ADVANCED SCIENCE
Open Access

Supporting Information

for *Adv. Sci.*, DOI 10.1002/advs.202305566

CAR-Aptamers Enable Traceless Enrichment and Monitoring of CAR-Positive Cells and Overcome Tumor Immune Escape

Hang Zhou, Tuersunayi Abudureheman, Wei-Wei Zheng, Li-Ting Yang, Jian-Min Zhu, Ai-Bin Liang, Cai-Wen Duan and Kaiming Chen**

Supporting Information

69451 Weinheim, Germany

CAR-aptamers Enable Traceless Enrichment and Monitoring of CAR-positive Cells and Overcome Tumor Immune Escape

Hang Zhou, Tuersunayi Abudureheman, Wei-Wei Zheng, Li-Ting Yang, Jian-Min Zhu, Ai-Bin Liang, Cai-Wen Duan*, Kaiming Chen*

Contents

Supporting Results

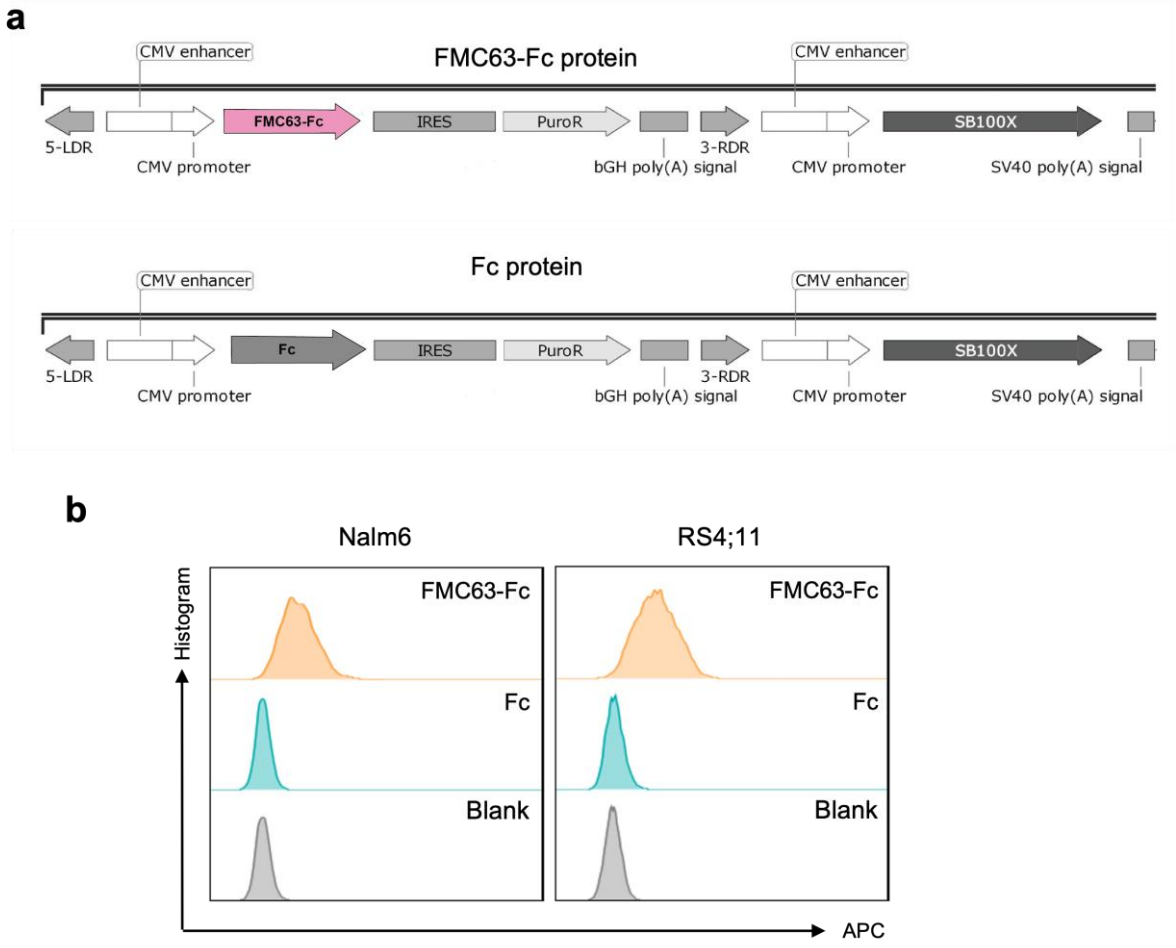


Figure S1. (a) The plasmids for FMC63-Fc and Fc protein expression. (b) Flow cytometry assays to verify binding to BALL cell lines of the expressed proteins.

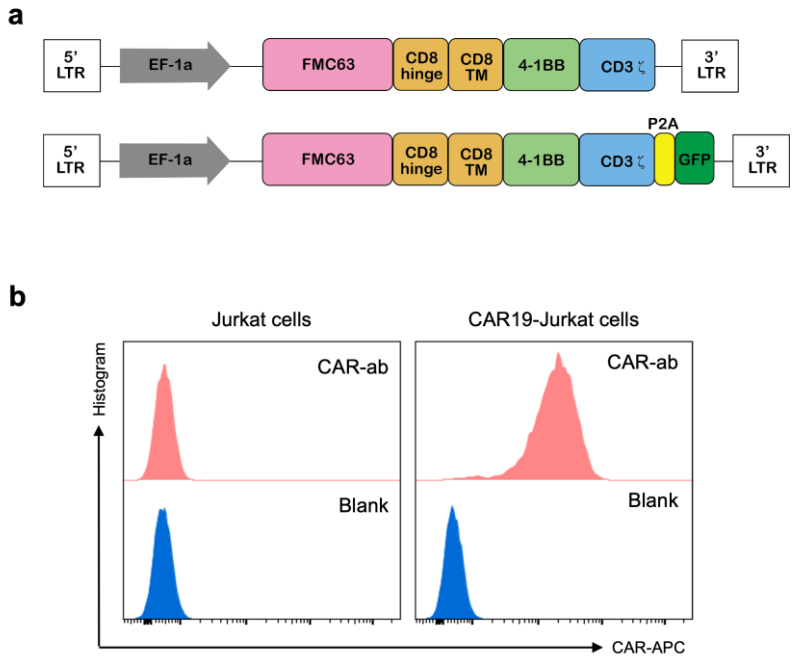


Figure S2. (a) The plasmids for CAR19 and CAR19-GFP. (b) Flow cytometry assays to validate the successful construction of CAR19-Jurkat cells.

