Supplementary Material For:

A Murine Model of Chemotherapy-induced ExPEC Translocation

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Running Title: Model of Bacterial Translocation

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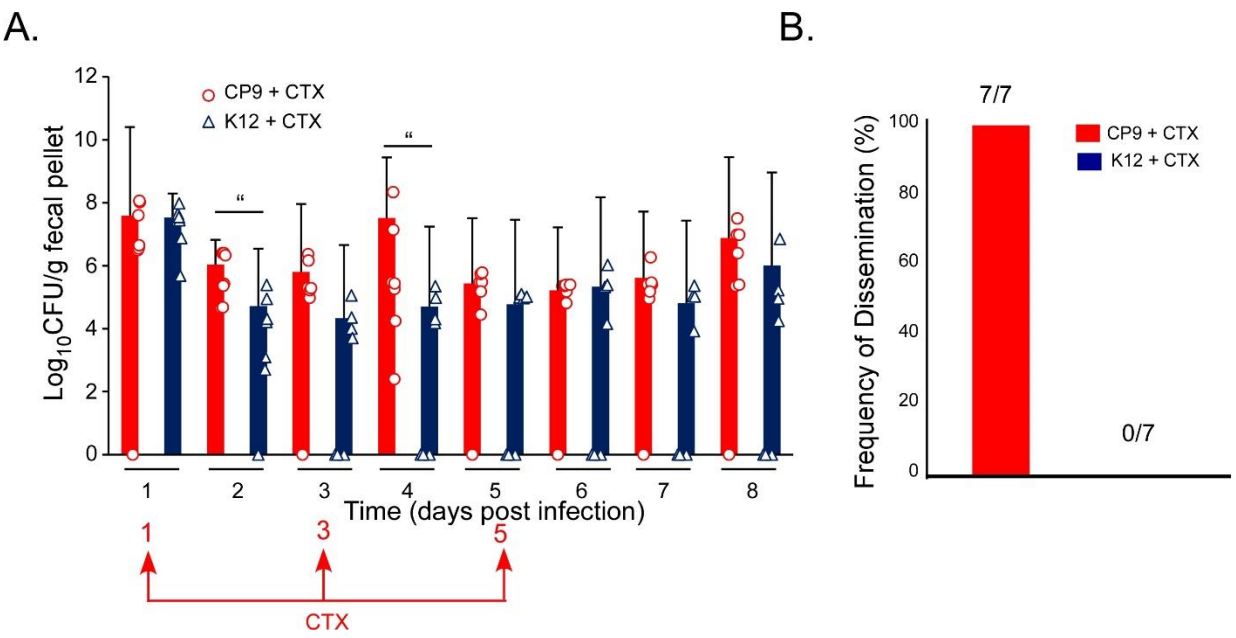

Figure S1 (A-B) Balb/c mice were gavaged with 10^9 CFU's of CP9 *E. coli* (red circles) or K12 MG1655 strain of *E. coli* (blue triangles). Mice were given cytoxan on days 1, 3 and 5 post-infection. (A). Colonization was monitored by plating fecal pellets on selective media. (B). Mice were euthanized on day 8 and bacteria enumerated by plating organ homogenates on selective media. Numbers above the bars represent mice with bacteria in organs (either K12 or CP9) over total mice gavaged with bacteria. Error bars represent ± standard deviation and (“) denotes a significant ($p \leq 0.05$) difference between groups (n equals 7).

