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**Novel method to prevent severe
postoperative pancreatic fistula
caused by lipolysis**

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**（脂肪分解による重症術後膵液瘻の
新しい予防法の開発）**

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主論文

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Title: Novel method to prevent severe postoperative pancreatic fistula caused by lipolysis

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1 Abstract

2 **Background:** Although several prophylactic strategies have been developed for
3 postoperative pancreatic fistula (POPF), research on its severe form is few. Recently, it has
4 been reported that severe POPF can be caused by intra-abdominal lipolysis. This study aimed
5 to establish a rat model of severe POPF by combining pancreatic juice leakage and lipolysis
6 and develop a prophylactic strategy for POPF.

7 **Methods:** Sprague-Dawley rats were subjected to pancreatic transection to induce pancreatic
8 juice leakage (PT group). Autologous fat tissue was thermally treated to prepare a fat solution,
9 which was intraperitoneally administered to the rats in the PT group (PT+F group). A water-
10 solubilized lipase inhibitor (cetilistat) was administered intraperitoneally to the rats in the
11 PT+F group. A polyethylene glycol-based hydrogel (PEG-HG) formulation of water-
12 solubilized cetilistat was equally administered. Ascitic and serum biochemical tests,
13 including free fatty acids (FFA) levels, macroscopic or microscopic examinations, and
14 survival analyses were performed.

15 **Results:** In the PT+F group, significantly elevated ascitic and serum FFA levels and serum
16 inflammatory cytokines levels were observed 24 h postoperatively ($P < 0.001$), and survival
17 rate was significantly exacerbated ($P < 0.0001$). Intraperitoneal administration of water-
18 solubilized cetilistat resulted in reduced inflammation and improved outcomes. Although
19 PEG hydrogel itself did not improve blood parameters or survival outcomes, the
20 incorporation of water-solubilized cetilistat into the PEG-HG enabled similar improvement.

- 1 **Conclusion:** Intraperitoneal administration of water-solubilized cetilistat prevented severe
- 2 inflammation and multiple failures associated with severe POPF. The incorporation of water-
- 3 solubilized cetilistat into the PEG-HG is a promising delivery system for clinical application.

1 Introduction

2 A postoperative pancreatic fistula (POPF) is a major complication of pancreatectomy
3 associated with significant postoperative morbidity, a possible cause of delayed
4 chemotherapy^{1, 2}, and a healthcare financial burden². Previously, POPF was defined as a
5 fluid output from operatively placed drainage tubes with an amylase activity greater than
6 three times the upper normal serum value³. In 2016, the diagnostic criteria for POPF were
7 revised by the International Study Group on Pancreatic Surgery (ISGPS)⁴. Namely,
8 conventional Grade A (without clinical symptoms) was reworded as biochemical leak and
9 was no longer considered a POPF, whereas those requiring clinical intervention were
10 defined as clinically relevant POPF (CR-POPF)⁴. According to the revised criteria,
11 pancreatic juice leakage is not a clinical problem; however, progression to CR-POPF,
12 which involves local tissue damage and multi-organ failure owing to unknown factors,
13 should be prevented. To date, various strategies have been attempted to prevent POPF,
14 including surgical technique modifications⁵⁻⁸, application of adhesives around pancreatic-
15 enteric anastomoses^{9, 10} or pancreatic transection margin¹⁰⁻¹⁶, and intravenous
16 administration of pancreatic secretion inhibitors¹⁷⁻¹⁹. However, few of these are reliable
17 because they mainly focus on preventing pancreatic juice leakage and not on preventing
18 progression to CR-POPF. A high incidence of CR-POPF occurs in patients with obesity²⁰⁻
19 ²³. Pecorelli et al.²¹ reported visceral fat area as an independent predictor of pancreatic
20 fistula (optimal cut-off 167 cm²). Patients with obesity usually have much visceral fat,
21 resulting in many triglyceride leaks due to heat damage during pancreatic surgery.
22 Triglycerides are broken down into free fatty acids (FFA) by leaked pancreatic lipase. We

1 previously reported that the FFA concentration in drain fluid is significantly associated
2 with the occurrence of CR-POPF and intra-abdominal lipolysis induced by lipases in leaked
3 pancreatic juice is a cause of CR-POPF²⁴. However, because purified triglycerides were
4 administered as a source of artificial FFA in our previous study, it was unclear whether this
5 was mimicking the phenomenon of human pancreatic surgery. In this study, we
6 hypothesized that intra-abdominal lipolysis of autologous adipose tissue is the cause of
7 severe POPF, and tested the possibility that it could be prevented by administering lipolysis
8 enzyme inhibitors. We aimed to create an animal model of severe POPF that is closer to
9 humans, to confirm that POPF is exacerbated by lipolysis, and to establish a new clinical
10 prevention method for severe POPF.

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1 **Methods**

2 **Experimental study**

3 All animal experiments were approved by the Institutional Animal Care and Use Committee
4 of Kyoto University. Male Sprague–Dawley rats (9–10 weeks old; Shimizu Laboratory
5 Supplies, Kyoto, Japan) were acclimatized for at least 7 days and were included when their
6 body weight was 280–370 g. Male rats were chosen to minimize variations in body weight
7 and biochemical indicators. The rats were housed under a 12-h light-dark cycle at 21–25°C,
8 fed with standard laboratory chow, and provided ad libitum access to water. To investigate
9 the effect of FFA on the abdominal cavity, an oleic acid model was established. Specifically,
10 after performing only a laparotomy, 1, 2, or 3 ml of oleic acid (Tokyo Chemical Industry Co.,
11 Ltd., Tokyo, Japan) was administered to the abdominal cavity (Figure S1). Next, a pancreatic
12 transection was performed (PT model), as previously described. Briefly, the rats were
13 anesthetized with sevoflurane and intraperitoneally injected with midazolam, butorphanol,
14 and medetomidine for sedation and analgesia. A midline laparotomy was performed, and the
15 pancreas was encircled on the ventral side of the portal vein after minimal mobilization. The
16 splenic artery and vein were preserved throughout the procedure. The rat pancreatic duct
17 comprises four branches: the gastric, duodenal, common, and splenic ducts (Figure 1a). The
18 gastric, splenic, and surrounding pancreatic parenchyma were transected using scissors
19 (Figure 1b). Finally, the pancreas was transected above the confluence of the portal and
20 splenic veins (Figure 1c). Bleeding from the pancreatic stump was controlled using gentle
21 compression. Considering the significant effect of visceral fat on CR-POPF, a fat injury
22 model (F model) was established using visceral fat. Briefly, fatty tissues around the bilateral

1 genital areas were dissected (Figure 1d) and coagulated for 5 min at 50 W output using a
2 bipolar electrocautery system (Figure 1e, f). The fat solution generated by this procedure
3 (Figure 1g) was cooled to room temperature to prevent thermal injury and then completely
4 reintroduced into the abdominal cavity of the rats without PT. The fat solution contained an
5 average of TG 238.8mg/dL and FFA37.7 μ Eq/L (n=4).

6 A PT+F model was established following PT by injecting the fat solution into the abdominal
7 cavity of the rats in the PT group. The sham group underwent a laparotomy alone. The rats
8 were euthanized 24 h post-surgery for analysis or observed for 7 days for survival analysis.
9 To minimize variability, the surgeries were conducted in the morning. Furthermore, in the
10 PT+F group, a significant number of deaths occurred between 24 and 36 h post-surgery.
11 Therefore, the analysis of the surviving rats in the PT+F group, along with comparisons to
12 other groups, was optimal 24 h after the surgery. Blood samples were collected from the
13 inferior vena cava immediately after euthanasia. Following the injection of 4 mL of saline
14 into the abdominal cavity, as much lavage fluid as possible was retrieved and centrifuged to
15 collect the supernatant, which was analyzed as the ascitic fluid. All samples were either
16 analyzed immediately or stored at -80°C for future analysis. Cetilistat (Cayman Chemical,
17 Michigan, USA) was used as the lipase inhibitor to inhibit lipolysis. Since cetilistat is
18 hydrophobic and easily aggregates when administered as a powder intraperitoneally,
19 resulting in the loss of efficacy, it was mixed with 10 μ L of polysorbate 80 (P80) (NOF
20 CORPORATION, Tokyo, Japan) and intraperitoneally administered after sufficient
21 dispersion was achieved to make it water-soluble. The intraperitoneal dose of cetilistat was
22 determined by converting the human oral dose based on body weight, and doses of 0, 1, 10,

and 50 mg/body were administered intraperitoneally to each rat. To retain the drug locally at the surgical site, the drug was incorporated in a water-solubilized solid polyethylene glycol (PEG)-based hydrogel (PEG-HG). The PEG-HG comprised two PEG derivatives (200-GS and 200-SH) provided by the NOF CORPORATION.

Sample size determination

For the analysis 24 h after the surgery, we calculated the required sample size based on an α error of 0.05 and a power ($1-\beta$ error) of 0.8. In preliminary experiments, the serum FFA values, which represent the most critical factor, showed a mean difference of 240 $\mu\text{Eq/L}$ between the two groups (PT/PT+F) and a common standard deviation of 230. Thus, the minimum required sample size was calculated to be 15, which we applied to each group. For the survival analysis, the 7-day survival rates in the Sham and PT groups were 0.9 and 0.3, respectively, in preliminary experiments. With a registration period (intervention day) of 1 day, an observation period of 7 days, an α error of 0.05, and a power ($1-\beta$ error) of 0.8, the minimum required sample size was calculated to be 13. No rats died in the Sham and F groups. We set the sample size to $n=5$ for animal welfare considerations.

Saponification score

To evaluate the saponification status of the fatty acids in the abdominal cavity, the amount of calcium salt of the FFA deposited was quantified and scored. Photographs were taken during repeat laparotomy after surgery, and the proportion of whitish deposits (calcium salts of free fatty acids) relative to the entire peritoneal area was quantified using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA). The scoring was as follows: 0, 0%–2.5%; 1, 2.5–5%; 2, 5.0–7.5%; 3, 7.5–10%; and 4, > 10%.

1 **Biochemical assay**

2 Biochemical assays, including the measurements of the levels of aspartate aminotransferase
3 (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinine, blood
4 urea nitrogen (BUN), amylase (AMY), lipase (LIP) and FFA were performed in a specialized
5 clinical laboratory (Japan Clinical Laboratories, Kyoto, Japan).

