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Supplementary Information for

Switchable control over *in vivo* CAR-T expansion, B cell depletion, and induction of memory

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Supplementary text and reference

Figs. S1 to S6

Table S1

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Materials and Methods

Expression and generation of anti-mouse CD19 switch

DNA fragments encoding the heavy and light variable regions of the rat 1D3 anti-mouse CD19 antibody were synthesized (IDT) and assembled in a pFUSE-based vector system (Invivogen) with the PNE peptide engraftment shown in Fig. 1A. Chimeric constructs were generated by fusing the 1D3 variable regions to human constant regions (chimeric, ch1D3) and likewise, the variable regions were paired with mouse constant regions for the fully murine version (murine, mu1D3) (Supplementary Table 1). Transient expression of the Fab switches was performed in the Expi293 Expression System (ThermoFisher Scientific). Briefly, Expi293F cells were transfected at a density of 2.9 x 10^6 cells/ml with a 1:2.67 ratio of plasmid DNA to Expifectamine. Heavy and light chain plasmids were used at ratio of 1:1 to screen expression, whereas a 3:2 plasmid ratio of heavy to light chain was used for production scale expressions. Enhancers were added to the culture medium 18 hours post-transfection. Expression cultures were harvested 72-96 hours post-transfection by centrifugation at 400 x g. Fab switches were purified by affinity chromatography using Protein G Sepharose 4 Fast Flow resin (GE Healthcare). Proteins were eluted with 100mM glycine pH 2.8 and subsequently neutralized by the addition of 1/10 v/v 1M Tris-HCl pH 9. Switches were buffer exchanged into phosphate buffered saline (PBS) with PD10 desalting columns (GE Healthcare) and filter sterilized for downstream applications. Switch integrity was confirmed by SDS-PAGE, ESI-QTOF mass spectrometry, and analytical size exclusion chromatography (Fig. S1A).

For in vitro and in vivo studies, after thawing, all switch molecules were filtered with low protein binding 0.22 µm filters (Millex-GV) and endotoxin levels were confirmed < 10 endotoxin units / ml using the Endosafe®-PTS™ system (Charles River) prior to use.

Construction of murine sCAR

The conventional CAR-19 murine construct (anti-mouse CD19 (1D3) scFv in MSGV1 1D3-28Z.1-3 plasmid (1D3 28z(1-3)) was generously provided by Dr. James N. Kochenderfer, National Cancer Institute. To generate the sCAR 28z(1-3), the rat anti-mouse CD19 (1D3) scFv was replaced with the mouse anti-PNE scFv. sCAR constructs used in this study are represented in Fig. 2A. DNA fragments encoding the different hinge, transmembrane, and co-stimulatory domains were generated by PCR or synthesized (IDT DNA) (Supplementary Table 1). The MSGV retroviral backbone was opened by restriction enzyme digest and the DNA fragments assembled modularly using NEBuilder HiFi DNA Assembly Mix (New England Biolabs). Clones were screened in the Stbl4 cell line (ThermoFisher Scientific) and verified by Sanger sequencing.

Culture media

The Platinum-E Retroviral Packaging Cell Line, Ecotropic (Cell Biolabs, Inc), was maintained in DMEM high glucose medium with 10% heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM MEM sodium pyruvate, 0.1 mM MEM non-essential amino acids (NEAA), 100 U/ml of penicillin and 100 µg/ml of streptomycin, 1 µg/ml of puromycin and 10 µg/ml of blasticidin S HCl (all from ThermoFisher Scientific).

Myc5 $CD19^+$ cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 1 mM MEM sodium pyruvate, 10 ng/ml LPS (Sigma Aldrich) and 0.4 mg/ml Geneticin selective antibiotic (G418 sulfate, ThermoFisher Scientific).

38c13 cells were cultured in RPMI 1640 medium containing 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM NEAA, 0.01 M HEPES and 50 µM 2-mercaptoethanol (all from ThermoFisher

Scientific). For reproducibility of results, 38c13 cells were kept in culture 2 weeks prior to inoculation to mice.

The mouse splenocytes and sCAR/CAR-T cells were cultured in RPMI 1640 medium (ATCC modification) (A10491, Gibco) with 10% FBS (Foundation B™), 50 µM 2-mercaptoethanol, 100 U/ml of penicillin and 100 µg/ml of streptomycin and 50 µg/ml gentamicin (Gibco). For cytotoxicity assays, the following culture medium was used: RPMI 1640 medium (ATCC modification) (A10491, Gibco) with 5% FBS (Foundation B™), 50 µM 2-mercaptoethanol and 100 U/ml of penicillin and 100 µg/ml of streptomycin.

Production of retroviral supernatants

To produce retroviral supernatants, Platinum-E cells were cultured in BioCoat™ Poly-D-lysine dishes (Corning) and transfected (with no puromycin and blasticidin) with Lipofectamine™ 2000 (ThermoFisher Scientific), pCL-Eco retrovirus packaging vector (Novusbio) and sCAR/CAR DNA plasmid. The 48 and 72-hour resulting supernatants were spun, pooled, aliquoted, snap-frozen in liquid nitrogen and stored at -80°C or concentrated 10X with Amicon® Ultra-15 tubes (Millipore) prior to aliquoting and freezing.

Enrichment of murine sCAR-T cells

To enrich for sCAR/CAR-T cells (for cytotoxicity assays) prior to seeding, cells were pre-incubated 10 min at 4°C in PBS + 0.5% BSA + 2 mM EDTA with biotin-SP AffiniPure goat anti-rat IgG, F(ab')2 (Jackson ImmunoResearch) for 1D3 28z(1-3) CAR-T cells or biotin-labelled PNE for sCAR-T cells. Labelled cells were then incubated with Anti-Biotin Microbeads (Miltenyi Biotec) and positively separated through MS columns (Miltenyi Biotec) according to the manufacturer's instructions.

Cytotoxicity colorimetric-based assay – LDH release

Ten thousand Myc5 $CD19^+$ target cells and 1×10^5 28z(1-3) sCAR-T cells or 1D3 28z(1-3) (10:1 ratio) with 1 nM of anti-mouse CD19 switches (rat/human) were cocultured for 6 hours at 37°C. The amount of lactate dehydrogenase (LDH) was quantified in the supernatants using the Cytotox 96® Non-Radioactive Cytotoxicity Assay kit (Promega). The percent specific kill of the target cells (lytic activity) was calculated with the following formula based on absorbance values (490 nm) subtracted from the culture medium background: % Cytotoxicity = 100 × [((Target cells + Effector cells + Switch) – (Target cells alone - Effector cells only)) / ((Maximum target cell lysis) – (Target cells only))]. The sCAR-T and 1D3 28z(1-3) conventional CAR-T cells used in these assays had a transduction efficiency of 65-70% and 51-54% respectively.

