SUPPLEMENTARY INFORMATION APPENDIX

- **Title:** A versatile strategy for controlling the specificity and activity of engineered T cells.

- **Running Title:** Switch-Mediated Control of CAR-T cell Activity.

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  Author contributions: J.S.Y.M, Y.C., J.Y.K., T.M.W., P.G.S., T.S.Y., and C.H.K. designed research; J.S.Y.M, Y.C., J.Y.K., M.S.K., D.T.R., and O.S. performed research; S.A.K., S.H.C., H.Y.Y., H.P., and S.B.S generated FITC linkers and antibody-switch intermediates; B.R.F. contributed CESI mass spectrometry tool and analysis; J.N.K. provided cells and constructs; J.S.Y.M., P.G.S, and C.H.K. wrote the paper.

  The authors declare no conflict of interest.

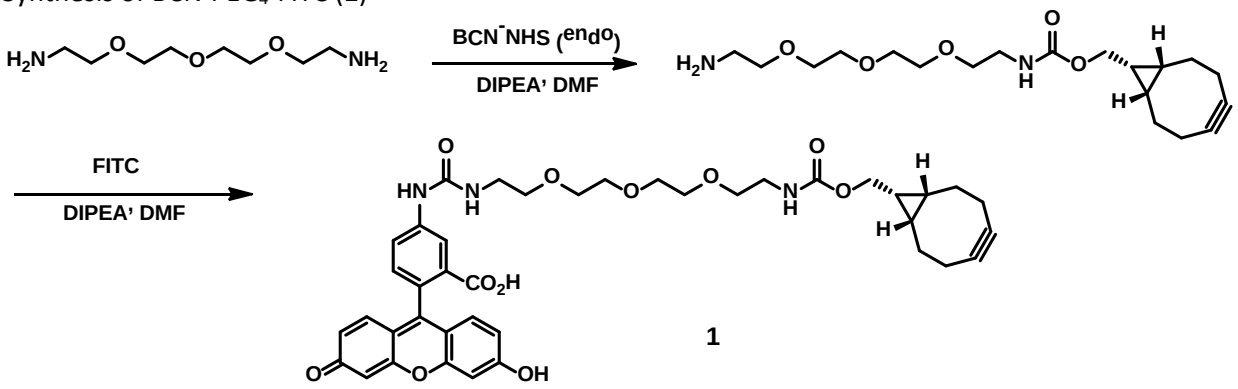
- **Keywords:**, Cancer immunotherapy, chimeric antigen receptor T cell, noncanonical amino acids, cytokine release syndrome, B-cell aplasia

Supplemental Methods

Synthesis

All chemicals were purchased from Sigma Aldrich. 1H- and 13C-NMR spectra were obtained on a Varian INOVA-399 (400 MHz) spectrophotometer. Chemical shift values were recorded as parts per million relative to tetramethylsilane as an internal standard. High resolution mass spectra were acquired at the Scripps Center for Mass Spectrometry (La Jolla, CA).

Synthesis of BCN-PEG_4-FITC (**1**)



A solution of (1R,8S,9s)-Bicyclo[6.1.0]non-4-yn-9-ylmethyl N-succinimidyl carbonate (130 mg, 0.446 mmol) in anhydrous DMF (3 mL) was added dropwise to a solution of 1,11-diamino-3,6,9-trioxaundecane (515 mg, 2.68 mmol) and *N,N*-diisopropylethylamine (0.39 mL, 2.23 mmol) in DMF (5 mL). After stirring for 15 min, the reaction mixture was concentrated *in vacuo*. The remaining residue was dissolved in dichloromethane (100 mL) and washed with 1N NaOH (10 mL × 2) and H_2O (10 mL) sequentially. The dichloromethane layer was dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*.

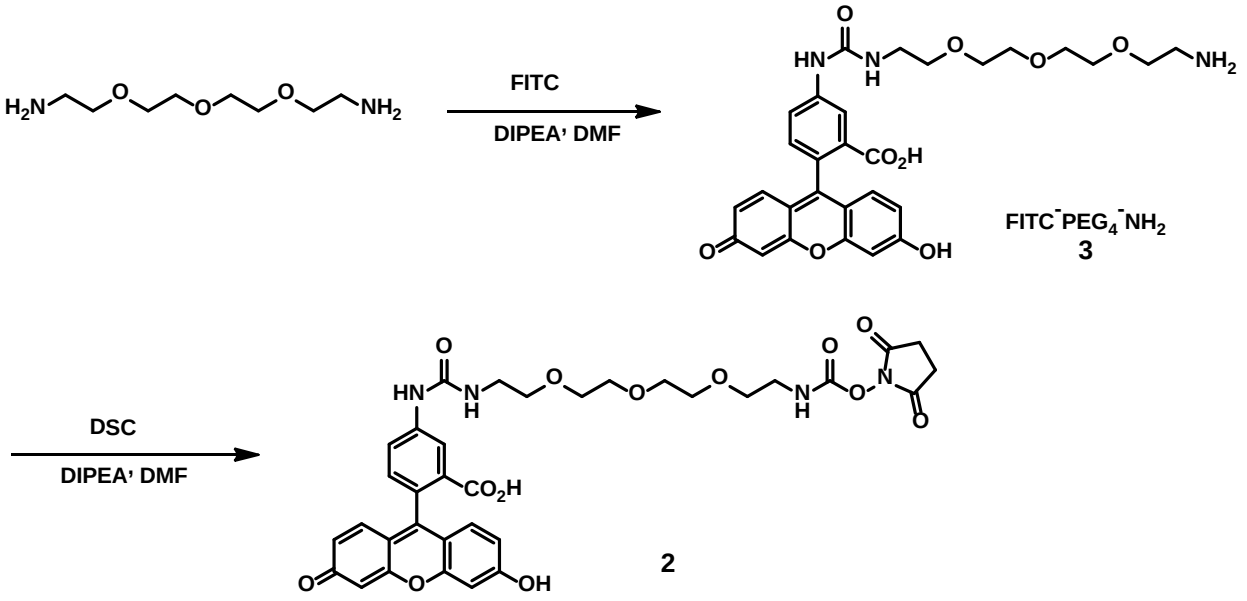
The crude residue was dissolved in DMF (5 mL), followed by the addition of *N,N*-diisopropylethylamine (0.23 mL, 1.32 mmol). A solution of FITC (171 mg, 0.40 mmol) in DMF (4 mL) was added dropwise to the mixture over 5 min. After 1 hr of stirring, the reaction mixture was concentrated *in vacuo* and purified by column chromatography to yield the desired product (215 mg, 0.284 mmol, 64% yield for 2 steps).

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 10.07 (s, br, 2H), 9.99 (s, br, 1H), 8.24 (s, 1H), 8.05 (s, br, 1H), 7.70 (d, 1H, *J* = 8 Hz), 7.14 (d, 1H, *J* = 8 Hz), 7.02 (s, br, 1H), 6.64 – 6.63 (m, 2H), 6.58 – 6.51 (m ,4H), 4.29 – 4.25 (m, 1H), 3.98 (d, 2H, *J* = 8 Hz), 3.68 – 3.63 (m, 2H), 3.58 – 3.55 (m, 2H), 3.49 – 3.45 (m, 4H), 3.36 – 3.29 (m, 4H), 3.17 – 3.14 (m, 1H), 3.09 – 3.05 (m, 2H), 2.21 – 2.08 (m, 5H), 1.70 – 1.68 (m, 1H), 1.48 – 1.46 (m, 2H), 1.22 -1.17 (m, 1H), 0.83 – 0.77 (m, 2H).

