

1 Inflammation and bacteriophages affect DNA inversion states and functionality 2 of the gut microbiota

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28 Summary

29 Reversible genomic DNA-inversions control expression of numerous bacterial molecules in the human
30 gut, but how this relates to disease remains uncertain. By analyzing metagenomic samples from six
31 human Inflammatory Bowel Disease (IBD) cohorts combined with mice experimentation, we identified

32 multiple invertible regions in which a particular orientation was correlated with disease. These include
33 the promoter of the anti-inflammatory polysaccharide-A (PSA) of *Bacteroides fragilis*, which is mostly
34 oriented 'OFF' during inflammation but switches to the 'ON' orientation when inflammation is
35 resolved. We further detected increased abundances of *B. fragilis* associated bacteriophages in
36 patients with the PSA 'OFF' orientation. Isolation and analysis of a *B. fragilis* associated bacteriophage
37 revealed that it induced the PSA 'OFF' switch, thereby altering the bacterial induced immune
38 modulation. Altogether, we reveal large-scale dynamic and reversible bacterial phase variations driven
39 both by bacteriophages and the host inflammatory state signifying bacterial functional plasticity and
40 suggesting potential clinical interventions.

41

42 **Keywords**

43 Phase variation, gut microbiome, bacteriophages, inflammatory bowel diseases, Crohn's disease,
44 ulcerative colitis, *Bacteroides*, immunomodulation

45

46 **Introduction**

47 Phase variation is the process by which bacteria undergo frequent and reversible alterations in specific
48 loci of their genome¹⁻⁴. In Bacteroidales, the dominant order of bacteria in the human gut, phase
49 variation is highly prevalent and is largely mediated by inversions of DNA segments between inverted
50 repeats. These inversions often involve promoter regions dictating transcription initiation of genes or
51 operons functioning as 'ON'\OFF' switches⁵. In addition, DNA inversions can occur so that new genes
52 are brought from an inactive to a transcriptionally active site by re-orientation or recombination of
53 genomic shufflons and thus altering the expressed protein⁶⁻⁸. Analysis of the orientations of bacterial
54 invertible regions in various host disease states can provide new insights into bacterial adaptation and
55 functional contributions to the disease pathogenesis or its resolution. Phase variation in the gut
56 Bacteroidales often modulates production of components presented on the bacterial surface⁹
57 dictating which surface molecules interact, for example, with neighboring microbes or with the host.
58 As such, phase variation confers altered functional phenotypes and bacterial functional plasticity.

59 *Bacteroides fragilis*, a common resident of the human gut, modulates its surface by the phase variable
60 expression of its capsular polysaccharides (PS, denoted PSA-PSH). The biosynthesis loci of seven of its
61 eight polysaccharides have invertible promoters that are orientated either 'ON' or 'OFF' in respect to
62 the downstream PS biosynthesis operon⁴. Studies have shown that the *B. fragilis* polysaccharide A
63 (PSA) modulates the host immune system by inducing regulatory T cells (Tregs) and secretion of the
64 anti-inflammatory cytokine interleukin (IL)-10^{10,11}. Moreover, PSA was shown to confer protection
65 against experimental colitis^{10,12-14}, and thus is regarded as an anti-inflammatory polysaccharide.

66 Ulcerative colitis (UC) and Crohn's disease (CD) are multifactorial inflammatory bowel diseases (IBD),
67 characterized by a compromised mucosal barrier, inappropriate immune activation, and
68 mislocalization of the gut microbiota^{15–18}. Genetic, immunological, and environmental factors all
69 contribute to the development and progression of IBD. The gut microbiota is a major environmental
70 factor in IBD, and as such, IBD has emerged as one of the most studied microbiota-linked diseases. A
71 mechanistic analysis of bacterial functions and potential functional alterations will allow us to better
72 understand the role of the gut microbiota in IBD.

73 Here we present an analysis of invertible DNA orientations in the gut microbiota of IBD patients from
74 multiple cohorts across the globe, focusing on Bacteroidales species. Our combined analysis of IBD
75 patient metagenomes and experimental mouse models reveals alterations in multiple DNA invertible
76 sites during gut inflammation, with the potential to modulate the host immune system. Importantly,
77 we show that filtered fecal extracts of IBD patients alter the orientation of the PSA promoter in *B.*
78 *fragilis* populations. We show that bacteriophages are correlated with the PSA 'OFF' state and we
79 show that a specific lytic bacteriophage of *B. fragilis* increases the PSA promoter 'OFF' population with
80 concurrent decline of host colonic Treg cells. These findings reveal a dynamic interplay between gut
81 inflammation, bacteriophage, and bacterial phase variation, with potential implications for the
82 diagnosis and treatment of IBD.

83

84 Results

85 Multiple Bacteroidales invertible DNA regions have altered orientations in association with IBD

86 As a means to determine if Bacteroidales phase variable molecules may be differentially produced in
87 IBD patients compared to controls, we used the PhaseFinder⁹ software to identify and quantify DNA
88 inversions in the gut metagenomes of cohorts of IBD patients and control subjects [Table 1]. To do
89 this, we first analyzed 39 sequenced genomes representing different human associated strains (36
90 species) from the Bacteroidales order and identified 311 invertible regions in these genomes. Analysis
91 of these regions in human gut metagenomes identified a total of 147 statistically significant DNA
92 inversions associated with the disease from 25 of the reference bacterial genomes. These DNA
93 inversions were located in both invertible promoters and intragenic regions of diverse genes, spanning
94 from regulatory genes to outer surface molecules. Table S1 details the locations of the inverted
95 repeats (IR) sequences and genes flanking the phase variable regions [Figure 1A]. The invertible
96 regions whose orientations differed most significantly between IBD patients and controls were within
97 or in proximity to SusC/SusD-like outer membrane transport systems¹⁹ and capsular polysaccharides
98 (PS) promoters [Figure 1B]. Four of the five invertible capsular polysaccharide promoters of

99 *Bacteroides thetaiotaomicron* were differentially oriented between the groups, and three of the seven
 100 invertible PS promoters of *B. fragilis* were differentially oriented, with the anti-inflammatory PSA
 101 promoter being the most distinct between healthy and UC patients. The PSA promoter showed a
 102 higher percentage (71%) of reverse oriented reads (compared to its reference genome sequence) in
 103 IBD patients, compared to 56.2% in the healthy controls [Figure 1C, 1D]. To note, In the reference
 104 genome of *B. fragilis* NCTC9343 the promoter of PSA is in its 'ON' orientation, hence, the reverse
 105 orientation, found in IBD patients, represents the PSA promoter's 'OFF' orientation. We further
 106 identified differential promoter orientations in healthy and IBD cohorts for PULs and polysaccharides
 107 promoters of *Phocaeicola dorei*, a bacteria shown to be present in healthy subjects^{20,21} and also
 108 correlated with disease activity in UC²² [Figure 1E].

109

110 **Table 1: Cohorts of IBD patients and control subjects included in DNA inversion analysis.**

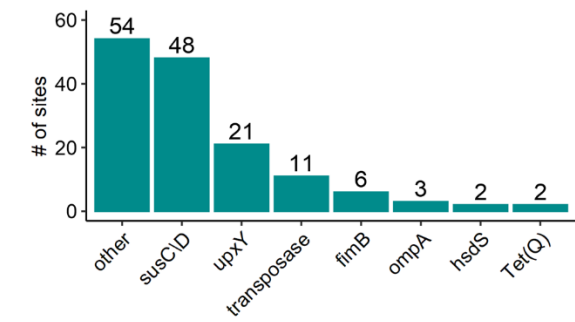
	N (Samples included in the analysis)	CD	UC	Non- IBD	Country	Notes	
IBDMDB	1283	574	349	360	United States	Longitudinal	ref ²³
HMP (3 phases)	320			320	United States		ref ²⁴
MetaHit	122	4	21	97	Denmark and Spain		ref ²⁵
1000IBD	331	205	126		the Netherlands		ref ²⁶
GeversD_2014	50	36		14	United States and Canada		ref ²⁷
LewisJD_2015	303	303			United States and Canada	Children with Crohn's Disease Longitudinal, treated	ref ²⁸
SUM	2409	1122	496	791			

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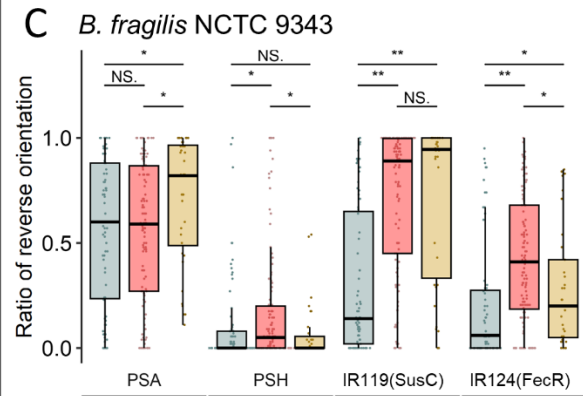
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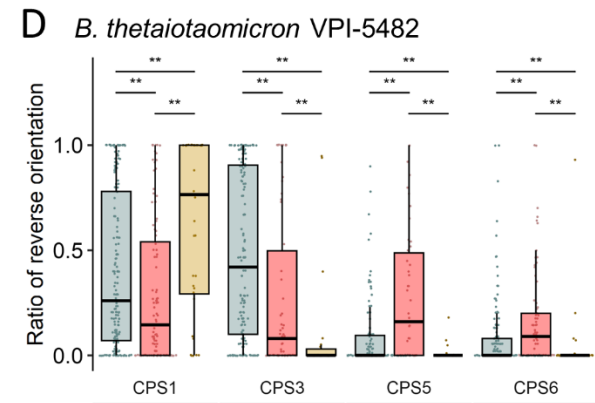
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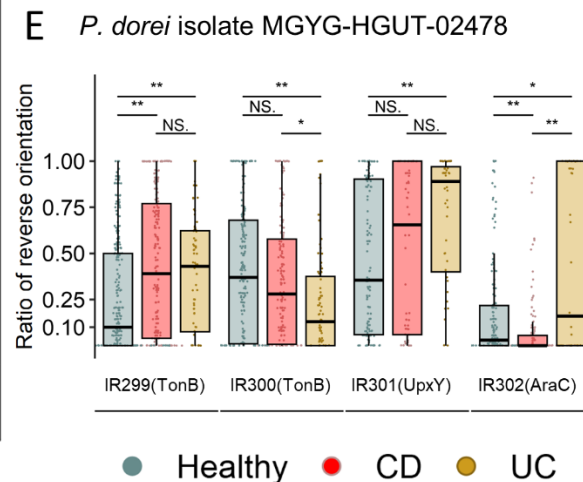
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D



E



112

113

114 **Figure 1: *Bacteroides* species exhibit phase variation in health and disease.**

115 **A.** Selected significantly differentially oriented invertible DNA regions (inverted repeats (IR) segments, see **Table S1**) (Wilcoxon rank sum
116 test, FDR $p < 0.05$) in at least one comparison between Healthy, CD (Crohn's Disease) and UC (Ulcerative Colitis). Blue indicates the Forward
117 orientation and red represents the Reverse orientation in comparison to the reference genome. **B.** Prevalence of functional genes in
118 proximity to invertible DNA regions significantly different between healthy individuals and IBD patients. **C.** Differentially oriented invertible
119 DNA regions, in *Bacteroides fragilis* NCTC 9343. PSA: Polysaccharide A, PSH: Polysaccharide H. **D.** Differentially oriented invertible DNA
120 regions in *Bacteroides thetaiotaomicron* VPI-5482. CPS: Capsular Polysaccharide. **E.** Differentially oriented invertible DNA regions in
121 *Phocaeicola dorei* MGYG-HGUT-02478. (Wilcoxon rank sum test, * $p < 0.05$; ** $p < 0.01$)

122 **Differential promoter orientation states are induced by inflammation in a dynamic and reversible**
123 **manner**

124 To assess the dynamics of the *B. fragilis* PSA promoter orientation under inflammatory conditions, we
125 designed a longitudinal experimental mouse model during which we analyzed the PSA promoter
126 orientation over time. Germ-free (GF) mice were colonized with a healthy human microbiota, which
127 was spiked with *B. fragilis* NCTC 9343, termed "humanized" mice. Experimental colitis was induced by
128 adding 3% Dextran sodium sulfate (DSS) to the drinking water²⁹ [**Figure 2A**] for 9 days after which DSS
129 was replaced with water until day 14, and as a result the inflammation was reduced. Inflammation
130 was confirmed by the levels of Calprotectin in the stool [**Figure 2C**], a commonly used biomarker for
131 inflammation, and with mouse weight loss [**Figure 2D**]. Stool was collected before, during and after
132 DSS treatment to analyze orientations of specific invertible DNA regions, using quantitative PCR
133 (qPCR). The *B. fragilis* PSA promoter orientation varied correlating with the inflammatory state [**Figure**
134 **2B**]. At the beginning of the experiment, ~45% of the population had the promoter in the 'ON'
135 orientation [**Figure 2B**]. Nine days after DSS was introduced, these percentages declined to 24%. By
136 day 14, the bacterial population returned to 40% 'ON', similar to the beginning of the experiment, and
137 to the control group, which did not receive DSS (mean of 58% of the *B. fragilis* population 'ON').

