

Supplementary Material

The Western dietary pattern combined with vancomycin-mediated changes to the gut microbiome exacerbates colitis severity and colon tumorigenesis

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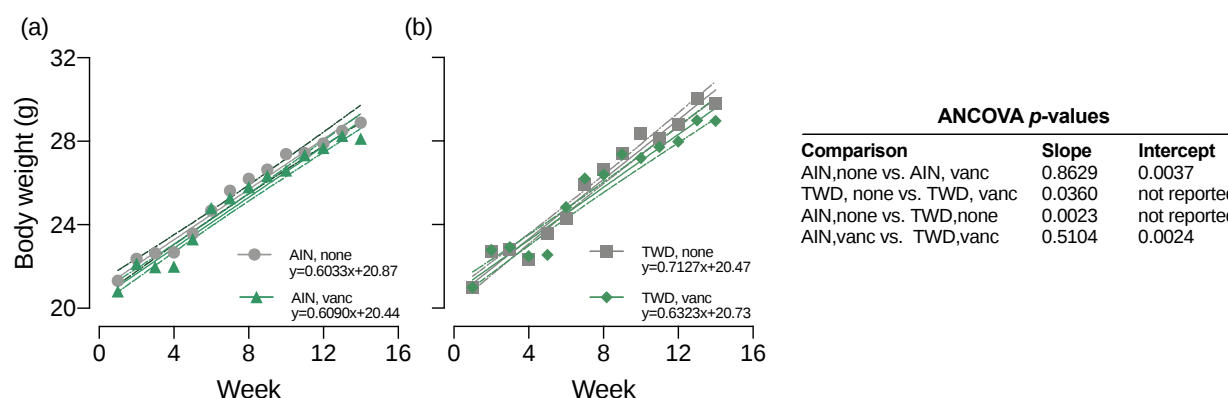


Figure S1. Body weight gain over time for (a) mice fed the standard AIN diet or (b) mice fed the TWD, with and without vancomycin treatment. The linear regression (solid line) and 95% confidence intervals are shown for each experimental group. Results of analysis of covariance (ANCOVA) are shown for specific comparisons to determine if the slope and/or intercepts for regressions are statistically significant among experimental groups. Abbreviations: AIN, the AIN93G diet; TWD, the total Western diet; Ab, antibiotic.

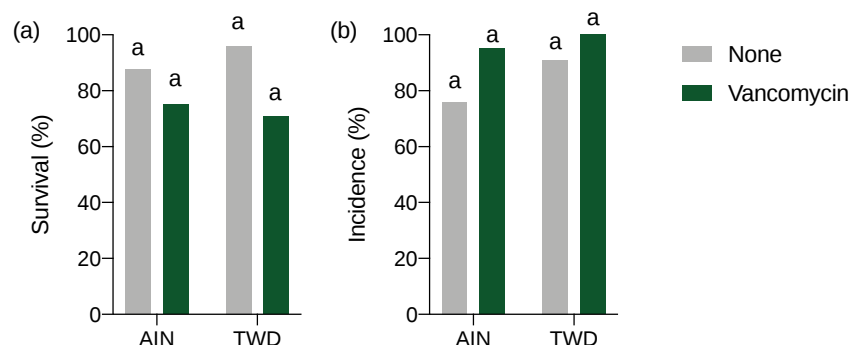


Figure S2. Overall survival (a) and tumor incidence (b) at the study end on day 98. Survival percentage shown for initial $n = 24$ per treatment group. Incidence percentage as fraction of mice with tumors for those mice that survived to day 98 ($n=17$ to 23). No significant differences in survival or tumor incidence percentage were found using the Fisher's exact test followed by the Bonferroni adjustment of p -values for multiple testing. Abbreviations: AIN, the AIN93G diet; TWD, the total Western diet; Ab, antibiotic.

