

1 **A TRADE-OFF BETWEEN RESISTANCE TO INFECTION AND REPRODUCTION IN PRIMATE**  
2 **EVOLUTION**

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15  
16 **SUMMARY**

17 Most mammals express a functional *GGTA1* gene encoding the N-  
18 acetyllactosaminide  $\alpha$ -1,3-galactosyltransferase enzyme, which synthesizes Gal $\alpha$ 1-  
19 3Gal $\beta$ 1-4GlcNAc ( $\alpha$ Gal) and are thus tolerant to this self-expressed glycan epitope.  
20 Old World primates including humans, however, carry *GGTA1* loss-of-function  
21 mutations and lack  $\alpha$ Gal. Presumably, fixation of such mutations was propelled by  
22 natural selection, favoring the emergence of  $\alpha$ Gal-specific immunity, which conferred  
23 resistance to  $\alpha$ Gal-expressing pathogens. Here we show that loss of *Ggta1* function  
24 in mice enhances resistance to bacterial sepsis, irrespectively of  $\alpha$ Gal-specific  
25 immunity. Rather, the absence of  $\alpha$ Gal from IgG-associated glycans increases IgG  
26 effector function, via a mechanism associated with enhanced IgG-Fc gamma  
27 receptor (Fc $\gamma$ R) binding. The ensuing survival advantage against sepsis comes  
28 alongside a cost of earlier onset of reproductive senescence. Mathematical modeling  
29 of this trade-off shows that under conditions of high exposure to virulent pathogens,  
30 selective pressure can fix *GGTA1* loss-of-function mutations, as likely occurred  
31 during the evolution of primates towards humans.

32 **KEYWORDS**

33 Infection; human evolution; microbiota; sepsis; natural antibodies; glycans; IgG  
34 effector function; trade-off; reproductive senescence;  $\alpha$ Gal.

35

## 36 INTRODUCTION

37 Mammals, including humans, generate relatively high levels of circulating anti-glycan  
38 antibodies (Ab) at steady state (Huflejt et al., 2009; Kearney et al., 2015; Schneider  
39 et al., 2015; Stowell et al., 2014). These are referred to as natural antibodies (NAb),  
40 to hint that their generation occurs in the absence of traceable immunization. It is  
41 becoming clear, however, that circulating anti-glycan NAb are generated to a large  
42 extent in response to glycans expressed by immunogenic bacteria (Palm et al., 2014)  
43 in the gut microbiota (Bunker et al., 2017; Soares and Yilmaz, 2016; Yilmaz et al.,  
44 2014).

45 Circulating NAb of the IgM isotype provide an immediate lytic response to  
46 pathogens via activation of the complement-cascade (Ochsenbein et al., 1999;  
47 Yilmaz et al., 2014). In contrast, circulating NAb from the IgG isotype confer  
48 protection against infection via cognate interaction with Fc $\gamma$  receptors (Fc $\gamma$ R) (Zeng et  
49 al., 2016) expressed by immune cells, a central driver of IgG effector function (Lu et  
50 al., 2018; Ravetch and Kinet, 1991).

51 The protective effect exerted by glycan-specific NAb is operational only when the  
52 targeted glycans are not expressed as self epitopes (Soares and Yilmaz, 2016).  
53 Remarkably, this fundamental principle of immune self/non-self discrimination was  
54 circumvented for several glycans, through the natural selection and fixation of loss-  
55 of-function mutations in genes synthesizing those glycans (Soares and Yilmaz, 2016;  
56 Springer and Gagneux, 2016). This is perhaps best illustrated for the loss of  $\alpha$ Gal  
57 expression in ancestral Old World primates, including humans, as originally  
58 described by K. Landsteiner and P. Miller (Galili et al., 1987; Galili and Swanson,  
59 1991; Landsteiner and Miller, 1925).

60 The  $\alpha$ Gal glycan is synthesized in most mammals, including New World  
61 monkeys, by GGTA1, a galactosyltransferase that catalyzes the transfer of a  
62 galactose (Gal) in  $\alpha$ 1-3 linkage, from a uridyl-diphosphate (UDP) donor onto the N-  
63 acetyllactosamine (Gal $\beta$ 1,4GlcNAc-R) of glycoproteins. This reaction does not occur  
64 in Old World primates, including humans (Galili et al., 1988b), which carry a *GGTA1*  
65 pseudogene (Galili et al., 1987; Galili and Swanson, 1991). Presumably, loss of  
66 *GGTA1* function in the ancestor of Old World primates contributed to shaping the  
67 human anti-glycan NAb repertoire (Huflejt et al., 2009), allowing for the emergence of  
68  $\alpha$ Gal-specific immunity (Galili et al., 1984; Macher and Galili, 2008; Soares and  
69 Yilmaz, 2016).

70 When considering infection as a major driving force in evolution (Haldane, 1949),  
71 it is reasonable to assume that protection from infection should act as a major driving

72 force in the natural selection and fixation of *GGTA1* loss-of-function mutations during  
73 primate evolution. In strong support of this notion, circulating  $\alpha$ Gal-specific NAb can  
74 account for up to 1-5% of all circulating IgM and IgG in healthy adult humans  
75 (Macher and Galili, 2008), providing protection against infection by pathogens  
76 expressing  $\alpha$ Gal-like glycans (Soares and Yilmaz, 2016; Takeuchi et al., 1996;  
77 Yilmaz et al., 2014).

78 Sepsis is a life-threatening organ dysfunction caused by a deregulated host  
79 response to infection (Singer et al., 2016), which can account for as much as 20% of  
80 global human mortality (Rudd et al., 2020). Presumably, therefore, infections by  
81 virulent pathogens that can trigger the development of sepsis are likely to have  
82 exerted a major selective pressure throughout primate and human evolution. Sepsis  
83 is thought to originate and progress, to at least some extent, from the immune  
84 response elicited upon translocation across epithelial barriers of bacterial pathobionts  
85 from the microbiota (Rudd et al., 2020; Vincent et al., 2009). As circulating  $\alpha$ Gal-  
86 specific NAb are generated, at steady state, in response to  $\alpha$ Gal-like glycans  
87 expressed by immunogenic bacteria in the microbiota (Galili et al., 1988a; Soares  
88 and Yilmaz, 2016; Springer and Horton, 1969), one might expect  $\alpha$ Gal-specific NAb  
89 to provide some level of protection against sepsis. While there is no epidemiological  
90 evidence to support this notion, there are other mechanisms via which loss of  
91 *GGTA1* function could enhance protection against bacterial sepsis.

92 Circulating IgG carry bi-antennary glycan structures, N-linked to an evolutionarily  
93 conserved asparagine-297 in their constant heavy chain of the Fc domain (Anthony  
94 et al., 2012). These glycan structures are composed of varying amounts of N-  
95 acetylglucosamine (GlcNAc), fucose, mannose, galactose (Gal) and sialic acid  
96 molecules, which modulate the binding affinity of the IgG Fc domain to different Fc $\gamma$ R  
97 and proteins of the complement cascade (Anthony et al., 2012; Dekkers et al., 2017;  
98 Wang and Ravetch, 2019). Given that  $\alpha$ Gal is part of these IgG-associated glycan  
99 structures in mammals that carry a functional *GGTA1* gene (de Haan et al., 2017),  
100 we hypothesized that  $\alpha$ Gal can modulate IgG binding to Fc $\gamma$ R and/or complement  
101 proteins (Anthony et al., 2012; Dekkers et al., 2017; Wang and Ravetch, 2019). In  
102 support of this hypothesis, when present in IgG-associated glycan structures terminal  
103 Gal residues can modulate IgG-Fc $\gamma$ R binding and complement activation  
104 (Nimmerjahn et al., 2007). This is also the case for other glycan residues, as  
105 demonstrated for example for fucose in an  $\alpha$ 1,6-linkage (Nimmerjahn and Ravetch,  
106 2005).

107

108 Here we hypothesized that the presence or absence of  $\alpha$ Gal from the glycan  
109 structure of IgG might modulate IgG NAb effector function in a manner that  
110 modulates resistance to bacterial sepsis. We report that loss of *Ggta1* function in  
111 mice confers robust protection from bacterial sepsis via a mechanism that acts  
112 irrespectively of  $\alpha$ Gal-specific immunity. This robust protective effect is mediated,  
113 instead, by enhancement of IgG NAb effector function, via a mechanism associated  
114 with increased IgG-Fc $\gamma$ R binding. The gained fitness advantage against infection is  
115 associated, however, with an accelerated onset of reproductive senescence.  
116 Mathematical modeling of this evolutionary trade-off suggests that under conditions  
117 of high exposure to virulent pathogens, the fitness gain prevails over the cost,  
118 providing a possible explanation for the natural selection and fixation of *GGTA1* loss-  
119 of-function mutations during primate evolution towards humans.

120

## 121 RESULTS

### 122 Loss of *Ggta1* function in mice enhances resistance to systemic infection by 123 symbiotic gut bacteria

124 We tested the hypothesis that the loss of *GGTA1* function during primate evolution  
125 (Galili and Swanson, 1991) might provide protection against bacterial sepsis in  
126 *Ggta1*-deficient (*Ggta1*<sup>-/-</sup>) mice, a well-established experimental model that mimics  
127 the absence of *GGTA1* function in modern humans. Polymicrobial infection was  
128 induced by cecal ligation and puncture (CLP), a well-established model of sepsis  
129 (Rittirsch et al., 2009), in which the gut epithelial barrier is breached in a controlled  
130 manner, leading to systemic dissemination of gut bacteria. *Ggta1*<sup>-/-</sup> mice showed a  
131 strong survival advantage against CLP, as compared to control wild type (*Ggta1*<sup>+/+</sup>)  
132 mice (*Fig. 1A*). This was associated with a 10-10<sup>5</sup>-fold reduction in bacterial load,  
133 dependent upon the organ examined (*Fig. 1B*), suggesting that loss of *Ggta1*  
134 function enhances resistance to bacterial sepsis.

135 The outcome of CLP is affected by the bacterial composition of the gut microbiota,  
136 which we found to be notably different between *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice, as  
137 determined by 16S rRNA gene sequencing analysis of the fecal microbiota for  
138 bacterial community structure (*Fig. 1C,D*), composition (*Fig. 1E*) and diversity (*Fig.*  
139 *S1A,B*). To dissect the contribution of host genotype vs. microbiota composition in  
140 conferring protection against sepsis, we used a previously established approach to  
141 normalize the microbiota between mice with different genotypes (Ubeda et al., 2012).  
142 We confirmed that vertical transmission from female *Ggta1*<sup>-/-</sup> mice (*Fig. 1F*),  
143 normalized the bacterial composition of the gut microbiota in F<sub>2</sub> littermate mice from

144 both genotypes (Fig. 1G-H, S1C-D). Nevertheless, F<sub>2</sub> *Ggta1*<sup>-/-</sup> mice retained a  
145 survival advantage following CLP, when compared to F<sub>2</sub> *Ggta1*<sup>+/+</sup> mice (Fig. 1I). This  
146 was associated with a 10<sup>1</sup>-10<sup>2</sup>-fold reduction in bacterial load (Fig. 1J), showing that  
147 loss of *Ggta1* function is sufficient *per se* to enhance resistance to bacterial sepsis.  
148 We note, however, that the relative survival advantage against CLP was higher in F<sub>0</sub>  
149 *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice (Fig. 1A,B) harboring distinct microbiota, as compared to F<sub>2</sub>  
150 *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice (Fig. 1I,J) harboring the same microbiota. This argues for a  
151 synergistic contribution of both the host genotype and its microbiota to enhanced  
152 protection from bacterial sepsis observed in *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice.

153

### 154 **Loss of *Ggta1* function in mice enhances IgG-mediated resistance to systemic** 155 **polymicrobial infection**

156 Different components of the adaptive immune systems (Kato et al., 2014), including  
157 circulating NAb that are generated in response to immunogenic bacteria in the gut  
158 microbiota (Kamada et al., 2015; Koch et al., 2016; Macpherson et al., 2018),  
159 restrain the growth of pathobionts and favor gut colonization by commensal bacteria  
160 while increasing overall microbiota diversity (Round and Palm, 2018). In keeping with  
161 this notion, the gut microbiota of *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice, inheriting the microbiota from  
162 *Ggta1*<sup>-/-</sup> mice in the absence of adaptive immunity (Fig. S1E), was remarkably distinct  
163 from that of *Ggta1*<sup>-/-</sup> mice (Fig. S1F,G). Moreover, the microbiota of *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup>  
164 mice exhibited a marked reduction in diversity (Fig. S1H,I) and an enrichment of  
165 several bacteria associated with pathobiont behavior, such as Proteobacteria,  
166 *Helicobacter* and *Bacteroides* (Palm et al., 2014) (Fig. S1J,K). This supports the view  
167 that the adaptive immune system of *Ggta1*<sup>-/-</sup> mice shapes the gut microbiota  
168 composition, which is consistent, albeit more pronounced, with studies in *Ggta1*<sup>+/+</sup>  
169 mice (Barroso-Batista et al., 2015).

170 Circulating NAb can limit the translocation of bacterial pathobionts across the gut  
171 epithelium and consequently prevent systemic infections from triggering the onset of  
172 sepsis (Kamada et al., 2015; Zeng et al., 2016). We therefore asked whether the loss  
173 of *Ggta1* function promotes NAb-driven resistance against pathobionts present in the  
174 gut microbiota from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice. As with CLP (Fig. 1A-B), *Ggta1*<sup>-/-</sup> mice were  
175 more resistant than *Ggta1*<sup>+/+</sup> mice to intra-peritoneal (i.p.) inoculation of cecal content  
176 from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice (Fig. 2A), showing a 10<sup>4</sup>-10<sup>7</sup>-fold reduction in bacterial load  
177 (Fig. 2B). Both genotypes survived when challenged (i.p.) with a paraformaldehyde-  
178 fixed cecal inoculum (Fig. S1L), suggesting that loss of *Ggta1* enhances anti-

179 bacterial resistance rather than providing protection against inflammation-driven  
180 immunopathology, a hallmark of sepsis (Rudd et al., 2020; Singer et al., 2016).

181 Confirming that loss of *Ggta1* enhances resistance to bacterial sepsis through  
182 mechanisms dependent upon adaptive immunity, *Rag2<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice were  
183 extremely susceptible to infection with the same cecal inoculum (Fig. 2C), carrying a  
184 10<sup>3</sup>-10<sup>6</sup>-fold higher bacterial load, as compared to *Ggta1<sup>-/-</sup>* mice (Fig. 2D). This was  
185 also observed in *J<sub>H</sub>t<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice lacking B cells (Fig. 2E,F), suggesting that anti-  
186 bacterial resistance is provided by a B cell-dependent mechanism.

187 Although protective against bacterial infection (Ochsenbein et al., 1999),  
188 circulating IgM was not essential in this experimental system to enhance anti-  
189 bacterial resistance, as demonstrated in *μS<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice lacking circulating IgM  
190 (Fig. S2A,B). Similarly, while IgA can be critical to support mucosal immunity  
191 (Macpherson et al., 2018; Sutherland et al., 2016) and prevent the development of  
192 sepsis (Wilmore et al., 2018), it was not essential to support anti-bacterial resistance  
193 in *Iga<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice lacking IgA (Fig. S2C,D). In contrast, protection against infection  
194 with the cecal inoculum isolated from *Rag2<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice was lost in *Aid<sup>-/-</sup>Ggta1<sup>-/-</sup>*  
195 mice lacking immunoglobulin (Ig) class-switch recombination, somatic hypermutation  
196 and affinity maturation (Muramatsu et al., 2000) (Fig. 2G). This was associated with a  
197 10<sup>3</sup>-10<sup>6</sup>-fold increase in bacterial load, as compared to *Ggta1<sup>-/-</sup>* mice (Fig. 2H),  
198 suggesting that the survival advantage against bacterial sepsis in *Ggta1<sup>-/-</sup>* mice relies  
199 on the presence of circulating NAb that must undergo class-switch recombination  
200 towards the IgG isotype.

201 Immunoglobulin class-switch, somatic hypermutation and affinity maturation are  
202 largely T cell-dependent, suggesting that T cells might support resistance to infection  
203 in *Ggta1<sup>-/-</sup>* mice. However, we observed that *Tcrβ<sup>-/-</sup>Ggta1<sup>-/-</sup>* (Fig. S2E,F) and *Tcrδ<sup>-/-</sup>*  
204 *Ggta1<sup>-/-</sup>* (Fig. S2G,H) mice, lacking α/β and γ/δ T cells respectively, were resistant to  
205 sepsis, similarly to *Ggta1<sup>-/-</sup>* mice. This suggests that the protective circulating IgG  
206 NAb in *Ggta1<sup>-/-</sup>* mice are generated in a T-cell independent manner.

