

Supplemental Tables

Table S1. Clinical metadata for biofilm-negative and biofilm-positive human colon tissue inocula.

Table S2. Leading significant genera associated with biofilm status.

Table S3. Microbial differential gene expression from mucosa of germ-free *Apc*^{MinΔ850/+} mice associated with human biofilm-positive tumor or biofilm-negative healthy colonoscopy biopsy inocula.

Table S4. 16S rRNA sequencing primers.

Supplemental Figures

Figure S1

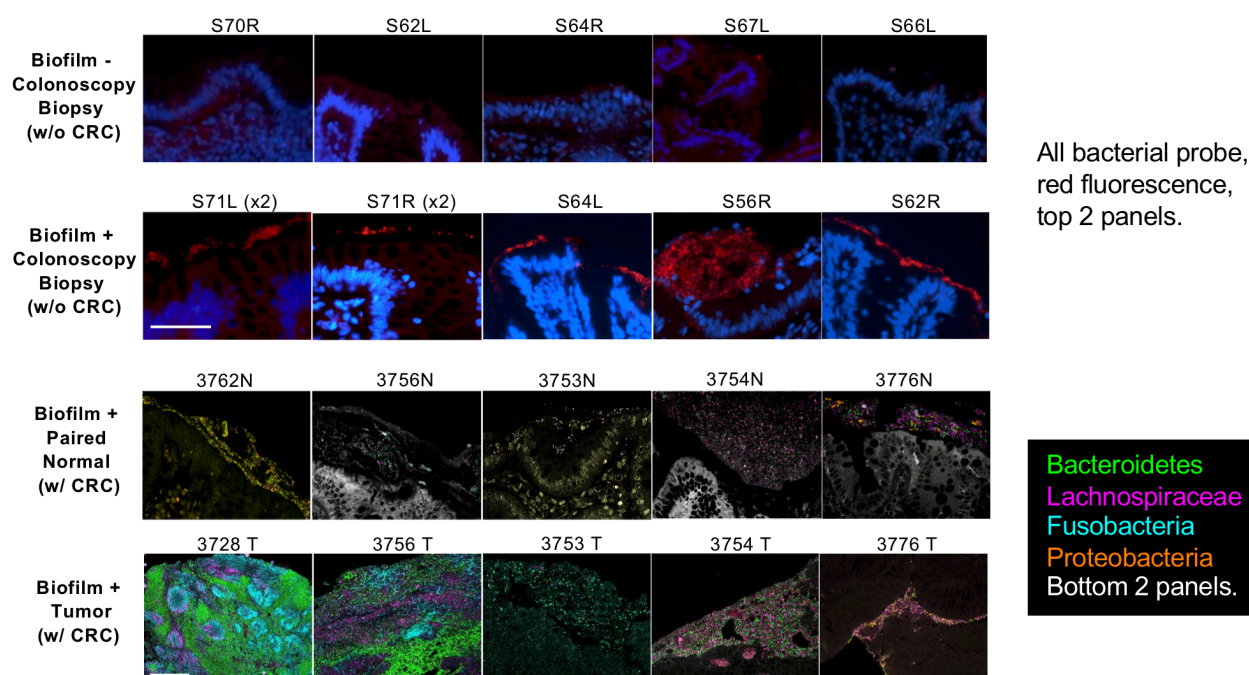


Figure S1. Fluorescent in situ hybridization (FISH) analysis of human colon biofilms present on tissues utilized for creation of biofilm-negative and biofilm-positive human colon tissue inocula. Biofilm-positive colonoscopy biopsy FISH using all bacterial probe; biofilm-positive paired normal and tumor using multiprobe FISH (see Methods); w/o and w/ CRC, without and with colorectal cancer. Scale bars, 50 μm .

