

Dear Professor Ma:

Manuscript NO: **84710**

Title: **Proteins in Tumor-derived Exosomes as Candidate Biomarkers for Colorectal Cancer Revealed by Proteomic Analysis**

Thank you for your letter and the reviewer's comments concerning our manuscript. We truly appreciate the reviewer's valuable comments and suggestions. We have studied these comments very carefully and have tried our best to improve the manuscript. The followings are our point-by-point responses to the original reviewer's remarks, which is attached to the end of the letter.

Revised portions are marked in yellow in the revised version of the manuscript. I hope that the adjustments made to the manuscript are satisfactory, and I am looking forward to your correspondence!

All the best.

Yours Sincerely,

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Answer reviewer 03004840

Thank you very much for your kind suggestions to this manuscript. We appreciate your comments and suggestions a lot. Those comments are all valuable and very helpful for revising and improving our paper. We have made revisions or explanations point by point. Revised portions are marked in yellow in the paper. It should also be noted that for the sake of consistency between the textual and graphical presentation, TIF1B is corrected to TRIM28, whereas the two names represent the same substance. Thank you again for your review and suggestions.

1. What is the filtration strategy of selected candidate genes? The authors are requested to provide the more details for the selection filter criterias instead of “ based on the results and other findings” expression.

Answer: We are deeply sorry for the ambiguous statement. According to your suggestion, we have described the filtration strategy in as much detail as we can. Please see the details in the revised manuscript.

2.The authors are requested to add the “Statistical analysis” part to the materials and methods section.

Answer: Thank you very much for your kind suggestion, and we have added the “Statistical analysis” in the penultimate part of “Materials and methods” section in the revised manuscript.

3.Did the authors use tools for gene ontology and pathway analysis? All bioinformatic analysis was conducted using the R platform? Authors should indicate the bioinformatic analysis clearly in the “Bioinformatic analysis” part of the materials and methods section.

Answer: Thank you for your careful review and helpful comment. More details have been modified and added in the “Bioinformatic analysis” part of the revised manuscript, highlighted in yellow.

4. What was the recorded video time duration and rate of frames per second information for the Brownian motion of the particles in the NTA analysis. Authors should add this information to the related part of materials and methods section. It would be informative for the readers.

Answer: Thank you a lot for the valuable suggestion. We have carefully searched the raw data of NTA analysis and reviewed the whole analysis process from scratch. The recorded video time duration was determined 90 seconds and rate of frames per second was 30, which has been supplemented in the NTA part.

5. How much protein did the Authors use for the WB analysis? Authors are requested to give this information in the western blot analysis part of the materials and methods section.

Answer: We are sorry for unclear description. In the WB analysis, cell lysates were set as the positive control and each signature marker was detected with 10 μ g of total protein. For exosome samples, each signature marker was detected with 30 μ g of total protein. The information has been added in the western blot analysis part of the materials and methods section, highlighted in yellow.

6. Authors are asked to analyze these six proteins in terms of prognostic importance such as overall survival analysis.

Answer: Thank you for your kind suggestion. We apologize for not successfully carrying out survival analysis of protein expression, but we have used GEPIA to perform survival analysis of the genes corresponding to the candidate proteins in the TCGA and GTEx databases. These have been supplemented and presented in the “Materials and methods” section and “Results” section respectively.