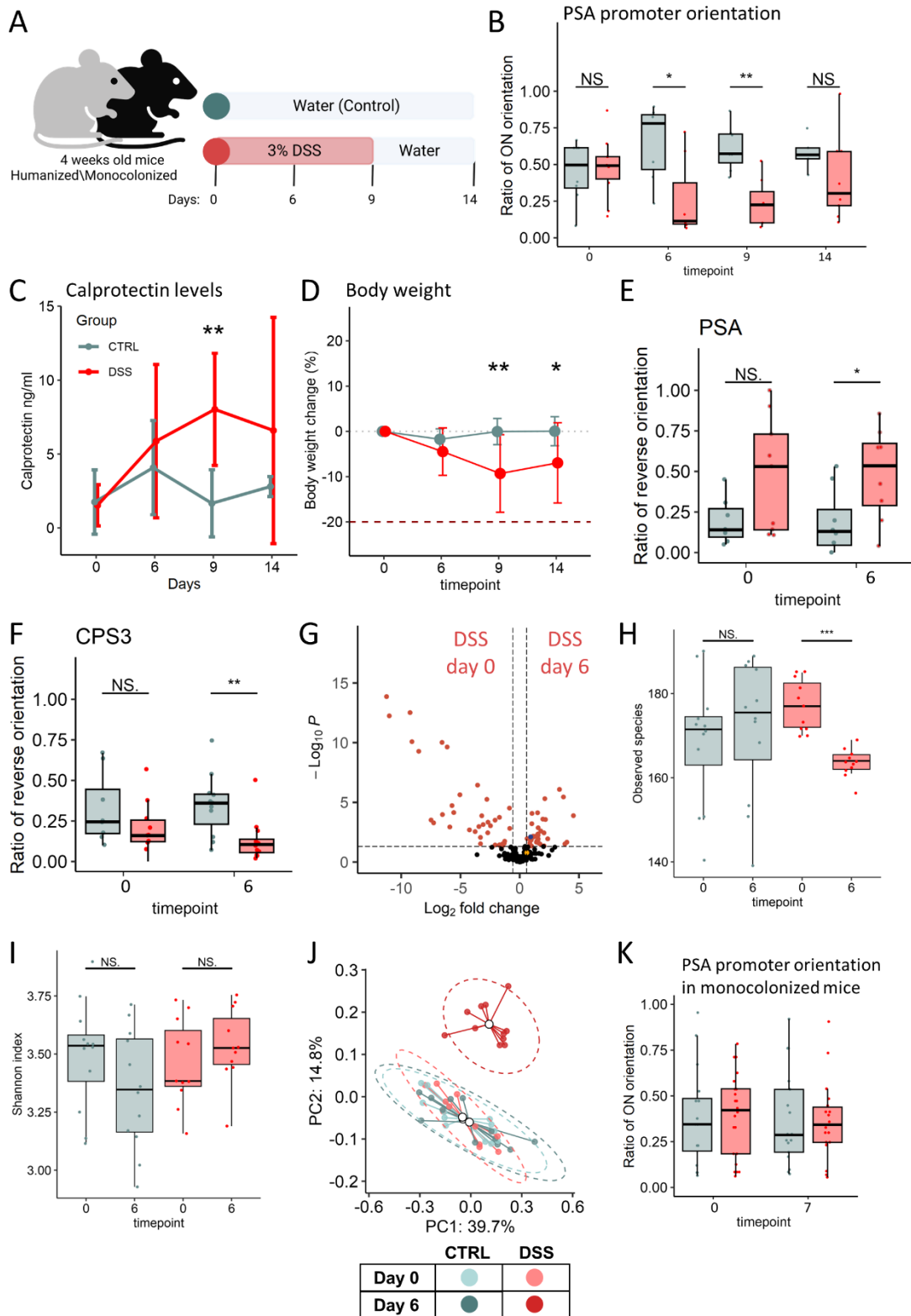
138 We further performed metagenomics analysis of these mice to study alterations in bacterial
139 composition and to apply the PhaseFinder⁹ algorithm for extensive phase variation analysis. This
140 analysis revealed phase variation in multiple bacteria and genomic regions, including the PSA
141 promoter of *B. fragilis* and the CPS3 promoter of *B. thetaiotaomicron*, overlapping with the results of
142 the human databases analysis [**Figure 2E, 2F, Supp. Table 2**].

143 Next, we identified alterations in bacterial composition of the DSS treated mice, whereas the control
144 mice were stable throughout. Bacterial richness (alpha-diversity, observed species) declined on day 6
145 under inflammatory conditions [**Figure 2H**], while evenness remained constant [**Figure 2I**]. Beta-
146 diversity (Bray-Curtis distance) was altered upon DSS treatment and each mouse had a different
147 bacterial composition five days after removal of the DSS [**Figure 2J**]. Inflamed mice showed a decrease
148 in bacterial abundances of *Blautia* species genera and *Eubacterium ventriosum*, while showing an
149 increase in *Barnesiella intestinhominis* and *Alistipes dispar* [**Figure 2G, Supp. table 2 and 3**]. The

150 relative abundances of *B. fragilis* and *B. thetaiotaomicron*, which exhibited differential orientations of
151 invertible DNA regions, increased in inflamed mice, although to a lesser extent **[Figure 2G]**.

152 To assess the role of inflammation on the orientation of the *B. fragilis* PSA promoter in the absence of
153 a complex microbiota, we repeated the experiment using gnotobiotic mice mono-colonized with *B.*
154 *fragilis* NCTC 9343. The PSA promoter orientation remained stable in the monocolonized mice over
155 the course of the experiment, suggesting a role of the microbiota in the relative 'OFF' orientation of
156 this promoter during inflammation. **[Figure 2K]**.

157



158

159 **Figure 2: Relative orientation of the PSA promoter of *B. fragilis* from the population is affected by inflamed state of the gut.**

160 **A.** Illustration of murine model of inflammation. Mice monocolonized with *B. fragilis* NCTC 9343 and mice colonized with a human microbiota
 161 spiked with *B. fragilis* NCTC 9343 - "Humanized", were exposed to 3% Dextran sodium sulfate (DSS) (Day=0 in the illustration) at four weeks
 162 of age; **B.** Ratio of *B. fragilis* PSA's promoter ON orientation measured by qPCR in different days of the experiment. Data represent the
 163 median (line in box), IQR (box), and minimum/maximum (whiskers). (Wilcoxon rank sum test, * $p < 0.05$; ** $p < 0.01$). Blue: Control group,
 164 Red: DSS treated mice. **C.** Calprotectin level (ng/ml) measured in different days of the experiment. Lines represent the standard deviations.
 165 Blue: Control group, Red: DSS treated mice. **D.** The body weight change of mice measured in different days of the experiment. Lines represent
 166 the standard deviations. Blue: Control group, Red: DSS treated mice. **E.** Ratio of *B. fragilis* PSA's promoter reverse orientation measured by
 167 the PhaseFinder tool, in different days of the experiment. Data represent the median (line in box), IQR (box), and minimum/maximum

168 (whiskers). (Wilcoxon rank sum test, $*p < 0.05$; $**p < 0.01$). Blue: Control group, Red: DSS treated mice. F. Ratio of *B. thetaiotaomicron*
169 CPS3's promoter reverse orientation measured by the PhaseFinder tool in different days of the experiment. Data represent the median (line
170 in box), IQR (box), and minimum/maximum (whiskers). (Wilcoxon rank sum test, $*p < 0.05$; $**p < 0.01$). Blue: Control group, Red: DSS treated
171 mice. G. Differential bacterial abundance between DSS treated mice in timepoint 0 and timepoint 6 detected by the Maaslin2 algorithm.
172 Red dots indicate differentially abundant bacteria that were determined by adjusted P value < 0.05 and \log_2 fold change >1 and <-1 ,
173 respectively. Yellow dot indicates *B. fragilis*, and blue dot indicates *Bacteroides thetaiotaomicron*. H. Alpha diversity (observed species)
174 between groups and timepoints. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). (t test, $*p < 0.05$;
175 $**p < 0.01$, $***p < 0.001$). Blue: Control group, Red: DSS treated mice. I. Alpha diversity (Shannon index) between groups and timepoints.
176 Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). (t test, $*p < 0.05$; $**p < 0.01$, $***p < 0.001$). Blue:
177 Control group, Red: DSS treated mice. J. PCoA on Bray–Curtis dissimilarity distances between groups and timepoints. Each point represents
178 a single sample, colored according to group and timepoints: light blue: Control at timepoint 0, blue: control at timepoint 6, light red: DSS
179 treated at timepoint 0, red: DSS treated at timepoint 6. The mean (centroid) of samples in each group is indicated with a blank circle. Ellipses
180 represent 0.95 confidence interval of each group. K. Ratio of *B. fragilis* PSA's promoter ON orientation measured by qPCR in different days
181 in monocolonized mice. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). (Wilcoxon rank sum test,
182 $*p < 0.05$; $**p < 0.01$). Blue: Control group, Red: DSS treated mice.

183
184

185 **The inflammatory milieu triggers DNA inversions in *B. fragilis***

186 We next sought to examine environmental factors of inflammation that result in a decreased
187 percentage of the *B. fragilis* population with the PSA promoter orientated ON. To do so, we exposed
188 *B. fragilis* NCTC 9343 to fecal filtrates from IBD patients. CD and UC patients were recruited from the
189 Rambam Health Care Campus (RHCC). Fecal samples were collected before and after Infliximab (HR)
190 or Humira (HuR) therapy, both are antibodies targeted against tumor necrosis factor- α (TNF- α), an
191 inflammatory cytokine increased in IBD patients. *B. fragilis* NCTC 9343 was cultured in fecal filtrates
192 until reaching mid-log phase, and subsequently DNA was extracted for qPCR analysis [Figure 3A]. *B.*
193 *fragilis* exposed to fecal filtrates of patients before treatment, during inflammation, showed higher
194 ratios of the population with the PSA promoter oriented 'OFF', while *B. fragilis* exposed to fecal
195 filtrates after treatment showed higher ratios of the PSA promoter 'ON' orientation [Figure 3B]. This
196 observation was in line with the fecal Calprotectin concentrations, which were reversely correlated
197 with PSA promoter 'ON' orientation [Figure 3C]. These results demonstrated that the population of
198 bacteria with the PSA promoter in each orientation vary in the inflamed and non-inflamed gut, with a
199 higher percentage of the population in the 'OFF' orientation under inflammatory conditions, and a
200 shift towards the 'ON' orientation following reduction in inflammation [Figure 3B, 3C]. This change
201 could be due to alterations of the inversion under these conditions or due to selection of a preferred
202 promoter state.

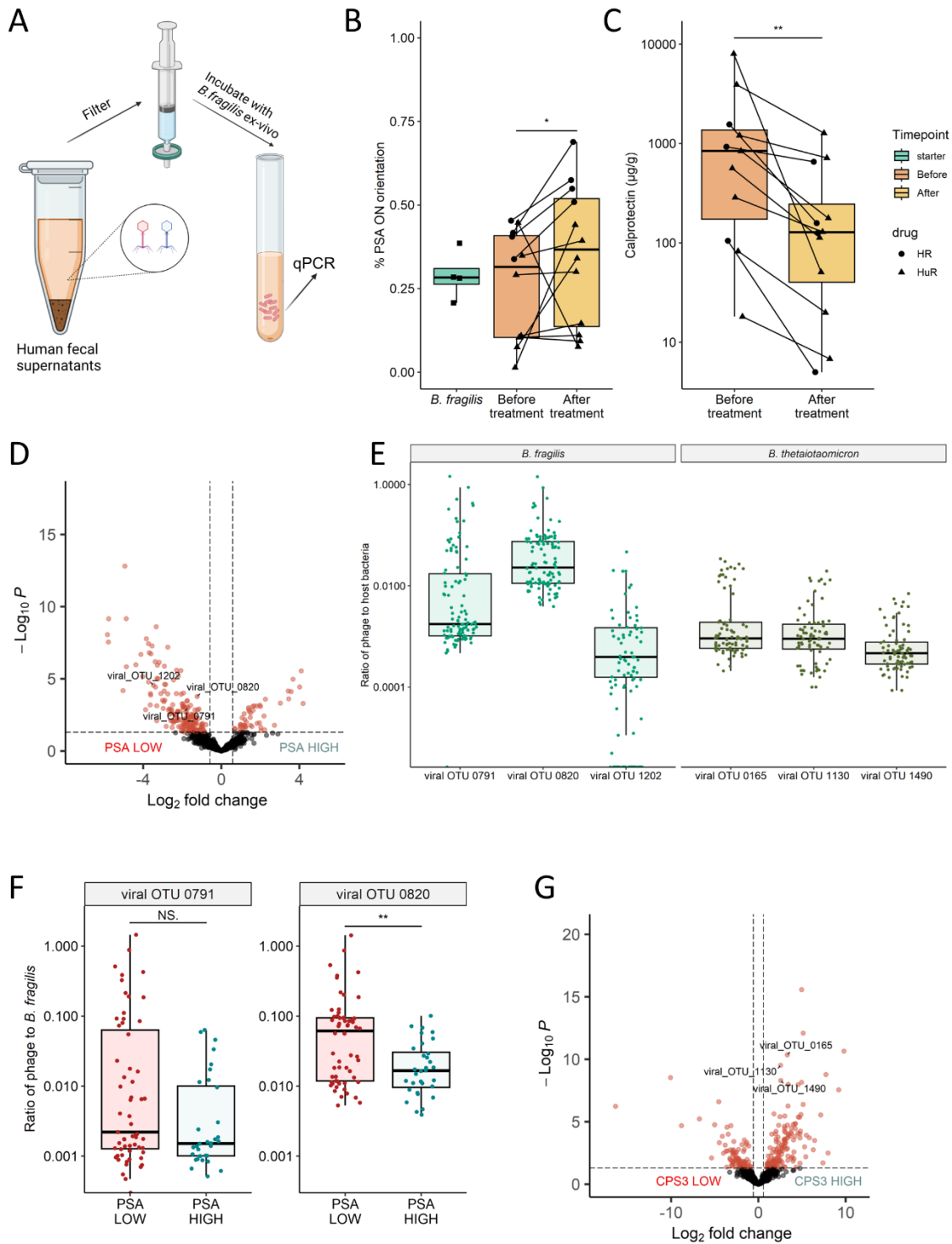
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204 **Investigating viral associations to orientation of the polysaccharide A promoter of *B. fragilis***

205 The fecal filtrates contain a mixture of bacterial and host metabolites, cytokines, antibodies, viruses,
206 bacteriophages, and other components. Bacteriophages have been observed to induce phase
207 variation in bacterial cells, leading to the switching 'OFF' of specific polysaccharides loci or increased

208 expression of alternative polysaccharides with possible protective effects³⁰. Moreover, they have been
209 shown to be associated with intestinal inflammation and IBD in several studies^{31,32}, including
210 Nishiyama et al.³³ who characterized temperate bacteriophages and their bacterial hosts in the
211 IBDMDB metagenomics database. Here, using phage sequences from IBDMDB database, and
212 compared the relative abundances of these phages between samples that displayed lower (<40%) or
213 higher (>60%) ratios of the PSA promoter 'ON' orientation. Differential abundance analysis revealed
214 that more bacteriophages were correlated with the PSA 'OFF' orientation (**Figure 3D, Supp. Table 3**),
215 three of which were predicted to infect *B. fragilis*³³. To assess whether the relative abundances of the
216 bacteriophages were accompanied with lower relative abundances of *B. fragilis*, we compared phage
217 to host for each *B. fragilis* associated bacteriophage. The two viral OTUs with the highest phage to
218 host ratios in patients, OTUs 0791 and 0820, were also correlated with low ratios of the PSA promoter
219 'ON' orientation (i.e., with the PSA promoter 'OFF' orientation) [**Figure 3E, 3F**]. Intriguingly, viral OTUs
220 0791 and 0820 were found to be more abundant in active CD and UC³³.

221 We conducted the same analysis comparing samples with high to low 'ON' orientation ratios of the
222 CPS3 promoter of *B. thetaiotaomicron*. Although there were differences in the virome compositions
223 between these groups, no *B. thetaiotaomicron* associated bacteriophages were correlated with the
224 'OFF' orientation of the CPS3 promoter (i.e., the orientation, which we identified as associated with
225 the disease) [**Figure 3G, Supp. Table 3**]. We found three *B. thetaiotaomicron* associated viral OTUs -
226 0165, 1130, and 1490, slightly associated with the 'ON' orientation of the CPS3 promoter (the
227 orientation that we identified as associated with healthy controls) [**Figure 3G**]. In addition, these
228 bacteriophages displayed low phage to host ratios [**Figure 3E**]. To note, these OTUs were not
229 previously associated with disease³³.