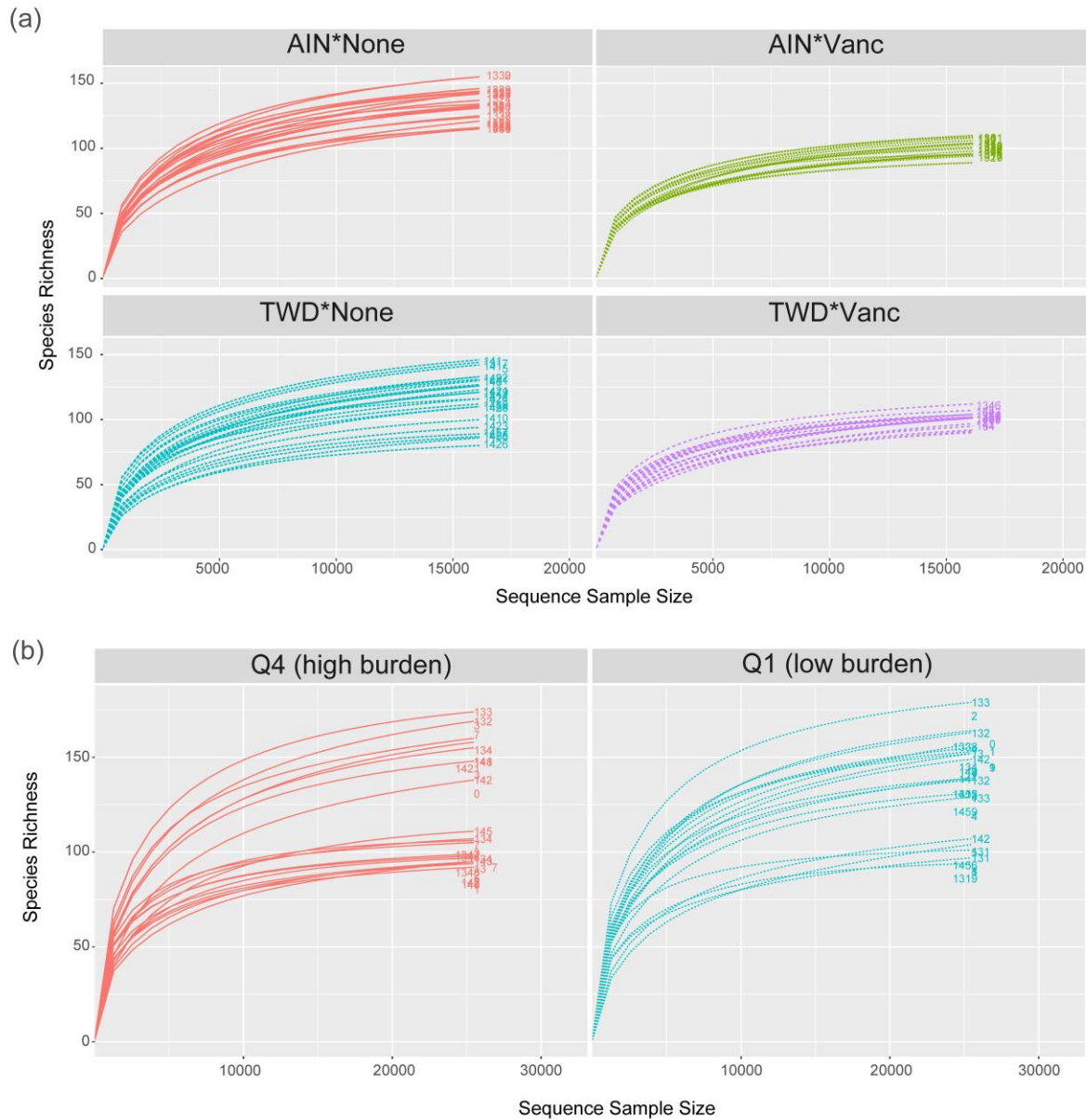


Figure S3. Rarefaction curve analysis by experimental group. Curves plot species richness as a function of sequence sample size. **(a)** For comparisons across experimental groups, data were rarefied to 16,459 sequences, the lowest total among all the samples. **(b)** For comparison of highest tumor burden (top 25%, Q4) versus lowest tumor burden (lowest 25%, Q1), rarefaction was to 26,519 sequences. These curves indicate that saturation was reached satisfactorily for the large majority of samples and that any additional sequence reads were unlikely to substantially increase the number of species detected.

