Supplementary Figure S1: In vitro characterization of T cells modified to express second generation CARs with different co-stimulatory domains. (A) CD4+ CAR T cells from several different donors were co-cultured with APC cells transduced with mesothelin (K562meso). Supernatants were obtained 24 hours after co-culture, and cytokine production was analyzed by ELISA. (B) CD4+ CAR-T cells and (C) CD8+ CAR-T cells were co-cultured with different tumor cell lines that express mesothelin. Supernatants were obtained 24 hours after co-culture, and cytokine production was analyzed by ELISA. (D) CD8+ CAR T cells were co-cultured with Capan-2 cells. Supernatants were obtained 24 hours after co-culture, and cytokine production was analyzed by Luminex. (E) CD4+ CAR-T cells were co-cultured with magnetic beads coated with recombinant mesothelin. Cell lysates were obtained at different time points and phosphorylation levels for AKT and ERK were analyzed by Western Blot. Basal phosphorylation was evaluated without stimulation (minute 0). Representative of 2 donors.
Supplementary Figure S2: Pattern of cytokine release by a third generation CAR containing ICOS and 4-1BB intracellular domains. CD4+ CAR T cells were co-cultured with pancreatic cancer cells (Capan-2) that express mesothelin. Supernatants were obtained 24 hours after co-culture, and cytokine production was analyzed by luminex.