Supplementary methods:

RNA-sequencing

Cell isolation: One to two minor salivary glands (MSGs) were collected in Dulbecco modified Eagle medium (DMEM) supplemented with antibiotics, sodium pyruvate, nonessential amino acids, and glutamine. Glands were first minced with scissors and enzymatically digested at 37°C under rotating agitation (100 rpm) in DMEM containing 232 U/mL collagenase II (Worthington Biochemical, Lakewood, NJ) and 8 U/mL DNAse I (Sigma, St Louis, MO). After 2 washes in calcium- and magnesium-free PBS containing 1 mmol/L EDTA, cellular aggregates were resuspended in 0.5 mL TrypLE Express Enzyme (Gibco, Carlsbad, CA) and incubated for 2 min at 37°C. Enzyme inactivation was achieved by dilution with 4 mL of DMEM and immediately gentle pipetting with a P1000 to facilitate cell dissociation. The resulting cell suspension was washed twice with medium supplemented with 0.8 U/mL DNAse I. Peripheral mononuclear cells were isolated by Ficoll gradient separation.

Cell sorting: After isolation, cells were stained with DAPI Pacific Blue (LifeTechnologies) to identify dead cells. After saturation of Fc receptors by incubation with Fcblock (Miltenyi Biotec). CD326 PE+ (Miltenyi Biotec) DAPI- cells [epithelial cells] in salivary gland and CD45+ CD19 PE Cy7+ (Biolegend) DAPI- cells [B lymphocytes], in salivary gland biopsies and in blood were sorted by using a FACS ARIA cell sorter (BD Biosciences). The list of antibodies used is in **Table S1.** Sorted cells were frozen on dry ice and stored at -80°C.

RNA extraction: Samples were thawed on ice and RNA extraction was performed according to the manufacturer's instructions (PicoPure RNA Isolation Kit (Arcturus, Applied Biosystems, Foster City, CA) with the following modifications. Because of low yield of RNA expected for this type of samples, linear acrylamide (Ambion) was diluted in extraction buffer to be

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spiked-in to a final concentration of 20 ng/uL. RNA quality control was performed on the Agilent 2100 Bioanalyzer with the RNA 6000 Pico Kit (Agilent).

RNA Sequencing of sorted cells, library Generation and Sequencing methods*:* The number of B cells sorted from biopsies difference between pSS ad controls (p=0.02). To avoid bias due to this initial difference, we performed RNA-seq with a normalized amount of RNA. An amount of 100pg total RNA was used as input for cDNA preparation by using the SMART-Seq v4 Ultra Low Input RNA Kit (Clontech catalog # 634892). Universal Human Reference RNA (UHRR) was included in the experiment as a positive control, and nuclease-free water was used to dilute the samples as a negative control or non-template control (NTC). Using the concentrations derived from the Ribogreen assay, a normalization plate was made with a final concentration of 10.53 pg/uL per sample. cDNA was prepared according to the manufacturer's instructions, except for the additional spike-in of 1uL diluted ERCC RNA Spike-In Mix (ThermoFisher catalog #4456740) in each sample. After subtracting the averaged concentration of the ERCC spiked NTC from the sample concentration from the Bioanalyzer trace, cDNA samples were normalized to 125pg in 5uL per sample. Library preparation used the Nextera XT DNA Sample Preparation Kit (Ilumina catalog # FC-131-1096), and the Nextera XT Index Kit v2 Set A (catalog # FC-131-2001) and Set D (catalog # FC-131-2004), according to the manufacturer's instructions. The High Sensitivity D1000 ScreenTape Assay (Agilent catalog # 5067-5585 and # 5067-5584) was used on the Agilent 4200 TapeStation system to check the libraries for base pair size and molarity. All samples were then pooled to a final concentration of 4nM in an DNA LoBind Microcentrifuge Tubes (Eppendorf catalog # 022431048). The High Sensitivity DNA Assay (Agilent catalog # 5067-4626) was used on the Agilent 2100 Bioanalyzer to analyze the pool, which was then adjusted with Buffer EB (Qiagen catalog # 19086). The 12.5pM denatured library with 1% Phix spike-in was run on

the MiSeq with MiSeq Reagent Kit v2 (50 cycles) (Illumina catalog # MS-102-2001), following the manufacturer's instructions. The frequency of identified reads per index was used to adjust individual samples concentration in the pool. The re-adjusted pool was then re-Bioanalyzed and rerun on the MiSeq using the same kit and protocol. The same process of re-adjusting the pool with the new set of MiSeq index data and validation with the Bioanalyzer was performed before running the pool on the Illumina HiSeq 2500 System. The 18pM denatured library with 1% Phix spike-in was clustered on the cBot with the HiSeq PE Cluster Kit v4 (Illumina catalog # PE-401-4001), following Illumina's HiSeq and GAIIx Systems Denature and Dilute Libraries Guide, and cBot System Guide, and run on the HiSeq with the HiSeq SBS Kit v4 (Illumina catalog # FC-401-4002), following the HiSeq 2500 System Guide.

Reads were first quality control-filtered and trimmed by using trimmomatic. Paired reads were aligned to the human reference genome (V38.79) by using STAR software (version 2.5.0c). Counting of reads involved use ofHTSeq.

The Interferome v2.01 database was used to identify and characterize interferon (IFN)-induced genes. Functional enrichment analysis of differentially expressed genes was performed for genes with absolute expression fold change ≥ 1.5, by using Ingenuity Pathway Analysis software (Qiagen). Volcano plot representation were performed by using R software and multidimensional scaling representations were generated using the SVD-MDS method.

Data are available upon reasonable request.

PCR validation of the differentially expressed genes

To confirm the results of RNA-seq on sorted cells, the residual complementary DNA (cDNA) were transferred to a 48.48 Dynamic Array primed chip, and real-time PCR (RT-PCR) was run according to the Fluidigm protocol. The primers tested are in **Table S2**. Data were analyzed by

using Fluidigm RT-PCR software. Relative mRNA expression was determined from normalized Ct values by using HPRT1 as housekeeping gene and the $2^{-\Delta Ct}$ method. To compare means the Mann-Whitney test was applied.

