nature research

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Last updated by author(s): Mar 18, 2022

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 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	BD Accuri C6 Software (version number, 1.0.264.21) were used to collect data of flow cytometry.
Data analysis	ImageJ (version number, 1.8.0) was used to analyze the data of western blot analysis and Coomassie blue staining. BD Accuri C6 Software (version number, 1.0.264.21) and FlowJo (version number, 10.0.0.0) were used to analyze the data of flow cytometry. GraphPad Prism (version number, 5.0 for Procedure 1 and 8.0 for Procedure 2) and IBM SPSS Statistics (version number, 19.0) were used for the statistical 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 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

The main data discussed in this protocol are available in the supporting primary research papers (ref.14,15). Source data for Figures 2-10 and Supp. Figure 1 is provided as Supplementary information.

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	No statistical methods were used to determine sample size. In vitro experiments were performed with at least 3 biologically independent samples. All in vivo experiments were performed with at least 3 independent animals. Sample sizes were sufficient to perform statistical analyses.
Data exclusions	No samples were excluded.
Replication	All attempts at replication were successful. Experimental repeat numbers are also reported in Figure Legends.
Randomization	All samples/organisms were numbered and randomly grouped by random number table method.
Blinding	All experimental procedures and quantification of results, including injections, isolation of the tumors or organs, tissue histological analysis and flow cytometry, were done by two independent researchers. Meanwhile, all researchers were blinded to group allocation.

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

Procedure 1:
Anti-HA tag antibody (Abcam, cat. no. ab236632, Clone: EPR22819-101, RRID: AB_2864361)
HRP-conjugated secondary antibody (goat anti rabbit IgG, Invitrogen, cat. no. A16096, Clone: polyclone, RRID: AB_2534770)
FITC-anti-mouse CD11c (BioLegend, cat. no. 117306, Clone: N418, RRID: AB_313775)
PE/Cy7-anti-mouse CD80 (BioLegend, cat. no. 104734, Clone: 16-10A1, RRID: AB_2563112)
APC-anti-mouse CD86 (BioLegend, cat. no. 105012, Clone: GL-1, RRID: AB_493342)
PE/Cy7-anti-mouse H-2Kb bound to SIINFEKL (BioLegend, cat. no. 141608, Clone: 25-D1.16, RRID: AB_11218593)
FITC-anti-mouse CD3 (BioLegend, cat. no. 100204, Clone: 17A2, RRID: AB_312661)
APC-anti-mouse CD8 (BioLegend, cat. no. 100712, Clone: 53-6.7, RRID: AB_312751)
PE/Cy7-anti-mouse IFNγ (BioLegend, cat. no. 505826, Clone: XMG1.2, RRID: AB_2295770)
PE-anti-mouse CD4 (Invitrogen, cat. no. 2013481, Clone: RM4-5, RRID: unknown)
APC-anti-mouse CD3 (BioLegend, cat. no. 100236, Clone: 17A2, RRID: AB_2561456)
FITC-anti-mouse CD8 (Invitrogen, cat. no. 2002714, Clone: 53-6.7, RRID: unknown)
FITC-anti-mouse CD4 (BioLegend, cat. no. 100406, Clone: GK1.5, RRID: AB_312691)
PE-anti-mouse Foxp3 (BioLegend, cat. no. 126404, Clone: MF-14, RRID: AB_1089117)
FITC-anti-mouse Ly6G (BioLegend, cat. no. 127606, Clone: 1A8, RRID: AB_1236494)
APC-anti-mouse CD11b (BioLegend, cat. no. 101212, Clone: M1/70, RRID: AB_312795)
PE/Cy7-anti-mouse F4/80 (BioLegend, cat. no. 123114, Clone: BM8, RRID: AB_893478)
PE-anti-mouse CD11b (BioLegend, cat. no. 101208, Clone: M1/70, RRID: AB_312791)
APC-anti-mouse Gr1 (BioLegend, cat. no. 108412, Clone: RB6-8C5, RRID: AB_313377)
PE-anti-mouse CD44 (BioLegend, cat. no. 103008, Clone: IM7, RRID: AB_312959)
PE/Cy7-anti-mouse CD62L (BioLegend, cat. no. 104418, Clone: MEL-14, RRID: AB_313103)

