Point-to-Point Response to Reviewers' Comments

We sincerely thank the editors and reviewers for their insightful and constructive comments on our manuscript titled " Extramural Venous Invasion in Gastric Cancer: Preclinical Evaluation via 9.4 T MRI and the Functional Role of Associated circRNAs ". We have carefully considered each suggestion and made the necessary revisions to improve the clarity, scientific rigor, and overall impact of our work. Below, we provide a detailed response to each comment.

Reviewer #1 Comments and Responses

1. Introduction:

- **Comment 1:** The introduction effectively sets the stage for the study; however, the transition from the EMVI imaging findings to the molecular role of hsa_circ_0097977 could be made smoother.

- **Response 1:** Thank you for your suggestion. We have revised the introduction to provide a smoother transition from EMVI imaging findings to the molecular role of hsa_circ_0097977.

- **Comment 2:** Clarify the rationale for selecting hsa_circ_0097977 among other circRNAs and how it specifically connects to EMVI.

- **Response 2:** Thank you for your valuable feedback. We appreciate your emphasis on clarifying the rationale for selecting hsa_circ_0097977. We would like to explain that hsa_circ_0097977 was identified as a novel circRNA in our study, and no prior literature directly links it to GC or EMVI.

  To address your comment, we have added an explanation in the DISCUSSION section regarding the differential expression of hsa_circ_0097977 between EMVI-positive and EMVI-negative cases, as well as its potential impact on cellular functions. Additionally, we have expanded the INTRODUCTION section to highlight the significant role of circRNAs in GC, including their involvement in tumor progression and metastasis, and their potential as diagnostic biomarkers and therapeutic targets.

  We believe that these revisions provide a clearer rationale for our study and emphasize the broader significance of circRNAs in GC research. Thank you again for your insightful comments.

2. Methods:

- **Comment 1:** Include more context on why a 9.4T MRI was chosen and whether this resolution is directly translatable to clinical imaging technologies.

- **Response 1:** We thank you for your suggestion and have added a new segment to the DISCUSSION section that explains why we used 9.4T MRI. This addition highlights the improved spatial resolution and signal-to-noise ratio of the technology, which are crucial for identifying the EMVI features. Given the significant size difference between nude mice and humans, 9.4T MRI is necessary for EMVI detection in nude mice. Although 9.4T MRI is not currently feasible in clinical settings, improving MRI protocols could enhance EMVI detection in everyday medical practice.

- **Comment 2:** Provide justification for the choice of Mann-Whitney U test in cell function assays.
- **Response 2:** Thank you for your suggestion. We chose the Mann-Whitney U test for cell function assays because our data failed the Shapiro-Wilk normality test and showed unequal variances, making the non-parametric Mann-Whitney U test a more appropriate choice than the t-test. This process is supplemented in the Statistical analysis section of MATERIALS AND METHODS.

3. Results:

- **Comment 1:** In "MR imaging-histopathological correlation analysis of EMVI in orthotropic models," clarify if there were any inter-observer discrepancies and how these were resolved.

- **Response 1:** We have clarified that there were no significant inter-observer discrepancies in the MR imaging-histopathological correlation analysis, as indicated by kappa coefficients or intraclass correlation coefficients greater than 0.75.

- **Comment 2:** In the cell function test results, explicitly state if the apoptosis and proliferation results are consistent across all cell lines or if there are notable differences.

- **Response 2:** We have explicitly stated in RESULT that cell apoptosis and invasion were consistent across all cell lines, with notable differences. However, differences in cell proliferation were only evident for some indicators and at certain time points. The figures and tables have been reformatted and enhanced, with the addition of a new Figure 6 and Table 2, and the reduction of complex technical data in Figure 7 to enhance the intuitiveness and readability of the results.

4. Figures and Tables:

- **Comment 1:** Figures 4 and 5 are visually informative but could benefit from clearer annotations and arrows indicating key findings (e.g., tumor boundaries, EMVI features).

