

Peer Review Information

Journal: Nature Immunology

Manuscript Title: Multi-omics analyses reveal that HIV-1 alters CD4 T cell immunometabolism to fuel virus replication

Corresponding author name(s): Jenny P.-Y. Ting, Lishan Su

Editorial Notes:

Redactions – published data Parts of this Peer Review File have been redacted as indicated to remove third-party material.

Reviewer Comments & Decisions:

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|--|
| Decision Letter, initial version: |
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Subject: Decision on Nature Immunology submission NI-A29484

Message: 4th May 2020

Dear Dr Ting,

Your Article, "Multi-omics analyses reveal immunometabolic reprogramming-dependent HIV-1 replication" has now been seen by 2 referees. You will see from their comments below that while they find your work of interest, some important points are raised. We are interested in the possibility of publishing your study in Nature Immunology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We therefore invite you to revise your manuscript taking into account all reviewer and editor comments. Please highlight all changes in the manuscript text file in Microsoft Word format. Please let us know if some issues will be challenging to address because of the lockdown - we can discuss whether there would be alternative paths forward. Ref. #2 raises some additional issues similar to the ones I'd discussed with you some time back - namely what's the USP of metaformin cf. CART? How could it be implemented in clinical practice? Is there any evidence that patients on metformin have lower incidence of infection/viremia? (you'd managed to fish out some studies which would be helpful to include).

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

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

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

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

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

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

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

Sincerely,

Zoltan Fehervari, Ph.D.
Senior Editor
Nature Immunology

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Referee expertise:

Referee #1: Immunometabolism, chronic infection

Referee #2: HIV, host response.

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

The authors performed transcriptomics and proteomics analyses and revealed an enhancement of metabolic activities including OXPHOS and glycolysis is associated with HIV-1 infection and disease progression. Treatment with metformin suppressed HIV-1 replication in both primary CD4 T cell cultures and humanized mice. The authors further found that the mitochondrial proteins NLRX1 and FASTDK5 interacted with each other and cooperatively promoted both OXPHOS and HIV-1 replication in CD4 T cells. Overall, this study has strong clinical importance, with clear functional and mechanistic insights. However, there are several important points to address.

1. The effects of metformin are complex and are clearly beyond complex I inhibition. Does metformin treatment affect the respiratory capacity of HIV-infected CD4 T cells? Also, is the expression level of NLRX1 affected by metformin treatment? Given the strong therapeutic effects of metformin showed in this study, the findings from metformin are highly significant (Fig. 2), but the authors should be cautious in interpreting the results. Additionally, they should try to better integrate the results from the first two figures with the rest of the manuscript that is mainly focused on NLRX1.
2. The authors showed HIV-1-dependent upregulation of NLRX1, but the regulation on FASTDK5 is unclear and should be investigated.
3. The authors clearly showed the effects of NLRX1 on the metabolism and function of CD4 T cells, but how NLRX1 affects OXPHOS is less clear. The authors should provide more mechanistic insights to strengthen how NLRX1, and by extension, FASTDK5, affect OXPHOS, for example, by in-depth analysis of their proteomics data.

Minor points:

1. The co-expression network described in Fig. 1D is derived from an old algorithm, GeneMania. The gene names listed in Fig. 1D are not clear and should be better described, for example, do these genes represent differentially expressed genes (DEGs) detected by the transcriptomics analysis? Also, can the authors comment how frequently this algorithm is currently used in metabolic and immunological studies, and can they apply alternative

bioinformatic tools to interrogate the link to OXPHOS (e.g. protein-protein-interaction network)?

2. The title of the manuscript does not accurately depict the main message of the study, and should be revised. The main highlight is about OXPHOS and NLRX1, not multi-omics. As a matter of fact, the authors only did transcriptomics analysis of human cells from patients infected with HIV-1, and proteomics analysis of wild-type and NLRX1-silenced cells. The revision of the title will also help distinguish the current study from the recent paper published in Cell Metabolism on HIV-1-induced metabolic reprogramming (Ref #6).