^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 181.23, 169.33, 160.33, 157.26, 152.71, 129.85, 113.41, 110.56, 103.06, 99.81, 70.57, 70.37, 69.97, 67.53, 62.20, 41.27, 29.81, 29.42, 26.01, 21.01, 20.38, 18.49.

HRMS Calcd. for $C_{40}H_{44}N_3O_{11}S$ ([M + H]$^+$): 742.2970. Found: 742.2971.

Synthesis of FITC-PEG$_4$-NHS (**2**)



A solution of FITC (400 mg, 0.925 mmol) in anhydrous DMF (20 mL) was added dropwise to a solution of 1,11-diamino-3,6,9-trioxaundecane (1.0 g, 5.20 mmol) and *N,N*-diisopropylethylamine (1.0 mL, 5.73 mmol) in DMF (15 mL). After stirring for 30 min, the reaction mixture was concentrated *in vacuo,* and purified by preparative HPLC to yield FITC-PEG$_4$-NH$_2$ as TFA salt (compound **3**, 574 mg, 0.826 mmol, 89%).

Compound **3** (TFA salt, 146 mg, 0.210 mmol) was dissolved in DMF (5 mL), followed by the addition of *N,N*-diisopropylethylamine (0.18 mL, 1.05 mmol). Next, *N,N'*-disuccinimidyl carbonate (81 mg, 0.315 mmol) was added to the reaction mixture. After stirring for 15 min, the reaction mixture was concentrated *in vacuo*, and purified by preparative HPLC to yield the desired product (**2**) (50.5 mg, 0.0699 mmol,33%).

^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm) 10.06 (s, br, 1H), 8.35 (s, br, 1H), 8.28 (s, 1H), 7.74 (d, 1H, J = 8 Hz), 7.18 (d, 1H, J = 8 Hz), 6.76 – 6.75 (m, 2H), 6.68 – 6.55 (m, 4H), 3.71 – 3.67 (m, 2H), 3.62 – 3.60 (m, 2H), 3.58 – 3.52 (m, 8H), 3.47 – 3.44 (m, 2H), 3.23 – 3.17 (m, 2H), 2.75 (s, 4H).

^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm) 182.32, 171.69, 169.33, 160.37, 152.71, 135.10, 129.86, 113.42, 110.55, 103.06, 70.60, 70.45, 70.40, 70.32, 70.20, 69.34, 41.84, 33.92, 26.09.

HRMS Calcd. for $C_{34}H_{35}N_4O_{13}S$ ($[M + H]^+$): 723.1967. Found: 723.1970.

Expression and generation of bifunctional FITC switch molecules

Anti-human CD19 Fab (clone FMC63), anti-human CD22 Fab (clone M971), and anti-mouse CD19 Fab (clone 1D3) sequences were cloned into pBAD vectors and site-specific mutations to introduce TAG amber nonsense codon were generated using Quikchange Site-directed Mutagenesis Kit (Stratagene). Antibodies were expressed in *Esherichia coli* (*E.coli*) with an orthogonal *Methanococcus jannaschii* tRNA/aminoacyl-tRNA synthetase specific for *p*-azido phenylalanine (pAzF) and purified as previously described (1). Purity and incorporation of UAA was confirmed by SDS-PAGE gel and mass spectrometry (QTOF).

Mutant antibodies containing pAzF (≤ 1 mg/mL) were conjugated with 30-fold molar excess of BCN-PEG_4-FITC (**1**) in phosphate-buffered saline (PBS) pH 7.4 and incubated overnight at 37˚C. The next day, completion of conjugation reaction was confirmed by QTOF, excess linkers were removed by size

filtration (Amicon, 10K and 30K), and the size and purity of the final products were confirmed by SDS-PAGE gel.

For random anti-CD19 FITC conjugates, wildtype anti-CD19 Fab were expressed in *E. coli* and purified as above. After size and purity were confirmed, antibodies were incubated with 48-fold molar excess of FITC-PEG_4-NHS in PBS at 37°C for 6 hours. Excess small molecules were removed by size filtration (Amicon, 10K and 30 K) and final product was analyzed on an Agilent Quadruple Time-of-Flight (QTOF) mass spectrometer and deconvoluted masses were obtained using Agilent Qualitative Analysis software. Random anti-CD19 FITC conjugates were also subject to CESI-MS analysis: Unmodified and random FITC labeled antibodies were prepared at 1 mg/mL using a 4-hour digestion protocol with RapiGest DTT, iodoacetamide, and trypsin, then diluted to 250 mg/mL in 125 mM ammonium acetate, pH 4. In parallel, intact antibodies were prepared at 1 mg/mL in 50 mM ammonium acetete, pH 4. CESI experiments were carried out on a SCIEX TripleTOF® 6600 system with a NanoSpray® III source and SCIEX CESI 8000 system. High resolution MS and MS/MS spectra were analyzed using SCIEX ProteinPilot™, PeakView®, and BioPharmaView™ softwares. Both MS strategies indicate that our random FITC conjugate has a drug-to-antibody ratio (DAR) of 2, where FITC conjugation was localized to the N-terminal glutamate of the heavy chain, and lysine residues at positions 31 and 145 within the light chain.

For *in vivo* studies, all anti-CD19 FITC switch molecules were filtered with Mustang Q membranes (Pall) and confirmed endotoxin levels were < 10 endotoxin units/mL using the Endosafe®-PTS system (Charles River) prior to injections.

Cell lines and culturing conditions

Leukemia and lymphoma cell lines (Nalm-6, Daudi, Raji, IM-9, and K562) were purchased from ATCC and maintained in RPMI 1640 media supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone) and 1 mM sodium pyruvate (Life Technologies). Virus producing cell lines, HEK293T and

Platinum E (Plat E, Cell Biolabs), were maintained in DMEM media with the following additions: 10% FBS, 2 mM Glutamax, MEM non-essential amino acids, and 1 mM sodium pyruvate. Human PBMC and transduced CAR-T cells were cultured in AIM V media (Life Technologies) with added 5% human AB serum (Valley Biomedical). Mouse splenocytes and transduced CAR-T cells were cultured in RPMI 1640 fully supplemented with 10% FBS (Gemini Bioproducts), 5 mM HEPES, 1.5 mM L-glutamine, 50 µM 2-mercaptoethanol, and 0.05 mg/mL Gentamicin (Life Technologies). All media contained 100 units/mL of penicillin and 100 µg/mL of streptomycin. Unless specified, all media and supplements were purchased from Life Technologies.

Binding Assays

Binding of anti-CD19 FITC conjugates was confirmed with Nalm-6 ($CD19^+$) and anti-FITC CAR-T cells by flow cytometry. Briefly, cells were incubated with indicated switch antibodies at 4˚C for 30-60 min and washed twice with staining buffer (1% BSA in PBS). Primary antibodies were revealed with Alexa Fluor®647 conjugated anti-human IgG or anti-human kappa secondary antibodies. After several washes, samples were acquired on a BD LSR II or BD Accuri C6 and analyzed using FlowJo 7.6.2 software. In each study, cells were incubated with secondary antibody alone and the observed mean fluorescence intensity (MFI) was used to subtract for background and non-specific staining.

