nature research

# Peer Review Information

**Journal:** Nature Cell Biology
**Manuscript Title:** Dapl1 controls NFATc2 activation to regulate CD8+ T cell exhaustion and responses in chronic infection and cancer
**Corresponding author names:** Shao-Cong Sun, Le-Le Zhu

## Reviewer Comments & Decisions:

**Decision Letter, initial version:**

Subject: Decision on Nature Cell Biology submission NCB-S45561
Message:

*Please delete the link to your author homepage if you wish to forward this email to co-authors.

Dear Dr. Sun,

Please first accept our apology for the delay getting back to you with a decision due to difficulties in retrieving reviewers' comments.

Your manuscript, "Dapl1 suppresses antitumor CD8 T cell responses by controlling NFAT1 activation", has now been seen by 3 referees, who are experts in T cells, tumor immunology (referee 1); and T cell biology (referee 2 and 3). As you will see from their comments (attached below) they find this work of potential interest, but have raised substantial concerns, which in our view would need to be addressed with considerable revisions before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

A) Strengthen the proposed molecular and cellular mechanism:

Reviewer 1
"The authors showed that Dapl1 regulates NFAT1 and that NFAT1 activation caused by Dapl1 deletion could increase T cells functions. A study from Martinez et al. published in Immunity in 2015 showed that NFAT drives the exhaustion program when constitutively active and unable to bind AP-1. Could the authors validate if this exhaustion program induced by NFAT is regulated in the absence of Dapl1? The overexpression of the CA-RIT-NFAT and CA-DBD-RIT-NFAT in Dapl1 and NFAT KO could help in answering this question. This is important to understand how sustained NFAT activation in Dapl1-deficienct T cells would not induce exhaustion. The information of this answer can largely strengthen the usage of Dapl1-targeting for cell therapy."

"The authors showed that the absence of Dapl1 increases Tim3 expression and the data suggest that the mechanism is controlled by NFAT with limited analyses. Investigating the mechanism by which Dapl1 deficiency drives Tim3 upregulation is needed."

"The authors suggest that Dapl1 regulates the dynamic of tumor infiltrating CD8 T cells by decreasing the progenitor population in cluster 1 and increasing the effector memory cells of cluster 3. However, as shown in suppl figure1, Dapl1 is expressed mainly in the progenitor-like T cells in cluster 1. Could the authors explain more in detail the role of Dapl1 in the progenitor population? Is the alteration residing in the progenitor population which leads to an increase in their differentiation into effectors, or the progenitors are formed less efficiently and therefore the effectors increase? A inducible targeting approach may provide a bit more details on this differentiation process and the answer for this critical question."

Reviewer 3
"Similarly, the mechanism by which Dapl1 enhances T cell function remain unclear. While fully resolving this would be beyond the scope of the present manuscript, a better quantification of the antigen-specific T-cell response would be very helpful in this respect. To date, most data stem from analyzing total TILs. A Better characterization of the kinetics/number and phenotype of OT-1 and pMel cells would be helpful."

"Figure 6: The interpretation that Nfat1 is needed for the DAP1 effect and for the effect on Tim-3 is quite far-stretched. Given that Nfat1 is involved in T cell activation, it is not overly surprising that it is required for the effects seen by the authors. Therefore, considering that the absence of nfat1 has a fairly global effect on T cell function, the interpretation should be significantly revised"

"Figure 7: Along the same lines, the negative regulatory function of Dapl1 on nfat1 is not well established. It should at least be complemented by an overexpression experiment."

B) Address the concern related to the intrinsic versus extrinsic role for Dapl1:

Reviewer 2
"The authors make sue of a full body Dapl1 KO mouse in many of their experiments. Although they make a strong point that Dapl1 acts specifically in CD8 T cells, it is difficult to rule out functions in other cells such as Tregs. Thus, key elements of the study should be performed in mixed bone marrow chimers, including LCMV infection and some tumor experiments."

"The authors have performed some studies using Dapl1-deficient TCR transgenic CD8 T cells. While their results support the conclusion that dapl1 acts CD8 T cell intrinsically, these experiments should be repeated with a mixed population approach, ie congenically marked WT and KO TCR transgenic T cells should be cotransferred and their phenotype and function should be examined within the same mouse, ie within the same tumor. Mixed bone marrow chimeric mice would serve the same purpose."

C) Provide further evidence for the claimed exclusive role for Dapl1 in regulating T cell exhaustion:

Reviewer 3
"Throughout the MS and abstract, the authors portray Dapl1 as a tumor- or T-cell exhaustion-specific repressor of T-cell function. While the repressive function is well established in this context, I am concerned about the proclaimed exclusive function. In fact, I believe that the data presented by the authors already show that similar findings can be made in acute Listeria infection. This would not make the finding less attractive but an exclusive function of Dapl1 in tumors is not clearly supported yet."

D) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes, as applicable should also be addressed.

E) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript. In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the

figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We would be happy to consider a revised manuscript that would satisfactorily address these points, unless a similar paper is published elsewhere, or is accepted for publication in Nature Cell Biology in the meantime.

When revising the manuscript please:

- ensure that it conforms to our format instructions and publication policies (see below and https://www.nature.com/nature/for-authors).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Reporting Summary (found here https://www.nature.com/documents/nr-reporting-summary.pdf). This is essential for reconsideration of the manuscript will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

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.

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This journal strongly supports public availability of data. Please place the data used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. Please note that for some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories appears below.

Please submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

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We would like to receive a revised submission within six months.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Zhe Wang

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Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
In this manuscript, the authors identified Dapl1 as a regulator of CD8+ T cell exhaustion. Dapl1 expression is restricted to the progenitor like subset important to maintain the antitumoral immunity. Indeed, Dapl1 depletion expanded a population of effector memory-like CD8 T cells, characterized by the concomitant expression of TCF7 and Tim3, and prevented their functional exhaustion which results in increased tumor control. The authors further showed that Dapl1 controls the activation of NFAT1 which is required for the T cells effector functions and that NFAT1 overactivation prevented functional exhaustion of CD8 TILS. In conclusion, Dapl1 depletion rescued NFAT1 activation and prevented the loss of effector function thereby providing a greater tumor control. Overall, the novelty of this work is highly appreciated. However, several points should be addressed to increase the significance and strength of the manuscript.

- The authors showed that Dapl1 regulates NFAT1 and that NFAT1 activation caused by Dapl1 deletion could increase T cells functions. A study from Martinez et al. published in Immunity in 2015 showed that NFAT drives the exhaustion program when constitutively active and unable to bind AP-1. Could the authors validate if this exhaustion program induced by NFAT is regulated in the absence of Dapl1? The overexpression of the CA-RIT-NFAT and CA-DBD-RIT-NFAT in Dapl1 and NFAT KO could help in answering this question. This is important to understand how sustained NFAT activation in Dapl1-deficienct T cells would not induce exhaustion. The information of this answer can largely strengthen the usage of Dapl1-targeting for cell therapy.

- The authors showed that the absence of Dapl1 increases Tim3 expression and the data suggest that the mechanism is controlled by NFAT with limited analyses. Investigating the mechanism by which Dapl1 deficiency drives Tim3 upregulation is needed.

- The authors showed in figure 2d that the percentage of TCF1+ CD8 T cells doesn't change in Dapl1 KO mice. However in figure 4a and 4b, they showed that the population of progenitor-like cells expressing TCF1 decreases in the absence of Dapl1. A discussion on that would clarify the interpretation of the data If lineage tracing experiment can not be done.

- The authors suggest that Dapl1 regulates the dynamic of tumor infiltrating CD8 T cells by decreasing the progenitor population in cluster 1 and increasing the effector memory cells of cluster 3. However, as shown in suppl figure1, Dapl1 is expressed mainly in the progenitor-like T cells in cluster 1. Could the authors explain more in detail the role of Dapl1 in the progenitor population? Is the alteration residing in the progenitor population which leads to an increase in their differentiation into effectors, or the progenitors are formed less efficiently and therefore the effectors increase? A inducible targeting

approach may provide a bit more details on this differentiation process and the answer for this critical question.

- The authors show that the KO of Dapl1 in human CD8 resemble the increased effector phenotype described in mice. However, is Dapl1 also expressed in human TILS and does its expression correlate with TCF expression? Dapl1 expression analysis in human TILs would also support this finding.

- In Figure 1, analyzing IFNg, TNFa and Il2 production in adoptively transferred Dapl1-deficient Pmel1 or WT T cells in melanoma bearing mice is needed.

Reviewer #2:
Remarks to the Author:
In this manuscript, Zhu et al identify death-associated protein like 1 (Dapl1) as a crucial regulator of CD8 T cell immunity. They show that Dapl1 is specifically expressed in CD8 T cells, and its expression in tumor-infiltrating lymphocytes (TILs) defines a progenitor-like CD8 T cell subpopulation known to be crucial for maintaining anticancer immunity. Dapl1 deletion expands effector memory-like CD8 TILs and prevents their functional exhaustion, coupled with profoundly increased antitumor immunity and improved efficacy of adoptive T cell therapy. Mechanistically, the authors show that Dapl1 controls activation of NFATC2, a transcription factor required for the effector function of CD8 T cells. They further show that although NFATC2 also mediates induction of the immune checkpoint Tim3, competent NFATC2 activation prevents functional exhaustion of CD8 TILs. Finally, they show that exhausted CD8 T cells display attenuated NFATC2 activation due to Tim3-mediated feedback inhibition, and Dapl1 deletion rescues NFATC2 activation, thereby unleashing the antitumor function of CD8 T cells. These findings establish Dapl1 as a pivotal regulator of antitumor immunity and implicates Dapl1 as a potential target for cancer immunotherapy.

Overall, this is a very thorough and comprehensive study that sheds light on an as yet unknown mechanisms of T cell exhaustion. The experiments were performed with a high degree of technical and scientific expertise and interpreted in a balanced way. Clearly, an important contribution to our understanding of T cell impairments in tumors and chronic infection.

However, there are a few points the authors should address to further clarify the functions of Dapl1 and NFATC2. One question that remains is how much of the phenotype of Dapl1-Ko cells is CD8 T cell intrinsic? Using full Ko makes it hard to rule out effects of other cell types. Similarly, reduced tumor growth will feed back to the phenotype of the infiltrating T cells. Thus, reduced exhaustion may be due to smaller tumor size.

Major points:
1. The authors make sue of a full body Dapl1 KO mouse in many of their experiments. Although they make a strong point that Dapl1 acts specifically in CD8 T cells, it is difficult to rule out functions in other cells such as Tregs. Thus, key elements of the study should be performed in mixed bone marrow chimers, including LCMV infection and some tumor experiments.
2. The authors have performed some studies using Dapl1-deficient TCR transgenic CD8 T cells. While their results support the conclusion that dapl1 acts CD8 T cell intrinsically, these experiments should be repeated with a mixed population approach, ie congenically marked WT and KO TCR transgenic T cells should be cotransferred and their phenotype and function should be examined within the same mouse, ie within the same tumor. Mixed bone marrow chimeric mice would serve the same purpose.
3. The LCMV clone 13 experiments are important experiments. Please expand on the analysis. A full kinetic analysis in WT and KO, including the analysis of progenitor and terminal exhausted population, including markers such as TCF1, CX3CR1 and additional inhibitory receptors would be critical.
4. The cytokine and granzyme B data in Fig. 3e look a bit odd to me. We never see as much Il-2 but have a much higher expression of granzyme B in our Tim3+ cells. Could the authors check this again. Please also comment whether CD4 T cells were deleted prior or infection or not? Is there a difference in viral control? Again, differences in viral burden may have secondary consequences for the T cell phenotype. Hence, chimeras are important. Overall, the LCMV data should be expanded as they allow a very clear view on the phenotype of Dapl1 deficiency.
5. Dapl1 also seems to play a critical role in acute infection. The authors show some data on Listeria infection. However, do not go into details of the phenotype. This should be expanded and clarified. What is the role of Dapl1 in acute infection (phenotype and function)? They also appear to have done experiments with mixed bone marrow chimeric mice; however, the data presented appear to not take that onto account and don't describe this. Could the authors please elaborate? Further to this, Tim3 does not play a major role in acute infection. How does Dapl1 act in acute infection? Purely via NFATC2?

