

Cell Reports Medicine, Volume 3

Supplemental information

**Trafficking and persistence
of alloantigen-specific chimeric antigen receptor
regulatory T cells in *Cynomolgus macaque***

Gavin I. Ellis, Kimberly E. Coker, Delaine W. Winn, Mosha Z. Deng, Divanshu Shukla, Vijay Bhoj, Michael C. Milone, Wei Wang, Chengyang Liu, Ali Naji, Raimon Duran-Struuck, and James L. Riley

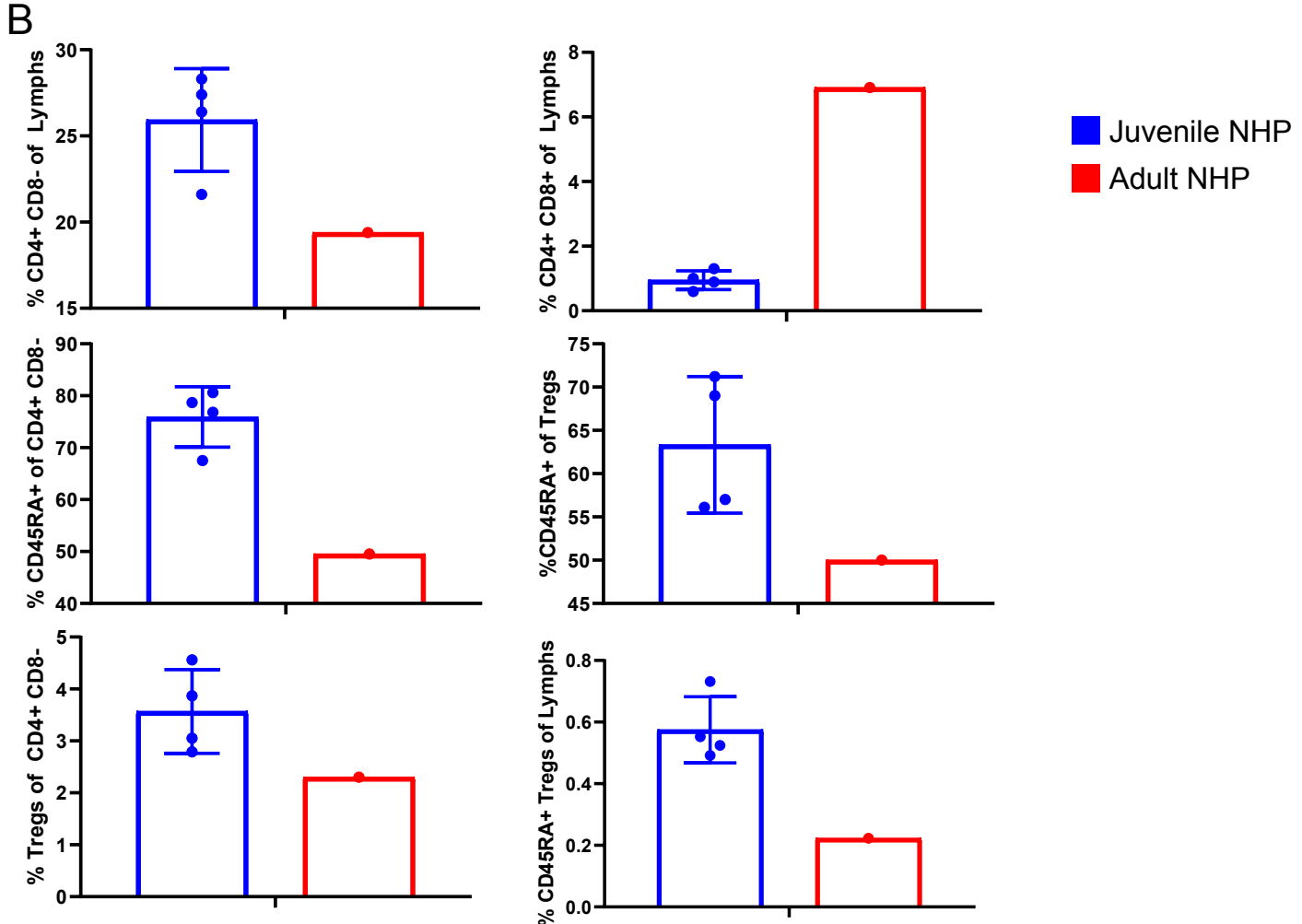
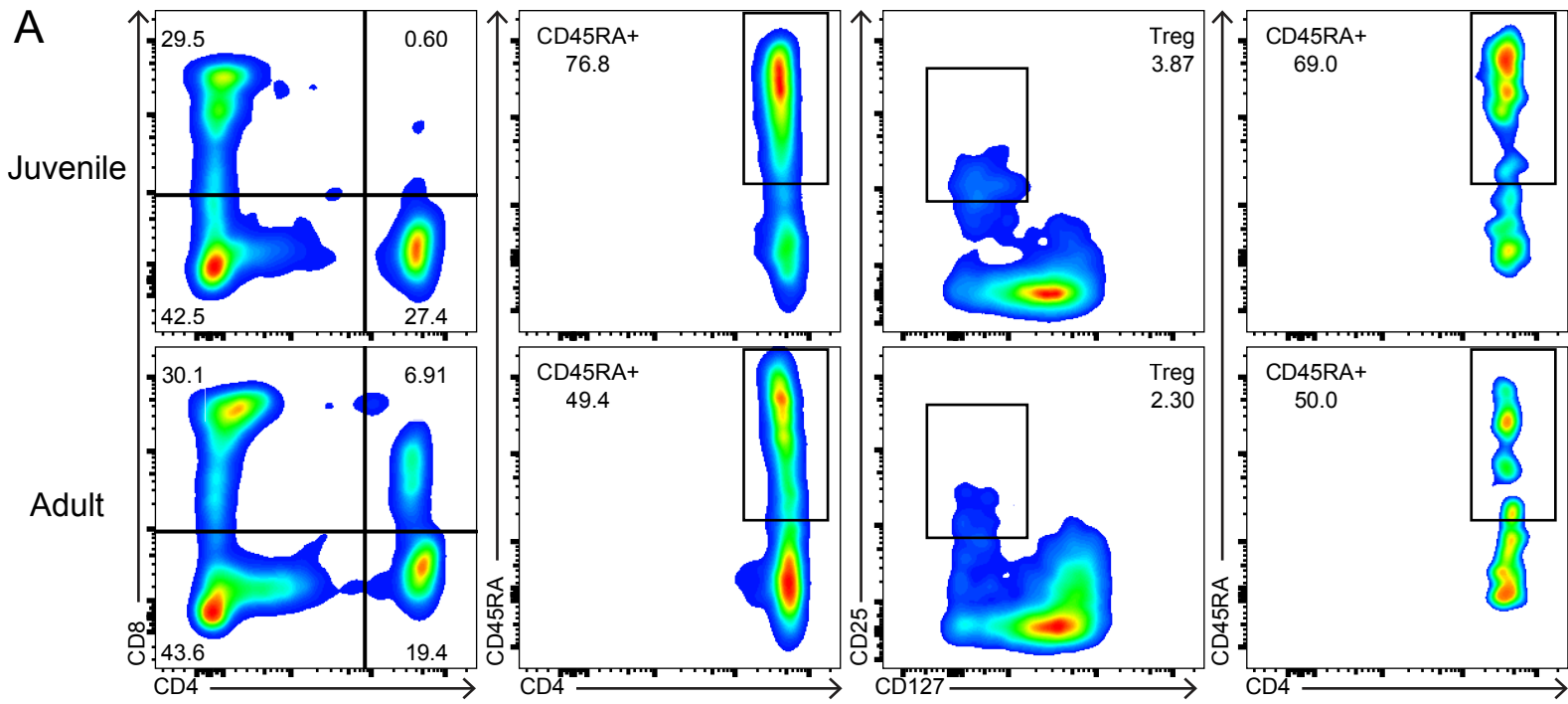


