Food Frontiers

WILEY

RESEARCH ARTICLE OPEN ACCESS

Innovative Food Intervention Containing Probiotics and Policosanols Counteracts Gut Barrier Breakdown and Inflammation Associated With Obesity via Butyrate-glial-IL1β Axis

Vanessa D'Antongiovanni[1] | Clarissa Pierucci[1] | Clelia Di Salvo[2] | Laura Benvenuti[2] | Cristina Segnani[1] | Chiara Ippolito[1] | Sara Angelini[1] | Gianfranca Carta[3] | Sebastiano Banni[3] | Carolina Pellegrini[1] | Luca Antonioli[2] | Nunzia Bernardini[1]

[1]Unit of Human Histology and Embryology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy | [2]Unit of Pharmacology and Pharmacovigilance, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy | [3]Department of Biomedical Science, University of Cagliari, Cagliari, Italy

Correspondence: Vanessa D'Antongiovanni (vanessa.dantongiovanni@med.unipi.it)

Received: 13 January 2026 | **Revised:** 10 June 2026 | **Accepted:** 26 June 2026

Keywords: enteric glia | food supplementation | intestinal epithelial barrier | intestinal inflammation | gut microbiota

ABSTRACT

The combined use of probiotics and policosanols has emerged as a promising therapeutic strategy for the management of obesity. Here, we investigated the efficacy of an innovative mixture, containing probiotics (*Bifidobacterium infantis* GM-21, *Bifidobacterium bifidum* GM-25, *Lactobacillus rhamnosus* GM-28) and policosanols in counteracting and preventing body weight gain, intestinal epithelial barrier (IEB) dysfunction, and enteric inflammation in a mouse model of high-fat diet (HFD)-induced obesity, also characterizing its molecular mechanism of action. C57BL/6J mice were fed with HFD or standard diet for 8 weeks. Supplementation with the mixture or each individual component was starting from the first day of the study (preventive) or from the fifth week (curative). Body weight gain, BMI, metabolic parameters, circulating lipopolysaccharide binding protein (LBP), colonic interleukin (IL)-1β, tight junction (TJ) protein expression and distribution, fecal butyrate, and butyrate SMCT1 transporter were analyzed. Enteric gliosis was assessed by glial fibrillary acidic protein (GFAP)$^+$ cells. After 8 weeks, HFD mice showed increased body weight and BMI, metabolic alterations, IEB disruption with elevated LBP levels and reduced TJ molecules, decreased colonic SMCT1 and butyrate bioavailability, increased GFAP$^+$ cells, and intestinal inflammation characterized by increased colonic IL-1β and GFAP$^+$/IL-1β^+ cells. Supplementation with the mixture improved all above-mentioned parameters, in both preventive and curative protocol, by enhancing butyrate bioavailability and reducing IL-1β release from reactive glial cells. Of note, each individual component only partially improved these parameters. Overall, our results suggest that mixture exerts anti-obesity properties, improving the IEB and blunting gut inflammation, likely through the modulation of the butyrate-glia-IL-1β axis.

Vanessa D'Antongiovanni and Clarissa Pierucci contributed equally to this work.

Luca Antonioli and Nunzia Bernardini jointly supervised this work.

1 | Introduction

Obesity is a multifactorial pathological condition characterized by an excess body fat often associated with several medical comorbidities, such as gastrointestinal disorders, type 2 diabetes mellitus, cardiovascular diseases, and cognitive impairment (D'Antongiovanni, Pellegrini, et al. 2020; Jarolimova et al. 2013; Khan et al. 2021). At present, obesity rates are increasing steadily and significantly worldwide; it is estimated that adult obesity has more than doubled since 1990 and that childhood/adolescence obesity (5-19 years old) has quadrupled (GBD 2021 Adolescent BMI Collaborators 2025). On these bases, increasing efforts have been addressed to better understand and characterize the molecular mechanisms underlying the onset and development of obesity, aimed at identifying novel strategy for therapeutic intervention. In this regard, alterations of intestinal epithelial barrier (IEB) have been identified as *primum movens* of a series of pathophysiologic events that precede the body weight gain, metabolic alterations, and systemic inflammation associated with obesity (D'Antongiovanni, Segnani, et al. 2023). Indeed, an imbalance in the IEB structure, besides altering intestinal absorptive/secretory functions, can facilitate the translation of toxins, bacteria, and their products into the mucosa, triggering the activation of enteric and circulating immune/inflammatory cells (including enteric glial cells—EGCs), which, in turn, contribute to low-grade enteric and systemic inflammation (D'Antongiovanni, Fornai et al. 2025; Massier et al. 2021; Nascimento et al. 2020). Of note, the presence of this chronic low-grade enteric and systemic inflammation, also called meta-inflammation, represents the pathophysiological link between obesity and related comorbidities (Gkrinia and Belančić 2025; Ruck et al. 2023).

In the past few years, the modulation of gut barrier functions through nutritional and other interventions, including the manipulation of gut microbiota via probiotics, has emerged as a potential therapeutic strategy for the management of obesity. In particular, human and preclinical studies in obese patients and murine models of obesity documented beneficial effects following probiotics supplementation on body weight control, metabolic parameters, and systemic inflammation along with an improvement of gut barrier permeability (Chandrasekaran et al. 2024; DiMattia et al. 2024). Indeed, *Lactobacillus* and *Bifidobacterium* strains exert strengthening effects on the gut barrier by increasing the expression of tight junction (TJ) proteins, enhancing mucin production and favoring the production of short-chain fatty acids (SCFAs) with consequent reductions in enteric and systemic inflammation (Deng et al. 2022; Han et al. 2019; Ho et al. 2024; Kou et al. 2023). In parallel, the dietary supplementation with natural compounds, such as policosanols–very-long-chain saturated fatty alcohols derived from rice bran, wheat, sugarcane, and bees wax—has recently emerged as an interest therapeutic option to manage obesity. For instance, the administration of octacosanol, the main component of policosanols, counteracted body weight gain and insulin resistance as well as reduced blood cholesterol levels and systemic inflammation in high-fat diet (HFD) mice (Sharma et al. 2019; Zhai et al. 2021). Similarly, hexacosanol reduced plasma and hepatic cholesterol in obese mice (Lee et al. 2017). In support of these findings, in a recent study, Mulè et al. (2025) demonstrated that the combination of probiotics and polycosanols improves the gut barrier integrity and reduces intestinal inflammation in an in vitro model of gut–liver–adipose axis, suggesting potential application in the context of obesity. Accordingly, based on this evidence, the present study was designed to evaluate the in vivo efficacy of an innovative mixture, containing probiotics and policosanols, in counteracting and preventing the body weight gain, IEB dysfunction, and enteric inflammation in a mouse model of HFD-induced obesity, characterizing also the molecular mechanisms underlying its beneficial effects.

2 | Materials and Methods

2.1 | Animal Model of Diet-Induced Obesity

Male C57BL-6J mice (20–25 g body weight) were purchased from ENVIGO Srl (San Pietro al Natisone UD) and employed throughout the study. To induce obesity, standard diet (SD, 18% calories from fat—TD.2018), administered during the adaptation period to all mice, was replaced with an HFD (60% calories from fat—TD.06414) for 8 weeks. In particular, HFD provided 18.3% of kcal as protein, 21.4% of kcal as carbohydrate, and 60.8% of kcal as fat (5.1 kcal/g), whereas the SD provided 24% of kcal as protein, 58% of kcal as carbohydrate, and 18% of kcal as fat (3.1 kcal/g). The animals had free access to tap water ad libitum and were not employed for at least 1 week after their delivery to the laboratory. They were housed two/three in a cage in a temperature-controlled room on a 12-h light cycle at 22–24°C and 50-60% humidity. All the procedures involving animals were compliant with ARRIVE (Animal Research Reporting of In Vivo Experiments) guidelines and were carried out following the guidelines of the European Community Council Directive 86-609 and in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki, EU Directive 2010/63/EU for animal experiments). The experiments were approved by the University of Pisa's Ethical Committee for Animal Experimentation and the Italian Ministry of Health (Authorization 48/2024-PR). All efforts were made to reduce and minimize the number of animals and their suffering.

HFD mice after 8 weeks are characterized by an increase in body and epididymal fat weight, blood cholesterol level, presence of systemic inflammation as well as intestinal bowel dysmotility (a typical condition complained by obese patients), without signs of insulin resistance, hypertension, and cardiovascular disorders (Antonioli et al. 2020; D'Antongiovanni et al. 2023; Jürgens et al. 2007; Pellegrini et al. 2021; Raybould 2012; Stenman et al. 2012). Of note, these metabolic alterations represent a condition that reflects those observed in obese patients who are not yet diabetic (Alshuweishi et al. 2024). Conversely, following 12–16 weeks of HFD diet, animals displayed an increase in insulin resistance and signs of cardiovascular alterations (Calligaris et al. 2013; Csóka et al. 2017; Ding et al. 2010). In addition, starting from week 2, mice fed with HFD showed alterations in IEB integrity and permeability along with the presence of enteric and systemic inflammation, features also observed in overweight and obese people (Antonioli et al. 2020; D'Antongiovanni et al. 2024; D'Antongiovanni et al. 2023).

2.2 | Experimental Design

In the present study, we examined the effect of a mixture, containing probiotics—(*Bifidobacterium infantis* GM-21 [1×10^9 CFU/mouse/day], *Bifidobacterium bifidum* GM-25 [1×10^9 CFU/mouse/day], *Lactobacillus rhamnosus* GM-28 [1×10^9 CFU/mouse/day]) and policosanols—(octacosanol [>60%] and hexacosanol [2%–15%])—in SD mice (control group) and HFD mice. In addition, each individual component (probiotics or policosanols) was also tested individually. The mixture and each individual component were provided by Nóos S.r.l., Rome, Italy. The mixture is covered by an Italian patent application (No. 102023000021603). Preliminary in vitro studies using a gut–liver–adipose axis model supported the selection and characterization of the final probiotic–polycosanol formulation included in the patent application (Mulè et al. 2025). Of note, the bacterial genera and species contained in the mixture were selected for their strong ability to release metabolites, including SCFAs, endowed with well-known beneficial effects on intestinal homeostasis, whereas policosanols were chosen for their ability to promote the release of SCFAs from the host gut microbiota and exert anti-oxidant and anti-inflammatory activities (Deng et al. 2022; Han et al. 2019; Ho et al. 2024; Kou et al. 2023; Lee et al. 2017; Sharma et al. 2019; Zhai et al. 2021). The mixture or each individual component was administered via oral gavage for: (a) 8 weeks, starting from the first day of the study (preventive protocol), or (b) 4 weeks, starting from the fifth week (curative protocol) (Figure 1A). Subgroups of animals received via oral gavage the drug vehicle (physiological solution) and served as controls. SD and HFD mice were treated simultaneously with the treatment groups, ensuring identical experimental conditions. Accordingly, since all experimental procedures were performed at the same time, SD and HFD mice served as common reference groups for both the preventive and curative protocols. Furthermore, the administration of the mixture and individual components to SD mice did not elicit any significant change in all parameters evaluated in the present study (Figure S1). Therefore, SD mice treated with drug vehicle were adopted as control group for all the evaluations on the drug under investigation. Body weight was measured once a week from the first day of the study. To calculate the food intake, all remnants of pellets were weighted at 11:00, including any spilled food in cages, and this value was subtracted from the initial weight. During these measurements, all mice were housed individually. At the end, animals were euthanized. Fecal content, blood samples, and tissue samples were collected and then stored at −80°C or fixed in formalin for the following analyses.

2.3 | Measurement of Body Mass Index and Evaluation of Metabolic Parameters

Body mass index (BMI) was calculated as body weight (g) divided by body length (mm) squared (BMI = body weight/body length^2). Blood samples were taken from the tail vein the day of sacrifice after overnight fast. Triglycerides and high-density lipoprotein (HDL) levels were evaluated by means of Multicare Insensor (BSI Srl, Arezzo, Italy), in accordance with the manufacturer's instructions.

