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Reviewer A

The manuscript entitled “Identification of N7-methylguanosine-related lncRNAs for the molecular subtyping and risk stratification of hepatocellular carcinoma” conducted by Li et al. aimed to determine lncRNAs related to m7G RNA modification that could be used as prognostic markers in HCC. In total, 32 m7G-related lncRNAs were identified as associated with overall survival (OS). Based on those 32 lncRNAs, two clusters were formed, in which cluster II was more related to poor OS and upregulated immune checkpoint genes (ICGs). The authors also proposed a signature for predicting risk based on 12 of 32 m7G-related lncRNAs. The paper is interesting, and I think it is well-designed; however, some points must be considered:

General points:

1) The major point that should be explained is the innovation of this study compared to others cited in the discussion. The work of Wei W. et al. (2022), Dai YY. Et al. (2022) and Wang T. et al. (2022) had the same objective as the present work, used the same database (TCGA), and most of the methodology was also shared between the works. Therefore, the innovation of the study should be highlighted.

Reply 1: Thanks for the editor’s comments. As you mentioned, the work of Wei W. et al. (2022), Dai YY. Et al. (2022) and Wang T. et al. (2022) had the same objective as the present work, used the same database (TCGA), and most of the methodology was also shared between the works. We have added the following sentence: **Importantly, AP003390.1 and SNHG26, which were found to be closely associated with the development of HCC in this study, have not been reported in the above-mentioned studies⁵²⁻⁵⁴**. The present study sought to generate a novel m7G-related prediction signature, elucidate its probable underlying molecular mechanisms, and assess its potential in predicting patient prognosis and determining the best therapy.

2) In the discussion section, although the authors cited the three works, it needs to be discussed the findings. From a quick look, 19 of the 32 lncRNAs found in this work were also identified in one of the other 3. Adding this information and discussing the others originally suggested in this study is important.

Reply 2: Thanks for the editor’s suggestion. According to your suggestion, we added the following sentence: **Previous studies have demonstrated that MKLN1-AS, AL031985.3, TMCC1-AS1, AC034229.4, LINC01224, POLH-AS1, and AC026356.1 were related to the development and progression of HCC, which is similar to our research^{53,54}**. Our study showed that MKLN1-AS, AL031985.3, TMCC1-AS1, AC034229.4, LINC01224, POLH-

AS1, and AC026356.1 were related to the development of HCC.

3) Another essential point is the possibility of performing the analysis based on the disease-specific survival (DSS) outcome. OS considers mortality of any cause, not necessarily for the disease that is being studied. This analysis could give a more precise list of related lncRNAs.

Reply 3: We show sincere thanks for the reviewer's comments and suggestions. We conducted a search for relevant data and didn't find disease-specific survival (DSS) related data.

4) The other works used a set of more than 20 m7G-related genes to perform this analysis. Why did the authors choose to work with 13 genes?

Reply 4: Thanks for the reviewer's comment. In our research, m7G genes were retrieved from the Molecular Signatures Database (MSigDB, <http://www.gseamsigdb.org>). We looked up the MSigDB database and found two gene sets. There are 13 genes in them, so there are 13 genes we analyzed.

5) Is it possible to test if the lncRNAs are also differentially expressed in cell lines or samples from HCC patients? That would enrich the results and discussion.

Reply 5: We show sincere thanks for the reviewer's comments and suggestions. We conducted a search for relevant data and didn't find related data for differentially expressed in cell lines or samples from HCC patients.

6) Overall, the quality of the figures is not satisfactory. It is hard to see the text.

Reply 6: Thanks for the reviewer's comment. We have replaced all figures of the paper.

7) The captions could be more descriptive, not only saying which type of graph it is presented. Specific comments:

Reply 7: We show sincere thanks for the reviewer's comments and suggestions. According to the reviewer's suggestion, in our revised manuscript, we have rewritten the captions.

8) Title: Based on the study's results, I think it is too much to say that a molecular subtyping was proposed. I would remove this term.

Reply 8: Thanks for the reviewer's comment. We changed the "Identification of N7-methylguanosine-related lncRNAs for the molecular subtyping and risk stratification of hepatocellular carcinoma." into "Identification of N7-methylguanosine-related lncRNAs for the risk stratification of hepatocellular carcinoma."

