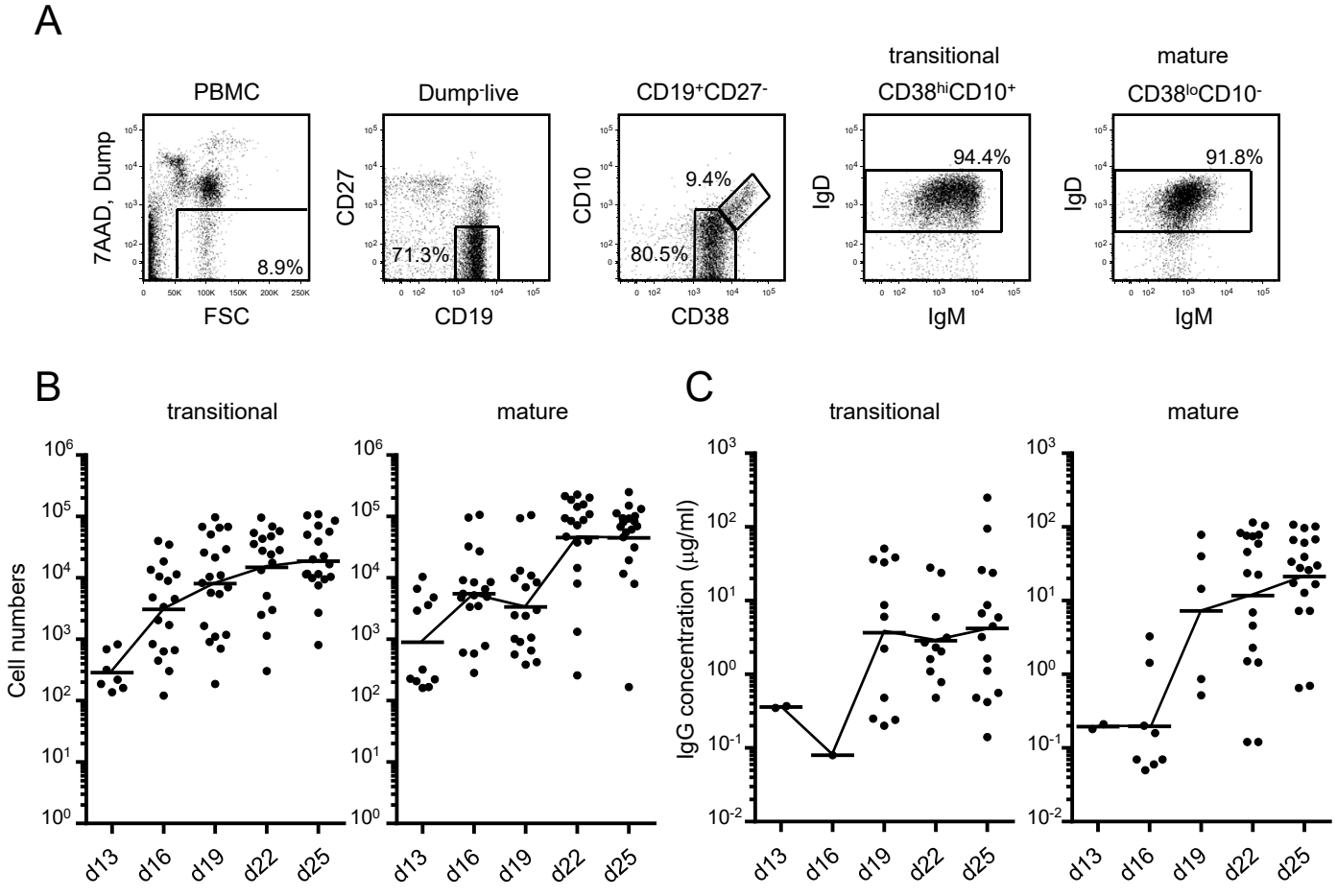

Figure S1. Kinetics of B-cell growth and IgG production from single sorted transitional and mature B cells.

Single B cells were sorted directly into individual wells and cultured in the presence of MS40L^{lo} feeder cells with exogenous recombinant human IL-2, IL-4, IL-21, and BAFF. (A) Representative flow plots showing the gating strategy used to isolate transitional B (CD19^{+}CD27^{-}CD38^{hi}CD10^{+}IgD^{+}) and mature naïve B cells (CD19^{+}CD27^{-}CD38^{lo}CD10^{-}IgD^{+}) from PBMCs of healthy controls. (B and C) Kinetics of B-cell numbers (B) and IgG concentrations in culture supernatants (C) during single B-cell cultures for transitional and mature B cells. We analyzed 22 individual cultures for each time point. Data shown are values for samples that exceeded the background for cell counting and IgG determinations.