Figure S2

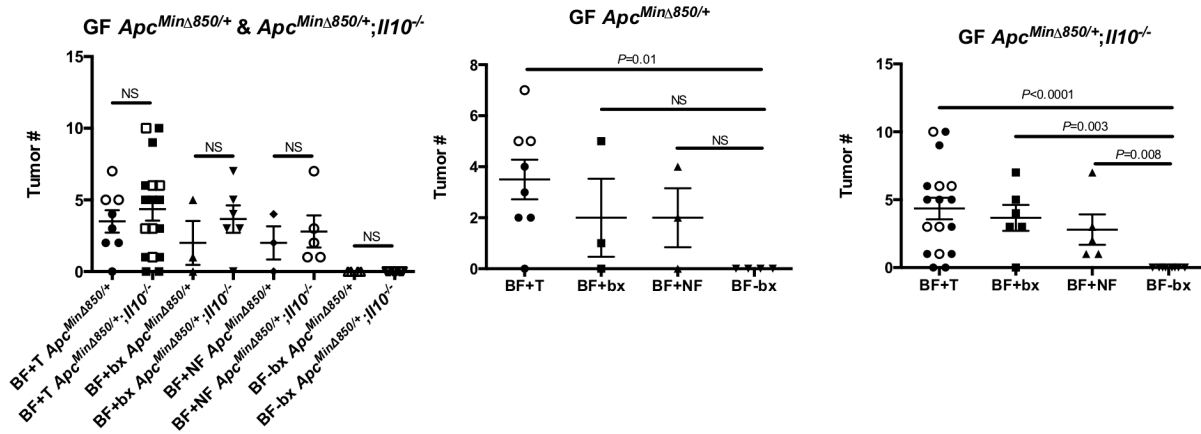


Figure S2. Colon tumors induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF mouse models (*Apc*^{MinΔ850/+}; *Il10*^{-/-} and *Apc*^{MinΔ850/+} mice). Left, comparison of tumor numbers induced in GF *Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice by type of human tissue inoculum; middle and right, comparisons of tumor numbers induced by type of inoculum within each mouse genotype. BF+T, biofilm-positive human colon tumor inoculum from CRC patients; BF+bx, biofilm-positive human colon mucosa biopsy inoculum from healthy subjects; BF+NF, biofilm-positive human colon mucosa inoculum from paired normal tissue from CRC patients (see Methods); BF-bx, biofilm-negative human colon mucosa biopsy inoculum from healthy subjects. For *Apc*^{MinΔ850/+} mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice, N=17, 5, 6 and 8 for BF+T, BF+NF, BF+bx and BF-bx, respectively. Closed circles represent mice analyzed at 12 weeks after inoculation. Open circles represent mice harvested at 13-20 weeks after inoculation (N=9 mice). Data displayed as mean +/- SEM analyzed by Mann-Whitney U test. For the middle and right panel, a P value of $P < 0.0167$ is considered significant based on Bonferroni correction.

