Age Associated B Cells Appear in Patients with Granulomatous Lung Diseases

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ONLINE DATA SUPPLEMENT

Online Data Supplement

Supplementary Figure Legends

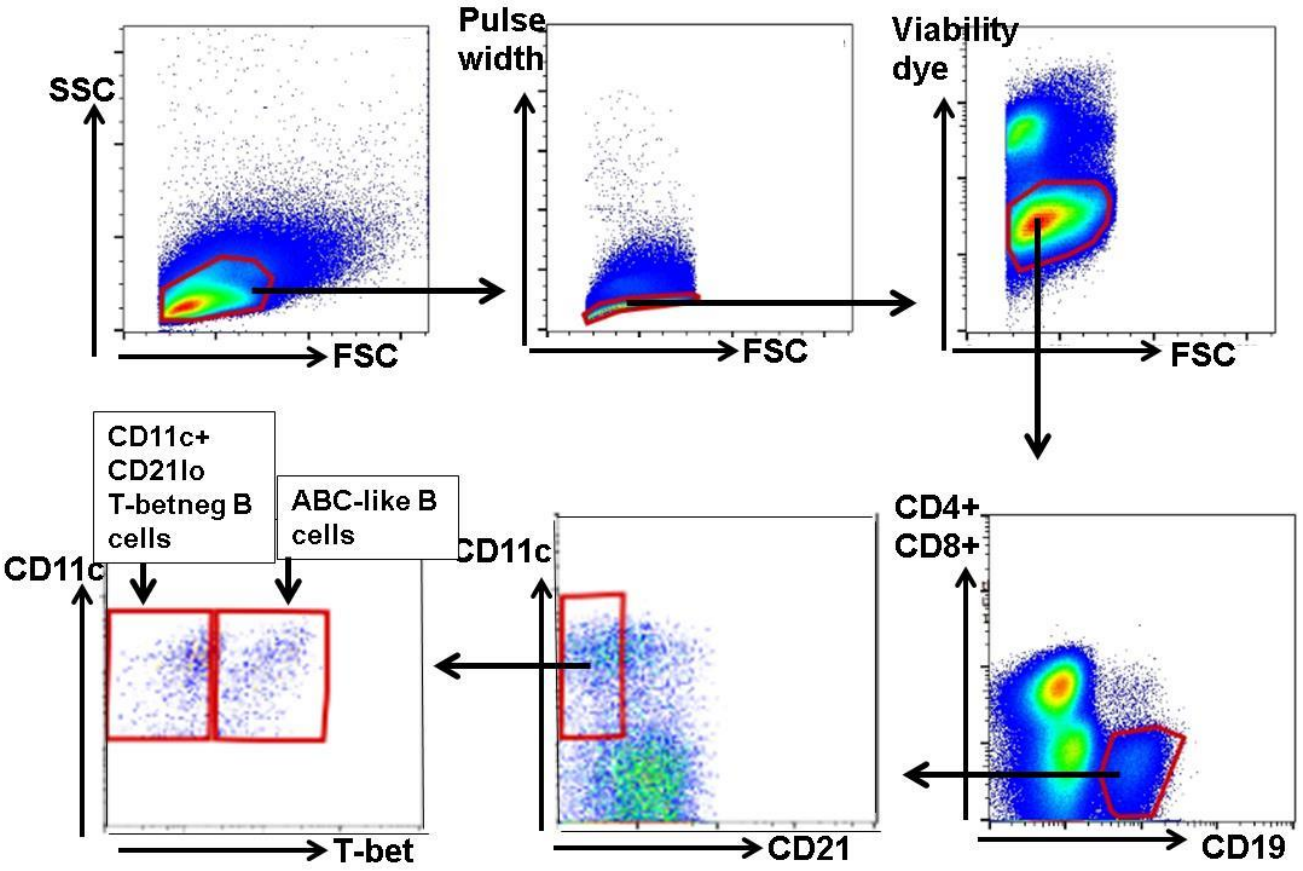
Supplementary Figure 1. Gating strategy to identify ABC-like cells. Shown is the gating strategy for different populations of B cells in peripheral blood cells. The example shown is from a SarcP. Antibodies used are listed in Table 1.

