EMBO reports

# Lactate induces metabolic and epigenetic reprogramming of pro-inflammatory Th17 cells

Aleksandra Lopez Krol, Hannah Nehring, Felix Krause, Anne Wempe, Hartmann Raifer, Andrea Nist, Thorsten Stiewe, Wilhelm Bertrams, Bernd Schmeck, Maik Luu, Hanna Leister, Ho-Ryun Chung, Uta-Maria Bauer, Till Adhikary, and Alexander Visekruna
**DOI: 10.15252/embr.202254685**

*Corresponding author(s): Alexander Visekruna (alexander.visekruna@staff.uni-marburg.de)*



## Review Timeline:

| | |
|---|---|
| Submission Date: | 18th Jan 22 |
| Editorial Decision: | 24th Jan 22 |
| Revision Received: | 17th Mar 22 |
| Editorial Decision: | 25th Apr 22 |
| Revision Received: | 10th May 22 |
| Editorial Decision: | 10th May 22 |
| Revision Received: | 26th Jul 22 |
| Editorial Decision: | 8th Sep 22 |
| Revision Received: | 22nd Sep 22 |
| Accepted: | 27th Sep 22 |

*Editor: Deniz Senyilmaz-Tiebe/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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

**1st Editorial Decision** **24th Jan 2022**

Dear Dr. Visekruna,

Thank you for submitting your manuscript to EMBO Reports. I have read your study carefully and discussed it with the other members of our editorial team including our chief editor Dr. Bernd Pulverer and sought advice from a good expert in the field whose opinion we trust. I regret to inform you that we have decided not to pursue publication of this manuscript in its current form, but we would be happy to reconsider it with some additional analysis as mentioned below. In its current form, we recommend a transfer to Life Science Alliance.

I apologize for this unusual delay in getting back to you, which was caused by the current high rate of new submissions to our office, affecting our usually much shorter editorial handling time. Also, we needed to wait for the expert's advice.

We appreciate your study demonstrating that CD4+ T cell activation is accompanied by an increase in histone lactylation. The findings further reveal that exogenous lactate promotes a phenotypic shift towards Treg phenotype in Th17 lymphocytes and a global increase in histone lactylation levels in particular around the promoter region of Foxp3. We realize that these findings are as such of interest to the field. However, we also find that, in our view, a causality between increased histone lactylation and the shift towards a Treg phenotype remains to be established. Moreover, functional relevance of the findings remains elusive as majority of the analyses were performed in vitro.

As mentioned above, I also sought advice from an external expert whose opinion we trust. The advisor mirrored our reservations.

We feel that these points would come up during peer-review as well. As such, we concluded that the advance provided is not sufficient for publication in EMBO Reports in the current form of the manuscript. That said, we would be happy to send the manuscript out for formal peer-review should you be willing to include additional data addressing at least one of these concerns in a reasonable timeframe.

That said, and as mentioned above, your work in its current form is an excellent candidate for of our partner journal Life Science Alliance (http://www.life-science-alliance.org/; our broad scope Open Access journal published in partnership between the EMBO-, Rockefeller University-, and Cold Spring Harbor Laboratory Presses). The editors of Life Science Alliance would be pleased to send your manuscript for in-depth peer review; no reformatting is required. We very much hope you will be interested in this option: please follow the link below for transfer. Eric Sawey, Executive Editor of Life Science Alliance (e.sawey@life-science-alliance.org), will be pleased to answer any questions.

I very much hope that you are interested in this option - please use the following link for transfer; no reformatting is required.

Yours sincerely,

Deniz Senyilmaz Tiebe, PhD
Scientific Editor
EMBO Reports

** As a service to authors, EMBO Press provides authors with the ability to transfer a manuscript that one journal cannot offer to publish to another journal, without the author having to upload the manuscript data again. To transfer your manuscript to another EMBO Press journal using this service, please click on Link Not Available

Dear Dr. Senyilmaz Tiebe,

Please find attached our revised manuscript EMBOR-2022-54685V1.

According to your comments and suggestions made by an expert in the field, we have generated additional /in vivo/ data. We do see that /in vivo/ treatment of mice with lactate suppresses the activity of inflammatory Th17 cells in the gut, resulting in amelioration of T cell-driven colonic inflammation. We think that these results (novel Fig. 4C-F) provide a functional relevance of the previous findings performed by us /in vitro/. Interestingly, we also observe that lactate-producing bacteria such as /Lactobacillus reuteri/, but not other common commensal bacteria (e.g. /Escherichia coli /or /Bacteroides fragilis/), strongly reduce the production of IL-17A in Th17 cells (novel Fig. 4A and B). All changes made in the revised version of our manuscript are marked in red color.

Just a few weeks ago, a novel manuscript published by Science Advances (Carlos Moreno-Yruela et al., Sci. Adv., 2022) has demonstrated that the enzymes histone acetyltransferase p300 and histone deacetylases HDAC1-3 regulate not only acetylation, but also lactylation of histones. We have cited and discussed this important paper in our revised manuscript.

We hope that our manuscript is now suitable for an external review process.

Should I re-submit our revised manuscript as a new submission via editorial menager ?

Thank you for your consideration.

Kind regards,

Alexander Visekruna

## 1st Revision - Editorial Decision 25th Apr 2022

Dear Prof. Visekruna,

Thank you for submitting your manuscript to EMBO Reports. We have now received three referee reports, which are included below.

We concur with the referees that the proposed role of histone lactylation in reprogramming of Th17 cells is in principle very interesting. However, referees also raise some concerns that need to be addressed to consider publication here. In particular,

- The links between extracellular lactate and the observed metabolic remodeling, histone lactylation and immune phenotypes need to be further investigated (referee #1 point 3, referee #3 point 2).

- The role of ROS in the process needs to be better demonstrated (referee #1 points 4, 5, referee #3 point 1). Of note, elucidation of the exact mechanism by which lactate induces ROS production (referee #3 point 1) is not required, but the link needs to be robustly demonstrated.

- Additional controls for the ChIP data are required (referee #1 point 2, referee #2 major points, referee #3 point 2).

As such, they do not recommend publication here. Given such input from these recognized experts who are also experienced referees, and the amount of work required to address these concerns, we cannot publish your manuscript in its current form.

However, in case you feel that you can address the referee concerns in a timely and thorough manner, and can obtain data that would considerably strengthen the study as in the referee reports, we would have no objection to consider a revised manuscript (along with a point-by-point response to the referee concerns) in the future. Please note that if you were to send a new manuscript this would be assessed again with respect to the literature and the novelty of your findings at the time of resubmission and in case of a positive editorial evaluation, the manuscript would be sent back to the original referees. I would like to emphasize that we will be reluctant to approach the referees again in the absence of major revisions, and we need strong support from the referees to consider publication here.

