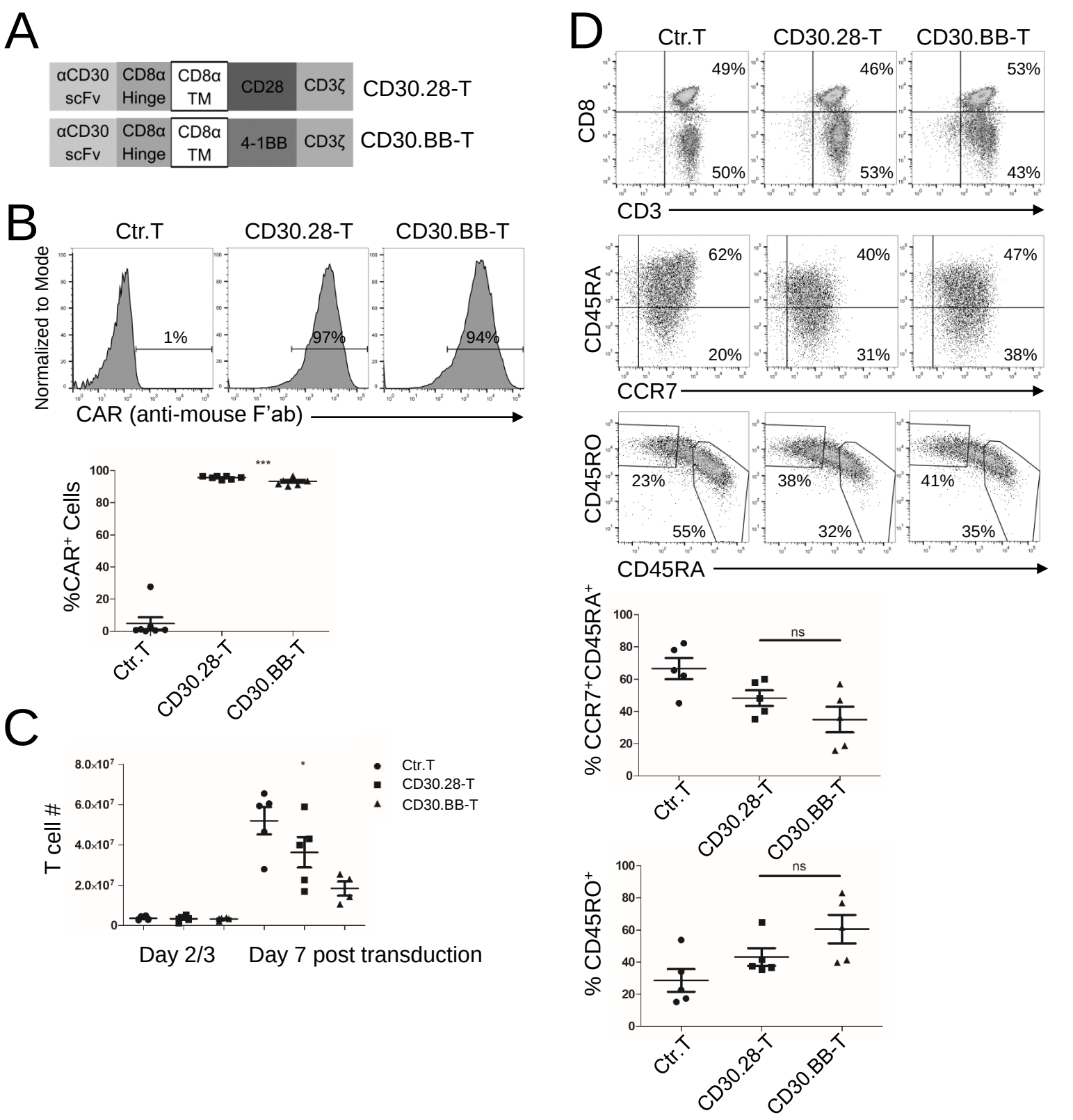
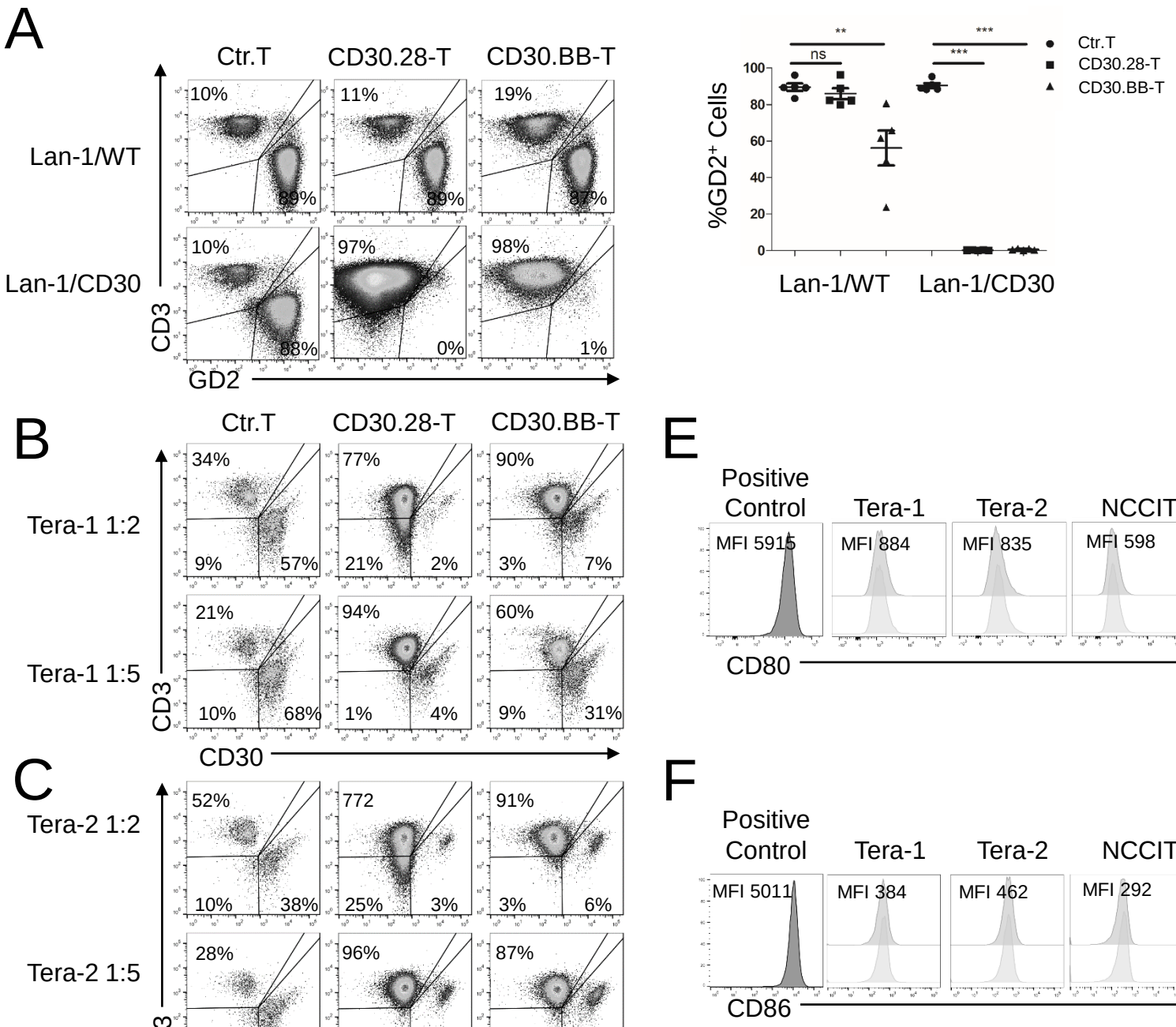
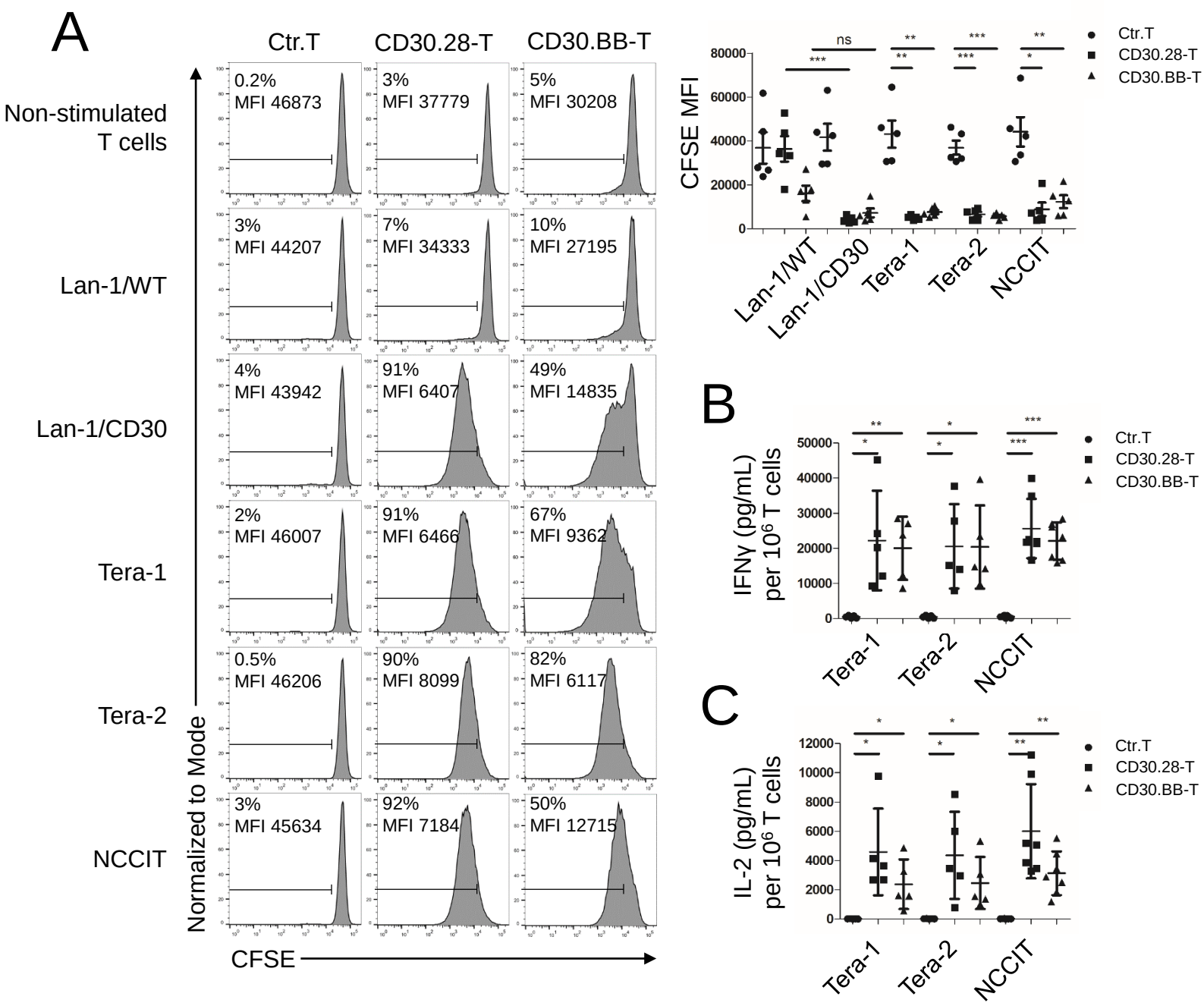


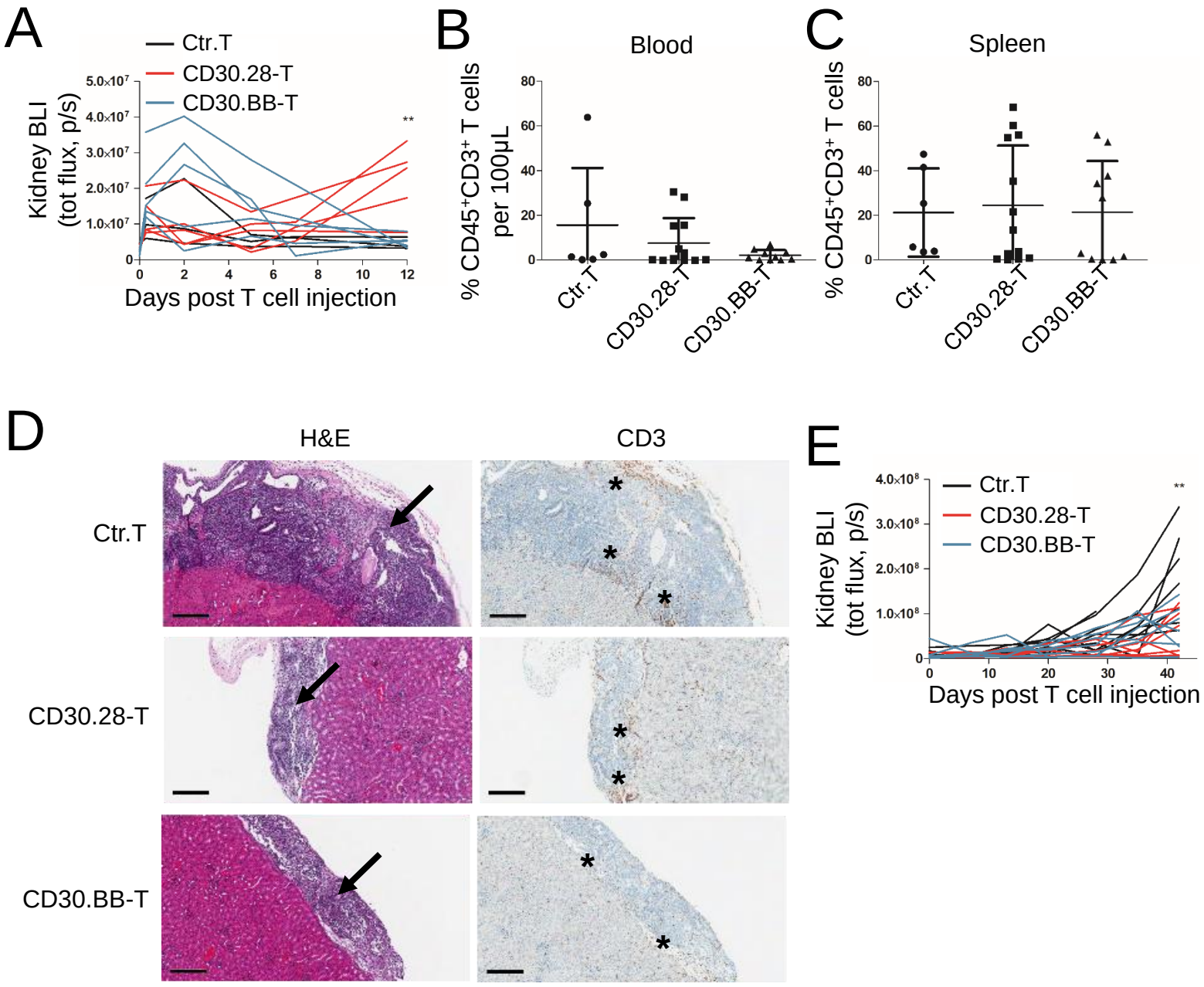
Supplementary Figure S1. CD30.CAR T cell characteristics. (A) Schematic of CD30.CARs encoding either the CD28 (CD30.28-T) or the 4-1BB (CD30.BB-T) endodomains. The anti-CD30 scFv domain (αCD30 scFv) was cloned in frame with CD8α hinge and transmembrane domain (TM) and either CD28 or 4-1BB endodomains with CD3ζ. (B) CAR transduction efficiency was detected by flow cytometry using an anti-mouse Alexa Fluor 647-conjugated F(ab')2 antibody. Representative