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Supplemental Information

Automated generation of gene-edited CAR T cells at clinical scale

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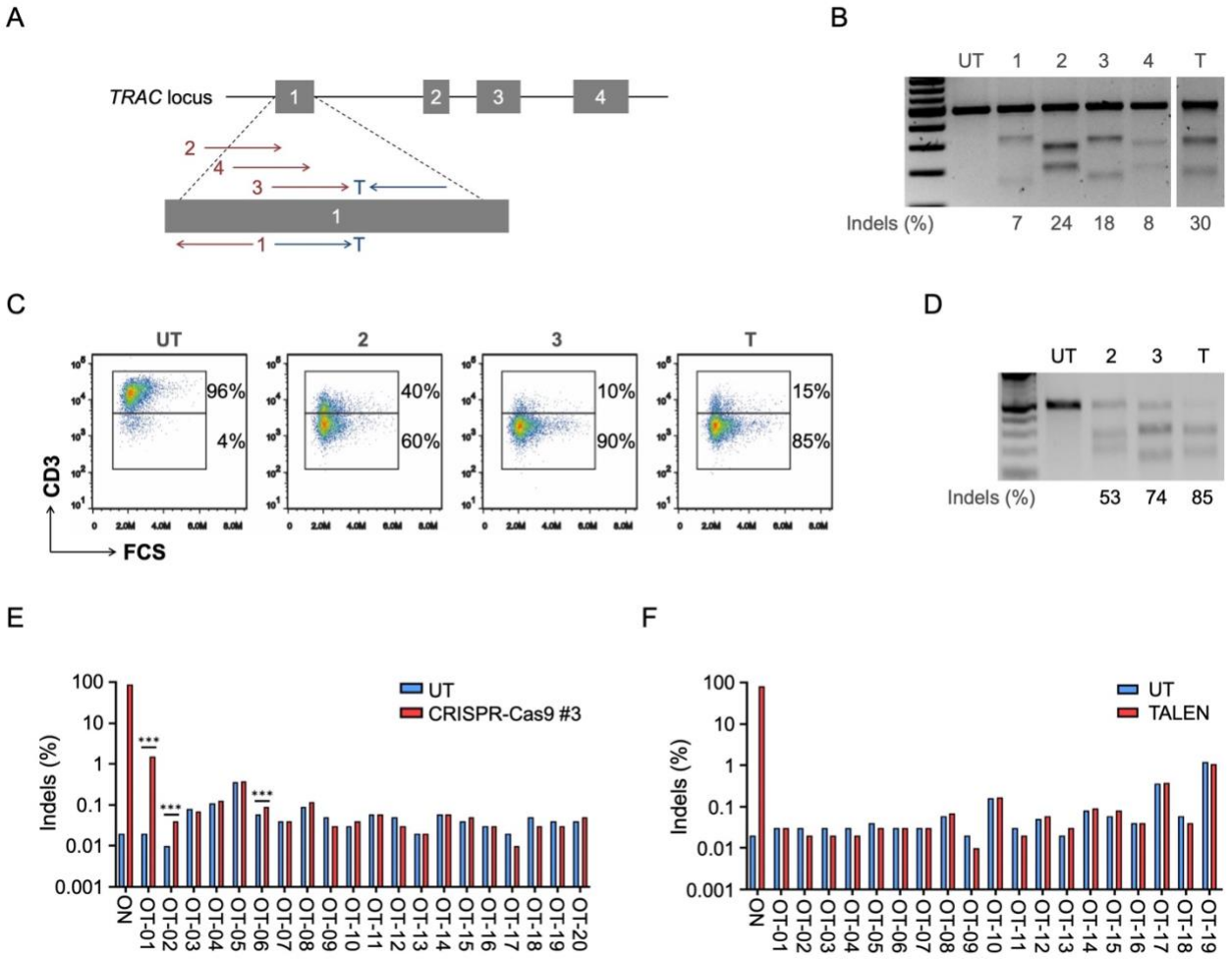