207

### 208 **The protective effect of IgG NAb acts irrespectively of αGal recognition**

209 We then asked whether circulating IgG NAb from *Ggta1<sup>-/-</sup>* mice are sufficient *per se*  
210 to confer resistance to infection. Upon adoptive transfer at the same dosage to *Rag2<sup>-/-</sup>*  
211 *Ggta1<sup>-/-</sup>* mice, only circulating IgG NAb purified from *Ggta1<sup>-/-</sup>* mice, but not from  
212 *Ggta1<sup>+/+</sup>* mice, were protective against infection (Fig. 3A). Since the concentration of  
213 circulating IgG in naive *Ggta1<sup>-/-</sup>* mice that are protected from infection, was similar to  
214 that of *Ggta1<sup>+/+</sup>* mice, that are not protected (Fig. S3A), this suggests that IgG NAb

215 from *Ggta1*<sup>-/-</sup> mice have an enhanced capacity to confer protection against bacterial  
216 infection, as compared to the IgG NAb from *Ggta1*<sup>+/+</sup> mice.

217 We reasoned that the enhanced capacity of IgG NAb from *Ggta1*<sup>-/-</sup> mice to confer  
218 protection against bacterial infection might be due to changes in IgG subclass  
219 composition, enhancing overall effector function (Lu et al., 2018). Alternatively,  
220 differences in the repertoire of circulating IgG NAb could enable the recognition of  
221 different bacterial antigens including, αGal-like glycans.

222 We found that the bacterium-binding IgG NAb from *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice  
223 were almost exclusively from the IgG2b subclass (*Fig. 3B*), analogous to previous  
224 reports in *Ggta1*<sup>+/+</sup> mice (Zeng et al., 2016). This excludes the possibility that  
225 increased resistance to bacterial infection provided by IgG NAb from *Ggta1*<sup>-/-</sup> vs.  
226 *Ggta1*<sup>+/+</sup> mice is due to differences in relative IgG subclass composition.

227 A number of bacteria in the gut microbiota express αGal-like glycans (Montassier  
228 et al., 2019), suggesting that αGal-specific NAb might be protective against systemic  
229 infection by these bacteria. When maintained under specific pathogen-free conditions  
230 however, circulating αGal-specific NAb accounted for less than 0.005% of circulating  
231 IgG2b NAb from *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice (*Fig. 3C*), which is consistent with  
232 previous reports (Galili et al., 1984; Yilmaz et al., 2014). In keeping with this  
233 observation, circulating IgG2b NAb from both *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice recognized  
234 only a negligible, but similar proportion of αGal-expressing bacteria in the infectious  
235 inoculum (*Fig. 3D,E, S3B*). Taken together, this suggests that loss of *Ggta1*  
236 enhances protection against bacterial sepsis via a mechanism that acts irrespectively  
237 of αGal-specific immunity.

238 We then asked whether circulating IgG NAb from *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice  
239 recognized to the same or different extent individual bacterial strains isolated from  
240 mouse microbiota. We found that circulating IgG NAb from *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice  
241 recognized these bacteria to the same extent (*Fig. 3F, S3C*), irrespectively of  
242 αGal expression by the targeted bacteria (*Fig. 3G, S3C,D*). This is illustrated for *E.*  
243 *faecalis*, which does not express αGal, and *C. bifermentans* that expresses relatively  
244 low levels of αGal, when compared to *E. coli* O86:B7 that expresses high levels of  
245 αGal (*Fig. 3G, S3C,D*). This suggests that the enhanced protective effect exerted by  
246 the circulating IgG NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice does not rely on the  
247 recognition of αGal-like glycans in the targeted bacteria.

248

249

## 250 **Loss of *Ggta1* function does not affect bacterial recognition by IgG NAb**

251 Having established that protection against bacterial sepsis was independent of  $\alpha$ Gal-  
252 specific immunity, we asked whether circulating IgG NAb from *Ggta1*<sup>-/-</sup> mice  
253 recognized distinct bacteria, when compared to IgG NAb from *Ggta1*<sup>+/+</sup> mice. The  
254 percentage of bacteria recognized in the cecal inoculum used for infection by IgG  
255 NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice was indistinguishable, as assessed *in vitro* by  
256 staining of bacteria (Fig. 4A) and *in vivo* by detecting IgG-bound bacteria recovered  
257 from the peritoneal cavity after infection with this inoculum (Fig. 4B). Relative amount  
258 of IgG bound *per* bacterium was also similar in both experimental settings (Fig.  
259 S4A,B). Co-staining of the same infectious inoculum with IgG NAb from *Ggta1*<sup>-/-</sup> and  
260 *Ggta1*<sup>+/+</sup> mice, conjugated to different fluorophores, demonstrated that these NAb  
261 recognized the same bacteria (*i.e.* >97% similar), as illustrated by flow cytometry  
262 (Fig. 4C,D). Moreover, the pattern of bacterial recognition obtained using IgG NAb  
263 from the same genotype, but conjugated to different fluorophores, was  
264 indistinguishable from that obtained using IgG NAb from different genotypes (S4C-F).  
265 Recognition of largely overlapping bacteria by IgG NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup>  
266 mice was confirmed by 16S rRNA analysis of IgG-bound (Fig. 4E-F, S4G) and non-  
267 IgG-bound bacteria (Fig. 4G-H, S4H). These observations suggest that circulating  
268 IgG NAb from both genotypes recognize the same bacteria in the infectious  
269 inoculum, and do so to the same extent. Therefore, the enhanced resistance to  
270 bacterial sepsis provided by the IgG NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice is likely not  
271 due to differences in the bacteria recognized.

272

## 273 **Loss of $\alpha$ Gal expression enhances IgG effector function**

274 We then reasoned that the enhanced protective effect exerted by the IgG NAb from  
275 *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice might be due to a corresponding enhancement of IgG  
276 effector function. To test this hypothesis, we used the bacterial strain, *E. faecalis* that  
277 lacks  $\alpha$ Gal but is recognized to a similar extent by IgG NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup>  
278 mice (Fig. 3F,G, S3C,D). Naïve *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice, lacking circulating Ig, were  
279 challenged (*i.p.*) with *E. faecalis*, opsonized, or not, by circulating IgG NAb purified  
280 from *Ggta1*<sup>-/-</sup> or *Ggta1*<sup>+/+</sup> mice. Recruitment of neutrophils (CD11b<sup>+</sup>Ly6G<sup>high</sup>) into the  
281 peritoneal cavity was enhanced only when *E. faecalis* was opsonized with the IgG  
282 from *Ggta1*<sup>-/-</sup>, but not from *Ggta1*<sup>+/+</sup> mice, as compared to naïve *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice  
283 (Fig. 5A, S5A). This was associated with an increase in the number of peritoneal  
284 neutrophils containing *E. faecalis* (Fig. 5B, S5B,C), probably due to increased, albeit  
285 borderline significant, IgG-dependent phagocytosis (Fig. 5C, S5C). This suggests  
286 that upon bacterial recognition, the relative capacity of IgG NAb from *Ggta1*<sup>-/-</sup> mice to



287 promote bacterial phagocytosis by neutrophils is enhanced, when compared to IgG  
288 from *Ggta1*<sup>+/+</sup> mice. Moreover, this effect acts independently of αGal-recognition in  
289 the targeted bacteria, suggesting that the loss of *GGTA1* function enhances the  
290 effector function of circulating IgG NAb, irrespectively of the epitope recognized.

291 It is well established that the relative composition of the glycan structures, N-  
292 linked to Asn297 of the constant heavy (H) chain of the IgG Fc domain, can have a  
293 considerable impact upon IgG-FcγR binding and downstream IgG effector functions  
294 (Anthony et al., 2012; Dekkers et al., 2017; Wang and Ravetch, 2019). Having  
295 confirmed the presence of αGal in the H chain of circulating IgG NAb from *Ggta1*<sup>+/+</sup>  
296 mice (de Haan et al., 2017), but not from *Ggta1*<sup>-/-</sup> mice (*Fig. 5D,E, Fig. S5D*), we  
297 asked whether αGal modulates IgG effector function in a manner that impacts on  
298 resistance to bacterial sepsis.

299 We found that the relative binding of mouse FcγRIV to IgG NAb from *Ggta1*<sup>-/-</sup> mice  
300 was enhanced, as compared to IgG NAb from *Ggta1*<sup>+/+</sup> mice (*Fig. 5F*). In contrast,  
301 there were no differences in the relative binding of an anti-IgG Fc Ab to IgG from  
302 either *Ggta1*<sup>-/-</sup> or *Ggta1*<sup>+/+</sup> mice in the same assay (*Fig. 5G*). This shows that  
303 increased binding of mouse FcγRIV to IgG from *Ggta1*<sup>-/-</sup> or *Ggta1*<sup>+/+</sup> mice in this  
304 assay is specific. Further strengthening this notion, the relative binding of other  
305 mouse FcγR to IgG NAb from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice was indistinguishable, as  
306 illustrated for FcγRI (*Fig. S5E*), FcγRIIb (*Fig. S5F*), FcγRIII (*Fig. S5G*) and FcRn (*Fig.*  
307 *S5H*). There was also no difference in the relative binding of mouse complement  
308 component 1q (C1q) to IgG from *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> mice (*Fig. 5H*). Overall, this  
309 suggests that when present in the glycan structure associated to the IgG H chain,  
310 αGal hinders IgG Fc recognition by FcγRIV. This is consistent with bacteria-reactive  
311 IgG NAb being exclusively IgG2b (*Fig. 3B*), an IgG sub-class recognized  
312 preferentially by FcγRIV (Nimmerjahn et al., 2010), the mouse orthologue of human  
313 FcγRIIIA (Nimmerjahn et al., 2005). We thus infer that the absence of αGal from the  
314 Fc-glycan structure of IgG enhances the effector function of circulating IgG NAb and  
315 presumably therefore, their protective effect against bacterial sepsis.

316

### 317 **Loss of *Ggta1* function precipitates reproductive senescence**

318 Loss of *GGTA1* function was a sporadic event in mammalian evolution, almost  
319 unique to Old World primates (Galili et al., 1988b; Galili and Swanson, 1991). This  
320 suggests that in most other mammals, even when considering its associated survival  
321 advantage against infection, the loss of *GGTA1* is associated with a major fitness  
322 cost, *i.e.* an evolutionary trade-off (Stearns and Medzhitov, 2015). Consistent with

323 this notion, we observed a marked reduction in the total number of offspring  
324 produced by *Ggta1*<sup>-/-</sup> compared to *Ggta1*<sup>+/+</sup> mice throughout their reproductive  
325 lifespan (*Fig. 6A*). While consistent with a previous report suggesting that αGal  
326 partakes in mammalian reproduction (Bleil and Wassarman, 1988), other studies do  
327 not report a conclusive, physiological role for αGal in this process (Thall et al., 1995).

328 We found that *Ggta1*<sup>-/-</sup> mice produced normal numbers of viable offspring *per*  
329 litter (*Fig. 6B*), which is in keeping with previously published data (Thall et al., 1995).  
330 However, we also found that *Ggta1*<sup>-/-</sup> mice had a reduced cumulative reproductive  
331 output compared to *Ggta1*<sup>+/+</sup> mice due to a reduction in the total number of litters  
332 produced during their reproductive lifespan (*Fig. 6C*). This phenotype had not  
333 previously been reported (Thall et al., 1995).

334 The birth rate as a function of age in *Ggta1*<sup>-/-</sup> females was reduced compared to  
335 that of *Ggta1*<sup>+/+</sup> females (*Fig. 6D*). Age-specific birth rate is used herein to define the  
336 product of: probability of birth at any age, mean number of pups produced *per*  
337 combined litter at any age, and number of combined litters produced *per* breeding  
338 group during its reproductive lifespan. Average age at which *Ggta1*<sup>-/-</sup> females  
339 produced their last viable litter was lower, when compared to *Ggta1*<sup>+/+</sup> females (*Fig.*  
340 *6E*), suggesting that loss of *Ggta1* function precipitates the onset of reproductive  
341 senescence.

342

### 343 **Protection against infection can outweigh the reproductive fitness cost** 344 **associated with loss of *Ggta1***

345 We then estimated the likelihood of *GGTA1* loss-of-function mutations being naturally  
346 selected and fixed in populations when taking into account their associated  
347 reproductive fitness cost. We developed a mathematical model (*See Methods*) that  
348 translates host survival advantage against infection and reproductive cost into a  
349 combined measure of fitness (*i.e.* lifetime reproductive success). This mathematical  
350 model takes into account the natural mortality rate of non-infected mice under  
351 laboratory conditions (Kunstyr and Leuenberger, 1975), and the empirically-observed  
352 survival advantage against infection associated with *Ggta1* deletion (*Fig. 1A*). This  
353 model predicts a marked increase in the probability of survival of *Ggta1*<sup>-/-</sup> compared  
354 to *Ggta1*<sup>+/+</sup> mice, under conditions of constant high exposure to infection (*i.e.*, ≥80%),  
355 if such an advantage is present at any given age (*Fig. 6F*).

356 To test whether such a survival advantage could outweigh the observed trade-off  
357 in reproductive output, we incorporated the reproductive fitness cost associated with  
358 *Ggta1* deletion into this model (*See Methods*). Computing host lifetime reproductive  
359 success as a combined integral of age-specific birth rate, weighted by age-specific

360 survival, we derived a global fitness measure that could be compared across the two  
361 host genotypes. Under defined biological scenarios, the positive fitness effect  
362 calculated from protection from infection over the lifetime outweighed the  
363 reproductive fitness cost, leading to a higher overall fitness (*Fig. 6G*). This is  
364 revealed by a positive selection coefficient ( $s > 0$ ) in some regions of the parameter  
365 space (*Fig. 6H*), indicating superior fitness of hosts carrying *Ggta1* deletion, relative  
366 to wild type. This was only achieved under conditions of high exposure to virulent  
367 pathogens against which the loss of *Ggta1* provides robust protection (*Fig. 6H, Fig.*  
368 *S6A-D*).

369

## 370 **DISCUSSION**

371 Humans carry a *GGTA1* pseudogene, carrying two frame-shift mutations and an  
372 exon deletion, which probably occurred in ancestral apes and Old World primates  
373 about 28 million years ago (Galili et al., 1987; Galili and Swanson, 1991). We  
374 propose that these loss-of-function mutations were naturally selected and fixed in  
375 different populations independently, owed to a robust associated fitness advantage  
376 against the development of sepsis, an often-lethal outcome of infection by different  
377 types of virulent pathogens that remains a major global cause of human morbidity  
378 and mortality (Rudd et al., 2020; Singer et al., 2016).

379 Our findings support the notion that the fitness advantage against infection,  
380 provided by the loss of *GGTA1* function, emerged from the “removal” of  $\alpha$ Gal from  
381 IgG-associated glycan structures, increasing IgG effector function and improving  
382 resistance to bacterial sepsis. This notion is supported by several independent  
383 observations in *Ggta1*-deficient mice, which similar to humans, fail to express  $\alpha$ Gal.  
384 First, *Ggta1*-deficient mice are more resistant to systemic bacterial infections  
385 originating from the gut microbiota and leading to sepsis, when compared to wild  
386 type controls (*Fig. 1,2*). Second, the survival advantage of *Ggta1*-deficient mice  
387 against bacterial sepsis relies on circulating IgG NAb recognizing bacteria in the gut  
388 microbiota (*Fig. 2,3*). Third, circulating IgG NAb confer protection against bacterial  
389 sepsis (*Fig. 3A*) irrespectively of  $\alpha$ Gal recognition (*Fig. 3D-G*). Fourth, bacterial  
390 recognition by circulating IgG NAb from *Ggta1*-deficient mice is indistinguishable  
391 from that of IgG NAb from wild type mice (*Fig. 4*). Fifth, lack of  $\alpha$ Gal in the glycan  
392 structure of IgG NAb from *Ggta1*-deficient mice increases IgG effector function, via a  
393 mechanism associated with enhanced binding to Fc $\gamma$ RIV (*Fig. 5*), the mouse  
394 orthologue of the human Fc $\gamma$ RIIIA (Nimmerjahn et al., 2005), which plays a critical  
395 role in driving IgG effector function (Shields et al., 2002).

396 Under the experimental conditions used in our study, the overwhelming majority of  
397 the circulating IgG NAb recognizing bacteria in the infectious cecal inoculum were  
398 from the IgG2b subclass (*Fig. 3B*). FcγRIV is the main FcγR recognizing IgG2b  
399 (Nimmerjahn et al., 2005), probably explaining why IgG NAb from *Ggta1*-deficient  
400 mice increase their binding specifically towards FcγRIV, but not to other FcγR (*Fig.*  
401 *5*). This does not preclude however, αGal from modulating the binding of different  
402 IgG subclasses to their corresponding FcγR (Anthony et al., 2012; Dekkers et al.,  
403 2017; Wang and Ravetch, 2019).

404 Our findings question to some extent the previous accepted notion that the  
405 evolutionary advantage conferred by the loss of *GGTA1* function was driven  
406 essentially by the emergence of protective immunity against αGal-expressing  
407 pathogens (Soares and Yilmaz, 2016; Takeuchi et al., 1996; Yilmaz et al., 2014).  
408 While this trait was likely naturally selected on the basis of improved resistance  
409 against a number of pathogens that express αGal provided by circulating IgM NAb  
410 (Soares and Yilmaz, 2016; Takeuchi et al., 1996; Yilmaz et al., 2014), the selective  
411 advantage provided by enhancing IgG NAb effector function probably improved  
412 resistance to a larger spectrum of pathogens, irrespectively of αGal recognition.  
413 Presumably, this is equally, if not more, important to explain the evolutionary  
414 advantage conferred by the loss of *GGTA1* function.

