SUPPLEMENTAL INFORMATION

Impaired TLR9 responses in B cells from patients with systemic lupus erythematosus

Vincent Gies,[1,2] Jean-Nicolas Schickel,[3*] Sophie Jung,[1,4*] Aurélie Joublin,[1] Salomé Glauzy,[3] Anne-Marie Knapp,[1] Anne Soley,[1] Vincent Poindron,[2] Aurélien Guffroy,[1,2] Jin-Young Choi,[3] Jacques-Eric Gottenberg,[1,5,6] Jennifer H. Anolik,[7] Thierry Martin,[1,2,5] Pauline Soulas-Sprauel,[1,2,8] Eric Meffre,[3**] Anne-Sophie Korganow,[1,2,5**]

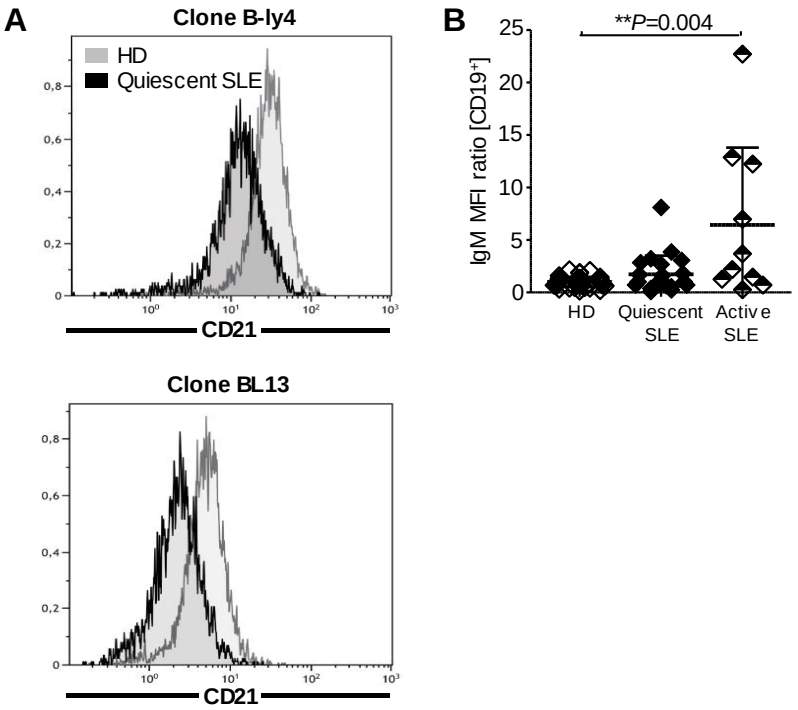
[1]CNRS UPR 3572 "Immunopathology and Therapeutic Chemistry"/Laboratory of Excellence Médalis, Institute of Molecular and Cellular Biology (IBMC), Strasbourg, France.
[2]Department of Clinical Immunology and Internal Medicine, National Reference Center for Autoimmune Diseases, University Hospital, Strasbourg, France.
[3]Departement of Immunobiology, Yale University School of Medicine, New Haven, Connecticut, USA.
[4]Department of Oral Medicine and Oral Surgery, University Hospital - Faculty of Dentistry, University of Strasbourg, Strasbourg, France.
[5]UFR Medicine, University of Strasbourg, Strasbourg, France.
[6]Department of Rheumatology, National Reference Center for Autoimmune Diseases, University Hospital, Strasbourg, France.
[7]Department of Medicine, Division of Allergy, Immunology and Rheumatology, University of Rochester Medical Center, Rochester, New York, 14642, USA.
[8]UFR Pharmaceutical Sciences, Strasbourg University, Illkirch, France.
*These authors contributed equally to the work
**These authors contributed equally to the work

Corresponding authors:
Eric Meffre, PhD
Yale University School of Medicine,
300 George Street, Room 353F, New Haven, CT 06511, USA
Phone: 203-737-4535 ; Fax: 1-203-785-7903,
E-mail: eric.meffre@yale.edu

