Supplemental Figure 1

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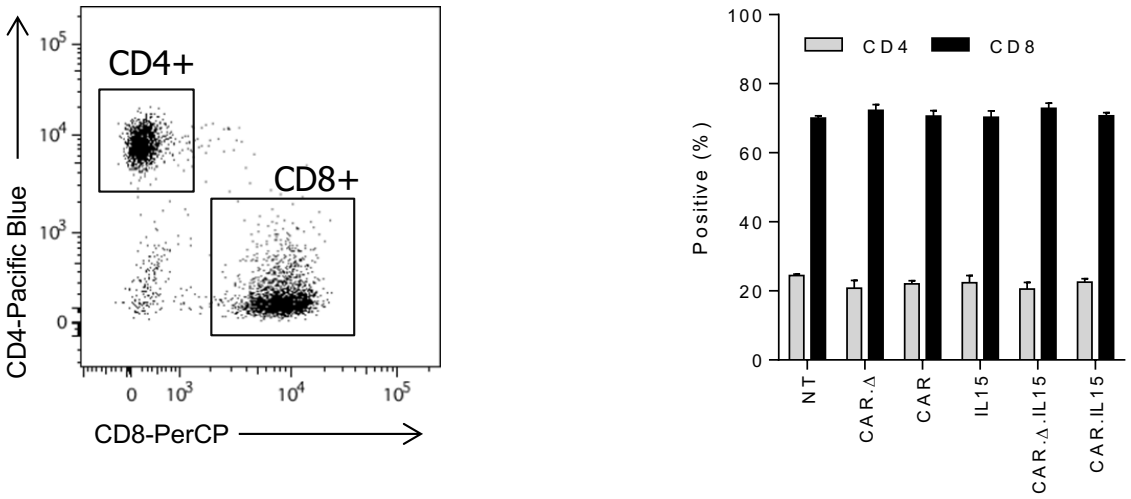

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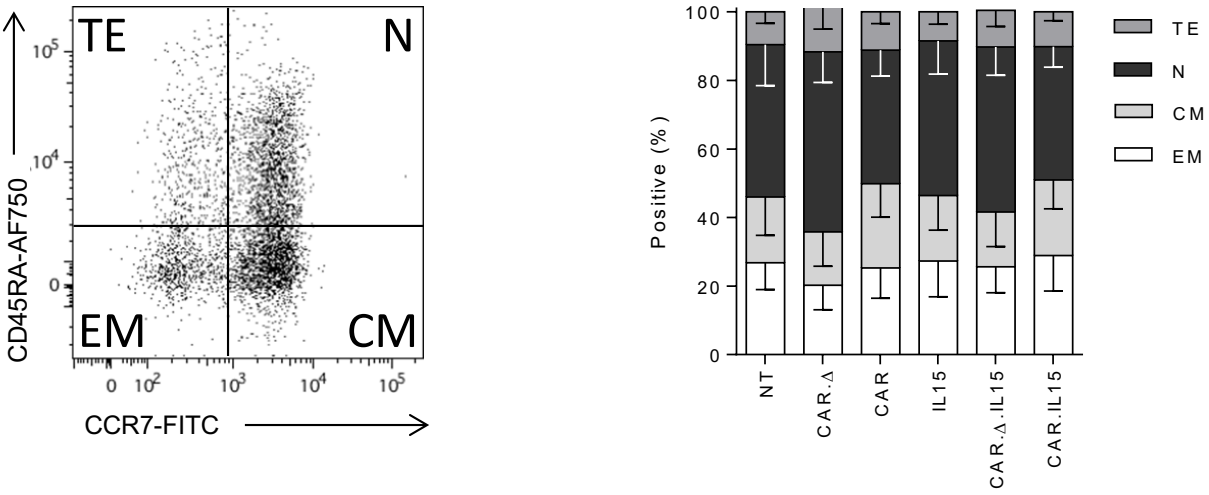
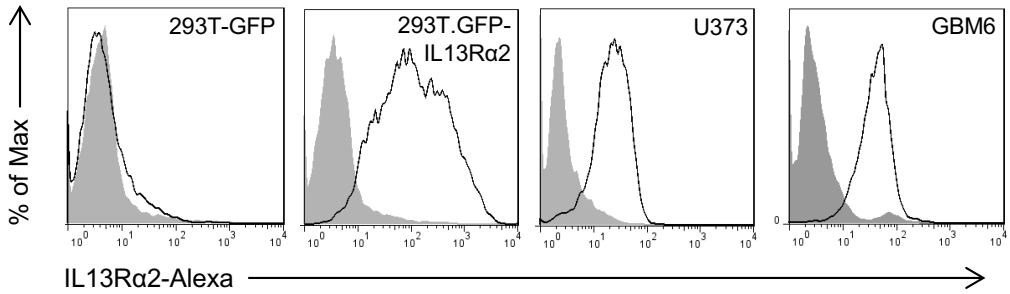
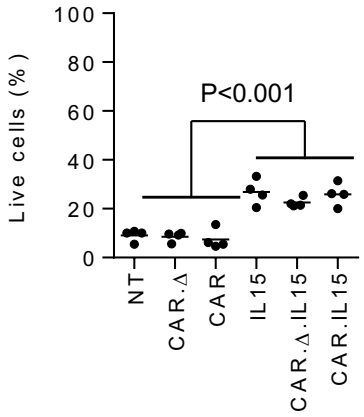


