Reviewer #1:

1. Although the data is sufficient to support the conclusions, the logic and readability of the manuscript is suffered by terminology usage and inadequate data description. I would suggest expanding some of the text in the result section.

Response: Thank you for your suggestion. We have gone through all the text and expand the text in the result section.

2. Authors assessed injury of pancreas, lung, and kidney following P-407+CAE administration. As liver is a key organ in regulating lipid metabolism. It will be helpful to examine the effect of P-407+CAE administration on liver function and insulin resistance.

Response: Thank you for your suggestion. Actually, we have detected the AST and ALT levels before. The AST and ALT levels have risen in caerulein (CAE)-treated groups, but there's no difference between CAE and P-407+CAE treated groups (Figure 1A). Hematoxylin eosin (HE) staining of liver tissues did not show obvious pathological changes (Figure 1B). The homeostasis model assessment insulin resistance (HOMA-IR) shows that there's no change among different groups (Figure 1C). All the results demonstrate P-407+CAE have no effect on liver compared with only CAE treated group. But our HTGP mouse model was induced by single intraperitoneal injection of P-407, we will use long-term animal models to examine the effect of P-407+CAE administration on liver

function and insulin resistance induced by the 7 and 28 days intraperitoneal injection of P-407.

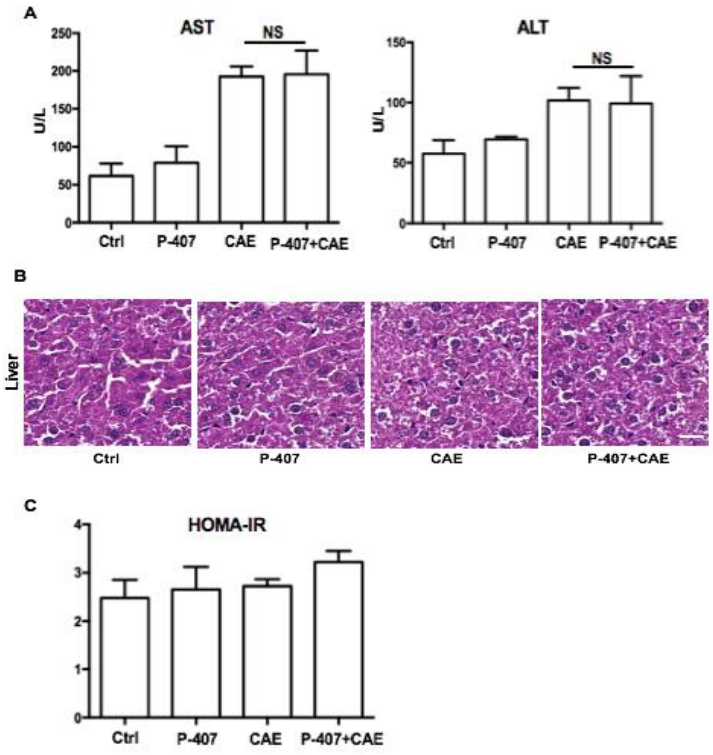

Figure 1

3. How does the proteomic analysis performed, there is no information in method part. What was the data base used to identify the protein?

Response: Thank you for your suggestion. We added the following information in method part. The data base is UniProt, the species-specific protein library is Mus_musculus_10090_SP_20210721.fasta.

Protein Extraction

The sample was grinded with liquid nitrogen into cell powder and then transferred to a 5-mL centrifuge tube. After that, four volumes of lysis buffer (8 M urea, 1% protease inhibitor cocktail) was added to the cell powder, followed by sonication three times on ice using a high intensity

ultrasonic processor (Scientz). The remaining debris was removed by centrifugation at 12,000 g at 4 °C for 10 min. Finally, the supernatant was collected and the protein concentration was determined with BCA kit according to the manufacturer's instructions.

Trypsin Digestion

For digestion, the protein solution was reduced with 5 mM dithiothreitol for 30 min at 56 °C and alkylated with 11 mM iodoacetamide for 15 min at room temperature in darkness. The protein sample was then diluted by adding 100 mM TEAB to urea concentration less than 2 M. Finally, trypsin was added at 1:50 trypsin-to-protein mass ratio for the first digestion overnight and 1:100 trypsin-to-protein mass ratio for a second 4 h-digestion. Finally, the peptides were desalted by C18 SPE column.