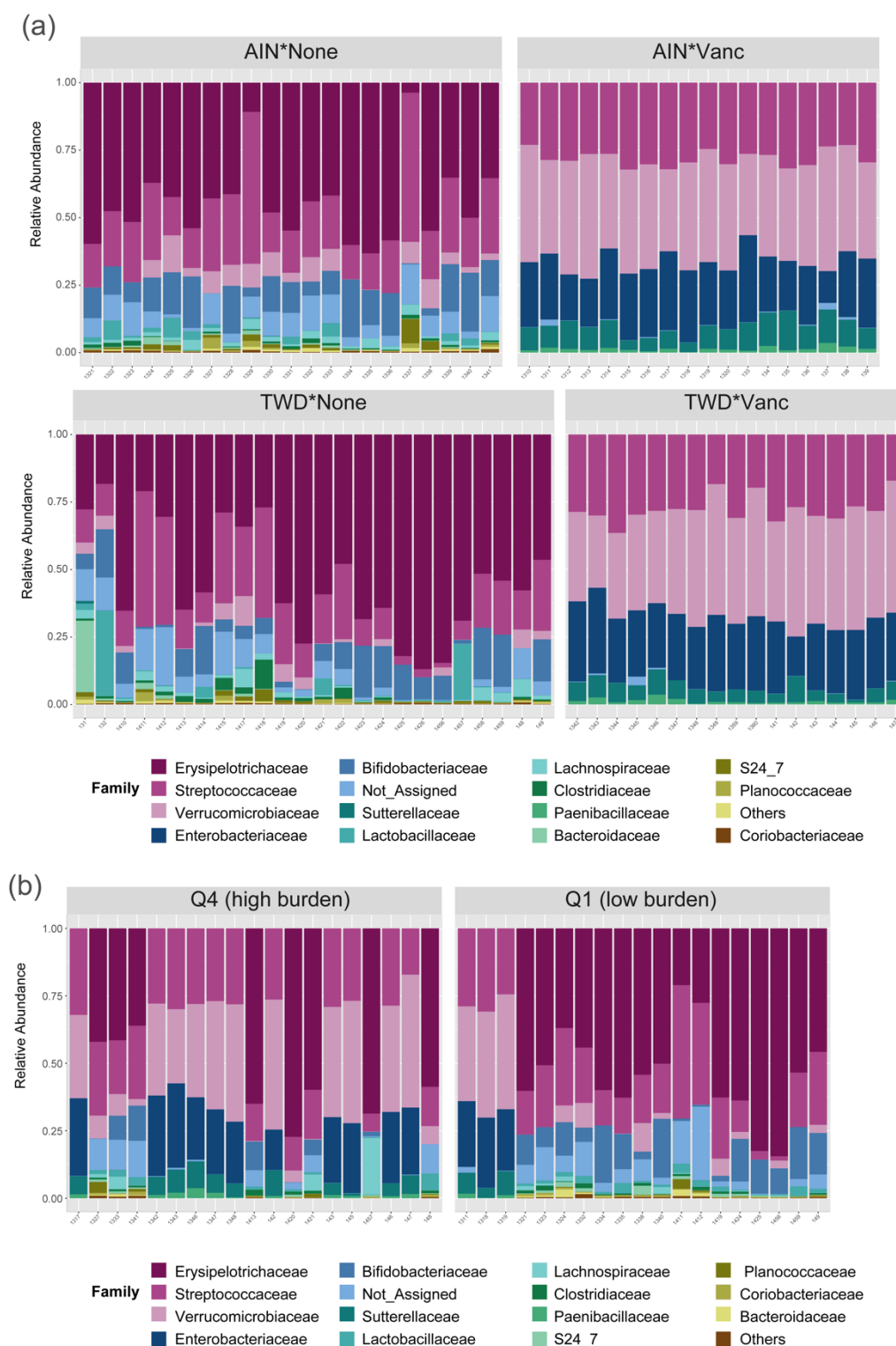


Figure S4. Taxonomic classification of mouse fecal bacteria by individual animal for all experimental groups (a) or for the highest and lowest tumor burden quartiles (b). Data shown are the relative normalized abundance of bacteria for each individual mouse annotated to the family taxonomic level for the top 15 most abundant taxa. Abbreviations: AIN, the AIN93G diet; TWD, the total Western diet; Vanc, vancomycin.

