SUPPLEMENTENTARY FIGURES AND TABLES

T follicular-like helper cells in the peripheral blood of patients with primary Sjögren's syndrome

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Supplementary Table 1. Fluorochrome-conjugated antibodies for flow cytometry.

Target	Fluoro-chrome	Clone	Isotype	Dilution	Voltage	Cat.No.*	Lot no.	Supplier
Live/Dead	BV510			1:800	375	L34957	1705825	Invitrogen
CD3	PE-CF594	UCHT1	Mouse IgG1, κ	1:200	556	562280	5205540	BD BioSciences
CD4	PerCP-Cy5.5	SK3	Mouse IgG1, κ	1:200	510	344608	B199343	Biolegend
CD19	BV605	HIB19	Mouse IgG1, κ	1:200	410	302244	B196360	Biolegend
CD20	APC-H7	2H7	Mouse IgG2b, κ	1:50	475	560853	5219809	BD BioSciences
CD27	PE-Cy7	LG.3A10	Armenian hamster IgG	1:100	650	124216	B185457	Biolegend
CD38	APC	AT13/5	Mouse IgG1, κ	1:80	495	MCA10 19A647	0304	AbD Serotec
CD138	FITC	DL-101	Mouse IgG1, κ	1:20	380	11-1389	E14545-103	eBioscience
CXCR5	PE	J252D4	Mouse IgG1, κ	1:200	510	356904	B188805	Biolegend
ICOS	BV421	DX29	Mouse IgG1, κ	1:20	310	562901	5190877	BD BioSciences
PD-1	BV711	EH12.1	Mouse IgG1, κ	1:100	480	564017	5156807	BD BioSciences

* Cat.No. = Catalogue Number

Supplementary Table 2. Serological levels of Ro52, Ro60 and La48 autoantibodies measured by ELISA compared with clinical data

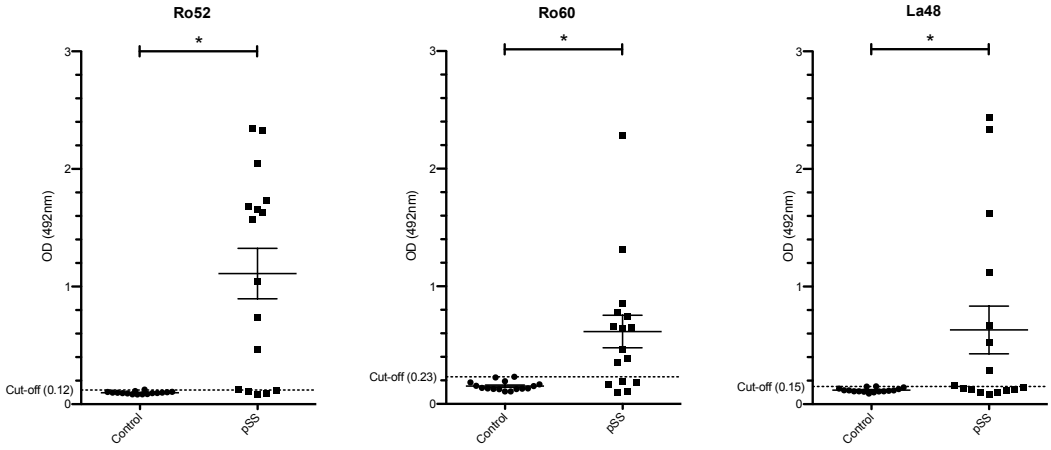
Patient	Ro52 (OD)	Ro60 (OD)	La48 (OD)	Inhouse analysis[1]	Clinical data[2] SSA	SSB
1	1.040	0.460	0.145	+/+/-	+	-
2	2.342	1.310	2.441	+/+/+	+	+
3	1.569	0.656	0.124	+/+/-	+	-
4	1.678	0.740	0.289	+/+/+	+	-[3]
5	1.634	0.640	0.525	+/+/+	+	-[3]
6	0.737	0.778	2.337	+/+/+	+	+
7	0.095	0.188	0.129	-/-/-	-	-
8	0.116	0.854	0.162	-/+/(+)	+	-[3]
9	0.109	0.100	0.097	-/-/-	-	-
10	2.331	2.280	0.672	+/+/+	+	+
11	0.128	0.165	0.121	(+)/-/-	(-)[3,4]	-
12	1.736	0.384	1.117	+/+/+	+	+
13	0.080	0.105	0.086	-/-/-	-	-
14	2.050	0.648	1.624	+/+/+	+	+
15	1.660	0.355	0.128	+/+/-	+	-
16	0.464	0.181	0.101	+/-/-	+	-
Cut-off[5]	0.120	0.230	0.150			