- **Response 1:** We are grateful for your detailed and implementable suggestions. Figure 4-5 has undergone a redesign to improve its readability. Furthermore, we have incorporated more explicit annotations and directional indicators to accentuate critical observations, such as the boundaries of tumors and EMVI characteristics.

- **Comment 2:** Ensure that tables, particularly Table 2, clearly highlight statistically significant results.

- **Response 2:** Thank you for your suggestion. The tables have undergone modifications. For enhanced clarity, we transformed the initial Table 2 into the current Figure 6, which now displays the information as a heatmap, making it easier to interpret. Table 2 Differences of Cell function test between knock-down and normal control groups has also been added.

5. Discussion:

- **Comment 1:** Expand on how hsa_circ_0097977 might act as a biomarker or therapeutic target in clinical settings.

- **Response 1:** Thank you for your suggestion. We have reconstructed and expanded the DISCUSSION to include the potential of hsa_circ_0097977 and its host gene, sponged miRNA as biomarkers or therapeutic target in clinical settings.

- **Comment 2:**Discuss whether hsa_circ_0097977's modulation has potential implications for treatment strategies, such as targeted therapies or personalized medicine approaches.

- **Response 2:** We have also discussed its implications for targeted therapies and personalized medicine, particularly in the context of EMVI.

6. Clinical Relevance:

- **Comment:** Elaborate on how findings could potentially guide clinical management of gastric cancer patients with EMVI. Provide hypothetical scenarios or clinical pathways where hsa_circ_0097977 detection and modulation could play a role in improving outcomes.

- **Response:** Thank you for your suggestion. We have added a section on the clinical relevance of our findings, proposing hypothetical scenarios where hsa_circ_0097977 detection could guide treatment decisions, such as the use of

targeted therapies or the stratification of patients for more aggressive treatment regimens.

7. Limitations:

- **Comment 1:** Explicitly state that findings from preclinical models may not fully replicate human pathology.
- **Response 1:** Thank you for your suggestion. We have expanded the limitations section to explicitly state that findings from preclinical models may not fully replicate human pathology.
- **Comment 2:** Highlight the small sample size and single-center design as factors limiting the generalizability of the results.
- **Response 2:** Thank you for your suggestion. We have also highlighted the small sample size and single-center design as limitations, acknowledging their impact on the generalizability of the results.

8. Future Directions:

- **Comment 1:** Propose specific follow-up studies, such as larger animal studies, clinical trials, or bioinformatics analyses, to validate the role of hsa_circ_0097977.
- **Response 1:** We have proposed specific follow-up studies, including larger animal studies, clinical trials, and bioinformatics analyses, to further validate the role of hsa_circ_0097977.
- **Comment 2:** Discuss whether hsa_circ_0097977 could be integrated into existing diagnostic or prognostic models for gastric cancer.
- **Response 2:** We have also discussed its potential integration into existing diagnostic or prognostic models for gastric cancer.

9. Language and Formatting:

- **Comment:** The manuscript is generally well-written, but minor grammatical inconsistencies are present. Conduct a thorough proofreading or consider professional language editing to improve clarity and readability.
- **Response:** Thank you for your reading and suggestion. We have conducted a thorough proofreading of the manuscript and made necessary grammatical corrections to improve clarity and readability. In addition, we will further improve clarity and readability through the article touch-up service provided by BPG Editorial Office.

Reviewer #2 Comments and Responses

1. Title:

- **Comment:** The title is specific but could be simplified to appeal to a broader audience. Consider simplifying the title to: "The Role of hsa_circ_0097977 in Extramural Venous Invasion of Gastric Cancer."

- **Response:** Thank you for your valuable suggestion regarding the title of the manuscript. We appreciate your input and agree that simplifying the title will enhance its appeal to a broader audience. In accordance with the journal's formatting requirements for article titles, we have revised the title of our manuscript to: " Extramural Venous Invasion in Gastric Cancer: Preclinical Evaluation via 9.4 T MRI and the Functional Role of Associated circRNAs"

2. Abstract:

- **Comment:** The abstract effectively summarizes the study's aims, methods, and findings but contains excessive technical details. Focus on the main findings and implications, reducing the emphasis on technical procedures to improve readability.