Of note, our sister journal Life Science Alliance (LSA) might be able to offer publication pending revision with a narrower scope. As per for your preference during submission, I could not discuss your manuscript and the reports with the editors of LSA, but I would be happy to do so should that be of interest. Eric Sawey, Executive Editor of Life Science Alliance (e.sawey@life-science-alliance.org), will be pleased to answer any questions.

Thank you in any case for the opportunity to consider this manuscript. I am sorry that I cannot communicate more positive news, but nevertheless hope that you will find our referees' comments helpful.

Kind regards,

Deniz Senyilmaz Tiebe

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

Referee #1:

This study addressed a crucial question in the field of tumor microenvironment, i.e. how the accumulated lactate regulates T cells in the niche.
They discovered that extracellular lactate modulates epigenetic status of Th17 cells, reducing IL-17A production and upregulating Foxp3 expression. Treatment of lactate leads to global alterations in gene expression, shifting Th17 signature gene expression patterns towards Treg cells. This shift coincides with an increase in ROS levels. Next they verified that it is ROS that drives the secretion of IL-2 and subsequently leads to the reduction of IL-17A, and reduction in ROS could partially rescue the decrease in IL-17A. In addition, they employed a T cell transfer model of colitis in mice, and demonstrated that lactate treatment suppresses Th17 pathogenicity by reducing IL-17A.
Overall, this work is an addition to the growing understanding of the role of lactate and histone lysine lactylation in tumor microenvironment and has potential therapeutic values. However, a few pieces of key evidence are still lacking to consolidate their conclusions. Below are a few major and minor comments that, if addressed properly, may further improve the quality of this study.

Major comments
1. Fig EV1B. They discovered LGSH, but not lactate, induced lactylation of purified histones without enzymes, and they

concluded that "this implies an enzyme-dependent mechanism underlying lactate-derived histone lactylation in T cells". Concentration of LGSH is very important in this experiment. What is the cellular concentration of LGSH? Your concentration is much higher than its cellular concentration. If this be the case, please clearly describe the issue. In addition, to strengthen this argument, you should stimulate the cellular LGSH to check out if K-L-La is elevated.
2. Fig EV3. Only three promoters regions were displayed, which raises the question what about the other regions. Peaks of additional promoter regions are expected to provide proper controls for this experiment. Without proper controls, the interpretation of their results is questionable.
3. A key aspect of this study is metabolic reprogramming of Th17, as indicated by the title and conclusions. However, the evidence provided is not sufficient to support this statement. Indeed, Foxp3 protein abundance and the transcripts of some metabolism related genes are altered, but it remains to be determined how these alterations translate to metabolic reprogramming.
4. Fig 3E. NAC, by itself has been shown to have immune-regulatory roles via ROS, increasing IL-2 production in human peripheral blood T cells, independent of lactate. The provided result suggests that NAC and lactate can both regulate IL-2 levels, but it does not necessarily confirm that lactate-induced IL-2 is via ROS. The authors are expected to test different reducing reagents and assess the activation of NFAT during the treatment.
5. Fig EV4. The authors hypothesized that DCA treatment would increase ROS levels without testing. This does not provide more evidence that ROS mediates lactated-induced immune-regulatory effect. ROS measurement is expected to support their hypothesis. It is of note that DCA treatment significantly elevates IFN-γ, but lactate does not, which suggests that these two treatments may trigger different outcomes.
6. this referee would suggest a Summary or Discussion section.

7. To establish the role of histone lysine lactylation, ChIP-seq is necessary for the experiments described in fig 3.

Minor comments
1. Some of the figure panels are not referred to properly, e.g. Fig 1H and Fig 2F)

Referee #2:

In this report Krol and colleagues investigate how intracellular and extracellular lactate pools influence histone modifications, transcriptional networks and T-cell identity. They find that metabolic switching to aerobic glycolysis leads to histone lactylation. Further, extracellular lactate also increased histone lactylation and altered gene expression programs in Th17 cells, consistent with identity switching. Finally, they demonstrate that lactate treatment of implanted T cells reduces the amount of inflammatory Th17 cells in a mouse colitis model.

Overall, this study is well written and investigates an important question of how metabolic rewiring, here of lactate levels, influences histone modifications and in turn expression patterns and cell identity. The authors raise the interesting possibility to use metabolic rewiring to influence inflammatory phenotypes. While the experiments are in general of good quality with biological replicates, there are some points which the authors have to address to improve the manuscript.

Major comments:

Antibodies for histone PTMs suffer from specificity problems especially for less well-established modifications. While the histone lactylation antibodies used in this study have been previously used in Zhang et al., 2019 where they were extensively characterized, it is unclear if the same batches were used. Polyclonal antibodies can show considerative batch to batch differences. Therefore, if a different batch was used, the authors should demonstrate the specificity of their antibodies.

Related to providing evidence for the specificity of the antibody, for the ChIP-qPCR in
Fig EV3 the authors should show more controls and include further detail about the sites investigated. In particular, a negative control where no histone lactylation would be expected should be included to judge the specificity of the antibody in the ChIP. It would benefit the figure to show a small diagram indicating the position of the primers in relation to the transcription start site of the genes.
There seems to be a substantial amount of variation for the different replicates of the ChIP. The authors should analyze if the differences described are statistically significant to determine if the replicates are indeed replicating the results.
Furthermore, as a pan-lactylation antibody was used in the ChIP (in addition to H3K18la) the authors should adjust their wording to reflect that the assay can detect any lactylated protein and not only histones.

The upregulation of Foxp3 (Fig2) and the appearance of a Treg specific expression signature (Fig3) upon lactate treatment is clearly demonstrated. However, the RNAseq analysis reveals "apoptosis" as one of the enriched pathways. The authors should quantify the amount of apoptotic cells upon lactate treatment of Th17 cells. Related to this, in Fig4 there is a significant reduction of intestinal IL-17A+ T cells for the lactate treated mice. What is the fate of the implanted T cells instead? Do they become

apoptotic? If a significant amount of apoptosis is detected in these experiments, it would be good to discuss the role of general stress caused by lactate treatment (leading to apoptosis) and therefore leading to altered gene expression programs instead of a specific effect on histone modifications and reprogramming.

Minor points:
As the authors have conducted multiple replicates for the immunoblots in Figure 1(B,C,E,F,G,H) it would strengthen the manuscript to either quantify the signal or show more replicates in the supplement. This seems particularly important where there are loading differences based on total Histone H3 levels.
In the legend of Fig3E: should untreated be gray instead of white?
In general, for the reproducibility of the findings it would be helpful if gating strategies for the Flow Cytometry data are shown in the supplement.
Related to FigEV3A: On Page 6 the sentence states "This suggests that the histone lactylation is a wide spread post-translational modification, which occurs in T cells as a consequence of activated TCR signalling pathway. "
However, if the ChIP signal is specific, there is also quite some signal for Th0 cells especially for some of the replicates, indicating that histone lactylation is present before TCR signalling at these sites. The authors should adjust their statements and conclusions accordingly.

