Dear reviewer,

Thanks for your letter and we have studied the comments carefully and have made corrections which we hope meet with approval.

We sincerely thanks for your kind enthusiasm and helpful comments regarding our paper.

Sincerely yours,
Bing Wang

Question : In later tests of this intrabody, the intrabody was made to express in the ER lumen, which is separated from the cytoplasmic side by the membrane and cannot interact with the cytoplasmic domains of BAP31 in an intact cell. This is a serious flaw in the study design, as the intrabody is not expected to work if it has no access to its epitope sequence. The authors need to test a different construct, expressing the intrabody in the cytosol and compare the effect with the construct they have in this submission. Without a convincing result of intrabody acting from the cytoplasmic side, all the observed effect can at best be attributed to a mechanism irrelevant to the one proposed in the manuscript.

Response: Thank you very much for the question raised by the reviewer. These comments are very valuable and helpful for revising and improving our paper, as well as the important guiding significance to my researches. This is also a challenge we faced during the experimental process. Because we found in our previous experiment that the expression level of VH-F12 was very low to the extent that bands were almost undetectable by western blotting. At the same time, we found that F12 did not have any positive function.

We speculate that F12 may be rapidly degraded in the cytoplasm and therefore have not enough time to effectively bind to BAP31. So we attempted to add signal peptides with the aim of guiding F12 into the ER lumen. Due to the fact that the translation, folding and maturation of BAP31 occurs within the ER lumen, guiding F12 entering the ER lumen could greatly increase its chances of binding to BAP31 (165-205). The experimental results confirm our hypothesis. The results showed that the addition of signal peptides enabled stable expression of F12 in cells and promoted F12 to effectively combine with BAP31 (165-205) (Figure 3A). Furthermore, our subsequent experimental results support our design for introduing signal peptides. The addition of signal peptides stabilized F12, promoted the binding of F12 to BAP31(165-205) , and thereby exerting its function of inhibiting gastric cancer.

The issues raised by the reviewer have sparked a rethinking of our mechanism. The new explanation is as follows: the combination of F12 with BAP31 in the ER lumen may affect the correct folding of BAP31 and thereby inhibiting the interaction between Sec61

and TRAP complexes. We have made corresponding modifications to the discussion section (line348-350) and schematic diagram (Figure 6).

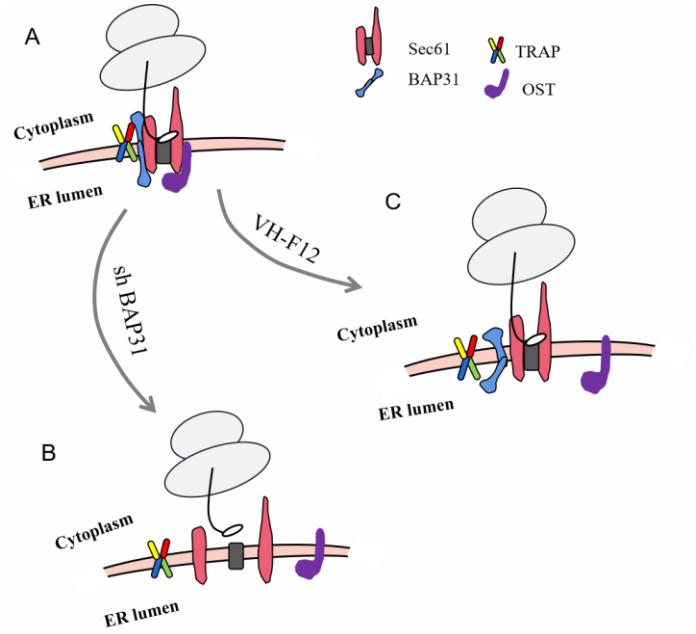


Figure 6. The simulation diagram of BAP31 participating in the process of EpCAM glycosylation.