Supplementary Materials for

Natural polyreactive IgA antibodies coat the intestinal microbiota

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This PDF file includes:

Figures S1 to S14
Table S1

Other Supplementary Material for this manuscript includes the following:

Database S1 (as Excel file)
References (*90-94*)

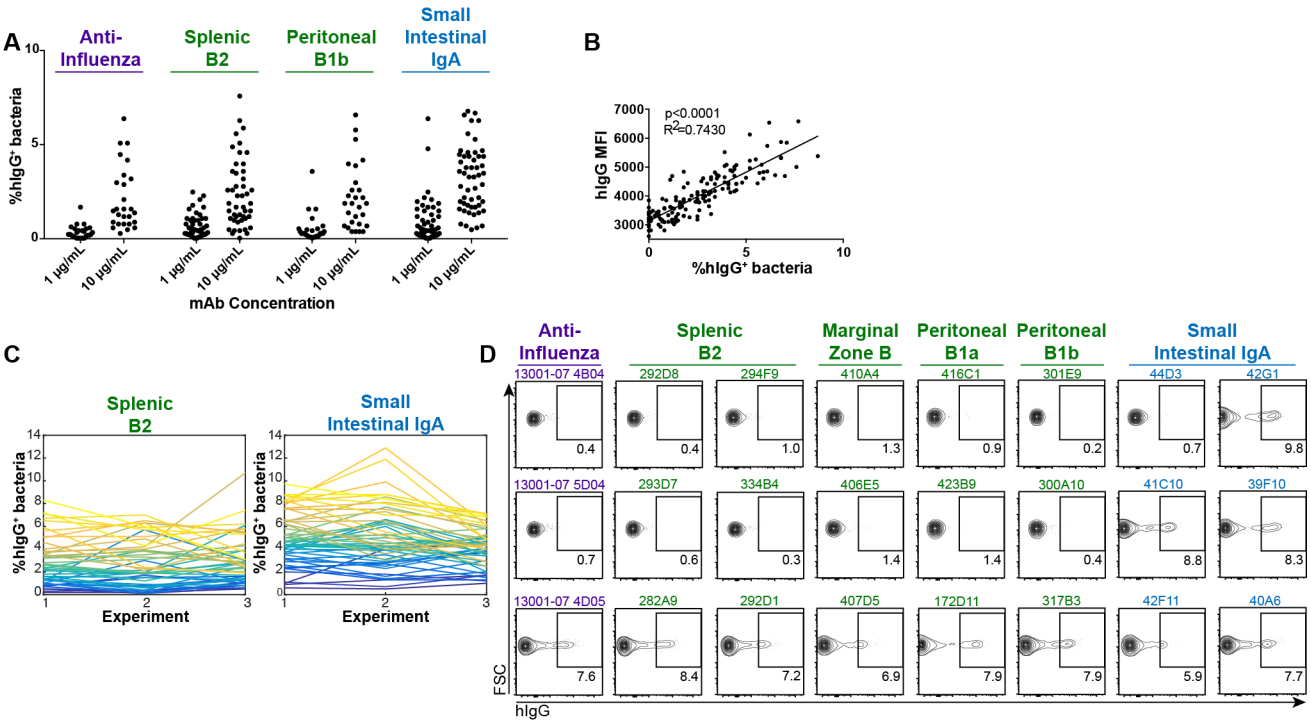


Figure S1. Reproducibility of mAb staining of microbiota. (A) Percent hIgG$^+$ of *Rag1*$^{-/-}$ SI microbiota stained with mAbs from indicated panels at indicated concentrations. Representative of multiple independent experiments. **(B)** Correlation of %hIgG$^+$ bacteria and median fluorescence intensity (MFI) within the positive gate. P and R^2 values calculated by linear regression. **(C)** Reproducibility of microbiota staining. Percent hIgG$^+$ bacteria of *Rag1*$^{-/-}$ SI microbiota stained with mAbs from indicated panels in three independent experiments. Independent preparations of SI microbiota were isolated from three distinct cohorts of *Rag1*$^{-/-}$ mice from our colony and stained on three separate days. The average standard deviation across experiments was 1.00% hIgG$^+$. Lines connect individual mAbs across experiments. Each mAb was assigned a unique color according to its rank order within its panel in experiment 1, with purple being the lowest rank and yellow the highest; color assignment was performed separately for B2 and IgA panels. **(D)** Representative flow cytometry plots depicting staining of *Rag1*$^{-/-}$ SI microbiota by indicated mAbs and panels. Gated on FSC$^+$SSC$^+$SYTO BC$^+$DAPI$^-$ cells. Plots are representative of multiple independent experiments.

