Online supplement to B Cell Depletion Therapy Normalizes Circulating Follicular Th Cells in Primary Sjögren Syndrome, *The Journal of Rheumatology*, doi:10.3899/jrheum.160313

Supplementary Table 1: Antibodies used for flow cytometry.

Antibody	**Fluorochrome**	**Clone**	**Company**
Surface marker expression analysis			
CD3	APC-eF780	UCHT1	eBioscience
CD4	AF700	OKT4	eBioscience
CD45RA	BV650	HI100	BD Biosciences
FoxP3	PE	236A/E7	eBioscience
CXCR3	BV711	1C6/CXCR3	BD Biosciences
CCR6	APC	11A9	BD Biosciences
CXCR5	PerCP-Cy5.5	RF8B2	BD Biosciences
CCR4	FITC	205410	R&D Systems
PD-1	BV786	EH12.1	BD Biosciences
Fixable viability dye	eF506	-	eBioscience
In vitro cytokine production analysis			
CD3	APC	UCHT1	BD Biosciences
CD8	PerCP	SK1	BD Biosciences
IL-21	PE	eBio3A3-N2	eBioscience
IL-17	AF488	eBio64DEC17	eBioscience
IFN-γ	AF700	B27	BD Biosciences
IL-4	PE-Cy7	MP4-25D2	Biolegend

Supplementary Table 2: Absolute numbers of different effector $CD4^+$ T-cell subsets at baseline, during B-cell depletion and after B-cell repopulation.

	Baseline	**wk 16**	**wk 24**	**p-value***	**wk 48**	**p-value****
Tfh-cells	1.02	0.64	0.84	**0.000**	1.01	0.080
Th17-cells	1.16	1.38	1.36	0.167	1.24	0.920
Th1-cells	3.63	3.44	3.78	0.401	4.55	0.324
Th2-cells	4.44	4.49	5.94	0.253	4.83	0.825
IL-21^+ T-cells	1.95	1.80	1.60	0.292	2.30	0.095
IL-17^+ T-cells	0.42	0.28	0.21	**0.000**	0.52	**0.000**
IFN-γ^+ T-cells	3.90	4.15	3.80	0.327	3.05	0.098
IL-4^+ T-cells	0.40	0.52	0.36	0.769	0.48	0.205

Values are presented as median absolute numbers (x10^4 cells/mL). *p-value from Generalized Estimating Equation model, including values at baseline, week 16 and week 24. **p-value from Wilcoxon matched pairs test, comparing week 24 with week 48.

