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The E3 ubiquitin ligase Peli1 regulates the metabolic actions of mTORC1 to suppress antitumor T cell responses

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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 14th Feb 2020

Thank you for submitting your manuscript entitled "Peli1 regulates T cell metabolism and antitumor immunity by regulating mTORC1 activation" to The EMBO Journal. Your study has been sent to three reviewers for evaluation, whose reports are enclosed below.

As you can see, the referees find the work potentially interesting. However, referee #1 stresses the lack of direct evidence linking Peli1 to metabolism regulation and asks you the test if autophagy mediates the effects of Peli1 knockout, and if AMPK and LKB1 are Peli1 targets. Reviewer #2 requests you to validate K63-linked polyubiquitination of TSC1 by using an anti-K63-Ub-specific antibody, whereas referee #3 asks you to address the impact of TORC2 on Peli1 function.

Given the overall interest of your study, I would like to invite you to submit a revised version that addresses these and the other referees' points. Note that solving all the issues raised by the referees is essential to warrant publication of your manuscript in The EMBO Journal. I should also add that it is our policy to allow only a single round of revision. Therefore, acceptance of your manuscript will depend on the completeness of your responses in this revised version.

We generally grant three months as standard revision time. Competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, please contact me as soon as possible upon publication of any related work in order to discuss how to proceed.

REFEREE REPORTS

Referee #1:

The paper focuses on understanding the role Peli1 in modulating T cell metabolism and function in the context of cancer. The authors demonstrate that Peli1 deficient T cells have enhanced metabolism (increased glycolysis and OXPHOS) and tumor clearance. They suggest deficiency in Peli1 reduces K63 ubiquitination of TSC1, reducing TSC1-TSC2 dimerization, and sensitizing these mTOR inhibitors for degradation.

Overall, this is an interesting study and for the most part the conclusions are supported by the data presented. The extensive use of Cre-mice gives me a lot of confidence on the cell-intrinsic effect of Peli1 on T cell phenotypes. In addition to this, the data are well-presented throughout the manuscript.

Major

The biggest issue that reduces my enthusiasm for the paper is the lack of direct evidence linking the effects of Peli1 on regulating metabolism; this is the central mechanistic part of the paper. For example, does the K30A mutant rescue the metabolic phenotype or effector function of the Peli1 KO T cells? Also, does rapamycin reverse the Peli1-mediated suppression of tumor growth.

Given the use of rapamycin and the identified Peli1 target as TSC1, are the effects of Peli1-KO

mediated through autophagy? Several recent papers show autophagy KO phenocopies Peli1 KO ih terms of anti-tumor immunity. These papers should be cited by the authors (PMID: 30429607, 29317452, 30245008, 30970253).

Given the potential for Peli1 to Ub other proteins in the mTOR pathway, another explanation for the observed effects of Peli1 KO on TSC1 is through the regulation of AMPK and LKB1. Are AMPK and LKB1 a target of Peli1? Does the activity, expression, or Ub patterns of AMPK and LKB1 change in Peli1KO T cells? These are important because both AMPK and LKB1 KO have consequences on T cell activation and effector function. These possibilities should be tested and shown as primary data.

Minor
Is there an effect on proliferation and/or survival of Peli1 KO T cells?

Is loss of Peli1 KO on antitumor activity simply due to stabilization of HIF-1?

The authors pointed out on several occasions that Peli1 KO affects effector T cells. However, as far as I can tell, there is no effector/memory phenotyping. Moreover, cells from the mice were analyzed >20 days after tumors were implanted into mice. This is likely too long after the initial tumor challenge to be considered an effector state. This needs to be addressed as shown as data.

Referee #2:

The manuscript by Ko, Sun et al expands upon this group's earlier identification of the E3 ligase Pellino as a negative regulator of T cell activation. In the current study, the authors demonstrate enhanced anti-tumor immunity by Peli1-deficient mice, which correlates in particular with increased numbers of locally-infiltrating CD8+ cells producing tumoricidal effector proteins. They additionally identify a role for Peli1 in controlling T cell metabolism by inhibition of mTORC1 activation, which appears to be mediated at least partially by stabilisation of its negative regulator complex TSC1/TSC2.

