RESEARCH ARTICLE

Figures and figure supplements

Compartmentalization and persistence of dominant (regulatory) T cell clones indicates antigen skewing in juvenile idiopathic arthritis

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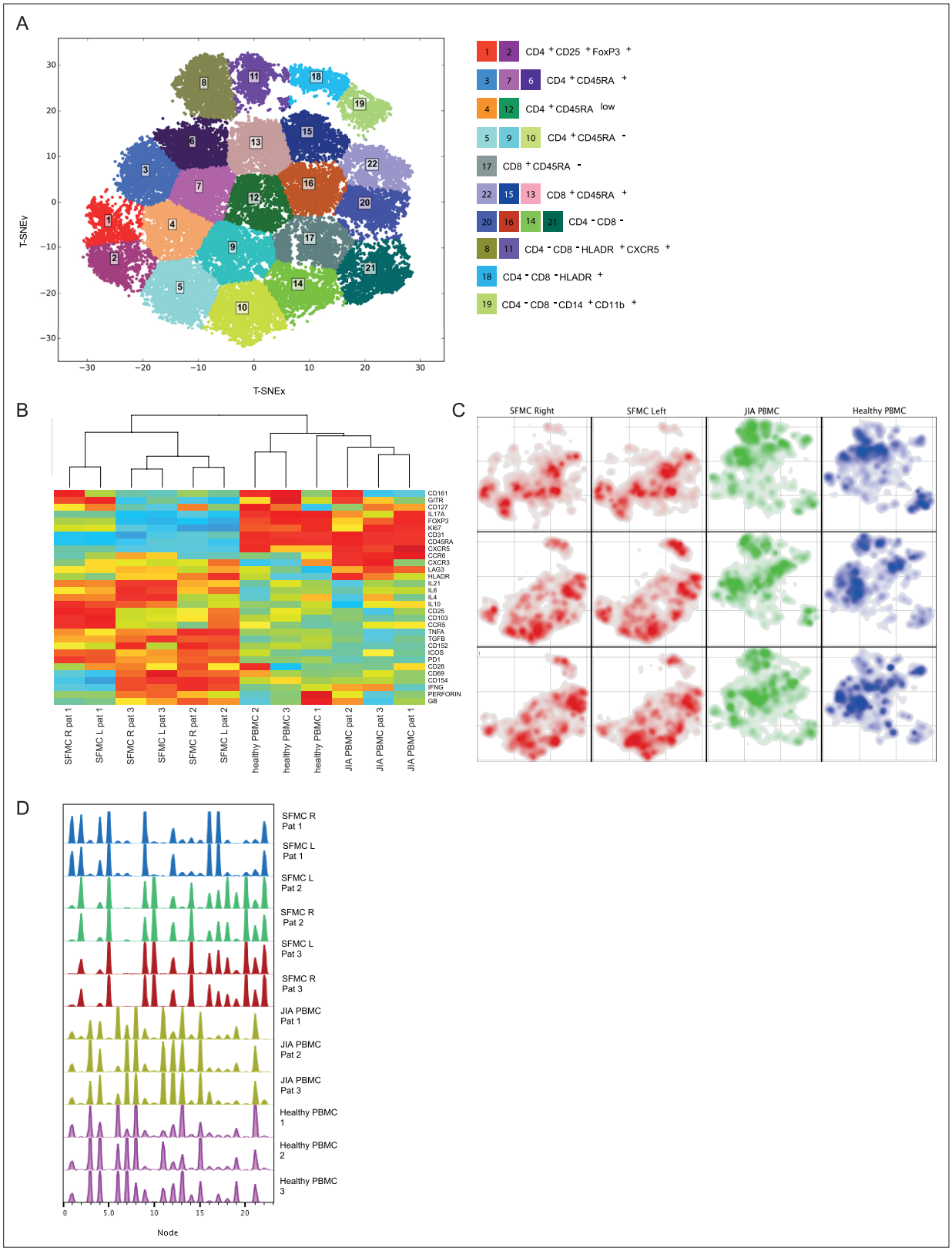

Figure 1. Overall immune architecture in left and right affected joints is very similar but distinct from peripheral blood. (**A**) Density maps based on t-SNE dimensional reduction and k-means clustering analysis on SF and PB samples, resulting in 22 cellular nodes. (**B**) Preliminary hierarchal clustering on the median expression of all markers, excluding lineage markers. (**C**) Density maps of immune cellular populations within the t-SNE maps. (**D**) Node frequency fingerprints showing the distribution across the nodes of SFMCs and PBMCs. PB, peripheral blood; PBMC, peripheral blood mononuclear cell; SF, synovial fluid; SFMC, synovial fluid mononuclear cell; t-SNE, t-distributed stochastic neighbor embedding.