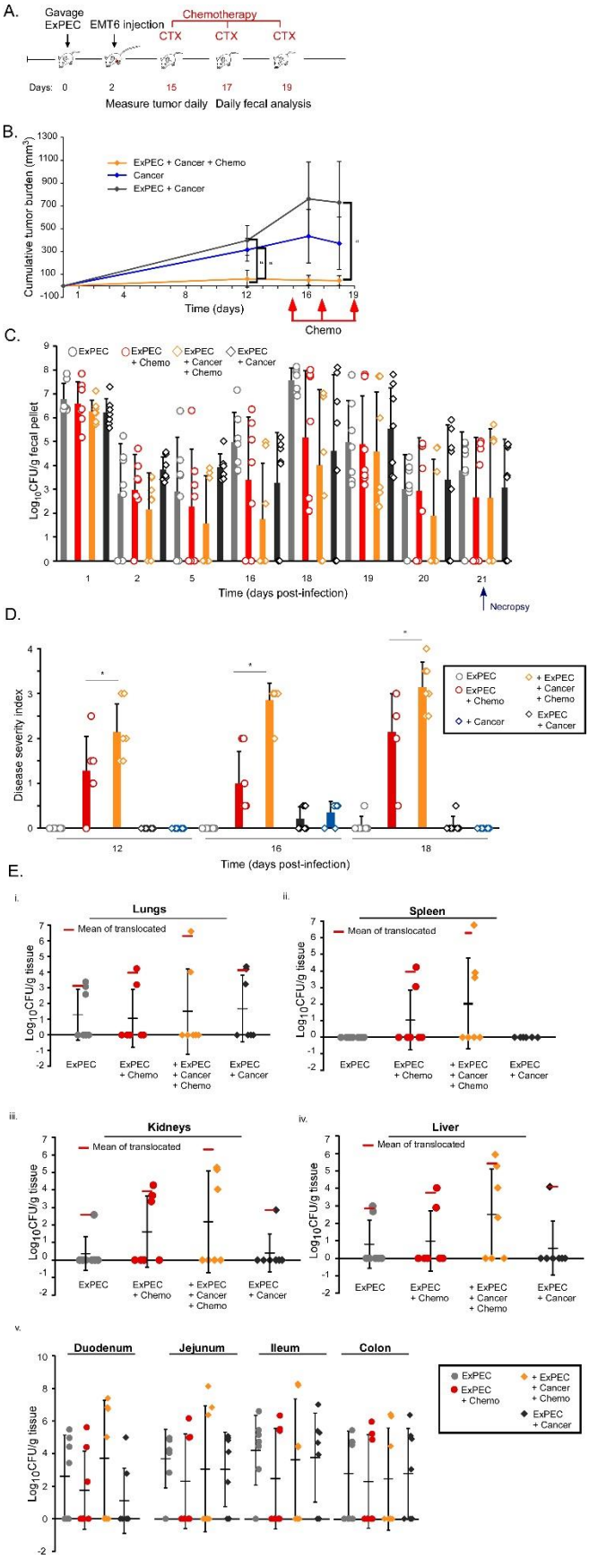
A.
Gavage
ExPEC
EMT6 injection
Chemotherapy
CTX
CTX
CTX
Days: 0
2
15
17
19
Measure tumor daily
Daily fecal analysis
B.
Cumulative tumor burden (mm3)
ExPEC + Cancer + Chemo
Cancer
ExPEC + Cancer
Time (days)
Chemo
C.
ExPEC
ExPEC + Chemo
ExPEC + Cancer + Chemo
ExPEC + Cancer
Log10CFU/g fecal pellet
Time (days post-infection)
Necropsy
D.
Disease severity index
Time (days post-infection)
ExPEC
ExPEC + Chemo
+ Cancer
+ ExPEC + Cancer + Chemo
ExPEC + Cancer
E.
i.
Lungs
ii.
Spleen
iii.
Kidneys
iv.
Liver
Mean of translocated
Log10CFU/g tissue
ExPEC
ExPEC + Chemo
+ ExPEC + Cancer + Chemo
ExPEC + Cancer
v.
Duodenum
Jejunum
Ileum
Colon

Figure S2. Chronically-colonized ExPEC translocates in cancerous mice receiving chemotherapy. (**A**) Schematic outlining the delivery of ExPEC and the development and treatment of cancer. BALB/c mice were gavaged with $1x10^9$ CFU's of CP9 or left uninfected and two days later mice were orthotopically injected with EMT-6 mammary carcinoma cells (1×10^5) on the right and left (**B**) mammary fat pad. Mice were treated with two rounds of cytoxan (150mg/kg, per day) for 6 days, as indicated. Tumor growth was monitored by measuring volume (mm^3) as described in the *Experimental Procedures*. (**C**) Fecal pellets were collected and plated on LB/Agar^{Chlor+} to enumerate shed CFUs. (**D**) Disease was monitored on days 12, 16 and 18 as described in the *Experimental Procedures*. (**E**) Bacteria translocation and intestinal colonization was determined by plating organs on LB/Agar^{Chlor+}. Error bars represent ± standard deviation and (") denotes a significant ($p \leq 0.05$) difference between groups.