4D Mass Spectrometer

The tryptic peptides were dissolved in solvent A (0.1% formic acid, 2% acetonitrile/in water), directly loaded onto a home-made reversed-phase analytical column (25-cm length, 75/100 μm i.d.). Peptides were separated with a gradient from 6% to 24% solvent B (0.1% formic acid in acetonitrile) over 70 min, 24% to 35% in 14 min and climbing to 80% in 3 min then holding at 80% for the last 3 min, all at a constant flow rate of 450 nL/min on a nanoElute UHPLC system (Bruker Daltonics).

The peptides were subjected to capillary source followed by the timsTOF Pro (Bruker Daltonics) mass spectrometry. The electrospray voltage

applied was 1.60 kV. Precursors and fragments were analyzed at the TOF detector, with a MS/MS scan range from 100 to 1700 m/z. The timsTOF Pro was operated in parallel accumulation serial fragmentation (PASEF) mode. Precursors with charge states 0 to 5 were selected for fragmentation, and 10 PASEF-MS/MS scans were acquired per cycle. The dynamic exclusion was set to 30 s.

Database Search

The resulting MS/MS data were processed using MaxQuant search engine (v.1.6.15.0). Tandem mass spectra were searched against the mouse SwissProt database (17089 entries) concatenated with reverse decoy database. Trypsin/P was specified as cleavage enzyme allowing up to 2 missing cleavages. The mass tolerance for precursor ions was set as 20 ppm in first search and 5 ppm in main search, and the mass tolerance for fragment ions was set as 0.02 Da. Carbamidomethyl on Cys was specified as fixed modification, and acetylation on protein N-terminal and oxidation on Met were specified as variable modifications. FDR was adjusted to < 1%.

4. Using proteomic data (in figure 2), authors showed that ferroptosis is a key biological process differentially regulated in pair-wise comparisons of 'AP vs WT' and 'HTGP vs AP'. However, there is lack of information regarding number and identity of ferroptosis-related protein in the study.

Response: Thank you for your suggestion. The number of ferroptosis-

related protein is 5 and the names as below.

KEGG pathway	Fisher's exact test p value	Fold Enrichment	Protein accession	Protein description	Gene name	P407+CAE/CAE Ratio	P407+CAE/CAE P value	Regulated Type
mmu04216 Ferroptosis	0.0010133	6.22	P14901	Heme oxygenase 1 OS=Mus musculus OX=10090 GN=Hmox1 PE=1 SV=1	Hmox1	2.106	0.00123204	Up
mmu04216 Ferroptosis	0.0010133	6.22	Q61093	Cytochrome b-245 heavy chain OS=Mus musculus OX=10090 GN=Cybb PE=1 SV=1	Cybb	1.57	0.000621303	Up
mmu04216 Ferroptosis	0.0010133	6.22	Q61147	Ceruloplasmin OS=Mus musculus OX=10090 GN=Cp PE=1 SV=2	Cp	1.758	1.09085E-07	Up
mmu04216 Ferroptosis	0.0010133	6.22	Q92111	Serotransferrin OS=Mus musculus OX=10090 GN=Tf PE=1 SV=1	Tf	1.557	1.58156E-06	Up
mmu04216 Ferroptosis	0.0010133	6.22	Q9DBD0	Inhibitor of carbonic anhydrase OS=Mus musculus OX=10090 GN=Ica PE=1 SV=1	Ica	1.766	8.80392E-05	Up

Figure 2

5. There is no information indicated on the Volcano plot (Fig 2D), where does NOX2 sit on the volcano plot. What are other proteins upregulated and downregulated.

Response: Thank you for your suggestion. The location of NOX2 has been marked on the figure. The other proteins upregulated and downregulated were listed in an excel file named "Differentially_expressed_statistics".

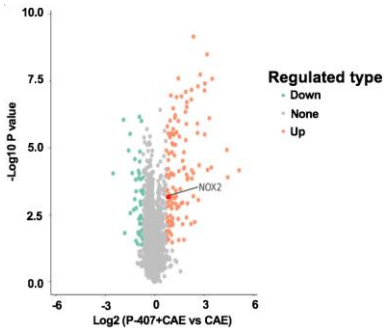

Figure 3

6. Hypertriglyceridemic pancreatitis (HTGP) and P-407+CAE used interchangeably, should be conspicuously used, otherwise, it may cause misunderstanding.

Response: Thank you for your suggestion. We intended to use P-407+CAE to represent the treatment group of mice; and use HTGP to represent the disease of mice. But it seems to cause reading problems. We have modified it to use "HTGP" only in the introduction and discussion for disease; and

"P-407+CAE group" in other part of the paper for treatment.

