Supporting Information

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SI Materials and Methods

Mice. *Myd88*$^{-/-}$ mice were kindly provided by S. Michalek (University of Alabama at Birmingham, Birmingham, AL).

Isolation of Colon Cells. Colon tissue was flushed, opened longitudinally, and then cut into strips 1 cm in length. Tissue pieces were incubated for 30–40 min at 37 °C with either collagenase D (2 mg/mL; Sigma) or collagenase B (2 mg/mL; Roche) and DNase (1 mg/mL; Sigma). Cells then were purified on a 40%/75% Percoll (vol/vol) gradient by centrifugation for 20 min at 25 °C and 600 × *g* with no brake.

Isolation of Bone Marrow Neutrophils. Bone marrow cells were harvested by flushing the tibia and femur bones with R10 medium (Roswell Park Memorial Institute) and pipetting up and down to break up the marrow into a single-cell suspension. Cells first were purified on a 50%/60% (vol/vol) Percoll gradient by centrifugation for 20 min at 25 °C and 600 × *g* with no brake, followed by FACS sorting on CD11b^{+}Ly6B.2^{+}FSC^{int}SSC^{int} cells. Donor bone marrow neutrophils were negative for CD4, NK1.1, and F4/80.

Isolation of Lung Neutrophils. Lungs were perfused with PBS, minced, and then incubated for 30 min at 37 °C with intermittent agitation in Roswell Park Memorial Institute (RPMI) 1640 with collagenase B (2 mg/mL; Roche) and DNase (1 mg/mL; Sigma). Cells were collected in R-20 (20% serum) and were FACS sorted on CD11b^{+}Ly6G^{+}F4/80^{-}CD11c^{-}FSC$^{\mathbf{int}}$SSC^{int} cells.

Real-Time PCR. RNA was isolated with the RNeasy kit (QIAGEN), and DNase treated with DNA-free (Ambion). cDNA synthesis was performed with the SuperScript III first-strand synthesis system (Invitrogen) and analyzed with Platinum qPCR Supermix UDG for Taqman primers per probe or Sybr GreenER qPCR supermix (Invitrogen) in a Bio-Rad iCycler. The primer sequences were purchased from Integrated DNA Technologies and are shown in Table S1.

ELISA. IL-6, IL-17, IL-22, and macrophage inflammatory protein 2 (MIP-2) ELISA kits were purchased from R&D Systems, and ELISA was performed according to the manufacturer's instructions.

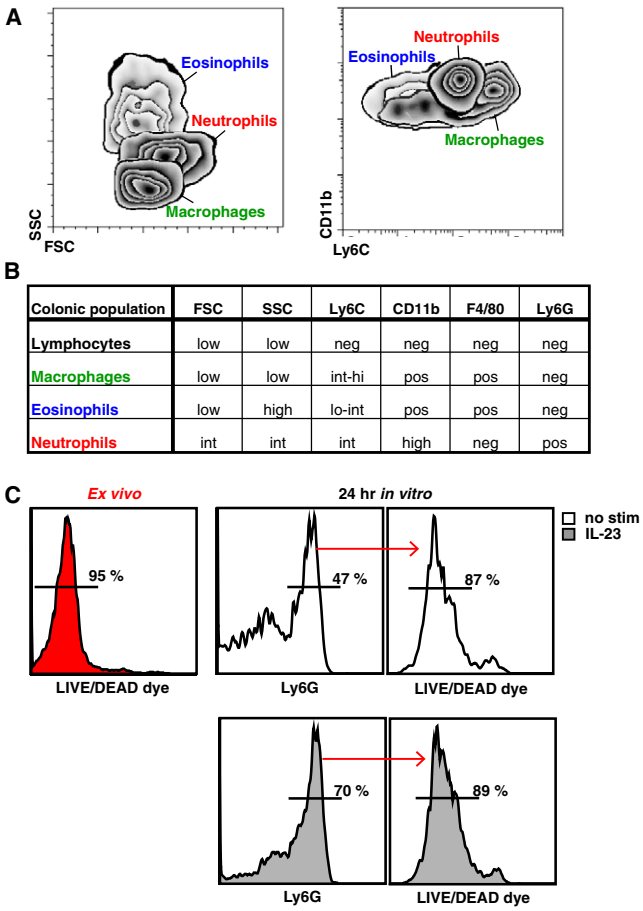
Flow Cytometry. Colon cells were stained with FcBlock (BD Biosciences) followed by staining with fluorescent-labeled antibodies. For intracellular cytokine staining, cells were stimulated for 4 h with recombinant mouse IL-23 (rmIL-23) (R&D Systems) and GolgiPlug (BD Biosciences) and then were processed using the FoxP3 staining buffer set (eBioscience). Samples were acquired on an LSRII flow cytometer (BD Biosciences) and analyzed by FlowJo software (Treestar).

