

1 **Title**

2 The spatial heterogeneity of the gut limits predation and fosters coexistence of bacteria and
3 bacteriophages

4

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27

28 **Summary**

29 The ecological dynamics underlying the coexistence between antagonistic populations of bacteria
30 and their viruses, bacteriophages (phages) in the mammalian gut microbiota remain poorly
31 understood. We challenged a murine synthetic bacterial community with phages to study the
32 factors allowing phages-bacteria coexistence. Coexistence was not dependent on the development
33 of phage-resistant clones, nor on the ability of phages to extend their host range. Instead, our data
34 suggest that phage-inaccessible sites in the mucosa serve as a spatial refuge for bacteria. From
35 there, bacteria disseminate in the gut lumen where they are predated by luminal phages fostering
36 the presence of intestinal phage populations. The heterogeneous biogeography of microbes
37 contributes to the long-term coexistence of phages with phage-susceptible bacteria. This
38 observation could explain the persistence of intestinal phages in humans as well as the low
39 efficiency of oral phage therapy against enteric pathogens in animal models and clinical trials.

40

41

42 **Introduction**

43 The mammalian gut is a highly complex and structured organ lined by a variety of eukaryotic cells
44 types that serve the establishment of a mutualistic relationship between the host and different
45 enteric microbes, including viruses. Bacteriophages (phages) are the most abundant viruses residing
46 in the gut, but their precise role in shaping the microbiome remains unclear (Manrique et al., 2017).
47 Changes in the viral and bacterial communities of the gut are increasingly reported to be associated
48 with pathological conditions in humans, including diabetes, inflammatory bowel diseases and
49 colorectal cancer (Hannigan et al., 2018b; Manrique et al., 2017; Zhao et al., 2017). Although
50 fluctuations in the viral communities have been reported in a recent longitudinal study in humans,
51 a large proportion of individual-specific viral contigs remain detectable over time (months to years),
52 suggesting that individuals possess their own viral fingerprint (Manrique et al., 2016; Shkoporov et
53 al., 2019). However, it remains understudied how phages and their corresponding bacterial targets
54 persist together in the gut.

55 Using phage-bacterial model systems, dynamics of the coexistence of predators and preys have
56 been the subject of theoretical and experimental studies, mostly performed *in vitro* and *in silico*
57 (Betts et al., 2014; Brockhurst et al., 2006; Hannigan et al., 2018a; Lenski and Levin, 1985; Weitz et
58 al., 2013). In the mammalian gut, the interaction of phages and bacteria has been explored in mice
59 and pigs (Galtier et al., 2016; Looft et al., 2014; Maura et al., 2012a; Maura et al., 2012b; Reyes et
60 al., 2013; Weiss et al., 2009; Yen et al., 2017), while human data are derived from metagenomics
61 studies (Manrique et al., 2017; Shkoporov et al., 2019) and few clinical trials of phage therapy
62 (Sarker and Brussow, 2016).

63 Studies in mice have shown that virulent phages have a limited effect on the targeted bacterial
64 populations within the gut (Bhandare et al., 2019; Galtier et al., 2017; Maura et al., 2012a; Weiss et
65 al., 2009). Nevertheless, both phage and bacterial populations could persist in the gut of animals for
66 several weeks (Maura and Debarbieux, 2012; Maura et al., 2012b). Likewise, a large randomized
67 phage therapy trial targeting *Escherichia coli* diarrhoea in Bangladeshi children showed no evidence
68 for *in vivo* amplification of oral phages in the gut despite their persistence (Sarker et al., 2016). A
69 similar situation was described in the human gut where the crAssphage coexists with its highly
70 abundant Bacteroidetes bacterial host (Guerin et al., 2018; Shkoporov et al., 2018; Yutin et al.,
71 2018). Three recent studies showed that administration of virulent phages can also strongly impact
72 the intestinal colonization of their targeted bacteria and nevertheless, still support long-term
73 coexistence (Duan et al., 2019a; Gogokhia et al., 2019; Hsu et al., 2019). The experimental settings

74 of these studies could account for differences in the amplitude of the impact, notably because of
75 the use of axenic mice, characterized by an immature immune system and thus unstable
76 colonization of bacteria of human origin. Moreover, the limited number of phages and bacteria
77 couples that have been studied in the gut environment does not allow yet drawing conclusions on
78 the type and prominence of factors that influence phage-bacterium interactions.

79 Since phages are proposed as a treatment for the major public health threat of antibiotic-resistant
80 bacterial infections (Roach and Debarbieux, 2017), as well as means to precisely engineer the
81 intestinal microbiota, further insight into the variety of phage-bacterium interactions and their
82 coexistence in the mammalian gut are deeply needed (Brussow, 2017). Several factors were shown
83 or proposed to be involved in this coexistence, such as (i) arms race dynamics with resistance
84 development to phage infection and viral counter-resistance, (ii) the inherent or evolved ability of
85 the phage to infect multiple hosts, and (iii) the distribution of these two antagonistic populations
86 into distinct anatomical structures (Brockhurst et al., 2006; Doron et al., 2018; Galtier et al., 2017;
87 Heilmann et al., 2012; Hilborn, 1975; Labrie et al., 2010). In this report, we investigated the
88 contribution of these factors using the synthetic Oligo-Mouse-Microbiota comprising 12 distinct
89 strains (OMM¹²) (Brugiroux et al., 2016).

90 This animal model allows following defined pairs of phages and bacteria in the gut without the need
91 of antibiotic treatments as a confounding factor commonly used to study enteric pathogens in
92 murine models (Croswell et al., 2009). In addition, it provides more realistic conditions than mono-
93 colonized mice that lack aspects of competitive and synergistic interspecies interactions of
94 microbes (Weiss et al., 2009). We established stable colonization of two *E. coli* strains in gnotobiotic
95 OMM¹² mice and studied the population dynamics of these strains in the presence of virulent
96 phages. We found that phages were less abundant in the mucosal part of the gut compared to *E.*
97 *coli* levels. Our data are in agreement with the ecological theory of source-sink dynamics (Holt,
98 1985) providing an explanation for the lack for phage-resistant mutant selection and the limited
99 efficacy of virulent phages in reducing intestinal bacterial loads.