- **Response:** Thank you for your suggestion. We have revised the abstract to reduce technical details and to emphasize the main findings and clinical implications. The revised abstract now focuses on the key results: 9.4T MRI can accurately identify EMVI in gastric cancer. the role of hsa_circ_0097977 in EMVI and its potential as a therapeutic target while maintaining scientific accuracy.

3. Keywords:

- **Comment:** The keywords are appropriate but could include more variety. Add terms like "circRNA," "mouse model," and "molecular imaging" to improve searchability.

- **Response:** We appreciate your recommendation and have incorporated the proposed keyword "CircRNA" into our list of keywords to enhance the manuscript's discoverability in relevant databases. It is worth noting that "mouse model" is already included as one of the keywords. Additionally, we believe that "radiogenomics" is more appropriate for this article than "molecular imaging." Therefore, we added "radiogenomics" to the keyword list.

4. Introduction:

- **Comment:** The introduction provides relevant background but is somewhat lengthy due to repeated references to the literature. Present background information more concisely and emphasize the novel contributions of the study earlier in the section.

- **Response:** We have streamlined the introduction by simplifying and removing redundant parts and focusing on the most relevant literature. Additionally, we have highlighted the innovative contributions, rationality, and importance of research related to EMVI-focused radiogenomics.

5. Methods:

- **Comment:** The methodology is thorough but includes an overwhelming level of detail that may detract from readability. Consider including a flowchart summarizing the experimental design and providing more detailed explanations of animal ethics compliance.

- **Response:** Thank you for your suggestion. We have revised a **flowchart (Figure 1)** summarizing the experimental design to improve readability. Additionally, we have expanded the section on animal ethics compliance to provide more details on adherence to international standards, including the ARRIVE guidelines and IACUC approval.

6. Results:

- **Comment:** The results section is comprehensive but dense with technical data, making interpretation challenging. Use summary tables to highlight key findings and simplify figure captions to make them more intuitive.

- **Response:** We appreciate your recommendation. The figures and tables have been reformatted and enhanced, with the addition of a new Figure 6 and Table 2, and the reduction of complex technical data in Figure 7 to enhance the intuitiveness and readability of the results . Additionally, we have restructured the results section to improve its clarity and logical flow.

7. Discussion:

- **Comment:** The discussion relates findings to existing literature but lacks sufficient emphasis on clinical and translational implications. Expand on the study's clinical significance and potential future applications, emphasizing how the findings could guide further research.

- **Response:** We have expanded the discussion to emphasize the clinical and translational implications of our findings. Specifically, we have added a section on how hsa_circ_0097977 could serve as a biomarker or therapeutic target in clinical settings, and we have proposed future research directions, including larger animal studies and clinical trials.

8. Figures and Tables:

- **Comment:** Figures and tables are relevant but lack consistency in format and clarity in descriptions. Standardize formatting across all figures and tables and ensure that captions provide sufficient context.

- **Response:** Thank you for your suggestion. We have standardized the formatting of all figures and tables and revised the captions to provide more context. The figures and tables have been reformatted and enhanced, with the addition of a new Figure 6 and Table 2 to enhance the intuitiveness and readability of the results. For example, in **Figures 4 and 5**, we have added arrows and annotations to highlight key findings such as tumor boundaries and EMVI features.

9. Biostatistics:

- **Comment:** Statistical analyses are appropriate, but details about power analysis and sample size justification are missing. Include information on power analysis and rationale for sample sizes to strengthen methodological transparency.

- **Response:** Thank you for your insightful comment regarding the need for power analysis and sample size justification. In response, we have added a sentence to the Methods section to address this point: "For animal experiments, a power analysis was performed based on previous studies to determine sample size." This addition clarifies that our sample size selection was informed by a power analysis grounded in relevant prior research, thereby enhancing the methodological transparency of our study. We are confident that this modification addresses your concern and enhances the quality of the manuscript.

