Supplemental Tables and Figures:

The *Bacteroides fragilis* toxin gene is prevalent in the colon mucosa of colorectal cancer patients

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Supplemental Table 1. PCR conditions

PCR	Primers	Primer sequence	Product	PCR conditions	Reference
bft 368	368F	GAACCTAAAACGGTATATGT	368 bp	20 cycles (94°C "30, 62 to 52°C "30, 72°C "30)	[22]
	368R	GTTGTAGACATCCCACTGGC		20 cycles (94°C "30, 52°C "30, 72°C "30)	
bft 281	281F	GCGAACTCGGTTTATGCAGT	281 bp	20 cycles (94°C "30, 62 to 52°C "30, 72°C "30)	[22, 23]
	281R	GTTGTAGACATCCCACTGGC		20 cycles (94°C "30, 52°C "30, 72°C "30)	

Supplemental Table 2A. Detailed Characteristics for Cases

CRC Patients												
Patient Identification	Age (yrs)	Sex	Race	Antibiotics	Bowel Preparation	Tumor Site	Tumor Size (cm)	Tumor Histology	Clinical Stage	T Status	N Status	M Status
3749	39	M	Caucasian	No	Mechanical prep	Sigmoid	5.0	Adenocarcinoma (low grade)	3	3	1	0
3752	73	F	Caucasian	No	No prep	Transverse	2.5	Adenocarcinoma (low grade)	2	3	0	0
3753	48	F	African American	No	Mechanical prep	Ascending	4.7	Adenocarcinoma (low grade)	4	3	0	1
3754	67	F	African American	No	No prep	Ascending	3.0	Adenocarcinoma (high grade)	2	3	0	0
3755	76	F	Caucasian	No	No Prep	Ascending	1.5	Tubular Adenoma (dysplasia)	na	na	na	na
3756	54	M	Caucasian	No	No Prep	Sigmoid	4.5	Adenocarcinoma (low grade)	4	3	0	1
3759	87	F	Caucasian	Yes	Mechanical prep	Rectum	4.5	Adenocarcinoma (low grade)	4	3	2	1
3760	29	F	Caucasian	No	No prep	Rectosigmoid	8.0	Adenocarcinoma (low grade)	2	4	0	0
3762	73	M	Caucasian	No	No prep	Ascending	5.4	Adenocarcinoma (low grade)	4	4	1	1
3763	66	F	Caucasian	No	No prep	Ascending	3.0	Adenocarcinoma (low grade)	2	3	0	0
3766	56	F	Caucasian	No	No prep	Sigmoid	5.5	Adenocarcinoma (low grade)	4	3	2	1
3768	45	M	African American	Yes	Mechanical prep	Transverse	4.2	Adenocarcinoma (low grade)	4	3	2	1
3769	78	F	African American	No	Mechanical prep	Splenic flexure	6.0	Adenocarcinoma (low grade)	3	3	1	0
3770	70	M	Caucasian	No	No prep	Hepatic flexure	3.5	Adenocarcinoma (high grade)	1	2	0	0
3773	58	F	African American	Yes	Mechanical prep	Cecum	3.5	Adenocarcinoma (low grade)	4	3	1	1
3774	45	M	Asian	No	No prep	Hepatic flexure	4.5	Adenocarcinoma (high grade)	2	3	0	0
3776	84	F	Caucasian	No	No prep	Ascending	3.5	Tubular Adenoma (no dysplasia)	na	na	na	na
3779A	74	F	Caucasian	Yes	Mechanical prep	Sigmoid	4.5	Adenocarcinoma (low grade)	3	3	1	0
3779B	--	--	--	--	--	Right colon[a]	4.2	Tubulovillous Adenoma (dysplasia)	na	na	na	na
3780	78	F	Caucasian	Yes	Mechanical prep	Ascending	4.0	Adenocarcinoma (low grade)	4	3	2	1
3781	62	M	Caucasian	Yes	Mechanical Prep	Descending	4.5	Adenocarcinoma (low grade)	4	3	1	1