Virus production and generation of CAR-T cells

A gene cassette containing the human anti-FITC scFv (2), CD8α hinge and transmembrane region, and the cytoplasmic domains of 41BB and CD3ζ was synthesize by Genescript and cloned into the LV-vector. Lentivirus production and transduction of human T cells were performed as previously described (3). Briefly, HEK293FT cells were transfected with anti-FITC CAR plasmid and viral packaging vectors and 48 hours later, supernatants containing lentivirus were harvested or frozen at -80°C until

ready for use. PBMCs were isolated from normal healthy blood (acquired from The Scripps Research Institute Normal Blood Donor Service) using Ficoll-Paque density gradient approach (GE healthcare), and activated with CD3/CD28 activation beads (Life Technologies) at 3:1 bead to cell ratio for 24 hours. Activated T cells were mixed with supernatant containing lentivirus in the presence of protamine sulfate (1 ug/ml), centrifuged at 1000 x g for 90 min, and incubated overnight at 37°C. The next day, viral supernatant were replaced with fresh media containing recombinant human IL-2 (300 IU/mL; R&D systems). Transduced T cells were maintained at 0.125-2 x 10^6 cells/mL in media containing IL-2, which was replenished every 2-3 days.

For mouse anti-FITC CAR-T cells, the anti-mouse CD19 (1D3) scFv within the MSGV1 1D3-28Z.1-3 plasmid (generously provided by Dr. James Kochenderfer, National Cancer Institute) was replaced with the human anti-FITC scFv. The mouse version of anti-FITC CAR consists of the human anti-FITC scFv, murine CD28 (excluding the N-terminus of the extracellular domain) and the cytoplasmic domain of murine CD3ζ (4). Retrovirus supernatants were produced using Plat E cells and used directly to transduce activated mouse splenocytes (C57BL/6) following a spinoculation protocol using retronectin (Takara) as previously described (5). Transduced mouse CAR-T cells were maintained at 0.5 x 10^6 cells/mL in media containing recombinant human IL-2 (60 IU/mL). The original anti-mouse CD19 CAR and an irrelevant CAR, which recognizes 2,4,6-trinitrophenyl, were transduced in parallel and used as positive and negative controls, respectively.

Transduction efficiency was verified by flow cytometry using Alexa Fluor®647 conjugated anti-mouse or anti-human IgG F(ab)'2 antibodies (Jackson ImmunoResearch). Anti-FITC CAR expression was also confirmed using a FITC-labeled mouse IgG1 isotype control antibody (Biolegend). Non-transduced T cells labeled with F(ab)'2 antibodies served as background controls.

Flow cytometry-based Cytotoxicity Assays

Target cells (1 x 10^4 cells), pre-labeled with CellVue® Claret Far Red Fluorescent Cell linker (Sigma), were co-cultured with CAR-T cells at indicated E:T (effector-to-target) ratios in 96-well round bottom plates supplemented with different concentrations of switch molecules, and incubated at 37°C. For competition assays, in addition to switch molecules, cultures also consisted of excess amounts of fluorescein (Sigma) or anti-CD19 antibody (or isotype control; Millipore). For CART-19 and anti-FITC CAR-T cell comparison, pre-labeled target cells were incubated with effector cells in the presence of 1nM anti-CD19 AB-FITC switch. After 24 hours, cells were labeled with 1:100 dilution of 7-AAD viability dye (BD Biosciences) in staining buffer, acquired on a BD Accuri C6 flow cytometer, and analyzed using FlowJo 7.6.2 software. Unstained and single color controls were acquired and compensation was done using FlowJo. The following formula was used to determine the percent cytotoxic activity: (values used represent percentage of Claret Red^+/$7AAD^-$ populations) % Cytotoxicity = 100 x [((Target cells + Effector cells only) – (Target cells + Effector cells + Switch))/(Target cells + Effector cells only)].

Colorimetric-based Cytotoxicity Assays

Co-cultures containing 1 x 10^4 target cells (murine CD19 overexpressing Myc5 cells, a kind gift from Dr. Andrei Thomas-Tikhonenko, University of Pennsylvania) and 1 x 10^5 anti-FITC CAR-T cells with different concentrations of anti-CD19 (1D3) FITC switch were incubated at 37˚C for 6 hours. Cytotoxicity was measured using the Cytotox-96 Nonradioactive cytotoxicity assay kit (Promega), which quantifies the amount of lactate dehydrogenase (LDH) released from lysed cells into the supernatant. The percent lytic activity was calculated with the following formula: (values used represent absorbance at 490nM) % Cytotoxicity = 100 x [((Target cells + Effector cells + Switch) - (Target cells + Effector cells only))/((Maximum target cell lysis) – (Target cells only))].

CD19 and CD22 expression quantification

Cell surface CD19 and CD22 expression levels were quantified on indicated leukemia and lymphoma cell lines using PE-conjugated anti-CD19 (HIB19, BD Biosciences) and anti-CD22 (4JB128, ebioscience). To quantify the number of antigens per cell, the observed MFI from test samples were compared with a standard curve established with BD Quanti-Brite PE beads (bead MFI vs. number of PE molecules per bead) according to product manual.

Enrichment of CAR-T cells

To enrich for anti-CD19 and anti-FITC CAR expressing T cells, cells were pre-incubated at 4˚C for 30 min with biotin-conjugated anti-mouse or anti-human IgG F(ab)'2 antibodies (Jackson Immunoresearch), respectively. Labeled cells were incubated with anti-Biotin microbeads (Milltenyi) and separated according to manufacturer's instructions. Purity of CAR-T cells were confirmed by flow cytometry and verified once a week using Alexa Fluor®647 conjugated anti-mouse IgG F(ab)'2 or anti-human IgG F(ab)'2 antibodies as described above. Enriched cells were used for experimentation after 1-2 passages, with each passage taking place every 2-3 days.

Activation and BcL-xL upregulation assay

Equal number (1×10^5) of target and enriched CAR-T cells were co-cultured in the presence of 1nM anti-CD19 AB-FITC switch in 96 well round bottom plates at 37°C for 24 hours. The next day, cultures were labeled with APC-conjugated anti-CD3 (OKT3), PerCP/Cy5.5-conjugated CD25 (BC96) and PE-conjugated CD69 (FN50) antibodies (all purchased from Biolegend). To evaluate the upregulation of BcL-xl, cells were surface stained with APC-conjugated anti-CD3 (OKT3), then fix and permeabilized using Cyotfix/Cytoperm kit (BD Biosciences) prior to intracellular staining with anti-Bcl-xl antibody (7B2.5,

Abcam). Appropriate isotype controls were included in each study to determine background and exclude non-specific staining. Unstained and single color controls were acquired and used for compensation.

Cytokine Release Assays

Cytokines in cultured media from activation studies were quantified using BD CBA Human Th1/Th2 Kit II (BD Biosciences) according to manufacturer's protocol.

Statistical Analysis

All graphs and statistics were generated using the Graphpad Prism 6.0 software.

