Dear Prof. Lian-Sheng Ma,

On behalf of my co-authors, we are very grateful to you for giving us an opportunity to revise our manuscript. We appreciate your positive and constructive comments and suggestions on our manuscript entitled **"Comprehensive Analysis of the Potential Role and Prognostic Value of the Sine Oculis Homeobox Homolog Family in Colorectal Cancers" (ID: 78685).** We have studied reviewers' comments carefully and tried our best to revise our manuscript according to the comments. The following are the responses and revisions we have made in response to the reviewers' questions and suggestions on an item-by-item basis. Our Manuscript was also polished by a native English speaker with biological background to make it easy understanding to readers. The revised portions are highlighted in yellow in the paper. Thank you again for the hard work of the editor and reviewers.

With many thanks and best wishes.

Jing Liu
Cancer Hospital of Shantou University Medical College

The main corrections are in the manuscript and the responds to the reviewers' comments are as follows point-to-point.

To Reviewer #1:

(1). The etiological and demographic information of the analyzed patient should be provided in a separate table. The expression pattern of SIX4 according to the stage may vary, and it will be useful to present it as a potential marker for an advanced stage of colorectal cancer.

Response: Thank you for your valuable and professional suggestions. The etiological and demographic information of the analyzed patients is now provided as Table 3 in Page 17. The reviewer's comments is critical that the expression of SIX4 may be vary in different stage. We used online dataset and clinical samples to analyze the expression characteristics of SIX4 in CRC, showing that SIX4 is an oncogenic gene in colorectal cancer, and the higher the pathological stage of colorectal cancer, the higher the degree of malignancy. We also discussed this in Discussion section in Page 22-23.

(2). The colorectal cancer-associated mechanism of SIX1 is well established rather to other types of SIXs. In this study, the rationale for selecting SIX4 as a major target for CRC prognosis is unclear. There was also no evidence suggesting that the expression of SIX4 was higher than that of other types of SIXs in the analyzed colorectal cancer tissues.

Response: Thank you for your in-depth analysis and practical comments. According to previous studies, studies on the SIX family in colorectal cancer are limited. SIX1 was the earliest discovered, and its cancer-promoting mechanism in colorectal cancer has been basically defined. However, there are few studies on

comprehensive analyses of SIXs in colorectal cancer. In this study, we tried to illustrate the potential expression pattern and prognostic values of SIXs in CRC. During the analysis processes, we found that although the mRNA expression levels of SIXs 1/2/4 were also increased in colorectal cancer, but the statistical significance was only found in the prognostic value of SIX4 on the OS and DFS in CRC patients. So our interesting was attracted by SIX4 in CRC after the comprehensive analysis of different SIXs. With the protein level of SIX4 in CRC tissues, the potential therapeutic target of SIX4 for CRC was confirmed. However, although this comprehensive analysis provided SIX4 as a potential biomarker for CRC, the underlying molecular mechanism needs further investigation, which may be involved in oxidative phosphorylation, respiratory chain activity and metabolism. We also discussed this part in Discussion section in Page 22-23.

(3). In Figure 3, further, the difference in OS by SIX1 is larger than in other types of SIXs.

Response: Thank you very much to the reviewer for your valuable comments. In Figure 3, although the CRC patients with high expression of SIX1 tends to have poor OS with hazard ratio (HR) = 2.11 (0.96 – 4.6), the statistical significance was not meet the criteria of $p < 0.05$. After analyzing different SIXs in OS of CRC patients, only high level of SIX4 predicts short OS in CRC patients, with HR = 2.28 (1.04 – 4.99). Interestingly, when analyzing the mortality rate of SIX1/4 in different periods, one patients with high SIX1 level was survived to the end, while no one with high SIX4 was survived at 120 months. All the results attracted our focus to SIX4 in CRC for further analysis. We also re-wrote the results of this part to make it clear in Page 11.

(4). The potential role of SIX4 in colorectal cancer as a potential biomarker has already been reported (PeerJ. 2017 May 30;5: e3394.). This is pointing out that it cannot be freed from the issue of novelty.

Response: Thank you for the critical comments. SIXs are a group of genes related to human organogenesis, and abnormal expression of SIXs was reported to involved in the oncogenesis and development of malignancies. However, the different expression pattern and prognostic value of SIXs in CRC was not analyzed and reported before. After comprehensive analyses of SIXs, among different family members, SIX4 attracted our interesting for further investigation, based on its high expression pattern and prognostic value in CRC patients. Our results evoked the potential value of SIX4 in CRC among different SIX members. Different from Li *et al*.'s research, our research provided the comprehensive analysis of SIXs in CRC, and further verified our findings at the clinical level. Furthermore, KEGG and GO analysis showed that SIX4 may be involved in oxidative phosphorylation, respiratory chain activity and metabolic processes, and we will further explore these aspects in the future. We also cited and discussed the difference and findings in our revised manuscript in Introduction section in Page 4-5, and in Discussion section in Page 22.

