Peer Review File

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Reviewer A

Comment 1) Based on this study, it is recommended to increase the identification of RMlncRNAs with clear prognostic value, and to further evaluate the association between prognostic model and tumor-infiltrating immune cells.
Reply 1: Dear reviewer, thank you for your positive comments and valuable suggestions. This study finally screened out six RMlncRNAs through differential expression analysis, univariate cox, LASSO and multivariate cox analysis screening steps, and constructed a prognostic model based on them. The constructed prognostic model proved its good prognostic value through ROC analysis, and it was also proved in the validation set. This shows that all six RMlncRNAs have good prognostic value. In addition, we obtained the differential immune cells between the disease group and the control group through immune infiltration analysis, and then performed correlation analysis on the six RMlncRNAs and differential immune cells. The specific methods and results have been supplemented in page 11, lines 230-236 and page 15, lines 310-317.
Changes in the text:
Page 11, lines 230-236: In addition, the Cibersort algorithm was employed to perform immune infiltration analysis on the lncRNA profiles from the TCGA database, comparing disease groups (524 samples) with control groups (59 samples) to identify differential immune cells. Perform correlation analysis between differentially expressed immune cells and RMlncRNAs. The marker genes for 22 types of immune cells were obtained from a designated reference (Table S2).
Page 15, lines 310-317: In addition, the expression results of the 22 types of immune cells in both the tumor and control groups revealed that B cells memory and Plasma cells were highly expressed in the tumor group (Figure S2A-B). Furthermore, with the exception of T cells CD4 naive, the expressions of the other 21 types of immune cells exhibited significant differences ($P<0.05$) between the tumor and control groups (Figure S2C). The correlation results between six RMlncRNAs (NFYC_AS1, OGFRP1, MIR4435_2HG, TDRKH_AS1, DANCR, TMPO_AS1) and the 21 types of immune cells are presented in Figure S2D.

Comment 2) The words in all the figures are very unclear, please adjust the figures and upload them again.
Reply 2: Dear reviewer, thank you for your reminder. We have adjusted the figures according to the requirements of the journal. We have adjusted the font size and increased the resolution of the figures. We hope that our modifications can improve the readability of the article.
Changes in the text: See updated figures for specific changes.

Comment 3) There have been many studies on LUAD. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 3: Dear reviewer, thank you for pointing this out. According to your suggestions, we have added the relevant background of lung adenocarcinoma (LUAD) research in page 4-5, lines 86-95 of the introduction and the innovativeness of the research in page 7-8, lines 152-163. We hope that our revisions can improve the readability of the article. Thank you again for your valuable suggestions.

Changes in the text:

Page 4-5, lines 86-95: Studies have shown that KLF5 can promote cell proliferation and migration of LUAD by upregulating STK24. STK24 may also be involved in the immune regulation process of LUAD (3). A novel circular RNA (circRNA) (circ_0004140) derived from the oncogene YAP1 is upregulated in LUAD. High expression of circ_0004140 is related to poor prognosis and CTL cell dysfunction in LUAD patients. Knocking down circ_0004140 can regulate the proliferation, migration and apoptosis of LUAD cells. Moreover, increased expression of circ_0004140 is associated with cytotoxic lymphocyte depletion, while C-021 (CCL22/CCR4 axis inhibitor) combined with anti-PD-1 therapy can attenuate the development of LUAD and immune resistance (4).

Page 7-8, lines 152-163: This study innovatively focused on lncRNA related to RNA methylation modification, screened and explored the expression of RMlncRNA in LUAD and its prognostic value, providing a new perspective for understanding the function of lncRNA in LUAD, especially its association with RNA methylation, an important epigenetic modification mechanism. Through bioinformatics analysis, a prognostic model of lncRNA was successfully constructed, providing a new tool for the prognosis assessment of LUAD patients. In addition, the study also deeply analyzed the correlation between the model and TMB, MSI, immune infiltration analysis, immunotherapy response, and conducted drug sensitivity analysis, comparing the half-inhibitory concentration of multiple drugs between different risk groups, providing an important basis for the personalized treatment of LUAD.

Comment 4) What are the expression patterns, biological functions, and diagnostic value of RMlncRNAs in LUAD? It is recommended to add relevant content.

Reply 4: Dear reviewer, thank you for your valuable suggestions. We have supplemented the relevant background of RMlncRNAs research in cancer in page18-19, lines 384-398 of the Discussion section according to your suggestions.

