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RESEARCH ARTICLE

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# Protein arginine methyltransferase 2 (PRMT2) promotes dextran sulfate sodium-induced colitis by inhibiting the SOCS3 promoter via histone H3R8 asymmetric dimethylation

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**Background and Purpose:** There is emerging evidence for a critical role for epigenetic modifiers in the development of inflammatory bowel disease (IBD). Protein arginine methyltransferase 2 (PRMT2) is responsible for the methylation of arginine residues on histones and targets transcription factors involved in many cellular processes, including gene transcription, mRNA splicing, cell proliferation, and cell differentiation. In this study, the role and underlying mechanisms of PRMT2 in colitis were studied.

**Experimental Approach:** A mouse dextran sulfate sodium (DSS)-induced experimental colitis model was used to study PRMT2 in colitis. Lentivirus-induced PRMT2 silencing or overexpression *in vivo* was applied to address the role of PRMT2 in colitis. Detailed western blot and expression analysis were done to understand epigenetic changes induced by PRMT2 in colitis.

**Key Results:** PRMT2 is highly expressed in inflammatory bowel disease patients, in inflamed murine colon and in TNF-α stimulated murine gut epithelial cells. PRMT2 overexpression aggravates, while knockdown alleviates DSS-induced colitis, suggesting that PRMT2 is a pivotal mediator of colitis in mice. Mechanistically, PRMT2 mediates colitis by increasing repressive histone mark H3R8 asymmetric methylation (H3R8me2a) at the promoter region of the suppressor of cytokine signalling 3 promoter (SOCS3). Resultant inhibition of SOCS3 expression and inhibition of SOCS3-mediated degradation of TNF receptor associated factor 5 (TRAF5) via ubiquitination led to elevated TRAF5 expression and TRAF5-mediated downstream NF-κB/MAPK activation.

**Conclusion and Implications:** Our study demonstrates that PRMT2 acts as a transcriptional co-activator for proinflammatory genes during colitis. Hence, targeting PRMT2 may provide a novel therapeutic approach for colitis.

## 1 | INTRODUCTION

Inflammatory bowel disease (IBD), consisting of ulcerative colitis and Crohn's disease, is a collection of remittent inflammatory disorders in the gastrointestinal tract and clinically characterized by recurrent and long-lasting episodes of diarrhoea and abdominal pain (Khor et al., 2011; Liu & Stappenbeck, 2016). The disease has a profound impact on quality of life but is also recognized as a leading causative risk factor for the progression of colorectal cancer, which is mediated through long-term chronic intestinal inflammation (Kim & Chang, 2014; Yashiro, 2014). Consequently, there is an urgent need for new targets and novel therapies for inflammatory bowel disease.

Despite the availability of an enormous amount of clinical and experimental data on the development of inflammatory bowel disease in both experimental animals and humans, the precise aetiology of inflammatory bowel disease remains elusive. It is generally believed that both genetic alterations and environmental factors contribute to the pathogenesis of inflammatory bowel disease. With the onset of inflammatory bowel disease, enhanced secretion of inflammatory cytokines, such as **TNF-α** and **IL-6**, is observed in the inflamed intestinal mucosa and is correlated with the disease severity (Strober & Fuss, 2011; Xiao et al., 2016). TNF-α overproduction is a hallmark of inflammatory bowel disease and secreted TNF-α can amplify the inflammatory response by stimulating downstream inflammatory mediators via the activation of transcription factors, such as **nuclear factor-κB (NF-κB)** and MAPKs. This in turn exacerbates mucosal injury and contributes to chronic intestinal inflammation (Biasi et al., 2013; Olesen et al., 2016). Moreover, recent studies have revealed that epigenetic modifications, such as DNA methylation, histone modifications and noncoding RNA interactions, promote the development of inflammatory bowel disease by regulating the expression of cytokines and tight junction proteins (Jabandziev et al., 2020; Kline et al., 2020; Nakanishi et al., 2018; Qi et al., 2019). Understanding these inflammatory bowel disease-specific epigenetic changes will provide new insights into new ways to treat inflammatory bowel disease.

Histone methylation is a common histone modification implicated in gene transcription. Arginine methylation mediated by the **protein arginine methyltransferase (PRMT)** family is a widespread post-translational modification in eukaryote cells and plays a key role in many biological processes, such as transcription, cell signalling, pre-mRNA splicing and DNA damage signalling (Blanc & Richard, 2017; Kim et al., 2016). Using S-adenosine-methionine as the methyl donor, PRMT transfers methyl to the nitrogen atom of protein arginine side chain and produces S-adenosy-L-homocysteine and methyl arginine (Yang & Bedford, 2013). **PRMT2** is a key member of the PRMT family and contains a highly conserved catalytic *S*-adenosyl methionine

### What is already known

- Inflammatory bowel disease (IBS) impacts on quality of life and epigenetic modifiers involve its pathogenesis.
- PRMT2 involves cellular signalling and gene expression, however its role in colitis remains unknown.

### What does this study adds

- Lentiviral overexpression of PRMT2 aggravates, while knockdown alleviates dextran sulfate sodium (DSS)-induced colitis.
- PRMT2 mediates colitis by increasing repressive histone mark H3R8me2a at the SOCS3 promoter region.

### What is the clinical significance

- Targeting PRMT2 may provide a novel therapeutic approach for colitis.

(Ado-Met) binding domain and a unique Src homology 3 domain that binds proteins with proline-rich motifs (Zhong et al., 2014). PRMT2 is important in the regulation of cellular signalling and gene expression by methylating histones and nonhistone proteins (Dong et al., 2018). So far, PRMT2 has been shown to be involved in breast cancer (Morettin et al., 2015) and tumorigenesis of glioblastoma (Dong et al., 2018), whereas higher PRMT2 expression was observed in intestinal specimens from both Crohn's disease and ulcerative colitis patients (Krzystek-Korpacka et al., 2020). However, the role of PRMT2 in colitis remain unclear.

In the current study, we identify PRMT2 as a proinflammatory mediator in colitis and provide evidence that the onset of colitis and inflammatory responses are sensitive to lentiviral PRMT2 over-expression or knockdown *in vivo* and *in vitro*. Our mechanistic studies demonstrate that PRMT2-mediated H3R8me2a methylation is responsible for the suppression of suppressor of cytokine signalling 3 (SOCS3) and therefore inhibits SOCS3-mediated ubiquitination and degradation of TNF receptor associated factor 5 (TRAF5), subsequently leading to elevated TRAF5 expression and TRAF5-mediated downstream NF-κB/MAPK activation. Our data also provide mechanistic insights into a PRMT2-mediated epigenetic regulatory mechanism of colitis and could lead to novel therapeutic strategies for the treatment of colitis by targeting PRMT2.

## 2 | METHODS

### 2.1 | Mice

All animal experiments were conducted according to protocols approved by the Institutional Animal Ethics Committee of Jiangnan University (JN. No20180115c0320430). Animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the *British Journal of Pharmacology* (Lilley et al., 2020). Male C57BL/6 mice (7–8 weeks old, 20 ± 2 g, RRID:IMSR_JAX:000664) were purchased from Su Pu Si Biotechnology Co. Ltd. (Suzhou, Jiangsu, China). Mice were housed in a specific pathogen-free animal facility of Jiangnan University (Wuxi, Jiangsu, China) under a 12-h light–dark cycle and standard conditions for temperature (24 ± 1°C) and were fed *ad libitum* (AIN93G). Mice were group-housed (4-5 mice per cage) in individually ventilated cages (IVC). Animal studies were designed to generate groups of equal size in a blinded and random fashion.

### 2.2 | Lentivirus production and transfection

The small hairpin RNA targeting mouse PRMT2 as listed in Table 1 was cloned into the pLKO.1 lentiviral vector by T4 DNA ligase enzyme according to the manufacturer's instructions (New England Biolabs, Ipswich, MA, USA). Lentivirus were produced by co-transfection of the PRMT2 lentiviral construct, the packaging plasmid ps-PAX2 and the envelope plasmid pMD2 (at a ratio of 4:3:1) into HEK-293T cells (RRID:CVCL_0063) using Lipofectamin 3000 (Invitrogen, Carlsbad, CA, USA). After incubation for 48 h, viral particles in the cell culture medium were collected and filtered through a 0.45-μm membrane filter, followed by ultracentrifugation at 100,000 g for 30 min. The pellet was washed once and resuspended with PBS. Viral titres were measured by QuickTiter™ Lentivirus Titer Kit (Cell Biolabs, San Diego, CA, USA). PRMT2 overexpression was constructed by cloning full-length mouse PRMT2 into the pLVX-Puro lentiviral expression vector (Clontech, Palo Alto, CA, USA), and then transfected into HEK-293T cells as above described and used to infect mice or colonic epithelial cells. Mice or cells transduced with pLKO.1-scramble shRNA or blank pLVX-Puro vector were used as negative controls. For *ex vivo* studies, intestinal epithelial cells (IECs)

from dextran sulfate sodium (DSS)-treated mice were isolated using isolation buffer (30-mM EDTA and 1-mM DTT) as previously described (Greten et al., 2004).

