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Berberine ameliorates dextran sulfate sodium -induced colitis through tuft cells and bitter taste signalling

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Abstract

Background Inflammatory bowel disease (IBD), a persistent gastrointestinal disease, is featured with impaired gut immunity. Previous studies have demonstrated that tuft cells can regulate the intestinal type 2 immune response by activating downstream ILC2 and Th2 cells and repair gut barrier upon invasion of parasitic helminths, bacteria, protozoans, and enteritis through different chemo-sensing receptors, such as bitter taste receptors. Berberine is a widely used in the treatment of diarrhea in clinic, however the mechanism underlying this effect is not clear. In this study, we aim to explore the relationship between berberine and tuft cells in dextran sulfate sodium (DSS) -induced colitis.

Results Our data showed that berberine significantly ameliorated DSS-induced colitis and regulating type 2 innate immune lymphocytes (ILC2) and Th2 immune cells via tuft cells in the gut. Furthermore, the effect of berberine on colitis was partially abolished by U73122, a bitter taste receptor inhibitor, suggesting that bitter taste signalling pathway played an important role in the effect of berberine on relieving colitis.

Conclusions Berberine ameliorates dextran sulfate sodium -induced colitis through tuft cells and bitter taste signalling. Our study reveals the unique pharmacological mechanisms of berberine in the context of colitis, laying the foundation for further clinical applications of this compound.

Keywords Tuft cell, Type 2 immune responses, Inflammatory bowel disease, Berberine, Bitter taste receptor

Background

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a persistent, immune-mediated gastrointestinal condition, which greatly reduces the quality of life of patients [1]. The pathogenesis of IBD remains not completely understood and without an effective treatment. Recent studies have suggested gut immune dysfunction and mucosal barrier damage as key factors in IBD progression [2]. $CD4^+$ T cells in the intestinal mucosa generate proinflammatory cytokines that are pivotal for the development and maintenance of the chronic inflammation in IBD [3, 4]. Many disorganized Th1/Th17 cells infiltrate the lamina propria of the gut, inducing high levels of pro-inflammatory cytokines, such as *Tnf*, *Il-1β*, *Ifn-γ* and *Il-17*. This

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infiltration is accompanied by a decrease in the number of Th2 cells that produce IL-4 and IL-13 [5–7]. Increased pro-inflammatory cytokines lead to an imbalance in Th1/Th2 responses, resulting in abnormal mucus production and defective gut barrier repair [8]. Therefore, maintaining the gut immunological homeostasis by ensuring the balance between Th1/2 immune responses might be determinant for the treatment of IBD.

Recent studies have suggested that tuft cells are critical for maintaining gut barrier and immune homeostasis; tuft cell is a rare type of epithelial cells in the gut, accounting for 0.4%−2% of all intestinal epithelial cells, that are characterized by their fusiform shape and special microvilli tubules [9–11]. Tuft cell is the only source of gut-derived IL-25, produced upon activation by succinate secreted by parasites, such as *Nippostrongylus brasiliensis*, *Trichinella spiralis* or *Tritrichomonas*. Parasite-induced IL-25 release activates type 2 innate immune lymphocytes (ILC2) and Th2 immune cells that produce IL-4 and IL-13, which further drive the differentiation of tuft and goblet cells, ultimately resisting pathogen invasion and regulating intestinal immune homeostasis [12–15]. Additionally, tuft cell-deficient mice with *Dclk1* (Double cortin-like kinase 1, a specific marker of tuft cells) knockout developed worsened colitis upon diverse stimuli, such as mucin-type O-glycan deficiency (DKO), Citrobacter rodentium (CR), and dextran sulfate sodium (DSS) [16–19]. Oral administration of exogenous succinate can reduce intestinal inflammation through the activation of Sucnr1 (Succinate Receptor 1) that promotes the growth of goblet and tuft cells [20, 21]. Therefore, tuft cells may be novel targets for relieving enteritis. Sucnr1 as well as bitter taste receptors, such as TAS2R143, TAS2R136, TAS2R117, TAS2R108, can be activated in tuft cells by *Trichinella spiralis* [12]. Tuft cells also specifically express bitter-taste receptors and its associated taste-signalling proteins, such as α-gustducin (Gnat3), ChAT (choline acetyl transferase), and Trpm5 (transient receptor potential cation channel subfamily M member 5) [22]. Therefore, the bitter taste receptors on the surface of tuft cells play a significant role in regulating their function.

Berberine (BBR), a quaternary ammonium alkaloid isolated from the traditional Chinese medicine, *Coptis Rhizoma*, is widely used to treat diarrhoea. However, the mechanism underlying its anti-colitis effect remains elusive [23, 24]. The bioavailability of berberine is limited [25], and it has the potential to interact with intestinal epithelial cells. For example, Berberine can activate bitter taste receptors on enteroendocrine cells in vitro; In addition, berberine can facilitate restitution of colonic epithelium by $Lgr5^{+}$ stem cell activation in DSS-induced colitis [26]. Our previous study reported that its oral administration in obese mice can activate TAS2Rs / Gα-gustducin / Gβ1γ13 / PLCβ2 (phospholipase C beta 2) / IP3 (inositol 1,4,5-trisphosphate receptor 1) signalling pathway, promoting gut barrier integrity [27, 28]. However, the correlation between the therapeutic efficacy of berberine and bitter taste receptors as well as tuft cells remains ambiguous.

In our study, we found that the efficacy of berberine treatment for enteritis is contingent upon tuft cell functionality and activation of bitter taste receptor signal, which provides a novel pharmacological mechanism of berberine in colitis and provides a foundation for further clinical applications of berberine.

Results

Oral berberine alleviates DSS-induced colitis

We started by examining the anti-colitis effects of oral berberine (200 mg/kg) in mice (Fig. 1A). In line with previous studies [23], advanced treatment of berberine (DSS + BBR) significantly ameliorated DSS-induced colitis as characterized by reduced DAI (Disease Activity Index) scores (including weight loss, stool consistency, and bleeding) (Fig. 1B-C), increased colon length (Fig. 1D-E), and improved histopathological scores (Fig. 1F-G). The expression of tight junction proteins Occludin and Claudin-1 was detected through immunohistochemistry. Berberine exhibited a significant capacity to enhance the expression of tight junction proteins in the colons of mice with colitis, suggesting its potential for repairing the compromised intestinal barrier (Fig. 1H-K).

To assess the therapeutic efficacy of berberine in colitis, we orally treated mice with berberine (200 mg/kg) on days 2 (DSS-2D + BBR), 4 (DSS-4D + BBR), and 6 (DSS-6D + BBR) respectively following DSS administration. As shown in the Additional file 1: Fig. S1A-D, berberine

