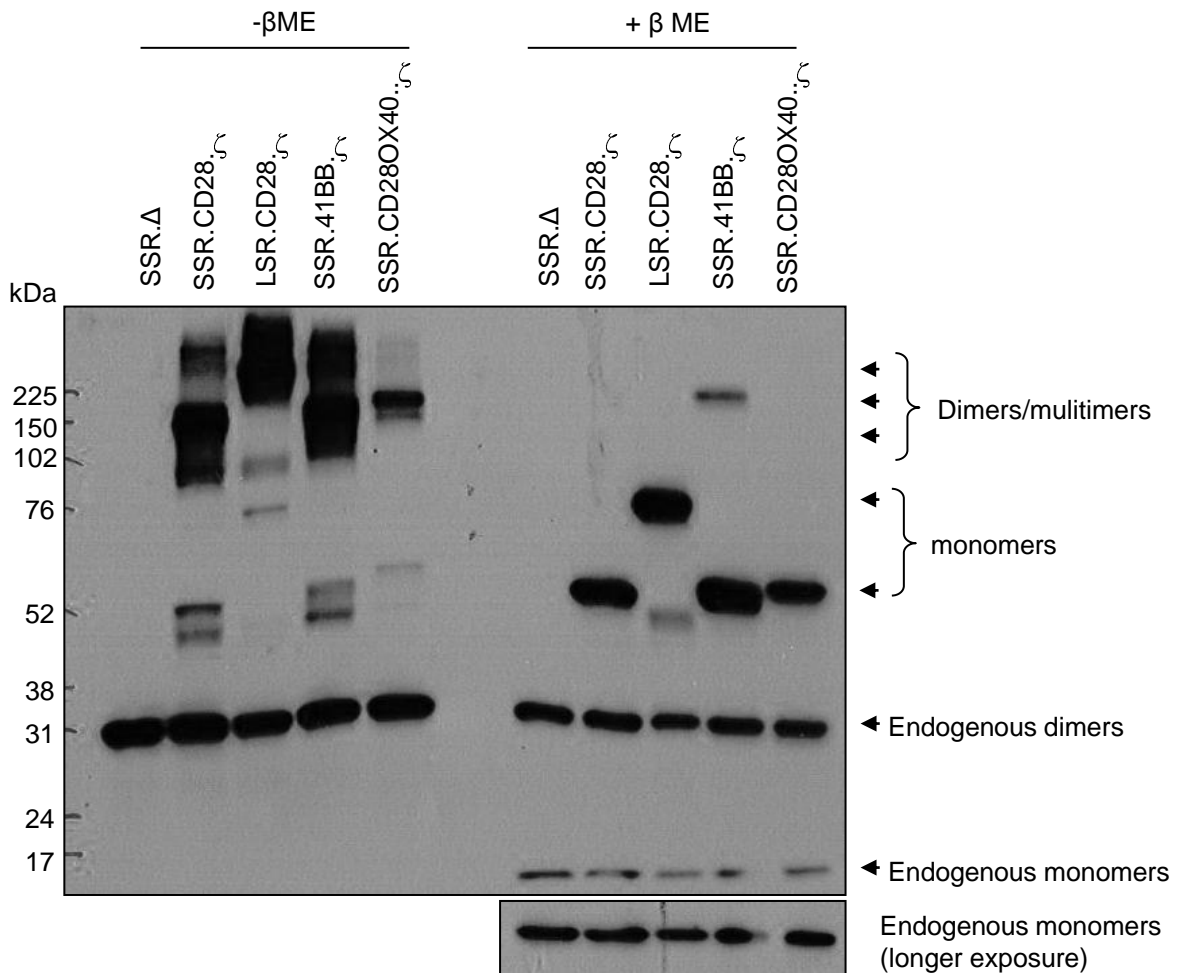
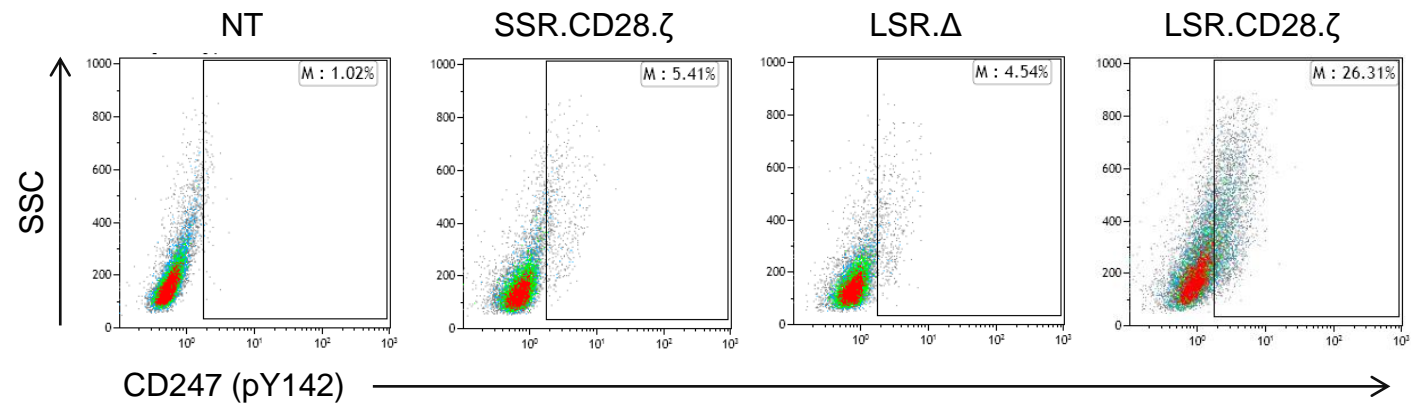


