

## Peer Review File

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### Review comments-Reviewer A

**Comment 1:** The reason why the predicted high activity of cuproptosis, a PCD, was associated with the poor prognosis of lung cancer patients should be discussed.

**Reply 1:** Our deepest gratitude goes to you for your suggestion. In this study, we identified 7 genes associated with LUAD prognosis based on the cuproptosis-related genes from a published study, through a series of analyses including ssGSEA and Cox regression analyses. Among them, the high expression of NMUR1, CLEC3B, METTL7A, DNAAF9, and CIQTNF7 were found to be associated with good prognosis, while the high expression of PTGES3 and RPE were found to be associated with poor prognosis. However, the expression levels of these several genes could not represent the activity of cuproptosis pathway. Therefore, it's hard to conclude that the high activity of cuproptosis is associated with poor prognosis of LUAD according to our results.

**Comment 2:** Genetic alterations of the cuproptosis genes: Although some probable inactivating mutations including frameshift indels, splice site mutations or a nonsense mutation, were found, majority of alterations were missense mutations. Information for these missense mutations, that is, VUSs or predicted as pathogenic or not is needed. In addition, to figure out the significance in lung cancer, analysis on and comparison with other types of cancer would be appreciated.

**Reply 2:** Thanks so much for your valuable suggestion. Here, we aimed to draw and compare the mutation landscape of the selected 7 cuproptosis-related genes. Therefore, we did not analyze these missense mutations in depth. However, your suggestion is really worthwhile, and in the future analyses, we will study the various mutation types in detail and compare the mutational landscape in LUAD with other cancers.

**Comment 3:** About pathway analyses for 238 hub genes identified: Th authors found some positively associated mitochondria-related cellular components which were in line with their hypothesis, they should have to refer apparently unrelated ones such as endothelial development or cell adhesion.

**Reply 3:** Thanks a lot for your precious comment. In this study, we listed some pathways that are not apparently related to cuproptosis, and expatiated the role of several pathways including cell adhesion molecules and extracellular matrix in lung cancer.

**Changes in the text:** We marked some apparently unrelated pathways in red. Please see page 10, lines 292-293 and page 11, lines 298-299.

**Comment 4:** Discussion on speculated mechanistic relationships between the selected 7 genes for the prediction model and cuproptosis or copper related metabolism would be appreciated.

**Reply 4:** As you suggested that it's better to discuss the potential mechanistic relationships between the model and cuproptosis. Indeed, we have been actively looking for the possible

relationships, but we only found an association between CLEC3B and copper metabolism, we also discussed this in the “Discussion” section.

**Changes in the text:** See page 16, lines 464-465. We added the following text: Furthermore, the protein encoded by CLEC3B may be related to calcium ion binding which can affect copper binding. And, the corresponding reference was added.

**Comment 5:** Discussion on drug sensitivity prediction analysis in relation to cuproptosis or copper related metabolism would be appreciated.

**Reply 5:** We agree with the comment regarding the limitations of our study and appreciate the suggestion. We have tried to explain the effects of therapeutic drugs on cuproptosis or copper metabolism. Unfortunately, we could not find an answer. Perhaps, more pharmacological studies are needed to understand the relationship.

**Comment 6:** All the authors belong to the thoracic surgery or the clinical laboratory departments. Who are finally responsible for the bioinformatics and statistical analyses used?

**Reply 6:** X Ling is currently doing his PhD in thoracic surgery. During this period, he learned some bioinformation analysis. J Zhu and J Ma designed and conceived the research, and X Ling finished the bioinformatics and statistical analysis.

**Minor concern:** “cuprotosis” should be corrected as “cuproptosis”.

**Reply:** Our deepest gratitude goes to you for your careful work. We have corrected the “cuprotosis” to “cuproptosis” in the manuscript.

