nature research

Peer Review Information

Journal: Nature Immunology
Manuscript Title: Tissue adaptation and clonal segregation of human memory T cells in barrier sites
Corresponding author name(s): Donna Farber

Reviewer Comments & Decisions:

Decision Letter, initial version:

Subject: Decision on Nature Immunology submission NI-A34405
Message: 31st Aug 2022

Dear Donna,

Thank you for sending your point-by-point response to the referees' comments on your manuscript entitled "Tissue adaptation and clonal segregation of human memory T cells in barrier sites". You will see from their comments below that while they find your work of interest, some important points are raised. We are very interested in the possibility of publishing your study in Nature Immunology, and it seems as if you have data in-hand that can readily address all of these points in a relatively short period of time. Hence, we invite you to submit a substantially revised manuscript, however please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

* Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

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* Please include a revised version of any required reporting checklist. It will be available to referees to aid in their evaluation of the manuscript goes back for peer review. They are

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We hope to receive your revised manuscript within four weeks. If you cannot send it within this time, please let us know. We will be happy to consider your revision so long as nothing similar has been accepted for publication at Nature Immunology or published elsewhere.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Immunology is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Kind regards,

Laurie

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Referee expertise:

Referee #1: T cell memory

Referee #2: Human T cells

Referee #3: Human T cells

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors investigate the phenotype, transcriptome, and TCR repertoire of T cells in multiple tissues of human cadavers, at the single-cell level. This allowed them to discern shared and distinct patterns of gene/protein expression between similar T cell subsets in distinct tissues, as well as address the degree of clonal "sharing" across sites, including resident memory (TRM) in barrier tissues. They report different tissue distributions of CD8 and CD4 clones, in which they find that CD8+ T cells with a TEM/TEMRA phenotype are often shared across multiple tissues, while most CD4+ T cell populations and CD8+ TRM were typically seen within a single tissue network. Using transcriptomics, the authors find shared and unique transcriptional profiles for TRM in certain tissue networks, while also highlighting a core transcriptional program of TRM across tissues.

The novelty of this report is somewhat limited by other recent publications that describe the phenotypes and transcriptomes of T cells from various tissues in both mice and humans (including some prior work by this group), with a focus on shared/distinct properties of TRM in distinct tissues. However, these findings provide a valuable resource by presenting integrated data from distinct single-cell approaches to address diversity and homology among human T cell populations in diverse organs.

There are, however, several concerns that need to be addressed.

1) The authors highlight the finding that certain CD8 T cell clones are shared across circulatory and barrier tissue networks (especially those of a TEM/TEMRA phenotype – Fig. 6) -- yet their analysis evidently fails to either interrogate or omit invariant CD8 T cells, such as mucosal associated invariant T (MAIT) cells, which have been described in blood and many mucosal sites. Indeed, the most shared CD8 TRBVs across tissues (Fig. S4) are those that have been associated with human MAIT cells in health (TRBV6, 20—Hinks, 2016, Immunology) and disease (TRBV19—Yao, et al., 2020, Front Immunol.). It will be

important for the authors to distinguish MAIT (and any other well defined invariant T cells) from the polyclonal peptide-Ag specific T cell pools (both CD4+ and CD8+).

On a related point, the authors do note detection of rare "innate T cells" in one subject (Fig. 1C), but it is not clear what CyTOF markers led to this designation, so this needs to be described.

2) In the paragraph beginning on line 247, the authors discuss gene expression traits that are shared among TRM, stating that:
"These genes included cell matrix and adhesion molecules (EZR, VIM, LGALS3, MCAM), transcription factors (AHR, KLF4), chemokine receptors (CCR6), and the residency marker CD101 (Fig. 4, Supplementary Fig. 8a,b)".

Aside from EZR and VIM (Fig. 4F), this reviewer could not find any data on expression of those genes in Fig. 4 or S8. Are alternative gene names being used? At a minimum, these should be more clearly identified in the figures.

On a related point, it is confusing that none of the genes being listed as shared among barrier site TRM in the text are evident in Fig. S8b – shouldn't those genes feature prominently among the ones listed as being shared among lung, skin and jejunum?

3) Fig. 4 is also confusing from the perspective of the genes listed as being uniquely expressed by TRM in certain sites (Fig 4a-c): it is difficult to know how comparisons such as "Lung TRM vs other" are weighted, since "other" will include all other T cell populations (correct?), including TRM. Some kind of weighting must be involved, otherwise it would be impossible for the same genes to arise as being "unique" to any two barrier tissues – yet multiple gene expression patterns ARE shared in panels a-c, such a low expression of TCF7, CCR7 and high expression of AHR. At a simplistic level then, these plots do not show unique gene expression characteristics in absolute terms, making their interpretation (and value) difficult to discern.

