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Supplemental material

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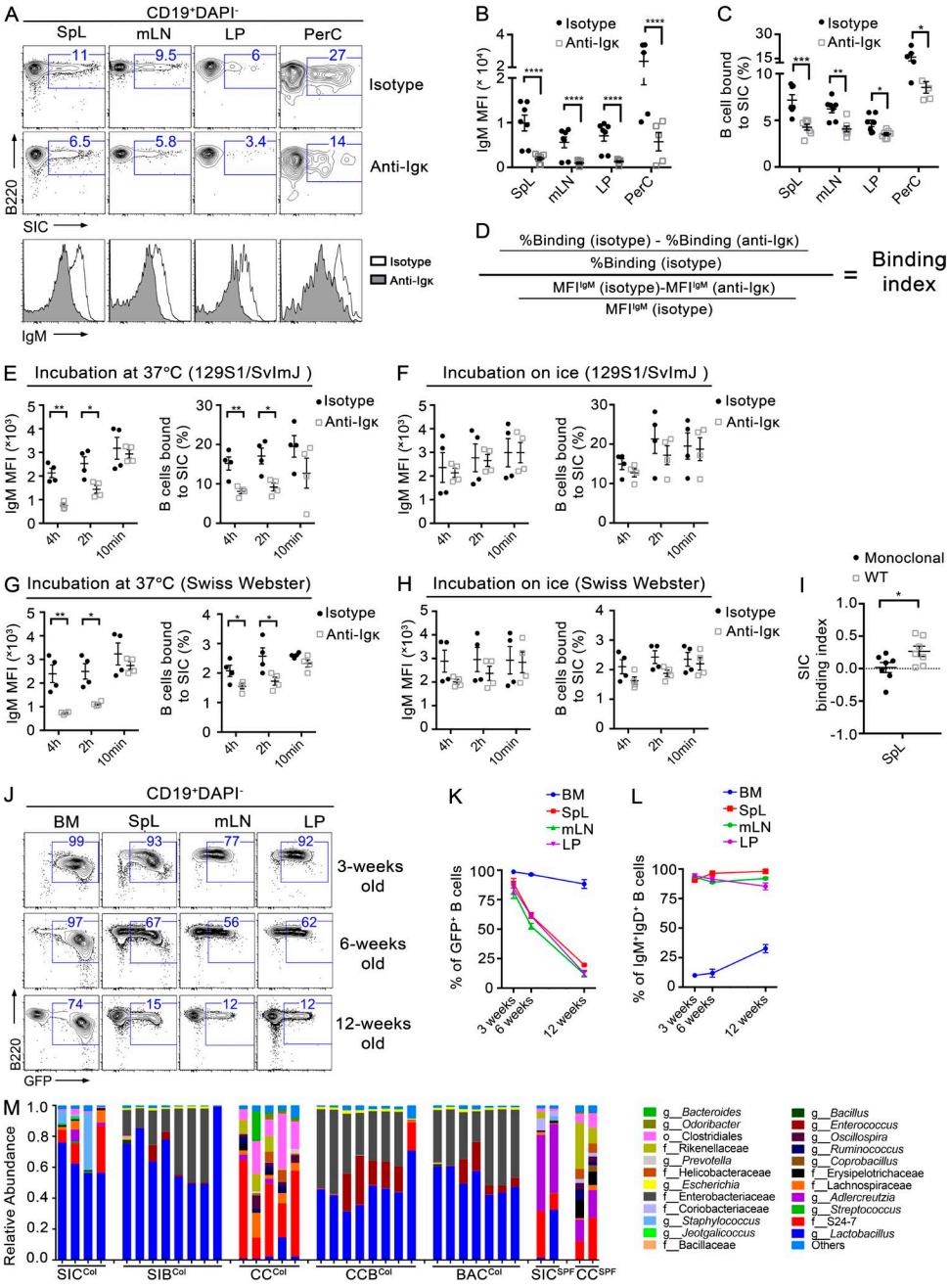