The tumor rejection data are conclusive, although not surprising given the hyperactivated T cell phenotype previously described. Similarly, the authors convincingly demonstrate that the remarkable increases in OXPHOS & aerobic glycolysis observed in activated CD8 T cells lacking Peli1 is mediated by mTORC1 activation downstream of intact Akt signalling. However, although the cumulative evidence for stabilisation of TSC2 by Peli1-mediated ubiquitination of TSC1 is compelling, several of the supporting blots are indistinct and it is usually not clear how many replicates of each were performed. The manuscript would benefit from quantification and statistical analysis of immunoblots throughout, but in particular for the key experiments in Fig 6a, d & k and Fig 7b & d, which are difficult to adequately assess without this information.

It is suggested that Peli1 mediates K63 polyubiquitination of TSC1. It would be important for the authors to confirm this by K63-Ub-specific immunoblot of the immunoprecipitated protein in Fig. 6g

The authors claim that naïve Peli1-deficient CD8 T cells display elevated mTORC1 activity as shown by increased phosphorylation of S6K & S6 (Fig 4a, Fig5a, b), yet this is contradicted by the starting time points in Figs 4b & f. The discrepancy should be explained. Again, details of replicates and quantitation would be desirable here.

It is not clear if Peli modifies TSC1 and TSC2 in a TCR activation-dependent manner. Showing the unstimulated controls Fig 6e and 6f would address this.

Minor points:
• Fig 4a & f: the authors should clarify which phospho-specific S6K antibody is used.
• Rapamycin & Torin 1 concentrations used are not stated; this should be rectified.
• The authors claim that Peli1 deficiency reduces the interaction between TSC1 & TSC2 in stimulated but not resting T cells (p12, line 13), but this is not clear in the relevant figure (6k); again, quantitation of replicates is needed.
• In Fig 3a, the symbol key for naïve KO cells is incorrectly labelled in the ECAR panel.
• The authors should provide a fuller description of the mass spectrometry analysis used to identify TSC1 ubiquitination sites

Referee #3:

The manuscript by Ko et al. describes a yet unappreciated function of the E3 ubiquitin ligase Peli1 as metabolic regulator in CD8 T cells. The authors show that deficiency for Peli1 increases anti-tumor immunity and effector functions of tumor infiltrating CD8 T cells. Mechanistically the authors showed that Peli1 acts as a positive regulator of the mTOR inhibitory TSC1/TSC2 complex. They linked the metabolic alterations found in Peli1-deficient T cells with the increased mTOR activation in these cells and even characterized the ubiquitin acceptor site in TSC1.

This is a well written article that includes novel and interesting findings. The authors combine physiological analyses with in depth signaling work. Their physiological claims are substantiated by a variety of genetic data that confirm the T cell specific effect of the authors claims (straight KO models, T cell-, B cell-, myeloid-specific conditional gene targeting and also TAM-inducible genetic deletion of Peli1). The authors also validate their tumor-specific claims in two distinct tumor models, which documents the overall relevance of their findings. The metabolic analyses could have worked out in more detailed, but are completely in accordance with the general setup of the study. In that respect, one minor detail might be of interest for the authors. The authors suggest a TORC1 dependent mechanism for Peli1 function, but what is the impact of TORC2? It would be interesting to show whether phosphorylation of AKT-P473 is impacted by Peli1 deficiency.

We would like to thank the reviewers for their critical evaluation of our manuscript entitled "Peli1 regulates T cell metabolism and antitumor immunity by regulating mTORC1 activation". We are pleased that the reviewers were enthusiastic about our work. Their constructive comments and suggestions have been extremely valuable for guiding our revisions to further improve the work. In the following, we provide point-by-point responses to the comments raised by the reviewers.

Referee #1:

Major

The biggest issue that reduces my enthusiasm for the paper is the lack of direct evidence linking the effects of Peli1 on regulating metabolism; this is the central mechanistic part of the paper. For example, does the K30A mutant rescue the metabolic phenotype or effector function of the Peli1 KO T cells? Also, does rapamycin reverse the Peli1-mediated suppression of tumor growth.

