Supplementary Figures

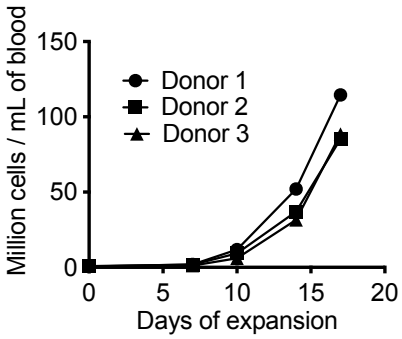

Figure S1. DNTs were obtained from 3 donors and expanded for 17 days. The number of DNTs derived from each mL of blood from each donor on varying days of expansion is shown.

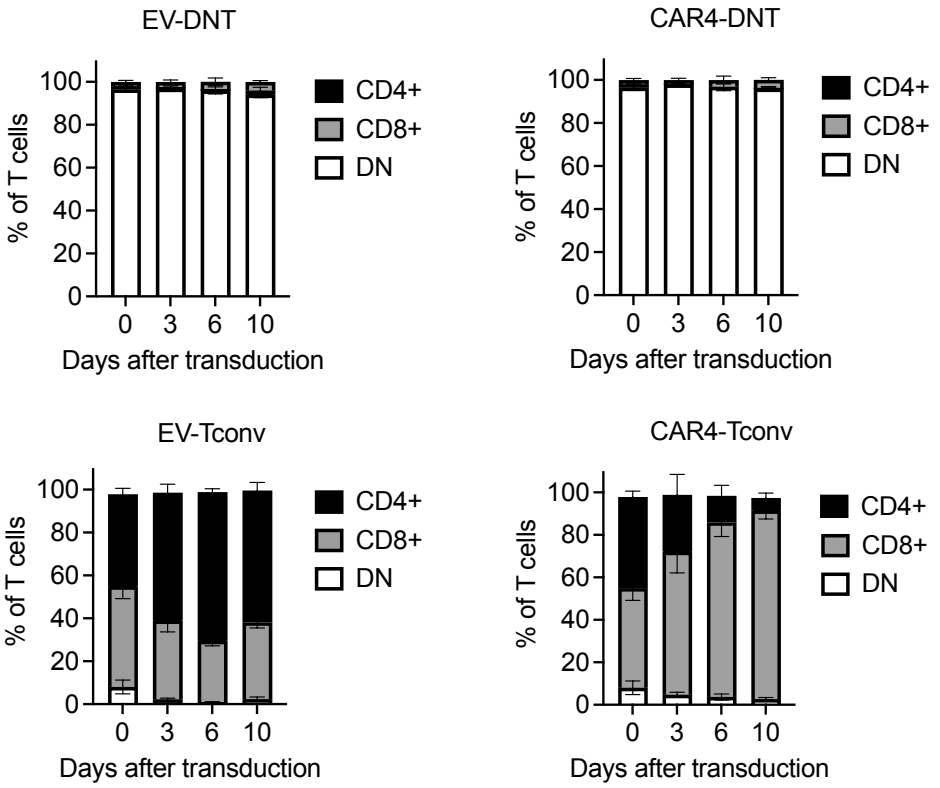

Figure S2. The composition of CD4$^+$, CD8$^+$, and double negative (DN) T cells within EV-DNTs, CAR4-DNTs, EV-Tconv, and CAR4-Tconv (*n*=3 per group) on varying days after transduction. Error bars represent SEM.