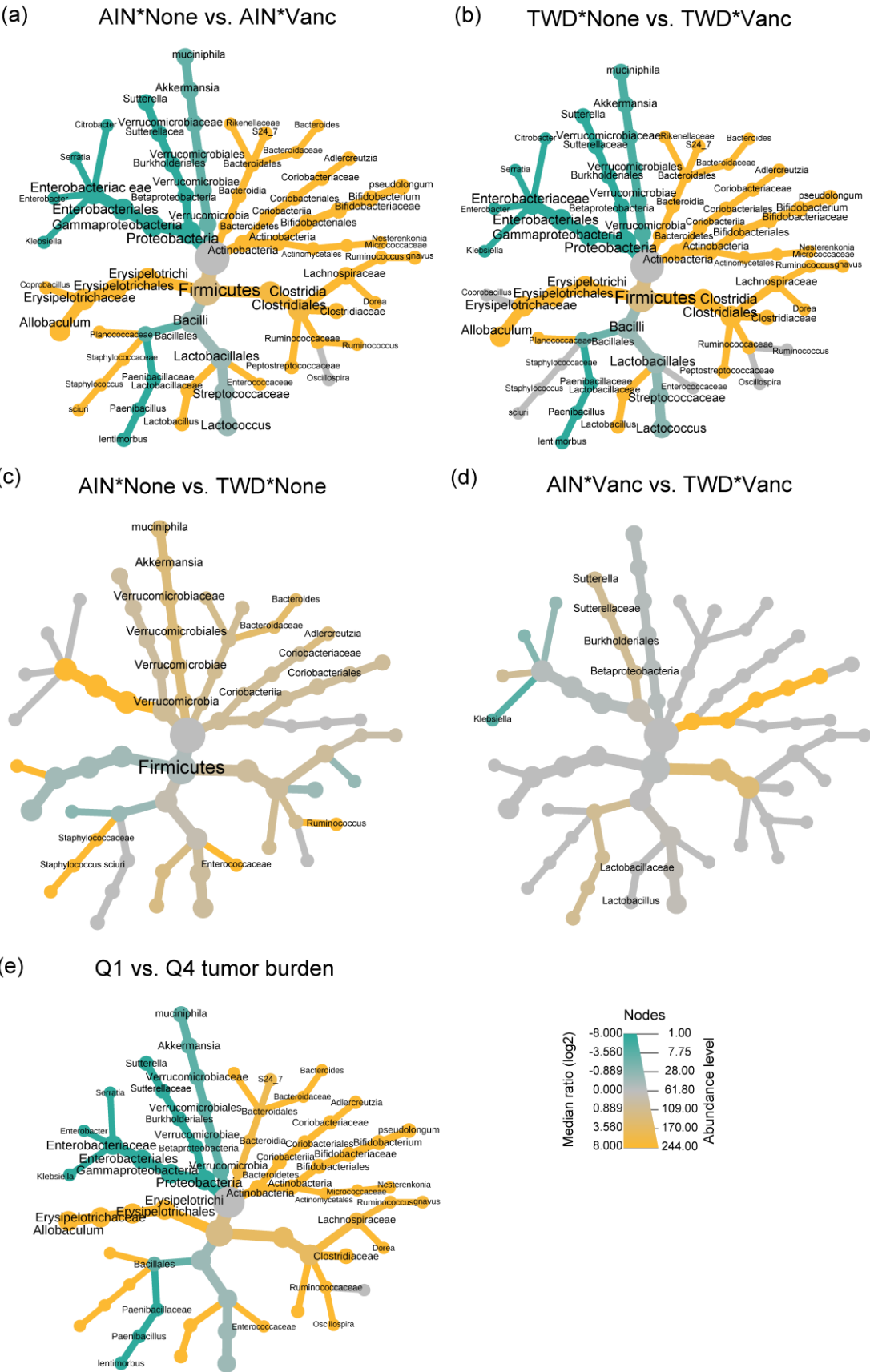


Figure S5. (preceding page) Phylogenetic heat trees for selected comparisons among experimental groups. The heat tree analysis leverages the hierarchical structure of taxonomic classifications to quantitatively (using the median abundance) and statistically (using the non-parametric Wilcoxon Rank Sum test) depict taxonomic differences between microbial communities. Comparisons are (diet*antibiotic): **(a)**, AIN*none vs. AIN*vanc, **(b)**, TWD*none vs. TWD*vanc, **(c)** AIN*none vs. TWD*none, **(d)** and AIN*vanc vs. TWD*vanc. Also shown is the heat tree comparing low tumor burden (Q1) versus high tumor burden (Q4) **(e)**. Tree nodes and branches are colored by the relative abundance level, with colors toward yellow indicating enrichment and colors toward green indicating depletion in the comparator group with respect to the reference (e.g., for panel a, yellow indicates enrichment of species *Akkermansia muciniphila* in the AIN*none group compared to the TWD*none group.) Nodes without labels were not significantly different by this nonparametric test. Abbreviations: AIN, the AIN93G diet; TWD, the total Western diet; Vanc, vancomycin.