415 Despite its significant benefit against infection, the loss of *GGTA1* function is a  
416 sporadic event in mammalian evolution, almost exclusive to Old World primates  
417 (Galili et al., 1988b; Galili and Swanson, 1991). This suggests that the fitness  
418 advantage against infection is linked to a decrease in fitness through a correlated  
419 trait (Stearns, 1989). At the population level, genetic trade-offs are explained by  
420 negative coupling of traits over life history, such that one trait increases fitness early  
421 in life, while another trait is detrimental later on (Williams, 1957). We propose that the  
422 trade-off associated with loss of *GGTA1* function is the emergence of reproductive  
423 senescence late in life (*Fig. 6*). Of note, reproductive senescence is a distinguishing  
424 feature of Old World primates, in which *GGTA1* function is impaired, compared to  
425 New World primates, in which *GGTA1* is functional (Hearn, 1983).

426 We have estimated the likelihood of *GGTA1* loss-of-function mutations being  
427 naturally selected and fixed in populations on the basis of their associated fitness  
428 advantage against infection vs. reproductive fitness cost. A mathematical model that  
429 computes lifetime reproductive success based on host survival advantage against  
430 infection and reproductive cost, suggests that the selective pressure imposed by  
431 sepsis can fix *GGTA1* loss-of-function when both exposure to and virulence of the  
432 pathogen are high (*Fig. 6*). This supports the hypothesis that protection from infection

433 by highly virulent pathogens associated with the development of sepsis might have  
434 led to a “catastrophic-selection” of ancestral primates, whereby mutant offspring  
435 lacking a functional *GGTA1* survived and replaced the parental populations (Galili,  
436 2019).

437 Although the mathematical model used in this study was parameterized by  
438 empirical data obtained from mice maintained under laboratory conditions, its  
439 biological structure speaks to general scenarios, where trade-offs between protection  
440 from infection and reproduction are at play. With such a quantitative framework of  
441 lifetime reproductive success, alternative combinations of functions and parameters  
442 can be integrated to explore selection of traits that simultaneously affect reproduction  
443 and survival. This includes protective immunity against virulent pathogens expressing  
444  $\alpha$ Gal (Soares and Yilmaz, 2016) or possibly female immunity against paternal  $\alpha$ Gal,  
445 as possible contributors to the rapid fixation of *GGTA1* loss-of-function mutations  
446 similar to other human specific loss-of-function mutations, such as in the *CMP-N-*  
447 *acetylneuraminic acid hydroxylase (CMAH)* gene (Ghaderi et al., 2011).

448 An evolutionary implication of our findings is that Old World primates, including  
449 humans, appear to be at a higher risk of developing sepsis in response to bacterial  
450 infection. In strong support of this notion, humans are more susceptible to develop  
451 septic shock when challenged by bacterial lipopolysaccharide (LPS), as compared to  
452 other mammalian lineages (Chen et al., 2019). Whether this can be explained by  
453 intrinsic characteristics of human immunity and/or the capacity to establish disease  
454 tolerance to infection (Martins et al., 2019; Medzhitov et al., 2012), is unclear. This is  
455 consistent however with the idea that, similar to *GGTA1*, humans carry other loss-of-  
456 function mutations associated with enhanced immune resistance to bacterial  
457 infection (Olson, 1999; Wang et al., 2006). Considering that genetic programs driving  
458 resistance and disease tolerance to infection are negatively correlated (Raberg et al.,  
459 2007), mutations increasing resistance might carry, as a trade-off, a reduced  
460 capacity to establish disease tolerance to infection.

461 In conclusion, our findings support the idea that the positive selection of *GGTA1*  
462 loss-of-function mutations in the common ancestor of Old World primates was  
463 propelled by an overall enhancement in IgG effector function, providing resistance  
464 against infection by gut bacteria pathobionts that would otherwise lead to the  
465 development of sepsis. This provided a survival advantage against infection by a  
466 broad range of pathogens, likely outweighing the trade-off imposed by the  
467 emergence of reproductive senescence and lower reproductive output, potentially

468 explaining why loss of *GGTA1* was a rare event, which occurred almost exclusively  
469 in Old World primates, including humans.

470

## 471 **ACKNOWLEDGEMENTS**

472 We thank L. Barreiro (U. Chicago), J. Howard, I. Gordo, K. Xavier, J. Demengeot, L.  
473 Chikhi, S. Ramos (Instituto Gulbenkian de Ciência) for critical review of the  
474 manuscript, M. Zeng (Weill Cornell Medicine) for providing mouse microbiota  
475 bacterial strains, M.E. Conner (Baylor College of Medicine) for the *Iga*-deficient mice,  
476 R. Knight, G. Humphrey and L. Marotz (University of California, San Diego) for help  
477 in bacterial sorting experiments, T. Paixão for data analysis and constructive  
478 reviewing of the mathematical model, K. Xavier and A.R. Oliveira for help in 16S  
479 sequencing, F. Braza, M. Monteiro and V. Martins for Flow Cytometry analyses, P.B.  
480 Amador for help with GF mice and Ab analyses, A. Regalado for IgG purification and  
481 conjugation, A. Vieira, J. Bom and A. Ribeiro (all IGC) for reproductive output data.  
482 S.S. is supported by Fundação para a Ciência e Tecnologia (FCT;  
483 SFRH/BD/52177/2013), J.A.T. by an ESCMID Research Grant and FCT  
484 (SFRH/BPD/112135/2015), S.W. by DFG Excellence Cluster EXS 2051 and CSCC,  
485 Jena University Hospital (BMBF 01EO1502), E.G by FLAD/NSF 274/2016 and  
486 M.P.S. by the Gulbenkian, “La Caixa” (HR18-00502) and Bill & Melinda Gates  
487 (OPP1148170) Foundations and FCT (5723/2014 and FEDER029411).

## 488 **AUTHOR CONTRIBUTIONS**

489 S.S. contributed critically to study design, and performed most experimental work  
490 and data analyses. J.A.T. contributed to study design, performed experimental work  
491 and data analyses. S.W. made the original observation that loss of *Ggta1* increases  
492 resistance to sepsis with B.Y. D.S. and M.T. performed 16S rRNA sequencing  
493 analysis. B.Y., S.R. and S.C. generated mouse strains, maintained breeding colonies  
494 and gathered reproductive output data with S.S. E.G. performed mathematical  
495 modeling for integrated fitness over lifespan. G.N. provided mouse microbiota  
496 bacterial strains and contributed intellectually. M.P.S. drove the study design and  
497 wrote the manuscript with S.S., with contributions from J.A.T.

## 498 **DECLARATION OF INTERESTS**

499 The authors declare no competing interests.

500 **MAIN FIGURE TITLES AND LEGENDS**

501 **Figure 1. Loss of *Ggta1* function in mice enhances resistance to systemic**  
502 **infection by symbiotic gut bacteria.**

503 **A)** Survival after CLP of *Ggta1*<sup>+/+</sup> (n=56) and *Ggta1*<sup>-/-</sup> (n=68) mice; 19 experiments.  
504 **B)** Colony forming units (CFU) of aerobic (Ae) and anaerobic (An) bacteria recovered  
505 from *Ggta1*<sup>+/+</sup> (n=5-15) and *Ggta1*<sup>-/-</sup> (n=6-15) mice, 24 hours after CLP; 11  
506 experiments. Principal Coordinate Analysis (PCoA) of **C)** Unweighted UniFrac  
507 distance and **D)** Weighted UniFrac distance of 16S rRNA amplicons, from fecal  
508 samples of *Ggta1*<sup>+/+</sup> (n=15) and *Ggta1*<sup>-/-</sup> (n=14) mice. **E)** Cladograms, from LEfSe  
509 analysis, representing taxa enriched in *Ggta1*<sup>+/+</sup> (green) or *Ggta1*<sup>-/-</sup> (red) mice in the  
510 same samples as (A-B). a: family\_Porphyrromonadaceae, b: genus\_  
511 Parabacteroides, c: species\_*Bacteroides acidifaciens*, d: species\_*B. ovatus*; 1  
512 experiment. **F)** Breeding strategy for the generation of F<sub>2</sub> *Ggta1*<sup>+/+</sup> vs. *Ggta1*<sup>-/-</sup>  
513 littermates with similar microbiota, maternally derived from *Ggta1*<sup>-/-</sup> mice. Microbiota  
514 PCoA of **G)** Unweighted UniFrac distance and **H)** Weighted UniFrac distance of 16S  
515 rRNA amplicons, in fecal samples from F<sub>2</sub> *Ggta1*<sup>+/+</sup> (n=22) and *Ggta1*<sup>-/-</sup> (n=18) mice  
516 generated as described in (F); 1 experiment. **I)** Survival after CLP of F<sub>2</sub> *Ggta1*<sup>+/+</sup>  
517 (n=12) and *Ggta1*<sup>-/-</sup> (n=10) mice; 3 experiments. **J)** CFU of Ae and An bacteria of F<sub>2</sub>  
518 *Ggta1*<sup>+/+</sup> (n=5-11) and *Ggta1*<sup>-/-</sup> (n=4-7) mice, 24 hours after CLP; 3 experiments.  
519 Symbols in (B, C, D, G, H, J) are individual mice. Red bars (B, J) show median  
520 values. P values in (A, I) calculated using log-rank test, in (C, D, G, H) using  
521 PERMANOVA test and in (B, J) using Mann-Whitney test. Peritoneal cavity (PC). \*P  
522 < 0.05; \*\*P < 0.01; \*\*\*P < 0.001; \*\*\*\*P < 0.0001.

523

524 **Figure 2. Loss of *Ggta1* function in mice enhances IgG-mediated resistance to**  
525 **systemic polymicrobial infection.**

526 **A)** Survival of *Ggta1*<sup>+/+</sup> (n=16) and *Ggta1*<sup>-/-</sup> (n=14) mice infected (*i.p.*) with a cecal  
527 inoculum from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice; 4 experiments. **B)** Colony forming units (CFU) of  
528 aerobic (Ae) and anaerobic (An) bacteria of *Ggta1*<sup>+/+</sup> (n=6) and *Ggta1*<sup>-/-</sup> (n=4) mice,  
529 24 hours after infection; 2 experiments. **C)** Survival of *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=15) and  
530 *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=13) mice infected as in (A); 3 experiments. **D)** CFU of Ae and An  
531 bacteria of *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=5-6) and *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=4) mice, 24 hours after  
532 infection; 3 experiments. **E)** Survival of *J<sub>h</sub>t*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=12) and *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=19)  
533 mice infected as in (A); 4 experiments. **F)** CFU of Ae and An bacteria of *J<sub>h</sub>t*<sup>+/+</sup>*Ggta1*<sup>-/-</sup>  
534 (n=4-6) and *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=7-11) mice, 24 hours after infection; 4 experiments. **G)**

535 Survival of *Aid*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=10) and *Aid*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=16) mice infected as in (A); 3  
536 experiments. **H**) CFU of Ae and An bacteria of *Aid*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=5) and *Aid*<sup>-/-</sup>*Ggta1*<sup>-/-</sup>  
537 (n=6-7) mice, 24 hours after infection; 4 experiments. Symbols (B, D, F, H) are  
538 individual mice. Red bars (B, D, F, H) are median values. P values in (A, C, E, G)  
539 calculated with log-rank test and in (B, D, F, H) with Mann-Whitney test. Peritoneal  
540 cavity (PC). \*P < 0.05; \*\*P < 0.01; ns: not significant.

541

542 **Figure 3. The protective effect of IgG NAb acts irrespectively of αGal**  
543 **recognition.**

544 **A**) Survival of *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice after transfer of IgG purified from *Ggta1*<sup>-/-</sup> mice  
545 (n=15), *Ggta1*<sup>+/+</sup> mice (n=10) or vehicle (PBS; n=6), 24 hours before infection (*i.p.*)  
546 with a cecal inoculum from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice; 4 experiments. **B**) Relative  
547 absorbance of IgG sub-classes in the serum of *Ggta1*<sup>+/+</sup> (n=11) and *Ggta1*<sup>-/-</sup> (n=11)  
548 mice, binding to cecal extract from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice, determined by ELISA;  
549 representative of 3 experiments. **C**) Concentration of total IgG2b and anti-αGal IgG2b  
550 in IgG from *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mice (n=3). **D**) Representative plots showing *Rag2*<sup>-/-</sup>  
551 *Ggta1*<sup>-/-</sup> cecal bacteria co-stained with IgG from *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mice and BSI-B4  
552 lectin (αGal). **E**) Quantification of IgG<sup>+</sup>αGal<sup>+</sup> bacteria in the same samples as in (C)  
553 (n=3); 3 experiments. **F**) Percentage of *in vitro*-grown bacteria isolated from the  
554 mouse microbiota bound by IgG purified *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup> mice. Data shown was  
555 pooled from N=3-4 independent experiments. **G**) Quantification of αGal expression  
556 by *in vitro*-grown species of bacteria as indicated; 3 independent experiments.  
557 Symbols in (B) are individual mice and in (C, E) are independent IgG preparations.  
558 Red lines in (B, C, E) are mean values. Error bars in (B, C, E, F, G) correspond to  
559 SD. P values in (A) calculated using log-rank test, in (B, E) using Mann-Whitney test  
560 and in (C, F) by 2-way ANOVA using Sidak's multiple comparison test. \*P < 0.05; \*\*P  
561 < 0.01; ns: not significant.

562

563 **Figure 4. Loss of *Ggta1* function does not alter bacterial recognition by IgG**  
564 **NAb.**

565 **A**) Percentage of IgG<sup>+</sup> bacteria in the *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> cecal content, after incubation  
566 with serum from *Ggta1*<sup>+/+</sup> (n=14) and *Ggta1*<sup>-/-</sup> (n=15) mice; 3 experiments. **B**)  
567 Percentage of IgG<sup>+</sup> bacteria in the peritoneal lavage of *Ggta1*<sup>+/+</sup> (n=9) and *Ggta1*<sup>-/-</sup>  
568 (n=14) mice, 3 hours after infection (*i.p.*) with a cecal inoculum from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup>  
569 mice; 3 experiments. **C**) Representative plot showing *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> cecal bacteria  
570 co-stained with IgG from *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup> mice. **D**) Percentage of double



571 positive (DP) vs. single positive (SP) bacteria among all IgG<sup>+</sup> bacteria in the same  
572 samples as in (C) (n=11); 6 experiments. **E-H**) Principal Coordinates Analysis of **E**)  
573 Unweighted UniFrac and **F**) Weighted UniFrac distance of IgG<sup>+</sup> bacteria and **G**)  
574 Unweighted UniFrac and **H**) Weighted UniFrac distance of IgG<sup>-</sup> bacteria in the  
575 peritoneal lavage of *Ggta1*<sup>+/+</sup> (n=10) and *Ggta1*<sup>-/-</sup> (n=10) mice, 3 hours after infection  
576 with a cecal inoculum from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice; 2 experiments. Symbols in (A, B, E,  
577 F, G, H) are individual mice and in (D) are independent *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> cecal  
578 preparations. Red lines (A, B, D) are mean values. Error bars in (A, B, D) correspond  
579 to SD. P values in (A, B) calculated using Mann-Whitney test and in (E-H) using  
580 PERMANOVA test. ns: not significant.

581

582 **Figure 5. Loss of αGal expression enhances IgG effector function.**

583 **A)** Total number of infiltrating CD11b<sup>+</sup>Ly6G<sup>high</sup> neutrophils recovered from the  
584 peritoneal cavity of *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice, either naive (dark gray circles; n=3) or 3 hours  
585 after injection of *E. faecalis*, unopsonized (light gray circles; n=2), or opsonized with  
586 IgG from *Ggta1*<sup>+/+</sup> (white circles; n=3) or *Ggta1*<sup>-/-</sup> (black circles; n=3) mice; 1  
587 experiment. **B)** Total number of bacteria-containing neutrophils in the same samples  
588 as in (A). **C)** IgG-dependent phagocytosis index calculated as a ratio of bacteria-  
589 containing neutrophils in IgG-opsonized groups over the unopsonized group, in the  
590 same samples as in (A). **D-E)** Detection of αGal in IgG from *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup>  
591 mice, using **D)** BSI-B4 lectin and **E)** Anti-αGal M86 mAb. SDS gel is shown as  
592 loading control. Representative of 6 independent IgG preparations. **F)** Relative  
593 binding to FcγRIV and **G)** Anti-IgG by *Ggta1*<sup>+/+</sup> (n=6) and *Ggta1*<sup>-/-</sup> (n=6) purified IgG.  
594 **H)** Relative binding to mouse C1q by *Ggta1*<sup>+/+</sup> (n=7) and *Ggta1*<sup>-/-</sup> (n=3) purified IgG,  
595 where n corresponds to independent IgG preparations. Data is representative of 1-3  
596 independent experiments. Symbols in (A, B, C) are individual mice. Red lines (A, B,  
597 C) are mean values. Error bars in (A, B, C) correspond to SD and in (F, G, H) to  
598 SEM. P values in (A) calculated using a one-tailed Kruskal-Wallis test with Dunn's  
599 correction for multiple comparisons, in (B, C) using Mann-Whitney test and in (F-H)  
600 using 2-Way ANOVA with Sidak's multiple comparison test. \*P < 0.05; ns: not  
601 significant.