100

101

102 **Results**

103 ***E. coli* commensal strain Mt1B1 colonizes the gut of OMM¹² mice**

104 Mice harbouring the OMM¹² consortium (*Acutalibacter muris*, *Akkermansia muciniphila*,
105 *Bacteroides caecimuris*, *Bifidobacterium longum subsp. animalis*, *Blautia coccoides*, *Clostridium*
106 *clostridioforme*, *Clostridium innocuum*, *Enterococcus faecalis*, *Flavonifractor plautii*, *Lactobacillus*
107 *reuteri*, *Muribaculum intestinale*, *Turicimonas muris*) were exposed to the murine *E. coli* commensal
108 strain Mt1B1 to test its capacity to establish in this synthetic community. Mice became colonized
109 with this strain within two to three days. The fecal levels of strain Mt1B1 remained stable over a
110 period of two weeks (Fig. 1A). Mice did not exhibit signs of discomfort or change in feces
111 consistency. Twelve days after inoculation of strain Mt1B1, intestinal sections (ileum and colon)
112 were examined and the location of strain Mt1B1 was determined by fluorescence *in situ*
113 hybridization (Fig. 1B and Fig. S1). Strain Mt1B1 was found in all sections of the gut, including the
114 ileum, consistent with the location from which it was isolated (mucosa from the ileum of
115 conventional laboratory mice) (Garzetti et al., 2018; Lagkouvardos et al., 2016).

116

117 **Selected virulent phages replicate only on strain Mt1B1 in the gut environment of OMM¹² mice**

118 We isolated several phages infecting strain Mt1B1 from the environment and selected three
119 (Mt1B1_P3, Mt1B1_P10, Mt1B1_P17) with different characteristics (host range, adsorption and
120 lysis kinetics, genomic content) (Table 1, Fig. S2 and Tables S1, S2, S3). Both, Podoviridae P3 and
121 P10 are close to *E. coli* phages K1F and K1E, respectively, which are capsule-specific phages.
122 Myoviridae P17 is closely related to *E. coli* phages phAPEC8 and ESCO13. Like ESCO13 and by
123 contrast to phAPEC8, Mt1B1_P17 does not carry an endo-N-acetylneuraminidase gene involved in
124 the degradation of the K1 capsule and therefore is most likely not capsule-specific (Trotureau et al.,
125 2017). In liquid broth phages P3 and P10 displayed similar infection kinetics patterns on strain
126 Mt1B1, with rapid lysis followed by a moderate bacterial regrowth at 1.5 hours. In contrast, phage
127 P17 halted the growth of strain Mt1B1 for several hours and slow bacterial regrowth resumed only
128 after more than 10 hours (Fig. 2A). When used in combination, these three phages caused rapid
129 lysis of strain Mt1B1 followed by very slow regrowth (Fig. 2A). Then, we assessed the capacity of
130 each phage to replicate in gut sections collected from Mt1B1-colonized OMM¹² mice, in an *ex-vivo*
131 assay previously used to reveal the activities of phages along different gut sections (Galtier et al.,
132 2017; Maura et al., 2012a). We collected samples of the ileum and colon from Mt1B1-colonized
133 OMM¹² mice. We then separated out the mucosal and luminal parts of the ileum and colonic

134 tissues. We compared the replication of the three phages in these samples *ex vivo* with their
135 replication on Mt1B1 planktonic cultures, at both exponential and stationary growth phases. All
136 three phages displayed similar patterns with efficient replication in all tested gut sections and in
137 exponential growth phase liquid culture, while no amplification was observed in cells at stationary
138 phase (Fig. 2B). As strain Mt1B1 did not multiply in the stationary growth phase (grey bars in Fig.
139 2B), we concluded that Mt1B1 cell growth is necessary for amplification of these three phages.
140 Next, we evaluated the transit time of Mt1B1 phages at 6, 24, 48 and 72 hr in axenic mice that
141 received a single oral dose (6×10^7 pfu) of a mixture of the three phages (equal amounts of each
142 phage). The fecal level of Mt1B1 phages was below the threshold at 6hr, maximum at 24 hr and
143 then decreased at 48 hr and finally reached the limit of detection at 72 hr (Fig. 3A). In gut sections,
144 we found that the level of phages at 6 hr reached 10^6 , 10^4 and 10^3 PFU/g in the luminal part of the
145 ileum, the mucosal part of the ileum and the luminal part of the colon, respectively, while it
146 remained undetected in the mucosal part of the colon. At 24 hr the level of phages in the ileum
147 dropped while it increased in the colon and at 48 hr it was barely detectable in the ileum while only
148 present in the luminal part of the colon. At 72 hr the level of phages was below the threshold of
149 detection in all gut sections tested (Fig 3B). Then, we performed a similar experiment in OMM¹²
150 mice, not inoculated with strain Mt1B1, by taking samples at 24 and 48 hr post-gavage (same dose
151 as above). Phage levels in feces were slightly lower than those observed in axenic mice. We could
152 detect phages only in the luminal part of the colon at both 24 and 48 hr post-gavage as anticipated
153 from experiments with axenic mice (Fig 3C). These findings indicate that none of the Mt1B1 phages
154 amplified *in vivo* on any of the 12 strains ruling out a possible off-target amplification. These data
155 are in agreement with previous results on phage safety from human volunteers and from
156 conventional mice not colonized with the targeted bacteria (Bruttin and Brussow, 2005; Maura et
157 al., 2012b; Weiss et al., 2009).

158 159 ***In vivo* infection of Mt1B1-colonized OMM¹² mice by a phage cocktail**

160 We next investigated how Mt1B1-colonized OMM¹² mice responded to an identical single oral dose
161 of the three phages (6×10^7 pfu). The fecal levels of phages and bacteria were monitored at short (4
162 and 6 hours post oral dose) and long (during two weeks) periods of time (Fig. S3A). At all time
163 points but one (day18) following phage administration, levels of Mt1B1 in the phage group were
164 lower than in the control group, despite remaining within one-log of variation and not reaching
165 significance. Within the two weeks of observation phage levels fluctuated within 2 logs (Fig. S3B).

166 Nevertheless, phage:bacteria ratios remained stable (less than 1 log variation) over time (Fig. S3C).
167 These data show that a single dose of these three phages is sufficient to initiate their long-term
168 replication in Mt1B1-colonized OMM¹² mice indicating that this animal model is suitable to study
169 the coexistence of phages and bacteria in the gut.

170 Afterwards, we asked whether repeating the phages administration during three consecutive days
171 could disturb this coexistence between phages and strain Mt1B1. Such a setting mimics a phage
172 therapy treatment targeting bacterial pathogens residing in the human gut (Corbellino et al., 2019).
173 In two independent experiments, we observed a small but significant decrease in the fecal levels of
174 strain Mt1B1 when comparing phage-treated and control groups (d15, p=0.0001; d16, p=0.002;
175 d17, p=0.023) (Fig. 4A and Table S4). Despite this significant impact, phage levels and
176 phage:bacteria ratios remained stable (within 1-log) (Fig. 4B, 4C) and were comparable to data
177 obtained with a single dose of phages showing that the three consecutive phage administrations
178 did not destabilize phage bacteria coexistence (Fig S3).