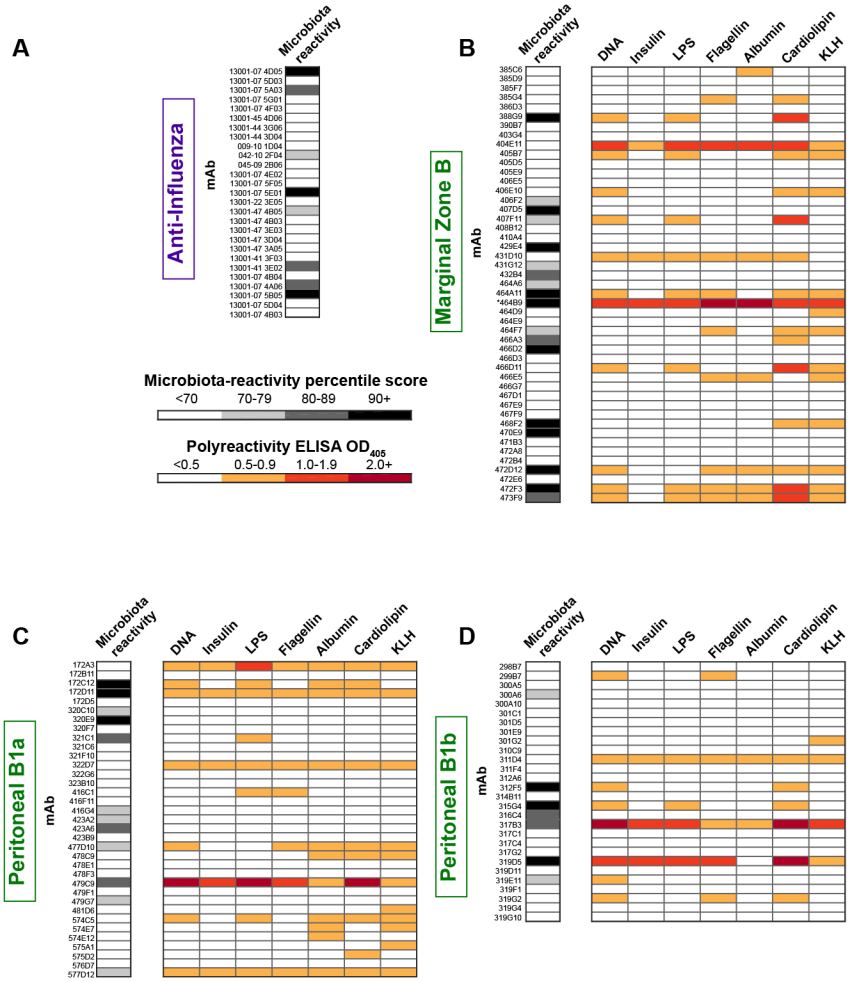
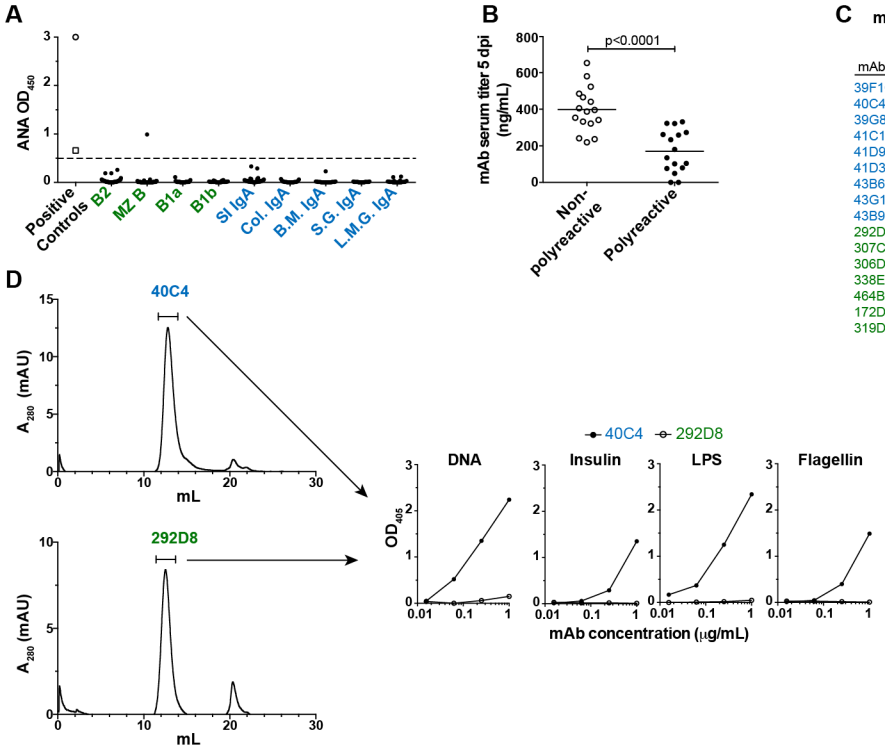


Figure S2. Reactivities of individual mAbs from naïve B cell subsets or anti-influenza panels. (A-D) Microbiota-reactivity percentile scores and polyreactivity ELISA OD_{405} values for individual mAbs from indicated panels. Data are summarized in Fig. 1C-D.



C **mAbs used in *Rag1*$^{-/-}$ in vivo serum retention experiments**

Polyreactive		Non-Polyreactive	
mAb	Origin	mAb	Origin
39F10	Small Intestinal IgA	35A2	Small Intestinal IgA
40C4	Small Intestinal IgA	44B1	Small Intestinal IgA
39G8	Small Intestinal IgA	280B11	Splenic B2
41C10	Small Intestinal IgA	280C9	Splenic B2
41D9	Small Intestinal IgA	277C4	Splenic B2
41D3	Small Intestinal IgA	284A8	Splenic B2
43B6	Small Intestinal IgA	292D8	Splenic B2
43G10	Small Intestinal IgA	277A2	Splenic B2
43B9	Small Intestinal IgA	277C1	Splenic B2
292D1	Splenic B2	277F7	Splenic B2
307C9	Splenic B2	335G4	Splenic B2
306D5	Splenic B2	403G4	MZ B
338E6	Splenic B2	172B11	PEC B1a
464B9	MZ B	320F7	PEC B1a
172D11	PEC B1a	300A10	PEC B1b
319D5	PEC B1b	317C1	PEC B1b

Figure S3. Anti-nuclear reactivities of individual mAbs, serum retention in vivo, and protein quality validation of polyreactive mAbs. (A) HEp2 lysate anti-nuclear antibody (ANA) ELISA. Filled circles indicate individual mAbs and all mAbs shown in Fig. 1 were assayed. Open circle in positive control column indicates 3H9 high positive control mAb; open square indicates low positive control serum provided by the manufacturer; these controls were included on all plates assayed. * in Fig S2B indicates the single HEp2-reactive MZ B mAb. Data compiled from five independent experiments. **(B)** Serum concentration of mAbs at 5 days-post-injection (dpi) of 2 μg into *Rag1*$^{-/-}$ mice. Data compiled from three independent experiments. P value calculated by unpaired t test. **(C)** List of mAbs used for in vivo serum retention measurements. **(D)** Size exclusion chromatography A_{280} trace of indicated mAbs. Brackets indicate monomeric fractions purified and immediately used in polyreactivity ELISAs against indicated antigens (right).

