# Supporting Information

## Kourepini et al. 10.1073/pnas.1316447111

### SI Materials and Methods

**Mice.** C57BL/6 (B6), B6 *Rag1*$^{-/-}$ (designated *Rag1*$^{-/-}$), BALB/c, BALB/c *Rag2*$^{-/-}$ (designated *Rag2*$^{-/-}$), transgenic (Tg) B6.129-*Gt*(*Rosa*)*26Sor*$^{tm1(cre/ERT)Nat}$/J (designated *CreER*$^{T1}$) and B6-Tg (Itgax-cre,-EGFP) 4097Ach/J (designated *Itgax*$^{cre}$) mice were purchased from the Jackson Laboratory. B6.129S6(Cg)-*Spp1*$^{tm1Blh}$/J mice (designated *Spp1*$^{-/-}$) were kindly provided by Lucy Liaw (Maine Medical Center Research Institute, Scarborough ME) and backcrossed onto the BALB/c mice and to the BALB/c *Rag2*$^{-/-}$ background for 10 generations. Mice were housed at the Animal Facility of the Biomedical Research Foundation of the Academy of Athens. Protocols were approved by the Bioethics Committee of Biomedical Research Foundation of the Academy of Athens and by the Greek Government. All procedures were in accordance with the US National Institutes of Health Statement of Compliance (Assurance) with Standards for Humane Care and Use of Laboratory Animals (A5736-01) and with the European Union Association for Assessment and Accreditation of Laboratory Animal Care. For the generation of Tg *Spp1-stop*$^{f/f}$ mice, we assessed transgene construction cloning of the full-length mouse *Spp1* (*mSpp1*) cDNA; the sense primer 5′-CTAACTACGACCATGAGATTGG-3′ and the antisense primer 5′-GGGTATAGTGATATAGACTG-3′ were used, and a 1,269-bp product was obtained. The cDNA product encoding osteopontin (Opn; NM_009263) was cloned as an EcoRI (New England BioLabs) fragment into the plasmid chicken actin LoxP stop LoxP (pCALSL) expression vector (a gift from R. Kelleher, Massachusetts General Hospital, Boston). The construct was created by subcloning the pCALSL vector and inserting a general eukaryotic promoter of chicken b-actin that drives the expression of a downstream intron inserted as a transcriptional enhancer. Downstream of the intron, there was inserted a stop cassette flanked by loxP sites. The *mSpp1* cDNA was cloned downstream of the last loxP site. The loxP-flanked stop sequence contains a neocassette and a triple-polyadenylation (tpA) sequence at the 3′ end of the neoexpression cassette. Transcriptional read-through is prevented with the use of a tpA (stop) sequence. The correct 5′-3′ orientation of the *mSpp1* cDNA was confirmed with HincII and NotI (New England BioLabs) digestion of the construct. An SV40 late polyadenylation signal (0.4 kbp) was added downstream of the *mSpp1* cDNA, and to verify the insertion, the product was digested with XbaI and SalI (New England BioLabs). The construct was isolated with an Endofree Kit (Qiagen), and SalI digestion followed to delete the prokaryotic backbone elements of the transgene construct. The final sequence (6.6-kbp fragment) was obtained with a Geneclean Gel Extraction Kit (Qiagen). The clean sequence containing the transgene construction was microinjected into mouse eggs (B6) to a final concentration of 50–100 ng/μL. The surviving eggs were transplanted into oviducts of foster mothers. The loxP-flanked DNA stop sequence prevents expression of the downstream *Spp1* gene. When Tg mice are crossed with a Cre-expressing strain, the stop sequence is removed in their litters and Opn is expressed in cells/tissues where Cre is expressed. All double-Tg mice, *Spp1-stop*$^{f/f}$/*CreER*$^{T1}$ and *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$, were healthy and bred well. Opn overexpression in the double-Tg *Spp1-stop*$^{f/f}$/*CreER*$^{T1}$ mouse was induced after daily i.p. administration of 1–3 mg of tamoxifen (Sigma) diluted in 200 μL of corn oil (Sigma) per mouse.

**In Vivo Experimental Protocols.** Colitis studies were performed in specific pathogen-free 8- to 10-wk-old male mice. For acute colitis induction, mice were lightly anesthetized with ketamine/xylazine (Pfizer, Bayer Healthcare) solution and administered 1–3 mg of trinitrobenzene sulfonic acid (TNBS; Sigma–Aldrich) dissolved in 50% (vol/vol) ethanol intrarectally via a 3.5-French catheter attached to a 1-mL syringe. The total volume of the enema was 150 μL. Control mice were administered ethanol in PBS. At the peak of disease (day 5), mice were euthanized with ketamine/xylazine. Mice received 20 μg i.p. of a polyclonal affinity-purified neutralizing Ab to mouse Opn (AF-808; R&D Systems) or 20 μg of goat IgG isotype control (R&D Systems) 1 d before (d − 1) and 1 d following (d + 1) administration of TNBS. Recombinant Opn (rOpn; 2.5 μg; R&D Systems) was administered i.p. daily. For the acute dextran sulfate sodium (DSS) colitis model, procedures have been described previously (1). Mice were analyzed on day 7.

For the CD4$^+$ T-cell transfer model of chronic colitis, naive CD4$^+$CD25$^-$CD44$^-$CD62L$^+$CD45RB$^{high}$ T cells were sorted from B6 mice after enrichment with a CD4$^+$ T-Cell Isolation Kit II (Miltenyi Biotec). The following anti-mouse mAbs were used for FACS sorting (FACSAria III; Becton Dickinson): CD3 (clone 145-2C11), CD4 (GK1.5), CD62L (MEL-14), CD25 (PC61.5), CD44 (IM7), and CD45RB (C363.16A). Sex-matched C57BL/6 *Rag1*$^{-/-}$ recipients were injected i.p. with $5 \times 10^5$ cells. Mice were euthanized at day 30 after cell transfer, and tissues were collected for cell populations or histological analysis.