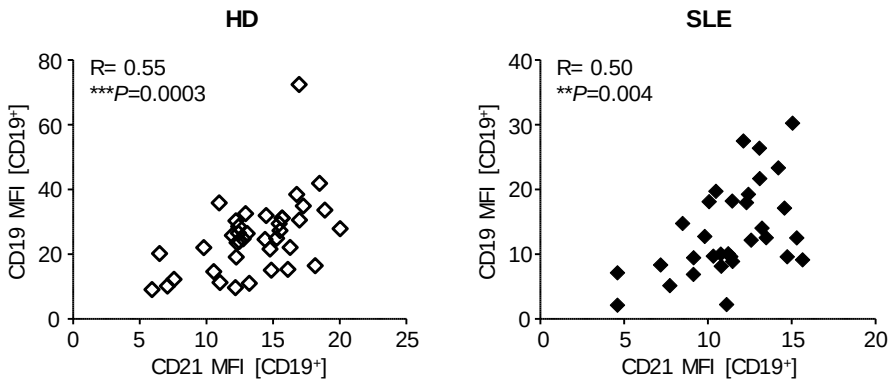
Prof. Anne-Sophie Korganow, MD PhD
CNRS UPR 3572 “Immunopathology and Therapeutic Chemistry”
Institute of Molecular and Cellular Biology (IBMC)
15 rue René Descartes, 67084 Strasbourg Cedex, FRANCE
Phone: + 33 3 88 41 70 25; Fax: + 33 3 88 61 06 80
E-mail: korganow@unistra.fr

Conflict of interest: The authors have declared that no conflict of interest exists.

