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Pterostilbene Ameliorates DSS-Induced Intestinal Epithelial Barrier Loss in Mice via Suppression of the NF-κB-Mediated MLCK-MLC Signaling Pathway

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ABSTRACT: The integrity of the intestinal barrier is critical for homeostasis. In this study, we investigated the protective effect of pterostilbene (PTE) on the intestinal epithelium barrier. *In vitro* results of transepithelial electrical resistance (TEER) in Caco-2 cells indicated that PTE counteracted tumor necrosis factor α (TNFα)-induced barrier damage. *In vivo* PTE pretreatment markedly ameliorated intestinal barrier dysfunction induced by dextran sulfate sodium (DSS). Notably, intestinal epithelial tight junction (TJ) molecules were restored by PTE in mice exposed to DSS. The mechanism study revealed that PTE prevented myosin light-chain kinase (MLCK) from driving phosphorylation of MLC (p-MLC), which is crucial for maintaining intestinal TJ stability. Furthermore, PTE blunted translocation of NF-κB subunit p65 into the nucleus to downregulate MLCK expression and then to safeguard TJs and barrier integrity. These findings suggest that PTE protected the intestinal epithelial barrier through the NF-κB-MLCK/p-MLC signal pathway.

KEYWORDS: *intestine, epithelial barrier, tight junction, pterostilbene, myosin light-chain kinase*

INTRODUCTION

The intestinal barrier is composed of multiple components including gut microbiota, unstirred water, mucus, intestinal epithelial cells (IECs), and immune cells.[1,2] Besides, the intestinal barrier acts as an intact interface to facilitate nutrients' intake and to prevent the transport of harmful entities from the intestinal lumen into the internal milieu.[3,4] Mounting evidence suggests that dysfunction of intestinal barrier homeostasis is linked to various pathological conditions. For example, unhealthy foods like alcohol and a high-fat diet increase intestinal permeability by creating aperture and easy penetration of toxic compounds through the damaged epithelial layer into the blood stream.[5−7] Indeed, the pathogenesis of a broad spectrum of diseases, including inflammatory bowel disease, obesity, diabetes, and cancer, is tightly associated with a leaky intestinal barrier.[8,9] Therefore, maintaining the integrity of the intestinal barrier is of great importance in disease prevention.

Among the components of the intestinal barrier, IECs are critical factors for intestinal homeostasis. Actually, the gut tract is fully covered by the IEC layer, which segregates gut microbiota and host immune cells and selectively controls nutrient absorption.[10] Furthermore, tight junctions (TJs) as the intercellular multiprotein complexes of IECs regulate the transportation of substances through paracellular pathways. A dysfunction of TJs results in augmented permeation of harmful entities such as bacteria, antigens, and toxins into the circulation.[11−13] A growing body of research in both animals and human beings indicated that various harmful factors including inflammation and oxidative stress target TJs to cause a leaky intestinal barrier.[14]

Given the crucial role of the intestinal barrier as an interface between health and disease, dietary approaches targeting inflammation and oxidative stress are preferentially recommended to mitigate intestinal barrier damage. For example, the Mediterranean diet has been well established as a health dietary pattern to prevent diseases including intestinal barrier dysfunction due to its high proportion of polyphenolic compounds such as resveratrol and flavonoids.[13] Pterostilbene (PTE) is a 3,5-dimethylated analogue of resveratrol and is frequently found in berries and grapes. PTE has higher intestinal absorption and bioavailability than resveratrol due to the partial methylation on 3,5-diphenols of resveratrol that leads to increased lipophilicity (Figure 1).[15−18] Given that resveratrol is recognized to maintain intestinal barrier homeostasis,[19] questions are raised regarding whether or not PTE can effectively prevent intestinal barrier dysfunction.

Therefore, in this study, we aimed to determine the preventive effects of PTE on the intestinal epithelial barrier and the possible underlying mechanisms. Our findings revealed that PTE maintained intestinal epithelial barrier homeostasis

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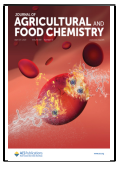

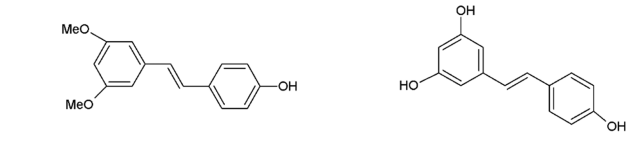

Trans-3,5-dimethoxy-4'-hydroxystilbene (Pterostilbene)

Trans-3,5,4'-trihydroxystilbene (Resveratrol)

Figure 1. Chemical structures of pterostilbene and resveratrol.

by alleviating the loss of epithelial tight junctions (TJs) mediated by a NF-κB-MLCK-p-MLC signal cascade.

MATERIALS AND METHODS

Materials and Cell Culture. For detailed materials used herein, please refer to Supporting Information Table S1. PTE, human colonic Caco-2 cells, and cell culture were used, as previously described.[12,20,21] Briefly, Caco-2 cells were inoculated on the collagen-coated permeable polycarbonate membrane of a transwell at a density of 1×10^5/mL. The cells were grown as monolayers for 21 days prior to experiments. During these days, the medium was changed every 2 days in the first week and then daily from the second week.

Transepithelial Electrical Resistance (TEER). The Caco-2 monolayer was treated with PTE (10 μM) for 6 h prior to TNFα (30 ng/mL) challenge, and then an epithelial voltammeter was used to measure the TEER of the cells at 2, 4, 8, 12, 24, and 48 h during the experiment. The electrode was placed in 75% alcohol for 15 min before measuring. To ensure the stability and accuracy of the resistance values, the culture plate was placed on an ultraclean bench for balance for 30 min, and then the resistance values were measured successively from three different directions of the transwell culture plate.