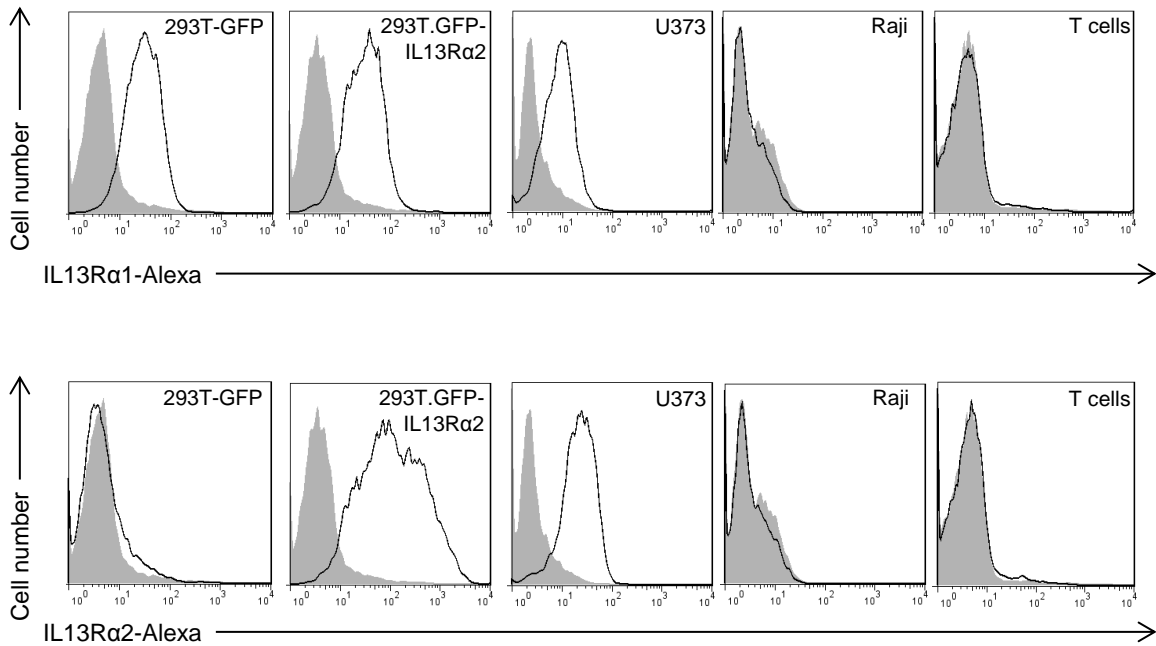
**Supplementary Figure 1: Phenotypic analysis of IL13R $\alpha$ 2-CAR T-cell lines.** CAR T cells were analyzed for CD4 and CD8 surface expression using CD4-PacBlue and CD8-PerCP antibodies (BD Biosciences).



**Supplementary Figure 2: Western blot of IL13Rα2-CAR T-cells.** Expression of full length IL13Rα2-CARs by Western blot using a CD3-ζ antibody. Experiment was done under non-reducing (-βME) and reducing (+βME) conditions to show formation of dimers/multimers. βME:2-Mercaptoethanol.