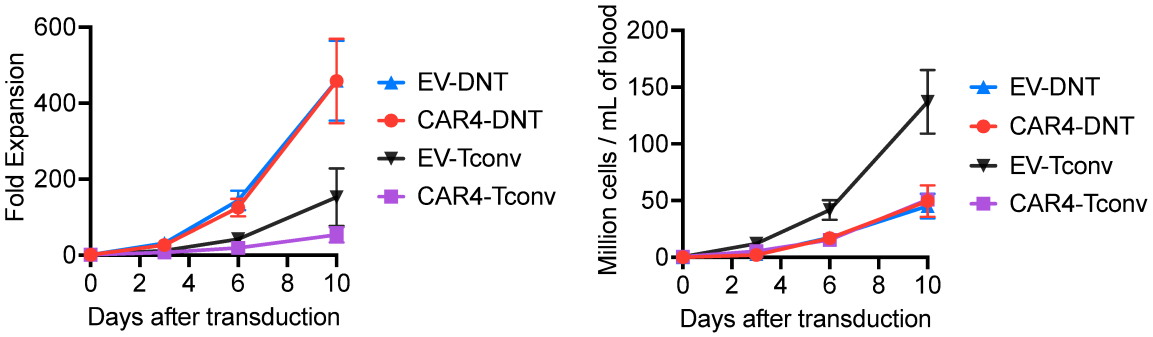

Figure S3. EV-DNTs, CAR4-DNTs, EV-Tconv, and CAR4-Tconv were expanded for 10 days after transduction. Left: Mean fold expansion of EV-DNTs (*n*=7), CAR4-DNTs (*n*=7), EV-Tconv (*n*=3), and CAR4-Tconv (*n*=3) on varying days after transduction. Right: The number of T cells derived from each mL of blood for EV-DNTs, CAR4-DNTs, EV-Tconv, and CAR4-Tconv (*n*=2 per group) on varying days after transduction. Error bars represent SEM.

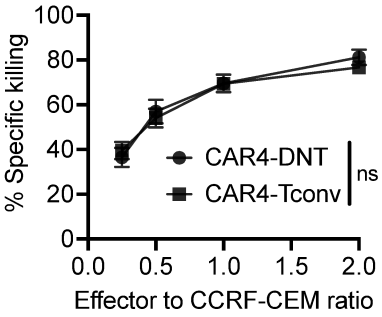

Figure S4. CAR4-DNTs (circles) and CAR4-Tconv (squares) derived from the same donor were cocultured with CCRF-CEM for 2 hours at indicated effector-to-target ratios. Percent specific killing of the target is shown. Symbols represent mean percent specific killing of triplicates, and error bars represent SEM. Data are a combination of 2 independently performed experiments. Two-way ANOVA was used for statistical analysis.

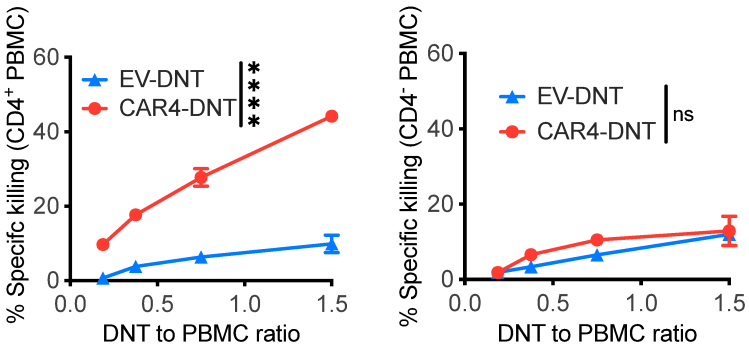

Figure S5. EV-DNTs and CAR4-DNTs were cocultured with PBMCs of healthy donors for 2 hours at indicated effector-to-target ratios. Percent specific killing of $CD4^+$ cells (left) and $CD4^-$ cells (right) within the PBMCs is shown. Symbols represent mean percent specific killing of triplicates and error bars represent SD. The experiment was independently performed 2 times, and representative data are shown.

