Supporting Information

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SI Materials and Methods

MNC Preparation. After euthanasia, mice were opened and guts were cut at the stomach and dissected out at the anal opening. The SB and colon were then separated at their cecal junctions. The fecal contents were flushed out using cold PBS. Both the SB and colon were then laid on paper and opened longitudinally using blunt scissors. Polyps, identified as elevated shouldered growths, were pinched using sharp tweezers, dissected using small sharp scissors, and pooled in a Petri dish. Surrounding nonpolyposis tissue and an adjacent healthy margin were put in a separate dish. Tissue was then minced using scalpels for about 3 min. Minced tissue pieces were then placed in 50-mL tube filled with 20 mL of RPMI medium (Lonza) supplemented with MEM, sodium pyruvate, L-glutamine, and 10% FBS. A digestion enzyme mixture of collagenase (C5138-1G; Sigma), hyaluronidase (H6254, 500 mg; Sigma), and DNaseI (D5025-15KU; Sigma) was added to the tissue suspension. The tubes were placed in a shaking incubator at 37 °C for 25 min. After digestion, the tubes were filled to 40 mL with complete RPMI and left to sediment for 8–10 min. Tissue remnants and cells were then filtered through 40-μm filters. The filtrate containing released cells was spun down at 216.3 × *g* for 10 min. The pellet was then reconstituted in 5 mL of 44% Percoll (GE Heathcare) diluted in HBSS and transferred to a 15-mL conical tube. The cell suspension was then underlaid with 67% Percoll. The cells were centrifuged for 18 min at 22 °C at 554 × *g*. MNCs at the interface of 44%/67% layers were collected and washed in PBS.

Flow Cytometry Staining. Cells were harvested from tissues of interest. A total of 2×10^6 cells per 100-μL staining volume were transferred to a 96-well, round-bottomed plate (Corning). Cells were pelleted by centrifugation for 5 min at 4 °C at 188.5 × *g*. Cells were then incubated for 20 min on ice with Fc block (clone 2.4G2) and washed with PBS. Cells were then incubated with extracellular antibodies and LIVE/DEAD Fixable Blue stain (Molecular Probes) for 30 min in the dark on ice, and washed twice with PBS. Cells were fixed using 2% PFA for 10 min in the dark on ice, and then washed once with PBS. For intracellular staining, cells were fixed and permeabilized using a Foxp3/Transcription Factor Staining Buffer kit (eBioscience). An intracellular antibody mixture, 100 μL per sample, was prepared in 1× wash/perm buffer and incubated for 2 h on ice or overnight at 4 °C. Cells were then centrifuged and washed twice using the wash/perm buffer, and samples were transferred to tubes for acquisition.

Flow Cytometry Acquisition and Analysis. Cells were suspended at 2×10^6 cells per 250-μL volume, and samples were run on a BD LSRII flow cytometer (BD Biosciences) and Accuri BD flow cytometer (BD Biosciences). Data were analyzed using FlowJo software (TreeStar).

