Supporting Information

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SI Materials and Methods

Animals. For dextran sodium sulfate (DSS)-treatment experiments, male mice for each genotype at age 7 wk were randomly divided into two groups. DSS-treated mice received 2% DSS (40–50 kD; USB; catalog no. 14489) in their drinking water for four cycles as shown in Fig. 1*A*. Control mice were given water without DSS. For azoxymethane (AOM)-treatment experiments, *Ppard*$^{-/-}$/*Il-10*$^{-/-}$ and *Ppard*$^{+/+}$/*Il-10*$^{-/-}$ were generated by conventionally crossing *Ppard*$^{-/-}$ mice with *Il-10*$^{-/-}$ mice. Genotypes of offspring were verified by PCR analysis with allele-specific primers. Eight-week-old male mice of each genotype were injected intraperitoneally with AOM (10 mg/kg) once weekly for 4 wk and then killed at 24 wk of age. At the end of the experiment, colon length was measured, and some of colons from each group were used for counting tumors and inflammation scores, and the rest were selected to examine immune cell profiles, proinflammatory genes, and prostaglandin E_2 (PGE_2) levels. After tumor burden was determined, colon tissues were embedded in paraffin. For histologic analysis, 5-μm-thick sections from all groups were stained with H&E to determine colonic inflammation scores and polyp morphology. The colonic inflammation score was quantified by using a described approach (1). The unstained sections were subjected to immunohistochemical staining.

Cell Culture and Reagents. All cancer cells were maintained in McCoy's 5A medium with 10% FBS. Colonic tumor epithelial cells and colonic macrophages were isolated from *Ppard*$^{+/+}$/*Apc*$^{Min/+}$ and *Ppard*$^{-/-}$/*Apc*$^{Min/+}$ mice treated with 2% DSS or water. To prepare THP-1–derived macrophages, monocytes were treated with 50 ng/mL phorbol 12-myristate 13-acetate in RPMI medium 1640 with 10% FBS overnight.

For bone marrow-derived macrophages (BMMs), bone marrow cells were flushed aseptically from the femurs of mice and cultured in Falcon Petri dishes (BD Biosciences) with DMEM supplemented with 10% FBS and 10 ng/mL macrophage colony-stimulating factor (M-CSF) for 5 d. These cells were continuously cultured in DMEM with 10% FBS, 10 ng/mL M-CSF, and 20 ng/mL IL-4 for 2 d before the experiments.

The conditionally immortalized Young Adult Mouse Colonic (YAMC) cell line was a gift from Robert Whitehead (Vanderbilt University). The YAMC cells were cultured as described (2). Briefly, cells were maintained under permissive conditions at 33 °C and 5% CO_2 with IFN-γ. Confluent cells were incubated in 10% FBS medium without IFN-γ under normal conditions, at 37 °C and 5% CO_2, for 48 h before all experiments.

All cell culture media and supplements were purchased from Gibco Life Technologies. Murine rIL-4, rM-CSF, and rIFN-γ were obtained from PeproTech. Human rWnt-3a was obtained from R&D Systems.

Establishment of Stable Cell Line. pBMN-I-GFP and pBMN-I-GFP-PPARδ retroviral vectors were transfected into Phoenix cells in 60-mm dishes by using Lipofectamine reagent (Invitrogen) according to the manufacturer's protocol. Culture medium containing virus particles was collected 48 h later and was added to HCT-116 cells. Infected cells were sorted by GFP positivity to eliminate uninfected cells.

TUNEL Assays. The fragmented DNA of apoptotic cells in tissue sections was end-labeled by using the DeadEnd colorimetric TUNEL system according to the manufacturer's instructions (Promega). Sections were deparaffinized, rehydrated, incubated with 20 μg/mL proteinase K, washed with PBS, and then immersed in a terminal deoxynucleotidyl transferase reaction mixture for 60 min at 37 °C. The slides were then washed in 2× SSC for 15 min to stop the reaction. Following another wash and blocking cycle, the slides were incubated with Streptavidin peroxidase for 30 min at room temperature and then stained with 3,3′-diaminobenzidine.

