Peer Review File

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Reviewer A

Comment 1: There seems to be an error in the labeling of Figure 7. Figure 7 has three panels labeled DAPI, Merged and P-AKT. It seems that the Merge and P-AKT labels are transposed. The red panel should be the P-AKT and the "purple" panel should be the merged images.
Reply 1: Thank you for your reminding. We've changed the labeling of Figure 7.
Changes in the text: Replacement of "Figure 7" with "Figure 7-revised".

Comment 2: Figure 8 is unclear. The graphs purport to show evidence that high expression of is associated with responsiveness to certain drugs. However, the y-axis is undefine. What do the numbers on the Y-axis means? What are the units?
Reply 2: Thank you for your kind reminder. IC50 represents the concentration at which a substance exerts half of its maximal inhibitory effect. In pharmacology, it is an important measure of potency for a given agent. Figure 8 shows Violin plots of the top 9 drugs with a significant difference in sensitivity between the high- and low-DNASE1L3 groups of ccRCC samples. The therapeutic target list for RCC was downloaded from a genomics database (1). The correlations between DNASE1L3 expression and drug therapeutic responses were further investigated and plotted with the oncoPredict R package (2). The Y-axis represents the drug sensitivity score, with a lower score indicating that the reorganization is more sensitive to the drug. The Y-axis is expressed as a numerical value with no units.
(1) Yang W, Soares J, Greninger P, et al. Genomics of Drug Sensitivity in Cancer (GDSC): a resource for therapeutic biomarker discovery in cancer cells. Nucleic Acids Res 2013;41:D955-61.
(2) Maeser D, Gruener RF, Huang RS. oncoPredict: an R package for predicting in vivo or cancer patient drug response and biomarkers from cell line screening data. Brief Bioinform 2021;22:bbab260.
Changes in the text: N/A

Comment 3: In order to truly assert that DNASE1L3 expression is associated with drug sensitivity, ROC analysis should be performed to determine the predictive strength of DNASE1L3 in relationship to therapy.
Reply 3: Many thanks for the valuable comments. In this study, our primary objective was to investigate the potential correlation between DNASE1L3 expression levels and clinical drugs. As suggested by the reviewer, it would indeed be essential to further assess the predictive strength of DNASE1L3 in relation to therapy using ROC curves. However, due to the constraints of our database-based clinical drug information, we were limited to integrating the expression levels of DNASE1L3 solely based on the TCGA database. As a result, we regrettably could not explore the effect of DNASE1L3

on the patients' survival rates in each drug category, precluding the generation of ROC curves in this context. Nevertheless, we are grateful for the reviewer's insightful comments, which have provided us with a promising research avenue.
Changes in the text: N/A

Reviewer B

Comment 1: First of all, biomarkers could be diagnostic and prognostic biomarkers but the authors did not clearly indicated this in the title and elsewhere of this paper. The title also did not indicate the research design of this study such as a bioinformatics analysis.
Reply 1: Thanks for the question. In Figure-1, our raw letter analysis using databases as well as some of our basic experiments demonstrated that the DNASE1L3 levels in the normal group were higher than those in the tumor group. This indicates that decreased DNASE1L3 has some value for the diagnosis of ccRCC. In Figure 2-3, we analyzed the correlations among DNASE1L3 expression and clinical characteristics in ccRCC patients. DNASE1L3 expression was found to be significantly positively related to patient prognosis. Meanwhile, DNASE1L3 expression was significantly related to tumor dimension, tumor weight, clinical stage and histological grade. In addition, we constructed a nomogram that integrated five independent significant clinicopathological factors (Figure 3A). The calibration curves of 1-, 2- and 3-year survival probabilities and the predictive accuracy of the nomogram indicated that the nomogram was in optimal agreement with an ideal model (Figure 3B,3C). Together, these results suggest that DNASE1L3 has certain value as a diagnostic and predictive biomarker for ccRCC. Since our study had both bioinformatics analysis and cellular experiments, this study is titled a “Integrative analysis” instead of a separate “Bioinformatics analyses”.
Changes in the text: N/A