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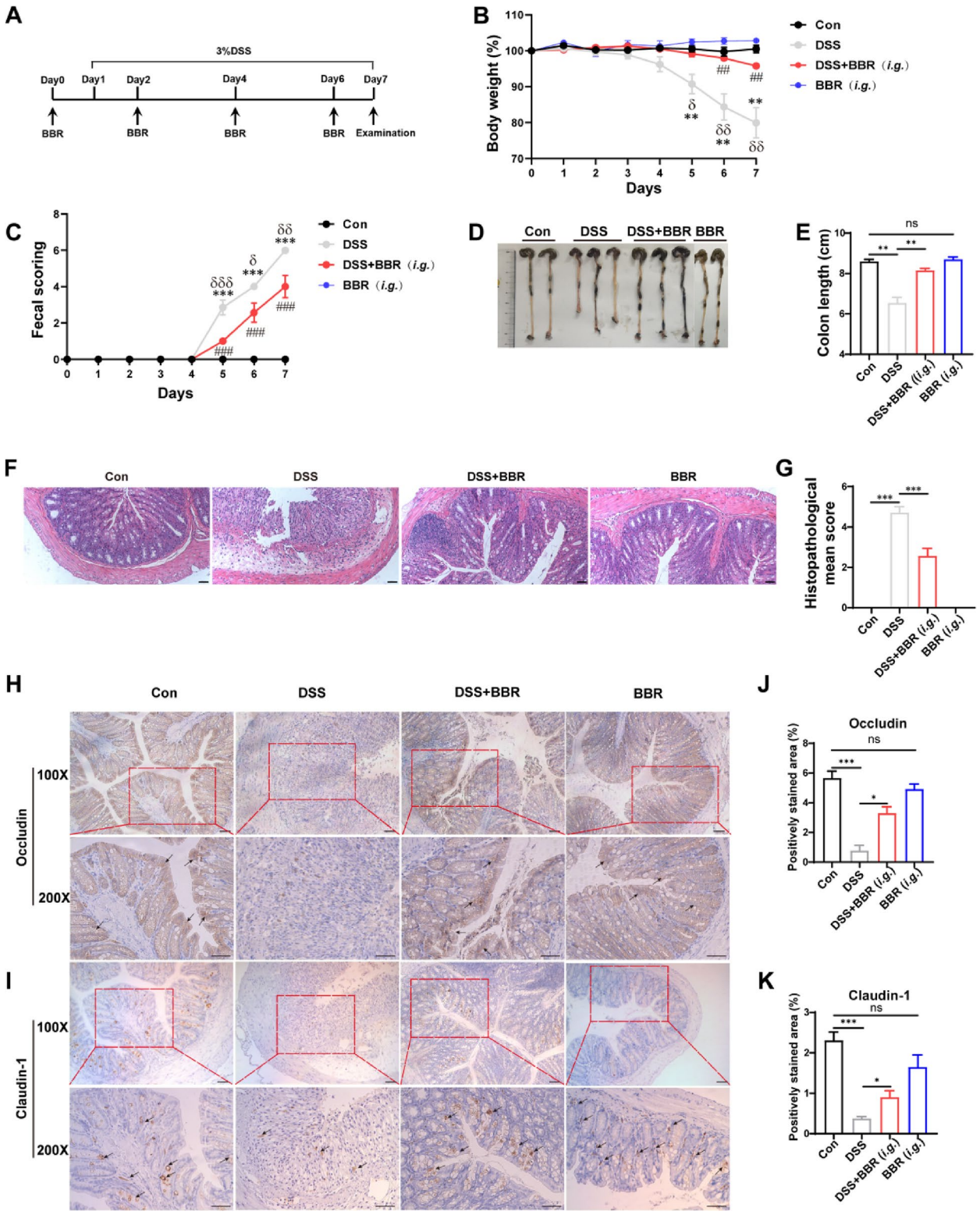
Fig. 1 Oral berberine alleviates DSS-induced colitis in mice. **A** Male mice were treated with 3% DSS in drinking water for consecutive 7 days to induce the colitis. Berberine at dosage of 200 mg kg^{-1} was orally administered on day 0, 2, 4 and 6 respectively ($n = 8$); **B** Body weight changes of each group during disease progression; **C** DAI score of each group; **D** Representative colon images of the four groups; **E** Colon length was analyzed; **F** H&E staining of colons (scale bars, 100 μm); **G** Histologic colitis scores. The expression of tight junction protein Occludin (**H**) and (**I**) Claudin-1 in colon were detected by immunohistochemical staining; **J**, **K** Statistics of the expression of Occludin and Claudin-1 ($n = 6$). All data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$; $^{\delta}P < 0.05$, $^{\delta\delta}P < 0.01$, $^{\delta\delta\delta}P < 0.001$



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significantly relived colitis, as evidenced by body weight, stool score, colon length, and H&E staining. Compared to the three treatment groups, the prevention group still exhibited superior therapeutic effects (Additional file 1: Fig. S1E-F).

Berberine promoted the proliferation of tuft cells and motivated type 2 immunity in the gut

Considering the crucial role of tuft cells in gut homeostasis and the beneficial effect of berberine on tuft cells in obese mice [14, 27], we investigated whether berberine affected tuft cell differentiation during colitis. The amount of $DCLK1^+$ tuft cells was decreased in the DSS-treated group, while berberine promoted the expansion of the tuft cell lineage in the colon of colitis mice (Fig. 2A-B and Additional file 1: Fig. S2A-B). As shown in Fig. 2C-D and Additional file 1: Fig S2C, berberine markedly increased the mRNA levels of *Pou2f3* (POU class 2 homeobox 3, a specific marker of tuft cells) and *Il-25* in the DSS-treated mice. As illustrated in Fig. 2E-G and Additional file 1: Fig S2D, DSS treatment elicited Th1/Th17 differentiation in the large intestine and spleen, which was effectively inhibited by berberine; Conversely, DSS treatment resulted in a reduction of Th2 cell population compared to the control group, whereas administration of berberine significantly upregulated the Th2 subset. As depicted in Fig. 2H-I and Additional file 1: Fig S2E-F, berberine treatment induced a substantial increase in colonic ILC2 cell abundance in DSS-treated mice. Furthermore, berberine treatment augmented mRNA expression levels of *Il-4*, *Il-10*, and *Il-13* while attenuating the expression of pro-inflammatory cytokines *Ifn-γ*, *Tnf-α*, and *Il-6* in DSS-treated mice (Fig. 2J-K and Additional file 1: Fig. S2G-H). Meanwhile, berberine treatment reduced the mRNA expression of Th17-related cytokines (*Il-17A*, *Il-17F*, *ROR-γt*) in DSS-treated mice (Fig. 2L and Additional file 1: Fig. S2I). To explore how long it takes berberine to initiate type 2 immune responses, we collected lamina propria of the colon at 2, 4, 8, 12, 24 and 48 h after berberine administration (200 mg/kg, *i.g*). Flow cytometry analysis showed that the relative proportions of ILC2 exhibited a slight alteration at 2 h and reached its peak at 24 h, which followed by a slight decrease at 48 h (Additional file 1: Fig. S2J).

To investigate the potential effects of berberine on other colonic epithelial cells, we examined goblet (MUC2) and enteroendocrine cell (ChgA) populations in the colon of mice using immunofluorescence staining. Both populations were found to be increased in the DSS + BBR group (Additional file 1: Fig. S3 and Additional file 1: Fig. S4A-D). To determine whether this increase is a direct result of berberine's effect on stem cell differentiation, we co-cultured colonic organoids with berberine (10 μM) for 48 h and assessed the number of tuft cells, goblet cells, and enteroendocrine cells through immunofluorescence staining. The results showed no significant effect of berberine on the number of tuft cells, goblet cells or enteroendocrine cells; however, there was an observed increase in IL-25 concentration following ex vivo berberine treatment (Additional file 1: Fig. S4E-K). Therefore, we conclude that the effect of berberine on epithelial cells is not directly mediated by stem cell differentiation. Instead, our findings suggest that direct activation of tuft cells by berberine serves as a primary factor initiating type 2 immune response and promoting tuft cell differentiation in vivo.

Berberine alleviates colitis by regulating gut type 2 immunity in a tuft cells-dependent manner

To verify whether the beneficial effect of berberine on colitis is related with tuft cells, Pou2f3 knockout mice, which are absent of tuft cells were used [13]. As shown in Fig. 3A-D, berberine failed to attenuate the DSS-induced symptoms in $Pou2f3^{-/-}$ mice. Meanwhile, $Pou2f3^{-/-}$ mice exhibited a more pronounced weight loss and an earlier onset of abnormal feces following colitis induction compared to the WT-DSS group. Moreover, there was a greater reduction in colon length. Additionally, H&E staining revealed that, in comparison to wild-type (WT) mice, berberine did not exhibit significant improvements in mucosal inflammation infiltration and epithelial damage in $Pou2f3^{-/-}$ mice within the colitis model (Fig. 3E-F). In the spleen, the $Pou2f3^{-/-}$ + DSS group exhibited a decreased population of Th2 cells and an increased population of Th17 cells compared to the WT + DSS group