Figure S3

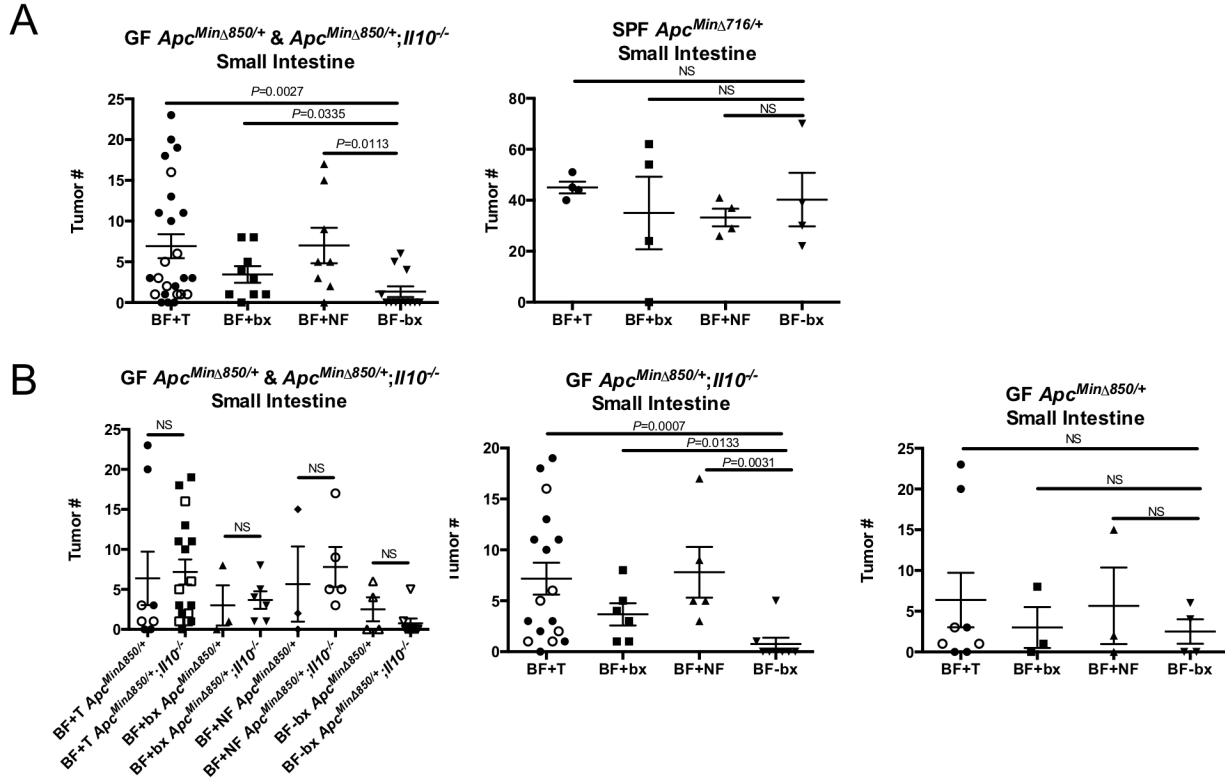


Figure S3. Small intestinal tumors induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF (*Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice) and SPF mouse models. **A.** Small intestinal tumor numbers induced by BF+ and BF- human colon tissue inocula in GF *Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice (left, combined analysis) and SPF mice (right). **B.** Small intestinal tumor numbers induced by BF+ and BF- human colon tissue inocula in GF *Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice. Left, comparison of small intestinal tumor numbers induced in *Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice by type of inoculum; middle and right, comparisons of small intestinal tumor numbers induced by type of inoculum within each mouse genotype. BF+T, biofilm-positive human colon tumor inoculum from CRC patients; BF+bx, biofilm-positive human colon mucosa biopsy inoculum from healthy subjects; BF+NF, biofilm-positive human colon mucosa inoculum from paired normal tissue from CRC patients (see Methods); BF-bx, biofilm-negative human colon mucosa biopsy inoculum from healthy

subjects. For *Apc*^{MinΔ850/+} mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for *Apc*^{MinΔ850/+;Il10^{-/-}} mice, N=17, 5, 6 and 8 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for SPF *Apc*^{MinΔ716/+} mice, N=4, 4, 4 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively. Closed circles represent mice analyzed at 12 weeks after inoculation. Open circles represent mice harvested at 13-20 weeks after inoculation (N=9 mice). Data displayed as mean +/- SEM analyzed by Mann-Whitney U test. For **A** and **B** (two panels on the right), a $P < 0.0167$ is considered significant based on Bonferroni correction.

