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 $Apc^{Min\Delta850/+}$ mice associated with human biofilm-positive tumor or biofilm-negative healthy colonoscopy biopsy inocula.

Table S4. 16S rRNA sequencing primers.
**Supplemental Figures**

**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**

Colon tumors induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF mouse models (Apc<sup>Min<sub>Δ850/+</sub></sup>;Il10<sup>−/−</sup> and Apc<sup>Min<sub>Δ850/+</sub></sup> mice). Left, comparison of tumor numbers induced in GF Apc<sup>Min<sub>Δ850/+</sub></sup> vs. Apc<sup>Min<sub>Δ850/+</sub></sup>;Il10<sup>−/−</sup> 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<sup>Min<sub>Δ850/+</sub></sup> mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for Apc<sup>Min<sub>Δ850/+</sub></sup>;Il10<sup>−/−</sup> 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. Small intestinal tumors induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF (Apc<sup>Min<850/+</sup> vs. Apc<sup>Min<850/+</sup>;Il10<sup>-/-</sup> mice) and SPF mouse models. A. Small intestinal tumor numbers induced by BF+ and BF- human colon tissue inocula in GF Apc<sup>Min<850/+</sup> vs. Apc<sup>Min<850/+</sup>;Il10<sup>-/-</sup> 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<sup>Min<850/+</sup> vs. Apc<sup>Min<850/+</sup>;Il10<sup>-/-</sup> mice. Left, comparison of small intestinal tumor numbers induced in Apc<sup>Min<850/+</sup> vs. Apc<sup>Min<850/+</sup>;Il10<sup>-/-</sup> 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 $Apc^{Min\Delta 850/+}$ mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for $Apc^{Min\Delta 850/+;Il10^{-/-}}$ mice, N=17, 5, 6 and 8 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for SPF $Apc^{Min\Delta 716/+}$ 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. Whole colon inflammation induced by biofilm-positive (BF+) or biofilm-negative (BF-) human colon tissue inocula in GF mouse models ($Apc^{Min\Delta850/+}$ vs. $Apc^{Min\Delta850/+};Il10^{-/-}$ mice). A. Comparison of whole colon inflammation scores induced in combined $Apc^{Min\Delta850/+}$ and $Apc^{Min\Delta850/+};Il10^{-/-}$ mice inoculated with BF+ or BF- human colon mucosal homogenates. B. Whole colon inflammation scores induced in combined $Apc^{Min\Delta850/+}$ and $Apc^{Min\Delta850/+};Il10^{-/-}$ 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 $Apc^{Min\Delta850/+}$ and $Apc^{Min\Delta850/+};Il10^{-/-}$ mice induced by inoculum type within each mouse genotype. For $Apc^{Min\Delta850/+}$ mice, N=8, 3, 3 and 4 for BF+T, BF+NF, BF+bx and BF-bx, respectively; for
$Apc^{Min^{850+}; Il10^{-/-}}$ 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. Periodic acid Schiff (PAS) stained murine tissues from $Apc^{Min\Delta850/+}$ or $Apc^{Min\Delta850/+;Il10^{-/-}}$ 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. 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 $Apc^{Min\Delta850/+}, II10^{-/-}$ stool (1 and 12 week time points) and DC tissue microbiota with inocula (top panel). Genus level PCoAs of the $Apc^{Min\Delta850/+}, II10^{-/-}$ stool (1 and
12 week time points) and DC tissue microbiota (bottom panel). **B.** Genus level PCoAs of the $Apc^{\text{Min}\Delta850/+}$ stool (1 and 12 week time points) and DC tissue microbiota with inocula (top panel). Genus level PCoAs of the $Apc^{\text{Min}\Delta850/+}$ 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 $Apc^{\text{Min}\Delta850/+;Il10^{-/-}}$ 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 $Apc^{\text{Min}\Delta850/+;Il10^{-/-}}$ 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 $\text{Apc}^{\text{Min}\Delta850/+;\text{Il10}^{-/-}}$ groups (A) and the 2 $\text{Apc}^{\text{Min}\Delta850/+}$ groups (B). Numbers in parentheses indicate the percent variation explained by that axis.
Figure S8. Microbiota analyses from GF \(Apc^{Min^{Δ850}/+};Il10^{−/−}\) and \(Apc^{Min^{Δ850}/+}\) 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 $Apc^{Min\Delta850/+;Il10^{-/-}}$ and $Apc^{Min\Delta850/+}$ 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 $Apc^{Min\Delta850/+;Il10^{-/-}}$ (B) and $Apc^{Min\Delta850/+}$ mice (C). D. Two of the KEGG Level 3 categories from PICRUSt analysis that were significantly increased in the stool of BF+ $Apc^{Min\Delta850/+;Il10^{-/-}}$ and $Apc^{Min\Delta850/+}$ mice with FDR-adjusted $P$ values shown.
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 –log10 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 $Apc^{Min, A850/+;Il10^{-/-}}$ mouse inoculated with biofilm-negative healthy subject colon mucosal biopsies.

**Video 2:** Colonoscopy of GF $Apc^{Min, A850/+;Il10^{-/-}}$ mouse inoculated with biofilm-positive healthy subject colon mucosal biopsies.

**Video 3:** Colonoscopy of GF $Apc^{Min, A850/+;Il10^{-/-}}$ mouse inoculated with biofilm-positive paired normal mucosa from CRC patients

**Video 4** Colonoscopy of GF $Apc^{Min, A850/+;Il10^{-/-}}$ mouse inoculated with human biofilm-positive tumors from CRC patients.