Additional points
1. Please use the correct nomenclature for NFATC2. NFAT1 is the old name and consistently leads to confusion with NADTC1.
2. Fig. 6h, i, j needs single KO data as controls.
3. How do the authors define progenitor cells within the tumor environment. Apparently, they are PD1-negative, TCF1+ and partly CD62L+. Indeed, the progenitor sort shown in Suppl. Fig. 9a looks like naïve T cells. Are they defined purely by location in the tumor or what criteria are being used?
4. Related to question 3, Fig. 7b seems to use naïve T cells as controls for exhausted T cells. However, the point the authors make is the progenitor cells have higher NFAT activity. This should be shown, ie a comparison between progenitor and effector cells.

5. The statement “NFAT1 mediates both effector function and ICR expression in T cells, but its role in regulating the functionality of exhausted T cells is unclear” (p 16) needs a reference. Overall, there is a lot of work published on the roles of NAFTC1 and NFATC2 in CD8 T cells. However, this is often somewhat confusing and should be placed into context of the authors work. How do the authors reconcile the literature on NFATC1 and NFATC2?
6. The authors state “overexpressed Dapl1 did not bind wildtype NFAT1 but strongly interacted with a constitutively active NFAT1”. This seems confusing to me as it would imply that Dapl1 does not interact with NFATC2 under normal circumstances. Perhaps reword.
7. In their methods, they describe retroviral overexpression in bone marrow cells. However, I could not find data related to such an experiment. Or was Dapl1 overexpression I T cell achieved by overexpressing it throughout development?
8. Can the authors comment on the expression of TOX, IRF4 and BATF in Dapl1 or NFATC2-deficient mice. All three molecules have been implicated in exhaustion and have been shown to be downstream of NFAT. Are they involved? At least TOX should be measured in key experiments.
9. The authors should show some supplementary data related to their in vitro exhausted T cell cultures in mouse and human T cells. These cultures are not well established, thus require some validation.

Reviewer #3:
Remarks to the Author:
Zhu at al. report on the interesting finding that the absence of Dap1 promotes survival, increases the number and IFNg production of tumor or pathogen-specific CD8 T cells, and potentiates checkpoint inhibition via PD-1. Furthermore, the authors link Dapl1 activity to reduced Nfat1 signaling and overall conclude that Dapl1 is a potent regulator of T cell effector function in tumors. While these aspects are well elaborated, there are critical points that need to be addressed.

Key points:

Throughout the MS and abstract, the authors portray Dapl1 as a tumor- or T-cell exhaustion-specific repressor of T-cell function. While the repressive function is well established in this context, I am concerned about the proclaimed exclusive function. In fact, I believe that the data presented by the authors already show that similar findings can be made in acute Listeria infection. This would not make the finding less attractive but an exclusive function of Dapl1 in tumors is not clearly supported yet.

Similarly, the mechanism by which Dapl1 enhances T cell function remain unclear. While fully resolving this would be beyond the scope of the present manuscript, a better quantification of the antigen-specific T-cell response would be very helpful in this respect. To date, most data stem from analyzing

total TILs. A Better characterization of the kinetics/number and phenotype of OT-1 and pMel cells would be helpful.

The authors begin the presentation of their results by claiming that they identified the expression of Dap1 in the Tcf-1+ population via scRNA sequencing and that this motivated them to generate a Dap1 KO-mouse. However. this timeline does not fit with the fact that Supp1A and Figure 4A are obviously from the same cluster analysis. Therefore, the authors should clarify the time when these datasets were obtained or explain what types of batch corrections (not mentioned in the methods so far) were applied if separately obtained datasets were merged afterwards. This issue does not call into question the main results, but it is not ideal if there is uncertainty about the dataset that provided the impetus for the project.

Figure 6: The interpretation that Nfat1 is needed for the DAP1 effect and for the effect on Tim-3 is quite far-stretched. Given that Nfat1 is involved in T cell activation, it is not overly surprising that it is required for the effects seen by the authors. Therefore, considering that the absence of nfat1 has a fairly global effect on T cell function, the interpretation should be significantly revised.

Figure 7: Along the same lines, the negative regulatory function of Dapl1 on nfat1 is not well established. It should at least be complemented by an overexpression experiment.

Minor points:

Figure 3f: The legend leaves unclear what is shown.

Figure 4c: The legend does not match what is shown. Legend says marker expression is shown in precursor, effector, and depleted clusters, but shown is the marker expression in 6 clusters.

Figure 4d: should include Tcf-7 data.

Figure 6d: no peptide control is missing.

GUIDELINES FOR MANUSCRIPT SUBMISSION TO NATURE CELL BIOLOGY

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

MANUSCRIPT FORMAT – please follow the guidelines listed in our Guide to Authors regarding manuscript formats at Nature Cell Biology.

TITLE – should be no more than 100 characters including spaces, without punctuation and avoiding technical terms, abbreviations, and active verbs..

AUTHOR NAMES – should be given in full.

AUTHOR AFFILIATIONS – should be denoted with numerical superscripts (not symbols) preceding the names. Full addresses should be included, with US states in full and providing zip/post codes. The corresponding author is denoted by: "Correspondence should be addressed to [initials]."

ABSTRACT AND MAIN TEXT – please follow the guidelines that are specific to the format of your manuscript, as listed in our Guide to Authors (http://www.nature.com/ncb/pdf/ncb_gta.pdf) Briefly, Nature Cell Biology Articles, Resources and Technical Reports have 3500 words, including a 150 word abstract, and the main text is subdivided in Introduction, Results, and Discussion sections. Nature Cell Biology Letters have up to 2500 words, including a 180 word introductory paragraph (abstract), and the text is not subdivided in sections.

ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

FINANCIAL AND NON-FINANCIAL COMPETING INTERESTS – the authors must include one of three declarations: (1) that they have no financial and non-financial competing interests; (2) that they have financial and non-financial competing interests; or (3) that they decline to respond, after the Author Contributions section. This statement will be published with the article, and in cases where financial and non-financial competing interests are declared, these will be itemized in a web supplement to the article. For further details please see https://www.nature.com/licenceforms/nrg/competing-interests.pdf.

REFERENCES – are limited to a total of 70 for Articles, Resources, Technical Reports; and 40 for Letters. This includes references in the main text and Methods combined. References must be numbered sequentially as they appear in the main text, tables and figure legends and Methods and must follow the precise style of Nature Cell Biology references. References only cited in the Methods should be numbered consecutively following the last reference cited in the main text. References only associated with Supplementary Information (e.g. in supplementary legends) do not count toward the total reference limit and do not need to be cited in numerical continuity with references in the main text. Only published papers can be cited, and each publication cited should be included in the numbered reference list, which should include the manuscript titles. Footnotes are not permitted.

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Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words. The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled “Statistics and Reproducibility”.

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policy see http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf. The Data availability statement should include:

• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

• Unique identifiers (accession codes, DOIs or other unique persistent identifier) and hyperlinks for datasets deposited in an approved repository, but for which data deposition is not mandated (see here for details http://www.nature.com/sdata/data-policies/repositories).

• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information), listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

• If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Methods.

We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

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All imaging data should be accompanied by scale bars, which should be defined in the legend. Cropped images of gels/blots are acceptable, but need to be accompanied by size markers, and to retain visible background signal within the linear range (i.e. should not be saturated). The boundaries of panels with low background have to be demarked with black lines. Splicing of panels should only be considered

if unavoidable, and must be clearly marked on the figure, and noted in the legend with a statement on whether the samples were obtained and processed simultaneously. Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, it should only be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

Figures should be provided at approximately the size that they are to be printed at (single column is 86 mm, double column is 170 mm) and should not exceed an A4 page (8.5 x 11"). Reduction to the scale that will be used on the page is not necessary, but multi-panel figures should be sized so that the whole figure can be reduced by the same amount at the smallest size at which essential details in each panel are visible. In the interest of our colour-blind readers we ask that you avoid using red and green for contrast in figures. Replacing red with magenta and green with turquoise are two possible colour-safe alternatives. Lines with widths of less than 1 point should be avoided. Sans serif typefaces, such as Helvetica (preferred) or Arial should be used. All text that forms part of a figure should be rewritable and removable.

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- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars

etc.). The only parts of the figure that should be in pixel raster/bitmap format are photographic images or 3D rendered graphics/complex technical illustrations.

All placed images (i.e. a photo incorporated into a figure) should be on a separate layer and independent from any superimposed scale bars or text. Individual photographic images must be a minimum of 300+ DPI (at actual size) or kept constant from the original picture acquisition and not decreased in resolution post image acquisition. All colour artwork should be RGB format.

FIGURE LEGENDS – must not exceed 350 words for each figure to allow fit on a single printed NCB page together with the figure. They must include a brief title for the whole figure, and short descriptions of each panel with definitions of the symbols used, but without detailing methodology.

TABLES – main tables should be provided as individual Word files, together with a brief title and legend. For supplementary tables see below.

SUPPLEMENTARY INFORMATION – Supplementary information is material directly relevant to the conclusion of a paper, but which cannot be included in the printed version in order to keep the manuscript concise and accessible to the general reader. Supplementary information is an integral part of a Nature Cell Biology publication and should be prepared and presented with as much care as the main display item, but it must not include non-essential data or text, which may be removed at the editor's discretion. All supplementary material is fully peer-reviewed and published online as part of the HTML version of the manuscript. Supplementary Figures and Supplementary Notes are appended at the end of the main PDF of the published manuscript.

Supplementary items should relate to a main text figure, wherever possible, and should be mentioned sequentially in the main manuscript, designated as Supplementary Figure, Table, Video, or Note, and numbered continuously (e.g. Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2 etc.).

Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labelled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

The total number of Supplementary Figures (not including the “unprocessed scans” Supplementary Figure) should not exceed the number of main display items (figures and/or tables (see our Guide to Authors and March 2012 editorial http://www.nature.com/ncb/authors/submit/index.html#suppinfo; http://www.nature.com/ncb/journal/v14/n3/index.html#ed). No restrictions apply to Supplementary Tables or Videos, but we advise authors to be selective in including supplemental data.

Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be accompanied by a separate Word document including titles and legends.

GUIDELINES FOR EXPERIMENTAL AND STATISTICAL REPORTING

REPORTING REQUIREMENTS – We are trying to improve the quality of methods and statistics reporting in our papers. To that end, we are now asking authors to complete a reporting summary that collects information on experimental design and reagents. The Reporting Summary can be found here https://www.nature.com/documents/nr-reporting-summary.pdf)If you would like to reference the guidance text as you complete the template, please access these flattened versions at http://www.nature.com/authors/policies/availability.html.

STATISTICS – Wherever statistics have been derived the legend needs to provide the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), and define what this value represents. Error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). Box plots need to be defined in terms of minima, maxima, centre, and percentiles. Ranges are more appropriate than standard errors for small data sets. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test used needs to be stated in the legend. Statistics such as error bars must not be derived from $n<3$. For sample sizes of $n<5$ please plot the individual data points rather than providing bar graphs. Deriving statistics from technical replicate samples, rather than biological replicates is strongly discouraged. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test stated in the legend.

Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled "Statistics Source Data", and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

**Author Rebuttal to Initial comments**

**Reviewer #1:**

Remarks to the Author:

In this manuscript, the authors identified Dapl1 as a regulator of CD8+ T cell exhaustion. Dapl1 expression is restricted to the progenitor like subset important to maintain the antitumoral immunity. Indeed, Dapl1 depletion expanded a population of effector memory-like CD8 T cells, characterized by the concomitant expression of TCF7 and Tim3, and prevented their functional exhaustion which results in increased tumor control. The authors further showed that Dapl1 controls the activation of NFAT1 which is required for the T cells effector functions and that NFAT1 overactivation prevented functional exhaustion of CD8 TILS. In conclusion, Dapl1 depletion rescued NFAT1 activation and prevented the loss of effector function thereby providing a greater tumor control. Overall, the novelty of this work is highly appreciated. However, several points should be addressed to increase the significance and strength of the manuscript.

**Response:**

We thank the reviewer for the positive comments and recognition of the novelty of our work. As detailed below, we have carefully addressed the reviewer's comments with substantial experiments.

(1) The authors showed that Dapl1 regulates NFAT1 and that NFAT1 activation caused by Dapl1 deletion could increase T cells functions. A study from Martinez et al. published in Immunity in 2015 showed that NFAT drives the exhaustion program when constitutively active and unable to bind AP-1. Could the authors validate if this exhaustion program induced by NFAT is

regulated in the absence of Dapl1? The overexpression of the CA-RIT-NFAT and CA-DBD-RIT-NFAT in Dapl1 and NFAT KO could help in answering this question. This is important to understand how sustained NFAT activation in Dapl1-deficienct T cells would not induce exhaustion. The information of this answer can largely strengthen the usage of Dapl1-targeting for cell therapy.