7. Minor comments (words used improperly) -Line 83, word "predict" -Line 128, word "screening" -Line 179, "proteome sequencing", suggestion proteomic profiling -Line 207, "rebounded"-Line 308, "significant evidence" -Line 443, "Calculation"

Response: Thank you for your careful suggestions.

Line 83: We change "predict" to "explore": In the present study, we tried to ~~predict~~ explore the role of ferroptosis in HTGP ~~by~~ through proteome sequencing.

Line 128: We change "Differential Protein Screening" to "Differentially Expressed Proteins Analysis":

Differentially Expressed Proteins Analysis

The samples to be compared were selected, and the relative quantitative values of each protein in the repeated samples were taken as the difference multiple (fold change, FC). In order to judge the significance of the difference, the relative quantitative value of each protein in the samples of the comparison group was subjected to a t-test. The corresponding P-value was calculated as the significance index. The default P-value was < 0.05.

Line 179: We change "proteome sequencing" to "proteomic profiling": *Proteome profiling predicts the role of ferroptosis in P-407+CAE-induced HTGP rather than AP.*

Line 207: We change "rebounded" to "increased": *We found that the*

expression of GPX4 in the P-407+CAE+Fer-1 group ~~rebounded~~ increased comparing to Ctrl group (Figure 4A).

Line 308: We change "significant evidence" to "strong evidence": *Our findings provides ~~significant~~ strong evidence that ferroptosis is involved in the process of HTGP and that NOX2 is a key point in the regulation of ferroptosis.*

Line 443: We deleted "Calculation of": *~~Calculation of~~ Changed protein numbers among the sample groups.*

Reviewer #2:

1. The materials and methods section doesn't describe clearly in Animal model processing and treatment. -The weight changes of experimental animals should also be described. -Any mortality or other exclusions should be fully documented. This should include a description of the procedure for replacing any animals that might have been lost from a group during the prolonged survival intervals. -It still should indicate the animal numbers used in each Figure. -There is lack of rational for using the protocols of P-407 and caerulein treatment. Are those treatments based on pilot experiments or previous reports?

Response: Thank you for your suggestion. Here are our answers and we have made corresponding changes in paper.

-The weight of experimental animals didn't have significant change during the experiment. -Because the treatments were within a week and we have

done a lot of pre-experiments, there was no animals lost during the prolonged survival intervals.

-We added the animal numbers used in each Figure, the animal number for experiments was n=4 and the animal number for proteome sequencing was n=3.

-The protocols of P-407 and caerulein treatment were based on pilot experiments and previous reports [1,2]. The P-407 exposure group was intraperitoneally injected with a dosage of 0.6 g/kg bodyweight (bw) P-407 only once [1]. The dose of Caerulein was derived from our own pilot experiments, and the previous literature started with the application of AP modeling from 50 μg/kg [3]. After HE staining of animal pancreas, we found that the pancreas of AP model would be significantly damaged when the dose of caerulein was 100 μg/kg [2]. So we chose to use this dose for modeling.

Reference:

[1] Pan Y, Li Y, Gao L, Tong Z, Ye B, Liu S, et al. Development of a novel model of hypertriglyceridemic acute pancreatitis in mice. Scientific reports. 2017; 7: 40799.

[2] Dai J, Jiang M, Hu Y, et al. Dysregulated SREBP1c/miR-153 signaling induced by hypertriglyceridemia worsens acute pancreatitis and delays tissue repair. JCI Insight. 2021 Jan 25; 6(2): e138584.

[3] Silva-Vaz P, Abrantes AM, Castelo-Branco M, et al. Murine Models of

Acute Pancreatitis: A Critical Appraisal of Clinical Relevance. Int J Mol Sci. 2019 Jun; 20(11): 2794.

2. The bioinformatics tools that have been used for the proteome sequencing analysis. The produced raw data should also be outlined and compared with other already published references.

Response: Thank you for your suggestion. We have misunderstood “raw data”. We used to think it includes the KEGG figues, excels and heat maps, etc. But When we consulted the sequencing company two days ago, we learned that "raw data" is a large file package, which needs to be sent to us by the sequencing company through the flash drive, and then we upload the data to a specific website. Therefore, we can only upload the data to the Internet in about 7 days. We hope you can wait for another week.