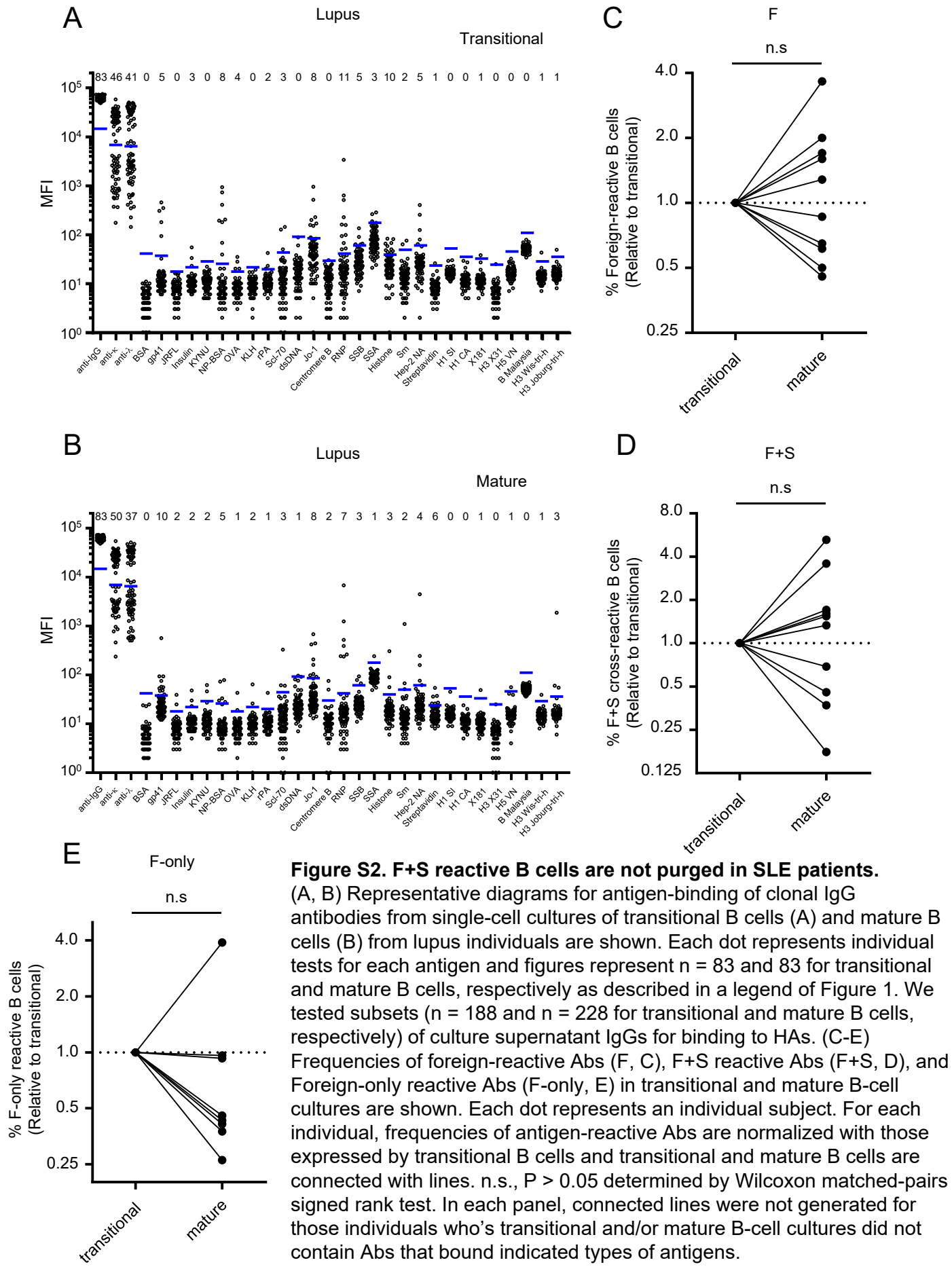

Figure S2. F+S reactive B cells are not purged in SLE patients.
(A, B) Representative diagrams for antigen-binding of clonal IgG antibodies from single-cell cultures of transitional B cells (A) and mature B cells (B) from lupus individuals are shown. Each dot represents individual tests for each antigen and figures represent n = 83 and 83 for transitional and mature B cells, respectively as described in a legend of Figure 1. We tested subsets (n = 188 and n = 228 for transitional and mature B cells, respectively) of culture supernatant IgGs for binding to HAs. (C-E) Frequencies of foreign-reactive Abs (F, C), F+S reactive Abs (F+S, D), and Foreign-only reactive Abs (F-only, E) in transitional and mature B-cell cultures are shown. Each dot represents an individual subject. For each individual, frequencies of antigen-reactive Abs are normalized with those