Supplementary figure legends

Supplementary figure 1. EGFR is overexpressed in TNBC and associated with poor prognosis.

(**a**) The expression of EGFR (FPKM, $\log_2$) in a cohort of clinical breast cancers from TCGA (The Cancer Genome Atlas) is shown by box plot. TNBC (triple-negative breast cancer) (n = 244); Non-TNBC (n = 3,412). *P*-value is shown at the top (**a**).

(**b-e**) The association between EGFR expression and prognosis in TNBC (**b**), LumA (**c**), LumB (**d**) and HER2 (**e**) subtypes of breast cancers.

EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer; FPKM, fragments per kilobase per million; LumA, luminal A; LumB, luminal B; HER2, human epidermal growth factor receptor 2; AE-frees survival, adverse events free survival; HR, hazard ratio; CI, chemotherapeutic index.

Supplementary figure 2. Cytotoxicity of EGFR CAR-T cells against primary TNBC cells *in vitro*.

(**a**) Primary TNBC cells as indicated were subjected to immunoblotting (IB) analysis with an anti-EGFR specific antibody. Actin served as a loading control. Molecular weight is indicated on the right. Experiments were repeated three times, and representative blots are shown.

(**b**) EGFR CAR-T cells were incubated with primary TNBC cells as described in (**a**) at a ratio of 1:1 for duration as indicated followed by cytotoxicity assay. Data were obtained from three replicates and are presented as mean ± s.e.m..

Patient 1, sample from Patient 1; Patient 2, sample from Patient 2; IB, immunoblotting; EGFR, epidermal growth factor receptor; TNBC, triple negative breast cancer.

Supplementary figure 3. UCSC Genome Browser views of representative genes associated

with naïve and effector T cell function.

(**a, b**) UCSC Genome Browser views of representative genes associated with effector (**a**) and naïve (**b**) T cell function from RNA-seq analysis.

CAR-T, chimeric antigen receptor-modified T cells; 231, MDA-MB-231; chr, chromosome.

Supplementary figure 4. The naïve-associated population in EGFR CAR-T cells expanded after TNBC stimulation.

(**a**) T cells after transduction and *ex vivo* expansion were incubated with or without MDA-MB-231 cells, and T cells were then collected and coated with CD3-FITC, CD8-APC, CD62L-PE and CCR7-Pacific Blue antibodies, followed by flow cytometry analysis. Experiments were repeated three times, and representative histograms are shown.

(b) T cells after transduction and *ex vivo* expansion were incubated with or without MDA-MB-231 cells, and T cells were then collected and coated with IgG-Fc-APC, CD62L-PE and CCR7-Pacific Blue antibodies, followed by flow cytometry analysis. Experiments were repeated three times, and representative histograms are shown.

EGFR, epidermal growth factor receptor; CAR-T, chimeric antigen receptor-modified T cells.

Supplementary figure 5. UCSC Genome Browser views of representative genes regulated by EGFR CAR-T treatment in MDA-MB-231 cells.

(**a, b**) UCSC Genome Browser views of representative genes that were up- (**a**) or down-regulated (**b**) by EGFR CAR-T treatment in MDA-MB-231 cells.

CAR-T, chimeric antigen receptor-modified T cells; CTL T, control T cells; 231, MDA-MB-231; chr, chromosome.

Supplementary figure 6. Quantification of band intensity as shown in Figure 5l.

MDA-MB-231 cells treated with control T (CTL T) or EGFR CAR-T cells were subjected to

immunoblotting analysis with antibodies as indicated in Figure 5l. Intensity of the bands was quantified by using Image J, and then normalized to actin loading control. Data were obtained from three replicates and are presented as mean ± s.e.m.. Statistical significance was determined using two-tailed *t*-test (**P* < 0.05, ***P* < 0.01 and ****P* < 0.001).

