Supplemental Data

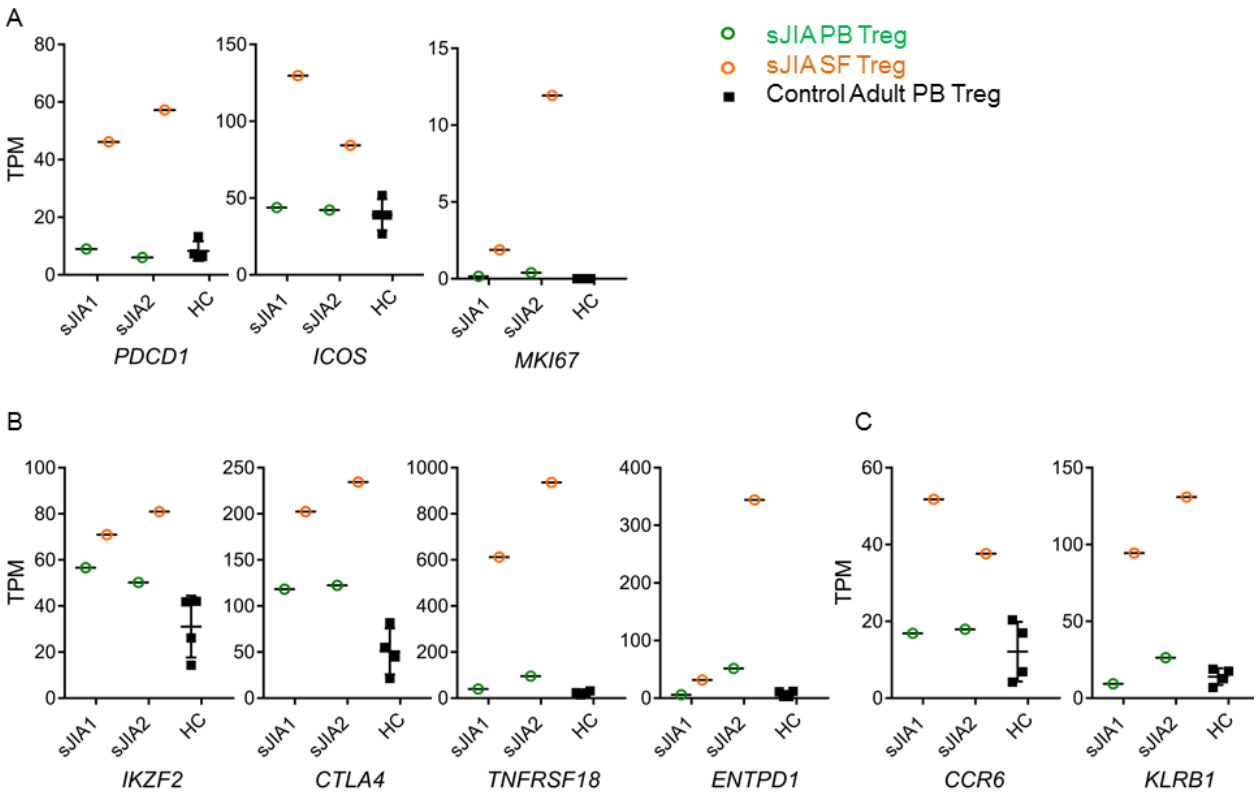

Figure S1. RNA sequencing analysis of Tregs in patients with chronic systemic juvenile idiopathic arthritis. Transcriptomic evaluation of PB and SF Tregs from two patients with chronic sJIA and PB Tregs from 4 healthy adult controls confirmed the findings of the mass cytometry analysis (mean ± SD). Tregs in the SF of sJIA patients with chronic arthritis upregulated genes related to A) T cell activation and proliferation, B) the Treg lineage, C) Th17 cell surface markers.

sJIA, systemic juvenile idiopathic arthritis; HC, healthy control; PB, peripheral blood; SF, synovial fluid; TPM, transcripts per million. *PDCD1* encodes for PD1, *MKI67* encodes for Ki67, *IKZF2* encodes for HELIOS, *TNFRSF18* encodes for GITR, *ENTPD1* encodes for CD39, KLRB1 encodes for CD161