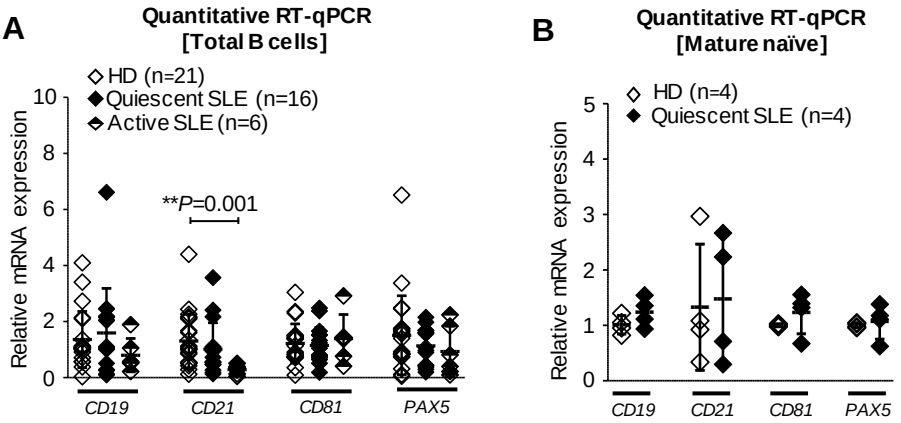
Supplemental Figures



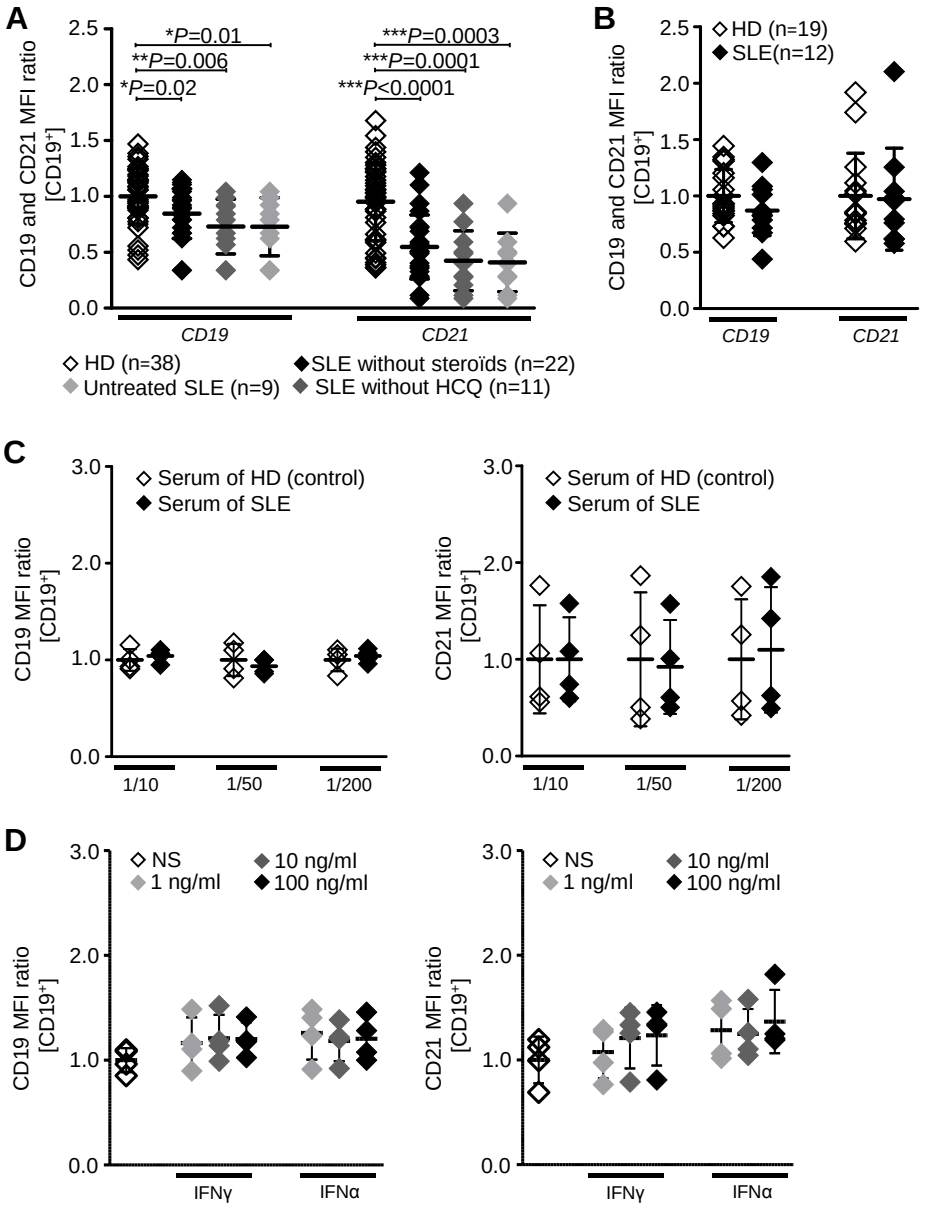
Supplemental Figure 1. Validation of decreased CD21 and increased IgM expression on SLE B cells. (**A**) Histograms represent CD21 MFI on $CD19^+$ B cells of SLE patients (black) and healthy donors (HD; grey) using 2 different anti-human CD21 antibodies (clones B-ly4 and BL13). (**B**) IgM MFI ratio on B cells from HD (n=41), quiescent (n=22) and active (n=10) SLE patients. MFI ratio was determined as follows: [MFI of the considered marker]/[mean of the MFI of the considered marker in HD group]. MFI: mean of fluorescence intensity. **P*<0.05, ***P*<0.005, ****P*<0.001. Two-tailed Mann-Whitney U-test.