Figure S1. Peripheral blood of juvenile NHPs contains more CD45RA⁺ Tregs than adult blood, Related to Figure 2. (A) Flow cytometry phenotype of peripheral blood from juvenile (top) or adult (bottom) NHPs. (B) Summary of peripheral blood flow phenotypes across 5 animals stratified by age.

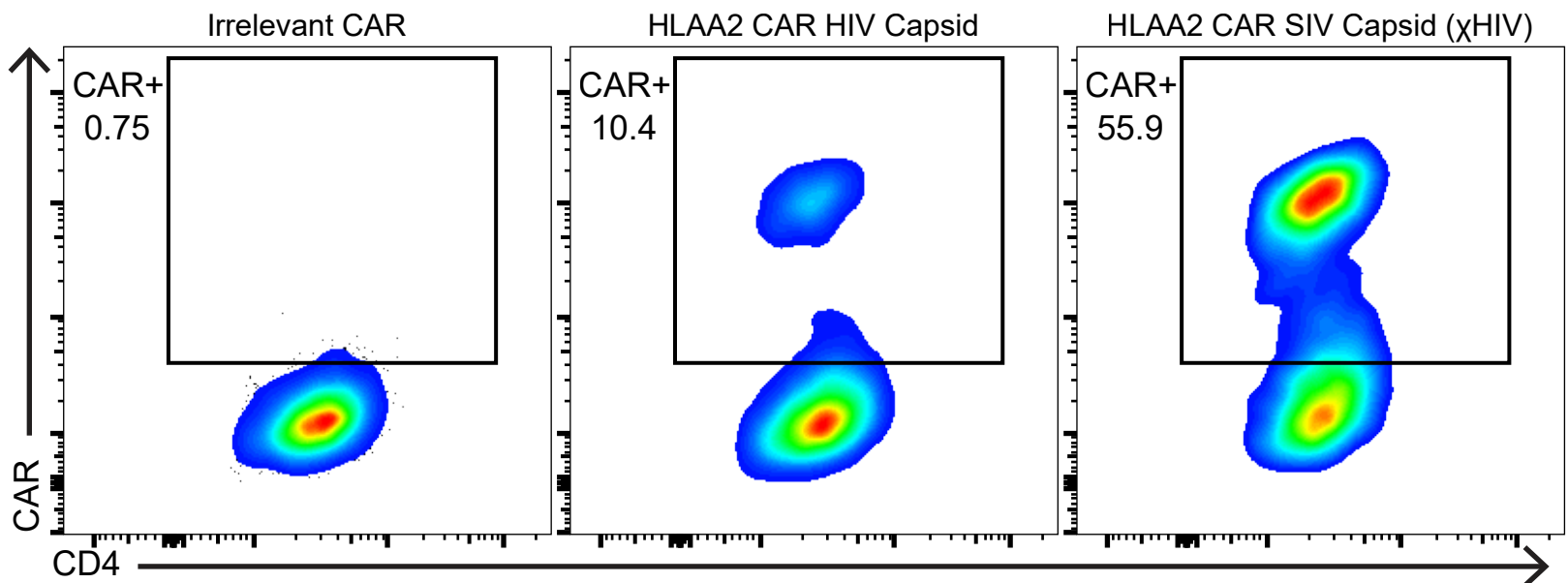


Figure S2. Lentiviral transduction with SIV capsid (χ HIV) virus outperforms HIV capsid virus, related to Figure 2. *Cynomolgus macaque* T cells were transduced with HLAA2 CAR lentivirus containing HIV or SIV capsid and stained for CAR expression with HLA-A2 tetramer.