**Histology.** Colons were excised for macroscopic damage evaluation and length measuring. Colon tissue was cut vertically or longitudinally, fixed in 10% (vol/vol) buffered formalin, and embedded in paraffin. Tissue sections that depict midcolon areas (5 μm) were stained with H&E, periodic acid–Schiff, and Giemsa. In H&E staining photomicrographs, the original magnification is 20×, with a 100-μm scale bar, unless otherwise stated. The degree of inflammation and the differential cell numbers on microscopic sections were graded blindly. The scoring systems used for acute TNBS colitis (2), acute DSS colitis (3), and chronic CD4$^+$ T-cell transfer colitis (4) were described previously.

**Cell Isolation and Culture, Cytokine Analysis, and Proliferation Assays.** We used a previously described method to isolate cells from draining lymph nodes (5) or colonic lamina propria (LP) (6). Mesenteric lymph node cells (MLNCs) from healthy or colitic mice were harvested, pooled from each mice group, stimulated ex vivo with 1 μg/μL LPS (Sigma) or 2 μg/mL soluble anti-CD3 (12A.2; BD Biosciences) and 3 μg/mL anti-CD28 (37.71; BD Biosciences). Cells were isolated with the CD4$^+$ T-Cell Isolation Kit II and stimulated with 2 μg/mL anti-CD3. MLN dendritic cells (DCs) were isolated with a CD11c Microbead Kit (Miltenyi Biotec); the CD103$^-$ DC fragment was obtained with negative selection and the CD103$^+$ DCs were obtained with positive selection from the whole DCs with a CD103 (integrin αE) mouse affinity-purified Ab (BD Pharmingen) and sheep anti-rat Dynabeads (Invitrogen). Alternatively, after DC enrichment with the CD11c Microbead Kit, the following anti-mouse Abs were used for purification of CD11c$^{high}$ DC subsets with FACS sorting: CD3, CD11c (N418), MHCII (M5/114.15.2, 39-10-8), CD11b (M1/70), and CD103 (2E7). DC populations were gated on 7-aminoactinomycin D$^-$ (7AAD$^-$) CD3$^-$MHCII$^{high}$CD11c$^{high}$ cells and then on CD103 expression (gating strategy in Fig. 1*B*) in all experiments. When CD103$^-$ and CD103$^+$ DC subsets are mentioned in experiments, they are always 7AAD$^-$ CD3$^-$MHCII$^{high}$CD11c$^{high}$ and include both CD11b$^+$ and

$CD11b^-$ subpopulations. DCs were stimulated 2–12 h with 1 μg/mL LPS, 0.5 μg/mL rOpn (R&D Systems), or 0.5 μg/mL of two synthetic secreted Opn fragments ($Opn_{134–153}$), RGD (Arg-Gly-Asp)-SLAYGLR (Ser-Leu-Ala-Tyr-Gly-Leu-Arg) sequence ($frOpn^{RGD}$) and RAA (Arg-Ala-Ala)-SLAYGLR ($frOpn^{RAA}$), or with a scrambled peptide ($frOpn^{scr}$). The $frOpn^{RGD}$ is a synthetic 20-aa peptide fragment of Opn extended between 134 and 153 aa of the normal Opn protein, the $frOpn^{RAA}$ has the RGD domain mutated to RAA, and the $frOpn^{scr}$ has both RGD mutated to RAA and SLAYGLR mutated to LRAGLRS (Leu-Arg-Ala-Gly-Leu-Arg-Ser) (CASLO Laboratory ApS). In some cases, DCs were treated with 10 μg/mL integrin α9 Ab or Ig isotype (R&D Systems) before frOpn treatment. Naive T cells for cocultures were isolated with the $CD4^+CD62L^+$ T-Cell Isolation Kit II. DCs for cocultures were obtained from MLNs of colitic mice, and the ratio of the cultured DC/T-cell numbers was 1:4. In cocultures, anti-CD3/CD28 was added. Measurements of cytokine levels were performed in MLN homogenate supernatants and MLNC culture supernatants, as previously described for lymph node cells (5). Commercially available ELISA kits were used for detection of mouse IFN-γ, IL-12p70, IL-4, and IL-10 (OptEIA; BD Biosciences) and IL-23, IL-17, IL-6, and Opn (R&D Systems). We performed in vitro proliferation assays with 1 μCi per well of $[^3H]$ pulsed thymidine as previously described (5). To monitor $CD3^+$ T-cell proliferation, MLNCs were stained with Celltrace Violet (Invitrogen); after 120 h of proliferation and stimulation with 2 μg/mL soluble anti-CD3 and 3 μg/mL anti-CD28, they were stained additionally with fluorochrome-conjugated Abs to CD3, CD4, CD8α (53-6.7), CD44, and CD62L and FACS was performed (described below). For in vivo proliferation studies, colitic mice were injected 24 h before colitis induction with 1 mg/g of BrdU, and the BrdU Flow Kit (BD Pharmingen) was used for assessment of BrdU incorporation with FACS.

**Flow Cytometry.** Freshly isolated MLNCs or LP cells were stained with combinations of fluorochrome-conjugated Abs to CD3, CD4, CD8α, CD44, CD45RB, CD62L, CD25, MHCII, CD11c, CD11b, CD103, E-cadherin (clone 36), lymphocyte antigen 6C (HK.1.4), and C-C chemokine receptor type 7 (CCR7) (4B12) (all from BD Biosciences). For dead cell exclusion, cells were stained with 7AAD (BD Biosciences). For intracellular cytokine staining, cells were stained with surface markers and then with Abs against IL-17 (eBio17B7), IFN-γ (XMG1.2), IL-10 (JES5-16E3), and forkhead box protein 3 (Foxp3) (FJK-16s) (all from eBiosciences). Intracellular cytokine expression was assessed by 25 ng/mL phorbol 12-myristate 13-acetate (Sigma) and 1 μg/mL ionomycin calcium salt (Sigma) for a 5-h incubation, as well as with a Cytofix/Cytoperm Kit Plus (Golgiplug; BD Biosciences). Intracellular staining for Foxp3 was performed with an eBiosciences kit. Stained samples were analyzed on a Coulter cytometer (FC 500; Cytomics), and the raw data were calculated and visualized with FlowJo software (TreeStar).