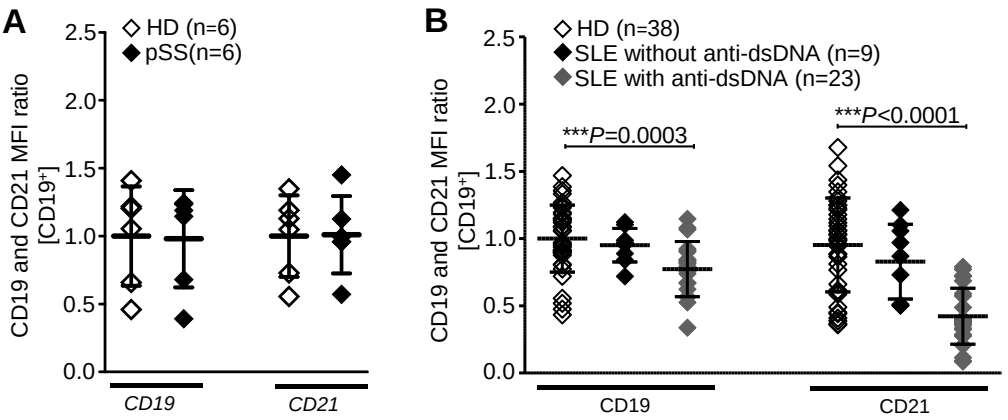
Supplemental Figure 2 Correlation between CD19 and CD21 cell surface expression. MFI of CD19 and CD21 staining on $CD19^+$ B cells from healthy donors (HD; n=41, left) and SLE patients (n=32, right). MFI: mean of fluorescence intensity. **P*<0.05, ***P*<0.005. Two-tailed Spearman rank correlation test**.**



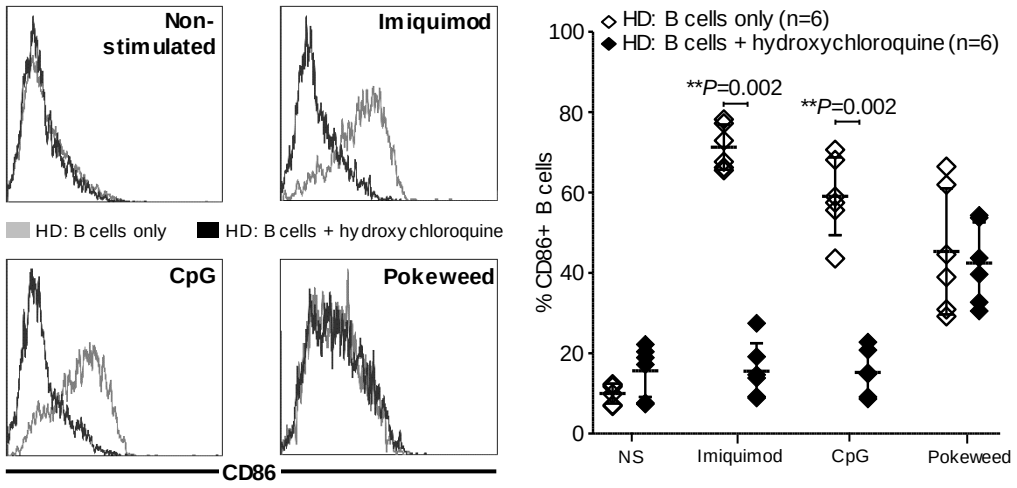
Supplemental Figure 3. Normal *CD19, CD21, CD81* and *PAX5* gene expression in B cells from quiescent SLE patients. (**A**) Real-time quantitative RT-qPCR of *CD19*, *CD21*, *CD81* and *PAX5* expression in sorted B cells from quiescent (n≥12) and active (n=6) SLE patients compared to healthy donors (HD; n≥16). (**B**) Real-time quantitative RT-qPCR of *CD19*, *CD21*, *CD81* and *PAX5* expression in mature naïve B cells from quiescent (n=4) SLE patients compared to HD (n=4). **P*<0.05, ***P*<0.005. Two-tailed Mann-Whitney U-test.