602

603 **Figure 6. The increase in fitness provided by the survival advantage against**  
604 **infection can outweigh the reproductive fitness cost associated with loss of**  
605 ***Ggta1*.**

606 **A)** Cumulative number of offspring (pups) produced over the reproductive lifespan of  
607 *Ggta1*<sup>+/+</sup> (n=432) and *Ggta1*<sup>-/-</sup> (n=135) trio breeding groups, over a period of five

608 years. **B)** Number of offspring at the time of weaning *per* combined litter from the  
609 same trio breeding groups as in (A). **C)** Number of combined litters produced over  
610 the reproductive lifespan of the same trio breeding groups as in (A). **D)** Birth rate as a  
611 function of age of females in the same trio breeding groups as in (A). **E)** Age of  
612 females at the time of the last living combined litter. **F)** Survival probability of non-  
613 infected vs. infected *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice. Dashed gray line depicts a scenario  
614 assuming high and constant exposure to infection (i.e. 80% probability at any age).  
615 **G)** Overall fitness of *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> mice, under conditions of no exposure or  
616 high exposure to infection. The reproductive cost of *Ggta1* deletion and survival  
617 advantage upon infection calculated in (F) is multiplied with the birth rate functions  
618 calculated in (D). **H)** Contour plot in which lifetime exposure to infection (*E*), assumed  
619 as constant over age, is varied along the x-axis and the magnitude of protection in  
620 *Ggta1*<sup>-/-</sup> relative to *Ggta1*<sup>+/+</sup> (*p*) is varied along the y-axis. The contour plot maps  
621 combinations of *E* and *p* to theoretical model-predictions for the fitness ratio (>1  
622 favoring *Ggta1*<sup>-/-</sup>, and <1 favoring *Ggta1*<sup>+/+</sup>). Black line indicating fitness ratio=1 sets  
623 the threshold for positive selective advantage (*s*>0) of *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> genotype.  
624 Red lines depict a scenario in which loss of *Ggta1*, conferring protection *p* of 64%,  
625 against highly virulent pathogens (*v*= 154) reaches a selective advantage (*s*) of 27%,  
626 despite the fitness cost shown in (D), when exposure is high (*E*=80%). Error bars in  
627 (A, B, C and E) correspond to mean ± SD. P values in (A, B, C and E) calculated with  
628 Mann-Whitney test. \*\*\*\*P < 0.0001, ns: not significant.

629

630

631 **STAR METHODS**

632 Detailed methods are provided in the online version of this paper and include the  
633 following:

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663 **SUPPLEMENTAL INFORMATION**

664 Supplemental Information includes six figures and can be found with this article.

665

## 666 CONTACT FOR REAGENTS AND RESOURCE SHARING

667 Further information and requests for reagents may be directed to, and will be fulfilled  
668 by, the lead contact, Miguel P. Soares ([mpsoares@igc.gulbenkian.pt](mailto:mpsoares@igc.gulbenkian.pt)).

669

## 670 EXPERIMENTAL MODEL AND SUBJECT DETAILS

### 671 Mice

672 Mice were used in accordance with protocols approved by the Ethics Committee of  
673 the Instituto Gulbenkian de Ciência (IGC) and Direção Geral de Alimentação e  
674 Veterinária (DGAV), following the Portuguese (Decreto-Lei no. 113/2013) and  
675 European (directive 2010/63/EU) legislation for animal housing, husbandry and  
676 welfare. C57BL/6J wild-type, *Ggta1*<sup>-/-</sup> (Tearle et al., 1996), *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (Gu et al.,  
677 1993), *Tcrβ*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (Yilmaz et al., 2014), *Aid*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (Yilmaz et al., 2014) and *μs*<sup>-/-</sup>  
678 *Ggta1*<sup>-/-</sup> (Yilmaz et al., 2014) mice were used. *Iga*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> and *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice  
679 were generated by crossing *Ggta1*<sup>-/-</sup> (Tearle et al., 1996) with C57BL/6 *Iga*<sup>-/-</sup> (Blutt et  
680 al., 2012) and *Rag2*<sup>-/-</sup> (Shinkai et al., 1992) mice, respectively. Mice were bred and  
681 maintained under specific pathogen-free (SPF) conditions (12 h day/night, fed *ad*  
682 *libitum*), as described (Yilmaz et al., 2014).

683 Germ-free (GF) C57BL/6J *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup> animals were bred and raised in  
684 the IGC gnotobiology facility in axenic isolators (La Calhene/ORM), as described  
685 (Yilmaz et al., 2014). Adult mice were transferred to sterile ISOcages (Tecniplast)  
686 and sterility of food, water, bedding, oral swab and feces were confirmed before each  
687 experiment by plating samples on Sabouraud Glucose Agar (BD #254039) for fungi,  
688 or Trypticase™ Soy Agar II plates with 5% Sheep Blood (BD #254053) for bacteria,  
689 and incubated (37°C, 5 days) in air with 5% CO<sub>2</sub> for aerobes and in air tight  
690 containers equipped with GasPak™ anaerobe container system (BD #11747194) for  
691 anaerobes. Anaerobic conditions were confirmed using BBL™ Dry Anaerobic  
692 Indicator Strips (Becton Dickinson #271051). Samples were added to Difco™  
693 Nutrient Broth medium (#234000), incubated (37°C, 5 days) and plated (~500  
694 μL/plate) on Sabouraud Glucose Agar and Trypticase™ Soy Agar II plates with 5%  
695 Sheep Blood and incubated (37°C, 5 days) under aerobic and anaerobic conditions.  
696 Plates and liquid medium were checked for absence of fungal and bacterial growth.  
697 Both male and female mice were used for all experiments. All animals were studied  
698 between 9-16 weeks of age unless otherwise indicated.

699

## 700 **Cecal Ligation and Puncture (CLP)**

701 CLP was performed as described (Rittirsch et al., 2009). Procedures were performed  
702 routinely at the same time of the day, starting at 9 am. Briefly, mice were  
703 anaesthetized (intraperitoneal, *i.p.*) using ketamine (75 mg/kg) and xylazine (15  
704 mg/kg) (~140  $\mu$ L/mouse, 1:1 vol/vol in sterile 0.9% saline). The lower left quadrant of  
705 the abdomen was disinfected with Betadine® solution. Under aseptic conditions, a 1-  
706 2 cm lower left quadrant laparotomy was performed and the cecum with the adjoining  
707 intestine was exposed. 20-30% of the cecum below the ileo-cecal valve, was tightly  
708 ligated with a silk suture (3-0 Mersilk #W212) and perforated twice (23G needle). The  
709 cecum was then gently squeezed to extrude a small amount of cecal content from  
710 the perforation sites, returned to the peritoneal cavity and the abdomen was closed  
711 with silk sutures (Virgin Silk #C0761214). The skin was closed with Reflex 9 mm clips  
712 (Cellpoint Scientific #201-1000). Mice were resuscitated by injecting 800  $\mu$ L (mice <  
713 25 g) or 1 mL (mice > 25 g) of sterile 0.9% saline solution (subcutaneous, 25G  
714 needle). Mice were placed on a heating pad (30 min. - 2 h) until recovery from  
715 anesthesia and provided with free access to food and water by placing hydrogel or  
716 food pellets on the bottom of the cage. Mice were monitored every 12 h for survival  
717 for 14 days or euthanized at various time points for analysis of different parameters.

## 718 **Cecal Slurry Injection**

719 Under aseptic conditions, 3-5 donor mice were sacrificed, and a 1-2 cm lower left  
720 quadrant laparotomy performed. The cecum was excised, contents extracted, pooled  
721 in pre-weighed sterile Eppendorf tubes and kept on ice. The cecal contents were  
722 weighed and homogenized in sterile PBS by vortexing (maximum speed, 1-5 min.).  
723 The resulting cecal slurry was filtered (BD Falcon™, 40  $\mu$ m cell strainer, # 352340)  
724 and injected to recipient mice (*i.p.* 1-1.25 mg/g body weight, 25G needle). Mice were  
725 monitored every 12 h for survival for 14 days or euthanized at various time points for  
726 analysis of different parameters.

727 To analyze mouse survival when exposed to killed cecal content, cecal slurry  
728 from *Rag2<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice was prepared as above, pelleted by centrifugation (4,000  
729 rpm, 4°C, 20 min.), supernatant discarded and material re-suspended in  
730 paraformaldehyde (PFA, 4% weight/vol in PBS). Fixation was left to proceed  
731 overnight, before centrifugation as above, and washing (2x, PBS). A lack of viable  
732 bacteria in the inoculum was confirmed by plating undiluted content on  
733 Trypticase™ Soy Agar II plates with 5% Sheep Blood (BD #254053) and incubating at  
734 37°C under anaerobic conditions for 3 days. Fixed cecal material was injected to

735 mice (*i.p.* 1.25 mg/g body weight, 25G needle) and survival was monitored every 12  
736 h for 14 days.

### 737 **Breeding experiments**

738 Segregation of *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup> genotypes carrying a similar microbiota derived  
739 from *Ggta1*<sup>-/-</sup> mice was achieved, as described (Ubeda et al., 2012). Briefly, two or  
740 more breeding pairs were established, consisting of two *Ggta1*<sup>-/-</sup> females and one  
741 *Ggta1*<sup>+/+</sup> male per cage. The male was removed after one week and the females  
742 were placed in a clean cage until delivery. F<sub>1</sub> *Ggta1*<sup>+/-</sup> pups were weaned at 3-4  
743 weeks of age and then co-housed until 8 weeks of age. F<sub>1</sub> *Ggta1*<sup>+/-</sup> breeding pairs  
744 were established randomly using one male and two females per cage. F<sub>2</sub> pups were  
745 weaned at 3-4 weeks of age, genotyped and segregated according to their *Ggta1*<sup>-/-</sup>  
746 vs. *Ggta1*<sup>+/+</sup> genotype in separate cages until adulthood. Fecal pellets were collected  
747 (10-12 weeks of age) for microbiota analysis.

### 748 **Reproductive output**

749 Breeding of *Ggta1*<sup>+/+</sup> and *Ggta1*<sup>-/-</sup> mice was performed under SPF conditions using  
750 trio breeding groups, composed of two females *per male per cage*. Breeding was  
751 established when mice reached 8-10 weeks of age. A total of n=432 of *Ggta1*<sup>+/+</sup> and  
752 n=135 of *Ggta1*<sup>-/-</sup> trio breeding groups were analyzed over a period of 5 years,  
753 spanning from 2012 to 2017. Breeding was monitored for: i) number of pups  
754 produced over the reproductive life-span of each breeding group, ii) number of pups  
755 per combined litter, whereby combined litter refers to the pool of offspring *per*  
756 breeding group, as accounted for at the time of weaning, iii) number of combined  
757 litters produced over the reproductive life-span of each breeding group and iv)  
758 reproductive senescence, as defined by the age at which females produced the last  
759 live combined litters. Breeding was followed until 2 months after the last viable litter.  
760 Pups were weaned at 3-4 weeks of age. Detection of dead progenitors and/or dead  
761 litters was a criterion for exclusion of the breeding group from the analysis.

762

## 763 **METHOD DETAILS**

### 764 **Genotyping**

765 Mice were genotyped from tail biopsies (0.5-1 cm) by PCR of genomic DNA using a  
766 standard protocol, as *per* manufacturer's protocols (KAPA mouse genotyping kit  
767 #KK7352). Samples were lysed in KAPA Express Extract Enzyme (1 µL), KAPA  
768 Express Extract Buffer (5 µL) and water (44 µL), heated (75°C, 15 min., and 95°C, 5  
769 min.), vortexed (3 sec.), centrifuged (16,000 g, 1 min., room temperature (RT)),  
770 diluted in water (1:4 vol/vol) and centrifuged (16,000 g, 1 min., RT). Extracted DNA (1

771  $\mu\text{L}$ ) was amplified in the PCR mix consisting of 2x KAPA2G Fast Genotyping Mix (5  
772  $\mu\text{L}$ ), each primer (0.5  $\mu\text{L}$ ) and water (2-2.5  $\mu\text{L}$ ). Visualization of PCR products was  
773 done on a 1-2% agarose gel (80-100 V, 1-2 h).

#### 774 **Pathogen Load**

775 Mice were sacrificed 24 h after CLP or cecal slurry injection, placed in a sterile  
776 surgical field and sprayed thoroughly with ethanol. A 5x5 cm window was created on  
777 the abdomen by excising the skin. Ice-cold sterile PBS was injected (*i.p.* 5 mL, 25G  
778 needle). The mouse was shaken vigorously (5x horizontally and vertically) to  
779 homogenize the peritoneal fluid and the peritoneal lavage was collected (3-4 mL,  
780 23G needle) and kept on ice. The abdominal and thoracic cavities were opened and  
781 blood was collected by intra-cardiac puncture through a 25G needle into a  
782 heparinized syringe. Mice were perfused (25 mL of ice-cold sterile PBS) through the  
783 left ventricle of the heart. The right atrium was cut after confirming blanching of the  
784 liver. Whole organs (*i.e.* lungs, liver, spleen and kidneys) were harvested, rinsed with  
785 sterile water and kept on ice in sterile Eppendorf tubes. Organs were homogenized  
786 under sterile conditions (2 mL dounce tissue grinder, Sigma #D8938-1SET) in 500  $\mu\text{L}$   
787 (lungs, kidneys and spleen) or 1 mL (liver) PBS. Serial dilutions were plated on  
788 Trypticase™ Soy Agar II plates with 5% Sheep Blood (BD #254053) and incubated  
789 (37°C) in air with 5% CO<sub>2</sub> for aerobes and in air tight containers equipped with  
790 GasPak™ anaerobe container system (Becton Dickinson #11747194) for anaerobes.  
791 Anaerobic conditions were confirmed using BBL™ Dry Anaerobic Indicator Strips  
792 (BD #271051). Colonies were counted after 24 h and quantified.

#### 793 **Serum collection**

794 Blood was collected from the submandibular vein of live mice (8-9 drops per mouse)  
795 or alternatively via intra-cardiac puncture of terminal mice. Coagulation was allowed  
796 to occur (1 h, RT), samples were centrifuged (2x, 2,000 g, 10 min., 4°C) and the  
797 supernatant was collected and stored at -20°C until use.

#### 798 **IgG purification**

799 IgG purification was performed using HiTrap™ Protein G HP (GE Healthcare #17-  
800 0404-01) according to manufacturer's instructions, with modifications. Briefly, serum  
801 was pooled from 20-30 *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mice, diluted in binding buffer (1:10  
802 vol/vol, Tris 20 mM, pH 8.0, 150 mM NaCl) and filtered (PALL Lifesciences #4612,  
803 0.2  $\mu\text{M}$ ). Samples were passed through the column (1 mL/min.) using a peristaltic  
804 pump (Gilson minipuls3) and the flow-through collected, followed by elution with  
805 elution buffer (100 mM glycine-HCl, pH 2.0, 1 mL/min.). The pH of the eluate (1 mL  
806 per Eppendorf tube) was neutralized (125  $\mu\text{L}$  of Tris 1M, pH 9.0). The initial flow-

807 through was passed through the column again and the process repeated whenever  
808 necessary. IgG concentration was initially measured by a spectrophotometer  
809 (NanoDrop™ 2000/2000C) and IgG fractions were pooled, dialyzed against PBS and  
810 concentrated (Amicon Ultra15 #UFC903024). Quality control was carried out by  
811 SDS-PAGE and the final IgG concentration was determined by ELISA, as described  
812 below.

### 813 **IgG conjugation**

814 Purified mouse IgG was labeled with either PE/Cy5® (Abcam #ab102893) or  
815 AlexaFluor®594 (Molecular Probes #A10239), according to the manufacturer's  
816 instructions. Concentration of the labeled IgG was determined by ELISA, as  
817 described below.

### 818 **Passive transfer of IgG**

819 Purified mouse IgG (300 µg in 240 µL PBS) was injected (*i.p.*) to *Rag2<sup>-/-</sup>Ggta1<sup>-/-</sup>* mice,  
820 24 h before cecal slurry injection.

### 821 **ELISA**

822 ELISA for IgG binding to cecal extracts was done, essentially as described (Zeng et  
823 al., 2016). Briefly, cecal content from 3-5 mice was collected, pooled and weighed in  
824 sterile pre-weighed Eppendorf tubes. The cecal content was then homogenized in  
825 sterile PBS by vortexing (maximum speed, 5 min., RT) and filtered (BD Falcon™, 40  
826 µm cell strainer # 352340). Larger debris were removed by centrifugation (1000 rpm,  
827 5 min., RT), the supernatant was collected and bacteria were pelleted by  
828 centrifugation (8,000 g, 5 min., RT). The pellets were washed in PBS (2x, 10,000 g, 1  
829 min., RT) and re-suspended in PBS (500 µL). Bacteria were heat-killed (85°C, 1 h)  
830 and suspended in Carbonate-Bicarbonate buffer (0.5 M, pH 9.5, 50 µL/mg),  
831 producing a cecal lysate.

