

# Differential peripheral B cell phenotype in patients with primary Sjögren's syndrome (pSS) compared to secondary Sjögren's syndrome associated with systemic lupus erythematosus (SS/SLE)

Nicolyn Thompson, David A. Isenberg, Elizabeth C. Jury and Coziana Ciurtin  
Centre for Rheumatology Research, Division of Medicine, University College London,



## INTRODUCTION:

Sjögren's syndrome (SS) is a chronic autoimmune disorder affecting approximately 0.1–0.4% of the general population with a female-to-male ratio of 9:1 usually diagnosed in the fourth and fifth decades of life [1]. Clinically, SS is typified by ocular and oral dryness developed as a consequence of the autoimmune process. It may occur either alone, as primary (p)SS, or secondary to other autoimmune disease, often rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) or systemic sclerosis, secondary (s)SS. There is an increased risk of developing non-Hodgkin's B cell lymphoma [1]. Altered B cell subpopulations have also been correlated with disease activity [2]. B cell activating factor (BAFF) and its receptor (BAFF-R) play an important role in activating a range of cell types. It is likely that their activation could be affected by sphingolipid/cholesterol enriched membrane microdomains lipid rafts [3–4]. T cells were initially identified as the predominant cells within the salivary gland infiltrates and have been shown to be involved in the generation and perpetuation of inflammatory infiltrates in salivary glands in SS [5–6].

### Aims

- 1.) Identify the peripheral B and T cell abnormalities in patients with pSS compared to SLE and SS/SLE patients.
- 2.) Correlate immune phenotype with clinical features and serological abnormalities.
- 3.) Investigate lipid rafts expression in different B cell populations and molecular mechanisms underlying B cell hyperactivity in patients with SS.

## METHODS:

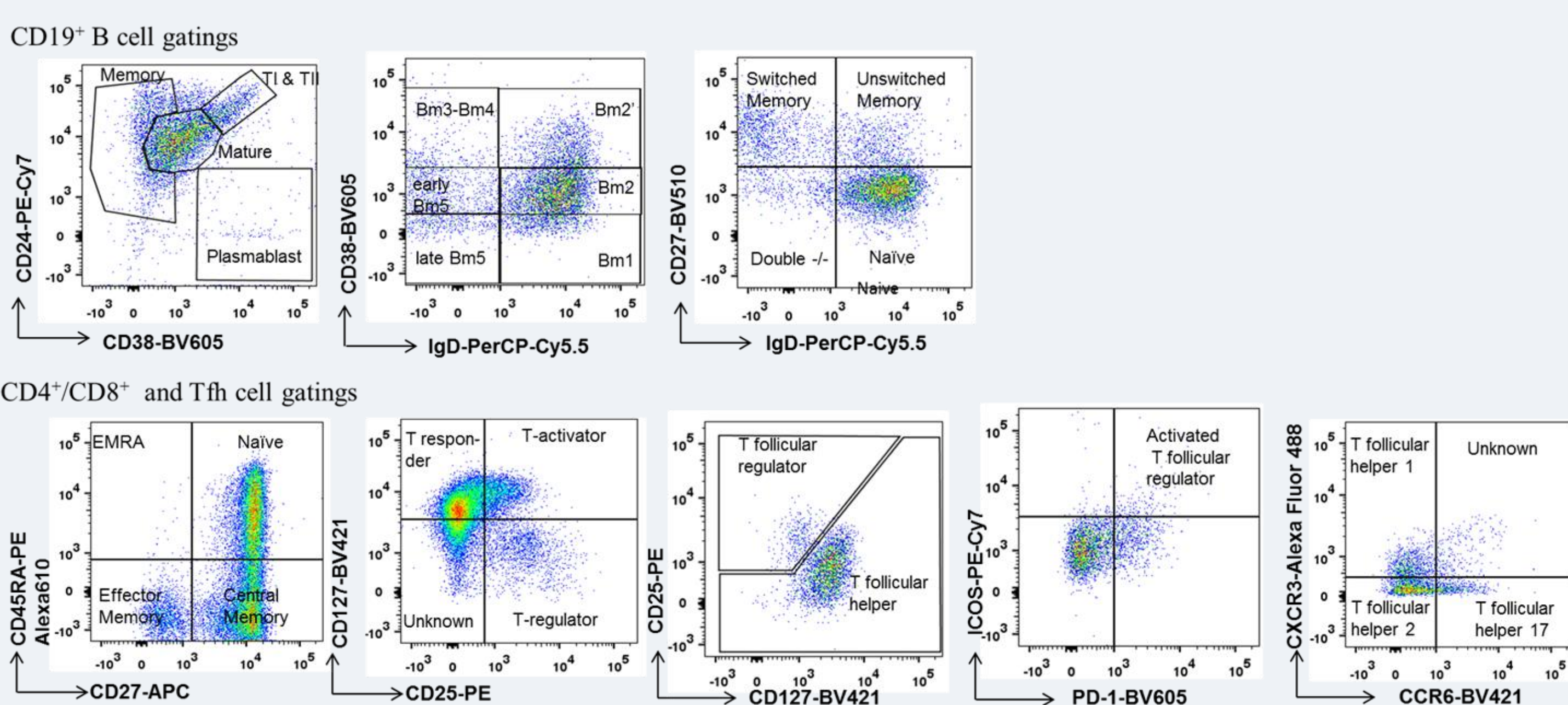
**Table 1: Study subjects demographic detail and clinical characteristics.**