histograms (top panels) and cumulative data (bottom panels) are shown (mean ± SEM, n=7 independent experiments). ***=p<0.001, repeated-measures ANOVA. (C) Cumulative data for CAR T-cell expansion *in vitro* at day 2/3 and day 7 post transduction (mean ± SEM, n=5 independent experiments). *=p<0.05, repeated-measures ANOVA. (D) CAR T-cell immunophenotype by day 7 post-transduction. Representative plots pre-gated on $CD3^+$ T cells (top panels) and cumulative data for $CCR7^+CD45RA^+$ stem cell-like memory T cells (top graph) (23) and $CD45RA^-CD45RO^+$ central and effector memory T cells (bottom graph) are shown (mean ± SEM, n=5 independent experiments). ns=not significant, two-sided, paired Student's T test.



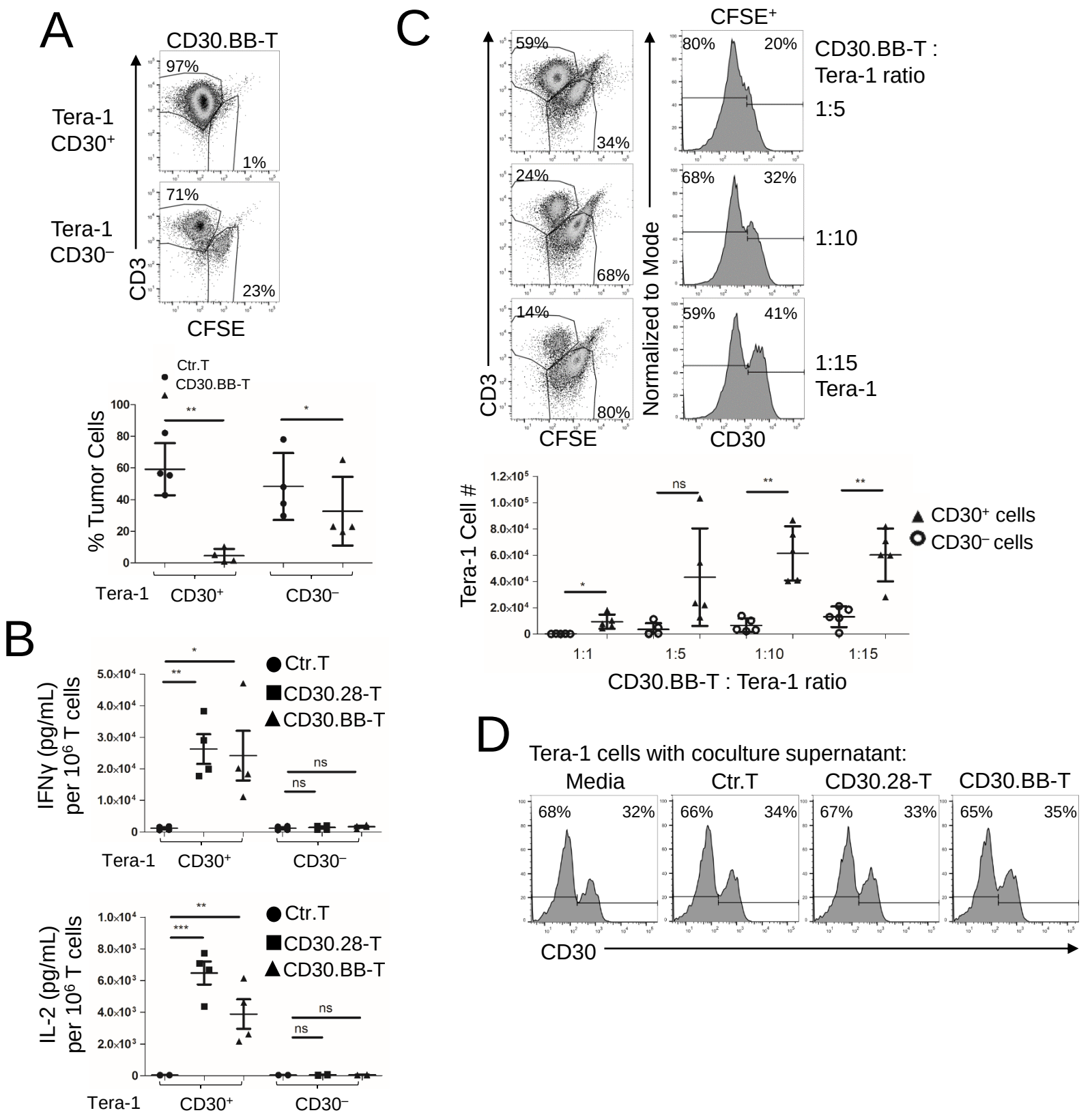
Supplementary Figure S2: Coculture of CD30.CAR T cells and tumor cells and tumor cell costimulatory antigen expression. (A) Representative flow plots (left panels) and cumulative data (right panels) of 5-day coculture assays of CD30.CAR T cells with Lan-1/WT ($CD30^-$) or Lan-1/CD30 ($CD30^+$) tumor cells labeled with anti-GD2 mAb (mean ± SEM, n=4 independent experiments). ns=not significant, **=p<0.01, ***=p<0.001, two-sided, paired Student's T test. (B-D) Representative flow plots of CD30.CAR