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Human IgA bind a diverse array of commensal bacteria

Running title: Human IgA bind a wide array of commensals.

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Abbreviation list

EI : Enrichment Index ; Gal : Galactose ; GlcNAc : N-Acetylglucosamine ; mAb : Monoclonal Antibody ; Neu5Ac : N-Acetylneuraminic acid ; Neu5Gc : N-Glycolylneuraminic acid

Short summary

Intestinal IgA are key players in host-commensals symbiosis. Sterlin *et al.* report that human IgA are microbiota cross-reactive at the clonal level. IgA1 and IgA2 mostly converge toward common gut microbiota targets, but possess distinct carbohydrate repertoires.

Abstract

In humans, several grams of IgA are secreted every day in the intestinal lumen. While only one IgA isotype exists in mice, humans secrete IgA1 and IgA2, whose respective relations with the microbiota remain elusive. We compared binding patterns of both polyclonal IgA sub-classes to commensals and glycan arrays, and also determined the reactivity profile of native human monoclonal IgA antibodies. While most commensals are dually targeted by IgA1 and IgA2 in the small intestine, IgA1⁺IgA2⁺ and IgA1⁻IgA2⁺ bacteria coexist in the colon lumen, where Bacteroidetes is preferentially targeted by IgA2. We also observed that galactose- α terminated glycans are almost exclusively recognized by IgA2. Although bearing signs of affinity maturation, gut-derived IgA monoclonal antibodies are cross-reactive in the sense that they bind to multiple bacterial targets. Private anti-carbohydrate binding patterns, observed at clonal level as well, could explain these apparently opposing features of IgA, being at the same time cross-reactive and selective in its interactions with the microbiota.

Introduction

IgA is the second most important isotype in serum, with secretory IgA being predominant in gut lumen, where its functions range from host-commensal symbiosis to protection against enteric pathogens. IgA contributes to intestinal homeostasis, either by directly inhibiting bacterial virulence features or by shaping microbiota composition and promoting symbiosis between bacteria (Forbes et al., 2008, 2011; Okai et al., 2016; Nakajima et al., 2018). Indeed, IgA-deficient mice and humans show increased susceptibility to gastrointestinal infections and inflammatory diseases, such as celiac disease or inflammatory bowel disease (Harriman et al., 1999; Johansen et al., 1999; Ludvigsson et al., 2016). Gut microbiota drives IgA production since germ-free mice show a drastic reduction in mucosal IgA-secreting B cells (Moreau et al., 1978). Most intestinal commensals are IgA-coated *in situ*, as shown by combined flow cytometry sorting and 16S ribosomal DNA sequencing (Kawamoto et al., 2012; Palm et al., 2014; Bunker et al., 2015; Kau et al., 2015). IgA predominantly binds colitogenic and commensal bacteria of the small intestine (Palm et al., 2014; Bunker et al., 2015). Mouse microbiota-reactive IgA antibodies were shown to target a broad pattern of commensals at the clonal level (Okai et al., 2016; Bunker et al., 2017).

Recent observations in mice highlighted a role for several commensals in the induction of serum IgA, binding in return a restricted fraction of gut microbiota and providing protection to polymicrobial sepsis (Wilmore et al., 2018). Humans, unlike mice, express two subclasses of IgA, IgA1 and IgA2. Whereas IgA1 is predominant in serum, IgA2 is mainly secreted in mucosal compartments (Chiba et al., 1987; Pakkanen et al., 2010). The abundance of the two IgA isotypes varies throughout the intestine (Chiba et al., 1987; Pakkanen et al., 2010). Peyer's patches contain more IgA1⁺ B cells than lamina propria, whereas, in contrast, colon contains mainly B cells that secrete IgA2, a subclass endowed with enhanced resistance to the action of bacterial proteases, as compared to IgA1 (He et al., 2007). In general, IgA2 class switching occurs through a CD4⁺ T cell-independent pathway, while IgA1 requires the activation of B cells by CD4⁺ T cells through CD40-CD40 ligand interaction and the secretion of TGF- β (He et al., 2007; Coffman et al., 1989). It is widely accepted that microbial capsular polysaccharides and lipopolysaccharides are major targets of commensal-specific antibodies derived from T-cell independent pathways (Bunker et al., 2015; Stowell et al., 2014). It could thus be speculated that the IgA2 repertoire might contain a broader spectrum of microbial carbohydrate-specific antibodies. Overall, the extent to which each of these two subclasses interacts with intestinal commensals along the intestine remains enigmatic.

We compared polyclonal reactivity of secretory IgA1 and IgA2 towards ileum and colon human microbiota, and further investigated the specificity of IgA2 at the clonal level. Our results argue that human IgA antibodies are both promiscuous and selective in their interaction with the microbiota.

Results

Broad but differential glycan recognition by serum IgA1 and IgA2

To decipher the repertoire of carbohydrate-specific IgA, we used glycan microarray technology. Comparison of the glycan recognition profiles of pooled human IgA1 and IgA2 revealed a broad recognition pattern of carbohydrate structures by both classes of antibodies, although only 15 glycans were co-targeted by both IgA1 and IgA2 (Figure 1A and B).

The terminal carbohydrate moiety has been shown to be a critical determinant of glycan-specific antibody responses (Schneider et al., 2015) which could explain the lack of overlap of IgA1 and IgA2 carbohydrate repertoires. Glycan microarray screening of pooled human serum-derived IgG revealed prominent recognition of galactose- α (Gal α)-terminated glycans, which include major xenoantigens (Griesemer et al., 2014), but limited binding to sialoglycans containing human N-Acetylneuraminic acid (Neu5Ac) or non-human N-Glycolylneuraminic acid (Neu5Gc) (Schneider et al., 2015). Strikingly, Gal α -terminated glycans were almost exclusively recognized by IgA2 (Figure 1C). Preferential recognition by IgA2 was observed for both Neu5Ac- and Neu5Gc-terminated sialoglycans, while N-Acetylglucosamine (GlcNAc)-terminal glycans were equally bound by IgA1 and IgA2. By consulting the Bacterial Carbohydrate Structure Database (BCSDB), we determined that among the 610 glycans on the microarray, 137 structures were identified as bacterial antigens, including 16 Gal α -terminated structures. We observed that bacterial antigens, excluding Gal α -terminated structures (n=121), had a similar recognition profile when compared to the entire glycan microarray dataset (Figure 1D). Thus, the overall structure of the glycans has an impact on the induced IgA1 and/or IgA2 immunogenicity. These results also demonstrate that IgA2 widely recognizes bacterial antigens and that this phenomenon is more evident for Gal α -structures.

Finally, we noticed that three out of the ten host glycans displayed on the microarray, known to serve as attachment sites for bacteria in various mucosal compartments (Schneider et al., 2015), were preferentially bound by IgA2, as compared to IgA1 (Figure 1E). The latter

findings support the conclusion that IgA tempers bacterial activity, not only through direct interaction with their surface antigens, but also by interfering with their host ligands. More host attachment sites should be tested in order to determine whether IgA2 is preferentially involved in recognizing host glycans used for bacterial adhesion.

The secretory component modestly contributes to glycan binding

The above study was conducted with serum IgA. However, in gut lumen, dimeric IgA is associated to the secretory component that mediates binding to Gram-positive bacteria irrespective of antibody specificity. Using glycan array technology, we assessed secretory component binding to carbohydrates, and found 27 glycans that were specifically recognized by the secretory component (Figure S1A). These results point out that, *in vivo*, the secretory component may further enlarge the spectrum of IgA binding to microbiota, however, the glycan binding pattern remains much wider for IgA alone, than for the secretory component.

Gut bacteria are predominantly targeted by IgA2

We observed partial overlap of microbial carbohydrate reactivity between serum IgA1 and IgA2, while IgA2 was found to exhibit a broader spectrum of microbial specificities. We attempted to test whether the carbohydrate reactivity patterns observed with IgA1 and IgA2 reflected microbial recognition *in vivo* using bacterial flow cytometry analysis (Moor et al., 2016). Because gut contains not only the highest concentration of microbial antigens, but also both subclasses of IgA, we investigated the extent of secretory IgA binding to gut commensals. We used isotype-specific secondary antibodies to probe naturally IgA-coated microbiota of healthy donors. We first ruled out cross-reactivity of anti-IgA1 and anti-IgA2 antibodies by probing human B cells that are known to express only one antibody isotype and determined that virtually no double-positive cells were present when B cells were incubated with fluorescent anti-IgA1 and anti-IgA2 antibodies (Figure S1B). Using these reagents, we observed that IgA2 accounted for most commensal binding in fecal microbiota of healthy donors (Figure 2A, upper panel).

Convergent IgA1 and IgA2 responses

While IgA1 bound the same commensal bacteria as IgA2, other commensals were targeted by the latter isotype only (Figure 2A). Whereas the frequency of IgA2⁺IgA1⁺ and IgA2⁺IgA1⁻ fractions varied among individuals (1.95[0.12-7.27]% vs 3.19[0.69-17.99]% of all fecal bacteria; Figure 2B), the IgA2⁺IgA1⁺ fraction was always negligible (0.08[0-1.00]%; Figure

2B). Flow cytometry analysis showed that IgA-coated bacteria segregated into two fractions, depending on median fluorescence intensity: IgA^{low} and IgA^{bright} staining. Dual staining by IgA1 and IgA2 was associated with the IgA^{bright} fraction, whereas the IgA^{low} fraction corresponded to IgA2⁺IgA1⁻ bacteria (Figure S1C). Because ileal Peyer's patches contained more IgA1⁺ than IgA2⁺ B cells (He et al., 2007), we also profiled total IgA and IgA subclass binding in ileal contents. As expected (Bunker et al., 2015), we found higher proportions of IgA-coated microbiota in the ileum than in the fecal compartment (53.6[44.6-79.4]% vs 9.15[1.61-34.6]%;- Figure S1D). A large proportion of ileal commensals were bound by both IgA1 and IgA2 (37.4[19.2-55.2]%; Figure 2A lower panel), indicating that the convergence of IgA1 and IgA2 responses is compartment-independent.

Convergent IgM and IgA responses

We observed significant IgM staining (IgM coated bacteria $\geq 1\%$) in the fecal microbiota of only five out of 20 healthy donors (0.32[0.04-7.51]%, Figure 2C) in line with observations suggesting that IgM binds mucosal (Magri et al., 2017), rather than luminal (Fadlallah et al., 2018), communities in colon. Among these five fecal samples and five other ileal samples, we searched for IgM, IgA1 or IgA2 overlapping binding. Like IgA-coated bacteria, IgM-coated bacteria were more abundant in the ileum. Indeed, the frequency of IgM-coated bacteria exceeded 20% in three out of five ileal specimens (24.10[0.65-74.71]%, Figure 2C). We found that IgM-bound bacteria were also largely bound by both IgA1 and IgA2 (Figure 2D-F). Hence, IgM converge toward IgA-bound microbes, both in the ileum and in the colon lumen.

IgA2 also predominates in early life

A constant ratio of IgA1 to IgA2 has been reported in serum throughout childhood, which implies that there is no delay in the production of one isotype as compared with the other (Schauer et al., 2003). However, little is known about the establishment of secretory IgA subclasses in early life. Because analysis of IgA-coated bacteria is complicated by the presence of breast milk-derived IgA during the first months of life, we selected fecal samples from 8 formula-fed neonates (age range 5[3-5] months) and quantified their proportions of IgA-, IgA1- and IgA2-bound microbiota. Like in adults, mostly IgA2⁺IgA1⁺ and IgA2⁺IgA1⁻ bacteria were detected, while IgA2⁻IgA1⁺ cells were rare among neonatal fecal microbiota (3.23[0.69-6.7]%, 3.96[2.94-32.8]% and 0.08[0.00-2.08]%, respectively; Figure 2G), suggesting that both autologous IgA1 and IgA2 are very early on involved in the coating of

the developing microbiota. To corroborate these findings, we took advantage of a single colon tissue specimen obtained from a three-month infant and verified its IgA1 and IgA2 B cell content. As expected, lamina propria IgA2⁺ B cells were more abundant than IgA1⁺ B cells in this sample (1.4% vs 0.4%, Figure 2H). Thus, like in adults, the IgA2 isotype predominates in infants, although IgA1 nevertheless co-binds IgA2-coated bacteria.

Inter-individual variability of IgA1 and IgA2 binding profiles

We next analyzed the composition of subclass-bound bacteria in fluorescent-activated cell-sorted IgA1⁺ and IgA2⁺ populations and identified bacterial species by 16S rRNA sequencing. Of note, as all IgA1-coated bacteria were also bound by IgA2, the IgA1-sorted fraction included only IgA2⁺IgA1⁺ coated bacteria, whereas the IgA2-sorted fraction both contained IgA2⁺IgA1⁺ and IgA2⁺IgA1⁻ (referred as IgA2⁺IgA1^{+/-} in the text), with IgA2⁺IgA1⁻ bacteria being predominant (IgA2⁺IgA1⁻/IgA2⁺IgA1⁺ ratio >2). We first examined the whole microbiota composition and found inter-individual variability at phylum and genus levels (Figure S2B-D). The microbial diversity, as well as bacterial composition, differed between donors, as shown by the Shannon index (2.98[2.85-3.30], Figure S2C). With respect to the sorted fractions, within a single donor IgA1 and IgA2 binding identified distinct patterns of bacteria at the family level. For example, in a given healthy donor, *Bifidobacteriaceae* were enriched in the IgA2⁺IgA1⁺-coated fraction, while *Ruminococcaceae* were predominant in the IgA2⁺IgA1^{+/-}-enriched fraction, as compared to the whole microbiota of the same donor (Figure S2E). We also noticed inter-individual variability of IgA1 and IgA2 binding profiles at the family level, suggesting that the preferred targets of IgA1 and IgA2 are dependent on microbiota composition rather than on microbial phylogeny (Figure 3A ; Figure S2E).

