**Supplementary Materials**

Fig. S1. Derivation of the B7-H3 CAR

Fig. S2. CAR efficacy is not limited by secreted soluble B7-H3 or antigen loss/downregulation in our models

Fig. S3. B7-H3 CAR T cells exert *in vivo* activity against NALM6-B7-H3-High expressing cells

Fig. S4. Differentiated dendritic cells but not monocytes express significant amounts of surface B7-H3

**Figure S1**

 **Figure S1: Derivation of the B7-H3 CAR**. (A) Structure of the CAR backbone used to create second generation anti-B7-H3 CARs combining six scFvs with a 4-1BB costimulatory domain and a short hinge-transmembrane region derived from CD8α. (B) CARs were expressed on T cells using a gamma retrovirus and expression was confirmed by flow cytometry after staining with Protein L. (C) T cells expressing different versions of the B7-H3 CAR were co-cultured with tumor cells and, 24h later, levels of interferon-gamma were measured by ELISA. (D) Structure of the CAR backbone reengineered to contain either an IgG1 spacer domain or (E) CD28 as an additional costimulatory domain. (F) Cytokine production of T cells bearing CD276.MG-41BB-ζ (CD276.MG) and CD276.3-41BB-ζ (CD276.3) CARs engineered using backbones shown in (A), (D) and (E) in response to co-culture with B7-H3 expressing osteosarcoma cell lines. (G) Flow cytometric analysis of the levels of expression of PD-1, LAG-3 and TIM-3 on the surface of T cells expressing CD276.MG-41BB-ζ or CD276.3-41BB-ζ on day 10 after activation with CD3/CD28 beads. (H) Flow cytometric analysis of the percentage of CD4 or CD8 positive population in transduced CD276.MG CAR or MOCK T cells.

**Figure S2**

**Figure S2: CAR efficacy is not limited by secreted soluble B7-H3 or antigen loss/downregulation in our models.** (A) Tumor culture supernatant was collected and measured for soluble B7-H3 by ELISA. (B) Flow cytometric analysis of the expression levels of B7-H3 on the surface of tumor cells maintained in culture (blue plots) or engrafted into mice (orange plots). (C) Flow cytometry mean fluorescence index (MFI) of B7-H3 expression on tumor cells co-cultured in the presence of B7-H3 CAR or MOCK T cells for 80 hours. Error bars represent SEM.

**Figure S3**

**Figure S3: B7-H3 CAR T cells exert *in vivo* activity against NALM6-B7-H3-High expressing cells.** 1e6 NALM6 cells expressing high amounts of surface B7-H3 were engrafted into mice by tail vein injection. Three days later, mice were injected with 1e7 B7-H3 CAR+ T cells or mock transduced control T cells. Tumor progression was measured by bioluminescence photometry and flux values (photons per second) were calculated using Living Image software. Values for individual mice are shown.

**Figure S4**

**Figure S4: Differentiated dendritic cells but not monocytes express significant amounts of surface B7-H3.** (A) Flow cytometric analysis of B7-H3 expression on the surface of MG63.3 tumor cell line, monocytes from a healthy donor and derived dendritic cells. (B) IL-2 secretion levels measured by ELISA in supernatants from B7-H3 CAR or MOCK T cells co-cultured with same cells as in (A) for 24h. Error bars represent SEM