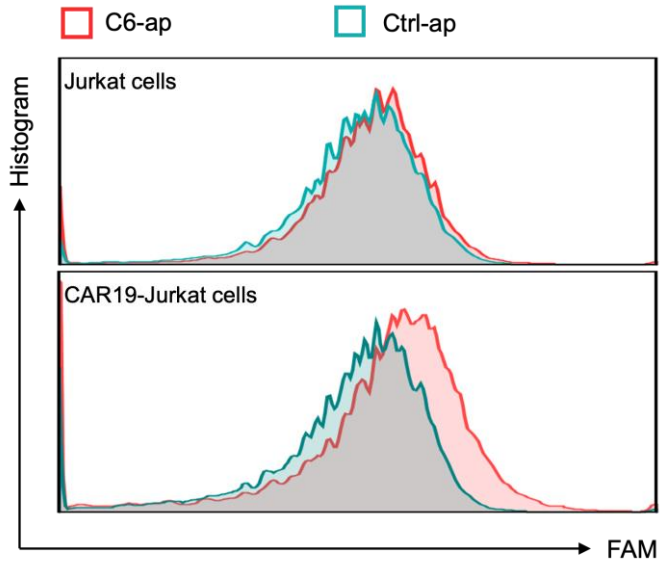


Figure S3. Flow cytometry assay to detect the binding of ssDNA pools (Ctrl-ap and c6-ap) to CAR19-Jurkat cells.

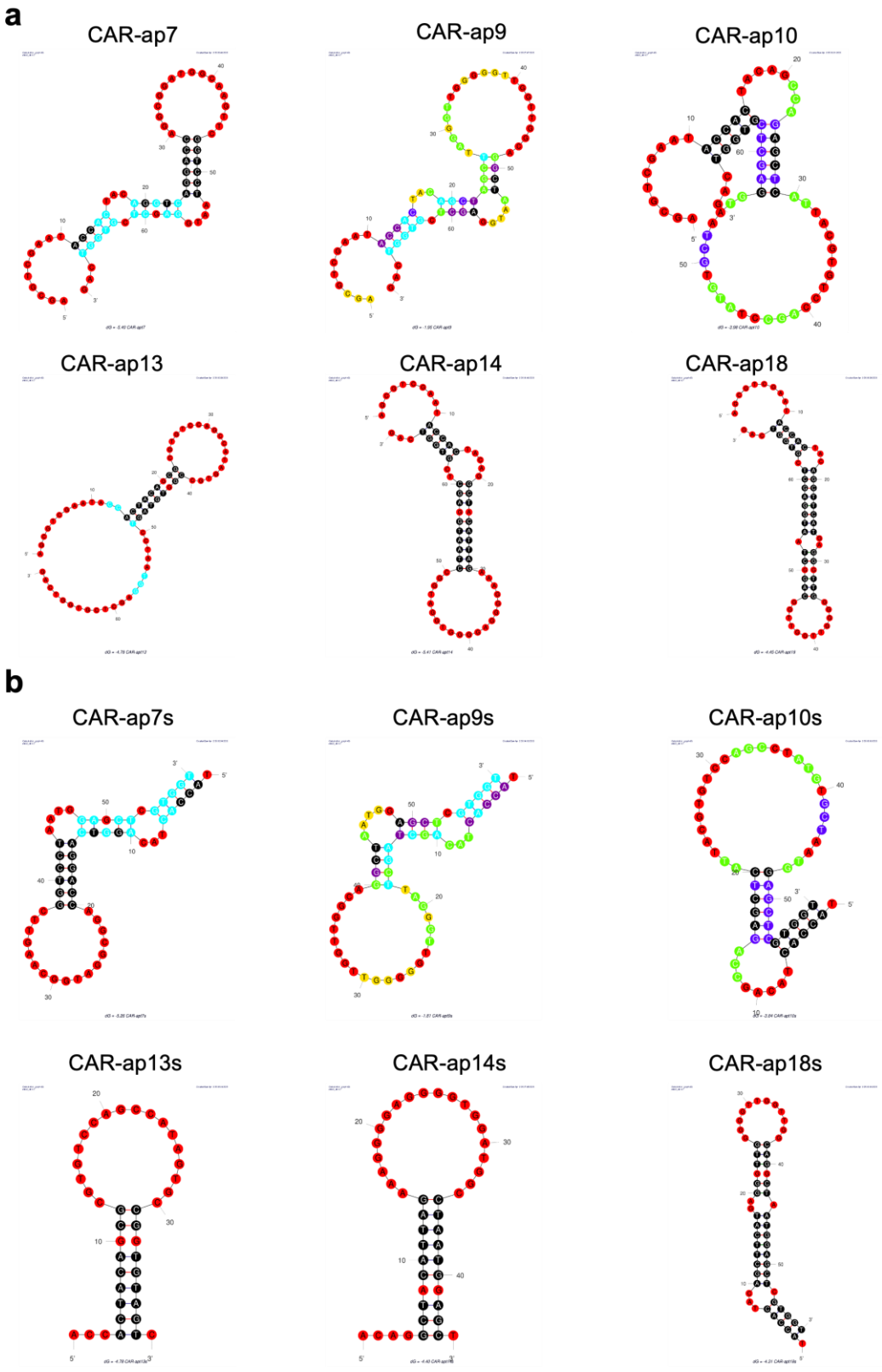

a
CAR-ap7
CAR-ap9
CAR-ap10
CAR-ap13
CAR-ap14
CAR-ap18
b
CAR-ap7s
CAR-ap9s
CAR-ap10s
CAR-ap13s
CAR-ap14s
CAR-ap18s

Figure S4. The predicted secondary structures of full-length CAR-ap (7, 9, 10, 13, 14, and 18) (a) and optimized CAR-ap (7s, 9s, 10s, 13s, 14s, and 18s) (b) aptamers by Mfold.

