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Supplemental Information

Treatment of Acute Myeloid Leukemia with T Cells Expressing Chimeric Antigen Receptors Directed to C-type Lectin-like Molecule 1

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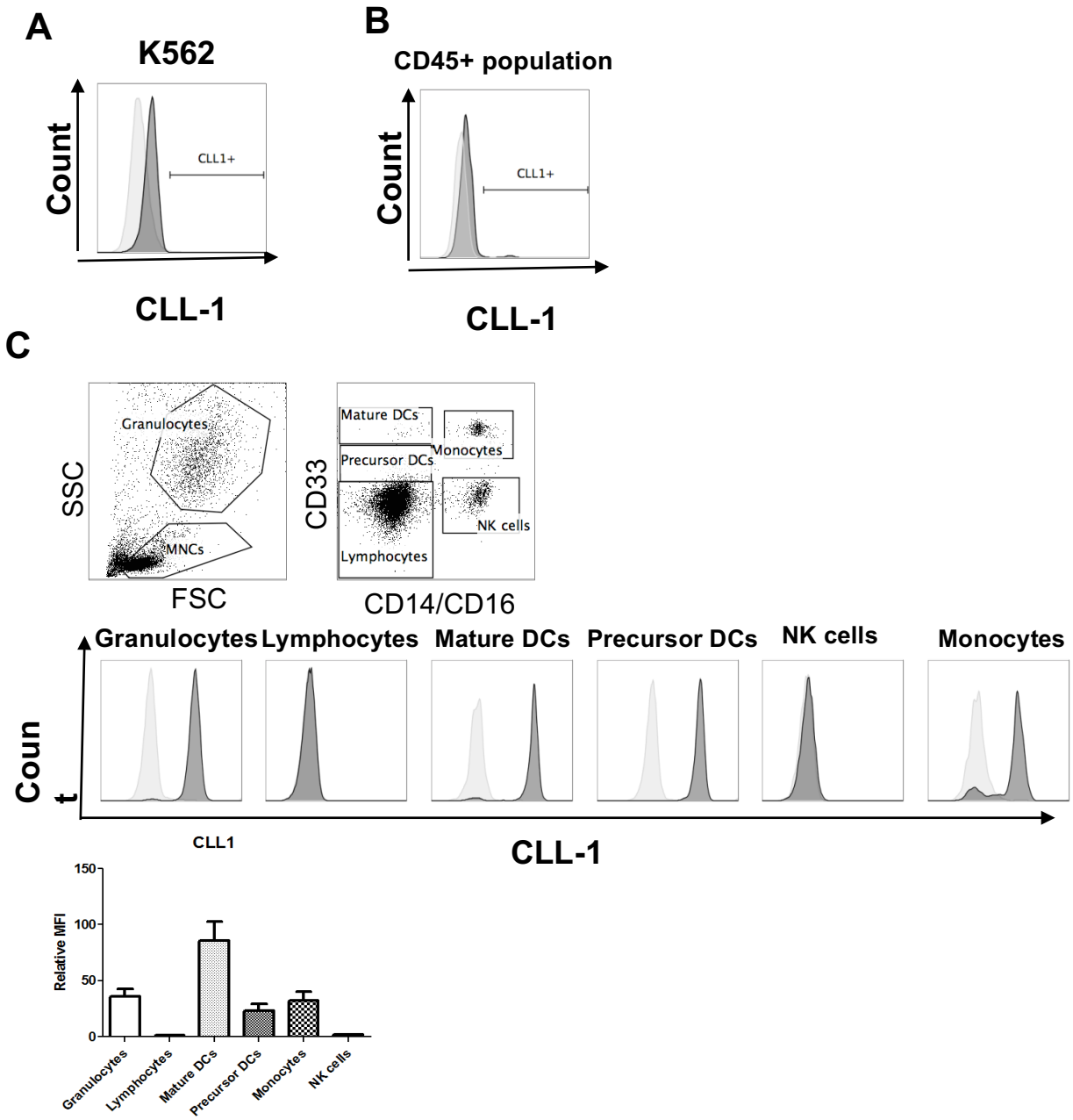
Supplementary Figure 1

