

Dear Editors and Reviewers:

Thank you for your letter and the reviewers' comments concerning our manuscript entitled "Inhibition of exosomal miR-191 accelerates ferroptosis and apoptosis of colorectal cancer cells by inducing ferroptosis in macrophages" (113524). Those further 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:

Reviewer #1

(1) Comment: Please specify the exact concentrations and durations for exosome treatment in co-culture experiments.

Response to comment: Thank you for your comment. In our experiment, the exosome treatment was applied at a concentration of 1×10^7 exosomes/mL with a treatment duration of 48 hours. The relevant details have been added in the corresponding section and are clearly marked.

(2) Comment: Also, provide catalog numbers for key reagents (antibodies, ELISA kits) to enhance reproducibility.

Response to comment: Thank you for your comment. We made the necessary revisions according to your suggestions and are clearly marked.

(3) Comment: In the discussion section, limitations of the study are necessary. The study lacks validation in patient-derived samples or clinical data, which is acceptable for a mechanistic study but could be noted as a limitation.

(4) Response to comment: Thank you for your comment. As requested, we have now added this content to the relevant section of the manuscript, and the changes have been clearly marked.

(5) Comment: Some pictures lack scale bar, such as Figure 3D, Figure 5D, Figure 6A and Figure 6D. It is suggested that they be improved.

Response to comment: Thank you for your comment. As requested, we have now added this content to the relevant section of the manuscript.

(6) Comment: -The results of Western blotting should be quantitatively statistically analyzed; otherwise, it will be difficult to accurately explain the results of this experiment

Response to comment: We thank the reviewer for this insightful comment. We agree that this information is important for supporting our conclusions. Following the reviewer's suggestion, we have now included these data as Supplementary Figure 1. We hope the revised manuscript with this addition fully addresses the reviewer's concern.

Reviewer #2

1. Comment: It is widely recognized that microRNAs (miRNAs) direct the RNA-induced silencing complex (RISC) to target mRNAs through imperfect base-pairing between their seed region (nucleotides 2-8 at the 5'end) and complementary sequences in the 3' untranslated region (3' UTR), thereby repressing translation or accelerating mRNA degradation and ultimately reducing protein output. Multiple studies have demonstrated oncogenic up-regulation of miR-191 in various malignancies, including colorectal cancer (CRC); nevertheless, its biological roles and molecular mechanisms within CRC-derived exosomes remain elusive. Consequently, the authors selected miR-191 as the focus of the present investigation. However, no systematic target prediction or functional validation of miR-191 regulated mRNAs was performed. The authors are requested to provide the specific rationale for omitting these analyses.

Response to comment: We thank the reviewer for their insightful comments. This study primarily focuses on the role of exosomal miR-191 in colorectal cancer (CRC), specifically investigating its ability to promote M2 macrophage polarization and its subsequent effects on ferroptosis, apoptosis, and tumor growth. Our work emphasizes the exploration of relevant phenotypic relationships rather than delving into the underlying molecular mechanisms. We greatly appreciate the reviewer's suggestion, and clarifying these mechanisms will indeed be a key focus of our future research.

2. Comment: In the animal experiments, the authors administered exosomal

miR-191 together with Erastin to mice, yet they did not employ miR-191-loss of function approaches (e.g., knock-down) or the ferroptosis inhibitor ferrostatin-1 for validation. We recommend establishing an miR-191 knock-down model to further clarify the functional role of miR-191 in vivo.

Response to comment: We thank the reviewer for their valuable suggestion. We agree that establishing an miR-191 knockdown model (e.g., via knockout or knockdown of miR-191 expression) would provide stronger evidence to further elucidate its function in vivo. Although the current study explored the role of miR-191 in ferroptosis through the combined application of exosomal miR-191 and Erastin, we acknowledge that validation via miR-191 knockdown was lacking.

To further verify the specific role of miR-191 in vivo, we plan to establish an miR-191 knockdown mouse model in subsequent experiments. This model will be used to evaluate the function of miR-191 in ferroptosis, tumorigenesis, and other related biological processes.

3. Comment: The flow-cytometry plot shown in Figure 3B is not readily matched to the corresponding bar graph. Please revise it to the same format used in Figure 4A and Figure 5B, or add the grouping and treatment labels to the flow cytometry result graph.

Response to comment: We have made the revisions according to your suggestions.

Reviewer #3:

1. Comment: The title is descriptive but overly long and slightly repetitive. Consider simplifying to: **“Exosomal miR-191 promotes colorectal cancer progression by inducing M2 macrophage polarization and inhibiting ferroptosis”**
Or: **“Inhibition of exosomal miR-191 suppresses CRC growth via macrophage ferroptosis and M2 polarization”**

Response to comment: We have made the revisions according to your suggestions.

2. The abstract should briefly mention the methods used for in vivo experiments (e.g., nude mouse model, Erastin treatment).

Response to comment: We believe this point has been adequately addressed and described in the section of our manuscript.

3. The conclusion could be strengthened by explicitly stating the novelty: e.g., **“This study reveals a novel mechanism by which exosomal miR-191 modulates the tumor microenvironment...”**

Response to comment: As you suggested, we have added this sentence to the relevant section.

4. The introduction is well-written and provides sufficient background. However, it would benefit from a clearer statement of the research gap and hypothesis at the end.

Response to comment: We have made the corresponding revisions to the relevant sections based on your suggestions.

5. Include scale bars in H&E images .

Response to comment: We have made the revisions according to your suggestions.

6. The discussion is thorough but could be more focused on the novelty of the findings—specifically, the dual role of exosomal miR-191 in regulating both macrophage polarization and ferroptosis.

Response to comment: We have provided corresponding descriptions in the relevant sections based on your suggestions.

7. The Western blot results require quantitative statistical analysis.

Response to comment: We thank the reviewer for this insightful comment. We agree that this information is important for supporting our conclusions. Following the reviewer's suggestion, we have now included these data as Supplementary Figure 1. We hope the revised manuscript with this addition fully addresses the reviewer's concern.

8. Some sentences are awkwardly phrased. Consider professional editing for fluency.

Example: “We succeeded in obtaining exosomes derived from CRC cells” → “We successfully isolated exosomes from CRC cells”

Response to comment: We have made the corresponding revisions to the relevant sections based on your suggestions.

9. Use either “miR-191” or “microRNA-191” consistently throughout.

Response to comment: We have made the corresponding revisions to the relevant sections based on your suggestions.