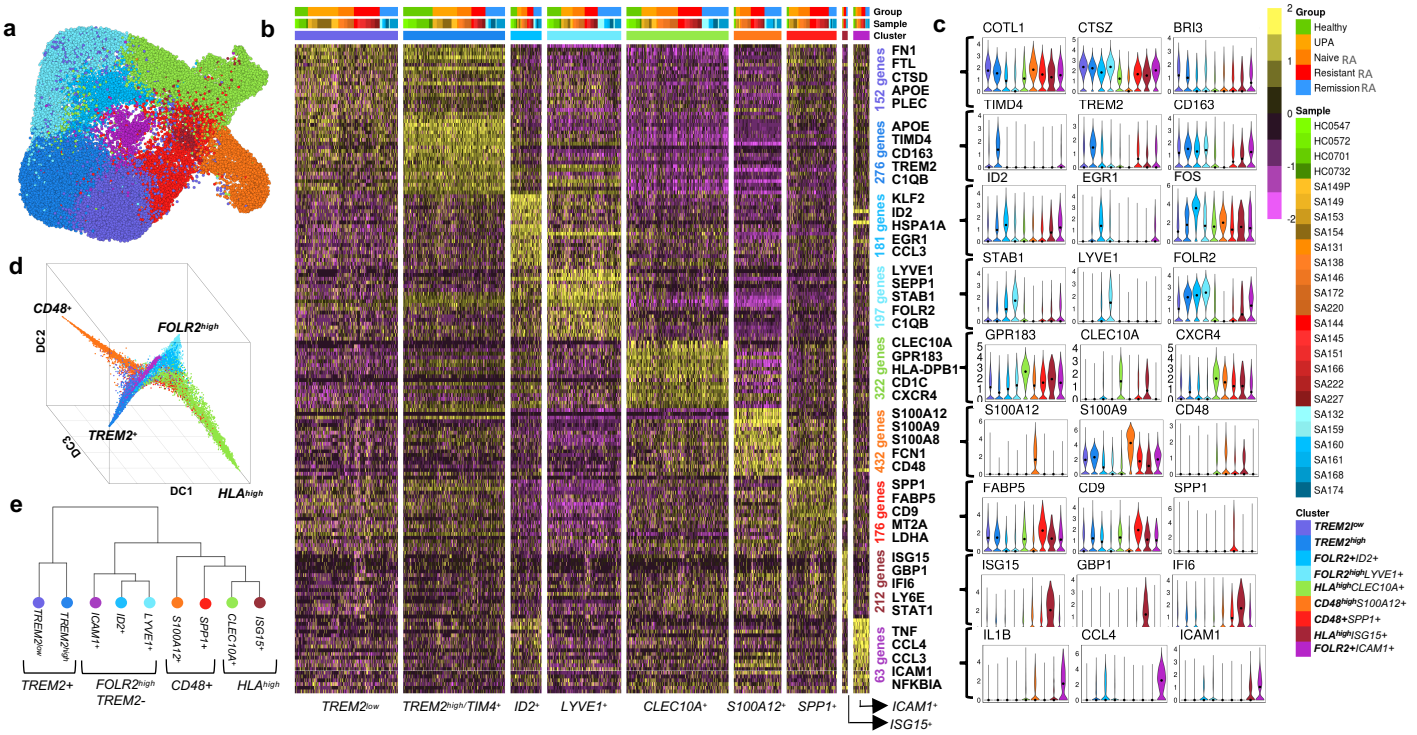

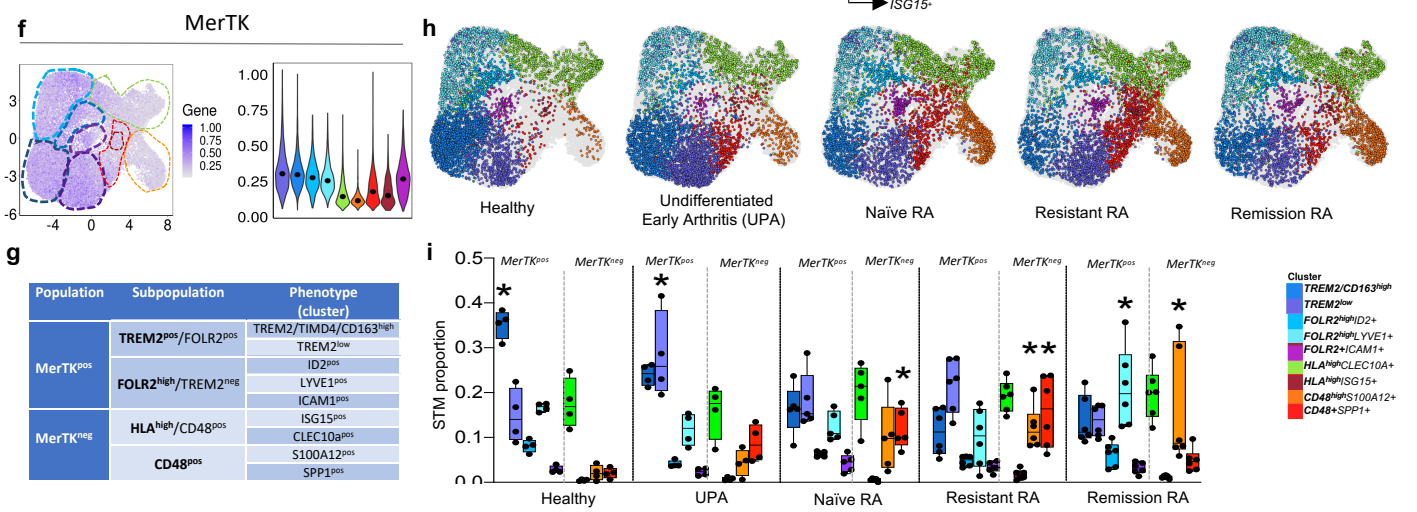

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Population	Subpopulation	Phenotype (cluster)
MerTK^{pos}	TREM2^{pos}/FOLR2^{pos}	TREM2/TIMD4/CD163^{high}
		TREM2^{low}
	FOLR2^{high}/TREM2^{neg}	ID2^{pos}
		LYVE1^{pos}
		ICAM1^{pos}
MerTK^{neg}	HLA^{high}/CD48^{pos}	ISG15^{pos}
		CLEC10a^{pos}
	CD48^{pos}	S100A12^{pos}
		SPP1^{pos}

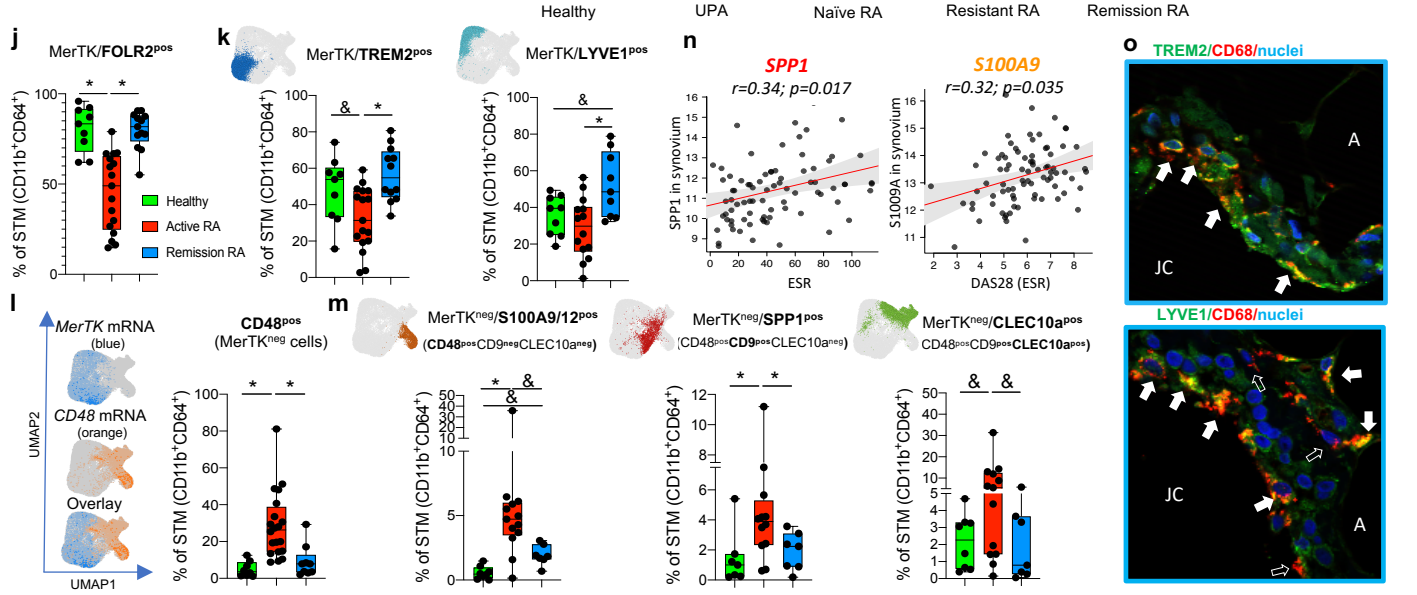

Figure 2. Single cell transcriptomics defines distinct STM subpopulations and phenotypes in different human joint immuno-conditions. (a) UMAP visualization of 9 clusters of synovial tissue macrophages identified by scRNAseq analysis; each cell represented by an individual point colored by cluster identity. (b) Heatmap illustrating scaled expression of the top 20 differentially expressed genes per cluster (rows are genes, columns are cells). Marker genes of interest are annotated, and the total number of genes differentially expressed by each cluster is written next to the cluster. All genes expressed in at least 40% cells