230

231 **Figure 3: Evidence of viral association to *B. fragilis* Polysaccharide A's promoter genomic orientation.**

232 **A.** Experimental design of culturing *B. fragilis* in patient's fecal filtrates. **B.** Ratio of the ON orientation of the PSA promoter of *B. fragilis*,
 233 measured by qPCR, after ex-vivo exposure to fecal filtrates of CD patients before and after treatment with anti-TNF (either Infliximab (HR)
 234 or Humira (HuR)). Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). (One-sided Wilcoxon rank sum
 235 test, $*p < 0.05$). green: *B. fragilis* not exposed to fecal filtrates, orange: *B. fragilis* exposed to fecal filtrates of patients before anti-TNF
 236 treatment, yellow: *B. fragilis* exposed to fecal filtrates of patients after treatment. Dots represent individual experiments; lines connect
 237 experiments from the same patient; Shapes are determined by the patients' treatments, circle: HR, triangle: HuR. **C.** Calprotectin levels
 238 ($\mu\text{g/g}$) measured in patients' feces. Data represent the median (line in box), IQR (box), and minimum/maximum (whiskers). (One-sided
 239 Wilcoxon rank sum test, $*p < 0.05$). Dots represent samples; lines connect samples from the same patient; Shapes are determined by the
 240 patients' treatments, circle: HR, triangle: HuR. **D.** Differential viral taxonomic units' abundance, (from the IBDMDB cohort, count table from
 241 Nishiyama et al. (2020).), between samples with low ON orientation of the PSA promoter (<40%) and high ON orientation (>60%), in the
 242 IBDMDB cohort count table. Differentially abundant viral taxonomic units were detected by the DeSeq2 algorithm (Wald test, $p < 0.01$). Red
 243 dots indicate differentially abundant bacteria that were determined by P value < 0.01 and fold change >1.5 and <-1.5, respectively. **E.** Phage
 244 to host ratios of viral OTUs detected in Figure 3D and 3F. **F.** Phage to host ratios of viral OTUs detected in Figure 3D against samples' levels

245 of PSA's promoter ON orientation analyzed from the IBDMDB cohort, based on the count table from Nishiyama et al. (2020). **G.** Differential
246 viral taxonomic units' abundance, (from the IBDMDB cohort, count table from Nishiyama et al. (2020).), between samples with low ON
247 orientation of the CPS3 promoter (<40%) and high ON orientation (>60%), in the IBDMDB cohort. Differentially abundant viral taxonomic
248 units were detected by the DeSeq2 algorithm (Wald test, $p < 0.01$). Red dots indicate differentially abundant bacteria that were determined
249 by P value < 0.01 and fold change > 1.5 and < -1.5 , respectively.
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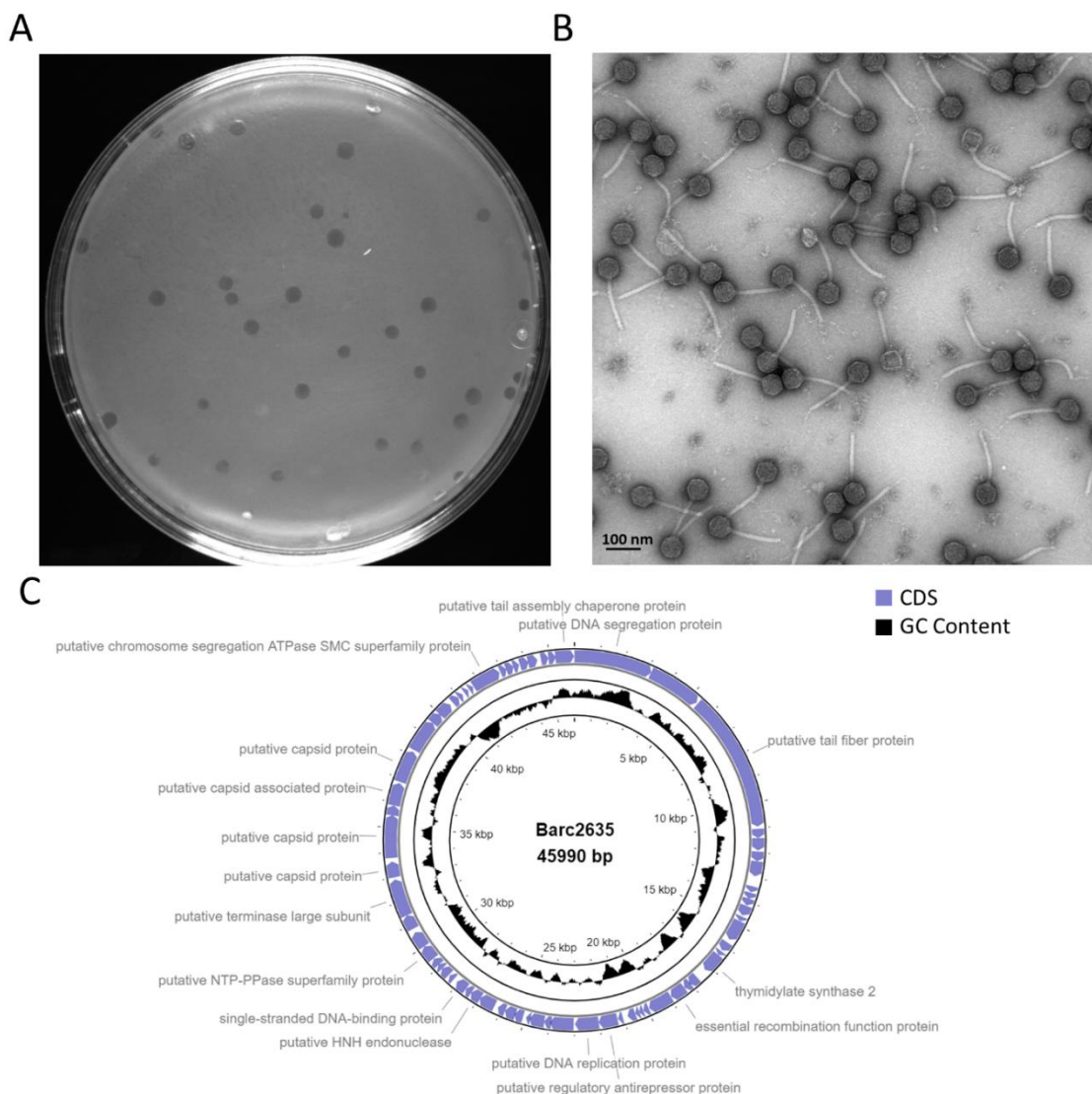
251 **Bacteriophage exposure drives phase variation and reduction in Treg cells population**

252 Since a higher abundance of bacteriophages were correlated with the PSA promoter in the 'OFF'
253 orientation, we sought to study whether encounter with bacteriophage can alter the orientation of
254 this promoter in *B. fragilis*. We started by isolating a bacteriophage from sewage that we designated
255 bacteriophage Barc2635 that makes plaques on a lawn of *B. fragilis* NCTC934 [**Supp Figure 1A**]. We
256 sequenced the phage genome (GenBank accession: MN078104) and characterized its morphology by
257 electron microscopy [**Supp. Figure 1B**]. Barc2635 is a double-stranded DNA lytic bacteriophage of
258 45990 bp with a GC content of 38.9%, containing 67 putative CDS belonging to the *Siphoviridae* family
259 and the *Caudovirales* order [**Supp. Figure 1C**]. Interestingly, increased abundance of bacteriophages
260 from the *Caudovirales* order were found to be correlated with IBD patients (UC and CD)³⁴. We
261 analyzed the sequence similarities of Barc2635, with the *B. fragilis* associated bacteriophages
262 identified in the IBDMDB³³ and found that Barc2635 is most similar to a cluster of bacteriophages,
263 including viral OTUs 0791 and 0820 which were associated with active disease³³ [**Figure 4A**].

264 To test whether this bacteriophage causes changes in relative orientations of the PSA promoter in
265 populations of *B. fragilis*, we monocolonized GF mice with *B. fragilis* NCTC 9343 in the presence or
266 absence of bacteriophage Barc2635 and analyzed genomic-wide DNA inversion states [**Figure 4B**]. In
267 the presence of Barc2635, *B. fragilis* exhibited prolonged alterations in the orientations of invertible
268 regions affecting outer surface components such as polysaccharide utilization loci (PULs), ABC
269 transporters and capsular polysaccharides, including PSA [**Supp. Table 4**]. In response to
270 bacteriophage, a higher percentage of the population had the PSA promoter in the 'OFF' state [**Supp.**
271 **Figure 2A**], with a concomitant increase in the percentage of the population with the PSF promoter
272 orientated 'ON' [**Supp. Figure 2B**]. Throughout the experiment (42 days), Barc2635 and *B. fragilis* co-
273 existed in gnotobiotic mice, with mild fluctuations in their abundances [**Supp. Figure 2C**]. Ten days
274 after the initial colonization, we quantified expression of the first gene of the PSA locus, *upaY*³⁵. *upaY*
275 expression requires that the PSA promoter be oriented 'ON', and that no inhibitory UpxZ products
276 from other loci are produced³⁶. **Figure 4C** shows a significant reduction in *upaY* expression in mice
277 containing Barc2635 in comparison to mice with bacteria alone. To directly measure the levels of PSA
278 on the surface of the *B. fragilis* population, we used specific antibodies and monitored surface PSA by
279 flow cytometry. The percentage of bacterial cells expressing PSA from mice with bacteriophage
280 Barc2635 was significantly lower in comparison to bacterial cells from mice without the bacteriophage

281 **[Figure 4D]**, in agreement with the *upaY* expression levels. In both groups of mice (i.e., *B. fragilis* alone
282 and *B. fragilis* with Barc2635), the CFU levels of *B. fragilis* were comparable, with a $10^{0.5}$ difference
283 **[Supp. Figure 2D]**, and no phages were detected in the *B. fragilis* monocolonized group (i.e., without
284 bacteriophage inoculation) **[Supp. Figure 2E]**.

285 In a previous study, we demonstrated that phase variation of specificity proteins for a Type I
286 restriction-modification system (R-M) of *B. fragilis* results in altered transcription of some capsular
287 polysaccharides with potential altered immunomodulatory functionality of these bacteria⁷. Since the
288 Type I R-M systems are known to provide protection against bacteriophage³⁷⁻⁴⁰, we used long-reads
289 sequencing to quantify the orientations of the genes encoding the Type I R-M specificity proteins upon
290 exposure to Barc2635. Our results demonstrate different patterns of specificity gene combination in
291 the expression locus in *B. fragilis* isolated from mice inoculated with the phage compared to those not
292 exposed to phage **[Figure 4E]**.



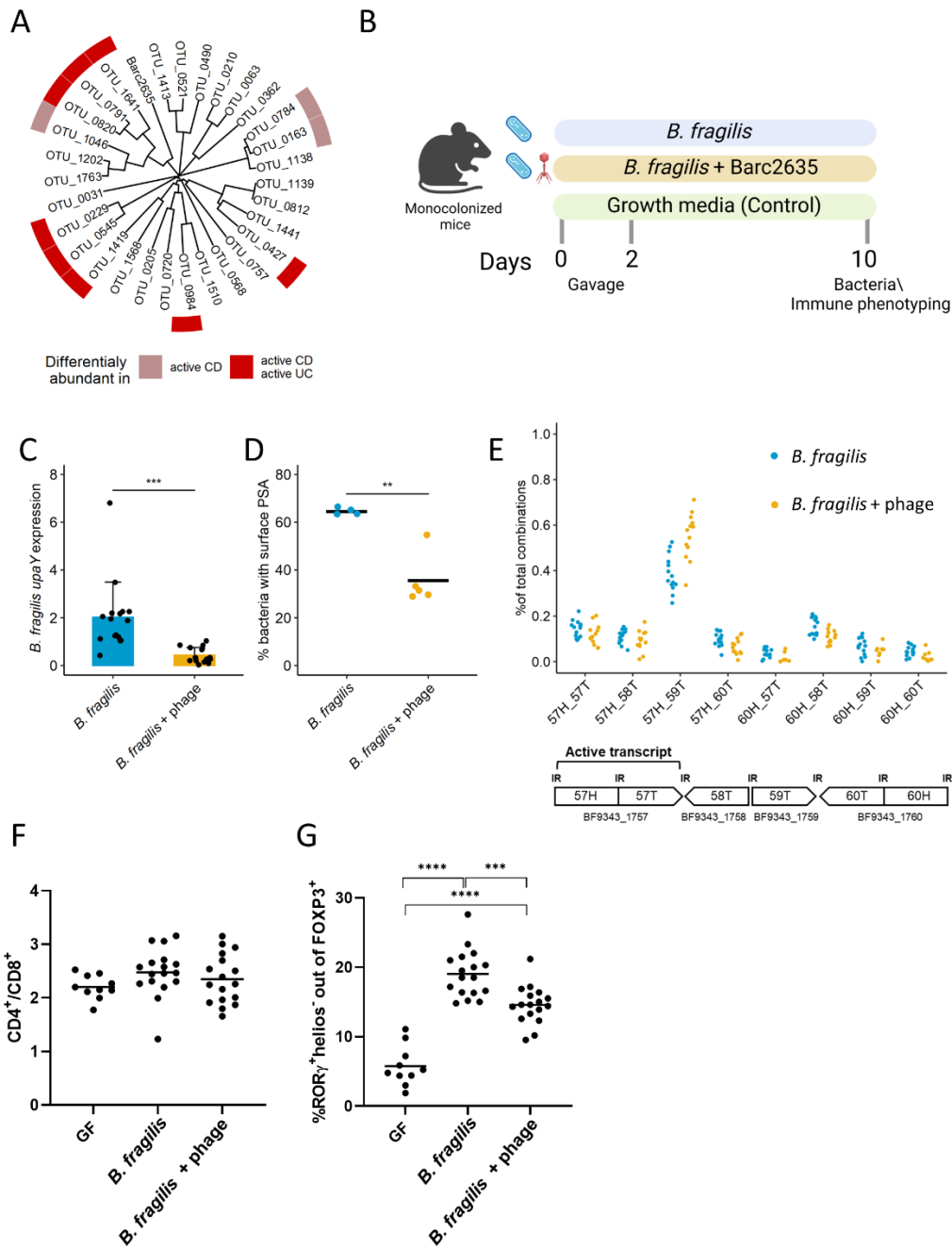
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294 **Supplemental Figure 1: Characterization of Bacteriophage Barc2635**

295 A. A representative transmission electron microscopy of Barc2635. B. Barc2635 plaques on a lawn *B. fragilis* NCTC 9343. C. Genome
296 structure, GC content, and putative annotations of Barc2635. CDS: coding sequence.

