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Supplemental Information

Optimizing EphA2-CAR T Cells for the Adoptive Immunotherapy of Glioma

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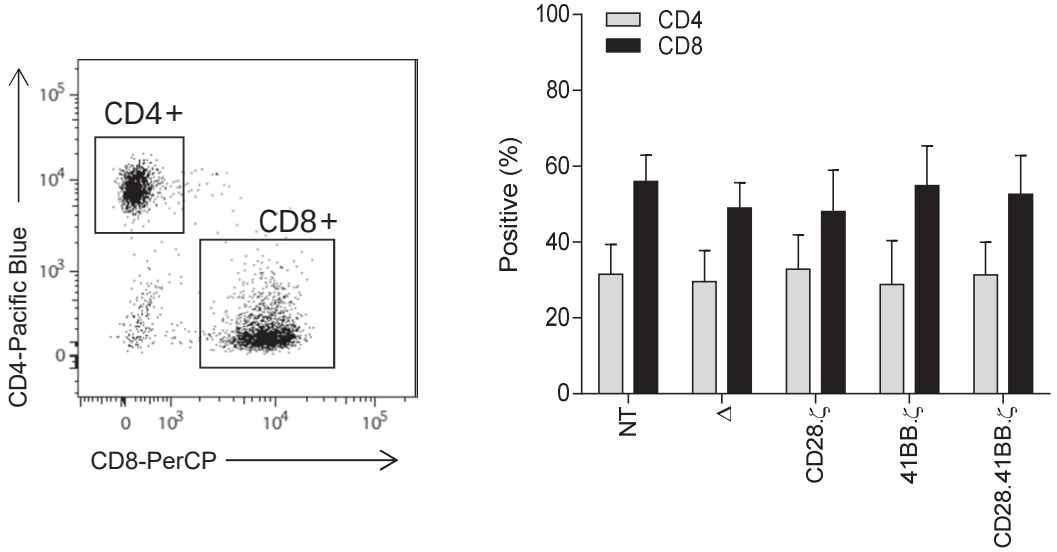
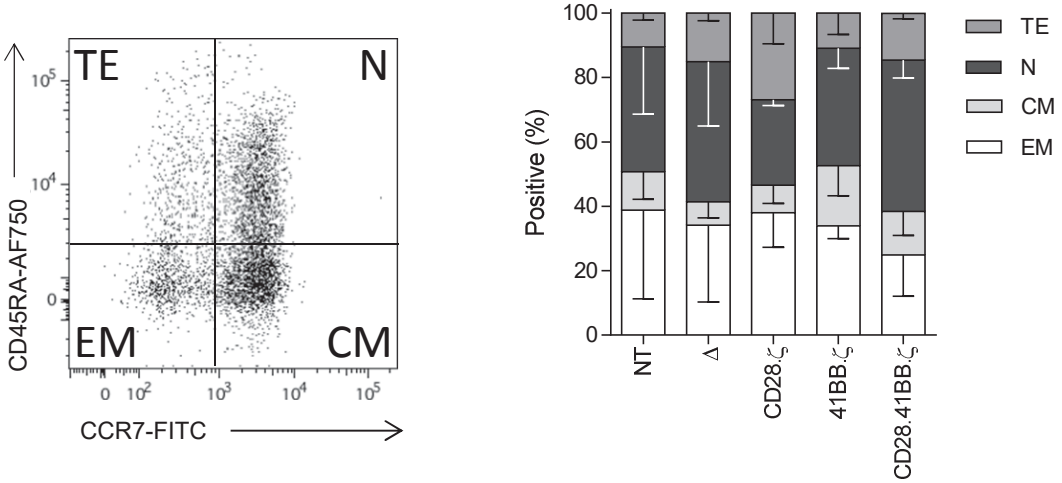
Supplementary Figure 1