### 2.3 | Dextran sulfate sodium (DSS)-induced colitis and delivery of lentivirus

DSS-induced colitis is the most widely used experimental model of acute colitis (Wirtz et al., 2007). To evaluate the role of PRMT2 in colitis, intracolonically administration of vehicle lentivector (shScr, scramble short hairpin RNA lentivector; Vector), PRMT2-shRNA lentivector (shPRMT2) or PRMT2-overexpression lentivector (PRMT2-OE) at a dose of $1 \times 10^9$ IU in a final volume of 100-μl PBS were first administered to pentobarbital sodium (30 mg·kg$^{-1}$; i.p.) anaesthetized mice. It should be noted that both epithelial cells and lamina propria cells could be affected by this procedure (Wirtz et al., 1999). The body temperature of spontaneously breathing anaesthetized mice was maintained on heated blankets. Mice were placed in separate cages, kept warm and monitored. All mice were fully recovery in 2 h. Two days later (Matsumoto et al., 2010), mice were given 3% (w/v) DSS (MW 36–50 kDa) in drinking water for 5 days followed by normal drinking water for 3 days. Negative control mice (CON) received normal drinking water (Li et al., 2020; Wirtz et al., 2017). Daily clinical evaluations, including the assessment of body weight, stool consistency and detection of rectal bleeding, were conducted to generate a disease activity index score. Each parameter was given a grade from 0 to 4 and then averaged as follows:- body weight loss (scored as 0, none; 1, 1%–5%; 2, 6%–10%; 3, 11%–15%; 4, >15%), stool consistency (scored as 0, well-formed pellets; 2, loose stools; 4, diarrhoea) and faecal blood (scored as 0, negative haemoccult test; 1, positive haemoccult test; 2, blood visibly present in the stool; 3, blood visible and blood clotting on the anus; 4, gross bleeding) as previously described (Kim et al., 2012). For permeability experiments, fluorescein isothiocyanate (FITC)-conjugated dextran (4000 MW) was gavaged at 0.6 g·kg$^{-1}$ of body weight (Sharma et al., 2018). After 4 h, mice were killed by an overdose of pentobarbitone (90 mg·kg$^{-1}$; i.p.) and serum concentrations of FITC-dextran were assessed. In addition, the colon epithelial permeability was determined using an Evans Blue assay according to Vargas Robles et al. (2017).

**TABLE 1**  The sequences of short hairpin (sh) RNA

| ID | Sequences (5′ → 3′) |
| --- | --- |
| PRMT2-shRNA#1 F | GATCGCCAAAGTCGAATCATATCTTCTCG AGAAGATATGATTCGACTTTGGCTTTTTG |
| PRMT2-shRNA#1 R | AATTCAAAAAGCCAAAGTCGAATCATATC TTCTCGAGAAGATATGATTCGACTTTGGC |
| Scramble shRNA F | GATCATCAGTAATCTTATTACCACAGTGC TTGGCCGTATTGGACTTACTTGTAGAGTA |
| Scramble shRNA R | AATTATTCATATAGGCATTATGGAATGTG TTGCTTATACGAAGATTCCGTCTCATGCC |

**TABLE 2**  The characteristics of human subjects (no. or mean ± SEM)

| Parameter | Control (N = 9) | UC (N = 10) | CD (N = 9) |
| --- | --- | --- | --- |
| Male number (%) | 5 (55.6%) | 4 (60.0%) | 4 (44.4%) |
| Age (mean ± SEM) | 52.6 ± 3.9 | 49.2 ± 9.0 | 54.2 ± 3.4 |
| Mayo score | 0 | 1.8 ± 0.2 | — |
| AI score | 0 | — | 54.9 ± 6.9 |

*Note*: Mayo score is a score used for evaluation of UC activity; Crohn's disease activity index (AI) score is a quantitative score for the assessment of activity.

Abbreviations: CD, Crohn's disease; UC, ulcerative colitis.

## 2.4 | Clinical samples

The human investigation protocol conformed to the WMA Declaration of Helsinki and the Ethical Review Methods for Biomedical Research involving Humans adopted by the National Health and Family Planning Commission of the People's Republic of China and was approved by the Ethics Committee of Affiliated Hospital of Jiangnan University (JN. No2019[029]). Written informed consent was obtained from all subjects. Colonoscopic biopsies were obtained from 9 Crohn's disease patients, 10 ulcerative colitis patients and 9 healthy individuals. The characteristics of human subjects were described in Table 2. The diagnosis of Crohn's disease or ulcerative colitis was confirmed by a gastroenterologist based on a standard combination of clinical, endoscopic, biochemical, stool and histological criteria. A Mayo score was used for evaluation of ulcerative colitis activity, which was based on four categories (bleeding, stool frequency, physician assessment and endoscopic appearance). The results correlate with disease severity: <2 remission; 3–5 mild; 6–10 moderate; 10–12 severe (Lewis et al., 2008). Crohn's disease activity index (CDAI) score was used for the assessment of Crohn's disease activity, which was based on clinically reported signs, laboratory results and patient-reported symptoms in a 7-day period. The three possible grades depending on the results are clinical remission <150; mild to moderate activity 150–450; and severe disease >450 (Freeman, 2008).

## 2.5 | Histopathological analysis

Fresh colonic tissues were fixed with 4% paraformaldehyde overnight and embedded in paraffin. After paraffin embedding, sections (5 μm) were cut and stained with haematoxylin and eosin (Hayakawa et al., 2010). Colonic injuries were examined under a DM2000 light microscope (Leica Microsystems GmbH, Wetzlar, Hesse, Germany) at 200× magnification. The stained tissues were histologically evaluated in a double-blind fashion as previously published, using a combined score for epithelial damage and infiltration of inflammatory cells. Histological scoring was performed as follows: epithelium (score 0, normal; score 1, crypt loss <10%; score 2, crypt loss 10%–50%;

score 3, crypt loss 50%–90%; score 4, crypt loss >90%; score 5, ulcer 1%–50%; and score 6, ulcer >50%); infiltration of mucosa (score 0, normal; score 1, <10%; score 2, 10%–50%; and score 3, >50%), submucosa (score 0, normal; score 1, 1%–50% and score 2, >50%) and muscle or serosa (score 0, normal and score 1, >1%). Histological scores were the sum of epithelial damage scores and inflammatory cell infiltration scores (Deng et al., 2019; Katakura et al., 2005).

## 2.6 | Isolation, culture and lentiviral infection of mouse colon epithelial cells

Mouse colonic epithelial cells were isolated and cultured as described previously (Booth & O'Shea, 2002; Di Claudio et al., 2017; Wang et al., 2017). Briefly, mice were killed by an overdose of pentobarbitone (90 mg·kg$^{-1}$; i.p.) and the colon was incubated with collagenase type XI (75 U·ml$^{-1}$) and dispase neutral protease (20 ug·ml$^{-1}$) for 2 h. After centrifugation, the pellets were suspended with DMEM–2% sorbitol (S-DMEM) and centrifuged again. S-DMEM washing/centrifugation procedure was carried out five times. The crypts were plated in dishes coated with collagen hydrogel (Corning, NY, USA) and cultured in medium supplemented with **Wnt-3A** (30 ng·ml$^{-1}$), **R-spondin**-2 (75 ng·ml$^{-1}$), **noggin** (70 ng·ml$^{-1}$) and EGF (50 ng·ml$^{-1}$). Passage was performed every 4 days. Colonic IECs (5 × 10$^5$ per well) were infected with PRMT2-OE, shPRMT2, or Vector and shScr at a multiplicity of infection of 50. At 48 h after infection, cells were stimulated with vehicle (PBS) or 50 ng·ml-1 TNF-α for 2 h.

## 2.7 | Gene expression

RNA samples were isolated using RNAiso Plus (Total RNA extraction reagent, TaKaRa, Kusatsu, Shiga, Japan) and then purified with LiCl precipitations as previously described (Viennois et al., 2018). Purified RNA was reversed by Prime-Script RT Reagent Kit (TaKaRa Bio) according to the manufacturer's instructions. The mRNA levels were detected by qPCR SYBR Green Mix kits (YEASEN Biotechnology Co., Ltd., Shanghai, China) and normalized to β-actin. $2^{-\triangle\triangle Ct}$ quantification method was conducted for calculations. The primers (Thermo Fisher Scientific, Waltham, MA, USA) used in this study are provided in Table 3.

**TABLE 3**  List of primers used for qPCR

| Gene | Forward (5′--3′) | Reverse (5′--3′) |
|------|------------------|------------------|
| β-Actin | GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT |
| IL-6 | ACCCCAATTTCCAATGCTCTC | AACGCACTAGGTTTGCCGAG |
| TNF-α | CCCTCACACTCAGATCATCTTCT | GCTACGACGTGGGCTACAG |
| CCL-5 | GCTGCTTTGCCTACCTCTCC | TCGAGTGACAAACACGACTGC |
| CCL-20 | AACTGGGTGAAAAGGGCTGT | GTCCAATTCCATCCCAAAAA |
| CXCL-12 | TGCATCAGTGACGGTAAACCA | TTCTTCAGCCGTGCAACAATC |
| CXCL-1 | CTGGGATTCACCTCAAGAACATC | CAGGGTCAAGGCAAGCCTC |
| TRAF-2 | AGAGAGTAGTTCGGCCTTTCC | GTGCATCCATCATTGGGACAG |
| TRAF-5 | TTTGAGCCCGACACCGAGTA | AGAGACCGGATGCACTGCT |
| SOCS3 (ChIP-1) | GACGAGCATGGGATGAGGG | TGGGTGCTGATGTCCTTTGG |

## 2.8 | Western blot analysis

Western blotting procedures and analysis complied with the *British Journal of Pharmacology* guidelines (Alexander et al., 2018). Colon tissues and cultured cells were homogenized in ice-cold RIPA buffer (P0013B; Beyotime Biotechnology, Shanghai, China) containing protease inhibitors and phosphatase inhibitors. Samples were centrifuged at 10,000 g for 20 min at 4°C, and the supernatant was quantified using a BCA protein assay kit (P0010; Beyotime Biotechnology). Samples were electrophoresed in SDS-PAGE and then transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk for 1 h at room temperature, further incubated with primary antibodies at 4°C overnight and then with secondary antibody for 2 h at room temperature. Rabbit anti-phospho-NF-κB p65 mAb (Cat# 3033, RRID:AB_331284), rabbit anti-suppressor of cytokine signalling 1 pAb (Cat# 3950T, RRID:AB_2192983), rabbit anti-ERK MAPK mAb (Cat# 4695, RRID:AB_390779), rabbit anti-phospho-ERK MAPK mAb (Cat# 4370, RRID:AB_2315112), rabbit anti-p38 MAPK mAb (Cat# 8690, RRID:AB_10999090), rabbit anti-phospho-p38 MAPK mAb (Cat# 4511T, RRID:AB_2139682), rabbit anti-SAPK/JNK Antibody pAb (Cat# 9252, RRID:AB_2250373) and rabbit anti-phospho-SAPK/JNK mAb (Cat# 9251S, RRID:AB_331659) were purchased from Cell Signaling Technology (Danvers, MA, USA). Mouse anti-NF-kB p65 mAb (Cat# ab13594, RRID:AB_300488) and rabbit anti-suppressor of cytokine signalling 3 pAb (Cat# ab3693, RRID:AB_304008) were from Abcam (Cambridge, MA, USA). Rabbit anti-PRMT2 mAb (Cat# sc-135010, RRID: AB_10610885) was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Goat anti-rabbit HRP secondary pAb (Cat# G-21234, RRID:AB_2536530) and goat anti-mouse HRP secondary pAb (Cat# G-21040, RRID:AB_2536527) were from Thermo Fisher Scientific. The densitometric analyses of protein expression by western blot were performed by AlphaView Software (ProteinSample, CA, USA, RRID:SCR_014549).

