**Table S1. Antibodies used in high dimensional flow cytometry panel for cellular composition and activation, proliferative and functional state of mononuclear cells**; *Antibodies used at appropriately tested titrations.*

| Fluorophore | Marker | Clone |
|---|---|---|
| BUV395 | CD4 | SK3 |
| BUV496 | HLA-DR | G46-6 |
| BUV563 | CD161 | DX12 |
| BUV615 | CD16 | 3G8 |
| BUV661 | 4-1BB (CD137) | 4B4-1 |
| BUV737 | CD25 | 2A3 |
| BUV805 | CD39 | TU66 |
| BV421 | TNFR2 (CD120b) | hTNFR-M1 |
| SB436 | CD123 | 6H6 |
| eFluor450 | ID2* | ILCID2 |
| BV480 | CD127 | HIL-7R-M21 |
| BV510 | CD71 | M-A712 |
| BV570 | CD56 | 5.1H11 |
| BV605 | CTLA-4*(CD152) | BNI3 |
| BV650 | Ki67* | B56 |
| BV711 | CD226 (DNAM-1) | 11A8 |
| BV750 | GARP | 7B11 |
| BV785 | CD69 | FN50 |
| Qdot 800 | CD14 | TuK4 |
| BB515 | CD96 | 6F9 |
| AF488 | GITR (CD357) | 108-17 |
| AF532 | CD45RA | HI100 |
| BB700 | PD-1 (CD279) | EH12.1 |
| PerCP-eFluor710 | Lap | FNLAP |
| PE | Foxp3* | 236A/E7 |
| PE-CF594 | CD11c | 3.9 |
| PE-Cy5 | CD19 | HIB19 |
| PC-Cy7 | CD112 | TX31 |
| APC | CD155 (PVR) | SKII.4 |
| AF647 | TIGIT | MBSA43 |
| AF700 | Helios* | 22F6 |
| eFluor 780 | FVD | --- |
| APC-Fire810 | CD3 | SK7 |

*stained intracellularly after FoxP3/transcription factor fixation/permeabilization buffer*