Supplementary Figure 1: IL13Rα2-CAR and/or IL15 expression does not change T-cell phenotype. (**A**) CAR T cells were analyzed for CD4 and CD8 surface expression using CD4-PacBlue and CD8-PerCP antibodies (BD Biosciences; n=4). (**B**) CAR T cells were analyzed for CD45RA and CCR7 surface expression using CD45RA-AF750 and CCR7-FITC antibodies (BD Biosciences; n=4). TE – terminal effector, N – Naïve, EM – effector memory, CM – central memory.



Supplementary Figure 2: Cell surface expression of IL13Rα2. Cell lines were analyzed for IL13Rα2 expression using primary goat anti-IL13Rα2 antibodies (AF146, R&D) followed by secondary rabbit anti-goat IgG Alexa647 antibody (Life Technologies). Grey: isotype control; white: antigen-specific antibody.



Supplementary Figure 3: IL15 improves cell viability in the absence of exogenous cytokines. NT, IL13Rα2-CAR (CAR).Δ, CAR, IL15, CAR.Δ.IL15, and CAR.IL15 T cells were cultured without cytokines for 2 weeks. On day 14, the percentage of live (Annexin-/7AAD-) cells was determined by FACS analysis.

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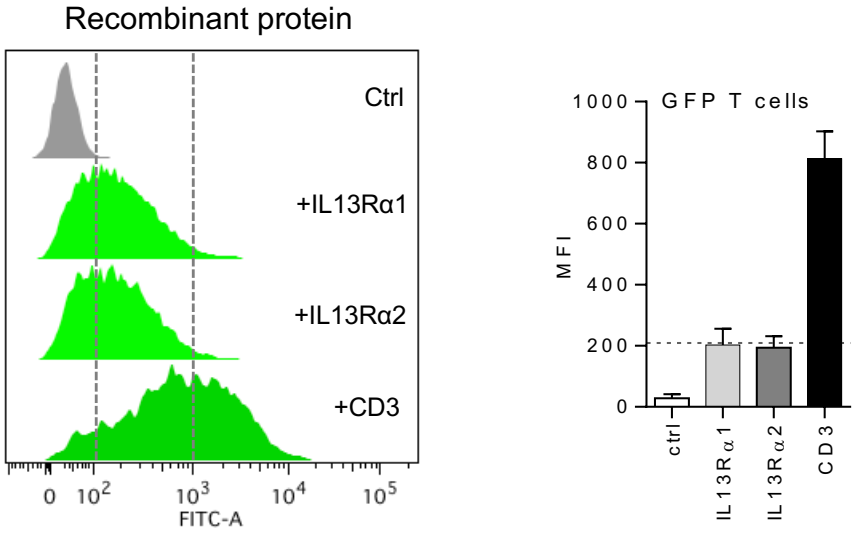