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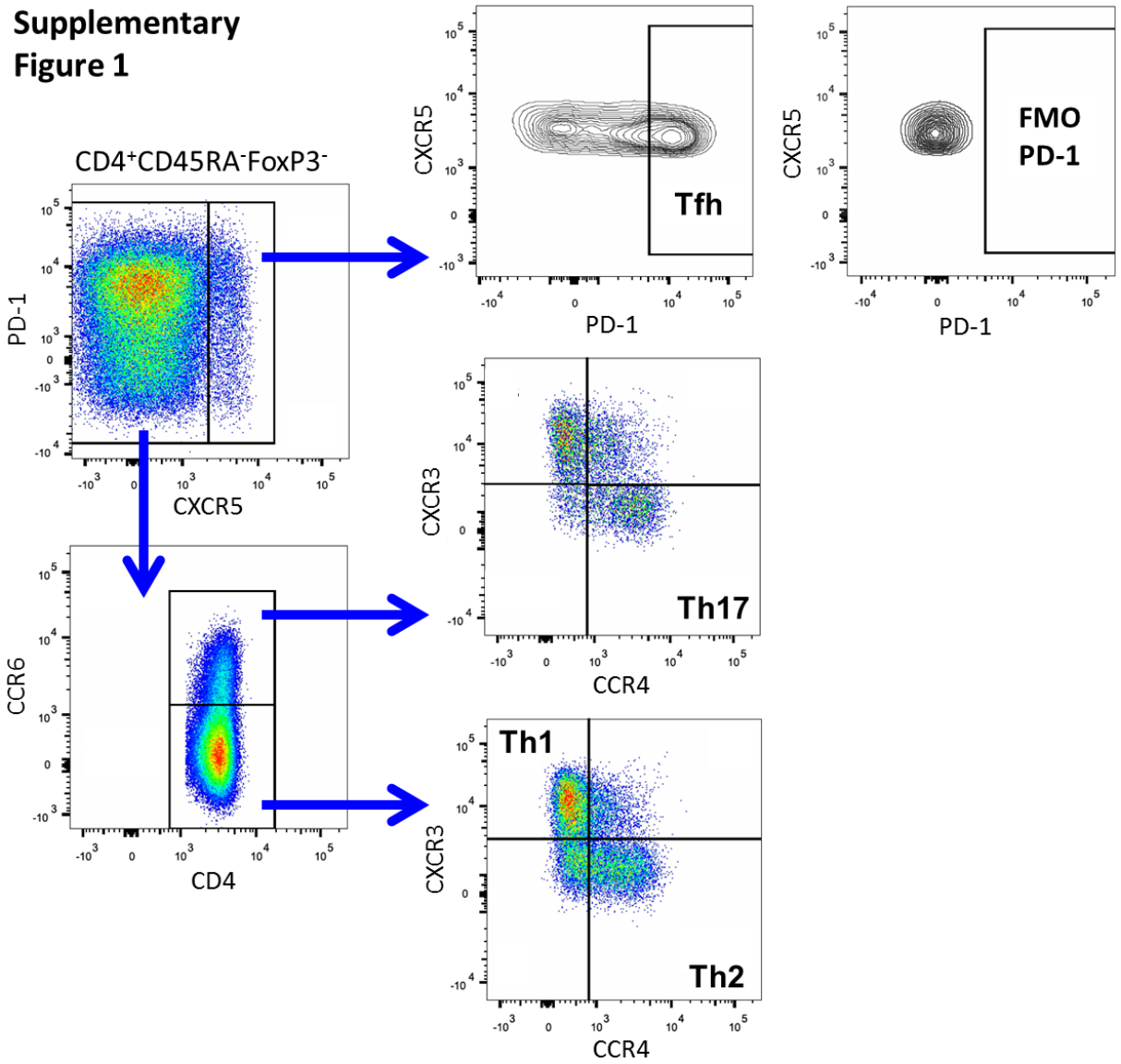

Supplementary Figure 1: Gating strategy to distinguish CD4+ T-cell subsets. First, memory CD4+ T-cells were gated (CD3+CD4+CD45RA-FoxP3-). Second, Th1-, Th2-, Tfh- and Th17-cells were gated based on the expression pattern of surface chemokine receptors. A gating plot for the PD-1 fluorescence minus one (FMO) control is also shown.

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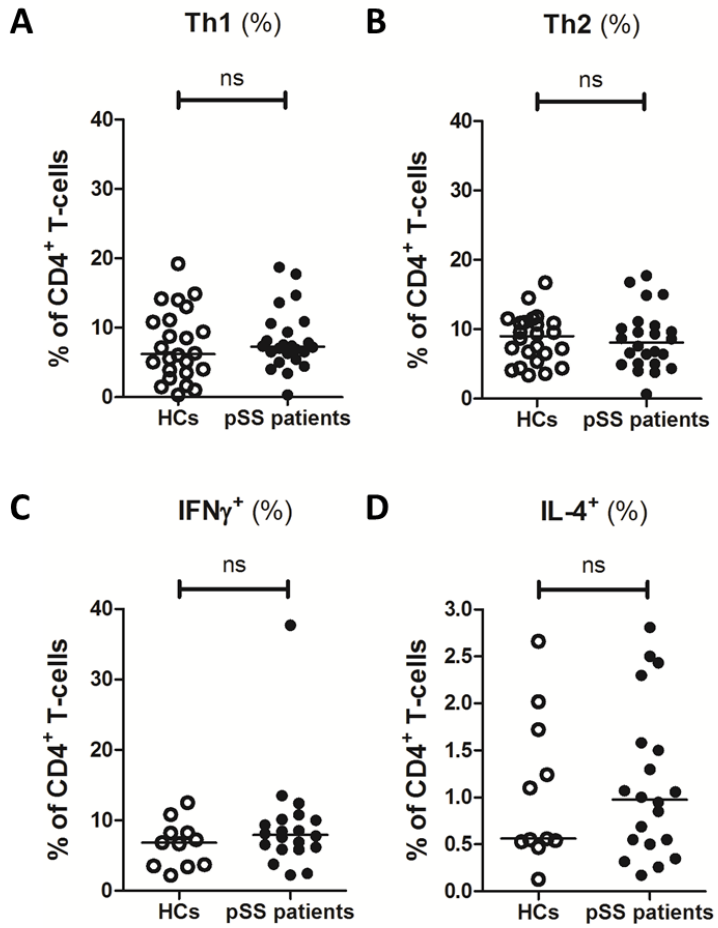
Supplementary Figure 2