Figure S4

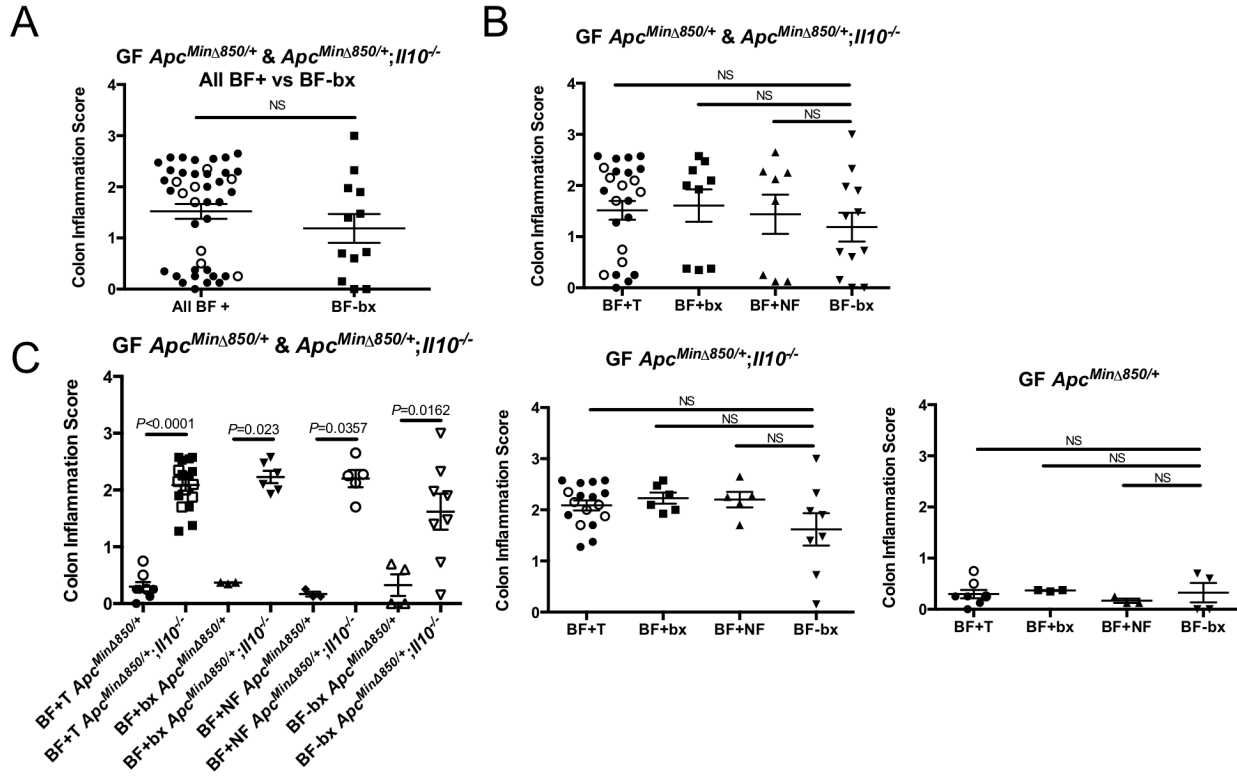


Figure S4. Whole colon inflammation induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF mouse models (*Apc*^{MinΔ850/+} vs. *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice). **A.** Comparison of whole colon inflammation scores induced in combined *Apc*^{MinΔ850/+} and *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice inoculated with BF+ or BF- human colon mucosal homogenates. **B.** Whole colon inflammation scores induced in combined *Apc*^{MinΔ850/+} and *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice inoculated with BF+T, biofilm-positive human colon mucosal tumor (CRC patients) inoculum; BF+bx, biofilm-positive human mucosal colonoscopy biopsy inoculum from healthy subjects; BF+NF, biofilm-positive human colon mucosal inoculum from paired normal tissue from CRC patients (see Methods); BF-bx, biofilm-negative human colonoscopy biopsy inoculum from healthy subjects. **C.** Comparisons of whole colon inflammation in GF *Apc*^{MinΔ850/+} and *Apc*^{MinΔ850/+}; *Il10*^{-/-} mice induced by inoculum type within each mouse genotype. For *Apc*^{MinΔ850/+} mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for

Apc^{Min Δ 850/+};*Il10*^{-/-} mice, N=17, 5, 6 and 8 for BF+T, BF+NF, BF+bx and BF-bx, respectively. Closed circles represent mice analyzed at 12 weeks after inoculation. Open circles represent mice harvested at 13-20 weeks after inoculation (N=9 mice). Data displayed as mean +/- SEM analyzed by Mann-Whitney U test.

Figure S5

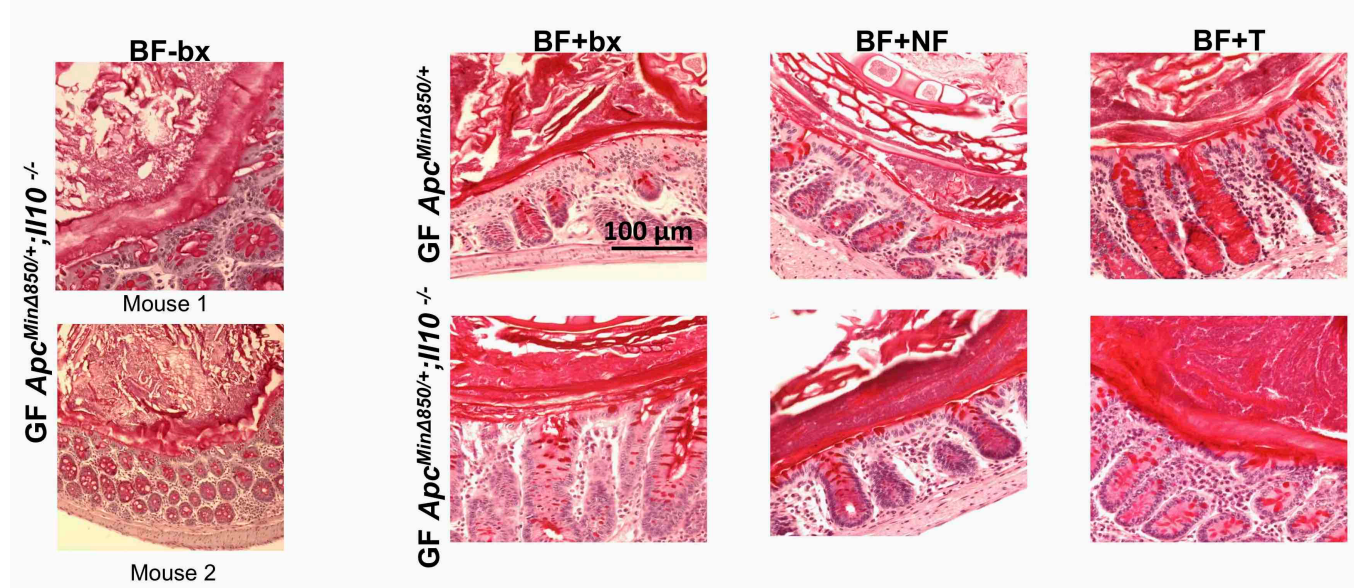


Figure S5. Periodic acid Schiff (PAS) stained murine tissues from *Apc*^{MinΔ850/+} or *Apc*^{MinΔ850/+;Il10^{-/-}} mice showing mucus layer in mouse colon tissues analyzed by FISH in **Figure 5**. Mice were inoculated with BF-bx, biofilm-negative human colonoscopy biopsy inoculum from healthy subjects; BF+bx, biofilm-positive human mucosal colonoscopy biopsy inoculum from healthy subjects; BF+NF, biofilm-positive human colon mucosal inoculum from paired normal tissue from CRC patients; or BF+T, biofilm-positive human colon mucosal tumor (CRC patients) inoculum (see Methods).