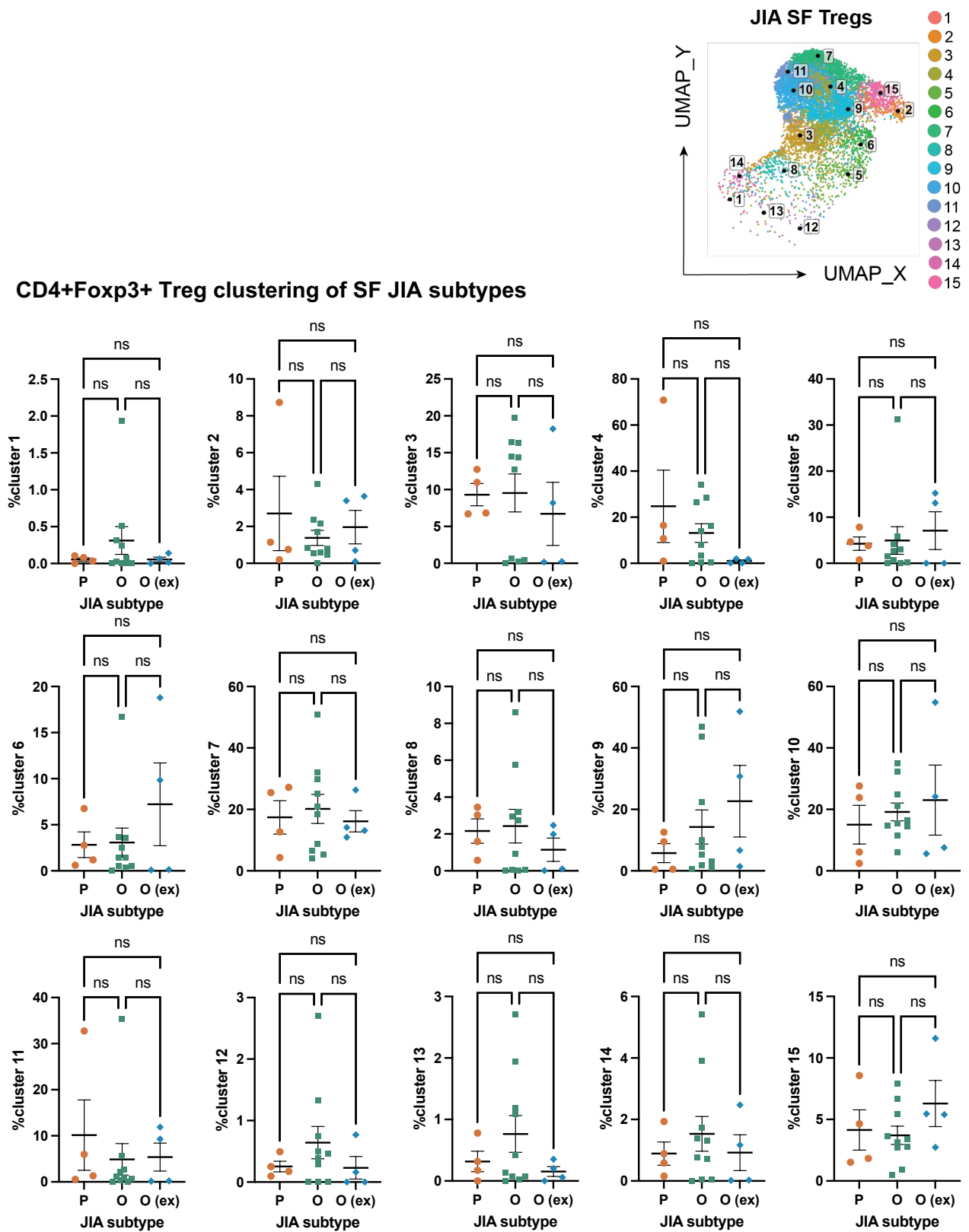# Cell composition clustering of SF JIA subtypes
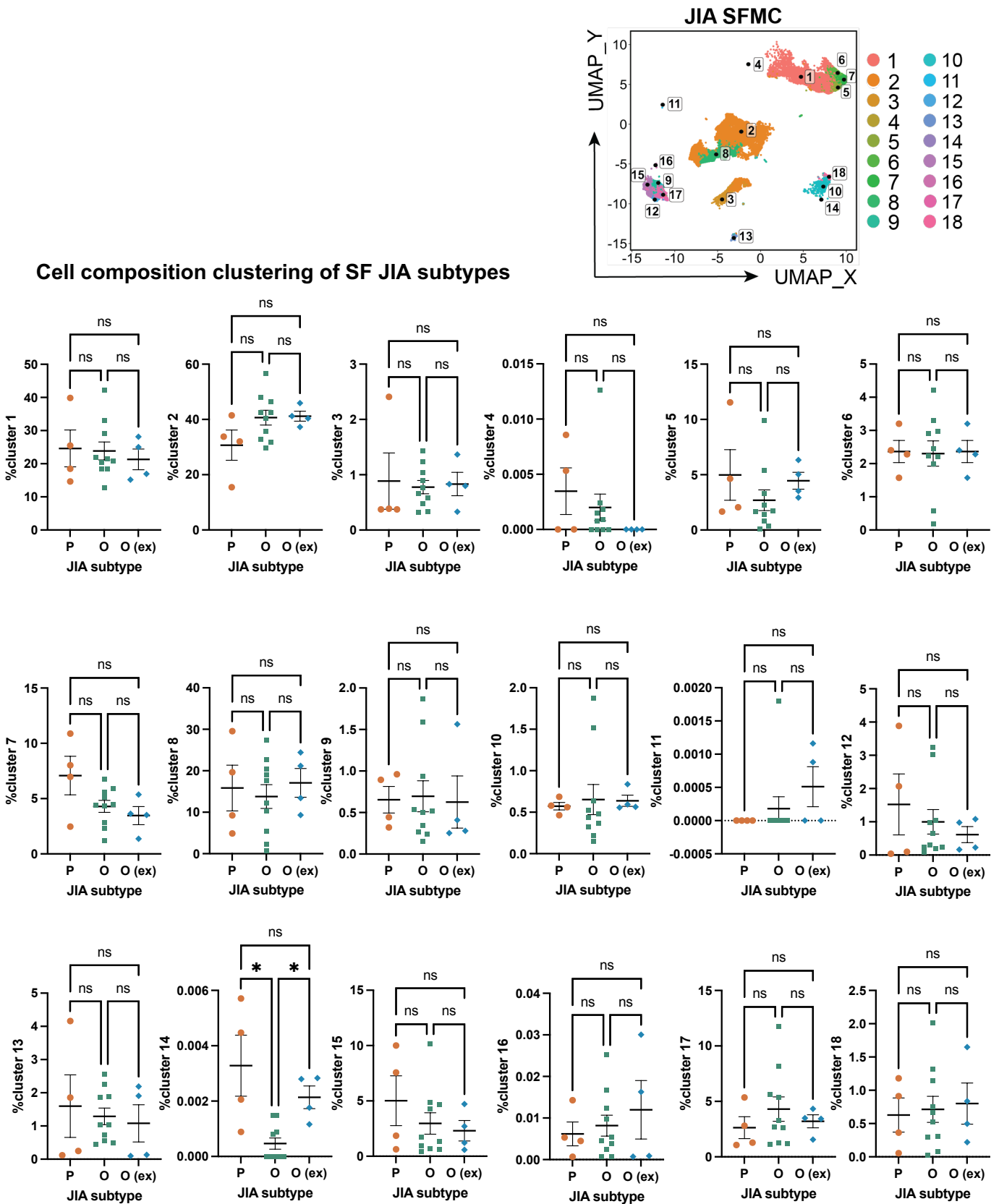


**Supplementary Figure S1. Cell composition cluster frequencies divided by JIA subtypes**
Significance determined by one-way ANOVA with Kruskal-Wallis multiple comparisons post hoc test. *p<0.05. non-significant (ns) p values classified as p≥0.05. mean±SEM displayed. P= Polyarticular RF-; O= Oligoarticular (persistent); O (ex)= Oligoarticular (extended).

