Supporting Information

Phosphodiesterase-4 inhibition attenuates murine ulcerative colitis via interfering with mucosal immunity

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DSS-induced colitis and drug treatment

Wide-type male C57BL/6J mice (8 weeks, 22-24g) were purchased from Shanghai Lingchang Biotechnology Co., Ltd. (Certificate No.2013-0018, China). The mice were housed under specific pathogen-free conditions with 12 h of light/12 h of dark cycle, 22±1 °C and 55±5% relative humidity. All mice were fed standard laboratory chow and water ad libitum and allowed to acclimatize in our facility for one week before any experiments started.

Mice were randomly divided into 5 groups, consisting of normal mice, normal mice treated with 50 $mg \cdot kg^{-1}$ apremilast, vehicle (DSS only), vehicle treated with 50 $mg \cdot kg^{-1}$ or 10 $mg \cdot kg^{-1}$ apremilast. Ulcerative colitis was induced by administration of 3% DSS for 7 days and further drinking water taken for another 4 days. During the treatment, weight loss, stool consistency and fecal blood, as indicators of disease activity index (DAI), were monitored by 3 investigators who were blinded to the experimental conditions. The DAI scores were calculated as the sum of the weight loss, stool consistency and rectal bleeding score shown in Table1. On day 11, mice were anesthetized with an intraperitoneal injection of 4% chloral hydrate. The spleen and colon were isolated for further measurement.

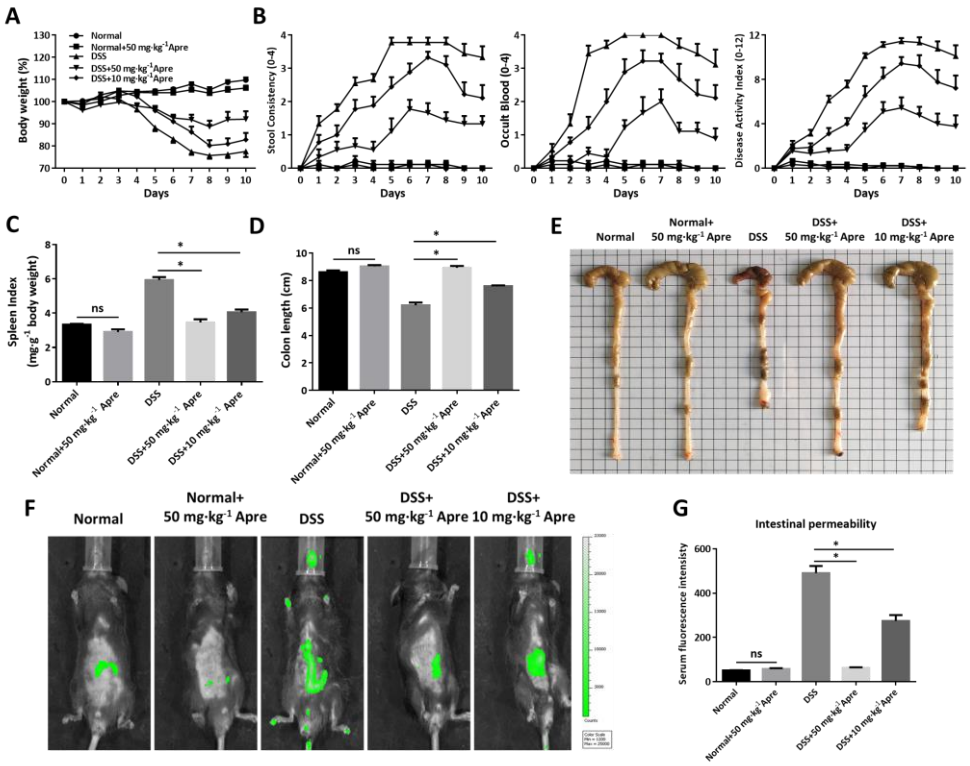

Figure S1. Apremilast dose-dependently attenuated DSS-induced ulcerative colitis without inflammation in control mice. (A) The percentage of body weight change during treatment. (B) The score of stool consistency and occult blood and DAI based on the criteria in Table1. (C) Spleen index calculated by spleen weight (mg)/body weight. (D) Colon length. (E) Representative colon images. (F) Fluorescence imaging with FITC-dextran administration and (G) serum fluorescence intensity. Data were shown as means±SEM; n=9 per group. *p<0.05, significantly different from vehicle group.