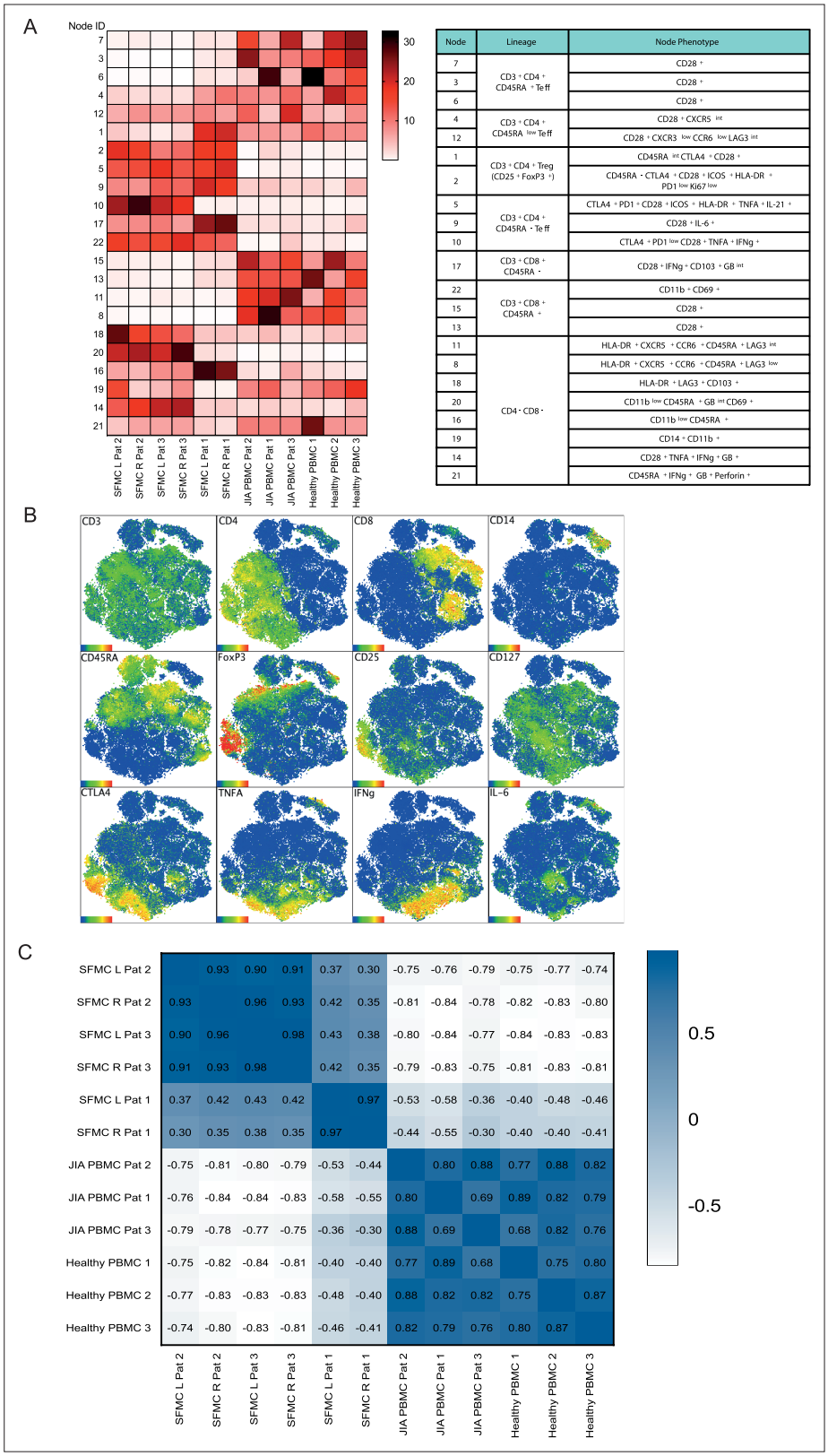

Figure 1—figure supplement 1. Preliminary analysis reveals correlation between SFMC from distinct joints. (**A**) Node frequency showing the distribution of T cell markers across the nodes of SFMCs an PBMCs in the CyTOF analysis. (**B**) Marker expression of t-SNE dimensional reduction and k-means clustering analysis on SFMC and PBMC samples (**C**) Correlation matrix using spearman correlation of the entire spectrum of node frequency given

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in (**A**). PBMC, peripheral blood mononuclear cell; SF, synovial fluid; SFMC, synovial fluid mononuclear cell; t-SNE, t-distributed stochastic neighbor embedding.

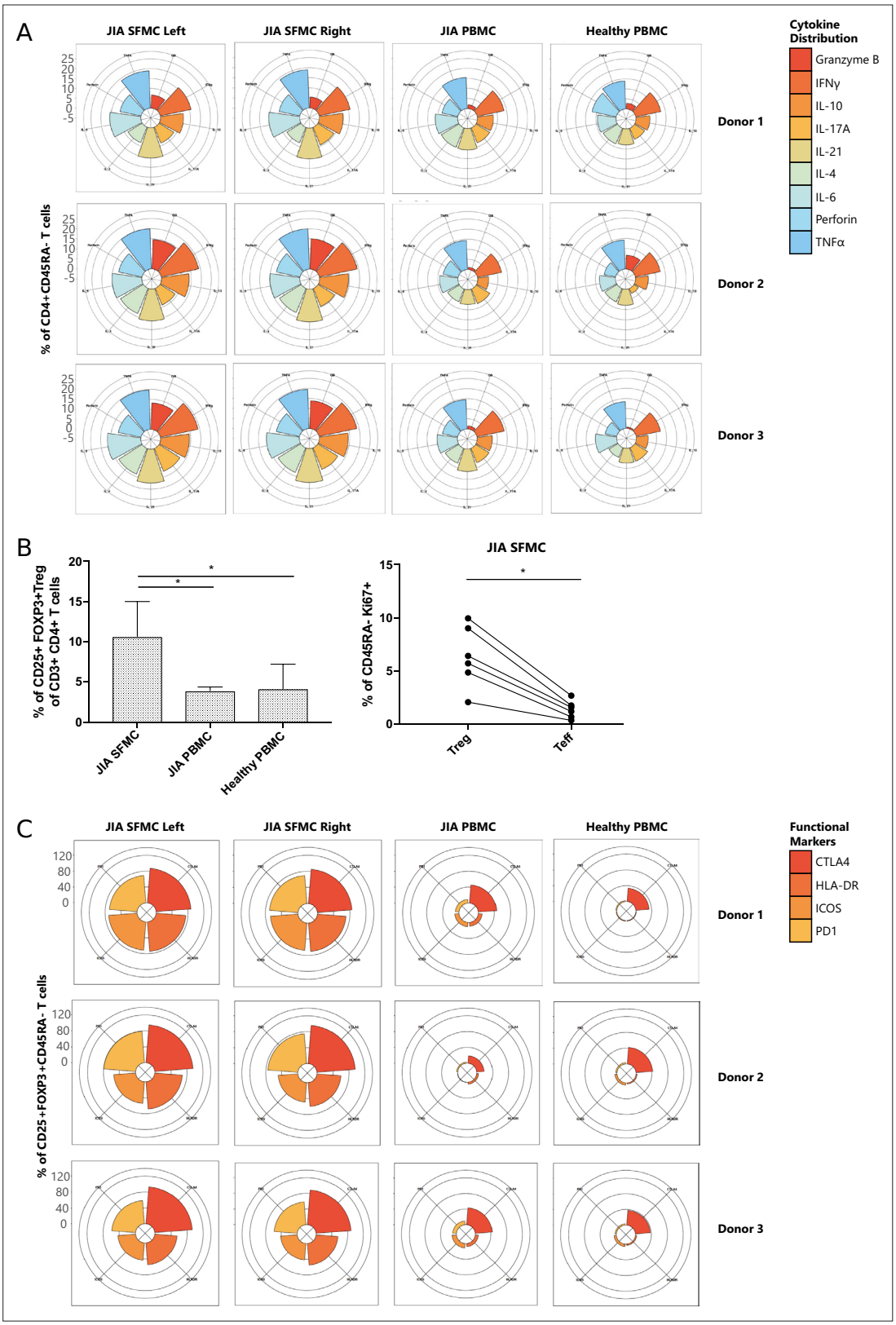

Figure 2. T cells display similar phenotypical and functional profiles at distinct inflamed locations. (**A**) Cytokine production of CD4⁺CD45RA⁻ memory T cells depicted in radar plots. Axis indicates the proportion of positive cells for individual cytokines (indicated by coloring) within the memory T cell fraction. (**B**) Percentage CD25⁺FOXP3⁺ Treg of CD3⁺CD4⁺ cells in SFMC and PBMC of JIA patients and healthy children, and percentage of Ki67⁺ cells within CD45RA⁻ cells in Treg and non-Treg in SFMC (nonparametric Mann-Whitney, *=p<0.05). For SFMCs, data from the right and left knee joints for

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all patients is shown. (**C**) Expression of functional markers by $CD25^{+}FOXP3^{+}CD45RA^{-}$ cells. JIA, juvenile idiopathic arthritis; PBMC, peripheral blood mononuclear cell; SFMC, synovial fluid mononuclear cell.