2.4 | Evaluation of Fecal SCFAs

Fecal samples were collected and stored at −80°C until analysis. Approximately 100 mg of frozen material was processed, derivatized with 1-butanol/boron trifluoride, and extracted into hexane as described previously (D'Antongiovanni, Antonioli et al. 2023). The organic phase containing butylated SCFAs was diluted in methanol and analyzed by LC-MS/MS (Agilent 1100 HPLC coupled to an Agilent 6420 triple quadrupole MS with ESI+). Separation was achieved on a C18 Zorbax Eclipse Plus column (50 × 4.6 mm, 5 μm) with a methanol/water/formic acid mobile phase (70:30:0.1, v/v/v). Quantification was performed in MRM mode using optimized transitions for butyl butyrate (m/z 145 → 89) and compared against authentic standard. Data were acquired and analyzed with the MassHunter Workstation software.

2.5 | Quantification of Colonic Interleukin-1β Levels

Colonic IL-1β levels were quantified, as previously described (Di Salvo et al. 2024), using a commercial ELISA Kit (ab100705; Abcam, Cambridge, UK). Colon tissues, previously collected and stored at −80°C, were briefly thawed, weighed, and homogenized in phosphate buffered saline (PBS; 0.4 mL/20 mg of tissue) at 4°C and centrifuged for 5 min at 10,000 × *g*. Aliquots of 100 μL were used to conduct the experiment. IL-1β levels were indicated as picograms per milligram of protein.

2.6 | Evaluation of Plasma Lipopolysaccharide Binding Protein

Circulating lipopolysaccharide binding protein (LBP) levels, an indirect index of increased gut permeability (Pellegrini et al. 2023), were measured by ELISA kit (ab213876; Abcam), as previously described (Di Salvo et al. 2024). For the procedure, blood samples were centrifuged for 5 min at 4000 rpm at 2–8°C, and after the centrifugation, supernatants were collected. Aliquots (100 μL) were used for the procedure, in accordance with the manufacturer's instructions.

2.7 | Western Blot Assays

Colonic tissues were weighed and then homogenized in lysis buffer using a polytron homogenizer (QIAGEN, Milan, Italy), as previously reported (D'Antongiovanni et al. 2025). Samples were then centrifuged at 12,000 rpm for 15 min at 4°C, and then the supernatants were separated from pellets and stored at −80°C. Bradford assay was used to quantify total proteins. Proteins (30 μg) were separated onto a pre-cast 4%–20% polyacrylamide gel (Mini-PROTEAN TGX gel, Biorad) and transferred to PVDF membranes (Trans-Blot TurboTM, PVDF Transfer packs, Biorad). Membranes were blocked with 3% bovine serum albumin diluted in Tris-buffered saline (20 mM Tris-HCl, pH 7.5, 150 mM NaCl) with 0.1% Tween 20. Primary antibodies against β-actin (ab8227; Abcam), occludin (ab167161; Abcam), zonulin-1 (ZO-1, Ab96587; Abcam), glial fibrillary acidic protein (GFAP, ab53554; Abcam), and sodium-coupled monocarboxylate transporter 1 (SMCT1, BS-

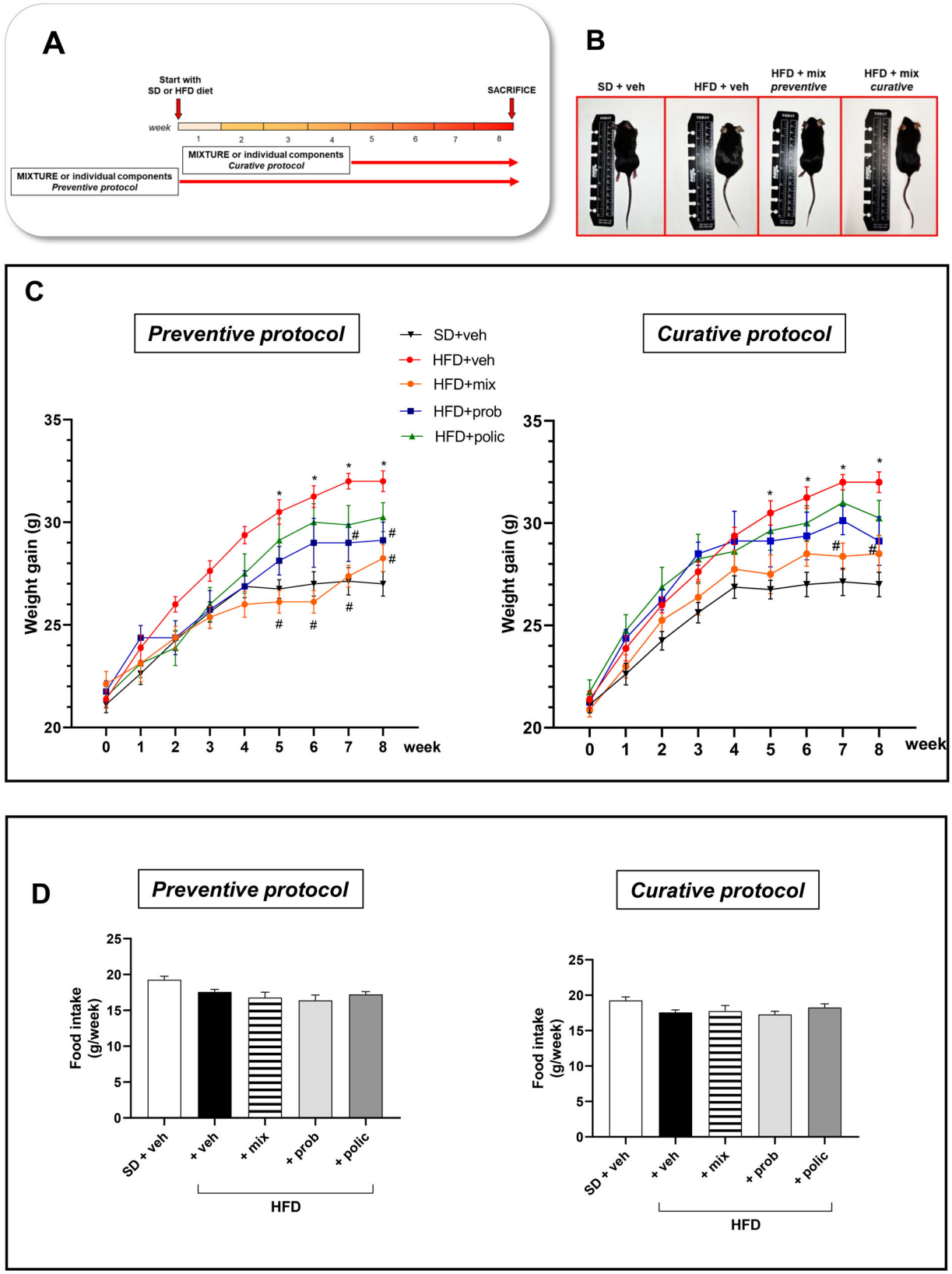

FIGURE 1 | (A) Schematic representation of in vivo treatment with placebo, mixture, or each individual components in C57BL-6J mice fed with SD or HFD for 8 weeks: preventive and curative protocol. (B) Representative images of C57BL-6J mice fed with SD or HFD for 8 weeks, with or without dietary supplementation with the mixture. Effects of the mixture and each individual components, in both preventive and curative protocol, on (C) body weight (g) and (D) food intake (g/week) in SD and HFD mice. Each column shows the mean ± SEM ($n = 8$/group). Two-way ANOVA, followed by Holm–Šídák's multiple comparisons test: results are as follows: *$p \leq 0.05$ for significant difference versus the SD group, and #$p \leq 0.05$ for significant difference versus the HFD group. HFD, high-fat diet; mix, mixture; polic, policosanols; prob, probiotics; SD, standard diet; veh, vehicle.

6106R, Bioss) were used. Secondary antibodies were obtained from Abcam (anti-mouse ab97040 and anti-rabbit ab6721). Protein bands were revealed with ECL reagents (Clarity Western ECL Blotting Substrate, Biorad), and subsequently the densitometric analysis, expressed as optical density, was performed with the iBright Analysis software.

2.8 | Confocal Immunofluorescence

Paraffin sections (7 μm thick) from full-thickness formalin-fixed samples of distal colon were processed for immunofluorescence analysis. In particular, the following markers were evaluated: GFAP-reactive glial cell marker alone or in combination with IL-1β (a pro-inflammatory cytokine released from reactive EGCs) in single and double immunofluorescence, respectively. In addition, double immunofluorescence was performed on colonic sections to detect ZO-1 and occludin, proteins involved in TJ complex. Briefly, sections were incubated overnight at 4°C with primary antibodies diluted in PBS: chicken anti-GFAP (1:400, ab4674; Abcam), rabbit anti-IL1β (1:500, ab283818; Abcam), rabbit anti-occludin (1:200, ab283818; Abcam), and rat anti-ZO-1 (1:200, MABT11; Merck Millipore), later with the appropriate fluorophore-conjugated secondary antibody and finally with TOPRO3 for nuclear counterstaining. Sections were examined under a Leica TCS SP8 confocal laser-scanning microscope (Leica Microsystems, Mannheim, Germany).

2.9 | Statistical Analysis

The statistical analysis of data complies with the requirements of good laboratory practices. The results are presented as mean ± standard error of the mean (SEM), with n referred to the number of mice/groups. The significance of differences was evaluated by one-way or two-way analysis of variance (ANOVA) followed by the appropriate post hoc test. p values ≤ 0.05 were considered representative of significant statistical differences. All statistical procedures were performed by commercial software (GraphPad Prism, version 8.0, RRID: SCR_002798, GraphPad Software Inc., San Diego, CA, USA).

3 | Results

3.1 | The Synergic Combination of Probiotics and Policosanols Counteracts the Body Weight Gain and BMI in Obese Mice

HFD mice showed a significant increase in body weight starting from the fifth week, as compared with SD mice (Figure 1B,C). Dietary supplementation with the mixture, in both preventive and curative protocols, significantly counteracted the body weight gain in HFD-fed mice starting from the fifth and seventh week, respectively (Figure 1B,C). In preventive protocol, probiotics administration alone counteracted the weight gain in obese mice starting only from seventh week, while no effects were observed in curative protocol. In addition, no effects were observed for policosanols alone on body weigh in both preventive and curative protocols (Figure 1C, Figure S2). Of note, no significant differences in food intake were detected in SD and HFD treated with vehicle, mixture, or individual components, in both preventive and curative protocol (Figure 1D, Figure S1).

At the end of week 8, a significant increase in BMI was observed in HFD mice, as compared with the SD group (Figure 2A). Supplementation with the mixture significantly counteracted such an increase in obese mice in both preventive and curative protocols (Figure 2A). No effects were observed for probiotics and policosanols alone on BMI of HFD mice, in both preventive and curative protocols (Figure 2A). Overall, these results highlight a synergistic combination of probiotics and policosanols in counteracting the increase in body weight and BMI associated with obesity.

3.2 | The Synergic Combination of Probiotics and Policosanols Ameliorates Metabolic Parameters in Obese Mice

Approximately 60%–70% of obese patients show a dyslipidemic phenotype, characterized by an increase in plasma levels of triglycerides (TG) along with a decrease in HDL involved in the removal of excess cholesterol levels (Ellulu et al. 2017; She et al. 2022). Therefore, the potential beneficial effect of the tested mixture on these metabolic parameters has been investigated.