Response:

We thank the reviewer for this important comment. Following the reviewer's suggestion, we overexpressed CA-RIT-NFATc2 or DBDmut-CA-RIT-NFATc2 in NFATc2 KO or Dapl1 KO OTI CD8 T cells and examined the effect on T cell function and exhaustion by adoptive transfer using B16OVA tumor model (Extended Data Fig. 10e). In line with the work by Martinez et al[1], expression of CA-RIT-NFATc2 in NFATc2 KO OTI CD8 T cells reduced their antitumor function, as evidenced by increased tumor growth and decreased rate of mouse survival, compared to the cells overexpressing a control NFATc2 mutant defective in DNA binding, DBDmut-CARIT-NFATc2 (Extended Data Fig. 10f-h). However, overexpression of CA-RIT-NFATc2 in Dapl1 KO OTI CD8 T cells only moderately reduced their antitumor function (Extended Data Fig. 10f-h). Furthermore, overexpression of CA-RIT-NFATc2 in NFATc2 KO OTI CD8 T cells, but not in Dapl1 KO OTI CD8 T cells, inhibited their function to generate effector CD8 TILs producing IFN-g, TNF-a, and Granzyme B (Extended Data Fig. 10i). These results suggest that that the exhaustion form of NFATc2 could not override the activation-function of the wildtype NFATc2 in Dapl1-deficient CD8 T cells.

(2) The authors showed that the absence of Dapl1 increases Tim3 expression and the data suggest that the mechanism is controlled by NFAT with limited analyses. Investigating the mechanism by which Dapl1 deficiency drives Tim3 upregulation is needed.

**Response:**

We thank the reviewer for this insightful comment. We performed new experiments to address the reviewer's comment. Since *in vivo* induction of Tim3 might involve indirect mechanisms, we performed acute activation of *in vitro* generated effector and exhausted CD8 T cells derived from Dapl1 KO, NFATc2 KO, Dapl1-NFATc2 dKO and wildtype control mice. Upon stimulation with anti-CD3/anti-CD28, both effector and exhausted CD8 T cells expressed Havcr2 mRNA, which was elevated in the Dapl1 KO CD8 T cells (Extended Data Fig. 10a,b). Importantly, NFATc2 deficiency blocked the induction of Havcr2 and erased the differences between Dapl1-sufficient and Dapl1-deficient CD8 T cells (Extended Data Fig. 10a,b). We also performed Chromatin Immunoprecipitation (ChIP) assays to examine whether NFATc2 could directly bind the *Havcr2*

promoter in exhausted antigen (gp33)-specific CD8 T cells isolated from day 30 LCMV clone 13-infected mice. We found that NFATc2 bound a region located between -432 and -5 of the Havcr2 promoter, in the LCMV-specific CD8 T cells, but not in naïve CD8 T cells (Extended Data Fig. 10c). Compared to the wildtype T cells, the Dapl1 KO T cells displayed an increased level of NFATc2 binding activity at the Havcr2 promoter (Extended Data Fig. 10d). These results further suggest that Dapl1 regulates Tim3 expression through controlling NFATc2 activation.

(3) The authors showed in figure 2d that the percentage of TCF1+ CD8 T cells doesn't change in Dapl1 KO mice. However in figure 4a and 4b, they showed that the population of progenitor-like cells expressing TCF1 decreases in the absence of Dapl1. A discussion on that would clarify the interpretation of the data If lineage tracing experiment can not be done.

**Response:**

The reviewer's point is well taken. Our flow cytometry revealed that CD8 TILs contain $TCF1^{hi}$ and $TCF1^{low}$ subpopulations, the latter of which also expressed Tim3 (Fig. 2d). Dapl1 deficiency reduced the $TCF^{hi}$ population and increased the $TCF1^{low}$ ($Tim3^{+}$) population (Fig. 2d). At the RNA level, revealed by scRNAseq (Fig. 4a,b), the $Tcf7(TCF1)^{hi}$ subpopulation (cluster 1) was reduced in Dapl1 KO TILs, whereas cluster 3 (expressing low levels of Tcf7) was increased. So, although protein and RNA expression may not always correlate, both the flow cytometry (Fig. 2d) and scRNAseq (Fig. 4a,b) data indicate that Dapl1 deficiency reduces $TCF1^{hi}$ TILs and increases $TCF1^{low}$ TILs. We discussed this point in the revised manuscript (page 15).

(4) The authors suggest that Dapl1 regulates the dynamic of tumor infiltrating CD8 T cells by decreasing the progenitor population in cluster 1 and increasing the effector memory cells of cluster 3. However, as shown in suppl figure1, Dapl1 is expressed mainly in the progenitor-like T cells in cluster 1. Could the authors explain more in detail the role of Dapl1 in the progenitor population? Is the alteration residing in the progenitor population which leads to an increase in their differentiation into effectors, or the progenitors are formed less efficiently and therefore the effectors increase? A inducible targeting approach may provide a bit more details on this differentiation process and the answer for this critical question.

**Response:**

This is again an excellent point. To address the reviewer's comment, we performed adoptive T cell transfer experiments. We sorted antigen-specific progenitor CD8 T cells ($CD8^{+}gp33^{+}Ly108^{+}$ $Tim\text{-}3^{-}$) from LCMV clone 13-infected WT or Dapl1 KO mice and adoptively transferred the cells to B6.SJL mice bearing B16F10 melanoma expressing an LCMV minigene (B16F10-LCMV mini)

(Extended Data Fig. 8d). We found that on days 3 and 10 after transfer, the Dapl1 KO CD8 T cells had a drastically reduced frequency of TCF1$^{hi}$ and TCF1$^{+}$Tim3$^{-}$ progenitor CD8 T cells (Extended Data Fig. 8e,f) and a concomitantly increased frequency of effector CD8 T cells (Extended Data Fig. 8g) in the tumor, compared to the wildtype CD8 T cells. Although the wildtype and Dapl1 KO progenitor CD8 T cells had comparable levels of apoptosis, the Dapl1 KO progenitor CD8 T cells displayed a significantly higher level Ki67 expression indicative of increased proliferation (Extended Data Fig. 8h,i). These data suggest that Dapl1 deficiency may promote progenitor-like CD8 T cell expansion and conversion to effector CD8 T cells.

(5) The authors show that the KO of Dapl1 in human CD8 resemble the increased effector phenotype described in mice. However, is Dapl1 also expressed in human TILS and does its expression correlate with TCF expression? Dapl1 expression analysis in human TILs would also support this finding.

**Response:**

Following the reviewer's suggestion, we analyzed a human uveal melanoma scRNAseq dataset (GSE139829). As seen in mouse CD8 TILs, Dapl1 was specifically expressed in the Tcf7$^{+}$ human CD8 progenitor TILs (Extended Data Fig. 8a-c).

(6)- In Figure 1, analyzing IFNg, TNFa and Il2 production in adoptively transferred Dapl1-deficient Pmel1 or WT T cells in melanoma bearing mice is needed.

**Response:**

This again an excellent suggestion. We have performed the suggested experiment and found that Dapl1-deficient Pmel1 CD8 cells showed an increased production of IFN-g, TNF-a and IL-2 in the tumor compared to the wildtype Pmel1 CD8 cells (Extended Data Fig. 2e,f). These data suggest that Dapl1 deficiency promote the antitumor effector function of CD8 T cells.

**Reviewer #2:**

Remarks to the Author:

In this manuscript, Zhu et al identify death-associated protein like 1 (Dapl1) as a crucial regulator of CD8 T cell immunity. They show that Dapl1 is specifically expressed in CD8 T cells, and its expression in tumor-infiltrating lymphocytes (TILs) defines a progenitor-like CD8 T cell subpopulation known to be crucial for maintaining anticancer immunity. Dapl1 deletion expands effector memory-like CD8 TILs and prevents their functional exhaustion, coupled with

profoundly increased antitumor immunity and improved efficacy of adoptive T cell therapy. Mechanistically, the authors show that Dapl1 controls activation of NFATC2, a transcription factor required for the effector function of CD8 T cells. They further show that although NFATC2 also mediates induction of the immune checkpoint Tim3, competent NFATC2 activation prevents functional exhaustion of CD8 TILs. Finally, they show that exhausted CD8 T cells display attenuated NFATC2 activation due to Tim3-mediated feedback inhibition, and Dapl1 deletion rescues NFATC2 activation, thereby unleashing the antitumor function of CD8 T cells. These findings establish Dapl1 as a pivotal regulator of antitumor immunity and implicates Dapl1 as a potential target for cancer immunotherapy.

Overall, this is a very thorough and comprehensive study that sheds light on an as yet unknown mechanisms of T cell exhaustion. The experiments were performed with a high degree of technical and scientific expertise and interpreted in a balanced way. Clearly, an important contribution to our understanding of T cell impairments in tumors and chronic infection.

However, there are a few points the authors should address to further clarify the functions of Dapl1 and NFATC2. One question that remains is how much of the phenotype of Dapl1-Ko cells is CD8 T cell intrinsic? Using full Ko makes it hard to rule out effects of other cell types. Similarly, reduced tumor growth will feed back to the phenotype of the infiltrating T cells. Thus, reduced exhaustion may be due to smaller tumor size.

**Response:**

We thank the reviewer for the positive comments as well as the insightful criticisms. As detailed below, we have carefully addressed the reviewer's specific points with substantial experiments.

Major points:

1. The authors make sue of a full body Dapl1 KO mouse in many of their experiments. Although they make a strong point that Dapl1 acts specifically in CD8 T cells, it is difficult to rule out functions in other cells such as Tregs. Thus, key elements of the study should be performed in mixed bone marrow chimers, including LCMV infection and some tumor experiments.

**Response:**

We thank the reviewer for this insightful and constructive comment. To address the reviewer's comment, we generated mixed bone marrow chimeric mice by transferring *Rag1*-KO mice with a mixture of WT (CD45.1$^+$) and Dapl1-KO (CD45.2$^+$) BM cells. We then examined the cell-intrinsic function of Dapl1 in regulating CD8 T cell responses using both B16-OVA tumorigenesis

model and LCMV infection model. Under such conditions, the Dapl1 KO CD8 T cells displayed similar phenotypes to those detected with non-transferred Dapl1 KO mice (Extended Data Fig. 5). These new data, described on page 12, further confirmed the cell-intrinsic role for Dapl1 in regulating CD8 T cell functions.

2. The authors have performed some studies using Dapl1-deficient TCR transgenic CD8 T cells. While their results support the conclusion that dapl1 acts CD8 T cell intrinsically, these experiments should be repeated with a mixed population approach, ie congenically marked WT and KO TCR transgenic T cells should be cotransferred and their phenotype and function should be examined within the same mouse, ie within the same tumor. Mixed bone marrow chimeric mice would serve the same purpose.

**Response:**

Following the reviewer's suggestion, we performed mixed T cell adoptive transfer by co-transferring wildtype ($CD45.1^+CD45.2^+$) and Dapl1 KO ($CD45.2^+$) Pmel1 CD8 T cells (in 1:1 ratio) into B16F10 tumor-bearing B6.SJL mice ($CD45.1^+$) (Extended Data Fig. 7). As described on page 13, these new data demonstrate that under mixed adoptive transfer conditions, the Dapl1 KO Pmel1 CD8 T cells still exhibit stronger proliferative ability, effector function, increased Tim3 expression (Extended Data Fig. 7). Furthermore, the Dapl1 KO $PD1^+Tim3^+$ Pmel1 TILs also displayed stronger proliferation and effector molecule production than their wildtype counterparts. These results further suggest a cell-intrinsic role for Dapl1 in regulating CD8 T cell functions.

3. The LCMV clone 13 experiments are important experiments. Please expand on the analysis. A full kinetic analysis in WT and KO, including the analysis of progenitor and terminal exhausted population, including markers such as TCF1, CX3CR1 and additional inhibitory receptors would be critical.

