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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	MACSQuantify Software, BD FACSDiva V8.0.1, Attune NxT Software v2.6, MiSeq Control Software v2.6.2.1, FortéBio Octet Data Acquisition Software, Bio-Plex Manager 6.1, Packard TopCount NXT Software, Living Image 4.5.
Data analysis	FlowJo V10, GraphPad Prism 7, Strawberry Perl 5.28.0.1, FASTAptamer v1.0.3, Ninja v1.2.1, FigTree v1.4.3, MEME Suite web application, NUPACK web application, FortéBio Octet Data Analysis 9.0 Software, Microsoft Excel 2016, NanoString nSolver 4.0, NanoString Advanced Analysis 2.0, R 3.3.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the main findings of this study are available within the paper and its Supplementary Information. All data generated for this study and relevant information are available from the corresponding authors upon reasonable request. The NanoString nCounter data have been deposited in the NCBI Gene Expression Omnibus (GEO), with accession code GSE130185.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Cells were derived from three donors for all T-cell studies, which is standard for the field given the cost and time associated with manufacturing CAR-T cells. No sample-size calculation was performed for animal studies. However, prior 'CAR stress tests' from the literature have used 5–14 mice per group (Zhao & Condomines et al., Cancer Cell, 2015), so 9 mice per treatment group (3 mice per donor) seemed reasonable. Furthermore, it was imperative to use the same number of mice per T-cell donor to prevent donor bias. Thus, the number of mice used per treatment group was restricted by the amount of CAR-T cells available in the donor with the lowest cell number after cell expansion.
Data exclusions	PBMC donors with low CD8 percentages were excluded from cell-isolation data analysis due to yielding insufficient cells required for flow-cytometry analysis, NanoString analysis, CAR-T cell production, and subsequent in vitro and in vivo effector function assays. A pre-exclusion criteria of yielding at least 15M CD8+ T cells from 200M PBMCs (both for antibody and traceless aptamer selection) was pre-established prior to carrying out the cell-isolation studies.
Replication	Attempts at replication were highly successful. Three independent experiments, or three biologically independent samples when appropriate, were performed for nearly all data presented in the main figures. Furthermore, three independent researchers performed the siRNA knockdown and flow-cytometry binding-curve experiments months apart with highly reproducible and consistent results.
Randomization	On day 6 post-tumour inoculation, mice were arranged into groups of 3 mice each for each donor and treatment group such that the average photon flux of the pre-established systemic tumours was approximately equal across all groups.
Blinding	Investigators were not blinded to group allocation during the assessment of tumour-imaging and tumour-survival endpoints; however, the efficacy of treatments and survival outcomes were readily apparent in tumour-flux quantification and in the behavior of mice.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

The SELEX library and all aptamers described in this study can be commercially synthesized by Integrated DNA Technologies with the necessary modifications using their 'Custom DNA oligos' service. The PLAT-02 lentiviral vector was kindly provided by City of Hope and is not commercially available. All other reagents are commercially available.

Antibodies

Antibodies used

The following dyes, antibodies, and secondaries were used to stain cells: Zombie Violet (1:500 in 100 µL/10^6 cells, BioLegend), Zombie Yellow (1:500 in 100 µL/10^6 cells, BioLegend), APC anti-human CD4 (1:100, 300514, BioLegend), PerCP/Cy5.5 anti-human CD8a (1:100, 301031, BioLegend), APC anti-human CD8a (rhesus cross-reactivity, 1:100, 301014, BioLegend), CD8-biotin (1:100, 130-098-556, Miltenyi), anti-mouse CD16/CD32 Fc block (1:100, 14-0161-86, eBioscience), FITC anti-mouse CD3e (1:50, 100305, BioLegend), BV421 anti-mouse CD8a (1:50, 100737, BioLegend), FITC mouse anti-human CD3e (rhesus cross-reactivity, 1:20, 55611, BD Biosciences), Purified anti-human CD3 (Clone UCHT1, 300402, BioLegend), Purified anti-human CD8a (Clone RPA-T8, 301002, BioLegend), Super Bright 600 anti-human CD19 (1:20, 63-0198-42, eBioscience), Super Bright 702 anti-human CD56 (1:100, 67-0566-42, eBioscience), PE anti-human CD3 (1:100, 300308, BioLegend), APC/Cy7 anti-human CD14 (1:40, 325619, BioLegend), FITC anti-human CD16 (1:50, 302006, BioLegend), Alexa Fluor 700 anti-human CD3 (1:50, 300424, BioLegend), Brilliant Violet 785 anti-human CD4 (1:50, 317442, BioLegend), PE/Cy7 anti-human CD8a (1:200, 300914, BioLegend), BUV737 mouse anti-human CD45RA (1:25, 564442, BD Biosciences), BUV395 mouse anti-human CD45RO (1:25, 564291, BD Biosciences), PE anti-human CD62L (1:400, 304806, BioLegend), Brilliant Violet 421 anti-human CCR7 (1:25, 353208, BioLegend), Erbitux-biotin (1:500, Jensen Lab), PE-Cy7 mouse anti-Ki-67 (1:20, 561283, BD Biosciences), BUV737 mouse anti-human PD-1 (1:20, 565299, BD Biosciences), Brilliant Violet 785 anti-human TIM-3 (1:20, 345032, BioLegend), PE mouse anti-human LAG-3 (1:20, 565616, BD Biosciences), Brilliant Violet 785 anti-human CD45RA (1:160, 304140, BioLegend), NeutrAvidin Protein DyLight 633 (1:500, 22844, Invitrogen), Alexa Fluor 647 Streptavidin (1:500, 405237, BioLegend), and PE Streptavidin (1:500, 405204, BioLegend). OneComp eBeads (Invitrogen) were used to prepare single-color controls for compensation, if needed. Stained samples were analyzed with either a MACSQuant Analyzer 10 (Miltenyi), Attune NxT (Invitrogen), or BD LSRFortessa (BD Biosciences) flow cytometer.