Animals and Treatment. Eight-week-old C57BL/C mice (male, 18−22 g) were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). The mice were kept at ambient temperature of 23 ± 3 °C and humidity of 50 ± 10% under a 12 h alternating light and dark cycle for a week to adapt to the environment prior to the experiment. Experimental procedures are illustrated in Figure 2. Briefly, the animals were grouped as the following with 10

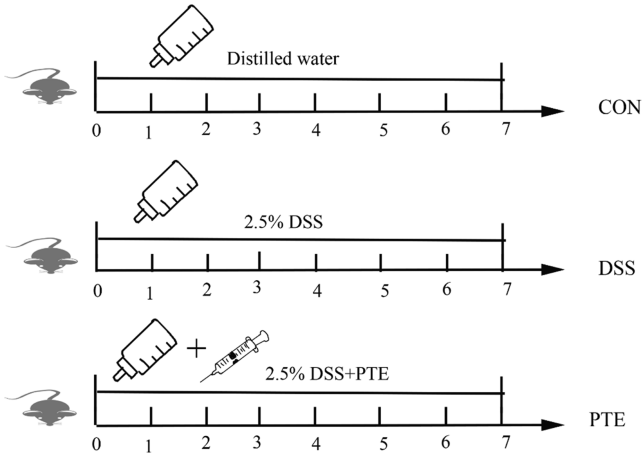

Figure 2. Schematic diagram of oral administration of PTE to mice.

mice per group: (i) control group (CON), mice were given free access to drinking water and food; (ii) DSS group (DSS), mice had free access to water containing 2.5% DSS, which was renewed daily for 7 days; and (iii) PTE group (PTE), mice were orally administrated with 10 mg/kg PTE dissolved in coconut oil in addition to the above DSS treatment. The dosage of PTE in the experiments is consistent with our previous report in which PTE at 10 mg/kg showed a protective effect on concanavalin A-induced acute lethal liver injury.[22] Accordingly, both the control group and the DSS group received an equal volume of coconut oil as the PTE group. All mice were sacrificed on day 8. All animal experimental procedures were strictly in line with the regulation of the Experimental Animals Ethics Committee of Tianjin University of Commerce (TKLFB-2020012) according to the Guide for the Care and Use of Laboratory Animals (order no. 2006-398, Ministry of Science and Technology, China).

Sample Collection. The venous blood was obtained from inner canthus and centrifuged at 2000*g* for 10 min at 4 °C. The supernatant was stored at −80 °C for subsequent experiments. The length of the colon was measured in each mouse. The colon was divided into two parts, one was placed in 4% paraformaldehyde for paraffin embedding and the other was stored at −80 °C.

Disease Activity Index (DAI) Score. The mice were monitored for their daily health, including weight loss, diarrhea, and bloody stool. The DAI was determined as described previously.[23]

Histological Analysis. The colon tissues were fixed in 4% paraformaldehyde for paraffin embedding. Colon paraffin-embedded sections were cut into 4 μm thickness. According to the standard procedure, the colon sections were deparaffinized, rehydrated, and stained. The slides were examined using light microscopy (Nikon CLIPSECi-L, Tokyo, Japan).

Measurement of Intestinal Epithelial Permeability. The mice were orally administered with 44 mg/kg of fluorescein isothiocyanate (FITC)-dextran 4 h prior to sacrifice. Subsequently, the serum samples obtained from venous blood were centrifuged at 630*g* and 4 °C for 10 min. Concentrations of FITC-dextran were determined according to the manufacturers. Briefly, 200 μL of serum samples was added to a completely black 96-well plate, and the absorbance was measured at 528 nm emission and 485 nm excitation.

Bacterial Translocation. The bacterial content in mesenteric lymph nodes (MLNs) was determined to assess the degree of bacterial translocation. The genomic DNA of *Escherichia coli Trans10* was extracted using the TIANamp Bacteria DNA Kit. The genomic DNA of *E. coli Trans10* was used as a standard curve calculating the weight of genomic DNA of bacteria in MLNs per unit weight.

Isolation of Total RNA and the Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The TRIzol reagent was used to isolate total RNA from colon tissue. The total RNA was transcribed into cDNA by a transcriptor cDNA synthesis kit. FastStart SYBRGreen Master Mix was used to perform quantitative real-time PCR, according to our previous report.[12] The primers are listed in Supporting Information Table S2.

Western Blot Analysis. Radioimmunoprecipitation assay buffer was used to extract colonic proteins. The bicinchoninic acid protein assay kit was used for determining protein concentrations. Protein samples were separated on 8, 10, and 12% sodium dodecyl sulfate-polyacrylamide gels, and then wet-transferred to poly(vinylidene fluoride) (PVDF) membranes. Skim milk in Tris-buffered saline with 0.5% Tween-20 (TBST) was used to block membranes for 1 h, and then the primary antibodies were incubated at 4 °C overnight. The following day, membranes were washed three times 10 min each using TBST. The membranes were then incubated with a secondary antibody at room temperature for 1 h, washed with TBST three times 10 min each, and then detected with an Amersham Imager 600 (General Electric Healthcare Life Sciences, Chicago, IL).

Immunofluorescence Staining. Immunofluorescence was detected using a tyramide signal amplification (TSA) kit, according to the instruction. The image of immunofluorescent was captured using a Leica confocal microscope (20× and 100× oil immersion objectives). Fluorescence quantification was performed using Image J. Statistical Analysis.

Statistical Analysis. Values were obtained from at least three independent experiments and presented as mean ± standard deviation (SD). One-way ANOVA testing was assessed using GraphPad Prism software (San Diego, CA) otherwise indicated. Data were considered statistically significant when the *p* value was less than 0.05.

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RESULTS

PTE Reversed the TNFα-Induced Intestinal Epithelial Barrier *in Vitro*. To explore the feasibility of the preventive effect of PTE on the intestinal barrier, the epithelial barrier function was first assessed by determining the TEER in Caco-2 cells. As shown in Figure 3, TNFα significantly decreased the TEER after 48 h of stimulation ($p < 0.0001$), compared with the control group. However, the TNFα-induced barrier loss was obviously restored by PTE pretreatment, indicating that PTE has the potential protection of the intestinal epithelial barrier.

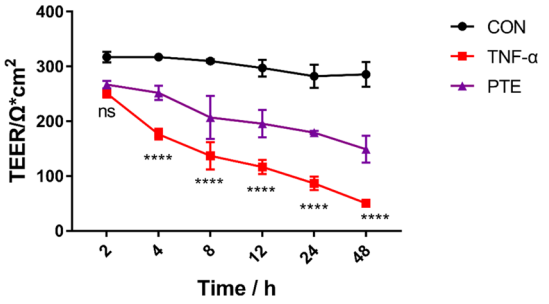

Figure 3. PTE counteracted the change of TEER on TNFα challenge. Two-way ANOVA testing was assessed using GraphPad Prism software. Values are shown as mean ± SD ($n = 3$) (ns = no significant; ****$p < 0.0001$ for DSS vs PTE groups).