Figure S1. Characterization of *TRAC*-targeting designer nucleases. (A) Target sites in *TRAC* locus. Shown are the target sites in *TRAC* exon 1 of all designer nucleases. Red arrows denote CRISPR-Cas9 target sites #1-4, blue arrows represent binding half-sites of the TALEN (T). **(B)** Activity of designer nucleases in U2OS cells. U2OS cells transfected with designer nuclease expression plasmids (#1-4, T) were subjected to T7E1 assay. Percentages of cleaved products are indicated. Shown are results from one experiment. **(C)** Phenotyping of TALEN-edited T cells. Primary T cells nucleofected with either CRISPR-Cas9 ribonucleoprotein (RNP) complexes (#2-3) or TALEN-encoding mRNA (T) were subjected to flow cytometry. Shown are fractions of CD3-positive or CD3-negative cells harvested 7 days post-nucleofection. **(D)** Genotyping of TALEN-edited T cells. Primary T cells nucleofected with RNPs (#2-3) or TALEN-mRNA (T) were subjected to T7E1 assay. Percentages of cleaved products are indicated. Shown are results from one experiment. **(E-F)** Specificity profiles of *TRAC*-targeting designer nucleases. Primary T cells nucleofected with RNP (E) or TALEN mRNA (F) were subjected to targeted amplicon sequencing. Indel frequencies at the *TRAC* on-target site (ON) and potential off-target (OT) sites are indicated in comparison to untreated (UT) T cells. Shown are results from one donor. *** $p \leq 0.001$; Indels, insertion/deletion mutations.

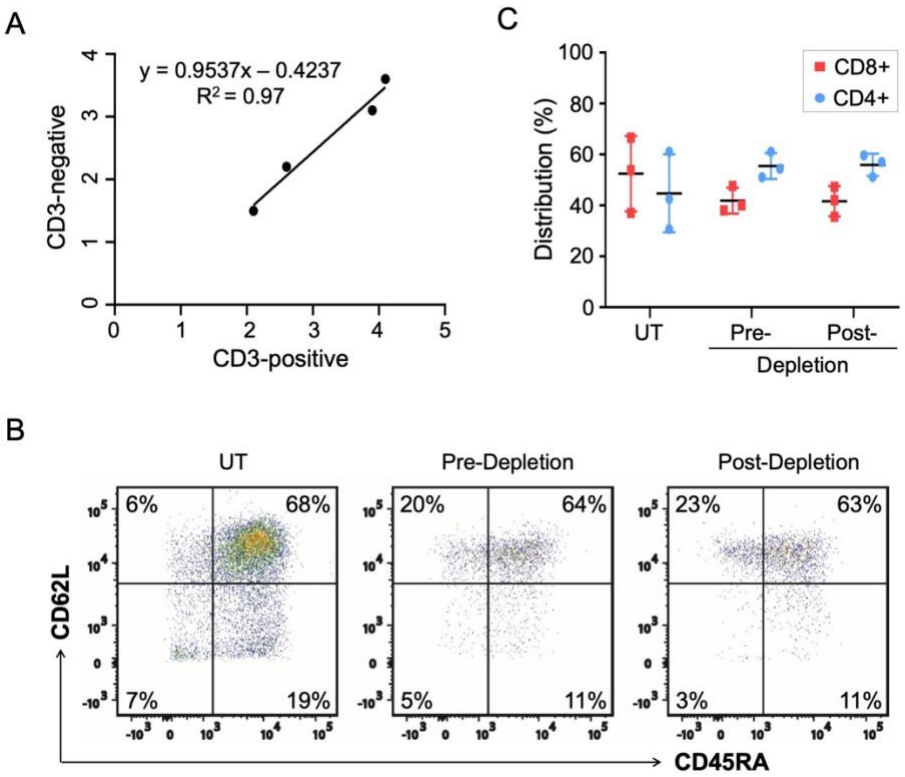

Figure S2. Quality assessment of *TRAC*-edited CAR T cells. (A) Comparison of cell division rates. Shown are the numbers of cell divisions for CD3-negative vs. CD3-positive cells determined at different time points during the manufacturing process (days 6, 7, 10, 12). Data were collected from all three manufacturing runs. **(B)** T cell phenotype analysis. T cell subsets were determined by assessing the expression of CD62L and CD45RA by flow cytometry. Edited CAR T cells were pre-gated for CD3 expression for UT, or CAR expression for the fraction of pre-depleted and post-depleted CAR T cells (not shown). Representative plots of one run are shown. **(C)** CD4 to CD8 ratio. Engineered T cells of three independent runs were harvested at indicated processing steps and CD4:CD8 ratio determined by flow cytometry.

