Dear editor:

Thank you for editor' and reviewers' opinions, these comments are very helpful to improve the quality of the manuscript. We have added the dual luciferase experiment to demonstrate the interaction between *COL5A2* and miR-144-3p, as well as between miR-144-3p and ENTPD1-AS1. We evaluated the changes in the transcription level of *COL5A2*, miR-144-3p and ENTPD1-AS1 after transfection of gastric cancer cells with si-ENTPD1-AS1 or miR-144-3p mimics. Words in bright yellow are the changes we have made in the manuscript. Now I response the reviewers' comments with a point by point and highlight the changes in revised manuscript. We sincerely hope that you find our responses and modifications satisfactory and that the manuscript is now acceptable for publication.

Reviewers' comments:

Reviewer #1:

Specific Comments to Authors: I have now reviewed your paper and recognize the importance of your research question. Manuscript NO. 81996 aimed to gain insight into the upstream competing endogenous RNA regulatory mechanism and immune microenvironment related to COL5A2 in Gastric Cancer (GC). Manuscript formatting should be revised according to BPG guidelines.

1. The main and short titles accurately reflect the major topic and content of the study. Overall, the Abstract should be further improved. Consider the Abstract as the section that will draw readers' attention to your manuscript. There is no clear delineation of the study's BACKGROUND. It would be interesting to bring data regarding GC itself and the already-established role of miRNAs and ncRNAs in this neoplasm. The "RESULTS" subsection should provide detailed important data from the research findings. Finally, the CONCLUSION subsection of the Abstract should further explore the limitations of the study and future prospects in the research field.

   Thank you for the above suggestion. Following your suggestion, we have made changes to the Abstract section. We highlight it in the revised manuscript.

2. The CoreTip should also contextualize the reader in the research question, present the most relevant findings and justify the relevance of the study (all in no more than 100 words).

   We have made corresponding modifications to CoreTip.

3. The INTRODUCTION should be improved. There is a gap between the need to find biomarkers that could benefit the detection and treatment of GC, the expression of COL5A2, and the aims of the study. The state-of-the-art of already known biomarkers in gastric cancer should be addressed. The study's AIM should also be clearly stated.
   We gratefully appreciate for your valuable suggestions. The AIM of the study is to explore the upstream competing endogenous RNA regulatory mechanism and immune microenvironment related to COL5A2 in Gastric Cancer (GC) instead of finding new biomarkers of GC. We have modified the abstract to make it more logical and consistent with the content of the article. These modifications are highlighted in the INTRODUCTION section of the revised manuscript.
4. Please remember that if you are using an acronym, you must introduce it with full terminology in the first instance so your reader knows what it means. You can do this by giving the full term first and the shortened version in parentheses: "The Cancer Genome Atlas (TCGA)".
   We have followed the recommendation to first write out the full name of an abbreviation when using it.
5. The MATERIALS AND METHODS could be further detailed. The use of specific approaches and platforms should be justified. Most of the information concerning this section is mentioned alongside the RESULTS section. Which was the threshold criteria for the statistical significance of gene expression? "
   We have supplemented and improved the MATERIALS AND METHODS section. $p<0.05$ represents a statistical difference, which we have added in the article.
6. [RESULTS section] In line with previous studies, significantly high expression of COL5A2 was found in TCGA data and 27 paired specimens (Figures 2A and 2B)" - please cite the studies. Which was the nominal p-value for each enriched signaling pathway in KEGG? This data should be presented in Supplementary Table S2 (interesting results, by the way). Please, also cite the nominal p-value for the correlation between miR-1443p expression and GC survival in the main text. This issue should be addressed in the other sections as well.
   We have cited references in Figures 2A and 2B, Reference 21. The p-value of KEGG analysis has been added to Supplementary Table S2. We have quoted the p-value in the main text.
7. The DISCUSSION section is well organized. This section describes your

main findings based on systematic theoretical analyses of the results. In the CONCLUSION, however, should further explore the limitations of the study and future prospects in the research field.