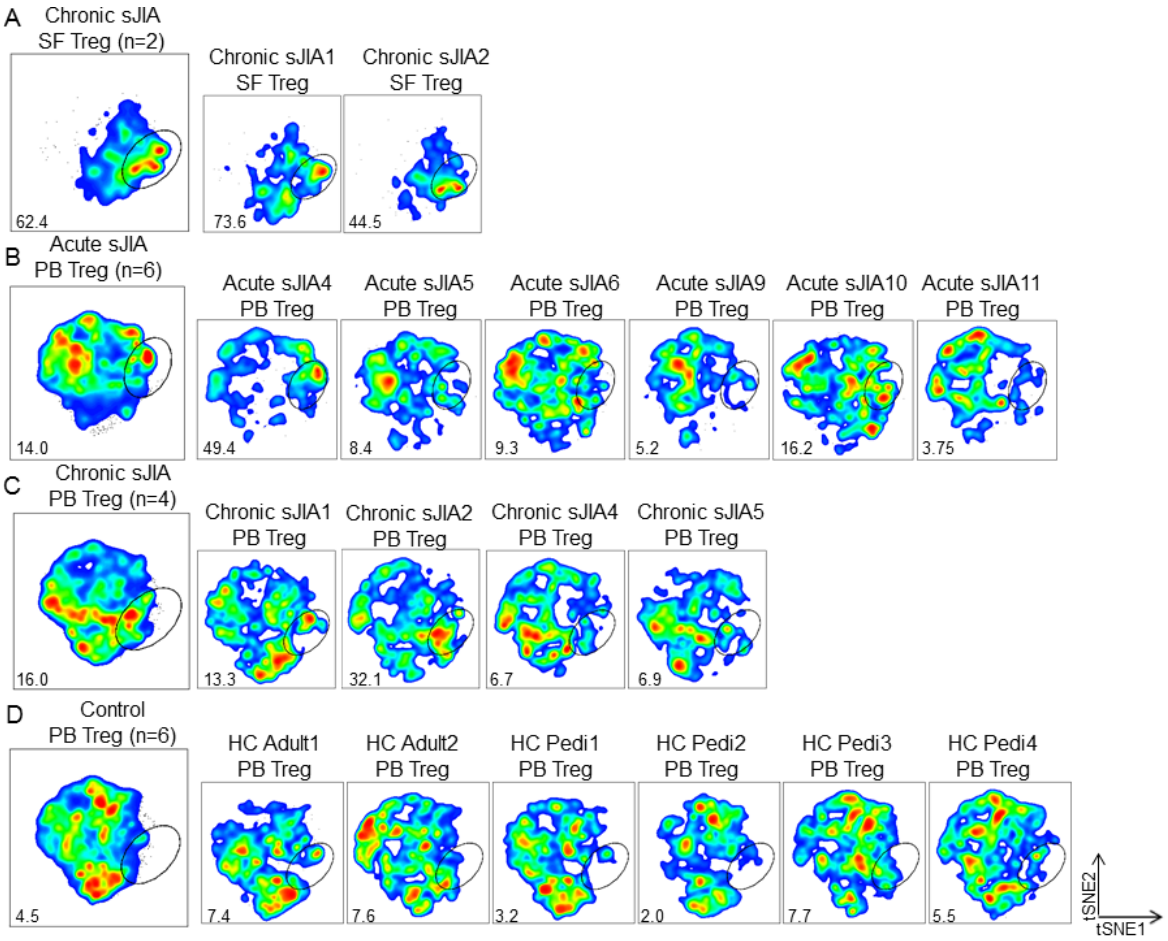

Figure S2. Individual viSNE plots of peripheral blood and synovial fluid Tregs in sJIA.
Concatenated and individual viSNE plots of mass cytometry of Tregs ($CD4^{+}CD25^{+}CD127^{lo}$) from A) chronic sJIA SF (n=2), B) acute sJIA PB (n=6), C) chronic sJIA PB (n=4), D) controls PB (n=6). viSNE analysis was performed by Cytobank and gating of the viSNE plots was done with Flowjo.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; pedi, pediatric; viSNE, visualization using t-Distributed Stochastic Neighbor Embedding; tSNE, t-Distributed Stochastic Neighbor Embedding

