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Reviewer A

1) First of all, the nomogram to predict the prognosis was not constructed based on the FRG genes only, so the authors should clearly indicate this in the title and elsewhere. In the title, please also indicate the development and validation of a prognosis prediction model based on FRG genes/risk score and clinical factors. My other major concern is the unsatisfactory predictive accuracy of the nomogram, with AUC values lower than 0.75, even lower than 0.70. The authors need to add predictors or change the algorithm to improve the accuracy, otherwise this is a failed nomgram.

Reply: Your suggestion is very good. I have changed the title accordingly, and the description of the body is accurate, so it has not been modified. **See page 25, line 674**. In addition, I fully understand your concern about the predictive accuracy of the risk score model. In the training set, the areas under the ROC curves (AUCs) for 1-, 3-, and 5-year OS were 0.714, 0.673, and 0.668, respectively. Similarly, the AUC curves for the test and whole set indicated that the model had moderate clinical prognostic significance. We are trying to improve the accuracy, but the AUC value is still around 0.7, so we can only say that the two-gene signature had a moderate predictive capacity for OS. Next, Our team predicted the prognosis of RCC patients at 1, 3, and 5 years by constructing a nomogram that included clinical characteristics and riskscores. The calibration curves showed good agreement between the nomogram and the predicted results. The consistency index (C-index) of the nomogram was 0.731 (95%CI: 0.672–0.790). It appeared that the model was stable over time, as the AUCs were 0.728, 0.704, and 0.898 at 1-, 3-, and 5-years, respectively, which shows that Nomogram has better prediction ability, **see page 10, line 287**.

2) Second, the abstract needs further revisions. In the background, the authors did not describe why the FRG genes based model can accurately predict the prognosis in RCC and what the clinical needs are for the current research focus. In the methods, please describe the databases used, the clinical factors and prognosis outcomes in the databases, the generation of training and validation samples, and the assessment methods of the predictive accuracy of the nomogram. In the results, please describe the predictors in the nomogram and the AUC values in both the training and validation samples. The current conclusion is vague and unclear. Please have comments for improving the accuracy of the nomogram and the clinical implications of this model.

Reply: We think this is an excellent suggestion. We have made some additions to the revised manuscript. In the abstract, we describe why the model based on FRG gene can accurately predict the prognosis of renal cell carcinoma, **see page 2, line 54**. We also added the clinical significance of this study in the background section, **see page 2, line 34**. With regard to the question of “In the methods, please describe the databases used, the clinical factors and prognosis outcomes in the databases, the generation of training and validation samples, and the assessment methods of the predictive accuracy of the nomogram”, I replied in detail in comment 4. Finally, We increased the C-index and AUC values of the nomogram and evaluated the

prediction performance of nomogram, **see page 10, line 287**.

3) Third, in the introduction of the main text, the authors did not review what has been known on the prognostic biomarkers in RCC and what prognosis predictive models are available for RCC, did not review what the limitations and accuracy of the available models are, did not analyze what the FRG-based models' strengths are, and did not explain why the FRG can accurately predict prognosis. In particular, why the FRG-based models need to add other clinical predictors to improve the accuracy.

Reply: Dear expert, thank you very much for your valuable advice. I can understand your concern. Although there are many prognostic biomarkers and prognostic prediction models for renal cell carcinoma, the prognosis of renal cell carcinoma is not satisfactory at present. It is necessary for us to develop new prognostic biomarkers to solve this problem. Ferroptosis-related genes have been proved to be a potential cancer treatment strategy, and their role in the occurrence and development of renal cell carcinoma is still unclear, so we try to construct a prognostic model related to iron death gene. This is the advantage of building a model based on FRGs. In addition, we did not review the known biomarkers and available prognostic prediction models of renal cell carcinoma, because there are so many predictive models and prognostic markers, such as copper death-related predictive models, autophagy-related predictive models, immune-related predictive models, etc. We do not know how to describe them, similar literature as DOI10.3389/fgene.2022.959456, Https://doi.org/10.1186/s12935-021-01782-6. As for the question of why FRG-based models need to add other clinical predictors to improve accuracy, I think the reasons are as follows: Nomogram integrates multiple predictive indicators on the basis of multivariate regression analysis, and then uses graduated segments to draw on the same plane according to a certain scale, so as to express the relationship between variables in the predictive model. Therefore, it is reasonable to add other clinical predictors to the FRG-based model.

