Peer Review File

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Reviewer A

The paper titled "Construction of subtype classifiers and a prognostic risk model based on hypoxia-associated lncRNAs for lung adenocarcinoma" is interesting. The results developed a signature based on hypolncRNAs, contributing to the development of personalized therapy for LUAD. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The introduction did not indicate the the potential role of hypoxia-associated lncRNAs, and needs further revisions.

Reply: Thank you for your precious comments. we added the potential role of hypoxia-associated lncRNAs in introduction part.

Changes in text: page3/4, line102-105.

2) What is the relationship of hypoxia-associated lncRNAs and immune microenvironment in lung adenocarcinoma? It is recommended to add relevant content.

Reply: Thank you for your precious comments. in the last para of results, we added hypoxia-associated lncRNAs and immune microenvironment correlation analysis:

MIR31HG, LINC00857, LINC01116 were obviously correlated with immune cells (Figure S2).

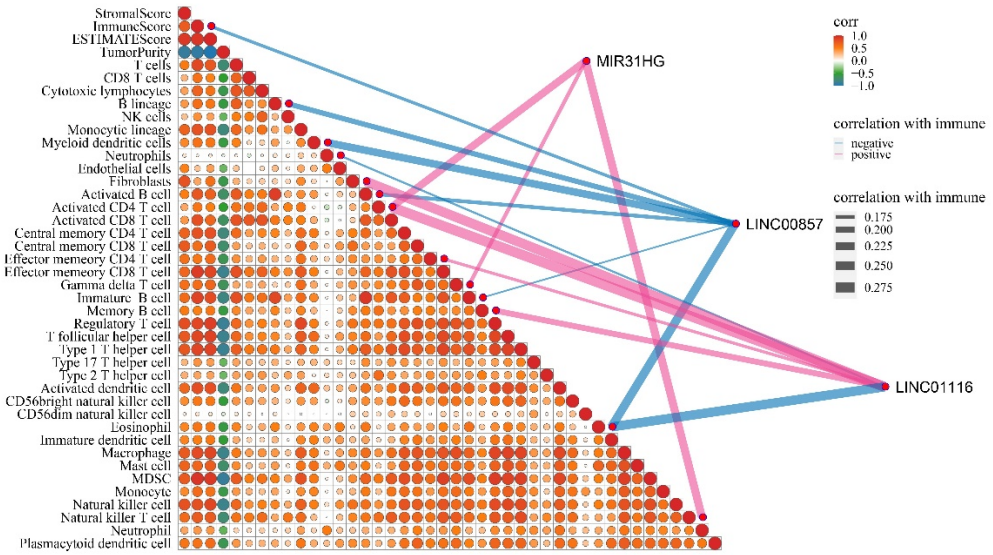


Figure S2

3) In this study, bioinformatics approaches were employed to construct the subtype classifiers. It is suggested to add further functional experiments to study its role in vivo and potential molecular mechanisms.

Reply: Thank you for your comments. Your comments are very meaningful. We will further study the function of these key genes in LUAD through basic experiments in the future, and we added this limitation in Discussion part. As showed: Finally, further functional experiments to study its role in vivo and potential molecular mechanisms are necessary.

4) All figures are not clear enough, especially Figure 3 is very blurry. It is recommended to

provide clearer figures again.

Reply: Thank you for your comments. we checked and revised figures, uploaded them again.

5) What are the roles of hypoxia-associated lncRNAs in predicting immunotherapy response? It is recommended to add relevant contents.

Response: Thank you for your comments. in the last para of results, we added immunotherapy response analysis: The low-risk score was featured by lower TIDE score in TCGA and GEO dataset in comparison to high-risk score group, suggested more suitable to immunotherapy (Figure S3).

