

Fitness advantage of *Bacteroides thetaiotaomicron* capsular polysaccharide is dependent on the resident microbiota

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1 **Summary**

2 Many microbiota-based therapeutics rely on our ability to introduce a microbe of choice into an
3 already-colonized intestine. However, we remain largely blind to the quantitative effects of
4 processes determining colonization success. In this study, we used genetically-barcoded
5 *Bacteroides thetaiotaomicron* (*B.theta*) strains in combination with mathematical modeling to
6 quantify population bottlenecks experienced by *B.theta* during gut colonization. Integrating
7 population bottlenecks sizes with careful quantification of net growth rates *in vivo* and *in vitro*
8 allows us to build models describing the events during intestinal colonization in the context of
9 gnotobiotic and complex microbiotas. Using these models, we estimated the decrease in niche
10 size for *B.theta* colonization with increasing microbiota complexity. In addition, our system can
11 be applied to mechanistically dissect colonization defects of mutant strains. As a proof of
12 concept, we demonstrated that the competitive disadvantage of a *B.theta* mutant lacking
13 capsular polysaccharide is due to a combination of an increased lag-phase before growth
14 initiation in the gut, combined with an increased clearance rate. Crucially, the requirement for
15 the *B.theta* capsule depended strongly on microbiota composition, suggesting that the
16 dominant role may be protection from bacterial or phage aggression rather than from host-
17 induced bactericidal mechanisms.

18 Introduction

19 From the moment that we first contact microbes at birth, we continuously encounter
20 environmental and food-borne microbes. Whether such encounters are transient or will lead to
21 long-term colonization, is influenced by complex ecological interactions between the invading
22 species and the existing consortium, as well as the host's dietary habits and the physiology of
23 the intestine (David et al., 2014; Wotzka et al., 2019). A better understanding of the factors
24 determining colonization efficiency is crucial in the development of microbiota engineering
25 strategies (Donia, 2015; Pham et al., 2017; Sheth et al., 2016) and in the use of bacterial
26 species as biosensors to probe microbiota function and stability (Goodman et al., 2009).

27 One established way of studying ecological processes within hosts is genetic barcode tagging
28 of otherwise isogenic microbes, which has previously been used to study population dynamics
29 of pathogens such as *Vibrio cholerae* or *Salmonella* Typhimurium within the infected host (Abel
30 et al., 2015b; Vlazaki et al., 2019). Based on barcode tagging and mathematical modelling, it
31 has been possible to infer parameters such as growth, clearance and migration rates
32 (Dybowski et al., 2015; Grant et al., 2008; Kaiser et al., 2014, 2013), as well as the size of
33 population bottlenecks imposed during colonization (Abel et al., 2015a; Li et al., 2013; Moor et
34 al., 2017), antibiotic treatment (Vlazaki et al., 2020) or immunity (Coward et al., 2014;
35 Hausmann et al., 2020; Lim et al., 2014; Maier et al., 2014; Moor et al., 2017). Furthermore,
36 combining neutral genetic barcodes with targeted mutant strains, this experimental tool can be
37 used to mechanistically analyze the fitness-effect of individual genes that regulate successful
38 gut colonization or tissue invasion (Di Martino et al., 2019; Nguyen et al., 2020).

39 In order to study the dynamics of invasion of a novel microbiota member, we chose to use
40 *Bacteroides thetaiotaomicron* (*B.theta*) as a model microbe. *B. theta* is a common commensal
41 member of the human intestinal microbiota (Porter et al., 2018), and a well-established toolbox
42 exists for engineering its genome (Porter et al., 2017; Whitaker et al., 2017). A common feature
43 of *Bacteroides* species is the ability use phase variation to modulate the expression of 3-10
44 capsular polysaccharide (CPS) operons, leading to the production of distinct capsule
45 structures (Porter and Martens, 2017). *B.theta* strains lacking a capsule have been shown to
46 engraft poorly in an existing microbiota when competing with CPS-expressing strains (Martens
47 et al., 2009; Porter et al., 2017). Critically for our studies, the deletion of all capsule gene
48 clusters is not expected to negatively influence the growth rate of *B.theta* per se (Rogers et al.,
49 2013) but is expected to affect survival on exposure to noxious stimuli, like bile acids, stomach
50 acid, antimicrobial peptides, or phage (Porter et al., 2020, 2017), making this a good model to
51 test our ability to quantify population dynamics *in vivo*. *B. theta* is also a relevant bacterium to
52 understand population dynamics of colonization, as its ability to colonize to very high densities

53 and the availability of precise tools for genetically engineering it (Lim et al., 2017; Mimee et al.,
54 2015) make it a strong candidate for introducing novel functions into microbiomes.

55 To quantify the processes determining success of *B.theta* colonization in the presence of
56 different resident microbiota, we generated genetically tagged *B.theta* strains with and without
57 capsule. Using a combination of mathematical modeling and experimental quantification of
58 population dynamics parameters, we estimated the probability of colonization success during
59 single-strain colonization and competition. This revealed that acapsular *B.theta* have similar
60 colonization success (i.e., encounter similar population bottlenecks) to wild-type strains when
61 invading gnotobiotic microbiota communities, but not when invading a more complete
62 microbiome. However, the acapsular strain still competes poorly with the wild-type strain in co-
63 colonization even in gnotobiotic settings. The underlying mechanisms depend upon the
64 increased clearance and extended lag-phase of the acapsular *B.theta* *in vivo*. Therefore,
65 neutral tagging and mathematical modeling can be used to infer behavior of *B.theta* and
66 protective functions of the polysaccharide capsule in a mouse model.

67 **Results**

68 **Genetically-tagged *B.theta* strains to study within-host population dynamics**

69 We first established tools that would allow us to quantify *B.theta* clonal loss *in vivo* and to test
70 the plausibility of our theoretical models. Briefly, we used the previously described pNBU2
71 integration plasmid carrying an antibiotic resistance cassette, a phage promoter and an
72 optimized ribosome binding site for fluorescent protein expression which allows visualization
73 of clones (Wang et al., 2000; Whitaker et al., 2017). Six barcode tags, previously developed
74 and validated for *Salmonella* (Wild-type Isogenic Tagged Strains, WITS (Grant et al., 2008;
75 Maier et al., 2013) were inserted into an erythromycin-resistant (*ermG* cassette (Cheng et al.,
76 2000) pNBU2 vector (Fig.1A). These six tags were inserted in both *B.theta* VPI-5482 Δtdk ,
77 which can phase vary the expression of eight different capsular polysaccharides (*B.theta* WT);
78 and a *B.theta* strain that cannot produce capsule, generated by sequentially deleting all CPS
79 gene clusters (*B.theta* acapsular) (Porter et al., 2017). A matched set of strains was produced
80 in which an identical pNBU2-derived plasmid was integrated carrying the *tetQb* resistant
81 cassette (Nikolich et al., 1992) in place of *ermG*, conferring instead tetracycline resistance
82 (TetR). All strains also express a fluorescent protein, either GFP or mCherry (Fig.1A,
83 Suppl.Table 1).

84 **Genetic barcode tags do not affect fitness of *B.theta* strains**

85 A critical assumption of any analysis using genetically barcoded strains is that the
86 chromosomal insertions, as well as the construction process, have not altered the fitness of
87 the strains compared to the wild-type, both in culture and when colonizing a host. When grown
88 individually in BHIS media, the *B.theta* strains carrying the different barcode tags had a similar
89 growth rate (median doubling time ranged from 78-90 min) (Fig.1B and Suppl. Fig.1A). Also,
90 they maintained their relative abundances, evaluated by qPCR (Fig.1C), when mixed and
91 grown overnight (Suppl.Fig.1B and Suppl.Fig.1C).

92 To test whether the tags confer a fitness effect upon colonization of a host, we colonized germ-
93 free mice with a uniform mixture of 10^7 colony forming units (CFU) of each tagged strain
94 (Fig.1D). At this tag abundance, stochastic loss of tags is highly unlikely. We compared the
95 relative abundance of each tag in the inoculum to that in the cecal content and feces after 48h
96 of colonization. These experiments revealed small, random deviations in the distribution,
97 consistent with uniform fitness (Fig.1E). Finally, as erythromycin- and tetracycline-resistance
98 were used to distinguish the tagged and untagged *B.theta* strains, we also tested whether the
99 antibiotic resistant cassettes alter competitive fitness and found no significant difference
100 between strains carrying the two resistance cassettes in culture (Fig.1F) or upon colonization
101 (Suppl. Fig.1D).

