nature research

Corresponding author(s): E.L. Pearce

Last updated by author(s): Jun 22, 2020

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	No software was used for data collection.
Data analysis	For statistical analysis and graphing we used Prism 8 (v.8.4.2). For the single cell RNA sequencing data samples were demultiplexed and aligned using Cell Ranger 2.2 (10X genomics) then processed and analyzed in R using Seurat and Uniform Manifold Approximation and Projection (UMAP) as a dimensionality reduction approach. Genes from specific metabolic pathways were retrieved from Kegg and expression scores were calculated using Seurat. Differentially expressed genes, with greater than a 1.2 fold change and an adjusted p value of less than 0.1, were analyzed for pathway enrichment using STRING.

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. 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

Single cell RNA sequencing data has been deposited in the Gene Ontology Omnibus under the accession number GSE152018.

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	Previously n=5 was of sufficient strength to determine significant differences in tumor growth of the E.G7-OVA tumor line used in the lab. Since we changed the standard lab and predicted less efficient tumor control, we doubled the number of animals per group to account for variation in growth within the groups. Previously n=5 was of sufficient strength to determine significant differences in tumor growth of the B16 tumor line used in the lab.
Data exclusions	N/A All animals treated were included in the analysis no exclusion criteria where established.
Replication	Replications of experiments and replicates per experiment are noted in the figure legends.
Randomization	The mice injected with E.G7-OVA tumors were housed in groups of 5 until D8 after tumor implantation. At D8, the mice were randomized before injection of the therapeutic T cell product.
Blinding	The investigator injecting the mice with in vitro generated T cells was blinded to the identity of the T cell pretreatments. Measurements of tumor size from day 15 onwards was performed by a blinded researcher.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	The following antibodies were used: anti-phospho-ACC1Ser79 (cell signaling catalogue #11818); anti-ACC1 (cell signaling catalogue #3676); anti-phospho-AMPKThr172 (Cell Signaling catalogue #2535); anti-AMPK (Cell Signaling catalogue #5831), anti-phospho-S6Ser235/236 (cell signaling catalogue #2211); anti-S6 (cell signaling catalogue #2317); anti-phospho-4E-BP1Thr37/45 (cell signaling catalogue #2855); anti-4E-BP1 (cell signaling catalogue #9644); anti-Glut1 (cell signaling catalogue #12939); and anti-tubulin (Sigma catalogue #T6199).
Validation	All antibodies were previously published, and for western blots were validated by the company we purchased from. The western blot antibodies were detecting proteins of predicted mass as shown in the uncropped blots in the source data files.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	B16-F10-OVA was a gift from Dr. Dietmar Zehn, E.G7-OVA was purchased directly from ATCC.
Authentication	N/A, we confirmed expression of OVA protein in both cell lines, as OT-I T cells reacted during co-culture and in-vivo immune responses respectively.
Mycoplasma contamination	N/A

Commonly misidentified lines (See ICLAC register)	no

Animals and other organisms

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

Laboratory animals	C57BL/6J (RRID: IMSR_JAX:000664), major histocompatibility complex (MHC) class I-restricted OVA specific TCR OT-I transgenic mice (RRID: IMSR_JAX:003831), and Thy1.1 congenic (Jax strain number 000406) mouse strains were purchased from The Jackson Laboratory. For in vitro T cell cultures animals of 8-12 weeks were used to isolate T cells, and they were not sex matched. For in vivo tumor experiments we used female donor OT-I mice 8-12 weeks of age and transplanted in Thy1.1 congenic female mice of 8-12 weeks old. We used female donors and recipients in the transplantation experiment to prevent rejection. Mice were sacrificed when tumor size reached 20mm average diameter or when the tumors ulcerated. Ulceration was the reason for sacrifice in the B16-OVA tumor experiments.
Wild animals	N/A
Field-collected samples	N/A
Ethics oversight	Animal studies were approved by the animal care committee of the Regierungspraesidium Freiburg.

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	Sample preparation is detailed in the materials and methods section. Staining was performed in 1% FBS/PBS for 30 min on ice, dead cells were excluded with the LIVE/DEAD Fixable Blue Dead Cell Stain Kit (Thermo scientific). Donor-derived CD8+ T cells from blood were quantified after bed blood cell lysis, by direct staining for CD90.1 and CD90.2 congenic markers. For intracellular cytokine staining cells were reactivated with phorbol 12-myristate 13-acetate (PMA, 50ng/ml, Sigma) and ionomycin (500ng/ml, Sigma), in the presence of Brefeldin A (0.1%, Biolegend) for 5 hours prior to fixation using Cytofix Cytoperm (BDBioscience), except for the spheroid cocultures, there Brefeldin was added directly into the coculture (without extra restimulation) 5 hours before staining.
Instrument	BD Fortessa cytometers were used for flow cytometry data in figures 1a-f, 5j-k and 6c-f; 6j-k, a BD LSRII was used to measure roGFP reported in figure 4, a BD cytoflex cytometer was used to measure ROX parameters reported in figures 4e-f.
Software	All data was collected with BD FACSDIVA software for the BD cytometers, except for the CytExpert software while using the Cytoflex cytometer. Data was gated and analyzed using FloJo software and bar graphs for quantification were made with Prism 8.
Cell population abundance	N/A
Gating strategy	Cells were gated on FSC v Live Dead to select live cells, followed by a FSC-w v FSC-a to remove doublets. For the data in figure 1 we selected the CD8-BV421 positive cells to analyze cytokine expression as shown in the panels in figure 1b. For the analysis in figure 6, we obtained live single cells as before, gated on CD8-FITC positive cells and plotted the data as effector molecule v donor derived congenic mark (CD45.2). Also see the Gating strategy file in the supplementary information.

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