PTE Improved the Health Status in DSS-Treated Mice. According to the published protocols, the DSS-induced colitis model in mice has been broadly used to assess intestinal epithelial injury because DSS causes characteristic damage on epithelial cells and subsequent barrier dysfunction and inflammation.[24,25] Therefore, to further understand the preventive effects of PTE on the intestinal epithelial barrier, we performed in vivo investigation using this model. The clinical signs including the survival rate (Figure 4A), body weight loss (Figure 4B), and disease activity index (Figure 4C) in the PTE group of mice were relatively normal compared to that in the DSS group. These observations suggested that PTE improved the quality of life of DSS-induced mice.

PTE Protected Colon Integrity in Mice. Given the above observations, we next investigated whether definite intestinal pathological changes are associated with clinical signs. As shown in Figure 5A, pretreatment with PTE broadly rescued the DSS-induced pathological gross changes including the reduced colon length, severe hyperemia and edema, thickened intestinal wall, and ulceration. Furthermore, according to the HE staining and histopathological scores of colon tissue (Figure 5B), severe crypt defect and extensive inflammation were easily observed in damaged colon of mice induced by DSS. In contrast, PTE evidently prevented the colon histopathological damage from DSS challenge.

To better understand the preventive impact of PTE on intestinal barrier loss, the FITC-dextran flux was determined. As shown in Figure 5C, DSS administration led to a significant increase of the FITC-dextran flux, whereas pretreatment with PTE clearly counteracted the increased flux induced by DSS. In accordance, further supporting evidence came from our observation on bacterial translocation. The bacteria normally colonize in the gut lumen. However, when the intestinal epithelial was dysfunctional, bacteria may migrate to mediastinal lymph nodes (MLNs).[26] As shown in Figure 5D, DSS treatment resulted in bulky bacteria in MLNs. However, pretreatment with PTE powerfully precluded bacteria from migrating to MLNs.

Taken together, these data indicated that PTE effectively prevented the DSS-induced intestinal epithelial barrier loss.

PTE Regulated the Expression of TJs. Having identified that PTE prevents DSS-induced barrier loss, we further investigated the underlying mechanisms. TJs are essential for maintaining the integrity of the intestinal epithelial barrier due

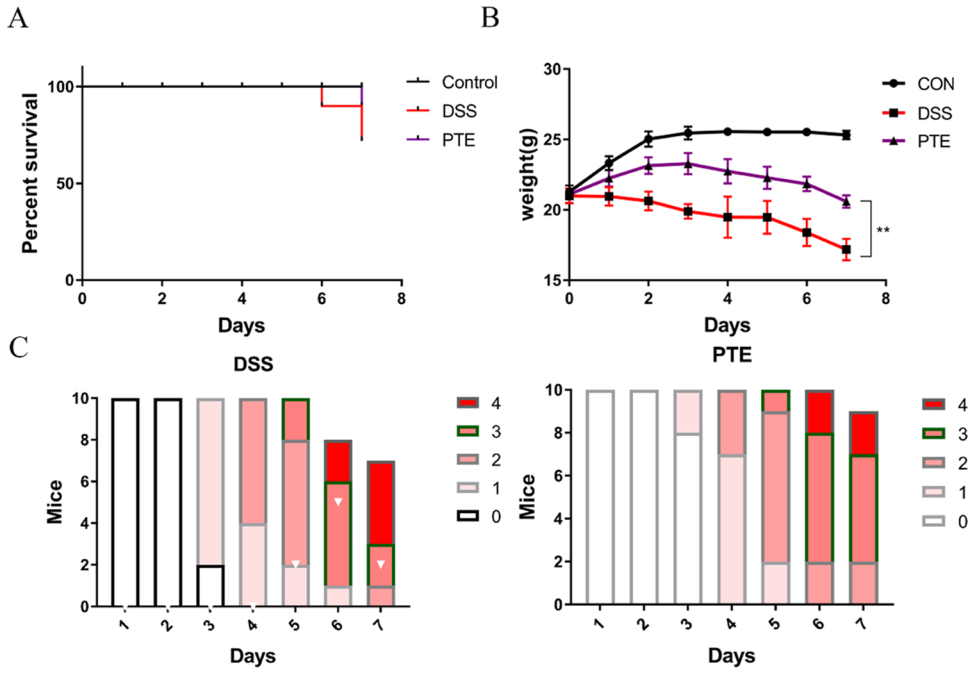

Figure 4. Protective effect of PTE on the intestinal epithelial barrier. (A) Survival rate of mice, (B) body weight alterations, and (C) DAI scores of mice during 7 days' observation. All error bars represent mean ± SD (**$p < 0.01$ for DSS vs PTE groups).

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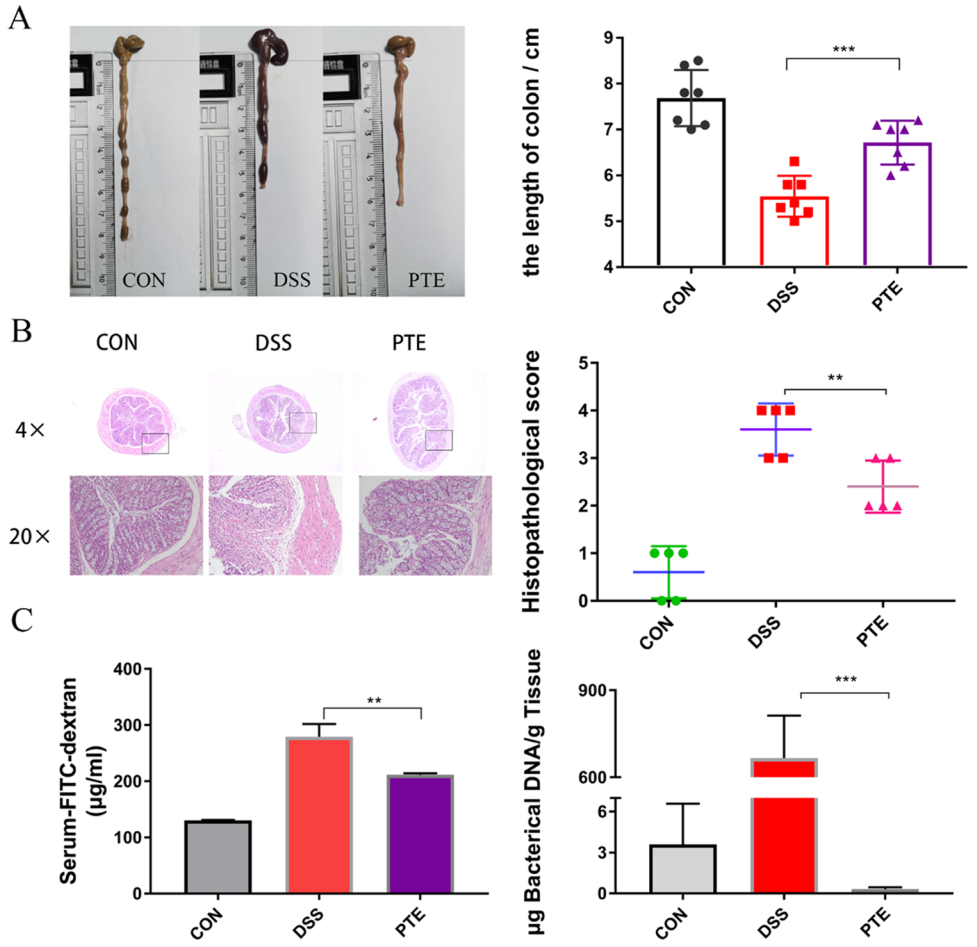