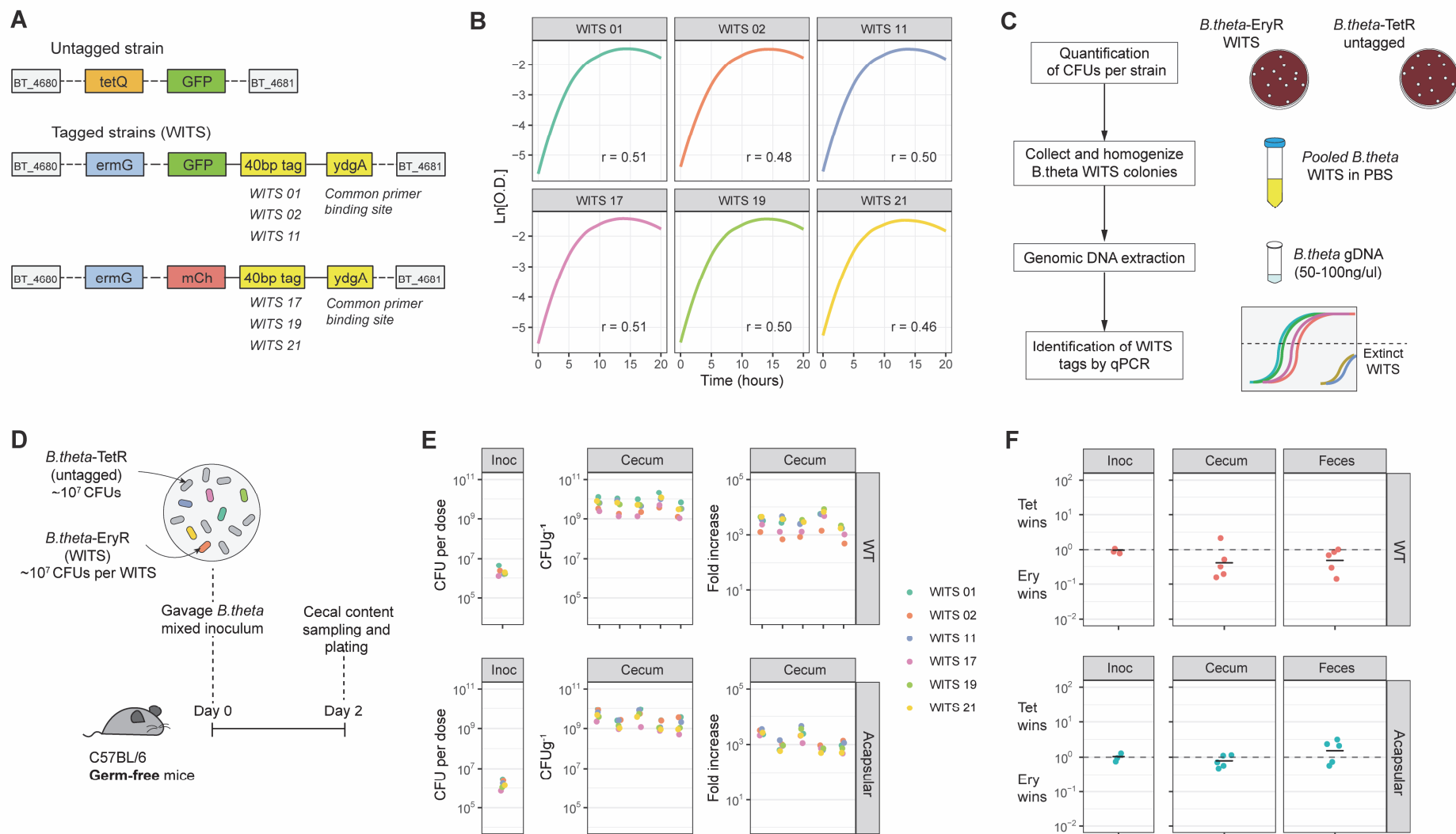
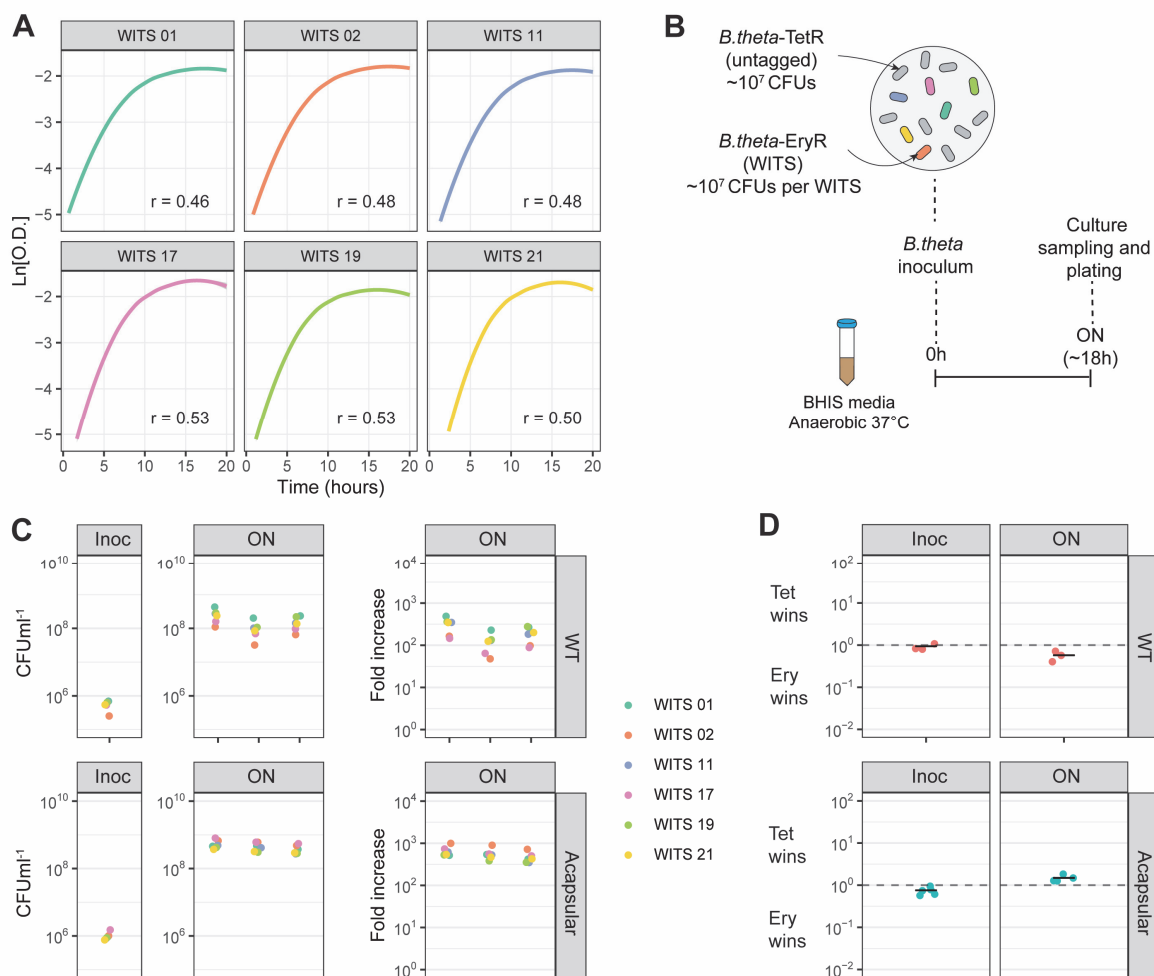


Figure 1: Tagged *B.theta* strains have similar fitness for growing *in vitro* and *in vivo*. (A) Schematic representation of insertions in *B.theta* genome. (B) Growth curves of *B.theta* WT in BHIS media ($n=3$ per strain) and growth rates (r , hours⁻¹) per tag. (C) Workflow for sample preparation and data acquisition. (D) Experimental design of *in vivo* competitions. All strains (untagged *B.theta*-TetR and all six tagged *B.theta*-EryR WITS) were mixed in a 1:1 ratio. (E) Tags distribution during colonization among six *B.theta* tagged strains either WT or acapsular. Plots show distribution of tags in the inoculum, in cecal content of individual mice after 48h of colonization and fold increase of each tag per mouse compared to the inoculum. (F) Competitive index of tetracycline (Tet)- over erythromycin (Ery)-resistant strains *in vivo* after 48h of colonization in *B.theta* WT and acapsular in cecal content and in feces.



Supplementary Figure 1: Tagged *B. theta* strains have similar fitness for growing *in vitro* (A)

Growth curves of *B. theta* acapsular in BHIS media and growth rates (r) per tag. **(B)** Experimental design of *in vitro* competitions. All strains (untagged *B. theta*-TetR and all six tagged *B. theta*-EryR WITS) were mixed in a 1:1 ratio. **(C)** Tags distribution after *in vitro* competition among six *B. theta* tagged strains either WT or acapsular to demonstrate neutrality *in vitro*. Plots show distribution of tags in the inoculum, in individual overnight (ON) cultures and fold increase of each tag per culture compared to the inoculum. **(D)** Competitive index of tetracycline (Tet)- over erythromycin (Ery)-resistant strains *in vitro* after ON cultures.

102 **Determining inoculum size of tagged strains that yields maximal information upon**
103 ***B.theta* colonization**

104 We then applied the neutrally tagged *B. theta* strains to estimate their invasion probabilities by
105 recovering *B. theta* cells after colonization and comparing the barcode distribution with that in
106 the inoculum. As invasion probabilities depend on the interaction with the resident microbiota
107 (Kurkjian et al., 2021), we probed *B.theta* colonization on three different communities: Low-
108 complexity microbiota (LCM) (Stecher et al., 2010); Oligo Mouse Microbiota (OligoMM12)
109 (Brugiroux et al., 2016) and specific-pathogen-free (SPF) complete microbiota (Suppl. Table
110 3). We evaluate recovering *B. theta* cells 48h after initial colonization, a timepoint shortly after
111 the *B. theta* population in the cecum reaches carrying capacity (Suppl.Fig.2A).

112 Assuming that the change in relative abundance of tags before and after the colonization
113 process is due to stochastic loss of *B. theta*, we formulated a mathematical model that
114 describes this process and that allows us to infer important parameters, namely a per-cell
115 colonization probability for *B. theta*. Mice are inoculated with n_0 copies of tagged *B.theta* cells,
116 which undergo random killing during their transition through the stomach and small intestine.
117 Surviving clones arriving in the cecum start growing and are detected 48h post-colonization
118 (Fig.2A). A simple calculation was carried out to estimate the probability for the lineage of a
119 bacterium clone spiked at n_0 copies in the inoculum to be found at the measurement time,
120 which can be assumed as the combination of making it alive to the cecum and not being lost
121 afterwards. As bacteria are not counted directly in the inoculum, but the inoculum is a sample
122 of known volume of a solution of a known concentration, the incoming distribution of tagged
123 bacteria is approximately Poisson, of mean n_0 . Then we assume each bacterium has an
124 independent probability of colonization β . Therefore, the probability of losing an individual tag
125 (which can be approximated to the fraction of tagged strains lost across different mice) is equal
126 to $e^{-\beta n_0}$. Finally, the colonization probability (β) can be approximated to negative natural
127 logarithm of the proportion of lost tags divided by the expected initial number of CFUs of each
128 tagged strain in the inoculum. As there are experiments with different initial inoculum sizes
129 (different n_0), we obtain β by maximizing the likelihood of the experimental observations (See
130 Methods, Mathematical modelling, section 1). Furthermore, a more complex calculation can
131 be carried out using the variance of the tagged population rather than defined loss/retention
132 (Suppl.Fig.2B and Suppl.Fig.2C; see Methods, Mathematical modelling, section 2), but the
133 overall obtained numbers remain similar.

134 It is important to note that if all tags are lost, or if all tags are recovered, only upper or lower
135 bounds for β can be determined, respectively, rather than numerical estimates. Thus,
136 experiments where some, but not all tags are lost from the final population yield maximum
137 information (Fig.2B). To find the inoculum sizes that lead to this outcome, we therefore titrated

138 the tagged strains into an untagged *B. theta* population at different ratios. We used these
139 mixtures to colonize mice carrying three different microbiota communities, and quantified tag
140 recovery from the cecum 48h post-colonization. We used the Pielou evenness (Pielou, 1966)
141 as a summary representation of the distributions of tagged population for both *B.theta* strains
142 (WT and acapsular) in mice carrying three different microbiota communities (Fig.2C). After an
143 analytical and numerical analysis, we found that the resulting estimates are most precise when
144 about half the tags are lost (see Supplementary Methods). In the case of LCM and OligoMM12
145 gnotobiotic mice, an n_0 of between 10-100 was optimal for both wild-type and acapsular
146 *B.theta*, whilst in SPF mice, an n_0 of around 500 was informative for wildtype, but 5000 CFU
147 were needed of each acapsular tagged strain.

148 Finally, the observed change in the distribution of tags after the colonization process can have
149 two explanations: stochastic loss of individual strains in this process, or mutation and selection
150 in a subset of the strains. In order to test whether mutations, followed by selective sweeps, can
151 explain observed changes, we recovered tagged *B. theta* strains from colonized mice, mixed
152 them with the ancestral strains carrying different tags in equal ratios, and used this mix to
153 inoculate a new group of mice. There was no consistent advantage of re-isolated strains over
154 ancestral strains (Suppl.Fig.2D), indicating that mutation and selection do not play a role in the
155 initial 48h after colonization.