CAR-T, chimeric antigen receptor-modified T cells; CTL T, control T cells; 231, MDA-MB-231; GZMB, granzyme B; Fas, factor associated suicide; PARP, poly (ADP-ribose) polymerase; pJAK1, phospho-Janus kinase 1; pSTAT1, phospho-signal transducer and activator of transcription 1; IRF1, interferon regulatory factor 1.

Supplementary figure 7. Histopathological evaluation of tissue sections from control T- or EGFR CAR-T-treated mice

(**a**) Lung sections from mice treated with control T (CTL T) or EGFR CAR-T cells (5×10^6 cells per injection) were subjected to histopathological evaluation. Images of lung metastasis (red arrows) from control T (CTL T)-treated mice were shown by H&E staining. Representative regions are enlarged from 200 × (small-sized black square) to 800 × magnification (big-sized black square) for clarity. Scale bars, 40 μm (black line) and 10 μm (red line). Representative data of three independent experiments are shown.

(**b, c**) Liver, lung and spleen sections from mice treated with control T (CTL T) or EGFR CAR-T cells were subjected to H&E staining (**b**) and TUNEL assay (**c**). Representative images of each tissue are shown at 200 × magnification (scale bar, 50 μm). Representative data of three independent experiments are shown.

EGFR, epidermal growth factor receptor; CAR-T, chimeric antigen receptor-modified T cells; CTL T, control T cells.

Supplementary figure 8. EGFR CAR-T cells were ineffective in ER-positive breast cancer

xenograft model in mouse

(**a**) SCID mice were injected subcutaneously with MCF7 cells (3.0×10^6) stably expressing a luciferase reporter (MCF7-fluc) and then brushed with estrogen (E_2, 10^{-2} M) every 3 days for the duration of the experiments. After one week of E_2 administration, control T (CTL T) or EGFR CAR-T cells (5.0×10^6 cells per injection) were administrated intravenously every other day (n = 3). The day mice received CAR-T treatment was counted as day 1. Tumor growth was monitored by using bioluminescence imaging for 20 days. Representative data of two independent experiments are shown.

(**b**) Tumor growth curve based on bioluminescence as described in (**a**). Data were obtained from two replicates and are presented as mean ± s.e.m.. Statistical significance across multiple comparisons was determined using two-way ANOVA (ns, non-significant, $P > 0.05$).

(**c**) Average bioluminescence after 20 days of observation as described in (**a**). Data were obtained from two replicates and are presented as mean ± s.e.m.. Statistical significance was determined using two-tailed *t*-test (ns, non-significant, $P > 0.05$).

CAR-T, chimeric antigen receptor-modified T cells; CTL T, control T cells; ns, non-significant.

Supplementary figure 9. Comparison of expansion rate and cytotoxicity between the second and third generation of EGFR CAR-T cells *in vitro*

