Cover Letter

Dear Editor and Reviewers:

We appreciated to receive the comments from the reviewers. Thank you for providing us the opportunity to further improve this manuscript. We have carefully revised our manuscript according to the suggestions made by the reviewers and editor and answer their questions as clear as possible. Revised portion are underlined in yellow. We believe that we have modified the manuscript in accordance with all additional issues in this letter. We hope the manuscript will now be acceptable for publication in ***Molecules***.

Thank you very much for your consideration!

Yours sincerely,

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Response to Reviewer 3 Comments

Point 1: Add more details for the reason of β-adrenergic activation in heart failure and upstream signaling.

Response 1: Thanks to reviewer for pointing out our shortcomings. We have added the reason of β-adrenergic activation in heart failure and upstream signaling in the Introduction section on page 1 line 39-46.

Point 2: How (TEC) can be tyrosine kinases 53 in hepatocellular carcinoma abbreviation?

Response 2: We thank the reviewer's comment. We have revised in the Introduction section on page 2 line 53-54.

Point 3: Why did the authors prefer do picrosirius red instead of Masson trichrome?

Response 3: Thank you very much for this critical question. Firstly, both Sirius red staining and Masson staining can reflect the severity of cardiac fibrosis by reflecting the collagen deposition in the heart. However, Sirius red staining was used more for quantification, and Masson staining was mainly used for pathological morphology observation (PMID：34349235, PMID：32611237). In our study, a quantitative analysis of the area of cardiac fibrosis was required. Besides, the relevant articles (PMID: 36449214, PMID: 28549109, PMID: 32246402) all chose to use Sirius red staining for quantification in recent years. Therefore, Sirius red staining was chosen to assess cardiac collagen deposition in our study.

Point 4: Why data are presented by Mean and SEM instead of SD?

Response 4: Thanks a lot for this critical question. Firstly, both SEM and SD can reflect the accuracy of data. However, SEM is used to describe the degree of dispersion of the sample mean from the population mean, which can reflect the mean of the two groups of samples; SD is used to describe the degree of dispersion of data, which can reflect the sample repeatability. In our study, we prefer to judge whether there is a statistical difference between groups by comparing the mean size of the two groups of samples. In addition, Mean±SEM analysis method is also widely used in recent articles (PMID: 34349235, PMID: 33965510, PMID: 34331017). Therefore, we chose Mean±SEM to present the data in our study.

Point 5: TGF-b is a main regulator in fibroblast activation. Why there is no any measurement of TGF in Fig.6?

Response 5: Thanks to reviewer for professional question. To begin with, previous research have shown the excessive activation of the sympathetic system is a key factor in heart failure. Indeed, β-adrenergic receptor (β-AR) plays a vital role in multiple cardiac diseases. Therefore, in our study, we mainly focused on the effect of BTK on the downstream mechanism of β-AR in heart failure. However, BTK belongs to the family of non-receptor tyrosine kinases (nRTKs). TGF-β is a serine-threonine kinase, which is located parallel or upstream of BTK. Besides, TGF-β has been mainly studied in heart disease models caused by ischemia and hypoxia, such as myocardial infarction model and ischemia-reperfusion injury

model (PMID: 32611237, PMID: 32542822, PMID: 33942481). So TGF-β-related indicators were not examined in our study.

Point 6: Please clarify limitation of this study?

Response 6: Thanks to reviewer for the constructive suggestions. We have added the limitations of this study in the Discussion section on page 18 line 497-506.

Point 7: Can ZB can be used in other model of heart failure?

Response 7: We thank the reviewer's comment. In our study, we mainly focus on the continuous activation of sympathetic nervous system induced heart failure, which is one of the most classical model in heart failure. However, in other models of heart failure caused by ischemia and hypoxia, the pathogenesis is not completely consistent with activation of sympathetic nervous system. The relevant content was also added in the Discussion section on page 18 line 497-506. Therefore, whether ZB has an effect still need to be proved.