Ovalbumin Immunization

Mice were immunized with an intraperitoneal injection of 100 µg OVA (ovalbumin/albumin from chicken egg white (Sigma-Aldrich)) admixed 1:1 with Imject Alum adjuvant (ThermoFisher Scientific). Two weeks later, blood was collected and plasma was subjected to an anti-OVA IgG1 mouse ELISA kit (Cayman chemical) to detect specific anti-OVA antibodies.

Anti-switch antibody assay

Mouse whole blood was collected in heparinized capillaries at day 139 for the experiment shown in Fig. S3A to F and at day 53 for Fig. S5. After centrifugation, plasma samples were collected and frozen at -80°C. ELISA high-binding microplates (Nunc) were coated with m1D3 LCNT switch at 5 µg/ml in PBS, pH 7.4, overnight at 4°C. Plates were blocked with 1% BSA in PBS for 60 minutes at room temperature, with shaking at 600 rpm. Thawed plasma was diluted 1/100, 1/1000, 1/10000, and 1/100000 in 1% BSA in PBS. After washing the plates with PBS containing 0.05% Tween 20, 20 µl of the diluted plasma (in

duplicate) was applied onto the coated plates. The murine anti-GCN4 antibody (Absolute Antibody, Ab00436-1.1) was spiked in mouse plasma as a positive control. The assay was developed by TMB/H_2O_2 colorimetry (R&D Systems) using HRP conjugated detection antibody. Optical density (O.D.) was measured at 450 nm (SpectraMax M3).

Flow cytometry analyses

F(ab')2-Goat anti-rat IgG (H+L) FITC (eBioscience) and AF488-conjugated PNE (Anaspec) were used to evaluate CAR expression on 1D3 28z(1-3) conventional CAR-T cells and sCAR-T cells respectively. Non-transduced cells were stained in parallel with sCAR/CAR-T cells and were used as background controls.

For analyses of lymphocytes from peripheral blood, 40 µl of heparinized blood were pre-incubated with TruStain fcX™ antibody (BioLegend) and then cells were stained first for 1D3 28z(1-3) CAR-T cell groups with biotin-SP AffiniPure goat anti-rat IgG, F(ab')2 (Jackson ImmunoResearch) for 30 min at RT, then washed twice with staining buffer. All the samples were then stained 30 min at RT with anti-mouse: CD3ε (BUV395) (145-2C11), CD4 (BUV805) (GK1.5), CD44 (BV786) (IM7), CD62L (PE) (MEL-14), CD45R/B220 (BUV661) (RA3-6B2), CD279 (BV421) (J43) (all from BD Biosciences), CD8α (BV711) (53-6.7), CD19 (APC-Cy7) (6D5), CD45 (PerCP-Cy5.5) (30-F11) (all from BioLegend), and AF488-conjugated PNE for sCAR groups or AF488 Streptavidin (BioLegend) for 1D3 28z(1-3) CAR groups. All the samples were then incubated with BD FACS™ Lysing solution 1X (BD Biosciences) for 20 min at RT, washed, filtered and analyzed on a BD LSRFortessa™ X-20 flow cytometer after adding CountBright™ Absolute Counting Beads for cell enumeration.

For analysis of lymphocytes from tissue, 1×10^6 cells were washed with PBS and then pre-incubated with TruStain fcX™ antibody and Zombie UV™ Fixable Viability marker (BioLegend). Cells were stained following the 1 or 2-step protocol described above, washed and fixed in 2% paraformaldehyde. After filtration, cells were analyzed on a BD LSRFortessa™ X-20 flow cytometer. Cell numbers were

determined based on % and total viable cell counts of the tissues after dissociation obtained with a Vi-Cell XR cell counter (Beckman Coulter).

Data analysis was performed using FlowJo V.10.1. software.

For data analysis, after gating the sCAR-T cells on viable single cells, $CD45^+$, $CD3^+$, $sCAR^+$, $CD4^+$ or $CD8^+$, $sCAR^+$ T E/EM cells were defined as $CD44^+CD62L^-$ and $sCAR^+$ T CM cells as $CD44^+CD62L^+$ (1).

Reference

1. Opata MM & Stephens R (2013) Early Decision: Effector and Effector Memory T Cell Differentiation in Chronic Infection. *Curr Immunol Rev* 9(3):190-206.

