

Dear Editor,

We greatly appreciate your review of our manuscript “Ca²⁺/Calmodulin Dependent Protein Kinase II Regulates Colon Cancer Proliferation and Migration via ERK1/2 and p38 Pathways”. Your suggestions are greatly helpful for our current and future researches on CaMKII signal in colon cancer. According to the reviewer’s comments, we revised our paper as following and highlighted all revised content in colors.

Reviewer 1:

1). First, in the Figure 1A, the methodology in quantitative analysis of DAB staining of paraffin block was not clearly depicted in material and method. The authors only depicted “----- was analyzed independently by two investigators and further processed with Adobe Photoshop CS5 extended computer software”. It is very confusing. The authors should make it clearly

Response: We revised this sentence as following:

Visualization was achieved with the DAB detection kit and the cell nuclei were counterstained using hematoxylin. After staining, five fields (at magnification of ×100) were randomly selected in each slide. Staining was quantified by Image J software and evaluated by two investigators following the principle of “blinded”.

2). Second, in Figure 1 B and C, the authors only got the paraffin block from the tissue bank. However the western blot and qPCR could be conducted in the tumor and surrounding tissue in the paraffin block.

Response: We changed the data of western blot and qPCR in our original fig. 1B and 1C. At the same time, western blot and qPCR data from paraffin block was used as replacement.

Reviewer 2:

1). According to my opinion important data about the IHC sampling are missing – were the samples of the same patient, were there 5 samples of each tissue (5paracancerous, 5 well dif.colon cancer, 5 poorly dif. Colon cancer) or 5 samples altogether. Who evaluated the samples and made histological assessment?

Response: Colon tissue samples were obtained from the Department of Gastrointestinal surgery at Renmin Hospital of Wuhan University. All samples were obtained from the primary colon sites of pretreatment cases of paracancerous lesions (n=5), well differentiated colon cancer (n=6) and poorly differentiated colon cancer tissues (n=6). All samples were analyzed and assessed by two histological specialists blindly.

2) minor written revision

Response: we revised our written and marked in red color.