Supplementary Data

Formylpeptide receptor2 contributes to colon epithelial homeostasis, inflammation, and tumorigenesis

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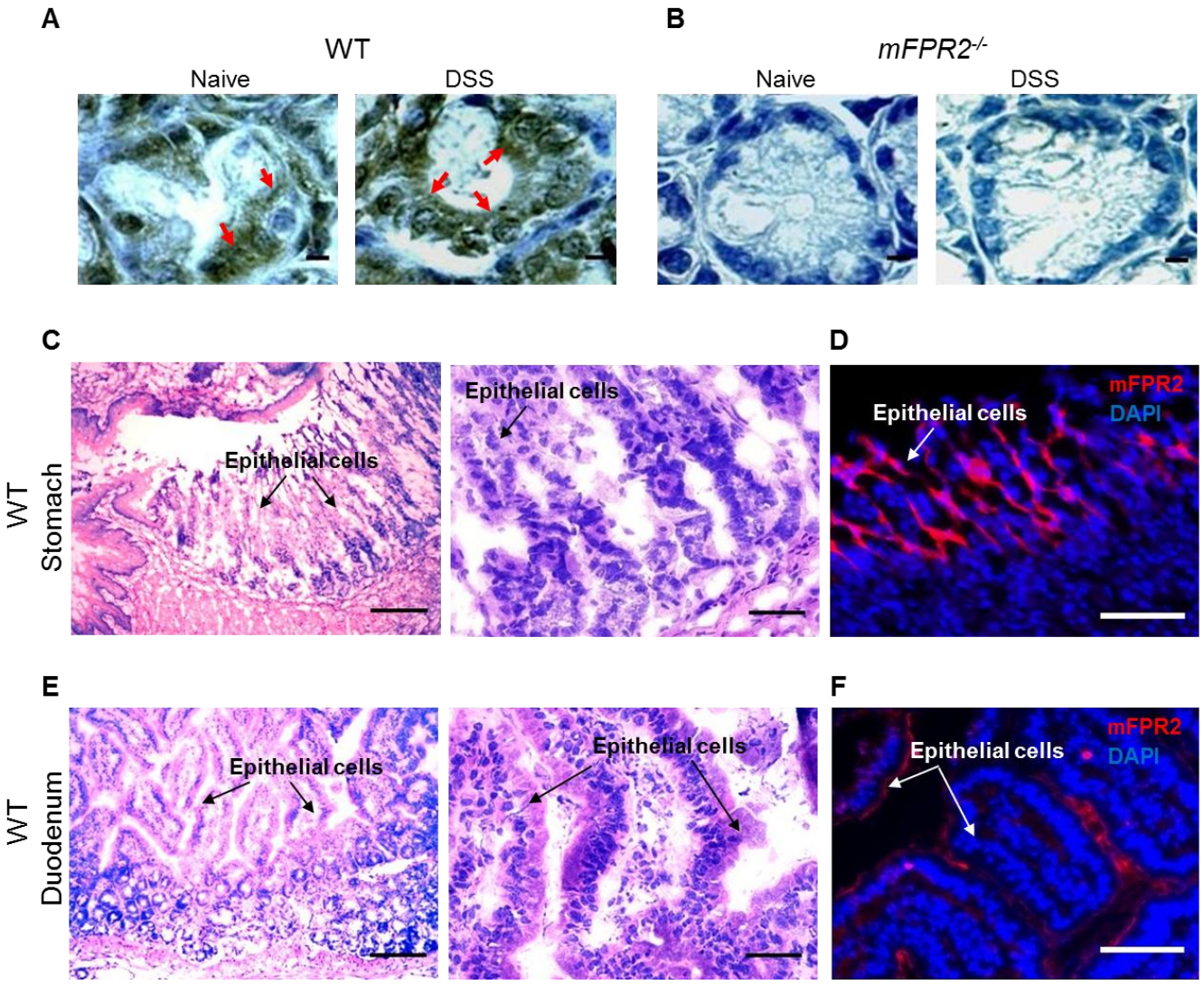
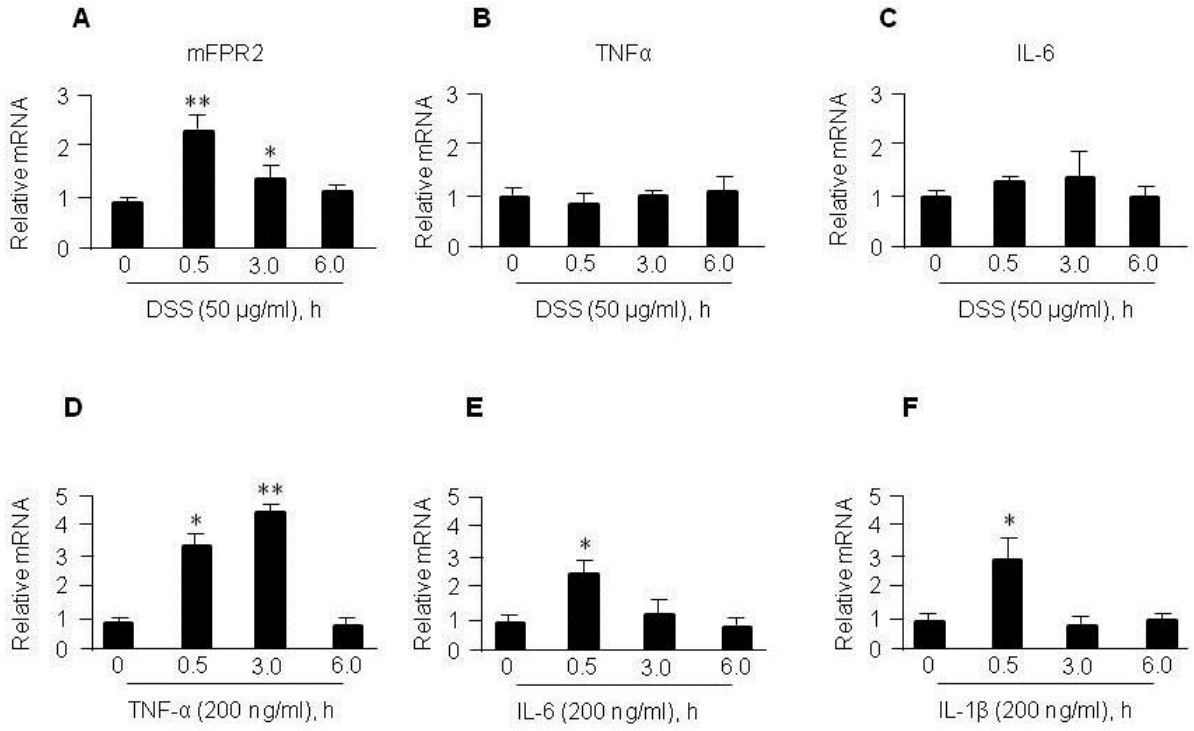


Supplementary Figure 1: The expression of mFPR2 protein in the epithelial cells of colon, stomach and duodenum.

