EMBO reports

STAT3 but not STAT4 is critical for γδT17 cell responses and skin inflammation

Rasmus Agerholm, John Rizk, Monica Torrellas Viñals, and Vasileios Bekiaris

Review timeline:

Submission date:	20 June 2019
Editorial Decision:	8 July 2019
Revision received:	31 July 2019
Editorial Decision:	12 August 2019
Revision received:	23 August 2019
Accepted:	4 September 2019

Editor: Martina Rembold/Achim Breiling

Transaction Report:

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

1st Editorial Decision 8 July 2019

Thank you for the submission of your research manuscript to our journal. We have now received the full set of referee reports that is copied below.

As you will see, the referees acknowledge that the findings are interesting and represent an advance in gd T cell biology. Nevertheless, the referees also raise a number of concerns that need to be addressed. In addition, the paper by Cai et al., 2019 should be cited and thoroughly discussed.

Given these constructive comments, we would like to invite you to revise your manuscript with the understanding that the referee concerns (as detailed above and in their reports) must be fully addressed and their suggestions taken on board. Please address all referee concerns in a complete point-by-point response. Acceptance of the manuscript will depend on a positive outcome of a second round of review. It is EMBO reports policy to allow a single round of revision only and acceptance or rejection of the manuscript will therefore depend on the completeness of your responses included in the next, final version of the manuscript.

Revised manuscripts should be submitted within three months of a request for revision; they will otherwise be treated as new submissions. Since timing matters in this case and since the referees ask for a minor revision only, I suggest to submit the revised manuscript as soon as possible. Would 2 months be sufficient? Please contact me in case you have any questions or comments.

When submitting your revised manuscript, please carefully review the instructions that follow below. Failure to include requested items will delay the evaluation of your revision.

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

See https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/EMBOPress_Figure_Guidelines_061115-1561436025777.pdf for more info on how to prepare your figures.

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide>). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript (<https://orcid.org/>). Please find instructions on how to link your ORCID ID to your account in our manuscript tracking system in our Author guidelines (<https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines>)

6) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview>

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

7) We would also encourage you to include the source data for figure panels that show essential data. Numerical data should be provided as individual .xls or .csv files (including a tab describing the data). For blots or microscopy, uncropped images should be submitted (using a zip archive if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

8) Regarding data quantification:
- Please ensure to specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments underlying each data point (not replicate measures of one sample), and the test used to calculate p-values in each figure legend. Discussion of statistical methodology can be reported in the materials and methods section, but figure legends should contain a basic description of n, P and the test applied.
IMPORTANT: Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates. If the data rely on a smaller number of replicates, scatter blots showing individual data points are recommended.
- Graphs must include a description of the bars and the error bars (s.d., s.e.m.).
- Please also include scale bars in all microscopy images.

9) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list,

data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at <https://www.embopress.org/page/journal/14693178/authorguide#referencesformat>.

10) As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. This File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

You are able to opt out of this by letting the editorial office know (emboreports@embo.org). If you do opt out, the Review Process File link will point to the following statement: "No Review Process File is available with this article, as the authors have chosen not to make the review process public in this case."

We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

I look forward to seeing a revised version of your manuscript when it is ready. Please let me know if you have questions or comments regarding the revision.

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REFEREE REPORTS

Referee #1:

This paper by Bekiaris and colleagues is a very straightforward study, based on conditional KO mouse technology, that delivers a clear and important message: STAT3 (but not STAT4) regulates IL-17+ γδ (γδT17) cell numbers, as well as their inflammation-driven production of IL-17A, IL-17F and IL-22, in the skin, thus dictating susceptibility to psoriasis-like disease.

Very recently, Cai et al. published in Cell Rep that "although the STAT3 pathway is critical for dermal Vγ4T17 effector function, it is not required for Vγ6T17 cells. (...) The absence of mTORC2 in dermal γδT cells, but not STAT3, ameliorates skin inflammation". While this publication could compromise the novelty of the study under evaluation here, the fact that they differ in their conclusions for STAT3 requirement (namely, STAT3-/- mice displaying distinct skin phenotypes in the two studies) justifies an increased interest in the current study. It is important for the community to gain access to both sets of data so that they can be challenged/ addressed by other researchers. Nonetheless, the paper by Cai et al. should be cited and thoroughly discussed - including potential reasons for the discrepancies between the two studies.