We next focused on the genus level and determined the breadth of bacteria coated by IgA1 and IgA2, by comparing the relative abundance of bacteria genera in each of the fractions. Genera were present at similar frequencies in both IgA2⁺IgA1⁻ and IgA2⁺IgA1^{+/-}-sorted fractions (median of genera relative abundance 0.00048[0.00018-0.0038] vs 0.0019[0.00041-0.00276], respectively, p=0.81; Figure 3B). Moreover, the two fractions did not significantly differ from input microbiota in terms of bacterial diversity (Shannon index 2.36[1.3-2.88], p=0.19 vs 2.31[1.97-2.86], p=0.13, respectively; Figure S2C), indicating that both IgA subclasses coat highly diverse commensals.

Core IgA1⁺IgA2⁺ microbiota among an extended IgA2⁺ bacterial repertoire

The results thus far indicate that IgA2⁺IgA1⁻ and IgA2⁺IgA1^{+/-}-sorted fractions are diverse. Their bacterial composition is not only different between donors (Figure 3A-B), but also distinguishes itself from the input fraction within a single donor (Figure 3C). Thereafter, we narrowed the analysis down to the 19 most frequent genera that were present in at least 80% of the IgA coated fractions. To unravel IgA subclass specificities for microbes, we compared IgA2⁺IgA1⁻ and IgA2⁺IgA1^{+/-}-sorted fractions by calculating log-based enrichment indexes (see calculation details in Methods). This calculation allows to deduce bacteria preferentially targeted by IgA2 alone, compared to dually coated bacteria. Eleven genera were preferentially counted in the IgA1⁺IgA2⁺ subset, while eight genera tended to be more frequent in the IgA2⁺IgA1^{+/-}-coated bacteria (Figure 3D). Interestingly, genera derived from Actinobacteria (*Rhodococcus*, *Bifidobacterium* and *Arthrobacter*) were all enriched in dually stained bacteria. Instead, genera derived from Bacteroidetes (*Prevotella*, *Flavobacterium* and *Bacteroides*) were preferentially found in the IgA2⁺IgA1^{+/-} fraction (Figure 3D), particularly *Flavobacterium* (IgA2⁺IgA1^{+/-} enrichment index 1.69[0.28-2.23] vs IgA2⁺IgA1⁺ enrichment index -0.11[-2.63-0]; p<0.05; Figure 3E).

We sought to validate these observations by staining microbiota and representative bacterial strains with IgA purified from maternal milk. The latter antibodies are more abundant and might be taken as representative of gut IgA, as in mammary glands, IgA plasma cells are mainly derived from gut B cells, in agreement with the observation that some breast milk antibodies recognize gut antigens (Lindner et al., 2015). Moreover, flow cytometry analysis is facilitated since breast milk contains equal quantities of both subclasses, in contrast to free fecal IgA that comprise mainly IgA2 (Ladjeva et al., 1989). We first confirmed that breast milk-IgA antibodies targeted microbiota from an IgA-deficient patient (Figure S3A). We then confirmed that both IgA1 and IgA2 bound *Bifidobacterium longum*, while *Bacteroides vulgatus* was only recognized by IgA2 (Figure 3F).

Taken together, these results demonstrate that both IgA subclasses bind a diverse spectrum of commensals. In the small intestine IgA1 targets the same commensal bacteria as IgA2 in a balanced manner, while in the colon IgA2 preferentially targets several genera (typically derived from *Bacteroidetes*).

Both IgA1 and IgA2 induce cytokine production

Because IgA1 simultaneously binds IgA2-coated bacteria, we investigated whether IgA1 and IgA2 differ in their function. Several compelling arguments support a role of IgA through

Fc α RI expressed on monocytes and macrophages in mucosal immunity (Aleyd et al., 2015; Hellwig et al., 2001; Balu et al., 2011). We therefore determined how IgA subclass opsonization affected the production of cytokines by macrophages. We selected *Staphylococcus haemolyticus* as a common commensal recognized by both IgA1 and IgA2 (Figure 3D) and incubated free or IgA-opsonized bacteria with monocyte-derived macrophages. In accordance with previous reports (Hansen et al., 2017), we observed that IgA opsonization strongly enhanced cytokine production by these cells (Figure S3B). Both IgA1 and IgA2 increased the production of IL-6 and TNF- α , yet IgA1 tended to induce more IL-10 than IgA2.

Human gut lamina propria-derived monoclonal IgA

Colonic bacteria are thus predominantly bound by the IgA2 isotype. To address the issue of IgA2 specificity for human gut microbiota at a single-cell level, we generated fully human monoclonal IgA antibodies (mAbs) derived from the colonic lamina propria of three healthy donors. We transduced highly purified intestinal IgA⁺ memory B cells with a Bcl-6/Bcl-xL-expressing retrovirus (Kwakkenbos et al., 2010), resulting in the establishment, under limiting dilution conditions, of long-term clonal B cell cultures (Figure S4A). Each B cell line was self-renewing and showed a stable expression of IgA and activation markers CD38 and CD95, for the duration of the study (Figure S4B). Eight monoclonal IgA antibodies of unrelated sequences (Table S1) were detected as dimeric IgA in culture supernatants (Figure S4C). Previously characterized monomeric human IgA2 clones directly obtained by single cell PCR amplification of the variable Ig genes of isolated colonic cells (Benckert et al., 2011) were also tested.

Discrete bacterial populations are brightly stained by gut-derived IgA antibodies

To characterize the commensal reactivity of these IgA mAbs in the absence of competing interference by naturally occurring IgA, we selected an IgA-deficient microbiota (coined #A) in order to probe antibody/bacteria interactions. It has previously been shown that microbiota complexity is maintained in IgA-deficient patients, without massive perturbations of gut luminal microbes (Fadlallah et al., 2018). All eight IgA mAbs bound restricted microbiota fractions (Figure 4A-B). However, both staining intensity (range of median fluorescence intensity: 2080-10724) and frequency of bound-bacteria (0.79-1.66% of mAb-coated bacteria) differed between mAbs. Neither the frequency of mAb-coated bacteria, nor that of somatic mutations, was correlated with the staining intensity (Figure 4C).

IgA binding to microbiota is mostly Fab-mediated

To test whether gut-derived IgA mAbs interacted in a Fab or Fc-dependent manner, we first tested an irrelevant human anti-trinitrophenol (TNP) IgA. The latter did not bind to microbiota, suggesting that gut-derived antibody binding might be mostly Fab-mediated (Figure 4A). However, we speculated that the anti-TNP IgA used could possibly bear a different glycosylation pattern compared with gut-derived IgA that may account for its lack of microbiota interaction. We therefore also investigated gut IgA2 mAbs bearing IgG1 constant domains (Benckert et al., 2011). Before measuring their reactivity to microbiota, we first ruled out the presence of IgG-bound bacteria in another IgA-deficient microbiota (microbiota #B), in agreement with the lack of IgG transport to the intestinal lumen (Figure S4D). Although their microbiota avidity might be lower as compared to parental dimeric IgA, all but one IgA2 Fab/IgG1 Fc monomeric chimeras bound fecal microbiota (0-1.13% of mAb-coated bacteria; Figure 4D-E). In line with results obtained with dimeric IgA mAbs (Figure 4C), the frequency of monomeric mAb-coated bacteria was not correlated with staining intensity (Figure 4F). Together, these results demonstrate that the IgA mAbs tested bind microbiota in a Fab-dependent manner.

Intestinal IgA clones target highly diverse commensal bacteria

To identify commensal bacteria bound by individual monoclonal IgA, we characterized mAb-bound (mAb⁺) sorted fractions by 16s rRNA gene sequencing (Figure 4G). All mAbs tested recognized the four major phyla, namely *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*, but each one exhibited a unique binding profile (Figure 4H). Genus-level analysis corroborated these distinct binding patterns and highlighted IgA cross-reactivity with numerous genera, irrespective of their microbial phylogeny (Figure 4I-J). Both series of mAbs, generated either with IgA2 or IgG1 Fc-domain, bound various genera (Figure 4I-J). These data therefore indicate that a single IgA might recognize a wide panel of bacteria. Because mAb reactivity extended to unrelated genera, we tested whether genus abundance might influence mAb binding patterns. We found that genera in mAb⁺ and mAb⁻ fractions did not differ in terms of relative abundance (median of genera relative abundance 0.00123[0.000284-0.0056] in the mAb⁺ fraction vs 0.0008[0.00026-0.00159] in the mAb⁻ fraction, p=0.27; Figure 4K). Moreover, 12 out of the 50 most abundant genera (*Eggerthella*, *Bacteroides*, *Parabacteroides*, *Anaerostipes*, *Kingella*, *Neisseria* and *Pelomonas* in microbiota A and *Tissierella*, *Peptostreptococcus*, *Paenibacillus*, *Microbacterium* and

Helcococcus in microbiota B) were not targeted by the tested mAbs (Figure S4E-F), thereby implying that IgA antibodies react with various commensal microbes regardless of their bacterial richness. As expected, mAb binding profiles included *Ruminococcus*, *Roseburia*, *Clostridium* and *Blautia* previously described as common IgA targets in humans (Figure 4I-J) (D'Auria et al., 2013; Palm et al., 2014; Magri et al., 2017).

IgA clones exhibit private microbiota binding patterns

The mAb binding patterns were characterized in a quantitative manner by calculating a log-based enrichment index (EI) at genus level, followed by the application of a hierarchical clustering algorithm (Figure S4E-F). All mAbs were highly cross-reactive against a median of 19 genera among the 50 most abundant (range: 8 to 27, Figure S4E-F). Neither frequency of somatic mutations, nor staining intensity, correlated with the number of target genera, according to positive enrichment indexes. In addition, enrichment indexes varied among the target genera of the mAbs, indicating that they might bind variant genera with different affinities. Higher enrichment indexes could also be accounted for, in case a mAb recognized more species within a genus.

To confirm the validity of the mAb targets identified by 16S rRNA sequencing, we also compared mAb IgA binding to representative bacterial strains isolated from human fecal samples. We previously calculated that only mAbs #1, #2, #3, #9 and #10 were associated with positive *Staphylococcus* enrichment indexes, while mAbs #4 and #7 were not. Accordingly, the latter two clones did not bind *Staphylococcus aureus*, nor *Staphylococcus haemolyticus* (Figure S4G). Interestingly, while mAbs #1, #2 and #3 indeed bound both bacteria, mAbs #9 and #10 only bound *Staphylococcus haemolyticus*, supporting the conclusion that some IgA, such as mAbs #9 and #10, target only distinct species within a genus.

In summary, we conclude that IgA clones recognize a broad spectrum of commensal species regardless of their phylogeny, while each of the mAbs generated in the present study exhibited a private binding profile.

Private glycan cross-reactive patterns of IgA clones

We hypothesized that shared structures between numerous bacterial species could contribute to the observed IgA cross-reactivity. Commensal bacteria express a wide diversity of glycans, and it is frequently assumed that bacterial surface carbohydrates are among the common targets of intestinal IgA (Bunker et al., 2017). Moreover, glycosylation is a very common

post-translational modification that might create shared epitopes between various bacterial species, independently of their phylogeny (Grangeasse et al., 2015; Macek et al., 2008; Bastos et al., 2017). We therefore extended our analysis by screening human IgA mAbs on a glycan microarray displaying 660 different synthetic carbohydrate structures. Figure 5A illustrates the binding profile for mAb#1 expressed as mean relative fluorescence units (RFU). For 17 glycans, for which binding was above background, the antibody binding ratio (ABR) of human IgA mAbs was calculated relative to an isotype-matched control mAb, followed by hierarchical clustering analysis (Schneider et al., 2015). Five glycan structures were specifically recognized by the mAbs tested (Figure 5B-C). It is noteworthy to mention that three out of the five structures are epitopes of known bacterial origin (Sugahara et al., 1979; Arbatsky et al., 2000; Bystrova et al., 2000; Tsepilov and Beloded, 2015). Only two glycans (804, (Gal β 1-1GlcNAc β -OCH₂CH₂)₂ ; 630, (GlcA β 1-3GlcNAc β 1-4)₂₀) were bound by all mAbs, albeit with different intensities. These results do not imply that IgA2 would only bind glycan antigens, but interestingly, all mAbs tested were glycan cross-reactive. Concurrently, each IgA2 clone tested exhibited a unique glycan-binding pattern.

Bacterial glycans contribute to IgA/microbiota interaction

The observations described above confirmed that IgA exhibits bacterial and glycan cross-reactivity, but do not infer that IgA binding to microbiota is mainly due to glycan recognition. In order to evaluate whether glycans indeed play a role at the IgA/microbiota interface, we sought to compare IgA binding to microbiota before and after deglycosylation. As shown in figure 5D, enzymatic procedure removal of glycans on gut bacteria resulted in a marked drop in the interaction with monoclonal IgA (median of decrease 23[15-28] %), indicating a contribution of carbohydrates in the binding. Since much remains to be uncovered concerning glycosylation in prokaryotes, we assumed that the panel of enzymes we used on whole microbiota likely lead to only partial deglycosylation of the latter, and a possible underestimation of glycan involvement with IgA binding. We therefore further explored the role of glycan in IgA binding at the bacterial strain level. Since mAb#9 and mAb#10 recognized *S. aureus* but not *S. haemolyticus* (Figure 5E), we purified their surface glycans and found that both mAbs bound purified peptidoglycan, but not teichoic acid from *S. aureus*. As expected, no binding to *S. haemolyticus* peptidoglycan was observed. Together, these observations confirm that glycans do play a role in IgA binding to microbiota.

Cross-reactive IgA bear features of antigen-driven affinity maturation

The above observations all support the view that IgA binds diverse bacterial species. This cross-reactive pattern raised the question of IgA affinity maturation. Cross-reactive antibodies are thought to derive from T cell-independent responses, which generate low affinity antibodies, referred to as “innate” antibodies, as compared to “classical” antibodies that are thought to originate from T cell-dependent responses, exhibiting both elevated numbers of somatic mutations and high affinity (Pabst, 2012). We observed high numbers of somatic mutations in the Ig gene encoding the variable heavy (VH) chain domain of the IgA clones tested. As shown, higher ratios of non-silent to silent mutations in the complementary determining regions (CDR) 1 and 2 (median 5.5[0-29.5]) than in the framework regions (FWR) 1-3 (median 2.3[0.3-15]; Figure S5A) are consistent with an antigen-mediated selection process. Thus, in the gut, the affinity maturation process gives rise to cross-reactive IgA.