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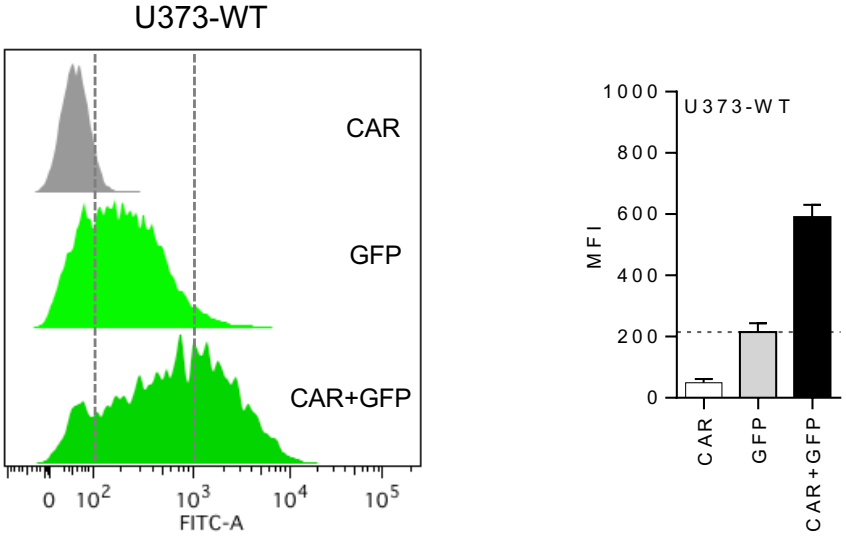
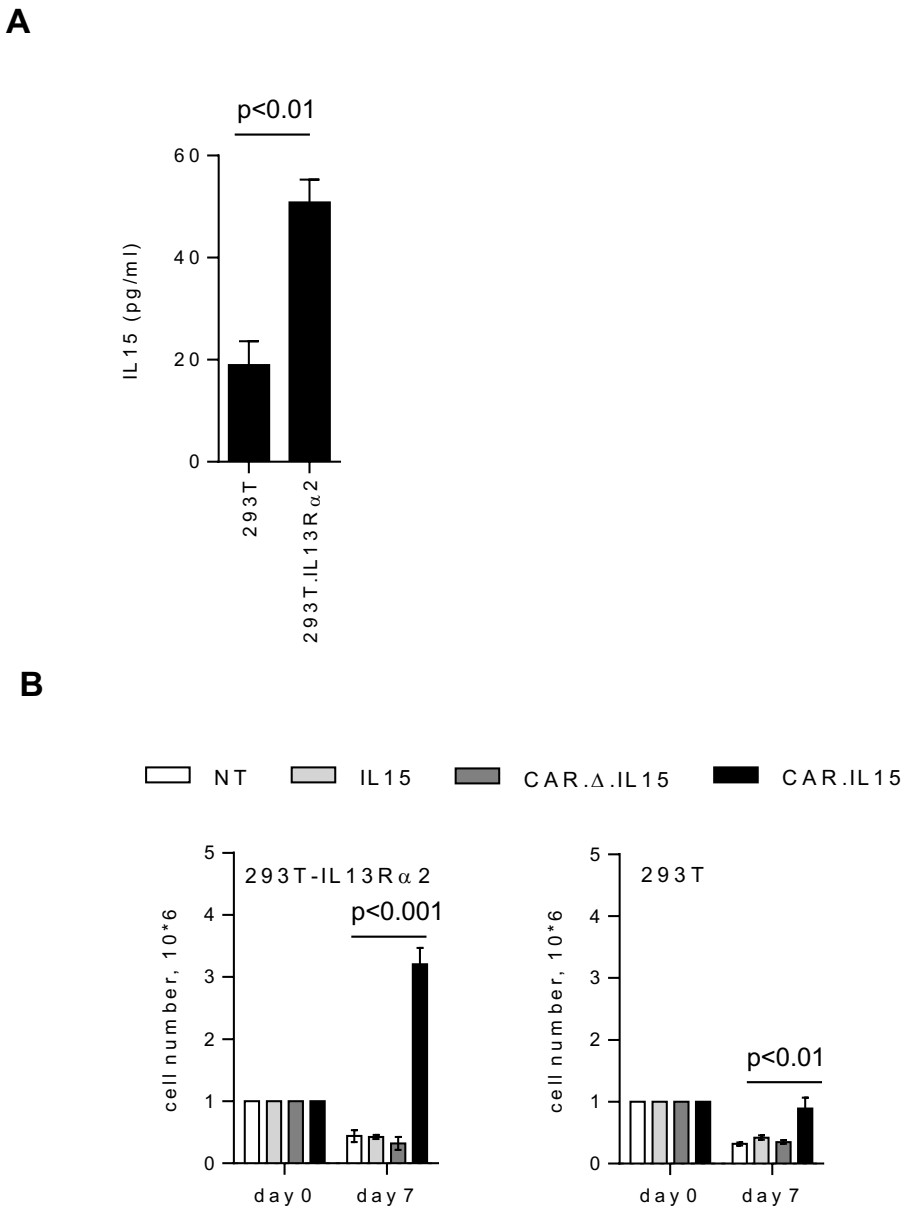