6 **Enzyme-linked immunosorbent assay**

7 The amount of serum tissue necrosis factor (TNF)- α and interleukin (IL)-6, pro-inflammatory
8 cytokines, was measured using enzyme-linked immunosorbent assay kits (R&D Systems Inc,
9 Minnesota, USA) according to the standard protocols.

10 **Histological analysis**

11 Tissue samples were collected 24 h postoperatively, fixed in 10% buffered formalin for 48–
12 72 h, and embedded in paraffin to create 4- μ m-thick sections. For the histological
13 examination, the entire pancreas, right median lobe of the liver, and right kidney were
14 analyzed. Standard protocols were followed for hematoxylin and eosin staining.

15 **Statistical analysis**

16 All the statistical data were expressed as the mean $\pm$ standard deviations. The data were
17 analyzed using a one-way analysis of variance with Kruskal–Wallis Analysis of Variance on
18 Ranks. Survival analysis was performed using Kaplan–Meier plots and evaluated using the
19 log-rank test using GraphPad Prism 10.3.1. (GraphPad Software, California, USA). A P-
20 value <0.05 was considered statistically significant.

Results

Severe POPF model with intraperitoneal fat

First, animal experiments were conducted to investigate whether FFA released from autologous adipose tissue during surgery could induce severe

-POPF. Four groups were included in this experiment: sham, F, PT, and PT + F. At 24 h postoperatively, widespread white deposits were observed only in the intraperitoneal cavity

of the rats in the PT+F group (Figure 2a). These white deposits were previously described as calcium fatty acid salts²⁴, which were degraded by pancreatic lipase from the fat solution

administered into the abdominal cavity. The saponification score was significantly higher in

the PT+F group than in the other three groups (Figure 2b). Ascitic FFA levels were

significantly elevated in the PT+F group, which correlated with the saponification score

(Figure 2c). The average composition of free fatty acid fractions in the ascitic fluid of the

PT+F group was as follows, in descending order: linoleic acid 33.7%, oleic acid 32.5%, and

palmitic acid 11.7% (Figure S3). Ascitic AMY and LIP levels were significantly higher in

the PT group than in the Sham and F groups, whereas no significant difference was observed

in the ascitic AMY and LIP levels between the PT+F and PT groups (Figures 2d, e). Similarly,

serum AMY and LIP levels were significantly higher in the PT group than in the Sham and

F groups, whereas no significant difference was observed in the serum AMY and LIP

between the PT+F and PT groups (Figures 2f, g). The levels of serum biochemical indicators

(AST, BUN, Cre, and FFA) increased significantly in the PT+F group compared with that in

the other three groups, suggesting liver and renal damage (Figures 2h–k). Microscopically,

the infiltration of inflammatory cells and destruction of pancreatic acinar cells were observed

only in the areas where FFA calcium had deposited on the surface of the pancreas, whereas no inflammatory changes or necrosis were observed in the interior of the pancreas (Figure 2l). Inflammatory cell infiltrations and deposition of calcium salts of FFA were observed in the peritoneum (Figure 2m). Serum TNF- α level was higher in the PT+F group (not significant), and serum IL-6 was significantly higher in the PT+F group than in the other three groups, suggesting a high level of inflammation in the PT+F group (Figures 2n, o). Survival curve analysis revealed that none of the rats in the Sham, PT, and F groups died within 7 postoperative days, whereas 15 of 16 rats (93.8%) in the PT+F group died, with a median survival time of 36 h and a significantly lower survival rate ($P < 0.0001$) (Figure 2p).

To investigate the systemic effects of intraperitoneal FFA alone, oleic acid, the most abundant FFA in the abdominal cavity²⁵, was intraperitoneally administered at doses of 1, 2, and 3 mL per rat without PT. Consequently, the saponification score and ascitic FFA levels increased with increasing oleic acid dose (Figures S1a, b). Similarly, ascitic AMY, LIP, serum LIP, AST, BUN, Cre, FFA, and IL-6 levels increased with increasing oleic acid dose (Figures S1c–l), resulting in reduced survival rate, suggesting that FFA alone (without lipase) caused systemic disorders (Figure S1m).

Prophylactic effect of lipase inhibitor for severe POPF model

We confirmed lipolysis inhibition by lipase inhibitors and the resulting local and systemic improvements in the PT+F group. Cetilistat with P80 was prepared to obtain water-solubilized cetilistat to prevent lipolysis. Up to 50 mg (0, 1, 10, and 50 mg) of water-solubilized cetilistat with P80 was concurrently injected into the abdominal cavity of the rat

in the PT+F group. Increasing the dosage of water-solubilized cetilistat resulted in decreases in saponification scores and ascitic FFA levels, with significant decreases observed at doses of 10 mg/body or higher (Figures 3a, b). The average composition of fatty acid fractions in the ascitic fluid of the PT+F+【C10mg+P80】 group was as follows, in descending order: linoleic acid 24.3%, oleic acid 23.6%, and palmitic acid 20.3% (Figure S3). However, ascitic AMY and LIP levels did not decrease with the administration of water-solubilized cetilistat (Figures 3c, d). Serum BUN and FFA levels were significantly decreased with doses of cetilistat ≥ 10 mg/body, and serum IL-6 was decreased significantly with doses of cetilistat ≥ 10 mg/body (Figures 3e–k). The survival curve showed improvement with increasing dose of cetilistat, reaching a plateau at 10 mg/body cetilistat (Figure 3l). Based on these results, water-solubilized cetilistat was effective in preventing severe POPF in rats, and the optimal dose of water-solubilized cetilistat was set at 10 mg/body.

Incorporation of lipase inhibitor into PEG-HG for adequate drug delivery

Water-solubilized cetilistat with P80 is liquid, so it was anticipated that it would be drained through the drainage tubes usually placed during pancreatic surgery or diffuse into the abdominal cavity, resulting in a loss of the drug effect. To prevent this, water-solubilized cetilistat with P80 was incorporated into a PEG-HG administered. To evaluate the degradability of the PEG-HG, it was placed in phosphate-buffered saline. It degraded within approximately 4h (data not shown). PEG-HG incorporating 10 mg water-solubilized cetilistat rapidly gelled immediately after the intra-abdominal administration and remained localized

(Figure 4a). At 24 h postoperatively, most of the PEG-HG had degraded, with little remaining visible to the naked eye. The water-solubilized cetilistat 10 mg and the PEG-HG formulation of 10 mg of water-solubilized cetilistat resulted in similar decreases in the saponification score and ascitic FFA, AMY, LIP, serum AMY, LIP, AST, BUN, FFA, and serum IL-6 levels (Figures 4b–l). Both groupss improved survival outcomes equally (Figure 4m). Furthermore, when PEG-HG without cetilistat was injected alone into the PT margin, there was no decrease in the saponification score or levels of ascitic FFA, AMY, and LIP, or in serum AMY, LIP, AST, BUN, and FFA compared with PEG-HG with water-solubilized cetilistat with P80 group (Figures S2a–j). No significant differences were observed in survival (Figure S2k), suggesting PEG-HG alone was ineffective in preventing severe POPF.

Discussion

In this study, the fat solution (F) from an autologous adipose tissue was administered into the abdominal cavity of rats following PT to establish a severe POPF model, and FFA was produced, which causes inflammation, resulting in reduced survival in PT+F rats. There have been reports using acute pancreatitis model mice demonstrating the association between elevated serum free fatty acids, increased inflammatory cytokines, subsequent deterioration of hepatic and renal function, and systemic disorders ²⁶. In the present study, a similar deterioration occurred due to FFA caused by pancreatic leakage. In fact, the FFA produced by fat decomposition in the abdominal cavity caused peritonitis and increased inflammatory cytokines (TNF- α , IL-6). Furthermore, serum FFA and inflammatory cytokines absorbed from the peritoneum caused liver damage (increased AST) and kidney

1 damage (increased BUN and creatinine), ultimately leading to systemic damage and a
2 decrease in survival rate. When water-solubilized cetilistat with P80 was concurrently
3 administered intraperitoneally, improvements in outcomes, including ascitic/serum
4 biochemistry, macroscopic peritoneal saponification, and survival, were observed. The
5 PEG hydrogel incorporating water-soluble cetilistat demonstrated a prophylactic effect
6 similar to that of water-soluble cetilistat alone.

7 Basic and clinical studies have attempted various measures to prevent POPF in the
8 remnant pancreas, including innovations in surgical techniques⁵⁻⁸, application of adhesives
9 or cell sheets or hydrogels to the pancreatic–digestive tract anastomosis^{9, 10} or pancreatic
10 resection margin¹⁰⁻¹⁶, and intravenous or subcutaneous administration of pancreatic
11 secretion inhibitors¹⁷⁻¹⁹. In previous animal experiments, a POPF model was established
12 using PT alone¹⁰⁻¹⁶. However, PT alone resulted in a 100% survival rate at 7 days post-
13 surgery based on the survival analysis result in our current study. Additionally, compared
14 with the sham group, no significant increase was observed in ascitic and serum levels of
15 biochemical indicators, except for ascitic and serum AMY.

16 Clinically, CR-POPF is highly associated with body mass index or visceral fat²⁰⁻²³, and we
17 observed a strong association between CR-POPF and ascitic FFA in patients undergoing
18 pancreatectomy²⁴. To mimic this situation in a rat POPF model, we previously administered
19 purified triolein intraperitoneally into the abdominal cavity of rats that underwent PT to
20 induce FFA²⁴. However, this process was artificial and does not correspond to human
21 pancreatic surgery. Therefore, we used a fat solution generated from autologous adipose
22 tissue by thermal injury instead of purified triglycerides in the current study. This approach

1 mimics the incidental thermal damage to fat caused by energy devices during surgery and
2 the subsequent exposure of the fat solution to the surgical site in human pancreatic surgery.
3 PT alone produced BL-like changes without causing severe adverse outcomes. Conversely,
4 the PT+F model group exhibited significant macroscopic saponification and inflammation,
5 with elevated ascites and serum FFA, AST, and BUN levels, resulting in reduced survival.
6 Thus, the PT+F model might be appropriate for simulating CR-POPF.