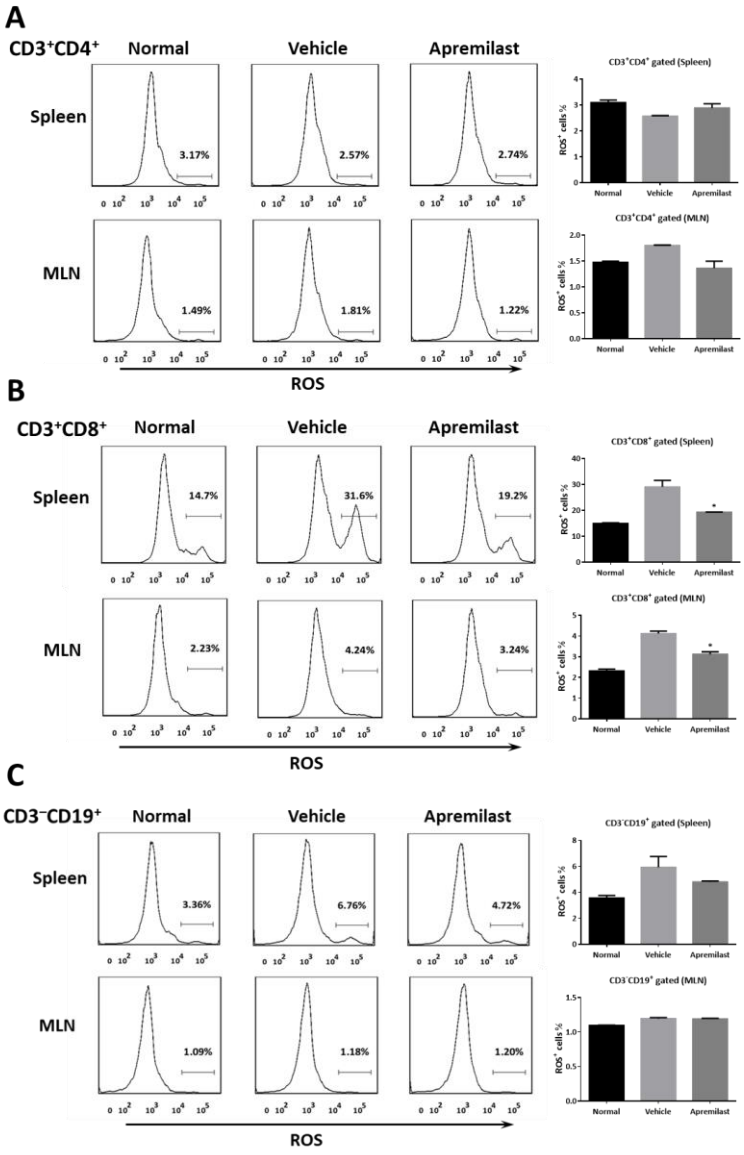

Figure S2. Flow cytometry analysis of ROS production from $CD3^+CD4^+$ (A), $CD3^+CD8^+$ (B), $CD3^-CD19^+$ (C) cells in spleen and mesenteric lymph nodes. Colitis was induced with 3% DSS as described in Methods. Splenocytes and mesenteric lymph node cells were isolated and labeled with fluorescent antibodies. Data correspond to experiments conducted on 8 mice.

