**Peer Review File**

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**Reviewer A**

1) First, the title also needs to indicate the functional experiments.
   Reply1: We sincerely appreciate your review of our paper and your valuable insights. Regarding your reference to the need for the title to reflect the functional experiment, we apologize for not highlighting it sufficiently in the title of the paper. We have modified the title of our text as advised. (see Page 1, lines 2-3 ).
   Changes in the text: Bioinformatics and functional experiments reveals that MRC2 inhibits atrial fibrillation via the PPAR signaling pathway
2) Second, the abstract is inadequate and needs further revisions. The background did not describe the needs for new biomarkers in AF and what the limitations of known biomarkers. The methods need to describe the data sources including the clinical samples and more details of the functional experiments. The results need to quantify the findings by using statistics such as the expression levels and accurate P values. The conclusion needs more detailed comments for the clinical and research implications of the findings.
   Reply2: Thank you for carefully reviewing our paper and providing valuable comments. Regarding the abstract section, we have reworked it to ensure that it is fuller and clearer, and better able to summarize the main points of the paper: in terms of the background of the literature, we have re-edited the paragraphs to emphasize the need for new biomarkers in atrial fibrillation and to explore in detail the limitations of the known biomarkers in order to better contextualize and motivate the study; and in the methodology section we have provided a more detailed description of the data sources are described in more detail, particularly the process of obtaining and using clinical samples. In addition, we have added more details about the functional experiments to ensure that readers can fully understand our research methodology; for the results section, we have performed statistical treatments, including the quantification of expression levels and the accurate calculation of p-values to support the reliability and significance of our findings; finally, in the conclusions section, we have added a more detailed discussion of the clinical and research implications of the paper .We have modified our text as advised(see Page 1-2, line 28-63)
   Changes in the text: The Background, Methods, Results, and Discussion sections of the Abstract have been revised and supplemented in accordance with the reviewer's suggestions, as described in line 28-63 on pages 1-2 of the article.

3) Third, the introduction of the main text is not adequate. The authors need to review what has been known on the pathological and physiological mechanisms of AF, biomarkers involved, and limitations of these known biomarkers to support the needs for the identification of new biomarkers.

   Reply3: The feedback provided by the reviewers is greatly appreciated. The fleshing out of the introduction is critical to setting the tone and context for the study. We apologize for any inconvenience in reading it. I have carefully reviewed and revised the Introduction section of the main text. A comprehensive and expanded overview of the current understanding of the pathophysiologic mechanisms of atrial fibrillation (AF) is provided. In addition, I have provided an in-depth discussion of established biomarkers associated with AF and their limitations in clinical practice, which in turn strengthens the theoretical basis of the study and enhances the clarity of the research objectives. We have modified our text as advised (see Page 3-4, line 89-99 and line113-115).

   Changes in the text: The introductory section of the text has been supplemented with the pathophysiologic mechanisms of AF as well as an increased discussion of relevant markers and their limitations, as detailed in the revised manuscript Page 3-4, line 89-99 and line113-115.

4) Fourth, in the methodology of the main text, please use a paragraph to briefly describe the research procedures in bioinformatics analysis and the functional experiments. The clinical and non-clinical samples in the datasets should be described. In statistics, please ensure $P<0.05$ is two-sided.

   Reply4: Thank you very much for your comments on the review of our paper. In the Methods section of the main text, we have added a paragraph with a brief description of the research procedures for the bioinformatics analysis and functional experiments. In this paragraph, we have provided a detailed description of the clinical and non-clinical samples in the dataset. In addition, in the statistical analysis, we have clearly stated that the two-sided P-value should be less than 0.05. We have modified our text as advised (see Page 7-8, line 234-257 and line263-265).

   Changes in the text: A brief description of the research process has been added to the Materials and Methods section of the manuscript, as well as additional clarification on the two-tailed test for p-values in the statistical analyses, as detailed in the revised manuscript Page 7-8, line 234-257 and line263-265.