**Adoptive Transfer of $CD4^+$ T Cells and/or DCs.** Naive $CD4^+$ $CD25^-CD44^-CD62^+$ T cells were sorted, and $10^6$ cells per mouse were transferred i.p. 10 d before acute colitis induction. A total of $10^5$ to $2 \times 10^5$ purified DCs ($CD103^-$ or $CD103^+$ DC subsets) per mouse were transferred i.p. in recipients 2 h before acute colitis induction or 15 d after chronic colitis induction. Mice were analyzed 5 d (acute TNBS), 7 d (acute DSS), or 30 d (chronic $CD4^+$ T-cell transfer) following colitis induction. In some experiments, DC subsets were BrdU-pulsed (10 μM) for 20 min and extensively washed before transfer.

**Quantitative PCR.** Total RNA extraction from pellets of MLNCs or isolated DC subsets was performed with the Nucleospin RNA II Kit (Macherey–Nagel). For RNA quantification, the Quant-iT RNA Assay Kit (Invitrogen) was used. One microgram of RNA was used for each reaction of cDNA synthesis with SuperScript II reverse transcriptase (Invitrogen) and RiboLock RNase inhibitor (Thermo Scientific). Primers were designed (Eurofins MWG) using the Primer3 program as follows: *Il6* sense: 5′-GGAAATCGTGGAAATGAGAA-3′, antisense: 5′-TGAAGGACTCTGGCTTTGTC-3′; *Itga9* sense: 5′-CGGAAAGAGAATGAAGATGG-3′, antisense: 5′-GGATGAGGAAGAGAACAGCA-3′; *Itga4* sense: 5′-TGCTTCTCTGCTGTTGTC-3′, antisense: 5′-GGGCTTATTTGGGATGGA-3′; *Il17*α sense: 5′-GCCCTCAGACTACCTCAACC-3′, antisense: 5′-CACACCCACCAGCATCTT-3′; *Il10* sense: 5′-AGCCTTATCGGAAATGATCC-3′, antisense: 5′-ACTCTTCACCTGCTCCAC-3′; *Il23p19* sense: 5′-GGACTTGTGCTGTTCTTGTTT-3′, antisense: 5′-TGATGCTCTGGGTTTTCTC-3′; *Il27p28* sense: 5′-ATCTCGATTGCCAGGAGT-3′, antisense: 5′-GTGGTAGCGAGGAAGCA-3′; *Foxp3* sense: 5′-CCTCCACTCCACCTAAAG-3′, antisense: 5′-TGAAACCAGACAACTAACAG-3′; and *Spp1* sense: 5′-GGTCAACTAAAGAAGAGGCAA-3′, antisense: 5′-ACAGGAAGAACAGAAGCAAAG-3′. Real-time PCR was performed with SYBR Green I (Molecular Probes) and Platinum Taq DNA polymerase (Invitrogen) in a StepOnePlus RT-PCR system (Applied Biosystems). PCR amplification of the housekeeping gene hypoxanthine phosphoribosyltransferase (*Hprt*) (sense primer: 5′-GTGAACTGGAAAGCCAAA-3′, antisense primer: 5′-GGACGCAGCAACTGACAT-3′) was performed as a control in each sample reaction.

**Statistics.** Data were analyzed using Prism Software (GraphPad) and the unpaired Student *t* test. *P* values of 0.05 or less were considered significant.


1. Okayasu I, et al. (1990) A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. *Gastroenterology* 98(3):694–702.
2. Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W (1995) Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J Exp Med* 182(5):1281–1290.
3. Cooper HS, Murthy SN, Shah RS, Sedergran DJ (1993) Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69(2):238–249.
4. Izcue A, et al. (2008) Interleukin-23 restrains regulatory T cell activity to drive T cell-dependent colitis. *Immunity* 28(4):559–570.
5. Panoutsakopoulou V, et al. (2001) Analysis of the relationship between viral infection and autoimmune disease. *Immunity* 15(1):137–147.
6. Zigmond E, et al. (2012) $Ly6C^{hi}$ monocytes in the inflamed colon give rise to proinflammatory effector cells and migratory antigen-presenting cells. *Immunity* 37(6):1076–1090.