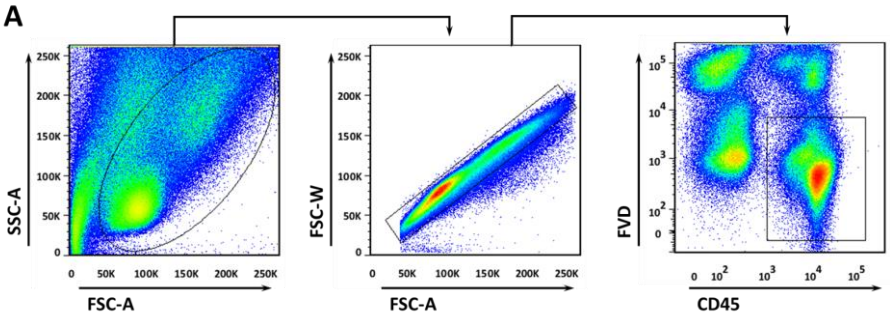

Figure S3. The gating strategy of flow cytometry. FSC-A plus SSC-A was used to identify the total cells for analysis. FSC-A plus FSC-W were used to identify the singlets. Subsequently, $CD45^{+}FVD^{int}$ cells were considered as living leukocytes for the further analysis.

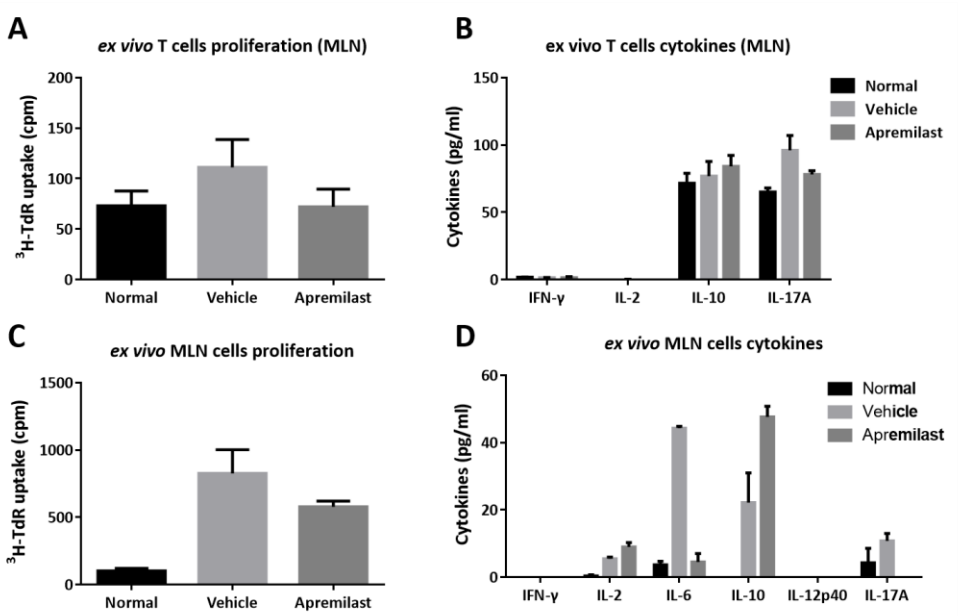

Figure S4. Baseline proliferation and cytokines release from $CD4^+$ T cell and whole MLN cells. $CD4^+$ T cells (A, and B), purified from MLNs, and whole MLN cells (C, and D) were cultured to measure the basal proliferation and cytokines levels in accord with Figure 5. Data were shown as means±SEM; n=8 per group.