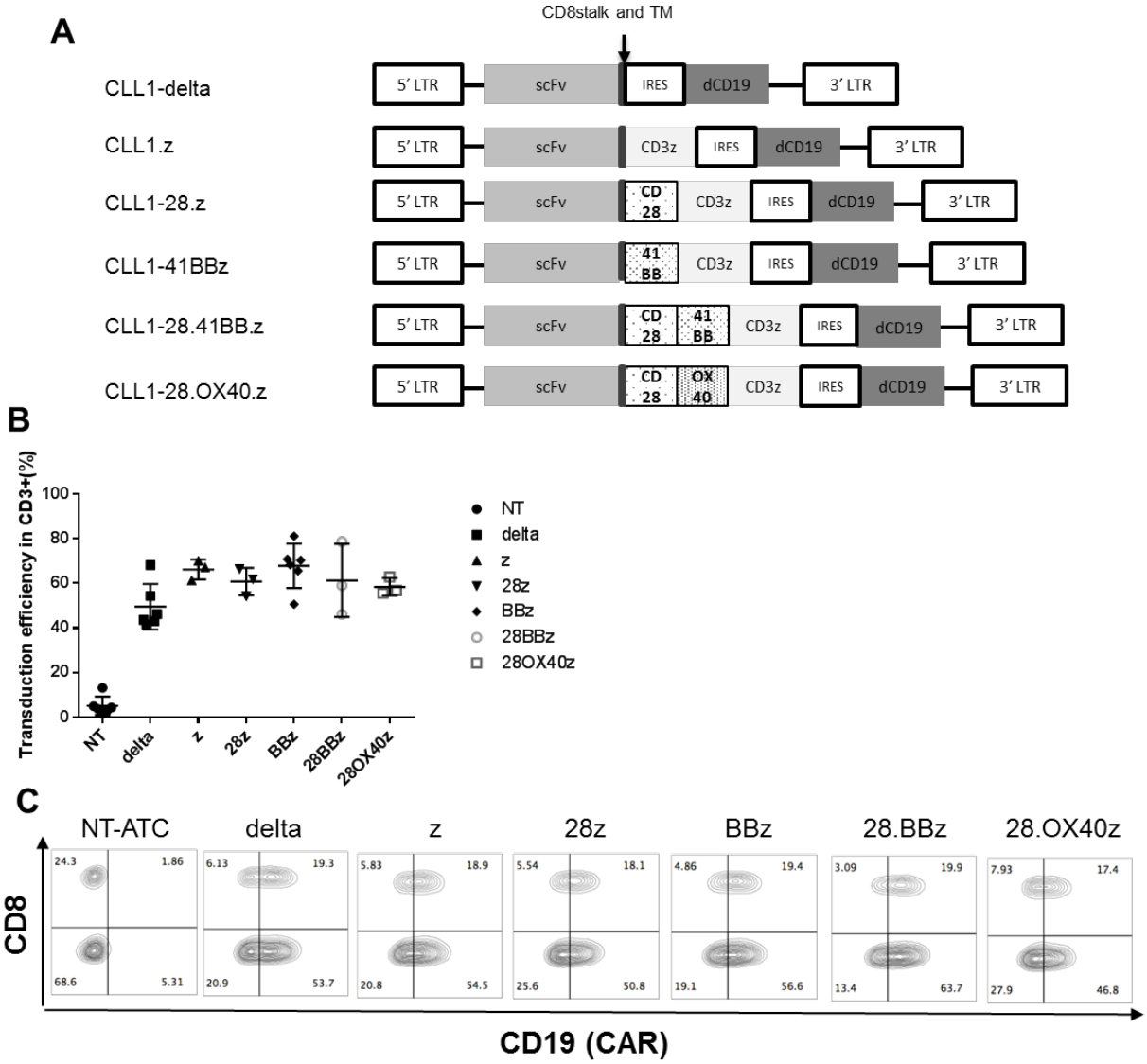


Supplementary Figure 1: CLL-1 expression on K562, CD45+ primary AML patient PBMC, and peripheral blood of healthy donors.