Procedure 2:
Anti-mouse IgG-gold conjugate (10 nm) (Fitzgerald, cat. no. 43R-IG084GD, Clone: unknown, RRID: AB_1286630, 1:20).
Anti-rabbit IgG-gold conjugate (5 nm) (Fitzgerald, cat. no. 43R-IG100GD, Clone: unknown, RRID: AB_1286646,1:20).
Anti-mouse Na+/K+ ATPse (Abcam, cat. no. ab76020, Clone: polyclone, RRID: AB_1310695, undiluted for Immunogold staining).
Anti-Ftsz (Agrisera, cat. no. AS10-715, Clone: polyclone, RRID: AB_10754647, https://www.agrisera.com/en/artiklar/ftsz-procaryotic-cell-division-gtpase-2.html).
Anti-mouse TLR4 (Abcam, cat. no. ab13867, Clone: polyclone, RRID: AB_300696, 1:200 for western blot)
Anti-mouse TLR1 (Abcam, cat. no. ab180798, Clone: polyclone, RRID: unknown, 1:1,000 for western blot)
Anti-mouse TLR2 (Abcam, cat. no. ab209216, Clone: EPR20302-119, RRID: unknown, 1:100 for western blot)
Anti-mouse TLR6 (CST, cat. no. 12717, Clone: D1Z8B, RRID: AB_2798005, 1:200 for western blot)
Anti-mouse NF-κB (Abcam, cat. no. ab16502, Clone: polyclone, RRID: AB_443394, 1:2,000 for western blot)
Anti β-actin (Abcam, cat. no. ab8226, Clone: Abcam 8226, RRID: AB_306371,1:5,000 for western blot)
FITC-anti-mouse CD11c (Biolegend, cat. no. 117305, Clone: N418, RRID: AB_313774, 1:100)
PE-anti-mouse CD80 (Biolegend, cat. no. 104707, Clone: 16-10A1, RRID: AB_313128,1:200)
PE-anti-mouse CD86 (Biolegend, cat. no. 105007, Clone: GL-1, RRID: AB_313150, 1:200)

Validation

All primary antibodies were purchased from the supplier as noted above and used without additional validation. The validation of all the antibodies could be found from manufacturers online:
Procedure 1:
Anti-HA tag antibody (ab236632): https://www.abcam.cn/ha-tag-antibody-epr22819-101-ab236632.html
HRP-conjugated secondary antibody (A16096): https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Secondary-Antibody-Polyclonal/A16096
FITC-anti-mouse CD11c (117306): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd11c-antibody-1815
PE/Cy7-anti-mouse CD80 (104734): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd80-antibody-9320
APC-anti-mouse CD86 (105012): https://www.biolegend.com/en-us/products/apc-anti-mouse-cd86-antibody-2896
PE/Cy7-anti-mouse H-2Kb bound to SIINFEKL (141608): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-h-2kb-bound-to-siinfekl-antibody-7883
FITC-anti-mouse CD3 (100204): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd3-antibody-45
APC-anti-mouse CD8 (100712): https://www.biolegend.com/en-us/products/apc-anti-mouse-cd8a-antibody-150
PE/Cy7-anti-mouse IFNγ (505826): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-ifn-gamma-antibody-5865
PE-anti-mouse CD4 (2013481): https://assets.thermofisher.cn/TFS-Assets/LSG/certificate/Certificates-of-Analysis/12004285_2013481.PDF
APC-anti-mouse CD3 (100236): https://www.biolegend.com/en-us/products/apc-anti-mouse-cd3-antibody-8055
FITC-anti-mouse CD8 (2002714): https://assets.thermofisher.cn/TFS-Assets/LSG/certificate/Certificates-of-Analysis/11008185_2002714.PDF
FITC-anti-mouse CD4 (100406): https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd4-antibody-248
PE-anti-mouse Foxp3 (126404): https://www.biolegend.com/en-us/products/pe-anti-mouse-foxp3-antibody-4660
FITC-anti-mouse Ly6G (127606): https://www.biolegend.com/en-us/products/fitc-anti-mouse-ly-6g-antibody-4775
APC-anti-mouse CD11b (101212): https://www.biolegend.com/en-us/products/apc-anti-mouse-human-cd11b-antibody-345
PE/Cy7-anti-mouse F4/80 (123114): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-f4-80-antibody-4070
PE-anti-mouse CD11b (101208): https://www.biolegend.com/en-us/products/pe-anti-mouse-human-cd11b-antibody-349
APC-anti-mouse Gr1 (108412): https://www.biolegend.com/en-us/products/apc-anti-mouse-ly-6g-ly-6c-gr-1-antibody-456
PE-anti-mouse CD44 (103008): https://www.biolegend.com/en-us/products/pe-anti-mouse-human-cd44-antibody-2206
PE/Cy7-anti-mouse CD62L (104418): https://www.biolegend.com/en-us/products/pe-cyanine7-anti-mouse-cd62l-antibody-1922