**JIA SF CD3+**



# CD3+ clustering of SF JIA subtypes
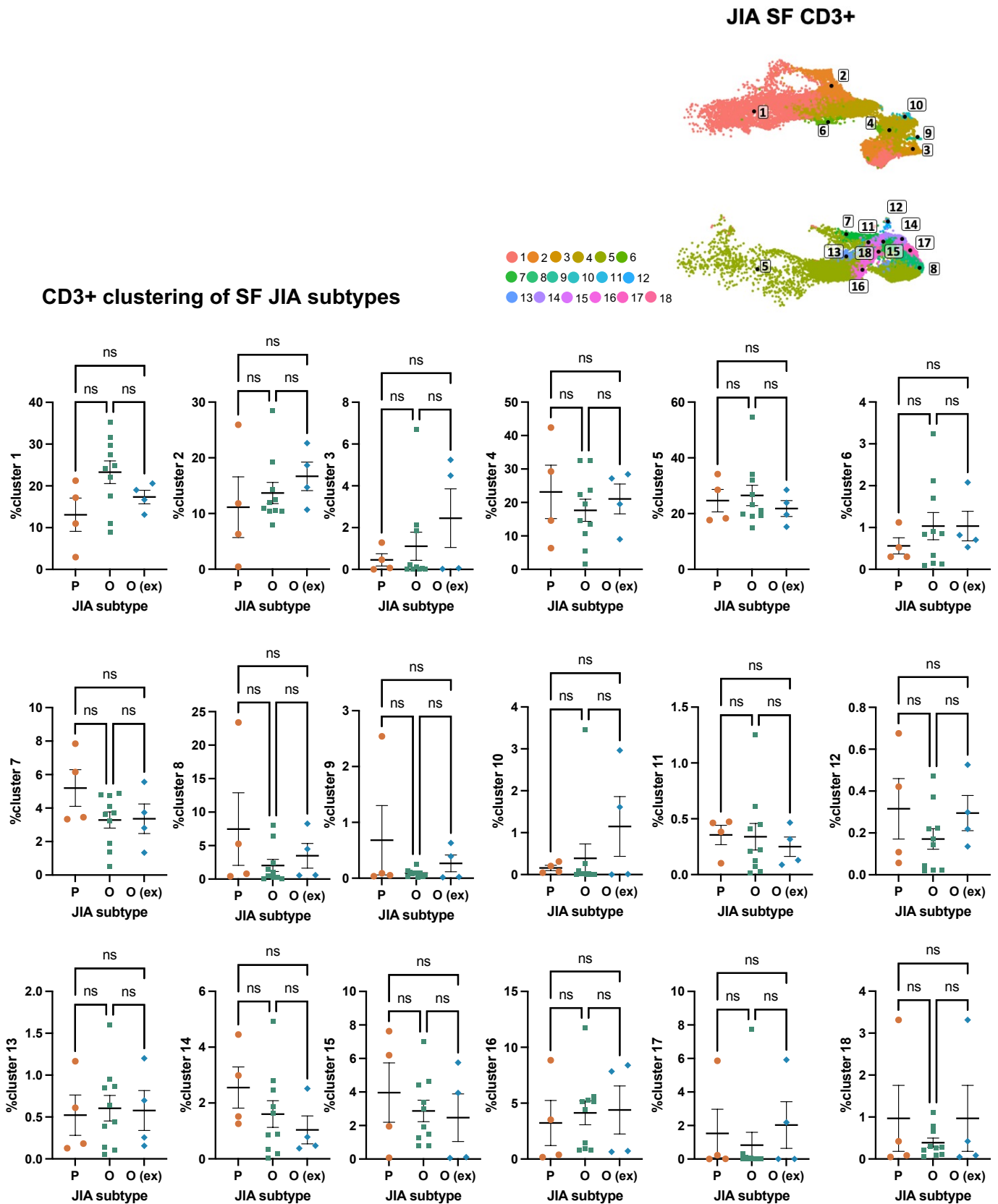


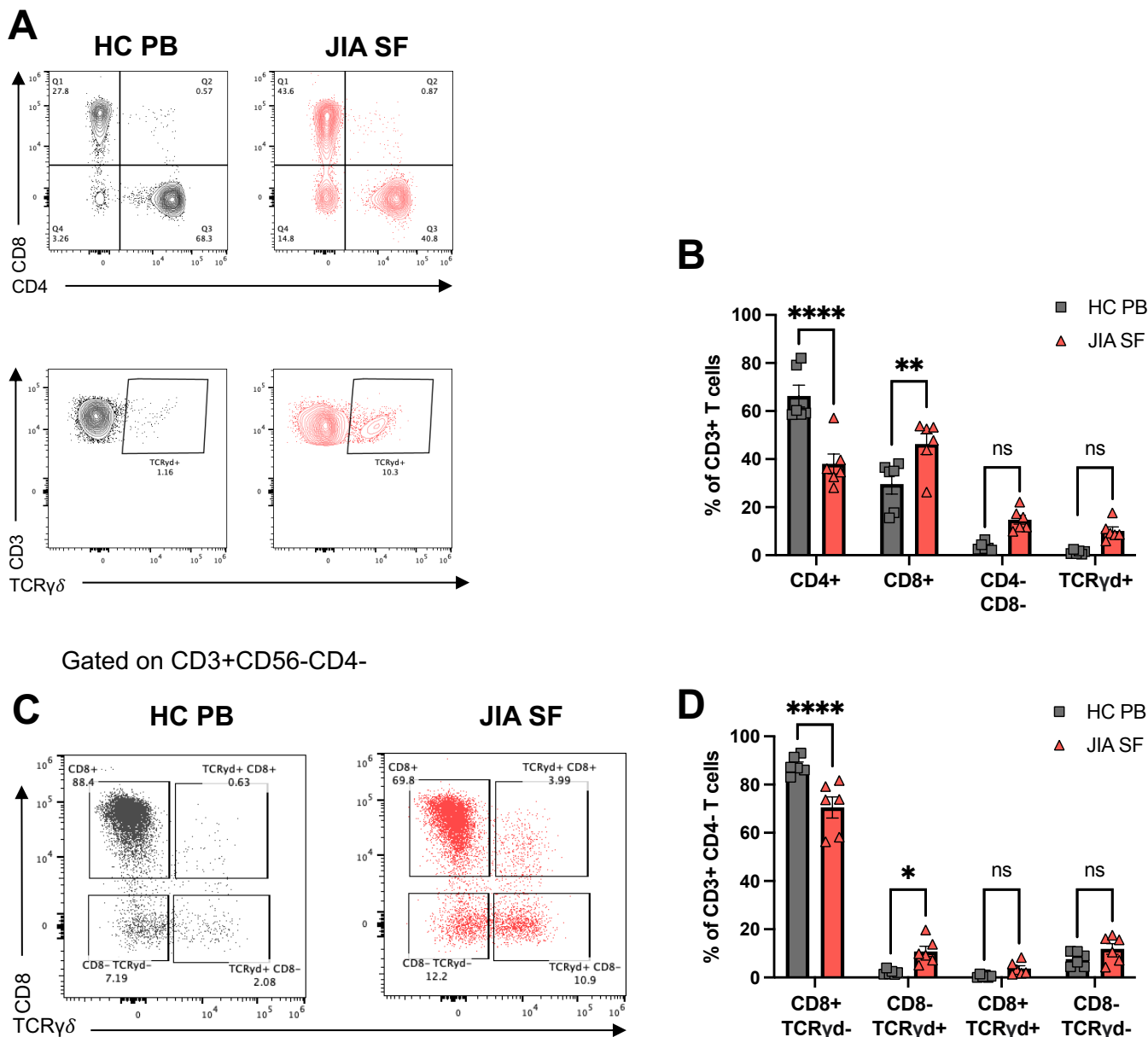**Supplementary Figure S2. CD3+ T cell cluster frequencies divided by JIA subtypes**

