry of HFs into anagen is extremely compelling and suggests and important function of LVs for normal HF cycling (Figure 6). Does ablation of LVs affect the normal cycling of HFs in non-pharmacologically induced growth and regression? And furthermore does it affect the normal differentiation mechanisms that HF cells utilize during growth?

We thank the reviewer for pointing out the relevance of addressing the functional connection between LV and normal HF cycling in non-pharmacologically induced conditions.
As the referee nicely suggested, we now include the new Fig panels 6G-J and new Fig EV5C-F. The data in these figures include the effect of depleting LV in mouse skin at the Anagen phase of the first HF cycle, and analyses of the presence of LV, HF apoptosis, and the expression of differentiation markers.

These new results show that the ablation of LV prompts to a collapse of growing HF (new Fig 6G and H), accompanied with cell death (Fig 6I and J) and loss of the growing HF differentiated layers (new Fig EV5C-F). Overall, the data support a role for LV in sustaining the proliferation of HF, in agreement with the findings observed during the pharmacological induction of HF growth.

3. Minor concerns: The abstract seems to focus on the relationship between LVs and HF stem cells, but this might undercut the overall novelty of the paper as many of the results point towards LVs being important for HF development, cycling and organization as a whole, a process that could involve more than the outlined HFSCs. We suggest reframing the abstract to state the importance of LVs to the process of HF regeneration.

We agree with the referee and modified the abstract accordingly.

4. Minor concerns: The finding that specific ablation of Wntless if K15 cells results in a reorganization of associated LVs is a very interesting point, suggesting that the HFSCs could be responsible for a niche for LVs that direct their organization within the dermis (Figure 1). The authors should include in the figure legends what age these mice were.

We have included the missing information in the Figure legend 1G.

5. Minor concerns: In Figure 2, the authors suggest that development of HFs from E17.5 onwards is coupled to recruitment of lymphatic capillaries. This is an interesting observation and makes one wonder what is the functional consequence of this early association of the HFs and LVs. We would suggest adding a section in the discussion to explore these possibilities.

We agree with the referee's suggestion and added a section in the discussion related to this important aspect.

Referee #2

1. The manuscript documents the distribution of lymphatic vessels relative to hair follicles in the back skin of mice. It would enhance potential clinical relevance to test some human skin samples to see if this relative distribution is similar in the human setting.

We thank this reviewer and reviewer 3 for making this point. Analyzing the distribution and functional implications of lymphatic vessels in human skin will enhance the potential clinical relevance of our findings. We mention this aspect in the discussion section; however, given that LV exhibit a differential distribution according to their anatomical location, we would like to document any future human studies as a complete study on its own.

2. Minor points: Figure 1B is labelled "P55" but the legend indicates the mice were P49. Were the mice in panel A P49 or P55? What does the labelling above the columns represent in panel F? What statistical test was used in F? Experimental Procedures refers to Student t test but surely multiple group testing was conducted.

Those mistakes are now corrected in the figure legends of Fig 1B and 1F, and the experimental procedures' section.

3. Minor points: What multiple group statistical testing was conducted for Figure 4B, Figure 6B and D and Figure EV2B and C?

We apologize for this oversight. We indeed conducted multiple comparisons between groups, using the statistical one-way Analyses of Variance (ANOVA), with Tukey's post hoc tests. The Tukey's multiple comparison test was selected over the Dunnett's test, as Tukey's determines which means amongst a set of means differ from the rest, while the Dunnett's test compares each sample with a single control.

This information has been indicated in all of the figure legends and the experimental procedures' section.

Referee #3

1. The authors investigated the transcriptome of LV cells by comparing two telogen phases. They found potentially interesting genes but the data analysis is relatively superficial and no specific molecular candidates were selected and validated (e.g by qPCR or immunostaining) or functionally tested (e.g to check the protein expression by staining or to perform in vitro experiments).

We thank the reviewer for raising this critical point, which has helped us to substantiate our findings.

To validate the expression changes of relevant candidates in tissue, double in situ hybridization analyses (RNAscope, Advanced Cell Diagnostics, USA) for LYVE1 and selected candidates were conducted in P55 and P70 mouse skin samples. Also, we performed immunofluorescence analyses of two membrane proteins.

We now include the quantification of those findings and representative images in the new Fig panels 5D-I, and the new Fig EV3. We have also described in detail in the methods section, how these analyses were conducted, and the quantification procedure.

2. It could be also interesting to reinforce the relevance of these findings on human skin (e.g to check the staining of some candidate genes on human skin).

We thank this reviewer and reviewer 2 for making this point. See answer to reviewer 2, point 1.

3. Minor comments: There are some typing errors that need to be corrected: Back skin instead of Backskin. In page 10, you should complete the sentence ... where 60% of the cells represent lymphatic endothelial cells..... Figure 1c: The line separating epidermis and dermis should be replaced correctly.

We corrected those mistakes and revised all the text and figures.

2nd Editorial Decision 5th Aug 2019

Thank you for submitting your revised manuscript for consideration by The EMBO Journal. Your amended study was sent back to two of the original referees for re-evaluation, and we have received comments from both of them, which I enclose below. As you will see the referees find that their concerns have been sufficiently addressed and they are now broadly in favour of publication.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal, pending some minor issues related to formatting and data representation, which need to be adjusted at re-submission.