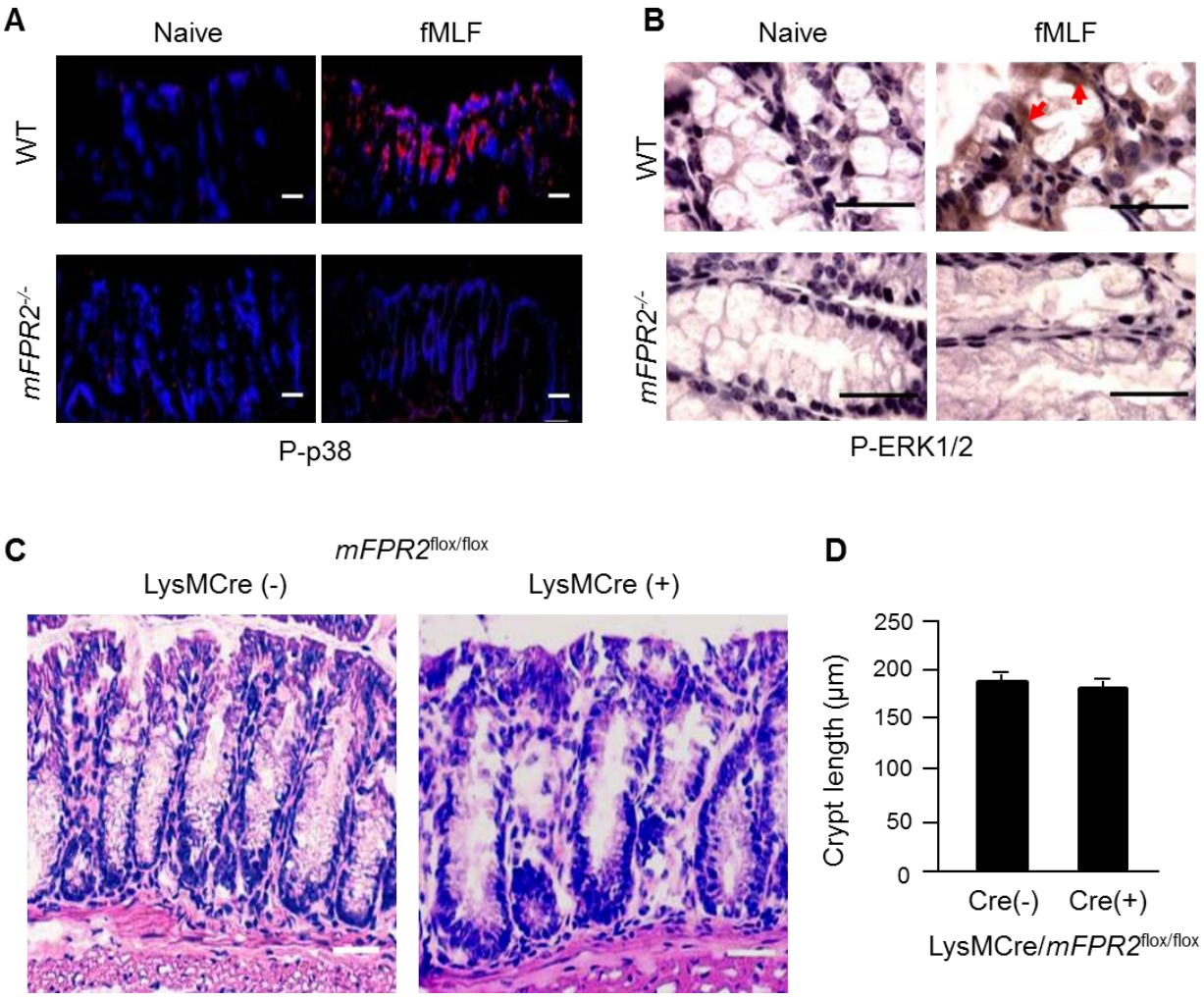
Colon from naïve or DSS-treated mice were paraffin sectioned (5 µm). The sections were stained with an anti-mFPR2 Ab followed by a biotinylated anti-Ig secondary Ab and streptavidin-HRP/DAB with hematoxylin counter staining. **A.** Colon sections from WT mice. **Left Panel**: Section from naïve mice; **Right Panel**: Section from DSS-treated mice. Arrows show mFPR2 expression in the crypt epithelial cells. *Scale bar* = 10 µm. **B.** Colon sections from *mFPR2*$^{-/-}$ mice, Left Panel: Section from naïve mice; Right Panel: Section from DSS-treated mice. *Scale bar* = 10 µm. **C.** Stomach tissues from naïve WT mice were paraffin sectioned and stained with H.E. **D.** Cryo-section of WT moue stomach stained with anti-mFPR2 Ab followed by a biotinylated anti-rabbit Ig secondary Ab-PE (Red). DAPI was used to

stain the nuclei (Blue). *Scale bar* = 50 μm. N = 5 mice. **E.** Duodenum tissues from naïve WT mice were paraffin sectioned and stained with H.E. **F.** Cryo-section of WT mouse duodenum was stained with anti-mFPR2 Ab followed by an anti-rabbit Ig secondary Ab-PE (Red). DAPI was used to stain the nuclei (Blue). *Scale bar* = 50 μm. N = 5 mice.



Supplementary Figure 2: Regulation of *mFPR2* mRNA in mouse colon epithelial cell line CT26 by DSS and proinflammatory cytokines.

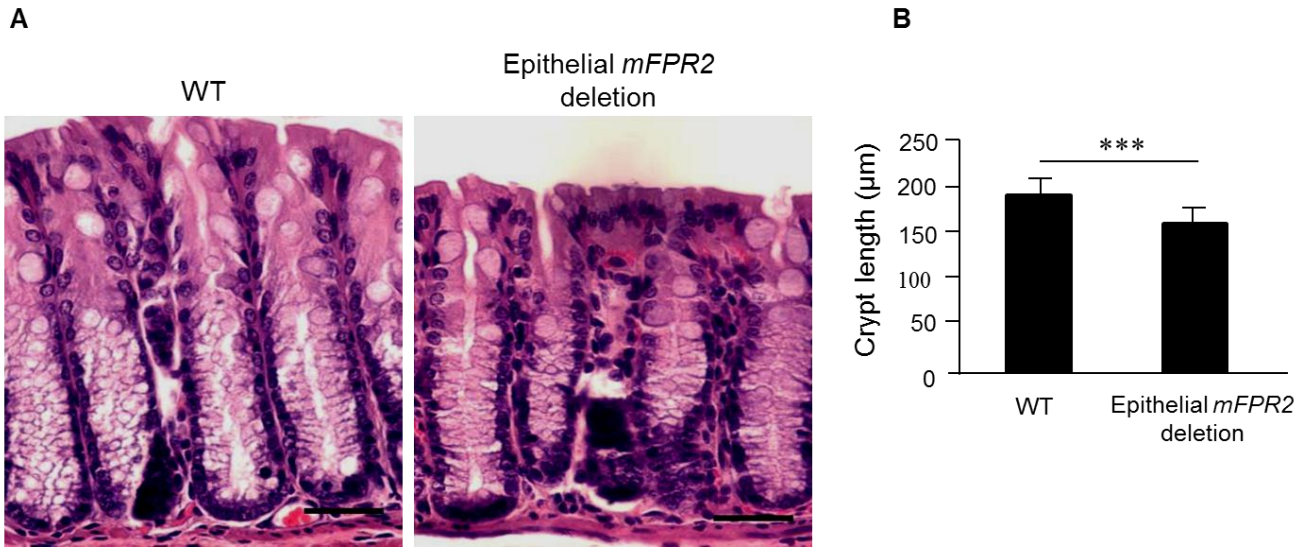
Mouse colon epithelial cell line CT26 was stimulated with DSS (50 μg/ml) or TNF-α (200 ng/ml), IL-1β (200 ng/ml), and IL-6 (200 ng/ml) at indicated time points. The expression of *mFPR2* and *β-actin* mRNA was examined by RT-PCR. The results were shown as relative mRNA by densitometry measurement. **A.** Up-regulation of *mFPR2* mRNA expression by DSS. **B-C**. DSS failed to induce TNF-α, and IL-6 mRNA expression by CT26 cells. **D-F**: Up-regulation of *mFPR2* mRNA expression by TNF-α, IL-1β, and IL-6. Results are expressed as the mean ± SEM. N = 3. * $P < 0.05$, ** $P < 0.01$.



Supplementary Figure 3: fMLF-induced phosphorylation of p38 and ERK1/2 in colon epithelial cells and the colon crypt length of mice with myeloid cell specific deletion of *mFPR2*.