Tissue and Cell Preparation. For immunostaining experiments, colons were fixed in 2% (vol/vol) paraformaldehyde (PFA) for 2 h at room temperature, were washed in PBS, and then were cushioned in cold, sterile 20% sucrose (wt/vol). Tissue then was rinsed briefly in cold PBS, cut into equal segments (proximal, middle, and distal), embedded in O.C.T. (Tissue-Tek), and frozen with 2-methyl butane chilled with liquid nitrogen. For histological analysis, colons were segmented as above, cut longitudinally, and placed immediately in 10% buffered formalin (Fisher) and processed for paraffin embedding and H&E staining. For cytospin preparations, sorted neutrophils were stimulated for 4 h with rmIL-23 and GolgiPlug and then were cytospun at 170 × *g* for 5 min. Cells were fixed with 4% (vol/vol) PFA for 15 min at room temperature, washed in PBS, and then placed in methanol with 0.3% H_2O_2 to quench endogenous peroxidase activity. To allow for sufficient permeabilization, 1% Triton X-100 and saponin were used in the staining buffer.

Electron Microscopy. Sorted colon cells and splenic red blood cells purified with anti–Ter-119 MACS beads (Miltenyi) (to help visualize pelleted cells) were fixed with Karnovsky's Fixative (Electron Microscopy Sciences). The cells were processed further for osmification, embedding, and analysis at the High Resolution Imaging Facility at the University ofAlabama at Birmingham (Birmingham, AL) using an FEI Tecnai 12 transmission electron microscope.

Antibodies and Reagents. The following antibodies and reagents were purchased from BD Biosciences or eBiosciences: anti-CD16/CD32 (2.4G2; FcBlock), anti-CD11b phycoerythrin (PE) or APC (M1/70), anti-Ly6C biotin (AL-21), anti-Ly6G FITC (1A8), anti–IL-22 PE (1H8PWSR), anti-rat Ig biotin, and anti-Epcam1 FITC. The following antibodies were purchased from Serotec: anti-F4/80 (CI:A3-1) and anti-Ly6B.2 (7/4). The following antibodies and cytokines were purchased from R&D Systems: anti-mouse S100A8, anti-mouse RegIIIβ, rmIL-23, and rmTNF. Streptavidin-PE/CY7 was purchased from Southern Biotech. Streptavidin-Alexa Fluor 594, Prolong Gold with DAPI, and LIVE/DEAD dye were from Invitrogen. Dextran sodium sulfate (DSS) salt (molecular weight = 36,000–50,000; MP Biomedicals) was purchased from Fisher Scientific. Control mouse Ig was purchased from Sigma, and the mouse anti-human IL-22–blocking antibody (clone 8E11) was kindly provided by W. Ouyang from Genentech (South San Francisco, CA).

Antibiotic Treatment. Mice were pretreated 1 wk before and during DSS treatment with 1 mg/mL ceftazidime, 1 mg/mL metronidazole, or 0.5 mg/mL vancomycin (Sigma) in their drinking water.



Colonic population	FSC	SSC	Ly6C	CD11b	F4/80	Ly6G
Lymphocytes	low	low	neg	neg	neg	neg
Macrophages	low	low	int-hi	pos	pos	neg
Eosinophils	low	high	lo-int	pos	pos	neg
Neutrophils	int	int	int	high	neg	pos

Fig. S1. Cellular profile and viability of colonic neutrophils isolated from DSS-treated mice. (*A*) Schematic of flow cytometry plots shows the differences in size (FSC), granularity (SSC), and levels of CD11b and Ly6C expression between colonic neutrophils, macrophages, and eosinophils. As summarized in *B,* colonic neutrophils typically have higher levels of CD11b and intermediate levels of Ly6C expression, but macrophage and eosinophil populations vary in their level of Ly6C expression. The varying levels of Ly6C expression in the eosinophil and macrophage populations suggest that there may be subsets of cells in different stages of differentiation. The cartoons were produced in Adobe Photoshop using FlowJo-designed contour plots. (*C*) Colonic neutrophils sorted from DSS-treated BL/6 mice were analyzed by flow cytometry for viability ex vivo or after in vitro culture in the presence or absence of IL-23. In the presence of IL-23, a higher percentage of colonic neutrophils maintain their phenotypic markers; however, their viability profiles were comparable. Data are representative of two independent sorting experiments.