Changes in the text:

Page18-19, lines 384-398: MIR4435-2HG promotes tumorigenesis by participating in six signaling pathways, including TGF-β, Wnt/β-catenin, MDM2/p53, PI3K/AKT, Hippo, and MAPK/ERK signaling pathways. It can competitively bind to a variety of miRNAs to form a complex ceRNA network, thereby regulating the expression of downstream target genes. High expression of MIR4435-2HG is closely related to the clinical pathological characteristics and poor prognosis of various tumors, and high expression in peripheral blood or serum is valuable for predicting the risk of various tumors. In addition, MIR4435-2HG is also involved in the mechanism of action of three anticancer drugs (resveratrol for lung

cancer, cisplatin for non-small cell lung cancer and colon cancer, and carboplatin for triple-negative breast cancer) (46).
As a lncRNA, MIR4435-2HG is also involved in the molecular cascade of other diseases such as coronary artery disease, osteonecrosis, osteoarthritis, osteoporosis and periodontitis. MIR4435-2HG exerts its functions through multiple mechanisms, including inhibiting cell apoptosis, adsorbing microRNAs (miRNAs), promoting cell proliferation, increasing cell invasion and migration ability, and enhancing EMT (47).

Comment 5) This study is based on the analysis of the bioinformatic study. It is suggested to add in vivo and in vitro experimental studies, which may be more meaningful.
Reply 5: Dear reviewer, thank you for pointing this out. You pointed out that our research is currently based on bioinformatics analysis, and suggested that in vivo and in vitro experimental studies be added to enhance the significance of the research. We strongly agree with this. Due to funding and time constraints, we were unable to perform additional experiments for verification, but we regard in vivo and in vitro experimental verification as an important research direction for our future research. We have supplemented the direction of our future research in page 21-22, lines 460-474 of the article. Thank you again for your valuable suggestions.
Changes in the text:
Page 21-22, lines 460-474: Additionally, our research is currently mainly based on bioinformatics analysis. In the future, we can conduct in vivo and in vitro experiments to further verify the research results. For example, culturing lung adenocarcinoma (LUAD) cell lines and normal lung epithelial cells for subsequent in vitro experiments will enable us to more directly observe the functional role of RMlncRNAs in lung cancer cells; using siRNA or plasmid technology to conduct gene knockdown or overexpression studies on identified RMlncRNAs; using qRT-PCR and Western Blot analysis techniques to verify the expression level of RMlncRNAs and their interaction with target proteins, which will provide us with important clues to reveal the molecular mechanism of RMlncRNAs in the occurrence and development of lung cancer; constructing mouse models (such as xenograft models) injected with LUAD cell lines manipulated by RMlncRNAs for in vivo experimental studies. This will enable us to observe the functional role of RMlncRNAs in an environment closer to the human body and provide strong support for its development as a potential therapeutic target.

Comment 6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “m5C RNA Methylation Regulators Predict Prognosis and Regulate the Immune Microenvironment in Lung Squamous Cell Carcinoma, Front Oncol, PMID: 34195072”. It is recommended to quote this article.
Reply 6: Dear reviewer, thank you for your careful review and valuable suggestions. We have added the relevant research background of m6A (page 5-6, lines 108-124), m5C (page 6, lines 127-130, the reference you mentioned is cited in article 16) and m7G (page 7, lines 135-138) in the introduction. Thank you again for your valuable suggestions.
Changes in the text:
page 5-6, lines 108-124: In eukaryotes, m6A methylation is the most abundant modification in mRNA. The basic process of m6A modification is installation by m6A methyltransferase,