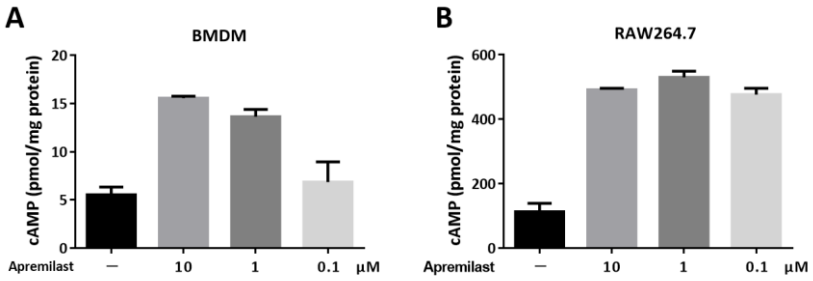

Figure S5. Effect of apremilast on cAMP elevation in BMDMs and macrophages. BMDMs and RAW264.7 cells were incubated with the indicated concentrations (10, 1, or 0.1 μM) of apremilast for 30 min. cAMP levels in BMDMs (A) and RAW264.7 cells (B) were detected by ELISA. Data were shown as means±SEM of three independent experiments.

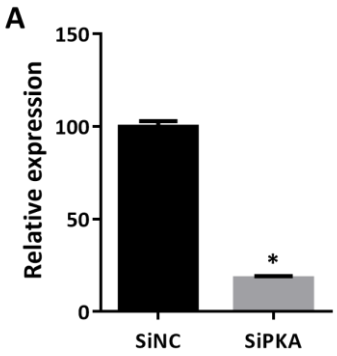

Figure S6. The percentage of knockdown for PKA C-α.

Table S1. Sequences of primers used for quantitative real-time PCR

Gene		**Sequence 5′-3′**
β-actin	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT
Tnf-α	Forward	CCCTCACACTCAGATCATCTTCT
	Reverse	GCTACGACGTGGGCTACAG
Ifn-γ	Forward	GCCACGGCACAGTCATTGA
	Reverse	TGCTGATGGCCTGATTGTCTT
Il-1β	Forward	GCAACTGTTCCTGAACTCAACT
	Reverse	ATCTTTTGGGGTCCGTCAACT
Il-2	Forward	TGAGCAGGATGGAGAATTACAGG
	Reverse	GTCCAAGTTCATCTTCTAGGCAC
Il-6	Forward	TAGTCCTTCCTACCCCAATTTCC
	Reverse	TTGGTCCTTAGCCACTCCTTC
Il-10	Forward	GCTCTTACTGACTGGCATGAG
	Reverse	CGCAGCTCTAGGAGCATGTG
Il-12p40	Forward	TGGTTTGCCATCGTTTTGCTG
	Reverse	ACAGGTGAGGTTCACTGTTTCT
Il-17a	Forward	TTTAACTCCCTTGGCGCAAAA
	Reverse	CTTTCCCTCCGCATTGACAC
Il-23	Forward	ATGCTGGATTGCAGAGCAGTA
	Reverse	ACGGGGCACATTATTTTTAGTCT
Inos	Forward	AAGTCAAATCCTACCAAAGTGA
	Reverse	CCATAATACTGGTTGATGAACT
Cox-2	Forward	GGGTGTGAAGGGAAATAAGG
	Reverse	TCTCCACCAATGACCTGAT
Nlrp3	Forward	ATTACCCGCCCGAGAAAGG
	Reverse	TCGCAGCAAAGATCCACACAG
Asc	Forward	CTTGTCAGGGGATGAACTCAAAA
	Reverse	GCCATACGACTCCAGATAGTAGC
Caspase-1	Forward	ACAAGGCACGGGACCTATG
	Reverse	TCCCAGTCAGTCCTGGAAATG
Il-18	Forward	GACTCTTGCGTCAACTTCAAGG
	Reverse	CAGGCTGTCTTTTGTCAACGA