Referee #3:

Krol et al. show that lactate promotes histone lactylation in CD4+ T cells. Upon lactate treatment, Th17 cells reduced IL-17A production but upregulated Foxp3 expression, which is potentially resulted from the ROS-driven IL-2 secretion. At the end of the manuscript, the authors showed that lactate treatment reduces the frequency of intestinal Th17 cells. Although the authors try to highlight the therapeutic benefit of lactate on manipulating the epigenetic and metabolic reprogramming of Th17 cells for targeting the inflammatory or autoimmune diseases, the results here is not sufficient to support the conclusion in the manuscript. Moreover, the main message of this manuscript is unclear.

• In Figure 3D-3G, the authors suggested that the reduced IL-17A expression in Th17 cells is mediated by lactate-induced ROS generation, which further leads to the increase of IL-2 production. However, key information is missing. The authors didn't further characterize the mechanism by which ROS is produced. In addition, whether the treatment of antioxidants can affect the lactylation of Th17 is unclear. Overall, the link between lactylation and ROS/IL-2 change is uncelar.
• In Figure 1, the authors showed that lactate induce histone lactylation in CD4+ T cells. With ChIP analysis of lactylated histone in the presence and absence of extracellular lactate, they identified an enrichment of lactylation at the proximal promoter region of FoxP3 under Th17-inducing condition. The authors should further confirm that a reduction for the lactylation can be also observed in the glucose-restricted condition. In addition, the authors should further address how the lactate-mediated histone lactylation contribute to the reduced IL-17A expression and the upregulated Foxp3 expression in Th17 cells. Overall, the link between lactylation and phenotype changes is not convincing.
• In Figure 2C-2F, the authors showed that the expression of Foxp3 is elevated in Th17 cells upon lactate treatment by flow cytometry analysis. In addition, the observation of increased Foxp3 expression upon lactate treatment is further supported by RNA sequencing results which is identified with enhanced Treg features. However, the authors should further perform the experiments to demonstrate the enhanced function of Treg cells upon lactate treatment both in vitro and in vivo.
• In Figure 4A-4B, the authors cultured CD4+ T cells with the supernatant derived from Lactobacillus reuteri, Escherichia coli, and Bacteroides fragilis to test whether bacteria-derived lactate can affect the IL-17A expression from Th17 cells. In this regard, the authors should evaluate the IL-17A expression by comparing the CD4+ T cells cultured with the indicated supernatants from the bacteria in the same family. While Lactobacillus reuteri can generate lactic acid in the supernatant, the authors should collect supernatant from another non-lactic acid producing bacteria as control and the control bacteria should belong to the same family with Lactobacillus reuteri. In an alternative choice, the authors should use the supernatant collected from the mutant of Lactobacillus reuteri as control which the mutated Lactobacillus reuteri is unable to generate lactate.

** As a service to authors, EMBO Press provides authors with the ability to transfer a manuscript that one journal cannot offer to publish to another journal, without the author having to upload the manuscript data again. To transfer your manuscript to another EMBO Press journal using this service, please click on
Link Not Available

Dear Dr. Senyilmaz Tiebe,

We thank you for your email and decision. We are also grateful for insightful comments and for constructive criticism raised by our referees.

I would like to briefly summarize how we have started to fully address the reviews´ critique and how we would like to reply to the short list of critical points made by you.

With regard to specific reviwers` comments, I think that we are able to completely address all issues raised by the referees 1 and 2 (and also most points suggested by the the referee 3 can be satisfactory addressed).

Just a few days ago, we have established the ChIP sequencing for histone lactylation in our lab (we will also includeadditional controls for ChIP q-PCR into the revised manuscript), which will allow us to better understand lactate-mediated link between epigenetic and metabolic remodeling of Th17 cells.

Moreover, we have identified the transcription factor downstream of lactate-mediated metabolic effects. Our novel data reveal that lactate particularly induces mitochondrial ROS (mitoSOX Red and Oxygen Consumption Rate measurement in Th17 cells, novel data) that activates the transcription factor c-Rel. The NF-kB protein c-Rel seems to be a dominant molecule acting downstream of lactate and ROS. c-Rel directly induces strong IL-2 production and a lactate-mediated switch towards regulatory T cells is strongly impaired in c-Rel-deficient Th17 cells.

In summary, the first journal we have chosen for this manuscript is the EMBO Reports. Instead of transferring the improved version of our manuscript to Nature Comms, PNAS or Cell Reports, I would like to resubmit this paper to EMBO Reports (we will need one month more for evaluation of ChIP sequencing data and for repetition of novel mechanistic data with ROS/c-Rel/IL-2 axis).

We think that we are able to address the reviewers´ concerns and that our manuscript might still be interesting for your journal.

Best regards,

Alexander Visekruna

## 2nd Revision - Editorial Decision 10th May 2022

Dear Prof. Visekruna,

Thank you for your email outlining your revision plan. I have now looked at your points carefully. I appreciate that you are willing to address many of the concerns raised and see that the proposed experiments will strengthen the manuscript.

Having looked at everything, I would like to invite you to submit a revised manuscript. However, I would like to point out that we need strong support from the referees to consider publication here. It is this aspect that is more difficult to assess at this stage.

Please see the guidelines for the revision below my signature.

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.

Kind regards,

Deniz Senyilmaz Tiebe

Deniz Senyilmaz Tiebe, PhD
Editor
EMBO Reports

--
Please revise your manuscript with the understanding that the referee concerns (as 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.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

*** Temporary update to EMBO Press scooping protection policy:
We are aware that many laboratories cannot function at full efficiency during the current COVID-19/SARS-CoV-2 pandemic and have therefore extended our 'scooping protection policy' to cover the period required for a full revision to address the experimental issues highlighted in the editorial decision letter. Please contact the scientific editor handling your manuscript to discuss a revision plan should you need additional time, and also if you see a paper with related content published elsewhere.***

IMPORTANT NOTE: we perform an initial quality control of all revised manuscripts before re-review. Your manuscript will FAIL this control and the handling will be DELAYED if the following APPLIES:
1. A data availability section providing access to data deposited in public databases is missing (where applicable).
2. Your manuscript contains statistics and error bars based on n=2. Please use scatter plots in these cases.

You can submit the revision either as a Scientific Report or as a Research Article. For Scientific Reports, the revised manuscript can contain up to 5 main figures and 5 Expanded View figures. If the revision leads to a manuscript with more than 5 main figures it will be published as a Research Article. In this case the Results and Discussion section should be separate. If a Scientific Report is submitted, these sections have to be combined. This will help to shorten the manuscript text by eliminating some redundancy that is inevitable when discussing the same experiments twice. In either case, all materials and methods should be included in the main manuscript file

Supplementary/additional data: The Expanded View format, which will be displayed in the main HTML of the paper in a collapsible format, has replaced the Supplementary information. You can submit up to 5 images as Expanded View. Please follow the nomenclature Figure EV1, Figure EV2 etc. The figure legend for these should be included in the main manuscript document file in a section called Expanded View Figure Legends after the main Figure Legends section. Additional Supplementary material should be supplied as a single pdf labeled Appendix. The Appendix includes a table of content on the first page with page numbers, all figures and their legends. Please follow the nomenclature Appendix Figure Sx throughout the text and also label the figures according to this nomenclature. For more details please refer to our guide to authors.