Mice fed with HFD for 8 weeks showed a significant increase in TG levels along with a decrease in HDL levels compared to SD mice (Figure 2B,C). The supplementation with the mixture, in both preventive and curative protocols, determined a normalization of these parameters in HFD animals (Figure 2B,C). Of note, the administration of each individual component did not affect TG and HDL levels in either preventive or curative protocols, except for policosanol supplementation, which increased HDL levels in the preventive protocol in HFD mice (Figure 2B,C). Taken together, these findings suggest potential synergistic benefits of a therapeutic combination of probiotics and policosanol in improving altered metabolic parameters associated with obesity.

3.3 | Food Supplementation With the Mixture Counteracts Enteric Inflammation in Obese Mice

Obesity is characterized by low-grade systemic inflammation, which seems to be a common root to the onset and progression of several medical comorbidities (D'Antongiovanni et al. 2021; L. Wang, Wang, et al. 2025). Consistently with this assumption, HFD mice displayed a significant increase in colonic IL-1β levels, compared with SD animals (Figure 3A), indicating the presence of enteric inflammation. Food supplementation with the mixture or with probiotics alone significantly reduced the colonic IL-1β levels in obese mice (Figure 3A). Of note, the administration of policosanols alone counteracted, although not significantly, the colonic IL-1β levels in HFD mice (Figure 3A). Overall, these results suggest that the mixture counteracts enteric inflammation in obese mice, and this effect is likely ascribable to its combined composition of probiotics and policosanols.

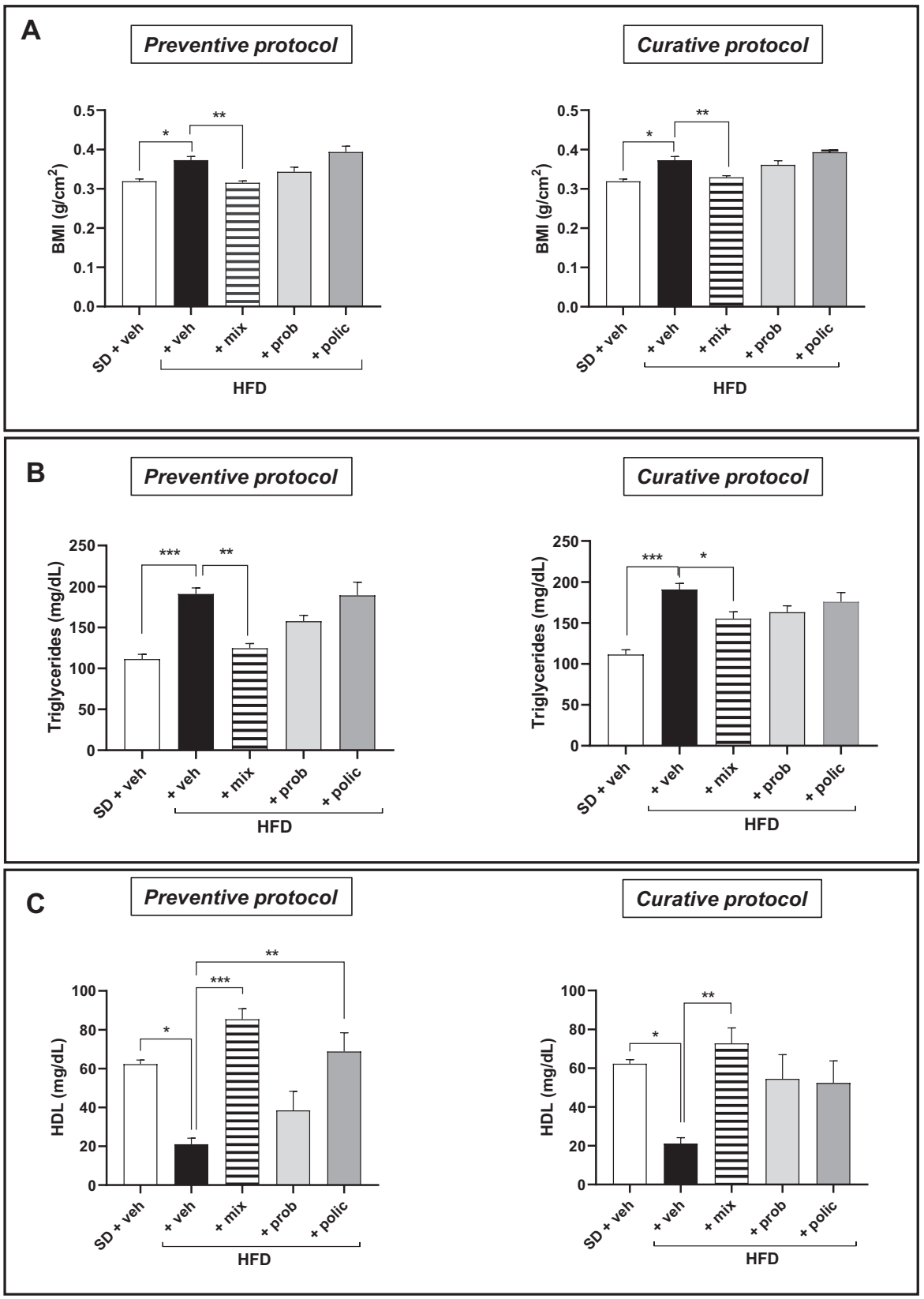

FIGURE 2 | Effects of the mixture and each individual components, in both preventive and curative protocol, on (A) BMI, (B) triglycerides, and (C) HDL in SD and HFD mice. Each column shows the mean ± SEM ($n = 8$/group). One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. BMI, body mass index; HFD, high-fat diet; mix, mixture; polic, policosanols; prob, probiotics; SD, standard diet; veh, vehicle.

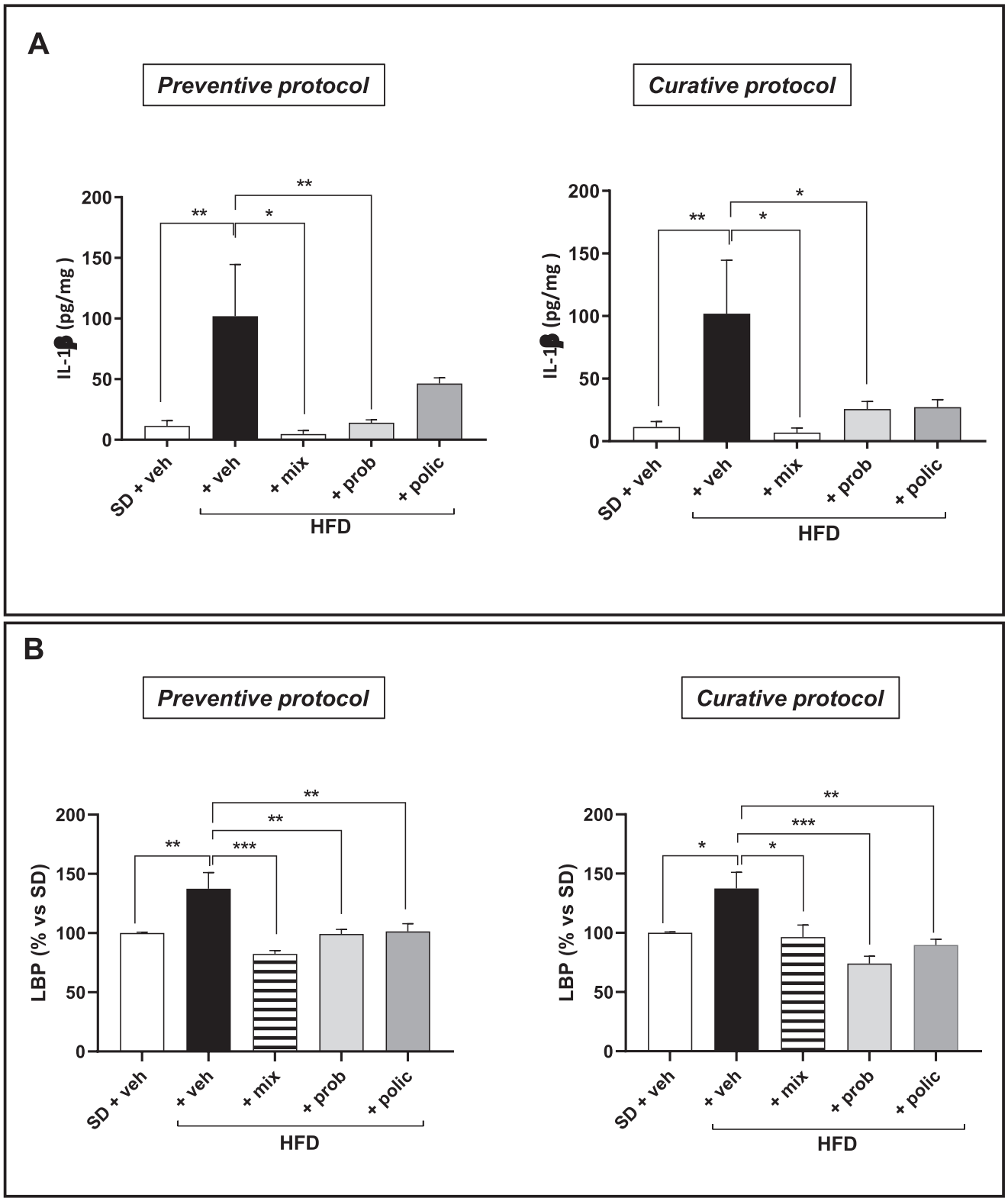

FIGURE 3 | (A) Colonic IL-1β levels and (B) circulating LPB levels in SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Each column shows the mean ± SEM ($n = 5$–8/group). One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. HFD, high-fat diet; IL-β, interleukin1-β; LBP, lipopolysaccharide binding protein; polic, policosanols; mix, mixture; prob, probiotics; SD, standard diet; veh, vehicle.

3.4 | The Synergic Combination of Probiotics and Policosanols Ameliorates the Intestinal Epithelial Barrier Integrity and Permeability in Obese Mice

Obesity is associated with a chronic low-grade systemic inflammation, which seems to contribute to further exacerbate the disruption of IEB integrity associated with a hypercaloric diet (D'Antongiovanni, Segnani, et al. 2023; Ellulu et al. 2017). In line with this view, mice fed with HFD for 8 weeks showed a significant increase in LBP plasma levels, an indirect index of increased gut barrier permeability (Bellini et al. 2022; D'Antongiovanni et al. 2023), along with a reduction in the expression levels of colonic TJ proteins, such as ZO-1 and occludin, as compared with SD animals (Figures 3B, 4A, and 5A). These findings in HFD-fed mice were corroborated by the TJ immunofluorescence staining, which showed a pathological remodeling of ZO-1 and occludin, characterized by a low, irregular and blurry signal (Figures 4B and 5B, Figure S3).

Of interest, dietary supplementation with the mixture, in both preventive and curative protocols, ameliorates the gut barrier integrity and permeability, as documented by a reduction in plasma LBP levels and an increment of TJ molecules (Figures 3B, 4A, and 5A). In particular, obese mice treated with the mixture showed a clear and sharp ZO-1 and occludin staining in specific cellular sites such as the cell boundaries, indicating that the TJ structure in these animals maintains its physiological morphology (Figures 4B and 5B, Figure S3). Of note, the administration of each individual component significantly improves LBP compared to HFD mice with vehicle but only partially improved TJ expression and distribution in HFD mice in both the preventive and curative protocols, with high variability (Figures 3B, 4, and 5, Figure S3).

3.5 | The Synergic Combination of Probiotics and Policosanols Reduced Fecal Butyrate Levels in HFD Mice

Alterations in fecal butyrate concentrations have been reported to contribute to gut barrier impairment in both obese patients and HFD mouse models (Benvenuti et al. 2023; Parada Venegas et al. 2019b; Peng et al. 2023). In line with these findings, mice fed with HFD for 8 weeks exhibited a significant increase in fecal butyrate, compared with SD mice (Figure 6A), indicating reduced butyrate bioavailability to colonocytes in these obese mice. Of interest, the supplementation with the mixture, in both preventive and curative protocols, significantly reduced the fecal butyrate concentration in HFD mice (Figure 6A). No effects were observed for probiotics and policosanols alone on fecal butyrate levels in HFD mice, in both preventive and curative protocols (Figure 6A). Taken together, these results highlight a synergistic combination of probiotics and policosanols in improving the butyrate bioavailability in the colon of obese mice.

