SUPPLEMENTARY MATERIALS

Supplementary Table 1. Demographic and clinical characteristics of the primary Sjögren's syndrome patient cohort

	Number	%
Females/males	73/5	93.6/6.4
Age, median (range) years	65 (31–84)	
Disease duration, median (range) years	20 (1–50)	
Methotrexate	4	5.1
Hydroxychloroquine	14	17.9
Corticosteroids	16	20.5
Rituximab	1	1.3
Extraglandular involvement	66	84.6
Focus score†, median (range)	1 (0–7)*	
Focus score ≥1	55*	83.3
GC-like structures present	14**	20.6
Tear flow Shirmer's I test, median (range) mm/5min	3.5 (0–31.5)§	
Ocular dryness, <5mm/5min	54§	71.1
Unstimulated salivary flow, median (range) g/15min	0.81 (0–4.3)§§	
Stimulated salivary flow, median (range) g/5min	3.86 (0–12.5) §§	
ESSDAI score, median (range)	2 (0–17)‡	
ESR, median (range) mm/h	20.5 (4–62)	
ESR, high levels††	26	33.3
CRP, median (range) mg/L	2 (1–17)	
CRP high levels (≥5 mg/L)	14	17.9
C3, median (range) g/L	1.12 (0.75–10.4)	
C3, low levels (<0.83 g/L) number (%)	6	7.7
C4, median (range) g/L	0.19 (0.06–1.24)	
C4, low levels (<0.13 g/L) number (%)	12	15.4
RF, median (range) IU/mL	11 (11–1040)	
RF positive (>25 IU/mL)	22	28.2
SSA antibodies	51	65.4
SSB antibodies	28	36.9

ANA	59	75.6
Anti-CCP	3	3.8
IgA, median (range) g/L	2.5 (0.25–16.4)	
Elevated levels (>4.1 g/L)	6	7.7
IgG, median (range) g/L	12.9 (6.2–29.5)	
Elevated levels (>15.3 g/L)	20	25.6
IgM, median (range) g/L	1.0 (0.3–10.4)	
Elevated levels (>2.5 g/L)	3	3.8
β2-microglobulin, median (range) mg/L	2.7 (0.0–7.7)	
Elevated levels (>2 mg/L)	65	83.3

Continuous data are expressed as the median (range). Categorical data are expressed as the absolute frequency (percentage).*, data not available for 12 patients; **, data not available for 10 patients; §, data not available for 2 patients; §§, data not available for 3 patients, ‡ data not available for 5 patients. †, Focus score indicates the number of inflammatory foci containing more than 50 mononuclear cells per 4 mm^2 biopsy tissue; ††, age and gender dependent. GC, germinal center-like structures; ESR, erythrocyte sedimentation rate; ESSDAI, the EULAR SS disease activity index; CRP, C-reactive protein; C, complement component; RF, rheumatoid factor; ANA, antinuclear antibodies; anti-CCP, anti-cyclic citrullinated peptide. The statistical significance was evaluated by the Mann–Whitney U test for continuous data and the χ^2 or Fisher's exact test as appropriate for categorical data.

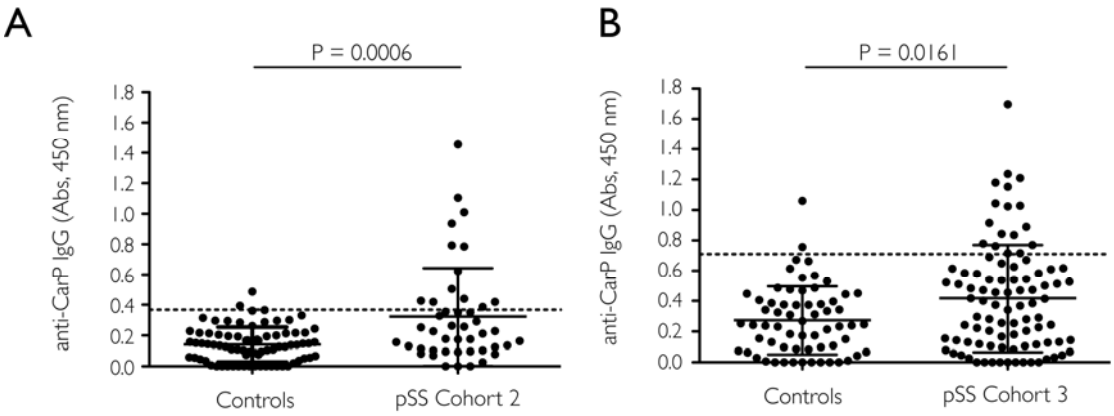
Supplementary Table 2. Association of the laboratory parameters with anti-CarP IgA-positivity and negativity in patients with primary Sjögren's syndrome