expressed by transitional B cells and transitional and mature B cells are connected with lines. n.s., P > 0.05 determined by Wilcoxon matched-pairs signed rank test. In each panel, connected lines were not generated for those individuals who's transitional and/or mature B-cell cultures did not contain Abs that bound indicated types of antigens.

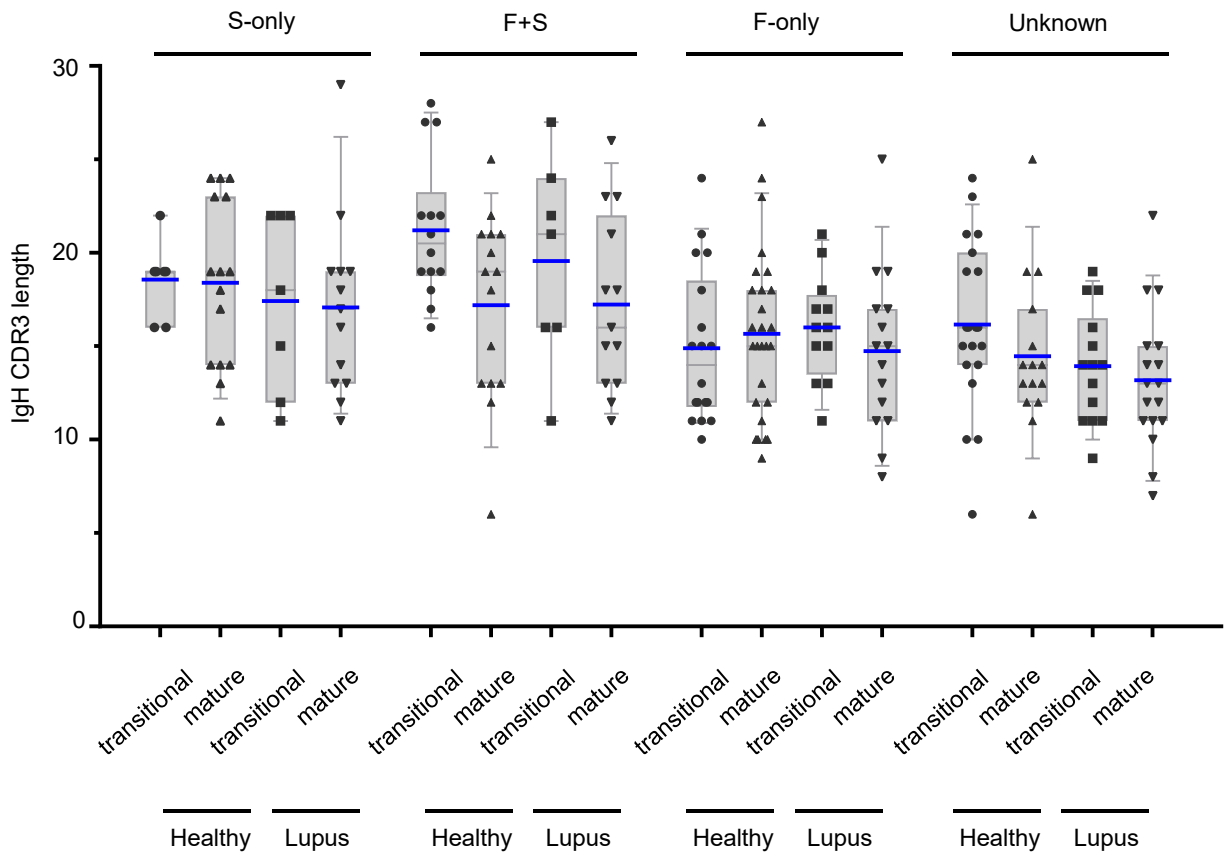

Figure S3. Self-reactive Abs exhibit long HCDR3.
V_HDJ_H rearrangements were recovered from transitional and mature B cells after single B-cell culture (see the legend of Fig. 6). S-only, n = 42; F+S, n = 49; F-only, n = 72; and Unknown, n = 67. Each dot represents an individual sample. Keys: transitional (●, ■) and mature B cells (▲, ▼) from healthy donors and from lupus patients, respectively. Blue bars, mean. Boxes extend from the 25th to 75th percentiles and bars represent the range (10th/90th percentile) of values.