Wang et al. [4] made a RNA sequence to analyze, at the hepatic level, the differentially expressed genes between acute pancreatitis (AP) with fatty liver rats and AP rats. They discovered the change in lipid metabolism and inflammation cytokines but not ferroptosis.

Reference:

[4] RNA sequence analysis of rat acute experimental pancreatitis with and without fatty liver: a gene expression pro ling comparative study.

3. How about the changes of TG、TC and α-AMY between P-407 group and P-407+CAE group in Figure 1?

Response: Thank you for your suggestion. There was no significant change

on TC and TG between P-407 group and P-407+CAE group, which proved that only P-407 caused a rise in TC and TG level rather than CAE. α-AMY significantly increased in P-407+CAE group comparing with P-407 group, which proved that only CAE caused a rise in α-AMY level rather than P-407.

4. The quantification criteria by HE images (Figure 1, Figure 4 and Figure5 were assessed should be clearly stated.

Response: Thank you for your suggestion. Tissue sections were evaluated blindly by two pathologists, based on previously reported criteria. The pathological injury of pancreas is scored according to the edema, inflammation, hemorrhage, and necrosis of pancreas (Table 1) [3]. The pathological score of lung were measured on the basis of edema, neutrophil infiltration and hemorrhage (Table 2) [4]. The renal pathological score is evaluated by the degree of tubulointerstitial injury based on previously reported criteria (Table 3) [5].

Reference:

Table 1. Histopathological Scoring of Pancreatic injury.

Score	Edema	Inflammation	Hemorrhage	Necrosis
0	No edema	0-1 neutrophil /HPF	No parenchyma hemorrhage	No necrosis
1	Regional interlobular edema	2-10 neutrophil /HPF	Parenchymal hemorrhage 0%-25%	Necrotic area 1%-10%
2	Diffuse interlobular edema	11-20 neutrophil /HPF	Parenchymal hemorrhage 25%-50%	Necrotic area 11%-20%
3	Acinus edema	21-30 neutrophil /HPF	Parenchymal hemorrhage 50%-75%	Necrotic area 21%-30%
4	Obvious lobule separation	> 30 neutrophil /HPF	Parenchymal hemorrhage >75%	Necrotic area >30%

Table 2. Histopathological Scoring of Lung injury.

Score	Edema	Infiltration of neutrophils	Hemorrhage	Thickness of Alveolar
0	No edema	Normal tissue	Normal tissue	Normal tissue
1	Interstitial and alveolar edema <25%	Little in interstitial	Alveolar hemorrhage 0%-25%	Mild
2	Interstitial thicken and alveolar edema 25%-50%	More in interstitial and partial alveolar	Alveolar hemorrhage 25%-50%	Moderate
3	Interstitial thicken evidently and alveolar edema 50%-75%	Neutrophil mass in interstitial and partial alveolar	Alveolar hemorrhage 50%-75%	Severe
4	Interstitial thicken evidently and alveolar edema >75%	Neutrophil mass in interstitial and alveolar	Alveolar hemorrhage >75%	Extremely severe

Table 3. Histopathological Scoring of Renal injury.

Score	**Histological changes were evaluated by quantitative measurement of tubulointerstitial injury, which was assessed by counting the number of necrotic and apoptotic cells, loss of tubular brush border, tubular dilatation, cast formation, and neutrophil infiltration.**
0	None
1	0-10%
2	11%-25%
3	26%-45%
4	46%-75%
5	76%-100%

[3] Schmidt J, Rattner DW, Lewandrowski K, et al. A better model of acute pancreatitis for evaluating therapy. Ann Surg. 1992 Jan;215(1):44-56.

[4] Matute-Bello G, Winn R, Jonas M, et al. Fas (CD95) induces alveolar epithelial cell apoptosis in vivo: implications for acute pulmonary inflammation. Am J Pathol. 2001; 158:153–61.

[5] Wu C, Zou L, Shi S, et al. The role of hypertriglyceridemia for acute kidney injury in the course of acute pancreatitis and an animal model. Pancreatology. 2017 ;17(4):561-566.

5. It was confused with the groups shown in Figure 3 (A and B) and Figure

5 (B and D). The four groups in each diagram should be clearly arranged.

Response: Thank you for your suggestion. We newly arranged the four groups in each diagram.