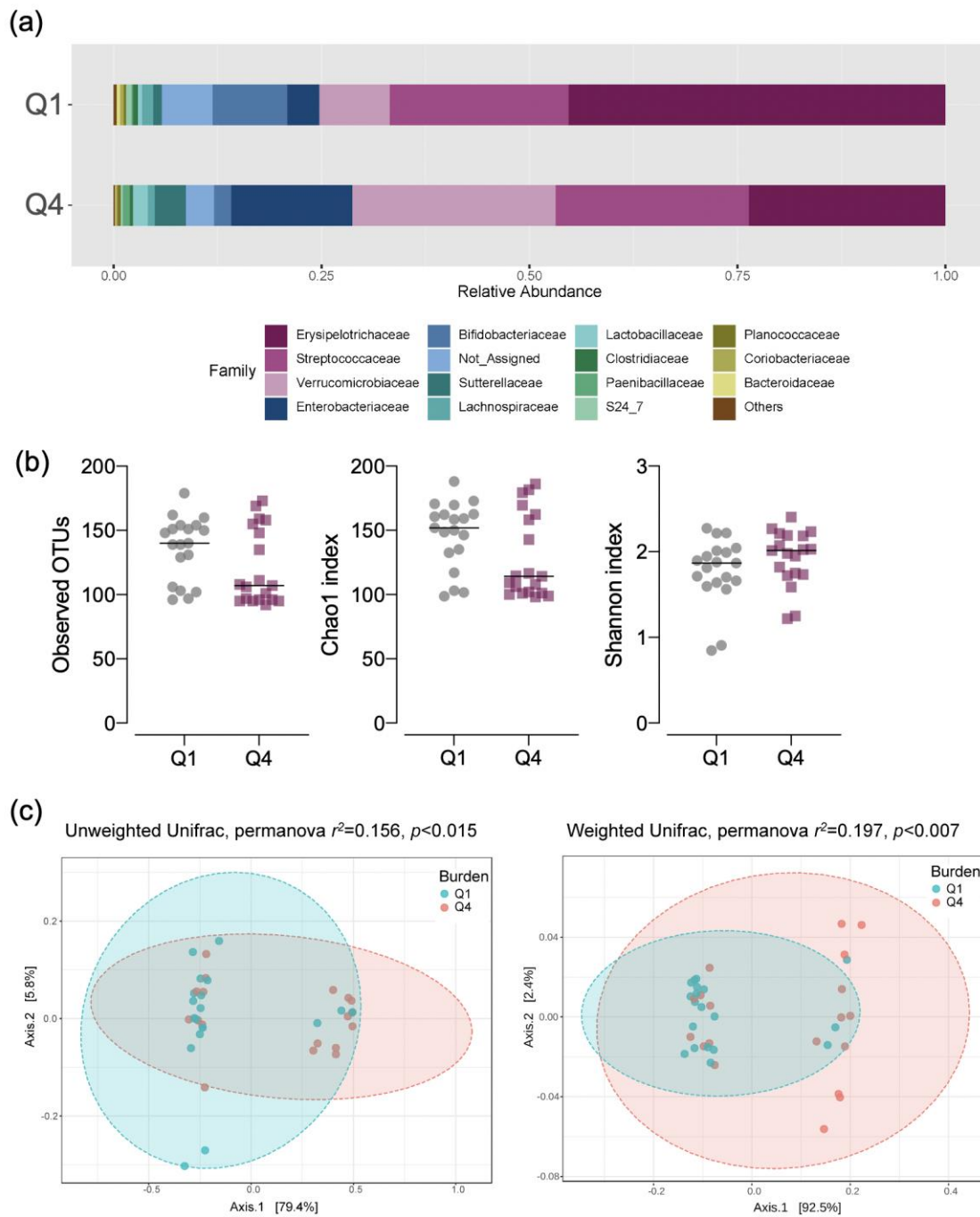


Figure S6. Taxonomic classification and diversity analyses for high and low tumor burden. Data were stratified into quartiles by tumor burden and the lowest 25% (Q1) and highest 25% (Q4) were compared. (a) Taxonomic classification at the family level. (b) Observed OTUs, Chao1 index, and Shannon index measures of alpha diversity. No significant differences between Q1 and Q4 were identified. (c) PCoA plots showing unweighted and weighted unifrac distances for beta diversity.

Table S1. Composition of experimental diets

Nutrient	AIN93G	TWD
Energy Density (kcal/g)	3.8	4.4
Macronutrients		
Carbohydrates (g/kg diet)		
Cellulose	50	30
Corn Starch	398	230
Maltodextrin	132	70
Sucrose	100	261
<i>kcal (% of total)</i>	<i>60.1%</i>	<i>54.5%</i>
Proteins (g/kg)		
Casein	200	190
L-cysteine	3	2.8
<i>kcal (% of total)</i>	<i>18.8%</i>	<i>15.4%</i>
Fats (g/kg)		
Anhydrous milk fat		36.3
Beef tallow		24.8
Cholesterol		0.4
Corn Oil		16.5
Lard		28
Olive oil		28
Soybean oil	70	31.4