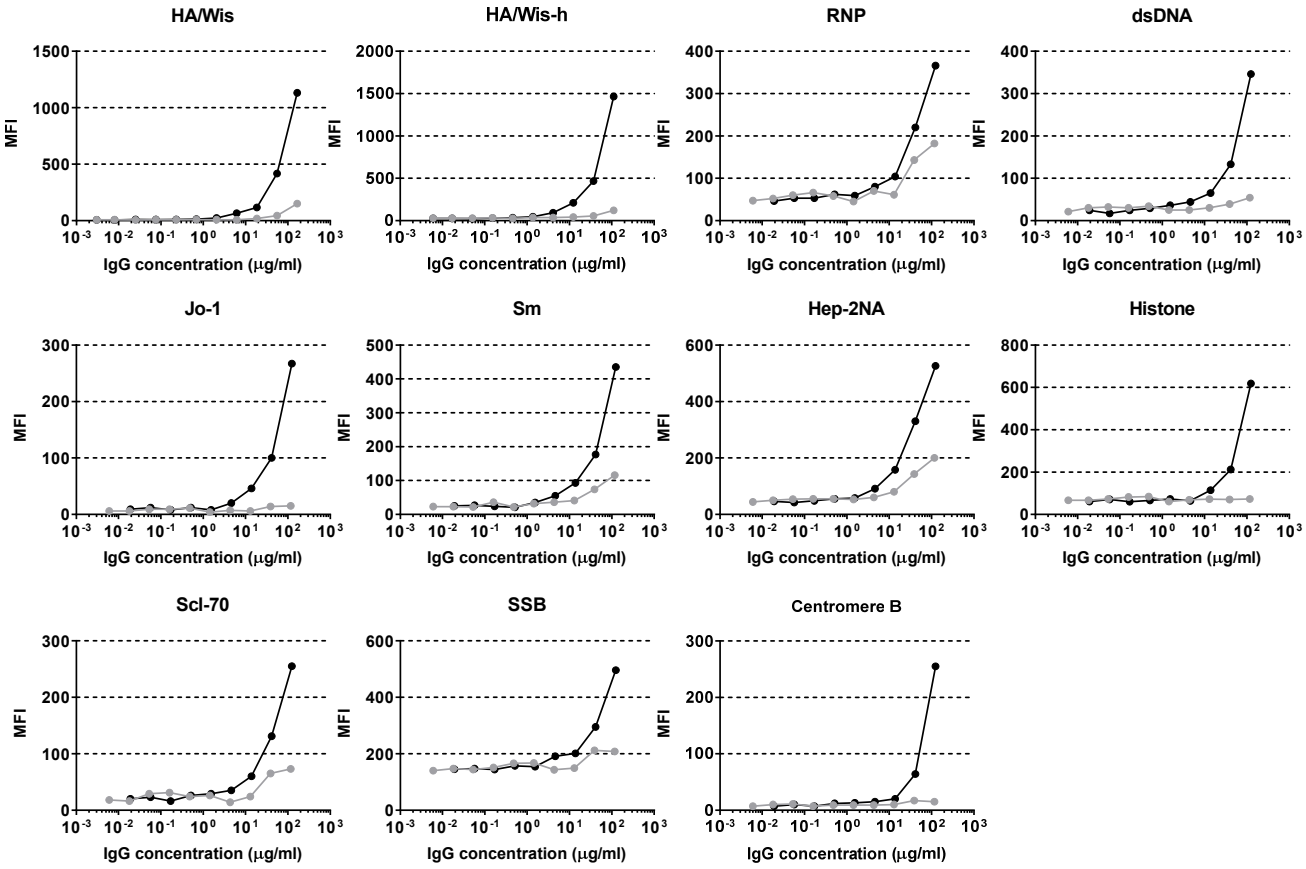

Figure S4. Binding to a panel of foreign and self-antigens by F+S reactive IgG (Ab5).
Various concentrations Ab5 (black) or isotype-matched, control IgGs (151K or K03.12) were incubated with a panel of foreign and self-antigens conjugated with beads and binding to individual antigens were determined by Luminex multiplex assay. As K03.12 avidly binds to HA/Wis and HA/Wis-h, 151K was used as negative controls for binding to HA/Wis and HA/Wis-h. Representative data from two independent experiments are shown.

