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Supplementary Materials for

Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria

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Materials and Methods

Patient selection and Sample acquisition

Polyps and paired normal tissues were collected from patients with a clinical FAP phenotype undergoing colon surgery at Johns Hopkins Hospital. A subset of patients underwent genetic counseling and mutational analysis confirming a mutation in the *APC* gene (table S1). Control subjects (colonoscopy and surgical) included individuals without a history of CRC, inflammatory bowel disease, or antibiotic usage within three months (table S2, fig 1C). Grossly normal colon mucosal biopsies (colonoscopy, samples S55-S71, table S2) and colon tissue from surgical resections (3714, 3723, 3724, 3730, 3734, 3737, table S2) not needed for pathologic diagnosis were collected. Normal colon tissue from an additional subject 3760, a patient with CRC, was used as a *bft*-negative control in Fig 1D. Bowel preparations prior to surgery or colonoscopy were determined by the attending surgeon or gastroenterologist. Two bowel preparations were typically used (mechanical bowel preparation [Miralax^{TM}], or Fleet Phospho-soda^{TM} enema). All colonoscopy patients received a bowel preparation whereas some surgical patients, both FAP and non-FAP patients, received no bowel preparation prior to surgery. Previous data suggested no relationship between bowel preparation and biofilm detection (*6*). Pre-operative intravenous antibiotics were administered in all surgical cases (cefotetan or clindamycin/gentamycin) immediately preceding surgery. One patient received oral antibiotics on the night prior to surgery as noted in the patient metadata (table S1). Biopsies and tissues were rapidly preserved in Carnoy's solution, RNAlater, anaerobic transport media or snap frozen for subsequent analysis. This study was approved by the Johns Hopkins Institutional Review Board. All samples were obtained in accordance with the Health Insurance Portability and Accountability Act (HIPAA).

Fluorescent in situ hybridization

Carnoy's fixed, paraffin-embedded tissues were sectioned to 5 μm thickness and de-waxed following standard procedures. Sections were stained with Periodic acid Schiff (PAS) to confirm mucus presence and preservation and successive sections were hybridized with the Eub338 universal bacterial probe (table S4). Slides were imaged using a Nikon E800 microscope with NIS elements software. Samples that were determined to have a bacterial presence by universal probe were next analyzed by the more specific probe set listed in table S4 (Life Technologies). Probes were applied to slides at a concentration of 2 pmol/ul in prewarmed hybridization buffer (900 mM NaCl, 20 mM Tris pH 7.5, 0.01% SDS, 20% formamide). Slides were incubated at 46°C in a humid chamber for 2 hours, and washed at 48°C for 15 minutes in wash buffer (215 mM NaCl, 20 mM Tris pH 7.5, 5 mM EDTA). Slides were mounted using ProLongGold antifade reagent (Life Technologies).

Biofilm quantification

As previously published (*6*), bacterial biofilms were defined as invasive bacterial aggregates within the colon mucus with a bacterial density $>10^9$ bacteria/mL. Bacterial biofilms were quantified for longitudinal distance along the epithelium and density using slides hybridized with the universal bacterial probe (table S4). When possible, up to five biofilm measurements were taken across the length of the histologic section (some samples did not have five patches of biofilm, in these cases all present biofilms were measured). Relative biofilm species quantification was performed using tissues hybridized with the universal bacterial probe along with group and *B. fragilis* and *E. coli* species specific probes (tables S3, S4). Because tissue sections were similar qualitatively within an individual patient, one tissue section per patient was selected for *E. coli* and *B. fragilis* quantification. For quantification, images were taken at 100x magnification and individual bacterial cells (all bacteria, *E. coli*, and *B. fragilis*) in a 10x10 μm

space were counted. Five 10x10 μm boxes on a single tissue section were counted per patient to determine the relative biofilm composition as a percentage.

Microbial Culture

Detection of *pks+E. coli* and ETBF by culture

Tissue stored at -80°C was utilized for microbial culture and selective amplification and identification of *E. coli* and *B. fragilis* isolates. Two to four mucosal samples per patient were available for microbiology culture analysis (metadata tables S1 and S2). An approximately 3mm diameter punch of mucosal samples from surgically-resected control tissue, FAP polyp, FAP paired normal, or colonoscopy biopsy was placed in tryptic soy broth (TSB) or peptone yeast glucose bile broth (PYGB) and grown in aerobic or anaerobic conditions, respectively, at 37°C for up to 48 hours. Microbial growth was pelleted and an aliquot preserved at -80°C for PCR detection of *clbB* and *E. coli* (TSB culture) and *bft* and *B. fragilis* (PYGB culture). The remaining pellet was diluted and plated on semi-selective agar (aerobic TSB culture plated on MacConkey agar and anaerobic PYGB culture plated on BBE agar) for single colony identification. A total of fifty Lac+ or bile-esculin+ colonies was selected for PCR from each sample. PCR detection was performed using *clbB* primers (163 bp product) or *bft* primers (281 bp product) (table S6).

Bacterial Strains and Mouse Experiments

Two mouse colon tumorigenesis models were utilized: $Apc^{+/\Delta 716}$ heterozygous multiple intestinal neoplasia (Min) mice (*9*) and specific pathogen free (SPF) C57BL/6J mice treated with AOM (10 mg/kg weekly for 6 weeks). In shorter experiments (<6 weeks) using the AOM model, AOM was given weekly until the time of mouse harvest. Prior to inoculation of bacterial strains

in either mouse model, 6-week-old mice were given water containing 500 mg/L cefoxitin for 48 hours. Cefoxitin treatment results in an absence of detectable bacteria by culture or 16S rRNA qPCR by 24 hours (*15*). After removal of antibiotic water for 24 hrs, mice were inoculated by oral gavage with 10^8 colony-forming units (cfu) ETBF (piglet 86-5443-2-2), 10^8 cfu *pks+ E. coli* (murine NC101, provided by Dr. Christian Jobin, University of Florida, expressing a fluorescent ampicillin resistance plasmid sfGFP-pBAD) or a mixture containing 10^8 cfu of each strain. *Akkermansia muciniphila* was obtained from ATCC (ATCC® BAA-835™). ETBF was grown as previously described (*9*); *pks+ E. coli* was grown overnight in LB broth in a shaking incubator; and *A. muciniphila* was grown anaerobically overnight in brain heart infusion broth. Colonization was confirmed and quantified by collection and cultivation of stool on selective media for NC101::sfGFP and ETBF (MacConkey plates with ampicillin or BHI plates with 10 μg/ml clindamycin and 200 μg/ml gentamicin, respectively) (fig S4). For fig S10, colonization by NC101::sfGFP and *A. muciniphila* was assessed using stool DNA extracted from endpoint stools using Zymo Quick-DNATM Fecal/Soil Microbe kit (Zymo Research), qPCR using 16S rRNA gene primer sets (table S6) to detect *pks+ E. coli* and *A. muciniphila* quantitated as copy numbers per 100 ng stool DNA.

