Supporting Information

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SI Materials and Methods

Endoscopic Procedures. Colonoscopy was performed at indicated time points to monitor for severity of colitis and tumorigenesis. According to the murine endoscopic index of colitis severity system (1), colitis was scored on five parameters: granularity of mucosal surface, stool consistence, vascular pattern, translucency of the colon, and fibrin visible (score between 0 and 3 for each parameter). Tumor sizes were graded from 1 to 5 as follows: Grade 1 (very small but detectable tumor), Grade 2 (tumor covering up to one-eighth of colonic circumference), Grade 3 (tumor covering up to one-fourth of the colonic circumference), Grade 4 (tumor covering up to half of the colonic circumference), and Grade 5 (tumor covering more than half of the colonic circumference) (1). Tumors observed during endoscopy were counted to obtain the total number of tumors per animal. The size of each tumor in every mouse was recorded, and the sum of all tumor sizes per mouse was calculated to be the tumor score.

Histopathology. After processing, colons were embedded lengthwise in paraffin (Blue RiBbon; Surgipath Medical Industries). Blocks were sectioned to the level of the lumen and then the next 5-μm section was stained with H&E, followed by placement of coverslips by routine methods. Colons were evaluated and were assigned scores by investigators blinded to experimental manipulation. Each section was evaluated for pathological changes in the mucosa, submucosa, muscularis externa, and serosa, including inflammation, edema, mucosal changes of ulceration, hyperplasia, and attenuation, with crypt loss or abscess noted by examination of H&E-stained slides assessed at low power and higher power and assigned scores for the presence and extent (overall severity) of the tissue changes by a semiquantitative criterion-based method (2). Severity scores ranged from 0 to 5 as follows: 0, within normal limits or absent; 1, minimal; 2, mild; 3, moderate; 4, marked; and 5, severe. Digital light microscopic images were recorded with a Zeiss Axio Imager.A1 microscope, AxioCam MRc5 camera, and AxioVision 4.7.1 imaging software (Carl Zeiss Microimaging).

Immunohistochemistry. Paraffin-embedded sections were dehydrated and incubated with the following antibodies: anti-BrdU (1:4,000; Sigma), anti-Ki67 (1:100, Thermo; Lab Vision), anti-CD3 (1:400, Biocare Medical), and anti-myeloperoxidase (MOP) (1:100, Thermo; Lab Vision), anti-Nk1.1 (1:100, Novus Biologicals). DAKO EnVision System was used for detection. All sections were counterstained with hematoxylin. For BrdU proliferation assay, mice were injected intraperitoneally with 1 mL BrdU (Sigma) solution, and killed 24 h after injection. TUNEL staining was performed using ApoAlert DNA Fragmentation Assay Kit (Clontech) and ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore) according to the manufacturer's instructions. Percentage of TUNEL-positive cells in tumor tissues were counted on slides from five sections of each slide and divided by the total cell number of each field at the magnification 10×40. $Ki67^+$ cells were quantitated from 30 crypts of each mouse at day 15 of the colitis-associated colorectal cancer (CAC) model regimen. $BrdU^+$ cells were quantitated from ≥ 25 crypts of each mouse at day 15. The percentage of $Ki67^+$ and $BrdU^+$ cells in tumor tissues were counted from six sections of each slide and divided by the total cell number of each field at the magnification of 10×100. The $CD3^+$ and $MOP\text{-}1^+$ cells in tumor tissues were counted from greater than or equal to six fields of tumor tissue from each slide at the magnification of 10×100.

Isolation of Epithelial Cells and Hematopoietic Cells from the Intestine. Epithelial cells and hematopoietic cells were isolated from the freshly dissected colon. After removal of the Payer's patches and the adventitial fat, the colon was cut longitudinally and washed with PBS. For disruption of the epithelial cells, the colon was incubated in HBSS/EDTA in a 37 °C water bath, manually shaking for 20 min. The supernatant was collected and further separated in $CD45^+$ (IEL) and negative cells using MACS. The remaining colon was cut into small pieces and digested using Collagenase/DNase incubation in a 37 °C water bath, manually shaking, and afterward filtered through a 100-μm cell strainer. $CD45^+$ cells (LPL) were purified using MACS. In a second step $CD45^+$ IEL and LPL cells were further purified using a MoFlo cell sorter. The purity of $CD45^+$ cells was > 95%, epithelial cells were > 99% $CD45^-$.

