

## Peer Review File

Article information: <https://dx.doi.org/10.21037/jgo-23-641>

### Reviewer A

1-In the introduction of the article, mention the role of TMPRSS2 gene in SARS-CoV-2 .

**Response:** According to your suggestion, we have described the role of TMPRSS2 in SARS-CoV-2 in the introduction part.

2- What package did you use to normalize GEO and TCGA data?

**Response:** We added the information in the method part.

3-In relation to the CIBERSORT algorithm, mention sufficient explanations in the method.

**Response:** According to your suggestion, we have explained more in detail in the method part.

4-In some places, FDR is mistakenly written as 0.5 instead of 0.05. Please corrected.

**Response:** Thank you for your notification and we have corrected it.

### Reviewer B

The authors investigated the role of TMPRSS2 expression in colorectal cancer and its potential implications for patient prognosis, molecular features, and immunotherapy response. The study is well-performed and deserves to be a publication. Here are some minor comments.

The study addresses a timely and relevant topic, linking the expression of TMPRSS2, a protein associated with SARS-CoV-2 infection, to colorectal cancer.

The study employs a multiomics approach multiple datasets, utilizing a combination of gene expression data from TCGA and GEO databases, along with functional annotations, miRNA prediction, immune cell infiltration analysis, and evaluation of immunotherapy response.

The study also discusses the potential clinical implications of TMPRSS2 expression in CRC, including its prognostic value and its potential role as a biomarker for immunotherapy response. However, I think this study mostly focuses on correlational analyses rather than establishing causative relationships. Therefore, mentioning in the conclusion 'CRC cells may be more resistant to SARS-CoV-2 infection.....' may not be relevant as the authors didn't investigate and that is not the focus of this study.

**Response:** Thank you very much for your suggestion. Indeed, no direct evidences have been obtained to support this conclusion and further research are urgently needed to be performed. However, the relationship between TMPRSS2 expression the SARS-CoV-2 infection has been widely accepted. In this study, it was confirmed that TMPRSS2 was highly expressed in normal

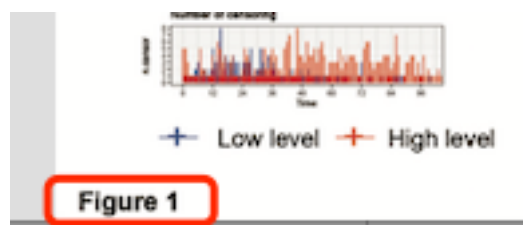
tissues compared with CRC tissues. Therefore, we speculated that CRC cells may be more resistant to SARS-CoV-2 infection. We have stated the limitations for lacking the direct and solid evidences to support this point in the discussion part.

### Reviewer C

1. When using abbreviations in table/figure or table/figure description, please mention the entire expression in a footnote below the corresponding table/figure. **Please check and revise.** Such as: PAAD, PRAD, SARC, STARD, THCA, UCEC, ..... (in figure S1); CR, PR, SD, PD (in figure 5), etc.

**Response:** We have added the abbreviations.

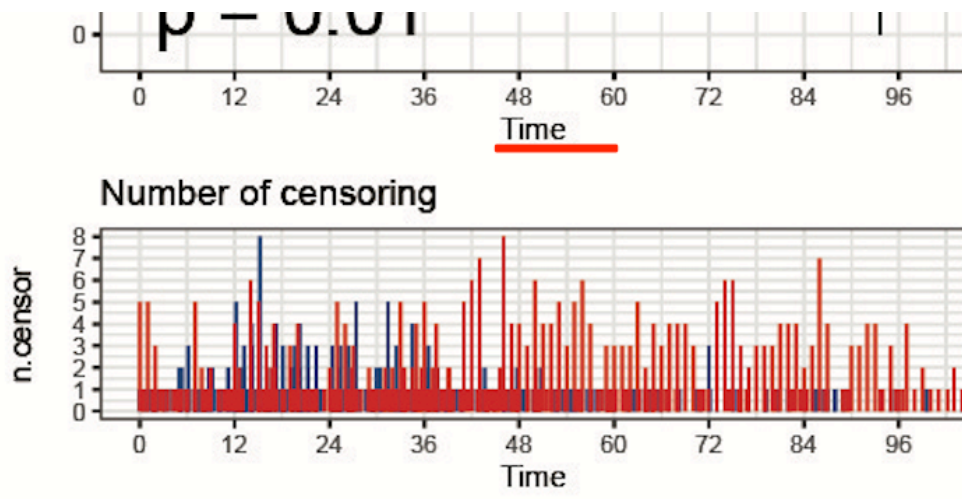
2. Please remove “Figure \*\*” from the figures (1-5).