Reviewer #2:

Remarks to the Author:

The manuscript submitted by Guo and colleagues uses a dataset of acute HIV cohort (RV217) to perform transcriptome analysis of CD4+ T cells searching for any correlation between gene expressions and the disease progression i.e. the viral load set-point. Their results indicated that the activation of OXPHOS pathway was the most correlated with the higher VL set point. This correlation was also confirmed in a different cohort (the Hallmark databases). The authors then went on to ask whether inhibition of OXPHOS by the FDA-approved drug -Metformin could affect HIV replications in HIV infected CD4 T cells in vitro or a humanized mice model in vivo. They showed that metformin or other similar drugs could suppress the replication of HIV both in vitro and in vivo experiments. Finally, the authors investigated the molecular pathway of OXPHOS regulated by the HIV and identified the mitochondrial protein NLRX1 was upregulated during the HIV infection of CD4 T cells. Further gene knockout and proteomic profiling of the virus infected CD4 T cells pointed to the fact that a potential new mechanism maybe the NLRX1 is a true regulator of OXPHOS through the interaction with the FASTKD5 to promote HIV replication.

This is a well written, clear and lucid paper that represents a potentially very important study. It has been well documented that immunometabolism plays a key role in the underlying mechanisms between metabolic reprogramming and immune response including immunity to HIV. However, there are few to directly address metabolism in the context of HIV infection per se. As such this study represents an important advance in the understanding how to regulate metabolic pathway to inhibit HIV replication and infection.

The study design is very unique and likely represents a new paradigm for how such studies can be carried out. The data are presented in a logical manner and are very clear, statistical analysis is appropriate. A major conclusion from this study is the fact that OXPHOS plays a critical role in HIV-1 replication and a novel NLRX1 and FASTKD5-dependent mechanism underlying immunometabolism regulation by HIV-1, suggesting the metformin originally used for the treatment of diabetes can be repurposed for targeting OXPHOS for the treatment of HIV-1 infection.

However, the authors appear to discount the role of cART quickly. While the correlative data between OXPHOS and HIV replication there is a number of questions that need to be addressed:

- 1) are there any data showing that diabetic patients treated with metformin have less HIV replications i.e. the lower VL?;
- 2) how to conduct the clinical trial for metformin in HIV infected individuals without cART?;

3) would it be a problem to take metformin for non-diabetic people who live with HIV?

Minor point: NLRX1 and PASTKD5 should be spelled out in the first appearance in the text. In Figure 1c, what is the difference between Glycolysis vs Gluconeogenesis? It seems quite opposite to each other in terms of the Asian vs African cohort, any explanations?

Author Rebuttal to Initial comments

Point-by-point response to the reviewer's comments

We have previously submitted a manuscript “Multi-omics analyses reveal immunometabolic reprogramming-dependent HIV-1 replication” (NI-A29484) to Nature Immunology. In response to the review, we have changed our title to “Integrative transcriptomic and proteomic analyses reveal that NLRX1 and FASTDK5 orchestrate immunometabolic fitness to fuel HIV-1 replication”.

We are grateful for the supportive and positive comments from both reviewers and appreciate their thoughtful critiques. Reviewer 1 states that “this study has strong clinical importance, with clear functional and mechanistic insights.”. Reviewer 2 states that this is a well written, clear and lucid paper that represents a potentially very important study.this study represents an important advance in the understanding how to regulate metabolic pathway to inhibit HIV replication and infection. The study design is very unique and likely represents a new paradigm for how such studies can be carried out. The data are presented in a logical manner and are very clear, statistical analysis is appropriate.”

We have addressed the concerns of the reviewers by performing new sets of experiments. **We have highlighted revisions and additions in the main text and supplemental data with the yellow color, and these changes are also noted in the point by point response below.** Responding to these queries has greatly strengthened our study, and we appreciate your effort to ensure a constructive and fair review process. We believe we have taken the reviewer's comments to heart and produced a thorough response to the critiques.