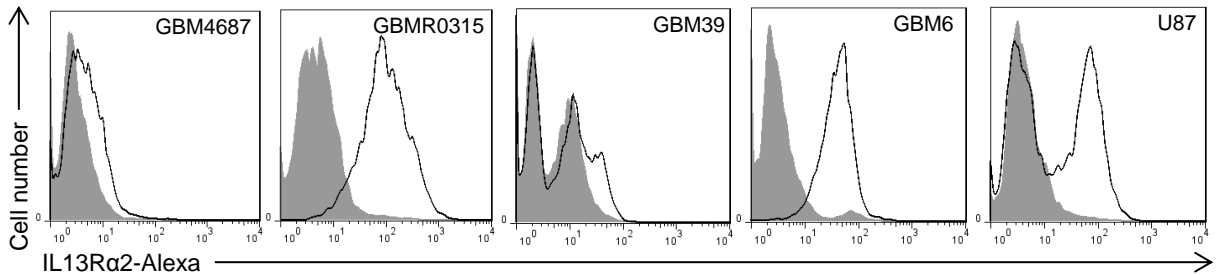


**Supplementary Figure 3: IL13R $\alpha$ 2-CAR.LSR.CD28.ζ induces constitutive CD3.ζ phosphorylation.** NT, SSR.CD28.ζ, LSR.CD28.ζ, and LSR.Δ T cells were analyzed for phospho-CD3.ζ using CD247 (pY142) - AF647 antibody (BD Biosciences).

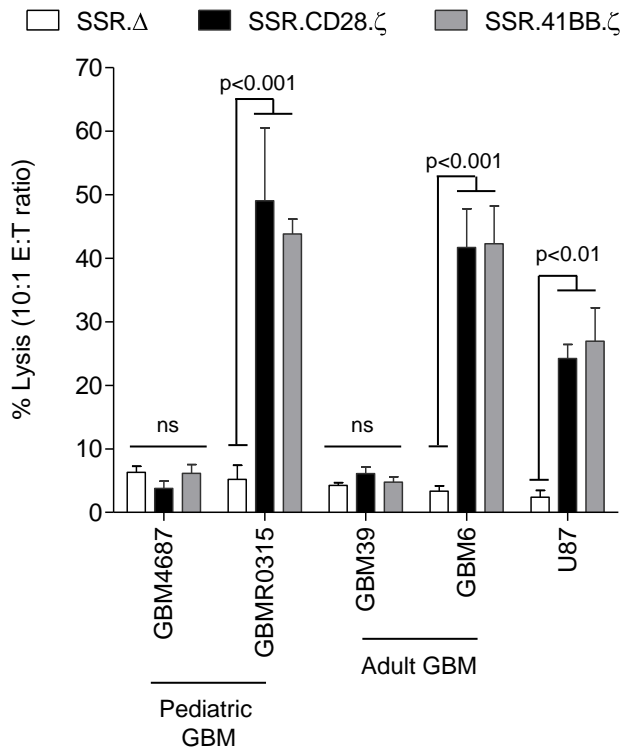


**Supplementary Figure 4: Cell surface expression of IL13Rα1 and IL13Rα2.** Cell lines were analyzed for IL13Rα1 and IL13Rα2 expression using primary goat anti-IL13Rα1 and anti-IL13Rα2 antibodies (AF152 and AF146 respectively, R&D) followed by secondary rabbit anti-goat IgG Alexa647 antibody (Life Technologies).

A



B



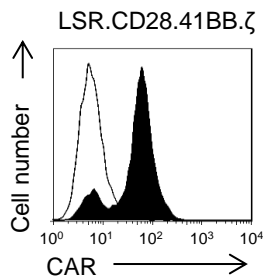
### Supplementary Figure 5: Cell surface expression of IL13Rα2 in brain tumor cell lines.

(A) Cell lines were analyzed for IL13Rα2 expression using primary goat anti-IL13Rα2 antibody (AF146, R&D) followed by secondary rabbit anti-goat IgG Alexa647 antibody (Life Technologies). (B) 4h cytotoxicity assay at an E:T ratio of 10:1 (n=2; each assay was performed in triplicates).