**Changes in the text:** There are 12 substitutions in the text, all marked in blue.

### Review comments-Reviewer B

**Comment 1:** Please check the full name of “SD” below. Is it correct?

165 and classified: as responders and non-responders: patients who showed a partial  
166 response (PR) or stable condition (SD) over than 6 months were classified as responders,

**Reply 1:** Thank you so much for pointing out this mistake. We have corrected “SD” to “SC” in the article.

**Changes in the text:** Please see page 6, lines 155-156.

**Comment 2:** The citation of reference 34 is missing in the main text; please have a check.

**Reply 2:** Reference 34 was added in our last revision and is cited in page 16, line 468 of the manuscript.

**Comment 3:** “Patel” is not the author of reference 40. Please check and revise.

574 stage LUAD(39). Patel et al. demonstrated that patients with LUAD and *MUC16*  
575 peptide mutants have worse outcomes(40). *TTN* encodes a large abundant protein of

**Reply 3:** We are so sorry for the mistake. We have corrected the authors of reference 40 in the

text.

**Changes in the text:** Please see page 17, line 504.

**Comment 4:** Please check if any more references need to be added in the below 3 sentences since you mentioned “Studies”, but only one reference was cited. If not, “studies” should be changed to “a study/a previous study”.

152 **Studies** have revealed diverse functions of mitochondria, such as producing  
153 biosynthetic intermediates and contributing to cellular stress responses(9). Since the  
162 ultimately cell death(11). **Several studies** have demonstrated the association of copper  
163 imbalances with tumor burden, progression, incidence, invasion, and reoccurrence of  
164 the disease(10). A previous study revealed a long non-coding RNA signature related to  
482 risk and low-risk groups (Fig. S6) **Previous studies** have revealed that the accumulation  
483 of copper inhibits the PI3K/AKT/mTOR pathway, which activates cellular autophagy  
484 and disrupts mitochondrial dynamics(29). Moreover, a high dose of copper can activate

**Reply 4:** Our deepest gratitude goes to you for your careful work. In the corresponding position of the text, we have modified the above three sentences.

**Changes in the text:** Page 4, line 84, “Studies have revealed diverse functions of mitochondria” was modified to “Diverse functions of mitochondria have been revealed”. Page 4, line 94, “Several studies have” was replaced by “A study has”. Page 13, line 367, “Previous studies have” was modified to “A previous study has”.

**Comment 5:** Table 1 :

Please indicate the full name of “Mut”, “WT” in the legend.

**Reply 5:** We are so sorry that we forgot explain the above words, and we have added the full names in the legend of Table 1.

**Changes in the text:** Please see page 22, line 672.

lung cancer; Mut, Mutation; WT, Wild Type;

**Comment 6:** Figure 1:

Please indicate the full name of “SNV” in the legend.

687 **Figure 1 Genetic alterations of the cuproptosis gene set in TCGA-LUAD cohort.**  
688 (A) The **SNV** frequencies of 10 cuproptosis genes within 42 LUAD samples from

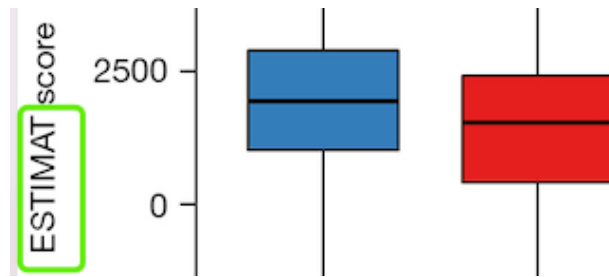
**Reply 6:** Thank you for your careful work. We have indicated the full name of “SNV” in the legend of Figure 1.

**Changes in the text:** Please see page 23, line 681.

681 adenocarcinoma; CNV, copy number variant; SNV, single nucleotide variations.