Histology and Immune Staining. Guts were harvested from mice, flushed with ice-cold PBS, cut into four pieces, and opened longitudinally using blunt scissors. Tissues were fixed using 10% formalin for 12–16 h. After that, guts were rolled and stored in 70% ethanol for processing, and embedding. Tissue sections (4–5 μm) were cut for staining. For immune staining, tissue sections were deparaffinized in xylene and then rehydrated in a series of dilutions of ethanol. Following rehydration, slides were immersed in target retrieval solution (Dako), and heat-induced epitope retrieval was performed in a Decloaking Chamber (Biocare Medical). Nonspecific background staining was blocked using Background Sniper (Biocare Medical). Nonspecific avidin/biotin was blocked when needed (Vector Labs). Primary antibodies were diluted in antibody diluent solution (Dako) and incubated for 2 h at room temperature or overnight at 4 °C. For MCs, proteases, and immunohistochemistry staining, biotinylated anti-rabbit antibodies (Vector Labs) were applied to the sections for 45 min, followed by prepared avidin/biotin alkaline phosphatase complex (Vector Labs) for 45 min. SIGMAFAST Fast red TR/Naphthol AS-MX tablets (Sigma) were used as a substrate for color development. Sections were then counterstained using Harris hematoxylin (Leica). Slides were mounted using CC/Mount (Sigma). For IL-33 staining, anti–IL-33 antibody was purchased from R&D Systems. For immunofluorescent staining, Alexa Fluor-conjugated secondary antibodies (Molecular Probes) were added to sections for 45 min, followed by washing in PBS for 5–10 min. Vectashield with DAPI (Vector Labs) was used for counterstaining and mounting. The GATA-3 staining method was adapted from Kim et al. (1). In brief, a series of secondary antibodies was used to amplify the signal. After overnight incubation with primary GATA3 antibody (clone TWAJ; eBioscience), secondary diluted FITC-conjugated Affini Pure Donkey α-Rat IgG (The Jackson Laboratories) was applied to sections for 30 min. After washing in PBS, tertiary Alexa Fluor 488-conjugated rabbit IgG fraction α Fluorescein/Oregon Green (Molecular Probes), together with anti-hamster anti-mouse CD3ε antibody (clone ebio500A2; eBioscience), was incubated for 45 min and then washed in PBS. Finally, the quaternary antibodies Alexa Fluor 488-conjugated donkey anti-rabbit and Alexa Fluor 594-conjugated goat anti-hamster were added and incubated for 30 min. Vectashield with DAPI (Vector Labs) was used for counterstaining.

Microscopy, Imaging, and Analysis. A Leica light microscope mounted with a Zeiss Axiocam 503 camera was used for imaging immunohistochemistry staining. For fluorescent staining, a Zeiss Observer.Z1 microscope mounted with an Axiocam 506 mono camera was used for imaging. Fiji software (2) was used for image analysis. The ImmunoRatio plug-in (3) was used for nuclear IL-33 ratio analysis.

Antibodies. The antibodies used for ILC flow cytometry staining are provided here. The lineage biotinylated antibodies used are as follows: anti-CD3ε (clone 145-2C11), anti-CD19 (clone 1D3), anti-CD45R (clone RA3-6B2), anti-CD11c (clone N418), anti-CD11b (cloneM1/70), anti-CD5 (clone 53-7.3), anti–TCR-β (clone H57), TER119 (clone TER119), anti-GR1 (clone RB6-8C5), and anti-CD8a (clone 53-6.7). BD Horizon Streptavidin V500 (BD Biosciences) and phycoerythrin/Cy7 Streptavidin (Biolegend) were used. Antibodies to CD45 (clone 30-F11), CD127 (clone SB/199), and CD4 (clone GK1.5) were purchased from Biolegend. Anti-RORγT (clone Q31-378) and anti-GATA3 (clone L50-823) antibodies were purchased from BD Biosciences. For MC flow cytometry staining (4), anti-CD117 (clone 2B8), anti-Thy1.2 (clone 30-H12), and anti-FcεR1 (clone MAR-1) antibodies were purchased from Biolegend. Anti-ST2 antibodies purchased from Biolegend (clone DIH9) and from Mdbiosciences (clone DJ8) were used. Antibodies specific to mMCP-2, mMCP-5, and MCP-6, which were generated by M.G. were used in immunohistochemistry. IL-33 antibody was purchased from R&D Systems. Anti-CD3ε (clone eBio500A2) was purchased from eBioscience.

MC Culture. Femurs from C57BL/6 mice were flushed with PBS using a 27-gauge syringe. Cells were pelleted, and RBCs were lysed using ACK lysis buffer (Lonza). Cells were then resuspended in

10 mL of RPMI media (Lonza) supplemented with FBS (Millipore), essential amino acids, sodium pyruvate, L-glutamine, Hepes buffer, and β-mercaptoethanol. Stem cell factor (Gibco), and IL-3 (Invitrogen) at 10 ng/mL and 20 ng/mL, respectively, were added to the media. Cells were transferred to a 25-cm^2 flask. The next day, cells were split into three new 25-cm^2 flasks filled with 5 mL of media. Five milliliters of media was added every 48 h until the volume reached 15 mL, and cells were then centrifuged at 96.2 × *g* and resuspended into 5 mL of media. After 3 wk, MCs were quantified by staining for FcεR1 and c-Kit and flow cytometry.