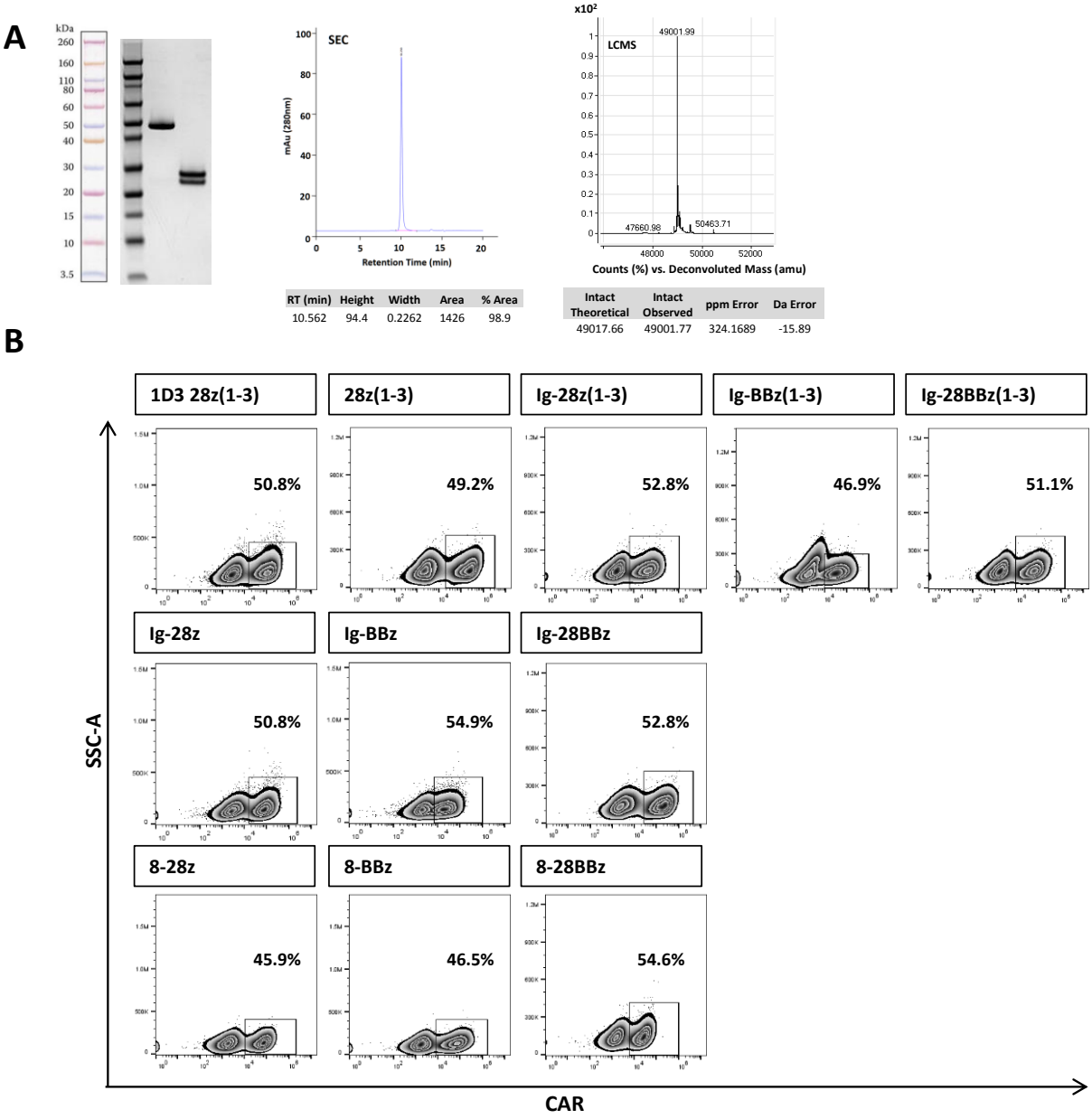

A
kDa
260
160
110
80
60
50
40
30
20
15
10
3.5
SEC
mAu (280nm)
Retention Time (min)
RT (min) Height Width Area % Area
10.562 94.4 0.2262 1426 98.9
x10²
LCMS
49001.99
47660.98
50463.71
Counts (%) vs. Deconvoluted Mass (amu)
Intact Theoretical
Intact Observed
ppm Error
Da Error
49017.66
49001.77
324.1689
-15.89
B
1D3 28z(1-3)
28z(1-3)
Ig-28z(1-3)
Ig-BBz(1-3)
Ig-28BBz(1-3)
50.8%
49.2%
52.8%
46.9%
51.1%
Ig-28z
Ig-BBz
Ig-28BBz
50.8%
54.9%
52.8%
8-28z
8-BBz
8-28BBz
45.9%
46.5%
54.6%
SSC-A
CAR
C
anti-PNE scFv
VL
VH
Hinge + TM
Costim
CD3ζ
% target killed
ns
*
8-28z
8-BBz
8-28BBz
1D3 28z(1-3)
NT
ng/ml
IFNγ
TNF
IL-2
ng/ml
8-28z
8-BBz
8-28BBz
1D3 28z(1-3)
NT

Fig. S1. Switch and sCAR-T cell quality controls and in vitro assessment of sCAR designs based on CD8 hinge. (A) Integrity evaluation of the murine 1D3 LCNT switch. From left to right: SDS-PAGE, intact and reduced; size exclusion elution profile on an Agilent BioAdvance SEC HPLC column; intact mass confirmation by LCMS on an ESI-QTOF. The mass difference when compared to the theoretical mass is a result of pyroglutamate formation on the N terminus of the heavy chain. (B) Representative flow plot of sCAR/CAR expression after normalization. sCAR-T and 1D3 28z(1-3) conventional CAR-T cells used in in vitro assays were normalized to 50±6% transduction efficiency with non-transduced cells prior to assays and sCAR/CAR expression in the resulting cells was verified. (C) Comparison of sCAR-T cells based on their cytotoxicity by flow cytometry against 38c13 target cells in the presence of 1 nM anti-mouse CD19 switch and on their cytokine secretion measured by CBA assay. The sCAR-T and 1D3 28z(1-3) conventional CAR-T cells used in these assays were previously enriched through sorting and normalized to 50±6% sCAR+/CAR^+ with non-transduced cells in the final assays. 1D3 28z(1-3) conventional CAR-T cells and non-transduced cells (NT) are used as a reference and a control, respectively. All graphs depict the data from 2 pooled experiments performed in triplicate. Statistical analyses were performed using the Kruskal-Wallis test completed with Dunn's non-parametric multiple comparisons test. Means and SEM are shown. * statistical significance at $P<0.05$; ns, not significant.

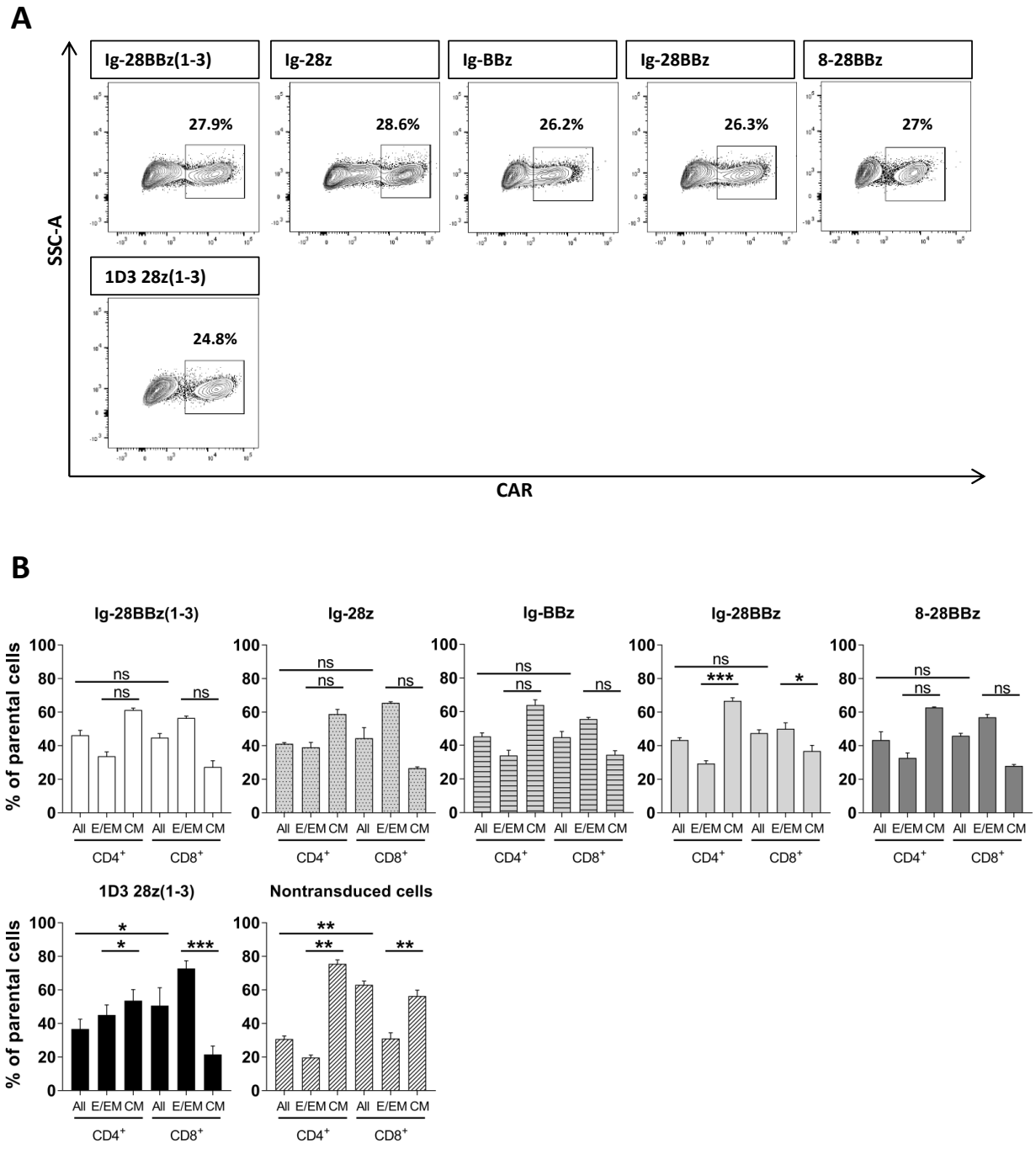
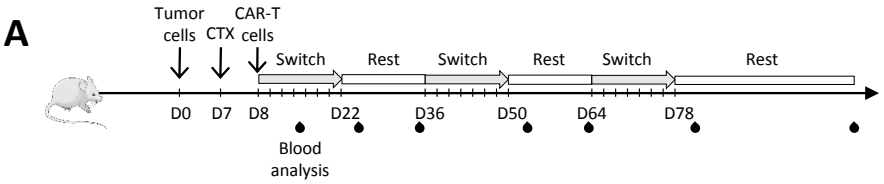
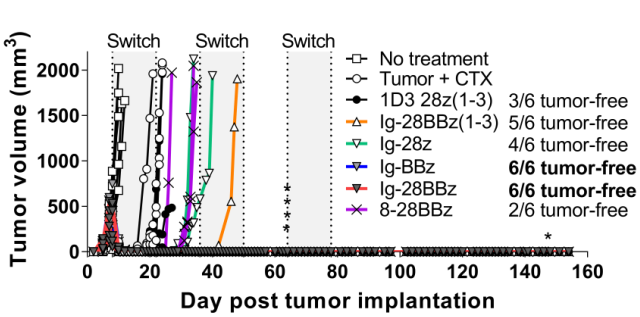
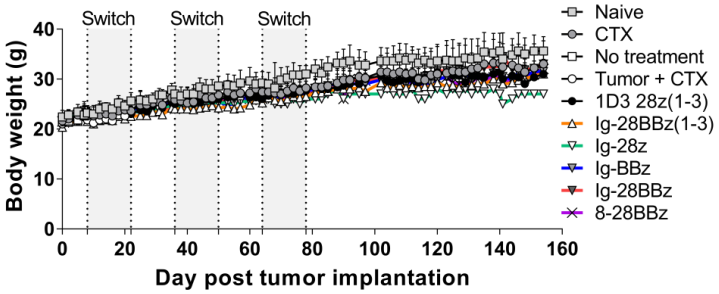