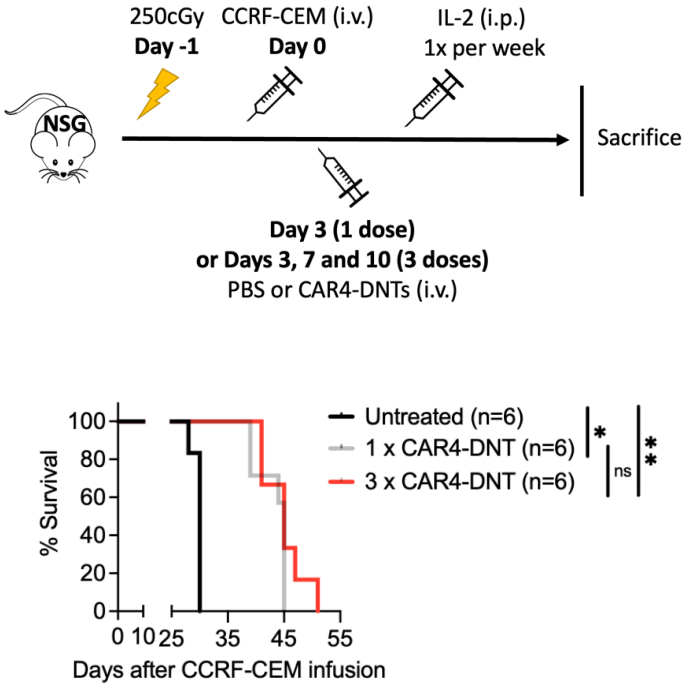

Figure S6. Top: Schematic outline of the *in vivo* experiment. NSG mice were sublethally irradiated (250cGy) on day -1 and intravenously (i.v.) injected with 2-5x10^5 CCRF-CEM cells on day 0. Mice were intravenously treated with PBS or CAR4-DNTs on day 3 (1 dose) or days 3, 7, and 10 (3 doses). All mice received intraperitoneal (i.p.) injections of IL-2 once per week. Mice were sacrificed when they reached the end point of the survival experiment. Bottom: Kaplan-Meier curve showing the percent survival of CCRF-CEM-engrafted mice treated with PBS (black, *n*=6), 1 dose of 2x10^6 CAR^+ CAR4-DNTs (gray, *n*=6), or 3 doses of 2x10^6 CAR^+ CAR4-DNTs (red, *n*=6). Log-rank test was used for statistical analysis.

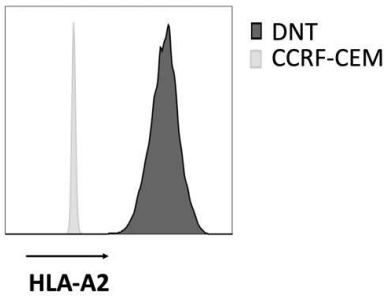

Figure S7. Representative flow plot showing the HLA-A2 staining on CCRF-CEM cells and DNTs from a HLA-A2^{+} donor.

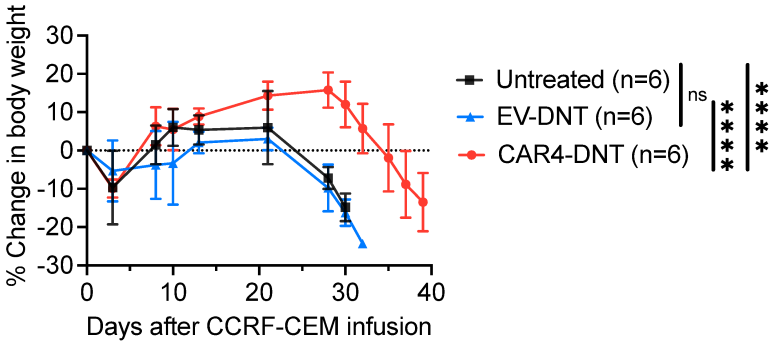

Figure S8. The percent change in body weight of CCRF-CEM engrafted mice treated with PBS (black, *n*=6), 2x10^6 EV-DNTs (blue, *n*=6) and 2x10^6 CAR4-DNTs (red, *n*=6). Two-way ANOVA Tukey's multiple comparisons test was used for statistical analysis.

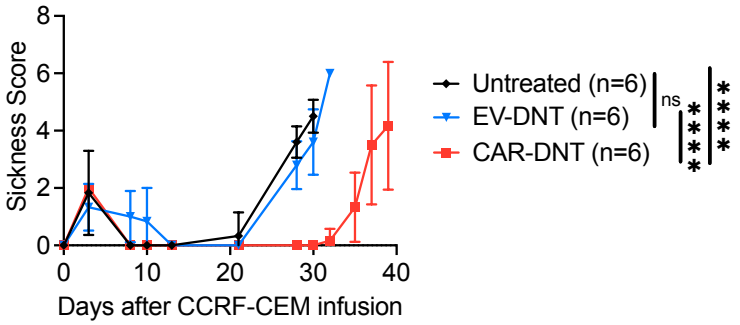

Figure S9. The sickness score of CCRF-CEM engrafted mice treated with PBS (black, *n*=6), 2x10^6 EV-DNTs (blue, *n*=6) and 2x10^6 CAR4-DNTs (red, *n*=6). Two-way ANOVA Tukey's multiple comparisons test was used for statistical analysis.