5) Finally, please consider to cite some potentially related papers to enrich this paper: 1. Chen H, Zhang F, Zhang YL, Yang XC. Relationship between circulating miRNA-21, atrial fibrosis, and atrial fibrillation in patients with atrial enlargement. Ann Palliat Med 2021;10(12):12742-12749. doi: 10.21037/apm-21-3518. 2. Zou T, Chen Q, Chen C, Liu G, Ling Y, Pang Y, Xu Y, Cheng K, Zhu W, Wang RX, Qian LL, Ge J. Moricizine prevents atrial fibrillation by late sodium current inhibition in

atrial myocytes. J Thorac Dis 2022;14(6):2187-2200. doi: 10.21037/jtd-22-534. 3. Uziębło-Życzkowska B, Krzesiński P, Maciorowska M, Gorczyca I, Jelonek O, Wójcik M, Błaszczyk R, Kapłon-Cieślicka A, Gawałko M, Tokarek T, Rajtar-Salwa R, Bil J, Wojewódzki M, Szpotowicz A, Krzciuk M, Bednarski J, Bakuła-Ostalska E, Tomaszuk-Kazberuk A, Szyszkowska A, Wełnicki M, Mamcarz A, Wożakowska-Kapłon B. Antithrombotic therapy in patients with atrial fibrillation undergoing percutaneous coronary intervention, including compliance with current guidelines—data from the POLish Atrial Fibrillation (POL-AF) Registry. Cardiovasc Diagn Ther 2021;11(1):14-27. doi: 10.21037/cdt-20-839. 4. Cui C, Zhou H, Xu J. ELABELA acts as a protective biomarker in patients with atrial fibrillation. J Thorac Dis 2021;13(12):6876-6884. doi: 10.21037/jtd-21-1728.

Reply5: Thank you very much for your comments and suggestions. We have enriched the paper by citing some of the references in your suggestions that are relevant to our research content. We have carefully reviewed the literature in the relevant fields to ensure that we cite, in the paper, quality literature that can further support and extend our research ideas. Thank you again for your guidance! as detailed in the revised manuscript Page 4, line 115-118 and page 11 line365-368.

Changes in the text: For the references given by the reviewers, after our careful reading and deliberate consideration, the first and fourth references were chosen to supplement the richness of the article, in the Introduction section (Page 4, line 115-118) and the Discussion section (page 11 line365-368), respectively.

**Reviewer B**

The paper titled “Bioinformatics study reveals that MRC2 inhibits atrial fibrillation via the PPAR signaling pathway” is interesting. MRC2, an inhibitory gene in AF, suppresses the development of AF through the PPAR signaling pathway. Besides, it has the potential to be a diagnostic indicator and therapeutic target for AF patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) It is recommended to increase the evaluation of the correlation between MRC2 expression and prognosis and clinicopathological factors in patients with atrial fibrillation.

Reply1: Thank you very much for reviewing our paper and providing your valuable comments. During the review process, we noted your concerns about the clinical samples and pathology data. We deeply regret that we were not able to cover these data. Our study focuses on utilizing bioinformatics methods to mine information about relevant genes. In our future studies, we will pay more attention to collecting information related to clinical samples and pathology data to support our research

content more comprehensively. Thank you again for your valuable comments, your suggestions are important for us to further improve the quality of the paper.

Changes in the text: Not have.

2) Some fonts need to be enlarged, as shown in Figures 1-3, and 5.

Reply2: Thank you very much for your valuable comments on our paper. We apologize for the inconvenience caused by the font size of the figures during the viewing process, and we have adjusted the fonts in the figures to ensure that the fonts in Figures 1-3 and 5 can be enlarged to improve readability and clarity. as detailed in the revised manuscript Page 19-21, fig 1-3 ;and page 23,fig5.

Changes in the text: Revised Figures 1-3 and 5 have been re-replaced with the original drawings of the original drafts

3) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply3: Thank you for your helpful suggestions. For the lack of richness in the abstract section we have made changes to make relevant additions and the background of the study also mentions the clinical needs of the study population, as detailed in the revised manuscript Page 1-2, line 28-61.

Changes in the text: Additional changes have been made to the abstract section and the clinical needs of the study population have been mentioned in the background of the study. See the abstract section of the revised manuscript for details.