Figure S1. **BCR-dependent binding of SIC and 16S data of bacterial populations. (A–C)** FACS plots (A) of B cells gated on CD19$^+$ DAPI$^-$ from indicated tissues stained with SIC and scatter plots of IgM geometric MFI (B) and percentage of B cells bound to SIC (C) after F(ab')$_2$ anti-Igκ Ab (Ab; n = 5–7) or isotype Ab treatment (n = 5–7). Data are from four to five independent experiments for SpL, mLN, LP, and PerC, respectively. Two-tailed t test. **(D)** The equation to calculate binding index. **(E–H)** Dot plots of IgM MFI and percentage of B cells bound to SIC CD19$^+$ DAPI$^-$ gated cells from SpL of 129S1/SvImJ WT mice (E and F; n = 4) and SW mice (G and H; n = 4) after F(ab')$_2$ anti-Igκ Ab or isotype Ab treatment at 37°C (E and G) or on ice (F and H) for the indicated times. Data are from two independent experiments. **(I)** Dot plot showing SIC binding index of CD19$^+$ DAPI$^-$ gated cells from SpL of WT BALB/c (n = 7) and monoclonal IgH/IgL (n = 7) mice. Data are from two independent experiments. Two tailed Mann–Whitney test. **(J–L)** FACS plots (J) and scatter plots (K and L) showing percentages of B220$^+$GFP$^+$ cells (K) and IgM$^+$ IgD$^+$ cells (L) gated on CD19$^+$DAPI$^-$ cells from indicated tissues of *BAC-Rag2pGFP* mice (n = 3–6) at the indicated ages. Data are from two to three independent experiments. Two-tailed t test. **(M)** Relative abundance of bacterial taxa as assessed by 16S rRNA gene sequencing. SIC^{Col} and CC^{Col} indicate small intestinal and cecal content, respectively, from GF mice that were conventionalized for 21 d. SIB^{Col} and CCB^{Col} indicate bacteria cultured from small intestine and cecum, respectively, from GF mice that were conventionalized for 21 d. BAC^{Col} indicates a 1:1 mixture of SIB^{Col} and CCB^{Col} used as the bacterial substrate in the ELISAs of the LDA experiments. For comparison, SIC^{SPF} and CC^{SPF} are also shown, which are small intestinal and cecal content, respectively, from SPF mice. Repeat experiments are shown as individual bars. The most abundant microbes are listed as genus (g), family (f), or order (o). Sparsely represented members of the same family or order are grouped together (others). The conventionalization of GF SW mice occurs during the interval beginning at the age of postnatal day 21 for 21 d. Error bars in the results indicate ± SEM *, P < 0.05; **, P < 0.01; ***, P < 0.0005; ****, P < 0.0001.