Primary cultures of salivary gland epithelial cells (SGECs)

After 2 to 3 weeks of culture, cells at 70% to 80% confluence were dissociated with 0.125% trypsin-EDTA. Cell suspension was suspended in basal epithelial medium and added at 80 000 cells/cm2 to a 6-well collagen type I (Institut de Biotechnologies, Reims, France) coated plate and incubated at 37°C and 5% CO2 in a humidified atmosphere. The basal epithelial medium was changed at day 1 to remove epithelial cells that were not adherent. The epithelial origin of cultured cells was routinely confirmed by staining with monoclonal antibodies against CD326 (Miltenyi Biotec, Paris) an epithelium-specific marker (**Figure S2A**).

Positive isolation of B lymphocytes for co-culture experiments

Peripheral mononuclear cells were isolated from residual blood of apheresis from healthy subjects (French blood donors) by Ficoll gradient separation. B lymphocytes were isolated by CD19 magnetic bead positive selection according to the manufacturer's instructions (CD19 Microbeads human and Fc-Block, Miltenyi Biotec) to achieve a purity of greater than 80% as assessed by FACS analysis (percentage of CD20+ cells).

Co-cultures of SGECs and B lymphocytes

The primary culture of salivary gland biopsy lasted 2 to 3 weeks until we obtained a cell layer. Then we performed only one passage of the SGECs that were harvested by using trypsin and seeded in wells. After 4 days, when cells were 70% confluent in the wells, we added the B

cells. The coculture lasted 5 days. B lymphocytes (1.5 x 10^6 cells) were cultured alone or with SGECs in 2mL RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X). Stimuli were added in the medium: IFNα 2400 U/mL (Roferon-A, Roche), IFNγ 5ng/mL (Sigma Aldrich) or poly(I:C) 10µg/mL (Invivogen). After 5 days, B lymphocytes were harvested for immunostaining and flow cytometry. Culture supernatants were collected for cytokine quantification by multiplex assay or ELISA. Schematic representation of co-cultures experimental design is presented in **Figure S1B**.

Transwell and inhibition experiments

Transwell assays were performed with 0.4µm pore sized inserts (Falcon Cell Culture Inserts, 6-well plates). B lymphocytes were placed in the upper chamber, and confluent SGECs in the lower in 4 mL of RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, and penicillin-streptomycin (1X). Poly(I:C) at 10 µg/mL (Invivogen) was added in the medium. After 5 days, B lymphocytes were harvested for immunostaining and flow cytometry. Several co-cultures were dedicated to inhibition experiments. Inhibitors or their control were added in the co-culture at day 0: belimumab (GSK) 10µg/mL or IgIV at the same concentration as control, tofacitinib (Pfizer) 50 or 100nM or DMSO at the same concentration as control, anti-APRIL (kindly provided by P. Schneider) or anti-EDA at the same concentration as control, tocilizumab (Roche) 5 or 50 µg/mL or IgIV at the higher concentration as control, ibrutinib (Selleckchem) 1µM, LY294002 (Selleckchem) 20µM, leflunomide (Selleckchem)100µM, hydroxychloroquine (Selleckchem) 10µM or DMSO at the same concentration as control. Concentrations of inhibitors were defined according to manufacturers recommendations or, when not available, were based on the serum levels measured in patients.

FACS analysis

To assess viability of B lymphocytes cultured alone or co-cultured with SGECs in the different conditions of stimulation, B lymphocytes were harvested and stained with Fixable viability Dye APC Vio770 (LifeTechnologies, Paisley, UK). Viability was assessed as the percentage of live B lymphocytes out of all events. The activation of B lymphocytes was assessed by CD38 + B lymphocytes percentage and CD38 MFI. CD27, IgD, CD40 and CD48 were also assessed in B lymphocytes. Then, after two PBS washing, adherent SGECs were harvested after dissociation with 0.125% trypsin-EDTA and stained with anti-BAFFR antibody**.** The list of the antibodies used in experiments is in **Table S1**. Samples were analyzed by using a BD FACS Canto flow cytometer and BD FACS Diva Software (BD, Becton Dickinson, Germany). The results were analyzed with FlowJo10 software. The percentage of alive cells was determined as the percentage of unstained cells with the Fixable viability Dye marker. The other percentages of positive cells were determined by comparison with the negative isotypic control. The gating strategy is presented in **Figure S2B**.

Cytokine assays

Cell-free supernatants were collected and assayed for IgG and Ig M production by enzyme-linked immunosorbent assay (ELISA) (IgG total uncoated ELISA and IgM uncoated ELISA, Life Technologies). The dosage of cytokines and chemokines (BAFF, APRIL, IL-6, CXCL10, CXCL12 and CXCL13) was performed using a multiplex assay (Thermo Fisher Scientific). BAFF and CCL28 levels were assessed by ELISA (R&D system and Thermo Fisher Scientific, respectively).

Anti-Ro/SSA antibodies were assessed by using the ALBIA method (Adressable laser bead immuno- assay) with Bioplex 2200 (Biorad). The confirmation was performed by immunodot (Euroimmun Ana3b).

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Supplementary figures:

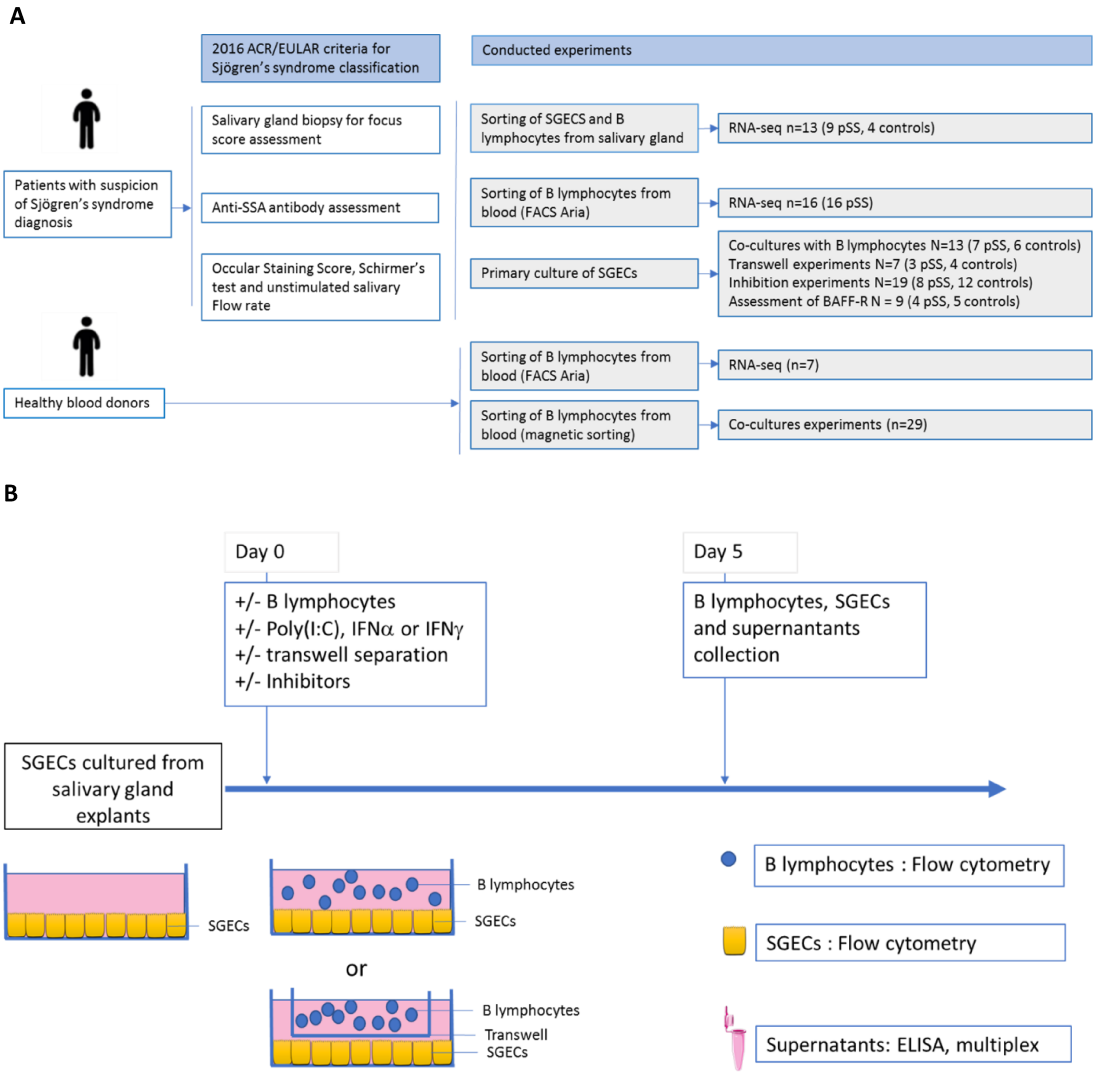

Figure S1: A: Experimental design of the study. SGECs, B lymphocytes sorted from biopsies and pSS B lymphocytes sorted from blood used for RNA-seq experiments as well as SGECs used for co-culture experiments were obtained from patients with suspected pSS. pSS was diagnosed according to 2016 ACR/EULAR criteria, and controls presented sicca symptoms without anti-SSA/SSB antibodies and had normal or sub-normal salivary glands. Control B lymphocytes sorted from blood used for RNA-seq experiments and B lymphocytes used for co-cultures, transwell and inhibition experiments were sorted from healthy blood-donors PBMCs. **B:** Schematic representation of co-culture experiments of SGECs and B lymphocytes.

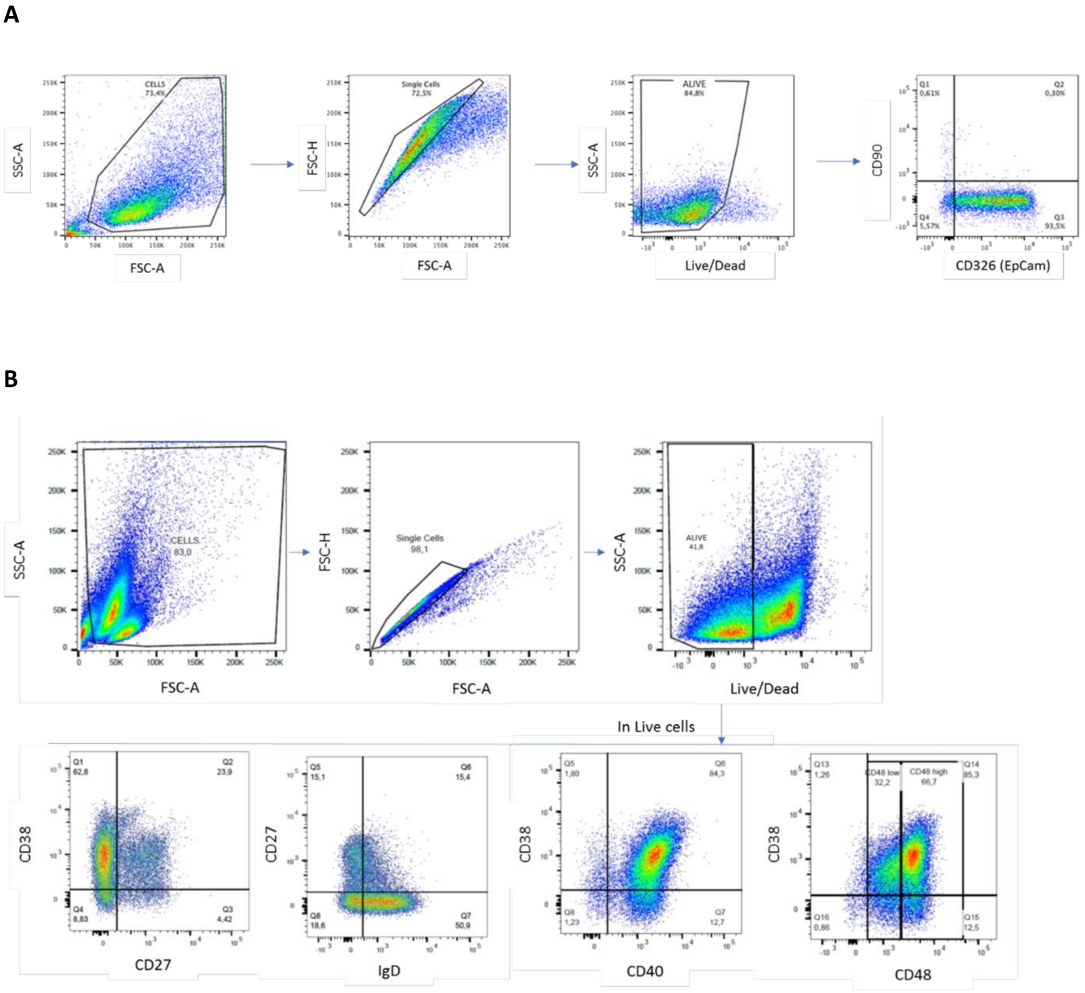

Figure S2: **A:** Gating strategy used to phenotype SGECs. CD326 and CD90 expression on SGECs after 2 weeks of primary culture. **B:** Gating strategy used to phenotype co-cultured B lymphocytes. Percentages of CD38, CD27, IgD, CD40 and CD48 B lymphocytes were determined among alive B cells. MFI was determined with the same strategy.