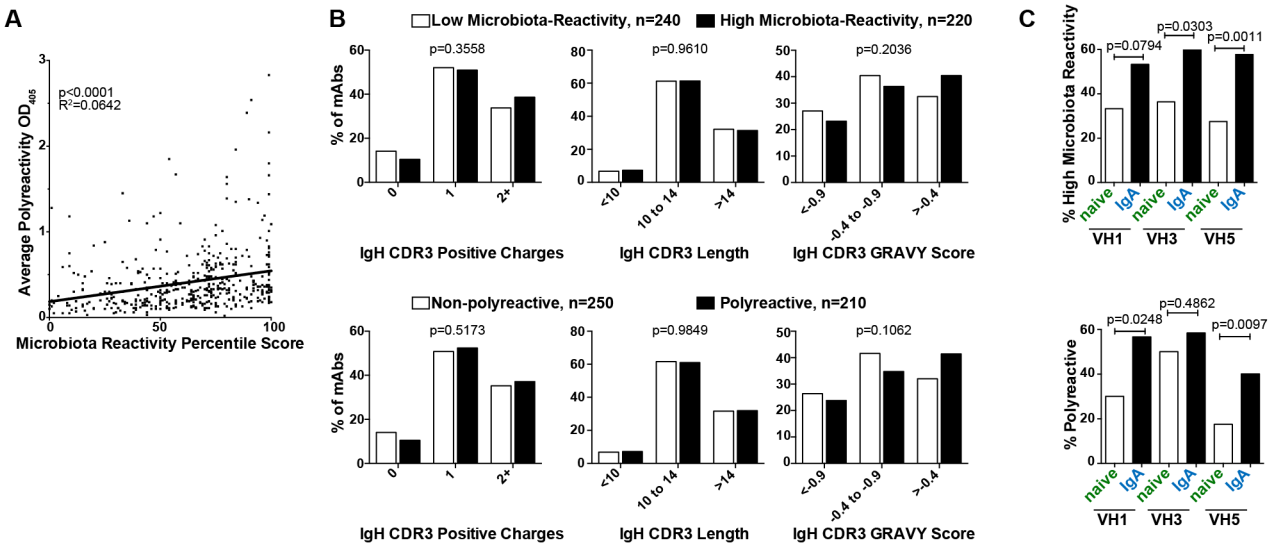


Figure S4. Correlates of microbiota-reactivity and polyreactivity. (A) Correlation of average polyreactivity ELISA OD_{405} across all seven antigens for individual mAbs with their microbiota reactivity percentile score. Plot includes all mAbs in this study except germline reverted mAbs and anti-influenza panels. P and R^2 values calculated by linear regression. **(B)** Percent of mAbs among low microbiota-reactivity and high microbiota-reactivity classifications (upper panels) or polyreactive and non-polyreactive classifications (lower panels) with indicated repertoire features. Plots include all mAbs in this study except germline reverted mAbs and anti-influenza panels. P values calculated by chi-square test. **(C)** Percent high microbiota reactivity (upper panel) or polyreactive mAbs (lower panel) among all naïve B cell or IgA-derived antibodies in this study, grouped according to VH gene usage. V genes other than 1, 3, and 5 were not included as sample numbers were inadequate for statistical comparison due to low or absent representation in many panels. P values calculated by 2x2 Fisher's exact test.

