Supporting Information

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SI Materials and Methods

Colonoscopy Procedures. Continuous monitoring of colitis and tumorigenesis was done using the Coloview high-resolution mouse endoscopic system (Karl Storz). In azoxymethane (AOM)-dextran sulfate sodium (DSS) experiments, tumors observed during endoscopy were counted to obtain the overall number of lesions. Tumor size was graded using the protocol described by Becker et al. (1) as grade 1, very small but detectable tumor; grade 2, tumor covering up to one-eighth of the colonic circumference; grade 3, tumor covering up to one-quarter of the colonic circumference; grade 4, tumor covering up to one-half of the colonic circumference; or grade 5, tumor covering one-more than half of the colonic circumference. The total tumor score per mouse was calculated as sum of all tumor sizes. In DSS experiments, colitis was scored as 1–3 for each of the following parameters: granularity of mucosal surface, stool consistency, vascular pattern, translucency of the colon, and fibrin. The colonoscopy scores were assigned by a scientist blinded to the experimental conditions.

Isolation of Colonic Lamina Propria Leukocytes. For isolation of colonic lamina propria leukocytes, entire colons from each group were longitudinally cut and washed to remove feces and debris. Colon pieces were incubated in HBSS containing 5 mM EDTA and 2% FBS at 37 °C for 25 min on a shaking platform. After removal of EDTA by three washes in PBS, the colon pieces were finely minced and then incubated in digestion media containing HBSS, 1 mg/mL collagenase VIII (Sigma-Aldrich), 0.01 mg/mL DNase I (Roche), and 1 M 2βME (Sigma-Aldrich) for 45 min at 37 °C on a shaking platform. After collagenase digestion, the medium containing the mononuclear cells was collected, filtered, and centrifuged at 300 × *g* for 5 min. The resulting cells were then used for flow cytometry analysis, cell sorting, and RNA extraction.

Histopathological Analysis. Colons were harvested and fixed by immersion in Bouin's fixative (Ricca Chemical), and then processed, embedded, sectioned, and stained with H&E following routine methods. Each section was evaluated using a semi-quantitative criterion-based method and scored as 0–5, as described previously (2). The histopathological scores were assigned by a pathologist blinded to the experimental manipulation.

Immunofluorescence. For immunofluorescence analysis, the intact colon was isolated, opened longitudinally, rinsed with PBS, and prepared as a colon roll. For TUNEL immunostaining, the colon was then fixed in 1% paraformaldehyde, immersed sequentially in 10%–20%–30% sucrose, embedded in optimal cutting temperature (OCT), and frozen in isopentane cooled with liquid nitrogen. Colon serial sections (7 μm thick) were blocked with BSA 5% and Triton 0.1% in PBS before incubation with the TUNEL reaction mixture (Roche), according to the manufacturer's instructions.

For Mer immunostaining, serial sections (7 μm thick) of isopentane-frozen colons were fixed in PFA 4% for 10 min at room temperature, then blocked in BSA 5% and Triton 0.2% in PBS for 1 h at room temperature. Sections were stained with anti–PE-conjugated CD11b (eBiosciences) and rabbit anti-Mer antibody (3) diluted 1:50 in blocking buffer and incubated overnight at 4 °C, followed by secondary staining with FITC-conjugated donkey α-rabbit IgG (Biolegend). Specimens were counterstained with Hoechst 33342 (Molecular Probes) and analyzed with a PerkinElmer Confocal-UltraVIEW screening microscope.

Generation of Bone Marrow Chimeras. Bone marrow (BM) chimeras were generated by lethally irradiating (two exposures to 300 rads, 3 h apart) B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ hosts using an X-RAD 320 irradiation system (Precision X-Ray). Irradiated mice were reconstituted by retro-orbital injection of 5×10^6 BM cells previously flushed from femurs, subjected to red blood cell lysis, and filtered through a 70-μm filter. Chimeras were maintained on water containing antibiotics (Sulfatrim; Roche) during the 2 wk after the BM transplantation. Over an 8-wk period, 90–95% of the donor BM cells completely repopulated the peripheral lymphoid system. Reconstitution was determined by flow cytometry analysis performed on peripheral blood mononuclear cells.