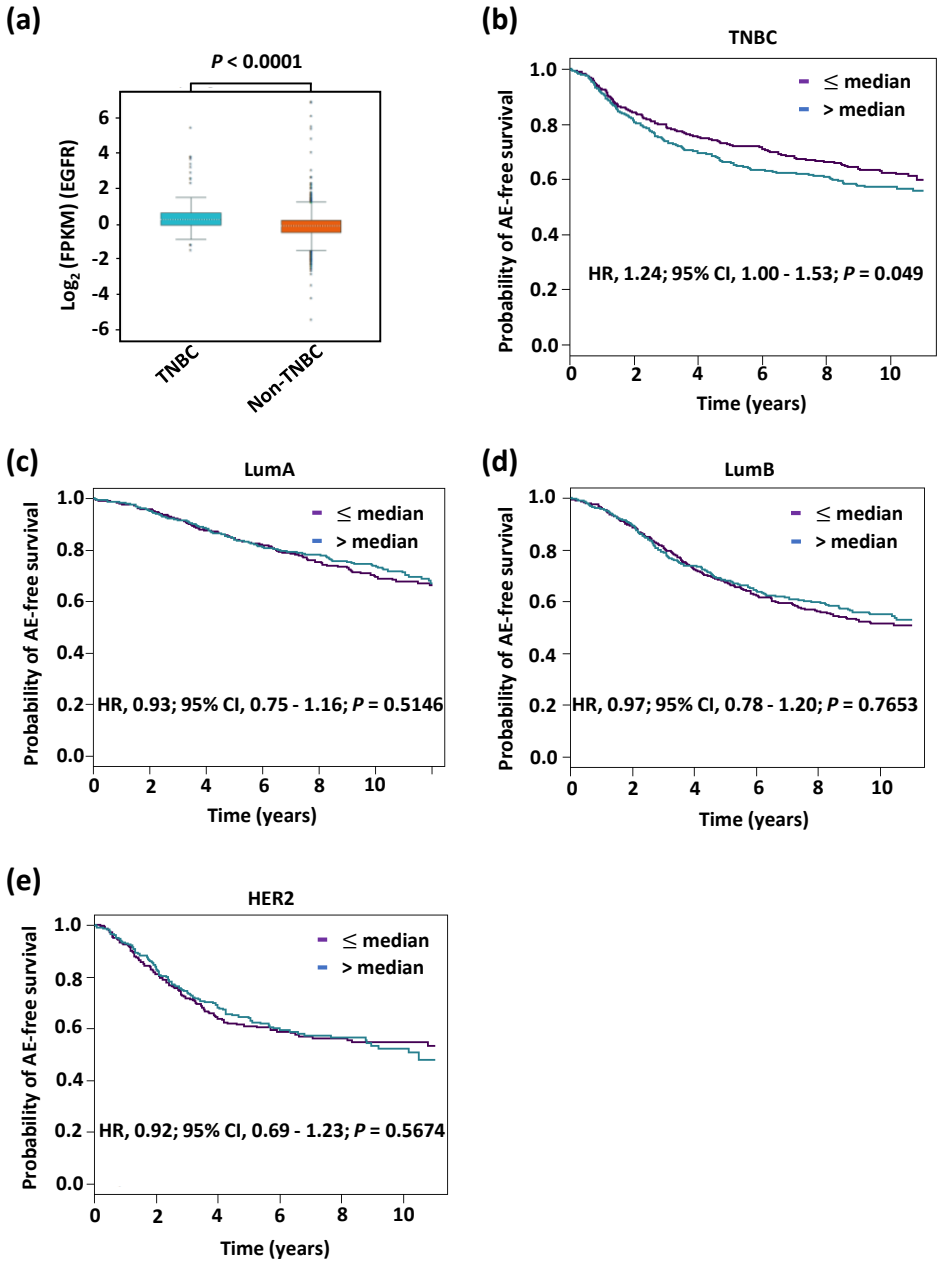
(**a**) Schematic illustration of the second generation of EGFR CAR. The second generation of EGFR CAR was constituted of a signal peptide of IL2 receptor (Sp1), an anti-EGFR scFv (single chain variable fragment) from cetuximab, a spacer (IgG1 Fc), a CD28 transmembrane domain (CD28 TM), and CD28 and CD3ζ intracellular signaling domains.

(**b**) Primary T lymphocytes infected with the second or third generation of EGFR CAR lentiviruses (2^{nd} EGFR CAR-T or 3^{rd} EGFR CAR-T) were maintained in culture medium and the number of

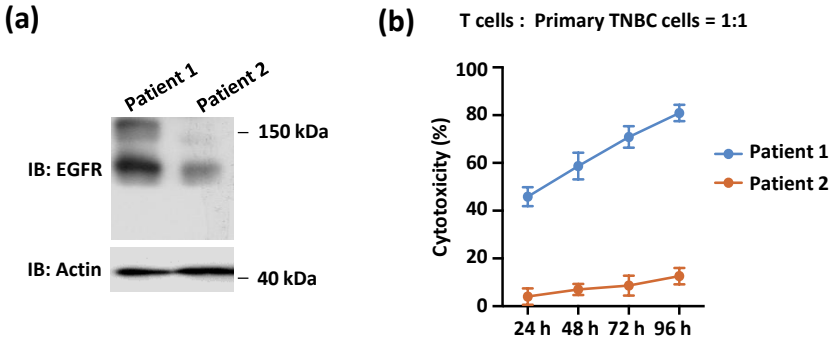
viable cells was counted at the time points as indicated. Initial number, 2.5×10^6. Data were obtained from three replicates and are presented as mean ± s.e.m..