3783	68	M	Caucasian	No	Mechanical Prep	Ascending	1.1	Tubular adenoma (dysplasia)	na	na	na	na
3784	47	M	African American	Yes	Mechanical Prep	Descending	5.5	Adenocarcinoma (high grade)	2	3	0	0
3785	54	F	Caucasian	No	No Prep	Rectosigmoid	4	Adenocarcinoma (low grade)	3	3	1	0
3786	56	M	Caucasian	Yes	Mechanical Prep	Sigmoid	1.0	Adenocarcinoma[b]	4	2	0	1
3788	52	M	Caucasian	No	Mechanical Prep	Rectosigmoid	4	Adenocarcinoma (low grade)	4	3	2	1
3789	55	M	Hispanic	No	No Prep	Descending	5.0	Adenocarcinoma (low grade)	3	3	1	0
3972	78	M	Caucasian	No	No Prep	Cecum	4	Adenocarcinoma (high grade)	3	4	2	0
3976	52	F	Caucasian	No	No Prep	Transverse	2.0	Adenocarcinoma (low grade)	1	2	0	0
3977	38	F	Caucasian	No	No Prep	Sigmoid	5	Adenocarcinoma (low grade)	1	2	0	0
3978	90	F	Caucasian	No	No Prep	Rectum	2.7	Adenocarcinoma (low grade)	1	2	0	0
3979	77	F	African American	No	Mechanical Prep	Cecum	3.5	Adenocarcinoma (low grade)	3	3	1	0
3982	62	M	Caucasian	No	No prep	Ascending	4.5	Adenocarcinoma (low grade)	2	4	0	0
3984	85	M	Caucasian	Yes	Mechanical Prep	Ascending	3.3	Tubular adenoma (no dysplasia)	na	na	na	na
3986	59	M	Caucasian	Yes	Mechanical Prep	Ascending	5.0	Adenocarcinoma (low grade)	2	3	0	0
3987	66	F	Caucasian	Yes	Mechanical Prep	Ascending	5	Adenocarcinoma (low grade)	2	3	0	0
3988	48	M	Caucasian	Yes	Mechanical Prep	Descending	3.5	Adenocarcinoma (low grade)	1	2	0	0
3989	41	F	Caucasian	Yes	Mechanical Prep	Cecum	8.5	Adenocarcinoma (low grade)	4	3	1	1
3990	61	F	Caucasian	Yes	Mechanical Prep	Ascending	3.0	Adenocarcinoma (low grade)	1	2	0	0
3991	70	M	Caucasian	Yes	Mechanical Prep	Ascending	3.1	Tubular adenoma (dysplasia)	na	na	na	na
3992	91	F	Caucasian	No	No prep	Splenic Flexure	4.5	Adenocarcinoma (high grade)	2	3	0	0
3994	82	F	Caucasian	Yes	Mechanical Prep	Ascending	3	Adenocarcinoma (low grade)	2	2	0	0
3996	67	F	Caucasian	Yes	Mechanical Prep	Ascending	2.0	Adenocarcinoma (low grade)	1	2	0	0
3997	28	F	Asian	Yes	Mechanical Prep	Descending	6.3	Adenocarcinoma (high grade)	4	3	2	1
3998	78	F	Caucasian	Yes	Mechanical Prep	Cecum	7.2	Adenocarcinoma (low grade)	2	3	0	0
3999A	59	M	Caucasian	Yes	Mechanical Prep	Ascending	10	Tubulovillous adenoma (no dysplasia)	na	na	na	na
3999B	--	--	--	--	--	Sigmoid	6.5	Tubulovillous adenoma (no dysplasia)	na	na	na	na
4002	85	F	Caucasian	Yes	Mechanical Prep	Sigmoid	4.5	Adenocarcinoma (low grade)	3	2	2	0
4003	71	M	African American	Yes	Mechanical Prep	Hepatic Flexure	8.0	Adenocarcinoma (low grade)	3	3	2	0
4008	48	M	Caucasian	Yes	Mechanical Prep	Cecum	6.5	Adenocarcinoma (high grade)	3	4	2	0
4009	53	M	Caucasian	No	No prep	Rectosigmoid	8.6	Adenocarcinoma (low grade)	3	4	1	0

[a]pathology report did not report a tumor site
[b]low and/or high grade
yrs = years, Mechanical bowel preparation = Polyethylene glycol and/or phosphate enema