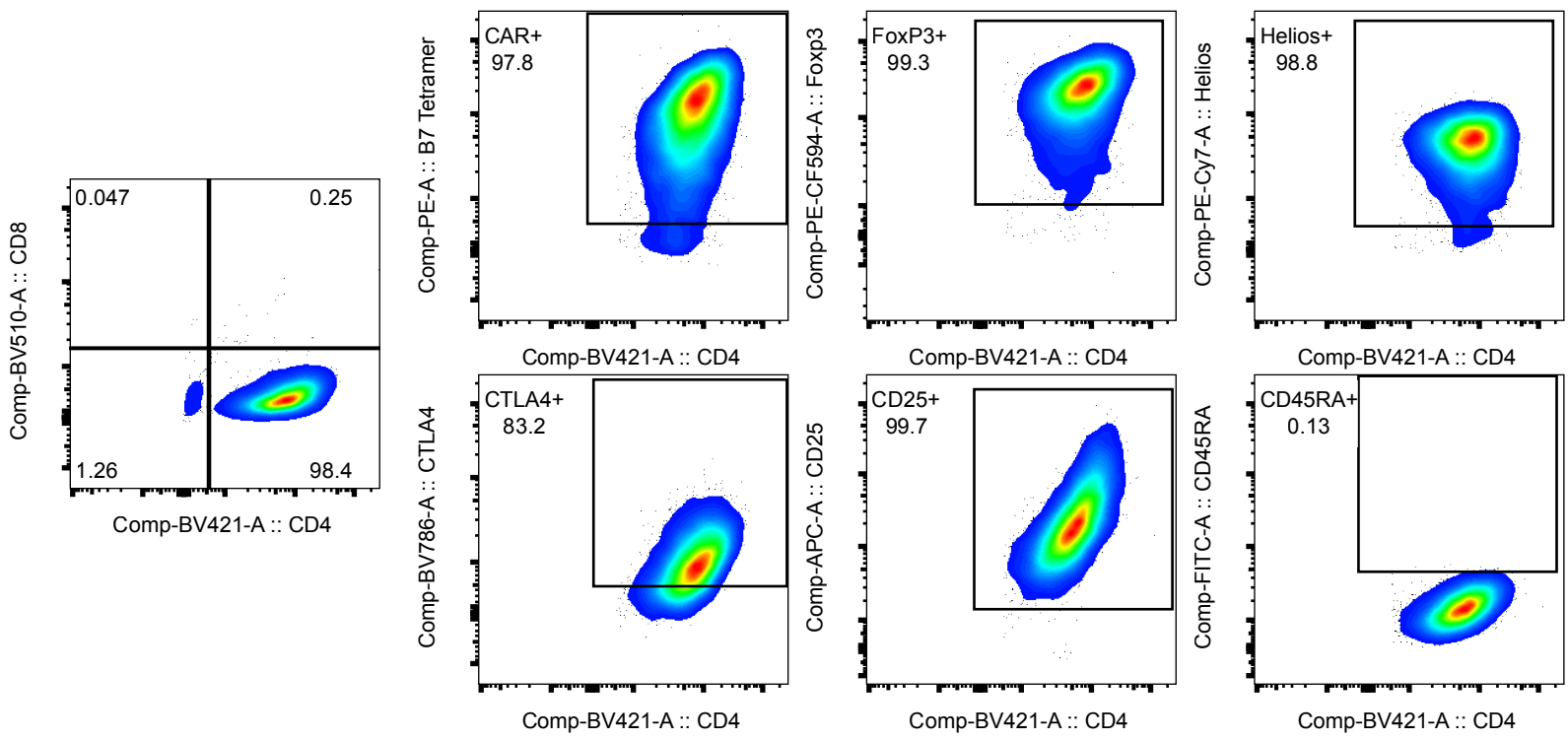


Figure S3. Expanded CAR Treg products express FoxP3, Helios, CTLA-4, and CD25, but lose CD45RA expression, Related to Figure 2. Flow cytometry phenotype of CAR Treg product following expansion. Data are representative of 8 similarly stained CAR Treg products.