Flow Cytometry Analysis. Lamina propria leukocytes were stained with surface-conjugated antibodies against CD45 (clone 30-F11; Biolegend), CD11b (clone Mm1/70; eBiosciences), F4/80 (clone BM8; Biolegend), CD11c (clone N418; Biolegend) CD4 (clone RM4-5; Biolegend), CD8 (clone 53-6.7; Biolegend), CD3 (clone 145-2C11; Biolegend) Ly6G (clone 1A8; Biolegend), B220 (clone RA3-6B2; Biolegend), Siglec-F (clone E50-2440; BD Biosciences), CD117 (clone 2B8; Biolegend), and Mer (clone 108928; R&D Systems) for 30 min at 4 °C, or stained for TUNEL detection (Roche) according to the manufacturer's instructions. Samples were acquired on a FACSCanto flow cytometer (BD Biosciences). Data were analyzed using FlowJo software (TreeStar).

IL-17α and IFN-γ were measured by intracellular staining on lamina propria leukocytes after stimulation in vitro for 4 h with phorbol 12-myristate 13-acetate and ionomycin. Cells were incubated with Golgi Plug (BD Biosciences) during the last 2 h of stimulation. Intracellular staining was performed with PE-conjugated anti–IL-17α antibody (clone eBio17B7; eBiosciences) and FITC-conjugated anti–IFN-γ (clone XMG1.2; eBiosciences) following the manufacturer's protocol.

The reconstitution of BM was determined by flow cytometry analysis at 8 wk after BM transplantation. Peripheral blood mononuclear cells obtained from all mice that received a BM transplant were stained using anti-CD45.1 (clone A20; Biolegend) and anti-CD45.2 (clone 104; Biolegend).

BM-Derived Macrophage Preparation. BM-derived macrophages (BMDMs) were generated from BM progenitor cells flushed from mouse femurs and tibias. Progenitor cells were incubated on 10-cm cell culture dishes at a concentration of 5×10^6 cells/mL in complete media containing 50% RPMI, 20% FBS, and 30% L929-conditioned media as the source of macrophage colony-stimulating factor. Macrophages were lifted on day 6 using ice-cold PBS and replated onto 12-well cell culture plates with 1.3×10^6 cells per well in complete media containing 65% RPMI, 15% FBS, and 20% L929 supernatant. BMDMs were subsequently used in phagocytosis assays on day 7 of in vitro differentiation.

Neutrophil Isolation and Apoptosis. Neutrophils were collected from BM cell suspensions using the EasySep Mouse Neutrophil Enrichment Kit (StemCell Technologies) to isolate neutrophils by negative selection. Biotinylated antibodies targeted CD4, CD5, CD11c, CD45R/B220, CD49b, CD117, TER119, and F4/80. After incubation with secondary antibodies bound to magnetic particles, cells were separated with EasySep magnets. Neutrophil purity was >90% as determined by CD11b and Ly6G staining. Neutrophils were aged for 20–24 h in complete media containing 90% RPMI and 10% FBS, and labeled with 0.5 μM CellTracker

Green CMFDA (Molecular Probes; Invitrogen). Apoptosis was verified by annexin V and propidium iodide staining.

Phagocytosis Assays. CellTracker-labeled apoptotic neutrophils and unlabeled BMDMs were cocultured at a 5:1 ratio. After 1 h, cells were washed and lifted using ice-cold PBS. Macrophages were then labeled with F4/80 antibodies. Macrophages with labeled apoptotic cargo were identified by CellTracker and F4/80 double-positive events using a FACSCalibur flow cytometer (BD Biosciences). Phagocytosis, not extracellular binding, was verified by coculturing macrophages with apoptotic cells at 4 °C or in the presence of 1 μM cytochalasin D to inhibit actin polymerization.