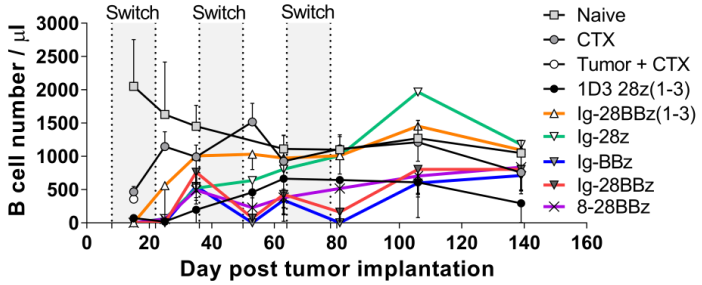
Fig. S2. Phenotype of the sCAR/CAR-T cells used in vivo for adoptive cell transfer. (A) (B) sCAR and CAR-T cells were normalized with non-transduced cells to 30±6% CAR$^+$ before being injected into mice. (A) sCAR/CAR expression was assessed by flow cytometry after staining the cells with either PNE conjugated with AF488 or F(ab')2-Goat anti-rat IgG (H+L) FITC antibody. These representative dot-plots

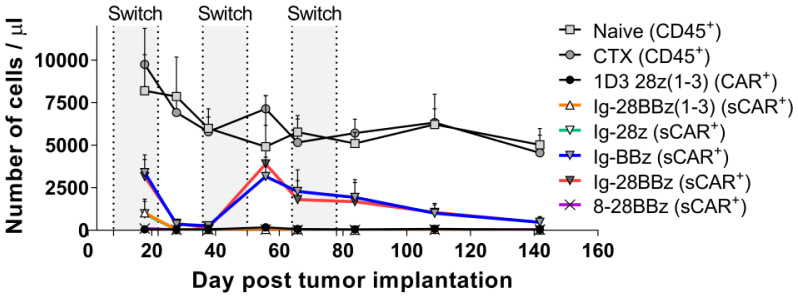
correspond to the study reported in Fig. S3. (B) The cell phenotype was analyzed by flow cytometry: $CD4^+$ and $CD8^+$ T total populations (All) (gated on viable single cells, $CD45^+$, $CD3^+$, $sCAR^+$ or CAR^+, $CD4^+$ or $CD8^+$), $CD4^+$ or $CD8^+$ T effector / effector memory (E/EM) ($CD44^+CD62L^-$) and central memory (CM) ($CD44^+CD62L^+$) subsets are shown. The results from 2 to 7 independent analyses are depicted. Statistical analyses using the Mann-Whitney test indicated significant differences at 95% CI. Data is shown as mean with SEM. * statistical significance at $P<0.05$; ** $P<0.01$; *** $P<0.001$; ns, not significant.


A
Tumor cells
CTX
CAR-T cells
Switch
Rest
Switch
Rest
Switch
Rest
D0
D7
D8
D22
D36
D50
D64
D78
Blood analysis


B
Switch
Switch
Switch
Tumor volume (mm3)
Day post tumor implantation
No treatment
Tumor + CTX
1D3 28z(1-3) 3/6 tumor-free
Ig-28BBz(1-3) 5/6 tumor-free
Ig-28z 4/6 tumor-free
Ig-BBz 6/6 tumor-free
Ig-28BBz 6/6 tumor-free
8-28BBz 2/6 tumor-free


C
Switch
Switch
Switch
Body weight (g)
Day post tumor implantation
Naive
CTX
No treatment
Tumor + CTX
1D3 28z(1-3)
Ig-28BBz(1-3)
Ig-28z
Ig-BBz
Ig-28BBz
8-28BBz


D
Switch
Switch
Switch
B cell number / µl
Day post tumor implantation
Naive
CTX
Tumor + CTX
1D3 28z(1-3)
Ig-28BBz(1-3)
Ig-28z
Ig-BBz
Ig-28BBz
8-28BBz


