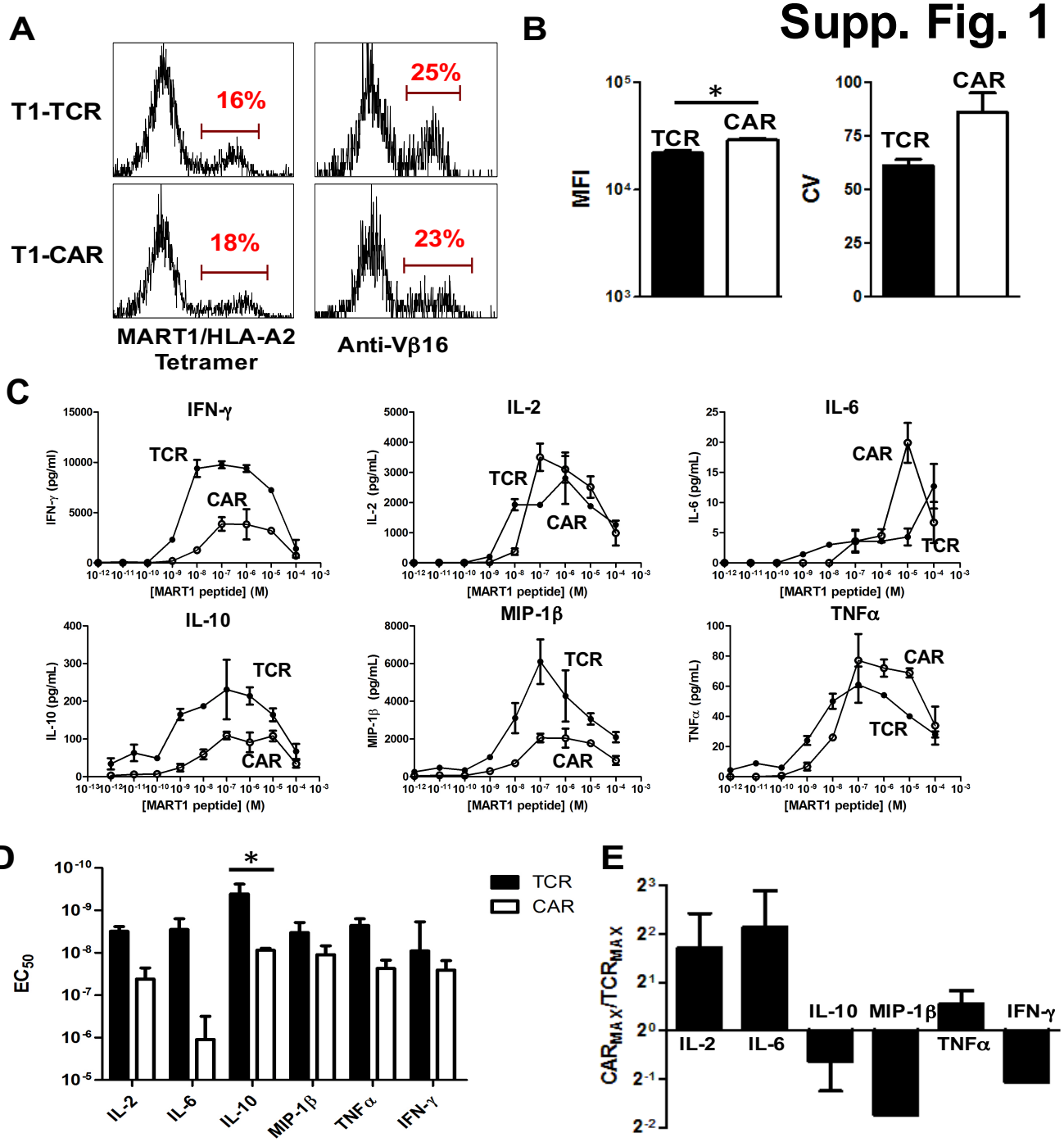
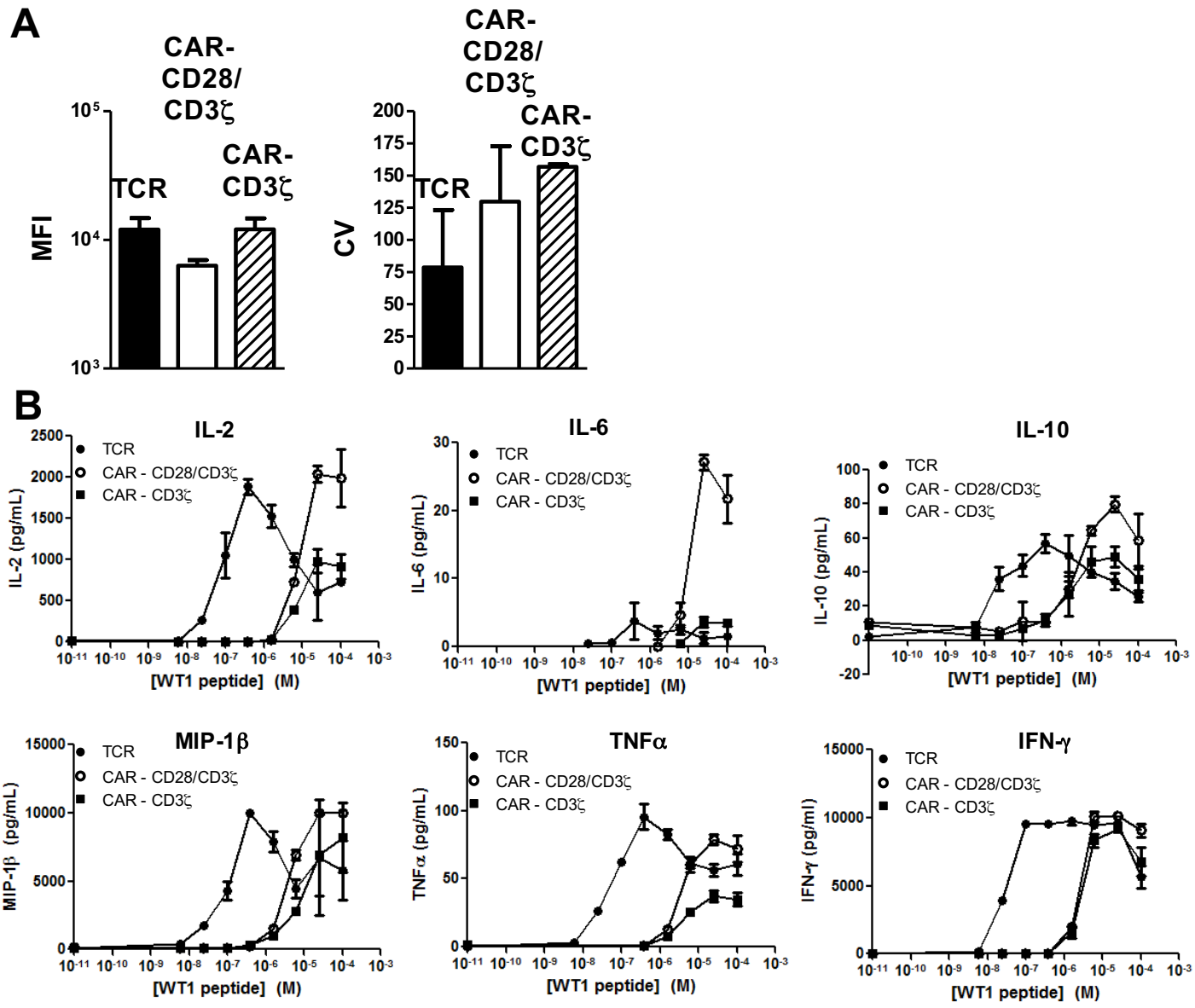
Supp. Fig. 1



