

## Peer Review File

Article information: <https://dx.doi.org/10.21037/tcr-24-1374>

### Reviewer A

Concern #1: The word “subtypes” should be removed from the paragraph discussing triple-negative breast cancer (TNBC).

Reply1: We have removed the word “subtypes” from the specified paragraph to enhance clarity. (see Page 4, line 71).

Concern #2: Additional details about the colony formation assay would enhance the manuscript.

Reply2: We have added a more detailed description of the colony formation assay, including the specific conditions and duration of the assay. (see Page 8, lines 198-204).

Concern #3: The methodology used for the analysis of TCGA data presented in Supplementary Table 2 is not described.

Reply3: We have included a detailed explanation of the methodology used for the TCGA data analysis in the Methods section. (see Page 10, lines 266-269).

Concern #4: A more detailed analysis of OPN3 stratified by TNBC subtypes using bcGenExMiner would be beneficial.

Reply4: Your suggestion is particularly good, we have analyzed different subtypes of breast cancer using several databases and finally chose triple-negative breast cancer for our follow up study, in the next study we will do more in-depth research on different TNBC subtypes.

Concern #5: A brief explanation of the analysis in Supplementary Table 2 would be helpful.

Reply5: We have added a brief explanation of the analysis presented in Supplementary Table 2 to the Methods section. (see Page 10, lines 266-269).

Concern #6: Figures 2G and 3L should have DIPA replaced by DAPI.

Reply6: We have corrected the labels in Figures 2G and 3L to replace DIPA with DAPI.

Concern #7: Replicating the knockdown in vitro results in MDA-MB-231 cells would be beneficial.

Reply7: Thank you for your suggestion, we chose to perform two-way validation in the same cell line is indeed not rigorous enough, due to time constraints, we only used PCR and WB to validate the knockdown in MDA-MB-231 cells, the picture has been submitted as an attachment (supplementary Fig2), in the next step of the study, we will carry out more in-depth research on different subtypes of TNBC cell lines.

Concern #8: Including proliferation assays such as EdU, BrdU, MTT, or live-cell analysis would be beneficial.

Reply8: We performed a CCK8 assay to assess cell proliferation and have included the results in the Methods, Results section of the text and added the data to the images.

Concern #9: Higher magnification images for the EMT phenotypes would help discern differences among conditions.

Reply9: We have provided higher magnification images in Supplementary Figure 1 to better illustrate the changes in EMT phenotypes.

Concern #10: Mentioning that the upregulation of OPN3 may not be exclusive to TNBC

in the discussion would be beneficial.

Reply10: We have added a discussion regarding the potential upregulation of OPN3 in HER2-enriched subtypes and its implications. (see Page 17, lines 465-467).

### **Reviewer B**

Concern #1: The abstract should be structured with Background, Methods, Results and Conclusions.

Reply1: The abstract has been restructured into Background, Methods, Results, and Conclusions as per your suggestion. (see Page 21, lines 35-53)

Concern #2: Please describe more background information.

Reply2: Additional background information has been included in the abstract to provide more context. (see Page 2, lines 36-38)

Concern #3: Please provide the full name of PCR.

Reply3: The full name of qRT-PCR (quantitative Real-time PCR) has been unified throughout the manuscript. (see Page2, lines 44)

Concern #4: Please unify the full name of qRT-PCR.

Reply4: The full name of qRT-PCR (quantitative Real-time PCR) has been unified throughout the manuscript. (see Page2, lines 44)

Concern #6: Please check all abbreviations in the main text, such as, FBS, PBS, TM, HRP, SYBR, GAPDH, PCR, RIPA, etc. All abbreviated terms should be full when they first appear.

Reply6: All abbreviations in the main text have been checked and defined upon their first appearance.

Concern #10: Please define all abbreviations in all figure legends.

Reply10: All abbreviations in the figure legends have been defined.

Concern #11: Figure 1: there is no \*\* in the figure. Please remove it.

Reply11: The \*\* has been removed from Figure 1.

Concern #12: Figure 1F-G: please indicate the meaning of red and black colors.

Reply12: The meanings of the red and black colors have been indicated in the figure legend for Figure 1F-G.

Concern #13: Figure 1H-I: please revise “HR” to “HR (95%CI)”.

Reply13: The term “HR” has been revised to “HR (95%CI)” in Figures 1H-I.

Concern #14: Figure 2: please indicate the meaning of \*\*\*\* in the legend.

Reply14: The meaning of \*\*\*\* has been defined in the legend for Figure 2.