|   | pSS<br>(n=23)     | SLE<br>(n=17) | SS/SLE<br>(n=8)   | HC<br>(n=16) |
|---|-------------------|---------------|-------------------|--------------|
| Sex (female/male)                       | 23/0              | 16/1          | 8/0               | 14/2         |
| Age (mean/range)                        | 63 (28 - 77)      | 53 (28 - 73)  | 52 (25 - 90)      | 34 (20 - 62) |
| RF (%)                                  | 35                | nd            | 66                | N/A          |
| ANA (%)                                 | 81                | 29            | 71                | N/A          |
| Ro (%)                                  | 75                | 18            | 66                | N/A          |
| ENA (%)                                 | 77                | 18            | 41                | N/A          |
| La (%)                                  | 55                | 12            | 29                | N/A          |
| IgG (>20 g/L%)                          | 17                | nd            | 43                | N/A          |
| CRP (> 5 mg/L%)                         | 17                | 17            | 29                | N/A          |
| ESSDAI                                  | 2.7 (0 - 18)      |               | 1.75 (0 - 6)      |              |
| BILAG (A=12, B=5, C=1)                  |                   | 4.8 (0 - 19)  | 0                 |              |
| Biopsy Confirmed SS (%)                 | 94                |               | 38                |              |
| Drugs (% of patients)                   |                   |               |                   |              |
| Prednisolone (<10 mg daily)             | 22                | 65            | 25                |              |
| Rituximab                               | 0.04 (RA overlap) |               | 12.5 (>12 months) |              |
| Immunosuppressive therapy (AZT/MMF/MTX) | 13                | 59            | 47                |              |
| Hydroxychloroquine                      | 35                | 59            | 38                |              |
| Statins (Simvastatin/Pravastatin)       | 17                | 6             | 25                |              |

