Supplementary Material

Transcriptomic profiling of programmed cell death (PD-1) expressing T cells in early rheumatoid arthritis identifies a decreased CD4+PD-1+ signature post-treatment

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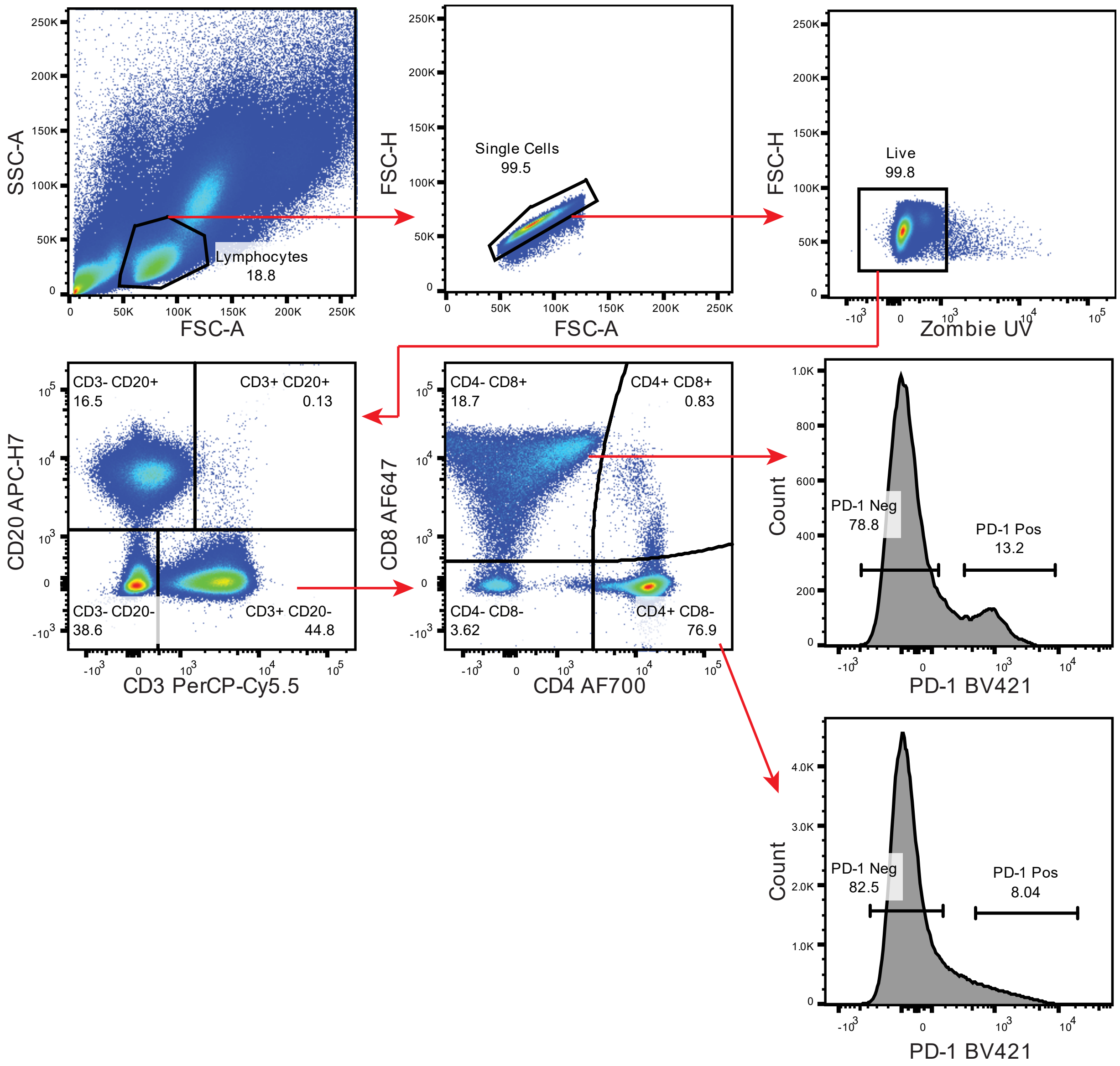
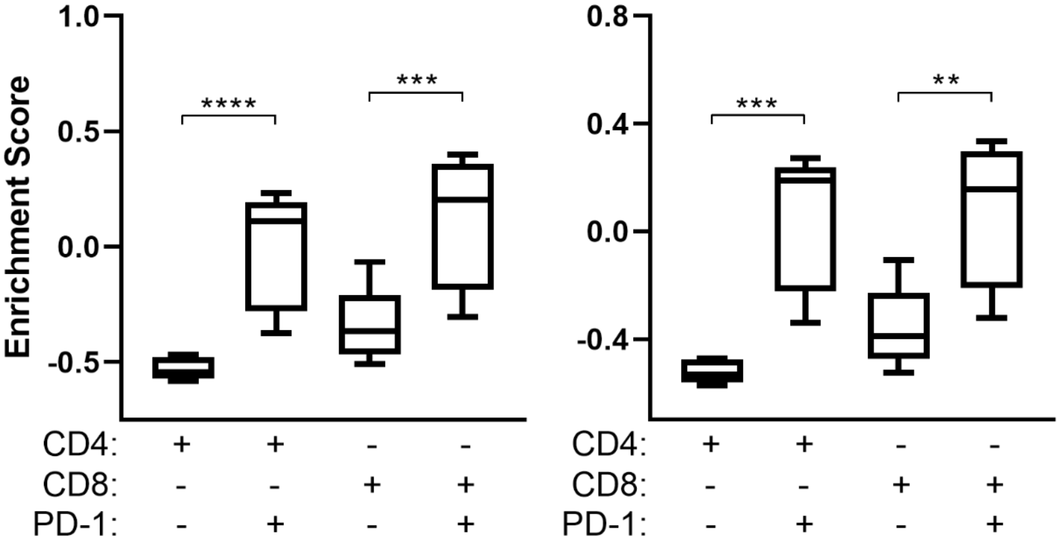
Number of tables: 1



