

Supplementary Information

Bacteriophages dynamically modulate the gut microbiota and metabolome

Bryan B. Hsu^{1,2}, Travis E. Gibson³, Vladimir Yelisseyev³, Qing Liu³, Lynn Bry³,
Pamela A. Silver^{1,2,*}, Georg K. Gerber^{3,*}

¹Department of Systems Biology
Harvard Medical School
Boston, MA 02115 USA

²Wyss Institute for Biologically Inspired Engineering
Harvard University
Boston, MA 02115 USA

³Massachusetts Host-Microbiome Center, Department of Pathology
Brigham and Women's Hospital, Harvard Medical School
Boston, MA 02115 USA

*Corresponding author:
pamela_silver@hms.harvard.edu, ggerber@bwh.harvard.edu

Lead contact: ggerber@bwh.harvard.edu

1 **Table S1 | Host range of phages against infant gut bacteria.** Phages were spotted onto lawns of
2 each bacteria and incubated anaerobically overnight 37°C. Zones of clearing indicated infectivity. (+) =
3 lysis; (-) = no lysis

<i>Bacteria</i>	<i>Phages</i>			
	T4	F1	B40-8	VD13
<i>A. muciniphila</i>	-	-	-	-
<i>B. fragilis</i>	-	-	+	-
<i>B. ovatus</i>	-	-	-	-
<i>B. vulgatus</i>	-	-	-	-
<i>C. sporogenes</i>	-	+	-	-
<i>E. faecalis</i>	-	-	-	+
<i>E. coli</i>	+	-	-	-
<i>K. oxytoca</i>	-	-	-	-
<i>P. distasonis</i>	-	-	-	-
<i>P. mirabilis</i>	-	-	-	-

4
5
6

Table S2 | Summarized effect of bacteria and phages on fecal metabolites. Metabolites with significant changes were categorized into KEGG pathway and direction of change (e.g., increase or decrease).

KEGG Pathway	Number of screened metabolites	Metabolites significantly changing (adj. <i>p</i> -value < 0.05)											
		Bacteria only versus GF				+T4/F1 Phages (+13d) versus Bacteria only				+VD13/B40-8 Phages (+13d) versus +T4/F1 Phages (+13d)			
		Decreasing		Increasing		Decreasing		Increasing		Decreasing		Increasing	
		Number	% of Pathway	Number	% of Pathway	Number	% of Pathway	Number	% of Pathway	Number	% of Pathway	Number	% of Pathway
Amino acids	203	21	10.3%	145	71.4%	3	1.5%	30	14.8%	5	2.5%	0	0.0%
Peptides	37	6	16.2%	22	59.5%	2	5.4%	3	8.1%	0	0.0%	0	0.0%
Carbohydrates	31	12	38.7%	13	41.9%	4	12.9%	6	19.4%	0	0.0%	0	0.0%
Energy	12	0	0.0%	7	58.3%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Lipids	356	49	13.8%	193	54.2%	40	11.2%	13	3.7%	1	0.3%	0	0.0%
Nucleotides	63	18	28.6%	33	52.4%	0	0.0%	9	14.3%	0	0.0%	0	0.0%
Cofactors/Vitamins	45	6	13.3%	25	55.6%	0	0.0%	11	24.4%	0	0.0%	0	0.0%
Xenobiotics	113	15	13.3%	76	67.3%	12	10.6%	13	11.5%	0	0.0%	0	0.0%
		127	14.8%	514	59.8%	61	7.1%	85	9.9%	6	0.7%	0	0.0%
Overall	860	641 (74.6%)				146 (17.0%)				6 (0.7%)			

Table S3 | Protein homologs in the defined consortia. Homologous sequences were identified by protein BLAST against each strain of bacteria in the defined consortia. When strain sequences were unavailable, homology was compared to all species in the non-redundant protein sequences database. (A) Tryptophan decarboxylases that produce tryptamine and (B) tyrosine decarboxylases that produce tyramine.