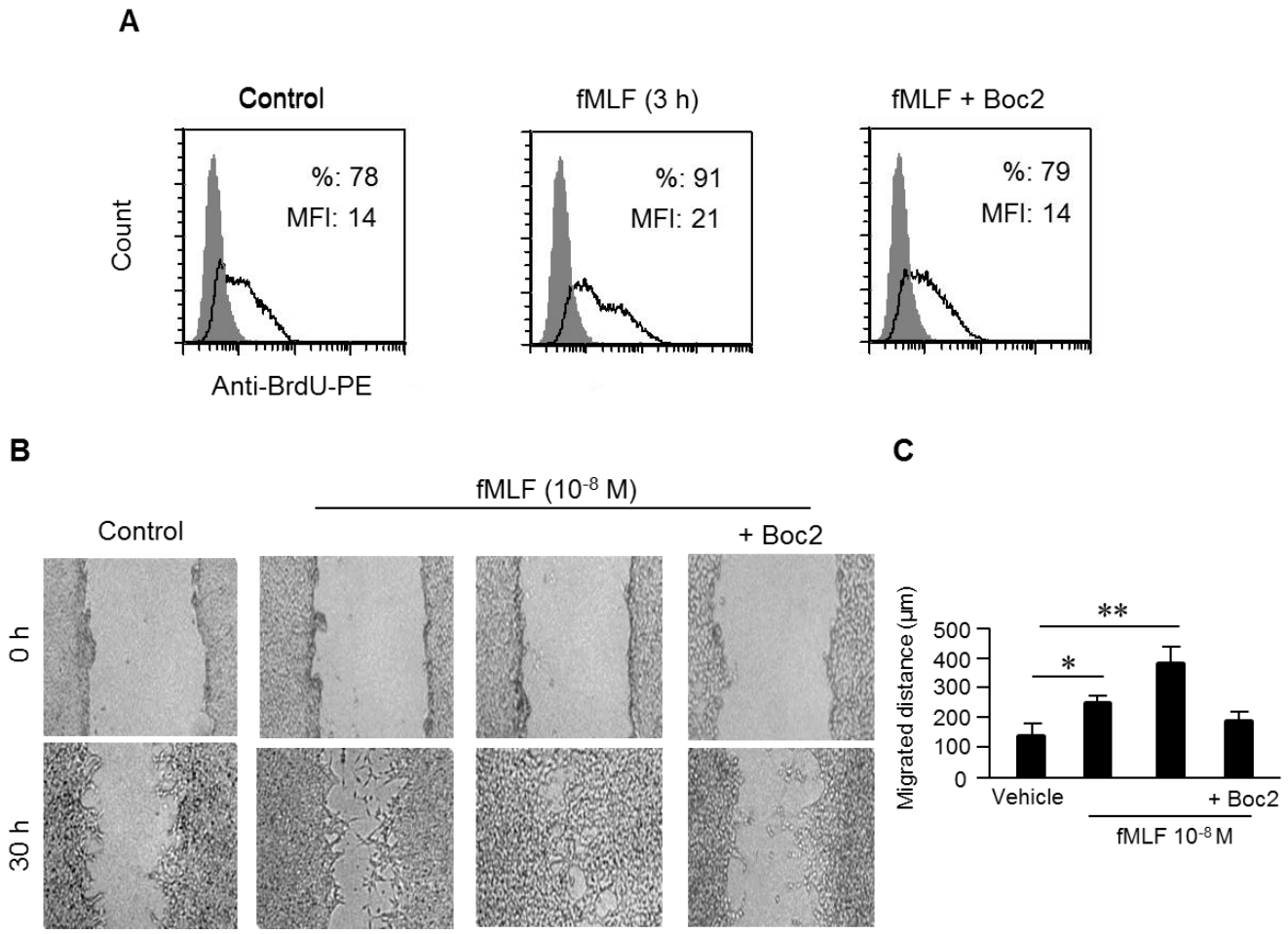
A. p38 phosphorylation. After intrarectal infusion of fMLF (5×10^{-5} M) for 5 min, mouse colon was cryo-sectioned and the phosphorylation of p38 was detected by an anti-phosphorylated p38 Ab followed by a biotinylated anti-rabbit Ig secondary Ab and Streptavidin-PE (Red). DAPI was used to stain the nuclei (Blue). *Scale bar* = 50 μm. N = 5 mice. **B.** ERK phosphorylation. Mouse colon was flushed with fMLF (5×10^{-5} M) and was then paraffin sectioned (5 μm). The phosphorylation of ERK1/2 was detected by an anti-phosphorylated ERK1/2 Ab followed by a biotinylated anti-IgG secondary Ab and streptavidin-HRP/DAB with hematoxylin counter staining. Arrows show phosphorylated ERK1/2 in colon epithelial cells. *Scale bar* = 50 μm. **C.** The length of colon crypts from myeloid cell specific

mFPR2 deletion mice. H&E stained sections of colons from naïve control (LysMCre (-)) or myeloid specific *mFPR2*$^{-/-}$ (LysMCre (+)) mice. *Scale bar* = 50 μm. **D.** Cumulative measurement of colon crypt length. Results are expressed as the mean ± SEM. N = 5 mice per group.



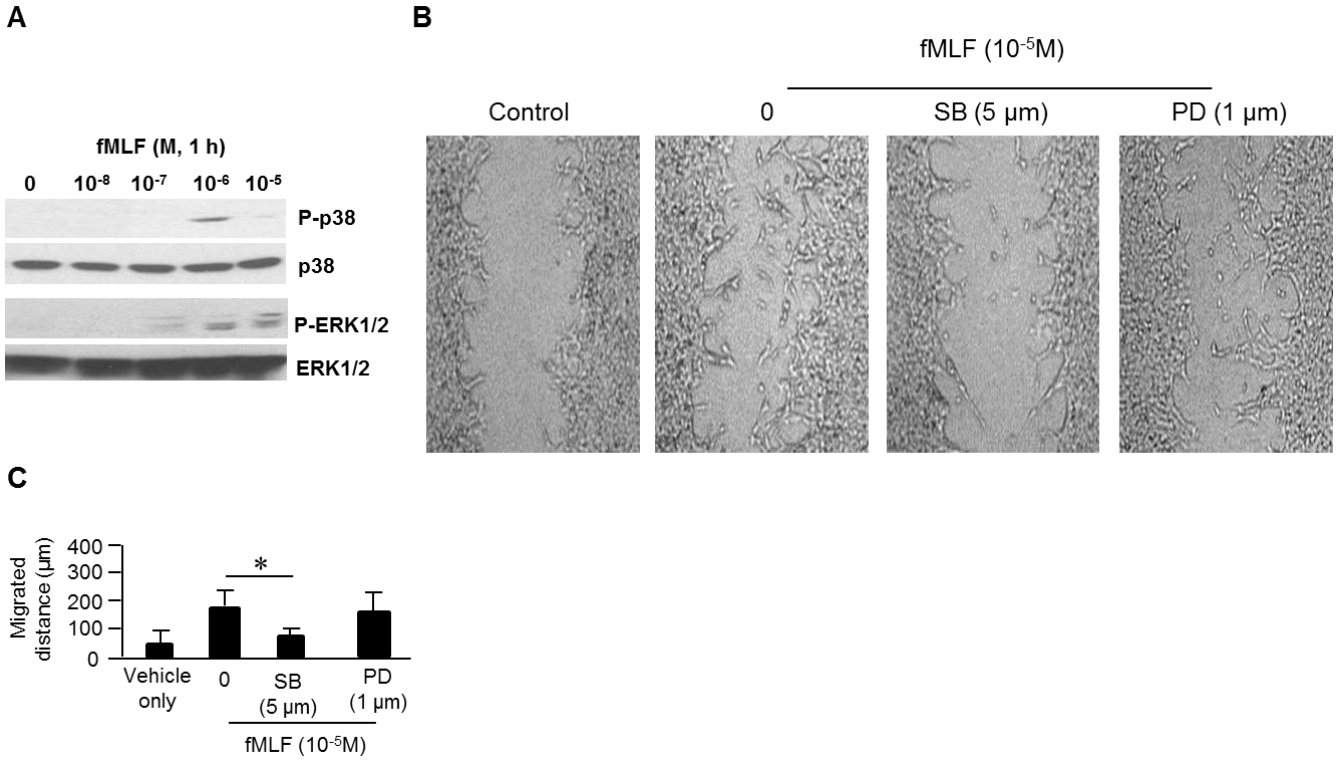
Supplementary Figure 4: The length of colon crypts from mice with epithelial cell specific *mFPR2* deletion.

A. The colon crypt length of mice with epithelial cell specific *mFPR2* deletion. H&E sections of colons from WT mice (Vallin-Cre (-) *mFPR2* $^{flox/flox}$) or mice with epithelial cell specific deletion of *mFPR2*(*Vallin*-Cre (+) *mFPR2*$^{flox/flox}$). *Scale bar* = 100 µm. **B.** Cumulative measurement of colon crypt length. Results are expressed as the mean ± SEM. N = 5 mice per group. *** $P < 0.001$.