Response: We thank the reviewer for this important point. To address the reviewer's comments, we have tested the effect of Rapamycin on Peli1-mediated regulation of antitumor immunity. Since rapamycin regulates both immune cells and tumor cells, we employed an adoptive T cell transfer model using WT or Peli1-KO OT1 CD8 T cells that were treated with either DMSO or Rapamycin (Fig. 4i). As expected, the Peli1-KO OT1 CD8 T cells were much more potent than wildtype OT1 CD8 T cells in suppressing tumor growth (Fig. 4j,k). Moreover, while rapamycin inhibited the antitumor function of both wildtype and *Peli1*-KO CD8 T cells, this effect was much more profound for the *Peli1*-KO CD8 T cells (Fig. 4j,k). These data are in line with our findings that rapamycin inhibits the hyper activation of Peli1-KO CD8 T cells in metabolism (Fig. 4d,e) and antitumor effector (IFNg and granzyme B) production (Fig EV3). The reviewer's suggestion to test the effect of K30 mutation on the metabolism/function of Peli1-KO T cells is also excellent. Unfortunately, this would need a ubiquitin-mimetic form of Tsc1, since Tsc1 K30A is equivalent to non-ubiquitinated Tsc1 and would not rescue the phenotype of Peli1 KO T cells. The technique for generating ubiquitin-mimetic form is currently unavailable. Nevertheless, we believe that our new data further strengthened the functional significance of Peli1-mediated regulation of mTORC1 activation and metabolism.

Given the use of rapamycin and the identified Peli1 target as TSC1, are the effects of Peli1-KO mediated through autophagy? Several recent papers show autophagy KO phenocopies Peli1 KO ih terms of anti-tumor immunity. These papers should be cited by the authors (PMID: 30429607, 29317452, 30245008, 30970253).

Response: We thank the reviewer for this insightful comment. In response to the reviewer's comment, we cited the recent papers on autophagy regulation of tumor growth and antitumor immunity. We also examined the effect of Peli1 KO on autophagy induction stimulated by TCR/CD28 signals based on generation of the lipidated isoform of LC3, LC3-II. We found that Peli1-KO T cells had a reduced level of LC3-II compared to wildtype T cells (Appendix Fig S5A). To assess the functional significance, we incubated the wildtype and Peli1-KO CD8 T cells with a cell-permeable beclin 1-activating peptide (Tat-Beclin 1) known to stimulate autophagy(Shoji-Kawata, Sumpter et al., 2013). Tat-Beclin 1 treatment increased the level of LC3-II and erased the differences between WT and Peli1-KO T cells (Appendix Fig S5B). Furthermore, consistent with previous studies, the Tat-Beclin 1 treatment reduced the level of IFNg induction in both WT and Peli1-KO T cells (Appendix Fig S5C). However, even under Tat-Beclin 1-treated conditions, Peli1 deficiency still significantly promoted IFNg induction (Appendix Fig S5C). These data suggest that reduced autophagy induction only partially contributes to the increased activation of Peli1-KO T cells.

Given the potential for Peli1 to Ub other proteins in the mTOR pathway, another explanation for the observed effects of Peli1 KO on TSC1 is through the regulation of AMPK and LKB1. Are AMPK and LKB1 a target of Peli1? Does the activity, expression, or Ub patterns of AMPK and LKB1 change in Peli1KO T cells? These are important because both AMPK and LKB1 KO have consequences on T cell activation and effector function. These possibilities should be tested and shown as primary data.

Response: These are again excellent suggestions. Following the reviewer's suggestion, we have analyzed the potential role of Peli1 in regulating AMPK and LKB1. We found that Peli1 deficiency did not affect TCR/CD28-stimulated AMPK activation (based on T172 phosphorylation) (Fig EV4A). Peli1 also did not promote ubiquitination of AMPK or LKB1 (Fig EV4B-D). These results suggest that Peli1 may not target these upstream kinases.

Minor

Is there an effect on proliferation and/or survival of Peli1 KO T cells?

Response:

We have previously shown that Peli1 KO T cells are hyper-proliferative upon in vitro stimulation by anti-CD3 plus anti-CD28 (Chang, Jin et al., 2011). To further address the reviewer's question, we performed apoptosis assays and found that the Peli1-KO T cells did not have a significant increase in the frequency of apoptosis (Appendix Fig S3).