E
Switch
Switch
Switch
Number of cells / µl
Day post tumor implantation
Naive (CD45+)
CTX (CD45+)
1D3 28z(1-3) (CAR+)
Ig-28BBz(1-3) (sCAR+)
Ig-28z (sCAR+)
Ig-BBz (sCAR+)
Ig-28BBz (sCAR+)
8-28BBz (sCAR+)

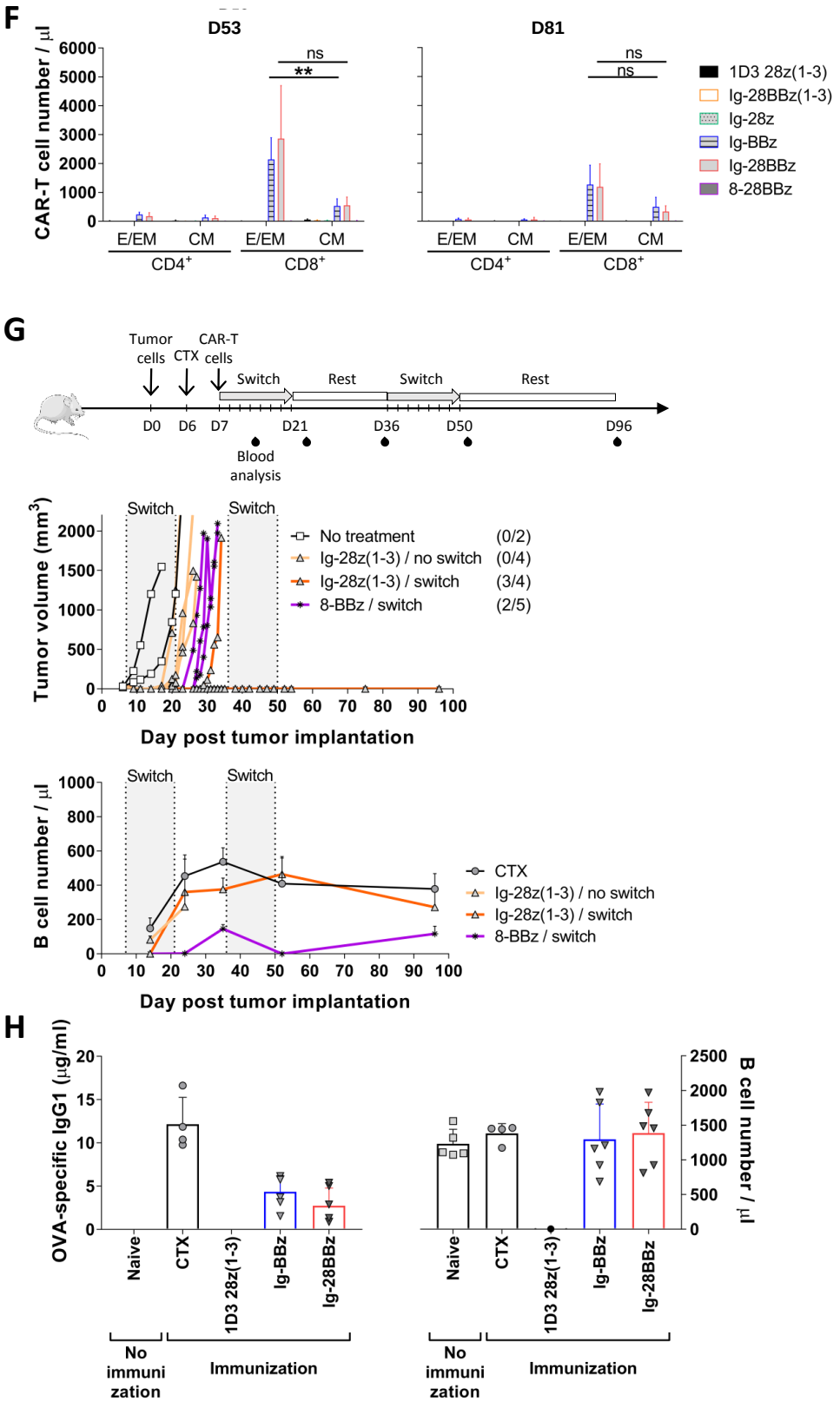


Fig. S3. In vivo efficacy of sCAR designs in a syngeneic murine tumor model. C3H mice were implanted subcutaneously on the flank with 1 × 10^6 38c13 cells at D0. At D7, mice were randomized on their tumor

volume and preconditioned with 100 mg/kg cyclophosphamide (CTX) intraperitoneally. The following day (D8) sCAR/CAR-T cells normalized to 30±6% $sCAR^+/CAR^+$ cells (Fig. S2a) were injected intravenously (10×10^6 cells 30±6% $sCAR^+/CAR^+$). Anti-murine CD19 switch doses (or PBS) were started at D8, D36 and D64 for 8 doses (gray shading) every other day at 1 mg/kg intravenously, in a 2-week on/off (rest) cycle. The study compared the efficacy of Ig-28BBz(1-3), Ig-28z, Ig-BBz, Ig-28BBz and 8-28BBz, through the evaluation of IgG4m hinge vs. murine CD8-based hinge (Ig-28BBz vs. 8-28BBz), murine CD28 vs. murine 4-1BB costimulatory molecule (Ig-28z vs. Ig-BBz) and murine CD3ζ signaling domain in its wild type form vs. with ITAMs 1 and 3 mutated (Ig-28BBz vs. Ig-28BBz(1-3)). The 1D3 28z(1-3) was used as a positive control. (n=5-6). This experiment is a combined experimental replicate of the 2 studies shown Fig. 3. (A) Experimental overview. (B) Tumor growth kinetic of tumor-bearing mice that received no treatment ('No treatment'), CTX only ('Tumor + CTX'), or were administered sCAR/CAR-T cells and switch following CTX (PBS for 1D3 28z(1-3) CAR-T group). The number of tumor-free mice in each treatment group is reported. * indicates at D64 when mice were found dead for an unknown reason: 1 in Ig-28BBz(1-3) and 3 in Ig-28z, and at D148 when 1 mouse in 8-28BBz was found dead with metastases. (C) Body weights over time. (D) Number of B cells / µl of peripheral blood over time as determined by flow cytometry. (E) Number of $CD45^+$ cells and $sCAR^+/CAR^+$-T cells / µl of peripheral blood over time as determined by flow cytometry. (F) sCAR/CAR-T cell phenotype analysis in the peripheral blood by flow cytometry at D53 and D81 (gated on viable single cells, $CD45^+$, $CD3^+$, $sCAR/CAR^+$, $CD4^+$ or $CD8^+$): $CD4^+$ or $CD8^+$ T effector / effector memory (E/EM) ($CD44^+CD62L^-$) and central memory (CM) ($CD44^+CD62L^+$) subsets. Refer to Fig. S6 for additional analysis regarding the outlier mouse in Ig-28BBz group. (G) This study compared the in vivo efficacy of Ig-28z(1-3) to 8-BBz (n=2-5) regarding their high in vitro cytotoxicity and cytokine secretion; top panel: experimental overview, middle panel: tumor growth kinetic and bottom panel: number of B cells / µl of peripheral blood over time as determined by flow cytometry. (H) B-cell functionality after repopulation due to switch cessation in the tumor-free mice from the experiment presented in Fig. 3A, B, C, D and E (6/6 Ig-BBz and Ig-28BBz, 1/6 1D3 28z(1-3), 4 CTX (no tumor) and 5

naïve). Mice were immunized following an injection of ovalbumin (OVA) admixed with Imject Alum adjuvant at D139, 2 months after last switch dose. B cells were analyzed in the peripheral blood of these mice by flow cytometry at D138 (right panel). Plasma was collected 2 weeks after immunization (D153) and an ELISA was performed to detect anti-OVA specific IgG1 (left panel). Mice that were not implanted with tumor but were only treated with CTX were used as a positive control of immunization. Means and SD are shown.

