

## 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  |
|-------------------------------------|--|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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 DLS data collection: Zetasizer Software v7.12. HPLC data collection: Empowe 3. Microscopy data collection: Carl Zeiss Zen 2.1 SP3 (black) v14.0 proprietary software. FACS data collection: BD FACS Diva software v8.0.1.1.

Data analysis GraphPad Prism 8.0, ImageJ open source software, OriginPro 9.0, Compass for SW software v6.3.0, FlowJo software v10.0.7.

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](#)

Provide your data availability statement here.

## 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

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

### Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

### Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, 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."

### Recruitment

Describe 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.

### Ethics oversight

Identify the organization(s) that approved the study protocol.

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](https://www.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

The sample size was determined based on previously published studies in similar areas. A minimum of three independent experiments were carried out for all ex vivo and in vitro studies.

### Data exclusions

No data was excluded from the analysis. Microscopic images are representative of typical experiments and all images are retained.

### Replication

All attempts at replication were successful.

### Randomization

Samples and animals were chosen at random for each experimental group.

### Blinding

Investigators were not blinded to group allocation except for organ section scanning and electron microscopy images.

## 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.*

Randomization

*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

## Ecological, evolutionary & environmental sciences study design

All studies must disclose on 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 field work?  Yes  No

## Field work, collection 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 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                                 | Involvement                         | Material/Method               |
|-------------------------------------|-------------------------------------|-------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Antibodies                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Eukaryotic cell lines         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Palaeontology and archaeology |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Animals and other organisms   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Clinical data                 |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Dual use research of concern  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | Plants                        |

## Methods

| n/a                                 | Involvement                         | Method                 |
|-------------------------------------|-------------------------------------|------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | ChIP-seq               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> | Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/>            | MRI-based neuroimaging |

## Antibodies

### Antibodies used

The following antibodies were used for nanoparticle preparation and treatment. They are listed as antigen first, following by supplier, catalog number and clone/lot number as applicable.

- Goat anti-rat IgG Fc antibody, Rockland, cat. no. 612-4103;
- Rabbit anti-human IgG Fc antibody, Rockland, cat. no. 609-4103;
- Anti-mouse NKG2A/C/E antibody, Bio X Cell, cat. no. BE0321, Clone: 20D5;
- Anti-mouse PDL1 antibody, Bio X Cell, cat. no. BE0101, Clone: 10F.9G2;
- Anti-mouse 4-1BB antibody, Bio X Cell, cat. no. BE0239, Clone: 3H3;
- Rat IgG2a isotype control (anti-trinitrophenol), Bio X Cell, cat. no. BE0089, Clone: 2A3;
- Rat IgG2b isotype control (anti-keyhole limpet hemocyanin), Bio X Cell, cat. no. BE0090, Clone: LTF-2;
- Humanized PDL1 antibody, 4-1BB antibody and TIGIT antibody were expressed by our research group;
- Anti-mouse CD8a antibody, Bio X Cell, cat. no. BE0061, Clone: 2.43;
- Anti-mouse NK1.1 antibody, Bio X Cell, cat. no. BE0036, Clone: PK136;
- Purified anti-mouse CD28, Biolegend, cat. no. 102102, Clone: 37.51;
- Purified anti-mouse CD3 $\epsilon$ , Biolegend, cat. no. 100302, Clone: 145-2C11.

The following primary antibodies were used for flow cytometry. They are listed as antigen first, following by supplier, catalog number and clone/lot number as applicable.

- Brilliant Violet 510™-conjugated  $\alpha$ CD45, Biolegend, cat. no. 103137, Clone: 30-F11;
- APC anti-mouse CD3 Antibody, Biolegend, cat. no. 100311, Clone: 145-2C11;
- APC/Cyanine7 anti-mouse CD4 Antibody, Biolegend, cat. no. 100413, Clone: GK1.5;
- Brilliant Violet 650™-conjugated  $\alpha$ CD8, Biolegend, cat. no. 100742, Clone: 53-6.7;
- Alexa Fluor 488 anti-mouse NK1.1 Antibody, Biolegend, cat. no.108717, Clone: PK136;
- Purified anti-mouse CD16/32 Antibody, Biolegend, cat. no. 101302, Clone: 93.