**Comment 7:** Figure 5:

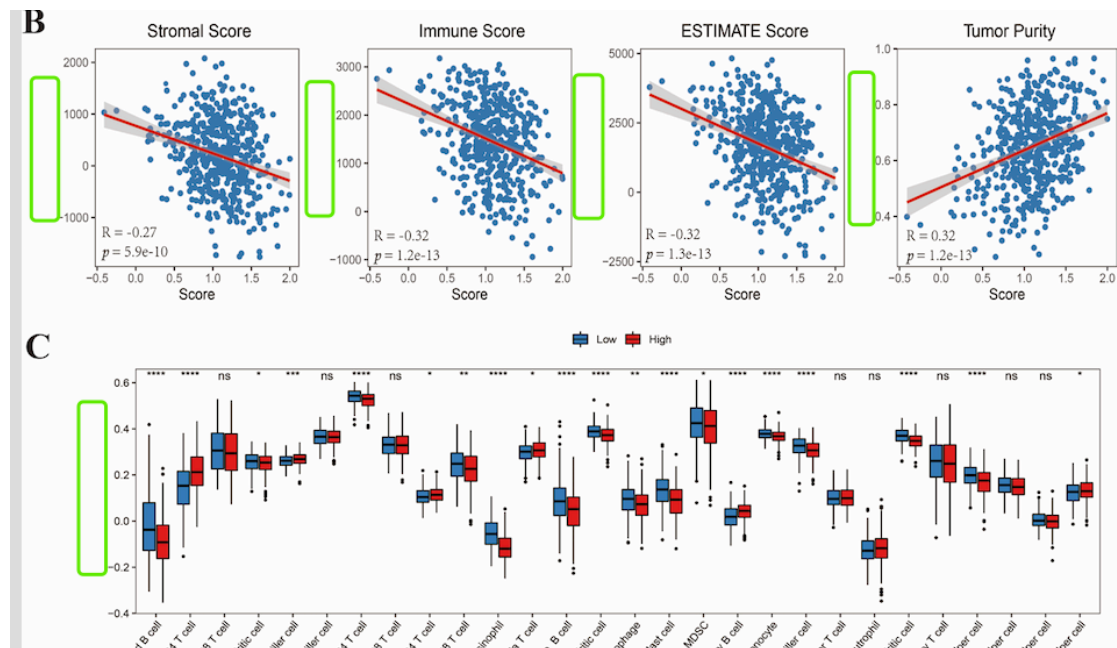
1) There is a spelling mistake in Figure 5A and B.



2) Should the below word be “**positively**” in your main text?

385 tumor purity was significantly higher than in the low-risk group (Fig. 5A). Spearman  
386 correlation analysis supported the risk score negatively associated with immune and  
387 stromal cell infiltration while negatively associated with tumor purity (Fig. 5B). Then

3) Please provide the description for the Y-axis in figure 5B and figure 5C, and resubmit the revised figure 5 to us as a separate file in jpg or tiff format.



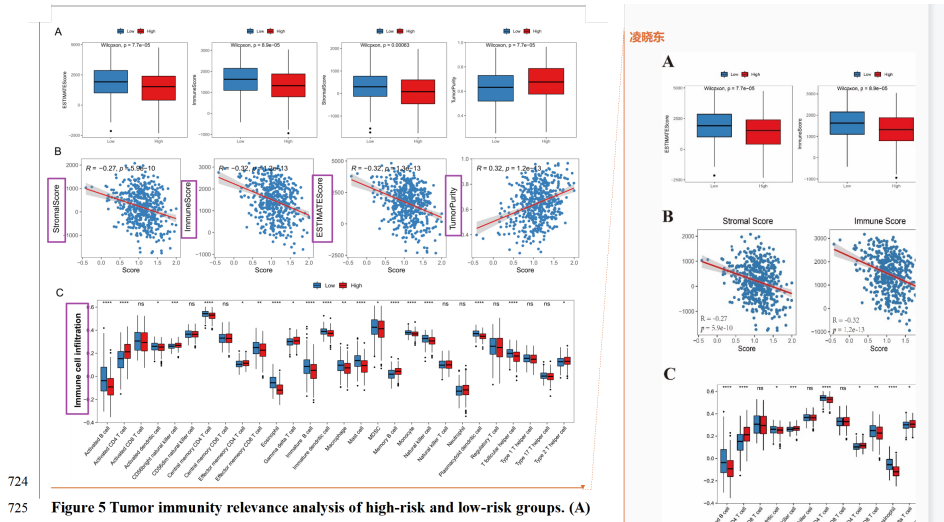
**Reply 7:** We appreciate your careful work,

1) “ESTIMATE” in the Figure 5 was corrected to “ESTIMATE”, we have replaced the Figure 5 in the main text, and sent the modified one to you by email.