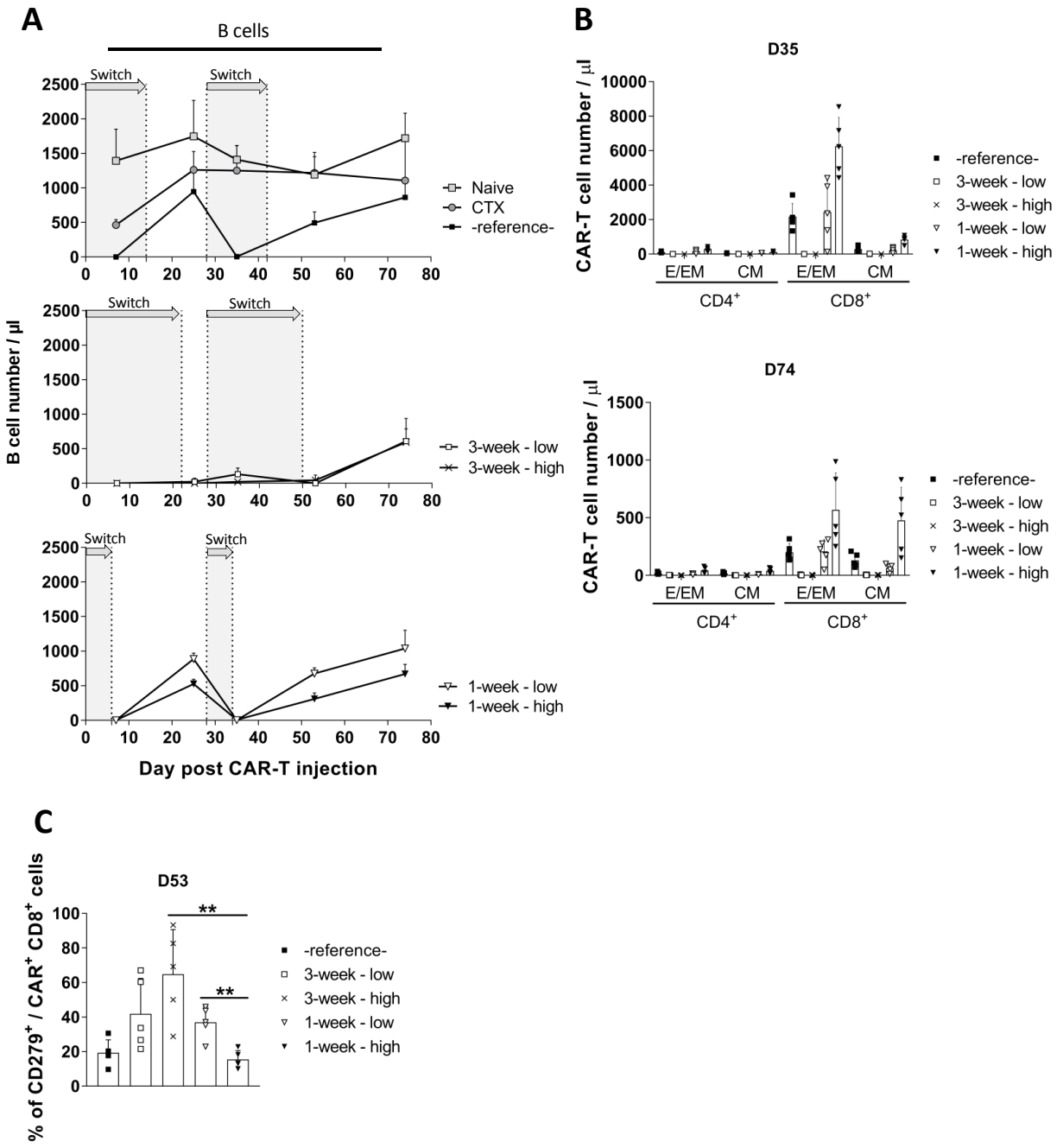


Fig. S4. Effect of switch dosing regimens on B cells and sCAR-T cell expansion and phenotype: B cell counts and sCAR-T cell phenotype analysis in the peripheral blood by flow cytometry. (A) Number of B cells / µl of peripheral blood over time as determined by flow cytometry. (B) (C) Numbers of sCAR-T cell subsets (gated on viable single cells, $CD45^+$, $CD3^+$, $sCAR^+$, $CD4^+$ or $CD8^+$) / µl blood are shown: $CD4^+$ or $CD8^+$ T effector / effector memory (E/EM) ($CD44^+CD62L^-$) and central memory (CM) ($CD44^+CD62L^+$)

subsets at D35 and D74 (B) and the percentage of $CD279^+$ ($PD\text{-}1^+$) cells among $sCAR^+$ $CD8^+$ T cells at D53 (C) are indicated. These results correspond to the study described in Fig. 4. A representative experiment of 2 experimental replicates is shown. Means and SD are shown. Statistical analyses using the Mann-Whitney test indicated significant differences at 95% CI. ** Statistical significance at $P<0.01$.

