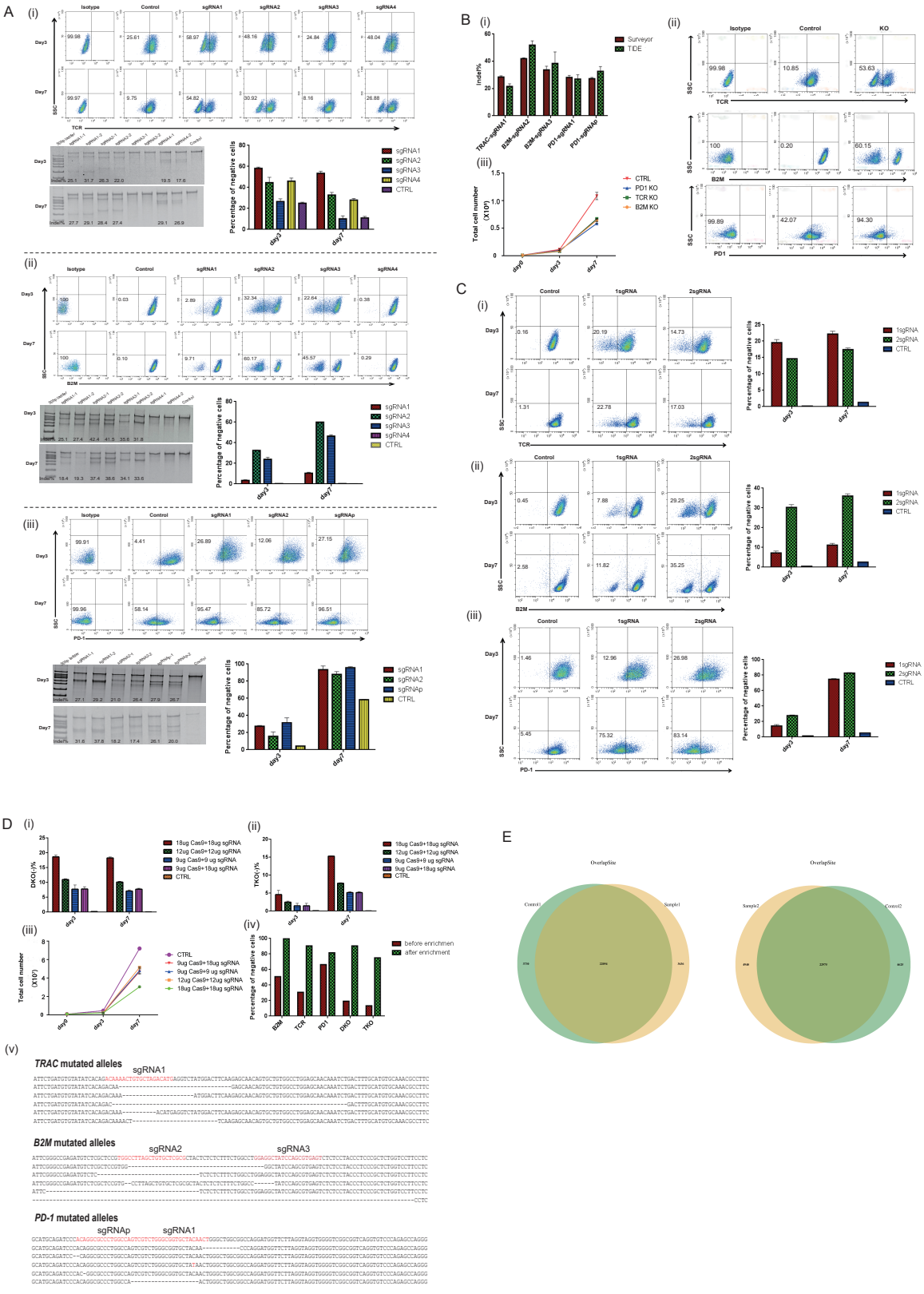
Liu et al. Figure S1



FigureS1. Multiplex gene editing mediated by CRISPR-Cas9 in primary T cells.

A. Screening for the efficient sgRNAs targeting human *B2M*, *TRAC* and *PD-1*. $1x10^6$ T cells were electroporated with RNP (3ug Cas9 protein and 3ug *in vitro* transcribed sgRNAs) 3 days post stimulation. 3 days and 7 days post-electroporation, cells were collected to quantify gene editing efficiency by Surveyor assay and surface expression of each gene was determined by FACS. (i) Targeting *TRAC*. Top panel is the expression of the target gene in RNP-treated T cells and control T cells shown as flow cytometric analysis plots. Bottom right panel is the quantification (mean±SEM, n=2). Bottom left panel is the Surveyor assay result. (ii) Targeting *B2M*. (iii) Targeting *PD-1*. **B.** Single gene editing of *TRAC*, *B2M* and *PD-1* in human primary T cells using CRISPR-Cas9. (i) Quantification of Cas9: single-guide RNA ribonucleoprotein (Cas9 RNP) mediated gene editing. Experiments are done in two biological repeats. Column plot shows (mean±SEM, n=2) the indel frequency of *TRAC*, *B2M* and *PD-1* analyzed by Surveyor assay and TIDE analysis. (ii) *TCR*, *B2M* and *PD-1* surface expression in human T cells 7 days post electroporation. (iii) Cell number count (mean±SEM, n=2) of RNP-treated T cells and control T cells. T cells were activated using anti-CD3/anti-CD28 beads. Three days after activation, T cells were electroperated, and the day of electroporation is indicated as day 0. **C.** Comparison of using one and two sgRNAs to target each gene. $1x10^6$ T cells were electroprorated with Cas9 protein and one sgRNA or two sgRNAs targeting each gene. Left panel shows the surface expression of target gene as the flow cytometric analysis plot. Right panel shows the percentage (mean±SEM, n=2) of target negative cells in RNP treated samples. (i) Targeting *TRAC*, for one sgRNA we used the most efficient sgRNA1. For two sgRNAs we used sgRNA1 and sgRNA2. (ii) Targeting *B2M*, for one sgRNA we used the most efficient sgRNA2, and for two sgRNAs we used sgRNA2 and sgRNA3. (iii) Targeting *PD-1*, for one sgRNA we used the most efficient sgRNA1, and for two sgRNAs we used sgRNA1 and sgRNAp. **D.** CRISPR-Cas9 multiplex gene editing of *TRAC*, *B2M* and *PD-1* in primary T cells. $1x10^6$ T cells were electroporated with RNP targeting three genes at different dosage. 3 days and 7 days post electroporation, cells were collected to analyze the frequency of *TRAC*, *B2M* and *PD-1* negative cells and cell viability. (i) Flow cytometric analysis (mean±SEM, n=2) of the frequency of *B2M* and *TRAC* double negative cells (DKO) at different RNP dosage. (ii) Flow cytometric analysis (mean±SEM, n=2) of the frequency of *B2M*, *TRAC* and *PD-1* negative cells (TKO) at different RNP dosage. (iii) Cell number count (mean±SEM, n=2) of RNP-treated T cells and control T cells. T cells were activated using anti-CD3/anti-CD28 beads. Three days after activation, T cells were electroperated, and the day of electroporation is indicated as day 0. (iv) The percentage of cells negative for specific targets

before and after magnetic bead enrichment. (v) Representative sequences of mutated alleles in RNP-transfected cells compared with wild-type sequence (top). sgRNA targeting sites are colored in red. **E.** Exome sequencing results shown as the Venn diagram of Indel mutations identified in sample edited using all five sgRNAs and their corresponding non-edited controls. Results of two donors are shown.