

Supplementary data Botta Gordon-Smith et al 2014**Supplementary Table 1.** Characteristics of patient population

	Persistent Oligoarticular JIA patients (n=27)	Extended Oligoarticular JIA patients (n=29)	Polyarticular JIA patients (n=16)
No. male/female	7/20	6/23	4/12
Age at sampling, mean \pm SD (years)	7.9 \pm 3.7	9.5 \pm 3.8	7.03 \pm 3.1
Age at disease onset, mean \pm SD (years)	5.1 \pm 3.2	3.2 \pm 2.3	4.5 \pm 3.8
Disease duration, mean \pm SD (years)	2.9 \pm 2.9	6.2 \pm 3.2	2.4 \pm 2.1
Treatment received: MTX (%)	*3/26 (11)	17/29 (59)	14/16 (88)
Treatment received: oral/i.v. steroids	*2/26 (8)	8/29 (27)	3/16 (19)
No. of swollen/tender joints involved at time of sampling, mean \pm SD	1.9 \pm 0.9	5.4 \pm 1.3	6.3 \pm 2.3

* Treatment data not available for one patient. MTX=Methotrexate

Figure S1

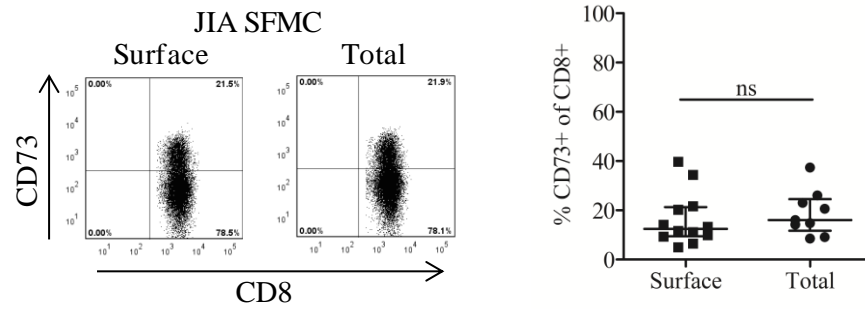


Figure S1) Comparison of CD73 surface protein expression with total [surface + intracellular] protein expression on CD8⁺ synovial lymphocytes. Representative FACS staining from one SFMC sample, gated on CD8 T cells [left]; scatter plot for surface and total expression summary data (n=12, 9) [right]. ns= not significant. Bars represent median and interquartile range (IQR).