Figure 5. PTE treatment attenuated DSS-induced intestinal barrier dysfunction. (A) Appearances and lengths of colon samples, (B) HE staining images and histopathological scores, (C) concentration of FITC in mice, and (D) bacterial translocation to the MLNs. All error bars represent mean ± SD. (*p < 0.05, **p < 0.01, and ***p < 0.001 for DSS vs PTE group).

to their paracellular location and permeability.[27] Therefore, we first screened TJs in mouse colon tissues. Indeed, whether at mRNA or protein levels, TJs associated with preventing the harmful substance from passing through the paracellular barriers, including occludin, ZO-1, and claudin-4, were markedly downregulated upon DSS stimuli (Figure 6A−C). We further compared the effect of treatment with and without PTE on TJs. Consistent with the above morphological and functional observation, treatment with PTE significantly restored the DSS-induced loss of intestinal TJs. In addition, we also tested claudin-2, a cation-channel-forming molecule, which is contrary to the previously mentioned TJs and acts as a vandal to form a leaky intestine.[28] As expected, PTE treatment reversed DSS-induced upregulation of intestinal claudin-2 (Figure 6D). To confirm the effect of PTE treatment on intestinal TJs visually, we performed immunofluorescence assay on the mice colon. The results showed that PTE treatment restored the intestinal epithelial ZO-1 (green) and occludin (red) decreased by DSS stimuli (Figure 6F,G). Taken together, these data indicated that PTE protects the intestinal epithelial barrier by regulating TJs.

PTE Prevented DSS-Induced Activation of the MLCK-p-MLC Pathway. Previous studies suggested that intestinal TJ loss is tightly dependent on MLCK-triggered phosphorylation of MLC (p-MLC) and inhibition of the MLCK-p-MLC signal pathway reverses the TJ loss and barrier dysregulation in DSS-induced intestinal injury models.[14,29] Therefore, we evaluated whether PTE treatment altered DSS-induced TJ loss involved in the MLCK-p-MLC pathway. As shown in Figure 7A, consistent with the changes of TJs, PTE treatment led to the definite reversal of DSS-induced upregulation of MLCK as well as corresponding phosphorylated MLC. Owing to the significance of MLCK on intestinal barrier homeostasis, our observation indicated that PTE-mediated disruption of the MLCK-p-MLC signal pathway prevented DSS-induced TJ barrier loss.

PTE Inhibited the Intestinal MLCK Expression Associated with Restraining NF-κB Activation. Having identified the preventive role of PTE associated with MLCK-dependent TJ dysregulation, we then asked how PTE modulated the expression of MLCK. Concerns were raised that NF-κB activation led to the initiation of the MLCK-p-MLC pathway in the inflamed intestine.[28,30] Our previous report suggested that PTE possesses remarkable anti-inflammatory potency associated with the inhibition of NF-κB activation.[31] Therefore, we hypothesized whether PTE altering the MLCK expression is associated with the NF-κB signaling pathway. To test this idea, immunoblotting was performed in mouse intestinal tissues to assess the relationship between PTE treatment and NF-κB activation. As shown in Figure 7B, increased phosphorylation of canonical NF-κB components, nuclear p65 (p-p65), and cytoplasmic IκBα (p-