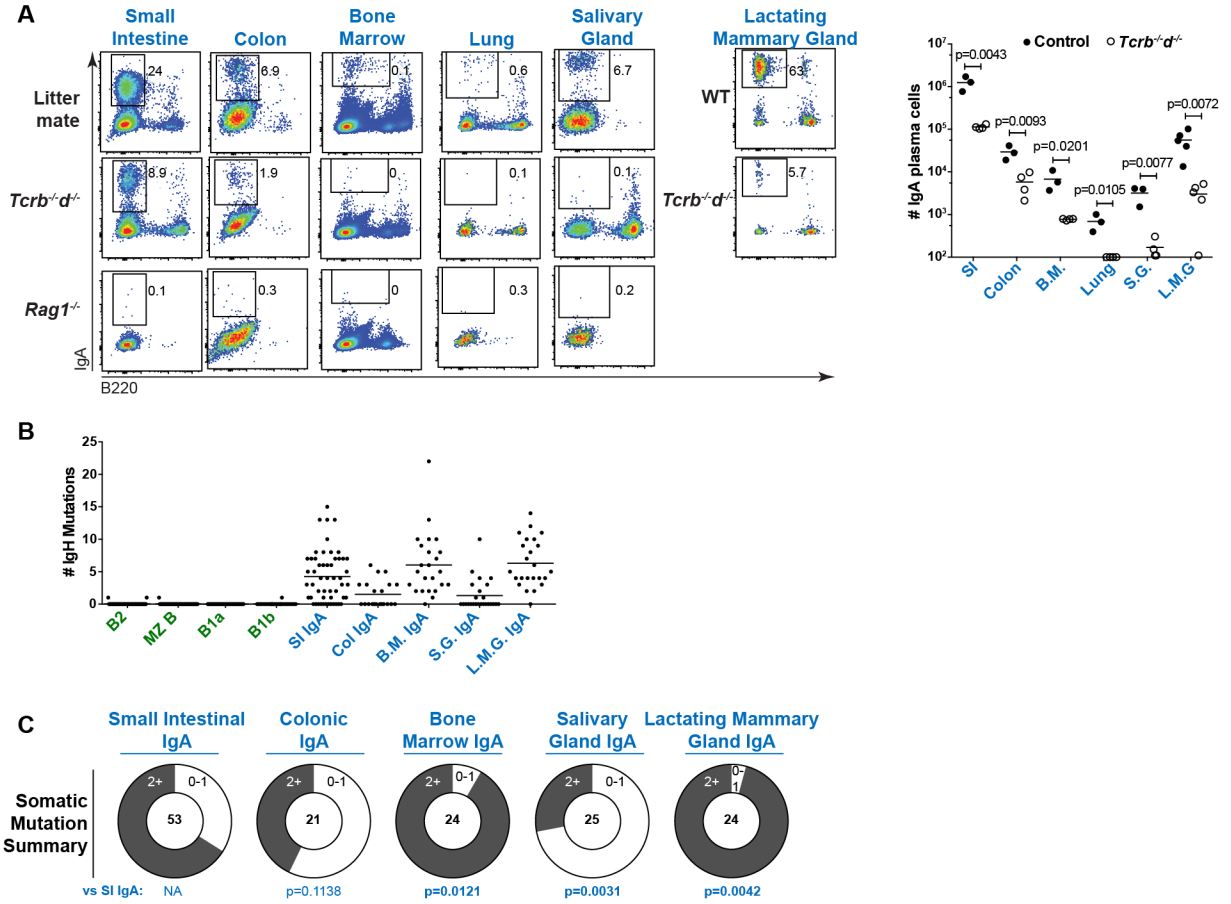


Figure S5. Contributions of TI or TD pathways to intestinal and extraintestinal IgA populations. (A) Representative staining and cell number summary of IgA plasma cells in indicated tissues of *Tcrb*$^{-/-}$*d*$^{-/-}$ mice, controls, or *Rag1*$^{-/-}$ mice. Mice were compared to co-housed *Tcrb*$^{+/-}$*d*$^{+/-}$ littermates for all tissues except for the lactating mammary gland; this analysis consisted of a separate cohort of 3-7d post-partum *Tcrb*$^{-/-}$*d*$^{-/-}$ females compared to age-matched and co-housed WT B6 females. P values calculated by unpaired t test. Data compiled from two independent experiments. **(B)** Number of IgH somatic mutations for individual mAbs of indicated cellular origin. **(C)** Summary of somatic mutations for indicated panels. P values calculated by Fisher's exact test.

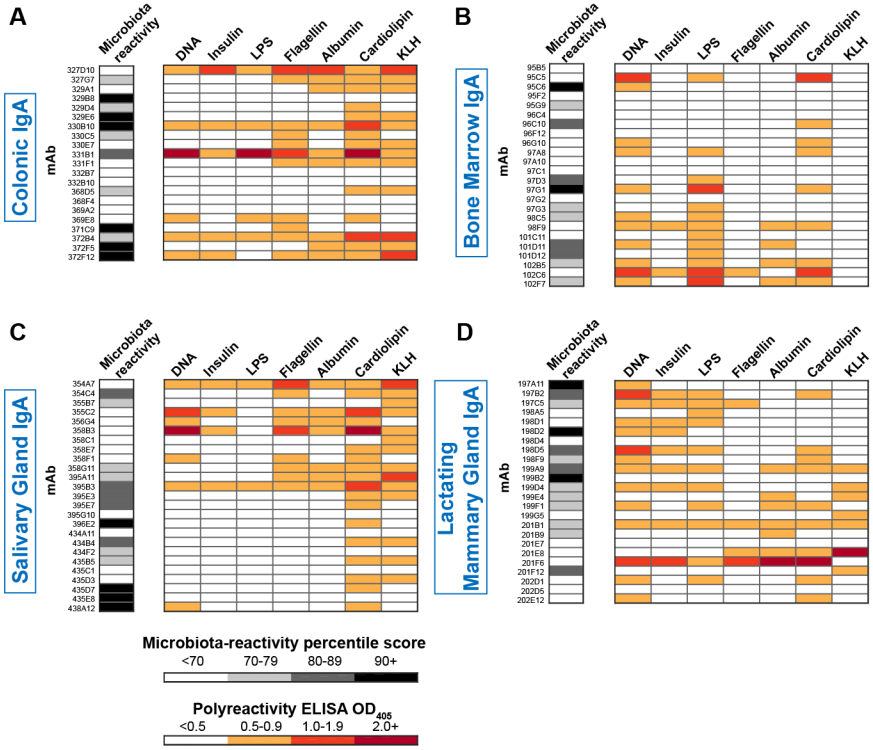


Figure S6. Reactivities of individual mAbs from colonic and extraintestinal IgA PC populations. (A-D) Microbiota-reactivity percentile scores and polyreactivity ELISA OD_{405} values for individual mAbs from indicated panels. Data are summarized in Fig. 1C-D.