## 2.9 | Immunofluorescence staining

The Immuno-related procedures used comply with the recommendations made by the *British Journal of Pharmacology* (Alexander et al., 2018). Fresh colon samples were frozen in liquid nitrogen immediately and embedded in OCT compound (Neg-50; Thermo Fisher Scientific), cryo-sectioned and fixed with acetone (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) for 15 min at 4°C. The sections were then permeabilized and blocked with blocking buffer (P0260; Beyotime Biotechnology) for 1 h at room temperature and incubated with fluorochrome primary antibody overnight at 4°C. After extensive washing with PBS, sections were incubated with goat anti-rabbit Alexa Flour 555 pAb (Invitrogen, Cat# A32732, RRID:AB_2633281) and goat anti-mouse-Alex Flour 488 pAb (Invitrogen, Cat# A32723TR, RRID:AB_2866489) for 1 h and stained with DAPI (P0131; Beyotime Biotechnology). Immunofluorescence was acquired using a Zeiss LSM880 microscope (Zeiss, Gottingen, Germany). The primary antibodies used for immunofluorescence were rabbit anti-mouse PRMT2 mAb (Santa Cruz Biotechnology, Cat# sc-135010, RRID:AB_10610885), rabbit anti-human PRMT2 pAb (Abcam, Cat# ab154154, RRID:AB_2891153) and mouse anti-E-Cadherin mAb (BD Biosciences, San Jose, CA, USA, Cat# 610181, RRID:AB_397580). The relative quantification of immunofluorescence staining was performed by comparing the mean grey value through ImageJ software and normalized to the control group (Arqués et al., 2012; Pan et al., 2020).

## 2.10 | Co-immunoprecipitation

Cell lysates were prepared with a RIPA (P0013D; Beyotime Biotechnology) supplemented with protease and phosphatase inhibitors. The cell extracts were incubated with rabbit anti-PRMT2 mAb (Santa Cruz Biotechnology, Cat# sc-135010, RRID:AB_10610885) at 4°C overnight, followed by incubation with protein A/G agarose (Santa Cruz Biotechnology) for 4 h. After washing five times with lysis buffer, PRMT2-interacting protein was eluted with $1\times$ loading buffer and resolved by SDS-PAGE.

## 2.11 | Chromatin immunoprecipitation (ChIP)

Chromatin fractions were prepared using a ChIP Assay Kit (P2078; Beyotime Biotechnology) according to the manufacturer's instructions and immunoprecipitated with primary antibody to H3R8me2a rabbit pAb (Active Motif, Carlsbad, CA, USA, Cat# 39651, RRID:AB_2793290). qPCR was carried out using primers specific for SOCS3 promoter (Table 3). Data are calculated as percentage of input.

## 2.12 | ELISA

Colon tissues were homogenized, and the concentration of TNF-α was analysed with a commercial ELISA kit (CUSABIO TECHNOLOGY LLC, Wuhan, Hubei, China) according to the manufacturer's instructions.

## 2.13 | Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2018). Data are expressed as means ± SEM. Statistical analysis was performed using GraphPad Prism (Version 7.04; GraphPad Software Inc., San Francisco, CA, USA, RRID:SCR_002798). The group size in different groups is the number of mice in each group or the number of separate experiments (*in vitro*). The group size for statistical analysis was $n = 6$. The distribution of the data was tested with the Shapiro–Wilk normality test. For the difference between two groups, a two-tailed, unpaired Student's $t$-test was used. Comparisons among groups were analysed using a one-way ANOVA followed by a Tukey's post hoc test. Post hoc tests were run only if $F$ achieved $P < 0.05$ and there was no significant variance inhomogeneity. A value of $P < 0.05$ was considered statistically significant.

## 2.14 | Materials

DSS was purchased from MP Biomedicals (Irvine, CA, USA). Evans Blue, paraffin and sorbitol were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). DMEM was from GE Healthcare (Chicago, IL, USA). Haematoxylin and eosin were purchased from Yulu Experimental Equipment Co., Ltd. (Nanchang, Jiangxi, China). RNAiso Plus and SYBR Green were described in Section 2.7. FITC-conjugated dextran, pentobarbital sodium, collagenase type XI and dispase neutral protease were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Wnt-3A, R-spondin, noggin, EGF and TNF-α were obtained from PeproTech (Rocky Hill, NJ, USA). Materials used for western blot, immunofluorescence staining, co-immunoprecipitation and chromatin immunoprecipitation were described, respectively.

## 2.15 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/2022 (Alexander, Fabbro, et al., 2021; Alexander, Kelly, et al., 2021).

## 3 | RESULTS

### 3.1 | Protein arginine methyltransferase 2 (PRMT2) expression is elevated in dextran sulfate sodium (DSS)-induced colitis

To address the relevance of PRMT family members in colitis, we analysed the protein expression of PRMTs in colon tissues of mice with DSS-induced colitis. As indicated in Figure 1a, PRMT2 and PRMT5 were significantly up-regulated in colitis mice compared with the control mice, whereas no significant differences were observed for other PRMT family members. Meanwhile, a more prominent PRMT2 expression than PRMT5 was observed during colitis. Therefore, we mainly focused on PRMT2 in the subsequent study. By immunofluorescence staining, we further confirmed that PRMT2 was highly expressed in the colonic tissues of mice with colitis (Figure 1b, c) and patients with inflammatory bowel disease (Figure 1d,e), and PRMT2 was mainly expressed in colonic epithelial cells (Figure 1b–e). Thus, these results suggest that aberrant PRMT2 up-regulation in colonic epithelium is associated with the development of colitis.

### 3.2 | Colonic PRMT2 overexpression aggravates DSS-induced colitis

To further address the function of PRMT2 in colitis, *in vivo* overexpression of PRMT2 in mouse colon was performed by intrarectal instillation with lentiviruses of either PRMT2-OE (PRMT2

overexpression lentivirus) or Vector (empty vehicle lentivirus) 2 days before DSS treatment. *In vivo*, a significant twofold increase in PRMT2 expression was observed after PRMT2-OE administration compared with the Vector + DSS-treated mice (Figure S1A). Overexpression of PRMT2 in the murine colon aggravated DSS-induced weight loss (Figure 2a); increased disease activity disease index score (DAI) based on body weight change, stool consistency and faecal blood (Figure 2b), and worsened shortening of the colon (Figure 2c). By further histological examination, we found that PRMT2 overexpression resulted in more severe colonic epithelial injury (white arrowheads), crypt loss (black arrows) and inflammatory infiltrates (black arrowheads) than in DSS-treated mice (Figure 2d,e). Overexpression of PRMT2 in steady state did not affect colon morphology (Figure S1B). Moreover, intestinal permeability was markedly increased in PRMT2-OE-treated mice, based on a serum FITC-dextran (Figure 2f) and Evans Blue assay (Figure 2g), which was further confirmed by decreased expression of ZO-1 and claudin-1 and increased expression of claudin-2 (Figure 2h). Together, these data indicate that PRMT2 overexpression aggravates DSS-induced colitis.

### 3.3 | Colonic PRMT2 knockdown attenuates DSS-induced colitis

We next determined whether colonic PRMT2 silencing could protect mice from colitis. Knocking down of PRMT2 in murine colon was achieved by intrarectal injection of shPRMT2 lentivirus. shPRMT2-treated mice exhibited a significant twofold decrease in PRMT2 expression level compared with the shScr + DSS-treated mice (Figure S1C). As expected, knockdown of PRMT2 in murine colon attenuated DSS-induced colitis, as evidenced by reduced weight loss (Figure 3a), DAI score (Figure 3b), alleviated shortening of colons (Figure 3c) and histological damage (Figure 3d,e). Knockdown of PRMT2 in steady state did not affect colon morphology (Figure S1B). Moreover, reduced intestinal permeability (Figure 3f,g) was observed in shPRMT2-treated mice. Knockdown of PRMT2 in colons increased expression of ZO-1 and claudin-1 and decreased expression of claudin-2 (Figure 3h). Collectively, our loss-of-function experiments complement the above findings obtained from the PRMT2-overexpressing mice, supporting that PRMT2 is critical in colitis development.

