nature research

Peer Review Information

Journal: Nature Immnuology
Manuscript Title: Distinct metabolic programs established in the thymus control effector functions of γδ T cell subsets in tumour microenvironments
Corresponding author name(s): Daniel Pennington

Reviewer Comments & Decisions:

Decision Letter, initial version:

Subject: Decision on Nature Immunology submission NI-A29750

Message: 9th Jun 2020

Dear Prof Pennington,

Your Article, "Distinct metabolic programmes control the effector fate of γδ T cell subsets and their activities in the tumour microenvironment" has now been seen by 2 referees. You will see from their comments copied below that while they find your work of considerable potential interest, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be very interested in considering a revised version that addresses these concerns.

We hope you will find the referees' comments useful as you decide how to proceed. If you wish to submit a substantially revised manuscript, please bear in mind that we will be reluctant to approach the referees again in the absence of major revisions.

If you choose to revise your manuscript taking into account all reviewer and editor comments, please highlight all changes in the manuscript text file.

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.

If 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.

* If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions at http://www.nature.com/ni/authors/index.html. Refer also to any guidelines provided in this letter.

* Include a revised version of any required reporting checklist. It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review. A revised checklist is essential for re-review of the paper.

The Reporting Summary can be found here:
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You may use the link below to submit your revised manuscript and related files:
[REDACTED]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

If you wish to submit a suitably revised manuscript we would hope to receive it within 6 months. 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. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

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.

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

Thank you for the opportunity to review your work.

Sincerely,

Zoltan Fehervari, Ph.D.
Senior Editor
Nature Immunology

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Referee expertise:

Referee #1:

Referee #2:

Referee #3:

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The manuscript by Lopes et al. reveals how distinct γδ T cells subsets appear to be differentially programmed metabolically, thus leading to distinct peripheral functional outcomes, which are shown to have clear implications for anti-tumour immune responses in mice. The authors find that IFNγ programming and anti-tumor function is dependent on glucose and glycolysis, whereas IL-17 programming is dependent on increased mitochondrial activity, as well as lipid uptake for the maintenance of IL-17+ γδ T cells. This is shown to be associated with transcriptional changes, using scRNAseq, and appears to be established intrathymically. The metabolic programming is shown to be initiated as γδ T cells differentiation from an immature CD24hi stage towards the CD44+CD45RB- (IL-17) or CD44+CD45RB+ (IFNγ) outcomes, with different levels of glycolytic and OXPHOS/FAO dependency, respectively. Remarkably, little is known about how γδ T cell metabolism is regulated and how metabolic changes affect their functional programming. Here, the authors provide a very timely and beautifully executed set of experiments showing that manipulating culture conditions, or their in vivo environment, of γδ T cells can have a direct influence in their function, such that a high fat diet increases IL17+ γδ T cell numbers, while high glucose cultures leads to increased IFNg+ anti-tumour function.

Overall, this is an impactful study that provides important and novel insights into how metabolic factors are implicated in γδ T cell functional programming in the thymus and periphery, and in addition reveal potential strategies for manipulating γδ T cell effector function to improve anti-tumor therapies.

A few points for further consideration (*major):

1) The authors argue that γδ17 (CD27-) cells are more dependent on mitochondrial activity, for example displaying higher mitotracker staining, an indication of higher mitochondria mass. However, it can be noticed in Figure 2b that γδ17 cells appear to be larger in cell size compared to γδIFN cells. Some quantification taking into consideration cell size ratios should be conducted and shown to reveal whether the increased mitochondrial requirement in γδ17 cells is merely due to their larger cell size.

2) The argument presented using CD73 to indicate TMRElo cells are the ones that experienced stronger TCR signaling is compelling, but the distinction is not obvious, as

both TMREhi and TMRElo cells clearly have both CD73- and CD73+ populations. Other markers such as CD5 can be assessed, or perhaps analyzing CD73 expression within CD24- cells.

3*) The authors perform an elegant precursor-product experiment as part of Figure 4, in which CD24- CD44- CD45RB- (γδTN) or CD24hi cells are placed in culture to assess their developmental outcomes. Curiously, the majority or nearly all of cells become CD44+CD45RB- (IL-17 lineage), which is an unusual outcome and could easily be due to the fact that most of these γδ T cells when placed in vitro no longer have a proper ligand to engage and default to an IL-17 fate. This can be further addressed by examining the levels of CD73 expression, which correlate with signaling, with the cells in culture. Additionally, the authors could simply add soluble anti-CD3 to mimic TCR engagement and show that the IFN fate is now favoured. This would be a beautiful way to link TCR signaling to metabolic programing to effector function adoption.

4) The authors indicate that γδIFN cells display anti-tumor properties while γδ17 cells display the opposite. Hence, the importance in establishing the metabolic requirements for these two distinct subsets which can have a profound impact on tumor immunity. The authors show a direct link between glucose uptake and the enhanced ability of γδIFN cells to reduce tumor volume. However, similar experiments were not performed showing the link between increased lipid intake and worse prognosis of tumor inoculation experiments. All is shown is that high-fat diet can increase γδ17 numbers in tumors, but whether this leads to a loss of tumor growth control or increased tumor growth is uncertain.