removal by m6A demethylase, and recognition by m6A recognition molecules, thereby regulating RNA metabolism. It can affect the expression of target genes and regulate a variety of physiological processes including self-renewal, invasion and proliferation. At the molecular mechanism level, m6A is involved in almost all RNA metabolic processes, including translation, degradation, splicing, export and folding (10). The mTOR signaling pathway is one of the signaling pathways that has been intensively studied in tumor research. Recent studies have found that the mTOR signaling pathway may be closely related to m6A methylation in regulating tumor metabolism. Inhibiting m6A methylation levels can activate the Wnt and PI3K-Akt-mTOR signaling pathways, thereby promoting cancer progression. Conversely, promoting m6A methylation expression can reverse these molecular changes in gastric cancer, indicating that the oncogenic phenotype and activation signal may be directly regulated by m6A-related enzymes rather than directly controlled by target genes regulated by m6A methylation (11,12).
Page 6, lines 127-130: The expression of most m5C and m1A regulators was significantly different between lung squamous cell carcinoma (LUSC) and normal samples, among which m5C regulators were associated with poor prognosis (16).
Page 7, lines 135-138: M7G regulators can affect the infiltration, maturation, and activation of T cells in the tumor immune microenvironment (TIME). Among these regulators, METTL1 and WDR4 are m7G methyltransferases that have been widely studied in the context of T cell biology (19,20).

Comment 7) Mechanisms of methylation regulation in the tumor microenvironment are suggested to be added to the discussion.
Reply 7: Dear reviewer, Thank you for your reminder.We have added the discussion on the methylation regulatory mechanism in page 16-17, lines 339-356.
Changes in the text:
Page 16-17, lines 339-356: Previous studies have found that METTL3 plays an important role in TGF-β-induced epithelial-mesenchymal transition (EMT) in lung cancer, mainly by promoting m6A modification, increasing total mRNA levels and enhancing the mRNA stability of JUNB, one of the most important transcriptional regulators of EMT (40). The study also found that YTHDF2 is upregulated in lung cancer tissues, which can promote the growth of lung cancer cells and directly bind to the m6A modification site in the 3′ untranslated region (UTR) of 6-phosphogluconate dehydrogenase (6PGD), thereby promoting the translation of 6PGD mRNA in lung cancer cells and enhancing the flow of the pentose phosphate pathway (PPP), which is crucial for promoting tumor growth (41). In addition, studies have shown that long noncoding RNAs (lncRNAs) modified by m6A can regulate biological processes. The effect of m6A modification on lncRNAs may involve multiple regulatory mechanisms: on the one hand, m6A modification can regulate the function of lncRNAs by providing binding sites for m6A reader proteins or regulating local RNA structures to induce the binding of RNA binding proteins (RBPs); on the other hand, m6A modification may also regulate the relationship between RNA and DNA and specific DNA sites by affecting the triple helix structure of lncRNA (12).

Reviewer B

1. Please provide the full name of “TCM” in Affiliation 2.

   Reply1: Thanks to the editor for reminding us that the full name of the second unit "TCM" is " Traditional Chinese Medicine ". We have completed this information in the affiliated institutions section. Thank you for your reminder.

   Changes in the text:
   Page 1, lines 11-12.

2. Please double check the full name of “IC50” in the Abstract.

   the immunotherapy response. Finally, to perform a drug sensitivity analysis, the half-maximal drug inhibitory concentration (IC50) of targeted drugs was calculated using

   Reply4: Thank you very much for your careful review and valuable comments on our paper. Regarding your concern about the expression of "IC_{50}", we have carefully checked and revised the entire paper to the standard "IC_{50}" expression.

   Changes in the text:
   Page 3, line 63; Page 4, line 72; Page 13, lines 278,280; Page 17, lines 358,359.

3. Please unify “LASSO” and “lasso”, “COX” and “Cox” in the manuscript.

   Reply6: Thank you for your reminder. We have thoroughly checked and revised the paper.

   Changes in the text:
   Page 3, line 57; Page 11, lines 229,231,234; Page 12, line 257; Page 15, line 312; Page 16, line 334.

4. Please check if any reference should be added since you mention “studies”.

   “Most lncRNAs have unclear functions and exact mechanisms, but various studies have shown their active roles in epigenetic modifications, transcription, and multiple regulatory layers (22), affecting tumor initiation, necrosis, apoptosis, proliferation, cell cycle, and metastasis.”

   “Studies have shown that the m5C methylation of lncRNA H19 mediated by NSUN2 may promote the development and growth of hepatocellular carcinoma by affecting its interaction with the cancer protein G3BP1 (23).”

   “For instance, studies have found that lncRNA lnc-H2AFV-1 alters m6A modification by modulating m6A methyltransferases METTL3/14 and FTO, mediating downstream target IFT80 to promote the progression of head and neck squamous cell carcinoma (39).”

   “Previous studies have found that METTL3 plays an important role in TGF-β-induced epithelial-mesenchymal transition (EMT) in lung cancer, mainly by promoting m6A modification, increasing total mRNA levels and enhancing the mRNA stability of JUNB, one of the most important transcriptional regulators of EMT (40).”