Reviewer 1

Major points:

1. *“The effects of metformin are complex and are clearly beyond complex I inhibition. Does metformin treatment affect the respiratory capacity of HIV-infected CD4 T cells?”*

We agree with the reviewer’s comments that the effects of metformin are complex, including activation of AMPK, induction of autophagy, suppression of gluconeogenesis, etc. (PMID: 28776086). However, all those effects caused by metformin administration could be explained by an increased AMP/ATP ratio due to inhibition of Complex I resultant decrease of ATP production. AMP/ATP decrease triggers AMPK activation (PMID: 11602624). The metformin-induced autophagy depends on AMPK activation (PMID: 22378068). Although inhibition of gluconeogenesis by metformin has AMPK-dependent and AMPK-independent mechanisms, both are AMP-dependent (PMID: 28776086). So it is very likely the molecular mechanism of metformin action is starting at complex I. However, we strongly agree with the reviewer that we should check if metformin affects the respiratory capacity of HIV-infected CD4 T cells, as this can directly address whether metformin acts on complex I in HIV infected cells. So we performed new experiments and found that in HIV-infected T cells, metformin suppressed basal and maximal respiration and reserved respiratory capacity (see **Extended Data Fig. 2b, c** in the revised manuscript).

“Also, is the expression level of NLRX1 affected by metformin treatment?”

To address this question, we assessed NLRX1 expression in metformin-treated primary human CD4 T cells in the context of HIV infection or non-infection. We found that, in primary CD4 T cells, metformin significantly induced NLRX1 expression, regardless of infection or not (see **Extended Data Fig. 5a-d** in the revised manuscript). These data suggest that a feedback mechanism likely exists to induce NLRX1 expression to compensate for the inhibition of mitochondrial respiration by metformin.

Given the strong therapeutic effects of metformin showed in this study, the findings from metformin are highly significant, but the authors should be cautious in interpreting the results. Additionally, they should try to better integrate the results from the first two figures with the rest of the manuscript that is mainly focused on NLRX1.

We greatly appreciate the reviewer’s insightful comments. The history of how we went from immunometabolism to NLRX1 is as follows. We analyzed the transcriptome of CD4 T cells from HIV-1 patients and revealed that elevated oxidative phosphorylation (OXPHOS) pathway is associated with

poor outcomes. We then used metformin, a drug that affects OXPHOS to assess its impact on HIV infection. To better interpret the mechanism of metformin action in HIV-infected cells and confirm it was linked to oxidative phosphorylation (OXPHOS), we assessed the effect of metformin on mitochondrial respiration in HIV-infected T cells, as the reviewer suggested in the first comment. We found metformin suppressed OXPHOS in those cells (see **Extended Data Fig. 2b, c** in the revised manuscript). Thus the first two figures focused on the role of immunometabolism in HIV replication. To better connect the whole manuscript, we performed new experiments to: (1) analyze the correlation between NLRX1 expression and OXPHOS pathway gene expression in CD4 T cells from HIV-1 infected individuals – in response we found that NLRX1 expression is positively correlated with OXPHOS pathway gene expression in HIV-1 infected individuals, regardless of the geographic location (see **Fig. 3b, c** in the revised manuscript), thus linking NLRX1 with the immunometabolism transcriptome in Figure 1; (2) assess whether NLRX1 has an effect on the effectiveness of metformin and other mitochondrial complexes inhibitors in HIV replication in T cells – in response we found that the inhibition of HIV-1 replication by metformin and other mitochondrial complexes inhibitors was dependent on NLRX1 in T cells (see **Fig. 5f, g** in the revised manuscript); (3) assess if induction of OXPHOS could restore the dampened HIV-1 replication in NLRX1-deficient T cells – in response, we found that the induction of OXPHOS by the small compound resveratrol successfully restored HIV-1 replication and OXPHOS in *NLRX1*-silenced T cells (see **Fig. 5h, i** in the revised manuscript). These data strengthened the connection between NLRX1 and OXPHOS. We also modified the result and discussion section of the manuscript to better link NLRX1 and OXPHOS.