**Response:**

We thank the reviewer for this important point. Following the reviewer's suggestion, we performed a detailed kinetic analysis of the effect of Dapl1 deficiency on CD8 T cell function during LCMV clone 13 infection. We found that Dapl1 deficiency profoundly increased the frequency of antigen (gp33)-specific CD8 T cells throughout a time course ranging from 7 days to 30 days of LCMV clone 13 infection (Extended Data Fig. 3a). The *Dapl1* KO mice also produced an increased frequency of antigen-specific CD8 T cells expressing Tim3, although not

the other ICRs analyzed, during this time course (Extended Data Fig. 3b,c). The Dapl1 deficiency resulted in a reduction in the frequency of $TCF1^+PD1^+Tim3^-$ progenitor exhausted CD8 T cells, coupled with an increase in the frequency of $TCF1^+PD1^+Tim3^+$ cells (Extended Data Fig. 3d). No significant differences in the frequency of $CX3CR1^+$ cytotoxic effector cells were detected between the wildtype and Dapl1 KO mice (Extended Data Fig. 3e). Taken together, these results suggest that Dapl1 deficiency promotes the expansion of antigen-specific CD8 T cells and the expression of Tim3 in a subpopulation of CD8 exhausted T cells that retains TCF1 expression. We described these results on page 10 of the text.

4. The cytokine and granzyme B data in Fig. 3e look a bit odd to me. We never see as much Il-2 but have a much higher expression of granzyme B in our Tim3+ cells. Could the authors check this again. Please also comment whether CD4 T cells were deleted prior or infection or not? Is there a difference in viral control? Again, differences in viral burden may have secondary consequences for the T cell phenotype. Hence, chimeras are important. Overall, the LCMV data should be expanded as they allow a very clear view on the phenotype of Dapl1 deficiency.

**Response:**

We thank you the reviewer for these insightful comments. We have performed new experiments to address the reviewer's concerns.

(1) Following the reviewer's suggestion, we repeated the LCMV infection experiment presented in original Fig.3e and improved the quality of the data. Consistent with the original data, splenic Dapl1 KO $PD1^+Tim3^+$ CD8 T cells exhibited hyper production of the effector-related molecules IFN-g and granzyme B. As stated by the reviewer, the overall level of IL-2 production was low; however, production of this cytokine was also increased in the Dapl1 KO cells (Fig. 3f).

(2) CD4 T cell depletion was not used in our LCMV clone 13 infection model, since CD4 T cell depletion is required for constant viral titers in several months or longer-term experiment[2].

(3) Following the reviewer's suggestion, we examined the titers of infectious virus in the spleen on day 30 after LCMV clone 13 infection by plaque assay on Vero cells as previously described[3] and found that Dapl1-deficient mice had a significantly lower virus load in the spleen (Fig. 3d). This result was consistent with the heightened CD8 T cell responses to LCMV clone 13 infection in Dapl1 KO mice.

(4) We also performed LCMV clone 13 infection using chimeric mice adoptively transferred with a mixture of WT ($CD45.1^+$) and Dapl1-KO ($CD45.2^+$) BM cells. Under these conditions, the Dapl1

KO CD8 T cells still displayed the hyperresponsive phenotypes as seen with the non-transferred Dapl1 KO mice (Extended Data Fig. 5h-l). These new data suggest a cell-intrinsic role for Dapl1 in regulating CD8 T cell functions.

5. Dapl1 also seems to play a critical role in acute infection. The authors show some data on Listeria infection. However, do not go into details of the phenotype. This should be expanded and clarified. What is the role of Dapl1 in acute infection (phenotype and function)? They also appear to have done experiments with mixed bone marrow chimeric mice; however, the data presented appear to not take that onto account and don't describe this. Could the authors please elaborate? Further to this, Tim3 does not play a major role in acute infection. How does Dapl1 act in acute infection? Purely via NFATC2?

**Response:**

We thank the reviewer for these important comments. We have now expanded the Listeria acute infection experiments (Fig. 1a-d). We have also performed Listeria infection using the mixed bone marrow chimeric mice, which demonstrated cell-intrinsic function of Dapl1 in controlling CD8 T cell responses to acute infections (Extended Data Fig. 6). Furthermore, we confirmed the role of NFATc2 in mediating the hyperresponsiveness of Dapl1 KO CD8 T cells by performing Listeria infection using Dapl1 and NFATc2 single KO and double KO mice (Fig. 6c,d). These data demonstrate that NFATc2 deletion attenuated the CD8 T cell responses to Listeria acute infection and erased the differences between wildtype and Dapl1 KO T cells (Fig. 6c,d). Together, these results suggested Dapl1 regulate primary CD8 T cell response to acute bacterial infection by regulating NFATc2 activation.

Additional points

1. Please use the correct nomenclature for NFATC2. NFAT1 is the old name and consistently leads to confusion with NADTC1.

**Response:**

We have replaced NFAT1 with NFATc2 and NFAT2 with NFATc1.

2. Fig. 6h, i, j needs single KO data as controls.

**Response:**

In response to the reviewer's comment, we repeated the experiment by including NFATc2 single KO mice as controls and replaced the old data in Fig. 6h-j.

3. How do the authors define progenitor cells within the tumor environment. Apparently, they are PD1-negative, TCF1+ and partly CD62L+. Indeed, the progenitor sort shown in Suppl. Fig. 9a looks like naïve T cells. Are they defined purely by location in the tumor or what criteria are being used?

**Response:**

In response to the reviewer's comment, we have included more detailed characterizations of the progenitor and exhausted CD8 T cells and also included the procedure in the Method section. Briefly, tumors were dissected from the surrounding fascia on the indicated day. Tumors were excised, minced and digested with collagenase IV and DNase I. Tumor infiltrating leukocytes were enriched using Lymphocyte Cell Separation Medium, followed by $CD8^+$ MACS positive selection (Miltenyi). We sorted the progenitor cells (gating strategies: $CD45^+$ $CD8^+$ $CD44^+$ $PD1^-$ $Tim3^-$) and exhausted CD8 cells (gating strategies: $CD45^+$ $CD8^+$ $CD44^+$ $PD1^+$ $Tim3^+$) using a BD FACS Aria II (BD Bioscience) to obtain more than 95% purity. After sorting, we also examined the TCF1 expression in progenitor cells and exhausted cells (Extended Data Fig. 11a, b). We found progenitor cells express high levels of TCF1, whereas exhausted cells express low levels of TCF1. Using these cells, we repeated the NFAT activation experiments and presented the data in Fig. 7a and Extended Data Fig. 11a-d.

4. Related to question 3, Fig. 7b seems to use naïve T cells as controls for exhausted T cells. However, the point the authors make is the progenitor cells have higher NFAT activity. This should be shown, ie a comparison between progenitor and effector cells.

**Response:**

In response to the reviewer's comments, we repeated the experiment by using progenitor and exhausted cells. We presented these new data in Fig. 7b and Extended Data Fig. 11e-h.

5. The statement "NFAT1 mediates both effector function and ICR expression in T cells, but its role in regulating the functionality of exhausted T cells is unclear" (p 16) needs a reference. Overall, there is a lot of work published on the roles of NAFTC1 and NFATC2 in CD8 T cells.

However, this is often somewhat confusing and should be placed into context of the authors work. How do the authors reconcile the literature on NFATC1 and NFATC2?

**Response:**

The reviewer's point is well taken. We have now cited a reference for the dual functions of NFAT in regulating effector function and ICR expression and also discussed our findings in the context of previous reports (see Discussion section). In addition, we have performed new experiments study the function of a NFATc2 mutant defective in AP1 binding (CA-RIT-NFATc2), which was previously shown to induce exhaustion program when overexpressed in NFATc2 KO CD8 T cells[1]. In line with the prior work by Martinez et al, overexpression of CA-RIT-NFATc2 in NFATc2 KO OTI CD8 T cells inhibited antitumor function compared to the overexpression of a control NFATc2 mutant lacking DNA binding function, DBDmut-CARIT-NFATc2 (Extended Data Fig. 10f-i). However, overexpression of CA-RIT-NFATc2 in the NFATc2-sufficient *Dapl1* KO OTI CD8 T cells only moderately reduced their antitumor function (Extended Data Fig. 10f-i). These results suggest that that the exhaustion form of NFATc2 (defective in AP1 binding) could not override the activation-function of the endogenous.

6. The authors state "overexpressed Dapl1 did not bind wildtype NFAT1 but strongly interacted with a constitutively active NFAT1". This seems confusing to me as it would imply that Dapl1 does not interact with NFATC2 under normal circumstances. Perhaps reword.

**Response:**

Wildtype NFATc2 is constitutively phosphorylated and retained in the cytoplasm, and its activation involves dephosphorylation by calcineurin. Dapl1 binds to an NFATc2 mutant mimicking the dephosphorylated NFATc2 but not the wildtype NFATc2. We clarified this point by rephrasing the sentence.

7. In their methods, they describe retroviral overexpression in bone marrow cells. However, I could not find data related to such an experiment. Or was Dapl1 overexpression I T cell achieved by overexpressing it throughout development?

**Response:**

We apologize for the mistake. We haven't done the retroviral overexpression in bone marrow cells in the manuscript and have deleted this part of the methods in the revised manuscript.

8. Can the authors comment on the expression of TOX, IRF4 and BATF in Dapl1 or NFATC2-deficient mice. All three molecules have been implicated in exhaustion and have been shown to be downstream of NFAT. Are they involved? At least TOX should be measured in key experiments.

**Response:**

We have performed the experiments suggested by the reviewer. We found the expression of Tox was moderately (but not significantly) increased in Dapl1 KO CD8 cells and slightly reduced in NFATc2 KO CD8 cells in the B16OVA tumor model (Fig. 6g). Similar results were observed with LCMV clone 13 infection model (data not shown). Emerging studies have reported Tox expression was regulated by NFAT[1, 4-7]. Overexpressing CA-RIT-NFATc2, a constitutively active version of NFATc2 that can't interact with AP-1, induced Tox mRNA expression[1, 4]. One recent study indicated NFATc1 KO CD8 T cells failed to express TOX during LCMV clone 13 infection and NFATc1 was required to induce TOX[6]. Calcineurin inhibitor FK506 inhibited TOX expression in antigen-specific CD8 T cells[5, 7]. These studies suggest that NFATc2 may be functionally redundant with other NFAT members in regulating Tox expression.

The expression of IRF4 and BATF were unaffected in Dapl1 deficient or NFATc2 KO CD8 cells (Fig. 6g). Similar results were obtained with LCMV clone 13 infection model (data not shown). A recent study showed NFATc1 single KO or NFATc2 single KO did not affect the expression of IRF4 and BATF. NFATc1 and NFATc2 double-deficiency had a substantial reduction in the expression of IRF4, although not BATF, in activated CD8 cells[8]. Cyclosporine, an inhibitor of Calcineurin/NFAT signaling, could block the expression of IRF4 and BATF in activated CD8 cells[8]. Together, these results suggest that NFAT proteins may have redundant roles in regulating the expression of Tox, IRF4 and BATF.

9. The authors should show some supplementary data related to their in vitro exhausted T cell cultures in mouse and human T cells. These cultures are not well established, thus require some validation.

**Response:**

The reviewer's comments are well taken. We have performed experiments to validate the *in vitro* exhausted mouse and human CD8 T cells by examine the cytokine production and the expression of inhibitory receptors and transcription factors. The new data are included in Extended Data Fig. 4.

**Reviewer #3:**

Remarks to the Author:

Zhu at al. report on the interesting finding that the absence of Dap1 promotes survival, increases the number and IFNg production of tumor or pathogen-specific CD8 T cells, and potentiates checkpoint inhibition via PD-1. Furthermore, the authors link Dapl1 activity to reduced Nfat1 signaling and overall conclude that Dapl1 is a potent regulator of T cell effector function in tumors. While these aspects are well elaborated, there are critical points that need to be addressed.

**Response:**

We thank the reviewer for these important comments. As detailed below, we have carefully addressed the reviewer's comments with substantial experiments.

Key points:

(1) Throughout the MS and abstract, the authors portray Dapl1 as a tumor- or T-cell exhaustion-specific repressor of T-cell function. While the repressive function is well established in this context, I am concerned about the proclaimed exclusive function. In fact, I believe that the data presented by the authors already show that similar findings can be made in acute Listeria infection. This would not make the finding less attractive but an exclusive function of Dapl1 in tumors is not clearly supported yet.

**Response:**

We thank the reviewer for this insightful comment. Following the reviewer's suggestion, we have included data from Listeria infections in the revised manuscript (Fig. 1a-d, Extended Data Fig. 6, and Fig. 6c,d). Indeed, Dapl1 deficiency promotes CD8 T cell responses to Listeria infections. These data suggest that Dapl1 regulates CD8 T cell responses under both chronic and acute conditions.

