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.

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Fora	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)
x	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
x	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), 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 <u>availability of computer code</u>

Data collection

 $We stern\,Blots\,data\,were\,acquired\,with\,Gel\,imaging\,system\,Gel\,DocTM\,XR+\,(Universal\,hood\,II,\,Bio-Rad).$

 $Confocal\ laser\ scanning\ microscope\ (CLSM)\ images\ and\ immunofluorescence\ (IFC)\ images\ were\ collected\ with\ LSM\ 510\ META\ (Olympus).$

Flow cytometer (FCM) data were obtained with FACS Calibur and Celesta (BD).

Photoacoustic bioimaging data were collected with iThera Medical MSOT in Vision 128 (iThera Medical).

Photographs of H&E staining were collected with BX53 (Olympus, Japan).

Data analysis

Origin 9.0 and Origin 2020 were used for all statistical assessments.

 $Image \textit{J}\ version\ software\ 1.45 f\ was\ used\ for\ blots\ quantification\ and\ colony\ intensity\ calculation.$

CellSens Entry was used for HE images analysis.

CellSens Dimension was used for CLSM images analysis.

FlowJo_V10 was used for FCM images analysis.

ViewMOST (Release 3.8.1.09) was used for Photoacoustic bioimaging 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 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 the data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding author upon reasonable request.

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Please select the	one below that is the best fit for your research.	If you are not sure,	read the appropriate sections b	pefore making your selection.
X Life sciences	Behavioural & social sciences	Fcological evo	olutionary & environmental scie	ences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size Details re

Details regarding the sample size of all experiments are provided in figure legends. Sample size was estimated to achieve about 90% power for detection of significant differences in tumor volume between groups based on means and standard deviations in preliminary studies. They were consistent with sample size of previously reported results in other studies

Data exclusions

No data was excluded from the analyses.

Replication

All experiments were performed with at least three technical replicates on more than one occasion to ensure reproducibility across experiments.

Randomization

For in vitro test, samples were randomly allocated to corresponding experimental groups. For in vivo test, mice were inoculated tumor at the same time and then randomly assigned to a group for similar average tumor sizes.

Blinding

The investigator was blinded to the group allocation during the tumor size measurement, tissue harvesting and processing.

Behavioural & social sciences study design

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

Study description

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample

State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.

Sampling strategy

Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.

Data collection

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.

Timing

Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.

Data exclusions

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.

Non-participation

State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.

Dual use research of concern

Ecological, evolutionary & environmental sciences study design

all studies must disclose or	n these points even when the disclosure is negative.			
Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.			
Research sample	Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.			
Sampling strategy	Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.			
Data collection	Describe the data collection procedure, including who recorded the data and how.			
Timing and spatial scale	Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken			
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.			
Reproducibility	Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.			
Randomization	Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.			
Blinding	Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.			
Did the study involve fiel	tion and transport			
Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).			
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).			
Access & import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).			
Disturbance	Describe any disturbance caused by the study and how it was minimized.			
Reporting fo	or specific materials, systems and methods			
Ve require information from	authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, evant 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 & experime				
n/a Involved in the study				
Antibodies	ChIP-seq			
Eukaryotic cell lines				
Palaeontology and				
Animals and other				
Human research pa				

Antibodies

Antibodies used

FITC anti-mouse/human CD44 Antibody (Biolegend, cat: 103005, lot: B278351) PE/Cyanine7 anti-mouse CD49b Antibody (Biolegend, cat: 103517, lot: B278409) APC/Cyanine7 anti-mouse CD3 Antibody (Biolegend, cat: 100222, lot: B334891) PE anti-mouse/human CD45R/B220 Antibody (Biolegend, cat: 103207, lot: B250170)

PerCP/Cyanine5.5 anti-mouse CD8a Antibody (Biolegend, cat: 100734, lot: B313041)

APC anti-mouse CD62L Antibody (Biolegend, cat: 104412, lot: B282479) FITC anti-mouse CD4 Antibody (Biolegend, cat: 100406, lot: B266671) PE anti-mouse FOXP3 Antibody (Biolegend, cat: 126404, lot:B320694)

FITC anti-mouse/human CD11b Antibody (Biolegend, cat: 101206, lot: B287569)