3.6 | The Beneficial Effects of the Mixture Are Likely Related to Its Ability to Modulate Butyrate–Glial–IL1β Axis in Obese Mice

In order to investigate the molecular mechanisms underlying the beneficial effects of the mixture, we investigate the expression levels of the apical butyrate transporter SMCT1, reactive gliotic processes, and GFAP$^+$/IL-1β^+ glial cells in colonic tissues from SD and HFD mice, treated with drug vehicle or the mixture, in both preventive and curative protocols.

After 8 weeks, mice fed with HFD showed a significant reduction in the colonic expression of the apical transporter, SMCT1, compared to SD mice (Figure 6B), indicating impaired absorption and utilization of butyrate by enterocytes. Of interest, dietary supplementation with the mixture restored the colonic expression of SMCT1 transporter, in both preventive and curative protocol (Figure 6B), suggesting an improvement in colonic butyrate bioavailability.

Next, we investigated the presence of enteric gliosis on the basis of the contribution of reactive EGCs in IEB impairment and immune/inflammatory processes, through the release of pro-inflammatory mediators, including IL-1β (D'Antongiovanni et al. 2023; Seguella and Gulbransen 2021). In this context, butyrate exerts a protective effect by inhibiting enteric glial reactivity (Feng et al. 2025). Consistently, HFD mice showed an increase in the colonic expression of the glial marker, GFAP, along with an increase in GFAP$^+$ cells mainly within myenteric plexus, compared with SD mice (Figure 7A,B), suggesting the presence of reactive gliotic processes. In particular, double immunofluorescence experiments revealed a high amount of IL-1β in GFAP$^+$ glial cells of enteric plexi (Figure 7C), suggesting an overproduction of this pro-inflammatory cytokine in reactive EGCs. Of interest, dietary supplementation with the mixture, in both preventive and curative protocols, significantly reduced the expression and the density of GFAP$^+$ glial cells as well as decreased the IL-1β^+ glial cells in HFD mice (Figure 7), suggesting that the mixture is able to counteract enteric gliosis in obese mice and, by reflex, the release of IL-1β from their reactive EGCs (Figure 3A).

4 | Discussion

Morphological and functional alterations of the IEB represent early events in obesity, occurring before body weight gain, metabolic alterations, and systemic inflammation (D'Antongiovanni et al. 2025; D'Antongiovanni, Segnani, et al. 2023; Nascimento et al. 2020). Accordingly, the modulation of the IEB, through the manipulation of gut microbiota with probiotics or dietary supplementation with natural extracts, including policosanols, has emerged as a promising strategy for the treatment of obesity and related comorbidities (Chandrasekaran et al. 2024; Deng et al. 2022; DiMattia et al. 2024; Han et al. 2019; Ho et al. 2024; Kou et al. 2023; Weng et al. 2021). However, only few studies have examined the beneficial effects of the combined use of probiotics and policosanols in this pathological context. For instance, in a recent in vitro study on intestinal (CaCo-2), hepatic (HepG2), and adipose (3T3-L1) cells, the authors reported a beneficial effect of a probiotics-polycosanols combination by improving IEB integrity, reducing inflammation and promoting the browning process (Mulè et al. 2025). In support of this view, a pilot clinical study reported positive epigenetic changes in miRNAs involved in the regulation of inflammation and adipogenesis in obese patients treated with probiotics plus octacosanol for 12 weeks, although no significant improvements in anthropometric or lipid parameters were recorded (Okuka et al. 2023). Despite this interesting evidence,

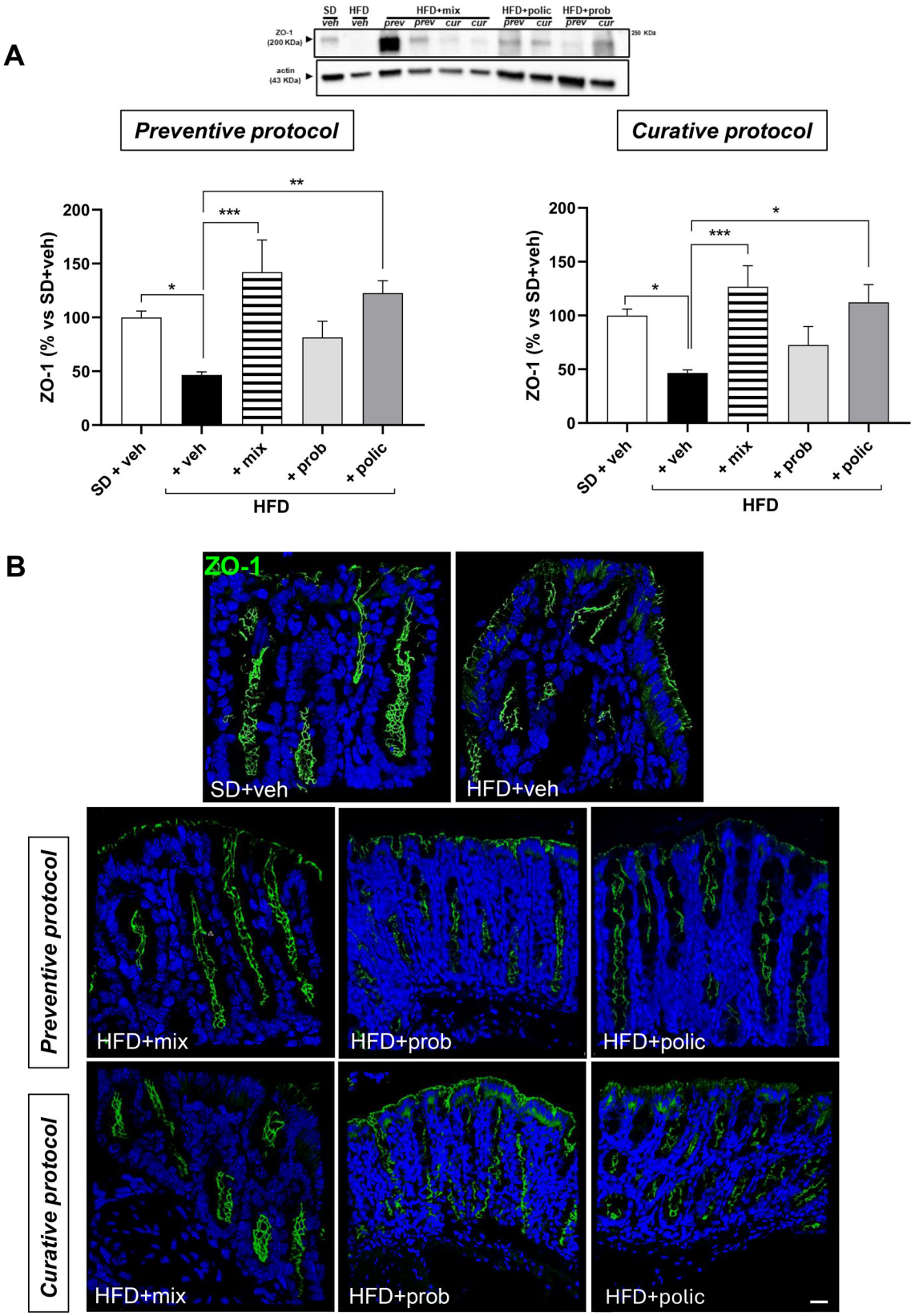

FIGURE 4 | (A) Densitometric analysis and related representative blot of ZO-1 expression in colonic tissues from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Each column shows the mean ± SEM ($n = 5–8$/group). One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. (B) Representative photomicrographs of ZO-1 (green) immunostaining in the cross-sectioned colon from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Original magnification 40×. Scale bar 20 μm. HFD, high-fat diet; mix, mixture; polic, policosanols; prob, probiotics; SD, standard diet; veh, vehicle; ZO-1, zonulin-1.

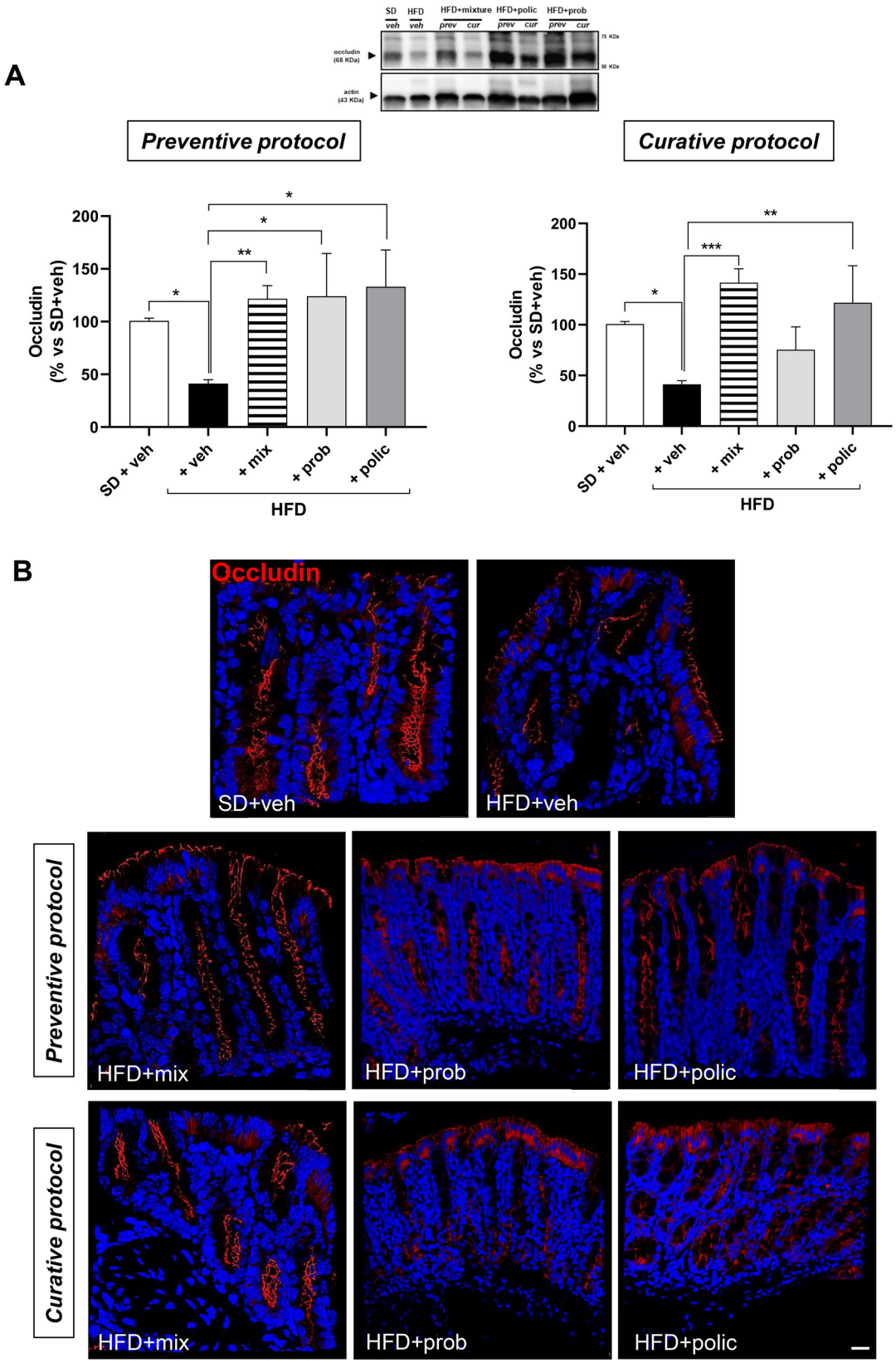

FIGURE 5 | (A) Densitometric analysis and related representative blot of occludin expression in colonic tissues from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Each column shows the mean ± SEM ($n = 5$–8/group). One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. (B) Representative photomicrographs of occludin (red) immunostaining in the cross-sectioned colon from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Original magnification 40×. Scale bar 20 μm. HFD, high-fat diet; mix, mixture; polic, policosanols; prob, probiotics; SD, standard diet; veh, vehicle.