Significance determined by one-way ANOVA with Kruskal-Wallis multiple comparisons post hoc test. *p<0.05. non-significant (ns) p values classified as p≥0.05. mean±SEM displayed. P= Polyarticular RF-; O= Oligoarticular (persistent); O (ex)= Oligoarticular (extended).

**JIA SF Tregs**

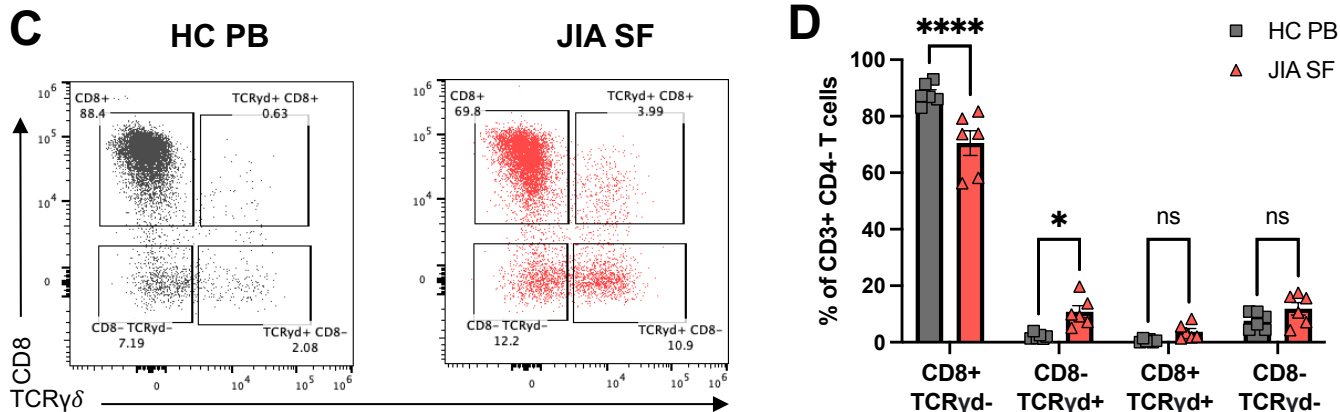# CD4+Foxp3+ Treg clustering of SF JIA subtypes



