Cancer, a major global cause of death, drives research into immunotherapy. In the present study, Tan et al focuses on the ANK3 gene, exploring its diverse roles and links to individual cancers. Using various databases, Tan et al assessed ANK3's impact on immunity, finding it differentially expressed in tumors and correlated with prognosis. ANK3 emerges as a potential biomarker for prognosis and immunotherapy, with implications for novel cancer treatment strategies. The article is dense with results and would benefit from small corrections:

Comment 1: The article will benefit from an English grammatical review by a native scientific editor.

Reply 1: Thank you for your valuable comments. We have modified the style problems in the article as required, and have invited native English speaking professionals to proofread it. Thank you again for your valuable advice.

Comment 2: The brand and catalog of antibodies used in the Western blot are not clear.

Reply 2: Thank you for your valuable comments. According to your request, we have supplemented the brand and catalog of antibodies used in the Western blot. Thank you again for your valuable advice.

Changes in the text: We have modified our text as advised (see Page 5 , line 157-158)

Comment 3: The item "Gene set enrichment analysis (GSEA)" is not described in sufficient detail to be reproduced by other researchers.

Reply 3: Thank you for your valuable comments. We have added a detailed description of GSEA in the article. Thank you again for your valuable advice.

Changes in the text: We have modified our text as advised (see Page 7 , line 212-218)

The paper titled "A comprehensive analysis of the prognostic and immunological role of ANK3 in pan-cancer" is interesting. ANK3 could serve as a new biomarker specific to prognosis and immunotherapy in various cancers. The findings could contribute to the development of novel strategies for treating malignancies. However, there are several minor issues that if addressed would significantly improve the manuscript.

Comment 1: What is the relationship between ANK3 and tumor-infiltrating immune cells?

What role does ANK3 play in prognosis in tumor? It is recommended to add relevant content.

Reply 1: Thank you for your valuable comments. By studying the relationship between the differential expression of ANK3 and immune cells, we found that the expression level of ANK3 in LIHC, COAD, KIRC was significantly positively correlated with macrophages, neutrophils, B cells, CD4+ T cells, CD8+ T cells, and dendritic cells. This is in much the same way that high levels of ANK3 expression are associated with higher immune scores in cancer. We describe this in the discussion section of this article. In addition, regarding the impact of differential expression of ANK3 in tumor tissues on the prognosis of cancer patients, we demonstrated through systematic OS,DSS and PFI analysis, which was reflected in the results and supplementary materials of the article. Thank you again for your valuable advice.

Changes in the text: We have modified our text as advised (see Page 15 , line 463-466; see Page 16 , line 492-501)

Comment 2: Some fonts need to be enlarged, as shown in Figures 9 and 14.

Reply 2: Thank you for your valuable comments. Since we put the images into the WORD document, the clarity of the images will be reduced, so we upload the original images of the two images to the revision system and send them to the editor by email. Thank you again for your valuable advice.

Comment 3: It is suggested to increase the in-depth study on the function of ANK3 in the occurrence and metastasis of different cancers, which may make this study more complete.

Reply 3: Thank you for your valuable comments. Since this is the first time to explore the correlation between ANK3 gene and pancarcinoma expression, prognosis and immunity, we will select closely related tumors for further analysis and experimental verification. Thank you again for your valuable advice.

Comment 4: In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply 4: Thank you for your valuable comments. Based on your suggestions, we have supplemented the limitations of the previous study and the clinical significance of this study in the introduction section. Thank you again for your valuable advice.

Changes in the text: We have modified our text as advised (see Page 14 , line 106-116)

Comment 5: It is recommended to increase the functional experimental study of the ANK3 gene.

Reply 5: Thank you for your valuable comments. Since this is the first time to explore the correlation between ANK3 and the expression, prognosis and immunity of pancarcinoma on the basis of previous studies, we preliminarily explored the functional pathways related to the progression and prognosis of pancarcinoma through GSEA analysis. The main purpose of this study is to verify the differences in the expression of ANK3 in different tumors through

detailed bioinformatics analysis and simple cell experiments. In the follow-up study, we will collect more clinical samples and conduct more cell function experiments to further explore the important role of ANK3 in pancarcinoma. Thank you again for your valuable advice.

Comment 6: Some letters in Figure 7B is not clear, please rearrange and upload the figure again.

Reply 6: Thank you for your valuable comments. Since we put the image into the WORD document, it will reduce the clarity of the image, so we upload the original image to the modification system and send it to the editor by email. Thank you again for your valuable comments.

Comment 7: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "A pan-cancer analysis of the expression of gasdermin genes in tumors and their relationship with the immune microenvironment, Transl Cancer Res, PMID: 35116710". It is recommended to quote the article.

Reply 7: Thank you for your valuable comments. At your request, we have included this reference in the article. Thank you again for your valuable comments.