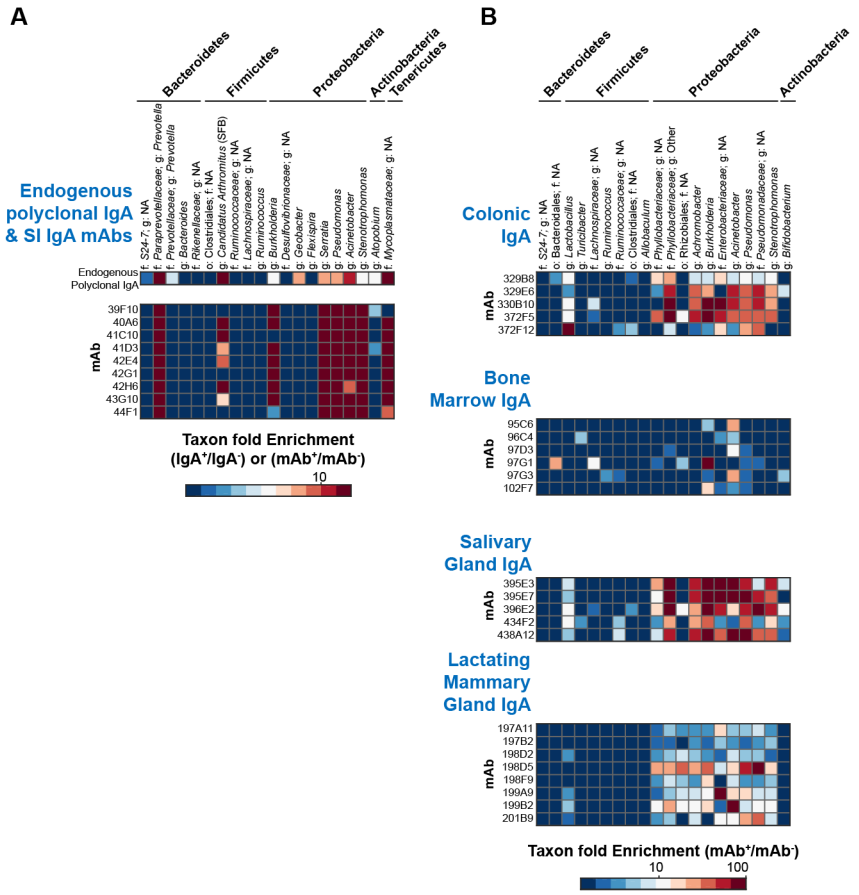


Figure S7. Microbial targets of individual microbiota-reactive IgA-derived mAbs. (A-B) Microbial taxa bound by individual mAbs from indicated panels. Enrichment calculated from 16S sequencing data as relative abundance in mAb^+/mAb^- or endogenous polyclonal IgA^+/IgA^- samples purified from WT B6 colonic microbiota in A or *Rag1*$^{-/-}$ SI microbiota in B. All mAbs in a given panel were co-purified in the same experiment.

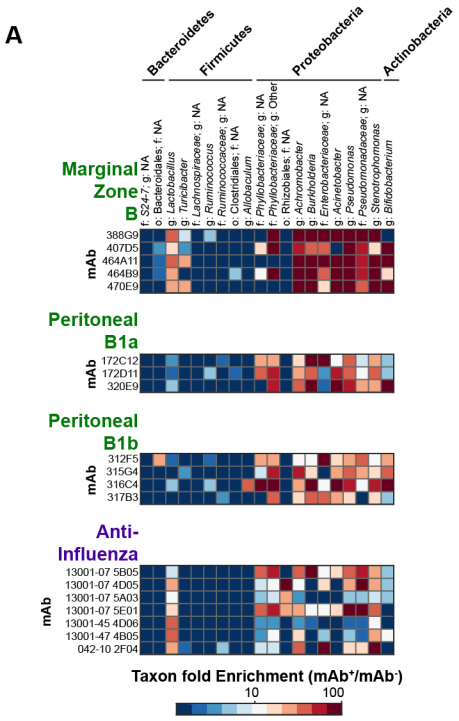


Figure S8. Microbial targets of individual microbiota-reactive naïve B cell-derived mAbs or anti-influenza controls. (A) Microbial taxa bound by individual mAbs from indicated panels. Enrichment calculated from 16S sequencing data as relative abundance in mAb^{+}/mAb^{-} samples purified from *Rag1*$^{-/-}$ SI microbiota. All mAbs in a given panel were co-purified in the same experiment.

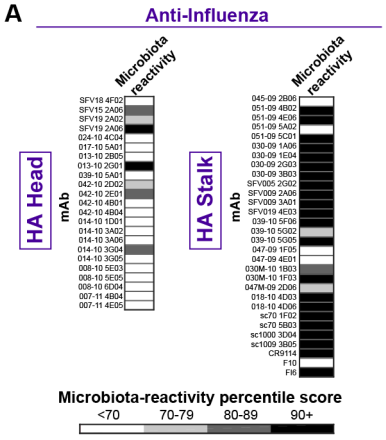


Figure S9. Microbiota-reactivity of individual mAbs from anti-influenza HA head strain-specific or HA stalk bnAb panels. (A) Microbiota-reactivity percentile scores assigned according to the HA head distribution and polyreactivity ELISA OD_{405} values for individual mAbs from indicated panels. Data are summarized in Fig. 2D.

