Peer Review File

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Reviewer A

The paper titled "Identification and validation of a novel cuproptosis-related lncRNA signature for predicting the prognosis of colorectal cancer" is interesting. The findings provided promising insights into the CRLs involved in CRC. The developed CRL-based signature may be used to predict the clinical outcomes and therapeutic responses of patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

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

Response: According to your suggestion, we have described the potential role of cuproptosis-related lncRNAs in CRC in the introduction part.

2) The description of some methods in this study is too simplistic, please describe in detail.

Response: We have described the methods in this study more in detail.

3) What is the relationship of cuproptosis-related lncRNAs and immune microenvironment in CRC? It is recommended to add relevant content.

Response: We cannot agree with you more about that the relationship between cuproptosis-related lncRNAs and immune microenvironment is interesting and important in CRC. In the present study, we focused on developing the prognostic signature and identifying key CRLs with regulating effect on cuproptosis. Actually, we are making more experiments to test the relationship between CRL and several other critical phenotypes including chemo-resistance and immunotherapy response. Therefore, we hope to elucidate the mentioned point more in depth in our future work.

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

Response: Thank you for your suggestion. We have discussed several established lncRNAs in the discussion part.

5) This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.

Response: We cannot agree with you more about it. We have displayed some preliminary basic results in this study. Actually, we are performing in depth studies aiming to reveal the mechanism of AC090116.1 regulating cuproptosis and we hope to publish it in the next story.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "A novel cuproptosis-related lncRNA signature to predict prognosis and immune landscape of lung adenocarcinoma, Transl Lung Cancer Res, PMID: 36895935". It is recommended to quote the article.
Response: According to your suggestion, we have quoted this article in the introduction part.

7) Figures 1,5 and 6 are not clear enough. It is recommended to provide clearer figures again.
Response: We have provided the PDF version of figures.

Reviewer B

In this article, Liu and Wu propose to construct a model in which cuproptosis-related lncRNAs (CRLs) control a novel form of cell deatch named cuproptosis and how this class of lncRNAs could serve as early-prognostic markers of colorrectal cancer (CRC). Based on the fact that copper (Cu) is an essential molecule in different cellular functions including energy production, disregulations in its extra- and intracellular levels can result in altered cell metabolism and therefore, in tumorogenesis.

Minor suggestions by page and line number in PDF:

1) Page 3 Line 87: In "RNA expression data sets", datasets is a single word.
Response: We have revised it.
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2) In the METHODS section, subsection "RNA expression datasets": please list the specific IDs for each one of the datasets you used for all your analyses.
Response: Actually, only TCGA dataset was used in this study. We have revised the description to avoid misunderstanding.

3) In the METHODS section, subsection "Somatic mutation analyses": please list the specific IDs for each one of the datasets you used for these analyses.
Response: All the data used in this study were obtained from TCGA.

4) In the METHODS section, subsection "Differential expression analysis": please be

more specific about all the used parameters. Write all them out in this section.
Response: The threshold of differential expression analysis has been described in this section.

5) In the METHODS section, subsection "Functional enrichment analysis": please explain why you used the linear for microarray method if all your datasets were generated by RNA-sequencing. Why did not you use the same tolos you explained in the "Differential expression analysis" subsection.
Response: We have made a wrong description in this section and we are extremely sorry for the misunderstanding. All the data are indeed generated by RNA-sequencing and we have revised the description in the method part.

6) In the METHODS section, subsection "Statistical analyses": please specify the statistical test you used for each of the analyzed datasets or data type. You can include this information in the figure legends if you prefer. In this sense, please specify the establisehd p-values in each figure legend. For example: p-values were defined as: ***p<0.001, **p<0.01, *p<0.05.
Response: According to your suggestion, we have added the information in each figure legend.

Major suggestions:

1) Due to the results and conclusions of the paper, I think it would be important to change the paper's title to: "Identification and validation of a novel cuproptosis-related lncRNA signature for predicting colorectal cancer patients survival".
Response: Thank you for your suggestion. We have changed it.

2) The study would be enriched if authors could include information about CRC-related and/or CRLs expression data in serum, or at least of some of the reported CRC-lncRNAs in patients' sera (NEAT1, Linc01836, FOXD2-AS1, NRIR, XLOC_009459, NNT-AS1, UCA1, etc.); eventhough they haven't been directly linked to cuproptosis. This, because they are suggesting the use of CRLs as prognostic tools for CRC and usually, non-coding RNAs that are used as early prognosis biomarkers are the ones present in serum exososomes, not in the tumors. Because of this, I also suggest autors to better describe the potential use of their 22 CRLs signature accordingly to the datasets they have used and the way they did their analyses. I mean, they should mention their main use as markers for CRC-tumors' reclassification and as survival markers, but not as early prognosis biomarkers of CRC development.
Response: Thank you for your suggestion. We have discussed several established lncRNAs incorporated in to predictive model in the discussion part.

