Peer Review File

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Reviewer A

The paper titled "Gene models of six coiled-coil domain-containing family members predict survival in hepatocellular carcinoma by regulating the hypoxia pathway and immune microenvironment" is interesting. The CCDC score based on 6 CCDC genes is a potential prognostic marker of HCC survival. A high CCDC score is associated with hypoxia pathway activation and an immunosuppressive TME in HCC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply: We thank the reviewer for carefully reading our manuscript. We have revised the content of the abstract and added information in the research background section regarding the focus on clinical needs. Please refer to the manuscript. (see Page 2-3, line 39-88)

2) What are the biggest strengths and weaknesses of this gene model? What is the biggest problem faced? It is recommended to add relevant content.

Reply: We are grateful to the reviewer for their insightful recommendation.

Strengths: One of the significant strengths of the CCDC family genes score is its potential as a prognostic marker for liver cancer outcomes. This can be a valuable tool for predicting patient prognosis, which is essential in clinical decision-making. The CCDC score also shows promise as a biomarker that can guide tailored therapeutic approaches. This is crucial in the era of personalized medicine, where treatments can be customized based on individual patient profiles. It has the capability to identify associations with immune infiltration and hypoxia pathway activation, shedding light on critical aspects of the tumor microenvironment. This knowledge can inform targeted therapies.

Weaknesses: Like many bioinformatics-based models, validation with extensive in vivo and in vitro experiments is required to confirm the clinical relevance and accuracy of the CCDC score. The absence of such validation can be considered a weakness.

The biggest problem faced by the CCDC family genes score is the need for further validation and mechanistic elucidation.

We added the strengths, weaknesses, and the biggest problem faced to the discussion section. (see Page 16, line 468-481)

3) What is the relationship of hypoxia-associated genes and immune microenvironment in HCC? It is recommended to add relevant content.

Reply: We appreciate the reviewer's suggestion. Hypoxia can impact the recruitment and function of immune cells within the tumor microenvironment. For instance, it can enhance the recruitment of tumor-associated macrophages (TAMs), which can have both pro-tumoral and anti-tumoral functions, depending on their polarization state. In summary, hypoxia-associated genes in HCC can contribute to an immunosuppressive microenvironment, which can hinder the body's natural immune response against the cancer. This complex relationship underscores the importance of developing therapies that target both the hypoxic aspects of the tumor and the immunosuppressive mechanisms to improve outcomes for HCC patients.
We added in the results section " Immune suppressive microenvironment in the CCDC score high group". (see Page 6, line 159-164; Page 11, line 330-350)

4) All figures are not clear enough. It is recommended to provide clearer figures again.
Reply: We thank this reviewer for his/her comments. We have provided clearer figures. (see Figures)

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: Thank you for the reviewer's suggestions and attention. We appreciate the reviewer's perspective. However, this study was designed with the aim of conducting bioinformatics analysis to explore relevant data and information. We acknowledge the importance of experimental validation for gaining a deeper understanding of the biological mechanisms underlying our research findings and their potential clinical applications. Nevertheless, considering the scope and resource constraints of this study, we have chosen to focus on leveraging the strengths of bioinformatics methods. We will make every effort to explain and address the limitations of our research, and we look forward to future studies delving further into this field, including experimental validation. Once again, we express our gratitude for the valuable suggestions from the reviewer.

6) It may be more meaningful to add functional research on key genes.
Reply: We thank the reviewer for raising this concern. The addition of functional research on key genes could indeed enhance the depth and meaning of the study. This kind of research would provide valuable insights into the specific roles and mechanisms of these genes in the context of the study's objectives. It would not only validate the bioinformatics findings but also offer a more comprehensive understanding of their biological significance. As previously noted, the limitation of this article lies in the absence of functional research. Nevertheless, we will provide an in-depth exploration of the functional roles of key genes from a bioinformatics standpoint. We would like to express our gratitude once more for the valuable suggestion provided by the reviewer.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Profiles of immune infiltration in the tumor microenvironment of hepatocellular carcinoma, J Gastrointest Oncol ，PMID: 34295564”. It is recommended to quote the article.

Reply: We thank this reviewer for raising this point. This paper has been cited. (see Page 5, line 121-127; Page 13, line 370-378)

8) It is recommended to increase the characteristics of HCC tumor microenvironment and the progress of treatment research in the discussion.