7 In the current study, ascitic AMY level was not significantly different between the PT and
8 PT+F model groups, which is consistent with the ISGPS definition of POPF. According to
9 their definition, the difference between BL and Grade B/C POPF (CR-POPF), not ascitic
10 AMY levels, is the need for intervention. In this study, intraperitoneal administration of
11 purified oleic acid (the most abundant free fatty acid in the blood ²⁵ARR) caused damage to
12 acinar cells, leading to increased ascitic AMY levels. However, although the AMY level in
13 the ascites fluid increased due to pancreatic leakage caused by PT, there was no significant
14 difference between the PT+F group. This indicates that the damage to acinar cells caused
15 by FFA is less than that caused by pancreatic resection. Given that PT represents a mild
16 POPF phenotype, ascitic AMY levels may not be related to the severity of the condition.
17 Our previous clinical studies revealed that inflammation as represented by CRP levels
18 rather than ascitic AMY levels, was the most relevant factor in the severity of POPF²⁷. In
19 fact, in our model, the PT+F group showed high levels of serum TNF-alpha or IL-6, and it
20 is highly likely that at least the triglycerides that have been broken down by lipase are a
21 contributing factor to severe POPF.

1 Post-pancreatectomy acute pancreatitis (PPAP) may contribute to the onset and severity of
2 POPF²⁸. The current severe POPF model should be distinguished from PPAP, and there
3 was no significant difference in serum AMY levels between the PT and PT+F models (Fig.
4 2f). In the areas where calcium salts of fatty acids had deposited on the surface of the
5 pancreas, we observed clusters of inflammatory cells and necrosis of islet cells, but in the
6 PT+F model, no inflammatory changes were observed in the interior of the pancreas (Fig.
7 2l). This indicated that pancreatitis was not caused throughout the pancreas in the PT+F
8 model.

9 To prevent severe POPF, lipase activity inhibition was investigated. Cetilistat (C:
10 C25H39NO3), a lipase inhibitor, was originally designed as an oral medication to inhibit
11 lipolysis in the gastrointestinal tract, thereby preventing and treating obesity. However,
12 cetilistat aggregates in water, making it unsuitable for direct intraperitoneal administration.
13 Therefore, P80 was selected to allow cetilistat to become soluble in water. When
14 intraperitoneal administration of water-solubilized cetilistat in various doses was performed
15 in the PT+F model, we observed better conditions in macroscopic peritoneal saponification,
16 decreased ascitic FFA, less inflammation, lower serum FFA, AST, BUN, IL-6, and TNF- α ,
17 and prolonged survival in relation to the dose of water-solubilized cetilistat. These results
18 indicated that intraperitoneal administration of water-solubilized cetilistat effectively
19 suppressed the generation of FFA and the progression of severe POPF. The inhibition of
20 severe POPF by targeting FFA derived from lipolysis is a novel approach, and there are no
21 prior reports on this method.

Water-solubilized cetilistat is liquid and so can be rapidly drained through drainage tubes or spread to unintended areas, such as the subdiaphragmatic or pelvic regions. Therefore, for clinical application to human, it is necessary to develop a method to maintain the efficacy of the drug while keeping the water-solubilized cetilistat at the affected area. PEGs with a molecular weight of 35 kDa (PEG35) injected into the abdominal cavity of rats with induce acute pancreatitis improved inflammatory responses and cell death²⁹. Additionally, physical prevention of POPF can be achieved by applying a hydrogel to the dissection margins³⁰. Thus, we prepared a PEG hydrogel for incorporating water-solubilized cetilistat. Our PEG-HG alone was ineffective at tightly covering the PT edge because of its low strength and rapid degradability. When administered to the rats in the PT+ F model group, the PEG-HG formulation of water-solubilized cetilistat was not visible to the naked eye nor ameliorated severe POPF on the first postoperative day. Although the PEG-HG formulation of water-solubilized cetilistat rapidly gelled and remained around the pancreas after its intraperitoneal administration, water-solubilized cetilistat alone and PEG-HG formulation showed similar macroscopic peritoneal saponification score, ascitic FFA levels, serum biochemical indicator levels, and inflammatory cytokine levels, and improved survival were observed. Thus, PEG-HG could be a suitable biomaterial for the clinical application of intraperitoneal lipase inhibitors. In the future, it is desirable to develop a biomaterial that securely covers the PT margin and enables the sustained release of the drug.

This study had some limitations. First, there were significant differences between rat and human POPF models in terms of anatomical structures, surgical techniques, and perioperative management. In particular, the spontaneous closure of the POPF in rats

1 prevents an accurate representation of the human POPF. Additionally, rats are resistant to
2 infections; therefore, the infection-induced deterioration of POPF observed in humans may
3 not be replicated. Second, the smaller volume of the rat abdominal cavity compared with
4 that of humans may have resulted in more pronounced effects of the water-solubilized
5 cetilistat, which might have masked the advantage of incorporating the water-solubilized
6 cetilistat in the PEG-HG. In order to address these challenges, experiments using larger
7 animals such as pigs, whose abdominal structures are closer to those of humans, are
8 necessary. Finally, in this study, we focused on the toxicity (lipotoxicity) of FFAs
9 generated from the fat solution administered intraperitoneally. Therefore, adipocytokines
10 such as adiponectin were not analyzed. Further experiments are needed to determine
11 whether these factors contribute to severe POPF.

12 In conclusion, a severe POPF rat model was designed using intraperitoneal lipolysis.
13 Intraperitoneal administration of the water-solubilized cetilistat with P80 prevented the
14 development of severe POPF. Furthermore, incorporating water-solubilized cetilistat into a
15 PEG-HG for stable drug delivery could be a novel method for preventing severe
16 -POPF. Further studies are required for its clinical application. The graphic abstract of this
17 article is available in Figure 5.

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1 **Conflict of interest:**

2 Authors declare no Conflict of Interest for this article.

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6

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Figure legends

Figure 1

A representative image of the main pancreatic ducts of rats and the procedures of PT and F.

(a) The rat pancreatic duct comprises four branches: the gastric, duodenal, common, and splenic ducts. Dashed line indicates the stream of the pancreatic ducts. (b) Schematic image of pancreatic parenchyma encircled just above the SMV-PV. Yellow dashed line indicates the PT line (transecting splenic duct and gastric duct with surrounding parenchyma). (c) Schematic image of after PT. The pancreas was transected just above the confluence of the SMV and SPV. (d–g) schematic image fat damage (F): (d) Arrows indicate adipose tissue around the testis of a rat. (e,f) The fat tissue was coagulated for 5 min at 50 W output using the bipolar electrocautery system. (g) The fat solution and debris created by the procedure.

Figure 2

Comparison of macroscopic findings, saponification score, ascitic and serum biochemistry, microscopical findings, and survival analysis among the Sham, F, PT, and PT+F groups.

(a) Microscopic findings of the abdominal cavity. (b) Saponification score. (c–e) Ascitic biochemistry: free fatty acid (FFA) (c), amylase (d), and lipase (e). (f–k) Serum biochemistry: amylase (f), lipase (g), aspartate aminotransferase (AST) (h), blood urea nitrogen (BUN) (i), creatinine (Cre) (j), and FFA (k) on postoperative day 1. (l) Microscopic findings of the surface of the pancreas. Dashed line indicates no inflammatory changes in the interior of the pancreas. Arrows indicate inflammatory cell infiltration and destruction of acinar cells. (m)

Microscopic findings of the surface of the peritoneum. Arrows indicate inflammatory cell infiltration and destruction of acinar cells. (n, o) Serum inflammatory cytokines: TNF- α (n) and IL-6 (o). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (Kruskal–Wallis test). (p) Kaplan–Meier survival curves. (n=5 for Sham, F and PT groups; n=16 for PT+F group). ****P < 0.0001 (log rank test).

Figure 3

Comparison of saponification score, ascitic and serum biochemistry, and survival analysis among the PT+F, PT+F+P80, PT+F+ 【C1mg+P80】 , PT+F+ 【C10mg+P80】 , and PT+F+ 【C50mg+P80】 groups. (a) Saponification score. (b–d) Ascitic biochemistry: FFA (b), amylase (c), and lipase (d). (e–i) Serum biochemistry: amylase (e), lipase (f), AST (g), BUN (h), and FFA (i). (j, k) Serum inflammatory cytokines: TNF- α (j), and IL-6 (k). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (Kruskal–Wallis test). (l) Kaplan–Meier survival curves (n=16 for PT+F+P80 group; n=14 for PT+F+(C1 mg+P80) and PT+F+(C10 mg+P80) groups; n=15 for PT+F+(C50 mg+P80) group).

Figure 4

The schemas of PEG hydrogel injection, comparison of saponification score, ascitic and serum biochemistry, and survival analysis, between the PT+F+ 【C10mg+P80】 and PT+F+ 【C10mg+P80/PEG-HG】 groups. (a) The schema of PEG-HG incorporating water-

solubilized cetilistat administered at the site of pancreatic transection. The white arrow indicates transected ends of the pancreas. (b) Saponification score. (c–e) Ascitic biochemistry: FFA (c), amylase (d), and lipase (e). (f–j) Serum biochemistry: amylase (f), lipase (g), AST (h), BUN (i), and FFA (j). * (k, l) Serum inflammatory cytokines: TNF- α (k) and IL-6 (l). $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ **** $P < 0.0001$ (Kruskal–Wallis test). (m) Kaplan–Meier survival curves ($n=14$ for PT+F+(C10 mg+P80) group; $n=16$ for PT+F+(C10 mg+P80/PEG-HG) group).

Figure 5

Graphic abstract of this article.

Figure S1

Comparison of saponification score, ascitic and serum biochemistry, and survival analysis among the sham, OA 1ml, OA 2ml, and OA 3ml groups. (a) Saponification score. (b–d) Ascitic biochemistry: FFA (b), amylase (c), and lipase (d). (e–j) Serum biochemistry: amylase (e), lipase (f), AST (g), BUN (h), Cre (i), FFA(j). (k, l) Serum inflammatory cytokines: TNF- α (k) and IL-6 (l). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ (Kruskal–Wailis test). (m) Kaplan–Meier survival curves ($n=5$ for Sham group; $n=13$ for OA 1 ml group and OA 3 ml groups; $n=14$ OA 2 ml group).