T-cell cocultures with Tera-1 (B) or Tera-2 (C) cells at the 1:2 and 1:5 E:T ratios, and of CD30.CAR T-cell coculture with NCCIT cells (D) at 1:1 and 2.5:1 E:T ratios. (E-F) CD80 (E) and CD86 (F) expression by flow cytometry with mean fluorescence intensity (MFI) among tumor cells (top) with isotype controls (bottom). HDLM-2 cells are used as positive controls.



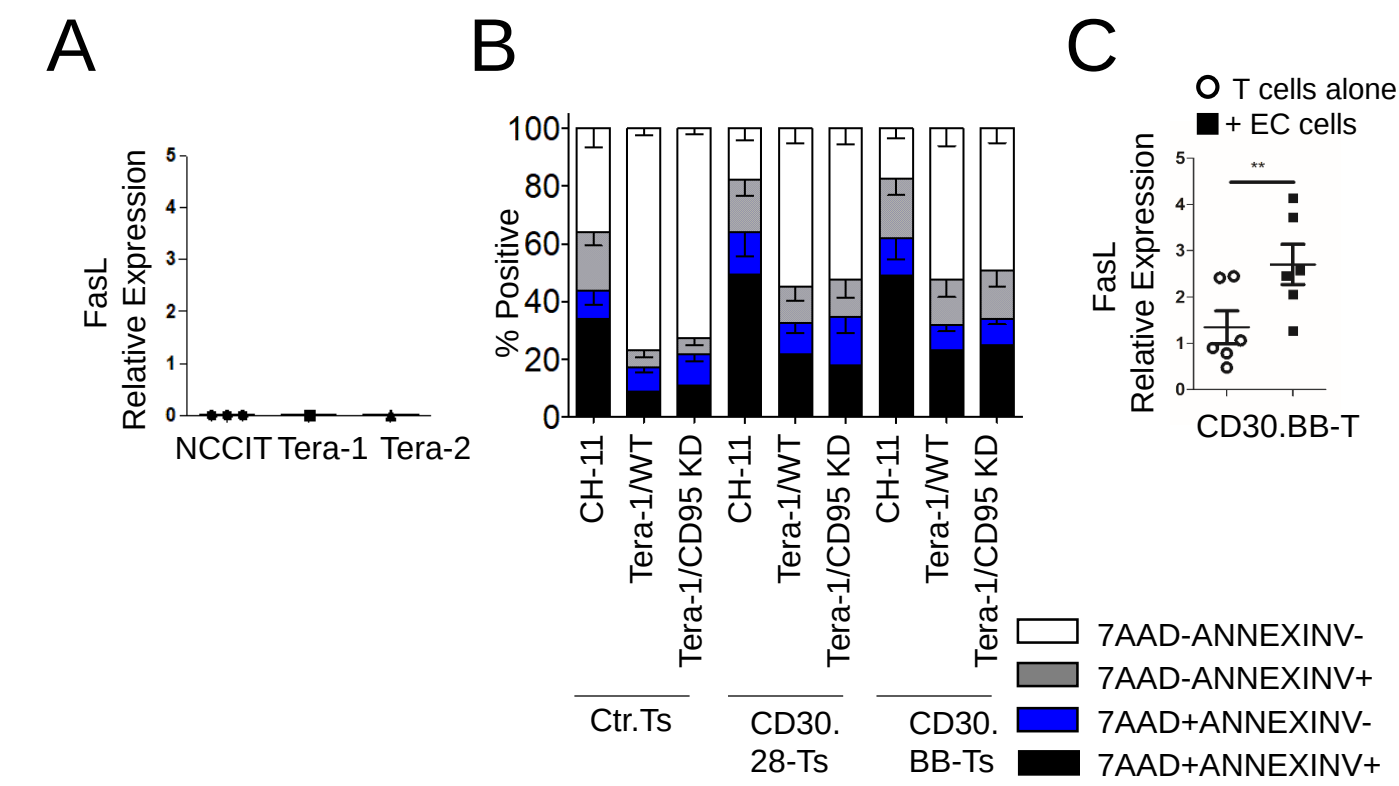
Supplementary Figure S3. CD30.CAR T cells proliferate and secrete pro-inflammatory cytokines in response to EC cell lines. (A) Representative histograms (left panels) and cumulative data (right panels) of CFSE dilution of CFSE-labeled $CD3^+$ control T cells, CD30.28-Ts and CD30.4-1BB-Ts after 5 days alone or in coculture with tumor cells (mean ± SEM of CFSE MFI, n=5 independent experiments). ns=not significant, *=p<0.05, **=p<0.01, ***=p<0.001, two-sided, paired Student's T test. (B-C) Cumulative quantification by ELISA of IFNγ (B) and IL-2 (C) released in coculture supernatant after 24 hours by control T cells, CD30.28-Ts and CD30.4-1BB-Ts with either $CD30^+$ or $CD30^-$ tumor cells at 1:1 E:T ratio (mean ± SEM, n=5 independent experiments). ns= not significant, *=p<0.05, **=p<0.01, ***=p<0.001, two-sided, paired Student's T test.

