Figure S1

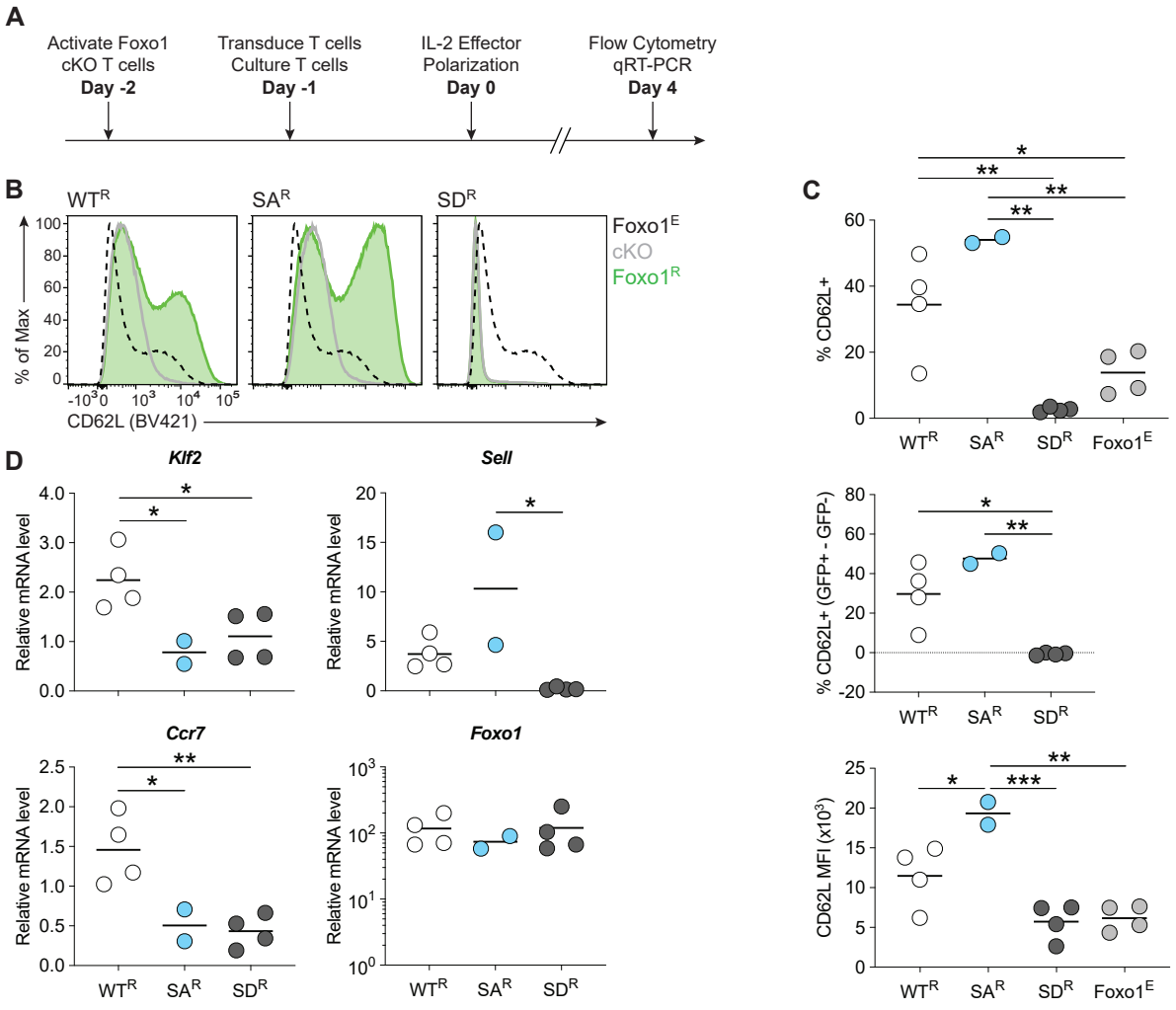

Figure S1. SD-Foxo1 fails to transactivate canonical target genes in effector-like CD8 T cells *in vitro*. **(A)** Diagram of experiment design. Foxo1-cKO-OT1 T cells were activated and then transduced with retroviruses encoding GFP-tagged WT-, SA-, or SD-Foxo1 and cultured in the presence of IL-2 for 4 days. Cells were harvested and prepared for analysis by flow cytometry or FACS sorted into GFP+ (Foxo1 variant reconstituted) and GFP- (Foxo1 null) populations for RNA isolation and qRT-PCR. Control "Foxo1^{E}" cells were either Foxo1-fl/+, GzmB-Cre+ or Foxo1-fl/fl, GzmB-Cre-. **(B)** Representative flow cytometry plots of WT^{R}, SA^{R}, SD^{R}, and control T cells stained for CD62L. **(C)** Quantification of CD62L expression in WT^{R}, SA^{R}, SD^{R}, and control T cells represented as the total percentage of CD62L+ cells (Top), the percentage of GFP+, C62L+ cells normalized to respective GFP- populations that lack both endogenous and retroviral Foxo1 (Middle), and CD62L mean fluorescence intensity (MFI) in the GFP+, CD62L+ populations (Bottom). **(D)** qRT-PCR analysis of Foxo1 target gene relative expression in WT^{R}, SA^{R}, and SD^{R} T cells. Values were normalized to control OT1 T cells expressing endogenous Foxo1 and *Actb* mRNA by the ΔΔCq method. All data are pooled from 4 independent experiments with $n = 4$ (WT^{R}, SD^{R}, and Foxo1^{E}) or $n = 2$ (SA^{R}) biological replicates. For all graphs, statistical analyses were performed with a 1-way ANOVA and Tukey's test to adjust for multiple comparisons. All data are the mean (bar) and all individual values (circles). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.