PE anti-mouse CD86 Antibody (Biolegend, cat: 105008, lot: B313366) APC anti-mouse CD80 Antibody (Invitrogen, cat: 17-0801-82, lot: 2193902)

Rabbit polyclonal anti-STAT3 (Cell Signaling Technology, cat: 9139T, lot: 12, 1:2000)

Rabbit polyclonal anti-Ki-67 (Cell Signaling Technology, cat: 9129T, lot: 3, 1:400) Rabbit polyclonal anti-mTOR (Cell Signaling Technology, cat: 2983T, lot: 16, 1:400)

Rabbit polyclonal anti-IDO1 (Proteintech, cat:13268-1-Ap, lot: 00024692, 1:2000)

Rabbit anti-Cytochrome C (Boster, cat: PB0291, lot: ZP1606BP06, 1:1000)

Rabbit polyclonal anti-Bcl-2 (Boster, cat: BA0412, lot: ZP7648BP48, 1:400)

Rabbit polyclonal anti-Bax (Boster, cat: BA0315-2, lot: 13CM400B, 1:400)

Rabbit polyclonal anti-Tubulin (Boster, cat: BM3877, lot: BST17393877, 1:400) Rabbit Polyclonal anti-MMP2 (Proteintech, cat:10373-2-AP, lot: 00090591, 1:800)

Rabbit Polyclonal anti-MMP9 (Proteintech, cat:10375-2-AP, lot: 00097961, 1:800)

Rabbit Polyclonal anti-E-cadherin (Proteintech, cat:20874-1-AP, lot: 00084161, 1:25000)

Cy3-labeled goat anti-rabbit IgG (Beyotime, cat: P0183, lot: 032019190703, 1:1000)

Alexa Fluor 488-labeled goat anti-rabbit IgG (Beyotime, cat: P0176, lot: 060321211213, 1:1000)

Validation

FITC anti-mouse/human CD44 Antibody (Biolegend): Flow cytometer (FCM), see manufacturer's website for references

PE/Cyanine7 anti-mouse CD49b Antibody (Biolegend): FCM, see manufacturer's website for references

APC/Cyanine7 anti-mouse CD3 Antibody (Biolegend): FCM, see manufacturer's website for references

PE anti-mouse/human CD45R/B220 Antibody (Biolegend): FCM, see manufacturer's website for references

PerCP/Cyanine5.5 anti-mouse CD8a Antibody (Biolegend): FCM, see manufacturer's website for references

APC anti-mouse CD62L Antibody (Biolegend): FCM, see manufacturer's website for references

FITC anti-mouse CD4 Antibody (Biolegend): FCM, see manufacturer's website for references

PE anti-mouse FOXP3 Antibody (Biolegend): FCM, see manufacturer's website for references

FITC anti-mouse/human CD11b Antibody (Biolegend): FCM, see manufacturer's website for references

PE anti-mouse CD86 Antibody (Biolegend): FCM, see manufacturer's website for references

APC anti-mouse CD80 Antibody (Invitrogen): FCM, see manufacturer's website for references

Rabbit polyclonal anti-STAT3 (Cell Signaling Technology): immunofluorescence (IFC), see manufacturer's website for references

Rabbit polyclonal anti-Ki-67 (Cell Signaling Technology): IFC, see manufacturer's website for references

Rabbit polyclonal anti-mTOR (Cell Signaling Technology): IFC, see manufacturer's website for references Rabbit polyclonal anti-IDO1 (Proteintech): western blot (WB) and IFC, see manufacturer's website for references

Rabbit anti-Cytochrome C (Boster): WB, see manufacturer's website for references

Rabbit polyclonal anti-Bcl-2 (Boster): WB, see manufacturer's website for references

Rabbit polyclonal anti-Bax (Boster): WB, see manufacturer's website for references

Rabbit polyclonal anti-Tubulin (Boster): WB, see manufacturer's website for references

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) The 4T1 mammary and B16F10 cell lines were obtained from Cell Bank, the Committee of Type Culture Collection of Chinese Academy of Sciences.

