Peer Review File

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Review Comments-Reviewer A

Lung cancer is a highly aggressive disease and the leading cause of cancer-related deaths. Lung adenocarcinoma (LUAD) is the most common histological subtype of lung cancer. As a type of programmed cell death, anoikis serves a key role in tumor metastasis. In the manuscript "A novel anoikis-related gene signature to predict the prognosis, immune infiltration, and therapeutic outcome of lung adenocarcinoma", authors constructed an anoikis-related risk model to explore how anoikis could influence the tumor microenvironment (TME), clinical treatment, and prognosis in LUAD patients.

Couple questions are required to be answered before it will be accepted.

(1) There was a similar report (DOI: 10.1111/1759-7714.14766) about the anoikis-related gene signature to predict the prognosis of LUAD in PubMed. What is the novel idea in the paper? Please elaborate in the introduction.

Reply: In the third paragraph of the introduction, we added the differences compared from the similar report. In fact, the other article was less extensive, and only included ARGs in LUAD and a predictive risk model. Among other things, our study constructed two subgroups and detected clinical response. (page 4, line 97-101.)

(2) The anoikis was the crucial topic in the study. What were the roles of anoikis in the prognosis of LUAD? Please state in the introduction.

Reply: Our additions in the third paragraph of the introduction. We found anoikis could regulate the immune function and metabolism procession which played great roles in prognosis in LUAD. (page 4, line 104-105.)

(3) In the introduction, it was proposed to add related reference (DOI: 10.21037/tcr-22-327) about the constructed gene signature in LUAD.

Reply: We added the reference in the third paragraph of the introduction. (page4, line102.)

(4) What were the differences between anoikis and apoptosis? Please state in the introduction.

Reply: Indeed, anoikis is one of the apoptosis, we described in detail in the second paragraph of introduction. (page 4, line 86-90.)

(5) What were the correlations between anoikis and tumor microenvironment (TME)? Please state in the discussion.

Reply: In the fifth paragraph of discussion, we discuss the correlations between anoikis and TME. Anoikis could greatly influence the regulation of the TME. (page 12, line 366-376.)

(6) It was better to validate the representative genes by experiments.

Reply: We know that adding experiments to verify the expression of ARGs in LUAD will make the study more credible and complete. But unfortunately, we currently do not have the sufficient funds or time to complete them at the moment, and we are sure to add experiments after the subsequent time and funds are available. (No modification in the text)

(7) Compared to other constructed related risk model, what were the advantages of anoikis-related risk model? Please state in the discussion.

Reply: Our answers are in the conclusion. (No modification in the text)

Review Comments-Reviewer B

In this study, the authors proposed an anoikis-related gene signature to predict the prognosis, immune infiltration, and therapeutic outcome of lung adenocarcinoma. Although the idea is of interest, some major points should be addressed as follows:

1. All experiments have been conducted on public data without further validation.

Reply: Thank you for your comments, due to lack of time and effort, we will add this aspect in a subsequent study.

Changes in the text: None.

2. It seems that several cancer types all showed higher expression of anoikis-related genes in the tumor compared to adjacent normal. Why only study lung cancer? If lung is a focus for some reason, they should also look at LUSC

Reply: The question has been explained in the text that LUAD is by far the most prevalent cancer in lung cancer and has the highest mortality rate, so LUAD was analyzed from this perspective instead of LUSC.

Changes in the text: we have modified our text as advised in line 70-line79.

3. DNA variation is completely ignored in this study. As a thorough investigation, the authors should look at somatic mutations or copy number variations in any of the anoikis-related gene family. These variants could also be related to clinical outcome.

Reply: This part is in line173 - line177.

4. The authors should implement nomogram prediction model.

Reply: This part is in line254-line257.

5. The authors should add analyses on DNA methylation and protein levels.

Reply: Due to the limited time and effort and the authors' poor understanding of methylation content, methylation analysis was not chosen to ensure the reliability of the results.

Changes in the text: None.

6. More references to bioinformatics workflow should be added to attract a broader readership i.e., PMID: 34572330, PMID: 35851932).

Reply: Thank you for your comments.

Changes in the text: line 181-line182, line 206-line 220.

7. How many AUC can be considered as a good model? The authors should have a standard.

Reply: The main text has clearly explained what is considered a good AUC can be, because the AUC of the model is the highest compared to the AUC of clinical features inherent to lung adenocarcinoma, which is clearly better than predicting patient prognosis by clinical features alone (line 385-line 388).