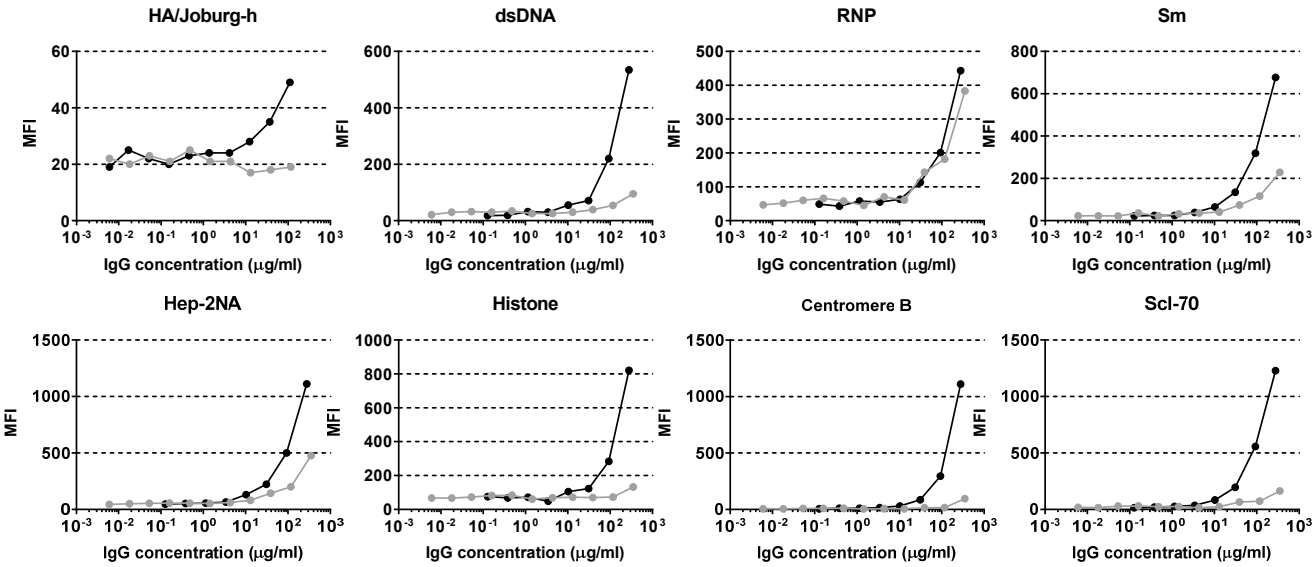

Figure S5. Binding to a panel of foreign and self-antigens by F+S reactive IgG (Ab6).
Various concentrations Ab6 (black) or isotype-matched, control IgGs (K03.12) were incubated with a panel of foreign and self-antigens conjugated with beads and binding to individual antigens were determined by Luminex multiplex assay. Representative data from two independent experiments are shown.