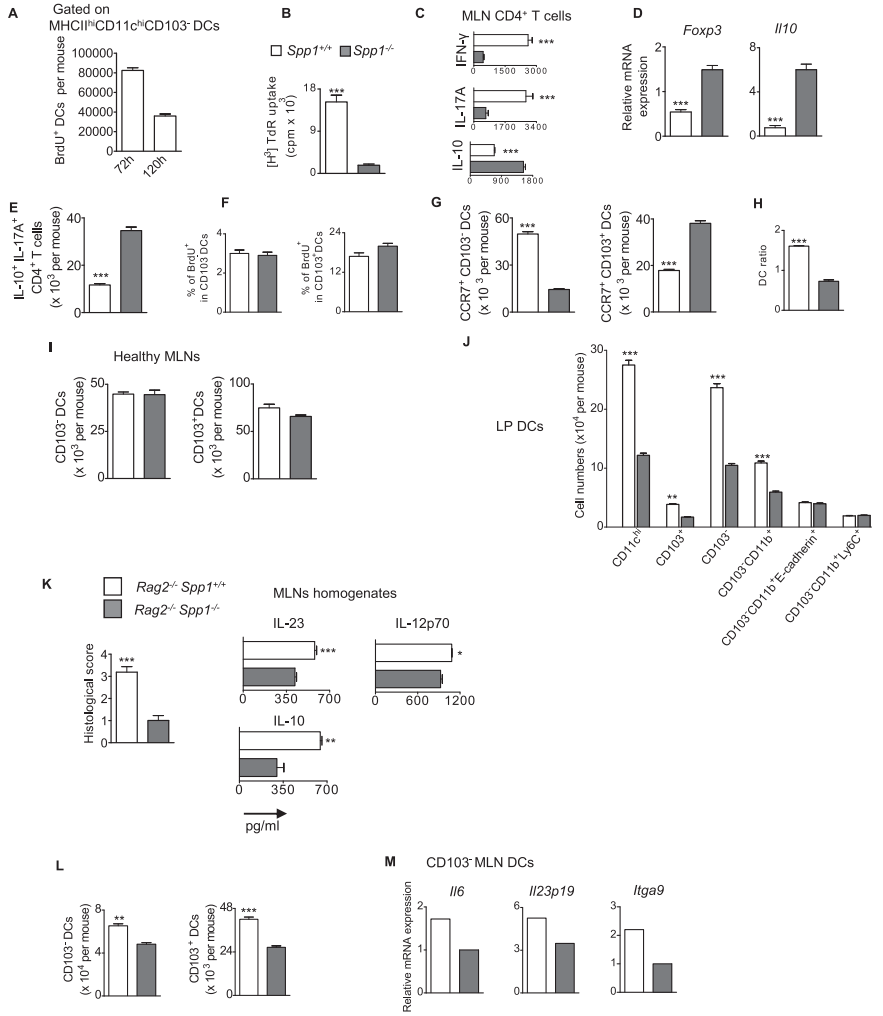


**Fig. S1.** Detection of transferred CD103$^-$ DCs. (*A*) MLNC numbers of BrdU-labeled CD103$^-$ DCs per BALB/c recipient 72 and 120 h after i.p. injection. BALB/c *Spp1*$^{-/-}$ and *Rag2*$^{-/-}$*Spp1*$^{-/-}$ mice are protected from acute colitis. (*B*) Proliferation of anti-CD3 stimulated (72 h) MLN CD4$^+$ T cells measured by [$^3$H] thymidine (TdR) incorporation (***$P = 0.0008$). The results are shown as mean ± SEM of triplicate wells. (*C*) IFN-γ (***$P = 0.0008$), IL-17A (***$P = 0.0007$), and IL-10 (***$P = 0.0002$) levels produced by $5 \times 10^5$ anti-CD3–stimulated (72 h) MLN CD4$^+$ T cells. Relative *Foxp3* (***$P = 0.001$) and *Il10* (***$P = 0.0007$) mRNA expression (*D*), numbers of IL-17A$^+$ IL-10$^+$ CD4$^+$ T cells (***$P = 0.0001$) (*E*), CD103$^-$ and CD103$^+$ DC percentages with BrdU incorporation in vivo (*F*), numbers of CCR7$^+$ CD103$^-$ (***$P = 0.0007$) and CCR7$^+$ CD103$^+$ (***$P < 0.0001$) MLN DCs in colitic mice (*G*), and DC ratios (***$P < 0.0001$) in colitic MLNCs (*H*) are shown. (*I*) Numbers of MLN CD103$^-$ and CD103$^+$ DCs in healthy mice. (*J*) Numbers of LP DCs (**$P = 0.0011$; ***$P < 0.0009$) in colitic mice. Values are expressed as mean ± SEM ($n = 6$ mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student *t* test. (*K*) Histological score of *Rag2*$^{-/-}$ mice (***$P <$ 0.0001, *Left*) and levels of IL-23 (***$P = 0.0003$), IL-12p70 (*$P = 0.022$), and IL-10 (**$P = 0.0046$) in supernatant homogenates of MLNs (*Right*). (*L*) Numbers of MLN CD103$^-$ (**$P = 0.0019$) and CD103$^+$ (***$P = 0.0004$) DCs in *Rag2*$^{-/-}$ colitic mice. Values are expressed as mean ± SEM ($n = 5$ mice per group) from two separate experiments. Statistical significance was obtained by an unpaired Student *t* test. (*M*) Relative *Il6, Il23p19*, and *Itga9* mRNA expression by MLN CD103$^-$ DCs isolated from colitic *Rag2*$^{-/-}$ mice. Data are presented as values normalized to hypoxanthine phosphoribosyltransferase of one representative experiment.

