Supporting Information

Mietzner *et al.* **10.1073/pnas.0803644105**

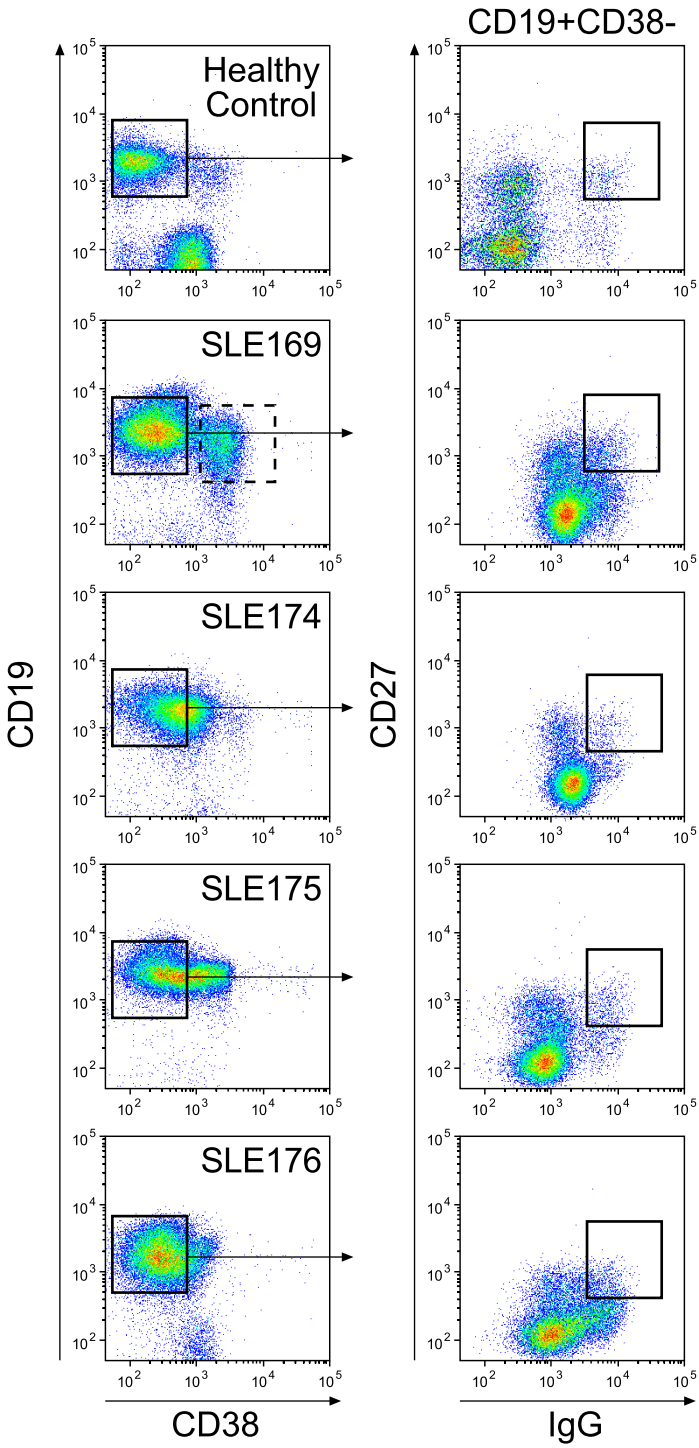

Fig. S1. Surface staining and gates for FACS purification of single IgG+ memory B cells from healthy controls and SLE patients. Preenriched B cells were stained for surface expression of CD19, CD38, CD27, and IgG. CD19+CD38− cells and CD19+CD38+ cells (*Left*) were further analyzed for CD19 and IgG expression (*Right*). Boxed CD38−CD19+IgG+CD27+ memory B cells from SLE169, SLE174, SLE175, and SLE176 and CD38+CD19+IgG+CD27+ plasmablasts from SLE169 were single-cell FACS sorted (*Right*) before amplification of mutated Igγ H chains and corresponding IgL chains (Table S2, Table S3, Table S4, Table S5, and Table S6).