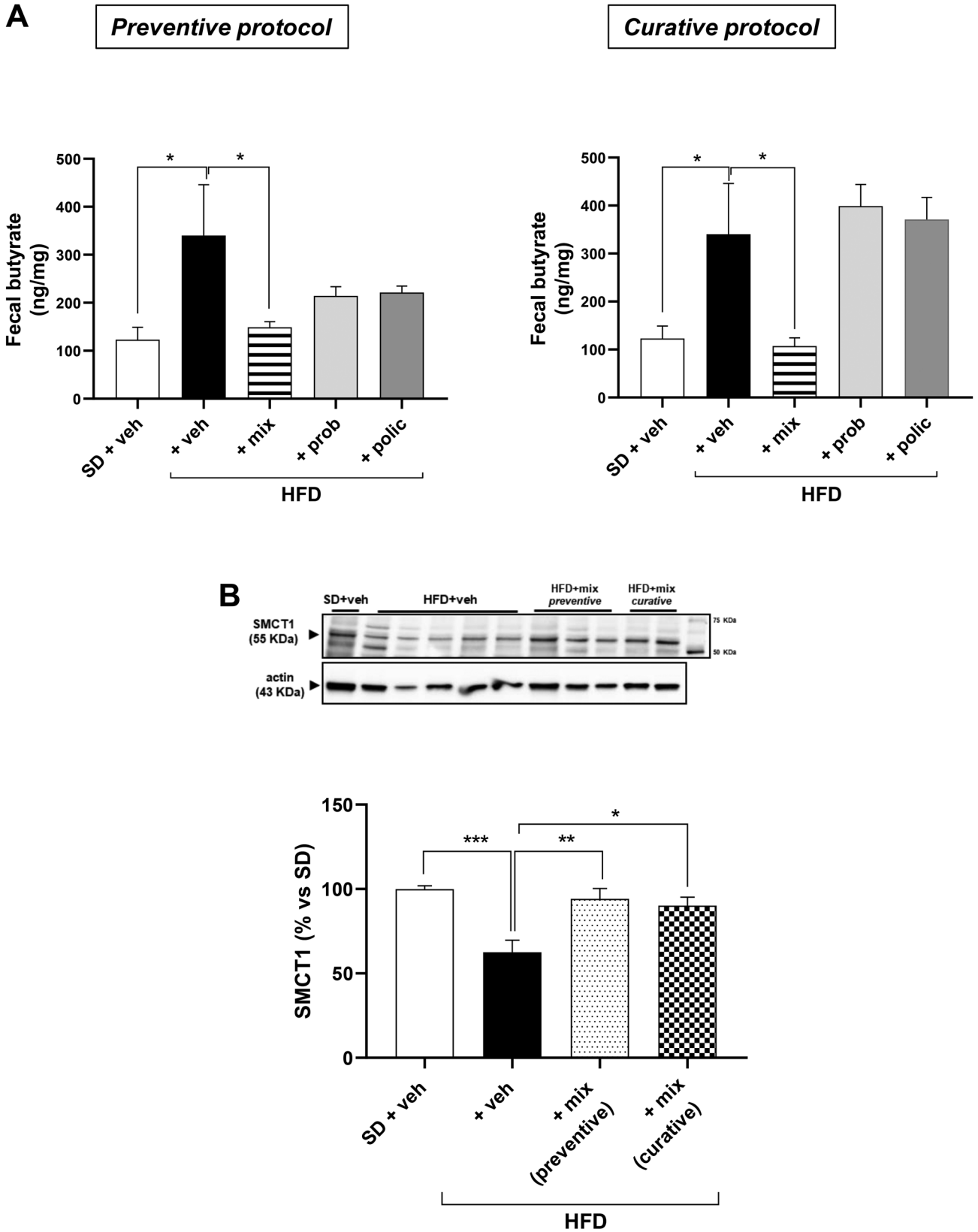

FIGURE 6 | (A) Fecal butyrate content in SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol (n = 5–8/group). (B) Densitometric analysis and related representative blot of the apical butyrate transporter, SMCT1, in colonic tissues from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol (n = 6–8/group). Each column shows the mean ± SEM. One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. HFD, high-fat diet; mix, mixture; SD, standard diet; SMCT1, sodium-coupled monocarboxylate transporter 1; veh, vehicle.

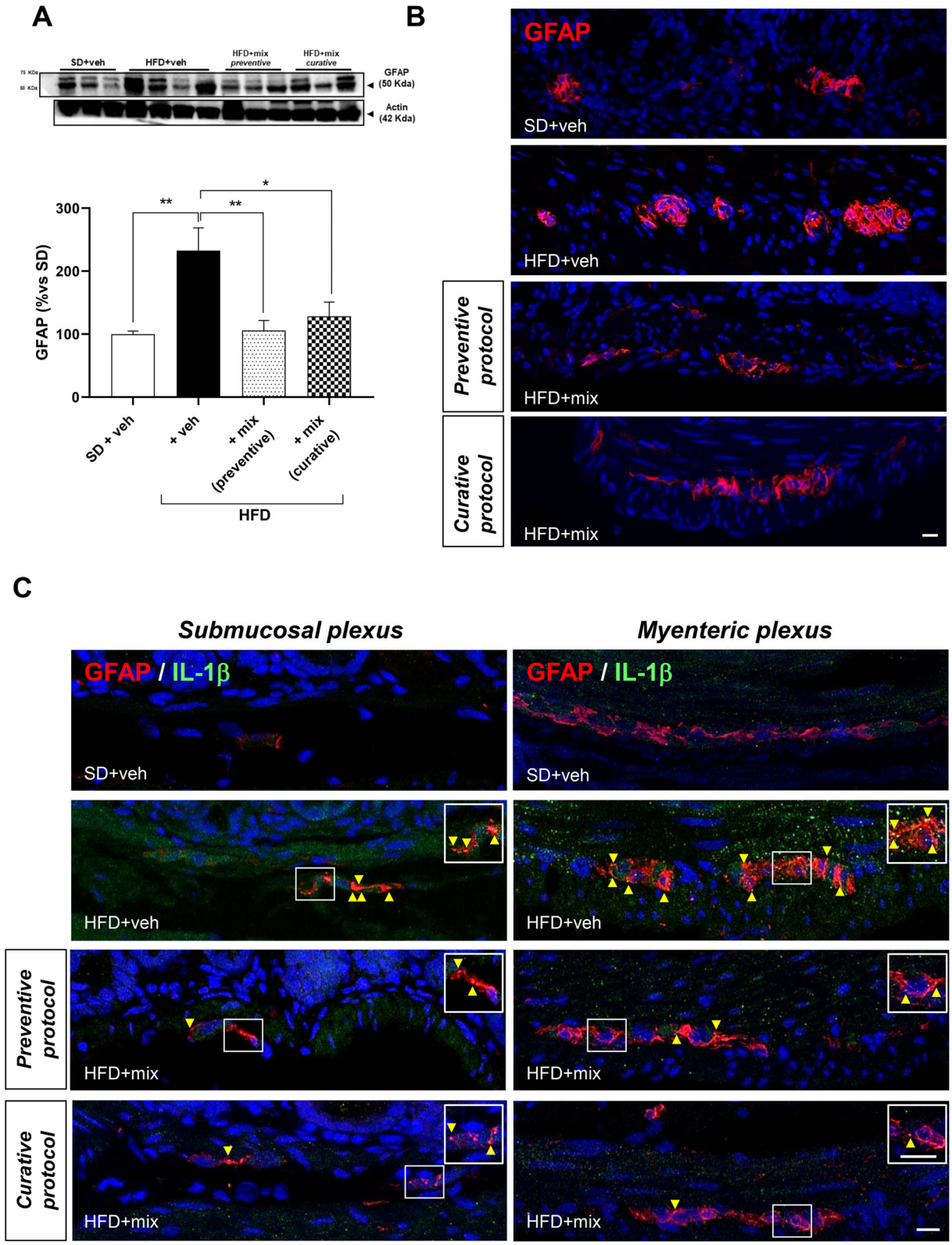

FIGURE 7 | (A) Densitometric analysis and related representative blot of GFAP in colonic tissues from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Each column shows the mean ± SEM (n = 6–7/group). One-way ANOVA, followed by Tukey post hoc test: results are as follows: *$p \leq 0.05$; **$p \leq 0.01$. (B) Representative confocal microscopy images of GFAP immunostaining of myenteric plexi from full-thickness, cross-sectioned colon obtained from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Scale bar: 10 μm. (C) Representative images of enteric plexi by double immunofluorescence confocal microscopy of IL-1β (green)-GFAP (red) in the cross-sectioned colon from SD and HFD mice treated with the mixture and each individual components, in both preventive and curative protocol. Original magnification 40×. Higher magnifications of selected areas are shown in the upper right corner. Scale bar 10 μm. HFD, high-fat diet; mix, mixture; SD, standard diet; veh, vehicle.

several aspects remain to be clarified, thus highlighting a strong need for further investigations. On these bases, the present study was designed to investigate the efficacy of a novel and innovative food supplement, containing a mixture of probiotics and policosanols, in counteracting and preventing the body weight gain, IEB dysfunction, and enteric inflammation in a mouse model of diet-induced obesity. In particular, the present research pointed out four major novel findings. The synergic combination of probiotics and policosanols (1) prevents and counteracts the increase in body weight and BMI as well as normalizes metabolic parameters, (2) exerts strengthening effects on the IEB integrity, (3) reduces enteric inflammation, and (4) exerts positive effects that are likely attributable to the ability of the mixture to modulate the butyrate–glial–IL1β axis in obese mice.

In line with previous studies (Antonioli et al. 2020; Benvenuti et al. 2023; D'Antongiovanni, Benvenuti, et al. 2020; D'Antongiovanni et al. 2023), mice fed with a hypercaloric diet for 8 weeks displayed a significant increment in body weight and BMI along with significant alterations of systemic metabolic parameters, such as triglycerides and HDL, thus confirming the suitability of the HFD-induced obesity model. Interestingly, dietary supplementation with the mixture, in both preventive and curative protocols, significantly counteracted the increase in body weight and BMI as well as normalized metabolic parameters in HFD mice. This is an interesting point, since the beneficial effects exerted by the mixture were also observed after the development of obesity, in contrast to most studies reported in the literature showing anti-obesity properties only after preventive treatment with probiotics or policosanols (Deng et al. 2022; Han et al. 2019; Ho et al. 2024; Kou et al. 2023; Sharma et al. 2019; Zhai et al. 2021). In addition, only minor or no effects were recorded after administration with each individual component in obese mice, highlighting a potential synergistic benefit deriving from the therapeutic combination of probiotics and policosanols.

Next, we investigated the presence of enteric inflammation, a condition commonly associated with hypercaloric diet-induced obesity (D'Antongiovanni et al. 2025; D'Antongiovanni, Pellegrini, et al. 2020; Khan et al. 2021; Rohm et al. 2021). Consistently, HFD mice showed a significant increase in colonic IL-1β levels, thus confirming gut inflammation in this experimental model. Food supplementation with the mixture or probiotics alone significantly counteracted intestinal inflammation. Of note, policosanol treatment also exerts anti-inflammatory properties by reducing, although not significantly, colonic IL-1β levels in HFD mice. These results corroborate previous in vitro anti-inflammatory properties of the mixture, as well as the anti-inflammatory properties of probiotics, including *Bifidobacterium* genera, and policosanols, such as octacosanol and hexacosanol, in counteracting inflammatory responses in several in vivo pathological contexts (Benvenuti et al. 2023; Cho et al. 2023; Mulè et al. 2025; N. Wang, Pei, et al. 2025; Yun et al. 2024). In particular, beneficial effects of probiotics are partly attributed to the release of metabolites, including SCFAs (acetate, propionate, and butyrate), which are well known to shape the differentiation of Treg cells (Furusawa et al. 2013; Hu et al. 2022; Mukhopadhya and Louis 2025), whereas policosanols exert their effects through the inhibition of pro-inflammatory MAPK signaling pathways and reduction in reactive oxygen species (Behl et al. 2021).