	Anti-CarP IgA positive (n = 29)	Anti-CarP IgA negative (n = 42)	P-value
Females, number (%)	27 (93.1)	40 (95.2)	1
Age, median (range) years	67 (48–84)	65 (31–85)	0.774
Disease duration, median (range) years	21 (1–38)	21.5 (1–50)	0.325
Methotrexate, number (%)	2 (6.9)	2 (4.7)	1
Hydroxychloroquine, number (%)	2 (6.9)	12 (28.6)	0.033
Corticosteroids, number (%)	3 (10.3)	13 (30.9)	0.048
Rituximab	0 (0)	1 (2.4)	1
Extraglandular involvement, number (%)	25 (86.2)	39 (92.8)	0.433
Focus score†, median (range)	1 (0–7)*	1 (0–7)**	0.398
Focus score ≥1, number (%)	19 (82.6)*	31 (81.57)**	0.919
GC-like structures present, number (%)	4 (16.0)**	7 (18.4)**	0.804

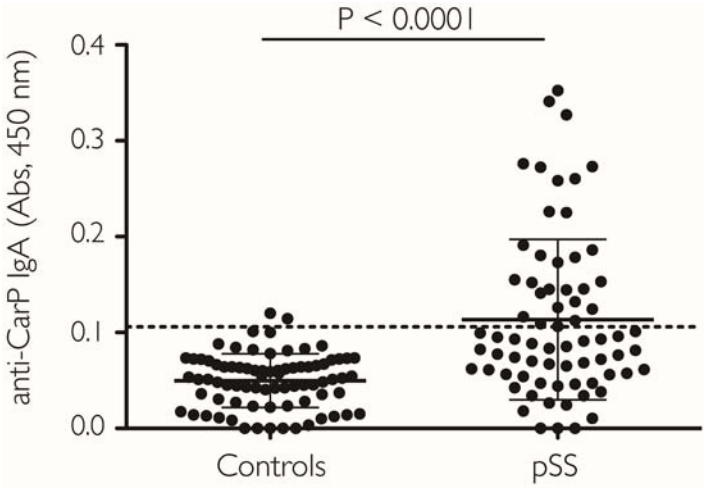
Tear flow Shirmer's I test, median (range) mm/5min	3.5 (0–31.5)	3.8 (0–31.5)‡	0.779
Ocular dryness, <5mm/5min	22 (75.9)	25 (62.5)‡	0.153
Unstimulated salivary flow, median (range) g/15min	0.56 (0–1.8) ‡	0.84 (0– 4.3)‡‡	0.940
Stimulated salivary flow, median (range) g/5min	3.4 (0–11.5)‡	4.16 (0–12.5)‡‡	0.290
ESSDAI score, median (range)	2 (0–17)‡‡‡	2 (0–15) ‡	0.961
ESR, median (range) mm/h	23 (4–58)	18 (4–47)	0.119
ESR, high levels††, number (%)	12 (41.4)	10 (23.8)	0.116
CRP, median (range) mg/L	2 (1 – 11)	2 (1–17)	0.552
CRP high levels (≥5 mg/L) (%)	4 (13.7)	8 (19.0)	0.750
C3, median (range) g/L	1.12 (0.79–10.4)	1.09 (0.7–8.5)	0.547
C3, low levels (<0.83 g/L) number (%)	1 (3.4)	4 (9.5)	0.642
C4, median (range) g/L	0.22 (0.06–0.33)	0.18 (0.06–1-24)	0.426
C4, low levels (<0.13 g/L) number (%)	5 (17.2)	4 (9.5)	0.470
RF, median (range) IU/mL	11 (11–246)	11 (1-1040)	0.008
RF positive	13 (44.8)	3 (7.1)	<0.0001
Anti-CarP IgG	0.95 (0.2–2.8)	0.82 (0.1–3.3)	0.085
Anti-CarP IgG positive	9 (31.0)	6 (14.3)	0.138
SSA antibodies, number (%)	18 (62.1)	29 (69.0)	0.541
SSB antibodies, number (%)	8 (27.6)	15 (35.7)	0.472
ANA, number (%)	20 (69.0)	32 (76.2)	0.499
Anti-CCP, number (%)	1 (3.4)	2 (4.8)	1
IgA, median (range) g/L	2.48 (0.9–16.4)	2.47 (0.3–16.8)	0.555
Elevated levels (>4.1 g/L), number (%)	1 (3.4)	2 (4.8)	1
IgG, median (range), g/L	12.7 (7.0–18.6)	12.4 (6.2 – 25.6)	0.256
Elevated levels (>15.3 g/L), number (%)	4 (13.8)	9 (21.4)	0.540
IgM, median (range) g/L	1.12 (0.6–10.4)	0.94 (0.3–3.4)	0.654
Elevated levels (>2.5 g/L) number (%)	2 (6.9)	1 (2.4)	0.563
β2-microglobulin, median (range) mg/L	2.6 (0–6.8)	2.7 (0–6.1)	0.930
Elevated levels (>2 mg/L), number (%)	27 (93.1)	34 (81.0)	0.183

Continuous data are expressed as the median (range). Categorical data are expressed as the absolute frequency (percentage). *, data not available for 6 patients; **, data not available for 4 patients;; ‡, data not available for 2 patients; ‡‡, data not available for 1 patient; ‡‡‡, data not available for 3 patients; †, Focus score indicates the number of inflammatory foci containing more than 50 mononuclear cells per 4 mm^2 biopsy tissue; ††, age and gender-dependent. GC, germinal center-like structures; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; C, complement component; RF, rheumatoid factor; ANA, antinuclear antibodies; anti-CCP, anti-cyclic citrullinated peptides. The statistical

significance was evaluated by the Mann–Whitney U test for continuous data and the χ^2 or Fisher's exact test as appropriate for categorical data.