(2) Similarly, the mechanism by which Dapl1 enhances T cell function remain unclear. While fully resolving this would be beyond the scope of the present manuscript, a better quantification of the antigen-specific T-cell response would be very helpful in this respect. To

date, most data stem from analyzing total TILs. A Better characterization of the kinetics/number and phenotype of OT-1 and pMel cells would be helpful.

**Response:**

We thank the reviewer for this important point. Following the reviewer's suggestion, we have now included more data on the analysis of antigen-specific T cell responses using Pmel1 model. In an adoptive transfer model, the Dapl1 KO Pmel1 CD8 T cells displayed heightened expansion and effector cytokine production in the tumor (Extended Data Fig. 2d-f), which was in line with stronger tumor suppression and increased mouse survival (Fig. 1p,q). To assure that this function of Dapl1 was cell-intrinsic, we performed mixed Pmel1 CD8 T cell adoptive transfer experiments by co-transferring wildtype ($CD45.1^+CD45.2^+$) and Dapl1 KO ($CD45.2^+$) Pmel1 CD8 T cells (in 1:1 ratio) into B16F10 tumor-bearing B6.SJL mice ($CD45.1^+$) (Extended Data Fig. 7a). Under these conditions, the Dapl1 KO Pmel1 T cells still displayed a profoundly higher level of proliferation (as revealed based on Ki67 expression) and effector molecule expression in the tumor (Extended Data Fig. 7b-d). Furthermore, as seen with the non-transferred Dapl1 KO mice, the transferred Dapl1 KO Pmel1 CD8 TILs also had an increased frequency of $Tim3^+$ and $PD1^+Tim3^+$ subsets (Extended Data Fig. 7e,f), and the Dapl1 KO $PD1^+Tim3^+$ Pmel1 TILs exhibited a markedly higher level of Ki67 expression and effector molecule production than the wildtype counterparts (Extended Data Fig. 7f). These results suggest a cell-intrinsic role for Dapl1 in regulating antigen-specific CD8 T cell functions.

We also performed mixed T cell adoptive transfer using wildtype and Dapl1 KO OT-I cells. Due to space limitations, we did not show these data. Instead, we have included new data on the analysis of OT-I T cells in an exhaustion experiment. In this experiment, we transferred *in vitro* generated wildtype or Dapl1 KO OVA-specific exhausted OT-I T cells into B16-OVA tumor-bearing mice. Compared to their wildtype counterpart, the Dapl1 KO exhausted OT-I T cells were markedly more potent in suppressing tumor growth (Fig. 3h,i), coupled with increased frequency in the tumor (Fig. 3j). Although both the wildtype and Dapl1 KO OT-I TILs were largely $PD1^+Tim3^+$, the *Dapl1* KO CD8 TILs expressed a much higher level of the cell proliferation marker Ki67 and effector-related molecules than their wildtype counterparts (Fig. 3k-m).

(3) The authors begin the presentation of their results by claiming that they identified the expression of Dap1 in the Tcf-1+ population via scRNA sequencing and that this motivated them to generate a Dap1 KO-mouse. However. this timeline does not fit with the fact that Supp1A and Figure 4A are obviously from the same cluster analysis. Therefore, the authors should

clarify the time when these datasets were obtained or explain what types of batch corrections (not mentioned in the methods so far) were applied if separately obtained datasets were merged afterwards. This issue does not call into question the main results, but it is not ideal if there is uncertainty about the dataset that provided the impetus for the project.

**Response:**

The reviewer's point is well taken. The original Extended Data Fig. 1a and Fig. 4a were from the same experiment. We have now revised the manuscript to present all of the scRNAseq data in Fig. 4. We also revised the first paragraph of the Result section to indicate our initial finding about Dapl1 in association with a study about the deubiquitinase Otub1. We have previously shown that CD8 T cells lacking Otub1 display increased function in immune responses against both infections and tumorigenesis[9]. Our RNA sequencing (RNAseq) analysis identified Dapl1 as a gene drastically downregulated in the Otub1-deficient CD8 T cells (GSE126777). Interestingly, Dapl1 was abundantly expressed in CD8 T cells, only weakly expressed in CD4 T cells, and barely detected in B cells, dendritic cells, or macrophages (Extended Data Fig. 1a,b). These findings, along with our previous observation that *Otub1*-deficient CD8 T cells had greatly improved functions against infections and cancer[9], prompted us to examine the role of Dapl1 in regulating T cell functions.

(4) Figure 6: The interpretation that Nfat1 is needed for the DAP1 effect and for the effect on Tim-3 is quite far-stretched. Given that Nfat1 is involved in T cell activation, it is not overly surprising that it is required for the effects seen by the authors. Therefore, considering that the absence of nfat1 has a fairly global effect on T cell function, the interpretation should be significantly revised.

**Response:**

We thank the reviewer for this insightful comment. Since NFATc2 deficiency did not affect the expression of other ICRs that are also associated with T cell activation (Fig. 6g), we further assessed the role of NFATc2 in regulating Tim-3 expression by performing more experiments. First of all, because *in vivo* induction of Tim3 might involve indirect mechanisms, we performed acute activation of *in vitro* generated effector and exhausted CD8 T cells derived from Dapl1 KO, NFATc2 KO, Dapl1-NFATc2 dKO and wildtype control mice. Upon stimulation with anti-CD3/anti-CD28, both effector and exhausted CD8 T cells expressed Havcr2 mRNA, which was elevated in the Dapl1 KO CD8 T cells (Extended Data Fig. 10a,b). Importantly, NFATc2 deficiency

blocked the induction of Havcr2 and erased the differences between Dapl1-sufficient and Dapl1-deficient CD8 T cells (Extended Data Fig. 10a,b).

To confirm the direct involvement of NFATc2 in *Havcr2* gene regulation, we performed Chromatin Immunoprecipitation (ChIP) assays to examine whether NFATc2 could directly bind the *Havcr2* promoter in exhausted antigen (gp33)-specific CD8 T cells isolated from day 30 LCMV clone 13-infected mice. We found that NFATc2 bound a region located between -432 and -5 of the Havcr2 promoter in the LCMV-specific CD8 T cells, but not in naïve CD8 T cells (Extended Data Fig. 10c). Compared to the wildtype T cells, the Dapl1 KO T cells displayed an increased level of NFATc2 binding activity at the Havcr2 promoter (Extended Data Fig. 10d). These results further suggest a role for NFATc2 in regulating Tim3 expression.

(5) Figure 7: Along the same lines, the negative regulatory function of Dapl1 on nfat1 is not well established. It should at least be complemented by an overexpression experiment.

**Response:**

Following the reviewer's suggestion, we examined the effect of Dapl1 overexpression on NFATc2 activation. We transduced *Dapl1* KO Pmel1 CD8 T cells with retroviruses carrying a *Dapl1* expression vector or an empty vector control (Extended Data Fig. 11i). We adoptively transferred these cells into B16F10 tumor-bearing B6.SJL mice and then sorted the $PD1^{+}Tim3^{+}$ exhausted and $PD1^{-}Tim3^{-}$ progenitor subpopulations from the tumor (Extended Data Fig. 11j,k). Dapl1 overexpression inhibited ionomycin-induced nuclear translocation of NFATc2 (Fig. 7c) but not NFATc1 (Extended Data Fig. 11l) in both the progenitor and exhausted Pmel1 CD8 T cells.

Minor points:

Figure 3f: The legend leaves unclear what is shown.

**Response:**

We revised the Fig. 3f legend in the revised manuscript.

Figure 4c: The legend does not match what is shown. Legend says marker expression is shown in precursor, effector, and depleted clusters, but shown is the marker expression in 6 clusters.

**Response:**

We revised the Fig. 4c legend in the revised manuscript.

Figure 4d: should include Tcf-7 data.

**Response:**

We added TCF-1 data in Fig. 4d of the revised manuscript.

Figure 6d: no peptide control is missing.

**Response:**

In response to the reviewer's comments, we added the no peptide control in Fig. 6d.

We believe that we have adequately addressed the reviewer's comments. We once again would like to thank the reviewers for their valuable comments and suggestions, which have guided us in the thorough revision to substantially improve our work.

**References**


1. Martinez, G.J. *et al.* The transcription factor NFAT promotes exhaustion of activated CD8(+) T cells. *Immunity* **42**, 265-278 (2015).
2. Matloubian, M., Concepcion, R.J. & Ahmed, R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol* **68**, 8056-8063 (1994).
3. Ahmed, R., Salmi, A., Butler, L.D., Chiller, J.M. & Oldstone, M.B. Selection of genetic variants of lymphocytic choriomeningitis virus in spleens of persistently infected mice. Role in suppression of cytotoxic T lymphocyte response and viral persistence. *J Exp Med* **160**, 521-540 (1984).
4. Seo, H. *et al.* TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8(+) T cell exhaustion. *Proc Natl Acad Sci U S A* **116**, 12410-12415 (2019).
5. Scott, A.C. *et al.* TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* **571**, 270-274 (2019).
6. Khan, O. *et al.* TOX transcriptionally and epigenetically programs CD8(+) T cell exhaustion. *Nature* **571**, 211-218 (2019).
7. Yao, C. *et al.* Single-cell RNA-seq reveals TOX as a key regulator of CD8(+) T cell persistence in chronic infection. *Nature immunology* **20**, 890-901 (2019).
8. Man, K. *et al.* Transcription Factor IRF4 Promotes CD8(+) T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection. *Immunity* **47**, 1129-1141 e1125 (2017).

9. Zhou, X. *et al.* The deubiquitinase Otub1 controls the activation of CD8(+) T cells and NK cells by regulating IL-15-mediated priming. *Nature immunology* **20**, 879-889 (2019).

**Decision Letter, first revision:**

Subject: Decision on Nature Cell Biology submission NCB-S45561A
Message: *Please delete the link to your author homepage if you wish to forward this email to co-authors.

Dear Professor Sun,

Your revised manuscript, "Dapl1 suppresses antitumor CD8 T cell responses by controlling NFATc2 activation", has now been seen by the original referees. As you will see from their comments (attached below) they find this work of interest, but have raised some important points. Although we are also very interested in this study, we believe that their concerns should be addressed before we can consider publication in Nature Cell Biology.

Nature Cell Biology editors discuss the referee reports in detail within the editorial team, including the chief editor, to identify key referee points that should be addressed with priority, and requests that are overruled as being beyond the scope of the current study. To guide the scope of the revisions, I have listed these points below. We are committed to providing a fair and constructive peer-review process, so please feel free to contact me if you would like to discuss any of the referee comments further.

In particular, it would be essential to:

A) Address the remaining reproducibility concern as raised by Reviewer 3:

"...Surprisingly, the authors only show data for two mice to support this claim. More data should be presented,.."

B) All other referee concerns pertaining to strengthening existing data, providing controls, methodological details, clarifications and textual changes as applicable should also be addressed.

C) Finally please pay close attention to our guidelines on statistical and methodological reporting (listed below) as failure to do so may delay the reconsideration of the revised manuscript.In particular please provide:

- a Supplementary Figure including unprocessed images of all gels/blots in the form of a multi-page pdf file. Please ensure that blots/gels are labeled and the sections presented in the figures are clearly indicated.

- a Supplementary Table including all numerical source data in Excel format, with data for different figures provided as different sheets within a single Excel file. The file should include source data giving rise to graphical representations and statistical descriptions in the paper and for all instances where the figures present representative experiments of multiple independent repeats, the source data of all repeats should be provided.

We therefore invite you to take these points into account when revising the manuscript. In addition, when preparing the revision please:

- ensure that it conforms to our format instructions and publication policies (see below and www.nature.com/nature/authors/).

- provide a point-by-point rebuttal to the full referee reports verbatim, as provided at the end of this letter.

- provide the completed Editorial Policy Checklist (found here https://www.nature.com/authors/policies/Policy.pdf),and Reporting Summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf). This is essential for reconsideration of the manuscript and these documents will be available to editors and referees in the event of peer review. For more information see http://www.nature.com/authors/policies/availability.html or contact me.

Nature Cell Biology 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 submit the revised manuscript files and the point-by-point rebuttal to the referee comments using this link:

***[REDACTED]***

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

We would like to receive the revision within four weeks. If submitted within this time period, reconsideration of the revised manuscript will not be affected by related studies published elsewhere, or accepted for publication in Nature Cell Biology in the meantime. We would be happy to consider a revision even after this timeframe, but in that case we will consider the published literature at the time of resubmission when assessing the file.

