Supporting Information

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SI Experimental Procedures

Histopathology, Cell Lineage Identification, and Immunohistochemistry. Colons were harvested from terminal ileum to distal rectum. After overnight fixation with 4% paraformaldehyde, samples were sent to the Harvard Cancer Center Rodent Histopathology Core for routine paraffin embedding, sectioning, and staining with H&E. The degree of colon inflammation was graded by one of the authors (J.N.G.), who was blinded to the genotype and experimental conditions of the mice. For these studies, a validated 20-point scoring system designed for experimental colitis (1) was used. Chloroacetate esterase histochemistry (2) and immunohistochemistry with the rat anti-mouse neutrophil antibody MCA771G (AbD Serotec) (3) were used to identify mast cells (MCs) and neutrophils, respectively. Eosinophils were visualized histochemically by staining tissue sections with Congo red, which preferentially recognizes these granulocytes (4, 5). Mouse mast cell protease (mMCP-6)-positive MCs were detected using an affinity-purified, rabbit anti–mMCP-6 antibody (6) (1:500 dilution) and fluorescently labeled secondary antibody (Alexa Fluor 568 goat anti-rabbit IgG, 1:1,000 dilution; Invitrogen). Briefly, sections were deparaffinized and rehydrated, and antigen retrieval was performed with Target Retrieval Solution (S1699; Dako) at 97 °C for 30 min. Nonspecific secondary antibody staining and synthetic fluorophore binding sites were blocked with 10% goat serum (Santa Cruz Biotechnology). Sections were mounted using VECTASHIELD mounting media containing DAPI (Vector Labs) and were photographed using an Eclipse 80i microscope (Nikon Instruments Inc.,), a Hamamatsu C10800 digital camera, and HCImage software 1.1.3.1 (Hamamatsu Corp.).

To demonstrate matrix metalloproteinase 3 (MMP3)-positive cells in the colon, paraffin-embedded, formalin-fixed sections were dewaxed and rehydrated through a series of graded alcohols. Sections were treated for 30 min in 0.6% H_2O_2 to quench endogenous peroxidase. Antigen retrieval was performed by microwaving sections in 10 mM NaOAc, pH 6.0, for 20 min. Nonspecific binding was inhibited by incubating sections in 5% goat serum in 1× Tris-buffered saline (TBS) for 1 h. Rabbit monoclonal anti-mouse and human MMP3 (clone EP1186Y; Epitomics) was diluted 1:150 and applied to sections overnight at room temperature. After thorough washing with TBS with Tween 20 (TBST), a 1:500 dilution of biotinylated goat anti-rabbit secondary antibody (Vector Labs) was applied to sections for 1 h at room temperature. Appropriate IgG control staining was performed on adjacent sections to evaluate background staining. Positive cells were visualized with an avidin-biotin peroxidase complex (Vectastain; Vector Labs) and 3,3′-diaminobenzidine tetrahydrochloride substrate (DAB) (Sigma). Nuclei were counterstained with Mayer's hematoxylin (Sigma), washed in TBS, dehydrated, cleared in xylenes, and mounted using Permount.

Solute carrier family 26 member 3 (SLC26A3)-positive colon cells were demonstrated in a similar manner. Briefly, paraffin-embedded, formalin-fixed sections were dewaxed and rehydrated through a series of graded alcohols. Endogenous peroxidase was quenched by incubating slides for 30 min in 0.6% H_2O_2. Antigen retrieval was performed by incubating sections in solution of proteinase K [4 mg/220 mL proteinase K, 5 mM EDTA (pH 8.0), 50 mM Tris HCl (pH 7.4)] for 10 min at room temperature, and nonspecific binding was inhibited by incubating sections in 5% rabbit serum diluted in 1× TBS for 1h. Goat polyclonal anti-SLC26A3 (sc-34939; Santa Cruz Biotechnology) was diluted 1:50 and applied to sections overnight at 4 °C. Biotinylated rabbit anti-goat secondary antibody (1:500; Vector Labs) was applied to sections for 1 h at room temperature. Appropriate IgG control staining was performed on adjacent sections to evaluate background staining. Positive cells were visualized with an avidin-biotin peroxidase complex (Vectastain; Vector Labs) and DAB (Sigma). Nuclei were counterstained with Mayer's hematoxylin (Sigma), washed in TBS, dehydrated, cleared in xylenes, and mounted using Permount (Fisher Scientific).