(**c**) Control T (CTL T), 2nd or 3rd EGFR CAR-T cells were incubated with MDA-MB-231 or MDA-MB-468 cells at different ratios for duration (24 to 96 hours) as indicated followed by cytotoxicity assay. Data were obtained from three replicates and are presented as mean ± s.e.m..

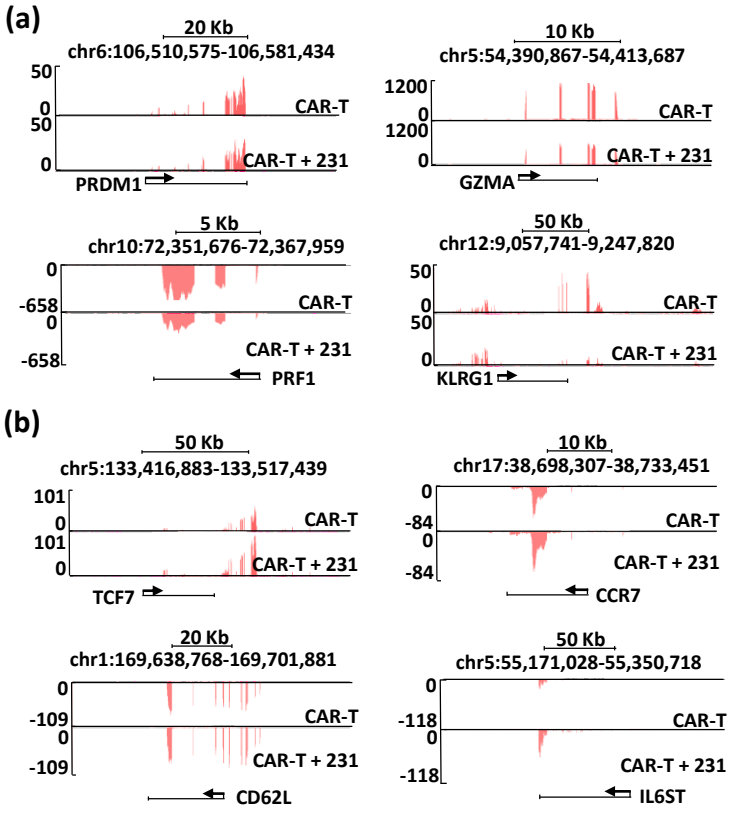
EGFR, epidermal growth factor receptor; CAR, chimeric antigen receptor; LTR, long terminal repeat; Sp1, signal peptide of IL2 receptor; EGFR scFv, single chain variable fragment against EGFR; IgG1, Immunoglobulin G1; Fc, fragment crystallizable; TM, transmembrane domain; CTL T, control T cell; CAR-T, chimeric antigen receptor-modified T cells.