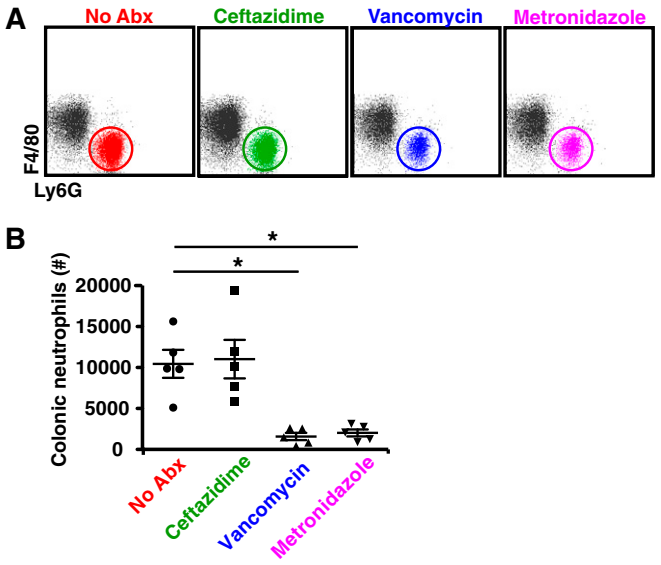


Fig. S2. Bacterial flora augment the recruitment of colonic neutrophils during DSS-induced colitis. (*A* and *B*) WT mice received no antibiotic (No Abx) or selective antibiotics (ceftazidime, vancomycin, or metronidazole) for 1 wk before and during DSS treatment. On day 7 of DSS treatment, colon cells were analyzed by flow cytometry (pregated on $CD11b^{+}Ly6C^{+}$ cells). Ceftazidime is a third-generation broad Gram-negative cephalosporin antibiotic. Vancomycin is a glycopeptides antibiotic that targets Gram-positive bacteria. Metronidazole is an antibiotic used for controlling anaerobic Gram-negative and Gram-positive bacteria. Together, these data indicate that certain commensal bacteria may enhance innate cell recruitment into the colon during acute colitis. Data are representative of two independent experiments with five mice per group. $*P < 0.05$.

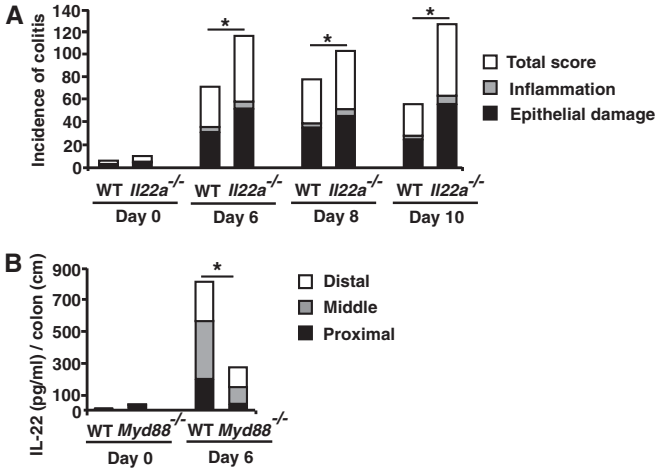


Fig. S3. IL-22 minimizes epithelial damage during DSS-induced colitis, which is partially controlled by MyD88 signals. (*A*) BL/6 and $Il22a^{-/-}$ mice were treated with DSS in their drinking water for 7 d, followed by normal drinking water. Colon tissue was collected at various times and stained with H&E for blinded histology scoring. (*B*) Colon tissue was collected from DSS-treated BL/6 and $Myd88^{-/-}$ mice and then was cultured for 2.5–3 d. Supernatant was analyzed for IL-22 protein levels by ELISA. Data are representative of two independent experiments with four mice per group. $*P < 0.05$.