IL-6 and IL-1β Protein Levels. For ELISA experiments, 1-cm sections of the distal colons were resected at day 9 of a dextran sodium sulfate (DSS) experiment and washed with RPMI-1640 medium supplemented with 1% penicillin and 1% streptomycin (Gibco). The colon segments were cultured in medium that contained 10% FCS (Sigma), 2-mercaptoethanol (Gibco), and 1% nonessential amino acids for 4 h at 37 °C with 5% CO_2. The resulting supernatants then were analyzed by an ELISA for levels of IL-6 proteins (eBioscience) following the manufacturer's protocol. For the IL-1β assay, resected colon segments were placed in 1% TBS and homogenized and spun down for 5 min at 10,000 × *g*. The supernatants were analyzed by an ELISA for IL-1β (eBioscience) following the manufacturer's protocol.

1. Garrett WS, et al. (2007) Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 131:33–45.
2. Friend DS, et al. (1996) Mast cells that reside at different locations in the jejunum of mice infected with *Trichinella spiralis* exhibit sequential changes in their granule ultrastructure and chymase phenotype. *J Cell Biol* 135:279–290.
3. Hirsch S, Gordon S (1983) Polymorphic expression of a neutrophil differentiation antigen revealed by monoclonal antibody 7/4. *Immunogenetics* 18:229–239.
4. Grouls V, Helpap B (1981) Selective staining of eosinophils and their immature precursors in tissue sections and autoradiographs with Congo red. *Stain Technol* 56: 323–325.
5. Friend DS, Gurish MF, Austen KF, Hunt J, Stevens RL (2000) Senescent jejunal mast cells and eosinophils in the mouse preferentially translocate to the spleen and draining lymph node, respectively, during the recovery phase of helminth infection. *J Immunol* 165:344–352.
6. Ghildyal N, et al. (1996) Fate of two mast cell tryptases in V3 mastocytosis and normal BALB/c mice undergoing passive systemic anaphylaxis: Prolonged retention of exocytosed mMCP-6 in connective tissues, and rapid accumulation of enzymatically active mMCP-7 in the blood. *J Exp Med* 184:1061–1073.

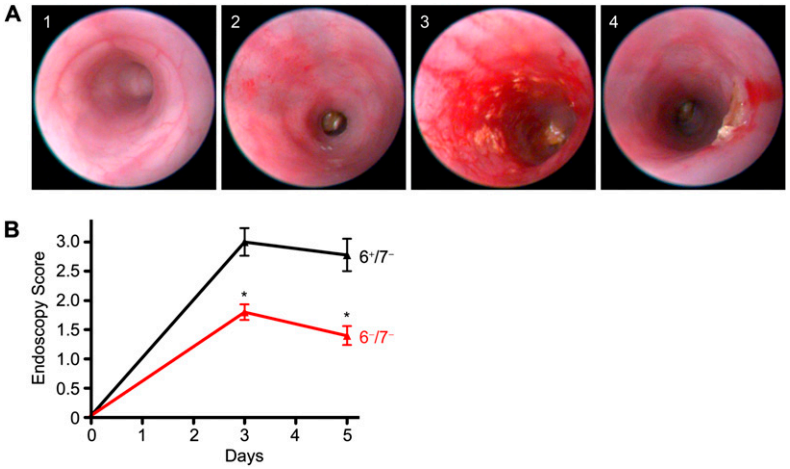
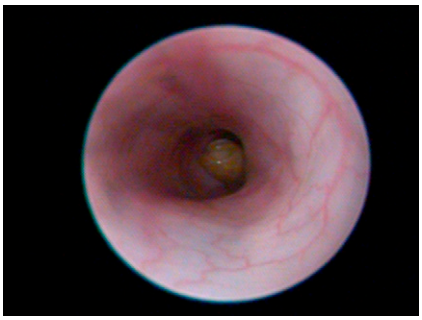
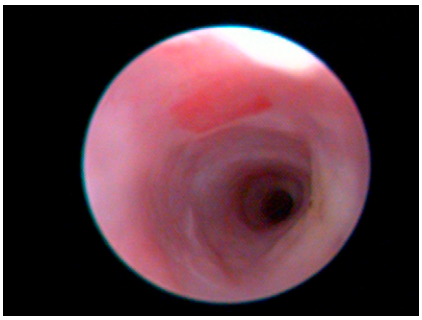


