Supplemental information

Clonal evolution and antigen recognition of anti-nuclear antibodies in acute systemic lupus erythematosus

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Supplementary Table 1. Patient characteristics

	Remission			Acute					
Patient ID	SLE2[a]	SLE3[b]	SLE4[c]	SLE5	SLE7	SLE9	SLE10	SLE11	SLE12
Date of blood collection	2012/7/3	2012/7/3	2012/7/3	2013/7/8	2014/2/5	2014/3/26	2014/7/9	2014/9/11	2015/12/8
Gender	F	F	F	F	F	F	F	F	M
Age (yr)	31	41	51	20	51	34	64	18	40
Malar rash	-	-	-	+	-	-	-	+	-
Discoid rash	-	-	-	-	+	-	-	+	-
Photosensitivity	-	-	-	-	-	-	-	-	+
Oral ulcers	-	-	-	-	-	-	-	-	-
Arthritis	-	-	-	+	+	-	+	-	+
Serositis	-	-	-	-	Pericarditis	Pericarditis Pleuritis	-	-	Pericarditis
Renal disorder	-	-	-	+	Class IV	Class IV	-	-	Class II
Neurologic disorder	-	-	-	-	-	-	+	-	-
Hematologic disorder	-	-	-	+	+	-	+	+	+
Immunologic disorders	-	-	-	+	+	+	+	+	+
Anti-nuclear antibody	*ND*[d]	*ND*[d]	*ND*[d]	+	+	+	+	+	+
Others	Prednisone, 11 mg/day	Prednisone, 5 mg/day	Prednisone, 2 mg/day	-	-	-	Anxiety disorder	-	-

[a] Diagnosed with SLE in 2010: Discoid rash, arthritis and neurologic symptoms.
[b] Diagnosed in 2007: Lupus nephritis (WHO class II).
[c] Diagnosed in 1993: Lupus nephritis (WHO class I).
[d] Not determined.

Supplementary Table 2. Ig genes and CDR3 AA sequences of the isolated ANAs

Clone ID	Donor	VH	DH	JH	HCDR3	Vκ/λ	Jκ/λ	LCDR3
51B12	SLE5	4-59	5-12	4	ARDGFDDYNLDY	Vκ2-30	2	MQGTHWYT
51C5	SLE5	1-46	6-19	4	ATRRGSGWYEGGFDY	Vκ2-28	3	MQALQTPRT
51F1	SLE5	3-15	6-19	4	TTDHIAVTGTDY	Vλ3-10	1	YSTDSSDNHGV
91E12	SLE9	5-10	2-21	4	AREEVGGGAIDY	Vκ4-1	4	QQYYTTPHT
113F3	SLE11	3-11	3-10	3	ARDFLDSAYGSGSFDM	Vλ2-8	2/3	SSYAGSNNLGV
71G1	SLE7	3-23	7-27	4	AKNARNWGSYYFDY	Vκ3-20	1	QQYGSSPPT
71F12	SLE7	4-59	1-20	4	ARHRNWLFDY	Vλ2-11	1	CSYAGSYTYV
72H11	SLE7	1-69	1-7	3	ARALGTTRFSAALGI	Vκ3-20	1	QQYGSSPWT
74F4	SLE7	3-21	1-26	4	ARRNSGSRFDY	Vλ2-14	2/3	SSYTSTNTFDGVV
74G9	SLE7	3-23	3-10	4	AKGITMVRGAPFDY	Vκ1-5	4	QQYNTYSPA
74H4	SLE7	4-59	3-10	3	ARRGWRSRSYYAFDI	Vκ3-20	3	QQYGSSLFT
10C3	SLE10	2-5	6-13	2	AHRPSGQQLGYWYFDL	Vκ3-20	4	QQYRSSPGLT
10C6	SLE10	4-39	3-10	4	ASGRANSYLRPFDY	Vκ3-20	1	QQYGRSPWT
121G9	SLE12	4-39	3-16	4	ARGVWGNYRYFDY	Vκ1-39	2	QQSYSPPLT