[1] Positive (+)/negative (-) serology against Ro52/Ro60/La48. () indicates weak results.
[2] SSA: anti-Ro60 and/or anti-Ro52. SSB: anti-La48. The +/- indicates a positive/negative serology and () indicates weak results.
[3] Inconsistent results between in-house ELISA and clinical data.
[4] Value at cut-off value
[5] Cut-off value (mean of ctrl+2SD)

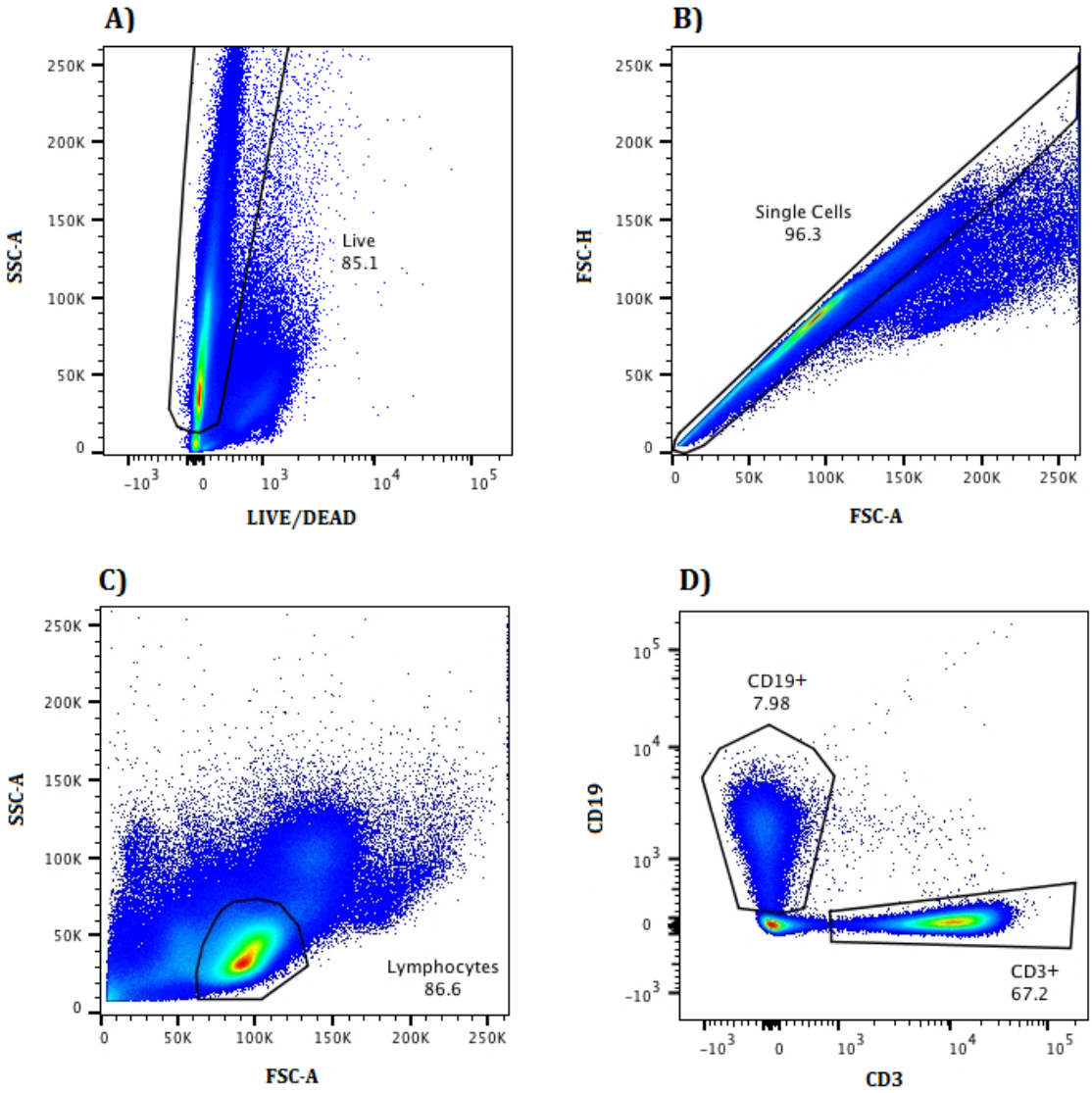
Supplementary Table 3. Distribution of Ro52, Ro60 and La48 autoantibodies in pSS patients

