Supplemental Methods

Generation of T cells for gene editing

Leukapheresis starting cell material used to produce gene edited T cells was obtained from healthy donor peripheral blood and sourced from HemaCare. The leukapheresis material was diluted with PBS + 10% HSA before isolation of T cells labeled with anti-CD4 and anti-CD8 magnetic microbeads using the CliniMACS Prodigy (Miltenyi Biotec) instrument. The isolated T-cells were then cryopreserved for use in experiments.

Generation of CBE mRNA

The sequence encoding CBE (rat APOBEC1-nSpCas9-2xUGI) was cloned into the mRNA template plasmid encoding a T7 promoter followed by a 5′ UTR, Kozak Sequence, editor open reading frame (ORF), 3′ UTR, a polyA tail, and a BbsI restriction site. Plasmids were purified using commercial midiprep kits (Zymo Research) and linearized using BbsI-HF (NEB). Linearized plasmid template was purified on a DNA Clean and Concentrate Column (Zymo Research). The NEB HiScribe High-Yield Kit was used as per the instruction manual but with co-transcriptional capping with CleanCap AG (TriLink). Isolation of mRNA from transcription reactions was achieved by lithium chloride precipitation.

Quantification of T cell yield following gene editing

Cryopreserved T cells were thawed on day 0 and activated using T cell TransAct at a 1:50 dilution in X-VIVO 15 serum free culture media supplemented with 300 IU/mL IL-2. On day 2, 1x10^6 T cells were electroporated with 1 μg of one, two, or three gRNAs and 2 μg mRNA encoding spCas9 nuclease or CBE using the Lonza 4D Nucleofector system (program DH-102).
Electroporated cells were then seeded into a 24-well G-Rex (Wilson Wolf) at a density of 5x10^5 cells/cm^2. Cell yields for each condition were quantified using a NucleoCounter NC-200 cell counter on day 9.

**7CAR8 lentiviral vector**

The anti-human CD7 CAR was created by cloning the 3A1e scFv sequence into a second-generation CAR backbone containing CD28 and CD3ζ endodomains and the CD8 spacer and transmembrane region. This sequence was placed downstream of the MND promoter in a third-generation self-inactivated lentiviral backbone. Because this construct was the eighth sequence evaluated, it was named 7CAR8.

**Generation of 7CAR8 CART cells**

The cryopreserved leukapheresis starting cell material used to produce 7CAR8 CART cells was obtained from healthy donor peripheral blood. The leukapheresis is thawed and diluted with PBS + 10% HSA, loaded on the CliniMACS Prodigy (Miltenyi Biotec) instrument and labeled with anti-CD4 and anti-CD8 magnetic microbeads. The labeled CD4+/CD8+ T cells are isolated using positive magnetic selection on the Prodigy system. The isolated T-cells are then cultured in X-VIVO 15 serum free culture media (Lonza) at 1x10^6 cells per mL in Permalife Cell Culture Bags. T Cell TransAct beads (Miltenyi) are added to the culture bag at a ratio of 1:100 (TransAct volume to culture volume) to activate and expand the cells before placing an incubator at 37°C / 5% CO2 for 2 days. On Day 2, the cells are washed with PBS and resuspended into Lonza P3 Primary Cell 4D-Nucleofactor Kit electroporation (EP) buffer at a target concentration of 50x10^6 cells/mL. The cells are electroporated using the Lonza 4D-Nucleofector LV Unit, where the
1µg/1x10^6 cells of each gRNA (CD52, PDCD1, TRAC, CD7) and 1µg/1x10^6 cells of mRNA (CBE) are delivered into the cells simultaneously using electroporation program DH-102. Following electroporation, the cells are immediately diluted in X-VIVO 15 serum-free culture media (Lonza) at a 1:5 volume ratio. The electroporated cells are then seeded into a 500M G-Rex (Wilson Wolf) at a target density of 1x10^6 cells/cm^2 before they are transduced with anti-CD7 CAR lentivirus. The lentiviral vector was added at a multiplicity of infection (MOI) of 2.5, along with Synperonic® F108 (Sigma Aldrich) a non-cytotoxic adjuvant at a concentration of 1mg/mL to enhance transduction efficiency. The edited and transduced cells are then cultured in an incubator at 37°C / 5% CO_2 for an additional 7 days to allow for cell expansion. On Day 9, the cells are concentrated using the GatheRex pump system to a final target volume of 1000 mL before being loaded onto the CliniMACS Prodigy instrument. The Prodigy instrument subsequently incubates the cells with biotin-tagged anti-TCRαβ immunomagnetic beads followed by washing with CliniMACS PBS/EDTA buffer. TCRαβ-expressing cells that are labeled are subsequently depleted from the bulk population through a negative selection immunomagnetic cell separation process using a customized program on the CliniMACS Prodigy instrument. Following TCRαβ depletion, cells are cultured in a new 500M G-Rex at a target concentration of 10x10^6 cells/cm^2 in an incubator at 37°C / 5% CO_2 for an additional 3 days before harvest. On the day of harvest, cells from the 500M G-Rex are washed and resuspended in a final formulation mixture containing 49% Plasmalyte A, 50% CryoStor 10, and 1% HSA at a final concentration of 50x10^6 cells/mL. The cryovials containing the final 7CAR8 cells are then cryopreserved and stored in vapor phase of a LN_2 tank at ≤ -160°C.