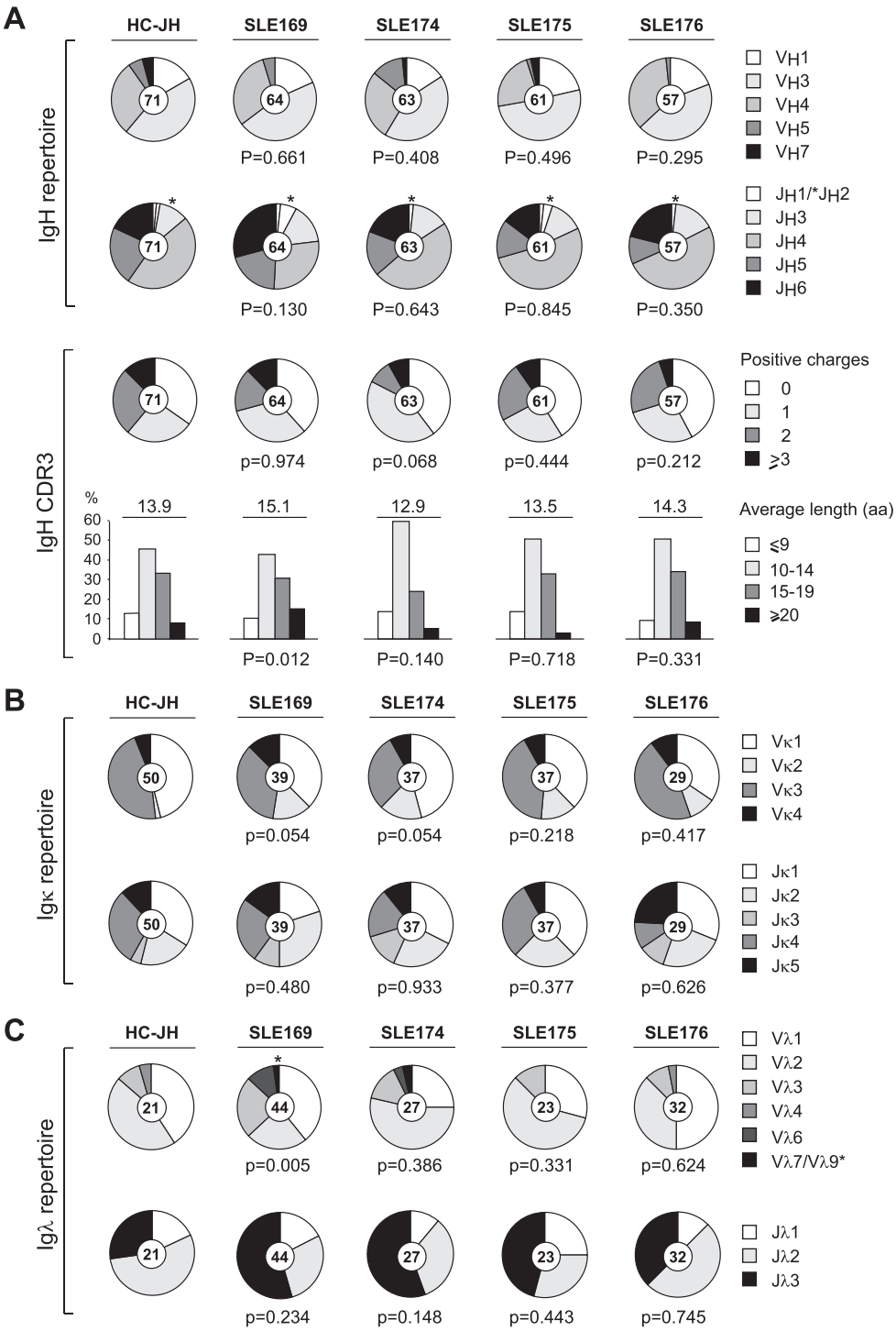

Fig. S2. Ig V and J gene usage and HCDR3 region features of IgG+ memory B cell antibodies from one unpublished HC and SLE patients. IgH and IgL chain gene repertoire and HCDR3 features of antibodies from IgG+ memory B cells of HC donor (JH) and four patients with SLE (SLE169, SLE174, SLE175, and SLE176). *P* values are in comparison with combined data from HC-JH and three additional published HCs (1). (*A*) Pie charts depict VH and JH family usage and the proportion of IgH CDR3s with 0, 1, 2, or ≥3 positive charges. Bar graphs show frequencies of IgH CDR3s with ≤9 aa (white bars), 10–14 aa (light gray bars), 15–19 aa (dark gray bars), and ≥20 aa (black bars). The absolute number of sequences analyzed is indicated in the center of each pie chart. Average IgH CDR3 amino acid length is indicated above the bar graphs. (*B* and *C*) Pie charts depict Vκ/Jκ (*B*) and Vλ/Jλ (*C*) gene family usage. The absolute number of sequences analyzed is indicated in the center of each pie chart.