To Reviewer #2:

This manuscript aimed to explore the expression pattern of 6 SIXs in colorectal cancers and their relationship with the clinicopathological parameters of CRC patients. This study suggested that SIX4 may be a potential therapeutic target for treatment of CRC patients. It is an interesting article; however, the manuscript needs some revisions that should be considered. There are

grammatical errors, please carefully revise the English language throughout the text and correct all the trivial imperfections.

(1). Introduction: In my opinion, the authors have addressed the gaps in the current literature to justify the current review very well and made a strong statement on how their study adds to the literature, however, I suggest trying to condense the third paragraph or reduce the amount discussed. It would be better to be more focused on the prognostic value of SIXs in CRC and explain the previous studies results about this topic.

Response: First of all, we thank the expert for the recognition and rigorous comments. According to our results and your professional suggestions, the introduction had been revised to focus on the expression pattern and prognostic value of the SIX family in colorectal cancer and previous studies in Page 4. We also reviewed the reported research about SIXs in the prognostic value in different types of malignancies in Page 5. We hope that our revised manuscript could attract readers and make the purpose more clear.

(2). Method: How did the authors calculate the sample size? Are 87 and 93 samples statistically enough? The authors did not provide references on how they evaluated the ranks of RNA and protein expression.

Response: Thank you for the reviewer's critical and professional comments. We used an online calculator to calculate the sample size, that is http://www.powerandsamplesize.com/. For analysis, the parameters were set as Power = 0.8 and Type I error rate = 5%, and the sample size was calculated as 67. According to our tissue microarray, there were 93 patients with colon cancer, of which 87 patients had corresponding adjacent tissues, which provided enough sample size for our analysis. For evaluating the ranks of

RNA and protein expression, the reference was cited accordingly in MM section in Page 5-6.

(3). Discussion: -The discussion is at a very superficial level. It is short and didn't compare the results with the findings from the other studies properly. In fact, "discussion" doesn't add any useful information to the manuscript. The first paragraph of the discussion should address the aim and important results of the study. The results of the study should be discussed one by one, and it is suggested to use the other studies to support your biochemistry claims and hypothesis and extend the objectives of your discussion. Why and under which mechanisms SIX4 can act as a tumor-promoting factor in the intestinal tract? and please discuss the expression pattern of the other SIXs clearly (the author can use more references for the raised claims and hypothesis). Moreover, the strengths and limitations of the present study should be mentioned and discussed. Also, it would be more interesting to read some sentences suggesting what factors should future studies consider?

Response: Thank you for your critical comments. According to the professional views and the results of our study, the first paragraph has focused on the purpose and main results of the article. Then we analyzed the results one by one sequentially. The expression pattern of different SIX was also described in the Discussion section to raise our claims and hypothesis. Based on the findings of different expression and prognostic value of SIXs, our interesting was focused on SIX4, which was evoked as a potential target for CRC treatment. Our manuscript first time provided a comprehensive analysis of SIXs in CRC, however, further investigation should be conducted to uncover the underlying molecular mechanism as SIX4 was predicted to be involved in oxidative phosphorylation, respiratory chain activity and metabolism. The revised Discussion

section was highlighted in Page 20-23.

To Reviewer #3:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: The author has done a reasonably good work. My only suggestion: Please include number of Ethical approval.

Response: Thank you for the reviewer's positive comments. According to the reviewer's suggestion, we added the number of Ethical approval (SUMC-2022-45) in MM section in Page 6. Our Manuscript was also polished by a native English speaker with biological background to make it easy understanding to readers.

To Editorial Office's comments #

I recommend the manuscript to be published in the World Journal of Gastrointestinal Oncology. Before final acceptance, when revising the manuscript, the author must supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript. To this end, authors are advised to apply a new tool, the Reference Citation Analysis (RCA). RCA is an artificial intelligence technology-based open multidisciplinary citation analysis database. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: https://www.referencecitationanalysis.com/.

Response: Thank you for the editors' valuable and professional suggestions

and comments. The RCA database has provided great help for us in finding the latest highlight articles, which were cited in our revised manuscript. We revised the manuscript according to reviewers' comments and suggestions. Our Manuscript was also polished by a native English speaker with biological background to make it easy understanding to readers.

To Revision reviewer #1:

Specific Comments to Authors: All concerns have been well addressed. There is no comment to raise.

Response: Thanks for your comments.

To Revision reviewer #2:

Specific Comments to Authors: I have no comments.

Response: Thanks for your comments.