ONLINE METHODS

Bacteriology. We grew ETBF strain 86-5443-2-2 (secretes BFT-2); and NTBF strain 9343 (American Type Culture Collection) overnight anaerobically at 37 °C.

Mice. Min$^{Apc716+/-}$ mice (expressing a mutant gene encoding an adenomatous polyposis coli protein truncated at amino acid 716), C57BL/6 mice (either wild-type littermates of Min$^{Apc716+/-}$ mice or from Jackson Laboratories) and conditional CD4 Stat3–KO mice (CD4-Cre × *Stat3*$^{flox/flox}$ mice) on a C57BL/6 background generated as described[22] were specific pathogen free. To enhance *B. fragilis* colonization, we administered clindamycin (0.1 g l^{-1}) and streptomycin (5 g l^{-1}) for 3–5 d before peroral bacterial inoculations (~1×10^8 bacteria in PBS) or PBS alone (sham control) at 4 weeks of age. We quantified fecal bacterial colonization as colony-forming units per g stool. To define visible colon adenomas, we stained 10% formalin–fixed colons with methylene blue, quantified the adenomas with a Leica ES2 dissecting scope (by S.W. and C.L.S.) and sized them with a Nikon SMZ2 1500 microscope with NIS-Elements AR2.30 software. For histopathology, we Swiss-rolled, paraffin-embedded, sectioned (5 μm) and stained colons with H&E. All mouse protocols were approved by the Johns Hopkins University Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International.

Histopathology. We scored inflammation on a 0–4 scale (0, normal mucosa; 1, minimal inflammation (occasional scattered granulocytes and leukocytes); 2, mild inflammation (scattered granulocytes with occasional mild infiltrates); 3, moderate inflammation (scattered granulocytes with patchy moderate infiltrates); and 4, severe inflammation (multiple extensive areas with abundant granulocytes and marked infiltrates)). We scored colonic proliferation on a 0–3 scale (0, normal mucosa; 1, mild proliferation (patchy distribution of mildly deepened crypts and slightly thicker mucosa); 2, moderate proliferation (regionally diffuse epithelial crowding, deep crypts and thickened mucosa); and 3, severe proliferation (extensive diffuse distribution of marked epithelial crowding, thickened mucosa and markedly elongated, branched crypts)). We quantified GIN on one 5-μm section of Swiss-rolled colon[46].

Flow cytometric analyses. We processed colons (3–5 mice per group) to obtain mucosal intraepithelial and lamina propria lymphocytes as previously described[22]. We stimulated mononuclear cells collected by Percoll gradient separation with phorbol 12-myristate 13-acetate (PMA) (30 nM), ionomycin (1 μM) and Golgiplug (BD Biosciences) and then stained them for cell surface markers and intracellular cytokines. We used FACSCalibur (BD Biosciences) for flow cytometry and analyzed data with FlowJo software (Tree Star Inc.). We also used flow cytometry to analyze mechanically dissociated splenic lymphocytes isolated by density gradient and stimulated with PMA, ionomycin and Golgiplug. We used antibodies to the following proteins: IFN-γ (clone XMG1.2), IL-17A (clone eBio17B7), CD4 (clone RM4.5), CD8a (clone 53-6.7), F4/80 (clone BM8), CD11c (clone N418), TCRβ (clone H57-597), TCRγ (clone eBioGL3) (eBiosciences) and IL-4 (clone 11B11), CD3e (clone 145-2C11), CD11b (clone M1/70), NK1.1 (clone PK136), CD16/CD32 (clone CD16/CD32) (BD Biosciences).

Depletion of T lymphocytes. We depleted $CD3^+CD4^+$ and $CD4^-CD8^-TCR\alpha\beta^-\gamma\delta^+$ T lymphocytes using the GK1.5 antibody ascites (75 μl per dose) and the TCRβ-$\gamma\delta^+$ depleting antibody (Clone UC7-13D5; 500 μg per dose), respectively, with rat or hamster IgG isotype antibody (0.5–1 g per dose; Sigma) as a control given intraperitoneally the day before, 1–2 d after then weekly after bacterial inoculations. We verified spleen and mucosal $CD4^+$ or TCRαβ-$\gamma\delta^+$ depletion by flow cytometry at 1 week and/or 4 weeks of age.

Cytokine blockade protocols. We administered monoclonal IL-17A–blocking antibody (clone 50104) and monoclonal IL-23R–blocking antibody (clone 258010) or isotype control antibodies (rat IgG2b, clone 141925; and IgG2a, clone 54447) (R&D Systems) intraperitoneally (500 μg) as described above. We administered monoclonal IFN-γ–blocking antibody (clone XMG1.2, eBioscience) or rat IgG control antibody similarly.

Real-time PCR. We extracted total RNA using the RNAeasy Kit (Invitrogen) and synthesized cDNA. All primers were from Applied Biosystems. We calculated relative gene expression by the $\Delta\Delta C_T$ method.

Detection of phosphorylated Stat proteins. We processed flash-frozen colonic tissue using phosphatase and protease inhibitors (Roche) to obtain nuclear protein extracts. We performed western blotting with antibodies specific for pStat1, pStat3, pStat5 (Cell Signaling), pStat4 (Zymed), pStat2 and pStat6 (Abcam) and detected pStat bands with horseradish peroxidase–conjugated goat secondary antibody to rabbit IgG (Jackson Immune Research) with Supersignal West Pico Chemiluminescent Substrate (Pierce). We verified antibody specificity with cytokine-stimulated cell lines expressing individual pStat proteins. We performed pStat3 immunohistochemistry by antigen retrieval (boiling 0.01 M citrate buffer and 0.025% trypsin) on deparaffinized tissues treated with hydrogen peroxide (0.3%) and 2% goat serum. We detected pStat3 staining with biotinylated goat secondary antibody to rabbit IgG (Southern Biotech), Avidin Biotin Complex (Vector Laboratories) and 3.3′-diaminobenzidine developer, counterstained with hematoxylin.

Statistical analyses. In general, we present data as box-and-whisker plots, where the line represents the median; the box, the interquartile range; the whiskers, the tenth and ninetieth percentiles; and the dots, individual data points beyond the tenth and ninetieth percentiles. To compare nonparametric distributions across experimental conditions, we used the Mann-Whitney *U* test. For analysis of graded associations between histology scores and tumor numbers (**Fig. 1d**), we used a parametric approach: the boxes represent means, the bars represent s.e.m.; the line was derived from a linear regression analysis; and *r* represents a Pearson correlation coefficient. We considered P values ≤ 0.05 to indicate statistical significance.

46. Boivin, G.P. *et al.* Pathology of mouse models of intestinal cancer: consensus report and recommendations. *Gastroenterology* **124**, 762–777 (2003).

doi:10.1038/nm.2015