1. Tiller T, *et al.* (2007) Autoreactivity in human IgG(+) memory B cells. *Immunity* 26:205–213.

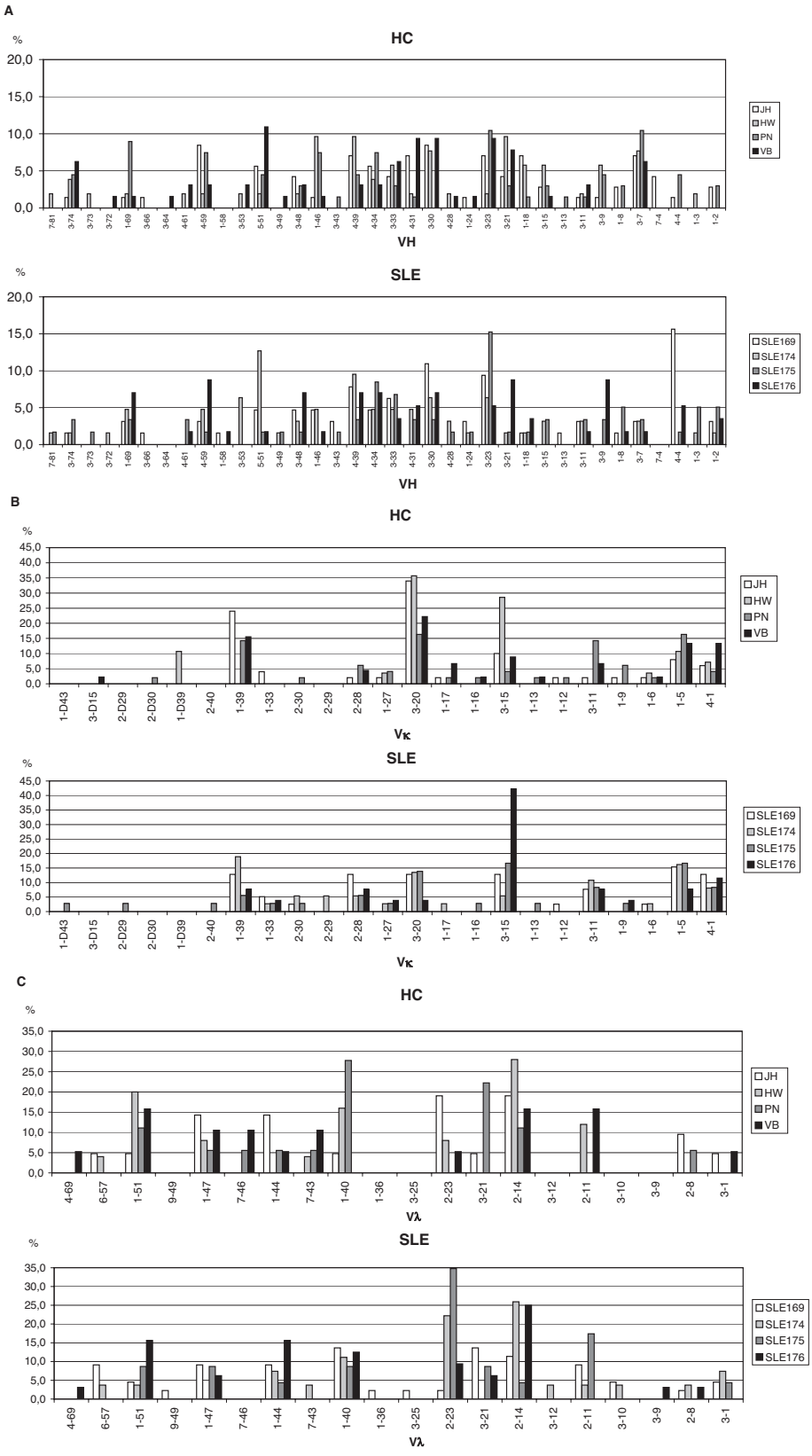

Fig. S3. VH (*A*), Vκ (*B*), and Vλ (*C*) gene usage in IgG+ memory B cells of healthy donors JH, HW, PN, and VB as compared with SLE patients 169, 174, 175, and 176. The frequency of individual V genes is shown for each individual.