Reply: We thank this reviewer for this comment. We appreciate the recommendation to enhance the discussion section by delving further into the characteristics of the HCC tumor microenvironment and the advancements in treatment research. We recognize the importance of these aspects in providing a well-rounded understanding of the research context and its potential clinical relevance. We will make the necessary additions to address these topics and enrich the discussion. (see Page 13, line 370-385)

Reviewer B

1) First, the current study did not test the predictive accuracy of six coiled-coil domain-containing family members, so the term “predict survival” in the title and elsewhere should not be used. The title also need to indicate the focus of the prognostic role and the bioinformatics analysis methodology.

   Reply: We thank the reviewer for raising this concern. We revised this concerning and using new title "Identification of potential prognostic biomarkers among gene models for coiled-coil domain-containing family members in hepatocellular carcinoma elucidates their influence on the hypoxia pathway and immune microenvironment". (see Page 1, line 6-8)

2) Second, the abstract needs further revisions. The authors did not explain the potential clinical significance of this research focus and what the current knowledge gap is. The methods need to describe the clinical sample in the datasets, the adjusted covariates, and how the independent prognostic role was tested. The results need to quantify the findings by reporting statistics and accurate P values, such as expression levels and HR values. The conclusion needs comments for the clinical implications of the findings.

   Reply: We appreciate the reviewer's suggestion. We have revised the abstract in accordance with the reviewer's suggestions. (see Page 3, line 71-72)

3) Third, the introduction of the main text is poor. The authors need to review what has been known on the prognostic biomarkers in HCC, analyze the limitations and knowledge gaps of prior studies, and clearly indicate the potential clinical significance of this study.

Reply: We thank the reviewer for carefully reading our manuscript. We have added new content, including information on known prognostic biomarkers for HCC, an analysis of previous research limitations and knowledge gaps, and a clear indication of the potential clinical significance of this study. Additionally, we have included the references mentioned by the reviewer. (see Page 16, line 468-481)

4) Fourth, in the methodology of the main text, please describe the research design, clinical samples in the datasets, baseline clinical factors, follow up procedures, and measurements of prognosis outcomes. In the statistical analysis, it is necessary to describe the test of the independent prognostic role in the multiple adjustment analysis. Please ensure P<0.05 is two-sided.

Reply: We thank this reviewer for raising this point. We used three independent datasets, including TCGA-LIHC, ICGC-LIRI-JP, and GSE14520. Relevant baseline information, follow-up data, and prognosis results can be accessed on the respective websites and articles. All data used in this study are sourced from publicly available datasets that have been previously published. The independent prognostic test method in the statistical analysis has been incorporated. We have confirmed that $P < 0.05$ is two-sided. (see Page 8, line 217-218)

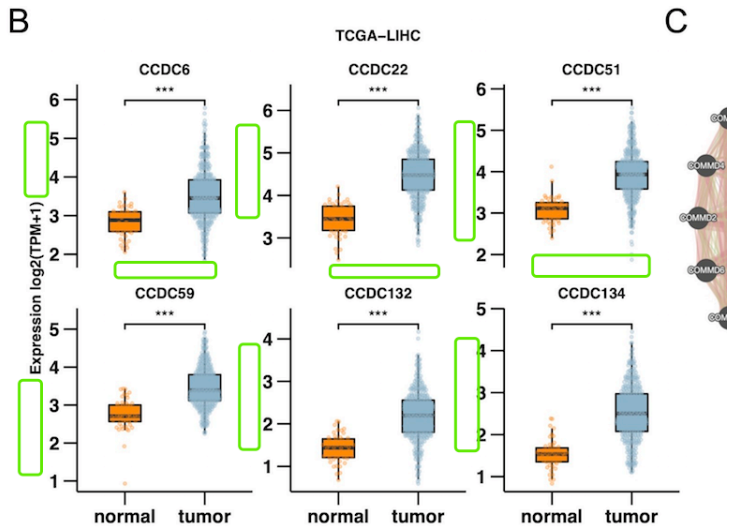
5) Finally, please consider to review and cite several related papers: 1. Xu ZP, Liu Y, Wu ZR, Gong JP, Wang YB. Prognostic and diagnostic value of SOX9 in cirrhotic and noncirrhotic hepatocellular carcinoma. Transl Cancer Res 2021;10(6):2738-2746. doi: 10.21037/tcr-20-3385. 2. Zhang L, Yuan L, Li D, Tian M, Sun S, Wang Q. Identification of potential prognostic biomarkers for hepatocellular carcinoma. J Gastrointest Oncol 2022;13(2):812-821. doi: 10.21037/jgo-22-303. 3. Chen W, Gao C, Shen J, Yao L, Liang X, Chen Z. The expression and prognostic value of REXO4 in hepatocellular carcinoma. J Gastrointest Oncol 2021;12(4):1704-1717. doi: 10.21037/jgo-21-98. 4. Feng X, Zhou Y, Pang S, Yang C, Wang S. Phosphotriesterase-related protein as a novel prognostic predictor for hepatocellular carcinoma patients. Chin Clin Oncol 2023;12(4):37. doi: 10.21037/cco-23-42.

