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## Supplemental Information

### **Non-signaling Chimeric Antigen Receptors Enhance Antigen-Directed Killing by $\gamma\delta$ T Cells in Contrast to $\alpha\beta$ T Cells**

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# Supplemental Figures

## ***Supplemental Figure Legends:***

**Supplemental Figure 1:** Variable expression of CD5-NSCAR and CD5 antigen in Jurkat T cells. Flow cytometry was performed on day 5 (black bars) and day 15 (white bars) to measure anti-CD5 and CD5-Fc binding. **(A)** Representative flow cytometry plots of CD5-NSCAR-modified Jurkat T cells (left) and CD5-edited Jurkat T cells (right). **(B)** The percentage of Jurkat T cells expressing the CD5-NSCAR on the cell surface on days 5 and 15 post-transduction. Experiments were performed in duplicate. Means and standard deviations are represented. **(C)** CD5 expression on Jurkat T cells on days 5 and 15 post- CD5-NSCAR transduction. Experiments were performed in duplicate. Means and standard deviations are represented. **(D)** Western blot analysis of naïve and CD5-NSCAR-modified Jurkat T-cell whole cell lysates on day 15 post-transduction. Membrane was blotted with anti-CD5 antibody. CD5 is detected at 54 kDa. Densitometry was performed using ImageJ.

**Supplemental Figure 2:** CD5-NSCAR-modified, CD5-edited Jurkat T cells cultured with non-modified Jurkat T cells for 14 hours at 0:1, 1:3 and 1:1 ratios of modified to non-modified cells. Flow cytometry was used to detect CD5-Fc binding and CD5 antigen expression. **(A)** CD5-NSCAR expression on CD5-edited Jurkat T cells cultured with either naïve Jurkat T cells (black curve) or CD5-edited Jurkat T cells (gray curve). **(B)** CD5 expression on non-modified Jurkat T cells when cultured with CD5-NSCAR-modified, CD5-edited Jurkat T cells. Data represent means and standard deviations of four independent replicates.

