Increased T helper 17.1 cells in sarcoidosis mediastinal lymph nodes

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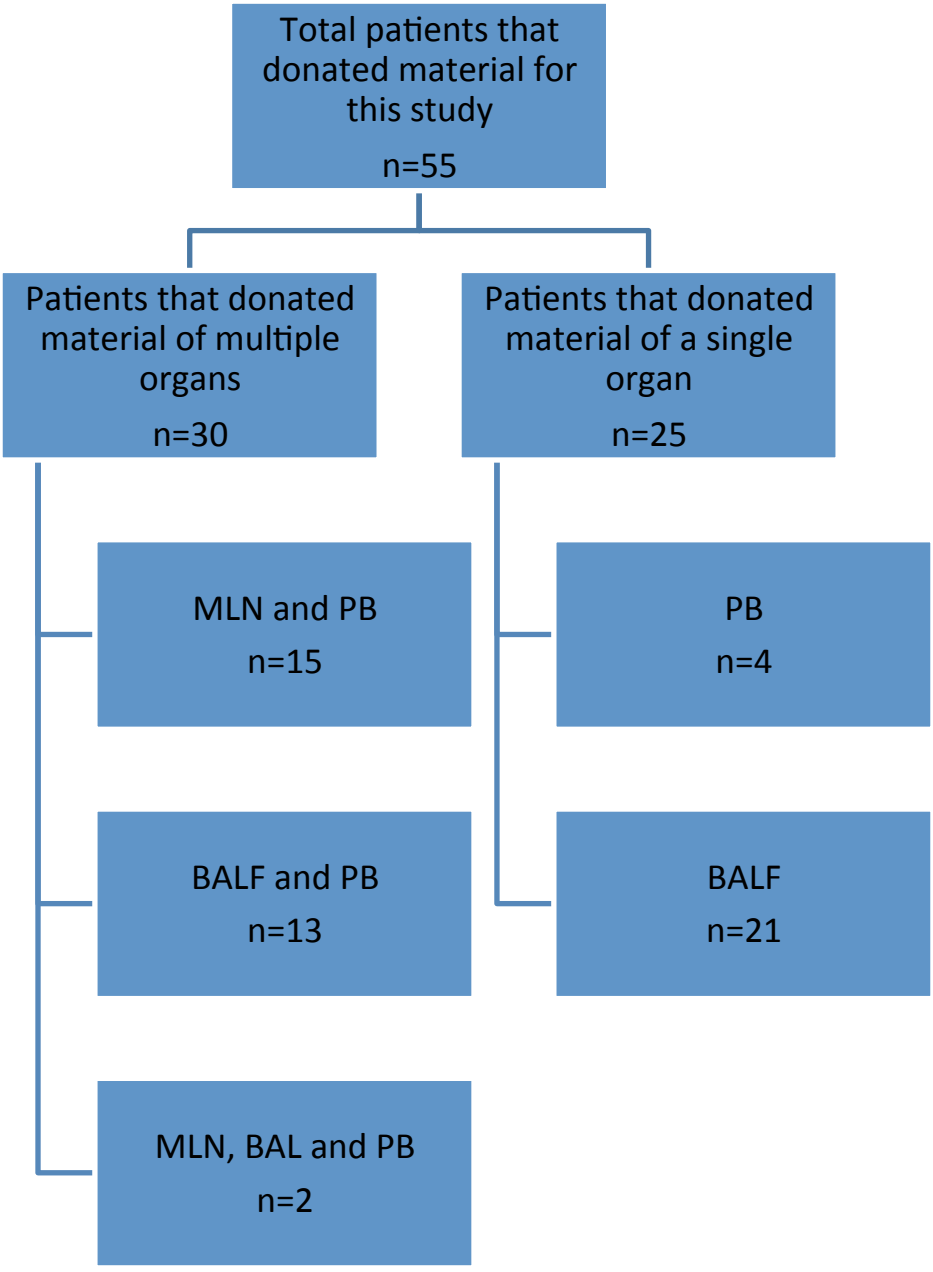
Online Data Supplement

Supplementary Figure E1.

Flowchart describing the donated material (BALF, MLN, and/or PB) by the 55 sarcoidosis patients in this study.