Supplementary Table 3. Summary of the HTS analysis of SLE7

Sample ID	SLE7 (VH3, Day 0)	SLE7 (VH3, Day 154)	SLE7 (VH3, Day 473)	SLE7 (VH4, Day 0)	SLE7 (VH4, Day 154)	SLE7 (VH4, Day 473)
Cell no. (PBMC)	1,000,000	2,000,000	2,000,000	1,000,000	2,000,000	2,000,000
Primers	VH3-JH	VH3-JH	VH3-JH	VH4-JH	VH4-JH	VH4-JH
Total read no.	463,981	465,684	434,731	242,704	252,560	288,164
Unique sequence no.	240,661	158,279	174,930	134,793	100,968	105,942
Clonal lineage no.	87,109	25,518	45,215	52,954	31,807	25,562
71G1 ln (ANA, VH3)	32 (0.0133%)	0	0	-	-	-
74F4 ln (ANA, VH3)	53 (0.0220%)	0	0	-	-	-
74G9 ln (ANA, VH3)	233 (0.0968%)	0	0	-	-	-
71B6 ln (Polyreactive, VH3)	20 (0.00831%)	0	0	-	-	-
71D10 ln (Polyreactive, VH3)	3 (0.00125%)	0	0	-	-	-
71F12 ln (ANA, VH4)	-	-	-	221 (0.164%)	3 (0.00297%)	0
74H4 ln (ANA, VH4)	-	-	-	2 (0.00148%)	0	0
71E6 ln (Polyreactive, VH4)	-	-	-	26 (0.0193%)	0	0
71E4 ln (polyreactive, VH4)	-	-	-	0	0	0