Supplementary Figure 4: Transgene expression is upregulated upon T cell activation. (**A**) GFP expressing T cells were co-cultures with IL13Rα1/α2 and CD3 recombinant proteins for 24 hrs. GFP expression was measured by FACS. IL13Rα1/α2 served as a negative controls; Ctrl – non-transduced T cells. (**B**) T cells expressing IL13Rα2-specific CAR (CAR) or GFP, or both (CAR+GFP) were co-cultured with U373 (positive for IL13Rα2) cells. GFP expression was assessed by FACS analysis 24 hrs after co-culture. Representative example and summary data (n=3) is shown.



Supplemental Figure 5. IL13Rα2-CAR.IL15 T cells display activation-dependent IL15 production and greater proliferative capacity. (**A**) IL13Rα2-CAR.IL15 T cells were co-cultured with 293T-IL13Rα2 cells at a 2:1 E:T ratio. After 24h IL15 was measured by ELISA (n=3; p=0.0079; unpaired t-test; error bars represent SEM). (**B**) CAR T cell expansion. CAR.IL15 T cells were stimulated with 293T-IL13Rα2 or 293T cells, and T cells were counted after 7 days of addition of target cells. (n=3; two-way ANOVA; error bars represent SEM).

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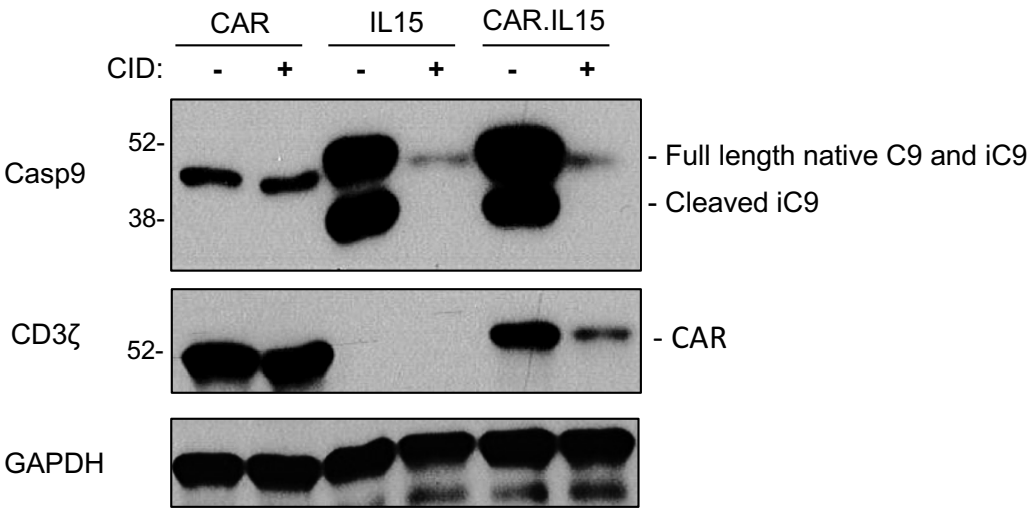

