

Figure S1

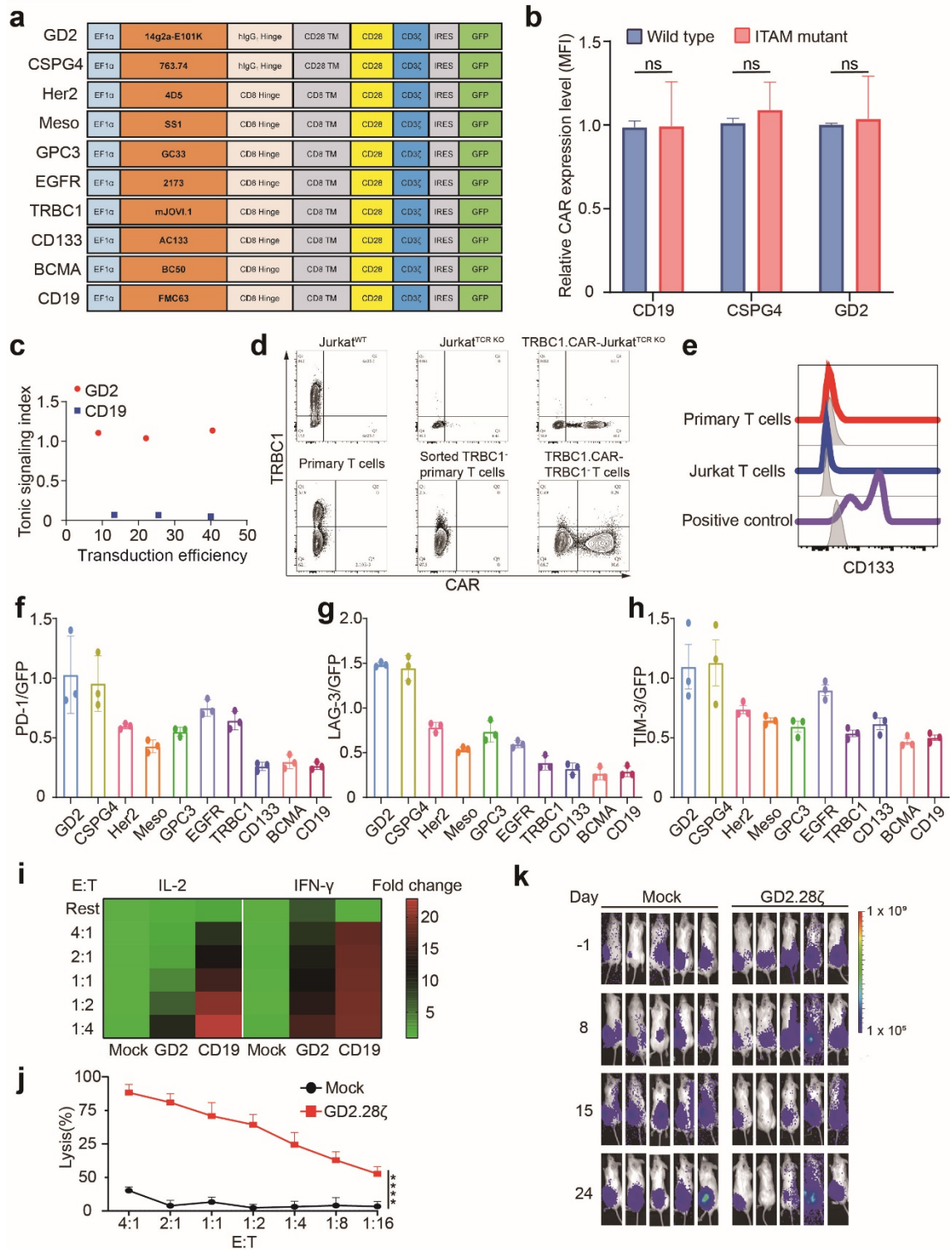


Figure S1. T cells incorporating many commonly used CARs express exhaustion markers and exhibit dysfunctional phenotypes.

- (a) The structures of the CARs used in this study.
- (b) Relative CAR expression levels after ITAM mutation in CD19, CSPG4, and GD2 CAR-T cells.
- (c) Correlation between the transduction efficiency and tonic signaling index.
- (d) Expressing TRBC1.CAR on TCR knockout Jurkat cells and sorted TRBC1⁺ primary T cells.
- (e) Surface expression of CD133 on Jurkat T cells and primary T cells. A CD133 transfected 293T cell sample was included as the positive control.
- (f-h) The GFP-normalized PD-1, LAG-3, and TIM-3 expression levels of ten commonly used CARs.
- (i) Cytokine secretion assay of GD2 and CD19 CAR-T cells after activation. Indicated CAR-T cells were harvested and co-incubated with their target tumor cells at the indicated E:T ratio. The levels of IL-2 and IFN- γ were detected by ELISA.
- (j) In vitro killing assay against GD2⁺ neuroblastoma cell line CHLA-255 of GD2 CAR-T cells.
- (k) In vivo killing assay against GD2⁺ neuroblastoma cell line CHLA-255 of GD2 CAR-T cells.

Data are presented as means \pm SEM; Comparisons were determined using unpaired student's t-tests (b) and two-way analysis of variance (j); **** $P < 0.0001$; ns not significant.