Reviewer #2:
Remarks to the Author:
The study from Pennington and colleagues starts with the observation that within TIL, the γδ T cell subsets making either IFN-γ or IL-17 have intrinsically distinct metabolic requirements. Next, they systematically employ the available toolbox to characterize oxphos metabolism in gdT17 versus a more glycolysis-dependent metabolic state of gdT1 lymphocytes. Finally, in vitro glucose supplementation enhanced IFN-g production and thus anti-tumour functions of gdT1 cells and reduced tumour growth upon adoptive transfer.
The study is novel and original. And the methodology employed is state-of-the-art, while the ZENITH protocol is particularly helpful to investigate small numbers of cells.
Still, some major issues should be clarified before publication.

Title: From the data presented, it does not directly follow that < Distinct metabolic programmes control the effector fate of γδ T cell subsets and their activities in the tumour microenvironment>. There is a strong association and correlation in the FTOC, but I am not convinced that the metabolic programmes do control the choice of effector fate. This is supported by their own conclusion line 233 < that γδTN cells have already committed to an effector fate>

Introduction: Only publications from Erica Pearce are cited, this appears a bit biased.

The authors suggest but do not show that γδ progenitors receiving agonist TCRγδ signals shift away from OXPHOS as indicated by their reduced ΔΨm. It would help to actually do the experiment and show whether TCR engagement , e.g. via plate-bound anti gdTCR or

anti- CD3e mAb would lead to lower TMRE levels (associated with adoption of Type 1 immunity phenotype).

In line 281 the reference 21 cited for the notion that <another marker that segregates γδ17 and γδIFN cells, the transcription factor PLZF (encoded by Zbtb16)> is a review. Are there original sources?

The study could benefit from the analysis of gdT17 cells (eventually compared vs gd T1?) in more tissues, at least in the skin (see e.g. PMID: 21339323) where these gdT17 cells are abundant. Similarly, how are gd T1 and gd T17 doing metabolically in fat tissue?

The description of adult (Fig. 3a) and newborn (Fig. 3b) thymus does ignore the idea that gdT17 cells in adult thymus are likely fetal thymus-derived and locally adapt to persist as effectors in the thymus (see e.g. PMID: 31216482)

To complement the data shown in figure 7, how are gd T17 cells doing when cultured ex vivo under high vs low glucose conditions?

minor:
Fig 6a axis labeling and description in Results section are suboptimal.

Fig S1 seems like copy and paste from Figure 1 of related and cited preprint of the ZENITH by Phillipe Piere's group?

In the discussion, the authors draw parallels with recent findings in ab T cells / Th17 cells (reference 34). A discussion about the metabolic requirements of ILC1 versus ILC3 could be inspiring, because those should differentiate independent of TCR signals.

Author Rebuttal to Initial comments

Point-by-point reply to the Reviewers' comments:

We thank both Reviewers for their positive and constructive comments that inspired new experiments and text changes that have undoubtedly improved our manuscript.

Reviewer #1:

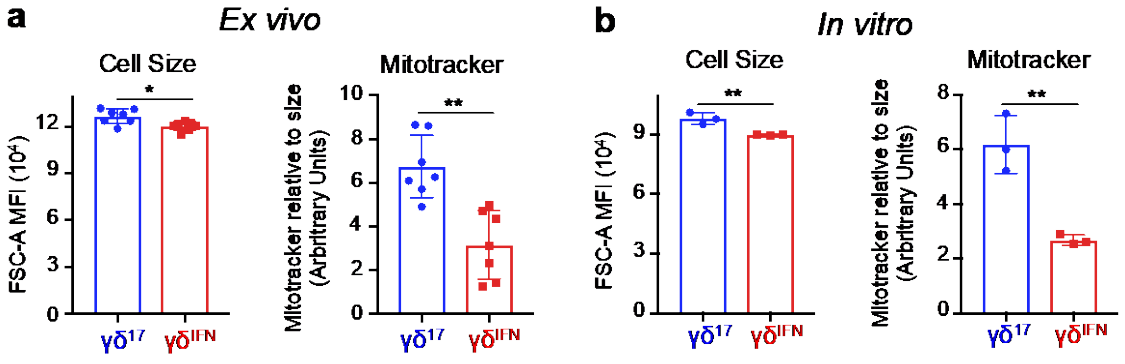
The manuscript by Lopes et al. reveals how distinct γδ T cells subsets appear to be differentially programmed metabolically, thus leading to distinct peripheral functional outcomes, which are shown to have clear implications for anti-tumour immune responses in mice. The authors find that IFNγ programming and anti-tumor function is dependent on glucose and glycolysis, whereas IL-17 programming is dependent on increased mitochondrial activity, as well as lipid uptake for the maintenance of IL-17+ γδ T cells. This is shown to be associated with transcriptional changes, using scRNAseq, and appears to be established intrathymically. The metabolic programming is shown to be initiated as γδ T cells differentiation from an immature CD24hi stage towards the