A fraction of gut-derived IgA is self-specific

Self-reactive IgA⁺ plasmablasts account for up to 15% of the normal intestinal plasmablast population (Benckert et al., 2011). To determine whether the microbiota-reactive mAb tested here might also react with self-antigens, we performed indirect immunofluorescence assays with the human epithelial cell line HEp-2000. We first established, as a positive control, that free fecal IgA reacted with nuclear antigens expressed by HEp-2000 cells (Figure S5B), thereby confirming that under homeostatic conditions some intestinal B cells do secrete antibodies with specificity for self-antigens. However, among the sixteen mAbs tested, only one showed a nuclear staining pattern (Figure S5B). Collectively, these results suggest that intestinal IgA favors microbiota reactivity over self-antigens.

Discussion

In the present study, we show that human intestinal IgA binds, at the clonal level, a wide subset of microbiota, including commensals from the four most frequent phyla. This reactivity extended to numerous genera, regardless of their phylogenetic distance or their relative abundance. One out of sixteen mAbs that were generated from intestinal B cells displayed both microbiota-reactivity and self-reactivity, resembling the proportion of 15% self-reactive plasmablasts described in the lamina propria (Benckert et al., 2011). While the molecular basis for this broad antibody reactivity spectrum remains unclear, it has been proposed that IgA binding may involve different affinity interactions with variant antigens of

commensal species (Pabst, 2012). Although we could not reliably measure the affinity of antibodies to bacterial cells, we observed (i) a wide scale of staining intensity and (ii) variations across enrichment indexes in mAb⁺ fractions that strongly support low to high-affinity interactions with commensals. Of note, IgA cross-reactivity does not imply random interactions: on the contrary, IgA interactions appeared rather selective since, for instance, *Staphylococcus haemolyticus* evaded some, but not all, IgA antibodies that target *Staphylococcus aureus*.

Recent studies report the identification of highly abundant clonotypes in peripheral memory, as well as lamina propria, B cells, that are cross-reactive (Lee et al., 2016; Rollenske et al., 2018). Quite remarkably, these antibodies are extensively mutated. Here, we found somatic mutation rates comparable to those of IgA⁺ memory B cells (Berkowska et al., 2011, 2015). Furthermore, replacement/silent ratios point to an antigen-mediated selection, indicating that intestinal cross-specific IgA are likely to originate from an affinity-maturation process. We therefore hypothesize that somatic mutations contribute to the breadth of commensals bound by intestinal IgA, even though reverted murine mAb were reported to remain poly- and microbiota-reactive (Bunker et al., 2017). Consistent with this hypothesis, germline counterparts of human cross-reactive antibodies against *K. pneumoniae* reportedly lose their binding signature (Rollenske et al., 2018).

It has been previously shown that mouse IgA antibodies react with carbohydrate epitopes (Peterson et al., 2007; Bunker et al., 2017). Our glycan microarray screening data corroborate and extend these observations to humans and further reveal that IgA, and in particular IgA2 antibodies, recognize a broad range of glycans. Although glycan microarray technology allows for a large number of carbohydrate moieties to be simultaneously assessed, it is still representative of a relatively narrow subset of naturally occurring glycans. In this light, it is remarkable that we observed specific positivity for all human IgA mAbs tested, and the observed glycan cross-reactivity is of considerable significance as well. Indeed, glycosylation is a key post-translational protein modification that has the capability to modulate protein interactions and the comprehension of this process might provide insight in evolutionary conservation. Studies relating to bacterial protein post-translational modifications have identified striking similarities across species, despite their phylogenetic distance (Macek et al., 2008; Grangeasse et al., 2015), reminiscent of our finding that IgA-derived mAbs bind bacteria across genera. While phylogenetically closer species present more overlapping post-translational modifications, environmental cues modulate them among bacteria (Macek et al., 2008; Grangeasse et al., 2015; Bastos et al., 2017). It seems likely that

intestinal conditions influence alterations in protein post-translational modifications with effects on bacterial fitness and bacterial immunogenicity (Ribet and Cossart, 2010; Butler et al., 2015). Likewise, the gut environment might favor post-translational pathways that enhance IgA-microbiota interactions.

Our observations raise the issue of the respective functional roles of IgA1 and IgA2 and the respective impact they may have on microbiota diversification along the gastrointestinal tract. We show that both IgA1 and IgA2 induce the production of pro-inflammatory cytokines, while the regulatory cytokine IL-10 was preferentially induced by IgA1-coated bacteria in vitro (Figure S3). While IgA2⁺IgA1⁺ bacteria are predominant in the ileum, we observe that IgA2 accounts for most colonic commensal binding. Three genera belonging to the phylum Bacteroidetes (*Bacteroides*, *Prevotella* and *Flavobacterium*) are preferentially recognized by IgA2 in the stool samples. One tentative explanation for this finding might be the longer transit time and the availability of complex polysaccharides in the colon that facilitate the growth of anaerobes such as *Bacteroidaceae* and *Prevotellaceae* (Donaldson et al., 2016). In contrast, bacteria coated by both IgA1 and IgA2 such as *Clostridium sp.*, and additional genera from *Actinobacteria* (e.g. *Bifidobacterium*) and *Proteobacteria* phyla (e.g. *Serratia*), are rather small intestine residents (Sekirov et al., 2010; Donaldson et al., 2016). These observations suggest that both IgA1 and IgA2 responses are elicited in the small intestine, while the colon would be the dedicated site for IgA2 responses. Bacteria coated by both subclasses in the colon may represent bacteria originating from the ileum. Indeed, while IgA1⁺ and IgA2⁺ B cells are found in similar proportions in the small intestine, IgA2⁺ B cells are preponderant in the colon (He et al., 2007). One could therefore speculate that IgA2 is essential for diversification of the colonic microbiota, whereas IgA1 is preferentially induced in the ileum.

IgA1 and IgA2 identified distinct patterns of bacteria within a single donor (Figure 3C), yet the five healthy donors analysed in this study only share isotypic binding trends (Figure 3D). The sorting strategy we adopted (*i.e.* comparing IgA1- and IgA2-sorted cells) introduced crude data noise, as IgA2-sorted cells are necessarily a mixture of IgA2⁺IgA1⁺ and IgA2⁺IgA1⁻ bacteria. Although the calculation of an enrichment index allows to deduce isotypic trends, not all subclass targets might have been described in this setting. The high inter-individual variability in microbiota composition might also have precluded the identification of more common IgA1 vs IgA2 targets. However, whereas this indirect sorting approach may, to a certain extent, dilute the signal, it does not invalidate any of the positive

results that have been detected. Future work combining more precise sorting (i.e. IgA2⁺IgA1⁺ vs IgA2⁺IgA1⁻ pure populations) in a larger healthy donor cohort might therefore identify more bacteria with differential isotype targeting.

Homeostatic secretory IgM responses have been described in humans but not in mice. Secretory IgM targets a fraction of mucus-embedded commensals that are also coated by IgA in human colon and ileum (Magri et al., 2017). These observations notwithstanding, we could detect fecal IgM-bound bacteria in luminal content of only five out of 20 healthy donors, underscoring the presence of an IgM-bound bacterial gradient from the epithelium to the lumen. IgM may help IgA to localize bacteria to be targeted in a favorable habitat in mucus. Indeed, IgM-bound bacteria are also recognized by IgA1 and IgA2. Consistent with this finding, Magri *et al.* noticed that IgM coats IgA^{bright} bacteria (Magri et al., 2017).

The mAbs tested for glycan reactivity were all of the IgA2 isotype. In an effort to better understand the molecular basis of the overlapping specificities of IgA1 and IgA2, we carried out a comprehensive investigation of the IgA anti-carbohydrate repertoire and highlighted a specialization for IgA2 in carbohydrate responses. Our analysis reveals a non-random association of glycan structural features with immunogenicity, especially directed against the monosaccharide moiety at the terminal position. High immunogenicity observed for glycans with terminal Gal α is interesting because this may reflect an important role for IgA2 in response to the microbiota. The finding that both Neu5Ac, a self-antigen, and Neu5Gc, a xenoantigen, showed similar binding profiles for IgA could possibly be explained by the structural similarity of the two sugars. It also supports the notion that terminal carbohydrate moieties influence the balance between immune response and tolerance, IgA being considered more tolerogenic than pro-inflammatory (Pasquier et al., 2005; Rossato et al., 2015). These results obtained by comparing serum IgA1 and IgA2, remain to be confirmed at the gut secretory IgA level.

In summary, we show that although IgA induction is antigen-dependent with highly mutated memory clonotypes, IgA microbiota binding patterns are broad, but nevertheless clonotype-specific. Similarly, at the polyclonal level, IgA2 and IgA1 targets are broad and partially overlapping, particularly in the ileum where IgA2⁺IgA1⁺ bacteria are prevalent among IgA-bound microbiota. In all cases, glycan reactivity did not only account for the observed cross-reactivity, but also for selectivity. IgA2 and IgA1 anti-commensal responses are observed as early as three months after birth, suggesting that both isotypes are induced during initial gut colonization by healthy microbiota.

Materials and Methods

Human specimens

Surgical samples from histologically normal ascending colon were obtained from colon cancer patients (Table S2) undergoing hemi-colectomy (Department of Surgery, Pitié-Salpêtrière hospital, Paris). Patients with a history of intestinal inflammation were excluded from the study. Ileostomy fluids were collected from intensive care unit patients (Table S2). Fresh stool and blood samples were collected from 20 healthy volunteers (Fadlallah et al., 2018). Fresh stools from three IgA deficient patients with undetectable serum IgA levels (<0.07 mg/ml) were obtained from the Department of Clinical Immunology at St Louis hospital, Paris (Fadlallah et al., 2018). Antibiotic therapy or diarrhea in the last three months were exclusion criteria in all instances. Maternal milk was obtained from three healthy donors. Stool samples, maternal milk and plasma were immediately frozen after collection and stored at -80°C until use. All individuals signed a written consent and the protocol was approved by the local ethical committee of the Pitié-Salpêtrière hospital.

Stool processing and microbiota purification

Fresh feces were aliquoted in a CO₂ rich – O₂ low atmosphere and stored at -80°C. Then microbiota were isolated by gradient purification under anaerobic conditions, as previously described (Juste et al., 2014). In brief, a density gradient of Nycodenz solution was prepared. Then, thawed stool was diluted in 1X-PBS (Eurobio), 60% Nycodenz, 0.03% sodium deoxycholate (NaDC) and loaded on the gradient. After ultracentrifugation (45min, 14567 x g, 4°C; Beckman Coulter ultracentrifuge), fecal bacteria were extracted and washed three times in 1X-PBS, 0.03% NaDC and centrifuged. The final bacterial pellet was diluted in 1X-PBS-10% glycerol, immediately frozen in liquid nitrogen and stored at -80°C.

Bacterial strains and culture conditions

Staphylococcus epidermidis, *Staphylococcus aureus* and *Staphylococcus haemolyticus* were isolated from human stool samples and identified by MALDI-TOF mass spectrometry (Microbiology department, Pitié Salpêtrière hospital, Paris). *Bifidobacterium longum* (E194v variantA) and *Bacteroides vulgatus* (NCTC11154) were collected and characterized at the Institut National de Recherche Agronomique (INRA ; Jouy en Josas, France). Bacterial strains

were cultured on sheep red blood agar plates at 37°C under aerobic (*Staphylococcus sp.*, for 24h) or anaerobic (*Bacteroides vulgatus* for 24h and *Bifidobacterium longum* for 48h) conditions. Individual colonies were picked, suspended in 1X-PBS – 10X glycerol (10^9 CFU/ml) and frozen at -80°C. Quantification of colony forming units (CFU) was performed by adding counting beads (Beckman Coulter) to bacterial suspensions on a flow cytometer (FACS Canto II™, BD).

Production of human mAbs

For IgA production from long-term clonal B cell cultures, B cells were isolated from colonic lamina propria dissected into 2 mm pieces and digested by the addition of collagenase in 1X-PBS (50mg/ml, Roche), then incubated at 37°C for 40 minutes (min). Lymphocytes were purified by centrifugation over Ficoll 400 (Eurobio) and stained with the following antibodies: anti-CD45 APC-H7, anti-CD19 BV421, anti-IgD FITC, anti-IgM BV605, anti-CD27 PE-Cy7 (all purchased from BD Biosciences) and anti-IgA PE (Jackson Immunoresearch). Dead cells were excluded with LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit (Invitrogen). CD45⁺CD19⁺CD27⁺IgD⁻IgM⁻IgA⁺ B cells were sorted on a FACS Aria II™ cytometer (BD). B cells were cultured for 4 days, in the presence of irradiated (50 Gy) mouse fibroblasts expressing CD40L, at a concentration of 10^4 cells per well (Arpin et al., 1995) and recombinant IL-21 (50 ng/ml, a kind gift of Dr Arjen Bakker, AIMM Therapeutics, Amsterdam, The Netherlands) in IMDM (LifeTechnologies), containing 10% FCS (Biowest) in a 96-well flat bottom plate. Retroviral transduction was performed with a Bcl-6 and Bcl-xL-expressing retroviral construct (AIMM Theapeutics), using the experimental conditions as described (Kwakkenbos et al., 2010). Briefly, activated B cells were incubated with retroviral supernatant and polybrene (Sigma, final concentration 4 µg/ml) in a 96-well flat bottom plate, coated with the recombinant human fibronectin fragment CH-296 (Takara) for 8h at 37°C. After washing, the cells were cultured for 4 days in the presence of irradiated CD40L-expressing mouse fibroblasts and recombinant IL-21 (50 ng/ml) in IMDM-10% FCS in 96-well flat bottom plates. Transduction efficiency based on GFP expression was monitored at day 4. B cells clones were then obtained by limiting dilution cultures and IgA were concentrated from culture supernatants with Amicon® centrifugal filters (Millipore).