Figure S6

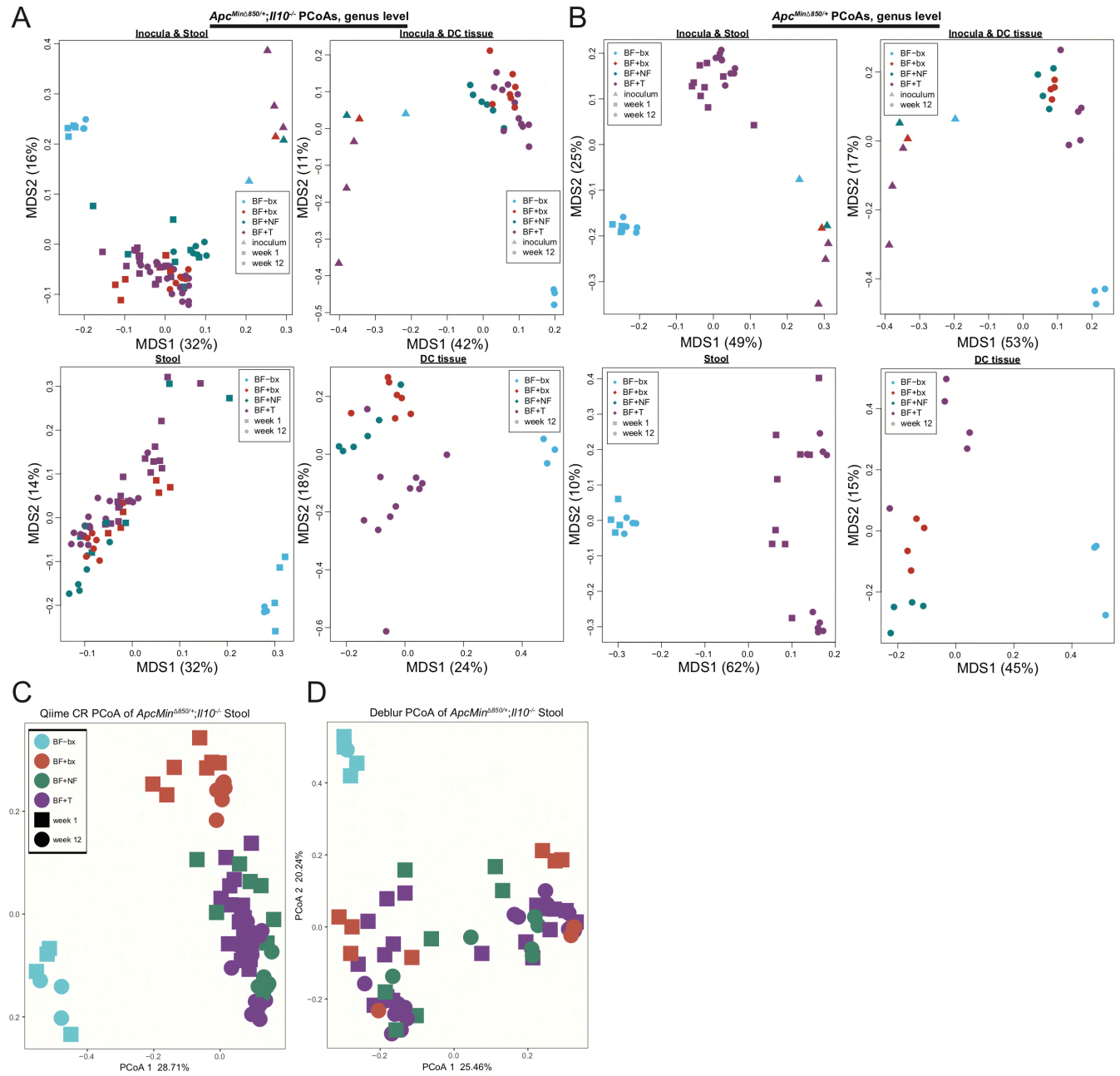


Figure S6. 16S rRNA amplicon sequencing microbiota analyses of stools and distal colon (DC) tissues from mouse models inoculated with biofilm-positive (BF+) and biofilm-negative (BF-) human colon mucosal tissues. **A.** Genus level PCoAs of the *Apc^{MinΔ850/+};Il10^{-/-}* stool (1 and 12 week time points) and DC tissue microbiota with inocula (top panel). Genus level PCoAs of the *Apc^{MinΔ850/+};Il10^{-/-}* stool (1 and

