Peer Review File

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Reviewer A

This is a well conceived carefully executed study identifying immune neoantigens. Study reveals PLEC is a novel immune antigen for adenocarcinoma of the pancreas, a previously unrecognized immune antigen. The methods and results are well presented. The work has clinical relevance for new treatment protocols and ability to identify other immune antigens. I recommend acceptance.

R: Thank you for your kind comments. We genuinely appreciate your efforts and are grateful for your positive feedback on our work.

Reviewer B

The paper titled "Identification of pancreatic adenocarcinoma immune subtype associated with tumor neoantigen from aberrant alternative splicing" is interesting. The present study used a transcriptome-guided approach to screen neoantigen candidates based on alternative splicing, NMD factors, and antigen-presenting signatures for PAAD. A prognosis model with guidance of immunotherapy will aid in patient selection for appropriate treatment. However, there are several minor issues that if addressed would significantly improve the manuscript.

R: Thank you for your kind comments on our manuscript. We appreciate your valuable feedback and have made revisions accordingly. These revisions are highlighted in blue or in track change in the revised manuscript. Below are our point-by-point responses:

1) What is the correlation between alternative splicing and immune cell infiltration events and patient prognosis? Suggest adding relevant content.

R: Thank you for your insightful question. Alternative splicing plays an important role in both immune cell infiltration events and patient prognosis. Recent studies have systematically explored the correlation between alternative splicing and immune cell infiltration in various cancers, including renal clear cell carcinoma (PMID: 35370291), colorectal carcinoma (PMID: 33996533), lung adenocarcinoma (PMID: 34864813), and malignant mesothelioma (PMID: 34294080). Additionally, studies have examined the impact of alternative splicing on patient prognosis in colon adenocarcinoma (PMID: 33099068), thyroid gland cancer (PMID: 33649373), and ovarian cancer (PMID: 34526089). This information has been supplemented in the updated manuscript.(See Page 4, line 87 to 91)

2) What is the impact on prognostic alternative splicing events of tumour immune microenvironment in PAAD? Suggest adding relevant content.

R: Dear Reviewer, thank you for your valuable comments on our manuscript. Specifically for PAAD, Wang et al. reported six prognostic splicing biomarkers, including UBA1, S100A13, SH3KBP1, COPSTA, GSE1, and NISCH. The risk model based on these six genes with AS events showed broad associations with the fractions of macrophages M1, resting mast cells, CD8 T cells, regulatory T cells, naive B cells, and memory B cells. This information has been updated in our latest manuscript (See Page 14, line 429 to 432). Thank you again for your helpful suggestion.

3) What is the greatest advantage of the prognosis model in this study? What is the biggest problem we are facing? It is suggested to add relevant content to the discussion.
R: Dear Reviewer, thank you for your insightful question. The greatest advantage of our prognosis model is its applicability for researchers aiming to investigate the immunogenicity of PAAD samples. The biggest challenge we currently face is the lack of clinical validation, which we plan to address in future studies. Relevant content has been added to the discussion section of the latest manuscript (See Page 15, line 463 to 465).

4) This study is based on bioinformatics analysis. It is recommended to increase in vivo experimental studies, which may be more meaningful.
R: Dear Reviewer, thank you for your thoughtful suggestion. As this is a systematic exploration study, we have reported many descriptive findings, and we do not have a specific point to validate at this stage. In the future, we plan to design basic research on how to target PLEC in the treatment of PAAD. At that stage, in vivo studies will be required to confirm our findings.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “A comprehensive characterization of alternative splicing events related to prognosis and the tumor microenvironment in lung adenocarcinoma, Ann Transl Med, PMID: 35571443”. It is recommended to quote this article.
R: Dear Reviewer, thank you for your valuable suggestion. Citing the recommended paper will significantly enhance the comprehensiveness of our introduction. In the updated manuscript, we have cited this paper as you suggested (See Page 4, line 90 to 91).

6) Please summarize recent advances in immune system associated tumor specific-antigens produced by alternative splicing in the discussion.
R: Dear Reviewer, thank you for your valuable suggestion. We have enriched the discussion by summarizing recent advances in immune system-associated tumor-specific antigens produced by alternative splicing (See Page 14, line 432 to 439).