**Table 2: B cell, T cell, lipid raft, BAFF-R and T follicular cell subpopulations and expression markers.**

| CD19 <sup>+</sup> B cell subpopulations | Surface marker                         | CD4 <sup>+</sup> or CD8 <sup>+</sup> T cell subpopulations | Surface marker                        | CXCR5 <sup>+</sup> T follicular cell subpopulations | Surface marker   |
|---|--|--|---------------------------------------|---|--|
| Total B cells                           | CD19 <sup>+</sup>                      | Total CD4 <sup>+</sup> or CD8 <sup>+</sup>                 | CD4 <sup>+</sup> or CD8 <sup>+</sup>  | Total Tfh   | CD3 <sup>+</sup> /CD4 <sup>+</sup> /CXCR5 <sup>+</sup>                         |
| Transitional                            | CD24 <sup>hi</sup> CD38 <sup>+</sup>   | T responder  | CD25 <sup>+</sup> CD127 <sup>+</sup>  | T follicular helper                                 | CD127 <sup>+</sup> CD25 <sup>+</sup>   |
| Mature                                  | CD24 <sup>lo</sup> CD38 <sup>int</sup> | T activator  | CD25 <sup>+</sup> CD127 <sup>+</sup>  | T follicular regulatory                             | CD127 <sup>+</sup> CD25 <sup>+</sup>   |
| Memory                                  | CD24 <sup>hi</sup> CD38 <sup>+</sup>   | T regulator  | CD25 <sup>+</sup> CD127 <sup>-</sup>  | Total activated Tregs                               | CXCR5 <sup>+</sup> Treg+ ICOS <sup>+</sup> PD-1 <sup>+</sup> ICOS <sup>+</sup> |
| Plasmablast                             | CD24 <sup>+</sup> CD38 <sup>+</sup>    | Unknown  | CD25 <sup>+</sup> CD127 <sup>-</sup>  | Total Activated Tfh                                 | CXCR5 <sup>+</sup> Tfh+ ICOS <sup>+</sup> PD-1 <sup>+</sup>                    |
| Bm1 - Naïve                             | IgD <sup>+</sup> CD38 <sup>-</sup>     | EMRA   | CD27 <sup>+</sup> CD45RA <sup>+</sup> | Tfh1  | CCR6 <sup>+</sup> CXCR3 <sup>-</sup>   |
| Bm2 - Naïve                             | IgD <sup>+</sup> CD38 <sup>+</sup>     | Naïve  | CD27 <sup>+</sup> CD45RA <sup>+</sup> | Tfh2  | CCR6 <sup>+</sup> CXCR3 <sup>+</sup>   |
| Bm2 - Bm2'                              |  |  |                                       |   |  |
| Transitional                            | IgD <sup>+</sup> CD38 <sup>++</sup>    | Central memory   | CD27 <sup>+</sup> CD45RA <sup>+</sup> | Tfh17   | CCR6 <sup>+</sup> CXCR3 <sup>-</sup>   |
| Bm3 - Bm4'                              |  |  |                                       |   |  |
| Plasmablast                             | IgD <sup>+</sup> CD38 <sup>++</sup>    | Effector memory  | CD27 <sup>+</sup> CD45RA <sup>-</sup> | Activated Tfh 1                                     | CCR6 <sup>+</sup> CXCR3 <sup>+</sup> ICOS <sup>+</sup> PD-1 <sup>+</sup>       |
| early Bm5                               | IgD <sup>+</sup> CD38 <sup>+</sup>     |  |                                       | Activated Tfh2                                      | CCR6 <sup>+</sup> CXCR3 <sup>-</sup> ICOS <sup>+</sup> PD-1 <sup>+</sup>       |
| late Bm5                                | IgD <sup>+</sup> CD38 <sup>-</sup>     |  |                                       | Tfh17   | CCR6 <sup>+</sup> CXCR3 <sup>-</sup> ICOS <sup>+</sup> PD-1 <sup>+</sup>       |
| Switched memory                         | IgD <sup>+</sup> CD27 <sup>+</sup>     |  |                                       |   |  |
| Unswitched memory                       | IgD <sup>+</sup> CD27 <sup>-</sup>     |  |                                       |   |  |
| Naïve                                   | IgD <sup>+</sup> CD27 <sup>-</sup>     |  |                                       |   |  |
| Double negative                         | IgD <sup>-</sup> CD27 <sup>-</sup>     |  |                                       |   |  |