Figure S2

Comparison of saponification score, ascitic and serum biochemistry, and survival analysis

between the between the PT+F+P80 and PT+F+P80+PEG-HG】 groups. (a) Saponification score. (b-d) Ascitic biochemistry: FFA(b), amylase(c), and lipase(d). (e-j) Serum biochemistry: amylase(e), lipase(f), AST(g), BUN(h), Cre(i) and FFA(j). *P< 0.05, **P< 0.01, ****P< 0.0001 (Kruskal-Wallis test). (k) Kaplan–Meier survival curves (*n*=16 for PT+F+P80 group; *n*=16 PT+F+P80+PEG-HG group).

Figure S3

The fractions of FFA in the ascites of the PT+F group and the PT+F+ 【C10mg+P80】 groups.

Fig 1

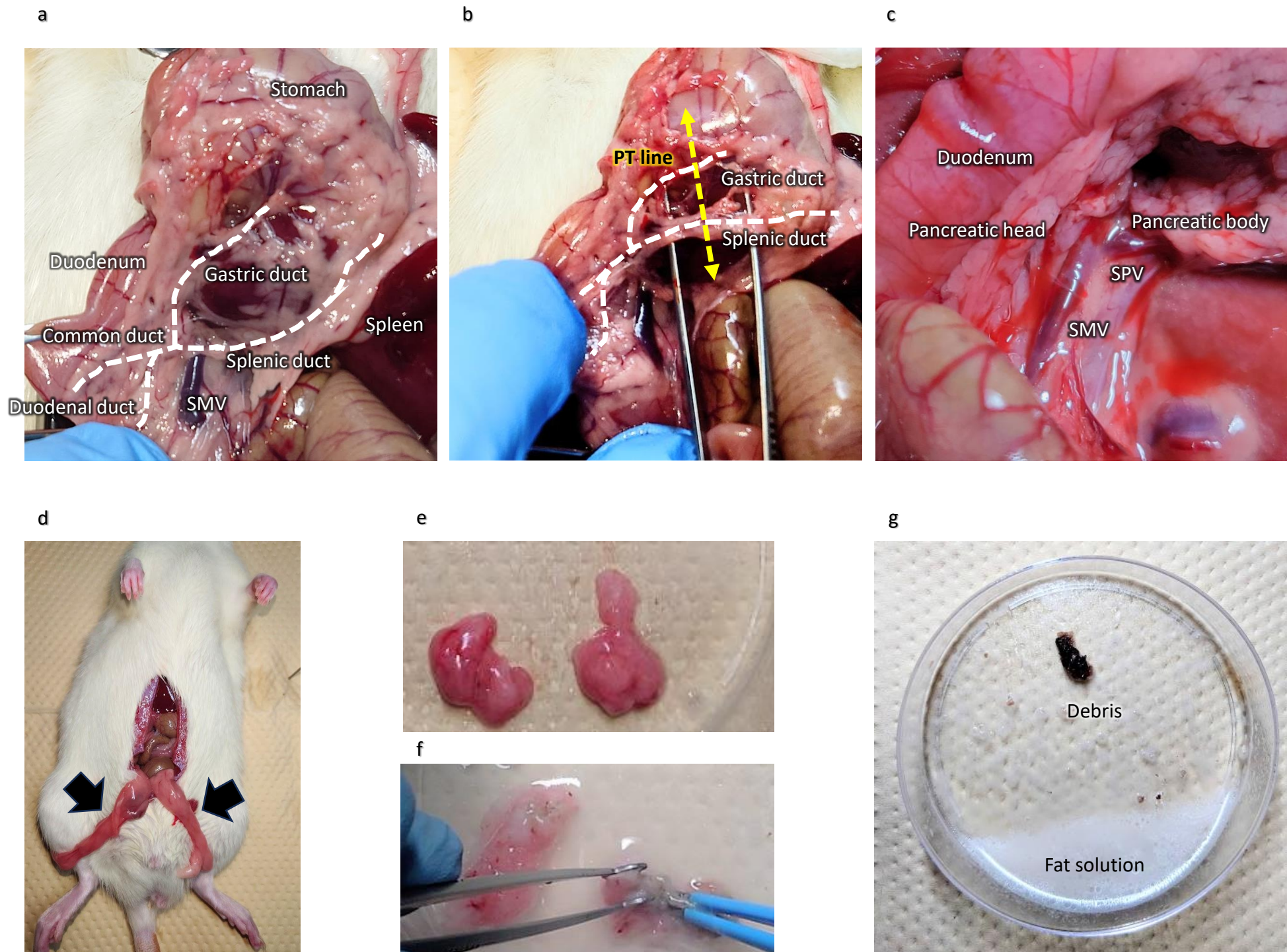
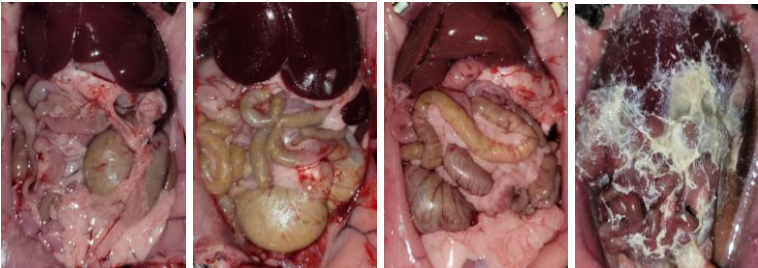
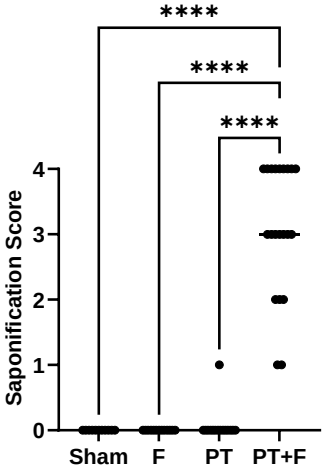


Fig 2

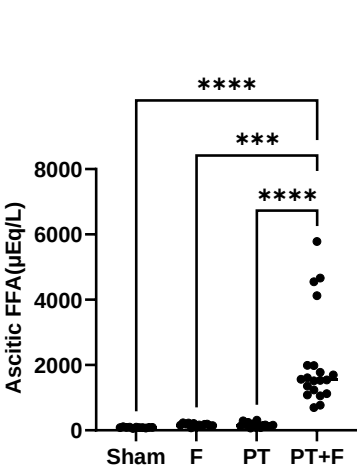
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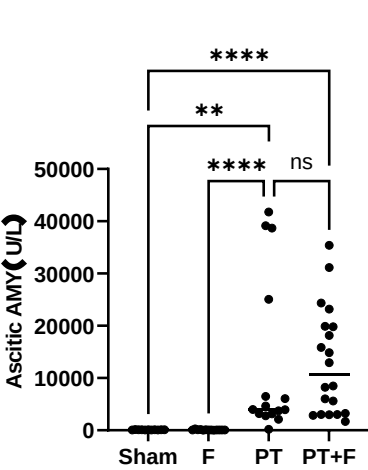
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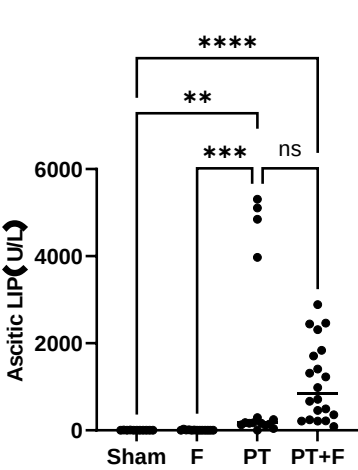
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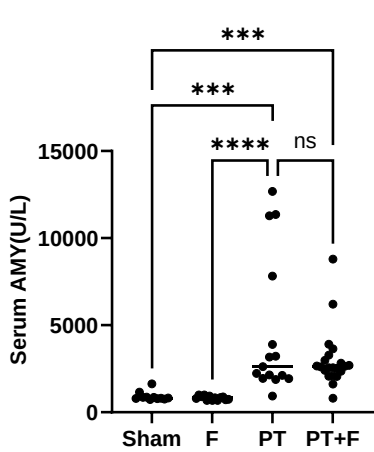
d Ascitic amylase



