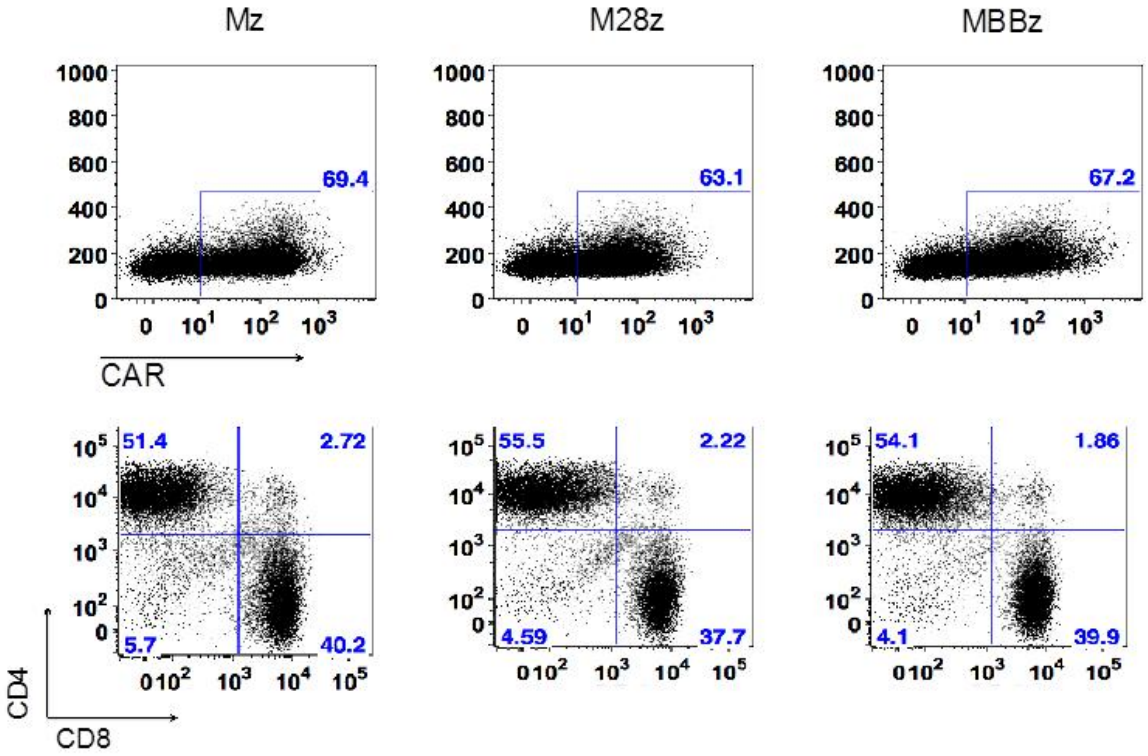
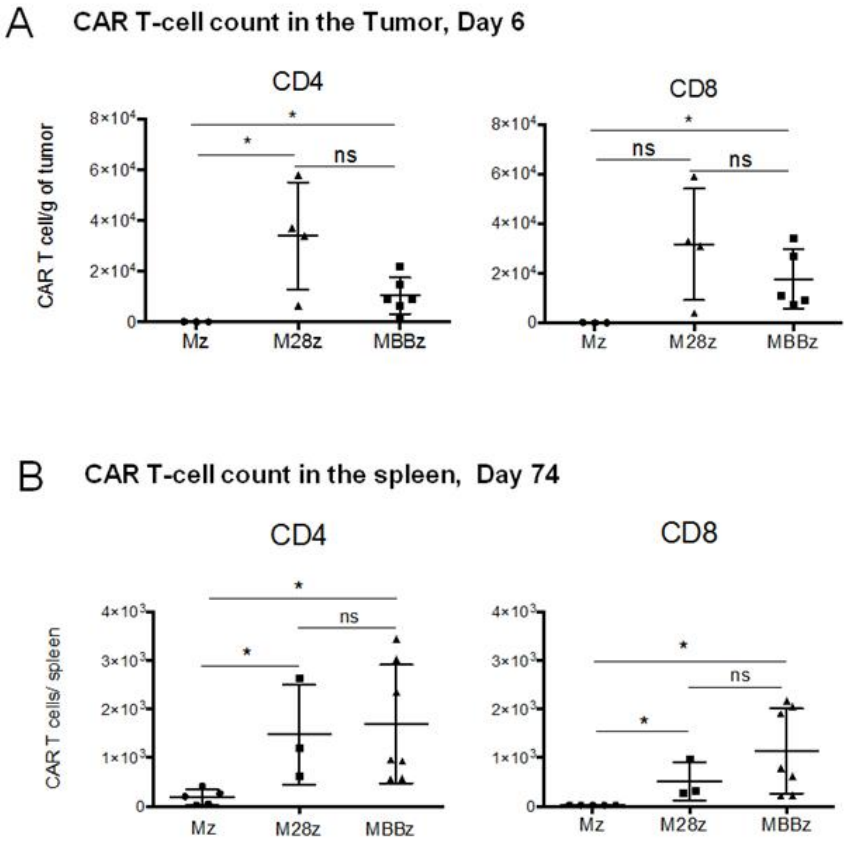
Supplementary Figure 1.



