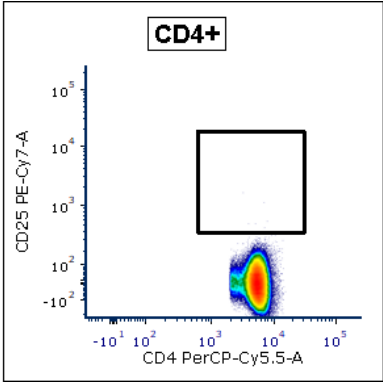
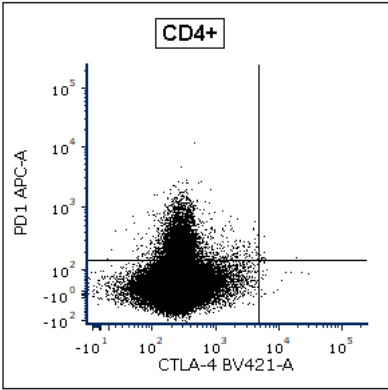
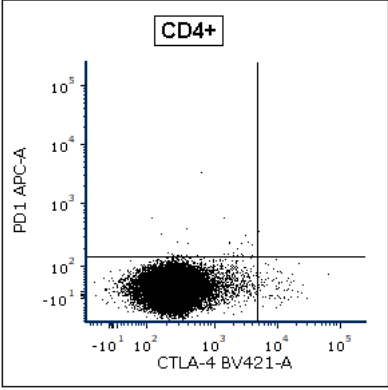
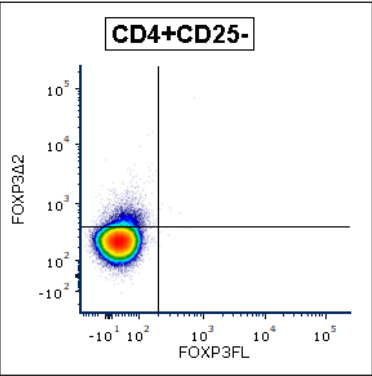
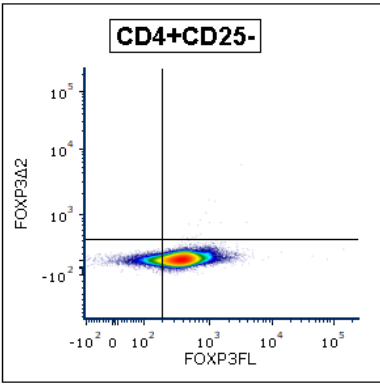
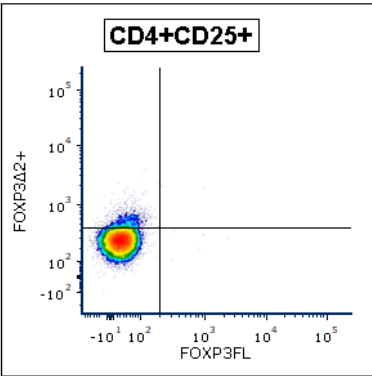
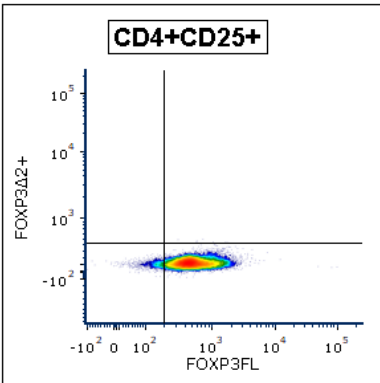
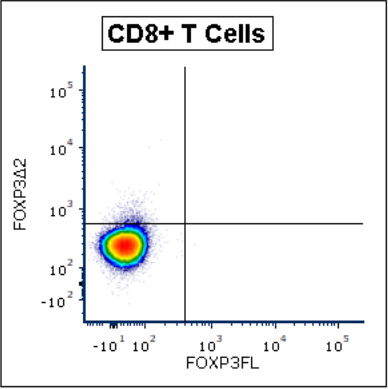
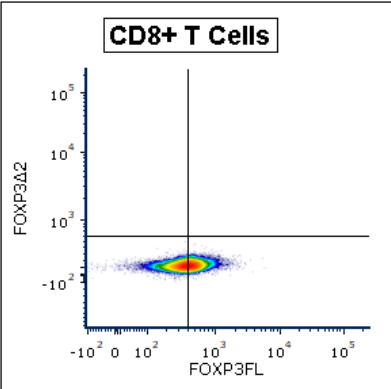
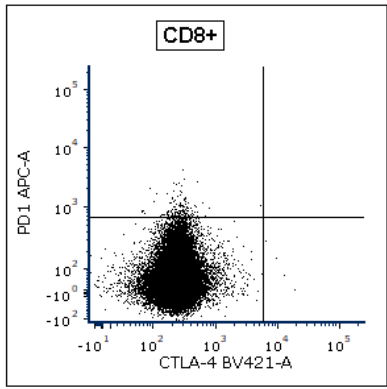
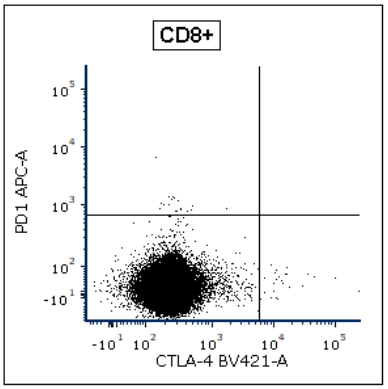
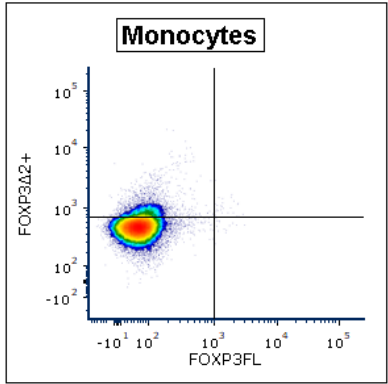
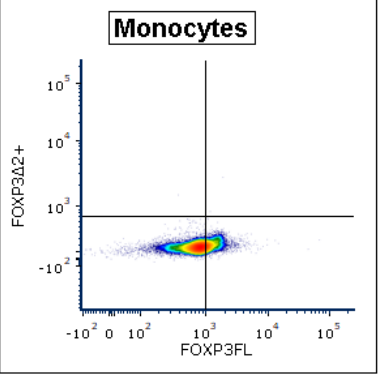
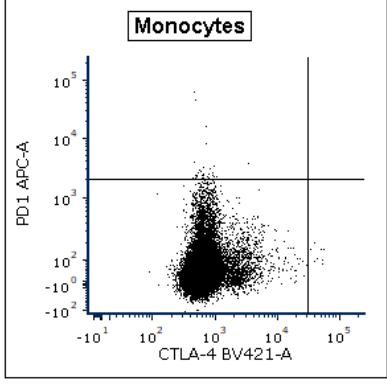
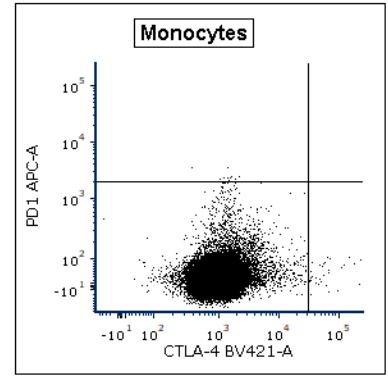
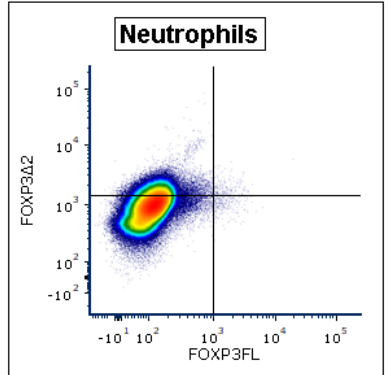
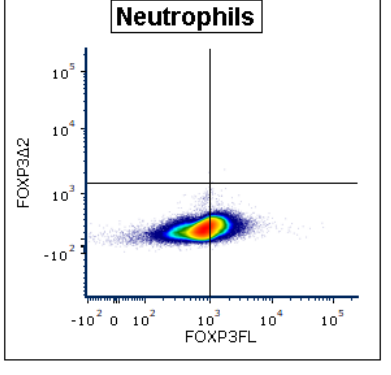
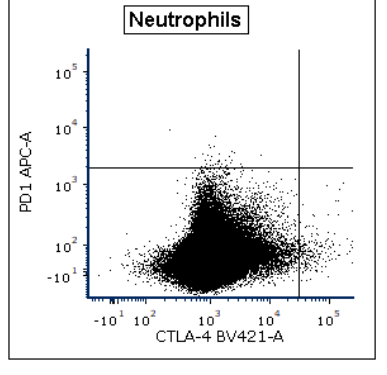
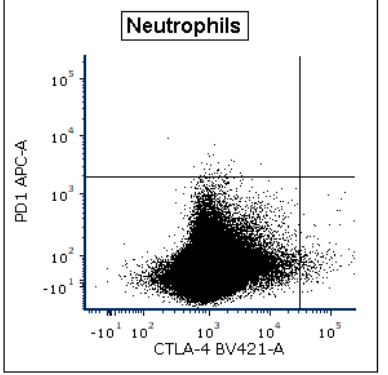