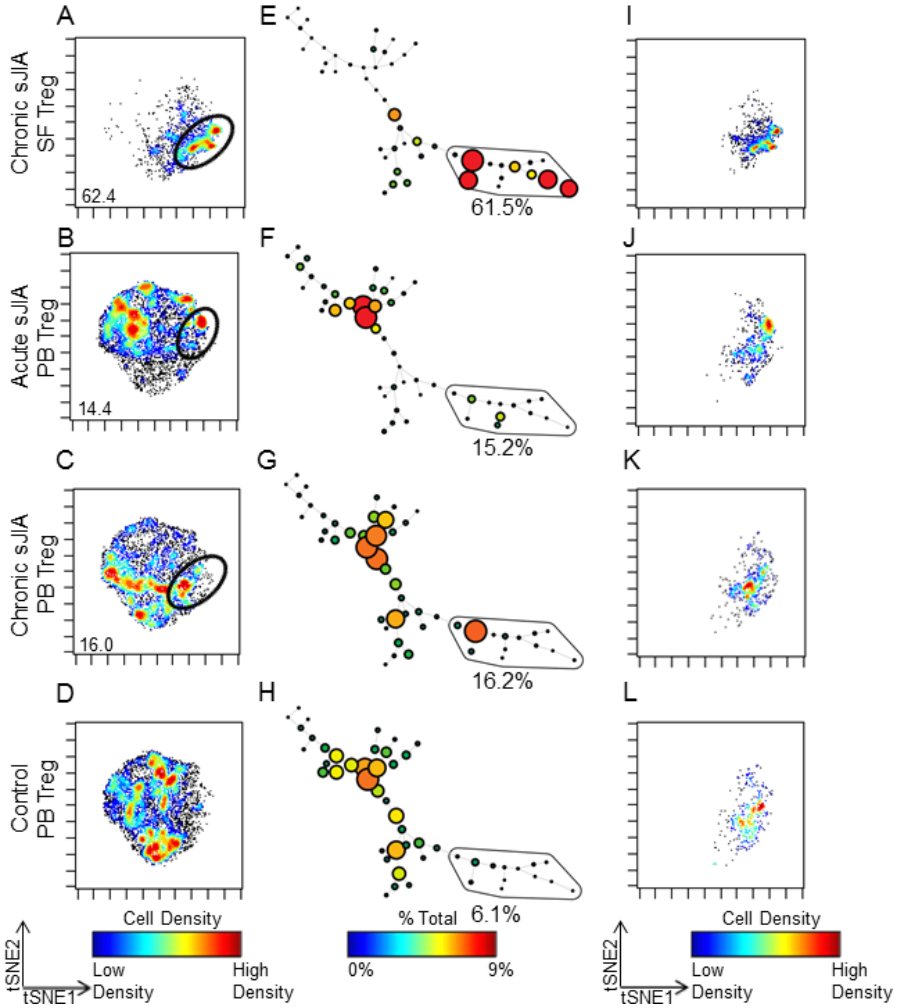

Figure S3. viSNE and SPADE identify a population of Tregs that is enriched in sJIA synovial fluid and peripheral blood. Mass cytometry data of Tregs ($CD4^{+}CD25^{+}CD127^{lo}$) was concatenated by study subject group, chronic sJIA SF (n=2), acute sJIA PB (n=6), chronic sJIA PB (n=4), and controls (n=6), and evaluated by A-D) viSNE and E-H) SPADE. The black circles were manually gated on the viSNE plots in B and C to highlight a population of Tregs that is cytometrically similar to SF Tregs depicted in panel A based on the location of the cells on the viSNE plot. The bubbled nodes (black circle) on the SPADE trees in E-H captures the dominate population of Tregs in sJIA SF as determined by hierarchical clustering. Tregs identified in the SPADE bubble were backgated onto the viSNE plot (I-L) and identify the same Treg subpopulations as the viSNE analysis. viSNE and SPADE analyses were performed with Cytobank.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; viSNE, visualization using t-Distributed Stochastic Neighbor Embedding; tSNE, t-Distributed Stochastic Neighbor Embedding; SPADE, spanning-tree progression analysis of density-normalized events

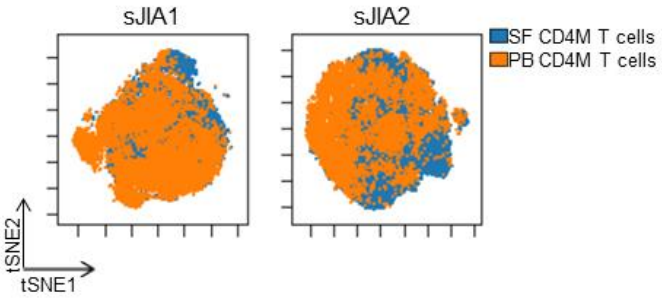

Figure S4. viSNE analysis of paired peripheral blood and synovial fluid memory CD4 T cells. Gated, live, single CD4 memory T cells ($CD4^{+}CD45RO^{+}$) with Tregs ($CD4^{+}CD25^{+}CD127^{lo}$) gated out of the analysis from the PB and SF of 2 chronic sJIA patients were analyzed together by viSNE and overlaid on the same viSNE plot. viSNE analysis was performed with Cytobank.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; M, memory; viSNE, visualization using t-Distributed Stochastic Neighbor Embedding; tSNE, t-Distributed Stochastic Neighbor Embedding

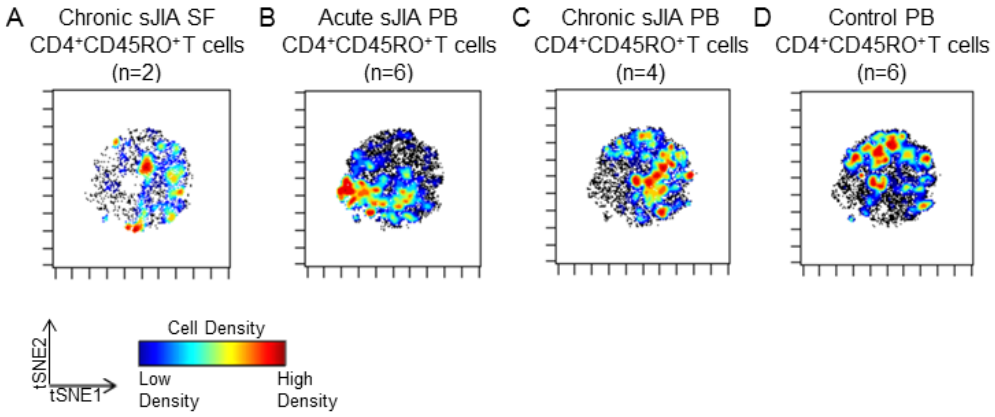

Figure S5. viSNE plots of CD4 memory T cells in sJIA patients and controls. Mass cytometry data of memory CD4 T cells ($CD4^+CD45RO^+$) with Tregs ($CD4^+CD25^+CD127^{lo}$) excluded from the analysis was concatenated by study subject group, A) chronic sJIA SF (n=2), B) acute sJIA PB (n=6), C) chronic sJIA PB (n=4), and D) controls (n=6), and evaluated by viSNE. viSNE analysis was performed with Cytobank.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; tSNE, t-Distributed Stochastic Neighbor Embedding