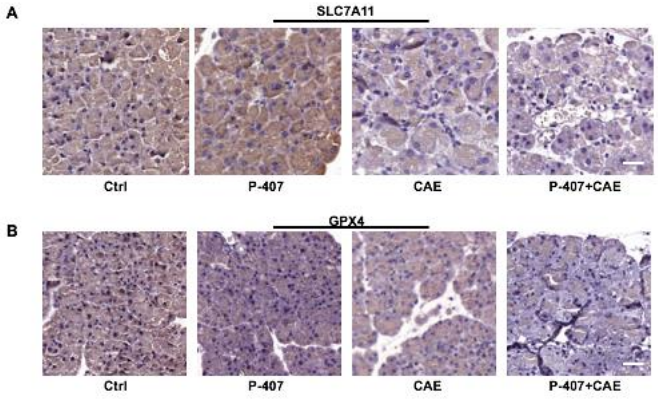

Figure 4 (A and B)

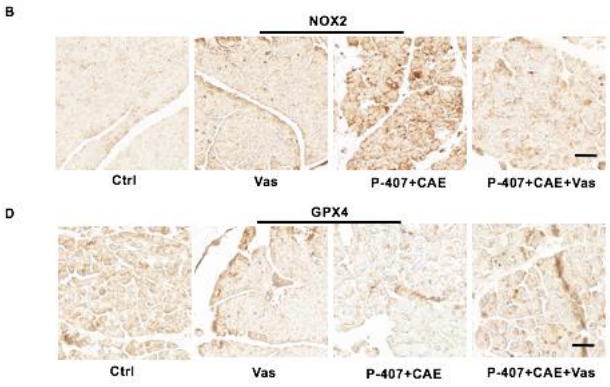

Figure 5 (B and D)

6. The authors indicate that X-ct is SLC7A11 (line 443), and the protein names should be consistent with the manuscript, Figure and legends.

Response: Thank you for your suggestion. We have gone through all the text and changed X-ct to SLC7A11.

7. It is difficult to estimate the changes of X-ct and GPX4 without statistical graphs of X-ct and GPX4. In addition, only the IHC of X-ct, GPX4 and MDA content could not draw a convincing conclusion that

HTGP specifically induces mouse pancreatic ferroptosis.

Response: Thank you for your suggestion. We have quantified the figures by Image J and made statistical analysis.

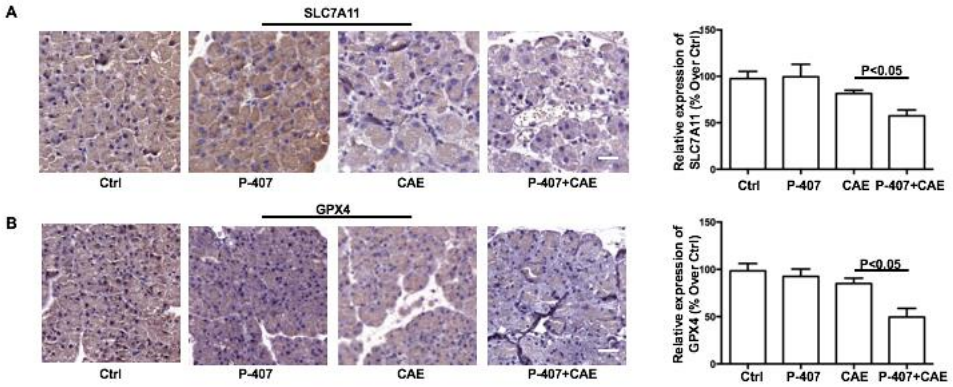

Figure 5

In addition, we added GSH and Fe^{2+} experiments to draw a convincing conclusion that HTGP specifically induces mouse pancreatic ferroptosis. The results showed P-407+CAE significantly induced GSH decrease and Fe^{2+} increase.

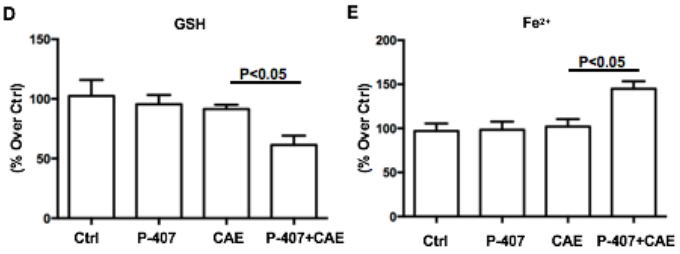

Figure 6

8. There are some grammatical and syntax errors in the manuscript, and should need more editing from this point of view.

Response: Thank you for your careful suggestions. We carefully checked the grammatical and syntactic errors of the paper and carefully revised it. After the paper was revised, we completed the language polishing service

again to meet the publication requirements.