in each cluster. Average log-fold change ≥0.25. (c) Expression of cluster marker genes on log-normalized violin plots; black dot (median). (d) Transcriptional relationship between clusters demonstrated by the top 50 PCs were embedded in the top 3 diffusion-map components. (e) Hierarchical clustering of STMs. (f) MerTK expression in the 9 STM clusters. (g) Proposed classification of human STMs based on scRNAseq and flow cytometry. (h) Split UMAP visualization demonstrating relative changes in the STM phenotypes between disease groups; cell numbers normalized to ~5,000/group. (i) Bar and dot plots illustrating changes in cluster distribution across conditions. Significant differences between the given condition and at least one other condition highlighted * (two-way ANOVA, with Tukey correction). Each dot represents individual patient. Flow cytometry validation of FOLR2/MerTKpos (j), TREM2/MerTKpos and LYVE1/MerTKpos (k) in healthy controls (n=10), active RA (n=17), and RA in remission (n=313) depicted by multi-panel flow cytometry. (l) An opposite expression of MerTK and CD48 mRNA in STMs clusters, suggesting that CD48 is a marker of MerTK negative STM clusters. An increased proportion of CD48pos cells in patients with active RA compared to healthy and RA in disease remission. (m) Flow cytometry validation of MerTKneg -S100A12pos cluster (defined by CD48pos/CD9neg/CLEC10aneg) (m), MerTKneg -SPP1pos cluster (defined by CD48pos/CD9pos/CLEC10aneg) and MerTKneg -CLEC10a cluster (defined by CD48pos/CD9pos/CLEC10apos) in healthy controls (n=8), active RA (n=13), and RA in remission (n=7) depicted by multi-panel flow cytometry. Dot plots show an increase in proportion of these clusters in active RA as compared to healthy and disease remission. (j-m) * p<0.05, one-way Anova adjusted for multiple comparison; &<0.05 Mann-Whitney test between two groups. (n) Correlation between synovial expression of S100A9 and SPP1 with disease activity in the validation PEAC cohort. **(o)** Representative confocal microscopy pictures of TREM2 or LYVE1 (green) and macrophage marker staining (CD68, red) of synovial tissue of RA in remission. A: Adipocytes; JC: Joint cavity.