nature portfolio

Corresponding author(s): Suman Paul

Last updated by author(s): Jan 4, 2024

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

iQue Forecyt Standard Edition v8.0.7430 (Sartorius): iQue Screener Plus Flow Cytometer data acquisition
BD FACSDiva v8.0.1 (BD Biosciences): LSR II Flow Cytometer data acquisition
Attune Cytometric Software v5.3.0 (Thermofisher Scientific): Attune NxT Flow Cytometer data acquisition
Biotek Gen5 v2.07 (Agilent): Synergy H1 microplate reader data acquisition software; used for luciferase & MTS Assays
Zeiss Zen v3.7 (Zeiss Group): Zeiss Confocal Microscope data acqusition software
Incucyte 2021A v20211.1.7745.21572 (Sartorius): Incucyte SX5 Live Cell Imaging data acquisition software
Living Image Software v.4.74.21053 (PerkinElmer): IVIS mouse Bioluminesence imaging data acquisition software
OpenLab CDS ChemStation Edition Rev C.01.10(287) (Agilent): 1260 Infinity HPLC data acquisition software

Data analysis	iQue Forecyt Standard Edition v8.0.7430 (Sartorius): Analysis of iQue Screener Plus flow cytometer data FlowJo v10.9.0 (BD Biosciences): Analysis of flow cytometry data Zeiss Zen v3.7 (Zeiss Group): Analysis of confocal microscopy images Living Image Software v4.7.3.20616 (PerkinElmer): Analysis of IVIS mouse Bioluminesence imaging data Unidec deconvolution Software v5.2 (University of Oxford): Mass spectrometry peak identification (service from Rapid Novor) Origin Pro Software v9.950171 (OriginLab Corporation): Mass spectrometry peak identification (service from Rapid Novor) OpenLab CDS ChemStation Edition Rev C.01.10(287) (Agilent): 1260 Infinity HPLC data analysis Spagene v6.1.2 (Dotmatics): Plasmid design & DNA sequence alignments Graphpad Prism v9.2.0 (Dotmatics): Data plotting & Statistical Analysis Python v3.8.16 was used for data analysis and plotting of HPLC chromatograms and mass spectra: the Plotly package v5.10.0 was used for data plotting, and HPLC chomatograms were baseline corrected using the ZhangFit Function using the BaselineRemoval package v0.1.5

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 anti-TRBC1 CAR amino acid sequence, sequences of reagents used for CRISPR editing of T cells, and sequence of the anti-TRBC1 chimeric antibody are available in Supplementary Tables 2-4. Plasmids generated for this study are available by request under a material transfer agreement with Johns Hopkins University. All source data for mouse experiments are available as Supplementary Table 1.

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	Reporting on sex and gender are not applicable to this study
Reporting on race, ethnicity, or other socially relevant groupings	Reporting on race, ethnicity, or other socially relevant groupings are not not applicable to this study
Population characteristics	Reporting on population characteristics are not relevant to this study
Recruitment	Study did not require a recruitment plan
Ethics oversight	Johns Hopkins Institutional Review Board

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	We performed no new sample size calculations for in vitro and cell-line mouse xenograft models, as they were based on our previous experience with these models [1]. In brief, for in vitro CAR T cell co-culture experiments, we used 25,000 CAR T cells and incubated them with an equal number of expected target cells in a 96 well plate for each sample. When assessing ADC performance, we incubated varying amounts of ADCs with 10,000 target cells in a 96 well plate for each sample. For mouse models, we used 3-5 mice in each group to account for higher variability with in vivo work. For mouse experiments involving patient derived samples, sample sizing was limited by the quantity of of available sample. Details regarding sample sizing are indicated in methods, figure legends, and main text. Any deviation from the above mentioned sample size are also indicated in the methods section. [1] Paul, S. et al. TCR B chain-directed bispecific antibodies for the treatment of T cell cancers. Sci Transl Med. 2021, 13(584): eabd3595
Data exclusions	No data were excluded.

Replication	The majority of experiments were performed with two or more biological replicates as indicated in the figure legends. Each measurement typically involved 3 or more technical replicates, and aggregate data of measurements are shown in the main figures and figure legends.
Randomization	Randomization was not applicable to in vitro experiments and during ADC synthesis/characterization, since it was necessary to know the identity of the samples to interpret the data. The exception is that for confocal microscopy experiments, images were taken at random locations on slides. For in vivo studies, mice were randomized into different treatment groups after tumor injection on day 0.
Blinding	Blinding was not undertaken in other experiments as objective measurements (eg. presence of absence of cell populations in flow cytometry, quantifiable fluorescent data, luminesence data, survival data, etc) were used to support the results.