179

180 **Phage replication does not trigger shifts in the OMM¹² bacterial community**

181 We next assessed the stability of the gut microbiota of both phage treated (n=6) and non-treated
182 (n=5) mice groups by two methods, 16S rRNA specific qPCR (Brugiroux et al., 2016) and 16S
183 amplicon sequencing. This quantification was performed at day0 (before colonization with strain
184 Mt1B1), day14 (before the gavage with the three phages) and day17 (one day after the third
185 phages gavage) and both methods showed similar results (Fig. S4). Changes in the bacterial
186 community (qPCR data) were analysed by performing a between-class principal component analysis
187 (PCA), taking into account days, cages and phage inoculation. Results identified daily fluctuations as
188 the main source of the observed variations regardless of the presence or absence of phages (Fig.
189 4D, Table S5). This finding is also consistent with the passive transit of these phages in OMM¹²
190 devoid of *E. coli* strain Mt1B1 (Fig. 3C). Therefore, the replication of phages observed in Mt1B1-
191 colonized OMM¹² mice result exclusively from their capacity to infect strain Mt1B1 in the gut
192 environment, confirming the lack of a possible off-target amplification to support phage
193 coexistence.

194

195 **Maintenance of bacterial populations is not caused by the emergence of phage-resistant clones**

196 Next, we tested whether the emergence of phage-resistant clones could explain how bacteria can
197 coexist with phages. A total of 280 isolated fecal clones of strain Mt1B1 from 13 mice exposed to

198 the three phages cocktail (from both short and long-term colonization) were susceptible to each of
199 the three phages. We also tested for the emergence of phage-resistant clones from several gut
200 sections (luminal and mucosal parts from ileum and colon). Again, all of these clones (n=800) were
201 susceptible to each of the three phages. Thus, phage resistance development does not explain the
202 maintenance of susceptible bacterial populations in presence of phages.

203

204 **Heterogeneous spatial localization of bacteria in the gut can explain phage-bacteria coexistence**

205 In the absence of evidence for off-target phage amplification on heterologous bacteria and a lack of
206 support for the emergence of phage-resistant bacteria, we wondered whether the spatial
207 heterogeneity of the mammalian gut might create niches where bacteria remain protected against
208 phage predation (Heilmann et al., 2012). We tested this hypothesis by examining luminal and
209 mucosal parts from ileum and colon sections from Mt1B1-colonized OMM¹² mice exposed or not to
210 phages. Compared to Mt1B1 titers in control mice, phage cocktail application was associated with
211 small but significant Mt1B1 titer decreases in both, the luminal and mucosal parts of the ileum and
212 the mucosal part of the colon (Fig. 4E and Table S4). This indicates that these regions harbour
213 Mt1B1 cells susceptible to phage infection. We observed an average of 2-log and 1-log lower phage
214 titers in the mucosal compared to the luminal parts of the ileum and colon, respectively (Fig. 4F).
215 Consequently, in mucosal parts we found a parallel reduction of the density of both phages and
216 bacteria. However, phage:bacteria ratios revealed a significant (p=0.006) lower relative abundance
217 of phages in the mucosal part of the ileum suggesting that at this location some Mt1B1 bacteria
218 might not be exposed to phages (Fig 4G and Table S4). We concluded that a heterogeneous spatial
219 localization of strain Mt1B1 in the ileum reduces accessibility to phages by providing phage-free
220 refuges.

221

222 **Coexistence of a single phage (CLB_P2) with its targeted bacterium (*E. coli* strain 55989) is also** 223 **explained by spatial refuges**

224 To challenge and strengthen the causal role of spatial localization of bacteria in their coexistence
225 with virulent phages, we investigated the behaviour of the Myoviridae phage CLB_P2 infecting the
226 pathobiont enteroaggregative *E. coli* strain 55989. This phage was previously shown to efficiently
227 infect strain 55989 in gut sections and to coexist with it over 3 weeks (Maura and Debarbieux,
228 2012; Maura et al., 2012b). First, we showed that strain 55989 colonizes the gut of OMM¹² mice
229 (Fig. S5A) as previously reported in conventional mice (Maura et al., 2012b), and did likewise not

230 result in any sign of intestinal disease. Second, when 55989-colonized OMM¹² mice received a
231 single oral dose of phage CLB_P2 (1×10^8 pfu) we observed that fecal levels of strain 55989 in
232 presence or absence of phage CLB_P2 were similar around 10^8 cfu/g and the phage titers remained
233 stable around 10^7 pfu/g (Fig 5A, 5B), showing that this model reproduces conditions for stable
234 phage:bacteria ratios (Fig 5C). No significant difference in the fecal levels of strain 55989 was
235 observed on individual days between phage CLB_P2-treated and control groups (Table S6).
236 However, the ANOVA comparison between these two groups, when including the three days,
237 reached significance ($p=0.0268$) (Table S7). Third, we examined the luminal and mucosal parts of
238 both ileum and colon from 55989-colonized OMM¹² mice three days after the administration of the
239 single dose of phage CLB_P2. In all sections, but the mucosal part of the colon, the level of strain
240 55989 was significantly lower in the phage CLB_P2 group than in the control group (Fig. 5D and
241 Table S6). Phage CLB_P2 levels were similar in all sections except in the mucosal part of the ileum in
242 which it was about 2-log lower (Fig. 5E). Phage CLB_P2:strain 55959 ratios in gut sections indicate a
243 significant difference between luminal and mucosal parts of the ileum, while no such difference
244 was observed in the colon (Fig 5F and Table S6). Fourth, we tested 165 and 822 colonies isolated
245 from feces and gut sections, respectively, for their susceptibility to phage CLB_P2 and found that
246 they were all susceptible, showing that development of phage resistance to a single phage could
247 not explain the maintenance of susceptible bacterial populations in presence of this phage (Fig.
248 S5B). In addition, we determined the frequency of phage-resistant clones in fecal pellets from
249 55989-colonized OMM12 mice that received either phage CLB_P2 or PBS and found that they were
250 at the same range for both groups and equivalent to the frequency obtained with a fresh culture of
251 strain 55989 (Table S7).

252 To support further our conclusions, we quantified the abundance of strain 55989 in unwashed gut
253 sections using immunohistochemistry (Fig. 6A and Fig. S6). We found no difference in the
254 abundance of strain 55989 in the mucosal parts of both ileal and colonic sections from phage
255 CLB_P2-treated mice compared to controls, which is in agreement with the presence of bacterial
256 refuges on the mucosal parts of the gut (Fig. 6B and Table S8). In the luminal parts of both ileum
257 and colonic sections the abundance of strain 55989 (quantified by fluorescence intensity) was not
258 different between the two conditions, while a significant difference was reached when comparing
259 CFU data (Fig. 5D). This underlines the difficulty to compare indirect (microscopy) with direct
260 (plating) quantifications. To conclude, the lower abundance of phages in the mucosal parts of the
261 ileum provides opportunities for bacteria to reside in phage-free niches by escaping to phage

262 predation. Taken together, data from two independent models (Mt1B1- and 55989-colonized
263 OMM¹² mice) using different phages show that the biogeography of bacteria participates to the
264 coexistence of phages and phage-susceptible bacteria in the gut.