Analysis of Flow Cytometry. The whole colon was weighed and cut into 2-mm pieces. Colon pieces were digested with 1 mg/mL dispase, 0.25 mg/mL collagenase A, and 25 U/mL DNase (Roche) at 37 °C for 20 min. The suspension was then passed through a 70-μm cell strainer (BD Biosciences). After cells were washed with PBS containing 4% FBS, 1×10^7 viable cells were suspended in the labeling buffer (0.05% BSA and 2 mM EDTA in PBS, pH 7.4) and labeled with the following mouse polyclonal antibodies: anti–Gr-1 conjugated with phycoerythrin (PE) (dilution 1:100), anti-CD3 conjugated with FITC (dilution 1:100), anti–CD-4 conjugated with PE-Cy5.5 (dilution 1:250), anti-F4/80 conjugated with allophycocyanin (APC) (dilution 1:200), anti-CD11c conjugated with APC–cyanine-7 (dilution 1:100), Alexa 700-conjugated anti-Ly6G (1:50), and FITC-conjugated anti-CD11b (1:50). After the cells were washed with 1 mL of the labeling buffer, they were analyzed by using a multicolor flow cytometry. All antibodies were purchased from BD Biosciences.

Quantitative PCR. RNA was extracted from mouse tissues and cells by using an RNeasy Mini Kit (Qiagen), and 5 μg of total RNA from each sample was reverse-transcribed with iScript Reverse Transcription Supermix (Qiagen). The mRNA levels of target genes were quantified by real-time PCR with an iCycler and iQ SYBR Green Supermix (both from Bio-Rad) as described (3). Primers for these genes and *mGapdh* were chosen by using the Beacon Designer 5 program (Premier BioSoft International). The relative expression of target gene is the average of triplicates that were normalized against the transcription levels of *mGapdh*.

Western Blot Analysis. After serum starvation for 24 h, colonic epithelial cells (1.5×10^6) were treated with the indicated concentration of GW501516 for 24 h, and THP-1–derived macrophages (1.5×10^6) were treated with the indicated dose of PGE_2 for 1 d. Antibody to COX-2 (Santa Cruz Biotechnology) was used in 1:200 dilutions. The blots were stripped and then reprobed with β-actin antibody (Sigma).

ELISA and Bio-Plex Assay. Total proteins were extracted from colonic tissues by homogenizing and subsequently sonicating in antiprotease buffer (50 mM Hepes, 150 mM NaCl, and 1 mM EDTA, pH 7.4) containing protease inhibitor mixture tablets (Boehringer Mannheim). THP-1–derived macrophages and BMMs (7×10^5) were cultured in serum-free medium for 24 h and then were treated with the indicated concentrations of PGE_2 for 48 h, respectively. The levels of CXCL1, CCL2, CCL3, CCL4, and IL-1β in mouse colonic tissues and cell-free supernatants were measured by using the Quantikine ELISA kit (R&D) according to the manufacturer's instructions. IL-6, -17, -22, and -23 protein levels were measured by using Bio-Plex Pro Mouse cytokine, chemokine, and growth factor assays (Bio-Rad) following a protocol established by the manufacturer.

Immunohistochemical and Immunofluorescent Staining. Tissue sections (5-μm thick; $n = 5$ per animal) were stained with anti–mCOX-2 rabbit antibody (1:250) (Cayman) and rabbit anti-Ki67

monoclonal antibody (SP6; 1:200) (Abcam). The immunohistochemical staining was completed by using a Zymed-Histostain-SP Kit (Zymed) as described (4).

Paraffin-embedded specimens were treated with xylene and ethanol to remove the paraffin. The slides (5-μm thick; $n = 5$ per animal) were immersed in Borg decloaker solution (Biocare Medical) and boiled in a pressure cooker at 125 °C for 5 min for antigen retrieval. The slides were blocked with 5% normal rabbit serum and incubated with rabbit anti–COX-2 (1:200), rabbit anti-CD45 (1:200), or rabbit anti-EpCAM (1:200) antibody at 4 °C overnight. After washing with PBS, the slides were incubated with 1:200 Alexa Fluor 488- or 564-conjugated secondary antibody. The nuclei were stained with DAPI.

In Situ Hybridization. In situ hybridization was performed as described (5). In brief, frozen sections (10 μm) were mounted onto poly(L-lysine)-coated slides and fixed in cold 4% paraformaldehyde in PBS. The sections were prehybridized and hybridized at 45 °C for 4 h in 50% formamide hybridization buffer containing the ^{35}S-labeled antisense or sense cRNA probes. Sections hybridized with sense probes served as negative controls.