A

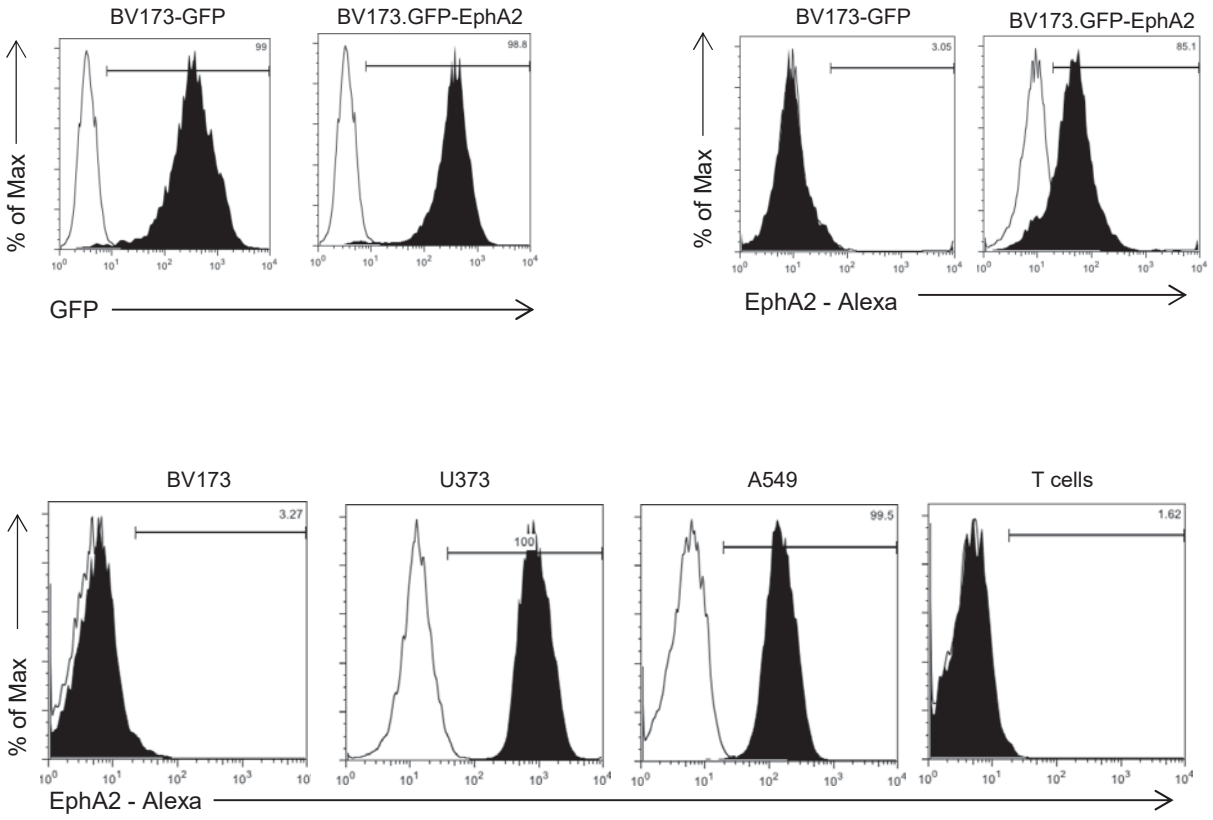


B



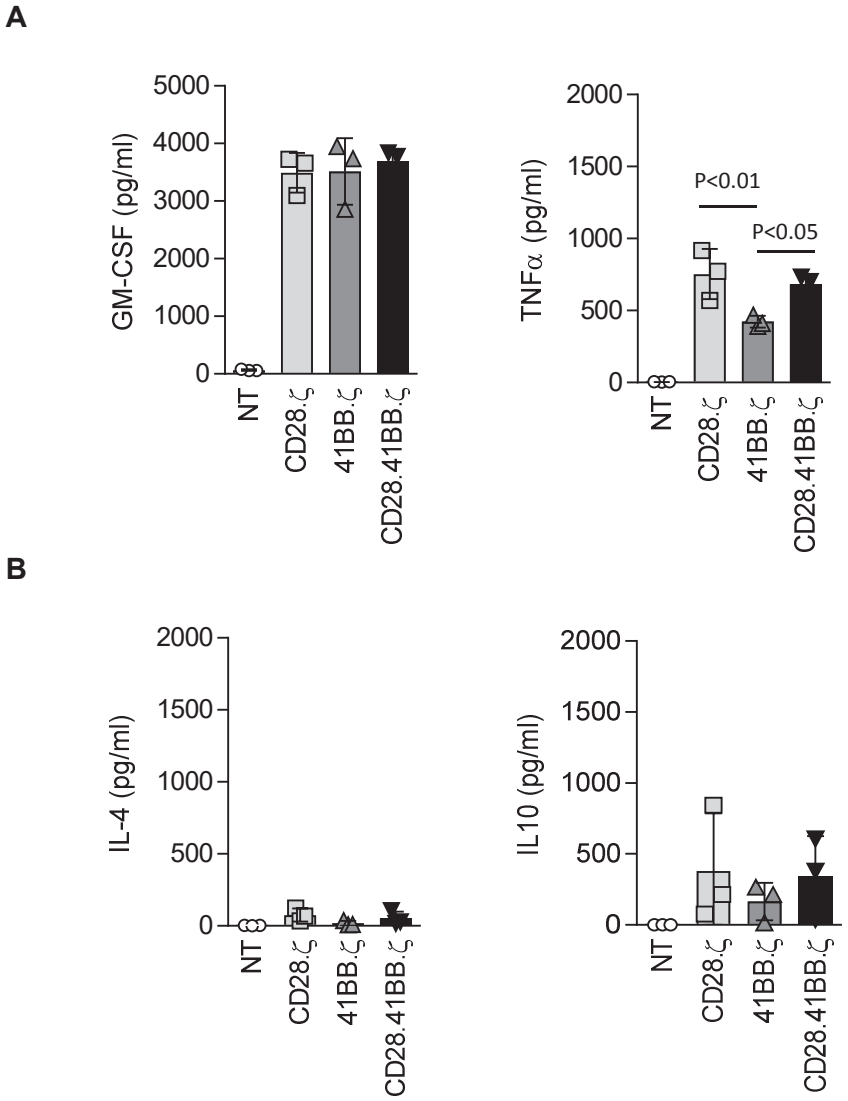
Supplementary Figure 1: Phenotypic analysis of EphA2-CAR T-cell lines. (**A**) CAR T cells were analyzed for CD4 and CD8 surface expression by using CD4-PacBlue and CD8-PerCP antibodies (BD Biosciences; n=4). (**B**) CAR T cells were analyzed for CD45RA and CCR7 surface expression by using CD45RA-AF750 and CCR7-FITC antibodies (BD Biosciences; n=3). TE – terminal effector, N – Naïve, EM – effector memory, CM – central memory.

Supplementary Figure 2



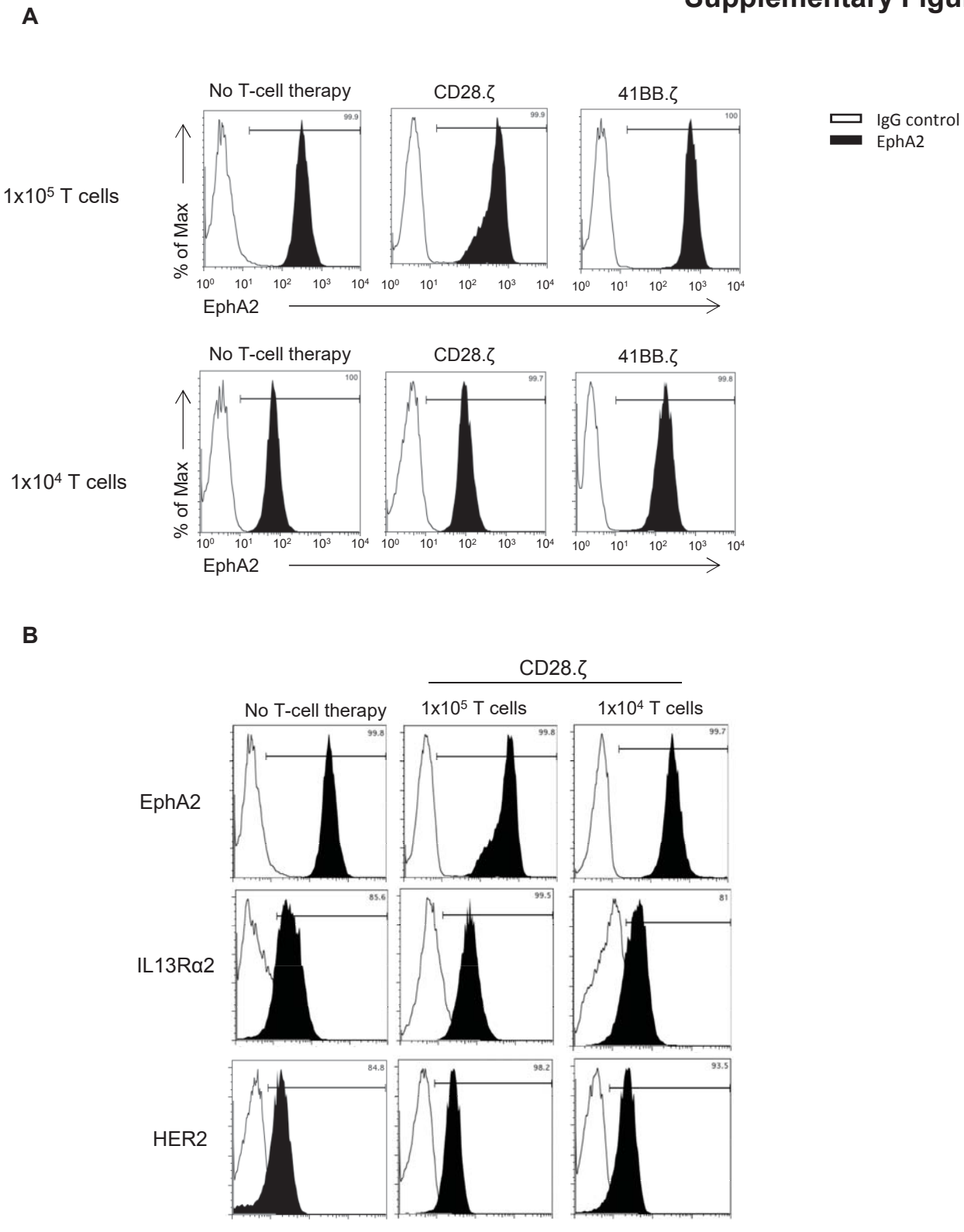
Supplementary Figure 2: Cell surface expression of EphA2. Cell lines were analyzed for EphA2 cell surface expression by using primary mouse anti-EphA2 antibody (MAB3035, R&D) followed by secondary goat anti-mouse IgG Alexa647 (Life Technologies).

