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1 **Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte**  
2 **survival and activation.**

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21

22 **ABSTRACT** (248 words)

23 **Objectives:** Primary Sjögren's syndrome (pSS) is characterized by chronic hyperactivation of B-  
24 lymphocytes. Salivary gland epithelial cells (SGECs) could play a role in promoting B-  
25 lymphocyte activation within the target tissue. We aimed to study the interactions between  
26 SGECs from pSS patients or controls and B-lymphocytes.

27 **Methods:** Patients had pSS according to 2016 EULAR/ACR criteria. Gene expression analysis of  
28 SGECs and B-lymphocytes from pSS and controls isolated from salivary gland biopsies and  
29 blood was performed by RNA-seq. SGECs from pSS and controls were co-cultured with B-  
30 lymphocytes sorted from healthy donor blood and stimulated. Transwell and inhibition  
31 experiments were performed.

32 **Results:** Gene expression analysis of SGECs identified an upregulation of interferon signaling  
33 pathway and genes involved in immune responses (*HLA-DRA*, *IL7*, *BAFFR*) in pSS. Activation  
34 genes *CD40* and *CD48* were upregulated in salivary gland sorted B-lymphocytes from pSS  
35 patients. SGECs induced an increase in B-lymphocyte survival, which was higher for SGECs  
36 from pSS patients than controls. Moreover, when stimulated with Poly(I:C), SGECs from pSS  
37 patients induced higher activation of B-lymphocytes than those from controls. This effect  
38 depended on soluble factors. Inhibition with anti-BAFF, anti-APRIL, anti-IL6-R antibodies  
39 JAK1/3 inhibitor, or hydroxychloroquine had no effect, conversely to leflunomide, BTK or PI3K  
40 inhibitors.

41 **Conclusions:** SGECs from patients with pSS had better ability than those from controls to  
42 induce survival and activation of B-lymphocytes. Targeting a single cytokine did not inhibit this  
43 effect, whereas, leflunomide, BTK or PI3K inhibitors partially decreased B-lymphocytes viability  
44 in this model. This gives indications for future therapeutic options in pSS.

45

46 **Key words:** Sjögren's syndrome, salivary gland epithelial cells, B lymphocytes, RNA-seq, co-  
47 culture

48 **Key Messages:**

49 - Primary Sjögren's syndrome (pSS) is characterized by a chronic hyperactivation of B  
50 lymphocytes, and salivary gland epithelial cells (SGECs) could be involved in pSS  
51 pathophysiology.

52 - RNA-seq analysis of SGECs sorted from salivary glands showed upregulated IFN signaling  
53 pathway, *HLA-DR*, *IL7* and *BAFFR*. RNA-seq analysis of B lymphocytes sorted from salivary  
54 glands showed upregulated *CD40* and *CD48*.

55 - Survival and activation of B lymphocytes was higher with SGECs from pSS patients than  
56 controls.

57 - SGECs interact with B lymphocytes mainly by secretion of soluble factors. Targeting a single  
58 cytokine is not sufficient to inhibit this effect, but leflunomide, BTK or PI3K inhibitors were  
59 successful, which suggests future therapeutic options in pSS.

60

61

62

63 **INTRODUCTION**

64 Primary Sjögren's syndrome (pSS) is a systemic auto-immune disorder characterized by a  
65 lymphocytic infiltrate of exocrine glands. Patients present sicca, fatigue, and pain, and in 30%  
66 to 40% of them, systemic manifestations including involvement of skin, joints, neurological  
67 system, lung or kidney[1]. B-lymphocyte hyperactivation is a hallmark of the disease[2], as  
68 illustrated by the presence of serum auto-antibodies, hypergammaglobulinemia, increased  
69 levels of free light chains, and increased risk of B-cell lymphoma. The peri-epithelial tropism  
70 of the lymphocytic infiltration is specific to pSS and justifies the term auto-immune  
71 epithelitis[3].

72 Several lines of evidence support that salivary gland epithelial cells (SGECs) are not only the  
73 target of autoimmunity in pSS patients but may also play a role in the pathology. They could  
74 act as non-professional antigen-presenting cells by expressing co-stimulatory molecules such  
75 as CD80 and CD86 [4]. In salivary glands from pSS patients, CyTOF immunophenotyping  
76 showed a significant up-regulation of HLA-DR in SGECs associated with disease activity [5].  
77 SGECs could be involved in local B-lymphocyte activation as they can secrete B-cell activating  
78 factor (BAFF) [6]. Of note, plasma cells are detected in salivary gland tissue in close contact  
79 with CXCL12-expressing ductal and acinar epithelium, which suggests potential interactions  
80 between these cells [7].

81 We hypothesized that SGECs could play a supportive role in B-lymphocyte survival and  
82 activation. We first focused on gene expression analysis of purified cellular populations sorted  
83 from salivary gland biopsies, then developed an in vitro co-culture model including SGECs and  
84 B lymphocytes to characterize the cross-talk between both.

85

## 86 **METHODS**

### 87 **Patients**

88 Minor salivary gland (MSG) biopsies were obtained from consecutive patients referred for  
89 suspected pSS to the Rheumatology Department of Bicêtre Hospital, a tertiary reference  
90 center for systemic auto-immune diseases. pSS was defined according to the 2016 ACR/EULAR  
91 criteria [8] and controls were patients presenting sicca symptoms without anti-Ro/SSA or anti-  
92 La/SSB antibodies and with normal or sub-normal MSGs (i.e., focus score <1). MSG biopsies  
93 were used for transcriptomic analysis of sorted cells and co-culture experiments. Peripheral  
94 blood mononuclear cells (PBMCs) used for transcriptomic analysis were isolated from whole  
95 blood of pSS patients and healthy blood donors. B lymphocytes sorted from blood of healthy  
96 donors were used for the co-culture experiments.

97 A total of 42 pSS patients and 62 controls were included in the study, among whom 26 pSS  
98 patients and 27 controls had an MSG biopsy. The characteristics of the patients and controls  
99 are presented in **Table 1**. Experimental design showing the number of patients included in  
100 each experiment is in **Figure S1A**.

101 The study received approval from the local ethics committee, and informed consent was  
102 obtained from all patients and control subjects. Patients or the public were not involved in the  
103 design, or conduct, or reporting, or dissemination plans of our research.

104 Methods of RNA-sequencing, primary cultures of SGECs [9] and co-cultures of SGECs and B  
105 lymphocytes, FACS analysis and assays are described in online **supplementary** text.

### 106 **Statistical methods**

107 *Statistical analysis of of RNA-seq*

108 Statistical analyses were performed using the DESeq2 package. Comparisons were performed  
109 between pSS patients and controls in each cell sub-types based on a non-parametric *t*-test.  
110 Cells sorted from biopsies and from blood in four pSS patients (with matched samples  
111 available) underwent paired comparison by non-parametric paired *t*-test. p-value < 0.05 was  
112 considered statistically significant to identify differentially expressed genes.

### 113 *Statistical analysis of co-culture experiments*

114 The percentage of surviving B lymphocytes was calculated for each culture condition as the  
115 difference between the percentage proportion of alive B lymphocytes co-cultured with SGECs  
116 and the percentage of alive B lymphocytes cultured alone. The same strategy was used to  
117 assess the percentage of activated B lymphocytes (CD38+ or CD48+ B-lymphocyte percentages  
118 and mean fluorescence intensity [MFI]). Statistical analyses were performed with Prism  
119 software using Mann-Whitney (unpaired data) and Wilcoxon (paired data) tests.

120 **RESULTS**

121 **SGECs from pSS patients over-express interferon (IFN)-induced genes, *HLA-DRA*, Interleukin**  
122 **7 (*IL7*) and BAFF receptor (*BAFFR*)**

123 Differential gene expression analysis of SGECs from 5 pSS patients versus 4 controls revealed  
124 494 genes differentially expressed, 280 upregulated and 214 downregulated. *IFI6*, *VGLL2* and  
125 *ZNF879* were the most significantly upregulated genes, and *CCL22* was one of the most  
126 significantly downregulated genes ( $-\log_{10}(\text{p-value}) > 4$ ) (**Figure 1A**). The identified  
127 transcriptional differences were associated with disease status as indicated by the distinct  
128 clustering of pSS and control samples in the multidimensional scaling (MDS) plot shown in  
129 **Figure 1B**. Functional enrichment analysis of differentially expressed genes highlighted an  
130 over-representation of the IFN signaling pathway, B-cell development pathway and IL7  
131 signaling pathway ( $-\log(\text{p-value}) = 3.52, 2.89$  and  $2.51$ , respectively) (**Table S3**). Several IFN-  
132 induced genes were up-regulated, including *IFI6*, *OAS2*, *STAT1* and *BST2* (bone marrow stromal  
133 cell antigen 2) (**Figure 1C**). Among the 280 upregulated genes in pSS SGECs, 18 were IFN-  
134 induced genes according to the Interferome database; 8 were induced by type I and II; 9 were  
135 induced by type I, II and III; and 1 was induced by type I only. We also observed an upregulation  
136 of *HLA-DRA* expression, coding for the alpha chain of the DR major histocompatibility complex,  
137 and *IL7* expression in SGECs from pSS patients versus controls ( $p = 0.04$ ,  $p = 0.02$ , respectively)  
138 (**Figure 1C and Table S4**). Of note, for all these genes, the highest gene expression values were  
139 obtained in anti-SSA positive patients who also had the highest salivary gland infiltration.  
140 These results were confirmed by RT-PCR, statistically significant for *BST2* and *IFI6* and with a  
141 trend for *HLA-DRA*, *OAS2*, *STAT1* and *IL7* (**Figure S3**). Lastly, an up-regulation of *BAFFR*  
142 expression was observed in SGECs from pSS patients versus controls ( $p = 0.01$ ) with RNA-seq



143 **(Figure 1C)** and confirmed by flow cytometry of unstimulated SGEs, with no change after  
144 Poly(I:C) stimulation **(Figure 1D and E )**.

145

## 146 **B-lymphocyte activation is observed at the transcriptomic level in salivary gland biopsies** 147 **and in blood**

148 When comparing gene expression in sorted B lymphocytes from salivary glands of pSS patients  
149 and controls, we detected 620 differentially expressed genes, 397 upregulated and 223  
150 downregulated. The most significantly up-regulated genes were *IGHG1* and *HADH* and the  
151 most significantly down-regulated genes were *MT-RNR2-L12*, *MARCO*, *S100A9*, *ELF4* and *ITGA1*  
152 ( $-\log_{10}(p\text{-value}) > 5$ ) **(Figure 2A)**. The identified differentially expressed genes were associated  
153 with the disease status as indicated by the distinct clustering of pSS and control samples in the  
154 MDS plot **(Figure 2B)**. Several genes involved in B-lymphocyte activation and differentiation  
155 such as *CD40*, *CD48*, and *CD22* ( $p=0.02$ ,  $p=0.01$  and  $p=0.048$ , respectively) were upregulated  
156 in pSS patients versus controls **(Figure 2C)**. *CD40* and *CD48*, but not *CD22* upregulation was  
157 confirmed by RT-PCR **(Figure S3)**. The IFN-induced gene, *OAS2*, and IFN $\gamma$  receptor 2 (*IFNGR2*)  
158 were also upregulated in B-lymphocytes from pSS patients versus controls ( $p=0.04$  and  $p=0.03$ ,  
159 respectively), but not confirmed by RT-PCR. The absence of RT-PCR confirmation may be due  
160 to a lack of power.

161 In sorted B lymphocytes from blood, 516 genes were differentially expressed between pSS  
162 patients and controls, 359 upregulated and 157 downregulated. Functional enrichment  
163 pathway analysis highlighted an over-representation of EIF2 signaling and IFN signaling  
164 pathways as well as a role for JAK signaling **(Table S3)**. *TLR7* and its downstream signaling  
165 molecule *IRF7* were upregulated in pSS; additionally, *IL6*, involved in B lymphocyte growth, was

166 upregulated (**Table S4**). The qPCR results showed a trend for a higher expression of TLR7, IRF7  
167 and IL6 in pSS compared to controls (**Figure S3C**).