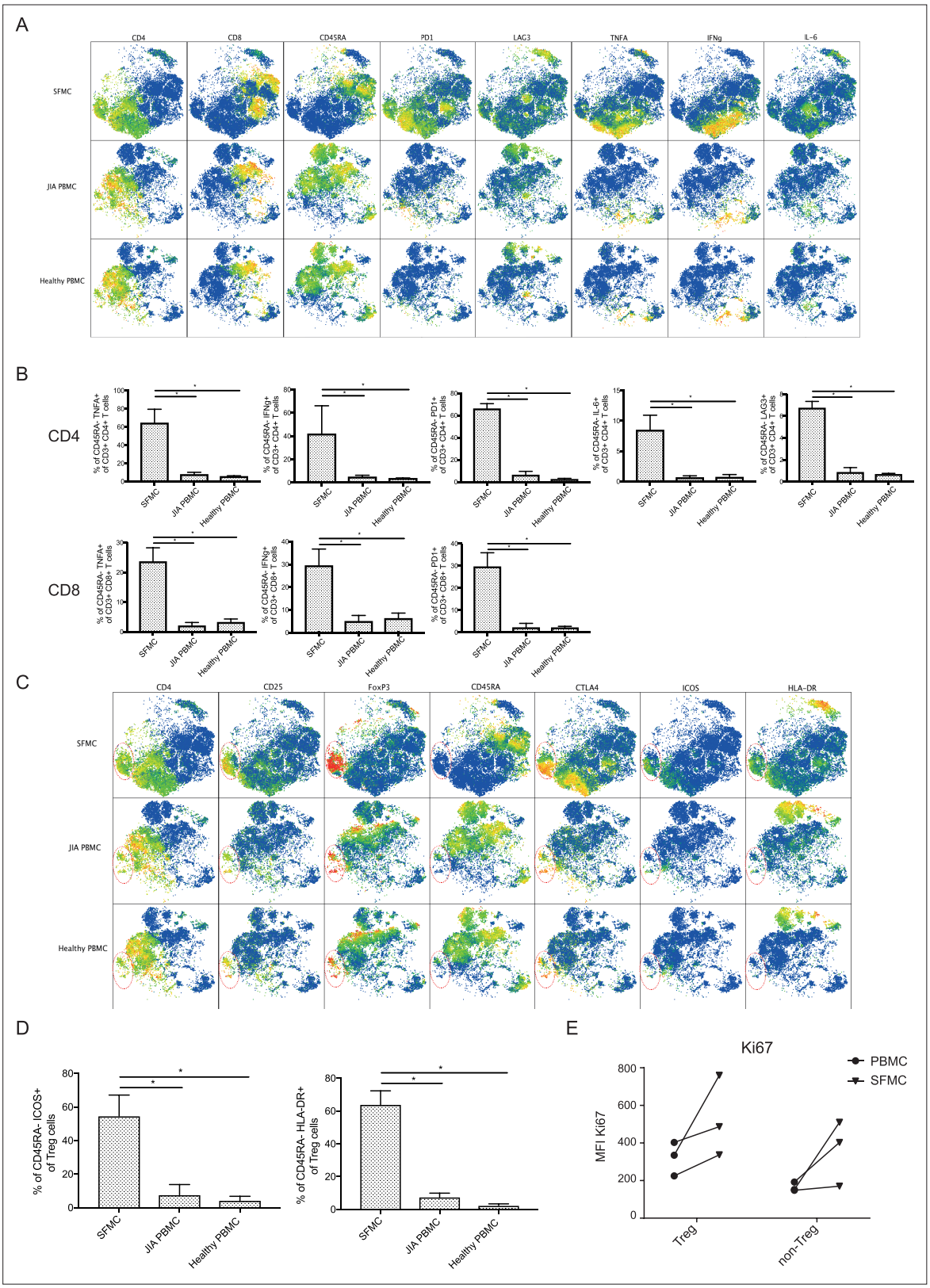

Figure 2—figure supplement 1. JIA SFMCs display an activated expression profile. (**A**) t-SNE plots showing the expression profile of phenotypical and functional markers in SFMC and PBMC from JIA patients and PBMC from healthy children. (**B**) Bar charts showing the percentage of specific cell populations within $CD4^{+}CD45RA^{-}$ and $CD8^{+}CD45RA^{-}$ cells (nonparametric Mann-Whitney, *=p<0.05). (**C**) TtSNE plots showing the expression profile of phenotypical and functional Treg markers in SFMC, PBMC from JIA patients and PBMC from healthy children. (**D**) Quantification of $CD45RA^{-}ICOS^{+}$ and

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CD45RA^{-}HLA^{-}DR^{+} expression on CD25^{+}FOXP3^{+} Treg (nonparametric Mann-Whitney, *=p<0.05). (**E**) MFI of Ki67 protein expression in Treg and non-Treg as determined by flow cytometry. JIA, juvenile idiopathic arthritis; PBMC, peripheral blood mononuclear cell; SFMC, synovial fluid mononuclear cell; t-SNE, t-distributed stochastic neighbor embedding.