A

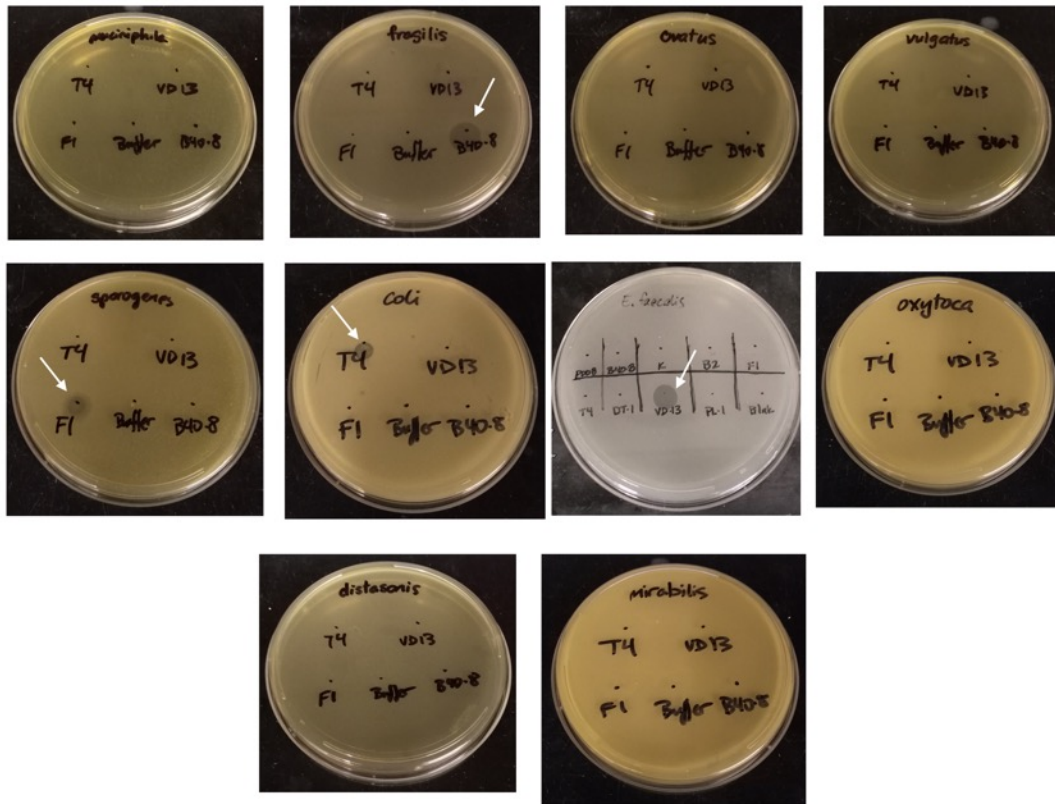
Bacteria	Consortia strain	Homology of tryptophan decarboxylases											
		<i>R. gnavus</i> derived (rumgna_01526)						<i>C. sporogenes</i> derived (clospo_02083)					
		Strain (or strain of closest homolog)	Sequence ID (or closest homolog)	Percent ID	AA length	Bit Score	E value	Strain (or strain of closest homolog)	Sequence ID (or closest homolog)	Percent ID	AA length	Bit Score	E value
<i>Bacteroides fragilis</i>	HSP40*	S6L5	EYE60564.1	28%	72	33.9 (76)	2.6	3_1_12	EFR53885.1	30%	168	52.8 (125)	2E-06
<i>Clostridium sporogenes</i>	213*	Isolate (Accession KRU25053)	KRU25053.1	27%	471	157 (398)	1E-41	ATCC 15579	EDU35915.1	100%	417	864 (2232)	0
<i>Enterococcus faecalis</i>	ATCC 29200	-	-	0%	-	-	-	ATCC 29200	EOJ09602.1	28%	21	53.9 (128)	1E-06
<i>Escherichia coli</i>	Nissle 1917	-	-	0%	-	-	-	Nissle 1917	AID78574.1	31%	86	49.7 (117)	2E-05
<i>Akkermansia muciniphila</i>	ATCC BAA-835	-	-	0%	-	-	-	ATCC BAA-835	ACD04211.1	25%	244	59.7 (143)	1E-08
<i>Bacteroides ovatus</i>	ATCC 8483	-	-	0%	-	-	-	ATCC 8483	EDO14168.1	30%	168	52.0 (123)	4E-06
<i>Bacteroides vulgatus</i>	ATCC 8482	-	-	0%	-	-	-	ATCC 8482	ABR41498.1	29%	165	53.5 (127)	1E-06
<i>Klebsiella oxytoca</i>	ATCC 700324*	Isolate (Accession SAP40428)	SAP40428.1	30%	410	208 (530)	1E-60	Isolate (Accession SAQ33394)	SAQ33394.1	28%	280	106 (256)	2E-24
<i>Parabacteroides distasonis</i>	ATCC 8503	-	-	0%	-	-	-	ATCC 8503	ABR42591.1	30%	168	52.0 (123)	4E-06
<i>Proteus mirabilis</i>	ATCC 29906	-	-	0%	-	-	-	ATCC 29906	EEI48912.1	22%	357	59.7 (143)	1E-08
<i>Ruminococcus gnavus</i>	-	ATCC 29149	EDN78222.1	100%	490	1024 (2647)	0	-	-	0%	-	-	-