Human mAbs obtained through single-cell PCR processing of isolated IgA-producing colonic lamina propria B cells expressed variable IgA domains fused to human IgG1 constant domains in HEK293T cells, as previously described (Benckert et al., 2011).

Ig gene sequence analysis

RNA extracted from B cell lines (Trizol, Ambion) was reverse-transcribed using random primers and SuperScript III (Invitrogen). Oligonucleotides used for VH and VL PCR are described in Table S3. Purified PCR products (GeneJET PCR Purification kit, ThermoFisher) were sequenced (ABI Prism 3730) and analysed using the ImMunoGeneTics information system® (<http://www.imgt.org>). Frequencies of non-silent to silent mutations in CDR1-CDR2 and FWR1-FWR3 were calculated for the *IGH* chain gene based on the total number of nucleotides analysed.

Bacterial flow cytometry

MAB binding to microbiota was assessed by bacterial flow cytometry assay, as previously described (Moor et al., 2016). All buffers were passed through sterile 0.22 µm-filters before use. Briefly, thawed microbiota or bacterial strains (10^6 / condition) were fixed in 500 µl paraformaldehyde (4% in 1X-PBS) and stained with Cell Proliferation Dye eFluor 450 (eBioscience) for 25 min at 4°C. After washing with 1X-PBS (10 min, 4000 x g, 4°C), bacteria were suspended in 1x-PBS, 2% BSA (Sigma), 0.02% sodium azide (Sigma) in a 96-well V-bottom plate. MAb or human monoclonal anti-TNP IgA (a kind gift of Dr Jönsson, Institut Pasteur, Paris, France) was added in a 96-well V-bottom plate at a final concentration of 1 µg/ml and the plates were incubated for 30 minutes at 4°C. After washing, goat anti-human IgA-FITC (1/400) or isotype control (both from Jackson ImmunoResearch Laboratories) were incubated for 20 minutes at 4°C. MAbs bearing a IgG-Fc portion were detected with goat anti-human IgG-A647 (1/400, Jackson ImmunoResearch). Quantification of *in vivo* IgA1 and IgA2 binding was performed in the same way. Fixed microbiota were incubated with mouse anti-human IgA1-FITC and mouse anti-human IgA2 Alexa Fluor 647 (Southern Biotech) or isotype controls for 20 minutes at 4°C. For IgA1 and IgA2 binding to pure bacterial strains, purified IgA from breast milk (final concentration 10 µg/ml) were added to 10^6 CFU of *Bacteroides vulgatus* and *Bifidobacterium longum* for 30 minutes at 4°C before the addition of anti-IgA1 and anti-IgA2 secondary antibodies. Then, bacteria were washed and re-suspended in sterile PBS. Samples were run using a BD FACS Canto II™ and 30000 bacterial events were acquired. Analysis was performed with Flow-Jo software (Treestar). Medians of fluorescence were used to measure mAb-binding levels for pure strains.

The same procedure was performed to assess the role of glycans in mAb-binding to microbiota. Prior to fixation, thawed microbiota (10^6 bacteria/ condition) were incubated with

a cocktail of enzymes (PNGase F, Endo-O-glycosidase, α -2(3,6,8,9)-Neuraminidase, β (1,4)-Galactosidase, β -N-Acetylglucosaminidase) that remove N- and O-linked carbohydrates for 1h30 at 37°C, according to manufacturer's recommendations (Enzymatic Protein Deglycosylation Kit, Sigma). After washing in 1X-PBS, microbiota was stained as described above.

Sorting of IgA-bound microbiota

Gut microbiota obtained from an IgA deficient patient was incubated with purified mAbs (final concentration 1 μ g/ml) in a 96-well V-bottom plate (10^7 bacteria/well, 10 wells/mAb) for 30 min at 4°C. After washing with 1X-PBS (10 min, 4000 x g, 4°C), cells were stained with goat anti-human IgA-FITC (1/200) or isotype control antibody (Jackson Immunoresearch Laboratories). For sorting of *in vivo* IgA1- or IgA2-bound commensal bacteria, purified fecal microbiota from 5 healthy donors were thawed, washed and directly stained with mouse anti-human IgA1-FITC or mouse anti-human IgA2 Alexa Fluor 647 (Southern Biotech) in a 96-well V-bottom plate (10^7 bacteria/well, 5 wells/subclass) for 20 min at 4°C. After washing, sorting was performed using a microbiota-dedicated single laser S3 cell sorter (Bio-Rad Laboratories, California, USA). Of note, while flow cytometry analysis is performed on fixed samples, combined flow sorting and DNA sequencing is performed on live bacteria in order to allow unbiased and optimal DNA extraction. Sorted bacteria (9.10^5) were collected in 1X-PBS at 4°C, centrifuged (8000 x g, 10 min, 4°C) and stored at -80°C until DNA extraction. Purity for both fractions was systematically verified after sorting. To check the absence of contaminants in flow cytometer fluid lines, sheath fluid was regularly incubated in Brain-Heart Infusion Broth (BioMérieux) at 37°C for 7 days.

16S rRNA gene sequencing and analysis

DNA was extracted using Trizol (Ambion), according to the manufacturer's instructions. Amplicons of V3-V4 regions of 16S rRNA genes were generated in a PCR mix containing, 5 μ l of extracted DNA, 500 units of MolTaq 16S (Molzym), 10mM dNTP (Invitrogen), 10 pmol/ μ l V3-external and V4-external primers (Table S3) in MolTaq buffer (Molzym) diluted in DNA-free water (Molzym) for a final volume of 50 μ l. 2 μ l of first round PCR reaction (95°C 10', 95°C 1'- 54°C 1'- 72°C 1' 10 times, final extension 72°C 10') were used as template in a second round PCR (95°C 10', 95°C 1'- 66°C 1'- 72°C 1' 10 times, final extension 72°C 10') with V3-V4 barcoded primers (Table S3). PCR products were purified

using paramagnetic beads (Agencourt® Ampure® XP, Beckman Coulter) and sequenced on a MiSeq instrument (Illumina) in a multiplexed sequencing run (paired-end 250 nucleotides reads) at iGenSeq ICM facility (Institut du Cerveau et de la Moelle Epinière, Paris, France).

De-multiplexed reads were processed using MG-RAST analysis pipeline. Sequencing artefacts, host DNA contamination and sequences less than 200 bp in length were removed. Insufficient quality reads were discarded (<5% of total reads). Sequences were then clustered into operational taxonomic units (OTUs) with a 97% homology using Greengenes database. OTUs containing only a single sequence were discarded. Previously published contaminant sequences (Salter et al., 2014) were removed if present in only one sorted fraction and absent from paired fractions. OTUs detected at >0.1% relative abundance in at least 2 samples were finally conserved. This process reduced the total OTU count from 313 down to 178. OTU table was rarefied to the minimum sample's depth (5000 reads). Shannon index was calculated according to the following equation: $Shannon\ index = - \sum p_i \ln(p_i)$ where p_i is the relative abundance of the i th OTU in the dataset. In calculating the enrichment index EI, we scored a pseudo-relative abundance equal to 0.0001, which was the lower limit of detection, if a taxon was not detected in a given fraction. Specificity of IgA1⁺IgA2⁺ targeting was calculated using the following formula: $\frac{Number\ of\ samples\ in\ which\ OTU\ had\ a\ positive\ IgA1IgA2\ Enrichment\ index}{Number\ of\ samples}$

while . Specificity of IgA2⁺ targeting was calculated with the formula: $\frac{Number\ of\ samples\ in\ which\ OTU\ had\ a\ negative\ IgA1IgA2\ Enrichment\ index}{Number\ of\ samples}$, in which IgA1IgA2 enrichment index refers to: $\log_{10}(\frac{IgA1+IgA2+taxon\ abundance}{IgA2+taxon\ abundance})$.

Purification and quantification of IgA

Breast milk was centrifuged at 4000 x g for 10 minutes to remove the fat content. Aqueous phase was diluted in PBS to achieve pH 7 and filtered through a 0.22 µm syringe filter. The breast milk suspension was loaded onto peptide M/agarose gravity flow column (InvivoGen) at a flow rate of 0.5ml/min. The column was washed with 20 column volumes of 1X-PBS. IgA were then eluted with 10ml of 0.1M glycine (pH 2-3, Sigma) and immediately neutralized to pH 7.5 with 1M Tris (pH 8.5, Sigma). Buffer exchange was performed by filtration through a 100 kDa membrane (Millipore). For free fecal IgA purification, fecal water were centrifuged twice at 13000 x g for 20 min at 4°C to eliminate debris and contaminants. After 0.22 µm filtration, IgA was isolated from fecal water as breast milk. Because IgA concentration is low in fecal water, the sample was loaded three times onto the column to increase efficiency.

For IgA1 and IgA2 purification, serum from 5 healthy donors was pooled and diluted in 1X-PBS (IgA final concentration: 0.5-1 mg/ml). First, IgA was isolated with a peptide M/agarose column, using the same process as that for breast milk. Then, purified IgA was loaded onto an immobilized Jacalin column (Thermoscientific). The column was washed with 10 column volumes of PBS: this fraction contained IgA2. The column was washed again with PBS until absorbance at 280nm reached the baseline (Nanovue, GE Healthcare) and 0.1 M α -D-galactose (Sigma) was added to the column to recover the bound IgA1. Buffer exchange was performed to remove galactose from IgA1 and to concentrate IgA2 with a 100 kDa ultrafiltration membrane (Millipore). The purity of the IgA1 and IgA2 fractions was assessed by ELISA. These antibody solutions were divided into aliquots and frozen at -20°C. The quantification of IgA in culture supernatants or after purification was determined using an ELISA quantitation set (Bethyl), according to the manufacturer's recommendations.

ELISA

Endocab[®] human IgA kit (Hycult[®]Biotech) was used to detect LPS reactivity of mAbs at 0.1 and 1 μ g/ml. Experimental procedure followed the manufacturer's recommendations.

500 ng of lipoteichoic acid from *S. aureus* (Sigma) was coated onto 96-well Nunc Maxisorp plate (Thermofisher) overnight. Free sites were blocked with 1X-PBS, 1% BSA for 30 min at room temperature. mAbs were incubated for 2 hours at room temperature at 0.1 and 1 μ g/ml. After 5 washes, HRP anti-human IgA (Bethyl) was added for 1h, followed by 3,3',5,5' Tetramethylbenzidine (TMB) substrate revealing reaction (Thermofisher). Absorbance was measured at 450nm.

Indirect Immunofluorescence assay

HEp-2000 slides (ImmunoConcepts) were incubated with 25 μ l of mAb at 2 μ g/ml for 25 minutes in a moist chamber at room temperature. A serum containing IgA anti-DNA served as a positive control and an irrelevant mAb culture supernatant as a negative control in all experiments. After washing with PBS, anti-human IgA-FITC (Inova Diagnostics) was added for 25 min in a moist chamber at room temperature. After washing, fluorescence patterns were immediately detected with a fluorescent microscope (Leica).

Glycan microarray analysis

Glycan microarray screening using Consortium for Functional Glycomics (CFG) version 5.3 slides or from Semiotik LLC, Moscow, Russia was performed as previously described (Schneider et al., 2015; von Gunten et al., 2009; Stowell et al., 2014). The CFG microarray screening was performed at the National Center for Functional Glycomics (NCFG) at Beth Israel Deaconess Medical Center (Boston, USA). Scanning of Semiotik slides was performed on a ScanRI Microarray Scanner (PerkinElmer, Waltham, MA, USA) with 5 μ m resolution. CFG glycan array data are published on the CFG website <http://www.functionalglycomics.org/glycomics/publicdata/primaryscreen.jsp>. mAbs were diluted in PBS and screened at 10 μ g/ml, if not stated otherwise; polyclonal IgA preparations were diluted in PBS and screened at 200 μ g/ml. Commercially available secretory component (Labforce, Muttens, Switzerland) was diluted in PBS and screened at 50 μ g/ml. Commercially available human myeloma IgA2 (400110, Sigma-Aldrich, Buchs, Switzerland) or mAb were used as controls. For the analysis of the CFG slides, the 90th percentile was used to indicate glycan significantly bound by IgA. For the analysis of the Semiotik slides, per recommendation of the manufacturer, RFU values five times higher than the empty spot negative control, were considered positive. The computed ABR is representative of the quotient of the respective sample RFU and the corresponding IgA control (Schneider et al., 2015; von Gunten et al., 2009). ABR intensities lower or equal to one were defined as unspecific binding. For purposes of analysis, ABR values lower than one were set to one. In order to assess preferential recognition by IgA1 or IgA2, the \log_{10} (IgA2/IgA1) was calculated. Glycans showing values less than -0.2 was characterized as being preferentially recognized by IgA1, while values higher than 0.2 was characterized as being preferentially recognized by IgA2 and values equal to or in between -0.2 and 0.2 was considered as showing no preferential reactivity.

Peptidoglycan (PG) and teichoic acid (TA) purification and dot blot assay

Five hundred mL of overnight culture of *S.aureus* or *S. haemolyticus* was centrifuged. The pellet was suspended and centrifuged twice in 40 mL 50 mM TrisHCl pH 7.4, 150 mM NaCl, 1% SDS and boiled for 10 min. The resulting pellet was suspended in 40 mL of 50 mM Tris pH 7.4, sonicated and centrifuged. The resulting pellet was again treated in 40 mL 50 mM TrisHCl pH 7.4, 150 mM NaCl, 1% SDS and boiled for 10 min. PG-TA was then further purified to eliminate all other known contaminants by the following successive treatments: digestion with DNase (1 mg/mL) and RNase (5 mg/mL) in MgSO₄ 20 mM followed by a trypsin (10mg/mL) digestion after addition of CaCl₂ (10 mM) for 12 hr. The resulting

suspension was boiled for 10 min in 1% SDS and centrifuged. The pellet was then suspended and centrifuged in (i) H₂O, (ii) 8M LiCl₂, (iii) 100 mM EDTA, (iv) twice in H₂O, (v) 46 % hydrofluoric acid and finally (vi) in H₂O. TA were recovered from the hydrofluoric acid supernatant after evaporation.