8. Literature review is insufficient. It should be improved using related bioinformatics works on LUAD or anoikis-related genes.

Reply: Thank you for your comments.

Changes in the text: line 181-line 182, line 206-line 220, line3 97-line 403, line 449-line 458.

Review Comments-Reviewer C

This research investigated the anoikis-related gene signature, and a risk model was well established to predict the prognosis, immune infiltration, and therapeutic outcome of lung adenocarcinoma (LUAD). The molecular alterations and clinical correlation of ARGs in LUAD, and the TME and drug sensitivity in relation to ARGs were revealed. This research provided new views of anoikis between the TME and LUAD progression, and contributed to the evidence base for tumor treatment, this research article was well prepared and recommended to be published after major revisions.

Major Revisions:

1) Labels in Figure 1 G, H; Figure 2 A,B,D,E; Figure 5 C,E,F; Figure 10 should be larger.

Reply: We resubmit the modified Figure.

2) Drug resistance or drug sensitivity in relation to ARGs should be analyzed in more detail, which chemotherapy was applied in the treatments of LUAD, and how the outcome be related to ARGs?

Reply: We add the corresponding analysis to the results.

Changes in the text: line336-line339.

3) For biological function pathways, any GO term could be shown, which pathway was

activated, upregulated or downregulated? Or alternation of the pathway was revealed between the high-risk and low-risk groups?

Reply: We add the corresponding analysis to the results.

Changes in the text: line 252-line 253.

4) In terms of novelty, were the subtypes of LUAD analyzed, and whether the disease severity was related to ARGs? Please discuss the subtypes and add analysis accordingly.

Reply: In the first step of this analysis, lung adenocarcinomas have been divided into two subgroups based on differential genes and analyzed accordingly.

Changes in the text: line 149-line 154, line 366-line 368.

5) TME in LUAD, despite cell population analysis, any checkpoint markers could be revealed in two risk groups as well as in relation to the prognosis?

Reply: This has been analyzed in the body of this article (line 323-lin 327).

As a similar study was published recently, adding the differences of ARGs between LUAD subtypes and adding pathway analysis with Gene Ontology Term Enrichment are strongly recommended.

Reply: Thank you for your comments, due to lack of time and effort, we will add this aspect in a subsequent study.

Changes in the text: None.

Review Comments-Reviewer D

The study by Wang et al., examines the role of a novel anoikis-related gene signature in the prognosis, immune infiltration, and therapeutic outcome of lung adenocarcinoma. In the conclusion, the authors claim that "The risk model constructed in this study can benefit to predict patient survival. Our results provided new potential treatment strategies."

In general, this is a well design study that provides insight into how anoikis plays a vital role in TME regulation and immune pathways in lung adenocarcinoma.

There are a few issues with the manuscript that should be addressed prior to publication.

1. Page 4, line 94, the authors mentioned "Multiple factors can regulate anoikis." Please specify what are the multiple factors that could regulate anoikis?

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: line 94.

2. Page 4, lines 96-97, “Resistance to anoikis may also be an important cause of resistance to radiotherapy and chemotherapy in tumors.” This sentence is confusing. Please revise it.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: line 96-97.

3. Page 4, lines 98-101, “There was similar article has been published recently however, what’s different was we not only constructed prediction risk model but also established two variously subtypes with “ConsensusClusterPlus” package and the clinical response was also detected, so our study may be more comprehensive.”

This sentence is full of grammatical mistakes and sentence construction is wrong. Please correct and revise it. Moreover, the authors mentioned that similar articles have been published recently, however, there is no reference or details of such studies.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: lines 98-101.

4. In the statistical section page 5, line 157 and line 162, the authors mentioned that they explore 29 immune cell fractions and 22 types of immune cells. I was wondering how and/or on what basis the authors selected these cell types.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: line 157 and line 162.

5. Page 6, line 185, authors mentioned that “We collected the ARGs from previous reports.” However, there are no references to support this statement.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: line 185.

6. In the result section, I would recommend specifying P-values to give more credibility to the observed data.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: 195-206.

7. Page 9, lines 276-277, authors stated “There is no doubt that immune checkpoint inhibitors (ICIs) can influence therapeutic effects of immunotherapy.” Please provide a reference to this statement.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: lines 276-277.