(A) Expression of CLL-1 on K562. (Light gray-isotype, dark gray-CLL-1) (B) Representative examples of CLL-1 expression on CD45+ population of primary AML patient PBMCs. Cells were gated on CD45+ and assessed CLL-1 expression (Light gray-isotype, dark gray-CLL-1). (C) Representative data of CLL-1 expression of peripheral blood from healthy donor. After lysis RBC, whole blood was stained with indicated antibodies. Leukocytes were divided into subsets based on their forward and side scatter. We then used this gating to measure CD33, CD14 and CD16. CLL-1 expression in each subset (Light gray

denotes isotype, dark gray denotes CLL-1) Summary of CLL-1 expression of peripheral blood from 6 healthy donors is shown in the bottom graph.

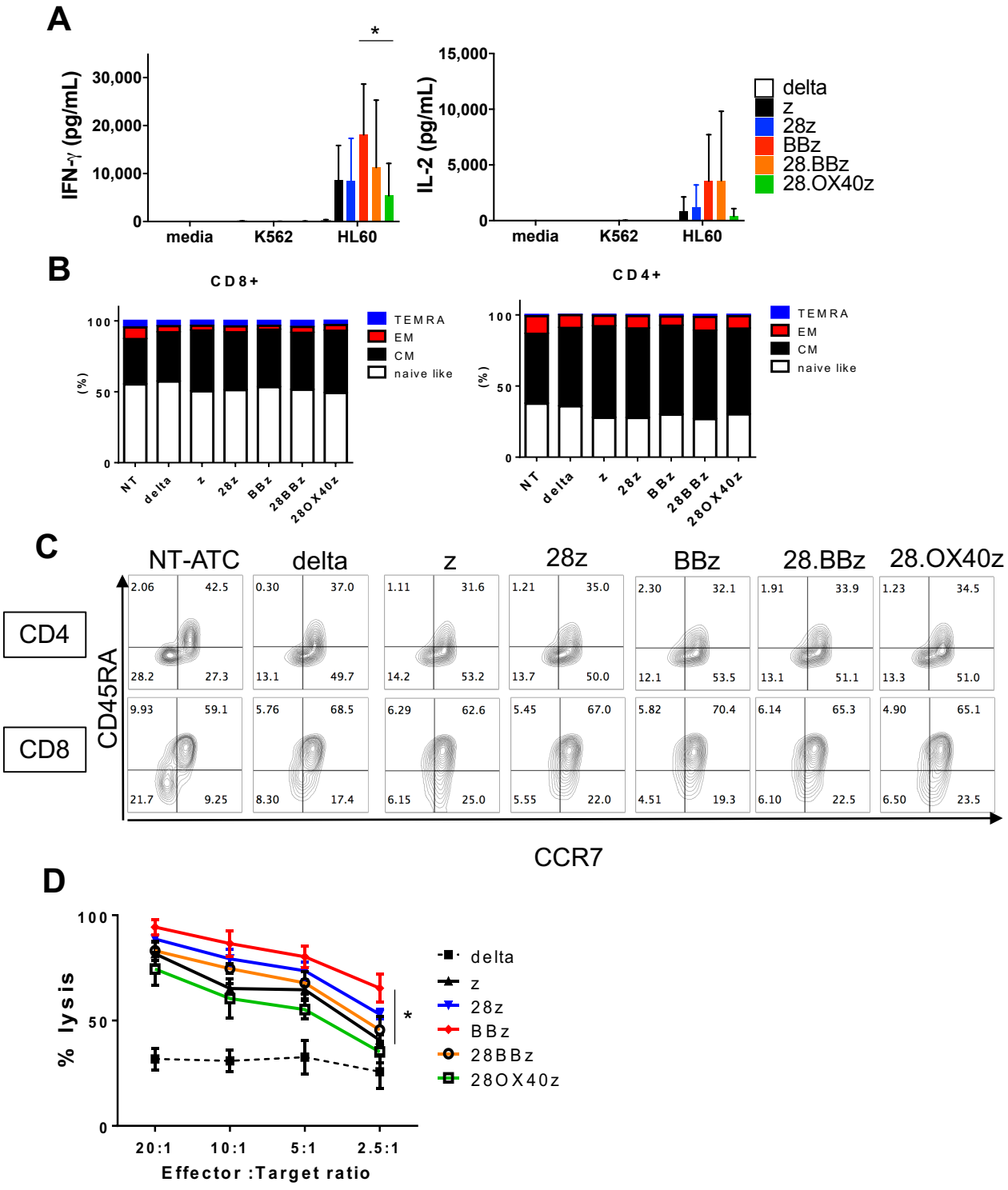
Supplementary Figure 2



Supplementary Figure 2: CLL-1 CAR constructions and expression in primary human T cells.

(A) Schematic of retroviral CAR expression vectors containing the anti-human CLL-1 scFv linked to CD8 stalk, transmembrane domain, and intracellular domains from CD3ζ alone (CLL-1.z) or with CD28 (CLL-1.28z), 4-1BB (CLL-1.BBz), CD28.4-1BB (CLL-1.28.BBz), or CD28.OX40 (CLL-1.28.OX40z). All constructs encode CD19 separated by internal ribosome entry site (IRES). Truncated version (without intracellular domains) was also created as a control (CLL-1.delta). (B) The summary of CAR expression in activated T cells confirmed by CD19 (n=3-6). (C) Representative CAR expression in activated T cells confirmed by CD19 expression.