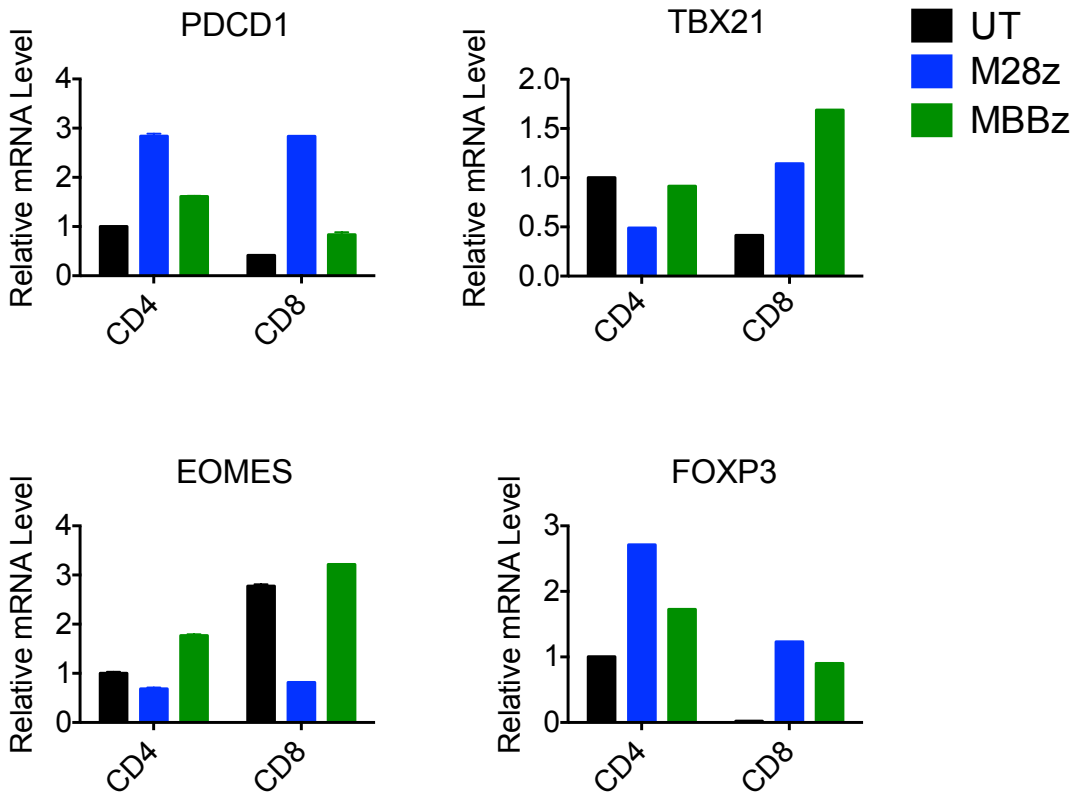
Efficient retroviral transduction of human T cells to express Mz, M28z, and MBBz CARs. (*Top*) Shown is representative FACS analysis 4 days after gene transfer. Fluorescence minus one staining was used to set positive gates after a live/dead stain excluded nonviable cells. All experiments used T cells with 50% to 70% CAR transduction efficiency; transduction percentages between T-cell groups were within 5% of each other. (*Bottom*) Both CD4+ and CD8+ T-cell subsets were efficiently transduced. CD4+ and CD8+ percentages after gating for CAR T cells are shown.

Supplementary Figure 2.