**CCRF-GFP-Luc T-ALL NSG Mouse Model**
All animal studies were performed according to the guidelines and approval of the Institutional Animal Care and Use Committee. Female NOD-\textit{scid} IL2Rgamma\textsuperscript{null} (NSG™, stock number 005557) mice were obtained from The Jackson Laboratory and were housed in a pathogen-free environment under controlled conditions and received food and water ad libitum. Tumors were established by intravenous injection of $1 \times 10^5$ cells, suspended in 100 uL of 0.1% BSA DPBS (Gibco). Tumor burden was quantified by in vivo whole-body bioluminescence imaging of luciferase-expressing tumor cells at Days 4, 7, 14, 18, 21, and 25 using the IVIS® Spectrum In Vivo System (Perkin Elmer, Inc.). The mice were administered an intraperitoneal injection of 0.2 mL luciferin (150 mg/kg) and were anesthetized and maintained under gaseous anesthesia through the duration of the imaging procedure. Images of the whole body were acquired 10 minutes after luciferin injection, with the IVIS set to pixel width and height of 1, binning factor of 4, f number of 8, exposure time of 0.2 seconds, object radius of 0.75 cm, subject height of 1.5 cm, and acquisition set to “auto” settings. Images were analyzed by measuring the total flux (p/s) within the ROI (Living Image® software Version 4.5.5.19626, Perkin Elmer, Inc.).

For efficacy studies, once the tumor burden became measurable mice were randomized into groups via matched distribution using StudyLog software, and treatment was administered as outlined in figure legends. Humane endpoints included, but were not limited to, body condition score of 2 or less, hind limb paralysis, pallor of the skin (notably ears, indicative of high leukemia tumor burden), weight loss equal to or greater than 20% of the first recorded body weight, and tumor burden with a total flux equal to or greater than $1.00 \times 10^{10}$ p/s.

\textbf{Patient Derived Xenografts}
Patient derived xenograft (PDX) models were created using nonobese diabetic/severe combined immunodeficiency (NOD/SCID/Il2rgtm1wjl/SzJ; NSG) mice. Patient samples were injected in to NSG mice and harvested from mouse spleens as has been previously described. PDX samples were modified using a self-inactivating lentivirus to express firefly luciferase. NSG mice were then injected intravenously with $1 \times 10^6$ live luciferase expressing blasts.

**In-Vivo Xenograft Experiments**

Anesthetized NSG mice that had been injected with luciferase expressing T-ALL blasts were imaged weekly using an IVIS Spectrum In Vivo Imaging System (Perkin Elmer, Massachusetts, USA). Mice were injected intraperitoneally with luciferase and imaged within 10 minutes of the injection in groups of 3-5. In situations where mice had significantly higher levels of disease than their group members they were imaged individually to avoid cross-saturation. Analysis was performed with Living Image version 4.7.4 software.

Mice were randomized to be treated with 7CAR8 or untransduced T-cells (UTD). Typically 3-5 mice were randomized to each arm; in one experiment (TH34) three mice died prior to receiving treatment so 2 mice were used in each of the untransduced and in the PD1 WT $1 \times 10^6$ arms (Supplementary Table 8). Treatment was started when average total flux was approximately $1-10 \times 10^6$. For dose-response experiments, mice were randomized to receive $1 \times 10^6$ or $5 \times 10^6$ 7CAR8 or UTD cells. Cell dose was corrected to account for percentage of T-cells with 7CAR8 expression. Following treatment, mice were imaged weekly for four to six weeks. To assess the efficacy of PD1 disruption on 7CAR8 cells, experiments were performed randomizing mice between 7CAR8 wild type cells (7CAR8 PD1 WT) and those with PD1 silenced (7CAR8 PD1
KO), compared to UTD. Survival experiments were also conducted. Following the initial period of imaging, mice were monitored for survival. Mice who appeared moribund were sacrificed in accordance with institutional animal policies.
Supplementary Figure 1. Multiplexed nuclease-mediated gene insertion into TRAC with simultaneous base editing of B2M. (A) The Cas12b nuclease was used to insert a GFP transgene into the TRAC locus with high efficiency in T cells. Insertion is mediated by homology-directed repair using an AAV donor template. The MFI of GFP protein expression can be modulated by the insertion site within the TRAC locus. (B) Simultaneous base editing-mediated knockout of B2M with Cas12b-mediated GFP insertion into TRAC.

Supplementary Figure 2. Cytokine secretion profiles of 7CAR8 cells with wild-type PDCD1 and PDCD1-edited 7CAR8 cells produced from two independent donors using an in vitro re-stimulation assay. (A) 7CAR8 cells with PD1 knockout and PD1 wildtype were assessed for polyfunctionality using the Isoplexis platform. Y axis depicts polyfunctionality expressed as a percentage of sample. (B) Contribution of individual cytokines to polyfunctionality for 7CAR8 with and without PD1 knockout as measured by Isoplexis. X-axis represents individual cytokines with Y-axis representing overall contribution to polyfunctional inflammation index.