IL-33 or IL-10 (10 ng/mL; Biolegend) was added to MC cultures 10 d after the start of culture, and cells were tested after 3 wk of culture.

Cytospin Tryptase Immunohistochemistry. MCs were cytospun at 500 RPM. Slides were dried for 5 min and then fixed with 2% paraformaldehyde for 10 min. Nonspecific avidin and biotin were blocked (Vector Labs). Nonspecific Fc-receptor binding was blocked by anti-mouse CD16/CD32 (clone 2.4G2). Primary anti-mouse MCP-6 antibody was added and incubated for 2 h. Secondary goat anti-rabbit antibody (Vector Labs) was incubated for 45 min, followed by addition of alkaline phosphatase conjugated avidin biotin complex (Vector Labs) for 30 min. SIGMAFAST Fast red TR/Naphthol AS-MX tablets (Sigma) were used as a substrate for color development.

Serum and Tissue Extracts. Polyps and healthy surrounding tissue were separately microdissected and minced, and then homogenized for 1 min using a 16-gauge needle and syringe on ice. Samples were centrifuged at 162.5 × *g* for 15 min, and the supernatant was collected and filtered. The protein concentration was determined using the Bradford assay.

Bone Reconstitution Experiments. At 2 mo of age, mice were irradiated with a 1,000-rad dose. Four hours following the irradiation, 2×10^6 lineage-depleted bone marrow cells obtained from WT or $MCP6^{-/-}$ mice were injected into the retroorbital sinus. Mice were kept on antibiotics supplied in drinking water for 2 mo. Mice were euthanized at 6 mo of age.

Statistical Analysis. The statistical analyses were performed with the use of Prism7 software (GraphPad). Unpaired one-tailed *t* tests with the Wells correction were used.

1. Kim S, et al. (2011) $CD117^+$ $CD3^-$ $CD56^-$ OX40Lhigh cells express IL-22 and display an LTi phenotype in human secondary lymphoid tissues. *Eur J Immunol* 41:1563–1572.
2. Schindelin J, et al. (2012) Fiji: An open-source platform for biological-image analysis. *Nat Methods* 9:676–682.
3. Tuominen VJ, Ruotoistenmäki S, Viitanen A, Jumppanen M, Isola J (2010) ImmunoRatio: A publicly available web application for quantitative image analysis of estrogen receptor (ER), progesterone receptor (PR), and Ki-67. *Breast Cancer Res* 12: R56.
4. Bankova LG, Dwyer DF, Liu AY, Austen KF, Gurish MF (2015) Maturation of mast cell progenitors to mucosal mast cells during allergic pulmonary inflammation in mice. *Mucosal Immunol* 8:596–606.