* No genome sequence available for this strain so the sequence with greatest homology was used

B

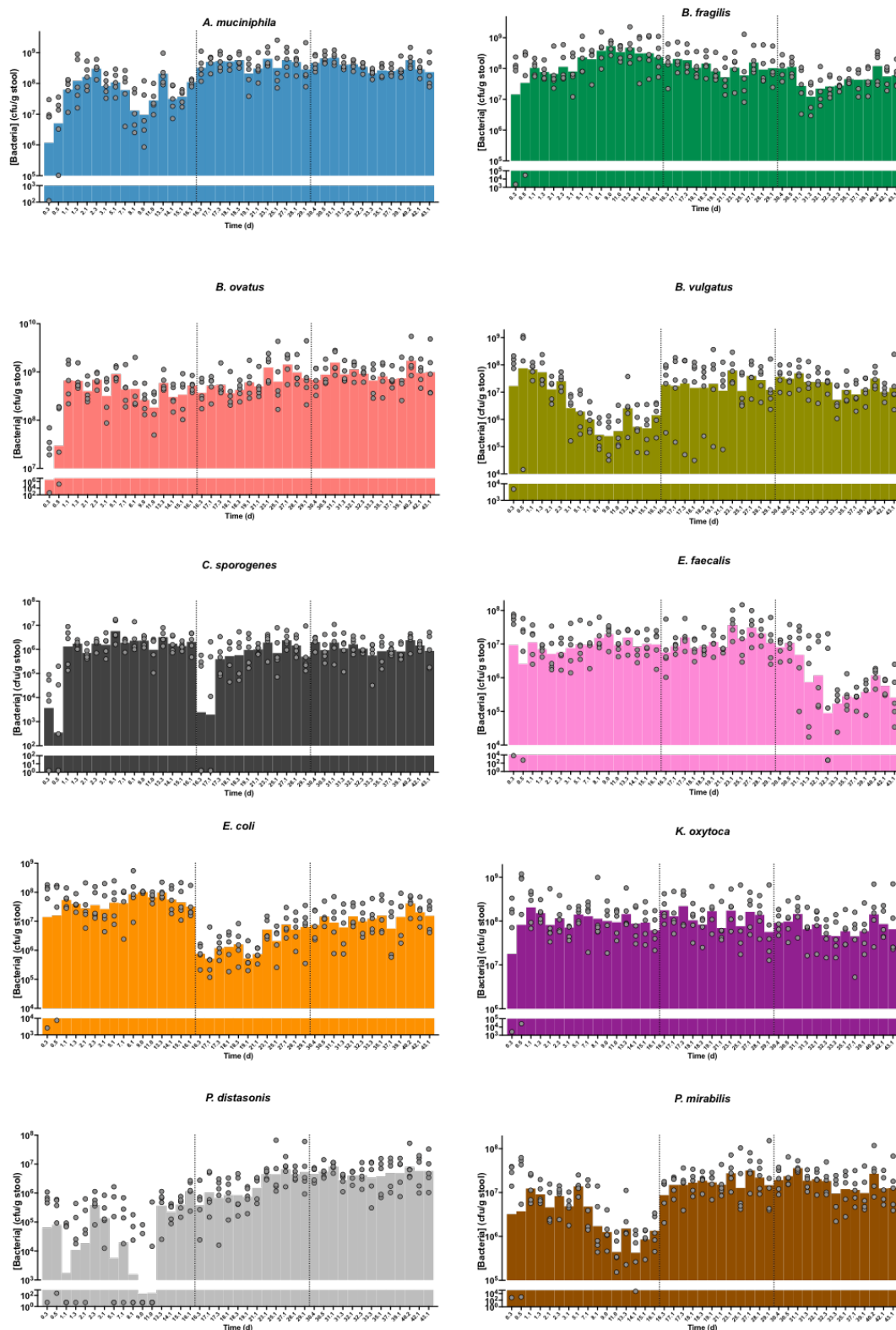
Bacteria	Consortia strain	Homology of tyrosine decarboxylases						
		<i>E. faecalis</i> derived (tyrDC)						
		Strain (or strain of closest homolog)	Sequence ID (or closest homolog)	Percent ID	AA length	Bit Score	E value	
<i>Bacteroides fragilis</i>	HSP40*	-	-	0%	-	-	-	
<i>Clostridium sporogenes</i>	213*	ATCC 15579	EDU35915.1	28%	417	60.8 (146)	1E-08	
<i>Enterococcus faecalis</i>	ATCC 29200	ATCC 29200	EOJ09602.1	100%	620	1274 (3298)	0	
<i>Escherichia coli</i>	Nissle 1917	-	-	0%	-	-	-	
<i>Akkermansia muciniphila</i>	ATCC BAA-835	-	-	0%	-	-	-	
<i>Bacteroides ovatus</i>	ATCC 8483	-	-	0%	-	-	-	
<i>Bacteroides vulgatus</i>	ATCC 8482	-	-	0%	-	-	-	
<i>Klebsiella oxytoca</i>	ATCC 700324*	Isolate (Accession FKZE01000017)	SAQ17440.1	29%	489	73.9 (180)	9E-13	
<i>Parabacteroides distasonis</i>	ATCC 8503	-	-	0%	-	-	-	
<i>Proteus mirabilis</i>	ATCC 29906	ATCC 29906	EEI49344.1	28%	610	231 (589)	1E-66	