**Response:** We have removed them.

### 3. Figure 1(E-I)

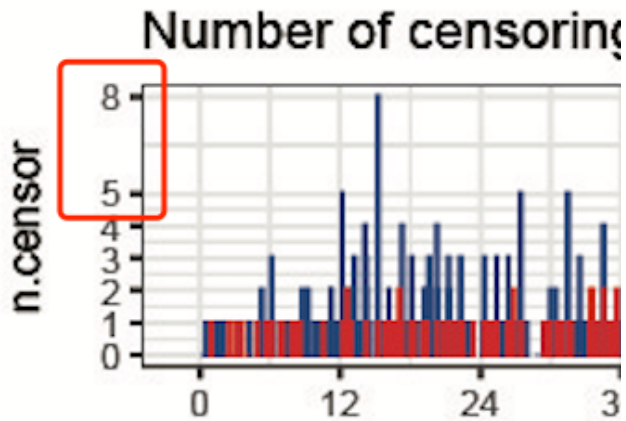
Please indicate the unit of Time.



**Response:** We have added the unit of time.

### 4. Figure 1E

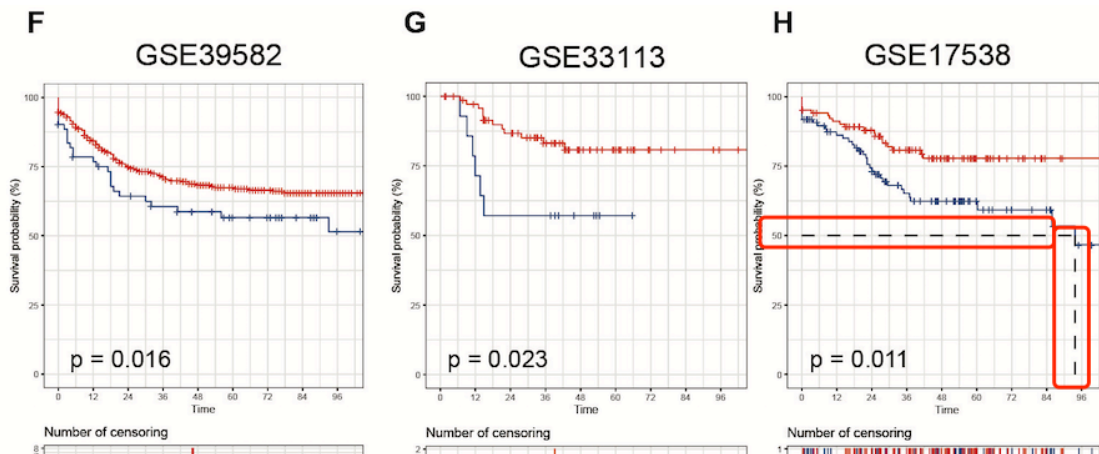
Numbers (6, 7) are missing in the Y-axis. Please add.



**Response:** We have added the missed numbers.

**5. Figure 1F, 1G**

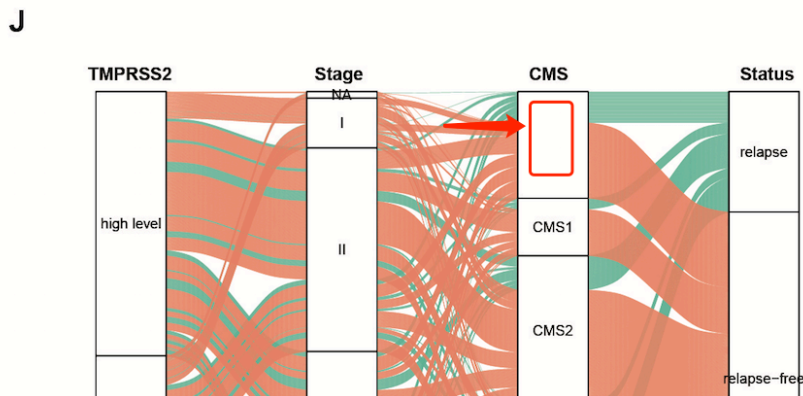
Please confirm if the median line is missing in figure 1F, G, as figure E, H has the median line.



**Response:** As median survival time was not reached in figure 1F and 1G during follow-up interval, the median line was not plotted.

**6. Figure 1J**

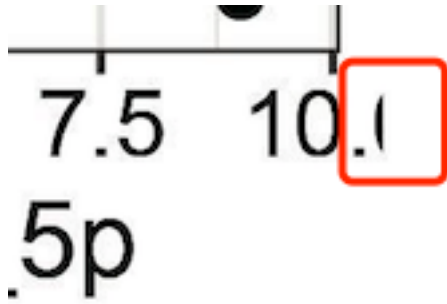
Please confirm if something missing in the box (pointed).