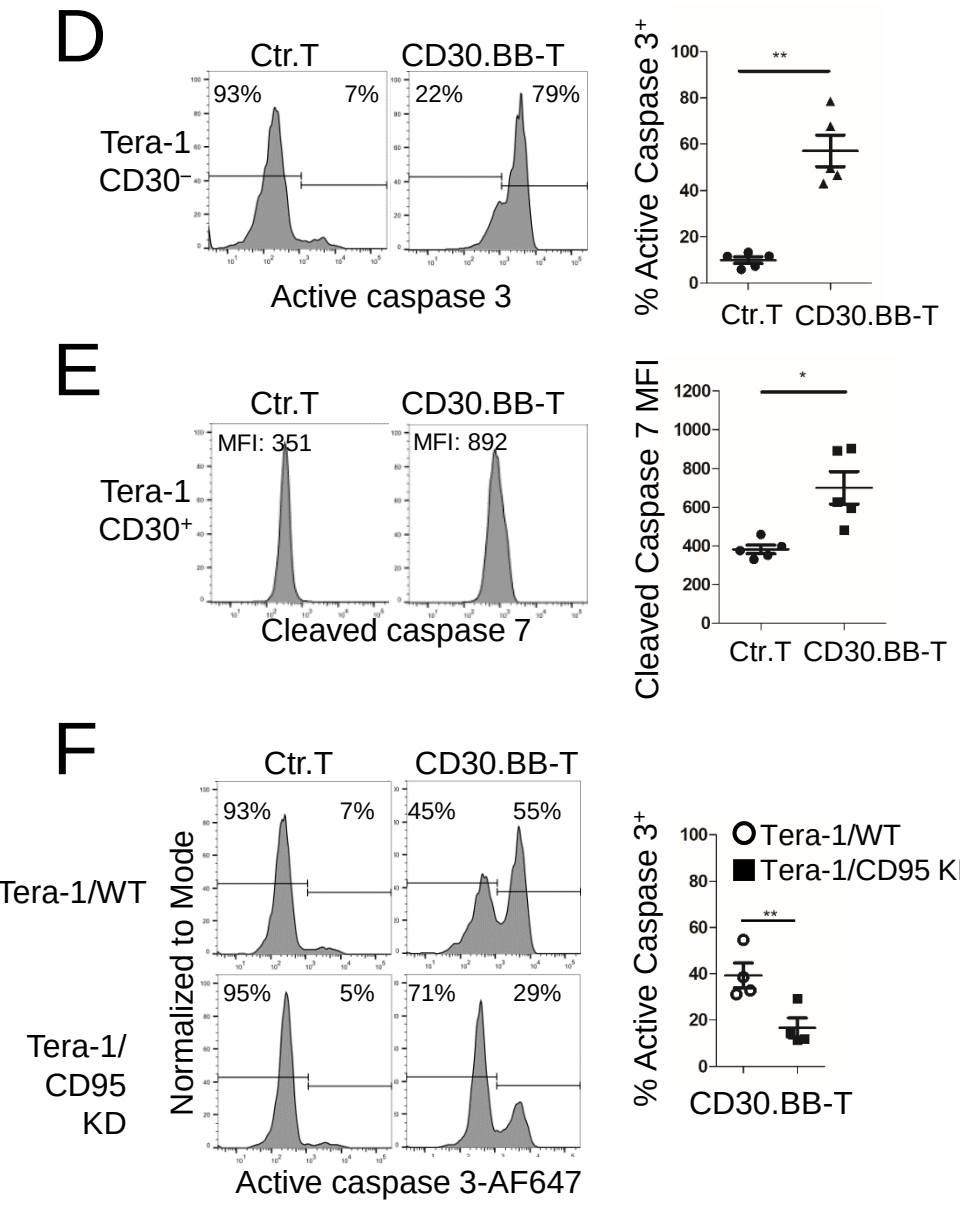


Supplementary Figure S4. CD30.CAR T-cell expansion and anti-tumor activity *in vivo*. (A) NSG mice were inoculated with Tera-2 cells under the left kidney capsule and, 21 days later, infused i.v. with 1 × 10^7 eGFP-FFLuc-labeled CD30.CAR T cells or CD19.CAR T cells (Ctr.T). BLI from individual mice are shown (n=3-4 per group, 2 independent experiments). **=p<0.001, one-way ANOVA at day 12. (B-C) T cell-treated mice were sacrificed at day 30-45 post tumor inoculation, and blood samples (B) and spleens (C) were collected to detect human $CD45^+CD3^+$ T cells. Blood samples were normalized to 100µL total volume. Cumulative quantification of $\%CD45^+CD3^+$ T cells are shown (mean ± SEM; n=1-5 per group, 4 independent experiments). (D) Tumors were harvested at day 30-45 post tumor inoculation and stained for hematoxylin/eosin (H&E) or anti-human CD3 mAb to detect tumor-infiltrating T lymphocytes. Positive samples displayed moderate to strong, diffuse cytoplasmic labeling for CD3, at 4X magnification, scale bar=200µm. Arrow=tumor, *=CD3 T cell clusters. (E) NSG mice were inoculated with Tera-2 cells labeled with eGFP-FFLuc under the left kidney capsule and received 1 × 10^7 CD30.CAR T cells or CD19.CAR T cells (Ctr.T) i.v. by day 15. BLI from individual mice are illustrated (n=4-5 per group, 2 independent experiments). **=p<0.001, one-way ANOVA at day 42.

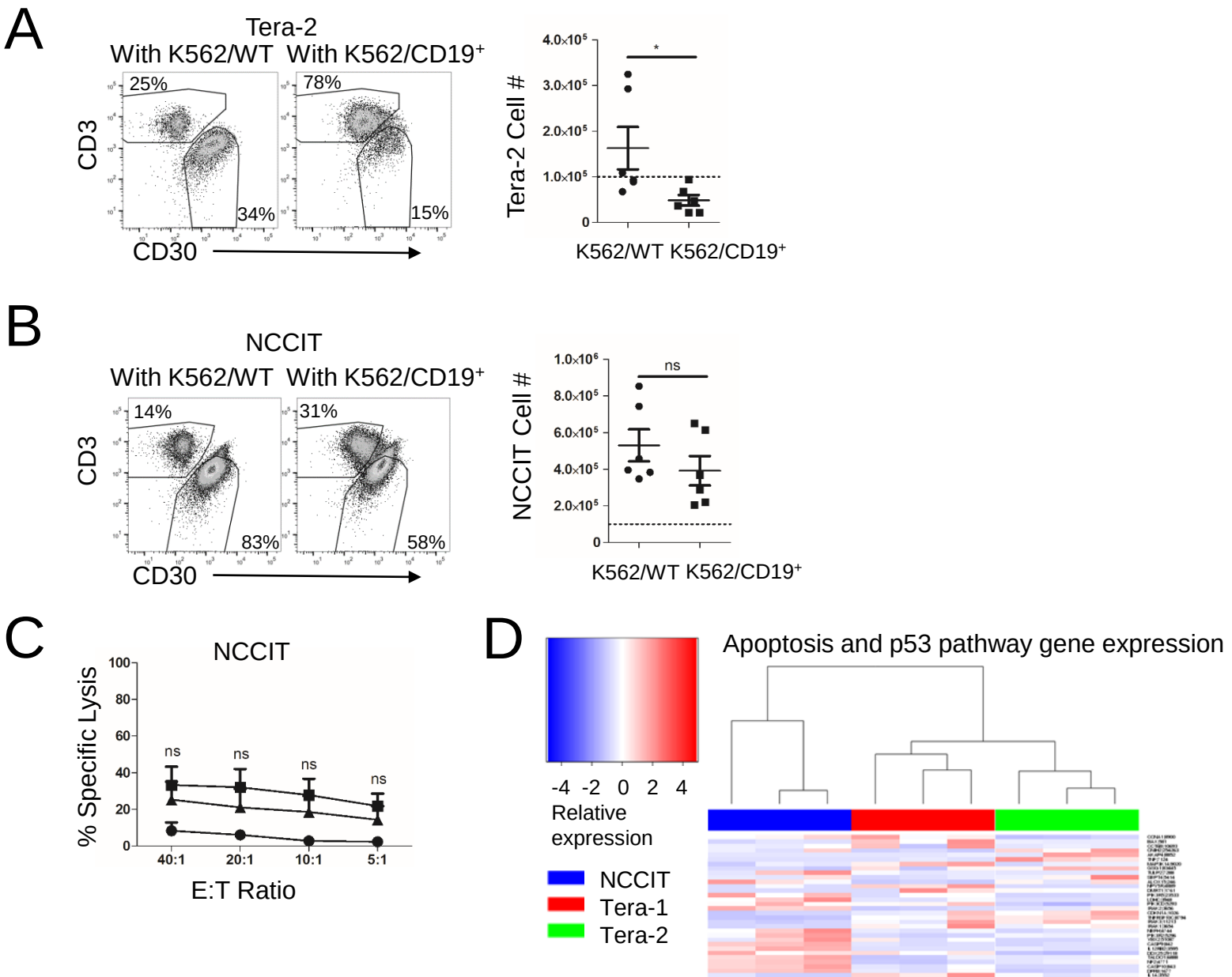


Supplementary Figure S5. Tera-1 CD30⁻ cells are eliminated by CAR T cells in a cell contact-dependent but antigen-independent manner. (A) Sorted Tera-1 CD30⁺ and CD30⁻ cells were labeled with CFSE and cocultured with either control T cells or CD30.BB-Ts for 5 days, and then analyzed by flow cytometry. Shown are representative flow plots (top panels) and cumulative data (bottom panels) summarizing tumor cell frequency (mean ± SEM, n=4 independent