Supplementary Figure E1



Supplementary Table E1. Overview antibodies

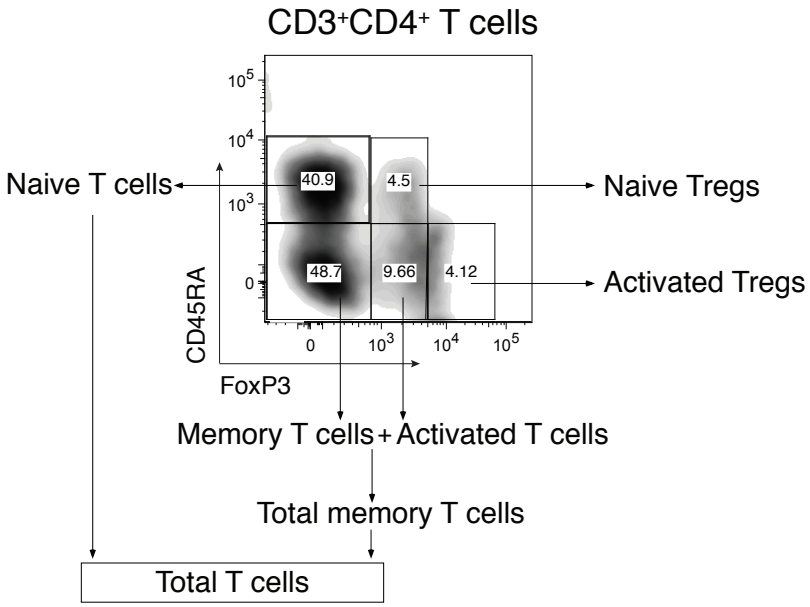
Company	Antigen	Fluor chrome	Clone
eBiosciences	CD3	APC-eFluor780	SK7
	CD4	AF700	OKT4
	FoxP3	PE	236A/E7
	CTLA4	PerCp-eFluor710	14D3
BD biosciences	CCR6	APC	11A9
	CXCR3	BV711	1C6/CXCR3
	Ki67	Pe-Cy7	B56
	CD28	BV605	CD28.2
	PD-1	BV786	EH12.1
	PD-1	BV711	EH12.1
	CD25	PE-Cy7	M-A251
	CD25	BV650	M-A251
	CD278 (ICOS)	BV650	DX29
R&D	CCR4	FITC	205410
Biolegend	CXCR3	BV421	G025H7
Invitrogen	CD45RA	PE-Texas Red	MEM-56