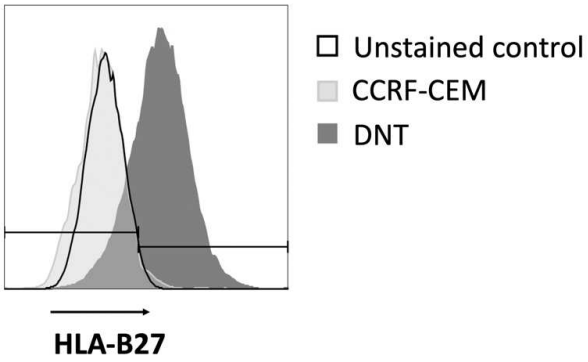

Figure S10. Representative flow plot showing the HLA-B27 staining on CCRF-CEM cells and DNTs from a HLA-B27^{+} donor.

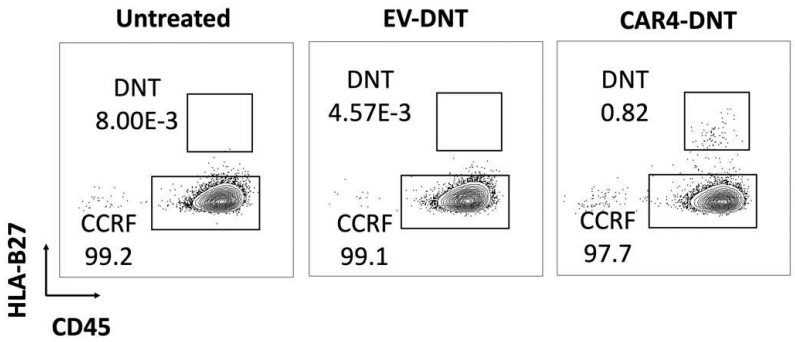

Figure S11. Representative flow plots for CD45 and HLA-B27 staining of liver cells from untreated, EV-DNT-treated, and CAR4-DNT-treated mice at the humane endpoint. CCRF-CEM cells are HLA-B27^{-}; an HLA-B27^{+} donor was used to manufacture EV-DNTs and CAR4-DNTs.

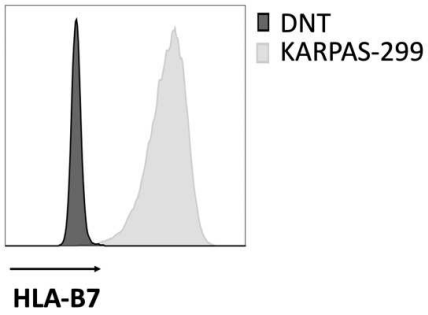

Figure S12. Representative flow plot showing the HLA-B7 staining on KARPAS-299 cells and DNTs from a HLA-B7$^-$ donor.

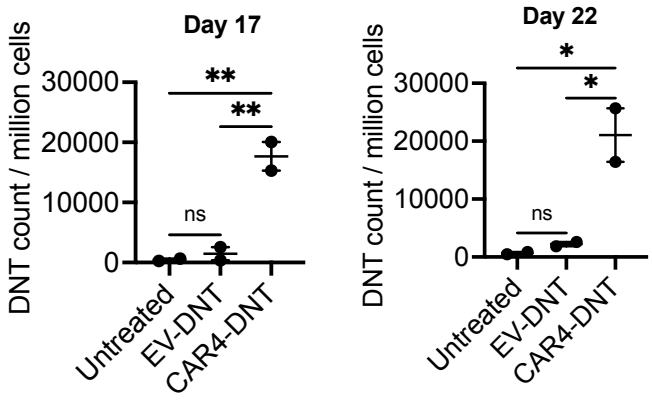

Figure S13. KARPAS-299 engrafted mice treated with PBS, EV-DNTs, or CAR4-DNTs were sacrificed on day 17 and day 22 and tumors were excised. Number of DNTs in resected tumor detected by flow cytometry on day 17 (left) and day 22 (right) after KARPAS-299 injection is shown. Each dot represents the DNT count in the tumor sample of one mouse, horizontal line represents the mean, and error bar represents SEM. One-way ANOVA Tukey's multiple comparison test was used for statistical analysis.

