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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	Flow cytometry data collection was performed with Attune NxT Software version 3 (Thermal Fischer Scientific) or LSRFortessa with FACSDiva Software version 8.0.1 (BD Biosciences). Transmission electron microscopy data collection was performed using Velox imaging software (Thermal Fischer Scientific). In vivo bioluminescence data collection was performed using Living Image Software (PerkinElmer). Seahorse data was collected using Seahorse Wave Desktop Software (Agilent). Metabolomics data was collected using the Agilent Quantitative analysis software (version B.07.00, MassHunter Agilent technologies). csRNA sequencing data was collected on a HiSeq4000 (Illumina).

Data analysis	Flow cytometry data were analyzed using FlowJo 10.8.1 (Tree Star, Oregon, USA). Statistical analysis was performed using GraphPad Prism 9 (GraphPad software, Inc, La Jolla, CA, USA). Relative quantification of metabolites was based on EIC (Extracted Ion Chromatogram) areas for the monitored MRM transitions. Signal intensity drift correction and noise filtering was done within the MRM PROBS software. The preprocessed data with peak areas were imported into Metaboanalyst 5.0 for further data analysis. csRNA sequencing raw data of mouse intratumoral HER2 CAR-T cells and human splenic CD19 CAR-T cells were demultiplexed by cellranger mkfastq from 10x Genomics (version 3.0.2) and primary data analysis performed with Cell Ranger (version 2.2.0) using a custom reference package based on mouse reference genome (mm10 or GRCh38) and GENCODE gene models. csRNA sequencing raw data of mouse splenic HER2 CAR-T cells CeleScope rna from Singleron (version 3.0.1) and primary data analysis was performed with CeleScope (version 1.10.0) using a custom reference package based on reference genome (Mus_musculus_ensembl_92). Unsupervised clustering was performed on the PC reduction using the FindNeighbors and FindClusters functions implemented in Seurat with parameters k.param=30 and resolution=0.35. Differentially expressed genes between clusters were determined using the FindMarkers function of Seurat, which applies a Wilcoxon test to determine significance. Differentially expressed genes were visualized using the EnhancedVolcano R package (https://github.com/kevinblighe/EnhancedVolcano) with cutoff on log2(fold-change)=0.5 and p-value=10-5. Gene sets for relevant biological processes and pathways were obtained from the database mSigDB, and signature scores for these gene sets were calculated using the UCell package. Gene set enrichment analysis between clusters was performed on average gene expression by cluster using the R package clusterProfiler and relevant signatures from mSigDB.40 and relevant signatures from mSigDB. Projection of HER2 CAR-T or IL-10 HER2 CAR-T cells into a reference atlas of TILs was performed using the ProjecTILs methodwith default parameters.

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

All data generated and supporting the findings of this study are available within the paper. The scRNA-seq data for murine CAR-T cells from this study have been deposited in the GEO under accession number 245517. The scRNA-seq data for the human CAR-T cells are available in the CNGB Sequence Archive (CNSA) of China National GeneBank Database with accession number CNP0003547. The R code required to reproduce the scRNA-seq data shown in Fig. 4, Fig. 6, Extended Data Fig. 5 Extended Data Fig.10 is alailable at the following repository: http://github.com/carmonalab/LiTang_IL10_CART. The reference genme for primary data analysis of HER2 CAR-T cells in tumors was mm10, GRCh38 for CD19 hCAR-T cells, and ensembl_92 for HER2 CAR-T cells in spleen. Source Data are provided with the online version of the paper. Additional information and materials will be made available upon reasonable request.

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	Group sizes for in vivo validation experiments were selected empirically based on previous results of the intragroup variation of tumor growth upon similar treatments. Similarly, group sizes in vitro were selected on the basis of prior knowledge of variation. Ref: Nat Immunol 2021 Jun; 22(6):746.756
Data exclusions	Rout outlier tests were run with default parameters (Q = 1%) in Prism on all mouse experimental data due to inherent variability within the model system.
Replication	All presented results were repeatable. Replicates were used in all experiments as noted in figure legend or methods.
Randomization	Age and sex-matched animals were used for each experiment. Mice were randomized prior to treatment. In the in vitro experiments, samples with same pretreatment conditions were randomly assigned to a treatment group with a pipette.
Blinding	No blinding was performed due to requirements for cage labeling and staffing needs.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

