Dear reviewers:

Thank you very much for your comments and professional advice. These opinions help to improve the academic rigor of our article. Based on your suggestion and request, we have corrected the revised manuscript's modifications. Furthermore, we would like to show the details as follows:

Response to the reviewer's comments:

Reviewer 1:

Comment 1: There were a total of 6 5'tiRNAs with differences in expression between SSLs and NCs?

Response: We would like to thank you for your careful reading and helpful comments, which have significantly improved the presentation of our manuscript. As you noticed, there were a total of six 5′tiRNAs with differences in expression between SSLs and NCs, which we incorrectly wrote as five in the manuscript. We are sorry for that. We have amended this to "Our previous screening criteria showed that six 5′tiRNAs (five upregulated and one downregulated) with different expression levels emerged between SSLs and NCs." (See page 11, lines 16-18), and we have added that "Since we previously reported increased levels of 5′tiRNA expression in SSLs (Figure 2A and B), we herein focus on the abovementioned five upregulated 5′tiRNAs in SSLs." in page 11, lines 24-25. Thanks again for your valuable comment.

Comment 2: The differential multiple of down-regulated genes is very high. Why is there no further study?

Response: We would like to thank you for your constructive suggestions, which have significantly improved the presentation of our manuscript. Since our study found at the beginning that the expression of 5′tiRNA increased in SSLs compared with that of NCs (Figure 2A and B), we further focus on 5′tiRNA, especially upregulated 5′tiRNA in SSL. As you say, tiRNA-1:33-Gly-CCC-3 is downregulated in SSLs at a high multiple. Therefore, tiRNA-1:33-Gly-CCC-3 may be worthy of further in-depth study in the future. Thanks again for your valuable comment.

Comment 3: The data and statistics of polyp size are not mentioned in the previous article. Please analyze how the gene locus is related to polyp size.

Response: We would like to thank you for your important tips and advice, which have significantly improved the presentation of our manuscript. As per your suggestion, we have added information on the sizes of all 16 SSL lesions in the manuscript, i.e., as written in the text that "The size of all collected lesions ranged from 4 to 15 mm, with an average of 6.31±3.07 mm." (see page 11, lines 28-29).

To further demonstrate the relationship between 5′tiRNA-Pro-TGG and lesion size, in addition to the previous correlation analysis between its expression level and lesion diameter, we added bar graphs to analyze the mean lesion diameter between high and low 5′tiRNA-Pro-TGG expression groups. The results showed that the lesion diameter in the 5′tiRNA-Pro-TGG high expression group

was significantly larger than that in the low expression group ($P < 0.05$). More description of the details has been added in our manuscript "We further analyzed the correlation between the lesion size and the expression levels of the three 5′tiRNAs that had been validated as significantly highly expressed in SSLs. It was tiRNA-1:33-Pro-TGG-1 (Figure 4A-B), not tiRNA-1:33-Gly-CCC-2 or tiRNA-1:34-Thr-TGT-4-M2 (Figure 4C-D), that positively correlated with lesion size." (see page 12, lines 7-10), and in Figure 4B as attached. Thanks again for your valuable comment.

