

## Peer Review File

Article Information: <https://dx.doi.org/10.21037/tcr-23-1886>

### Reviewer A

**Comment 1:** Please summarize the current knowledge on chromatin regulators with tumor suppressor properties, as well as their aberrant alterations in human cancers.

**Reply 1:** Thanks for the suggestion, it makes the manuscript much more standardized. We have added relevant content in the introduction section.

Changes in the text: Line 139-147

**Comment 2:** The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

**Reply 2:** Thanks for the suggestion, we recognize the problem. We have enriched the content of the abstract and hope it will reach your approval.

Changes in the text: abstract

**Comment 3:** Figures 5,8 and 9 are not clear enough. It is recommended to provide clearer figures again.

**Reply 3:** Thank you for your suggestion, it was our mistake. We changed the font of the image to make it clear.

Changes in the text: Figures 5,8 and 9

**Comment 4:** What are the biggest strengths and weaknesses of the clinical prediction model? What is the biggest problem faced? It is recommended to add relevant content.

**Reply 4:** Thanks for the suggestion, it got us thinking about this. We made a clarification in the introduction section.

Changes in the text: Line 103-114

**Comment 5:** This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.

**Reply 5:** Thank you for your suggestion. Due to the author had a fever and the limitations of the experimental conditions, we have only completed the viral packaging of the PKM

knockdown plasmid at this time. However, your suggestion makes us think deeply. We further reviewed the related papers on PKM and found that the function of PKM has been explained a lot in colon cancer. However, we found that in the results of this paper, PKM was correlated with drug therapy, a phenomenon that remains unexplained in the literature. We think that the next step could be to experimentally verify the relationship between PKM and drug treatment, however, perhaps we are not able to do so due to the limitation of experimental conditions in our laboratory. Again, we apologize to you because we didn't address your question well.

Changes in the text:

**Comment 6:** The description of some methods in this study is too simplistic, please describe in detail.

**Reply 6:** Thank you for your careful review. We have made additions to the Methods section and hope to have your approval.

Changes in the text: Methods section, Line 174, 178, 210-211, 218-222, 238-239, 257-260

**Comment 7:** The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Bioinformatic identification and analysis of immune-related chromatin regulatory genes as potential biomarkers in idiopathic pulmonary fibrosis, *Ann Transl Med*, PMID: 36111015”. It is recommended to quote the article.

**Reply 7:** Thank you for your careful review. We have added relevant content to the introduction section.

Changes in the text: Line 131-138

**Comment 8:** What is the relationship of chromatin regulators and immune microenvironment in colon cancer? It is recommended to add relevant content.

**Reply 8:** Thanks for the advice. We have added the relevant content to the introduction section and hope to get your approval.

Changes in the text: Line 124-131

## **Reviewer B**

1. Please provide the full name of “WGCNA/qRT-PCR” in the Abstract. Please also check

the whole article to make sure all the abbreviations have been defined when they first appear both in the Abstract and the main text.

**Reply:** Thanks for the suggestion, we made the changes as requested.

Changes in the text: Page 2

2. Please confirm whether the genes (not proteins) in the text have been italicized.

**Reply:** Thanks for the suggestion, we made the changes as requested.

3. References

- The name you mentioned in the following sentence does not match the corresponding reference. Please check and revise.

124 Epigenetic regulation is one of the key mechanisms of immune checkpoint expression  
125 in the tumor microenvironment(20). Leping Li et al. identified DNASE1L3, an  
126 enzyme that regulates autoimmune responses to its own DNA and chromatin, as a

*21. Li W, Nakano H, Fan W, et al.; DNASE1L3 enhances antitumor immunity and suppresses tumor progression in colon cancer. JCI Insight 2023;8(17). doi: 10.1172/jci.insight.168161.*

**Reply:** Thanks for the suggestion, we made the changes as requested.

4. Figures:

- 1) Please check if any unit/description of x/y-axis should be added to the Figures.
  - 2) Please indicate the meaning of lines in Figure 2E.
- **Changes in the text: Page 2**

- 1) When consecutive subparts of a figure are cited, they should be cited as: Figure 3A-3I, Figure 4A-4I, etc.

In your text, Figure 3 contains 9 parts, these parts should also be cited consecutively, unless Figure 3 is already cited before Figure 3A, 3B, 3C, 3D...etc.

The citation of Figure 9C,9D should be before Figure 9E,9H. Please check and revise.