Supplementary Figure E2

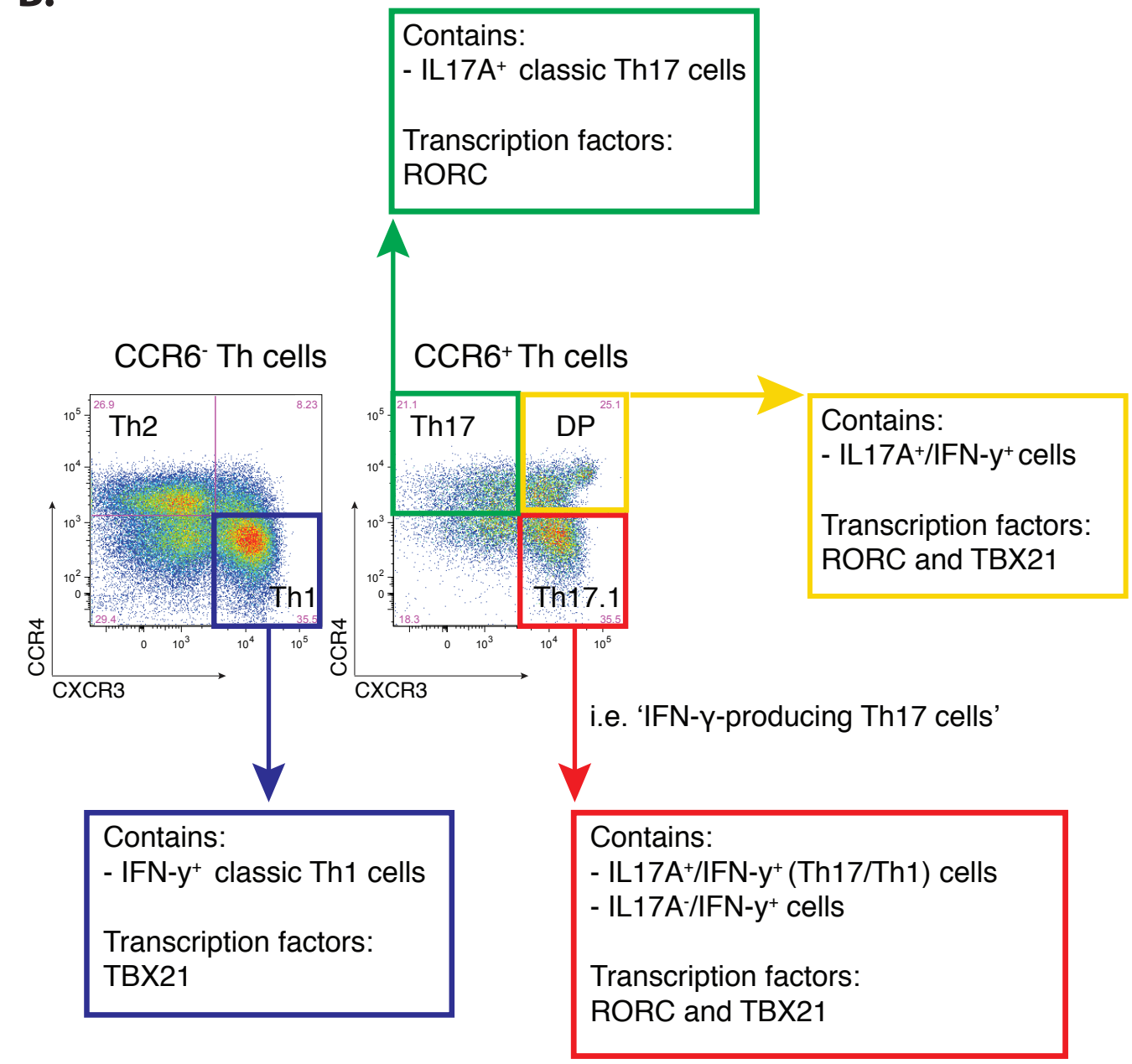
A. Five $CD3^+CD4^+$ T cell populations were characterized according to CD45RA and intracellular FoxP3 expression[1], i.e. $CD45RA^+FoxP3^-$ naïve T cells, $CD45RA^-FoxP3^-$ memory T cells, $CD45RA^-FoxP3^{int}$ activated (non-suppressive/-regulatory) T cells, $CD45RA^+FoxP3^{int}$ naïve regulatory T cells and $CD45RA^-FoxP3^{high}$ activated Tregs. The total memory T cell pool consists of memory T cells plus activated T cells. The total (non-suppressive/-regulatory) T cell pool consists of naïve T cells plus total memory T cells. B. Th cell subsets can be classified according to chemokine-receptor expression[2-6]. $CCR6^-$ memory T cells subsets, including $CCR4^-CXCR3^+$ Th1 cells and $CCR4^+CXCR3^-$ Th2 cells; and $CCR6^+$ T cell subsets, which include $CCR4^+CXCR3^-$ Th17 cells, $CCR4^+CXCR3^+$ DP Th cells and $CCR4^-CXCR3^+$ Th17.1 cells. Th17.1 cells (i.e. IFN-γ-producing Th17 cells) contain both $IL\text{-}17A^+/IFN\text{-}\gamma^+$ (Th17/Th1) cells and IFN-γ-single-positive cells. $CCR6^+CCR4^+CXCR3^+$ DP cells are thought to reflect an intermediate Th17 and Th17.1 cell population[4 5 7], expressing both RORγt/IL-17A and T-bet/IFN-γ in patients with rheumatoid arthritis (RA)[7]. *Abbreviations:* CCR: C-C chemokine receptor, CXCR: CXC chemokine receptor, DP: double-positive, Th: T helper, Treg: regulatory T cells.

Supplementary Figure E2.

A.