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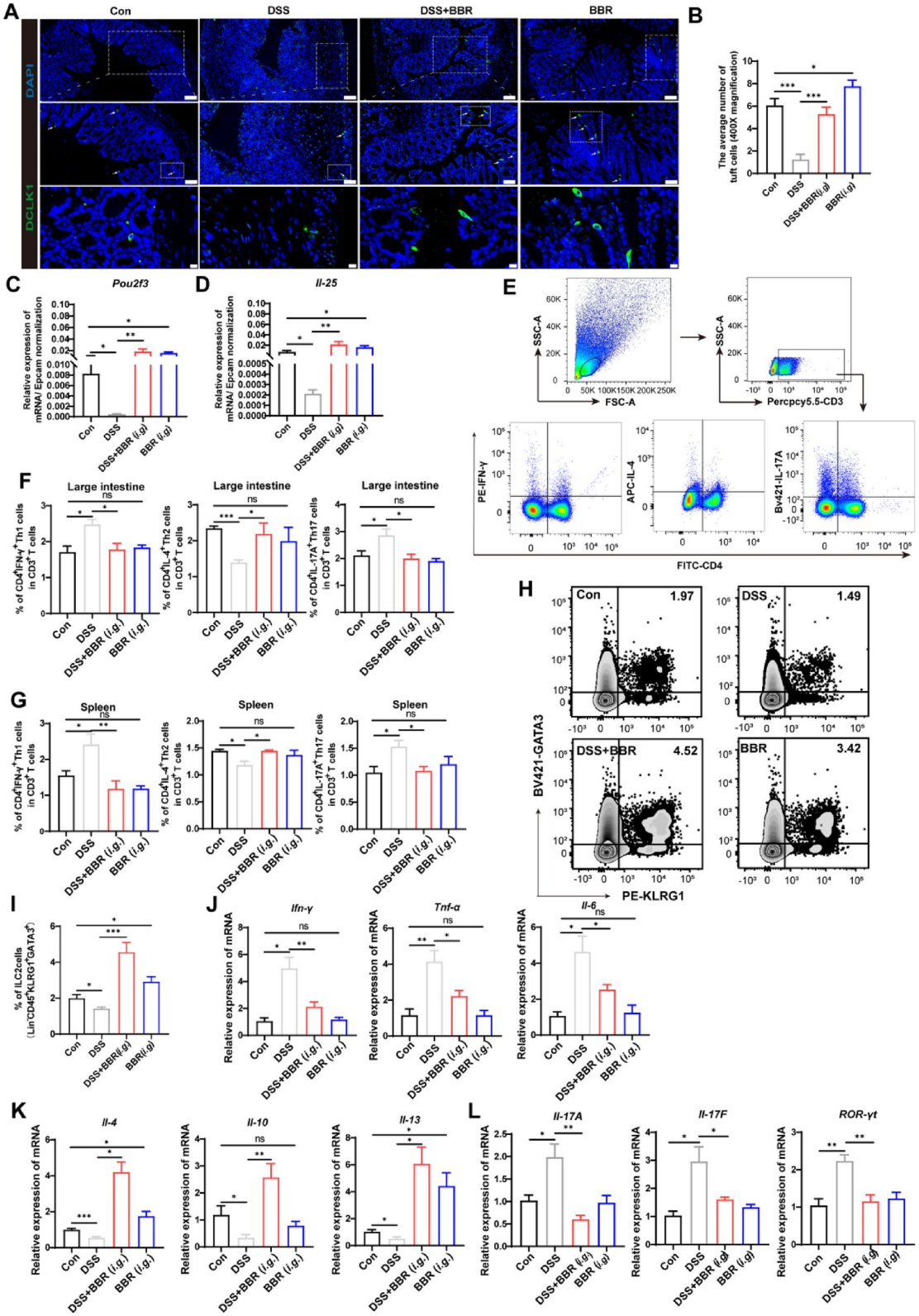
Fig. 2 Berberine promoted the proliferation of tuft cells and motivated type 2 immunity in the gut. **A** Immunofluorescence staining of DCLK1 in the colonic tissues (scale bars, 100 μm, 50 μm and 20 μm respectively). **B** Statistics of the number of tuft cells ($n = 8$). **C**, **D** The mRNA expression level of *Pou2f3* and *Il-25* in the colonic epithelial cell (*Epcam* normalization) by qRT-PCR ($n = 8$); **E** Splenic and colonic lamina propria lymphocytes collected from each group were analyzed by flow cytometry; **F**, **G** Flow cytometric analysis of IFN-γ^+ (Th1), IL-4^+ (Th2) and IL-$17A^+$ (Th17) in $CD4^+$ T-lymphocyte of spleen and large intestine from 4 groups of male mice ($n = 6$); **H-I** Flow cytometric analysis of the number of $Lineage^-CD45^+KLRG1^+GATA3^+$ ILC2 of large intestine from 4 groups of male mice ($n = 6$); **J** The mRNA expression of Th1 associated pro-inflammatory cytokines, **K** Th2 associated anti-inflammatory cytokines and **L** Th17 associated cytokines were measured by qRT-PCR in the colon ($n = 8$). All data were presented as mean ± SEM. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$



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(Fig. 3G). No significant alterations in the percentage of Th1 or Th2 cells were observed in the colon between WT + DSS and Pou2f3$^{-/-}$ + DSS group mice; however, there was a strong tendency towards a higher population of Th17 cells in Pou2f3$^{-/-}$ + DSS group mice (Fig. 3H). The berberine-mediated regulation of Th1/Th2/Th17 immune responses was partially abolished in Pou2f3$^{-/-}$ mice. Similarly, in Pou2f3$^{-/-}$ + DSS mice, administration of berberine had no effect on mRNA expression levels of inflammatory cytokines including *Ifn-γ*, *Tnf-α*, *Il-6*, *Il-4*, *Il-10*, and *Il-13* when compared with WT + DSS mice (Fig. 3I-J). These results demonstrate that tuft cells are essential for both the anti-colitis effect of berberine and its induction of type 2 immune responses in the colon.

Berberine activates bitter taste signalling pathway

To investigate whether berberine could activate the bitter taste receptor signalling pathway during enteritis, we examined the expression of genes related to this signalling pathway. Berberine treatment differentially increased the expression of *Tas2r143*, *Tas2r136*, *Tas2r117*, *Tas2r108* in the epithelial cells of the colon (Fig. 4A-D). In addition, berberine can also increase the expression of α, β, and γ subunits of bitter taste receptors and their downstream effector *Trpm5* in the epithelial cells of the colon (Fig. 4E-H). To further explore the activation of bitter taste signalling, we co-localized Dclk1, α-gustducin and Trpm5 by immunofluorescence staining, and found that berberine effectively upregulated the expression of those two proteins in tuft cells (Fig. 4I).

Inhibition of bitter taste receptor partially abolishes the anti-enteritis effect of berberine

To determine whether the preventive effect of berberine on tuft cells is dependent on bitter taste signalling, U73122, a PLCβ2 specific inhibitor, was used to block bitter taste signalling. Our data indicated that U73122 partially reduced the protection of berberine against colitis (Fig. 5A-F). Meanwhile, administration of U73122 partially blocked the berberine-induced increase in the percentage of Th2 cells and decrease in the percentage of Th1/Th17 cells (Fig. 5G-H). Similarly, U73122 significantly decreased the population of gut ILC2s as quantified by flow cytometry (Fig. 5I). Additionally, the mRNA expression of *Il-25* and *Pou2f3* suggested a decreased tuft cell function in the U73122 + BBR + DSS group (Fig. 5J-K), and the number of DCLK1^{+} tuft cells was also decreased in the U73122-treated group (Fig. 5L-M). Overall, these results indicate that U73122 partially abolishes the anti-enteritis effects of berberine and support that the berberine-induced beneficial effects on colitis are dependent on bitter taste signalling.

U73122 inhibits berberine-induced bitter-taste receptor activation in enteritis

Previous studies have suggested that the immune regulation of tuft cells is a regulatory process with negative feedback [14, 29]; therefore, to verify whether U73122 could inhibit the berberine-induced activation of Tas2rs, we analysed the gene expression of the effectors in the bitter taste receptor signalling pathway. U73122 partially decreased the expression of the bitter taste receptor (Fig. 6A-D), and the β and γ subunits of the G protein (Fig. 6E-G). The decreased expression of *Trpm5* suggested that ion channel activation was also partially suppressed (Fig. 6H). Furthermore, immunofluorescence colocalization of tuft cells with α-gustducin and Trpm5 was employed to ascertain the activation of bitter taste signalling in tuft cells. As showed in Fig. 6I, U73122 markedly suppressed the expression of these two proteins. Additionally, The immunohistochemical results demonstrated a significant reduction in the expression of tight junction proteins Occludin and Claudin-1, as well as an inhibitory effect on berberine-mediated intestinal injury repair by U73122. (Additional file 1: Fig. S5A-D). The mechanism diagram of berberine acting on tuft cells to relieve colitis was showed in Fig. 7.

Discussion

IBD, as a type of intestinal disease characterized by dysregulated immune response in the intestinal mucosa, has garnered significant attention. Currently, the clinical manifestation of IBD primarily manifests as chronic inflammation in the intestines; however, its pathogenesis encompasses occult inflammation, acute exacerbations, and a chronic active phase [30–32]. In this study, we employed the DSS-induced acute colitis model to simulate the acute exacerbation of IBD in order to evaluate