4) Finally on this figure, for all of Figure 4 and part of Fig. 5, the authors appear not to separate TRM based on CD4 vs CD8 expression. Since part of the authors' conclusion from Fig. 4 is that there are tissue-specific programs imposed on TRM, it would be important to know a) whether these characteristics are shared among both CD4+ and CD8+ TRM and b) how the authors control for distinct representation of CD4+ vs CD8+ cells in those tissues (Fig. 1d) during the analysis.

5) I have some hesitation towards the authors' discussion starting at line 342:

Interestingly, deviation of these steady-state functional states for TRM is associated with pathologic states. Diseases and dysregulation in skin T cells can manifest as a Th17 response in cutaneous candidiasis23 and psoriasis58 and a Th1 response in rejection of facial transplants59. Conversely in the lung, a Th2 response is pathogenic and promotes allergic airway disease60,61. In this way, altering the tissue environment through introduction of T cells with functions distinct from the barrier causes pathology.

While thought provoking, this argument seemed to entail picking and choosing Th responses that don't "fit" the transcriptional data observed and connecting them to known examples of disease states. Presumably, the authors would not argue that a Th1 immune response in the skin is always detrimental for immunity from pathogens, nor that a

sustained Th1 response in the lung is always a safe way to avoid immunopathology? While the authors point about the populations in tissues at steady-state may be valid, this argument comes off as being too rigid for the critical flexibility of local immune responses.

Reviewer #2:
Remarks to the Author:
In this study, Farber and colleagues provide a detailed analysis of human T cells across different tissues, including major barrier sites (skin, lung, jejunum), lymph nodes, lymphoid organs and peripheral blood, using single cell proteomic, gene expression and TCR repertoire analysis. The authors show that barrier sites contain predominant tissue resident memory T cells (TRM) populations, which are transcriptionally and clonally segregated from circulating memory T cells in lymphoid organs and blood. They also identify core barrier and site-specific signatures for TRM cells. Interesting findings came from the analysis of T cell clone overlap across different sites, which was first performed by sequencing the CDR3β chain of sorted CD4+ and CD8+ T cells and then sequencing the TRA and TRB transcripts on single T cells. This analyses showed that circulating memory T cells are highly expanded with extensive overlap between tissue sites, while TRM clones are expanded but restricted mainly to the barrier site. Overall, the study is well performed and takes advantage of a unique tissue resource and of validated protocols. Although the data are clearly presented and discussed, the novelty is somehow limited by previous studies and in particular by the recent article from the Teichmann laboratory on the cross-tissue atlas of human immune cells (Domínguez Conde et al., Science, 2022), which is also co-authored by Donna Farber. It would be therefore important for the authors to cite this latter study and to discuss their findings in the context of the previously published work.

Reviewer #3:
Remarks to the Author:
The authors have assembled completely unique data on lymphoid and non lymphoid tissue from 7 inidividuals, including CYtoF, scRNAseq, TCRHTS, and not all individuals contribute to each. Cyotof identifies a broad collection of cell types, though some inconsistencies are present. Current literature does not support the finding that skin TRM are exclusivily TH2/c2; in fact, cytokine production studies find TH2 cytokines spare. Either further study, or discussion of the uniqueness of this result, are required. Clonality studies are very interesting and powerful, and the observations are sound. scRNA seq data is interesting but again the skin scRNA seq results are curious--CD3e is most sparse in the skin cluster, suggesting lack of purity (doublets) in this population. Similarly, CD103 is abundant in jejeunum and lung TRM but not skin. These are technically difficult experminents.
Having said that, the data overall are so unique and one of a kind that I would recommend that this data be shared broadly.
The interpretation needs to be approached with more caution, but the extreme relevance is crystal clear.

Author Rebuttal to Initial comments

Point-by Point Response

"Tissue adaptation and clonal segregation of human memory T cells in barrier sites"

NI-A34405

Corresponding Author: Donna L. Farber

We appreciate the helpful and insightful comments from each reviewer about our manuscript. To address the reviewers' comments, we have performed additional data analyses and revised the text accordingly. The revised manuscript contains revisions to Figures 1-6 and Supplementary Figures 6, 7, 8, and 10 and a new Supplementary Figure 9, along with corresponding revisions to the text throughout. Our response to each reviewer comment describing how we revised our manuscript to address each comment with references to the appropriate figures and pages in the revised text is presented below. Major revisions to the manuscript are indicated by underlining.