Supplementary figure 1. Representative gating strategy used for the flow cytometric sorting of CD4$^+$/CD8$^+$ PD-1$^+$/PD-1$^+$ populations from isolated, fresh PBMCs. Lymphocytes were gated based on size and complexity, single cells were gated and judged as viable based on their ability to exclude Zombie UV viability dye, CD3$^+$ lymphocytes were gated, and from these, CD4$^+$ and CD8$^+$ populations were gated. Cells were then sorted based on their expression of PD-1 (positive vs. negative), resulting in the four sorted populations.

	Early RA baseline PBMC samples	**Early RA baseline ST samples**	**Early RA 6-month ST sample**
Number of subjects	5	19	19
Sex—female (%)	2 (40%)	8 (42%)	
Age (years)	Mean: 55 Range: 37-73	Mean: 50.84 Range: 33-72	
Disease duration at baseline (weeks)	Mean: 19.86 Range: 11-48	Mean: 17.58 Range: 4-40	
CCP (U/mL)	Min: 40 (3/5 with value >100)	Min: 11 (11/19 with value >100)	
RF (IU/mL)	Mean: 100.4 Range: 52-280 (1/5>900)	Mean: 130 Range:16-570 (2/19 RF negative)	
CRP (mg/dl)	Mean: 2 Range: 0.14-5.58	Mean: 2.41 Range: 0.069-9.2	Mean: 0.60 Range: 0.034-4.6
ESR (mm/hr)	Mean: 36.4 Range: 7-79	Mean: 39.42 Range: 7-108	Mean: 14.42 Range: 1-52
DAS-28 (ESR)	Mean: 5.19 Range: 4.03-6.55	Mean: 5.80 Range: 4.80-7.40	Mean: 3.10 Range: 1.08-5.56

Supplementary table 1. Patient demographics for the two cohorts used in this study. Early RA baseline PBMC samples (column 2, n=5) were sorted into CD4^{+}/CD8^{+} PD-1^{+} and PD-1^{-} T cell populations and subjected to bulk RNA sequencing. Early RA ST samples (n=19) baseline and post- 6-months of tDMARD treatment are shown in columns 3 and 4 (adapted from [1]).



Supplementary figure 2. Exhaustion signatures are enriched in both CD4$^+$ and CD8$^+$ PD-1$^+$ cells from the peripheral blood of patients with early RA compared to PD-1$^-$ cells. Identified genes in sorted CD4$^+$/CD8$^+$ PD-1$^+$ and PD-1$^-$ cells from five patients with early RA were statistically compared with existing exhaustion gene signatures from CD4$^+$ T cells (left graph) and CD8$^+$ T cells from patients with hepatocellular carcinoma (right graph) [2]. Enrichment for exhaustion signatures are shown. Data is expressed as box and whisker plots from min to max, with box representing 25th to 75th percentile ± median. Comparisons were made by one-way ANOVA followed by Tukey's multiple comparison test, **$p<0.01$; ***$p<0.001$; ****$p<0.0001$.

References

1. Walsh, A.M., et al., *Triple DMARD treatment in early rheumatoid arthritis modulates synovial T cell activation and plasmablast/plasma cell differentiation pathways.* PloS one, 2017. **12**(9): p. e0183928-e0183928.
2. Zheng, C., et al., *Landscape of Infiltrating T Cells in Liver Cancer Revealed by Single-Cell Sequencing.* Cell, 2017. **169**(7): p. 1342-1356.e16.