Major issues:

1. Whereas the role of IL-17A in this model well established, what is the relative relevance of IL-17F and IL-22? And even if the cytokines themselves may be important, what is the relevance of gd T cells as their source? Reverse gating strategies for the 3 cytokines, showing the fraction of cytokine-producing cells specifically accounted by gd T cells, should be shown and discussed.
2. The in vivo data of Figure 3, showing that "production of IL-17A and IL-17F is driven by STAT3 during inflammation but not at steady-state" should be complemented by in vitro experiments to demonstrate a defect of STAT3-/- gd T cells (best to be enriched for CD27- CD44+) to respond to Th17-driving cytokines (such as IL-1b or IL-23). This point could be made out of experiments such as the one shown on Fig 4C - although there, IL-17A production seems very abundant upon deletion of STAT3??
3. Given that the previous report of Cai et al. suggests a different impact of STAT3 on Vg4+ versus Vg6+ gd T cells, Bekiaris and colleagues should attempt to specifically address this issue in their manuscript.

Minor: When discussing the phenotype of gd17 T cells (referring to figure EV1), authors should cite

the papers that established the relevant markers: CCR6, CD44, CD27 (Haas et al. EJI 2009; Ribot et al. Nat Immunol 2009).

Referee #2:

In their manuscript entitled "Importance of STAT3 and STAT4 in regulation γδT17 cell responses and skin inflammation", Agerholm et al. provide data on the role of JAK/STAT signaling in γδ T cells during homeostasis and dermal inflammatory disease. The authors use Rorc-Cre;Stat3F/F mice and Stat4-/- mice to show that STAT3 (but not STAT4) regulates γδ T cell numbers in skin and lymph nodes, after imiquimod (IMQ)-induced psoriasis. Deletion of STAT3 in RORγt-expressing cells also reduces skin disease pathology, as demonstrated by decreased epidermal thickness. The authors show that STAT3 regulates IL-17A, IL-17F and IL-22 production by γδ T cells in lymph nodes of IMQ-treated mice, while STAT4 regulates only IL-17F.

Overall, this study represents an advance in γδ T cell biology by contributing important information on the affect of STAT3 signaling on IL-17-related molecules in skin inflammation. Although the role of STAT3-regulated IL-17 has been established for some time in Th17 cells, the novelty of the study lies within the methodology where specific depletion of STAT3 in RORγt-expressing cells was used to show phenotype and function. The authors show a causal relationship between STAT3 signaling, IL-17-producing γδ T cells and psoriasis. The experiments are nicely controlled with adequate power and the conclusions are not overstated. Below are some minor comments that should be addressed to strengthen the manuscript:

1. Several lymphocyte populations - including some CD4 T cells, innate lymphoid cells and NK cells - express RORγt, whose promoter was used to drive Cre expression. Therefore, the statement in the Abstract stating "...only mice lacking STAT3 expression in γδT17 cells..." is not entirely true.

2. What is the effect of STAT3 depletion in these other populations (i.e. Th17 cells which can be analyzed by gating on TCRβ)?

3. The use of the word "resistant" in the Abstract should be reconsidered, since Rorc-Cre;Stat3F/F mice still developed psoriasis, albeit to a lesser degree than Stat3F/F mice.

4. Please remove "for the first time" from the Abstract. Other studies have shown the importance of STAT3 in γδ T cells under homeostatic conditions (Shibata et al., Blood 2011).

5. In the Introduction, the statement "...although γδT17 cells originate in the embryonic thymus..." should be reworded. IL-17-producing γδ T cells can be derived from thymus but also extra-thymically from yolk sac (see Gentek et al., JEM 2018).

6. How are Vγ5 cells affected in IMQ-treated Rorc-Cre;Stat3F/F mice? The data on STAT3 and IL-17-producing γδ T cells would be further supported by showing that other γδ T cell populations are normal in this mouse model.