Fig. S1. Trinitrobenzene sulfonic acid (TNBS)-treated $6^{-}/7^{-}$ mice have less inflammation, as assessed by endoscopy, than TNBS-treated $6^{+}/7^{-}$ mice. (*A*) Representative images from the endoscopy grading scale for the scores 1, 2, 3, and 4. (*B*) Inflammation was assessed using the endoscopy scale at days 3 and 5. TNBS-treated $6^{-}/7^{-}$ mice had significantly lower endoscopy scores at days 3 and 5 than TNBS-treated 6+/7- mice. Endoscopy scores are reported as mean ± SEM. *$P < 0.0001$.



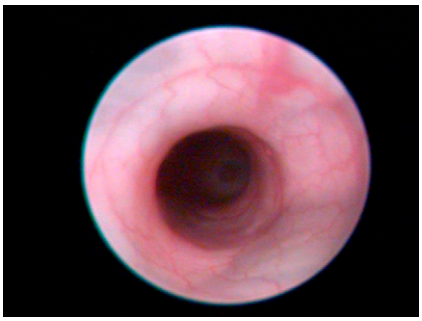
Movie S1. Colon endoscopy of a representative WT $6^{+}/7^{-}$ B6 mouse that had not received DSS or TNBS.

Movie S1



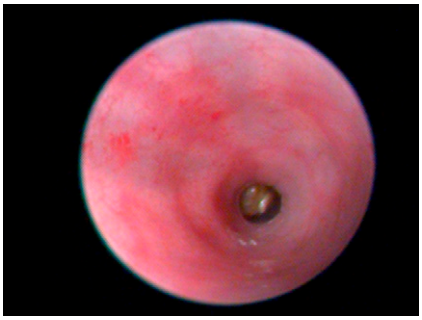
Movie S2. Colon endoscopy of a representative WT $6^{+}/7^{-}$ B6 mouse on day 9 after the 7-d treatment with DSS.

Movie S2



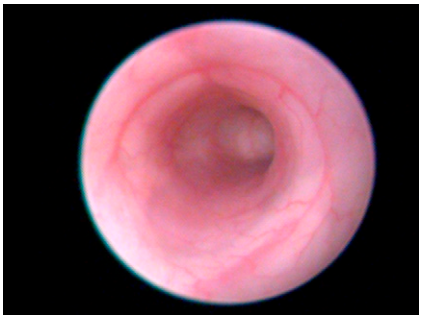
Movie S3. Colon endoscopy of a representative mast cell tryptase-null $6^{-}/7^{-}$ B6 mouse on day 9 after the same 7-d treatment with DSS.

Movie S3



Movie S4. Colon endoscopy of a representative WT $6^{+}/7^{-}$ B6 mouse on day 5 after treatment with TNBS.

Movie S4



Movie S5. Colon endoscopy of a representative MC tryptase-null $6^{-}/7^{-}$ B6 mouse on day 5 after treatment with TNBS.

Movie S5

Other Supporting Information Files

Dataset S1 (XLSX)