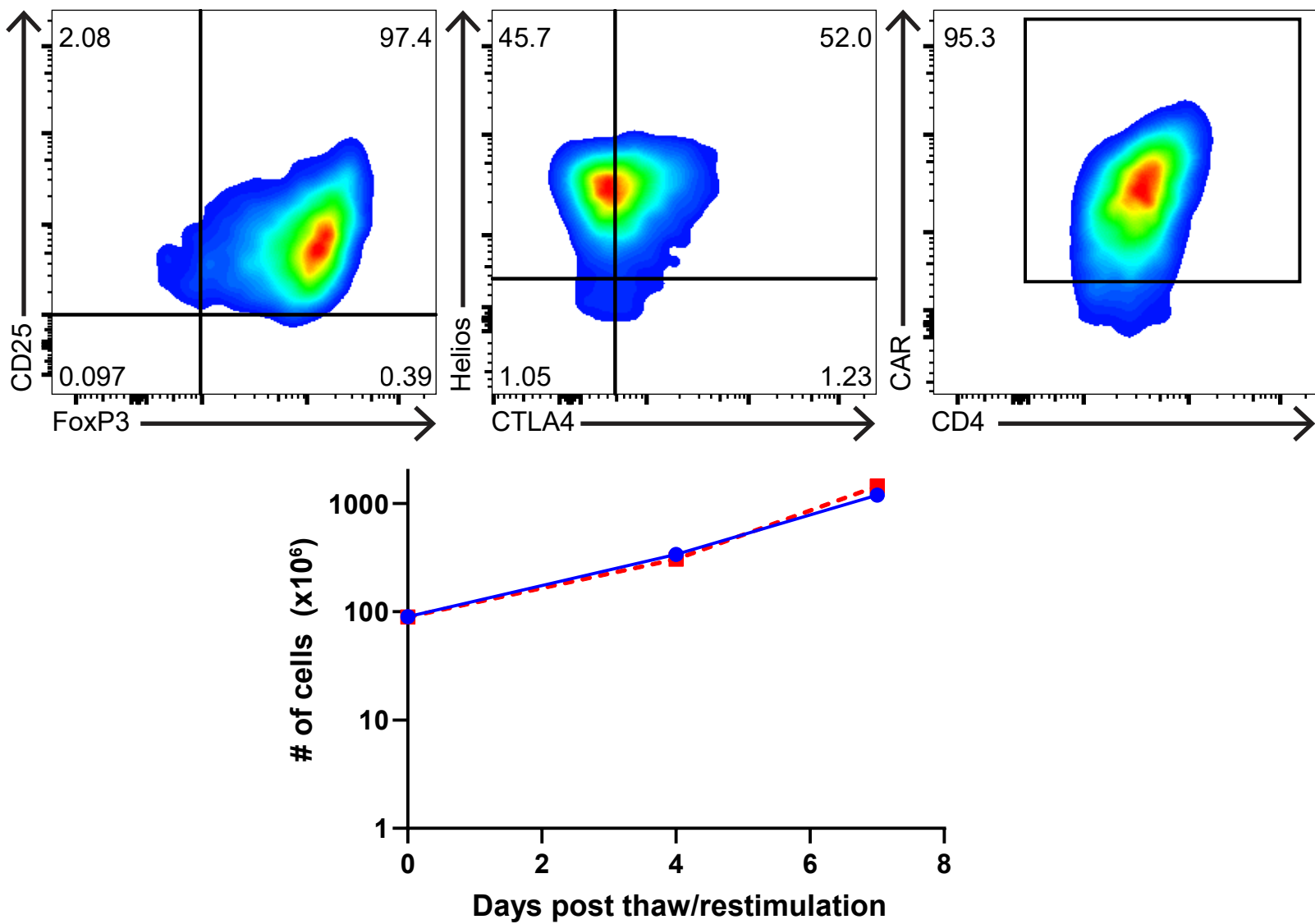


Figure S4. CAR Treg products survive cryopreservation and can be further expanded, Related to Figure 2. Flow cytometry phenotype (top) and expansion kinetics (bottom) 7 days following thawing and restimulation of cryopreserved CAR Treg product. Each line represents 1 of 2 separate expanded products. Gates are based on FMO controls.

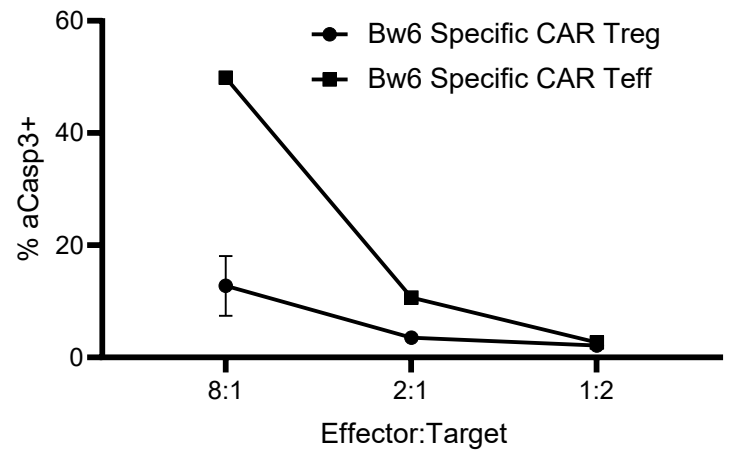