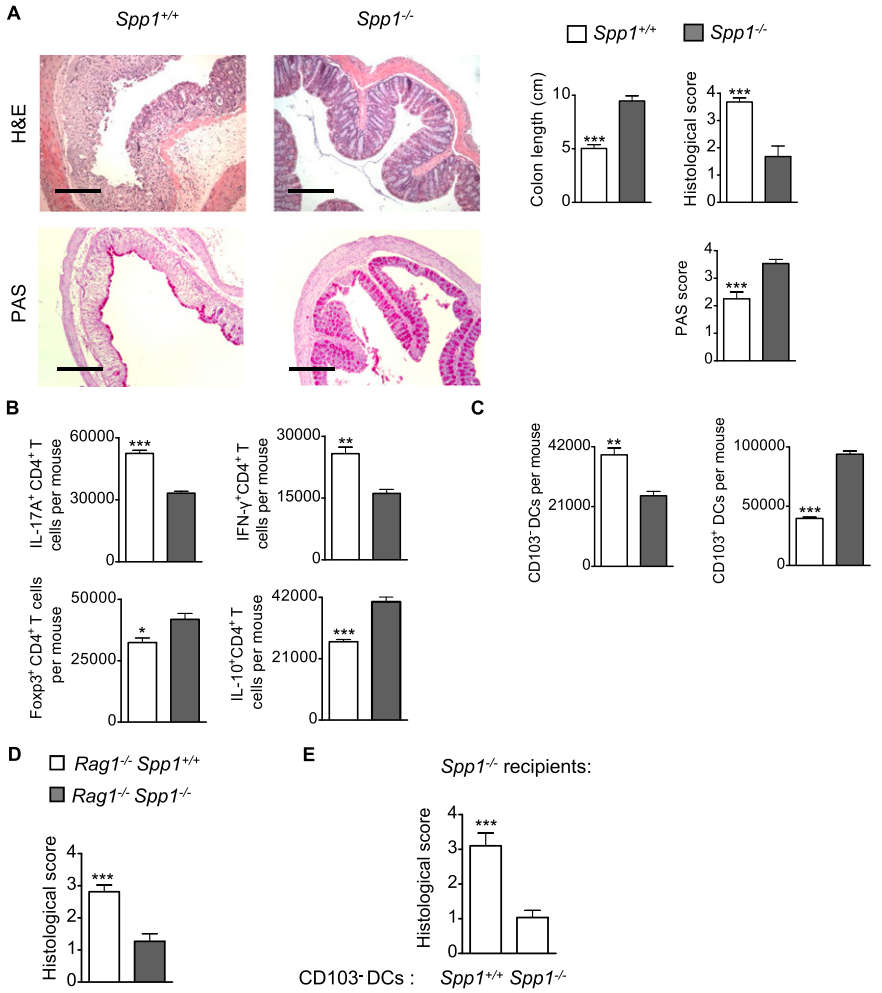


**Fig. S2.** C57BL/6 *Spp1*$^{-/-}$ and *Rag1*$^{-/-}$*Spp1*$^{-/-}$ mice are protected from DSS acute colitis. (*A*) Representative photomicrographs of colonic sections [*Left*, H&E and periodic acid–Schiff (PAS)], colon length (*Center*, ***$P < 0.0001$), histological score (*Right Upper*, ***$P < 0.0001$), and PAS score of mucus secretion (*Right Lower*, ***$P = 0.0004$) of *Spp1*$^{+/+}$ and *Spp1*$^{-/-}$ colitic mice. (Original magnification: 10×.) (Scale bars: 200 μm.) (*B*) Numbers of IL-17A$^+$ (***$P = 0.0004$), IFN-γ$^+$ (**$P = 0.0021$), Foxp3$^+$ (*$P = 0.0363$), and IL-10$^+$ (***$P = 0.0006$) MLN CD4$^+$ T cells. (*C*) Numbers of total CD103$^-$ (**$P = 0.0024$) and CD103$^+$ (***$P < 0.0001$) DCs per mouse. (*D*) Histological score of *Rag1*$^{-/-}$ mice with acute DSS colitis (***$P = 0.0002$). (*E*) Histological score of *Spp1*$^{-/-}$ recipients of $10^5$ colitic MLN CD103$^-$ DCs injected i.p. 2 h before DSS administration (***$P = 0.0006$). All values are expressed as mean ± SEM ($n = 5$ mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student *t* test.

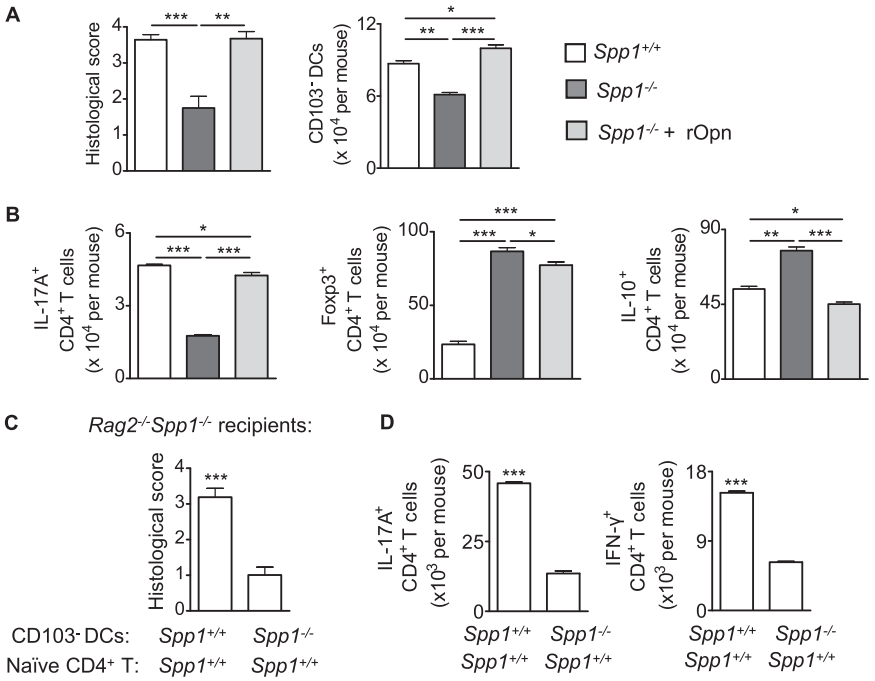


**Fig. S3.** Opn promotes pathogenicity. Histological score of BALB/c *Spp1*$^{+/+}$, *Spp1*$^{-/-}$, and *Spp1*$^{-/-}$ mice administered rOpn daily (**$P = 0.0022$; ***$P = 0.0002$) and MLN CD103$^-$ DC numbers (*$P = 0.285$; **$P = 0.0011$; ***$P = 0.0003$) (*A*) and numbers of IL-17A$^+$ (*$P = 0.0349$; ***$P < 0.0001$), Foxp3$^+$ (*$P = 0.484$; ***$P <$ 0.0001), and IL-10$^+$ (*$P = 0.011$; **$P = 0.0011$; ***$P = 0.0002$) MLN CD4$^+$ T cells (*B*). Values are expressed as mean ± SEM ($n$ = 5–6 mice per group) from two separate experiments. Statistical significance was obtained by an unpaired Student $t$ test. Opn-expressing CD103$^-$ DCs are proinflammatory in vivo. (*C*) Histological score of BALB/c *Rag2*$^{-/-}$*Opn*$^{-/-}$ mice adoptively transferred with $10^5$ colitic *Spp1*$^{-/-}$ or *Spp1*$^{+/+}$ CD103$^-$ DCs, together with $10^6$ naive WT CD4$^+$ T cells before colitis induction (***$P < 0.0001$). (*D*) Numbers of IL-17A$^+$ and IFN-γ$^+$ (***$P < 0.0001$) MLN CD4$^+$ T cells per *Rag2*$^{-/-}$*Spp1*$^{-/-}$ recipient. All values are expressed as mean ± SEM ($n$ = 6 mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student $t$ test.