297

298 The PSA of *B. fragilis* induces regulatory T cells (Tregs, CD4⁺Foxp3⁺Rorg⁺) in the colonic lamina propria
299 of mice^{41–44}. To monitor the effect of intestinal Barc2635 during *B. fragilis* colonization on the
300 CD4⁺Foxp3⁺Rorg⁺ Tregs population, we extracted cells from the colonic lamina propria and
301 immunophenotyped them by flow cytometry. We found that in the presence of Barc2635, the
302 CD4⁺Foxp3⁺Rorg⁺ Tregs population is decreased in concert with the decrease in the *upaY* transcription,
303 coinciding with less surface production of the immunomodulatory PSA [**Figure 4F**, **Figure 4G**]. These
304 data link phage to alterations in bacterial functionality with concomitant effects on host physiology.



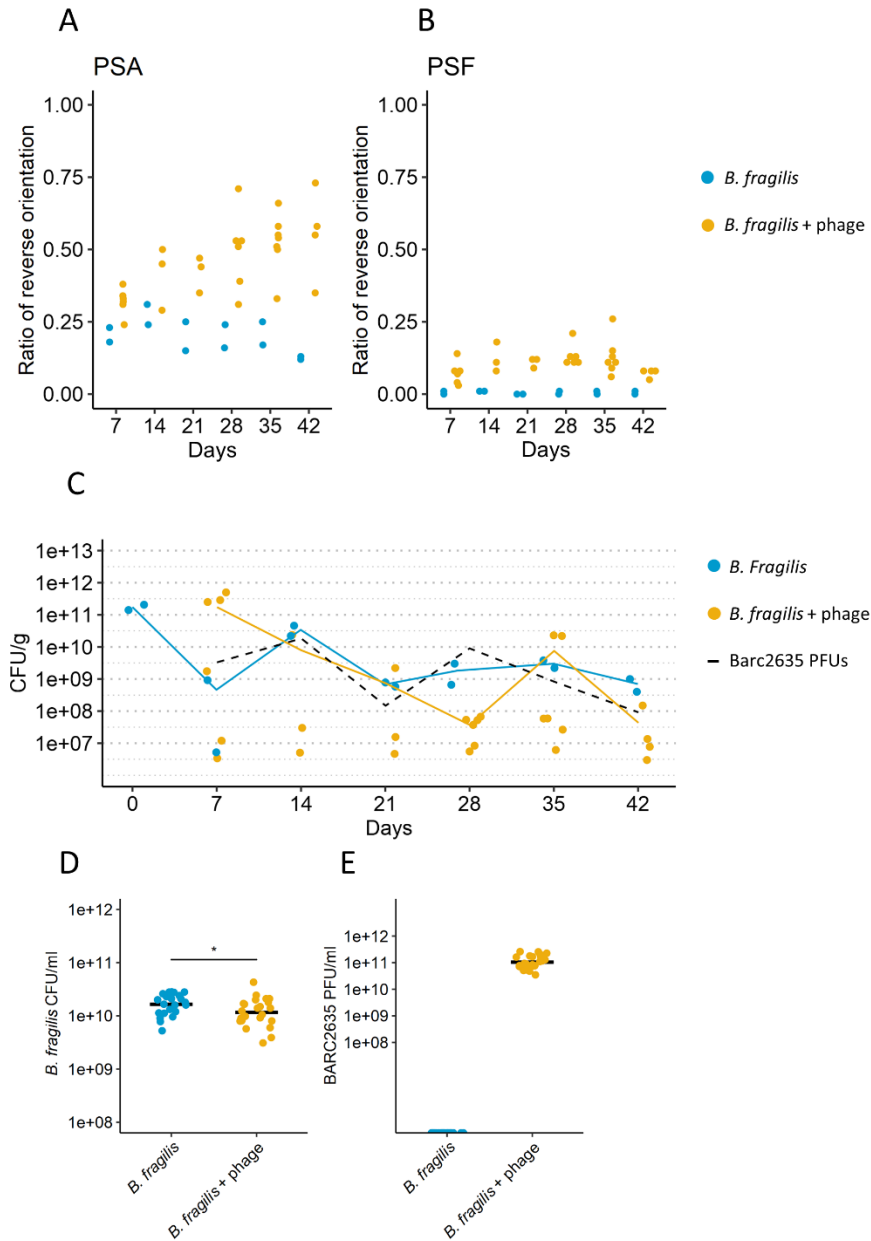
305

306 **Figure 4: Phage exposure alters expression of the PSA locus and PSA surface**

307 **A.** Phylogenetic tree based on the whole genome of viral OTUs identified as bacteriophages against *B. fragilis* as well as bacteriophage
 308 Barc2635. MAFFT was used to perform multiple sequence alignment. The average-linkage method was used to construct the phylogenetic
 309 tree with 1000 bootstrap replicates. Colors denote association between the viral OTUs abundances in Nishiyama et al. (2020), IBDMDB
 310 cohort, with active Crohn's disease (light red) or with both active Ulcerative colitis and active Crohn's disease (red). **B.** Experimental design
 311 of *in vivo* experiments. **C.** Expression levels of *upaY* in ceca of mice monocolonized with *B. fragilis* vs. mice monocolonized and infected with the
 312 Barc2635 bacteriophage. Levels are shown as $2^{(-\Delta\Delta CT)}$ with *rpsL* as a reference gene. Each dot represents a mouse. **** $P < 0.0001$,
 313 Mann-Whitney test. **D.** PSA presence on the surface of *B. fragilis* exposed to the Barc2635 bacteriophage, detected by anti-PSA antibodies.
 314 Bacteria were analyzed by flow cytometry for the expression of PSA, using Rabbit anti PSA antibodies. Each dot represents a mouse. **E.**
 315 Percent of the bacterial population with each of the phase-variable Type-I R-M specificity gene combinations at the expression locus. Each
 316 dot represents a mouse. IR: inverted repeat. **F.** CD4⁺ to CD8⁺ ratio out of CD45⁺TCR β^+ live cells in colon. Single cells were isolated from colon
 317 lamina propria. Immune cells were analyzed by flow cytometry. Each dot represents a mouse. **G.** ROR γ^+ helios⁻ percentages out of FOXP3⁺
 318 cells in colon. Single cells were isolated from colon lamina propria. Immune cells were analyzed by flow cytometry. Each dot represents a
 319 mouse. *** $P < 0.001$, **** $P < 0.0001$, one-way analysis of variance (ANOVA).

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Supplemental Figure 2

A. Ratio of *B. fragilis* PSA promoter reverse orientation in mice fecal samples measured by the PhaseFinder tool, on different days of the experiment. Blue: *B. fragilis*, yellow: *B. fragilis* + Barc2635. **B.** Ratio of *B. fragilis* PSF promoter reverse orientation in mice fecal samples measured by the PhaseFinder tool, on different days of the experiment. Blue: *B. fragilis*, yellow: *B. fragilis* + Barc2635. **C.** CFUs of *B. fragilis* in fecal samples of mice monocolonized with *B. fragilis* (blue) or colonized with *B. fragilis* + phage (yellow); PFUs of Barc2635 in fecal samples of mice colonized with *B. fragilis* + phage (dashed black line). **D.** CFUs of *B. fragilis* in fecal samples of mice monocolonized with *B. fragilis* (blue) or colonized with *B. fragilis* + phage (yellow) (Wilcoxon rank sum test, $*p < 0.05$). **E.** PFUs of Barc2635 in fecal samples of mice monocolonized with *B. fragilis* (blue) or colonized with *B. fragilis* + phage (yellow).

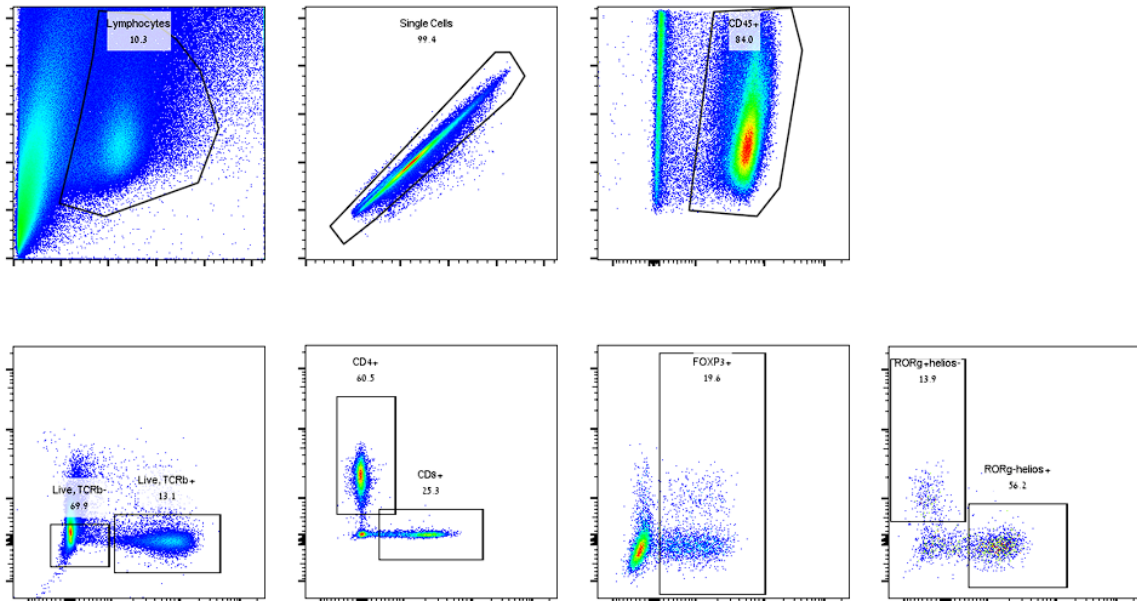
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338 **Supplemental Figure 3 – gating strategy**

339 Representative flow cytometry plots demonstrating the gating strategy for the staining panel.

340

341 **Discussion**

342 Phase variations, prevalent in host-associated species, especially in the abundant gut Bacteroidales
343 order⁹, contribute to bacterial fitness in changing ecosystems, such as the human gut. Reversible DNA
344 inversions lead to phase variable synthesis of numerous molecules (e.g. surface, regulatory, and other
345 molecules), and as such, confer functional plasticity. Our study reveals that phase variable states of
346 certain molecules correlate with gut inflammation, with potential implications on host physiology. By
347 analyzing six different public databases of IBD patients (CD and UC) and controlled mice experiments,
348 we identify multiple invertible genomic regions that map to 25 different Bacteroidales strains.
349 Notably, we find that both intergenic regions (e.g. promoters) and intragenic regions (e.g. genomic
350 shufflons) exhibit altered genomic phase variable states in gut inflammatory conditions - both
351 affecting gene expression.

352 The most prevalent genomic regions that demonstrated differential orientations of invertible regions
353 were in *susC/susD* homologs and polysaccharide (PS) promoters - both with immunomodulatory
354 potential. An epitope of *SusC*-like proteins, part of the starch-utilization system in *Bacteroidetes*, was
355 recently shown to elicit T cell responses in IBD patients and healthy controls⁴⁵, suggesting that operons
356 that include *SusC* homologs might confer immunomodulatory properties to the bacteria, and phase
357 variation in such genes might alter bacteria-host interactions. Among the phase-variable PSs, the anti-

358 inflammatory polysaccharide A (PSA) promoter of *B. fragilis* showed a higher percentage of reverse
359 oriented reads in IBD patients compared to healthy controls, indicating that the 'OFF' orientation was
360 more prevalent in IBD patients, potentially limiting the protective, anti-inflammatory, effects of PSA.
361 These results align with a previous study⁴⁶ that focused on the PSA promoter of *B. fragilis* in IBD
362 patients using PCR digestion of biopsy samples.

363 We further find that some DNA inversion states, like the PSA promoter of *B. fragilis*, are dynamic and
364 can respond to changes in their local environment, specifically during inflammation. Our analysis
365 focused on the bacterial population level, and therefore the DNA inversions could either occur in
366 individual bacterial cells in response to inflammation, or as a selection process on the bacterial
367 population level, where those with the promoter OFF orientation are enriched under inflammatory
368 conditions.

369 Intriguingly, the preferential 'OFF' orientation of the PSA promoter of *B. fragilis* did not occur to the
370 same extent in inflamed monocolonized mice as it did in both the inflamed mice colonized with a
371 human microbiota and when bacteria are grown in patients' fecal filtrates. These results suggest that
372 environmental factors in humanized mice and patients are necessary for this effect. Our analysis of
373 phage OTUs in the IBDMDB cohorts revealed enrichment of *B. fragilis* associated viral OTUs in samples
374 where the PSA promoter was present in the 'OFF' orientation. In addition, exposure of *B. fragilis* to
375 Barc2635 resulted in the PSA promoter 'OFF' orientation, suggesting a role for gut bacteriophages in
376 bacterial phase variation.

377 Type I restriction-modification (R-M) systems provide protection against bacteriophages by identifying
378 and restricting foreign DNA. We previously characterized a phase variable Type I R-M system of *B.*
379 *fragilis* that regulates distinct transcriptional programs affecting polysaccharide synthesis. Along with
380 the effects on the PSA promoter orientation, infection with Barc2635 also triggered genomic inversion
381 of this Type I R-M system towards inhibition of PSA synthesis^{7,36}.

382 The anti-inflammatory effects of PSA are mediated by upregulation of Tregs and subsequent induction
383 of IL-10 secretion^{12,13}. We found that the Treg cells populations decreased in *B. fragilis* monocolonized
384 mice infected with Barc2635, suggesting that the PSA 'OFF' trigger of Barc2635 has implications on
385 the host immune system¹¹. Applying the same analysis to *B. thetaiotaomicron*'s CPS3, we observed
386 bacteriophages that are more abundant in samples with a higher ratio of CPS3 'ON' oriented
387 promoters, however, the bacteriophage to host ratios per sample were too low to conclude that *B.*
388 *thetaitaomicron* bacteriophages lead to populations with altered CPS3 synthesis. Altogether, we
389 demonstrate differential orientations of invertible regions in IBD and elucidate the bacteriophages as

390 a potential trigger. These phase variations result in bacterial functional plasticity affecting the host
391 immune system. Bacteriophages are an example of an environmental factor that can alter phase
392 variable states. However, within the inflamed gut there are numerous factors that can potentially alter
393 phase variable states. These include the microbiome^{33,47-50} (e.g. neighboring bacteria and viruses), the
394 gut metabolome⁴⁷, an altered immune system⁵¹ and physical alterations in the intestine such as
395 abnormal pH concentrations⁵², osmotic^{53,54} and oxidative stress^{55,56}. For example, Tropini et al.⁵⁷
396 showed that PEG induced osmotic perturbation resulted in an increased ratio of CPS4 to CPS5 in *B.*
397 *thetaiotaomicron*.