9) Introduction: based on the introduction, the relationship between lncRNAs and m7G modification needs to be clarified. This methylation can result in what kind of alterations in lncRNAs? Are there experimental works that showed that this modification resulted in the dysregulation of lncRNAs or other mechanisms in cancer? I know this was not the focus of the work, but it is important for the reader to understand the study's relevance.

Reply 9: We show sincere thanks for the reviewer's comments and suggestions. According to your suggestion, we clarified the relationship between lncRNAs and m7G modification. Long non-coding RNAs (lncRNAs) are non-protein-coding RNAs over 200 nucleotides in length²⁰. There is increasing evidence that lncRNAs play a role in a wide range of biological activities, including disease pathogenesis²¹. Further, certain lncRNAs have been linked to the initiation and development of HCC. For example, lncRNA-ANRIL (antisense non coding RNA in the INK4 locus) has been shown to increase HCC proliferation²², and lncRNA-HULC (highly upregulated in liver cancer) can act as a driver to promote HCC proliferation, migration, and invasion²³. Additionally, other lncRNAs have been shown to influence HCC prognosis. For example, lncRNA-MVIH (microvascular invasion in hepatocellular carcinoma) overexpression has been linked to poor overall survival (OS) in HCC patients²⁴. Meanwhile, lncRNA-PTTG3P (pituitary tumour-transforming 3, pseudogene) expression in HCC patients has been associated with poor survival and TNM stage²⁴. According to recent research, several lncRNAs may be useful predictive biomarkers for HCC²⁵⁻²⁷. **Wang et al. revealed that m7G-related lncRNAs (LOC102555374 and LOC102554730) were significantly upregulated and exacerbated disease progression in a mouse model of hypoxia-induced pulmonary hypertension²⁸. Several studies have revealed that m7G-related lncRNAs are linked to tumor therapy and prognosis²⁹⁻³³.** However, the prognostic value and precise role of m7G-related lncRNAs in HCC are still unclear.

10) Results: In subsection "Tumor immune cell infiltration" the authors only describe that a difference between groups exists, but what differences were observed is important to be described as well. For example, what has higher expression and in which group?

Reply 10: Thanks for the reviewer's comment. We have corrected the subsection "Tumor immune cell infiltration". A heatmap of immune cell infiltration was constructed based on the TIMER, CIBERSORT, QUANTISEQ, MCP-counter, XCELL, and EPIC algorithms (Figure 8A and Table S7). A comparative analysis of the immune cell-related functions revealed differences in cytolytic activity, MHC class I (Major Histocompatibility Complex (MHC) Class I), type I IFN (Interferon) response, and type II IFN response between the two risk groups ($P < 0.05$, Figure 8B). **Compared to the low-risk group, cytolytic activity, type I IFN response, and type II IFN response have lower expression in the high-risk group, while MHC class I has higher expression in the high-risk group.** Differences in immune checkpoint expression were also observed between the two risk groups (Figure 8C). **Compared to the low-risk group, CD80, TNFRSF14, TNFSF18, CD48, CTLA4, CD200, TNFRSF18, CD276, CD28, ICOS, CD44, TNFRSF25, CD160, LAG3, HAVCR2, TNFRSF9, CD70, VTCN1, TNFRSF8, HHLA2, TNFSF4, TNFSF9, LAIR1, TIGIT, LGALS9, TNFRSF4,**

TNFSF15, CD200R1, CD27, CD86, PDCD1, and NRP1 have higher expression in the high-risk group.

11) Results: Describe in the text the name of the 12 lncRNAs that were selected to determine the risk score. Figure 5I is cut and this part is missing, therefore is not possible to see which lncRNA was selected in this part.

Reply 11: Thanks for the reviewer's comment. We have replaced Figure 5I of the paper.

12) Discussion: In line 357 is not clear in what context AP003390.1 was not previously reported. This also happens in line 368, "4 of these lncRNAs have never been observed", but in what context? In HCC, or in literature in general?