Twenty µg of purified PG and TA from *S. aureus* and *S. haemolyticus* were coated on nitrocellulose membrane (Bio-Rad). The membrane was blocked in 1X-PBS, 5% non fat milk for 30 min at room temperature and washed in 1X-PBS, 0.05% Tween 20. MAbs were then incubated for 30 min at room temperature at 2 µg/ml. After washing, mAbs were detected with horseradish peroxidase-conjugated goat anti-human IgG used at 1:40,000 (A0170; Sigma-Aldrich), followed by enhanced chemi-luminescence revealing reaction (Clarity™ Western ECL, Bio-Rad). All incubations were in 1X-PBS with 5% non fat milk and washing steps in 1X-PBS with 0.05% Tween.

Functional cellular assays

Peripheral blood mononuclear cells were isolated from blood samples by density gradient centrifugation over Ficoll-Paque (Eurobio). Monocytes for macrophage differentiation were isolated by plastic adherence as follows: PBMC were distributed into 25 cm² flask and allowed to adhere for 1 h at 37°C in RPMI (Gibco) supplemented with 10% fetal calf serum (FCS, Biowest). Non-adherent cells were removed and the adherent monocytes were washed twice with medium, detached with Versene (Invitrogen), counted, seeded in 96-well U-bottom plates at a density of 5.10⁴ cells/well and cultured in RPMI-10% FCS in the presence of 100 ng/mL hGM-CSF (Miltenyi Biotec, Paris, France) in a total volume of 100 µL. At day 6, macrophages were stimulated with 5.10⁵ *S. aureus*, which had been pre-incubated with 0.5 mg/mL of purified IgA1 or IgA2 and washed. After 24h, cytokine levels in supernatants were measured using the Cytokine 3-Plex A (IL-6, IL-10, TNF-α) immunoassay (Quanterix) relying on a Single Molecule Array (Simoa) technology run on a HD-1 Analyzer (Quanterix). Working dilutions were 1:500 in diluent sample. Lower limits of quantification for IL-6, IL-10 and TNF-α were 5.5, 3.65 and 25.5 pg/mL, respectively.

Statistical analysis

Statistical analysis was performed using Graphpad Prism® v6. Non parametric tests were used whenever necessary: Wilcoxon paired rank test was used when comparing paired groups, Mann-Whitney test when comparing two independent groups, Kruskal Wallis for

multiple comparisons. Significant p values ($p < 0.05$) are indicated on plots (* $p < 0.05$; ** $p < 0.01$; *** <0.001). Hierarchical clustering algorithms were run with Partek® software or “R” (The R Foundation for Statistical Computing, version 3.4.3).

Supplemental material

Fig. S1 presents secretory component glycan-binding pattern and additional characterization of gut-derived IgA reactivity. Fig. S2 further explores inter-individual microbiota variability. Fig. S3 presents milk-derived IgA reactivity and functional assays. Fig. S4 shows additional characterization of monoclonal binding patterns. Fig. S5 describes monoclonal self-reactivity. Table S1 presents Ig gene sequence analysis of dimeric antibodies. Table S2 lists additional demographic data of human specimens used in this study. Table S3 lists primers used for Ig and 16s rDNA genes amplification.

Author contributions

J.F., C.C., C.F., H.B., C.T. recruited patients. D.S, J.F, C.J, M.L., prepared the specimens from healthy donors. A.A. and F.J. provided bacterial strains or control antibodies. H.W. provided previously characterized monomeric human IgA clones bearing IgG1 Fc portion. D.S., J.F., C.P., K.D., A.R., G.A., J.L.C., F.J, T.C. designed and performed experiments. D.S., J.F., O.A., H.E.K performed data analysis. R.D.C. supervised glycan microarray analysis and reviewed the manuscript. D.S., O.A, H.Y., S.V.G. and G.G. designed the study, prepared the figures and wrote the manuscript.

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interests.

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Figure legends

Figure 1: Carbohydrate binding profile of polyclonal IgA1 and IgA2 antibodies

- A. Glycan reactivity of serum polyclonal IgA1 and IgA2 (n=5 healthy donors pooled in one experiment). Each peak represents an individual glycan recognized by IgA1 (blue line) or IgA2 (red line).
- B. Heatmap diagram depicting glycans recognized by IgA1 or IgA2. Each row represents an individual glycan.
- C. Preferential recognition of distinct terminal carbohydrate moieties by serum polyclonal IgA1, IgA2 or both. Terminal carbohydrate moieties equally recognized by both IgA1 and IgA2 are depicted in white. Terminal moieties preferentially recognized by IgA1 or IgA2 are showed in grey or in black respectively.
- D. Isotype-dependent recognition of Gala-terminal structures of bacterial origin and other bacterial antigens excluding Gala-terminal antigens (w/o Gala) by serum polyclonal IgA, as compared to entire glycan microarray data set. Terminal carbohydrate moieties equally recognized by both IgA1 and IgA2 are depicted in white. Terminal moieties preferentially recognized by IgA1 or IgA2 are showed in grey or in black respectively.
- E. Differential isotype binding to bacterial attachment sites.

Figure 2: IgA1 target IgA2-coated bacteria

- A. Representative flow cytometry analysis of endogenous IgA1 and IgA2 fecal or ileal microbiota coating.
- B. Endogenous IgA1 and IgA2 microbiota coating levels in 20 fecal and 5 ileal healthy samples. Data are cumulative from three independent experiments. Error bars represent minimum and maximum values. P values were calculated using Mann-Whitney test (colon vs ileum) or Wilcoxon test (* p<0.05; ***p<0.001).
- C. Endogenous IgM microbiota coating levels in 20 fecal and 5 ileal healthy samples (two independent experiments). Dark bars indicate medians. Red dotted line represent significant the positive cut-off. P value was calculated using the Mann-Whitney test (***p<0.001).
- D. Left panels: representative flow cytometric analysis of endogenous IgM-coated fecal or ileal microbiota. Right panels: representative flow cytometric analysis of

endogenous IgA1 and IgA2 binding in IgM-coated fecal or ileal microbiota (two independent experiments).

- E. Endogenous IgM microbiota coating levels in 20 fecal and 5 ileal healthy samples (two independent experiments). Error bars represent minimum and maximum values. P values were calculated using the Mann-Whitney test (** $p < 0.01$; * $p < 0.05$)
- F. Model Venn diagram showing overlap between endogenous IgA1, IgA2 and IgM binding in IgA-coated microbiota.
- G. Endogenous IgA1 and IgA2 microbiota coating levels in eight fecal samples from formula-fed neonates (two independent experiments). Error bars represent minimum and maximum values. P values were calculated using Wilcoxon test (* $p < 0.05$; ** $p < 0.01$).
- H. Flow cytometric analysis of IgA1 and IgA2 expression on colonic lamina propria B cells from a three-month-old infant (one experiment).

Figure 3: IgA1 coordinates with IgA2 to coat distinct commensal bacteria.

- A. Median relative abundance of the 4 most frequent families in sorted fractions from five healthy donors. Each dot represents one donor.
- B. Median relative abundance of genera from IgA1⁺IgA2⁺ and IgA2⁺ fractions from five healthy donors. Each dot represents one donor.
- C. Relative abundance of indicated top 19 most abundant genera in sorted fractions from one healthy donor.
- D. Specificity of IgA subclass targeting for all individuals analyzed (n=5). Number of samples in which a given genera had a positive indicated IgA subclass enrichment index, divided by the total number of samples. The formula is detailed in the Material and Methods section.
- E. Flavobacterium enrichment in IgA2⁺, as compared to IgA1⁺IgA2⁺ fractions. Each dot represents one donor. P-value defined using Wilcoxon test (* $p < 0.05$).
- F. Binding of purified breast milk IgA to indicated bacterial strains. Monoclonal IgA isotype control (grey-filled histogram) was included as negative flow cytometry control. The same experiment was repeated twice.

Figure 4: Human monoclonal IgA target highly diverse commensal bacteria.

- A. Representative flow cytometry plot of microbiota-reactivity for mAb#2 and human IgA2 anti-TNP. MAb#1 to #8 are gut monoclonal IgA2 expressed as dimeric IgA.

- B. MAb coating of IgA-free microbiota. MAbs are classified in increasing fluorescence intensity order. Median Fluorescence Intensity (MFI, range 2080-10724). The same experiment was repeated twice.
- C. Somatic mutations of mAbs are not correlated to IgA staining intensity. Somatic mutations in V-region of *IGH* gene were analysed. Non-parametric Spearman correlation was calculated.
- D. Representative flow cytometry plot of microbiota-reactivity for mAb#10 and human IgG1 anti-TNF α . MAb#9 to #16 are gut monoclonal IgA2 expressed as IgG1 (Benckert et al., 2011).
- E. MAb coating of IgA and IgG-free microbiota. MAbs are classified in increasing fluorescence intensity order. Median Fluorescence Intensity (MFI, range 3585-17683). The same experiment was repeated twice.
- F. Somatic mutations of mAbs are not correlated to mAb staining intensity. Somatic mutations in V-region of *IGH* gene were analysed. Non-parametric Spearman correlation was calculated.
- G. MAb⁺ and mAb⁻ fractions of IgA-free gut microbiota were sorted by flow cytometry and their composition was analysed by 16S rRNA sequencing.
- H. Relative abundance of phyla in whole microbiota (input) and mAb⁺ fractions. Microbiota #A is IgA-free, while Microbiota #B is IgA- and IgG-free. 16S rRNA sequencing data were from two independent experiments.
- I. Relative abundance of genera in whole microbiota #A (input) and mAb⁺ fractions.
- J. Relative abundance of genera in whole microbiota #B (input) and mAb⁺ fractions.
- K. Scatter dot plot of median relative abundance of genera from mAb⁺ and mAb⁻ fractions.

Figure 5: Microbial surface glycans are common targets of gut human IgA

- A. Glycan reactivity for five mAbs (5 μ g/ml) was assessed using glycan microarray technology (660 structures). Representative Median relative fluorescence units (RFU) of mAb1 is shown. Glycan ID numbers of top-bound glycans are indicated.
- B. The median RFU for top-bound glycans (above background) are shown for all five mAbs tested.
- C. The antibody ratio (ABR) of glycans showing positive reactivity (above background, as in I) was calculated using an isotype control and visualised in an ordered (by

dendrogram algorithm) reactivity matrix (heatmap). Each column represents a glycan. Color key is shown.

- D. Representative flow cytometric dot plot of microbiota-reactivity for mAb#6 after whole microbiota enzymatic deglycosylation (left). Decrease of mAb binding to microbiota after enzymatic deglycosylation (right). The same experiment was repeated twice.
- E. Representative dot blots of purified peptidoglycan (PG) and lipotechoic acid (TA) from *S. aureus* and *S. haemolyticus* probed with irrelevant IgG1 (anti-TNF α IgG1), mAb#9 and mAb#10 (grey, red and green circles respectively). MAb#9 and #10 were generated with IgG1 Fc domain. The same experiment was repeated twice.

Figure S1 (related to figures 1 & 2): Gut bacteria segregate into IgA^{bright} and IgA^{low} fractions in healthy humans

- A. The secretory component binds a modest range of carbohydrates. Glycan reactivity with the secretory component was assessed using glycan microarray technology (660 structures). Representative Median relative fluorescence units (RFU) is shown. Glycans specifically recognized by secretory component are colored in red.
- B. Anti-IgA1 and anti-IgA2 antibodies do not cross-react. Flow cytometry analysis of IgA1 and IgA2 expression on peripheral B cells from a healthy donor.
- C. Quantification of IgA-coated bacteria in stool (n=20) and ileum (n=5). Flow cytometry analysis of human gut microbiota stained with anti-IgA FITC or isotype-matched control. Numbers indicate % of positive cells. Data are cumulative from three independent experiments. Error bars represent minimum and maximum values. P-value was defined using Mann-Whitney test. ***p<0.001.
- D. IgA-coated bacteria split into IgA^{bright} and IgA^{low} fractions depending on IgA1 and IgA2 coating. Representative flow cytometric analysis of IgA1 and IgA2 coating in IgA^{bright} coated bacteria (red lines), IgA^{low} coated bacteria (blue lines), IgA-unbound bacteria (grey lines).

Figure S2 (related to Figure 4): IgA1⁺IgA2⁻ and IgA2⁺-sorted fractions show distinct compositions within the same donor and between donors.

- A. Sorting strategy of IgA1- and IgA2-coated bacteria, representative of five independent experiments.

- B. Relative composition of phyla in fecal samples (input). Each bar corresponds to one sample.
- C. Genera diversity of input, IgA1⁺ and IgA2⁺ fractions calculated using the Shannon index.
- D. Relative composition of genera in fecal samples (input). Each bar corresponds to one sample. The 25 most abundant genera are shown.
- E. Relative abundance of families in input and sorted fractions from five healthy donors. Top 16 most abundant families are shown.

Figure S3: IgA1 and IgA2-coated bacteria promote cytokine production by macrophages

- A. One out of three representative flow cytometric analyses of human gut microbiota purified from an IgA-deficient donor incubated with breast milk IgA and subsequently with anti-IgA FITC (three independent experiments). Numbers indicate % of positive cells.
- B. Cytokine levels measured using Simoa technology in supernatants of macrophages incubated for 24 h with heat-killed *S. haemolyticus* opsonized with IgA1 (blue), IgA2 (red) or without IgA (grey). Mann-Whitney test was used to calculate *P* values. *ns*, not significant. (n=3 healthy donors, two independent measurements)

Figure S4 (related to figure 4): Human monoclonal IgA bind a broad but nevertheless private pattern of commensals.

