
Peer Review File

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Reviewer A

1. In Figure 2 panel 6, the authors have shown the P value of the K-M curve analysis. There is a significant difference in OS between cluster A and cluster B, and compared to cluster B, cluster A has a higher risk. But what are the specific risks between them? The authors may need to calculate the hazard ratio between these two groups using the method of Cox regression analysis. The following K-M analysis in other parts (figures) should also check the specific risk between the two groups.

Reply 1: We sincerely appreciate the valuable comments. We have added Cox regression analysis for Figure 2 panel D. Cox regression analysis for following two risk groups was in Figure 7.

Changes in the text: line 312-316.

2. In Figure 5 panel C, we can see that the number of genes corresponding to the lambda on the left (λ_{\min}), is more than ten, and the number of genes corresponding to the right one is just one. But it's very confusing that the number of genes used to construct the model is five. Would the authors please give a detailed description of the process of the variables' shrinkage?

Reply 2: We agree with your assessment. We calculated the AUC values of the corresponding gene combinations in the range of (λ_{\min}) to ($\lambda_{\min} + \text{standard error}$). The combination with the highest AUC value was used for subsequent analysis.

3. According to the method of unsupervised consensus clustering, "limma" R package was used to identify differential expression genes between different subtypes according to a P value of 0.05 and a $|\log_{2}FC|$ of 0.5. Generally, the fold change over two is considered to be a significant change. So, the $|\log_{2}FC|$ of DEGs should be more than 1.

Reply 3: We totally understand the reviewer's concern. In most cases, the standard of $|\log_{2}FC|$ of DEGs should be more than 1. But in this study, if we set the threshold of $|\log_{2}FC|$ to 1, we were unable to obtain enough DEGs for subsequent analysis. A similar study has set the similar threshold of $|\log_{2}FC|$ ¹.

4. The below reference might be able to help the authors to address the above comments/suggestions:

<https://doi.org/10.3390/biology11101488>

Reply 4: Thank you so much for your great suggestion. And we have cited the relevant literature in our study.

Changes in the text: line 249

Reviewer B

The paper is devoted to the significant issue of the oncology. The lack on data highlighted the inner mechanisms of the cancer heterogeneity leads to the failure in the anti-cancer therapy. it is found the way of application of the results of the transcriptome analysis. The data is novel and could be used in the future fundamental studies.

Reply: We sincerely appreciate your comments.

Reviewer C

In this review of the TCGA and Gene Expression Omnibus database, the investigators performed differing analyses of infiltration, drug, and molecular features to determine clinical implications of different subtypes.

Patients were stratified into groups A and B. Group B had a better prognosis and more likely to respond to immunotherapy.

Then, a model was built on 5 methylation-related genes. Areas under the curve based on survival were good.

Plans for stratifying by Group A and Group B needs to be clearly described in the methods. Group A needs to be clearly described in the results. We are only informed that subtypes were chosen based on the least interference between clusters. The unsupervised consensus clustering approach is poorly described and should include a reference.

Overall, the manuscript is interesting and hypothesis generating.

Reply: We greatly appreciate your valuable suggestions. We have made modifications to the corresponding description according to your suggestion

Changes in the text: line 196-204; line 328-330

1. Ye, X., Wang, R., Yu, X., Wang, Z., Hu, H., and Zhang, H. (2022). m(6)A/ m(1)A /m(5)C/m(7)G-related methylation modification patterns and immune characterization in prostate cancer. *Front Pharmacol* 13, 1030766. 10.3389/fphar.2022.1030766.

Reviewer D

1. Citation of **Figure S1B** was missing in the main text. Please revise. Figures should be **cited consecutively** in the text and numbered in the order in which they are discussed. (*example: Figure 1 contains 4 parts, such as Figure 1A, 1B, 1C, 1D, these parts should*

also be cited consecutively, unless Figure 1 is already cited before Figure 1A, 1B, 1C, 1D.)

Reply: We have revised in line 316.

2. Reference/citation

a. There are **two reference lists**. Please check which version is correct and remove the unnecessary one.

Reply: We have removed the unnecessary one.

b. If available, please update your reference list by including related literatures published within a year (2023). Some of the references are outdated. References should be cited consecutively and consistently according to the order in which they first appear in the main text.

Format of reference list should be: Author 1, Author 2, Author 3, et al. Title of the article. Journal Abbreviation name Year; Volume: Page numbers.

Reply: We have deleted outdated references.

c. The authors mentioned “**studies...**”, while only one reference was cited. Change “Studies” to “A study” or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

In recent years, many studies have demonstrated that the dysregulation of methylation is related to human cancer progression, especially in gastrointestinal cancers (22).