168 The paired comparison between B lymphocytes from salivary glands and from blood in four  
169 pSS patients revealed the upregulated genes *CD138*, a plasma cell marker; *RANK*; and *IL6*.  
170 Moreover, *CCR10*, which is the receptor for CCL28, a mucosal homing chemokine, was  
171 upregulated in B lymphocytes sorted from biopsies versus blood (**Table S4**). These results were  
172 confirmed for *RANK* and *IL6* by RT-PCR ( $p=0.03$  and  $p=0.03$ ).

173

#### 174 **SGECs increase B-lymphocyte survival and activation**

175 To analyze the potential interaction between SGECs and B lymphocytes, we used 5-days co-  
176 cultures of SGECs from pSS patients or controls with B lymphocytes from healthy donors with  
177 or without IFN $\alpha$ , IFN $\gamma$  or poly(I:C) stimulation. We observed an increase of B cells viability  
178 under all stimulation conditions ( $p<0.001$ ) (**Figure 3A and B**). The increase in B-lymphocyte  
179 survival was higher in the poly(I:C) than in the IFN $\alpha$  or IFN $\gamma$  stimulated co-cultures (**Figure 3A**  
180 **and Figure S4**). Conversely, poly(I:C) did not drive survival of B lymphocytes cultured alone  
181 (**Figure 3A and B**). More importantly, survival of B lymphocytes was higher in co-culture with  
182 SGECs from pSS patients (stimulated or not with poly(I:C)) than in co-culture with SGECs from  
183 controls ( $p<0.05$ ) (**Figure 3C**).

184 Co-cultures of SGECs with B lymphocytes also increased B-lymphocyte activation, as assessed  
185 by increased expression of CD38 on B lymphocytes under all stimulation conditions (**Figure 3D**  
186 **and E**), with a difference between SGECs from patients and controls after poly(I:C) stimulation

187 **(Figure 3F and H)**. Poly(I:C) had a minor impact on the percentage and the MFI of CD38 on B  
188 lymphocytes cultured alone **(Figure 3E and 3G)**.

189 The effect of SGECs from pSS patients on B-lymphocyte viability and CD38 expression was  
190 increased by poly(I:C) but not IFN stimulation. Thus, we wondered whether Toll-like receptor  
191 3 (TLR3) signaling might differ between patients and controls. However, on looking at the  
192 poly(I:C) signaling pathways in SGECs, we found no difference between pSS and controls in  
193 expression of *TLR3*, *TRAF3*, *IRF3*, *RIG1* and *MDA5* (data not shown). However, *PKR* and *IRF7*  
194 were upregulated but not significantly in SGECs from pSS patients versus controls **(Figure 1C)**.  
195 Of note, no expression of *TLR3* in B lymphocytes sorted from salivary gland biopsies and from  
196 blood was detected.

197 After co-culture with SGECs, the proportion of CD27+ B lymphocytes was increased, and  
198 notably switched memory B lymphocytes defined as CD27+ IgD- cells on co-culture with  
199 poly(I:C) stimulation **(Figure S5A and S5B)**. The co-culture with SGECs did not achieve  
200 differentiation of B lymphocytes into antibody-secreting cells, as supported by the lack of  
201 increase in immunoglobulins (IgM and IgG) production in the co-culture supernatants **(Figure**  
202 **S6A and S6B)**.

203 To sum up, the co-culture experiments demonstrated that SGECs from pSS patients had a  
204 higher ability than SGECs from controls to increase survival and activation of B lymphocytes.

205

### 206 **Interactions between SGECs and B lymphocytes require mostly soluble factors**

207 To determine whether the survival and activation of B lymphocytes required direct cell contact  
208 or not, we performed transwell experiments. In absence of stimulation, the transwell

209 separation did not change the percentage of surviving B lymphocytes co-cultured with SGECs,  
210 which suggests that B-lymphocytes survival mostly depended on soluble factors (**Figure 4A**).  
211 Similar results were obtained for B-lymphocyte activation as assessed by CD38, CD48, and  
212 CD40 markers (**Figure 4B-4F**). However, under poly(I:C) stimulation, part of the effect of SGECs  
213 on B-lymphocyte survival and activation was reduced with transwell separation, which  
214 suggests the additional role of cell contact in this specific condition (**Figure 4A, C, D and E**).

215 Then, in order to determine which factors could play a role in the interactions between SGECs  
216 and B lymphocytes, we tested successively potential involved factors (including BAFF, APRIL,  
217 IL6) and signaling pathways (including JAK, BTK, PI3K and pathways involved in B lymphocytes  
218 activation and proliferation) by adding specific inhibitors in the co-cultures. In spite of  
219 detection of numbers of cytokines in the supernatant of co-cultures (**Figure 5A, B, C, D**),  
220 especially in pSS patients, none of the tested anti-cytokines (belimumab, anti-APRIL antibody,  
221 tocilizumab) was effective for decreasing B-lymphocyte survival and activation by SGECs.  
222 Likewise, tofacitinib was unsuccessful (**Figure 5I and J**). Several chemokines were detected in  
223 co-cultures supernatants, which suggests a potential role for homing factors secreted by SGECs  
224 supporting B-lymphocyte survival and activation. CXCL10, also known as IFN $\gamma$ -inducible  
225 protein 10 (IP-10), and CXCL13 were detected in SGECs cultures and co-cultures supernatants  
226 (**Figure 5E and 5G**). At day 5, CXCL12 was detected at very low levels in the supernatant of only  
227 3 pSS SGECs co-cultures (**Figure 5F**) but not in control SGECs co-cultures. CXCL12 was detected  
228 in SGECs cultures supernatants at day 1 and day 2 (**Figure 57**). Lastly, CCL28, another  
229 chemokine involved in mucosal homing was detected in co-culture supernatant, with no  
230 difference between pSS patients and controls (**Figure 5H**).

231 Given these results, we tried to target multiple immunological pathways at the same time by  
232 using BTK and PI3K inhibitors. BTK was targeted by adding ibrutinib, a BTK inhibitor, in the co-  
233 cultures. Ibrutinib decreased B-lymphocyte viability in co-culture ( $p < 0.05$ ), but this effect was  
234 lost in the presence of poly(I:C) (**Figure 5I and J**). LY294002, a PI3K inhibitor, decreased B-  
235 lymphocyte viability in co-culture ( $p < 0.05$ ) (**Figure 5I**). This effect was maintained but not  
236 significantly with poly(I:C) stimulation (**Figure 5J**). LY294002 could also decrease co-cultured  
237 B-lymphocyte activation, as assessed by the decrease in CD38 MFI (**Figure 5K**). Of note, there  
238 was no effect of the leflunomide, the BTK inhibitor and the PI3K inhibitor on SGEC viability as  
239 compared with the control condition (DMSO). LY294002, but not leflunomide or ibrutinib also  
240 affected B-lymphocyte viability when B lymphocytes were cultured alone (**Figure S8**).

241 Given some recent clinical results [10], we also looked at the effect of the association of  
242 leflunomide and hydroxychloroquine. Leflunomide decreased B-lymphocyte viability in co-  
243 culture, but this effect was lost in the presence of poly(I:C) (**Figure 5I and J**).  
244 Hydroxychloroquine alone or combined with leflunomide had no effect on co-cultured B-  
245 lymphocyte viability (**Figure 5I**).

246

## 247 **DISCUSSION**

248 Transcriptome analysis of sorted cells from salivary-gland biopsies showed an increased IFN  
249 signature in SGECs and a higher activation of tissue and circulating B lymphocytes in pSS  
250 patients than controls. Using a co-culture model, SGECs from pSS patients were more efficient  
251 to promote survival and activation of B lymphocytes than SGECs from controls. These effects  
252 were mainly mediated by soluble factors. Targeting a single cytokine (BAFF, APRIL, IL6 and  
253 JAK1/3) did not inhibit this effect, whereas, leflunomide, BTK or PI3K inhibitors partially  
254 decreased B-lymphocytes viability induced by SGECs. A schematic representation of this  
255 hypothesis is presented in **Figure S9**. Important roles for B lymphocytes, SGECs and IFN have  
256 been suggested by previous studies in pSS pathophysiology. This work brings new elements to  
257 better characterize the interactions between these actors. Moreover, the results we obtained  
258 in this model could have therapeutic consequences by suggesting that directly targeting B  
259 lymphocytes activation pathways would be more efficient than targeting a single cytokine.  
260 However, our model with only two cellular actors is over-simplified and we cannot eliminate  
261 that, in vivo, anti-cytokine therapy might have an indirect action on microenvironment.

262 RNA-seq analysis of sorted SGECs demonstrated an IFN signature within these cells. The IFN  
263 signature is well known in PBMCs and salivary gland biopsies of pSS patients[11,12] ; however,  
264 this is the first time that this IFN signature has been demonstrated in sorted SGECs. The chronic  
265 expression of endogenous retroviral (ERV) sequences could explain the over-representation of  
266 the IFN signaling pathway in SGECs from pSS patients[13]. Overexpression of *BST2*, a  
267 transmembrane protein that inhibits viral particles budding from infected cells, could suggest  
268 an overexpression of some ERVs. A recent work highlighted increased mRNA and protein levels  
269 of *BST2* in salivary glands from pSS patients[14]. However, in our study, *BST2* overexpression

270 was only the consequence of IFN overexpression since we did not find a differential expression  
271 of coding ERV between MSG from pSS patients and controls (*data not shown*).

272 SGECs could act as non-professional presenting cells by expressing HLA-DR but also adhesion  
273 and co-stimulatory molecules [15]. We observed an upregulation of *HLA-DRA*, which codes for  
274 one subunit of HLA-DR. This observation agree with previously published works showing an  
275 aberrant expression of HLA-DR by SGECs[16], which can be induced by a type II IFN  
276 stimulation[17].

277 Type I and II IFN are also able to induce BAFF production by SGECs [6]. BAFF could represent  
278 the link between the innate and adaptive immunity in pSS. BAFFR is the most specific receptor  
279 of BAFF and is mainly present on B lymphocytes. By using RT-PCR and immunofluorescence,  
280 Lahiri et al. showed that BAFFR was expressed by SGECs [18] and that this receptor was  
281 involved in SGECs survival owing to an autocrine effect of BAFF. We showed a differential  
282 expression of BAFFR on SGECs between pSS and controls. Thus, the BAFF cytokine could play  
283 a dual role, having a direct effect on B-lymphocyte survival and also supporting SGECs survival,  
284 which in turn may have a feeder effect on B lymphocytes.

285 B lymphocytes sorted from salivary glands from pSS patients overexpressed activating genes,  
286 as expected based on the pSS pathophysiology. Of note, the co-culture between SGECs and B  
287 lymphocytes increased the expression of activation markers such as CD38, CD48 and CD40 as  
288 compared with B lymphocytes cultured alone. CD40, a co-stimulatory pathway receptor  
289 essential for germinal center reactions seems of particular interest [19-21]. Moreover, a phase  
290 2 therapeutic trial of an anti-CD40 antibody recently yielded positive results [22]. In our study,  
291 CD48, which may enhance the CD40-mediated activating signal for B lymphocytes[23], was  
292 upregulated in pSS versus controls. This result was congruent with a work describing soluble

293 CD48 as a biomarker of pSS which was correlated with the biological domain of the ESSDAI  
294 [24].

295 B lymphocytes sorted from blood overexpressed TLR7 and the downstream signaling molecule  
296 IRF7. Interestingly, the upregulation of IRF7 was confirmed by Jin-Lei Sun et al. who recently  
297 performed RNAseq analysis of B lymphocytes sorted from blood comparing 3 pSS patients to  
298 3 controls (log2FC = 1.39 and p-value =  $8.59 \cdot 10^{-7}$ ) (**Figure S10**) [25]. Moreover, Imgenberg-  
299 Kreuz et al. showed in a RNA-seq analysis of B-lymphocytes sorted from blood an upregulation  
300 of *TLR7* as well as *IRF7* in pSS compared to controls (FC = 2.8, p-value=  $5.09 \cdot 10^{-5}$  and FC=2.8,  
301 p-value=  $5.09 \cdot 10^{-5}$ , respectively) [26].