Mucosal colonization was confirmed and quantified on approximately 200 μg of tissue collected from the terminal 2 cm of the distal mouse colon. Tissue samples were washed by placing the tissue in 500 μl of 0.016% DTT saline followed by 30 sec vortex (speed 7-8), pelleting the tissue and discarding the DTT saline. After two washes, the tissue was homogenized and plated on selective media as described previously *(13).* Additional experiments were conducted with strains ETBF::*Δbft* (*16*) and NC101::*Δpks* (provided by Dr. Christian Jobin) (*10*). Mice were sacrificed at specified time-points, unless otherwise noted for poor health and/or excessive

weight loss (defined as ≥20% total body weight). Germ-free C57BL/6 mice were similarly inoculated with ETBF or *pks+ E. coli* (mono-colonization) or a mixture of ETBF/*pks+ E. coli* (co-colonization) and harvested at ~48-60 hours after inoculation. Preliminary experiments determined that mono-colonized or co-colonized germ-free mice expired by ~72 hrs after bacterial inoculation.

Lamina propria lymphocyte isolation

Mouse colon was removed, flushed, and tissue was minced before enzymatic digestion with 400 U/ml liberase and 0.1 mg/ml DNAse1 (Roche Diagnostics). Lymphocytes were isolated by percoll gradient separation (GE Healthcare life Science).

Flow cytometry

For surface marker staining, 1-2x10^6 cells were stained in 1mL of 1X PBS with the LIVE/DEAD Fixable Aqua viability kit according to manufacturer's instructions (ThermoFisher Scientific), washed in an additional 2mL of 1X PBS by centrifugation at 1500 rpm for 3 min, and then resuspended in 100 μL of 1X PBS containing 2% fetal calf serum and 1 μg of Mouse Fc Block™ (BD Biosciences) for 10 minutes prior to the addition of the following fluorochrome-conjugated anti-mouse antibodies at the manufacturers' recommended concentrations for 30 min on ice: CD11b-PerCP/Cy5.5 (Clone M1/70, Biologend), CD11c-APC (Clone N418, Miltenyi Biotec), MHC II (I-A/I-E)-APC/Cy7 (Clone M5/114.15.2, Biolegend), Ly6C-BV421 (Clone HK1.4, Biolegend), CD64-PE/Cy7 (Clone X54-5/7.1, Biolegend), F4/80-BV650 (Clone BM8, Biolegend), Ly6G-AF700 (Clone 1A8, Biolegend) or CD3-PerCP/Cy5.5 (Clone 145-2C11, eBioscience), CD4-BV605 (Clone RM4-5, Biolegend), CD8-PE (Clone 53-6.7, Biolegend), γδTCR-APC (Clone eBioGL3, eBioscience) and CD19-BV421 (Clone 6D5, Biolegend),. Stained cells were washed in 2 mL of 1X PBS containing 2% fetal calf serum by centrifugation at 1500

rpm for 3 min prior to resuspension in buffer and acquisition on a flow cytometer. Flow cytometry analysis of IL-17-producing cells (Fig 3E) was performed using intracellular cytokine staining (ICS), following 3.5 hour *in vitro* stimulation of LP leukocytes (LPL) in presence of eBioscience stimulation plus protein transport inhibitor cocktail (PMA/Ionomycin/BrefeldinA/Monensin; Thermofisher) as previously described *(9).* A BD LSR II instrument equipped with FACSDiva software (BD Biosciences) was used for data acquisition and FlowJo software (Tree Star Inc.) was utilized for analysis. Instrument compensation was performed prior to data acquisition using anti-rat/hamster or anti-mouse BDCompBeads^{TM} (BD Biosciences) according to the manufacturer's recommendations. Positive staining of live cells was determined against fluorescence-minus-one (FMO) controls after first gating away from debris, doublets, and dead cells. Conventional dendritic cells were defined as CD11c^{Hi}/MHC II^{Hi}, macrophages as CD64^{+}/F4/80^{+}, inflammatory monocytes as CD11b^{Hi}/Ly6C^{Hi}, neutrophils as CD11b^{Hi}/Ly6G^{Hi}, CD4 T cells as CD3^{+}/CD4^{+}, CD8 T cells as CD3^{+}/CD8^{+}, γδ T cells as CD3^{+}/ γδTCR^{+}, and B cells as CD3^{-}/CD19^{+} cells. The gating strategies are detailed in fig S11.

Quantitative real-time PCR (IL-17a)

An approximately 200 mg segment of distal mouse colon was processed for RNA isolation immediately following colon removal. Tissue was homogenized by bead beating in buffer ALS (Qiagen) and then run through a tissuelyzer column (Qiagen). The resultant solution was utilized for RNA extraction with RNeasy kit according to the manufacturer's recommended procedures. Transcription to complementary DNA was carried out using superscript III (Invitrogen). All qPCRs were performed in triplicate with TaqMan primer/probe for IL-17a and18s (as reference gene)(Applied Biosystems), and TaqMan 2x mastermix (Applied Biosystems). The level of target mRNA was determined by the delta delta CT method.

ELISA for Fecal IgA

Nunc maxisorp plates were coated with 50 μg/μl Collagen I, Rat tail (Gibco) in 0.2 acetic acid overnight on a shaker at 4°C. Plates were subsequently washed twice with PBS before adding 10^9 bacteria/ml in PBS (bacterial cultures were grown overnight and rinsed three times in PBS). Plates were incubated overnight at 37°C in aerobic or anaerobic conditions (for NC101 and ETBF respectively). After incubation, plates were rinsed three times with PBS and fixed in 4% paraformaldehyde. Fixative was removed and plates were rinsed three times with PBS before blocking with 5% milk in PBS 0.05% tween 20 (PBST) for 1 hour at room temperature on a shaker. Fecal supernatants were prepared from 4 week stool samples resuspended at a concentration of 1 g/ml and centrifuged at 50 g for 10 minutes. Fecal supernatant was applied at 1:100 dilution in 2% milk PBST for 2 hours at room temperature on a shaker. Plates were rinsed three times in PBS followed by application of HRP-Goat Anti-mouse IgA (Life Technologies) at 1:1000 in 2% milk PBST for 1 hour at room temperature. Plates were rinsed three times in PBS and developed with TMB substrate (KPL).

Cell culture and mucus digestion assay

HT29-MTX-E12 cells were grown on transwell inserts (12mm diameter, pore size 0.4 μm-Costar) in Dulbecco's modified Eagles Medium (DMEM) supplemented with 10% FCS at a density of $7.5x10^4$ cells per insert for 21 days. After confluence, the cells were treated with 10 μm DAPT (Sigma Aldrich) in the basolateral compartment for 6 days to stimulate mucus production, the apical compartment contained DMEM only. The media were refreshed every 2-3 days. After 21 days, tight junction formation was confirmed with trans-epithelial resistance measurements (TEER).

For mucus assays ETBF was grown in BHI (supplemented with hemin, vitamin K and L-cysteine) in an anaerobic jar for 24-48 hours until an OD of ~1.0 *(9). Akkermansia mucinophila* was grown in BHI with N2 overlayed gas while shaking at 37°C for 48 hours until ~OD 1.0. *Escherichia coli* NC101-GFP was grown in BHI overnight while shaking at 37°C until ~OD 1.0. Next, bacteria were pelleted and resuspended at OD 0.2 in DMEM supplemented with 1% FCS and 10% BHI. Sterile DMEM with 1% FCS was added to the basolateral compartment and resuspended bacteria or medium control (DMEM with 1% FCS and 10% BHI) was added to the apical compartment at time 0h. At 24 hours, cells were washed in warm PBS twice, fixed in Carnoy's solution for 10 minutes, dehydrated in 100% ethanol and cleared with xylene for 10 minutes. Next, liquid paraffin (58°C) was added to the bottom and top of the transwell insert. Paraffinized membranes were embedded in the right orientation for sectioning at 5 μm. Sections were stained with alcian blue for mucus visualization.