265

266 **Discussion**

267 The intestinal microbiota of mammals is relatively stable over time in healthy subjects while it
268 contains large antagonistic populations of bacteria and phages. The mechanisms that underlie this
269 apparently peaceful coexistence are unknown. Several hypotheses can be drawn from years of *in*
270 *vitro* studies of isolated systems (one phage/one bacterium) or more recently from environmental
271 studies (Buckling and Rainey, 2002; Fortuna et al., 2019; Horne, 1970; Laanto et al., 2017; Meyer et
272 al., 2016). However, none of them has yet been tested in a relevant intestinal environment. Using a
273 gnotobiotic mouse model, two *E. coli* strains and four different virulent phages, we showed that the
274 emergence of phage-resistant bacteria and the possibility to infect off-target bacteria can be
275 excluded as major factors supporting coexistence. Instead, we found that the spatial distribution of
276 phages and bacteria along the radial axis of the gut fits the classical ecological theory of source-sink
277 dynamics (Holt, 1985). Our data suggest that refuges for bacteria in the mucosal layer serve as a
278 source, with the lumen acting as a sink in which the phages can infect their target. As phages do not
279 exert a major direct selective pressure on the source, the bacteria reaching the gut lumen remain
280 susceptible enabling the phages to maintain their density in the lumen over time (Fig 6C). This
281 different luminal/mucosal distribution of phages was significant in ileal but not in colonic sections.
282 This might not be the case in the human colon that displays organisational differences compared to
283 mice colon, the latter being essentially filled with structured fecal pellets in which phage-bacteria
284 interactions are likely to be less intense (Nguyen et al., 2015).

285

286 In phage bacteria interaction studies, the prey-predator dynamics have been classically studied
287 through the prism of phage-resistant bacteria and counter resistant phages. Recently, an *in vitro*
288 study has proposed the model named “leaky resistance” to explain the maintenance of virulent
289 phages by the high rate of transitions between phage-resistant and phage-susceptible bacteria
290 within the populations (Chaudhry et al., 2018; Silveira and Rohwer, 2016). However, we failed to
291 isolate phage-resistant bacteria within the entire gut during this work performed with two couples
292 of phages and bacteria. This suggests that phage-resistant bacteria may, *in vivo*, either carry a high
293 cost or have a low selective value in both single and repeated phage application (Gomez and

294 Buckling, 2011; Koskella et al., 2012). Coupled with the concept of costly phage resistance
295 mutations, our results are in agreement with observations from *in silico* individual based stochastic
296 spatial models, which showed that structured environments can create spatial refuges that lead to
297 coexistence between bacteria and phages without the emergence of resistant clones (Heilmann et
298 al., 2012; Sousa and Rocha, 2019).

299

300 The lower abundance of phages in the mucosal parts could be triggered by the reduced bacterial
301 density compared to luminal parts, according to a density-dependent phenomenon that was
302 previously observed in several *in vitro* structured environments (Eriksen et al., 2018). This density-
303 dependent phenomenon was linked to the “proliferation threshold” that proposes that a minimum
304 number of bacteria is needed for the phage to initiate infection and amplify (Payne et al., 2000;
305 Wiggins and Alexander, 1985). Note that the source-sink and the “proliferation threshold”
306 hypotheses are not mutually exclusive to support the presence of bacterial refuges driving the long-
307 term coexistence of phages and susceptible bacteria. We propose that the gut displays a mucosal-
308 luminal gradient of phage density exposing bacterial populations to different phage concentrations
309 (Fig. 6C). Such a gradient can explain the persistence for months and years of phage populations in
310 human fecal samples without major variations of the microbiota composition.

311

312 Nevertheless, the heterogeneous spatial distribution of bacteria in the gut does not exclude other
313 factors or processes that could also participate to the long-term coexistence of phage with
314 susceptible bacteria. Some of these processes were not directly tackled in this study like the
315 influence of abiotic factors (Lourenco et al., 2018; Scanlan et al., 2019). In particular, phenotypic
316 resistance that is described as the ability of bacteria to oscillate between phage-susceptible and
317 phage-resistant cells could be involved in some of our observations. This phenotypic resistance is
318 led by environmental or cellular stochasticity that modulate the expression of genes, for example
319 those encoding for phage receptors (Bull et al., 2014; Chapman-McQuiston and Wu, 2008). Phage
320 diffusion may be limited by mucins and other glycoproteins, lipids and DNA molecules (Johansson
321 et al., 2011; Qi et al., 2017). Immunoglobulin motifs in structural phage proteins were also shown to
322 favour phage binding to mucus (Barr et al., 2013). However, only ORF118 of phage Mt1B1_P17
323 possesses such a motif (identified by a specific *in silico* search) homologous to the bacterial Ig-like
324 domain (Big_2) (bit score=29.5, individual E-value=5.1x10⁻⁹ and conditional E-value=2.8x10⁻¹¹)
325 (Fraser et al., 2006). The absence of such motifs in three out of the four phages and in particular in

326 CLB_P2 is consistent with our *in vivo* observation that these phages are less abundant in the
327 mucosal parts of gut sections.

328

329 Our data show that the gnotobiotic OMM¹² model is particularly well suited to study the
330 mechanisms involved in phage bacteria dynamics in the gut. OMM¹² community establishes a long-
331 term stable composition over several mouse generations and can be used as platform to flexibly
332 incorporate additional bacterial strains (Brugiroux et al., 2016; Herp et al., 2019; Studer et al.,
333 2016). Interestingly, Hsu and colleagues recently reported the use of similar synthetic microbiota
334 models to study phage bacteria interactions with two major differences. First, axenic mice were
335 inoculated with bacterial strains during two weeks before the inoculation of phages, compared to
336 stably colonized OMM¹² mice. Second, the 10 bacterial strains chosen are from human origin,
337 instead of mouse origin for OMM¹², and may rapidly undergo genetic adaptation to the mouse
338 environment (Barroso-Batista et al., 2014; Lourenco et al., 2016). Despite these differences, both
339 mouse models confirm that the inoculation of virulent phages in the gut generally leads to the
340 coexistence of phages and bacteria. Hsu et al. found that *Enterococcus faecalis* phage resistant
341 mutants increased over time and that the microbial community structure was affected. Here, we
342 could not detect any of these two events. In addition, they found that some phages have strong
343 impact on abundance of cognate host bacteria, while such impact was not previously observed in
344 several mouse models (Maura et al., 2012a; Weiss et al., 2009). This suggests that different phage-
345 bacteria couples may exhibit distinct eco-evolutionary dynamics, increasing the complexity of
346 studying these interactions in natural environments (Shkoporov and Hill, 2019). A recent example
347 of such complexity was reported when studying the *in vivo* prophage induction of *Roseburia*
348 *intestinalis*, a dominant bacterium of the human gut, which leads to the coexistence of phages and
349 bacteria with the selection of CRISPR-mediated bacterial resistance along with hypervirulent phage
350 variants (Cornuault et al., 2019).