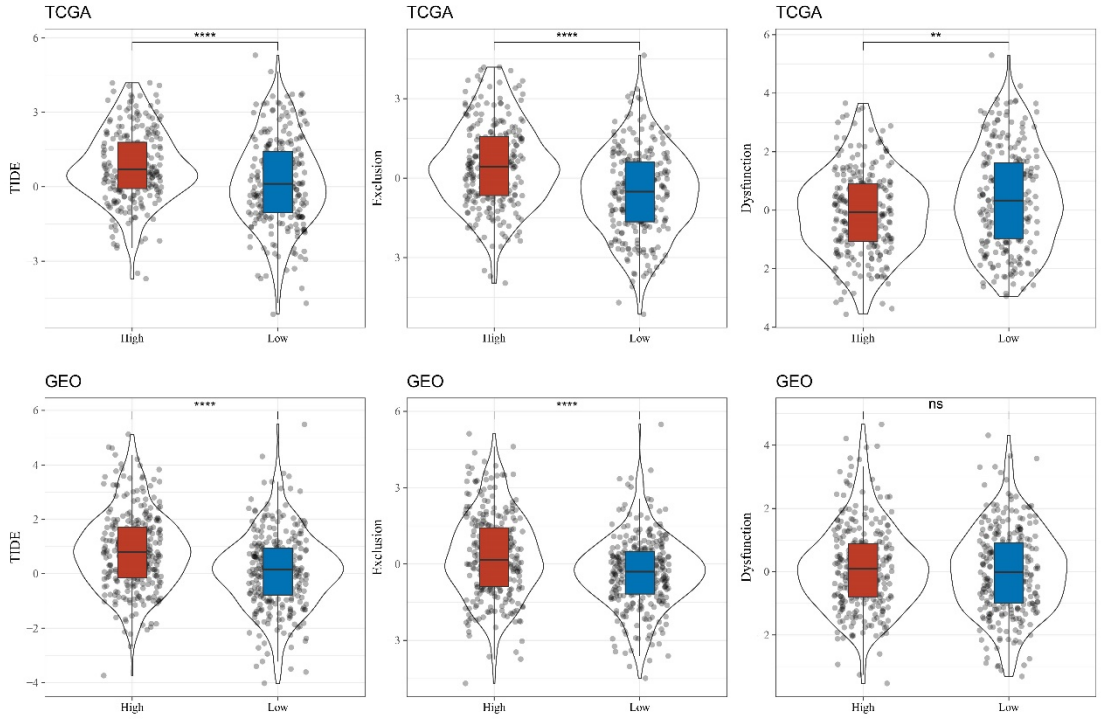


Figure S3

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Development and verification of a hypoxia- and immune-associated prognosis signature for esophageal squamous cell carcinoma, J Gastrointest Oncol, PMID: 35557566","Identification of immune-associated lncRNAs as a prognostic marker for lung adenocarcinoma, Transl Cancer Res, PMID: 35116427". It is recommended to quote the articles.

Reply: Thank you for your comments. we cited those articles in introduction part.

Changes in text: page3/4, line98-101.

7) The biological characteristics of hypoxia-associated lncRNAs and its research progress in tumors should be added to the discussion.

Reply: Thank you for your comments. we added more information about hypoxia-associated lncRNAs in tumors in discussion.

Changes in text: page11, line338-342.

8) How does lncRNA interact with other signal networks in the progression of lung adenocarcinoma? What dual role does it play in increasing/inhibiting tumor progression? It is recommended to add relevant contents.

Reply: Thank you for your comments. in discussion part, we added the possible mechanisms

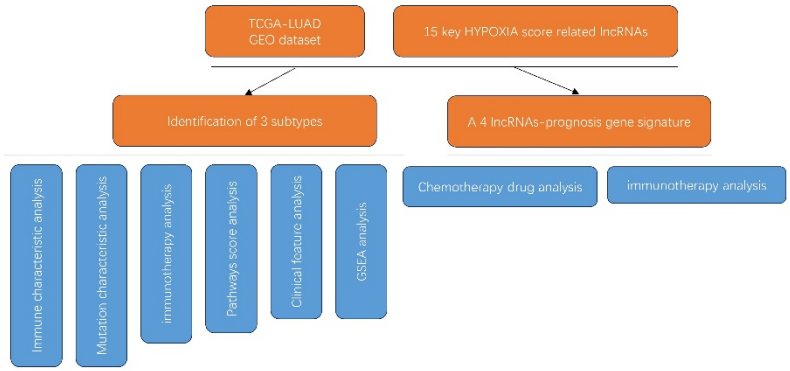
of action regulate cancer. As far as the literature is concerned, ceRNA is the most common method.