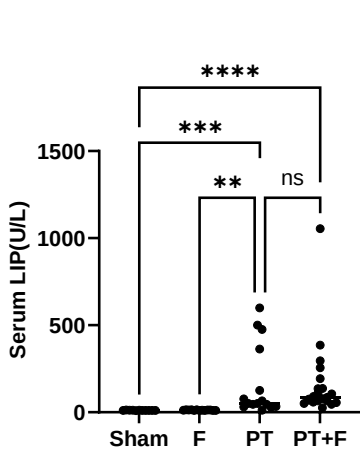
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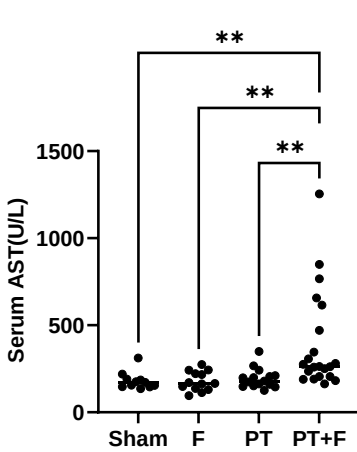
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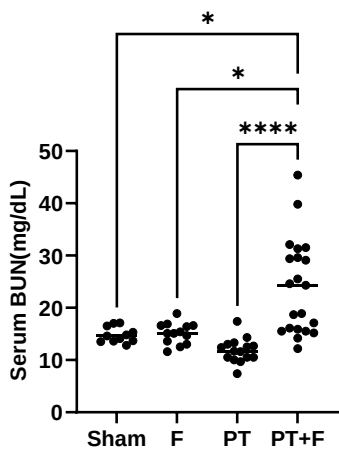
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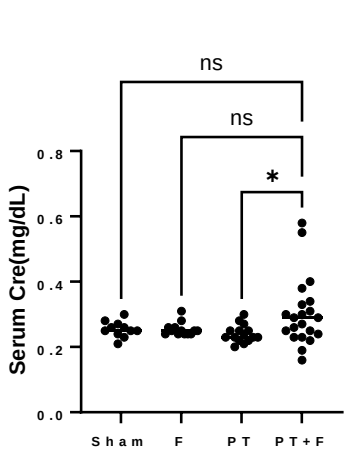
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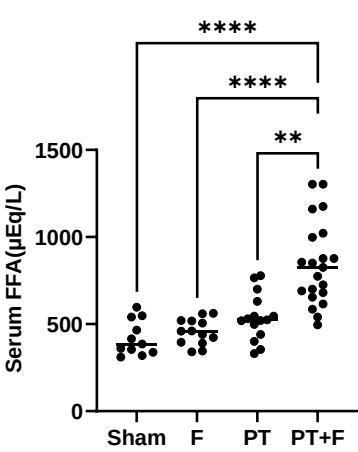
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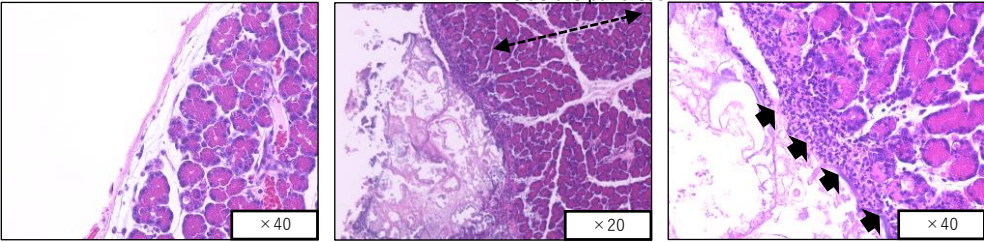
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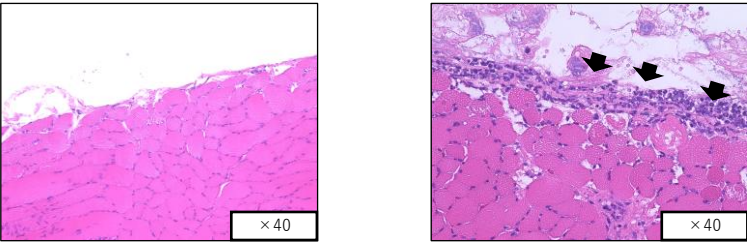
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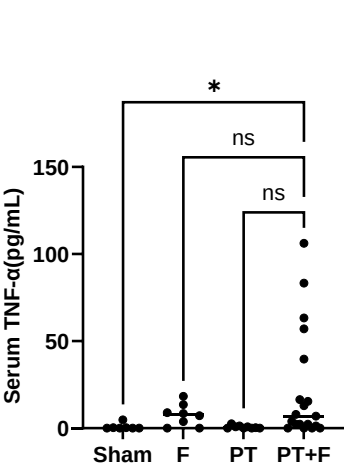
l Microscopic findings of pancreas



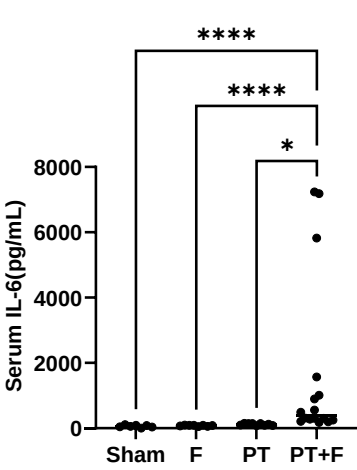
m Microscopic findings of peritoneum



n Serum TNF-α



o Serum IL-6



p Survival

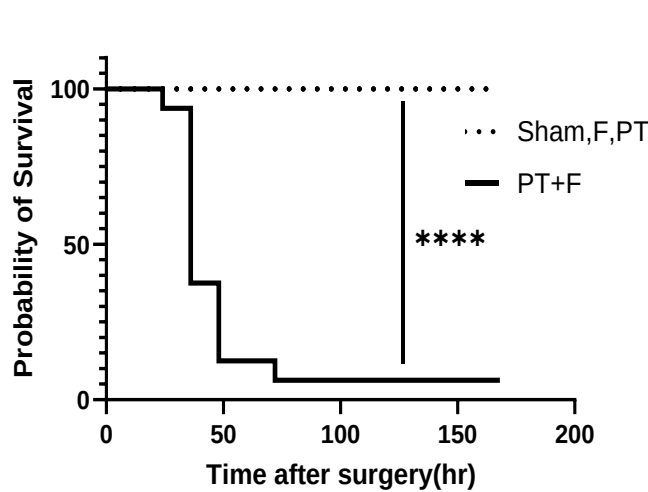
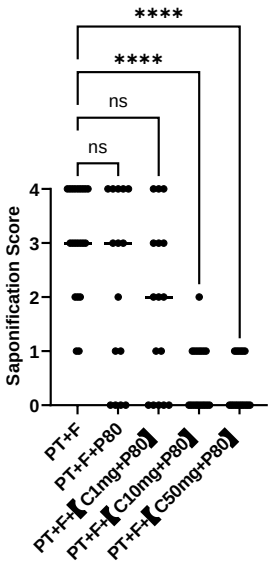
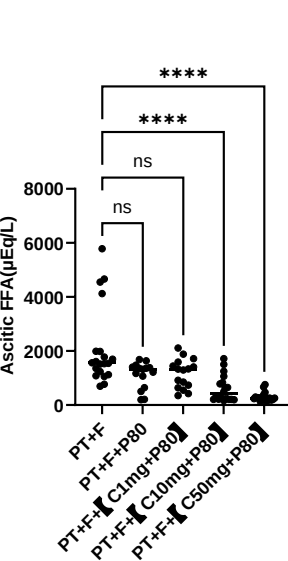


Fig 3

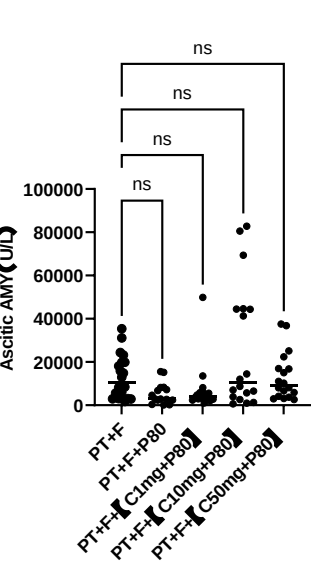
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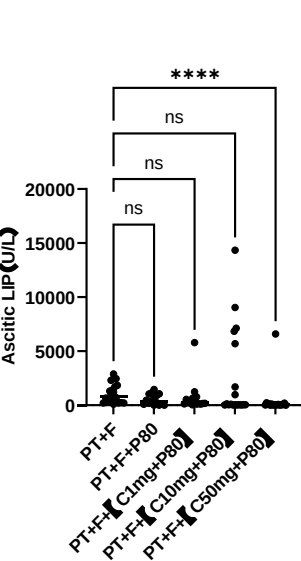
b Ascitic FFA



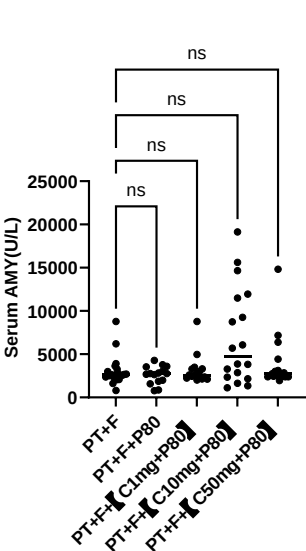
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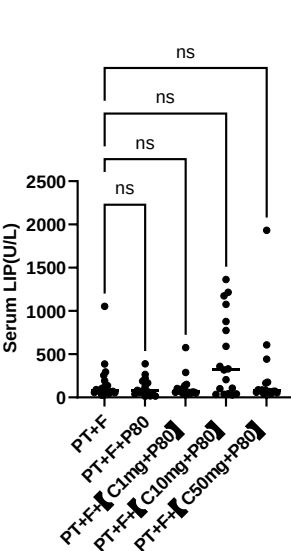
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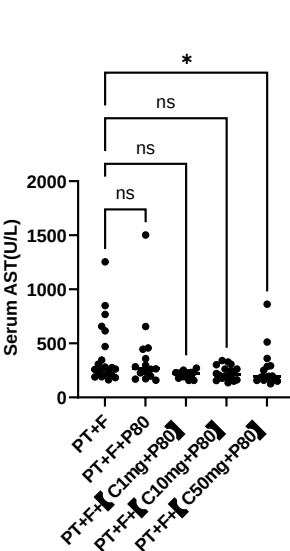
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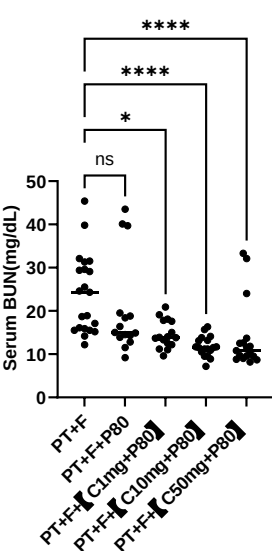
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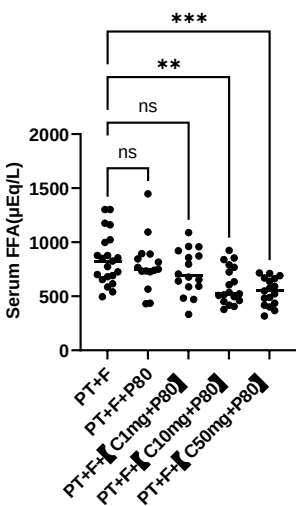
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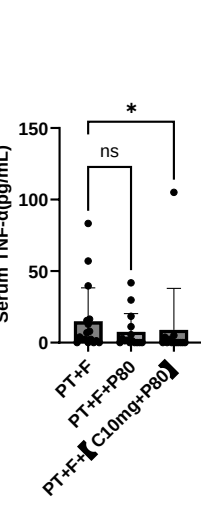
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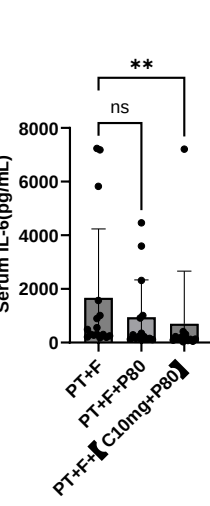
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j Serum TNF-α