Reviewer C

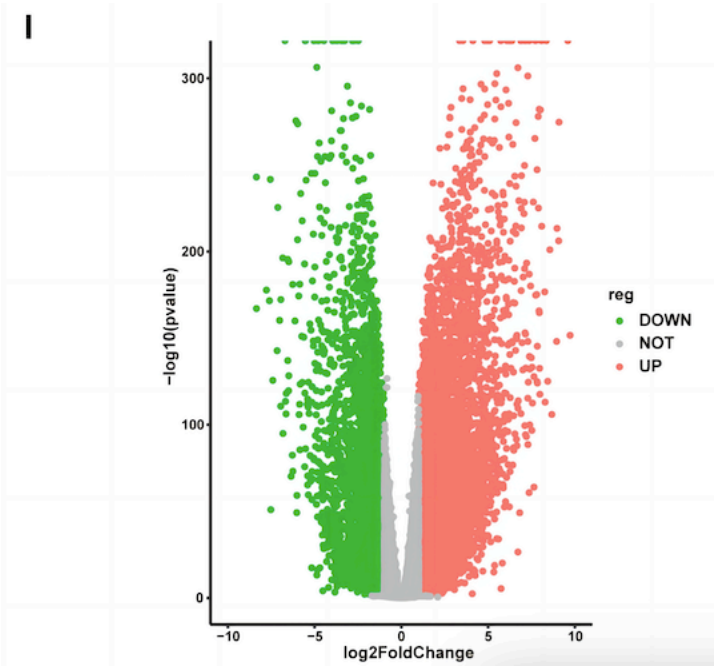
1. Please cite the related reference for the below sentence accordingly.

Notably, Bailey et al. identified a PDAC subtype designated as immunogenic tumors with altered immune pathways.

R: We have cited this reference in the revised manuscript (Line 86).

2. Fig 1I is "Volcano plot", not "Heat map". Please check.

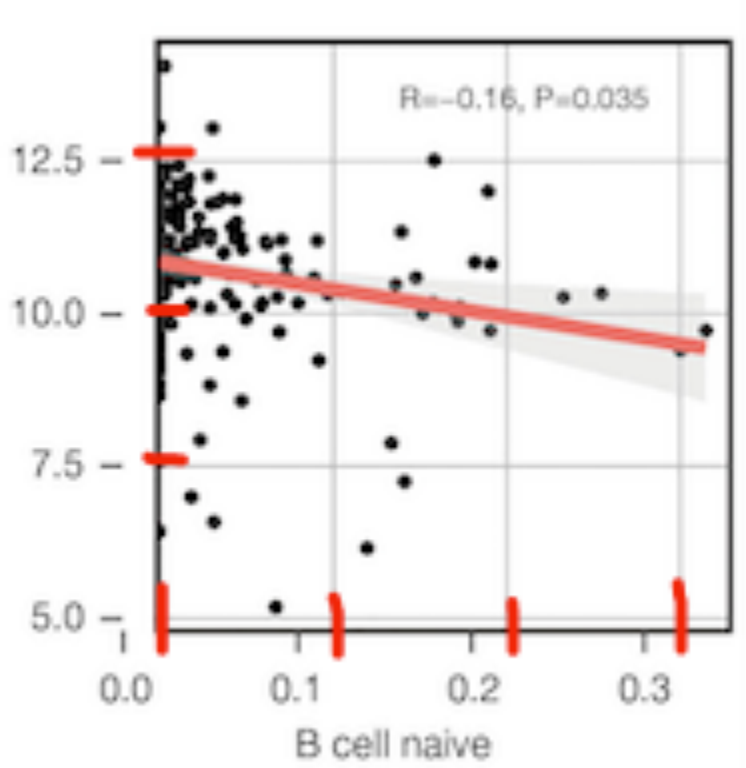
645 mutations. (I) Heatmap showing differential genes between PAAD and adjacent pancreas. (J)



R: Thank you for your reminder. We have corrected the "Heat map" into "Volcano plot" (Line 662). We are really sorry for our careless mistake.

3. Figure 2C

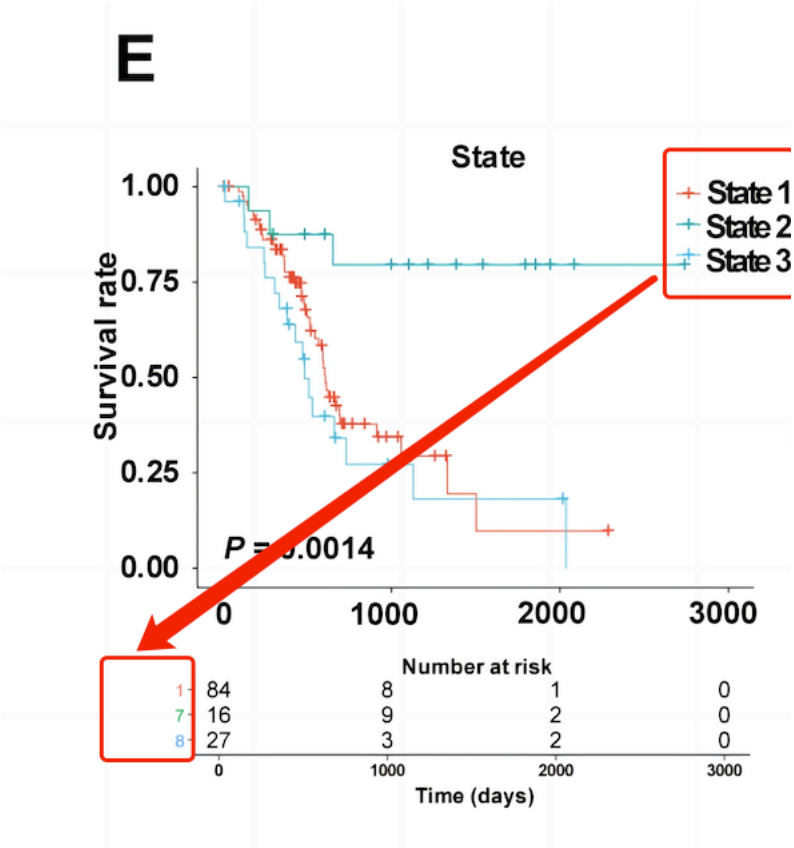
One bar should be corresponded to one data. It seems that the data are misplaced. Should them be moved to the right? Please check and revise.