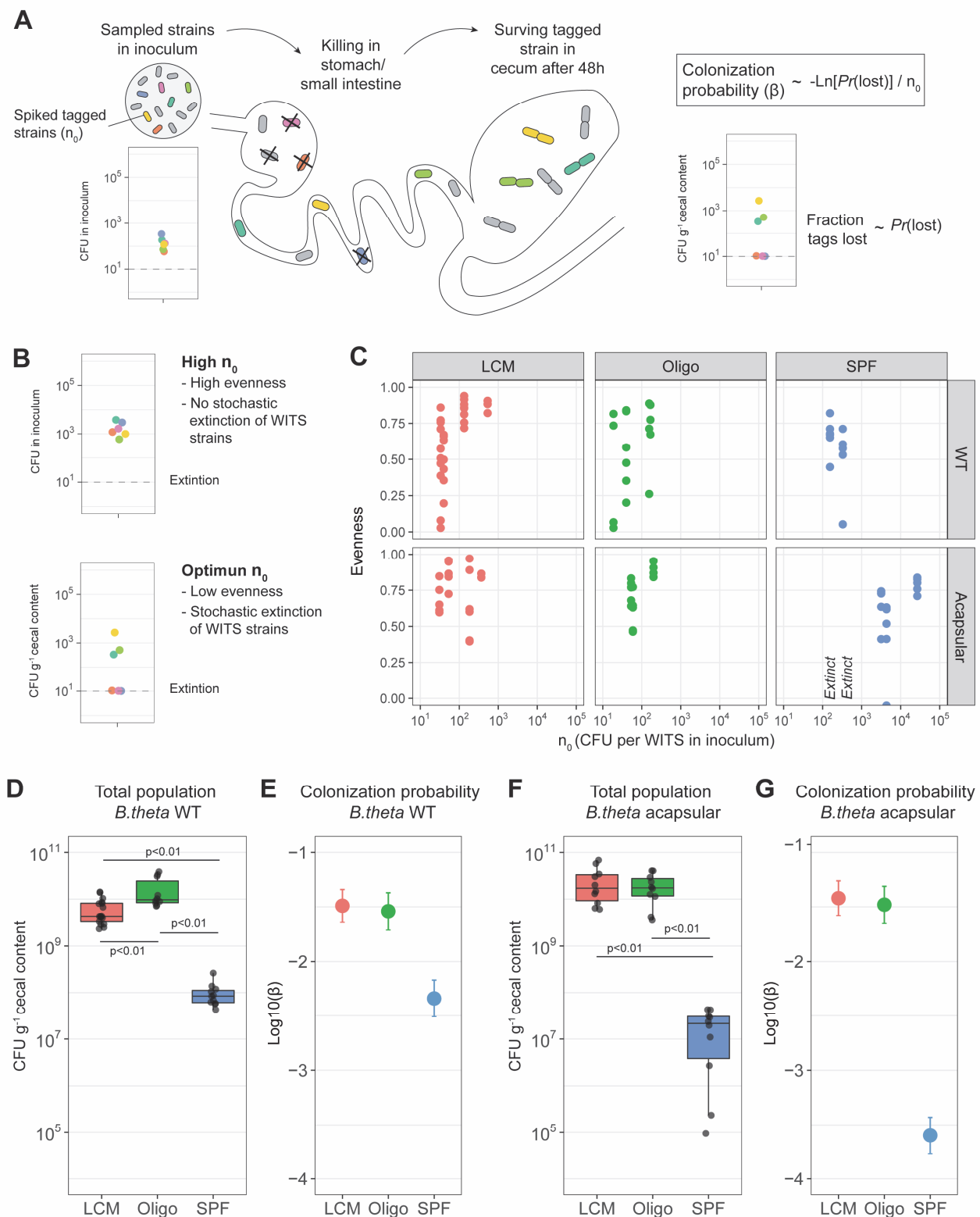
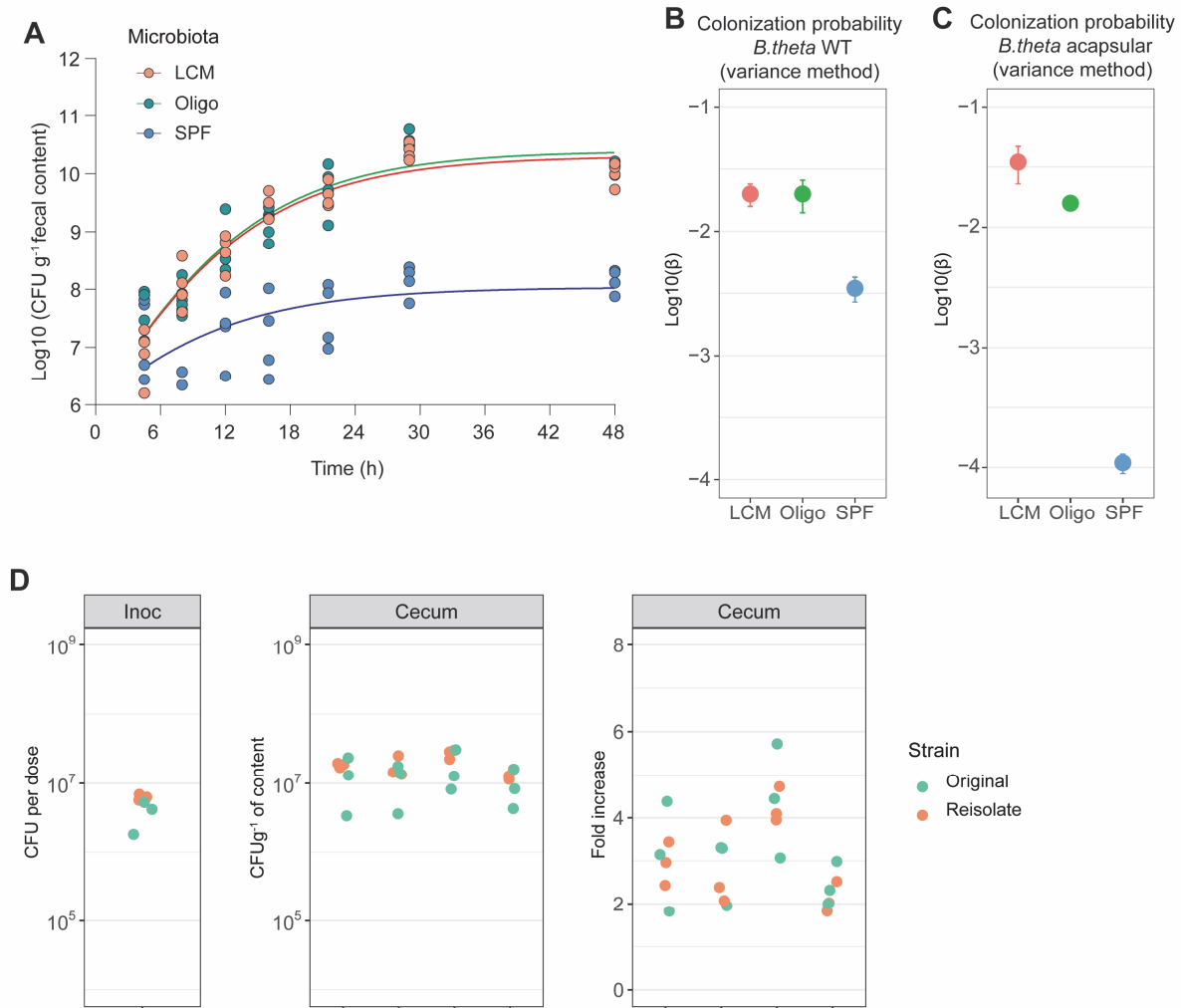


Figure 2: Colonization probability of *B.theta* strains in LCM, Oligo-MM12 and SPF mice. (A) Schematic representation of experimental estimation of colonization probability **(B)** Two different scenarios of n_0 and distribution of tagged strains: n_0 in which no extinction of tags is observed (“High n_0 ”) and n_0 in which tags are randomly lost (“Optimal n_0 ”). **(C)** Pielou’s evenness vs. n_0 . Pielou’s evenness was estimated with an maximum possible value of $H_{\max} = \ln(6)$ for all data points (six tagged strains). Each dot represents the evenness calculated per mouse. Values of n_0 for which all tags were lost, and therefore no evenness could be estimated, were marked with “Extinct” in the graph. **(D and F)** Total *B.theta* population in the cecum at 48h post-colonization for **(D)** WT and **(F)** acapsular strains. **(E and G)** Probability of colonization (β) in **(E)** WT and **(G)** acapsular in the cecum after 48h of colonization using the loss method. Estimation based on 6 tags times the total number of mice (LCM=17, OligoMM12=10, SPF=11) and on lost tags.



Supplementary Figure 2. (A) *In vivo* growth curve of WT *B.theta* in three different microbiota backgrounds. (B and C) Probability of colonization (β) for (B) WT and (C) acapsular in the cecum after 48h of colonization. Estimation based on 6 tags x m mice (LCM=17, OligoMM12=10, SPF=11) and on variance on tags before (inoculum) and after colonization (cecum). (D) *In vivo* competition among *B.theta* strains reisolated from SPF mice vs. original stock strains. Plots show distribution of *B.theta* original and isolated strains in the inoculum, in cecal content of individual mice after 48h of colonization and fold increase of each tag per culture compared to the inoculum.

156 ***B.theta* colonization probability in LCM, OligoMM12 and SPF mice**

157 The resident microbiota composition in the mammalian gut is one of the main factors
158 constraining the colonization of newly arriving species. This can happen through various
159 mechanisms such as competition for nutrients (Baumler and Sperandio, 2016; Maier et al.,
160 2013), modification of the intestinal environment (Cremer et al., 2017) or via direct suppression
161 of the invaders by phages (Almeida et al., 2019; Barr et al., 2013) or Type VI secretion systems
162 (Chatzidaki-Livanis et al., 2016; Wexler et al., 2016). Therefore, we used the previously
163 described mathematical model to estimate the suppressive potential of different microbiotas,
164 gnotobiotic (LCM, OligoMM12) and SPF, on *B. theta* invasion.

165 *B.theta* load in the cecal content after 48h of post-inoculation were similar in LCM and
166 OligoMM12 mice, but significantly lower in SPF mice (Fig.2D). We then used the measured
167 tag loss to determine colonization probabilities as described above, for the different *B. theta*
168 strains in the different microbiota backgrounds. The colonization probability of *B.theta* WT was
169 lower in SPF mice ($\text{Log}_{10}(\beta, \text{colonization probability}) \pm 2 \text{ standard deviations} = -2.35 \pm 0.14$)
170 compared to the two gnotobiotic microbiotas (LCM, -1.50 ± 0.10 ; and Oligo, -1.54 ± 0.13)
171 (Fig.2E). This additionally reveals that there is a discrepancy between the relative size of the
172 final population (100-fold lower in SPF mice) and the relative probability to recover a specific
173 clone from the inoculum at 48h (only 10-fold lower in SPF mice), indicating that size of the
174 open niche does not linearly translate into colonization probability. For these two observations
175 to agree, the founding population of *B.theta* has to be smaller in SPF mice, and the net growth
176 during the first 48h post-inoculation has to be lower than in the gnotobiotic mice (Suppl.Fig.2A).