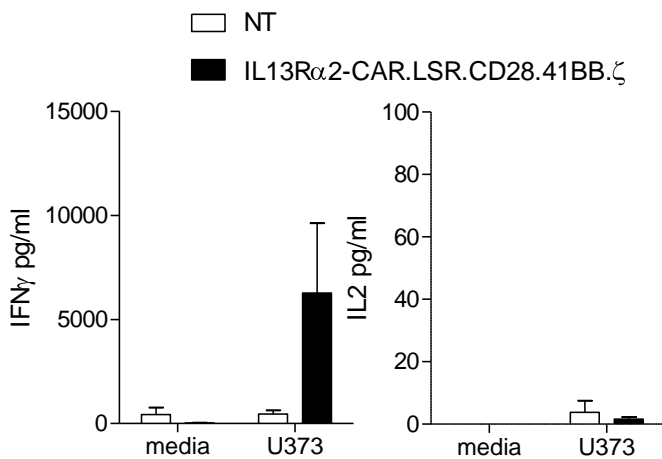
A



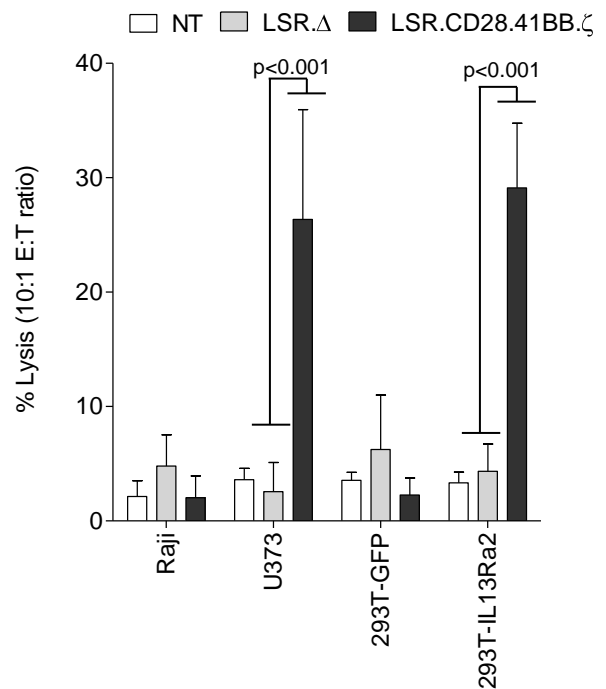
B



C

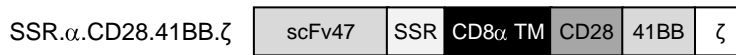


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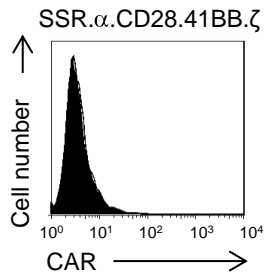


**Supplementary Figure 6: Generation and characterization of LSR.CD28.41BB.ζ CAR T cells.** (A) Scheme of LSR.CD28.41BB.ζ CAR construct. (B) CAR expression was confirmed using FACS analysis. Representative plot. (C) LSR.CD28.41BB.ζ CAR T cells were cocultured with U373 cells at a 1:2 E:T ratio. NT T cells served as controls. After 24h IFN $\gamma$  or IL2 was measured by ELISA (n=3). (D) Standard 4h cytotoxicity assay at an E:T ratio of 10:1 (n=4).

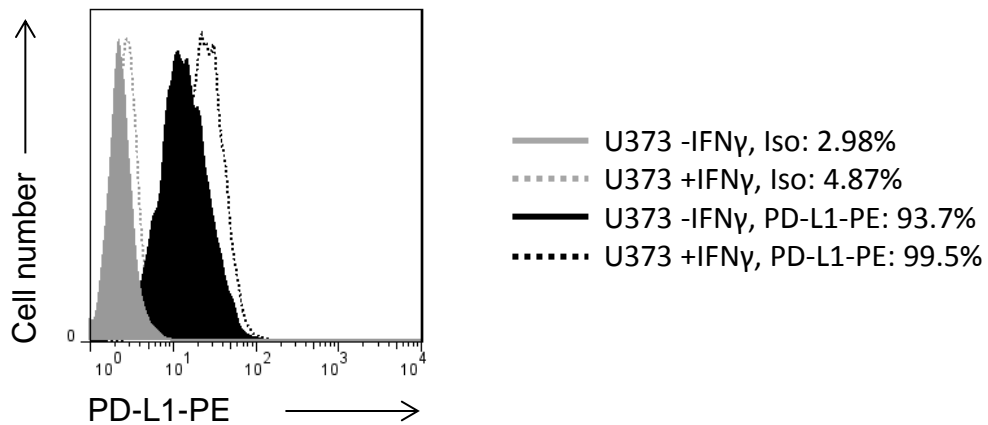
A



B



**Supplementary Figure 7: Generation of SSR. $\alpha$ .CD28.41BB. $\zeta$  CAR T cells.** (A) Scheme of SSR. $\alpha$ .CD28.41BB. $\zeta$  CAR construct. (B) CAR expression was tested using FACS analysis (representative plot is shown).



**Supplementary Figure 8: FACS analysis of PD-L1 expression on U373 cell surface with and without IFN $\gamma$  stimulation.** U373 cells were cultured with or without IFN $\gamma$  (100units/mL). After 24 hours U373 cells were analyzed for PD-L1 expression using a CD271 PE antibody (BD Biosciences).