832 96-well ELISA plates (Nunc MaxiSorp #442404) were coated with the cecal  
833 lysate (100 µL/well, 4°C, overnight), washed (3x, PBS 0.05% Tween-20, Sigma-  
834 Aldrich #P7949-500ML), blocked (200 µL, PBS 1% BSA wt/vol, Calbiochem #12659-  
835 100GM, 3 h, RT) and washed (3x, PBS 0.05% Tween-20). Plates were incubated  
836 with serially diluted (50 µL) mouse sera (PBS 1% BSA, wt/vol), starting at 1:20  
837 (vol/vol) for detection of IgG1, IgG2c and IgG3 and at 1:200 (vol/vol) for detection of  
838 IgG2b (2 h, RT) and washed (5x, PBS/0.05% Tween-20). Immunoglobulins were  
839 detected using horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG1  
840 (Southern Biotech #1070-05), IgG2b (Southern Biotech #1090-05), IgG2c (Southern  
841 Biotech #1079-05) or IgG3 (Southern Biotech #1100-05) in PBS/1%BSA/0.01%



842 Tween-20 (100  $\mu$ L, 1:4,000 vol/vol, 1 h, RT) and plates were washed (5x, PBS/0.05%  
843 Tween-20).

844 For quantification of total serum IgG, 96-well ELISA plates (Nunc MaxiSorp  
845 #442404) were coated with goat anti-mouse IgG (Southern Biotech #1030-01, 2  
846  $\mu$ g/mL in Carbonate-Bicarbonate buffer, 100  $\mu$ L/well, overnight, 4°C), washed (3x,  
847 PBS 0.05% Tween-20, Sigma-Aldrich #P7949-500ML), blocked (200  $\mu$ L, PBS 1%  
848 BSA wt/vol, 2 h, RT) and washed (3x, PBS 0.05% Tween-20). Plates were incubated  
849 with serially diluted mouse sera (50  $\mu$ L, PBS 1% BSA, wt/vol, 2 h, RT) and standard  
850 mouse IgG (Southern Biotech #0107-10, prepared in duplicates) and washed (5x,  
851 PBS/0.05% Tween-20). IgG was detected using HRP-conjugated goat anti-mouse  
852 IgG (Southern Biotech #1030-05) in PBS/1%BSA/0.01% Tween-20 (100  $\mu$ L, 1:4,000  
853 vol/vol, 1 h, RT) and plates were washed (5x, PBS/0.05% Tween-20).

854 For quantification of anti- $\alpha$ Gal IgG, 96-well ELISA plates (Nunc MaxiSorp  
855 #442404) were coated with either  $\alpha$ Gal-BSA (Dextra) or goat anti-mouse Ig(H+L)  
856 (Southern Biotech #1010-01) (5  $\mu$ g/mL in Carbonate-Bicarbonate buffer, 50  $\mu$ L/well,  
857 overnight, 4°C). Wells were washed (3x, PBS 0.05% Tween-20, Sigma-Aldrich  
858 #P7949-500ML), blocked (200  $\mu$ L, PBS 1% BSA wt/vol, 2 h, RT) and washed (3x,  
859 PBS 0.05% Tween-20). Plates were incubated with serially diluted IgG purified from  
860 *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mice (50  $\mu$ L, PBS 1% BSA, wt/vol, 2 h, RT) and standard mouse  
861 anti- $\alpha$ Gal IgG2b (derived from GT6-27 (Ding et al., 2008) and washed (5x,  
862 PBS/0.05% Tween-20). IgG was detected using HRP-conjugated goat anti-mouse  
863 IgG2b (Southern Biotech #1090-05) in PBS/1%BSA (100  $\mu$ L, 1:4000 vol/vol, 1.5 h,  
864 RT) and plates were washed (5x, PBS/0.05% Tween-20).

865 For IgG binding to Fc $\gamma$ Rs, 96-well ELISA plates (Nunc MaxiSorp #442404) were  
866 coated with purified IgG (5  $\mu$ g/mL in Carbonate-Bicarbonate buffer, 50  $\mu$ L/well,  
867 overnight, 4°C), washed (3x, PBS 0.05% Tween-20, Sigma-Aldrich #P7949-500ML),  
868 blocked (200  $\mu$ L, PBS 1% BSA wt/vol, 2 h, RT) and washed (3x, PBS 0.05% Tween-  
869 20). Plates were incubated with serially diluted biotinylated mouse Fc $\gamma$ RI  
870 (Acrobiosystems #CD4-M5227), Fc $\gamma$ RIIb (Acrobiosystems #CDB-M82E8), Fc $\gamma$ RIII  
871 (Acrobiosystems #CDA-M52H8), Fc $\gamma$ RIV (Acrobiosystems #FC4-M82E8) or FcRn  
872 (Acrobiosystems #FCM-M82W5) (50  $\mu$ L, PBS 1% BSA, wt/vol, 2 h, RT) and washed  
873 (5x, PBS/0.05% Tween-20). Signal was detected using HRP-conjugated Streptavidin  
874 in PBS/1%BSA/0.01% Tween-20 (100  $\mu$ L, 1:2,000 vol/vol, 1 h, RT) and plates were  
875 washed (5x, PBS/0.05% Tween-20).

876 For IgG binding to C1q, 96-well ELISA plates (Nunc MaxiSorp #442404) were  
877 coated with purified IgG (5  $\mu$ g/mL in Carbonate-Bicarbonate buffer, 50  $\mu$ L/well,  
878 overnight, 4°C), washed (3x, PBS 0.05% Tween-20, Sigma-Aldrich #P7949-500ML),

879 blocked (200  $\mu$ L, PBS 1% BSA wt/vol, 2 h, RT) and washed (3x, PBS 0.05% Tween-  
880 20). Plates were incubated with serially diluted mouse C1q (CompTech #M099),  
881 washed (5x, PBS/0.05% Tween-20), incubated with biotinylated anti-mouse C1q  
882 (Cedarlane #CL7501B, 1:50,000 vol/vol, PBS/1%BSA/0.01% Tween-20, 1 h, RT)  
883 and washed (5x, PBS/0.05% Tween-20). Signal was detected using HRP-conjugated  
884 Streptavidin in PBS/1%BSA/0.01% Tween-20 (100  $\mu$ L, 1:2,000 vol/vol, 1 h, RT) and  
885 plates were washed (5x, PBS/0.05% Tween-20).

886 To control for IgG binding, 96-well ELISA plates (Nunc MaxiSorp #442404) were  
887 coated with purified IgG (5  $\mu$ g/mL in Carbonate-Bicarbonate buffer, 50  $\mu$ L/well,  
888 overnight, 4°C), washed (3x, PBS 0.05% Tween-20, Sigma-Aldrich #P7949-500ML),  
889 blocked (200  $\mu$ L, PBS 1% BSA wt/vol, 2 h, RT) and washed (3x, PBS 0.05% Tween-  
890 20). Plates were incubated with serially diluted HRP-conjugated goat anti-mouse IgG  
891 (Southern Biotech #1030-05) in PBS/1%BSA/0.01% Tween-20 (100  $\mu$ L, 1 h, RT) and  
892 washed (5x, PBS/0.05% Tween-20).

893 HRP activity was detected with 3,3',5,5'-Tetramethylbenzidine (TMB) Substrate  
894 Reagent (BD Biosciences #555214, 50  $\mu$ L, 20-25 min., dark, RT) and the reaction  
895 was stopped using sulfuric acid (2N, 50  $\mu$ L). Optical densities (OD) were measured  
896 using a MultiSkan Go spectrophotometer (ThermoFisher) at  $\lambda$ =450 nm and  
897 normalized by subtracting background OD values ( $\lambda$ = 600 nm). Concentrations of  
898 IgG2b in purified serum IgG samples were calculated from the absorbance obtained  
899 with reference to the standard curve determined for total and  $\alpha$ Gal-specific IgG2b,  
900 respectively.

#### 901 **Western blot for detection of $\alpha$ Gal**

902 Purified IgG, BSA conjugated to  $\alpha$ Gal (Dextra # NGP1334) and unconjugated BSA  
903 (New England Biolabs #174B9000S) were denatured in Laemmli buffer (1%  $\beta$ -  
904 mercaptoethanol, 2% SDS, 70°C, 10 min.) and separated by SDS-PAGE (12%  
905 acrylamide/bisacrylamide gel, 29:1; 100V; 2 h). Proteins were transferred onto a  
906 PVDF membrane (50 min, 12 V), blocked (5% BSA in 20 mM Tris/HCl pH 7.5, 150  
907 mM NaCl and 0.1% Tween-20 or TBST buffer, 2 h) and incubated (4°C, overnight)  
908 with biotinylated BSI-B4 lectin from *Bandeiraea (Griffonia) simplicifolia* (Sigma-  
909 Aldrich, #L3759-1MG, 1 mg/mL, 5% BSA in TBST buffer, 5 mL) or with Anti- $\alpha$ Gal  
910 M86 mAb (1:1000, 5% BSA in TBST buffer, 5 mL). Membranes were washed with  
911 TBST (1x, 5 min., RT) and incubated with Streptavidin-HRP (10 mL, 1:5,000, 1 h,  
912 RT) for detection of BSI-B4, or with goat anti-mouse IgM-HRP (Southern Biotech  
913 #1021-05, 10 mL, 1:5,000, 1 h, RT) for detection of M86 mAb. Membranes were  
914 washed with TBST (3x, 20 min., RT) and developed (SuperSignal®

915 West FEMTO Max. Sensitivity Substrate #11859290). As a loading control, SDS-  
916 PAGE gel was stained with InstantBlue™ Safe Coomassie Stain (Sigma # ISB1L-1L).

### 917 **Bacterial Strains and Culture Conditions**

918 *E. coli* O86:B7 (ATCC12701) were streaked from -80°C glycerol stocks onto Luria-  
919 Bertani (LB) 1.5% agar plates, incubated at 37°C overnight. For liquid culture, a  
920 single colony was inoculated into 5-10 mL LB liquid medium and incubated (12-16 h,  
921 37°C) with aeration (180-200 rpm). For analysis of  $\alpha$ Gal expression, *E. coli* O86:B7  
922 was grown in NB medium (BD Difco). Optical Density at 600 nm (OD<sub>600</sub>) of the  
923 overnight culture was measured by spectrophotometry (Bio-Rad SmartSpec™3000).  
924 To harvest bacteria during exponential growth phase, sub-culturing was done in NB  
925 medium with a starting OD<sub>600</sub> of 0.05 by incubation (3 h, 37°C) with aeration (180-200  
926 rpm). OD<sub>600</sub> of the bacterial sub-culture was measured (OD=2 corresponding to  
927 approximately 10<sup>9</sup> CFU/mL). The culture was incubated to stop growth (5 min., 4°C)  
928 and bacteria were pelleted by centrifugation (4,000 rpm, 20 min., 4°C). The pellet  
929 was suspended in sterile PBS (5 mL). OD<sub>600</sub> was measured in PBS (1:10 vol/vol) in  
930 triplicate. A volume of bacterial culture corresponding to approximately 10<sup>6</sup>-10<sup>7</sup> cells  
931 per condition was harvested, fixed with 4% PFA/1xPBS and stained for flow  
932 cytometry as described.

933 *E. coli* M6L4, *E. coli* M5S5 and *Klebsiella pneumoniae* were cultured as  
934 described above. Cells from -80°C frozen stocks were streaked onto LB agar plates,  
935 incubated overnight at 37°C under aerobic conditions, and cells from the resulting  
936 colonies used to inoculate 5-10 mL LB medium. Bacterial cultures were incubated at  
937 37°C, with aeration (180-200 rpm), for 12-16 h. *Staphylococcus sciuri*, *Clostridium*  
938 *bifermentans*, *Enterococcus faecalis* and *E. gallinarum* were grown as above using  
939 solid or liquid brain heart infusion (BHI) media supplemented with 100 mM vitamin K1  
940 and 1.9  $\mu$ M hematin; and cultured anaerobically at 37°C for 2 days. *E. faecalis* and *E.*  
941 *gallinarum* were also grown under aerobic conditions on BHI solid or liquid media  
942 overnight for flow cytometry analysis. *Parabacteroides goldsteinii* was cultured  
943 anaerobically on solid or liquid BHI medium supplemented with 1.2  $\mu$ M histidine, 1.9  
944  $\mu$ M hematin, 1  $\mu$ g/mL menadione, and 500  $\mu$ g/mL cysteine at 37°C for 2 days.

### 945 **Flow cytometry of bacterial IgG binding and $\alpha$ Gal expression**

946 Overnight cultures of bacteria were prepared as described above. Samples of 5-20  
947  $\mu$ L of each bacterial culture depending on OD<sub>600</sub> measurements, and corresponding  
948 to approximately 10<sup>6</sup>-10<sup>7</sup> cells, were fixed in PFA (4% wt/vol in PBS) and washed  
949 with filter-sterilized PBS. Bacteria were incubated in either Fluorescein  
950 Isothiocyanate (FITC)-conjugated BSI-B4 lectin from *Bandeiraea* (*Griffonia*)

951 *simplicifolia* (Sigma-Aldrich, #L2895-1MG, 50  $\mu$ L, 40  $\mu$ g/mL in PBS, 2 h) for detection  
952 of  $\alpha$ Gal, or IgG purified from *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mouse serum (60  $\mu$ g/mL, PBS 2%  
953 BSA, wt/vol, 30 min.) followed by anti-mouse IgG-PE (Southern Biotech #1030-09,  
954 1:100 in PBS 2% BSA wt/vol, 30 min.). Cells were washed and analyzed by flow  
955 cytometry using an LSR Fortessa SORP (BD Biosciences), as described below. Data  
956 from at least 10,000 single cell events were measured and analyzed using FlowJo  
957 software (v.10).

### 958 ***In vivo* phagocytosis assay**

959 *In vivo* phagocytosis assays were performed upon injection (i.p.) of FITC-labeled *E.*  
960 *faecalis* (10<sup>8</sup> CFU, 4% PFA, in 100  $\mu$ L PBS) to *J<sub>h</sub>t*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice. Bacteria were either  
961 unopsonized or opsonized with IgG (300  $\mu$ g/mL, 30 min., 37°C) purified from *Ggta1*<sup>+/+</sup>  
962 or *Ggta1*<sup>-/-</sup> mice. Peritoneal lavage was done 3 h after injection (5 mL, ice-cold PBS)  
963 using naïve mice as controls. Samples were prepared as follows: 500  $\mu$ L aliquots of  
964 the lavage were centrifuged (500 g, 2 min., 4°C) and viable cells were stained using  
965 Live/dead fixable yellow stain (1:1000 in 1% FBS/PBS, Molecular Probes, 15 min.,  
966 on ice). Cells were washed and incubated (15-20 min., on ice) with Fc Block (1:100  
967 in 1% FBS/PBS, Clone 2.4G2, BD Biosciences), followed by anti-CD45-PE-Cy5.5  
968 (1:100, clone 30-F11, Life Technologies), anti-Ly6G-PE-Cy7 (1:100, clone 1A8,  
969 Biolegend) and anti-CD11b-BV421 (1:100, clone M1/70, Biolegend). Cells were  
970 analyzed using LSR Fortessa X20 (BD Biosciences) and FACSDiva software  
971 (BDv.6.2). Cell numbers were calculated using PerfectCount Microspheres  
972 (Cytognos). Data from at least 10,000 single viable CD45<sup>+</sup> cells were acquired and  
973 analyzed by FlowJo software (v10.0.7).

### 974 **Flow cytometry for bacterial staining**

975 Bacterial staining of cecal content was performed essentially as described (Bunker et  
976 al., 2017; Koch et al., 2016; Zeng et al., 2016). Briefly, cecal slurry was prepared as  
977 described above, homogenized, filtered through a 40  $\mu$ M cell strainer and diluted to a  
978 concentration of 5 mg/mL in sterile PBS. Large debris were pelleted by centrifugation  
979 (600 g, 4°C, 5 min.). 50  $\mu$ L supernatant (containing bacteria) per condition was  
980 added to a 96-well v-bottom plate (Corning Costar #3894) for staining. Bacteria were  
981 pelleted by centrifugation (3,700 g, 10 min., 4°C) and suspended in flow cytometry  
982 buffer (filter-sterilized 1xPBS, 2% BSA, wt/vol). Bacterial DNA was stained using  
983 SYTO®41 Blue Fluorescent Nucleic Acid Stain (Molecular Probes #S11352, 1:200  
984 vol/vol, wt/vol) or with SYBR® Green I Nucleic Acid Gel Stain (Invitrogen #S-7563,  
985 1:1000 vol/vol) in flow cytometry buffer (100  $\mu$ L, 30 min., RT). Cecal content from  
986 germ free (GF) mice was used as control. Bacteria were washed in flow cytometry

987 buffer (200  $\mu$ L), centrifuged (4000 g, 10 min., 4°C) and supernatant was removed by  
988 flicking the plate. Bacteria were incubated with mouse serum (1:20, vol/vol), or  
989 purified IgG from *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup> mice (60  $\mu$ g/mL) in flow cytometry buffer (100  
990  $\mu$ L, 30 min., RT) and washed, as above. IgG was detected using Phycoerythrin (PE)-  
991 labeled goat anti-mouse IgG (Southern Biotech #1030-09, 100  $\mu$ L, 1:100 vol/vol, 30  
992 min., RT or 4°C, 1 h) and washed as above. Samples were re-suspended in flow  
993 cytometry buffer (300  $\mu$ L), transferred to round-bottom tubes (BD Falcon™ #352235)  
994 and centrifuged (300 g, 1 min., RT).

