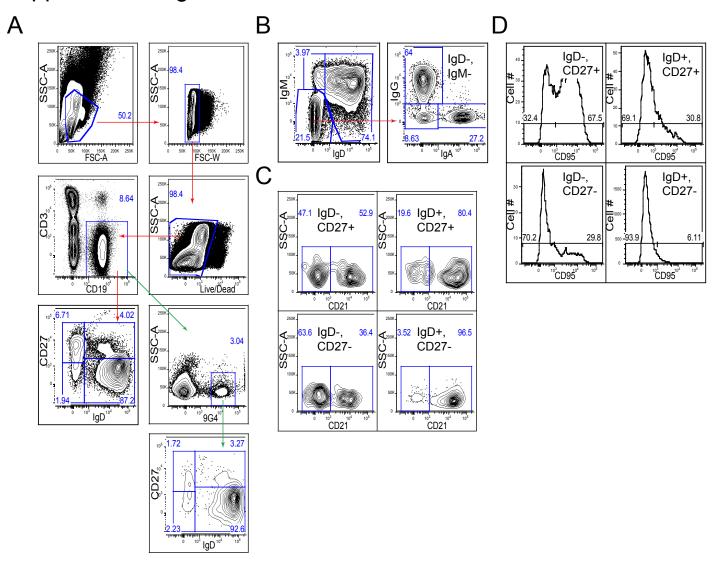
Supplemental Figure 1



Supplemental Figure 1. Gating strategy for assessing bulk and 9G4+ B cell subsets. Antibodies for the analysis are listed in supplemental table 1. A. Lymphocytes were first gated on by forward side scatter – area and side scatter – area. Next doublet cells were excluded by using the side scatter width 'low' gated population. Living cells were then gated as low for the live/dead dye. Next CD3 and CD19 antibodies were used to gate on bulk B cells as CD3-, CD19+. Bulk B cells were separated into naïve and memory B cell subsets by using IgD and CD27. Separately, the bulk B cells were gated on for 9G4+ity, and then the 9G4+ cells were separated by IgD and CD27 as well. B. Both bulk and 9G4+ B cells were further gated as IgD+, IgM, IgD-, or switched (IgD-, IgM-). The switched population was then further separated by IgG and IgA to decipher IgG+ (IgA-), IgA+ (IgG-), or 'other' unlabeled cells. C. Both bulk B cells or 9G4+ B cells were further gated on for being positive or negative for CD21 for the IgD, CD27 naïve/memory B cell subsets. D. Both bulk and 9G4+ B cells were assessed for CD95 by gating on IgD and CD27 memory subsets and using a histogram positive gate for CD95.

Supplemental Table 1.

Adult Panel

Marker	Color	Company	Catalog #
CD19	BV421	BioLegend	302234
Live/Dead	Aqua	Invitrogen	L34957
IgM	BV570	Biolegend	314517
CD3	BV605	Biolegend	317321
CD27	BV650	BioLegend	302827
IgG	Fitc	BD	560952
CD95	PE-CF594	BD	562395
CD21	PE-Cy5.5	BD	551064
lgD	PE-Cy7	Biolegend	348210
IgA	APC	MiltenyBiotec	130-093-113
9G4	Biotin	(From Ignacio	Sanz)
Streptavidin	AF680	Invitrogen	S-32358

6 year old panel

Marker	Color	Company	Catalog #
CD19	BV421	BioLegend	302234
Live/Dead	Aqua	Invitrogen	L34957
CD3	BV605	Biolegend	317321
CD27	PE	Biolegend	356406
lgD	PE-Cy5.5	Biolegend	348208
9G4	Biotin	(From Ignacio Sanz)	
Streptavidin	AF680	Invitrogen	S-32358

Biolegend: San Diego, CA
Beckman Dickinson (BD) Franklin Lakes, NJ
Invitrogen Carlsbad, CA
MiltenyiBiotec San Diego, CA

Supplemental Table 1. Antibody panels used in cellular flow cytometric analysis.

Two B cell flow panels with markers, fluorochrome colors, companies, catalog numbers, and company locations listed.