CD44+CD45RB- (IL-17) or CD44+CD45RB+ (IFNγ) outcomes, with different levels of glycolytic and OXPHOS/FAO dependency, respectively. Remarkably, little is known about how γδ T cell metabolism is regulated and how metabolic changes affect their functional programming. Here, the authors provide a very timely and beautifully executed set of experiments showing that manipulating culture conditions, or their in vivo environment, of γδ T cells can have a direct influence in their function, such that a high fat diet increases IL17+ γδ T cell numbers, while high glucose cultures leads to increased IFNg+ anti-tumour function. Overall, this is an impactful study that provides important and novel insights into how metabolic factors are implicated in γδ T cell functional programming in the thymus and periphery, and in addition reveal potential strategies for manipulating γδ T cell effector function to improve anti-tumor therapies.

*A few points for further consideration (*major):*

1) *The authors argue that γδ17 (CD27-) cells are more dependent on mitochondrial activity, for example displaying higher mitotracker staining, an indication of higher mitochondria mass. However, it can be noticed in Figure 2b that γδ17 cells appear to be larger in cell size compared to γδIFN cells. Some quantification taking into consideration cell size ratios should be conducted and shown to reveal whether the increased mitochondrial requirement in γδ17 cells is merely due to their larger cell size.*

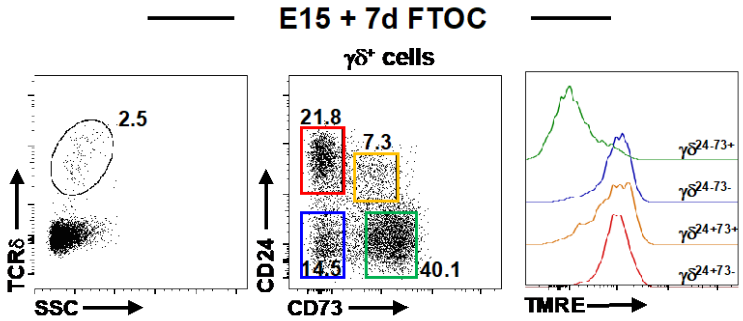
Indeed, γδ17 cells are slightly bigger than γδIFN cells (please see FSC data in the figure below). However, even when the Mitotracker staining is normalized to size (Mitotracker MFI divided by FSC MFI x100), the increased mitochondrial mass in γδ17 still holds true:



We have now added the *ex vivo* normalized Mitotracker data to the revised **Fig. 2b**.

2) *The argument presented using CD73 to indicate TMRElo cells are the ones that experienced stronger TCR signaling is compelling, but the distinction is not obvious, as both TMREhi and TMRElo cells clearly have both CD73- and CD73+ populations. Other markers such as CD5 can be assessed, or perhaps analyzing CD73 expression within CD24- cells.*

We have replaced **Fig. 4e** by the one shown below, clearly showing that **CD73+** CD24- γδ cells are the only substantially **TMRElo** γδ thymocyte subset, and thus supporting the link between strong TCR signalling and TMRE downregulation:



In our experience, at this stage of thymic development, CD5 staining is not consistent, which in fact underlines the utility of CD73 as a useful indicator of TCR signalling in γδ T cell development.

3*) *The authors perform an elegant precursor-product experiment as part of Figure 4, in which CD24- CD44- CD45RB- (γδTN) or CD24hi cells are placed in culture to assess their developmental outcomes. Curiously, the majority or nearly all of cells become CD44+CD45RB- (IL-17 lineage), which is an unusual outcome and could easily be due to the fact that most of these γδ T cells when placed in vitro no longer have a proper ligand to engage and default to an IL-17 fate. This can be further addressed by examining the levels of CD73 expression, which correlate with signaling, with the cells in culture.*

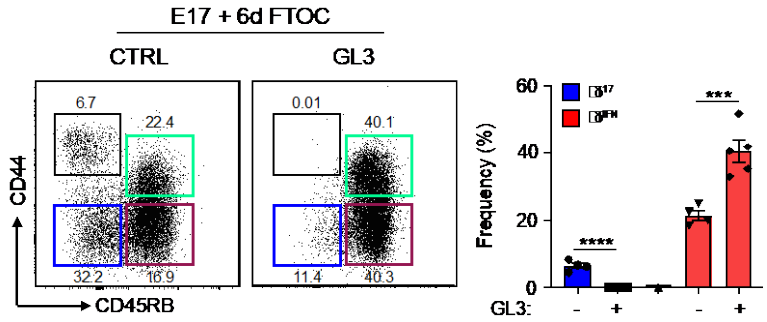
Please allow us to clarify that in these experiments (**Fig. 4a,b**), we did not culture *total* γδTN or CD24+ γδ thymocytes, but instead, these were *sorted* into **TMRElo** (upper panels) versus

TMREhi (lower panels) subpopulations. Whereas TMREhi cells indeed selectively generated γδ17 cells (quantified in the bottom graphs), please note that the former (TMRElo cells) selectively differentiated into γδIFN cells. Thus, sorting on **TMRE levels** clearly segregates the effector fates, which demonstrates an early, **metabolism-based**, divergence of the two developmental pathways.