4) Fourth, in the methodology of the main text, please have an overview paragraph to describe the research design and procedures of this study. The authors need to describe the clinical samples, clinical factors, and prognosis outcomes in the databases used, describe details for generating training and validation samples, and provide threshold AUC/C-index value for a good predictive model.

Reply: Thank you very much for your valuable advice to improve our method. In the part of methods, we use a paragraph to summarize the research design of this study, **see page 4, line 118**. The clinical samples and clinical sample information of the database used are shown in **Table S1**. The details of generating training sets and verification sets have been added in the methodology section, **see page 6, line 156**, and the corresponding clinical features can be found in **Table S3**. Finally, we add the C-index value of the prediction model in the results, **see page 10, line 287**.

Reviewer B

The paper titled "Construction of a two-gene prognostic model related to ferroptosis in renal cell carcinoma" is interesting. The results constructed a prognostic model associated with

ferroptosis, which may provide clinicians with a reliable predictive assessment tool and offer new perspectives for the future clinical management of RCC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the correlations between ferroptosis-related genes and the tumor microenvironment? How valuable are ferroptosis-related genes in predicting survival and drug sensitivity in renal cell carcinoma patients? It is recommended to add relevant content.

Reply: Your suggestion is very critical, and our discussion in these two parts is really not enough. We add the correlations between ferroptosis-related genes and the tumor microenvironment as advised, **see page 13, line 390**. And we also discussed the significance of iron death related genes in predicting drug sensitivity, **see page 14, line 411**.

2) The abstract is not adequate and needs further revisions. The research background does not indicate the clinical needs of this research focus. The study results need to show the clinical characteristics of the two groups of patients.

Reply: There are indeed some deficiencies in our abstract. Thank you for your valuable comments. We have modified the abstract as advised. We added the clinical significance of this study in the background section, **see page 2, line 34**. We added the clinical characteristics of the two groups of patients in the part of the results, **see page 2, line 49**.

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

Reply: Thank you for your valuable advice. In this study, we obtained two ferroptosis-related genes through bioinformatics analysis, and verified their expression in renal cell carcinoma. We understand that the functional research of related key genes may better reveal the role of iron death in renal cell carcinoma. However, in the present study, we mainly focused on the construction of the model. Importantly, we are currently conducting a detailed functional study and mechanism study of key genes in another study.

4) In this study, bioinformatics approaches were employed to develop the model. It is suggested to add further functional experiments to study its role in vivo and potential molecular mechanisms.

Reply: Dear expert, thank you very much for your valuable suggestions which will help us a lot. I know that adding this part of the experiment will improve the overall level of this article. Our study developed a prognostic risk model based on the genetic association of ferroptosis and identified low- and high-risk RCC groups. Besides, we simply validated the expression of key genes NCOA4 and CDKN1A in RCC patients. However, we did not conduct further functional experiments and study their possible molecular mechanisms in this paper, because we studied their functions in detail in another article, at present, we are doing research in this area. And these results will be published in the near future.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Targeting ferroptosis in breast cancer, Biomark Res, PMID: 33292585”. It is recommended to quote this article.

Reply: We appreciate your valuable comments and recommended references on our manuscript, as well as your suggestion to cite the reference you have provided. I think this will enrich our

introduction, so we have revised it accordingly as required. **See page 3, line 90**.

6) The biological characteristics of ferroptosis-related genes and its research progress in tumors should be added to the discussion.

Reply: Thank you very much for your advice. Following your guidance, we have increased the biological characteristics of ferroptosis-related genes (CDKN1A, DPP4, MT1G, and NCOA4) in tumors in the discussion section, **see page 12, line 353**. And we will continue to explore the molecular mechanism of FRGS in renal cell carcinoma and continue to contribute.