351

352 Deciphering the dynamics of phage bacteria interactions in the gut is required to develop efficient
353 phage-guided therapeutic strategies and ultimately obtain firm clinical evidences that are still
354 lacking for intestinal infections (Brussow, 2017; Duan et al., 2019b; Sarker et al., 2016). A recent
355 case report showed that the *in vitro* isolation of a single virulent phage, used to target a multidrug
356 resistant strain of *Klebsiella pneumoniae*, led to the eradication of this pathogen from the patient's

357 gut (Corbellino et al., 2019). While lacking mechanistic insights, this work confirms the medical
358 potential of phages to selectively target bacteria residing in the gut.
359

360 **Author contribution**

361 Conceptualization, L.D., L.D.S. and M.L.; Methodology, B.S., L.D., L.D.S., M.B. and M.L.;
362 Investigation, C.E., L.C., L.D.S., M.L., Q.L.B. and T.P.; Formal Analysis, L.C., M.L. and P.C.; Writing –
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384

385

386 **Declaration of Interests**

387 The authors declare no competing interests.

388

389

390 **Main figures titles and legends**

391

392 **Figure 1. The *E. coli* strain Mt1B1 stably colonizes the gut of the OMM¹² mice.**

393 **A.** Fecal levels of the *E. coli* strain Mt1B1 at the indicated time points for each OMM¹² mouse (n=11)
394 receiving a single dose of 10⁸ cfu by oral gavage at day 0. Red dots, individual values; horizontal bar,
395 median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval).

396 **B.** Localization by FISH of the strain Mt1B1 in the ileal section of Mt1B1-colonized OMM¹² mice.
397 Intestinal cells (nuclei) were stained with DAPI (purple), and Mt1B1 (red+green=yellow) and
398 Eubacteria (red) were stained with specific FISH probes. A representative image from a group of
399 four mice is presented. Scale bar, 50µm. See also Figure S1.

400

401 **Figure 2. Mt1B1 phages P3, P10 and P17 infect strain Mt1B1 both *in vitro* and *ex-vivo*.**

402 **A.** Growth curves for strain Mt1B1 (n=3 for each condition) measured via OD_{600nm} reading in liquid
403 broth in the absence (grey) or presence of phage P3 (orange), P10 (blue) or P17 (green), or of a
404 cocktail of these three phages (purple; equal proportions of each) added at t=0 at MOI of 1 x 10⁻².
405 The inset shows an enlargement for early time points. (error bars represent standard error of the
406 mean, SEM). **B.** Amplification over 5h (n=3 biological replicates) of individual phages (P3, orange;
407 P10, blue; P17, green; each at an MOI of 1 x 10⁻²) and Mt1B1 cells (grey) in indicated homogenized
408 gut sections (lu., luminal; mu, mucosal) from Mt1B1-colonized OMM¹² mice (*ex vivo*) and flasks with
409 Mt1B1 cells in exponential (exp.) (OD_{600nm}=0.5) or stationary (stat.; 24hr) growth phases (*in vitro*).
410 N-fold multiplication relative to the initial number of phages added at t=0, which was
411 approximately 100 fold lower than the amount of Mt1B1 cells, is shown. The n-fold multiplication
412 of strain Mt1B1 was calculated by plating samples before and after the 5hr incubation (means with
413 standard deviation).

414

415 **Figure 3. Passive phage transit in axenic and OMM¹² mice.**

416 Axenic (n=8) and OMM¹² (n=4) mice received a single dose of the three Mt1B1 phages (3x10⁷ pfu;
417 phages mixed in equal proportions). Phage titers were determined in fecal pellets and intestinal
418 sections at indicated time points. **A.** Fecal phage titers from axenic mice. **B.** Phage titers in organs
419 from axenic mice. **C.** Phage titers from OMM¹² mice feces and organs. Dots represent individual
420 data and horizontal bars represent the average.

421

422 **Figure 4. Virulent Mt1B1 phages do not affect the microbiota composition of the OMM¹² mice**
423 **and display an heterogeneous repartition in gut sections supporting their coexistence with strain**
424 **Mt1B1.**

425 **A.** Mt1B1-colonized OMM¹² mice (n=25) received at day14, 15 and 16 PBS (red, n=11) or the three
426 phages P3, P10 and P17 together (blue, n=14; 6×10^7 pfu per dose made of the same amount of each
427 phage) by oral gavage. Levels of *E. coli* strain Mt1B1 in the feces. **B.** Phage titers from the fecal
428 samples reported in panel A. **C.** Phage:bacteria ratios for fecal samples collected on days15, 16 and
429 17. Dots, individual values; horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max
430 values (within 1.5 x interquartile interval). **D.** Between-group PCA (BCA, axes 1 and 2) for the 16S
431 rRNA qPCR data for mice receiving PBS (n=5, filled circle) or the phage cocktail (n=6, filled triangle)
432 by oral gavage at the indicated time points (see the colors indicated) for 10 bacteria from the fecal
433 microbiota of OMM¹² mice (strains YL2 and KB18 were not detected). On day 17 mice were
434 sacrificed and gut section analyzed. **E.** Titers of strain Mt1B1 in indicated gut sections (lu., luminal;
435 mu., mucosal). **F.** Titers of Mt1B1 phages in the samples reported in panel E. **G.** Phage:bacteria
436 ratios for the indicated gut sections in panels A and B. Dots, individual values; horizontal bar,
437 median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x interquartile interval).
438 See also Table S4 and S5.

439

440 **Figure 5. Spatial heterogeneity of the *E. coli* strain 55989 in the gut sections participates to the**
441 **coexistence with virulent phage CLB_P2.**

442 **A.** 55989-colonized OMM¹² mice (n=28) were colonized during 7 days before receiving a single
443 administration of PBS (red, n=12) or phage CLB_P2 (blue, n=16; 1×10^8 pfu) by oral gavage. **A.** Fecal
444 titers of strain 55989. **B.** Fecal titers of phage CLB_P2. **C.** Phage:bacteria ratios for fecal samples
445 collected on days 8, 9 and 10. On day 10 mice were sacrificed and gut section analyzed. **D.** Titers of
446 strain 55989 in indicated gut sections. **E.** Titers of phage CLB_P2 in the samples reported in panel D.
447 **F.** Phage:bacteria ratios for the indicated gut sections in panels D and E. Dots, individual values;
448 horizontal bar, median; box, 25th-75th quantiles, vertical bars, min/max values (within 1.5 x
449 interquartile interval). See also Table S6.