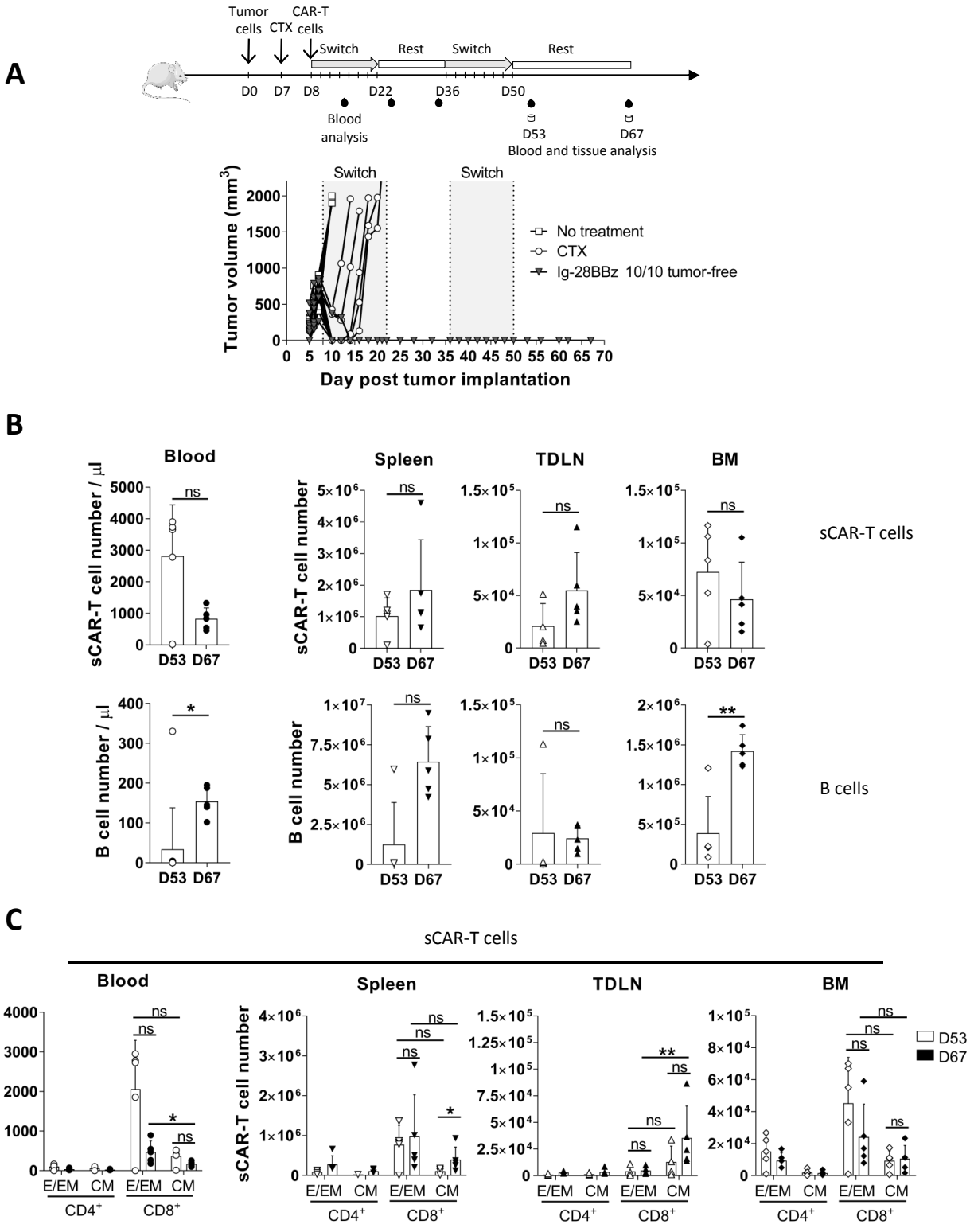

A
Tumor cells
CTX
CAR-T cells
Switch
Rest
Switch
Rest
D0
D7
D8
D22
D36
D50
Blood analysis
D53
D67
Blood and tissue analysis
Switch
Switch
Tumor volume (mm3)
No treatment
CTX
Ig-28BBz 10/10 tumor-free
Day post tumor implantation
B
Blood
Spleen
TDLN
BM
sCAR-T cell number / µl
sCAR-T cell number
B cell number / µl
B cell number
sCAR-T cells
B cells
D53 D67
ns
*
**
C
sCAR-T cells
Blood
Spleen
TDLN
BM
sCAR-T cell number / µl
sCAR-T cell number
E/EM CM E/EM CM
CD4+ CD8+
D53
D67

Fig. S5. sCAR-T cell trafficking in tissues. 38c13 tumor-bearing C3H mice were treated with cyclophosphamide prior to adoptive cell transfer with Ig-28BBz sCAR-T cells. Anti-murine CD19 switch injections (or PBS) were started at D8 and D36 for 8 doses (gray shading) every other day at 1 mg/kg, with a 2-week rest period (instead of 4- week rest period as in Fig. 5). Peripheral blood and tissues (spleen, tumor-draining inguinal lymph node (TDLN), bone marrow (BM)) were analyzed for B and sCAR-T cells at D15, D25, D35 for blood only, at D53 and D67 for blood and tissues (n=4-5). (A) The experimental setting is shown as well as the tumor growth kinetic. (B) Number of sCAR-T (top graphs) and B cells (bottom graphs) / µl of peripheral blood and in the spleen, TDLN node and BM of the Ig-28BBz treated mice as determined by flow cytometry at D53 and D67. (C) sCAR-T cell phenotype analysis in the peripheral blood, spleen, TDLN and BM, by flow cytometry at D53 and D67 (gated on viable single cells, $CD45^+$, $CD3^+$, $sCAR^+$, $CD4^+$ or $CD8^+$): $CD4^+$ or $CD8^+$ T effector / effector memory (E/EM) ($CD44^+CD62L^-$) and central memory (CM) ($CD44^+CD62L^+$) subsets. Data is shown as mean with SD. The results from one study are depicted. Refer to Fig. S6 for additional analysis regarding the outlier mouse at D53. Statistical analyses using the Mann-Whitney test indicated significant differences at 95% CI. * statistical significance at $P<0.05$; ** $P<0.01$; ns, not significant.

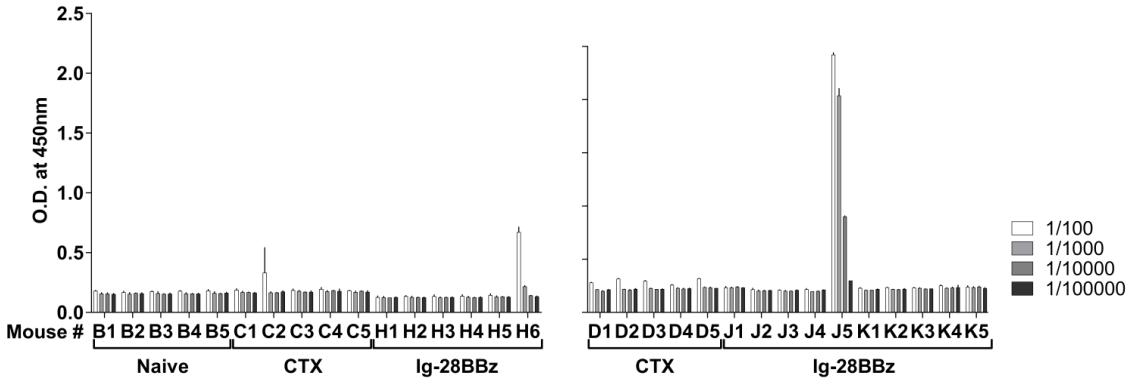


Fig. S6. Anti-switch antibody response in treated animals. Plasma from the experiments depicted in Fig. S3 (panels A-F) collected at day 139 (shown here in left panel) and from Fig. S5 at day 53 (shown here in the right panel) were tested for an antibody-drug-antibody response. Plasma samples from individual animals were subjected to a dilution of 1/100, 1/1000, 1/10000 or 1/100000 and incubated on plates coated with the anti-murine CD19 switch, followed by detection with an anti-murine antibody. The assay was developed by colorimetric readout and response shown as optical density (O.D.) at 450 nm. Mouse H6 from Ig-28BBz group in Fig. S3 in the 1:100 dilution and mouse J5 from Ig-28BBz group in Fig. S6 in the 1:10000 dilution exhibit an anti-switch titer (>2x above background). Notably these two mice were outliers in Fig. S3 and in Fig. S5 with regards to their inability to maintain B-cell depletion.