Figure 4

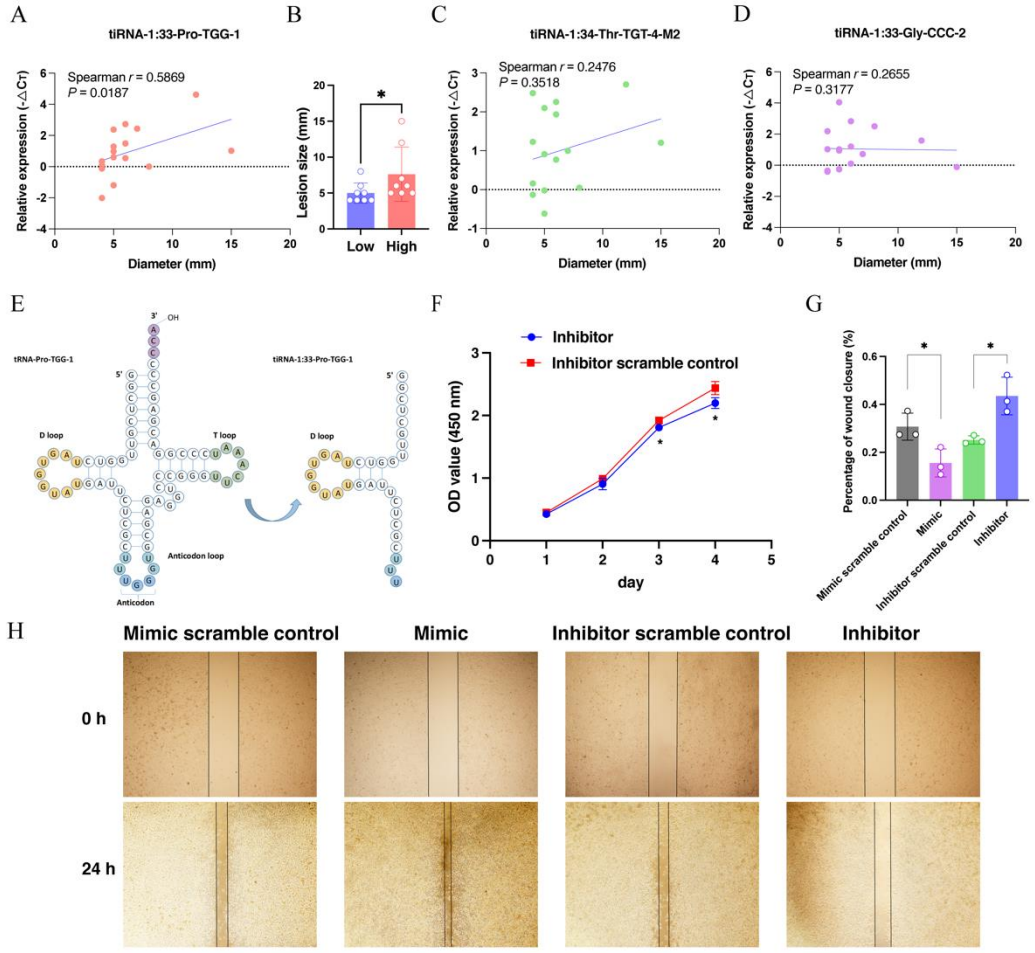

Figure 4. tiRNA-1:33-Pro-TGG-1 is associated with lesion size and promotes oncogenesis in CRC cells. (A) Correlation analysis of the lesion sizes and expressions of tiRNA-1:33-Pro-TGG-1 of SSLs. The X-axis represents the diameter of the lesion and the Y-axis represents the expression level of tiRNA-1:33-Pro-TGG-1. (B) Lesions in the tiRNA-1:33-Pro-TGG-1 high-expression group are larger than that of the low-expression group. Lesions are divided into high- and low-expression groups based on the median tiRNA-1:33-Pro-TGG-1 expression levels. The lesion sizes are compared between the two groups. (C-D) Correlation analysis of lesion sizes and the expressions of tiRNA-1:34-Thr-TGT-4-M2 (C) and tiRNA-1:33-Gly-CCC-2 (D) of SSLs. (E) 5′tiRNA-Pro-TGG is a type of 5′tiRNA that originated in tRNA-Pro-TGG-1. (F) 5′tiRNA-Pro-TGG knockdown suppressed the proliferation of RKO cells compared to that of the scramble control as determined by CCK-8 assays. (G-H) Wound-healing assays demonstrated that the overexpression of 5′tiRNA-Pro-TGG promotes cell migration and the knockdown of 5′tiRNA-Pro-TGG inhibits cell migration when compared to those of the corresponding scramble control, respectively. The asterisk indicates

a significant difference between the two groups (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$). SSLs, sessile serrated lesions.

Comment 4: There is no data on survival outcomes in the previous article. Please analyze how to compare survival outcomes of patients with polyps?

Response: Thank you for pointing out this problem in our manuscript. In this study, we analyzed the prognosis of colorectal cancer patients with high and low levels of HPSE2 expression using overall survival but did not analyze the survival outcomes of patients with SSL. This is a shortcoming of our study. Survival outcomes in patients with polyps were usually measured by the rate of recurrence of polyps and the time of the progression of polyps to advanced neoplastic lesions (ANLs). ANLs included advanced adenomas and cancers. It was reported that SSL was associated with the formation of concurrent advanced tumors. Meanwhile, SSL was thought to have the malignant potential to rapidly progress to CRC in a short period of time [1,2], with two case reports reporting rapid progression of SSL to invasive tumors at 8 and 13 months respectively[3,4]. We will follow up and monitor SSL patients for recurrence and disease progression in subsequent studies.

This section is a limitation of our article and we have included in the limitations section of our article that "In addition, the expression level of 5′tiRNA-Pro-TGG and its association with recurrence and prognosis in SSL patients require further studies in large samples.". (see page 15, lines 25-27).

References

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2 Hazewinkel Y, de Wijkerslooth TR, Stoop EM, Bossuyt PM, Biermann K, van de Vijver MJ, Fockens P, van Leerdam ME, Kuipers EJ, Dekker E. Prevalence of serrated polyps and association with synchronous advanced neoplasia in screening colonoscopy. *Endoscopy* 2014; **46**: 219–224. [PMID: 24254386 DOI: 10.1055/s-0033-1358800]

3 Oono Y, Fu K, Nakamura H, Iriguchi Y, Yamamura A, Tomino Y, Oda J, Mizutani M, Takayanagi S, Kishi D, Shinohara T, Yamada K, Matumoto J, Imamura K. Progression of a sessile serrated adenoma to an early invasive cancer within 8 months. *Dig Dis Sci* 2009; **54**: 906–909. [PMID: 18688718 DOI: 10.1007/s10620-008-0407-7]

4 Amemori S, Yamano H-O, Tanaka Y, Yoshikawa K, Matsushita H-O, Takagi R, Harada E, Yoshida Y, Tsuda K, Kato B, Tamura E, Eizuka M, Sugai T, Adachi Y, Yamamoto E, Suzuki H, Nakase H. Sessile serrated adenoma/polyp showed rapid malignant transformation in the final 13 months. *Dig Endosc* 2020; **32**: 979–983. [PMID: 31677187 DOI: 10.1111/den.13572]

Reviewer 2:

Comments: (A)An overview of the manuscript In this study, the specific role of tRNA halves (tiRNAs), a subcategory of Transfer ribonucleic acid (tRNA)-derived small RNAs (tsRNAs) in sessile serrated lesions (SSLs) in the colon, was investigated. Results suggest that tiRNAs could promote the development of SSLs and CRC progression via immune pathways. (B) Introduction and discussion: All relevant previously published studies have been cited The work's aims, significance, and novelty are clearly outlined in the manuscript. (C) Materials and methods: The experimental methods and statistical analyses are appropriate. Moreover, the authors should avoid using alternative methods or adding additional experiments to their current work. The manuscript complies with relevant national or international ethics guidelines. Any misidentified cell lines have been used. (D) Results The data presented is convincing regarding the reliability and validity of the results and figures. The authors present the relevant controls. The data support all the conclusions made by the authors. (E) The level of the English The manuscript does not require further revisions by a language editing company or a native English speaker. However, minor revision is required.

Response: Thank you for your comments and for recognizing our work. We have revised our manuscript according to your advice.