### 3.4 | PRMT2 mediates NF-κB/MAPK activation in DSS-induced colitis

To further explore the underlying mechanism of PRMT2-induced colitis, we analysed the expression of several colitis-associated inflammatory cytokines (TNF-α, IL-6 and IL-4) and inflammatory-related chemokines (chemokine [C–X–C motif] ligand [CXCL1, CXCL12, and chemokine [C–C motif] ligand CCL2, CCL20) in murine colon. As shown in Figure S2, PRMT2-OE increased the levels of TNF-α (Figure S2A) and knockdown of PRMT2 reduced TNF-α levels
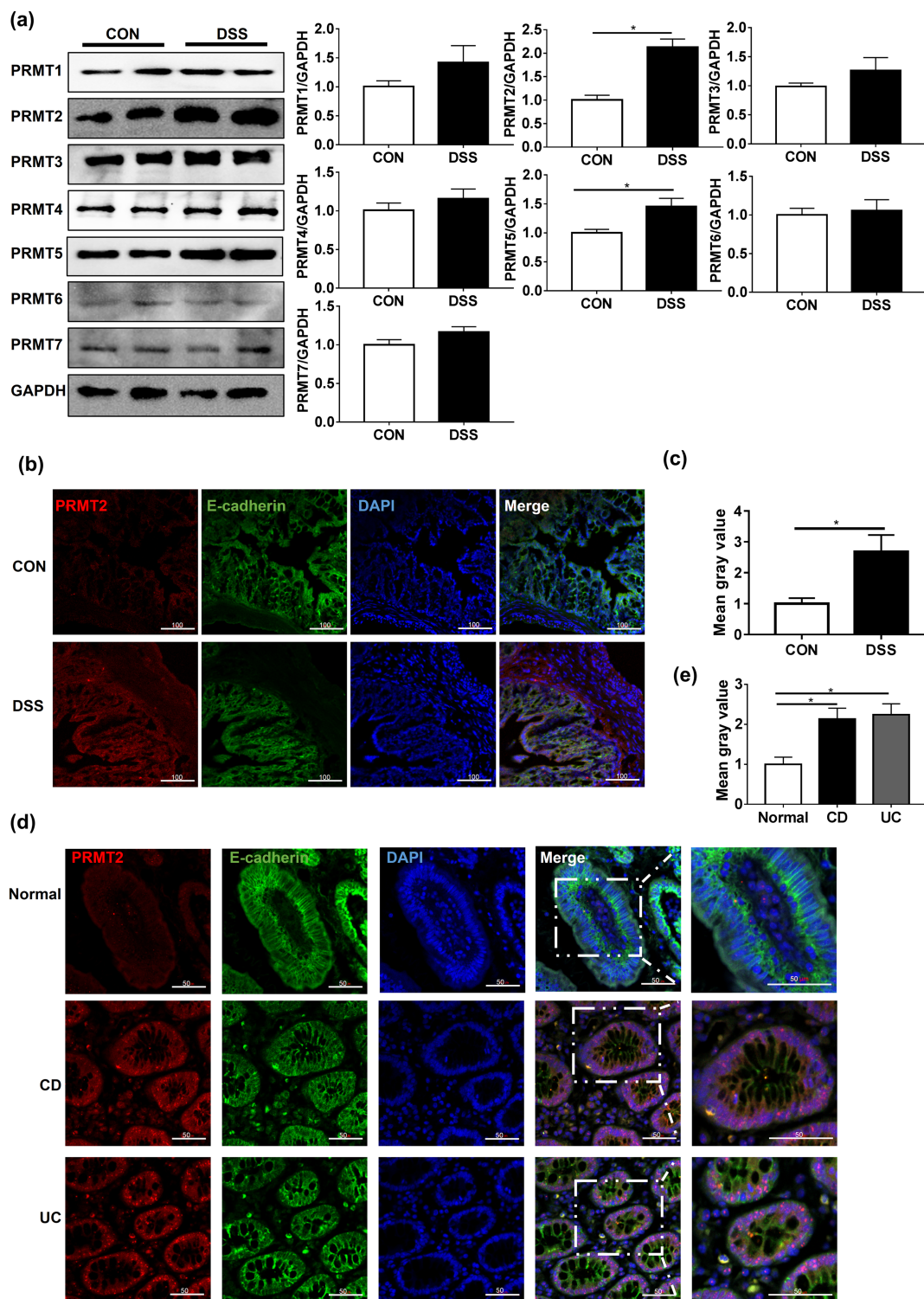
**FIGURE 1**   Protein arginine methyltransferase 2 (PRMT2) expression is elevated in dextran sulfate sodium (DSS)-induced colitis. (a) Western blot analysis of PRMT family members in murine colon. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control (CON) and the grey value and area of each band were normalized to GAPDH ($n = 6$ per group). (b,c) Representative immunofluorescence images (b) and fold change in immunofluorescence relative to control group (c) of PRMT2 (red) in colonic tissues of mice. Blue, DAPI; green, E-cadherin; $n = 6$ per group, scale bar: 100 μm. (d,e) Representative images (d) and fold change in immunofluorescence relative to normal group (e) of PRMT2 in health control ($n = 9$), Crohn's disease (C; $n = 9$) and ulcerative colitis (UC) patients ($n = 10$). Blue, DAPI; green, E-cadherin; scale bar: 50 μm. The scale bar of zoom-in immunofluorescent imaging is 50 μm. Data are means ± SEM. *$P < 0.05$
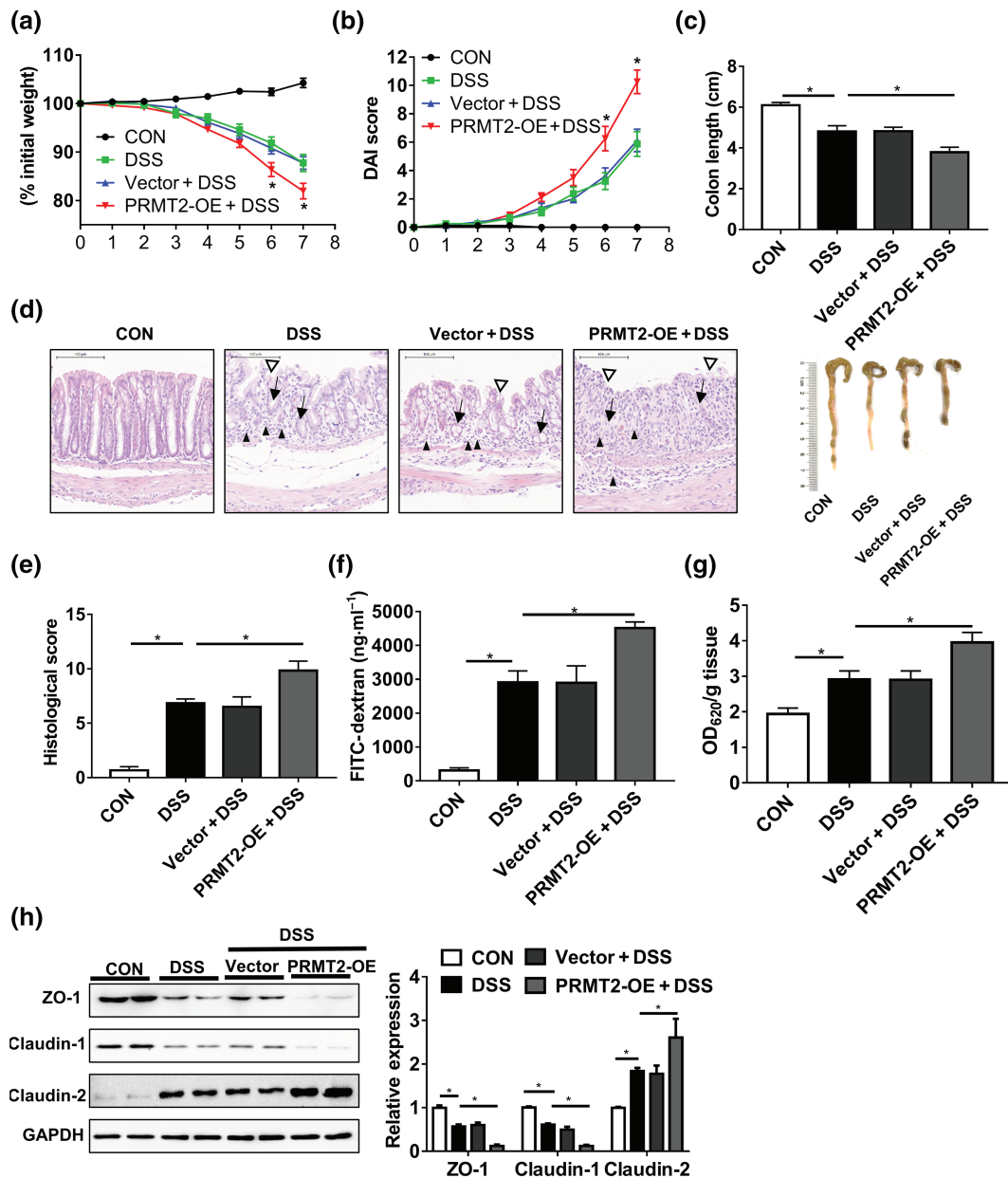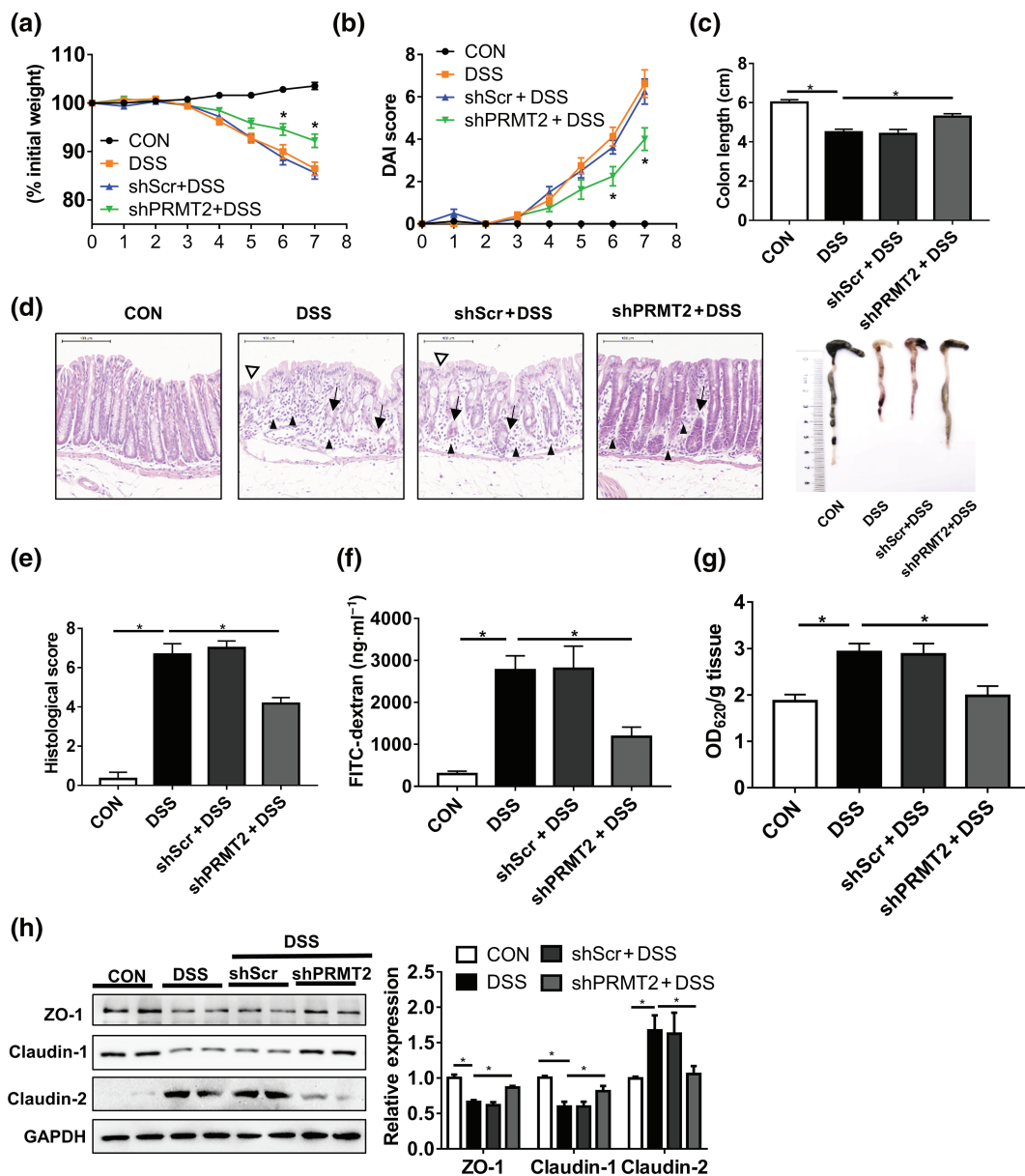
**FIGURE 2** Colonic protein arginine methyltransferase 2 (PRMT2) overexpression aggravates dextran sulfate sodium (DSS)-induced colitis. (a) Weight change and (b) disease activity index (DAI) were determined. The statistical significance was indicated for the difference between DSS group and PRMT2-OE + DSS group. (c) Colon length was determined. (d,e) Histological analysis of colonic tissues by haematoxylin–eosin staining (d) and scoring (e). Scale bar: 100 μm. Epithelial injury, crypt loss and infiltrated inflammatory cells were indicated by white arrowheads, black arrows and black arrowheads, respectively. (f) Intestinal permeability determined by the concentration of serum FITC-dextran. (g) Colon epithelial permeability determined using Evans Blue assay. (h) Western blot analysis of ZO-1, claudin-1 and claudin-2 in colons; GAPDH was used as an internal control (CON) and the grey value and area of each band were normalized to GAPDH. Data are means ± SEM, $n = 6$. *$P < 0.05$