Reply7: Thank you for pointing this out. We have reviewed the sentence you mentioned and modified the description of the "studies" mentioned to make the quoted sentence more accurate. Thank you again for your review and valuable comments.

Changes in the text:
Page 8, lines 167-169,175; Page 18, lines 375,379.

5. Figures

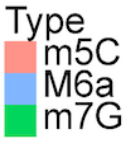
- **All abbreviations** in figures and legends should be explained (including the supplementary materials). “TMB” “NA” in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legend.

  Reply: Thank you very much for your careful review of our paper and for pointing out the issues regarding the interpretation of abbreviations in the figures and legends. We attach great importance to your comments and have thoroughly checked and revised the paper. We have ensured that all abbreviations appearing in the figures and legends are fully explained. Specifically, including the "TMB" and "NA" in Figure 1 that you mentioned, we have added their full names in the corresponding legends. We believe that by following the above requirements, we have improved the clarity and readability of the paper. Thank you again for your review and valuable comments.

- **A summarized legend** for a figure with different parts should be provided, followed by legends for each part. Please provide the summarized legends for Figure 5-8.

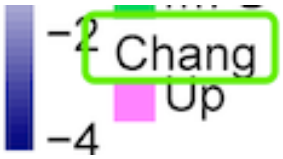
  Reply: Thank you very much for your careful review of our paper and for pointing out the accuracy of the legend titles and content of Figures 5 to 8. We attach great importance to your comments and have added a legend title that summarizes the content of the entire figure to Figures 5 to 8.

- Please revise “M6a” to “m6A” in Figure 1A.



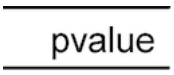
  Reply:"M6a" in Figure 1A was changed to "m6A": This has been modified to ensure that the label text in Figure 1A is consistent with standard terminology.

- Figure 1A: “Change”?



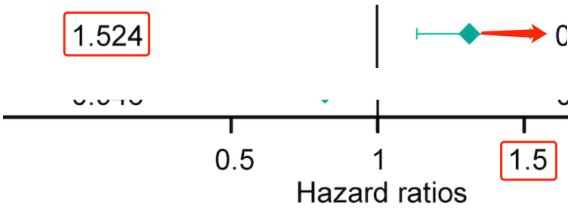
  Reply: The label of Figure 1A has been corrected to ensure that it is spelled correctly.

- Please revise “pvalue” to “P value” in Figure 3B and 3D, 6A and 6B.



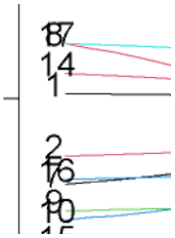
Reply: "pvalue" in Figures 3B, 3D, 6A, and 6B has been changed to "P value": "pvalue" in all designated figures has been changed to conform to the standard writing of statistical terms.

- Figure 3B, 3D; 6A, 6B: To standardize the results, the part that exceeds the horizontal coordinates should be indicated by **arrows** as below.



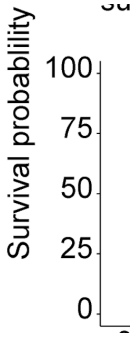
Reply: Red arrows are used in Figures 3B, 3D; 6A, 6B to indicate the portion exceeding the horizontal coordinate. As requested, red arrows have been added to the specified figures to clearly indicate the portion of data exceeding the horizontal coordinate.

- The numbers are covered in Figure 3C.



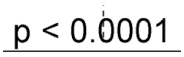
Reply: Overlapping text labels in Figure 3C: The text labels in Figure 3C have been adjusted to ensure that they are clearly readable and do not overlap.

- Please add unit “%” in the y-axis of Figure 3E, 4A, 5C.



Reply: The unit "%" has been added to the y-axis of Figures 3E, 4A, and 5C: The unit "%" has been added to the y-axis of the designated figures to conform to the standard format for data presentation.

- Please revise “p” to “P” in Figure 3E, 4A, 5C.

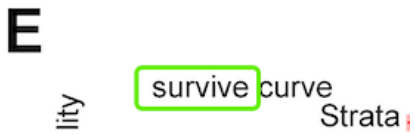


Reply: "P" in Figures 3E, 4A, and 5C has been changed to uppercase "P": The capitalization of "P" in the designated figures has been corrected to ensure consistency with the standard writing of statistical terms.