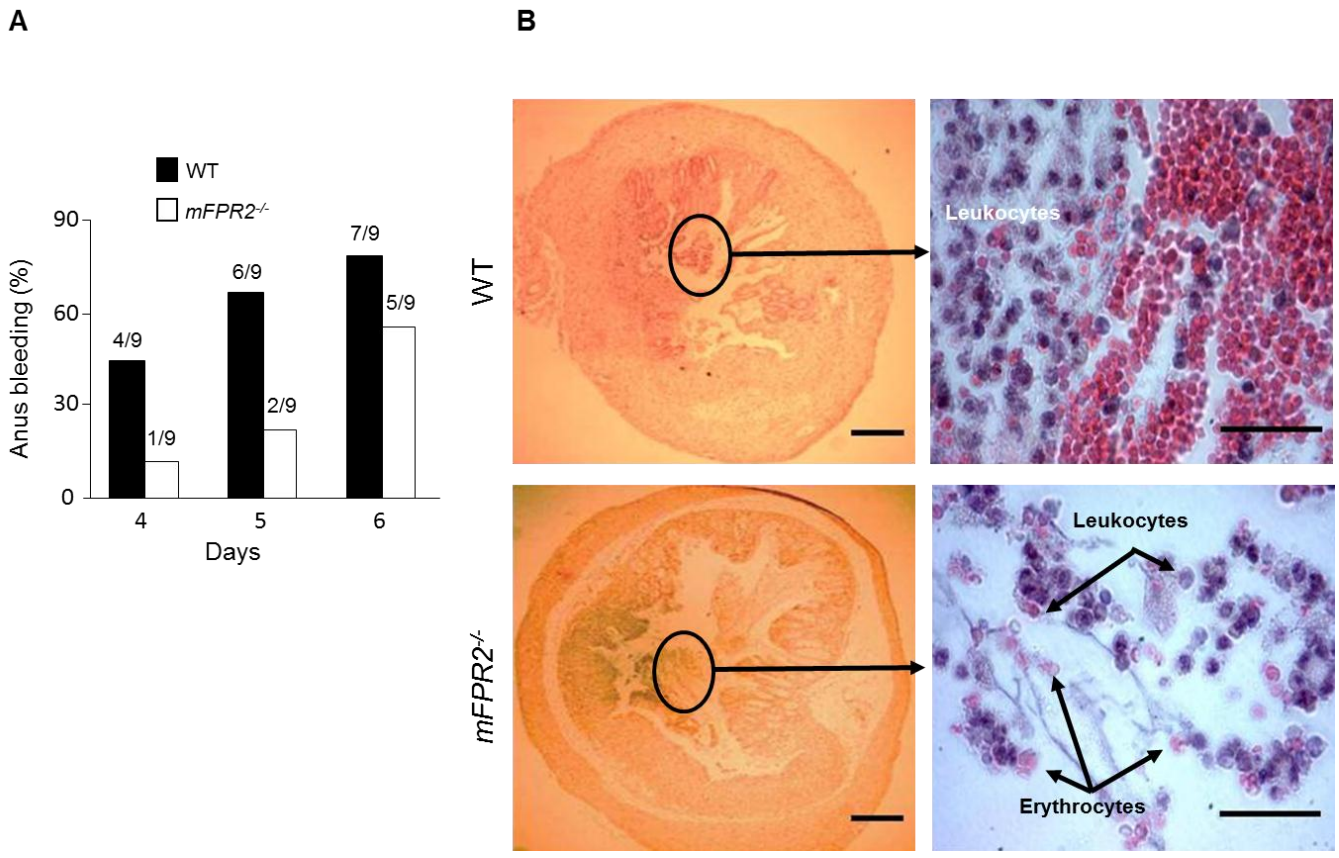
Supplementary Figure 5: In vitro proliferation of human colon epithelial cell line in vitro induced by fMLF.

A. DNA synthesis in the colon epithelial cell line T84. The cells were plated in 6 well cell culture cluster (1.5×10^6 cells/ml) with 10 µl of 1 mM BrdU solution, then were incubated with fMLF or fMLF plus Boc2 (5 µg/ml) for 3 h at 37°C before analysis by FACS. Results are presented as the percentage of $BrdU^+$ cells and MFI. **B.** fMLF promotes the closure of scratching wound on T84 cell monolayer. A single linear wound was created through the confluent T84 cell monolayer using a sterile pipette tip followed by incubation with fMLF (10^{-8}M, 10^{-5}M) in the presence or absence of Boc2 (5µg/ml). The distance from the wound edges that cells migrated into the wound space were determined at 30 h. **C.** Distance migrated by T84 cells. $*P < 0.05$, $**P < 0.01$. Results are expressed as the mean ± SEM. Data shown is representative of 3 independent experiments.



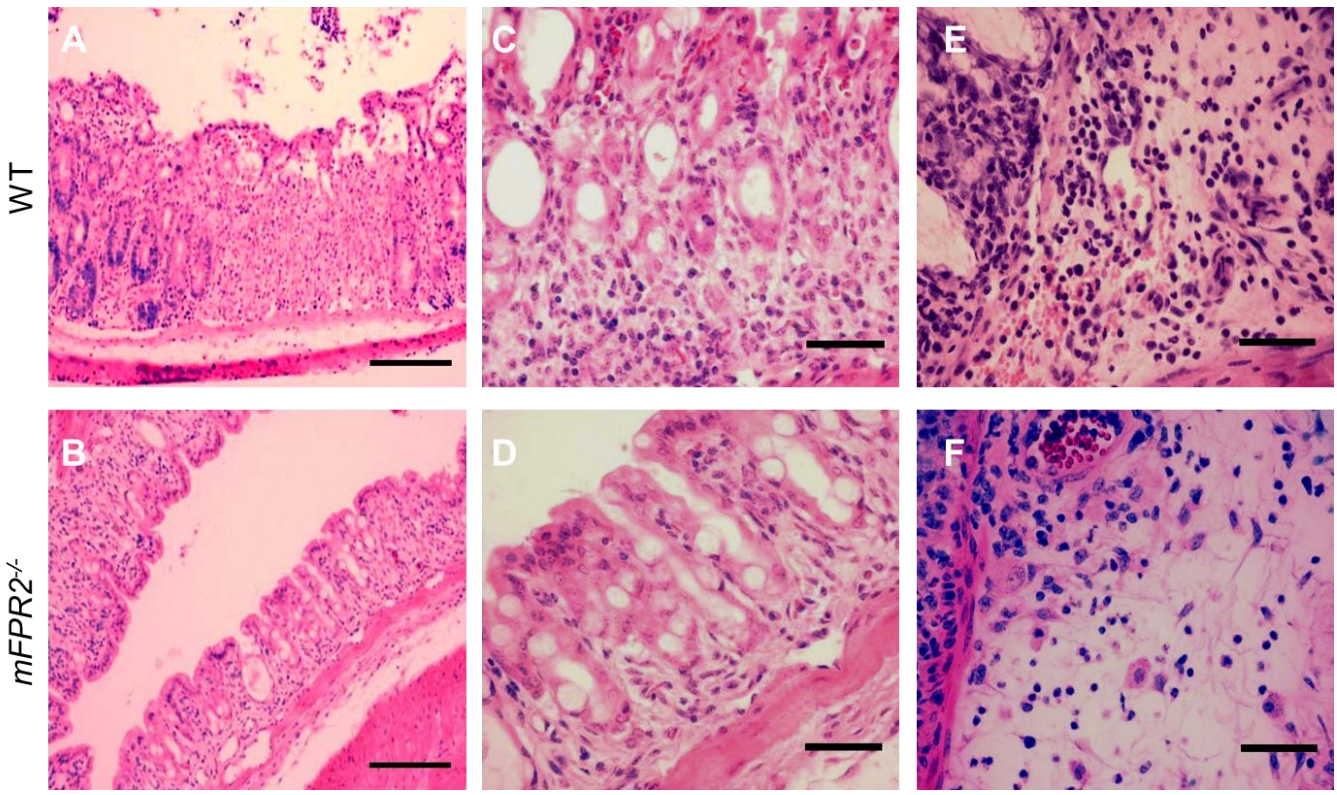
Supplementary Figure 6: fMLF-induced phosphorylation of MAPKs in T84 cells and the effect of MAPK inhibitors on fMLF-promoted wound closure.

A. fMLF-induced phosphorylation of MAPKs in T84 cells. T84 cells were stimulated with fMLF at indicated concentrations for 1 h. Phosphorylated p38 and ERK1/2 were detected by Western blotting. Membranes were stripped for detection of total p38 and ERK1/2. **B.** The effect of MAPK inhibitors on fMLF-promoted scratch wound closure. A single linear wound was created on confluent T84 cell monolayer. The cells were then incubated with fMLF (10^{-5}M) in the presence or absence of the p38 inhibitor SB203580 (5 μm) or the ERK inhibitor PD98059 (1 μm). The distance shown by cells migrating from the wound edges towards the center was determined at 10 h. **C.** Blockage of the effect of fMLF on T84 cell wound closure by the p38 inhibitor SB203580 (SB). N = 3. *$P < 0.05$. Results are expressed as the mean ± SEM. Data shown is representative of 3 independent experiments.