Autoantibody[1]	Number	Frequency (%)
None	3	18.75
Only Ro52	2	12.50
Only Ro60	0	0
Only La48	0	0
Ro52+Ro60	3	18.75
Ro52+La48	0	0
Ro60+La48	1	6.25
All	7	43.75
Total Ro52	12	75.00
Total Ro60	11	68.75
Total La48	8	50.00

[1] (-): Serum negative for Ro/La autoantibodies; (+): Serum positive for either Ro52, Ro60 or La48; (++): Serum positive for 2/3 Ro/La autoantibodies; (+++): Serum positive for all three autoantibodies

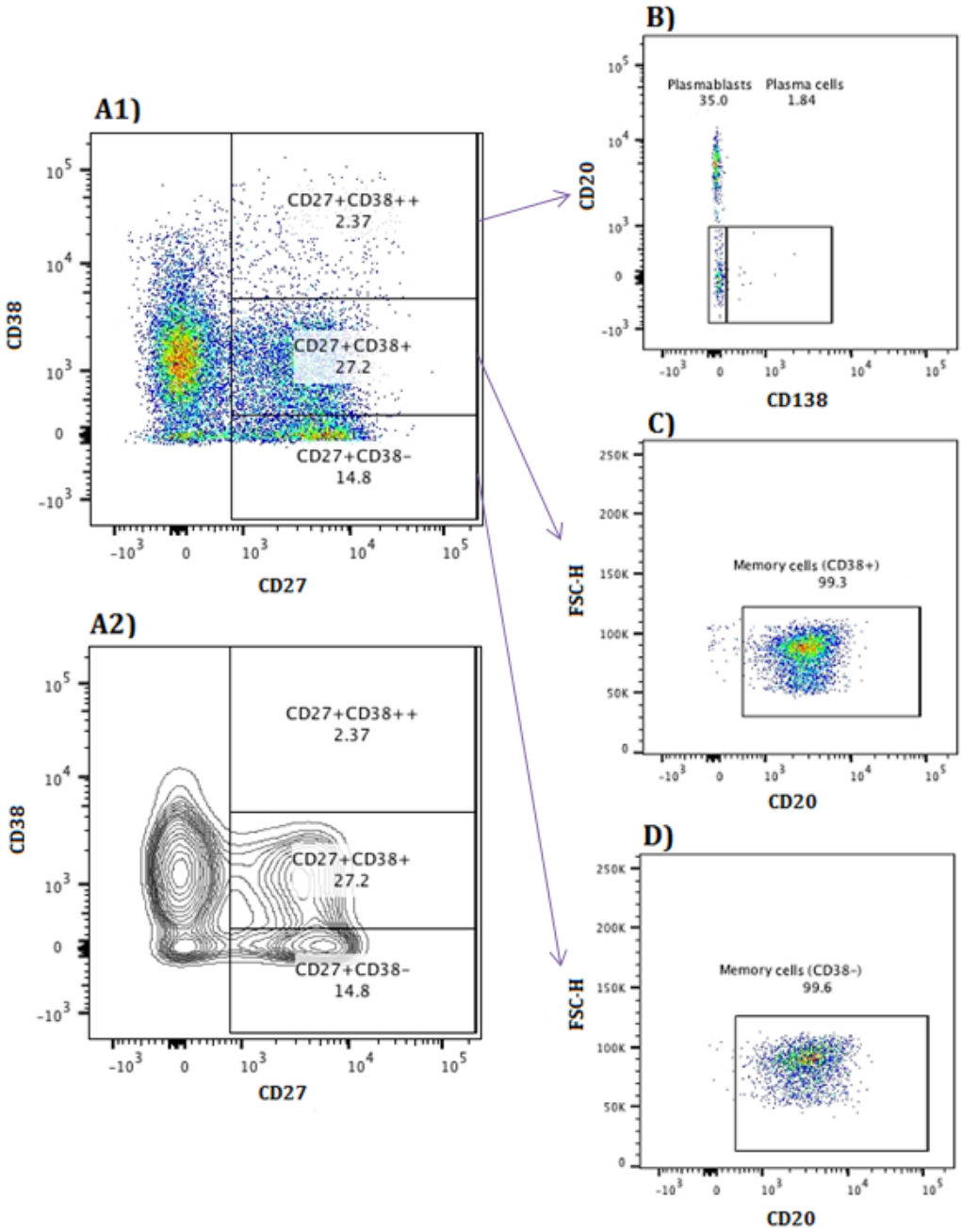


Supplementary Figure 1. Serological detection of Ro52, Ro60 and La48 autoantibodies in samples from pSS patients and control subjects. The graphs show the OD values of plasma samples of pSS patients (n=16) and controls (n=16) diluted 1:1000 measured by indirect ELISA. The lines represent mean±SEM (Standard error of mean), and statistical significant results are labeled with an asterisk (*).



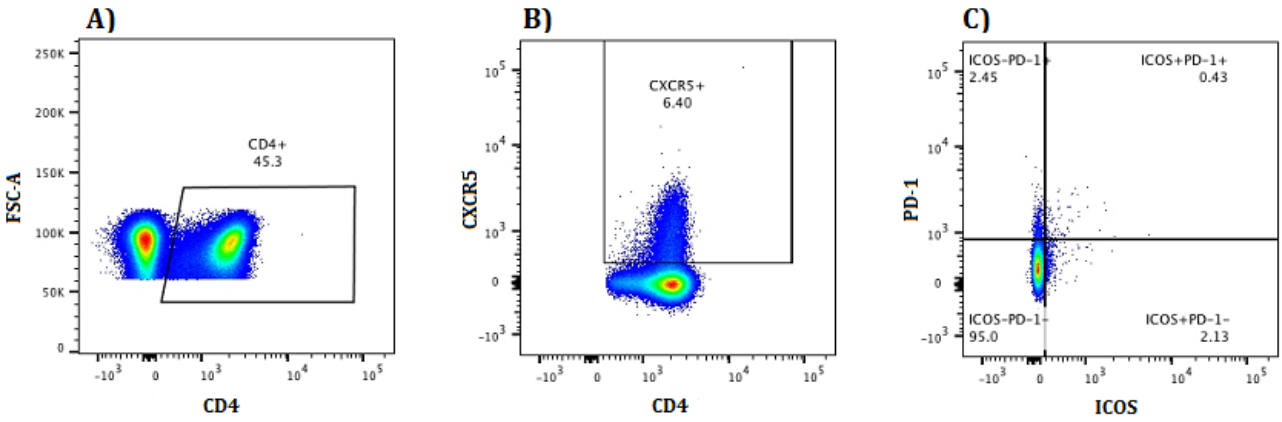
Supplementary Figure 2. Gating strategy B and T cells

A) First dead cells were excluded as these might produce false positive signals by binding non-specifically to many reagents. B) Within the live cells the singlets were identified, and the rest were excluded due to the possibility of false positives (as a duplicate will be interpreted as a single cell by the flow cytometer). C) As only lymphocytes were of interest in this study, a gate was drawn around this cell population. D) The lymphocytes were then divided into CD3+ cells and CD19+ cells.



Supplementary Figure 3. Gating strategy B cell sup-populations

The figure shows how different B-cell subsets were defined within the CD19+ subset, identified in figure 4.7 D). A1) shows how the cells were divided in three subsets based on the markers CD27 and CD38. This is also shown as a contour plot (A2), which more easily shows the distinguishment of subsets. B) Within the CD27+CD38++ population, subsets were identified based on the markers CD20 and CD138– plasma blasts as CD20-CD138- and plasma cells as CD20-CD138+. C) and D) show how two subsets of memory B cells were identified within the CD27+CD38+ cells and CD27+CD38- cells, respectively. They both had the classification criteria of being CD20+.



Supplementary Figure 4. Gating Strategy T cell sup-populations

The figure shows how different T-cell subsets were defined within the CD3+ subset, identified in figure 4.7 D). A) shows how the CD4+ cells were gated, and B) shows how the CXCR5+ cells were identified within this gating. C) The defined CXCR5+ population was separated into four subsets based on the markers ICOS and PD-1. The four subsets were ICOS-PD-1-, ICOS+PD-1-, ICOS-PD-1+ and ICOS+PD-1+ cells.