384 deterioration of HCC[39] and pancreatic cancer[40] conditions. LINC00857 has been
385 shown to recruit the serine/arginine-rich splicing factor 1 (SRSF1) to promote
386 alternative splicing (AS) of CLDN12, thereby affecting the phenotype of pancreatic
387 adenocarcinoma cells [41]. LINC01116 promotes tumor proliferation and metastasis in
388 LUAD[42]. LINC01116 was highly expressed in small cell lung cancer and promoting
389 cell invasion and migration in small cell lung cancer through ceRNA method [43]. We

Reviewer B

1) First, the title needs to indicate the development and validation of a prognosis prediction model.
   Reply: Thank you for your comments. we checked and revised the title as: Construction of subtype classifiers and validation of a prognostic risk model based on hypoxia-associated lncRNAs for lung adenocarcinoma.
   Changes in text: Page1, line3-4.
2) Second, the abstract needs some revisions. The background did not describe the knowledge gaps on the needs for prognosis prediction model in LUAD and why hypolncRNA-based model could accurately predict prognosis. The methods need to describe the clinical samples and prognosis outcomes in the databases, the generation of training and validation samples, and statistical methods for assessing the predictive accuracy. The results need to provide data to support the accuracy of the prognosis prediction model. The conclusion needs to have comments for the clinical implications of the findings.
   Reply: Thank you for your comments. in abstract part, we checked and revised the four parts according to your suggestions.
   Change in text: Page1, line39-70.
3) Third, in the introduction of the main text, the authors need to have a brief review on what has been known on the prognostic biomarkers and prognosis prediction models in LUAD, analyze their limitations and knowledge gaps, and explain why hypolncRNA-based model could accurately predict prognosis.
   Reply: Thank you for your comments. in introduction part, we added the brief review on prognostic biomarkers and prognosis prediction models in LUAD, and The potential value of lncRNA associated with hypoxia was characterized.
   Changes in text: page3/4, line105-112.
4) Fourth, in the methodology of the main text, please clearly describe the research design and procedures of this study. The authors need to describe the statistical methods for assessing the accuracy of the predictive models including how the AUC was calculated and what its threshold values for a good predictive model is.

Reply: Thank you for your comments. we added the workflow of this study in method part, as showed in figure1.



And in method, we added more information.

Reviewer C

1. The authors mentioned "studies...", while no reference was cited. Please add citations or revise this sentence.

97 outcomes of targeted therapies, including immunotherapy[9]. Recent studies have
98 reported three molecular subtypes of triple-negative breast cancer based on hypoxia-
99 related genes. Additionally, by consistent clustering of 397 hypoxia-related genes and

Reply: Thank you for your comment. After carefully checking, we decided to delete it.

2. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise.

395 while those in C2 had the least favorable survival outcome. Several studies have
396 suggested that hypoxia affects the TME in its entirety[30]. Differences in the degrees

Reply: Thank you for your comment. We added more reference in here

while those in C2 had the least favorable survival outcome. Several studies have suggested that hypoxia affects the TME in its entirety[8, 30, 31]. Differences in the

3. "representing" would be suitable, please check and revise.

70 different TMEs. We developed a signature based on hypolncRNAs, contributing to the
71 development of personalized therapy and represent a new potential therapeutic target
72 for LUAD.

Reply: Thank you for your comment. Yes, we agree it, and revised it as representing.

4. Please check all abbreviations in the abstract and the main text, such as "HCC". Abbreviated terms should be full when they first appear.

122 HCC hypoxia-associated noncoding RNAs can also classify cancer specimens. Chen et

Reply: Thank you for your comment. We added full name. Hepatocellular carcinoma (HCC).