REFEREE REPORTS:

Referee #1:

This revised manuscript has incorporated all requested edits and includes additional experiments that have provided an important distinction between the relationship of lymphatic vessels compared to blood vessels with regards to hair follicle cycling. Additional experiments involving ablation of lymphatic vessels further corroborate the importance of these vessels for normal entry into growth phase. We recommend no further experiments or edits as this manuscript is ready for publication and will make an important contribution to the field.

Referee #3:

The authors adequately addressed my initial questions and I recommend publication of this paper in EMBOJ.

2nd Revision - authors' response 5th Aug 2019

The authors performed the requested editorial changes.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Mirna Perez-Moreno
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ 2019-101688R

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

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

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

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

B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	See answers below
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The number of animals used in the experiments was calculated considering the minimum number of animals needed to perform a statistical analysis to determine if there were differences between groups with a confidence level of 95% (k = 1.96) (p <0.05) , considering the type of study and the deviations / standard population errors. A typical experiment includes 8 mice (4 controls, 4 samples), all data is representative of at least two independent experiments performed in triplicates. For statistical analysis of quantitative data, the data normality was evaluated and data that presented a Gaussian distribution was analyzed using Student's t-test. For multiple comparisons between groups, one way Analyses of Variance (ANOVA) with Tukey's post hoc tests were conducted. Data that did not present a Gaussian distribution were analyzed using the Mann-Whitney U and Krusal-Wallis tests. Statistical analyses were done using GraphPad Software (La Jolla, Ca). All data is representative of at least two independent experiments performed in triplicates.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	No animals were excluded from the analyses.
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.	Since animals were from different genotypes, littermates were randomly subjected to treatment regimens for control or sample groups.
For animal studies, include a statement about randomization even if no randomization was used.	Mice of the same age (littermates) were randomly assigned into different groups based on the respective genotype, maintaining a balanced gender distribution between treatment groups and time points.
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.	Blinding of the investigators
4.b. For animal studies, include a statement about blinding even if no blinding was done	Blinding of genotypes and treatments was maintained when analysing samples, to minimize the effects of subjective bias.
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Data normality. D´Agostino-Person omnibus normality test was used to analyse the values from the Gaussian distribution. When data did not present a normal distribution, nonparametric version methods were used in the statistical analysis.
Is there an estimate of variation within each group of data?	Yes, One-way analysis of variance (ANOVA) tests were used to determine whether there are more than two group means that are statistically significantly different from each other and to perform within-group variation analysis.
Is the variance similar between the groups that are being statistically compared?	Yes, Bartlett's test was used to test whether the variances differ among groups.

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html	Biosecurity Documents from NIH
http://www.selectagents.gov/	List of Select Agents

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibody, Reference, Company AE15 (Ab58755, Abcam); BrdU (AB6326, Abcam); Cleaved Caspase3 (9661, Cell signaling); CD34 (553731, BD Biosciences); Emilin1 (103-M80, ReliaTech); GATA3 (SC-268, Santa Cruz Biotechnology); K6 (PRP-169P, Covance); Ki67 (MAD-000310QD, Master Diagnostica); Lhx2/9 (Gift, Thomas Jessel lab, Columbia University); LYVE1 (ab149117, Abcam); P-cadherin (13-2000Z, Invitrogen); Smooth Muscle Actin (MS-113-P0, ThermoFisher Scientific); Tenascin C (NB110-68136, Novus Biologicals); Anti-Rabbit FITC (711-095-152, Jackson immunoresearch); Anti-Rabbit Alexa Fluor 594 (711-585-152, Jackson immunoresearch); Anti-Rat Alexa Fluor 594 (712-585-150, Jackson immunoresearch); Anti-Mouse Alexa Fluor 488 (715-545-151, Jackson immunoresearch). Most antibodies used are commercially available and are validated by the vendor for the assay and species used in this study. The Lhx2/9 antibody has been used broadly by the scientific community and validated in several studies.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	NA

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The following strains were used in this study, without gender segregation for analyses: C57Bl/6 mice (Jackson); age, embryonic (E15.5, E16.6 and E17.5) and postnatal (P) days (P5, P12, P16, P23, P35, P45, P49, P55, P69 and P85). The Prox1-CreERT2 mouse model (Tg(Prox1-cre/ERT2)#aTmak, gift from Dr. Taija Mäkinen, Uppsala University)(Bazigou et al., 2011) was crossed under the background of the ROSA26-LSL-eYFP (Jakson) reporter mice. Age 6-12 weeks. The Prox1-CreERT2 (Tg(Prox1-cre/ERT2)#aTmak, gift from Dr. Taija Mäkinen, Uppsala University)(Bazigou et al., 2011) was crossed under the background of the ROSA26-LSL-iDTR (Gt(ROSA)26Sortm1(HBEGF)Awai/J, Jackson) mice. Age. 6-12 weeks. The K15-CrePR1 mice [(Krt1-15-cre/PGR)22Cot/J], and the Wlstm1.1Lan/J mice were acquired from Jackson Labs. Age 1-12 weeks. All animals were housed under SPF conditions, with food and water provided ad libitum on a 12 hour-based light/dark cycle.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All mouse experiments were approved and performed according to international, institutional and ethical regulations, with the approval of the local authorities.
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.	Compliance with respective guidelines is confirmed.

E- Human Subjects

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

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	RNAseq data have been deposited in the GEO database, GEO number GSE102463.
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).	RNAseq data have been deposited.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No