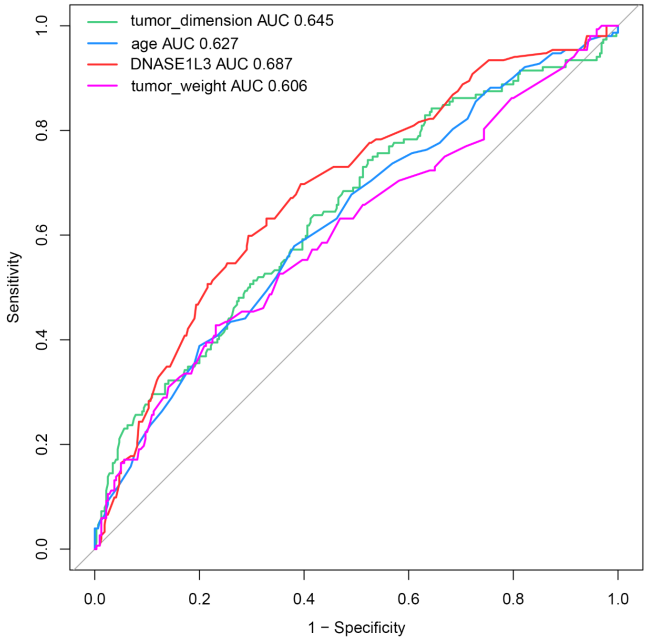
Comment 2: Second, the abstract needs further revisions. The background did not indicate the significance of this research focus and why there is a need to focus on DNASE1L3. The methods did not describe the clinical variables and prognosis outcomes in the databases used, as well as more details for analyzing the “role and potential mechanism”. The results did not quantify the findings by reporting expression levels, HR values, accurate P values, and other important statistics. The conclusion is overstated since there is no external validation in real-world clinical samples. Please also have a few comments for the limitations of this study.
Reply 2: Thank you for your kind reminder. We have undertaken revisions to the Abstract section to provide further clarity regarding our rationale for focusing on DNASE1L3. Additionally, in the methods section, we have incorporated the clinical variable, specifically whether the tissue was RCC (renal cell carcinoma). Furthermore, in the results section, we have included corresponding expression values along with their respective p-values. Although we acknowledge the absence of clinical samples for validation, it is crucial to highlight that our study incorporates a comprehensive

approach encompassing both bioinformatics and basic experimental validation. As such, it is appropriate to suggest that DNASE1L3 may be a potential biomarker. To ensure comprehensive and transparent reporting, we have also added appropriate limitations at the end of the article. By making these modifications, we aspire to enhance the scientific rigor and clarity of our study, thus contributing to a better understanding of the potential significance of DNASE1L3 in the context of RCC.
Changes in the text: Abstract (page 1-2, line 26-57) and limitation (page 13, line 384-389).

Comment 3: Third, the introduction of the main text did not have an overview regarding known diagnostic and prognostic biomarkers of ccRCC, did not have comments on the limitations and knowledge gaps, and did not explain why the DNASE1L3 is potentially important for the diagnosis and treatment of ccRCC.
Reply 3: Many thanks to the reviewer for the reminder. In the introduction section, we have succinctly outlined known diagnostic and prognostic biomarkers for ccRCC. The limitations and knowledge gaps of this research are also discussed in the final section of the discussion. Our research shows that the expression level of DNASE1L3 was significantly down-regulated in ccRCC compared to normal tissue, and the DNASE1L3 expression level was noticeably correlated with the severity of ccRCC patient. The survival analysis revealed that DNASE1L3 was the independent predictor of overall survival of ccRCC patients. In addition, the functional experiment showed that DNASE1L3 overexpression inhibited the proliferation and invasion of RCC cells. Finally, the immune infiltration analysis and the response to drug therapy analysis suggested that the expression of DNASE1L3 was significantly correlated with the tumor immune microenvironment and drug sensitivity in ccRCC. Therefore, we speculated that the DNASE1L3 is potentially important for the diagnosis and treatment of ccRCC.
Changes in the text: Introduction (page 4, line 86-90) and limitation (page 13, line 384-389).