3) In the same direction, I think that including expression data about microRNAs in CRC could enrich their prediction tool for CRC survival. Especially because many CRC-related lncRNAs regulate different pathways through sponging microRNAs and

therefore, changing the expression of their target genes, which are usually involved in cell proliferation, cell transofrmation, etc. This information could be crossed with that the authors got from both their KEGG pathway enrichment and differential gene expression analyses. Thus, the study will be more robust and informative.

Response: Thank you very much for your suggestion. We cannot agree with you more about that incorporating more information into predictive models will enhance its robustness. However, in this study, we focused on the lncRNAs and we extremely hope to develop a more informative prognostic signature in our future work.

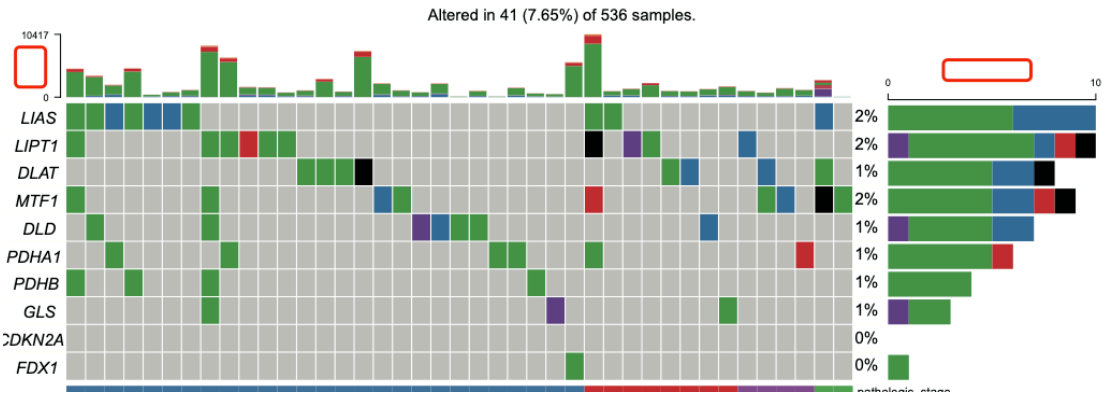
4) Why didn't you include rescue experiments in which you overexpressed AC090116.1? I think it would be good to repeat some of the key experiments (cell proliferation and viability, elesclonol-induced ROS production) including not only the silencing, but the overexpression of this lncRNA. In addition, it would be also good to include at least experiments with other 2 CRLs (at least 3 lncRNAs).

Response: In this study, we focused on the lncRNA AC090116.1 which showed that highest expression fold change in cancer tissues compared with normal tissue. Actually, we are making more experiments to test the relationship between CRL and several other critical phenotypes including chemo-resistance and immunotherapy response. Therefore, we will elucidate the value CRLs in our next study.

Reviewer C

1. Figure 1

a. Please supplement the descriptions of X- and Y-axis in the figure.