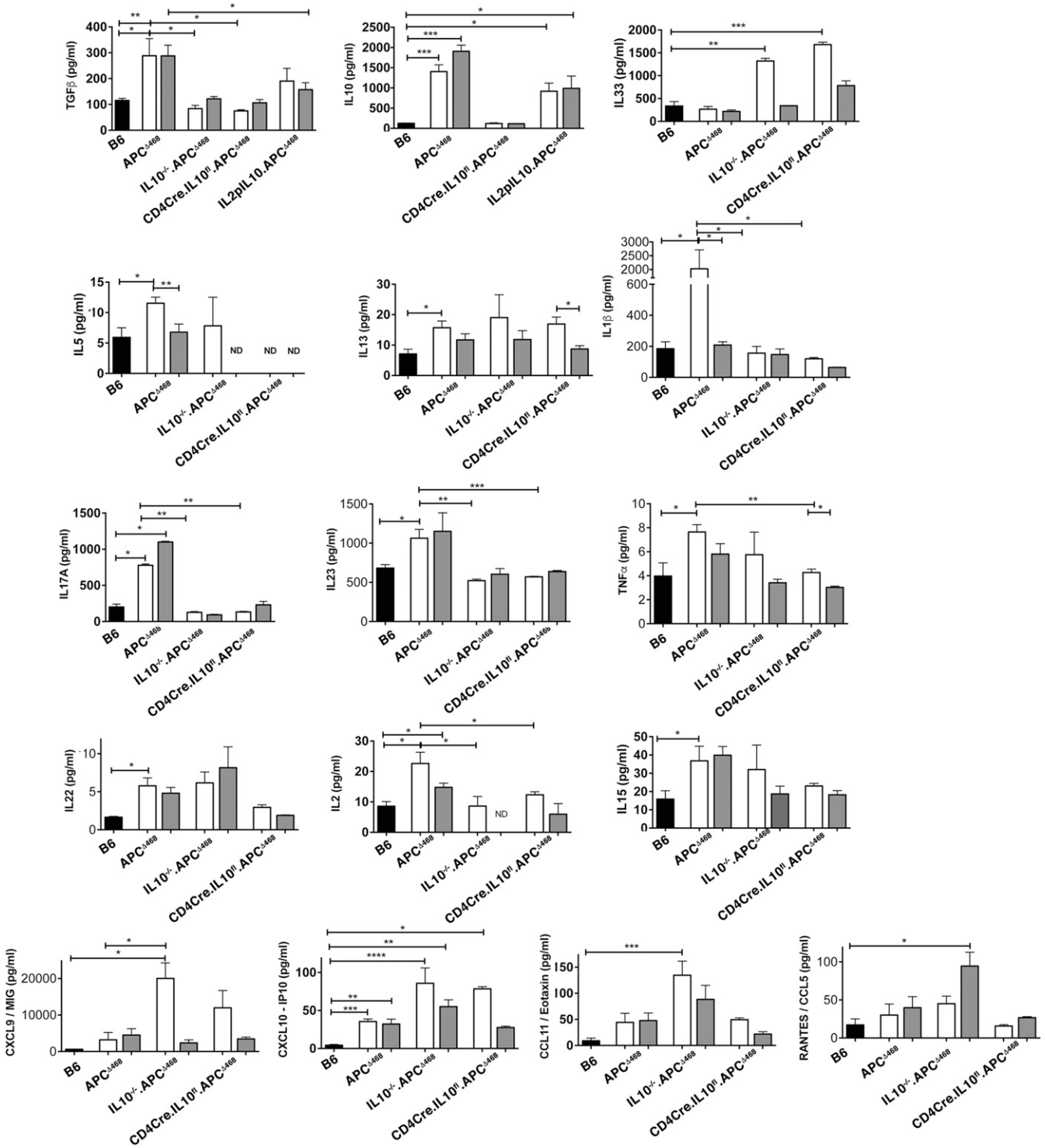

Fig. S1. Expression of cytokines and chemokines by the SB of IL-10–proficient and –deficient polyposis-prone mice. Tissue extracts of polyps or healthy surrounding tissue from 4-mo-old mice were analyzed with multiplex ELISA for cytokines. The results of three independent experiments conducted in triplicate were used to calculate the positive or negative changes. White bars are polyps, and gray bars are healthy distant tissue. Unpaired *t* test: $****P < 0.0001$; $***P < 0.001$; $**P < 0.005$; $*P < 0.05$.