Supplementary Table 4. Summary of the HTS analysis of FV and FI

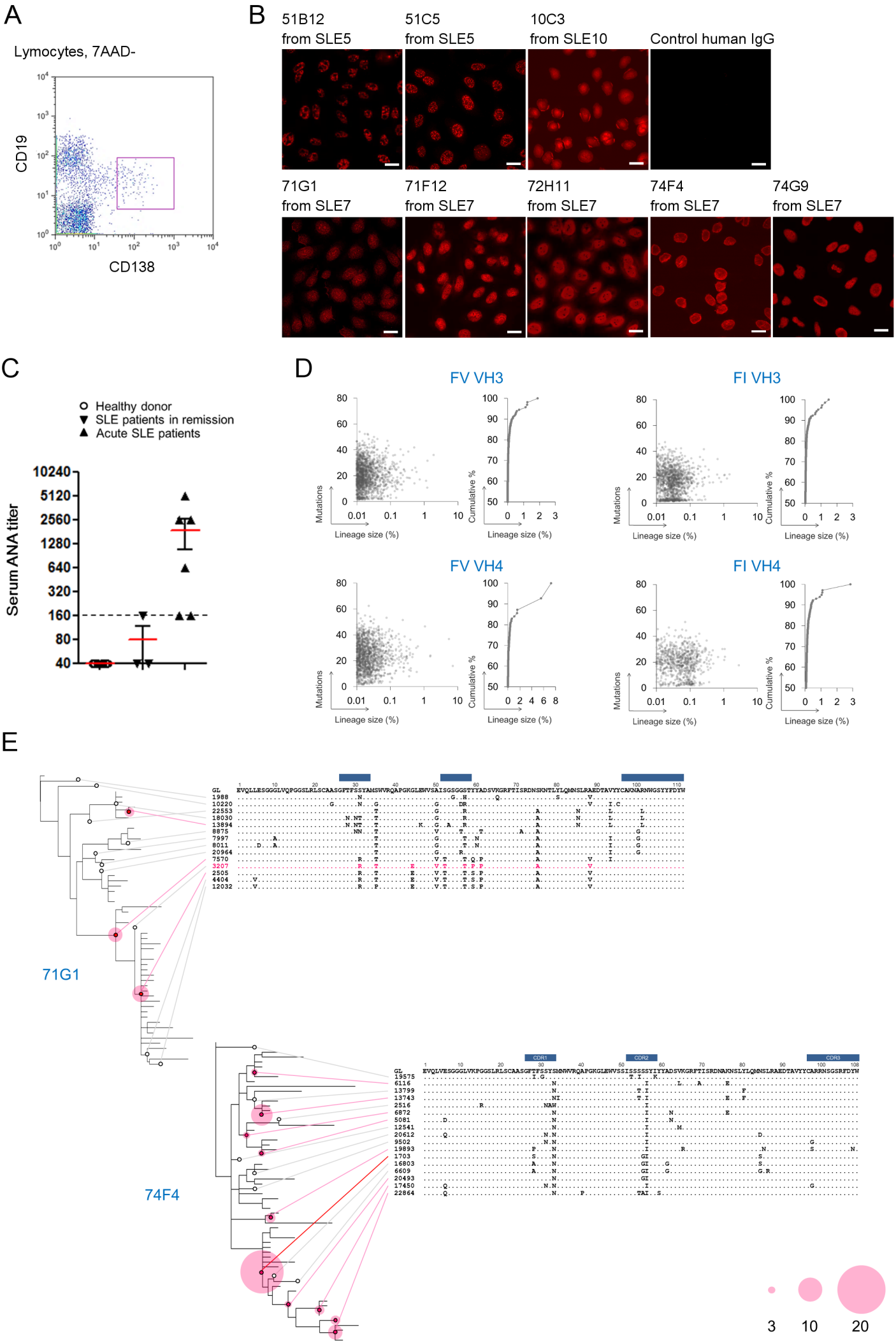
Sample ID	FV (VH3)	FV (VH4)	FI (VH3)	FI (VH4)
Cell no. (PBMC)	1,000,000	1,000,000	1,000,000	1,000,000
Primers	VH3-JH	VH4-JH	VH3-JH	VH4-JH
Unique sequence no.	149,985	133,354	133,425	125,412
Clonal lineage no.	49,255	27,289	43,463	26,983

Supplementary Figure legends

Supplementary Fig. 1. ANA isolation and HTS analysis. (A) Sorting strategy for single cell antibody cloning. Blood PBs (7-AAD^{-} CD19^{low} CD138^{+}) were sorted from freshly isolated PBMCs. (B) IFA on Hep2 cells with ANA clones isolated from acute SLE patients. Unrelated human IgG was used as negative control. Representative results are shown. Bars = 20 μm. (C) Serum ANA titers. Serum ANA titer was determined by IFA with serial diluted sera. (D) VH3 and VH4 Ig repertoires derived from a donor who received a multivalent influenza HA vaccine 7 days before collecting blood (FV) and a convalescent donor who was infected with influenza virus (FI). (E) Phyogenetic analysis of the 71G1 and 74F4 ANA lineages (day 0). The identical sequence to the originally isolated 71G1 is colored in red (#3297). The lineage sequences were analyzed by Maximum-likelihood method. Red circles represent the number of reads (≧ 3).

Supplementary Fig. 2. Structural basis of antigen recognition by 71F12. (A) Binding models of various bases. The models in which a cytosine, adenine, or guanine base is bound at site 1 were built by replacing the thymine base in the crystal structure with other bases without changing other atomic coordination. (B) *in silico* model of the unmutated germline (GL) precursor (upper and bottom left). The D72H mutation of the 71F12 heavy chain disrupts the intramolecular interaction with K75, instead generates the H72-K75 diad (right). (C) Predicted structure model of a longer DNA-71F12 Fab interaction. 3'-end of ssDNA could attach to the outer loop of 71F12 H chain.

Supplementary Figure 1



Supplementary Figure 2