Supplemental Figures

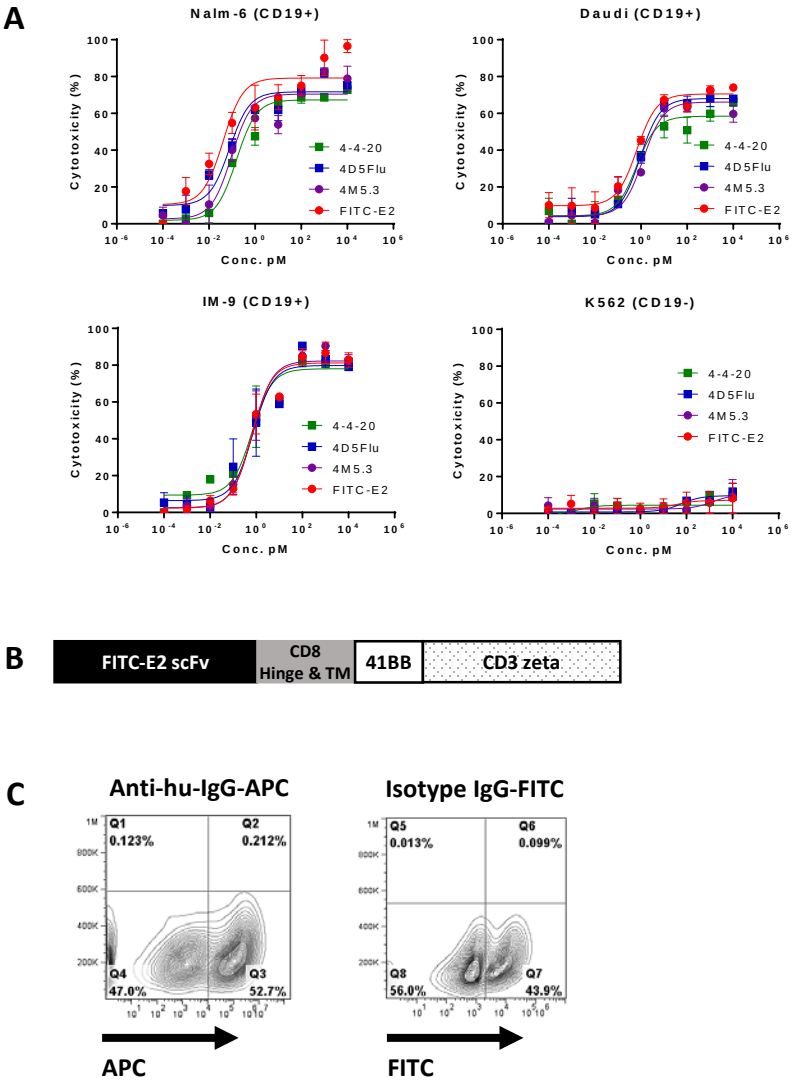
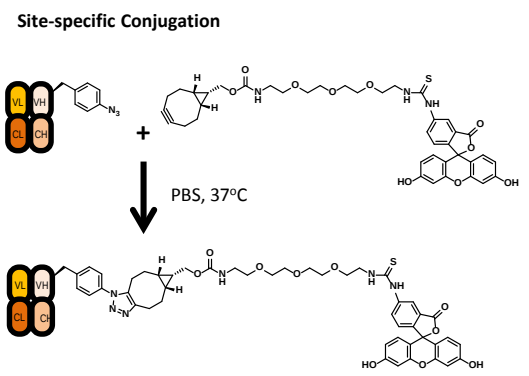


Figure S1. (A) Cytotoxicity assays comparing CAR-T cells derived from different anti-FITC scFvs against various CD19-positive target cell lines. Anti-FITC CAR-T cells and CD19-positive cells were co-cultured at a 5:1 ratio, respectively, with different concentrations of anti-CD19 AB-FITC. A CD19-negative target cell line (K562) was included as a negative control. Each data point represents a mean of duplicate samples, and error bars represent SD. Results presented are a representative of three independent experiments. **(B)** Second generation CAR construct consisting of the fully human anti-FITC scFv (FITC-E2) and signaling domains of 41BB and CD3ζ. **(C)** Cell surface CAR expression levels on transduced human T cells. Transduction efficiency was evaluated weekly by flow cytometry with APC-conjugated anti-human IgG F(ab')2 antibody or FITC-labeled isotype antibodies.

A



C

FITC switches	Expected Mass (Da)	Observed Mass (Da)
A-FITC	48793.59	48795.03
B-FITC	48763.57	48764.05
C-FITC	48749.54	48750.97
D-FITC	48779.57	48780.95
E-FITC	48763.57	48764.62
F-FITC	48722.47	48722.90
EF-FITC	49581.44	49582.78
AB-FITC	49652.56	49654.32

B

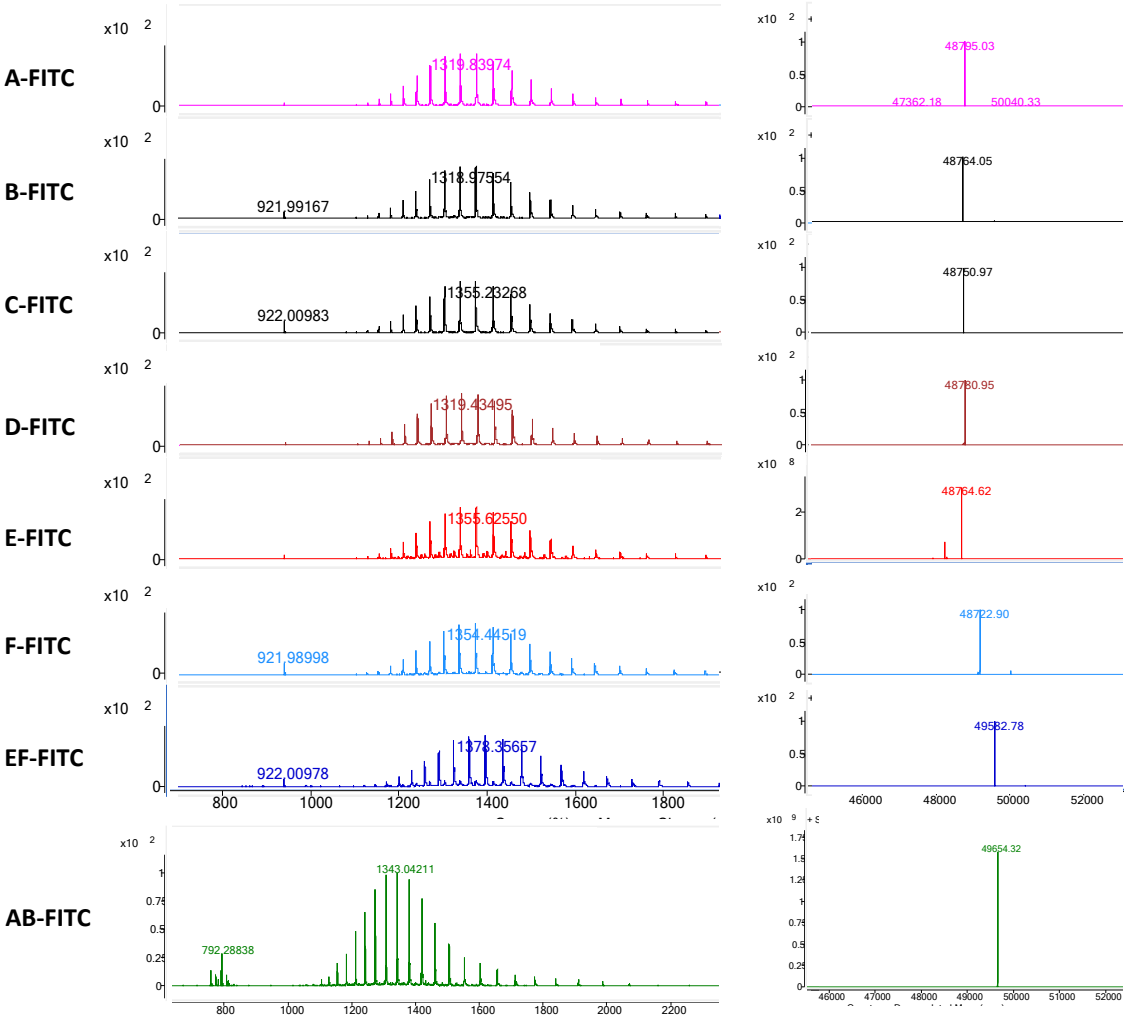


Figure S2. (A) General scheme to generate site-specific FITC-antibody conjugates. Mutant antibodies incorporated with pAzF were conjugated with BCN-PEG$_4$-FITC by "Click" reaction. **(B)** Mass spectrometry analysis of site-specific anti-CD19-FITC conjugates obtained on an Agilent Quadruple Time-of-Flight (QTOF) mass spectrometer. Deconvoluted masses were obtained using Agilent Qualitative Analysis software. **(C)** Table of expected and observed masses of site-specific anti-CD19 FITC switches.