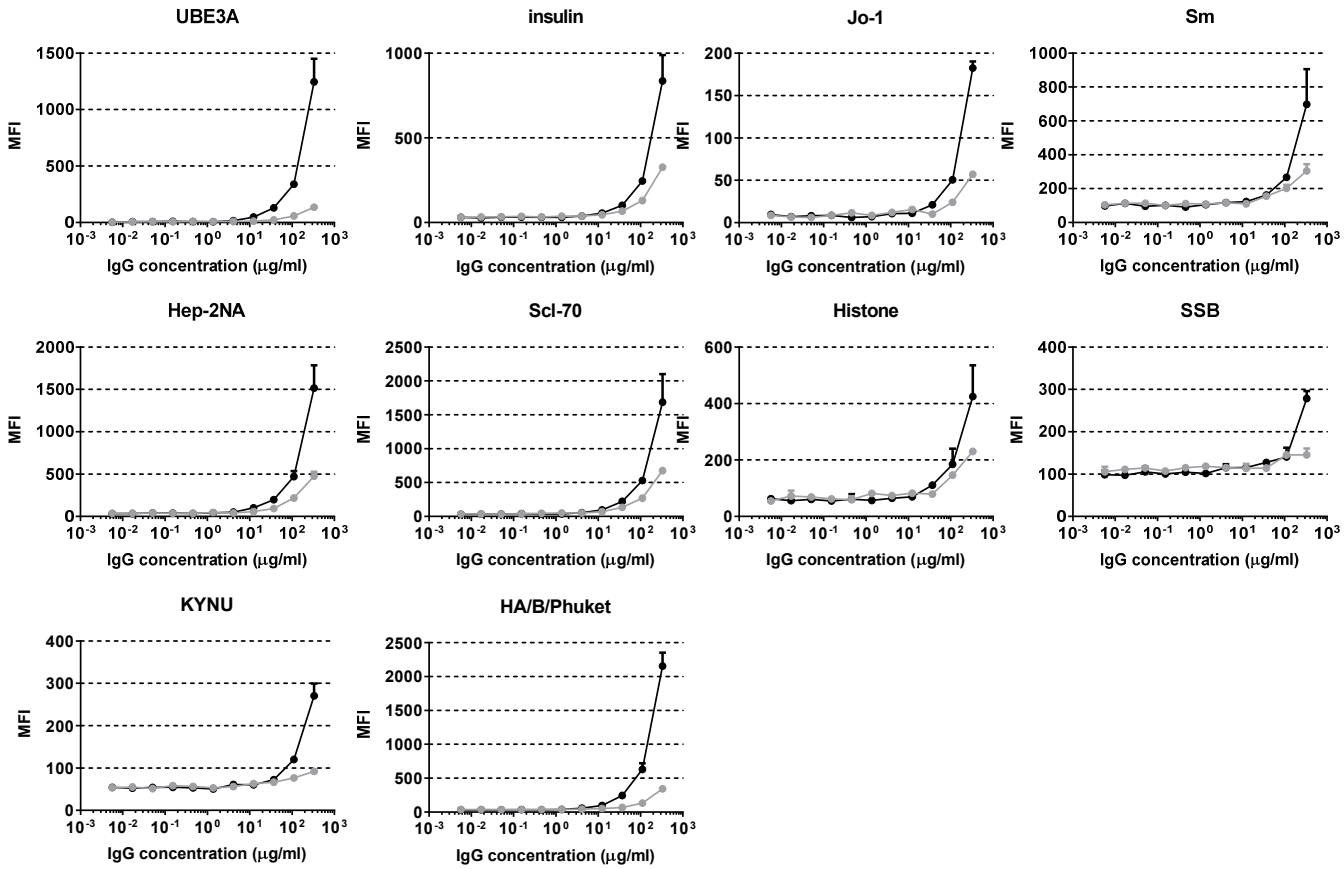

Figure S6. Binding to a panel of foreign and self-antigens by F+S reactive IgG (Ab9).
Various concentrations Ab9 (black) or isotype-matched, control IgGs (K03.12) were incubated with a panel of foreign and self-antigens conjugated with beads and binding to individual antigens were determined by Luminex multiplex assay. Representative data from two independent experiments are shown.

