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Dietary inulin ameliorates obesity-induced severe acute pancreatitis via gut-pancreas axis

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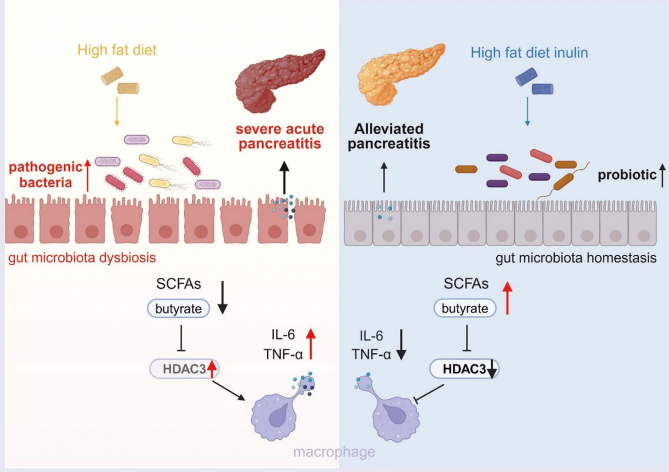
ABSTRACT

Obesity is a definitive factor of severity and mortality of acute pancreatitis (AP), and gut microbiota dysbiosis is involved in its pathogenesis. However, the effect of gut microbiota modulation by dietary components on high fat diet (HFD)-induced severe AP remains unclear. Here, we found that the inulin, a soluble dietary fiber, mitigated pancreatic injury and systematic inflammation in mice fed HFD, which was dependent on gut microbiota as this protective effect was attenuated in germ-free mice. Inulin treatment suppressed the overgrowth of pathogenic bacteria *Escherichia Shigella*, *Enterococcus*, *Klebsiella*, while increased the abundance of probiotics *Akkermansia*. Fecal microbiota transplantation from inulin-treated mice to recipient mice reduced pancreatic damage and remodeled intestinal homeostasis. Additionally, inulin increased fecal short chain fatty acids (SCFAs), strengthened gut barrier and restored Paneth cells. The beneficial effect of inulin on improving pancreatic damage and leaky gut was diminished after the suppression of SCFAs. Notably, SCFAs administration, especially butyrate, to HFD mice blocked pancreatic and intestinal injury with the inhibition of histone deacetylase 3 (HDAC3), and pharmacological HDAC3 inhibition mimicked the ameliorative effect of SCFAs. Mechanically, butyrate modulated macrophage M1/M2 polarization balance by suppressing HDAC3 and subsequent acetylation of histone H3K27. Collectively, our data offer new insights into the gut microbiota-pancreas axis that may be leveraged to augment the potential supplementation of prebiotic inulin in the management of obesity associated severe AP.



Graphical summary. A high fiber diet, which supplements with inulin, mitigates obesity-induced severe acute pancreatitis and gut barrier impairment via mediating gut microbiota-SCFA-pancreas axis. The inhibition of HDAC3 by SCFA modulates the polarization of macrophages and protects against pancreatic and intestinal injury.

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1. Introduction

Acute pancreatitis (AP) is a common gastrointestinal disorder that caused by inappropriate release and activation of trypsin resulting in autodigestion of the pancreatic parenchyma. It is a complex disease that varies in severity and course, with one-fifth of patients develop severe AP (SAP) and the mortality rate is approximately 20%.[1] Emerging evidence suggest that worldwide obesity epidemic is an important risk factor for the development of AP.[2] Obesity is associated with an amplified systemic inflammatory response that increases the incidence of inpatient hospitalization, large areas of pancreatic necrosis, multisystem organ failure and finally leads to higher mortality rate.[3] Studies have elucidated mechanisms of unsaturated fatty acids-mediated lipotoxicity, endoplasmic reticulum stress, and mitochondrial dysfunction, etc.[4,5] Therefore, understanding the key mechanisms involved in aggravation of AP in obesity and identification of potential prevention and treatment targets are necessary.

Gut dysfunction is a common and critical complication in patients with SAP, playing an active role in the development of systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS).[6] The severity of gut injury was associated with poor outcome and can be used as a predictor for mortality in AP.[7] Multiple studies have reported the dysbiosis of gut microbiota immediately after the onset of AP, with lower diversity and the enrichment of pathogenic *Escherichia shigella*, *Enterococcus* and *Klebsiella*.[8,9] The culture positive of these enteric bacteria in patients with infectious pancreatic necrosis supports the idea of bacterial translocation from impaired mucosal barrier.[10] Moreover, the causal relationship between gut microbiota dysbiosis and AP deterioration has been demonstrated by fecal microbiota transplantation in germ-free or antibiotics-treated mice.[11] Current studies have shown that several probiotics, such as *Bifidobacterium, Parabacteroides, Lactobacillus*, are capable to protect against AP through microbial modulation and inflammation suppression, which indicates gut microbiota as a promising therapeutic target.[12–14]

The probiotic prophylaxis in patients with AP, especially SAP, remains controversial as the strains and dosage are both uncertain.[15] Prebiotics are non-digestible compounds that through metabolization by microorganisms in the gut, modulate the composition of the gut microbiota, thereby conferring a beneficial physiological effect on host. Studies have shown that prebiotics, including lactulose and chitosan oligosaccharides, improved intestinal homeostasis and inhibited inflammation response in AP.[16,17] Inulin, a polysaccharide dietary fiber found in chicory root and Jerusalem artichoke, has been proposed to suppress obesity associated metabolic disorders through promoting the growth of *Bifidobacterium*, *Lactobacillus* and the production of the metabolites short chain fatty acids (SCFAs).[18] Gut microbiota members, including *Parabacteroides distasonis*, are capable of utilizing dietary inulin to generate beneficial metabolites that suppress nonalcoholic steatohepatitis.[19] In contrast, microbiota-derived bile acids by inulin are associated with intestinal inflammation and cholestatic liver cancer.[20,21] Multiple studies reveal a benefit of early enteral nutrition in reducing morbidity and mortality in obese patients with AP.[22] As one of the main components of the enteral nutrition formulas, the impact of inulin on obesity associated SAP and its interaction with gut microbiota remain unexplored.

In this work, we discovered that supplementation with inulin alleviated SAP induced by the consumption of high fat diet (HFD), and this effect was dependent on the gut microbiota, as confirmed by germ-free mice and fecal microbiota transplantation (FMT). Consumption of an inulin-enriched diet reshaped the structure of gut microbiota and promoted the biosynthesis of SCFAs. Inulin also contributed to the restoration of gut barrier function, thereby inhibiting local and systematic inflammation. This beneficial effect of inulin was weakened with the inhibition of SCFAs. Furthermore, we found SCFAs administration, especially butyrate, improved pancreatic and intestinal injury by inhibiting histone deacetylase 3 (HDAC3). Together, this study revealed the protective role of inulin in obesity-induced SAP through modulating gut-pancreas axis.

2. Materials and methods

2.1. Mice

All animal experiments were approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Nanchang University and complied with the national and international guidelines for the Care and Use of Laboratory Animals (CDYFY-IACUC -202302QR076). Both specific-pathogen-free (SPF) and germ-free (GF) C57BL/6 mice (male, 6–8 weeks old) were obtained from GemPharmatech Company (Jiangsu, Nanjing, China) at 6 weeks of age. GF mice were bred within sterile vinyl isolators and maintained at the gnotobiotic mouse facility. Mice were randomly allocated to one of the three following diets: control diet (CD, LAD 3001 G, Trophic Animal Feed High-tech, Nantong, China), high-fat diet (HFD, TP 23,300, Trophic Animal Feed High-tech, Nantong, China), a HFD with 37 g inulin per 1000 kcal as a source of fiber (FFD, TP23300-X2, Trophic Animal Feed High-tech, Nantong, China) for 4 weeks.[23] The CD diet consisted of 16.7% kcal from fat, 63.9% kcal from carbohydrate, and 19.4% kcal from protein. The HFD and FFD are isocaloric, consisting of 60% kcal from fat, 20.6% kcal from carbohydrates, and 19.4% kcal from protein. To suppress SCFAs production by inhibiting bacterial fermentation, mice were treated with either a vehicle control (Propylene Glycol) or 20 ppm β-acid (extracted from the hops plant, S.S. Steiner Inc., New York, USA).[24] All mice had free access to food and water under a strict 12 h light/dark cycle at a controlled temperature
(23 ± 2℃).

2.2. Experimental AP model

The induction of AP was performed by administering ten hourly intraperitoneal injections of caerulein (Sigma-Aldrich, St. Louis, Missouri, USA,100 μg/kg). The control group was intraperitoneally injected with saline. Mice were sacrificed 24 h after the first injection. Fecal samples, peripheral blood, pancreatic, and intestinal tissue were collected.

2.3. Microbiota depletion and fecal microbiota transplantation

In brief, mice were treated with an antibiotic solution (Abx) containing ampicillin 1 g/L, neomycin sulfate 1 g/L, metronidazole 1 g/L, and vancomycin 0.5 g/L added into drinking water for 4 weeks as previously described.[8] Fresh fecal pellets (200 mg) were collected and resuspended in 2 ml sterile PBS. After filtering through a sterile 70 μm strainer, the fecal microbial suspension was administered by gavage to Abx and GF mice at a dose of 200 μl per mouse for 4 consecutive days.