Supplementary Figure 3



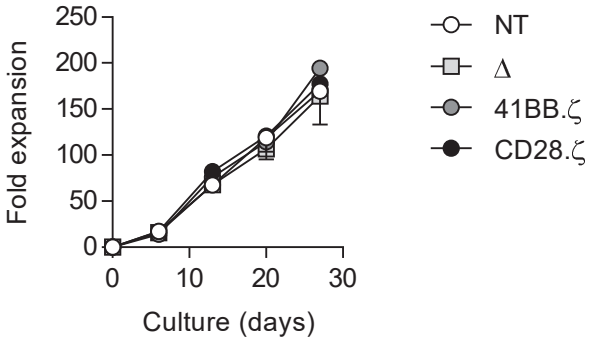
Supplementary Figure 3: Cytokine secretion analysis when EphA2-specific CAR T cells were co-cultured with BV173.EphA2 tumor cells. EphA2-specific CAR T cells were co-cultured with BV173.EphA2 cells at a 2:1 E:T ratio and 24 hours later a small aliquot of media was removed to determine the concentrations of cytokines by Multiplex assay (HSTCMAG28SPMX13, EMD Millipore, Billerica, MA). NT served as controls. (n=4; for TNFα: CD28.ζ vs 41BB.ζ, $p<0.01$, 41BB.ζ vs CD28.41BB.ζ, $p<0.05$).