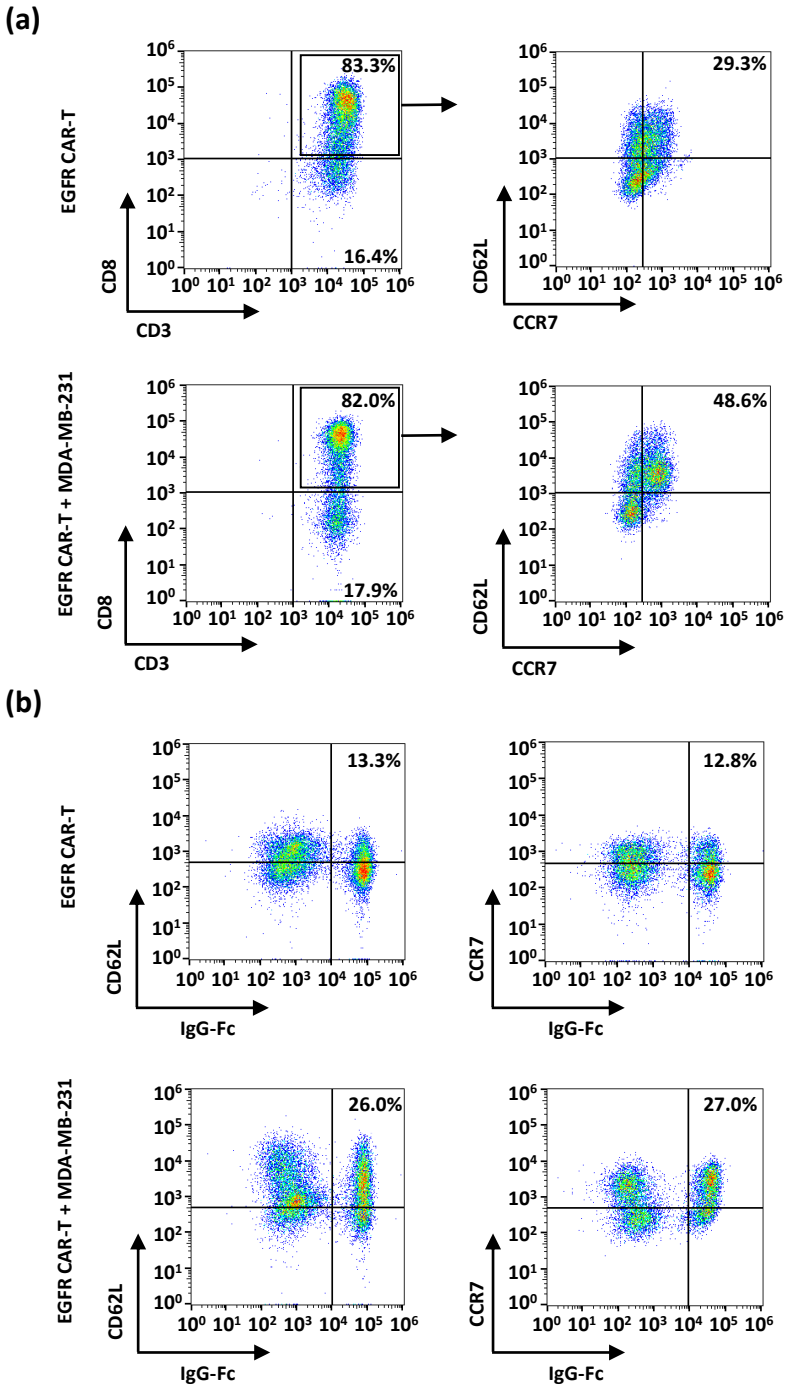
Supplementary figure 1



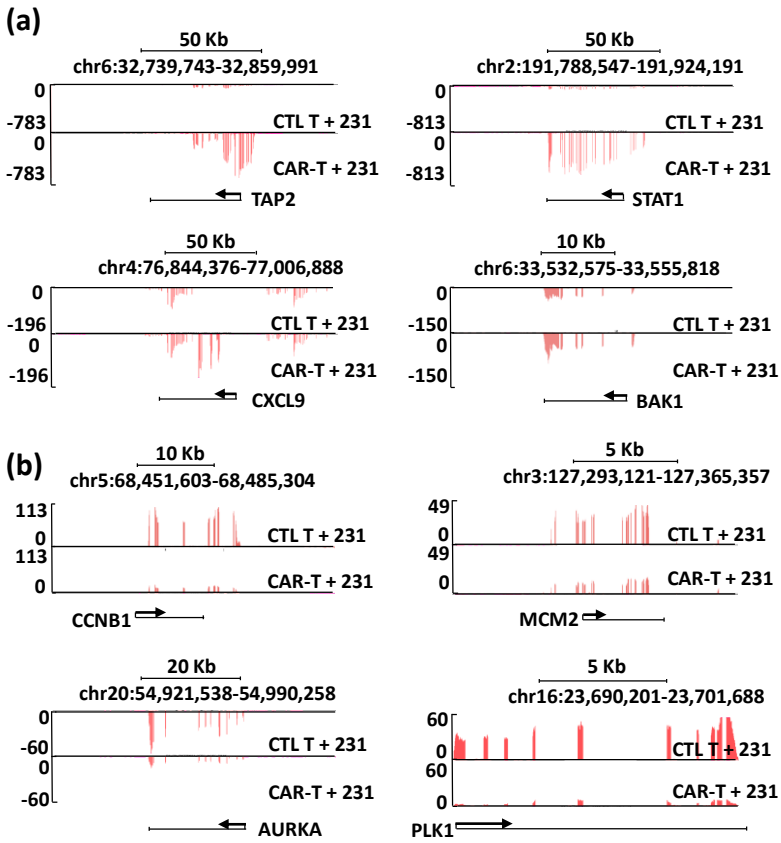
