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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.

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	Flow cytometer BD LSR II was used to run samples and data was acquired and analyzed by BD FACS Diva or FlowJo software. Epoch (BioTek) plate reader was used for requiring absorbance and data was analyzed by Gen5 software. LMax II384 (Molecular Devices) plate reader was used for the assays requiring luminescence quantification and data was analyzed by SoftMax Pro software. GloMax Discover System (Promega) was used for requiring luminescence or fluorescence quantifications. Oxidized phospholipids in cell lysate were analyzed on a HPLC system (ExionLC, Sciex, Framingham, MA) connected to a triple quadrupole mass spectrometer (Triple Quad 6500+, Sciex). Real-time PCR was run on StepOnePlus system (Thermo fisher) and data was analyzed by StepOne Software v2.2.2. Microscopy pictures were acquired by Leica DMI4000B microscope and analyzed by LAS AF software (Leica Biosystems). Automated slide-scanning was done on Aperio AT2 platform (Leica Biosytems). The images were analyzed with ImageScope software (Leica Biosystems). In vivo bioluminescence signal was assessed and analyzed with the IVIS Spectrum In Vivo Imaging System (PerkinElmer). Microsoft Excel and GraphPad Prism were also used software for data collection and analysis.
Data analysis	GraphPad Prism version 7 and R were used for analysis.

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

RNA sequencing data that support the findings of this study have been deposited in NCBI Gene Expression Omnibus (GEO) under accession number GSE128392. All other data that supported the findings of this study are available from the corresponding author upon request.

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	No statistical method was used to calculate sample size.
Data exclusions	No data was excluded for all in vitro experiments. For animal experiments, if a mouse died due to other condition than tumor burden, it was excluded from the analysis.
Replication	As reported in the figure legends, the findings were reliably reproduced. The anti-PDL1 therapy in ID8-Erastin resistant cells was performed once (Extended Data Fig. 2c). The effect of liproxstatin-1 on the combination of anti-CTLA-4 and anti-PD-L1 (Fig. 1g) was performed once. The anti-PDL1 therapy in ACSL4 knockout ID8 cells was performed once (Extended Data Fig. 2k, l). The effect of liproxstatin-1 on the combination of cyst(e)inase and anti-PDL1 (Extended Data Fig. 7h) was performed once.
Randomization	For all in vivo experiments, animals were randomly assigned into a treatment group after tumor inoculation. The starting tumor burden in the treatment and control groups was similar before treatment.
Blinding	Immunohistochemistry scoring was performed in a blinded way.

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	Cyst(e)inase can be requested from George Georgiou (gg@che.utexas.edu)

Antibodies

Antibodies used

InVivoMAb anti-mouse CTLA-4 (clone 9H10) and anti-mouse PD-L1 (clone 10F. 9G2) were from Bio X Cell.

Antibodies for functional studies: anti-human CD3 (Clone HIT3α, BD Biosciences) and anti-human CD28 (Clone CD28.2, BD Biosciences) ; anti-mouse CD3 (Clone 145-2C11, BD Biosciences) and anti-mouse CD28 (Clone 37.51, BD Biosciences);

Antibodies used for FACS: anti-CD45 (30-F11,Thermo Fisher Scientific), anti-OVA257-264-H2Kb (25-D1.16, Thermo Fisher Scientific); anti-CD90 (53-2.1, Thermo Fisher Scientific), anti-CD4 (RM4-5, Thermo Fisher Scientific), anti-CD8 (53-6.7, Thermo Fisher Scientific), anti-TNF (MP6-XT22, Thermo Fisher Scientific), anti-IFNγ (XMG1.2, Thermo Fisher Scientific)

Antibodies used for immunoblot: anti-human SLC7A11 (CST, Cat#12691), anti-human SLC3A2 (CST, Cat#13180), anti-mouse SLC7A11 (Thermo Fisher Scientific, Cat#711589), anti-IRF1 (CST, Cat#8478), anti-ACSL4 (abcam, Cat#ab155282),anti-GAPDH (CST, Cat#5174), anti-β-actin(CST, Cat#5125), anti-STAT1 (CST, Cat#9175).

Validation

All antibodies for FACS were well-recognized clones in the field and validated by the manufacturers. These antibodies are further validated and routinely used in our lab.
Antibodies targeting SLC7A11, SLC3A2 and STAT1 were validated by suppression or knockout of the intended target gene by RNAi or CRISPR/Cas9, and verification of the loss of a band of the predicted molecular weight by immunoblotting.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HT-1080, A375 and B16 are from ATCC. The original source of ID8 is cited.

Authentication

Cell lines were not authenticated.

Mycoplasma contamination

All cell lines in our laboratory are routinely tested for mycoplasma contamination and cells used in this study are negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No cell line used in the paper is listed in ICLAC database.