Comment 4: Fourth, in the methodology of the main text, the authors needs to indicate the research design, procedures and the questions to be tested by them, and clinical and prognosis outcomes in the databases. Because of the focuses of the diagnostic accuracy and prognosis prediction accuracy of DNASE1L3, the authors need to examine DNASE1L3's accuracy alone, not the combination of DNASE1L3 with other clinical factors. Without these results, the authors cannot conclude that DNASE1L3 is a potentially useful diagnostic and prognostic biomarkers. This is my major concern regarding the statistical methods of this study. Please also ensure $P<0.05$ is two-sided.
Reply 4: Thank you for the invaluable feedback. Our investigation into the role of DNASE1L3 in kidney cancer builds upon our team's preliminary findings in liver cancer [1], which confirmed its potential to impede the progression of hepatocellular carcinoma. Encouraged by these results, we proceeded to explore DNASE1L3 in the context of kidney cancer. In the preliminary study, we download the TCGA database to obtain crucial clinical information of patients with kidney cancer. In this initial

exploration, we conducted DNASE1L3 survival analysis, revealing a significant correlation between DNASE1L3 expression and the survival probabilities of ccRCC patients ($P<0.05$). However, in light of your insightful comments, we recognized the importance of considering the factor of survival time in our analysis. Consequently, we performed ROC analysis based on the survival status of patients, as shown in the following figure. Notably, DNASE1L3 exhibited the highest AUC curve among these clinical parameters. Although the AUC value was less than 0.8, indicating an average predictive effect, it is essential to acknowledge potential outliers of DNASE1L3 in some tumor samples within the TCGA data, which might have influenced the AUC value. We anticipate that employing the STEP model in pROC package in R for further optimization may obtain a higher AUC value for DNASE1L3. But, our current data convincingly demonstrates the superiority of DNASE1L3 compared to other clinical parameters, suggesting its potential as a diagnostic biomarker. Building upon your recommendations, our next study involves conducting a retrospective study encompassing patients included in the hospital's renal cancer clinical database. Through this comprehensive approach, we aim to further validate the clinical significance of DNASE1L3. We sincerely appreciate your valuable insights and look forward to sharing the results of this ongoing research. Please stay tuned for updates on our progress.



[1] Xiao Y, Yang K, Liu P, Ma D, Lei P, Liu Q. Deoxyribonuclease 1-like 3 Inhibits Hepatocellular Carcinoma Progression by Inducing Apoptosis and Reprogramming Glucose Metabolism. Int J Biol Sci. 2022 Jan 1;18(1):82-95. doi: 10.7150/ijbs.57919. PMID: 34975319; PMCID: PMC8692146.

Changes in the text: N/A

Comment 5: Finally, some potentially important papers are ignored by the authors but I suggest the authors to briefly review and cite them accordingly: 1. Shao Y, Wu B, Yang Z, Liu Z, Ma Y, Huang H, Liu Y, Wang Z, Hu W, Wang Y, Niu Y. ALDOB represents a potential prognostic biomarker for patients with clear cell renal cell carcinoma. Transl Androl Urol 2023;12(4):549-571. doi: 10.21037/tau-22-743. 2. Zhang Z, Guan B, Li Y, He Q, Li X, Zhou L. Increased phosphorylated CREB1 protein correlates with poor prognosis in clear cell renal cell carcinoma. Transl Androl Urol 2021;10(8):3348-3357. doi: 10.21037/tau-21-371. 3. Chen J, Ye Z, Liu L, Xuan B. Assessment of the prognostic value of SPOCK1 in clear cell renal cell carcinoma: a bioinformatics analysis. Transl Androl Urol 2022;11(4):509-518. doi: 10.21037/tau-22-161.

Reply 5: Many thanks for reminding. In the introduction section, we have cited these important papers.

Changes in the text: Introduction (page 4, line 86-90).

Reviewer C

1. Please define below abbreviations in Abstract.

compared with control group (TCGA: 7.98 vs. 10.87, P<0.001). Meanwhile, DNASE1L3 expression correlated with the clinical characteristics of patients. Patients with low DNASE1L3 expression had worse survival (P<0.001) and larger (r=-0.32, P<0.001) and heavier tumors (r=-0.17, P<0.001). DNASE1L3 overexpression inhibited the proliferation (786O: 0.135±0.014 vs. 0.322±0.027, P<0.001) and invasion (786O: 1479±134 vs. 832±67, P<0.05) of RCC cells. The expression of DNASE1L3 was significantly correlated with the tumor immune microenvironment and drug sensitivity in ccRCC. Moreover, the level of the key PI3K/AKT signaling pathway protein P-AKT

Reply 3: Thanks so much for the heads up. We've defined the abbreviations in Abstract.