Please note that for all articles published beginning 1 July 2020, the EMBO Reports reference style will change to the Harvard style for all article types. Details and examples are provided at

https://www.embopress.org/page/journal/14693178/authorguide#referencesformat

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).

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. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14693178/authorguide#transparentprocess
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."

4) a complete author checklist, which you can download from our author guidelines (<http://embor.embopress.org/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 (<http://embor.embopress.org/authorguide>).

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: <http://embor.embopress.org/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 <http://embor.embopress.org/authorguide#sourcedata>.

8) 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 <http://embor.embopress.org/authorguide#datacitation>.

9) Please make sure to include a Data Availability Section before submitting your revision - if it is not applicable, make a statement that no data were deposited in a public database. Primary datasets (and computer code, where appropriate) produced in this study need to be deposited in an appropriate public database (see <http://embor.embopress.org/authorguide#dataavailability>).

Please remember to provide a reviewer password if the datasets are not yet public.

The accession numbers and database should be listed in a formal "Data Availability " section (placed after Materials & Method)

that follows the model below. Please note that the Data Availability Section is restricted to new primary data that are part of this study.

# Data availability

The datasets (and computer code) produced in this study are available in the following databases:

- RNA-Seq data: Gene Expression Omnibus GSE46843 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE46843)
- [data type]: [name of the resource] [accession number/identifier/doi] ([URL or identifiers.org/DATABASE:ACCESSION])

*** Note - All links should resolve to a page where the data can be accessed. ***

10) 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.
Please note that error bars and statistical comparisons may only be applied to data obtained from at least three independent biological replicates.
Please also include scale bars in all microscopy images.

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

11) The journal requires a statement specifying whether or not authors have competing interests (defined as all potential or actual interests that could be perceived to influence the presentation or interpretation of an article). In case of competing interests, this must be specified in your disclosure statement. Further information: https://www.embopress.org/competing-interests

**Referee #1:**

**Major comments**

1. Fig EV1B. They discovered LGSH, but not lactate, induced lactylation of purified histones without enzymes, and they concluded that "this implies an enzyme-dependent mechanism underlying lactate-derived histone lactylation in T cells". Concentration of LGSH is very important in this experiment. What is the cellular concentration of LGSH? Your concentration is much higher than its cellular concentration. If this be the case, please clearly describe the issue. In addition, to strengthen this argument, you should stimulate the cellular LGSH to check out if K-L-La is elevated.

**AUTHOR RESPONSE:**

We thank the Reviewer for raising this important issue. We share the concern expressed by the referee. Although the lactylation of lysine residues on histones can be achieved by providing the LGSH in the absence of enzymes, the cellular levels of this molecule are kept in the low micromolar range via the glyoxalase cycle (mainly localized in the cytosol). As LGSH is hardly present in the nucleus and this alternative mechanism for histone lysine lactylation is physiologically likely not relevant, we decided to delete these findings from the Fig. EV1 and manuscript text.

2. Fig EV3. Only three promoters regions were displayed, which raises the question what about the other regions. Peaks of additional promoter regions are expected to provide proper controls for this experiment. Without proper controls, the interpretation of their results is questionable.

**AUTHOR RESPONSE:**

We agree with the referee and apologize for omitting to provide additional control. We have tested several other genes and included an example into the manuscript (Fig EV4). We have also established the ChIP-Seq for H3K18 lactylation. These novel and interesting data are now the part of the novel Fig. 4.

3. A key aspect of this study is metabolic reprogramming of Th17, as indicated by the title and conclusions. However, the evidence provided is not sufficient to support this statement. Indeed, Foxp3 protein abundance and the transcripts of some metabolism related genes are altered, but it remains to be determined how these alterations translate to metabolic reprogramming.

**AUTHOR RESPONSE:**

We highly appreciate the suggestion of the reviewer and now provide the additional experiments indicating that metabolic rewiring of Th17 cells induced by lactate results in increased mitochondrial

ROS generation. This, in turn, activates the NF-κB transcription factor c-Rel, which is the crucial regulator of IL-2 expression and induction of Foxp3. We also show novel data demonstrating that lactate induce elevated oxygen consumption rate (OCR) and an increased spare respiratory capacity (SRC). We provide the evidence that the diverting pyruvate towards mitochondria (but not the complete disruption of glycolysis) drives the observed phenotypical switch.

4. Fig 3E. NAC, by itself has been shown to have immune-regulatory roles via ROS, increasing IL-2 production in human peripheral blood T cells, independent of lactate. The provided result suggests that NAC and lactate can both regulate IL-2 levels, but it does not necessarily confirm that lactate-induced IL-2 is via ROS. The authors are expected to test different reducing reagents and assess the activation of NFAT during the treatment.

**AUTHOR RESPONSE:**

According to Reviewer`s suggestion, we have performed additional experiments. We do see that other antioxidants (e.g. mitoTEMPO, a mitochondria-targeted antioxidant, as well as glutathione) counteract lactate-mediated IL-2 secretion and induction of Foxp3 expression. Importantly, we have observed that c-Rel, a transcription factor that binds together with NFAT to *Il2* locus, is crucially involved in IL-2-mediated Foxp3 induction in lactate-treated Th17 cells.

5. Fig EV4. The authors hypothesized that DCA treatment would increase ROS levels without testing. This does not provide more evidence that ROS mediates lactated-induced immune-regulatory effect. ROS measurement is expected to support their hypothesis. It is of note that DCA treatment significantly elevates IFN-γ, but lactate does not, which suggests that these two treatments may trigger different outcomes.

**AUTHOR RESPONSE:**

We thank the reviewer for this comment. We have now included additional data showing an increased mitochondrial ROS production mediated by DCA, Moreover, DCA (but not 2-DG) was also able to increase the expression of Foxp3 in Th17 cells (novel Fig 3). The significantly increased levels of IFN-g were observed only for $CD8^+$ Tc17 cells, but not for $CD4^+$ Th17 cells (please see the Fig EV3). This can be explained by constant and robust production of IFN-g by $CD8^+$ Tc17 cells. A similar increase in IFN-g production was observed for lactate-treated Tc17 cells (Fig EV1), but not for lactate-treated Th17 cells (Fig EV1).

6. this referee would suggest a Summary or Discussion section.

**AUTHOR RESPONSE:**

We have to stick to the requirements of the journal. For this manuscript type (“Short Report”), a separate Discussion part is not intended.