302 Previously, co-culture experimental models with SGECs used allogenic PBMCs and failed  
303 because of allogenic reactions[17]. In our co-culture model, we used sorted allogenic B  
304 lymphocytes, avoiding the T-lymphocyte allogenic reaction. Moreover, the effect of SGECs on  
305 B-lymphocyte survival and activation was maintained even with transwell separation that  
306 would have prevented antigen presentation. Co-culture with a human salivary gland cell line  
307 and tonsillar B lymphocytes revealed increased apoptosis in epithelial cells [27]. This model  
308 using a cancer cell line was more artificial than primary cultured SGECs from patients or  
309 controls. One limitation of this model is that we used B-lymphocytes sorted from blood, which  
310 might have different characteristics than B-lymphocytes sorted from biopsies. However, the  
311 use of B-lymphocytes sorted from biopsies would have been not technically feasible, due to  
312 the low number of B-lymphocytes present in salivary gland biopsies.

313 In our co-culture model, the ability of SGECs from pSS patients to increase B-lymphocyte  
314 survival was increased by poly(I:C) rather than  $IFN\alpha$  or  $IFN\gamma$  stimulation. The transcriptomic  
315 analysis showed a higher expression of *PKR* and *IRF7*, although not significant, in SGECs from



316 pSS patients than controls, whereas the expression of other downstream signaling factors of  
317 TLR3 were similar. Thus, poly(I:C) stimulation could mimic a viral trigger and signal through  
318 several pathways inducing a positive feedback loop.

319 We hypothesized that SGECs could provide a favorable environment enhancing B-lymphocyte  
320 survival and activation. We observed an increase in memory B-lymphocyte differentiation in  
321 co-cultures; however, the SGECs did not achieve differentiation of B-lymphocytes in antibody-  
322 secreting cells. Thus, this co-culture model remains partial, and other actors such as CD40L,  
323 IL2, IL4, IL21, as showed by McCarthy, using single-cell Nojima cultures could be required [28].  
324 T follicular helper lymphocytes might also be required to obtain differentiated plasma cells.

325 By analogy with bone-marrow plasma cells niches, in which several survival factors including  
326 BAFF, APRIL, IL6 and CXCL12 are required [29], we assessed BAFF, APRIL, IL6, CXCL10, CXCL12,  
327 CXCL13, and CCL28 in co-culture supernatants. All were present in co-culture supernatant and  
328 could participate to survival and activation of B lymphocytes, CXCL12 at very low level and  
329 BAFF only after poly(I:C) stimulation, which agrees with our previous studies [6, 30]. Of note,  
330 CXCL12 was detected in the SGECs supernatants at day 1 and day 2 of culture, but not at day  
331 5 (**Figure S7**). This chemokine could be involved in B lymphocytes recruitment and organization  
332 of the tertiary lymphoid structures in the salivary glands [31]. We did not use any specific  
333 inhibitor of the CXCL12 signaling but this could be of interest. Our experiments with inhibitory  
334 molecules showed that a single pathway inhibition (anti-BAFF, anti-APRIL, anti-IL6R antibody)  
335 was not sufficient. Even tofacitinib, which targets numbers of pro-inflammatory cytokines, was  
336 not successful. Leflunomide, a pyrimidine synthesis inhibitor that inhibit B-lymphocyte  
337 proliferation and cell cycle progression [10, 32], as well as PI3K and BTK inhibitors (targeting at  
338 the same time CXCL13, BAFF and IL6 signaling [33]) decreased B-lymphocyte viability in co-

339 cultures. Lastly, PI3K was the only inhibitor to maintain an inhibitory effect under poly(I:C)  
340 stimulation, which suggests that in a local IFN-rich environment, such as pSS salivary glands,  
341 PI3K inhibition might be the most efficient option.

342 In conclusion, the analysis of SGECs and B lymphocytes sorted from salivary glands from pSS  
343 patients showed an up-regulation of several genes and pathways that could be involved in  
344 their interactions. Our co-culture model showed that SGECs from pSS patients had better  
345 ability than controls to stimulate survival and activation of B lymphocytes. This effect was  
346 mediated mostly by soluble factors. Targeting a single cytokine did not inhibit this effect,  
347 whereas, leflunomide, BTK or PI3K inhibitors partially decreased B-lymphocytes viability in this  
348 model. These findings may give indications for the choice of future therapeutic options in pSS.

349

350 **Competing interests:** A. T., N.A. and M. M. are employed by Biogen. X.M. received an  
351 honorarium for consultancy advice on Sjögren's syndrome from BMS; GSK; Novartis and  
352 Servier and a research grant from Servier. The rest of the authors declare that they have no  
353 relevant conflicts of interest.

354 **Contributorship:** E.R. participated in designing research studies, conducting experiments,  
355 acquiring data, analyzing data and writing the manuscript. J.P. participated in designing  
356 research studies, conducting experiments, acquiring data and analyzing data. N.T. participated  
357 in analyzing data and writing the manuscript. S.B. participated in designing research studies,  
358 conducting experiments and acquiring data. A.P., B.L. and A.D. participated in designing  
359 research studies, conducting experiments and acquiring data. H.C., A.T. and N.A. participated  
360 in conducting experiments and acquiring data. B.J. participated in acquiring data, analyzing  
361 data and writing the manuscript. M.M. participated in designing research studies, acquiring  
362 data, analyzing data and writing the manuscript. G.N. and X.M. participated in designing  
363 research studies, analyzing data and writing the manuscript.

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381 **Ethical approval:** The study received approval from the local ethics committee, and informed  
382 consent was obtained from all participants.

383 **Data availability statement:** Data are available on reasonable request. All data relevant to the  
384 study are included in the article or uploaded as online supplementary information. Additional  
385 data are available on reasonable request.

386 **Patients and public involvement:** Patients or the public were not involved in the design, or  
387 conduct, or reporting, or dissemination plans of our research.

388

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473 **Table1: Characteristics of the patients with primary Sjögren’s syndrome (pSS) and controls.**

	RNA-seq experiments				SGECs used for co-cultures experiments (including transwell and inhibition experiments)	
	SGECs and B lymphocytes sorted from biopsies		B lymphocytes sorted from blood		pSS (n=18)	control (n=21)
	pSS (n=9)	control (n=4)	pSS (n=16)	control (n=7)		
<b>Age (years)</b>	51 (47-71)	64 (55-66)	55 (47-68)	61 (46-82)	52 (42-71)	56 (47-70)
<b>Female sex, no (%)</b>	8 (89)	4 (100)	15 (94)	7 (100)	16 (88)	18(86)
<b>Pathologic Shirmer, n (%)</b>	1 (12.5)	2 (50)	2 (33)	NA	14 (82)	9 (56)
<b>Pathologic salivary flow, n(%)</b>	7 (87.5)	3 (75)	6 (86)	NA	15 (88)	7 (44)
<b>Focus score ≥ 1, n (%)</b>	4 (44)	0 (0)	3 (50)	NA	12(66)	0 (0)
<b>SSA, n (%)</b>	7 (78)	0 (0)	13 (81)	NA	13 (72)	0 (0)
<b>ESSDAI</b>	2 (0-4.5)		0.5 (0-2.75)		2 (1-4)	

474 Data are expressed as median (25th-75th percentiles), unless otherwise specified. All percent-  
 475 ages are calculated for patients with available data. SGECs, salivary gland epithelial cells; SSA,  
 476 Sjögren’s-syndrome-related antigen; ESSDAI: EULAR Sjögren Syndrome Disease Activity Index;  
 477 NA: data not available.



478 **Figure legends:**

479 **Figure 1: Salivary gland epithelial cells (SGECs) sorted from salivary gland biopsies express**  
480 **interferon (IFN)-induced genes, *HLA-DRA*, *BAFFR*, *PKR* and *IRF7*.** **A:** Volcano plot of  
481 differentially expressed genes in sorted SGECs from patients with primary Sjögren's syndrome  
482 (pSS) (n=5) versus controls (n=4). The horizontal red dashed line indicates the cutoff for  
483 significance at  $p < 0.05$  and the vertical lines for fold change (FC) in expression  $\geq 1.5$  and  $\leq -1.5$   
484 **B:** Multidimensional scaling (MDS) representation of the similarities between transcriptomic  
485 profiles. Each dot in the representation corresponds to a transcriptomic profile, and the  
486 distances between the dots are proportional to the transcriptomic distances computed based  
487 on the list of 494 differentially expressed genes. **C:** Normalized counts of genes expressed by  
488 SGECs in controls (HC) and pSS patients. Results of RNA-seq of sorted cells: IFN-stimulated  
489 genes (*IFI6*, *OAS2*, *STAT1*, *BST2*), *HLA-DRA*, *IL7*, *BAFFR*, *PKR* and *IRF7*. P-values were determined  
490 by using DESeq2 software. **D:** Flow cytometry example of BAFFR expression in unstimulated  
491 alive SGECs and isotype control from a pSS patient. **E:** Expression of BAFFR evaluated by flow  
492 cytometry on alive SGECs with or without poly(I:C) stimulation. Statistical analysis was  
493 performed using the Mann-Whitney test, one star corresponds to a p-value  $\leq 0.05$ . SGECs from  
494 controls are in green, SGECs from pSS patients are in orange.

495

496 **Figure 2: B lymphocytes sorted from salivary gland biopsies from pSS overexpress activation**  
497 **and differentiation genes as compared with controls.** **A:** Volcano plot of differentially  
498 expressed genes in sorted B lymphocytes from pSS patients (n=9) versus controls (n=4). The  
499 horizontal red line indicates the cutoff for significance at  $p < 0.05$  and the vertical lines  $FC \geq 1.5$   
500 and  $\leq -1.5$ . **B:** MDS representation of similarities between transcriptomic profiles. Each dot

501 corresponds to a transcriptomic profile and distances between the dots are proportional to  
502 the transcriptomic distances computed based on the 620 differentially expressed genes. **C:**  
503 Normalized counts of *CD40*, *CD22*, *CD48*, *OAS2*, and *IFNGR2* in B lymphocytes sorted from  
504 biopsies from controls (HC) and in pSS patients. P-values were determined by using DESeq2.

505

506 **Figure 3. SGECs from pSS patients increase B-lymphocyte survival and activation. A:**  
507 Representative dot plot of B-lymphocyte viability stained with Live/Dead (Fixable Viability Dye)  
508 cultured alone and or co-cultured with SGECs with or without poly(I:C). **B:** Percentage of alive  
509 B lymphocytes on day 5, cultured alone or co-cultured with SGECs with or without IFN $\alpha$ , IFN $\gamma$   
510 or poly(I:C). **C:** Difference between the percentage of alive B lymphocytes co-cultured with  
511 SGECs from pSS patients and controls and the percentage of alive B lymphocytes cultured  
512 alone on day 5. **D:** Representative dot plot of B lymphocytes cultured alone and co-cultured  
513 with SGECs with or without poly(I:C) and stained with for CD38 and CD27. **E:** Percentage of  
514 CD38+ B lymphocytes at day 5 in all conditions of culture and stimulation. **F:** Difference  
515 between the percentage of CD38+ B lymphocytes in co-culture with SGECs and the percentage  
516 of CD38+ B lymphocytes cultured alone at day 5. **G:** Mean fluorescence intensity (MFI) of CD38  
517 in B lymphocytes cultured alone with or without poly(I:C), or B lymphocytes co-cultured with  
518 SGECs with or without poly(I:C). **H:** Difference between the MFI for CD38 for B lymphocytes  
519 co-cultured with SGECs or cultured alone on day 5.

520 \* p-value  $\leq 0.05$ , \*\* p-value  $\leq 0.01$  and \*\*\* p-value  $\leq 0.001$ .

521

522 **Figure 4. Increases of B-lymphocyte viability and activation were mainly due to soluble**  
523 **factors.** B lymphocytes were examined on day 5 with and without transwell, with and without

524 poly(I:C), in co-cultures with SGECs from pSS patients and controls. **A:** Percentage of alive B  
525 lymphocytes. **B:** Percentage of CD38+ B lymphocytes **C:** CD38 MFI of B lymphocytes **D:**  
526 Percentage of CD48<sup>high</sup> B lymphocytes. **E:** CD48 MFI of B lymphocytes. **F:** CD40 MFI of B \* p-  
527 value  $\leq 0.05$ .