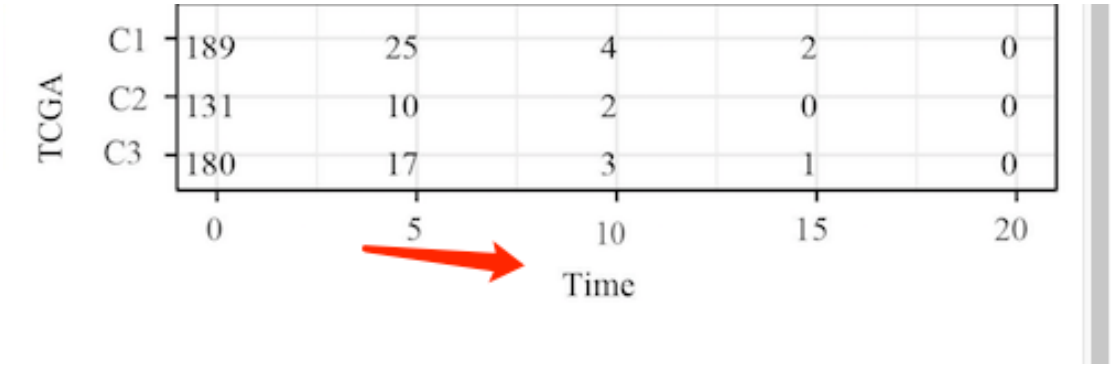
5. Please indicate in the main text the citation of Figure 6E

(Figure 6A,6B). Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells with an immunosuppressive phenotype that renders tumors resistant to immunotherapy[22]. MDSC scores differed significantly among the three subtypes classified by hypoloncRNA expression, with C1 having the lowest score and C2 having the highest score, which implied that immunotherapy was most likely to be effective in C1 relative to C2 (Figure 6C,6D). Patients with true response had prolonged survival compared with those with false response in TCGA-LUAD (Figure 5E). Among the patients in the GEO cohorts, the difference in OS rate between the two states was not significant (Figure 6F).

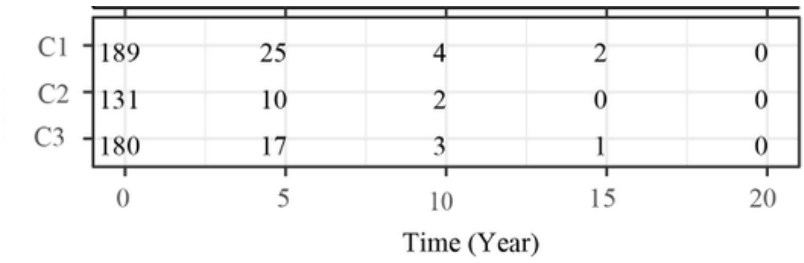
Reply: Thank you for your comment. The 5E should be 6E, and we revised it. Thank you.

6. Figure 2E, 2F

Please provide the unit.



Reply: Thank you for your comment. It is year, and we added it



7. Figure 2

*, **, ***, ****: please explain their meaning in the legend

Reply: Thank you for your comment. We added it.

8. Figure 3

*, **, ***, ****, ns: please explain their meaning in the legend

Reply: Thank you for your comment. We added it.

