# natureresearch

Corresponding author(s):	Jun-O Jin
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# **Reporting Summary**

X Life sciences

Behavioural & social sciences

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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Statistics							
For all statistic	al analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.						
n/a Confirme	/a Confirmed						
The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement							
A sta	tement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly						
The s	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 des	scription of all covariates tested						
A des	scription of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons						
☐ ☐ A full AND	description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)						
	ull 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>P</i> values as exact values whenever suitable.						
For B	ayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings						
For h	ierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes						
Estim	nates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated						
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.						
Software	and code						
Policy informa	tion about <u>availability of computer code</u>						
Data collecti	on Used Microsoft Excel for collection of data						
Data analysi:	For flow cytometry analysis, we used FlowJo 8.6 software (Tree Star, San Diego, CA, US)						
	tilizing 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. Irage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.						
Data							
All manuscrip - Accession - A list of fig	tion about <u>availability of data</u> ots must include a <u>data availability statement</u> . This statement should provide the following information, where applicable: codes, unique identifiers, or web links for publicly available datasets cures that have associated raw data ion of any restrictions on data availability						
	The authors declare that all data supporting the findings of this study are available within the article and its supplementary information files or from the corresponding author upon reasonable request.						
	pecific reporting						
Diagra calact t	he one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection						

Ecological, evolutionary & environmental sciences

# Life sciences study design

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

For statistical analysis, six independent samples were used (two-three mice per experiment, two-tree experiments). Sample size

Data exclusions No data were excluded

Research sample

Data collection

Replication For replication of the experimental findings, we repeated measurement for two to three times. All attempts at replication were successful.

Randomization We randomly divided experimental group for immune stimulation and anti-tumor study.

Blinding The investigators were blinded to group allocation during data collection and/or analysis.

# Behavioural & social sciences study design

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

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

quantitative experimental, mixed-methods case study).

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. Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to Sampling strategy

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.

Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper,

predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale

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.

If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale Data exclusions

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.

If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if Randomization 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.

describe the data and its source.

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.

Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Research sample 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,

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.

Describe the data collection procedure, including who recorded the data and how. Data collection

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, collect	ion 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 and 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.						
We require information from a	n/a Involved in the study  ChIP-seq  Flow cytometry  MRI-based neuroimaging						
Antibodies							
Antibodies used	Mouse Abs: CD11c (N418)-B117324, CD8α (53-6.7)-B100734, CD40 (HM40-3)- B102911, CD80 (16-10A1)-B104707, CD86 (GL-1)-B105013, anti-MHC class I (AF6-88.5)-B116511, anti-MHC class II (M5/114.15.2)-B107620, anti-IFN-γ (XMG1.2)-B505825, anti-TNF-α (MP6-XT22)-B506323, anti-PD-L1 (B7-H1 )-BBE0101, anti-B220 (RA3-6B2)-B103205, anti-CD3 (17A2)-B100203, anti-CD49b (DX5)-B108905, anti-Gr1 (RB6-8C5)-B108405, anti-Thγ-1.1 (OX-7)-B202503, and anti-TER-119 (TER-119)-B116205 Human Abs: CD11c (3.9)-B254813, CD80 (2D10)-B207831, CD83 (HB15e)-B147674, CD86 (BU63)-B202906, anti-HLA-A, B, C (W6/32)-B212641, anti-HLA-DR, DP, DQ (Tü39)-B211013, anti-CD1c (L161)-B331510, anti-CD141 (M80)-B344112, anti-IFN-γ (4S.B3)-B193274, anti-CD3 (HIT3a)-B300306, anti-CD14 (63D3)-B367115, anti-CD16 (3G8)-B302006, anti-CD19 (HIB19)-B302206, anti-CD20 (2H7)-B302304, and anti-CD56 (5.1H11)-B362545.						
Validation	In this study, we used commercially available antibodies. Antibody profiles provided online databases.						
Eukaryotic cell line	es Es						
Policy information about <u>cel</u>	<u>l lines</u>						
Cell line source(s)	B16-F10 (ATCC, CRL-6475) and CT26 (ATCC, CRL-2638)						
Authentication	Purchased from ATCC						

Confirmed that all cell lines were negative for mycoplasma contamination.