8. The results of figure 10 need to be explained in more detail.

Reply: Thanks for the reminder, we corrected it in the text.

Changes in the text: line302-305.

9. The manuscript needs to be revised extensively for English and scientific expressions.
Reply: Thanks for the reminder, we corrected it in the text.

Review Comments-Reviewer E

The author aimed to investigate the molecular alterations and clinical relevance of ARGs in LUAD, and to discuss the relationship between ARGs and LUAD prognosis， they found anoikis could regulate the immune function and metabolism procession which played great roles in prognosis in LUAD. Albeit, I consider these findings to provide new insight into cancer-related fields, I still have some suggestions.
1, Most figures and tables are highly professional; however, the authors should guide the readers to the meaning of the images and tables appropriately; otherwise, it is likely to cause misunderstandings. Therefore, I suggest the author consider revising these figures and table legends again.
Reply: We resubmit the modified Figure.

2, In Figure 2, the author conducted to construct the prognostic model, indicating 21 genes including MCL1, TLE1, ANGPTL4, TIMP1, ITGA6, PIK3CG, FADD, CDKN3, EDA2R, EIF2AK3, CEMIP, LTB4R2, PPP1R13B, FGF2, PIK3R2, COL4A2, SHC1, CDC25C, SLCO1B3, LDHA, RHOQ. Since the authors gave a general answer on gene expression, is there any evidence of different roles in cancer phenotypes of 21 genes? Please perform pertinent bioinformatic analyses and provide examples of studies investigating miRNA alteration or DNA methylation (https://biit.cs.ut.ee/methsurv/) (PMID: 29264942, 34834441, 33437202).
Reply: Due to the limited time and effort and the authors' poor understanding of methylation content, methylation analysis was not chosen to ensure the reliability of the results.
Changes in the text: None.

3, In figure 10, the author presented the expression of 21 ARGs in LUAD tissues and normal tissues. However, It would be much better if the authors could validate their data via proteinatlas database (PMID: 25613900, 36536279, 34329194)
Reply: Thanks, we have proved it with the GEPIA database which is completely different from TCGA
Changes in the text: line 123-125, line 312-314, line 419-424.

4, Since Connectivity Map (CMap) can be used to discover the mechanism of action of small molecules, functionally annotate genetic variants of disease genes, and inform clinical trials. It would be fascinating if these data could be correlated with other clinical databases. Therefore, I suggest the authors can validate their data via CMap or proteinatlas, and discuss these methodologies and literature as well as

the validated data for cancer recurrence or metastasis in the manuscript (PMID: 17008526, 29195078, 32064155).

Reply: Thank you, we have supplemented the GEPIA database with a different one from the full TCGA, and the effect of different genes on the mechanism is addressed in the discussion.

Changes in the text: line 123-125, line 312-314, line 419-424.

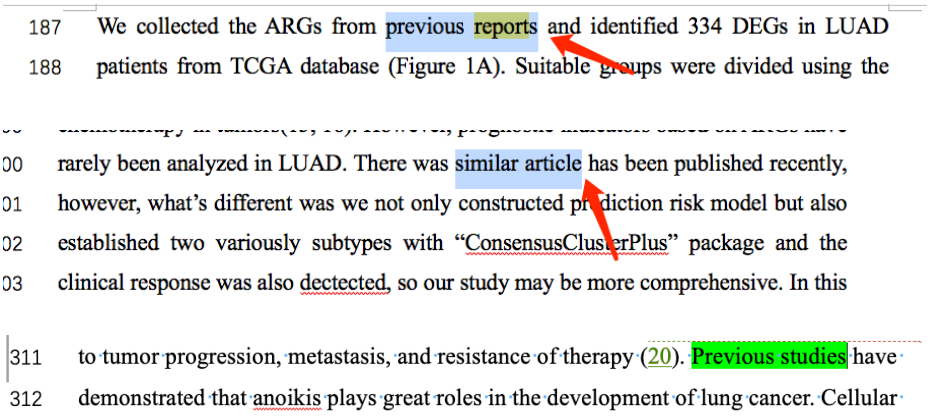
5, There are few typo issues for the authors to pay attention to; please also unify the writing of scientific terms. “Italic, capital”? The font is too small for some of the current figures; meanwhile, the manuscript also needs English proofreading.

Reply: Thanks for the reminder, we corrected it in the text.

Review comments-Reviewer F

1. References/Citations

a) Please double-check if citations should be added as you mentioned “previous reports/stidues” and “similar article”.