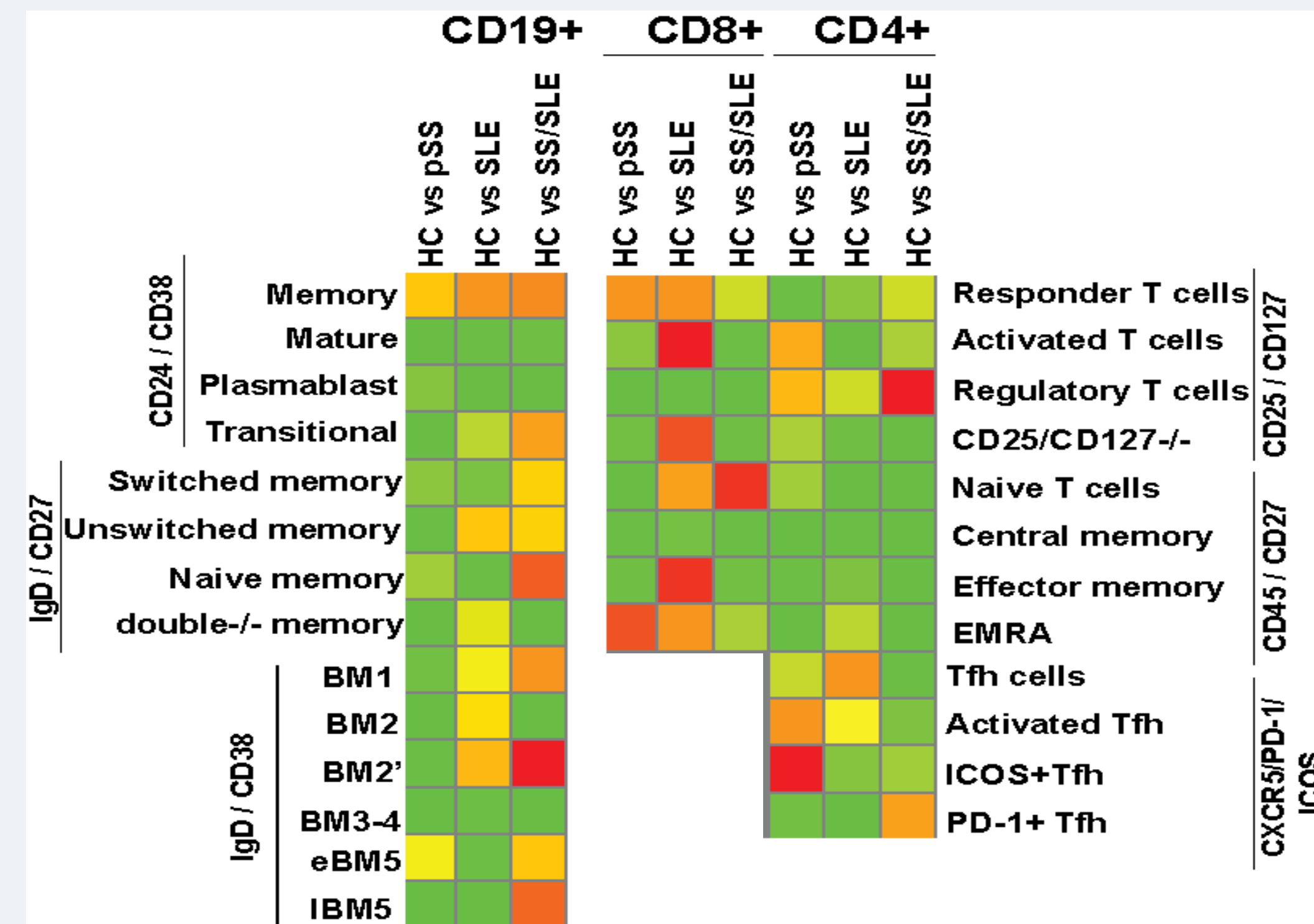
## CB19<sup>+</sup> B cells, CD4<sup>+</sup>/CD8<sup>+</sup> T cells and T follicular cells subpopulation representative plots from peripheral blood mononuclear cells (PBMCs).



Representative flow cytometry dotplots showing CB19<sup>+</sup> B cells, CD4<sup>+</sup>/CD8<sup>+</sup> T cells and T follicular cell subpopulations.