450 **Figure 6. Source-sink dynamics support the coexistence of virulent phages and bacteria in the gut.**
451 **A.** Representative photograph (scale bar, 200µm) of immunofluorescence staining of strain 55989
452 (red) and mucus (green) in unwashed ileum section with nuclei of epithelial cells stained with DAPI
453 (blue). **B.** Quantification of the red fluorescence per pixel in mucosal and luminal parts of ileal and
454 colonic sections from 55989-colonized OMM¹² mice at day 10 (n=4 to 5). **C.** Schematic
455 representation of the source-sink dynamics between virulent phage and bacteria populations in the
456 gut. Bacteria located close to the mucosal layer (in yellow) form refuges that phages cannot
457 reached. Bacteria located in the intestinal lumen are killed (dotted lines) by phages that can persist
458 through the gut. Bacteria and phage populations coexist without the strong selection of phage-
459 resistant bacteria.

460

461

462

463 **Main tables and legends**

464

465 **Table 1.** Main characteristics of Mt1B1 phages P3, P10 and P17

466

467

468

469 **STAR Methods**

470 **RESOURCE AVAILABILITY**

471 **Lead contact**

472 Further information and requests for resources and reagents should be directed to and will be
473 fulfilled by the Lead Contact, Laurent Debarbieux (laurent.debarbieux@pasteur.fr).

474 **Materials Availability**

475 This study did not generate new unique reagents

476 **Data and Code Availability**

477 Accession numbers for phage genomes are: MT496969 for Mt1B1_P3, MT496971 for Mt1B1_P10
478 and MT496970 for for Mt1B1_P17.

479

480 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

481 **Microorganisms**

482 Bacterial strains including Mt1B1 (DSM-28618) and 55989 are listed in Table S1. Bacteriophage
483 CLB_P2 isolation and characterization is described on Maura *et al.* (Maura et al., 2012b).

484 Bacteriophage Mt1B1_P3, P10 and P17 were isolated and purified from sewage water, with an
485 enrichment technique (explained in method details below) using E. coli strain Mt1B1. All phages
486 used were amplified in exponential growing cultures of the respective host strain for approximately
487 4 hours. Cell lysate supernatants containing amplified phages were after 0.22 µm filter sterilized
488 and stored at 4°C.

489 Strains were routinely cultured in lysogeny broth (LB), or on LB agar or Drigalski agar (Bio-Rad,
490 Hercules, CA) plates, at 37°C.

491

492 **Animals and ethics**

493 C57Bl/6J mice (seven to nine-week-old) OMM¹² were bred at Institut Pasteur (Paris, France). A total
494 of 72 OMM¹² and 8 C57Bl/6J axenic healthy mice were used. For all the experiments littermates of
495 the same sex were randomly assigned to experimental groups, being used both female and male
496 mice. Food and drinking water were provided ad libitum. All mice were housed in isocages and in an
497 animal facility in accordance with Institut Pasteur guidelines and European recommendations. All
498 animal experiments were approved by the committee on animal experimentation of the Institut
499 Pasteur and by the French Ministry of Research.

500

501 **METHOD DETAILS**

502 **Phage isolation**

503 First, sewage water from four locations was filtered at 0.45µm and mixed with an equal volume of
504 2X Luria- Bertani (LB) medium. Second, these four mixtures were inoculated with a fresh growing
505 culture of Mt1B1 (OD_{600nm} of 0.4 at 600nm, final dilution 1/200) and incubated on a shaker at 37°C
506 overnight. The next day chloroform (1/10 vol./vol.) was added to the flasks and incubated at room
507 temperature for one hour. Following centrifugation at 8000g for 10min 1 mL of the supernatant
508 was mixed with 1/10 vol./vol. of chloroform and centrifuged at 8000g for 5min. A 100-fold dilution
509 in TN buffer (10mM Tris HCl pH7.5, 100mM NaCl) of the aqueous phase was prepared. 10µL of the
510 undiluted and diluted solutions were spread with an inoculation loop on the top of two separate LB
511 agar plates and allowed to dry for 30min under a safety cabinet. Subsequently, 1mL of an
512 exponentially growing culture of Mt1B1 was applied to fully cover each plate; the excess of liquid
513 culture was removed and plates were incubated at 37°C overnight. The next day, individual plaques
514 were picked and resuspended in tubes containing 200µL TN buffer. 1/10 vol./vol. chloroform was
515 mixed and tubes were centrifuged at 8000g for 5min. These steps of plaque purification were
516 performed three times. Finally, 10µL of the last resuspended plaque were added to 1mL of a liquid
517 culture of Mt1B1 (OD_{600nm} of 0.4 at 600nm) and incubated at 37°C in a shaker for 5hr. 1/10 vol./vol.
518 of chloroform was mixed and after centrifugation at 8000g for 5min this stock was stored at 4°C
519 and served as starting solution for large scale lysates (Henry et al., 2013).

520

521 **Host range tests**

522 Host range tests were performed as follows: 3µL of PBS-diluted phage solutions (0.2µm filtered
523 sterilized crude lysates adjusted to 10⁷ pfu/mL) were deposited side by side on the lawn of each
524 tested bacterium on agar LB plates. Plates were incubated at 37°C overnight. Phages were grouped
525 according to their host range and three representative phages (Mt1B1_P3, Mt1B1_P10 and
526 Mt1B1_P17) of the main groups were chosen (Table S1).

527

528 **Adsorption assays and phage growth**

529 Three independent adsorption assays were performed for each phage according to the protocol
530 previously described (Chevallereau et al., 2016). Data were fitted using an exponential function and
531 adsorption times were defined as the time required to reach a threshold of 10% of non-adsorbed

532 phage particles. To record phage growth and bacteria lysis, an overnight culture of strain Mt1B1
533 was diluted in LB broth and grown to an OD_{600nm} of 0.2 from which 150µL were distributed into
534 each of the wells of a 96-well plate (Microtest 96 plates, Falcon). 10 µL of sterile phage lysates
535 diluted in PBS to obtain a multiplicity of infection (MOI) of 1×10^{-2} in each well. Plates were
536 incubated in a microplate reader at 37°C, with a shaking step of 30sec before the automatic
537 recording of OD_{600nm} every 15min over 20hr (Glomax MultiDetection System, Promega, USA).

538

539 **Phage genomes sequencing and analysis**

540 Sterile phage lysates were treated by DNase (120 U) and RNase (240 µg/mL) and incubated for 30
541 min at 37°C before adding EDTA (20 mM). Lysates were treated with proteinase K (100 µg/mL) and
542 SDS (0.5%) and incubated at 55°C for 30min. DNA was extracted by a phenol-chloroform protocol
543 modified from Pickard (Pickard, 2009). Sequencing was performed using Illumina technology
544 (Illumina Inc., San Diego, CA) MiSeq Nano with paired-end reads of 250bp. Quality of reads was
545 visualised by FastQC v0.10.1 Brabraham Bioinformatics
546 (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Assembly was performed using a
547 workflow implemented in Galaxy-Institut Pasteur using clc_assembler v4.4.2 and clc_mapper v4.4.2
548 (CLC Bio, Qiagen). Phage termini were determined by PhageTerm (Garneau et al., 2017) and
549 annotations were performed by the RAST v2.0 server (Aziz et al., 2008). According to the
550 nomenclature proposed for naming viruses (Adriaenssens and Brister, 2017), the full names for
551 phages P3, P10 and P17 are vB_EcoP_Mt1B1_P3, vB_EcoP_Mt1B1_P10 and vB_EcoM_Mt1B1_P17,
552 respectively. The Accession numbers for the phage genomes are pending.