Validation

Antibodies were either titrated on an appropriate cell type prior to studies reported herein to determine the optimal dilution for staining or diluted according to the manufacturer's instructions.

Links to manufacturer's validation statements of species and application:
Zombie Violet (BioLegend, Cat# 423114): https://www.biolegend.com/en-us/products/zombie-violet-fixable-viability-kit-9341
Zombie Yellow (BioLegend, Cat# 423104): https://www.biolegend.com/en-us/products/zombie-yellow-fixable-viability-kit-8514
APC anti-human CD4 (BioLegend, Cat# 300514): https://www.biolegend.com/en-us/products/apc-anti-human-cd4-antibody-823
PerCP/Cy5.5 anti-human CD8a (BioLegend, Cat# 301031): https://www.biolegend.com/en-us/products/percpcyanine55-anti-human-cd8a-antibody-4222
APC anti-human CD8a (BioLegend, Cat# 301014): https://www.biolegend.com/en-us/products/apc-anti-human-cd8a-antibody-831
CD8-biotin (Miltenyi, Cat# 130-098-556): https://www.miltenyibiotec.com/_Resources/Persistent/b0ddfd194c359e08b38b5d29e1dd21e1510e2017/80.pdf
anti-mouse CD16/CD32 Fc block (eBioscience, Cat# 14-0161-86): https://www.thermofisher.com/antibody/product/CD16-CD32-Antibody-clone-93-Monoclonal/14-0161-86
FITC anti-mouse CD3e (BioLegend, Cat# 100305): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd3epsilon-antibody-23
BV421 anti-mouse CD8a BioLegend, Cat# 100737): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd8a-antibody-7138
FITC mouse anti-human CD3e (BD Biosciences, Cat# 55611): http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-non-human-primate-antibodies/cell-surface-antigens/fitc-mouse-anti-human-cd3-sp34/p/556611
Purified anti-human CD3 (BioLegend, Cat# 300402): https://www.biolegend.com/en-us/products/purified-anti-human-cd3-antibody-867
Purified anti-human CD8a (BioLegend, Cat# 301002): https://www.biolegend.com/en-us/products/purified-anti-human-cd8a-antibody-839
Super Bright 600 anti-human CD19 (eBioscience, Cat# 63-0198-42): https://www.thermofisher.com/antibody/product/CD19-Antibody-clone-SJ25C1-Monoclonal/63-0198-42
Super Bright 702 anti-human CD56 (eBioscience, Cat# 67-0566-42): https://www.thermofisher.com/antibody/product/CD56-NCAM-Antibody-clone-TULY56-Monoclonal/67-0566-42
PE anti-human CD3 (BioLegend, Cat# 300308): https://www.biolegend.com/en-us/products/pe-anti-human-cd3-antibody-753
APC/Cy7 anti-human CD14 (BioLegend, Cat# 325619): https://www.biolegend.com/en-us/products/apc-cy7-anti-human-cd14-antibody-3959
FITC anti-human CD16 (BioLegend, Cat# 302006): https://www.biolegend.com/en-us/products/fitc-anti-human-cd16-antibody-567
Alexa Fluor 700 anti-human CD3 (BioLegend, Cat# 300424): https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-human-cd3-antibody-3394
Brilliant Violet 785 anti-human CD4 (BioLegend, Cat# 317442): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd4-antibody-7978
PE/Cy7 anti-human CD8a (BioLegend, Cat# 300914): https://www.biolegend.com/en-us/products/pe-cy7-anti-human-cd8a-antibody-1916
BUV737 mouse anti-human CD45RA (BD Biosciences, Cat# 564442): https://www.bdbiosciences.com/eu/reagents/research/

antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv737-mouse-anti-human-cd45ra-hi100/p/564442
BUV395 mouse anti-human CD45RO (BD Biosciences, Cat# 564291): http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv395-mouse-anti-human-cd45ro-uchl1/p/564291
PE anti-human CD62L (BioLegend, Cat# 304806): https://www.biolegend.com/en-us/products/pe-anti-human-cd62l-antibody-653
Brilliant Violet 421 anti-human CCR7 (BioLegend, Cat# 353208): https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-cd197-ccr7-antibody-7497
Erbitux-biotin (Jensen Lab): Reactivity to human EGFR. Validated applications are flow cytometry and MACS (see main text).
PE-Cy7 mouse anti-Ki-67 (BD Biosciences, Cat# 561283): http://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/pe-cy7-mouse-anti-ki-67-b56/p/561283
BUV737 mouse anti-human PD-1 (BD Biosciences, Cat# 565299): https://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-human-antibodies/cell-surface-antigens/buv737-mouse-anti-human-cd279-pd-1-eh121-also-known-as-eh12/p/565299
Brilliant Violet 785 anti-human TIM-3 (BioLegend, Cat# 345032): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd366-tim-3-antibody-11965
PE mouse anti-human LAG-3 (BD Biosciences, Cat# 565616): http://www.bdbiosciences.com/eu/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/pe-mouse-anti-human-lag-3-cd223-t47-530/p/565616
Brilliant Violet 785 anti-human CD45RA (BioLegend, Cat# 304140): https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-human-cd45ra-antibody-7972
NeutrAvidin Protein DyLight 633 (Invitrogen, Cat# 22844): https://www.thermofisher.com/antibody/product/NeutrAvidin-Protein/22844
Alexa Fluor 647 Streptavidin (BioLegend, Cat# 405237): https://www.biolegend.com/en-us/products/alexa-fluor-647-streptavidin-9305
PE Streptavidin (BioLegend, Cat# 405204): https://www.biolegend.com/en-us/products/pe-streptavidin-1475
OneComp eBeads (Invitrogen): https://www.thermofisher.com/order/catalog/product/01-1111-41

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	J.RT3-T3.5, Jurkat Clone E6-1, K562, K562 + OKT3, K562 + CD19, and Raji cell lines were purchased from ATCC or derived from cell lines purchased from ATCC. TM-LCLs were made from PBMCs as previously described (Pelloquin et al. 1986). PBMCs were isolated from TRIMA LRS Chambers that were purchased from Bloodworks Northwest. Mixed and pure CD8+ T cells used in non-isolation experiments were a generous gift from Juno Therapeutics. Rhesus PBMCs were a generous gift from Dr. Agne Taraseviciute (Seattle Children's Research Institute).
Authentication	Cell-line authentication information was provided by the vendor, and authentication was conducted using short-tandem repeat genotyping.
Mycoplasma contamination	The cell lines were tested by the vendor and also tested monthly. The cell lines were found to be negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Tg(Aldh1l1-EGFP,-DTA)D8Rth/J stock breeders were purchased from The Jackson Laboratory (JAX Stock No. 026033), and the mice used for spleen harvesting were male mice 20-weeks old. All animal experiment protocols, handling and reporting for these mice were conducted in compliance with the Institutional Animal Care and Use Committee (IACUC) at the University of Washington (UW) under protocol number 4053-01. NSG mice stock breeders were purchased from The Jackson Laboratory (JAX Stock No. 005557), and the mice used in the in vivo study were F1 produced female mice 9-to-11-weeks old at the start of the experiment. All animal experiment protocols, handling, and reporting for these mice were conducted in compliance with the IACUC at Seattle Children's Research Institute (SCRI) under protocol number 13853. Details are described in Methods section.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Sample preparation is described in detail in Methods.
Instrument	MACSQuant Analyzer 10 (Miltenyi), Attune NxT (Invitrogen), or BD LSRFortessa (BD Biosciences)
Software	Flow-cytometry data were collected with either MACSQuantify Software, BD FACSDiva V8.0.1, or Attune NxT Software v2.6. Flow-cytometry data were analyzed with FlowJo V10.
Cell population abundance	Flow cytometry was used for quantification purposes only; no post-sorting fractions were collected.
Gating strategy	Briefly, the desired cell population was selected by using forward and side scatter. Single cells were selected by using forward scatter area and height linearity. Live cells were selected as defined by Zombie Violet or Zombie Yellow negativity. Supplementary Fig. 10 illustrates further downstream gating strategies for select samples.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.