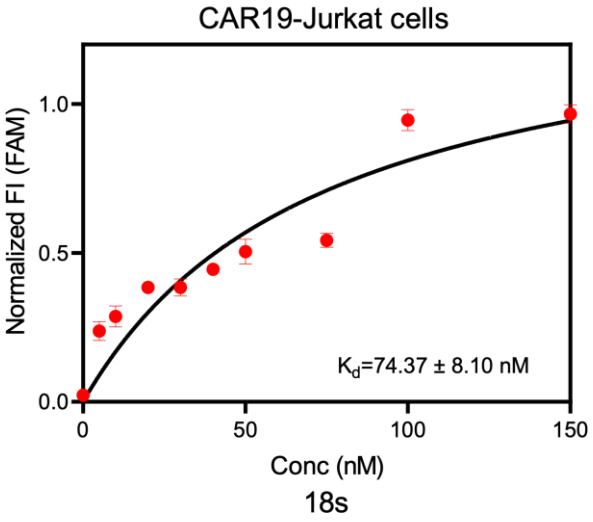


Figure S5. The binding affinity of CAR-ap18s to CAR19-Jurkat cells.

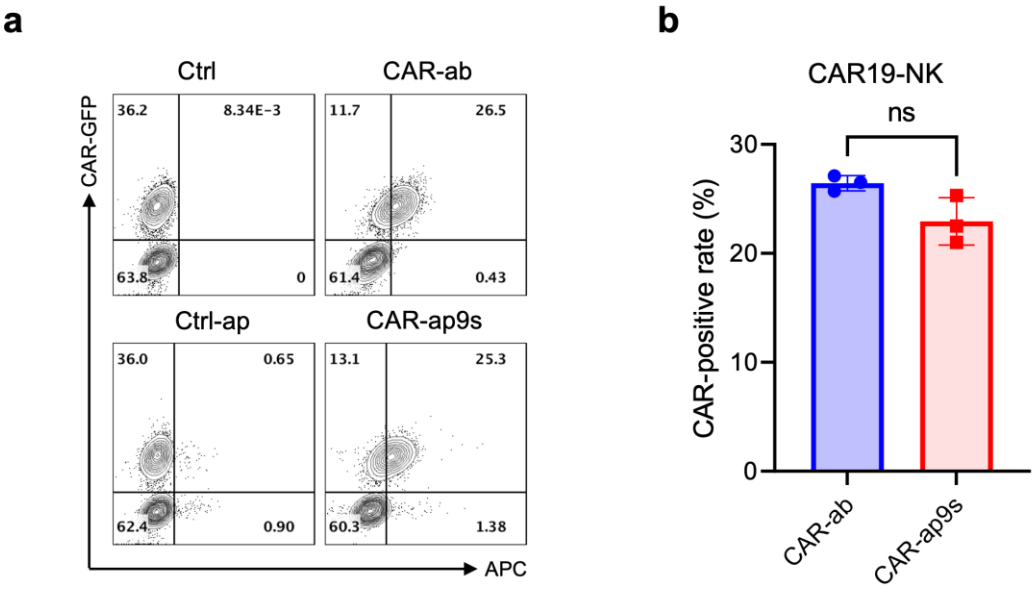


Figure S6. Flow cytometry assay of CAR-ab and CAR-ap9s aptamer binding to CAR19-NK92 cells. Left, flow cytometry plots representing three replicates (a). Right, the percentage of GFP^+ NK92 cells that were also positive for CAR-ab or CAR-ap9s aptamer binding (b).

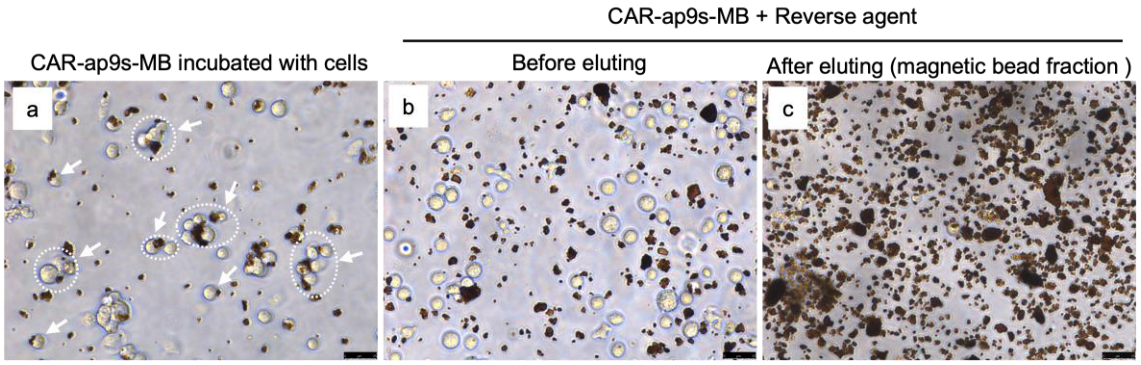


Figure S7. Microscopic observation of CAR19-T cell binding to the magnetic beads during cell sorting. (a) Magnetic beads loaded with aptamers bind to CAR-positive T cells and form cell clusters (white circles and arrows). (b) CAR-positive T cells are released from magnetic beads after adding the reverse agent and the cell clusters disappear. (c) CAR-positive T cells are eluted and completely separated from the magnetic bead fraction.