(Figure S2B) in the murine colon with DSS-induced colitis. By further qPCR detection, we found that overexpression of PRMT2 significantly increased TNF-α expression and enhanced downstream expression of the chemokines CXCL1, CXCL12, CCL20 and CCL5 (Figure 4a). Conversely, knockdown of PRMT2 significantly suppressed TNF-α and downstream chemokine expression (Figure 4b). In addition, primary colonic mouse epithelial cells were isolated and transfected with PRMT2-OE or shPRMT2 followed by TNF-α stimulation, to further determine whether PRMT2 regulates TNF-α signalling *in vitro*. Consistently, PRMT2-OE transfection elevated TNF-α-induced downstream genes expression (Figure 4c), while knockdown of PRMT2 diminished TNF-α-induced downstream genes expression (Figure 4d) in epithelial cells. These results demonstrate that PRMT2 regulates TNF-α signalling to promote colitis.
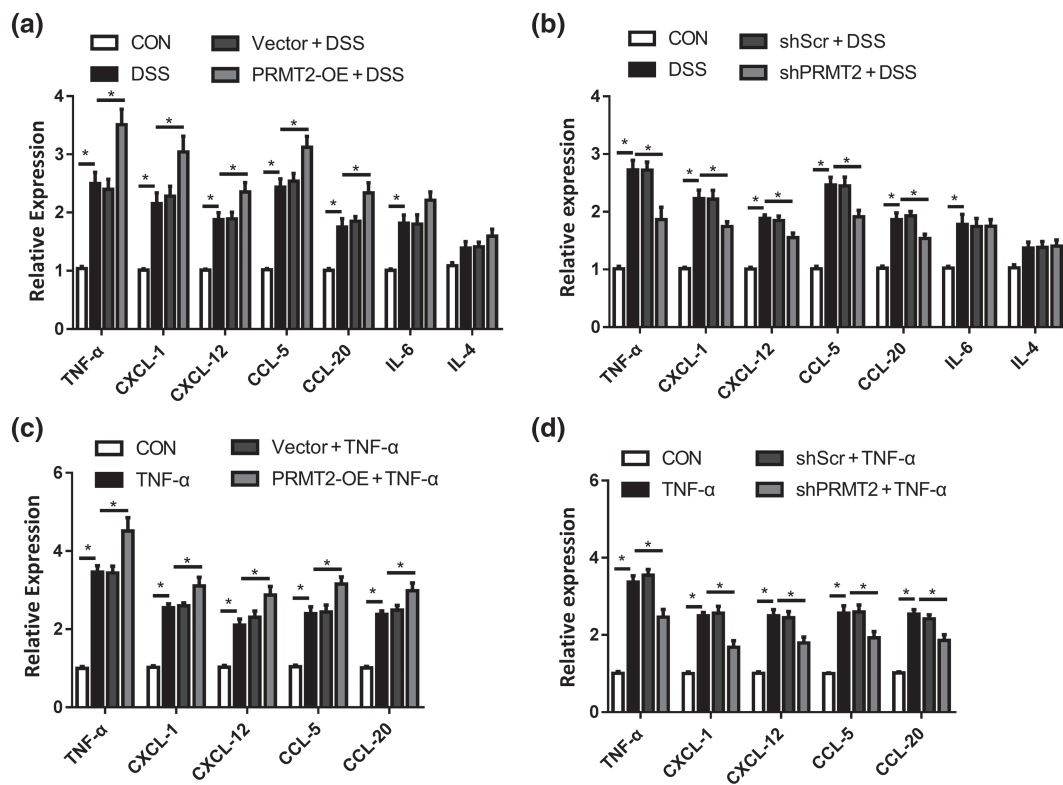
**FIGURE 3** Colonic protein arginine methyltransferase 2 (PRMT2) knockdown attenuates dextran sulfate sodium (DSS)-induced colitis. (a) Weight change and (b) disease activity index (DAI) were determined. The statistical significance was indicated for the difference between DSS group and PRMT2-short hairpin RNA lentivector (shPRMT2) + DSS group. (c) Colon length was determined. (d,e) Histological analysis of colonic tissues by haematoxylin–eosin staining (d) and scoring (e). Scale bar: 100 μm. Epithelial injury, crypt loss and infiltrated inflammatory cells were indicated by white arrowheads, black arrows and black arrowheads, respectively. (f) Intestinal permeability determined by the concentration of serum FITC-dextran. (g) Colon epithelial cell permeability determined using Evans Blue assay. (h) Western blot analysis of ZO-1, claudin-1 and claudin-2 in colons; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control (CON) and the grey value and area of each band were normalized to GAPDH. Data are means ± SEM, $n = 6$. *$P < 0.05$

A positive feedback regulation loop has been reported between NF-κB and TNF-α (Kagoya et al., 2014). To investigate the underlying mechanism of PRMT2 on the induction of TNF-α modulated genes, we next examined the effect of PRMT2 on the activation of NF-κB and MAPKs. By determining phosphorylation levels of nuclear factor-κB (NF-κB) p65 and MAPKs in murine colon or colonic epithelial cells transfected with shPRMT2 or PRMT2-OE, we found