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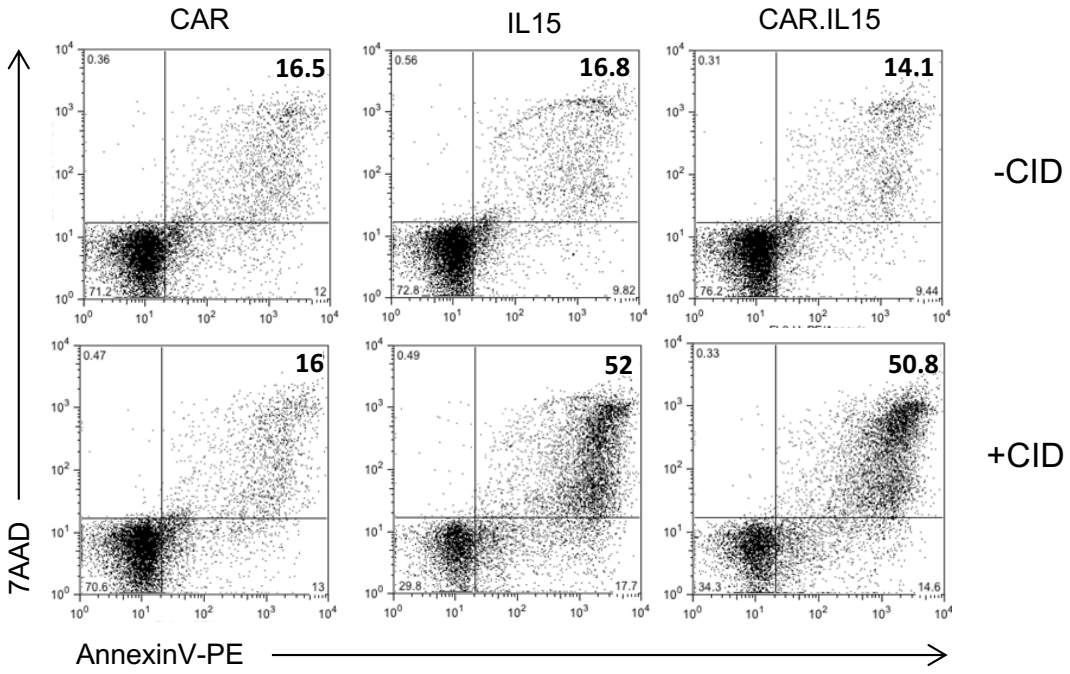
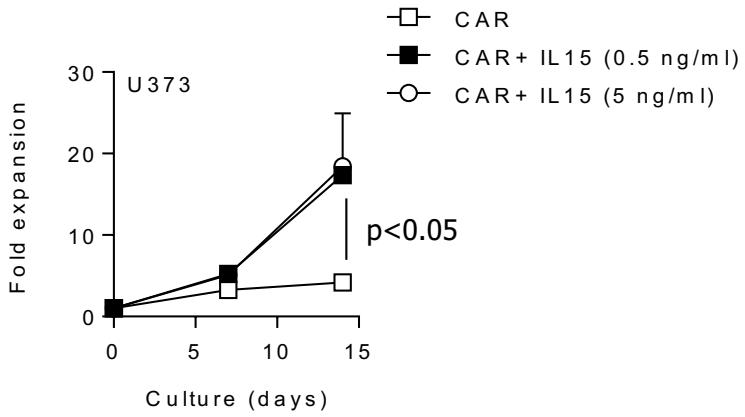
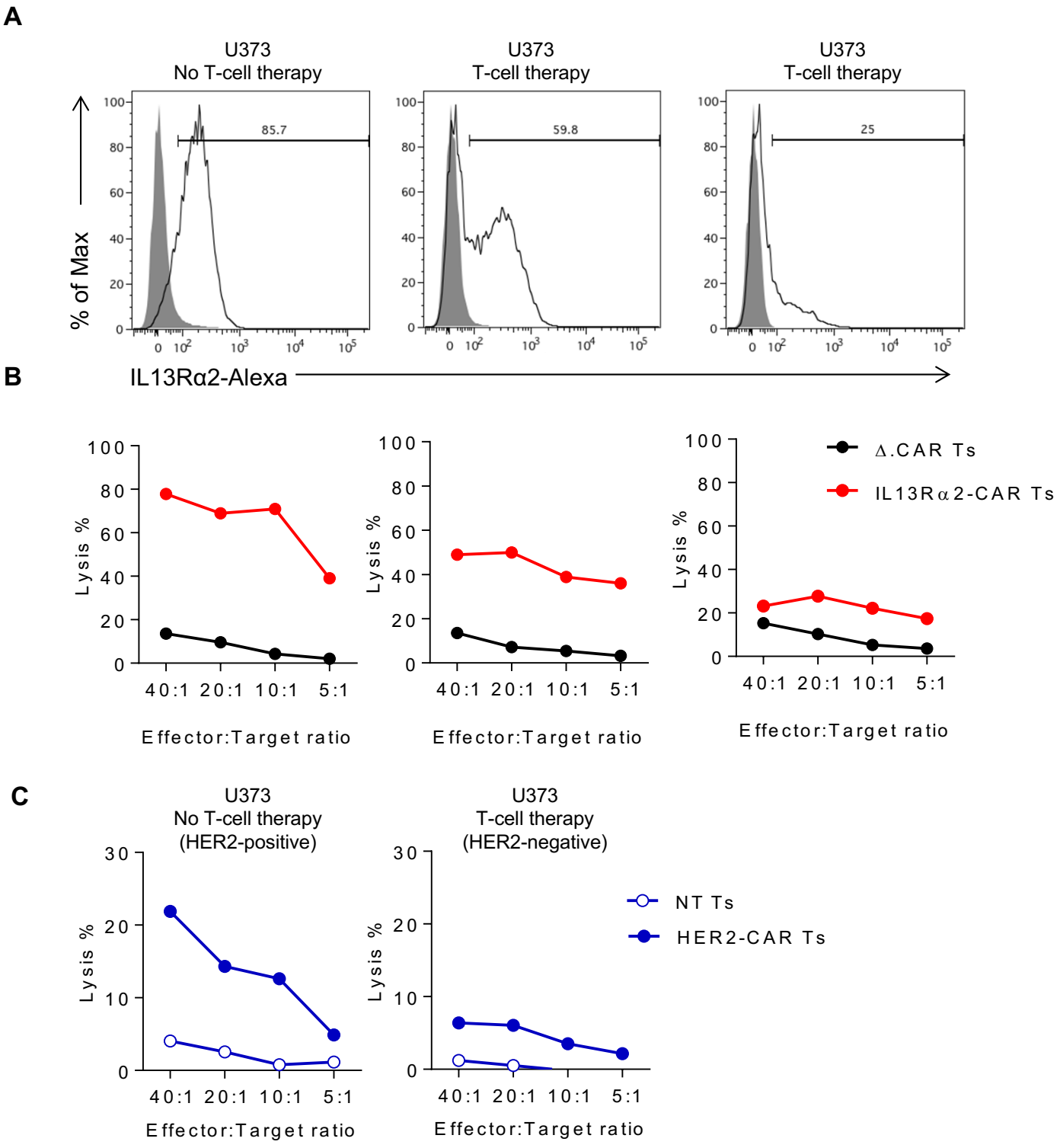


