CD28 Co-Stimulus Achieves Superior CAR T Cell Effector Function against Solid Tumors Than 4-1BB Co-Stimulus

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Supplementary Materials:

**Figure S1.** Transduction efficacy of CAR constructs. (A) Mouse splenocytes derived from ChRLuc/OT-1/Rag\(^+\) mice were transduced with retroviruses encoding L1CAM-specific SS-4-1BB/ζ, LS-4-1BB/ζ, SS-28/ζ or LS-28/ζ CARs. Cells were stained with cetuximab (anti-EGFR antibody) and anti-CD8 antibody to assess transduction efficacy by flow cytometry. Untransduced CD8\(^+\) T cells served as negative control. (B) Representative flow cytometry plots showing CD8 and EGFRt expression on CD8\(^+\) human T cells transduced with L1CAM-specific SS-4-1BB/ζ, LS-4-1BB/ζ, SS-28/ζ or LS-28/ζ constructs after MACS enrichment. Untransduced T cells served as negative control. (C) Mouse splenocytes derived from ChRLuc/OT-1/Rag\(^+\) mice were transduced with retroviruses encoding HER2-SS-4-1BB/ζ, HER2-LS-4-1BB/ζ, HER2-SS-28/ζ, or HER2-LS-28/ζ CARs and stained with cetuximab (anti-EGFR antibody) and anti-CD8 antibody to determine transduction efficacy by flow cytometry.
Figure S2. Target antigen surface expression on tumor cells. Flow cytometry analysis of L1CAM expression on SK-N-BE(2) neuroblastoma cells (A) and single cells prepared from a PDX stained with anti-L1CAM monoclonal antibody (B). (C) Flow cytometry analysis of HER2 expression on SKOV3 ovarian carcinoma cells stained with anti-HER2 antibody. Cells stained with the isotype control antibody served as control.

Figure S3. Flow cytometry gating strategy to identify T cells within co-cultures with tumors cells. Single cell suspension was stained with anti-GD2 (to discriminate from GD2+ tumor cells), anti-CD8 and annexin V conjugated with different fluorochromes and analyzed by flow cytometry. Among single cells, T cells (CD8+, GD2-) were selected to assess annexin V positivity.
Figure S4. PDX mouse model treated with single dose of $1 \times 10^6$ human L1CAM-specific CAR T cells. NOG mice were engrafted with a neuroblastoma PDX and treated 40 days later with a single dose of $1 \times 10^6$ human L1CAM-SS-28/ζ (n=5), L1CAM-SS-28/ζ (n=5) or untransduced T cells (n=5).