2.4. Short chain fatty acids treatment

Five types of high-fat diets-a control high-fat diet (HFD) and four HFD with acetate, propionate, butyrate and their admixture (SCFAs)-were designed according to the high-fat diet formula (60% kcal from fat) from SYSE Bio-tec (Changzhou, Jiangsu, China). These five HFD contained the same amount of lard and soy soil as the main source of fat in each diet. Sodium acetate, sodium propionate, sodium butyrate (Sigma-Aldrich, St. Louis, MO) or their admixture (ratio at 3:1:1) were incorporated into the diet at a proportion of 5%.[25] These diets were given for 4 weeks prior to the induction of AP.

2.5. Statistical analysis

The data were expressed as the mean ± SEM. Statistical analysis was performed using GraphPad Prism 7.0 (GraphPad Software) and the SPSS 26.0 software. Comparison between two groups was determined by Student's t test or Mann-Whitney U test. For comparisons involving more than two groups, one-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) was used. Correlation between bacteria and metabolites was performed by Spearman. Histological evaluation was assessed by two pathologists in a blinded manner. The value of $p < 0.05$ was regarded as the cutoff for statistical significance.

Additional methods were provided in the supplementary materials.

3. Results

3.1. Inulin ameliorated high fat diet-associated severe acute pancreatitis in mice

To explore the effects of dietary fiber on diet-induced SAP, mice were fed with one of the three following diets: control diet (CD), high-fat diet (HFD), high fat/high fiber diet (a high fat diet with inulin as a source of fiber, FFD) for 4 weeks and then underwent 10 times intraperitoneal injection of caerulein for AP establishment (Figure 1a). Histological assessment identified the marked reduction of inflammation, edema and necrosis in the pancreatic tissues of FFD-treated mice compared with HFD (Figure 1b,c). Consistently, serum levels of pancreatic damage markers amylase and lipase in FFD mice were lower than those in HFD mice (Figure 1d). To further elucidate the impact of inulin on inflammatory response, we observed a decrease in mRNA transcripts of IL-1β, TNF-α, and IL-6 in the pancreas of FFD mice compared to HFD mice using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), while the expression of anti-inflammatory cytokine IL-10 was increased (Figure 1e). The pancreatic infiltration of neutrophils and macrophages was suppressed after FFD treatment (Figure 1f,i). The percentage of TUNEL positive cells in the pancreas of FFD mice was dramatically diminished compared to HFD mice (Figure 1h). We also found that dietary fiber suppressed the systematic inflammatory response with a consequent reduction in the serum concentrations of IL-1β, TNF-α, and IL-6 (Figure 1g). Thus, these findings clearly demonstrated that the consumption of dietary fiber provided protection against HFD-induced acinar cell injury and excessive inflammation.

3.2. Inulin reshaped the gut microbiome and enhanced the production of short chain fatty acids

Dysbiosis of gut microbiota caused by HFD has been reported to involve in the progression of SAP.[26] Inulin, widely acknowledged as a potent prebiotic, is known to stimulate the proliferation of beneficial intestinal bacteria. Hence, we performed 16S rRNA gene sequencing to evaluate how inulin influenced the gut microbiota. Notably, the increased alpha diversity induced by HFD was significantly restored after FFD (Figure 2a). Determination of beta diversity by principal coordinates analysis (PCoA) based on Bray-curtis and unweighted UniFrac distance revealed significant differences in microbiota composition between HFD and FFD (Figure 2b). The proportion of proteobacteria phylum, represented by the *Escherichia_Shigella* genus, was markedly reduced in FFD mice compared to HFD mice (Figure 2c,d). The abundance of well-characterized probiotics, including *Akkermansia*, *Muribaculaceae*, *Anaerostipes*, was enriched in FFD mice by linear discriminant analysis (LDA). In contrast, pathogenic bacteria including *Escherichia_Shigella*, *Enterococcus*, *Klebsiella*, were depleted in FFD mice compared to HFD mice (Figure 2e,f). LEfSe analysis at the species level identified an enhancement of the probiotic species *Bifidobacterium pseudolongum* and *Akkermansia muciniphila* in FFD-treated mice compared to the HFD group, which was further supported by the qPCR results (Supplement fig. S1A-1B).

Given the possibility that gut-derived metabolites were involved in the regulation of AP progression, we performed targeted metabolomic analysis to identify the influence of dietary fiber on the SCFAs profiles of gut microbiota. As expected, principal component analysis (PCA) displayed a prominent shift in the SCFAs profiles of gut microbiota in FFD mice (Figure 2g). Dietary fiber treatment improved the generation of total SCFAs, especially acetate, propionate and butyrate, which were impaired by HFD (Figure 2h–o). To assess these metabolites with potential metabolic activities of the gut microbes, we performed integrative analyses of altered bacteria and metabolites. We observed that the probiotic *Parabacteroides* had the most positive correlation with total SCFAs, especially acetate. Additionally, *Akkermansia* and *Muribaculaceae* were positively correlated with butyrate (Figure 2p). Collectively, the gut microbiota and its production of SCFAs may work together to mitigate the severity of AP.

3.3. Gut microbiota mediated the protective effect of inulin on pancreatic injury

To determine whether the beneficial effect of inulin on diet-induced SAP was dependent on gut

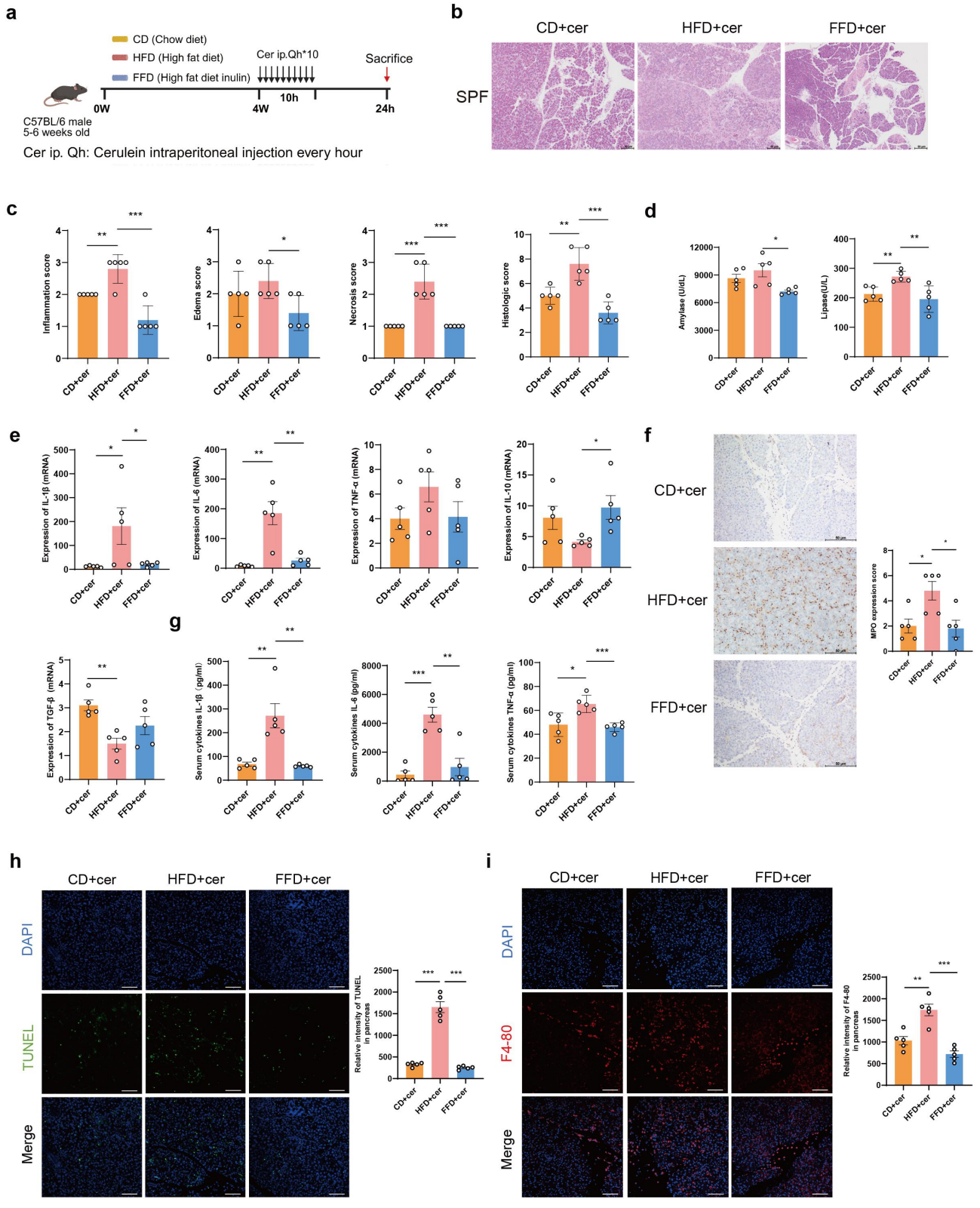

Figure 1. Inulin alleviated the pancreatic damage and systemic inflammation caused by a high fat diet. (a) Experimental design for the dietary treatment in AP mouse model. (b) Representative H&E staining of pancreatic tissue from each group showing pancreatic injury. Scale bar: 50 μm. (c) Histopathological scoring of pancreatic damage. (d) Serum amylase and lipase activity. (e) Real-time PCR was performed to detect the mRNA expression of cytokines in pancreatic tissue. (f) Representative immunohistochemical staining of myeloperoxidase (MPO). Scale bar: 50 μm. (g) Serum levels of cytokines were lower in CD and FFD mice than in HFD mice. (h) Pancreatic staining of TUNEL (green staining) and DAPI (blue) to visualize nuclei (×200). (i) Pancreatic staining of F4/80 (red staining) and DAPI (blue) to visualize nuclei (×200). CD, chow diet. HFD, high fat diet. FFD, fat fiber diet. Cer, cerulein. *$p < .05$, **$p < .01$, ***$p < .001$.