7. The use of the terminology "Type 3 cytokines" is not common and may need further explanation.

8. The authors may reconsider using "γδT17" as STAT3 may regulate other cytokines that were not measured. IL-17 can't be the only molecule regulated by STAT3.

9. The data displayed in Figure 3C, F and Figure 5B, D are not represented correctly. Cre- mice and Cre+ mice cannot be analyzed by a paired t test because these are different mice. They are not the same units measured under different experimental conditions - they are different units measured under the same experimental conditions. The data should be shown differently and Mann-Whitney should be used to analyze the differences. My guess is that there is no difference between the groups, so the interpretation will have to be restated as well.

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Very recently, Cai et al. published in Cell Rep that "although the STAT3 pathway is critical for dermal Vγ4T17 effector function, it is not required for Vγ6T17 cells. (...) The absence of mTORC2 in dermal γδT cells, but not STAT3, ameliorates skin inflammation". While this publication could compromise the novelty of the study under evaluation here, the fact that they differ in their conclusions for STAT3 requirement (namely, STAT3-/- mice displaying distinct skin phenotypes in the two studies) justifies an increased interest in the current study. It is important for the community to gain access to both sets of data so that they can be challenged/ addressed by other researchers. Nonetheless, the paper by Cai et al. should be cited and thoroughly discussed - including potential reasons for the discrepancies between the two studies.

- We thank the reviewer for pointing this out. We have now thoroughly discussed the findings by Cai et al in relation to our data. In relation to this, we now present data on the activity of the RORγt^{CRE} in different populations after we crossed RORγt^{CRE} with ROSA26-STOPflox-RFP mice. Please refer to amended text in lines 119-131 and amended Figure EV1 panel C with its corresponding title and legend.

Major issues:

1. Whereas the role of IL-17A in this model well established, what is the relative relevance of IL-17F and IL-22? And even if the cytokines themselves may be important, what is the relevance of gd T cells as their source? Reverse gating strategies for the 3 cytokines, showing the fraction of cytokine-producing cells specifically accounted by gd T cells, should be shown and discussed.

- Both IL-17F and IL-22 have been shown to be important for induction of epidermal thickening during IMQ-driven psoriasis. Burkhard Becker and colleagues used IL-17A, IL-17F and IL-22 knockout mice and showed that both IL-17F and IL-22 deficiency protects from IMQ-induced psoriasis. In fact, IL17F$^{-/-}$ mice were better protected than IL-17A$^{-/-}$ mice (Pantelyushin et al, 2012, JCI). We now include this reference in our manuscript and have amended our text in lines 135-137.

- We now show reverse gating. We gated on all IL-17A$^+$, IL-17F$^+$ or IL-22$^+$ live cells and displayed the frequencies of TCRγδ$^+$ versus TCRβ$^+$. We found that the significant majority of IL-17A- and IL-17F-producing cells are TCRγδ$^+$. Due to the very low frequency of detectable IL-22$^+$ cells directly ex vivo, we first pre-gated on IL-17A$^+$ cells and then on IL-22$^+$. We could not detect IL-22$^+$ cells that were not IL-17A$^+$. See our new figure EV5 and its corresponding legend and amended text lines 153-158 and 161-164.

2. The in vivo data of Figure 3, showing that "production of IL-17A and IL-17F is driven by STAT3 during inflammation but not at steady-state" should be complemented by in vitro experiments to demonstrate a defect of STAT3-/- gd T cells (best to be enriched for CD27- CD44+) to respond to Th17-driving cytokines (such as IL-1b or IL-23). This point could be made out of experiments such as the one shown on Fig 4C - although there, IL-17A production seems very abundant upon deletion of STAT3??

- As clearly pointed out by the reviewer STAT3 does not impact steady-state production of IL-17A or IL-17F, which we show in Figure 3 and is also evident in Figure 4. In our experience in vitro cultures often cannot replicate in vivo results and therefore measurement of cytokines directly after cell isolation reflects what happens in the organism much closer. Thus, our data clearly show that at steady-state and with as minimum cell manipulation as possible, STAT3 does not impact on cytokine production.