The following primary antibodies were used for Western Blot, immunofluorescence experiment. They are listed as antigen first, following by supplier, catalog number and clone/lot number as applicable.

- Phospho-NF-kappaB p65(Ser536) Rabbit mAb, Cell Signaling Technology, cat. no. 3033, Clone: 93H1;
- Phospho-p44/42 MARK(Erk1/2)(Thr202/Tyr204) XP Rabbit mAb, Cell Signaling Technology, cat. no. 4370, Clone: D13.14.4E;
- Alpha Tubulin Polyclonal antibody, Proteintech, cat. no. 11224-1-AP;
- Beta Actin Recombinant antibody, Proteintech, cat. no. 81115-1-RR, Clone: 4H1;
- PTPN6 Polyclonal antibody, Proteintech, cat. no. 24546-1-APR;
- Brilliant Violet 421™-conjugated CD8a, Biolegend, cat. no. 100737, Clone: 53-6.7;
- Alexa Fluor 647 anti-mouse NK1.1 antibody, Biolegend, cat. no. 108719, Clone: PK136;
- Alexa Fluor™ 488 phalloidin, Thermo Fisher Scientific, cat. no. , A12379.
- Anti-NCAM1/CD56 polyclonal antibody, Proteintech, cat. no. 14255-1-AP;
- Recombinant anti-CD3 antibody, Servicebio, cat. no. GB150004-100;
- Anti-Rabbit Secondary HRP Antibody, ProteinSimple, cat. no. 042-206.

### Validation

These commercially available flow cytometry antibodies have not been further validated by our laboratory. Certificates of Analysis can be found here:

- Goat anti-rat IgG Fc antibody:  
[https://rockland-inc.com/store/Whole-IgG-Affinity-Purified-Secondary-Antibodies-612-1103-O4L\\_23062.aspx](https://rockland-inc.com/store/Whole-IgG-Affinity-Purified-Secondary-Antibodies-612-1103-O4L_23062.aspx);
- Rabbit anti-human IgG Fc antibody:  
<https://www.rockland.com/categories/secondary-antibodies/human-igg-fc-antibody-109-4103/>;
- Anti-mouse NKG2A/C/E antibody:  
<https://bioxcell.com/invivomab-anti-mouse-nkg2a-c-e-be0321>;
- Anti-mouse PDL1 antibody:  
[https://bioxcell.com/catalogsearch/result/?q=InVivoMAB%20anti-mouse%20PD-L1%20\(B7-H1\);](https://bioxcell.com/catalogsearch/result/?q=InVivoMAB%20anti-mouse%20PD-L1%20(B7-H1);)
- Anti-mouse 4-1BB antibody:  
<https://bioxcell.com/invivomab-anti-mouse-4-1bb-cd137-be0239?>

queryID=c11681b11149389440dd6527179a4718&objectID=30674&indexName=bioxcell\_live\_default\_products;

6. Rat IgG2a isotype control (anti-trinitrophenol):  
<https://bioxcell.com/product/invivoplus-rat-igg2a-isotype-control-anti-trinitrophenol/>;

7. Rat IgG2b isotype control (anti-keyhole limpet hemocyanin):  
<https://bioxcell.com/invivomab-rat-igg2b-isotype-control-anti-keyhole-limpet-hemocyanin-be0090>;

8. Humanized PDL1 antibody, 4-1BB antibody and TIGIT antibody were expressed and purity and functional activities were confirmed;

9. Anti-mouse CD8a antibody:  
[https://bioxcell.com/invivomab-anti-mouse-cd8-alpha-be0061?queryID=f928d820a5b0701165a7d7b24f3280e5&objectID=30539&indexName=bioxcell\\_live\\_default\\_products](https://bioxcell.com/invivomab-anti-mouse-cd8-alpha-be0061?queryID=f928d820a5b0701165a7d7b24f3280e5&objectID=30539&indexName=bioxcell_live_default_products;);