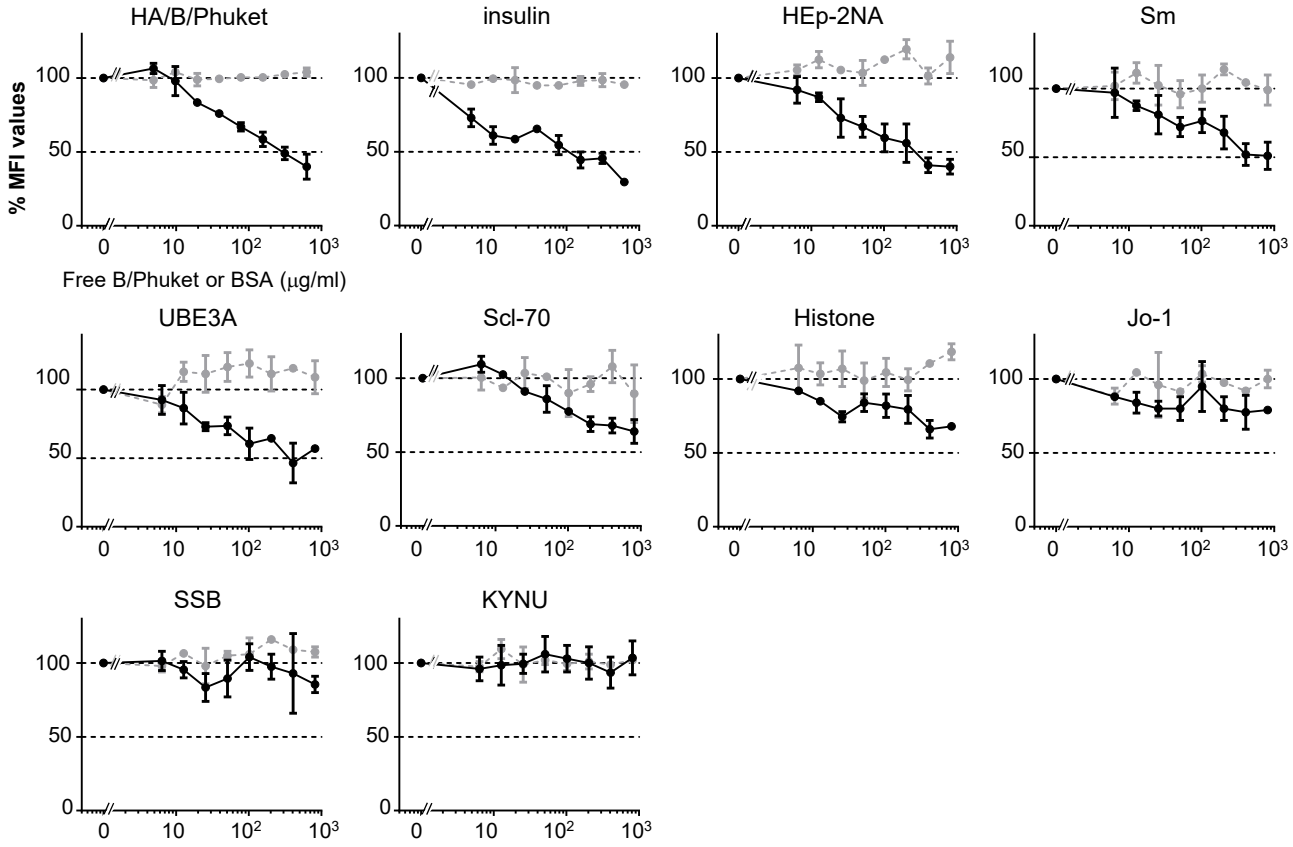

Figure S7. Inhibition of F+S reactive IgGs by free foreign antigens.
F+S reactive IgG (Ab9) were incubated with various concentrations of free, either specific or irrelevant (BSA) foreign antigens, and then bindings to foreign antigens (homologous inhibition) and self-antigens (heterologous inhibition) were assessed by Luminex multiplex assay. Fixed concentrations of Ab9 (400 μg/ml) were first incubated with indicated concentrations of free HA/B Phuket (black lines) or BSA (gray lines), and then these mixtures were added to a panel of antigen-bead conjugates. MFI values on each antigen/bead conjugate were normalized with those without free antigens that were set as 100%. The Ab9 bound HA/B Phuket, and human self-antigens, including insulin, HEp-2 NA, Sm, UBE3A, Scl-70, Histone, Jo-1, SSB, and KYNU (Fig. S6).