Quantitative Real-Time PCR. At the indicated time points, cells were harvested and washed, and RNA was isolated using the Qiagen RNeasy Mini Kit following the manufacturer's instructions. Reverse transcription was performed with RT SuperScript III (Invitrogen) or the iScript cDNA Synthesis Kit (Bio-Rad). Quantitative real-time PCR (qPCR) reactions were performed on a Stratagene Mx3000 system using a KAPA SYBR Fast qPCR Kit (Kapa Biosystems). The reactions were normalized to housekeeping gene, and the specificity of the amplified products was verified by dissociation curves. The following primers were used: for IFN-γ: forward, GGATGCATTCATGAGTATTGC; reverse, CCTTTTCCGCTTCCTGAGG; for IL-6: forward, TACCACTTCACAAGTCGGAGGC; reverse, CTGCAAGTGCATCATCGTTGTTC; for IL12p35: forward, ACGAGAGTTGCCTGGCTACTAG; reverse, CCTCATAGATGCTACCAAGGCAC; for IL-17α: forward, ATCCCTCAAAGCTCAGCGT; reverse, GGGTCTTCATTGCGGTGGAGAG; for Nos2: forward, CCCTTCCGAAGTTTCTGGCAGCAGC; reverse, GGCTGTCAGAGCCTCGTGGCTTTGG; for GAPDH: forward, TCCCACTCTTCCACCTTCGA; reverse, AGTTGGGATAGGGCCTCTCTT; for Axl: forward, ATGCCAGTCAAGTGGATTGCT; reverse, CACACATCGCTCTTGCTGGT; for Mer: forward, GTAGATTACGCACCCTCGTCAAC; reverse, GCCGAGGATGATGAACATAGAGT; for RELMα: forward, CCAATCCAGCTAACTATCCCTCC; reverse, CCAGTCAACGAGTAAGCACAG; for TNF-α: forward, CCCTCACACTCAGATCATCTTCT; reverse, GCTACGACGTGGGCTACAG; for TGF-β: forward, TGATACGCCTGAGTGGCTGTCT; reverse, CACAAGAGCAGTGAGCGCTGAA; for IL-10: forward, CGGGAAGACAATAACTGCACCC; reverse, CGGTTAGCAGTATGTTGTCCAGC.

Statistical Analysis. Differences in mean values of the experimental groups were analyzed with the two-tailed Student *t* test or two-way ANOVA, using GraphPad Prism. The Bonferroni multiple comparison test was used after two-way ANOVA. A P value ≤ 0.05 was considered significant.

1. Becker C, Fantini MC, Neurath MF (2006) High-resolution colonoscopy in live mice. *Nat Protoc* 1(6):2900–2904.
2. O'Connor W, Jr., et al. (2009) A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat Immunol* 10(6):603–609.
3. Prasad D, et al. (2006) TAM receptor function in the retinal pigment epithelium. *Mol Cell Neurosci* 33(1):96–108.

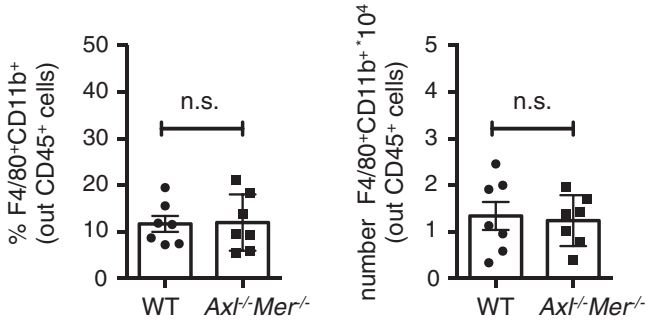

Fig. S1. Frequency (*Left*) and absolute number (*Right*) of F4/80$^+$CD11b$^+$ colonic lamina propria macrophages per colon assessed in WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice at steady state. Results represent mean ± SEM of seven mice per group. n.s., not significant.