Supplementary figure 2



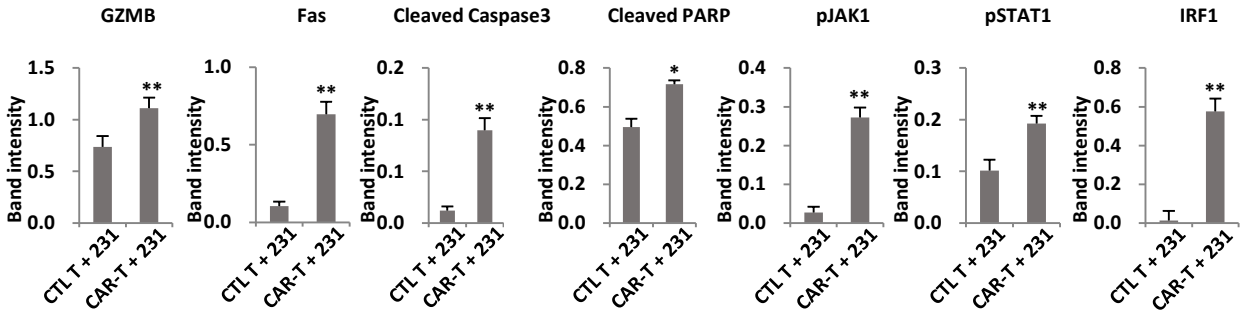
Supplementary figure 3



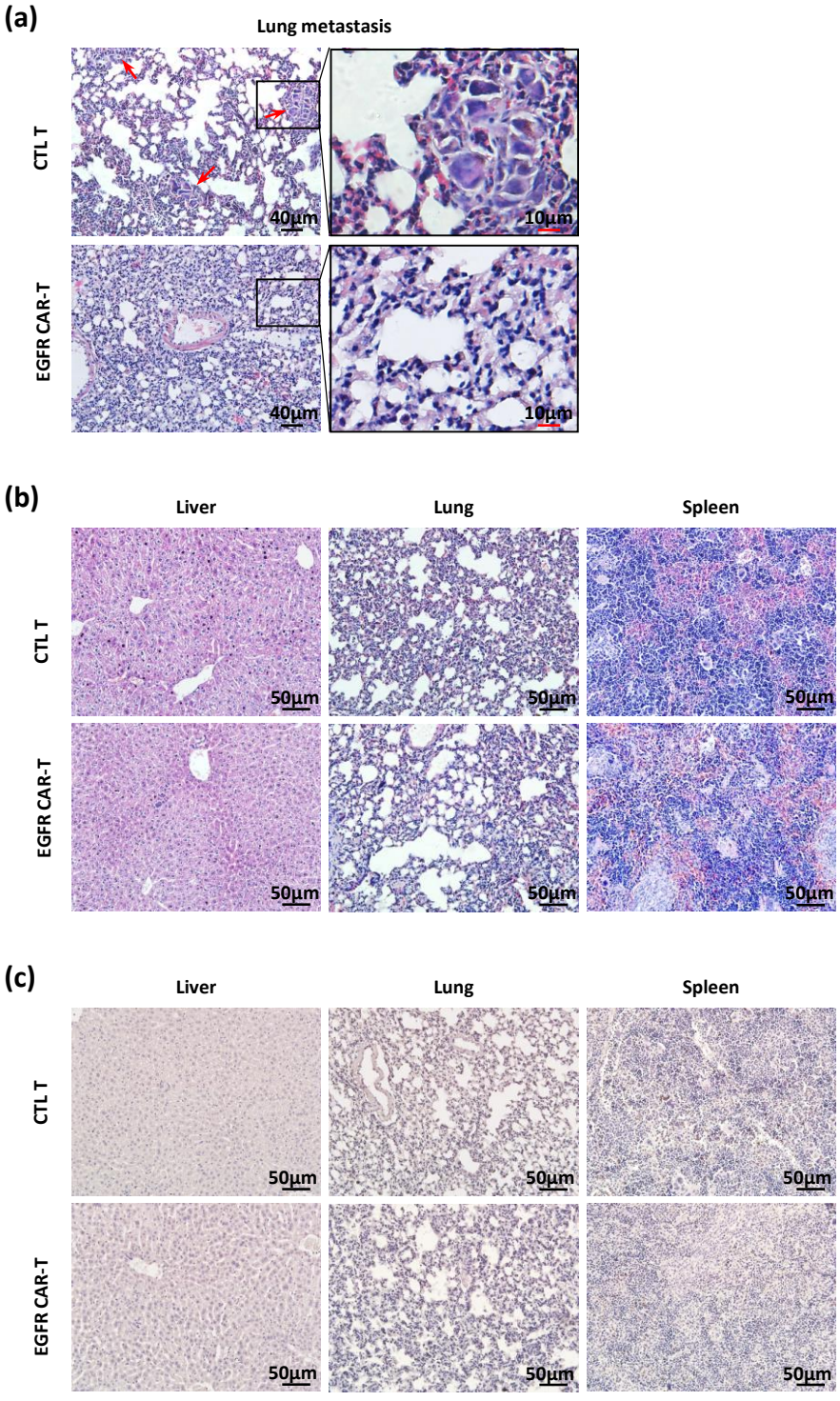