**Supplementary Figure S3. CD4+Foxp3+ Treg cluster frequencies divided by JIA subtypes**

Significance determined by one-way ANOVA with Kruskal-Wallis multiple comparisons post hoc test. *p<0.05. non-significant (ns) p values classified as p≥0.05. mean±SEM displayed. P= Polyarticular RF-; O= Oligoarticular (persistent); O (ex)= Oligoarticular (extended).
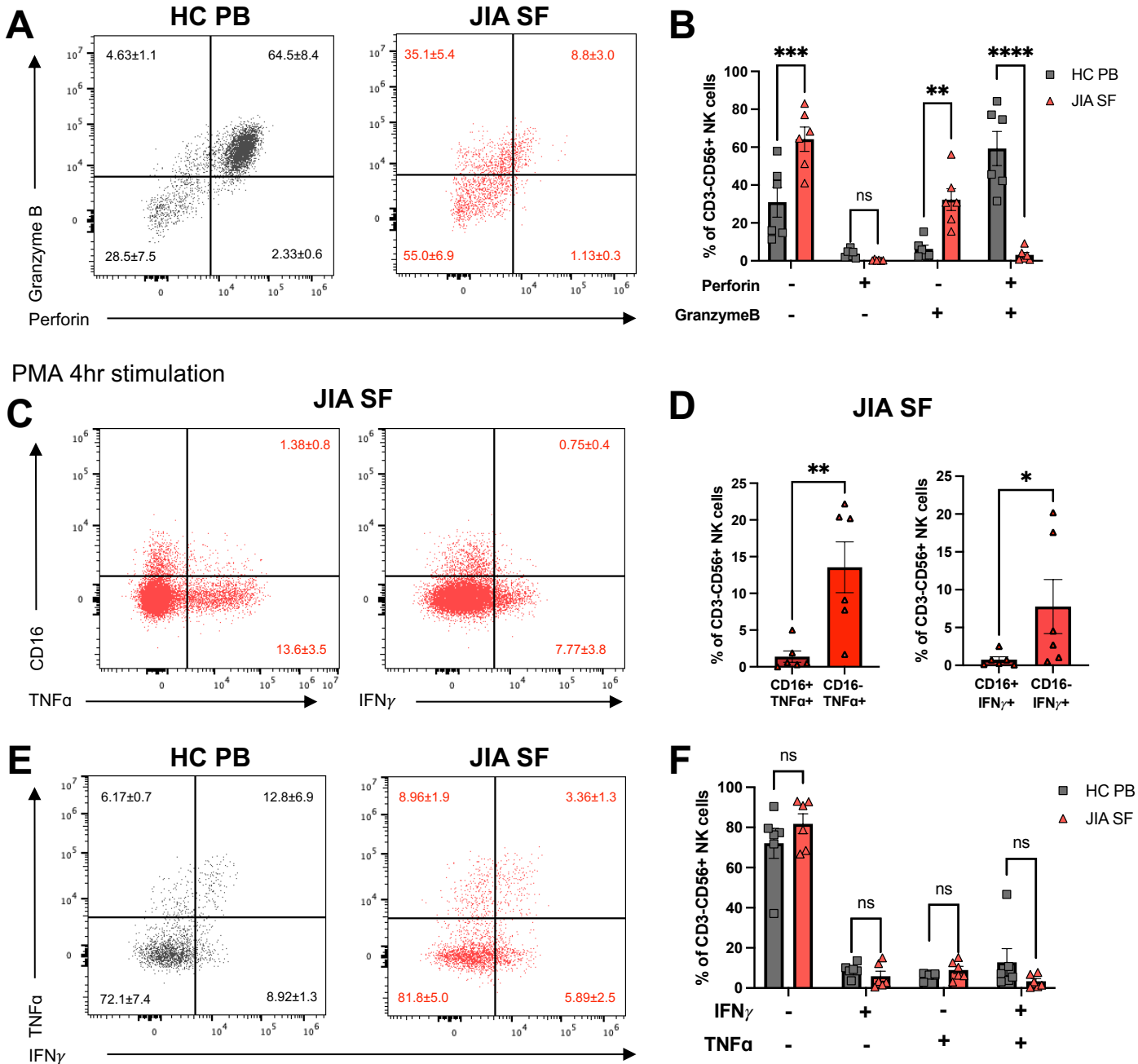
Gated on CD3+CD56- T cells