Related to this, we now also show (in response also to **point 7** raised by Reviewer #2), that TCR signals, as provided by activating anti-TCRδ monoclonal antibody GL3 in a dose-dependent manner, downregulate TMRE (concomitant with CD24) in a population of γδ progenitors (new **Fig. 4h**).

Additionally, the authors could simply add soluble anti-CD3 to mimic TCR engagement and show that the IFN fate is now favoured. This would be a beautiful way to link TCR signaling to metabolic programing to effector function adoption.

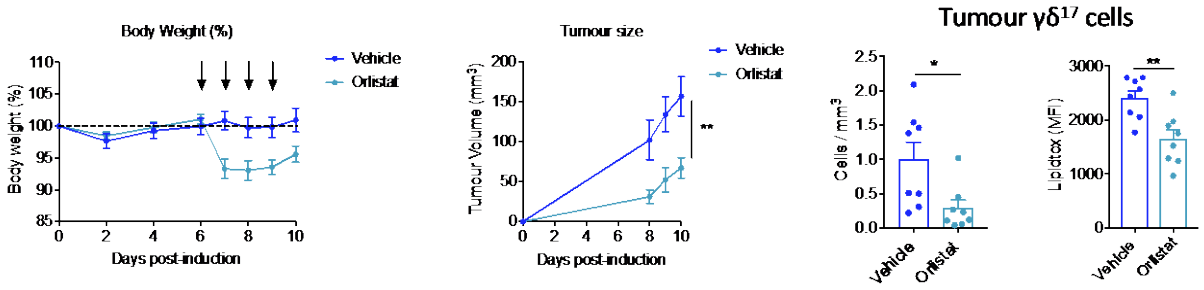
To address this point, we have now performed 6-day FOTC of E17 thymic lobes +/- GL3 mAb stimulation, which, as anticipated by the Reviewer, shows promotion of the γδIFN pathway, in parallel with full inhibition of the γδ17 pathway. These new data have been added to the revised paper as **Fig. 4g**.



Of note, using E17 instead of E15 lobes significantly increases γδIFN cell output (not shown), which better demonstrates the positive affect of TCR stimulation on this subset.

4) *The authors indicate that γδIFN cells display anti-tumor properties while γδ17 cells display the opposite. Hence, the importance in establishing the metabolic requirements for these two distinct subsets which can have a profound impact on tumor immunity. The authors show a direct link between glucose uptake and the enhanced ability of γδIFN cells to reduce tumor volume. However, similar experiments were not performed showing the link between increased lipid intake and worse prognosis of tumor inoculation experiments. All is shown is that high-fat diet can increase γδ17 numbers in tumors, but whether this leads to a loss of tumor growth control or increased tumor growth is uncertain.*

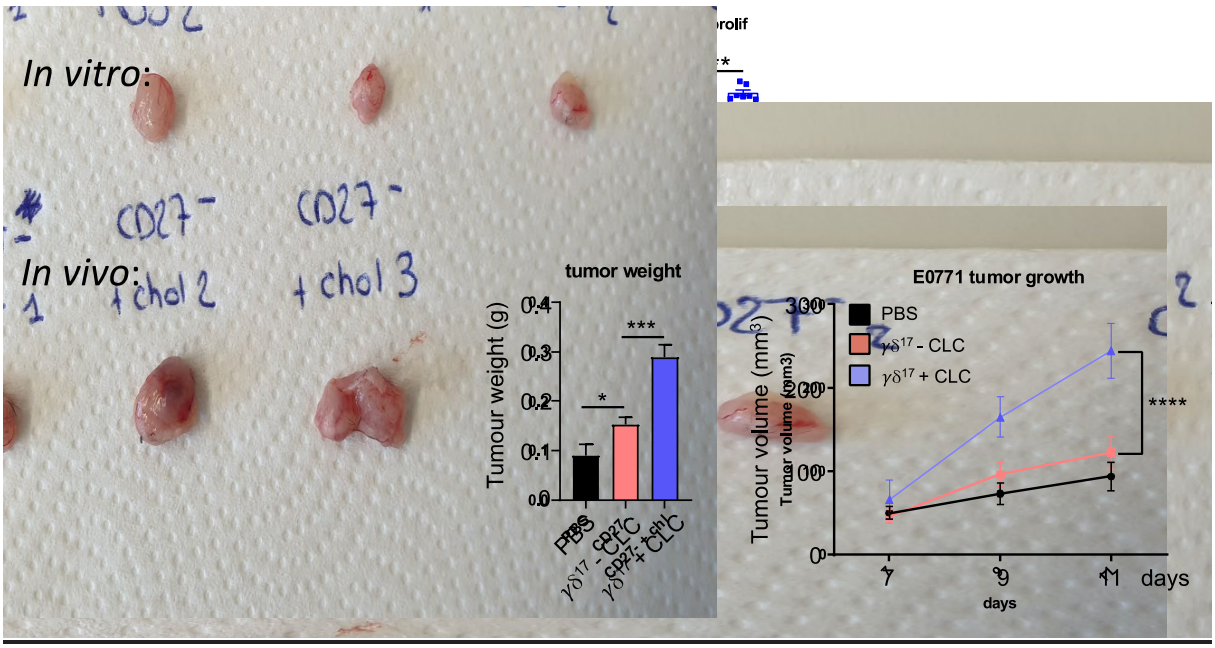
We thank the Reviewer for this comment that has led us to add of a series of new data sets to the revised paper. First, we have added data showing that tumour growth is enhanced by a high fat diet (**Fig. 6g**). Second, we have performed the converse experiment of reducing lipid uptake *in vivo* using orlistat (which inhibits lipases and thus prevents uptake of dietary fat). Orlistat reduced B16 tumour growth and decreased γδ17 cells within the tumour lesions, as well as their lipid content. These data we added as a new **Supplementary Fig. 7**.