12 week time points) and DC tissue microbiota (bottom panel). **B.** Genus level PCoAs of the *Apc^{MinΔ850/+}* stool (1 and 12 week time points) and DC tissue microbiota with inocula (top panel). Genus level PCoAs of the *Apc^{MinΔ850/+}* stool (1 and 12 week time points) and DC tissue microbiota (bottom panel). Numbers in parentheses indicate the percent variation explained by that axis. **C.** OTU level PCoA of the *Apc^{MinΔ850/+};Il10^{-/-}* stool (1 and 12 week time points) generated from rarefied QIIME closed-reference OTUs using unweighted UniFrac distance metric. **D.** Sub Operational Taxonomic Unit (sOTU) level PCoA of the *Apc^{MinΔ850/+};Il10^{-/-}* stool (1 and 12 week time points) generated from rarefied Deblur sOTUs using unweighted UniFrac distance metric.

Figure S7

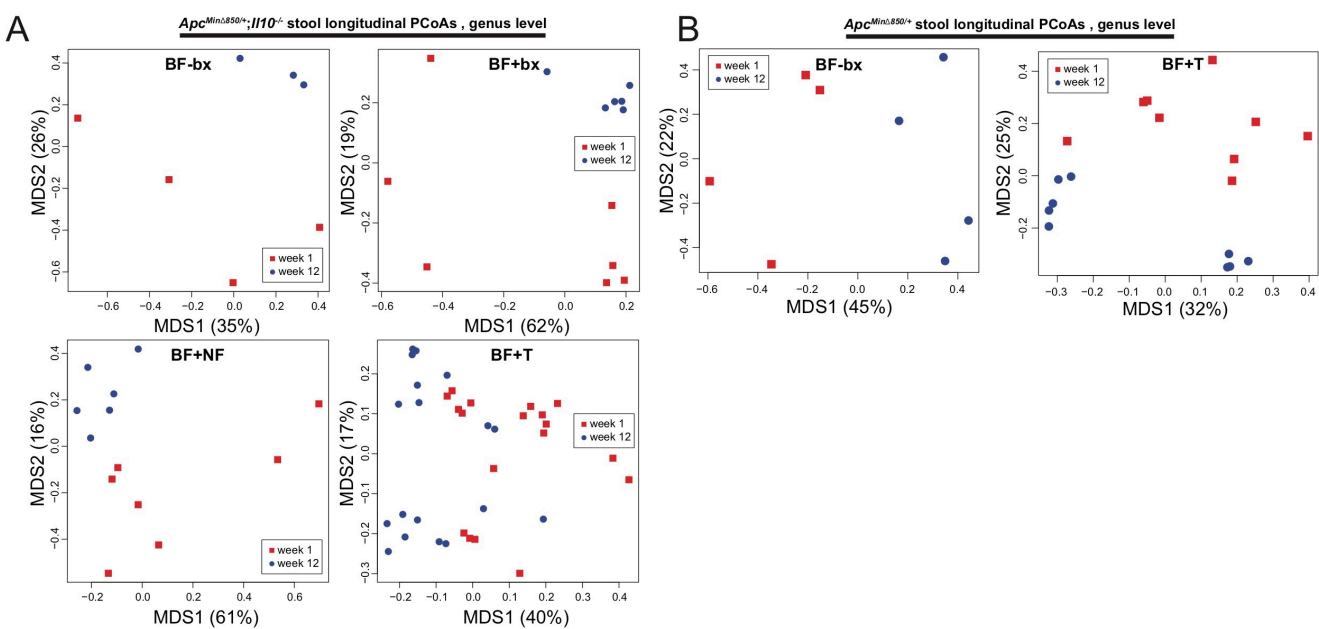


Figure S7. Genus level PCoAs showing the effect of time on stool microbiota composition in the 4 *Apc^{MinΔ850/+};Il10^{-/-}* groups (**A**) and the 2 *Apc^{MinΔ850/+}* groups (**B**). Numbers in parentheses indicate the percent variation explained by that axis.