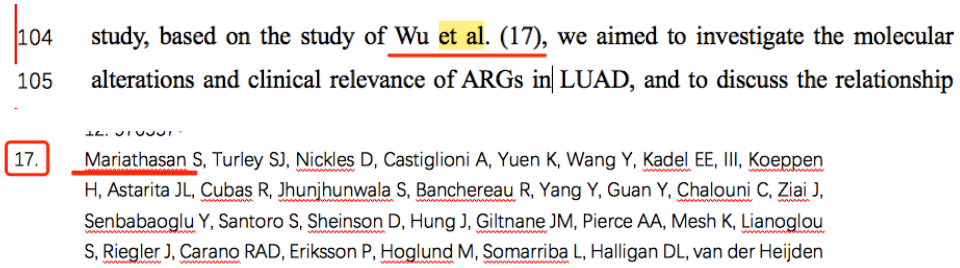


Reply: Thanks for the reminder, we corrected it in the text.

b) There are 2 reference lists in the file, please keep the correct one and delete another one.
*Please note that there are 30 citations in the text.

Reply: We have modified it.

c Please check if the author’s name matches with the citation.



Reply: Thanks for the reminder, we corrected it in the text.

369 report by Wang et al. who constructed a risk model according to immune-related genes
370 (30). In contrast to Li et al. who reported that the low tumor purity group was mainly
371 enriched in immune-related pathways, such as T cell, B cell, and macrophage pathways,
372 leading to higher OS (31), we found that high tumor purity suggested poor prognosis.

30. Qu Y, Cheng B, Shao N, Jia Y, Song Q, Tan B, Wang J. 2020. Prognostic value of immune-related genes in the tumor microenvironment of lung adenocarcinoma and lung squamous cell carcinoma. *Aging (Albany NY)* 12: 4757-77

31. Deng Y, Song Z, Huang L, Guo Z, Tong B, Sun M, Zhao J, Zhang H, Zhang Z, Li G. 2021. Tumor purity as a prognosis and immunotherapy relevant feature in cervical cancer. *Aging (Albany NY)* 13: 24768-85

Reply: Thanks for the reminder, we corrected it in the text.

d) Please double-check if more studies should be cited as you mentioned "studies". OR use "study" rather than "studies".

366 molecules, in LUAD, several published studies have shown that mast cells abundance
367 is correlated with prolonged survival in early-stage LUAD patients (27). In our opinion,

Reply: We have added one reference 28 at line 357, so there are finally two references, at line 305.

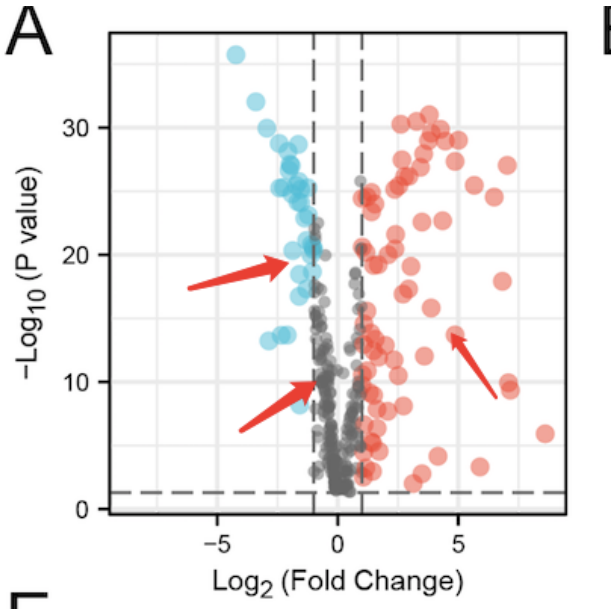
2. Please confirm if more studies should be cited here as you mentioned "previous reports".

352 memory resting were enriched in low-risk groups. Previous reports have demonstrated
353 that increased CD4+ T cells are a favorable independent prognostic factor for lung
354 cancer (26). Mast cells are a major component of the immune microenvironment and

Reply: Thanks for the reminder, we corrected it in the text.