2. *“The authors showed HIV-1-dependent upregulation of NLRX1, but the regulation on FASTKD5 is unclear and should be investigated.”*

We appreciate the reviewer’s constructive suggestion. To address this, we assessed the mRNA level of *FASTKD5* in the Jurkat T cell and human primary CD4 T cells in both HIV-1 infection and non-infection conditions. We found that HIV-1 did not alter the *FASTKD* transcript levels (see **Extended Data Fig. 8b, c** in the revised manuscript). We also found that the protein level of *FASTKD5* was not altered upon HIV-1 infection of T cells (see **Fig. 6c** in the revised manuscript). Thus, we conclude that the expression of *FASTKD5* is not regulated by HIV-1 infection.

3. *“The authors clearly showed the effects of NLRX1 on the metabolism and function of CD4 T cells, but how NLRX1 affects OXPHOS is less clear. The authors should provide more mechanistic insights to strengthen how NLRX1, and by extension, FASTKD5, affect OXPHOS, for example, by in-depth analysis of their proteomics data.”*

This is an extremely challenging question. We performed a large number of new experiments to explore how NLRX1 and FASTKD5 affect OXPHOS. First, we provide new data that HIV-1 infection enhanced NLRX1 and FASTKD5 association (see **Fig. 6c** in the revised manuscript). Then, we explored how FASTKD5 affect OXPHOS by comparing the proteome between *FASTKD5*-silenced cells with its parental cells in the context of HIV-1 infection (see **Fig. 7a, b** in the revised manuscript). We found a general decreased expression of mitochondrial electron complexes components with the most significant difference at complex I and complex IV (see **Fig. 7c-e** in the revised manuscript). The observed decrease of mitochondrial complexes components in *FASTKD5*-silenced cells was similar to what we observed in *NLRX1*-silenced cells (**Fig. 4d** in the revised manuscript). Therefore, we believe that the new data strengthened the mechanism that NLRX1 and FASTKD5 promote OXPHOS by upregulating the expression of mitochondrial respiratory complexes components. We have included these new data in the revised manuscript and discussed the mechanism in both result and discussion sections.

Minor points:

1. *“The co-expression network described in Fig. 1D is derived from an old algorithm, GeneMania. The gene names listed in Fig. 1D are not clear and should be better described, for example, do these genes represent differentially expressed genes (DEGs) detected by the transcriptomics analysis?”*

We have now better explained the co-expression network. What we have done here is correlate gene expression with the outcome (set-point viral load). We fit a linear regression model between gene expression and set-point viral load values as a continuous variable. We calculate a correlation coefficient for every gene (Tables are provided as the source data in the revised manuscript). After that step, what we do is use the GSEA algorithm to test the enrichment of the positively and negatively correlated genes with outcomes and map them to pathways. We focused on the metabolic pathways and observed a significant positive enrichment with OXPHOS in Asia, Africa, and Asia+Africa cohort. Leading edge genes, which are the genes from a specific pathway contributing to its correlation with the outcome, are well represented by OXPHOS genes presented in the network. Those genes are from the OXPHOS pathway that are correlated with set-point viral load levels. The network was visualized by GeneMania plugin within Cytoscape. We have explained this more clearly in the Results, Figure legend, Method sections of the revised manuscript.

“Also, can the authors comment how frequently this algorithm is currently used in metabolic and immunological studies, and can they apply alternative bioinformatic tools to interrogate the link to OXPHOS (e.g. protein-protein-interaction network)?”