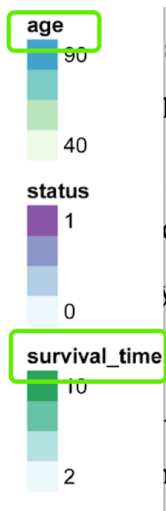
- 2) Figure 3B-C, 4B-C: there is a spelling error.

100

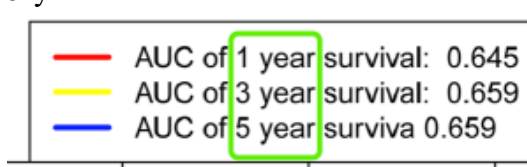
200

Patients (increasing risk score)

- 3) Figure 3D, 4D: Please add unit for Age and survival time.



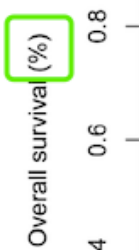
4) Figure 3E, 4E, Supplementary Figure 3B: Please revise “1/3/5 year” to “1-year, 3-year, 5-year”.



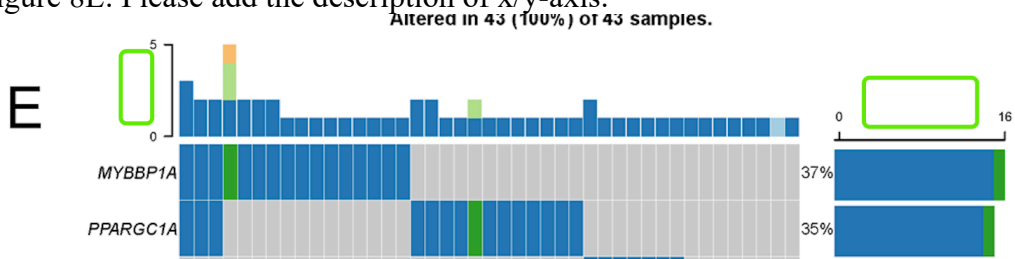
5) Figure 3F-G, 4F-G: Please revise “pvalue” to “P value” and “Hazard ratio” to “Hazard ratio (95% CI)”.



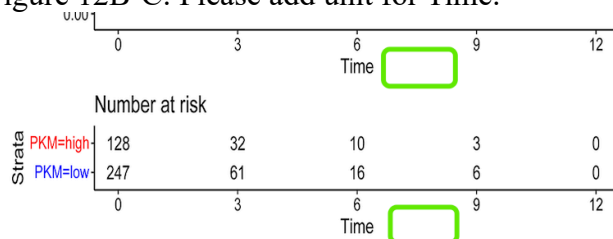
6) Figure 3I, 4I, Supplementary Figure 3C: Please remove (%) from the x/y-axis.



7) Figure 8E: Please add the description of x/y-axis.



8) Figure 12B-C: Please add unit for Time.



- 9) Please indicate the meaning of asterisk (\*/\*\*/\*\*\*\*/\*\*\*\*/ns) in Figures 3,4, 7, 11,12.
- 10) All abbreviations in figures and legends should be explained. GO/KEGG/SWI/SNF/CRs/COAD/ in Figure 1 for example. Please check all your figures.

- **Reply:** Thank you for your careful review, we made the changes as requested.

5. It is suggested to rename your “Supplementary File 1” to “Supplementary Table X” and provide a Table title for it.

**Reply:** Thanks for the suggestion, we made the changes as requested.

## 6. Tables

- 1) Table 1: Please add a header for the first column.
- 2) It is suggested to add “year” after “Age” in Table 1.
- 3) The citation of Supplementary Table 2 is missing in the main text. Please check and revise.
- 4) **All** abbreviations in tables and legends should be explained. TCGA/T/N/M in Table 1 for example. Please check all your tables.

**Reply:** Thanks for the suggestion, we made the changes as requested.

7. These two descriptions are contradictory, please check and revise.

**Consent to Publication**↵

542 Not applicable.↵

543 ↵

544 *Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that

545 questions related to the accuracy or integrity of any part of the work are appropriately

546 investigated and resolved. This study was conducted in accordance with the Declaration of

547 Helsinki (as revised in 2013). The Medical Ethics Committee of Northern Jiangsu People's

548 Hospital approved the study on March 17th, 2021 (*No. the registration number of the ethics*

549 *board*). The patient participating in the study gave informed consent.↵

**Reply:** Thanks for the suggestion, we made the changes as requested.