10. References:

- **Comment:** References are current and relevant but exhibit inconsistencies in formatting. Ensure all references comply with the journal's required style and consider including additional recent studies for broader context.

- **Response:** Thank you for your suggestion. We have reformatted all references to comply with the journal's style guidelines. We have also added recent studies to provide broader context, particularly in the discussion of circRNAs and their role in cancer progression.

11. Ethics Statement:

- **Comment:** Ethical approval is mentioned but briefly explained. Elaborate on adherence to international standards for animal experiments to reassure readers of ethical compliance.

- **Response:** We have expanded the ethics statement to provide more comprehensive details on our adherence to international standards, including

the ARRIVE guidelines and IACUC approval. This enhancement underscores our commitment to ethical research practices, assuring readers of our compliance with these critical standards. Furthermore, we have supplemented the ethics-related details in accordance with the format required by the guidelines.

Editorial Office's comments.

(1) ***Science Editor:***

- **Comment 1: Country/Territory of origin:** China.

- **Comment 2:** Please provide the latest language certificate after Return the Manuscript to Author for Revision. Please visit the following website for the professional English language editing companies that we recommend: https://www.wjgnet.com/bpg/gerinfo/240.
- **Response 2:** Thank you for your suggestion. Considering the expertise, quality assurance, and consistency, we will further enhance the clarity and readability of our manuscript through the professional article touch-up service provided by the BPG Editorial Office.

- **Comment 3:** Author names (unabbreviated) should be given as **first name, middle name initial (with no period)** and **family (sur)name**, and typed in bold with the first letter of each capitalized. A hyphen should be included between the syllables of **Chinese names**. For example: Yi-Fan Chang, Jia-Jing Li, Tao Liu, Chong-Qing Wei, Li-Wei Ma, Vladimir N Nikolenko, Wei-Long Chang.
- **Response 3:** Thank you for your suggestion. We have revised Author names.

- **Comment 4:** Policy of allowing co-first authors and co-corresponding authors who made equal contribution to a manuscript (https://www.wjgnet.com/bpg/GerInfo/310).
- **Response 4:** Thank you. We have revised this manuscript and the bibliographic format of co-first or co–corresponding authors according to this policy.

- **Comment 5: Author contributions:** The authors' names will be listed in the following format: full family (sur)name, followed by abbreviated first and middle names. For example, a full multi-author example is: Wang CL, Liang L, Fu JF, Zou

CC, Hong F and Wu XM designed the research study; Wang CL, Zou CC, Hong F and Wu XM performed the research.

- **Response 5:** Thank you. We have revised the format of author's names in Author contributions.

- **Comment 6: Please add the "Core Tip".** The Core Tip is a short paragraph that is independent of the content of the Abstract. The 'Core Tip' will provide a succinct summary of the study that outlines its most innovative and important arguments. This section should be less than 100 words. Abbreviations must be defined upon first appearance in the Core Tip. Do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition.
- **Response 6:** Thank you. We have added the Core Tip： This study demonstrates that extramural venous invasion (EMVI), a risk factor for distant metastasis in gastric cancer (GC), can be accurately detected using 9.4T magnetic resonance imaging (MRI) in orthotopic GC mouse models, with histopathological confirmation. The expression of hsa_circ_0097977, a circular RNA, was found to regulate EMVI, and its knockdown reduced tumor cell invasion and promoted apoptosis, suggesting its potential as a therapeutic target in GC. These findings highlight the clinical relevance of EMVI detection and the role of hsa_circ_0097977 in GC progression.

- **Comment 7: Audio Core Tip.** In order to attract readers to read the full-text article, we request that the first author make an audio file describing the final core tip. This audio file will be published online, along with the article. **The author can invite English language editing company to assist in resolving the language issues of Audio Core Tip.**
- **Response 7:** Thank you for this suggestion. The first author will prepare and submit an audio file describing the final core tip for publication along with the article.