Studies have shown that demethylase ALKBH5 regulates m6A modification of downstream target PKMYT1 to suppress the invasion and metastasis of GC (26).

Reply: It has been revised.

3. **When using abbreviations** in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. **Please check and revise**. Such as: MSS, (figure 3, 8).

4. Figure 5

Please add (95% CI) after HR.

Hazard ratio
1.864(1.027–3.381)
0.274(0.099–0.760)
2.165(1.159–4.044)

Reply: It has been revised.

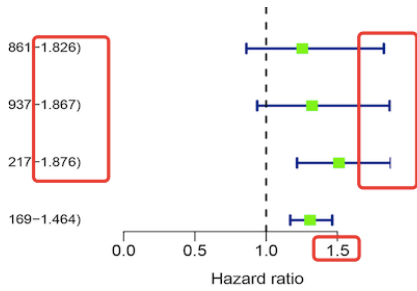
5. Figure 7A, B and Figure S1A, B

a. Please revise “pvalue” to “p value”; and add (95% CI) after HR.

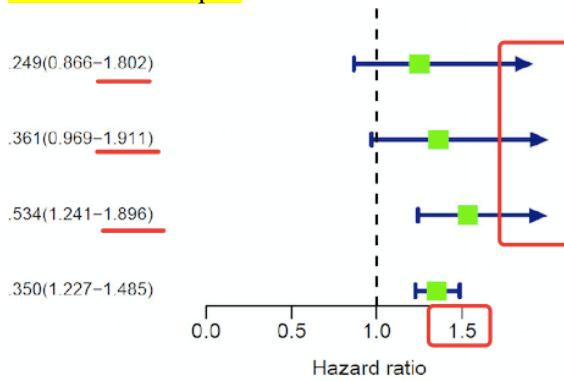
pvalue Hazard ratio

Reply: It has been revised.

b. To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows, or please extend the X-axis.



Here is an example:



Reply: It has been revised.

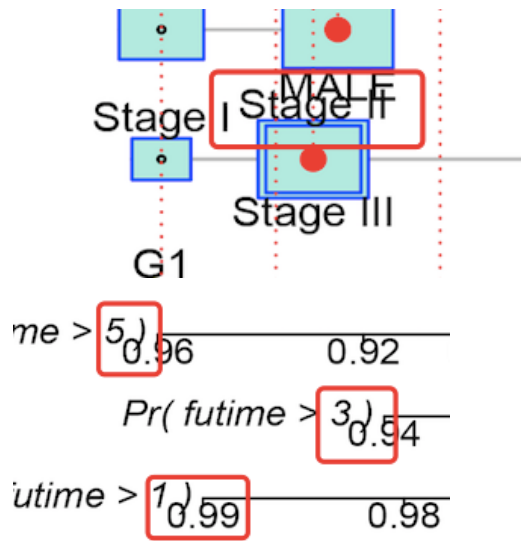
6. Figure 7

a. There are no “*, **” in figure 7, but they were explained in the legend. Please check and revise.

Reply: It has been revised.

821 calibration curves for 1-, 3-, and 5-year overall survival. *, P<0.05; **, P<0.01; ***,
822 P<0.001. AUC, area under the ROC curve; OS, overall survival; ROC, receiver
823 operating characteristic