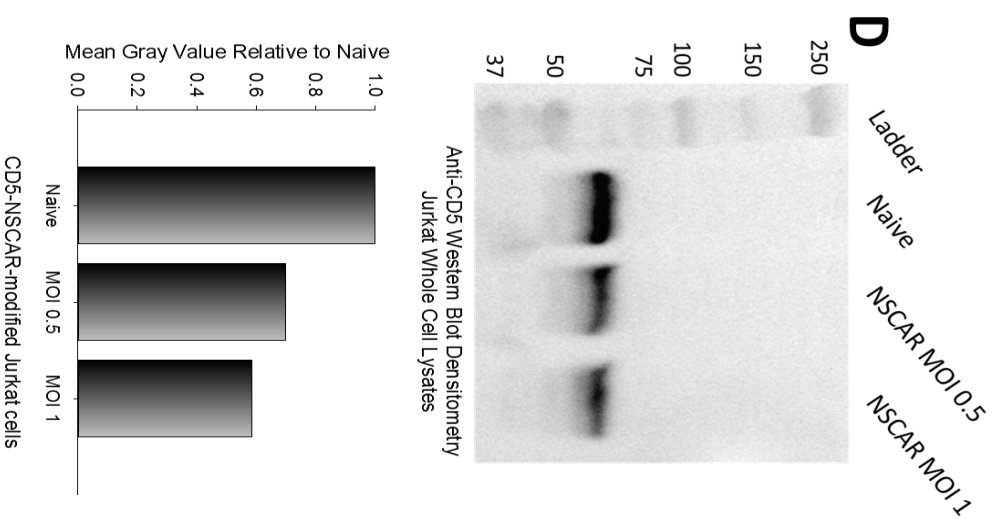
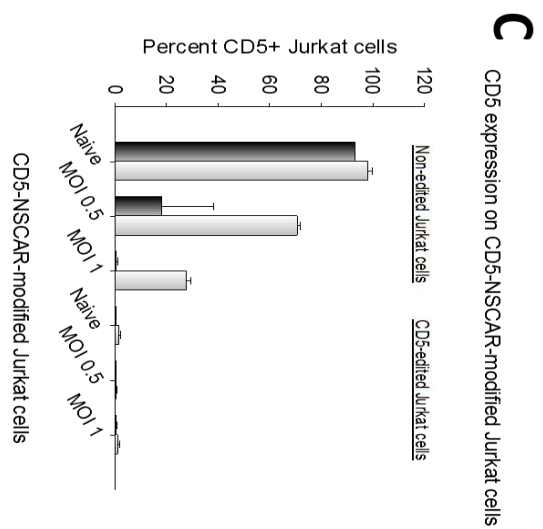
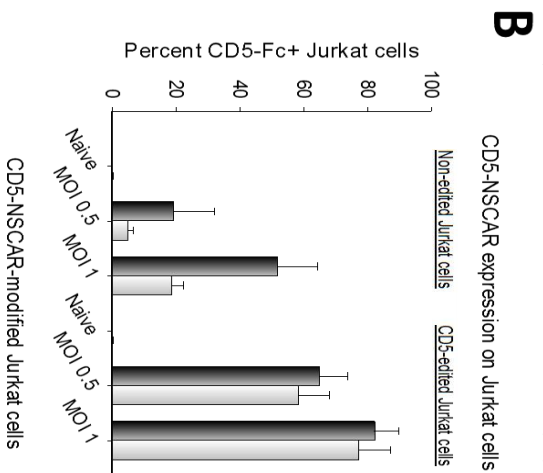
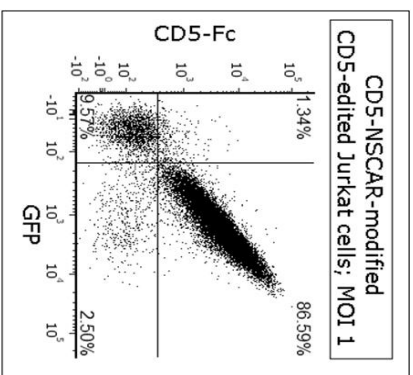
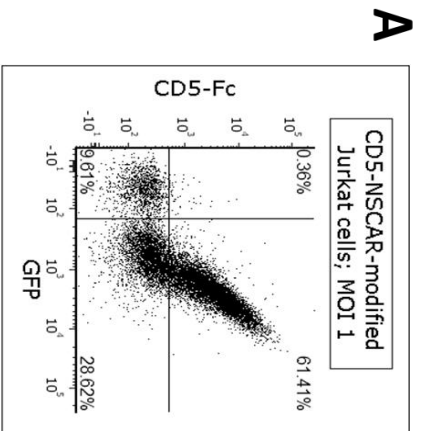
**Supplemental Figure 3:** CD19-NSCAR- and CD19-CAR-modified  $\gamma\delta$  T-cell cytotoxicity against 697 cells at 3:1 (black bars) and 5:1 (white bars) E:T ratios. Means and standard deviations are

represented. N=3 for CAR-modified cells, however one donor was also assessed in duplicate at 5:1 ratio. N=2 for NSCAR-modified cells, however, at the 5:1 ratio, one donor was also assessed in duplicate. Statistics were performed using a 2-tailed Student's t test.

**Supplemental Figure 4:** CD5-NSCAR- and CD5-CAR-modified  $\alpha\beta$  T cells. **(A)** GFP expression in CD5-NSCAR-modified  $\alpha\beta$  T cells from donor 1 (left) and donor 2 (middle) and in CD5-CAR-modified  $\alpha\beta$  T cells from donor 1 (right) on day 6 post-transduction. **(B)** CD5-CAR-modified  $\alpha\beta$  T-cell cytotoxicity assay against Jurkat T cells at a 3:1 E:T ratio.