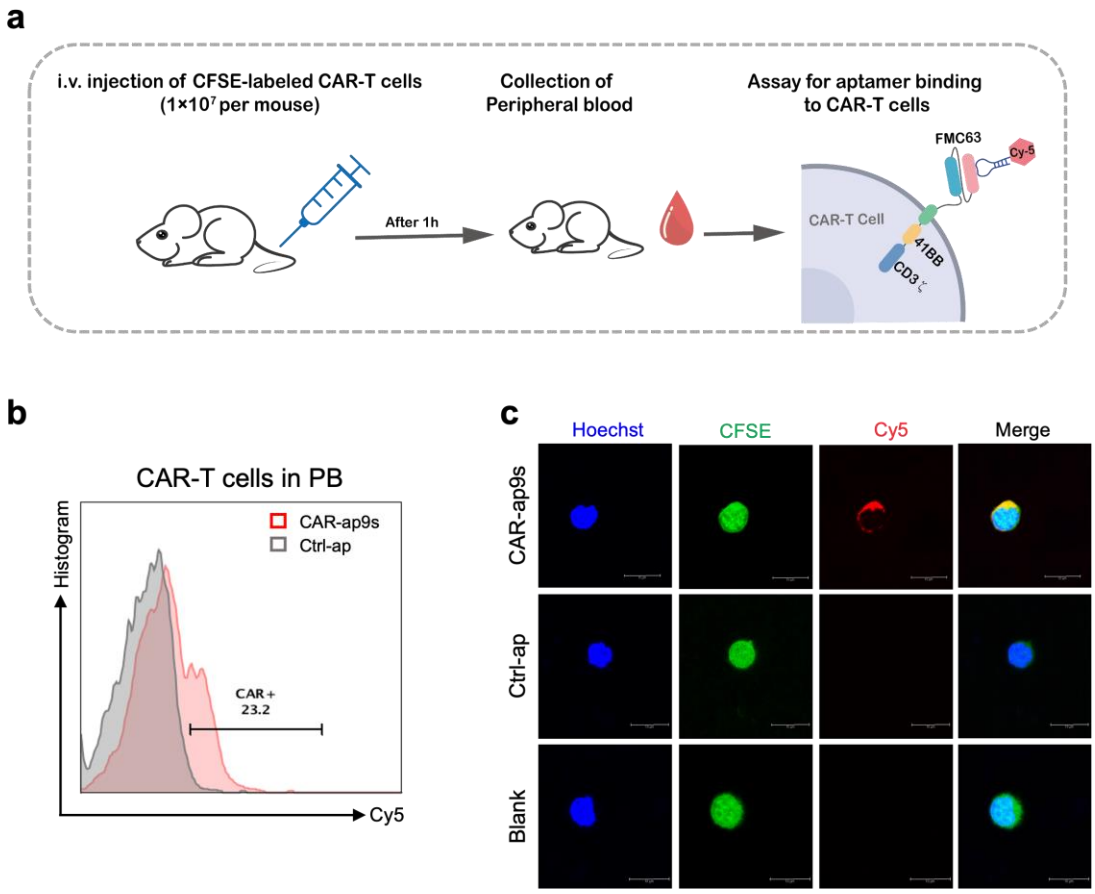


Figure S8. (a) Schematic of CAR-ap9s aptamer binding to CFSE-labeled CAR19-T cells in peripheral blood (PB). (b) Flow cytometry assay to detect CAR-ap9s aptamer and Ctrl aptamer binding to $CFSE^{+}$ CAR19-T cells in PB. (c) Fluorescence confocal microscopy of PB cells from CFSE-labeled CAR19-T cells injected into a mouse stained with CAR-ap9s-cy5 aptamer or Ctrl-ap-cy5 aptamer.

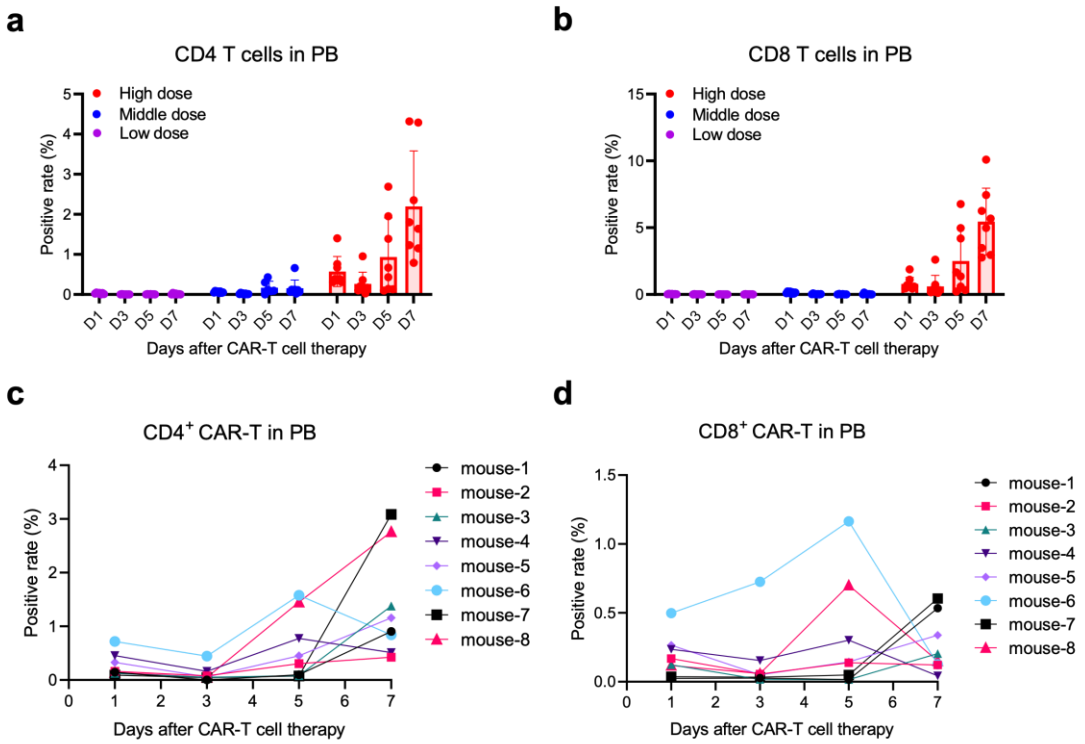


Figure S9. (a-b) Flow cytometry assay of the percentage of $CD4^+$ T cells (a) and $CD8^+$ T cells (b) in the PB of Nalm6-modeled mice treated with low-doses (purple), middle-doses (blue), and high-doses (red) of CAR19-T cells. (c-d) Flow cytometry detection of the expansion of $CD4^+CAR^+$ T cells (c) and $CD8^+CAR^+$ T cells (d) in the PB of each mouse in the high-dose group at different time points.