4) There are a variety of genes that can regulate the atrial fibrillation. Why did the author choose MRC2 for research? Please add relevant content to the discussion.

Reply4: Thank you for your helpful suggestion. We chose MRC2 as the hub gene based on the consistency of the results of multiple analyses showing its association with AF. In gene overlap analysis, MRC2 was found to have 10 overlapping genes with tan module genes and GSE143924-DEG, suggesting its relevance in specific biological contexts. By GSEA analysis, MRC2 was enriched in several functional pathways such as bile acid biosynthesis, RAS, cell membrane DNA sensing pathway, and PPAR signaling pathway, implying its important role in AF biological processes. Gene expression analysis TCGA data and GEPIA tools showed that MRC2 expression was

significantly reduced in AF patients, suggesting that it may have an inhibitory function in AF. It may be related to the pathophysiologic process of AF. In addition, MRC2 showed potential diagnostic value in biomarker analysis, with its ROC curve analysis determining that MRC2 has a high AUC value (0.8), results suggesting that it may be a biomarker for AF. And the role of MRC2 in AF was also demonstrated by subsequent experiments. Combining these findings, we chose MRC2 as a research subject, which is expected to be a potential target for future treatment or diagnosis, as detailed in the revised manuscript Page 12, line 373-385.

Changes in the text: The reasons for the selection of pivotal genes have been added in the discussion section of the text. See the Discussion section of the revised manuscript for details.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Screening of atrial fibrillation diagnostic markers based on a GEO database chip and bioinformatics analysis, J Thorac Dis, PMID: 36647491”. It is recommended to quote this article.

Reply5: Thank you very much for your valuable comments on our paper. We have reviewed and carefully analyzed this paper you provided to ensure that our introduction section is more comprehensive and covers relevant literature and background. We have cited the paper in our revised version and have adjusted the introduction section accordingly to ensure that our paper is more complete and accurate, as detailed in the revised manuscript Page 4, line 129-131.

Changes in the text: A citation for this document has been added to the introductory section of the manuscript: The potential diagnostic key genes unearthed can also provide a theoretical basis for drug sensitivity analysis and targeted drug development for AF.( Page 4, line 129-131.)

6) This study is based on bioinformatics analysis. It is recommended to increase in vivo experimental studies, which may be more meaningful.

Reply6: Thank you very much for your valuable comments and suggestions. The suggestion of adding in vivo experiments will enrich the scientific value of our study even more. However, it is a limitation of our study that we are currently unable to

conduct in vivo experiments due to various technical, resource and ethical constraints. A description of the limitations has been made in the text. In future studies, we will fill in the gaps in in vivo experiments so as to support and strengthen our findings more comprehensively, as detailed in the revised manuscript Page 14, line 448-454.

Changes in the text: The absence of additional in vivo experiments is a limitation of our study, and the limitation has been described in the discussion section of the revised article: In the present study, a potential key hub gene, MRC2, was identified by mining DEGs. it should be noted that this study has some limitations. First, AF pathogenesis is caused by a combination of multiple genetic and environmental factors. Second, due to the lack of clinical samples, we were unable to perform gene expression validation, and other basic experiments are needed to validate the function and regulatory mechanisms of these genes. Third, without the added validation of in vivo experiments, there may be limitations in clinical application. (Page 14, line 448-454.)

7) It is recommended to increase the study of lncRNA or miRNA regulating MRC2, which may make the whole study more complete.

Reply7: Thank you very much for your valuable suggestions on our paper. lncRNAs and miRNAs have important roles in cellular processes, especially in gene regulation and signaling. However, at present, our research directions and resources are fixed and we cannot add these additions to the current study. In our future studies, we will further explore their relationship with MRC2 in depth in order to gain a more comprehensive understanding of the relevant molecular mechanisms. We plan to focus on exploring how these molecules affect the expression and function of MRC2, as well as their potential roles in related disease mechanisms, in our subsequent research programs. Thank you again for your valuable suggestions, your comments are very helpful for our future research directions.