10. Anti-mouse NK1.1 antibody:  
<https://bioxcell.com/invivomab-anti-mouse-nk1-1-be0036>;

11. Purified anti-mouse CD28:  
<https://www.biolegend.com/en-us/products/purified-anti-mouse-cd28-antibody-117?GroupID=GROUP20>;

12. Purified anti-mouse CD3ε:  
<https://www.biolegend.com/en-us/products/purified-anti-mouse-cd3epsilon-antibody-28>;

13. Brilliant Violet 510™-conjugated αCD45:  
<https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-cd45-antibody-7995>;

14. APC anti-mouse CD3 Antibody:  
<https://www.biolegend.com/en-us/products/apc-anti-mouse-cd3epsilon-antibody-21>;

15. APC/Cyanine7 anti-mouse CD4 Antibody:  
<https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd4-antibody-1964>;

16. Brilliant Violet 650-conjugated αCD8:  
<https://www.biolegend.com/en-us/products/brilliant-violet-650-anti-mouse-cd8a-antibody-7635>;

17. Alexa Fluor 488 anti-mouse NK1.1 Antibody:  
<https://www.biolegend.com/en-us/products/alexa-fluor-488-anti-mouse-nk-1-1-antibody-3143>;

18. Purified anti-mouse CD16/32 Antibody:  
<https://www.biolegend.com/en-us/search-results/purified-anti-mouse-cd16-32-antibody-190>;

19. Phospho-NF-kappaB p65(Ser536) Rabbit mAb:  
<https://www.cellsignal.com/products/primary-antibodies/phospho-nf-kb-p65-ser536-93h1-rabbit-mab/3033>;

20. Phospho-p44/42 MARK(Erk1/2)(Thr202/Tyr204) XP Rabbit mAb:  
<https://www.cellsignal.cn/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370>;

21. Alpha Tubulin Polyclonal antibody:  
<https://www.ptgcn.com/products/TUBA1B-Antibody-11224-1-AP.htm>;

22. Beta Actin Recombinant antibody:  
<https://www.ptgcn.com/products/beta-actin-Antibody-81115-1-RR.htm>;

23. PTPN6 Polyclonal antibody:  
<https://www.ptgcn.com/products/PTPN6-Antibody-24546-1-AP.htm>;

24. Brilliant Violet 421™-conjugated CD8a:  
<https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-mouse-cd8a-antibody-7138>;

25. Alexa Fluor 647 anti-mouse NK1.1 antibody:  
<https://www.biolegend.com/en-gb/products/alexa-fluor-647-anti-mouse-nk-1-1-antibody-3144>;

26. Alexa Fluor™ 488 phalloidin:  
<https://www.thermofisher.cn/order/catalog/product/A12379?SID=srch-srp-A12379>;

27. Anti-NCAM1/CD56 polyclonal antibody:  
<https://www.ptgcn.com/products/NCAM1-Antibody-14255-1-AP.htm>;

28. Recombinant anti-CD3 antibody:  
<https://www.servicebio.cn/goodsdetail?id=21832>;

29. Anti-Rabbit Secondary HRP Antibody:  
[https://www.bio-techne.com/p/simple-western/anti-rabbit-secondary-hrp-antibody\\_042-206](https://www.bio-techne.com/p/simple-western/anti-rabbit-secondary-hrp-antibody_042-206).

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

|   |  |
|---|--|
| Cell line source(s)   | B16-F10 and MC38 cell lines were obtained from American Type Culture Collection (ATCC). B16-F10-OVA, MC38-luc, and MC38-GFP cell lines were constructed by transfecting OVA-,fluc- or GFP-encoding lentiviral vectors (VectorBuilder) into B16-F10 or MC38 cells. The human cell line 293F was obtained from the National Collection of Authenticated Cell Cultures. |
| Authentication  | Authentication was performed by ATCC for B16-F10, MC38 cell lines and by National Collection of Authenticated Cell Cultures for 293F cell line (Method: STR profiling).  |
| Mycoplasma contamination  | All cell lines were tested for mycoplasma contamination. No mycoplasma contamination was found.  |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used in the study.   |

## Palaeontology and Archaeology

|                     |  |
|---------------------|--|
| Specimen provenance | <i>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.</i> |
|---------------------|--|