Reply 12: We show sincere thanks for the reviewer's comments and suggestions. We have corrected the original sentences as follows: The proposed signature contained 12 m7G-related lncRNAs. The high expression of MKLN1-AS was previously reported to aggravate the progression of HCC and was associated with shorter OS and disease-free survival in HCC patients⁶². MKLN1-AS affects HCC progression by regulating microRNAs⁶². Several studies have also reported that AL031985.3, TMCC1-AS1, LINC01224, SNHG10, **RNF216P1**, and AC025176.1 are potential prognostic predictors in HCC⁶³⁻⁶⁹. **An immune-related and autophagy-related lncRNA, AL031985.3, had been shown to predict HCC prognosis^{63,64}. Chen et al. revealed that high expression of TMCC1-AS1 in HCC patients may lead to shorter survival⁶⁵. Gu et al. found that suppression of LINC01224 inhibited colorectal cancer cell proliferation, migration, and invasion while increasing apoptosis via the LINC01224/miR-485-5p axis⁷⁰. LINC01224 and TMCC1-AS1 have been implemented in multiple prognostic models for HCC⁷¹⁻⁷³. SNHG26 may be a potential biomarker for predicting survival in tongue squamous cell carcinoma⁷⁴. The POLH-AS1 mutation is correlated with skin cancer development⁷⁵. Based on these findings, we speculate that the m7G-related lncRNAs are also associated with cancer progression. Importantly, **AP003390.1 and SNHG26 have never been reported in HCC**, and thus additional studies need to be conducted to assess their value in the early detection of HCC and the development of novel prognostic signatures.**

Reviewer B

The paper titled "Identification of N7-methylguanosine-related lncRNAs for the molecular subtyping and risk stratification of hepatocellular carcinoma" is interesting. The findings demonstrated that m7G-related lncRNAs are associated with the tumor immune landscape and prognosis and can serve as independent prognostic markers for HCC. These findings provide new insights into the functions of m7G-related lncRNAs in HCC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The display in Figure 5 is incomplete. Please carefully check and make corrections.

Reply 1: Thanks for the reviewer's comment. We have replaced Figure 5 of the paper.

2) It is recommended to increase the research of the potential value of the m7G-related lncRNA

risk model in predicting immunotherapy response and drug sensitivity in HCC patients.

Reply 2: We show sincere thanks for the reviewer's comments and suggestions. We will increase the research of the potential value of the m7G-related lncRNA risk model in predicting immunotherapy response and drug sensitivity in HCC patients next time.

3) It is suggested to increase in vivo and in vitro experimental verification, which may be more meaningful.

Reply 3: Thanks for the reviewer's comment. Due to the conditions, we could not perform the experiment. We will increase in vivo and in vitro experimental verification next time.

4) How does m7G-related lncRNAs interact with other signal networks in the progression of HCC? What dual role does it play in increasing/inhibiting tumor progression? It is recommended to add relevant contents.

Reply 4: Thanks for the reviewer's comment. We have corrected the original sentences as follows: For example, Peng et al. revealed that m7G methyltransferase *WDR4* (WD repeat domain 4) promotes HCC progression and thus may be a potential therapeutic target for HCC¹⁴. **WDR4 can enhance HCC progression by promoting cyclin B1 mRNA stability and translation and is a candidate HCC therapeutic target¹⁴.** The abnormal tRNA m7G modification by **METTL1 (methyltransferase-like 1)/WDR4** has been linked to the progression and incidence of various cancers, including lung cancer¹⁵, intrahepatic cholangiocarcinoma¹⁶, and head and neck squamous cell carcinoma¹⁷. **Another study showed that METTL1 is shown to be upregulated in HCC and promotes HCC migration and proliferation through the phosphatase and tensin homolog (PTEN) as well as the AKT signaling pathway¹⁸. METTL1 exerts oncogenic activity by inhibiting PTEN signaling, and the METTL1/PTEN axis has potential for the treatment of HCC^{18,19}. These results indicate m7G modification play an important role in HCC.**

5) All figures are not clear enough. It is recommended to provide clearer figures again.

Reply 5: Thanks for the reviewer's comment. We have replaced all figures of the paper.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Constructing and validating of m7G-related genes prognostic signature for hepatocellular carcinoma and immune infiltration: potential biomarkers for predicting the overall survival, J Gastrointest Oncol, PMID: 366360517". It is recommended to quote this article.

Reply 6: We show sincere thanks for the reviewer's comments and suggestions. We have cite the paper "Constructing and validating of m7G-related genes prognostic signature for hepatocellular carcinoma and immune infiltration: potential biomarkers for predicting the overall survival, J Gastrointest Oncol, PMID: 366360517" in the part of the introduction.

7) The biological characteristics of m7G-related lncRNAs and its research progress in tumors should be added to the discussion.