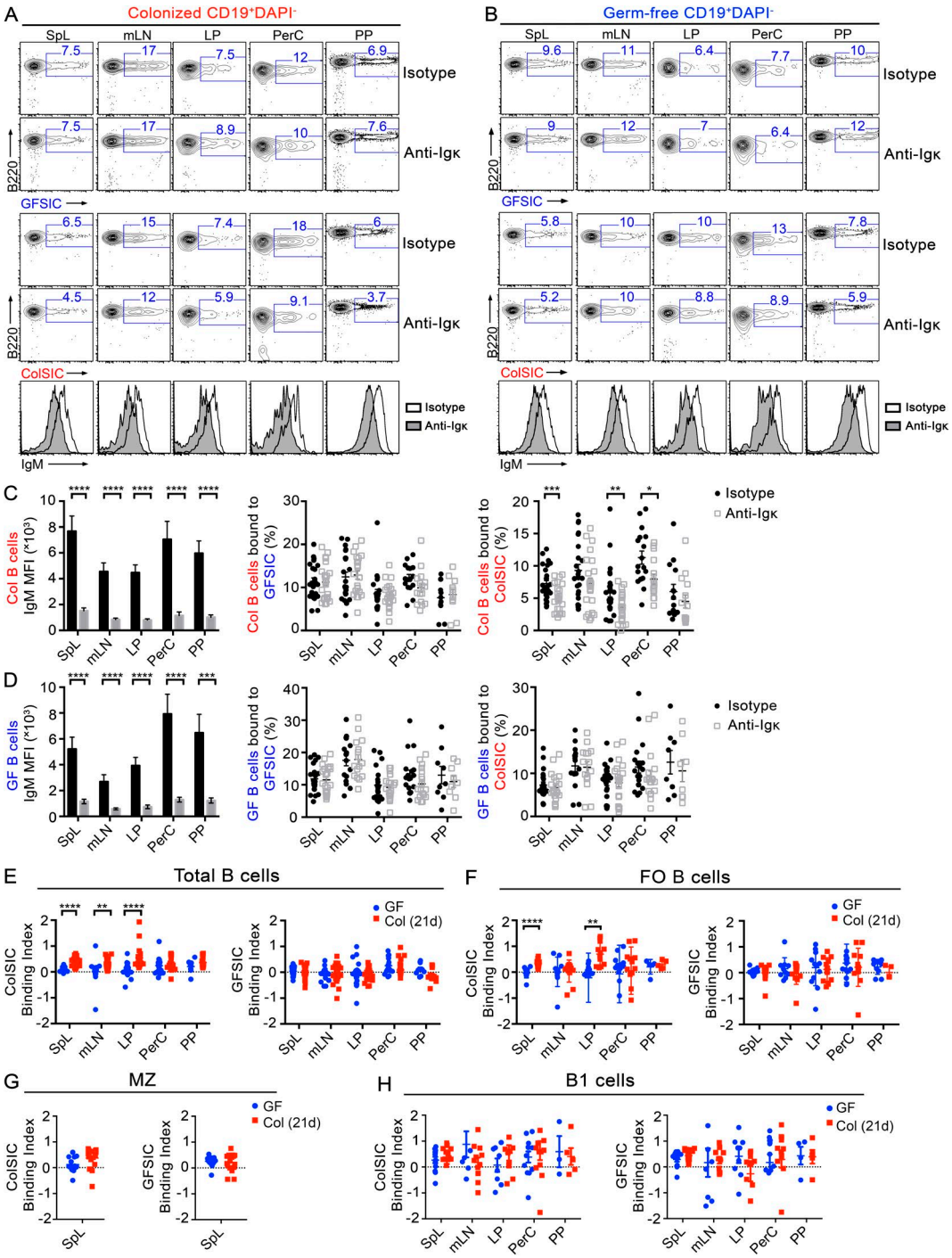

Figure S2. **BCR-dependent binding of SIC to B cell surfaces in GF and conventionalized mice. (A and B)** Representative FACS plots of B cells gated on CD19⁺ DAPI⁻ from indicated tissues binding to GFSIC or colonized SIC (ColSIC) after F(ab')$_2$ anti-Igκ Ab or isotype Ab treatment. Tissues were taken from colonized SW littermates (A) or GF SW littermate mice (B). **(C and D)** Bar graphs showing IgM geometric MFI and dot plots showing percentage of CD19⁺ DAPI⁻ B cells from indicated tissues bound to GFSIC and ColSIC after F(ab')$_2$ anti-Igκ Ab or isotype Ab treatment. Two-tailed *t* test. Tissues were taken from conventionalized (Col; C; *n* = 11–27) or GF SW littermates (C; *n* = 8–22). Data are from four to eight independent experiments. **(E)** Binding index measurements of ColSIC and GFSIC to DAPI⁻ CD19⁺ B220⁺ B cells of the indicated tissues from GF (*n* = 8–22) and conventionalized (Col; *n* = 11–27) SW littermates. For the GFSIC binding index plot, one outlier (−4.07) in mLN and one outlier (−2.30) in PerC from Col SW mice were included in the statistics calculation but not depicted in the scatter plots. Data are from four to eight independent experiments. **(F–H)** Binding index (Fig. S1 D) of ColSIC and GFSIC to B cells with a follicular (FO) B cell phenotype (DAPI⁻ CD19⁺ B220⁺ CD93⁻ CD23⁺ CD21^{int}; F), MZ B cell phenotype (DAPI⁻ CD19⁺ B220⁺ IgM⁺ CD93⁻ CD23⁻ CD21^{hi}; G), and B1 B cell phenotype (H) in indicated tissues of GF SW (*n* = 3–14) and colonized littermates (Col; *n* = 3–14). Data are from two to three independent experiments. The following markers defined B1 cells from SpL and mLN: DAPI⁻ CD19⁺ B220⁺ IgM⁺ CD93⁻ CD23⁻ CD43⁺ (splenic B1 cell phenotype). The following markers defined B1 cells from nonsplenic tissues: DAPI⁻ CD19⁺ B220⁺ CD93⁻ CD23⁻ CD11b⁺ (PerC B1 cell phenotype). Error bars in the results indicate ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.0005; ****, P < 0.0001. Two tailed Mann–Whitney test. Conventionalization experiments were conducted by cohousing GF mice with SPF mice for 21 d from the age of postnatal day 21.