398 This study demonstrates that alterations in invertible genomic regions, and consequently molecular
399 phase variations in gut bacteria, during IBD, can potentially influence the host immune system and
400 inflammatory state. These phase variations can occur in response to changes in gut environmental
401 factors during inflammation, including, but not limited to bacteriophages. Future studies integrating
402 bacterial DNA inversions and phase variation analyses may illuminate the role of bacterial functional
403 plasticity in additional physiological states, possibly resulting from different environmental factors,
404 which may drive alterations in microbe-host interactions.

405 **Limitations of the Study:**

406 The six databases analyzed in this study included adults and children, healthy controls and inflamed
407 patients (before and after treatment) and longitudinal samples. The impact of each of these factors,
408 and the impact of diet and lifestyle, merit further research. The PhaseFinder⁹ algorithm that we
409 applied identifies DNA inversions in short reads. As such, our analysis does not include other phase
410 variation mechanisms like duplications or insertions. Also, analysis of short reads of metagenomics
411 sequencing data might overlook inversions resulting from long structural genomic alterations^{3,7,58}.
412 Furthermore, metagenomic sequencing data averages the whole bacterial population of samples,
413 implying a dilution factor for low abundant bacteria. Moreover, genomic inversion sites might
414 interfere with genome sequence assemblies, leading to incomplete genomes, and thus could be
415 overlooked when applying tools that search inversions in reference genomes. This study used an
416 identified and isolated lytic bacteriophage for *B. fragilis*, further studies are required to elucidate the
417 functional effects of IBD associated lytic and temperate bacteriophages. With these limitations
418 notwithstanding, our study highlights the importance of considering bacterial phase variation in the
419 context of IBD and its potential impact on inflammation and on altered microbe-host interactions.

420 Methods

421 Mice

422 All mouse work was in accordance with protocols approved by the local IACUC committee under
423 approval numbers: IL-151-10-21 and IL-105-06-21.

424 Males, 4-5 weeks-old Germ-Free (GF) C57BL/6 or Swiss Webster mice from the Technion colony were
425 used. Mice were housed and maintained in a GF care facility and were provided with food and water
426 *ad libitum*; they were exposed to a 12:12 h light-dark cycle at room temperature.

427 Humanized mice were created by oral gavage of GF C57BL/6 mice with human feces from a single
428 healthy human donor, spiked with *B. fragilis* NCTC 9343.

429 Monocolonized mice were created by oral gavage of GF C57BL/6 mice with *B. fragilis* NCTC 9343.

430

431 For the prolonged experiments of Barc2635 and *B. fragilis* co-existence in gnotobiotic mice, female
432 Swiss Webster GF mice were administered with *B. fragilis* NCTC 9343 and bacteriophage Barc2635, or
433 *B. fragilis* only. All administrations were performed by oral gavage. On day 49 mice were sacrificed for
434 bacteria and immune phenotyping.

435

436 For phage immunomodulation studies, GF mice were gavaged twice (on day 0 and on day 2) with *B.*
437 *fragilis* NCTC 9343 and bacteriophage Barc2635, *B. fragilis* only, or growth media as control. On day
438 10 mice were sacrificed for bacteria and immune phenotyping.

439

440 DSS model

441 For acute DSS-induced colitis, humanized mice were treated with 3% Dextran sodium sulfate in their
442 drinking water for 9 days followed by 5 days recovery before sacrificing. The control animals were
443 administered distilled water.

444 Fecal samples were obtained on days 0, 6, 9 and 14 (before DSS treatment, during and after
445 discontinuing DSS treatments), for further analyses.

446

447 Mice Inflammation measurement

448 Fecal supernatants were prepared after suspension in 1:10 sterile PBS and centrifugation at 4,500xg
449 for 15 minutes. Enzyme-linked immunosorbent assay (ELISA) to measure calprotectin concentrations
450 was performed using Mouse S100A8/S100A9 Heterodimer kit according to the manufacturer protocol
451 [R&D systems].

452 Mice weights were assessed using an electronic scale at the same daytime.

453

454 qPCR and primers

455 DNA was extracted from fecal samples using ZymoBIOMICS DNA Miniprep Kit [Zymo research]. The
456 'ON'/'OFF' orientation of the PSA promoter in the extracted DNA samples was determined by
457 quantitative polymerase chain reaction (qPCR) using SYBR® Green mix [Thermo Fisher Scientific]. Two
458 sets of primers were designed to target the PSA locus. One set was used as a proxy to the number of
459 bacteria in the samples and targeted *UpaY*, the first gene immediately downstream to the promoter
460 region. The second set of primers targeted the promoter region and would only produce a product
461 when the orientation is 'ON'. The ratio of 'ON'/'OFF' PSA orientation in the samples was calculated
462 using the $2^{-\Delta\Delta CT}$ method calculated against the PSA locked 'ON' results (100% 'ON' orientation).

463

464 *upaY* gene expression was determined by RT-qPCR. RNA was extracted from fecal samples using
465 zymoBIOMICS RNA miniprep kit [Zymo research]. Reverse transcription of RNA to cDNA was
466 performed using the qScript cDNA Synthesis Kit [Quantabio]. *upaY* mRNA levels were determined by
467 qPCR using SYBR® Green mix [Thermo Fisher Scientific] with primers against *rpsL* as a reference gene.
468 The $2^{-\Delta\Delta CT}$ method was employed for the specificity fold change tests.

469 Primers:

B_Frag_upaY_F	CGCTCGGACAAAGAAGGACC
B_Frag_upaY_R	ACTTCTACCCTACGACGACGA
B_Frag_PSA_M	GGTGTTCCTCAAAGACGAACGT
B_Frag_PSA_F	TGTGTAAATGATAGGAGGCTAGGG
rpsL_F	CCGAACTCTGCAATGCGTAA
rpsL_R	CGCGAACCAGTACGATTGAG

470

471 Mice feces sequencing

472 DNA samples underwent quality control by Qubit fluorescence analysis to determine concentration of
473 DNA for downstream analysis (ThermoFisher, Cat. Q32850). Libraries were prepared using the Illumina
474 Tagmentation DNA prep streamlined library preparation protocol according to manufacturer's
475 instructions with a minimum of 50 ng of DNA starting mass and 8 cycles of PCR enrichment, ending
476 with a fragment size of 550 bp. IDT for Illumina DNA/RNA UD indexes and Nextera DNA CD indexes
477 were used (Illumina IDT, Cat. 20027213; Illumina Nextera, Cat. 20018708).

478 All libraries were diluted to 15 pM in 96-plex pools and validated on 100-cycle paired-ends read Miseq
479 V2 runs (Illumina, Cat. MS-102-2002), before shipping to the US at 4 nM for sequencing on the
480 Novaseq 6000 in S4 mode at 96-plex in a 300-cycle paired-end reads run, with an estimated read depth
481 of 30 Gbp per sample (Illumina, Cat. 20028312). Final loading concentration of 600 pM. All sequencing
482 runs were performed with a spike-in of 1% PhiX control library V3 (Illumina, Cat. FC-110-3001).

483

484 Taxonomic profiling

485 Community profiling was performed using metaphlan4 v4.0.0⁵⁹ with mpa database vJan21. For each
486 sample, the forward reads were first aligned against the mpa database using bowtie2 v2.3.5.1⁶⁰
487 (flags `--sam-no-hd --sam-no-sq --no-unal --very-sensitive`). Next, the resulting
488 sam file was analyzed by metaphlan4 with default parameters. In each analysis, species at abundance
489 $\leq 0.1\%$ were ignored.

490

491 Microbiome analysis

492 Statistical analysis of sequenced data was initially performed using MicrobiomeAnalyst⁶¹ followed by
493 comprehensive analysis using R packages: Phyloseq⁶², Vegan⁶³ and DESeq2⁶⁴. Differences in microbial
494 taxa and functional modules were assessed by differential abundance analyses using DESeq2⁶⁴. Alpha
495 diversity indices (observed species and Shannon) were compared using the Kruskal–Wallis rank sum
496 test. Beta diversity distance matrices (Bray-Curtis) were compared using the vegan package's function
497 ADONIS, a multivariate ANOVA based on dissimilarity tests and visualized using PCoA. Results were
498 visualized using the ggplot2 R package⁶⁵.

499

500 Identification of DNA inversion sites

501 Representative *Bacteroides* species were selected from an in-house database of human-associated
502 microbial species. The database was constructed from 118K metagenomics assembled genomes
503 (MAGs) recovered from human-associated metagenomics samples. Taxonomy assignment was
504 performed using gtdbtk v2.0.0 and GTDB release 207 with the classify_wf program and default
505 parameters⁶⁶. Overall, 36 *Bacteroides* species were identified of which 35 had species-level GTDB
506 taxonomy assignments. For each of these species, the representative genome from GTDB was used
507 for the PhaseFinder analysis. We have also included the genomes of two additional *B. fragilis* strains,
508 *Phocaeicola dorei*, and *Parabacteroides distasonis*. Overall, 40 genomes were included. Information
509 about the genomes is provided in Table S1.

510 PhaseFinder⁹ (v1.0) was used to identify invertible sites in the metagenomics samples. The default
511 parameters of PhaseFinder were used. We filtered the results by removing identified sites with < 20

512 reads supporting either the forward or reverse orientations combined from the paired-end method,
513 mean $Pe_ratio < 1\%$ across all samples, and sites within coding regions of rRNA products. To compare
514 DNA orientation patterns between CD and UC patients with those of healthy controls, we focused on
515 sites that displayed a difference of over 10% between at least one of the groups. The Wilcoxon rank
516 sum test was employed to conduct these comparisons, and the Benjamini-Hochberg method was
517 utilized to correct for multiple comparisons, with a false discovery rate (FDR) set at less than 0.1. Each
518 invertible region was manually curated to assess its coding regions, gene annotations, and their
519 putative functions. Briefly, genomic regions were visualized online using the NCBI Graphical Sequence
520 Viewer (version 3.47.0). Invertible regions with coding sequences (CDS) within them, were annotated
521 according to the CDS name(s). Invertible regions lacking CDS within them were searched for CDSs that
522 start in proximity to the invertible DNA sites ($< 200 \sim bp$). CDSs in the region (four upstream and four
523 downstream) were used to assess the functionality of the region. Regions containing or in proximity
524 to rRNA and tRNA genes were filtered from the comparisons as well as invertible regions with no CDSs
525 start in proximity to the inverted repeats.

526

527 Bacteriophage isolation

528 For isolation of bacteriophages, inflowing raw sewage for a waste-water treatment plant (WWTP)
529 from Barcelona (Spain) was filtered through low protein binding 0.22 μm pore size polyethersulfone
530 (PES) membrane filters (Millex- GP, Millipore, Bedford, Massachusetts) to remove bacteria. Isolated
531 plaques were obtained by the double-agar layer technique⁶⁷. Briefly, tubes containing 2.5 ml of soft
532 BPRM-agar kept at 45°C were inoculated with 1 ml of an exponential growth phase culture
533 ($OD_{600}=0.3$, corresponding to ca 2×10^8 CFU / ml) of the host bacteria grown in BPRM broth and 1 ml
534 of the filtered sewage sample. After gently mixing, the contents of each tube were poured onto a plate
535 of BRPM-agar and incubated inside GasPak (BBL) jars at 37°C. Plaques were clearly spotted after 18 h
536 of incubation.

537 For phage isolation, discrete well-isolated plaques were stabbed with a sterile needle and inoculated
538 in a tube containing 5 ml of BRPM broth. Then 1-ml of a culture of *B. fragilis* NCTC 9343 in exponential
539 growth was inoculated into the tube, which was then incubated for 18h at 37 °C. After incubation, an
540 aliquot of the culture was treated with chloroform (1:10 (v:v), vigorously mixed for 5 minutes and
541 centrifuged at 16,000 $\times g$ for 5 minutes⁶⁸. The supernatant containing the phage suspensions were
542 further filtered through low protein binding 0.22 μm pore size polyethersulfone (PES) membrane
543 filters (Millex-GP, Millipore, Bedford, Massachusetts), diluted and plated as indicated in the previous
544 paragraph to verify the uniformity of the plaques. Then, one well differentiated plaque was stabbed

545 and the whole operation repeated to obtain a high titer, over 1×10^9 plaque forming units (PFU), phage
546 suspensions.

547

548 Phage genome sequencing

549 5hrs induction of phage DNA, phage particles were PEG-precipitated (6,000-12,000 MW, 8%), and
550 isolated by a CsCl gradient; 33 g, 41 g, 55 g in 50 ml TM buffer (50mM Tris-Cl pH8.0, 10mM MgCl₂),
551 ultracentrifuged at 40,000 rpm for 1.5hrs, and dialyzed overnight in 100mM Tris pH7.5, 1M NaCl,
552 1mM EDTA 56. Genomic DNA was extracted using phenol-chloroform as described 57.

553 Illumina sequencing of Bacteroides phage Barc2635 was performed at the Biopolymers Facility,
554 Harvard Medical School, Department of Genetics, producing paired-end reads of 150 bp. Adapter
555 sequence removal and quality trimming was performed using BBDuk, part of the BBTools (v 37.50)
556 suite of programs. The reads were further screened against NCBI's UniVec_Core database (build
557 10.0) and the *B. fragilis* NCTC 9343 genome sequence using blastn and reads that returned a
558 significant hit to either were removed. The phage genome was assembled de novo using Velvet
559 1.2.10 under a k-value determined by Velvet Optimizer (v. 2.2.5). The genome was annotated using
560 an in-house customized version of Prokka v1.12, submitted to NCBI, and assigned GenBank accession
561 MN078104. Phage Genome map [**Supp. Figure 1C**] was visualized using the online tool Proksee
562 (<https://proksee.ca/>, accessed on 21 January 2023).

563 Viral OTUs multiple alignment

564 Alignments and phylogenetic tree of whole genomes of viral OTUs identified as bacteriophages against
565 *B. fragilis*³³ as well as bacteriophage Barc2635 were generated using MAFFT online service (version 7).
566 Multiple sequence alignment was done using the default settings of the site. Phylogenetic tree was
567 constructed using the average-linkage method with 1000 bootstrap replicates and was visualized with
568 R package ggtree⁶⁹ (version 3.4.4).

569 MinION library preparation and sequencing

570 The specificity region of BF9343_1757–1760 was amplified by PCR from a population of bacteria
571 grown in vitro and in vivo from fecal content as described above. The primers annealed outside the
572 invertible region. The amplicons were purified by Wizard SV Gel and PCR Clean-Up System (Promega)
573 and measured by nanodrop.