Reply 7: We show sincere thanks for the reviewer's comments and suggestions. According to your suggestion, we added the biological characteristics of m7G-related lncRNAs and its research progress in tumors. **Wang et al. revealed that m7G-related lncRNAs (LOC102555374 and LOC102554730) were significantly upregulated and exacerbated disease progression in a mouse model of hypoxia-induced pulmonary hypertension²⁸. Several studies have revealed that m7G-related lncRNAs are linked to tumor therapy and prognosis²⁹⁻³³.**

8) It may be more meaningful to suggest to increase the functional research of related key lncRNAs.

Reply 8: We show sincere thanks for the reviewer's comments and suggestions. We have corrected the original sentences as follows: "The proposed signature contained 12 m7G-related lncRNAs. The high expression of MKLN1-AS was previously reported to aggravate the progression of HCC and was associated with shorter OS and disease-free survival in HCC patients⁶². MKLN1-AS affects HCC progression by regulating microRNAs⁶². Several studies have also reported that AL031985.3, TMCC1-AS1, LINC01224, SNHG10, **RNF216P1**, and AC025176.1 are potential prognostic predictors in HCC⁶³⁻⁶⁹. **An immune-related and autophagy-related lncRNA, AL031985.3, had been shown to predict HCC prognosis^{63,64}. Chen et al. revealed that high expression of TMCC1-AS1 in HCC patients may lead to shorter survival⁶⁵. Gu et al. found that suppression of LINC01224 inhibited colorectal cancer cell proliferation, migration, and invasion while increasing apoptosis via the LINC01224/miR-485-5p axis⁷⁰. LINC01224 and TMCC1-AS1 have been implemented in multiple prognostic models for HCC⁷¹⁻⁷³. SNHG26 may be a potential biomarker for predicting survival in tongue squamous cell carcinoma⁷⁴. The POLH-AS1 mutation is correlated with skin cancer development⁷⁵. Based on these findings, we speculate that the m7G-related lncRNAs are also associated with cancer progression. Importantly, **AP003390.1 and SNHG26 have never been reported in HCC**, and thus additional studies need to be conducted to assess their value in the early detection of HCC and the development of novel prognostic signatures".**

Reviewer C

1. Please check if the author's name matches the reference. Please note that you should use the last name.

example **Peng et al.** revealed that m7G methyltransferase *WDR4* (WD repeat domain

14. Xia P, Zhang H, Xu K, Jiang X, Gao M, Wang G, et al. MYC-targeted WDR4 promotes proliferation, metastasis, and sorafenib resistance by inducing CCNB1 translation in hepatocellular carcinoma. *Cell death & disease* 2021; 12 (7):691. ↵

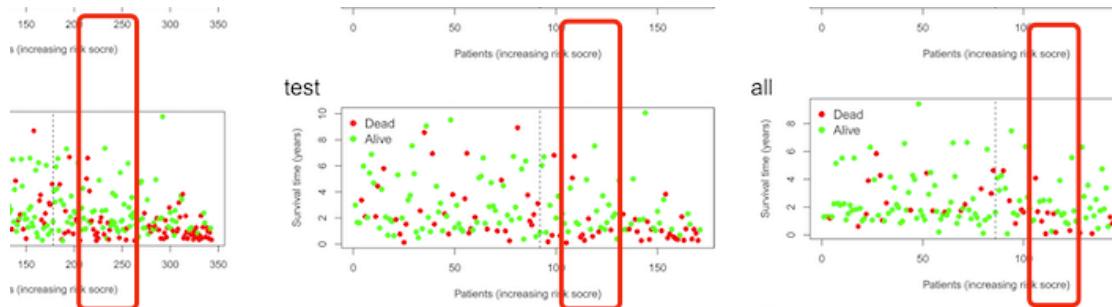
Reply 1: The name of the author here should be Xia et al. We have made changes in the manuscript.

2. Figure 5

a. Please revise them to “1 year”.