1. Dieleman LA, et al. (1998) Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* 114(3): 385–391.
2. Whitehead RH, VanEeden PE, Noble MD, Ataliotis P, Jat PS (1993) Establishment of conditionally immortalized epithelial cell lines from both colon and small intestine of adult H-2Kb-tsA58 transgenic mice. *Proc Natl Acad Sci USA* 90(2):587–591.
3. Wang D, et al. (2006) CXCL1 induced by prostaglandin E2 promotes angiogenesis in colorectal cancer. *J Exp Med* 203(4):941–951.
4. Wang D, et al. (2004) Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. *Cancer Cell* 6(3):285–295.
5. Daikoku T, et al. (2005) Cyclooxygenase-1 is a potential target for prevention and treatment of ovarian epithelial cancer. *Cancer Res* 65(9):3735–3744.

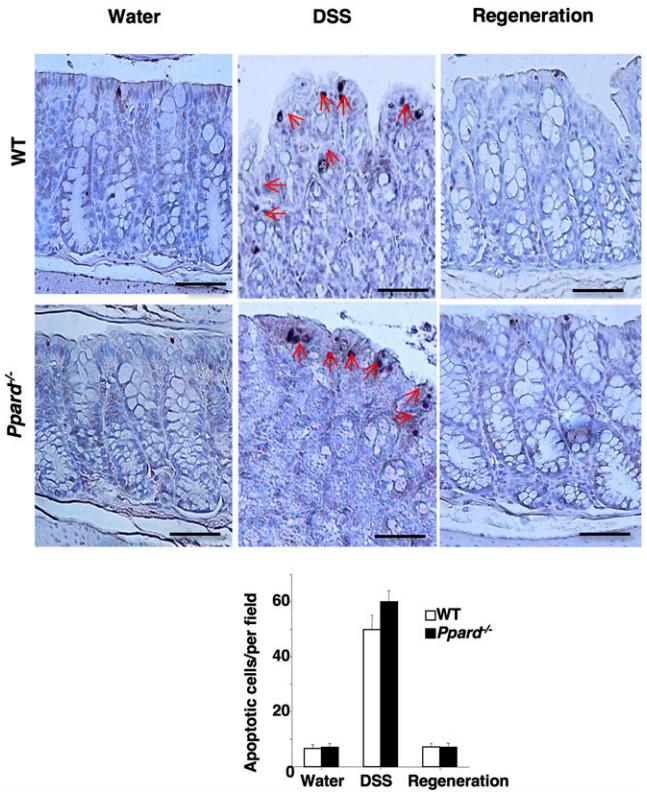

Fig. S1. TUNEL staining of colon tissues taken from WT and peroxisome proliferator-activated receptor δ (PPARδ)-deficient mice treated with water or DSS. (*Upper*) A set of representative images from WT and PPARδ-deficient mice treated with water as control (*Left*), 2% DSS for 3 d (*Center*), or 2% DSS for 1 cycle (*Right*) shows that apoptotic nuclei are stained as dark brown by the DeadEnd colorimetric TUNEL system as described in *SI Materials and Methods*. The red arrows denote a group of TUNEL-positive stained epithelial cells undergoing apoptosis. (Scale bars, 100 μm.) (*Lower*) The bar graph represents mean ± SEM of apoptotic cells in five fields per section of four sections per mouse from five mice for each group. $*P < 0.05$.