- A. Flow cytometric sorting of intestinal IgA⁺ memory B cells (defined as CD19⁺, cell surface IgA⁺ IgD⁻). Sort gate among CD19⁺ B cells is shown. Doublets and dead cells were excluded before CD19 gating, CD19⁺ cells were gated among CD45⁺ cells (not shown). Representative images from three independent sorts.
- B. Transduced B cells exhibited a stable germinal center-like phenotype and maintained IgA expression. Transduced B cells were surface-labelled with anti-CD38, anti-CD95, anti-IgA (orange lines) or appropriate isotype antibody controls (grey dotted lines). Representative images of eight monoclonal B cell lines, evaluated in three independent experiments.
- C. Monoclonal B cell lines produced dimeric IgA. Representative immunoblotting showing high molecular weight dimeric mAb in non-reducing conditions for mAb#8. Representative image of eight mAbs and two independent experiments.

- D. Representative flow cytometric plot of microbiota #B stained with anti-IgG Alexa647 and anti-IgA FITC. The same experiment was repeated twice.
- E. Heatmap diagram of enrichment index (EI) of the 50 most frequent genera from Microbiota #A. Hierarchical clustering grouped mAb⁺ fractions and genera.
- F. Heatmap diagram of enrichment index (EI) of the 50 most frequent genera from Microbiota #B. Hierarchical clustering grouped mAb⁺ fractions and genera.
- G. Flow cytometric analysis of mAb or negative control (mAb⁻ supernatant (left) or irrelevant IgG (right, anti-TNF α IgG1)) staining of pure bacterial strains (two independent experiments).

Figure S5: Self-reactivity and glycan reactivity of antigen-selected secretory IgA.

- A. Median frequency of non-silent (black) and silent (grey) somatic mutations in CDRs and VH FWRs in mAb *IGH* genes (n=16 mAbs, four independent experiments).
- B. Self-reactivity was tested by IFA with HEP-2000 cells. (1) negative control, (2) positive control: auto-immune human serum containing anti-DNA, (3) purified IgA from fecal water (20 μ g/ml), (4) negative staining representative of 15 nonreactive mAbs, (5) mAb#4. Representative images of three independent experiments.

	Donor	Isotype	VH	DH	JH	H-CDR3	VL	JL	L-CDR3
mAb#1	HD1	IgA2 κ	4-30	6-13*01	3*02	CAKDTRYSSYDAFDIW	1-27*01	3*01	CQKYDSVPPTF
mAb#2	HD1	IgA2 κ	3-23	6-13	2	CAKDLLAGGGRGYWYFDLW	3-11	4	CQQRNWPALTF
mAb#3	HD1	IgA2 κ	7-4	6-6	4	CVREGPMPGRPGVEWW	4-1*01	5*01	CQQHYTSPPTF
mAb#4	HD1	IgA2 κ	1-69	5-12	4	CAKVPGYSGFGIDYW	1-39	1	CQQSYNTPWTF
mAb#5	HD1	IgA2 κ	1-18*01	3-10*01	5*02	CARDLKEIRGSFITVGGFDPW	1-17*01	1*01	CLQHKVTSTF
mAb#6	HD2	IgA2 κ	4-34	3-10*01	3*01	CARAPRGIRAFDFW	ND	ND	ND
mAb#7	HD3	IgA2 κ	1-2*02	2-2*02	4*02	CARVAGDLEYCSRVS CYAFD YW	1-39	1*01	CQQLPYHPQTF
mAb#8	HD3	IgA2 κ	1-2*02	6-19	4*03	CARESDSSGWYGYW	1-39	1*01	CQQGWSTSPNTF

Table S1 : Ig gene analysis of dimeric monoclonal antibodies

ND : not determined

Patient ID	Age	Sexe	Specimen	Clinical details
#1	51y	M	Colon	Hemicoloectomy / colonic tumor
#2	80y	M	Colon	Hemicoloectomy / colonic tumor
#3	75y	F	Colon	Hemicoloectomy / colonic tumor
#4	3 m	M	Colon	Colostomy reversal surgery
#5	86y	F	Ileal fluid	Ileostomy
#6	60y	M	Ileal fluid	Ileostomy
#7	72y	F	Ileal fluid	Ileostomy
#8	42y	F	Ileal fluid	Ileostomy
#9	80y	M	Ileal fluid	Ileostomy
#10	28y	F	Breast milk/feces	3 months postpartum
#11	30y	F	Breast milk/feces	4 months postpartum
#12	29y	F	Breast milk/feces	3 months postpartum
#13	62y	F	Feces	healthy donor
#14	24y	F	Feces	healthy donor
#15	26y	M	Feces	healthy donor
#16	35y	F	Feces	healthy donor
#17	33y	F	Feces	healthy donor
#18	33y	F	Feces	healthy donor
#19	27y	F	Feces	healthy donor
#20	37y	F	Feces	healthy donor
#21	25y	F	Feces	healthy donor
#22	25y	M	Feces	healthy donor
#23	27y	F	Feces	healthy donor
#24	33y	F	Feces	healthy donor
#25	31y	M	Feces	healthy donor
#26	32y	M	Feces	healthy donor
#27	34y	M	Feces	healthy donor
#28	33y	M	Feces	healthy donor
#29	24y	F	Feces	healthy donor
#30	41y	M	Feces	healthy donor
#41	46y	M	Feces	healthy donor
#42	30y	F	Serum	healthy donor
#43	27y	M	Serum	healthy donor
#44	48y	F	Serum	healthy donor
#45	52y	F	Serum	healthy donor
#46	29y	F	Serum	healthy donor
#47	51y	F	Feces	IgA deficient patient
#48	25y	F	Feces	IgA deficient patient
#49	37y	M	Feces	IgA deficient patient
#50	3m	F	Feces	Formula fed neonate
#51	3m	F	Feces	Formula fed neonate
#52	5m	F	Feces	Formula fed neonate
#53	5m	F	Feces	Formula fed neonate
#54	5m	F	Feces	Formula fed neonate
#55	5m	M	Feces	Formula fed neonate

#56	5m	M	Feces	Formula fed neonate
#57	5m	M	Feces	Formula fed neonate

Table S2 : Demographic data of human samples

Primers	Target gene	Primer sequence
C α 1	C α 1	ACGTCGTGGGTCCGGTCTA
C α 2	C α 2	CTGTCGTGGGGGGTCTA
C α int	C α	AYGACCACGTTCCCATCT
C κ int	C κ	CAGATTTCAACTGTCATCAGATGG
C κ ext	C κ	GCAGGCACACAACAGAGGCA
C λ	C λ	CCTTGTTGGCTTGRAGCTCCTCAGA
VH ext	VH1	CCATGGACTGGACCTGGA
	VH2	ATGGACATACTTTGTTCCAC
	VH3	ATGGAGTTKGKCTGARCTGG
	VH4	ATGAAACACCTGTGGTTCTT
	VH5	ATGGGGTCAACCGYCATCCT
	VH6	ATGTCTGTCTCCTTCCTCAT
V κ ext	VK1	CRTGAGGDYCCCCRCTCA
	VK2	CTCCCTGCTCAGCTCCTG
	VK3	CAGCTTCTCTCCTCCTG
	VK4	TCTCTGTTGCTCTGGATCTCT
	VK5	TCACCTCCTCAGCTTCCTCCT
	VK6	GCTSCTCTGGGTTCAGCCT
V λ ext	VL1	ATGACCTGSTCYCCTCTCCTC
	VL1	ATGGCCRGCTTCCCTCTCCTC
	VL3.7	ATGGCCTGGACCCCTCTCTKKCT
	VL2	CTGCTCCTCACTCTCCTCACTC
	VL4	ATGACTTGGACCCCACTCCTCTTC
	VL6	ATGGCCTGGGCTCCACTACTT
VH int	VH1	TGGCTGTAGCTCCAGGTGCYCA
	VH1	TGGCAGCAGCCACAGKWGCCYA
	VH1	TGGCAGCAGCTACAGGYGYCCA
	VH2	CTGACTGTCCCGTCCGGGGTCTTA
	VH3	GCTMTTTTAARAGGTGTCCAGTGT
	VH3	GTTATTTTACAAGGTGTCCAGTGT
	VH4	CTGGTGGCRGCTCCAGATGGGTC
	VH5	CCTCSTCCTRGCTRRTCTCCAAGG
	VH6	GGCCTCCCATGTGTCTCTGTCA
V κ int	VK1	GACATCCAGATGACCAGTCTCC
	VK2	GATATTGTGATGACCCAGACTCCAC
	VK3	GAAATTGTGWTGACRCAGTCTC
	VK4	GACATCGTGATGACCCAGTCTCC
	VK5	GAAACGACATCACGCAGTCTCC
V3-external	16S rDNA	TAC-GGR-AGG-CAG-CAG
V4-external		CCG-TCA-ATT-CMT-TTR-AGT
V3-barcoded		<i>CTT-TCC-CTA-CAC-GAC-GCT-CTT-CCG-ATC-TAC-GGR-AGG-CAG-CAG</i>
V4-barcoded		<i>GGA-GTT-CAG-ACG-TGT-GCT-CTT-CCG-ATC-TTA-CCA-GGG-TAT-CTA-ATC-CT</i>

Table S3 : Primers for Ig and 16s rDNA gene sequence analysis

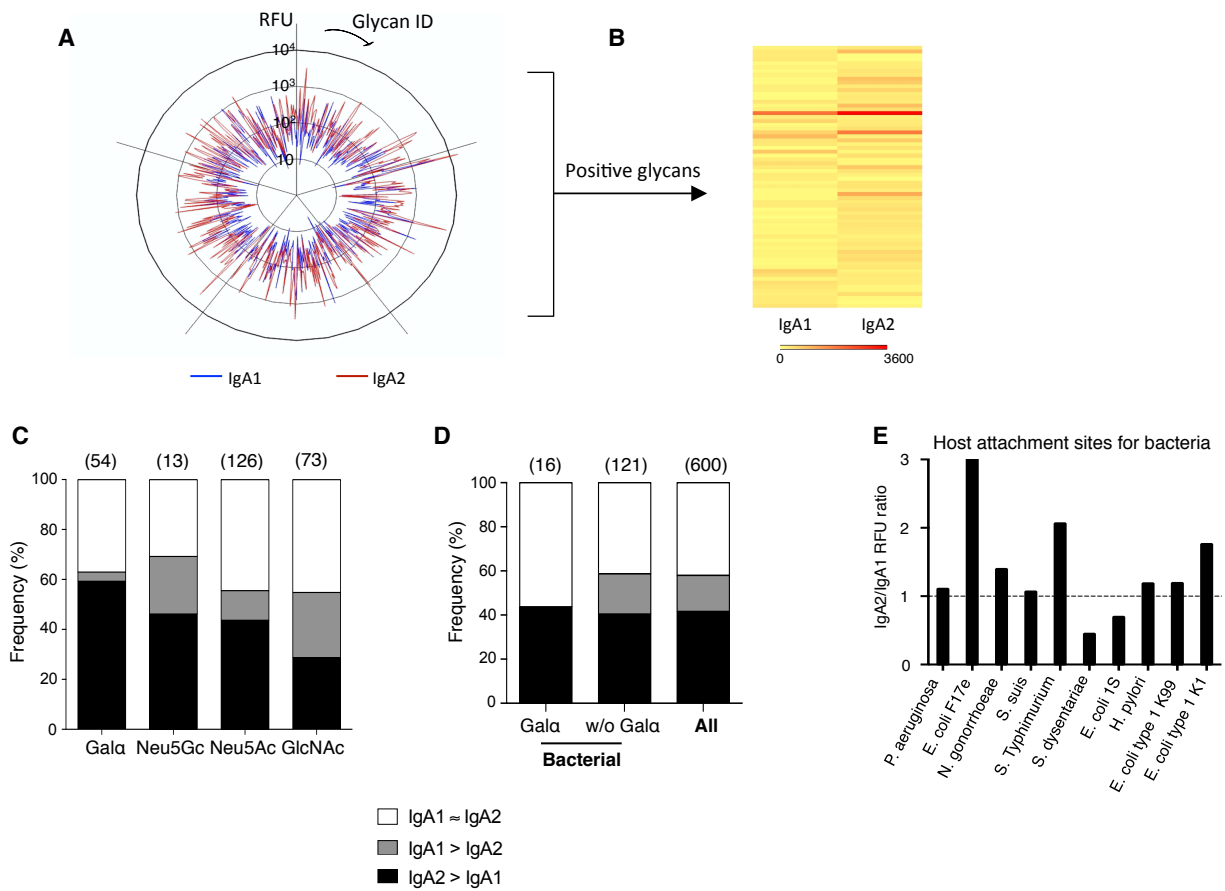
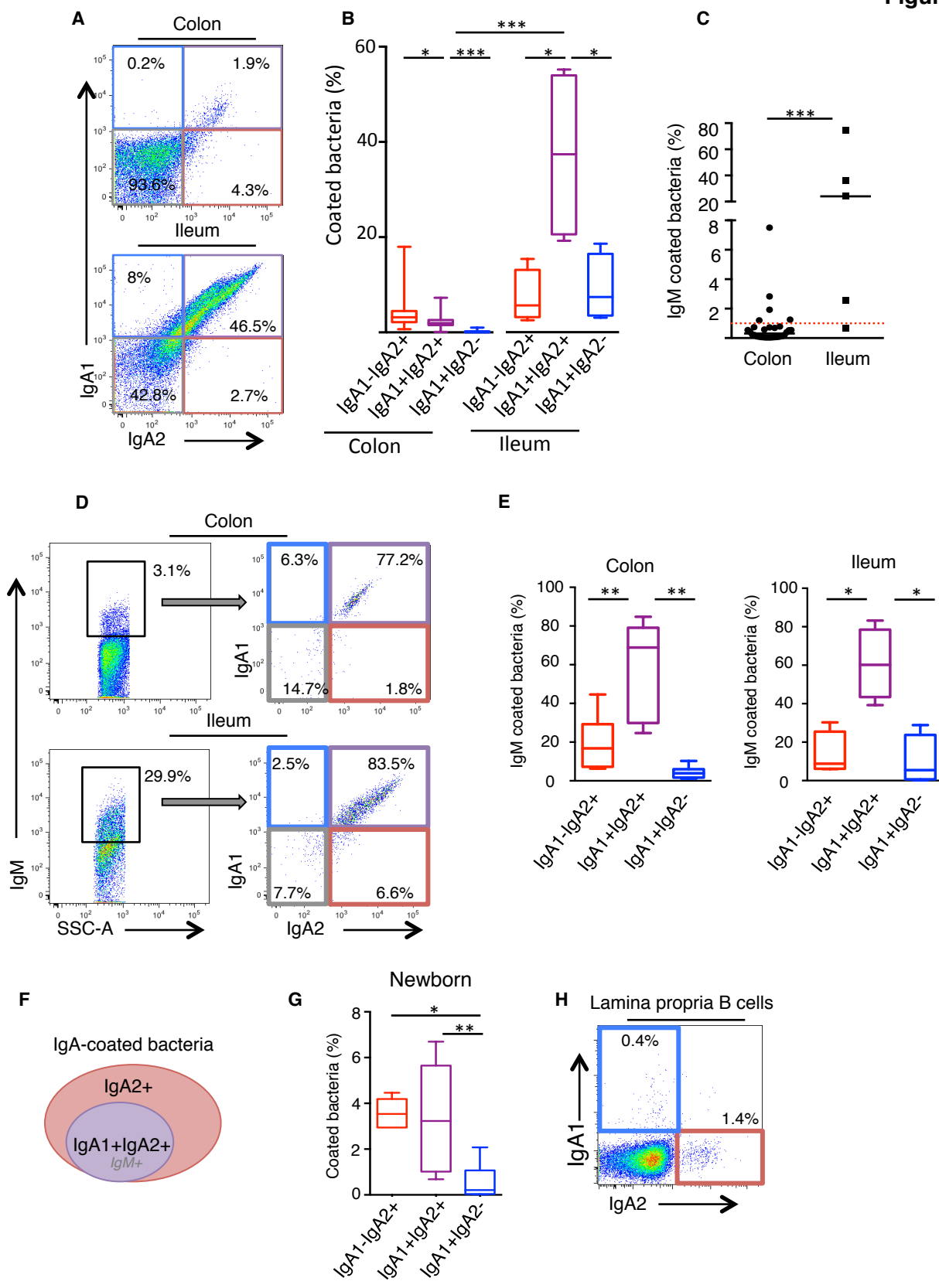