Supplemental Table 2B. Detailed Characteristics for Controls

Patient Identification	Age (yrs)	Sex	Race	Antibiotics	Antibiotic History Details	Bowel Preparation[a]	Reason for Colonoscopy	Biopsy Sites	Gross Colonoscopy Findings
S1	51	F	African American	NA	NA	GoLYTELY	Screening	Left only	Polyps[c]
S2	66	F	Caucasian	NA	NA	GoLYTELY	Workup (Diarrhea)	Left, Right	Polyps, Diverticulosis
S3	66	M	African American	NA	NA	GoLYTELY	Screening	Left, Right	Polyp, Diverticulosis
S4	66	M	African American	NA	NA	GoLYTELY	Workup (Anemia)	Left, Right	Diverticulosis
S5	44	F	Caucasian	NA	NA	GoLYTELY	Workup (Diarrhea)	Left, Right	Polyp, Diverticulosis
S6	52	F	Caucasian	NA	NA	GoLYTELY	Screening	Left, Right	Normal
S7	74	M	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Normal
S8	51	F	African American	No	NA	GoLYTELY & MiraLAX	Screening	Left, Right	Polyps[c]
S9	73	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Polyps[c]
S10	40	F	Caucasian	No	NA	GoLYTELY	Workup (Anemia)	Left only	Normal
S11	42	F	Hispanic	No	NA	GoLYTELY	Workup (Constipation)	Left only	Normal
S12	43	F	Caucasian	No	NA	MiraLAX	Workup (Abdominal pain)	Left, Right	Polyp[c]
S13	62	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Possible arteriovenous malformation
S14	75	M	Caucasian	Yes	PCN, past 6 mo	GaviLyte Prep	Workup (Diarrhea)	Left, Right	Polyps[c], Radiation Proctitis
S15	44	M	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Polyps[c], Diverticulosis
S17	71	F	African American	Yes	PCN, current	GoLYTELY	Screening	Left, Right	Normal
S18	74	F	African American	No	NA	NuLYTELY	Screening	Left, Right	Normal
S19	62	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Normal
S20	59	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Normal
S21	49	F	Asian	Yes	FQ, past 3 mo	NuLYTELY	Workup (Constipation)	Left, Right	Polyp[c]
S22	47	M	African American	Yes	Unknown[b], past 3 mo	GoLYTELY	Workup (Anemia)	Left, Right	Diverticulosis
S23	78	F	Caucasian	No	NA	Mag Citrate & Bisacodyl	Workup (Abdominal pain)	Left, Right	Diverticulosis
S24	47	M	African American	No	NA	MiraLAX	Workup (Abdominal pain)	Left, Right	Diverticulosis
S25	63	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Polyp
S26	39	F	African American	Yes	Unknown[b], past 3 mo	MiraLAX	Workup (Constipation)	Left, Right	Normal
S27	51	F	African American	Yes	FQ,PCN & FLZ, past 3 mo	GoLYTELY	Screening	Left, Right	Polyp
S28	47	F	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Normal
S29	64	F	Caucasian	No	NA	GoLYTELY	Workup (Constipation)	Left, Right	Diverticulosis
S31	65	M	African American	Yes	Unknown[b], past 6 mo	MiraLAX	Workup (Blood in stool)	Left, Right	Diverticulosis, Hemorrhoids
S32	92	F	Caucasian	Yes	DOXY, past 3 mo	MiraLAX	Workup (Abdominal pain)	Left only	Diverticulosis, Polyp
S33	58	F	African American	No	NA	MiraLAX	Screening	Left, Right	Normal
S34	36	M	Asian	Yes	DOXY, past 12 mo	MiraLAX	Screening	Left, Right	Normal
S35	66	F	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Diverticulosis
S36	60	F	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Normal
S37	79	F	African American	No	NA	GoLYTELY	Screening	Left, Right	Diverticulosis
S38	66	M	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Diverticulosis
S39	59	M	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Polyps[c]
S40	72	F	African American	No	NA	MiraLAX	Screening	Left, Right	Polyps[c]
S41	58	F	African American	No	NA	GoLYTELY	Screening	Left, Right	Polyps[c]
S42	46	F	Asian	No	NA	GoLYTELY	Workup (Blood in stool)	Left, Right	Normal
S43	69	M	African American	No	NA	MiraLAX	Screening	Left, Right	Polyps
S44	62	F	African American	No	NA	GoLYTELY	Workup (+FOBT)	Left, Right	Diverticulosis, Polyps
S45	47	F	Caucasian	Yes	Unknown[b], past 3 mo	GoLYTELY	Workup (Diarrhea)	Left, Right	Normal
S46	73	M	African American	No	NA	GoLYTELY	Screening	Left, Right	Diverticulosis, Polyps[c]
S47	63	F	African American	Yes	FQ, past 3 mo	GoLYTELY	Workup (Melena)	Left, Right	Normal
S48	66	M	Caucasian	No	NA	MiraLAX	Screening	Left, Right	Polyps
S49	56	F	African American	No	NA	GaviLyte	Screening	Left, Right	Normal
S50	63	F	African American	Yes	FQ & MTZ, current	GoLYTELY	Screening	Left, Right	Inadequate prep
S51	64	M	Other	No	NA	GoLYTELY	Screening	Left, Right	Normal