Supplemental Figure 4. Normalization in vitro of CD19 and CD21 expression on SLE B cells. (**A**) CD19 and CD21 MFI ratios of B cells from SLE patients without steroïds (n=22), without hydroxychlroroquine (HCQ; n=11) or without any treatments (n=9) compared to healthy donors (HD; n=38). MFI ratio was determined as follows: [MFI of the considered marker]/[mean of the MFI of the considered marker in HD group]. (**B**) CD19 and CD21 MFI ratios of B cells from SLE patients (n=12) compared to HD (n=19) after 2 days in culture with medium only. (**C**) CD19 and CD21 MFI ratios of sorted B cells from HD (n=4) after in vitro stimulation with pooled HD or SLE patients serum for 48 hours. MFI ratio was determined as follows: [MFI of the considered marker]/[mean of the MFI of the considered marker at the same dilution with pooled HD serum] (**D**) CD19 and CD21 MFI ratios of sorted B cells from HD (n=4) after no stimulation (NS) or in vitro stimulation with interferon γ (IFNγ) or IFNα at different concentrations for 48 hours. MFI ratio was determined as follows: [MFI of the considered marker]/[mean of the MFI of the considered marker in NS samples]. MFI: mean of fluorescence intensity. **P*<0.05, ***P*<0.005, ****P*<0.001. Two-tailed Mann-Whitney U-test.



Supplemental Figure 5. Decreased CD19 and CD21 expression observed on SLE B cells is not found on B cells from patients with Sjögren syndrom and correlates with serum anti-dsDNA autoantibodies. (**A**) CD19 and CD21 MFI ratios of B cells from primary Sjögren's syndrome (pSS) patients (n=6) compared to healthy donors (HD; n=6). (**B**) CD19 and CD21 MFI ratios of B cells from SLE patients with (n=23) or without (n=9) anti-dsDNA compared to HD (n=38). MFI ratio was determined as follows: [MFI of the considered marker]/[mean of the MFI of the considered marker in HD group]. **P*<0.05, ***P*<0.005, ****P*<0.001. Two-tailed Mann-Whitney U-test.



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89 **Supplemental Figure 6. Hydroxychloroquine inhibits TLR7- and TLR9-induced B cell**
90 **activation.** Histograms represent CD86 molecules MFI on B cells from healthy donors (HD), in the
91 presence or absence of hydroxychloroquine, after no stimulation (NS) or in vitro stimulation with
92 imiquimod (TLR7 ligand), CpG (TLR9 ligand) or pokeweed for 2 days. The graphic represent the
93 frequency of $CD19^{+}CD86^{+}$ B cells from HD, in the presence (n=6) or absence (n=6) of
94 hydroxychloroquine, after no stimulation or in vitro stimulation with imiquimod, CpG or pokeweed
95 for 2 days. **P*<0.05, ***P*<0.005. Two-tailed Mann-Whitney U-test

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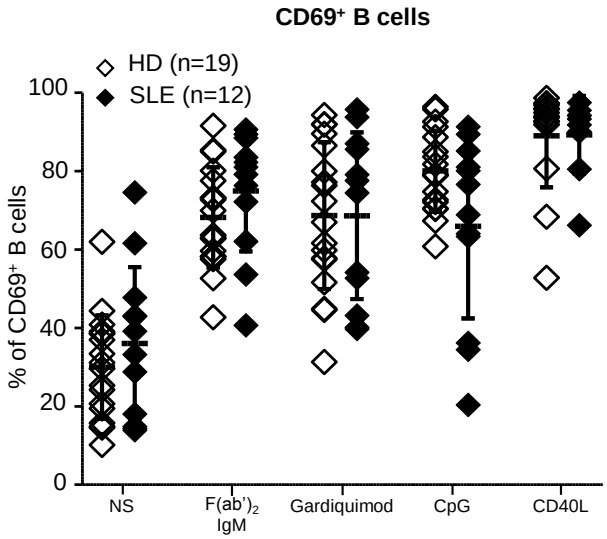
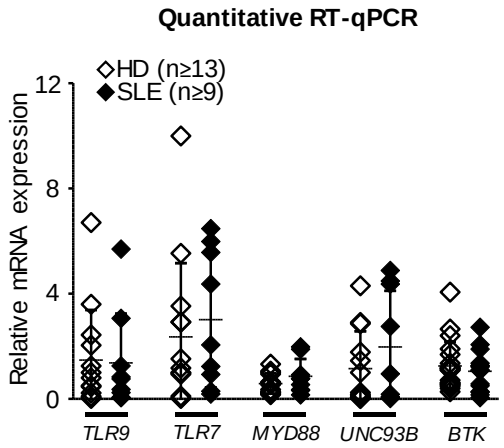


