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.

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	1.RNA-seq data were sequenced by Illumina Hiseq ×10 platform; 2.RiboTag-seq data were sequenced by Illumina Novaseq6000 platform; 3.Flow cytometry data were collected with BD LSRFortessa×20; 4.Immunoblots were collected with Bio-Rad ChemiDoc MP Imaging System(12003153); 5.RT-qPCR data were collected with Bio-Rad CFX Connect Real-Time System(Bio-Rad CFX Maestro 1.1).
Data analysis	1. GraphPad Prism 8 (version 8.4.1) and Excel(16.54) were used to analyze the RT-qPCR data; 2.Sequencing data was analyzed using the Snapgene(6.0.2); 3.For Flow cytometry, Flowjo (vX.0.7) was used. Numerical data were exported to Excel(16.54)and were further analyzed by GraphPad Prism 8 (version 8.4.1) ; 4.For RNA-seq,RNA-seq reads were aligned to the Mus musculus genome (UCSC genome mm10) by HISAT2 (v2.1.0) with '--rna-strandness rf –fr'. Read counts were calculated by htseq-count (0.11.2) using mapped reads, and differentially expressed genes were identified by DESeq2 (v1.36.0) . Volcano plots and principal component analysis (PCA) plots were generated using the ggplot2 package (v3.4.0). 5.For RiboTag-seq Fastq files from RNA sequencing and Ribo-Tag RNA sequencing were aligned to the mm10 genome using HISAT2 version 2.2.1, and StringTie version 2.2.1 was employed to count reads mapped to each gene. 6.For m6Amseq,MultiQuantTM(version3.0.3),Trim_galore(version0.6.6),HISAT2(version2.1.0),IGV(version2.9.4),Homer(version4.10), MEME(version 4.12.0). All R packages used are available online.

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

The raw sequence data of bulk RNA-seq/Ribo-Tag-seq data generated in this study have been deposited to the BioProject database under BioProject ID：PRJNA1188217. Reviewers can access through the following link: https://dataview.ncbi.nlm.nih.gov/object/PRJNA1188217?reviewer=jnrpj02ckf4nvfdqd2r116v05n. The remaining data are available within the Article, Supplementary Information or Source Data file. Source data are provided with this paper.

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

Life sciences study design

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

Sample size	Sample size is indicated in the figure legend for each experiments. Sample sizes were chosen based on previous publications, which were sufficient for statistical analysis, For in vivo studies, n ≥5 mice per group is sufficient to detect meaningful biological differences with good reproducibility. in vitro experiments, results of two or three independent biological replicates were used.
Data exclusions	No data exclusion
Replication	All experiments were replicated as indicated in the respective figure legends.
Randomization	Samples or mice were grouped according to treatment or genotypes,thus not randomized.
Blinding	Data collection and analysis were not performed blind to conditions of the experiments.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

For mice in vivo treatment:
InVivoMAb anti-mouse CD4(BioXCell, catalog no. BE0003-1,Clone:GK1.5);
InVivo MAb anti-mouse CD8(BioXCell, catalog no. BE0061,Clone:2.43);
InVivoMAb anti-mouse NK1.1(BioXCell, catalog no. BE0036,Clone:PK136);
InVivoMAb anti-mouse PD-1(BioXCell, catalog no. BE146,Clone:Clone:RMP1-14);
InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin(BioXCell, catalog no. BE0090,Clone:Clone:LTF-2);

For western blot:
Stat1 (D1K9Y) (1:1000, CST, catalog no. 14994S);
P-Stat1(1:1000, CST, catalog no. 9167S);
PCIF1(1:1000, Abcam, catalog no. ab205016);
Beta-Actin(1:1000, CST, catalog no. 3700S);
GAPDH(1:1000, CST, catalog no. 2118);
Tubulin(1:1000, Servicebio, catalog no. GB15201-100);
T-bet （1：1500，CST, catalog no.97135);
m6A （1：50，abclonal, catalog no. A17924);
GATA3 （1：1000，abclonal, catalog no. A21809);
FOXP3 （1：1000，abclonal, catalog no. A27170);
HRP-conjugated Affinipure Donkey Anti-Mouse IgG(H+L)(1:5000, Proteintech, no. SA00001-8);
HRP-conjugated Affinipure Donkey Anti-Rabbit IgG(H+L)(1:5000, Proteintech, no. SA00001-9);

