Figure S1. TROP2 is highly expressed in breast cancer tissues and cells

(A-B) H&E and IHC staining of TROP2, CD3 and KI67 in HER2$^+$ tumor (A, n=12) and luminal tumor (B, n=12) and para-tumor tissues and representative images were shown. Scale bar, 100 μm. (C) Immunoblotting analysis of TROP2 expression in breast tumor cell lines. Experiment (C) was repeated for twice.

Figure S2. The characterizations of F7AK3

(A) F7AK3 was purified by cation exchange chromatography. (B) Different amounts of purified monomeric F7AK3 were separated by SDS-PAGE and stained with Coomassie Blue. (C-E) Surface plasmon resonance analysis of F7AK3 binding to immobilized CD3 fusion protein (C) and TROP2 protein (D), and the affinities of F7AK3 were shown (E). (F) The binding capacities of F7AK3 (1μg/ml) to TROP2 in different breast tumor cell lines were determined by flow cytometry. The experiment was repeated for three times (F).

Figure S3. F7AK3 induced enhanced activation of T cells in HCC1395 compared to MCF7

(A-D) PBMCs isolated from healthy donors were cultured with either MCF-7 or HCC1395 cells at an E:T ratio of 3:1, in the presence or absence of 1μg/ml F7AK3 for 72 hours. Afterwards, surface expression of CD25 (A), CD69 (B) on CD4$^+$ and CD8$^+$ T cells was assessed by flow cytometry. (C-D) After 72 hours co-culture, cells were stimulated with PMA, ionomycin and Golgiplug for another 4 hours and Granzyme B production in CD8$^+$ T cells and expression of IL-2 (F) in CD4$^+$ and CD8$^+$ T cells were measured by intracellular staining. The experiments were repeated for three times. Data were analyzed with unpaired *t* test. Mean ± SEM; **p*<0.05; ***p*<0.01; ****p*<0.001.

Figure S4. Binding capacity and cytotoxicity of F7AK3-mediated T cells towards 4T1 murine breast cancer cells

(A) The binding of bispecific antibody to 4T1 cells was determined by flow cytometry. 4T1 cells were incubated with 1μg/ml F7AK3 for 30 mins, followed by staining with PE-conjugated anti-human IgG Fc for 30 mins. Blank contains no first antibody. (B) 4T1 cells were cultured with activated T cells in the presence of increasing amounts

of F7AK3 for 24 hours. The released amounts of LDH were measured to determine the T cell-mediated tumor cell killing. All experiments were repeated twice with similar results.

Figure S5. F7AK3 does not affect breast tumor cells proliferation and apoptosis

(A-D) Tumor cells were cultured and treated with or without 1μg/ml F7AK3 for 48 hours. The numbers of live cells were determined by PI/Annexin V staining. (E-H) Tumor cells were cultured with or without 1μg/ml F7AK3. Cell proliferation was measured with CCK8 assay after tumor cells were treated with for indicated days. Experiments were repeated for twice with triplicates and are plotted as Mean ± SEM. (A-D) The significance was determined by *t* test. (E-H) Data were analyzed with one-way *ANOVA* analysis with Brown-Forsythe test. **p*<0.05; ***p*<0.01; ****p*<0.001; ns, not significant.

Figure S6. F7AK3 activates T cells and reduces tumor burden in a xenogeneic tumor model

(A-G) NOD/ShiLtJGpt-*Prkdc*^{em26}*Il2rg*^{em26}/Gpt (NCG) immunodeficient mice were subcutaneously injected with 2.5×10^6 MDA-MB-231 cells. After 7 days, mice were injected with 5×10^6 human PBMCs intravenously and starting at day 8, mice were received without or with varying doses of F7AK3 twice a week for four consecutive weeks (n=7 per group). At day 42, all mice were sacrificed. (A) Mice weights were measured twice a week and represented as Mean ± SEM. (B) Tumor weights were measured at day 42. (C-F) Flow cytometric analysis of the percentages of CD45$^+$ (C), CD3$^+$ cells, CD8$^+$ and CD4$^+$ T cells (E) in spleen, CD8$^+$ and CD4$^+$ T cells (F) in the tumor tissues. (G-H) CD69 expression of CD8$^+$ and CD4$^+$ T cells (G) and the percentage of PD1$^+$ cells of CD8$^+$ T cells in tumor tissues (H) were determined by flow cytometry. Data were assessed by one-way *ANOVA* analysis with Brown-Forsythe test compared with control group. Mean ± SEM; **p*<0.05; ***p*<0.01; ****p*<0.001; nd, not detected.