Animals and other organisms

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

Laboratory animals

Six- to eight-week-old female NOD-scid IL2Rgnull (NSG) ,C57BL/6-Tg (TcraTcrb) 1100Mjb/J (OT-1 mice) and wild type C57BL/6 mice were obtained from the Jackson Laboratory. All mice were maintained under pathogen-free conditions.

Wild animals

The study did not involve wild animals.

Field-collected samples

The study did not involve samples collected from 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

For lipid ROS analysis, tumor cells were treated and harvested by trypsinization, resuspended in 1 mL Hanks Balanced Salt Solution (HBSS, Gibco) containing 5 μM BODIPY 581/591 C11 (Thermo Fisher), and incubated for 15 min at 37 °C in a tissue culture incubator. When using LiperFluo, cells were preloaded with Liperfluo and treated with ferroptosis inducers. Cells were then washed and resuspended in 200 μl fresh HBSS and analyzed immediately on a flow cytometer (LSR II, BD Biosciences). For the co-culture of OVA+ tumor cells and OT-I cells, the mixture was collected and resuspended in 100 μl FACS buffer. Cells were firstly stained with anti-CD45 (30-F11) and anti-OVA257-264-H2Kb (25-D1.16) antibodies for 10 minutes at room temperature. To perform BODIPY-C11 staining, cells were resuspended in 1 mL HBSS containing 5 μM BODIPY 581/591 C11 and incubated for 15 minutes at 37°C in a tissue culture incubator. Cells were then washed and resuspended in 200 μl fresh HBSS and analyzed immediately with a flow cytometer.

For cell death analysis, cells were treated, collected and initially stained with specific antibodies, and then resuspended in PBS containing 1 µg/ml Propidium Iodide (PI) or 7-Aminoactinomycin D (7-AAD) for 5 minutes and directly run on a flow cytometer. For cells expressing intracellular fluorescence proteins, cells were resuspended in PBS containing 1 µl LIVE/DEAD Fixable Blue Dead Cell Stain (Thermo Fisher Scientific) for 20 minutes and then analyzed.

To quantify the lipid peroxidation in samples from animals that received immunotherapy, single cell suspension was firstly prepared. For ID8 tumor-bearing mouse, peritoneal cavity was washed with 5 - 10 ml PBS to collect tumor and immune cells. A small fraction of cell pellet was resuspended in 1 ml Red Blood Cell Lysis Buffer (Sigma-Aldrich) for 1 min, washed and stained with anti-CD45 antibody following with BODIPY 581/591 C11. For B16 tumor-bearing mouse, subcutaneous tumor tissue was resected and cut into small pieces, then mechanically disaggregated the minced tumor tissue against a 100 µM cell strainer, washed with PBS to collect the cell mixture. Tumor and inflammatory cells were pre-enriched using density gradient centrifugation (Ficoll, Sigma-Aldrich). Cell pellet was then stained with anti-CD45 and anti-OVA257-264-H2Kb antibodies following with BODIPY 581/591 C11. Cells were strained through a 40 µM cell strainer and analyzed.

To quantify T cell and cytokine production, single-cell suspensions were prepared from fresh tumor tissues and lymphocytes were enriched by density gradient centrifugation. For cytokine staining, lymphocytes were incubated in culture medium containing PMA (5 ng/ml), Ionomycin (500 ng/ml), Brefeldin A (1: 1000) and Monensin (1: 1000) at 37°C for 4 hours. Anti-CD45 (30-F11), anti-CD90 (53-2.1), anti-CD4 (RM4-5) and anti-CD8 (53-6.7) were added for 20 minute for surface staining. The cells were then washed and resuspended in 1 ml of freshly prepared Fix/Perm solution (BD Biosciences) at 4°C for overnight. After being washed with Perm/Wash buffer (BD Biosciences), the cells were stained with anti-TNF (MP6-XT22) and anti-IFNγ (XMG1.2) for 30 min, washed, and fixed in 4% formaldehyde (Sigma Aldrich).

Instrument

All samples were read on an LSR II cytometer (BD Biosciences).

Software

All data were analyzed with FACS DIVA software v. 8.0 (BD Biosciences).

Cell population abundance

When cells were sorted or enriched, the purity was confirmed by flow cytometry and in each case was above 90% purity.

Gating strategy

The cells were gated on FSC-A/SSC-A basis on the location known to contain lymphoid cells and tumor cells. To analyze cell death or lipid peroxidaiton, CD45 negative population was gated and considered as tumor enriched; when tumor cells expressing OVA, CD45- H2kb/OVA+ population was gated; The percentage of PI+ population or mean fluorescence intensity of ROS probe were then analyzed in either CD45- or CD45+ population. To analyze cytokine production by T cells, CD45+ population was first gated; and then select CD90+CD8+ or CD90+CD4+ population. In CD8 and CD4 gate, the percentage of TNF+ or IFNg+ cells were analyzed.

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