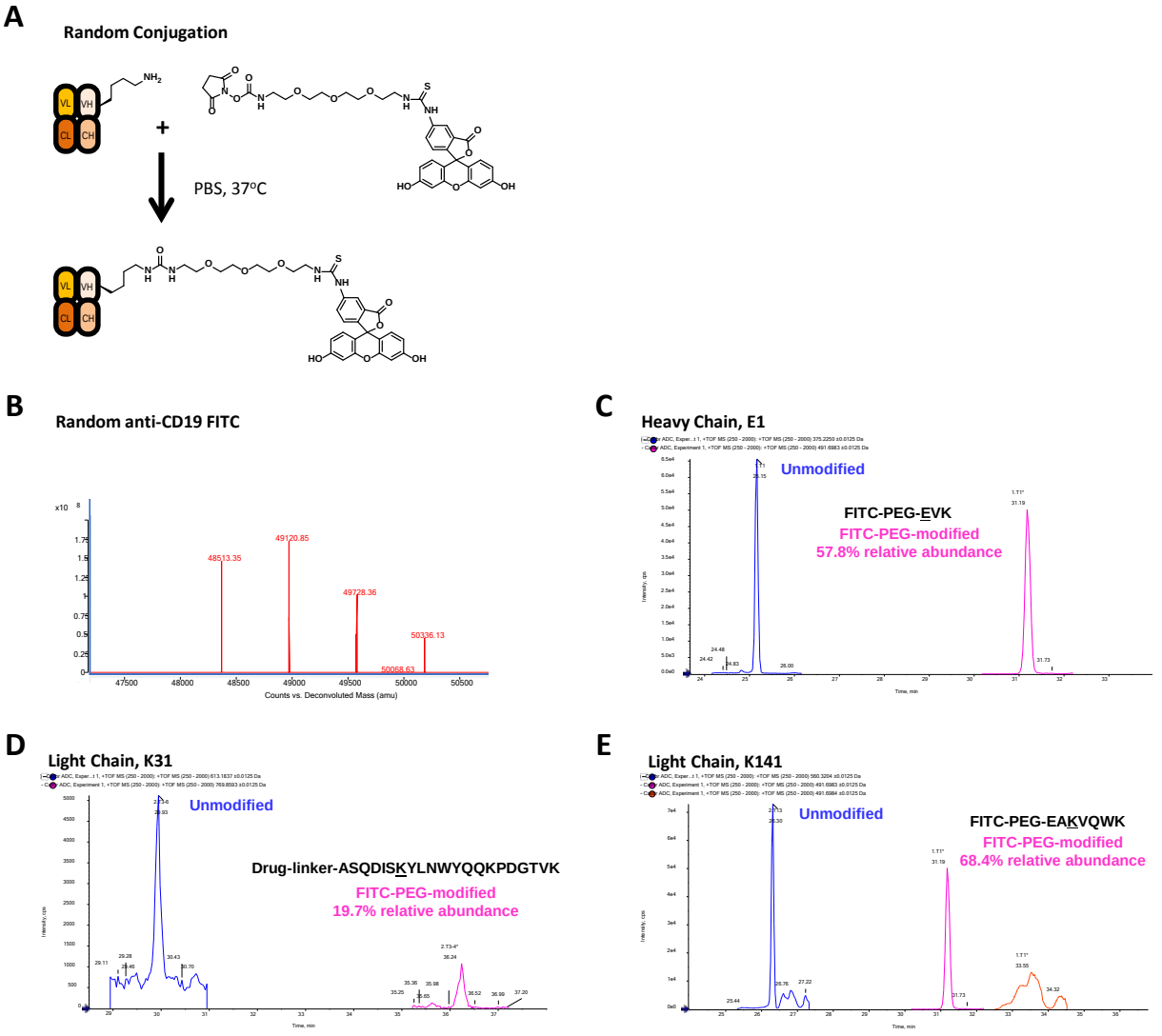


Figure S3. (A) General scheme to generate random FITC-antibody conjugates with FITC-PEG$_4$-NHS. **(B-E)** Mass spectrometry analysis of random anti-CD19-FITC conjugate. **(B)** Deconvolution profiles of random anti-CD19 FITC conjugate obtained on an Agilent QTOF mass spectrometer. **(C-E)** Butterfly plots of FITC-conjugated and unconjugated peptides from a single CESI-MS IDA peptide mapping run to identify modified sites found on random FITC switches generated by NHS chemistry as described in **(A)**.

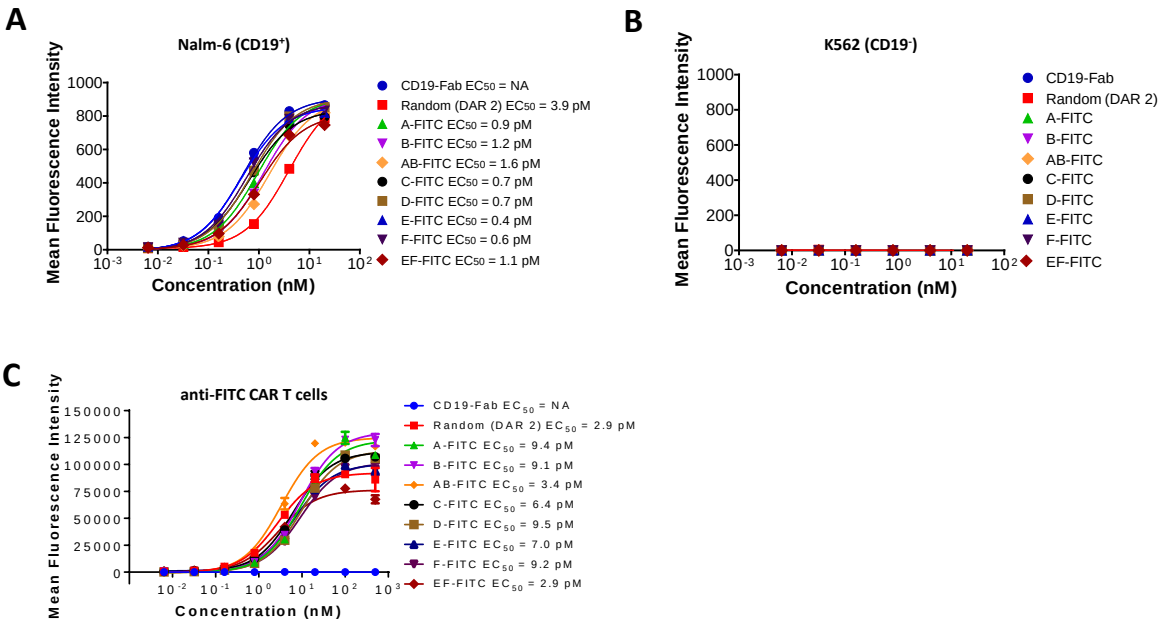
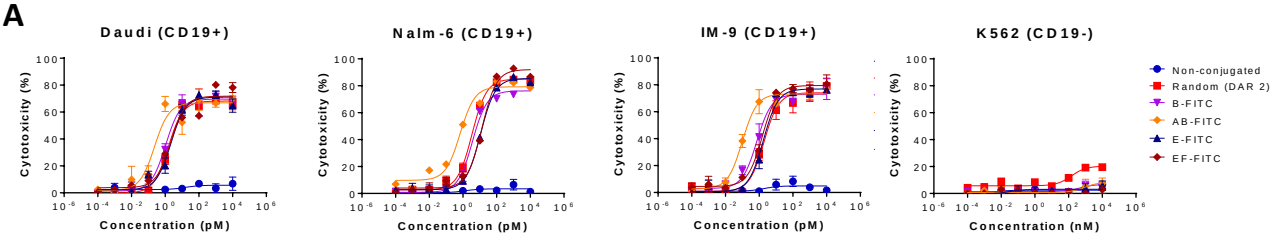