Reviewer #1:

1. The authors highlight the finding that certain CD8 T cell clones are shared across circulatory and barrier tissue networks (especially those of a TEM/TEMRA phenotype – Fig. 6) -- yet their analysis evidently fails to either interrogate or omit invariant CD8 T cells, such as mucosal associated invariant T (MAIT) cells, which have been described in blood and many mucosal sites. Indeed, the most shared CD8 TRBVs across tissues (Fig. S4) are those that have been associated with human MAIT cells in health (TRBV6, 20—Hinks, 2016, Immunology) and disease (TRBV19—Yao, et al., 2020, Front Immunol.). It will be important for the authors to distinguish MAIT (and any other well defined invariant T cells) from the polyclonal peptide-Ag specific T cell pools (both CD4+ and CD8+). On a related point, the authors do note detection of rare "innate T cells" in one subject (Fig. 1C), but it is not clear what CyTOF markers led to this designation, so this needs to be described.

Response: MAIT T cells are distinguished from conventional ab T cells by expression of certain cell surface markers and specific TCR α and β chain genes[1, 2]. In our single cell RNAseq dataset, we re-analyzed and visualized the data to distinguish MAIT cells by co-expression of marker genes (*TRAV1-2*, *SLC4A10*, *KLRB1*)[3], which we further confirmed using the scRNAseq TCR analysis as expressing TCR α and β genes (*TRAV1-2* + *TRAJ33/20/12*; *TRBV6/19/20*) expressed by MAIT cells[4]. The revised UMAP showing the cluster of MAIT cells, its distribution by tissue for each donor is presented in the revised Fig. 3, and described the identification of this subset as MAIT in the results (p. 9) and method which details these annotation strategies.

In our dataset, T cells identified as MAIT represent 1.7% of the dataset, corresponding to a small cluster of $CD8^+$ memory T cells, designated by Leiden clusters 34 and 36 (Supplementary Fig. 7). The identification of this MAIT cluster does not affect the overall frequencies and tissue distribution of the different conventional T cell subsets, or their tissue-specific features presented in Fig. 4. We also include information on MAIT cell clonality in the revised Fig. 5a-c, showing that while a few MAIT cells are clonally expanded, most of them are not. MAIT clones were also included in the single cell clonal overlap analysis (Fig. 6 and Supplementary Fig. 11), but were not among the most highly disseminated. In the CYTOF analysis, we did not have all of the markers necessary to identify MAIT cells; however, we have markers for other innate T cells. In the revised Fig. 1 and in the results (p. 6), we have added a statement to clarify that the majority of innate-like T cells identified are $\gamma\delta$ T cells identified by expression of the gdTCR.

2. In the paragraph beginning on line 247, the authors discuss gene expression traits that are shared among TRM, stating that:
"These genes included cell matrix and adhesion molecules (EZR, VIM, LGALS3, MCAM), transcription factors (AHR, KLF4), chemokine receptors (CCR6), and the residency marker CD101 (Fig. 4, Supplementary Fig. 8a,b)".

Aside from EZR and VIM (Fig. 4F), this reviewer could not find any data on expression of those genes in Fig. 4 or S8. Are alternative gene names being used? At a minimum, these should be more clearly identified in the figures.

On a related point, it is confusing that none of the genes being listed as shared among barrier site TRM in the text are evident in Fig. S8b – shouldn't those genes feature prominently among the ones listed as being shared among lung, skin and jejunum?

Response: We have revised the presentation of the analysis of barrier-site-specific genes in Fig. 4 to include annotation of the significantly differentially expressed genes between barrier sites and non-barrier site T cells in the volcano plots in Fig. 4b-d, in the dot plots in Fig. 4f, g, and by cell in the heatmaps in the revised Supplementary Fig. 8. No alternative gene names are being used. The genes annotated and mentioned in the text include genes that are differentially expressed by all barrier site TRM in lung, gut and skin, those differentially expressed by two barrier sites, and those which are differentially expressed by a single site (lung, gut, or skin). The text in the results (pp. 10-11) has been revised to more clearly describe the different classifications. The heatmaps in the revised Supplementary Fig. 8 are now filtered to include only protein-coding genes, so that genes we discuss in the text are displayed more prominently. Non-protein coding genes are still included in Supplementary Table 7.