Supp. Fig. 1- Flow cytometry and activity analysis of T1 TCR and CAR constructs transduced in primary CD4 T cells. **(A)** CD4 T cells were isolated from AAD mice, activated *in vitro* with anti-CD3/CD28 beads, and transduced with T1 TCR and CAR constructs. Transduced cells were stained with 50 nM MART1/HLA-A2 tetramer and anti-Vb16 antibody. **(B)** The median fluorescence intensity (MFI) (n = 2, p = 0.04) and coefficient of variation (CV) values (n = 2, p = 0.1) were calculated for the TCR and CAR constructs in CD4 transduced cells. **(C)** CD4 T cells transduced with the T1 TCR and CAR were incubated in duplicate with T2 cells (HLA-A2$^+$) at a 1:1 ratio and various concentrations of MART1 peptide. Supernatants were assayed for IFN-g, IL-2, IL-6, IL-10, MIP-1b and TNFa, concentrations were calculated using the Luminex Multiplex system. **(D)** EC_{50} values from two experiments with CD4 T cells were calculated for each cytokine (p = 0.2 (IL-2), p = 0.3 (IL-6), p = 0.01 (IL-10), p = 0.2 (MIP-1b), p = 0.1 (TNFa), p = 0.3 (IFN-g)). **(E)** The maximum concentration of each cytokine released for the CAR was calculated relative to the respective maximum concentration for the TCR (p = 0.9 for IL-2 vs. IL-6, p = 0.7 for IL-2 vs. IL-10, p = 0.9 for IL-2 vs. TNFa, p = 0.5 for IL-6 vs. IL-10, p = 0.7 for IL-6 vs. TNFa, p = 0.9 for IL-10 vs. TNFa) For MIP-1b and IFN-g, n=1 due to saturation at high ligand concentrations.

Supp. Fig. 2



Supp. Fig. 2- Flow cytometry and activity analysis of D13.1.1 TCR, CAR-CD28/CD3z and CAR-CD3z constructs transduced in primary CD4 AAD T cells. CD4 T cells were isolated from AAD mice, activated *in vitro* with anti-CD3/CD28 beads, and transduced with D13.1.1 TCR, CAR-CD28/CD3z and CD3z CAR constructs. Transduced cells were stained with 50 nM WT1/HLA-A2 tetramer as shown in **Fig. 5**. Viable cells were gated based on a FSC vs. SSC plot. **(A)** The median fluorescence intensity (MFI) and coefficient of variation (CV) values were calculated for the TCR, CAR-CD28/CD3z and CAR-CD3z constructs in CD4 transduced cells (n=2, p= 0.3 for TCR vs. CAR-CD28/CD3z (MFI), p = 0.9 for TCR vs. CAR-CD3z (MFI), p = 0.3 for CAR-CD28/CD3z vs. CAR-CD3z (MFI), p = 0.6 for TCR vs. CAR-CD28/CD3z (CV), p 0.4 = for TCR vs. CAR-CD3z (CV), p = 0.9 for CAR-CD28/CD3z vs. CAR-CD3z (CV). **(B)** CD4 T cells transduced with the T1 TCR and CAR-CD28/CD3z were incubated in duplicate with T2 cells (HLA-A2$^+$) at a 1:1 ratio and various concentrations of WT1 peptide. Supernatants were assayed for IFN-g, IL-2, IL-6, IL-10, MIP-1b, TNFa, and IFN-g concentrations using the Luminex Multiplex system. * Indicates statistical significance ($p < 0.05$) determined using one-way ANOVA followed by Tukey's test (maximum cytokine ratios) in Prism 6. P values generated from Tukey's test are adjusted for multiple comparisons.