**Response:** We have added the missing information.

**7. Figure 3A**

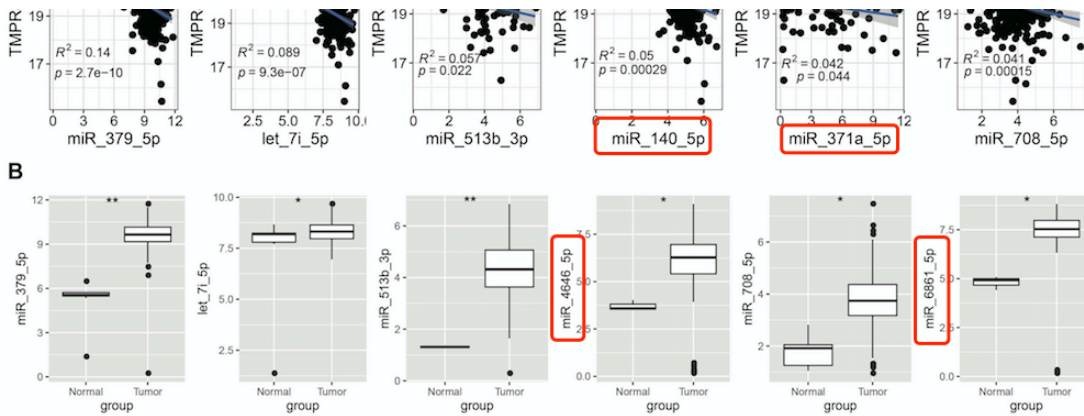
This number is incomplete in the X-axis. Please revise.



**Response:** We have revised it.

**8. Figure 3A, 3B**

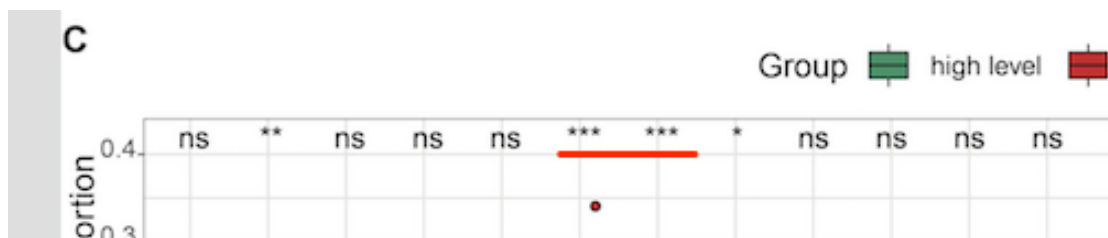
Please confirm if figure 3A and 3B are correct, as some miRNAs are not the same.



**Response:** We have re-checked the data and revised the legend.

**9. Figure 4**

Please indicate the meaning of “\*\*\*\*” in the figure legend.