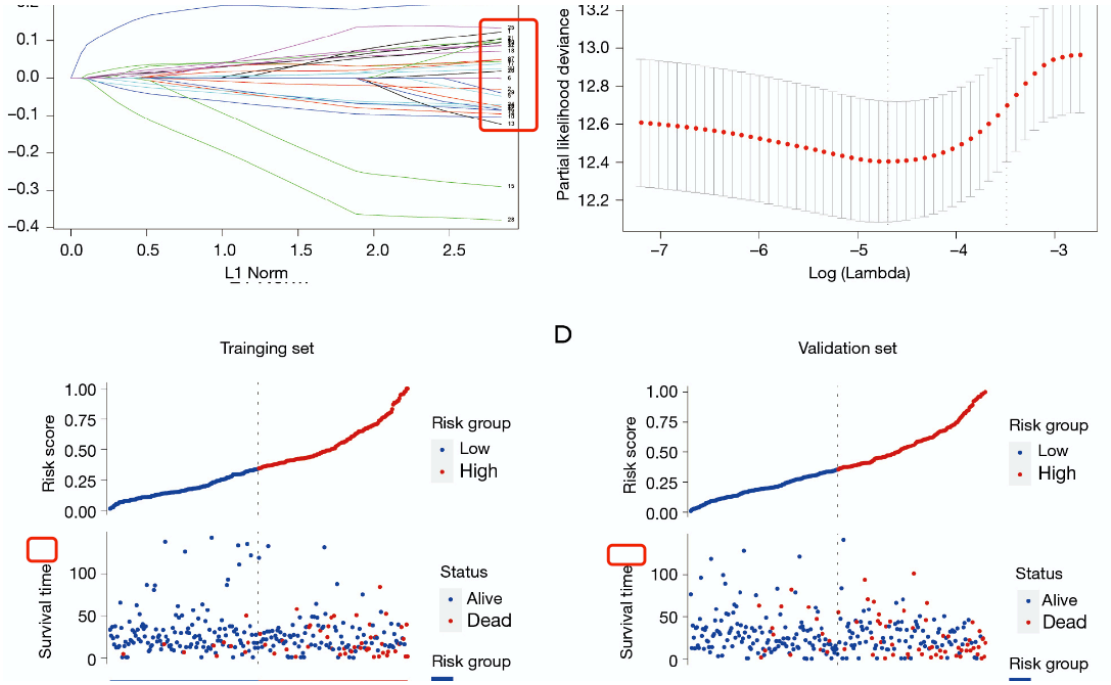
b. Please define "**, ***, ****, ns" in figure legends.

Response: We have added the description and defined the "**, ***, ****, ns".

2. Figure 3

a. Figure 3A: Some numbers got covered, please revise to make them clearer.

b. Figure 3C-D: Please provide the units for Y-axis.



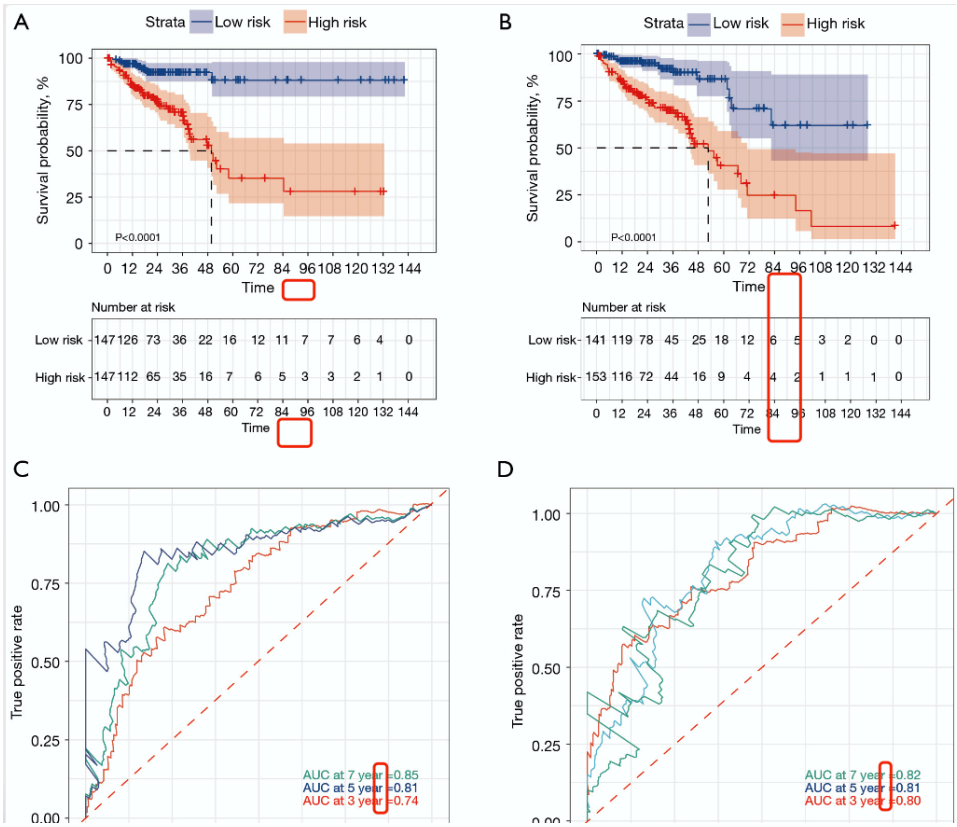
c. Please check and revise the typo.

Response: We have revised them according to your suggestions.

3. Figure 4

a. Figure 4A/4B: Please supplement the units for Figure 4A and 4B.

b. Figure 4C/4D: Please revise “year” to “years”.



Response: We have revised them according to your suggestions.