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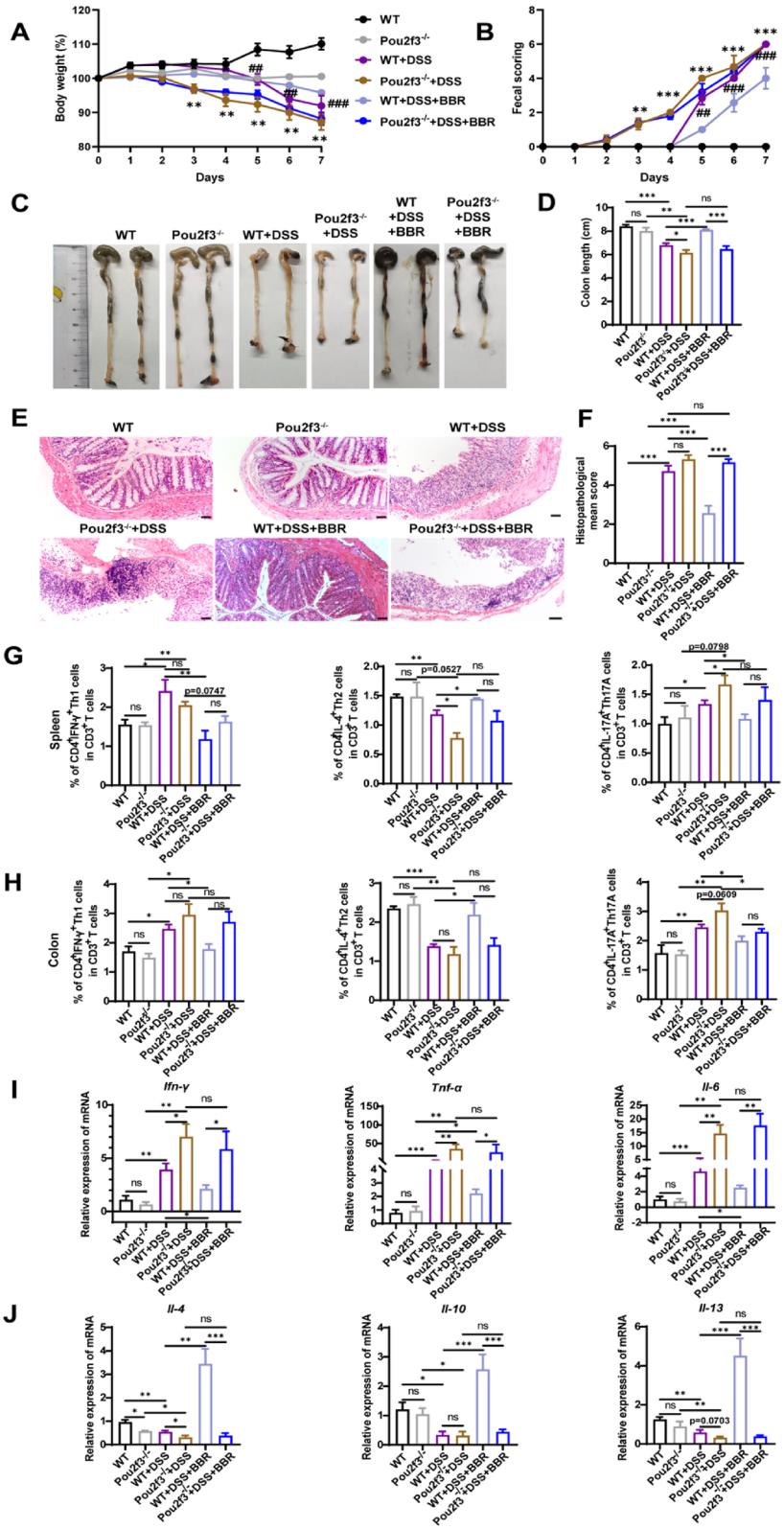
Fig. 3 Berberine alleviates colitis by regulating gut type 2 immunity in a tuft cells-dependent manner. **A** Body weight changes of each group during disease progression ($n = 8$); **B** DAI score of each group; **C** Representative colon images of the six groups; **D** Colon length was analyzed; **E** Representative H&E images of colons (scale bars, 100 μm); **F** Statistics of histological score; **G**, **H** Flow cytometric analysis of IFN-γ^{+} (Th1), IL-4^{+} (Th2) and IL-17A^{+} (Th17) in CD4^{+} T-lymphocyte of spleen and large intestine from 6 groups of male mice ($n = 6$); **I** The mRNA expression level of pro-inflammatory cytokines (*Ifn-γ/Tnf-α/Il-6*) and **J** anti-inflammatory cytokines (*Il-4/Il-10/Il-13*) in the colon ($n = 8$). All data were presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$



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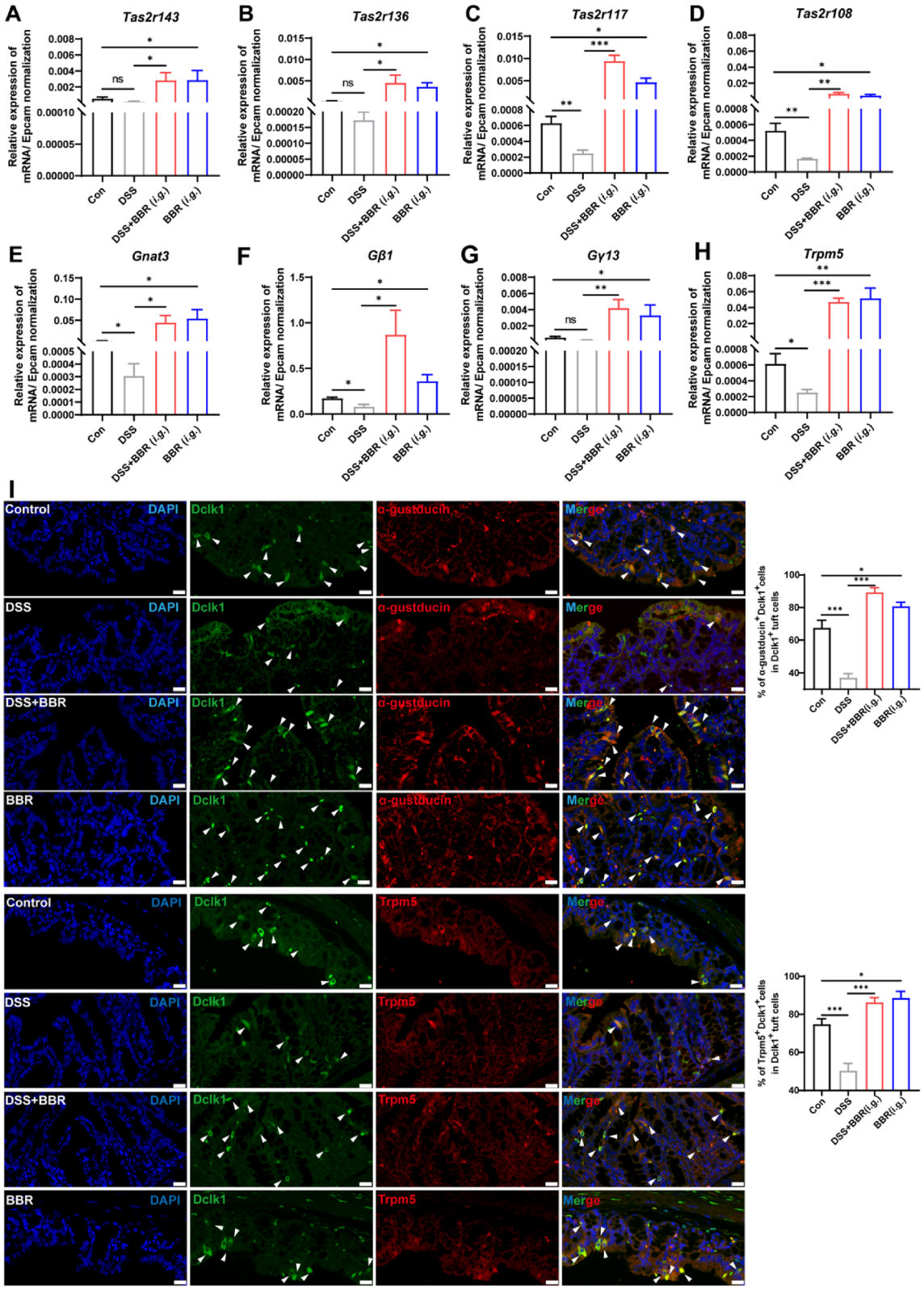

Fig. 4 Berberine activates bitter taste signalling pathway. **A**-**D** The mRNA expression level of bitter taste receptor (*Tas2r143/Tas2r136/Tas2r117/Tas2r108*) and **E**-**H** signalling pathways downstream of bitter taste receptors in the colonic epithelial cell (*Epcam* normalization) by qRT-PCR ($n=8$); **I** Immunofluorescence co-localization of tuft cells (Dclk1) with α-gustducin and Trpm5 (scale bars, 20 μm) ($n=6$) and statistics of the percentage of Dclk1$^+$ α-gustducin$^+$ or Dclk1$^+$ Trpm5$^+$ cells in Dclk1$^+$ tuft cells. All data were presented as mean ± SEM. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

the preventive and therapeutic effects of berberine (BBR). Notably, BBR has been extensively utilized in clinical practice for treating diarrhea including acute-onset cases of IBD with remarkable efficacy. Nevertheless, despite its widespread use, the specific mechanism underlying berberine's treatment effect remains unclear. In the present study, we found that BBR exert beneficial effect on DSS-induced colitis, which was strongly associated with tuft cells-mediated type 2 immune responses and bitter taste signalling pathway.