Mucus depth was quantified in each condition in 5 random fields (40x magnification) of 100 μm length by measuring mucus depth every 10 μm on a VisionTek® (Sakura). Median mucus depth for each condition was calculated.

Immunohistochemistry

Formalin-fixed (10%), paraffin-embedded tissues were sectioned (5 μm) and stained. Slides were de-paraffinized and rehydrated following standard procedures. Slides were steamed in citrate buffer pH 6.0 for 45 minutes for antigen retrieval, and allowed to cool to room temperature, followed by blocking of endogenous peroxidase activity for 10 minutes with 3% hydrogen peroxide. Slides were blocked for 30 minutes in 10% normal goat serum, followed by primary antibody application overnight (1/500 rabbit anti-γH2AX [Bethyl Laboratories, IHC00008]). Slides were incubated with HRP-conjugated anti-rabbit IgG (Leica Biosystems,

PV6119) for 30 minutes followed by DAB chromogen development for 10 minutes. All sections were counterstained with hematoxylin prior to mounting.

Immunohistochemistry (IHC) quantification

Because no regional differences (proximal, mid, distal colon) in γH2AX staining were identified, six crypts were selected for γH2AX quantification from each mouse; 2 each from the proximal, middle, and distal mouse colon. Only crypts displaying proper colon surface to crypt cross-sectional orientation, permitting counting of all cells and those cells with γH2AX staining, were selected for quantification. Positive cells (containing 3 or more nuclear foci) were counted along with total number of cells in the crypt and a resulting percentage was determined. H2AX IHC staining shown for distal colon (fig 4D, fig S9). Quantified results display all crypts examined from 4-5 mice per condition at day four after AOM administration and bacterial inoculation (fig 4D, fig S9). Cells were counted blinded by PF and CMD.

Statistical Analysis

Data were analyzed using the nonparametric unpaired, two-tailed Mann-Whitney *U* test for two group comparisons, nonparametric Kruskal-Wallis test for multiple group comparisons, log rank test or X^2 tests as labeled for each figure. For multiple group comparison achieving statistical significance with Kruskal-Wallis testing, we then performed subsequent two group comparisons of biological interest using Mann-Whitney U testing. For assessment across the 6 cell groups displayed in Fig 3E (see also fig S11C), we used log-transformed data and two-way ANOVA to take into account cell types when comparing treatment groups. Data are presented as mean +/- s.e.m or as box-and-whisker plots where the line represents the median, the box the interquartile range and the whiskers, the tenth and ninetieth percentiles. *P*-values <0.05 were considered statistically significant.

Fig S1

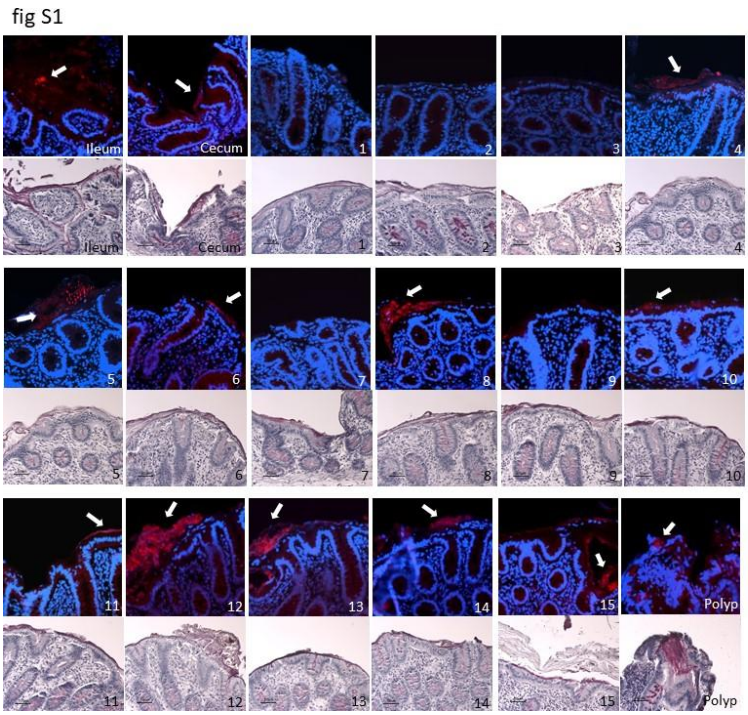


Fig S1. Fluorescent *in situ* hybridization (FISH) of all bacteria (red) on colon specimens collected from an individual with FAP (3775). Tissue specimens were collected approximately every 3-5 centimeters starting in the right colon (sample 1) and ending in the rectum (sample 15). Patchy biofilms (delineated by white arrows) detected throughout the colon on both polyp and grossly normal tissues. PAS-stained histopathology images for each FISH image are displayed. Images are representative of at least ten 5 μm sections examined per tissue specimen shown and were obtained at 40X magnification. Scale bar is 50 μm.

Fig S2

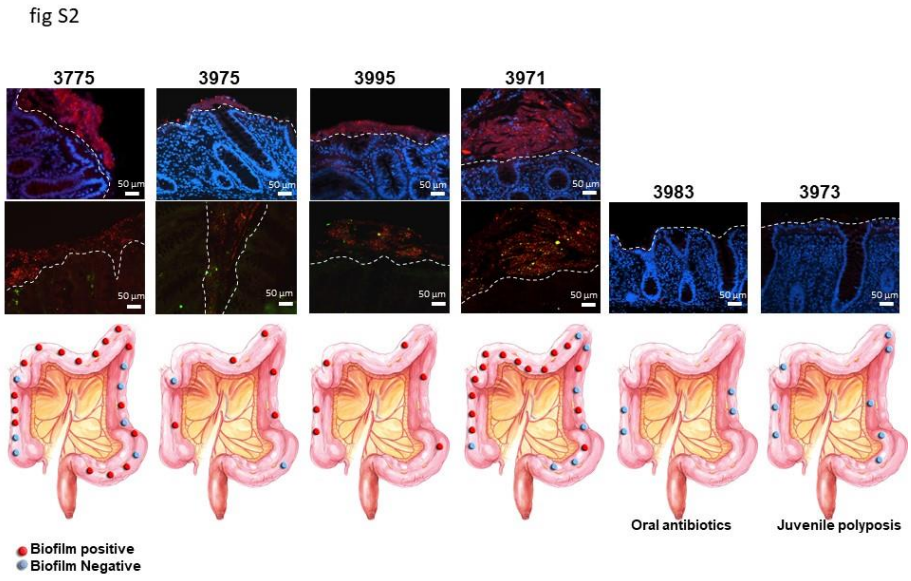


Fig S2. Biofilm characterization of FAP colons. Specimens from five prospectively collected FAP colons and one juvenile polyposis (JP) colon were available for FISH analysis. Colon tissue from four patients (3775, 3975, 3995 and 3971) contained a biofilm, while the JP and antibiotic-treated patient colon tissues had no biofilms. FISH of all bacteria (red) top panels, displaying representative biofilms from each patient and species specific FISH probes (below) of *E. coli* (red) and *B. fragilis* (green) biofilm composition from each patient. Bottom panel displays colon specimen sites with biofilm designations (red=biofilm, blue=no biofilm). Images are representative of n=4-23 tissue samples per patient screened (at least ten 5 μm sections screened per patient). Images were obtained at 40X magnification. Scale bar is 50 μm.