2. Please define AKT in Introduction section, please note that you should also ensure all abbreviations are defined when they first appear in the main text.

treatment of metastatic RCC, thus providing new therapeutic opportunities for patients.

The phosphatidylinositol 3 kinase (PI3K)/AKT pathway is a highly conserved and

Reply 4: Thanks so much for the heads up. We've defined the abbreviations in Introduction.

3. For a study involving **human gene pool**, a statement that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013) is needed, according to our journal policy.

Please confirm and indicate in your manuscript (in both Methods section and Ethical Statement in Footnote) that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013), available at: https://www.wma.net/wp-content/uploads/2016/11/DoH-Oct2013-JAMA.pdf.

- *Suggested wording: "**The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).***

Reply 5: Thanks so much for the heads up. We've indicated in the manuscript that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013) in both Methods section and Ethical Statement in Footnote.

4. There are two references lists included in your paper, please check and just keep the final version in your manuscript.

Reply 6: Thank you for the heads up. We've made changes.

5. You've mentioned "studies", while only one reference was cited in the below sentences. Please check. (You could either choose to revise them to "study" or to give **more than one reference** in those sentences. In the latter case, please keep the citations consecutively in text.)

107 end-motif frequencies (15). Studies have shown that genetic alterations of
108 DNASLE1L3 resulting in lower endonuclease activity are linked to systemic rheumatic
109 diseases (16). The absence of DNASLE1L3 in serum results in a series of autoimmune

364 Many studies have revealed that tumor-infiltrating immune cells (TICs), one of the most
365 important components in the tumor environment, are potential biomarkers (33). High

Reply 7: Thank you for the heads up. We've revised them to "study".

6. Figure 1

a. They are not included in the figure, please check and revise the legends.

540 to determine the statistical significance of differences between two groups. *P<0.05,
541 **P<0.01, and ***P<0.001 vs. the normal group.

Reply 8a: Thank you for reminding. We have made changes.

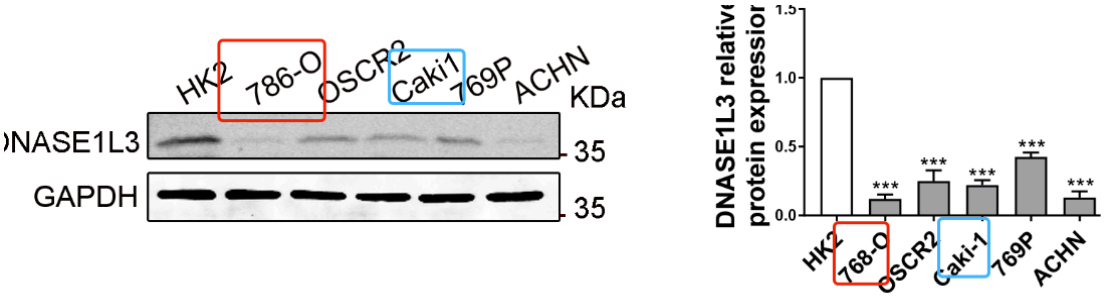
b. And Figure 1E was from HPA database, please kindly provide the **websites that directly link to the figures** in figure legends, according to HPA policy.

Reply 8b: Thank you for reminding. We have provided the websites that directly link to the figures.

c. Please indicate the scale bar (with the numbers) in Figure 1E.

Reply 8c: Thank you for reminding. We have provided the scale bar.

d. “786-O” or “768-O”? “Caki-1” or “Caki1”? Please check and unify them in the figure.

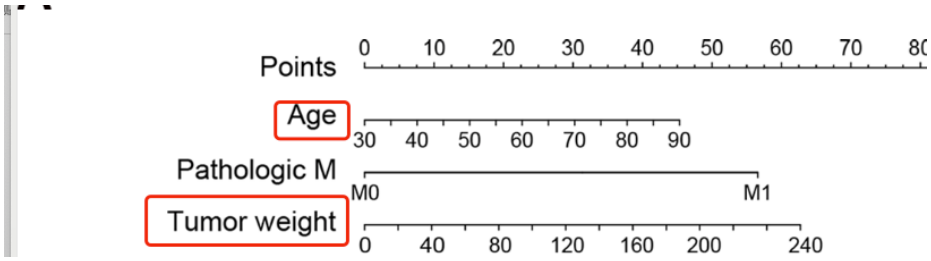


Reply 8d: Thank you for reminding. we have made changes. 786-O and Caki-1 are right.