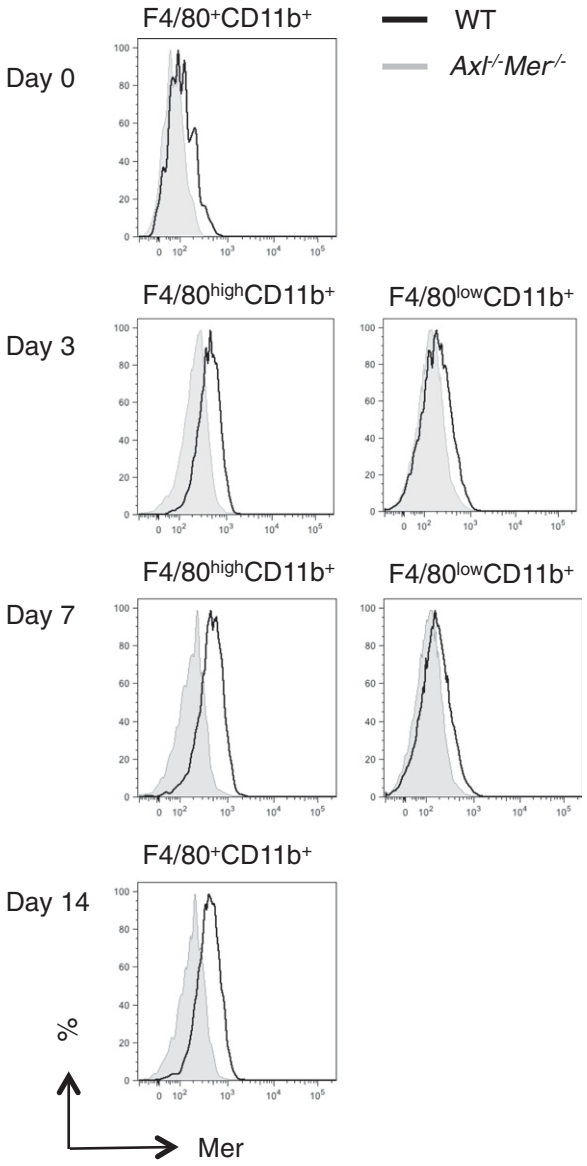

Fig. S2. Histograms showing the expression of Mer receptor on the different macrophage populations isolated from colonic lamina propria of WT mice (black histograms) at steady state (day 0) or after treatment with DSS for 3 d (day 3), 7 d (day 7), or 12 d followed by 2 d of normal water (day 14). Macrophages isolated from colonic lamina propria of $Axl^{-/-}Mer^{-/-}$ mice were used as negative controls (shaded histograms). The histograms are representative of at least three independent experiments per time point.