Changes in the text: We have modified our text as advised (see Page 5, line 132)

1. Figure 3

a) Please explain the meaning of different colors in 3C.

Reply: Thank you for your valuable comments, we have supplemented the meanings of the colors in Figure 3C in the legend section.
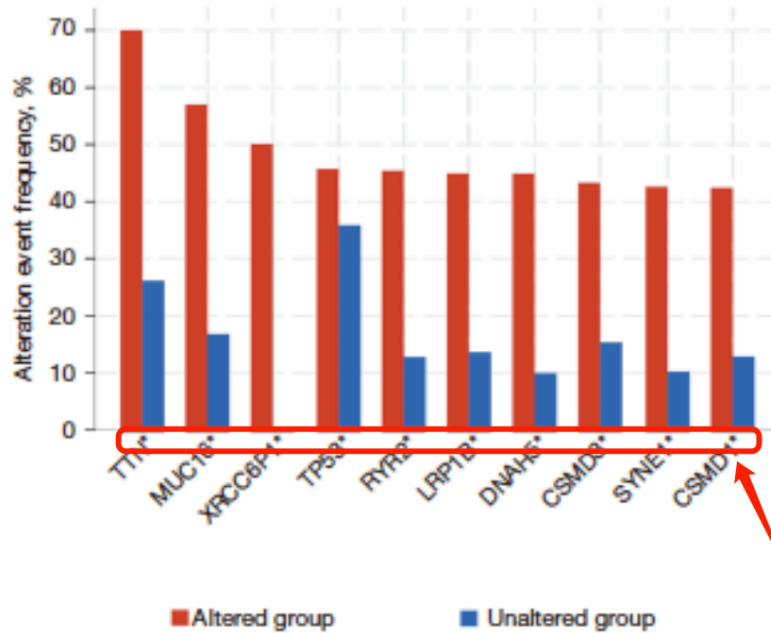
Change in the text: We have modified our text as advised (see Page 25, line 723-725).

b) Please explain the meaning of the symbols in 3D.

Reply: Thank you for your valuable comments, we have supplemented the meanings of the "*" in Figure 3D in the legend section.

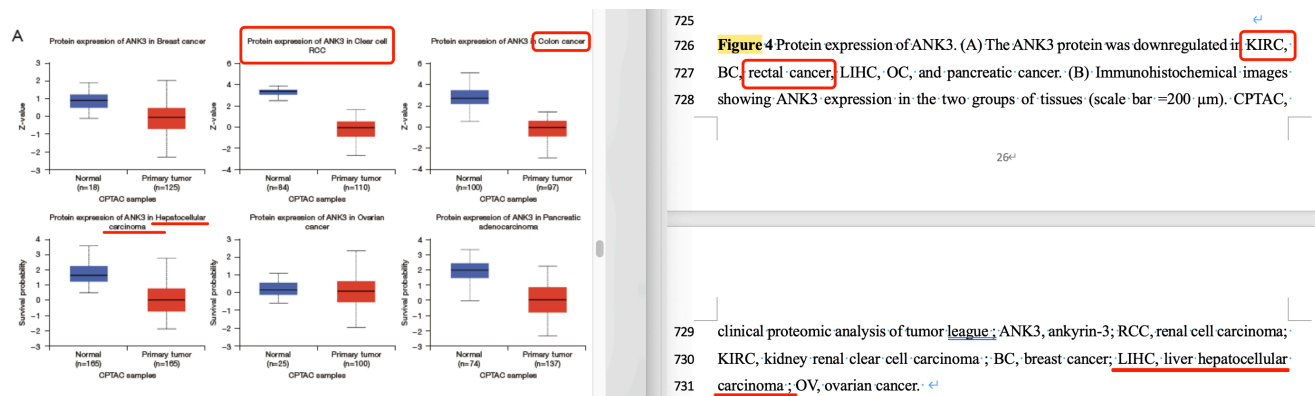**Change in the text:** We have modified our text as advised (see Page 25, line 725).

D

2. Figure 4

Please check if the figure matches the legend.

Reply: Thank you for your valuable comments, we have modified and checked the legend part of Figure 4A in detail according to your requirements.

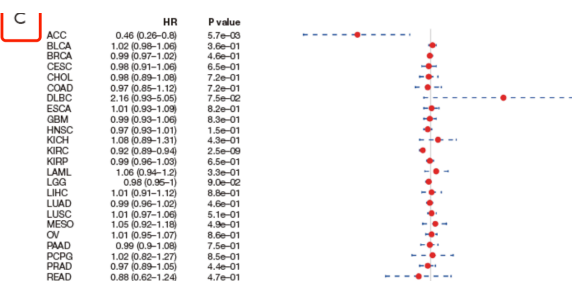**Change in the text:** We have modified our text as advised (see Page 27, line 734-735).