TaqMan Gene Expression Assays' Catalog Numbers. Caspase-1: Mm00438023_m1; NLRC4: Mm01233150_m1; NLRP3: Mm00840904_m1; IL-17a: Mm00439618_m1; IFN-γ: Mm00801778_m1; L-18: Mm00434225_m1; IL-11: Mm00434162_m1; IL-1β: Mm01336189_m1; TNF-α: Mm00443258_m1; IL-22: Mm00444241_m1; IL-6: Mm00446190_m1.

1. Becker C, Fantini MC, Neurath MF (2006) High resolution colonoscopy in live mice. *Nat Protoc* 1:2900–2904.
2. O'Connor W, Jr., et al. (2009) A protective function for interleukin 17A in T cell-mediated intestinal inflammation. *Nat Immunol* 10:603–609.

Fig. S1. Cytokine quantification in WT and Casp1$^{-/-}$ mice in the acute and chronic phases of colitis. TNF-α, IFN-γ, IL-1β, IL-18, IL-6, IL-22, IL-11, and IL-17A mRNA expression in WT and Casp1$^{-/-}$ colons was measured at day 15 of the CAC regimen (*A*) or 10 d following the cessation of the last cycle of dextran sodium sulfate (DSS) treatment (*B*). For protein measurement, 1-cm halved colon explants were incubated for 24 h and ELISA was performed on the supernatant during acute and chronic phases of colitis (*C* and *D*, respectively).

Fig. S2. Bone-marrow chimera experiments. WT or Casp1$^{-/-}$ bone marrow was transferred to WT or Casp1$^{-/-}$ mice following sublethal irradiation (two doses of 550 rad). Azoxymethane (AOM)-DSS CAC regimen was induced 8 wk later. Tumor load and tumor numbers/mouse were determined by colonoscopy on day 65 of the CAC regimen.

Fig. S3. Enhanced colon epithelial cell proliferation during the acute inflammatory phase and tumor cell proliferation in Casp1$^{-/-}$ mice. BrdU^{+} cells were quantitated from 30 crypts of each mouse at baseline (*A*) and day 15 of the CAC regimen (*B*, *Left*). BrdU^{+} cells were quantitated from ≥ 25 crypts of each mouse at day 15 of the CAC regimen (*B*, *Right*). The percentage of Ki67^{+} and BrdU^{+} cells (*C*) in tumor tissues were counted from six sections of each slide and divided by the total cell number of each field at the magnification 10 × 100. *P* values < 0.05 were considered statistically significant.

Fig. S4. NLRC4$^{-/-}$ mice develop aggressive locally invasive tumors. Representative histopathologic sections of colon adenocarcinomas and foci of tumor invasion from WT and NLRC4$^{-/-}$ mice. Invasive tumor foci (arrows) were not found in WT mice, but they were present in 66% of NLRC4$^{-/-}$ mice, where tumor foci were surrounded by abundant amounts of pale blue mucin. H&E staining; *muscularis mucosae; m, mascularis externa. (Scale bars, 200 μm.)

Fig. S5. Enhanced colon epithelial cell proliferation during the acute inflammatory phase and tumor cell proliferation in NLRC4$^{-/-}$ mice. BrdU^{+} cells were quantified from ≥ 25 crypts of each mouse at steady-state conditions (*A*); Ki67^{+} and BrdU^{+} cells were quantitated from ≥ 25 crypts of each mouse at day 15 of the CAC regimen (*B*). TUNEL-positive cells in tumor tissues were counted from greater than or equal to six fields of each slide at the magnification 10×40 (*C*). *P* values < 0.05 were considered statistically significant.