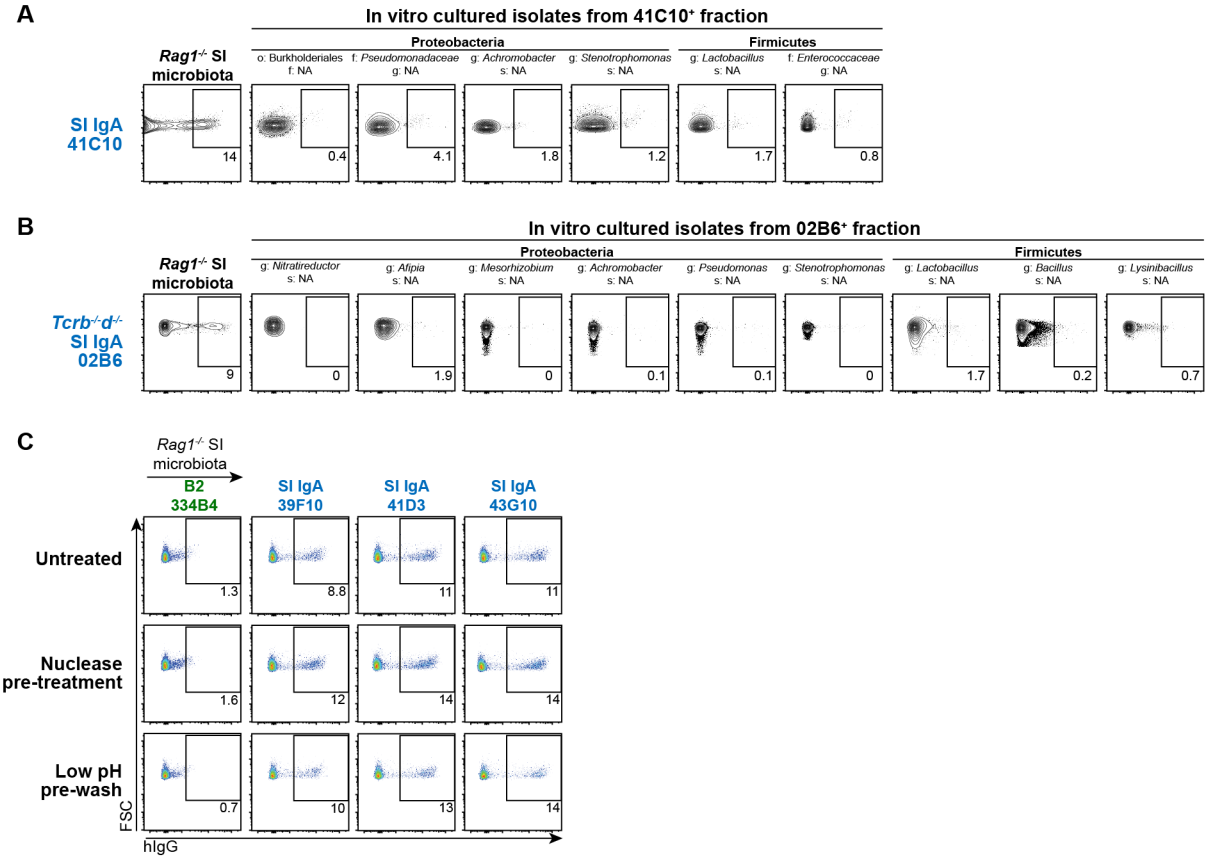


Figure S10. Reactivity of mAbs to in vitro cultured bacterial strains and ex vivo staining after nuclease pre-treatment or low pH pre-washing. (A) Flow cytometry staining with SI IgA mAb 41C10 of ex vivo *Rag1*$^{-/-}$ SI microbiota or individual strains cultured in vitro from the 41C10 mAb^{+} fraction. All staining was performed at 10 µg/mL. Taxonomy abbreviations: o= order; f=family; g=genus; s=species. Similar negative staining of these in vitro cultured isolates was obtained with an additional 27 microbiota-reactive SI IgA mAbs. **(B)** Flow cytometry staining with mAb 02B6 of ex vivo *Rag1*$^{-/-}$ SI microbiota or individual strains cultured in vitro from the 02B6 mAb^{+} fraction. All staining was performed at 10 µg/mL. **(C)** mAb staining of ex vivo *Rag1*$^{-/-}$ SI microbiota that was untreated, pre-treated with nuclease, or pre-washed with glycine-HCl pH 3.0.

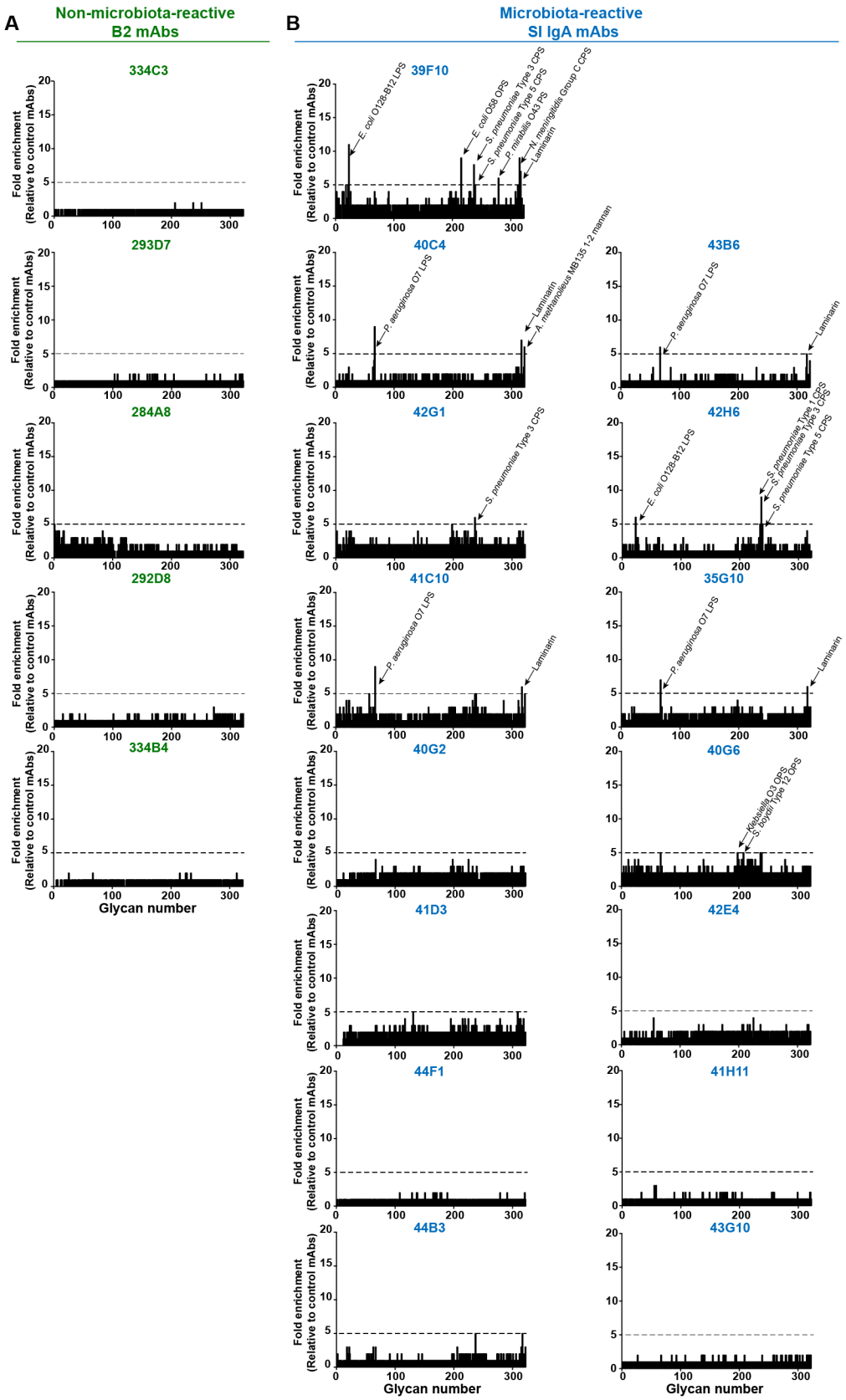


Figure S11. Microbial glycan microarray analysis of individual mAbs. Microbial glycan microarray data for **(A)** Indicated negative control B2 mAbs selected based on lack of microbiota-reactivity or **(B)** microbiota-reactive SI IgA mAbs. Data expressed as enrichment over average background of six B2 mAb negative controls. Annotated peaks showed >5-fold enrichment. Data compiled from two independent experiments.