Zo-1	Forward	GCCGCTAAGAGCACAGCAA
	Reverse	TCCCCACTCTGAAAATGAGGA
E-cadherin	Forward	CAGGTCTCCTCATGGCTTTGC
	Reverse	CTTCCGAAAAGAAGGCTGTCC
Occludin	Forward	TTGAAAGTCCACCTCCTTACAGA
	Reverse	CCGGATAAAAAGAGTACGCTGG
Mmp2	Forward	CAAGTTCCCCGGCGATGTC
	Reverse	TTCTGGTCAAGGTCACCTGTC
Mmp3	Forward	ACATGGAGACTTTGTCCCTTTTG
	Reverse	TTGGCTGAGTGGTAGAGTCCC
Mmp9	Forward	CTGGACAGCCAGACACTAAAG
	Reverse	CTCGCGGCAAGTCTTCAGAG
Ccr2	Forward	ATCCACGGCATACTATCAACATC
	Reverse	CAAGGCTCACCATCATCGTAG
Ccr4	Forward	GGAAGGTATCAAGGCATTTGGG
	Reverse	GTACACGTCCGTCATGGACTT
Ccr5	Forward	TTTTCAAGGGTCAGTTCCGAC
	Reverse	GGAAGACCATCATGTTACCCAC
Ccr6	Forward	CCTGGGCAACATTATGGTGGT
	Reverse	CAGAACGGTAGGGTGAGGACA
Ccr9	Forward	CTTCAGCTATGACTCCACTGC
	Reverse	CAAGGTGCCCACAATGAACA
Cxcr2	Forward	ATGCCCTCTATTCTGCCAGAT
	Reverse	GTGCTCCGGTTGTATAAGATGAC
Cxcr3	Forward	TACCTTGAGGTTAGTGAACGTCA
	Reverse	CGCTCTCGTTTTCCCCATAATC
Icam	Forward	GTGATGCTCAGGTATCCATCCA
	Reverse	CACAGTTCTCAAAGCACAGCG
Ip-10	Forward	CCAAGTGCTGCCGTCATTTTC
	Reverse	GGCTCGCAGGGATGATTTCAA
Kc	Forward	CTGGGATTCACCTCAAGAACATC
	Reverse	CAGGGTCAAGGCAAGCCTC
Mcp-1	Forward	TTAAAAACCTGGATCGGAACCAA
	Reverse	GCATTAGCTTCAGATTTACGGGT

Mdc	Forward	AGGTCCCTATGGTGCCAATGT
	Reverse	CGGCAGGATTTTGAGGTCCA
Mig	Forward	TCCTTTTGGGCATCATCTTCC
	Reverse	TTTGTAGTGGATCGTGCCTCG
Mip-1α	Forward	TTCTCTGTACCATGACACTCTGC
	Reverse	CGTGGAATCTTCCGGCTGTAG
Mip-1β	Forward	TTCCTGCTGTTTCTCTTACACCT
	Reverse	CTGTCTGCCTCTTTTGGTCAG
Mip-3α	Forward	ACTGTTGCCTCTCGTACATACA
	Reverse	GAGGAGGTTCACAGCCCTTTT
Rantes	Forward	GCTGCTTTGCCTACCTCTCC
	Reverse	TCGAGTGACAAACACGACTGC
Osm	Forward	ATGCAGACACGGCTTCTAAGA
	Reverse	TTGGAGCAGCCACGATTGG
Osmr	Forward	CATCCCGAAGCGAAGTCTTGG
	Reverse	GGCTGGGACAGTCCATTCTAAA
Fap	Forward	GTCACCTGATCGGCAATTTGT
	Reverse	CCCCATTCTGAAGGTCGTAGAT
Pdpn	Forward	ACCGTGCCAGTGTTGTTCTG
	Reverse	AGCACCTGTGGTTGTTATTTTGT
Col1a1	Forward	GCTCCTCTTAGGGGCCACT
	Reverse	CCACGTCTCACCATTGGGG
Pde4a	Forward	GAACCGGGAACTCACACACC
	Reverse	GTACTCTGAGACCTGGTTTCCT
Pde4b	Forward	CGCAGGGAGTCGTTCCTCTA
	Reverse	CTCCTGTGGTCGCACACTTG
Pde4c	Forward	TCCGAGAGCCAGTGGATTCT
	Reverse	CCTTGAGTTCCAATCGTGAAGAC
Pde4d	Forward	TTTTGCCAGTGCAATACATGATG
	Reverse	CAGAGCGAGTTCCGAGTTTGT