* No genome sequence available for this strain so the sequence with greatest homology was used



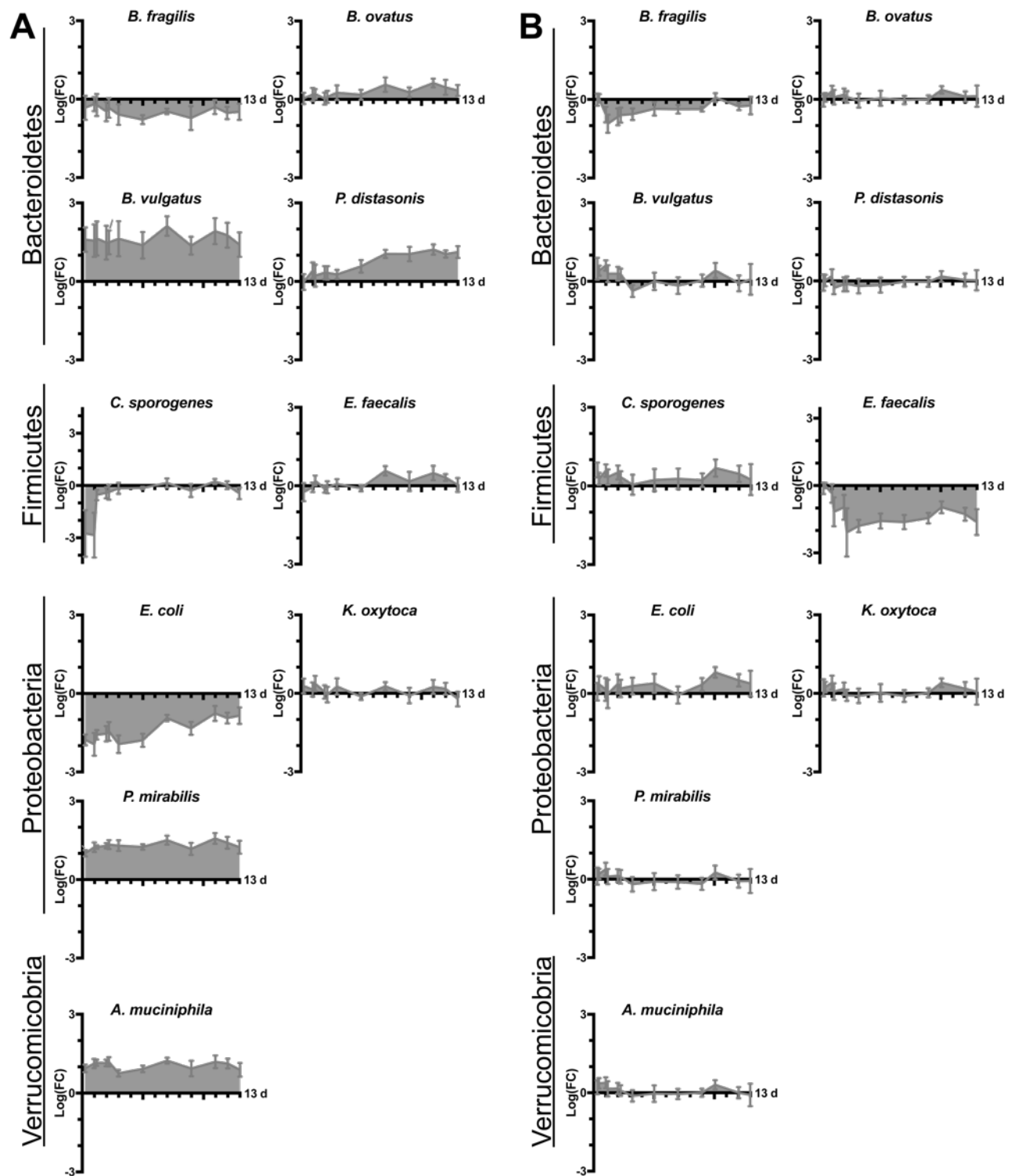
Supplementary Figure S1 | Spot tests of phage against each bacteria in the defined consortia.