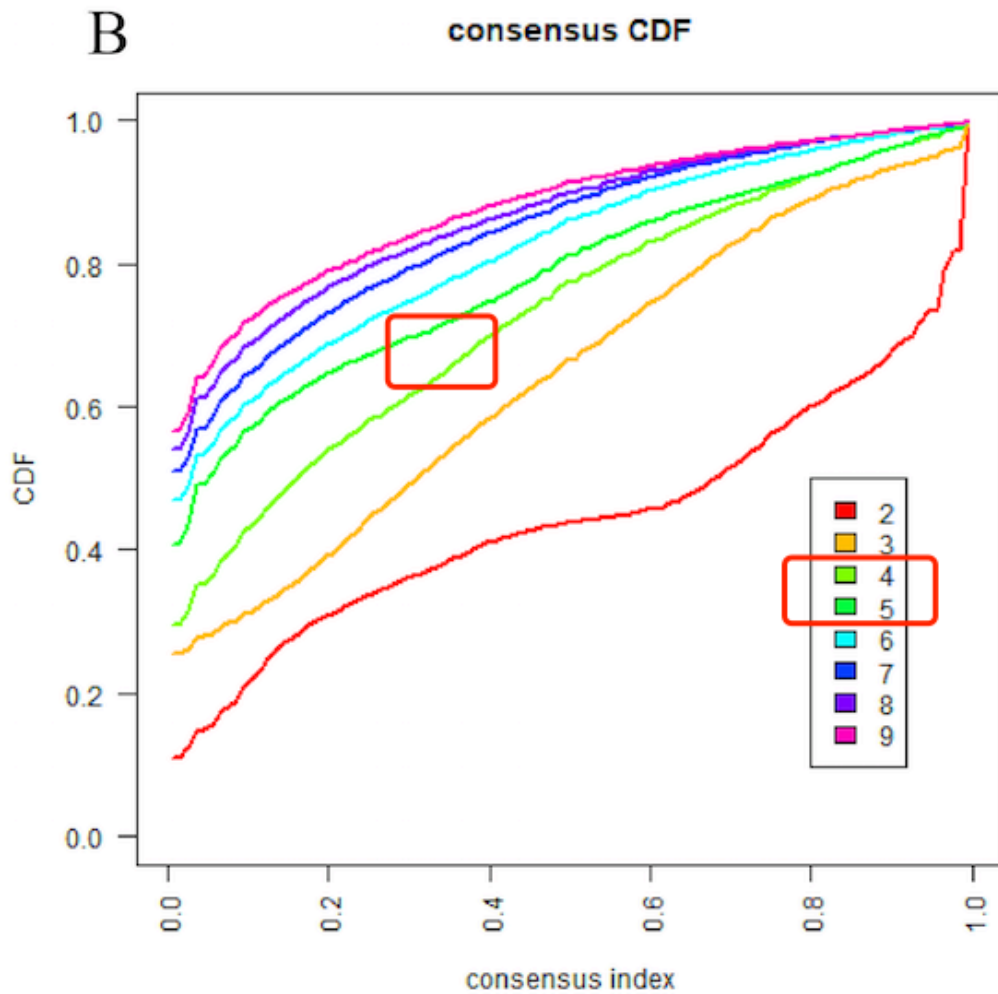
b. Some words and numbers are overlapped. Please revise.



Reply: It has been revised.

7. Figure 2B

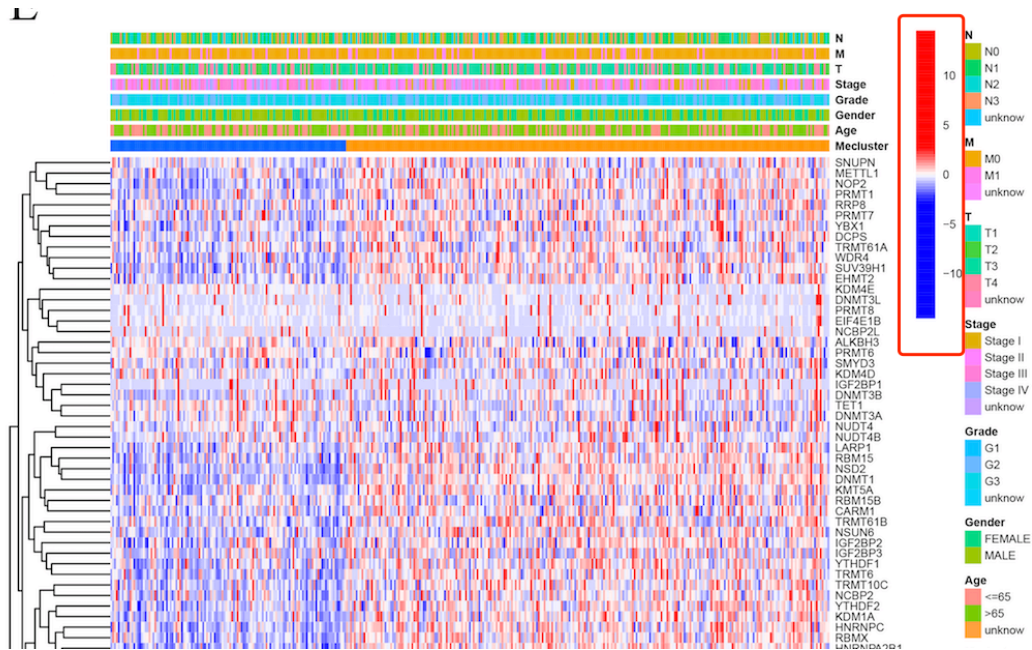
Lines for “4” and “5” are difficult to identify. Please revise.



Reply: It's difficult to revise the color because the color is fixed in "ConsensusClusterPlus" R package. So we removed the Figure 2B.