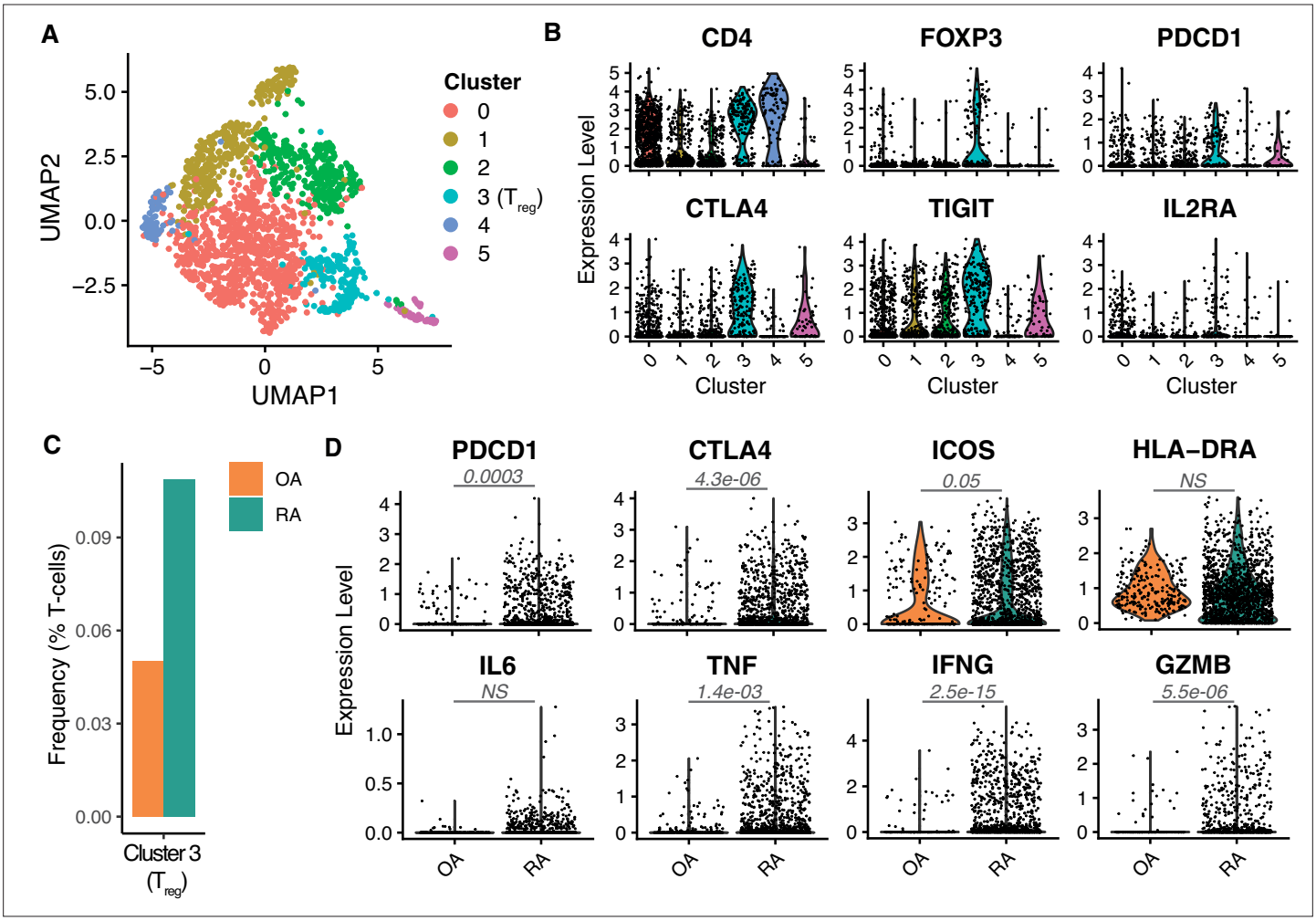
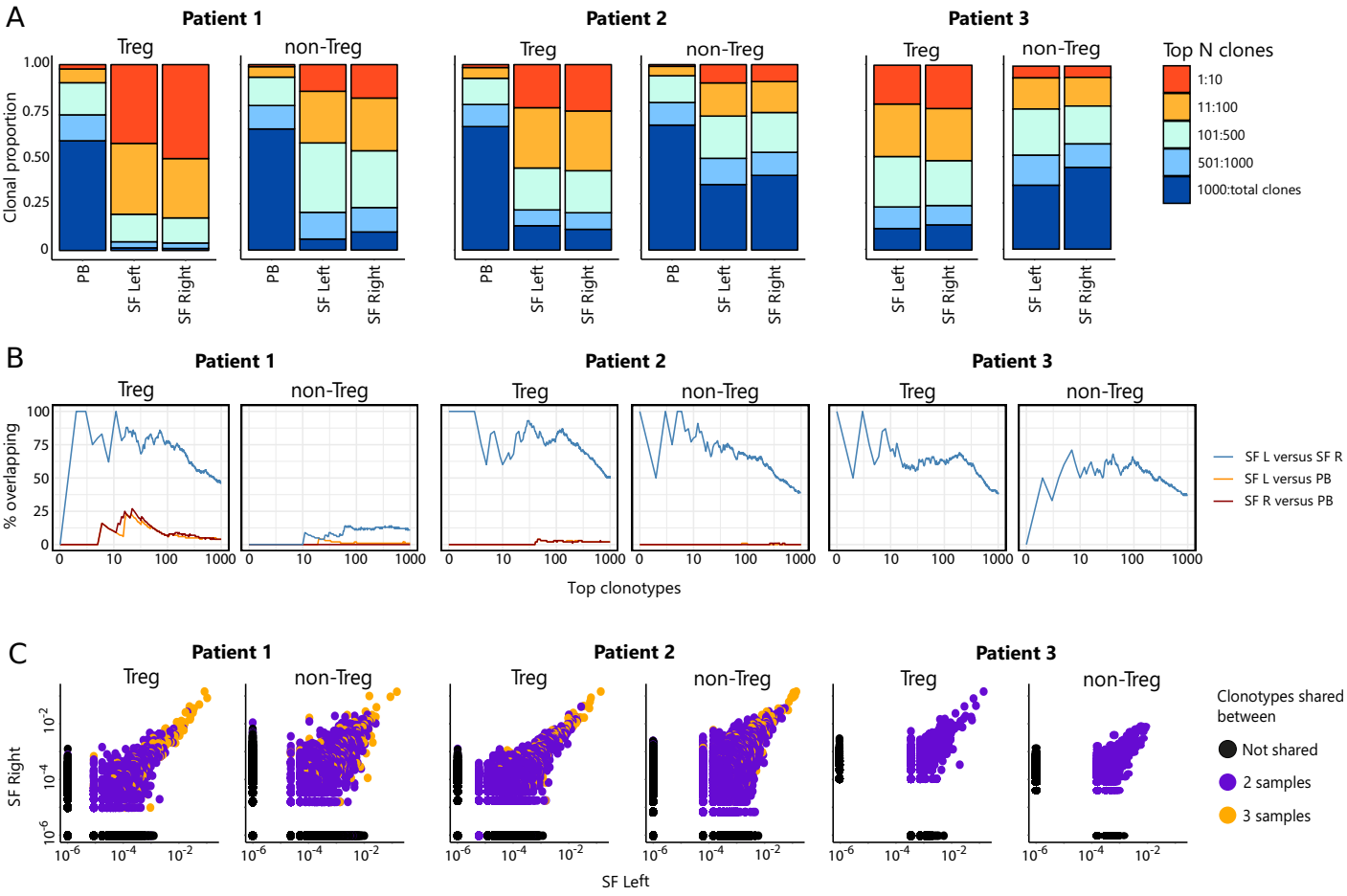
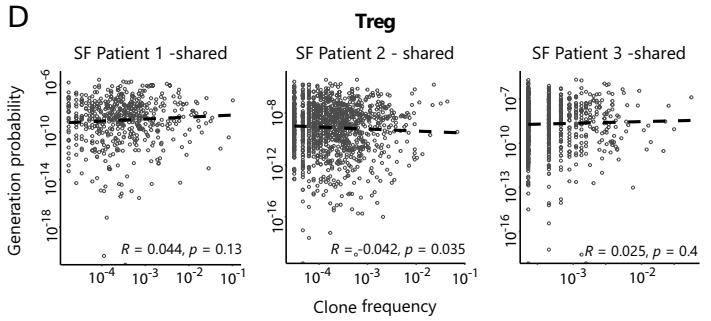


Figure 2—figure supplement 2. Tregs are increased in autoimmune rheumatic disease and express markers of enhanced activation. (**A**) UMAP of scRNA-seq data of T cells obtained from RA and OA patients. Colors indicate different clusters, with cluster 3 being classified as Tregs. (**B**) Expression of prototypical Treg markers across different clusters identified in (**A**). (**C**) Frequency of Tregs as a percentage of the total number of T cells (y-axis) in OA (orange) and RA (green) patients. (**D**) Expression of markers associated with chronic TCR activation (*PDCD1*, *CTLA4*, and *ICOS*), and cytokines (*TNF*, *IFNG*, and *GZMB*) across OA (orange) and RA (green) patients. Numbers indicate p values (Wilcoxon rank-sum test) of the comparison between OA and RA. OA, osteoarthritis; NS, not significant; RA, rheumatoid arthritis.





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Pat.	Cell type	Overlap	*R*	*p*
1	Treg SF	Shared	0.044	0.13
1	Treg SF	Only right	0.068	0.021
1	Treg SF	Only left	0.044	0.1
1	non-Treg SF	Shared	0.0094	0.71
1	non-Treg SF	Only right	0.27	<2.2e−16
1	non-Treg SF	Only left	0.32	<2.2e−16
2	Treg SF	Shared	-0.042	0.035
2	Treg SF	Only right	0.017	0.37
2	Treg SF	Only left	-0.001	0.95
2	non-Treg SF	Shared	0.0076	0.61
2	non-Treg SF	Only right	0.27	<2.2e−16
2	non-Treg SF	Only left	0.16	<2.2e−16
3	Treg SF	Shared	0.025	0.4
3	Treg SF	Only right	0.063	0.00042
3	Treg SF	Only left	0.044	0.094
3	non-Treg SF	Shared	0.039	0.064
3	non-Treg SF	Only right	0.08	2.9e−16
3	non-Treg SF	Only left	0.086	2.2e−06

Figure 3. Highly dominant T cell clones are shared in synovial fluid (SF) from left and right joints and peripheral blood (PB). (**A**) Clonal proportions of the TCRβ clones as detected in Treg and non-Treg sorted from PBMC, SF left joint, SF right joint of two different JIA patients. (**B**) Sequential intersection of abundant TCRβ clonotypes (based on amino acid sequence) across samples. Top clonotypes (ranging from 1 to 1000) are given on the x-axis, with the percentage of sequences overlapping between two given samples on the y-axis. For patient 3, no PB sample was available. (**C**) Frequency plots showing the overlapping Treg and non-Treg clones between left joint derived SF (x-axis) and right joint derived SF (y-axis), with color coding highlighting the

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clones that are shared with none of the other samples (black circle), shared in two samples (purple) and all three samples (PB, SF left, SF right; yellow). (**D**) Correlation (linear regression, dashed line) between frequency (x-axis) and generation probability (y-axis) of TCR clones shared across SF two samples. (**E**) Results of correlation between frequency and generation probability across all samples. p, p value; Pat., patient; PBMC, peripheral blood mononuclear cell; R, Spearman's Rho; SF, synovial fluid.