7. To establish the role of histone lysine lactylation, ChIP-seq is necessary for the experiments described in fig 3.

**AUTHOR RESPONSE:**

We are grateful for this important comment. We have now established a ChIP sequencing for H3K18 lactylation (novel Fig. 3). We hope that we were able to address most referee`s comments.

**Minor comments**

1. Some of the figure panels are not referred to properly, e.g. Fig 1H and Fig 2F)

**AUTHOR RESPONSE:**

We thank for this comment. We adjusted the referring of figure panels accordingly.

**Referee #2:**

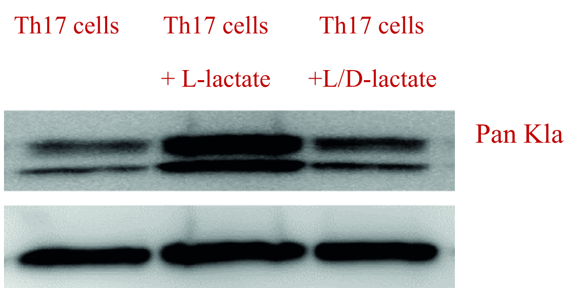
**Major comments:**

Antibodies for histone PTMs suffer from specificity problems especially for less well-established modifications. While the histone lactylation antibodies used in this study have been previously used in Zhang et al., 2019 where they were extensively characterized, it is unclear if the same batches were used. Polyclonal antibodies can show considerative batch to batch differences. Therefore, if a different batch was used, the authors should demonstrate the specificity of their antibodies.

**AUTHOR RESPONSE:**

We are grateful for this comments that we have tried to approach adequately. We have contacted the manufacturer, who stated that the batch we purchased is the same as used by Zhang et al., 2019. Manufacturer has also provided us with information that this Ab can also discriminate between both lactate enantiomers, D-forma and L-form. We tested this statement, by treating Th17 cells with D-lactate and L-lactate. According to the new paper from the Yinnming Zhao lab (Zhang et al., Nature 2019, and now Moreno-Yruela et al., Science Advances, 2022), both lactate isoforms are able to act as precursor molecules for histone lactyl modifications. We do see a competition between both enantiomers in lactate-treated T cells. Of note, the elevated histone lactylation in WB caused by L-lactate treatment decreases afterco- treating the Th17 cells with D-lactate (Fig 1. for the Reviewer 2). Moreover, the co-treatment of T cells with SCFAs butyrate or propionate (histone butyrylation and propionylation were recently described) did not influence histone lactylation levels. In turn, the co-treatment of butyrate-treated Th17 cells with L-lactate did not affect the levels of histone butyrylation in T cells. These experiments suggests a high sensitivity of used antibodies.

Th17 cells | Th17 cells + L-lactate | Th17 cells +L/D-lactate



Pan Kla

H3

**Fig 1**: Western blots of acid-extracted histones from Th17 cells in the presence of either only L-lactate (25 mM), or both L-Lactate + D-lactate (both, 25 mM). Untreated Th17 cells were used as control lymphocytes. Three similar experiments were performed.

Related to providing evidence for the specificity of the antibody, for the ChIP-qPCR in Fig EV3 the authors should show more controls and include further detail about the sites investigated. In particular, a negative control where no histone lactylation would be expected should be included to judge the specificity of the antibody in the ChIP. It would benefit the figure to show a small diagram indicating the position of the primers in relation to the transcription start site of the genes.
There seems to be a substantial amount of variation for the different replicates of the ChIP. The authors should analyze if the differences described are statistically significant to determine if the replicates are indeed replicating the results.
Furthermore, as a pan-lactylation antibody was used in the ChIP (in addition to H3K18la) the authors should adjust their wording to reflect that the assay can detect any lactylated protein and not only histones.

**AUTHOR RESPONSE:**

We thank the Reviewer for raising these important issues and for giving us the opportunity to address his concerns. According to Reviewer`s suggestions, we have included more controls for ChIP results in the manuscript. Moreover, we have established the ChIP-sequencing for H3K18 lactylation. We have also adjusted the wording for a pan-lactylation antibody (please see thnovel page 8 of the manuscript).

The upregulation of Foxp3 (Fig2) and the appearance of a Treg specific expression signature (Fig3) upon lactate treatment is clearly demonstrated. However, the RNAseq analysis reveals "apoptosis" as one of the enriched pathways. The authors should quantify the amount of apoptotic cells upon lactate treatment of Th17 cells. Related to this, in Fig4 there is a significant reduction of intestinal IL-17A+ T cells for the lactate treated mice. What is the fate of the implanted T cells instead? Do they become apoptotic? If a significant amount of apoptosis is detected in these experiments, it would be good to discuss the role of general stress caused by lactate treatment (leading to apoptosis) and therefore leading to altered gene expression programs instead of a specific effect on histone modifications and reprogramming.

**AUTHOR RESPONSE:**

We agree with the referee that apoptosis should always be carefully analyzed. In the Fig. 3C, under the term “apoptosis” both, pro-apoptotic and anti-apoptotic genes are included. We re-analyzed all genes related to “apoptosis” in KEGG-pathways and found almost the same number of pro-and anti-apoptotic genes differentially regulated in lactate-treated Th17 cells. For example, we do observe an increase in *RelA* (NF-kB p65) expression, which is a dominant anti-apoptotic factor (mice deficient in *RelA* die at embryonic days 14–15 with massive liver apoptosis). Moreover, we already performed titration for lactate-treated Th17 cells at the beginning of this project. We now provide these titration results for the reviewer (Fig. 2 for the Referee 2). A slightly increased apoptosis was detected for cells treated with 50 mM lactate, but not for those simulated with 25 mM lactate. Based on these data, we

decided to perform all functional analysis with 25 mM lactate. With regard to in vivo experiments, we always counted the absolute cell numbers for $CD4^+$ T cells in the intestine. We were not able to find a decreased number of T cells in the gut after lactate treatment. Probably, the differentiation of Th17 cells is impaired in the intestinal lamaina propria. However, we cannot exclude the possibility that the general stress contributes to the pathology. We are grateful for this important comment.

