**Supplementary Figure 1: Murine SAR expression and immunophenotyping of transduced T cells**

(A) Schematic overview of the therapeutic concept: a BiAb specific for both the SAR transduced-T cells and the tumor cell expressing the associated antigen mediates on-target activation of the T cell and redirected tumor cell lysis. (B) Applied gating strategy to determine transduction efficiency of murine and human T cells. (C) Transduction efficiencies of murine SAR and CAR constructs. (D, E, F, G, H, I, J) Transduced T cells were co-cultured with Panc02-OVA-EpCAM+ tumor cells and 2 + 1 BiAb (1 μg/ml). The absolute number of the T cells was determined before and after co-culture (D). For phenotypic analysis, T cells were stained for CD8, CCR7 and CD62L surface expression. CD8+CCR7+CD62L+ T cells were defined as central memory T cells (E) and CD8+CCR7-CD62L- T cells as effector memory T cells (F). Activation, proliferation rate and exhaustion of the T cells was determined by staining cells for intracellular Ki67 and surface CD69, LAG3 and PD-1 (G, H, I, J).

All graphs show mean values of experiments performed at least in triplicates. Experiments are representative of at least three independent experiments. p-values are shown resulting from two-sided unpaired t-test. p < 0.05 was considered statistically significant.

**Supplementary Figure 2: Soluble and bound EGFR ligands do not trigger unwanted SAR T cell activation, while antibodies monovalent and bivalent for SAR can trigger T cell activation to the same extent.**

(A) E3 T cells (red) and wild-type T cells (green) were mixed in a 1:1 ratio and co-cultured with 4T1 tumor cells for 8 to 10 h. Cluster formation was analyzed by confocal microscopy. (B, C) Quantification of T cell stimulation as measured by IL-2 (B) and IFN-y (C), following coculture of E3 T cells with known EGFR ligands in their soluble as well as Fc-bound forms. Plate-bound cetuximab (5 μg/ml) was used as a positive control. (D) Quantification of T cell stimulation following coculture of E3 T cells with A375 tumor cells (E:T 10:1). The metalloprotease inhibitor Batimastat was added (10uM) to prevent EGFR ligand shedding. (E, F) Quantification of 4T1 (E) or B16-EpCAM+ (F) tumor cell lysis when co-cultured with E3 T cells and decreasing concentrations (1, 0.5, 0.25, 0.125 or 0.062 μg/ml) of either 2 + 2 or 2 + 1 BiAb. (G, H) Saturation curve analysis of E3 T cells for 2 + 2 BiAb and 2 + 1 BiAb in relation to the lysis curves of 4T1 (G) and B16-EpCAM+ (H) tumor cells.

All graphs show mean values of experiments performed at least in triplicates. p-values are shown resulting from two-sided unpaired t-test. p < 0.05 was considered statistically significant.

**Supplementary Figure 3: In contrast to SAR T cells, CAR T cells rely on the release of perforin for efficient killing.**

(A) Real-time killing of Panc02-OVA-EpCAM+ cells when co- cultured with E3 and aEpCAM- CAR wild-type or perforin knock-out T cells (E:T 10:1). (B, C) Co-culture of 4T1 (B) or B16- EpCAM+ (C) tumor cells with E3 T cells and anti-FasL blocking antibody (10 mg/ml, E:T 10:1). (D) Panc02-OVA-EpCAM, B16EpCam and 4T1 tumor cells were stimulated with 100 U/mL recombinant murine IFNγ. Gene expression levels of CD95 were analyzed by qRT PCR. (E, F, G) Co-culture of Panc02-OVA-EpCAM+ (E), 4T1 (F) or B16-EpCAM+ (G) tumor cells with aEpCAM-CAR T cells and anti-FasL blocking antibody (10 mg/ml, E:T 10:1). (H) Co-culture of Panc02-OVA-EpCAM+ tumor cells with C3 T cells and anti-FasL blocking antibody (10 mg/ml, E:T 10:1).

All graphs show mean values of experiments performed at least in triplicates. Impedance- based cytotoxicity assays were performed in duplicates for technical reasons. Experiments are representative of at least three independent experiments. Exact p-values are shown resulting from two-sided unpaired t-test. For impedance-base cytotoxicity assays the total curve over time was compared. p < 0.05 was considered statistically significant.