Mycoplasma contamination

Commonly misidentified	line
(See ICLAC register)	

We	did	not	used	misidentified	cell	lines

### Palaeontology

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).

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.

autes are provided.

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

### Animals and other organisms

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

Laboratory animals

C57BL/6, TLR2-KO, TLR4-KO, OT-I, OT-II TCR transgenic mice, C57BL/6-Ly5.1 (CD45.1) congenic mice, and BLAB/c mice were obtained from Shanghai Public Health Clinical Center (SPHCC), MD2-KO mice (B6.129P2-Ly96 KO) on C57BL/6 background were kindly provided by Professor Guang Liang (Wenzhou Medical University, Wenzhou, Zhejiang, China)

Wild animals

We did not use wild animals

Field-collected samples

the mice were kept under pathogen-free conditions. Female mice were used in all experiments. The mice were housed in a room at 20–22°C and 50–60% humidity, and fed with standard rodent chow and water.

Ethics oversight

The study was carried out using the guidelines of the Institutional Animal Care and Use committee at the SPHCC. The mouse protocol approved by the Ethics of Animal Experiments committee of the SPHCC is 2018-A049-01.

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

### Human research participants

Policy information about studies involving human research participants

Population characteristics

Health donor, 25~35 years old male and female.

Recruitment

volunteer

Ethics oversight

This study has been conducted according to principles of the Declaration of Helsinki. Peripheral blood samples were harvested from healthy donors at the SPHCC. Written informed consent was obtained from all volunteers. The study is approved by the Institutional Review Board at SPHCC (IRB number: 2017-Y037).

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

#### Clinical data

Policy information about <u>clinical studies</u>

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.

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

Study protocol

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.

#### ChIP-seq

#### Data deposition

Confirm that y					

Data access links

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document,

Data access links

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 (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 and whether they were paired- or single-end.

redus und whether they were palied- 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.

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.

illucx 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

Lymph node cells were incubated with unlabeled isotype control Abs and Fc-block Abs for 15 min (BioLegend, San Diego, CA, USA). Later, fluorescence-conjugated Abs were added, and the cells were further incubated on ice for 30 min. After washing with PBS, the cells were analyzed on FACS Fortessa (Becton Dickinson, Franklin Lakes, New Jersey, US) using FlowJo 8.6 software (Tree Star, San Diego, CA, US). Cellular debris and dead cells were excluded by forward- and side-scatter gating and 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, St. Louis, Missouri, US) staining.

Instrument

FACS Fortessa (Becton Dickinson, Franklin Lakes, New Jersey, US)

Software

FlowJo 8.6 software (Tree Star, San Diego, CA, US).

Cell population abundance

DCs were analyzed more than 5,000 cells after gating. Total tumor infiltrated OT-I and OT-II cells were analyzed by flow cytometry.

Gating strategy

Mouse DCs: The leukocytes were stained with FITC-labeled monoclonal Abs (mAbs) for 30 min. Anti-B220 (RA3-6B2), anti-CD3 (17A2), anti-CD49b (DX5), anti-Gr1 (RB68C5), anti-Thy1.1 (OX-7), and anti-TER-119 (TER-119) were added as lineage markers. Lymphoid DCs were defined as a lineage-CD11c+ cells in live leukocyte population, the populations of which were further separated and represented as CD8 $\alpha$ + and CD8 $\alpha$ - DCs.

Human PBDCs: The PBMCs isolated from blood were stained with FITC-conjugated lineage Abs, anti-CD3 (HIT3a), anti-CD14 (63D3), anti-CD16 (3G8), anti-CD19 (HIB19), anti-CD20 (2H7), and anti-CD56 (5.1H11). The lingeage-CD11c+ cells in live leukocytes were gated as PBDCs using flow cytometry. The PBDCs were further fractionated into BDCA1+ (L161) and BDCA3+ (M80) DC cells by FACS Fortessa (Becton Dickinson, Franklin Lakes, New Jersey, US).

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).					
cquisition						
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					
reprocessing						
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.					
tatistical 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 (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 cor  Graph analysis  Multivariate modeling or predi						
Functional and/or effective connecti	unctional 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,					

Multivariate modeling and predictive analysis

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