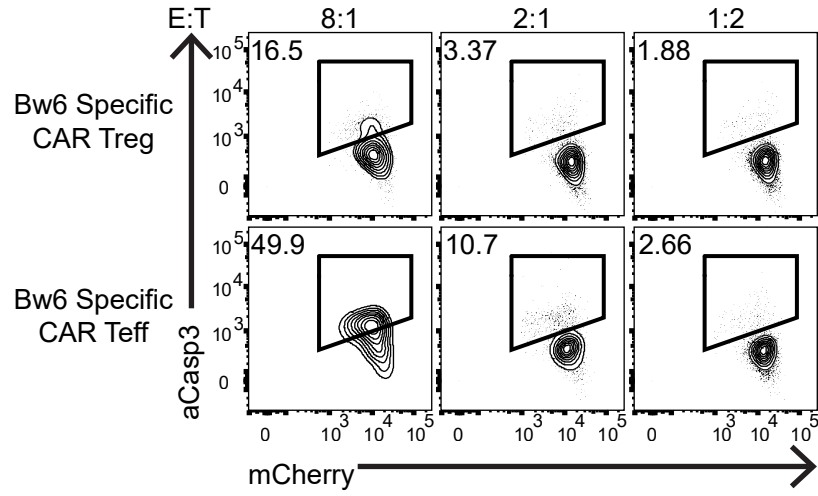


Figure S5. Bw6 specific CAR Tregs induce minimal active caspase 3 on target cells, Related to Figure 4. Bw6 specific CAR Tregs or CAR Teffs were mixed at the indicated effector to target ratio (E:T) with Bw6+ target aAPCs overnight before intracellular staining with active caspase-3 antibody. Target cells were transduced with mCherry to differentiate them from CAR T cells.