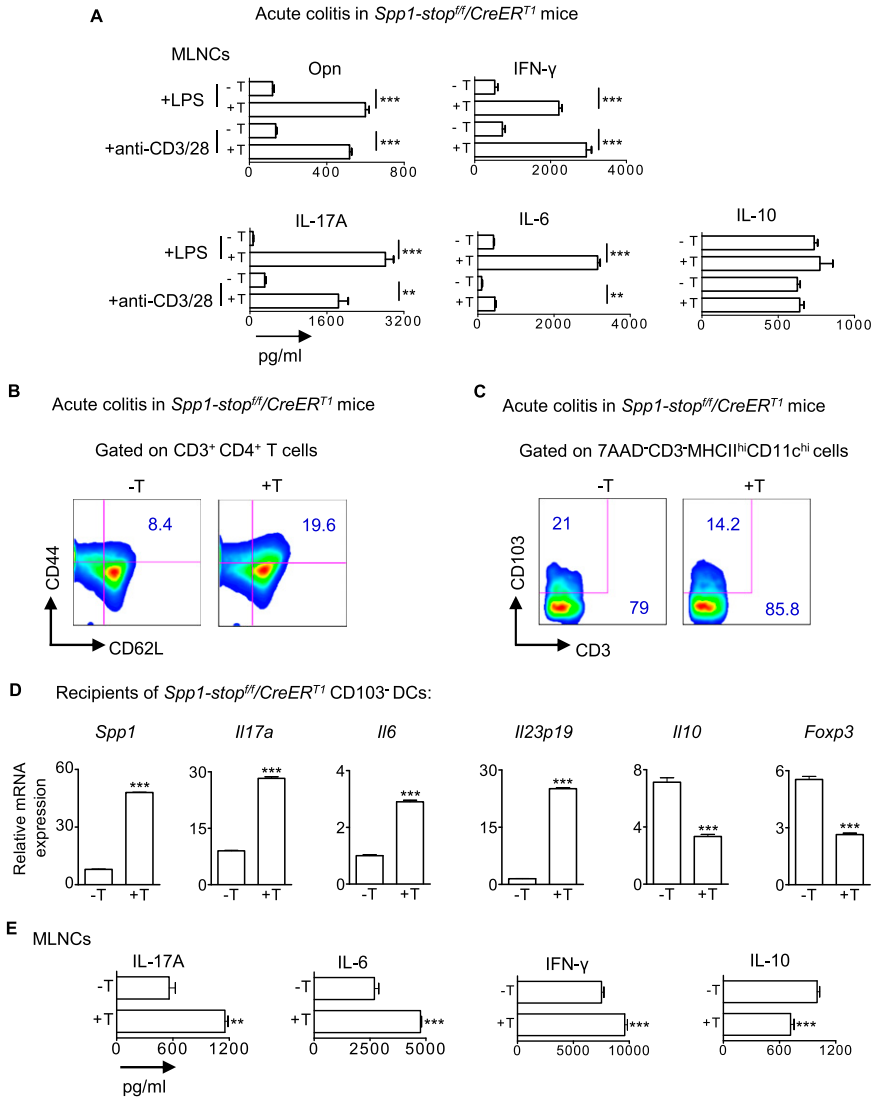


**Fig. S4.** Opn-overexpressing CD103$^-$ DCs are extra proinflammatory in vivo. Opn (***$P = 0.0003$; ***$P = 0.0001$), IFN-γ (***$P = 0.0002$; ***$P = 0.0001$), IL-17A (**$P = 0.0017$; ***$P < 0.0001$), IL-6 (**$P = 0.002$; ***$P < 0.0001$), and IL-10 secretion by LPS- and anti-CD3/28– stimulated (72 h) MLNCs (*A*) and percentages of activated effector CD44$^{high}$CD62L$^+$CD4$^+$ T cells (*B*) and CD103$^-$ or CD103$^+$ DCs (*C*) in MLNs of C57BL/6 *Spp1-stop$^{f/f}$CreER$^{T1}$* colitic mice (–T/+T). Relative *Spp1*, *Il17a*, *Il6*, *Il23p19* (***$P < 0.0001$), *Il10* (***$P = 0.0004$), and *Foxp3* (***$P < 0.0001$) mRNA expression (*D*) and levels of IL-17A (**$P = 0.0013$), IL-6 (***$P = 0.0006$), IFN-γ (***$P = 0.0007$), and IL-10 (***$P = 0.0003$) by $10^6$ anti-CD3/28–stimulated (72 h) MLNCs (*E*) from colitic C57BL/6 recipients of *Spp1-stop$^{f/f}$CreER$^{T1}$* CD103$^-$ DCs (–T/+T). Values are expressed as mean ± SEM ($n = 6$ mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student *t* test.