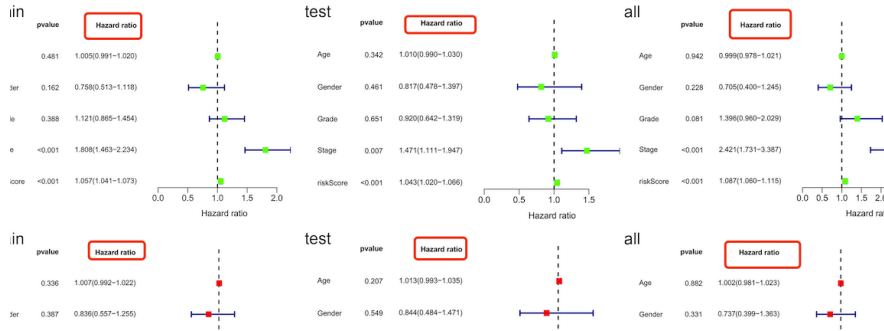
b. Please revise them to “score”.



Reply 2: We have modified the image as requested and uploaded the modified image file.

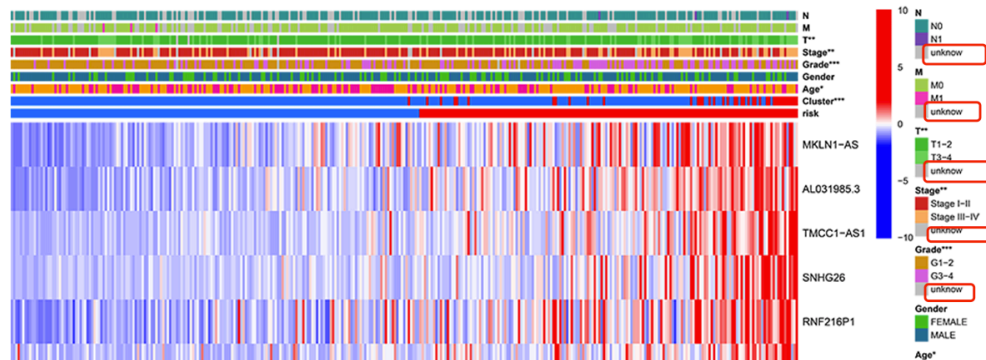
3. Figure 6:

a. “Hazard ratio” should be revised to “Hazard ratio (95%)”

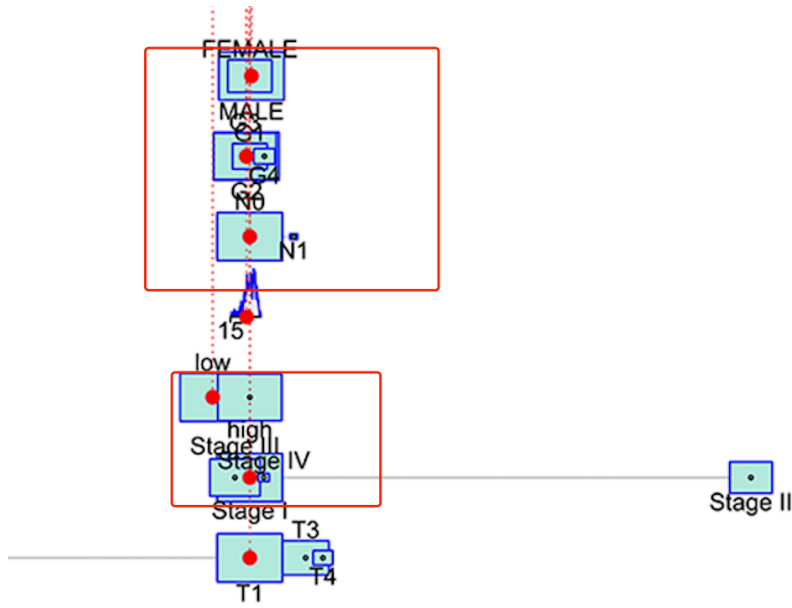


b. Please revise them to “unknown”.