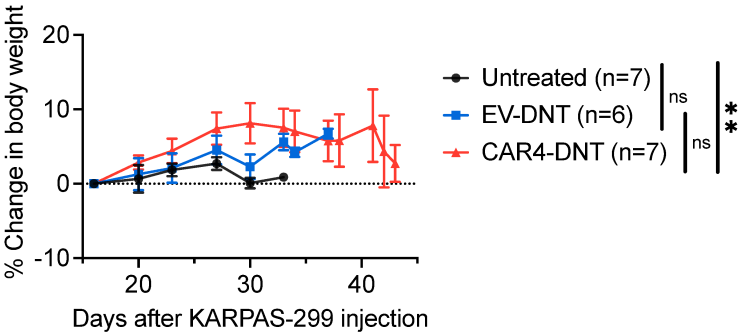

Figure S14. The percent change in body weight of KARPAS-299 engrafted mice treated with PBS (black, *n*=7), EV-DNTs (blue, *n*=6) and CAR4-DNTs (red, *n*=7).

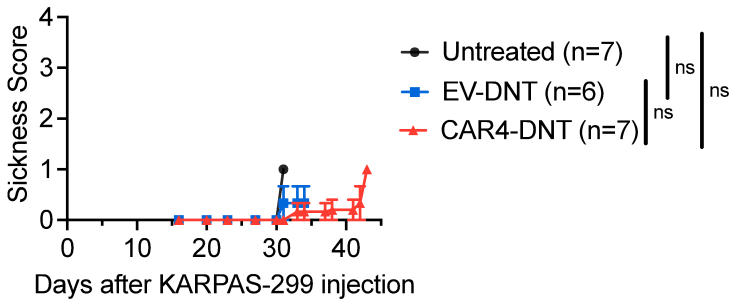

Figure S15. The sickness score of CCRF-CEM engrafted mice treated with PBS (black, *n*=7), EV-DNTs (blue, *n*=6) and CAR4-DNTs (red, *n*=7). Two-way ANOVA Tukey's multiple comparisons test was used for statistical analysis.

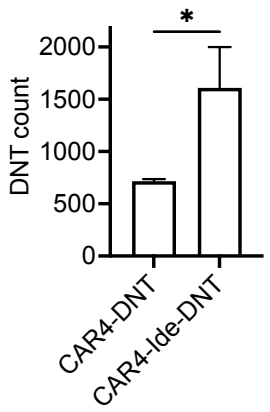

Figure S16. Fresh CCRF-CEM cells were added every 24 hours to CAR4-DNT or CAR4-Ide-DNT culture. The number of persisting DNTs in the CAR4-DNT and CAR4-DNT culture prior to the 12th round is shown. Bars represent mean of technical triplicates, and error bars represent SD. Student *t* test was used for statistical analysis.