Supplementary figure 4



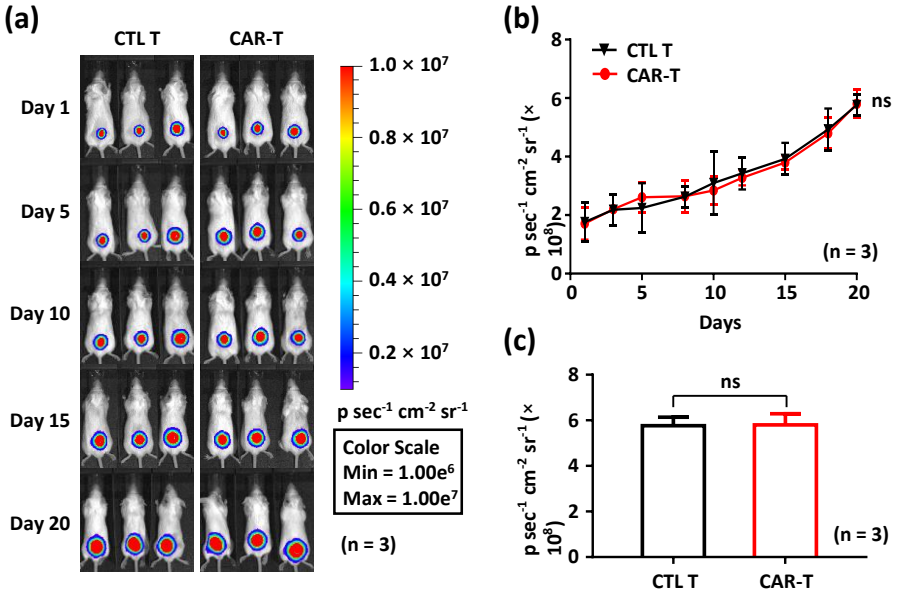
Supplementary figure 5



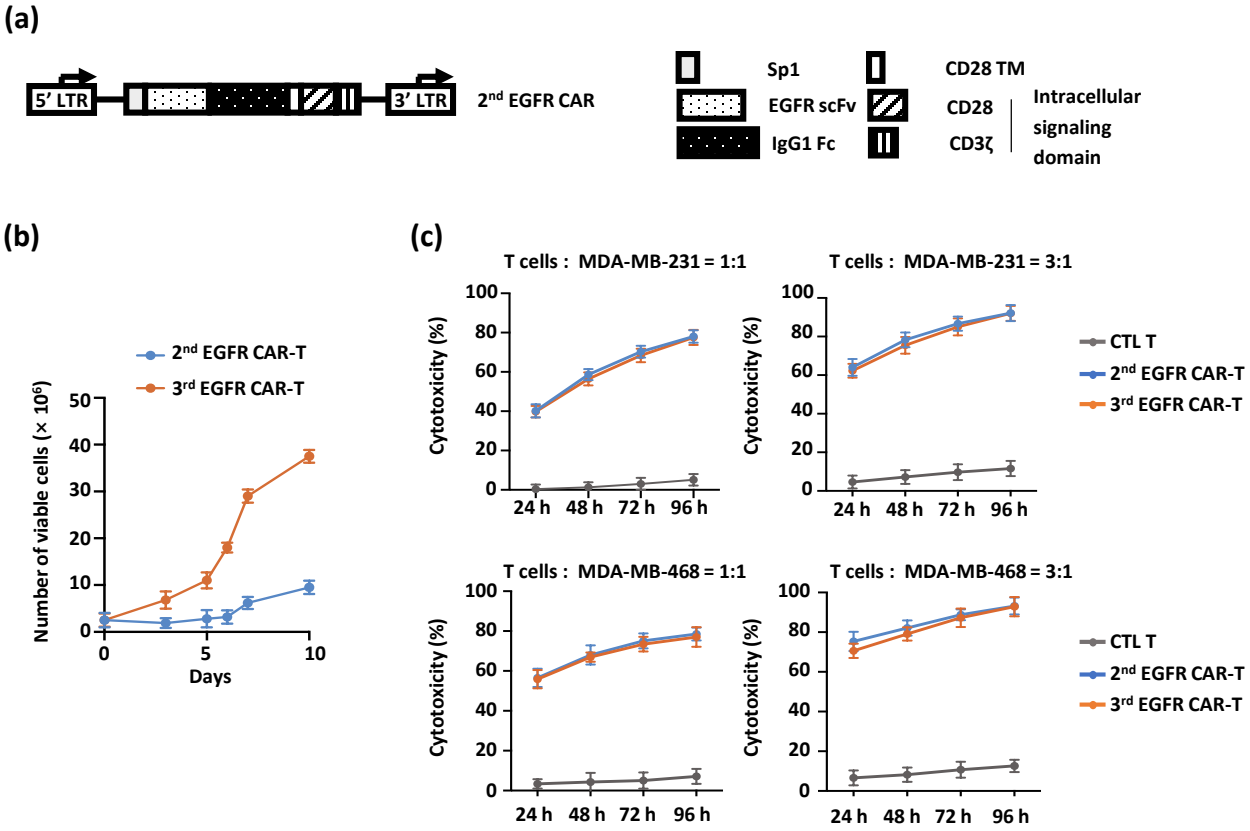
Supplementary figure 6



Supplementary figure 7



Supplementary figure 8



Supplementary figure 9