Supplementary Figure 3. Statistical comparison of in vivo antileukemic activity between three lots of BEAM-201 in the CCRF-CEM model of TALL. (A) Mice implanted with 1x10^5 CCEF-CEM cells IV on study day 0 were 10 randomized on day 10 to groups of comparable tumor burden via matched distribution using StudyLog software, then subsequently treated as indicated on day 11 with either a preparation of negative control untransduced (UTD) cells, 15x10^6, or 3x10^6 viable CAR positive BEAM-201 cells. Plots represent tumor growth of individual mice from day of randomization to the day when the first treated mouse reached a humane endpoint. (B and C) Areas under the curve (AUC) from the data in A were calculated using flux data from day of
randomization (day 10) through the day at which the first mouse in these groups reached a humane endpoint (day 25). A one-way ANOVA (ANalysis Of VAriance) with post-hoc Tukey HSD (Honestly Significant Difference) test was then used to assess whether any observed differences between low dose lots or high dose lots over this time period are significant. No significant differences were observed.

Supplementary Figure 4. Flow cytometric evaluation of PD-1 expression on T-ALL PDX samples. Histograms of surface PD-1 expression measured by flow cytometry for all PDX models of T-ALL. PD-1 expression is shown in blue with isotype control shown in red. X-axis represents mean-flourescence index (MFI) and Y-axis represents percentage of cells.

Supplementary Figure 5. Flow cytometric evaluation of PD-L1 expression on T-ALL PDX samples. Histograms of surface PDL-1 expression measured by flow cytometry for all PDX models of T-ALL. PDL-1 expression is shown in blue with isotype control shown in red. X-axis represents mean-flourescence index (MFI) and Y-axis represents percentage of cells.

Supplementary Figure 6. Evaluation of the efficacy of 7CAR8 in PDX models of T-ALL via bioluminescent imaging. Mean total flux over time in mice treated with untransduced T-cells (UTD), 7CAR8 cells, and 7CAR8 cells with PD-1 knocked out (PD1 KO) at varying dose levels. Y-axis represents log-transformed total flux and X-axis represents days from cell infusion. Data are shown for each PDX.
Supplementary Table 1.

| Clone   | Number of cells | Karyotypic abnormality                | Description                                                                                                                                 |
|---------|-----------------|--------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|
| Clone 1 | 11/100          | TRAC-CD52                            | The cells in the primary clone (eleven of one hundred cells examined) contain an apparently balanced translocation between the short (p) arm of chromosome 1 and the long (q) arm of chromosome 14, an apparently balanced translocation between the short (p) arm of chromosome 1 and the long (q) arm of chromosome 17, and an unbalanced structural aberration in the long (q) arm of chromosome 17. This abnormality, in which additional material of unknown origin translocated to chromosome 17q, cannot be characterized by G-banded chromosome analysis. |
|         |                 | CD7-CD52                             | Structural aberration with translocated material of unknown origin at CD7.                                                                                                           |
| Clone 2 | 9/100           | CD52-PDCD1                           | The cells in the secondary clone (nine of one hundred cells examined) contain an apparently balanced translocation between the short (p) arm of chromosome 1 and the long (q) arm of chromosome 2.                                      |
| Clone 3 | 2/100           | TRAC-PDCD1                           | The cells in the tertiary clone (two of one hundred cells examined) contain an unbalanced rearrangement of chromosome 2 in which a copy of the long (q) arm of chromosome 14 was translocated to the long arm of chromosome 2 and the 14 centromere and short (p) arm were lost. The derivative chromosome 2 results in loss of chromosome 2q and loss of 14p and part of the q arm. |

Supplementary Table 1: primary human T cells produced from one donor simultaneously multiplex edited at four target sites (TRAC, CD7, CD52, PDCD1) were analyzed by G-banded karyotyping, resulting in the characterization of three primary clonal populations with karyotypic abnormalities. All clonal karyotypic abnormalities were mapped to on-target editing sites.

Supplementary Table 2.