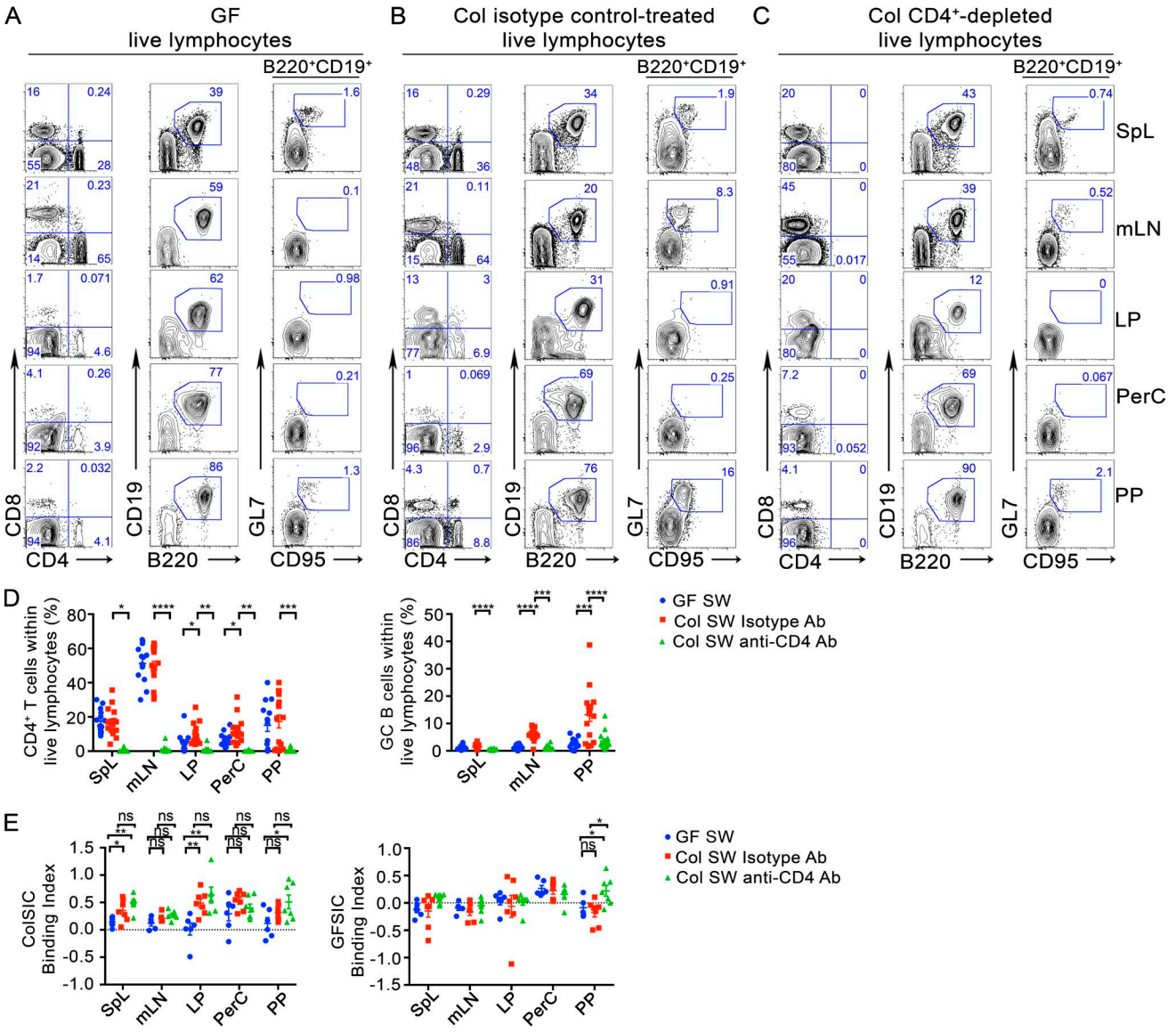

Figure S3. **CD4 T cell depletion in anti-CD4 Ab–injected colonized mice. (A–D)** FACS plots (A–C) and scatter plots (D) showing the percentage of $CD4^+$ T cells, GC B cells ($DAPI^-CD19^+B220^+GL7^+CD95^+$) in the indicated tissues from the GF SW ($n = 14$), isotype Ab–injected ($n = 16$), and anti-CD4 Ab–injected ($n = 16$) SW GF littermates colonized for 14 or 21 d. Data are from five independent experiments. Multiple *t* test. **(E)** Dot plots showing binding index (see text) of ColSIC and GFSIC of $CD19^+B220^+$ B cells gated on the live lymphocytes from indicated tissues from GF ($n = 4–6$), isotype Ab–injected ($n = 6–7$), and anti-CD4 Ab–injected ($n = 6–7$) SW GF littermates conventionalized for 14 or 21 d. Data are from two independent experiments. Error bars in the results indicate ± SEM. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0005$; ****, $P < 0.0001$. Two tailed Mann–Whitney test. Colonization experiments were initiated in GF mice beginning at the age of postnatal day 21.