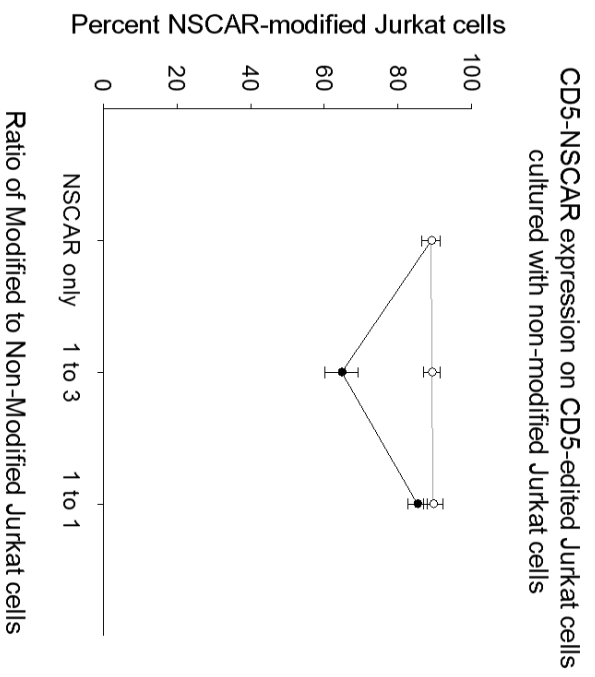
**Supplemental Figure 5:** CD5 and CD19 expression on Jurkat T cells **(A and B)** and 697 cells **(C and D)**, respectively, when cultured in  $\gamma\delta$  T-cell supernatant.  $\gamma\delta$  T cells were cultured for 48 hours prior to supernatant collection. Jurkat T cells and 697 cells were subsequently cultured in  $\gamma\delta$  T-cell supernatant for four hours. Flow cytometry was used to measure antigen expression. **(A)** CD5 expression on Jurkat T cells cultured in  $\gamma\delta$  T-cell supernatant. Sample size of groups: none and naïve: n=5; GFP and CD5-NSCAR: n=4; CD19-CAR and CD5-CAR: n=2. **(B)** CD5 expression on Jurkat T cells when  $\gamma\delta$  T-cell supernatant was pre-incubated with CD5-Fc prior to Jurkat T-cell culture in the supernatant. These experiments were performed using cells from two donors. **(C)** CD19 expression on 697 cells cultured in  $\gamma\delta$  T-cell supernatant; n=2. **(D)** CD19 expression on 697 cells when  $\gamma\delta$  T-cell supernatant was pre-incubated with CD19-Fc prior to 697 cell culture in the supernatant; n=2. One-way ANOVA was performed using the Holm-Sidak method to compare to the naïve control group **(A)** or pairwise **(B)**. One-way ANOVA with Dunnett's method was used to compare to the naïve control group **(C and D)**. Each replicate represents an independent donor.