Fig S3

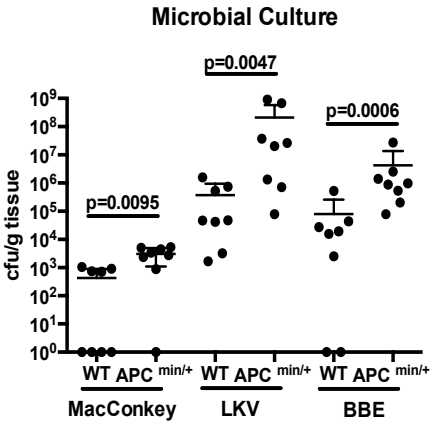


Fig S3. Microbiology culture analysis of distal colon mucosal tissues from 6-week old wild-type C57Bl/6 (n=8) and *Apc*$^{Min\Delta716/+}$ (n=8) mouse littermates (2 litters). *Apc*$^{Min\Delta716/+}$ mice displayed significantly more cultivatable *Enterobacteriaceae* (MacConkey), Gram-negative obligate anaerobes (Brucella laked blood agar with kanamycin and vancomycin, LKV) and *B. fragilis* group (BBE) than their WT littermates. Data displayed as colony-forming units per gram of tissue cultured (8 mice per group, mean +/-SEM) and significance was calculated using the Mann-Whitney U test.

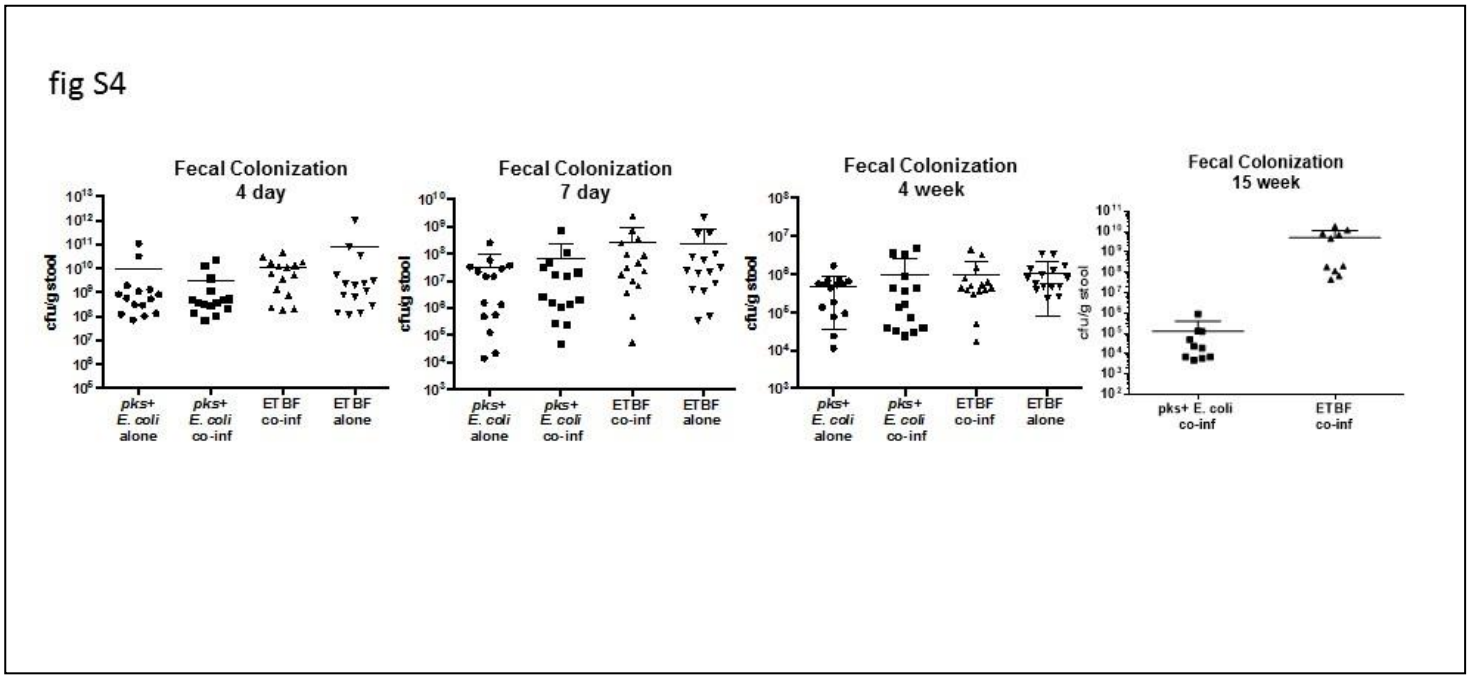


Fig S4. Fecal colonization is present among all colonization conditions over time in the AOM mouse model. Fecal colonization is plotted as mean +/- SEM of colony-forming units (cfu) per gram of stool collected from mono-colonized (with *pks+ E. coli* or ETBF) or co-colonized (with *pks+ E. coli*/ETBF) mice at 4 days, 7 days and 4 weeks after bacterial inoculation. At 15 weeks culture data shown for co-colonization condition only. At 15 weeks, colonization for mono-colonized mice (*pks+ E. coli* or ETBF) was determined by qPCR yielding 7.6-8.7 X 10^6 cfu/gm for *pks+ E. coli* or 7.8-9.1 X 10^6 cfu/gm for ETBF. Data represent 3 independent experiments.