Figure 1



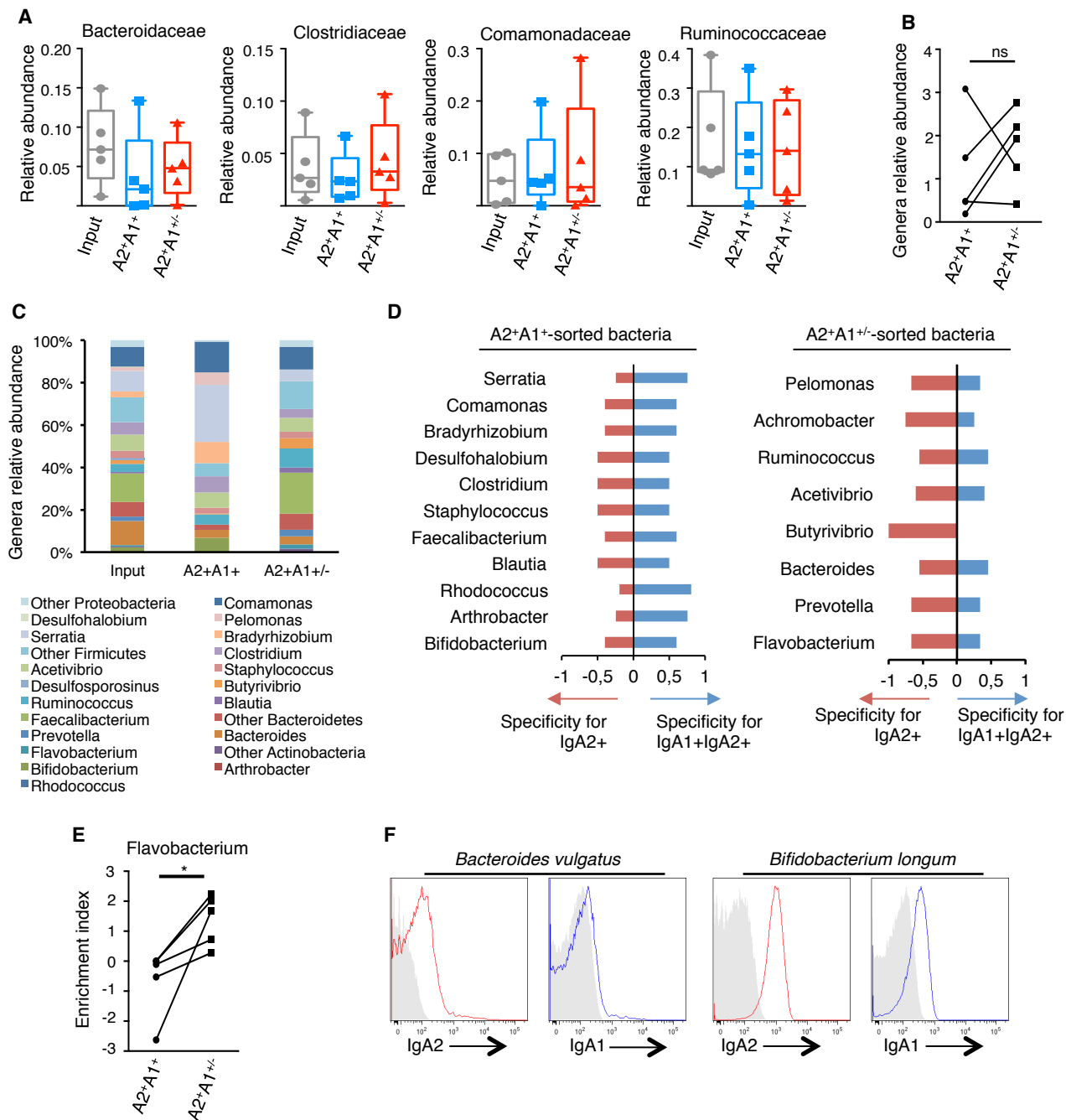


Figure 3

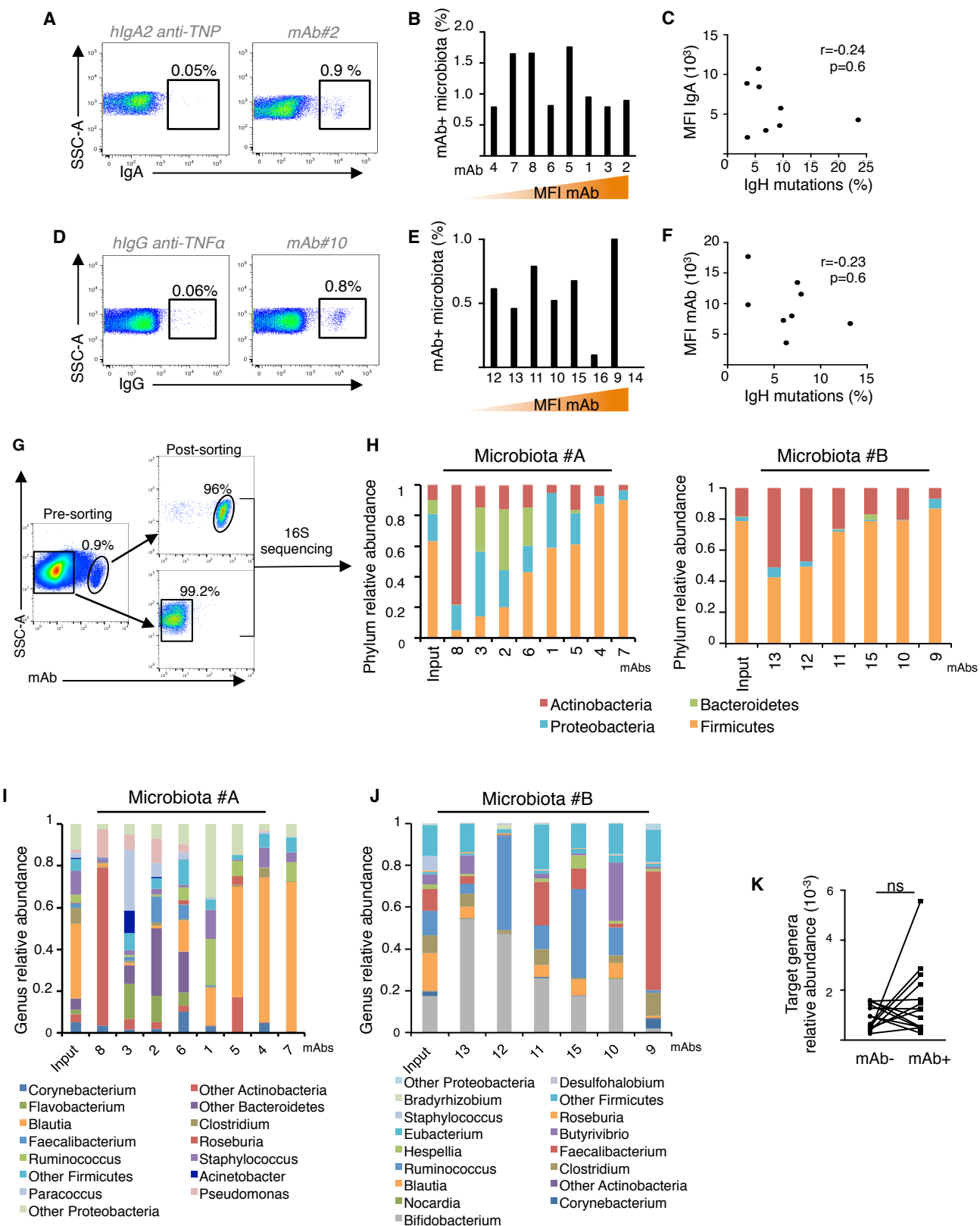


Figure 4

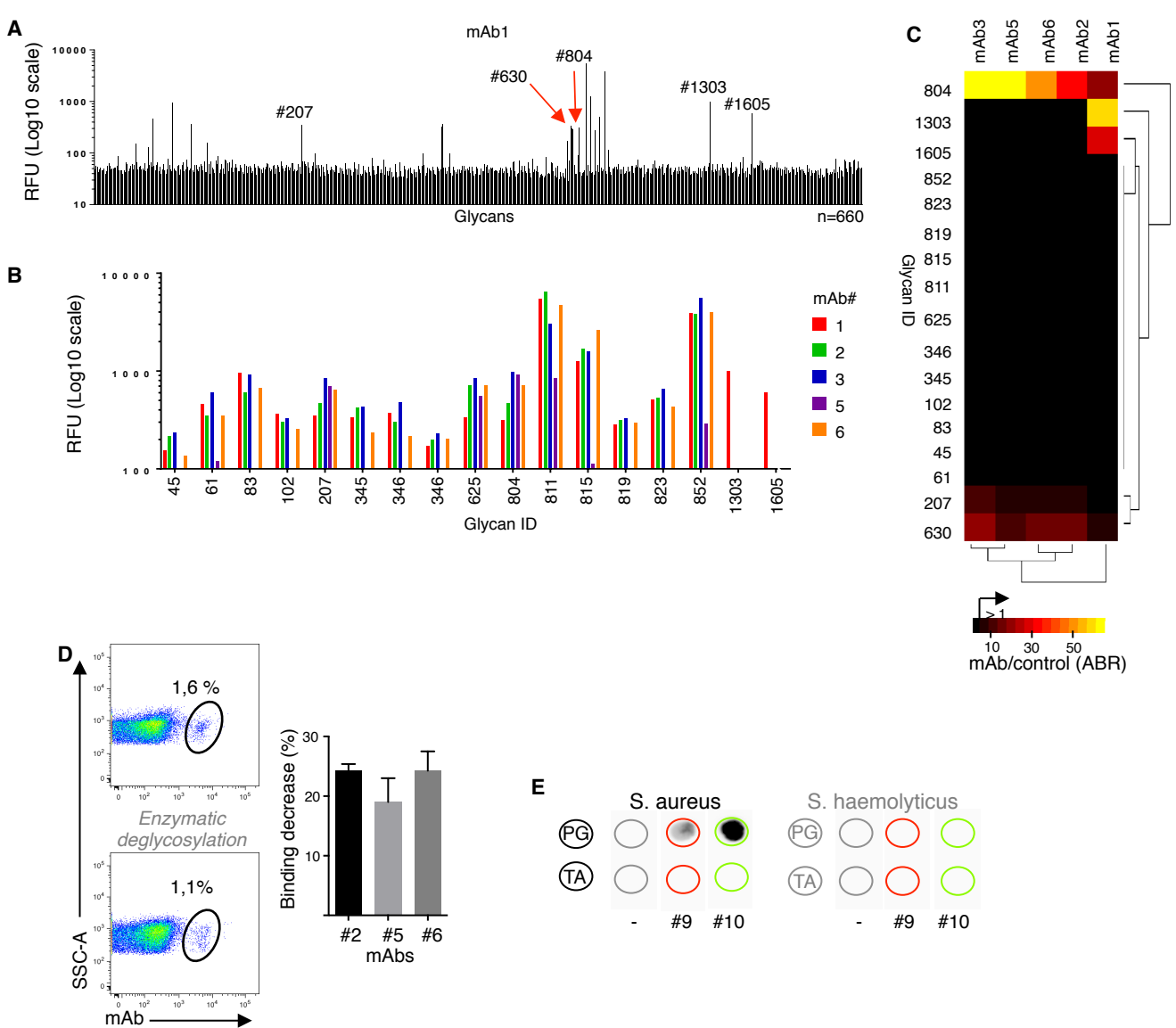


Figure 5

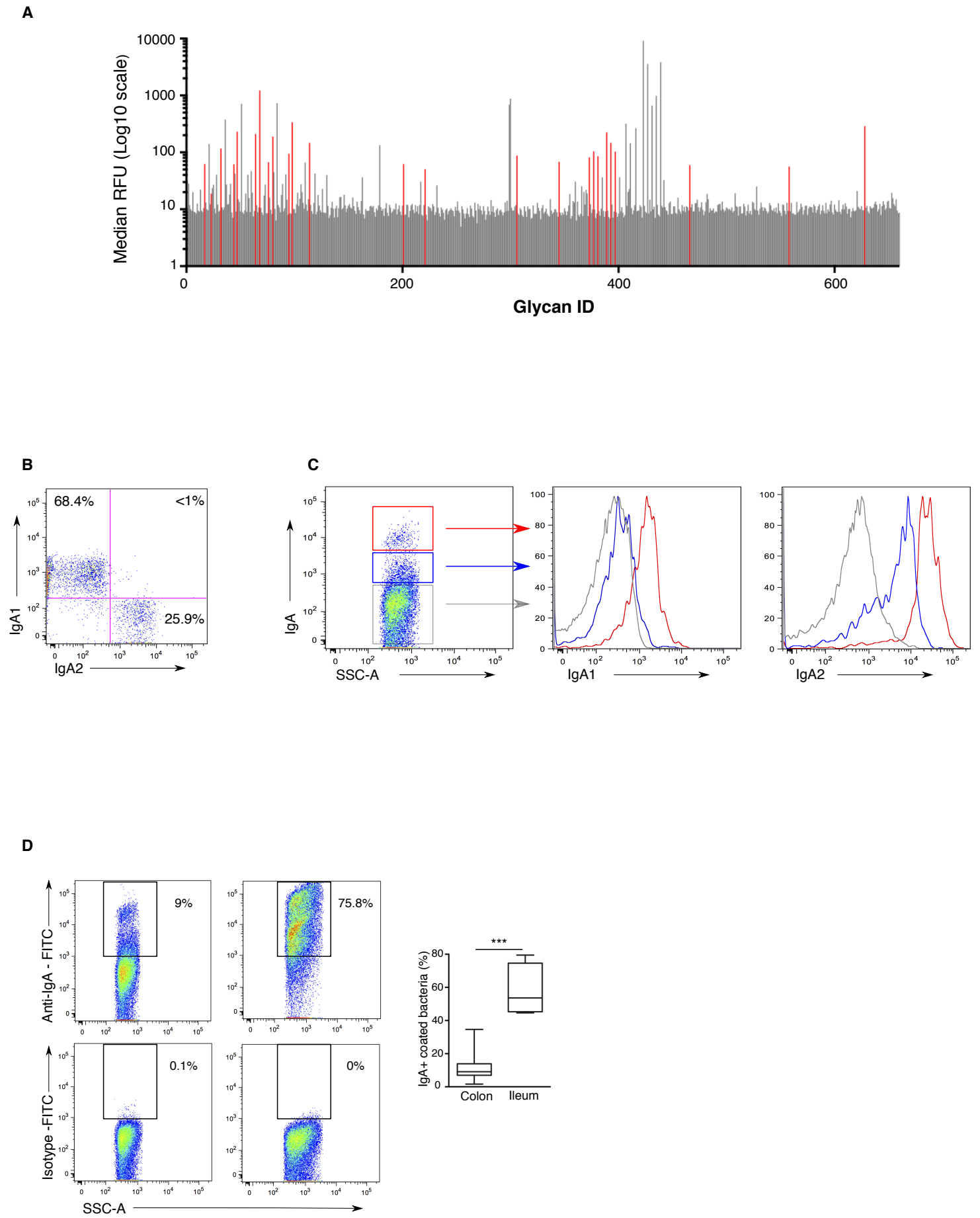


Figure S1

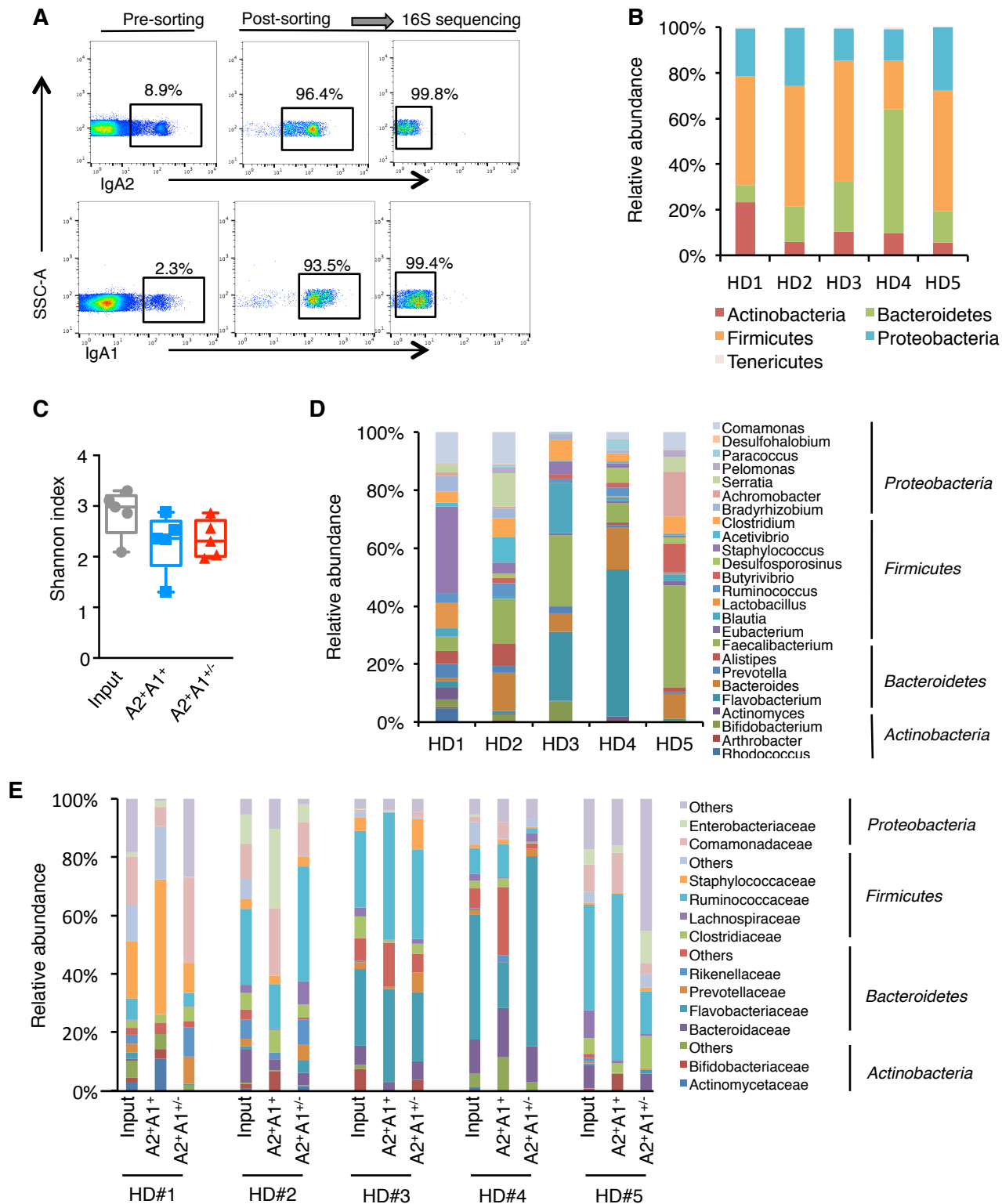