Supplemental Figure 7. Normal CD69 upregulation in SLE B cells after TLR9 stimulation. Frequency of $CD69^+$ in gated $CD20^+CD27^-$ naïve B cells from healthy donors (HD; n=19) or SLE patients without hydroxychloroquine (HCQ) (n=12) after no stimulation (NS) or in vitro stimulation of B cells with polyclonal $F(ab')_2$ anti-human IgM, gardiquimod (TLR7 ligand), CpG (TLR9 ligand) or CD40 ligand (CD40L) for 2 days. Two-tailed Mann-Whitney U-test.



Supplemental Figure 8. Normal *TLR9, TLR7, MYD88, UNC93B* and *BTK* gene expresstion in B cells from quiescent SLE patients. Real-time quantitative RT-qPCR of *TLR9*, *TLR7*, *MYD88*, *UNC93B* and *BTK* expression in sorted total B cells of quiescent SLE patients (n≥9) and healthy donors (HD; n≥13) compared to HD (n≥13). Two-tailed Mann-Whitney U-test.

Supplemental Tables

Supplemental Table 1. Clinical features and disease activity index of the 49 SLE patients.

Patient n°	Age	Sex	SLEDAI*	Ig levels (g/l)	ACAN	Anti-dsDNA	Steroids	Hydroxy chloroquine
1	36	F	0	ND	1/160	-	-	+
2	29	F	0	13	1/1280	-	-	+
3	65	F	3	10.4	1/1280	+	-	+
4	42	F	3	18.9	1/1280	+	-	+
5	38	F	2	10.3	1/1280	+	+	+
6	32	F	0	17.1	1/1280	-	-	+
7	26	F	3	12	1/1280	+	-	+
8	42	F	2	13.4	1/1280	+	+	+
9	50	F	0	12	1/1280	-	-	+
10	43	F	2	8.4	1/1280	+	-	+
11	37	F	0	14.8	1/160	-	-	+
12	22	F	0	11.8	1/320	-	-	-
13	31	F	2	10.2	1/1280	+	+	+
14	60	F	2	ND	1/640	+	+	+
15	41	F	2	ND	1/1280	+	+	-
16	39	F	2	ND	1/320	+	+	+
17	48	F	0	19	1/1280	+	-	+
18	31	F	0	15.1	1/640	-	-	-
19	64	F	2	ND	1/1280	+	-	-
20	30	F	2-4	10.5	1/1280	+	-	+
21	28	F	0	14.6	1/1280	-	-	+
22	79	M	1	19.2	1/1280	+	-	-
23	28	F	0	8.1	1/320	-	+	+
24	32	F	2	19.9	1/1280	+	-	-
25	31	F	0	13.78	1/1280	+	+	-
26	37	F	0	ND	1/1280	+	-	+
27	28	F	15	21.5	1/360	+	-	-
28	24	F	26	21.4	1/1280	+	-	-
29	71	M	17	21	1/1280	+	+	-
30	55	F	8	19.8	1/1280	+	+	+
31	24	F	14	20.3	1/1280	+	-	+
32	29	F	8	ND	1/1280	+	-	-
33	45	F	12	13.2	1/1280	+	-	+
34	60	F	8	17	1/1280	+	+	+

35	39	F	14	17	1/640	+	-	-
36	42	M	8	ND	1/1280	-	-	+
37	32	M	25	17.2	1/1280	+	-	-
38	39	F	11	ND	1/640	+	+	+
39	25	M	4	18.1	1/1280	+	-	+
40	68	F	0	8.8	ND	-	+	-
41	83	F	6	9.9	1/1280	+	-	+
42	66	F	14	10	1/1280	+	+	+
43	35	F	0	9.5	1/1280	+	-	+
44	46	M	2	10.8	1/1280	-	-	-
45	45	F	7	15.2	1/1280	+	-	-
46	75	M	0	17.9	ND	-	+	-
47	77	F	0	ND	ND	+	+	+
48	42	F	16	15.5	1/1280	-	-	-
49	32	F	3	13.8	1/1280	+	+	+

*SLEDAI: Systemic Lupus erythematosus disease activity index

Supplemental Table 2. Clinical features and serological parameters of the 49 SLE patients.