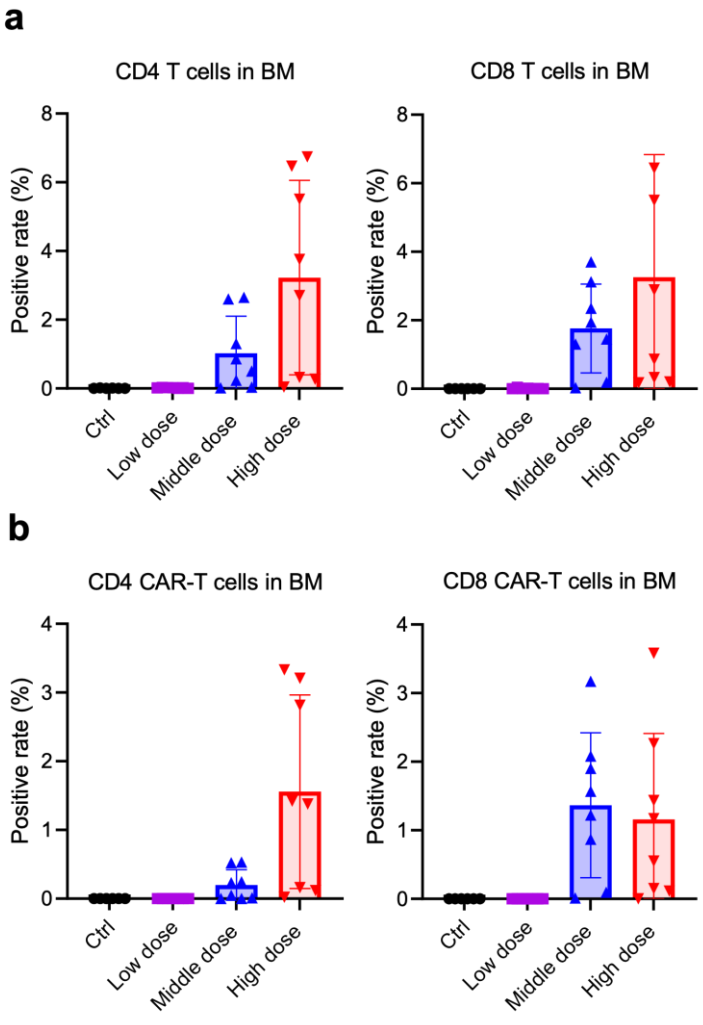


Figure S10. Flow cytometry assay of the percentage of $CD4^+$ T cells, $CD8^+$ T cells (a), $CD4^+CAR^+$ T cells, and $CD8^+CAR^+$ T cells (b) in the bone marrow of Nalm6-modeled mice on the eighth day after treatment with low-dose (purple), middle-dose (blue), and high-dose (red) CAR19-T cells.

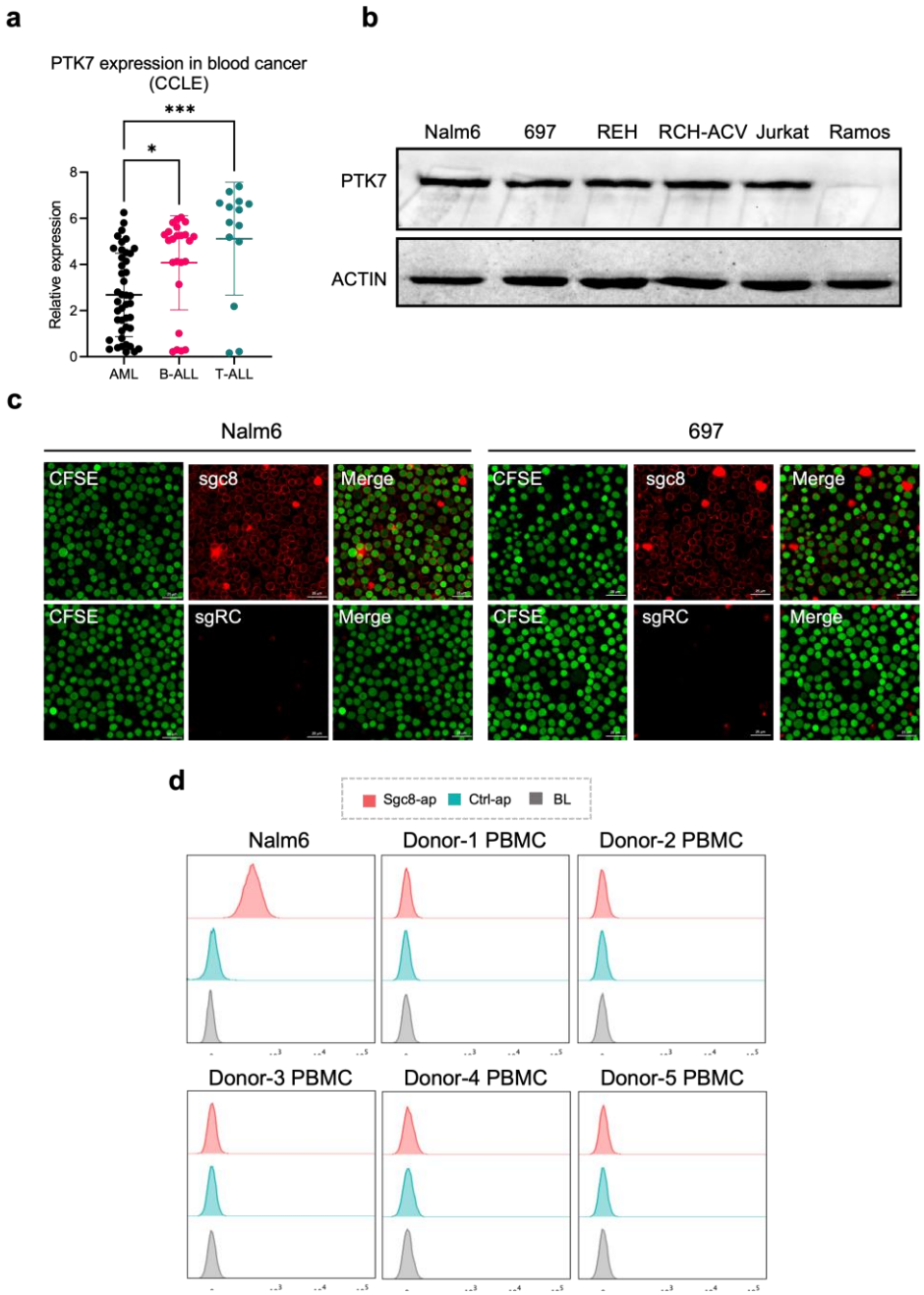


Figure S11. (a) Expression of PTK7 in AML, B-ALL, and T-ALL tumor cell lines and data from the Cancer Cell Line Encyclopedia (CCLE). (b) PTK7 expression in B-ALL cell lines was detected by Western blot. Jurkat cells and Ramos cells were used as positive and negative control, respectively. (c) Fluorescence confocal microscopy of Sgc8-cy5 aptamer binding to Nalm6 cells and 697 cells. (d) Flow cytometry assay of sgc8 aptamer (sgc8-ap) binding to Nalm6 cells and healthy donors' PBMCs.