that overexpression of PRMT2 enhanced the phosphorylation of p65, p38, ERK and JNK both in the colon (Figure 5a) and in isolated colonic epithelial cells (Figure 5b). In contrast, the knockdown of PRMT2 suppressed the activation of p65, p38, ERK and JNK in colons (Figure 5c) and epithelial cells (Figure 5d). Taken together, these data suggest that PRMT2 acts upstream of NF-κB/MAPK to stimulate TNF-α signalling in experimental colitis.
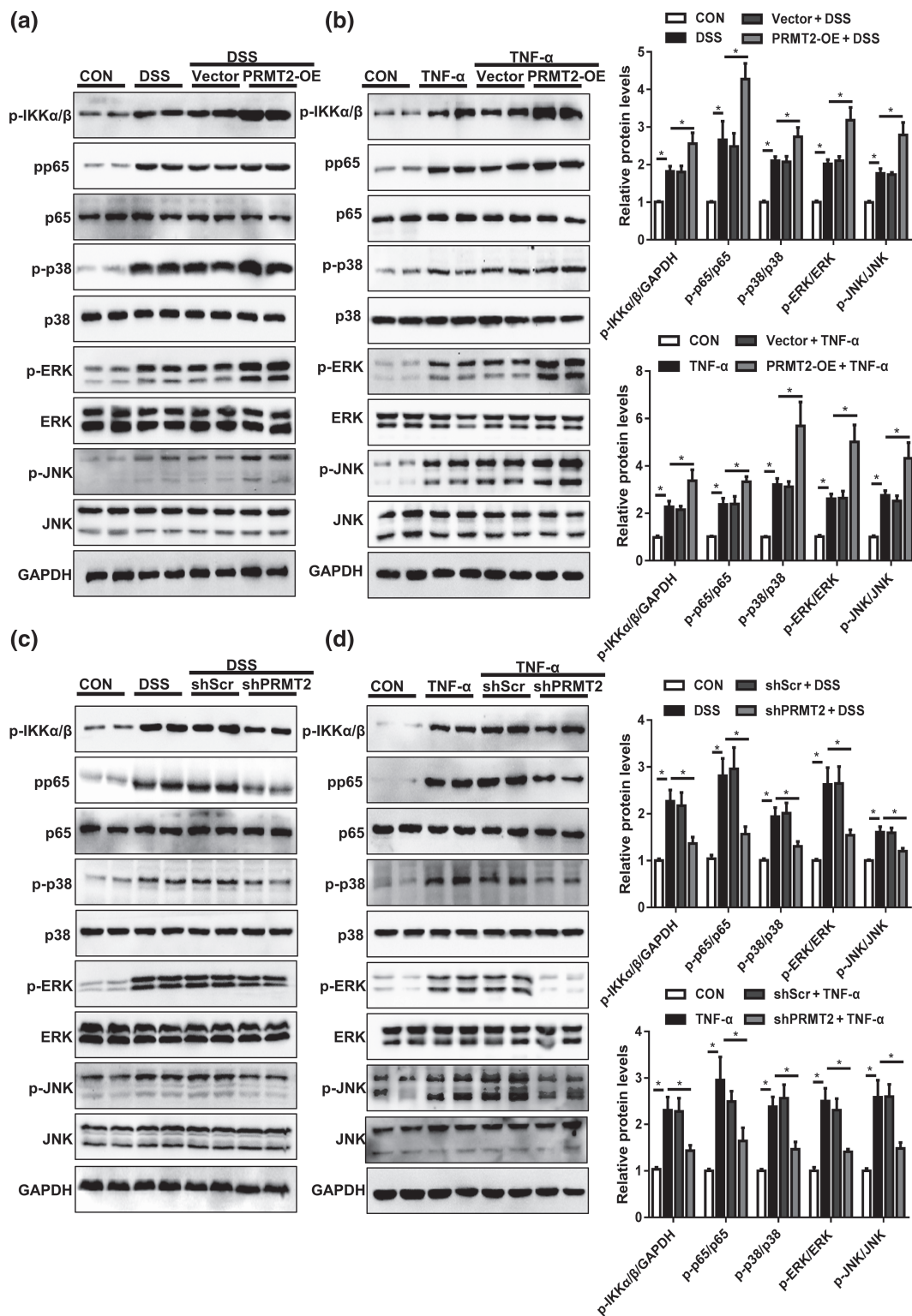
**FIGURE 4** Protein arginine methyltransferase 2 (PRMT2) regulates TNF-α signalling. (a,b) Relative mRNA levels of cytokines in murine colon were measured by qPCR. (c,d) Relative mRNA levels of TNF-α-induced cytokines in primary colonic epithelial cells were measured by qPCR. Data are means ± SEM, $n = 6$. *$P < 0.05$

## 3.5 | PRMT2 enhances NF-κB/MAPK activation by stimulating TRAF5 expression in DSS-induced colitis

TNF receptor associated factor (TRAF) proteins, located downstream of TNF-α signalling and especially TRAF2/TRAF5 participate in mediating downstream NF-κB and MAPK signalling pathways (Häcker et al., 2011; Liu et al., 2017). To further dissect the mechanism underlying how PRMT2 mediated NF-κB/MAPK activation, we next analysed the consequences of gain/loss of function of PRMT2 on TRAF2/TRAF5 expression in colon samples. As shown in Figure 6, the expression of TRAF5 was up-regulated in DSS-treated mice, and overexpression of PRMT2 in colons further enhanced DSS-induced TRAF5 expression at both mRNA (Figure 6a) and protein levels (Figure 6b). Accordingly, knockdown of PRMT2 blunted DSS-induced TRAF5 expression (Figure 6c,d). TRAF2 expression was elevated during experimental colitis. Its expression was reduced by shPRMT2 but not affected by PRMT2 overexpression (Figure S3A,B). Together, these results demonstrate that PRMT2 modulates NF-κB/MAPK activation by inducing TRAF5 expression in experimental colitis.

## 3.6 | PRMT2 inhibits the binding of SOCS3 to TRAF5 and the degradation of TRAF5 protein via ubiquitination

Previous studies suggest that suppressor of cytokine signalling 3 (SOCS3) may directly mediate the Lys48-linked ubiquitination and degradation of TRAF proteins (Zhang et al., 2018; Zhou et al., 2015). Thus, we explored whether PRMT2 stimulates TRAF5 expression by inhibiting SOCS3-mediated ubiquitination. We found that SOCS3 was up-regulated in the colon of mice with experimental colitis (Figure 7a, b), and its expression was markedly decreased by PRMT2 over-expression (Figure 7a) and correspondingly increased by PRMT2 knockdown (Figure 7b). This suggests that SOCS3 may be involved in the PRMT2-mediated regulation of colitis. By subsequent immunoprecipitation assays, we confirmed that SOCS3 interacted with TRAF5 (Figure 7c,d) in primary colonic epithelia of DSS-treated mice, which were consistent with the findings in TNF-α-treated epithelial cells (Figure 7e,f). Moreover, the Lys48-linked ubiquitination of TRAF5 by SOCS3 was down-regulated with overexpression of PRMT2 (Figure S4A) and up-regulated by the knockdown of PRMT2 (Figure S4B). These results collectively demonstrate that PRMT2

**FIGURE 5** Protein arginine methyltransferase 2 (PRMT2) mediates MAPK and NF-κB activation. (a) Western blot analysis of MAPKs and NF-κB p65 in murine colon administered with PRMT2-overexpression lentivector (PRMT2-OE). (b) Western blot analysis of MAPKs and NF-κB p65 in primary colonic epithelial cells transfected with PRMT2-OE. (c) Western blot analysis of MAPKs and NF-κB p65 in murine colon treated with PRMT2-short hairpin RNA lentivector (shPRMT2). (d) Western blot analysis of MAPKs and NF-κB p65 in primary colonic epithelial cells transfected with shPRMT2. For p-IKKα/β, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control and the grey value and area of each band were normalized to GAPDH. For p-p65, p-p38 and p-JNK, total p65, p38 and JNK were used as loading controls, respectively, and the grey value and area of each band were normalized to loading controls, respectively. Data are means ± SEM, $n = 6$. *$P < 0.05$
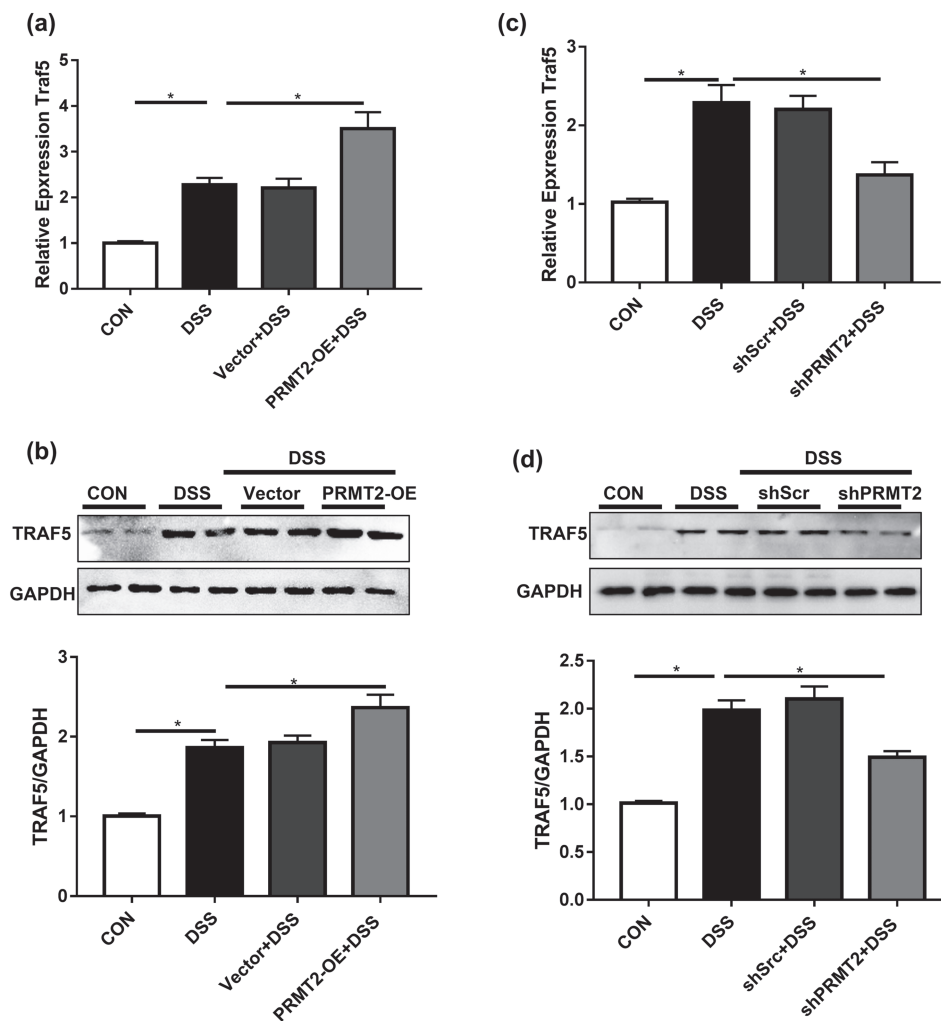
**FIGURE 6** Protein arginine methyltransferase 2 (PRMT2) promotes TNF receptor associated factor 5 (TRAF5) expression in DSS-induced colitis. (a) Relative mRNA levels of TRAF5 in murine colon administered with PRMT2-overexpression lentivector (PRMT2-OE). (b) Western blot analysis of TRAF5 in murine colon administered with PRMT2-OE. (c) Relative mRNA levels of TRAF5 in murine colon administered with PRMT2-short hairpin RNA lentivector (shPRMT2). (d) Western blot analysis of TRAF5 in murine colon administered with shPRMT2. GAPDH was used as an internal control and the grey value and area of each band were normalized to GAPDH for (b) and (d). CON =control; Data are means ± SEM, $n = 6$. *$P < 0.05$

negatively regulates SOCS3 to inhibit the Lys48-linked ubiquitination of TRAF5, resulting in suppressed degradation of TRAF5.

