Supplementary Figure 1. Overview of CAR constructs used and their transduction efficacy after enrichment.

A. Scheme of L1CAM-targeting second generation CAR constructs. B. After enrichment, detection of CAR positive CD8+ cells was performed with fluorochrome-conjugated cetuximab antibody. Untransduced T cells serve as negative control (labeled as mock).

Supplementary Figure 2. PD-L1 induction in neuroblastoma cells is independent of direct cell-cell interaction.

A. Schematic overview of experiment set-up with conditioned media (CM). Media was conditioned by culturing SK-N-BE(2) neuroblastoma cells alone or with either L1CAM-CAR or control T cells at a 1:5 E:T ratio for 24 hours. B. PD-L1 expression on SK-N-BE(2) neuroblastoma cells obtained by flow cytometry after incubation with CM for 24 hours. C. IFNG levels assessed via ELISA after culturing SK-N-BE(2) neuroblastoma cells alone or with either L1CAM-CAR or control T cells at a 2:1 E:T ratio for 24 hours. D. PD-L1 expression on L1CAM-CAR T cells obtained by flow cytometry 24 hours after adding 1ng/ml IFNG or CD3/CD28 Beads at a 1:1 bead-to-T cell ratio in relation to untreated L1CAM-CAR T cells.

Supplementary Figure 3. Higher PD-1 expression in CD4+ TCM-derived L1CAM-CAR T cells.

A. PD-1 expression of bulk- or TCM-derived CD4+ control and CD4+ L1CAM-4-1BB-CAR T cells before and after co-culture with SK-N-BE(2) at a 1:1 E:T ratio for 24h. B. PD-L1 expression of SK-N-BE(2) neuroblastoma cells alone and after co-culture with either bulk- or TCM-derived CD4+ L1CAM-4-1BB-CAR T cells at a 1:5 E:T ratio for 24h. Data depicts 3 independent experiments assessed for each donor. \*P < 0.05 and \*\*P < 0.01 were assessed by two-tailed unpaired t test, comparing TCM-derived in relation to bulk-derived control and CAR T cell PD-1 and PD-L1 expression.