experiments). *=p<0.05, **=p<0.01, two-sided, paired Student's T test. (B) Cumulative data for IFNγ (top panels) and IL-2 (bottom panels) release by control T cells, CD30.28-Ts and CD30.4-1BB-Ts in the supernatant of coculture with Tera-1 CD30⁺ or CD30⁻ cells at 1:1 E:T ratio are shown (mean ± SEM, n=4 independent experiments). ns= not significant, *=p<0.05, **=p<0.01, ***=p<0.001, two-sided paired Student's T test. (C) Representative plots (left columns) and CD30 histograms (right columns) of CFSE⁺ Tera-1 cells cocultured for 5 days with CD30.BB-Ts at decreasing E:T ratios and cumulative data (right graph) for remaining tumor cells calculated with flow cytometry-based counting beads are shown (mean ± SEM, n=5 independent experiments). ns=not significant, *=p<0.05, **=p<0.01, two-sided, paired Student's T test. (D) CD30 expression by flow cytometry among Tera-1 cells cocultured with supernatant from cocultures with control T cells or CD30.CAR T cells for 48hrs.





Supplementary Figure S6. Functional Fas-FasL interaction is critical for the elimination of CD30⁻ EC cells by CD30.CAR T cells. (A) FasL mRNA expression measured by RT-qPCR of EC cells normalized to housekeeping gene β-actin. (B) Quantification of the composition of T cells or CAR T cells 72 hrs after coculture with wild type or CD95 knock down Tera-1 cells at E:T ratio of 1:1 identified by 7AAD and Annexin V co-staining. T cells with CH-11 anti-Fas agonistic antibody alone were used as control (n=5). (C) FasL mRNA expression measured by RT-qPCR of CD30.BB-Ts cultured alone or with EC cells at a 5:1 E:T ratio for 4 hours at 37°C. Expression is normalized to housekeeping gene β-actin. n=6 independent experiments; **=p<0.001 by two-sided, paired Student's T test. (C-D) Representative flow plots (left panels) of active caspase 3 (C) or cleaved caspase 7 (D) staining pre-gated on Tera-1 CD30⁻ or CD30⁺ cells after coculture