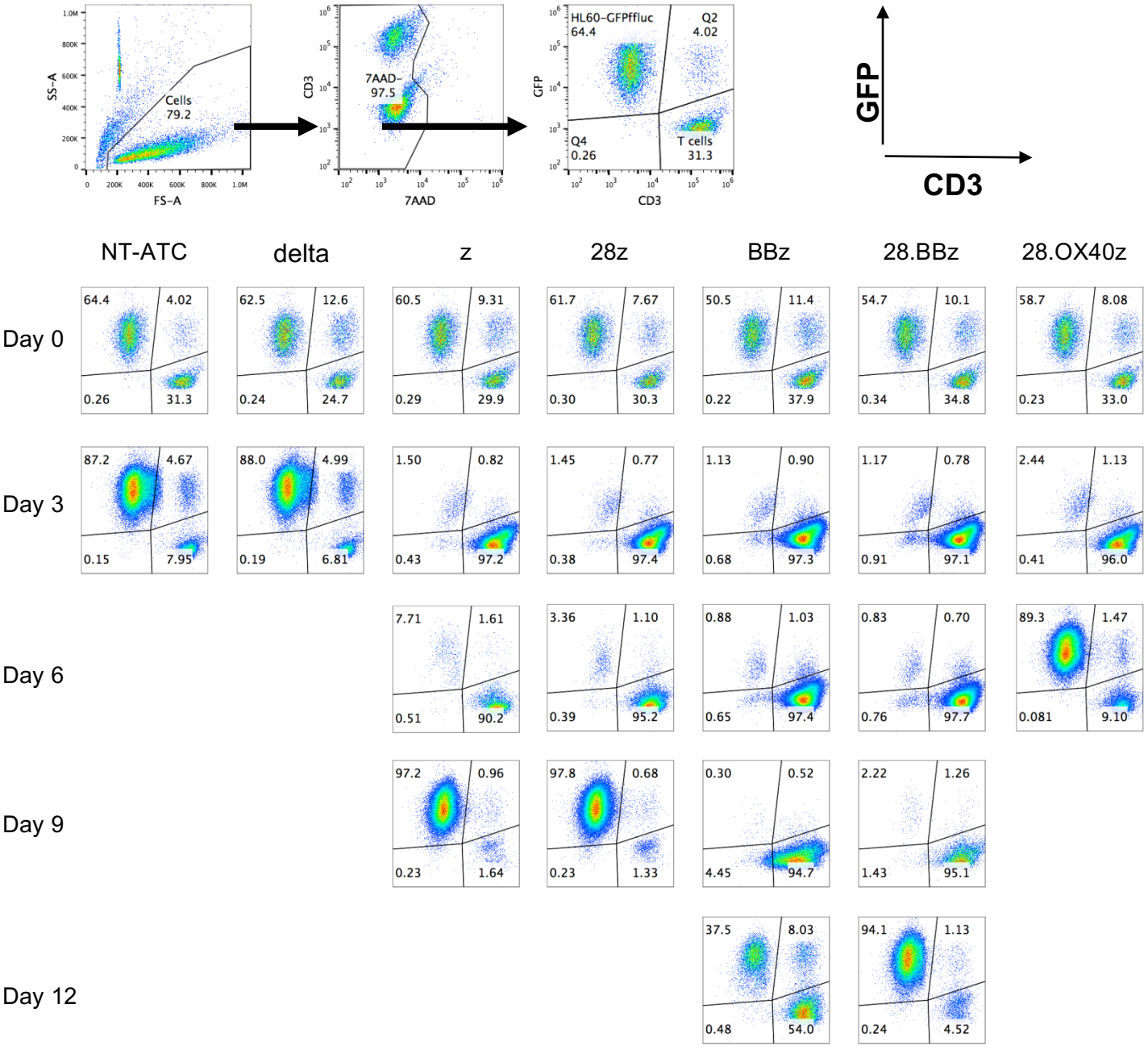
Supplementary Figure 3



Supplementary Figure 3: The functional and phenotypic comparison of different endodomain CLL-1 CAR.

(A) CLL-1.CAR-T were incubated with HL60 and K562 at E:T 1: 1 ratio or without any target cells for 24h. Supernatant was harvested and IFN-Ɣ and IL-2 measured by ELISA. Data denote mean ± SD from 3 donors and their replicated experiments. (B) Memory phenotype of CLL-1.CAR-T was analyzed by CD45RA and CCR7 expression on day 7 after transduction. Cells were gated on CD4+ or CD8+ CD3+CAR+. Cells