Supplementary Figure 6: CID induces cell death in T cells genetically modified with retroviral vector encoding iC9.dNFGR.IL15. IL13Rα2-CAR (CAR), IL15, or CAR.IL15 T cells were generated by transduction with retroviral particles encoding iC9.dNFGR.IL15 and/or CAR. Transduction efficiency for iC9.ΔNFGR.IL15 was 50.4% for IL15 T cells and 49% for CAR.IL15 T cells. T cells were treated with CID (10 nM; A/C Heterodimerizer, Clontech, Mountain View, CA) and after 24 hrs cells were processed for western blot or FACS analysis. (**A**) Equal amounts of total protein were analyzed by western blot for C9, CD3ζ or GAPDH (loading control). (**B**) T-cell lines were analyzed by FACS analysis for the presence of AnnexinV-PE+/7AAD+ (apoptotic) cells. ~51% of IL15 and IL13Rα2-CAR.IL15 T-cell lines were apoptotic, indicating that all ΔNFGR-positive T cells were killed when treated with CID (10nM, 24 hrs).



Supplemental Figure 7. Addition of exogenous IL15 increases CAR T-cell expansion *in vitro*. CAR T cells were co-cultured with U373 cells at a 2:1 E:T ratio with or without addition of exogenous IL15. T cells were stimulated weekly with fresh U373 cells, and T cells were counted before addition of fresh target cells. Graph shows cumulative data of CAR T-cell expansion (n=3; post 2rd stimulation with U373: CAR vs CAR+IL15 (0.5 and 5 ng/ml) $p<0.05$; t-test; error bars represent SD).



Supplementary Figure 8: IL13Rα2 or HER2 antigen loss variants are not killed by IL13Rα2- or HER2-CAR T cells. (**A**) Cell surface expression of IL13Rα2 in recurrent tumors. Cell lines were analyzed for IL13Rα2 expression using primary goat anti-IL13Rα2 (AF146, R&D) followed by secondary rabbit anti-goat IgG Alexa647 (Life Technologies). (**B**) U373 cells isolated from recurrent tumors are killed in an IL13Rα2 expression level dependent manner in a standard 4h cytotoxicity assay. (**C**) U373 cells isolated from recurrent tumor post T-cell treatment (HER2-negative as shown in Fig. 6A, lower left FACS plot) are not killed by HER2-specific CAR T cells in a standard 4h cytotoxicity assay. U373 cells with no T cell therapy (HER2-positive) served as control.