Figure S4. Binding capacity of anti-CD19 FITC conjugates were evaluated using **(A)** Nalm-6, **(B)** K562, and **(C)** anti-FITC CAR-T cells. Cells were incubated with indicated switch antibodies at 4˚C for 30-60 min and washed twice with staining buffer (1% BSA in PBS). Primary antibodies were revealed with Alexa Fluor®647 conjugated anti-human IgG or anti-human kappa secondary antibodies. After several washes, samples were acquired on a BD LSR II or BD Accuri C6 and analyzed using FlowJo 7.6.2 software. In each study, cells were incubated with secondary antibody alone and the observed mean fluorescence intensity (MFI) was used to subtract for background and non-specific staining.

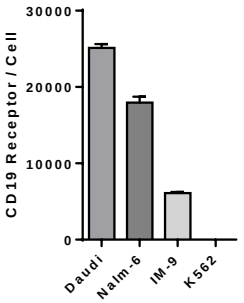
A



B

EC50 (pM)	Daudi	Nalm-6	IM-9
CD19 Fab	15.3 ± NA	1.4 ± 0	1.9 ± 1.6
Random (DAR 2)	1.5 ± 0.4	3.4 ± 1.0	1.9 ± 0.1
B-FITC	1.1 ± 0.3	3.7 ± 0.5	0.7 ± 0.8
AB-FITC	0.2 ± 0.0	0.7 ± 0.0	0.1 ± 0.0
E-FITC	2.2 ± 0.8	11.1 ± 0.9	1.8 ± 0.5
EF-FITC	2.1 ± 0.01	13.1 ± 0.5	1.5 ± 0.3
Fold Change (B-FITC vs AB-FITC)	4.6	5	7.3

C



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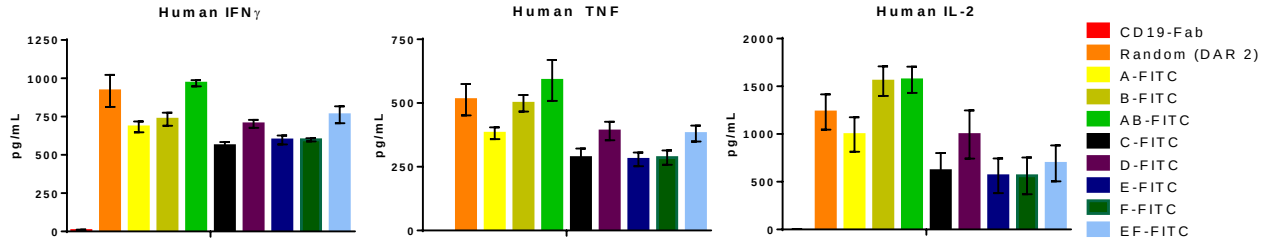

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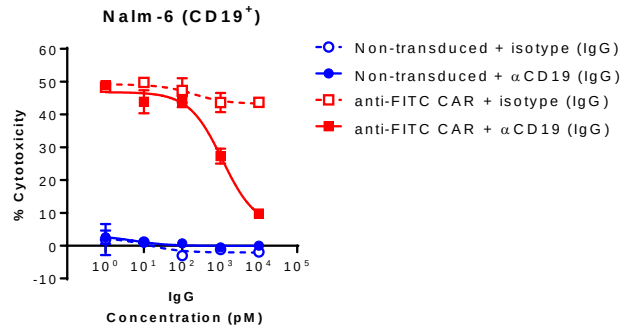
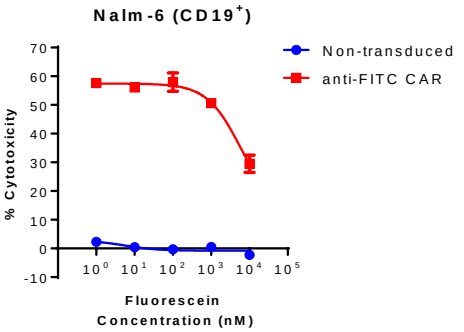


F



G

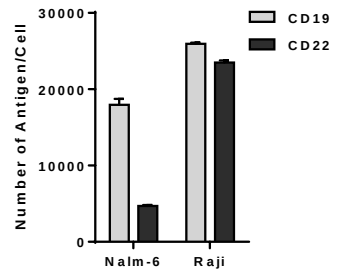


Figure S5.

Figure S5. (A) Cytotoxicity assay comparing indicated anti-CD19 FITC against target cells with different CD19 expression levels. Data points depict a mean of duplicate samples, and error bars represent SD. **(B)** Table of EC_{50} values from cytotoxicity assays described in **(A)**. **(C)** Surface CD19 expression on indicated cell lines was quantified with PE-conjugated CD19 flow cytometry antibodies using Quanti-Brite PE beads. **(D)** Quantification of indicated cytokines in co-cultures containing equal numbers (1 x 10^5) of anti-FITC CAR-T cells and Nalm-6 (CD19+) cells in the presence of 1 nM anti-CD19 AB-FITC switch. The next day, cultured media was harvested and cytokines were quantified using BD Cytometric Bead Array (CBA) Human Th1/Th2 II. **(E, F)** Cytotoxicity assays consisting of 10 pM anti-CD19 AB-FITC with anti-FITC CAR-T cells and Nalm-6 (CD19+) cells co-cultured at a 5:1 ratio supplemented with excess amounts of anti-CD19 antibody (FMC63 IgG) or isotype control (Millipore) or fluorescein (Sigma). **(G)** Surface antigen expression was quantified with PE-conjugated CD19 and CD22 flow cytometry antibodies using Quanti-Brite PE beads. All results are a representative or summary of independent experiments with CAR-T cells generated from three different donors. Data shown are an average of duplicate or triplicate samples, and error bars represent SD.