k Serum IL-6



l Survival

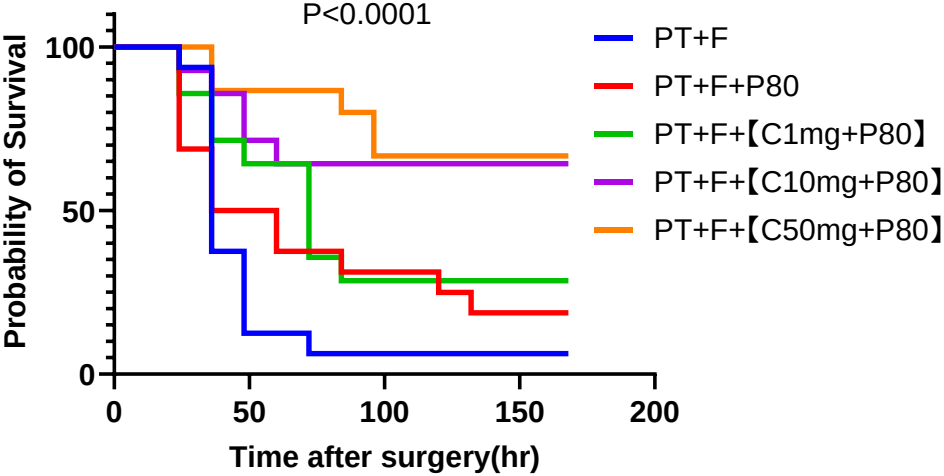
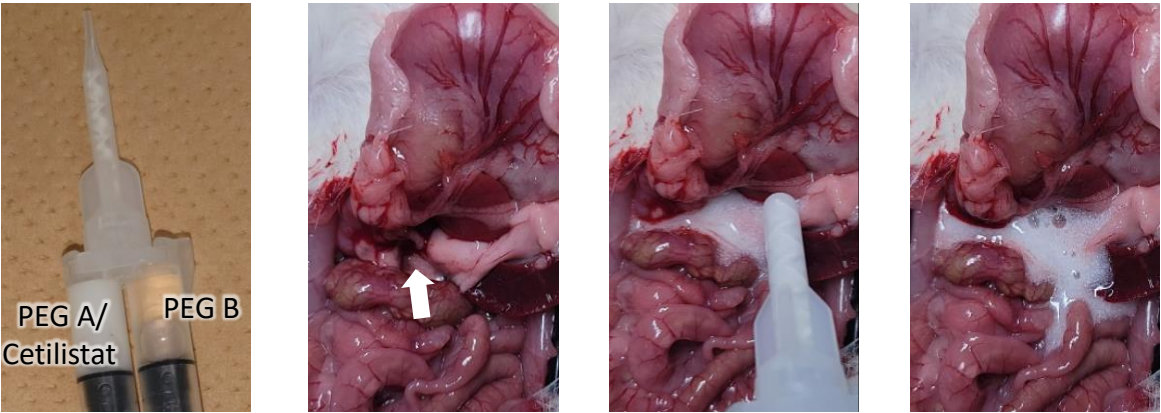
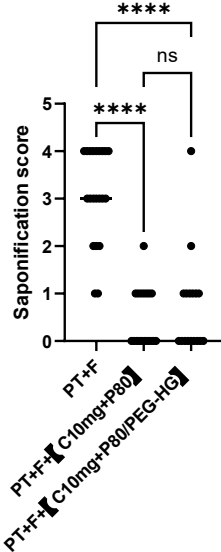


Fig 4

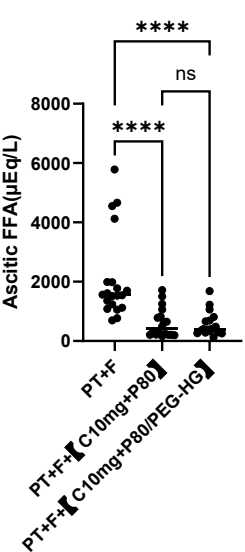
a The schema of PEG hydrogel injection



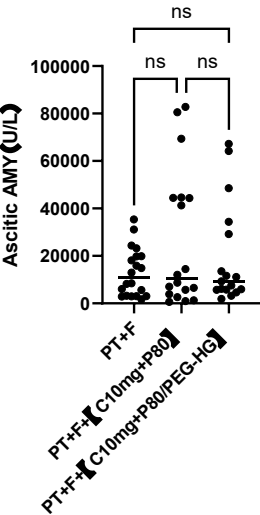
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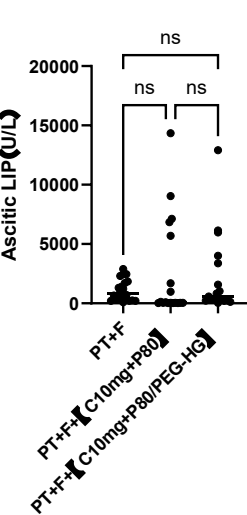
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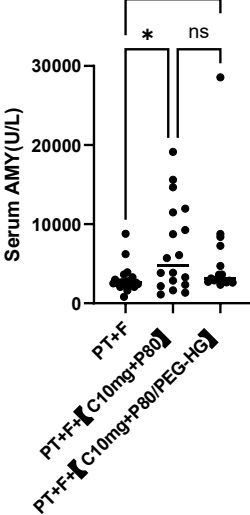
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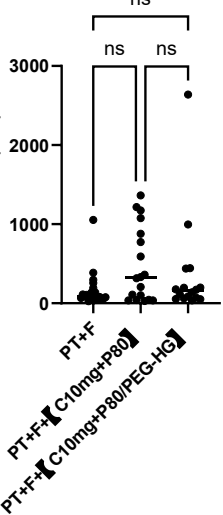
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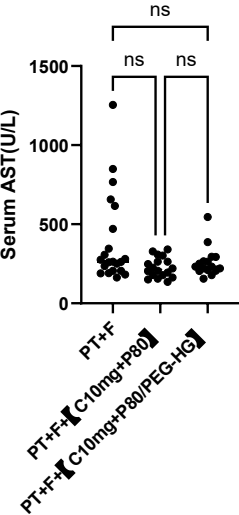
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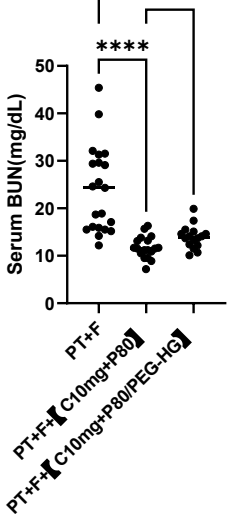
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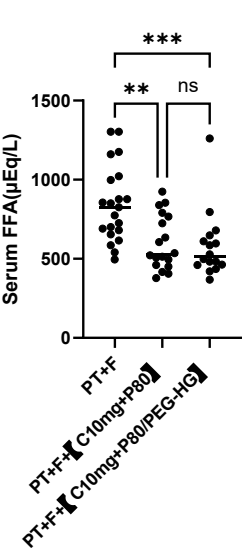
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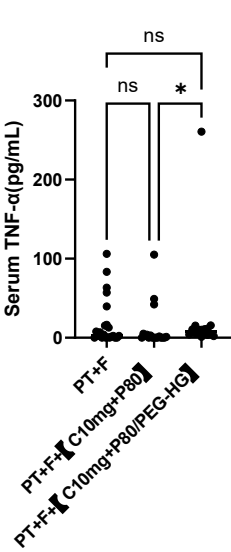
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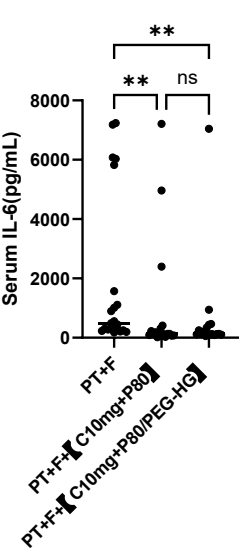
j Serum FFA