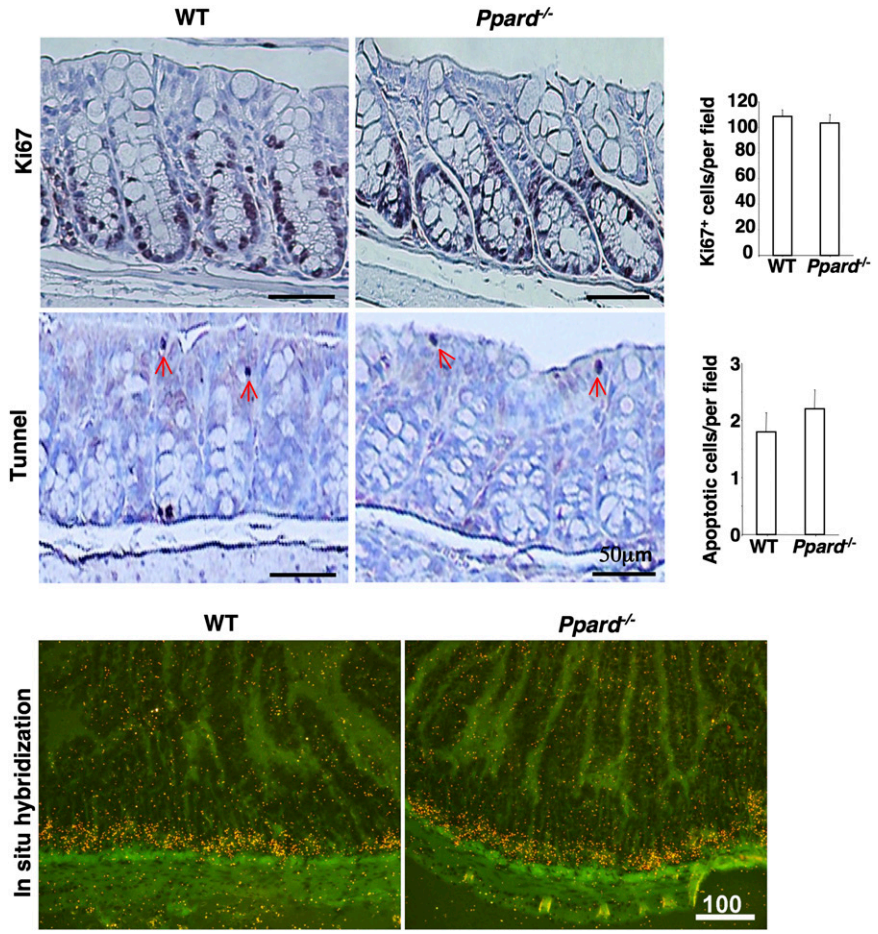

Fig. S2. Loss of PPARδ does not affect intestinal epithelial cell proliferation, survival, and Lgr5-positive cell numbers. Three mice for each genotype were used to examine rates of colonic epithelial cell proliferation and survival as well as Lgr5-expression levels in the intestine. (*Top*) *Left* represents immunoreactive staining (brown) for Ki-67. (Scale bars, 50 μm.) *Right* represents the average numbers of $Ki67^+$ cells in five fields per section of four sections per mouse from three mice for each group. (*Middle*) *Left* represents TUNEL-positive stained epithelial cells undergoing apoptosis. (Scale bars, 50 μm.) *Right* represents the average numbers of apoptotic cells in five fields per section of four sections per mouse from three mice for each group. (*Bottom*) In situ hybridization for *Lrg5* mRNAs in the small intestine taken from WT and PPARδ-deficient mice. (Scale bar, 100 μm.)