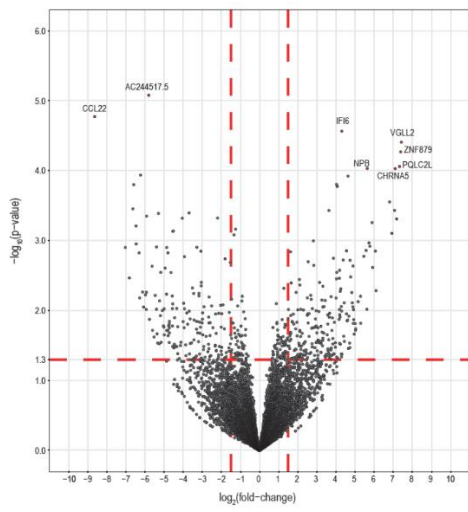
528

529 **Figure 5: Assessment of soluble factors in co-cultures supernatants and inhibition**  
530 **experiments. A:** Level of APRIL in the supernatants of B lymphocytes cultured alone, SGECs  
531 from pSS and controls cultured alone and SGECs co-cultured with B lymphocytes. **B:** Dosage of  
532 APRIL in supernatants of SGECs cultured alone or co-cultured with B lymphocytes (after  
533 subtraction of APRIL level in supernatants of B lymphocytes cultured alone). **C:** Level of IL6 in  
534 supernatants of B lymphocytes cultured alone, SGECs from pSS and controls cultured alone  
535 and SGECs co-cultured with B lymphocytes. **D:** Level of IL6 in supernatants of SGECs cultured  
536 alone or co-cultured with B lymphocytes (after subtraction of IL6 level in supernatants of B  
537 lymphocytes cultured alone). **E, F, G and H:** Level of CXCL10 (**E**), CXCL12 (**F**), CXCL13 (**G**) and  
538 CCL28 (**H**) in supernatants of B lymphocytes cultured alone, SGECs from pSS and controls  
539 cultured alone and SGECs co-cultured with B lymphocytes. Dashed line represents the lower  
540 limit of detection of the assay. **I, J:** Viability of B lymphocytes co-cultured with SGECs and  
541 different inhibitors relative to the condition without the inhibitor and without poly(I:C) (**I**), and  
542 with poly(I:C)(**J**). **K:** Effect of leflunomide, ibrutinib (BTK inhibitor) and LY294002 (PI3K  
543 inhibitor) on CD38 MFI of B lymphocytes cultured alone and in co-culture with SGECs. \* p-  
544 value  $\leq 0.05$ , \*\* p-value  $\leq 0.01$  and \*\*\* p-value  $\leq 0.001$ .

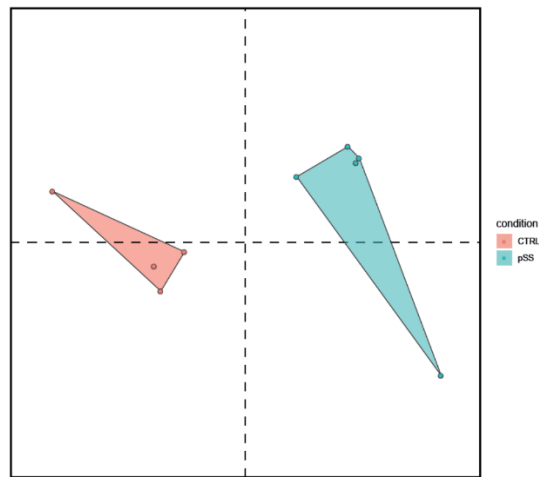
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547 **Figure 1 A**

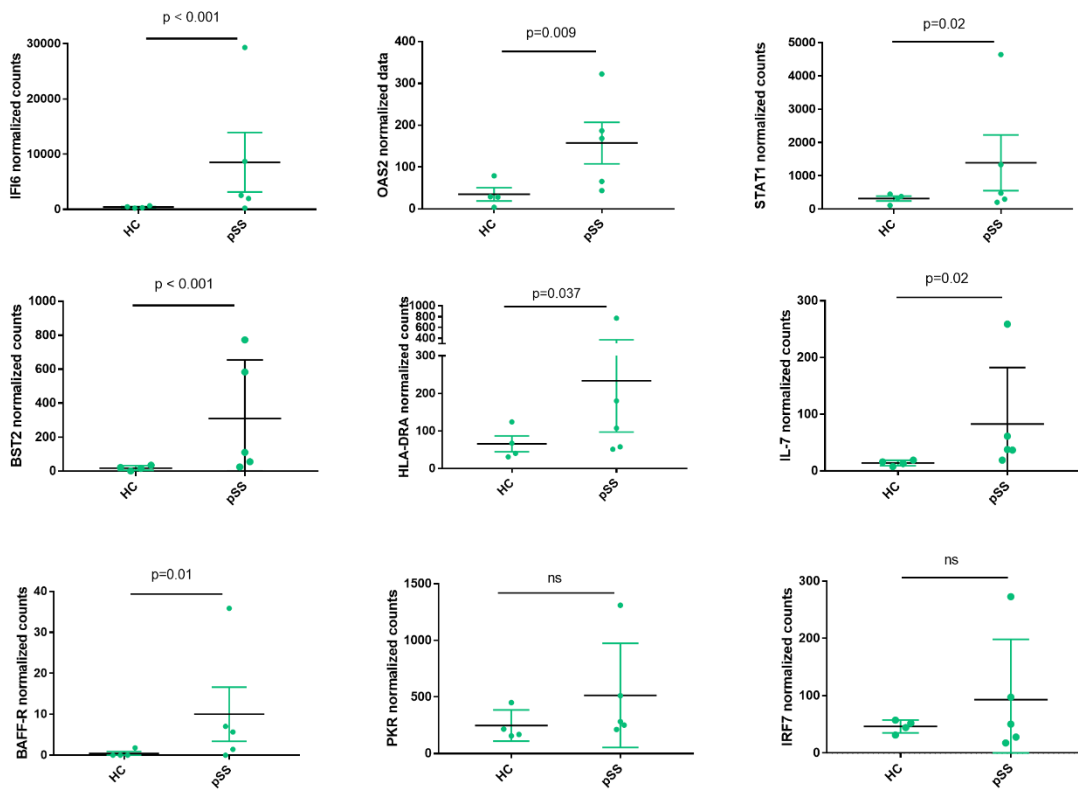


**B**



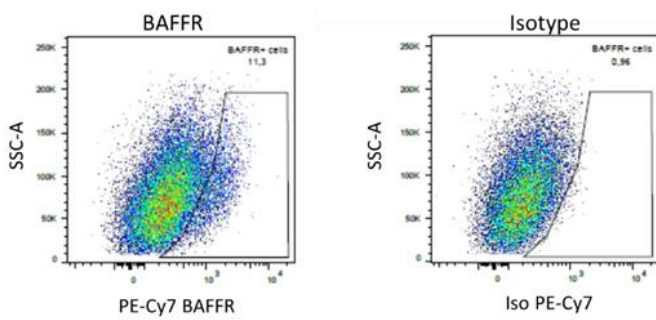
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549 **C**



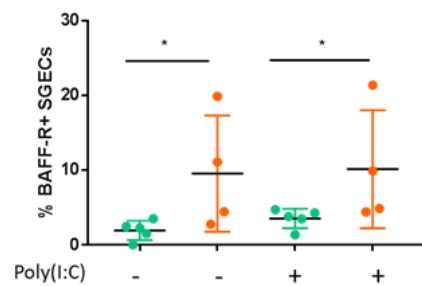
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551 **D**

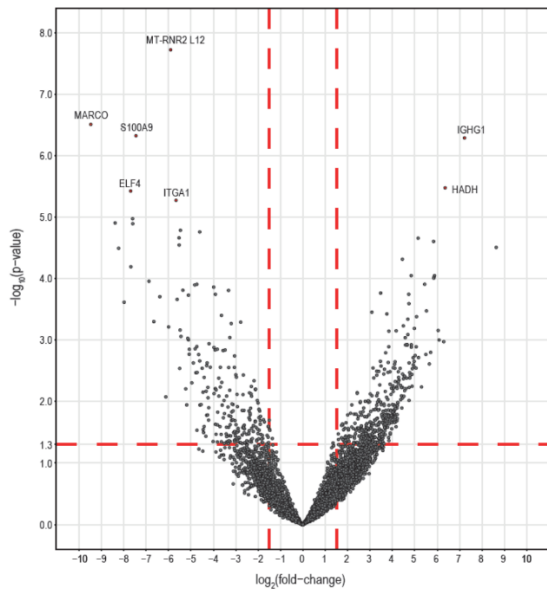


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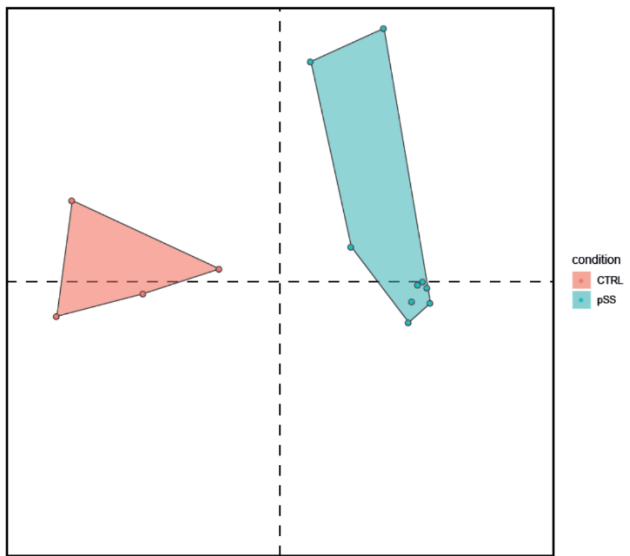
**E**



553 **Figure 2 A**



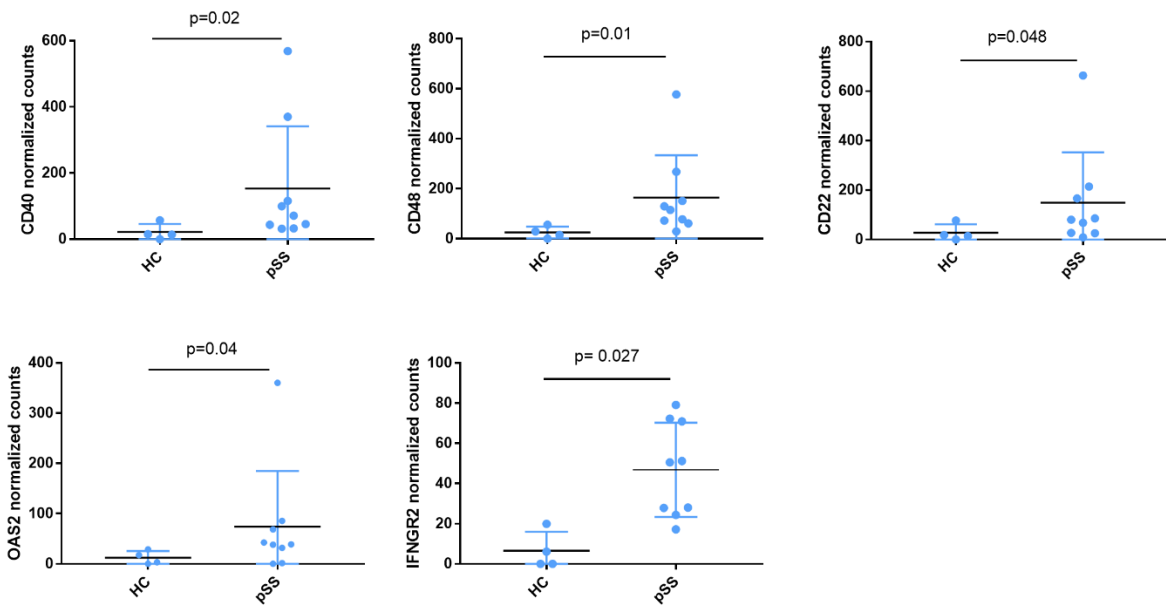
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555 **C**