Supplementary Figure 2. CD19 is at higher levels on ABC-like cells than on mature naive B cells or CD11c+ CD21lo cells that are T-betneg. Peripheral blood cells from HS, SarcP and SarcPT were gated as described in Figure 1. Shown are; A The percentage of PBMCs that were B cells in HS and SarcP, the two groups stained and analyzed in a single experiment B The percentage of PBMCs that were B cells in HS and SarcPT, the two groups stained and analyzed in a single experiment, C-E CD19 levels on CD11c- CD21med-hi B cells (other B cells) CD11c+ Cd21lo T-betneg-lo B cells and CD11c+ Cd21lo T-bethi B cells. C B cell populations from HS analyzed with Kruskal-Wallis test with Dunns post test. * $p<0.05$. D B cell populations from SarcP analyzed as described in C. , ** $p<0.01$, *** $p<0.001$ E Comparison of CD19 levels on CD11c+ CD21lo T-bethi cells from HS versus SarcP. Data were analyzed with a Mann Whitney test. * $p<0.05$.

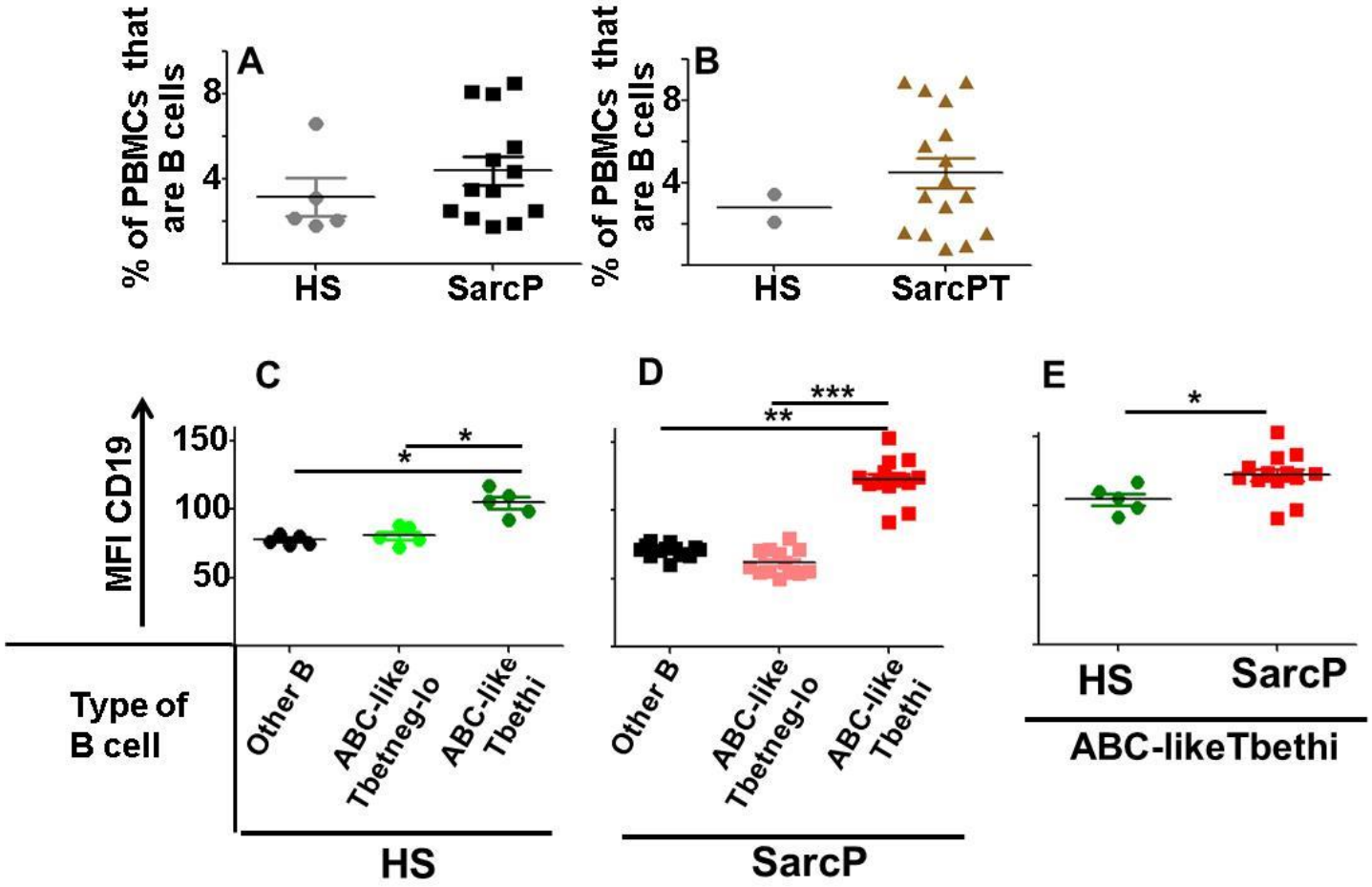
Supplementary Figure 3. Analysis of the levels of FcRL1-5 on several B cell populations in HS or SarcP on therapy *A* Peripheral blood cells from HS were stained to identify B cells (CD19+) and these cells were stained with antibodies to CD11c and CD21 and, separately, with antibodies against FcRL1-5 or isotype control antibodies *B* cells were gated for expression of CD11c and low levels of CD21 (ABC-