995 For co-staining of cecal content with purified mouse IgG from both genotypes, the  
996 bacteria were collected, stained for DNA and washed, as described above. Bacteria  
997 were incubated with Alexa-Fluor 594 (AF594)-conjugated mouse IgG and PeCy5-  
998 conjugated mouse IgG (100  $\mu$ L, 60  $\mu$ g/mL, 30 min., RT). Samples were washed and  
999 collected before analysis, as described above.

1000 For co-staining of cecal content with purified mouse IgG and  $\alpha$ Gal, bacteria from  
1001 the cecal content were collected, stained for DNA and washed, as described above.  
1002 Bacteria were incubated in Fluorescein Isothiocyanate (FITC)-conjugated BSI-B4  
1003 lectin from *Bandeiraea (Griffonia) simplicifolia* (Sigma-Aldrich, #L2895-1MG, 50  $\mu$ L,  
1004 40  $\mu$ g/mL in PBS, 2 h) for detection of  $\alpha$ Gal and IgG purified from *Ggta1*<sup>+/+</sup> or *Ggta1*<sup>-/-</sup>  
1005 mouse serum (60  $\mu$ g/mL, PBS 2% BSA, wt/vol, 30 min.) followed by anti-mouse IgG-  
1006 PE (Southern Biotech #1030-09, 1:100 in PBS 2% BSA wt/vol, 30 min.). Supernatant  
1007 was removed by aspiration and the stained bacteria were suspended in sterile  
1008 filtered PBS (500  $\mu$ L) and collected before analysis as described above. *E coli*  
1009 O86:B7 (about 10<sup>7</sup> per tube) were also stained with FITC-conjugated BSI-B4 lectin  
1010 as described above, as a positive control for bacterial  $\alpha$ Gal expression.

1011 For bacterial staining in the peritoneal cavity, the bacteria were harvested by  
1012 peritoneal lavage as described above, and centrifuged (900 g, 5 min., 4°C). The  
1013 supernatant containing the bacteria was collected (500  $\mu$ L), centrifuged (10,000 g, 1  
1014 min., 4°C) and suspended in flow cytometry buffer. The remainder of the procedure  
1015 was similar to that detailed above. An additional step to exclude host leukocytes was  
1016 included in which the suspension was co-stained with PECy5.5-labeled rat anti-  
1017 mouse CD45 (eBioscience, Clone 30-F11, 1:100 vol/vol, 15 min., RT or 4°C, 1 h) and  
1018 washed as above. For staining  $\alpha$ Gal, the pellet was suspended in freshly prepared  
1019 BSI-B4-FITC conjugate (50  $\mu$ L, 40  $\mu$ g/mL, 2 h, RT). Samples were washed and  
1020 collected before analysis as described above.

1021 Samples were analyzed in LSR Fortessa SORP (BD Biosciences) equipped with  
1022 a high-throughput sampler (HTS) using the FACSDiva Software (BD v.6.2). SYBR®  
1023 Green and BSI-B4-FITC were excited with blue laser (488 nm, filters: 530/30,

1024 502LP), IgG-PE with Yellow-green laser (561 nm, detection filter: 590/20), CD45-  
1025 PECy5.5 with Yellow-green laser (561 nm, detection filters: 705/70, 685LP), Syto41  
1026 with Blue-violet laser (442 nm, detection filters: 480/40, 455LP), PeCy5 with Blue  
1027 laser (488 nm, detection filters: 695/40, 660LP) and AF594 with Yellow-green laser  
1028 (561 nm, detection filters: 630/30, 610LP). Compensations were done using anti-  
1029 rat/hamster Igk compensation beads (BD™ CompBead #552845). Before acquisition  
1030 of samples, laser voltages were standardized using SPHERO™ Ultra Rainbow  
1031 Fluorescent Particles (Spherotech #URFP01-30-2K). Data from at least 10,000  
1032 single bacterial events were measured and analyzed by FlowJo software (v10.0.7).

### 1033 **Flow cytometry for bacterial sorting**

1034 Bacterial staining for IgG in the peritoneal cavity was done as described above.  
1035 Sorting was performed in FACSAria IIu (BD Biosciences, 70 µm nozzle). SYBR®  
1036 Green, IgG-PE and CD45-PE-Cy5.5 were excited with blue laser (488 nm) and  
1037 fluorescence detected using the following filters, respectively: 530/30, 502LP,  
1038 585/42, 550LP, 695/40, 655LP. The gating strategy was set using Fluorescence  
1039 Minus One (FMO) controls for all fluorochromes, as well as biological controls that  
1040 specifically lack target populations. 5,000-10,000 events of IgG-positive and IgG-  
1041 negative bacterial populations were sorted into tubes containing filter-sterilized PBS  
1042 (50 µL) and stored at -80°C until further analysis.

### 1043 **Extraction of bacterial DNA from feces**

1044 Fecal pellets (4-5/mouse) were collected in sterile Eppendorfs and stored at -80°C.  
1045 DNA extraction was done according to manufacturer's instructions (QIAamp Fast  
1046 DNA Stool Mini Kit #50951604). Briefly, individual samples were thawed and  
1047 mechanically disrupted in InhibitEX Buffer (1 mL) with a motorized pestle using  
1048 sterile glass beads (Disrupter Genie # 9730100, about 0.4 g/sample). Disruption of  
1049 microbial cells was enhanced using TissueLyser II (QIAGEN, 30 shakes/sec., 1 min.,  
1050 2x). To lyse Gram-negative bacteria, the suspensions were heated (12 min., 95°C),  
1051 vortexed (15 s) and larger particles were pelleted by centrifugation (20,000 g, 1 min.,  
1052 RT). The supernatant (200 µL) was lysed by incubation (12 min., 70°C) in a mixture  
1053 of Proteinase K (15 µL) and AL buffer (200 µL) and vortexed (15 s). DNA in the  
1054 lysate was precipitated with ethanol (96–100%, 200 µL) and washed in AW buffer  
1055 (20,000 g, 1min., RT, 2x). DNA was incubated in ATE buffer (100 µL, 1 min., RT),  
1056 eluted by centrifugation (20,000 g, 1 min., RT) and frozen at -80°C until further use.

### 1057 **Extraction of sorted bacterial DNA**

1058 DNA was extracted from flow cytometry sorted bacterial samples using  
1059 manufacturer's protocols (DNeasy PowerSoil Kit #12888-50). Briefly, samples

1060 (5x10<sup>3</sup>-1x10<sup>4</sup> bacteria) were lysed in Solution C1 (60 µL) in PowerBead tubes,  
1061 vortexed briefly and heated (10 min., 65°C). Mechanical disruption of microbial cells  
1062 was done using TissueLyser II (QIAGEN, 30 shakes/sec., 10-20 min.) and the  
1063 samples were centrifuged (10,000 g, 30 sec., RT). The supernatant was cleaned by  
1064 incubation (5 min., 2–8°C) in Solution C2 (250 µL) followed by Solution C3 (200 µL),  
1065 vortexed (5 sec.), and centrifuged (10,000 g, 1 min., RT). To bind DNA to the MB  
1066 Spin Column, the supernatant was mixed with Solution C4 (1,000 µL), vortexed (5  
1067 sec.), loaded onto the column and centrifuged (10,000 g, 1 min., RT). DNA was  
1068 washed in Solution C5 (500 µL) by centrifugation (10,000 g, 30 sec., RT, 2x). DNA  
1069 was eluted in Solution C6 (50 µL) by centrifugation (10,000 g, 30 sec., RT) and  
1070 stored at -80°C until further use.

### 1071 **Metagenomics: 16S amplicons production and sequencing**

1072 The 16S rRNA V4 region was amplified in triplicate by PCR following the Earth  
1073 Microbiome Project (<http://www.earthmicrobiome.org/emp-standard-protocols/>).  
1074 Briefly, the mix for each sample consisted of DNA (1 µL), water (9 µL), PCR  
1075 Mastermix (5PRIME HotMasterMix-1000R #733-2474, 2x, 10 µL), Primer\_barcode  
1076 (2 µM, 2.5 µL) and Primer\_universal (2 µM, 2.5 µL). For samples extracted from  
1077 bacterial sorting (low biomass), 10 µL of DNA and no water were used. The PCR  
1078 conditions were: 94°C (3 min.); 35 cycles of 94°C (45 sec.), 50°C (60 sec.), 72°C (90  
1079 sec.); 72°C (10 min.), 4°C (1 h) for 96 wells and 94°C (3 min.); 35 cycles of 94°C (60  
1080 sec.), 50°C (60 sec.), 72°C (105 sec.); 72°C (10 min.), 4°C (1 h). After amplification,  
1081 the triplicates of each sample were pooled (75 µL) and quantified with Quant-iT™  
1082 PicoGreen® dsDNA Assay Kit (Invitrogen™#P7589). Equal amounts of amplicons  
1083 from each sample containing individual barcodes were pooled (240-300 ng for low  
1084 biomass) and cleaned (MoBio UltraClean PCR Clean-Up Kit #12500). DNA purity  
1085 and concentration were assessed using a spectrophotometer (NanoDrop™) and  
1086 Qubit® dsDNA HS Assay Kit (Invitrogen™) #Q32854). DNA library was prepared by  
1087 denaturing with NaOH (0.2 M, 5 min., RT) and mixed with Illumina Phix (10-15%) to  
1088 balance the nucleotide representation.

1089 Sequencing of the 16S rRNA region was done using custom primers from the  
1090 Earth Microbiome Project that was adapted for the Illumina MiSeq (MiSeq Reagent  
1091 Kit v2, 500 cycle #MS-102-2003, Illumina) (Caporaso et al., 2010b; Zhang et al.,  
1092 2014). The custom primers were:

1093 Read 1 primer (TATGGTAATTGTGTGYCAGCMGCCGCGGTAA), Read 2 primer  
1094 (AGTCAGCCAGCCGGACTACNVGGGTWTCTAAT) and Index primer  
1095 (AATGATACGGCGACCACCGAGATCTACACGCT). The denatured DNA library and

1096 the custom primers were loaded on specific reservoirs on the MiSeq cartridge and  
1097 sequenced on a 2x250 cycles run.

### 1098 **Metagenomics: Sequences and Operational Taxonomic Unit (OTU) Quality** 1099 **Control**

1100 The raw sequencing reads obtained from Illumina MiSeq were demultiplexed and  
1101 quality filtered with SeqTK (v.1.2-r94) using  $q=0.01$ . Filtered paired reads were  
1102 merged with PEAR (v.0.9.6) (Zhang et al., 2014) using default parameters. Merged  
1103 reads were then processed using the QIIME package (v.1.9.1) (Caporaso et al.,  
1104 2010b). Reads included had a quality score above 19, a median length of 250 bp,  
1105 maximum 1 mismatch on the primer and default values for other quality parameters.  
1106 Quality filtered reads were clustered into OTUs at 97% similarity using the default  
1107 UCLUST (Edgar, 2010) method with an open reference approach. Subsequent  
1108 taxonomic assignment was performed using the UCLUST classifier against the  
1109 Greengenes database (v.13.8) (DeSantis et al., 2006). Sequence alignment and  
1110 open-reference OTU picking (Rideout et al., 2014) were performed using the default  
1111 Pynast (Caporaso et al., 2010a). Tree building was done with (Price et al., 2010) and  
1112 taxonomic assignment with the RDP classifier (Wang et al., 2007). Two extra filtering  
1113 steps were applied to taxonomy-assigned OTUs to remove outliers, eliminating  
1114 sequences with less than a total of 10 counts across all samples and sequences with  
1115 more than 50 counts for 3 samples or less.

### 1116 **Metagenomics: Downstream Bioinformatics Analyses**

1117 To calculate alpha-diversity measures, samples were rarefied to match the least  
1118 abundant sample. Using QIIME 1.9.1 (Caporaso et al., 2010b), the Chao1 and  
1119 Shannon diversity measures were obtained for each sample and the mean of 10  
1120 independent rarefactions was taken. To estimate the significance of differences of  
1121 alpha-diversity, the Mann-Whitney test was used to compare two groups and  
1122 Kruskal-Wallis to compare multiple groups followed by Dunn post hoc test (Dunn,  
1123 1964) and Bonferroni correction was done to estimate significance of pairwise  
1124 differences. The Unweighted and Weighted UniFrac distances (Lozupone et al.,  
1125 2011) between samples were calculated, Principal Coordinates Analysis (PCoA) was  
1126 performed and group significance estimated by using PERMANOVA test to obtain a  
1127 pseudo-F statistic and a p-value for the statistic. In the case of multivalued factors,  
1128 PERMANOVA was executed on all pairwise comparisons, followed by Bonferroni  
1129 correction. Alternatively, the Mann-Whitney or Kruskal-Wallis test were used to  
1130 compare mean Unweighted and Weighted UniFrac distances between elements of  
1131 different groups. Ellipses are centered on the categorical averages of the metric



1132 distances with a 95% confidence interval for the first two coordinates of each group,  
1133 drawn on the associated PCoA.

1134 Linear discriminant analysis effect size (LEfSe) analysis (Segata et al., 2011) was  
1135 performed to represent taxa distinguishing different groups. Cladograms, generated  
1136 from LEfSe analysis, represent taxa enriched in each genotype. The central point  
1137 represents the root of the tree (Bacteria), and each ring represents the next lower  
1138 taxonomic level (phylum through genus). The diameter of each circle represents the  
1139 relative abundance of the taxon.

#### 1140 **Mathematical Modeling: Model structure and assumptions**

1141 The model computes the lifetime reproductive success of a typical host from a given  
1142 genotype. To obtain the reproductive output over lifetime, the birth rate at each age,  
1143  $B(a)$ , was weighted by the survival probability,  $S(a)$ , at that age, and integrated over  
1144 the entire lifespan. Denoting this lifetime reproductive success as fitness  $W$ , this  
1145 quantity was formalized as follows:

$$W = \int_0^{A_{max}} B(a) \times S(a) da$$

1146

1147 where  $A_{max}$  is the maximum lifespan of a typical host. In the absence of infection, the  
1148 survival probability over ages follows a simple exponential with rate equal to the  
1149 natural host mortality rate ( $m$ ):

$$S(a) = e^{-ma}$$

1150

1151 However, in the presence of exposure to infection, the survival probability becomes a  
1152 weighted average between probability of getting infected/exposed ( $E$ ) and  
1153 subsequently dying from infection, and the probability of escaping infection ( $1-E$ ) and  
1154 dying from natural mortality. The parameter  $E$  denoting the lifetime infection risk of  
1155  $0 \leq E \leq 1$ , constant over age, is assumed equal for both genotypes and driven by the  
1156 environment. Its influence on the survival function of a typical host from the first  
1157 genotype was formalized as follows:

$$S(a) = E \times e^{-m(1+v)a} + (1 - E)e^{-ma}$$

1158

1159 where  $v$  is the fold-increase in mortality due to infection, an indicator of pathogen  
1160 virulence. For example if  $v = 5$ , a typical infected host will experience a 5-fold  
1161 additional increase in mortality relative to an uninfected host per unit of time.

1162 Protection of a second host genotype from infection was modeled through a  
1163 factor  $q$  ( $0 \leq q < 1$ ), relative to the reference genotype, reducing the fraction of hosts  
1164 that experience infection-induced mortality. Motivated by the data, it was assumed  
1165 that individuals dying of infection experience the same mortality rate. Thus, the age-  
1166 specific survival function of a typical host from the second genotype is given by:

$$S'(a) = qE \times e^{-m(1+v)a} + (1 - qE)e^{-ma}$$

1167

1168 The protective effect is defined as  $p = 1 - q$ , namely the relative gain in proportion  
1169 survival of infected hosts in the second genotype relative to the first. When  
1170 comparing the two host genotypes (0/1) in terms of their lifetime reproductive  
1171 success, their difference in birth rate given by  $B_0(a)$  for host genotype 0 and  $B_1(a)$  for  
1172 host genotype 1, was combined with their difference in survival given by  $S_0(a)$  and  
1173  $S_1(a)$ , accounting for infection. This led to the numerical comparison of  $W_0$  and  $W_1$ :

$$W_0 = \int_0^{A_{max}} B_0(a) \times \left( E \times e^{-m(1+v)a} + (1 - E)e^{-ma} \right) da$$
$$W_1 = \int_0^{A_{max}} B_1(a) \times \left( qE \times e^{-m(1+v)a} + (1 - qE)e^{-ma} \right) da$$

1174

1175 With this model for lifetime reproductive output, the fitness ratio was given by:

$$\frac{W_1}{W_0},$$

1176

1177 which if above 1, indicates a relative advantage of host genotype 0, and if below 1,  
1178 indicates an advantage of host genotype 1. The selective advantage of 1 to 0 was  
1179 then defined as:

$$s = \frac{W_1}{W_0} - 1$$

1180

1181 If  $s$  is positive ( $s > 0$ ), the higher it will be, the more host genotype 1 are favored by  
1182 natural selection relative to host genotype 0. Conversely, if  $s$  is negative ( $s < 0$ ), then  
1183 host genotype 1 suffer overall a fitness disadvantage relative to host genotype 0.  
1184 Variation in model parameters common to or different between the two host  
1185 genotypes in corresponding survival/birth rate functions, leads to explicit  
1186 mathematical variation in  $s$ , and provides insights on biological conditions favoring  
1187 selection.