k Serum TNF-α



l Serum IL-6



m Survival

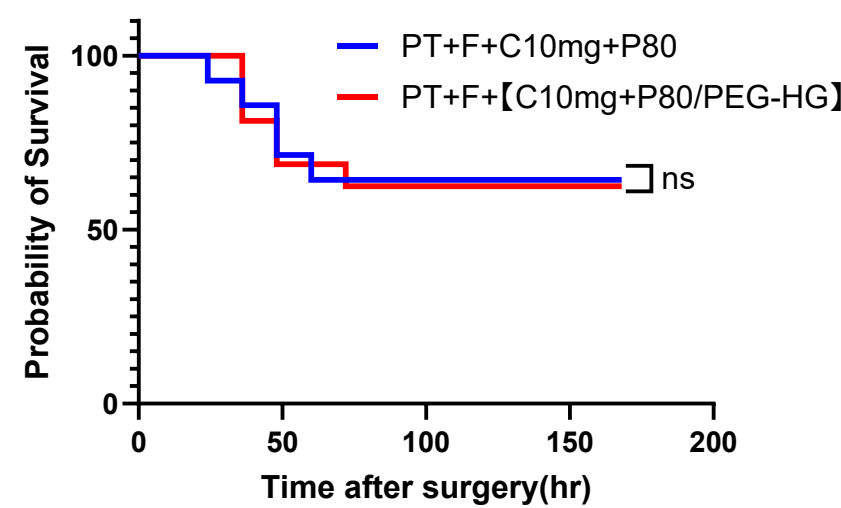


Fig 5

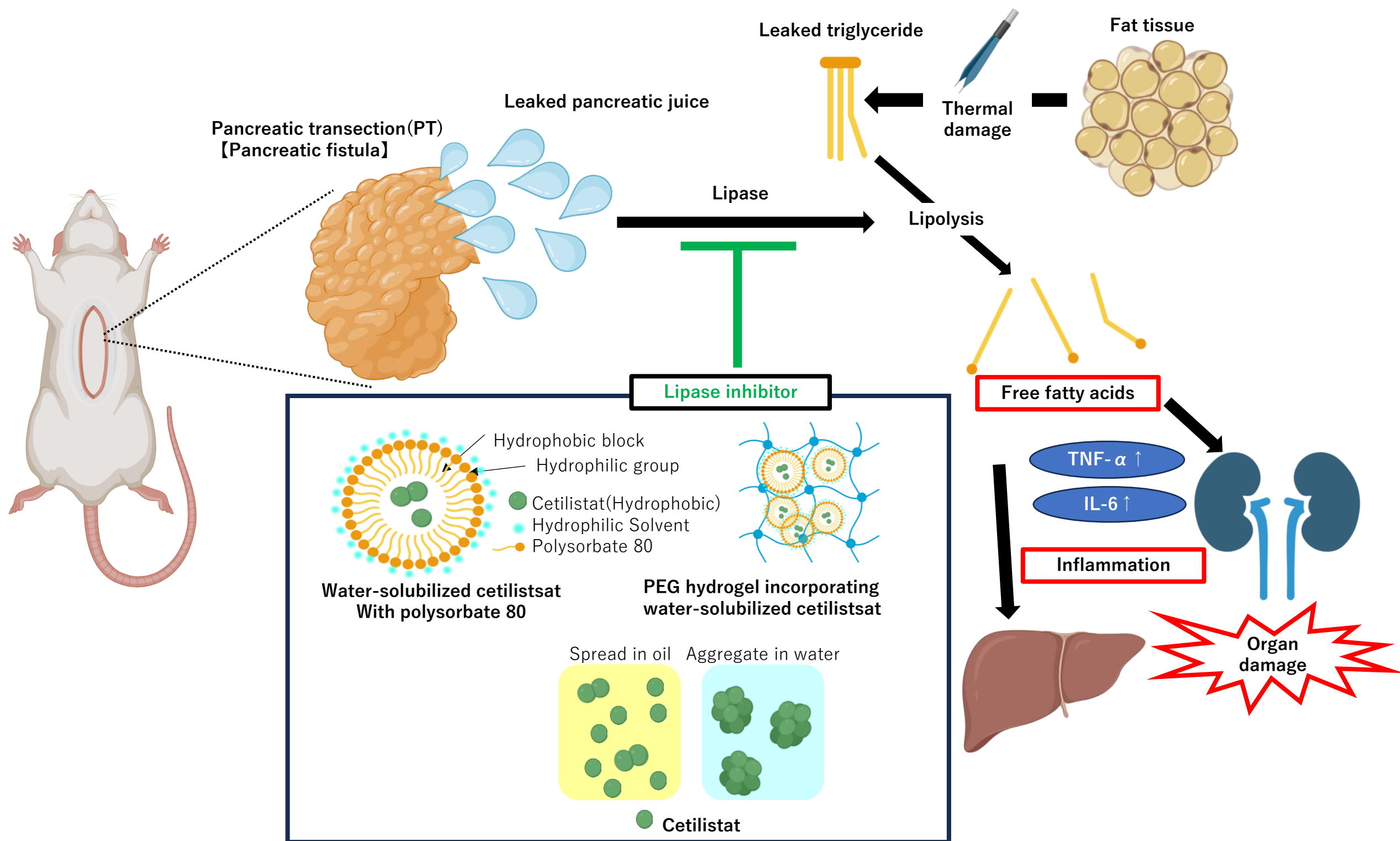
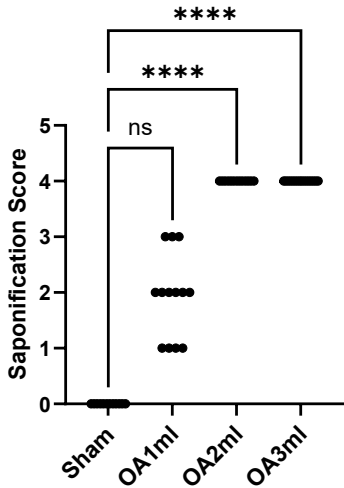
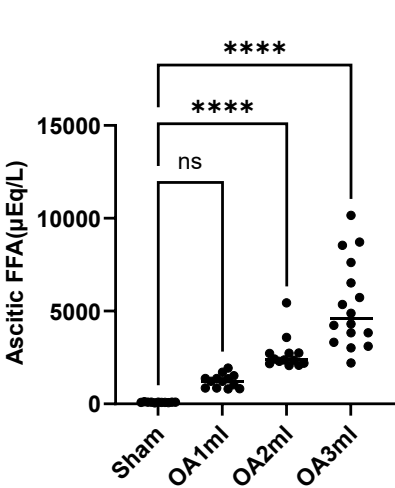


Fig S1

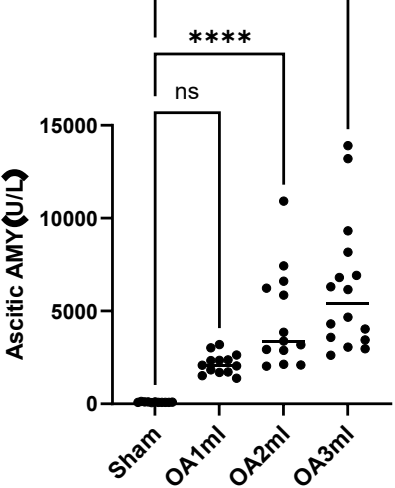
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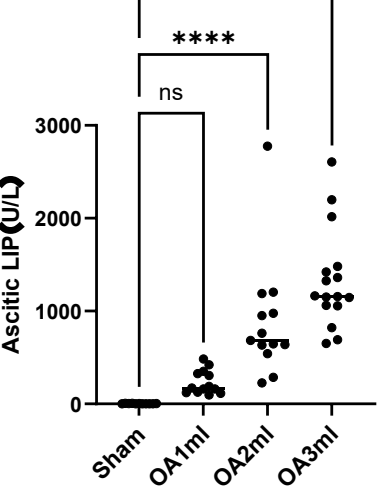
b Ascitic FFA



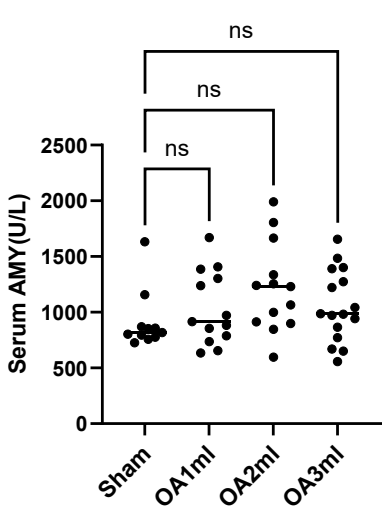
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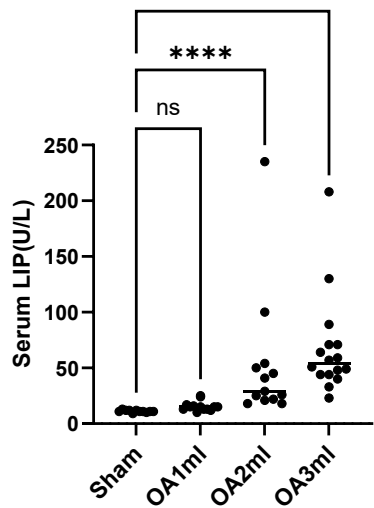
d Ascitic lipase



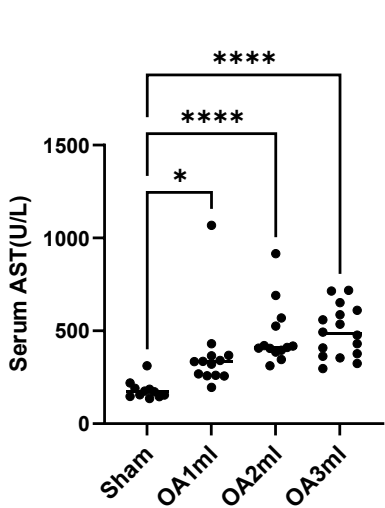
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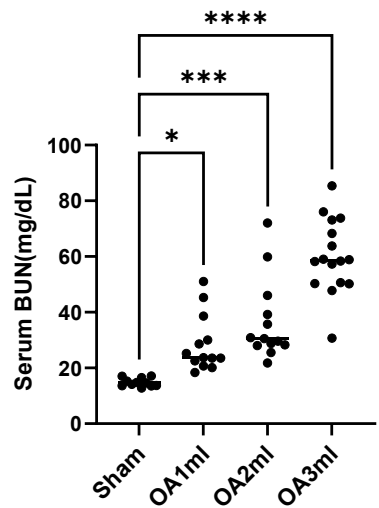
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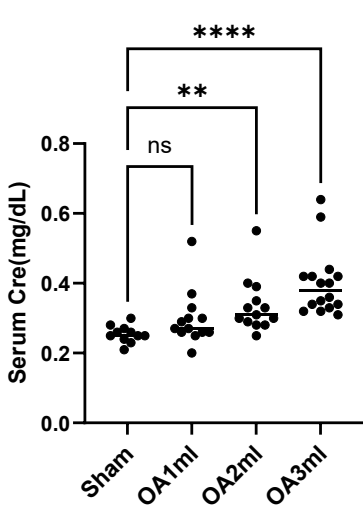
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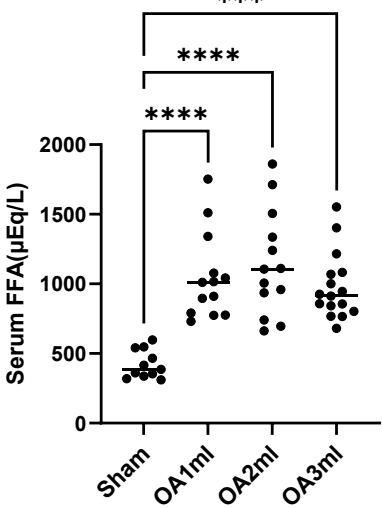
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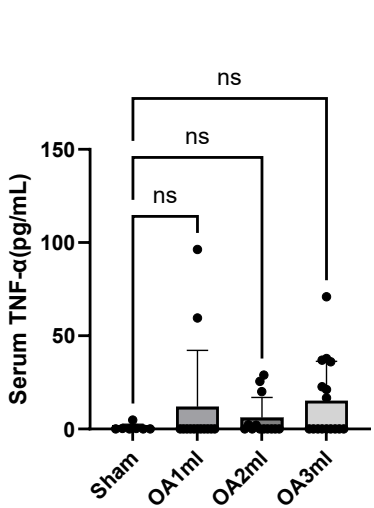
i Serum Cre