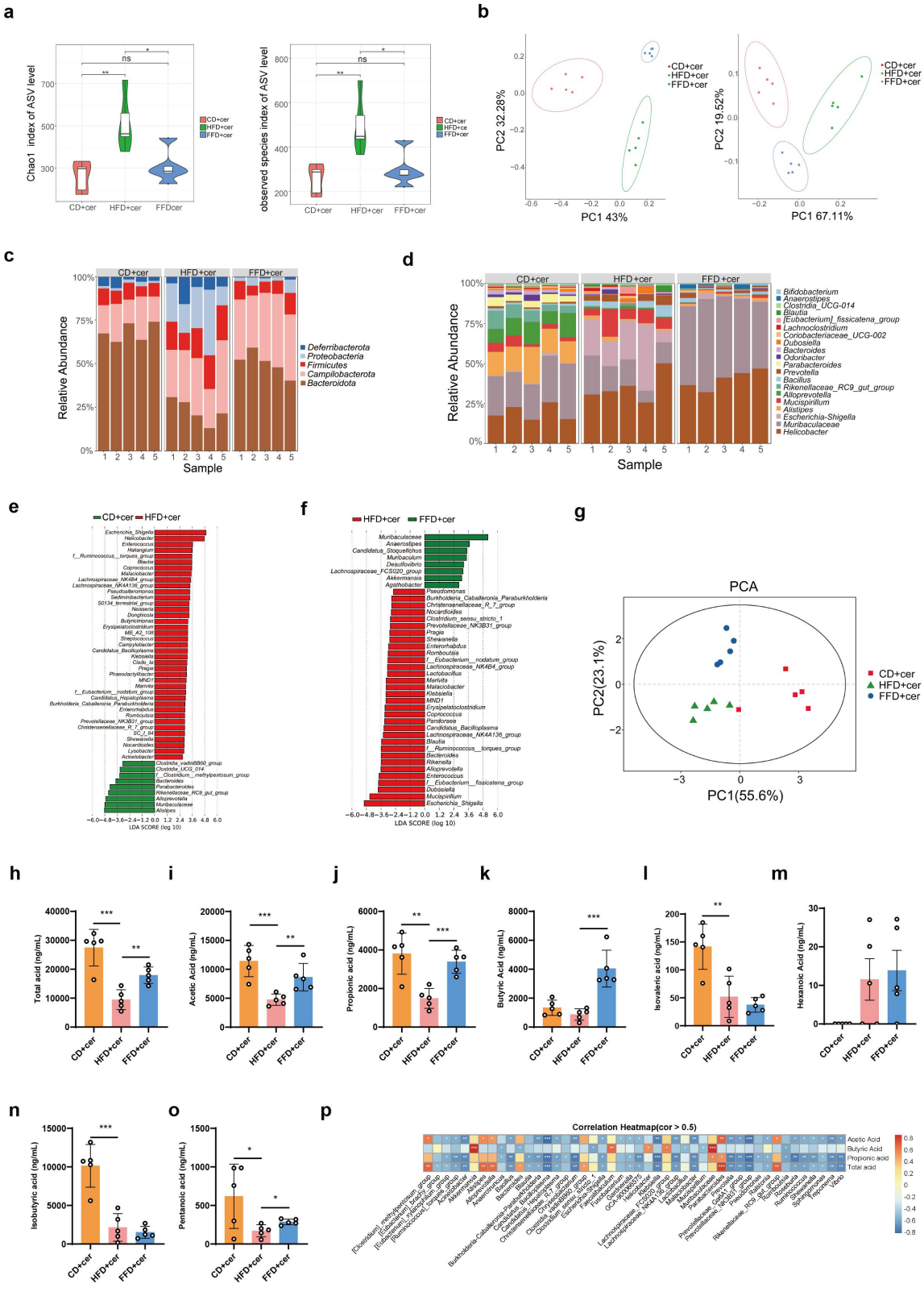

Figure 2. Inulin modulated gut microbiota and promoted short chain fatty acids production in obese AP mice. (a) Alpha diversities of microbiota. (b) Principal coordinates analysis based on Bray-Curtis (left) and weighted UniFrac (right) distances. Relative abundance of bacterial phyla (c) and genus (d). Differential genera between HFD and CD (e), HFD and FFD (f) were identified by LEfSe analysis. (g) Principal coordinate analysis (PCA) showing the differences in the gut short chain fatty acids (SCFAs) profile of CD, HFD and FFD group. (h-o) the concentrations of SCFAs were examined by GC-MS. (p) Correlation between the top 50 genera and SCFAs by Pearson analysis. * $p < .05$, ** $p < .01$, *** $p < .001$.

microbiota, we administered germ-free mice with different diets followed by the construction of AP (Figure 3a). Hematoxylin and eosin (H&E) staining showed comparable histopathological scores for AP in HFD and FFD mice (Figure 3b,c). The serum levels of amylase and lipase from HFD and FFD groups were statistically indistinguishable in germ-free mice (Figure 3d). In addition, both HFD and FFD mice showed similar expression of inflammatory cytokines and MPO-positive cells in the pancreas (Figure 3e,f). Gut microbiota depletion did not result in any changes of acinar cell death between HFD and FFD mice (Figure 3g). We failed to detect any differences in cytokines, including IL-1β, IL-6, TNF-α and LPS in the serum of germ-free mice (Figure 3h).

To further verify the causality between FFD-alleviated AP and the altered gut microbiota, the fecal microbiota from donor mice (CD, HFD, and FFD) were transplanted into germ-free mice as well as antibiotic-treatment mice (Figure 3i, Supplement fig. S2A). Histopathological examination showed that gut microbiota-depleted mice that received feces from FFD donors developed less severe pancreatic damage compared with those that received feces from HFD donors in response to AP induction (Figure 3j,k, Supplement fig. S2B-2C). In agreement, immunohistochemistry revealed that transplantation of fecal microbiota from FFD individuals could inhibit infiltration of MPO-positive cells in the pancreas of recipient mice subjected to caerulein (Figure 3n, Supplement fig. S2F). The pancreatic mRNA transcripts, as well as the serum concentrations of cytokines were decreased in FFD feces recipients (Figure 3m,p, Supplement fig. S2E). In line with these results, fecal transplantation from FFD individuals reduced the death of acinar cells in comparison with mice that received feces from the HFD ones (Figure 3o, Supplement fig. S2G). These findings indicate that the gut microbiota shaped by dietary fiber contributed to the mitigation of AP.

3.4. Inulin restored hfd-induced gut dysfunction in AP

Given that SAP is associated with leaky gut via the gut-pancreas-axis,[27] we then examined the effect of dietary fiber inulin on gut barrier functions in AP mice. The mRNA transcripts of pro-inflammatory cytokines IL-1β, IL-6, and TNF-α tended lower in FFD mice compared to HFD mice, whereas the levels of anti-inflammatory cytokines IL-10 and TGF-β were elevated (Figure 4a). Transplantation of FFD fecal microbiota also inhibited intestinal inflammation and strengthened gut barrier in microbiota-depleted mice as compared to those received HFD feces (Supplement fig. S3A-3B). The expressions of tight junction proteins, including ZO-1, Occludin, Claudin-1 and E-cadherin, were enhanced in FFD mice compared with HFD mice as determined by Western blot and qRT-PCR (Figure 4b,d). In keeping with this, the levels of lipopolysaccharide (LPS) and D-lac, which are markers of gut barrier integrity, were consistently reduced in FFD mice (Figure 4f). Paneth cells have been shown to play a critical role in maintaining intestinal homeostasis by synthesizing and releasing antimicrobial peptides such as lysozyme and defensins. Interestingly, we found that the number of Paneth cells (labeled by lysozyme) was significantly increased after FFD treatment (Figure 4e). Accordingly, the mRNA expression levels of Paneth cells-related genes, Lysozyme1 (Lyz1) and defensins, exhibited a similar upward trend to that of Paneth cells (Figure 4c). Additionally, transplantation of stools from FFD mice significantly promoted the proliferation of Paneth cells in the recipient antibiotics-treated mice, as evidenced by increased Lyz1-positive cells and defensins expression (Supplement fig. S3C-3D). Taken together, these data indicate that dietary fiber-gut microbiota axis might contribute to the restoration of gut barrier function.

To further clarify the role of Paneth cells in inulin-mediated pancreatic protection, mice were treated with either the inhibitor or the agonist of Paneth cells prior to AP induction, respectively. As expected, histological analysis revealed that the depletion of Paneth cells with dithizone worsened pathological changes, particularly pancreatic necrosis, in FFD-treated mice (Supplement fig. S4). This implies that the beneficial impact of inulin is associated with Paneth cells. However, mice treated with lysozyme, known as a Paneth cells agonist, still developed severe pancreatic injury following HFD, suggesting that activating Paneth cells alone does not fully prevent the progression of severe AP (Supplement fig. S4).