# Supplemental Figure 1

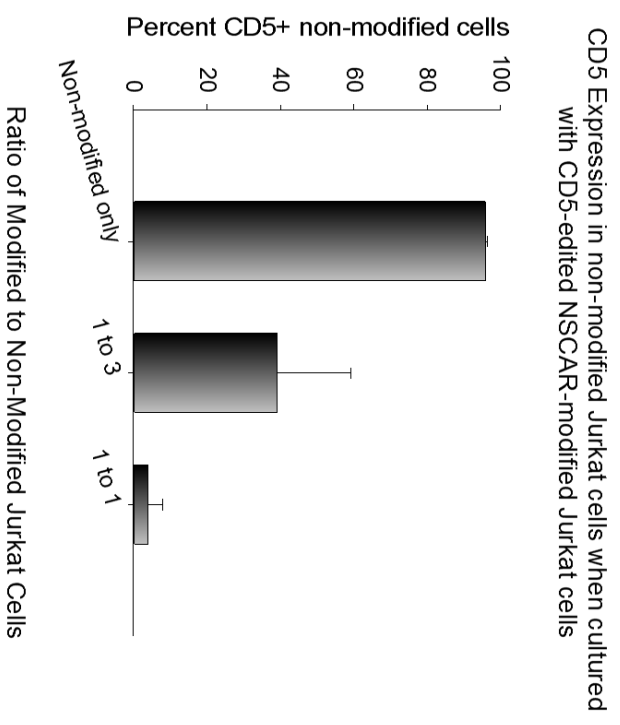


## Supplemental Figure 2

### A

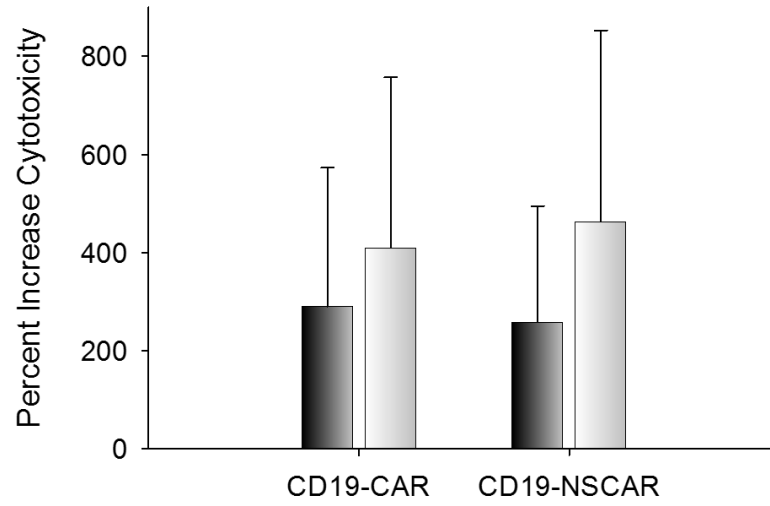


### B



### Supplemental Figure 3

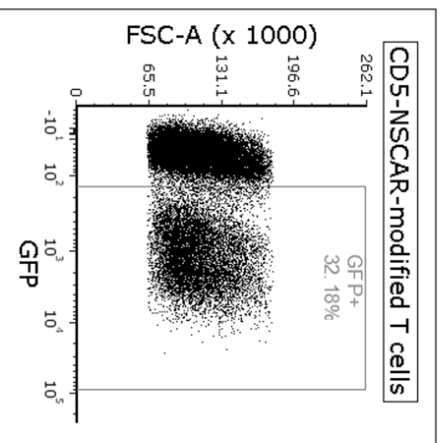
NSCAR-modified  $\gamma\delta$  T-cell cytotoxicity against 697 cells