<i>kcal (% of total)</i>	<i>17.2%</i>	<i>34.5%</i>
Micronutrients		
Minerals (mg/kg)		
Calcium	5000	2011
Copper	6	2.6
Iron	35	31
Magnesium	507	589
Phosphorus	3000	2757
Potassium	3600	5333
Selenium	0.15	0.2
Sodium	1019	7078
Zinc	30	25
Vitamins (unit/kg)		
Choline (mg)	1027	648
Folate (mg)	2	1.3
Niacin (mg)	30	50.6
Pyridoxine (mg)	6	3.9
Riboflavin (mg)	6	4.4
Thiamin (mg)	5	3.5
Vitamin A (IU)	4000	4300
Vitamin B12 (µg)	25	11
Vitamin D (IU)	1000	391
Vitamin E (IU)	75	24.6
Vitamin K (µg)	750	189

Notes: Composition of the TWD was previously published.¹ NHANES data do not provide intakes of biotin, chloride, iodine, manganese, pantothenic acid, or ultra-trace minerals. The levels of these micronutrients in the AIN93G diet were used in the formulation of the TWD. Abbreviations: AIN, American institute of Nutrition; TWD, total Western diet

¹ Hintze, K.J.; Benninghoff, A.D.; Ward, R.E. Formulation of the total western diet (TWD) as a basal diet for rodent cancer studies. *J. Agric. Food Chem.* **2012**, *60*, 6736-6742, doi:10.1021/jf204509a.