Since the presence of a chronic low-grade inflammatory condition in the gut contributes to further exacerbate the disruption of IEB associated with a hypercaloric diet, thus creating a vicious cycle that paves the way for systemic dissemination (Bona et al. 2021; Ding et al. 2010; Ellulu et al. 2017; Raybould 2012; Sanz and Moya-Pérez 2014; Schroeder et al. 2020), we assessed the IEB integrity and permeability. In line with this view, HFD mice displayed an increase in circulating LBP levels (an indirect index of increased gut permeability; Pellegrini et al. (2023)) along with a reduction in ZO-1 and occludin expression as well as an altered subcellular distribution of TJs, thus indicating an altered integrity and permeability of the gut barrier. Of interest, the administration of the dietary supplement, both in preventive and curative protocol, significantly reinforced the IEB structure and integrity, normalizing the LBP levels and ZO-1 and occludin expression as well as favoring the correct distribution of TJ on cell membrane of enterocytes. Differently, the administration of each individual component normalized LBP but only partially improved TJ expression and distribution in HFD mice. These results highlight a strengthening effect of synergistic combination of probiotics and policosanols, in accordance with the previous in vitro study (Mulè et al. 2025), that may be ascribed to the ability of probiotics to improve the butyrate bioavailability, which is strategic in the correct assembling of TJs on enterocyte cell membranes (Parada Venegas et al. 2019a), as well as to policosanols able to promote the release of SCFAs, especially butyrate, from the gut microbiota, thus favoring a correct gut barrier integrity and permeability (Miao et al. 2022). To confirm this view, fecal butyrate levels and its apical SMCT1 transporter were investigated. Our results displayed a significant increase in fecal butyrate concentration along with a reduction in the expression of the apical butyrate transporter SMCT1 in colonic tissues of HFD mice, indicating a reduced transport and utilization of butyrate by the enteric mucosa. Of note, these results may explain the altered TJ distribution observed in obese mice and are consistent with human studies showing fecal butyrate accumulation along with an increase in circulating zonulin, an index of increased permeability, in obese patients (Kim et al. 2019; Moreno-Navarrete et al. 2012). Interestingly, food supplementation with the mixture, both in preventive and curative protocol, significantly reduced fecal butyrate concentration as well as increase SMCT1 expression in HFD mice, indicating an increase in the butyrate bioavailability in the colon, which may be involved in the improvement of gut barrier integrity and permeability. Of note, no significant effects on fecal butyrate concentration were recorded after administration with each individual component in HFD mice; accordingly, the evaluation of SMCT1 expression was not performed in these experimental groups.

Taken together, our results showed synergistic benefits of a therapeutic combination of probiotics and policosanols in preventing and counteracting body weight gain, intestinal inflammation, and gut barrier impairment associated with hypercaloric diet intake. On this basis, the last part of the present study was designed to investigate one of potential mechanical mechanisms underlying these beneficial effects of the mixture.

As a first step, we investigated the presence of enteric reactive gliosis, a pathological process in which EGCs acquire pro-inflammatory phenotypes with consequent release of inflammatory mediators, such as IL-1β, that contribute to gut barrier

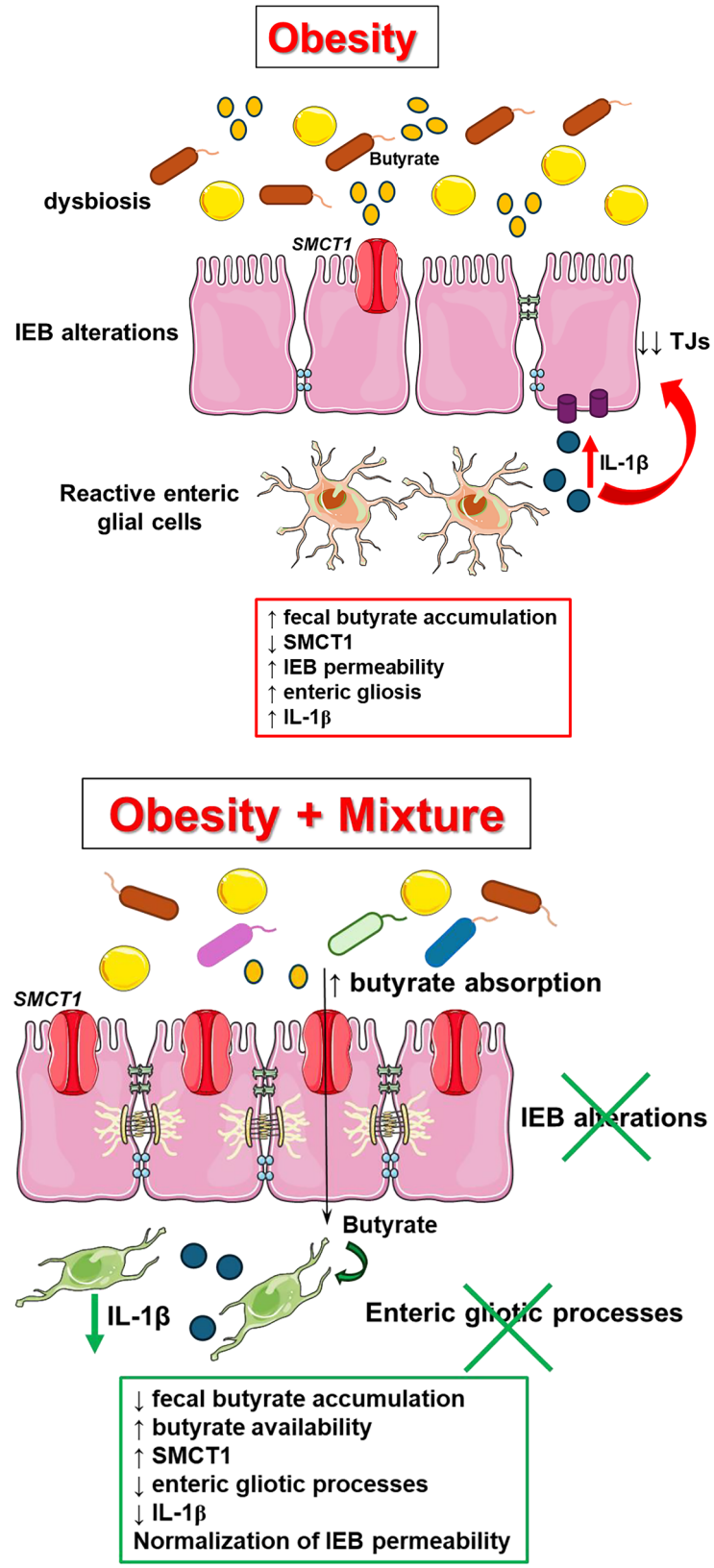

FIGURE 8 | Hypothesis on the intervention mechanism of the mixture in obese mice. Top panel: HFD mice show an altered butyrate bioavailability due to a reduced SMCT1 expression, increased IEB permeability, and enteric inflammation with high amount of IL-1β in GFAP^{+} glial cells. Bottom panel: the mixture exerts anti-obesity properties through the modulation of the butyrate–glial–IL1β axis. In particular, the mixture favors a correct absorption and utilization of butyrate by the enteric mucosa. This increased bioavailability of butyrate may inhibit enteric gliotic processes with consequent reduction of IL-1β release from EGCs, thus leading to decreased enteric inflammation and improved IEB integrity and permeability.

impairment and enteric inflammatory responses in obesity conditions (Antonioli et al. 2020; D'Antongiovanni et al. 2025; D'Antongiovanni et al. 2023; Seguella and Gulbransen 2021). In particular, we focused the attention on the glial marker GFAP, whose expression is modulated by cell differentiation, inflammation, and tissue injury (D'Antongiovanni et al. 2023). Accordingly, GFAP levels correlate with the functional state of EGCs and represent a useful marker for tracking the morphological changes associated with enteric gliosis. By contrast, other glial markers, such as Sox10 and S100β, are more suitable for glial cell quantification but do not provide information on glial morphology (Boesmans et al. 2021; Grubišić and Gulbransen 2017). In this context, it has been reported that butyrate exerts an inhibitory effect on reactive GFAP$^+$-EGCs, resulting in a reduction in intestinal inflammation and consequent improvement of IEB integrity in a murine model of obesity (Feng et al. 2025). In accordance with these findings, we observed a significant increase in the colonic expression of glial marker, GFAP, along with an increase in colonic GFAP$^+$ glial cells in obese mice, thus indicating that HFD mice, characterized by reduced butyrate bioavailability, IEB alterations, and gut inflammation, display enteric gliotic processes. Interestingly, dietary supplementation with the mixture, both in preventive and curative protocol, counteracted enteric gliosis, as documented by the reduction in colonic GFAP$^+$ glial cells and its expression. Of note, this is an interesting point since these data suggest that the mixture is able to inhibit the reactive EGCs, likely through the increase in butyrate bioavailability, in accordance with previous data (Feng et al. 2025). To corroborate this evidence, we performed a double-staining immunofluorescence analysis to investigate IL-1β specks, reflecting a reactive status of the glial cells, at level of enteric GFAP$^+$ glial cells. In particular, we focused our attention on the cytokine IL-1β, abundantly involved in the onset and amplification of the inflammatory response as well as in the development of IEB dysfunctions (R. Al-Sadi et al. 2013; R. M. Al-Sadi and Ma 2007; D'Antongiovanni et al. 2025). Our results showed that in HFD mice reactive glial cells displayed an upregulation of IL-1β levels, thus confirming a pro-inflammatory phenotypic shift of EGCs in this pathological condition. Of note, an appreciable number of IL-1β specks were also observed throughout the colonic layers of HFD mice, in accordance with molecular results demonstrating the presence of colonic inflammation. Of interest, in HFD mice, food supplementation with the mixture, both in preventive and curative protocol, reduced IL-1β specks in EGCs and surrounding colonic tissues, suggesting the ability of the mixture to inhibit IL-1β release by reactive EGCs. It is worthy to note that this finding represents the first evidence on the potentiality of the synergistic combination of probiotics and policosanols to exert their anti-inflammatory properties on gut barrier through the modulation of GFAP$^+$ glial cells/IL-1β axis in the context of obesity. Undoubtedly, other processes and cellular mechanisms may be involved in the regulatory activity of the mixture on to IEB dysfunctions, which deserve to be investigated in the future as a whole.

5 | Conclusion

Our results suggest that mixture, containing probiotics (*B. infantis* GM-21, *B. bifidum* GM-25, *L. rhamnosus* GM-28) and polycosanols, exerts anti-obesity properties, improving the IEB integrity and permeability as well as blunting the gut inflammation, likely due, at least in part, to its ability to modulate the butyrate–glial–IL1β axis. Indeed, it is conceivable that the combination of probiotics and policosanols favors a correct absorption and utilization of butyrate by the enteric mucosa. This increased bioavailability of butyrate, besides exerting a trophic effect on enterocytes, may inhibit enteric gliotic processes with consequent reduction of IL-1β release from EGCs, thus leading to decreased enteric inflammation and improved IEB integrity and permeability (Figure 8). Of note, this represents an innovative aspect since at present no studies have investigated the effects of these components on enteric glia. In addition, another novel point of the present paper is that the beneficial effects exerted by the mixture administration were also observed in curative protocol, allowing us to hypothesize that its administration in overweight or obese patients could be effective. Accordingly, based on these promising experimental results, a clinical study on overweight/obese patients will begin in 2026.