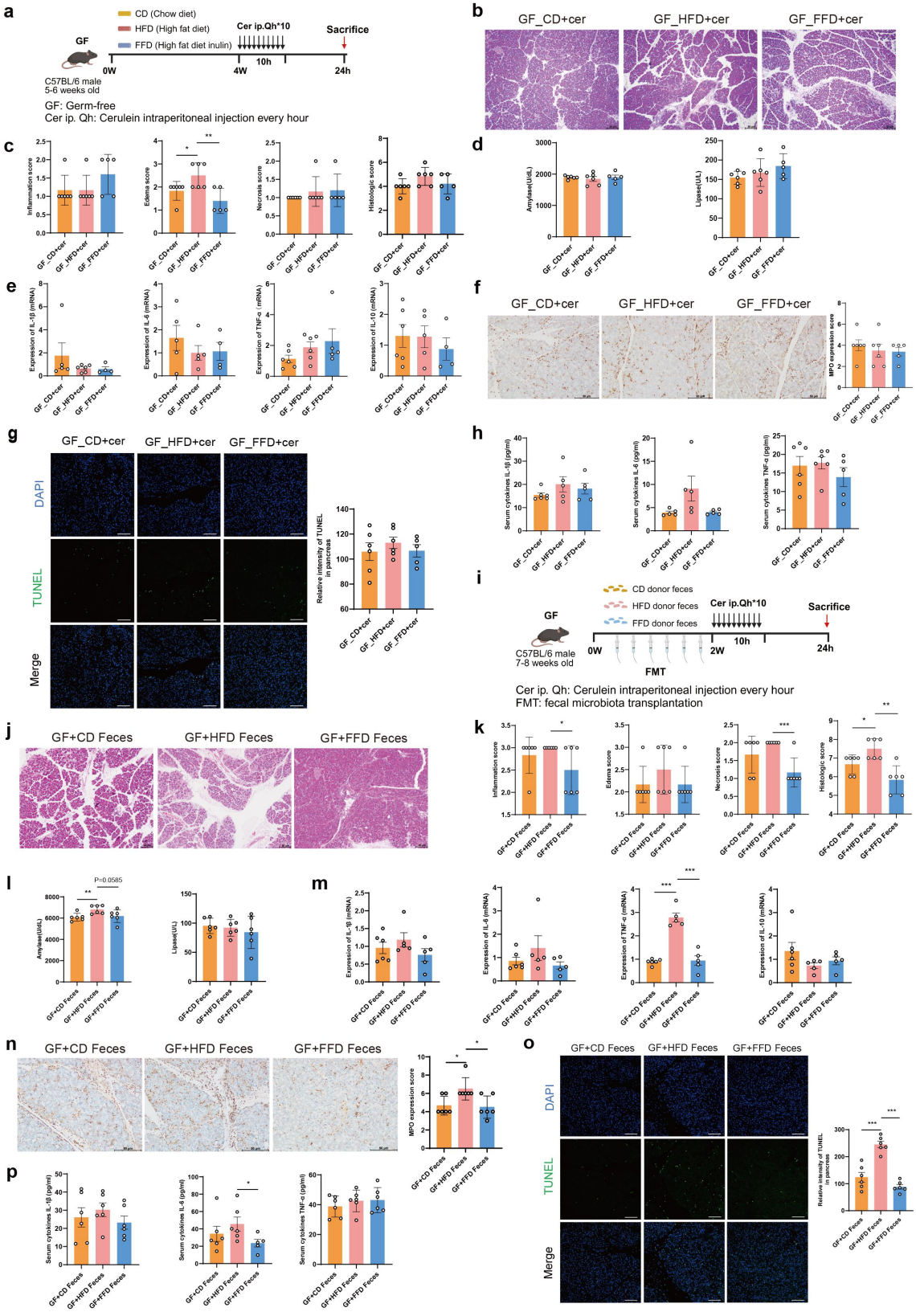

Figure 3. The protective effect of inulin on obesity-associated severe AP was mediated by gut microbiota. (a) Study design of germfree mice with dietary treatment followed by AP induction. (b, j) Representative H&E staining of pancreatic tissue. Scale bar: 50 μm. (c, k) histopathological scoring of pancreatic damage. (d, l) serum levels of amylase and lipase activity. (e, m) the mRNA expression of cytokines in pancreatic tissue was detected by real-time PCR. (f, n) immunohistochemical staining of MPO which reflects pancreatic inflammation. Scale bar: 50 μm. (g, o) pancreatic staining of TUNEL (green staining) and DAPI (blue) to visualize nuclei (×200). (h, p) serum levels of cytokines. (i) Schematic of fecal microbiota transplant to germfree mice. *$p < .05$, **$p < .01$, ***$p < .001$.

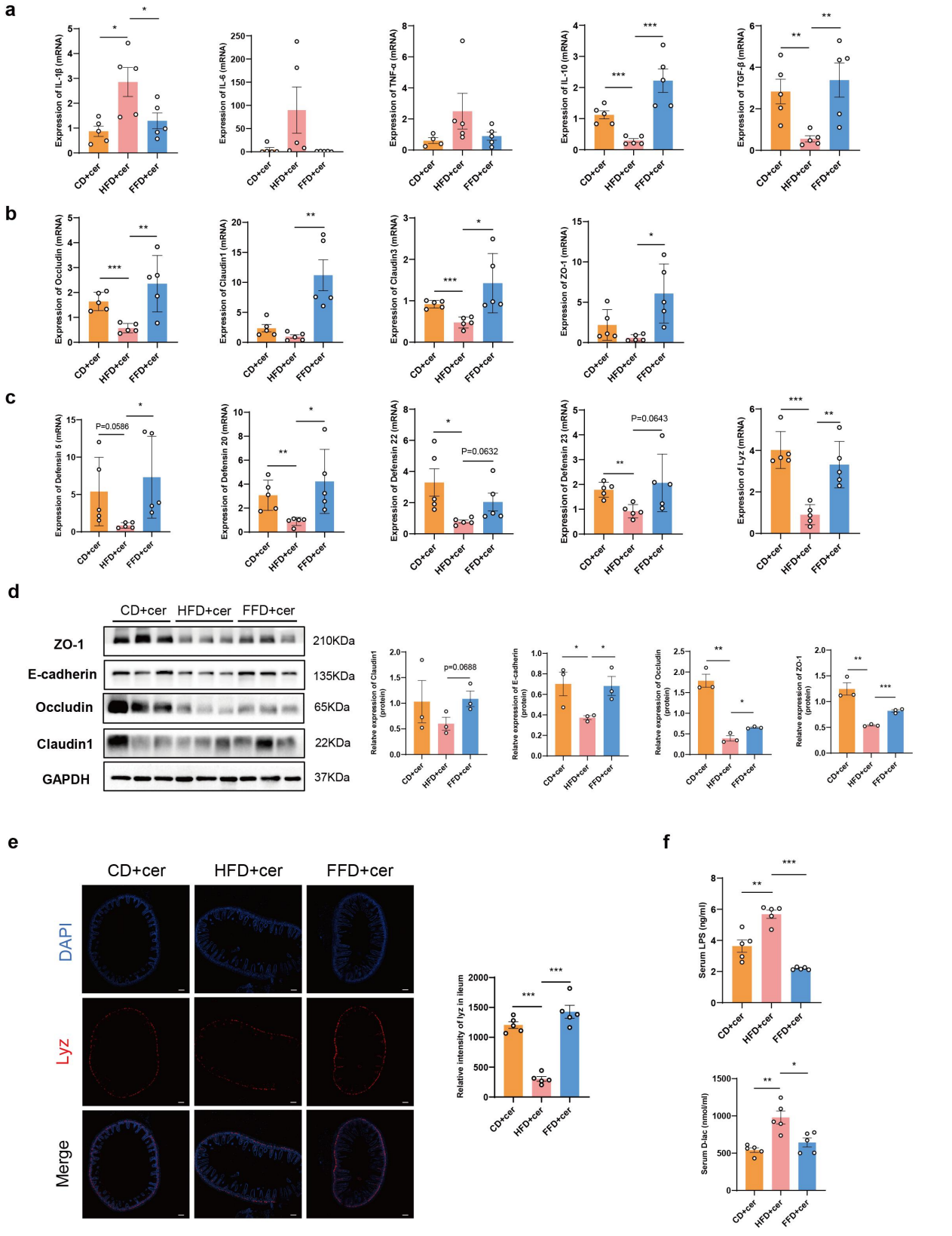

Figure 4. Inulin restored gut barrier dysfunction in obese AP mice. Real-time PCR was performed to detect the mRNA expression of cytokines (a) adhesion molecules (b) and defensins (c) in ileum tissue. (d) Western blot analysis showed the expression of intestinal tight junction proteins. (e) Ileum expression of Lyz by immunofluorescene. (f) Serum levels of gut permeability indexes LPS and D-lac. *$p < .05$, **$p < .01$, ***$p < .001$.

3.5. SCFAs mitigate severe pancreatitis and gut dysfunction caused by HFD

Considering that metabolites mediate the crosstalk between gut and pancreas, we hypothesized that the production of SCFAs in the gut of FFD mice was causally related to their protection against pancreatic damage. To determine this hypothesis, acetate, propionate, butyrate and their mixture were supplemented into the high fat diet for 4 weeks, after which caerulein was injected (Figure 5a, Supplement fig. S5A). Histopathological examination showed that treatment with a mixture of SCFAs and butyrate, but not acetate and propionate, significantly improved pancreatic injury, especially necrosis and inflammation (Figure 5b,c, Supplement fig. S5B-5C). In agreement, we observed that pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, were down-regulated by SCFAs and butyrate, while anti-inflammatory cytokine IL-10 and TGF-β was up-regulated (Figure 5e). The proportion of MPO- and F4/80-positive cells was lower in mice supplement with SCFAs and butyrate by IHC and immunofluorescence (Figure 5f,j). Significantly reduced apoptosis of acinar cells was also observed in mice administered SCFA and butyrate (Figure 5i). Moreover, SCFAs and butyrate suppressed the concentrations of serum lipase, IL-1β, and IL-6 (Figure 5d,h).