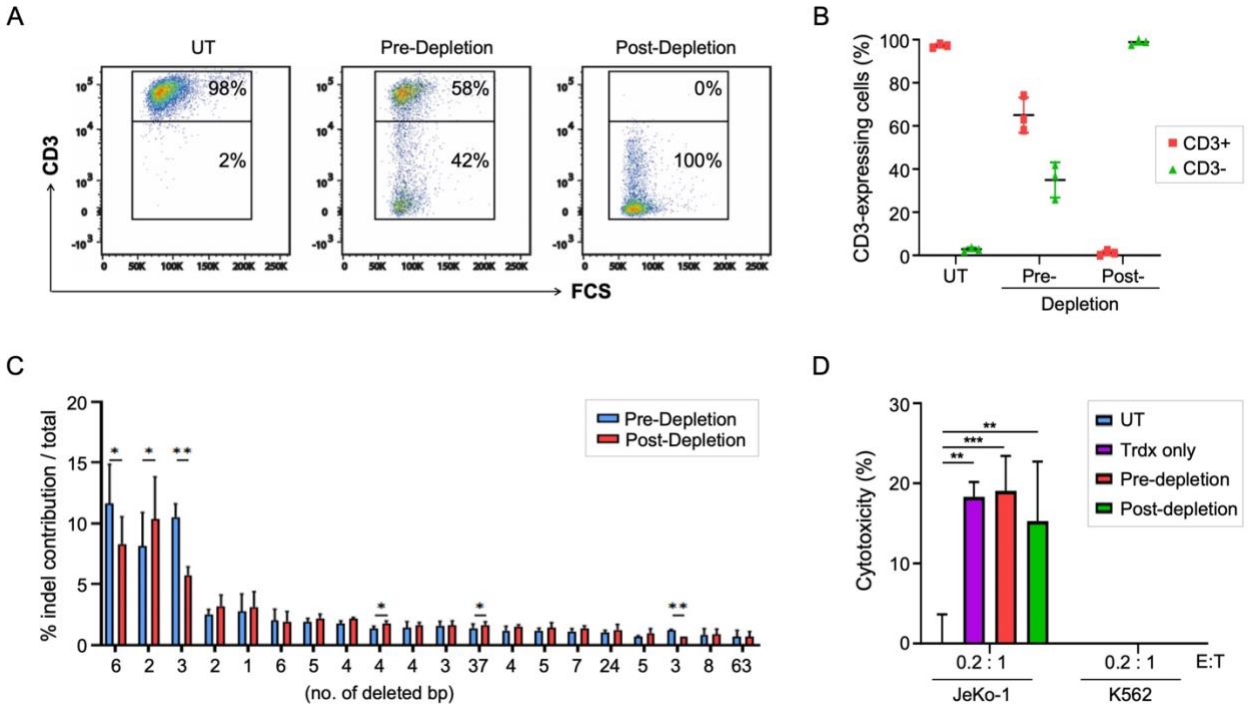

Figure S3. Characterization of *TRAC*-edited CAR T cells. (A) Phenotypic analysis of *TRAC* knockout. Engineered T cells were harvested at indicated processing steps. *TRAC* disruption was evaluated by flow cytometry analysis of CD3 expression. Representative plots of one run are shown. **(B)** Synopsis of three manufacturing runs. Shown are the fractions of CD3-positive and CD3-negative cells at indicated processing steps. **(C)** Indel distribution. Engineered T cells of pre and post-depletion steps were subjected to targeted amplicon sequencing of the *TRAC* locus. Shown are results of three manufacturing runs. Numbers on x-axis indicate the number of deleted bp. Indel, insertion/deletion; UT, untreated T cells. **(D)** Cytolytic activity. CAR T cells from indicated groups were co-cultured at an E:T ratio of 0.2:1 with CD19-positive JeKo-1 cells or CD19-negative K562 cells. Cytotoxicity was determined by flow cytometry. Shown are results of two or three independent runs, each in duplicate. UT, untreated T cells; Trdx only, CAR T cells retrieved directly after lentiviral transduction; Pre-depletion, CAR T cells harvested before TCRα/β depletion; Post-depletion, CAR T cells harvested after TCRα/β depletion.