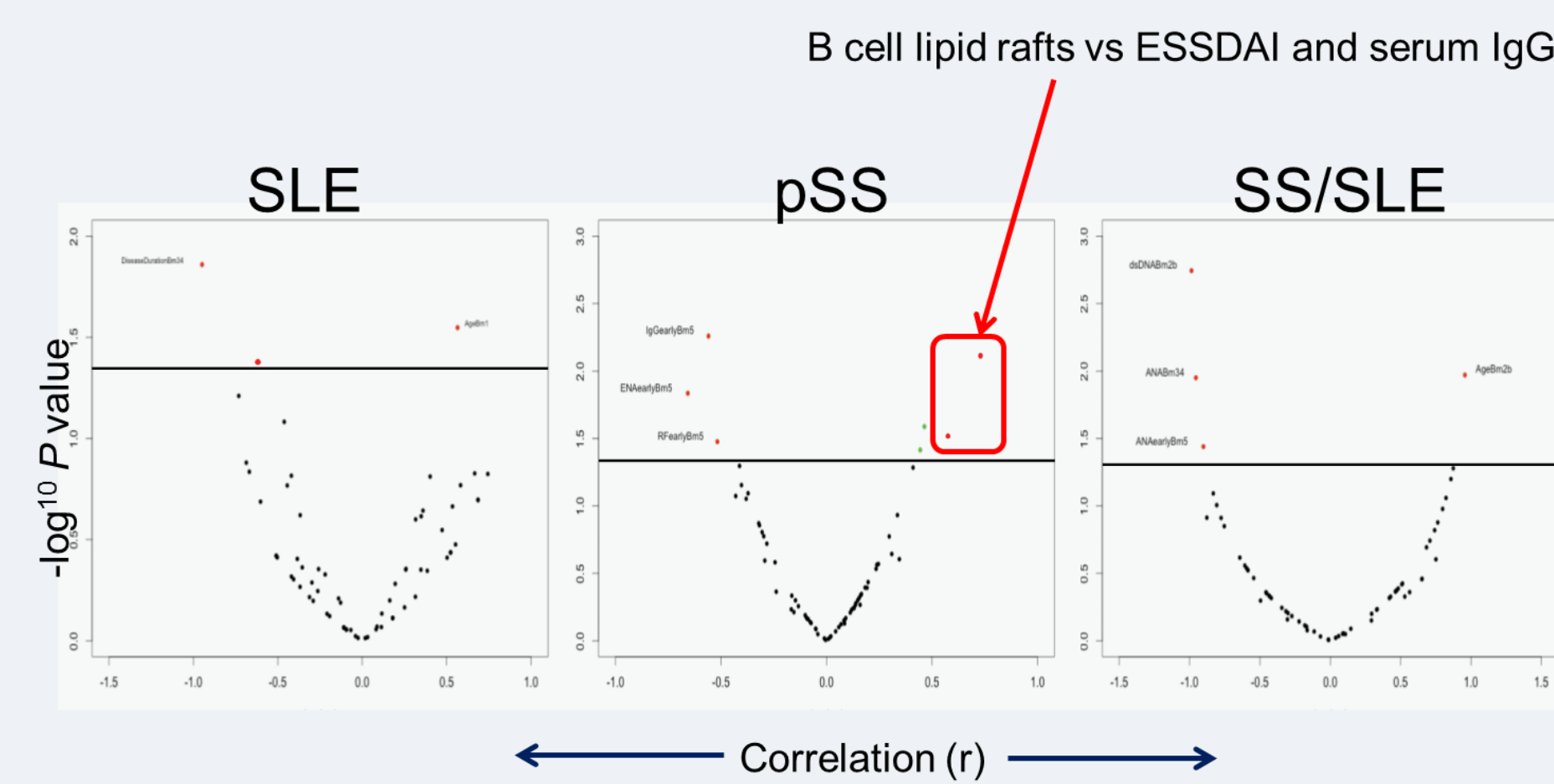
## RESULTS:

### Distinct distribution of B cell, T cell and T follicular cell subpopulations in pSS, SLE and SS/SLE compared to healthy donors.