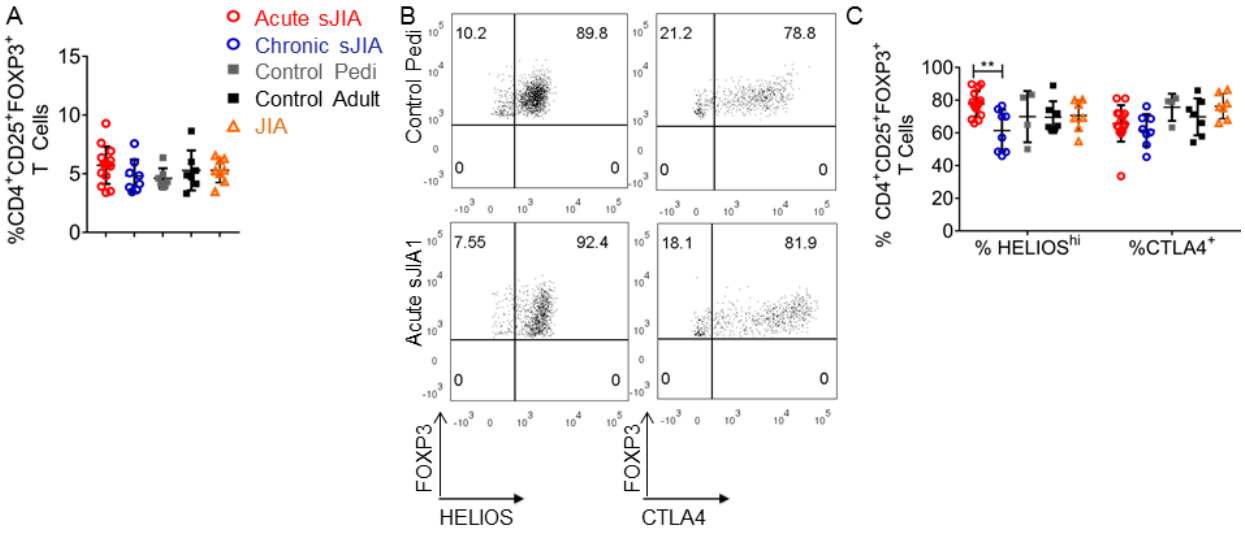

Figure S6. sJIA Tregs express HELIOS and CTLA4. The mean percentage +SD of A) Tregs ($CD4^+CD25^+FOXP3^+$) among $CD4^+$ T cells in the peripheral blood as assessed by flow cytometry in acute sJIA (n=14), chronic sJIA (n=8), pediatric control (n=7), adult control (n=7), and non-systemic JIA (n=8) study subjects. B) Representative flow cytometry dot plots gated on Tregs ($CD4^+CD25^+FOXP3^+$). C) Mean percentage +SD of $HELIOS^{hi}$ (unstimulated) and $CTLA4^+$ (stimulated) Tregs in the peripheral blood as assessed by flow cytometry in acute sJIA (n=15), chronic sJIA (n=8), pediatric control (n=4), adult control (n=7), and non-systemic JIA (n=7) study subjects (ANOVA corrected for multiple comparisons, ** $p \leq 0.01$).

sJIA, systemic juvenile idiopathic arthritis; JIA, juvenile idiopathic arthritis; pedi, pediatric

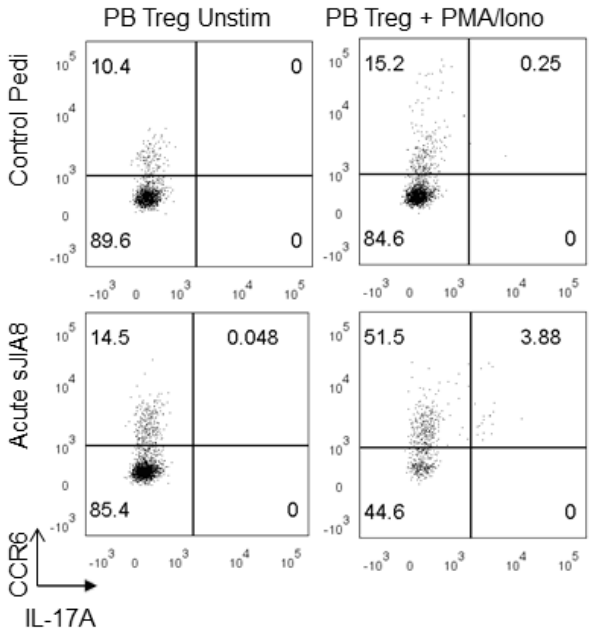

Figure S7. IL-17 and CCR6 expression in sJIA Tregs. Representative flow cytometry dot plots gated on Tregs ($CD4^+CD25^+FOXP3^+$).