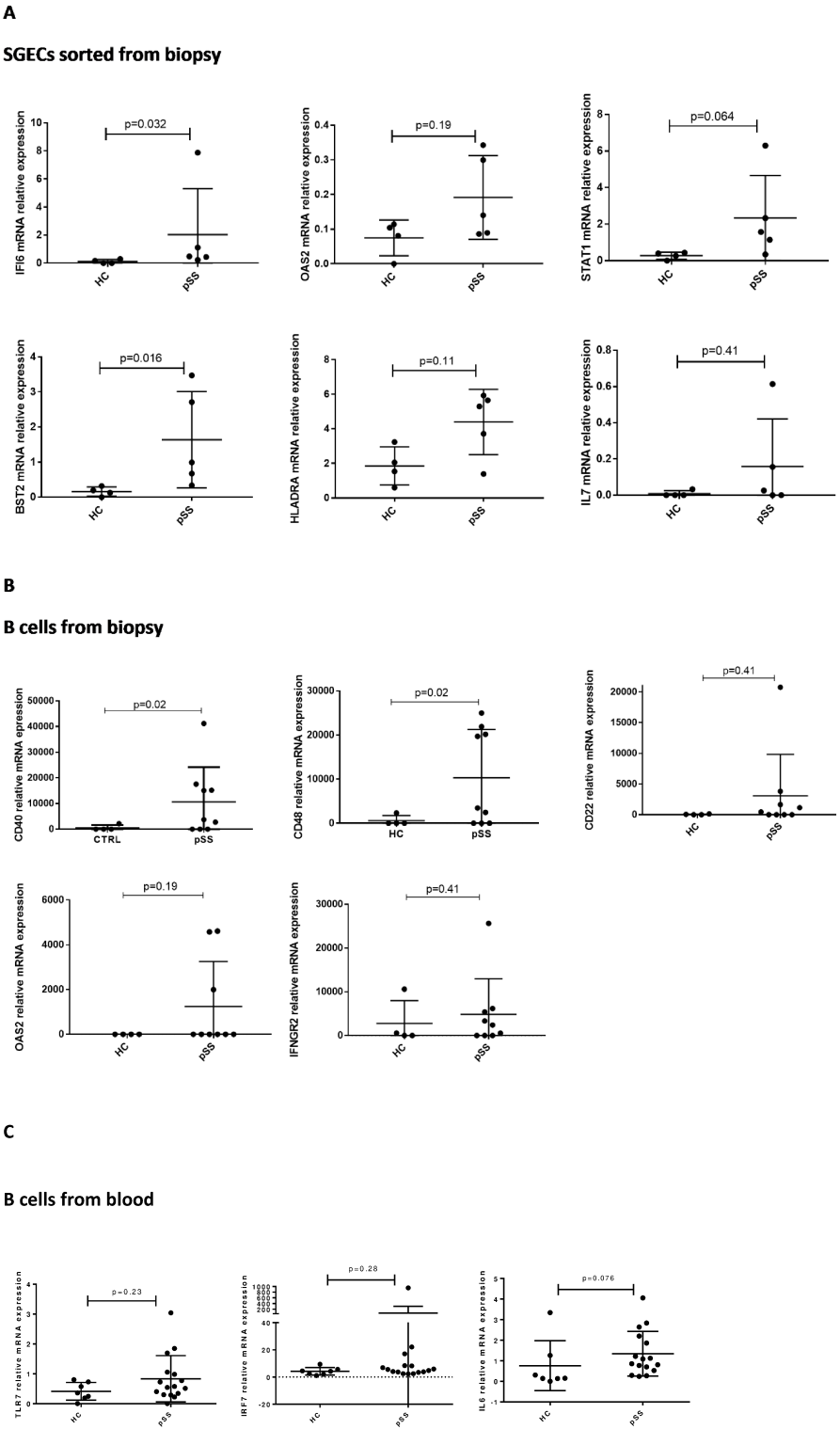

Figure S3: A: Validation of the RNA-seq results by RT-PCR. Relative mRNA expression of *IFI6*, *OAS2*, *STAT1*, *BST2*, *HLA DRA* and *IL-7* in SGECs. **B:** Relative mRNA expression of *CD40*, *CD48*, *CD22*, *OAS2*, and *IFNGR2* in B lymphocytes sorted from salivary gland biopsy**. C:** Relative mRNA expression of *TLR7*, *IRF7*and *IL-6* in B lymphocytes sorted from blood. To compare groups the Mann-Whitney test was applied.