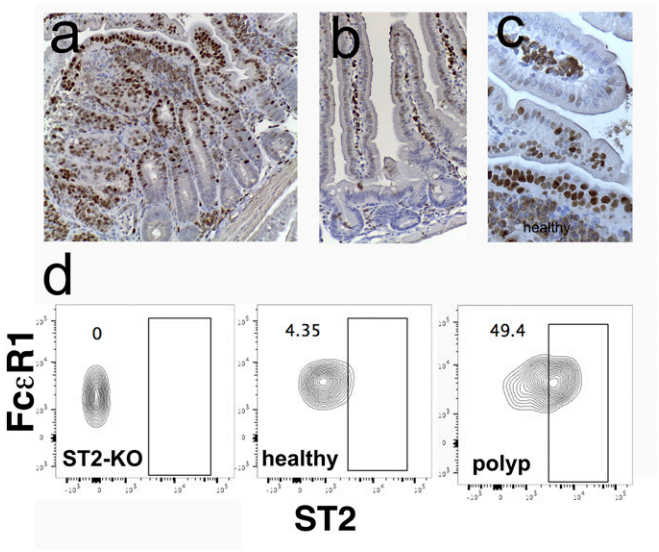

Fig. S2. Time course of the polyp-to-carcinoma transition and MC expansion. APC$^{\Delta 468}$ mice were euthanized at 4 mo, and polyps, together with healthy surrounding SB, were analyzed by histology and immune staining. (*A*) Expression of nuclear IL-33 by transformed polyp epithelial cells; note that hyperproliferative crypts underneath the aberrant crypts are not stained. (Magnification: 200×.) (*B*) IL-33 stain of healthy ileum. (Magnification: 200×.) (*C*) IL-33 stain of distant healthy villus (not stained), immediate neighboring villus (a few cells stained), and polyp epithelium (strong staining). (Magnification: 400×.) (*D*) Microdissected SB polyps were collagenase-digested, and total MNCs were analyzed by FACS. Representative FACS dot plots of ST2^{+} MCs among cKit^{+}FceR1^{+} MCs derived from the SB of an ST2 KO mouse, healthy SB of an APC$^{\Delta 468}$ mouse, and polyps of an APC$^{\Delta 468}$ mouse are shown.