Table S2. MetagenomeSeq analyses of differential abundance of family- and genus-level taxa by experimental group and tumor burden

Family, Genus, Species	Diet main effect	AB main effect	Pairwise comparisons for diet and antibiotic treatment				High (Q4) vs. Low (Q1) Tumor Burden
			AIN: None vs. Vancomycin	TWD: None vs. Vancomycin	None: AIN vs. TWD	Vancomycin: AIN vs. TWD	
Actinobacteria	1.07E-01	5.61E-14	1.84E-04	6.35E-10	4.58E-01	1.00E+00	1.59E-02
Bifidobacteriaceae	8.36E-01	6.98E-27	1.87E-15	5.74E-13	3.91E-01	1.00E+00	6.41E-02
Bifidobacterium	7.13E-01	5.31E-29	1.67E-14	5.24E-16	6.08E-01	1.00E+00	4.08E-02
Coriobacteriaceae	5.88E-03	9.45E-28	2.58E-15	4.47E-17	4.68E-02	1.00E+00	4.95E-01
Adlercreutzia	1.00E-03	2.45E-19	1.46E-13	1.70E-09	2.91E-04	1.00E+00	4.34E-01
Micrococcaceae	9.24E-01	2.55E-06	3.50E-04	9.33E-04	9.43E-01	1.00E+00	6.34E-02
Nesterenkonia	8.77E-01	3.36E-07	4.63E-04	6.82E-04	7.54E-01	1.00E+00	7.63E-02
Bacteroidetes	1.07E-01	1.08E-09	1.91E-07	2.80E-04	9.14E-01	1.00E+00	7.22E-02
Bacteroidaceae	7.56E-02	2.18E-12	5.79E-10	1.08E-06	1.90E-01	1.00E+00	1.78E-01
Bacteroides	1.20E-01	4.65E-13	1.73E-07	2.90E-07	1.50E-01	1.00E+00	1.75E-01
Bacteroidales S24_7	1.00E+00	1.00E+00	3.94E-09	3.95E-09	9.00E-01	1.00E+00	6.41E-02
Rikenellaceae	2.22E-01	3.58E-07	7.65E-06	1.50E-04	2.11E-01	1.00E+00	2.06E-01
Firmicutes	4.18E-01	2.55E-16	3.81E-05	1.11E-09	4.58E-01	9.28E-01	1.95E-01
Clostridiaceae	9.24E-01	3.77E-17	5.36E-11	3.62E-09	5.53E-01	1.00E+00	7.90E-01
Christensenellaceae	1.00E+00	1.00E+00	1.00E+00	1.00E+00	2.43E-01	1.00E+00	
Enterococcaceae	2.60E-04	3.41E-06	1.88E-05	1.00E+00	7.34E-03	1.00E+00	6.30E-02
Erysipelotrichaceae	9.96E-01	1.87E-52	1.35E-25	2.27E-32	6.54E-01	2.42E-01	1.78E-01
Allobaculum	7.13E-01	5.01E-52	6.80E-21	8.30E-28	5.87E-01	3.11E-01	1.83E-01
Coprobacillus	1.28E-01	2.46E-12	3.08E-05	1.00E+00	2.65E-01	1.00E+00	
Lachnospiraceae	2.22E-01	1.59E-26	3.41E-18	9.19E-15	4.15E-01	1.00E+00	1.78E-01
Dorea	7.51E-02	8.07E-10	7.59E-06	5.02E-06	8.73E-02	1.00E+00	9.04E-01
Lactobacillaceae	9.75E-01	6.50E-10	2.99E-06	1.20E-07	8.30E-01	5.85E-03	4.95E-01
Lactobacillus	9.11E-01	4.22E-12	5.65E-06	1.88E-07	7.54E-01	1.00E+00	5.42E-01
Leuconostocaceae	1.00E+00	1.00E+00	1.00E+00	4.45E-05	2.11E-01	1.00E+00	
Paenibacillaceae	6.11E-01	1.21E-27	1.17E-13	6.12E-16	1.00E+00	4.04E-01	6.41E-02
Paenibacillus	9.11E-01	4.97E-30	1.67E-14	4.88E-16	1.00E+00	9.67E-01	4.08E-02
Peptostreptococcaceae	2.22E-01	4.87E-14	4.70E-09	1.36E-07	9.84E-01	1.00E+00	7.90E-01
Planococcaceae	9.75E-01	1.77E-08	1.09E-05	3.38E-07	3.26E-01	1.00E+00	3.82E-01