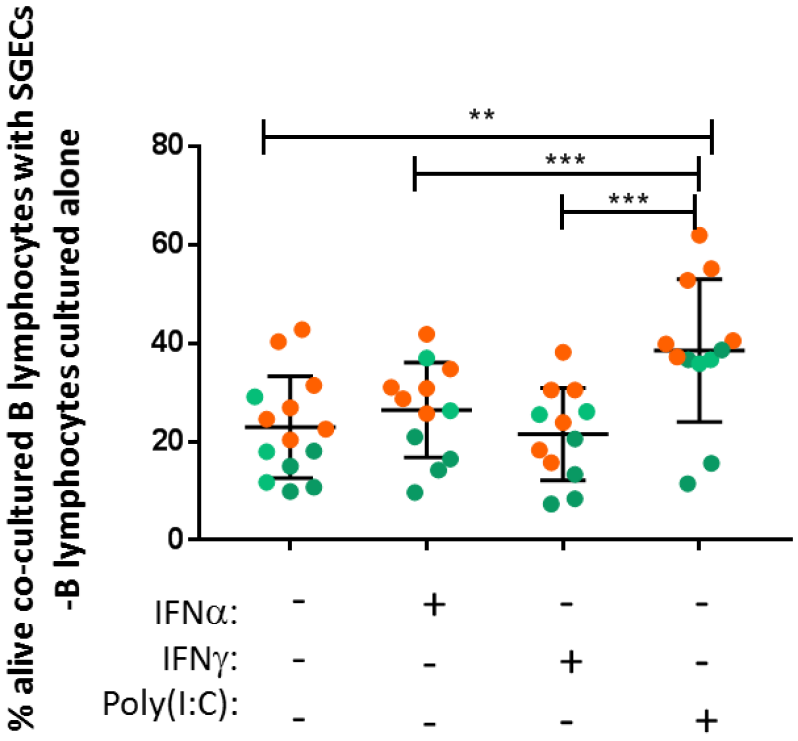

Figure S4: Difference between the percentage of alive B lymphocytes on co-culture with SGECs and the percentage of alive B lymphocytes cultured alone on day 5 stimulated with IFNα, IFNγ or poly(I:C). **P<0.01, ***P<0.001

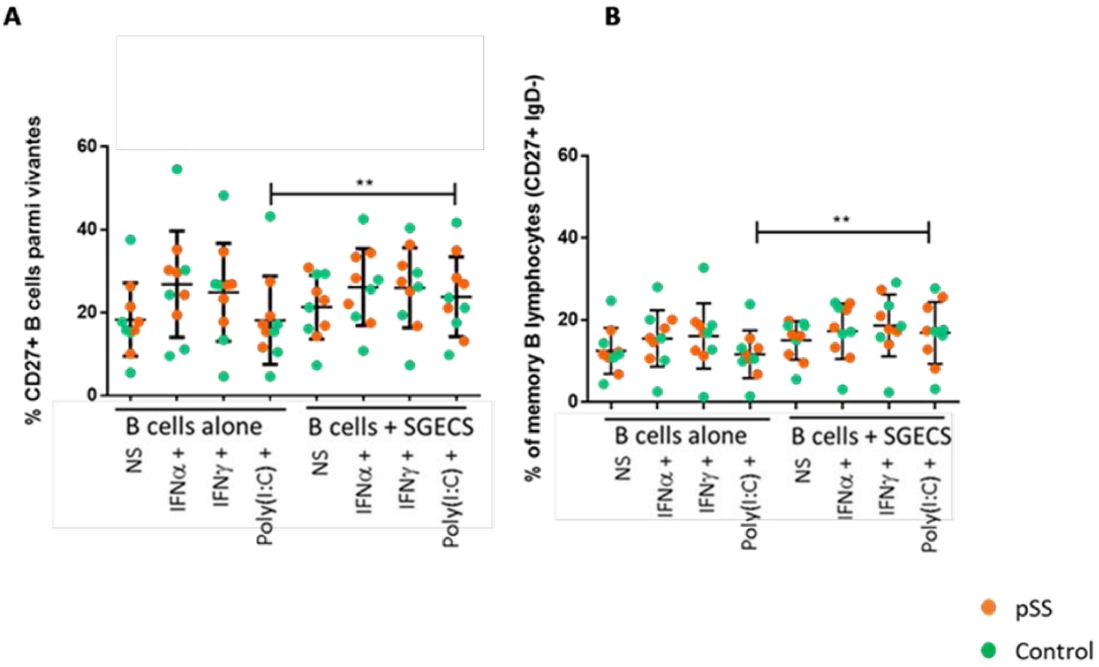

Figure S5: A: Percentage of memory B lymphocytes (CD27+) on day 5 under all conditions of culture and stimulation. **B:** Percentage of switched memory B lymphocytes (CD27+ IgD-) on day 5 under all conditions of culture and stimulation. **P<0.01

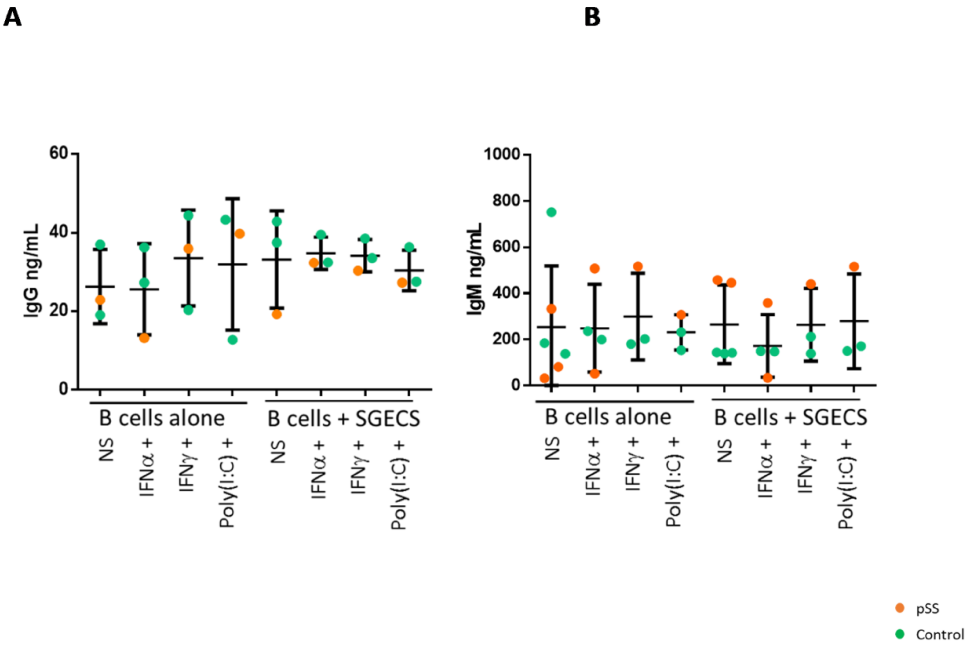

Figure S6: Level of IgG (**A**) and IgM (**B**) in supernatant of B lymphocytes cultured alone and co-cultured with SGECs for 5 days.