like cells) or for no expression of CD11c and expression of C21med-hi and the levels of staining with the various anti-FcRL antibodies analyzed. *A.* Cells from HS *B* Cells from SarcP all of whom were on therapy except SarcP 8 who was not being treated for disease.

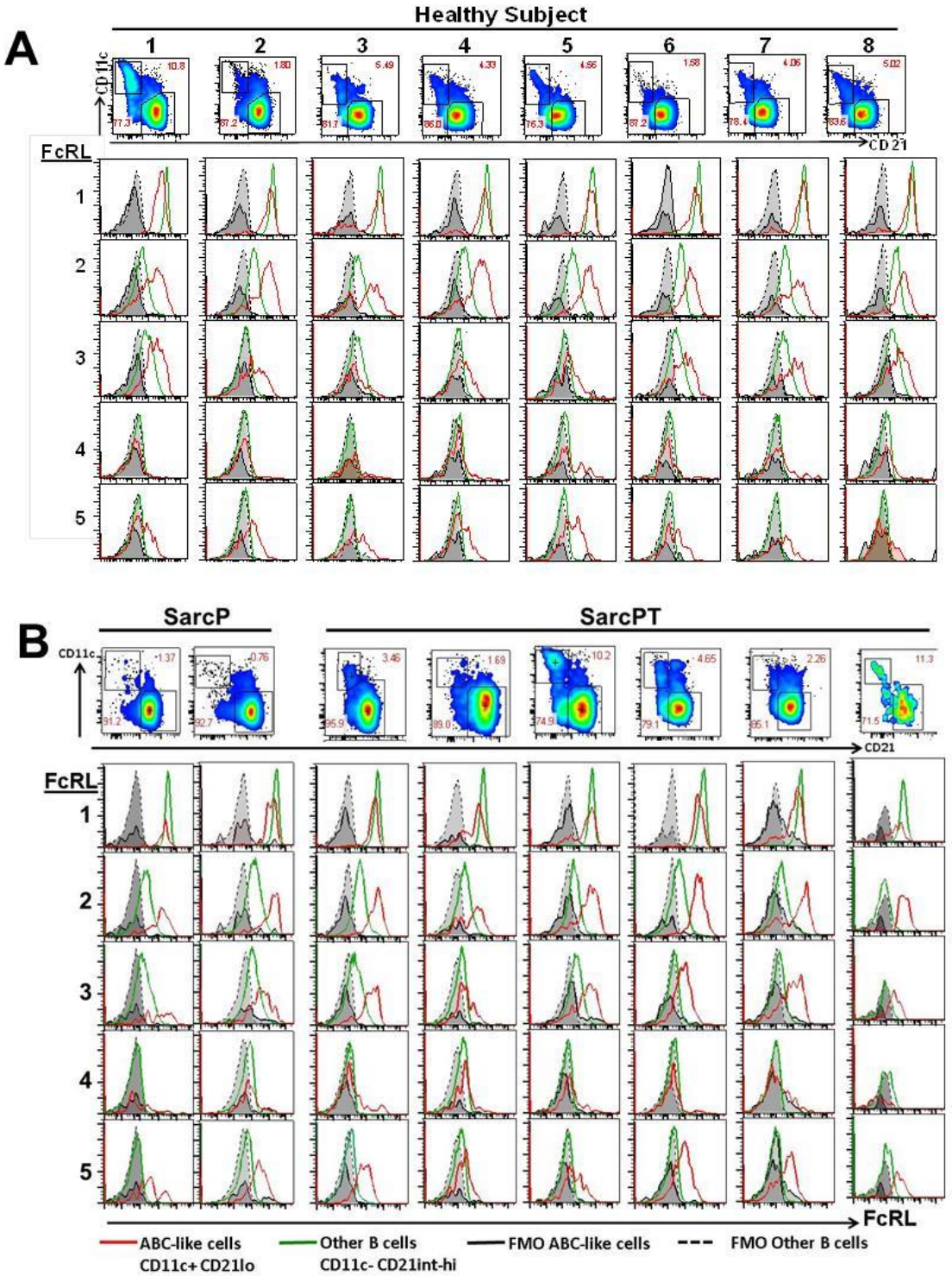
Supplementary Figure 4. Analysis of FcRL1-5 expression on B cells expressing different amounts of CD11c suggests that B cells that are destined to convert to ABC-like cells are at different stages of conversion. ABC-like cells from the peripheral blood B cell samples shown in Supplementary Figure 4 were divided into 3 categories depending upon the level of CD11c they bore. The levels of FcRLs expressed by each category in each HS (Supplementary Figure 5A) or in each SarcP +/- therapy (Supplementary Figure 5B) were then analyzed.