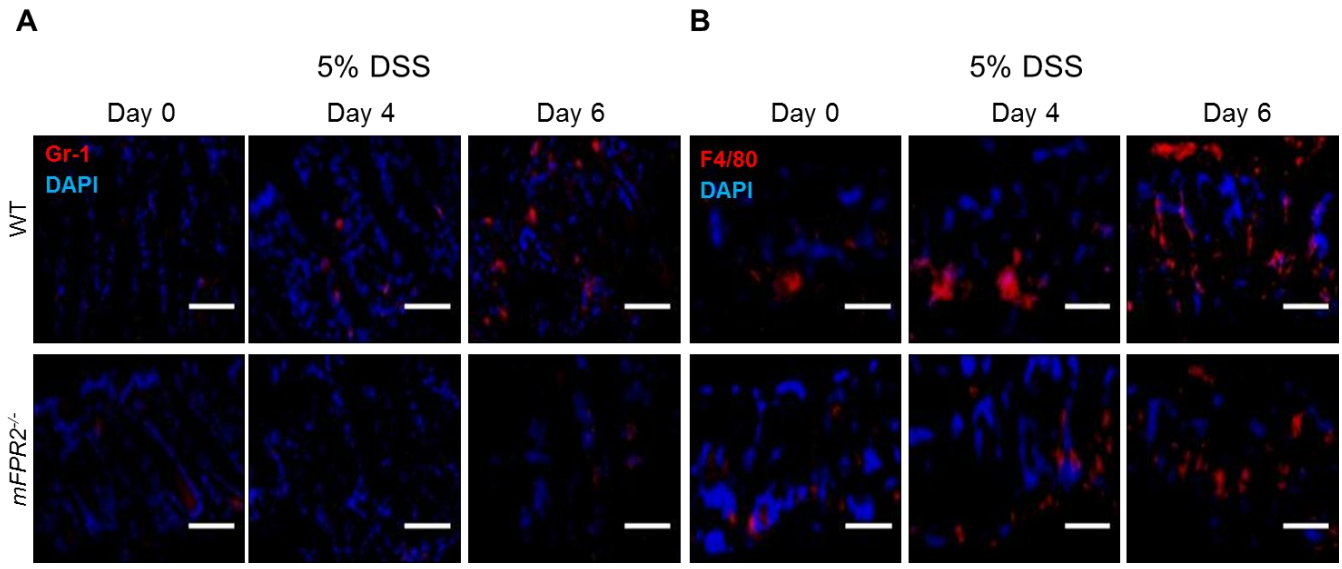
Supplementary Figure 7: Mouse colon bleeding in DSS-induced acute colitis.

A. Anus bleeding. Mice were given 5% DSS in drinking water for 6 days. Anus bleeding was monitored daily. **B.** Bleeding in the colon lumen. Mouse colon was harvested at day 5 after 5% DSS intake in drinking water. The colon was paraffin-sectioned (5 µm) and stained with H&E to reveal erythrocytes and leukocytes (arrows). N = 9 mice per group. *Scale bar* = 200 µm and 50 µm, respectively.



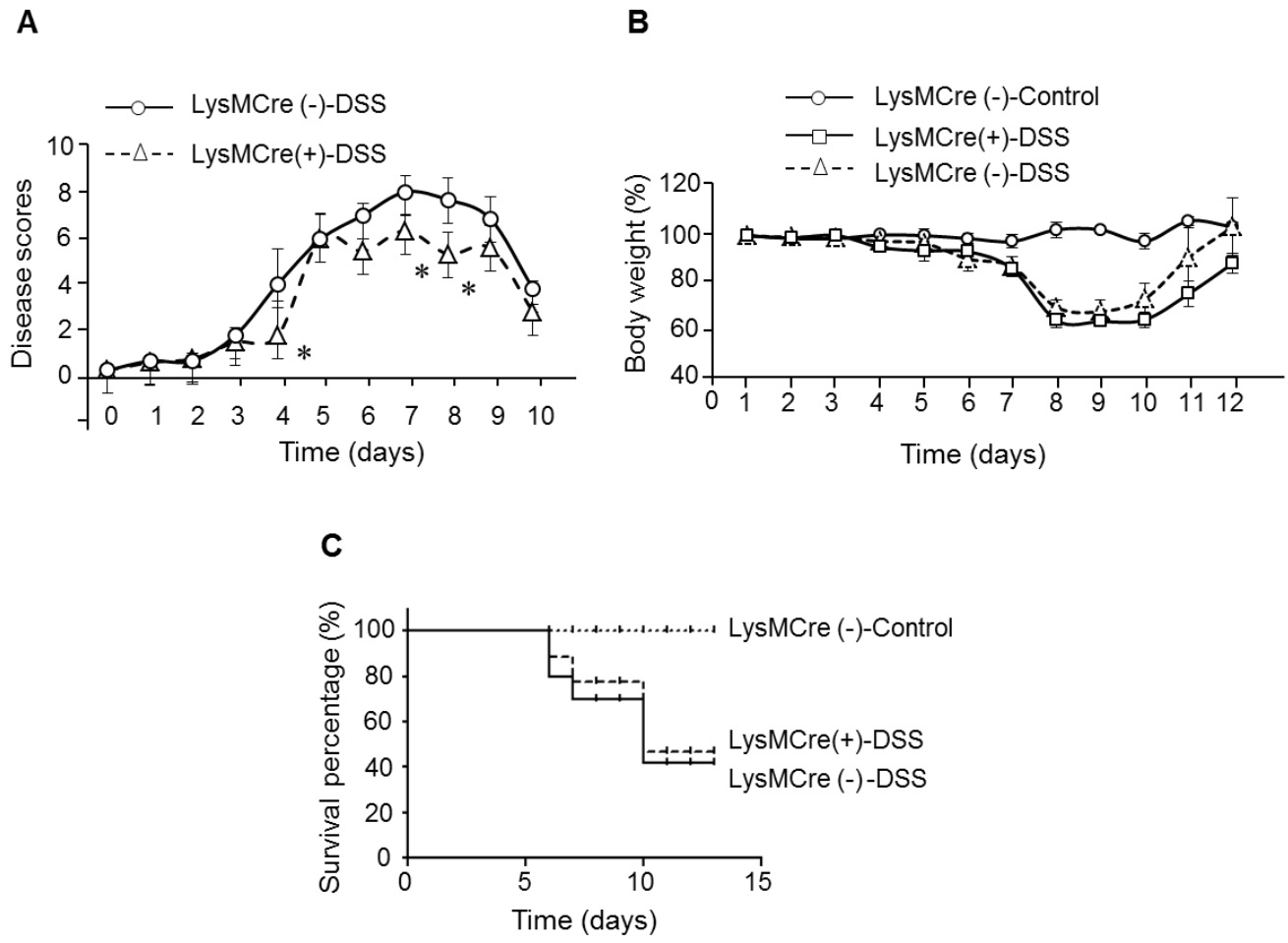
Supplementary Figure 8: DSS-induced acute colon inflammation.

Ascending colon from WT and *mFPR2*$^{-/-}$ littermates treated with 5% DSS for 4 days was sectioned (5 µm) and stained with H&E. **A-B.** Injuries of crypts. **C-D.** Infiltration of leukocytes in the mucosa. **E-F.** Accumulation of leukocytes in the submucosa. N = 9 mice per group. *Scale bar* = 100 µm (A-B) and 50 µm (C-F), respectively.