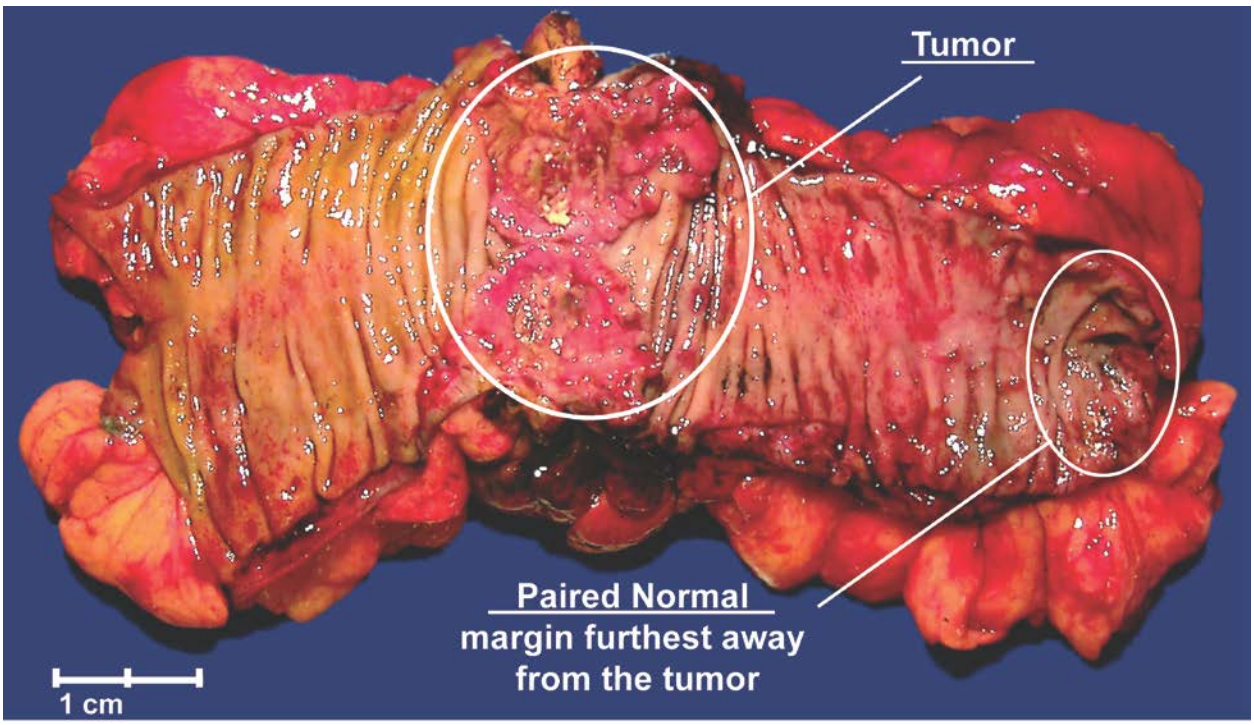
[a]Bowel preparation included Polethylene glycol (PEG) with electrolyte additives (GoLYTELY®, GaviLyte®, NuLYTELY®), PEG without electrolyte additives (MiraLAX®), saline laxative (magnesium citrate) and stimulant laxative (Bisacodyl)

[b] Could not recall type of antibiotic taken

[c]11 had tubular adenomas removed at the time of their procedure, all other polyps were either hyperplastic or inflammatory polyps

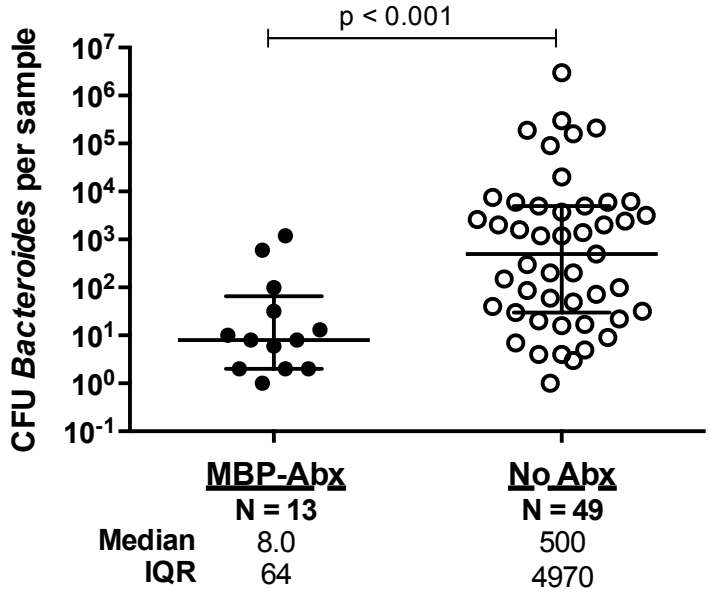
yrs = years, mo = month, NA = not applicable, PCN = penicillin, FQ = Fluoroquinolone, FLZ = Fluconazole, DOXY = doxycycline, MTZ = Metronidazole