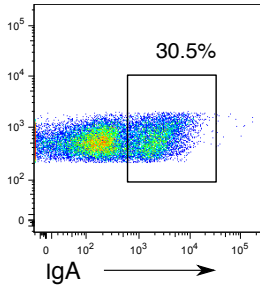
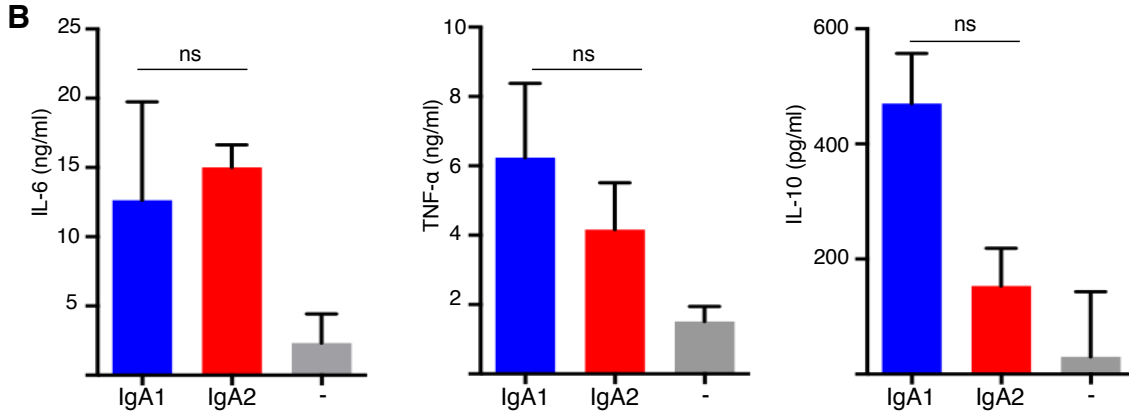


Figure S2

A**Figure S3***Staphylococcus haemolyticus*

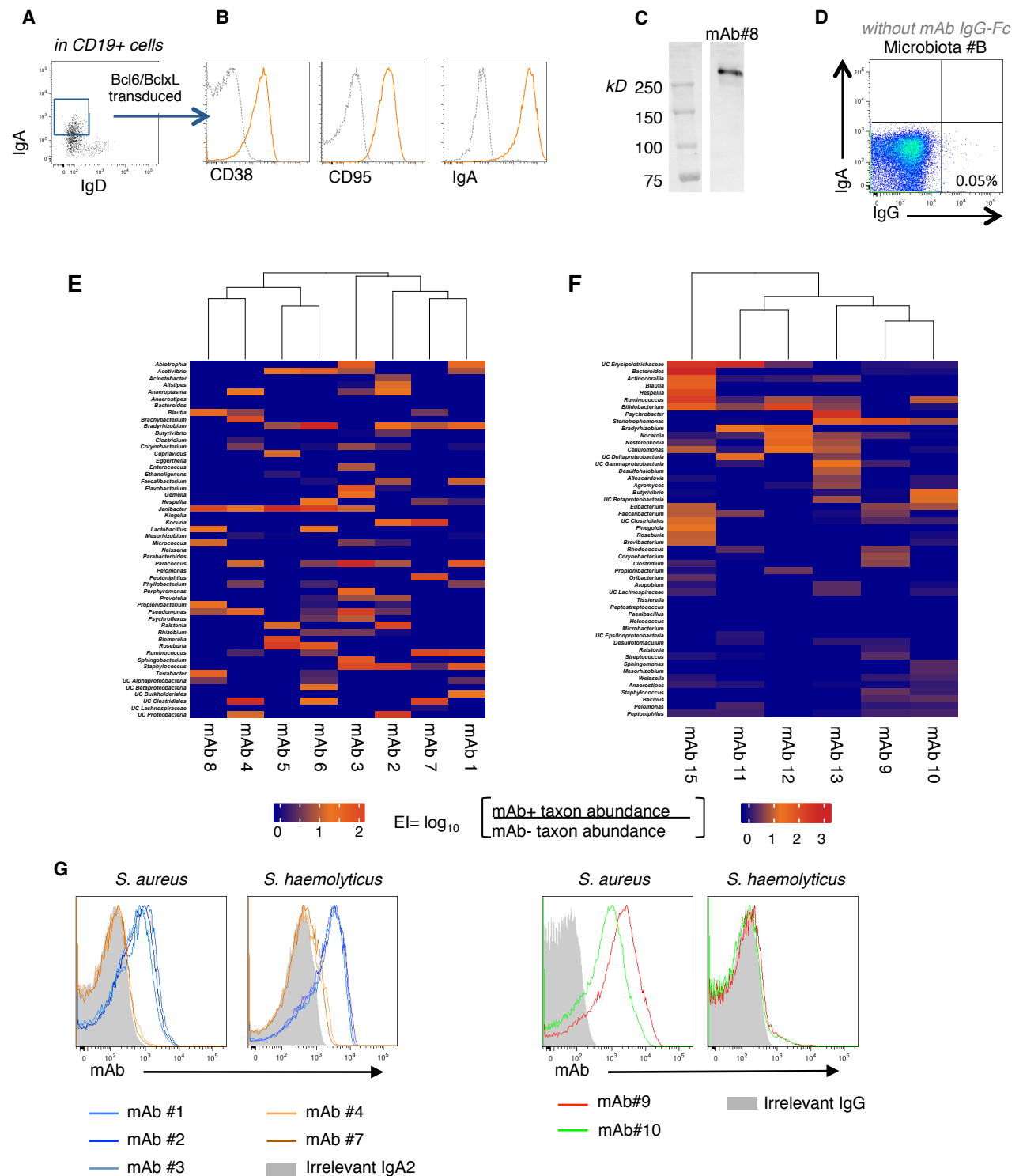
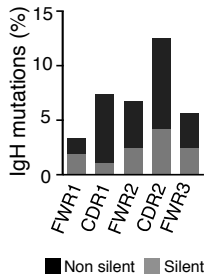
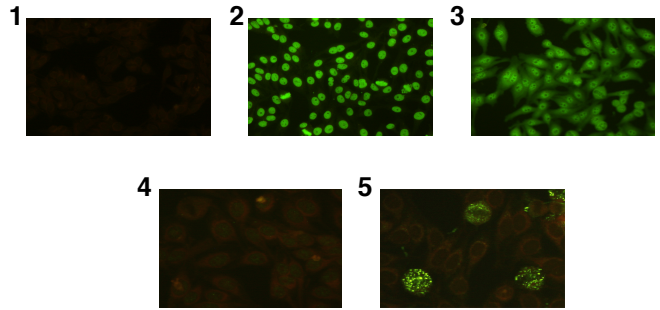


Figure S4

A**B****Figure S5**