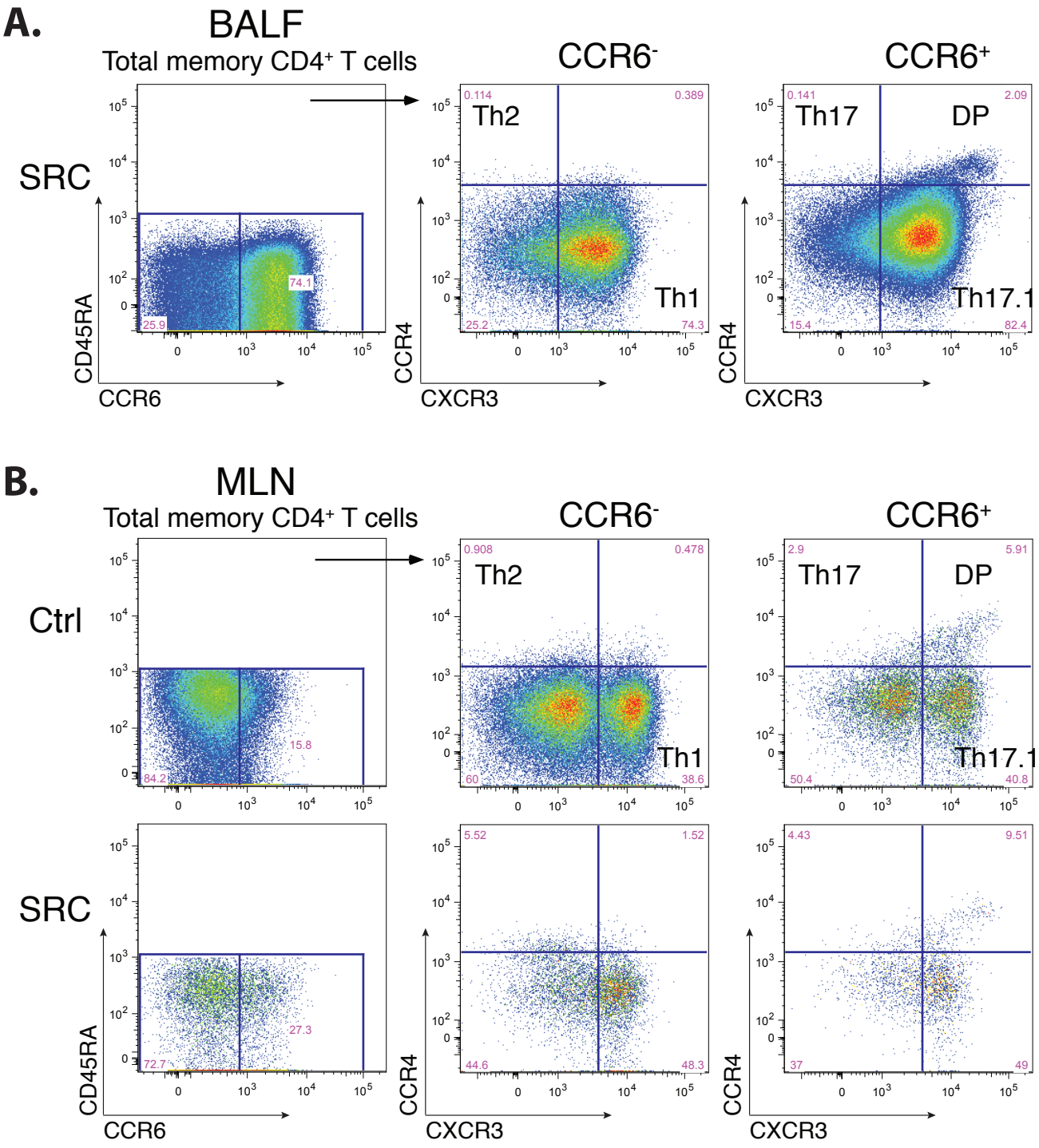
B.



Supplementary Figure E3

Representative flow cytometry analysis of BALF (A) and MLN (B) from one sarcoidosis patient and one control. *Abbreviations:* CCR: C-C chemokine receptor, BALF: broncho-alveolar lavage fluid, MLN: mediastinal lymph nodes, SRC: sarcoidosis, Ctrl: control.

Supplementary Figure E3.