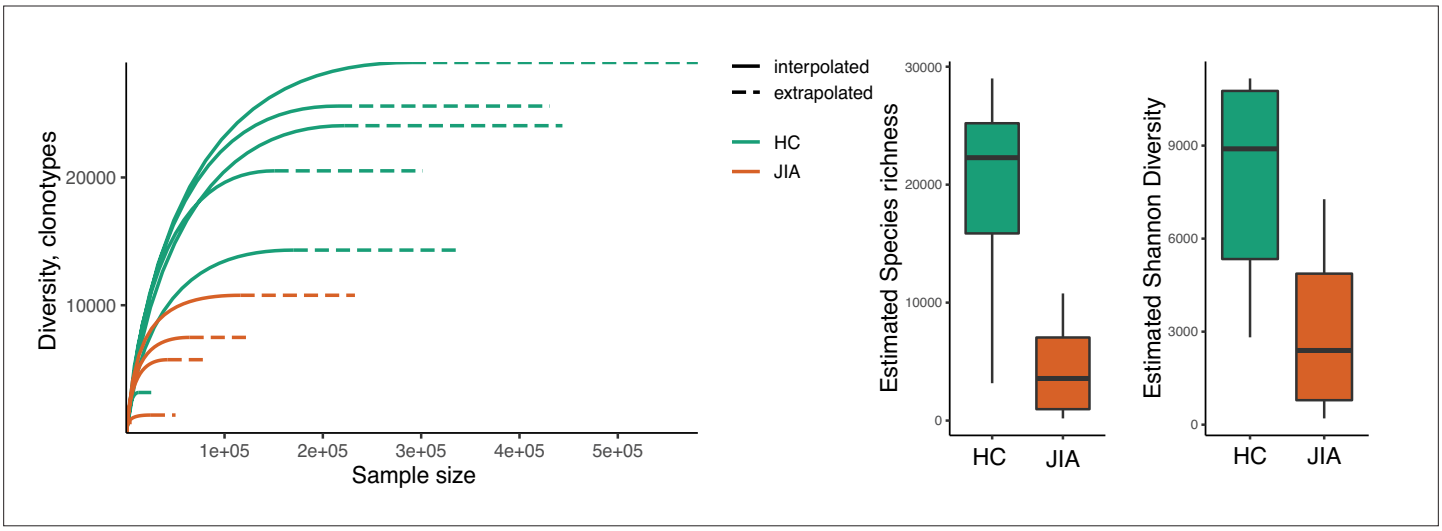

Figure 3—figure supplement 1. The JIA peripheral Treg repertoire is less diverse than healthy. Sample-based rarefaction and extrapolation curves. Solid lines depict observed data, dashed lines depict extrapolated data. Calculated for all healthy samples (green), and JIA samples (orange). Every line represents one individual sample. Boxplots show median of estimated species richness (left) and estimated Shannon diversity (right) calculated from the rarefied and extrapolated data shown in (**A**). JIA, juvenile idiopathic arthritis; HC, healthy control.

Figure 4. Persistence of Treg clones over the course of relapse remitting disease. (**A**) Heatmap showing overlap (Jaccard index, light blue = limited overlap, darkblue = high overlap) of Treg derived TCRβ sequences obtained from SF or PB from JIA patients over time. L=left knee, R=right knee. (**B**) Venn diagrams displaying the 100 most abundant unique TCRβ clones, defined by amino acid sequence, for longitudinal SF samples from all patients. (**C**) Frequency plots showing the overlapping Treg clones between visits for SF and PB, with color coding and shapes highlighting the number

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of samples in which unique clones are found. L=left; R=right. (**D**) Correlation (linear regression, dashed line) between frequency (x-axis) and generation probability (y-axis) of TCR clones shared across two visits for SF samples. PB, peripheral blood; SF, synovial fluid; TCR, T cell receptor.

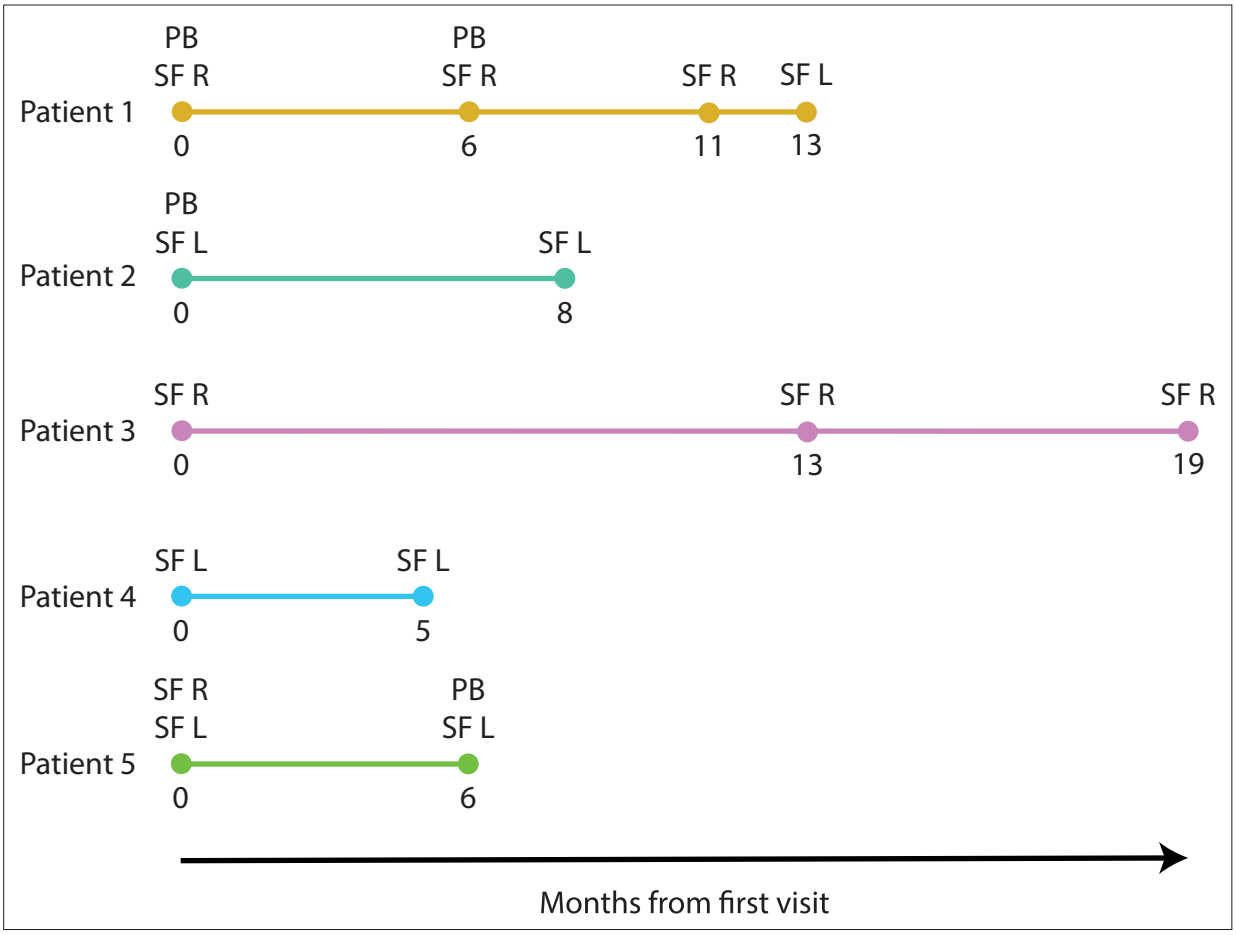

Figure 4—figure supplement 1. Longitudinal sampling timelines of JIA patients. JIA, juvenile idiopathic arthritis; L, left; PB, peripheral blood; R, right; SF, synovial fluid.