Supplementary Figure 4



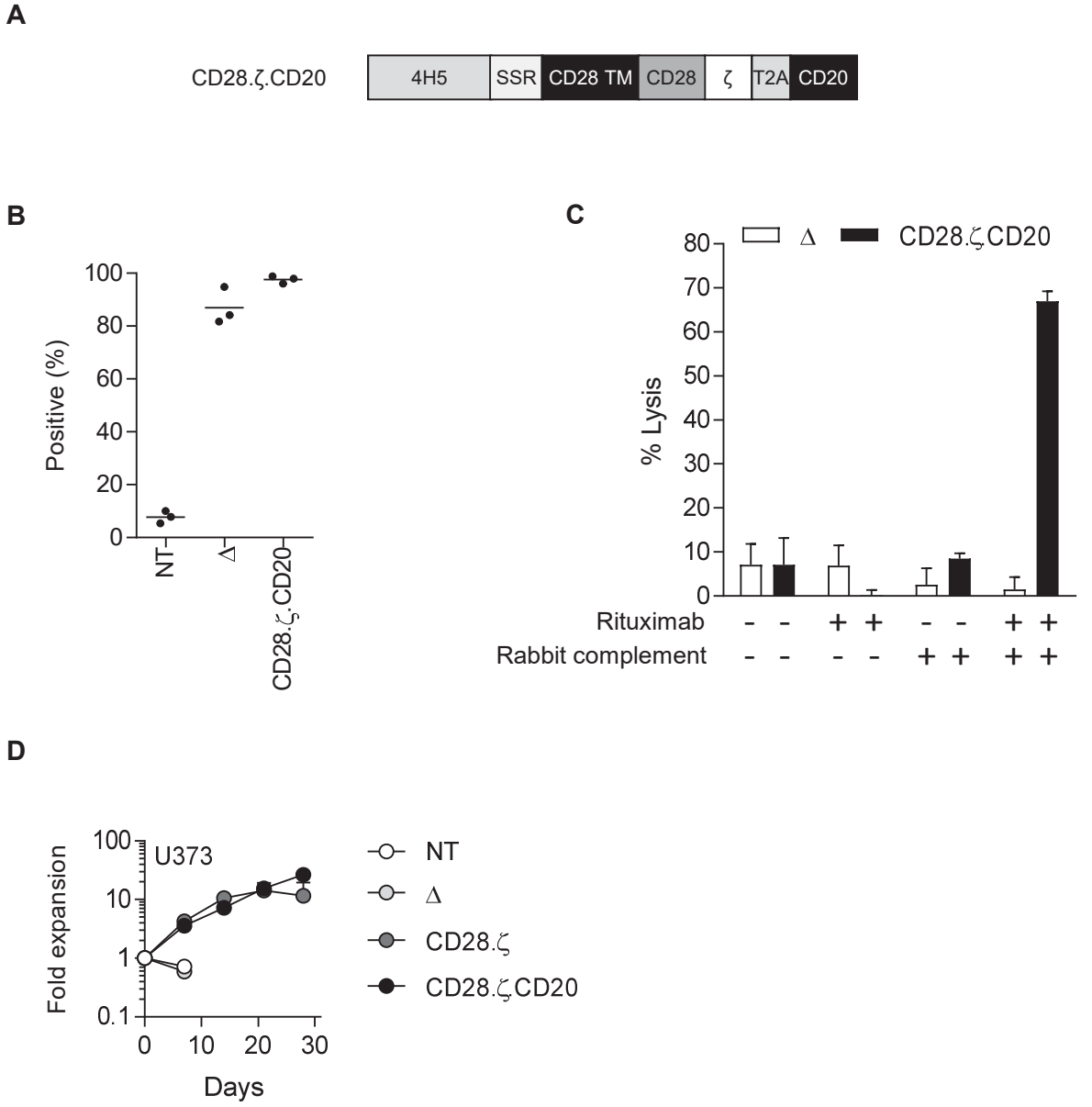
Supplementary Figure 4: Analysis of U373 cells isolated from recurrent tumors. U373 cells were isolated from recurrent tumor of mice that were treated with EphA2-CAR T cells. After short-term culture (2 to 7 days), FACS analysis was performed. (**A**) FACS analysis for EphA2. Representative plots, n=2-3 in each group.

Supplementary Figure 5



Supplementary Figure 5. CD28.ζ and 41BB.ζ CAR T cell expansion. CAR T cells were plated at $0.25x10^6$ cell per well of 24-well plate and subsequently expanded in a regular T cell culture media with IL-7 and IL15. CAR T cells were counted every 7 days. Figure shows cumulative data of CAR T cell expansion.

Supplementary Figure 6



Supplementary Figure 6: Schematic representation of EphA2-specific CD28.ζ CAR containing CD20. (**A**) Scheme of the CD28.ζ.CD20 CAR. tCD19 in CD28.ζ CAR was replaced with CD20 and will serve as a safety switch and CAR detection molecule. (**B**) Expression of the CAR was determined by the FACS analysis; summary data. (**C**) CD20 functionality test using ^{51}Cr release assay. CD28.ζ.CD20 and Δ CAR T cells were labeled with ^{51}Chromium and treated with rituximab and/or complement in a standard cytotoxicity assay. (n=3; assay was performed in triplicate; (**D**) EphA2-specific CD28.ζ and CD28.ζ.CD20 CAR T cells were co-cultured with U373 at a 2:1 E:T ratio. T cells were stimulated weekly with fresh U373 cells, and T cells were counted before addition of fresh target cells. Figure shows cumulative data of CAR T cell expansion (n=2-3; error bars represent SEM); NT and Δ served as controls.