7. Figure 3

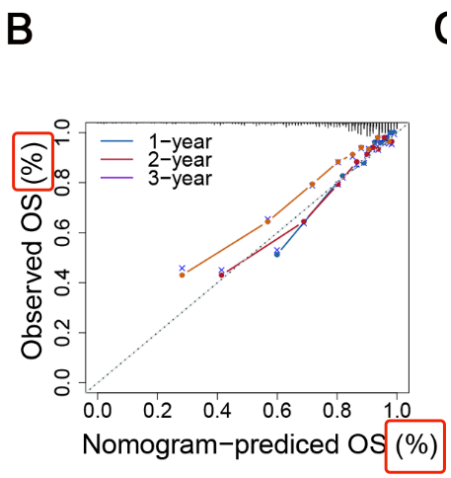
a. Figure 3A: Please also provide the units.

Reply 9a: Thank you for reminding. We have made changes.



b. Figure 3B: Please remove “(%)” from the figure and resend us updated one.

Reply 9b: Thank you for reminding. We have made changes.



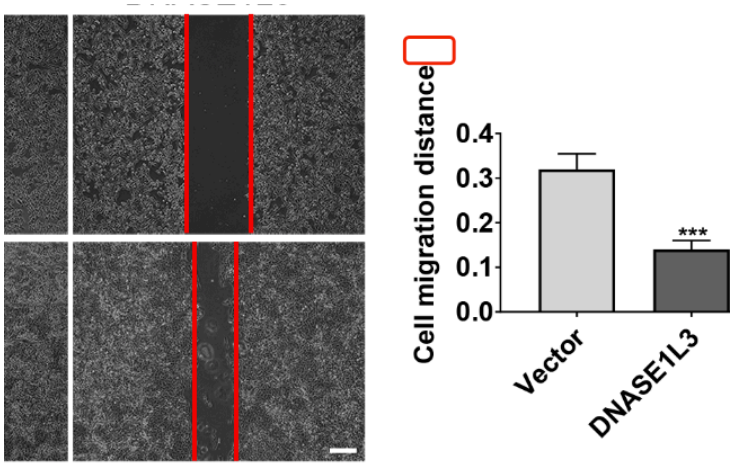
8. Figure 4

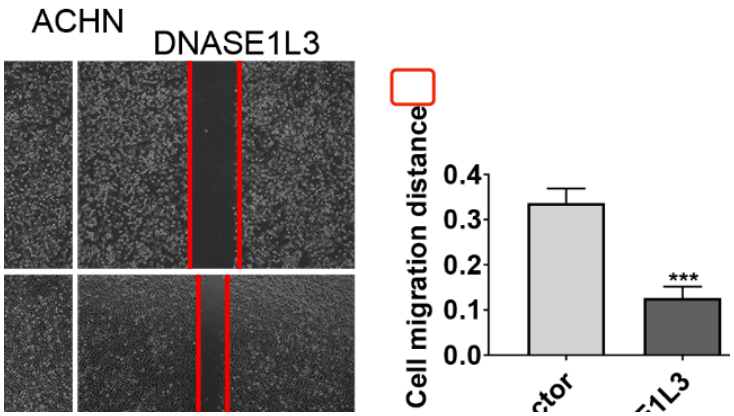
a. “**” was not shown in your figure, please check and revise the legends.

563 different treatments. Scale bar: 100 μm. Data are shown as the mean ± SE values from
564 three independent experiments. *P<0.05, ** P<0.01, and ***P<0.001 vs. the vector

Reply 10a: Thank you for reminding. We have made changes.

b. Check if units are missing in the Y-axis since it indicates the distance.
Reply 10b: Thank you for reminding. We have added units.