PB, peripheral blood; unstim, unstimulated; pedi, pediatric; sJIA, systemic juvenile idiopathic arthritis

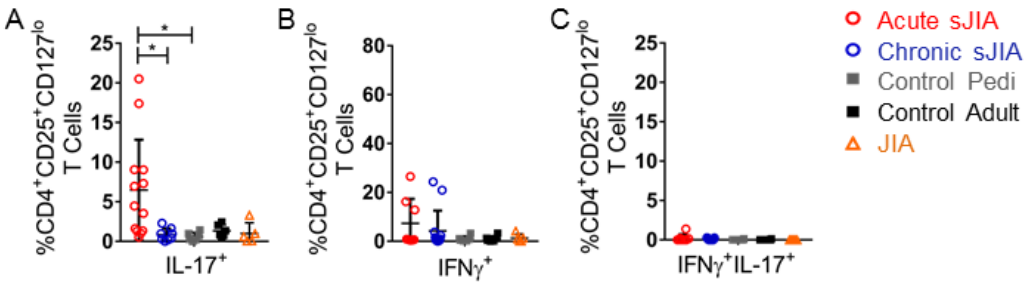

Figure S8. Acute sJIA Tregs defined by cell surface markers express IL-17. The mean percentage +SD of A) IL-17^{+}, B) IFNγ^{+}, and C) IL-17^{+}IFNγ^{+} Tregs (CD4^{+}CD25^{+}CD127^{lo}) in the peripheral blood of acute sJIA (n=13), chronic sJIA (n=8), pediatric control (n=6), adult control (n=6), and non-systemic JIA (n=5) study subjects as assessed by flow cytometry (ANOVA corrected for multiple comparisons, * p≤0.05).

sJIA, systemic juvenile idiopathic arthritis; JIA, juvenile idiopathic arthritis; pedi, pediatric

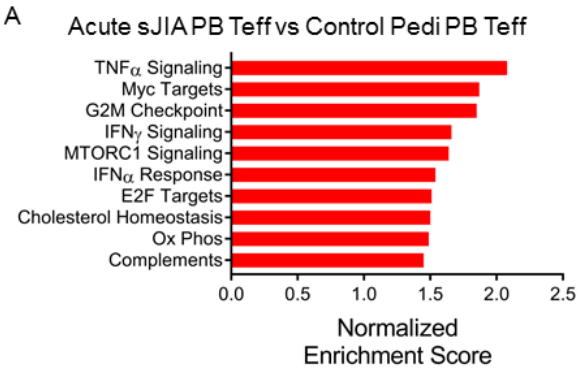

Figure S9. Top enriched gene sets in acute sJIA Teff cells from the peripheral blood. A). Top enriched gene sets in PB Teff cells ($CD4^+CD25^-$) from patients with acute sJIA (n=7) compared to pediatric healthy controls (n=4) based on the normalized enrichment score (NES) from Gene Set Enrichment Analysis (GSEA). All displayed gene sets have an FDR<0.1.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; Teff, effector T cell; pedi, pediatric; E2F, E2 factor family of transcription factors; Ox Phos, oxidative phosphorylation