| gene_symbol | logFC_CBE | logCPM_CBE | F_CBE  | PValue_CBE | FDR_CBE | logFC_cas9 | logCPM_cas9 | F_cas9 | PValue_cas9 | FDR_cas9 |
|-------------|-----------|------------|--------|------------|---------|------------|------------|--------|------------|---------|
| CD52        | -3.320    | 3.906      | 405.5  | 2.08E-07   | 0.00272 | -2.63      | 3.91       | 271.66 | 8E-07      | 5E-03   |
| CD7         | -1.840    | 6.567      | 168.9  | 4.03E-06   | 0.02642 | -2.52      | 6.57       | 300.22 | 6E-07      | 5E-03   |
| TRIM22      | NA        | NA         | NA     | NA         | NA      | 1.19       | 5.57       | 216.18 | 2E-06      | 8E-03   |
| KIF1A       | NA        | NA         | NA     | NA         | NA      | 4.08       | 1.51       | 182.68 | 3E-06      | 1E-02   |
| TPS3INP1    | NA        | NA         | NA     | NA         | NA      | 1.58       | 1.61       | 141.29 | 7E-06      | 2E-02   |
| ZMAT3       | NA        | NA         | NA     | NA         | NA      | 1.12       | 2.80       | 140.34 | 7E-06      | 2E-02   |
| SESN1       | NA        | NA         | NA     | NA         | NA      | 0.94       | 3.83       | 121.78 | 1E-05      | 2E-02   |
| SIGLEC10    | NA        | NA         | NA     | NA         | NA      | 1.40       | 1.57       | 114.85 | 1E-05      | 2E-02   |
| RPS27L      | NA        | NA         | NA     | NA         | NA      | 0.88       | 6.76       | 115.57 | 1E-05      | 2E-02   |
| BDNF-AS     | NA        | NA         | NA     | NA         | NA      | 1.61       | 1.10       | 113.13 | 2E-05      | 2E-02   |
| PACSN1      | NA        | NA         | NA     | NA         | NA      | 2.00       | 0.49       | 105.34 | 2E-05      | 2E-02   |
| ANKMY1      | NA        | NA         | NA     | NA         | NA      | -0.87      | 3.62       | 93.518 | 3E-05      | 2E-02   |
| GPR155      | NA        | NA         | NA     | NA         | NA      | 1.00       | 2.59       | 97.732 | 2E-05      | 2E-02   |
| CMBL        | NA        | NA         | NA     | NA         | NA      | 1.76       | 0.95       | 93.283 | 3E-05      | 2E-02   |
| SULF2       | NA        | NA         | NA     | NA         | NA      | 1.52       | 1.02       | 93.984 | 3E-05      | 2E-02   |
| KIAA1671    | NA        | NA         | NA     | NA         | NA      | 0.79       | 5.34       | 98.485 | 2E-05      | 2E-02   |
| IFIT3       | NA        | NA         | NA     | NA         | NA      | 1.15       | 1.29       | 85.916 | 4E-05      | 3E-02   |
| Gene     | TMEM169 | CPM   | ING5   | PLKSN2 | APOBE3H | FRMD4B | KLHL24 | TNFSF11 | PDE1B | CDKN1A | SCN3A | HAAO | DENND2B | HEXT | P2RX7 | HDLBP | SYT1L2 | HMGN2 | TNFRSF10B | GSN | CH3L2 | PPP1R7 | DRAM1 | SLC47A1 | PBXIP1 | LAMB3 | CD24 | EDA2R | HERC5 | CDH1 | CEACAM1 | BBC3 | PTPRS | TFPI2 | OTOF | GADD45A | CALD1 | MDM2 | TBCD |
|----------|---------|-------|--------|--------|---------|--------|--------|--------|-------|--------|-------|------|---------|------|-------|--------|--------|-------|---------|------|-------|--------|-------|--------|-------|-------|------|-------|--------|-------|-------|-------|
|          | NA      | NA    | NA     | NA     | NA      | NA     | NA     | NA     | NA    | NA     | NA    | NA   | NA      | NA   | NA    | NA     | NA     | NA    | NA      | NA   | NA    | NA     | NA    | NA     | NA    | NA    | NA    | NA    | NA     | NA    | NA    | NA    | NA    |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
|          |         |       |        |        |         |        |        |        |       |        |        |      |         |      |       |        |        |       |         |      |       |        |        |        |       |        |      |       |        |        |       |       |       |       |
| Term                                      | Number of Genes Up | Number of Genes Down | P.Up       | P.Down       |
|-------------------------------------------|--------------------|----------------------|------------|--------------|
| GO:0006915 apoptotic process              | 21                 | 2                    | 6.61E-07   | 0.33190873   |
| GO:0012501 programmed cell death          | 21                 | 2                    | 1.18E-06   | 0.34806899   |
| GO:0008219 cell death                     | 21                 | 2                    | 3.22E-06   | 0.37772441   |
| GO:0044783 G1 DNA damage checkpoint       | 5                  | 0                    | 7.25E-06   | 1            |
| GO:0031571 mitotic G1 DNA damage checkpoint| 5                  | 0                    | 7.25E-06   | 1            |
| GO:0044819 mitotic G1/S transition checkpoint| 5                  | 0                    | 7.25E-06   | 1            |
| GO:0072332 intrinsic apoptotic signaling pathway by p53 class mediator | 5                  | 0                    | 8.69E-06   | 1            |
| GO:0097190 apoptotic signaling pathway    | 11                 | 1                    | 1.00E-05   | 0.33374085   |

Supplementary Table 2: primary human T cells produced from one donor simultaneously multiplex edited at four target sites (TRAC, CD7, CD52, PDCD1) using either spCas9 or CBE were analyzed for transcriptomic changes using whole-transcriptome RNA-esq.
| GO:0097193 | intrinsic apoptotic signaling pathway | 8 | 0 | 1.09E-05 | 1 |
| GO:0006950 | response to stress | 29 | 0 | 1.10E-05 | 1 |
| GO:0043281 | regulation of cysteine-type endopeptidase activity involved in apoptotic process | 7 | 0 | 1.12E-05 | 1 |
| GO:2000116 | regulation of cysteine-type endopeptidase activity | 7 | 0 | 2.15E-05 | 1 |
| GO:0042981 | regulation of apoptotic process | 16 | 2 | 2.41E-05 | 0.22923251 |
| GO:0052548 | regulation of endopeptidase activity | 8 | 0 | 2.82E-05 | 1 |
| GO:0043067 | regulation of programmed cell death | 16 | 2 | 2.82E-05 | 0.23375003 |
| GO:0052547 | regulation of peptidase activity | 8 | 0 | 4.57E-05 | 1 |
| GO:0009605 | response to external stimulus | 19 | 1 | 5.13E-05 | 0.74958632 |
| GO:006978 | DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator | 3 | 0 | 5.31E-05 | 1 |
| GO:0044773 | mitotic DNA damage checkpoint | 5 | 0 | 5.61E-05 | 1 |
| GO:2000134 | negative regulation of G1/S transition of mitotic cell cycle | 5 | 0 | 6.30E-05 | 1 |
| GO:0042772 | DNA damage response, signal transduction resulting in transcription | 3 | 0 | 6.51E-05 | 1 |
| GO:0030330 | DNA damage response, signal transduction by p53 class mediator | 5 | 0 | 6.67E-05 | 1 |
| GO:1902807 | negative regulation of cell cycle G1/S phase transition | 5 | 0 | 6.67E-05 | 1 |
| GO:0010941 | regulation of cell death | 16 | 2 | 7.06E-05 | 0.26138717 |
| GO:0044774 | mitotic DNA integrity checkpoint | 5 | 0 | 8.31E-05 | 1 |
| GO:0072331 | signal transduction by p53 class mediator | 7 | 1 | 8.56E-05 | 0.17623728 |
| GO:0003008 | system process | 14 | 1 | 8.63E-05 | 0.56107843 |
| GO:1903035 | negative regulation of response to wounding | 4 | 0 | 8.88E-05 | 1 |
| GO:0043068 | positive regulation of programmed cell death | 10 | 1 | 0.00010159 | 0.35181553 |
| GO:0043280 | positive regulation of cysteine-type endopeptidase activity involved in apoptotic process | 5 | 0 | 0.00010242 | 1 |