were broadly divided into “Naïve” (CD45RA+CCR7+), “central memory” (CD45RA-CCR7+), “effector memory” (CD45RA-CCR7-) and “terminally differentiated” CD45RA+CCR7-, subsets. (C) One representative dot plot of 3 donors data are shown. (D) GFPffluc expressing HL60 were co-cultured with CLL-1.CAR-T for 5 hours at the indicated effector: target ratios. CLL-1.∆-T were used as a control (n=3).

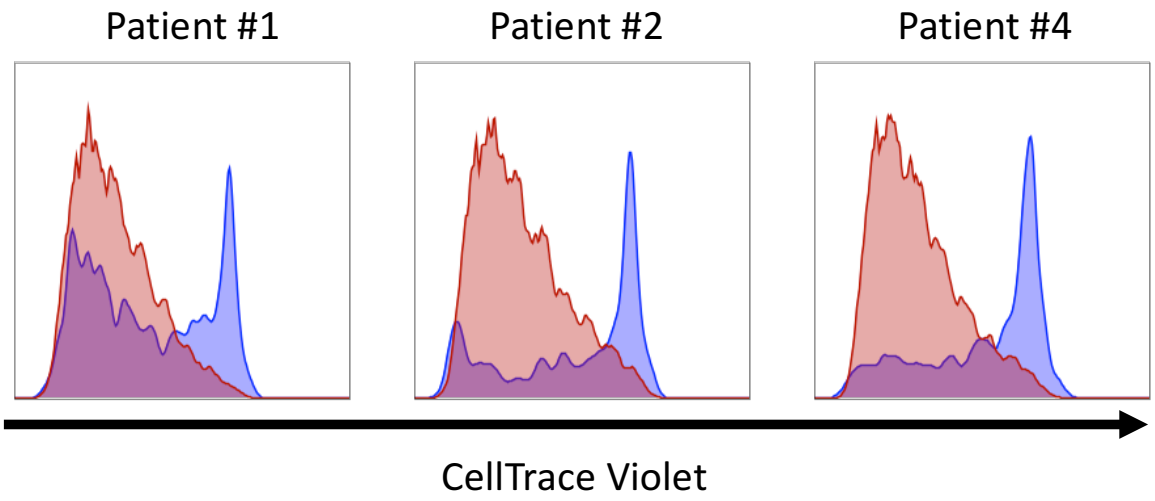
Supplementary Figure 4



Supplementary Figure 4: Representative serial coculture assay of CLL-1.CAR-T against HL60 GFPffluc