Procedure 2:
Anti-mouse IgG-gold conjugate (43R-IG084GD): https://www.fitzgerald-fii.com/goat-anti-mouse-igg-10-nm-gold-colloid-43r-ig084gd.html
Anti-rabbit IgG-gold conjugate (43R-IG100GD): https://www.fitzgerald-fii.com/goat-anti-rabbit-igg-5-nm-gold-colloid-43r-ig100gd.html
Anti-mouse Na+/K+ ATPse (ab76020): https://www.abcam.com/sodium-potassium-atpase-antibody-ep1845y-plasma-membrane-loading-control-ab76020.html
Anti-Ftsz (AS10-715): https://www.agrisera.com/en/artiklar/ftsz-procaryotic-cell-division-gtpase-2.html
Anti-mouse TLR4 (ab13867): https://www.abcam.com/tlr4-antibody-ab13867.html
Anti-mouse TLR1 (ab180798): https://www.abcam.cn/tiltlr1-antibody-ab180798.html
Anti-mouse TLR2 (ab209216): https://www.abcam.com/tlr2-antibody-epr20302-119-ab209216.html
Anti-mouse TLR6 (12717): https://www.cellsignal.com/products/primary-antibodies/toll-like-receptor-6-d1z8b-rabbit-mab/12717
Anti-mouse NF-κB (ab16502): https://www.abcam.cn/nf-kb-p65-antibody-ab16502.html
Anti β-actin (ab8226): https://www.abcam.cn/beta-actin-antibody-mabcam-8226-loading-control-ab8226.html
FITC-anti-mouse CD11c (117305): https://www.biolegend.com/ja-jp/products/fitc-anti-mouse-cd11c-antibody-1815?GroupID=BLG11937
PE-anti-mouse CD80 (104707):https://www.biolegend.com/en-us/products/pe-anti-mouse-cd80-antibody-43
PE-anti-mouse CD86 (105007): https://www.biolegend.com/en-us/products/pe-anti-mouse-cd86-antibody-256?GroupID=BLG10719

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Procedure 1:
The B16-F10 murine melanoma cell line (ATCC, Manassas, USA Cat. no. CRL-6475, RRID: CVCL_0159, sex chromosomes: XY), American Type Culture Collection (ATCC, Manassas, USA).
The MC38 murine colon cancer cell line (CRC cat. no. 1101MOU-PUMC000523, RRID: CVCL_B288, sex chromosomes: XX), Cell Resource Center, IBMS, CAMS/PUMC.

	The B16-OVA cell is generously provided by Prof. Wang Hao at the National Center for Nanoscience and Technology, which was constructed from B16-F10 that was originally purchased from American Type Culture Collection (Manassas, VA, USA) through stable transfection of the ovalbumin gene (Gene ID: 396058).. Procedure 2: Murine 4T1 breast tumor cell (ATCC, Manassas, USA, cat. no. CRL-2539, RRID: CVCL_0125, sex chromosomes: XX) Murine B16-F10 melanoma cell (ATCC, Manassas, USA cat. no. CRL-6475, RRID: CVCL_0159, sex chromosomes: XY) Murine CT26 colon carcinoma cell (ATCC, Manassas, USA cat. no. CRL-2638, RRID: CVCL_7256, sex chromosomes: unknown)
Authentication	All cell lines were validated with cell line authentication by the short tandem-repeat DNA profiling.
Mycoplasma contamination	All cell lines were carried out with mycoplasma detection and were negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	All cell lines are not listed in the database.

Animals and other organisms

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

Laboratory animals	All animal studies were performed in accordance with ARRIVE guidelines. The mice were fed in a room at 20-22 °C with a 12-h light/dark cycle and a humidity of 30–70%. Provide food and water ad libitum. Procedure 1: C57BL/6 mice (6-8 week old), Vital River Laboratory Animal Technology Co. Ltd (Beijing, China). Procedure 2: C57BL/6 and BALB/c Mice (6-8 week old), Vital River Laboratory Animal Technology Co. Ltd (Beijing, China).
Wild animals	This study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal studies were approved by the Institutional Animal Care and Use Committee of National Center for Nanoscience and Technology.

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	The sample preparation method was described in the Protocol.
Instrument	BD Accuri C6 (BD Biosciences, USA) was used to analyze samples.
Software	BD Accuri C6 Software (version number, 1.0.264.21) and FlowJo (version number, 10.0.0.0) were used to analyze the data of flow cytometry.
Cell population abundance	Over 10000 cells were analyzed for fluorescent intensity in the defined gate.
Gating strategy	A gate is drawn around the cells. Single cells are determined with the area and the height of the side scatter (SSC). The analysis was carried out in this gate.

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