**A**



**B**



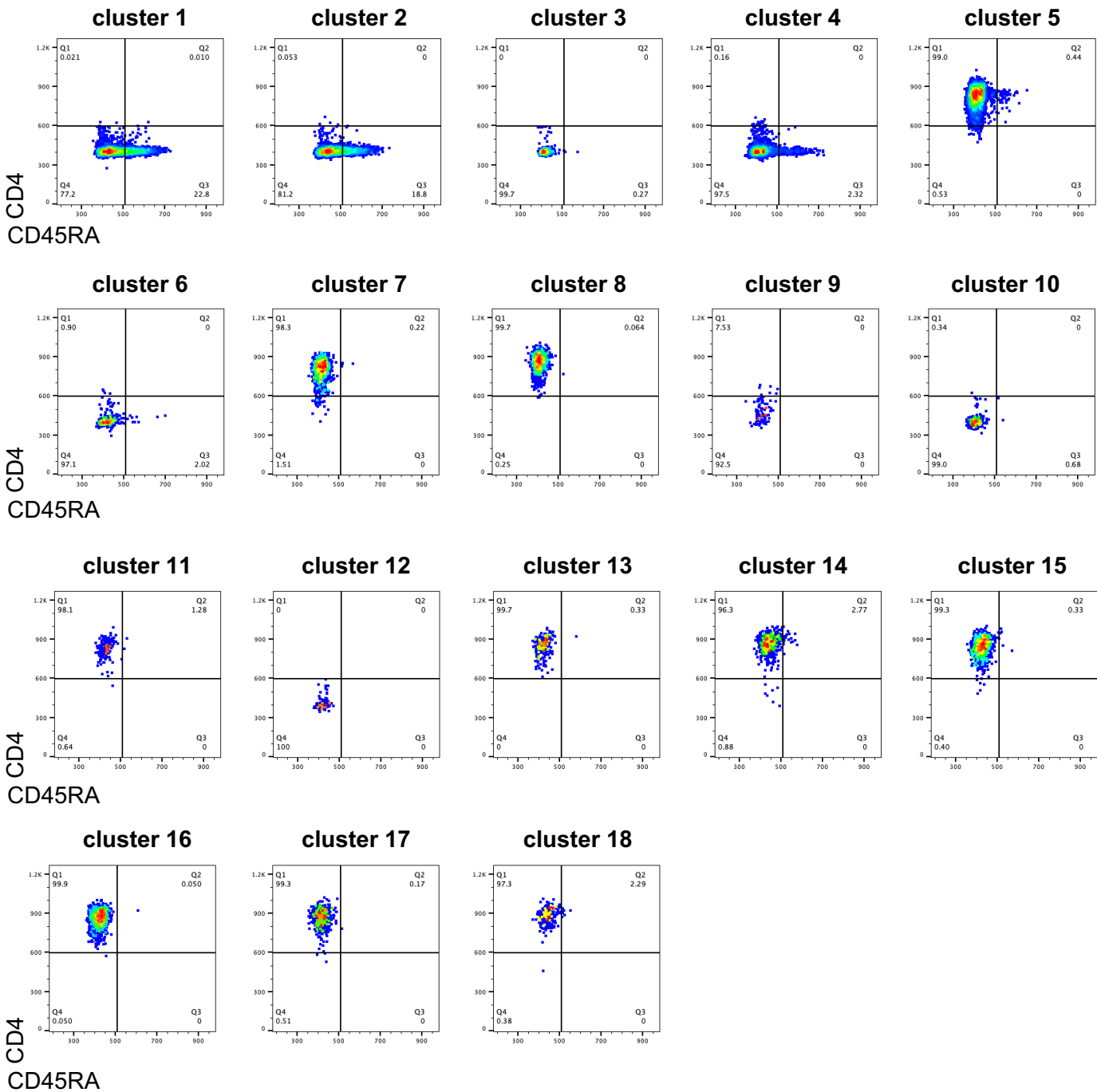Gated on CD3+CD56-CD4-

**C**



**D**



**Supplementary Figure S4. Lineage identities of CD3+ CD4- T cells in blood and synovial fluid**
Additional spectral flow cytometry panel run on healthy control peripheral blood (HC PB, n=6) and Juvenile Idiopathic Arthritis synovial fluid (JIA SF, n=6) to identify additional lineages of CD4- T cell populations described in cell composition and CD3+ clustering. A) Percentages of CD4+, CD8+, CD4-CD8- and TCRγ$\delta$ of all live CD3+ T cells in HC PB and JIA SF, concatenated plot of all samples for each group with percentages shown. B) Differences in frequencies of T cell lineages of all live CD3+ between HC PB and JIA SF. C) Identities of CD3+CD4- T cells by CD8 and/or TCRγ$\delta$ expression in HC PB and JIA SF. Concatenated plots of all samples for each group with percentages shown.
mean±SEM displayed. Significance determined by two-way ANOVA with Tukey's multiple comparison post hoc test.
*p<0.05, **p<0.01, ****p<0.0001, ns= not significant (p≥0.05).

Gated on CD3-CD56+



PMA 4hr stimulation



**Supplementary Figure S5. Cytotoxicity and cytokine production of NK cells in blood and SF**
Additional spectral flow cytometry panels run on healthy control peripheral blood (HC PB, n=6) and Juvenile Idiopathic Arthritis synovial fluid (JIA SF, n=6). A) Expression of cytotoxic molecules perforin and granzymeB in NK cells (CD3-CD56+) in HC PB and SF, incubated for 4 hours with brefeldin A. Singular representative plot with mean±SEM of frequencies of all samples displayed. B) Differences in frequencies of cytotoxic molecule expression of NK cells between HC PB and JIA SF. C-D) TNFα and IFNγ expression in CD16+ and CD16- SF NK cells (CD3-CD56+) after 4 hours stimulation with PMA, ionomycin and brefeldin A. Concatenated plot of all samples with mean±SEM of frequencies of all samples displayed. E) Expression of TNFα and IFNγ in NK cells (CD3-CD56+) in HC PB and SF, incubated for 4 hours with PMA, ionomycin and brefeldin A. Singular representative plot with mean±SEM of frequencies of all samples displayed. F) Differences in frequencies of cytokine expression of NK cells between HC PB and JIA SF. mean±SEM displayed. Significance determined by Mann-Whitney test (D) or by two-way ANOVA with Tukey's multiple comparison post hoc test (B and F). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns= not significant (p≥0.05).
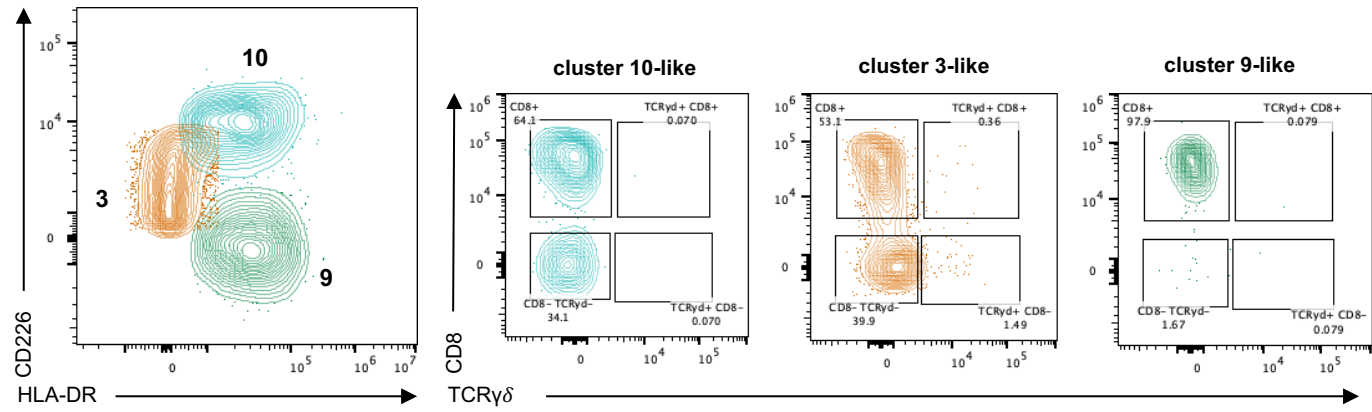
# JIA SF CD3+