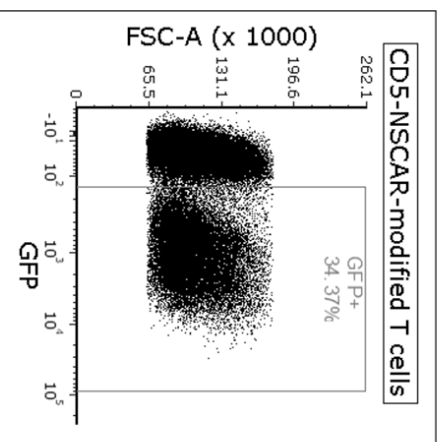
# Supplemental Figure 4

**A**

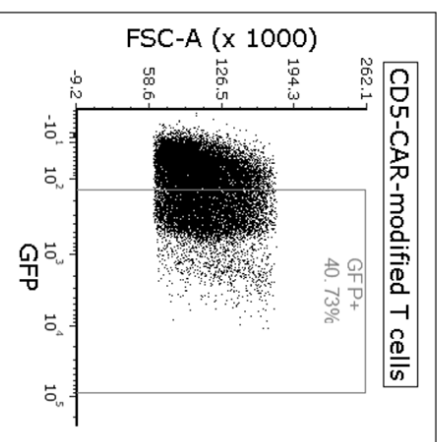
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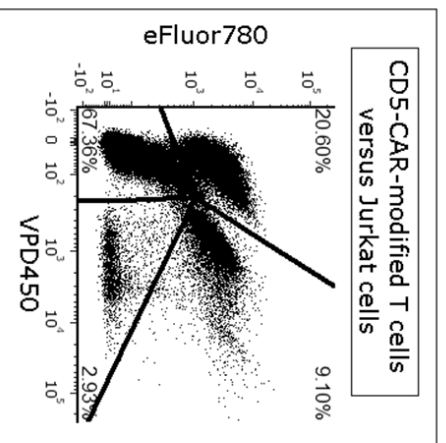
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Donor 1

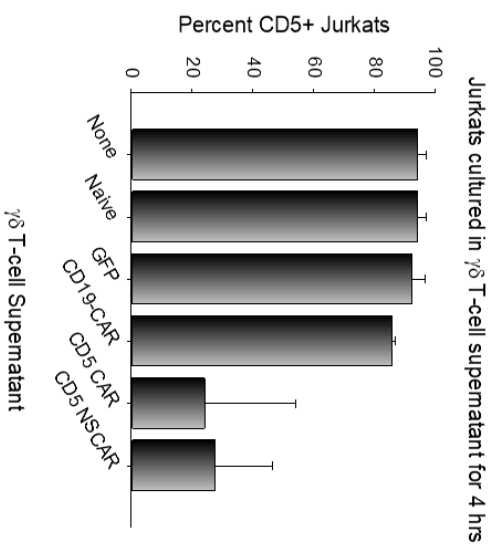


**B**

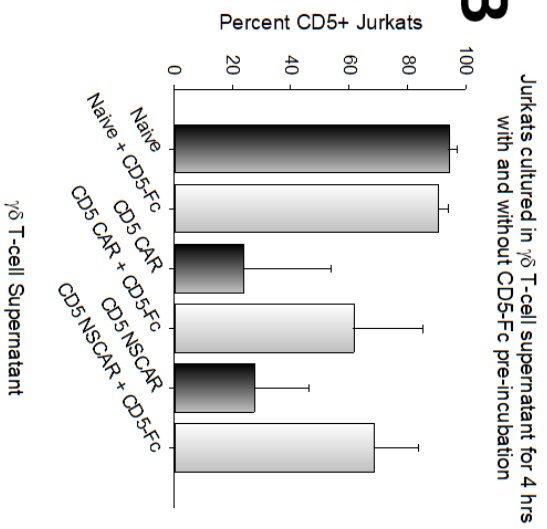


# Supplemental Figure 5

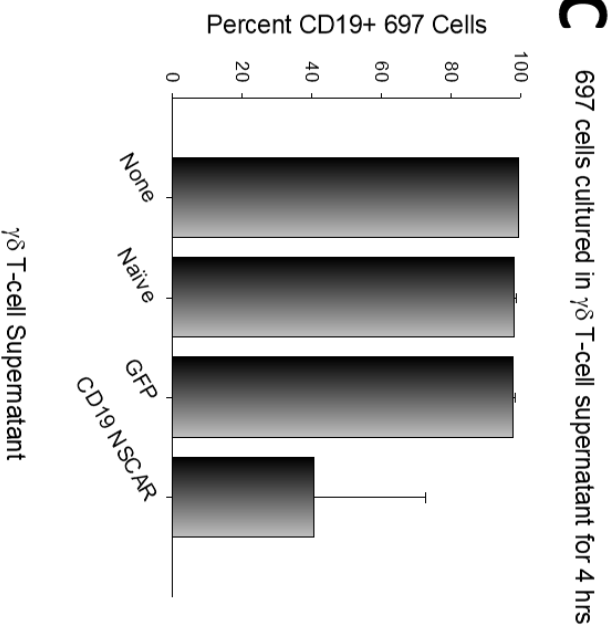
**A**



**B**



**C**



**D**