- Please revise “TP” to “TPR”, “FP” to “FPR” in Figure 3F, 4B.

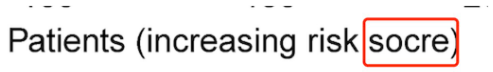
Reply: "TP" in Figures 3F and 4B was changed to "TPR", and "FP" was changed to "FPR": As requested, the terms in the designated figures have been modified to conform to the standard writing of professional terms.

- Figure 3E: “survival”?



Reply: Figure 3E text label changed from “survive” to “survival”: The text label in Figure 3E has been corrected to ensure accurate terminology.

- There are spelling mistakes in Figure 3G and H, 4C and D.



Reply: The typo “Socre” was changed to “Score” in Figures 3G and H and 4C and D: The typo has been corrected in all indicated figures.

- Please unify “MIR4435-2HG” and “MIR4435_2HG”, “NFYC-AS1” and “NFYC_AS1”, “TDRKH-AS1” and “TDRKH_AS1”, “TMPO-AS1” and “TMPO_AS1” in Figures and the text.

Reply: Standardization of the written form of text in the manuscript and figures: The designated terms in the manuscript and figures have been unified to ensure consistency.

- Please unify the P value in Figure 3E and the text.

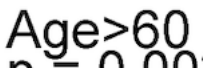
“The overall survival (OS) curve showed statistically significant differences in survival rates between the high-risk and low-risk groups (P < 0.001), with lower survival rates observed in the high-risk group (Figure 3E).”

Reply: Harmonize P values and text in Figure 3E: The inconsistencies between the P values in Figure 3E and the text description have been checked and corrected to ensure that they match.

- Please add unit for “Age” in Figure 5A and 5C.

Reply: Units added for “Age” in Figures 5A and 5C: Units (years) have been added for “Age” in the indicated figures to conform to the standard format for data presentation.

- Please check whether it should be “>=60” in Figure 5C.



Reply: Check whether the text label in Figure 5C is ">=60": The text label in Figure 5C has been modified to ">=60".

- There is no “**” in Figure 5, while it is explained in Figure 5 legend.

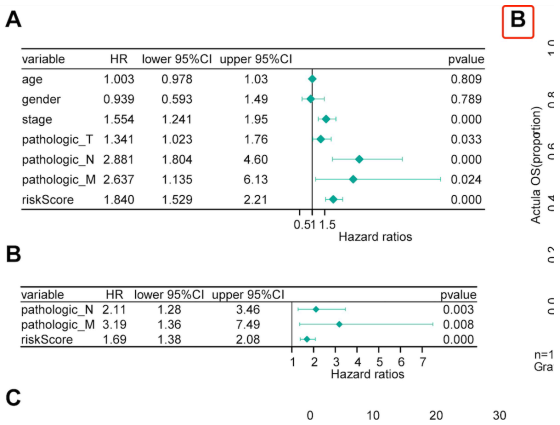
Reply: The explanation of "**" has been deleted in the legend of Figure 5.

- Please double check the highlighted content in the following sentence.

“Analysis of risk scores for different clinical features showed differences in stage and pathological T stage (Figure 5A).”

Reply: Harmonize the results in Figure 5A and the main text: Inconsistencies between the figure and the text description have been checked and corrected to ensure that the two match.

- Please check and revise the labels in Figure 6.



Reply: Check and modify the labels in Figure 6: The labeling errors in Figure 6 have been checked and corrected to ensure that each label is correct and not repeated.

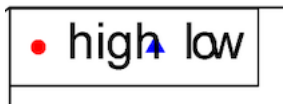
- There is spelling mistake in Figure 6B and some letters are covered.

Reply: Correction of spelling error and overlapping issues in label text in Figure 6B: The spelling error in Figure 6B has been corrected, and the label position has been adjusted to avoid overlapping.

- And please remove the space in Figure 6B.

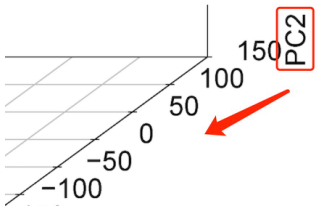
Reply: The spaces between the text labels in Figure 6B have been removed to ensure that the labels are clear and concise.

- The symbol and word are covered in Figure 7A.