**Supplementary Table 3.** Gene ontology analysis of whole transcriptome sequencing of T-cells simultaneously edited at four loci using spCas9.

**Supplementary Table 4.**
| Assay                                      | Test Method | Run 1 | Run 2 | Run 3 |
|--------------------------------------------|-------------|-------|-------|-------|
| Anti-CD7 CAR+ cells                        | Flow cytometer | 90.3% | 91.2% | 81.3% |
| Residual TCRαβ+ T cells                    | Flow cytometry | 0.3%  | 0.1%  | 0.3%  |
| Percent CD4+/CD8+ cells                    | Flow cytometry | 48.3%/47.0% | 68.7%/27.1% | 57.6%/41.5% |
| CD7, CD52, PD1 negative cell populations  | Flow Cytometry | CD7 99.9% | 100.0% | 100.0% |
|                                           |             | CD52 96.2% | 96.5% | 97.6% |
|                                           |             | PD-1 92.6% | 92.7% | 90.4% |
| Residual B cells, monocytes, NK cells      | Flow Cytometry | B cells 0.5% | 0.3% | 0.1% |
|                                           |             | Monocytes 0.4% | 0.0% | 0.0% |
|                                           |             | NK cells 0.6% | 0.8% | 2.2% |
| Memory Phenotype                           | Flow Cytometry | Naïve/Stem Central Memory (CD45RA+,CCR7+, CD62L+) 20.9% | 21.7% | 33.5% |
|                                           |             | Central Memory (CD45RA-,CCR7+, CD62L+) 9.9% | 15.6% | 7.1% |
|                                           |             | Effector Memory (CD45RA-,CCR7-, CD62L-) 18.8% | 15.9% | 8.1% |
|                                           |             | Terminal Effector (CD45RA+,CCR7-, CD62L-) 50.4% | 46.8% | 51.3% |
| Population Doubling Levels*                | Automated cell counter | 5.73 | 5.84 | 5.98 |
| Total viable cell yield per manufacturing run* | Automated cell counter | 17.7x10⁶ | 18.3x10⁶ | 21.1x10⁶ |
| Viability                                  | Automated cell counter | 92.00% | 91.20% | 94.10% |
| Karyotypic abnormalities                   | G-banded karyotyping | None detected | None detected | None detected |
| Interferon-γ production                    | ELISA       | 87,700 pg/mL | 103,548 pg/mL | 64,339 pg/mL |
| Cytokine independent growth expansion factor | Flow cytometry | 0.2 | 0.1 | 0.1 |

Supplementary Table 4: Data from three representative manufacturing runs demonstrates consistency and reproducibility of the 7CAR8 clinical manufacturing process. Each run was performed using leukapheresis starting material from a different healthy donor. All results reported are obtained from post-thaw analysis of the final 7CAR8 drug product cells.

*Population doubling levels and total viable cells produced for each manufacturing run were assessed on the day of harvest prior to final formulation.

Supplementary Table 5.
Supplementary Table 5: Whole transcriptome RNA sequencing was performed on CBE-edited T cells to determine the identities of the mature mRNAs produced from edited alleles.

| Target Gene | Class | Region | Sequence | Chr Coordinates | Edit Class | mRNA outcome | Protein outcome |
|-------------|-------|--------|----------|-----------------|------------|--------------|----------------|
| CD52        | On-target | CD52 (exon-intron boundary) | CTCTTAACCTGTA CCATAACCAGG | 1 26318056-26318078 | Splice donor disruption | Probable NMD | No protein detected |
| CD7         | On-target | CD7 (exon-intron boundary) | CCTACCTGTCAC CAGGACCAGGG | 17 82316662-82316684 | Splice donor disruption | intron retention; 7 bp exon 2 skipped; 13 bp exon 2 skipped | No protein detected |
| PDCD1       | On-target | PDCD1 (exon-intron boundary) | CACCTACCTAAG ACCATCCTGG | 2 241858756-241858778 | Splice donor disruption | Probable NMD | No protein detected |
| TRAC        | On-target | TRAC (exon-intron boundary) | TTCTGTATCTGTA AAAACCAAGGG | 14 22550541-22550563 | Splice acceptor disruption | Intron retention | No protein detected |