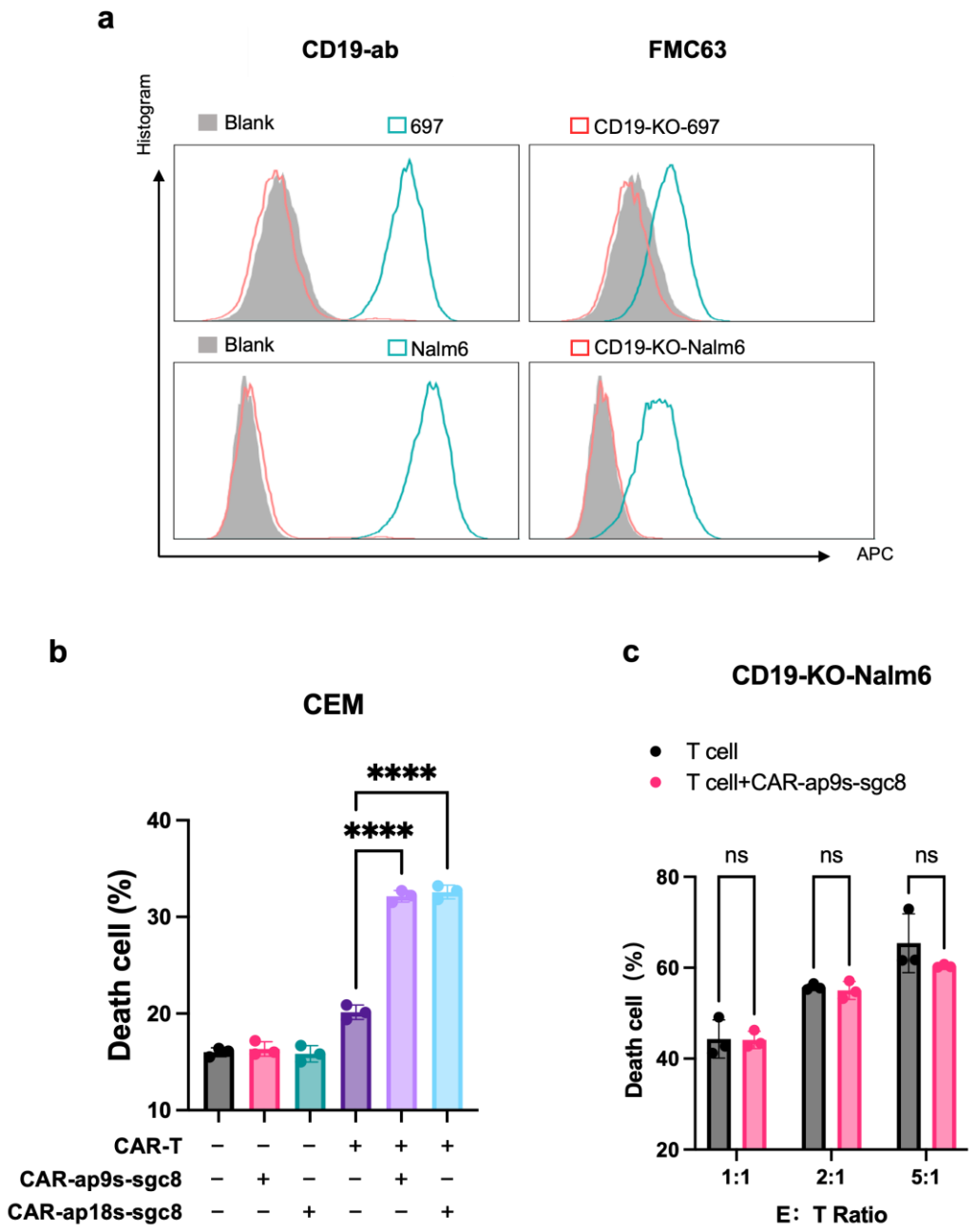
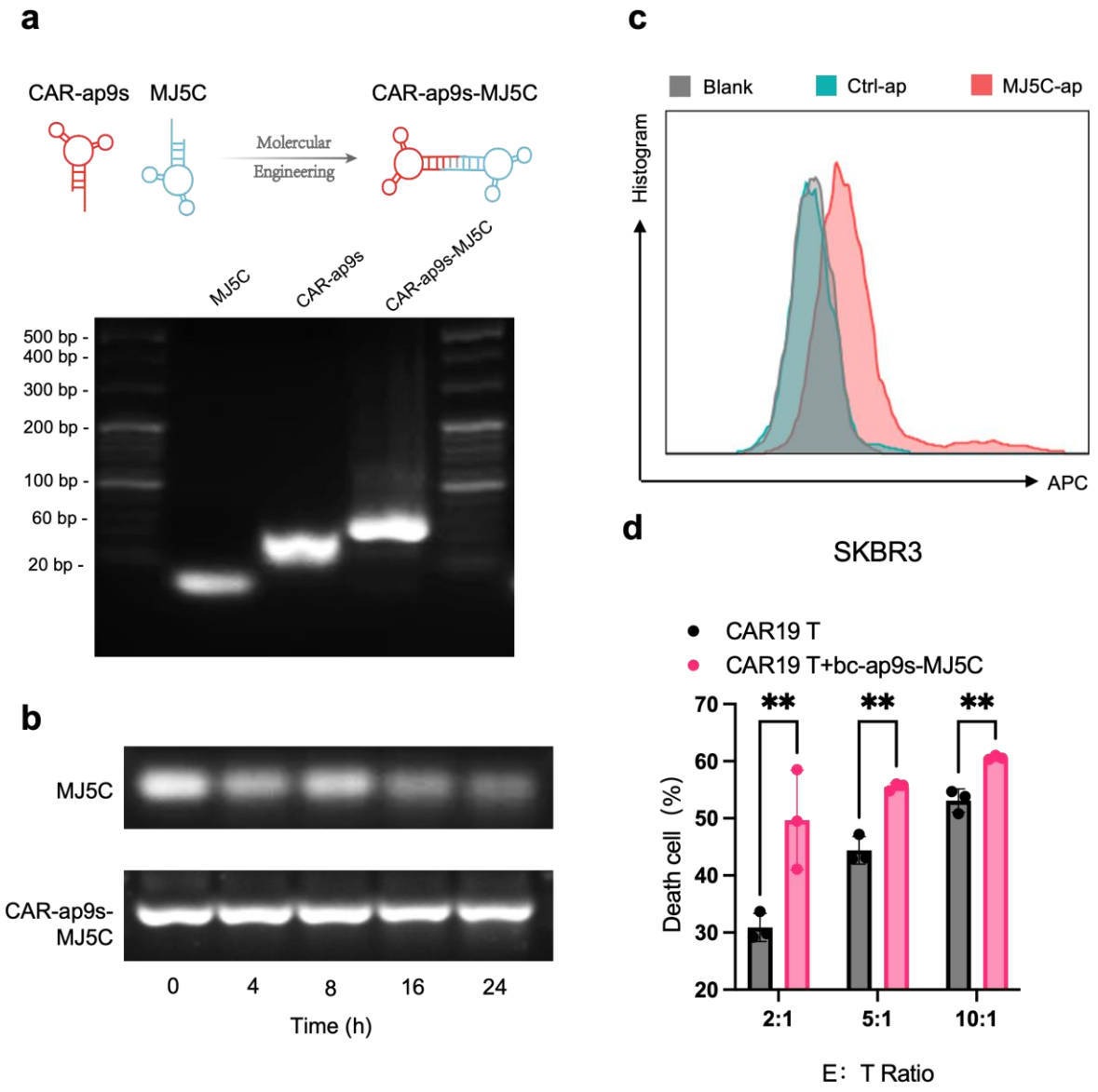