- **Comment 8: Reference numbers in the main text.** The format of in-text citation of references should be [References Number]. Please change the format of the cited references from "( )" to "[ ]". **Example:** The pathophysiology is thought to be due to an increased arterial flow that leads to secondary hepatocellular hyperplasia[1,2].

There are issues with the references: Please provide the PMID numbers (https://pubmed.ncbi.nlm.nih.gov/) and DOI citation numbers (https://doi.crossref.org/simpleTextQuery) to the reference list and list all authors of the references. If a reference has no PMID and DOI, please provide the source website address of this reference. To ensure the accuracy of the references, please use **"Edit References by Auto-Analyser"** (https://www.f6publishing.com/Forms/main/ArticleReferenceTool.aspx) to edit the references of the manuscript.

- **Response 8:** Thank you for your detailed instructions regarding the reference format and citation standards. We have made the necessary revisions.

- **Comment 9: Figures.** The numbered in the figures should match the numbered in the legends (such as a and (a) are not written in the same way). Uniform presentation should be used for figures showing the same or similar contents; for example, "**Figure 1 Pathological changes of atrophic gastritis after treatment.** A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Abbreviations must be defined upon first appearance in the **Figure Legends.** Do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition.
- **Response 9:** Thank you for your detailed feedback. We have carefully reviewed and revised the figures to address the issues raised.

- **Comment 10: Original figure documents.** *In the meantime, authors should provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor, and upload it to the file destination of "**Image File**" in the F6Pubishing system.*
- **Response 10:** Thank you for your guidance. The revised figures have been carefully arranged and checked to meet the publication standards. These files have now been uploaded to the "Image File" destination within the F6Publishing system.

- **Comment 11: Figure Color:** When using different color markings on the Figures, please avoid using **red or green**, including the **arrow colors** on the Figures.

- **Response 11:** Thank you for your suggestion. We have updated the Figures to enhance their readability and eliminated the simultaneous use of red and green colors.

- **Comment 12: Tables.** The Table title needs to be bolded. Abbreviations are not allowed in table titles.
- **Response 12:** Thanks for your suggestion. We have checked and revised Table1-2.

- **Comment 13:** Please upload the approved grant application form(s) or funding agency copy(ies) of any approval document(s). **[Supported by the National Natural Science Foundation of China under Grant Number 81901819 and Beijing Natural Science Foundation under Grant Number 7232187.]**
- **Response 13:** Thank you. We have added another approved grant **【Peking University People's Hospital Scientific Research Development Funds under Grant Number RDX2024-08】**. And we will upload all approved grant application files.

- **Comment 14:** Regarding abbreviations, please do not list them separately. Abbreviations must be defined at the first occurrence. For example, when CT first appears in the manuscript, please use the format "computed tomography (CT)".
- **Response 14:** Thank you for your suggestion. We have removed the abbreviations list and examined the manuscript to verify that all abbreviations are properly defined upon their initial appearance.

- **Comment 15: Abstract.** The 5 sections of the structured abstract are: **BACKGROUND; AIM; METHODS; RESULTS**; **CONCLUSION. Please add "BACKGROUND".**
- **Response 15:** Thank you. As requested, we have incorporated a "BACKGROUND" section into the abstract and adjusted the abstract's structure accordingly.

- **Comment 16: Main text.** The main text contains **INTRODUCTION; MATERIALS AND METHODS; RESULTS; DISCUSSION; and CONCLUSION**. **Please add "CONCLUSION".**
- **Response 16:** Thank you. We have added "CONCLUSION" .

Conclusion:

We believe that the revisions made in response to the reviewers' comments have significantly improved the manuscript. We are grateful for the reviewers' thoughtful feedback and hope that the revised version meets the journal's standards for publication.

Sincerely,
Jin Cheng
On behalf of the authors