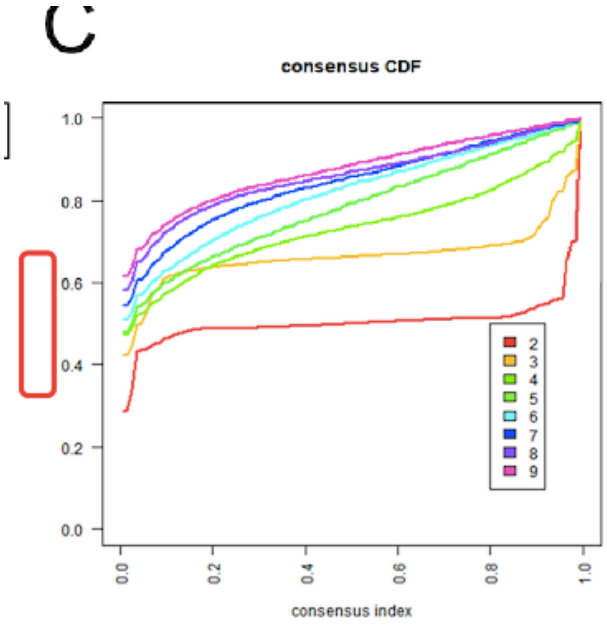
3. Figure 1

1) Please indicate the meaning of blue, red and grey dot in the figure legend (1A).



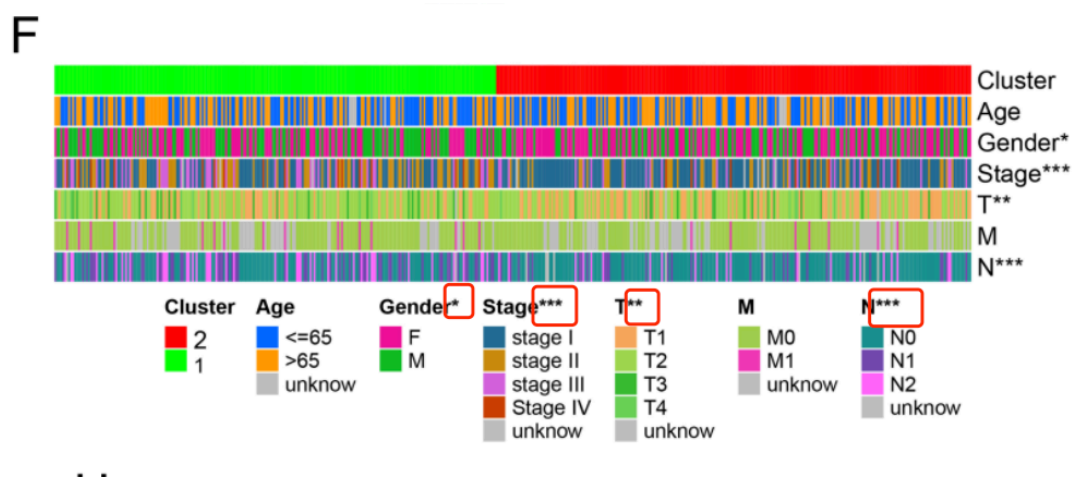
Reply: Thanks for the reminder, we corrected it in the text.

2) Please provide a description for the Y-axis in figure 1C.



Reply: Thanks for the reminder, we corrected it in the text.

3) Please explain the meaning of *, **, *** in the legend.



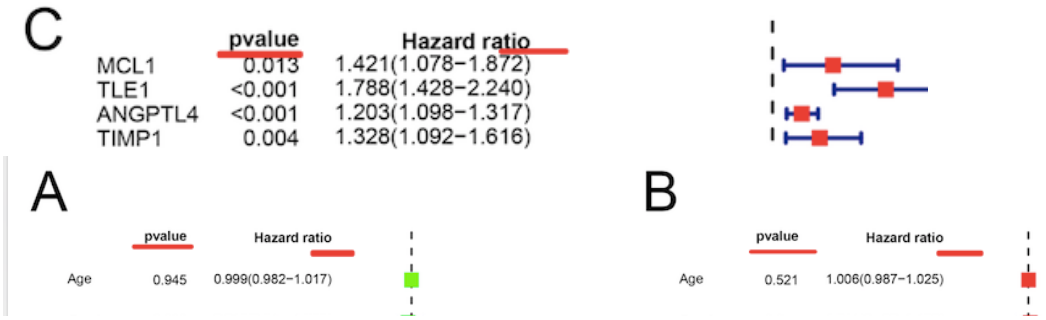
Reply: We have modified it.