Table S2. MetagenomeSeq analyses of differential abundance of family- and genus-level taxa by experimental group and tumor burden

Family, Genus, Species	Diet main effect	AB main effect	Pairwise comparisons for diet and antibiotic treatment				High (Q4) vs. Low (Q1) Tumor Burden
			AIN: None vs. Vancomycin	TWD: None vs. Vancomycin	None: AIN vs. TWD	Vancomycin: AIN vs. TWD	
Ruminococcaceae	2.22E-01	7.22E-12	1.29E-06	4.75E-06	9.84E-01	1.00E+00	1.05E-01
Oscillospira	1.20E-01	8.24E-07	7.69E-04	1.64E-05	5.87E-01	1.00E+00	4.34E-01
Ruminococcus	3.86E-01	1.21E-23	1.06E-12	2.91E-10	6.08E-01	1.00E+00	1.83E-01
Staphylococcaceae	2.22E-01	3.39E-09	5.38E-06	1.15E-04	2.11E-01	1.00E+00	5.70E-01
Staphylococcus	1.70E-01	5.97E-09	1.29E-05	1.27E-04	1.45E-02	1.00E+00	6.32E-01
Streptococcaceae	2.22E-01	1.14E-02	8.45E-02	1.32E-02	2.52E-01	4.70E-01	7.91E-01
Lactococcus	1.35E-01	2.19E-03	6.24E-02	2.15E-02	1.76E-01	6.05E-01	8.24E-01
Proteobacteria	1.07E-01	4.47E-17	3.03E-05	2.63E-10	1.28E-02	9.28E-01	5.52E-02
Enterobacteriaceae	8.25E-01	2.83E-56	1.30E-26	2.98E-27	1.00E+00	4.70E-01	1.78E-01
Citrobacter	7.13E-01	2.42E-09	3.08E-05	1.62E-07	1.00E+00	1.67E-01	
Enterobacter	7.51E-02	4.93E-15	7.03E-06	1.07E-12	1.00E+00	4.21E-01	1.63E-01
Klebsiella	3.06E-02	4.27E-10	1.00E+00	2.79E-06	1.00E+00	4.55E-02	4.08E-02
Serratia	9.11E-01	9.25E-19	1.86E-07	3.88E-12	1.00E+00	3.99E-01	1.83E-01
Pseudomonadaceae	1.00E+00	1.00E+00	1.00E+00	1.59E-05	1.00E+00	8.86E-02	
Pseudomonas	1.00E+00	1.00E+00	1.00E+00	2.73E-04	1.00E+00	4.61E-02	
Sutterellaceae	9.75E-01	1.18E-32	1.79E-21	1.66E-16	3.29E-01	2.42E-01	1.78E-01
Sutterella ^a	4.17E-01	1.00E-35	5.08E-19	4.88E-16	7.21E-01	4.55E-02	1.63E-01
Teneriutes	1.00E+00	1.00E+00	7.26E-04	1.00E+00	1.28E-02	1.00E+00	
Verrucomicrobia	3.24E-01	2.89E-01	1.75E-03	3.11E-01	7.00E-01	9.28E-01	1.32E-01
Verrucomicrobiaceae	5.17E-01	5.05E-09	8.57E-05	1.36E-07	4.15E-01	9.52E-01	2.06E-01
Akkermansia	2.27E-01	1.23E-10	1.23E-04	2.94E-06	5.53E-01	6.05E-01	1.77E-01

Values above are the FDR-corrected p -values obtained using Microbiome Analyst metagenomeSeq with $\alpha = 0.05$ and the zero-inflated gaussian fit ($n=17$ to 23). Abbreviations: AIN, the AIN93G diet; TWD, the total Western diet; AB, antibiotic; Q1, lowest 25% quartile; Q4, highest 25% quartile. Empty cells indicate taxa was not included in the metagenomeSeq report for that specific comparison due to low counts.

^a The *Sutterella* genus was classified incorrectly as belonging to family Alcaligenaceae by GreenGenes database. The correct classification is shown here