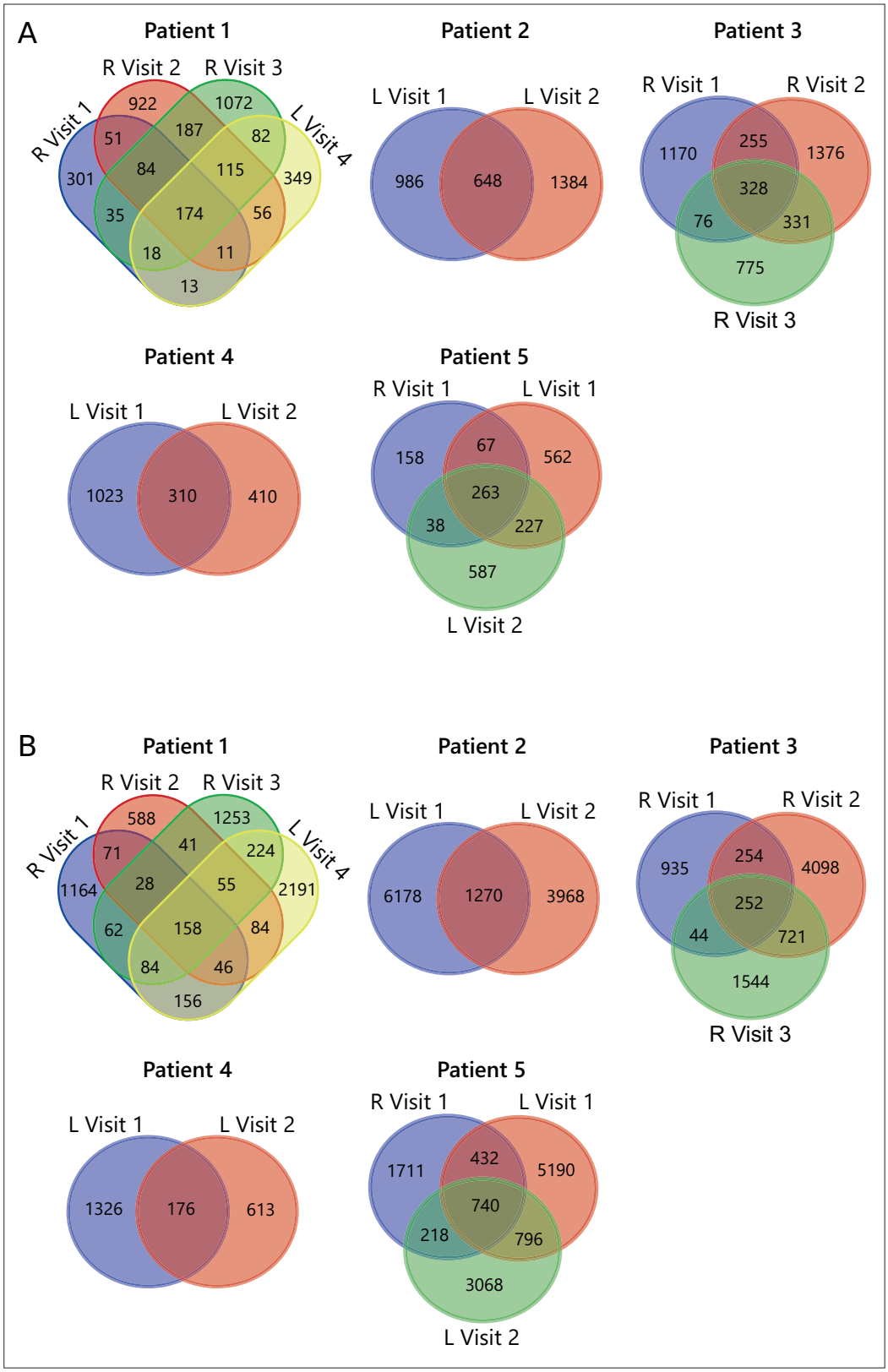

Figure 4—figure supplement 2. TCR overlap analysis. (**A**) Venn diagrams displaying the overlap of all unique TCRβ clones, defined by amino acid sequence, for longitudinal SF samples from all patients for Tregs and (**B**) non-Tregs. SF, synovial fluid; TCR, T cell receptor.