Supplemental Figure 1. Collection of Tumor and Paired Normal Samples from Surgical Specimens



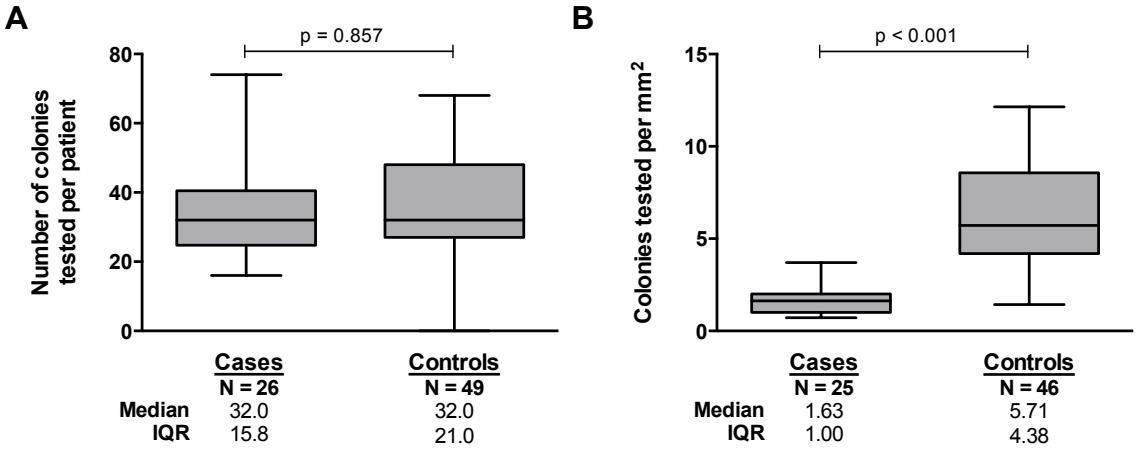
Entire specimen is roughly 23 centimeters in length. Tumor site is indicated. Normal tissue was routinely collected from a site on the specimen most distant from the tumor.

Supplemental Figure 2. *Bacteroides* colonies recovered from tumor and normal samples from cases with and without exposure to oral antibiotics



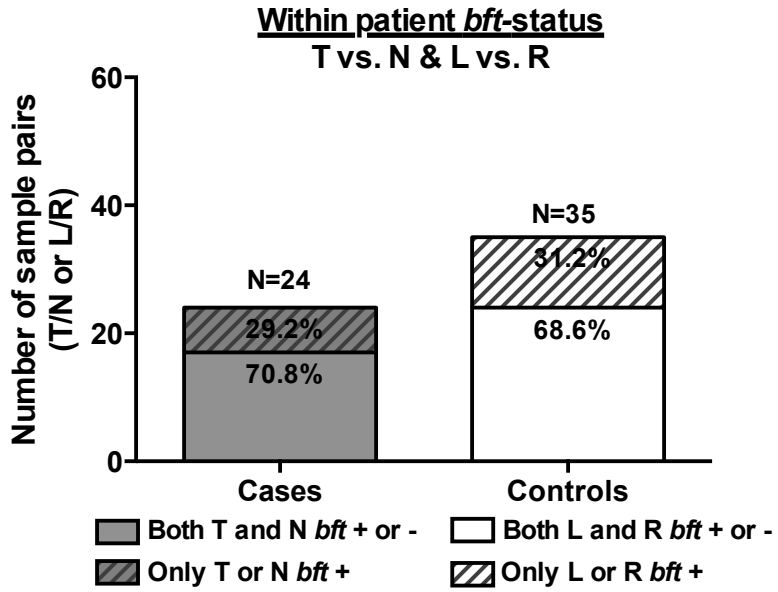
Of 44 tissue samples from 23 MBP-Abx cases, 13 tissue samples (8 tumors,5 normal tissues) grew colonies on BBE. Of 49 tissue samples from 26 No Abx cases, 49 tissue samples (24 tumor samples, 25 normal tissues) grew colonies on BBE. Oral antibiotic exposure as part of the mechanical bowel preparation prior to surgery significantly decreased recovered *Bacteroides* CFUs ($p<0.001$). MBP-Abx= mechanical bowel preparation with oral antibiotics, No Abx = no preoperative bowel preparation or mechanical bowel preparation without oral antibiotics, CFU= colony forming units