We hope that you will find our referees' comments, and editorial guidance helpful. Please do not hesitate to contact me if there is anything you would like to discuss.

Best wishes,

Zhe Wang

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Senior Editor
Nature Cell Biology

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Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors have addressed all the questions and concerns, including deciphering the role of NFAT in T cell exhaustion and Tim3 expression in Dapl1-deficient background. However, there are still some minor concerns about the revised manuscript mentioned below:
1. In this study, the authors found that Dapl1 deletion could expand a population of TCF1+PD1+TIM3+ int. exhausted CD8 T cells in both tumor and LMCV model. And in the bone marrow chimeric mice model, the results revealed that the proportion of TCF1+PD1+TIM3+ CD8 T cells in Dapl1 KO group was higher than that of wide type CD8+ T cell in LVMC clone 13 infection model (Extended Data Fig 5). Since TCF1 expression is an important parameter for the whole study, it would be better to analyze the TCF1

expression either in Listeria-OVA infection model (Extended Data Fig 6) or B16F10 tumor model (Extended Data Fig 7). In addition, in Extended Data Fig 5e, the proportion of TCF1+TIM3+PD1+ CD8 T cell should be statistically analyzed.
2. In the Introduction Section, NFAT and NFATc2 should be introduced.
3. In line 69, should it be “NFATc2 mediates Tim3 induction”?
4. In line 196, authors used CX3CR1+ as the marker of cytotoxic effector cell. Please introduce CX3CR1 and replenish the reference.
5. In line 226, Extended Data Fig.4a, b, what is the cell group of “Unsti”? Unstimulated effector-like or exhausted-like T cells? This might mislead.
6. In line 240, the expression of IL-2 and IFN-γ were shown in Fig 3n and 3o, but not in Extended Data Fig. 4d-g.
7. In Fig 5h, please check if the label of the first line is correct.

Reviewer #2:
Remarks to the Author:
The authors have done a fine job addressing my comments and requests for additional experiments. The new data have made the manuscript stronger and more convincing.

One remaining point that should be addressed is the question how Dapl1 might increase the proliferation and expansion of Tim3+ effector cells? This is not obvious, given that Dapl1 expression is limited to the precursor population of exhausted T cells. In this context, Fig. 7 shows that WT TEX are impaired in activating NFATC2, while Dapl1-KO TEX show much improved NFATC2 activation. If that is indeed the case, how does it work if Dapl1 is not expressed in TEX in the first place? The authors should really provide the data for precursor T cell as well (not just TEX). Do they also show improved signalling? Conceptually, this is an important point.

Finally, the authors introduced a new experimental approach, ie the overexpression of Dapl1, which results in reduced NFATC2 signalling (Fig. 7). These experiments need to be analysed more thoroughly and the data displayed completely. What is the phenotype of the Dapl1-overexpressing cells (TCF1+ vs TCF1-, Tim3 expression etc).

Reviewer #3:
Remarks to the Author:
I must admit that the revisions and the new data have confirmed a concern that another reviewer and I had previously expressed. The question is whether Dapl1 has a specific function in the immune response

directed against tumors or whether it affects T cell function in general. The authors now provide compelling data for a general function, showing, for example, massively impaired control of Listeria infection in the absence of Dapl1. In stark contrast to this observation, the title, abstract, and essentially most of the MS still place Dapl1 in a tumor-specific context. In my opinion, this is misleading and the manuscript should not be published as is. I think the overall data and results are fine and worth publishing, but the biological function should be correctly reflected/interpreted.

I had also asked the authors to better characterize the "kinetics/number of OT-1 and pMel." The authors have now included a statement about "enhanced expansion" of adoptively transferred Dapl1 T cells and refer to data 2d-f. Surprisingly, the authors only show data for two mice to support this claim. More data should be presented, and perhaps a link could be provided to ExtData 7b, which shows a slight preference for the KO cells in the tumor.

GUIDELINES FOR SUBMISSION OF NATURE CELL BIOLOGY ARTICLES

READABILITY OF MANUSCRIPTS – Nature Cell Biology is read by cell biologists from diverse backgrounds, many of whom are not native English speakers. Authors should aim to communicate their findings clearly, explaining technical jargon that might be unfamiliar to non-specialists, and avoiding non-standard abbreviations. Titles and abstracts should concisely communicate the main findings of the study, and the background, rationale, results and conclusions should be clearly explained in the manuscript in a manner accessible to a broad cell biology audience. Nature Cell Biology uses British spelling.

ARTICLE FORMAT

TITLE – should be no more than 100 characters including spaces, without punctuation and avoiding technical terms, abbreviations, and active verbs..

AUTHOR NAMES – should be given in full.

AUTHOR AFFILIATIONS – should be denoted with numerical superscripts (not symbols) preceding the names. Full addresses should be included, with US states in full and providing zip/post codes. The corresponding author is denoted by: "Correspondence should be addressed to [initials]."

ABSTRACT – should not exceed 150 words and should be unreferenced. This paragraph is the most visible part of the paper and should briefly outline the background and rationale for the work, and accurately summarize the main results and conclusions. Key genes, proteins and organisms should be specified to ensure discoverability of the paper in online searches.

TEXT – the main text consists of the Introduction, Results, and Discussion sections and must not exceed 3500 words including the abstract. The Introduction should expand on the background relating to the work. The Results should be divided in subsections with subheadings, and should provide a concise and accurate description of the experimental findings. The Discussion should expand on the findings and their implications. All relevant primary literature should be cited, in particular when discussing the background and specific findings.

ACKNOWLEDGEMENTS – should be kept brief. Professional titles and affiliations are unnecessary. Grant numbers can be listed.

AUTHOR CONTRIBUTIONS – must be included after the Acknowledgements, detailing the contributions of each author to the paper (e.g. experimental work, project planning, data analysis etc.). Each author should be listed by his/her initials.

FINANCIAL AND NON-FINANCIAL COMPETING INTERESTS – the authors must include one of three declarations: (1) that they have no financial and non-financial competing interests; (2) that they have financial and non-financial competing interests; or (3) that they decline to respond, after the Author Contributions section. This statement will be published with the article, and in cases where financial and non-financial competing interests are declared, these will be itemized in a web supplement to the article. For further details please see https://www.nature.com/licenceforms/nrg/competing-interests.pdf.

REFERENCES – are limited to a total of 70 in the main text and Methods combined,. They must be numbered sequentially as they appear in the main text, tables and figure legends and Methods and must follow the precise style of Nature Cell Biology references. References only cited in the Methods should be numbered consecutively following the last reference cited in the main text. References only associated with Supplementary Information (e.g. in supplementary legends) do not count toward the total reference limit and do not need to be cited in numerical continuity with references in the main text. Only published papers can be cited, and each publication cited should be included in the numbered reference list, which should include the manuscript titles. Footnotes are not permitted.

METHODS – Nature Cell Biology publishes methods online. The methods section should be provided as a separate Word document, which will be copyedited and appended to the manuscript PDF, and incorporated within the HTML format of the paper.

Methods should be written concisely, but should contain all elements necessary to allow interpretation and replication of the results. As a guideline, Methods sections typically do not exceed 3,000 words. The Methods should be divided into subsections listing reagents and techniques. When citing previous methods, accurate references should be provided and any alterations should be noted. Information must be provided about: antibody dilutions, company names, catalogue numbers and clone numbers for monoclonal antibodies; sequences of RNAi and cDNA probes/primers or company names and catalogue numbers if reagents are commercial; cell line names, sources and information on cell line identity and authentication. Animal studies and experiments involving human subjects must be reported in detail, identifying the committees approving the protocols. For studies involving human subjects/samples, a statement must be included confirming that informed consent was obtained. Statistical analyses and information on the reproducibility of experimental results should be provided in a section titled “Statistics and Reproducibility”.

All Nature Cell Biology manuscripts submitted on or after March 21 2016, must include a Data availability statement as a separate section after Methods but before references, under the heading "Data Availability”. For Springer Nature policies on data availability see http://www.nature.com/authors/policies/availability.html; for more information on this particular policy see http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf. The Data availability statement should include:

• Accession codes for primary datasets (generated during the study under consideration and designated as "primary accessions") and secondary datasets (published datasets reanalysed during the study under consideration, designated as "referenced accessions"). For primary accessions data should be made public to coincide with publication of the manuscript. A list of data types for which submission to community-endorsed public repositories is mandated (including sequence, structure, microarray, deep sequencing data) can be found here http://www.nature.com/authors/policies/availability.html#data.

• Unique identifiers (accession codes, DOIs or other unique persistent identifier) and hyperlinks for datasets deposited in an approved repository, but for which data deposition is not mandated (see here for details http://www.nature.com/sdata/data-policies/repositories).

• At a minimum, please include a statement confirming that all relevant data are available from the authors, and/or are included with the manuscript (e.g. as source data or supplementary information),

listing which data are included (e.g. by figure panels and data types) and mentioning any restrictions on availability.

• If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Methods.

We recommend that you upload the step-by-step protocols used in this manuscript to the Protocol Exchange. More details can found at www.nature.com/protocolexchange/about.

DISPLAY ITEMS – main display items are limited to 6-8 main figures and/or main tables. For Supplementary Information see below.

FIGURES – Colour figure publication costs $395 per colour figure. All panels of a multi-panel figure must be logically connected and arranged as they would appear in the final version. Unnecessary figures and figure panels should be avoided (e.g. data presented in small tables could be stated briefly in the text instead).

All imaging data should be accompanied by scale bars, which should be defined in the legend. Cropped images of gels/blots are acceptable, but need to be accompanied by size markers, and to retain visible background signal within the linear range (i.e. should not be saturated). The boundaries of panels with low background have to be demarked with black lines. Splicing of panels should only be considered if unavoidable, and must be clearly marked on the figure, and noted in the legend with a statement on whether the samples were obtained and processed simultaneously. Quantitative comparisons between samples on different gels/blots are discouraged; if this is unavoidable, it has be performed for samples derived from the same experiment with gels/blots were processed in parallel, which needs to be stated in the legend.

Figures should be provided at approximately the size that they are to be printed at (single column is 86 mm, double column is 170 mm) and should not exceed an A4 page (8.5 x 11"). Reduction to the scale that will be used on the page is not necessary, but multi-panel figures should be sized so that the whole figure can be reduced by the same amount at the smallest size at which essential details in each panel are visible. In the interest of our colour-blind readers we ask that you avoid using red and green for contrast in figures. Replacing red with magenta and green with turquoise are two possible colour-safe alternatives. Lines with widths of less than 1 point should be avoided. Sans serif typefaces, such as Helvetica (preferred) or Arial should be used. All text that forms part of a figure should be rewritable and removable.

We accept files from the following graphics packages in either PC or Macintosh format:

- For line art, graphs, charts and schematics we prefer Adobe Illustrator (.AI), Encapsulated PostScript (.EPS) or Portable Document Format (.PDF). Files should be saved or exported as such directly from the application in which they were made, to allow us to restyle them according to our journal house style.

- We accept PowerPoint (.PPT) files if they are fully editable. However, please refrain from adding PowerPoint graphical effects to objects, as this results in them outputting poor quality raster art. Text used for PowerPoint figures should be Helvetica (preferred) or Arial.

- We do not recommend using Adobe Photoshop for designing figures, but we can accept Photoshop generated (.PSD or .TIFF) files only if each element included in the figure (text, labels, pictures, graphs, arrows and scale bars) are on separate layers. All text should be editable in 'type layers' and line-art such as graphs and other simple schematics should be preserved and embedded within 'vector smart objects' - not flattened raster/bitmap graphics.

- Some programs can generate Postscript by 'printing to file' (found in the Print dialogue). If using an application not listed above, save the file in PostScript format or email our Art Editor, Allen Beattie for advice (a.beattie@nature.com).

Regardless of format, all figures must be vector graphic compatible files, not supplied in a flattened raster/bitmap graphics format, but should be fully editable, allowing us to highlight/copy/paste all text and move individual parts of the figures (i.e. arrows, lines, x and y axes, graphs, tick marks, scale bars etc). The only parts of the figure that should be in pixel raster/bitmap format are photographic images or 3D rendered graphics/complex technical illustrations.