Supplementary Figure 2: The proportion of Th1- and Th2-cells in pSS patients at baseline compared with HCs. Frequencies of circulating (A) Th1- (CD45RA-CXCR5-CXCR3+CCR4-CCR6-) and (B) Th2-cells (CD45RA-CXCR5-CXCR3-CCR4+CCR6-) in pSS patients (n=24) and HCs (n=24) are displayed. Additionally, frequencies of (C) IFNγ- and (D) IL-4-producing CD4+ T-cells in pSS patients (n=20) and HCs (n=11) are displayed. Horizontal lines indicate the median. P-value < 0.05 was considered statistically significant. P-values were calculated using the nonparametric Mann-Whitney U-test.

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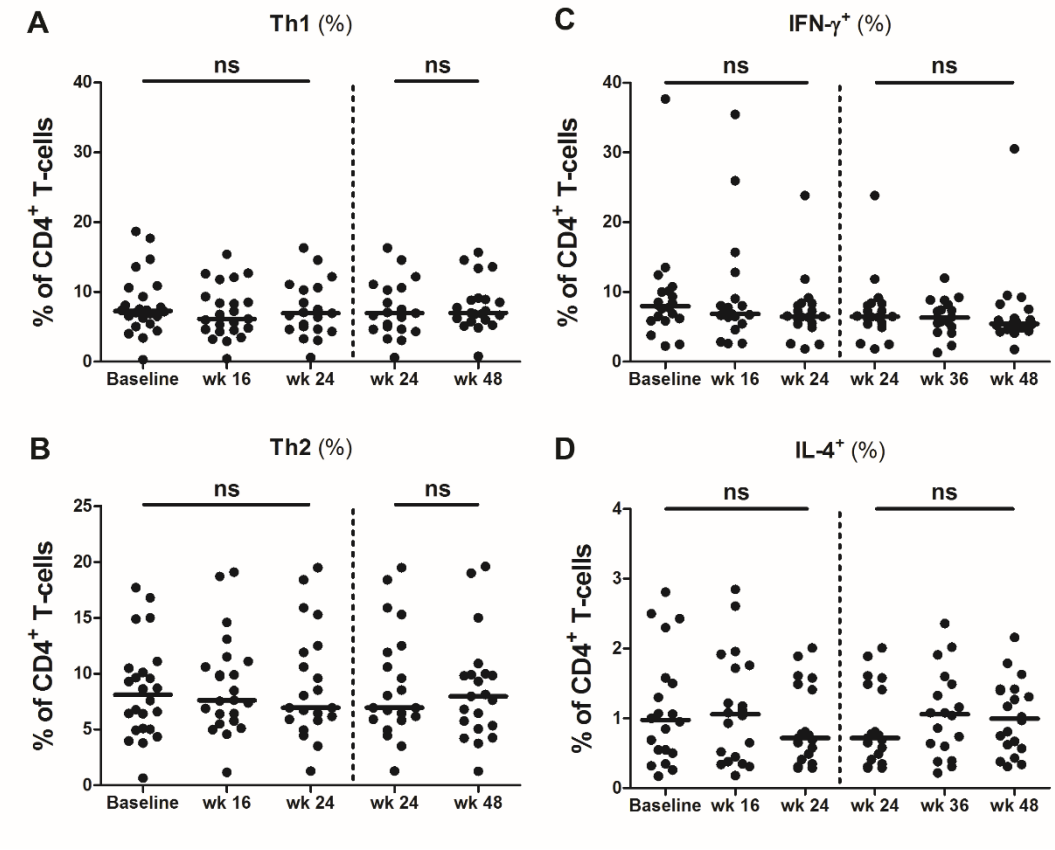
Supplementary Figure 3