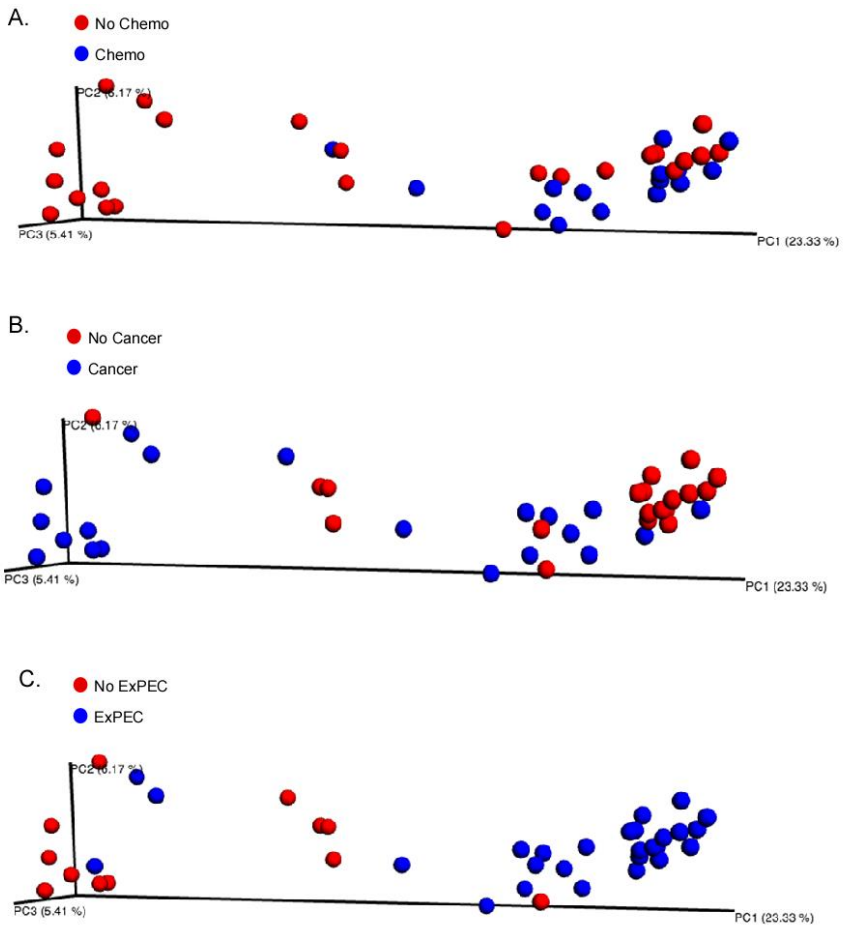

Figure S3. Diversity of the blood microbiota. Principal Coordinate Analysis of bacterial blood communities by unweighted UniFrac distances in (**A**) No Chemo and Chemo (p = 0.021), (**B**) No Cancer and Cancer (p = 0.004), and (**C**) No ExPEC and ExPEC (p = 0.001) groups. Coordinates are scaled to the percent of variability explained.

GROUP	Mouse #	Metastasis (+/-)
Cancer + Chemotherapy	1	-
	2	-
	3	-
	4	-
	5	-
	6	-
	7	-
Cancer No Chemotherapy	8	-
	9	+
	10	+
	11	-
	12	+
	13	+
	14	+

Table S1: Assessment of tumor metastasis to the lungs. Mice were implanted with EMT-6 mammary cancer cells, infected with ExPEC, and treated with chemotherapy. Following necropsy of the mice, the lungs of cancerous mice treated with chemotherapy and those not treated with chemotherapy were examined histologically for the presence of EMT-6 cells into the mammary epithelium. No metastasis identified is indicated with a minus sign whereas lung tissue with metastasis is indicated with a plus sign.