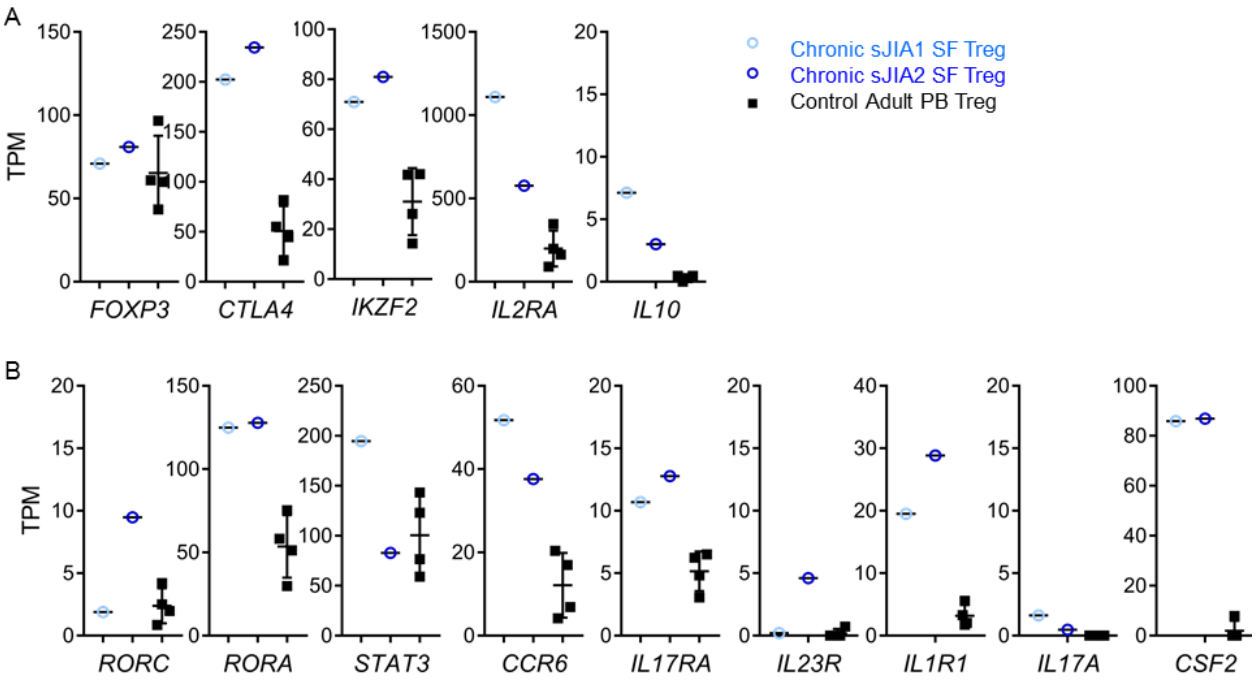

Figure S10. RNAseq analysis of Tregs in the synovial fluid of patients with chronic arthritis. Transcriptomic evaluation of SF Tregs from two patients with chronic sJIA and PB Teff cells from 4 healthy adult controls (mean ± SD). Gene expression is depicted for A) Treg-related genes and B) Th17-related genes.

sJIA, systemic juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; TPM, transcripts per million; *IKZF2* encodes for HELIOS and *CSF2* encodes from GMCSF

Table S1. Mass cytometry panel.

Metal	Target	Clone
113In	Granzyme B	GB11
115In	CD3	UCHT1
141Pr	CCR6	G034E3
143Nd	RANKL	MIH24
144Nd	CD39	A1
145Nd	CD4	RPA-T4
146Nd	CD8	RPA T8
147Sm	HLADR	L243
148Nd	TCRgd	B1
149Sm	CD45RO	UCHL1
150Nd	CCR4	L291H4
151Eu	PD1	EH12.2H7
152Sm	CTLA4	L3D10
153Eu	CD62L	DREG-56
154Sm	CXCR5	J252D4
156Gd	Neuropilin	12C2
158Gd	T-Bet	4B10
159Tb	CCR7	G043H7
160Gd	ICOS	C398.4A
161Dy	Ki67	8D5
162Dy	CXCR3	G025H7
165Ho	FoxP3	PCH101
166Er	GITR	621
167Er	GATA3	TWAJ
168Er	Helios	22F6
170Er	RORyt	AFKJS-9
171Yb	CD127	eBioRDR5
172Yb	CD38	HIT2
173Yb	CD25	M-A251
191Ir	DNA1	
193Ir	DNA2	
195Pt	Viability	
209Bi	CD161	HP-3G10

Table S2. Differentially expressed genes in peripheral blood Tregs between acute sJIA patients and pediatric controls.

Gene	LFC	P-Value	Adjusted P-Value
	Acute sJIA vs. Pedi HC		
OSMR	0.48	5.43E-14	8.29E-10
CBS	0.74	4.46E-12	2.27E-08
HRH1	0.13	1.21E-11	4.60E-08
DBN1	0.02	1.58E-11	4.81E-08
PTCHD1	0.14	1.79E-09	4.54E-06
TMEM225B	0.00	4.31E-09	9.40E-06
RRH	0.19	1.14E-08	2.17E-05
VASN	0.19	8.14E-07	1.38E-03
CALCRL	0.10	2.18E-06	3.32E-03
KLHL5	2.17	9.67E-06	1.23E-02
HAL	0.34	1.10E-05	1.29E-02
RRM2B	1.86	2.75E-05	3.00E-02
VPS26A	1.39	4.17E-05	4.24E-02
WWC1	0.47	4.55E-05	4.34E-02
ENPP1	-0.84	2.10E-12	1.60E-08
SIM1	-0.41	3.54E-06	4.91E-03

LFC, log2 fold change; sJIA, systemic juvenile idiopathic arthritis; Pedi, pediatric; HC, healthy control

Table S3. Differentially expressed genes in peripheral blood Effector T cells between chronic sJIA patients and adult controls.

Gene	LFC Chronic sJIA vs. Adult HC	P-Value	Adjusted P-Value
C6orf62	1.20	4.76E-08	1.69E-04
JUN	2.27	5.20E-08	1.69E-04
MYO7A	0.01	4.45E-07	6.53E-04
ZNF614	1.67	2.93E-07	6.53E-04
SF3B2	0.91	2.55E-06	2.26E-03
NIPBL	0.95	3.16E-06	2.53E-03
CDK6	1.85	4.99E-06	2.91E-03
MTDH	1.37	5.39E-06	2.92E-03
ABHD17A	1.84	6.07E-06	3.12E-03
SEC31A	0.89	8.83E-06	4.04E-03
OVCA2	1.67	8.37E-06	4.04E-03
APBB3	1.42	1.09E-05	4.16E-03
SERP1	1.12	1.06E-05	4.16E-03
DNAJC27	1.56	1.45E-05	5.04E-03
KIAA1191	1.31	2.99E-05	9.11E-03
ZNF845	1.37	3.22E-05	9.54E-03
PIM1	1.25	3.80E-05	1.09E-02
AKAP10	1.41	4.16E-05	1.13E-02
HMGA1	1.46	4.37E-05	1.13E-02
TTC3	0.99	4.12E-05	1.13E-02
TRIB3	2.20	5.88E-05	1.40E-02
DR1	0.84	6.03E-05	1.40E-02
UBE2B	1.34	6.98E-05	1.41E-02
CAPRIN1	0.86	7.16E-05	1.41E-02
FPGS	1.13	7.65E-05	1.41E-02
NFKBIZ	1.68	6.81E-05	1.41E-02
ATF7IP	0.99	6.77E-05	1.41E-02
ATP6V0C	1.74	7.56E-05	1.41E-02
UBE4A	0.75	9.32E-05	1.62E-02
CCNL2	1.19	9.55E-05	1.62E-02
STAG1	1.10	9.98E-05	1.65E-02
TSPYL2	1.47	1.09E-04	1.72E-02
EMD	1.31	1.11E-04	1.72E-02
PCNT	1.05	1.20E-04	1.76E-02
ZNF565	1.48	1.18E-04	1.76E-02
AC008758.1	0.93	1.21E-04	1.76E-02