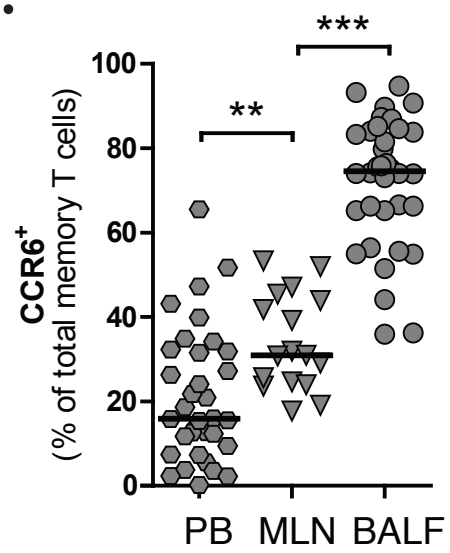


Supplementary Figure E4

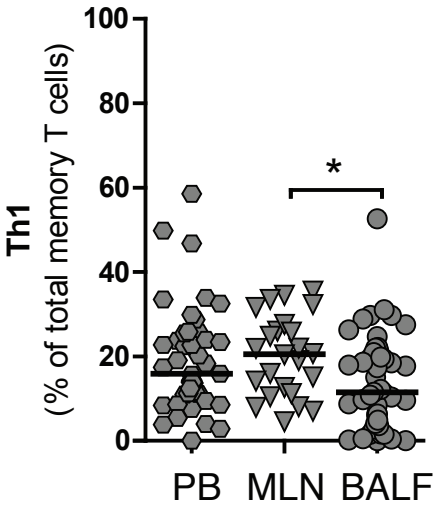
Proportions of total $CCR6^+$ and Th1 cells were determined in total memory $CD4^+$ T cells from sarcoidosis PB, MLN and BALF. A-B. Proportions of total $CCR6^+$ and Th1 cells of total memory $CD4^+$ T cells. *Statistics:* Horizontal lines indicate median values and significance was determined using a Mann-Whitney U test, * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Data are from 34 (PB), 17 (MLN) and 36 (BALF) patients. *Abbreviations:* CCR: C-C chemokine receptor, PB: peripheral blood, MLN: mediastinal lymph nodes, BALF: broncho-alveolar lavage fluid, DP: double-positive, Th: T helper.

Supplementary Figure E4.

A.



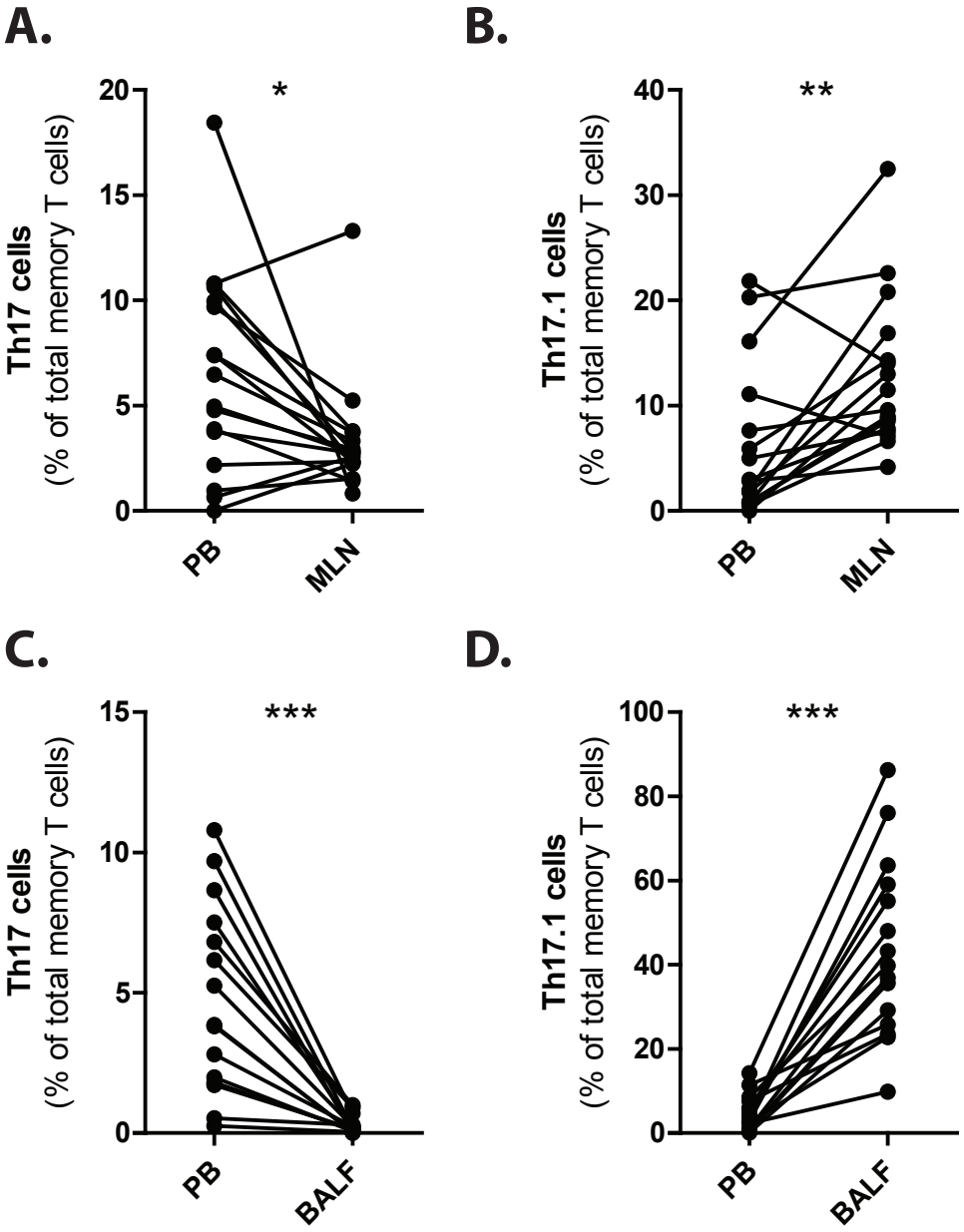
B.



