Dear Editor,

Thank you very much for your advice regarding our manuscript entitled "Comparative transcriptomic analysis reveals the molecular changes of acute pancreatitis in experimental models" (90586). We also greatly appreciate the comments and suggestions by the reviewers, which have significantly improved the content and presentation of this manuscript. We have added keywords, core type, conclusion, limitations, reformatted references, and written all explanations of abbreviations in the revised manuscript, as suggested by the reviewers. We have revised the manuscript accordingly and the amendments were highlighted in red in the revised version of the manuscript. In addition, point-by-point responses to the reviewers' comments are listed in the following text below.

This revised manuscript has been edited and proofread. We hope that the revision now is suitable for publication in your journal and look forward to hearing from you soon.

Yours sincerely,

Yin Zhu

Firstly, we would like to express our sincere gratitude to the Reviewers for their constructive and positive comments.

Replies to Reviewer 1:

The purpose of this study is to investigate the shared molecular changes underlying the development of AP across varying severity levels. Methods: Acute pancreatitis was induced in animal models through treatment with caerulein alone or in combination with LPS. Additionally, transgenic C57BL/6J- hM3/Ptf1α(cre) mice were administered Clozapine N-oxide (CNO) to induce AP. Subsequently, we conducted RNA sequencing of pancreatic tissues and validated the expression of significantly different genes using the Gene Expression Omnibus (GEO) database. Results: Caerulein-induced AP showed severe inflammation and edema, which were exacerbated when combined with LPS and accompanied by partial pancreatic tissue necrosis. Compared with control group, RNA sequencing analysis revealed 880 significantly differentially expressed genes in the caerulein model and 885 in the caerulein combined with LPS model. KEGG enrichment analysis and Gene Set Enrichment Analysis (GSEA) indicated substantial enrichment of the Toll-like receptor (TLR) and NOD-like receptor signaling pathway, TLR signaling pathway, and NF-κB signaling pathway, alongside elevated levels of apoptosis-related pathways, such as apoptosis, P53 pathway and

phagosome pathway. The significantly elevated genes in the TLR and NOD-like receptor signaling pathways, as well as in the apoptosis pathway, were validated through qRT-PCR experiments in animal models.

Thank you very much for your valuable advise.

Replies to Reviewer 2:

1: Keywords are not written

Response: Many thanks for your comments and questions. We have added the keywords' part, and highlighted them in red in the revised version of the manuscript. The details are as follows: "Key Words: acute pancreatitis; RNA-sequencing; experimental AP models; TLR and NOD-like signaling pathways; apoptosis.

Core type: unwritten

Response: Many thanks for your comments and questions. We have added the core tip part in the revised version of the manuscript. The specific contents are as follows: "Core tip: AP is a critical emergency condition with no effective targeted therapeutic interventions currently available. Therefore, RNA sequencing (RNA-seq) was employed to investigate the molecular alterations in AP, aiming to identify novel therapeutic strategies. The RNA-seq analysis showed a significant upregulation of TLR and NOD-like signaling pathways in AP, with

crucial involvement of genes such as *TLR7*, *IRF7*, and *SPP1*. Notably, the Tuba1a and Gadd45a genes were identified as key players in the apoptosis signaling pathway. Substantial evidence was provided through comprehensive validation using GSE datasets from human peripheral blood and mouse pancreatic tissues, as well as transgenic mouse models to examine inflammation and apoptosis-related molecules. This study offers fresh insights for future therapeutic approaches in managing AP and establishes new directions for subsequent fundamental investigations."

Conclusion part is not written.

Response: Many thanks for your comments and questions. We added the conclusion part at the end of the article as follows: "Conclusion: This study investigated the molecular changes associated with the development of AP. RNA-seq analysis identified a significant overlap in the gene expression patterns between AP and normal pancreas, primarily involving inflammatory, immune, and apoptotic pathways. The validation of the TLR and NOD-like receptor signaling pathways using animal tissues and two GEO datasets highlighted several potential key genes, including *TLR7*, *IRF7*, *SPP1*, *OAS2,* and *PIPK3*. Moreover, we emphasized the importance of apoptotic pathways in AP. By incorporating transgenic animal models into the validation process,

Tuba1a and *Gadd45a* were identified as the most important expressed genes, suggesting their potential as critical targets for future interventions and therapies in AP. Both the wild type and the *hM3/Ptf1α* $^{(cre)}$ mice shared the same pattern of inflammation. These discoveries provide new avenues for the treatment of necrosis in AP."

Limitations of the study were not mentioned.

Response: Many thanks for your comments and questions. Limitations of the study were mentioned in the last paragraph of the discussion part as follows: "The etiology of AP was diverse, and we have yet to explore other causative factors leading to AP, such as hyperlipidemic pancreatitis and alcoholic pancreatitis. Furthermore, there was a wide range of animal models available for AP, including models induced by L-arginine, pancreatic duct ligation, and retrograde ductal infusion, but comparative experiments in this area were lacking. Despite the identification of numerous genes playing important roles in inflammation and apoptosis, further validation experiments in animal models and in-depth exploration of their mechanisms will be finished in future work. Transgenic mouse models of AP certainly exhibit unique molecular changes, but we have not extensively investigated the differences between these models and the commonly used cerulenin model. The aforementioned unfinished aspects will be the focus of our subsequent work. Nonetheless, our study provides

insights into the molecular alterations in AP and identifies genes that play crucial roles in inflammation and apoptosis processes, offering potential therapeutic targets."

References are not written in accordance with the journal writing rules.

Response: Many thanks for your comments and questions. References have been changed into the correct format. You can check these changed parts in the revised version of the manuscript.

All explanations of abbreviations are not written. These deficiencies need to be completed. Kind Regards.

Response: Many thanks for your comments and questions. The explanations of abbreviations that we missed have been added and highlighted in red, you can find in the revised version of the manuscript.