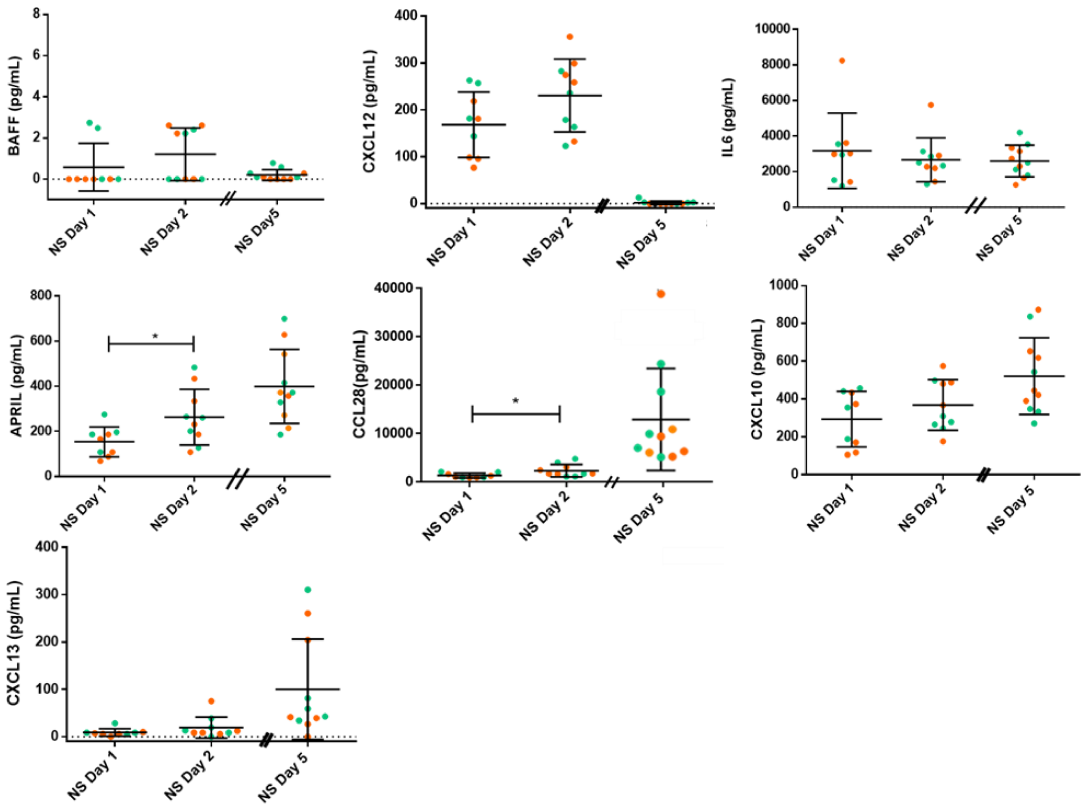

Figure S7 : Levels of BAFF, CXCL12, APRIL, IL-6, CCL28, CXCL10 and CXCL13 in supernatants from cultured SGECS from 5 pSS and 5 controls at day 1 and day 2. The results obtained at day 5 are also reported on the graphs (similar to the results presented on Figure 5). Controls are in green and pSS are in orange. NS : not stimulated.

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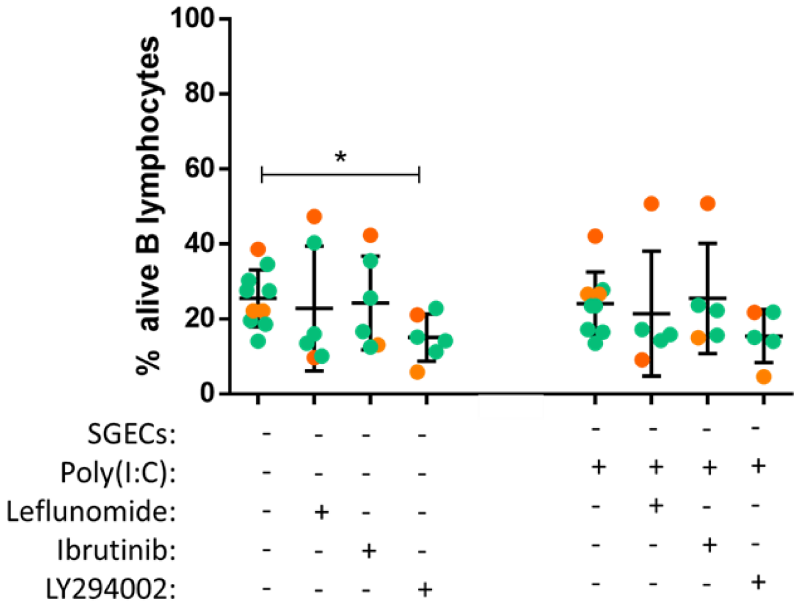

Figure S8 : Percentage of alive B lymphocytes cultured alone after 5 days in the presence of leflunomide, ibrutinib (BTK inhibitor) or LY294002 (PI3K inhibitor). * p<0.05.