3. Fig. 4 is also confusing from the perspective of the genes listed as being uniquely expressed by TRM in certain sites (Fig 4a-c): it is difficult to know how comparisons such as "Lung TRM vs other" are weighted, since "other" will include all other T cell populations (correct?), including TRM. Some kind of weighting must be involved, otherwise it would be impossible for the same genes to arise as being "unique" to any two barrier tissues – yet multiple gene expression patterns ARE shared in panels a-c, such a low expression of TCF7, CCR7 and high expression of AHR. At a simplistic level then, these plots do not show unique gene expression characteristics in absolute terms, making their interpretation (and value) difficult to discern.

Response: The comparisons in Fig. 4 were done for each barrier-tissue TRM (lung, skin, and intestine) versus all other T cells (non-TRM of all sites and TRM of non-barrier sites). In the revised Fig. 4a, we added a schematic to clarify these groups and these comparisons. The only weighting used in the differential expression analysis was subsampling cell counts and down sampling total counts to be equivalent across these four groups (lung TRM, skin TRM, jejunum TRM, all other T cells—i.e. T cells from all sites that were not barrier site TRM), as described in Methods. The analysis in Fig. 4 revealed genes shared across these multiple barrier site TRM populations (e.g. upregulated in each of the barrier site TRM populations when compared to all other T cells), those shared by TRM in two barrier sites, and those differentially expressed by individual barrier TRM populations (Fig. 4f,g) as described in the results (pp.9-10).

4. Finally on this figure, for all of Figure 4 and part of Fig. 5, the authors appear not to separate TRM based on CD4 vs CD8 expression. Since part of the authors' conclusion from Fig. 4 is that there are tissue-specific programs imposed on TRM, it would be important to know a) whether these characteristics are shared among both CD4+ and CD8+ TRM and b) how the authors control for distinct representation of CD4+ vs CD8+ cells in those tissues (Fig. 1d) during the analysis.

Response: Genes identified as differentially expressed in specific tissues (Fig. 4) are largely expressed by both $CD4^+$ and $CD8^+$TRM, albeit at somewhat different levels as shown in the new Supplementary Fig. 8 and described in the revised results (p. 11, lines 243-6). These results show that the site-specific gene expression profiles obtained from the analysis were not due to differential representation of specific lineages in the different sites.

5. I have some hesitation towards the authors' discussion starting at line 342:

Interestingly, deviation of these steady-state functional states for TRM is associated with pathologic states. Diseases and dysregulation in skin T cells can manifest as a Th17

response in cutaneous candidiasis23 and psoriasis58 and a Th1 response in rejection of facial transplants59. Conversely in the lung, a Th2 response is pathogenic and promotes allergic airway disease60,61. In this way, altering the tissue environment through introduction of T cells with functions distinct from the barrier causes pathology.

While thought provoking, this argument seemed to entail picking and choosing Th responses that don't "fit" the transcriptional data observed and connecting them to known examples of disease states. Presumably, the authors would not argue that a Th1 immune response in the skin is always detrimental for immunity from pathogens, nor that a sustained Th1 response in the lung is always a safe way to avoid immunopathology? While the authors point about the populations in tissues at steady-state may be valid, this argument comes off as being too rigid for the critical flexibility of local immune responses.

Response: We have revised our discussion about the potential importance and utility of defining the functional profiles of T cells within a particular site in steady-state conditions, to gain new insights into protective or pathological immune responses in tissues. In the revised discussion, we provide examples of how understanding of whether responses are typical of TRM or circulating T cells can provide insights into the origin of the tissue response. Conversely, deviations from typical functional responses in steady state can also indicate alterations in the tissue environment. We present these more focused ideas in the revised discussion (p. 15):

"Defining tissue T cell functions in steady state can provide insights into the origin of protective or pathological responses in tissues. For example, skin T cells can manifest as a Th17 response in cutaneous candidiasis[5] and psoriasis[6], consistent with a TRM-mediated response. Conversely, Th1 and cytotoxic responses are associated with rejection of facial transplants[7], suggesting potential infiltration from circulation. In the lung where TRM are Th1-like or regulatory, an overactive Th2 response promotes allergic airway disease[8, 9], suggesting aberrant TRM, consistent with mouse studies[10, 11]. In this way, prolonged alteration of tissue environments can promote pathological TRM functions."