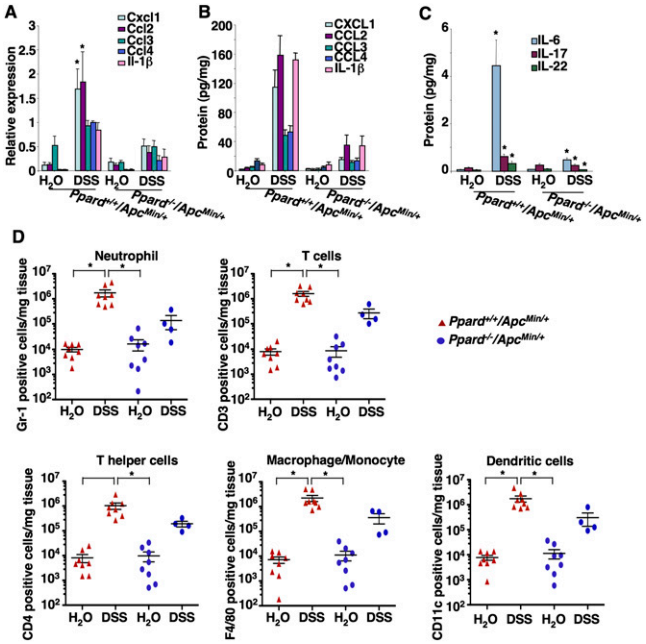

Fig. S3. Loss of PPARδ attenuated DSS-induced proinflammatory mediators and massive infiltration of immune cells in the colon mucosa of $Apc^{Min/+}$ mice. A cohort of 12 mice for each genotype was treated with DSS, and a cohort of 7 mice for each genotype was fed with water as control as described in Fig. 1*A*. (*A*–*C*) The levels of indicated genes at mRNA (*A*) and protein (*B* and *C*) in mouse colonic tissue samples were analyzed as described in Fig. 2 *B* and *C*. (*D*) The profiles of immune cells in the colon mucosa of indicated genotypic mice treated with either DSS or were determined as described in Fig. 2*A*.

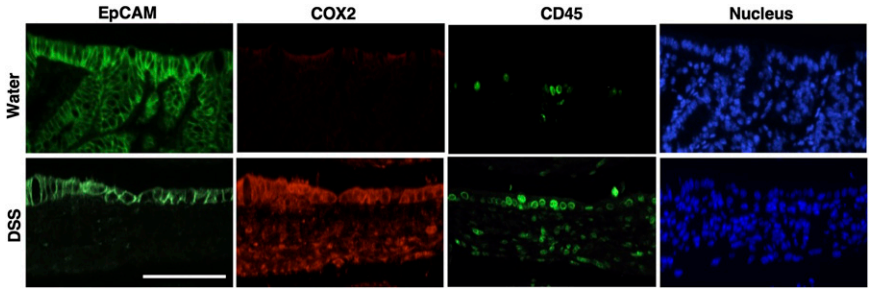

Fig. S4. Immunofluorescent staining of colon tissues taken from WT and PPARδ-deficient mice treated with water or DSS. A set of representative images from colons taken from WT mice treated with water and 2% DSS for four cycles shows that COX-2 (red), CD45 (green), and EpCAM (green) immunofluorescence was detected by a fluorescence microscope (Nikon ECLIPSE TE300; 10×). (Scale bar, 100 μm.)

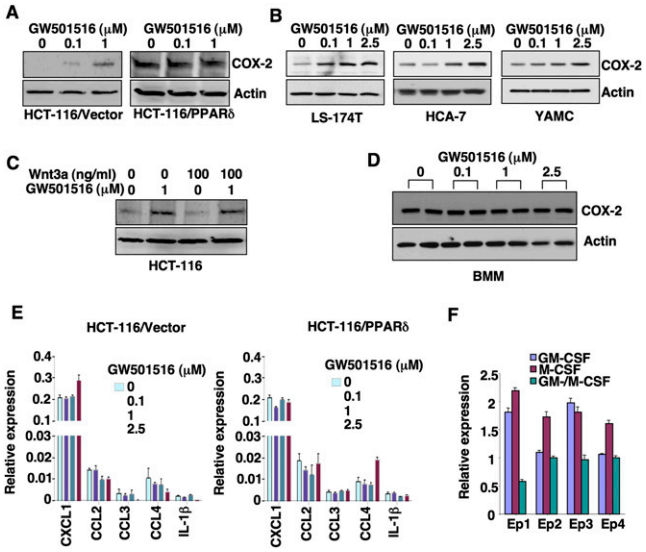

Fig. S5. The effect of PPARδ agonist or PGE_2 on induction of COX-2 or chemokines and cytokines as well as the profiles of prostaglandin E receptors (EP) in BMMs. (*A*) PPARδ-overexpressing HCT-116 (HCT-116/PPARδ) and control cells (HCT-116/vector) were treated with the indicated dose of GW501516 for 24 h following serum starvation for 24 h. (*B–D*) LS-174T, HCA-7, YAMC (*B*), and HCT-116 cells (*C*) as well as mouse BMM cells (*D*) were treated with the indicated dose of GW501516 and/or Wnt3a for 24 h after serum starvation for 24 h. COX-2 protein expression was analyzed by Western blotting. (*E*) The mRNA levels of indicated genes in HCT-116/PPARδ and HCT-116/vector cells treated with vehicle or the indicated dose of GW501516 were determined by q-PCR. (*F*) The mRNA levels of indicated genes in BMM cells were quantified by quantitative PCR as mentioned above.