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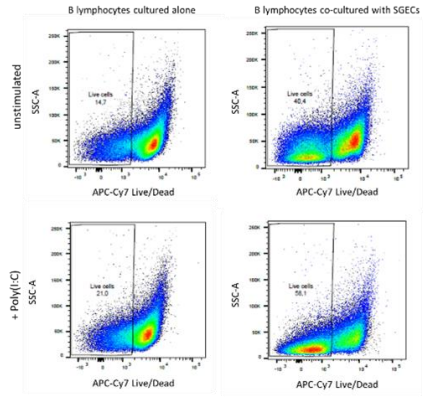
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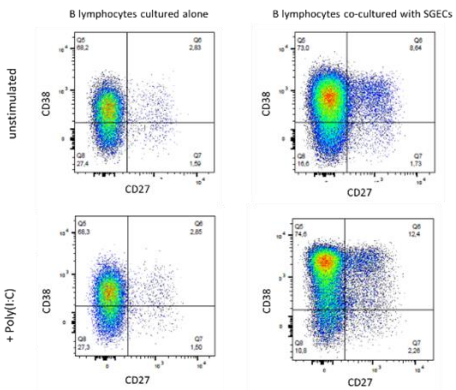
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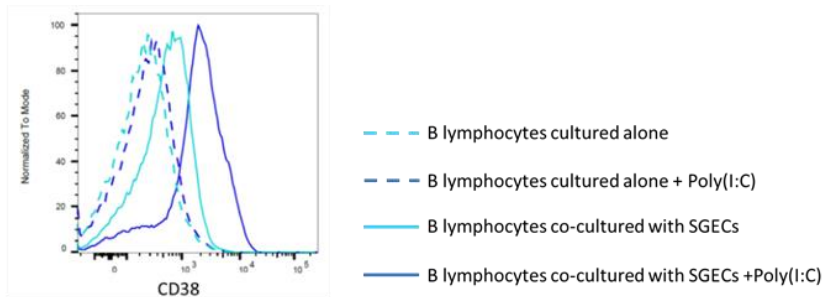
**Figure 3 A**



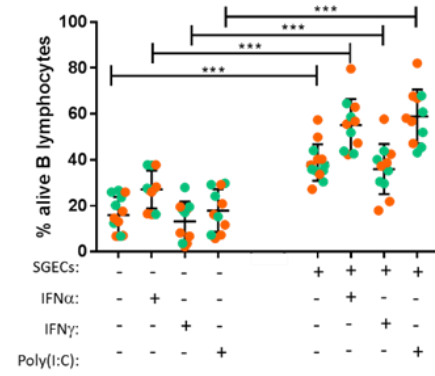
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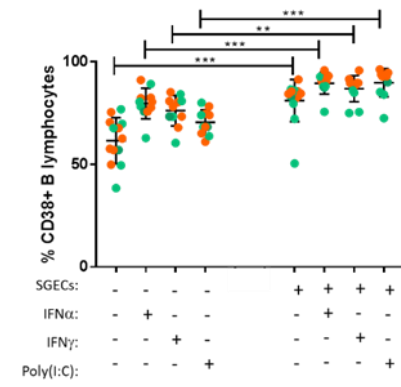
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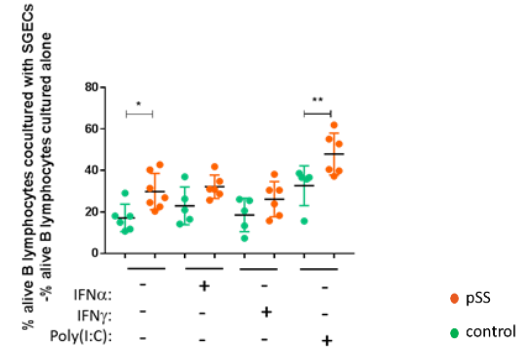
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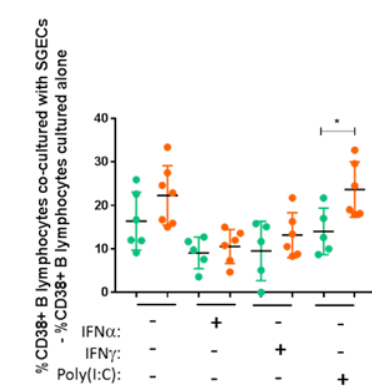
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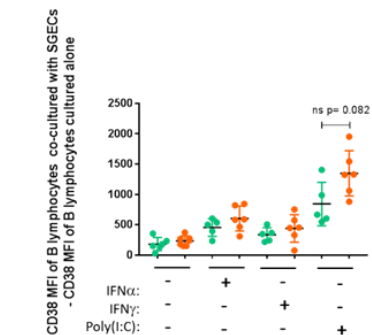
**C**



**F**

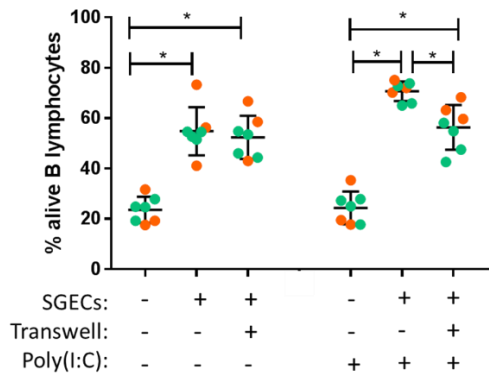


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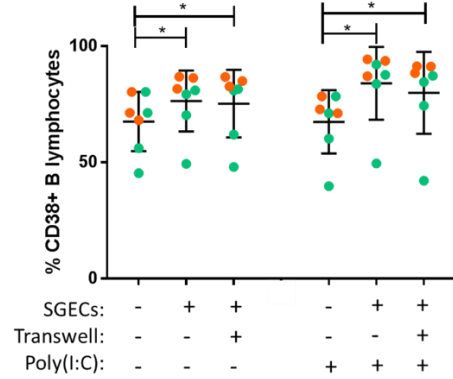


**Figure 4**

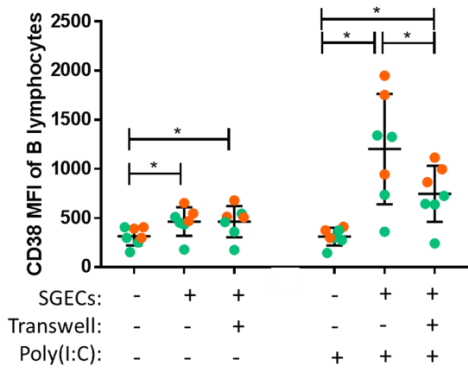
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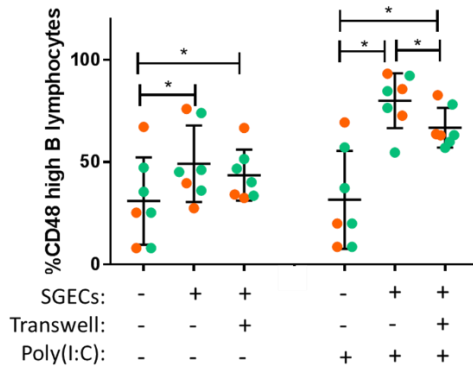
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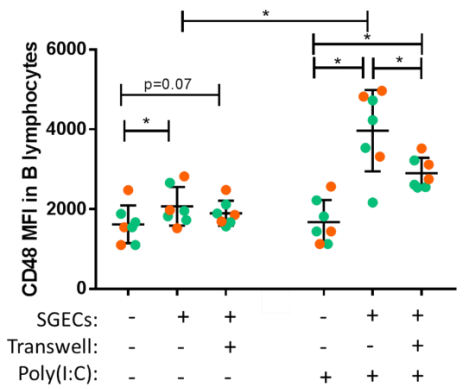
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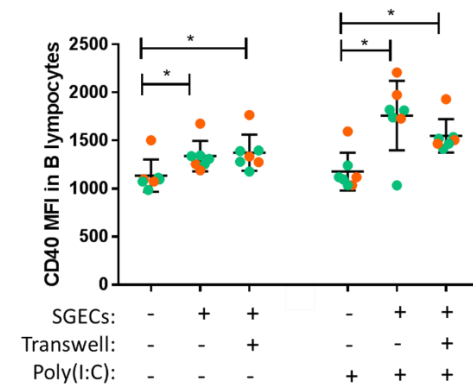
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**E**

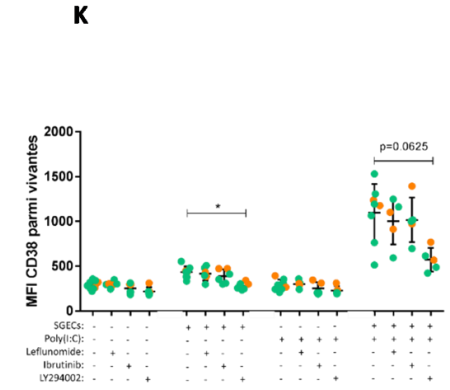
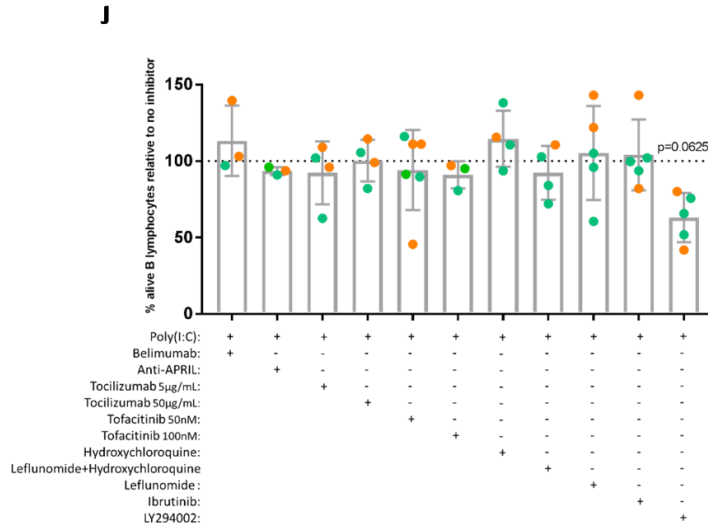
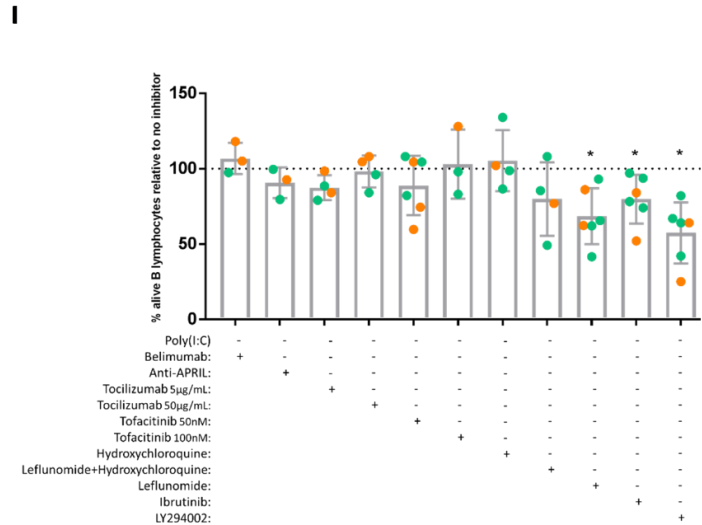
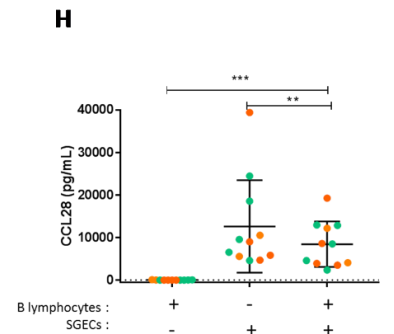
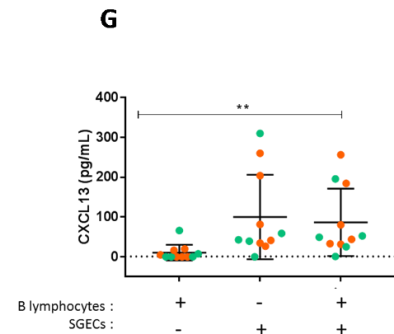
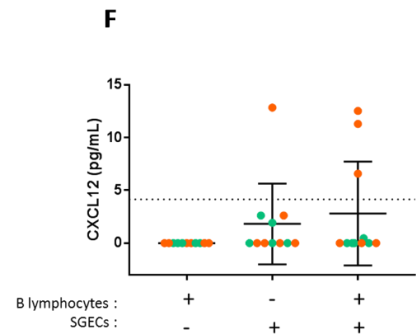
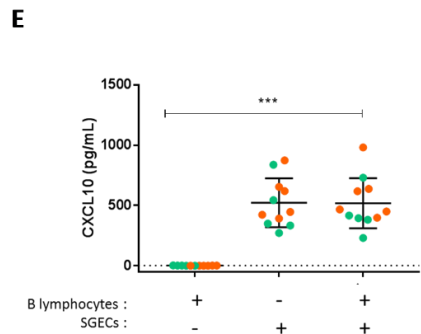
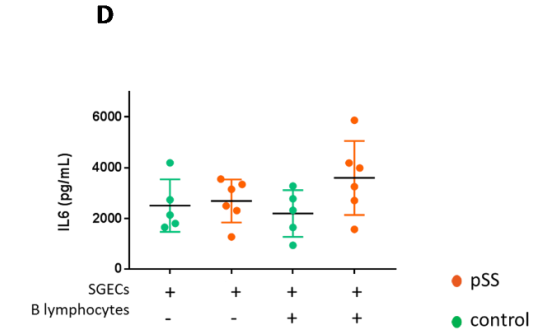
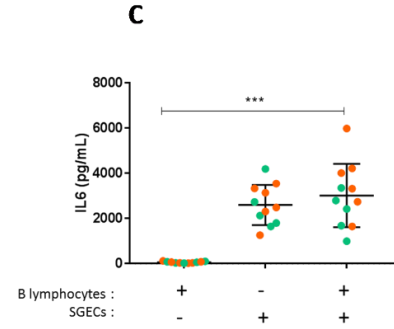
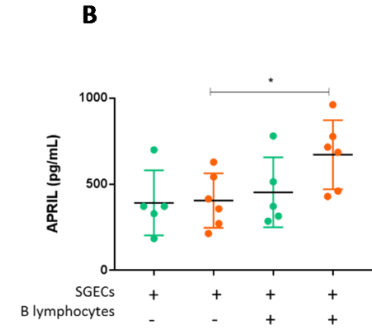
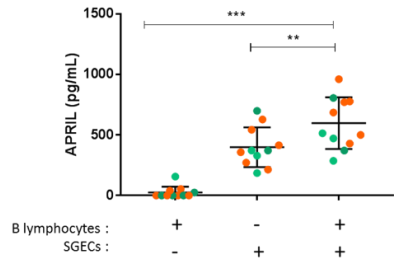