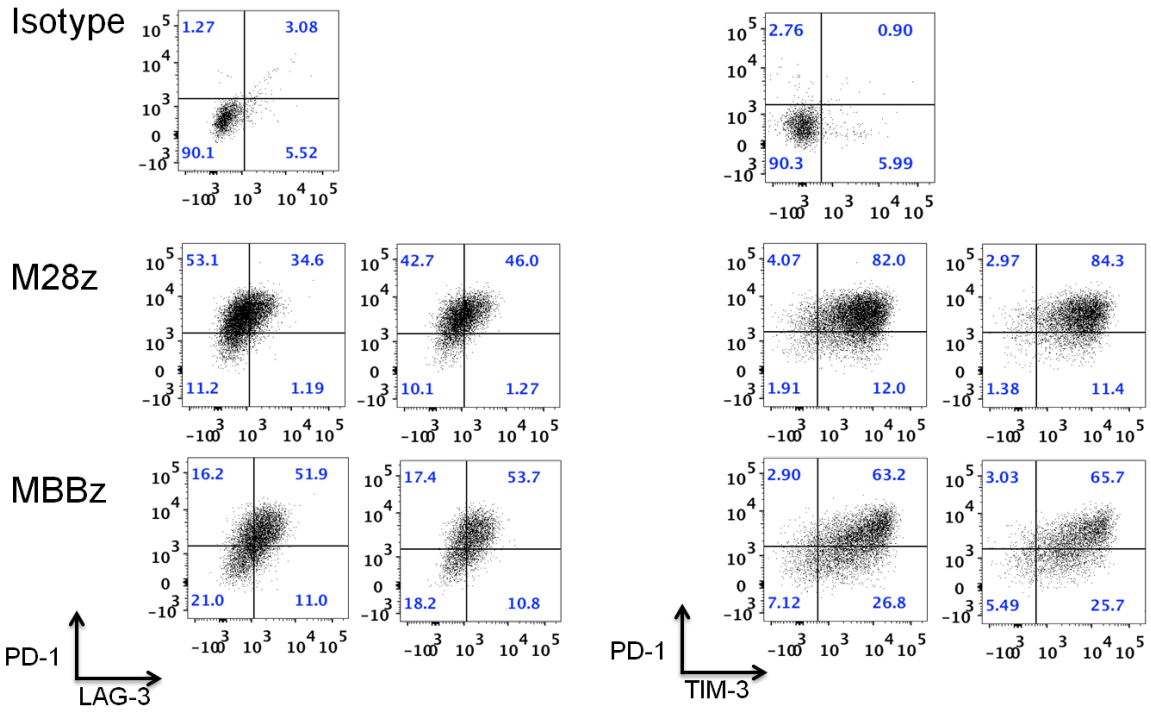
M28z and MBBz CAR+ T cells demonstrated a similar early intratumoral accumulation and long-term persistence of both CD4+ and CD8+ T cells. Mz, M28z, and MBBz CAR T cells were harvested from the tumor at Day 6 and spleen at Day 74 following T-cell administration into mice with pleural tumor. The absolute number (right panels) of CD4+ or CD8+ CAR T cells per gram of tumor (Panel A) or per spleen (Panel B) was quantified by flow cytometry using countbright absolute counting beads. Student's *t* tests were performed for statistical significance (*$P < 0.05$).

Supplementary Figure 3.