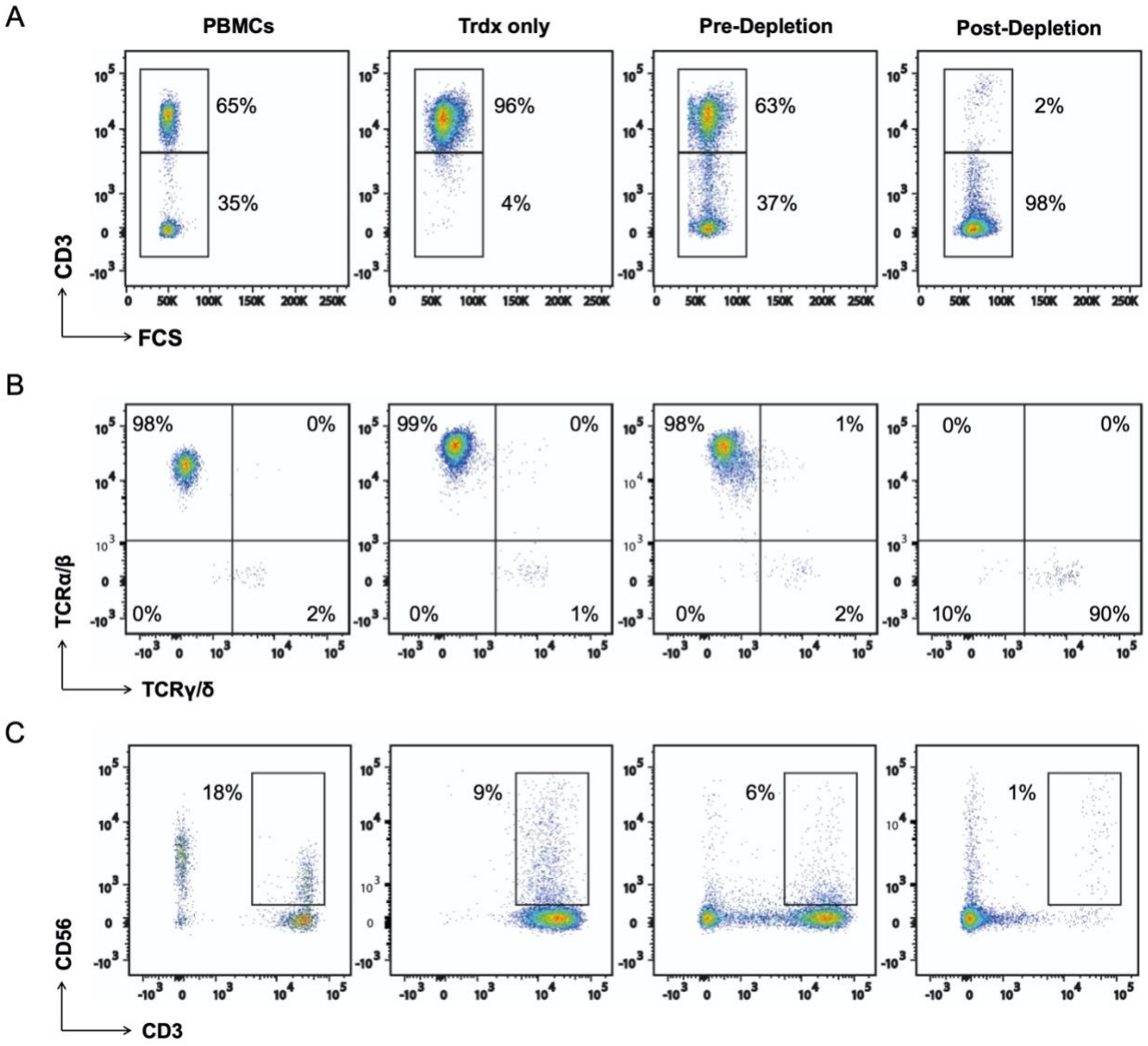

Figure S4. Characterization of final CAR T cell product. Engineered T cells were harvested at indicated processing steps and characterized in terms of **(A)** CD3 expression, **(B)** TCRα/β and TCRγ/δ expression, and **(C)** CD56 expression to identify the presence of NK and NKT cells. Shown are results of one representative run. PBMCs, peripheral blood mononuclear cells; Trdx only, T cells transduced with CD19-CAR encoding lentiviral particles only.