Supplementary figure 1. IgG antibodies against carbamylated fetal calf serum (cFSC) in healthy controls and patients with primary Sjögren's syndrome (pSS). The cohorts were recruited from Haukeland University hospital in Bergen, Norway (consequtive, 2004-2009) (A) and Federal University Hospital of Espírito Santo and Federal University Hospital of Minas Gerais, Brazil (B). The data is shown as the mean ± SD. The dashed line represent the cutoff value for patients considered positive for antibodies against carbamylated proteins, which was calculated as the man plus two times the SD of the healthy controls. No outliers were removed. The statistical significance was evaluated using Mann-Whitney U test.



Supplementary figure 2. IgA antibodies against carbamylated fetal calf serum (cFCS) in healthy controls and patients with primary Sjögren's syndrome (pSS). The cohort was recruited from Haukeland University hospital in Bergen, Norway (consecutive, 2011-2015). The data is shown as the mean ± SD. The dashed line represent the cut-off value for patients considered positive for antibodies against carbamylated proteins, which was calculated as the mean plus two times the SD of the healthy controls. The statistical significance was evaluated by the Mann-Whitney U test.

Material and Methods

Study sample. The Bergen Cohort 2 comprises of patients diagnosed with pSS (n = 45) who had been consecutively recruited and evaluated at the Department of

Rheumatology, Haukeland University Hospital in Bergen, Norway between 2004 and 2009. The patients fulfilled the American-European Consensus Group criteria for classification of pSS and had their medical history recorded. Existing control cohort with sex- and approximately age-matched healthy controls (n = 77) recruited from the same geographical area through the Haukeland University Hospital Blood Bank was used. The study was approved by the Regional Committee of Medical and Health Research Ethics (REK 3.2006.3085). All patients provided written informed consent. Cohort 3 comprises of pSS patients (n=92) and controls (n=63) recruited from 2 Brazilian University hospitals (Federal University of Espírito Santo and Federal University of Minas Gerais. Exclusion criteria were any other autoimmune inflammatory disease, chronic infection, cardiovascular disease, and heart, kidney, respiratory and/or liver insufficiency. Project was approved by the Ethic Committee of UFES (407.199/2013).

Detection of anti-CarP IgA antibodies. Using BD Vacutainer SST II Advanced plus blood collection tubes (BD), serum samples were obtained from peripheral blood. To allow clotting, the tubes were incubated at room temperature for 30 min. Following centrifugation at 1800 × g for 10 min, the serum layer was collected, aliquoted and stored at -70°C until analysis. To investigate the levels of anti-CarP antibodies in serum, a modified ELISA protocol developed by Shi et al.[11] was used. Fetal calf serum (FCS) was carbamylated by incubation with a final concentration of 1 M KCNO in distilled water for 48 h at 37°C. Following incubation, the KCNO-containing buffer was exchanged with water using Float-a-lyzer G2 (5kDa) (Spectrum Labs, Breda, the Netherlands). Native and carbamylated FCS (cFCS) was coated on Nunc Maxisorp 96-well plates (Thermo Scientific, Oslo, Norway) overnight at a concentration of 10 µg/mL in 0.1M carbonate-bicarbonate buffer. Following washing (0.05% Tween in PBS) and blocking (1% BSA in PBS), the wells were incubated with serum at a 1:50 dilution in duplicate overnight at 4°C on ice. Bound IgA was detected after incubation with an HRP-conjugated rabbit anti-human IgA antibody (ab8510, Abcam, Cambridge, UK) for 3.5 h at 4°C on ice. After the last wash, HRP enzyme activity was detected using ortho-phenylenediamine (OPD) tablets (Sigma, St.Louis, USA). The optical density (OD) was measured at 450 nm using an E_{max} precision microplate reader (Molecular Devices, Sunnyvale, California, USA) and analysed using Soft Max Pro software. To ensure that all results would be

comparable, all samples were analysed simultaneously. The background signal of FCS was subtracted from the signal of cFCS in order to analyse the specific anti-CarP reactivity. Samples yielding OD values more than two standard deviations (SD) above the mean of the anti-CarP measurement of the healthy controls were considered positive for anti-CarP antibodies.

Laboratory analyses. Laboratory tests were carried out by the Haukeland University Hospital's routine laboratory. Anti-Ro/SSA, anti-La/SSB, ANA and anti-CCP antibodies were reported as either present or absent per individual, whereas all other serum and blood parameters were reported as continuous values. These included erythrocyte sedimentation rate (ESR) and serum levels of C-reactive protein (CRP), complement component 3 and 4 (C3 and C4), non-specific RF, IgA, IgG, IgM, and β2-microblobulin. The salivary gland focus score, information on the formation of germinal center (GC)-like structures in the minor salivary glands, and information regarding extraglandular involvement were obtained from medical records.