Despite its poor in vivo bioavailability, berberine exerts considerable therapeutic effects against colitis in both patients and mice [27]. The proposed mechanisms for berberine therapeutic action in enteritis include reducing neutrophil infiltration and gut epithelial cell apoptosis, regulating gut flora dysregulation to alter microbial metabolites, and repairing the gut barrier damage [23, 24, 33]. Berberine can significantly recover dysregulated microbial tryptophan catabolites such as IAA (Indoleacetic acid), IPA (indolepopionic acid), and IA (indoleacrylic acid), which have been reported to regulate gut flora composition and reduce inflammation in mice by improving gut epithelial barrier function. Most tryptophan metabolites are AhR ligands, which can act synergistically with AhR to restore gut microbiota imbalance and improve gut barrier function. These metabolites can also promote the expression of the downstream target gene, *Il-22*, which mediates the inflammatory response during the development of IBD [23]. The enteric nervous system (ENS), has been linked to the pathogenesis of colitis and other gut inflammatory diseases. The neuroimmune axis of the ENS is a complex and broad network of sensory neurons, interneurons, and glial cells within the digestive system, of which enteric glial cells (EGCs) are the major constituents. Decreased EGC levels or functional abnormalities can result in gut barrier failure. The administration of berberine can improve mucosal barrier homeostasis in colitis, preserving EGC residency, reducing inflammatory infiltration, and stabilizing overactive immune cells. Furthermore, in co-culture setups, berberine modified the interactions between gut EGCs, IECs (Intestinal epithelial cells), and immune cells. These findings offer a novel concept for the use of berberine in the treatment of enteric neurogenic enteritis [24]. However, these findings only partially explain the multi-pharmacological effects of berberine, and a more comprehensive and in-depth understanding of the mechanism of action of berberine in the gut is required for its safe clinical administration.

Tuft cells were first hypothesised to be involved in chemosensory sensation because of their structural characteristics, which are similar to those of tongue taste bud cells [9, 34]. Our findings showed that berberine treatment dramatically enhanced the transcription of *Tas2r143, Tas2r136, Tas2r117, Tas2r108* in the colon (Fig. 4A-D). Berberine also increased the mRNA expression of Gα, β and γ subunits of the G protein-coupled receptors, which was consistent with an increased expression of *Trpm5* (Fig. 4E-H). At the same time, the expression of α-gustducin and Trpm5 on tuft cells was also significantly enhanced by berberine treatment (Fig. 4I). Trpm5 is a key cation channel for taste sensation and signal transmission, stimulated by bitter, sweet, and umami tastants. *Trichinella spiralis* infection is sensed by tuft cells, through the TAS2Rs-Trpm5-mediated taste signalling, increasing the amplification of the tuft cell lineage, along with the release of IL-25 [14]. Furthermore, we observed berberine-induced promotion of *Pou2f3* and *Il-25* expression (Fig. 5I-J), suggesting that the bitter taste receptors are crucial for sensing and initiating type 2 immunity. Recent studies have found that taste receptor signalling goes beyond sensing food flavour, showing a determinant role in the modulation of the immune responses to microbes and parasites [13, 14]. Both excretory–secretory products (E–S) and extract of *Trichinella spiralis* can stimulate the release of IL-25 from tuft cells in intestinal organoids through the activation of bitter taste receptor [12]. Additionally, research has shown that α-gustducin, the crucial G-protein α subunit involved in sweet, umami and bitter taste receptor signalling, played an important role in gut inflammation. α-gustducin knockout mice exhibit aggravated inflammation and increased immune cell infiltration in the gut [35]. Furthermore, bitter taste receptor signalling in the oral cavity also promotes anti-inflammatory effects. Studies have found that the activation of TAS2R16 signalling by salicin inhibits the release of pro-inflammatory cytokines by partly suppressing HGFs' NF-B/p65 nuclear translocation of HGFs and LPS-induced increase in intracellular cAMP [36]. Aberrant TAS2Rs signalling was suggested as a potential promotor of cardiac inflammatory and oxidative stress diseases, defined by contractile dysfunction and arrhythmia [37]. Additionally, TAS2Rs activation shows beneficial anti-asthma effects by reducing allergic airway inflammation and preventing neuroinflammatory reactions [38]. Increasing evidence suggested that berberine regulates the TAS2Rs-GLP-1 secretion pathway in mouse enteroendocrine cells, which have therapeutic value for hyperglycaemia. Furthermore, as mentioned, oral berberine ameliorates HFD-induced obesity through the TAS2R-IL-25 signalling pathway in tuft cells in the gut [27, 28]. Our findings also showed the beneficial effects of berberine in colitis were decreased upon suppression of the bitter taste receptor signalling (Fig. 5A-F). Collectively, bitter taste receptor signalling plays an

important role in modulating inflammation and may be an effective target for gut inflammation treatment.

Besides bitter-taste receptors (TAS2Rs), tuft cells have been shown to be highly enriched with other GPCRs, such as Gpr91/Sucnr1 and Gpr41/Ffar3 [39]. These receptors can potentially identify pathogens through their reaction with compounds produced by luminal microorganisms. Studies have shown that succinate derived from the commensal microbiome can reduce gut inflammation by driving ATOH1-independent tuft cell gene expression and expansion [40]. Copious evidence identified Sucnr1 as a critical sensor of the succinate acid secreted from intestinal parasites, such as the protist *tritrichomonad*. Furthermore, this recognition is usually followed by a type 2 immune response via the tuft-ILC2 circuit [13, 40]. The helminth *Nippostrongylus brasiliensis* also secretes succinate, however, the consequent activation of tuft cells is not dependent on Sucnr1 but on Trpm5, which suggests that either tuft cells do not sense helminth-derived succinate or that sensing is redundant [12, 14, 29]. STAT6 is required for anti-helminth immunity; the helminth induced-O-GlcNAcylation activates epithelial STAT6 signalling to promote *Pou2f3* transcription and tuft cell differentiation [41]. Furthermore, tuft-2 cells regulate gut immunity against bacterial infections through the recognition of bacterial metabolites by Vmn2r26 [42]. In our previous study we found that stimulating tuft cells through the free fatty acid receptor 3 (Ffar3) pathway resulted in IL-25 secretion, IPA-mediated repair of intestinal barrier damage, and amelioration of metabolic dysfunction and high-fat diet-induced obesity [43]. These results suggest that tuft cells are crucial for gut immunity and inflammation, and that these effects are mediated by different receptors.

Disturbances in gut immunity are concurrent with the progression of colitis. An adaptive immune response can be triggered in the intestinal lamina propria resulting from the delivery of foreign antigens to T and B cells by expert antigen-presenting cells. The initiation and maintenance of inflammation in patients is correlated with an increased number of activated and mature T cells. Naïve T cells can differentiate into functional populations, such as Th1, Th2, Th9, Th17, or Treg cells. This procedure aids in the elimination of specific pathogens; however, a dysregulated T cell response, with aberrant activation of T cell subsets and excessive release of cytokines and chemokines, can cause persistent inflammation [3, 44]. With the progression of UC (Ulcerative Colitis) T regulatory cells (Treg), which suppress Th17 activity, typically decrease, whereas the T helper cell 17 (Th17) population, which leads to inflammation, typically increases. Previous studies have reported that berberine can alleviate enteritis through regulation of Treg/Th17 [7]. Furthermore, in UC patients the gut type 1 immune response is enhanced as well as the expression levels of TNF-α, IFN-γ, IL-1β and IL-6 in the serum [3]. Studies have shown that inducing a Th2 response could be advantageous when mucosal inflammation is mainly mediated by the Th1 or Th17 pathways. TNBS (trinitro-benzene-sulfonic acid)-induced colitis is significantly reduced with *Il-4* gene therapy. Meanwhile, IL-4 and IL-10, as type 2 cytokines, can suppress the Th1 pro-inflammatory response, which alleviated the intestinal tissue damage and decreased the levels of IFN-γ and TNF-α in the mucosa [45–47]. Furthermore, IL-21, a pro-inflammatory cytokine secreted by Th17 cells, also contributes to gut inflammation in patients with IBD, and the inhibition of IL-21 can protect mice against Th1/Th17 cell-driven colitis [6]. Studies have also showed that in the mucosa of patients with IBD, Smad7 downregulation can restore the TGF-β signal that is pivotal for Treg cells-mediated maintenance of intestinal immunological homeostasis [5, 48, 49]. In our study, we also demonstrated that DSS promoted Th1/Th17 differentiation, while berberine reversed this effect; berberine-treated mice exhibited higher Th2 percentages than the DSS group in a tuft cell-dependent manner (Fig. 3H-I). In addition, we also found a bit of IL-17A-positive cells in non-CD4-positive T cells, which have no significant changes after berberine administration (Additional file 1: Fig. S6). Hence, by controlling the intestinal type 2 immune response, tuft cells can reduce the upregulated Th1/Th17 response during enteritis, improving enteritis, reducing pathological damage, and becoming a viable target for enteritis treatment.