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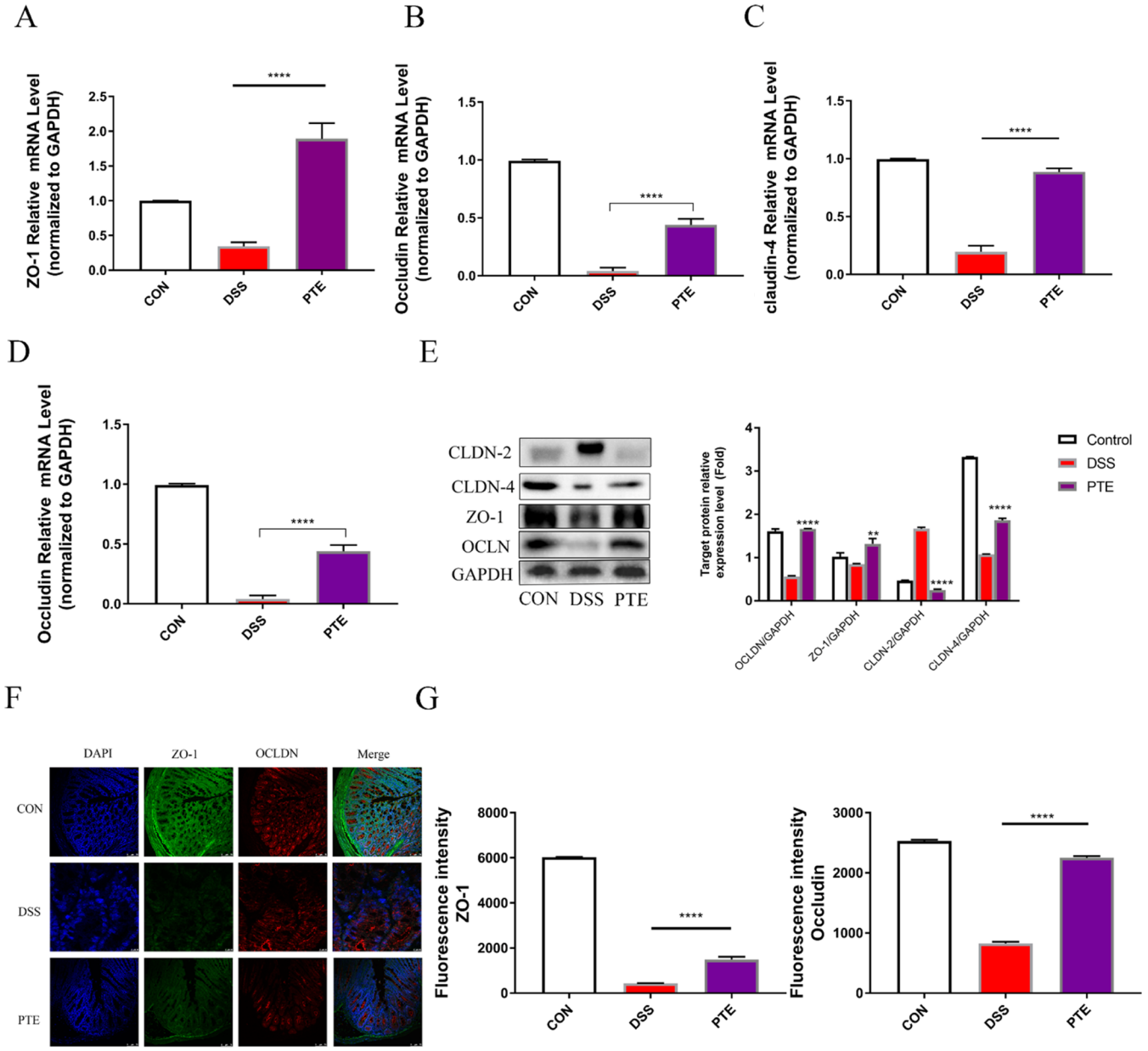

Figure 6. Effects of PTE on loss of intestinal tight junctions in DSS-induced mice. Intestinal mRNA levels of TJs: occludin (A), ZO-1 (B), claudin-4 (C), and claudin-2 (D). (E) Intestinal TJ proteins. (F) and (G) Immunofluorescence assessment of intestinal TJ molecules ZO-1(green) and occludin (red). All error bars represent SD (ns = no significant; $^{**}p < 0.01$ and $^{****}p < 0.0001$ for DSS vs PTE groups).

IκBα), upon DSS stimuli were notably inhibited by PTE treatment, indicating that PTE inactivated the NF-κB signal in an inflamed intestine barrier. The observation was further confirmed by immunofluorescence assays in which the results showed that the alterations of intestinal epithelial cytoplasmic MLCK (green) were in line with nuclear NF-κB/p65 (red) responding to with or without PTE treatment (Figure 7C,D). Indeed, the phenomena are subjected to the well-established mechanism that p65 as a transcription factor shifts into the nucleus to regulate the intestinal MLCK expression and TJ permeability.[32,33] Taken together, these data indicate that PTE inhibited the intestinal MLCK expression associated with restraining NF-κB activation.

DISCUSSION

As the number one discussed hallmark of health, the integrity of the barrier, definitely including the intestine, is indispensable for physiologically handling body homeostasis.[3,34] Evidence from vast experimental or medicinal research studies indicates that intestinal barrier dysfunction resulted from risk factors related to a wide range of illnesses.[35,36] For example, diets high in saturated fat impair tight junction-mediated paracellular barrier preceding the onset of diabetes and IBD.[37,38] However, a healthy diet such as high intake of fruits is beneficial for our health.[39] Defect of intestinal permeability-facilitating gut translocation of microbiota might enhance susceptibility and severity to systemic inflammation such as sepsis and even the ongoing Coronavirus Disease 2019 (COVID-19).[40,41]

Although the underlying molecular mechanisms of the initiation of intestinal barrier loss have not yet been thoroughly

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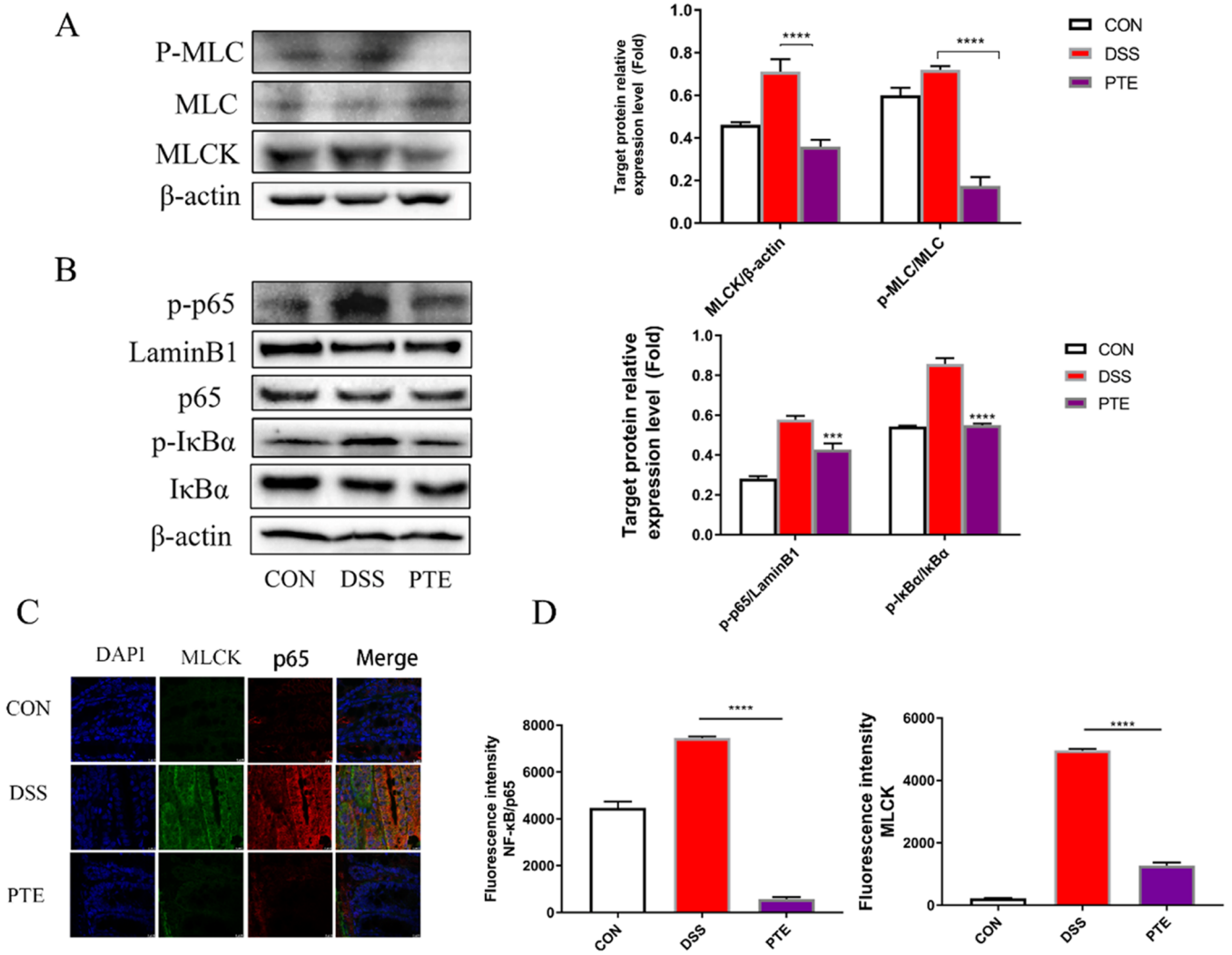