The network was not derived from GeneMania. As described, regression analysis was performed, followed by GSEA, and then the GeneMania plugin within Cytoscape was used to plot the leading edge genes as nodes based on the GSEA output. We did not infer or derive the biological function via GeneMania (which we agree would be an old and not robust technique); the annotation is from KEGG as indicated by the pathway tested, and the network was generated via Cytoscape. We have explained this more clearly in the Results and Method sections of the revised manuscript. As the reviewer’s request, we made additional networks of the leading edge genes of the OXPHOS pathway using ClueGO (PMID: 19237447) and STRING v11 (PMID: 30476243) - two popular and highly cited network visualization tools (see **Extended Data Fig.1** in the revised manuscript).

2. “The title of the manuscript does not accurately depict the main message of the study, and should be revised. The main highlight is about OXPHOS and NLRX1, not multi-omics. As a matter of fact, the authors only did transcriptomics analysis of human cells from patients infected with HIV-1, and proteomics analysis of wild-type and NLRX1-silenced cells. The revision of the title will also help distinguish the current study from the recent paper published in Cell Metabolism on HIV-1-induced metabolic reprogramming (Ref #6).”

We appreciate the reviewer’s great suggestion and take this to heart. We changed the title to “Integrative transcriptomic and proteomic analyses reveal that NLRX1 and FASTDK5 orchestrate immunometabolic fitness to fuel HIV-1 replication”, which we believe adequately expressed our key findings and distinguishes from the previous publication.

Reviewer 2

We thank the reviewer for the positive comment that *“This is a well written, clear and lucid paper that represents a potentially very important study..... As such this study represents an important advance in the understanding how to regulate metabolic pathway to inhibit HIV replication and infection..... The study design is very unique and likely represents a new paradigm for how such studies can be carried out”*. We list the concerns raised by this reviewer below.

Major points:

1. *“Are there any data showing that diabetic patients treated with metformin have less HIV replications i.e. the lower VL?”*

We appreciate the reviewer’s insightful suggestion. There are clinical data showing that HIV-1 positive individuals with type-2 diabetes mellitus morbidity (HIV-T2DM) has an average 1.33 fold ($P = 0.07$) lower HIV-1 viral load than non-diabetic HIV patients among the early cART treated cohort (6 months) (PMID: 28661233; the original data from this paper is shown in Table 1 as below). In congruent with the viral load data, HIV-T2DM patients had a higher CD4 T cell baseline and faster recovery of CD4 T cells number after cART, which was found to be correlated with metformin use (PMID: 28661233 and PMID: 24485344; the original data from these two papers [REDACTED]). We have add these findings to the discussion of the revised manuscript.

2. *“How to conduct the clinical trial for metformin in HIV infected individuals without cART?”*

The reviewer brought out an important question. So far, cART is the most efficient and successful treatment to suppress HIV-1 viremia and is the first-line treatment for HIV-1 infection. The current guidance for the initiation of cART is as an immediate use after HIV-1 diagnosis, no matter the CD4 T cell count. Thus it would be difficult to conduct a clinical trial for treating HIV-1 infected individuals without cART. However, we believe that there are potential benefits of supplementing cART with metformin. First, cART cannot suppress the transcription of HIV-1 RNA and translation of HIV-1 proteins. HIV Env and Tat proteins can be secreted and cause toxicity in bystander cells, which may account for inflammatory pathogenesis such as neuro-AIDS (PMID: 24359561 and PMID: 31320730). Metformin can suppress metabolism (see **Extended Data Fig. 2b, c** in the revised manuscript), which will subsequently inhibit HIV-1 RNA and protein synthesis by limiting the ATP, nucleotide, and amino acid supplies. This distinct anti-viral mechanism may help cART further reduce the viral load, thus reducing the size of the established HIV-1 reservoir size. Second, the toxic side effect of cART usually leads to treatment interruption (PMID: 12904812), which results in a rebound of HIV-1 viremia and the decline of CD4 T cells. Administration of metformin during the cART interruption may delay the rebound of HIV-1 viremia and offer a longer period before the next round of cART. We have discussed this in the revised manuscript.