Butyrate has been reported to modulate the immune response through inhibition of histone deacetylase 3 (HDAC3).[28–30] We observed that HDAC3 was downregulated by SCFAs and butyrate, accompanied with the upregulation of acetylated (ac)-H3 (Figure 5g). Consistently, SCFAs showed inhibition on HDAC3 transcription and the enhanced acetylation of H3 in THP-1 cells (Supplement fig. S6B-6C). However, the mRNA transcripts of other HDAC subtypes were not inhibited by SCFAs treatment (Supplement fig. S7A). In vitro study further demonstrated that SCFAs, especially butyrate, adjusted the macrophage polarization from pro-inflammatory M1 to anti-inflammatory M2, as revealed by lower mRNA transcripts of CD86, IL-6, TNF-α, IL-1β, along with higher levels of CD163 (Supplement fig. S6A). Further analysis revealed that among the five HDAC subtypes, the inhibition of class I HDACs (HDAC1/2/3) by entinostat exhibited the most robust effects on the promotion of TGF-β and IL-10, known as anti-inflammatory cytokines, whereas the inhibition of other HDAC classes had either minor effect or no effect (Supplement fig. S7C). Additionally, the expression of pro-inflammatory TNF-α and CCL2 was downregulated by entinostat and rocilinostat, which selectively inhibit class IIb HDACs, but also class I HDACs (Supplement fig.S 7B). Moreover, the beneficial effect of butyrate on macrophage polarization and the acetylation of H3K27 was largely impeded by HDAC3 agonist (Supplement fig. S6D-6E). These observations highlight the importance of SCFAs, especially butyrate, which rendered resistance to HFD-induced pancreatic damage in association with HDAC3 inhibition.

It has been established that SCFAs maintain gut homeostasis via regulating host immunity and inflammation.[31] As shown in Figure 6a, the intestinal expressions of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, were reduced by SCFAs and butyrate, while anti-inflammatory cytokine TGF-β was increased. Of note, SCFAs and butyrate restored the expressions of intestinal tight junctions, including Claudin 1, ZO-1, and Occludin, that were impaired by HFD (Figure 6b,d). The permeability of gut barrier was decreased in mice treated with SCFAs and butyrate, as evidenced by lower levels of LPS and D-lac in the serum (Figure 6f). Administration of SCFAs and butyrate enhanced the density of Lyz+ Paneth cells, with elevated mRNA expression of antimicrobial peptide genes (Lyz, Defensins) (Figure 6c,e). In short, these findings suggest that SCFAs protected against AP-induced gut injury.

3.6. Blocking SCFAs production abrogates the protective effects of inulin on severe pancreatitis and gut dysfunction

To explore the role of inulin-induced SCFAs in strengthening gut barrier and mitigating pancreatitis, we employed β-acids, a compound derived from hops, to reduce bacterial-mediated SCFAs production without significantly affecting bacterial loads per se.[24] The administration of β-acids to mice effectively blocked the inulin-induced rise in SCFAs in vivo (Figure 8d). Histopathological and biochemical assessment showed that β-acids exacerbated indices of pancreatitis regardless of inulin administration, evidenced by increased pancreatic necrosis, acinar cell death, and higher levels of serum lipase (Figure 7b,d,h).

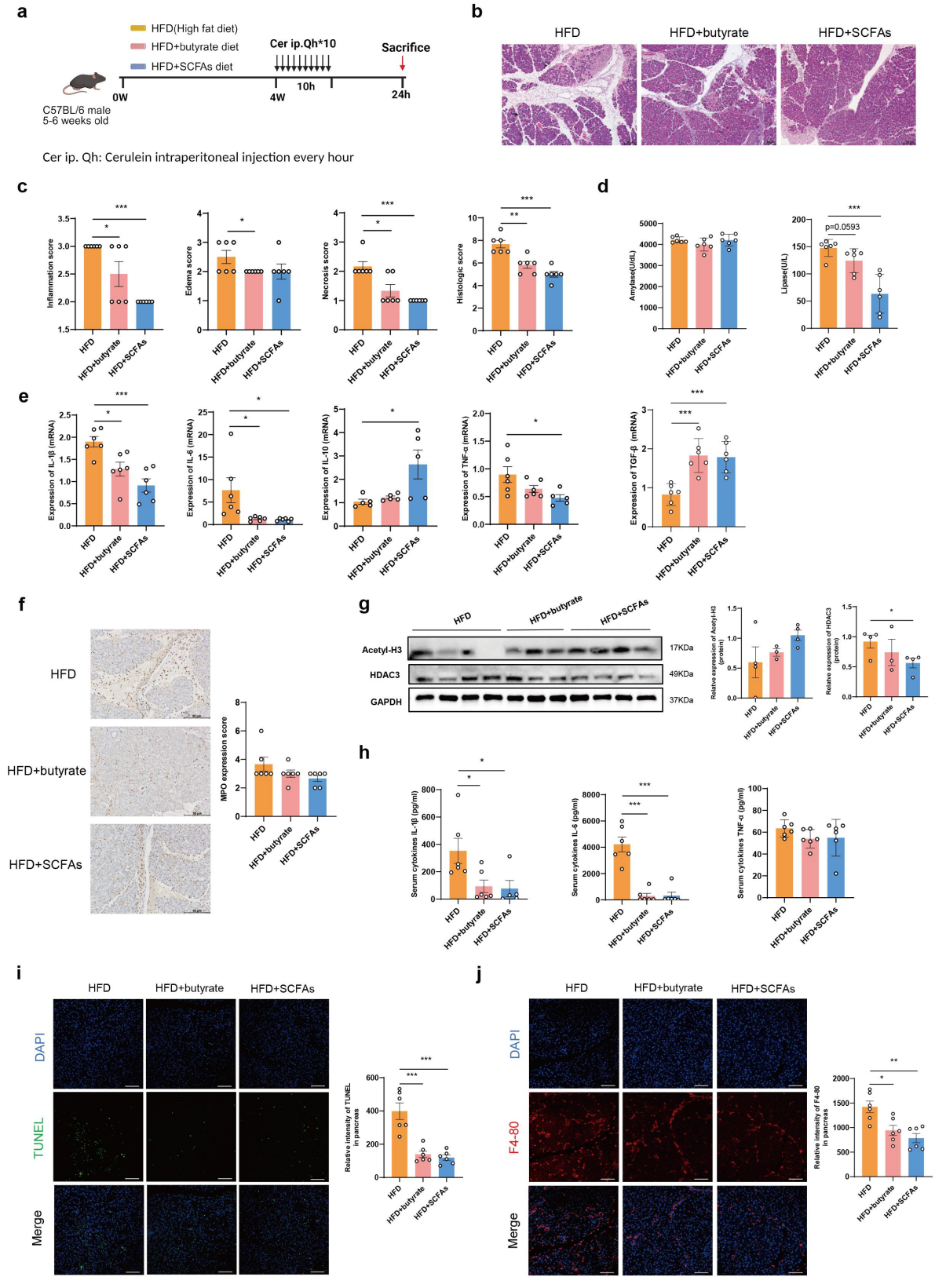

Figure 5. SCFAs, especially butyrate, alleviated the severity of pancreatic injury induced by a high fat diet. (a) Schematic of the SCFAs and butyrate administration and AP induction. (b) Representative H&E staining of pancreatic tissue. Scale bar: 50 μm. (c) Histopathological scoring of pancreatic damage. (d) Activity of amylase and lipase in the serum. (e) The mRNA expression of cytokines in pancreatic tissue. (f) Immunohistochemical staining of pancreatic MPO. Scale bar: 50 μm. (g) The expression of pancreatic HDAC3 and acetyl histone 3 proteins by Western blot. (h) Serum levels of cytokines. Pancreatic staining of TUNEL (green staining) (I) and F4/80 (red staining) (j) and DAPI (blue) to visualize nuclei (×200). *$p < .05$, **$p < .01$, ***$p < .001$.