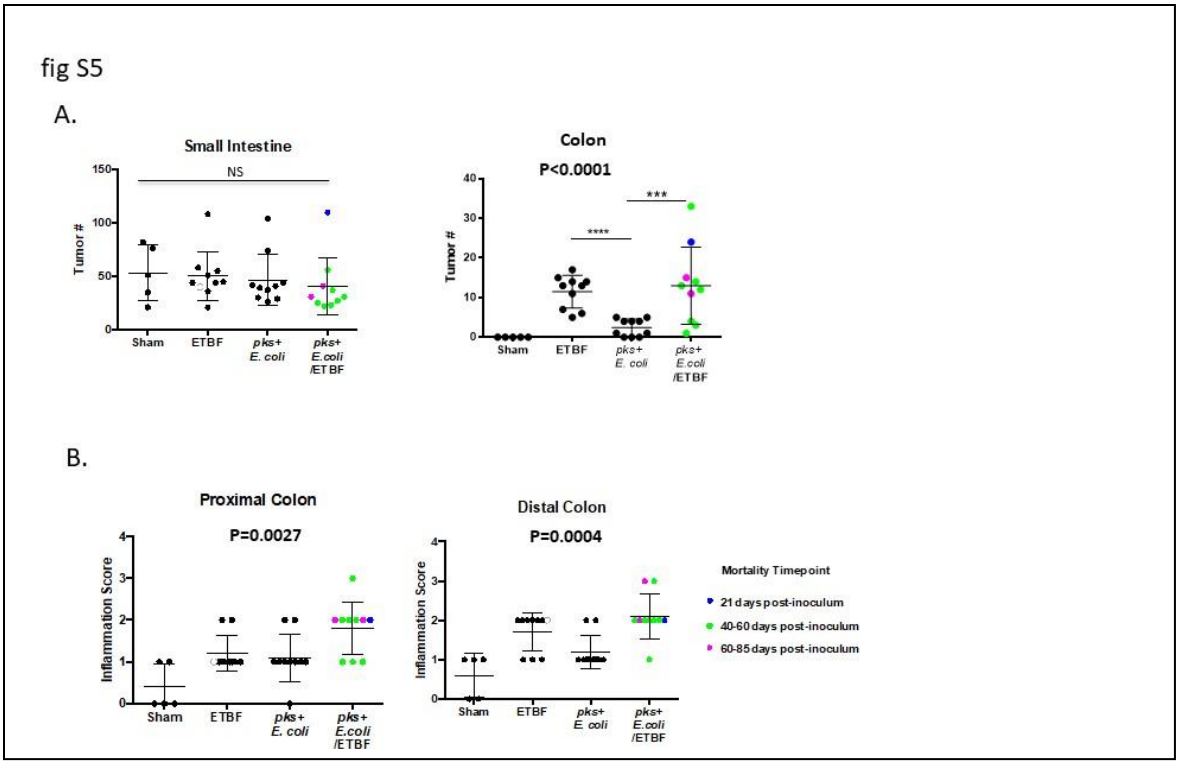


Fig S5. Gross tumor counts (**A**) and inflammation colon scores (**B**) of 12 week $Apc^{Min_{\Delta 716/+}}$ sham (n=5), and mice mono-colonized with *pks+ E. coli* (n=10), ETBF (n=10) or co-colonized with *pks+ E. coli*/ETBF (n=10; mortality time points noted; only 2 co-colonized mice survived to 12 weeks). No significant difference was detected between mice mono-colonized with ETBF and co-colonized with *pks+ E. coli*/ETBF in gross small intestinal tumors (NS, non-significant). Data

represent mean +/- SEM of 3 independent experiments. Overall significance was calculated using the Kruskal-Wallis test and the overall p values are shown; Mann-Whitney U was used for two group comparisons, p values: ***, p<0.006; ****, p<0.0001.

fig S6

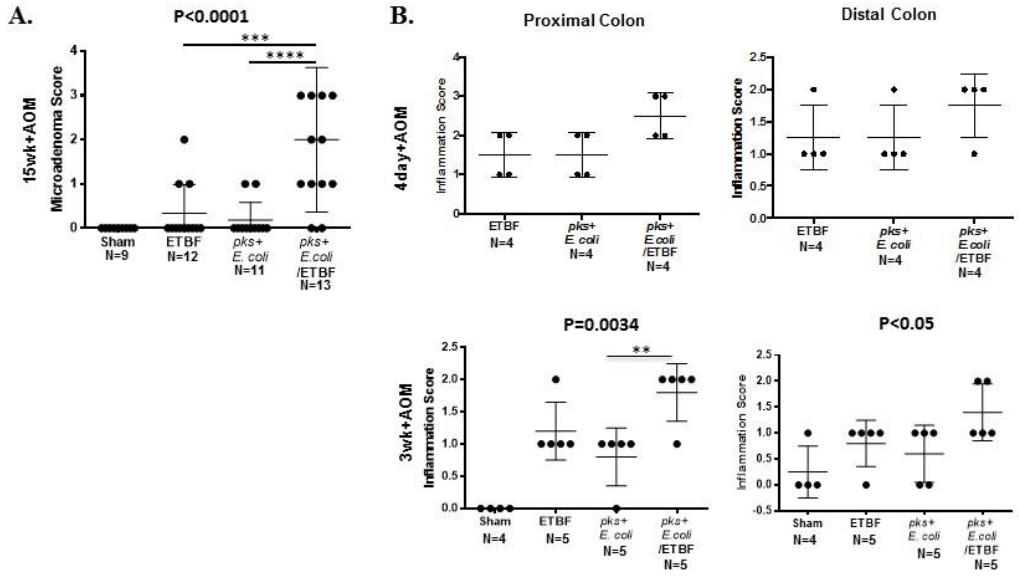


Fig. S6 Histopathology microadenoma (**A**) and inflammation (**B**) scores of AOM mice mono-colonized with *pks+ E. coli* or ETBF and co-colonized with *pks+ E. coli*/ETBF. At 15 weeks post-inoculation, microadenomas (**A**) were significantly increased under co-colonization conditions. For **(B)**, no significant difference was noted between colonization groups at 4-days (top panel) in either the proximal (left panel) or distal (right panel) colons. At 3-weeks (bottom panel) post inoculation, inflammation was significantly increased in both proximal colon (left panel) and distal colon (right panel). For A, data represent mean +/- SEM of 3 independent experiments (total 9-13 mice per group). For B (top), data represent mean +/- SEM of one

independent experiment (total 4 mice per group). For B (bottom), data represent mean +/- SEM of 2 independent experiments (total 4-5 mice per group). For A and B, overall significance was calculated using the Kruskal-Wallis test and the overall p value is shown; Mann-Whitney U was used for two group comparisons, p values: **, p=0.04; ***p<0.002; ****p=0.0006.

Fig S7

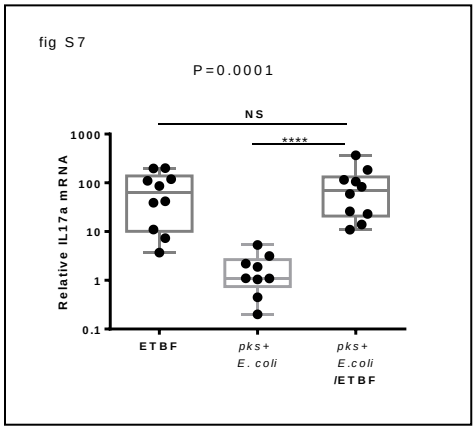


Fig S7**.** Distal colon normal (non-tumor) mucosal IL-17 mRNA expression relative to sham in ETBF and *pks+ E. coli* mono-colonized and co-colonized AOM mice at 15 weeks. Data represent box-and-whisker plot (line, median; box, interquartile range; whiskers, 10^{th} and 90^{th} percentiles) of 3 independent experiments (total 9-10 mice per group). Overall significance was calculated using the Kruskal-Wallis test and the overall p value is shown; Mann-Whitney U was used for two group comparisons, p value: ****, P<0.0001. NS=non-significant.

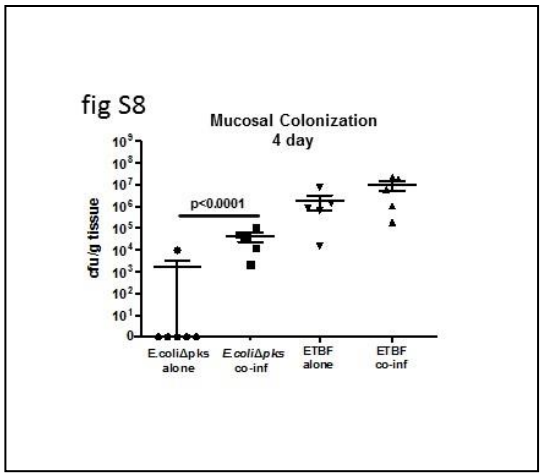


Fig S8. Mucosal colonization is enhanced for *E. coli*Δ*pks* under co-colonization conditions in AOM mice. Mucosal colonization is displayed as colony-forming units (cfu) per gram of tissue. Data represent mean +/- SEM of one independent experiment (total 5-6 mice per group). Significance was calculated using the Mann-Whitney U test.