3. *“Would it be a problem to take metformin for non-diabetic people who live with HIV?”*

We appreciate the reviewer’s comments. Although it is very safe for the diabetic patient to take metformin for treatment, the safety of administration of metformin for non-diabetic HIV patients, especially combined with cART, remains to be explored. Interestingly a clinical trial (NCT02659306) has been registered to treat non-diabetic HIV-1 patients with metformin combined with anti-HIV drugs. This will shed light on the safety and effectiveness of metformin in non-diabetic HIV patients once there are some results released (PMID: 31005944). We have discussed this in the revised manuscript.

Minor points:

“NLRX1 and PASTKD5 should be spelled out in the first appearance in the text.”

We have spelled out NLRX1 and FASTKD5 in the first appearance in the Introduction section in the revised manuscript.

“In Figure 1c, what is the difference between Glycolysis vs Gluconeogenesis? It seems quite opposite to each other in terms of the Asian vs African cohort, any explanations?”

Glycolysis is breaking down glucose to produce energy, while gluconeogenesis is an opposite event that synthesizes glucose. We have provided a better explanation of the difference between these two processes in this revision. Although the correlation data for glycolysis and gluconeogenesis seem opposite to each other in terms of the Asian vs. African cohort, the *P*-values for these correlations did not reach significance, especially when combining the Asian and African cohorts together. Only the OXPHOS pathway was significantly correlated with set-point viral load in Asian, African, and combined cohort with a *P*-Value < 0.05. We emphasized this point in the revised manuscript and revised **Supplementary Table 1** to include the *P*-value data for every pathway.

Decision Letter, first revision:

Subject: Nature Immunology - NI-A29484A pre-edit

Message: Our ref: NI-A29484A

8th Jan 2021

Dear Dr. Ting,

Thank you for your patience as we’ve prepared the guidelines for final submission of your Nature Immunology manuscript, "Integrative transcriptomic and proteomic analyses reveal that NLRX1 and FASTDK5 orchestrate immunometabolic fitness to fuel HIV-1

replication" (NI-A29484A). Please follow the instructions provided here and in the attached files, as the formal acceptance of your manuscript will be delayed if these issues are not addressed.

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

General formatting:

1. Online methods do not have a strict limit but we suggest 3000 words as a target. Your methods section is currently 4178 words.
2. Please include a separate "Data availability" subsection at the end of your Online Methods. This section should inform our readers about the availability of the data used to support the conclusions of your study and should include references to source data, accession codes to public repositories, URLs to data repository entries, dataset DOIs, and any other statement about data availability. We strongly encourage submission of source data (see below) for all your figures. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, these should be included in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see: <http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf>.
3. The title should provide a clear and compelling summary of the main findings in fewer than 100 characters including spaces and without punctuation.
4. Your abstract must be fewer than 150 words and should not include citations.
5. As a guideline, Articles allow up to 50 references in the main text. An additional 20 references can be included in the Online Methods. Only papers that have been published or accepted by a named publication or recognized preprint server should be in the numbered list. Published conference abstracts, numbered patents and research data sets that have been assigned a digital object identifier may be included in the reference list.
6. All references must be cited in numerical order. Place Methods-only references after the Methods section and continue the numbering of the main reference list (i.e., do not start at 1).
7. Genes must be clearly distinguished from gene products (e.g., "gene *Abc* encodes a kinase," not "gene *Abc* is a kinase"). For genes, provide database-approved official symbols (e.g., NCBI Gene, <http://www.ncbi.nlm.nih.gov/gene>) for the relevant species the first time each is mentioned; gene aliases may be used thereafter. Italicize gene symbols and functionally defined locus symbols; do not use italics for proteins, noncoding gene products and spelled-out gene names.

Figures and Tables:

8. You have exceeded the limit of 8 display items. [Ed: give suggestions for combining/cuts]

9. All figures and tables, including Extended Data, must be cited in the text in numerical order. Please correct the following: Fig 7c not cited.

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

11. Shadings or symbols in graphs must be defined in some fashion. We prefer that you use a key within the image; do not include colored symbols in the legend.