The cell lines were morphologically confirmed according to the information provided by their supplier. Authentication

Mycoplasma contamination All cell lines were tested negative for mycoplasma contamination. No mycoplasma contamination was found before use.

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

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information). Permits should encompass collection and, where applicable, export.

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

Animals and other organisms

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

Laboratory animals

C57BL/6 mice and Balb/c mice (about 6 weeks old) were purchased from Beijing Institution for Drug Control (China). All animals were bred in the pathogen-free facility with a 12 h light/dark cycle and relative humidity (40-70%) at 21±2°C. All the mice had access to

food and water ad libitum.

Wild animals The study did not involve wild animals.

Field-collected samples The study did not involve samples collected from field.

Ethics oversight All animal studies were operated according to guidelines of the Institutional Animal Care and Use Committee at Northwestern

Polytechnical University.

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

Human research participants

Population characteristics

Policy information about studies involving human research participants

oney information about <u>studies involving number research participants</u>

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study

design questions and have nothing to add here, write "See above."

RecruitmentDescribe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

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 ICMJEguidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration | Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.

Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

Outcomes Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

Dual use research of concern

Policy information about dual use research of concern

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No Yes Public health National security Crops and/or livest Ecosystems Any other significa Experiments of concer	nt area		
Does the work involve an	y of these experiments of concern:		
Confer resistance t Enhance the virule Increase transmiss Alter the host rang Enable evasion of o	to render a vaccine ineffective of therapeutically useful antibiotics or antiviral agents ince of a pathogen or render a nonpathogen virulent bility of a pathogen e of a pathogen liagnostic/detection modalities ization of a biological agent or toxin lly harmful combination of experiments and agents		
	and final processed data have been deposited in a public database such as <u>GEO</u> .		
Confirm that you have	deposited or provided access to graph files (e.g. BED files) for the called peaks.		
Data access links May remain private before public	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.		
Files in database submiss	on Provide a list of all files available in the database submission.		
Genome browser session (e.g. <u>UCSC</u>)	Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.		
Methodology			
Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.		
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads as whether they were paired- or single-end.		
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.		
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.		
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.		

Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community

Software

repository, provide accession details.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- x 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).
- **X** 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

Depending on the experiment, B16F10 cells were grown to 80-90% confluence, harvested, and seeded in a 6 well plate and allowed to adhere overnight. Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM) supplementing with 10 % (v/v) FBS and 1 % (w/v) penicillin (100 U/mL)/streptomycin (100 μ g/mL) with 5% CO2 at 37 °C. For all flow cytometry assays, following treatment or post-treatment incubation, cells were washed with PBS, centrifuged (2000 rpm × 10 min, 4 °C), collected, resuspended in cell binding solution (300 μ L) and analyzed by FCM.

After various administrations, tumor tissues were excised and digested using tumor tissue dissociation kit (Miltenyi, Germany) at 37 °C for 40-50 min. The resulting solution was isolated by passing 200 μ m and 75 μ m filter, and then harvested into mono-dispersive lymphocytes. The obtained cells were first stained with live/dead for 15-20 min in dark, stained with suitable antibodies at 4 °C for 30 min and then detected by FCM.

Instrument

Celesta (BD) and FACS Calibur (BD).

Software

Data analysis was done on FlowJo software (v10, FlowJo).

Cell population abundance

Cells were run to achieve > 10,000 events in the gated cell population.

Gating strategy

Gating was performed based on identifying a distinct population in FSC vs SSC plots.

 $\boxed{\mathbf{x}}$ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type

Indicate task or resting state; event-related or block design.

Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

Acquisition

Imaging type(s)

Specify: functional, structural, diffusion, perfusion.

Field strength

Specify in Tesla

Sequence & imaging parameters

Used

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

Area of acquisition

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

Diffusion MRI

Not used

Preprocessing

Preprocessing software

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

Normalization

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.			
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).			
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.			
Statistical modeling & infe	rence			
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).			
Effect(s) tested	Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.			
Specify type of analysis:	Whole brain ROI-based Both			
Statistic type for inference (See <u>Eklund et al. 2016</u>)	Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.			
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).			
Models & analysis n/a Involved in the study				
Functional and/or effective co	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).			

Multivariate modeling and predictive analysis

etc.).

Graph analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency,