Supplementary Data

Supplementary Table 1.

CLL PBMC #	CD19+	CD37+	CD5+	CD20+	CD79b+
2	95.6	98.9	73.3	87.6	13.3
3	90.7	99.7	94.4	91.3	11.4
4	79.2	99.9	44.7	96.8	59.7
5	94.2	99.8	84.5	87.1	6.09
6	67.9	99.3	49.8	63.7	10.5
7	88.4	97.6	15.3	91.2	11.5
8	82.7	99.6	93.2	95.2	27.8
9	90.7	99.4	2.22	97.3	10.5
10	78.6	94.9	85.6	18	10.6
11	95.2	97.6	96.3	95.2	11.7
12	90.2	99.6	73.6	85.4	31.4
13	92.6	99.7	92.5	81.8	29.1
14	62.3	99.1	54.6	94.8	41.7
15	91.8	99.2	93.2	98.8	39.6
16	66.4	99.5	38.3	75.3	18.1
17	96.3	99.7	95.5	96.6	42.9
18	95.6	97.9	80.9	97.6	65.8
19	92.9	97.8	93.8	65	11.7
20	83.4	95.4	84.6	92.3	12.6
21	96.6	99.1	89.7	90.5	7.14

Supplementary Table 2.

Specimen Type	Clinical Findings From Path Report	CD37 Expression	Immunophenotype	Other Notes
PBL	**B-Cell Lymphoma**: Splenic Marginal Zone Lymphoma	+	**B cells**: CD19+ CD20+ CD5-/dim CD10- CD23- CD38- CD200dim CD25- CD11c-/+ CD103- with monotypic moderate surface lambda immunoglobulin light chain expression	Asymptomatic, non-bulky disease with a modestly enlarged spleen (17.5cm), referred for evaluation in the context of leukocytosis up to 29.97 K/uL at time of testing. No prior lines of therapy.
BMA	**Myelofibrosis**: primary myelofibrosis vs post-essential thrombocythemia myelofibrosis with CALR and ASXL1 mutations	-	**Myeloid blasts**: CD33+ CD13- MPO- CD117+ CD34+ HLA-DR+	*Normal B cells +, patient actively on JAK2 inhibitor at time of testing.
BMA	**B-Cell Lymphoma**: CLL with 17P deletion	+	**B-Cells**: CD19+ CD20-/+ CD5+ CD10- CD23+/- CD38- CD200+ with monotypic dim surface lambda immunoglobulin light chain expression	Previously treated with ibrutinib and allogeneic stem cell transplant with residual disease of 30-40% at time of testing (day +82 post transplant).
BMA	**AML arising from MDS**: recurrence, SRSF2, DNMT3A, RUNX1, NOTCH1 mutated.	-	**Myeloid blasts**: CD33- CD13- MPO- CD117+/dim CD34+ HLA-DR+.	*Normal B cells +, patient actively on azacitdine and venetoclax at time of testing.
PBL	**B-Cell Lymphocytosis**: CLL/SLL with trisomy 12	+	**B-Cells**: CD19+ CD20+ CD5+ CD10- CD23+ CD38- CD200+) with monotypic moderate surfae immunoglobulin kappa light chain expression	No prior therapy, on observation.
PBL	**B-Cell lymphoma**: Mantle cell lymphoma.	+	**1. 40% B-Cells**: CD19+ CD20+ CD5dim/- CD10- CD23- CD38- CD200dim/- with monotypic strong surface kappa immunoglobulin light chain expression **2. 9% B-Cells**: CD19+ CD20+ CD5- CD10+ CD23dim CD38- CD200+ TdT- with no detectible surface or cytoplasmic light chain expression	No prior therapy, on observation.
PBL	**B-Cell Lymphoma**: Splenic Marginal Zone Lymphoma	+	**B-Cells**: CD19+ CD20+ CD5- CD10- CD23- CD38, CD200- CD25- CD11b CD11c+/-with monotypic moderate surface lambda immunoglobulin light chain expression	Patient previously treated with rituxan monotherapy.
PBL	**B-Cell Lymphoma**: CLL with trisomy 12	+	**B-Cells**: CD19+, CD20+, CD5+, CD10-, CD23-/+, CD38-, CD200+ with monotypic moderate surface kappa immunoglobulin light chain expression	No prior therapy, on observation.
BMA	**AML**: In remssion but now has multilineage displasia with increased blasts.	-	**Myeloid blasts**: CD33+ CD13+/- MPO - CD117+/- CD34+ HLA-DR+ CD7 -	*Normal B cells +
BMA	**AML**: therapy- related	-	**Myeloid Blasts**: CD33+ CD13- MPO- CD117+ CD34+ HLA-DR+ CD56dim CD2- CD7- CD4-	*Normal B cells +
PBL	**B-ALL:** JAK2 fusion	-	**Lymphoid Blasts**: CD19+ CD20+/- CD5- CD10+ CD45 dim CD10+ CD34+ CD33- TdT+ CD38+ without surface light chain expression	*Normal B cells +, no prior therapy, newly diagnosed
BMA	**APL in remission**: t(15;17) confirmed, also WT1 and FLT3-ITD	-	**Hematogones**: CD19+ CD10+ CD20variable+	*Normal B cells +, sample following ATRA/Arsenic therapy, PML/RARA undetectable by PCR
BMA	**B-Cell Lymphoma**: HCL, BRAF V600E and SF3B1 mutated	+	**B-Cells**: CD20+ CD19+ CD5- CD23- clonal population with co-expression of CD25 (dim), CD103 and CD11c	No prior therapy.
L AXILLARY LN	**B-Cell Lymphoma:** MCL	+	**B-Cells**: CD19+ CD20+ CD5+ CD10- CD23- CD38+ CD200- with monotypic strong surface kappa immunoglobulin light chain expression	No prior therapy.
AXILLARY LN	**B-Cell Lymphoma:** MZL	+	**B cells**: CD19+ CD20+ CD5-/+ CD10- CD23- CD38- CD200+ with monotypic marked excess of surface lambda immunoglobulin light chain compared to kappa.	Previously treated with R-bendamustine and R-CHOP.
L NECK LN FNA	B-cell lymphoma with plasmacytic differentiation (IgM kappa+).	+	**B cells**: CD19+ CD20+ CD5- CD10- CD23-/+ CD38- CD200 dim with monotypic moderate surface kappa immunoglobulin light chain expression	
BMA	**AML:** recurrence, TET2, STAG2, ASXL1 and CEBPa mutated	-	**Myeloid blasts**: CD33+ CD13+/- MPO+ CD117+ CD34+ HLA-DR+ CD56dim CD7+	*Normal B cells +
PBL	**B-Cell Lymphoma:** MCL	+	**B cells**: CD19+ CD20+ CD5+ CD10- CD23- CD38- CD200-) with monotypic moderate to strong surface cytoplasmic kappa immunoglobulin light chain expression. CD5+ but with associated t(11;14).	Patient previously treated with R-CVP.
PBL	**B-Cell Lymphoma:** CLL	+	**B-Cells**: CD19+ CD20- CD5+ CD10- CD23+ CD38- CD200+ with monotypic dim lambda immunoglobulin light chain expression	No prior therapy, on observation.
L PELVIC MASS BX	**B-Cell Lymphoma:** DLBCL	+	**B cells:** CD19+ CD20+ CD5- CD10- CD23- CD38- CD200- CD103- CD25- CD11c- with monotypic strong surface lambda immunoglobulin light chain expression.	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
PBL	Normal	+	**B-Cells:** CD19+ CD20+	*Normal B cells +
BMA	**AML:** Normal cytogenetics, no associated mutations.	-	**Myeloid Blasts:** CD33+ CD13+ MPO-/dim CD117+ CD34+ HLA-DR+	*Normal B cells +, noprior therapy.

Supplementary Table 3.

PTCL PDX	CLASSIFICATION	CD37
WCTL-81162-Q13	Anaplastic large cell lymphoma, ALK positive	-
DFTL-78024-V4	Angioimmunoblastic T-cell lymphoma	+
DFTL-28776-V1	T-cell prolymphocytic leukemia	+
DFTL-22685-V4	Primary cutaneous CD30+ T-cell lymphoproliferative disorder	+
DFTL-47880-V1	Angioimmunoblastic T-cell lymphoma	+
CBTL-81777-V2	Hepatosplenic T-cell lymphoma	+
DFTL-85005-V4	Extranodal NK/T-cell lymphoma	-

Supplementary Materials and methods

Primary human T cells culture

For primary T-lymphocyte expansions, bulk human T-cells were activated (day 0) using anti-CD3/CD28 Dynabeads (LifeTechnologies), followed by transduction with a lentiviral vector encoding the CAR 24-hours later. T cells were cultured in media supplemented with 20 IU/ml rhIL-2 beginning on day 0 of culture and were maintained at a constant cell concentration of 0.5 $x10^6$/mL by counting every 2-3 days. For functional assays, CAR T cells were cryopreserved at day 8-10 of culture, and upon thawing, were immediately stimulated with antigen or injected into mice.

Cell lines and culture conditions

The JEKO-1, RAJI and wild-type parental K562 cells were purchased from American Type Culture Collection (ATCC). K562 cells were engineered to express CD37 and CD19 (K562-CD37-CD19). For some assays, cell lines were engineered to constitutively express click beetle green (CBG) luciferase/enhanced GFP (eGFP) and then sorted on a FACSAria (BD) to obtain a ≥99% pure population (CBG-GFP+). The cell lines were cultured in RPMI media containing 10% fetal bovine serum (FBS), penicillin, and streptomycin.

Flow cytometry

The following antibodies were used: CD37-APC (clone MB-1, eBioscience), CD37-BV711 (clone MB-371, BD), CD19-Pacific Blue (clone HIB19, Biolegend), CD19-FITC (clone 4G7, BD), CD5-BUV737 (clone UCHT2, BD), CD20-APC Cy7 (clone 2H7, Biolegend), CD79b-PE (clone CB3-1, eBioscience), CD3-BV786 (clone SK7, BD), CD3-BV605 (clone OKT3, Biolegend), CD45-PeCy7 (clone HI30, Biolegend), CD16-PE (clone B73.1, Biolegend), CD14-Pacific Blue (clone HCD14, Biolegend), CD56-APC (clone HCD56, Biolegend), CD33-BV510 (clone P67.6, Biolegend), CD107a-AF700 (clone H4A3, BD), CD69-APC (clone

FN50, Biolegend), IFNγ-FITC (clone GZ-4, eBioscience). Cells were stained for 30 min in the dark at 4°C and washed twice in PBS with 2% FBS. DAPI was added to gate on viable cells before acquisition. Antigen density was measured using Antibodies bound per cell (ABC) and was calculated using Quantum™ Simply Cellular (Bangs Laboratories).

Fratricide assay

Human T cells purified from anonymous human healthy donor leukopaks were activated with Cell Stimulation Cocktail (eBioscience™, Catalog#00-4970-03) for 6 hours. Activated and non-activated T cells were labelled with CFSE (ThermoFisher, Catalog#C34554) following manufacturer's instructions and co-cultured with CAR-37, CAR-19 or untransduced T cells generated form the same normal donors. After 24 hours flow cytometry was used to count the number of CFSE positive cells in each condition.

Immune cells isolation, differentiation and co-culture assay

PBMCs from three normal donors were isolated with Ficoll-Paque PLUS (GE Healthcare, C987R36) and monocytes were purified with StemCell kit (Catalog#19359). We generate M1/M2 macrophages *in vitro* as previously described [1]. Monocytes, macrophages, NK and T cells were cultured at 1:1 E:T ratio with CAR-37 CAR-19 or untransduced T cells for 6 hours and CD107a and IFNγ production was measured by flow cytometry. PMA/ionomycin was used as positive control. Values were normalized on media and graphs represent fold change.

Cellular cytotoxicity and cytokines assay

CAR-37 and CAR-19 T cells were normalized for CAR expression by adding untransduced but cultured and activated T cells from the same donor to achieve the same proportion of CAR+ T cells in each sample. For cytotoxicity assays, percent specific lysis was calculated by the following equation: %specific lysis = (total RLU / target cells only RLU) x100. For

cytokine assays, cell-free supernatants were analyzed for cytokine expression using a Luminex array (Luminex Corp, FLEXMAP 3D) according to the manufacturer's instructions. All samples were measured in technical duplicates. Duplicates were averaged before graphing with GraphPad Prism 7 (version 7.0). In addition, all assays were performed with biologic duplicates or triplicates or more, as indicated by the N in each experiment, which is based on the number of unique healthy donors T cells tested.

Jurkat reporter activation assay

Jurkat (NFAT-Luc) reporter cells (Signosis, SL-0032) were transduced with the different CAR constructs. We co-cultured them at 1:1 E:T ratio for 24 hours with B cell lymphoma tumuor cells or Nalm6 leukemia cells; anti-CD3/CD28 beads were used as positive control and media as negative control. Luciferase activity was measured after 16 hours with a Synergy Neo2 luminescence microplate reader (Biotek). Relative activation was calculated on PMA.

Immunohistochemistry

Paraffin sections were deparaffinized with xylene and then rehydrated with a series of ethanol washes followed by H2O. Antigen retrieval was conducted by microwaving slides for 15 min in 0.01M sodium citrate buffer, pH 6.0. After washing with phosphate-buffered saline containing 0.1 % Tween-20 (PBS-T), endogenous peroxidase activity was quenched with 3% H2O2 for 10 min. Slides were then washed again with PBS-T and blocked with Novolink Protein Block for 30 min at 25^{o} C. After additional washing with PBS-T, slides were incubated with PBS-T containing 5% goat serum and mouse anti-CD37 (Invitrogen catalog # MA5-15492) diluted 1:150 for 1 hr at 25^{o} C. Following washing with PBS-T, slides were incubated with Cell Signaling Technology Signal Stain Boost IHC murine detection reagent for 30 min

at 25^{o} C, washed again with PBS-T, and incubated with DAB diluent (Vector Labs) containing DAB chromogen. After stain development, slides were again washed with PBS-T and counterstained with hematoxylin.

TMA construction

Formalin-fixed paraffin embedded tissues involved by peripheral T cell lymphoma were retrieved from the archives of the Department of Pathology at Brigham and Women's Hospital. Cores (0.6 mm in diameter) from donor blocks were transferred to recipient blocks to create a tissue microarray in the Tissue Microarray Core of the Dana Farber/Harvard Cancer Center and used to prepare 4 micron sections for immunohistochemical staining studies.

In vivo studies

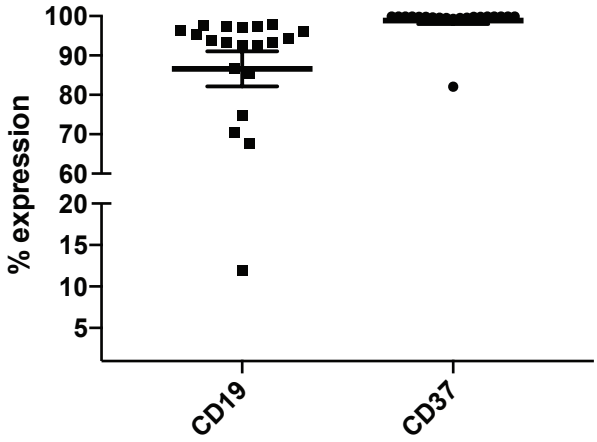
NOD-SCID-γ chain-/- (NSG) (Jackson Laboratories) were engrafted with JEKO-1 cell line or patient derived tumor cells via the route of administration described. Cryopreserved CAR-37, CAR-19, or untransduced T cells were injected intravenously after engraftment of tumor was confirmed by luminescence. Tumor burden was regularly monitored using an Ami spectral imaging apparatus and analyzed with IDL software v. 4.3.1 following an intraperitoneal injection of D-Luciferin substrate solution (30 mg/mL). Animals were euthanized as per the experimental protocol or when they met pre-specified endpoints defined by the IACUC.

Statistical analysis

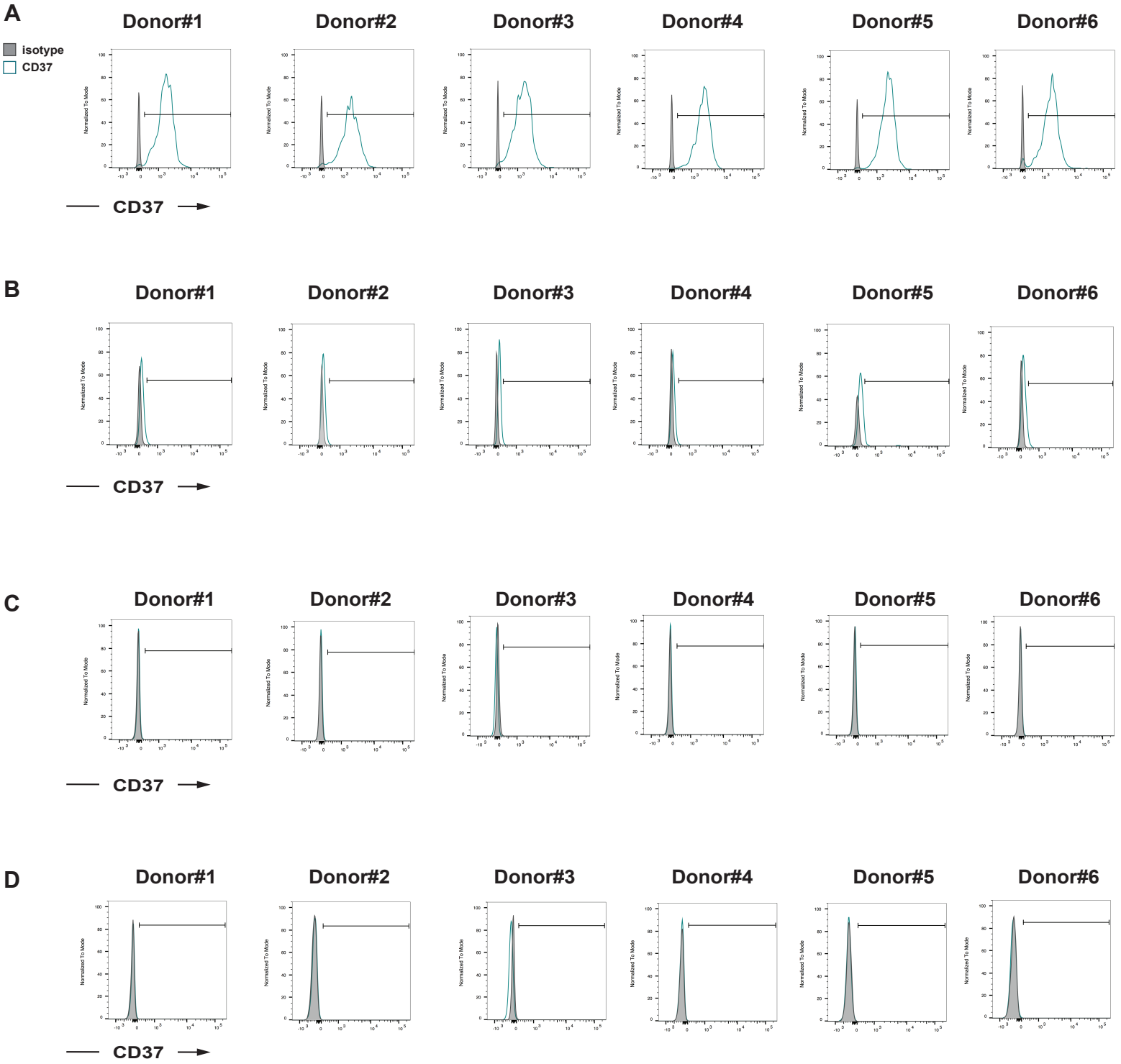
Unless otherwise stated, a 2-tailed Student t test or 2-way Anova test were used for normal data at equal variance. Significance was considered for $P< 0.05$. Analyses were performed with GraphPad Prism 7 (version 7.0).

References

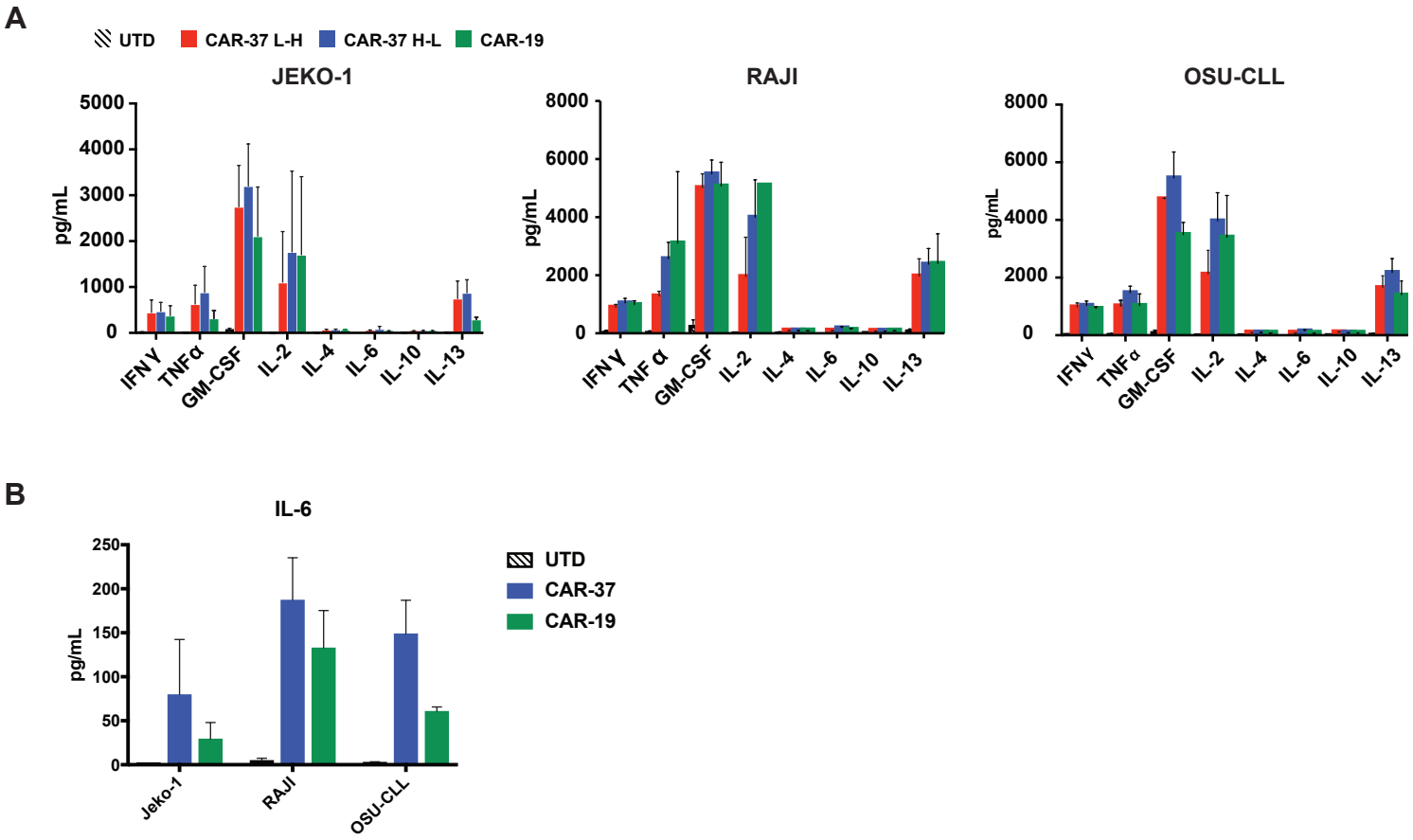
1. Zhang, M., et al., *Anti-CD47 Treatment Stimulates Phagocytosis of Glioblastoma by M1 and M2 Polarized Macrophages and Promotes M1 Polarized Macrophages In Vivo.* PLoS One, 2016. **11**(4): p. e0153550.



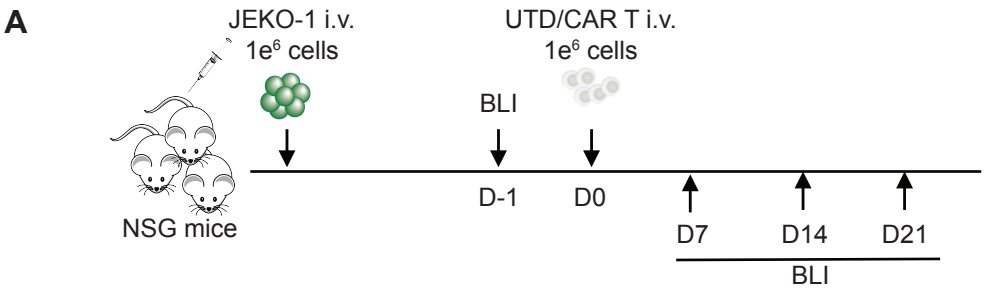
Supplementary Figure 1. CD19 and CD37 expression on CLL samples. Expression level of CD19 and CD37 on 21 patients with chronic lymphocytic leukemia by flow cytometry gated on the CD3-CD20+ B cells. Mean ±SD shown.

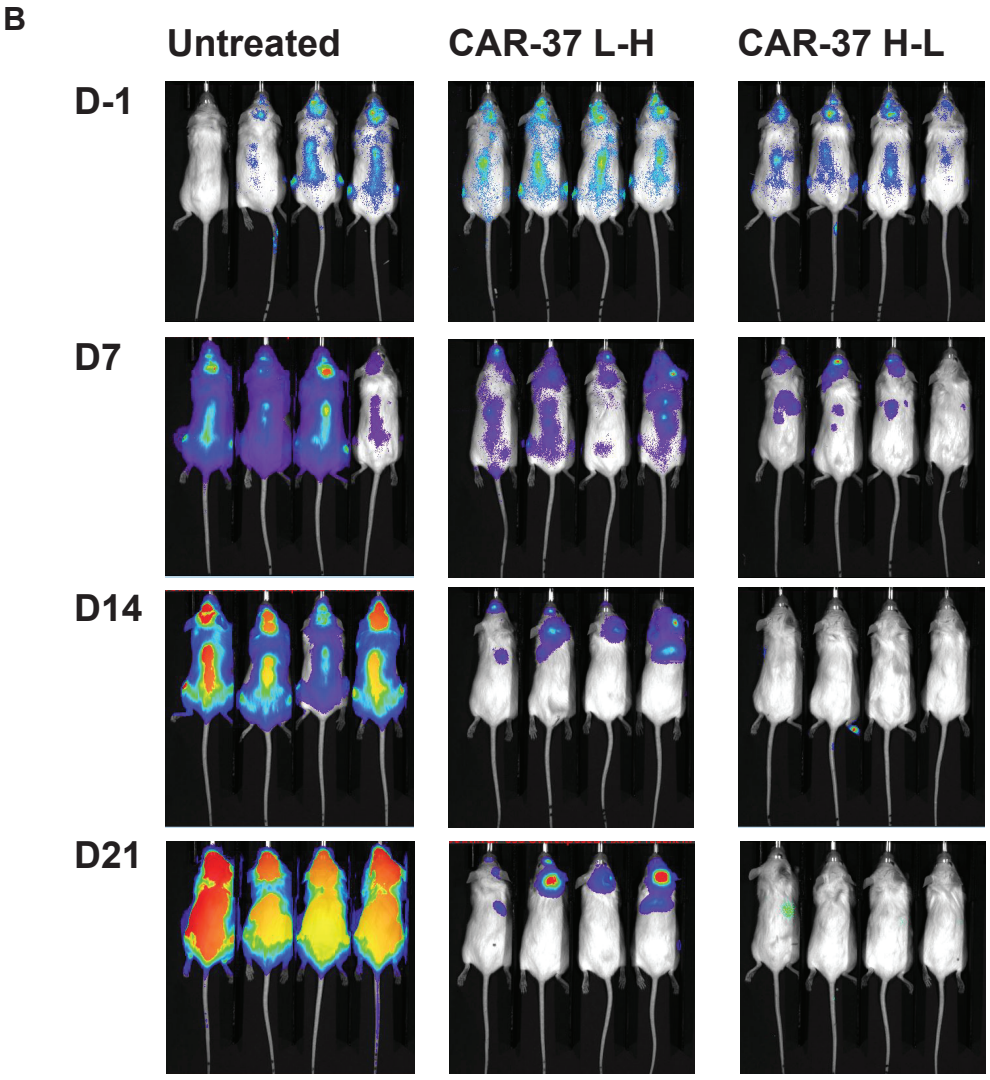


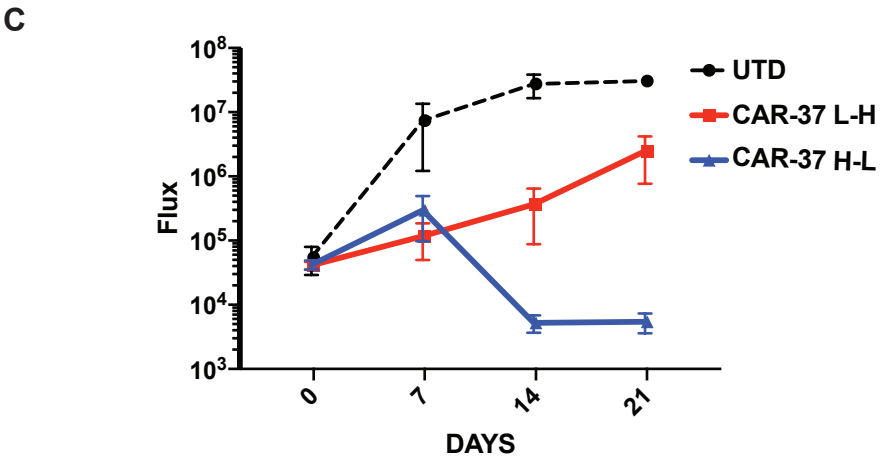
Supplementary Fig. 2. Whole blood from six normal donors was stained with CD45, CD3, CD19, CD16, CD14, CD56 and CD37 antibodies or isotype control. (A) Histograms represent CD37 expression on gated CD45+CD19+ B cells. (B) Histograms represent CD37 expression on gated CD45+CD16+CD14+ monocyte population. (C) Histograms represent CD37 expression on gated T cells (CD45+CD3+). (D) Histograms represent CD37 expression on gated CD45+CD16+CD56+ NK cells.



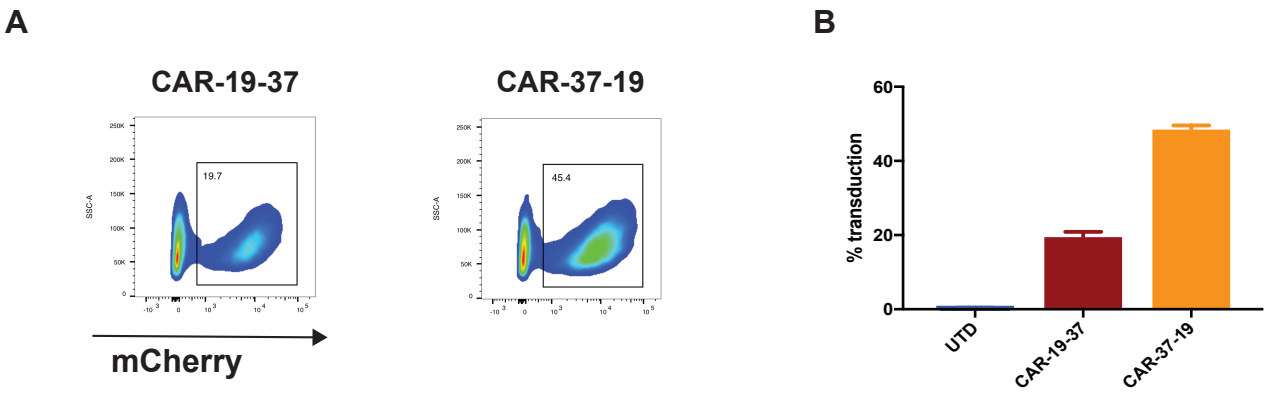
Supplementary Figure 3. (A) Cytokine production by CAR-37, CAR-19, or UTD T cells incubated with indicated tumor cell lines for 24 hours at 1:1 E:T ratio was analyzed in the culture supernatants by Luminex assay. Significant production of several cytokines is noted in CAR-37 and CAR-19 groups, but not in UTD. (B) IL-6 production by CAR-37, CAR-19, or UTD T cells incubated with indicated tumor cell lines for 24 hours at 1:1 E:T ratio was analyzed in the culture supernatants by Luminex assay. Three normal donors analyzed, mean±SEM is shown.







Supplementary Figure 4. Direct comparison of anti MCL-activity of CAR-37 T cells in MCL tumor model. (A) Experiment schematic: NSG mice were injected IV with 1x106 JEKO-1(CBG-GFP) cells and monitored by BLI for tumour burden at different time points. At day 0, mice were randomized based on tumor burden (BLI) to receive 1x106 control T cells (UTD), CAR-37 L-H or CAR-37 H-L. (B) Representative bioluminescent images of JEKO-1 growth over time. (C) Average radiance [p/s/cm2/sr] of whole mice in the 3 groups at different time points. Graph is representative of 1 experiment with 5 mice per group. Mean±SD shown.



Supplementary Figure 5. (A) Representative flow plots of primary human T cells transduction efficiency after 10 days of activation with CD3/CD28 beads. (B) Expanded T cells from 2 healthy donors included variable CAR expression with a mean of 19% (CAR-19-37) and 48% (CAR-37-19).