8. Figure 2E

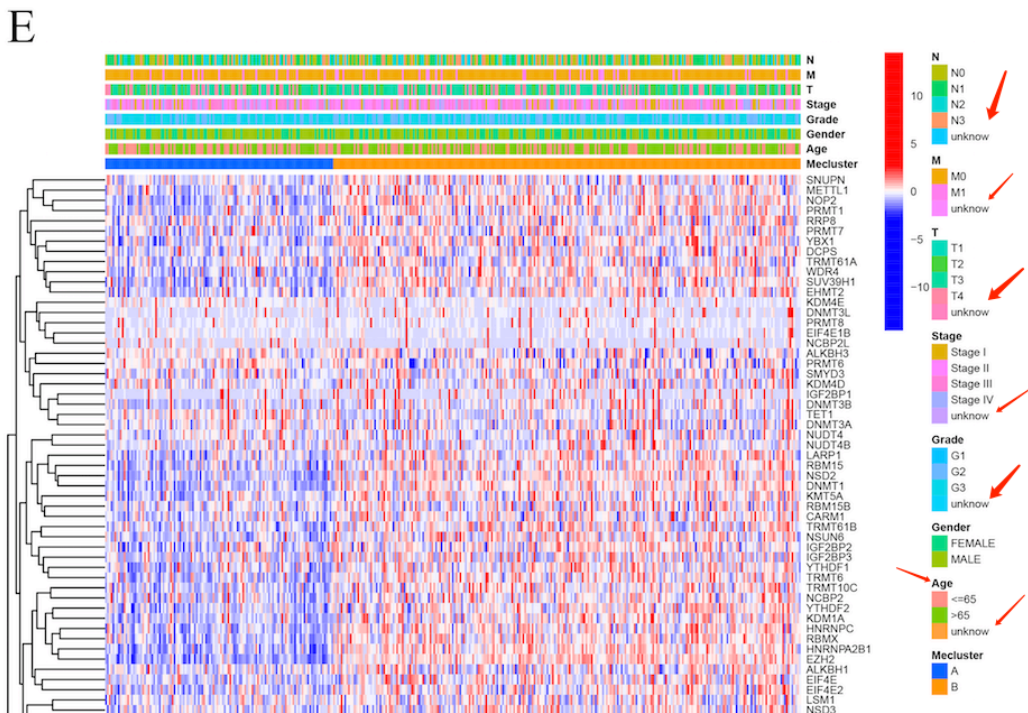
Please provide the meaning of the bar.



Reply: We have revised it.

9. Figure 2E

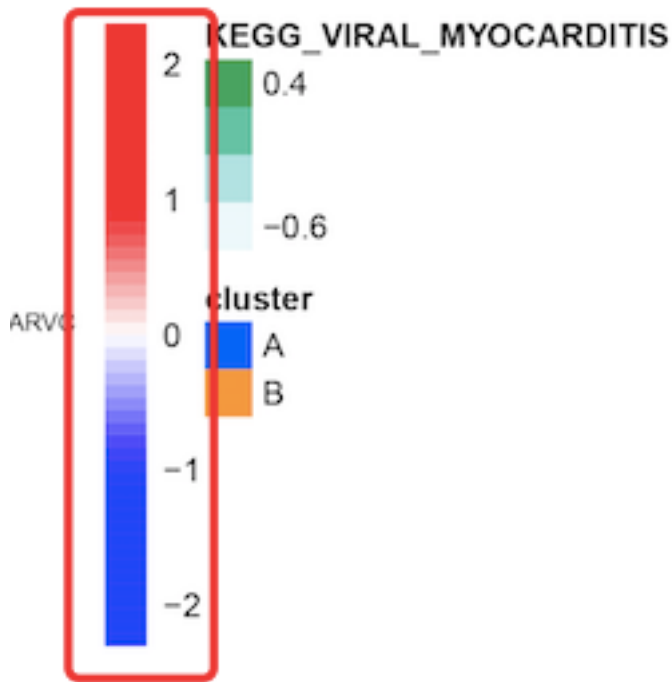
- 1) Please check and revise the typo.
- 2) Please provide the unit of "Age".



Reply: We have revised it.