Supplementary Figure 3: Frequencies of Th1-cells, Th2-cells, IFN-γ- and IL-4-producing CD4$^+$ T-cells after rituximab. (A) Frequencies of Th1-cells (CD45RA$^-$CXCR5$^-$CXCR3$^+$CCR4$^-$CCR6$^-$) and (B) Th2-cells (CD45RA$^-$CXCR5$^-$CXCR3$^-$CCR4$^+$CCR6$^-$) are displayed (n=24). (C) Frequencies of IFN-γ- and (D) IL-4-producing CD4$^+$ T cells are displayed (n=20). Values within subjects over time were analyzed with generalized estimating equations during B-cell depletion (week 0-24) and B-cell repopulation (week 24-48). Horizontal lines indicate the median. P-value <0.05 was considered statistically significant.

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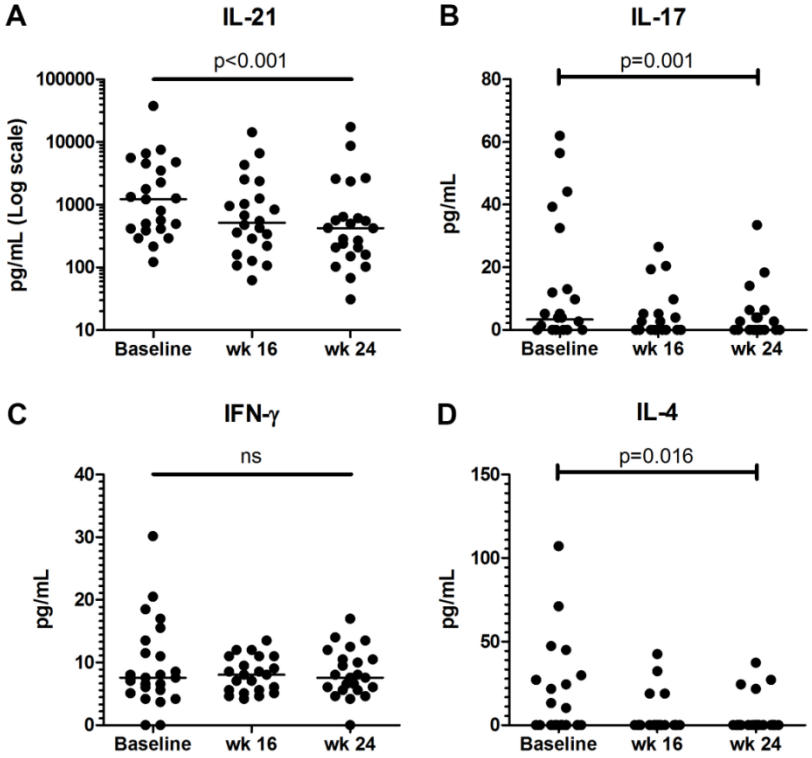
Supplementary Figure 4



Supplementary Figure 4: Serum cytokines levels change after rituximab. Serum levels of IL-21, IL-17, IFN-γ and IL-4 were measured by a multiplex bead immunoassay. Values from 24 pSS patients during B-cell depletion (week 0-24) are displayed. Changes in IL-21 and IFN-γ within patients over time were analyzed with generalized estimating equations (GEE). IL-17 and IL-4 could not be modelled using GEE and therefore values at baseline and week 24 were compared using Wilcoxon matched pairs tests. Lines indicate the median. P-value <0.05 was considered statistically significant.