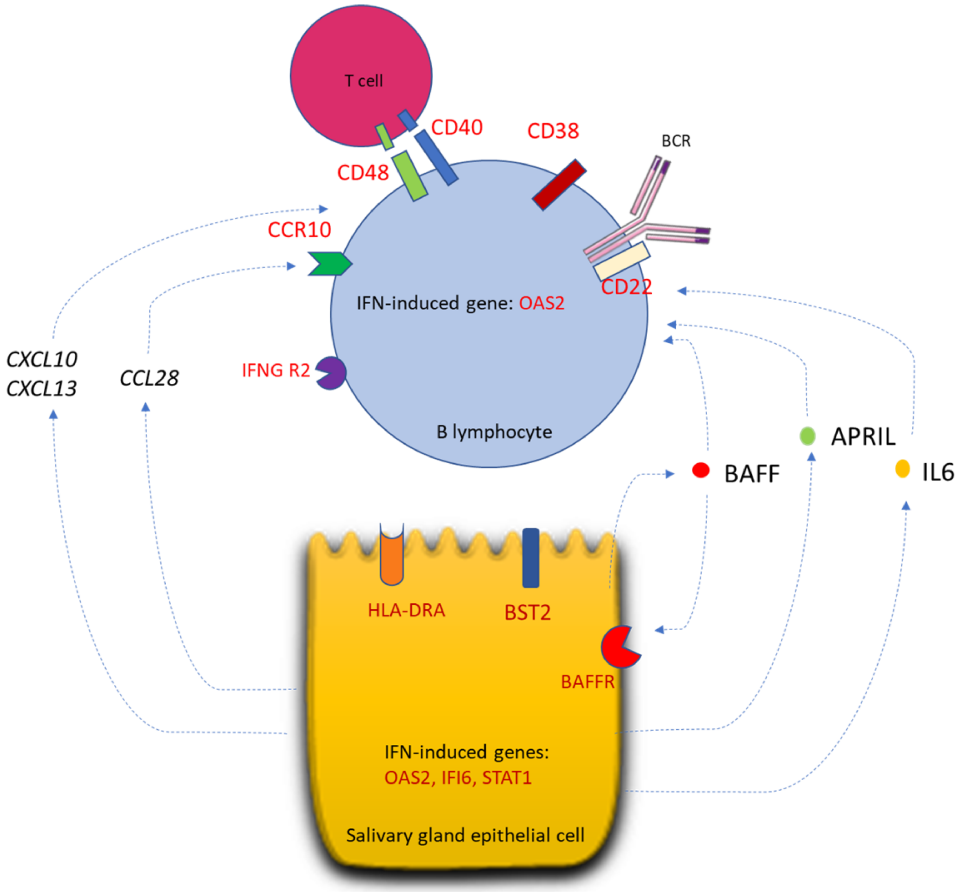

Figure S9: Figure 8: Schematic representation of hypothesis for mechanisms of interaction between SGECs and B lymphocytes. Summary of the genes and pathways that were highlighted by transcriptome analysis (CD40, CD48, CD22, CCR10, IFNGR2 and OAS2 for B cells ; HLADRA, BST2, BAFFR, OAS2, IFI6, STAT1 for SGECs) or flow cytometry (CD38, CD48 for B cells ; BAFFR for SGECs) or measured in SGEC supernatant (CXCL10, CXCL13, CCL28, APRIL, IL-6 and BAFF after poly(I:C) simulation. SGECs secrete cytokines and chemokines potentially involved in B-lymphocytes activation or homing.

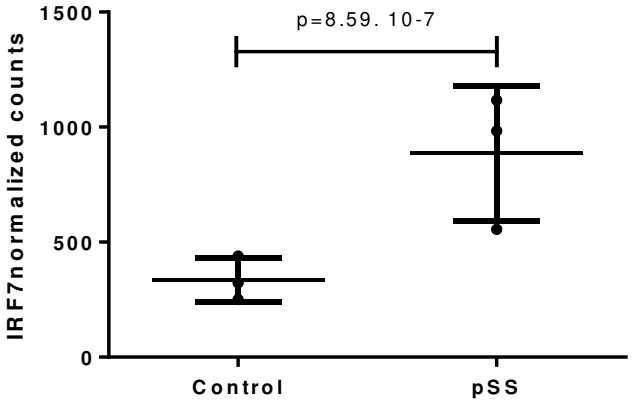

Figure S10: Normalized counts of IRF7 in the RNAseq dataset from Jin-Lei Sun et al. in B lymphocytes sorted from blood from 3 controls and 3 pSS patients

Supplementary tables:

Table S1: List of antibodies used for FACS experiments on co-cultured cells

ANTIBODY	CONJUGATED FLUOROCHROME	**CLONE**	**TYPE OF ANTIBODY**	SOURCE
Sorting of SGECs and B lymphocytes from salivary gland biopsies				
496-diamidino-2-phenylindole dihydrochloride (DAPI)	Pacific Blue			Life Technologies
CD45	APC Vio770	HI30	mouse, monoclonal	BioLegend
CD19	PE Cy7	HIB19	mouse, monoclonal	BioLegend
CD326	PE	HEA-125	mouse, monoclonal	Miltenyi Biotec
Co-culture experiments				
Fixable viability Dye	APC Vio770			LifeTechnologies
anti-CD20	PerCpVio700	REA780	recombinant human IgG1	Miltenyi Biotec
anti-CD38	APC	REA572	recombinant human IgG1	Miltenyi Biotec
anti-CD27	PE	REA499	recombinant human IgG1	Miltenyi Biotec
anti-IgD	PEVio770	REA740	recombinant human IgG1	Miltenyi Biotec
anti-CD40	PE	REA733	recombinant human IgG1	Miltenyi Biotec
anti-CD48	PEVio770	REA426	recombinant human IgG1	Miltenyi Biotec
anti-CD326	PE	REA764	recombinant human IgG1	Miltenyi Biotec
anti-HLA-DR	FITC	REA805	recombinant human IgG1	Miltenyi Biotec
anti-BST2	APC	REA202	recombinant human IgG1	Miltenyi Biotec
anti-BAFFR	PEVio770	REA1115	recombinant human IgG1	Miltenyi Biotec
REA-control	PerCpVio700	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	APC	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	PE	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	PEVio770	REA293	recombinant human IgG1	Miltenyi Biotec
REA-control	FITC	REA293	recombinant human IgG1	Miltenyi Biotec

Table S2: List of primers used for RT-PCR validation of RNA-seq results with Biomark technology purchased from Fluidigm