12. All relevant figures must have defined error bars.

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

14. Cropping of gel and/or blot images must be mentioned in the figure legend. Gel pieces should be separated with white space (do not add borders). Please ensure that all blots and gels are accompanied by the locations of molecular weight/size markers; at least one marker position must be present in all cropped images. Please also supply full scans of all the blots and gels as Source Data, as instructed below.

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

16. When submitting the revised version of your manuscript, please pay close attention to our [Digital Image Integrity Guidelines](https://www.nature.com/nature-research/editorial-policies/image-integrity) and to the following points below:

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

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

Statistics and Reproducibility:

17. The Methods must include a statistics section where you describe the statistical tests used. For all statistics (including error bars), provide the EXACT n values used to calculate the statistics (reporting individual values rather than a range if n varied among experiments) AND define type of replicates (e.g., cell cultures, technical replicates). Please avoid use of the ambiguous term "biological replicates"; instead state what constituted the replicates (e.g., cell cultures, independent experiments, etc.). For all representative results, indicate number of times experiments were repeated, number of images collected,

etc. Indicate statistical tests used, whether the test was one- or two-tailed, exact values for both significant and non-significant P values where relevant, F values and degrees of freedom for all ANOVAs and t-values and degrees of freedom for t-tests.

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

Please provide an updated version of the Reporting Summary and Editorial Policy Checklist with your final files and include the following statement in the Methods section to indicate where this information can be found: "Further information on research design is available in the Nature Research Reporting Summary linked to this article."

The Reporting Summary and Editorial Policy Checklist can be found here:

<https://www.nature.com/authors/policies/ReportingSummary.pdf>

<https://www.nature.com/documents/nr-editorial-policy-checklist.pdf>

Note that these forms are smart "dynamic" PDFs which cannot be opened by most web browsers. Download them or right-click and choose "save as" in order to save them to your computer desktop and fill them in using Adobe Acrobat.

Supplementary Information:

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

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

20 SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that

we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

All Extended Data must be called out in your manuscript and cited as Extended Data 1, Extended Data 2, etc. Additional Supplementary Figures (if permitted) and other items are not required to be called out in your manuscript text, but should be numerically numbered, starting at one, as Supplementary Figure 1, not SI1, etc.

21 SOURCE DATA: We encourage you to provide source data for your figures whenever possible. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Statistics source data should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. For imaging source data, we encourage deposition to a relevant repository, such as figshare (<https://figshare.com/>) or the Image Data Resource (<https://idr.openmicroscopy.org>).

Other

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

23 Nature Research journals [encourage authors to share their step-by-step experimental protocols](https://www.nature.com/nature-research/editorial-policies/reporting-standards#protocols) on a protocol sharing platform of their choice. Nature Research's Protocol Exchange is a free-to-use and open resource for protocols; protocols deposited in Protocol Exchange are citable and can be linked from the published article. More details can found at www.nature.com/protocolexchange/about.

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

Best regards,

Zoltan Fehervari, Ph.D.
Senior Editor
Nature Immunology

The Macmillan Building
4 Crinan Street
Tel: 212-726-9207
Fax: 212-696-9752
z.fehervari@nature.com

Reviewer #1:

Remarks to the Author:

The authors have successfully addressed my questions. This is an important contribution to the field.

Reviewer #2:

Remarks to the Author:

The authors have addressed all my concerns and comments and I am now recommending for publishing this study in NI.

Final Decision Letter:

Subject: Decision on Nature Immunology submission NI-A29484B

Message: In reply please quote: NI-A29484B

Dear Dr. Ting,

I am delighted to accept your manuscript entitled "Multi-omics analyses reveal that HIV-1 alters CD4 T cell immunometabolism to fuel virus replication" for publication in an upcoming issue of Nature Immunology.

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

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

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Sincerely,

Zoltan Fehervari, Ph.D.
Senior Editor
Nature Immunology

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