Patient n°	Cutaneous disorder	Joint disorder	Renal disorder	Neurological disorder	Other systemic disorder	Haematological disorder	APL syndrome*	anti-nuclear Abs	anti-DNA Abs	APL
1	+	+	-	-	-	-	-	1/1280	+	+
2	+	+	-	+	-	-	-	1/1280	-	-
3	+	+	-	+	-	+	-	1/5120	+	+
4	+	+	-	-	-	-	-	1/1280	+	+
5	+	+	+	-	-	-	-	1/1280	+	+
6	+	+	-	-	+	+	-	1/1280	+	-
7	+	+	-	+	-	+	+	1/1280	+	+
8	+	+	-	-	-	+	-	1/1280	+	-
9	+	+	-	+	+	-	+	1/1280	+	+
10	+	+	-	+	-	+	+	1/1280	+	+
11	+	+	-	-	-	-	-	1/320	-	+
12	-	+	-	-	-	-	-	1/1280	+	+
13	+	+	-	-	-	-	+	1/1280	+	+
14	+	+	-	-	-	-	-	1/1280	+	-
15	-	+	-	-	-	-	-	1/1280	+	-
16	-	+	-	-	+	-	-	1/1280	+	-
17	+	+	-	-	-	-	-	1/1280	+	+
18	+	-	-	-	+	+	-	1/640	-	-
19	+	-	-	-	-	+	-	1/1280	+	+
20	+	+	+	-	-	-	-	1/1280	+	-
21	-	+	-	-	+	-	-	1/1280	+	-
22	-	+	-	-	-	+	-	1/1280	+	+
23	+	+	+	+	+	+	-	1/640	-	+
24	+	+	-	-	-	+	-	1/1280	+	+
25	+	+	+	-	-	+	-	1/1280	+	-
26	+	+	-	-	-	+	-	1/1280	+	+
27	+	+	-	-	-	-	-	1/1280	+	-
28	+	+	+	-	-	+	+	1/1280	+	+
29	+	+	-	-	+	-	-	1/1280	+	+
30	+	+	-	-	+	-	-	1/1280	+	-
31	+	+	-	-	+	-	-	1/1280	+	-
32	-	+	-	-	-	-	-	1/1280	+	+
33	+	+	-	-	-	-	-	1/1280	+	-
34	+	+	-	-	+	-	-	1/1280	+	+
35	+	+	+	-	+	+	+	1/1280	+	+
36	+	-	+	-	-	-	-	1/1280	-	+
37	-	-	+	-	+	+	-	1/1280	+	+

38	+	+	-	-	+	+	-	1/640	+	+
39	+	-	-	-	-	+	-	1/1280	-	-
40	-	+	-	-	-	+	-	1/1280	+	+
41	+	+	-	-	-	+	-	1/1280	+	+
42	+	+	-	-	-	+	+	1/1280	+	+
43	-	+	-	-	-	-	-	1/640	+	-
44	+	+	-	-	-	-	-	1/640	-	-
45	-	+	-	-	+	-	-	1/1280	+	-
46	+	+	+	-	-	-	-	1/1280	-	-
47	+	+	-	-	+	+	-	1/320	+	+
48	-	+	+	-	-	+	-	1/1280	-	-
49	+	+	+	+	-	+	-	1/1280	+	+

*APL syndrome: antiphospholipid syndrome according to ACR criteria

Supplemental Table 3. Biological features of the 6 patients with primary Sjögren syndrome.

Patient n°	Age	Sex	Ig levels (g/l)	ACAN
1	49	F	26.3	1/1280
2	41	F	12.9	1/640
3	44	F	11.3	1/1280
4	61	F	13.4	1/1280
5	38	M	18.1	1/1280
6	71	F	40.4	1/1280