**F**



● pSS  
● control

**A** Figure 5





## 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 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 (Illumina 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 GAllx 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  $\geq 1.5$ , 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/cm<sup>2</sup> to a 6-well collagen type I (Institut de Biotechnologies, Reims, France) coated plate and incubated at 37°C and 5% CO<sub>2</sub> 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 ( $1.5 \times 10^6$  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: IFN $\alpha$  2400 U/mL (Roferon-A, Roche), IFN $\gamma$  5ng/mL (Sigma Aldrich) or poly(I:C) 10 $\mu$ g/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 cocultures experimental design is presented in **Figure S1B**.

### **Transwell and inhibition experiments**

Transwell assays were performed with 0.4 $\mu$ m 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  $\mu$ g/mL (Invivogen) was added in the medium. After 5 days, B lymphocytes were harvested for immunostaining and flow cytometry. Several cocultures were dedicated to inhibition experiments. Inhibitors or their control were added in the co-culture at day 0: belimumab (GSK) 10 $\mu$ g/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  $\mu$ g/mL or IgIV at the higher concentration as control, ibrutinib (Selleckchem) 1 $\mu$ M, LY294002 (Selleckchem) 20 $\mu$ M, leflunomide (Selleckchem)100 $\mu$ M, hydroxychloroquine (Selleckchem) 10 $\mu$ M 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**

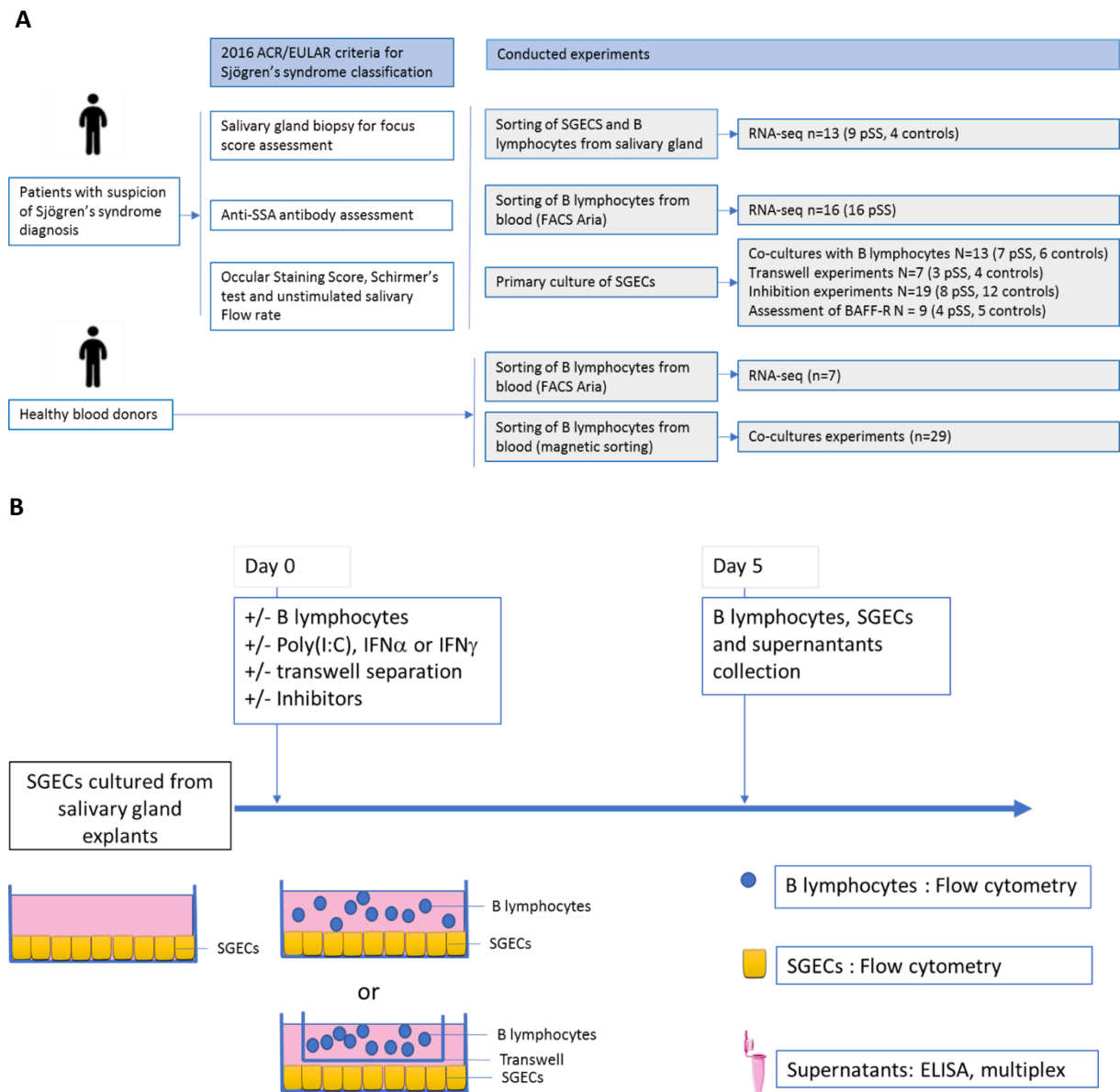
To assess viability of B lymphocytes cultured alone or co-cultured with SGECs in the different conditions of stimulation, B lymphocytes were harvested and stained with Fixable viability Dye APC Vio770 (LifeTechnologies, Paisley, UK). Viability was assessed as the percentage of live B lymphocytes out 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).

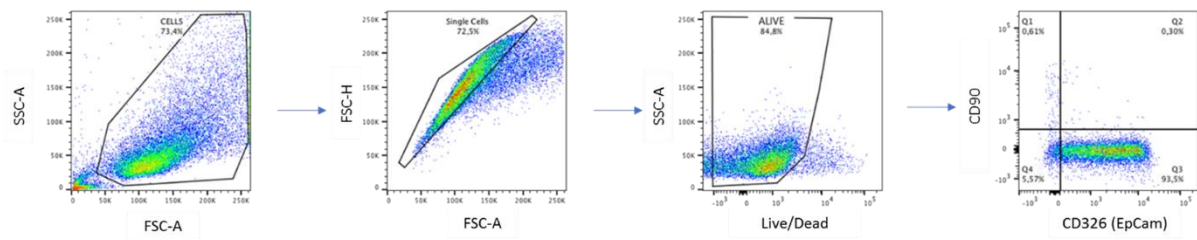
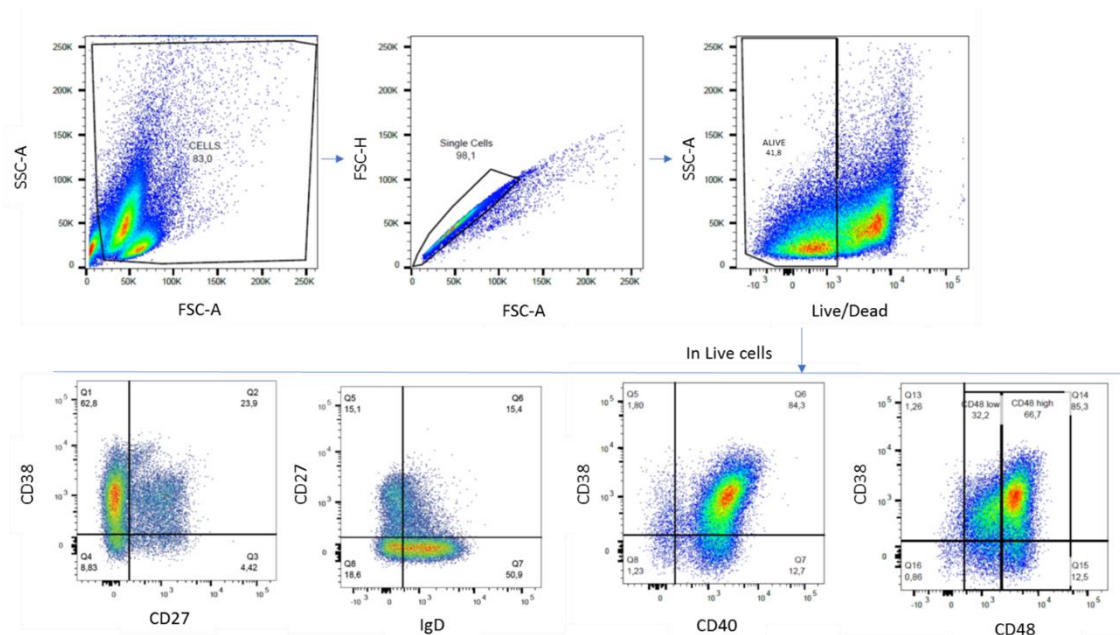
Anti-Ro/SSA antibodies were assessed by using the ALBIA method (Addressable laser bead immuno- assay) with Bioplex 2200 (Biorad). The confirmation was performed by immunodot (Euroimmun Ana3b).