The following antibodies or staining reagents were purchased from BioLegend: CD16/32 (93, 101302), CD45.2 (104, 109814), CD8β (YTS256.7.7, 126606), CD4 (RM4-5, 100526), NK1.1 (PK136, 108740), F4/80 (BM8,123108), CD3ε (17A2, 100306), CD11c (N418, 117348), I-A/I-E (MHC-II, M5/114.15.2, 107643), Siglec-F (S17007L, 155508), CD80 (16-10A1, 104734), CD86 (GL-1, 105006), CD11b (M1/70, 101228), Ki67 (16A8, 652424), Granzyme B (GB11, 515403), IFNγ (XMG1.2, 505826), TNFα (MP6-XT22, 506308), IL-2 (JES6-5H4, 503822), Gr-1 (RB6-8C5, 202519), PD-1 (29F.1A12, 135216), TIM-3 (RMT3-23, 119706). CD107a (H4A3, 328608), CD44 (IM7, 103028), CD62L (MEL-14, 104432), CD122 (5H4, 105906), Sca-1 (D7, 108106), KLRG1 (2F1/KLRG1, 138410), IL-7Rα (SB/199, 121111), human CD95 (DX2, 305606), Streptavidin-PE/Cyanine7 (405206), human Granzyme B (QA16A02, 372208), human IFN-γ (B2, 552887). The following antibodies or staining reagents were purchased from BD Biosciences: human CD4 (SK3, 563550), human CD8 (SK1, 557834). The following antibodies or staining reagents were purchased from Invitrogen: human CD62L(DREG56, 48-0629-42), human CD45RA (H100, 69-0458-42), human CD27 (O323, 56-0279-42), CCR7 (3D12, 61-1979-42). Biotinylated Human Her2/ErbB2 Protein (HE2-H822R) was purchased from ACROBiosystems. Myc-tag antibody (9B11, 3739) was obtained from Cell Signaling Technology. Goat anti-mouse IgG was purchased from Jackson ImmunoResearch (Polyclonal, 115-606-003), In VivoMAb anti-mouse CD3 (BE0002, 17A2) and In VivoMab anti-mouse CD28 (BE0015-1, 37.51) were purchased from BioXcell.

Validation

Validated by manufacturer as indicated on the websites. In Vivo anti-mouse CD3 and CD28 were used for stimulate the proliferation and cytokine production of murine T cells. The other antibodies described above are applied to Flowcytometry in this study, and thier validation can be found on the manufacturer website. Antibodies with human reactivity are labeled as such, remaining antibodies without labels have mouse reactivity.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HER2 transduced MC38 mouse colon cancer cells (MC38-HER2) were provided by Prof. Pedro Romero (University of Lausanne). B16F10 melanoma cells, Phoenix-Eco cell sand Raji cells were originally obtained from the American Type Culture Collection. 4T1-EGFRvIII-Luc mouse breast cancer cells were provided by Prof. Darrell J. Irvine (Massachusetts Institute of Technology). NIH/3T3-CD19 cells, PANC1-CD19 cells and PANC1-CD19-Luc cells were generated by Prof. Jie Sun (Zhejiang University).

Authentication

None of the cell lines were authenticated in these studies. In all related studies, cell lines with low passage number were used.

Mycoplasma contamination

All cell lines were confirmed mycoplasma negative.

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

Five to six-week old female C57BL/6 (C57BL/6J) mice, BALB/c (BALB/cByJ) mice were purchased from Charles River Laboratories (Lyon, France). Six to twelve-week old NOD/SCID/IL-2Rγ null mice were purchased from GemPharmatech (Nanjing, China). T-cell receptor (TCR)-transgenic OT-I mice (C57BL/6-Tg(TcraTcrb)1100Mjb/J) were originally purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained in École Polytechnique Fédérale de Lausanne (EPFL)-Center of PhenoGenomics (CPG) animal facility. Mpc1fl/fl mice were crossed to Cd4cre mice on an OT-I background to generate MPC1 knock out (MPC1-KO) OT-I mice. Mpc1 fl/fl breeder mice and OT-I breeder mice range in age from six to 40 weeks. Six to twelve-week OT-I T mice and MPC1-KO OT-I mice were used for T cell isolation. Mice were housed under a controlled 12-hour light and 12-hour dark cycle, with tempratures maintained between 22-24 degree and humidity levels between 40-60% to ensure a comfortable environment.

Wild animals

Study did not involve wild animals.

Field-collected samples

Study did not involve field-collected samples.