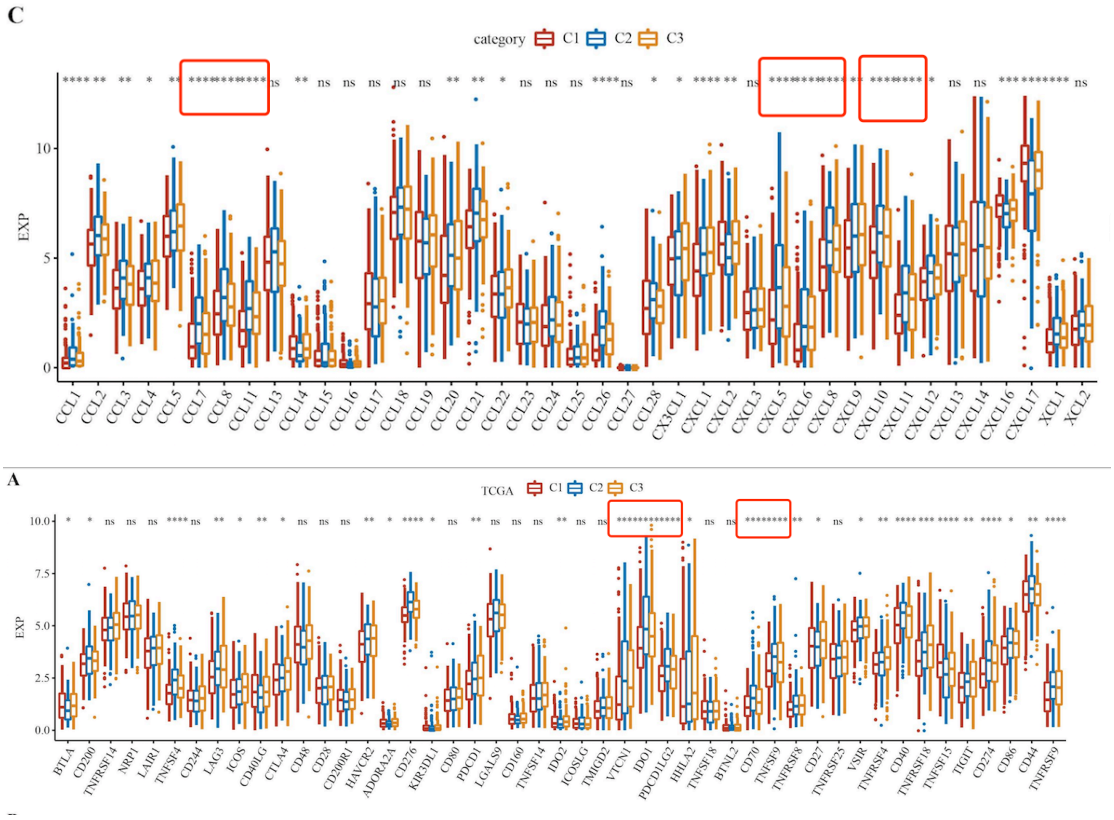
9. Figure 4

*, **, ***, ****, ns: please explain their meaning in the legend

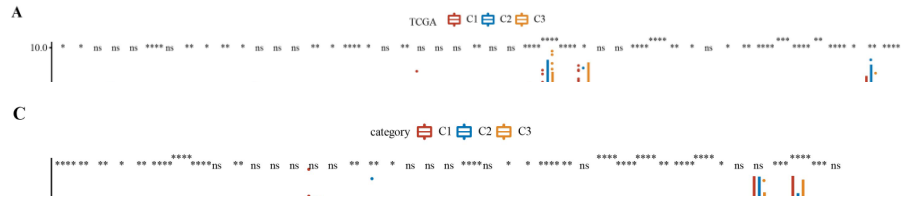
Reply: Thank you for your comment. We added it.

10. Figure 4A & Figure 4C

The special symbols are so close and unclear. Please check and revise.