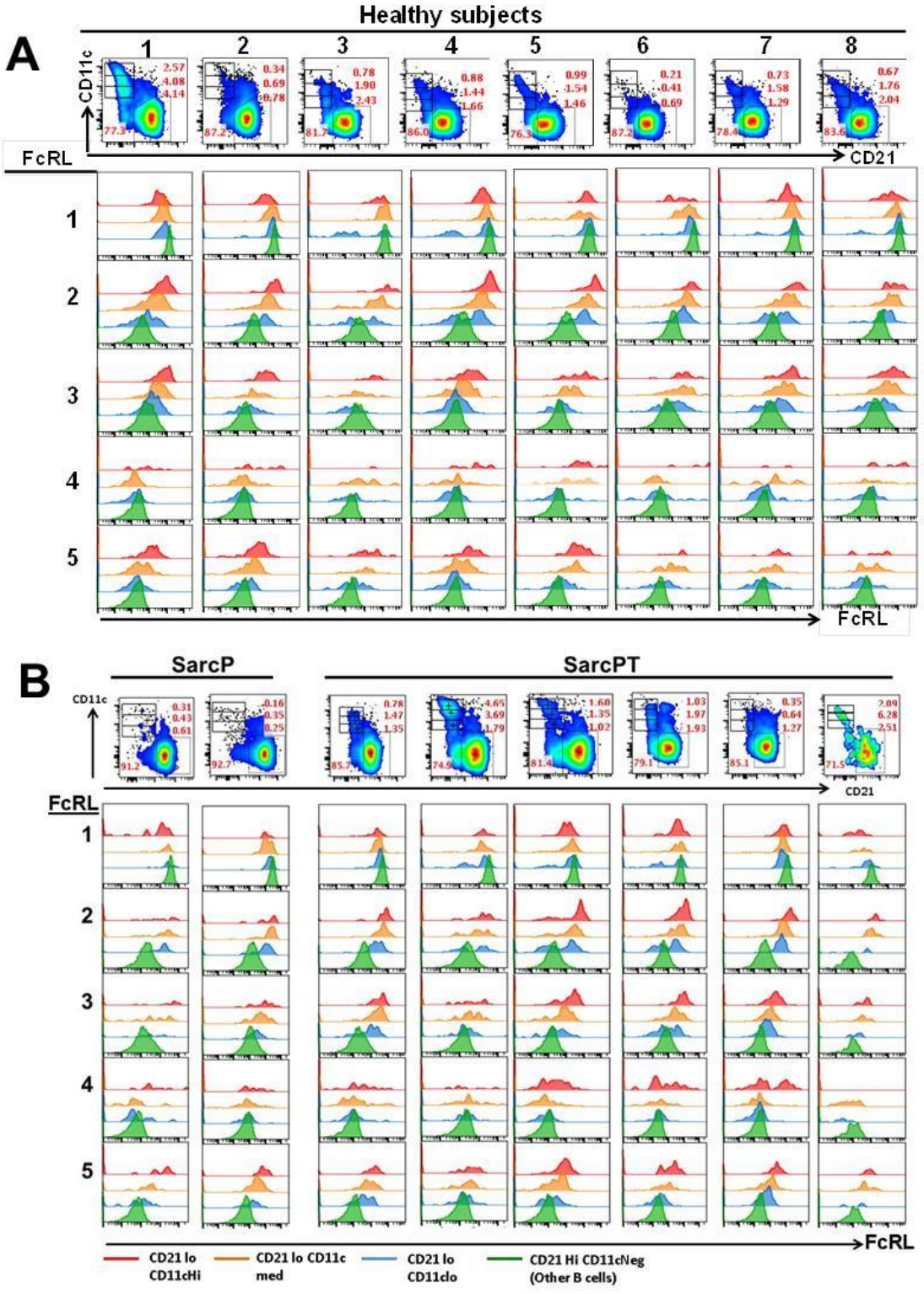
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4

Supplementary Table 1 Antibodies used in flow cytometry analyses				
Target	**Fluorochrome**	**Clone**	**Source**	**Catalogue number**
live cells	PB		BioLegend	423113
CD3	BV605	OKT3	BioLegend	317322
CD4	FITC	OKT4	BioLegend	317408
CD4	PerCP	L200	BDPharmingen	550631
CD8	FITC	HIT8a	BioLegend	300906
CD8	PerCP	SK1	BioLegend	344708
CD11b	BV421	ICRF44	BioLegend	301324
CD11b	PE	ICRF44	BD Biosciences	561001
CD11c	PECy7	3.9	Invitrogen	25-0116-42
CD19	FITC	HIB19	eBioscience	11-0199-42
CD19	APCCy7	HIB19	BioLegend	302217
CD21	APC	HB5	eBioscience	17-0219-42
CD307a/FcRL1	PE	E3	BD Pharmingen	566457
CD307b/FcRL2	PE	296902	BioLegend	FAB2048P
CD307c/FcRL3	PE	H5/FcRL3	BioLegend	374405
CD307d/FcRL4	PE	413D12	BioLegend	340203
CD307e/FcRL5	PE	509f6	BioLegend	340304
Tbet	PE	eBio4B10	eBioscience	12-5825-82
Tbet	AF488	4B10	BioLegend	644830
Mouse IgG1 control	PE	MOPC21	BD	554680
Mouse IgG2a control	PE	G155-178	BD	556653
Mouse IgG2b control	PE	27-35	BioLegend	402204