Reviewer C

1. Reference/citation

The word "Studies" was used, yet only one reference was cited in the below sentence. Please either choose to revise them to "study" or to give more than one reference in this sentence.

**Please note that the references should be cited in order of their appearance in the text.*

"some studies have discussed the sensitivity of different subtypes of breast cancer to iron death, suggesting that iron death related genes may provide a new direction for the development of biomarkers and treatment strategies for breast cancer (16)."

Response: This is indeed our negligence. I have revised "studies" to "the study" as advised, see page 4, line 95.

2. Abstract

a. The Abstract should be 200 ~ 350 words, but the current one has 270 words. Please update.

b. Please indicate the full name of "AUC" in the line 59.

58 nomogram that predicted the OS in RCC patients, the consistency index (C-index) of
59 the nomogram was 0.731 (95% CI: 0.672–0.790), the AUCs were 0.728, 0.704, and
60 0.898 at 1-, 3-, and 5-years, respectively, which shows that nomogram has good

Response: I have changed the number of words of the abstract to 339 and added the full name of AUC, see page 2, line 60.

3. Figure 1

a. Please check if this sentence matches figure 1A.

647 **Figure 1** Differential expression analyses of FRGs in RCC. (A) The expression
648 distribution of ferroptosis-related mRNA in tumor tissues and normal tissues; the
649 horizontal axis represents different mRNA, the vertical axis represents the mRNA
650 expression distribution, the different colors represent different groups, and the upper
651 left corner represents the significant P value test method. Asterisks represent the levels

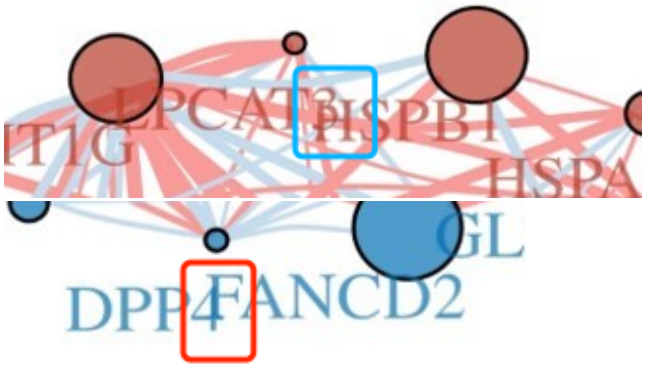
Response: This is my mistake. Please forgive me for the inconvenience. I have deleted the sentence, see page 22, line 638.

b. Please check if "Small;" is appropriate here.

659 prognosis log-rank P value. Small; the different colors of the circles represent the
660 different cluster categories. Here, there are two categories by default. (D) List 1

Response: I have delete the word “small”, see page 22, line 647.

c. figure 1D: The words are overlapped, please revise.



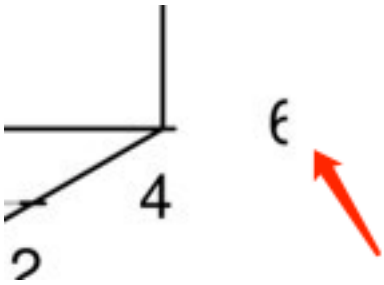
Response: Dear editor, this picture is drawn with an online database. I don't seem to be able to get the code or modify it. The most important thing is to enlarge the picture so that you can see the details clearly. I hope you can understand.

4. Figure 3D, E, F

Please revise ‘progression free’ to ‘progression-free’.

5. Figure 3G, H, I

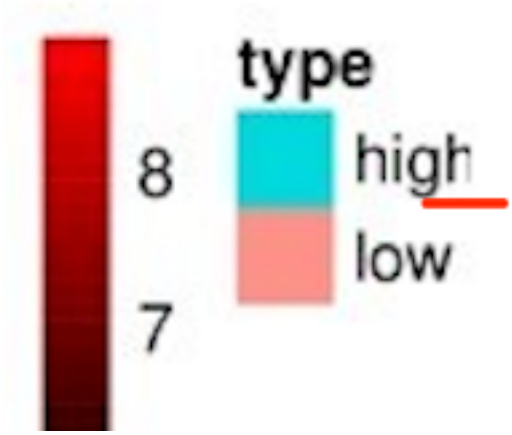
The number is incomplete, please revise.