Reply: We thank this reviewer for this comment. All papers have been cited and added in introduction. (see Page 5, line 127-144)

Reviewer C

1. Figure 1B

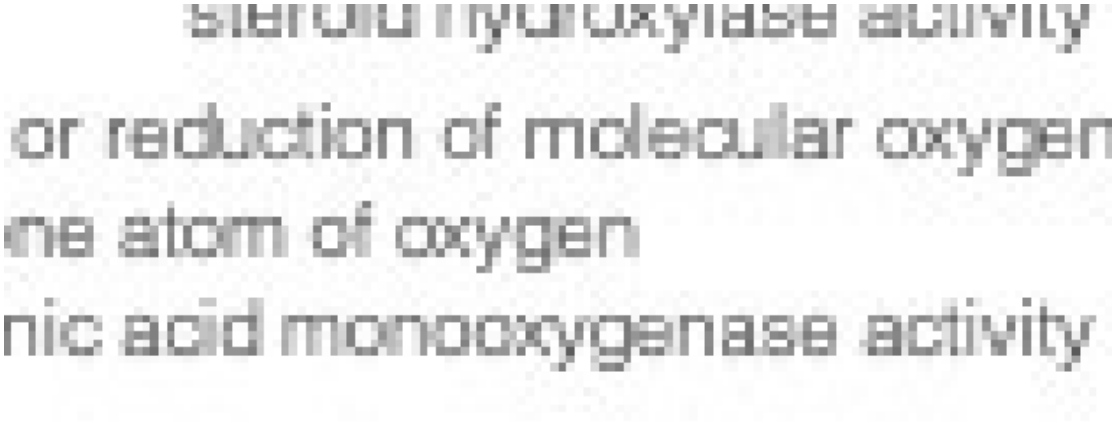
Caption of coordinate axis should be indicated respectively in each plot. Please revise.



Reply: Thank you for your suggestion. We have added the caption of coordinate axis in Figure 1B. (See page22, line710)

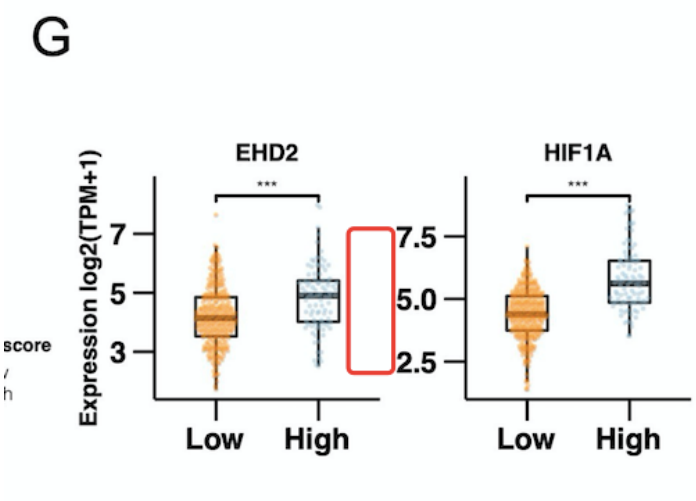
2. Figure 4

a. Please send us Figure 4(C, D, E) with higher resolution in JPG/TIFF, as the current one is not clear enough.



Reply: Thank you for raising this point. We have provided the Figure 4(C, D, E) with higher resolution in the "Figure-resived" file.