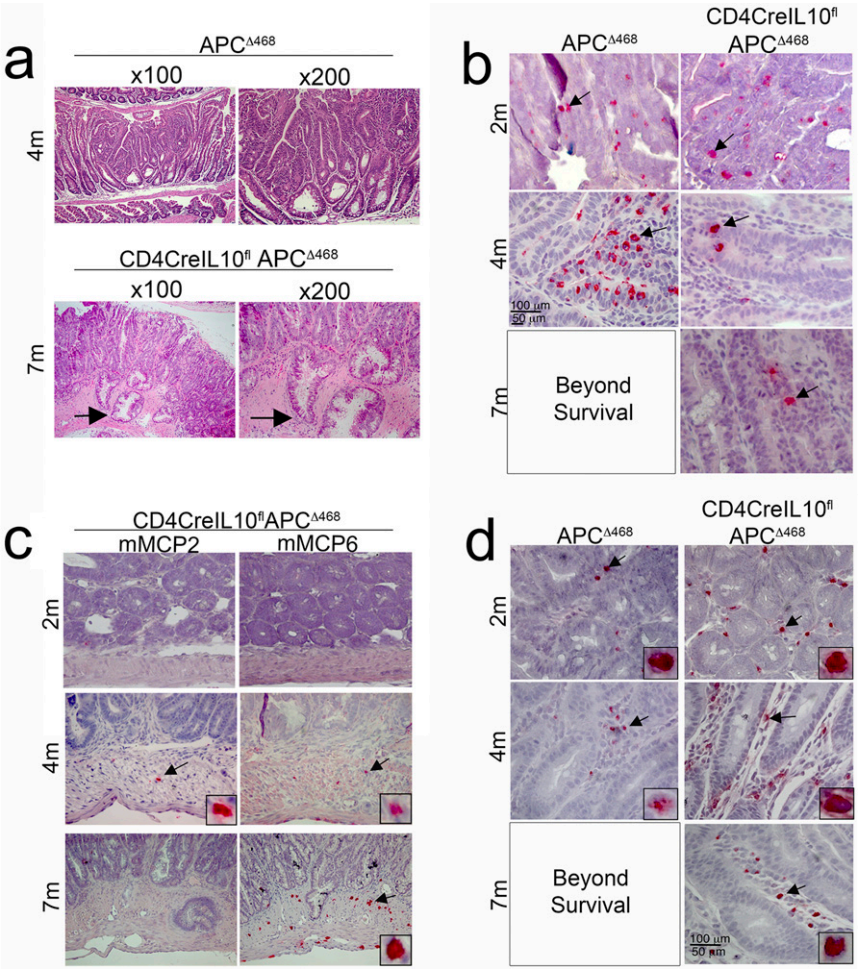

Fig. S3. Time course of the polyp-to-carcinoma transition and MC expansion. APC$^{\Delta 468}$ mice were euthanized at 2 mo (m), 4 mo, and 7 mo of age, and the SB was analyzed by histology and immune staining. (*A*) H&E stain of small intestine at 4 mo and 7 mo of age. (*B*) Intraepithelial mMCP2^{+} MCs in the indicated mice. (*C*) Submucosal mMCP2^{+} and mMCP6^{+} MCs in the indicated mice. (Magnification: *Top* and *Middle*, 200×; *Bottom*, 100×.) (*D*) Stromal mMCP6^{+} MCs in the indicated mice. Arrows point to MCs. *Insets* are enlarged images of the MCs.

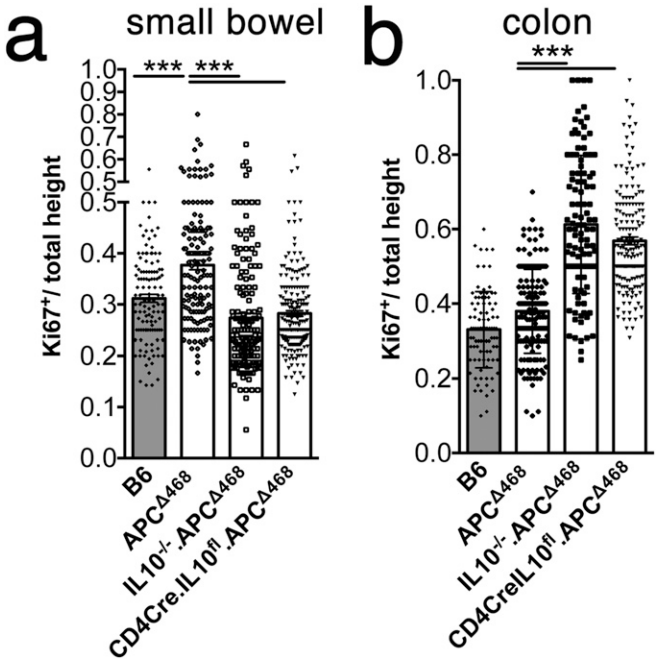

Fig. S4. Mitotic activity of healthy enterocytes in the SB (*A*) and colon (*B*) of indicated mice ($n = 3$ mice). Unpaired *t* test: ***$P < 0.001$.

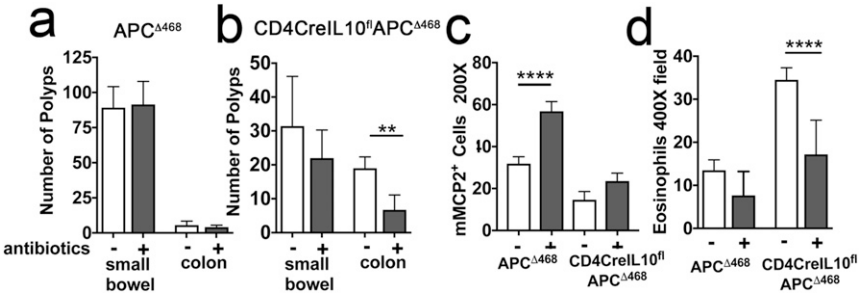

Fig. S5. SB polyps and infiltrating MCs are resistant to broad-spectrum antibiotics. The SB polyp load of $APC^{\Delta 468}$ and $CD4CreI10^{fl}APC^{\Delta 468}$ mice following antibiotic treatment is shown; gray bars indicate the treated groups. Antibiotic treatment: From 2.5 mo of age, mice received an i.p. injection of 200 μL of kanamycin (4 mg/mL), gentamycin (0.35 mg/mL), colistin (8,500 U/mL), metronidazole (2.12 mg/mL), and vancomycin (0.45 mg/mL), repeated every other day for 3 wk; they were allowed 1 wk of rest and then analyzed. (*A* and *B*) Polyp numbers in the SB of indicated mice. Frequencies of infiltrating MCs (*C*) and frequency of infiltrating eosinophils (*D*) before (white bars) and after (gray bars) treatment are shown ($n \geq 3$ mice). Unpaired *t* test: ****$P < 0.0001$; **$P < 0.005$.

