nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the 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
☐	☒ A statement on 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 statistical parameters 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

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Confocal images were collected using NIS-Elements AR v 5.11.01. Microscopy images from TIMING were collected using ZEN PRO v2.6. Flow cytometry readings were collected using FlowJo v10.8. Mice images using IVIS were collected using Living Image v2.50.1.
Data analysis	Confocal images were analyzed using the following ImageJ plugins: Subtract background, 3D objects counter, TrackMate. ScRNAseq FASTQ files obtained from sequencing core were processed using CellRanger v6.0. ScRNAseq data was analyzed in R v4.0.1 with the following packages: Seurat v4.1, SAVER v1.1.2, GSVA v0.3.4, Monocle v2.26. TIMING tracking and images were analyzed using a custom pipeline developed by the Varadarajan lab.

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The scRNA-seq of the infusion products is available under GEO accession number GSE208052 and migratory CAR T-cell scRNA-seq is available under GEO accession number GSE253872. External datasets used for CD8-fit signature validation were accessed under GEO accession numbers GSE197268 and GSE151511 and EMBL-EBI accession number E-MTAB-11536. The external dataset used for T-cell persistence was accessed via dbGaP under Study Accession phs002966.v1.p1. The healthy donor T-cell scRNA-seq was accessed under GSE201035. Source data for Fig. 2, 4, 5, 6 and Extended Data Fig. 1-2, 7-10 have been provided as Source Data files. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	No sex or gender analysis was performed.
Reporting on race, ethnicity, or other socially relevant groupings	No reporting on race, ethnicity, or other socially relevant groupings was conducted for this study.
Population characteristics	For the infusion products, patients had pre-existing DLBCL prior to standard of care treatment. For human blood donations, whole blood was coded and negative for viral markers. For TILs, information regarding population characteristics can be found at https://clinicaltrials.gov/study/NCT00338377.
Recruitment	For the infusion products, patients with DLBCL were selected. For human blood donations, healthy donors need to meet the criteria established by the Gulf Coast Regional Blood Center. For TILs, information regarding recruitment of patients can be found in detail at https://clinicaltrials.gov/study/NCT00338377.
Ethics oversight	All work pertaining to the infusion products outlined in this report was performed according to protocols approved by the Institutional Review Boards at the University of Houston and the University of Texas MD Anderson Cancer Center. Patients provided written informed consent. Blood donations via the Gulf Coast Regional Blood Center are subject to FDA regulation and state agencies. Donors provide written informed consent. For TILs, protocol (2004–0069) was approved by the Institutional Review Board (IRB) of the University of Texas MD Anderson Cancer Center (Houston, TX) and an FDA-approved Investigational New Drug (IND) application (NCT00338377). More information can be found at can be found in detail at https://clinicaltrials.gov/study/NCT00338377. Patients provided written informed consent for use of their samples.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

Sample size	No statistical methods were used to predetermine sample sizes for the infusion products or animal studies.
Data exclusions	No data was excluded from the analysis except for TIMING results. Time of conjugation and time of death events lower than 10 minutes were omitted from analysis; time of conjugation lower than 10 minutes assumes no synapse formation and time of death lower than 10 minutes can be attributed to spontaneous cell death.
Replication	Experiments using infusion products were not subject to technical replications due to limited number of cells. Mice models used biological replicates. TIMING experiments using in-house CAR T cells were repeated three times with all data presented.
Randomization	Animal studies used were subject to randomization.
Blinding	The researchers were not blinded to allocation during experiments and outcome assessment. Data collection and analysis were not performed blind to the conditions of the experiments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used: Anti-CD62L (Clone:DREG-56, Cat. 304827) [Dilution 1:20], Anti-CD45RA (Clone:HI100, Cat. 304111)[Dilution 1:20], Anti-CD3 (Clone: SK7, Cat. 981004)[Dilution 1:20], Anti-CD4 (Clone:OKT4, Cat. 317415)[Dilution 1:20], Anti-CD8 (Clone:RPA-T8, Cat. 301008)[Dilution 1:20] and Anti-Granzyme B (Clone: QA16A02, Cat. 372203)[Dilution 1:20] from Biolegend. CAR was produced in-house.

Validation: Per the Biolegend website:
To ensure they are both specific and sensitive, we validate our antibodies through a variety of methods including:

1) Testing on multiple cell and tissue types with a variety of known expression levels.
2) Validation in multiple applications as a cross-check for specificity and to provide additional clarity for researchers.
3) Comparison to existing antibody clones.
4) Using cell treatments to modulate target expression, such as phosphatase treatment to ensure phospho-antibody specificity.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): NALM6-from ATCC, T cells isolated from whole blood

Authentication: None of the cells were authenticated

Mycoplasma contamination: All cell lines used tested negative for mycoplasma contamination

Commonly misidentified lines (See ICLAC register): No commonly misidentified cell line was used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: 7-week-old NOD.Cg-Prkdcscidll2rgtm1wjl/SzJ (NSG) female mice. Mice were housed at room temperature (20-24C), had 12 hours light-dark cycles with low intensity, and humidity between 40-60%. Cages were at least 12in^2 in size. The limit for maximal tumor size is 2000 mm^3 according to IAUCU guidelines and the limit was not exceeded for this study.

Wild animals: This study did not involve wild animals.

Reporting on sex: Findings from the mouse tumor models are applicable only to female mice. NSG female mice can randomized and change groups, but males will kill each other if mixed from other groups.

Field-collected samples: Study did not involve collecting samples from the field.

Ethics oversight: All experiments were approved and in accordance with the Institutional Animal Care and Use Committee at the University of Houston.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT00338377
Study protocol	Full trial protocol can be found in https://clinicaltrials.gov/study/NCT00338377
Data collection	Clinical trial is ongoing and started in 2006. More data is available at https://clinicaltrials.gov/study/NCT00338377
Outcomes	Outcomes defined can be found in detail at https://clinicaltrials.gov/study/NCT00338377

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Isolated cells were washed with FACS buffer two times, blocked with human plasma for 30 minutes, then stained with respective antibodies for 1 hour.
Instrument	BD LSRFortessa X-20 cell analyzer
Software	FlowJo v10
Cell population abundance	In vitro populations expressed >40% of markers of interest; in vivo populations expressed <5% of markers of interest.
Gating strategy	Gating strategy was performed on the preliminary FSC/SSC plots. Boundary of the gating strategy is shown in Extended Data Fig. 8D.

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