### 1188 **Mathematical Modeling: Model application to the dataset**

1189 We numerically parameterized the model based on our study with laboratory mice,  
1190 taking  $Ggta1^{+/+}$  as reference host genotype 0 and  $Ggta1^{-/-}$  as host genotype 1. Natural  
1191 lifespan was assumed to be 128 weeks (Kunstyr and Leuenberger, 1975), leading to  
1192 a natural mortality rate  $m=0.0078$  per week. The survival data from the CLP  
1193 experiments with the two genotypes (Fig. 1A) were used for the estimation of  $v$  (the  
1194 fold-increase in mortality rate due to infection) and  $p$  (relative reduction in fraction of  
1195  $Ggta1^{-/-}$  hosts that die of infection). Since all mortality occurred within 2 weeks, it was  
1196 assumed that this corresponds to all mortality due to infection in each group, (1-  
1197 0.676) for  $Ggta1^{-/-}$ , and (1-0.089) for  $Ggta1^{+/+}$ , leading to a relative protective effect of

1198 64% ( $p=0.64$ ). The infection-induced mortality via an additional factor  $v$  relative to  
1199 natural mortality, was calculated as:

$$v = -\frac{\ln(0.089)}{2m} - 1$$

1200

1201 which in our particular case resulted in  $v=154$ , indicating substantial virulence. Our  
1202 data support similar death kinetics in both genotypes, motivating the same  $v$   
1203 parameter in their survival functions. While capturing the difference between  
1204 genotypes with a single parameter  $p$  is appropriate and sufficient in our context, in  
1205 other systems,  $v$  could also vary. Exposure to infection  $E$  was assumed to be  
1206 constant over age in this model. However, an age-dependent exposure  $E(a)$  could  
1207 also be used, informed by empirical evidence or theoretical assumptions. Increasing  
1208 exposure risk differentially in younger ages should amplify the selection potential for  
1209 protection against infection. In contrast, increasing exposure risk in the post-  
1210 reproductive period should reduce the selection potential for protection, given that  
1211 host fitness would be more strongly affected by the reproductive fitness cost in that  
1212 case. In the current formalism, epidemiological and co-evolutionary loops between  
1213 host and pathogens were not modeled. The source of infection was assumed to be  
1214 environmental and not explicitly driven by transmission between hosts. Similarly,  
1215 details of pathogen-immunity dynamics within the host were not included. More  
1216 complex modeling frameworks capturing such nested and bidirectional population  
1217 feedbacks (Gilchrist and Sasaki, 2002), were considered to be beyond the scope of  
1218 the current work.

### 1219 **Mathematical Modeling: quantification and statistical analyzes**

1220 All statistical tests were performed using GraphPad Prism Software (v.6.0). To  
1221 assess differences in binding of purified IgG to each of the Fc $\gamma$  receptors and C1q,  
1222 the sigmoidal curves of the form:

$$a + v_{\max} \frac{x^h}{x^h + K^h}$$

1223

1224 where  $x$  is the concentration, to each of the OD curves, were fitted using least-  
1225 squares regression. One initial fit to the aggregate data was used to provide initial  
1226 estimates to the fitting algorithm. Estimates for  $K$  (concentration at which OD is half-  
1227 maximum) were compared between  $Ggta1^{+/+}$  and  $Ggta1^{-/-}$  IgG preparations using  
1228 Mann-Whitney tests for differences in the median. Curve fitting and statistical tests  
1229 were performed using scipy 1.2.1.

1230

1231 **SUPPLEMENTAL FIGURE TITLES AND LEGENDS**

1232 **Figure S1. *Ggta1* and *Rag2* deletion are associated with changes in microbiota**  
1233 **composition. Related to Figure 1.**

1234 **A-B)** Microbiota **A)** richness (Chao index) and **B)** diversity (Shannon index) in the  
1235 same samples as in *Fig. 1C-E*. **C-D)** Microbiota **C)** richness (Chao index) and **D)**  
1236 diversity (Shannon index) in the same samples as in *Fig. 1G-H*. **E)** Breeding strategy  
1237 for the generation of *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice from *Ggta1*<sup>-/-</sup> females. F<sub>0</sub> *Rag2*<sup>-/-</sup>*Ggta1*<sup>+/+</sup>  
1238 males crossed with *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> females to generate F<sub>1</sub> *Rag2*<sup>+/-</sup>*Ggta1*<sup>+/-</sup> mice,  
1239 which were bred to generate F<sub>2</sub> *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> vs. *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> littermates. F<sub>2</sub>  
1240 *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice were bred subsequently for 7 generations. **F-I)** Microbiota PCoA  
1241 of **F)** Unweighted UniFrac, **G)** Weighted UniFrac distance, **H)** richness (Chao index)  
1242 and **I)** diversity (Shannon index) from 16S rRNA amplicons in fecal samples from  
1243 *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=15) and *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=14) mice. **J)** LDA scores and **K)**  
1244 Cladogram, generated from LEfSe analysis, showing taxa enriched in *Rag2*<sup>+/+</sup>*Ggta1*<sup>-/-</sup>  
1245 (red) vs. *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (green) fecal microbiota in the same samples as (F-I); a:  
1246 family\_Prevotellaceae, b: family\_Rikenellaceae, c: family\_24-7, d:  
1247 class\_Alphaproteobacteria, e: class\_Betaproteobacteria, f: order\_RF32, g:  
1248 order\_Burkholderiales, h: order\_RF39, i: order\_Anaeroplasmatales. Data from one  
1249 experiment. **L)** Survival of *Ggta1*<sup>+/+</sup> (n=5) and *Ggta1*<sup>-/-</sup> (n=4) mice infected (*i.p.*) with  
1250 paraformaldehyde-treated cecal content from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice. Data from one  
1251 experiment. Symbols in (A, B, C, D, F, G, H, I) are individual mice. Red bars (A, B, C,  
1252 D, H, I) correspond to mean values. Error bars (A, B, C, D, H, I) correspond to SD. P  
1253 values in (A, B, C, D, H, I) are calculated using Mann-Whitney test, P values and Fs  
1254 in (F, G) using PERMANOVA test and in (L) using log-rank test. \*P < 0.05, \*\*P <  
1255 0.01, \*\*\*P < 0.005; ns: not significant

1256

1257 **Figure S2. Loss of *Ggta1* enhances resistance to systemic polymicrobial**  
1258 **infection via a mechanism independent of IgM, IgA, or T cells. Related to**  
1259 **Figure 2.**

1260 **A)** Survival of  $\mu$ S<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=6) and  $\mu$ S<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=10) mice infected (*i.p.*) with a  
1261 cecal inoculum from *Rag2*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> mice; 2 experiments. **B)** Colony forming units  
1262 (CFU) of aerobic (Ae) and anaerobic (An) bacteria of  $\mu$ S<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=5) and  $\mu$ S<sup>-/-</sup>  
1263 *Ggta1*<sup>-/-</sup> (n=4-5) mice, 24 hours after infection; 2 experiments. **C)** Survival of  
1264 *Iga*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=9) and *Iga*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=11) mice infected as in (A); 2 experiments. **D)**  
1265 CFU of Ae and An bacteria of *Iga*<sup>+/+</sup>*Ggta1*<sup>-/-</sup> (n=5) and *Iga*<sup>-/-</sup>*Ggta1*<sup>-/-</sup> (n=6) mice, 24

1266 hours after infection; 2 experiments. **E**) Survival of  $Tcr\beta^{+/+}Ggta1^{-/-}$  (n=7) and  $Tcr\beta^{-/-}$   
1267  $Ggta1^{-/-}$  (n=8) mice infected as in (A); 2 experiments. **F**) CFU of Ae and An bacteria  
1268 of  $Tcr\beta^{+/+}Ggta1^{-/-}$  (n=3) and  $Tcr\beta^{-/-}Ggta1^{-/-}$  (n=6) mice, 24 hours after infection; 2  
1269 experiments. **G**) Survival of  $Tcr\delta^{+/+}Ggta1^{-/-}$  (n=7) and  $Tcr\delta^{-/-}Ggta1^{-/-}$  (n=8) mice  
1270 infected as in (A); 2 experiments. **H**) CFU of Ae and An bacteria of  $Tcr\delta^{+/+}Ggta1^{-/-}$   
1271 (n=5) and  $Tcr\delta^{-/-}Ggta1^{-/-}$  (n=5) mice, 24 hours after infection; 2 experiments. Symbols  
1272 in (B, D, F, H) are individual mice. Red bars in (B, D, F, H) are median values. P  
1273 values in (A, C, E, G) calculated using log-rank test and in (B, D, F, H) using Mann-  
1274 Whitney test. Peritoneal cavity (PC). \*P < 0.05; ns: not significant

1275

1276 **Figure S3. The protective effect of IgG NAb acts irrespectively of  $\alpha$ Gal**  
1277 **recognition. Related to Figure 3.**

1278 **A**) Concentration of IgG in serum from  $Ggta1^{+/+}$  (n=10) and  $Ggta1^{-/-}$  (n=10) mice; 2  
1279 experiments. **B**) Representative single stained control plots for [Fig. 3D,E](#) showing  
1280  $Rag2^{-/-}Ggta1^{-/-}$  cecal bacteria stained with BSI-B4 lectin for  $\alpha$ Gal, purified IgG from  
1281  $Ggta1^{+/+}$  and purified IgG from  $Ggta1^{-/-}$  mice. **C**) Representative flow cytometry plots  
1282 showing IgG-binding of *in vitro*-grown species of bacteria isolated from the mouse  
1283 microbiota incubated with IgG purified from  $Ggta1^{+/+}$  or  $Ggta1^{-/-}$  mice, as indicated in  
1284 [Fig. 3F](#). **D**) Representative flow cytometry plots of  $\alpha$ Gal expression by *in vitro*-grown  
1285 species of bacteria isolated from the mouse microbiota, as indicated in [Fig. 3G](#). Plots  
1286 for *E. faecalis* (C, D) are highlighted in blue. Symbols in (A) are individual mice. Red  
1287 bars in (A) are mean values. Error bars (A) correspond to SD. P values in (A)  
1288 calculated using Mann-Whitney test. ns: not significant

1289

1290 **Figure S4. IgG from  $Ggta1^{+/+}$  and  $Ggta1^{-/-}$  mice recognize cecal bacteria to the**  
1291 **same extent. Related to Figure 4.**

1292 **A**) Median Fluorescence Intensity (MFI) of IgG<sup>+</sup> bacteria from the same samples as in  
1293 [Fig. 4A](#). **B**) MFI of IgG<sup>+</sup> bacteria from the same samples as in [Fig. 4B](#). **C-F**)  
1294 Representative controls plots for data shown in [Fig. 4C,D](#) showing  $Rag2^{-/-}Ggta1^{-/-}$   
1295 cecal bacteria stained with **C**) PECy5-conjugated  $Ggta1^{+/+}$  IgG co-stained with AF594-  
1296 conjugated  $Ggta1^{-/-}$  IgG, **D**) PECy5-conjugated  $Ggta1^{-/-}$  IgG co-stained with AF594-  
1297 conjugated  $Ggta1^{+/+}$  IgG, **E**) PECy5-conjugated  $Ggta1^{+/+}$  IgG co-stained with AF594-  
1298 conjugated  $Ggta1^{+/+}$  IgG and **F**) PECy5-conjugated  $Ggta1^{-/-}$  IgG co-stained with  
1299 AF594-conjugated  $Ggta1^{-/-}$  IgG. **G-H**) Relative abundance of **G**) IgG<sup>+</sup> bacteria and **H**)  
1300 IgG<sup>-</sup> bacteria at >2% frequency in the same samples as in [Fig. 4E-H](#). Stacked bars

1301 represent the mean and colors represent the relative fraction of each taxon. Symbols  
1302 (A, B) are individual mice. Red lines (A, B) are mean values. Error bars (A, B)  
1303 correspond to SD. P values in (A, B) calculated using Mann-Whitney test, ns: not  
1304 significant.

1305

1306 **Figure S5. Controls for *in-vivo* phagocytosis assay, detection of  $\alpha$ Gal on**  
1307 **purified IgG and IgG binding to other recombinant mouse Fc $\gamma$ R. Related to**  
1308 **Figure 5.**

1309 **A)** Analysis of peritoneal neutrophils based on the expression of CD11b and Ly6G in  
1310 the same samples as in [Fig. 5A-C](#). **B)** Bacterial uptake by peritoneal CD45<sup>+</sup>  
1311 leukocytes recovered from mice detailed in (A). **C)** Analysis of phenotype of  
1312 phagocytic CD45<sup>+</sup> cells from (B). **D)** Control of [Fig. 5D,E](#) for detection of  $\alpha$ Gal with  
1313 BSI-B4 lectin in BSA conjugated to  $\alpha$ Gal and unconjugated BSA. SDS gel is shown  
1314 as loading control. **E-H)** Relative binding to mouse **E)** Fc $\gamma$ RI, **F)** Fc $\gamma$ RIIb, **G)** Fc $\gamma$ RIII  
1315 and **H)** FcRn by *Ggta1*<sup>+/+</sup> (n=4-7) and *Ggta1*<sup>-/-</sup> (n=3-6) purified IgG, where n  
1316 corresponds to independent IgG preparations. Data is representative of 1-3  
1317 independent experiments. Error bars correspond to SEM. P values calculated using  
1318 2-Way ANOVA with Sidak's multiple comparison test.

1319

1320 **Figure S6. Epidemiological contexts where survival advantage against**  
1321 **infection outweighs the reproductive fitness cost associated with loss of**  
1322 ***Ggta1*. Related to Figure 6.**

1323 Contour plots showing fitness ratios of *Ggta1*<sup>-/-</sup> and *Ggta1*<sup>+/+</sup> genotypes. Lifetime  
1324 exposure to infection (*E*), assumed as constant over age, is plotted on the x-axis.  
1325 The magnitude of protection (*p*) is plotted on the y-axis. Black line indicates the  
1326 threshold for positive selective advantage (*s*>0) of *Ggta1*<sup>-/-</sup> vs. *Ggta1*<sup>+/+</sup> genotype. **A)**  
1327 Condition of low virulence (*v*) relative to natural mortality. The selective forces for  
1328 protection are lower than the cost of reproduction for the *Ggta1*<sup>-/-</sup> genotype. **B)**  
1329 Condition of higher virulence relative to natural mortality. Here, a parameter region  
1330 above the black line emerges where the survival advantage exceeds the cost. **C)**  
1331 Condition of further increase in virulence, increasing the possibility of selection even  
1332 for lower range of exposure and protection. **D)** Condition of very high virulence,  
1333 which favors selection for an even smaller protective effect.

1334

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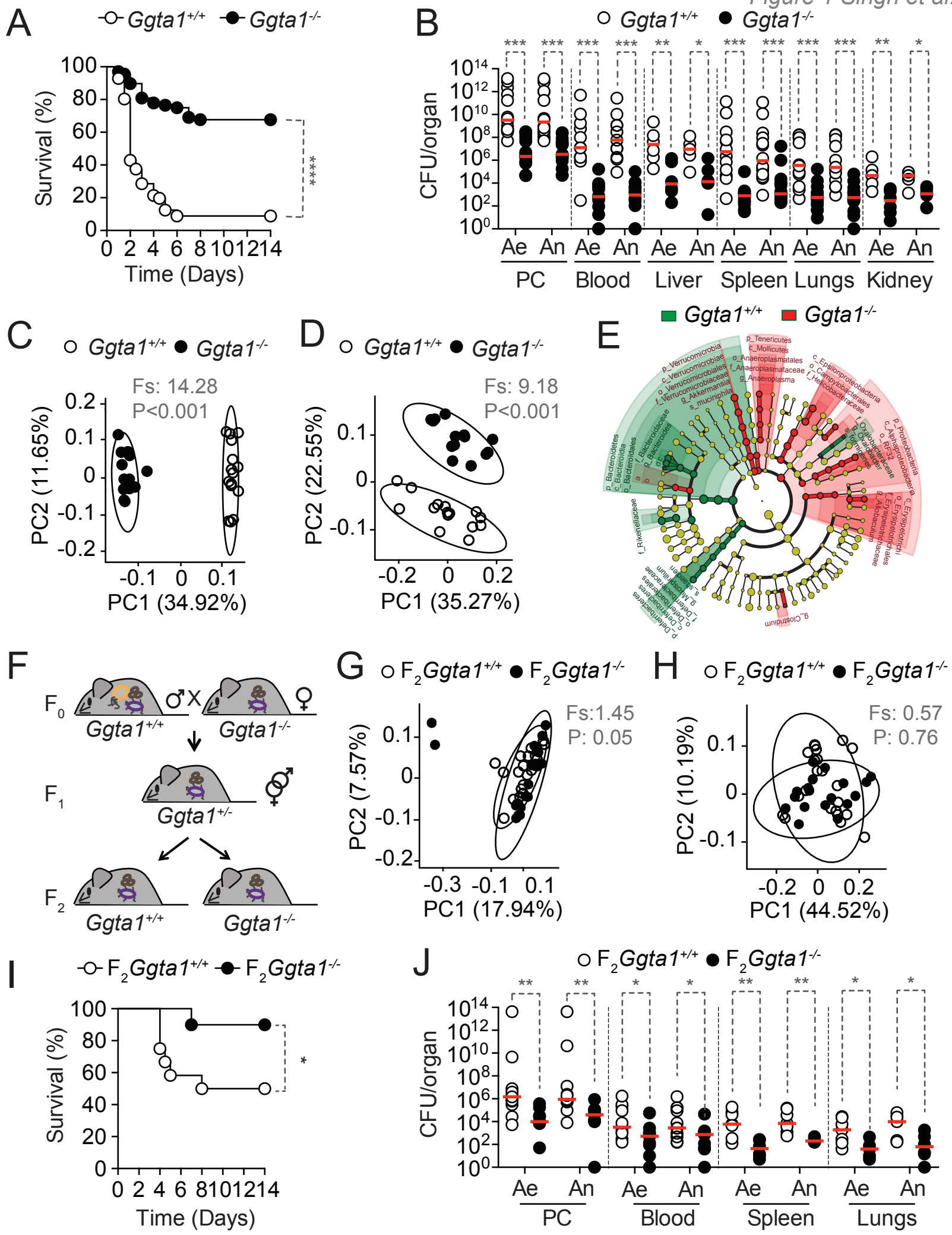