Table S2. Antibodies for immunoblotting

Antibody	Source	Vendor	Catalog No.
anti-Occludin	Rabbit	Thermo Fisher Scientific	71-1500
anti-ZO-1	Rabbit	Proteintech	21773-1-AP
anti-α Tubulin	Mouse	Proteintech	HRP-66031
anti-SOCS3	Rabbit	Proteintech	14025-1-AP
anti-PDE4D	Rabbit	Proteintech	12918-1-AP
anti-PDE4C	Rabbit	Proteintech	21754-1-AP
anti-PDE4A	Rabbit	Proteintech	16226-1-AP
anti-E-cadherin	Rabbit	Cell Signaling Technology	3195
anti-PDE4B	Rabbit	Cell Signaling Technology	72096
anti-Rap1A/Rap1B	Rabbit	Cell Signaling Technology	4938
anti-Epac1	Mouse	Cell Signaling Technology	4155
anti-EPAC2	Mouse	Cell Signaling Technology	4156
anti-p-CREB	Rabbit	Cell Signaling Technology	9198
anti-CREB	Mouse	Cell Signaling Technology	9104
anti-PKA C-α	Rabbit	Cell Signaling Technology	5842
anti-p-stat3	Rabbit	Cell Signaling Technology	98543
anti-p-IκBα	Mouse	Cell Signaling Technology	9246
anti-p-p38	Rabbit	Cell Signaling Technology	9215
anti-IκBα	Rabbit	Cell Signaling Technology	4812
anti-p-NF-κB	Rabbit	Cell Signaling Technology	4806
anti-p65	Rabbit	Cell Signaling Technology	4764
anti-p-SAPK/JNK	Rabbit	Cell Signaling Technology	4671
anti-p-Akt	Rabbit	Cell Signaling Technology	4056
anti-SAPK/JNK	Rabbit	Cell Signaling Technology	9252
anti-p38	Rabbit	Cell Signaling Technology	8690
anti-HMGB1	Rabbit	Cell Signaling Technology	6893
anti-p44/42	Rabbit	Cell Signaling Technology	4695
anti-p-p44/42	Rabbit	Cell Signaling Technology	4377
anti-Akt	Rabbit	Cell Signaling Technology	9272
anti-Stat5	Rabbit	Cell Signaling Technology	94205
anti-p-Stat5	Rabbit	Cell Signaling Technology	9351
anti-Stat4	Rabbit	Cell Signaling Technology	2653

anti-p-Stat4	Rabbit	Cell Signaling Technology	5267
anti-MyD88	Rabbit	Cell Signaling Technology	4283
anti-Stat6	Rabbit	Cell Signaling Technology	9362
anti-p-Stat6	Rabbit	Cell Signaling Technology	56554
anti-Stat1	Rabbit	Cell Signaling Technology	9172
anti-p-Stat1	Rabbit	Cell Signaling Technology	7649
anti-Stat3	Rabbit	Cell Signaling Technology	4904
anti-p85	Rabbit	Cell Signaling Technology	4257
anti-p-p85	Rabbit	Cell Signaling Technology	4228
anti-p-mTOR	Rabbit	Cell Signaling Technology	5536
anti-p-MEK1/2	Rabbit	Cell Signaling Technology	2338
anti-p-IκBα	Rabbit	Cell Signaling Technology	2859
anti-mTOR	Rabbit	Cell Signaling Technology	2983
anti-MEK1/2	Rabbit	Cell Signaling Technology	8727
anti-IkB-α	Rabbit	Cell Signaling Technology	9242