177 As capsular polysaccharides (CPS) are thought to play a crucial role in phage evasion/infection
178 (Porter et al., 2020), immune evasion (Fanning et al., 2012; Hsieh et al., 2020; Porter et al.,
179 2017) and protection from other environmental stressors, we expected to see a tighter
180 bottleneck (decreased colonization probability) for the acapsular *B.theta* strain in all microbiota
181 backgrounds. Surprisingly, in gnotobiotic mice, the total population size of acapsular *B.theta*
182 and the probability to colonize ($\text{Log}_{10}\beta$: LCM, -1.43 ± 0.13 ; and OligoMM12, -1.49 ± 0.14) were
183 not different to the WT strain (Fig.2F and Fig.2G). Thus, there is no measurable fitness benefit
184 of CPS in gut colonization up to 48h post-inoculation in these settings. However, we observed
185 a different scenario when we inoculated acapsular *B.theta* into mice carrying a SPF microbiota.
186 Both the total population size of acapsular *B.theta* (Fig.2F) and the colonization probability
187 ($\text{Log}_{10}\beta$: SPF, -3.65 ± 0.13 ; Fig.2G) were 10-fold lower compared to the wildtype strain,
188 indicating a fitness benefit of CPS in the context of a more diverse microbiota and a smaller
189 niche. Given the previously observed absence of a growth defect for acapsular *B.theta*, we
190 hypothesized that increased clearance of acapsular *B. theta* cells causes the difference,

191 potentially attributable to competition/aggression from other microbiota species or from more
192 active host immunity in SPF mice.

193 **Competitive colonization with acapsular and WT *B.theta* reveals a role of CPS in**
194 **gnotobiotic mice**

195 The absence of an increased bottleneck during colonization of gnotobiotic mice with acapsular
196 *B.theta* is in conflict with previous studies showing a fitness defect of this strain (Coyne et al.,
197 2008; Porter et al., 2017). We therefore carried out competitive colonization with *B.theta* WT
198 and acapsular *B.theta*. Starting at a 1:1 ratio we inoculated mice with erythromycin-resistant
199 WT and tetracycline-resistant acapsular *B.theta* and quantified the cecal bacterial load 48h
200 post-inoculation (Suppl.Fig.3A). This reveals a gradient of outcompetition of the acapsular
201 strain ranging from a competitive index (abundance of WT over acapsular *B.theta* at the end
202 of the experiment) of approx. 20 in germ-free mice, 100 in gnotobiotic mice and 10⁴ in SPF
203 mice (Fig.3A). This demonstrates a competitive fitness benefit of CPS, that increases in
204 proportion to microbiota complexity.

205 To confirm these findings, we performed a competition experiment in the same microbiota
206 backgrounds, but this time, using tetracycline-resistant *B.theta* WT and barcoded
207 erythromycin-resistant acapsular *B.theta* strains. WT strain density (Suppl.Fig.3B) and the
208 average increase in the WT relative to the acapsular strains (after adjusting for the initial ratio
209 in the inoculum, Suppl.Fig.3C) were similar between the two experiments. Interestingly, when
210 calculating the colonization probability β based on tag loss as described before, this is lower
211 for the acapsular *B.theta* strain when co-colonizing with the WT strain than when colonizing
212 alone (Fig.3B). This indicates that the competition with wild-type results in both a lower total
213 population size and increased clonal extinction in the acapsular strains.

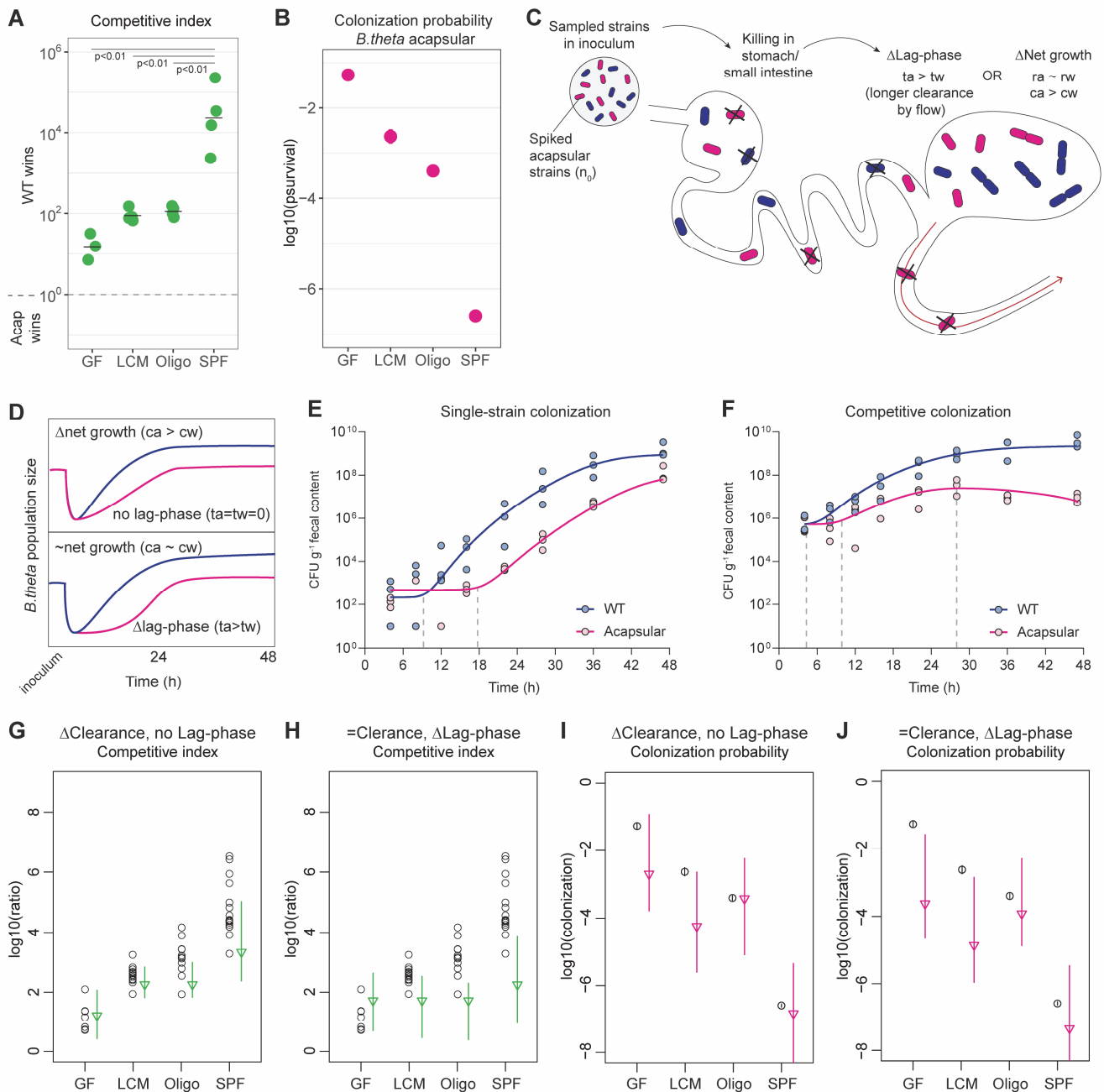
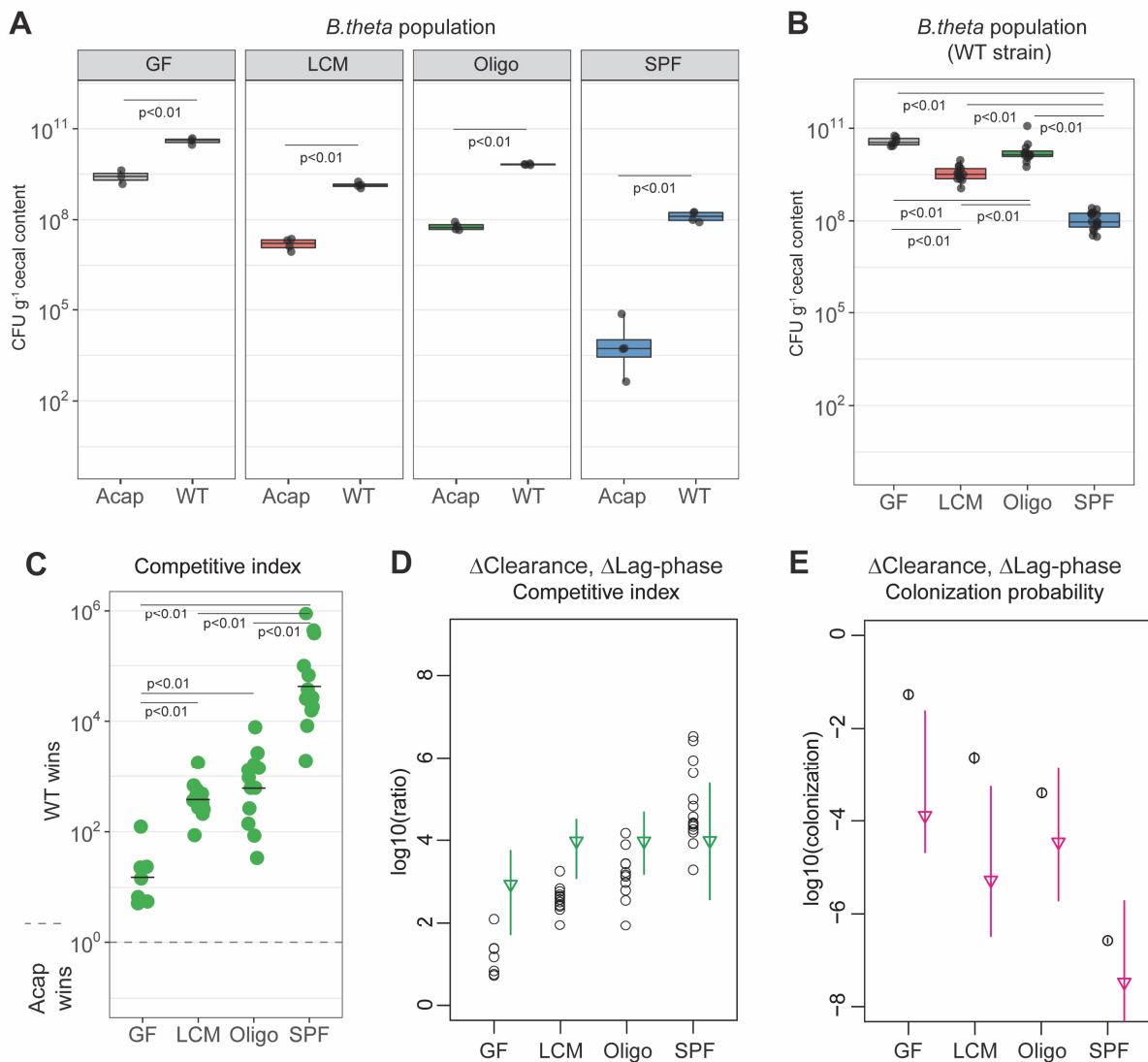


Figure 3. Competition colonization with acapsular and WT strains. (A) Competition index (ratio between WT over acapsular in the cecum) after 48h of colonization starting at a 1:1 ratio. (B) Probability of colonization by the acapsular strain during competition with the WT strain. Tagged acapsular strains were spiked into a WT-strain inoculum (GF=7, LCM=13, OligoMM12=12, SPF=16). (C) Schematic representation of two scenarios of competitive advantage of the WT over the acapsular *B.theta* having a similar initial probability of colonization of the cecum: difference in lag-phase (mean time to growth commencement in acapsular (t_a) and WT (t_w)) and difference in net growth rate (growth rate in acapsular (r_a) and WT (r_w); clearance rate in acapsular (c_a) and WT (c_w)). Clearance can be due to both flow/loss in the fecal stream and death. (D) Growth curves representing the two scenarios in (C). (E) Fecal bacterial load of *B.theta* WT or acapsular during single-strain colonization in OligoMM12 mice. Overlay graph of individual experiments with starting inoculum 10^2 - 10^3 CFU each. (F) Fecal bacterial load of *B.theta* WT or acapsular during competitive colonization in OligoMM12 mice. Overlay graph with starting inoculum 10^6 CFU each (ratio 1:1). (G-J) Estimation of the competitive index and colonization probability of the acapsular strain (see Supplementary Methods): (G and I) assuming no lag-phase and estimated difference in clearance rate between the WT and acapsular strains and (H and J) assuming a mean 6h difference in lag-phase and identical clearance rate between the WT and acapsular strains (circles: experimental data; triangles: mean parameter estimation and error).