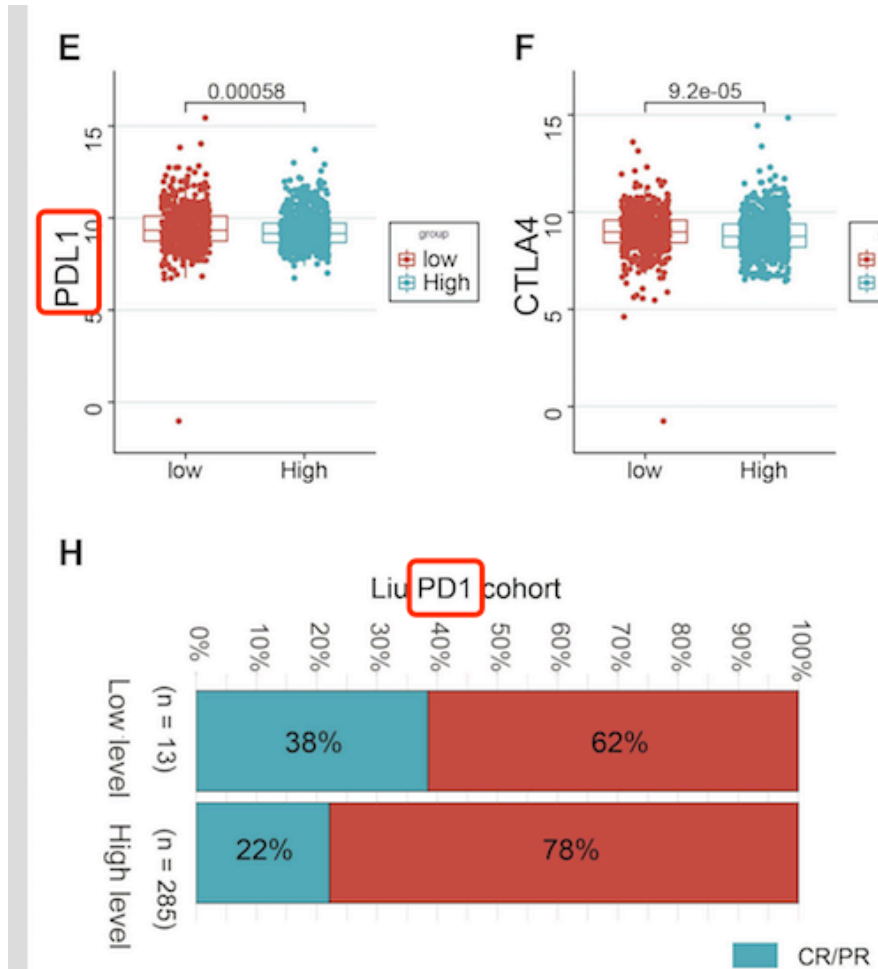


**Response:** We have added it.

**10. Figure 5E, H**

Please unify the expression. PD-L1 or PDL1

15 **Figure 5** Box plots for the differences of immune score (A), stromal score (B), estimate  
 16 score (C), MATH score (D), **PD-L1** expression (E), and CTLA-4 expression (F)  
 17 between the low and high TMPRSS2 expression group. Comparison of TMPRSS2

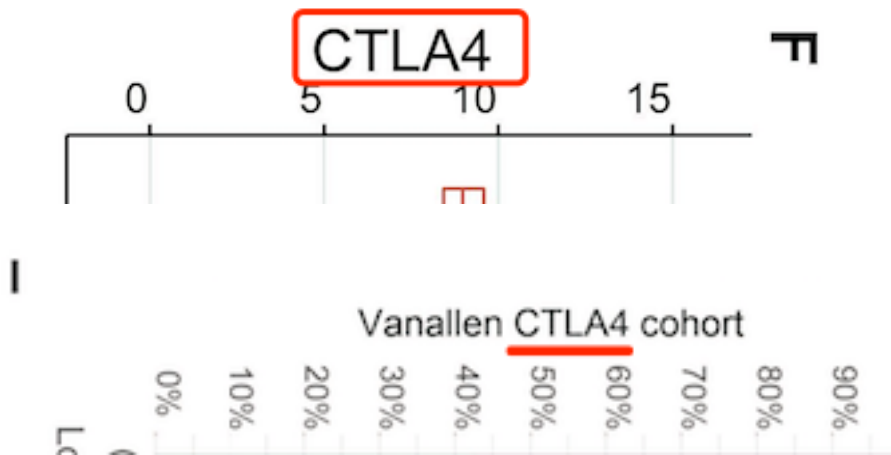


**Response:** We have revised it. In figure 5H, it is indeed PD-1 instead of PD-L1.

**11. Figure 5F, 5I**

Please unify the expression. CTLA-4 or CTLA4

15 **Figure 5** Box plots for the differences of immune score (A), stromal score (B), estimate  
 16 score (C), MATH score (D), PD-L1 expression (E), and **CTLA-4** expression (F)  
 17 between the low and high TMBPSS2 expression group. Comparison of TMBPSS2



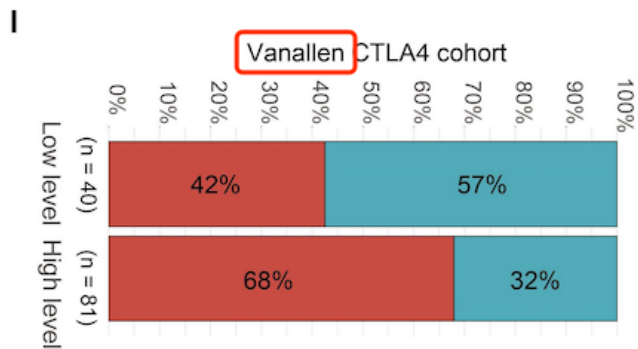
**Response:** We have revised it.

## 12. Figure 5I

The first author's name of ref. (22) is not "Vanallen". Please check and revise.

22. Miao D, Margolis CA, Gao W, et al. Genomic correlates of response to immune checkpoint therapies in clear cell renal cell carcinoma. Science 2018;359:801-6.

- 14 anti-CTLA-4 cohort (22), patients with low *TMPRSS2* expression displayed more  
15 effective clinical response to anti-PD-1 or CTLA-4 immunotherapies as compared to



**Response:** We have revised it.