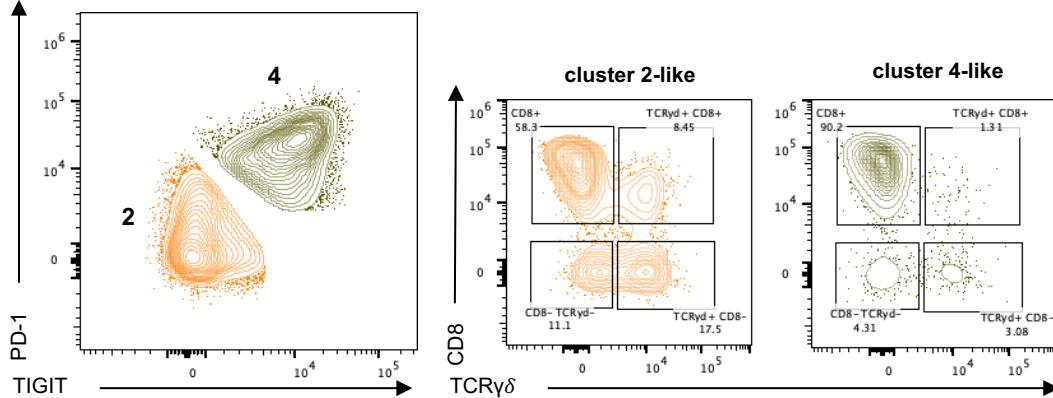**Supplementary Figure S6. The majority of SF CD3+ T cells have a memory phenotype**
Representative FACS plots of each cluster, normalised and concatenated from all JIA SF CD3+ T cell samples (n=18) in CD3+ T cell clustering (clusters 1-18). Frequencies of quadrants displayed as a proportion of each cluster.
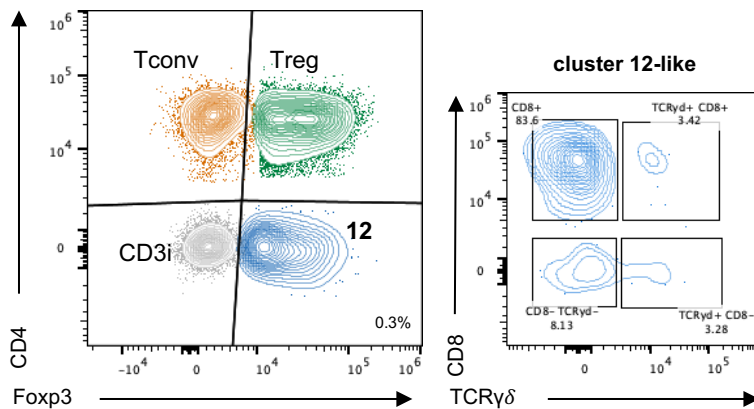
Gated on CD4- from JIA SF CD3+ T cells

**A**



**B**



**C**  Gated on JIA SF CD3+ T cells



**Supplementary Figure S7. Lineage identities of CD3+ CD4- T cell clusters in SF**
Additional spectral flow cytometry panel run on Juvenile Idiopathic Arthritis synovial fluid (JIA SF, n=6) to identify additional lineages of CD4- SF T cell populations described in CD3+ clustering. 'Cluster-like' populations were identified by the following gating strategies on CD3+CD56-CD4-: CD161-CD69+TIGIT-PD-1low (cluster 2); CD161+CD39-HLA-DR-CD226int (cluster 3); CD161-CD69+PD-1+TIGIT+ (cluster 4); CD161+CD39+HLA-DR+CD226- (cluster 9); CD161+CD39+HLA-DR+CD226high (cluster 10). Cluster 12 was gated on CD4-Foxp3+ from all live CD3+ T cells. CD8 and TCRγδ expression of A) cluster3,9 and 10-like, B) cluster2 and 4-like, and C) cluster 12-like CD4- T cells in SF. Concatenated plots of all samples for each group with percentages shown.