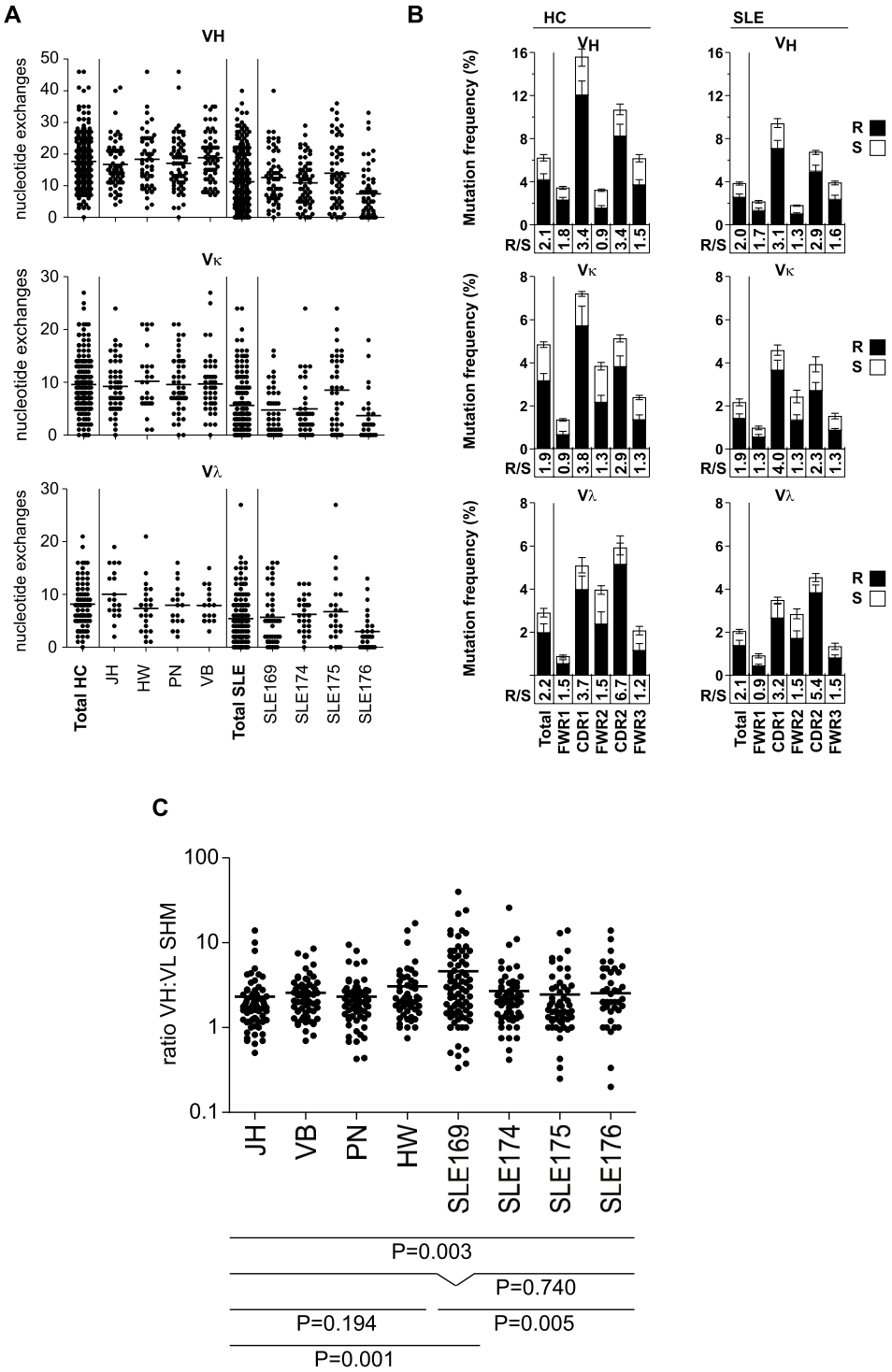

Fig. S4. The absolute number of mutations (nucleotide exchanges as compared with germ line) and the frequency of replacement (R) and silent (S) mutations in individual VH, Vκ , and Vλ genes (FWR1-FWR3) and ratios of VH to VL mutations of antibodies from IgG+ memory B cells of healthy donors JH, HW, PN, and VB and SLE patients 169, 174, 175, and 176 are shown. (*A*) Dots depict individual VH, Vκ and Vλ genes of healthy controls and SLE patients. Pooled data from healthy donors (Total HC) and SLE patients (Total SLE) is shown for comparison. The horizontal lines represent the average number of mutations in each individual. (*B*) Bar graphs indicate the frequency of R (black bar) and S (white bar) nucleotide exchanges per base pair in FWRs and CDRs in VH, Vκ, and Vλ genes. Standard deviations are indicated. The R/S ratio for each region is shown below the graphs. (*C*) Dots show ratios of VH to VL mutations of single antibodies cloned from single IgG+ memory B cells from healthy donors JH, HW, PN, and VB and SLE patients 169, 174, 175, and 176. Absolute numbers of V gene FWR1-FWR3 nucleotide exchanges as compared with germ line were used for the calculation. In cases were mutated IgH chains were associated with unmutated IgL chains (Table S2, Table S3, Table S4, Table S5, and Table S6), VL mutation values were artificially set from 0 to 1. *P* values indicate variance and were calculated by using one-way ANOVA.