For flow cytometry:
anti-mouse CD45(Biolegend, catalog no. 103154, clone: 30-F11);
anti-mouse CD3ε(Biolegend, catalog no. 100355, clone: 145-2C11);
anti-mouse TCR β chain(Biolegend, catalog no. 109222, clone: H57-597);
anti-mouse CD4(Biolegend, catalog no. 100428, clone: GK1.5);
anti-mouse CD8a(Biolegend, catalog no. 100734, clone: 53-6.7);
anti-mouse NK-1.1(Biolegend, catalog no. 108710, clone: PK136);
anti-mouse IFN-γ(Biolegend, catalog no. 505808, clone: XMG1.2);
anti-mouse IL-2(Biolegend, catalog no. 503806, clone: JES6-5H4);
anti-mouse/human CD44(Biolegend, catalog no. 103006, clone: IM7);
anti-mouse CD62L(Biolegend, catalog no. 104412, clone: MEL-14);
anti-human IFN-γ(Biolegend, catalog no. 502509, clone: 4S.B3);
anti-human IL-2(Biolegend, catalog no. 500310, clone: MQ1-17H12);
anti-mouse GZMB(Biolegend, catalog no. 372206, clone: QA16A02);
anti-human CD4(Biolegend, catalog no. 344646, clone:SK3);
anti-human IFN-γ(Biolegend, catalog no. 502526, clone:4S.B3);
anti-mouse Perforin(Biolegend, catalog no. 5154306, clone:S16009B);
anti-T-bet Antibody(Biolegend, catalog no. 644812, clone:4B10);
anti-mouse IL-4(Biolegend, catalog no. 504104, clone:11B11);
anti-mouse Ki-67(Biolegend, catalog no. 652406, clone:16A8);
anti-mouse IL-17A(Biolegend, catalog no. 506915, clone:TC11-18H10.1);
anti-mouse FOXP3(Biolegend, catalog no. 126403, clone:MF-14);

Validation

All of the antibodies used have been extensively utilized in the literature and have been validated by manufacturers. The information can be found at the following websites:

For mice in vivo treatment:
InVivoMAb anti-mouse CD4(https://bioxcell.com/invivomab-anti-mouse-cd4-be0003-1)
InVivo MAb anti-mouse CD8(https://bioxcell.com/invivomab-anti-mouse-cd8-alpha-be0061)

InVivoMAb anti-mouse NK1.1(https://bioxcell.com/invivomab-anti-mouse-nk1-1-be0036)
InVivoMAb anti-mouse PD-1(https://bioxcell.com/invivomab-anti-mouse-pd-1-cd279-be0146)
InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin(https://bioxcell.com/invivomab-rat-igg2b-isotype-control-anti-keyhole-limpet-hemocyanin-be0090)

For western blot:
Stat1 (D1K9Y) (https://www.cellsignal.cn/products/primary-antibodies/stat1-d1k9y-rabbit-mab/14994);
P-Stat1(https://www.cellsignal.cn/products/primary-antibodies/phospho-stat1-tyr701-58d6-rabbit-mab/9167P-Stat1);
PCIF1(https://www.abcam.cn/products/primary-antibodies/pcif1-antibody-ab205016.html);
Beta-Actin(https://www.cellsignal.cn/products/primary-antibodies/b-actin-8h10d10-mouse-mab/3700);
GAPDH(https://www.cellsignal.cn/products/primary-antibodies/gapdh-14c10-rabbit-mab/2118);
Tubulin(https://afsbio.com/products/recombinant-anti-alpha-tubulin-antibody-rabbit-mab-gb15201);
T-bet(https://www.cellsignal.cn/products/primary-antibodies/t-bet-tbx21-e4i2k-rabbit-monoclonal-antibody/97135);
m6A（https://abclonal.com.cn/catalog/A17924);
GATA3（https://www.abclonal.com.cn/catalog/A21809);
FOXP3（https://abclonal.com.cn/catalog/A12051);
HRP-conjugated Affinipure Donkey Anti-Mouse IgG(H+L)(https://www.ptgcn.com/results?category=&filter=&q=SA00001-8);
HRP-conjugated Affinipure Donkey Anti-Rabbit IgG(H+L)(https://www.ptgcn.com/products/Peroxidase-conjugated-Affinipure-Donkey-Anti-Rabbit-IgG-H-L.htm);