Third, to directly address the Reviewer's comment, we sorted CD27- γδ T cells (from peripheral LN) and incubated them (or not) with **cholesterol** for 5hr, before injecting them twice into E0771 tumour lesions (as previously done with glucose for CD27+ γδ cells in Fig. 7). Strikingly, we found that **cholesterol supplementation**:

> increased CD27- γδ cell proliferation *in vitro* (new **Fig. 6k**);

> **increased tumour growth** upon transfer of CD27- γδ cells ***in vivo*** (new **Fig. 6l-n**).



Reviewer #2:

The study from Pennington and colleagues starts with the observation that within TIL, the γδ T cell subsets making either IFN-γ or IL-17 have intrinsically distinct metabolic requirements. Next, they systematically employ the available toolbox to characterize oxphos metabolism in gdT17 versus a more glycolysis-dependent metabolic state of gdT1 lymphocytes. Finally, in vitro glucose supplementation enhanced IFN-g production and thus anti-tumour functions of gdT1 cells and reduced tumour growth upon adoptive transfer.

The study is novel and original. And the methodology employed is state-of-the-art, while the ZENITH protocol is particularly helpful to investigate small numbers of cells. Still, some major issues should be clarified before publication.

5) *Title: From the data presented, it does not directly follow that <Distinct metabolic programmes control the effector fate of γδ T cell subsets and their activities in the tumour microenvironment>. There is a strong association and correlation in the FTOC, but I am not convinced that the*

metabolic programmes do control the choice of effector fate. This is supported by their own conclusion line 233 < that γδTN cells have already committed to an effector fate>.

We agree that the original title was not as clear as it should have been. It was our intention to emphasize that the metabolic programs are established in the thymus, and that these metabolic programs then influence (as demonstrated by the manipulations of these programs with glucose and cholesterol) γδ T cell effector functions in tumour microenvironments. Thus, to address this, we have modified the title to:

"**Distinct metabolic programmes established in the thymus control effector functions of γδ T cell subsets in tumour microenvironments**"

6) *Introduction: Only publications from Erica Pearce are cited, this appears a bit biased.*

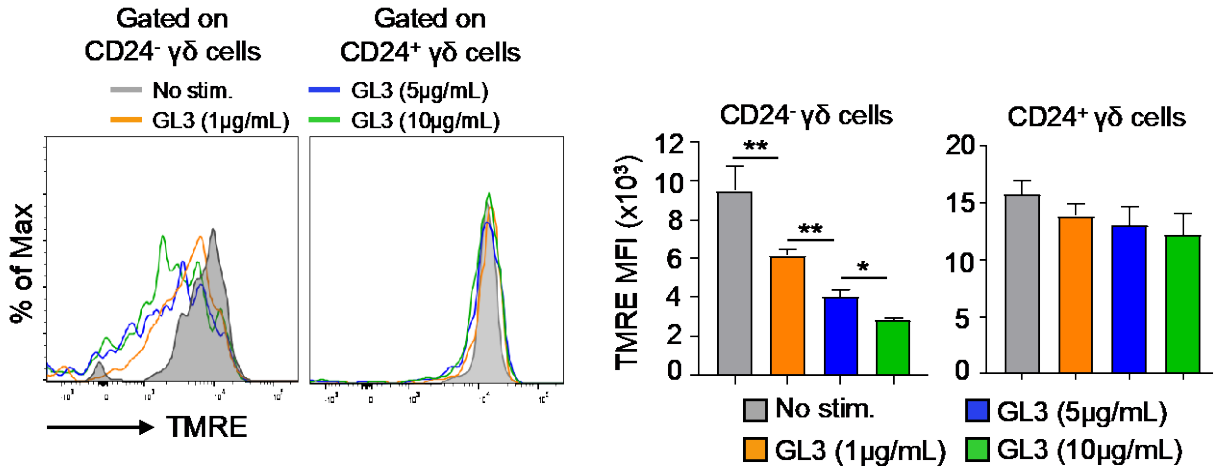
We have replaced previous **ref. 1** from the Pearce lab by the following review from the Sparwasser lab:

Almeida L, Lochner M, Berod L, Sparwasser T. 2016. Metabolic pathways in T cell activation and lineage differentiation. *Semin Immunol* 28(5):514-524.

We feel the remaining references from the Pearce lab are necessary on the basis of specific content.

7) *The authors suggest but do not show that γδ progenitors receiving agonist TCRγδ signals shift away from OXPHOS as indicated by their reduced ΔΨm. It would help to actually do the experiment and show whether TCR engagement, e.g. via plate-bound anti gdTCR or anti- CD3e mAb would lead to lower TMRE levels (associated with adoption of Type 1 immunity phenotype).*

We thank the Reviewer for making this important point and suggestion. We have now performed triplicate independent experiments that demonstrate that a small population of **CD24+ TMREhi** γδ progenitors indeed downregulates TMRE, concomitant with CD24, upon TCR stimulation and in a GL3 mAb dose-dependent manner – these data were added to the revised paper as new **Fig. 4h**:



8) *In line 281 the reference 21 cited for the notion is a review. Are there original sources?*

We have now also cited the following original papers:

Lu Y, Cao X, Zhang X, Kovalovsky D. 2015. PLZF Controls the Development of Fetal-Derived IL-17^{+}Vγ6^{+} γδ T Cells. *J Immunol* 195(9): 4273-4281.

Kohlgruber AC, Gal-Oz ST, LaMarche NM, et al. 2018. γδ T cells producing interleukin-17A regulate adipose regulatory T cell homeostasis and thermogenesis. *Nat Immunol* 19(5): 464-474.

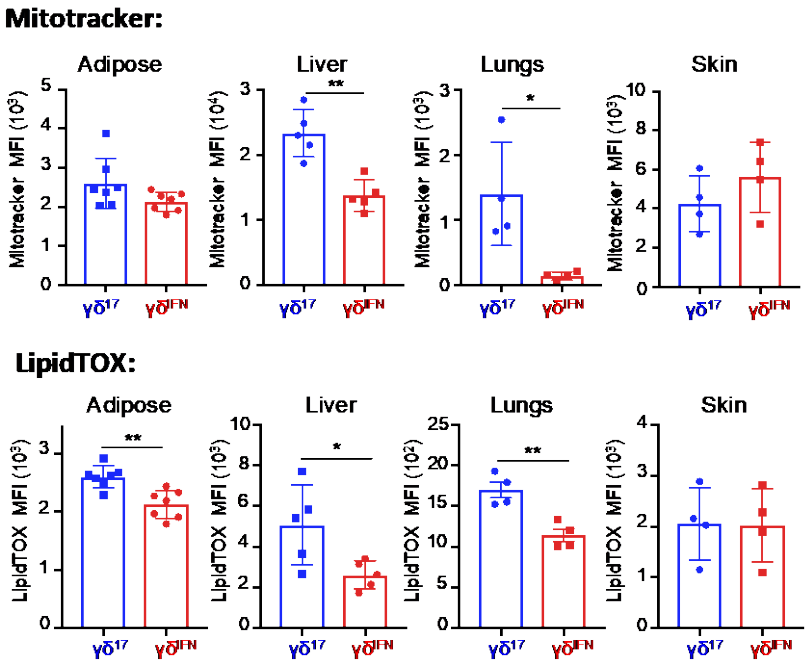
9) *The study could benefit from the analysis of gdT17 cells (eventually compared vs gd T1?) in more tissues, at least in the skin (see e.g. PMID: 21339323) where these gdT17 cells are abundant. Similarly, how are gd T1 and gd T17 doing metabolically in fat tissue?*

In the figure below, we show mitotracker and lipidTOX staining in γδ17 versus γδIFN cells isolated from adipose tissue, liver, lungs and skin. We have added the lipidTOX data as **Fig. 5e** in the revised paper.

Regarding adipose tissue, we have found that all cell types, including CD4+, CD8+ and NKT cells, have higher mitotracker than their counterparts in other organs, which is interesting but out of the scope of the present study. In terms of lipidTOX, the adipose tissue behaves similarly to the other organs (i.e. γδ17 > γδIFN).

As specifically anticipated by the Reviewer, the skin (total skin prep, combining dermis and epidermis) is the truly different organ, since we do not find differences between γδ17 and γδIFN cells, neither in terms of mitotracker nor lipidTOX. We have now commented on this….