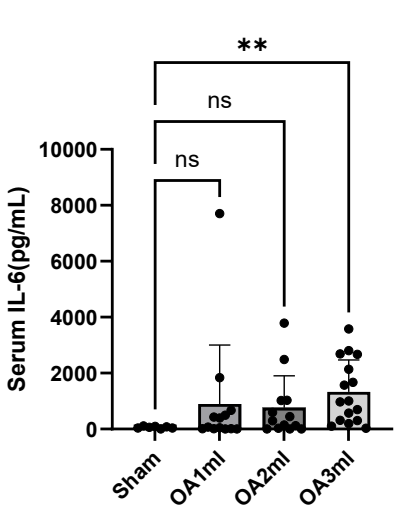
j Serum FFA



k Serum TNF-α



l Serum IL-6



m Survival

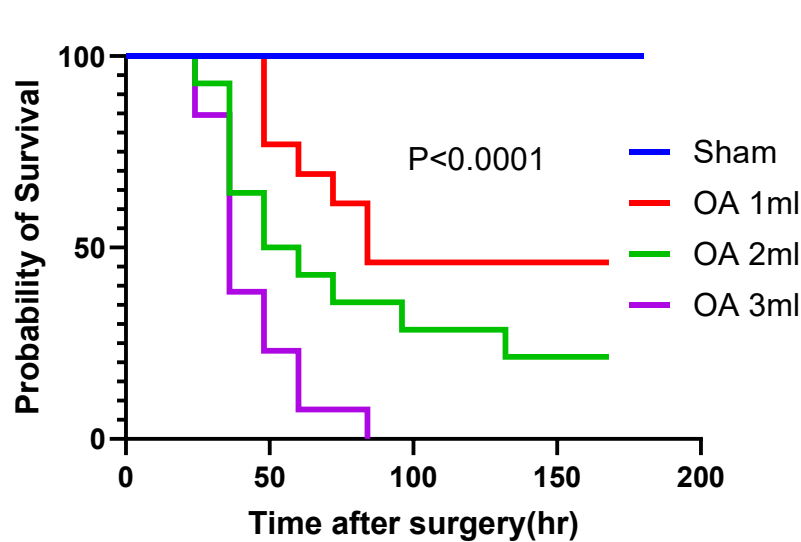
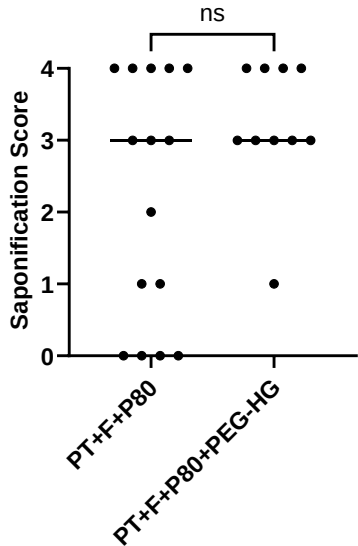
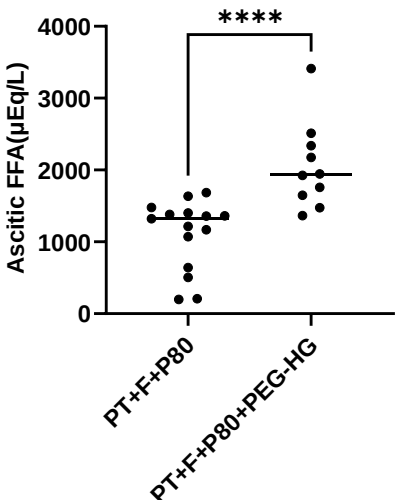


Fig S2

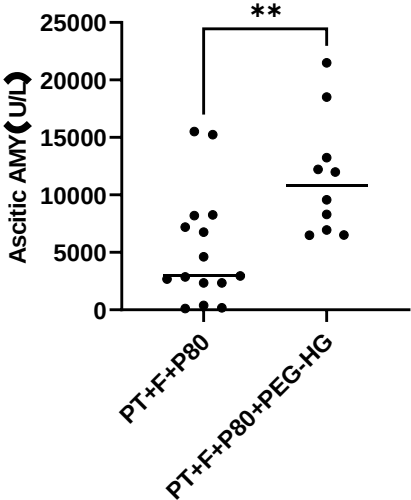
a Saponification score



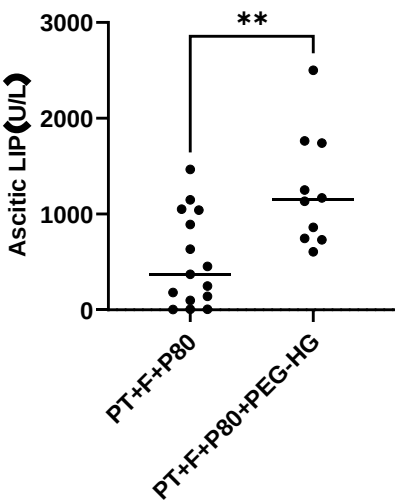
b Ascitic FFA



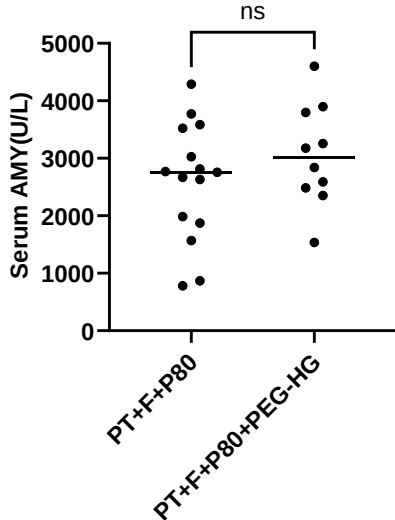
c Ascitic amylase



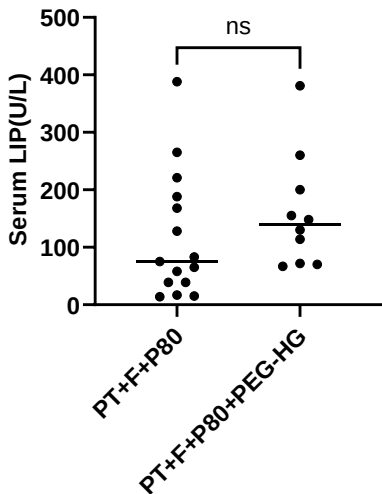
d Ascitic lipase



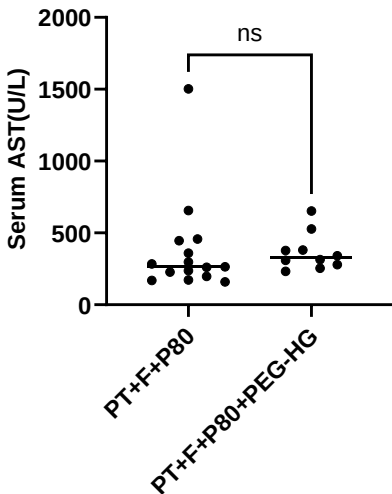
e Serum amylase



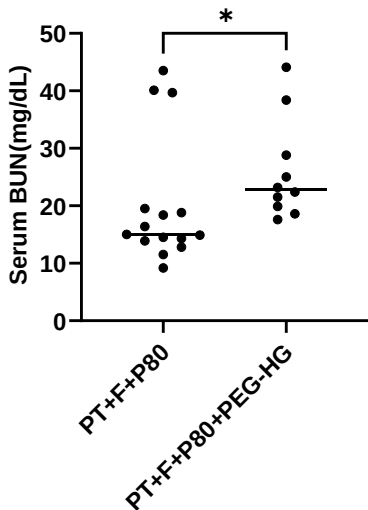
f Serum lipase



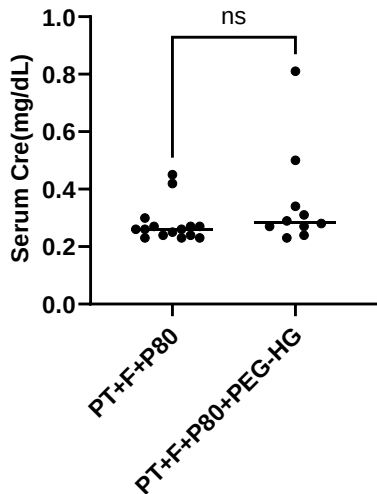
g Serum AST



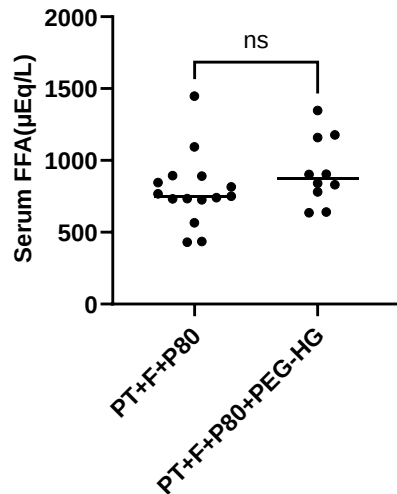
h Serum BUN



i Serum Cre



j Serum FFA



k Survival

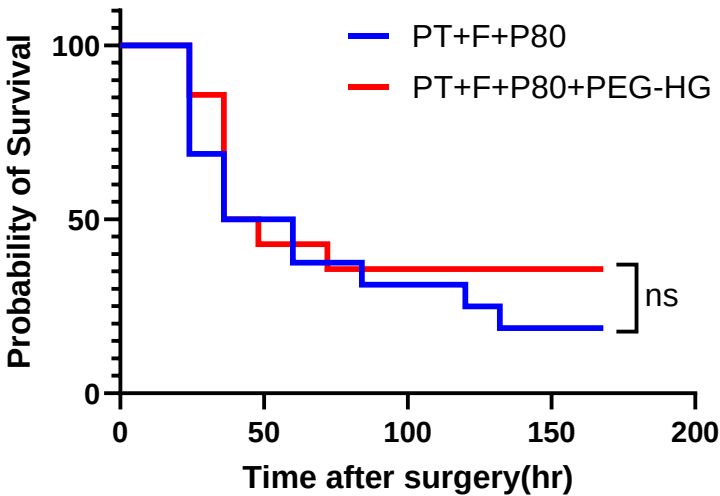


Fig S3