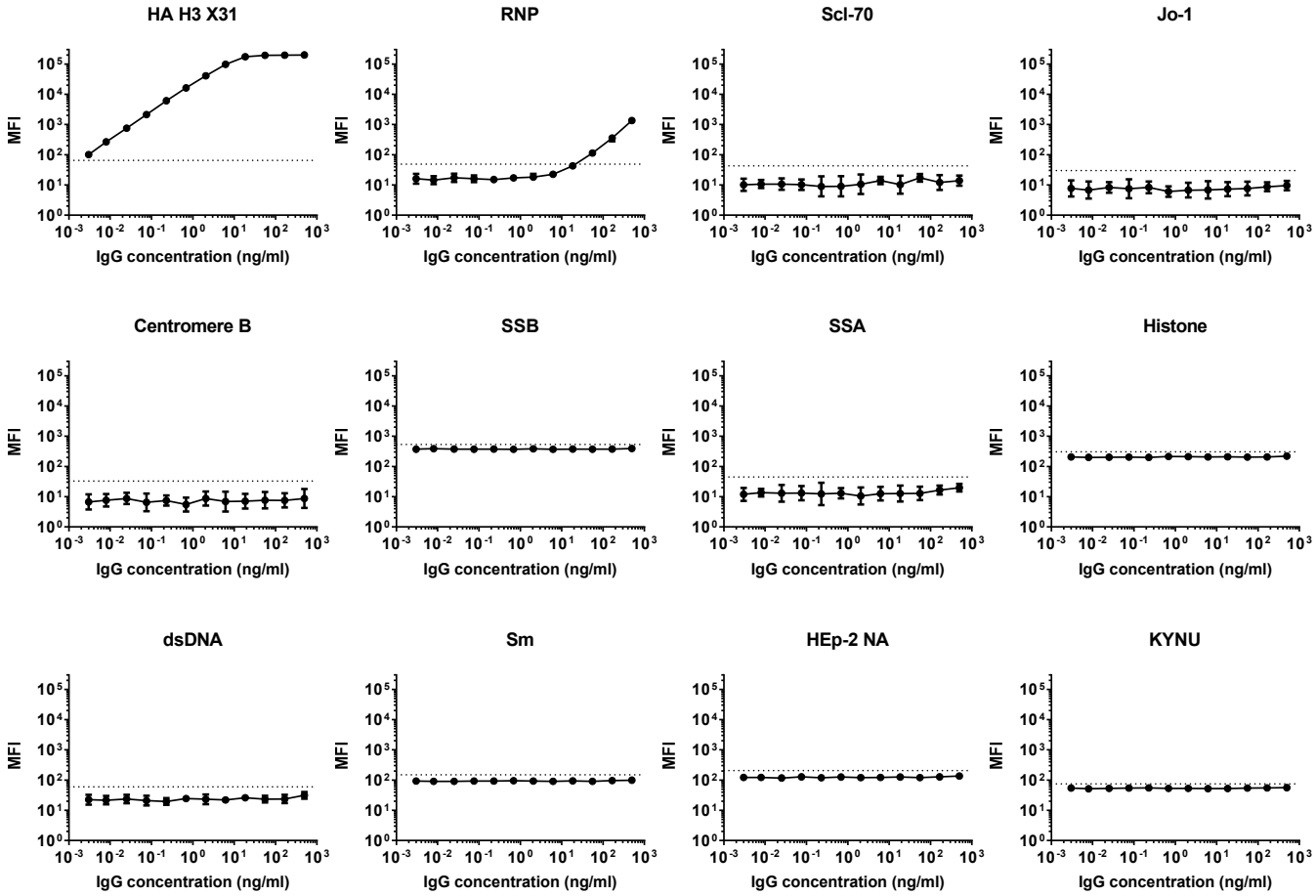

Figure S8. HC19 binds HA H3 X31 and RNP.
Serially diluted, influenza HA-specific monoclonal antibody (HC19, black lines, n = 14) were tested for binding to self-antigens, including AtheNA autoantigens and KYNU. Doted lines (gray) represent threshold median fluorescence intensity (MFI) values established for individual antigens (mean + 6SD MFI of B-cell negative mock cultures). HC19, starting at 0.5 μg/ml and 3-fold, 12 serial dilutions. Shown are geometric mean of individual MFI values ± geometric SD.

Table S1. Number of antigen-reactive BCRs/antibodies.

Antigen	Healthy		Lupus	
	Transitional (n = 429)	Mature (n = 654)	Transitional (n = 506)	Mature (n = 742)
Scl-70	2	2	7	7
dsDNA	3	7	1	9
Jo-1	40	39	35	44
Centromere B	6	4	4	7
RNP	52	63	31	47
SSB	25	9	12	14
SSA	17	13	6	6
Histone	40	20	21	21
Sm	5	3	3	9
Hep2NA	13	9	8	17
KYNU	3	5	1	11
Insulin	10	3	8	15
gp41 mn	53	54	31	43
Streptavidin	2	7	1	18
gp140 JR-FL	3	3	1	10
NP-BSA	38	35	31	36
KLH	7	3	0	8
OVA	8	5	5	12
rPA	14	11	9	18
HAs	6	3	4	6

Culture supernatant IgGs from single-cell cultures of indicated B-cell subsets were screened against a panel of self-antigens and foreign antigens in Luminex assay (see also legends of Figures 1 and S2. For each antigen and for each B-cell type, number of antigen-reactive BCRs (as culture supernatant IgGs) is shown.