Thanks for your suggestion. In the CONCLUSION section, we have added the shortcomings of this article and the content that needs further research.

Reviewer #2:

Specific Comments to Authors:

1. What is the content of ENTPD1-AS1 -- miR-144-3p and COL5A2 in cells?

   Thank for your comments. We supplemented the dual luciferase experiment to verify the direct interaction between ENTPD1-AS1, miR-144-3p and *COL5A2*. After transfection with miR-144-3p mimics or si-ENTPD1-AS1, we detected the expression of ENTPD1-AS1, miR-144-3p and *COL5A2* in AGS cell. The results are shown in Figure 5.
2. The discussion focuses on the mechanism research.

   Thank for your comments. We have added some shortcomings and areas for further research.
3. The analysis of supplementary Table 1 is too simple.

   Considering the Reviewer's suggestion, we have added more analysis on supplementary Table 1. The supplementary content is: Excluding patients with missing or incomplete information, 322 of the 433 patients were included in the cox regression analysis. From clinical information, it can be seen that patients with gastric cancer are mainly in the middle and late stages, with a high rate of lymph node metastasis, less distant metastasis, and a high mortality rate (Supplementary Table S1).
4. Small sample size and sample inclusion criteria.

   We thank the reviewer for pointing out this issue. Currently, we can only collect a small number of paired gastric cancer specimens and perform simple verification. We will continue to collect more specimens in the future. Sample inclusion criteria have been added to the MATERIALS AND METHODS section, "sample collection".

Reviewer #3:

Specific Comments to Authors: In this manuscript, authors combined bioinformatics with wet lab experiments validation to reveal an ENTPD1-AS1/miR-144-3p/COL5A2 axis in gastric cancer. Firstly, they

checked the expression level of COL5A2 in diverse cancer types and found COL5A was upregulated in GC and predicted a worse survival of GC patients. Then, TCGA data proved this point. Next, they identified miR-144-3p was an upstream regulator of COL5A and could be sponged by a lncRNA ENTPD1-AS1. Finally, they demonstrated that COL5A expression was associated with macrophage infiltration. Here I have several comments for the authors.

1. A major issue is that the main regulatory relationships between miR-144-3p/COL5A2, and ENTPD1-AS1/miR-144-3p need to be validated by experiments. Solely in-silico analysis is not enough.

   We totally understand the reviewer's concern. We validated the interaction between miR-144-3p/COL5A2, and ENTPD1-AS1/miR-144-3p, in 293 T cells and AGS cells using a dual luciferase assay. We also detected the expression of ENTPD1-AS1, miR-144-3p and COL5A2 in AGS cells after transfection with miR-144-3p or si-ENTPD1-AS1. The results are shown in Figure 5.

2. How is the physiological function of COL5A in immune cell (particularly macrophage) infiltration? This should be introduced in the background section.

   Thanks for your suggestion. We added the physiological function of *COL5A* in immune cell (particularly macrophage) infiltration in the background section, as follows: *COL5A* is also found closely associated with immune cell infiltration, which may be related to the inhibitory effect of collagen on the production of CCL2[5]. In proliferative diabetic retinopathy, *COL5A2* is closely related to the infiltration of M2 macrophages[11].

3. The basic information and function of ENTPD1-AS1 and miR-144-3p need to be mentioned.

   Thanks for your suggestion. There is relatively little research on ENTPD1-AS1. We added "ENTPD1-AS1 is an antisense lncRNA that may be associated with short stature[18]. Recently, ENTPD1-AS1 is considered to be a new ncRNA that regulates the proliferation and apoptosis of cancer cells and also serves as a prognostic marker in Glioblastoma Multiforme[19, 20]." to the background section. We also added the information and function for miR-144-3p to the background section.

4. In the sentence of Abstract: The high expression of COL5A2was positively linked to macrophage infiltration in GC. There should be a space between "COL5A" and "was".

Thank you so much for your careful check. We have made corrections.