Figure S2

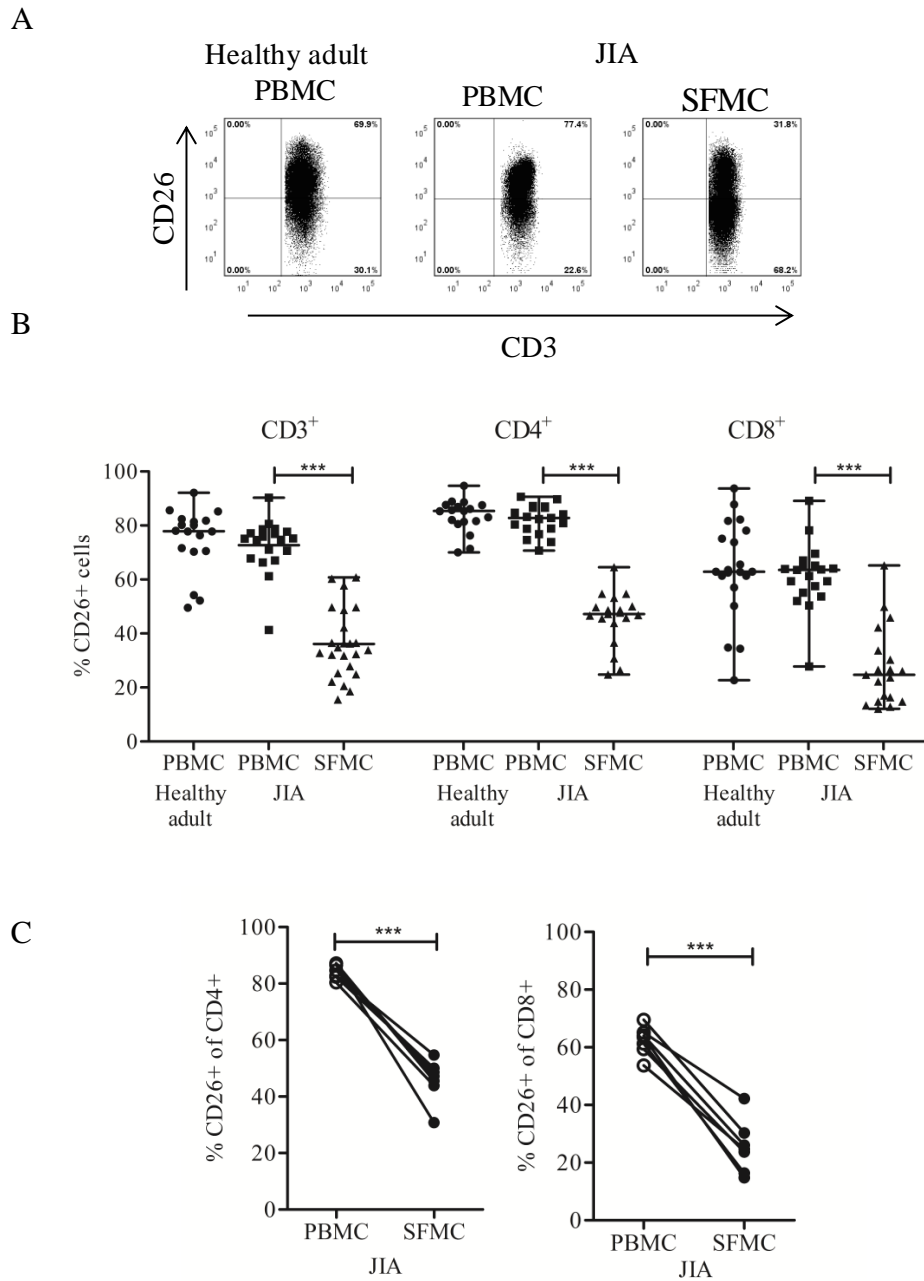


Figure S2) A) Representative flow cytometry plots of CD26 expression of CD3⁺ T cells from healthy control blood, and paired blood and synovial fluid from a JIA patient. B) Scatter plots summarising percentages of CD3, CD4 and CD8 T cells positive for CD26 from healthy blood (n=18), patient blood (n=19) and synovial fluid (n=24). Bars represent median and IQR. C) Comparison of percentage of CD4 and CD8 T cells positive for CD26 expression from paired samples of JIA patient blood and synovial fluid (n=7). ***=p <0.0001.

Figure S3

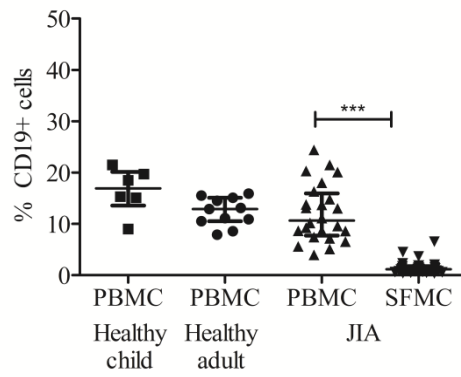


Figure S3) Frequency of CD19⁺ B cells within healthy child PBMC (n=6), adult PBMC (n=11), JIA PBMC (n=24) and SFMC (n=30.) Bars represent median and IQR. ***=p <0.0001.

Figure S4

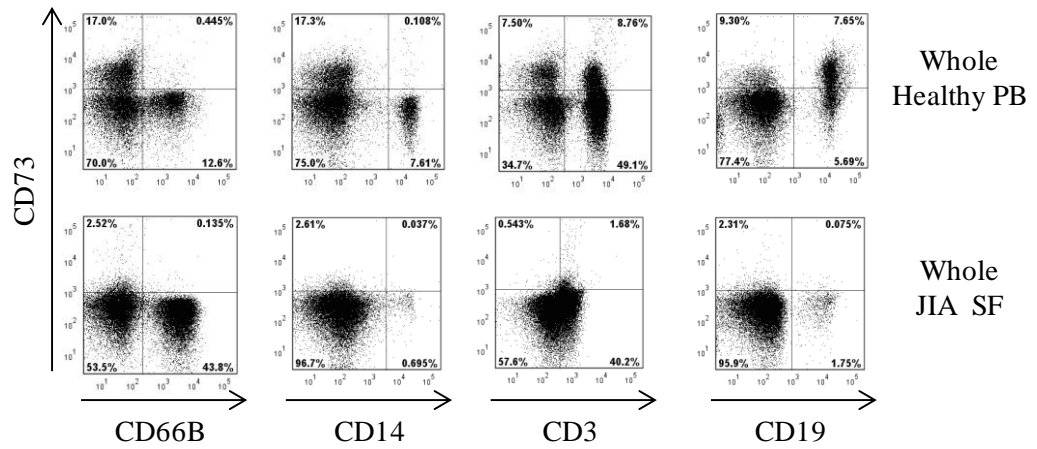


Figure S4) Expression of CD73 on cells analysed direct from blood (whole healthy PB) or synovial fluid (whole JIA SF), after lysis of red blood cells. CD73 expression was still significantly higher on healthy blood CD3⁺ T cells and CD19⁺ B cells compared to those infiltrating the synovial fluid [bottom and top right hand plots]. CD73 expression was absent on monocytes (CD14⁺) and granulocytes (CD66B⁺) in both whole peripheral blood (upper panels) and freshly isolated synovial fluid cells (lower panels). All plots gated on live cells.