725

726 **Figure** 4 Protein expression of ANK3. (A) The ANK3 protein was downregulated in KIRC,

727 BC, rectal cancer, LIHC, OC, and pancreatic cancer. (B) Immunohistochemical images

728 showing ANK3 expression in the two groups of tissues (scale bar =200 μm). CPTAC,

26

729 clinical proteomic analysis of tumor league ; ANK3, ankyrin-3; RCC, renal cell carcinoma;

730 KIRC, kidney renal clear cell carcinoma ; BC, breast cancer; LIHC, liver hepatocellular

731 carcinoma ; OV, ovarian cancer.

3. Figure 5

Please check if the figure matches the legend. Only ABC in the figure, but there's D in the legend.
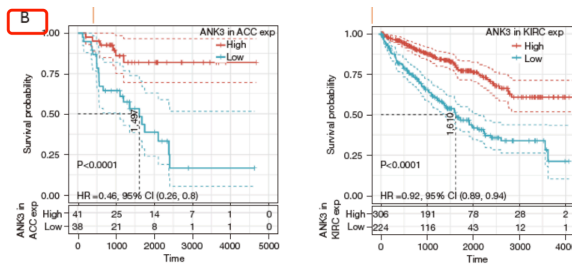
Figure 5 ANK3 expression in pan-cancer and its relevance to OS. (A) The GEPIA2 tool was used to analyze ANK3 gene expression in tumors in TCGA. (B) The K-M test was used to analyze the ANK3/OS correlation in ACC. (C) The K-M test was used to analyze the ANK3/OS correlation in KIRC. (D) Forest map based on prognosis of different types of cancer in TCGA. ANK3, ankyrin-3; HR, hazard ratio; ACC, adrenocortical carcinoma; CI, confidence interval; KIRC, kidney renal clear cell carcinoma ; OS, overall survival; TCGA, The Cancer Genome Atlas; K-M, Kaplan-Meier.

Reply: Thank you for your valuable comments. First of all, we are sorry for our wrong behavior and we have modified it according to your request.

Change in the text: We have modified our text as advised (see Page 28, line 754).

## 4. Supplementary Materials

a) Please cite figure S1 and S2 in the main text in order.

Reply: Thank you for your valuable comments, we have referenced S1 and S2 in the article and highlighted them in italics.

Change in the text: We have modified our text as advised (see Page 10, line 307).

b) Please provide the unit of the x-axis in figure S1A and S2A.

Reply: Thank you for your valuable comments, the unit of the x-axis in figure S1A and S2A "days".
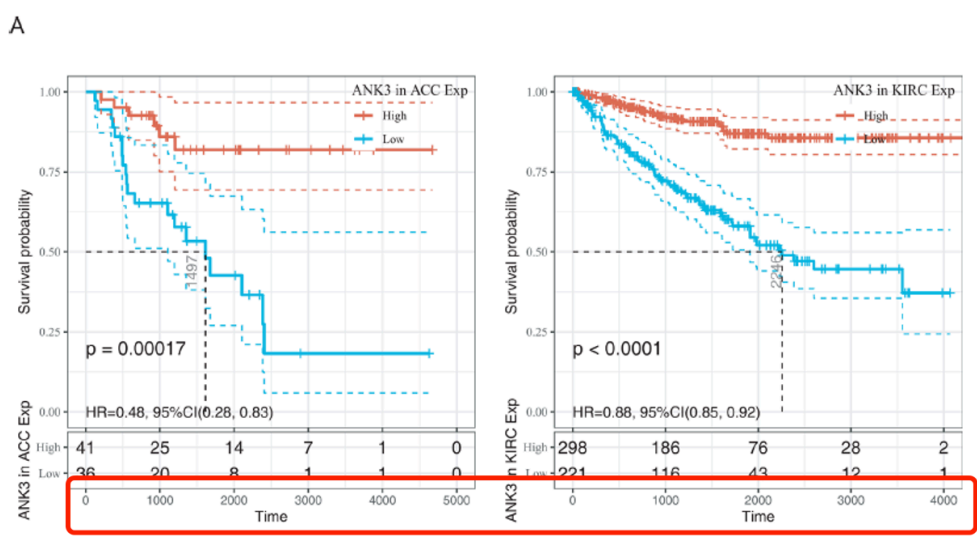
5. References/Citations

Please double-check if more studies should be cited as you mentioned "studies". OR use "study" rather than "studies".

104   (11). Recent studies have examined the relevance of ANK3 to the androgen receptor
105   signaling pathway and its effect on the prognosis of breast cancer (BC) patients (11).

154

155   The cells were lysed with lysis buffer as described in previous studies (14), and protein

450   Subsequently, other studies(11) have shown its extensive involvement in tumor
451   proliferation, invasion, and metastasis, and its different roles in tumors.

Reply: Thank you for your valuable comments, we have carefully checked the spelling of the quoted content in this section and have made changes.

Change in the text: We have modified our text as advised (see Page 4, line 104-105; see Page 4, line 159; see Page 15, line 456).