Is loss of Peli1 KO on antitumor activity simply due to stabilization of HIF-1?

Response:

Based on the known function of HIF1a, we agree with the reviewer that the increased HIF1a expression in Peli1 KO T cells (Fig. 4g) suggests its involvement in the increased metabolic activity and antitumor function of the Peli1-deficient CD8 T cells. However, we think that other mTORC1-downstream factors also contribute to this functional phenotype. In fact, the Peli1 deficiency drastically enhances the induction of c-Myc, which is critical for T cell metabolic reprograming. We emphasized the importance of HIF1a and c-Myc in the Discussion section of the revised text.

The authors pointed out on several occasions that Peli1 KO affects effector T cells. However, as far as I can tell, there is no effector/memory phenotyping. Moreover, cells from the mice were analyzed >20 days after tumors were implanted into mice. This is likely too long after the initial tumor challenge to be considered an effector state. This needs to be addressed as shown as data.

Response:

We agree with the reviewer that our data could not distinguish effector and memory T cells. We have changed the "tumor-infiltrating CD8 effector T cells" to "tumor-infiltrating CD8 T cells".

Referee #2:

The tumor rejection data are conclusive, although not surprising given the hyperactivated T cell phenotype previously described. Similarly, the authors convincingly demonstrate that the remarkable increases in OXPHOS & aerobic glycolysis observed in activated CD8 T cells lacking Peli1 is mediated by mTORC1 activation downstream of intact Akt signalling. However, although the cumulative evidence for stabilisation of TSC2 by Peli1-mediated ubiquitination of TSC1 is compelling, several of the supporting blots are indistinct and it is usually not clear how many replicates of each were performed. The manuscript would benefit from quantification and statistical analysis of immunoblots throughout, but in particular for the key experiments in Fig 6a, d & k and Fig 7b & d, which are difficult to adequately assess without this information.

Response: We thank the reviewer for this important point. We have now performed densitometric quantification of the immunoblots based on three different experiments (Fig. 6a,d,l; Fig. 7b,d).

It is suggested that Peli1 mediates K63 polyubiquitination of TSC1. It would be important for the authors to confirm this by K63-Ub-specific immunoblot of the immunoprecipitated protein in Fig. 6g

Response: To address the reviewer's question, we employed ubiquitin mutants harboring lysine (K)-to-arginine (R) substitutions at K48 (Ub K48R) or K63 (Ub K63R). Peli1 induced strong conjugation of ubiquitin K48R to TSC1 but barely detectable conjugation of K63R (Fig. 6h). We also employed ubiquitin mutants harboring K-to-R mutation in all Ks, except K48 (Ub K48) or K63 (Ub K63). Peli1 induced much stronger conjugation of Ub K63 than Ub K48 (Fig. 6h). These results further suggest that Peli1 predominantly conjugates K63-linked ubiquitin chains to TSC1.

The authors claim that naïve Peli1-deficient CD8 T cells display elevated mTORC1 activity as shown by increased phosphorylation of S6K & S6 (Fig 4a, Fig5a, b), yet this is contradicted by the starting time points in Figs 4b & f. The discrepancy should be explained. Again, details of replicates and quantitation would be desirable here.

Response: We apologize for not clearly describing the experimental conditions. As noted in the revised text, mTORC1 activation in naïve CD8 T cells was detected using freshly isolated CD8 T cells. For in vitro T cell activation with anti-CD3/anti-CD28 (Fig. 4b,f), we first rested the cells on ice to reduce the steady-state mTORC1 activation and then stimulated with anti-CD3/CD28 for

the indicated time points. We have revised both the main text (page 9) and figure legend to make this clarification. We have also quantified the results based on three independent experiments (Fig. 4a, right panel).

It is not clear if Peli modifies TSC1 and TSC2 in a TCR activation-dependent manner. Showing the unstimulated controls Fig 6e and 6f would address this.
Response: In response to the reviewer's comment, we have performed TSC1 and TSC2 ubiquitination assays under unstimulated conditions and included the data in Appendix Fig S6. Peli1 deficiency also increased TSC2 ubiquitination and decreased TSC1 ubiquitination in freshly isolated and unstimulated CD8 T cells, albeit less strikingly compared to the activated CD8 T cells.