SF3B1	0.78	1.63E-04	2.21E-02
RBM39	0.95	1.70E-04	2.27E-02
PRELID1	1.09	1.76E-04	2.28E-02
JADE1	1.01	1.87E-04	2.28E-02
SREBF2	1.24	1.97E-04	2.37E-02
GTPBP1	1.37	2.16E-04	2.51E-02
SND1	0.71	2.31E-04	2.53E-02
NPIPB2	1.77	2.29E-04	2.53E-02
LRRC59	1.31	2.61E-04	2.71E-02
IER2	1.57	2.66E-04	2.71E-02
ZBED6	1.12	2.57E-04	2.71E-02
JAK2	1.50	2.71E-04	2.72E-02
ACSL3	1.27	2.78E-04	2.77E-02
PITHD1	1.46	2.91E-04	2.79E-02
IRF7	1.48	2.92E-04	2.79E-02
NFATC1	0.96	3.04E-04	2.80E-02
UBXN7	0.96	3.26E-04	2.92E-02
ITSN2	1.09	3.26E-04	2.92E-02
GDI1	0.93	3.22E-04	2.92E-02
GPCPD1	1.24	3.67E-04	3.14E-02
FNBP4	1.21	3.85E-04	3.19E-02
OSBPL11	1.57	3.75E-04	3.19E-02
DDX19A	0.91	3.84E-04	3.19E-02
LPCAT1	1.58	3.98E-04	3.21E-02
COMTD1	1.68	4.48E-04	3.45E-02
QRICH1	1.03	4.60E-04	3.51E-02
CLN8	1.46	4.95E-04	3.75E-02
UBE2G1	1.45	5.03E-04	3.78E-02
SRSF6	0.86	5.20E-04	3.82E-02
TSSC4	1.17	5.41E-04	3.89E-02
ULK3	1.08	5.61E-04	3.94E-02
JAK1	0.92	5.71E-04	3.98E-02
PGGHG	1.16	5.86E-04	4.06E-02
ITGA6	1.27	6.06E-04	4.06E-02
SLC1A5	1.51	5.98E-04	4.06E-02
SAP130	1.07	6.02E-04	4.06E-02
USP54	1.62	6.07E-04	4.06E-02
RB1CC1	1.18	6.24E-04	4.15E-02
PRMT1	0.89	6.41E-04	4.22E-02
STRIP1	0.97	6.58E-04	4.23E-02
PPP1R21	1.17	6.72E-04	4.29E-02
CKAP5	1.24	7.44E-04	4.57E-02
MSH6	1.18	7.49E-04	4.57E-02

SYVN1	0.84	7.93E-04	4.74E-02
EAF1	1.24	8.04E-04	4.76E-02
CACUL1	0.96	8.15E-04	4.80E-02
COLGALT1	0.86	8.32E-04	4.86E-02
MED17	1.04	8.67E-04	4.88E-02
SLC23A2	1.15	8.72E-04	4.88E-02
LMF2	0.93	8.65E-04	4.88E-02
TP53INP1	1.36	8.67E-04	4.88E-02
ATXN2L	0.99	8.79E-04	4.88E-02
HMGCS1	1.40	8.87E-04	4.89E-02
SLC7A1	1.49	9.01E-04	4.94E-02
TRAF4	1.59	9.38E-04	4.95E-02
ARHGEF10	1.59	9.26E-04	4.95E-02
PIK3R5	0.90	9.54E-04	4.95E-02
SPIDR	1.11	9.50E-04	4.95E-02
NPIPB3	0.96	9.32E-04	4.95E-02
UBE2H	1.06	9.15E-04	4.95E-02
ZNF26	1.18	9.64E-04	4.98E-02
EIF5AL1	-3.02	8.69E-09	8.48E-05
ABRACL	-1.21	4.68E-07	6.53E-04
AC006030.1	-3.16	3.35E-07	6.53E-04
BMPR1A	-2.19	1.21E-06	1.31E-03
RPLP2	-1.53	1.20E-06	1.31E-03
RPS27	-1.47	1.92E-06	1.88E-03
HIST1H4C	-1.57	3.36E-06	2.53E-03
CEBPZOS	-1.46	3.84E-06	2.68E-03
ALDH1L2	-3.32	4.83E-06	2.91E-03
ZBTB8B	-2.47	5.07E-06	2.91E-03
PGAM1	-1.19	9.43E-06	4.04E-03
PTMA	-1.27	9.52E-06	4.04E-03
HLA-B	-1.43	1.11E-05	4.16E-03
B2M	-1.37	1.18E-05	4.25E-03
RPL36	-1.17	1.85E-05	6.22E-03
ARPC5	-1.24	1.93E-05	6.28E-03
MYCBP	-1.49	2.57E-05	8.09E-03
DRAXIN	-2.67	4.38E-05	1.13E-02
SRP9	-0.89	4.99E-05	1.25E-02
NAA20	-1.11	5.94E-05	1.40E-02
GABARAPL2	-0.81	7.53E-05	1.41E-02
RPL22	-1.04	7.24E-05	1.41E-02
CD2	-0.94	7.43E-05	1.41E-02
C9orf16	-1.24	6.86E-05	1.41E-02
AC112229.3	-2.92	7.08E-05	1.41E-02