with control T cells or CD30.BBTs for 18 hours at 37°C and cumulative data (right panels) are shown (mean ± SEM, n=5 independent experiments). *=p<0.05, **=p<0.001, two-sided, paired Student's T test. (E) Tera-1/WT or Tera-1/CD95 KO cells were cocultured with control T cells or CD30.BB-Ts at 1:5 E:T ratio for 18 hours. Representative flow plots (left panels) and cumulative data (right panels) for active caspase 3 among Tera-1 CD30⁻ cells are shown (mean ± SEM, n=4 independent experiments). **=p<0.001, two-sided, paired Student's T test.



Supplementary Figure S7. Tera-2 cells are susceptible to antigen-independent killing by CD30.CAR T cells, while NCCIT cells are resistant to CD30.CAR T-cell targeting. (A-B) Tera-2 (A) and NCCIT (B) cells were cocultured with CD19.CAR T cells in the presence of either K562/WT or K562/CD19$^+$ cells at 1:1:1 ratio for 5 days, and then analyzed by flow cytometry to detect T cells (CD3$^+$) and CD30$^+$ EC cells after excluding CD33$^+$ K562 cells. Cell numbers were calculated using flow cytometry-based counting beads. Representative flow plots (left panels) and cumulative data (right panels) summarizing tumor cell numbers with the dashed lines indicating initial tumor cell numbers are shown (mean ± SEM, n=5 independent experiments). ns=not significant, *=p<0.05, two-sided, paired Student's T test. (C) ^{51}Cr release assay of control T cells (Ctr.T), CD30.28-Ts and CD30.BB-Ts against NCCIT cells (mean ± SEM, n=4 independent experiments). ns=not significant, repeated measures one-way ANOVA comparing individual effector:target (E:T) ratios. (D) Heatmap showing expression of differentially expressed genes between NCCIT, Tera-1, and Tera-2 cells within the MSigDB KEGG Apoptosis and Biocarta c2 p53 pathways, scaled by samples. Dendrogram represents hierarchical clustering based upon complete linkage of the Euclidean distances of gene expression.