Table S1. Protein sequences of the switch heavy (HC) and light (LC) chains and of the different CAR/sCAR constructs used in the study.

Identity	Protein sequence
ch1D3 HC	QVQLVQSGAELVRPGTSVKLSCKVSGDTITFYYMHFVKQRPGQGLEWIGRIDPEDESTKYSEKFKNKATLTADTSSNTAYLKLSSLTSEDTATYFCIYGGY YFDYWGPGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKKVEPKSC
ch1D3 LC	NYHLENEVARLKKLGGGGSDIQLTQSPASLSTSLGETVTIQCQASEDIYSGLAWYQQKPGKSPQLLIYGASDLQDGVPSRFSGSGSGTQYSLKITSMQTEDE GVYFCQQGLTYPRTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKA DYEKHKVYACEVTHQGLSSPVTKSFNRGEC
mu1D3 HC	QVQLVQSGAELVRPGTSVKLSCKVSGDTITFYYMHFVKQRPGQGLEWIGRIDPEDESTKYSEKFKNKATLTADTSSNTAYLKLSSLTSEDTATYFCIYGGY YFDYWGPGTMVTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA HPASSTKVDKKIVPRDC
mu1D3 LC	NYHLENEVARLKKLGGGGSDIQLTQSPASLSTSLGETVTIQCQASEDIYSGLAWYQQKPGKSPQLLIYGASDLQDGVPSRFSGSGSGTQYSLKITSMQTEDE GVYFCQQGLTYPRTFGGGTKLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKD EYERHNSYTCEATHKTSTSPIVKSFNRNEC
1D3 28z(1-3)	DIQMTQSPASLSTSLGETVTIQCQASEDIYSGLAWYQQKPGKSPQLLIYGASDLQDGVPSRFSGSGSGTQYSLKITSMQTEDEGVYFCQQGLTYPRTFGGGT KLELKGGGGSGGGGSGGGGSEVQLQQSGAELVRPGTSVKLSCKVSGDTITFYYMHFVKQRPGQGLEWIGRIDPEDESTKYSEKFKNKATLTADTSSNTAY LKLSSLTSEDTATYFCIYGGYYFDYWGQGVMVTVSSIEFMYPPPYLDNERSNGTIIHIKEKHLCHTQSSPKLFWALVVVAGVLFCYGLLVTVALCVIWTNS RRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAYRPRAKFSRSAETAANLQDPNQLFNELNLGRREEFDVLEKKRARDPEMGGKQQRRRNPQEGV YNALQKDKMAEAYSEIGTKGERRRGKGHDGLFQGLSTATKDTFDALHMQTLAPR
28z(1-3)	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSIEFMYPPPYLDNERSNGTIIHIKEKHLCHTQSSPKLFWALVVVAGVLFCYGLLVTVA LCVIWTNSRRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAYRPRAKFSRSAETAANLQDPNQLFNELNLGRREEFDVLEKKRARDPEMGGKQQR RRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLFQGLSTATKDTFDALHMQTLAPR
Ig-28z(1-3)	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSESKYGPPCPPCPFWALVVVAGVLFCYGLLVTVALCVIWTNSRRNRGGQSDYMNMT PRRPGLTRKPYQPYAPARDFAAYRPRAKFSRSAETAANLQDPNQLFNELNLGRREEFDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAEAYS EIGTKGERRRGKGHDGLFQGLSTATKDTFDALHMQTLAPR
Ig-BBz(1-3)	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSESKYGPPCPPCPFWALVVVAGVLFCYGLLVTVALCVIWTKWIRKKFPHIFKQPFKK TTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSRSAETAANLQDPNQLFNELNLGRREEFDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAE AYSEIGTKGERRRGKGHDGLFQGLSTATKDTFDALHMQTLAPR
Ig-28BBz(1-3)	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSESKYGPPCPPCPFWALVVVAGVLFCYGLLVTVALCVIWTNSRRNRGGQSDYMNMT PRRPGLTRKPYQPYAPARDFAAYRPKWIRKKFPHIFKQPFKKTTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSRSAETAANLQDPNQLYNELNLGRREEFDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLFQGLSTATKDTFDALHMQTLAPR
Ig-BBz	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSESKYGPPCPPCPFWALVVVAGVLFCYGLLVTVALCVIWTKWIRKKFPHIFKQPFKK TTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMA EAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR
Ig-28BBz	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSESKYGPPCPPCPFWALVVVAGVLFCYGLLVTVALCVIWTNSRRNRGGQSDYMNMT PRRPGLTRKPYQPYAPARDFAAYRPKWIRKKFPHIFKQPFKKTTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSRSAETAANLQDPNQLYNELNLGRRE EYDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR
8-28z	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSTTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALL LSLIITLICNSRRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAYRPRAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQ QRRRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR
8-BBz	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSTTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALL LSLIITLICKWIRKKFPHIFKQPFKKTTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSRSAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGG KQQRRRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYDALHMQTLAPR
8-28BBz	DAVVTQESALTSSPGETVTLTCRSSTGAVTTSNYASWVQEKPDHLFTGLIGGTNNRAPGVPARFSGSLIGDKAALTITGAQTEDEAIYFCVLWYSDHWVFG GGTKLTVLGGGGGSGGGGSGGGGSGGGGSDVQLQESGPGLVAPSQSLSITCTVSGFLLTDYGVNWVRQSPGKGLEWLGVIWGDGITDYNSALKSRLSVT KDNSKSQVFLKMNSLQSGDSARYYCVTGLFDYWGQGTTLTVSSTTTKPVLRTPSPVHPTGTSQPQRPEDCRPRGSVKGTGLDFACDIYIWAPLAGICVALL LSLIITLICNSRRNRGGQSDYMNMTPRRPGLTRKPYQPYAPARDFAAYRPKWIRKKFPHIFKQPFKKTTGAAQEEDACSCRCPQEEEGGGGGYELRAKFSR SAETAANLQDPNQLYNELNLGRREEYDVLEKKRARDPEMGGKQQRRRNPQEGVYNALQKDKMAEAYSEIGTKGERRRGKGHDGLYQGLSTATKDTYD ALHMQTLAPR