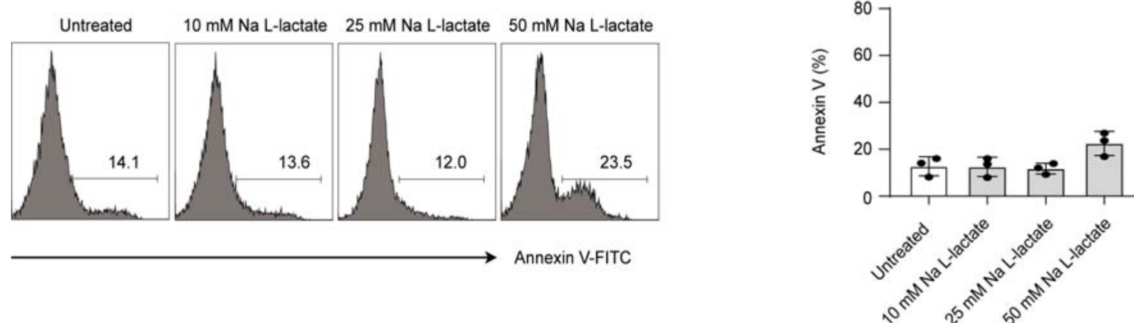



**Fig 2**: Murine Th17 cells were cultured for three days in the presence of increasing L-lactate concentrations. The frequency of annexin $V^+$ cells was analyzed by flow cytometry. A representative of three experiments is shown (left side). Results (right side) are expressed as mean ± SEM.

**Minor points:**

As the authors have conducted multiple replicates for the immunoblots in Figure 1(B,C,E,F,G,H) it would strengthen the manuscript to either quantify the signal or show more replicates in the supplement. This seems particularly important where there are loading differences based on total Histone H3 levels.

**AUTHOR RESPONSE:**

We thank the reviewer for this point. All western blot replicates will be provided in a separate file by the journal ("source file").

In the legend of Fig3E: should untreated be gray instead of white?

**AUTHOR RESPONSE:**

We thank the reviewer. We have changed the color.

In general, for the reproducibility of the findings it would be helpful if gating strategies for the Flow Cytometry data are shown in the supplement.

**AUTHOR RESPONSE:**

The gating strategy for the flow cytometry is now included into the novel Fig EV5.

Related to FigEV3A: On Page 6 the sentence states "This suggests that the histone lactylation is a wide spread post- translational modification, which occurs in T cells as a consequence of activated TCR signalling pathway. "
However, if the ChIP signal is specific, there is also quite some signal for Th0 cells especially for some of the replicates, indicating that histone lactylation is present before TCR signalling at these sites. The authors should adjust their statements and conclusions accordingly.

**AUTHOR RESPONSE:**

We thank the Reviewer for raising this issue. Th0 cells are always stimulated with anti-CD3/anti CD28 antibodies (but without cytokine cocktail). Therefore, they are similar to Tregs, Th17 cells and Th1 cells with regard to the "basic" histone lactylation. The only cells that have never seen TCR signaling stimulation are the "ex vivo" purified $CD4^{+}$ T cells. They are shown as controls for all western blots.

**Referee #3:**

• In Figure 3D-3G, the authors suggested that the reduced IL-17A expression in Th17 cells is mediated by lactate-induced ROS generation, which further leads to the increase of IL-2 production. However, key information is missing. The authors didn't further characterize the mechanism by which ROS is produced. In addition, whether the treatment of antioxidants can affect the lactylation of Th17 is unclear. Overall, the link between lactylation and ROS/IL-2 change is uncelar.

**AUTHOR RESPONSE:**

We thank the reviewer for constructive criticism. We have performed several additional experiments in order to make the main massage of the manuscript more clear. The redirection of metabolic flow by lactate leads to increased mitochondrial ROS generation as shown in the novel Fig. 3. The antioxidant MitTEMPO is able to partially abolish lactate-induced IL-2 production. Furthermore, the NF-kB transcription factor c-Rel seems to be involved in increased IL-2 production mediated by ROS. Lactate-treated c-Rel-deficient Th17 cells are not able to increase IL-2 levels, and they only partially induce Foxp3 expression following lactate treatment.

• In Figure 1, the authors showed that lactate induce histone lactylation in CD4+ T cells. With ChIP analysis of lactylated histone in the presence and absence of extracellular lactate, they identified an enrichment of lactylation at the proximal promoter region of FoxP3 under Th17-inducing condition. The authors should further confirm that a reduction for the lactylation can be also observed in the glucose-restricted condition. In addition, the authors should further address how the lactate-mediated histone lactylation contribute to the reduced IL-17A expression and the upregulated Foxp3 expression in Th17 cells. Overall, the link between lactylation and phenotype changes is not convincing.

**AUTHOR RESPONSE:**

We have now established ChIP-Seq for H3K18 lactylation in order to get a deeper insights and details. We think that histone lactylation is a process induces by lactate which accompanies and strengthen

metabolic effects. As seen in the novel Fig 4., genes displaying increased H3K18 lactylation following lactate treatment of Th17 cells belong to the T cell receptor signaling, as well as NF-κB and MAPK signaling pathways, and some other signaling cascades related to Treg induction such as FoxO signaling pathway.

• In Figure 2C-2F, the authors showed that the expression of Foxp3 is elevated in Th17 cells upon lactate treatment by flow cytometry analysis. In addition, the observation of increased Foxp3 expression upon lactate treatment is further supported by RNA sequencing results which is identified with enhanced Treg features. However, the authors should further perform the experiments to demonstrate the enhanced function of Treg cells upon lactate treatment both in vitro and in vivo.

**AUTHOR RESPONSE:**

We thank the Reviewer for raising this important issue. We have performed additional experiments showing that lactate-treated Th17 cell change their functionality. They are able to suppress proliferation of T responder cells similar to Foxp3$^{+}$ Tregs (novel Fig 4H. We hope that the reviewer will understand that we have some limitations made by the type of this manuscript. As the manuscript format is the "Short report", we are allowed to display only 4 Figures.

• In Figure 4A-4B, the authors cultured CD4+ T cells with the supernatant derived from Lactobacillus reuteri, Escherichia coli, and Bacteroides fragilis to test whether bacteria-derived lactate can affect the IL-17A expression from Th17 cells. In this regard, the authors should evaluate the IL-17A expression by comparing the CD4+ T cells cultured with the indicated supernatants from the bacteria in the same family. While Lactobacillus reuteri can generate lactic acid in the supernatant, the authors should collect supernatant from another non-lactic acid producing bacteria as control and the control bacteria should belong to the same family with Lactobacillus reuteri. In an alternative choice, the authors should use the supernatant collected from the mutant of Lactobacillus reuteri as control which the mutated Lactobacillus reuteri is unable to generate lactate.

**AUTHOR RESPONSE:**

We thank the Reviewer for this very important comment. We agree with the referee that this kind of experiments needs much more control bacterial strains or mutated bacteria. By testing approximately 20 commensals as controls, we realized that many other potent compounds contained in various supernatants are capable of influencing IL-17A production. Firstly, lactate producing bacteria secrete not only L-lactate, but also D-lactate. It turned out that both substances can potently reduce IL-17A production. Secondly, not only Lactobacillus species, but also many other commensals (which have been used by us as control bacteria) produce SCFAs. SCFAs propionate, butyrate and valerate are able to suppress IL-17A synthesis in a concentration-dependent manner. Finally, some Clostridia strains produce Indole-3-propionic acid (IPA), a tryptophan catabolite. In our *in vitro* settings, both *Clostridium sporogenes* supernatants and IPA alone strongly inhibited the differentiation of Th17 cells. In the course of this revision, we realized that diverse bacterial metabolites have a capacity to modulate the expression of IL-17A in Th17 cells, which led us to the conclusion that these data (Fig. 4, A and B) should be removed from the manuscript.