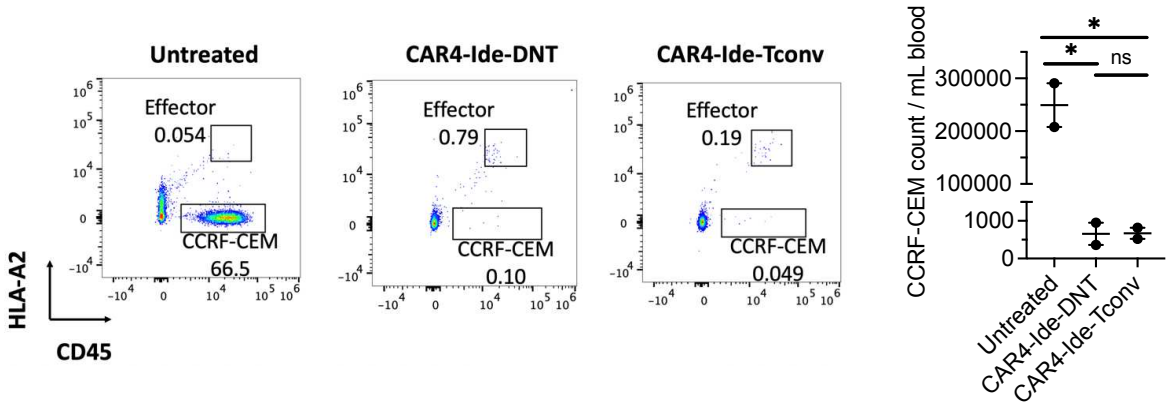

Figure S17. Left: Representative flow plots for HLA-A2 and CD45 staining of blood cells from PBS-, CAR4-Ide-DNT-, and CAR4-Ide-Tconv-treated mice on day 26 post CCRF-CEM engraftment. An HLA-A2-mistmatched donor was used to manufacture CAR4-DNTs and CAR4-Tconv to distinguish from CCRF-CEM cells. Right: CCRF-CEM cell counts in the peripheral blood of mice after treatment with PBS, CAR4-Ide-DNTs and CAR4-Ide-Tconv (*n=2* for each treatment group). Each dot represents CCRF-CEM cell count per mL of blood in one mouse, horizontal lines represent the mean, and the error bars represent SEM.

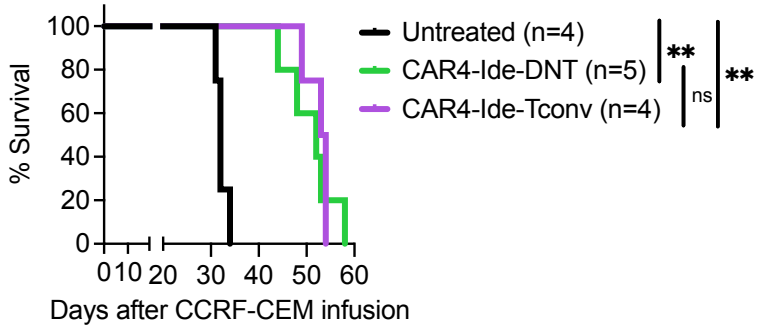

Figure S18. Kaplan-Meier curve showing the percent survival of CCRF-CEM-engrafted mice treated with PBS (black, *n*=4), 2x10^6 CAR$^+$ CAR4-Ide-DNTs (green, *n*=5), or 2x10^6 CAR$^+$ CAR4-Ide-Tconv (purple, *n*=4). Log-rank test was used for statistical analysis.

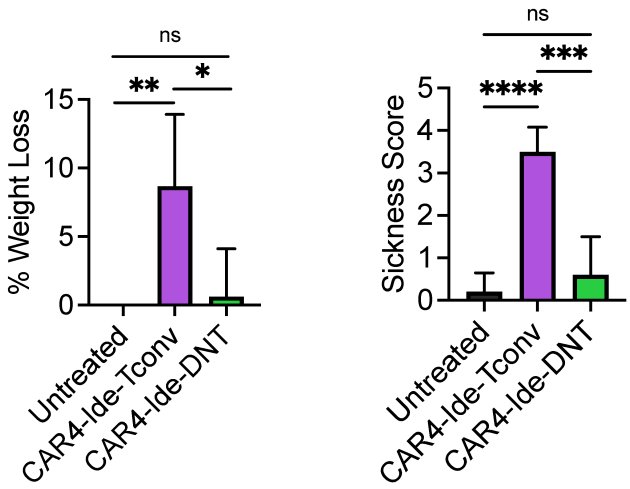

Figure S19. The percent weight loss (left) and sickness score (right) of CCRF-CEM engrafted mice 10 days after treatment with PBS (black, *n*=5), CAR4-Ide-DNTs (green, *n*=5), and CAR4-Ide-Tconv (purple, *n*=4). Error bars represent SD. One-way ANOVA Tukey's multiple comparisons test was used for statistical analysis.