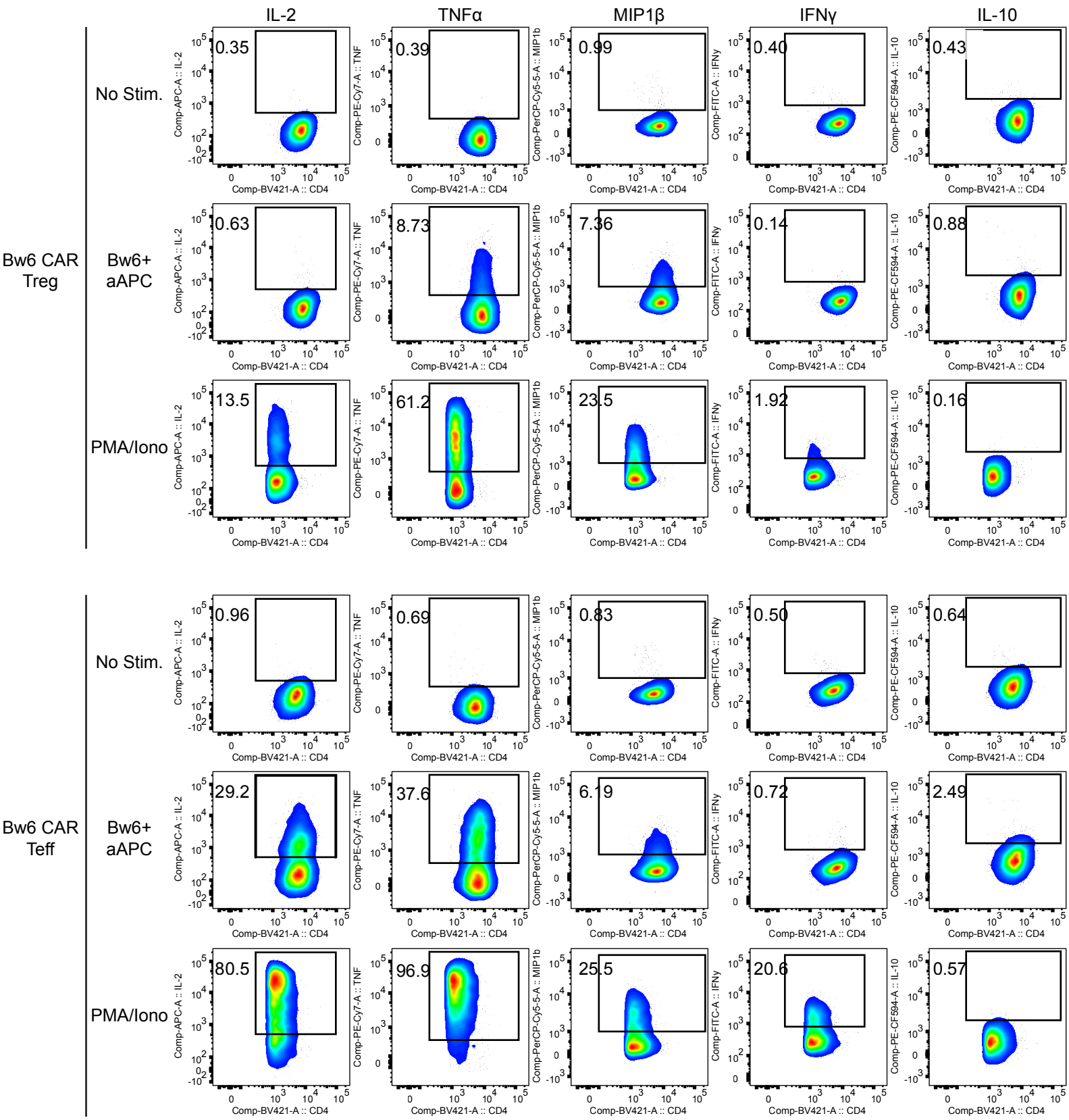


Figure S6. CAR Treg products secrete some TNF α and MIP1 β , but no IL-2, IFN γ , or IL-10, Related to Figure 4. Flow plots displaying cytokine secretion following 5 hour co-culture between indicated cell types and Bw6+ aAPCs or PMA/ionomycin. Data are representative of 4 independent experiments.