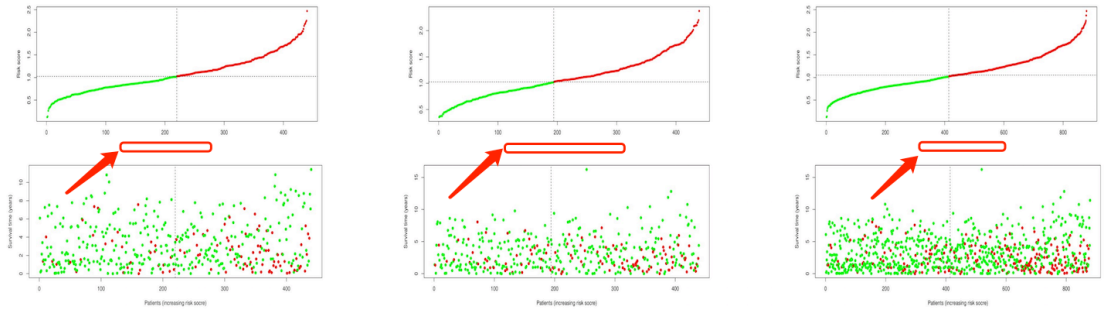
Response: I have modified the figure 3D,E,F,G,H,I as required, see page 24, line 666.

6. Figure 4A, B, C

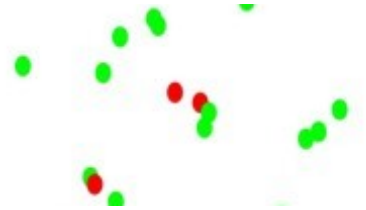
a. This word is incomplete, please revise.



b. Please add the description to the X-axis.



c. Please indicate the meaning of red and green dots in figure or in figure legend.



d. Please correct this typo to ‘score’.

asing risk socre)

7. Figure 4D, E, F

Please revise ‘1 years’ to ‘1 year’.

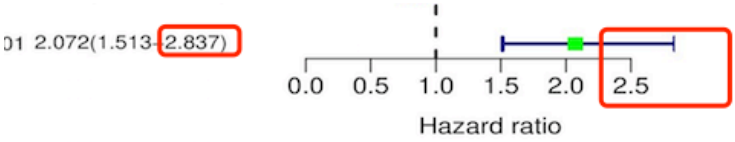
UC at 1 years: 0.714
UC at 3 years: 0.673

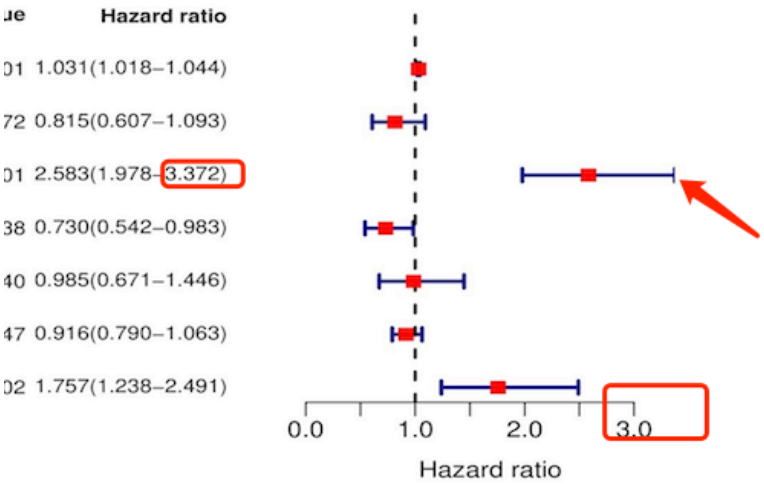
Response: I have modified the figure 4A, B, C, D, E, F as required, see page 25, line 674.

8. Figure 6A

a. Please revise ‘pvalue’ to ‘p value’.
b. Please add “95% CI” after HR.

c. Please extend the length of the X-axis and also indicate the number.