4. Figure 2C and Figure 5AB

1) Please revise “Pvalue” to “P value”;

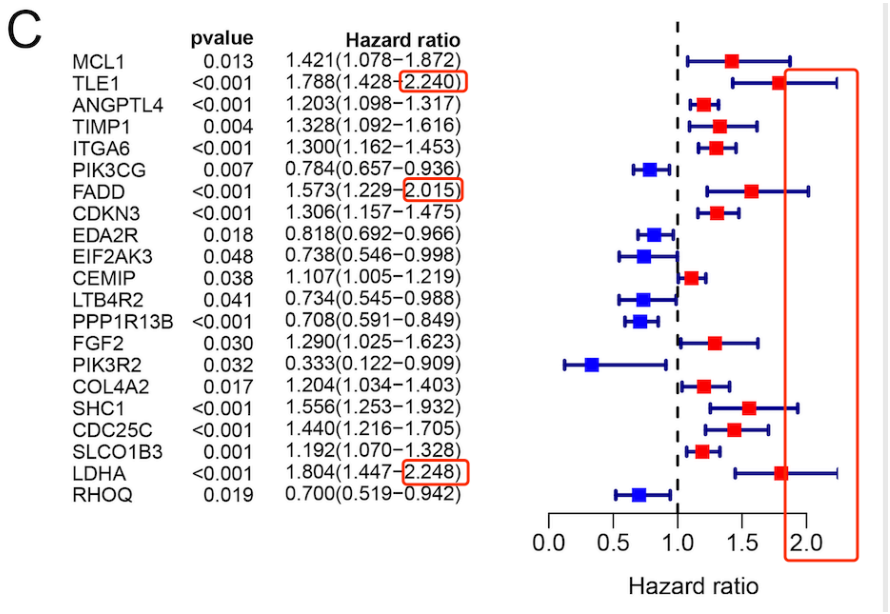
2) Please add (95% CI) after HR.

Reply: Thanks for the reminder, we corrected it in the text.



5. Figure 2

To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.



Reply: The figure is automatically generated by the code, and there is no need to modify it.

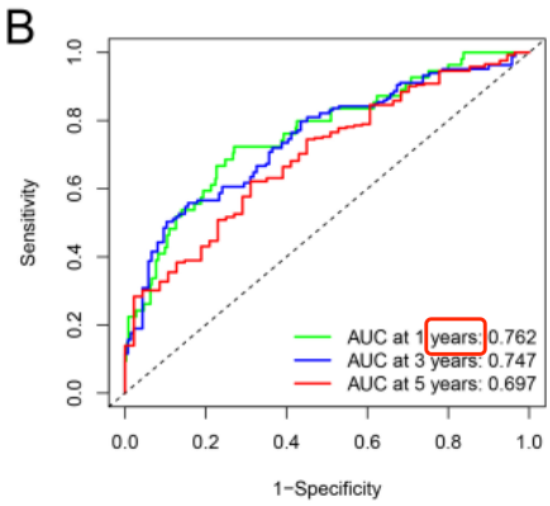
6. Figure 3

a) Please provide the full term of ROC, K-M and OS in the legend.

Reply: We have modified it.

b) Please revise “1 years” to “1 year”.

Reply: We have modified it.



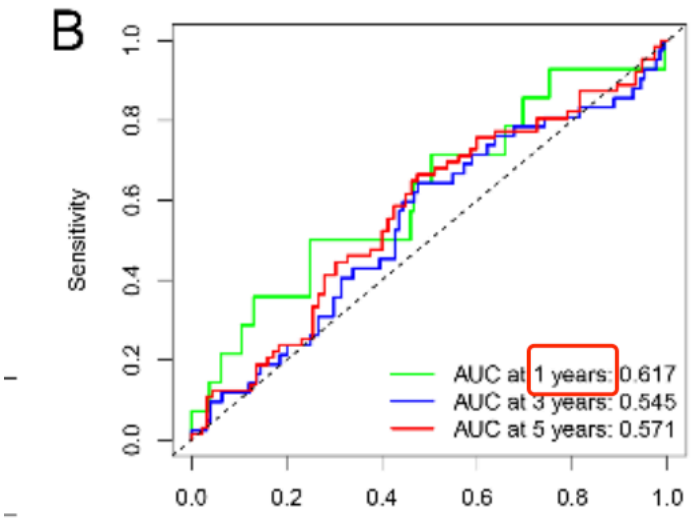
Reply: We have modified it.