Reviewer #2:

1. *In this study, Farber and colleagues provide a detailed analysis of human T cells across different tissues, including major barrier sites (skin, lung, jejunum), lymph nodes, lymphoid organs and peripheral blood, using single cell proteomic, gene expression and TCR repertoire analysis. The authors show that barrier sites contain predominant tissue resident*

memory T cells (TRM) populations, which are transcriptionally and clonally segregated from circulating memory T cells in lymphoid organs and blood. They also identify core barrier and site-specific signatures for TRM cells. Interesting findings came from the analysis of T cell clone overlap across different sites, which was first performed by sequencing the CDR3β chain of sorted CD4+ and CD8+ T cells and then sequencing the TRA and TRB transcripts on single T cells. This analyses showed that circulating memory T cells are highly expanded with extensive overlap between tissue sites, while TRM clones are expanded but restricted mainly to the barrier site. Overall, the study is well performed and takes advantage of a unique tissue resource and of validated protocols. Although the data are clearly presented and discussed, the novelty is somehow limited by previous studies and in particular by the recent article from the Teichmann laboratory on the cross-tissue atlas of human immune cells (Domínguez Conde et al., Science, 2022), which is also co-authored by Donna Farber. It would be therefore important for the authors to cite this latter study and to discuss their findings in the context of the previously published work.

Response: Our study is novel in that it provides a comprehensive assessment of T cells in multiple sites using orthogonal single-cell approaches including analysis of protein expression by CyTOF, high throughput TCR clonal analysis by TCR-seq, and in-depth scRNAseq + paired *TRA* and *TRB* chain analysis. From these different approaches, we found that TRM in barrier sites are distinct from each other and clonally segregated, that tissue-specific features and function mark TRM in barrier sites, and we revealed how T cells are clonally related across the body in different networks, including segregated TRM in the lungs, gut, and skin. The recent publication by Teichmann and colleagues (Science, 2022), presents scRNAseq analysis of total CD45+ immune cells from multiple tissues of organ donors integrating immune cell scRNAseq data from different tissue sources, processing strategies, and sequencing technologies for which we contributed 3'scRNAseq data from 8 tissues of 2 donors (no TCR, no skin). The major focus of the Teichmann study was to implement a newly developed classifier (CellTypist) to computationally annotate immune cell subsets—both innate and adaptive—across different sites. The study and dataset were not set up to identify tissue-specific properties of the immune cell subsets and single cell TCR data (from a small portion of the dataset) did not allow for tracking across sites within individuals. The findings in our study are therefore distinct and not repetitive of the findings in the published dataset.

In the revised version of our manuscript, we mention and cite this study in the context of our findings of tissue-specific adaptations for barrier site TRM in the revised discussion (pp. 14-15). In particular, we raise the question of whether the barrier site adaptations identified for TRM apply to other resident immune cells in these sites*: "The extent to which other immune cells in these sites acquire tissue adaptations remains to be determined. Recent single cell profiling efforts for human immune cells combined with advanced computational tools for annotation*

initiated by our group and others[12] will enable a precise dissection of the effects of tissue, lineage, and age on resident immune cells."

Reviewer #3:

1. *The authors have assembled completely unique data on lymphoid and non lymphoid tissue from 7 individuals, including CYtoF, scRNAseq, TCRHTS, and not all individuals contribute to each. Cyotof identifies a broad collection of cell types, though some inconsistencies are present. Current literature does not support the finding that skin TRM are exclusively TH2/c2; in fact, cytokine production studies find TH2 cytokines spare. Either further study, or discussion of the uniqueness of this result, are required. Clonality studies are very interesting and powerful, and the observations are sound. scRNA seq data is interesting but again the skin scRNA seq results are curious--CD3e is most sparse in the skin cluster, suggesting lack of purity (doublets) in this population. Similarly, CD103 is abundant in jejenum and lung TRM but not skin. These are technically difficult experiments. Having said that, the data overall are so unique and one of a kind that I would recommend that this data be shared broadly.*
The interpretation needs to be approached with more caution, but the extreme relevance is crystal clear.

Response: The designation of a Th2-like profile for skin TRM was based largely on the elevated expression of GATA3 transcripts in skin TRM compared to other sites in the scRNAseq results, and the surface expression of CRTH2 by a subset of skin TRM by CyTOF. However, these markers do not themselves exclusively define Th2 cells in humans. In order to dissect heterogeneity in skin T cells in our dataset, we performed additional analysis of the scRNAseq data, and re-evaluated the designation of clusters in the CyTOF data. For the scRNAseq data, we performed clustering analysis of skin TRM as presented in a new figure (Supplementary Fig. 9), identifying Th1-, Th2-, and Th17-like TRM populations by gene expression within the skin, as well as other cytotoxic and effector TRM. We have described these results on functional heterogeneity and profiles within skin TRM in the revised results (p. 11), which are consistent with other studies on human skin T cells[13, 14, 15].