## Supplementary figures:

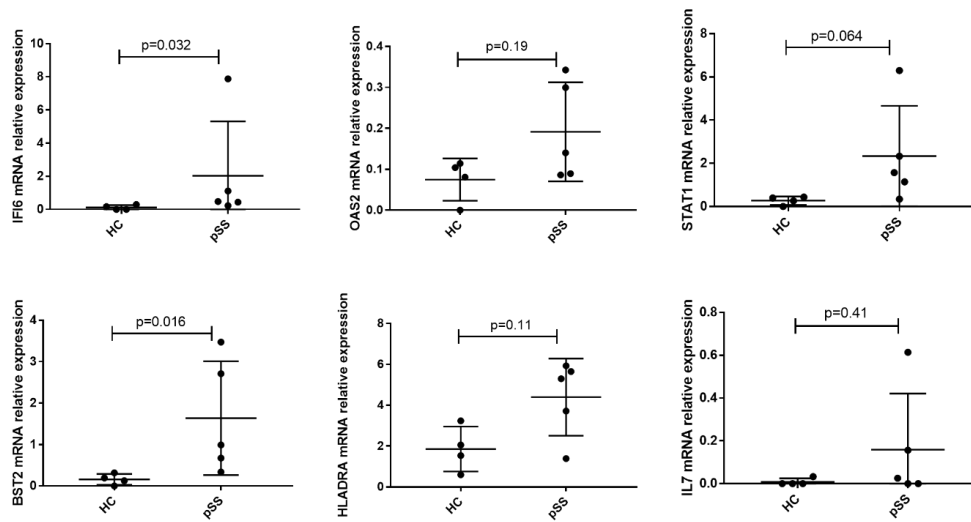
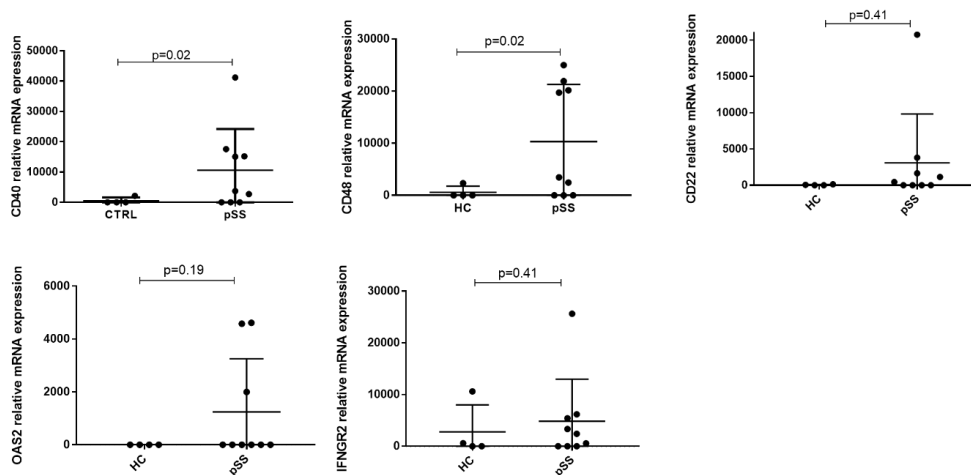
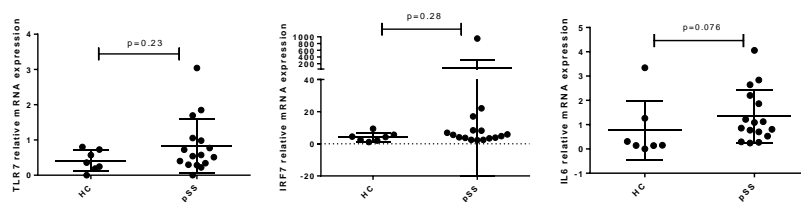


**Figure S1: A:** Experimental design of the study. SGECs, B lymphocytes sorted from biopsies and pSS B lymphocytes sorted from blood used for RNA-seq experiments as well as SGECs used for co-culture experiments were obtained from patients with suspected pSS. pSS was diagnosed according to 2016 ACR/EULAR 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.

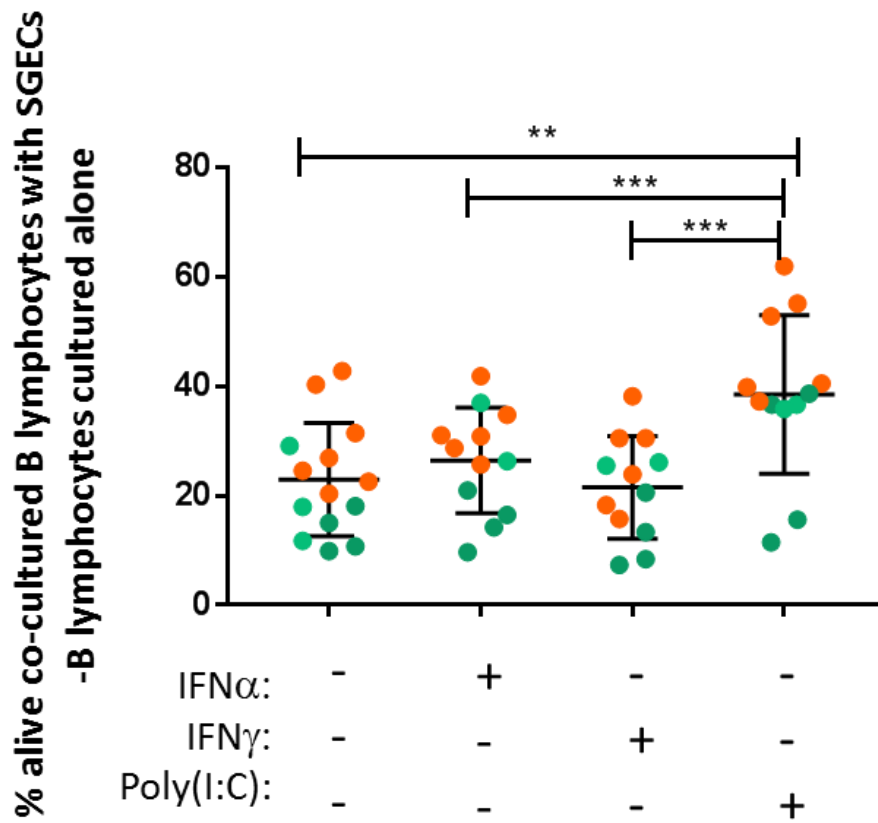


**A****B**

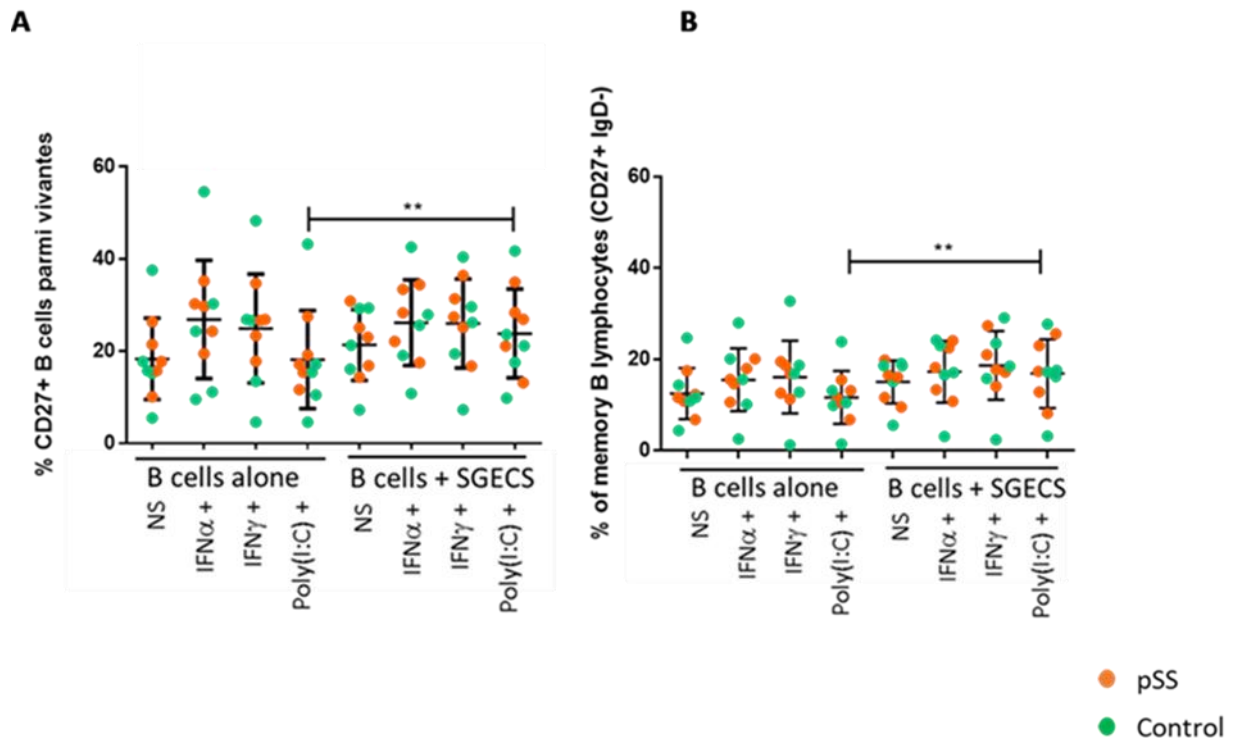
**Figure S2: A:** Gating strategy used to phenotype SGEs. CD326 and CD90 expression on SGEs after 2 weeks of primary culture. **B:** Gating strategy used to phenotype co-cultured B lymphocytes. Percentages of CD38, CD27, IgD, CD40 and CD48 B lymphocytes were determined among alive B cells. MFI was determined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

**A****SGECs sorted from biopsy****B****B cells from biopsy****C****B cells from blood**

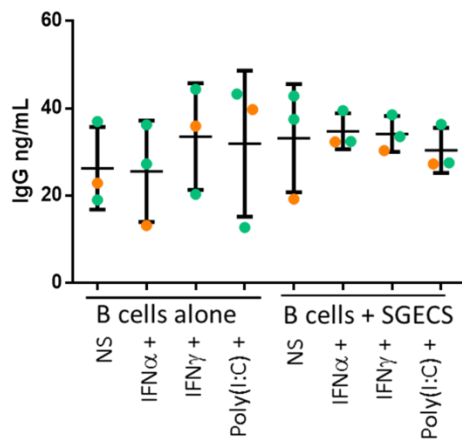
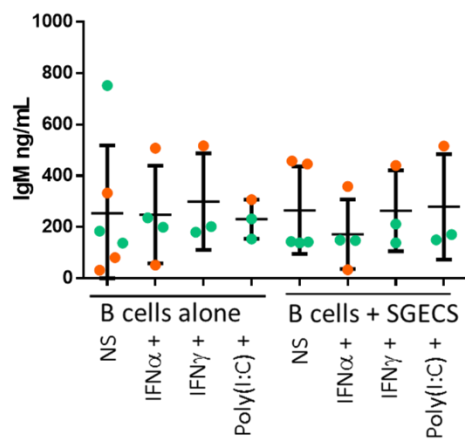
**Figure S3: A:** Validation of the RNA-seq results by RT-PCR. Relative mRNA expression of *IFI6*, *OAS2*, *STAT1*, *BST2*, *HLA DRA* and *IL-7* in SGECs. **B:** Relative mRNA expression of *CD40*, *CD48*, *CD22*, *OAS2*, and *IFNGR2* in B lymphocytes sorted from salivary gland biopsy. **C:** Relative 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 IFN $\alpha$ , IFN $\gamma$  or poly(I:C). \*\*P<0.01, \*\*\*P<0.001

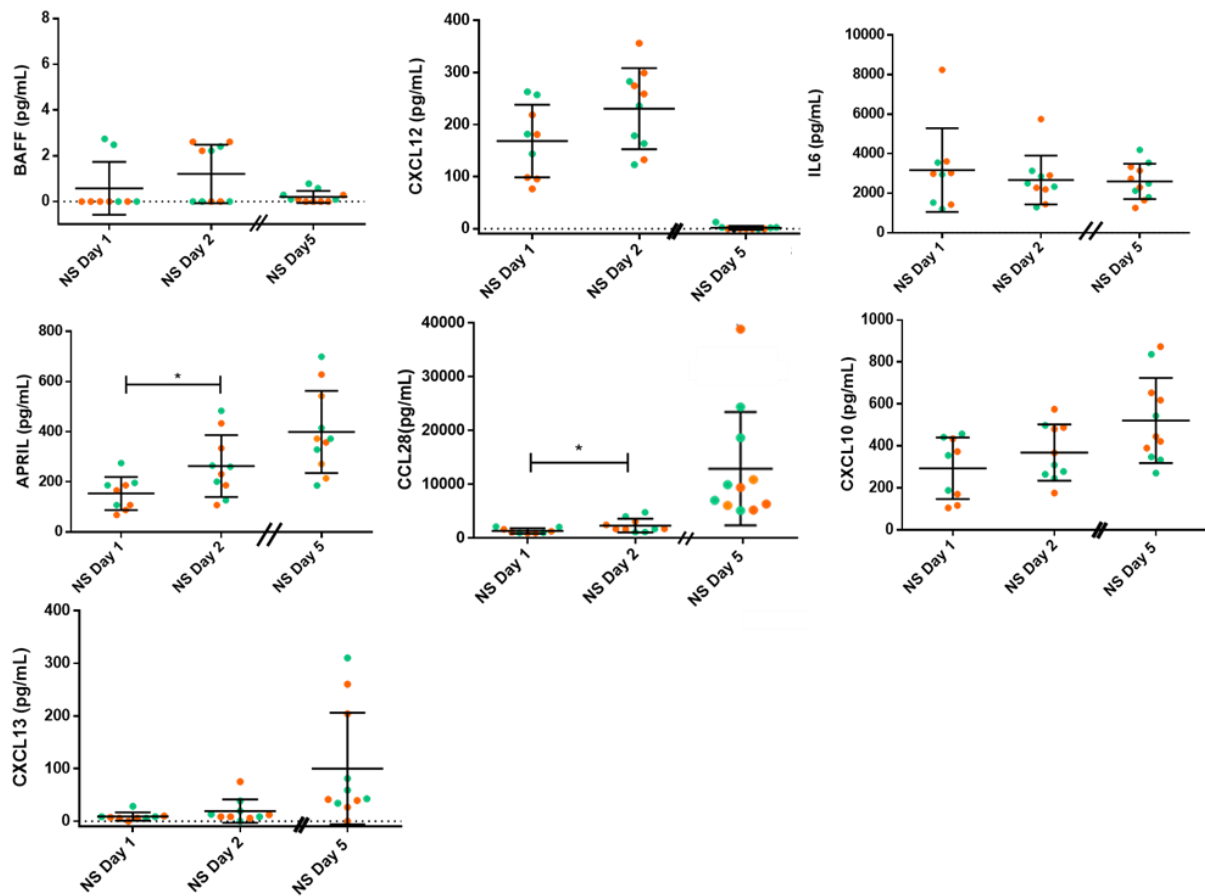


**Figure S5: A:** Percentage of memory B lymphocytes (CD27+) on day 5 under all conditions of culture and stimulation. **B:** Percentage of switched memory B lymphocytes (CD27+ IgD-) on day 5 under all conditions of culture and stimulation. \*\*P<0.01