Supplementary Table 6: Name, USI and patient characteristics of patient derived xenografts (PDX) used for in vivo mouse experiments testing 7CAR8.

| Name   | USI     | Sex | Age at Dx (years) | WBC at Dx (u/L) | Phenotype |
|--------|---------|-----|-------------------|-----------------|-----------|
| ETP1   | PARIKN  | M   | 8                 | 66,000          | ETP       |
| ETP5   | PARXNE  | M   | 5                 | 42,000          | ETP       |
| ETP27  | PATRAP  | F   | 7                 | 92,000          | ETP       |
| ALL8   |         | M   | 13                | UNK             | non-ETP   |
| ALL33  |         | M   | 4                 | 216,000         | non-ETP   |
| TH34   | PAXAAC  | F   | 15                | 54,000          | non-ETP   |
| TH75   | PAXHIT  | M   | 4                 | 241,000         | non-ETP   |

Supplementary Table 7.

| ALL8   | Treatment Condition | UTD 1x10^6 | UTD 5x10^6 | PD1 WT 1x10^6 | PD1 WT 5x10^6 | PD1 KO 1x10^6 | PD1 KO 5x10^6 |
|--------|---------------------|-------------|-------------|----------------|----------------|----------------|----------------|
| Number of Mice | 5 | 5 | 5 | 5 | 5 | 5 |
| Median Survival (Weeks) | 10.9 | 8 | 14.9 | 15.9 | 19.3 | 20.9 |

| ETP5   | Treatment Condition | UTD 5x10^6 | 7CAR8 5x10^6 |
|--------|---------------------|------------|--------------|
| Number of Mice | 3 | 3 |
| Median Survival (Weeks) | 8.9 | 26.7 |
| Treatment Condition | UTD 1x10⁶ | UTD 5x10⁶ | PD1 WT 1x10⁶ | PD1 WT 5x10⁶ | PD1 KO 1x10⁶ | PD1 KO 5x10⁶ |
|----------------------|-----------|-----------|--------------|--------------|--------------|--------------|
| Number of Mice       | 3         | 3         | 3            | 3            | 3            | 3            |
| Median Survival (Weeks) | 4         | 4         | 11.9         | 17.3         | 11           | 13.3         |

| Treatment Condition | UTD 1x10⁶ | UTD 5x10⁶ | PD1 WT 1x10⁶ | PD1 WT 5x10⁶ | PD1 KO 1x10⁶ | PD1 KO 5x10⁶ |
|----------------------|-----------|-----------|--------------|--------------|--------------|--------------|
| Number of Mice       | 2         | 2         | 2            | 3            | 3            | 3            |
| Median Survival (Weeks) | 5.2       | 4.9       | 16.7         | 26.7         | 17.3         | 20.4         |

| Treatment Condition | UTD 5x10⁶ | 7CAR8 5x10⁶ |
|----------------------|-----------|-------------|
| Number of Mice       | 3         | 3           |
| Median Survival (Weeks) | 2.3       | 6.1         |

**Supplementary Table 7.** Median Survival of patient derived xenografts (PDX) treated with untransduced T Cells or 7CAR8.

**Supplementary Table 8.**

### TH75

| Weeks | UTD 1x10⁶ | UTD 5x10⁶ | PD1 WT 1x10⁶ | PD1 WT 5x10⁶ | PD1 KO 1x10⁶ | PD1 KO 5x10⁶ |
|-------|-----------|-----------|--------------|--------------|--------------|--------------|
| 0.0   | 100.0     | 100.0     | 100.0        | 100.0        | 100.0        | 100.0        |
| 3.4   |           | 66.7      | 66.7         |              |              |              |
| 4.0   |           | 33.3      | 33.3         |              |              |              |
| 5.0   |           | 0.0       | 0.0          |              |              |              |
| 9.9   |           |           |              |              | 66.7         |              |
| 11.0  |           |           |              | 66.7         | 33.3         |              |
| 11.9  |           |           |              | 33.3         |              |              |
| 12.4  |           |           |              |              | 0.0          | 0.0          |
| 13.3  |           |           |              |              |              | 33.3         |
| 17.3  |           |           |              |              | 33.3         | 0.0          |
| 20.0  |           |           |              |              |              | 0.0          |

### ETP5

| Weeks | UTD 5x10⁶ | 7CAR8 5x10⁶ |
|-------|-----------|-------------|
| 0.0   | 100.0     | 100.0       |
| 8.1   | 66.7      |              |
| 8.9   | 0.0       |              |
| 22.1  |           | 66.7        |
| 26.7  |           | 33.3        |
| 36.7  |           | 0.0         |
### ALL33

| Weeks | UTD $5\times10^6$ | 7CAR8 $5\times10^6$ |
|-------|-------------------|------------------|
| 0.0   | 100.0             | 100.0            |
| 2.3   | 0.0               |                  |
| 5.9   |                   | 66.7             |
| 6.1   |                   | 33.3             |
| 7.3   |                   | 0.0              |

### PATRAP

| Weeks | UTD $5\times10^6$ | 7CAR8 $5\times10^6$ |
|-------|-------------------|------------------|
| 0.0   | 100.0             | 100.0            |
| 15.7  |                   | 66.7             |
| 16.7  |                   | 33.3             |
| 18.1  |                   | 0.0              |
| 27.6  |                   | 66.7             |
| 34.7  |                   | 33.3             |
| 37.9  |                   | 0.0              |