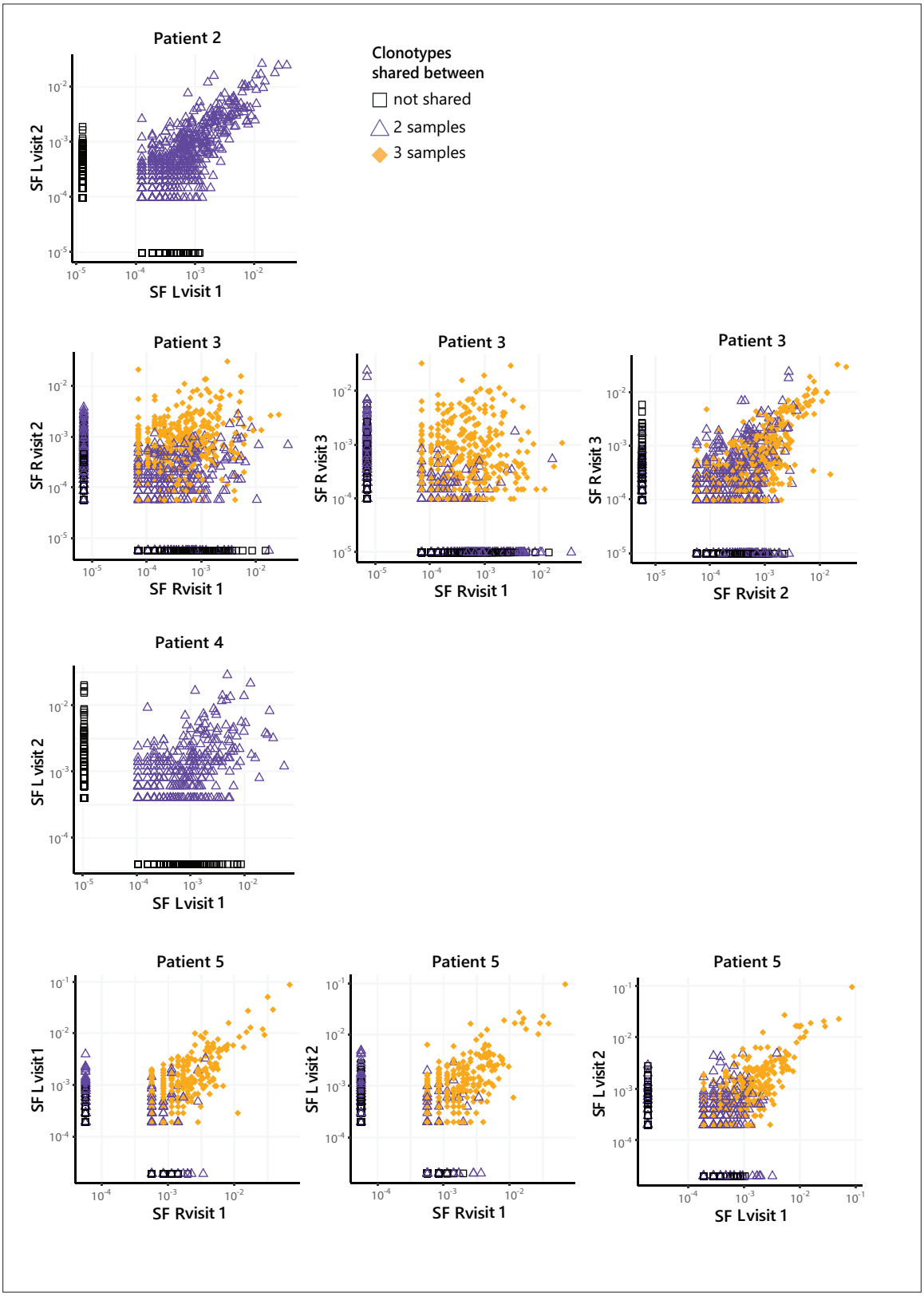

Figure 4—figure supplement 3. JIA Treg TCR frequencies over time in the remaining four patients. Frequency plots showing the overlapping Treg clones between visits for SF and PB, with color coding and shapes highlighting the number of samples in which unique clones are found. JIA, juvenile idiopathic arthritis; L, left; PB, peripheral blood; R, right; SF, synovial fluid; TCR, T cell receptor.

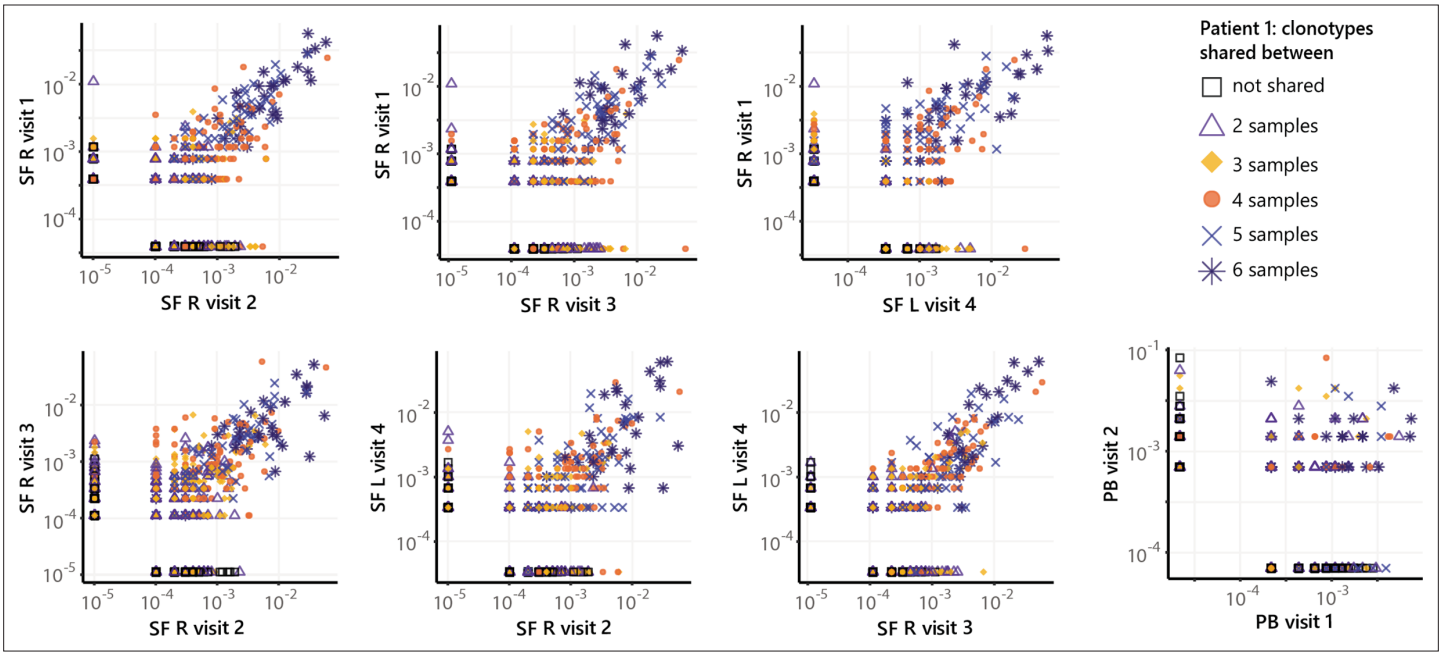

Figure 4—figure supplement 4. Frequencies of TCRs from persistent Tregs shared across SF and PB samples. Frequency plots showing the overlapping Treg clones between visits for SF and PB for patient 1, with color coding and shapes highlighting the number of samples in which unique clones are found. PB, peripheral blood; SF, synovial fluid; TCR, T cell receptor.

Figure 5. Persistence of non-Treg clones over the course of relapse remitting disease. (**A**) Heatmap showing overlap (Jaccard index, light blue = limited overlap, darkblue = high overlap) of non-Treg derived TCRβ sequences obtained from SF or PB from JIA patients over time. L, left knee, R, right knee. (**B**) Venn diagrams displaying the 100 most abundant unique TCRβ clones, defined by amino acid sequence, for longitudinal SF samples from all patients. (**C**) Frequency plots showing the overlapping non-Treg clones between visits for SF and PB, with color coding and shapes highlighting the number of samples in which unique clones are found. L, left; R, right. (**D**) Correlation (linear regression, dashed line) between frequency (x-axis) and

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generation probability (y-axis) of TCR clones shared across two visits for SF samples. JIA, juvenile idiopathic arthritis; PB, peripheral blood; SF, synovial fluid; TCR, T cell receptor.