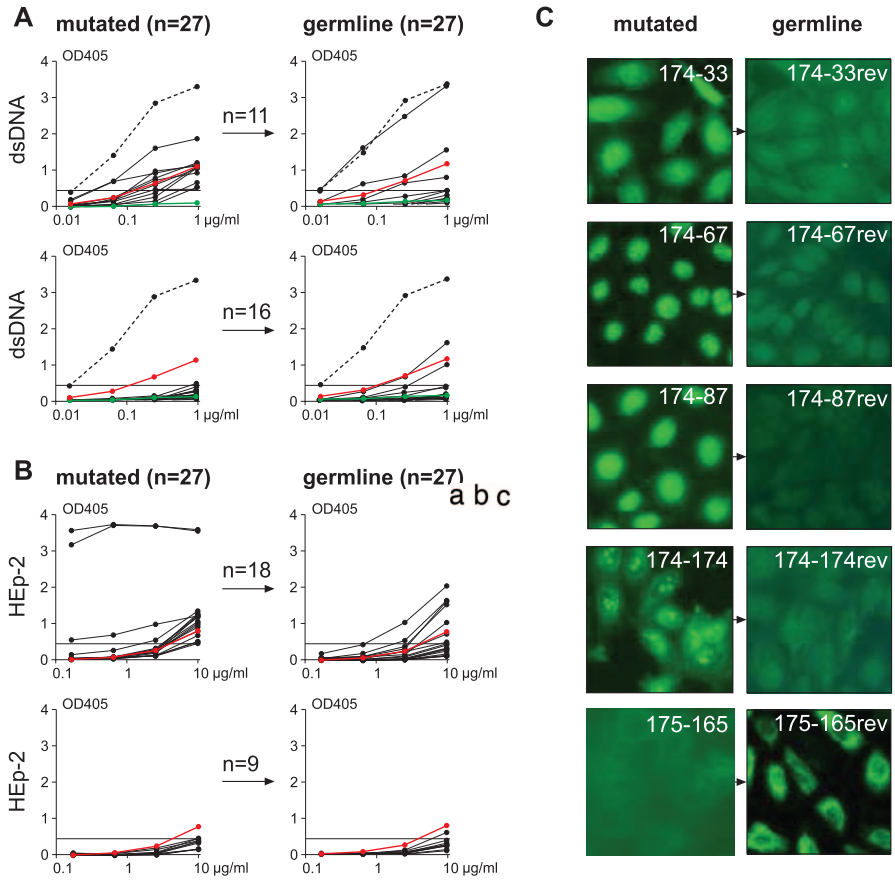

Fig. S5. Somatic hypermutation contributes to polyreactivity and self-reactivity in IgG memory B cell antibodies in SLE. (*A*) IgH and IgL chains from IgG memory B cell antibodies of SLE patients were reverted into their germ-line counterparts by PCR. Recombinant mutated (*Left*) IgG memory B cell antibodies and their germ-line counterparts (*Right*) were tested for polyreactivity (*A*) and self-reactivity by HEp-2 cell ELISA (*B*) and IFA (*C*). (*A*) Polyreactive mutated (*Upper Left*) antibodies and mutated nonpolyreactive (*Lower Left*) antibodies were tested for polyreactivity by ELISA and compared with their germ-line counterparts (*Right*). Representative polyreactivity graphs with dsDNA as antigen are shown. Dotted lines represent the high positive control antibody ED38 (1), red lines represent the low positive control antibody eiJB40, and green lines represent the negative control antibody mGO53 (2). Horizontal lines show cut-off OD_{405} for positive reactivity. (*B*) HEp-2 cell self-reactive mutated (*Upper Left*) antibodies and mutated non-self-reactive (*Lower Left*) antibodies were tested for HEp-2 cell self-reactivity by ELISA and compared with their germ-line counterparts (*Right*). Red lines represent low serum controls, and horizontal lines show cut-off OD_{405} for positive reactivity. (*C*) Typical HEp-2 cell IFA staining patterns of mutated IgG memory B cell antibodies (*Left*) and their germ-line counterparts (*Right*).

1. Meffre E, *et al.* (2004) Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med* 199:145–150.
2. Wardemann H, *et al.* (2003) Predominant autoantibody production by early human B cell precursors. *Science* 301:1374–1377.

Table S1. SLE patient criteria at first presentation

Patient	SLE169	SLE174	SLE175	SLE176
Gender	F	F	F	F
Age, yr	12	17	11	13
Treatment	None	None	None	None
Clinical features	Arthritis	Malar rash, arthritis	Pain, hypothyroidism	Rash
Lupus nephritis	IV	/	/	V
ANA	+	+	+	+
Serology	D, CL	ENA/RNP, Sm	ENA/RNP, Sm	D, ENA/RNP, Sm

D, anti-dsDNA antibodies; CL, anti-cardiolipin antibodies; Sm, anti-Smith antibodies; ENA/RNP, anti-extractable nuclear antigen/anti-ribonucleoprotein antibodies

Other Supporting Information Files

Table S2 (XLS)

Table S3 (XLS)

Table S4 (XLS)

Table S5 (XLS)

Table S6 (XLS)

Table S7 (XLS)

Table S8 (XLS)