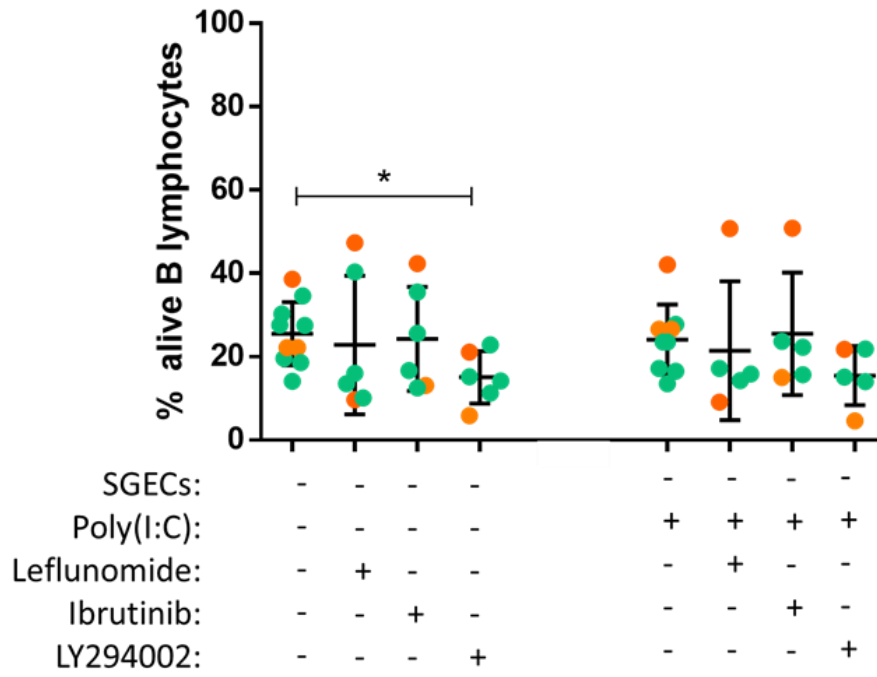
**A****B**

● pSS  
● Control

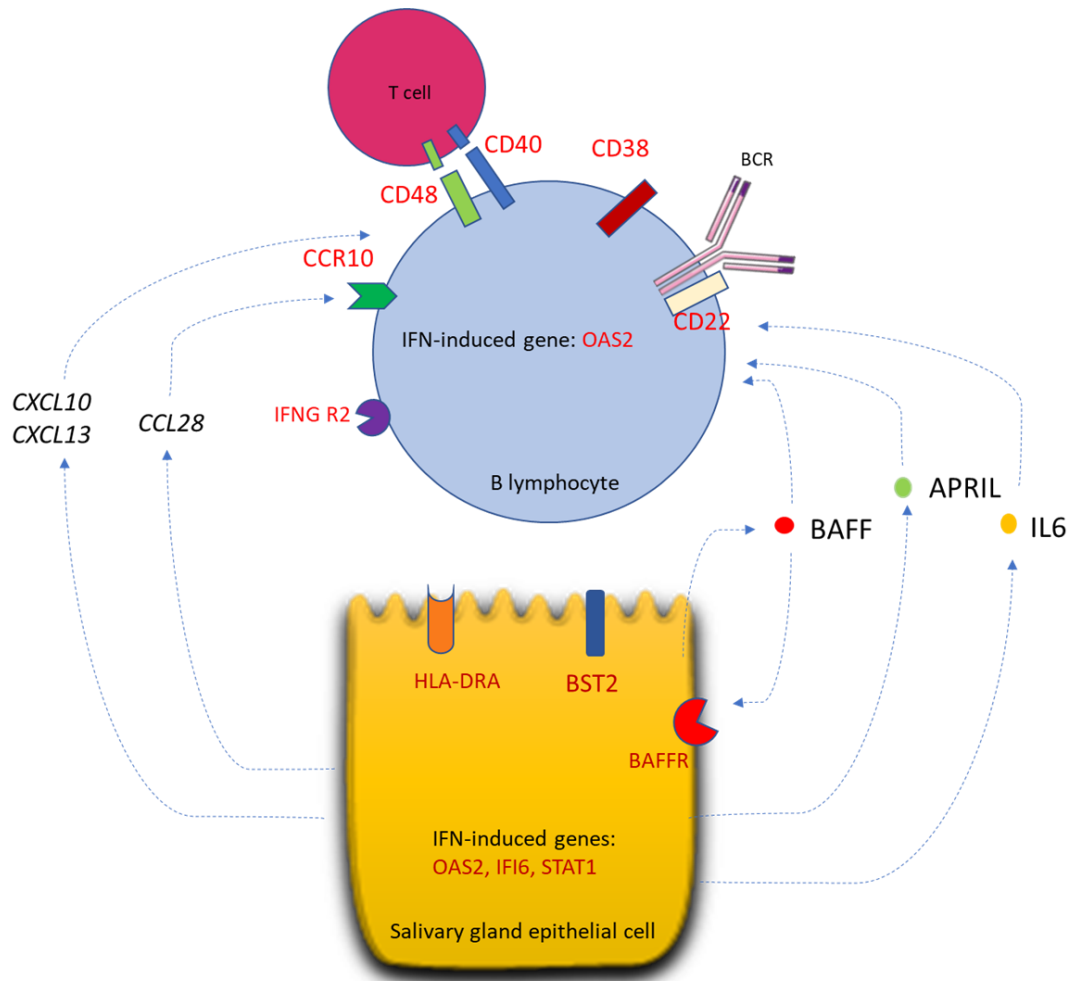
**Figure S6:** Level of IgG (A) and IgM (B) in supernatant of B lymphocytes cultured alone and co-cultured with SGECs for 5 days.



**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.

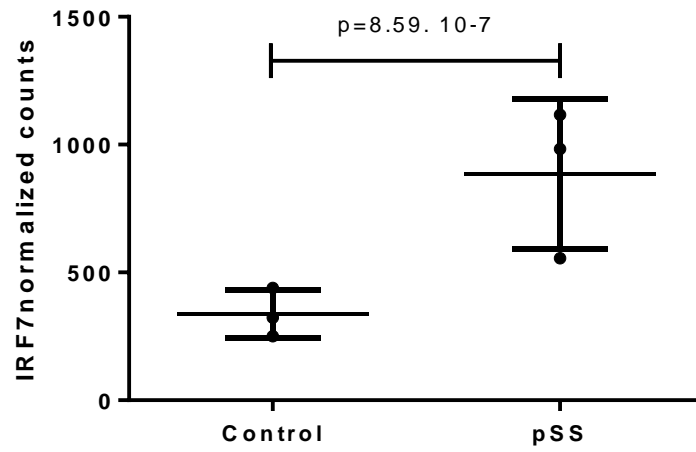


**Figure S8** : Percentage of alive B lymphocytes cultured alone after 5 days in the presence of leflunomide, ibrutinib (BTK inhibitor) or LY294002 (PI3K inhibitor). \*  $p < 0.05$ .



**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 ; HLA-DRA, 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
<b>Sorting of SGECs and B lymphocytes from salivary gland biopsies</b>				
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
<b>Co-culture experiments</b>				
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
<b>BST2</b>	BST2_90198_i2	ACATTAAACCATAAGCTTCAGGAC	GCGATTCTCACGCTTAAGAC	NM_004335.N
<b>CCL28</b>	CCL28_90212_i1	GAGCTGATGGGATTGTGAC	TTGGCAGCTTGCACTTCA	NM_001301875.N
<b>CCR10</b>	CCR10_90205_i0	GGACGGAGGCCACAGA	AGTGGCTCAGCCGAGTAT	NM_016602.N
<b>CD22</b>	CD22_59645_i9	CTGCCTCGCCATCCTCA	TGGCTGTGTCTCTTCC	NM_001771.3
<b>CD40</b>	CD40_55716_i2	ACTGCCACCAGCACAATAC	TACAGTGCCAGCCTTCTCA	NM_152854.2
<b>CD48</b>	CD48_86944_i0	ATTCGTGTCTGGCTCTGGAA	CGGAGACCACGGTCATATGTA	NM_001256030.N
<b>PKR/EIF2AK2</b>	EIF2AK2_90200_i13	GCGAACAGGAGTAAGGGAA	AGAGGTCCACTTCTTTCCA	NM_002759.N
<b>HLA-DRA</b>	HLA-DRA_54516_i0	CGCTCAGGAATCATGGGCTA	CGCCTGATTGGTCAGGATTCA	NM_019111.4
<b>HPRT1</b>	HPRT1_5093_i5	GCTTTCCTTGGTCAGGCAGTA	ACTTCGTGGGGTCTTTTCCAC	NM_000194.2
<b>IFI16</b>	IFI16_26848_i8	GTGAATGGGGTGTGAGGTAC	CACCACTTCCATCTCCCTGTA	NM_005531.2
<b>IFI6</b>	IFI6_90203_i1	TGCTACCTGCTGCTTCA	TCAGGGCCTCCAGAACC	NM_022873.N
<b>IFNGR2</b>	IFNGR2_87575_i2	GTGGCCCTGAGCAATAGCA	TGTCGGCCGTAACCATTTA	NM_001329128.N
<b>IL6</b>	IL6_12521_i3	AGAGCTGTGCAGATGAGTACAA	GTTGGGTCAAGGGTGGTTA	NM_000600.3
<b>IL7</b>	IL7_60520_i1	ATTGAAGGTAAGATGGCAAACA	TCATTATTCAGGCAATTGCTACC	NM_000880.3
<b>IRF7</b>	IRF7_25580_i5	GGCAGAGCCGTACCTGTCA	ACCGTGCGGCCCTTGTGA	NM_004031.2
<b>OAS2</b>	OAS2_83324_i0	TGGTGAACACCATCTGTGAC	CCATCGGAGTTGCCTCTTAA	NM_001032731.N
<b>SDC1 (CD138)</b>	SDC1_90204_i2	AAGATGGCTCTGGGGATGAC	GAGCTGCGTGCCTTCCA	NM_001006946.N
<b>STAT1</b>	STAT1_56036_i14	ATGCTGGCACCAGAACGAA	GCTGGCACAATTGGGTTTCAA	NM_007315.3
<b>TLR7</b>	TLR7_67595_i1	TCTTCAACCAGACCTCTACATCC	AGCCCAAGGAGTTTGGAAA	NM_016562.3
<b>TNFRSF13C (BAFFR)</b>	TNFRSF13C_90207_i1	CCCCGACGGAGACAAG	CTGTGGCATCAGAGATTCCC	NM_052945.N
<b>TNFRSF11A (RANK)</b>	TNFRSF11A_90208_i1	TGGGACGGTGTGTAACAAA	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 software**

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

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

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