All placed images (i.e. a photo incorporated into a figure) should be on a separate layer and independent from any superimposed scale bars or text. Individual photographic images must be a minimum of 300+ DPI (at actual size) or kept constant from the original picture acquisition and not decreased in resolution post image acquisition. All colour artwork should be RGB format.

FIGURE LEGENDS – must not exceed 350 words for each figure to allow fit on a single printed NCB page together with the figure. They must include a brief title for the whole figure, and short descriptions of each panel with definitions of the symbols used, but without detailing methodology.

TABLES – main tables should be provided as individual Word files, together with a brief title and legend. For supplementary tables see below.

SUPPLEMENTARY INFORMATION – Supplementary information is material directly relevant to the conclusion of a paper, but which cannot be included in the printed version in order to keep the manuscript concise and accessible to the general reader. Supplementary information is an integral part of a Nature Cell Biology publication and should be prepared and presented with as much care as the main display item, but it must not include non-essential data or text, which may be removed at the editor's discretion. All supplementary material is fully peer-reviewed and published online as part of the HTML version of the manuscript. Supplementary Figures and Supplementary Notes are appended at the end of the main PDF of the published manuscript.

Supplementary items should relate to a main text figure, wherever possible, and should be mentioned sequentially in the main manuscript, designated as Supplementary Figure, Table, Video, or Note, and numbered continuously (e.g. Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2 etc.).

Unprocessed scans of all key data generated through electrophoretic separation techniques need to be presented in a supplementary figure that should be labeled and numbered as the final supplementary figure, and should be mentioned in every relevant figure legend. This figure does not count towards the total number of figures and is the only figure that can be displayed over multiple pages, but should be provided as a single file, in PDF or TIFF format. Data in this figure can be displayed in a relatively informal style, but size markers and the figures panels corresponding to the presented data must be indicated.

The total number of Supplementary Figures (not including the “unprocessed scans” Supplementary Figure) should not exceed the number of main display items (figures and/or tables (see our Guide to Authors and March 2012 editorial http://www.nature.com/ncb/authors/submit/index.html#suppinfo; http://www.nature.com/ncb/journal/v14/n3/index.html#ed). No restrictions apply to Supplementary Tables or Videos, but we advise authors to be selective in including supplemental data.

Each Supplementary Figure should be provided as a single page and as an individual file in one of our accepted figure formats and should be presented according to our figure guidelines (see above). Supplementary Tables should be provided as individual Excel files. Supplementary Videos should be provided as .avi or .mov files up to 50 MB in size. Supplementary Figures, Tables and Videos much be accompanied by a separate Word document including titles and legends.

GUIDELINES FOR EXPERIMENTAL AND STATISTICAL REPORTING

REPORTING REQUIREMENTS – To improve the quality of methods and statistics reporting in our papers we have recently revised the reporting checklist we introduced in 2013. We are now asking all life sciences authors to complete two items: an Editorial Policy Checklist (found here

https://www.nature.com/authors/policies/Policy.pdf) that verifies compliance with all required editorial policies and a Reporting Summary (found here https://www.nature.com/authors/policies/ReportingSummary.pdf) that collects information on experimental design and reagents. These documents are available to referees to aid the evaluation of the manuscript. Please note that these forms are dynamic 'smart pdfs' and must therefore be downloaded and completed in Adobe Reader. We will then flatten them for ease of use by the reviewers. If you would like to reference the guidance text as you complete the template, please access these flattened versions at http://www.nature.com/authors/policies/availability.html.

STATISTICS – Wherever statistics have been derived the legend needs to provide the n number (i.e. the sample size used to derive statistics) as a precise value (not a range), and define what this value represents. Error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). Box plots need to be defined in terms of minima, maxima, centre, and percentiles. Ranges are more appropriate than standard errors for small data sets. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test used needs to be stated in the legend. Statistics such as error bars must not be derived from n<3. For sample sizes of n<5 please plot the individual data points rather than providing bar graphs. Deriving statistics from technical replicate samples, rather than biological replicates is strongly discouraged. Wherever statistical significance has been derived, precise p values need to be provided and the statistical test stated in the legend.

Information on how many times each experiment was repeated independently with similar results needs to be provided in the legends and/or Methods for all experiments, and in particular wherever representative experiments are shown.

We strongly recommend the presentation of source data for graphical and statistical analyses as a separate Supplementary Table, and request that source data for all independent repeats are provided when representative experiments of multiple independent repeats, or averages of two independent experiments are presented. This supplementary table should be in Excel format, with data for different figures provided as different sheets within a single Excel file. It should be labelled and numbered as one of the supplementary tables, titled "Statistics Source Data", and mentioned in all relevant figure legends.

--------- Please don't hesitate to contact NCB@nature.com should you have queries about any of the above requirements ---------

**Author Rebuttal, first revision:**

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

The authors have addressed all the questions and concerns, including deciphering the role of NFAT in T cell exhaustion and Tim3 expression in Dapl1-deficient background. However, there are still some minor concerns about the revised manuscript mentioned below:

1. In this study, the authors found that Dapl1 deletion could expand a population of TCF1+PD1+TIM3+ int. exhausted CD8 T cells in both tumor and LMCV model. And in the bone marrow chimeric mice model, the results revealed that the proportion of TCF1+PD1+TIM3+ CD8 T cells in Dapl1 KO group was higher than that of wide type CD8+ T cell in LVMC clone 13 infection model (Extended Data Fig 5). Since TCF1 expression is an important parameter for the whole study, it would be better to analyze the TCF1 expression either in Listeria-OVA infection model (Extended Data Fig 6) or B16F10 tumor model (Extended Data Fig 7). In addition, in Extended Data Fig 5e, the proportion of TCF1+TIM3+PD1+ CD8 T cell should be statistically analyzed.

**Response:**

(1) We thank the reviewer for this important comment. Following the reviewer's suggestion, we have examined TCF1 expression in the bone marrow chimeric mice infected with Listeria-OVA. We found the Dapl1 deficiency decreased the frequency of $TCF1^+$ $CD8^+$ T cells and concomitantly increased the frequency of $TCF1^-$ $CD8^+$ T cells (Extended Data Fig. 6c). In acute infections, naive $CD8^+$ T cells are activated and differentiate into effector T cells. The expression of TCF-1 is highly expressed in naive T cells, down-regulated in effector T cells, and re-enhanced in memory T cells[1, 2]. The increased frequency of $TCF1^-$ $CD8^+$ effector T cells in Dapl1 KO cells were consistent with the much strong immune response against LM-OVA infection in Dapl1 KO mice (Fig. 1a-c).

(2) We have included TCF1 data from B16F10 tumor-bearing B6.SJL mice ($CD45.1^+$) adoptively transferred with a mixture (1:1) of wildtype ($CD45.1^+CD45.2^+$) and Dapl1 KO ($CD45.2^+$) Pmel1 CD8 T cells. We found the Dapl1 KO Pmel1 T cells still displayed the reduced the $TCF^{hi}$ $Tim3^-$ population and the increased the $TCF1^{hi}Tim3^+$ population (Extended Data Fig 7f).

(3) In response to the reviewer's comment, we have added statistical analysis of $TCF1^+TIM3^+PD1^+$ CD8 T cell in Extended Data Fig.5e.

2. In the Introduction Section, NFAT and NFATc2 should be introduced.

**Response:**

We have included NFAT and NFATc2 in the Introduction Section (page 4).

3. In line 69, should it be “NFATc2 mediates Tim3 induction”?

**Response:**

Thank you for picking up this error. We have changed this sentence to “NFATc2 mediates Tim3 induction” (page 4).

4. In line 196, authors used CX3CR1+ as the marker of cytotoxic effector cell. Please introduce CX3CR1 and replenish the reference.

**Response:**

Following the reviewer’s suggestion, we have added the introduction and reference for $CX3CR1^+$ $CD8^+$ cytotoxic effector cell (page 10).

5. In line 226, Extended Data Fig.4a, b, what is the cell group of “Unsti”? Unstimulated effector- like or exhausted-like T cells? This might mislead.

**Response:**

Following the reviewer’s suggestion, we have changed “Unsti” to “Mock” in Extended Data Fig.4a, b. We have added more details of control cells (Mock), effector-like (effector) and exhausted-like (exhausted) CD8 T cells and also included the procedure in the Method section.

Effector CD8 cells generation, OT-I CD8 T cells (97.5% of total cells) were cocultured with $CD11b^+$ $CD11c^-$ monocytes (1.5% of total cells) and $CD11b^+CD11c^+$ dendritic cells (1% of total cells) with IL-15 and IL-7 plus $OVA_{257–264}$ peptide for 48 h. The cells were washed to remove the peptide and were cultured in the complete medium with 5 ng/ml IL-15 and 5 ng/ml IL-7 for an additional three days.

For generation of exhausted CD8 T cells, OT-I CD8 T cells were cocultured with $CD11b^+$ $CD11c^-$ monocytes and $CD11b^+CD11c^+$ dendritic cells IL-15 and IL-7 plus $OVA_{257–264}$ peptide for 48 h. The cells were washed and then subjected to daily repeated stimulations with $OVA_{257–264}$ peptide in the presence of IL-15 and IL-7 for an additional 3 days.

Control cells (Mock) were cultured in complete medium with IL-15 and IL-7 for 5 days without adding peptide.

6. In line 240, the expression of IL-2 and IFN-γ were shown in Fig 3n and 3o, but not in

Extended
Data Fig. 4d-g.

**Response:**

Thank you for pointing out this error. We have corrected it (page 12).

7. In Fig 5h, please check if the label of the first line is correct.

**Response:**

Thank you for spotting this error. We have fixed the label mistake in Fig. 5h.

Reviewer #2:

Remarks to the Author:

The authors have done a fine job addressing my comments and requests for additional experiments. The new data have made the manuscript stronger and more convincing.

One remaining point that should be addressed is the question how Dapl1 might increase the proliferation and expansion of Tim3+ effector cells? This is not obvious, given that Dapl1 expression is limited to the precursor population of exhausted T cells. In this context, Fig. 7 shows that WT TEX are impaired in activating NFATC2, while Dapl1-KO TEX show much improved NFATC2 activation. If that is indeed the case, how does it work if Dapl1 is not expressed in TEX in the first place? The authors should really provide the data for precursor T cell as well (not just TEX). Do they also show improved signalling? Conceptually, this is an important point.

**Response:**

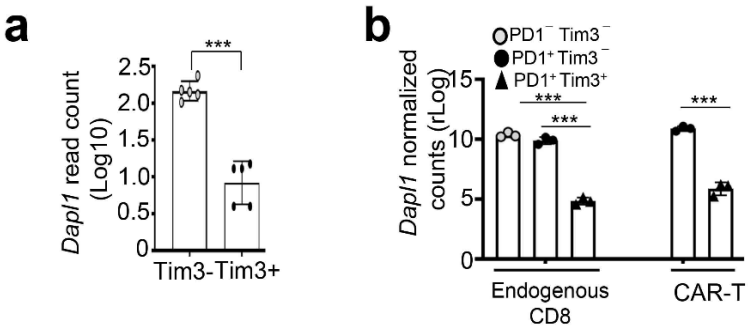
We thank the reviewer for these insightful comments. We performed new experiments to address the reviewer's comments. We sorted progenitor CD8 T cells ($PD1^{-}Tim3^{-}$) and exhausted CD8 T cells ($PD1^{+}Tim3^{+}$) from LCMV infected wildtype and Dapl1 KO mice. We examined the expression of Dapl1 mRNA in progenitor CD8 T cells and exhausted CD8 T cells. Although wildtype exhausted CD8 cells displayed reduced level of Dapl1 mRNA compared with the wildtype progenitor CD8 T cells, we could detect the expression of Dapl1 in wildtype exhausted CD8 cells (Extended Data Fig. 8d). Our scRNA-Seq data found Dapl1 was highly

expressed in the TCF1$^{hi}$ cluster, and weakly expressed in TCF1$^{low}$ cluster, but not unexpressed (Fig. 4d). In addition, detecting low-expression genes can require an increase in read depth, which generates more informational reads. Furthermore, we also analyzed two bulk RNA-seq datasets (GSE132033; GSE123738) to examine the expression of Dapl1 mRNA in Tim3$^{+}$ cells and Tim3$^{-}$ cells from LCMV clone 13 model and B16-OVA tumor model, and found Dapl1 mRNA is expressed at a high level in precursor cells (Tim3$^{-}$ cells), whereas it is expressed at a reduced level in exhausted cells (Tim3$^{+}$ cells) (See Figure. R1 below). These results suggest that Dapl1 was highly expressed in precursor population, moderately expressed in exhausted cell population.