For flow cytometry:
anti-mouse CD45(https://www.biolegend.com/en-gb/products/apc-fire-750-anti-mouse-cd45-antibody-13049);
anti-mouse CD3ε(https://www.biolegend.com/en-gb/products/brilliant-violet-785-anti-mouse-cd3epsilon-antibody-12081);
anti-mouse TCR β chain(https://www.biolegend.com/en-gb/products/pe-cyanine7-anti-mouse-tcr-beta-chain-antibody-4144);
anti-mouse CD4(https://www.biolegend.com/en-gb/products/pacific-blue-anti-mouse-cd4-antibody-3316);
anti-mouse CD8a(https://www.biolegend.com/en-gb/products/percp-cyanine5-5-anti-mouse-cd8a-antibody-4255);
anti-mouse NK-1.1(https://www.biolegend.com/en-gb/products/apc-anti-mouse-nk-1-1-antibody-427);
anti-mouse IFN-γ(https://www.biolegend.com/en-gb/products/pe-anti-mouse-ifn-gamma-antibody-997);
anti-mouse IL-2(https://www.biolegend.com/en-gb/products/fitc-anti-mouse-il-2-antibody-952);
anti-mouse/human CD44(https://www.biolegend.com/en-gb/products/fitc-anti-mouse-human-cd44-antibody-314);
anti-mouse CD62L(https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd62l-antibody-381);
anti-human IFN-γ(https://www.biolegend.com/en-gb/products/pe-anti-human-ifn-gamma-antibody-1011);
anti-human IL-2(https://www.biolegend.com/en-gb/products/apc-anti-human-il-2-antibody-1348);
anti-mouse GZMB(https://www.biolegend.com/en-gb/products/fitc-anti-human-mouse-granzyme-b-recombinant-antibody-14430?GroupID=GROUP28);
anti-human CD4(https://www.biolegend.com/en-gb/product-preview/brilliant-violet-605-anti-human-cd4-antibody-15992?GroupID=GROUP28);
anti-human IFN-γ(https://www.biolegend.com/en-gb/products/pe-anti-human-ifn-gamma-antibody-1011?GroupID=BLG10006);
anti-mouse Perforin(https://www.biolegend.com/en-gb/products/apc-anti-mouse-perforin-antibody-15281?GroupID=ImportedGROUP1);
anti-T-bet Antibody(https://www.biolegend.com/en-gb/products/fitc-anti-t-bet-antibody-6435?GroupID=BLG6433);
anti-mouse IL-4(https://www.biolegend.com/en-gb/sean-tuckers-tests/pe-anti-mouse-il-4-antibody-893?GroupID=BLG1753);
anti-mouse Ki-67(https://www.biolegend.com/en-gb/products/apc-anti-mouse-ki-67-antibody-8447?GroupID=GROUP26);
anti-mouse IL-17A(https://www.biolegend.com/en-gb/products/apc-anti-mouse-il-17a-antibody-3540?GroupID=GROUP24);
anti-mouse FOXP3(https://www.biolegend.com/en-gb/products/pe-anti-mouse-foxp3-antibody-4660?GroupID=BLG5706);

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Murine melanoma B16/F10 cells, MC38 cells , Jurkat , Raji and HEK293T cells were acquired from the Cell Bank of the Shanghai Academy of Chinese Sciences.
Authentication	cell lines have been authenticated by STR analysis, as declared by cell Bank of the Shanghai Academy of Chinese Sciences.
Mycoplasma contamination	cell lines have been tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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	Pcif1flox/flox mice, with a C57BL/6 genetic background, were created utilizing a LoxP-targeting system by Cyagen Company. These mice were subsequently crossed with Cd4Cre mice obtained from The Jackson Laboratory to generate Pcif1flox/floCd4Cre conditional knockout (cKO) mice. Concurrently, Pcif1flox/flox mice were used as wild-type (WT) littermate controls.
Wild animals	No wild animals were used in the study.
Reporting on sex	Eight- to Twelve-week-old mice (both sexes) were used for all experiments. Mice of the same gender were used in the experiment.

Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	Animal procedures were approved by Institutional Animal care and Use Committee(IACUC) of Shanghai jiao Tong University School of Medicine.

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

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, mosiacism, off-target gene editing) were examined.*

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 thymus, spleen, and lymph nodes were collected and passed through a 100 µm cell strainer. Spleen cells were prepared using a red blood cell lysis buffer (Thermo Fisher Scientific). To extract colonic lymphocytes, the colon was first sectioned and rinsed with cold PBS. It was then divided into 1-centimeter segments and placed in an extraction solution (comprising 2% fetal bovine serum, 2 mM EDTA, and 1 mM dithiothreitol in PBS), and agitated at 200 revolutions per minute (rpm) at a temperature of 37°C for 30 minutes. Following thorough cleansing to eliminate intraepithelial lymphocytes, the lamina propria was finely diced and further incubated at 37°C with agitation at 200 rpm for 60 minutes in a digestion medium consisting of RPMI supplemented with 2% fetal bovine serum, type II collagenase at a concentration of 1 mg/ml, and dispase at 0.5 mg/ml. The resulting supernatant was harvested, and the lymphocytes were isolated through centrifugation over a Percoll density gradient. To extract tumor infiltrating lymphocytes (TILs), tumor specimens obtained from mice harboring tumors were minced into fine fragments, subjected to enzymatic digestion in RPMI 1640 medium (comprising 10% FBS, DNase I and type II collagenase), and agitated at 200 rpm at a temperature of 37°C for 30 minutes. Subsequently, the mixture was forced through a 100 µm cell strainer to create a cell suspension. The resulting supernatant was harvested, and the TILs were isolated through centrifugation over a Percoll density gradient.
Instrument	Flow cytometry data were collected with BD LSRFortessa×20
Software	For Flow cytometry, Flowjo (vX.0.7) was used.
Cell population abundance	20,000-100,000 cells were acquired and analyzed for Tissue preparation(thymus, spleen, and lymph nodes) For tumor infiltrated immune cells ,tumor infiltrating lymphocytes (TILs)were isolated through centrifugation over a Percoll density gradient, enrich immune cells from tumor tissues.
Gating strategy	1.Forward versus side scatter(FSC vs SSC) gating was used to identify cells and exclude cell debris and dead cells.2.Cells were gated on CD45+population.3.T cell were identified by gating CD3+ TCRb+ cells. 4.CD4 and CD8 T cells were were identified by gating CD4 +and CD8+ events within the CD3+ TCRb+ gating.5. Cytokine expression were gating within CD4 and CD8 T cells .

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