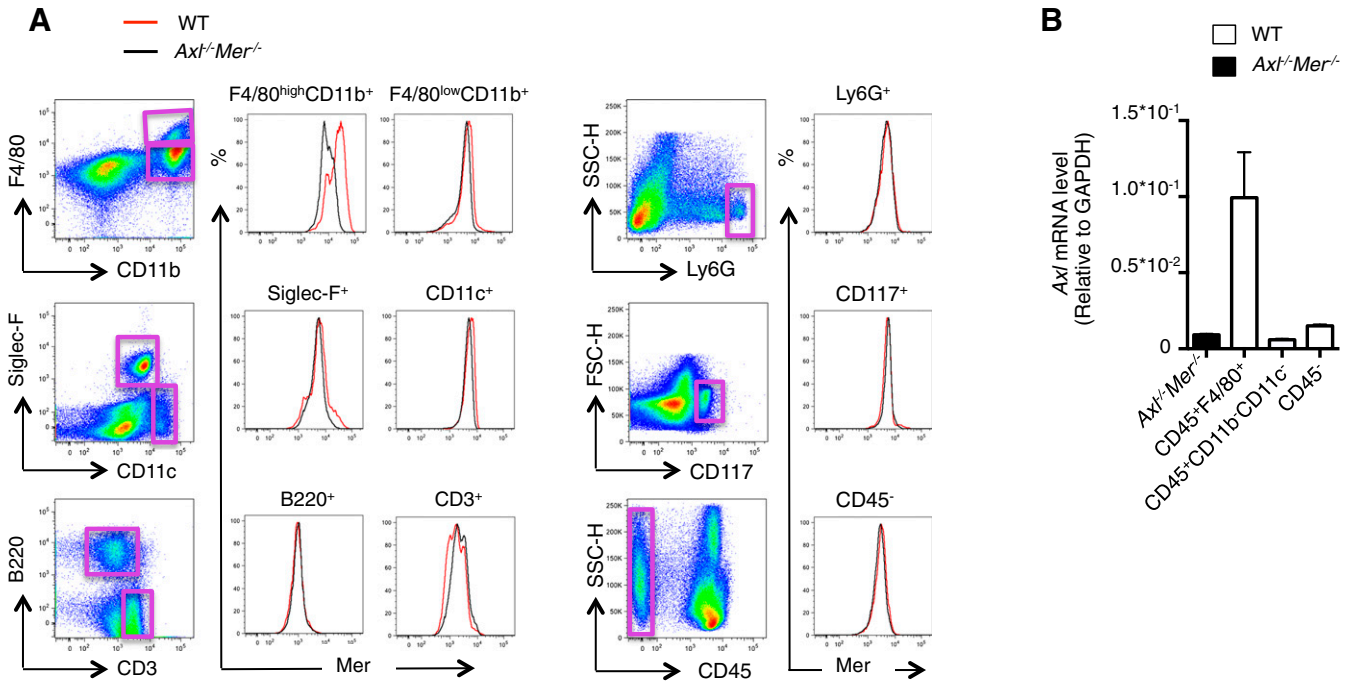

Fig. S3. (*A*) WT and *Axl*$^{-/-}$*Mer*$^{-/-}$ mice were fed 1.5% DSS in the drinking water for 7 d, after which cells from colonic lamina propria were isolated and analyzed for Mer expression. Representative dot plots show the gating strategy on live CD45^{+} cells. Histograms show Mer expression in the selected populations from WT mice (red), with *Axl*$^{-/-}$*Mer*$^{-/-}$ samples used as negative controls (black). (*B*) Expression of *Axl* in the indicated cell-sorted populations from tumor-free regions of the lamina propria of WT mice after the completion of AOM-DSS treatment, as detected by qPCR. Cells sorted from *Axl*$^{-/-}$*Mer*$^{-/-}$ mice were used as an internal negative control.

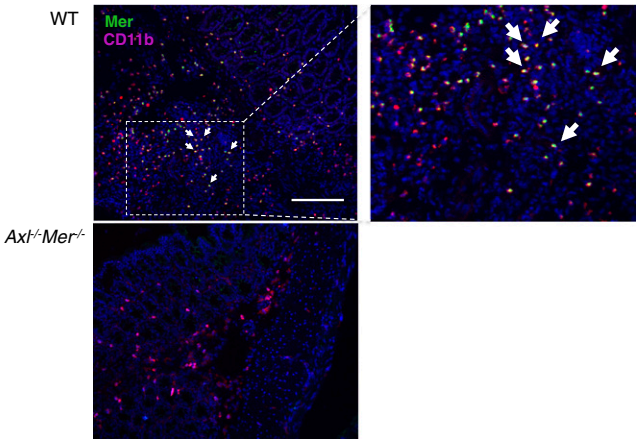

Fig. S4. (*Upper*) (*Left*) Mer (green) expression in the colon of WT mice fed 1.5% DSS in drinking water for 7 d. (*Right*) Magnified view showing colocalization of Mer staining in CD11b^{+} cells (arrows). (*Lower*) Colon sections from *Axl*$^{-/-}$*Mer*$^{-/-}$ mice used as negative controls. (Scale bar: 100 μm.)