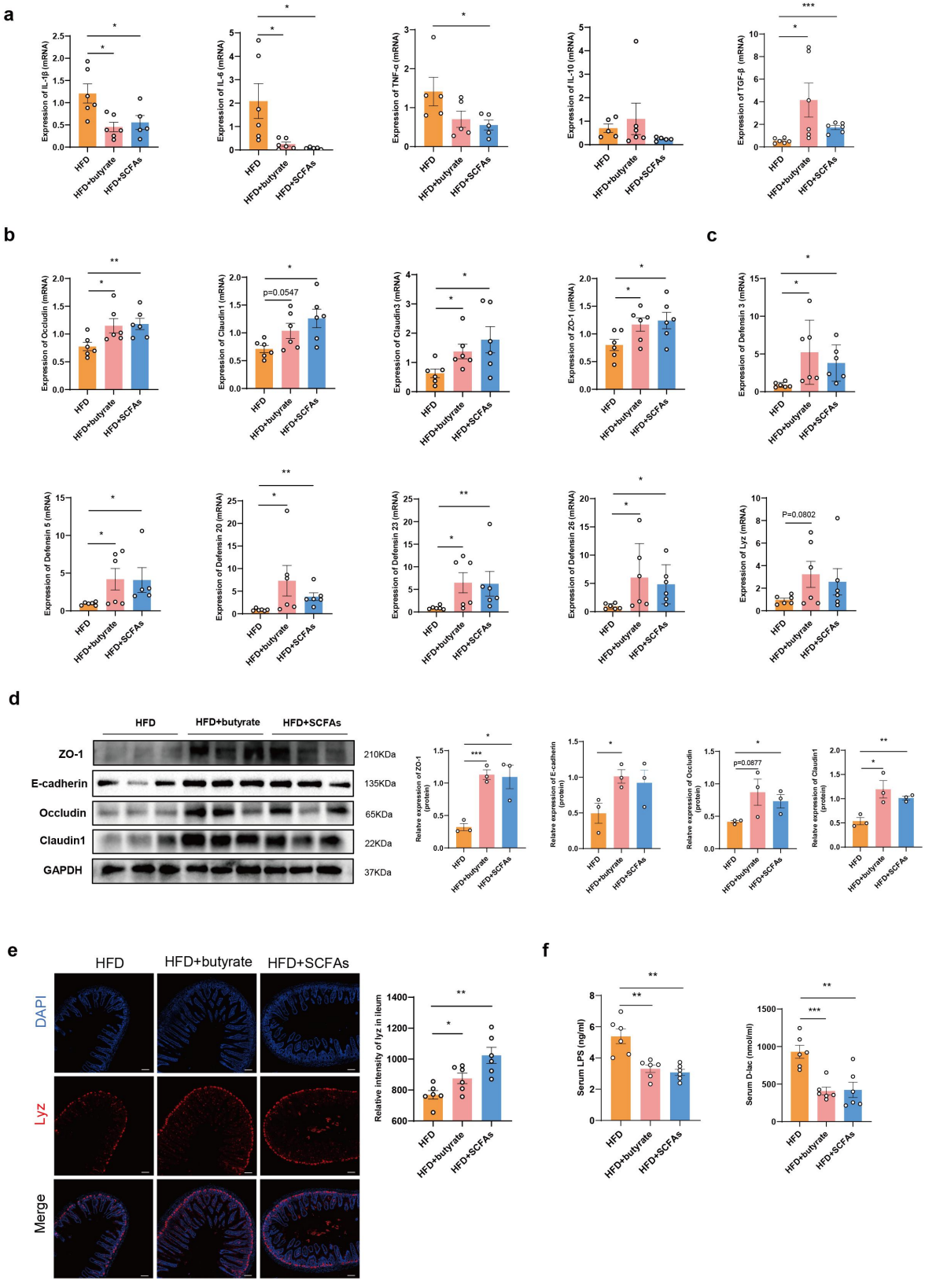

Figure 6. SCFAs improved gut barrier injury induced by a high fat diet in AP. The mRNA expression of cytokines (a), adhesion molecules (b) and defensins (c) in ileum tissue was detected by real-time PCR. (d) Western blot showed the expression of intestinal tight junction proteins. (e) Ileum expression of Lyz by immunofluorescene. (f) Serum levels of LPS and D-lac. *$p < .05$, **$p < .01$, ***$p < .001$.

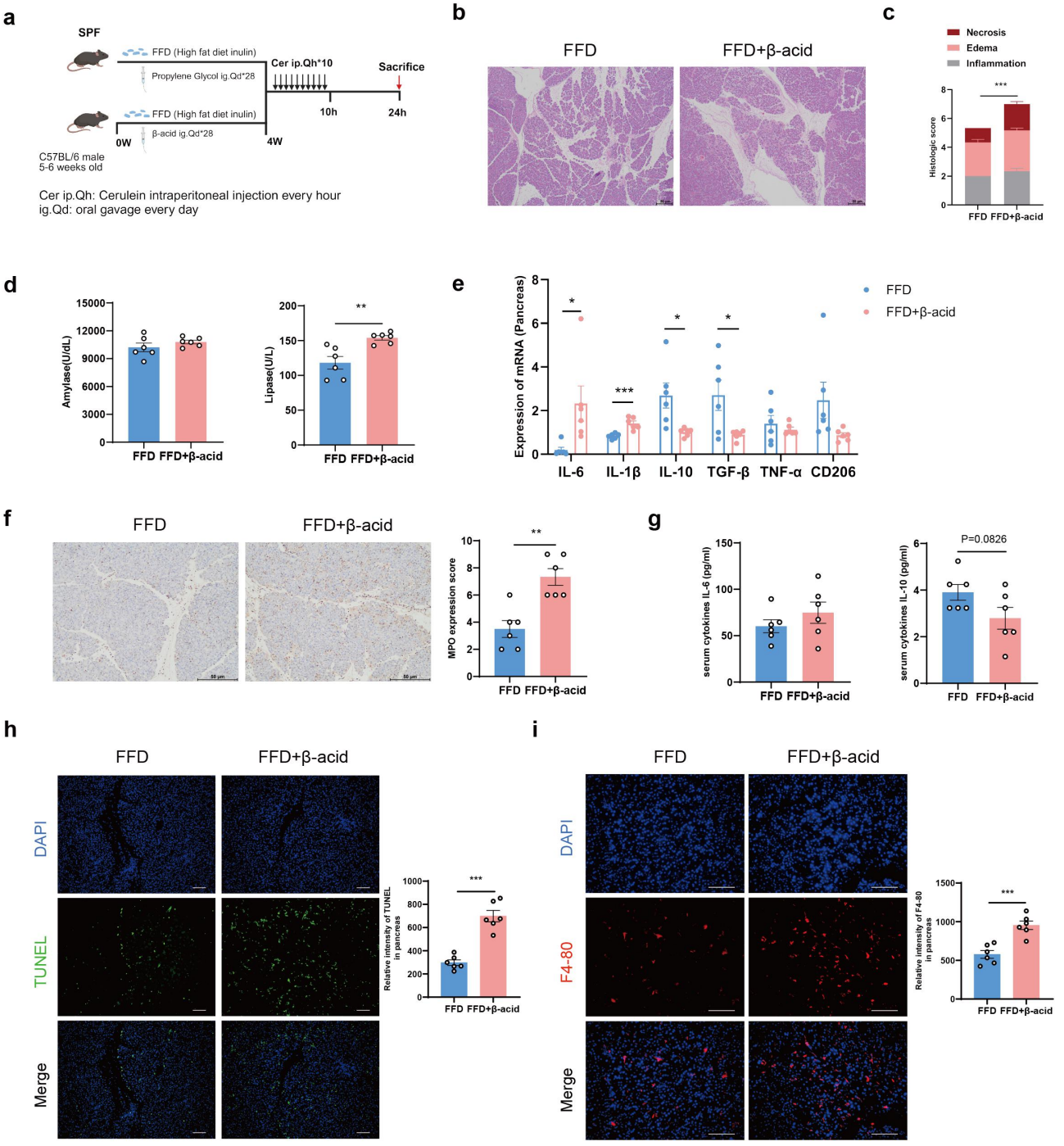

Figure 7. The alleviation of pancreatitis by inulin was dependent on SCFAs. (a) Experimental schema for β-acids administration and FFD diet before AP induction. (b) Representative H&E staining of pancreatic tissue. Scale bar: 50 μm. (c) Histopathological scoring of pancreatic damage. (d) Serum amylase and lipase activity. (e) The mRNA expressions of cytokines in the pancreas. (f) Immunohistochemical staining of MPO. Scale bar: 50 μm. (g) Serum levels of cytokines detected by ELISA. Pancreatic staining of TUNEL (green staining) (h) and F4/80 (red staining) (i) and DAPI (blue) to visualize nuclei (×200). $*p < .05$, $**p < .01$, $***p < .001$.

The inulin-induced suppression of neutrophils and macrophages infiltration was reversed by treatment with β-acids (Figure 7f,i). Consistently, levels of pro-inflammatory cytokines (IL-6, IL-1β) in serum and pancreas were elevated in β-acids-treated group, while anti-inflammatory cytokines (IL-10, TGF-β) were reduced (Figure 7e,g).

Considering the gut-pancreas axis in the progression of severe pancreatitis, we also examined the impact of β-acids on intestinal function in FFD-fed mice. The mRNA transcripts of IL-6, IL-1β in the intestine were higher in β-acids group compared to the vehicle group, whereas a decreasing trend was noted in IL-10, TGF-β (Figure 8a). Additionally, the gut barrier was disrupted and its permeability was heighted following β-acids administration, as shown by decreased expression of tight junction proteins, elevated serum levels of LPS and D-lactate (Figure 8b,e,g). Mice treated with β-acids exhibited a reduced number of Lyz-positive cells, along with decreased expression of defensins, indicating dysfunction in Paneth cells (Figure 8c,f). Collectively, these results demonstrated that SCFAs were the key contributor to inulin-mediated pancreatic and intestinal protection.