"Skin-resident γδ T cells, in particular the Vγ6+ subset have been shown to be transcriptionally distinct from those in pLNs (Tan et al. 2019 Cell Reports). In the skin this subset of γδ T cells displays a highly activated effector phenotype and proliferates at a reduced level compared to those in the pLN. We propose this tissue-specific adaptation changes the metabolic requirements of γδ T cells and therefore may explain the similar levels of mitotracker and lipidTOX staining between the γδ17 and γδIFN cells. Additionally, γδ T cells may adapt to utilize specific metabolites present in the skin, as has been shown for memory T cells (McCully et al. 2015 J. Immunol)."

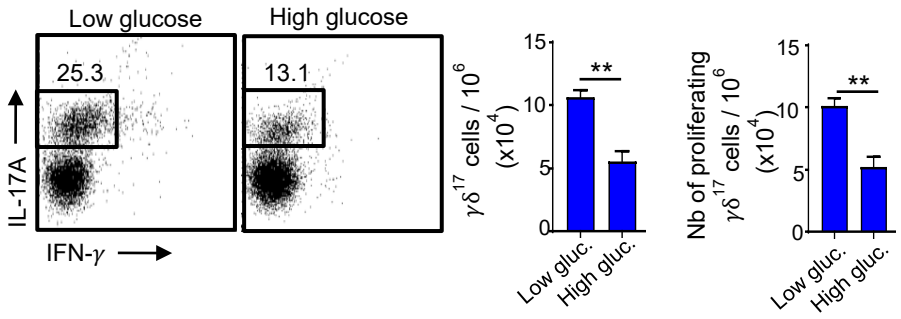


10) *The description of adult (Fig. 3a) and newborn (Fig. 3b) thymus does ignore the idea that gdT17 cells in adult thymus are likely fetal thymus-derived and locally adapt to persist as effectors in the thymus (see e.g. PMID: 31216482).*

We fully agree, but don't think it's necessary to cite this paper (now discussed in response to point 9 above) again at this stage of the results, given that we are highlighting metabolic differences between γδ17 versus γδIFN cells that are conserved throughout ontogeny and that are important with respect to the tumour microenvironment.

11) *To complement the data shown in figure 7, how are gd T17 cells doing when cultured ex vivo under high vs low glucose conditions?*

We thank the Reviewer for pointing this out; we have now added data that demonstrate a reduction in IL-17-producing γδ T cells, and specifically in their proliferation, upon glucose supplementation; now included as a new **Supplementary Fig. 8**:



Minor issues:

- Fig 6a axis labeling and description in Results section are suboptimal.

We have modified the axis labels on the graph and added more labels to make it clearer. We have also expanded our explanation in the results section to improve clarity.

- Fig S1 seems like copy and paste from Figure 1 of related and cited preprint of the ZENITH by Phillipe Pierre's group?

Thank you for spotting this; we apologize. We have now prepared a different **Supplementary Fig. 1** for the revised paper.

- In the discussion, the authors draw parallels with recent findings in ab T cells / Th17 cells (ref. 34). A discussion about the metabolic requirements of ILC1 versus ILC3 could be inspiring, because those should differentiate independent of TCR signals.

Metabolic differences between ILC1 and ILC3 have not been specifically reported. A recent paper on ILC3 (Di Luccia et al. *J Exp Med* 2019) showed a mixed phenotype in the sense of

reprogramming towards glycolysis upon activation while requiring mitochondrial production of ROS to stabilize the ILC3 programme, namely RORγt expression. We have now briefly mentioned this study in the discussion of the revised manuscript (**page 16**).

Decision Letter, first revision:

Subject: Nature Immunology - NI-A29750A pre-edit

Message: Our ref: NI-A29750A

30th Oct 2020

Dear Dr. Pennington,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Immunology manuscript, "Distinct metabolic programmes established in the thymus control effector functions of γδ T cell subsets in tumour microenvironments" (NI-A29750A). Please follow the instructions provided here and in the attached files, as the formal acceptance of your manuscript will be delayed if these issues are not addressed.

When you upload your final materials, please include a point-by-point response to the points below. We won't be able to proceed further without this detailed response.

General formatting:
1. Our standard word limit is 4500 words for the Introduction, Results and Discussion. Please cut accordingly.

2. Please include a separate "Data availability" subsection at the end of your Online Methods. This section should inform our readers about the availability of the data used to support the conclusions of your study and should include references to source data, accession codes to public repositories, URLs to data repository entries, dataset DOIs, and any other statement about data availability. We strongly encourage submission of source data (see below) for all your figures. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, these should be included in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see: http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf.

3. As a guideline, Articles allow up to 50 references in the main text. An additional 20 references can be included in the Online Methods. Only papers that have been published or accepted by a named publication or recognized preprint server should be in the numbered list. Published conference abstracts, numbered patents and research data sets that have been assigned a digital object identifier may be included in the reference list.