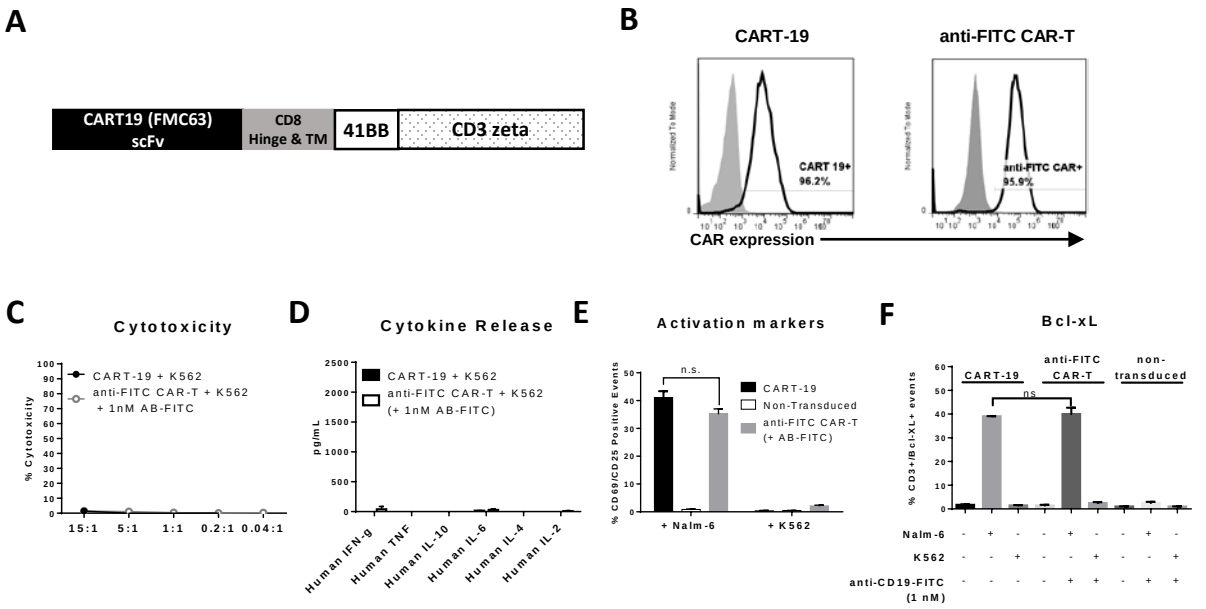


Figure S6. (A) Second generation CAR construct consisting of anti-CD19 scFv (clone FMC63) and signaling domains of 41BB and CD3ζ. **(B)** Cell surface CAR expression levels on enriched transduced human T cells. Transduction efficiency was evaluated weekly by flow cytometry with APC-conjugated anti-mouse or anti-human IgG F(ab')2 antibody. **(C, D)** Cytotoxicity and cytokine release assays comparing CART-19 and anti-FITC CAR-T cell activity against K562 (CD19-) target cells. **(E)** 1 x 10^5 anti-FITC CAR-T cells and Nalm-6 (CD19+) or K562 (CD19-) cells were cultured in the presence of 1 nM of anti-CD19 AB-FITC switch. The next day, CD3-positive cells were evaluated for upregulation of activation markers (CD69 and CD25) by flow cytometry. **(F)** Equal number (1 x 10^5) of anti-FITC CAR-T cells and target cells were co-cultured in the presence of 1 nM anti-CD19 AB-FITC switch. The next day, cell mixtures were surface labeled with anti-CD3, fixed, and permeabilized prior to anti-Bcl-xl staining. Data shown represent an average of duplicate samples and error bars represent SD. All results are a representative or summary of independent experiments with CAR-T cells generated from three different donors. n.s. = $p>0.05$ using one-tailed Student's t-test.

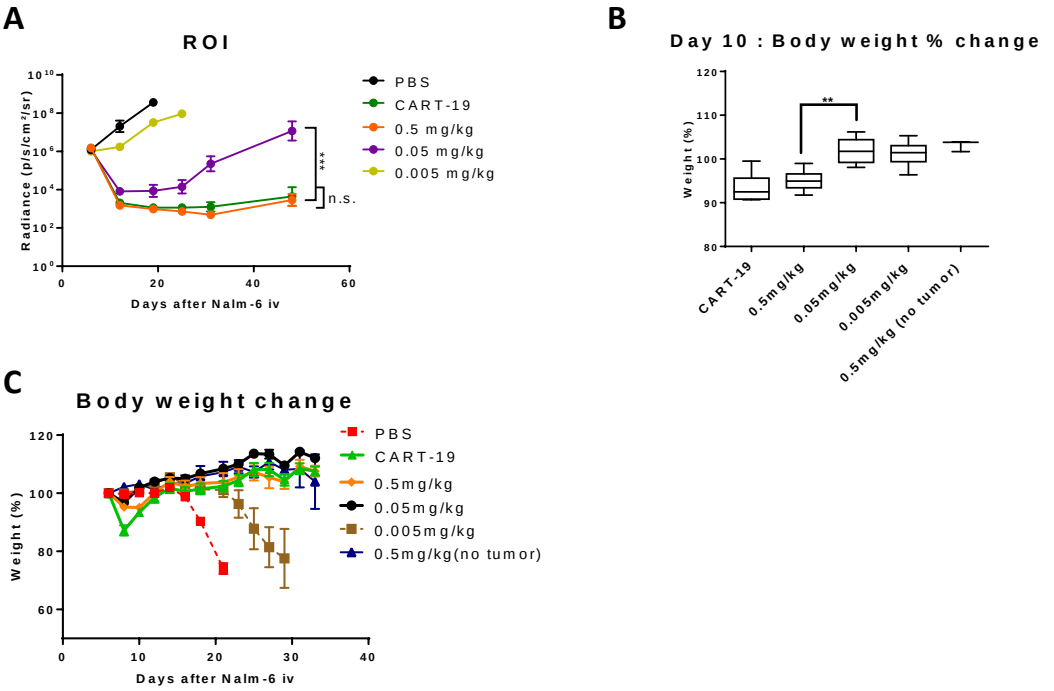


Figure S7. (A) Tumor burden quantified using bioluminescent signal intensity from switch dose titration study as described in **Fig. 3A**. No significant difference was found between CART-19 and sCAR-T with 0.5 mg/kg treatment groups; n.s. = $p > 0.05$. However, significance was observed when comparing sCAR-T with 0.05mg/kg and CART-19 or sCAR-T with 0.5 mg/kg treatment; *** = $p<0.0005$. Significance was determined using 2-way ANOVA with Turkey's multiple comparisons test (shown for final time-point only). **(B)** Graphical representation of body weight change (percent) observed after initiation of CART-19 or anti-FITC CAR-T cells with anti-CD19 AB-FITC therapy on day 10 of the study. ** = $p<0.005$ using one-tailed Student's t-test. **(C)** Summary of body weight change observed throughout the study. Data points and error bars represent average and SD derived from 6 mice/group, respectively.