3.7. HDAC3 inhibition ameliorates severe pancreatitis induced by HFD

To determine the role of HDAC3 in AP progression, we treated HFD-fed mice with RGFP966, a selective inhibitor of HDAC3, for 1 week followed by caerulein injection (Figure 9a). We observed that RGFP966 restrained pancreatic damage and the release of amylase in the serum (Figure 9b–d). IHC and immunofluorescence showed that the neutrophils and macrophages populations were decreased in RGFP966-treated mice (Figure 9e,h). The expressions of macrophage M1 markers (IL-1β, IL-6, TNF-α) were reduced in both pancreatic and intestinal tissues, while the expressions of M2 markers (TGF-β, CD206) tended to increase following RGFP966 administration (Figure 9f,j). RGFP966 also effectively prevented the apoptosis of acinar cells (Figure 9g). ELISA analysis revealed that lower concentrations of inflammatory cytokines in the serum of mice treated with RGFP966 compared to the control group (Figure 9i). RGFP966 significantly increased the acetylation of H3 at H3K27 residues in AP mice (Figure 9k). Administration of RGFP966 decreased intestinal permeability as evidenced by reduced levels of LPS and D-lac in the serum (Figure 9i). In vitro study further demonstrated that macrophages were transformed from the M1 to M2 phenotype by RGFP966, showing up-regulated expressions of TGF-β, CD206, IL-10, and down-regulated expressions of IL-1β, TNF-α, CCL3 (Supplement fig. S8A-8B). As expected, RGFP966 markedly elevated H3K27 acetylation in THP-1 cells (Supplement fig. S8C). These findings illustrated that inhibition of HDAC3 modulated macrophage polarization, thereby ablating SAP development.

4. Discussion

With changes in people's dietary structure and an increase in sedentary lifestyles, obesity has become a global epidemic public health issue. Obesity is not only a risk factor for AP, but also closely associated with disease severity and poor prognosis.[32,33] Preventing the progression of SAP associated with obesity is a key focus of clinical research. Here we identified that high-fiber intake improved pancreatic injury, suppressed systemic inflammation, and strengthened gut barrier in obesity mice with SAP. Mechanistically, we demonstrated that the high-fiber shaped gut microenvironment played a vital role.

Accumulating evidence suggest that the disruption of gut-pancreas axis is correlated with the development of SAP.[11] In this study, we found that a high-fat diet induced gut microbiota dysbiosis, including the overgrowth of pathogenic *Escherichia Shigella*, *Enterococcus* and *Klebsiella*, ultimately exacerbating pancreatic damage. This was consistent with an earlier study that highlighted the detrimental impact of western-type diet on the mortality of acute necrotizing pancreatitis in mice.[26] Imbalances of gut microbiota have also been observed in hypertriglyceridemic pancreatitis patients, characterized by an increase in *Escherichia Shigella*, *Enterococcus* and *Klebsiella*. Moreover, the transplantation of their fecal microbiota has been found to exacerbate disease progression in recipient mice.[9] Additionally, we observed an augmentation of gut inflammation and permeability in obese AP mice. Of note, these mice also displayed a significant loss in Paneth cells, which directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. The vital role of gut barrier injury in pancreatic necrosis have also been documented in other studies where researchers found that, the elimination of Paneth cells worsened intestinal inflammation and subsequent pancreatic damage.[34]

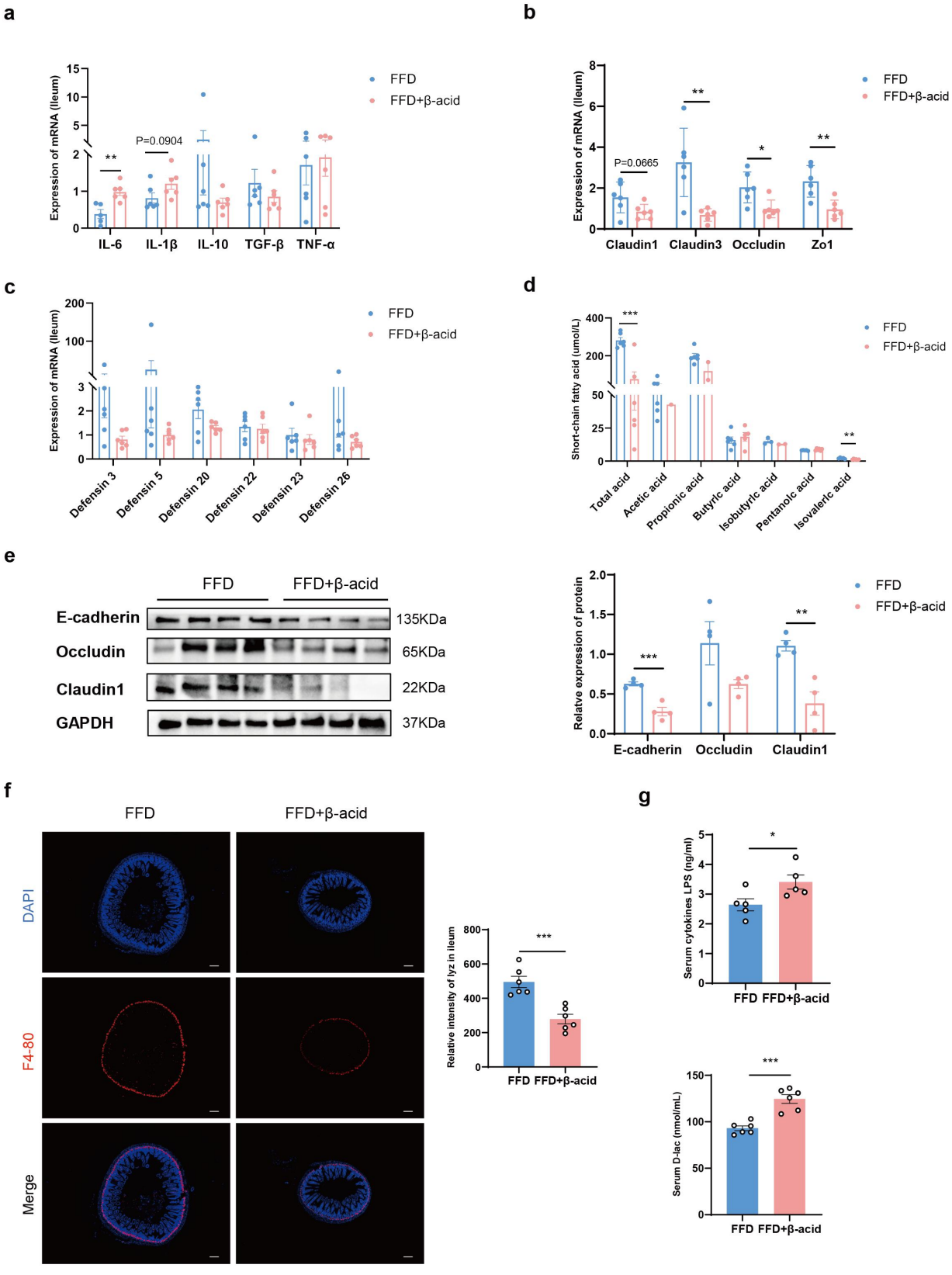

Figure 8. The restoration of gut dysfunction by inulin was abolished after inhibiting SCFAs production. The mRNA expression of cytokines (a), adhesion molecules (b) and defensins (c) in ileum tissue was detected by real-time PCR. (d) The concentrations of SCFAs in fecal samples were detected by GC-MS. (e) Western blot showed the expression of intestinal tight junction proteins. (f) Ileum expression of Lyz by immunofluorescene. (g) Serum levels of LPS and D-lac. *$p < .05$, **$p < .01$, ***$p < .001$.