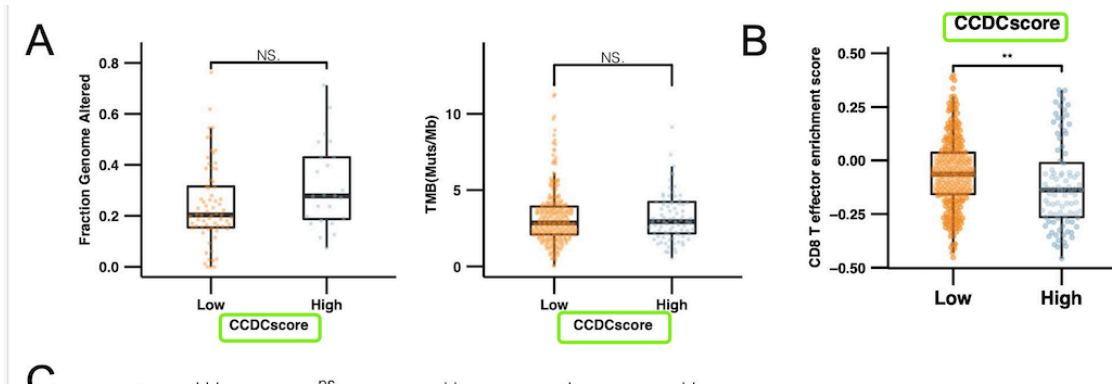
b. Caption of Y-axis should be indicated in each plot respectively.



Reply: Thank you for your comments. We have revised it. (See page25, line755)

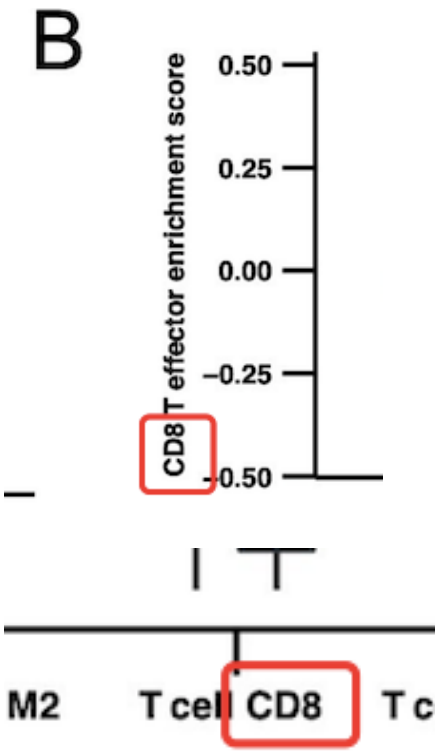
3. Figure 5

a. Please revise 'CCDCscore' to 'CCDC score'.



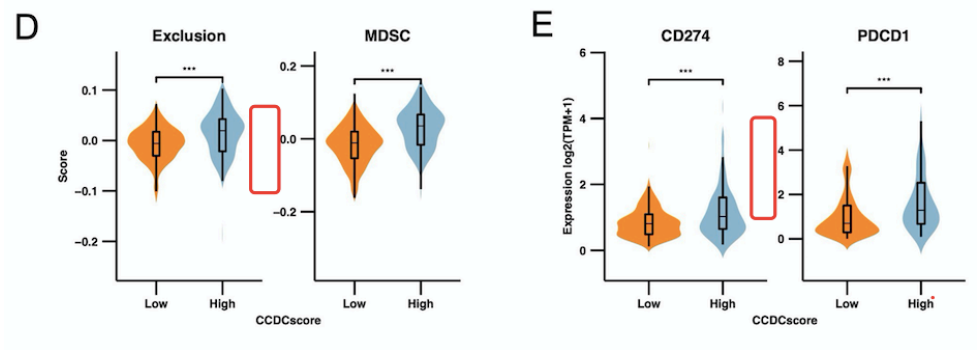
Reply: Thank you for your comments. We have revised it. (See page26, line770)

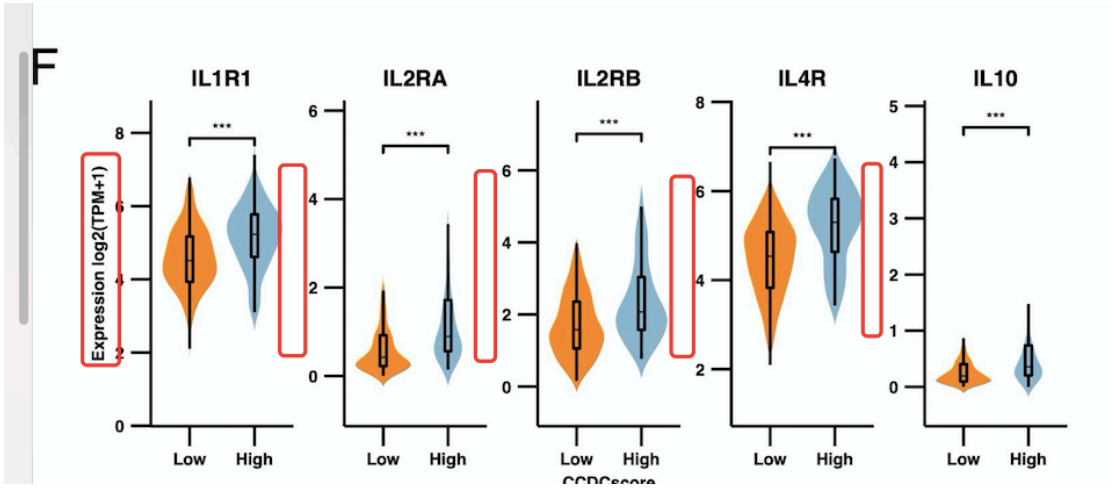
b. It is "$CD8^+$" in figure legend. Please check and unify.