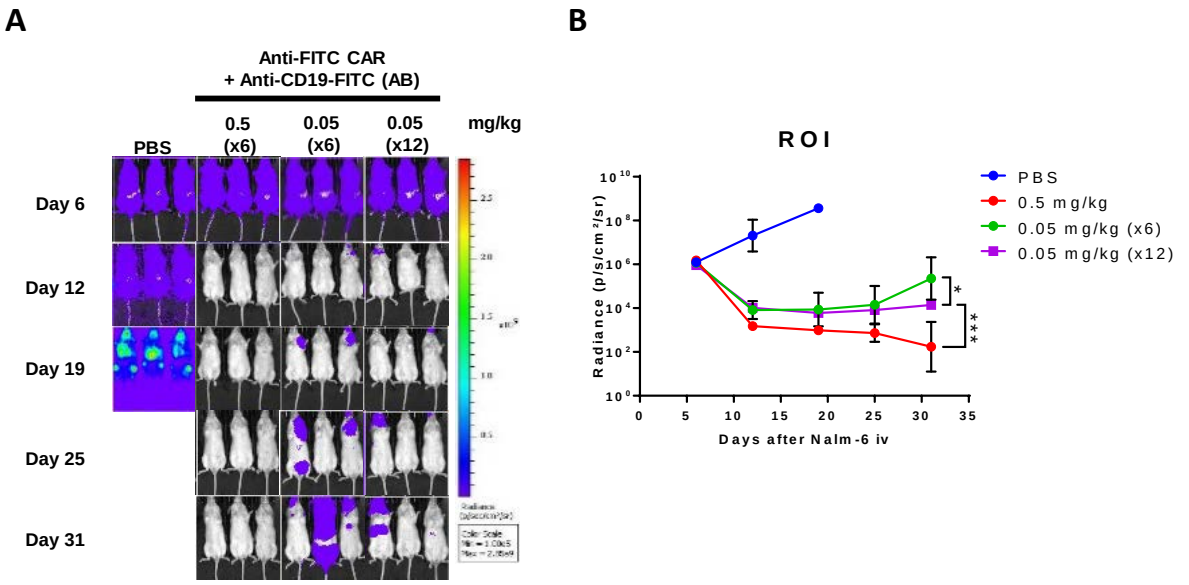


Figure S8. (A) Nalm-6 xenograft model. 0.5 x 10^6 luciferized Nalm-6 cells were intravenously (IV) injected into 6-8 weeks-old female NSG mice. Seven days later, mice were IV infused with 40 x 10^6 CAR-T cells and switch treatment was initiated with anti-CD19 AB-FITC conjugate at indicated concentrations or PBS every other day. Parentheses indicate the total number of doses that each group received. **(B)** Tumor burden was monitored by weekly bioluminescence imaging (BLI). Results are derived from 6 mice/group and error bars represent SD. * = $p<0.05$ and *** = $p<0.0005$ was determined using 2-way ANOVA with Turkey's multiple comparisons test (shown for final time-point only).

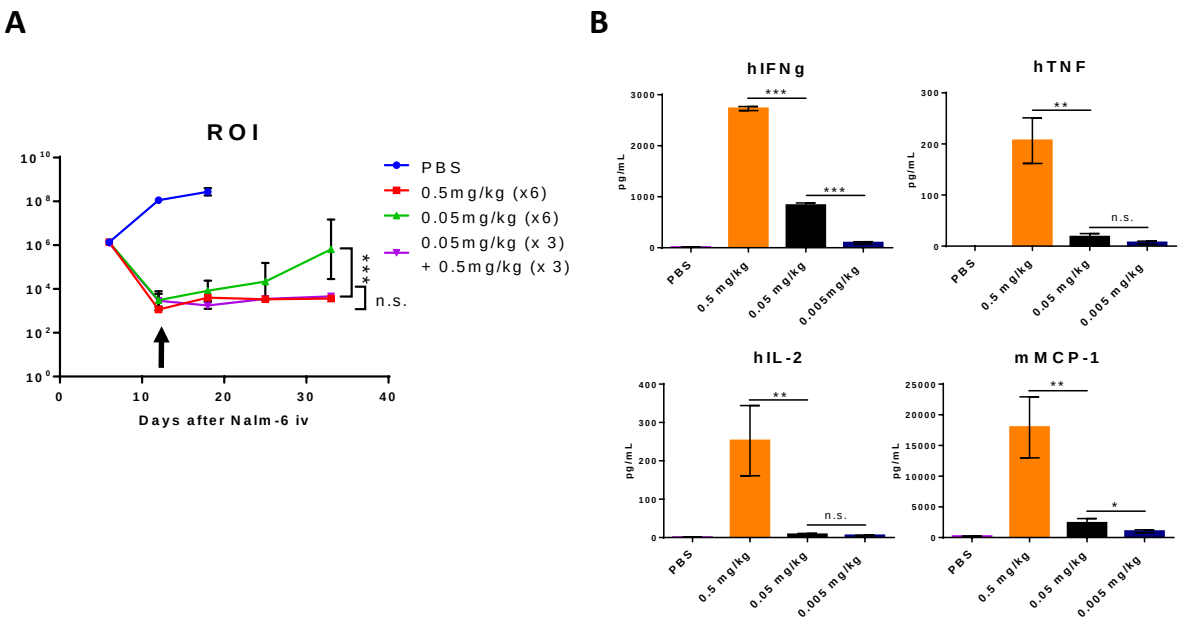


Figure S9. (A) Tumor burden quantified by bioluminescent signal intensity from **(Fig. 3C)**. Parentheses indicate the total number of doses that each group received. Arrow specifies time of increase in switch dose from 0.05 to 0.5 mg/kg. No significant difference was found between 0.5 mg/kg (x6) and dose escalation treatment groups; n.s. = $p>0.05$. However, significance was observed when comparing 0.05mg/kg (x6) and 0.5 mg/kg (x6) or dose escalation regimen; *** = $p<0.0005$.Significance was determined using 2-way ANOVA with Turkey's multiple comparisons test (shown for final time-point only). **(B)** Serum cytokine levels 24 hours after initiation of sCAR-T cell therapy with indicated switch dose. Cytokines were quantified with CBA Human Th1/Th2 Kit II and Mouse Inflammation Kit (BD). Averages and SD values were derived from 6 mice/group. Significance was calculated using 2-way ANOVA with Turkey's multiple comparisons test: n.s. = $p>0.05$, * = $p<0.05$, ** = $p<0.005$, *** = $p<0.0005$.

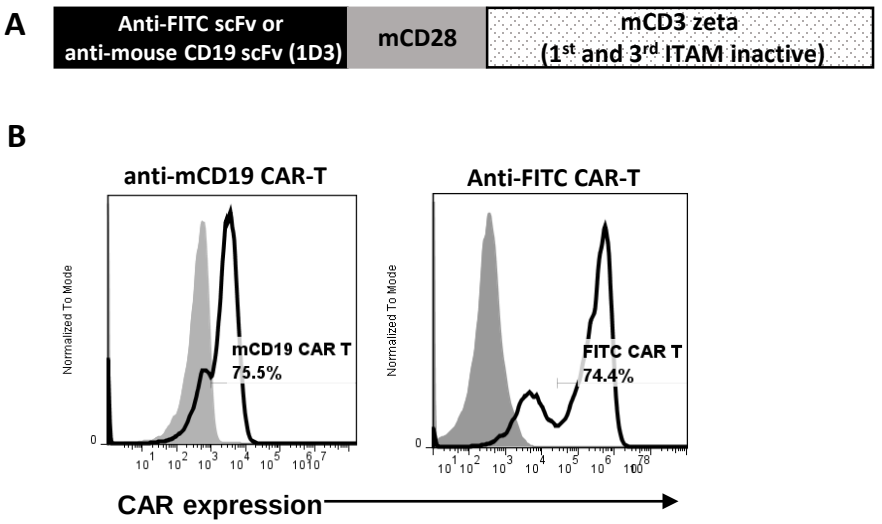


Figure S10. (A) Second generation retroviral construct consisting of anti-mouse CD19 (1D3) or anti-FITC (FITC-E2) scFV, and signaling domains of CD28 and CD3ζ. **(B)** Cell surface CAR expression levels on transduced mouse T cells. Transduction efficiency of anti-mouse CD19 CAR T cells and anti-FITC CAR-T cells were evaluated by flow cytometry using PE-conjugated anti-rat or APC-conjugated anti-human IgG F(ab)'2 antibody, respectively.

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