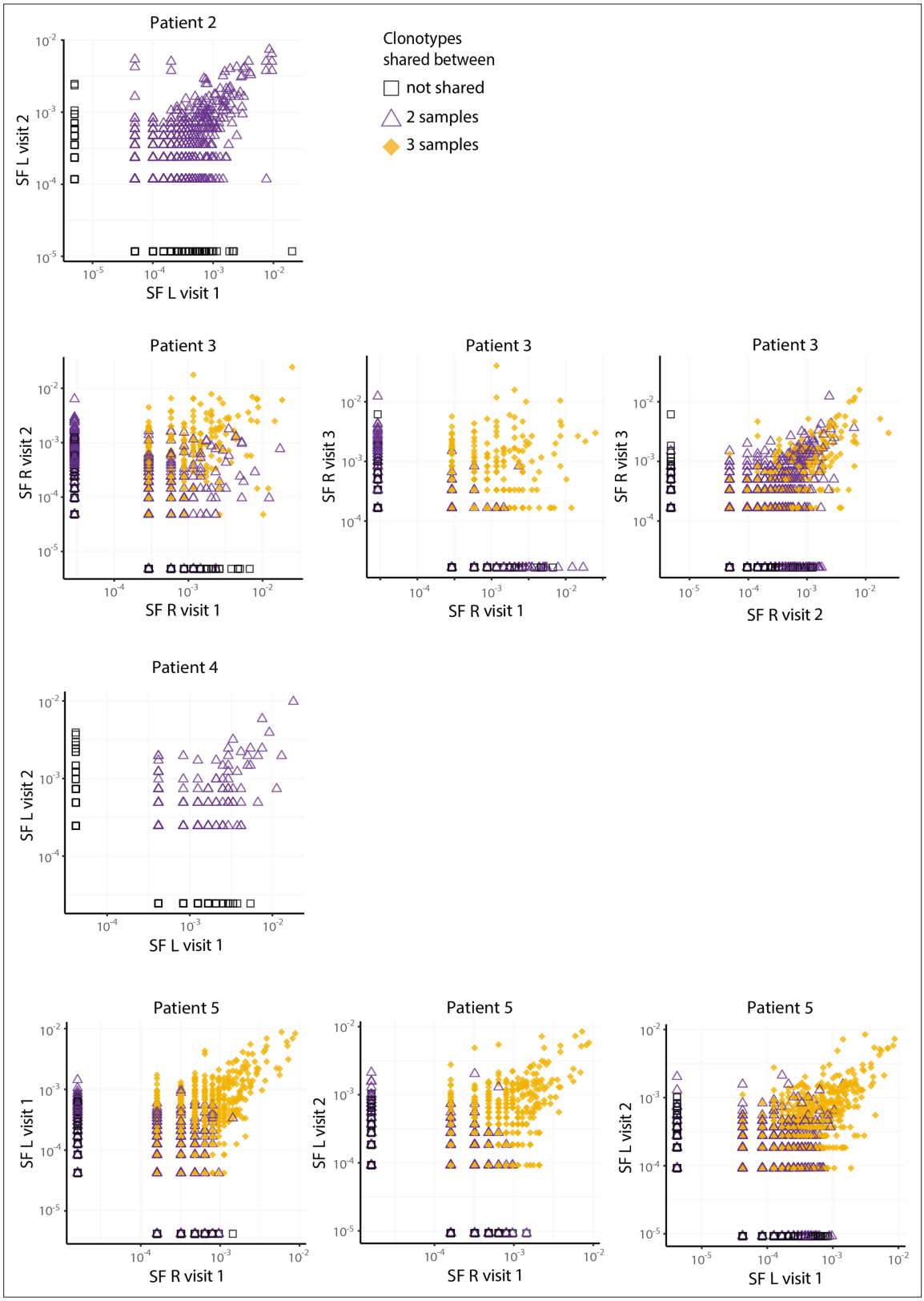

Figure 5—figure supplement 1. JIA non-Treg TCRβ frequencies over time in the remaining four patients. Frequency plots showing the overlapping non-Treg clones between visits for SF and PB, with color coding and shapes highlighting the number of samples in which unique clones are found. JIA, juvenile idiopathic arthritis; L, left; PB, peripheral blood; R, right; SF, synovial fluid; TCR, T cell receptor.

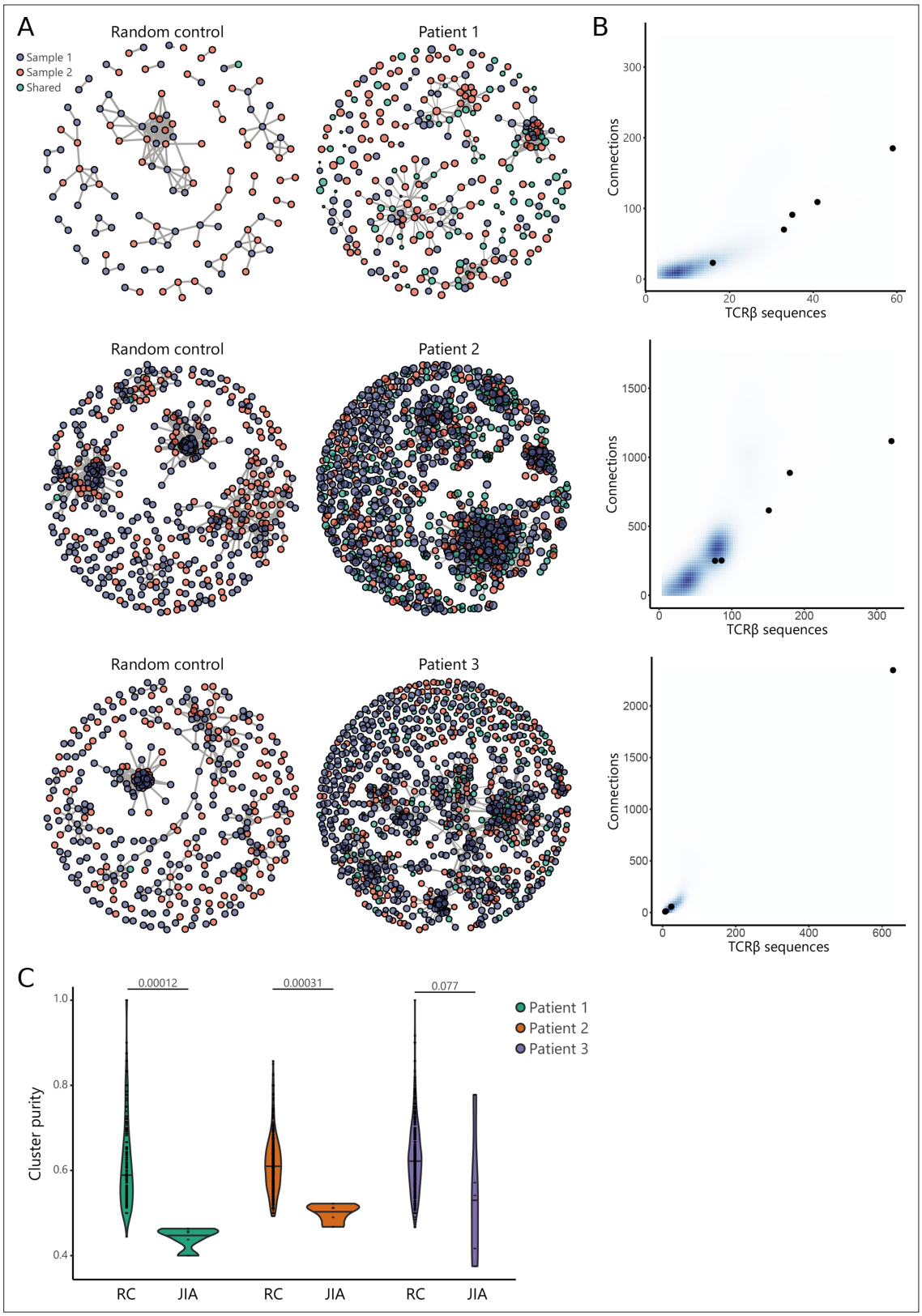

Figure 6. TCR similarity analysis of sequences found across distinct JIA patient knees. (**A**) TCR similarity networks based on amino acid k-mer sharing (k=3) between TCR sequences. Every node represents one TCRβ sequence, with sequences present in one sample (SF from left or right knees) highlighted in blue and orange, and sequences shared across two samples highlighted in green. Nodes are connected if TCRs share at least eight k-mers. Networks from JIA patient repertoires (right) are compared to random repertoires (left), with the same repertoire size. (**B**) Number of TCR

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sequences (x-axis) and their connections (y-axis) to other TCR sequences of the top five similarity clusters identified in (**A**). Blue density maps depict clusters identified in random repertoires (N=100), while black circles depict clusters identified in JIA patients. (**C**) Cluster purity (y-axis, %) for the top five clusters identified in random repertoires (RC), and JIA patient TCR similarity networks. Numbers indicate p-value of difference between RC and JIA (Mann-Whitney). JIA, juvenile idiopathic arthritis; RC, random repertoires; SF, synovial fluid; TCR, T cell receptor.