Figure S12. (a) The binding of commercial antibody CD19-ab or self-prepared FMC63 protein to CD19-KO-697 or CD19-KO-Nalm6 cells was determined by flow cytometry. (b) Evaluation of CAR19-T cell killing against CEM cells mediated by CAR-ap9s-sgc8 and CAR-ap18s-sgc8. (c) Detection of basal nonspecific killing of CD19-KO-Nalm6 cells by T cells.



Figures S13. (a) Schematic illustrating the construction of bispecific circular aptamer, CAR-ap-MJ5C. Agarose gel electrophoresis of ssDNA CAR-ap9s, ssDNA MJ5C, and CAR-ap9s-MJ5C. (b) The stability of ssDNA MJ5C and CAR-ap9s-MJ5C in FBS was determined by agarose gel electrophoresis at 37 ℃ for the different incubation times. (c) Flow cytometry assay of the MJ5C aptamer (MJ5C-ap) binding to SKBR3 cells. (d) *In vitro* antitumor cytotoxicity of CAR19-T cells to SKBR3 cells at different E : T ratios in the presence or absence of CAR-ap-MJ5C.

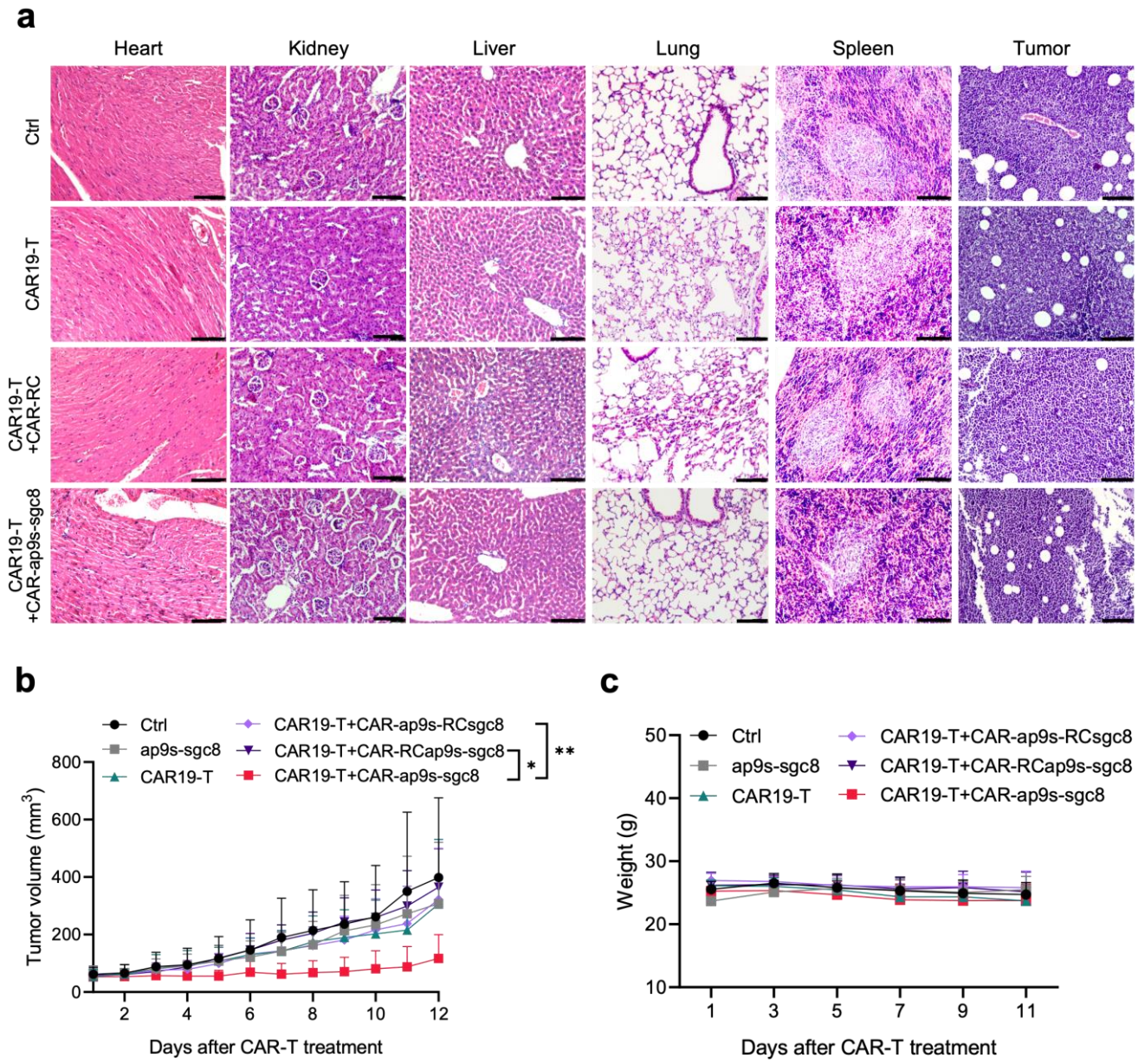


Figure S14. (a) H&E staining of tissue sections from CEM tumor xenografts after treatment. Scale bar: 100 μm. (b) Average tumor growth kinetics of mice under different treatments. Tumor volume (mm^3) was monitored daily using the caliper method. Data are presented as means ± s.d. (n=5). Statistical analyses were performed using two-tailed paired Student's t-tests (*p <0.05, **p<0.01, ***p <0.001, n.s., not significant). NSG mice (n=5 per group) were inoculated in the flank with CD19-KO-Nalm6 tumor cells (1×10^7) on day 14. After two weeks of tumor establishment, CAR19-T cells (1×10^7) were administered intravenously (i.v.) to tumor-bearing NSG mice on day 0. Different groups of mice received different intratumoral (i.t.) treatments on day 1 to day 5 (50μL of PBS for CTRL and CAR19-T groups, bc-RCap9s-sgc8, ap9s-RCsgc8, or bc-ap9s-sgc8). (c) Body weight was monitored and recorded. Data are presented as means ± s.d. (n=5).

SUPPORTING INFORMATION

Table S1. Sequences in this work

Name	Sequence(5'-3')
ssDNA pool	AGCGTCGAATACCACTACAGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTAATGGAGCTCGTGGTCAG
Primer-F	AGCGTCGAATACCACTACAG
Primer-R	Biotin-CTGACCACGAGCTCCATTAG
CAR-ap7	AGCGTCGAATACCACTACAGGTCAGGACCAGGCGGATGGCAAGTTCGGTCCTAATGGAGCTCGTGGTCAG
CAR-ap9	AGCGTCGAATACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGTCAG
CAR-ap10	AGCGTCGAATACCACTACAGCCAGAGCTCATTACGTGTCCAGCCTATGTGCTAATGGAGCTCGTGGTCAG