Phages were spotted onto lawns of each bacteria and incubated anaerobically overnight 37°C. Zones of clearing indicated infectivity.

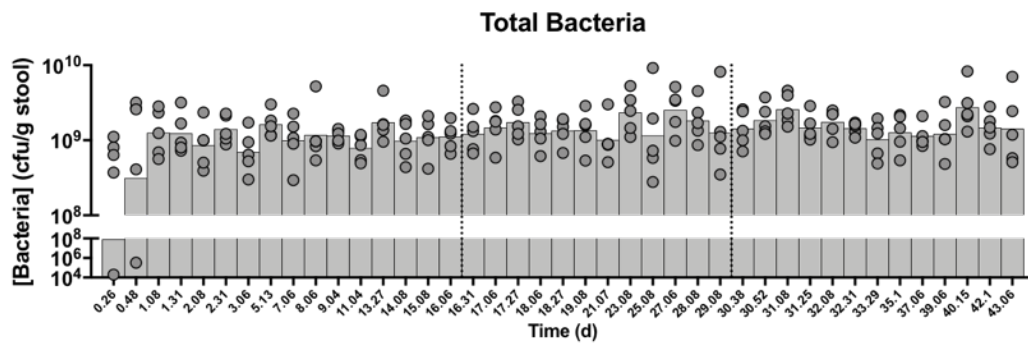


Supplementary Figure S2 | Fecal bacterial concentrations of each strain over time.

Concentrations of each strain for each mouse is represented by each circle while the geometric means are shown as bars. Vertical dashes represent administration of T4 and F1 phages, then B40-8 and VD13 phages, respectively.

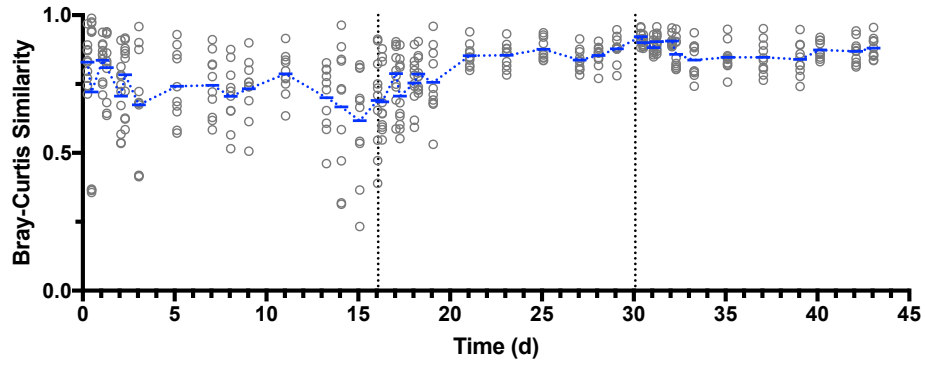


Supplementary Figure S3 | Relative change of each strain after phage perturbation. Relative fecal concentrations of each bacteria over time after perturbation by phages (A) T4 and F1 phages targeting *E. coli* and *C. sporogenes*, respectively, and (B) VD13 and B40-8 phages targeting *E. faecalis* and *B. fragilis*, respectively.