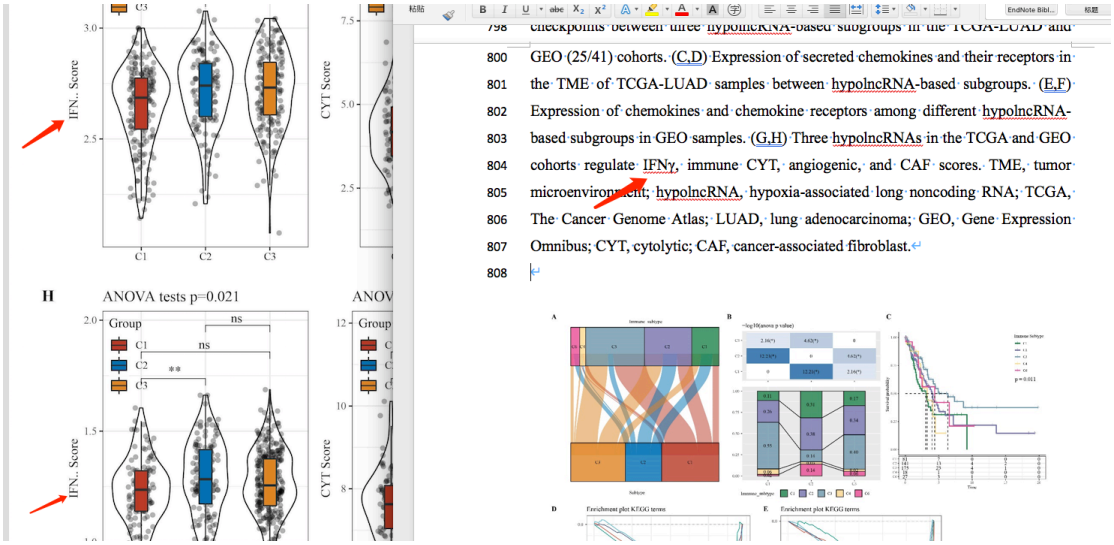


Reply: Thank you for your comment. We revised it



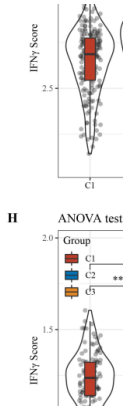
11. Figure 4G & Figure 4H

IFNγ or IFN? Which one is correct? Please check and revise.



checkpoints between three hypolncRNA-based subgroups in the TCGA-LUAD and
GEO (25/41) cohorts. (C,D) Expression of secreted chemokines and their receptors in the TME of TCGA-LUAD samples between hypolncRNA-based subgroups. (E,F) Expression of chemokines and chemokine receptors among different hypolncRNA-based subgroups in GEO samples. (G,H) Three hypolncRNAs in the TCGA and GEO cohorts regulate IFNγ, immune CYT, angiogenic, and CAF scores. TME, tumor microenvironment; hypolncRNA, hypoxia-associated long noncoding RNA; TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; GEO, Gene Expression Omnibus; CYT, cytolytic; CAF, cancer-associated fibroblast.

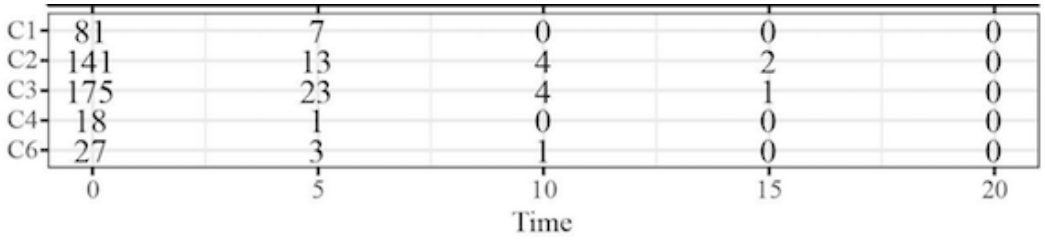
Reply: Thank you for your comment. It is IFNγ, and we revised it in figure.



12. Figure 5C

Please provide the unit.

Reply: Thank you for your comment. It is year, and we added it



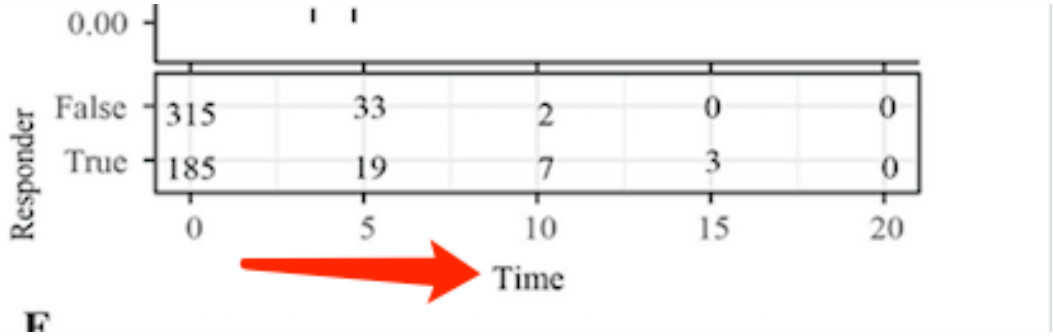
13. Figure 5B

*: please explain its meaning in the legend

Reply: Thank you for your comment. We added it.