Since we only investigated the Th1, Th2 and Th17 immune response in colitis, we did not address

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Fig. 5 Inhibition of bitter taste receptor partially abolishes the anti-enteritis effect of berberine. **A** Body weight changes of each group during disease progression ($n=8$); **B** DAI score of each group; **C** Representative colon images of the four groups; **D** Colon length was analyzed; **E** Representative H&E images of colons (scale bars, 100 μm); **F** Statistics of histological score; **G**, **H** Flow cytometric analysis of IFN-γ^+ (Th1), IL-4^+ (Th2) and IL-$17A^+$ (Th17) in $CD4^+$ T-lymphocyte of spleen and large intestine from 4 groups of male mice ($n=6$); **I** Flow cytometric analysis of the number of Lineage$^-$CD45$^+$KLRG1$^+$GATA3$^+$ ILC2 of large intestine from 4 groups of male mice ($n=6$); **J**, **K** The mRNA expression level of *Pou2f3* and *Il-25* in the colonic epithelial cell (*Epcam* normalization) by qRT-PCR ($n=8$); **L** Immunofluorescence staining of DCLK1 of colonic tissue (scale bars, 100 μm, 50 μm and 20 μm respectively) ($n=6$); **M** Statistics of the number of tuft cells. All data were presented as mean ± SEM. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$

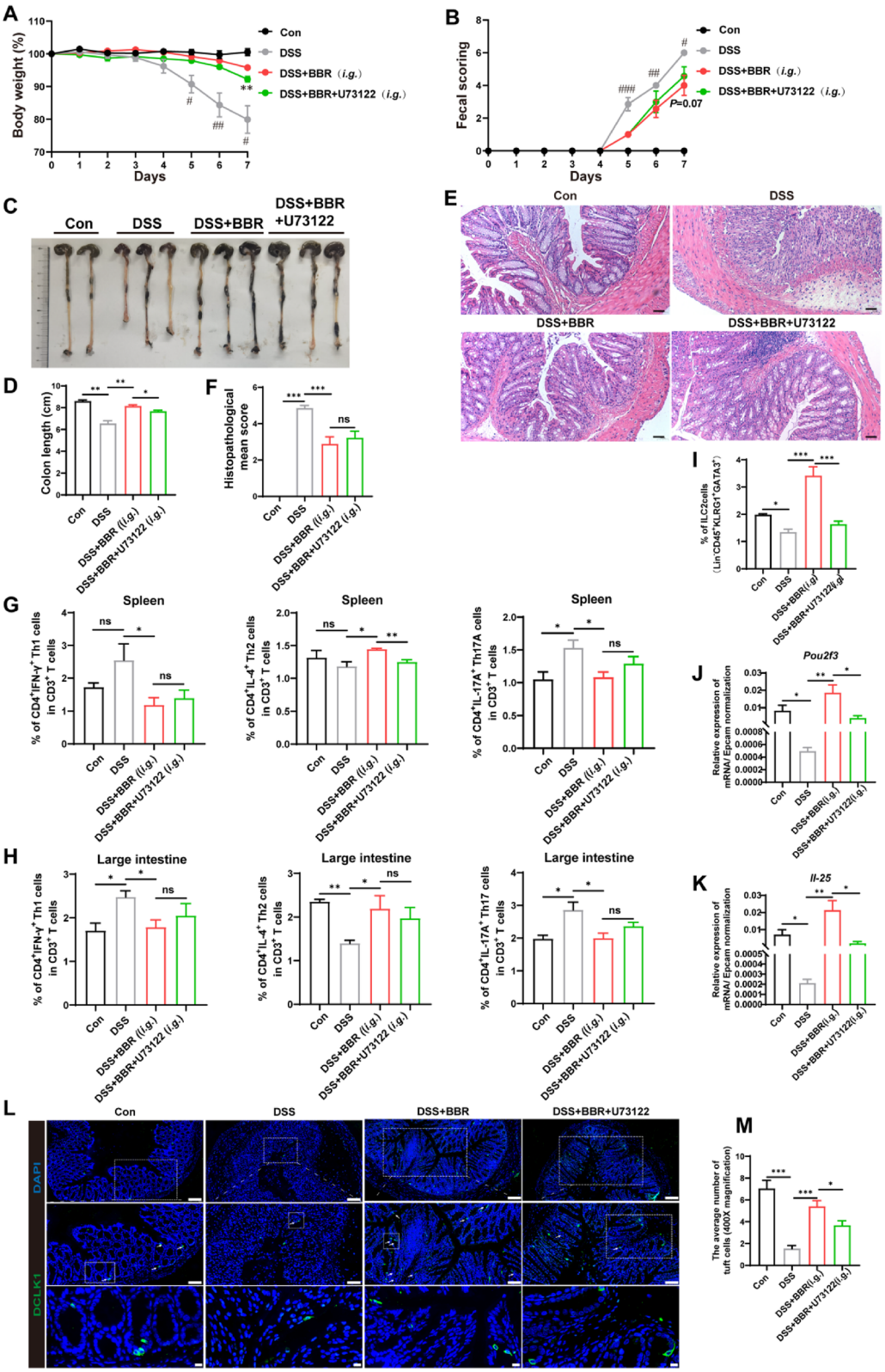

Fig. 5 (See legend on previous page.)

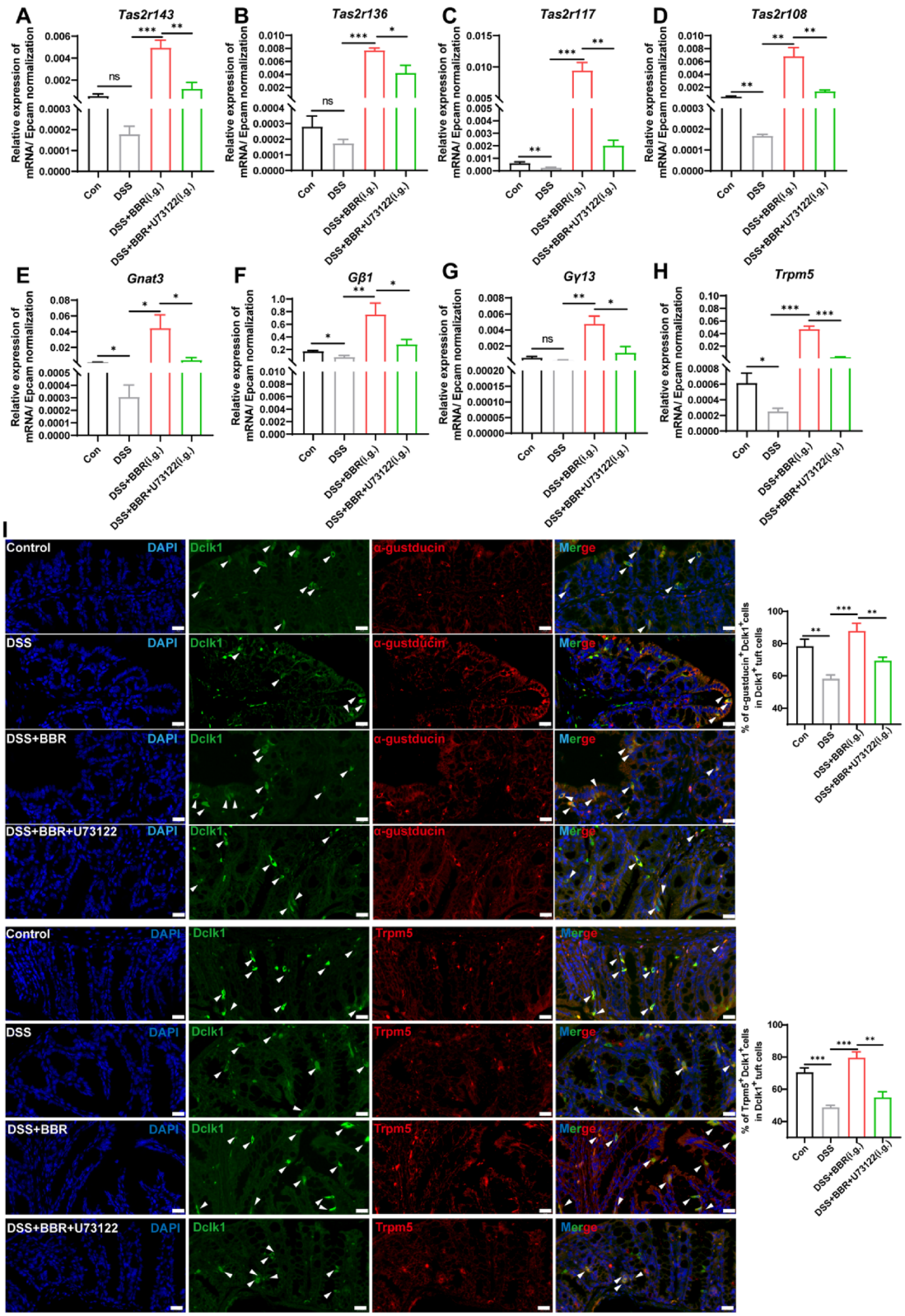

Fig. 6 U73122 inhibits berberine-induced bitter-taste receptor activation in enteritis. **A**-**D** The mRNA expression level of bitter taste receptor and **E**-**H** signalling pathways downstream of bitter taste receptors in the colonic epithelial cell (*Epcam* normalization) by qRT-PCR ($n=8$); **I** Immunofluorescence co-localization of tuft cells (Dclk1) with α-gustducin and Trpm5 (scale bars, 20 μm) ($n=6$) and statistics of the percentage of Dclk1$^+$ α-gustducin$^+$ or Dclk1$^+$ Trpm5$^+$ cells in Dclk1$^+$ tuft cells. All data were presented as mean ± SEM. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

macrophages, dendritic cells and so on that regulate inflammatory response. In addition, our data have shown that U73122 partially inhibited the therapeutic effect of berberine, indicating that berberine may regulate tuft cells function through other receptors, which remains to be further studied.