KNL1	-1.99	8.47E-05	1.53E-02
RNF44	-1.31	9.06E-05	1.61E-02
ATP5E	-1.10	9.63E-05	1.62E-02
MEAF6	-0.80	1.02E-04	1.66E-02
HLA-DPA1	-1.59	1.07E-04	1.71E-02
ARHGAP19-SLIT1	-2.05	1.21E-04	1.76E-02
CNPY3	-0.77	1.32E-04	1.90E-02
ATP6V1G1	-0.71	1.40E-04	1.98E-02
COX18	-1.53	1.44E-04	2.01E-02
RPL41	-1.20	1.54E-04	2.11E-02
HNRNPA2B1	-0.67	1.75E-04	2.28E-02
RPL37A	-1.16	1.77E-04	2.28E-02
RPL31	-1.12	1.87E-04	2.28E-02
PTPN14	-1.76	1.86E-04	2.28E-02
JAM2	-2.78	1.87E-04	2.28E-02
SH3BGRL	-0.96	2.12E-04	2.51E-02
HMGN1	-0.64	2.16E-04	2.51E-02
OAZ1	-0.84	2.24E-04	2.53E-02
SCOC	-1.12	2.25E-04	2.53E-02
MRPL40	-1.22	2.28E-04	2.53E-02
FRMD4B	-1.47	2.65E-04	2.71E-02
NSRP1	-1.30	2.57E-04	2.71E-02
CCR5	-1.26	2.58E-04	2.71E-02
TOMM7	-1.20	2.51E-04	2.71E-02
NUCKS1	-0.84	2.85E-04	2.79E-02
LAMTOR1	-0.91	2.88E-04	2.79E-02
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NCEH1	-1.19	3.56E-04	3.10E-02
YWHAB	-0.87	3.61E-04	3.12E-02
TAF3	-0.69	3.82E-04	3.19E-02
SCP2	-0.91	3.93E-04	3.21E-02
KLF13	-1.30	3.94E-04	3.21E-02
NDUFC1	-1.05	4.16E-04	3.33E-02
MAP3K20	-1.72	4.22E-04	3.35E-02
RPS19	-0.94	4.29E-04	3.35E-02
GNG5	-0.83	4.26E-04	3.35E-02
C19orf53	-0.77	4.42E-04	3.43E-02
SEC62	-0.94	5.20E-04	3.82E-02

LDHB	-0.98	5.14E-04	3.82E-02
TMEM55A	-1.20	5.25E-04	3.83E-02
COX6B1	-0.74	5.42E-04	3.89E-02
ZNF554	-1.61	5.61E-04	3.94E-02
RPS29	-1.37	5.58E-04	3.94E-02
RBX1	-0.74	6.07E-04	4.06E-02
ARHGEF18	-1.03	6.44E-04	4.22E-02
TMX1	-0.89	6.54E-04	4.23E-02
LYAR	-0.93	6.58E-04	4.23E-02
BIRC3	-1.19	6.81E-04	4.30E-02
CD3G	-0.88	6.83E-04	4.30E-02
ATP5F1	-0.67	7.00E-04	4.38E-02
ENOPH1	-0.87	7.10E-04	4.42E-02
SUCLG1	-0.68	7.34E-04	4.53E-02
C4orf3	-0.91	7.71E-04	4.67E-02
RPLP1	-0.89	7.85E-04	4.73E-02
AGFG2	-1.16	7.96E-04	4.74E-02
TMEM230	-0.84	8.73E-04	4.88E-02
RPL28	-0.93	8.70E-04	4.88E-02
CRIPT	-1.07	8.71E-04	4.88E-02
ALKBH7	-0.71	8.80E-04	4.88E-02
PAPLN	-2.02	9.49E-04	4.95E-02
VSIR	-1.08	9.09E-04	4.95E-02
BBS7	-1.27	9.43E-04	4.95E-02
RPS27L	-1.29	9.20E-04	4.95E-02
HIRIP3	-1.10	9.68E-04	4.98E-02

LFC, log2 fold change; sJIA, systemic juvenile idiopathic arthritis; HC, healthy control