However, we performed cultures with IL-23 and IL-1β of LN cells from RORγt^{CRE}-STAT3$^{F/F}$ and littermate control mice as suggested. We found that the frequency of IL17A^{+} or IL-17F^{+} cells did not change irrespective of treatment (Fig 3G-H). However, we observed that induction of IL-17A and IL-17F MFI by both IL-23 and IL-1β was halted in STAT3 deficient cells (Fig 3G-H). Induction of IL-22^{+} cells was also impaired in STAT3-deficient irrespective of stimulus (Fig 4E). See amended text lines 140-146 and 169-170 and amended Figures 3 and 4 with their corresponding legends.

3. Given that the previous report of Cai et al. suggests a different impact of STAT3 on Vg4+ versus Vg6+ gd T cells, Bekiaris and colleagues should attempt to specifically address this issue in their manuscript.

- This is a valid argument and we would like to point out that Figure EV3 is dedicated on analyzing the impact of STAT3 and STAT4 in Vγ4^{+} and Vγ4^{-} (Vγ6^{+}) cells. Based on this evidence we concluded that there is not differential impact of STAT3 or STAT4 on these two subsets of γδ T cells. We have now amended the text lines 103-104 to make this clearer.

Minor: When discussing the phenotype of gd17 T cells (referring to figure EV1), authors should cite the papers that established the relevant markers: CCR6, CD44, CD27 (Haas et al. EJI 2009; Ribot et al. Nat Immunol 2009).

- This was a major citation omission on our behalf !! Thank you for pointing this out. Both papers are now cited.

Referee #2:

In their manuscript entitled "Importance of STAT3 and STAT4 in regulation γδT17 cell responses and skin inflammation", Agerholm et al. provide data on the role of JAK/STAT signaling in γδ T cells during homeostasis and dermal inflammatory disease. The authors use Rorc-Cre;Stat3F/F mice and Stat4-/- mice to show that STAT3 (but not STAT4) regulates γδ T cell numbers in skin and lymph nodes, after imiquimod (IMQ)-induced psoriasis. Deletion of STAT3 in RORγt-expressing cells also reduces skin disease pathology, as demonstrated by decreased epidermal thickness. The authors show that STAT3 regulates IL-17A, IL-17F and IL-22 production by γδ T cells in lymph nodes of IMQ-treated mice, while STAT4 regulates only IL-17F.

Overall, this study represents an advance in γδ T cell biology by contributing important information on the affect of STAT3 signaling on IL-17-related molecules in skin inflammation. Although the role of STAT3-regulated IL-17 has been established for some time in Th17 cells, the novelty of the study lies within the methodology where specific depletion of STAT3 in RORγt-expressing cells was used to show phenotype and function. The authors show a causal relationship between STAT3 signaling, IL-17-producing γδ T cells and psoriasis. The experiments are nicely controlled with adequate power and the conclusions are not overstated. Below are some minor comments that should be addressed to strengthen the manuscript:

1. Several lymphocyte populations - including some CD4 T cells, innate lymphoid cells and NK cells - express RORγt, whose promoter was used to drive Cre expression. Therefore, the statement in the Abstract stating "...only mice lacking STAT3 expression in γδT17 cells..." is not entirely true.

- The reviewer is right. However, the word “only” in this case refers to the comparison between STAT3 and STAT4 deficient animals and not on which cells STAT3 is missing. We have now removed “only” to avoid this confusion (see line 14). In addition, we have added data showing the RORγt^{CRE} activity in γδ T cells and other populations (see Fig EV1C).

2. What is the effect of STAT3 depletion in these other populations (i.e. Th17 cells which can be analyzed by gating on TCRβ)?