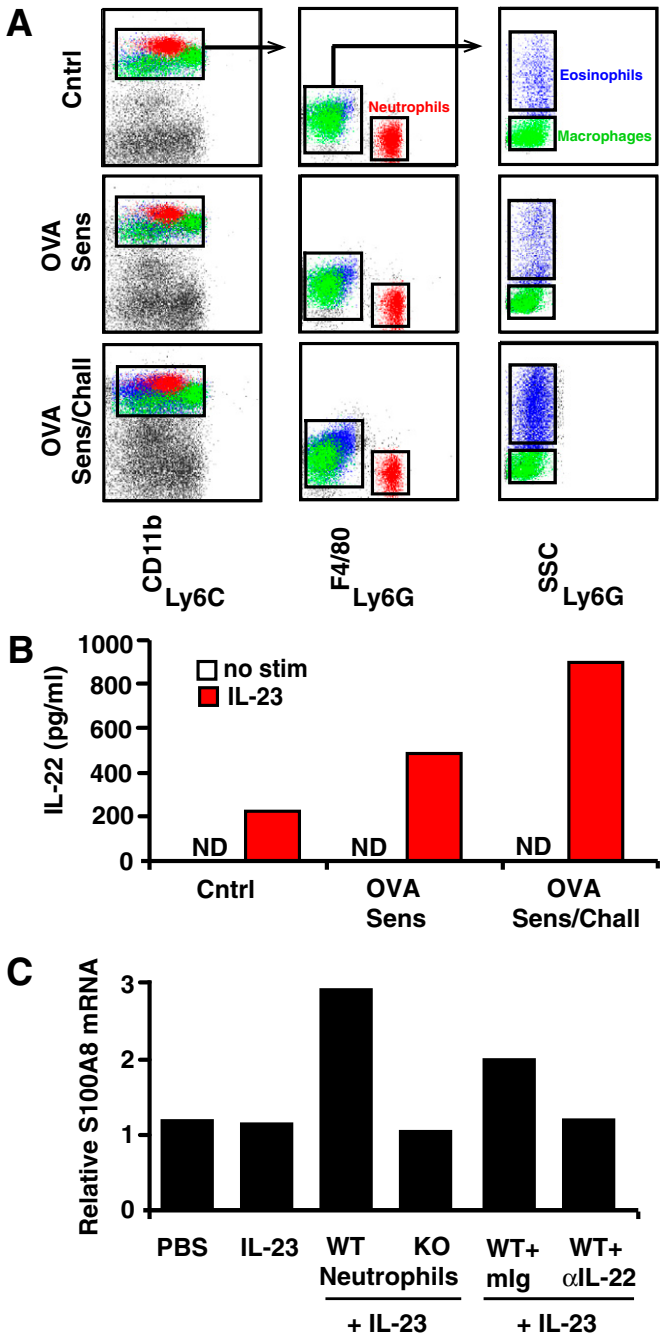


Fig. S4. Neutrophils isolated from the lungs of asthmatic mice produce IL-22 protein. C57BL/6J or BALB/c mice were sensitized with i.p. injections of ovalbumin (OVA) with alum on days 1 and 7, followed by a PBS (OVA sens) or OVA (OVA Sens/Chall) challenge via intranasal instillation of OVA on day 14. (*A*) Four to five days later, cells were isolated from the lungs, were stained with anti-CD11b, anti-Ly6C, anti-Ly6G, and anti-F4/80, and were analyzed by flow cytometry. (*B*) Lung neutrophils ($FSC^{int}SSC^{int}CD11b^{+}Ly6C^{+}Ly6G^{+}F4/80^{-}CD11c^{-}$) were sorted and cultured in the absence or presence of rmIL-23 for 2.5–3 d. Supernatant was analyzed for IL-22 protein levels by ELISA. ND, not detected. (*C*) Lung neutrophils from WT or *Il22*$^{-/-}$ (KO) mice were sorted and cultured with cells derived from the colons of young adult mice (YAMC cells) for 48 h. Cells were stimulated with rmIL-23 in the presence or absence of either a control mouse antibody or blocking anti-IL22 antibody. Cell lysate was analyzed for S100A8 mRNA levels by real-time PCR. S100A mRNA levels were normalized to GAPDH. Data are representative of three independent sorting experiments.

Table S1. Primer sequences used in real-time PCR

IL-22 FOR	TCC GAG GAG TCA GTG CTA A
IL-22 REV	AGA ACG TCT TCC AGG GTG AA
IL-22 probe	FAM-TGC TAC CTG ATG AAG CAG TGC T
IL-23R FOR	TCA GTG CTA CAA TCT TCA GAG GAC A
IL-23R REV	GCC AAG AAG ACC ATT CCC GA
IL-23R probe	FAM-CCT GCT TCA GGT AAT CAT CAA GAC
RegIIIβ FOR	ATA CCC TCC GCA CGC ATT AGT T
RegIIIβ REV	AGG CCA GTT CTG CAT CAA ACC A
S100A8 FOR	TGA GTG TCC TCA GTT TGT GCA G
S100A8 REV	TGT GAG ATG CCA CAC CCA CTT T
S100A9 FOR	CAA ATG GTG GAA GCA CAG TTG GCA
S100A9 REV	TTG TGT CCA GGT CCT CCA TGA TGT
Ahr FOR	CCT GAG GGC CAA GAG CTT CT
Ahr REV	CCT GGC CTC CAT TTC TGT CA
RORγt FOR	GGA GGA CAG GGA GCC AAG TT
RORγt REV	TTG TCC CCA CAG ATC TTG CA
GAPDH FOR	TCC ATG ACA ACT TTG GCA TTG
GAPDH REV	CAG TCT GGG TGG CAG TGA
GAPDH probe	TxRd-AGG GCT CAT GAC CAC AGT CCA TGC C-BHQ2

FOR, forward; IL-23R, IL-23 receptor ; REV, reverse. RORγt, retinoic acid-related orphan receptor gT. Ahr, aryl hydrocarbon receptor.