Changes in the text: Not have.

**Reviewer C**

1. The authors mentioned “studies...”, while only one reference was cited. Change “Studies” to “A study” or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

*Studies have shown that the disease is closely related to diseases like coronary and congenital heart disease and is regarded as an invisible “killer” of cardiovascular and cerebrovascular diseases (6).*

*And there are studies that prove ELABELA (peptide hormone essential for cardiac development) is a protective factor in patients with cardiac arrhythmias and can be used as a prognostic marker for atrial fibrillation and its associated complications(12).*

*Several studies have shown that AF harms the quality of life by limiting physical activity, dying of social interactions and emotions, reducing happiness, increasing dependence and early retirement (32).*

*Recent studies have shown that microRNAs (miRNAs) are important regulators of several cardiovascular disease processes associated with the heart, and that atrial fibrosis is a hallmark of cardiac remodeling that perpetuates atrial fibrillation (AF)(36).*

*Moreover, some previous studies have shown that in the blood, liver, muscle, and other organ systems, arachidonic acid functions as a structural lipid linked to phospholipids (37).*

*Studies by López-Guisa et al. show that by slowing the pace of interstitial collagen formation, MRC2 is crucial in the development of solid organ fibrosis, thereby protecting the parenchyma surrounding the kidney from fibrosis-related damage (40).*

Response: Thank you for your suggestion. We apologized for the problem of citing only one document but mentioning "studies", which is an oversight in our writing. In order to more accurately reflect the content of our study, we will follow your suggestion and replace "Studies" with "A study" to emphasize the specific studies that we have cited in the introduction or related sections. In addition, regarding your second comment, which asked us to number the citations in the order in which the literature was first mentioned in the text, we will follow your suggestion and ensure that the literature citations are numbered in the order in which they are presented in order to improve the

readability and consistency of the paper. Once again, thank you for your valuable comments, we have followed your suggestions and ensured that our paper meets high standards in terms of academic quality and accuracy.

2. Figure 4 & Figure 6

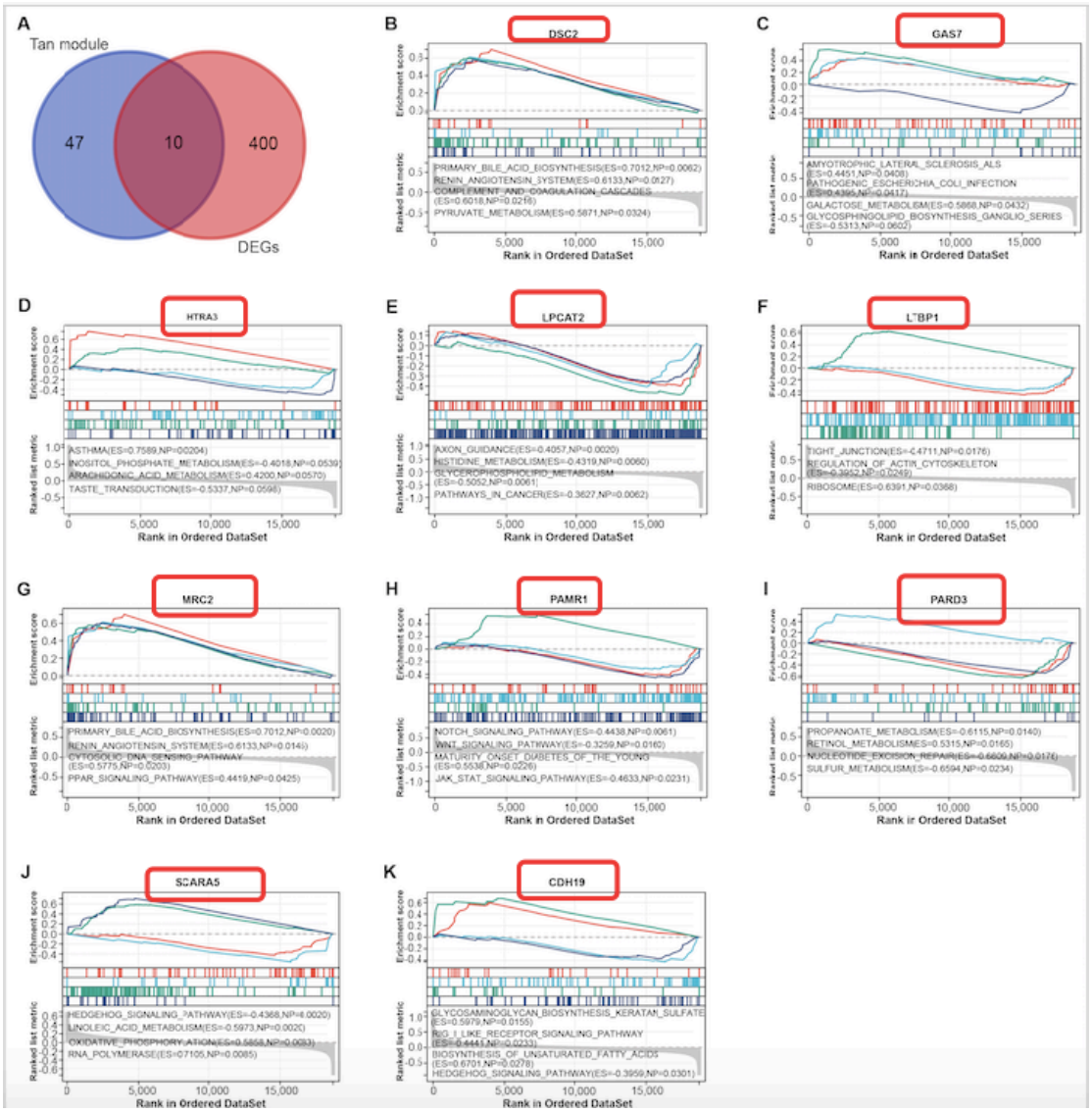
Please send us the figures with higher resolution in JPG/TIFF, as the current ones are not clear enough.