7. Figure 4

a) Please explain ROC in the legend.

Reply: We have modified it.

b) Please revise “1 years” to “1 year”.

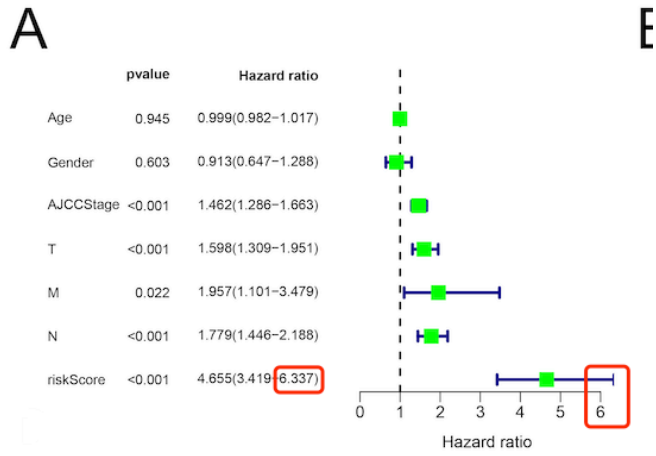


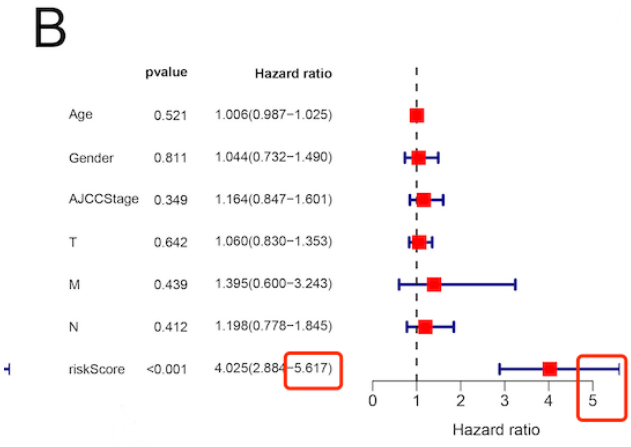
Reply: We have modified it.

8. Figure 5

a) To standardize the results, the part that exceeds the horizontal coordinates should be indicated by arrows.

Reply: The figure is automatically generated by the code, and there is no need to modify it.

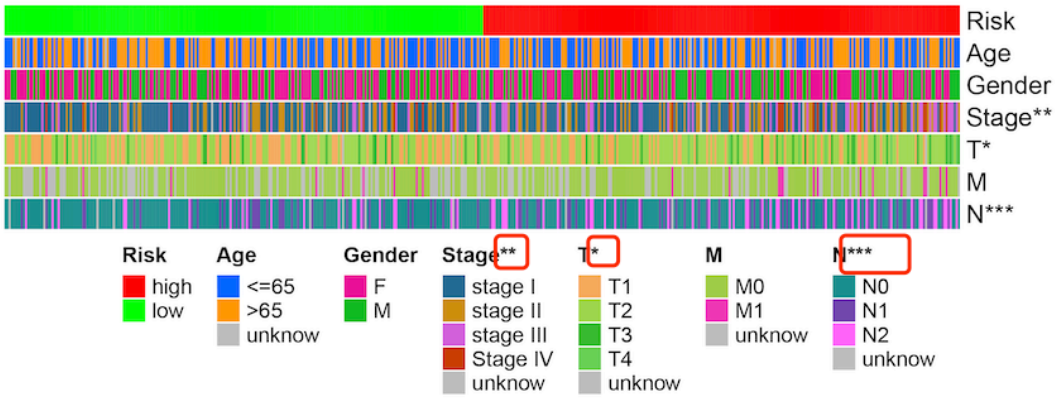




b) Please explain AUC and OS in the legend.

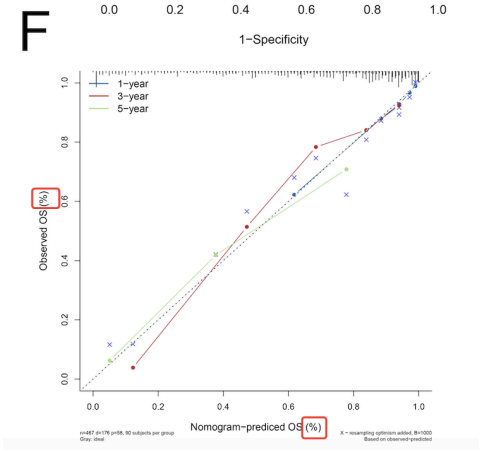
Reply: We have modified it.

c) Please explain the meaning of *, **, *** in the legend.