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

For ADC Synthesis:
-anti-TRBC1 antibody: clone JOVI.1 (Custom order from BD Biosciences)

For T cell activation:
-anti-human CD3 antibody (clone OKT3, BioLegend, #317347)

For flow cytometry:
-PE-anti-human Cβ1 TCR (clone JOVI.1, BD Biosciences, #565776)
-Brilliant Violet (BV)–711 anti-human CD3 (clone OKT3, BioLegend, Catalog #317328)
-Phycoerythrin (PE)–anti-human CD4 (clone RPA-T4, BioLegend, Catalog #300508)
-BV-421 anti-human CD3 (clone UCHT1, BioLegend, #300434)
-APC-anti-human CD7 (clone CD7-6B7, BioLegend, Catalog #343108)
-APC-anti-human CD26 (clone BA5b, BioLegend, Catalog #302710)
-APC-anti-human TCR α/β antibody (clone IP26, BioLegend, Catalog #306718)
-APC-anti-human NGFR (clone ME20.4, BioLegend, Catalog #345108)
-R-PE AffiniPure F(ab')$_2$ Fragment Goat Anti-Mouse IgG, F(ab')$_2$ fragment specific (RRID: AB_2338627, Jackson ImmunoResearch, Catalog #115-116-072)

For confocal microscopy:
-Anti-LAMP1-Alexa647 antibody (clone D2D11, Cell Signaling Technology, Catalog #73589)
-anti-pH2Ax antibody (clone #2572, Cell Signaling Technology, Catalog #2577)
-anti-mouse IgG2a-Alexa568 antibody (RRID: AB_2535773, ThermoFisher Scientific, Catalog #A-21134)
-anti-rabbit-Alexa647 antibody (RRID: AB_2536101, ThermoFisher Scientific, Catalog #A27040)

Validation

The JOVI.1 antibody used for anti-TRBC1-SG3249 production was previously validated in: Maciocia, P.M. et al. Targeting the T cell receptor β-chain constant region for immunotherapy of T cell malignancies. Nature Medicine. 2017, 23, 1416-1423.

All commercial antibodies were previously validated by the manufacturer. Manufacturer validation and peer-reviewed references where these antibodies are provided in the following links:
-anti-human CD3 antibody: https://www.biolegend.com/fr-fr/cell-health/ultra-leaf-purified-anti-human-cd3-antibody-7745?GroupID=BLG4203
-PE-anti-human Cβ1 TCR: https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-c-1-tcr.565776
-Brilliant Violet (BV)–711 anti-human CD3: https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd3-antibody-7941
-Phycoerythrin (PE)–anti-human CD4: https://www.biolegend.com/en-us/products/pe-anti-human-cd4-antibody-827
-BV-421 anti-human CD3: https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-cd3-antibody-7153
-APC-anti-human CD7: https://www.biolegend.com/en-us/products/apc-anti-human-cd7-antibody-6088
-APC-anti-human CD26: https://www.biolegend.com/en-us/products/apc-anti-human-cd26-antibody-7525
-APC-anti-human TCR α/β antibody: https://www.biolegend.com/en-us/products/apc-anti-human-tcr-alpha-beta-antibody-6704

-APC-anti-human NGFR: https://www.biolegend.com/en-us/products/apc-anti-human-cd271-ngfr-antibody-6877
-R-PE AffiniPure F(ab')$_2$ Fragment Goat Anti-Mouse IgG, F(ab')$_2$ fragment specific: https://www.jacksonimmuno.com/catalog/products/115-116-072
-Anti-LAMP1-Alexa647 antibody: https://www.cellsignal.com/products/antibody-conjugates/lamp1-d2d11-xp-rabbit-mab-alexa-fluor-647-conjugate/73589
-anti-pH2Ax antibody: https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h2a-x-ser139-antibody/2577
-anti-mouse IgG2a-Alexa568 antibody: https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG2a-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21134
-anti-rabbit-Alexa647 antibody: https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-Heavy-chain-Secondary-Antibody-Recombinant-Polyclonal/A27040

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Jurkat (Clone E6–1, [American Type Culture Collection (ATCC)], H9 (ATCC), SUP-T1 (ATCC), HPB-ALL [Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ)], CML-T1 (DSMZ), EMT6 cells (ATCC), ExpiCHO (Geneart, ThermoFisher Scientific)
Authentication	Cell lines were directly sourced from ATCC and DSMZ. ATCC and DSMZ authenticated the cell lines with short tandem repeat profiling. In addition, we confirmed the cell line immunophenotype that matched the report available at ATCC and DSMZ. Cell line immunophenotype matched the report available at ATCC and DSMZ.
Mycoplasma contamination	Cell lines tested negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this 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	6-8 week old NSG mice were used in the study. Mice were housed at 70-75°F and 45-50% humidity with a 12 hour light/dark cycle.
Wild animals	Study did not involve wild animals
Reporting on sex	Only female NSG mice were used in the study. We do not expect any sex-based variable to influence the study results.
Field-collected samples	Study did not involve field-collected samples
Ethics oversight	Study was approved by the JHU Animal Care and Use Committee

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Cells were suspended in flow sorting buffer [PBS (phosphate buffered saline containing 4% FBS] or flow stain buffer (PBS and 0.5% bovine serum albumin, 2 mM EDTA, 0.1% sodium azide), and incubated with desired antibodies using manufacturer recommended dilutions, for 20 min at 4°C, protected from light. Cells were washed three times using flow stain buffer, followed by flow cytometry analysis.
Instrument	BD LSR II (BD Biosciences), Intellicyte iQue Screener Plus (Sartorius), or Attune NxT (ThermoFisher Scientific)
Software	Data was analyzed using the iQue Forecyt Software (Sartorius) or Flowjo software, or BD FACSDiva™ Software.
Cell population abundance	Cell population abundance within each gate is noted in the figures
Gating strategy	Cells were separated from debris using FSC-A & SSC-A gates, followed by selection of single cells using FSC-A & FSC-H gates. Subsequent gating was conducted to select the desired cell populations based on the expression of known cell markers as shown in the figures

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