Figure S8

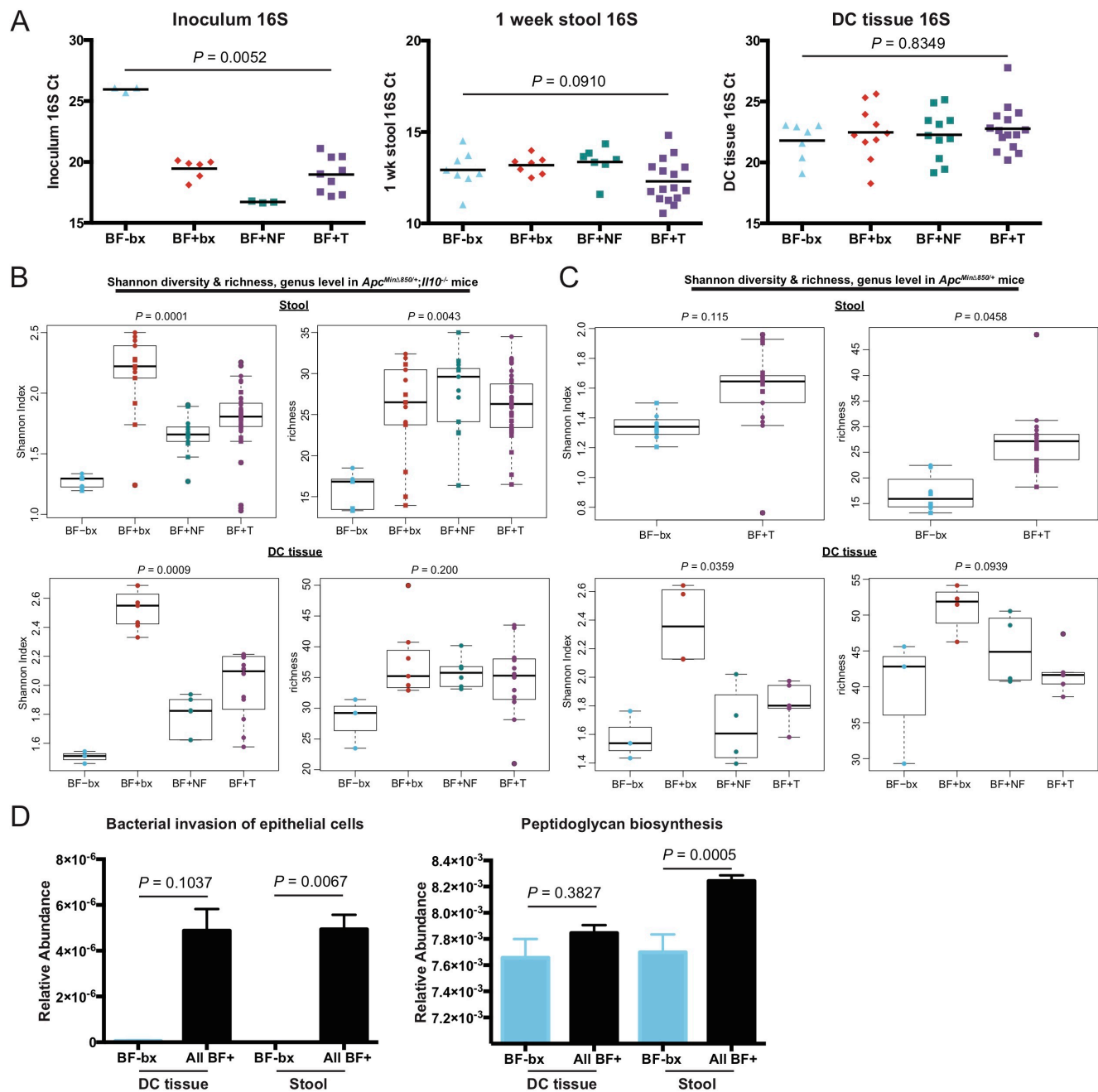


Figure S8. Microbiota analyses from GF *Apc^{MinΔ850/+};Il10^{-/-}* and *Apc^{MinΔ850/+}* mice inoculated with biofilm-positive (BF+) and biofilm-negative (BF-) human colon mucosal tissues. **A.** qRT-PCR of universal bacterial 16S rRNA of inoculum (each symbol represents one triplicate from inoculum; BF-bx and BF+NF, 1 inoculum; BF+bx, 2 inoculum samples, BF+T, 3 inoculum samples), 1 week stool and 12

week distal colon tissue samples from *Apc^{MinΔ850/+};Il10^{-/-}* and *Apc^{MinΔ850/+}* mice (each symbol represents an individual mouse). *P* values shown calculated with the Kruskal-Wallis test followed by Dunn's multiple comparisons test. **B-C.** Shannon diversity and richness from 16S rRNA amplicon sequencing of stool (top panels) and DC tissue (bottom panels) from *Apc^{MinΔ850/+};Il10^{-/-}* (**B**) and *Apc^{MinΔ850/+}* mice (**C**). **D.** Two of the KEGG Level 3 categories from PICRUSt analysis that were significantly increased in the stool of BF+ *Apc^{MinΔ850/+};Il10^{-/-}* and *Apc^{MinΔ850/+}* mice with FDR-adjusted *P* values shown.