Reply: We have modified it.

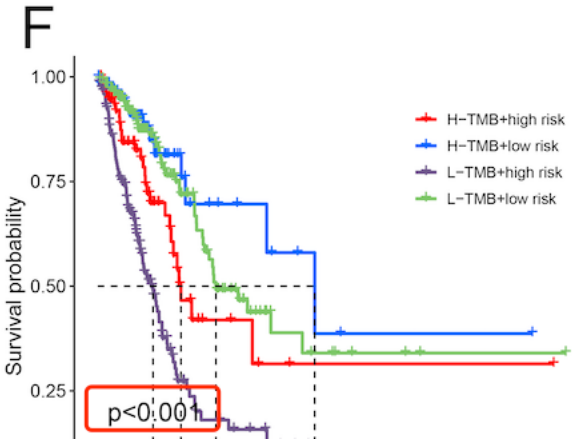
d) Figure 5F: Please remove (%) from the Y-axis and X-axis.



Reply: Thanks for the reminder, we corrected it in the text.

9. Figure 6

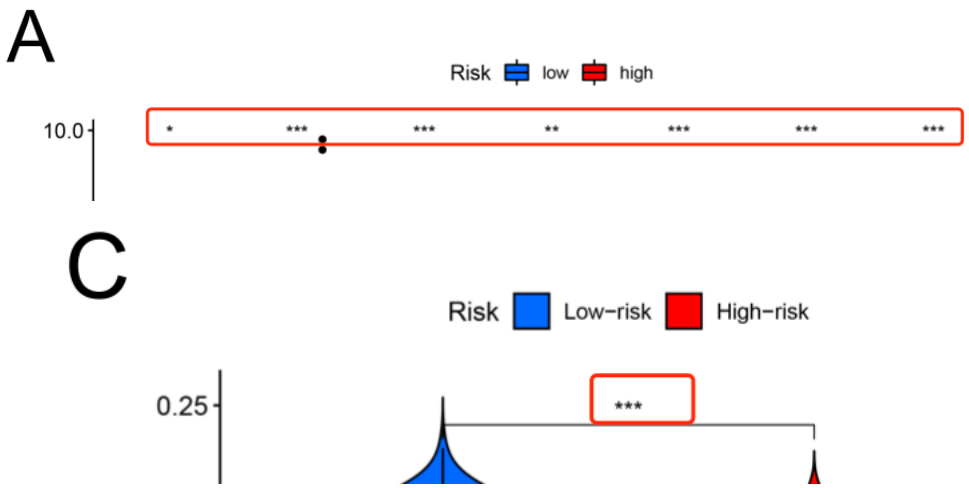
The P value was not clear in the figure, please revise.



Reply: We have modified it.

10. Figure 8

Please provide the meaning of the symbol "*, **, ***" in the legend.

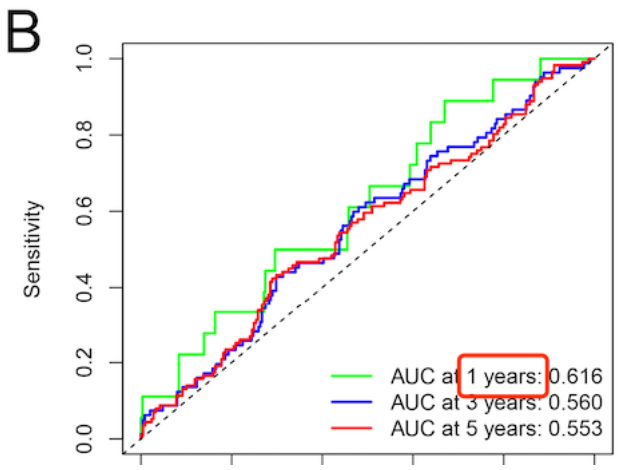


Reply: We have modified it.

11. Figure 9

a) Please explain OS, CR, PR, SD, and PD in the legend.

b) Please revise "1 years" to "1 year".



Reply: We have modified it.

13. Figure 10

Please provide the meaning of the symbol "*, ***" in the legend.

Reply: We have modified it.