574 Primers:

Type1RM_hsdS_F GACAATCGAGATGAAGAACAAC
Type1RM_hsdS_R CCATAGGCGTATGATTCCTG

575

576 DNA quantity was measured again using Qubit fluorometry (Thermo Fisher Scientific, Waltham, MA,
577 USA). Nanopore sequencing libraries were prepared from 200 fmol purified amplicons using Ligation
578 Sequencing Kit 1D (SQK-LSK109) and PCR-free Native Barcoding Expansion Kit (EXP-NBD104) (Oxford
579 Nanopore Technologies, Oxford, England). The barcoded libraries were loaded and sequenced on the
580 MinION device controlled by MinKNOW software (v.19.12.5) using MinION flow cells (FLO-MIN106D
581 R9.4.1, Oxford Nanopore Technologies, Oxford, England) after quality control runs. The raw data were
582 base called and demultiplexed by Guppy Basecalling Software (v. 3.3.3+fa743a6).

583 MinION data analysis

584 Adapters and barcodes sequences were removed from the reads using Porechop (v0.2.4, available
585 from <https://github.com/rrwick/Porechop>). Reads were oriented using the 'Preparing Reads for
586 Stranded Mapping' protocol (Eccles, D.A. (2019). *Protocols.io*⁷⁰). We aligned the reads to the PCR
587 forward primer using LASTAL (v.1060)⁷¹, and then reverse-complemented the reverse-oriented reads.
588 The reads were combined to an all forward oriented file and cropped to the first 1300 bases using
589 Trimmomatic (v.0.39)⁷². The reads were then split according to their alignment to the 1757-57 or 1757-
590 60 5" half sequences using LASTAL. Reads were mapped to the full sequences with Minimap2 (v.2.17-
591 r941)⁷³ using the `-for-only` and `asm20` options. Mapped read counts were extracted from the
592 Minimap2 SAM output using SAMtools (v.1.7)⁷⁴.

593 MinION sequence data has been deposited in the NCBI sequence read archive (SRA) under the
594 BioProject accession number: PRJNA948162

595 Fecal filtrates of patients

596 Recruitment of IBD patients for this study was conducted at the Rambam Health Care Campus (RHCC).
597 The study was approved by the local institutional review boards with study numbers 0052-17 and
598 0075-09 in which all patients consented to be included in it. During therapy, patients were treated
599 either with Infliximab or Humira for at least two weeks.

600 Fecal samples were collected by patients at home prior to their clinic visit and collected from each at
601 the hospital. The samples were then stored at -80°C until they were shipped to the laboratory for
602 analysis.

603 Calprotectin levels were measured in each fecal sample using LIAISON Calprotectin (catalogue No.
604 318960) according to the manufacturer's instructions. The levels of calprotectin in the fecal samples
605 were used as a measure of disease activity in IBD patients.

606

607 In vitro assays with fecal filtrates from IBD patients

608 *Bacteroides fragilis* NCTC 9343 was grown in Brain heart infusion (BHIS) supplemented with 5 mg/l
609 hemin and 2.5 µg/l vitamin K to OD₆₀₀~0.6 and centrifuged at 4,500xg for 5 minutes. Bacterial pellets
610 were washed twice with sterile PBS to remove BHIS components and then suspended with 1mL
611 supplemented M9 minimal media. Patients' fecal samples were suspended in sterile PBS (1:5),
612 centrifuged at 4,500xg for 15 minutes, and supernatants were collected and filtered using the Medical
613 Millex-VV Syringe Filter Unit, 0.22 µm, PVDF membrane. For the *in vitro* assay, *B. fragilis* was cultured
614 in patients' supernatants and diluted in M9 minimal media in a 1:25:25 ratio (bacteria: fecal
615 supernatants: media). Subsequently, 200µl of each culture was collected for DNA extraction at
616 OD₆₀₀~0.6.

617

618 M9 Medium was prepared as follows:

619 Per L:

620 1g NH₄Cl

621 6g Na₂HPO₄

622 3g KH₂PO₄

623 0.5g NaCl

624 Supplements:

625 14.7mg CaCl₂·2H₂O (0.1ml 1M stock)

626 246mg MgSO₄·7H₂O (1ml 1M stock)

627 0.5% glucose (20ml 25%)

628 0.05% L-cysteine (10ml 5% stock)

629 5mg/ml Hemin (1ml 0.5% stock)

630 2.5mg/ml VitK₁ (0.5ml 0.5% stock)

631 2mg/ml FeSO₄·7H₂O (0.1ml 2% stock)

632 5ng/ml VitB12 (0.05ml 0.01% stock)

633

634 Gut lamina propria preparation

635 For lamina propria immunophenotyping, mice colons were removed by cutting the colon from the
636 cecum-colon junction to the anus. Fat tissue was carefully removed from colon tissue and further
637 proceeded for single cell suspension preparation using lamina propria dissociation kit (Miltenyi),
638 according to the manufacturer's protocol.

639

640

641 Flow cytometry

642 Cell preparations for flow cytometry analysis were performed in 5 ml tubes or U shape 96 wells plates.
643 Single cells were washed with PBS and stained for live/dead staining using 1:1,000 in PBS, Zombie
644 fixable viability dye (Biolegend) for 10 minutes, at room temperature, and washed once with FACS
645 buffer, by centrifuge at 300xg for 5 minutes. For FcR blocking, cells were incubated with 0.5 µg
646 CD16/CD32 antibody for 10 minutes on ice and proceeded to further staining without a washing step.
647 Extracellular markers were stained with the relevant antibody panels for 30 minutes on ice and
648 washed twice with FACS buffer, by centrifuge at 300xg for 5 minutes. After the last wash, cells were
649 fixed with Foxp3 Fixation/Permeabilization working solution (Thermo) for 16 hours at 4 °C in the dark.
650 For Intracellular staining, cells were permeabilized using 1X Foxp3 permeabilization buffer (Thermo)
651 according to the manufacturer protocol. For intracellular blocking, 2 µl of 2% rat serum (Stemcell
652 technologies) was added to each well for 15 minutes at room temperature and proceeded to further
653 staining without a washing step.

654 To quantify the percentage of *B. fragilis* from monocolonized mice with PSA on their surface, feces
655 from monocolonized mice with *B. fragilis* with or without phage were suspended 1:10 in ice cold PBS
656 (mg/µl) and centrifuged at 300xg for 5 minutes, 4°C. Supernatants were separated from pellets and
657 further centrifuged at 4,500xg for 5 minutes, 4°C. Bacterial pellets were resuspended in an ice cold
658 FACS buffer, 1:10 from initial PBS suspension. 100 µl of resuspended bacteria were incubated with
659 1:1,000 Rabbit anti *B. fragilis* PSA for 30 minutes at 4°C. Bacteria were washed twice using an ice cold
660 FACS buffer by centrifuge at 4,500xg for 5 minutes and then incubated with a donkey anti rabbit
661 fluorophore conjugated secondary antibody. After staining steps, the bacteria were washed twice with
662 an ice cold FACS buffer and finally resuspended in 500 µl ice cold PBS plus 1:1,000 Hoechst dye and
663 analyzed by flow cytometry using FSC and SSC thresholds of 1,000, and logarithmic scale. Gating
664 strategy is detailed in **Supplemental Figure 3**.

665

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692

693 [Author contributions](#)

694 SC HH RZ DKK TG IS and NGZ conceived and designed the project. SC HH RZ DKK and TG performed
695 and analyzed the experiments. SC, IS and NGZ planned the computational and analytical aspects; SC
696 and IS performed the computational analyses. MJC and LC contributed to the computational analysis
697 and interpretation of the results. SP and YC designed, lead, and executed the clinical study. KJ
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702 interpreted the experiments, and wrote the manuscript.

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707 **References**

- 708 1. Moxon R, Bayliss C, Hood D. Bacterial contingency loci: The role of simple sequence DNA
709 repeats in bacterial adaptation. *Annu Rev Genet.* 2006;40:307-333.
710 doi:10.1146/annurev.genet.40.110405.090442
- 711 2. Phillips ZN, Tram G, Seib KL, Attack JM. Phase-variable bacterial loci: how bacteria gamble to
712 maximise fitness in changing environments. *Biochem Soc Trans.* 2019;47(4):1131-1141.
713 doi:10.1042/BST20180633
- 714 3. West PT, Chanin RB, Bhatt AS. From genome structure to function: insights into structural
715 variation in microbiology. *Curr Opin Microbiol.* 2022;69:102192.
716 doi:10.1016/J.MIB.2022.102192
- 717 4. Goldberg A, Fridman O, Ronin I, Balaban NQ. Systematic identification and quantification of
718 phase variation in commensal and pathogenic Escherichia coli. *Genome Med.* 2014;6(11):112.
719 doi:10.1186/s13073-014-0112-4
- 720 5. Krinos CM, Coyne MJ, Weinacht KG, Tzianabos AO, Kasper DL, Comstock LE. Extensive surface
721 diversity of a commensal microorganism by multiple DNA inversions. *Nature.*
722 2001;414(6863):555-558. doi:10.1038/35107092
- 723 6. Van Der Woude MW, Bäumlér AJ. Phase and Antigenic Variation in Bacteria. *Clin Microbiol*
724 *Rev.* 2004;17(3):581. doi:10.1128/CMR.17.3.581-611.2004
- 725 7. Ben-Assa N, Coyne MJ, Fomenkov A, et al. Analysis of a phase-variable restriction
726 modification system of the human gut symbiont *Bacteroides fragilis*. *Nucleic Acids Res.*
727 2020;48(19):11040-11053. doi:10.1093/nar/gkaa824
- 728 8. Porter NT, Hryckowian AJ, Merrill BD, et al. Phase-variable capsular polysaccharides and
729 lipoproteins modify bacteriophage susceptibility in *Bacteroides thetaiotaomicron*. *Nat*
730 *Microbiol.* 2020;5(9):1170-1181. doi:10.1038/s41564-020-0746-5
- 731 9. Jiang X, Brantley Hall A, Arthur TD, et al. Invertible promoters mediate bacterial phase
732 variation, antibiotic resistance, and host adaptation in the gut. *Science (1979).*
733 2019;363(6423):181-187. doi:10.1126/science.aau5238
- 734 10. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal
735 inflammatory disease. *Nature.* 2008;453(7195):620-625. doi:10.1038/nature07008
- 736 11. Surana NK, Kasper DL. The yin yang of bacterial polysaccharides: lessons learned from *B.*
737 *fragilis* PSA. *Immunol Rev.* 2012;245(1):13-26. doi:10.1111/j.1600-065X.2011.01075.x
- 738 12. Dasgupta S, Erturk-Hasdemir D, Ochoa-Reparaz J, Reinecker HC, Kasper DL. Plasmacytoid
739 dendritic cells mediate anti-inflammatory responses to a gut commensal molecule via both
740 innate and adaptive mechanisms. *Cell Host Microbe.* 2014;15(4):413-423.
741 doi:10.1016/j.chom.2014.03.006
- 742 13. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal
743 bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A.* 2010;107(27):12204-12209.
744 doi:10.1073/PNAS.0909122107