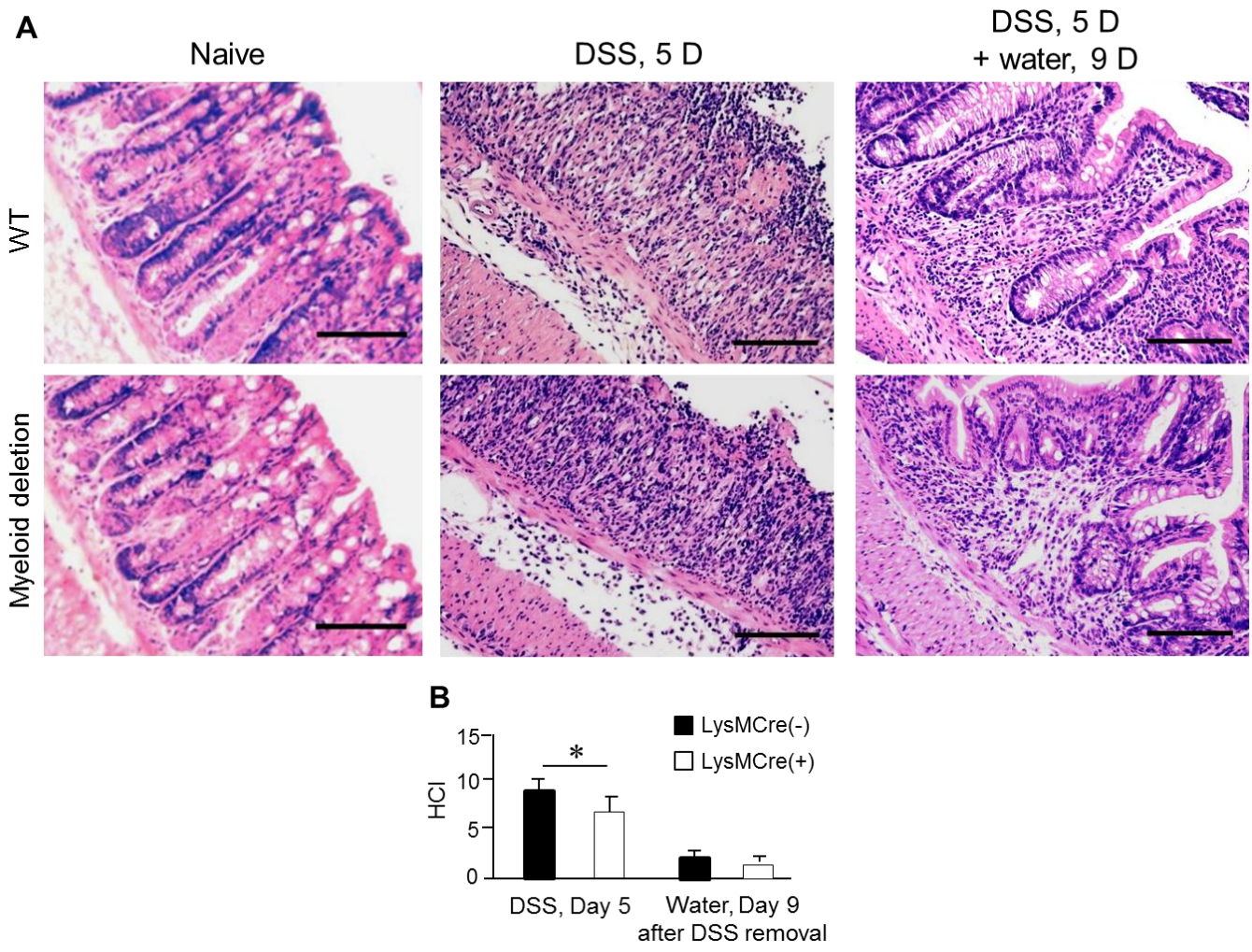
Supplementary Figure 9: Leukocyte infiltration in DSS-induced acute colon inflammation.

Mice were given 5% DSS in drinking water for 4 days. Colons were cryo-sectioned (5 μm) and stained with anti Gr-1 or anti F4/80 Abs. **A.** Infiltration of Gr-1$^+$ cells in the colon mucosa. **B.** Infiltration of F4/80$^+$ cells in the colon mucosa. *Scale bar* = 50 μm.



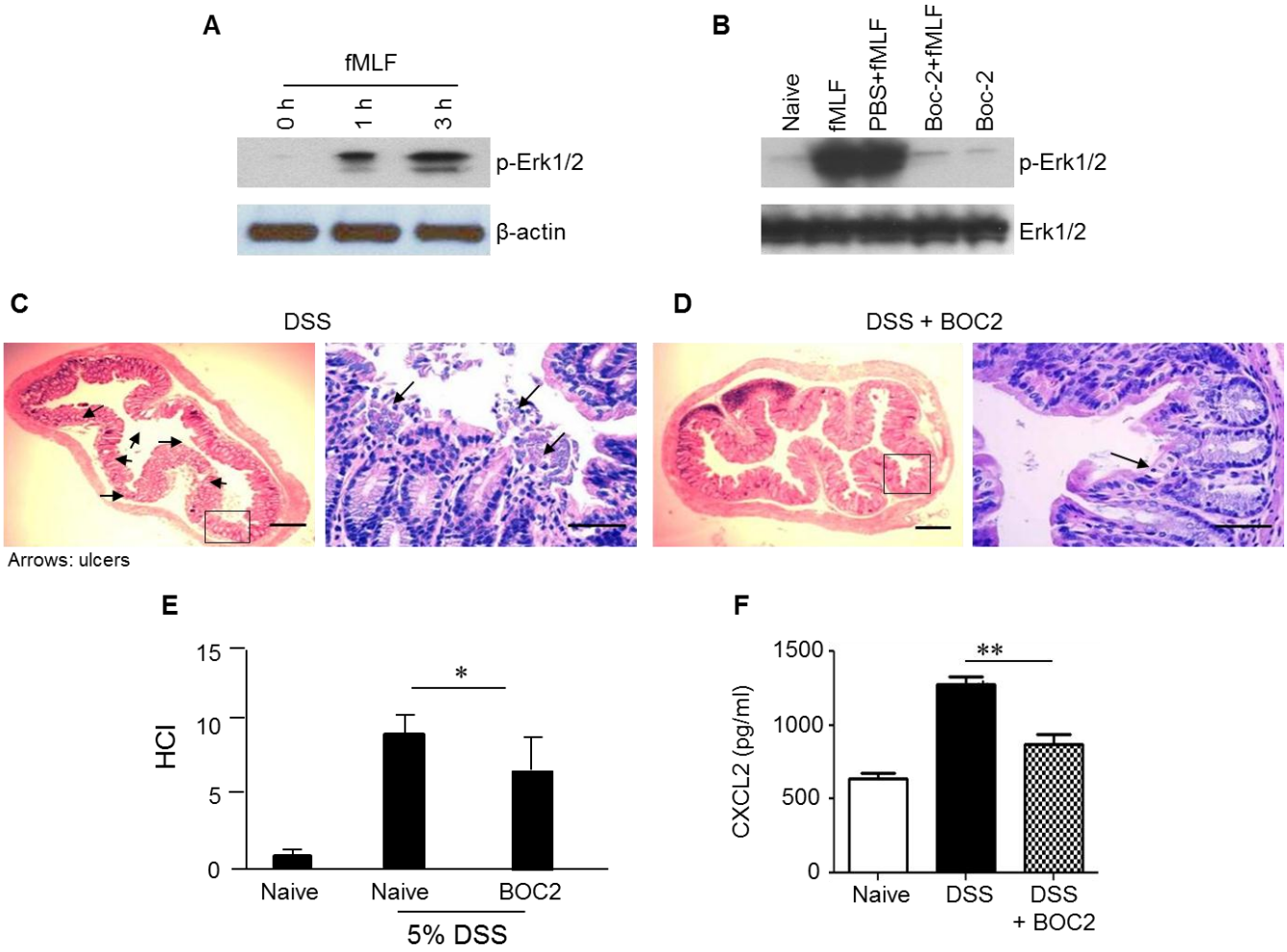
Supplementary Figure 10: DSS-induced acute colitis in mice with myeloid cell specific deletion of *mFPR2*.

Mice with myeloid cell specific deletion of *mFPR2* (LysMCre (+)) and WT mice (LysMCre (-)) were given 5% DSS in drinking water for 5 days followed by normal water. **A.** Disease scores. N = 9 mice per group. **B.** Death rate of DSS-treated mice. N = 9 mice per group. **C.** Body weight. N = 9 mice per group. * indicates a significant difference in disease scores in mice with myeloid cell specific deletion of *mFPR2* (LysMCre (+)) compared to WT mice (LysMCre (-)) ($P < 0.05$).



Supplementary Figure 11: Histological changes in DSS-induced acute colitis in mice with myeloid cell specific deletion of *mFPR2*.