Following the reviewer's suggestion, we have examined the NFATc2 activation in precursor CD8 T cells and found that Dapl1 deficiency also promoted ionomycin-induced nuclear translocation of NFATc2 in precursor CD8 T cells (Extended Data Fig. 11c and 11h). These results suggest that Dapl1 negatively regulates NFATc2 activation in both precursor and exhausted CD8 T cells.

Together, these findings suggest that Dapl1 deficiency promotes NFATc2 activation, which enhance the proliferative capacity and effector functions of CD8 TILs.

**Figure R1**




**a**, Dapl1 read counts for the sorted TIM-3$^{-}$ and TIM-3$^{+}$ populations in transferred wild-type P14 T cells isolated from C57BL/6 mice on day 8 after infection with clone 13 (Data from GSE132033). **b,** Dapl1 normalized counts for PD1$^{-}$Tim3$^{-}$, PD1$^{+}$Tim3$^{-}$ and PD1$^{+}$Tim3$^{+}$ subsets sorted from endogenous CD8 TILs or CAR T TILs from B16-OVA-hCD19 tumour bearing C57BL/6 mice (Data from GSE123738). Summary data are shown as the mean ± s.d. with P values determined using a two-tailed unpaired Student's t-test, ***P< 0.001.

Finally, the authors introduced a new experimental approach, ie the overexpression of Dapl1, which results in reduced NFATC2 signalling (Fig. 7). These experiments need to be analysed more thoroughly and the data displayed completely. What is the phenotype of the Dapl1-overexpressing cells (TCF1+ vs TCF1-, Tim3 expression etc).

**Response:**

These are again excellent suggestions. We transduced Dapl1 KO Pmel1 CD8 T cells with retroviruses carrying a Dapl1 expression vector or an empty vector control. We adoptively transferred these cells into B16F10 tumor-bearing B6.SJL mice and analyzed the effect of Dapl1 overexpression on CD8 T cell phenotype in tumor model (Extended Data Fig.2k,l). We found that Dapl1 overexpression profoundly suppressed the frequency of Tim3$^{+}$ CD8 T cells (Fig. 2d), TCF1$^{+}$PD1$^{+}$Tim3$^{+}$ cells, coupled with an increased frequency of TCF1$^{+}$PD1$^{+}$Tim3$^{-}$ progenitor exhausted CD8 T cells (Fig. 2e), and attenuated effector function (Extended Data Fig. 2m). We demonstrated that ectopic expression of Dapl1 suppressed Tim3 expression and effector function.

Reviewer #3:

Remarks to the Author:

I must admit that the revisions and the new data have confirmed a concern that another reviewer and I had previously expressed. The question is whether Dapl1 has a specific function in the immune response directed against tumors or whether it affects T cell function in general. The authors now provide compelling data for a general function, showing, for example, massively impaired control of Listeria infection in the absence of Dapl1. In stark contrast to this observation, the title, abstract, and essentially most of the MS still place Dapl1 in a tumor- specific context. In my opinion, this is misleading and the manuscript should not be published as is. I think the overall data and results are fine and worth publishing, but the biological function should be correctly reflected/interpreted.

**Response:**

We thank the reviewer for this important suggestion. We have now carefully revised the title, abstract and main text and indicated that Dapl1 affects T cell response against pathogens and cancer. The major changes in the text are highlighted in red.

I had also asked the authors to better characterize the "kinetics/number of OT-1 and pMel." The authors have now included a statement about "enhanced expansion" of adoptively transferred Dapl1 T cells and refer to data 2d-f. Surprisingly, the authors only show data for two mice to support this claim. More data should be presented, and perhaps a link could be provided to ExtData 7b, which shows a slight preference for the KO cells in the tumor.

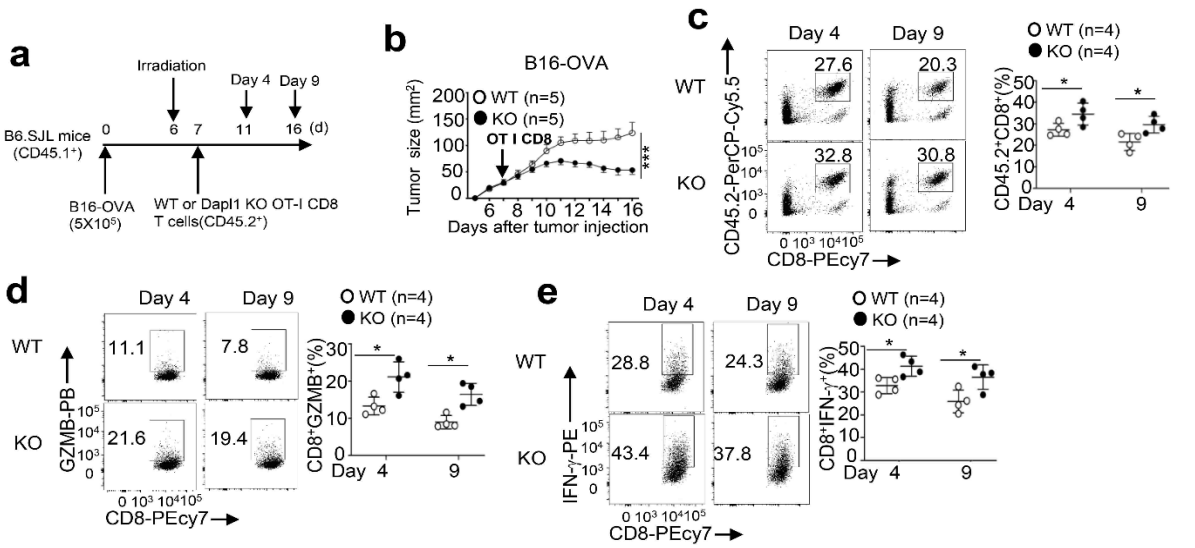
**Response:**

We have performed the experiments suggested by the reviewer. We performed a detailed kinetic analysis of the number of OT-1 or Pmel1 CD8 cells adoptively transferred into B16-OVA or B16F10 tumor bearing mice.

Pmel1 CD8 T cell adoptive transfer model, wildtype B6.SJL mice (CD45.1$^+$) were injected subcutaneously with B16F10 melanoma cells, and after 4 days, the tumor-bearing mice were subjected to whole-body irradiation. One day after the irradiation, the mice were adoptively transferred with *in vitro*-activated wildtype or Dapl1 KO Pmel1 CD8 T cells (CD45.2$^+$). The tumor-bearing mice were euthanized for flow cytometry analysis of tumor-infiltrating CD8 T cells on day 5, 10 and 15 after Pmel1 cell transfer (Extended Data Fig. 2d). We found that Dapl1 deficiency profoundly increased the frequency Pmel1 CD8 T cells throughout a time course (Extended Data Fig. 2e). Dapl1-deficient Pmel1 CD8 cells showed an increased

production of IFN-g, TNF-a and IL-2 in the tumor compared to the wildtype Pmel1 CD8 cells (Extended Data Fig. 2f,g) in the tumor of day 5, 10 and 15 post-transfer, n=5 in each group. Similar results were observed in OTI adoptively transfer tumor model (See Figure R2 below). Together, these results suggest that Dapl1 deficiency promotes the expansion and antitumor effector function of CD8 TILs.

**Figure R2**




**a-e**, Schematic of experimental design (**a**), tumor growth curve (**b**), flow cytometry analysis of the frequency of donor OTI CD8 T cells (CD45.2$^+$) (**c**) and donor (CD45.2$^+$) IFN-γ- or Granzyme B-producing OTI CD8 T cells (**d,e**) in the tumor of B16F10-implanted B6.SJL mice (CD45.1$^+$) adoptively transferred with *in vitro* activated wildtype or *Dapl1* KO OTI CD8 T cells (CD45.2$^+$, 5x10$^5$ cells/mouse) (collected on day 4 and 9 after OTI CD8 cell transfer). Data are presented as representative FACS plots and summary graphs. Summary data are shown as the mean±s.d. with P values determined using a two-tailed unpaired Student's t-test (**c-e**) and two-way ANOVA with Bonferroni correction (**b**). *P< 0.05; ***P< 0.001.

## References


1. Zhao, D.M. *et al.* Constitutive activation of Wnt signaling favors generation of memory CD8 T cells. *J Immunol* **184**, 1191-1199 (2010).
2. Willinger, T. *et al.* Human naive CD8 T cells down-regulate expression of the WNT pathway transcription factors lymphoid enhancer binding factor 1 and transcription factor 7 (T cell factor- 1) following antigen encounter in vitro and in vivo. *J Immunol* **176**, 1439-1446 (2006).


**Decision Letter, second revision:**

nature research

Subject: Your manuscript, NCB-S45561B
Message: Our ref: NCB-S45561B

10th March 2022

Dear Dr. Sun,

Thank you for submitting your revised manuscript "Dapl1 suppresses CD8 T cell responses by controlling NFATc2 activation" (NCB-S45561B). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Cell Biology, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in about a week. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Cell Biology Please do not hesitate to contact me if you have any questions.

Sincerely,

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: zhe.wang@nature.com

Reviewer #1 (Remarks to the Author):

The authors address my concerns. This revised manuscript meets with the high impact and standard for Nature Cell Biology.

Reviewer #2 (Remarks to the Author):

The authors have addressed my remaining concerns in a satisfying manner.

Reviewer #3 (Remarks to the Author):

The authors addressed all the points I had raised.

**Decision letter, final requests:**

Subject: NCB: Your manuscript, NCB-S45561B
Message: Our ref: NCB-S45561B

12th April 2022

Dear Dr. Sun,

Thank you for your patience as we've prepared the guidelines for final submission of your Nature Cell Biology manuscript, "Dapl1 suppresses CD8 T cell responses by controlling NFATc2 activation" (NCB-S45561B). 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. 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 one week). Please get in contact with us if you anticipate delays.

When you upload your final materials, please include a point-by-point response to any remaining reviewer comments.

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-research/editorial-policies/plagiarism#policy-on-duplicate-publication for details).

In recognition of the time and expertise our reviewers provide to Nature Cell Biology's editorial process, we would like to formally acknowledge their contribution to the external peer review of your

manuscript entitled "Dapl1 suppresses CD8 T cell responses by controlling NFATc2 activation". For those reviewers who give their assent, we will be publishing their names alongside the published article.

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If you have any further questions, please feel free to contact us. Thank you!

Best regards,

Ziqian Li
Editorial Assistant
Nature Cell Biology

On behalf of

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

Tel: +44 (0) 207 843 4924
email: zhe.wang@nature.com

Reviewer #1:
Remarks to the Author:
The authors address my concerns. This revised manuscript meets with the high impact and standard for Nature Cell Biology.

Reviewer #2:
Remarks to the Author:
The authors have addressed my remaining concerns in a satisfying manner.

Reviewer #3:
Remarks to the Author:
The authors addressed all the points I had raised.

**Author Rebuttal, second revision:**

Reviewer #1:
Remarks to the Author:
The authors address my concerns. This revised manuscript meets with the high impact and standard for Nature Cell Biology.
Response: We thank the reviewer for approving our revised manuscript.

Reviewer #2:
Remarks to the Author:
The authors have addressed my remaining concerns in a satisfying manner.
Response: We thank the reviewer for approving our revised manuscript.

Reviewer #3:
Remarks to the Author:
The authors addressed all the points I had raised.
Response: We thank the reviewer for approving our revised manuscript.

Sincerely,

Shao-Cong Sun, corresponding author
Lele Zhu, corresponding author

**Final Decision Letter:**

Subject: Decision on Nature Cell Biology submission NCB-S45561C
Message:

Dear Dr Sun,

I am pleased to inform you that your manuscript, "Dapl1 controls NFATc2 activation to regulate CD8+ T cell exhaustion and responses in chronic infection and cancer", has now been accepted for publication in Nature Cell Biology.

Thank you for sending us the final manuscript files to be processed for print and online production, and for returning the manuscript checklists and other forms. Your manuscript will now be passed to our production team who will be in contact with you if there are any questions with the production quality of supplied figures and text.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Cell Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Please feel free to contact us if you have any questions.

With kind regards,

Zhe Wang, PhD
Senior Editor
Nature Cell Biology

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