Minor points:
• *Fig 4a & f: the authors should clarify which phospho-specific S6K antibody is used.*
Response: we have added the details of the phospho-S6K as well as other phospho-antibodies to the different panels in Fig. 4.

• *Rapamycin & Torin 1 concentrations used are not stated; this should be rectified.*
Response: we have added the dose of rapamycin and Torin 1 to the figure legends.

• The authors claim that Peli1 deficiency reduces the interaction between TSC1 & TSC2 in stimulated but not resting T cells (p12, line 13), but this is not clear in the relevant figure (6k); again, quantitation of replicates is needed.
Response: We have quantified the bands based on three independent experiments and included quantify the bands and make a summary graph.

• In Fig 3a, the symbol key for naïve KO cells is incorrectly labelled in the ECAR panel.
Response: We have corrected the error in Fig. 3a.

• The authors should provide a fuller description of the mass spectrometry analysis used to identify TSC1 ubiquitination sites
Response: we have included a detailed description of the mass spectrometry analysis in the Method section.

Referee #3:

The metabolic analyses could have worked out in more detailed, but are completely in accordance with the general setup of the study. In that respect, one minor detail might be of interest for the authors. The authors suggest a TORC1 dependent mechanism for Peli1 function, but what is the impact of TORC2? It would be interesting to show whether phosphorylation of AKT-P473 is impacted by Peli1 deficiency.

Response:

We thank the reviewer for this insightful comment. We apologize for not labeling the phosphorylation residue of the p-AKT in original Fig. 4b. It was actually S473. To further address the reviewer question, we repeated this experiment by detecting p-AKT based both S473 and T308 phosphorylation (Appendix Fig S4). These new data revealed that Peli1 deficiency had no appreciable effect on AKT phosphorylation at either S473 or T308. Since S473 is the target of mTORC2, these data suggest that Peli1 may not be important for regulation of mTORC2.

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

References

Chang M, Jin W, Chang JH, Xiao Y, Brittain GC, Yu J, Zhou X, Wang YH, Cheng X, Li P, Rabinovich BA, Hwu P, Sun SC (2011) The ubiquitin ligase Peli1 negatively regulates T cell activation and prevents autoimmunity. Nat Immunol 12: 1002-1009

Shoji-Kawata S, Sumpter R, Leveno M, Campbell GR, Zou Z, Kinch L, Wilkins AD, Sun Q, Pallauf K, MacDuff D, Huerta C, Virgin HW, Helms JB, Eerland R, Tooze SA, Xavier R, Lenschow DJ, Yamamoto A, King D, Lichtarge O et al. (2013) Identification of a candidate therapeutic autophagy-inducing peptide. Nature 494: 201-6

2nd Editorial Decision 17th Sep 2020

Thank you for submitting your revised manuscript. The study has been seen by two of the original referees, whose comments are shown below.

As you can see, the reviewers find that their criticisms have been sufficiently addressed. However, they request you to rephrase and tone down certain statements in order for them to more accurately reflect the data presented.

In addition to solving this remaining point, there are a few editorial issues concerning the text and the figures that I need you to address before we can officially accept your manuscript.

REFEREE REPORTS

Referee #1:

The revised manuscript is much improved and has largely addressed all of my concerns. I believe that the substantive data characterizing the effects of Peli1 on T cells represents a novel body of work and is deserving of publication. Thus, the authors should be commended for their excellent revisions.

There is one remaining issue that has not been experimentally addressed from my previous review,

"....is the lack of direct evidence linking the effects of Peli1 on regulating metabolism; this is the central mechanistic part of the paper...". I am convinced that Peli1 is acting in some manner to reprogram metabolism. By these effects on metabolism are secondary to the mTORC1 changes (and potentially other factors like HIF-1 and c-myc, as the authors pointed out in the response to reviewers). While this may seem like a subtle point, the title, abstract, and structure of the manuscript gives a different impression. For example, only 1 or 2 of the subheadings in the body of the text as well as the figure legends speak to Peli1's impact on metabolism. Therefore, I strongly recommend some minor changes.