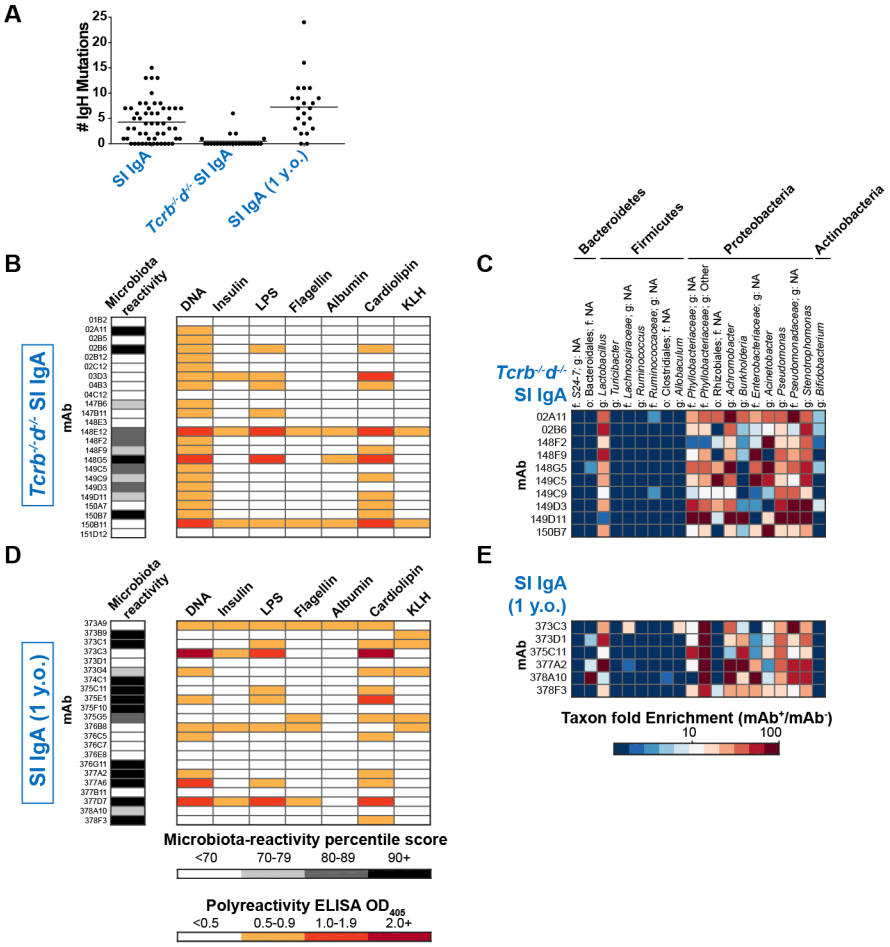


Figure S12. Reactivities of individual T-dependent or T-independent SI IgA mAbs. (A) Number of IgH somatic mutations for individual mAbs from indicated SI IgA panels. **(B, D)** Microbiota-reactivity percentile scores and polyreactivity ELISA OD_{405} values for indicated mAbs and panels. Data summarized in Fig. 3B-C. **(C, E)** Microbial taxa bound by indicated mAbs and panels. Enrichment calculated from 16S sequencing data as relative abundance in mAb^{+}/mAb^{-} samples purified from *Rag1*$^{-/-}$ SI microbiota. All mAbs in a given panel were co-purified in the same experiment.

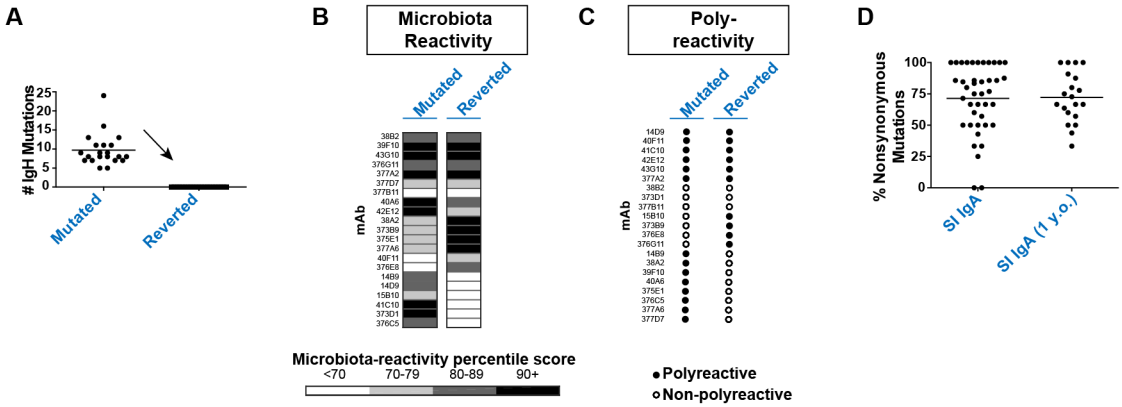


Figure S13. Reactivities of individual mutated or germline reverted SI IgA mAbs. (A) IgH somatic mutations in mAbs selected for reversion to germline. IgK chains were similarly reverted. **(B)** Microbiota-reactivity percentile scores and **(C)** polyreactivity ELISA scoring of indicated mutated or germline reverted mAbs. **(D)** Percent nonsynonymous mutations among individual mutated mAbs in indicated panels.