14. Figure 6E & Figure 6F

Please provide the unit.



Reply: Thank you for your comment. It is year, and we added it.

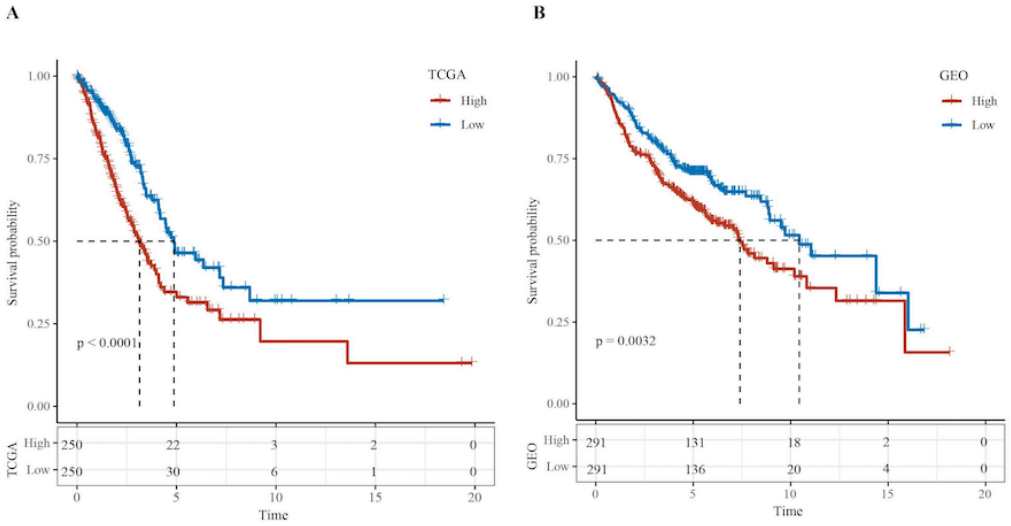
15. Figure 6

, *, ****, ns: please explain their meaning in the legend

Reply: Thank you for your comment. We added it.

16. Figure 7A & Figure 7B

Please provide the unit.



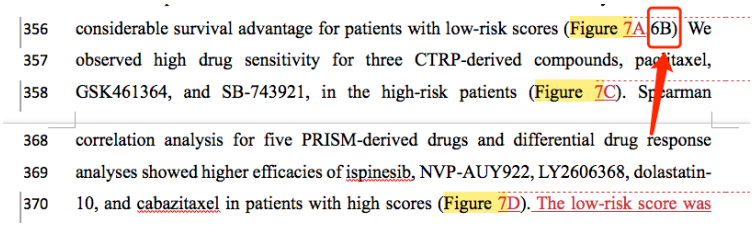
Reply: Thank you for your comment. It is year, and we added it.

17. Figure 7

***: please explain its meaning in the legend

Reply: Thank you for your comment. We added it.

18. Please check if "6B" should be "7B", as figure 7B was not cited in the main text.



Reply: Thank you. Yes, and we revised it

19. Figure S1

Two descriptions for “GEO”. Please delete one.

647 Figure S1 Concurrent presence of hypoxia pathway-derived lncRNAs in TCGA-LUAD
648 and GEO specimens. (A) Venn diagram showing the intersection of hypolncRNAs in
649 TCGA-LUAD and GEO cohorts; (B) GSEA results of hypolncRNAs in the hypoxia
650 pathways in TCGA. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma;
651 GEO, Gene Expression Omnibus; hypolncRNA, hypoxia-associated long noncoding
652 RNA; GEO, Gene Expression Omnibus; GSEA, Gene Set Enrichment Analysis.

Reply: Thank you for your comment. We deleted the one.

20. Figure S3

, **, ns: please explain their meaning in the legend

Reply: Thank you for your comment. We added it.