Target	Assay Name	Forward Primer	Reverse Primer	Design RefSeq
BST2	BST2_90198_i2	ACATTAAACCATAAGCTTCAGGAC	GCGATTCTCACGCTTAAGAC	NM_004335.N
CCL28	CCL28_90212_i1	GAGCTGATGGGGATTGTGAC	TTGGCAGCTTGCACTTTCA	NM_001301875.N
CCR10	CCR10_90205_i0	GGACGGAGGCCACAGA	AGTGGCTCAGCCGAGTAT	NM_016602.N
CD22	CD22_59645_i9	CTGCCTCGCCATCCTCA	TGGCTCTGTGTCCTCTTCC	NM_001771.3
CD40	CD40_55716_i2	ACTGCCACCAGCACAAATAC	TACAGTGCCAGCCTTCTTCA	NM_152854.2
CD48	CD48_86944_i0	ATTCGTGTCTGGCTCTGGAA	CGGAGACCACGGTCATATGTA	NM_001256030.N
PKR/EIF2AK2	EIF2AK2_90200_i13	GCGAACAAGGAGTAAGGGAA	AGAGGTCCACTTCCTTTCCA	NM_002759.N
HLA-DRA	HLA-DRA_54516_i0	CGCTCAGGAATCATGGGCTA	CGCCTGATTGGTCAGGATTCA	NM_019111.4
HPRT1	HPRT1_5093_i5	GCTTTCCTTGGTCAGGCAGTA	ACTTCGTGGGGTCCTTTTCAC	NM_000194.2
IFI16	IFI16_26848_i8	GTGAATGGGGTGTTTGAGGTAC	CACCACTTCCATCTTCCCTGTA	NM_005531.2
IFI6	IFI6_90203_i1	TGCTACCTGCTGCTCTTCA	TCAGGGCCTTCCAGAACC	NM_022873.N
IFNGR2	IFNGR2_87575_i2	GTGGCCCTGAGCAATAGCA	TGTCGGCCGTGAACCATTTA	NM_001329128.N
IL6	IL6_12521_i3	AGAGCTGTGCAGATGAGTACAA	GTTGGGTCAGGGGTGGTTA	NM_000600.3
IL7	IL7_60520_i1	ATTGAAGGTAAAGATGGCAAACA	TCATTATTCAGGCAATTGCTACC	NM_000880.3
IRF7	IRF7_25580_i5	GGCAGAGCCGTACCTGTCA	ACCGTGCGGCCCTTGTA	NM_004031.2
OAS2	OAS2_83324_i0	TGGTGAACACCATCTGTGAC	CCATCGGAGTTGCCTCTTAA	NM_001032731.N
SDC1 (CD138)	SDC1_90204_i2	AAGATGGCTCTGGGGATGAC	GAGCTGCGTGTCCTTCCA	NM_001006946.N
STAT1	STAT1_56036_i14	ATGCTGGCACCAGAACGAA	GCTGGCACAATTGGGTTTCAA	NM_007315.3
TLR7	TLR7_67595_i1	TCTTCAACCAGACCTCTACATTCC	AGCCCCAAGGAGTTTGGAAA	NM_016562.3
TNFRSF13C (BAFFR)	TNFRSF13C_90207_i1	CCCCGACGGAGACAAG	CTGTGGCATCAGAGATTCCC	NM_052945.N
TNFRSF11A (RANK)	TNFRSF11A_90208_i1	TGGGACGGTGCTGTAACAAA	CAGGGCAGACATACACTGTCA	NM_003839.N

Table S3: Pathways over-represented in salivary gland epithelial cells (SGECs) sorted from salivary glands and B lymphocytes sorted from blood, analysis performed with Ingenuity pathway analysis

Pathway	-log p-value	Genes
SGECs sorted from salivary gland		
Primary Immunodeficiency Signaling	4.08	*PTPRC, BTK, IGHG1, CD8A, TAP1, TNFRSF13C*
Interferon Signaling	3.52	*IFIT3, OAS1, IFI6, STAT1, TAP1*
B Cell Development	2.89	*PTPRC, HLA-DRA, CD86, IL7*
Role of JAK2 in Hormone-like Cytokine Signaling	2.73	*STAT5A, IRS1, SH2B3, STAT1*
IL7 Signaling Pathway	2.51	*STAT5A, SLC2A1, IRS1, IGHG1, STAT1, IL7*
B lymphocytes sorted from blood		
EIF2 Signaling	8,6	*PIK3CA,RPLP1,RPL3,RPS23,RPLP2,EIF4G3,EIF4G1,RPL28,PPP1CC,RPL8,EIF4G2,RPL13,UBA52,PIK3C3,RPS9,RPL10,RPS25,RPS2,EIF2AK2,RPS3,RPS12,RPL18,RPL13A,RPL41,RPLP0,RPS14*
Interferon Signaling	7,02	*IFIT3,OAS1,MX1,TYK2,IFI6,IFI35,STAT2,IFNAR2,STAT1,IFITM1*
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	4,24	*IFIH1,PIK3CA,IRF7,OAS1,OAS2,TICAM1,PIK3C3,TLR7,MAVS,PRKCH,NFKB2,IL6,CCL5,EIF2AK2*
Activation of IRF by Cytosolic Pattern Recognition Receptors	3,98	*IFIH1,IRF7,MAVS,IKBKE,STAT2,IL6,NFKB2,ADAR,STAT1*
mTOR Signaling	3,35	*PIK3CA,RPS23,VEGFB,EIF4G3,EIF4G1,RHOG,EIF4G2,PIK3C3,RPS9,RPS6KB2,RPS25,PRKCH,RPS2,RPS12,RPS3,RPS14*
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	3,13	*TYK2,STAT2,NFKB2,IFNAR2,STAT1*

software

Table S4: Fold change and p-value for differential gene expression between pSS patients and controls in SGECs and B lymphocytes sorted from salivary gland biopsies and from blood.

Gene Symbol	Gene ID	log2 fold-change	p-value
SGECs from salivary gland			
HLA-DRA	ENSG00000204287	1.84	0.04
IFI6	ENSG00000126709	4.32	$2.7\ 10^{-5}$
STAT1	ENSG00000115415	2.13	0.02
BST2	ENSG00000130303	4.08	<0.01
BAFFR	ENSG00000159958	4.94	0.01
IL7	ENSG00000104432	2.56	<0.01
OAS2	ENSG00000111335	2.18	<0.01
B lymphocytes from salivary gland			
CD48	ENSG00000117091	2.59	0.01
CD22	ENSG00000012124	2.29	.048
CD40	ENSG00000101017	2.64	0.02
IFNGR2	ENSG00000159128	2.28	0.03
OAS2	ENSG00000111335	2.91	0.04
B lymphocytes from salivary gland			
TLR7	ENSG00000196664	1.40	<0.01
IRF7	ENSG00000185507	0.76	0.04
IL6	ENSG00000136244	1.54	<0.01
Salivary gland vs blood B lymphocytes in pSS			
CD138	ENSG00000115884	6.92	$9.6\ 10^{-5}$
IL6	ENSG00000136244	3.05	<0.01
CCR10	ENSG00000184451	2,73	<0.01
RANK	ENSG00000141655	6.14	$3.7\ 10^{-5}$