Response: Thank you for your suggestions on our paper. We apologize for the resolution issue with the images in the article and the inconvenience it caused you in reading it. We have taken steps to resolve this issue to ensure that our images Figure 4 & Figure 6 are made available to you again in the highest resolution JPG/TIFF format. Ensuring our papers are of a high standard in terms of imagery.

3. Figure 3B-3K

The legend did not match the figure. Please check and revise.

631 (B-K) GSEA of 10 overlapping genes: (B) *CDH19*, (C) *DSC2*, (D) *GAS7*, (E) *HTRA3*,
632 (F) *LPCAT2*, (G) *LTBP1*, (H) *MRC2*, (I) *PAMR1*, (J) *PARD3*, (K) *SCARA5*. DEGs,




Response: Thank you very much for your valuable comments on our paper. We have examined the relevant figures Fig. 3B-Fig. 3K of the paper in detail and found that there is indeed a mismatch between the figures and the annotations. This is our mistake and we are very sorry for it. We have re-examined the relevant text and diagrams and revised the data and text therein to ensure that they accurately reflect the information presented in the diagrams. Ensure consistency and accuracy of the article. Thank you again for your valuable comments.

4. Figure 5

"MOCK, MOCK" is confusing. Please check and revise.

655 dehydrogenase; over-, over-expressed-; OD, optical density; MOCK, MOCK; APC,

Response: Thank you for taking the time to scrutinize my paper and providing valuable

feedback. Regarding the confusion caused by the note "MOCK, MOCK" in Figure 5, we have revisited Figure 5 and there is no mention of MOCK in it, this was an inadvertent error on our part and we apologize for it, we have removed the word from the article to ensure that the accuracy of Figure 5 is improved. Once again, thank you for your valuable advice, it is crucial to improve the quality of my paper.

5. No “APC” in Figure 5 while it is explained in the legend. Please check and revise.

Response：Thank you for carefully reviewing my paper and providing feedback. Regarding the issue of "APC" which is not mentioned in Figure 5, this is our problem in writing the paper, we apologize for this and I have already checked and revised the deletion to ensure the accuracy and clarity of the paper.