Supplementary Figure 3. (A) Competition with acapsular and WT strains during colonization starting at a 1:1 ratio. Population density of the WT and acapsular strain in cecum after 48h of colonization. **(B and C)** Estimation of probability of colonization of the cecum by the acapsular strain using a WT strain inoculum spiked with tagged acapsular strains. **(B)** Population density of the WT strain and **(C)** Competitive index after correction with the initial WT/acapsular ratio in the inoculum. **(D and E)** Estimation of the **(D)** competitive index and **(E)** colonization probability of the acapsular strain assuming a mean 6h difference in lag-phase and the estimated difference in clearance rate between the WT and acapsular strains (circles: experimental data; triangles: mean parameter estimation and error).

214 **Longer lag-phase and higher clearance rate explains fitness defect of acapsular**

215 ***B.theta* in competitive colonization**

216 In order to understand how acapsular *B.theta* can have an indistinguishable colonization
217 probability when inoculated alone, but a major fitness defect in competition with wildtype
218 *B.theta*, we extended our simple, one-step colonization model to include a lag-phase after
219 arrival in the cecum (Fig.3C). Based on this model, two different but non-exclusive scenarios
220 can explain the competitive disadvantage of the acapsular strain during competition. In the first
221 scenario, there is a difference in the net growth rate, therefore explaining the ratio between
222 acapsular and WT strains at the end of the experiment (Fig.3D, top). In the second scenario,
223 both strains have similar net growth rates but a different lag-time, which delays initial growth
224 of the acapsular such that the wild-type will occupy a larger fraction of the available niche
225 during competition (Fig.3D, bottom).

226 To evaluate these two different scenarios, we carried out a time-course analysis of the fecal
227 bacterial load of *B.theta* during colonization in OligoMM12 mice for the acapsular and WT
228 strains alone and in competition. This allows us to extract the *in vivo* net growth rates, i.e.,
229 growth rate minus clearance rate, as well as lag-phases between strains. Interestingly, the
230 acapsular strain does exhibit an initial lag-phase of approximately 4-8h when compared to the
231 WT, even in single colonization (Fig.3E and Fig.3F). Based on the CFU data alone, this delay
232 in colonization could either be attributed to a tighter population bottleneck during small
233 intestinal transit, or to a longer lag-phase of clones of the acapsular strain after arrival in the
234 large intestine, or potentially to both. However, since our neutral tagging data indicates an
235 identical bottleneck of wild-type and acapsular *B.theta* strains when colonized individually in
236 OligoMM12 (Fig.2E and Fig.2G), we can exclude increased killing of the acapsular strain prior
237 to seeding of the cecum. Once in the cecum, the net growth rate during the exponential growth
238 phase is lower for the acapsular strain (WT: 0.53 ± 0.05 and acapsular: 0.40 ± 0.02 ; estimated
239 from Fig.3E). As the growth rates of these strains in culture is identical, it is reasonable to
240 assume that the slightly lower net growth rate is due to an increased clearance rate for the
241 acapsular *B.theta* in the gut lumen. This is further supported by the observed decrease in the
242 total population of the acapsular strain after the WT has reached carrying capacity (Fig.3F).

243 We then use these experimentally-measured parameters to theoretically estimate the
244 competitive index and the colonization probability of the acapsular strain during competition.
245 We modelled three different scenarios assuming either a difference in clearance rate or lag-
246 phase, or a combination of both (see Supplementary Methods for the description of all
247 parameters used). Comparison of the modeled values with the observed data will then indicate
248 whether the model can capture the dominant part of the phenomenon. We found that the
249 competitive index (abundance of WT over acapsular strain) can be mostly explained by a

250 model in which only clearance rate differs between strains (Fig.3G). It was not possible to fit a
251 model in which only the lag-phase differed for the observed competitive index (Fig.3H). In
252 contrast, models based either on an increased clearance rate (Fig.3I) or on a prolonged lag-
253 phase (Fig.3J), could explain the observed colonization probability of the acapsular during
254 competition. Combining both parameters showed a similar tendency to the initial model without
255 a lag-phase (Suppl.Fig.3D and Suppl.Fig.3E). Therefore, while we clearly observe a delayed
256 start of growth in the acapsular strain in vivo, the increased clearance rate clearly has a
257 stronger effect on the overall abundance of the acapsular strain during competitive
258 colonization.

259 **Acute challenges modify *B.theta* population dynamics in vivo**

260 Finally, as a proof of concept for neutral tagging in the study of established microbiota, we
261 used our system to probe clonal extinction when an established *B.theta* population in the gut
262 is challenged by two major environmental perturbations: 1) Shifting from standard chow to a
263 high-fat no-fiber diet (HFD) (David et al., 2014; Wotzka et al., 2019)) and 2) infectious
264 inflammation driven by *Salmonella* Typhimurium (Stm) (Maier et al., 2014; Wotzka et al., 2017).

265 In order to have a highly simplified system to study host-*B.theta* interactions, we
266 monocolonized germ-free C57BL/6J mice with WT *B.theta* spiked with tagged strains in such
267 a way to maintain all tags with a roughly uniform distribution, i.e., minimum loss, in the gut
268 lumen prior to challenge (Fig.4A). After 4 days of colonization, we exposed mice to either HFD
269 or oral infection with 10^6 - 10^7 CFUs of attenuated Stm SL1344^{ΔSPI-2}. This attenuated strain was
270 used to induce moderate intestinal inflammation in the gnotobiotic mice but to avoid the rapid
271 lethal infections associated with wildtype *Salmonella* in germ-free animals (Hapfelmeier et al.,
272 2005; Stecher et al., 2005). *B.theta* populations were monitored in feces before the challenge
273 was administered and three days after the challenge in feces and cecum.

274 Despite published reports that *B.theta* is sensitive to bile acids (Wotzka et al., 2019), HFD did
275 not significantly change the cecum population size (Fig.4B) or increased loss of tagged *B.theta*
276 (Fig.4C) in the diet-shift experiments. We can still set a lower-bound for the probability of an
277 established *B.theta* clone to survive the acute dietary challenge by estimating the value in case
278 that one tagged strain was lost during the challenge. In this case, HFD imposed a similar
279 survival probability ($\text{Log}_{10}(\beta) = -1$ to 0) to the one observed in the control group ($\text{Log}_{10}(\beta) = -$
280 0.39 to 0). Estimating that the initial *B.theta* population is in the order of 10^{10} CFUg⁻¹ in the
281 cecum, the bottleneck effect imposed by HFD would reduce the population minimally to a range
282 between 10^9 to 10^{10} clones, which are densities normally observed in the cecum (Fig.4B).

283 In contrast, after three days of infection, *Salmonella* induced-inflammation reduced the total
284 *B.theta* population approx. 100-fold in feces at day 3 post-infection (Fig.4B). In addition, we

285 estimated the probability of an established clone to survive this acute inflammation challenge
286 is approximately 1 out of 500 in cecum (Fig.4C) while competing with the *Salmonella*
287 population (Fig.4D). This means that the initial estimated population of 10^{10} CFUg⁻¹ in the
288 cecum is reduced to a population of between 10^7 to 10^8 clones by the bottleneck effect induced
289 by inflammation and then bouncing back to the observed levels between 10^9 to 10^{10} CFUg⁻¹
290 (Fig.4B). Therefore, the bottleneck effect on the population induced by acute inflammation not
291 only reduces the total population size but also diminishes the diversity of clones in the
292 population.

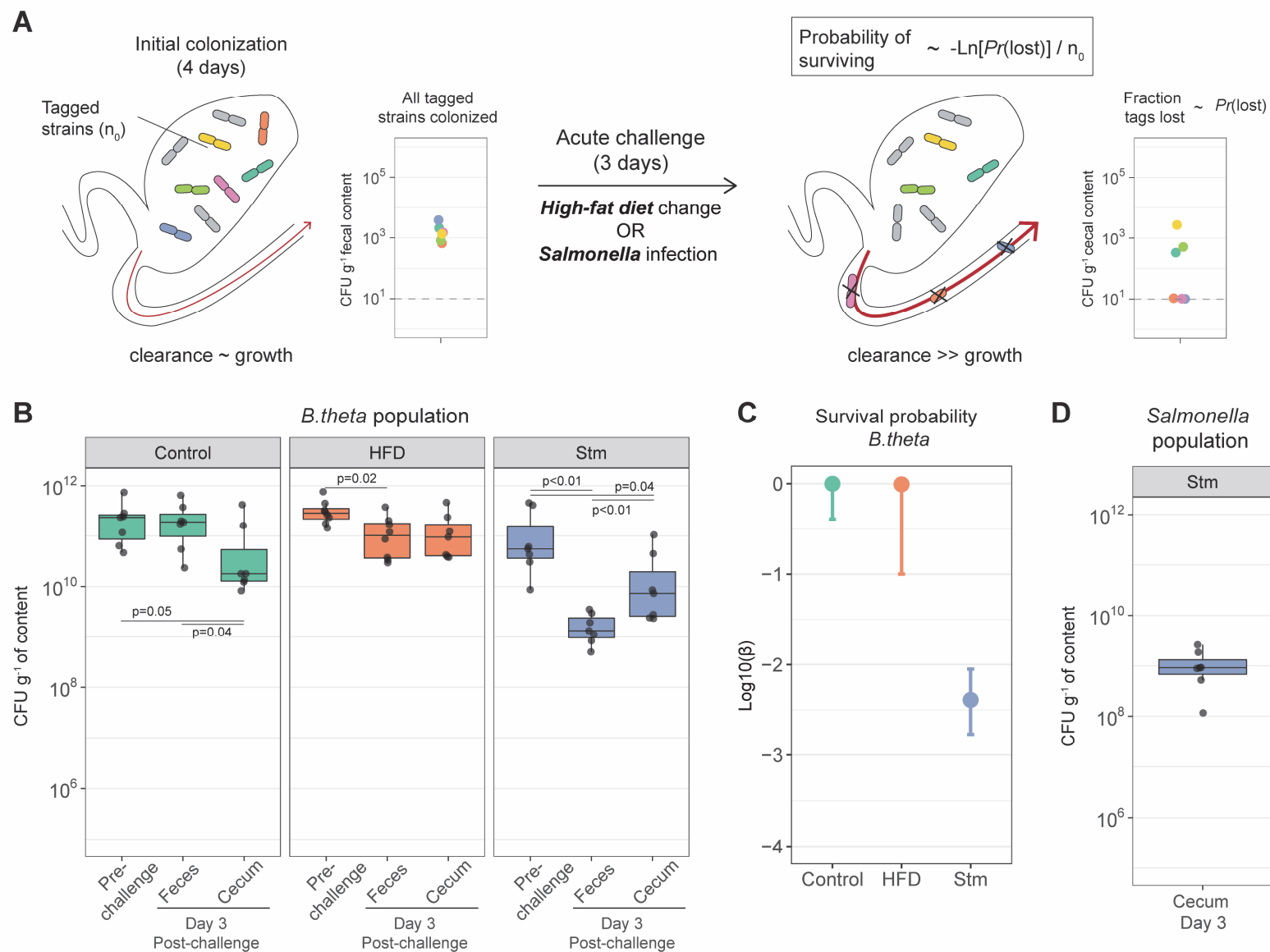


Figure 4. Acute challenges imposed a population bottleneck in the resident *B.theta* population. (A) Schematic representation of experimental estimation of colonization survival probability after acute challenges. **(B)** Population of *B.theta* in monocolonized ex-GF mice kept under standard chow (control) and during acute challenge with high-fat diet (HFD) or attenuated *Salmonella* infection. **(C)** Probability of surviving in the cecum after 3 days of the acute challenge. Estimation based on 6 tags timer the total number of mice (Control=7, HFD=8, Stm=7). **(D)** Population of attenuated *Salmonella* in cecal content.