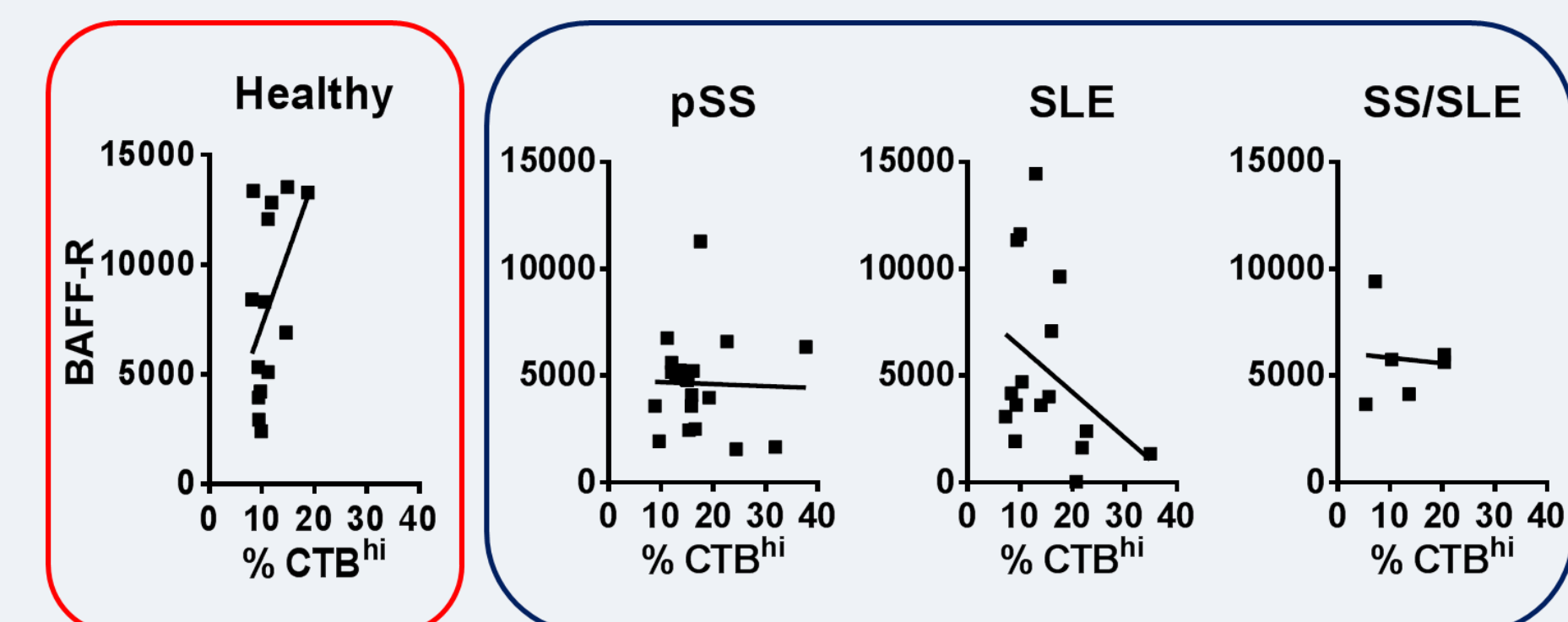
Heatmaps comparing expression of T and B cell populations in healthy donors compared to patients with SS, SLE and SS/SLE. Lipid rafts were also significantly elevated in B cells from patients with SS and SLE but not SLE/SS.