Supplementary Figure E5

Proportions Th17 and Th17.1 cells were determined in total memory $CD4^+$ T cells from sarcoidosis patient that donated either both PB and MLN (n=17) or PB and BALF (n=15). A-B**.** Proportions of Th17 and Th17.1 cells of total memory $CD4^+$ T cells in PB and MLN of 17 sarcoidosis patients. C-D**.** Proportions of Th17 and Th17.1 cells of total memory $CD4^+$ T cells in PB and BALF of 15 sarcoidosis patients. *Statistics:* Significance between median values of paired samples was determined using a Wilcoxon signed rank test, * $p < 0.05$ ** $p<0.01$ *** $p < 0.001$. *Abbreviations:* PB: peripheral blood, MLN: mediastinal lymph nodes, BALF: broncho-alveolar lavage fluid, Th: T helper.

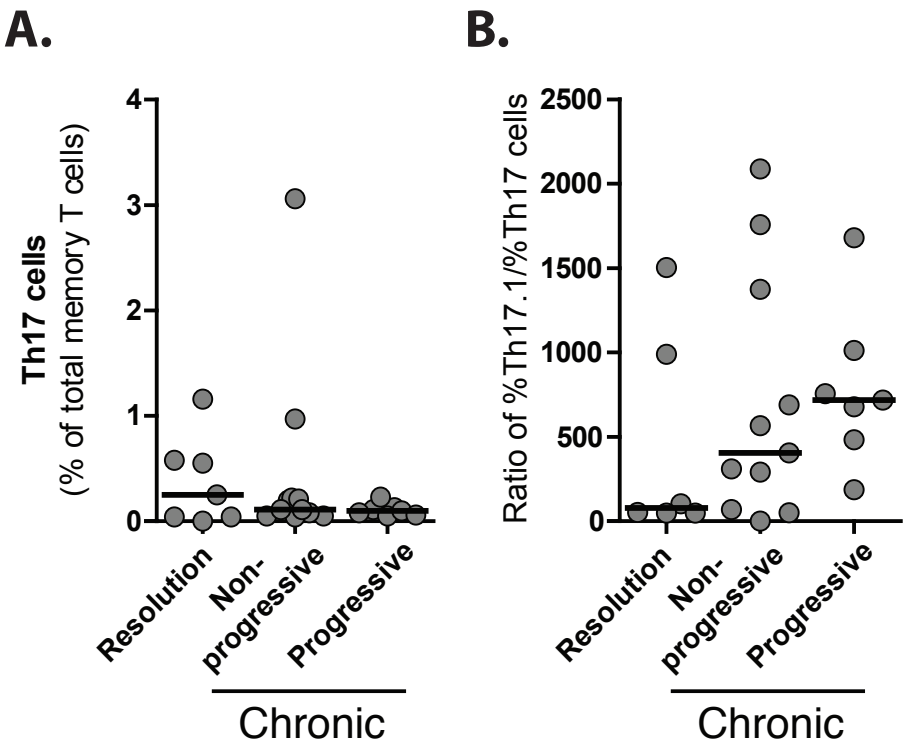
Supplementary Figure E5.



Supplementary Figure E6

Disease course of a subgroup (n=25) of patients was determined 2 years after study inclusion. Resolution of disease (n=7) was defined by the absence of abnormalities on the chest X-ray and clinical symptoms. Patients with residual abnormalities on chest X-ray, but without need for treatment were designated as non-progressive chronic (n=11); and patients with need for treatment as progressive chronic (n=7)[8]. A. Proportions Th17 cells of total memory $CD4^+$ T cells at time of diagnosis. B. Ratio of proportions Th17.1 versus Th17 cells. *Statistics:* Data are presented as boxplots and whiskers that show the 10-90 percentile of the data. Significance was determined using a Mann-Whitney U test. *Abbreviations:* BALF: broncho-alveolar lavage fluid, Th: T helper, SRC: sarcoidosis.

Supplementary Figure E6.



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