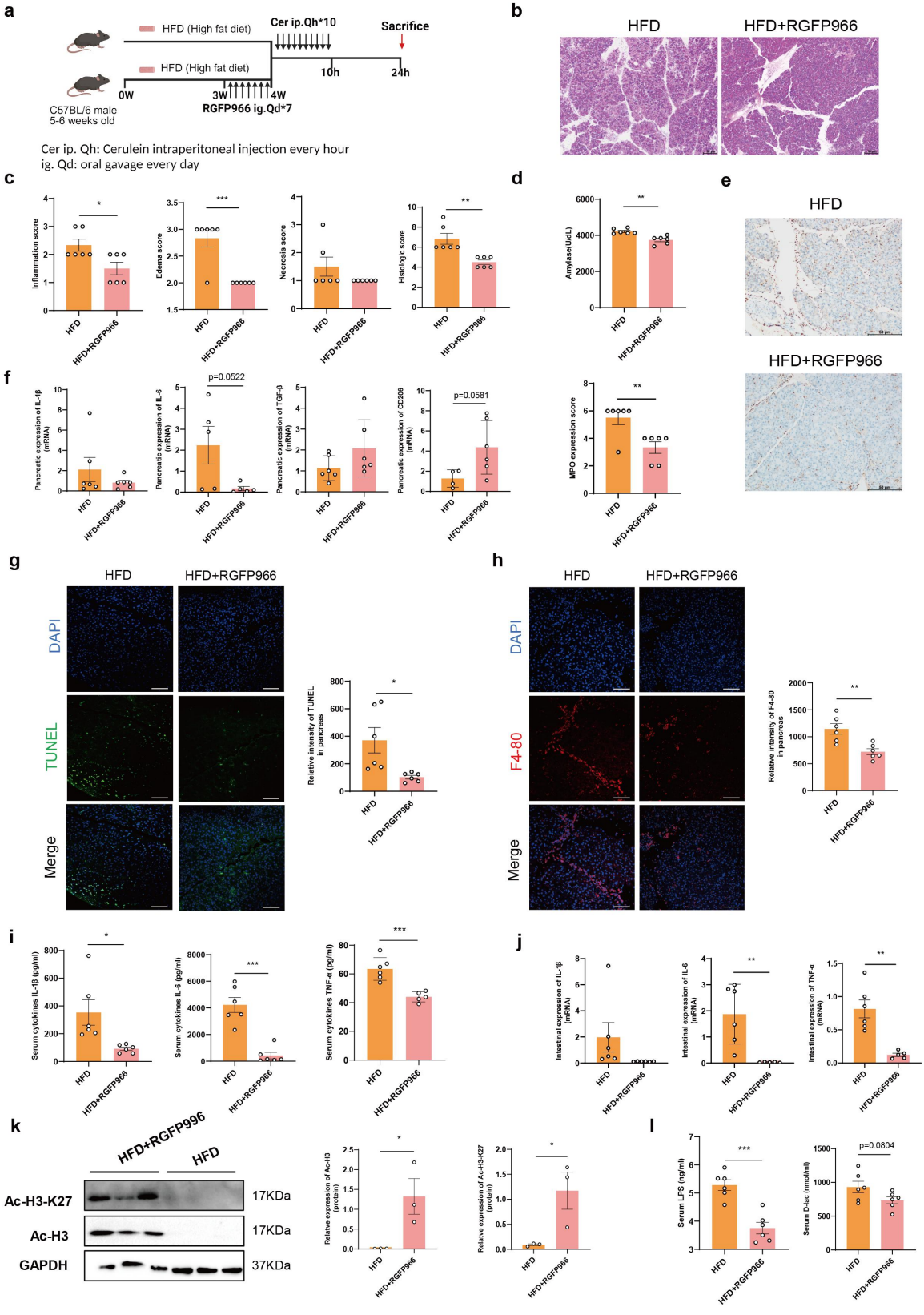

Figure 9. Inhibition of HDAC3 protected against obesity-associated pancreatic and intestinal injury. (a) The administration of a specific HDAC3 inhibitor to high fat fed-mice followed by AP induction. (b) Representative H&E staining of pancreatic tissue. Scale bar: 50 μm. (c) Histopathological scoring of pancreatic damage. (d) Serum amylase activity. (e) Immunohistochemical staining of MPO. Scale bar: 50 μm. The mRNA expressions of cytokines in the pancreas (f) and ileum (j) tissue were detected by RT-PCR. Pancreatic staining of TUNEL (green staining) (g) and F4/80 (red staining) (h) and DAPI (blue) to visualize nuclei (×200). Serum levels of cytokines (i) and gut barrier indexes (l) were detected by ELISA. (k) The expression of intestinal acetyl histone 3 and H3K27ac proteins was examined by Western blot. *$p < .05$, **$p < .01$, ***$p < .001$.

The gut microbiota is regarded as a promising target for the treatment of AP.[35] Dietary fiber, such as inulin, has garnered much attention for its capability to preserve gut ecology and modulate immune response through the regulation of gut microbiota.[36] Previous studies have shown that the consumption of inulin induced global gut microbiota alterations with the specific changes of SCFA-producing bacteria, including *Anaerostipes*, *Bifidobacterium* and *Akkermansia*.[24,37,38] In this study, we demonstrated that high-fiber intake rectified the dysbiosis of gut microbiota in obese AP mice, including the depletion of pathogenic *Escherichia Shigella*, *Enterococcus*, *Klebsiella* and the proliferation of beneficial *Akkermansia*. Notably, the protective effect of dietary fiber against pancreatic injury and systematic inflammation induced by a high-fat diet was attenuated in germ-free mice but reinstated in mice received microbiota transplantation from high-fiber donors. These results underscored the causal relationship between dietary fiber-induced reshaping of gut microbiota and the mitigation of AP severity. *Akkermansia*, which was enriched by high fiber intake, has been reported to lessen the severity of AP through its membrane protein.[39] While dietary inulin is generally recognized for its health-promoting benefits through fermentation into metabolites by gut bacteria, there have also been reports of adverse effects of inulin contributing to the development of liver cancer and allergic inflammation.[20,21] Thus, we speculate that whether inulin is beneficial or harmful is highly context-dependent, with the dose and duration also playing a significant role.

Impaired gut barrier function, characterized by the loss of tight junction protein and disturbance of immunological intestinal defense, is a common hallmark of AP.[40] As anticipated, our findings revealed that dietary fiber supplementation effectively modulated the immune balance within the gut, leading to a decrease in pro-inflammatory cytokines and an increase in anti-inflammatory cytokines. Decreased serum levels of D-lactate and lipopolysaccharide, and increased expressions of epithelial tight proteins ZO-1, Occludin and E-cadherin were observed in high-fiber treated mice, indicating that gut barrier function was restored. Furthermore, dietary fiber, together with its reshaped microbiota, induced the elevation of Paneth cell counts and the expression levels of antimicrobial peptides, which act as guardians in maintaining the homeostasis of intestinal environment. This aligns with previous studies that reported the role of prebiotic fiber in preventing leaky gut, which facilitates the translocation of bacteria.[41] Recent studies suggest that gut bacteria, including *Akkermansia muciniphila*, *Lactobacillus reuteri*, may modulate the mitochondrial function of Paneth cells, stimulate cell proliferation, and boost the secretion of antimicrobial peptides through the production of metabolites, such as SCFAs.[42–44]

One possible explanation of how gut microbiota can influence AP is by their generation of various metabolites. Our previous study found that SCFAs, as one of the most common metabolites of gut microbiota, were depleted in AP patients.[8] The current study revealed that concentrations of SCFAs, which were correlated with alterations in gut microbiota, were significantly elevated by a high-fiber diet. Furthermore, SCFAs treatment, specifically butyrate, also attenuated obesity-induced severe pancreatic damage and inflammatory response, mirroring the effect of dietary fiber. This observation is consistent with a prior study that reported butyrate supplementation reduced mortality and bacterial dissemination in AP mice fed western-type diet.[26] Our findings demonstrated that the protective effect of a combination of SCFAs was superior to that of a single acid, indicating a synergistic action among the three acids. Conversely, a recent study revealed an increased abundance of species that are known as SCFAs producers in SAP patients through metagenomic sequencing of rectal swab samples, with functional profiling indicating elevated SCFA production.[45] We speculate that differences in sampling sites and the use of medications like antibiotics may contribute to the discrepancies observed in various studies. Thus, a longitudinal study is required to investigate not only the onset but also the dynamic changes of SCFAs during disease progression to establish causality. The ongoing proof-of-concept randomized trial involving prophylactic butyrate supplementation in AP patients will likely elucidate the role of SCFAs (NCT06147635).

Additionally, we illustrated that SCFAs inhibited the intestinal inflammation and enhanced immune defense capability in obese mice with AP. To further elucidate the underlying mechanism, we investigated the effect of SCFAs on HDAC3, given that SCFAs, particularly butyrate, modulate gut immunity through the inhibition of HDAC3.[28] Interestingly, we observed that the expression of HDAC3 was down-regulated, while acetylation of H3 was up-regulated by SCFAs and butyrate treatment both in vitro and in vivo. Moreover, HDAC3 inhibitor protected against pancreatic injury and systemic inflammation in obese mice, similar to the effects of SCFAs. This is consistent with a previous study that presented the protective role of HDAC3 inhibition in AP by regulating intestinal function.[46] Aligns with prior studies, we found that SCFAs modulated the polarization of macrophages from a pro-inflammatory M1 phenotype to an anti-inflammatory M2 phenotype, which was mimicked by HDAC3 inhibitor and impeded by its agonist.[47,48]

Our study has some limitations that should be noted. First, we demonstrated that the beneficial effect of dietary fiber in mitigating obesity-associated SAP was dependent on gut microbiota, although the specific bacterial species have not been identified. Shotgun metagenome sequencing could be applied to explore the alterations of gut microbiota at the species level, as well as their potential functions. Second, the impacts of dietary fiber on microbial metabolites other than SCFAs are unclear. Further studies could use DNA-stable isotope probing technique to track the flow of metabolites synthesized directly through microbial fermentation of fiber.[19] Another limitation is that the precise molecular mechanisms and signaling pathways involved in SCFAs-mediated HDAC3 inhibition in macrophages remain to be determined.

5. Conclusions

To summarize, our study demonstrated that the dysbiosis of gut microbiota induced by HFD was associated with the progression of SAP. Furthermore, we identified dietary fiber intake can prevent against obesity-associated SAP by regulating the bacterial composition and SCFA formation. Beyond its effect on pancreatic damage, dietary fiber also improved gut barrier function and repressed systemic inflammation. SCFAs, especially butyrate, exhibited protective effects on pancreatic injury that may be linked to the inhibition of HDAC3 and the modulation of macrophage polarization. Overall, gut microbiota-targeted prebiotics supplementation is a potential prophylactic approach against SAP, warranting further clinical investigation in patients.

Highlights

- Inulin alleviates high fat diet-induced severe acute pancreatitis (SAP) and improves gut barrier dysfunction.
- Inulin modulates the dysbiosis of gut microbiome in SAP, including the suppression of potential pathogens and the enrichment of short chain fatty acids (SCFAs)-producing probiotics.
- SCFAs, especially butyrate, protect against pancreatic and intestinal injury in obesity-associated SAP through the inhibition of HDAC3.
- The acetylation of H3K27 by HDAC3 is involved in modulating macrophage M1/M2 polarization by butyrate.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Author contributions

Xin Li performed the experiments, analyzed data and assisted in manuscript writing. Pan Zheng, Yaoyu Zou, Liangyi Guan assisted in animal experiments and 16S rRNA analysis. Nianshuang Li, Jianping Liu, Nonghua Lu assisted in methodology. Cong He acquired funding and wrote the manuscript. Cong He and Yin Zhu designed the study, interpreted the data and supervised the study.

Data availability statement

The raw sequencing data of gut microbiota have been uploaded to the NCBI Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/sra.) under accession number PRJNA1062127. The raw sequencing data of SCFAs have been uploaded to China National Center for Bioinformation (OMIX005615).

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