Ethics oversight

Experimental procedures in syngeneic mouse studies were approved by the Swiss authorities (Canton of Vaud, animal protocol ID 3206 and 3533) and NSG mouse studies were approved by Institutional Animal Care and Use Committee of Zhejiang University

(Hangzhou, China, animal protocol NO.2021914). Animal studies were performed in accordance with the guidelines from CPG of EPFL, the animal facility of University of Lausanne, and Zhejiang University. Mice were euthanized when body weight loss was beyond 15% of baseline weight, tumor area reached 150 mm2 (syngeneic mouse models) or the maximum diameter reached 20 mm (NSG mouse models), or any signs of discomfort were detected by the investigators or as recommended by the veterinarian who monitored the mice every other day.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Whole blood samples from healthy volunteers (female/male, 19-32 years old) were collected by a doctor of the First Affiliated Hospital of Zhejiang University School of Medicine
Recruitment	Volunteers recruitment occurred through campus public media. Healthy volunteers aged 18-35 with written informed consent were recruited and the protocol was approved by the Ethics Committee of Zhejiang University School of Medicine (Hangzhou, China, NO.2020-067). Donors were compensated according to Institute policy of Zhejiang University School of Medicine. Other than ensuring good health, no other bias were identifed. While variability among different donors may affect batch-to batch variability, it impact on comparing treatment groups within the same batch of T cells is minor.
Ethics oversight	Ethics Committee of Zhejiang University School of Medicine (Hangzhou, China, NO.2020-067).

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

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	Tumors were dissected from the surrounding tissues, weighed, mechanically minced, and stirred at 1000 rpm in RPMI-1640 medium with Collagenase Type IV (1 mg/mL, Gibco / Thermo Fisher Scientific), Dispase II (100 µg/mL, Sigma-Aldrich, St. Louis, Missouri, USA), Hyalurondase (100 µg/mL, Sigma-Aldrich), and DNase I (100 µg/mL, Sigma-Aldrich) at 37 °C for 60 min for digestion. Red blood cell lysis was performed on the tumor digestion samples with ACK Lysing Buffer (Gibco / Thermo Fisher Scientific), and then tumor-infiltrating leukocytes were then enriched by density gradient centrifugation against Percoll (GE healthcare) and resuspended in PBS with bovine serum albumin (0.2%, wt/v, Sigma-Aldrich). Spleen were dissected from the surrounding tissues, grinned, and filtered with strainers. Red blood cell lysis was performed on the spleen samples with ACK Lysing Buffer and then resuspended in PBS with bovine serum albumin (0.2%, wt/v, Sigma-Aldrich). Blood samples were collected from abdominal aorta, and resuspend in PBS with 2mM EDTA. Red blood cell was removed by ACK lysis buffer(Gibco / Thermo Fisher Scientific), then red blood cell debris were removed by density gradient centrifugation against Percoll (GE healthcare), and then resuspended in PBS with bovine serum albumin (0.2%, wt/v, Sigma-Aldrich). Bone marrow was harvested from freshly isolated femurs and tibiae. Bones were crushed in 5 ml of PBS with ethylenediaminetetraacetic acid (2mM, Sigma-Aldrich) and filtered with strainers. Remaining RBCs were lysed with ACK buffer.
Instrument	Data were collected using Attune NxT Flow Cytometer (Invitrogen / Thermal Fischer Scientific) or LSRFortessa Flow Cytometer (BD Biosciences). Cell sorting was performed with FACS Aria II (BD Biosciences).
Software	Flow cytometry data were analyzed using FlowJo 10.8.1 (Tree Star, Oregon, USA). Flow cytometry data collection was performed using Attune NxT Software version 3 (Invitrogen / Thermal Fischer Scientific) or LSRFortessa with FACSDiva Software (BD Biosciences).
Cell population abundance	Purity was determined by flow cytometry for CAR positive T cells (> 95 %).
Gating strategy	We used standard gating strategies: gating on the typical lymphocyte population based on FSC-SSC signals, doublet exclusion based on FSC-H and FSC-A comparison, Live/Dead discrimination based on DAPI or fixable Aqua dye signals. Cell populations were identified based on the expression markers listed below. CAR-T cells: CD45+/CD3+/CAR+; natural killer cells: CD45+/CD3-/NK1.1+; dendritic cells: CD45+/Gr-1-/CD11b+/CD11c+/MHCII+; Tumor-associated macrophages: CD45+/Gr-1-/CD11b+/F4/80+/MHCII+; Gr-1+CD11b+ Myeloid-Derived Suppressor Cells: CD45+/Gr-1+/CD11b+; Eosinophils: CD45+/CD11b+/Siglec-F+. All the representative FACS gating strategies were described in Supplemental Fig. 1.

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