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 research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals *Female/male C57BL/6 mice and female ICR mice were purchased from Hunan Silaikejingda Laboratory Animal Technology Co. Ltd. OT-1 (C57BL/6-Tg (TcraTcrb) 1100Mjb/J) TCR transgenic mice were a munificent gift from Prof. Tian-Meng Sun from Jilin University. NOD/ShiLtJGpt-Prkdcem26Cd52 Il2rgem26Cd22 Il15em1Cin(hlL15)/Gpt mice (NCG-hlL15, Strain NO. T004886) and B6-Rag1-KO mice (Strain NO. T004753) were purchased from GemPharmatech (Nanjing, China).*

Wild animals *The study did not involve wild animals.*

Reporting on sex *This finding applies to both sexes:  
For MC38 murine colon cancer treatment and tumor penetration experiments, female C57BL/6 mice were used.  
For B16-F10 murine melanoma treatment experiment, both female and male C57BL/6 mice were used.  
For immune cells in vivo depletion and interaction experiment, male C57BL/6 mice were used.  
For immuno-deficient mice experiments, female NCG-hlL15 and female B6-Rag1-KO mice were used.*

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

Ethics oversight *All the animal experiments were approved by the Animal Care and Use Committee of South China University of Technology (SCUT) , and every effort was made to minimize suffering from experiments (official approval number: 2019012).*

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 ICMJE [guidelines 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.*

Data collection *Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.*

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                      |                            |
|--------------------------|--------------------------|----------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | Public health              |
| <input type="checkbox"/> | <input type="checkbox"/> | National security          |
| <input type="checkbox"/> | <input type="checkbox"/> | Crops and/or livestock     |
| <input type="checkbox"/> | <input type="checkbox"/> | Ecosystems                 |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other significant area |

## Experiments of concern

Does the work involve any of these experiments of concern:

- | No                       | Yes                      |
|--------------------------|--------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
| <input type="checkbox"/> | <input type="checkbox"/> |
- Demonstrate how to render a vaccine ineffective
  - Confer resistance to therapeutically useful antibiotics or antiviral agents
  - Enhance the virulence of a pathogen or render a nonpathogen virulent
  - Increase transmissibility of a pathogen
  - Alter the host range of a pathogen
  - Enable evasion of diagnostic/detection modalities
  - Enable the weaponization of a biological agent or toxin
  - Any other potentially harmful combination of experiments and agents

## Plants

|                       |   |
|-----------------------|---|
| Seed stocks           | Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.  |
| Novel plant genotypes | Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied. |
| Authentication        | Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.   |

## ChIP-seq

### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

|   |   |
|---|---|
| Data access links<br>May remain private before publication. | 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 submission                                | Provide a list of all files available in the database submission.   |
| Genome browser session<br>(e.g. <a href="#">UCSC</a> )      | 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 and 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.  |
| Software                | Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.        |

## 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

The tumors were cut into small pieces and digested using a cocktail of enzymes containing collagenase (1 mg/mL), hyaluronidase (100 µg/mL) and DNase I (100 µg/mL), at 37 °C for 30 min. Then digested cells were passed through a 40-µm nylon mesh, and the red blood cells were lysed. Through counting, 100 µL of cell suspension (20 million cells/mL) was obtained for subsequent antibody staining and analysis via flow cytometry.

Instrument

BD FACSCelesta™ flow cytometer

Software

Data collection: BD FACS Diva software v8.0.1.1  
Data analysis: FlowJo software v10.0.7

Cell population abundance

No sorting was performed.

Gating strategy

Initial cell populations were gated for removing cell debris using an FSC and SSC plot. DAPI negative cells were gated to identify live cells. Then CD45 positive, CD3 positive, CD8 positive cells were gated in turn to differentiate CD8 T cell lineage. CD45 positive, CD3 negative, and NK1.1 positive cells were gated to receive NK cell populations.

- 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

*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

Used

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 & inference

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

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See [Eklund et al. 2016](#))

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 connectivity  Graph analysis  Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

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, etc.).

Multivariate modeling and predictive analysis

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