Figure 7. PTE inhibited NF-κB activating intestinal MLCK. (A) PTE treatment inhibited intestinal MLCK and *p*-MLC, (B) PTE treatment inhibited intestinal p-IκBα and p-p65, and (C) and (D) immunofluorescence assessment of colocation of cytoplasm MLCK (green) and nuclear p65 (red). Values are shown as mean ± SD (n = 3) (***p < 0.001 and ****p < 0.0001 for DSS vs PTE groups).

elucidated, the loss of gut epithelial MLCK-mediated TJ molecules is an agreeable potential therapeutic target. Indeed, while TJs located on both membrane sides of epithelial cells regulate paracellular permeability and the epithelial barrier dysfunction superficially manifests leaky gut and TJ defect, accumulating evidence had established convergence that epithelial MLCK is a critical upstream regulator of increased TJ permeability.[42,43] According to this idea, Graham et al. developed a specific intestinal epithelial MLCK inhibitor, which restored TJ loss and corrected barrier dysfunction in experimental rodent IBD.[29]

In addition to directly targeting MLCK, intestinal epithelial NF-κB is considered to modulate MLCK activity. Specifically, activated NF-κB releases p65, which undergoes cytoplasmic-to-nuclear translocation and combines with the MLCK promoter to amplify MLCK.[44] Accordingly, MLCK mediates MLC phosphorylation, which causes myosin contraction leading to TJ dysregulation.[45] Our present study revealed that, as a dimethylated analogue of resveratrol (Figure 1), PTE targeted intestinal MLCK-mediated TJ barrier dysfunction related to inhibition of the activation of NF-κB. Notably, notwithstanding PTE and resveratrol exhibit many pharmacological similarities. Reports from our observation and other groups suggested that the pharmacological features of the PTE chemical structure are more metabolically stable and bioavailable, thus PTE possesses stronger antioxidant and anti-inflammatory activities than resveratrol.[46] Therefore, given increasing reports regarding the ameliorative effect of dietary resveratrol on intestinal mucosal barrier defects,[22,47] whether dietary PTE might represent a higher-priority strategy to the application of functional food in preventing intestinal barrier loss than resveratrol will be worth investigating in future. Interestingly, Chen et al. found that glycosylated PTE, pterostilbene 4′-β-glucoside, ameliorated DSS-induced colitis by targeting an AU-rich element-binding protein tristetraprolin, which plays an important role in promoting degradation of proinflammatory mediators and subsequently limiting the inflammatory response.[48] By complement and parallel to their proposed mechanisms, our study suggested that PTE inhibited activation of the master inflammatory regulator NF-κB and subsequently MLCK-mediated barrier loss.

In summary, our present study has demonstrated that PTE safeguards the intestinal epithelial integrity in mice via targeting MLCK-mediated TJ defect and barrier loss. This study based on dietary stilbene PTE provides a potential

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solution for the protection of the integrity of the intestinal epithelial barrier.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c00274.

Information of reagents and antibodies (Table S1) and primers (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Turner, J. R. Intestinal mucosal barrier function in health and disease. *Nat. Rev. Immunol.* **2009**, *9*, 799−809.

(2) Ray, K. Intestinal tract: A breach in the intestinal barrier during hyperglycaemia. *Nat. Rev. Gastroenterol. Hepatol.* **2018**, *15*, 255.

(3) Odenwald, M. A.; Turner, J. R. The intestinal epithelial barrier: a therapeutic target? *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 9−21.

(4) Ma, T. Y.; Iwamoto, G. K.; Hoa, N. T.; Akotia, V.; Pedram, A.; Boivin, M. A.; Said, H. M. TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2004**, *286*, G367−G376.

(5) Massey, V. L.; Arteel, G. E. Acute alcohol-induced liver injury. *Front Physiol.* **2012**, *3*, No. 193.

(6) Moreira, A. P. B.; Texeira, T. F.; Ferreira, A. B.; Peluzio Mdo, C.; Alfenas Rde, C. Influence of a high-fat diet on gut microbiota, intestinal permeability and metabolic endotoxaemia. *Br. J. Nutr.* **2012**, *108*, 801−809.

(7) Pendyala, S.; Walker, J. M.; Holt, P. R. A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology* **2012**, *142*, 1100−1101.e2.

(8) Salim, S. Y.; Söderholm, J. D. Importance of disrupted intestinal barrier in inflammatory bowel diseases. *Inflammatory Bowel Dis.* **2011**, *17*, 362−381.