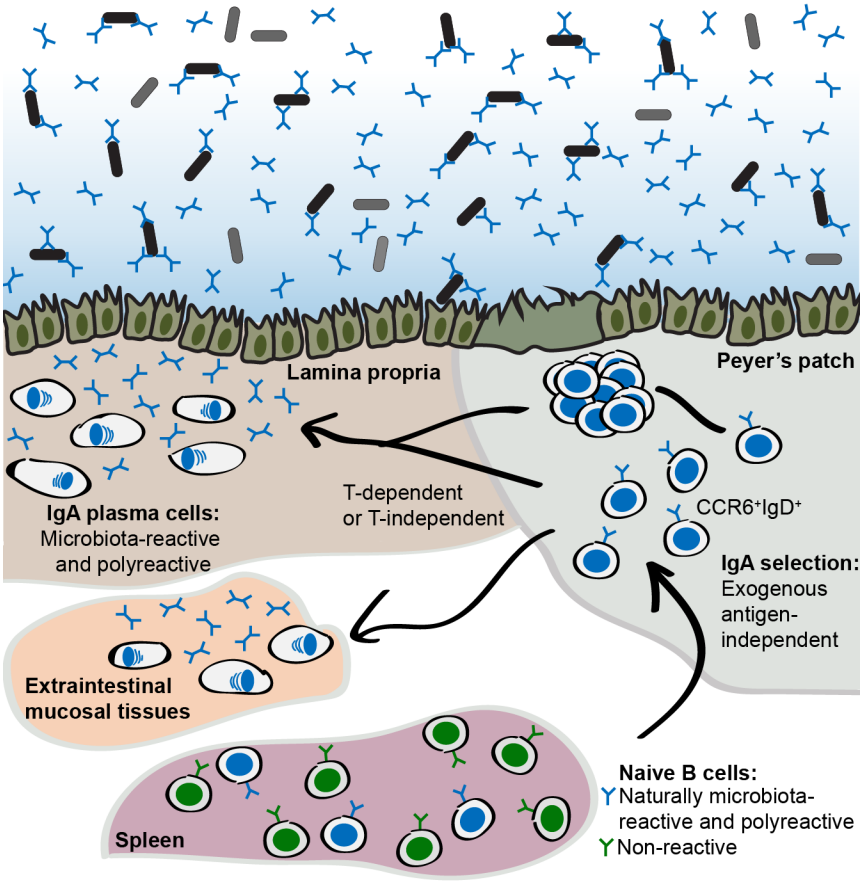


Figure S14. Summary model for selection of microbiota-reactive and polyreactive IgAs. Microbiota-reactive and polyreactive specificities occur naturally at frequencies of ~30% in the repertoires of all naïve B cell subsets. Upon trafficking through Peyer's patches, cells expressing these specificities are selected to divide via a natural, exogenous antigen-independent mechanism. Cells that undergo IgA selection upregulate CCR6, which facilitates receipt of local factors that direct IgA class-switch recombination and gut imprinting. Cells then proceed through either T-dependent or T-independent pathways that instruct differentiation to IgA plasma cells. Cells migrate to the intestinal lamina propria or to extraintestinal tissues and secrete specificities that naturally bind a diverse but defined subset of commensal microbiota.

Table S1. Recipe for antigen-free diet.

Aqueous Solution 1	
Add to sterile nanopure water at 70 ºC	g/L
0.36 L H_2O	
Dextrose (Sigma D9434)	220
Aqueous Solution 2	
Add to sterile nanopure water at 70 ºC	
0.64 L H_20	
Leucine (Sigma L8000)	5.775
Phenylalanine (Sigma P2126)	2.25
Isoleucine (Sigma I2752)	3.3
Methionine (Sigma M9625)	4.8
Tryptophan (Sigma T0254)	1.125
Valine (Sigma V0500)	4.1
Asparagine (Sigma A0884)	3.75
Arginine-HCl (Sigma A5131)	2.775
Threonine (Sigma T8625)	2.25
Lysine-HCl (Sigma L5626)	5.4
Histidine-HCl*H2O (Sigma H5659)	2.25
Cool to 45C, add the following:	
Glycine (Sigma G7126)	1.8
Proline (Sigma P0380)	4.5
Serine (Sigma A0884)	4.05
Alanine (Sigma A7627)	1.8
Na-Glutamate (Sigma G1626)	10.35
Ethyl L-tyrosinate HCl (Sigma T4879)	1.875
Ferrous gluconate (Sigma 344427)	0.15
Mn acetate tetrahydrate (Sigma 229776)	0.1662
ZnSO4*H2O (Sigma 96495)	0.1218
Cu acetate*H2O (Sigma 217557)	1.11E-02
Cr acetate*H2O (Santa Cruz sc-227648)	7.50E-03
NaF (Sigma 201154)	6.30E-03
SnSO4*2H2O (Sigma 244635)	1.11E-03
Ammonium molybdate tetrahydrate (Sigma 09880)	1.11E-03
NiCl2 hydrate (Sigma 364304)	1.11E-03
Cobalt acetate tetrahydrate (Sigma 403024)	3.30E-04
Sodium orthovanadate (Sigma 450243)	6.60E-04
Sodium selenate (Sigma S5261)	2.88E-04

Cool to 4 ºC, add the following:	
Ca glycerophosphate (Sigma G6626)	15.9
Mg glycerophosphate (Sigma 17766)	4.35
CaCl2*2H2O (Sigma 3881)	0.5625
NaCl (Sigma S9888)	0.1778
KI (Sigma 207969)	1.42E-03
K acetate (Sigma P1190)	5.625
Choline HCl (Sigma C1879)	0.95
Vitamin B12 (Sigma V2876)	4.72E-03
Biotin (Sigma B4501)	8.20E-04
Folic acid (Sigma 7876)	1.23E-03
Thiamine HCl (Sigma T4625)	4.10E-03
Pyridoxine HCl (Sigma 9755)	5.13E-03
Riboflavin (Sigma R4500)	6.15E-03
Niacinamide (Sigma N5535)	3.08E-02
D-Pantothenic acid (Sigma 21210)	4.10E-02
myo-Inositol (Sigma I5125)	2.05E-01
Combine both solutions at 4 ºC *& sterile filter*	
Lipid Solution	
Soybean oil (Sigma S7381)	
Retinyl palmitate (Sigma R3375)	1.87E-01
Cholecalciferol (Sigma C9756)	5.00E-04
Vitamin K (Sigma V3501)	0.675
DL-tocopherol acetate (Sigma T3376)	20
DL-tocopherol (Sigma 258024)	10
Warm to 50 ºC prior to sterile filtration	

Online supplementary files

Database S1. List of mAbs. Excel file listing name, cellular origin, microbiota-reactivity, polyreactivity, variable gene usage, and sequences of antibodies used in this study. Sequence characteristics were determined using IMGT (www.imgt.org).