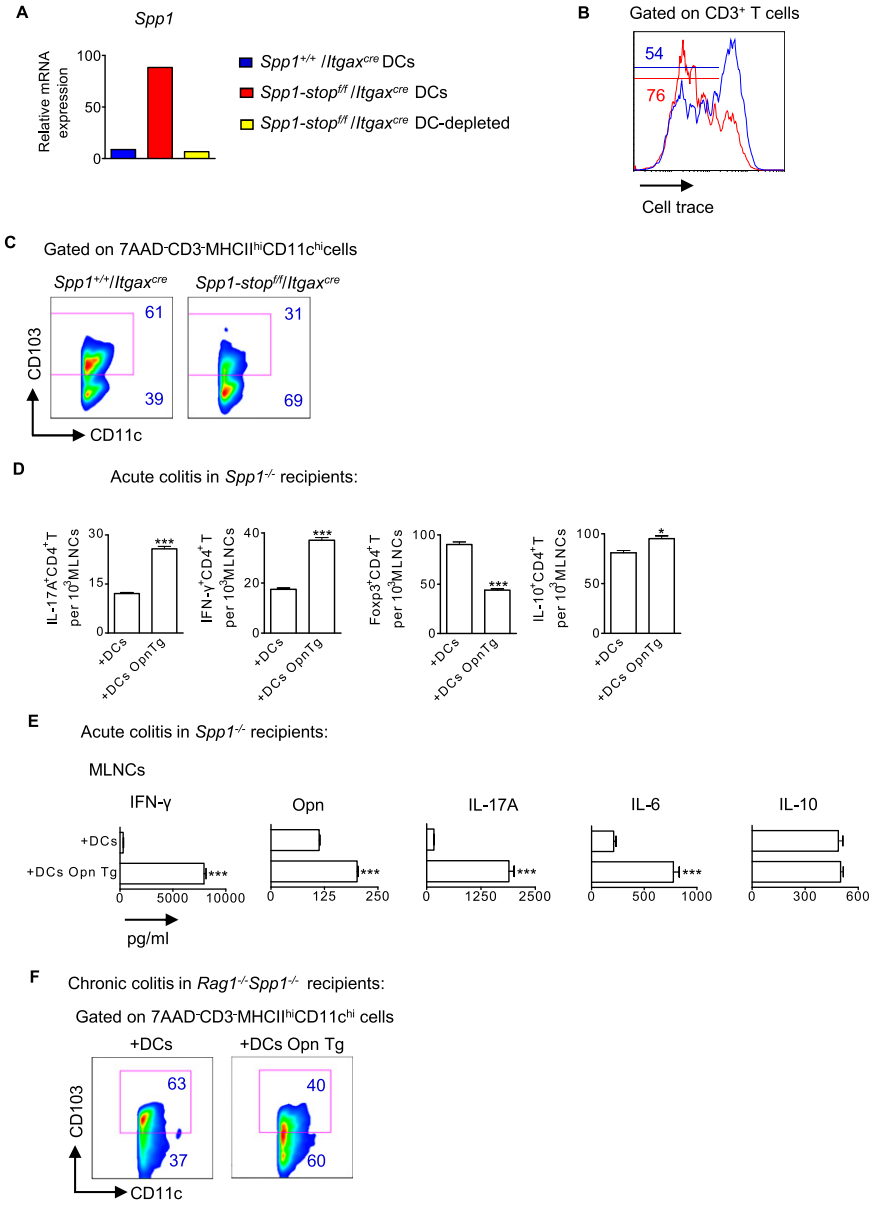


**Fig. S5.** Opn overexpression by CD103$^-$ DCs promotes pathogenicity in acute and chronic colitis. (*A*) Relative *Spp1* mRNA expression in naive *Spp1*$^{+/+}$/*Itgax*$^{cre}$ MLN DCs, *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$ MLN DCs, and DC-depleted MLNCs. (*B*) Percentages of proliferated anti-CD3/28–stimulated (120 h) MLN CD3$^+$ T cells from naive *Spp1*$^{+/+}$/*Itgax*$^{cre}$ (blue line) and *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$ (red line) mice, evaluated by the percentage of the CellTrace violet dilution. (*C*) Percentages of CD103$^-$ or CD103$^+$ DCs among MLN DCs of C57BL/6 *Spp1*$^{+/+}$/*Itgax*$^{cre}$ and *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$ colitic littermates. (*D*) Numbers of IL-17A$^+$ (***$P < 0.0001$), IFN-γ$^+$ (***$P < 0.0001$), Foxp3$^+$ (***$P < 0.0001$), and IL-10$^+$ (*$P = 0.0168$) CD4$^+$ T MLNCs per *Spp1*$^{-/-}$ recipient. (*E*) IFN-γ (***$P < 0.0001$), Opn (***$P < 0.0001$), IL-17A (***$P < 0.0001$), IL-6 (***$P < 0.0001$), and IL-10 secretion by anti-CD3/28–stimulated (72 h) MLNCs from C57BL/6 *Spp1*$^{-/-}$ recipients of $10^5$ naive *Spp1*$^{+/+}$/*Itgax*$^{cre}$ (+DCs) or *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$ (+DCs Opn Tg) CD103$^-$ DCs. Values are expressed as mean ± SEM ($n$ = 4–5 mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student $t$ test. (*F*) Percentages of MLN CD103$^-$ or CD103$^+$ DCs in C57BL/6 *Rag1*$^{-/-}$*Spp1*$^{-/-}$ mice injected i.p. with 5 × $10^5$ *Spp1*$^{-/-}$ CD45RB$^{high}$ CD4$^+$ T cells for chronic colitis induction and adoptively transferred with $10^5$ naive *Spp1*$^{+/+}$/*Itgax*$^{cre}$ (+DCs) or *Spp1-stop*$^{f/f}$/*Itgax*$^{cre}$ (+DCs Opn Tg) CD103$^-$ DCs 15 d later. Values are expressed as mean ± SEM ($n$ = 4–5 mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student $t$ test.