Response: I have modified the figure 6A as required, see page 27, line 695.

9. Figure 8

Please unify the number.

725 **Figure 8** The exploration of the immune profile of the high- and low-risk groups. (A)
726 Barplot shows the proportion of 22 kinds of TILs in RCC tumor samples. Column
727 names of the plot were sample IDs. (B) In TCGA, a heat map of the correlation between

316 tumor-infiltrating immune subpopulations and construct 21 immune cell profiles in the
317 RCC samples (*Figure 8A*). Correlation analysis of the immune cell populations and

Response: I have changed 21 to 22 in the main text, see page 11, line 306.

10. Table S1

a. Please add a table header.

Table S1 Clinical information of 883 patients with renal cell carcinoma

	Character	TCGA-RCC (n=883)
Status	Alive	656

Response: I have add a table header named "Variables".

b. The sum-up of 'Race' and 'pTNM_stage' and 'Grade' is not 883. Please check.

Table S1 Clinical information of 883 patients with renal cell carcinoma

	Character	TCGA-RCC (n=883)
Status	Alive	656
	Dead	227
Age	Mean (SD)	60.2 (12.4)
	Median [MIN, MAX]	60 [17,90]
Gender	FEMALE	288
	MALE	595
Race	AMERICAN INDIAN	2
	ASIAN	16
	BLACK	120
	WHITE	721
pT_stage	T1	79
pTNM_stage	I	464
	II	107
	III	188
	IV	103
Grade	G1	14
	G2	227
	G3	206
	G4	75
	GX	5

Response: The clinical features of some patients in TCGA are missing, for example, some patients do not have TNM stage or race, so the total is not enough.

11. Table S3

Please revise the column of p value as follows:

Age					0.0497
	<=65	576(65.53%)	302(68.79%)	274(62.27%)	
	>65	303(34.47%)	137(31.21%)	166(37.73%)	

Table S3 Clinical features of RCC patients in the training set and testing set

Covariates	Type	Total	Test	Train	P-value
Age	<=65	576(65.53%)	302(68.79%)	274(62.27%)	0.0497
	>65	303(34.47%)	137(31.21%)	166(37.73%)	
Gender	FEMALE	287(32.65%)	141(32.12%)	146(33.18%)	0.7916

Response: I have modified the tableS3 according to the format.

12. Table S4

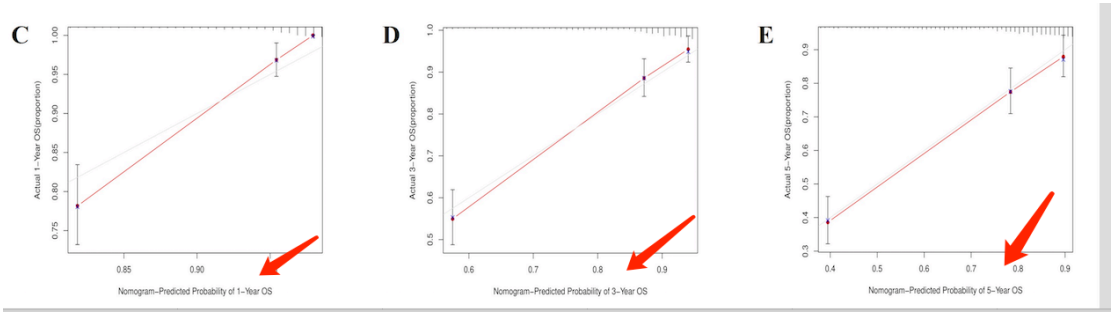
Please confirm if data are missing in the empty boxes.

Vinorelbine	KIN001-102	LFM-A13
WZ3105		
YM155		

Response: I have confirmed that there is nothing missing here, because the numbers on both sides are not the same.

13. Figure 6C-E

"1-,3-,5-" or "1-, 2-, 3-"? Which one is correct? Please check and revise.



742 5-year OS of RCC patients. (C-E) In the calibration curve of the 1-, 2-, and 3-year OS

Response: I've confirmed that "1-, 3-, and 5-" is correct, and I've corrected the figure legends, see page 27, line 700.