Reply: Thank you for your comments. We have revised it. (See page26, line770)

c. Caption of Y-axis should be indicated in each plot respectively.

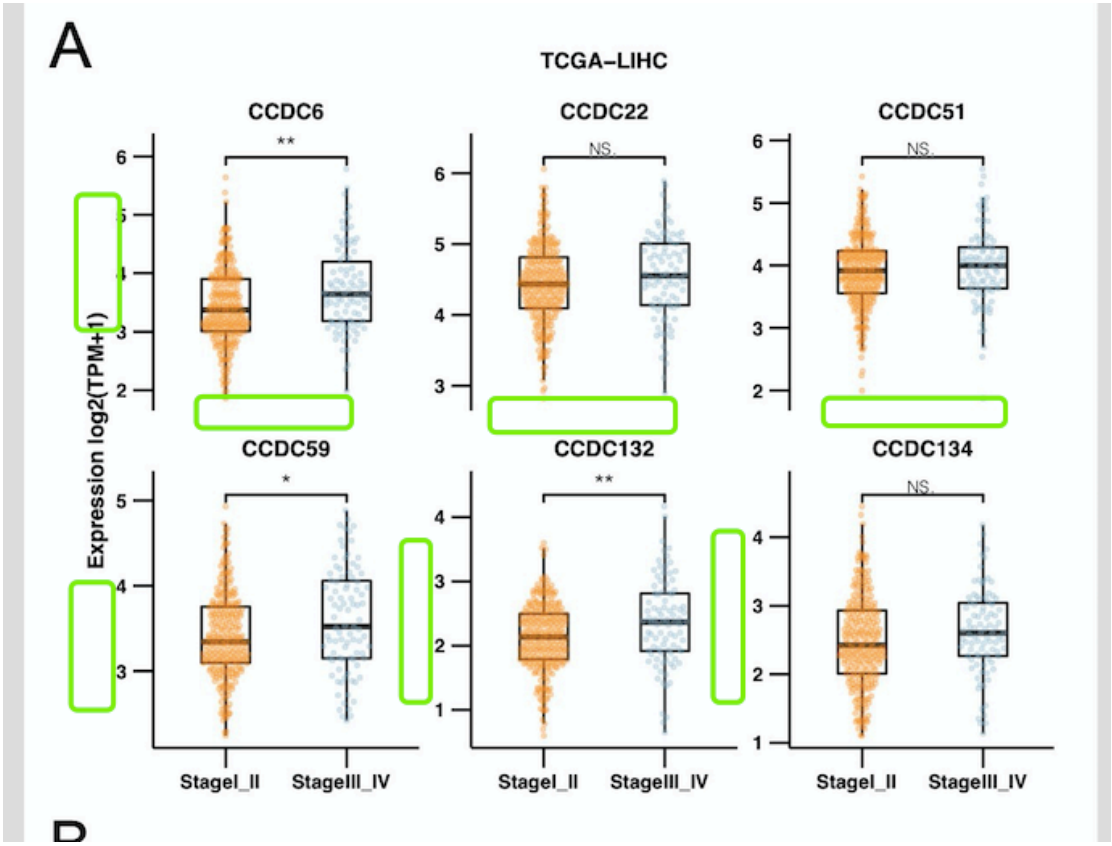




Reply: Thank you for your comments. We have revised it. (See page26, line770)

4. Figure S1A-C

Caption of coordinate axis should be indicated respectively in each plot. Please revise.



Reply: Thank you for your comments. We have revised it. (See page28, line800)