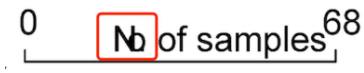
Reply: Correction of overlapping symbols and words in Figure 7: The symbols and words in Figure 7 have been adjusted to ensure that they are clearly readable and do not overlap.

- It is suggested to adjust the position of “PC2” in Figure 7A.



Reply: The label position of “PC2” in Figure 7A has been adjusted as suggested to improve readability.

- The letters are covered in Figure 7B.



Reply: The labels in Figure 7B have been adjusted to ensure that they are clearly readable and do not overlap.

- Please check if “Dysfunction” needs to be added in Figure 7D legend.

  “(D) Differences of TIDE scores in high and low risk groups.”

  Reply: The legend of Figure 7D has been modified as suggested by adding “Dysfunction” to more fully describe the data.

- There is no “***” in Figure 7, while it is explained in Figure 7 legend.

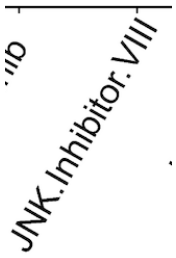
  Reply: The explanation of "***" has been deleted in the legend of Figure 7.

- Please indicate the meaning of “ns” “*” “***” in Figure 8 legend.

  Reply: The explanations of “ns”, “*” and “***” have been added to the legend of Figure 8 to improve the readability of the figure.

- It is suggested to unify the description in Figure 8A and the text.

BIBW2992, Bryostatin.1, EHT.1864, GDC0941, Gefitinib, JNK Inhibitor, VIII,



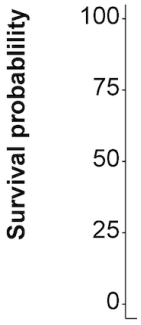
Reply: Unified description and text in Figure 8A: The description and text in Figure 8A have been unified to ensure consistency.

6. Supplementary

- The word is incomplete in Figure S1A. And please revise “p” to “P” and revise the P value.

Reply: It has been confirmed that the words in Figure S1A are presented in full, and the “p” has been modified to “P”.

- Please add unit “%” in the y-axis of Figure S1A.

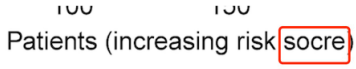


Reply: The unit “%” has been added to the y-axis of Figure S1A to conform to the standard format for data presentation.

- Please revise “TP” to “TPR”, “FP” to “FPR” in Figure S1B.

Reply: The term “TP” in Figure S1B has been changed to “TPR” and “FP” has been changed to “FPR” to ensure consistency with the standard writing of statistical terms.

- There are spelling mistakes in Figure S1C and D.



Reply: The spelling errors in Figure S1C and D have been corrected to ensure accurate terminology.

- Please double check Figure S2B and its legend.

  "(B) Heatmap of the expression levels of 22 types of immune cells in the tumor group and the control group."

  Reply: The number of immune cell types in the image of Figure S2B has been reviewed and replaced with the correct image, which is consistent with the description in the article.

- Please indicate the meaning of "****" "ns" in Figure S2C legend.

  Reply: The P values in Supplementary Figure S2C have been recalculated and verified, and the accurate P values are reported in the article.

- It is suggested to report the exact P value.

  "Furthermore, with the exception of T cells CD4 naive, the expressions of the other 21 types of immune cells exhibited significant differences (P<0.05) between the tumor and control groups (Figure S2C)."

  Reply: The P values in Supplementary Figure S2C have been recalculated and verified, and the accurate P values are reported in the article.

- Please check the labels. There is no colored dot in Figure S2D.

  Reply: Figure S2D has been redrawn to ensure that the colored dots appear correctly, and a new combined figure has been submitted.

- Please revise "M6a" to "m6A" in Table S1.

  Reply: "M6a" in Table S1 has been modified to "m6A" to ensure consistency with standard terminology.

- Please add unit for "Age" in Table S3.

  Reply: The unit "year" for "age" has been added in Table S3 to conform to the standard format for data presentation.

- Please uppercase the first letter of each column in Table S3.

Reply: The first letter of each column in Table S3 has been capitalized to improve the readability of the table.

- Table S3: Please indicate how the data are presented. “mean ± SD” for example.

  Reply: The description of the data presentation format, i.e., “mean ± SD”, has been added below Table S3 to clarify the statistical methods used for the data.