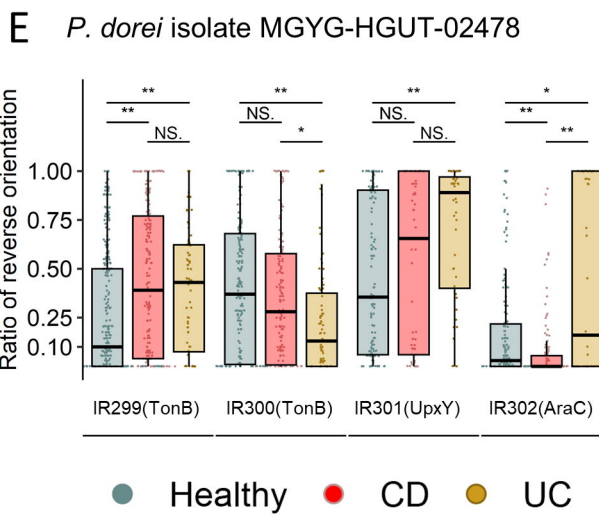
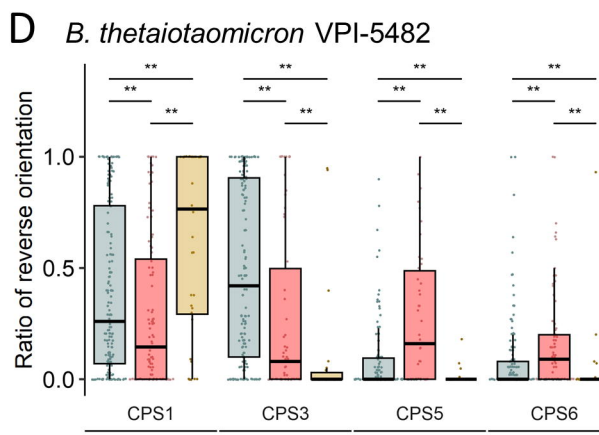
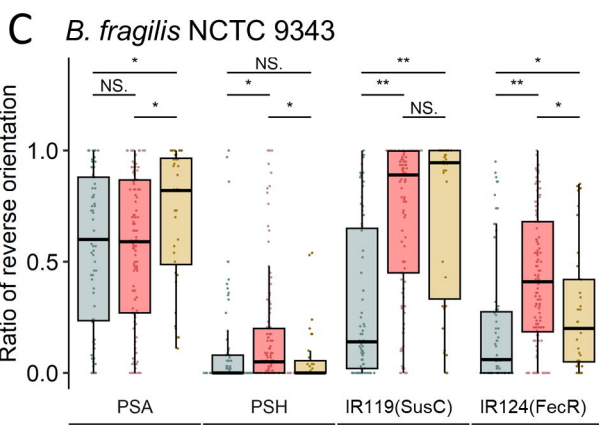
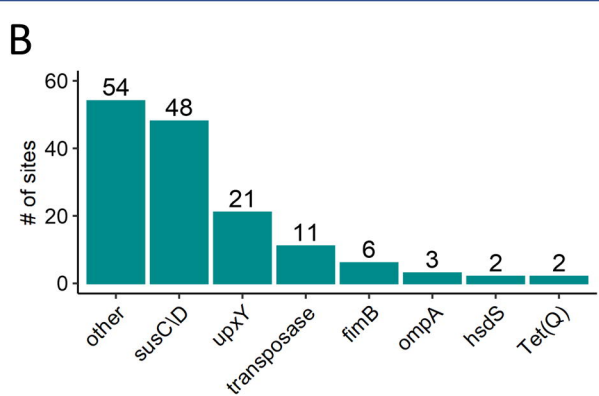
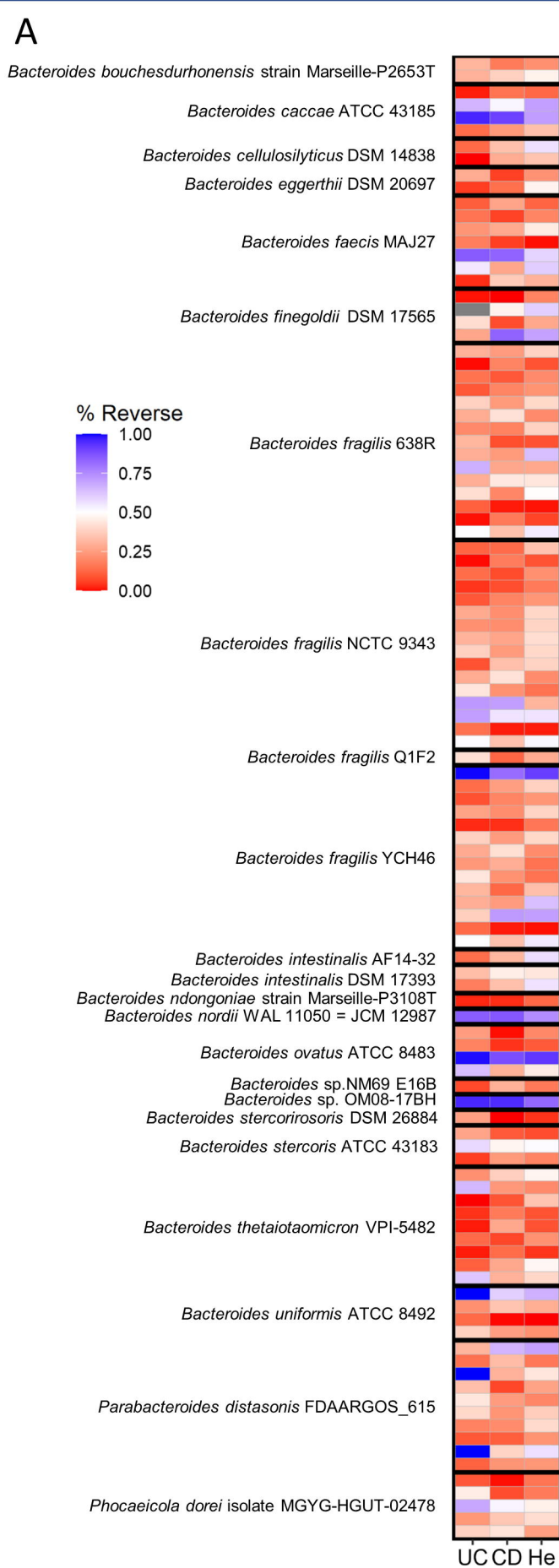
- 745 14. Chang YC, Ching YH, Chiu CC, et al. TLR2 and interleukin-10 are involved in *Bacteroides*
746 *fragilis*-mediated prevention of DSS-induced colitis in gnotobiotic mice. *PLoS One*. 2017;12(7).
747 doi:10.1371/JOURNAL.PONE.0180025
- 748 15. Ramos GP, Papadakis KA. Mechanisms of Disease: Inflammatory Bowel Diseases. *Mayo Clin*
749 *Proc*. 2019;94(1):155-165. doi:10.1016/J.MAYOCP.2018.09.013
- 750 16. Guan Q. A Comprehensive Review and Update on the Pathogenesis of Inflammatory Bowel
751 Disease. *J Immunol Res*. 2019;2019. doi:10.1155/2019/7247238
- 752 17. Corridoni D, Arseneau KO, Cominelli F. Inflammatory bowel disease. *Immunol Lett*.
753 2014;161(2):231-235. doi:10.1016/J.IMLET.2014.04.004
- 754 18. Ha CWY, Martin A, Sepich-Poore GD, et al. Translocation of Viable Gut Microbiota to
755 Mesenteric Adipose Drives Formation of Creeping Fat in Humans. *Cell*. 2020;183(3):666-
756 683.e17. doi:10.1016/J.CELL.2020.09.009
- 757 19. Bolam DN, Koropatkin NM. Glycan recognition by the Bacteroidetes Sus-like systems. *Curr*
758 *Opin Struct Biol*. 2012;22(5):563-569. doi:10.1016/J.SBI.2012.06.006
- 759 20. Kulagina E v., Efimov BA, Maximov PY, Kafarskaia LI, Chaplin A v., Shkoporov AN. Species
760 composition of Bacteroidales order bacteria in the feces of healthy people of various ages.
761 *Biosci Biotechnol Biochem*. 2012;76(1):169-171. doi:10.1271/BBB.110434
- 762 21. Kraal L, Abubucker S, Kota K, Fischbach MA, Mitreva M. The Prevalence of Species and Strains
763 in the Human Microbiome: A Resource for Experimental Efforts. *PLoS One*. 2014;9(5):e97279.
764 doi:10.1371/JOURNAL.PONE.0097279
- 765 22. Nomura K, Ishikawa D, Okahara K, et al. Bacteroidetes species are correlated with disease
766 activity in ulcerative colitis. *J Clin Med*. 2021;10(8):1749. doi:10.3390/JCM10081749/S1
- 767 23. Proctor LM, Creasy HH, Fettweis JM, et al. The Integrative Human Microbiome Project.
768 *Nature* 2019 569:7758. 2019;569(7758):641-648. doi:10.1038/s41586-019-1238-8
- 769 24. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The Human
770 Microbiome Project. *Nature* 2007 449:7164. 2007;449(7164):804-810.
771 doi:10.1038/nature06244
- 772 25. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic
773 sequencing. *Nature* 2010 464:7285. 2010;464(7285):59-65. doi:10.1038/nature08821
- 774 26. Imhann F, van der Velde KJ, Barbieri R, et al. The 1000IBD project: Multi-omics data of 1000
775 inflammatory bowel disease patients; Data release 1. *BMC Gastroenterol*. 2019;19(1):1-10.
776 doi:10.1186/S12876-018-0917-5/TABLES/2
- 777 27. Gevers D, Kugathasan S, Denson LA, et al. The treatment-naive microbiome in new-onset
778 Crohn's disease. *Cell Host Microbe*. 2014;15(3):382-392. doi:10.1016/J.CHOM.2014.02.005
- 779 28. Lewis JD, Chen EZ, Baldassano RN, et al. Inflammation, Antibiotics, and Diet as Environmental
780 Stressors of the Gut Microbiome in Pediatric Crohn's Disease. *Cell Host Microbe*.
781 2015;18(4):489-500. doi:10.1016/J.CHOM.2015.09.008

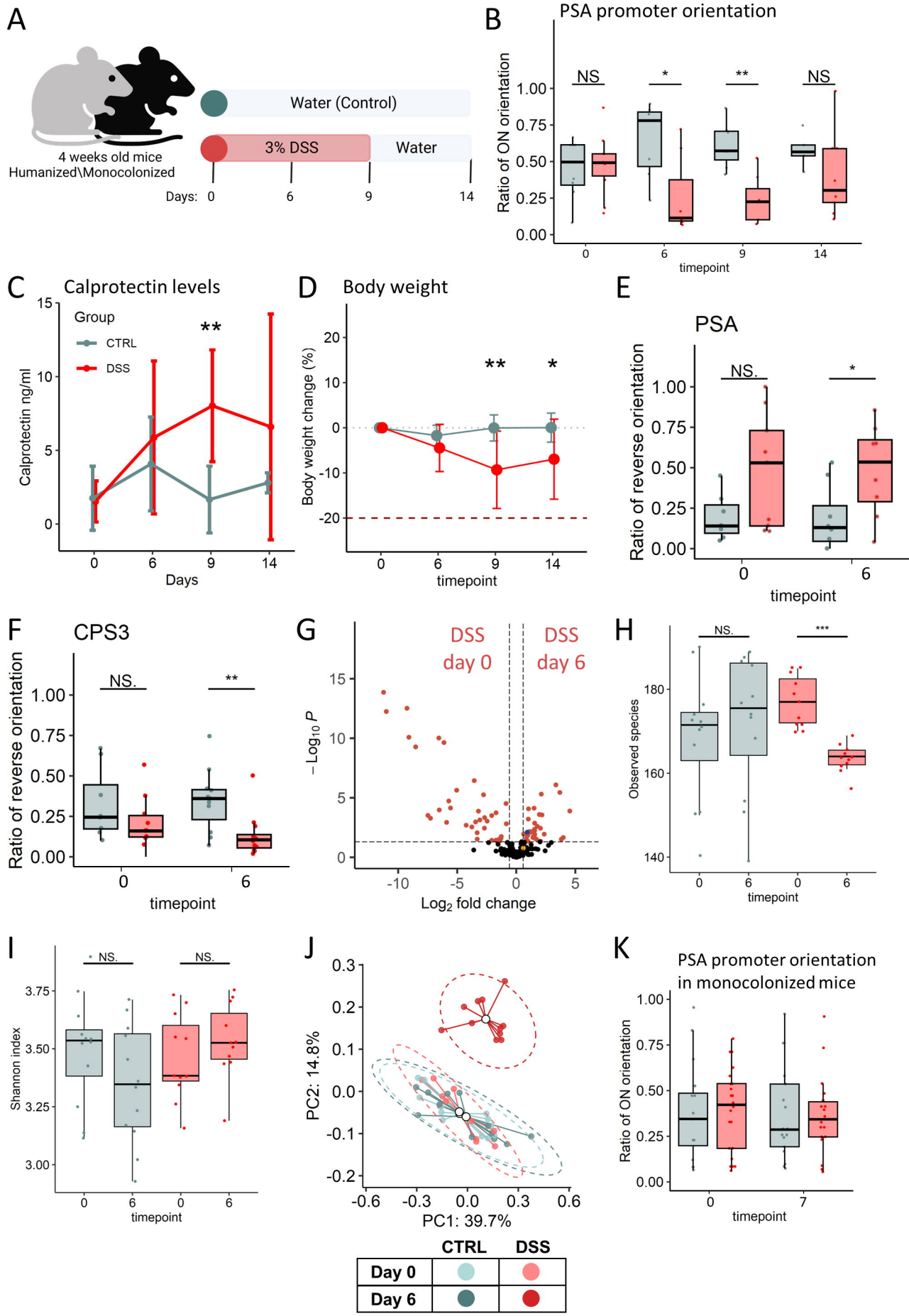
- 782 29. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran Sulfate Sodium (DSS)-
783 Induced Colitis in Mice. *Current protocols in immunology / edited by John E Coligan . [et al].*
784 2014;104(SUPPL.104):Unit. doi:10.1002/0471142735.IM1525S104
- 785 30. Shkoporov AN, Khokhlova E V., Stephens N, et al. Long-term persistence of crAss-like phage
786 crAss001 is associated with phase variation in *Bacteroides intestinalis*. *BMC Biol.* 2021;19(1).
787 doi:10.1186/S12915-021-01084-3
- 788 31. Qv L, Mao S, Li Y, Zhang J, Li L. Roles of Gut Bacteriophages in the Pathogenesis and
789 Treatment of Inflammatory Bowel Disease. *Front Cell Infect Microbiol.* 2021;11.
790 doi:10.3389/FCIMB.2021.755650
- 791 32. Adiliaghdam F, Amatullah H, Digumarthi S, et al. Human enteric viruses autonomously shape
792 inflammatory bowel disease phenotype through divergent innate immunomodulation. *Sci*
793 *Immunol.* 2022;7(70):eabn6660. doi:10.1126/SCIIMMUNOL.ABN6660
- 794 33. Nishiyama H, Endo H, Blanc-Mathieu R, Ogata H. Ecological Structuring of Temperate
795 Bacteriophages in the Inflammatory Bowel Disease-Affected Gut. *Microorganisms.*
796 2020;8(11):1-15. doi:10.3390/MICROORGANISMS8111663
- 797 34. Norman JM, Handley SA, Baldrige MT, et al. Disease-specific Alterations in the Enteric
798 Virome in Inflammatory Bowel Disease. *Cell.* 2015;160(3):447.
799 doi:10.1016/J.CELL.2015.01.002
- 800 35. Chatzidaki-Livanis M, Coyne MJ, Comstock LE. A family of transcriptional antitermination
801 factors necessary for synthesis of the capsular polysaccharides of *Bacteroides fragilis*. *J*
802 *Bacteriol.* 2009;191(23):7288-7295. doi:10.1128/JB.00500-09
- 803 36. Chatzidaki-Livanis M, Weinacht KG, Comstock LE. Trans locus inhibitors limit concomitant
804 polysaccharide synthesis in the human gut symbiont *Bacteroides fragilis*. *Proc Natl Acad Sci U*
805 *S A.* 2010;107(26):11976-11980. doi:10.1073/PNAS.1005039107
- 806 37. Noyer-Weidner M, Trautner TA. Methylation of DNA in prokaryotes. *EXS.* 1993;64:39-108.
807 doi:10.1007/978-3-0348-9118-9_4/COVER
- 808 38. Bickle TA, Krüger DH. Biology of DNA restriction. *Microbiol Rev.* 1993;57(2):434-450.
809 doi:10.1128/MR.57.2.434-450.1993
- 810 39. Murray NE, Daniel AS, Cowan GM, Sharp PM. Conservation of motifs within the unusually
811 variable polypeptide sequences of type I restriction and modification enzymes. *Mol*
812 *Microbiol.* 1993;9(1):133-143. doi:10.1111/J.1365-2958.1993.TB01675.X
- 813 40. Sistla S, Rao DN. S-Adenosyl-L-methionine-dependent restriction enzymes. *Crit Rev Biochem*
814 *Mol Biol.* 2004;39(1):1-19. doi:10.1080/10409230490440532
- 815 41. Round JL, Mazmanian SK. Inducible Foxp3+ regulatory T-cell development by a commensal
816 bacterium of the intestinal microbiota. *Proc Natl Acad Sci U S A.* 2010;107(27):12204-12209.
817 doi:10.1073/PNAS.0909122107
- 818 42. Erturk-Hasdemir D, Oh SF, Okan NA, et al. Symbionts exploit complex signaling to educate the
819 immune system. *Proc Natl Acad Sci U S A.* 2019;116(52):26157-26166.
820 doi:10.1073/PNAS.1915978116