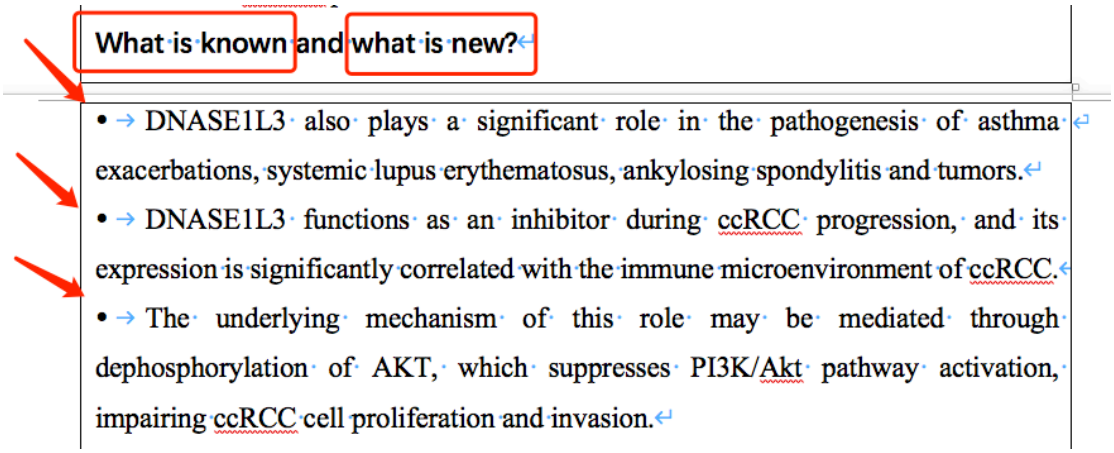




c. Please also provide the staining methods of Figure 4E-4F in figure legends.
Reply 10c: Thank you for reminding. We have added staining methods in Figure 4 legends.

9. Figure 6: They are not shown in the figure, please check and revise the legends.
577 between the high- and low-DNASE1L3 groups; (C) correlations between DNASE1L3
578 expression and the expression levels of the top 20 significant immune genes and
579 enrichment scores of the predicted immune-related pathways. *P<0.05, **P<0.01, and
Reply 11: Thank you for reminding. We have made changes.

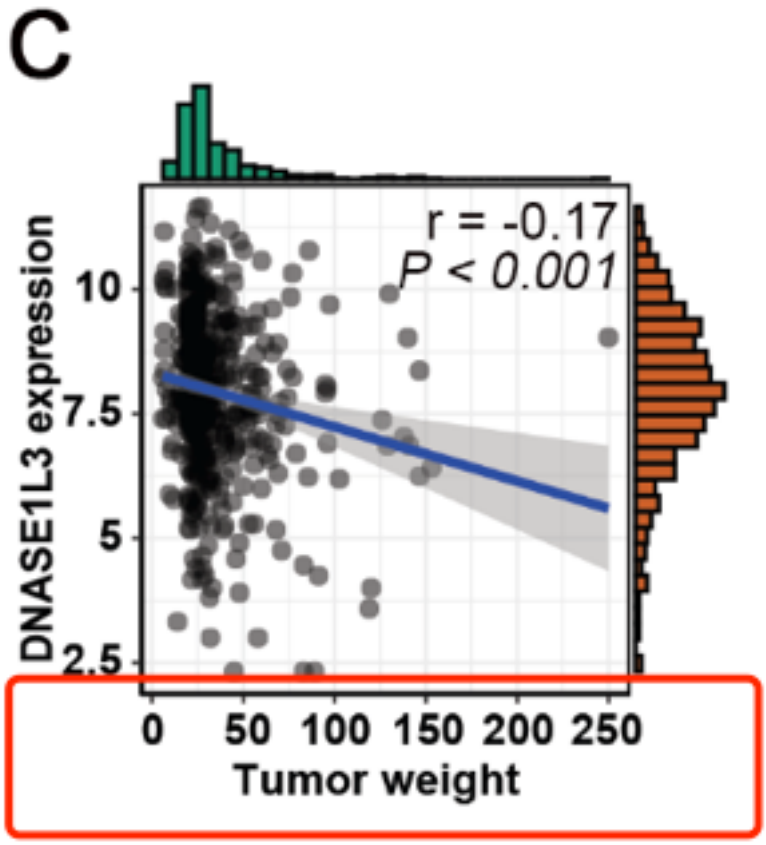
10. Highlight box
Only two points are needed. One for "What is known" and the other for "what is new".
Please revise.



Reply 3: Thank you very much. We have deleted the first point.

11. Figure 2C

Please check whether the data on the x-axis have units.



Reply 4: Thank you very much. We have added the units.

12. Figure 6

It seems that “NS” was not presented in Figure 6 while the authors provided its explanation. Please revise.

Reply 5: Thank you very much. We have deleted the NS explanation.