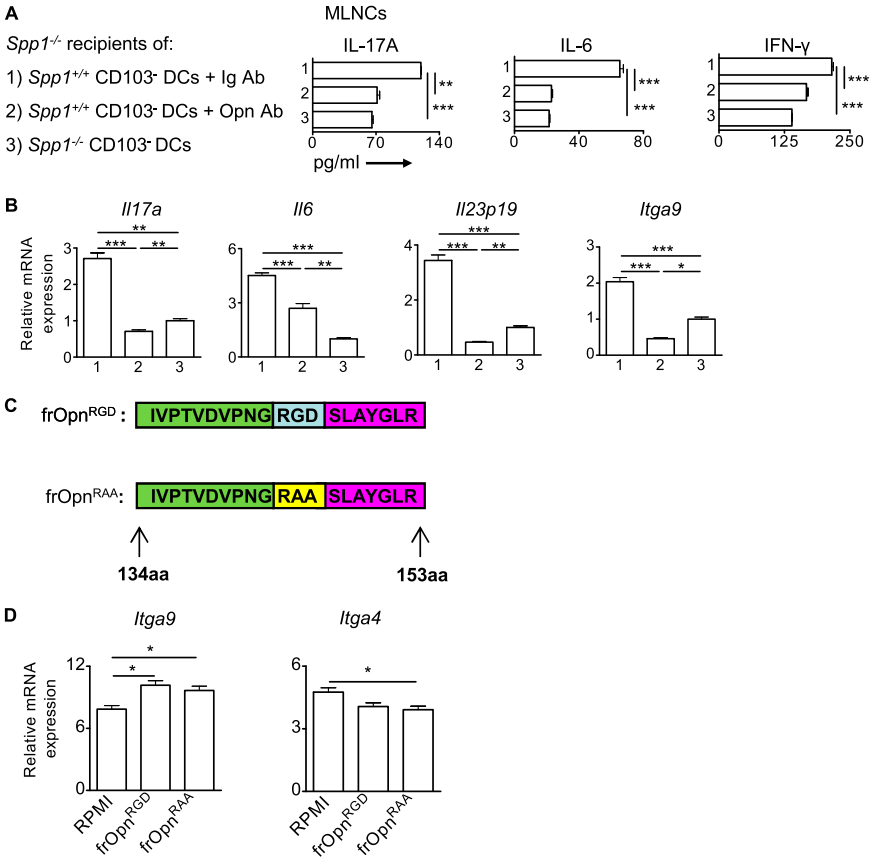


**Fig. S6.** Opn secreted by CD103$^{-}$ DCs mediates colitis exacerbation. (*A*) Levels of IL-17A (**$P$ = 0.0036; ***$P$ = 0.0006), IL-6 (***$P$ = 0.0002; ***$P$ = 0.0001), and IFN-γ (***$P$ = 0.0003; ***$P$ < 0.0001) in $10^5$ anti-CD3/28–stimulated (96 h) colitic MLNCs. (*B*) Relative mRNA expression of *Il17a* (**$P$ = 0.0014; **$P$ = 0.001; ***$P$ = 0.0002), *Il6* (**$P$ = 0.003; ***$P$ < 0.0001; ***$P$ = 0.0003), *Il23p19* (**$P$ = 0.0011; ***$P$ = 0.0003; ***$P$ = 0.0001) and *Itga9* (*$P$ = 0.0147; ***$P$ = 0.0005; ***$P$ = 0.0002) in colitic MLNCs. Values are expressed as mean ± SEM ($n$ = 5 mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student $t$ test. (*C*) Schematic representation of the 134–153-aa mouse Opn peptide fragments containing SLAYGLR and RGD (frOpn$^{RGD}$) or mutated in RAA (frOpn$^{RAA}$) domains. Binding of frOpn$^{RGD}$ or frOpn$^{RAA}$ on the CD103$^{-}$ DC surface integrin α9 changes the integrin expression. (*D*) *Itga9* (*$P$ = 0.0144; *$P$ = 0.0291) and *Itga4* (*$P$ = 0.343) mRNA expression of RPMI-treated, frOpn$^{RGD}$-treated, frOpn$^{RAA}$-treated, and LPS-stimulated *Spp1*$^{-/-}$ CD103$^{-}$ DCs in vitro. Values are expressed as mean ± SEM ($n$ = 4–5 mice per group) from three separate experiments. Statistical significance was obtained by unpaired an Student $t$ test.

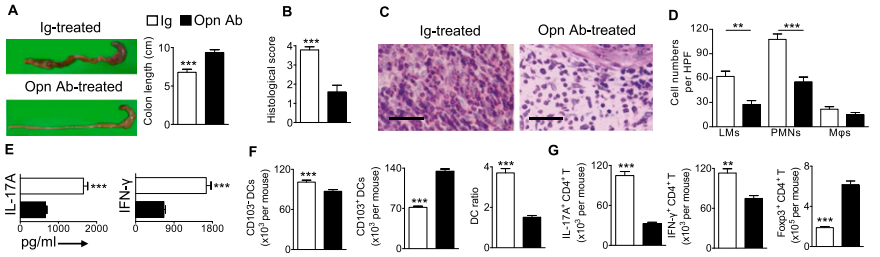


**Fig. S7.** Neutralization of secreted Opn (Opn-s) at colitis induction attenuates acute colitis. Representative colons, colonic length (***$P$ = 0.0002) (*A*), histological score (***$P$ = 0.0002) (*B*), and characteristic neutrophil infiltration of the submucosa (*C*) of Ig- and Opn Ab-treated BALB/c colitic mice (Giemsa staining). (Magnification: 62×.) (Scale bars: 32 μm.) (*D*) Differential cell counts in colonic tissue (**$P$ = 0.0026; ***$P$ = 0.0004). For each sample, data were obtained measuring at least two high-power fields (HPFs). LMs, lymphomononuclear cells; Mφs, macrophages; PMNs, polymorphonuclear cells. (*E*) Levels of IL-17A (***$P$ < 0.0001) and IFN-γ (***$P$ < 0.0001) in 5 × $10^5$ anti-CD3/28–stimulated (72 h) colitic MLNCs. Numbers of MLN CD103$^{-}$ DCs (***$P$ < 0.0001), CD103$^{+}$ DCs (***$P$ < 0.0001), and DC ratios (***$P$ = 0.0007) (*F*) and numbers of IL-17A$^{+}$ (***$P$ = 0.0003), IFN-γ$^{+}$ (**$P$ = 0.0082), and Foxp3$^{+}$ (***$P$ = 0.0003) MLN CD4$^{+}$ T cells (*G*) are shown. Values are expressed as mean ± SEM ($n$ = 6 mice per group) from three separate experiments. Statistical significance was obtained by an unpaired Student $t$ test.

**Table S1. MLNC numbers of healthy and colitic Spp1$^{+/+}$ BALB/c mice**

| Mice | CD11c$^{high}$ | CD103$^{+}$ | CD103$^{-}$ | CD103$^{-}$ DCs<br>CD11b$^{+}$<br>CD11b$^{-}$ | CD103$^{-}$ DCs<br>CD11b$^{+}$ Ly6C$^{+}$<br>CD11b$^{-}$ Ly6C$^{+}$ | CD103$^{-}$ DCs<br>CD11b$^{+}$ E-cadherin$^{+}$<br>CD11b$^{-}$ E-cadherin$^{+}$ |
|---|---|---|---|---|---|---|
| Healthy | 105,300 ± 4,261 | 68,050 ± 3,689 | 49,420 ± 1,427 | 35,010 ± 1,011<br>14,410 ± 416 | 5,214 ± 150<br>410 ± 11 | 2,884 ± 83<br>3,671 ± 106 |
| Colitic | 123,300 ± 3,907 | 47,130 ± 1,707** | 75,670 ± 2,185*** | 62,410 ± 1,802***<br>13,260 ± 383 | 4,420 ± 127*<br>1,067 ± 30*** | 12,220 ± 353***<br>13,240 ± 382*** |

Numbers of total CD11c$^{high}$ DCs, CD103$^{+}$ and CD103$^{-}$ DC subsets, and subpopulations of CD103$^{-}$ DCs per mouse. All cells were gated on 7AAD$^{-}$ CD3$^{-}$MHCII$^{high}$CD11c$^{high}$ DCs (as in Fig. 1*B*) (*$P < 0.0354$; **$P < 0.0068$; ***$P < 0.0005$). All values are expressed as mean ± SEM ($n = 4$ mice per group) from two separate experiments. Ly6C, lymphocyte antigen 6C.