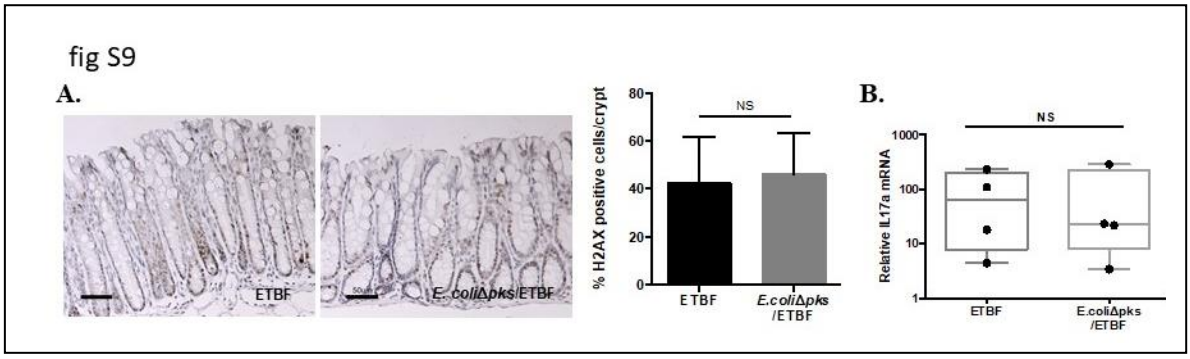


Fig S9. Colonic epithelial cell DNA damage is not enhanced in co-colonized *E. coli*Δ*pks*/ETBF mice at day 4 after bacterial inoculation and administration of AOM. **A.** H2AX immunohistochemical staining of distal colon (left) and quantification (right) in mice mono-colonized with ETBF and co-colonized with *E. coli*Δ*pks*/ETBF. Data (right) represent mean +/- SEM. **B.** Mucosal IL17A is similar in monocolonized ETBF and co-colonized *E. coli*Δ*pks*/ETBF

mice. Data represent box-and-whisker plot (line, median; box, interquartile range; whiskers, 10th and 90th percentiles). Results represent one independent experiment (total 4 mice per group). Significance was calculated using the Mann-Whitney U test.

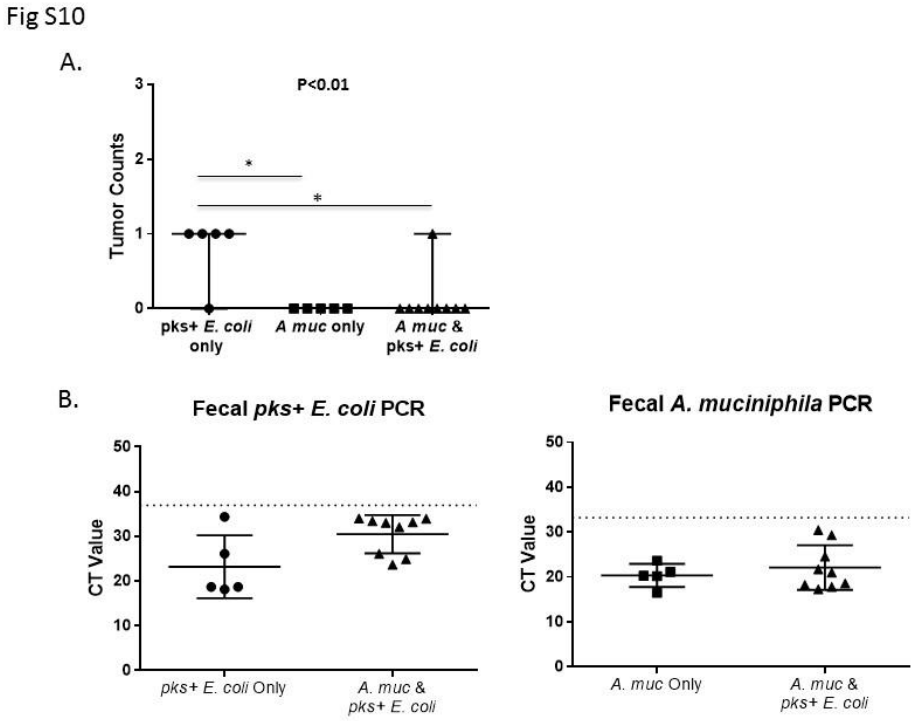
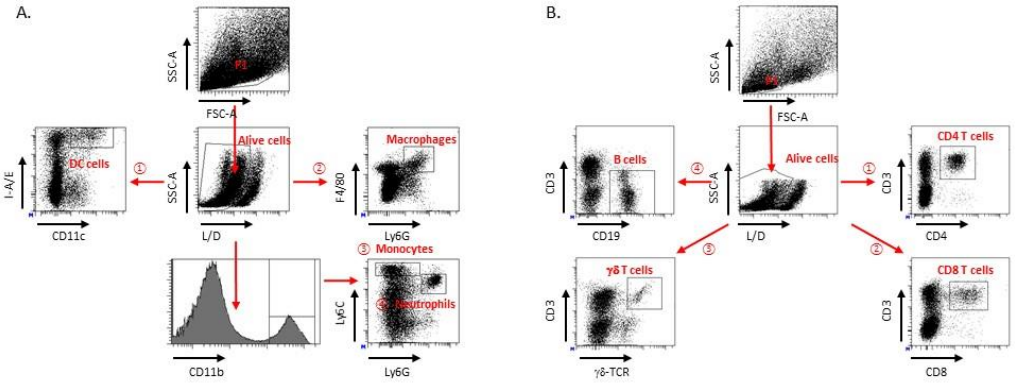


Fig S10. AOM-treated mice co-colonized with *pks+ E. coli* and *A. muciniphila* (*A muc*) for 15 weeks do not display increased colon tumorigenesis. **A**. Colon tumor counts in mice mono-colonized with *pks+ E. coli* or *A muciniphila* or co-colonized with *A. muciniphila*/*pks+ E. coli*. Overall significance was calculated using the Kruskal-Wallis test and the overall p value is shown; Mann-Whitney U was used for two group comparisons, p value: *, $p<0.05$.

B. Detection of *pks+ E. coli* or *A. muciniphila* by fecal qPCR. Dotted line reflects limit of fecal detection (10^2 gene copies for *pks+ E. coli* and 10^2 gene copies for *A. muciniphila*). Data represent mean +/- SEM of 2 independent experiments (total 5-9 mice per group).