We thank the review for his/her valuable assessment of our manuscript and hope that the revised version of the manuscript could be suggested for a publication.

Dear Prof. Visekruna,

Thank you for the submission of your revised manuscript to our editorial offices. I have now received the reports from the two of the three referees that I asked to re-evaluate your study, you will find below. Referee #1 declined to look into the manuscript again. But going through your point-by-point-response and the revised manuscript, I consider his/her points as adequately addressed. As you will see, the remaining referees now support the publication of your study. However, both referees, in particular referee #3, have some remaining points and suggestions to improve the study I ask you to address in a final revised manuscript. Please also provide a final p-b-p-response to these remaining concerns.

Please note that for the report format we allow up to 5 figures (and also 5EV figures). Moreover, the text can have up to 25,000 (+/- 2,000) characters, excluding references and materials and methods. Thus, there is room to expand the manuscript, to add even one more figure, also to expand the conclusion section of the 'results & discussion part'. I would thus ask you to use this space, adding the data requested by referee #3.

Moreover, I have these editorial requests I ask you to address:

- In the abstract you mention the therapeutic value of your findings. However, there is no data indicating clinical relevance in the manuscript. I would thus ask you to remove the last sentence from the abstract or to add relevant data or to discuss this in much more detail in the manuscript text (see above and the second point of referee #3).

- Please upload individual production quality figure files as .eps, .tif, .jpg, .pdf (one file per figure), of main figures AND EV figures. Please upload these as separate, individual files upon re-submission. Please also add a dedicated legend section for the EV figures.

- We updated our journal's competing interests policy in January 2022 and request authors to consider both actual and perceived competing interests. Please review the policy https://www.embopress.org/competing-interests and update your competing interests if necessary. Please name this section 'Disclosure and Competing Interests Statement' and put it after the Acknowledgements section.

- We now use CRediT to specify the contributions of each author in the journal submission system. CRediT replaces the author contribution section. Please use the free text box to provide more detailed descriptions. Thus, please remove the author contributions section from the manuscript text file. See also guide to authors:
https://www.embopress.org/page/journal/14693178/authorguide#authorshipguidelines

- Please order the manuscript sections like this (using these names):
Title page - Abstract - Keywords - Introduction - Results & Discussion - Materials & Methods - DAS (data availability section) - Acknowledgements - Disclosure and Competing Interests Statement - References - Figure legends - EV Figure legends.

- In the "Data Availability section" (DAS) please add direct links to the two datasets and make sure these are public latest upon publication of the study.

- Please make sure that the number "n" for how many independent experiments were performed, their nature (biological versus technical replicates), the bars and error bars (e.g. SEM, SD) and the test used to calculate p-values is indicated in the respective figure legends (main and EV figures), and that statistical testing has been done where applicable. Please avoid phrases like 'independent experiment', but clearly state if these were biological or technical replicates. Please add complete statistical testing to all diagrams (main and EV figures). Please also indicate (e.g. with n.s.) if testing was performed, but the differences are not significant. In case n=2, please show the data as separate datapoints without error bars and statistics.

- Please make sure that all the funding information is also entered into the online submission system and that it is complete and similar to the one in the acknowledgement section of the manuscript text file.

- Please use our reference format:
http://www.embopress.org/page/journal/14693178/authorguide#referencesformat

- Finally, please find attached a word file of the manuscript text (provided by our publisher) with changes we ask you to include in your final manuscript text, and some queries, we ask you to address. Please provide your final manuscript file (using the attached file as basis) with track changes, in order that we can see any modifications done.

In addition, I would need from you:
- a short, two-sentence summary of the manuscript (not more than 35 words).
- two to four short (!) bullet points highlighting the key findings of your study (two lines each).

- a schematic summary figure (in jpeg, png or tiff format with the exact width of 550 pixels and a height of not more than 400 pixels) that can be used as a visual synopsis on our website.

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

Please use this link to submit your revision: https://embor.msubmit.net/cgi-bin/main.plex

Yours sincerely,

Achim Breiling
Senior Editor
EMBO Reports

--------------
Referee #2:

The authors addressed the major concerns. However, information on on the batch/lot numbers of all antibodies used should be provided in the material and methods section.

--------------
Referee #3:

Krol et al. show that lactate treatment promotes metabolic and epigenetic reprogramming in Th17 cells. Upon lactate treatment, Th17 cells are shown with IL-17A reduction while Foxp3 up-regulation, which is promoted from the ROS-driven IL-2 secretion. In the revised manuscript, they further addressed the observation for the identification of increased histone lactylation H3K18 in the Th17 cells upon lactate treatment. Although new data of the metabolic assay and ChIP-seq was added in the manuscript, there are still several points unresolved. The results still do not sufficiently support the conclusion of the manuscript.
• In Figure 2C-2F, the authors showed that the expression of Foxp3 is elevated in Th17 cells upon lactate treatment. We suggested that the authors should further perform experiments to demonstrate the enhanced function of Treg cells upon lactate treatment both in vitro and in vivo. The author replied with updated results for the suppressed proliferation for the T cells from lactate-treated Th17 cell in new Fig 4H. However, in the revised manuscript, there is no Fig. 4H.
• The authors have removed the results from the cultured cells treated with supernatants from different bacteria and have newly added the ChIP-seq results. In the ChIP-seq results, they found an enrichment of H3K18 lactylation at the proximal promoter of Foxp3. However, the results in the updated version are still not addressing how we can manipulate the epigenetic and metabolic status of Th17 cells for improving the function of T cells for targeting the inflammatory or autoimmune diseases. The main message is still obscure for the readers.

**Referee #2:**

The authors addressed the major concerns. However, information on on the batch/lot numbers of all antibodies used should be provided in the material and methods section.

**AUTHOR RESPONSE:**

We thank the Reviewer for raising this important point. For completion of the missing information, we have included the lot numbers into the "Material and methods" for all antibodies used in the manuscript.

**Referee #3:**

Krol et al. show that lactate treatment promotes metabolic and epigenetic reprogramming in Th17 cells. Upon lactate treatment, Th17 cells are shown with IL-17A reduction while Foxp3 up-regulation, which is promoted from the ROS-driven IL-2 secretion. In the revised manuscript, they further addressed the observation for the identification of increased histone lactylation H3K18 in the Th17 cells upon lactate treatment. Although new data of the metabolic assay and ChIP-seq was added in the manuscript, there are still several points unresolved. The results still do not sufficiently support the conclusion of the manuscript.