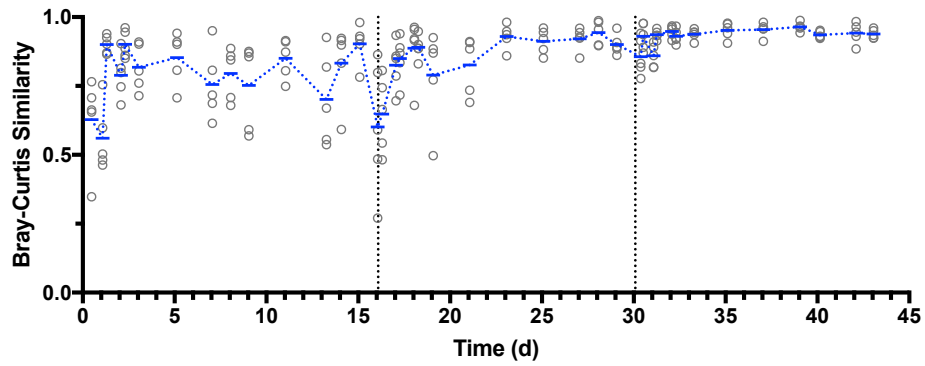


Supplementary Figure S4 | Total fecal bacteria over time. Concentration of fecal bacteria for each mouse is represented by each circle while the geometric means are shown as bars. Vertical dashes represent administration of T4 and F1 phages, then B40-8 and VD13 phages, respectively.

A

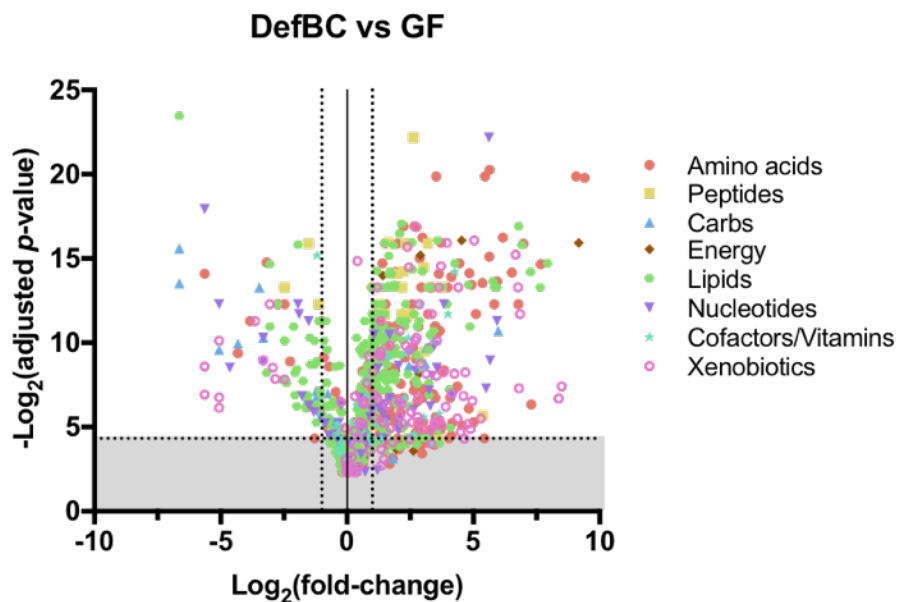


B



Supplementary Figure S5 | Bray-Curtis measures of similarity. The similarity in bacterial microbiota compositions was determined (A) between mice and (B) between timepoints.

1



2

3

4 **Supplementary Figure S6 | Volcano plot of the effect of bacterial colonization on the fecal**
5 **metabolome.** Points above the horizontal dashed line indicate significant changes with adjusted p -
6 values < 0.05 .

7

8

9