	CD25	FOXP3-FL	FOXP3-Δ2	CTLA-4	PD-1
CD4		—	—		
CD4 ⁺ CD25 ⁻	—			—	—
CD4 ⁺ CD25 ⁺	—			—	—

Supplementary Figure S1. FMO controls for CD4⁺ T-cell subpopulations. Representative fluorescence-minus-one (FMO) controls used to define positivity thresholds for each marker. FOXP3-FL and FOXP3-Δ2 FMO controls are shown within the CD4⁺CD25⁺ and CD4⁺CD25⁻ gates, respectively. CTLA-4 and PD-1 FMO controls were acquired and gated on the total CD4⁺ population and applied uniformly to both CD4⁺CD25⁺ and CD4⁺CD25⁻ subsets. Dashes (–) indicate that no independent FMO control was required for that marker–population combination. Gates shown are representative of one donor and were applied consistently across all six donors.

	CD25	FOXP3-FL	FOXP3-Δ2	CTLA-4	PD-1
CD8 ⁺	-				
MONOCYTE	-				
NEUTROPHIL	-				

Supplementary Figure S2. FMO controls for CD8⁺ T cells, monocyte and neutrophil CTLA-4 and PD-1 FMO controls were performed within each specific cell population (CD8⁺ T cells, monocytes, and neutrophils). Given the inherently low expression of these markers in these populations under basal conditions, the resulting FMO gates provide highly stringent positivity thresholds, minimizing the risk of false-positive assignment.