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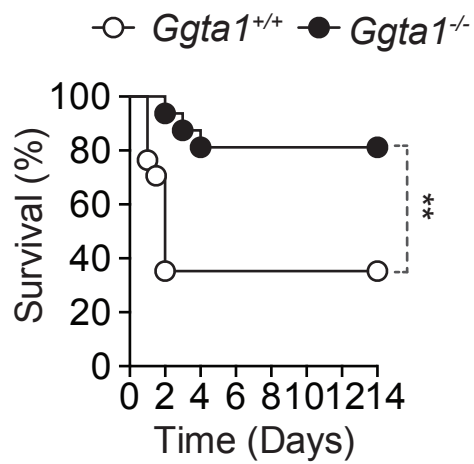
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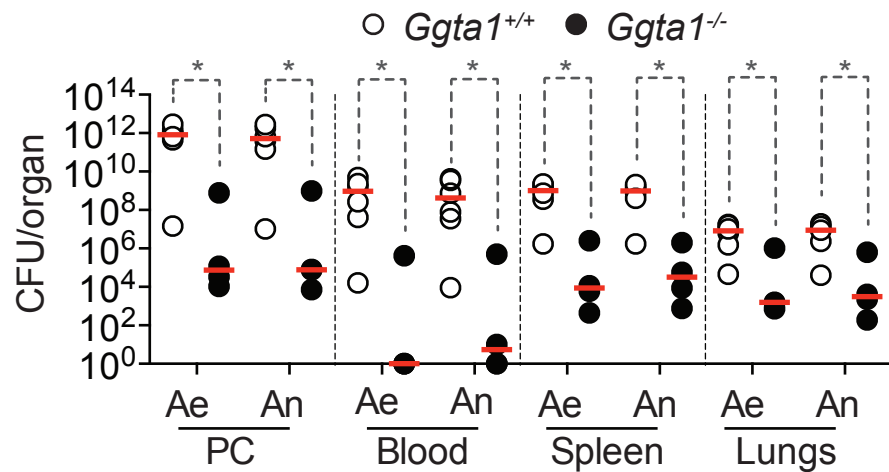
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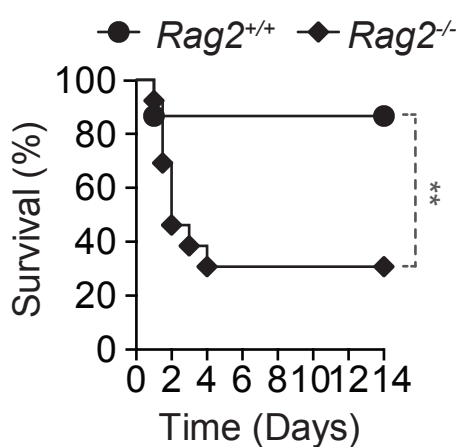
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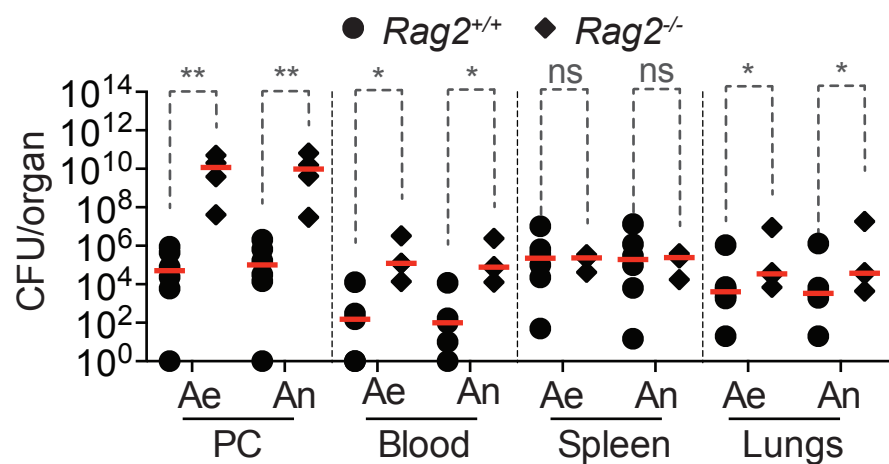
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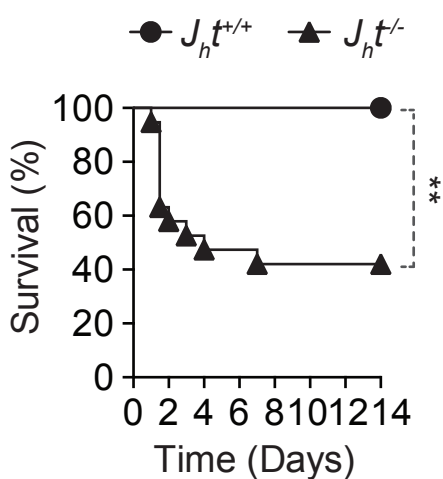
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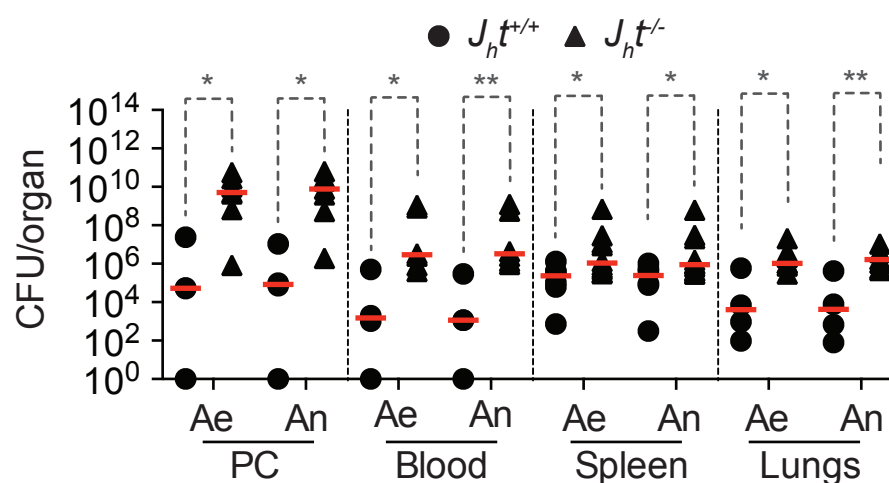
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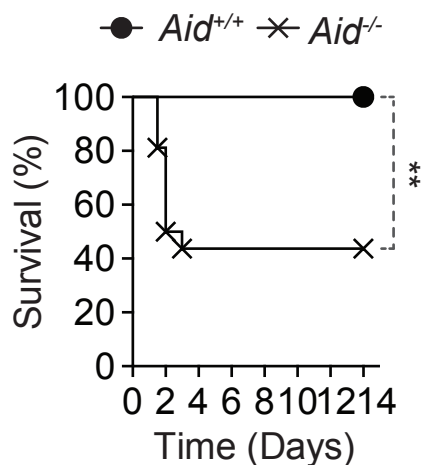
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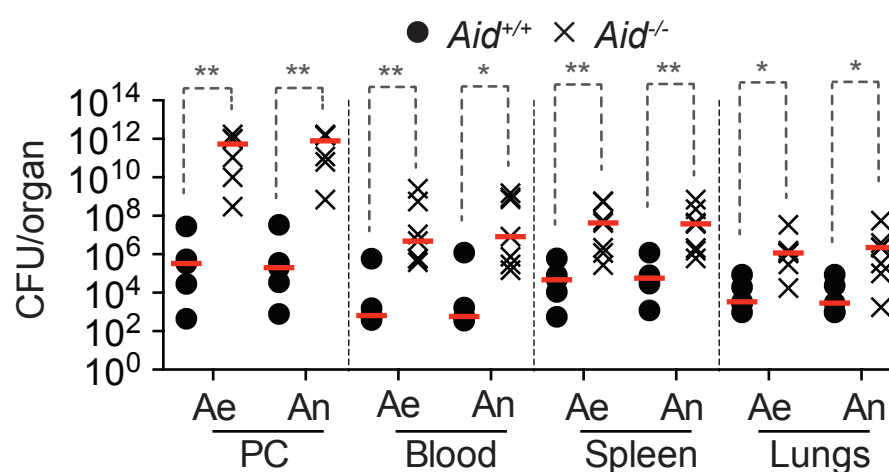
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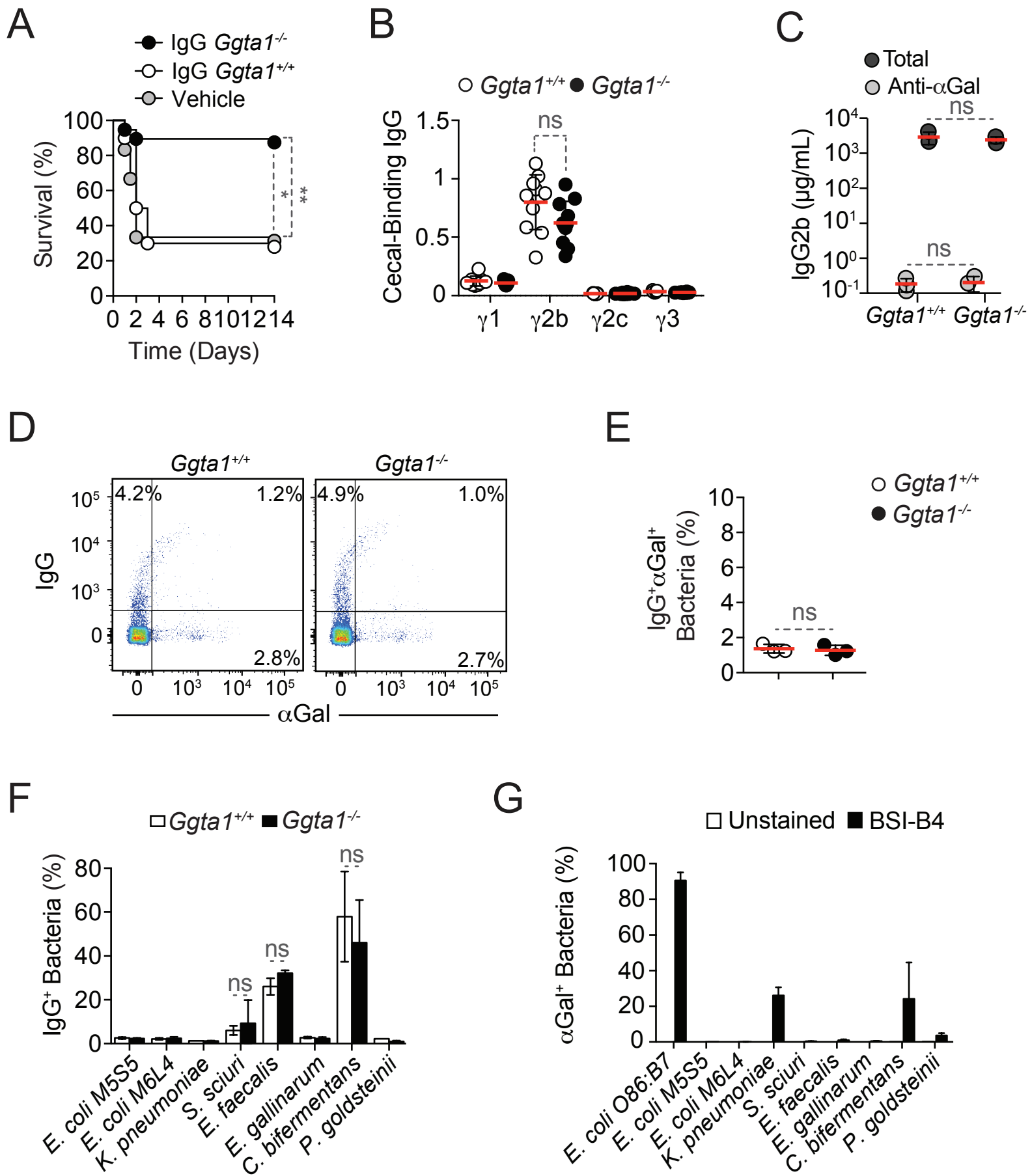


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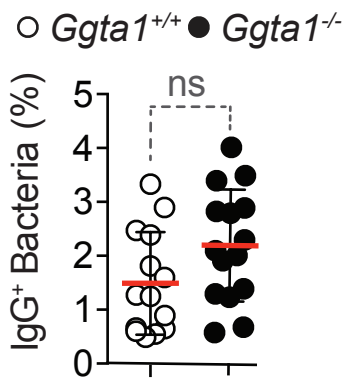


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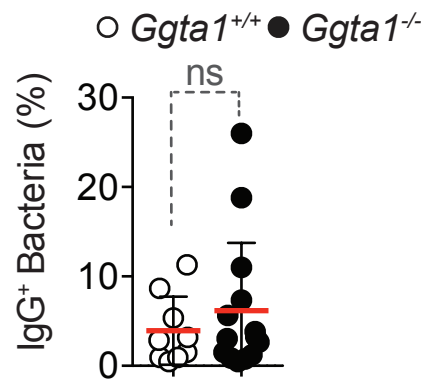




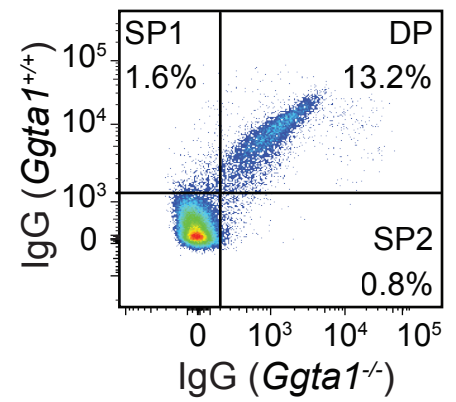
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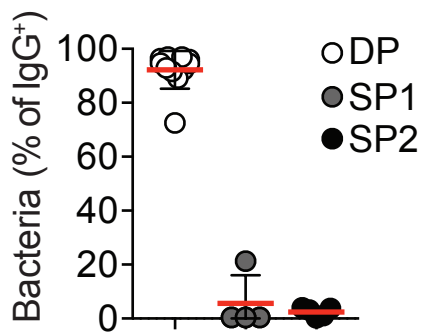
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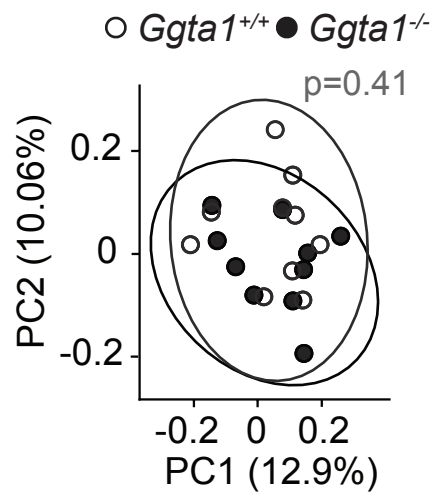
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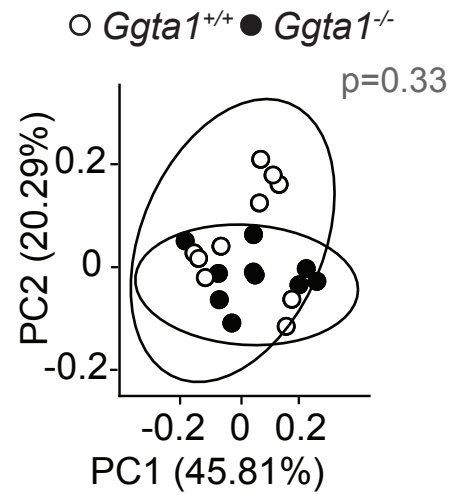
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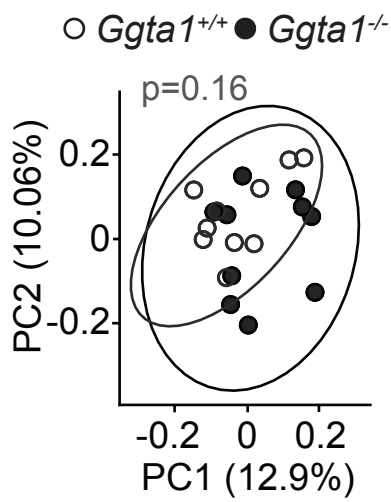
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