(9) Zhuang, S.; Zhong, J.; Zhou, Q.; Zhong, Y.; Liu, P.; Liu, Z. Rhein protects against barrier disruption and inhibits inflammation in intestinal epithelial cells. *Int. Immunopharmacol.* **2019**, *71*, 321−327.

(10) Okumura, R.; Takeda, K. Maintenance of intestinal homeostasis by mucosal barriers. *Inflammation Regener.* **2018**, *38*, No. 5.

(11) Dokladny, K.; Zuhl, M. N.; Moseley, P. L. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. *J. Appl. Physiol.* **2016**, *120*, 692−701.

(12) Wen, X.; Zhao, H.; Wang, L.; Wang, L.; Du, G.; Guan, W.; Liu, J.; Cao, X.; Jiang, X.; Tian, J.; Wang, M.; Ho, C. T.; Li, S. Nobiletin Attenuates DSS-Induced Intestinal Barrier Damage through the HNF4α-Claudin-7 Signaling Pathway. *J. Agric. Food Chem.* **2020**, *68*, 4641−4649.

(13) Valdez, J. C.; Bolling, B. W. Anthocyanins and intestinal barrier function: a review. *J. Food Bioact.* **2019**, *5*, 18−30.

(14) Su, L.; Nalle, S. C.; Shen, L.; Turner, E. S.; Singh, G.; Breskin, L. A.; Khramtsova, E. A.; Khramtsova, G.; Tsai, P. Y.; Fu, Y. X.; Abraham, C.; Turner, J. R. TNFR2 activates MLCK-dependent tight junction dysregulation to cause apoptosis-mediated barrier loss and experimental colitis. *Gastroenterology* **2013**, *145*, 407−15.

(15) Yeo, S. C. M.; Ho, P. C.; Lin, H.-S. Pharmacokinetics of pterostilbene in Sprague-Dawley rats: the impacts of aqueous solubility, fasting, dose escalation, and dosing route on bioavailability. *Mol. Nutr. Food Res.* **2013**, *57*, 1015−1025.

(16) Lin, H. S.; Yue, B. D.; Ho, P. C. Determination of pterostilbene in rat plasma by a simple HPLC-UV method and its application in pre-clinical pharmacokinetic study. *Biomed. Chromatogr.* **2009**, *23*, 1308−1315.

(17) Perečko, T.; Drabikova, K.; Rackova, L.; Ciz, M.; Podborska, M.; Lojek, A.; Harmatha, J.; Smidrkal, J.; Nosal, R.; Jancinova, V. Molecular targets of the natural antioxidant pterostilbene: effect on protein kinase C, caspase-3 and apoptosis in human neutrophils in vitro. *Neuroendocrinol. Lett.* **2010**, *31*, 84−90.

(18) Kapetanovic, I. M.; Muzzio, M.; Huang, Z.; Thompson, T. N.; McCormick, D. L. Pharmacokinetics, oral bioavailability, and metabolic profile of resveratrol and its dimethylether analog, pterostilbene, in rats. *Cancer Chemother. Pharmacol.* **2011**, *68*, 593−601.