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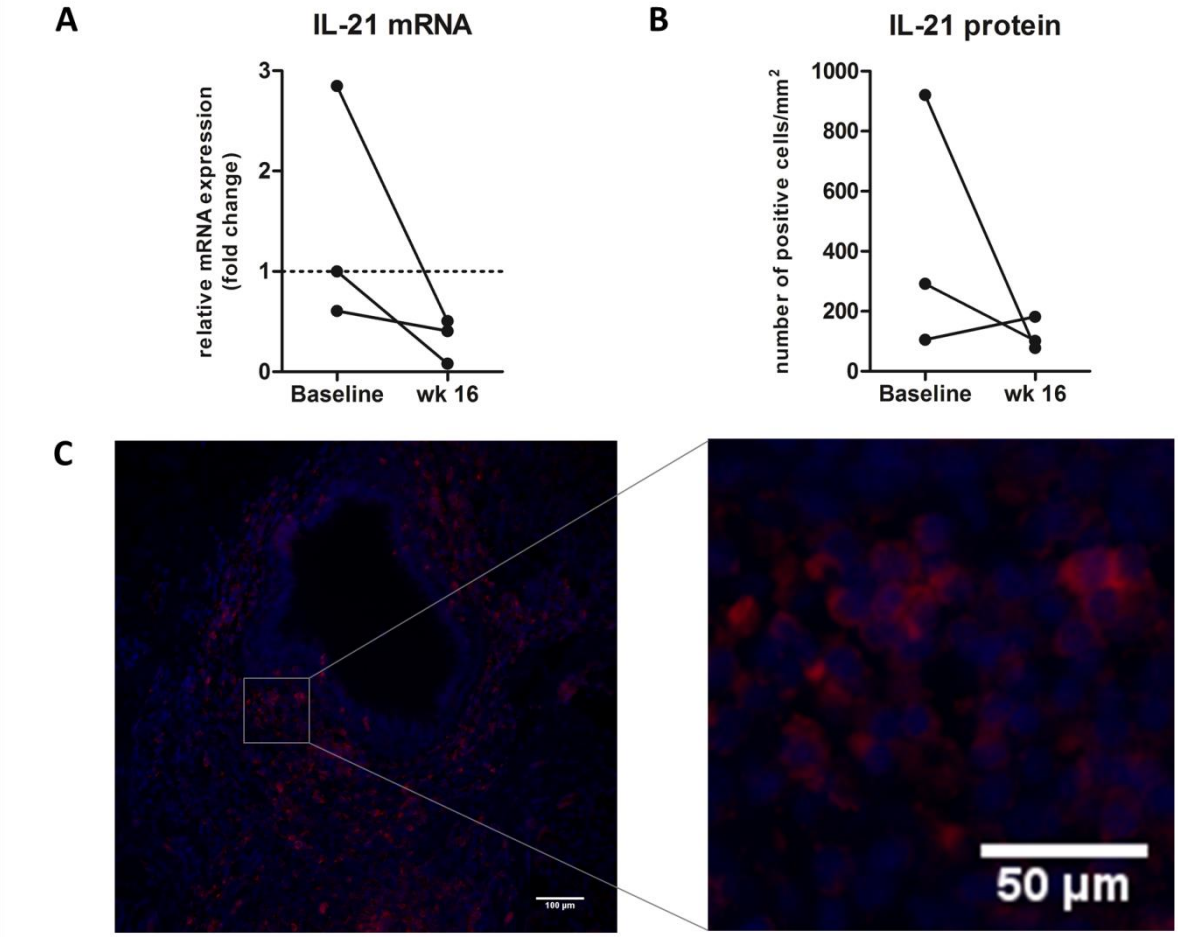
Supplementary Figure 5



Supplementary Figure 5: IL-21 expression in parotid gland tissue before and after rituximab. (A) IL-21 mRNA expression levels plotted as the fold change relative to median baseline expression of IL-21 mRNA and (B) IL-21 protein expression quantified by the number of IL-21-positive cells per mm^2 of parotid gland tissue before and after B-cell depletion therapy. (C) Representative immunofluorescence image from a patient with high numbers of IL-21-positive cells in the parotid gland at baseline. Red staining represents IL-21. DAPI (blue) staining is used to label nuclei of cells.