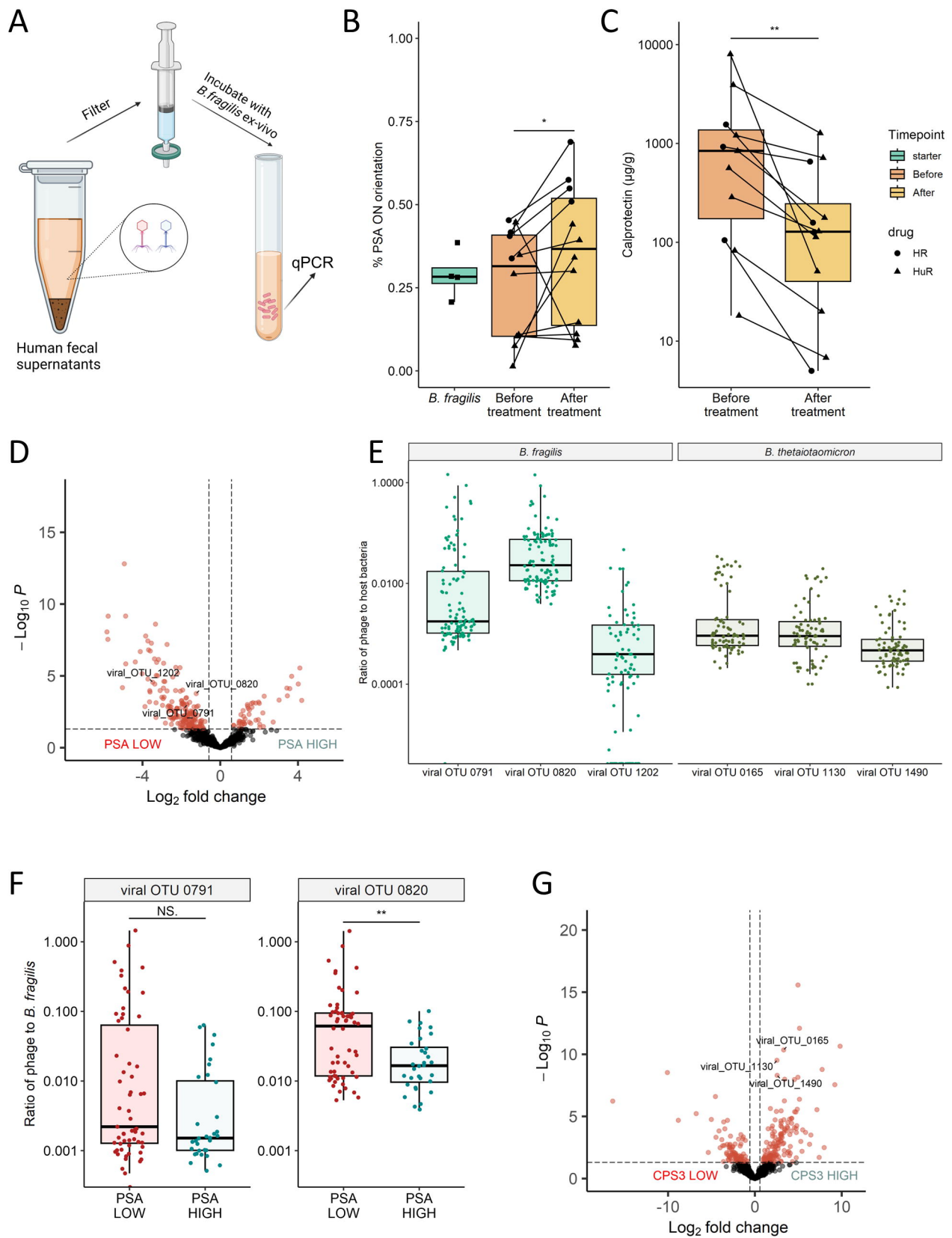
- 821 43. Johnson JL, Jones MB, Cobb BA. Polysaccharide-experienced effector T cells induce IL-10 in
822 FoxP3+ regulatory T cells to prevent pulmonary inflammation. *Glycobiology*. 2018;28(1):50-
823 58. doi:10.1093/GLYCOB/CWX093
- 824 44. Telesford KM, Yan W, Ochoa-Reparaz J, et al. A commensal symbiotic factor derived from
825 *Bacteroides fragilis* promotes human CD39(+)Foxp3(+) T cells and Treg function. *Gut*
826 *Microbes*. 2015;6(4):234-242. doi:10.1080/19490976.2015.1056973
- 827 45. Pedersen TK, Brown EM, Plichta DR, et al. The CD4+ T cell response to a commensal-derived
828 epitope transitions from a tolerant to an inflammatory state in Crohn's disease. *Immunity*.
829 2022;55(10):1909-1923.e6. doi:10.1016/J.IMMUNI.2022.08.016
- 830 46. Blandford LE, Johnston EL, Sanderson JD, Wade WG, Lax AJ. Promoter orientation of the
831 immunomodulatory *Bacteroides fragilis* capsular polysaccharide A (PSA) is off in individuals
832 with inflammatory bowel disease (IBD). *Gut Microbes*. Published online February 7, 2019:1-9.
833 doi:10.1080/19490976.2018.1560755
- 834 47. Lloyd-Price J, Arze, C Ananthakrishnan, AN Schirmer M, Avila-Pacheco J, Poon T, Andrews, E
835 Ajami N, Bonham, KS Brislawn C. Multi-omics of the gut microbial ecosystem in inflammatory
836 bowel diseases. *Nature*. 2019;569(7758):655–662. Accessed March 18, 2021.
837 <https://www.nature.com/articles/s41586-019-1237-9>
- 838 48. Federici S, Kredo-Russo S, Valdés-Mas R, et al. Targeted suppression of human IBD-associated
839 gut microbiota commensals by phage consortia for treatment of intestinal inflammation. *Cell*.
840 2022;185(16):2879-2898.e24. doi:10.1016/J.CELL.2022.07.003
- 841 49. Norman JM, Handley SA, Baldridge MT, et al. Disease-specific alterations in the enteric
842 virome in inflammatory bowel disease. *Cell*. 2015;160(3):447-460.
843 doi:10.1016/J.CELL.2015.01.002
- 844 50. Duerkop BA, Kleiner M, Paez-Espino D, et al. Murine colitis reveals a disease-associated
845 bacteriophage community. *Nat Microbiol*. 2018;3(9):1023-1031. doi:10.1038/s41564-018-
846 0210-y
- 847 51. Wallace KL, Zheng LB, Kanazawa Y, Shih DQ. Immunopathology of inflammatory bowel
848 disease. *World Journal of Gastroenterology : WJG*. 2014;20(1):6. doi:10.3748/WJG.V20.I1.6
- 849 52. Nugent SG, Kumar D, Rampton DS, Evans DF. Intestinal luminal pH in inflammatory bowel
850 disease: possible determinants and implications for therapy with aminosaliculates and other
851 drugs. *Gut*. 2001;48(4):571-577. doi:10.1136/GUT.48.4.571
- 852 53. Vertzoni M, Koulouri C, Poulou A, Goumas K, Reppas C. Exploring the impact of Crohn's
853 disease on the intragastric environment of fasted adults. *ADMET DMPK*. 2020;8(2):122.
854 doi:10.5599/ADMET.846
- 855 54. Barkas F, Liberopoulos E, Kei A, Elisaf M. Electrolyte and acid-base disorders in inflammatory
856 bowel disease. *Annals of Gastroenterology : Quarterly Publication of the Hellenic Society of*
857 *Gastroenterology*. 2013;26(1):23. Accessed February 11, 2023. /pmc/articles/PMC3959504/
- 858 55. Tian T, Wang Z, Zhang J. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease
859 and Potential Antioxidant Therapies. *Oxid Med Cell Longev*. 2017;2017.
860 doi:10.1155/2017/4535194

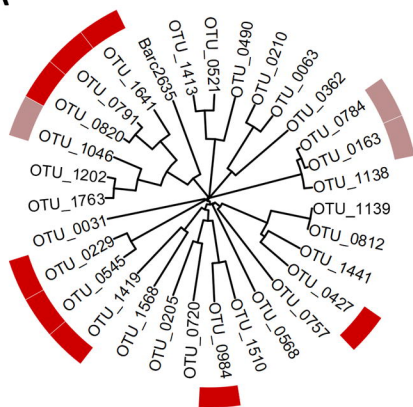
- 861 56. Bourgonje AR, Feelisch M, Faber KN, Pasch A, Dijkstra G, van Goor H. Oxidative Stress and
862 Redox-Modulating Therapeutics in Inflammatory Bowel Disease. *Trends Mol Med*.
863 2020;26(11):1034-1046. doi:10.1016/J.MOLMED.2020.06.006
- 864 57. Tropini C, Moss EL, Merrill BD, et al. Transient osmotic perturbation causes long-term
865 alteration to the gut microbiota. *Cell*. 2018;173(7):1742. doi:10.1016/J.CELL.2018.05.008
- 866 58. Milman O, Yelin I, Kishony R. Systematic identification of gene-altering programmed
867 inversions across the bacterial domain. *Nucleic Acids Res*. 2023;51(2):553-573.
868 doi:10.1093/NAR/GKAC1166
- 869 59. Blanco-Míguez A, Beghini F, Cumbo F, et al. Extending and improving metagenomic
870 taxonomic profiling with uncharacterized species using MetaPhlAn 4. *Nature Biotechnology*
871 2023. Published online February 23, 2023:1-12. doi:10.1038/s41587-023-01688-w
- 872 60. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*.
873 2012;9(4):357. doi:10.1038/NMETH.1923
- 874 61. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. MicrobiomeAnalyst: A web-based tool
875 for comprehensive statistical, visual and meta-analysis of microbiome data. *Nucleic Acids Res*.
876 2017;45(W1):W180-W188. doi:10.1093/nar/gkx295
- 877 62. McMurdie PJ, Holmes S. phyloseq: An R Package for Reproducible Interactive Analysis and
878 Graphics of Microbiome Census Data. *PLoS One*. 2013;8(4):e61217.
879 doi:10.1371/JOURNAL.PONE.0061217
- 880 63. Simpson GL. *The Vegan Package*.; 2009. Accessed December 29, 2020. [http://cran.r-](http://cran.r-project.org/)
881 [project.org/](http://cran.r-project.org/),
- 882 64. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-
883 seq data with DESeq2. *Genome Biol*. 2014;15(12). doi:10.1186/s13059-014-0550-8
- 884 65. Wickham H. ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*. Published
885 online 2016.
- 886 66. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk v2: memory friendly classification
887 with the genome taxonomy database. *Bioinformatics*. 2022;38(23):5315-5316.
888 doi:10.1093/BIOINFORMATICS/BTAC672
- 889 67. Adams MH. Bacteriophages. *Bacteriophages*. Published online 1959.
- 890 68. García-Aljaro C, Muniesa M, Jofre J. Isolation of Bacteriophages of the Anaerobic Bacteria
891 Bacteroides. *Methods Mol Biol*. 2018;1693:11-22. doi:10.1007/978-1-4939-7395-8_2
- 892 69. Yu G. Using ggtree to Visualize Data on Tree-Like Structures. *Curr Protoc Bioinformatics*.
893 2020;69(1):e96. doi:10.1002/CPBI.96
- 894 70. Eccles DA. Preparing Reads for Stranded Mapping. Published 2022. Accessed February 11,
895 2023. [https://www.protocols.io/view/preparing-reads-for-stranded-mapping-](https://www.protocols.io/view/preparing-reads-for-stranded-mapping-5qpvon2zzl4o/v7)
896 [5qpvon2zzl4o/v7](https://www.protocols.io/view/preparing-reads-for-stranded-mapping-5qpvon2zzl4o/v7)
- 897 71. Frith MC, Hamada M, Horton P. Parameters for accurate genome alignment. *BMC*
898 *Bioinformatics*. 2010;11(1):1-14. doi:10.1186/1471-2105-11-80/FIGURES/7

- 899 72. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data.
900 *Bioinformatics*. 2014;30(15):2114-2120. doi:10.1093/BIOINFORMATICS/BTU170
- 901 73. Li H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*.
902 2018;34(18):3094-3100. doi:10.1093/BIOINFORMATICS/BTY191
- 903 74. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools.
904 *Bioinformatics*. 2009;25(16):2078-2079. doi:10.1093/BIOINFORMATICS/BTP352
- 905



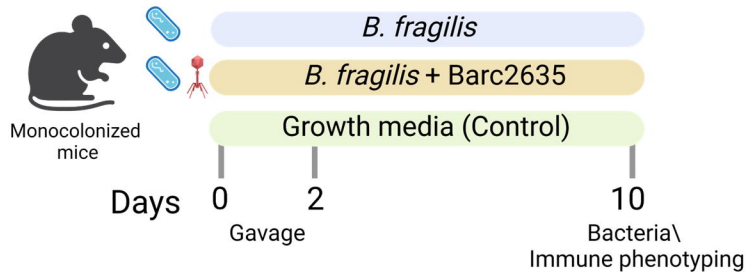
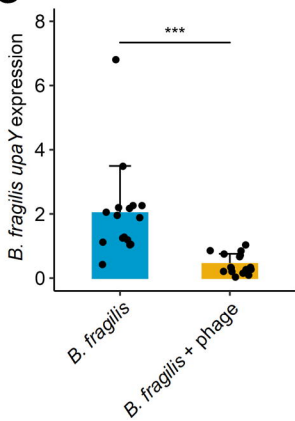
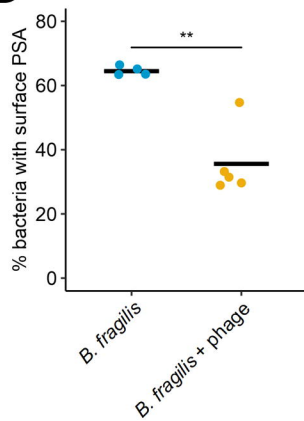
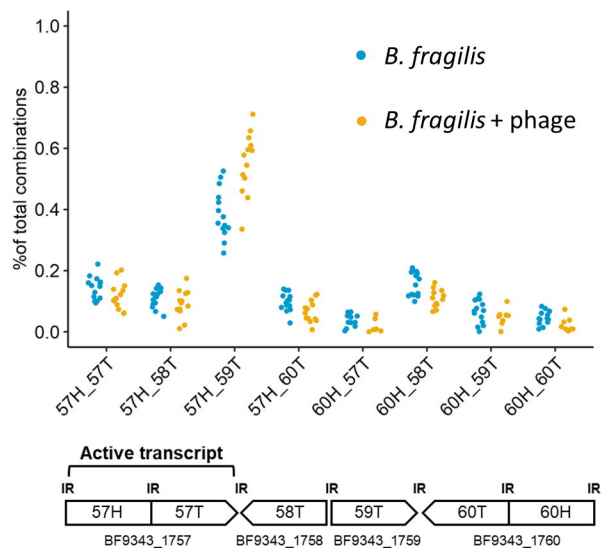
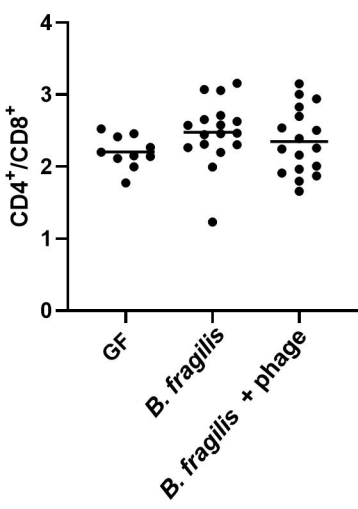




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- active CD
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B**C****D****E****F****G**