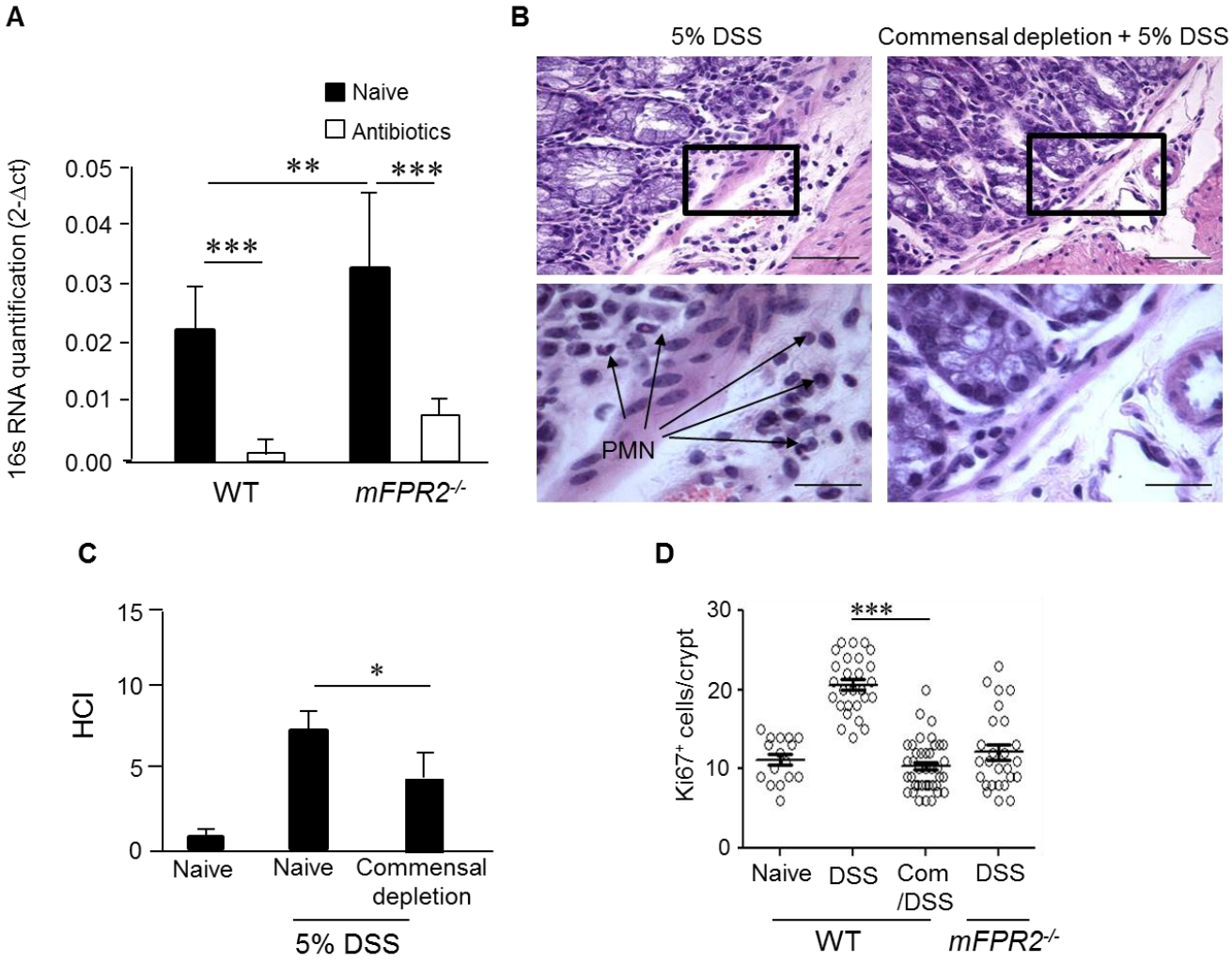
A. Left Panels: Histology of the colon from naïve myeloid cell specific *mFPR2* deletion mice (LysMCre (+)) and WT (LysMCre (-)) mice. **Middle Panels:** Histological changes in the colon mucosa of mice given 5% DSS in drinking water for 5 days. **Right Panels:** Histological changes in the colon mucosa of mice given 5% DSS in drinking water for 5 days followed by normal water for 9 days. *Scale bar* = 100 µm. **B.** HCI in mouse colon. * $P < 0.05$.



Supplementary Figure 12: The effect of Boc2 on DSS-induced acute colitis.

A. Phosphorylation of Erk1/2 in colon epithelial cells after I.P. injection of fMLF. WT mice were I.P. injected with 100 µl fMLF (10^{-3} M) and the colons were harvested at indicated time points. Colon mucosa was scraped, lysed and ERK1/2 phosphorylation was measured by Western blotting. The membrane was stripped for detection of β-actin. **B.** Inhibition of the effect of fMLF by I.P. injection of Boc2. WT mice were I.P. injected with Boc-2 (1 µg) or PBS prior to injection of fMLF (10^{-3} M, 100 µl) and colon epithelial layer was harvested 3 h later to examine the phosphorylation of Erk1/2 **C-F.** WT mice were given 5% DSS in drinking water (**C**) or combined with I.P. injection of Boc2 (100 µg/mouse) (**D**) for 5 days. The colon was paraffin-sectioned (5 µm) and stained with H&E. Arrows indicate ulcers

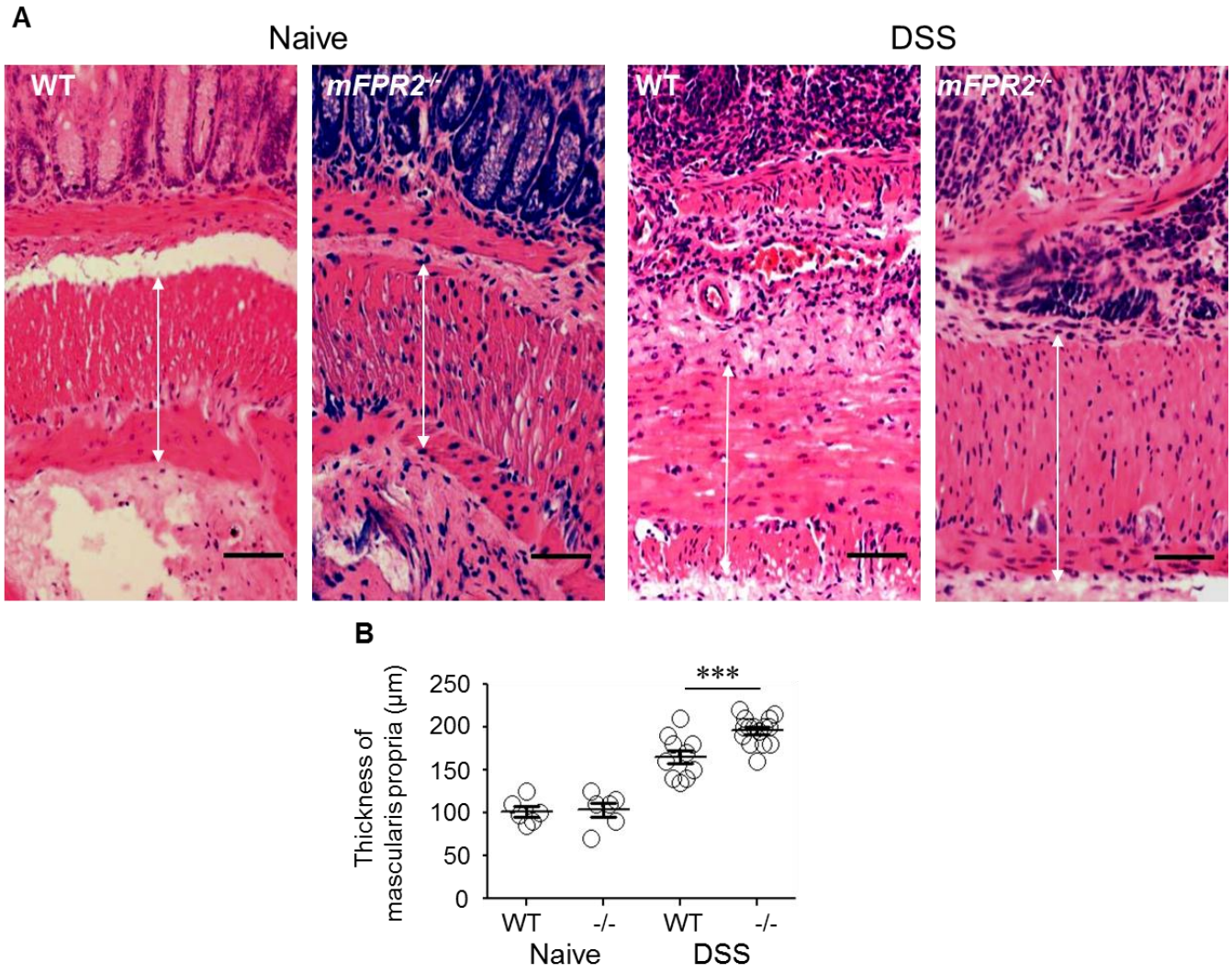
in the colon mucosa layer. *Scale bar* = 200 µm and 100 µm, respectively. **E.** HCI. N = 5 mice per group. **F.** CXCL2 production by colon mucosal epithelial cells. Mucosal fragments (3 cm) were cultured in 12-well flat bottom culture plates in serum-free RPMI-1640 at 37^{o}C for 24 h. The supernatant was then collected to measure CXCL2 by ELISA. Results are expressed as the mean ± SEM. N = 5 mice per group. * $P < 0.05$, ** $P < 0.01$.



Supplementary Figure 13: The effect of depletion of commensal bacteria on DSS-induced acute colitis.