Cells were plated at E:T ratio of 1:2. CD3 T cells were collected and counted by flow cytometry using CountBright beads every three days. T cells were then replated and reconstituted with a fresh HL60-GFPffluc at the same E:T ratio. Co-cultures were carried on until tumor cells outgrew.

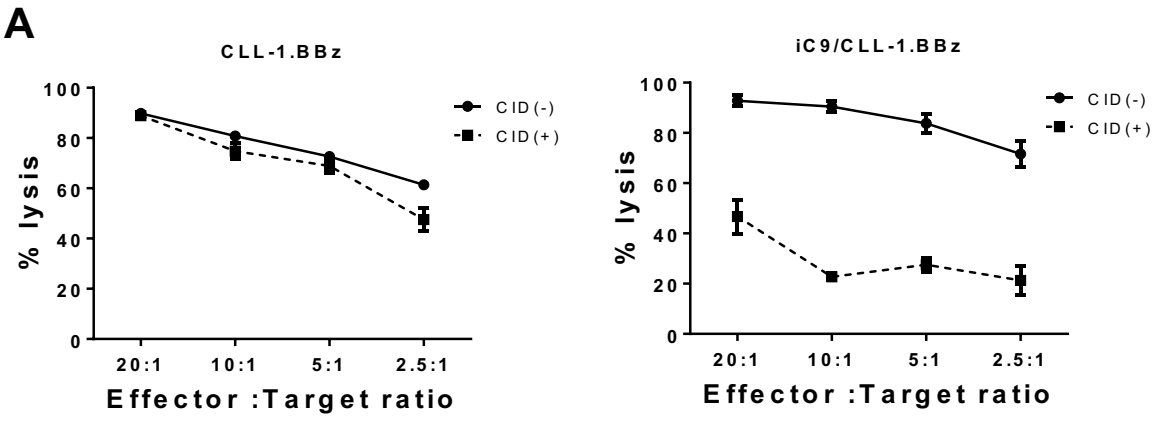
Supplementary Figure 5

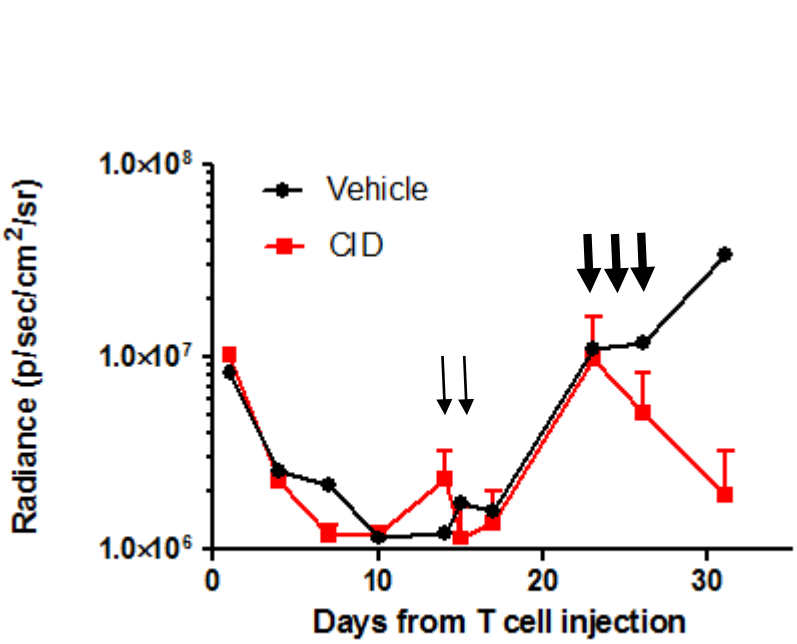


Supplementary Figure 5: CLL-1.BBζ CAR-T proliferate in response to primary AML blasts.