553

554 **Search for Ig-like domains on phage genomes**

555 Protein sequences of the four phages were scanned for homologs on Pfam database using the
556 HMMER website (Potter et al., 2018). The results were compared to a comprehensive HMM
557 (Hidden Markov Models) database of Ig-like domains found on Pfam that was kindly provided by Dr.
558 Sean Benler.

559

560 **Ex vivo phage replication assay**

561 Oligo-MM¹² mice received 200µL of strain Mt1B1 (10^7 CFU prepared from an overnight culture in LB
562 at 37°C) in sterile sucrose sodium bicarbonate solution (20% sucrose and 2.6% sodium bicarbonate,
563 pH 8) by oral gavage and three days after were sacrificed to collect and weight intestinal sections

564 (ileum and colon). PBS was added to each sample (1.75mL) before homogenization (Oligo-Macs,
565 Milteny Biotech). A volume of 150µL of each homogenized gut sample was distributed in the wells
566 of a 96-well plate and 10µL of each individual phage were added to reach an MOI of 1×10^{-2} , and
567 the plate was incubated at 37°C. A fraction of the homogenized samples was also serially diluted in
568 PBS and plated on Drigalski medium to count Mt1B1 colonies at t=0. Following five hours of
569 incubation, samples were serially diluted in PBS and plated on Drigalski medium as well as on LB
570 agar plates overlaid with strain Mt1B1. Both set of plates were incubated at 37°C overnight. The
571 same procedure was followed for *in vitro* growth assays with bacteria taken during exponential
572 (OD_{600nm} 0.5) or stationary (24hr) growth phase at 37°C with shaking.

573

574 **Passive phages transit in axenic and OMM¹² mice**

575 Axenic (n=8 divided in 4 cages) or OMM¹² (n=4 divided in 2 cages) mice were orally gavaged once by
576 200µL of a cocktail of Mt1B1 phages (1×10^7 pfu of each of the three phages) diluted in PBS. Fecal
577 pellets were collected before phage gavage and at 6, 24, 48 and 72hr post-gavage for axenic mice
578 and at 24 and 48hr post-gavage for OMM¹² mice. Pellets were transferred in pre-weighted, sterile,
579 2mL tubes, weighted and resuspended in 1mL of PBS. Serial dilutions in PBS were performed and
580 plated onto LB plates overlaid with strain Mt1B1 to assess phage titers. Two mice were sacrificed
581 on each time point to collect intestinal sections, which were homogenized in PBS using
582 gentleMACSTM OTO dissociator (Miltenyi Biotec) and then plated on LB plates overlaid with strain
583 Mt1B1. The luminal part corresponds to the gut content that was recovered by squeezing the
584 intestinal tube with the back of a scalpel, which was subsequently homogenized in 1mL of PBS. The
585 mucosal part corresponds to the remaining tissues (empty intestinal tube) that were washed in
586 10mL of PBS before being transferred into a new tube with 1.75mL of fresh PBS and then
587 homogenized.

588

589 **Murine model of *E. coli* colonization**

590 The long-term coexistence experiment with strain Mt1B1 included 7 mice (5 that received phages
591 and 2 that did not) and lasted 23 days. At day0 mice feces were collected prior to Mt1B1
592 administration by oral gavage (200 µL of bacteria resuspended in sodium bicarbonate buffer (see
593 above). Fecal pellets were transferred in pre-weighted, sterile, 2mL tubes, weighted and
594 resuspended in 1mL of PBS. Serial dilutions in PBS were performed and plated onto Drigalski plates.
595 The three phages (2×10^7 pfu of each phage in 200µL of PBS) were administered as a mixture once

596 by oral gavage at day9. The level of phages was assessed from serial dilutions in PBS spotted on LB
597 plates overlaid with strain Mt1B1.

598 Two shorter independent experiments with repeated phage administration were performed with
599 11 (6 with phages and 5 without divided in 4 cages) and 14 (8 with phages and 6 without divided in
600 4 cages) Mt1B1-colonized mice respectively. At day0 mice feces were collected prior to Mt1B1
601 administration (as described above) by oral gavage. Fecal pellets were prepared as above. The
602 three phages (2×10^7 pfu of each phage in 200 μ L of PBS) were administered altogether once by oral
603 gavage at day14, 15 and 16. The level of phages was assessed as above. Each mouse was sacrificed
604 at day17 to collect feces and intestinal sections, the latest being homogenized in PBS using
605 gentleMACS™ OTO dissociator (Miltenyi Biotec) and all samples were plated on both Drigalski
606 plates and LB plates overlaid with strain Mt1B1. Luminal and mucosal part of gut sections were
607 recovered as described above.

608 For experiments with *E. coli* strain 55989 fecal samples were collected on days0, 1, 3, 7, 8, 9 and 10.
609 On day0, after collecting fecal samples, oral gavage of *E. coli* strain 55989 was performed as
610 described above for strain Mt1B1. At day7, after collecting fecal sample, 200 μ L of either phage
611 CLB_P2 (2×10^8 pfu/mL) (16 mice divided in 6 cages) or PBS (12 mice divided in 5 cages) were
612 administered to the mice by oral gavage (two independent experiments were performed). At
613 day10, feces were collected before sacrifice and dissection of the mice and assessment of the levels
614 of bacteria and phages as above.

615

616 **Identification of resistant clones**

617 For the experiments with Mt1B1-colonized OMM¹² mice and the phage cocktail, 20 clones from
618 each gut section from mice (n=8) exposed to the cocktail during three days and sacrificed at day17,
619 as well as from fecal samples from mice (n=5) exposed to a single dose of the cocktail at days10, 16
620 and 24, were streaked vertically in LB agar plates and subsequently each of the three phages was
621 horizontally streaked across. Plates were incubated at 37°C and phenotype was checked after 5h
622 and overnight.