### Correlation between clinical parameters and Bm1-Bm5 subpopulations in patients with pSS, SLE and SS/SLE.

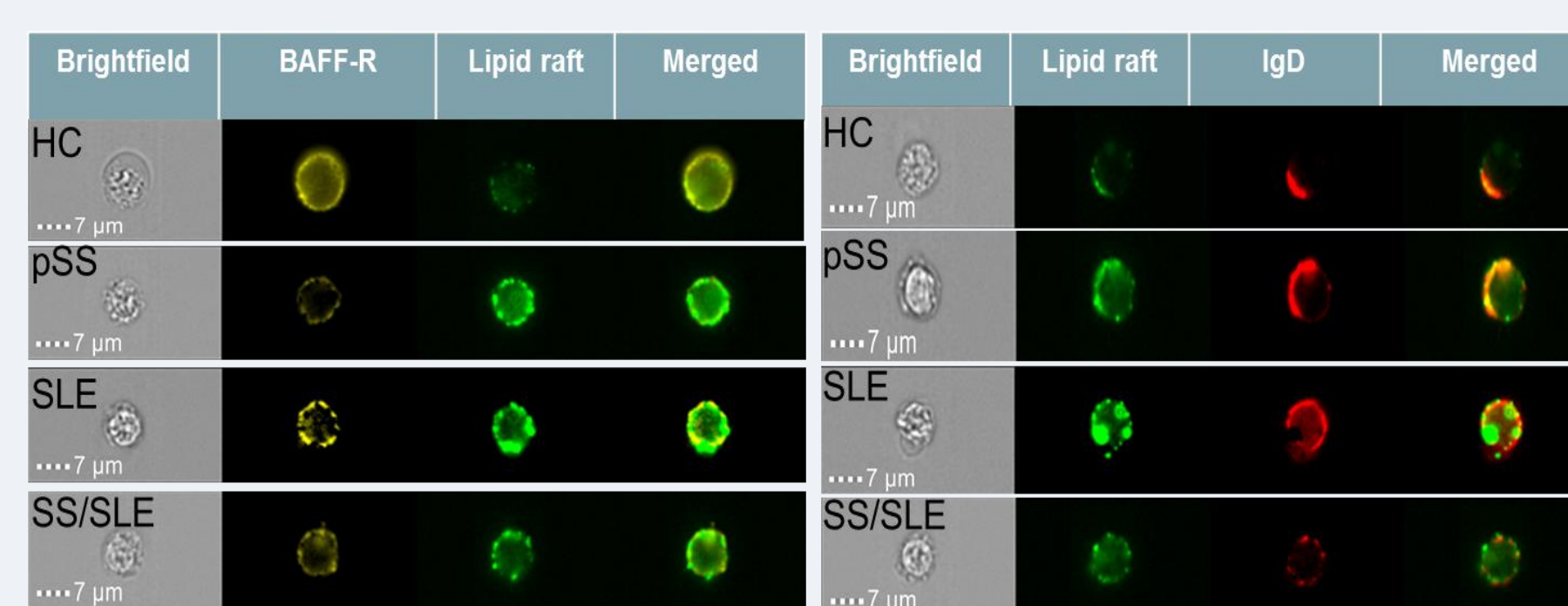


A significant positive correlation was seen between B cell lipid raft expression and disease activity in patients with SS.

### Significant positive correlation between B cell BAFF-R and lipid raft expression in healthy donors but not patients with SS, SLE and SS/SLE.



### Altered association of BAFF-R and IgD with lipid rafts in patients with pSS, SLE and SS/SLE compared to healthy donors.



Representative ImageStream images

## DISCUSSION/CONCLUSIONS:

- SS/SLE have the most striking B cell phenotype abnormalities than patients with pSS or SLE (increased Bm2 cells and decreased early and late Bm5 cells).
- SS/SLE patients also shared B cell phenotype abnormalities with pSS (decreased early Bm5 cells and SLE (increased Bm2' cells).
- These abnormalities suggest disturbance of B cell trafficking in the patient groups, and a possible bias towards plasma cell differentiation (as they all had low memory B cells)
- Significant correlations between lipid rafts expression and disease activity in patients with pSS, which suggest abnormal B cell signalling (never explored before).
- Altered colocalisation of BAFF-R and lipid rafts in patients with pSS, SLE and SS/SLE compared to healthy donors.
- This is the first comprehensive immunophenotype analysis performed patients with pSS and SS/SLE, which identified that the SS/SLE patient group is immunologically distinct from pSS and SLE patients.
- Lipid raft abnormalities could be relevant for the variability of patients' response to biologic treatments in SLE compared to pSS, as B cell targeted monoclonal antibodies are internalised within the lipid rafts.

### REFERENCES:

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- 2.) Roberts, M. et al. Primary Sjögren's Syndrome Is Characterized by Distinct Phenotypic and Transcriptional Profiles of IgD<sup>+</sup>Unswitched Memory B Cells. *Arthritis & Rheumatism.* 9, 2558-2569 (2014).
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- 6.) Isenberg, A.D. et al. "An immunohistological study of secondary Sjögren's syndrome." *Ann Rheum Dis.* 43, 470-6, (1984).