Conclusions

Collectively, our research uncovered a novel mechanism of berberine in the treatment of enteritis: tuft cells sense berberine through their bitter taste receptors, secreting IL-25 to stimulate type 2 immune responses, which ultimately promote the regeneration of tuft cells, maintaining the integrity of the gut barrier and the homeostasis of Th1/Th2/Th17. This study has significant implications for our understanding of gut immunity and homeostasis and proposes berberine as a potential new therapeutic intervention for colitis.

Methods

Animals

Male Wild-type C57BL/6 mice (6–8 weeks, 23 ± 2 g) and Male tuft cell deficient (Pou2f3$^{-/-}$) C57BL/6 mice (6–8 weeks old, 23 ± 2 g) came from the Laboratory Animal Center of Nanjing Medical University (Jiangsu Province, China). Mice were housed under specific pathogen-free (SPF) conditions (21 ± 2 ℃, 12 h: 12 h dark–light cycle. Wild type mice were divided into five groups: Control (Con), DSS, DSS + 200 mg/kg berberine, DSS + berberine + 20 mg/kg U73122 and berberine (n = 8 in each group). Tuft cell deficient mice were divided into three groups: Pou2f3$^{-/-}$, Pou2f3$^{-/-}$ + DSS, Pou2f3$^{-/-}$ + DSS + berberine (n = 8 in each group).

Berberine ($C_{20}H_{18}NO_4^+$, purity: 99.70%, Selleck Chemicals, 2086–83-1, Houston, TX, USA) was administered orally one day in advance and once every two days. Mice were effectively given 3% DSS (Material Pioneer Inc, 160,110, Oklahoma, USA) orally by drinking water for seven consecutive days to create an established mouse model of acute colitis, which displayed symptoms such diarrhea, prostration, and bloody stools. The experimental intervention of U73122 (MedChemExpress, New Jersey, USA) was performed by gavage. Berberine and U73122 were dissolved in carboxymethyl cellulose sodium (CMC-Na, 0.5%, Macklin, 9004–32-4). The control mice were orally administered with solvent as a control.

Disease activity index score

Weight loss, stool consistency, and bleeding were assessed according to Millar et al. Methods [50, 51].

Histopathological examination

Colons were collected and fixed in 4% PFA for 48 h prior to paraffin embedding. Haematoxylin and eosin (H&E) were used to stain three-μm-thick sections following standard protocols and the degree of inflammation was assessed through a previously described histological colitis score. The scores ranged from 0 to 6 (total score) representing the degree of tissue damage and inflammatory infiltration. 0 = no inflammation or mucosal injury, 1 = slight accumulation of inflammatory cells or discrete mucosal injury, 2 = inflammatory infiltration of submucosa or surface mucosal erosions and focal ulcers, 3 = aggravated infiltration or extensive mucosal damage.

Immunofluorescence and immunohistochemistry

Colons were cut into 3 μm slices for immunohistochemistry. The sections were blocked with donkey serum containing 0.3% triton X-100 for 2 h at 21–25 ℃, after which antigens were unmasked with a 0.01 mol L^{-1} citrate buffer solution at 91–95 ℃ for 3 min. Slices were then incubated with anti-Dclk1 antibody (Abcam, Cambridge, ab37994, MA, USA), anti-MUC2 antibody (Proteintech, 27,675–1-AP), anti-ChgA antibody (Abcam, Cambridge, ab283265, MA, USA), anti-α-gustducin antibody (Santa cruz, sc-518163), anti-Dclk1 antibody (Proteintech, 68,234–1-Ig) and anti-Trpm5 antibody (Proteintech, 18,027–1-AP), overnight at 4 ℃. After incubation, slices were washed thrice (5 min per wash) with phosphate-buffered saline (PBS) and incubated with fluorescein isothiocyanate (FITC)-labelled anti-rabbit IgG antibody (Thermo Fisher Scientific, A-21206, PA, USA) for 2 h at 22–25 ℃. Finally, the sections were sealed with an anti-fluorescence quenching tablet containing DAPI (4′,6-diamidino-2-phenylindole, Solarbio, Beijing, China) for nuclei staining and photographed under a fluorescence microscope (400 × magnification, ZEISS, Jena, Germany). The frequency of DCLK1$^+$ tuft cells and ChgA$^+$ enteroendocrine cell were determined by employing Image J software. The positive staining area of MUC2$^+$ goblet cells was counted using Image J software. The percentage of α-gustducin$^+$Dclk1$^+$ cells and Trpm5$^+$Dclk1$^+$ cells in Dclk1$^+$ positive cells in the colon was enumerated and subjected to analysis.

Colon sections were de-paraffinized in xylene, rehydrated using a graduated alcohol-to-water ratio and blocked as previously mentioned. The slides were then incubated with the primary antibodies anti-Occludin (abcam, ab216327, MA, USA) and Claudin-1 (Santa Cruz, sc81796, MA, USA), overnight at 4 °C. After primary antibody incubation, slides were washed thrice with PBS-T and then incubated with streptavidin–horseradish peroxidase (Absin Bioscience Inc, Shanghai, China) for 40 min at 22–25 ℃, followed by an incubation with the

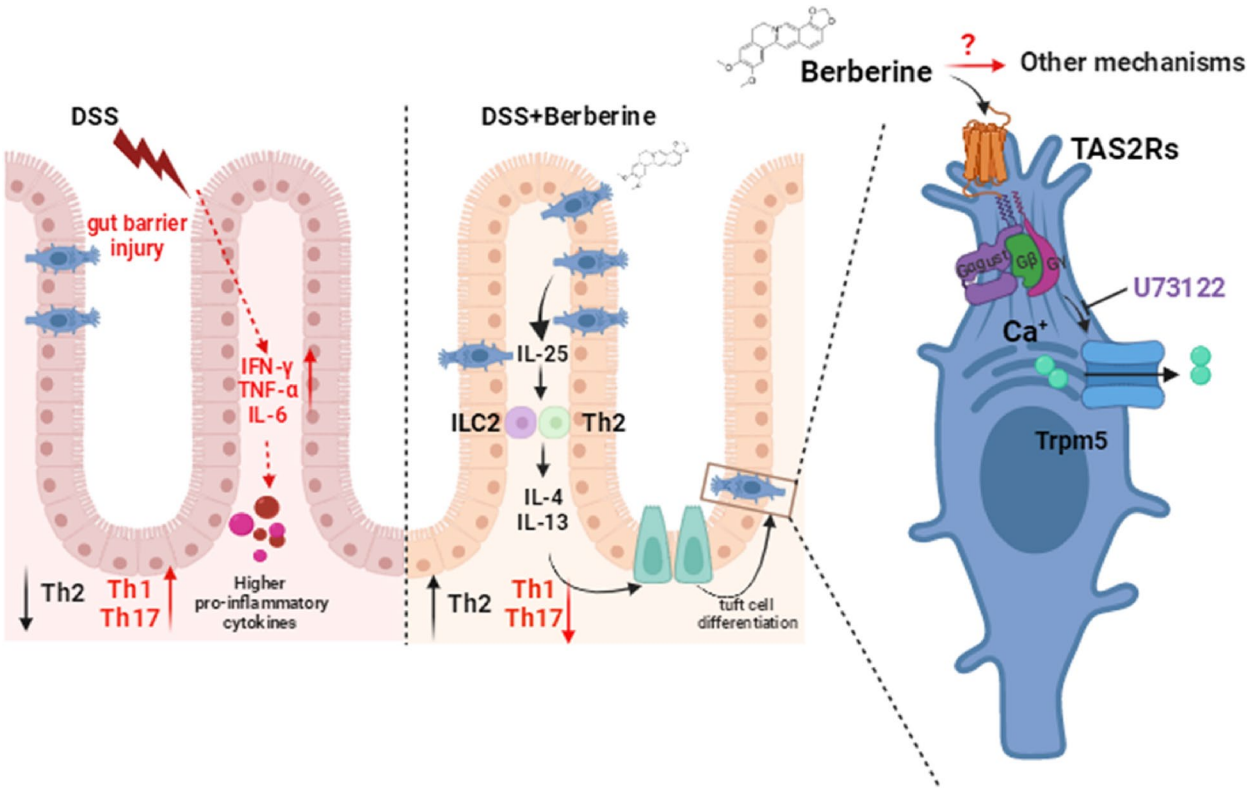

Fig. 7 Mechanism diagram of berberine acting on tuft cells to relieve colitis. During the development of colitis, gut barrier injury is accompanied by an increase in Th1/17 cells as well as a plethora of pro-inflammatory cytokines. Concurrent administration of berberine activates the bitter taste signaling pathway, thereby enhancing tuft cell functionality in regulating type 2 immune response and suppressing inflammation in the colon

substrate 3,3′-diaminobenzidine (Shanghai Gene Company, Shanghai, China) for 5–15 min. Haematoxylin was used as a counterstain. Image acquisition was performed using a Zeiss microscope (ZEISS, Jena, Germany).