2) The “negatively” should be “positively”, we have corrected it.

**Changes in the text:** Please see page 13, line 380.

3) We appreciate your careful work, we have corrected it. We have revised Figure 5 in the manuscript and sent it to you by email.



Changes in the text: Please see page 27, line 724.

**Comment 8:** Figure 7B:

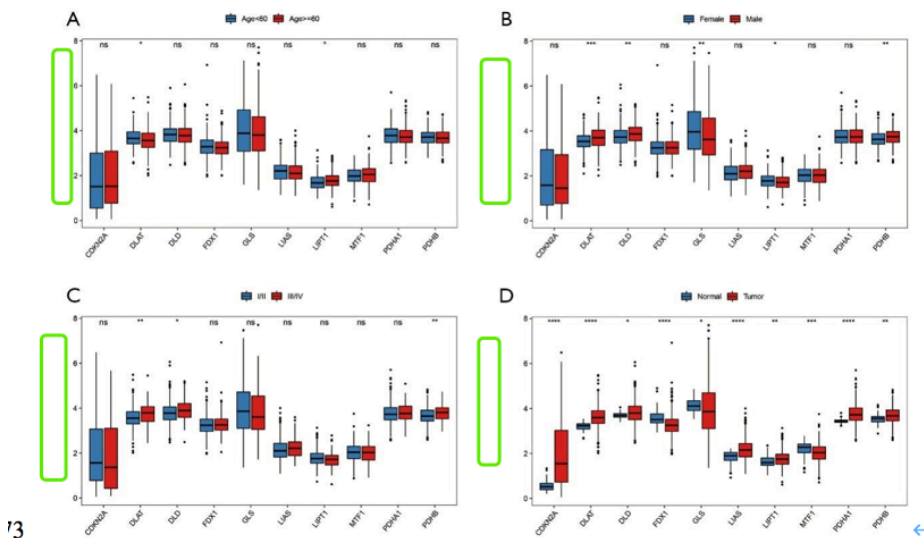
The symbols \*\*\*\* are not complete and not clear.



**Reply 8:** We apologize for our carelessness. We have revised Figure 7 in the manuscript and sent it to you by email.

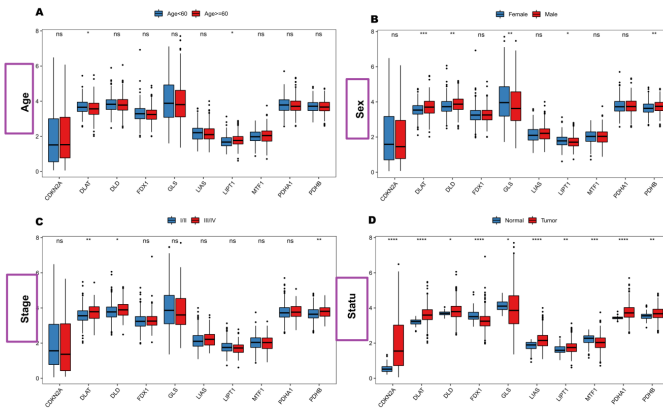
**Comment 9:** Figure S1:

Please provide the description for the Y-axis, and resubmit the revised figure 1 to us as a separate file in jpg or tiff format.



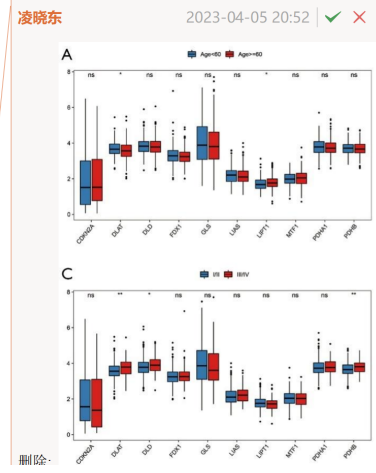
**Reply:** We apologize for our carelessness. We have revised Figure S1 in the manuscript and sent it to you by email.

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750 **Figure S1 Association between the expression of 10 cuproptosis genes and clinical**



Changes in the text: Please see page 30, line 749.