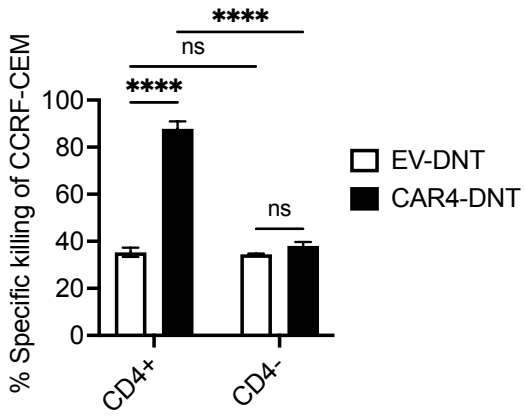

Figure S20. CD4^{+} and CD4-negative CCRF-CEM cells harvested from mice were cocultured with fresh EV-DNTs and CAR4-DNTs for 2 hours at a 4:1 effector-to-target ratio. Percent specific killing of the target is shown, and error bars represent SD. The experiment was independently performed 2 times, and representative data are shown. Two-way ANOVA multiple comparisons test was used for statistical analysis.

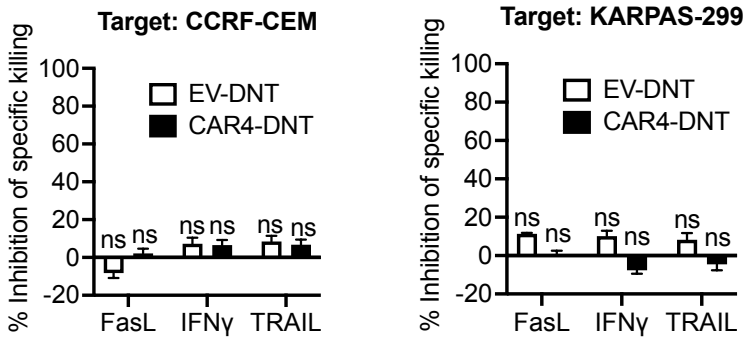

Figure S21. An *in vitro* cytotoxicity assay was conducted using EV-DNTs (white bars) and CAR4-DNTs (black bars) against CCRF-CEM and KARPAS-299, in the presence of FasL, IFNγ, and TRAIL blocking antibody, or isotype controls. Percent inhibition of specific killing by each blocking antibody was determined relative to isotype control. Each experiment was done in triplicates, and data are a combination of 2 independently performed experiments. One-way ANOVA Dunnett's multiple comparisons test was used for statistical analysis.

Supplementary Table

Patient Sample ID	Source	Sample Collection Status	Patient Diagnosis	Blasts	Flow Cytometry Results
160236	PB	diagnosis	T-ALL	89%	Positive: CD38, CD7, CD8, CD4, CD3, cCD3, CD2, CD71, CD58, CD1a, CD5, cCD79a, and partly TdT.
846785	PB	diagnosis	T-ALL		Positive: cCD3 CD1a/2/4/7/8/99/TdT. Weak: CD10. Negative: sCD3/5/34/79a/117/MPO
286069	PB	diagnosis	T-ALL	98%	Positive: CD1a, CD2, cCD3, CD4, CD5, CD7, CD8 (dim), CD10, CD99 and TdT. Partial variably positive for sCD3. The following antigens are analyzed: Negative: CD19, CD34, CD45, CD117, CyMPO, CyCD79a.
100104	PB	diagnosis	T-ALL	86%	The blasts express CD1a/3/4/5/8/7 and TdT and CyCD3 and CycCD79a. Co-expression of CD4 and CD8 is noted. Negative: myeloid markers, CD34, and HLA-DR.
090693	PB	diagnosis	T-ALL	92%	Positive: CD3/4/5/7/8 and CyCD3. Partial expression of CD1a and CD117. Negative: TdT, CD34, HLA-DR, and B cell markers.

Table S1. Characteristics of patient samples.

Scoring system	Activity	Posture	Fur	Skin	Weight loss
0	normal, interest in enrichment	normal, no hunch	smooth coat	normal	none
1	slightly reduced activity	mild hunch	slight to moderate ruffling	reddish, small irritated areas, slight pallor	up to 5%
2	reduced activity, less frequent movements, no interest in enrichment	moderate hunch	marked ruffling, slight loss of fur	scaling/ dry skin <20% BSA, moderate pallor	5-15%
3	no activity, stationary, +/- tremors	marked hunch, appears puffy	severe ruffling, fur staining, moderate + fur loss	scaling / dry skin > 20% BSA, marked pallor or cyanosis	15-20%

Table S2. Criteria for mouse sickness scoring.