### ALL33 (Repeat)

| Weeks | UTD $1\times10^6$ | UTD $5\times10^6$ | PD1 WT $1\times10^6$ | PD1 WT $5\times10^6$ | PD1 KO $1\times10^6$ | PD1 KO $5\times10^6$ |
|-------|-------------------|-------------------|---------------------|---------------------|---------------------|---------------------|
| 0.0   | 100.0             | 100.0             | 100.0               | 100.0               | 100.0               | 100.0               |
| 1.9   |                   |                   |                     |                     |                     | 80.0                |
| 2.3   |                   | 80.0              | 60.0                |                     |                     |                     |
| 2.4   |                   |                   |                     |                     |                     | 60.0                |
| 2.7   |                   |                   |                     |                     |                     | 40.0                |
| 2.9   |                   | 0.0               | 0.0                 |                     |                     | 80.0                |
| 5.9   |                   |                   |                     |                     |                     | 80.0                |
| 6.3   |                   |                   |                     |                     |                     | 60.0                |
| 7.0   |                   |                   |                     |                     |                     | 80.0                |
| 7.9   |                   |                   |                     |                     |                     | 40.0                |
| 10.0  |                   |                   |                     |                     |                     | 60.0                |
| 11.4  |                   |                   |                     |                     |                     | 20.0                |
| 12.9  |                   |                   |                     |                     |                     | 40.0                |
| 13.1  |                   |                   |                     |                     |                     | 20.0                |
| 14.4  |                   |                   |                     |                     |                     | 0.0                 |
| 14.9  |                   |                   |                     |                     |                     | 60.0                |
| 16.0  |                   |                   |                     |                     |                     | 40.0                |
| 20.4  |                   |                   |                     |                     |                     | 20.0                |
| 22.1  |                   |                   |                     |                     |                     | 0.0                 |
### ALL8

| Weeks | UTD 1x10⁶ | UTD 5x10⁶ | PD1 WT 1x10⁶ | PD1 WT 5x10⁶ | PD1 KO 1x10⁶ | PD1 KO 5x10⁶ |
|-------|-----------|-----------|--------------|--------------|--------------|--------------|
| 0.0   | 100.0     | 100.0     | 100.0        | 100.0        | 100.0        | 100.0        |
| 5.3   |           | 80.0      |              |              |              |              |
| 7.0   |           |           |              | 60.0         |              |              |
| 8.0   |           |           |              | 40.0         |              |              |
| 8.4   |           |           |              |              |              | 80.0         |
| 10.1  |           |           |              |              | 80.0         |              |
| 10.4  |           |           |              | 60.0         |              |              |
| 10.9  |           |           |              | 40.0         |              | 80.0         |
| 11.3  |           |           |              |              | 20.0         |              |
| 11.9  |           |           |              |              | 20.0         |              |
| 12.9  |           |           |              |              | 60.0         |              |
| 13.4  |           |           |              |              |              | 80.0         |
| 13.9  |           |           |              |              | 80.0         |              |
| 14.9  |           |           |              | 40.0         |              |              |
| 15.9  |           |           |              | 40.0         |              |              |
| 16.3  |           |           |              | 20.0         |              |              |
| 17.3  |           |           |              | 20.0         |              |              |
| 17.9  |           |           |              |              |              | 0.0          |
| 18.0  |           |           |              |              |              | 60.0         |
| 19.0  |           |           |              |              |              | 60.0         |
| 19.1  |           |           |              |              | 0.0          |              |
| 19.3  |           |           |              |              | 40.0         |              |
| 20.9  |           |           |              |              | 40.0         |              |
| 22.3  |           |           |              |              | 0.0          |              |
| 22.9  |           |           |              |              |              | 40.0         |

### TH34

| Weeks | UTD 1x10⁶ | UTD 5x10⁶ | PD1 WT 1x10⁶ | PD1 WT 5x10⁶ | PD1 KO 1x10⁶ | PD1 KO 5x10⁶ |
|-------|-----------|-----------|--------------|--------------|--------------|--------------|
| 0     | 100       | 100       | 100          | 100          | 100          | 100          |
| 4.3   |           |           |              | 50           |              |              |
| 5     |           |           |              | 50           |              |              |
| 5.4   |           |           |              | 0            | 0            |              |
| 5.9   |           |           |              |              |              | 75           |
Supplementary Table 8. Survival Proportions of patient derived xenografts (PDX) treated with untransduced T cells or 7CAR8.

|    |        |        |    |        |
|----|--------|--------|----|--------|
| 12.4 |        |        |    |        |
| 16.1 |        | 50 |        | 66.7 |
| 17.3 |        | 0 |        | 33.3 |
| 20.4 |        |    |        |        |
| 26.4 |        | 50 |        |        |
| 27.1 |        | 25 |        | 0     |
| 44.4 |        |    |        |        |
| 49.3 |        | 25 |        |        |

Supplementary Table 9. Primers used for genomic DNA amplification for use in next generation sequencing.