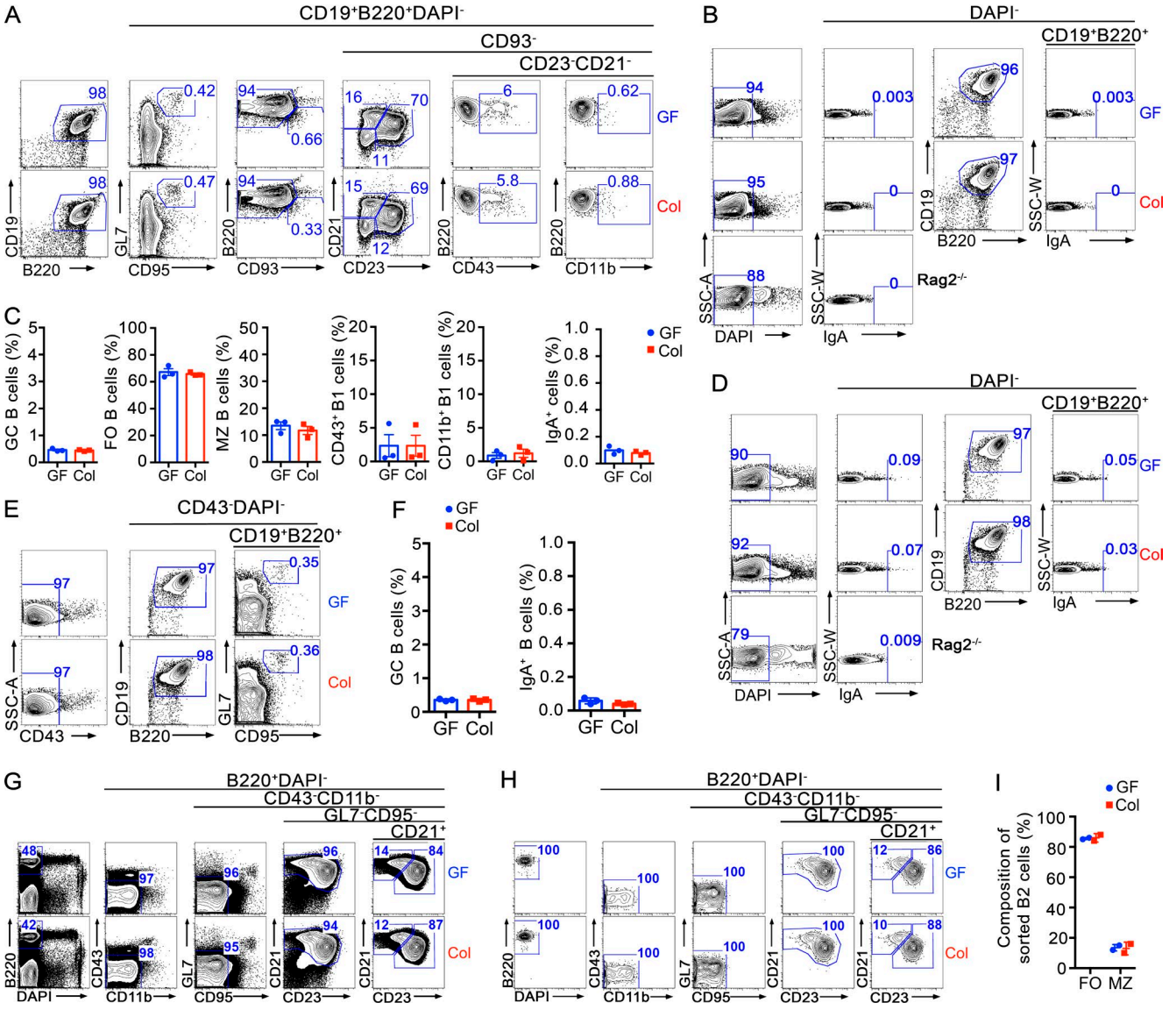

Figure S4. **Adoptive Transfer of GF and Conventionalized B Cells to *Rag2*$^{-/-}$ Mice. (A–C)** FACS plots (A and B) and bar graphs (C) showing the percentage of the indicated B cell composition of the B220^{+} magnetically purified splenic B cells from GF SW and conventionalized (Col) littermates. Under the CD19^{+} B220^{+} DAPI^{-} B cell gate, the GC cells, follicular (FO) B cells, MZ B cells, CD43^{+} B1 cells, and CD11b^{+} B1 cells were further gated on CD95^{+} GL7^{+}, CD93^{-} CD23^{+} CD21^{int}, CD93^{-} CD23^{-} CD21^{hi}, CD93^{-} CD23^{-} CD21^{-} CD43^{+}, and CD93^{-} CD23^{-} CD21^{-} CD11b^{+}, respectively (C). Three independent experiments of eight SW mice donors for each are shown. **(D–F)** FACS plots (D and E) and bar graphs (F) showing the percentage of the indicated B cell composition of the CD43^{-} magnetically purified splenic B cells from GF SW and conventionalized (Col) littermates. Two independent experiments of eight SW mice donors for each are shown. **(G–I)** FACS plots of sorting strategy (G), post-sort purity (H), and the scatter plot (I) of