Fig S11


A.
SSC-A
FSC-A
P1
Alive cells
DC cells
I-A/E
CD11c
SSC-A
L/D
F4/80
Ly6G
Macrophages
③ Monocytes
② Neutrophils
CD11b
Ly6C
Ly6G
B.
SSC-A
FSC-A
P1
Alive cells
B cells
CD19
SSC-A
L/D
CD3
CD4 T cells
CD4
γδ T cells
γδ-TCR
CD8 T cells
CD8

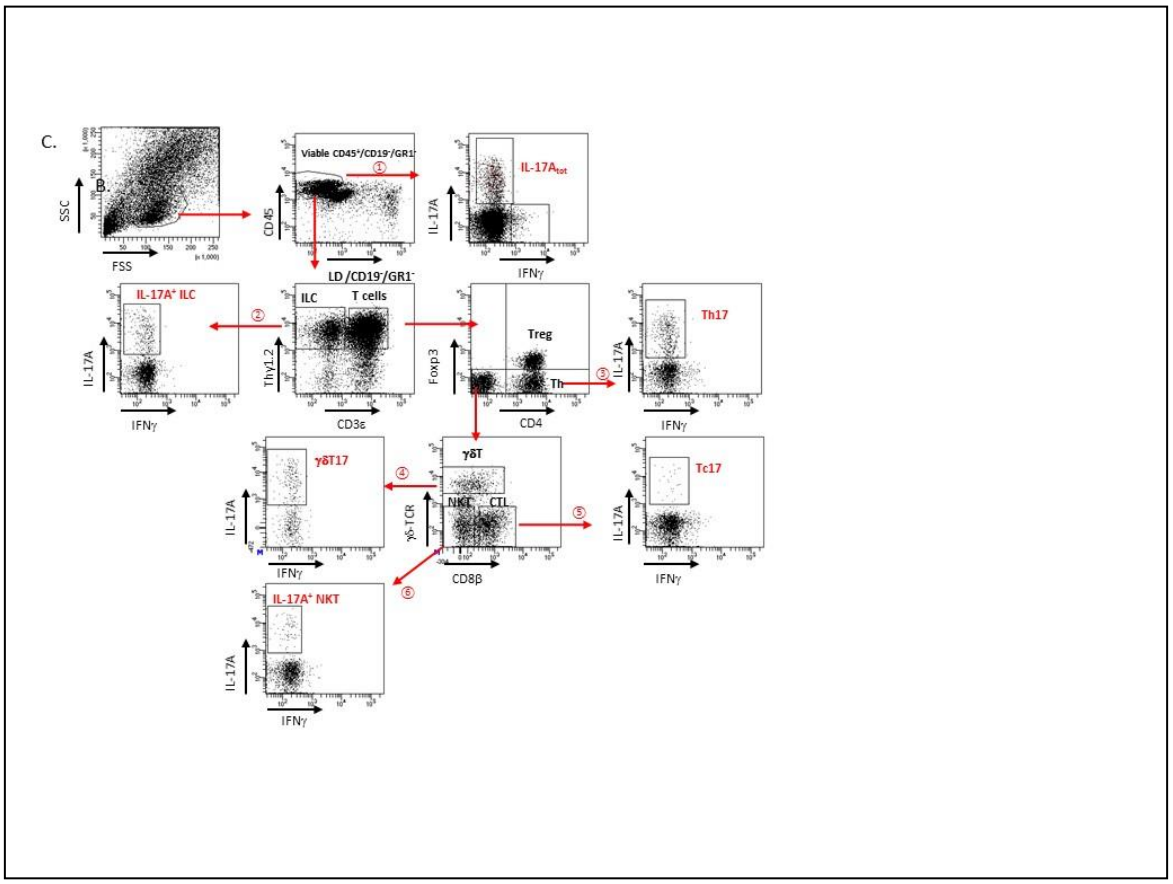


Fig S11. Flow cytometry gating strategy to delineate lymphoid, myeloid and IL-17-producing lamina propria lymphocytes. **A**, Myeloid cell gating strategy; ① Dendritic cells (DC) are identified as CD11c+I-A/E+ after gating out dead cells (viable cell gate). ② Macrophages are identified from the viable cell gate as F4/80+CD64+. ③ Monocytes are identified from the CD11b+ gate as Ly6-C^{hi}Ly6-G-. ④ Neutrophils are identified from the CD11b+ gate as Ly6-C+Ly6-G+. **B**, Lymphoid cell gating strategy; ① CD4+ T cells are identified as CD3+CD4+ after gating out dead cells (viable cell gate). ② CD8+ T cells are identified from the viable cell gate as CD3+CD8+. ③ γδT cells are identified from the viable cell gate as CD3+γδTCR+. ④ B cells are identified from the viable cell gate as CD3-CD19+. **C**, Intracellular Cytokine Staining to

detect IL-17-producing cells; ① IL-17+ total cells are identified as CD45+ after gating out dead cells, viable B cells and neutrophils (viable $CD19^-$ $GR\text{-}1^-$ gate). ② IL-17-producing innate lymphoid cells (IL-17+ ILC) are identified from the viable CD45+ cell gate as IL-17+CD3-Thy1.2+. ③ IL-17-producing CD4+ cells (Th17) are identified from the CD3+Thy1.2+ gate as IL-17+CD4+Foxp3-. ④ γδT17 cells are identified from the CD4-Foxp3- T cell gate as IL-17+γδ from the TCR+. ⑤ IL-17-producing cytotoxic T cells (Tc17) are identified from the CD4-Foxp3- T cell gate as IL-17+γδTCR-CD8β+. ⑥ IL-17-producing natural killer cells (IL-17+-NKT cells) are identified from CD4-Foxp3- T cell gate as IL-17+γδTCR-CD8β−.

Table S1

table S1 FAP patient metadata

Patient ID	Age	Sex	Race	Operation	Mutation	Analyses
365	39	M	Caucasian	Proctocolectomy	ND	Micro culture
1420	16	F	Caucasian	Colectomy	ND	Micro culture
1679	27	F	Caucasian	Colectomy	ND	Micro culture
2017	8	F	Caucasian	Proctocolectomy	ND	Micro culture
2215	16	M	Caucasian	Proctocolectomy	ND	Micro culture
2544	7	F	Caucasian	Proctocolectomy	ND	Micro culture
2732	25	F	Caucasian	Proctocolectomy	ND	Micro culture
2735	35	M	Caucasian	Proctocolectomy	ND	Micro culture
2891	65	F	African American	Partial Proctolectomy	ND	Micro culture
2904	18	F	Caucasian	Proctocolectomy	ND	Micro culture
2927	22	M	Caucasian	Proctocolectomy	ND	Micro culture
3024	53	M	Caucasian	Proctocolectomy	ND	Micro culture
3026	38	M	Caucasian	Proctocolectomy	ND	Micro culture
3037	9	M	Caucasian	Proctocolectomy	ND	Micro culture
3166	43	F	Caucasian	Proctocolectomy	ND	Micro culture
3174	17	F	Caucasian	Colectomy	ND	Micro culture
3233	27	M	African American	Proctocolectomy	ND	Micro culture
3345	35	F	Caucasian	Colectomy	ND	Micro culture
3381	25	M	Caucasian	Proctocolectomy	ND	Micro culture
3775*	51	F	Caucasian	Colectomy	MYH	Seq, FISH, Micro culture
3971	27	M	African American	Proctocolectomy	ND	Seq, FISH, Micro culture
3973*a	27	F	Caucasian	Colectomy	ND	Seq, FISH, Micro culture
3975	50	M	Caucasian	Colectomy	APC	Seq, FISH, Micro culture
3983[b]	*51*	F	African American	Colectomy	APC	Seq, FISH, Micro culture
3995	39	M	Caucasian	Proctocolectomy	APC	Seq, FISH, Micro culture