â ¢ In Figure 2C-2F, the authors showed that the expression of Foxp3 is elevated in Th17 cells upon lactate treatment. We suggested that the authors should further perform experiments to demonstrate the enhanced function of Treg cells upon lactate treatment both in vitro and in vivo. The author replied with updated results for the suppressed proliferation for the T cells from lactate-treated Th17 cell in new Fig 4H. However, in the revised manuscript, there is no Fig. 4H.

â ¢ The authors have removed the results from the cultured cells treated with supernatants from different bacteria and have newly added the ChIP-seq results. In the ChIP-seq results, they found an enrichment of H3K18 lactylation at the proximal promoter of Foxp3. However, the results in the updated version are still not addressing how we can manipulate the epigenetic and metabolic status of Th17 cells for improving the function of T cells for targeting the inflammatory or autoimmune diseases. The main message is still obscure for the readers.

**AUTHOR RESPONSE:**

We thank the reviewer for these important comments. We apologize for inaccuracy. The results showing the suppressed proliferation of responder T cells, mediated by lactate-treated Th17 cells, are presented in the new Figure 3I (and not in the Figure 4H). We also thank the reviewer for the suggestion that more autoimmune models could possibly be tested. We share his/her enthusiasm; we hope that there will be a follow up to this study and that researchers working on other autoimmune models will extrapolate these findings to other relevant models. In order to make the main message of the manuscript more clear, we (together with the editor) decided to remove the last sentence of the abstract (the speculative sentence on the therapeutic potential of metabolic/epigenetic reprogramming of Th17 lymphocytes for targeting the inflammatory or autoimmune diseases). In this study, we focus on novel observations unveiling the role for lactate in plasticity of Th17 cells.

## 4th Revision - Editorial Decision 27th Sep 2022

Prof. Alexander Visekruna
Phillips-Universität Marburg
Hans Meerwein Strasse 2
Hesse 35042
Germany

Dear Prof. Visekruna,

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, 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.

If you do NOT want this File to be published, please inform the editorial office within 2 days, if you have not done so already, otherwise the File will be published by default [contact: 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." Please note that the author checklist will still be published even if you opt out of the transparent process.

Thank you again for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Achim Breiling
Editor
EMBO Reports

**********************************************************************************

THINGS TO DO NOW:

Please note that you will be contacted by Wiley Author Services to complete licensing and payment information. The required 'Page Charges Authorization Form' is available here: https://www.embopress.org/pb-assets/embo-site/er_apc.pdf - please download and complete the form and return to embopressproduction@wiley.com

EMBO Press participates in many Publish and Read agreements that allow authors to publish Open Access with reduced/no publication charges. Check your eligibility: https://authorservices.wiley.com/author-resources/Journal-Authors/open-access/affiliation-policies-payments/index.html

You will receive proofs by e-mail approximately 2-3 weeks after all relevant files have been sent to our Production Office; you should return your corrections within 2 days of receiving the proofs.

Please inform us if there is likely to be any difficulty in reaching you at the above address at that time. Failure to meet our deadlines may result in a delay of publication, or publication without your corrections.

All further communications concerning your paper should quote reference number EMBOR-2022-54685V5 and be addressed to emboreports@wiley.com.

Should you be planning a Press Release on your article, please get in contact with emboreports@wiley.com as early as possible, in order to coordinate publication and release dates.

## EMBO Press Author Checklist

Corresponding Author Name: Alexander Visekruna
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2022-54685V5

**USEFUL LINKS FOR COMPLETING THIS FORM**
The EMBO Journal - Author Guidelines
EMBO Reports - Author Guidelines
Molecular Systems Biology - Author Guidelines
EMBO Molecular Medicine - Author Guidelines

**Reporting Checklist for Life Science Articles (updated January 2022)**
This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.
**Please note that a copy of this checklist will be published alongside your article.**

**Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots 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. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship 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 χ2 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.

**Please complete ALL of the questions below.**
**Select "Not Applicable" only when the requested information is not relevant for your study.**

**Materials**

| Newly Created Materials | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| New materials and reagents need to be available; do any restrictions apply? | Not Applicable | |

| Antibodies | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| For **antibodies** provide the following information:<br>- Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number<br>- Non-commercial: RRID or citation | Yes | Materials and Methods |

| DNA and RNA sequences | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| **Short novel DNA or RNA including primers, probes**: provide the sequences. | Yes | Materials and Metods |

| Cell materials | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| **Cell lines:** Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID. | Not Applicable | |
| **Primary cultures:** Provide species, strain, sex of origin, genetic modification status. | Yes | Materials and Methods |
| Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination. | Not Applicable | |

| Experimental animals | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| **Laboratory animals or Model organisms:** Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID. | Yes | Material and Methods |
| **Animal observed in or captured from the field:** Provide species, sex, and age where possible. | Not Applicable | |
| Please detail **housing and husbandry conditions**. | Yes | Material and Methods |

| Plants and microbes | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| **Plants:** provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens). | Not Applicable | |
| **Microbes:** provide species and strain, unique accession number if available, and source. | Not Applicable | |

| Human research participants | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants. | Not Applicable | |

| Core facilities | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| If your work benefited from core facilities, was their service mentioned in the acknowledgments section? | Yes | Acknowledgments |

**Design**

| Study protocol | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI. | Not Applicable | |
| Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable. | Not Applicable | |

| Laboratory protocol | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| Provide DOI OR other citation details if **external detailed step-by-step protocols** are available. | Not Applicable | |

| Experimental study design and statistics | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| Include a statement about **sample size** estimate even if no statistical methods were used. | Yes | Materials and Methods, Figures |
| Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described? | Not Applicable | |
| Include a statement about **blinding** even if no blinding was done. | Yes | Materials and Methods |
| Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established?<br><br>If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification. | Not Applicable | |
| For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared? | Yes | |

| Sample definition and in-laboratory replication | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| In the figure legends: state number of times the experiment was **replicated** in laboratory. | Yes | Figures |
| In the figure legends: define whether data describe **technical or biological replicates**. | Yes | Figures |

## Ethics

| Ethics | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. | Not Applicable | |
| Studies involving **human participants**: 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. | Not Applicable | |
| Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained. | Not Applicable | |
| Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations. | Yes | Mterial and Methods |
| Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why. | Not Applicable | |

| Dual Use Research of Concern (DURC) | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm | Not Applicable | |
| If you used a select agent, is the security level of the lab appropriate and reported in the manuscript? | Not Applicable | |
| If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript? | Not Applicable | |

## Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

| Adherence to community standards | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided. | Not Applicable | |
| 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. | Not Applicable | |
| 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. | Not Applicable | |

## Data Availability

| Data availability | Information included in the manuscript? | In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section) |
|---|---|---|
| Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section? | Yes | Materials and Methods |
| Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement? | Not Applicable | |
| Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided? | Not Applicable | |
| If publicly available data were reused, provide the respective **data citations in the reference list**. | Not Applicable | |