Figure S5

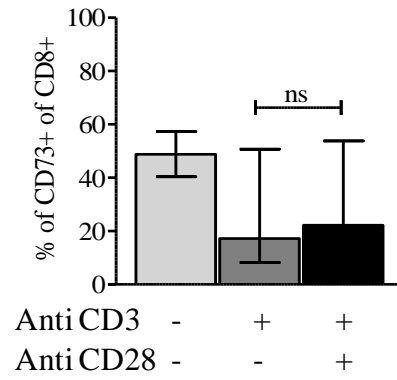


Figure S5) Costimulation is not required for CD73 downregulation. Summary data for 3 experiments showing CD73 expression on CD8 T cells after culture in medium, anti-CD3 or anti CD3/CD28 for 5 days (n=3). ns= not significant. Bars represent median and IQR.

Figure S6

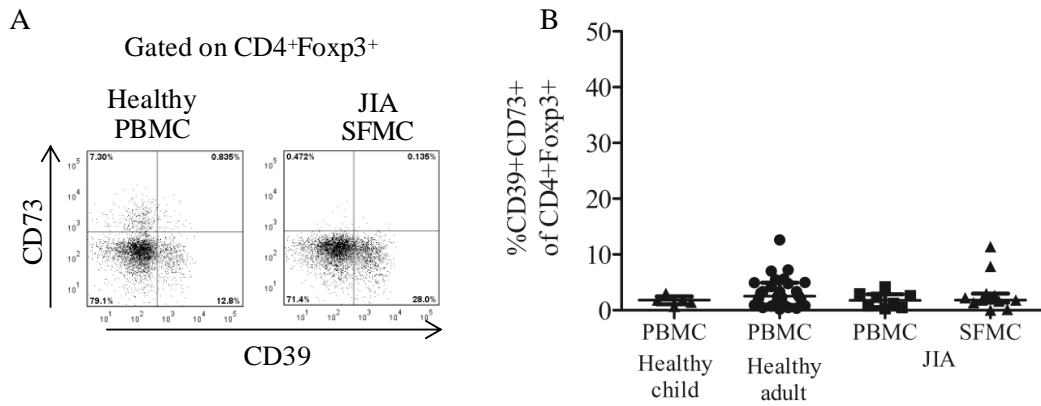
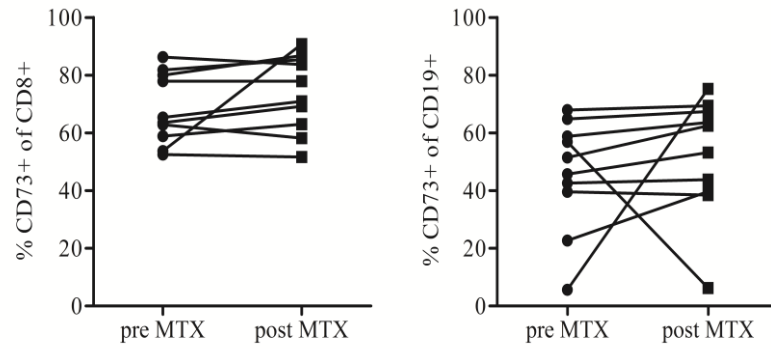


Figure S6) A) Human Treg do not co-express CD39 and CD73. Flow cytometry plots gated on CD4⁺ Foxp3⁺ T cells show lack of coordinated expression of CD73 and CD39 on CD4⁺ Foxp3⁺ cells from healthy blood (left) and the JIA synovial cells (right). B) Proportion of CD39/CD73 coexpression on CD4⁺ Foxp3⁺ PBMC from healthy children (n=5), adults (n=27) and JIA patient PBMC (n=8) and SFMC (n=11). Bars represent median and IQR.

Figure S7

A



B

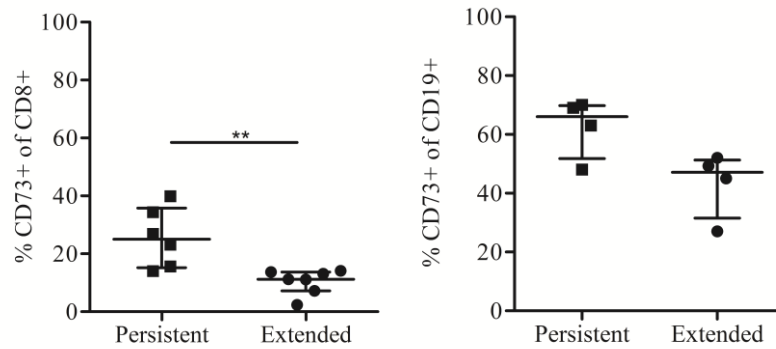


Figure S7) A) Frequency of CD73 positive cells within CD8 T cells [left] and CD19 B cells [right] from peripheral blood of JIA patients, prior to treatment (pre MTX) or after 6 months of treatment with methotrexate (post MTX) (n=10). B) Comparison of the frequency of synovial fluid CD8 T cells [left] and CD19 B cells [right] from methotrexate- and steroid-naïve extended oligoarticular (n=7,4) and persistent oligoarticular arthritis patients (n=6,4). **= $p < 0.001$. Bars represent median and IQR.