623 For the experiments using 55989-colonized OMM¹² mice and phage CLB_P2, 75 to 94 isolated
624 colonies per sample (gut sections and feces from n=8 mice sacrificed at day10) were randomly
625 chosen by a robot (Qpix 420; Molecular Devices, Sunnyvale, USA) and cultured overnight in 150 μ L
626 of LB in 96 well microplates. The next day, 10 μ L were added to a new plate filled with 140 μ L of LB
627 and incubated at 37°C for 2hr. Then, 8 μ L of each clone were spotted on two separated LB agar

628 plates and let dry for 20 minutes. Then, on one plate spots of 4 μ L of phage CLB_P2 (400 pfu) were
629 spotted on top of each bacterial spot. On the second plate phage spots were 100 fold more
630 concentrated (40,000 pfu). Susceptible clones are defined by the clearance of the bacterial spot at
631 one or both phage concentrations (often full clearance was observed with 40,000 pfu and partial
632 clearance with 400 pfu), while clones not affected by the phage at any concentration are defined as
633 resistant. The frequency of phage-resistant clones in fecal samples from 55989-colonized OMM¹²
634 mice was assessed by plating 2.25x10⁴ to 3.42x10⁵ 55989 cells on Drigalski plates inundated with
635 3x10⁷ pfu of phage CLB_P2. The number of phage-resistant clones was recorded after overnight
636 incubation at 37°C and compared to plates inundated with the same amount of phage CLB_P2 and
637 on which 3.83x10⁶ cells from an exponential growing culture of strain 55989 was spread.

638

639 **Histological analysis by FISH and immunochemistry**

640 Fluorescence in situ hybridization was performed as previously described on intestinal samples
641 from *E. coli* Mt1B1 colonized OMM¹² mice bred at the LMU Munich where the fecal level of strain
642 Mt1B1 reached 10⁹ CFU/g (Brugiroux et al., 2016). Ileal and colonic tissues were fixed in 4%
643 paraformaldehyde (4°C overnight), washed in 20% sucrose (4°C overnight), embedded in O.C.T
644 (Sakura), and flash frozen in liquid nitrogen. FISH was performed on 7 μ m sections, using double
645 3' and 5'-labelled 16S rRNA targeted probes specific for Enterobacteriaceae (Ent186-2xCy3 (CCC
646 CCW CTT TGG TCT TGC)) and Eubacteria (1:1 mix of Eub338I-2xCy5 (GCT GCC TCC CGT AGG AGT)
647 and Eub338III-2xCy5 (GCT GCC ACC CGT AGG TGT)). 1 μ g/mL-1 4',6-diamidino-2-phenylindole (Roth)
648 was used for DNA staining. Images were recorded with a Leica TCS SP5 confocal microscope (Leica,
649 Wetzlar).

650 For immunofluorescence staining unflushed ileal and colonic tissues from *E. coli* 55989-colonized
651 OMM¹² mice bred at Institut Pasteur were fixed in Carnoy (ethanol/chloroform/acetic acid
652 60:30:10) for mucus preservation, dehydrated, and embedded in paraffin according to the standard
653 protocol. All the stainings were done on dewaxed 8 μ m sections. Antigen retrieval was performed
654 with Retrieval A (BD BIOSCIENCES) during 10min at 97°C, followed by 20min at room
655 temperature. After blocking with Protein Block Serum Free (DAKO) during 15min at room
656 temperature, immunofluorescence staining was performed using mouse monoclonal antibody anti
657 Muc2-A488, 1/100 (Santa Cruz Biotechnology) and rabbit anti *E. coli* O104, 1/50 (SSI diagnostica)
658 during 4hr at room temperature, then overnight at 4°C. After washings in PBS, the following
659 fluorescence-labeled secondary antibodies were used: anti-mouse-A488, 1/200 (Life Technologies)

660 and anti-rabbit-A555, 1/200 (Life Technologies) during 4.30hr at room temperature, and nuclei
661 stained with DAPI. Slides were examined under an Olympus IX81 microscope equipped with a
662 charge-coupled device (CCD) camera, and red fluorescence (*E. coli* O104) per pixel was quantified
663 with ImageJ software. An example of the different regions delimited for the quantifications is
664 shown in Fig. S6.

665

666 **OMM¹² community composition**

667 From homogenized fecal samples, 500µL were centrifuged at 8.000g for 10min and the supernatant
668 was removed. Pellets were diluted in 500µL of lysis buffer (500 mM NaCl, 50 mM Tris-HCl, pH 8.0,
669 50 mM EDTA, 4% sodium dodecyl sulfate (SDS) and incubated for 15min at 50°C (Yu and Morrison,
670 2004). Then, 100µL of lysozyme (25mg/ml) was added and samples were incubated at 37°C for 2 hr.
671 DNA extraction was performed using the Maxwell Cell tissue kit (Promega) and samples were
672 frozen at -20°C until use. The primers, probes and qPCR protocol were used in conformity with
673 previously described methods (Brugiroux et al., 2016) with the exception of the SsoAdvancedTM
674 Universal Probes Supermix (BioRad). The qPCR reactions were performed in duplicate and in two
675 independent runs using MasterCycler realplex4 from Eppendorf. Amplicon libraries targeting the
676 V3-V4 16S region were amplified by PCR (25 cycles) using Illumina primers (forward primer: 5'-
677 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', reverse primer: 5'-
678 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') and then
679 sequenced (Illumina MiSeq 2x300 bp). Sequences and statistical analyses were performed with
680 SHAMAN (shaman.c3bi.pasteur.fr) (Quereda et al., 2016).

681

682 **QUANTIFICATION AND STATISTICAL ANALYSIS**

683 For the growth curves for strain Mt1B1 (n=3 for each condition) error bars represent standard error
684 of the mean (SEM). The n-fold multiplication of strain Mt1B1 on the *ex vivo* experiment,
685 represented on figure 2 was calculated by plating samples before and after the 5hr incubation and
686 the error bars represent means with standard deviation.

687 Statistical analysis on the number of bacteria and phages generated by the animal experiments
688 were carried out using the lme4, lmerTest and car packages of R (Bates et al., 2015; Fox and
689 Weisberg, 2019; Kuznetsova et al., 2017). Both cfu and pfu were log₁₀-transformed prior to
690 analysis. In each experiment, two groups of mice were considered, a group exposed to phages and

691 an unexposed control group. The impact of phages could be assessed based on the abundance of
692 phages (log-pfu). Given the non-linearity of responses, the day at which a measure was performed
693 was considered as a categorical variable. Linear mixed-models were used to account for random
694 experimental effects (i.e., individuals, experiments and cage effects).

695 Overall effects were assessed with Analysis of Variance (ANOVA) and post-hoc Tukey's comparisons
696 and were performed using the lsmeans R package (Lenth, 2016). $p < 0.05$ was considered
697 statistically significant.

698 16S-quantification data were analysed using multivariate analysis after standard normalization. A
699 principal component analysis (PCA) was performed with the R package ade4 on the matrix of ΔC_t
700 values of 10 bacterial strains (strains YL2 and KB18 were not detected) (Dray and Dufour, 2007). In
701 addition, a between-group PCA was done in order to assess experimental effects, based on 12
702 groups of observations: 3 days (0, 14, and 17) and 4 cages (2 exposed, 2 unexposed).

703

704

705 **Supplemental Table**

706 **Table S3, related to Table 1. Genome annotations of Mt1B1 phages P3, P10 and P17.**

707

708

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