1) Title: Peli1 regulates the metabolic actions of mTORC1 to enhance antitumor immunity.

2) Last sentence of the abstract: These results establish Peli1 as a novel regulator of mTORC1 and downstream mTORC1-mediated actions on T cell metabolism and antitumor immunity.

3) Pg 16. "Our data demonstrated a crucial role for Peli1 in controlling the metabolic reprograming of CD8 T cells." This sentence should be modified to reflect the abstract/title and my comments above.

Referee #2:

The revised manuscript by Ko, Sun et al addresses most of the issues raised in our previous report. In particular, new experiments using Ub mutants in a transfection system suggest that Peli1 predominantly conjugates non-degradative K63-linked polyUb chains to TSC1. The additional experiment included in Appendix Fig S6 looks at ubiquitination of TSC1 and 2 in unstimulated cells. However, this does not address the query of whether Peli modifies TSCs in a TCR activation-dependent manner. The unstimulated and stimulated samples from WT and KO would have to be run on the same gel.

The revised manuscript includes additional experiments designed to test whether reduced autophagy contributes to the increased activation phenotype of Peli1 KO CD8 T cells. Surprisingly, given the data presented in Appendix Fig S5A-C, the authors suggest that it does! The

immunoblots are again unsupported by quantification or statistical analysis of replicate experiments, but nonetheless do not clearly show reduced LC3-II in Peli1-deficient cells as is claimed. Unless they can provide convincing evidence to the contrary, the authors should rewrite this section to more accurately reflect the data presented.

Minor:
The reference to Fig S1B on p5, line 10 should be to Fig 1B.

2nd Authors' Response to Reviewers 24th Sep 2020

Comments from referees

Referee #1:

There is one remaining issue that has not been experimentally addressed from my previous review, "....is the lack of direct evidence linking the effects of Peli1 on regulating metabolism; this is the central mechanistic part of the paper...". I am convinced that Peli1 is acting in some manner to reprogram metabolism. By these effects on metabolism are secondary to the mTORC1 changes (and potentially other factors like HIF-1 and c-myc, as the authors pointed out in the response to reviewers). While this may seem like a subtle point, the title, abstract, and structure of the manuscript gives a different impression. For example, only 1 or 2 of the subheadings in the body of the text as well as the figure legends speak to Peli1's impact on metabolism. Therefore, I strongly recommend some minor changes.

1) Title: Peli1 regulates the metabolic actions of mTORC1 to enhance antitumor immunity.
Response: we have changed the title to "Peli1 regulates the metabolic actions of mTORC1 to suppress antitumor T cell responses".

2) Last sentence of the abstract: These results establish Peli1 as a novel regulator of mTORC1 and downstream mTORC1-mediated actions on T cell metabolism and antitumor immunity.
Response: we have made this change in the abstract.

3) Pg 16. "Our data demonstrated a crucial role for Peli1 in controlling the metabolic reprograming of CD8 T cells." This sentence should be modified to reflect the abstract/title and my comments above.
Response: we have changed this sentence to "Our data demonstrated a crucial role for Peli1 in regulating mTORC1 activation and mTORC1-mediated actions on T cell metabolism and antitumor immunity".

Referee #2:

The revised manuscript by Ko, Sun et al addresses most of the issues raised in our previous report. In particular, new experiments using Ub mutants in a transfection system suggest that Peli1 predominantly conjugates non-degradative K63-linked polyUb chains to TSC1. The additional experiment included in Appendix Fig S6 looks at ubiquitination of TSC1 and 2 in unstimulated cells. However, this does not address the query of whether Peli modifies TSCs in a TCR activation-dependent manner. The unstimulated and stimulated samples from WT and KO would have to be run on the same gel.
Response: we have revised Appendix Fig. S6 to include data from both unstimulated and stimulated samples run on the same gel.