Figure S9

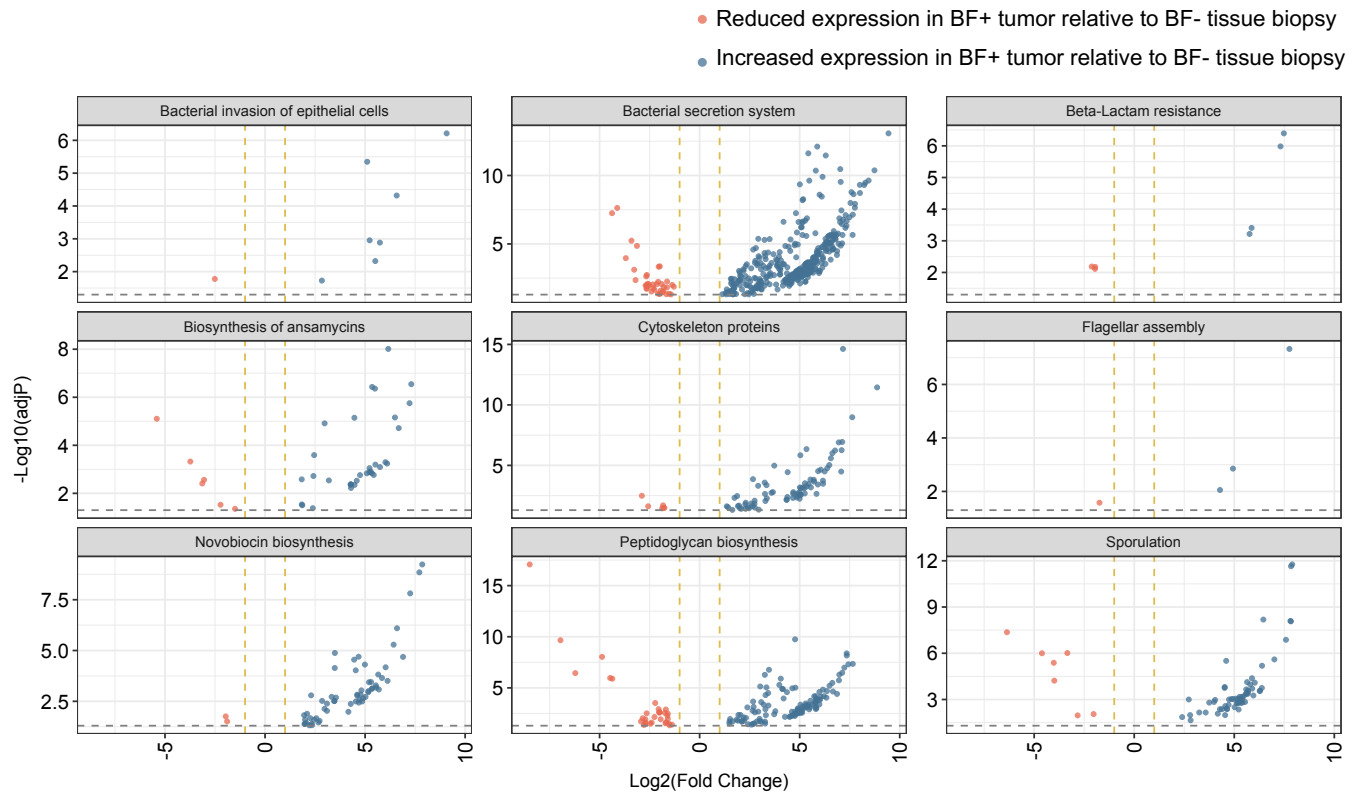


Figure S9. Differentially expressed microbial genes by KEGG sub-category. For each functional category, a volcano plot is displayed showing the log2 fold change (x-axis) versus the $-\log_{10}$ adjusted p-value (y-axis). Blue dots show genes enriched in biofilm+ tumor samples, while red dots show genes enriched in biofilm- tissue biopsies. Yellow vertical dashed lines mark fold-change values of -2 and +2. The gray horizontal dashed line indicates an adjusted p-value of 0.05. Microbial genes associated with bacterial invasion of epithelial cells, bacterial secretion systems, cytoskeleton proteins, peptidoglycan biosynthesis, novobiocin biosynthesis and sporulation showed increased numbers of upregulated genes in biofilm+ tumor samples relative to biofilm- biopsies. Limited differences were observed among microbial genes associated with beta-lactam resistance, biosynthesis of ansamycins and flagellar assembly.

Videos

Video 1: Colonoscopy of GF *Apc*^{MinΔ850/+};*Il10*^{-/-} mouse inoculated with biofilm-negative healthy subject colon mucosal biopsies.

Video 2: Colonoscopy of GF *Apc*^{MinΔ850/+};*Il10*^{-/-} mouse inoculated with biofilm-positive healthy subject colon mucosal biopsies.

Video 3: Colonoscopy of GF *Apc*^{MinΔ850/+};*Il10*^{-/-} mouse inoculated with biofilm-positive paired normal mucosa from CRC patients

Video 4 Colonoscopy of GF *Apc*^{MinΔ850/+};*Il10*^{-/-} mouse inoculated with human biofilm-positive tumors from CRC patients.