4. All references must be cited in numerical order. Place Methods-only references after the Methods section and continue the numbering of the main reference list (i.e., do not start at 1).

5. Genes must be clearly distinguished from gene products (e.g., "gene Abc encodes a kinase," not "gene Abc is a kinase"). For genes, provide database-approved official symbols (e.g., NCBI Gene, http://www.ncbi.nlm.nih.gov/gene) for the relevant species the first time each is mentioned; gene aliases may be used thereafter. Italicize gene symbols and functionally defined locus symbols; do not use italics for proteins, noncoding gene products and spelled-out gene names.

Figures and Tables:

6. All figures and tables, including Extended Data, must be cited in the text in numerical order.

7. Figure legends should be concise. Begin with a brief title and then describe what is presented in the figure and detail all relevant statistical information, avoiding inappropriate methodological detail.

8. All relevant figures must have scale bars (rather than numerical descriptions of magnification).

9. All relevant figures must have defined error bars.

10. Graph axes should start at zero and not be altered in scale to exaggerate effects. A 'broken' graph can be used if absolutely necessary due to sizing constraints, but the break must be visually evident and should not impinge on any data points.

11. All bar graphs should be converted to a dot-plot format or to a box-and-whisker format to show data distribution. All box-plot elements (center line, limits, whiskers, points) should be defined.

12. When submitting the revised version of your manuscript, please pay close attention to our href="https://www.nature.com/nature-research/editorial-policies/image-integrity">Digital Image Integrity Guidelines. and to the following points below:

-- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
-- that control panels for gels and western blots are appropriately described as loading on sample processing controls
-- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Statistics and Reproducibility:

13. The Methods must include a statistics section where you describe the statistical tests used. For all statistics (including error bars), provide the EXACT n values used to calculate the statistics (reporting individual values rather than a range if n varied among experiments) AND define type of replicates (e.g., cell cultures, technical replicates). Please

avoid use of the ambiguous term "biological replicates"; instead state what constituted the replicates (e.g., cell cultures, independent experiments, etc.). For all representative results, indicate number of times experiments were repeated, number of images collected, etc. Indicate statistical tests used, whether the test was one- or two-tailed, exact values for both significant and non-significant P values where relevant, F values and degrees of freedom for all ANOVAs and t-values and degrees of freedom for t-tests.

14. Reporting Guidelines– Attached you will find an annotated version of the Reporting Summary you submitted, along with a Word document indicating revisions that need to be made in compliance with our reproducibility requirements. These documents detail any changes that will need to be made to the text, and particularly the main and supplementary figure legends, including (but not limited to) details regarding sample sizes, replication, scale and error bars, and statistics. Please use these documents as a guide when preparing your revision and submit an updated Reporting Summary with your revised manuscript. The Reporting Summary will be published as supplementary material when your manuscript is published.

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All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

25 EXTENDED DATA: Extended Data are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data figures, and each must be referred to in the main text. Each Extended Data figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

26 SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Tables containing large data sets should be in Excel format, with

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Other
28 As mentioned in our previous letter, all corresponding authors on a manuscript should have an ORCID – please visit your account in our manuscript system to link your ORCID to your profile, or to create one if necessary. For more information please see our previous letter or visit www.springernature.com/orcid.

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We ask that you aim to return your revised paper within 7 days. If you have any further questions, please feel free to contact me.

Best regards,

Zoltan Fehervari, Ph.D.
Senior Editor
Nature Immunology

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z.fehervari@nature.com

Reviewer #1:
Remarks to the Author:

The authors have fully addressed all the initials concerns and have significantly strengthened the conclusions and impact of their work.

Reviewer #2:
Remarks to the Author:
The authors have convincingly addressed and replied to the concerns of both reviewers. Furthermore, they performed additional experiments that support the stimulating hypothesis that diet might influence anti-tumor immunity via balancing gd17 vs. gd1 activity and homeostasis in TIL.

Final Decision Letter:

Subject: Decision on Nature Immunology submission NI-A29750B

Message: In reply please quote: NI-A29750B

Dear Dr. Pennington,

I am delighted to accept your manuscript entitled "Distinct metabolic programs established in the thymus control effector functions of γδ T cell subsets in tumour microenvironments" for publication in an upcoming issue of Nature Immunology.

The manuscript will now be copy-edited and prepared for the printer. Please check your calendar: if you will be unavailable to check the galley for some portion of the next month, we need the contact information of whom will be making corrections in your stead. When you receive your galleys, please examine them carefully to ensure that we have not inadvertently altered the sense of your text.

Acceptance is conditional on the data in the manuscript not being published elsewhere, or announced in the print or electronic media, until the embargo/publication date. These restrictions are not intended to deter you from presenting your data at academic meetings and conferences, but any enquiries from the media about papers not yet scheduled for publication should be referred to us.

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Sincerely,

Zoltan Fehervari, Ph.D.
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