CAR-ap13	AGCGTCGAATACCACTACAGCGCGTGTCCAGCCATAGTGCCGGTGTAGTCCTAATGGAGCTCGTGGTCAG
CAR-ap14	AGCGTCGAATACCACTACAGGCTACATTAGAAAGGGGAGGGGTGGATGGCCTAATGGAGCTCGTGGTCAG
CAR-ap18	AGCGTCGAATACCACTACAGCTTCATGAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGTCAG
CAR-ap7s	TACCACTACAGGTCAGGACCAGGCGGATGGCAAGTTCGGTCCTAATGGAGCTCGTGGT
CAR-ap9s	TACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
CAR-ap10s	TACCACTACAGCCAGAGCTCATTACGTGTCCAGCCTATGTGCTAATGGAGCTCGTGGT
CAR-ap13s	ACCACTACAGCGCGTGTCCAGCCATAGTGCCGGTGTAGTC
CAR-ap14s	ACAGGCTACATTAGAAAGGGGAGGGGTGGATGGCCTAATGGAGCT
CAR-ap18s	TACCACTACAGCTTCATGAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT

Sgc8	GTCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAC
Ctrl-ap（sgRC）	GTCTAACCGTACAGTATTTTCCCGGCGGCGCAGCAGTTAGAC
CAR-ap9s-FAM/Cy5	FAM/Cy5-TACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
CAR-ap18s-FAM/Cy5	FAM/Cy5-TACCACTACAGCTTCATGAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
Sgc8-FAM/Cy5	FAM/Cy5-GTCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAC
Ctrl-ap-FAM/Cy5	FAM/Cy5-GTCTAACCGTACAGTATTTTCCCGGCGGCGCAGCAGTTAGAC
（sgRC-FAM/Cy5）	
sgc8-13L-2-5P-INBIO	Phosphate- TGACTGAT/iBiodT/TACG GTCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAC
CAR-ap9s-13L-1-5P	Phosphate- CGTAAATCAGTCA ACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
MJ5C-13L-1-5P	Phosphate- CGTAAATCAGTCA ACAGGTTCTGGGGGGTGGGTGGGGAACCTGT

sgc8-13L-1-5P	Phosphate-CGTAAATCAGTCAGTCTAACTGCTGCGCCGCCGGGAAAATACTGTACGGTTAGAC
CAR-ap9S-13L-2-5P	Phosphate-TGACTGATTTACGACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
RC9S-13L-2-5P	Phosphate-TGACTGATTTACGACCACGAGCTCCATTAGCCTGCCAACCAACCCCCAACCCTAAGCTAGCTGTAGTGGT
RCsgc8-13L-1-5P	Phosphate-CGTAAATCAGTCAGTCTAACCGTACAGTATTTTCCCGGCGGCGCAGCAGTTAGAC

Table S2. Sequences of aptamers and reversal agent (RA) used in the cell sorting experiments

Name	Sequence(5'-3')
CAR-ap9s-biotin	Biotin-TACCACTACAGCTAGCTTAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
CAR-ap18s-biotin	Biotin-TACCACTACAGCTTCATGAGGGTTGGGGGTTGGTTGGCAGGCTAATGGAGCTCGTGGT
RA-CAR-ap9s	ACCACGAGCTCCATTAGCCTGCCAACCAACCCCCAACCCTAAGCTAGCTGTAGTGGTA

RA-CAR-ap18s	ACCACGAGCTCCATTAGCCTGCCAACCAACCCCCAACCCTCATGAAGCTGTAGTGGTA

Table S3. Sequences of CRISPR/cas9 sgRNA for CD19 knockout

Name	Sequence
sgRNA-CD19-Oligo 1	CACCGTTCCTCGGGCCTGACTTCCA
sgRNA-CD19-Oligo 2	CTGGAAGTCAGGCCCGAGGAACAAA