293 Discussion

294 Understanding the different mechanistic factors determining how microbes can invade a
295 resident gut microbiota is of considerable importance for combating mucosal infections
296 (Kreuzer and Hardt, 2020; Stecher, 2021), but also for rationally introducing new functions into
297 existing communities (Cubillos-Ruiz et al., 2021). These factors can include, among others,
298 nutrient/energy availability, environmental factors such as pH and flow/dilution rate (Arnoldini
299 et al., 2018), and the presence of directly toxic or aggressive activities derived from the host
300 (Cullen et al., 2015) or from other microbiota species (García-Bayona and Comstock, 2018),
301 all of which can affect different microbes in different ways. Here, we condense all these
302 mechanisms into three processes: factors affecting immigration rate (i.e., arrival into growth-
303 permissive sites in the gut), factors affecting growth, and factors affecting clearance/death
304 (Hoces et al 2020). Combining well-designed experiments using genetically barcoded strains
305 with mathematical modeling, we were able to empirically (e.g., net growth rates through plating)
306 or deductively (e.g., probability of colonization) estimate the relative importance of these three
307 processes for colonization of *B. theta* under different conditions. In addition, we were able to
308 study mechanistically how fitness-relevant genetic changes in *B. theta* (production of capsular
309 polysaccharide) affect colonization success.

310 When analyzing the effect of capsular polysaccharide expression in the process of colonizing
311 mice with different resident microbiotas, we found that the previously shown fitness
312 disadvantage of acapsular strains (Coyne et al., 2008; Porter et al., 2017) depends on the
313 microbiota context, rather than on host effects. Both, the wild type and the acapsular strains
314 colonize mice with a relatively simple microbiota (LCM, Oligo) similarly well. However, in mice
315 with more complex microbiota (SPF), the acapsular strain engrafted significantly less well than
316 the wild type. While a possible explanation includes more robust intestinal immunity in fully
317 colonized SPF mice, the simplest explanation is that expression of CPS is primarily important
318 for interaction with or protection against other microbes. Possible microbe-inflicted processes
319 against which CPS can protect include microbe-on-microbe killing (Chatzidaki-Livanis et al.,
320 2016; Wexler et al., 2016) and susceptibility to phages (De Sordi et al., 2019; Porter et al.,
321 2020). Co-colonization experiments with mixed *B. theta* inoculums consisting of wild type and
322 acapsular strains revealed the specific disadvantages of the acapsular strain: a longer lag-
323 phase before it starts growing in the gut and a higher clearance rate.

324 The effect of two important challenges with known effects on resident commensals, high-fat
325 feeding (Wotzka et al, 2019) and inflammation (Maier et al, 2014), differentially affect *B. theta*
326 and seem to depend on the ecological context in different ways. Feeding mice which are stably
327 monocolonized with *B. theta* a fiber-less high-fat diet imposes a surprisingly mild bottleneck on
328 the *B. theta* population 3 days after treatment, even though this intervention has been shown

329 to increase bile acid concentrations to levels that inhibit *B. theta* growth (Wotzka et al, 2019).
330 As it has been shown that *B.theta* rapidly evolves to adapt to dietary challenges in the context
331 of a resident microbiota (Dapa et al., 2022), the observed mild population bottleneck imposed
332 by high-fat diet feeding might only manifest if *B. theta* competes against other gut microbiota
333 members, potentially ones that are more resistant to bile salts (e.g., *E. coli*, see Wotzka et al,
334 2019). When infecting mice that are stably monocolonized with *B. theta* with Salmonella, we
335 observed a larger decrease in *B. theta* clonal survival probability, which is consistent with the
336 sensitivity of commensal species to gut inflammation (cite Stecher et al, 2007). Interestingly,
337 inflammation also causes a population bottleneck in the infecting *Salmonella* population (Maier
338 et al, 2014), but it is less pronounced than the one we observe for *B. theta*, and the total
339 population size of *Salmonella* rapidly recovers after this bottleneck. Therefore, the rapid
340 killing/clearance of gut luminal *B. theta* seems to be representative of microbiota suppression
341 that underlies the loss of colonization resistance observed in Salmonella-induced colitis.

342 Of course, our mathematical models are based on certain assumptions which are useful to
343 simplify calculations but always risk introducing bias. Most notably, we have made estimates
344 for a single population of bacteria that has a constant growth and clearance rate. Necessarily
345 the reality is more complex than this – the nutrient profile and motility of the intestine will vary
346 with circadian rhythm. Also previous work has demonstrated that particular CPS-expressing
347 clones may have an advantage colonizing the mucus (Donaldson et al., 2018), although
348 gnotobiotic studies suggest that at the population level *B.theta* grows at a similar rate in the
349 mucus layer and lumen (Li et al., 2015). Nevertheless, recognizing these limitations, our
350 estimates of colonization probability, growth and clearance rates still give a good overview of
351 the harsh processes with strong effects on the *total* intestinal *B.theta* population. Future works
352 should include individual-based models that can evaluate the impact of bacterial clones with
353 different distributions of growth/clearance rates, as well as working with experimental models
354 (microfluidics, for example) that would allow experimental investigation of the impact of single-
355 cell level variation on total population behavior.

356 By combining mathematical modelling with direct quantification of bacterial population
357 dynamics, we can gain insight into the major phenomena influencing colonization efficiency.
358 Not only do these results help us understand the different steps of *B. theta* colonization, but
359 they also serve as a proof of concept for studying other complex, multi-step biological
360 processes using the set of experimental and data-analysis tools we are describing here. This
361 raises the possibility to optimize colonization conditions in order to promote the efficient
362 engraftment of beneficial species into target microbiota, or to better understand the processes
363 of invasion of pathogens and the functional basis of colonization resistance.

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380 **Author Contributions**

381 Conceptualization, D.H., M.A., C.L. and E.S.; Methodology, D.H., G.G., M.A., I.K., F.B., A.W.,
382 E.M., C.L. and E.S.; Formal Analysis, D.H., M.A., C.L., and E.S.; Investigation, D.H., G.G.,
383 C.M., S.B., C.L.; Resources, I.K., J.H., E.M., W-D.H., C.L. and E.S.; Writing – Original draft,
384 D.H. and E.S.; Writing – Review and Editing D.H., G.G., M.A., C.M., S.B., I.K., F.B., A.W., J.H.,
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386 E.S.; Funding acquisition, C.L. and E.S.

387 **Declaration of Interest**

388 The authors declare no competing interests.

389 **Methods**

390 **Bacterial strains and cultures**

391 *B. theta* strains were grown anaerobically (5% H₂, 10% CO₂, rest N₂) at 37°C, overnight in brain
392 heart infusion (BHI) supplemented media (BHIS: 37g/L BHI (Sigma); 1g/L-cysteine (Sigma);
393 1mg/L Hemin (Sigma)). For enrichment cultures in plates, we used BHI-blood agar plates
394 (37g/L BHI (Sigma); 1g/L-cysteine (Sigma); 10% v/v defibrinated sheep blood (Sigma)).
395 Antibiotics were added to liquid cultures or plates as required for strain selection: erythromycin
396 25µg/L or tetracycline 2µg/L. In the case of BHI-blood agar plates used for cloning or gut
397 content enrichment, we additionally added gentamycin 200µg/L to all plates. Plates were
398 incubated for 48-72h at 37°C in anaerobic conditions. For a complete list of the bacterial strains
399 used in this study, see Suppl. Table 1.

400 **Isogenic tag construction and integration**

401 Genetic tags, fluorescent proteins and antibiotic resistance were introduced by using the
402 mobilizable *Bacteroides* element NBU2, which integrates into the *Bacteroides* genomes at a
403 conserved location (Wang et al., 2000). Gene fragments containing a unique 40bp sequence
404 (binding site for forward primer) and a 609bp sequence with the *ydgA* pseudogene (common
405 binding site for reverse primer) were synthesized (gBlocks, Integrated DNA Technologies) and
406 cloned by Gibson Assembly Master Mix (NEB) into an NBU2 plasmid carrying the erythromycin
407 resistant cassette *ermG* (tagged *B.theta*-EryR strains). A similar NBU2 plasmid carrying the
408 tetracycline resistant cassette *tetQb* was used to construct the untagged strains (*B.theta*-
409 TetR). For both, we used 10µL of desalted assembly reaction products to transform competent
410 *E.coli* S17-1 cells (mid-log cells, washed three times in deionized ice-cold water) by
411 electroporation (V=1.8kV; MicroPulser, BioRad). After 1h recovery at 37°C in 1mL of LB, cells
412 were plated on LB plates with chloramphenicol (12µg/mL) and grown overnight. Plasmid-
413 carrying *E.coli* S17-1 and *B.theta* strains were cultured overnight in 5mL of liquid media. *E.coli*
414 S17-1 and *B.theta* were washed with PBS, pooled in 1mL of PBS, plated BHI-blood agar plates
415 without antibiotics, and grown aerobically at 37°C for at least 16 hours. The lawn of *E.coli* S17-
416 1 and *B.theta* was collected in 5mL of PBS, homogenized by vortex and 100µL were plated in
417 BHI-blood agar plates supplemented with erythromycin 25µg/L and gentamycin 200µg/L. After
418 48 hours, single colonies were streaked in fresh BHI-blood agar plates with antibiotics, to avoid
419 potential contamination with WT strains. Successful insertion in the BTt70 or BTt71 sites was
420 evaluated by PCR.

421 ***In vitro* growth curves and competition**

422 Individual *B.theta* strains were grown overnight on BHIS. Stationary-phase cultures were
423 washed with PBS, and O.D. was quantified and adjusted to 0.05 in 200 μ L of fresh BHIS in a
424 round 96-well tissue culture plates. Plates were transferred into the anaerobic tent and growth
425 was quantified at 37°C with shaking, using a plate reader (Infinite PRO 200, Tecan).