For the CyTOF data, we re-evaluated the designation of clusters and found that CXCR4 expression was more significantly enhanced on the major skin TRM subsets compared to other sites, with lower level expression of CRTH2, while CRTH2 is more highly expressed on the TEM-like clusters in skin. We have designated the skin clusters as CXCR4+ and CRTH2+, rather than group them together as Th2- like to more precisely describe the different skin

subsets. The description of the CyTOF results for skin T cells is presented in the revised results (p. 7).

Regarding potential impurities and doublets in the skin preparation for scRNA-Seq, skin TRM express CD3E, rearranged TCR, were *PTPRC*+ (CD45^{+}) and did not express other lineage markers (*CD19*, *CD1A*, *CD14*, *SDC-1*, *FCGR1A*) across the cluster, suggesting these events are indeed T cells and not doublets contaminated by other immune or non-immune subsets. These points are highlighted in the revised Methods. Differences in *CD3E* expression in the skin is more likely due to varied sequencing depth across batches, which we control for during differential expression analysis by down sampling counts across each group. In addition, we and others have found that CD103 (*ITGAE*) expression is limited to a subset of TRM in the skin[13, 14](Fig. 1), accounting for variations in *ITGAE* expression across sites.

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2. Klenerman, P., Hinks, T.S.C. & Ussher, J.E. Biological functions of MAIT cells in tissues. *Mol Immunol* **130**, 154-158 (2021).
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8. Yeon, S.M. *et al.* IL-7 plays a critical role for the homeostasis of allergen-specific memory CD4 T cells in the lung and airways. *Sci Rep* **7**, 11155 (2017).
9. Rahimi, R.A., Nepal, K., Cetinbas, M., Sadreyev, R.I. & Luster, A.D. Distinct functions of tissue-resident and circulating memory Th2 cells in allergic airway disease. *J Exp Med* **217** (2020).
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14. Watanabe, R. *et al.* Human skin is protected by four functionally and phenotypically discrete populations of resident and recirculating memory T cells. *Sci Transl Med* **7**, 279ra239 (2015).
15. Strobl, J. *et al.* Human resident memory T cells exit the skin and mediate systemic Th2-driven inflammation. *J Exp Med* **218** (2021).

Decision Letter, first revision:

Subject: Your manuscript, NI-A34405A

Message: Our ref: NI-A34405A

14th Nov 2022

Dear Dr. Farber,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Immunology manuscript, "Tissue adaptation and clonal segregation of human memory T cells in barrier sites" (NI-A34405A). Please carefully follow the step-by-step instructions provided in the attached file, and add a response in each row of the table to indicate the changes that you have made. Please also check and comment on any additional marked-up edits we have proposed within the text. Ensuring that each point is addressed will help to ensure that your revised manuscript can be swiftly handed over to our production team.

We would like to start working on your revised paper, with all of the requested files and forms, as soon as possible (preferably within two weeks). Please get in contact with us if you anticipate delays.

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If you have not done so already, please alert us to any related manuscripts from your group that are under consideration or in press at other journals, or are being written up for submission to other journals (see: https://www.nature.com/nature-portfolio/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Immunology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your manuscript entitled "Tissue adaptation and clonal segregation of human memory T cells in barrier sites". For those reviewers who give their assent, we will be publishing their names alongside the published article.

Nature Immunology offers a Transparent Peer Review option for new original research manuscripts submitted after December 1st, 2019. As part of this initiative, we encourage our authors to support increased transparency into the peer review process by agreeing to have the reviewer comments, author rebuttal letters, and editorial decision letters

published as a Supplementary item. When you submit your final files please clearly state in your cover letter whether or not you would like to participate in this initiative. Please note that failure to state your preference will result in delays in accepting your manuscript for publication.

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Best regards,

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On behalf of

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Reviewer #1:
Remarks to the Author:
The authors have responded appropriately to my previous concerns, and the new data shown strengthen the authors' conclusions.

Reviewer #3:
Remarks to the Author:
I accept the point by point rebuttal of the authors.
accept!

Final Decision Letter:

Subject: Decision on Nature Immunology submission NI-A34405B

Message: In reply please quote: NI-A34405B

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Kind regards & Happy Thanksgiving,

Laurie

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