10. Figure 3A

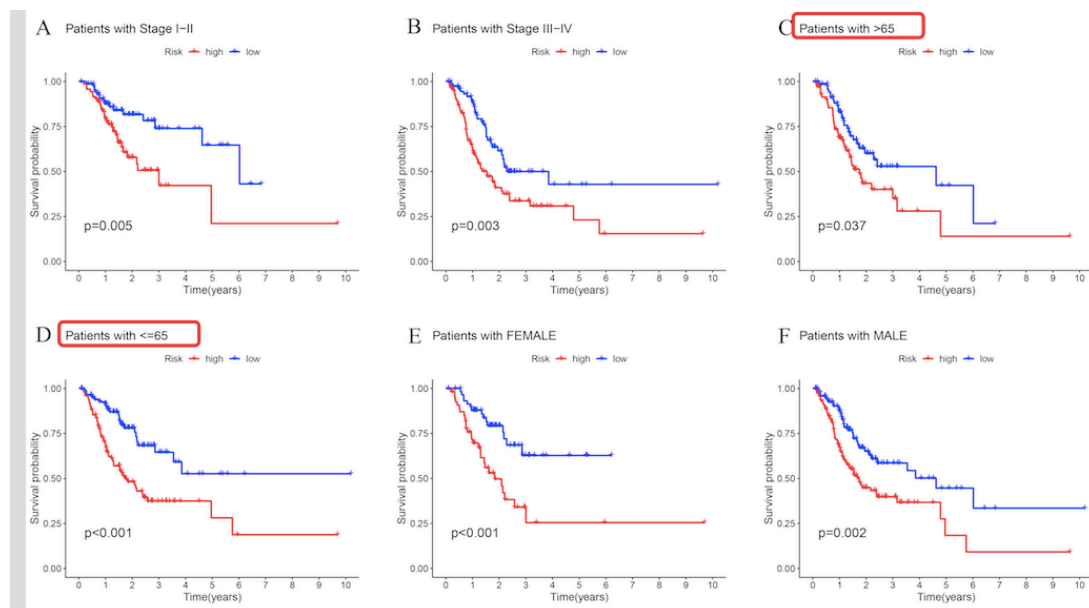
Please provide the meaning of the bar.



Reply: We have revised it.

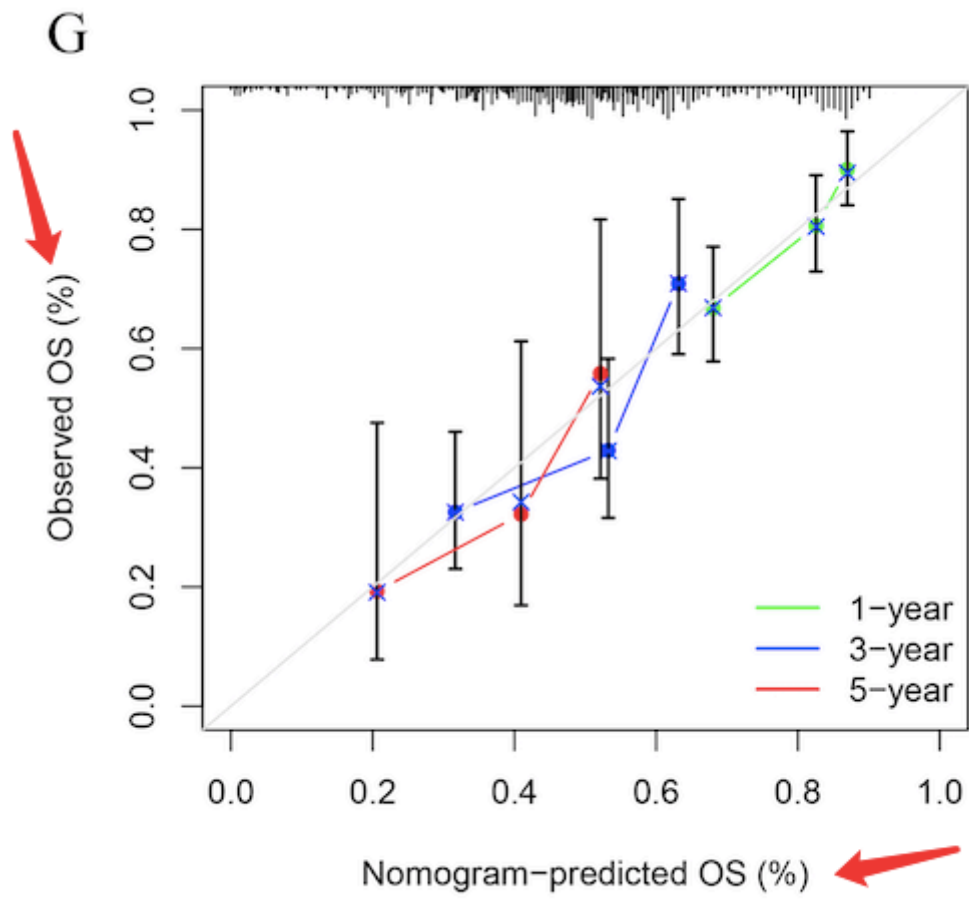
11. Figure 6

Please provide the unit of "Age"



Reply: We have revised it.

12. Figure 7G



Reply: We have revised it.