426 For competition experiments, stationary-phase cultures *B.theta* WT or acapsular strains were
427 washed with PBS, O.D. was quantified and adjusted to approximately 5x10⁶ CFU/mL per strain
428 (one *B.theta*-TetR and six *B.theta*-EryR tagged strains) in 10mL of fresh BHIS. An aliquot of
429 this mix was serially diluted and plated in BHI-blood agar plates with the respective antibiotics
430 for CFU quantification. Cultures were kept overnight, with shaking (800rpm) at 37°C in the
431 anaerobic tent. Afterwards, an aliquot was plated as described before. For assessing the
432 competition between *B.theta* tagged strains, we isolated DNA from one of the dilutions used
433 for quantification and assessed the relative distribution of the tags by qPCR (see Quantitative
434 PCR section). For assessing the competition among strains with different antibiotic
435 resistances, we calculated the competition index by dividing the CFU/mL of the untagged
436 *B.theta*-TetR strain (tetracycline-resistant) by the adjusted number of *B.theta*-EryR tagged
437 strains (erythromycin-resistant; CFU/mL divided by six, as all the tagged strains were present
438 in the culture).

439 **Mice**

440 All animal experiments were performed with approval from the Zürich Cantonal Authority. In all
441 experiments, we used mice with C57BL/6J genetic background, between 12-15 weeks old and
442 of variable gender. C57BL/6J germ-free and gnotobiotic mouse lines (Low-complexity
443 microbiota (LCM) (Stecher et al., 2010); Oligo Mouse Microbiota (OligoMM12) (Brugiroux et
444 al., 2016)) were raised in surgical isolators under high hygiene standards at the ETH Phenomic
445 Center. C57BL/6J SPF mouse line was also raised in the same facility. Mice were transferred
446 to our experimental facility in sterile, tight closed cages and house into the IsoCage P-
447 Bioexclusion System (Tecniplast) for 24h-48h before the experiment to adapt to new housing
448 conditions. In all experiments, standard chow and water was supplemented under strict sterile
449 conditions to avoid potential contaminations.

450 ***In vivo* growth curves and competition**

451 *B.theta* WT WITS 01 strain was grown overnight on BHIS. Stationary-phase cultures were
452 washed with PBS, and an inoculum of ~5x10⁷ CFUs/100 μ L dose was prepared. C57BL/6J
453 mice carrying the described microbiota composition (LCM, OligoMM12, SPF, 4 mice per group)
454 were gavaged with the inoculum and fecal pellets were collected approximately every 4h for
455 the first 20h, followed by two extra time points at 29h and 48h post-inoculation. Fecal pellets

456 were weighted and homogenized in 500 μ L of PBS with steel ball by mixing (25Hz, 2.5min) in a
457 TissueLyser (Qiagen). Serial dilutions were plated for quantification on BHI-blood agar plates
458 supplemented with gentamycin 200 μ g/L and erythromycin 25 μ g/L.

459 For competition experiments, *B.theta* strains were grown overnight in 8mL of BHIS with
460 corresponding antibiotics (erythromycin 25 μ g/L or tetracycline 2 μ g/L). Each culture was spun
461 down at 3000g for 20min and resuspended in 10mL of PBS and individual O.D. was measured
462 (cell number estimation 1 O.D.= \sim 4 \times 10⁸ cells/mL). Each strain was adjusted to approximately
463 5 \times 10⁶ CFU/100 μ L dose per strain in the inoculum mix (one *B.theta*-TetR and six *B.theta*-EryR
464 tagged strains). Germ-free mice were gavaged with 100 μ L of the inoculum. After 48h, fecal
465 and cecal contents were collected. Fecal content was homogenized and plated for
466 quantification as described before. Cecal content was resuspended in 1mL of PBS and
467 homogenized with steel ball by mixing with the same protocol (25Hz, 2.5min). Serial dilutions
468 were prepared and plated in BHI-blood agar plates supplemented with gentamycin plus either
469 erythromycin or tetracycline for CFU quantification of each strain. Similar to the in vitro
470 competition experiment, we isolated DNA from one of the dilutions used for quantification to
471 assess the competition between *B.theta* tagged strains. Relative distribution of the tags was
472 obtained by qPCR. For calculating the competition index among strains with different antibiotic
473 resistances, we divided the bacteria density of the untagged *B.theta*-TetR strain by a sixth of
474 the bacteria density of the total *B.theta*-EryR tagged strains (as all six tagged strains were
475 present in the culture).

476 **Colonization experiments**

477 Stationary-phase *B.theta* strains were grown overnight as described before. Each culture was
478 washed once with PBS and adjusted in the inoculum based on its O.D. For the untagged strain
479 (*B.theta*-TetR), \sim 5 \times 10⁷ CFUs/100 μ L dose were transferred to the inoculum. For the *B.theta*-
480 EryR tagged strains, we prepared an initial mix of all tagged strains in 50mL of PBS to a
481 concentration of 10⁵ CFU/mL of each strain. After mixing by vortex for 1min, the required
482 amount of *B.theta*-EryR tagged strains was spiked into the inoculum (between 30 - 5 \times 10⁴ CFUs
483 depending on the experiment). Gnotobiotic (LCM, Oligo) and SPF C57BL/6J mice were
484 gavaged with the 100 μ L inoculum. Inoculum was serially diluted and plated for quantification
485 of CFUs in BHI-blood agar plates supplemented with gentamycin 200 μ g/L plus either
486 erythromycin 25 μ g/L or tetracycline 2 μ g/L. In addition, 2-3 doses (100 μ L) were directly plated
487 in BHI-blood agar plates with gentamycin plus erythromycin to address initial distribution of
488 *B.theta*-EryR tagged strains in the inoculum by quantitative PCR (qPCR). Two days after
489 colonization, mice were euthanized and cecal content was collected in 2mL Eppendorf tubes
490 and weighted. Cecal content was homogenized as described before. Serial dilutions were
491 prepared and plated in BHI-blood agar plates supplemented gentamycin plus either

492 erythromycin or tetracycline for CFU quantification. In addition, 100 μ L of homogenized content
493 was plated directly in BHI-blood agar plates with gentamycin plus erythromycin for further
494 assessment of the distribution of *B.theta*-EryR tagged strains by qPCR.

495 **In vivo competition of post-colonization versus original strains**

496 To discard potential increased colonization fitness in the tagged strains that were present in
497 the cecum content after 48h, we isolated single *B.theta* WT tagged strains that were present
498 in the cecal content of SPF during a colonization experiment. Single colonies were expanded
499 in liquid media, and the presence of a single strain was confirmed by qPCR analysis of the
500 tagged tag. We randomly selected three of the *B.theta* WT tagged strains isolated from the
501 cecal content. We prepared an inoculum as described before for the in vivo competition
502 experiments with approximately 5x10⁶ CFU/100 μ L dose per strain in the inoculum mix. We
503 complemented the inoculum with the remaining three *B.theta* WT tagged strains coming from
504 the original stock. SPF mice were inoculated by gavage and cecal content was collected 48h
505 later. Cecal content was processed as described before for CFU quantification and relative tag
506 distribution by qPCR.

507 **Diet modification and Infection challenge experiments**

508 In accordance with what we described before, *B.theta* WT strains were grown overnight in
509 BHIS with corresponding antibiotics. As the untagged strain *B.theta*-TetR was used in higher
510 concentrations, we prepared between 50-100mL of liquid culture depending on the number of
511 mice to colonize. Inoculum was prepared as previously described with a concentration of 10⁸-
512 10⁹ CFUs/100 μ L dose of untagged *B.theta*-TetR, spiked with approximately 30 CFU of each
513 *B.theta*-EryR tagged strains. Germ-free mice were gavaged with 100 μ L of the inoculum. Mice
514 were maintained on standard chow diet (Kliba Nafag, 3537; autoclaved; per weight: 4.5% fat,
515 18.5% protein, ~50% carbohydrates, 4.5% fiber) for 4 days. Afterwards, mice were housed on
516 fresh IsoCages and challenges were applied as follows: 1) Control group (continuation of
517 standard chow diet); 2) Western-type diet without fiber (BioServ, S3282; 60% kcal fat;
518 irradiated; per weight: 36% fat, 20.5% protein, 35.7% carbohydrates, 0% fiber); or 3) infection
519 with 5x10⁷ CFU of attenuated *Salmonella* Typhimurium (Stm SL1344^{ΔSPI-2}). Fecal pellets were
520 collected pre-challenge (day 0) and during the following three day. On the day 3, mice were
521 euthanized and cecal content was collected. Fecal pellets were weighted and homogenized in
522 500 μ L of PBS as described before. Serial dilutions were prepared and plated in BHI-blood agar
523 plates supplemented with corresponding antibiotics for CFU quantification. In addition, 100-
524 300 μ L of homogenized content was plated directly for further assessment of the distribution of
525 *B.theta*-EryR tagged strains by qPCR. Cecal content was processed as previously described.

526 Quantitative PCR

527 Colonies from enrichment plates were pooled in 5ml of PBS and homogenized by vortex.
 528 Genomic DNA was isolated with the QIAamp DNA Mini Kit (Qiagen). qPCR was performed
 529 using with FastStart Universal SYBR Green Master Mix (Roche, Cat. N° 4385610). Primers
 530 (Supplementary Table 2) were mixed to a final concentration of 1µM. Between 160-200ng of
 531 DNA was amplified using StepOne Plus or QuantStudio 7 Flex instruments (Applied
 532 Biosystems) using the following protocol: initial denaturation at 95°C for 14min followed by 40
 533 cycles of 94°C for 15sec, 61°C for 30sec, and 72°C for 20sec as described previously.

534 Mathematical modelling

535 1. Estimation of colonization probability based on lost tags

536 Let us denote C the bacterial concentration in the prepared solution. Then, if there is a
 537 volume V of solution, then there are $N = CV$ bacteria. Therefore, the probability to have taken
 538 n_0 bacteria in a volume v_0 is:

$$539 \quad p(n_0) = \text{Binomial distribution}(N, n_0/V) = \left(\frac{v_0}{V}\right)^{n_0} \left(1 - \frac{v_0}{V}\right)^{N-n_0} \frac{N!}{(N-n_0)!n_0!} \quad (1)$$

540 In the limit of $N = cV$ large and $v_0 \ll V$,

$$541 \quad p(n_0) \cong \text{Poisson distribution}(N v_0/V) = \frac{(N v_0/V)^{n_0} \exp(-N v_0/V)}{n_0!} \quad (2)$$

542 Let us define β as the probability for each bacterium to get to the cecum alive, and then have
 543 its lineage survive until measurement. There are a priori no interactions early on between
 544 incoming bacteria, as their concentration is initially low enough to limit the competition between
 545 them before arriving to the cecum. Then, the probability for a tagged *B.theta* strain not to be
 546 present at measurement time, if started with an average of n_0 bacteria (Poisson distributed) is
 547 the zero of the Poisson distribution of average βn_0 , and thus:

$$548 \quad p_{loss} = \exp(-\beta n_0) \quad (3)$$

549 As n_0 is estimated via the concentration and volume of the inoculum, and p_{loss} is best estimated
 550 via the number of tags lost divided by the total number of tags, then β is estimated as:

$$551 \quad \beta \cong \frac{-\log(n_{loss} \text{ tags}/n_{tags})}{n_0} \quad (4)$$

552 To consider the fact that not all tags have the same n_0 , β is actually estimated by its value
 553 maximizing the probability of the experimental observations, thus β maximizing:

$$554 \quad LL = \sum_{i=1}^{\omega} \log \left((\exp(-\beta n_i))^{l_i} (1 - \exp(-\beta n_i))^{1-l_i} \right) \quad (5)$$

555 This expression is also used for calculating the confidence interval, as detailed in appendix.

556 *2. Estimation of colonization probability based on variance*

557 The variance on the proportions is:

558
$$var(p) = \frac{1}{h_1} \sum (p_i - \frac{1}{h})^2 = \frac{1}{h_1} \sum (\frac{n_i}{\sum n_j} - \frac{1}{h})^2 \quad (6)$$

559 In the limit where the initial number of bacteria are of the same order of magnitude, we find:

560
$$\langle var(p) \rangle - var(p_0) \cong \frac{1}{h \sum n_{j,0}} \frac{var1}{m1^2} \quad (7)$$

561 with $var(p_0)$ the variance in proportions in the inoculum, $\sum n_{j,0}$ the total number of tags in the
 562 inoculum, and $var1/m1^2$ the relative variance starting from one bacterium. We find (see
 563 Supplementary Methods) that $var1/m1^2$ is $2/(\text{colonization probability})$. Then $var1/m1^2$ is
 564 estimated for each mouse using equation (7), and the average variance is used to estimate
 565 $var1/m1^2$. The standard error on $var1/m1^2$ is used to obtain the confidence interval for the
 566 colonization probability.