Supplemental Figure 3. Number of Single Colonies Tested per Case or Control and per mm^2 of Tissue Sampled



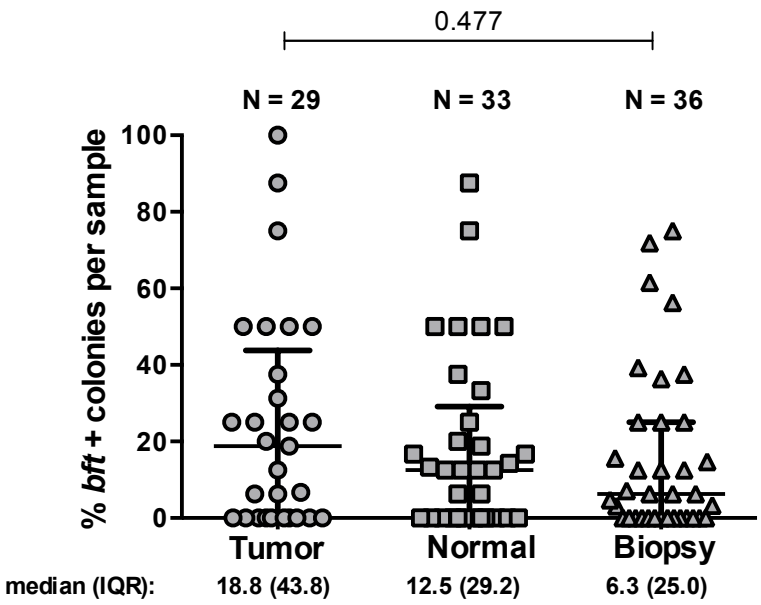
Box whisker plots of number of colonies analyzed per case or control and per mm^2 tissue A) There was no difference in the number of colonies tested when No Abx cases were compared to all controls (Mann-Whitney U test; p=0.857). B) When the number of colonies tested per No Abx case or control was corrected for tissue size sampled, the number of colonies tested per mm^2 of tissue was significantly lower in No Abx cases compared to controls (Mann-Whitney U test; p<0.001). One No Abx case had an unknown biopsy size and was excluded. 3 controls had no growth and were also excluded.
IQR = Interquartile range

Supplemental Figure 4. Consistency of *bft* Status for Tissue Sample Pairs from the Same Case or Control



Consistency of *bft* status within cases and controls. *bft* status was analyzed by paired tumor (T) and normal (N) samples from the same case or left (L) and right (R) biopsy pairs from the same control. Tissue pairs (T/N or L/R) were processed using the direct single colony method to determine consistency between paired samples (see Materials and Methods). A total of 24 T/N sample pairs from 26 No Abx cases revealed that 17/24 pairs (70.8%) had consistent *bft* results (13 pairs both *bft*-positive and 4 pairs both *bft*-negative). In 4/24 sample pairs (16.7%), N was *bft*-negative while T was *bft*-positive; in 3/24 sample pairs (12.5%), N was *bft*-positive while T was *bft*-negative. A total of 35 L/R pairs from 45 controls demonstrated consistent *bft* results in 24/35 samples pairs (68.6%) while in 11/35 biopsy pairs (31.2%) only the L or R biopsy was *bft*-positive.
N = normal tissue; T = tumor tissue; Ctrl = controls

Supplemental Figure 5. Median Percentage of *bft*-Positive Colonies in Tumor, Normal and Colonoscopy Biopsy Samples



Number of bft positive colonies per tissue sample analyzed. For all cases with growth on BBE plates, a total of 29 tumor samples (5 adenomas, 52 adenocarcinomas) and 33 normal tissues were analyzed. A total of 36 biopsies were analyzed from colonoscopy controls. The percentage of *bft*+ colonies was higher in tumors compared to normal tissues and biopsies from controls (Kruskall-Wallis; p=0.477). Tissues were analyzed by the direct single colony method.
IQR = Interquartile range