Author Contributions

Vanessa D'Antongiovanni: writing – original draft, methodology, investigation, conceptualization. **Clarissa Pierucci, Gianfranca Carta** and **Sebastiano Banni**: methodology, data curation and formal analysis. **Clelia Di Salvo** and **Laura Benvenuti**: methodology. **Cristina Segnani**, **Chiara Ippolito** and **Sara Angelini**: acquisition and analysis. **Carolina Pellegrini**: methodology, supervision. **Luca Antonioli**: screening the search results, funding. **Nunzia Bernardini**: writing – review and editing, project administration, funding.

Acknowledgments

The authors thank Nóos S.r.l. for donating the probiotics and policosanols used to evaluate their effectiveness in this study

Open access publishing facilitated by Universita degli Studi di Pisa, as part of the Wiley - CRUI-CARE agreement.

Funding

The authors have nothing to report.

Ethics Statement

The animal study was reviewed and approved by the University of Pisa's Ethical Committee for Animal Experimentation and the Italian Ministry of Health (Authorization 48/2024-PR).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

References

Al-Sadi, R., S. Guo, D. Ye, et al. 2013. "Mechanism of IL-1β Modulation of Intestinal Epithelial Barrier Involves p38 Kinase and Activating Transcription Factor-2 Activation." *The Journal of Immunology* 190, no. 12: 6596–6606. https://doi.org/10.4049/jimmunol.1201876.

Al-Sadi, R. M., and T. Y. Ma. 2007. "IL-1β Causes an Increase in Intestinal Epithelial Tight Junction Permeability." *The Journal of Immunology* 178, no. 7: 4641–4649. https://doi.org/10.4049/jimmunol.178.7.4641.

Alshuweishi, Y., A. A. Almufarrih, A. Abudawood, et al. 2024. "Patterns of Lipid Abnormalities in Obesity: A Comparative Analysis in Normoglycemic and Prediabetic Obese Individuals." *Journal of Personalized Medicine* 14, no. 9: 980. https://doi.org/10.3390/jpm14090980.

Antonioli, L., V. D'Antongiovanni, C. Pellegrini, et al. 2020. "Colonic Dysmotility Associated With High-Fat Diet-Induced Obesity: Role of Enteric Glia." *The FASEB Journal* 34, no. 4: 5512–5524. https://doi.org/10.1096/fj.201901844R.

Behl, T., T. Upadhyay, S. Singh, et al. 2021. "Polyphenols Targeting MAPK Mediated Oxidative Stress and Inflammation in Rheumatoid Arthritis." *Molecules* 26, no. 21: 6570. https://doi.org/10.3390/molecules26216570.

Bellini, G., L. Benvenuti, C. Ippolito, et al. 2022. "Intestinal Histomorphological and Molecular Alterations in Patients With Parkinson's Disease." *European Journal of Neurology* 30: 3440–3450. https://doi.org/10.1111/ENE.15607.

Benvenuti, L., V. D'Antongiovanni, C. Pellegrini, et al. 2023. "Dietary Supplementation With the Probiotic SF68 Reinforces Intestinal Epithelial Barrier in Obese Mice by Improving Butyrate Bioavailability." *Molecular Nutrition & Food Research* 67, no. 13: e2200442. https://doi.org/10.1002/mnfr.202200442.

Boesmans, W., A. Nash, K. R. Tasnády, W. Yang, L. A. Stamp, and M. M. Hao. 2021. "Development, Diversity, and Neurogenic Capacity of Enteric Glia." *Frontiers in Cell and Developmental Biology* 9: 775102. https://doi.org/10.3389/fcell.2021.775102.

Bona, M., C. Torres, S. Lima, A. Lima, and B. Maciel. 2021. "Intestinal Barrier Function in Obesity With or Without Metabolic Syndrome: A Systematic Review Protocol." *BMJ Open* 11, no. 5: e043959. https://doi.org/10.1136/BMJOPEN-2020-043959.

Calligaris, S. D., M. Lecanda, F. Solis, et al. 2013. "Mice Long-Term High-Fat Diet Feeding Recapitulates Human Cardiovascular Alterations: An Animal Model to Study the Early Phases of Diabetic Cardiomyopathy." *PLoS ONE* 8, no. 4: e60931. https://doi.org/10.1371/journal.pone.0060931.

Chandrasekaran, P., S. Weiskirchen, and R. Weiskirchen. 2024. "Effects of Probiotics on Gut Microbiota: An Overview." *International Journal of Molecular Sciences* 25, no. 11: 6022. https://doi.org/10.3390/ijms25116022.

Cho, K.-H., J.-E. Kim, H.-S. Nam, D.-J. Kang, and S.-H. Baek. 2023. "Comparison of Policosanols via Incorporation Into Reconstituted High-Density Lipoproteins: Cuban Policosanol (Raydel®) Exerts the Highest Antioxidant, Anti-Glycation, and Anti-Inflammatory Activity." *Molecules* 28, no. 18: 6715. https://doi.org/10.3390/molecules28186715.

Csóka, B., G. Töro, J. Vindeirinho, et al. 2017. "A2A Adenosine Receptors Control Pancreatic Dysfunction in High-Fat-Diet-Induced Obesity." *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology* 31, no. 11: 4985–4997. https://doi.org/10.1096/FJ.201700398R.

D'Antongiovanni, V., L. Benvenuti, M. Fornai, et al. 2020. "Glial A2B Adenosine Receptors Modulate Abnormal Tachykininergic Responses and Prevent Enteric Inflammation Associated With High Fat Diet-Induced Obesity." *Cells* 9, no. 5: 1245. https://doi.org/10.3390/cells9051245.

D'Antongiovanni, V., L. Antonioli, L. Benvenuti, et al. 2023. "Use of *Saccharomyces boulardii* CNCM I-745 as Therapeutic Strategy for Prevention of Nonsteroidal Anti-Inflammatory Drug-Induced Intestinal Injury." *British Journal of Pharmacology* 180, no. 24: 3215–3233. https://doi.org/10.1111/bph.16200.

D'Antongiovanni, V., M. Fornai, L. Benvenuti, et al. 2023. "Dietary Supplement, Containing the Dry Extract of Curcumin, Emblica and Cassia, Counteracts Intestinal Inflammation and Enteric Dysmotility Associated With Obesity." *Metabolites* 13, no. 3: 410. https://doi.org/10.3390/metabo13030410.

D'Antongiovanni, V., M. Fornai, R. Colucci, et al. 2024. "Enteric Glial NLRP3 Inflammasome Contributes to Gut Mucosal Barrier Alterations in a Mouse Model of Diet-Induced Obesity." *Acta Physiologica* 241, no. 1: e14232. https://doi.org/10.1111/apha.14232.

D'Antongiovanni, V., M. Fornai, R. Colucci, et al. 2025. "Enteric Glial NLRP3 Inflammasome Contributes to Gut Mucosal Barrier Alterations in a Mouse Model of Diet-Induced Obesity." *Acta Physiologica* 241, no. 1: e14232. https://doi.org/10.1111/apha.14232.

D'Antongiovanni, V., M. Fornai, C. Pellegrini, C. Blandizzi, and L. Antonioli. 2021. "Managing Obesity and Related Comorbidities: A Potential Pharmacological Target in the Adenosine System?" *Frontiers in Pharmacology* 11: 621955. https://doi.org/10.3389/FPHAR.2020.621955.

D'Antongiovanni, V., C. Pellegrini, L. Antonioli, et al. 2023. "Enteric Glia and Brain Astroglia: Complex Communication in Health and Disease Along the Gut-Brain Axis." *The Neuroscientist* 30, no. 4: 493–510. https://doi.org/10.1177/10738584231163460.

D'Antongiovanni, V., C. Pellegrini, M. Fornai, et al. 2020. "Intestinal Epithelial Barrier and Neuromuscular Compartment in Health and Disease." *World Journal of Gastroenterology* 26, no. 14: 1564–1597. https://doi.org/10.3748/wjg.v26.i14.1564.

D'Antongiovanni, V., C. Pierucci, L. Benvenuti, et al. 2025. "Prolonged Treatment With Probiotics Mitigates Intestinal Dysfunction and Enteric Alzheimer's-Related Pathology in Senescence-Accelerated Mice." *Journal of Functional Foods* 133, no. September: 107016. https://doi.org/10.1016/j.jff.2025.107016.

D'Antongiovanni, V., C. Segnani, C. Ippolito, et al. 2023. "Pathological Remodeling of the Gut Barrier as a Prodromal Event of High-Fat Diet-Induced Obesity." *Laboratory Investigation* 103, no. 10: 100194. https://doi.org/10.1016/j.labinv.2023.100194.

Deng, Z., J. Dai, Y. Wei, et al. 2022. "Comparison Between *Lactobacillus rhamnosus* GG and LuxS-Deficient Strain in Regulating Gut Barrier Function and Inflammation in Early-Weaned Piglets." *Frontiers in Immunology* 13: 1080789. https://doi.org/10.3389/fimmu.2022.1080789.

Di Salvo, C., V. D'Antongiovanni, L. Benvenuti, et al. 2024. "*Lactiplantibacillus plantarum* HEAL9 Attenuates Cognitive Impairment and Progression of Alzheimer's Disease and Related Bowel Symptoms in SAMP8 Mice by Modulating Microbiota-Gut-Inflammasome-Brain Axis." *Food & Function* 15, no. 20: 10323–10338. https://doi.org/10.1039/d4fo02075h.

DiMattia, Z., J. J. Damani, E. Van Syoc, and C. J. Rogers. 2024. "Effect of Probiotic Supplementation on Intestinal Permeability in Overweight and Obesity: A Systematic Review of Randomized Controlled Trials and Animal Studies." *Advances in Nutrition* 15, no. 1: 100162. https://doi.org/10.1016/j.advnut.2023.100162.

Ding, S., M. Chi, B. Scull, et al. 2010. "High-Fat Diet: Bacteria Interactions Promote Intestinal Inflammation Which Precedes and Correlates With Obesity and Insulin Resistance in Mouse." *PLoS ONE* 5, no. 8: e12191. https://doi.org/10.1371/JOURNAL.PONE.0012191.

Ellulu, M. S., I. Patimah, H. Khaza'ai, A. Rahmat, and Y. Abed. 2017. "Obesity and Inflammation: The Linking Mechanism and the Complications." *Archives of Medical Science* 13, no. 4: 851–863. https://doi.org/10.5114/aoms.2016.58928.

Feng, A., S. Su, Q. Li, C. Li, Y. Liu, and J. Qiu. 2025. "1,25-Dihydroxyvitamin D3 Regulates Enteroglial Bioactivity Through Butyric Acid Pathway in a High-Fat Diet Mouse Model." *The Journal of Steroid Biochemistry and Molecular Biology* 247: 106655. https://doi.org/10.1016/j.jsbmb.2024.106655.

Furusawa, Y., Y. Obata, S. Fukuda, et al. 2013. "Commensal Microbe-Derived Butyrate Induces the Differentiation of Colonic Regulatory T Cells." *Nature* 504, no. 7480: 446–450. https://doi.org/10.1038/nature12721.

Gkrinia, E. M. M., and A. Belančić. 2025. "The Mechanisms of Chronic Inflammation in Obesity and Potential Therapeutic Strategies: A Narrative Review." *Current Issues in Molecular Biology* 47, no. 5: 357. https://doi.org/10.3390/cimb47050357.

GBD 2021 Adolescent BMI Collaborators. 2025. "Global, Regional, and National Prevalence of Child and Adolescent Overweight and Obesity, 1990–2021, With Forecasts to 2050: A Forecasting Study for the Global

Burden of Disease Study 2021." *The Lancet* 405, no. 10481: 785–812. https://doi.org/10.1016/S0140-6736(25)00397-6.