E

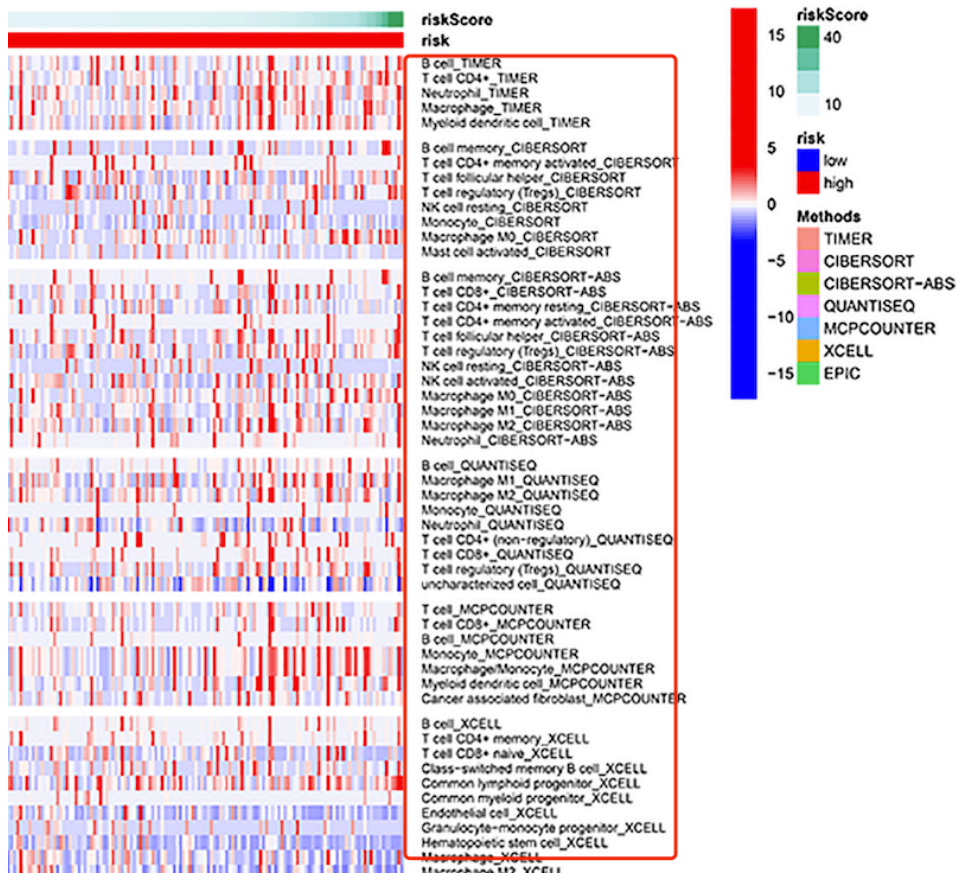


c. Some words got covered, please revise the figure.



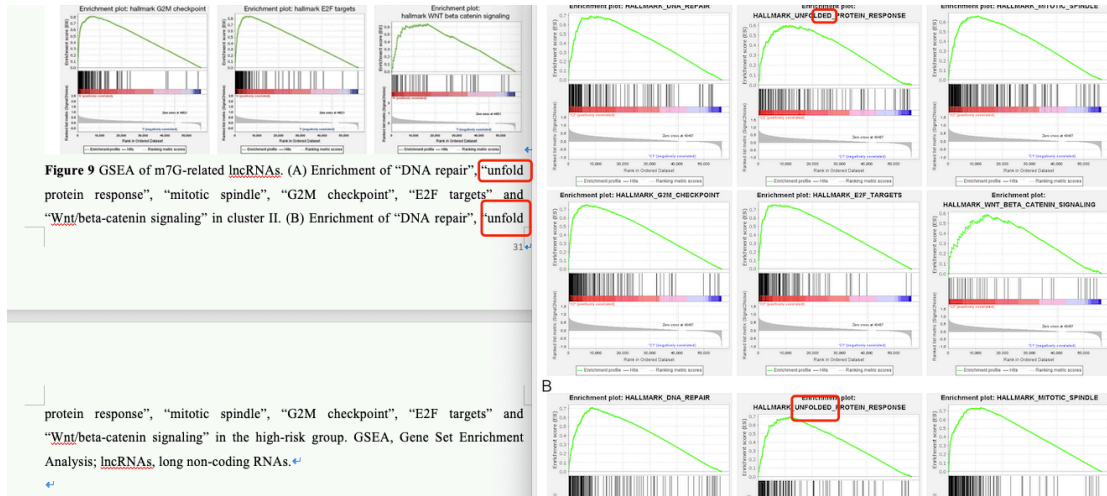
Reply 3: We have modified the image as requested and uploaded the modified image file.

4. Figure 8: Those words could not be clearly identified, please revise the figure and resend us a higher resolution version.



Reply 4: We have modified the image as requested and uploaded the modified image file.

5. Figure 9: Check if they should be “unfold” or “unfolded”.



Reply 5: This should be "unfolded", which we have also modified in the manuscript.