- We and others have shown before that in the presence of γδ T cells, CD4 or other αβ T cells do not contribute to IMQ pathology (Pantelysushin et al. J Clin Invest, 2012, Bekiaris et al. Immunity 2013, Sandrock et al. J Exp Med) and we therefore did not originally investigate

further. However, we have now re-analyzed our flow cytometry data by focusing on $TCR\beta^+CD4^-$ and $TCR\beta^+CD4^+$ populations in lymph node and skin. Indeed we found that STAT3 deficiency impacted on $TCR\beta^+$ cells, which responded to IMQ, as previously shown (Sandrock et al. J Exp Med). However, the contribution of γδT17 cells was significantly higher. See amended text lines 104-109 and 153-158 and new figures EV4 and EV5 with their corresponding legends.

3. The use of the word "resistant" in the Abstract should be reconsidered, since Rorc-Cre;Stat3F/F mice still developed psoriasis, albeit to a lesser degree than Stat3F/F mice.

- We have now changed this statement. See lines 14-15.

4. Please remove "for the first time" from the Abstract. Other studies have shown the importance of STAT3 in γδ T cells under homeostatic conditions (Shibata et al., Blood 2011).

- Shibata et al showed no role for STAT3 in γδ T cells, however they did not study these cells in the skin or during inflammatory conditions. We therefore think that it is appropriate to use this phrase.

5. In the Introduction, the statement "...although γδT17 cells originate in the embryonic thymus..." should be reworded. IL-17-producing γδ T cells can be derived from thymus but also extra-thymically from yolk sac (see Gentek et al., JEM 2018).

- To the best of our knowledge Gentek et al, showed that DETCs derive from yolk sac and did not investigate γδT17 cells. Spidale et al., Immunty, 2018, displayed some evidence that γδT17 cells may have their earliest progenitors in the yolk sac, however the authors could definitively conclude this. The authors summarized these results as follows: "These results supported the possibility that Tgd17 cells may originate from embryonic hematopoietic tissues, rather than the conventional lymphopoietic pathway. A definitive demonstration waits physiological testing of YS developmental potential in utero and/or improved culture system to favor lymphopoiesis from defined YS cell subsets". Therefore, we cannot state that γδT17 cells can be derived from YS.

6. How are Vγ5 cells affected in IMQ-treated Rorc-Cre;Stat3F/F mice? The data on STAT3 and IL-17-producing γδ T cells would be further supported by showing that other γδ T cell populations are normal in this mouse model.

- We are not aware of any published data reporting the importance of Vγ5 cells in the IMQ response. Furthermore, Vγ5 cells do not express RORγt at any point during their ontogeny (Turkinovich and Hayday, Immunity 2011). We therefore did not analyze them. Moreover, our methodology for isolating skin lymphocytes favors dermal γδ T cells and not the epidermal-specific Vγ5 population. Despite this, we analyzed lymph node $CD27^+$ γδ T cells in $ROR\gamma t^{CRE}$-$STAT3^{F/F}$ mice and found that although they expand during IMQ treatment, STAT3 deficiency in $ROR\gamma t^+$ cells does not affect them (see new Figure EV4E). Finally, by using $ROR\gamma t^{CRE}$-$RFP^{STP-F/F}$ mice, we show in Fig EV1C that $CD27^+$ γδ T cells do not express reporter activity suggesting a minimal impact of the $ROR\gamma t^{CRE}$ in this population.

7. The use of the terminology "Type 3 cytokines" is not common and may need further explanation.

- We have now amended the text in lines 52, 101, 113 without using this terminology to avoid confusion.

8. The authors may reconsider using "γδT17" as STAT3 may regulate other cytokines that were not measured. IL-17 can't be the only molecule regulated by STAT3.

- We are not entirely sure what the reviewer is referring to here. STAT3 certainly does not only regulate IL-17 but also IL-22 as we show and others have shown for Th17 and group 3 innate lymphoid cells. The nomenclature γδT17 is common within the field to refer to the γδ T cell population that expresses IL-17, similar to Th17, which refers to CD4 T-helper cells that produce IL-17.

9. The data displayed in Figure 3C, F and Figure 5B, D are not represented correctly. Cre- mice and Cre+ mice cannot be analyzed by a paired t test because these are different mice. They are not the same units measured under different experimental conditions - they are different units measured under the same experimental conditions. The data should be shown differently and Mann-Whitney should be used to analyze the differences. My guess is that there is no difference between the groups, so the interpretation will have to be restated as well.