**Supplementary Figure 4: Human SAR T cells are specifically stimulated by anti-human mesothelin x anti-EGFRv3 2 + 1 BiAb and redirect lysis to mesothelin+ target cells in vitro and in vivo**

(A) Representative transduction efficiencies of SAR constructs used in human characterization, and their relevant controls. (B) CD8 and CD4 T cell ratios of transduced T cells during in vitro expansion. (C) Quantification of E3, E3del or untransduced T cell activation when co-cultured with HEK293-MSLN cells and 2 + 1 BiAb (5 μg/ml, E:T 10:1). (D, F) Real-time quantification of HEK293-MSLN (D) or MiaPaca-MSLN (F) tumor cell lysis by E3 or E3del T cells and 2 + 1 BiAb. (E) Quantification of E3, E3del or untransduced T cell activation when co-cultured with MiaPaca-MSLN tumor cell and decreasing concentrations of 2 + 1 BiAb (0.001, 0.005, 0.01, 0.05, 1 or 5 μg/ml, E:T 10:1). (G) NSG mice inoculated s.c. with Suit-2-MSLN (n = 5 / group) were treated with a single i. v. injection of human aMSLN- CD28-CD3z CAR T cells or untransduced T cells as indicated. (H) NSG mice inoculated s.c. with 1 x 106 MSTO-MSLN-LUC cells. When tumors reached the size of < 25 mm2, mice were treated with a single i. v. injection of E3 T cells only (n = 5), E3 T cells + 10 μg BiAb (n = 5), 10 μg BiAb only, (n = 5), and PBS (n = 5), followed by a total of four BiAb injections, as indicated. (I) FACS-based quantification of E3 T cell compartments before therapy administration, and the intra-tumoral infiltrate following tumor harvest at day 25 of MSTO- MSLN-LUC xenograft treatment experiment. Inner circle, CD4 and CD8 ratios. Outer circle, ratio of transduced and un-transduced T cells within each compartment. (J) Representative NSG mice from MSTO-MSLN-LUC xenograft treatment experiment are depicted. Tumor persistence in both treated mouse (right hand side) and control treated mouse (left hand side). Differences in luminescence shown in radiance scales underneath. (K, L, M, N) LDH (K), urea (L), AST (M) and ALT (N) serum levels from MSTO-MSLN-LUC xenograft treatment experiment.

All graphs show mean values of experiments performed at least in triplicates. Impedance- based cytotoxicity assays were performed in duplicates for technical reasons. Experiments are representative of at least three independent experiments. p-values are shown resulting from two-sided unpaired t-test. For impedance-base cytotoxicity assays the total curve over time was compared. p < 0.05 was considered statistically significant.

**Supplementary Figure 5: Effect of pre-activated SAR T cells on hepatocellular carcinoma and glioblastoma cells**

(A) Coculture of pre-activated murine E3 SAR T cells with murine hepatocellular carcinoma cell lines RIL, Hepa 1.6 and Hep55.1c (E:T 5:1). T cells were either redosed or non-redosed with BiAb (5 μg/ml). (B) Coculture of activated or resting human E3 SAR T cells with human hepatocellular carcinoma cell line HEP3B (E:T 10:1). T cells were either redosed or non- redosed with BiAb (5 μg/ml). (C, D) Real-time quantification of MiaPaca-MSLN (C) or U251- EGFRvIII (D) tumor cell lysis by E3 or WT T cells and 2 + 1 BiAb. (E, F) Quantification of T cell stimulation as measured by IL-2 (E) and IFN-y (F), following coculture of E3 or WT T cells with MSLN-expressing MiaPaca-MSLN or EGFRvIII-expressing U251-EGFRvIII and 2 + 1 BiAb (5 μg/ml).

All graphs show mean values of experiments performed at least in triplicates. Experiments are representative of at least three independent experiments. p-values are shown resulting from two-sided unpaired t-test. For impedance-base cytotoxicity assays the total curve over time was compared. p < 0.05 was considered statistically significant