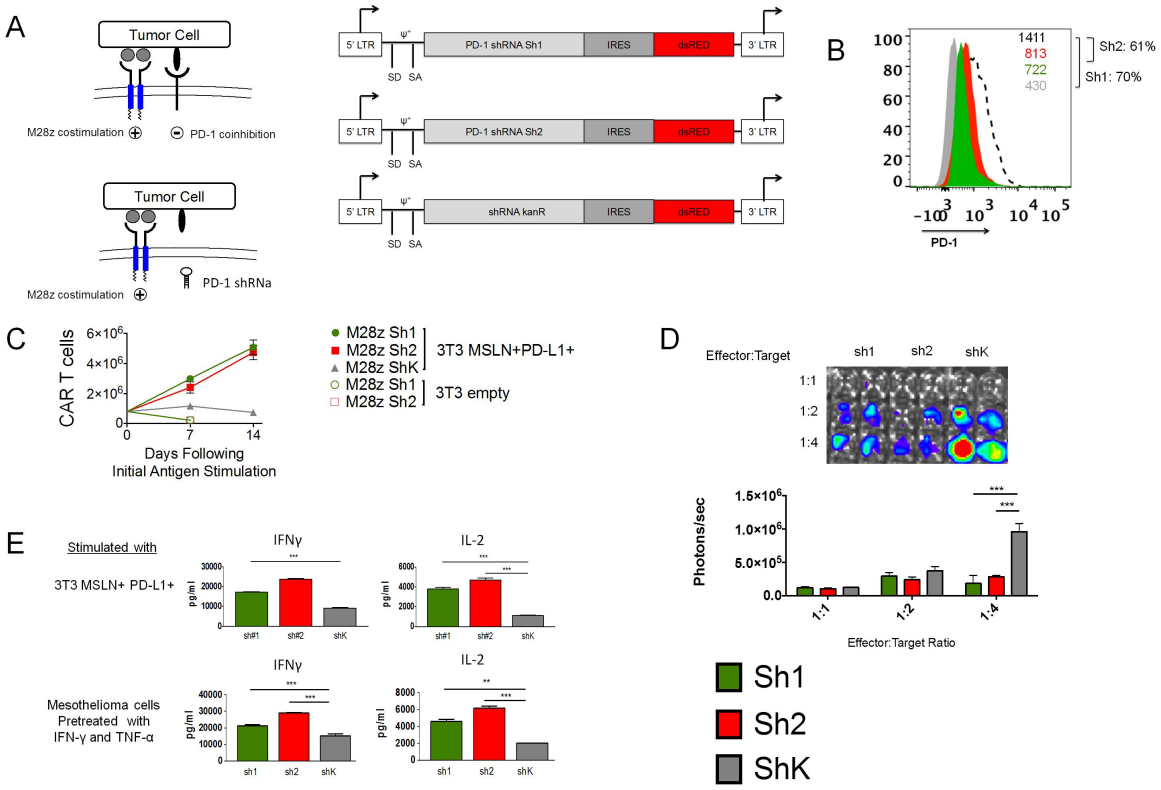
MBBz CAR T cells express a less exhausted, more potent phenotype compared to M28z CAR T cells. 4-1BB- and CD28-costimulated T cells were expanded with repeated antigen stimulation, and mRNA was extracted and subjected to RT-PCR analysis 20 h after the third stimulation. Data are represented in fold change relative to the mRNA expression of CD4+ unstransduced T cells. MBBz CAR T cells express higher levels of *EOMES* (Eomesodermin) and *TBX21* (T-bet), and lower levels of *PDCD1* (PD-1) and *FOXP3* (Foxp3). All comparisons were significant at $P<0.001$. Data are representative of at least 2 independent experiments.

Supplementary Figure 4.



Tumor-infiltrating M28z and MBBz CAR T cells coexpress PD-1 along with other inhibitory receptors. At Day 6 after the administration of CAR T cells, the pleural tumor were harvested and CAR T cells were stained with CD3, CD45, LNGFR, PD-1 and Lag-3 (left panel) or Tim-3 (right panel). Isotype staining (top) was used as control to established positive gates

Supplementary Figure 5.



Cotransduction of PD-1 receptor–targeting shRNAs rescues M28z CAR T cells from PD-L1/PD-1–mediated inhibition *in vitro*. (A) (*Left*) Schematic representation of CD28-costimulated T cells binding tumor-expressed PD-L1 via endogenous PD-1 receptor, with or without coexpression of PD-1–targeting shRNA. (*Right*) All experiments included M28z CAR T cells cotransduced with one of two PD-1–targeting shRNAs (sh1 or sh2 coexpressing a dsRED reporter) or with an shRNA targeting a bacterial sequence (KanR). **(B)** Compared with KanR-transduced cells, M28z CAR T cells cotransduced with PD-1–targeting shRNAs demonstrated a 60% to 70% knockdown in PD-1 receptor protein expression upon stimulation with phytohemagglutinin. Cells were incubated with either 3T3 fibroblasts overexpressing PD-L1 (3T3 MSLN+ PD-L1+)

or mesothelioma tumor cells that had been treated with IFN-g and TNF-a in order to upregulate PD-L1 and PD-L2. M28z PD1 shRNA CAR T cells demonstrate enhanced accumulation upon repeated antigen stimulation **(C)**, enhanced cytolytic function at low effector to target ratios, as measured by luciferase activity of remaining live tumor cells **(D)**, and increased Th1 cytokine secretion **(E)** (**P<0.01; ***P<0.001). Student's *t* tests were performed and statistical significance was determined using the Sidak-Bonferonni correction for multiple comparisons.for multiple comparisons. Data represent the mean ± SEM of three replicates and are representative of at least 3 independent experiments.