Grubišić, V., and B. Gulbransen. 2017. "Enteric Glia: The Most Alimentary of all Glia." *The Journal of Physiology* 595, no. 2: 557–570. https://doi.org/10.1113/JP271021.

Han, X., A. Lee, S. Huang, J. Gao, J. R. Spence, and C. Owyang. 2019. "*Lactobacillus rhamnosus* GG Prevents Epithelial Barrier Dysfunction Induced by Interferon-Gamma and Fecal Supernatants From Irritable Bowel Syndrome Patients in Human Intestinal Enteroids and Colonoids." *Gut Microbes* 10, no. 1: 59–76. https://doi.org/10.1080/19490976.2018.1479625.

Ho, P.-Y., Y.-C. Chou, Y.-C. Koh, et al. 2024. "*Lactobacillus rhamnosus* 069 and *Lactobacillus brevis* 031: Unraveling Strain-Specific Pathways for Modulating Lipid Metabolism and Attenuating High-Fat-Diet-Induced Obesity in Mice." *ACS Omega* 9, no. 26: 28520–28533. https://doi.org/10.1021/acsomega.4c02514.

Hu, M., B. Alashkar Alhamwe, B. Santner-Nanan, et al. 2022. "Short-Chain Fatty Acids Augment Differentiation and Function of Human Induced Regulatory T Cells." *International Journal of Molecular Sciences* 23, no. 10: 5740. https://doi.org/10.3390/ijms23105740.

Jarolimova, J., J. Tagoni, and T. A. Stern. 2013. "Obesity: Its Epidemiology, Comorbidities, and Management." *Primary Care Companion to the Journal of Clinical Psychiatry* 15, no. 5: 27045. https://doi.org/10.4088/PCC.12f01475.

Jürgens, H. S., S. Neschen, S. Ortmann, et al. 2007. "Development of Diabetes in Obese, Insulin-Resistant Mice: Essential Role of Dietary Carbohydrate in Beta Cell Destruction." *Diabetologia* 50, no. 7: 1481–1489. https://doi.org/10.1007/s00125-007-0662-8.

Khan, S., H. Luck, S. Winer, and D. A. Winer. 2021. "Emerging Concepts in Intestinal Immune Control of Obesity-Related Metabolic Disease." *Nature Communications* 12, no. 1: 2598. https://doi.org/10.1038/s41467-021-22727-7.

Kim, K. N., Y. Yao, and S. Y. Ju. 2019. "Short Chain Fatty Acids and Fecal Microbiota Abundance in Humans With Obesity: A Systematic Review and Meta-Analysis." *Nutrients* 11, no. 10: 2512. https://doi.org/10.3390/nu11102512.

Kou, R., J. Wang, A. Li, et al. 2023. "Ameliorating Effects of *Bifidobacterium longum* subsp. *infantis* FB3-14 Against High-Fat-Diet-Induced Obesity and Gut Microbiota Disorder." *Nutrients* 15, no. 19: 4104. https://doi.org/10.3390/nu15194104.

Lee, J. H., Y. Jia, T. T. Thach, et al. 2017. "Hexacosanol Reduces Plasma and Hepatic Cholesterol by Activation of AMP-Activated Protein Kinase and Suppression of Sterol Regulatory Element-Binding Protein-2 in HepG2 and C57BL/6J Mice." *Nutrition Research* 43: 89–99. https://doi.org/10.1016/j.nutres.2017.05.013.

Massier, L., M. Blüher, P. Kovacs, and R. M. Chakaroun. 2021. "Impaired Intestinal Barrier and Tissue Bacteria: Pathomechanisms for Metabolic Diseases." *Frontiers in Endocrinology* 12: 616506. https://doi.org/10.3389/FENDO.2021.616506.

Miao, S.-T., Q.-S. Lu, Y.-J. Zhou, Y.-N. Chang, T. Xu, and M.-Y. Zhu. 2022. "Oral Administration of Octacosanol Modulates the Gut Bacteria and Protects the Intestinal Barrier in Ulcerative Colitis Mice." *Journal of Food Biochemistry* 46, no. 10: e14284. https://doi.org/10.1111/jfbc.14284.

Moreno-Navarrete, J. M., M. Sabater, F. Ortega, W. Ricart, and J. M. Fernández-Real. 2012. "Circulating Zonulin, a Marker of Intestinal Permeability, Is Increased in Association With Obesity-Associated Insulin Resistance." *PLoS ONE* 7, no. 5: e37160. https://doi.org/10.1371/journal.pone.0037160.

Mukhopadhya, I., and P. Louis. 2025. "Gut Microbiota-Derived Short-Chain Fatty Acids and Their Role in Human Health and Disease." *Nature Reviews Microbiology* 23: 635–651. https://doi.org/10.1038/s41579-025-01183-w.

Mulè, S., R. Galla, F. Parini, M. Botta, S. Ferrari, and F. Uberti. 2025. "An in Vitro Gut-Liver-Adipose Axis Model to Evaluate the Anti-Obesity Potential of a Novel Probiotic-Polycosanol Combination." *Foods* 14, no. 11: 2003. https://doi.org/10.3390/foods14112003.

Nascimento, J. C., V. A. Matheus, R. B. Oliveira, S. F. S. Tada, and C. B. Collares-Buzato. 2020. "High-Fat Diet Induces Disruption of the Tight Junction-Mediated Paracellular Barrier in the Proximal Small Intestine before the Onset of Type 2 Diabetes and Endotoxemia." *Digestive Diseases and Sciences* 66: 3359–3374. https://doi.org/10.1007/s10620-020-06664-x.

Okuka, N., V. Schuh, U. Krammer, et al. 2023. "Epigenetic Aspects of a New Probiotic Concept—A Pilot Study." *Life* 13, no. 9: 1912. https://doi.org/10.3390/life13091912.

Parada Venegas, D., M. K. De la Fuente, G. Landskron, et al. 2019a. "Corrigendum: Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases." *Frontiers in Immunology* 10: 1486. https://doi.org/10.3389/fimmu.2019.01486.

Parada Venegas, D., M. K. De la Fuente, G. Landskron, et al. 2019b. "Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases." *Frontiers in Immunology* 10: 277. https://doi.org/10.3389/fimmu.2019.00277.

Pellegrini, C., M. Fornai, L. Benvenuti, et al. 2021. "NLRP3 at the Crossroads Between Immune/Inflammatory Responses and Enteric Neuroplastic Remodelling in a Mouse Model of Diet-Induced Obesity." *British Journal of Pharmacology* 178: 3924–3942. https://doi.org/10.1111/bph.15532.

Pellegrini, C., M. Fornai, V. D'Antongiovanni, L. Antonioli, N. Bernardini, and P. Derkinderen. 2023. "The Intestinal Barrier in Disorders of the Central Nervous System." *The Lancet Gastroenterology & Hepatology* 8, no. 1: 66–80. https://doi.org/10.1016/S2468-1253(22)00241-2.

Peng, K., W. Dong, T. Luo, et al. 2023. "Butyrate and Obesity: Current Research Status and Future Prospect." *Frontiers in Endocrinology* 14: 1098881. https://doi.org/10.3389/fendo.2023.1098881.

Raybould, H. E. 2012. "Gut Microbiota, Epithelial Function and Derangements in Obesity." *The Journal of Physiology* 590, no. 3: 441–446. https://doi.org/10.1113/JPHYSIOL.2011.222133.

Rohm, T. V., R. Fuchs, R. L. Müller, et al. 2021. "Obesity in Humans Is Characterized by Gut Inflammation as Shown by Pro-Inflammatory Intestinal Macrophage Accumulation." *Frontiers in Immunology* 12: 668654. https://doi.org/10.3389/fimmu.2021.668654.

Ruck, L., S. Wiegand, and P. Kühnen. 2023. "Relevance and Consequence of Chronic Inflammation for Obesity Development." *Molecular and Cellular Pediatrics* 10, no. 1: 16. https://doi.org/10.1186/s40348-023-00170-6.

Sanz, Y., and A. Moya-Pérez. 2014. "Microbiota, Inflammation and Obesity." *Advances in Experimental Medicine and Biology* 817: 291–317. https://doi.org/10.1007/978-1-4939-0897-4_14.

Schroeder, B. O., G. M. H. Birchenough, M. Pradhan, et al. 2020. "Obesity-Associated Microbiota Contributes to Mucus Layer Defects in Genetically Obese Mice." *Journal of Biological Chemistry* 295, no. 46: 15712–15726. https://doi.org/10.1074/JBC.RA120.015771.

Seguella, L., and B. D. Gulbransen. 2021. "Enteric Glial Biology, Intercellular Signalling and Roles in Gastrointestinal Disease." *Nature Reviews Gastroenterology & Hepatology* 18, no. 8: 571–587. https://doi.org/10.1038/s41575-021-00423-7.

Sharma, R., T. Matsuzaka, M. K. Kaushik, et al. 2019. "Octacosanol and Policosanol Prevent High-Fat Diet-Induced Obesity and Metabolic Disorders by Activating Brown Adipose Tissue and Improving Liver Metabolism." *Scientific Reports* 9, no. 1: 5169. https://doi.org/10.1038/s41598-019-41631-1.

She, Y., R. Mangat, S. Tsai, S. D. Proctor, and C. Richard. 2022. "The Interplay of Obesity, Dyslipidemia and Immune Dysfunction: A Brief Overview on Pathophysiology, Animal Models, and Nutritional Modulation." *Frontiers in Nutrition* 9: 840209. https://doi.org/10.3389/FNUT.2022.840209.

Stenman, L. K., R. Holma, and R. Korpela. 2012. "High-Fat-Induced Intestinal Permeability Dysfunction Associated With Altered Fecal Bile Acids." *World Journal of Gastroenterology* 18, no. 9: 923. https://doi.org/10.3748/wjg.v18.i9.923.

Wang, L., Q. Wang, Y. Xiong, W. Shi, and X. Qi. 2025. "Obesity and Its Comorbidities: Current Treatment Options, Emerging Biological Mechanisms, Future Perspectives and Challenges." *Diabetes, Metabolic Syndrome and Obesity : Targets and Therapy* 18: 3427–3445. https://doi.org/10.2147/DMSO.S540103.

Wang, N., Z. Pei, H. Wang, J. Zhao, and W. Lu. 2025. "*Bifidobacterium longum* Ameliorates Intestinal Inflammation and Metabolic Biomarkers in Mice Fed a High-Fat Diet With Gliadin by Indoleacrylic Acid." *Probiotics and Antimicrobial Proteins* 17: 3614–3629. https://doi.org/10.1007/s12602-025-10486-6.

Weng, G., Y. Duan, Y. Zhong, et al. 2021. "Plant Extracts in Obesity: A Role of Gut Microbiota." *Frontiers in Nutrition* 8: 727951. https://doi.org/10.3389/fnut.2021.727951.

Yun, S.-W., Y.-J. Shin, X. Ma, and D.-H. Kim. 2024. "*Lactobacillus plantarum* and *Bifidobacterium longum* Alleviate High-Fat Diet-Induced Obesity and Depression/Cognitive Impairment-Like Behavior in Mice by Upregulating AMPK Activation and Downregulating Adipogenesis and Gut Dysbiosis." *Nutrients* 16, no. 22: 3810. https://doi.org/10.3390/nu16223810.

Zhai, Z., J. Liu, K.-M. Niu, et al. 2021. "Integrated Metagenomics and Metabolomics to Reveal the Effects of Policosanol on Modulating the Gut Microbiota and Lipid Metabolism in Hyperlipidemic C57BL/6 Mice." *Frontiers in Endocrinology* 12: 722055. https://doi.org/10.3389/fendo.2021.722055.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supplementary Figures: fft270329-sup-0001-FigureS1-S3.pdf