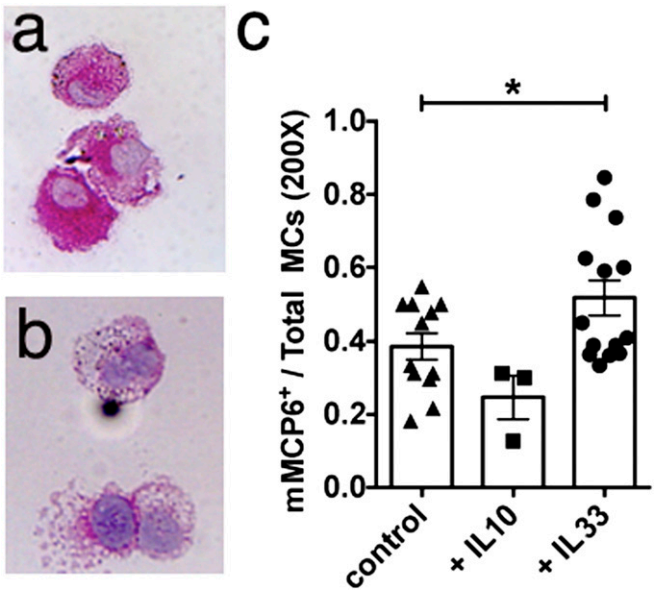

Fig. S6. IL-33 induces expression of mMCP6 by MC. Bone marrow-derived MC progenitors were cultured in medium containing stem cell factor (SCF) without or with IL-33 or IL-10. After 3 wk, matured MCs were cytospun and stained with mMCP6 antibody. (*A*) MCs cultured with IL-33. (*B*) MCs cultured with IL-10. (*C*) Frequencies of mMCP6^{+} MCs counted by eye (control, $n = 12$; IL-10, $n = 3$; IL-33, $n = 14$). Unpaired t test: $^{*}P < 0.005$. (Magnification in *A* and *B*: 1,000×.)

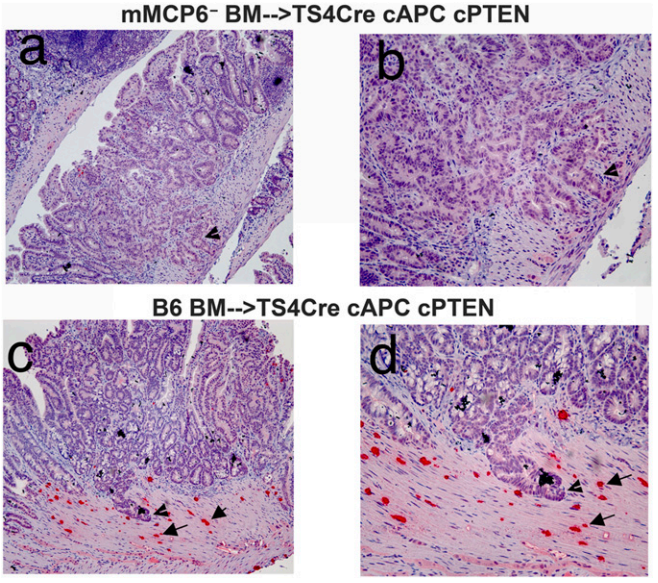

Fig. S7. Depleting mMCP6^{+} MCs does not hinder the polyp-to-carcinoma transition. (*A* and *B*) SB polyp in a 5-mo-old TS4CrecAPC mouse with mMCP6$^{-/-}$ bone marrow; note the absence of mMCP6 staining and the presence of invasive crypts. (*C* and *D*) SB polyp in a 5-mo-old TS4CrecAPC mouse with control B6 bone marrow; note the presence of mMCP6-stained MCs and also of invasive crypts. Arrows point to MCs, and arrowheads point to invasion. (Magnification: *A*–*C*, 200×; *D*, 400×.)