- As it is evident from Figures 3C, F and 5B, D, the differences in the MFIs between cells from Cre^- and Cre^+ mice within the same experiment are consistent; thus for example in 3C IL-17A MFI is always lower in Cre^+ cells when compared to Cre^- cells of the same experiment (indicated by the lines in original figure). However, there is great variation in MFI measurements simply because the experiments were done over the course of months and thus the strength of the fluorescence detection and any given fluorochrome varies according to the flow cytometer's baseline. Because a Mann-Whitney cannot account for this variation we dealt with this data using a statistical analysis that we have used in the past (Bekiaris et al., Immunity, 2013). Therefore, to correct for the technical variations of MFI measurement between experiments we used a 2-way ANOVA test in R that was defined as such: lm(MFI ~ Var1 + Var2 ,data=data), where Var1 represents the experiment (i.e. exp#1, exp#2 etc) and Var2 represents the experimental condition (i.e. Cre^-, Cre^+). We have now included this in our Methods section lines 253-256 and in the corresponding figure legends. Mind that in the figure legends, if significant, we additionally display p-values calculated by the Mann-Whitney test. Furthermore, we have changed the display of our Figure 3C, F and 5B, D panels so that each color represents a different experiment.

2nd Editorial Decision 12 August 2019

Thank you for the submission of your revised manuscript to our editorial offices. We have now received the reports from the two referees that were asked to re-evaluate your study, you will find below. As you will see, both referees now support the publication of your study in EMBO reports.

Before we can proceed with formal acceptance, I have a few editorial requests.

REFEREE REPORTS

Referee #1:

Authors have made adequate revisions that improved the manuscript.

Referee #2:

The authors have satisfactorily addressed my comments and suggestions. This is an important study for the gamma delta T cell field.

2nd Revision - authors' response 23 August 2019

The authors performed all minor editorial changes.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Vasilieos Bekiaris
Journal Submitted to: Embo Reports
Manuscript Number: 48647

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

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

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

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

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

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B- Statistics and general methods

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

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	At least 4 samples were analyzed to ensure adequate power.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	at least 5 mice/ condition were analyzed to ensure adequate power.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	no samples were exlucded.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	mixed groups of male and female mice were assigned to groups before designating treatment or control conditions to minimize bias
For animal studies, include a statement about randomization even if no randomization was used.	mixed groups of male and female mice were assigned to groups before designating treatment or control conditions to minimize bias
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	no
4.b. For animal studies, include a statement about blinding even if no blinding was done	animal experiments were not blind.
5. For every figure, are statistical tests justified as appropriate?	yes.
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	yes the data meets the assumptions of the tests

Is there an estimate of variation within each group of data?	yes
Is the variance similar between the groups that are being statistically compared?	no

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	CD4-FITC (RM4-4), CD19-FITC (6D5), CD8-FITC (53-6.7), TCRβ-APCeF780 (H57-597; eBioscience), TCRγδ-BV421 (GL3), CD44-V500 (IM7), CCR6-AF647 (140706), Vγ4-PerCPeF710 (UC3-10A6), CD27-PECy7 (LG.3A10), Vγ5-FITC (536), CD3-PE (145-2C11; BioLegend), CD45-V500 (30-F11), IL-17A-BV786 (TC11-18H10), IL-17F-PECF594 (O79-289), IL-22-PE (1H8PWSR; eBioscience), IFNγ-APC (XMG1.2; BioLegend), CD3-PECF594 (145-2C11), CD4-BUV395 (RM4-5)
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	specied: mus musculus. Strain: RORgtCre- STAT3f/f, RORgtCre+ STAT3f/f ,STAT4 -/+, STAT4 -/- . Age: at least 8 weeks. Gender: both females and males. Housing and husbandary: mice were housed at the animal facility in DTU health tech, in individually ventilated cages under SPF conditions.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Animal studies herein are in line with the ARRIVE guidelines.

E- Human Subjects

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

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	no, the study does not fall under dual use research restriction