the composition of the sorted live B2 (B220^{+} DAPI^{-} CD43^{-} CD11b^{-} GL7^{-} CD95^{-} CD21^{+}) splenic B cells from GF SW and colonized littermates (Col). Two independent experiments of eight SW mice donors for each are shown. Conventionalization of GF mice was for 21 d beginning at the age of postnatal day 21. Error bars indicate ± SEM.

Table S1. **Summary of Ig repertoire sequencing of sorted B cell subsets from germ-free versus conventionalized SW littermates for 7 or 21 days.**

	Germ-free mice			Conventionalized mice		
Cell types	**Total sequences analyzed**	**No. of libraries included**	**No. of unique CDR3s**	**Total sequences analyzed**	**No. of libraries included**	**No. of unique CDR3s**
	Postnatal day 28			**7-d conventionalization**		
SpL T1	87,759	6	35,852	190,496	5	50,620
SpL FO	402,856	6	158,621	438,818	7	142,802
LP	12,353	5	3,426	22,491	5	6,962
	Postnatal day 42			**21-d conventionalization**		
SpL T1	149,654	7	26,855	110,207	7	25,472
SpL FO	455,613	7	140,313	563,464	7	166,032
LP	138,866	12	47,114	62,163	8	18,576

Each library was generated from the indicated sorted B cell subtype of one mouse.