Gated on CD8+ within CD4- clusters from JIA SF CD3+ T cells
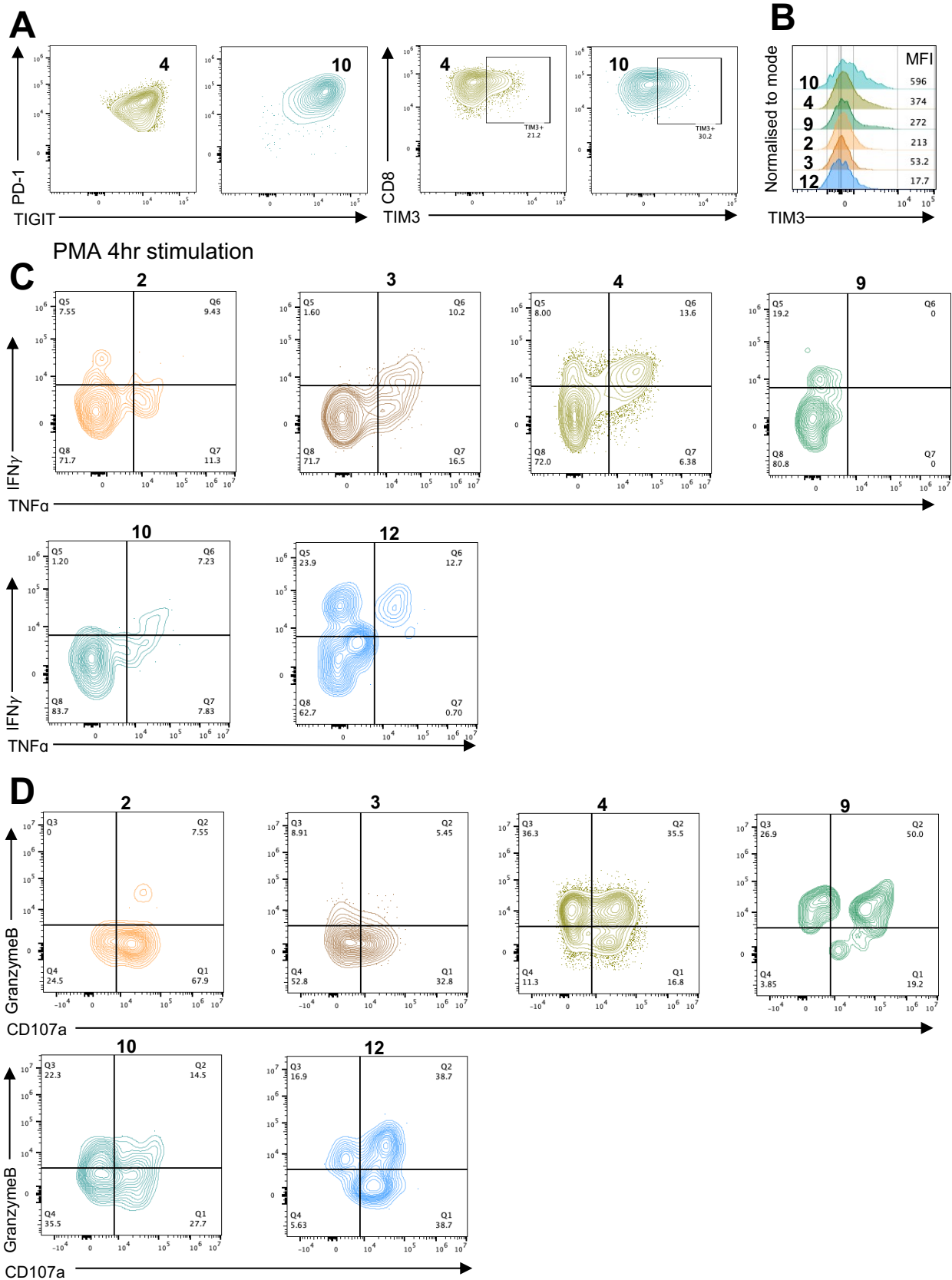


**Supplementary Figure S8. Cytokine production and cytotoxic capabilities of CD8+ T cells within CD4- SF clusters**
Additional spectral flow cytometry panels run on Juvenile Idiopathic Arthritis synovial fluid (JIA SF, n=6) to identify additional lineages of CD4- SF T cell populations described CD3+ clustering. 'Cluster-like' populations were identified by gating strategies as described in supplementary figure S5, with further gating on CD8+. A) TIM3 expression on SF TIGIThighPD-1high clusters (cluster 4 and 10-like) with B) median fluorescence intensities (MFI) compared to other SF cluster-like populations. Cytokine (C) production and cytotoxic molecules (D) produced by each cluster-like CD4- T cell population in SF assessed after 4 hours PMA, ionomycin and brefeldin A stimulation. Concatenated plots of all samples for each group with percentages shown.

**Table S2. JIA PB cohort divided by clinically active or inactive disease**
Of all JIA PBMC samples, 'Active' Juvenile Idiopathic Arthritis (JIA) PB classified by one or more clinically active joint; 'Inactive' JIA PB classified by no clinically active joints at time of sample of those where AJC data was available. Shown as mean (range) unless otherwise specified.

| | JIA PBMC 'Inactive' | JIA PBMC 'Active' |
|---|---|---|
| **Number of participants, n** | 17 | 29 |
| **Gender, % Female** | 88.2 | 65.5 |
| **Age at sample in years, mean (range)** | 9.6 (2-18) | 5.7 (1-16) |
| **Ethnicity, % Caucasian** | 82.3 | 62.1 |
| **Disease duration:** | | |
| **Time since diagnosis, months (range)** ◊ | 47.1 (3-125) | 31.6 (2-105) |
| **JIA subtype:** | | |
| **% RF- Polyarticular** | 64.7 | 21.4 |
| **% Oligoarticular** | 35.3 | 78.6 |
| **Medication at time of sample:** | | |
| **Methotrexate, n** | Yes= 11 No= 6 | Yes= 15 No= 14 |
| **Steroids, n** ▽ | Yes= 10 No= 7 | Yes= 13 No= 15 |
| **Biologics, n** | Yes= 1 No= 16 | Yes= 3 No= 26 |
| **Clinical information at time of sample:** | | |
| **ANA, n** # | positive = 7 negative = 4 | positive = 21 negative = 6 |
| **AJC, mean (range)** | 0 (0) | 2.03 (1-5) |
| **cJADAS, mean (range)** ⌃ | 1.8 (0-8.6) | 7.9 (1.7-17.3) |

PBMC= Peripheral Blood Mononuclear Cells; RF-: Rheumatoid factor negative; ANA: Antinuclear Antibodies (positive classified as titre ≥1:160); AJC: Active Joint Count; cJADAS: clinical Juvenile Arthritis Disease Activity Score
◊ 5.9% of Inactive JIA PBMC samples missing data; ▽ 2.4% of Active JIA PBMC samples missing data; # 35.2% of Inactive JIA PBMC and 6.9% of Active JIA PBMC samples missing data; ♦11.5% of JIA PBMC samples missing data; ⌃11.8% of Inactive JIA PBMC and 13.8% of Active JIA PBMC samples missing data