## 3.7 | PRMT2 epigenetically inhibits SOCS3 expression by increasing H3R8 asymmetric methylation (H3R8me2a) level in the SOCS3 promoter

PRMT2 epigenetically regulates gene expression through the methylation of histones (Blythe et al., 2010). To reveal whether the altered gene expression is a consequence of PRMT2-mediated histone arginine methylation, we examined several histone methylation levels in murine colon. As shown in Figure 8a, the levels of asymmetric demethylation on H3R8 (H3R8me2a) were specifically increased in PRMT2-overexpressing mice. Conversely, knockdown of PRMT2 significantly decreased H3R8me2a methylation levels (Figure 8a). By further ChIP-qPCR analysis, we found that levels of H3R8me2a in the promoter region of SOCS3 were higher in primary colonic epithelia of DSS-treated mice than in normal colonic epithelia (Figure 8b). Overexpression of PRMT2 in mice enhanced

H3R8me2a levels at the promoter of SOCS3 (Figure 8b). Correspondingly, the H3R8me2a level at the promoter of SOCS3 was reduced by PRMT2 knockdown (Figure 8b). Moreover, the above PRMT2-mediated H3R8me2a methylation at the SOCS3 promoter was further demonstrated in TNF-α-treated epithelial cells (Figure 8c–e). These results suggest that PRMT2 increases repressive histone mark H3R8me2a at the SOCS3 promoter, thus inhibiting the expression of SOCS3 and its downstream gene expression.

## 4 | DISCUSSION

In this study, we demonstrate that PRMT2 expression is elevated in murine colon with experimental colitis. Overexpression of PRMT2 aggravates while knockdown of PRMT2 attenuates DSS-induced colitis, supporting that PRMT2 is a pivotal and essential mediator of colitis. Additional mechanistic investigations reveal that PRMT2 promotes colitis by increasing repressive histone mark H3R8me2a at the SOCS3 promoter, thus inhibiting SOCS3 expression and its
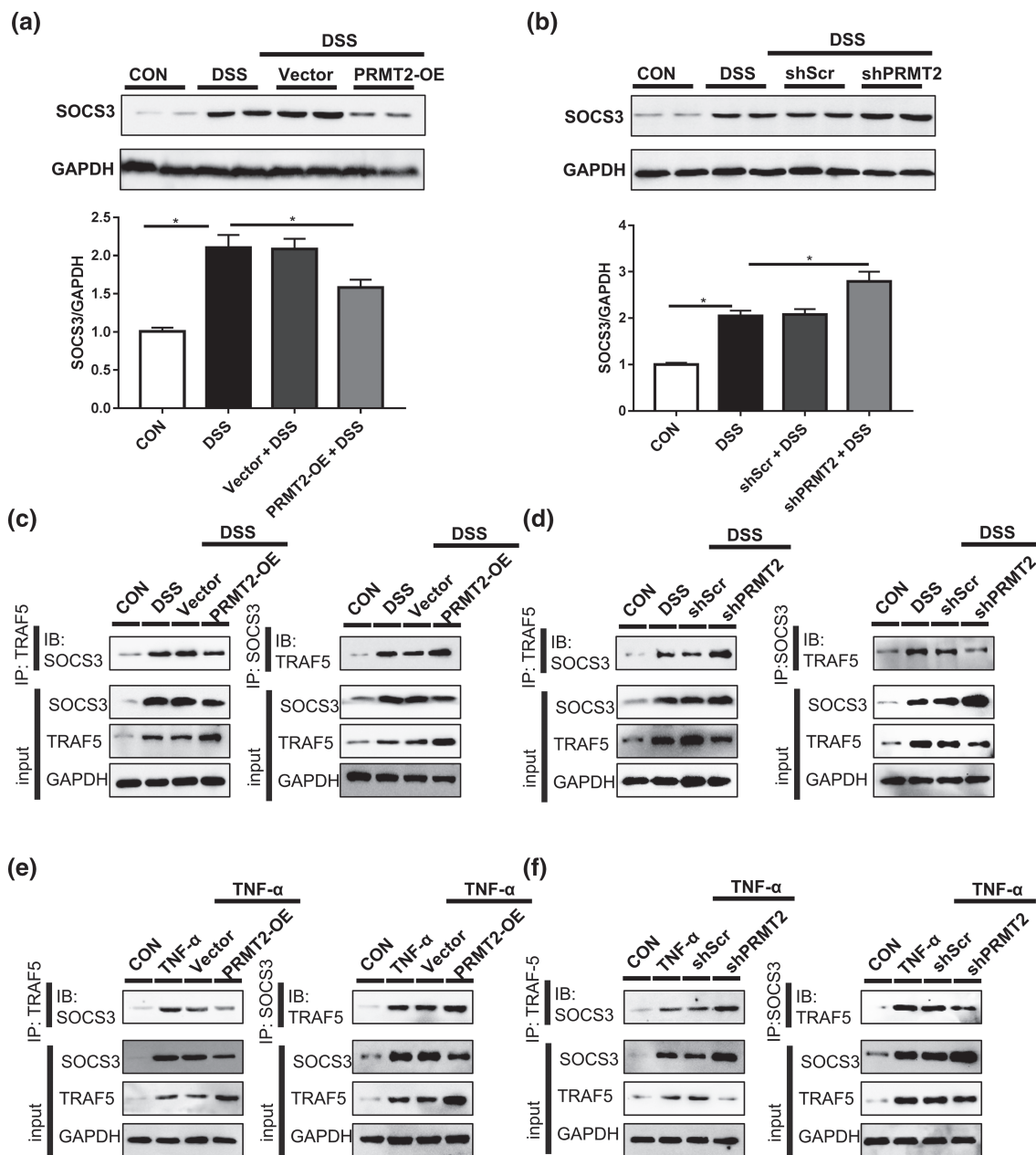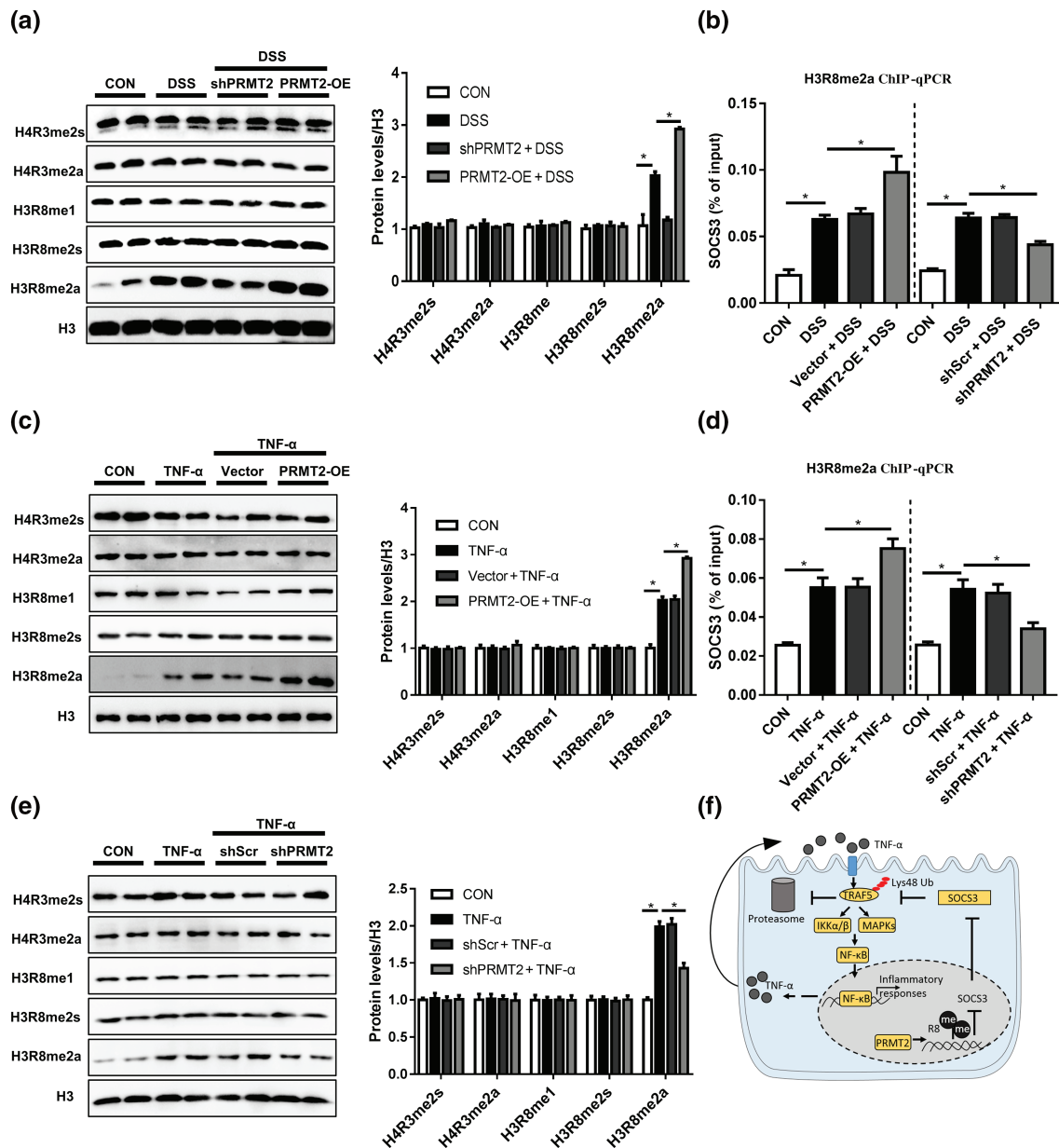
**FIGURE 7** Protein arginine methyltransferase 2 (PRMT2) inhibits the interaction between suppressor of cytokine signalling 3 (SOCS3) and TNF receptor associated factor 5 (TRAF5). (a,b) Western blot analysis of SOCS3 expression in murine colon. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the loading control and the grey value and area of each band were normalized to GAPDH. (c,d) Co-immunoprecipitation analysis of the interaction between SOCS3 and TRAF-5 in primary colonic epithelia of dextran sulfate sodium (DSS)-treated mice administered with PRMT2-overexpression lentivector (PRMT2-OE) (c) or PRMT2-short hairpin RNA lentivector (shPRMT2) (d). (e,f) Co-immunoprecipitation analysis of the interaction between SOCS3 and TRAF-5 in TNF-α-treated epithelial cells transfected with PRMT2-OE (e) or shPRMT2 (f). Con = control; Data are means ± SEM, $n = 6$. *$P < 0.05$

degradation of TRAF5 protein via ubiquitination, subsequently leading to elevated TRAF5 expression and TRAF5-mediated downstream NF-κB/MAPK activation (Figure 8f). Altogether, our study identifies PRMT2 as a critical protein in the development colitis. Pharmacological targeting at PRMT2 may therefore provide a novel approach for the treatment of colitis.