Concern #15: Figure 2A-C: please unify the normal mammary epithelial cells. Should it be HBL100 or BHL100?

Reply15: The term has been unified to HBL100 throughout the figures.

Concern #16: The citation of Figure 2C is missing in the text. Please check and revise.

Reply16: The citation for Figure 2C has been added in the main text. (see Page11, lines 344)

Concern #17: Figure 2D: please check the following number.

Reply17: The number in Figure 2D has been checked and corrected as necessary.

Concern #18: Figure 2E: Please check the citation of Figure 2E in your main text.

Reply18: The citation for Figure 2E has been corrected to ensure it appears in the proper order in the text.

Concern #19: Figure 2I-K: Please indicate the staining method.

Reply19: The staining method has been indicated in the legend for Figures 2I-K.

Concern #20: Figure 2J-K: Please provide the scale bar or magnification.

Reply20: The scale bar has been added to Figures 2J-K.

Concern #21: Figure 3: please indicate the meaning of \*\*\*\* in the legend.

Reply21: The meaning of \*\*\*\* has been defined in the legend for Figure 3.

Concern #22: Figure 3G-I, K: Please indicate the staining method.

Reply22: The staining method has been indicated in the legend for Figures 3G-I, K.

Concern #23: Figure 3H, I, K, M: Please provide the scale value for the scale bar.

Reply23: The scale values have been provided for the scale bars in Figures 3H, I, K, M.

Concern #24: Figure 3N: Please check the citation of Figure 3N in your main text.

Reply24: The citation for Figure 3N has been corrected to ensure it appears in the proper order in the text.

Concern #25: Figure 4: please indicate the meaning of ns, \*\*\*\* in the legend.

Reply25: The meanings of ns and \*\*\*\* have been defined in the legend for Figure 4.

Concern #26: Figure 4D: Please check the citation of Figure 4D in your main text.

Reply26: The citation for Figure 4D has been corrected to ensure it appears in the proper order in the text.

Concern #27: Figure 4G-H: Please provide the scale value for the scale bar.

Reply27: The scale values have been provided for the scale bars in Figures 4G-H.

Concern #28: Figure 4F-H: Please indicate the staining method.

Reply28: The staining method has been indicated in the legend for Figures 4F-H.

Concern #29: Figure 5: the citation of Figure 5 is missing in the text.

Reply29: The citation for Figure 5 has been added in the main text.

Concern #30: Figure S1: Please provide a summarized legend for Figure S1.

Reply30: A summarized legend for Figure S1 has been provided in the requested format.

Concern #31: Figure S1: there is no \*, \*\*, \*\*\* in the figure. Please remove the following sentence.

Reply31: The sentence indicating \*, \*\*, \*\*\* has been removed from Figure S1.

Concern #32: Figure S1C-D: Please provide the scale value for the scale bar.

Reply32: The scale values have been provided for the scale bars in Figures S1C-D.

Concern #33: Figure S1A-B: Please add unit (%) in the y-axis.

Reply33: The unit (%) has been added to the y-axis in Figures S1A-B.

Concern #34: Figure S2: the citation of Figure S2 is missing in the text.

Reply34: The citation for Figure S2 has been added in the main text.

Concern #35: Figure S2: please provide the figure legend of figure S2.

Reply35: The figure legend for Figure S2 has been provided.

Concern #36: Figure S2: please indicate the meaning of \*, \*\*, \*\*\* in the legend.

Reply36: The meanings of \*, \*\*, \*\*\* have been defined in the legend for Figure S2.

Concern #38: Table 1 and 2: please define TNBC, OPN3, TNM in the table footnote.

Reply38: The definitions for TNBC, OPN3, TNM have been added to the table footnotes.

Concern #39: Table S2: please add unit for age.

Reply39: The unit for age has been added in Table S2.

Concern #40: Table S2: please define OPN3, BRCA, TCGA, T, M, N, OS in the table footnote.

Reply40: The definitions for OPN3, BRCA, TCGA, T, M, N, OS have been added to the table footnotes.

Concern #41: Table S2: please revise " $\leq$ " to " $\leq$ ".

Reply41: The symbol " $\leq$ " has been revised to " $\leq$ " in Table S2.

Concern #42: Table S2: please revise "pvalue" to "P value".

Reply42: The term "pvalue" has been revised to "P value" in Table S2.

Concern #43: There is no \* in Table S2, but it is indicated in the foot.

Reply43: The indication of \* has been removed from the footnote of Table S2.

Concern #44: The overall language is acceptable. Some suggested changes have been marked below.

Reply44: We have carefully reviewed and revised the entire manuscript.