(19) Mayangsari, Y.; Suzuki, T. Resveratrol enhances intestinal barrier function by ameliorating barrier disruption in Caco-2 cell monolayers. *J. Funct. Foods* **2018**, *51*, 39−46.
(20) Kim, J. Y.; Le, T. A. N.; Lee, S. Y.; Song, D. G.; Hong, S. C.; Cha, K. H.; Lee, J. W.; Pan, C. H.; Kang, K. 3,3′-Diindolylmethane Improves Intestinal Permeability Dysfunction in Cultured Human Intestinal Cells and the Model Animal Caenorhabditis elegans. *J. Agric. Food Chem.* **2019**, *67*, 9277−9285.
(21) Polewski, M. A.; Esquivel-Alvarado, D.; Wedde, N. S.; Kruger, C. G.; Reed, J. D. Isolation and Characterization of Blueberry Polyphenolic Components and Their Effects on Gut Barrier Dysfunction. *J. Agric. Food Chem.* **2020**, *68*, 2940−2947.
(22) Wu, J.; Li, M.; He, J.; Lv, K.; Wang, M.; Guan, W.; Liu, J.; Tao, Y.; Li, S.; Ho, C. T.; Zhao, H. Protective effect of pterostilbene on concanavalin A-induced acute liver injury. *Food Funct.* **2019**, *10*, 7308−7314.
(23) Gonzalez-Rey, E.; Delgado, M. Therapeutic application of mesenchymal stromal cells in murine models of inflammatory bowel disease. *Methods Mol. Biol.* **2014**, *1213*, 331−339.
(24) Hoffman, J. M.; Baritaki, S.; Ruiz, J. J.; Sideri, A.; Pothoulakis, C. Corticotropin-Releasing Hormone Receptor 2 Signaling Promotes Mucosal Repair Responses after Colitis. *Am. J. Pathol.* **2016**, *186*, 134−144.
(25) Hovhannisyan, Z.; Liu, N.; Khalil-Aguero, S.; Panea, C.; VanValkenburgh, J.; Zhang, R.; Lim, W. K.; Bai, Y.; Fury, W.; Huang, T.; Garnova, E.; Fairhurst, J.; Kim, J.; Aryal, S.; Ajithdoss, D.; Oyejide, A.; Del Pilar Molina-Portela, M.; E, H.; Poueymirou, W.; Oristian, N. S.; Brydges, S.; Liu, X.; Olson, W.; Yancopoulos, G.; Murphy, A. J.; Sleeman, M. A.; Haxhinasto, S. Enhanced IL-36R signaling promotes barrier impairment and inflammation in skin and intestine. *Sci. Immunol.* **2020**, *5*, No. eaax1686.
(26) Stevenson, B. R. Understanding tight junction clinical physiology at the molecular level. *J. Clin. Invest.* **1999**, *104*, 3−4.
(27) Sawada, N. Tight junction-related human diseases. *Pathol. Int.* **2013**, *63*, 1−12.
(28) Raju, P.; Shashikanth, N.; Tsai, P. Y.; Pongkorpsakol, P.; Chanez-Paredes, S.; Steinhagen, P. R.; Kuo, W. T.; Singh, G.; Tsukita, S.; Turner, J. R. Inactivation of paracellular cation-selective claudin-2 channels attenuates immune-mediated experimental colitis in mice. *J. Clin. Invest.* **2020**, *130*, 5197−5208.
(29) Graham, W. V.; He, W.; Marchiando, A. M.; Zha, J.; Singh, G.; Li, H. S.; Biswas, A.; Ong, M.; Jiang, Z. H.; Choi, W.; Zuccola, H.; Wang, Y.; Griffith, J.; Wu, J.; Rosenberg, H. J.; Wang, Y.; Snapper, S. B.; Ostrov, D.; Meredith, S. C.; Miller, L. W.; Turner, J. R. Intracellular MLCK1 diversion reverses barrier loss to restore mucosal homeostasis. *Nat. Med.* **2019**, *25*, 690−700.
(30) Cao, M.; Wang, P.; Sun, C.; He, W.; Wang, F. Amelioration of IFN-γ and TNF-α-induced intestinal epithelial barrier dysfunction by berberine via suppression of MLCK-MLC phosphorylation signaling pathway. *PLoS One* **2013**, *8*, No. e61944.
(31) Suzuki, M.; Nagaishi, T.; Yamazaki, M.; Onizawa, M.; Watabe, T.; Sakamaki, Y.; Ichinose, S.; Totsuka, M.; Oshima, S.; Okamoto, R.; Shimonaka, M.; Yagita, H.; Nakamura, T.; Watanabe, M. Myosin light chain kinase expression induced via tumor necrosis factor receptor 2 signaling in the epithelial cells regulates the development of colitis-associated carcinogenesis. *PLoS One* **2014**, *9*, No. e88369.
(32) Al-Sadi, R.; Guo, S.; Ye, D.; Rawat, M.; Ma, T. Y. TNF-α Modulation of Intestinal Tight Junction Permeability Is Mediated by NIK/IKK-α Axis Activation of the Canonical NF-κB Pathway. *Am. J. Pathol.* **2016**, *186*, 1151−1165.
(33) Graham, W. V.; Wang, F.; Clayburgh, D. R.; Cheng, J. X.; Yoon, B.; Wang, Y.; Lin, A.; Turner, J. R. Tumor necrosis factor-induced long myosin light chain kinase transcription is regulated by differentiation-dependent signaling events. Characterization of the human long myosin light chain kinase promoter. *J. Biol. Chem.* **2006**, *281*, 26205−26215.
(34) López-Otín, C.; Kroemer, G. Hallmarks of Health. *Cell* **2020**, *184*, 33−63.
(35) Hering, N. A.; Fromm, M.; Schulzke, J. D. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J. Physiol.* **2012**, *590*, 1035−1044.
(36) Oshima, T.; Miwa, H. Gastrointestinal mucosal barrier function and diseases. *J. Gastroenterol.* **2016**, *51*, 768−78.
(37) Li, X.; Wei, X.; Sun, Y.; Du, J.; Li, X.; Xun, Z.; Li, Y. C. High-fat diet promotes experimental colitis by inducing oxidative stress in the colon. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2019**, *317*, G453−g462.
(38) Nascimento, J. C.; Matheus, V. A.; Oliveira, R. B.; Tada, S. F. S.; Collares-Buzato, C. B. 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. *Dig. Dis. Sci.* **2020**, 1−16.
(39) Dreher, M. L. Whole Fruits and Fruit Fiber Emerging Health Effects. *Nutrients* **2018**, *10*, No. 1833.
(40) Visitchanakun, P.; Saisorn, W.; Wongphoom, J.; Chatthanathon, P.; Somboonna, N.; Svasti, S.; Fucharoen, S.; Leelahavanichkul, A. Gut leakage enhances sepsis susceptibility in iron-overloaded β-thalassemia mice through macrophage hyper-inflammatory responses. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2020**, *318*, G966−G979.
(41) Sharma, L.; Riva, A. Intestinal Barrier Function in Health and Disease-Any role of SARS-CoV-2? *Microorganisms* **2020**, *8*, No. 1744.
(42) Marchiando, A. M.; Shen, L.; Graham, W. V.; Edelblum, K. L.; Duckworth, C. A.; Guan, Y.; Montrose, M. H.; Turner, J. R.; Watson, A. J. The epithelial barrier is maintained by in vivo tight junction expansion during pathologic intestinal epithelial shedding. *Gastroenterology* **2011**, *140*, 1208−1218.e2.
(43) Yu, D.; Marchiando, A. M.; Weber, C. R.; Raleigh, D. R.; Wang, Y.; Shen, L.; Turner, J. R. MLCK-dependent exchange and actin binding region-dependent anchoring of ZO-1 regulate tight junction barrier function. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 8237−41.
(44) Arita, Y.; Ito, T.; Oono, T.; Kawabe, K.; Hisano, T.; Takayanagi, R. Lysophosphatidic acid induced nuclear translocation of nuclear factor-kappaB in Panc-1 cells by mobilizing cytosolic free calcium. *World J. Gastroenterol.* **2008**, *14*, 4473−9.
(45) Ma, T. Y.; Boivin, M. A.; Ye, D.; Pedram, A.; Said, H. M. Mechanism of TNF-{alpha} modulation of Caco-2 intestinal epithelial tight junction barrier: role of myosin light-chain kinase protein expression. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **2005**, *288*, G422−G430.
(46) Wang, L.; Zhao, H.; Wang, L.; Tao, Y.; Du, G.; Guan, W.; Liu, J.; Brennan, C.; Ho, C. T.; Li, S. Effects of Selected Resveratrol Analogues on Activation and Polarization of Lipopolysaccharide-Stimulated BV-2 Microglial Cells. *J. Agric. Food Chem.* **2020**, *68*, 3750−3757.
(47) Li, F.; Han, Y.; Cai, X.; Gu, M.; Sun, J.; Qi, C.; Goulette, T.; Song, M.; Li, Z.; Xiao, H. Dietary resveratrol attenuated colitis and modulated gut microbiota in dextran sulfate sodium-treated mice. *Food Funct.* **2020**, *11*, 1063−1073.
(48) Chen, Y.; Park, J.; Joe, Y.; Park, H. J.; Jekal, S. J.; Sato, D.; Hamada, H.; Chung, H. T. Pterostilbene 4′-β-Glucoside Protects against DSS-Induced Colitis via Induction of Tristetraprolin. *Oxid. Med. Cell. Longevity* **2017**, *2017*, No. 9427583.

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