Dear Editor of World Journal of Gastroenterology,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled "Involvement of methylation-associated silencing of FMN2 in colorectal carcinogenesis" [41540]. We have studied reviewer's comments carefully and have made revision in the paper. We have tried our best to revise our manuscript according to the comments. Attached please find the revised version, which we would like to submit for your kind consideration. We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,

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List of Responses

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Involvement of methylation-associated silencing of FMN2 in colorectal carcinogenesis"[41540]. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. The main corrections in the paper and the responds to the reviewer's comments are as flowing:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: (*My comment concerns the RT-PCR. Is there any data that demonstrate whether the RNA used is DNA free? Trizol isolation is not necessarily going to get rid of all the DNA, and I do not see any indication that any subsequent DNase step was performed. Therefore, is there any negative control, such as doing the RT-PCR but skipping the RT step? If the RNA is really DNA free, then performing PCR directly on the RNA should not yield a band. Can the authors comment on this, at least? If they have any of these RNA samples left over, showing that no product is formed without the RT step would be helpful.*)

Response: We would like to thank you for your review. In our article, the reverse transcription reaction was performed using ReverTra Ace qPCR RT Master Mix with **gDNA Remover** (TOYOBO). The kit of ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Code No. FSQ-301 200 reactions) includes reagents for reverse transcription and for the removal of genomic DNA [DNase I treatment]. The description of the official website is as follows: *In many cases, total RNA prepared using spin-columns or acid guanidium-phenolchloroform (AGPC) extraction methods contains small amount of genomic DNA. Any contaminating genomic DNA will be amplified along*

with cDNA, especially when primer pairs are designed within the same exon or from pseudogenes. Amplification from genomic DNA can result in qualitative and quantitative inaccuracies. The protocol consists of (i) a genomic DNA degradation step using "gDNA remover" and (ii) a reverse transcription step. The two steps can be achieved sequentially without purification or heat inactivation of DNase I. So in our article, the "genomic DNA degradation step" and "cDNA synthesis step" can be achieved sequentially in approximately 30 min by using this kit. More details and manual about this product (ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Code No. FSQ-301 200 reactions) can be found in official website of TOYOBO (http://www.toyobo-global.com/seihin/xr/lifescience/). At the same time, we also added a simple description in the method of our article.

Reviewer #2:

1. Response to comment: (*This study was well examined and written logically. There is no need of additional information to publish this article.*)

Response: We are very grateful to the reviewer for giving us such a high evaluation; we will continue to spend more time and energy to study the relationship between FMN2 and colorectal cancer.

Reviewer #3:

1. Response to comment: (*In this paper, authors found that promoter hypermethylation of FMN2 gene at CpG islands induce down-regulation of mRNA FMN2 expression in CRC at early stages of the disease. The manuscript is well written and the state-of-the-art is adequate to the field of research. The paper is also original because FMN2 expression in CRC was previously studied; however this is the first work however this is the first paper relating it to promoter hypermethylation. The study was conducted in cultured CRC cell lines, samples from patients and public base data from patients. The number of samples from patients is too small (only 9). The results are very interesting because they suggest that DNA hypermethylation may be an important early event in CRC, most likely playing a critical role in cancer initiation, and can serve as an ideal diagnostic biomarker in patients with early-stage colon cancer, although not for survival.*)

Response: We are very grateful to the reviewer for giving us such a high evaluation; we will continue to spend more time and energy to study the relationship between FMN2 and colorectal cancer. The reason about why we only selected 9 paired colorectal tumor samples and adjacent nontumor tissue samples for study listed below: because this is the first paper about FMN2 and promoter hypermethylation in CRC, so in this article we mainly want to know the features of promoter hypermethylation of 4 CpG regions (FMN2-1, FMN2-2, FMN2-3 and FMN2-4) and each CG site in CpG island regions through MethylTargetTM assays (targeted bisulfite sequencing) developed by Genesky BioTech (Shanghai, China), the result identified 3 CpG regions, namely, FMN2-2, FMN2-3 and FMN2-4, with a statistically significant difference (p=0.0069, p=0.0094 and p=0.0005, respectively), and all CG sites in FMN2-2 (13/13), FMN2-3 (23/23) and FMN2-4 (27/27) revealed a significantly stronger methylation pattern in the tumor tissues than in the corresponding noncancerous tissues. These results narrowed the scope of research and provided direction for our future research.