Abbreviations: ND, not determined; micro=aerobic and anaaerobic microbiology culture (see methods); FISH=fluorescent in situ hybridization

*attenuated phenotype
a Juvenille polyposis
b Patient received oral antbiotics 24 hours prior to surgery

Table S2

table S2 Control Subject metadata

Patient ID	Age	Sex	Race	Analyses
3714	33	F	Caucasian	Micro culture
3723	49	F	Caucasian	Micro culture
3724	66	M	Hispanic	Micro culture
3730	61	F	Caucasian	FISH, Micro culture
3734	57	F	Caucasian	FISH, Micro culture
3737	52	F	Caucasian	FISH, Micro culture
S55	60	F	African American	FISH, Micro culture
S56	52	F	African American	FISH, Micro culture
S57	52	M	Caucasian	FISH, Micro culture
S58	57	F	African American	FISH, Micro culture
S59	77	M	African American	FISH, Micro culture
S60	45	F	Caucasian	FISH, Micro culture
S61	58	M	Caucasian	FISH, Micro culture
S62	64	M	African American	FISH, Micro culture
S63	74	F	African American	FISH, Micro culture
S64	61	M	African American	FISH, Micro culture
S65	67	F	African American	FISH, Micro culture
S66	49	F	African American	FISH, Micro culture
S67	67	F	Caucasian	FISH, Micro culture
S68	47	F	African American	FISH, Micro culture
S69	57	M	Caucasian	FISH, Micro culture
S70	59	F	Caucasian	FISH, Micro culture
S71	52	F	African American	FISH, Micro culture

Abbreviations: Micro=as for table S1; FISH=as for table S1

Table S3

table S3 FISH Analysis of Bacterial Biofilms on FAP Mucosa

Patient ID	# Tissue Samples Collected	# Samples with Biofilms	Bacterial Biofilm Location	Biofilm Density	Gammaproteobacteria & Betaproteobacteria (% of bf population)	Bacteroides (% of bf population)	Lachnospiraceae (% of bf population)	Fusobacteria (% of population)	E. coli (% of bf population)	B. fragilis (% of bf population
3775*	23	16, 69%	3 Right (1 polyp, 2 paired normal) 13 Left (1 polyp, 12 paired normal)	2.60E+11	67%	12%	3%	0%	49%	9%
3971	22	14, 61%	4 Right (1 polyp, 3 paired normal) 11 Left (2 polyp, 9 paired normal)	8.02E+10	59%	32%	2%	0%	51%	29%
3975	7	5, 71%	1 Right (polyp) 4 Left (2 polyps, 2 paired normal)	2.80E+11	69%	10%	0%	0%	66%	7%
3995	6	6, 100%	3 Right (1 polyp, 2 paired normal) 3 Left (2 polyps, 1 paired normal)	6.40E+11	64%	11%	3%	0%	59%	5%
3973* [a]	8	0	NA	NA	NA	NA	NA	NA	NA	NA
3983 [b]	4	0	NA	NA	NA	NA	NA	NA	NA	NA

Abbreviations: bf=biofilm; NA=not applicable

*Attenuated phenotype
[a] Juvenille polyposis
[b] Patient received oral antibiotics 24 hours prior to surgery

Table S4

Table S4 FISH Probes				
Probe Target(s)	**Probe Name**	**Fluorophore**	**Probe Sequence (5'-3')**	**Reference**
Kingdom Bacteria except Planctomycetales and Verrucomicrobia	Eub338	Cy3, Alexa 405	GCTGCCTCCCGTAGGAGT	1
Fusobacteria	Fus714	Alexa 488	GGCTTCCCCATCGGCATT	2
Prevotella, Bacteroides	PRV392	Rhodamine Red X	GCACGCTACTTGGCTGG	3
Bacteroidetes (Bacteroides, Parabacteroides, Prevotella)	CFB286	Alexa 514	TCCTCTCAGAACCCCTAC	4
Betaproteobacteria	Bet42a	Alexa 647	GCCTTCCCACTTCGTTT	5
Gammaproteobacteria	Gam42a	Alexa 647	GCCTTCCCACATCGTTT	5
Lachnospiraceae	Lac435	Texas Red X	TCTTCCCTGCTGATAGA	6
Enterobacteriaceae except *Proteus* spp	Ent186	Alexa 555	CCCCCWCTTTGGTCTTGC	7
Bacteroides fragilis	S-S-Bfrag-998-a-A-20	Alexa 633	GTTTCCACATCATTCCACTG	8
Escherichia coli	Eco1531	HRP-tyamide488	CACCGTAGTGCCTCGTCATCA	9
Escherichia coli	Eco1161	HRP-tyamide488	GCATAAGCGTCGCTGCCG	10

Table S5

table S5 FISH of FAP Remnants				
Patient	Colonoscopy timepoint	Biofilm	*E. coli*	*B. Fragilis*
1	1	+	+	+
	2	+	+	-
	3	+	-	+
2	1	+	+	-
	2	+	+	-
	3	-	-	-
3	1	+	+	-
	2	-	+	+
	3	+	+	-
4	1	+	+	-
5	1	-	-	-
6	1	-	-	-
7	1	-	-	-
8	1	-	-	-
	2	-	+	-
9	1	-	-	-
10	1	-	-	-
11	1	-	-	-
	2	+	+	+
12	1	-	-	-
13	1	-	-	-
	2	-	+	-
14	1	-	-	-

Colonoscopy time point represents serial sampling of colon mucosa over 16 months in post-colectomy FAP patients.

Table S6

Primer target	Primer Name	Sequence (5'-3')	Reference	Product size (bp)
Akkermansia muciniphila 16S (Taqman) (fig S10)	AM1 (S-St-Muc-1129-a-a-20)	CAGCACGTGAAGGTGGGGAC	11	NA
	AM2 (S-St-Muc-1437-a-a-20)	CCTTGCGGTTGGCTTCAGAT		
	A. muc 16S Probe	HEX]ACTGGGCATTGTAGTACGTGTGCA[BHQ1]	This Study	
E. coli pks (Taqman) (fig S10)	clbB-F	GCGCATCCTCAAGAGTAAATA	12	NA
	clbB-R	GCGCTCTATGCTCATCAACC		
	pks Probe	FAM]TATTCGACACAGAACAACGCCGGT[BHQ1]	This study	
E. coli pks (fig 1D)	clbB-F	GCAACATACTCGCCCAGCT	This study	163
	clbB-R	TCTCAAGGCGTTGTTGTTTG		
B. fragilis toxin (bft) (fig 1D)	bft-F	GCGAACTCGGTTTATGCAGT	This study	280
	bft-R	GTTGTAGACATCCCACTGGC		

Table S7

Table S7 P-value of Mann-Whitney test (figure 3E)

Comparasion	ILC	Th17	Tc17	Yδ T17	NKT	IL-17 tot
ETBF vs. pks+ E.coli	0.0159	0.0159	0.0571	0.0159	0.0159	0.0159
pks+ E.coli vs. co-infection	0.0159	0.0159	0.0159	0.0159	0.0571	0.0159
ETBF vs. Co-infection	>0.9999	0.2222	0.5556	0.0079	0.3929	0.0556

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