

Figure S1. Immunomagnetic selection of various T-cell lines lentivirally transduced with huEGFRt containing constructs. Pre- and post-selection flow cytometric analyses for surface huEGFRt expression are depicted underneath their corresponding schematics of the CD19CAR-T2A-EGFRt (**a**) and CD19CAR-T2A-EGFRt-IMP2DM (**b**) constructs contained in lentiviral vectors. Codon optimized sequence portions of the CD19-specific, CD28 co-stimulatory CAR (CD19CAR), followed by the self-cleavable T2A, and selection markers huEGFRt and IMPDH2dm (a double mutant of the inosine monophosphate dehydrogenase 2 gene that allows for cell survival upon addition of mycophenolate ²⁷) are indicated, along with the Elongation Factor 1 promoter sequences (EF-1p), the GM-CSF receptor alpha chain signal sequences (GMCSFRss), and the 3 nucleotide stop codon. Flow cytometric analysis of lentivirally transduced T cell lines that had been stained with biotinylated-cetuximab and either PE-conjugated streptavidin or PE-conjugated anti-biotin Ab (black histograms) was performed on both the input T cells (PRE SLXN) and the positive fraction obtained from AutoMACS® (POS FRXN). Open histograms represent staining with PE-conjugated reagent alone, and the percent positive cells are indicated in each histogram. Note that selection of CD19CAR⁺EGFRt⁺ cells (**a**, top to bottom) occurred either 3 days after transduction of OKT3 blasts (for cJ05525), after 1 rapid expansion cycle of transduced effector memory (T_{EM}; i.e., autoMACS® selected CD62L-CD45RO⁺)-derived cells (for cJ05478), after 3 rapid expansion cycles of transduced CMVpp65-specific T_{CM}-derived cells (for cJ05257), or after 2 rapid expansion cycles of transduced CD8⁺T_{CM}-derived cells (for cJ05435); and selection of CD19CAR⁺EGFRt⁺IMP2DM⁺ cells (**b**, cJ05521) occurred after 1 rapid expansion cycle of transduced T_{CM}-derived cells.

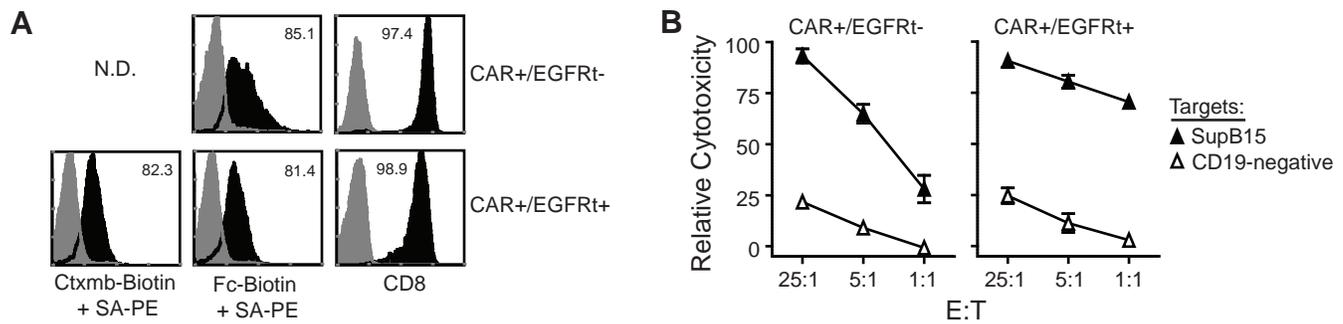


Figure S2. Non-EGFRt-selected CD19CAR-expressing effector T cells as an alternative control. **A**, Surface EGFRt, Fc (i.e., CD19-specific CAR) and CD8 expression on T_{CM} -derived cells that were transduced with CD19Rop_epHIV7 (top, CAR+/EGFRt-) or transduced with CD19Rop-T2A-EGFRt_epHIV7 and selected for the EGFRt+ population (bottom, CAR+/EGFRt+) was determined by flow cytometry. Percent positive staining compared to SA-PE or isotype controls alone (grey) is indicated. N.D., not done. **B**, The same T cells as in **A** were incubated for 4hrs with ^{51}Cr -labeled CD19+ SupB15 cells, CD19-negative control cells or OKT3-expressing LCL positive control cells as targets at the indicated E:T ratios. Relative cytotoxicity (\pm S.E. of triplicate wells) was determined by normalizing the chromium release to that obtained with OKT3-expressing LCL targets at a 25:1 ratio, which was set at 100.