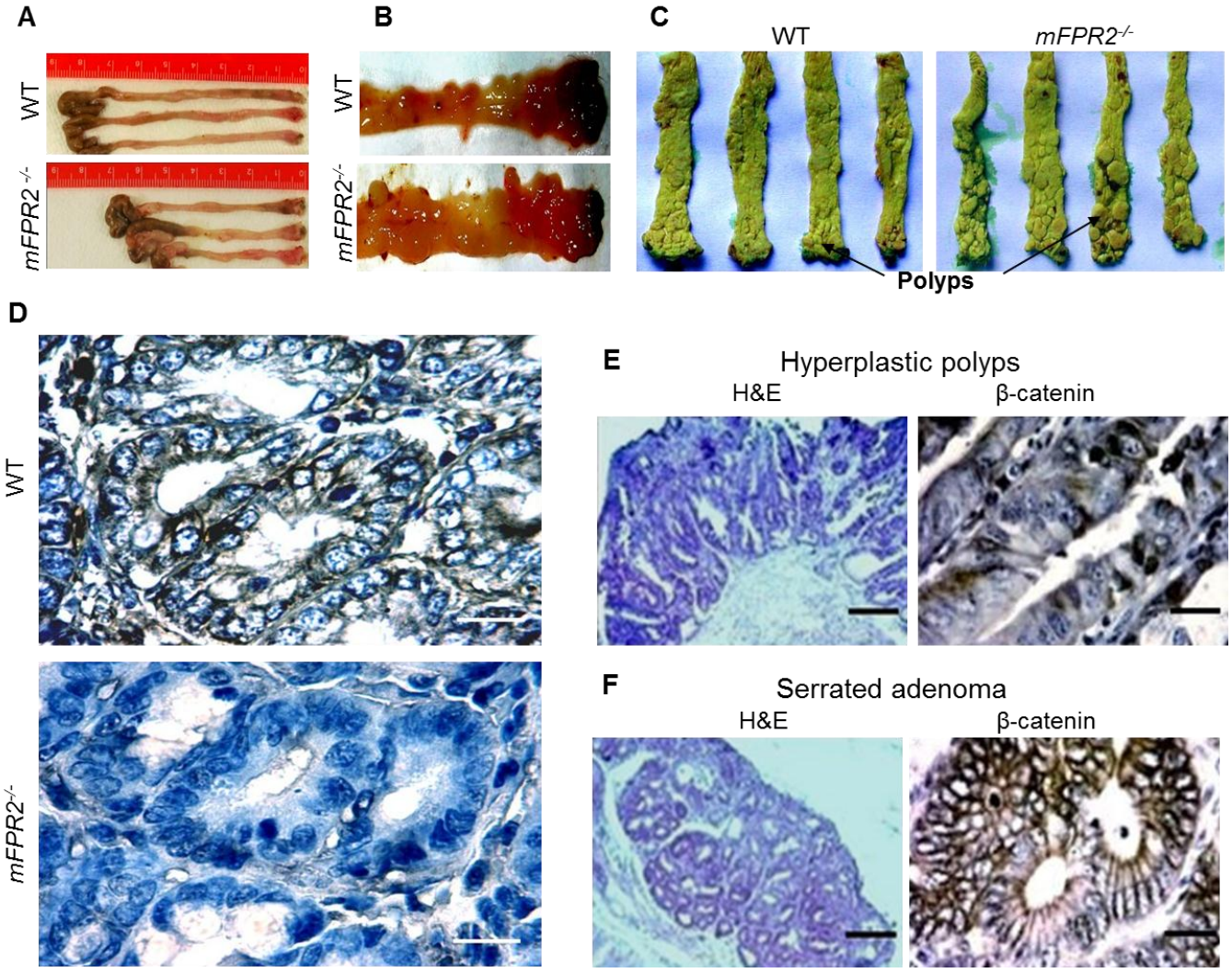
A. The effect of antibiotics on gut microbiome. Mice were given a cocktail of ampicillin (A, 1 g/l,), vancomycine (V, 500 mg/l), neomycin sulfate (N, 1 g/l), and metronidazole (M, 1g/l) in drinking water for 4 weeks. Stool from the cecum was collected and DNA was extracted using a QIAamp DNA stool Mini Kit. The quantity of 16sRNA for gut bacteria was assayed by real time PCR. ** $P < 0.01$, *** $P < 0.001$. **B-D.** Reduced inflammatory responses and colon crypt epithelial cell proliferation after commensal bacterial depletion in WT mouse colon. Mice were given antibiotics cocktail for 4 weeks followed by 5% DSS in drinking water for 5 days. **B.** The colon was paraffin-sectioned (5 μm) and

stained with H&E. Arrows indicate infiltrating neutrophils. *Scale bar* = 100 µm and 50 µm, respectively. **C.** HCI. N = 10 mice per group. *$P < 0.05$. **D.** Cumulative number of $Ki67^+$ cells in colon crypts. A mean value of 5 crypts was calculated from each mouse. N = 10 mice per group. Results are representative of 2 independent experiments. ***P < 0.001**.** Com: Commensal depletion**.**



Supplementary Figure 14: DSS-induced chronic colon inflammation in WT and *mFPR2*$^{-/-}$ mice.

A. Thickness of muscularis propria. The distal colon tissue section approximately 10 mm from the anus was analyzed to calculate a mean value of the thickness from 3 different points. **B.** Cumulative results of the thickness of muscularis propria. Results are expressed as the mean ± SEM. N = 5-7 mice per group. *** $P < 0.001$.



Supplementary Figure 15: Chronic colitis-associated tumorigenesis.

Mice treated with AOM were given 2.5% DSS for 1 week followed by 2 weeks of regular drinking water. The DSS and regular water treatment was repeated for three additional cycles. **A.** The length of colon after AOM + DSS treatment. **B-C.** Macroscopic view of adenomas in mouse colon. **D.** Detection of mFPR2 in colon adenoma cells of WT mice. Colon tissues with adenoma were paraffin sectioned (5

μm). The sections were stained with an anti-mFPR2 Ab followed by a biotinylated anti-Ig secondary Ab and streptavidin-HRP/DAB with hematoxylin counter staining. **E.** Hyperplastic polyps in the colon. **F.** Serrated adenomas in the colon. **Left Panels**: H&E stained sections of colon adenomas. **Right Panels:** Immunohistology of β-catenin in colon adenomas. *Scale bar* = 100 μm.

Supplementary Table 1. Disease score indexes (DAI)

Score	Weight loss (%)	Stool consistency	Occult/gross rectal bleeding
0	< 1	Normal	Negative
1	1-5	Loose stool	
2	5-10		Hemo-occult positive
3	11-15	Diarrhea	
4	15-20		Gross bleeding
5	>20		

Total DAI score = 15

Supplementary Table 2. Histopathological change index (HCI)

Score	Crypt damage	PMN infiltration	Range of crypt damage and inflammation
0	Intact	Few PMN	Normal
1	Loss of the basal 1/3 of crypt	1-3 PMN/field in the submucosa	1/5 colon
2	Loss of basal 1/2 of the crypt	>3 PMN/field in the submucosa	2/5 colon
3	Loss of basal 2/3 of the crypt	1-3 PMN/field in the mucosa	3/5 colon
4	Entire loss of the crypt	>3 PMN/field in the mucosa	4/5 colon
5	Loss of crypt and surface epithelia	numerous PMN in the epithelium	whole colon

Total HCI: 15

Supplementary Table 3. Primer pairs for RT-PCR

Gene		Primers
mFPR2	Sense:	5'- CTT CTT TAT CTG CTG GTT TCC CTT-3'
	Antisense:	5'-CTG GTG CTT GAA TCA CTG GTT TG-3'
mFPR1	Sense:	5'-CCT TGG CTT TCT TCA ACA GC-3'
	Antisense:	5'- GCC CGT TCT TTA CAT TGC AT-3'
TNF-α	Sense:	5'-ATG AGC ACA GAA AGC ATG ATC-3'
	Antisense:	5'-TAC AGG CTT GTC ACT CGA ATT-3'
IL-1β	Sense:	5'-CAG GAT GAG GAC ATG AGC ACC-3'
	Antisense:	5'-CTC TGC AGA CTC AAA CTC CAC-3'
IL-6	Sense:	5'-AGTTGC CTT CTT GGG ACT GA-3'
	Antisense:	5'-TCC ACG ATT TCC CAG AGA AC-3'
iNOS	Sense:	5'-GCA TTT GGG AAT GGA GAC TG-3'
	Antisense:	5'-GTT GCA TTG GAA GTG AAG CGT TTC-3
β-actin	Sense:	5'-TGT GAT GGT GGG AAT GGG TCA-3'
	Antisense:	5'-TTT GAT GTG ACG CAC GAT TTC CC-3'

RT-PCR was performed with Verso 1-Step RT-PCR ReddyMix Kit (Thermo Fisher Scientific, Surrey, UK), consisting of a 15-min reverse transcription at 50^{o}C, a 2-min inactivation of reverse transcriptase at 95^{o}C, and 40 cycles of denaturing at 95^{o}C (20 s), annealing at 60^{o}C (30 s), and extension at 72^{o}C (1 min), with a final extension for 10 min at 72^{o}C.