R: Thank you for your careful checks. We are sorry for our carelessness. We have revised the figure.

4. Figure 6E

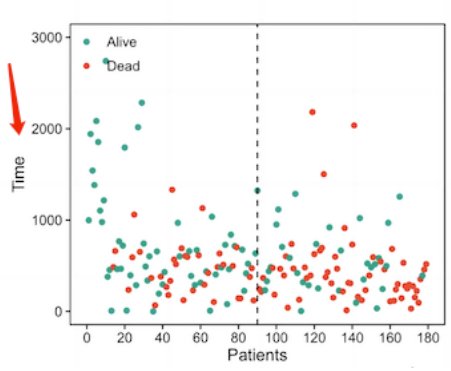
Should the pointed "1, 7, 8" be "State 1, 2, 3"? Please check and revise.



R: Thank you for your careful checks. We are sorry for our carelessness. We have revised the figure.

5. Figure 7H

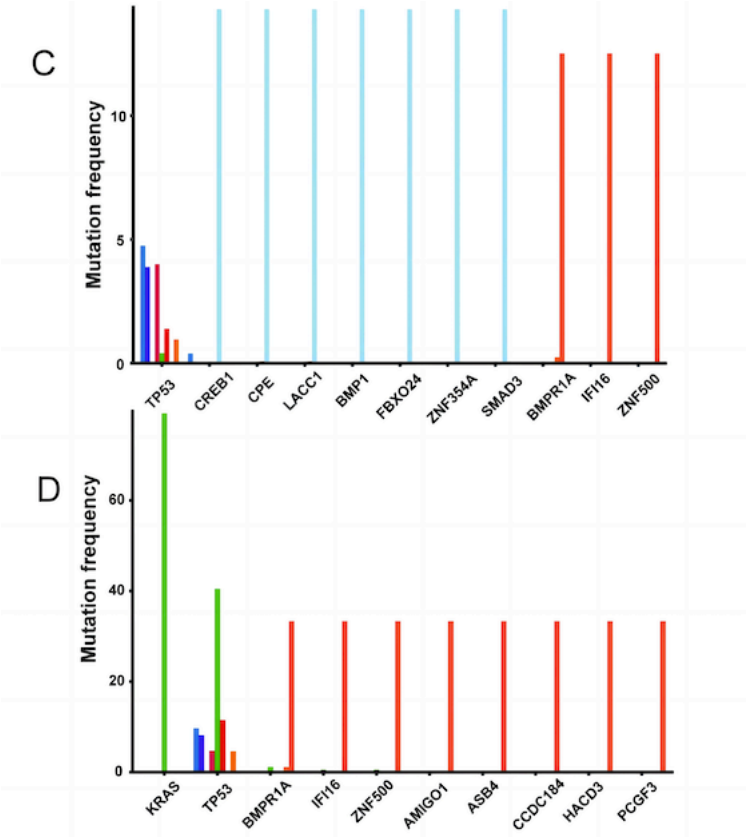
Please provide unit for "Time"



R: Thank you for your careful checks. We have added the unit for Y-axis.

6. Figure S1C, S1D

Please explain the meaning of different colors for the bars.



R: Thank you for your careful checks. We are sorry for our carelessness. We have revised the figure.

7. As for the special symbols "*, **, ***" in Figure S1, please explain their meaning in the legend

R: Thank you for your careful checks. “*”, “**”, “***” in Figure S1 respectively represent “P<0.05”, “P<0.01”, “P<0.001”.

8. As for the special symbols “*, **, ***” in Figure S2, please explain their meaning in the legend

R: Thank you for your careful checks. “*”, “**”, “***” in Figure S2 respectively represent “P<0.05”, “P<0.01”, “P<0.001”.

9. As for the special symbols “*, **, ***” in Figure S3, please explain their meaning in the legend

R: Thank you for your careful checks. “*”, “**”, “***” in Figure S3 respectively represent “P<0.05”, “P<0.01”, “P<0.001”.