| Target Gene | Forward Primer | Reverse Primer |
|-------------|----------------|----------------|
| CD52        | ACACTCTTCCCTACACGACGC | TGGAGTCAGAGGTGCTCTTCC |
| CD7         | ACACTCTTCCCTACACGACGC | TGGAGTCAGAGGTGCTCTTCC |
| PDCD1       | ACACTCTTCCCTACACGACGC | TGGAGTCAGAGGTGCTCTTCC |
| TRAC        | ACACTCTTCCCTACACGACGC | TGGAGTCAGAGGTGCTCTTCC |

Supplementary Table 10. 7CAR8 purity flow assay (a table of the flow panel)

| Antibody | Fluorophore | Clone | Vendor         | Cat. No.    |
|----------|-------------|-------|----------------|-------------|
| CD45     | Viogreen    | REA747| Miltenyi Biotech | 130-110-638 |
| CD14     | Vioblue     | REA599| Miltenyi Biotech | 130-110-524 |
| CD4      | Viobright B515 | REA623| Miltenyi Biotech | 130-114-535 |
| CD8      | PerCP-Vio700| REA734| Miltenyi Biotech | 130-110-682 |
| CD56     | VioBright R667 | REA196| Miltenyi Biotech | 130-114-553 |
| CD19     | PE          | REA675| Miltenyi Biotech | 130-113-646 |
| CD2      | PE-Vio770   | REA972| Miltenyi Biotech | 130-116-151 |

**Staining procedure in brief**

7CAR8 cells were thawed and washed with PBS. Cells were stained for live dead in PBS and then washed and resuspended in staining buffer before being stained with these antibodies at
1:50 concentrations. CD2+ T cells, CD14+ Monocytes, CD56+ NK cells, and CD19+ B cells were detected.

2. Editing panel
   a. TRAC, CD52

| Antibody | Fluorophore | Clone | Vendor             | Cat. No.  |
|----------|-------------|-------|--------------------|-----------|
| CD45     | Viogreen    | REA747| Miltenyi Biotech   | 130-110-638|
| CD52     | PE          | REA164| Miltenyi Biotech   | 130-123-743|
| TCRαβ    | PerCP-Vio700| REA652| Miltenyi Biotech   | 130-113-540|
| CD2      | PE-Vio770   | REA972| Miltenyi Biotech   | 130-116-151|

Staining procedure in brief
7CAR8 cells were thawed and washed with PBS. Cells were stained for live dead in PBS and then washed and resuspended in staining buffer before being stained with these antibodies at 1:50 concentrations. TCRαβ+ and CD52+ T cells were detected.

b. PD1

| Antibody | Fluorophore | Clone    | Vendor             | Cat. No.  |
|----------|-------------|----------|--------------------|-----------|
| CD45     | Viogreen    | REA747   | Miltenyi Biotech   | 130-110-638|
| PD1      | Viobright B515| REA1165 | Miltenyi Biotech   | 130-120-386|
| CD2      | PE-Vio770   | REA972   | Miltenyi Biotech   | 130-116-151|

Staining procedure in brief
7CAR8 as well as unedited cells were thawed and stimulated with a cell activation cocktail (Biolegend 423301) at 37°C for 24 hours. Cells were then washed with PBS. Cells were stained for live dead in PBS and then washed and resuspended in staining buffer before being stained with these antibodies at 1:50 concentrations.

Supplementary Table 10. Flow cytometry antibodies and procedures used to assess CAR expression and base editing efficiencies.
Supplementary Figure 1

A

AAV (MOI \(1 \times 10^6\)) control

Cas9 AAV (MOI \(2 \times 10^5\))

Cas12b TRAC1 AAV (MOI \(2 \times 10^5\))

Cas12b TRAC10 AAV (MOI \(2 \times 10^5\))

Cas12b TRAC11 AAV (MOI \(2 \times 10^5\))

Supplementary Figure 1

B

Day 0

Thaw PBMCs and activate with soluble anti-CD3/28 antibodies

Day 3

EP BE4 mRNA, Cas12b mRNA, and two guide RNAs (B2M, TRAC)

Day 3

Seed cells into media or media + AAV donor

Day 9

Assess editing by flow cytometry

\[
\begin{align*}
\text{No edit} & & \text{Cas9} + \text{AAV (MOI } 2 \times 10^5) & & \text{Cas12b TRAC1} + \text{AAV (MOI } 2 \times 10^5) & & \text{Cas12b TRAC10} + \text{AAV (MOI } 2 \times 10^5) & & \text{Cas12b TRAC11} + \text{AAV (MOI } 2 \times 10^5)
\end{align*}
\]
Supplementary Figure 2

Donor 1 Polyfunctionality

Polyfunctionality (% of sample)

7CAR8 PD1 KO 7CAR8 PD1 WT

2 proteins 3 proteins 4 proteins 5+ proteins

Contribution to Overall Polyfunctional Inflammation Index

GM-CSF GDX/PMB IFN-y IL-2 IL-4 IL-9 IL-10 IL-19 IL-21 IFN-γ TNF-α

Donor 2 Polyfunctionality

Polyfunctionality (% of sample)

7CAR8 PD1 KO 7CAR8 PD1 WT

2 proteins 3 proteins 4 proteins 5+ proteins

Contribution to Overall Polyfunctional Inflammation Index

GM-CSF GDX/PMB IFN-y IL-2 IL-4 IL-9 IL-10 IL-19 IFN-γ TNF-α
Supplementary Figure 5

PDL-1 Expression
Supplementary Figure 6