567 *3. Estimation of clearance rate due to flow*

568 We examined the expected magnitude of the effect of an extended lag phase in the cecum on
 569 colonization probability to determine whether this is consistent with our observed neutral
 570 tagging data. It should also be noted that the cecum is a dynamic environment with pulsatile
 571 arrival of material from the small intestine and loss of material to the feces. This generates a
 572 clearance rate due to flow, on top of any clearance rate due to bacterial death. Assuming that
 573 the main site of growth of *B.theta* is the cecum/upper colon, the parameter for clearance due
 574 to flow can be estimated by quantifying the volume of cecum content lost per day. This can be
 575 empirically estimated by measuring 1) fecal dry mass produced per day, and 2) the water
 576 content of cecum content. Assuming minimal change in dry mass during colon transit in the
 577 mice, this infers a dilution rate of cecal content in the order of 0.12 volumes/h in a germ-free
 578 mouse and 0.18 volumes/h in an SPF mouse; gnotobiotic mice will have a value in between
 579 these two. Bacterial clearance due to killing will contribute over and above these values. Of
 580 note, bacteria with a long lag-phase after introduction into the cecum will be cleared by the
 581 flow before growth can start, i.e., during the early phase of colonization this will be a
 582 determinant of colonization probability.

Estimation of cecum turnover rates

	Water fraction in	Dry fecal excretion (gram/day)	Estimated wet cecal mass	Wet cecal mass (gram)	Estimated cecum	Estimated cecum

	cecal mass (%)		excretion (gram/day)		turnover rate (volume/day)	turnover rate (volume/h)
Germ-free	80.9 (0.4)	1.55 (0.27)	8.12 (1.42)	2.83 (0.59)	2.87 (0.78)	0.12 (0.03)
SPF	76.2 (1.2)	0.81 (0.09)	3.40 (0.42)	0.77 (0.32)	4.42 (1.91)	0.18 (0.08)

583 4. Estimation of the competitive index

584 We assume that bacteria have first a probability of survival q_i (with $i = w$ for the WT strain, and
585 $i = a$ for the acapsular strain). Then once the cecum is reached, they have a loss rate c_i .

586 During an initial lag-phase τ_i , they do not grow. Then, each bacterial strain grows logistically,
587 initially at a rate r_i , which saturates when approaching carrying capacity with a factor $(1 - (A +$
588 $W)/K)$. When carrying capacity is reached, the total number of bacteria remains constant until
589 the end of the experiment at time t_{tot} , with both loss and replication ongoing and compensating
590 each other. Given the growth rates for WT and acapsular are similar in vitro, we assume $r_w =$
591 r_a , so that the difference in the initial net growth rates $(r_i - c_i)$ originates from $c_a > c_w$.
592 Therefore, in the competition setting, the population is mostly composed of WT when carrying
593 capacity is reached, so that the global population size is depleted with rate approximately c_w .
594 As a result, the effective residual replication rate of both types exactly compensates the loss
595 rate, c_w , because they compete for the same resources and have the same growth ability. As
596 acapsular keep being lost at a larger rate c_a , their net growth rate at carrying capacity is
597 negative, so that they keep decreasing in frequency in the population.

598 With this model (see detailed calculations in Supplementary Methods), we find that the relative
599 ratio between WT and acapsular is:

$$600 \quad \frac{q_w}{q_a} \exp((net_w + c_w)(\tau_a - \tau_w)) (\exp((net_w - net_a)t_{tot})) \quad (8)$$

601 For all the microbiota except SPF, as the colonization probabilities were similar for the WT and
602 acapsular, then we assume $q_a = q_w$. For SPF, we use the ratio of q_w / q_a in the colonization
603 experiments, adjusted for the fact that the full colonization probability also includes steps after
604 the initial death before reaching the cecum.

605 All the parameters used are determined using experiments other than the competition
606 experiment (except for t_{tot} , the total experimental time).

607 5. Estimation of colonization probability during competition

608 We find that the overall survival probability for the acapsular in the competition experiment is
609 the colonization probability from the colonization experiment (alone), multiplied by a factor

610 considering later loss (when the carrying capacity is reached by the WT and the acapsular
611 decreases). The complete expression can be found in the corresponding section of the
612 Supplementary Methods.

613 *6. Estimation of survival probability after challenge*

614 In these experiments, at the time of the start of the challenge, the bacterial population is at
615 carrying capacity, so the effective growth rate (likely limited by availability of nutrients) is about
616 the same as the loss rate. Also, let us assume that the population the population size is known
617 at this time. Then, a challenge is applied. A challenge may have different effects: it may impose
618 a temporary bottleneck in the population (loss becomes higher than reproduction), or it may
619 increase the loss rate (with the reproduction rate increasing enough to compensate), and thus
620 the turnover of the population. In any case, we can calculate β as the probability that a bacteria
621 present at day=0 of the challenge has its lineage still alive at day 3. Then, if there are n_0
622 bacteria of a given type at day=0, then:

$$623 \quad p_{loss} = (1 - \beta)^{n_0} \quad (6)$$

624 To estimate the total population size in the cecum at before the challenge (n_0), we assume
625 that (1) all the animals are in the control conditions at day=0, and (2) the cecum mass and
626 bacteria concentration is the same on day=0 and day=3 in the control group. Therefore, the
627 average ratio of the concentration of bacteria in feces relative to cecum at day=3 in the control
628 animals is used to estimate the bacterial concentration in the cecum at day=0. Then, we
629 estimate the proportion of each tag strain in from the total tagged-bacteria CFU counts using
630 the relative distribution the qPCR data. In addition, p_{loss} is best estimated via the number of
631 tags lost divided by the total number of tags.

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Supplementary Information

Supplementary Table 1: Bacterial strains

Strain	Genotype	Description	Reference
<i>B. theta</i> WT	ATCC 29148 <i>tdk-</i>	Parent strain of <i>B. theta</i> . Used to generate wild-type CPS mutants in this study.	(Porter et al., 2017)
<i>B. theta</i> acapsular	<i>tdk- Δcps1-8</i>	Acapsular <i>B. theta</i> with deletion of capsular polysaccharide locus. Used to generate acapsular mutants in this study.	(Porter et al., 2017)
<i>B. theta</i> -EryR WT tagged1/2/11	<i>tdk- :: pNBU2-cat-ermG-GFP-WITS1/2/11</i>	<i>B. theta</i> WT strain isogenic tag insert; fluorescent tag; erythromycin resistant	This study
<i>B. theta</i> -EryR WT tagged17/19/21	<i>tdk- :: pNBU2-cat-ermG-mCherry-WITS17/19/21</i>	Wild type CPS strain with isogenic tag insert; fluorescent tag; erythromycin resistant	This study
<i>B. theta</i> -EryR acapsular tagged 1/2/11	<i>tdk- Δcps1-8 :: pNBU2-cat-ermG-GFP-WITS1/2/11</i>	Acapsular strain with isogenic tag insert; fluorescent tag; erythromycin resistant	This study
<i>B. theta</i> -EryR acapsular tagged17/19/21	<i>tdk- Δcps1-8 :: pNBU2-cat-ermG-mCherry-WITS17/19/21</i>	Acapsular strain with isogenic tag insert; fluorescent tag; erythromycin resistant	This study
<i>B. theta</i> -TetR WT	<i>tdk- :: pNBU2-bla-tetQb</i>	Untagged strain. Wild type strain with fluorescent insert; tetracycline resistant	This study
<i>B. theta</i> -TetR acapsular	<i>tdk- Δcps1-8 :: pNBU2-bla-tetQb</i>	Untagged strain. Acapsular strain with fluorescent insert; tetracycline resistant	This study
Stm SL1344 ^{ΔSPI-2} (M3103)	<i>ΔssaV</i>	Attenuated <i>Salmonella enterica</i> serovar Typhimurium; SPI-2 KO	

Supplementary Table 2: Primers

Primer	Sequence	Use
WITS01	acgacaccactccacaccta	Forward primer. Quantification of tagged strain
WITS02	acccgcaataccaacaactc	Forward primer. Quantification of tagged strain
WITS11	atcccacacactcgatctca	Forward primer. Quantification of tagged strain
WITS17	tcaccagcccaccccctca	Forward primer. Quantification of tagged strain
WITS19	gcactatccagcccataac	Forward primer. Quantification of tagged strain
WITS21	acaaccaccgatcactctcc	Forward primer. Quantification of tagged strain
ydgA	ggctgtccgcaatgggtc	Common reverse primer. Quantification of tagged strain

Supplementary Table 3: Microbiota composition in gnotobiotic mice LCM, OligoMM-12

Low complexity microbiota

OTU	Relative abundance	8 ASF species match	SILVA best match
1	6,43E-01	Bacteroides sp. ASF519	Parabacteroides goldsteinii dnLKV18
2	1,65E-01	Clostridium sp. ASF502	Clostridiales bacterium
3	9,60E-02	Eubacterium plexicaudatum strain ASF 492	Lachnospiraceae bacterium 6_1_63FAA
4	5,56E-02	Eubacterium plexicaudatum strain ASF 492	Blautia
5	2,00E-02	*	Turicibacter sp. LA61
6	8,52E-03	Eubacterium plexicaudatum strain ASF 492	Lachnoclostridium;bacterium NLAE-zl-411
7	7,40E-03	Bacterium ASF500	Flavonifractor;human gut metagenome

OligoMM12

OTU	Strain ID	DSM number	Phyla	Classification
1	YL2	26074	Actinobacteria	<i>Bifidobacterium longum subsp. animalis</i>
2	YL27	28989	Bacteroidetes	'Muribaculum intestinale'
3	I48	26085	Bacteroidetes	'Bacteroides caecimuris'
4	YL45	26109	Proteobacteria	'Turicimonas muris'
5	YL44	26127	Verrucomicrobia	<i>Akkermansia muciniphila</i>
6	KB1	32036	Firmicutes	<i>Enterococcus faecalis</i>
7	I49	32035	Firmicutes	<i>Lactobacillus reuteri</i>
8	YL32	26114	Firmicutes	<i>Clostridium clostridioforme</i>
9	YL58	26115	Firmicutes	<i>Blautia coccoides</i>
10	YL31	26117	Firmicutes	<i>Flavonifractor plautii</i>
11	KB18	26090	Firmicutes	'Acutalibacter muris'
12	I46	26113	Firmicutes	<i>Clostridium innocuum</i>