The PRMT enzyme family regulate diverse cellular programmes, including RNA splicing, signalling transduction, DNA damage repair

and protein–protein interactions (Blanc & Richard, 2017). While the other four PRMTs including PRMT1, PRMT4, PRMT5 and PRMT6 have been documented to correlate with inflammatory responses (Kim et al., 2016), the role of PRMT2 in inflammatory diseases remains unclear. Here, we demonstrate that PRMT2 is expressed at a low basal level in normal colon tissues of mice but increases during colitis development. Our study demonstrated that PRMT2 was more prominently up-regulated than other PRMT proteins during inflammatory bowel

**FIGURE 8** Protein arginine methyltransferase 2 (PRMT2) epigenetically inhibits suppressor of cytokine signalling 3 (SOCS3) expression by increasing H3R8 asymmetric methylation (H3R8me2a) level in the SOCS3 promoter. (a) Western blot analysis of designated histone modification levels in murine colon. (b) ChIP-qPCR analysis of H3R8me2a enrichment on the promoter regions of SOCS3 in primary colonic epithelia of DSS-treated mice administered with PRMT2-overexpression lentivector (PRMT2-OE) (left) or PRMT2-short hairpin RNA lentivector (shPRMT2) (right). (c,d) Western blotting analysis of designated histone modification levels in TNF-α-treated epithelia transfected with PRMT2-OE (c) or shPRMT2 (d). (e) ChIP-qPCR analysis of H3R8me2a enrichment on the promoter regions of SOCS3 in TNF-α-treated epithelia transfected with PRMT2-OE (left) or shPRMT2 (right). (f) Graphical summary for the role and mechanism of PRMT2 in mediating the development of colitis in mice. PRMT2-mediated H3R8me2a directly suppresses the expression of SOCS3 and SOCS3-mediated degradation of TRAF5, subsequently leading to downstream NF-κB/MAPK activation, proinflammatory cytokine production and propagation of inflammatory responses during colitis. GAPDH was used as an internal control (CON) and the grey value and area of each band were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for (a), (c) and (d). Data are means ± SEM, $n = 6$. *$P < 0.05$

disease, which is consistent with the recent publication (Krzystek-Korpacka et al., 2020). Meanwhile, colonic PRMT2 overexpression aggravates while PRMT2 knockdown protects against DSS-induced colitis in mice, proving that PRMT2 acts as a proinflammatory mediator in colitis. Our observation is in agreement with an earlier clinical

study showing elevated PRMT2 expression in inflamed tissues of ulcerative colitis patients, suggesting that PRMT2 positively correlates with clinical disease severity (Krzystek-Korpacka et al., 2020).

TNF-α signalling and TNF-α-induced inflammatory responses have an important role in the initiation and development of colitis

(Khalil et al., 2016; Liu et al., 2017; Sharma et al., 2019). Therefore, in this study, we determined the regulatory effects of PRMT2 on TNF-α signalling and its regulatory transcriptional factor NF-κB. Our results demonstrated that colonic expression of TNF-α and its downstream chemokines CXCL1, CXCL12, CCL20 and CCL5 increased concomitantly with the up-regulation of PRMT2 in DSS-induced colitis mice and their expression levels were tightly regulated by PRMT2 knockdown, further supporting the proinflammatory role of PRMT2 in the development of colitis. Furthermore, overexpression of PRMT2 enhanced the phosphorylation of NF-κB p65 subunit, both in murine colon and in murine colonic epithelial cells. In contrast, PRMT2 knockdown suppressed the activation of NF-κB p65 in the colon and epithelial cells. It has been suggested that a positive feedback loop exists between NF-κB and TNF-α (Kagoya et al., 2014). NF-κB and MAPKs are transcriptional factors and signalling molecules that regulate the expression of TNF-α. TNF-α derived from destroyed epithelial or immune cells can activate NF-κB and MAPKs to produce more proinflammatory cytokines including TNF-α itself (Kagoya et al., 2014; Sabio & Davis, 2014). Thus, our findings suggested that PRMT2 may promote colitis by targeting at the positive feedback loop between NF-κB and TNF-α.

TNF-α-induced apoptosis and necrosis in epithelium contribute to the pathogenesis of colitis. It has been demonstrated that PRMT2 inhibited cell activation and promoted apoptosis in mouse embryo fibroblasts (Ganesh et al., 2006). PRMT2 interacted with splicing factors to regulate the alternative splicing of **Bcl-2-like 1 (Bcl-xl/BCLX)** in TNF-α-treated HeLa and HEK293T cells (Vhuiyan et al., 2017). Additionally, PRMT2 rendered cells more susceptible to apoptosis by cytokines or cytotoxic drugs through reducing E2F transcription factor 1 (E2F1) expression (Yoshimoto et al., 2006). The above findings suggest that PRMT2 may participate in inflammatory bowel disease by stimulating epithelial apoptosis. While our study demonstrated the damage effects of PRMT2 on intestinal epithelium, whether and how apoptosis/necrosis pathways are involved needs to be further revealed in the follow-up study.

Arginine methylation plays a major role in transcriptional regulation because of the ability of the PRMTs to deposit key activating (histone H4R3me2a, H3R2me2s, H3R17me2a and H3R26me2a) or repressive (H3R2me2a, H3R8me2a, H3R8me2s and H4R3me2s) histone marks (Blanc & Richard, 2017). In this study, we analysed the regulatory effects of PRMT2 on H4R3me2a, H4R3me2s, H3R8me2a and H3R8me2s histone marks and identified that PRMT2 is specifically responsible for H3R8me2a levels. Moreover, PRMT2-mediated H3R8me2a directly targets SOCS3 for its transcriptional inhibition to promote downstream proinflammatory gene expression, suggesting that a PRMT2-mediated epigenetic mechanism critically regulates the development of colitis. Similarly, Dong et al. (2018) demonstrated that PRMT2 is the main source for H3R8me2a and PRMT2-mediated H3R8me2a is responsible for the activation of oncogenic transcriptional programmes (Dong et al., 2018). While our study is the first to indicate that PRMT2-mediated H3R8me2a is involved in mediating colitis, whether other histone marks by PRMT2 may participate in colitis progression remains to be identified. In addition, most of the

histone-modifying enzymes including PRMTs have multiple nonhistone substrates. For example, PRMT2 has been implicated in the methylation of STAT3 (Iwasaki et al., 2010) and E1B-AP5 (Kzhyshkowska et al., 2001). Thus, it would be interesting to identify potential nonhistone substrates of PRMT2 in the context of colitis.

SOCS3, a well-known anti-inflammatory mediator, has been shown to negatively regulate NF-κB and ERK/MAPK-dependent gene expression of proinflammatory mediators (Hovsepian et al., 2013; Mahony et al., 2016). The expression of SOCS3 is directly regulated by promoter methylation, and its expression may be inhibited or greatly reduced due to hypermethylation of the CpG islands in its promoter region (Boosani & Agrawal, 2015). In our study, we demonstrate that PRMT2-mediated H3R8me2a directly targets the promoter region of SOCS3 for its transcriptional inhibition. Thus, PRMT2 overexpression suppresses SOCS3 expression in the inflamed colons, which explains the enhanced activation of NF-κB and MAPK and the subsequent aggravation of colitis. Previously, studies have reported that SOCS3 degrades TRAF6 via polyubiquitination (Zhang et al., 2018; Zhou et al., 2015), and elevated SOCS3 promotes Lys48-linked ubiquitination and degradation of TRAF6, consequently impairing NF-κB activation (Zhang et al., 2018). Here, we demonstrate that SOCS3 could also bind to TRAF5 and mediate Lys48-linked ubiquitination of TRAF5, thereby promoting TRAF5 degradation. In contrast, TRAF2 elevated during experimental colitis was reduced by shPRMT2. However, its expression was not affected by PRMT2 overexpression, suggesting that TRAF2 may be influenced by other regulators and thus not as important as TRAF5 for PRMT2-mediated inflammatory response. TRAF5 has been reported to mediate NF-κB/MAPK activation in response to TNF and other proinflammatory cytokines (Au & Yeh, 2007; Oeckinghaus et al., 2011). As a result, PRMT2 overexpression induces the expression of NF-κB/MAPK responsive proinflammatory mediators is by preventing TRAF5 degradation, contributing to aggravated colitis development.

In summary, our study demonstrates that PRMT2 promotes the development of colitis by acting as a proinflammatory mediator. Mechanistically, PRMT2-mediated H3R8me2a directly suppresses the expression of SOCS3 and SOCS3-mediated degradation of TRAF5, subsequently leading to downstream NF-κB/MAPK activation, proinflammatory cytokine production and propagation of inflammatory responses during colitis. Thus, the development of therapeutic strategies to specifically target PRMT2 might be useful for manipulating inflammatory diseases including colitis.

## AUTHOR CONTRIBUTIONS

JS and L-LP designed and interpreted experiments. JL performed experiments and analysed data. XP, ZR, BL, HL, CW and XD assisted the experiments and helped analyse data. JL and XP drafted the manuscript. PdV, JS and L-LP critically reviewed the manuscript. All authors approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, Immunoblotting and Immunochemistry and Animal Experimentation, and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

## DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author. Some data may not be made available because of privacy or ethical restrictions.

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