The revised manuscript includes additional experiments designed to test whether reduced autophagy contributes to the increased activation phenotype of Peli1 KO CD8 T cells. Surprisingly, given the data presented in Appendix Fig S5A-C, the authors suggest that it does! The immunoblots are again unsupported by quantification or statistical analysis of replicate experiments, but nonetheless do not clearly show reduced LC3-II in Peli1-deficient cells as is claimed. Unless they can provide convincing evidence to the contrary, the authors should rewrite this section to more accurately reflect the data presented.
Response: The reviewer's point is well taken. To further confirm our finding, we quantified the LC3-I and LC3-II bands based on three sets of data and included the graph in Appendix Fig. S5A. The LC3-II reduction in Peli1 KO T cells is statistically significant, although it is quite moderate. Based on these data, we revised the statement in the text (page 10) to indicate that "Peli1-deficient T cells had a moderately reduced level of LC3-II generation". We also think that our conclusion regarding the function of autophagy is overstated, since even in the presence of Tat-Beclin 1, Peli1 KO T cells still produced much higher level of IFNg (Appendix Fig. S5C). Thus, we have revised the conclusion to "These data suggest that autophagy regulation is not a major mechanism by which Peli1 suppresses T cell activation".

Minor:
The reference to Fig S1B on p5, line 10 should be to Fig 1B.
Response: We have made this correction.

We believe that we have addressed all of the concerns raised by the reviewers and also addressed the editorial issues.

Accepted 5th Oct 2020

I am pleased to inform you that your manuscript has been accepted for publication in The EMBO Journal.

Congratulations!

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Shso-Cong Sun

Journal Submitted to: EMBO J

Manuscript Number: EMBOJ-2020-104532

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http://oba.od.nih.gov/biosecurity/biosecurity_documents.html
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Reporting Checklist For Life Sciences Articles (Rev. June 2017)

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

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

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

2. Captions

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

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

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple 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.

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

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

B- Statistics and general methods

	Please fill out these boxes (Do not worry if you cannot see all your text once you press return)
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For mouse in vivo experiments, sample sizes were chosen according to the basis of previous publications in the immunology field. More than four mice per group were usually used to ensure the statistically significant difference which could be obtained from unpaired two-tailed Student's t-test or ANOVA analysis followed by multiple-comparisons test. For in vivo experiments, the conclusive results were obtained from at least three independent experiments.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	It was described on page 25, paragraph 2 and in figure legends.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	There were no excluded data or samples from the analysis in this study.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	For Adoptive transfer experiment, animals were assigned randomly to treatment and control groups, and within animal controls were performed wherever possible. We also randomly chose the mice from the same littermates for each experiment group and control groups.
For animal studies, include a statement about randomization even if no randomization was used.	We randomly chose the mice from the same age- and sex- matched littermates for each experiment group. All these mice were randomly allocated into experimental groups. It was also described on page 18 and page 34.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
4.b. For animal studies, include a statement about blinding even if no blinding was done	For animal experiments and cell-based experiments Western blotting and FACS, cell types were known when prepare the samples or start to treat cells at the beginning of experiments. Investigators performed, acquired and analyzed experiments were not blinded. Because treatments used made it difficult to blind and there was no human bias given all the data were collected independently using instrumentation.
5. For every figure, are statistical tests justified as appropriate?	Yes, statistical test methods and p values were described on page 25 paragraph 2, and in figure legends on manuscript page 31-39).
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Each statistical test is defined in the figure legends (page31-39) and is also described in methods (page 25 paragraph 2).
Is there an estimate of variation within each group of data?	N/A

Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	The reagents and antibodies used in this study were described in material and methods (page 17-24), including catalog number, clone number and vendor.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	No mycoplasma contamination was observed. 293T, B16F10 and E.G7-OVA were originally obtained from the American Type Culture Collection (ATCC). TSC1–/– MEFs were provided by Boyi Gan (MD Anderson cancer center) .

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

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	The information was provided in the methods section (page 17-18 and page 24) and corresponding figure legends (page31-39).
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	All animal experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of the University of Texas MD Anderson Cancer Center. (page17 and 18)
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	confirm

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	No human subject was involved in this study.
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The mass spectrometry data from this publication have been deposited to the PRIDE database [https://www.ebi.ac.uk/pride/] and assigned the identifier [accession: PXD020734]).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A