CLL-1.BBz or CLL-1.Δ were labeled with CellTrace Violet at 5uM and incubated with primary AML blasts at 1:1 ratio. After 5 days of incubation, CAR+ cells that had divided were detected by dilution of CellTrace Violet. Red, CLL-1.BBz; Blue, CLL-1.Δ.

Supplementary Figure 6





Supplementary Figure 6: CLL-1.BBζ CAR-T expressing inducible caspase-9 gene retain their anti-leukemic activity and are eliminated by a chemical inducer of dimerization.

(A) Luciferase based cytotoxicity assay against HL60-GFPffluc. CLL-1.BBζ (left) and iC9/CLL-1.BBζ (right) were incubated without CID (solid line) or with 10nM CID for 2 hours (dot line) then target cells were added. After 5 hours incubation at the indicated E;T ratios, cytotoxicity was assessed by the loss of the target cell luminescence. (B) NSG mice were injected with 50,000 of WT-HL60 on day 1 after irradiation at 200cGy. Then day 7 after tumor injection, mice were treated with $2x10e^6$ iC9-CLL1.BBζ labeled with GFPffluc . Day 14 and 16, 3 mice were treated with small dose of CID (thin arrow). Day 23, 25, and 27 after T cell injection, CID treated mice were given 50ug of CID (bold arrow). The mean radiance of CID treated mice is shown in red line and vehicle is shown in black line (n=2).

Supplementary Information

Generation and evaluation of CLL-1 CAR-T cells

Tested CAR constructs (i.e CLL-1.ζ, CLL-1.28ζ, CLL-1.BBζ, CLL-1.28.BBζ, CLL-1.28.OX40ζ, CLL-1.Δ) are shown in Supplementary Figure 2A. Each construct was fused to an IRES and truncated ΔCD19 for detection of transduced cells. To determine the functionally optimal construct, we compared the in vitro proliferation, cytokine production, and cytolytic ability of all 5 CLL-1.CAR-T. After OKT3 and CD28 antibody stimulation of unselected PBMCs from healthy donors (n=6 for CLL-1.Δ and CLL-1.BBζ, n=3 for all other constructs), we retrovirally transduced the cells with each CAR and evaluated expression of ΔCD19 on the T cells by flow cytometry. There was no significant difference in transduction efficiency between constructs (Supplementary Figure 2B). Both CD3+CD4+ and CD3+CD8+ T-cell populations were transduced equally (Supplementary Figure 2C). All CLL-1.CAR-T expanded similarly to non-transduced activated T cells (NT-ATC) (data not shown). To assess antigen specific cytokine production, we co-cultured all CLL-1.CAR-T with/or without stimulation by CLL-1 expressing HL60 or CLL-1 negative K562 and compared IFN-γ and IL-2 production by ELISA. CLL-1.BBζ showed the greatest IFN-γ production in response to CLL-1+ HL60 with a significant difference to 28.OX40ζ (Supplementary Figure 3A). By contrast, there was no secretion in response to CLL-1 negative K562 or other control cells. Although we also measured IL-2, the production of this cytokine was similar regardless of the co-stimulatory domain in the construct (Supplementary Figure 3A). We saw no construct-dependent differences in the memory phenotype (CD45RA and CCR7) on day 7 post transduction (Supplementary Figure 3B and C).

We used luciferase-based cytotoxic assays against HL-60-GFP-ffluc to compare the cytolytic ability of all CLL-1 CAR-T cells. Compared to CLL-1.Δ as a control, all CLL-1.CARs demonstrated significantly greater cytotoxicity against HL-60GFP-ffluc (Supplemental Figure 3D). There was no significant difference between the constructs with the exception of between CLL-1.BBζ and CLL-1.28.OX40ζ in serial co-culture killing assays in which fresh HL60-GFPffluc cells were added to T cells every 3 days. In these studies, CLL-1.BBζ showed the greatest specific cytokine release and the most sustained cytolytic activity and was therefore used in all further studies.