Culture of colonic organoids and immunofluorescence staining

The cultivation and handling of colonic organoids were performed as described [52]. Colon tissues were taken from mice with 4 weeks age, cut longitudinally, remove feces, clean with cold PBS solution at least 15 times. Incubate the tissue at 4 °C on a rotator at 20 rpm for 30 min in 25 mL 2.5 mmol/L cold EDTA solution. Then, resuspend the tissue in 10 mL cold PBS and filter the upper liquid through a 70 μm filter membrane into a 15 mL centrifuge tube. Repeat the previous step six times, labeling the tubes 1–6. Take 500 μL of each tube and transfer it to 12-well plates under a microscope to observe the quality and number of crypts. Count and record the best result, which is 500 crypts per well. Resuspend the Matrigel (Corning, NY, USA) mixed culture medium and form a hemispherical shape on the plate. Add 500 μL of culture medium (Stem Cell Technologies, Vancouver, BC, Canada) to each well after the gel solidifies. Crypts usually form within 5–7 days. On day 6, the organoids were treated with berberine at concentrations of 10 μM for 48 h. For immunofluorescence staining, the Matrigel-containing organoids were placed in a refrigerator for 15 min to dissolve the Matrigel. Then they were centrifuged at 3000 g for 10 min and fixed in 1 mL PFA for 45 min. Staining was performed using anti-DCLK1/MUC2/ChgA antibody, DAPI stain (Vazyme, Nanjing, China), following the aforementioned methods.

ELISA

The levels of IL-25 in the mouse colonic organoid culture supernatants were determined using a mouse IL-25 ELISA kit (MULTI SCIENCES, EK2179, Hangzhou, China).

RNA extraction and quantitative real-time polymerase chain reaction(qRT-PCR)

Colon tissues were homogenised in TRIzol before total RNA extraction using a RNA extraction kit (Thermo Fisher Scientific, Waltham, MA, USA), according to manufacturer's instructions. The RNA purity and

concentration was determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific) and RNA was converted in cDNA by reverse transcription. Thereafter, quantitative real-time PCR was carried out using a iQTM SYBR® Green Supermix (Vazyme Biotech, Nanjing, China) on a BioRad CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Relative gene expression was calculated using the comparative cycle threshold (CT) method. β-actin was used as a housekeeping gene to normalise gene expression. The primer sequences used in this study are listed in Additional File 2: Table 1.

Extraction of lymphocytes from the colonic *lamina* propria and spleen

The large intestine was prepared for analysis by removal of fat and lymph nodes from its surface, followed by a longitudinal incision to extract its content. The tissue was then cut to 1–2 cm pieces, incubated for 10 min at 22–25 ℃ with 1 mmol L^{-1} DTT (Ditiotreitol, Aladdin, 3483–12-3, USA) at 250 rpm, and then further digested for 10 min at 37 °C at 200 rpm with PBS containing 30 mmol L^{-1} EDTA (Aladdin, 139–3–33, USA) and 10 mmol L^{-1} HEPES (Aladdin, H109408, USA). An additional digestion step was conducted using RPMI1640 media (Sigma R8758, USA) containing 150 g/mL of DNase I (Sigma, 9003–98-9, USA) and 150 μg/mL of collagenase VIII (Sigma, 9001–12-1, USA), in an incubator with 5% CO_2 at 37 °C for 75 min. The homogenate was collected, shaken vigorously until fragmentation by a vortex, and centrifuged at 1800 rpm for 5 min at 22–25 ℃. The supernatant was removed. The cells were reconstituted in 4 mL of 40% Percoll, and 80% Percoll (2.5 mL) (univ, 17,089,109–1, Shanghai, China) was gradually added to the bottom of the tube while rotating at 2500 g at 22–25 ℃. Twenty minutes of centrifugation was spent using a gradient centrifugation. The cells in the middle layer were collected, counted, and characterized using flow cytometry. Splenic lymphocytes were extracted with reference to previous studies [53].

Flow cytometry analysis

The lymphocytes collected from the large intestine were incubated with PerCP-Cy5.5 anti-mouse CD3 (BD, 551,163, New York, USA), FITC anti-mouse CD4 (Biolegend, 100,406, Beijing, China), PE anti-mouse IFN-γ (eBioscience™, 12–7311-82, USA), APC anti-mouse IL-4 (eBioscience™, 17–7041-82, USA) and BV421 anti-mouse IL-17A (BD, 563,354, New York, USA); APC anti-mouse CD45 (Biolegend, 157,606, Beijing, China), FITC anti-mouse lineage (eBioscience™, 22–7770-72, USA), PE anti-mouse KLRG1 (BD, 561,621, New York, USA), BV421 anti-mouse GATA3 (Biolegend, 653,814, Beijing, China) and acquired in a BD FACSVerseTM cytometer (BD Biosciences, Heidelberg, Germany). Results were analysed using the FlowJo® software (Treestar Inc., San Carlos, CA, USA).

Statistical analysis

All of the experiments were performed at least three times independently. Data were presented as mean ± SEM. Paired two-tailed Student's t-test was utilized for the comparison of two groups; one-way or two-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used for the comparison of more than two groups. Statistical significance was defined as $P < 0.05$, and was performed using GraphPad Prism software (version 8.0, San Diego, CA).

Abbreviations

BBR	Berberine
IBD	Infammatory bowel disease
UC	Ulcerative colitis
CD	Crohn's disease
Pou2f3	POU domain, class 2, transcription factor 3
ILC2	Type 2 innate immune lymphocytes
DSS	Dextran sulfate sodium
Dclk1	Doublecortin like kinase 1
DKO	Mucin-type O-glycan deficiency
CR	Citrobacter rodentium
Sucnr1	Succinate receptor 1
Gnat3	α-Gustducin
ChAT	Choline acetyl transferase
Trpm5	Transient receptor potential cation channel subfamily M member 5
PLCβ2	Phospholipase C beta 2
IP3	Inositol 1,4,5-trisphosphate
ENS	Enteric nervous system
EGCs	Enteric glial cells
IECs	Intestinal epithelial cells
TNBS	Trinitro-benzene-sulfonic acid
Ffar3	Free fatty acid receptor 3

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12915-024-02078-7.

Additional file 1: Fig. S1 - [Comparison of the effect of berberine on enteritis at different time]. Fig. S2 - [Immune changes of tuft-ILC2 circuit during different periods of treatment of enteritis with berberine]. Fig. S3 - [Changes of colonic goblet cells and enteroendocrine cells with berberine treatment time]. Fig. S4 - [Berberine does not affect the proliferation of colonic epithelial cells in stem cells]. Fig. S5 - [Inhibition of bitter receptor signaling affects gut barrier damage repair]. Fig. S6 - [The bitter receptor signaling does not affect the number of non-CD4+ Th17 cells].

Additional file 2: Table 1 - [Primers sequences used for qRT-PCR].

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Authors' contributions

Y.X.Y., L.C.2 and M.J.J. designed and conceived the study; Y.X.Y., W.Q.L., K.N.S., S.Y.S., Y.Z., Y.Y.N., L.C.1., M.H., and Z.P.X. performed the experiment; Y.X.Y., W.Q.L. and S.Y.S., analyzed the data; Y.X.Y. wrote the paper; L.C.2. amended the paper. All authors read and approved the final manuscript.

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Data availability
All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate
All animal experiments were examined and approved by the Nanjing Medical University Ethical Research Committee and carried out strictly in compliance with the guidelines for managing experimental animals (experimental animal ethics number IACUC—2107035).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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