nature portfolio

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Last updated by author(s): Sep 1, 2024

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	Flow cytometry (FACS) ;BD FACS Celesta machine (BD FACS Diva Software V8.0); RNA sequencing:NovaSeq 6000 platform; ELISA:BioTek Synergy HTX ; QPCR:QuantStudioTM Flex Real-Time PCR System,96-well.
Data analysis	Flow cytometry (FACS) :FlowJo software V10.6.2; RNA sequencing:RNA-sequencing raw read counts were used for differential gene expression analysis by DESeq2. Genes with p value < 0.05 and log2 Fold change (FC) > 1 were counted as upregulated genes, and genes with p value < 0.05 and log2 FC < -1 were counted as downregulated genes.; ELISA:Gen5 CHS 3.11; QPCR:QuantStudioTM Flex Real-Time PCR Software V1.2.

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 RNA-seq raw data have been deposited in the Gene Expression Omnibus (GEO) database under the accession code GSE244939 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE244939]. All data are included in the Supplementary Information or available from the authors, as are unique reagents used in this Article. The raw numbers for charts and graphs are available in the Source Data file whenever possible. 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	Male and female lung cancer samples were used in our study, and this information is provided in Supplementary Table 4.
Reporting on race, ethnicity, or other socially relevant groupings	We worked to ensure sex and gender balance in the recruitment of the participants. We worked to ensure recial and ethnic or other types of diversity in the recruitment of the participants.
Population characteristics	Information about the human lung cancer samples is provided in Supplementary Table 4.
Recruitment	All of the patients were enrolled with written informed consent.
Ethics oversight	The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Ethical Review Board of Jiangsu Cancer Hospital and complied with all relevant ethical regulations.

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 sizes were chosen based on previous publications, which were sufficient for statistical analysis.
Data exclusions	No data were excluded from the analysis.
Replication	All attempts of replication were successful.The replication numbers were described in the corresponding figure legends.
Randomization	For all in vivo studies, gender and age-matched mice or equal genotype were randomly assigned to cages. Cages were placed on the same rack in the same room and were supplemented with equal food and water.
Blinding	Data collection of most mouse tumor experiments (Figure 6A,Figure 6I, Figure 7C and Supplementary Figure 7B) were performed in a double blinding manner. Cellular and biochemical experiments were not performed in a blinding manner.

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 human and mouse T cell activation, anti-mouse CD3ε (clone 145-2C11) and anti-mouse CD28 (clone 37.51) were purchased from Biolegend, anti-human CD3 (clone UCHT-1) and anti-human CD28 (clone CD28.2) were purchased from BD Biosciences. Phorbol 12-myristate 13-acetate (PMA) and PHA were purchased from Sigma-Aldrich. Ionomycin was purchased from AbMole BioScience.

For the flow cytometry analysis, anti-mouse CD45 (clone 30-F11), anti-mouse CD3ε (clone 500A2), anti-mouse CD4 (clone GK1.5), anti-mouse CD8a (clone 53-6.7 dilution 1:40), anti-mouse CD127 (clone A7R34 dilution 1:40), anti-granzyme B (clone QA18A28 dilution 1:100), anti-mouse IFNγ (clone XMG1.2 dilution 1:40), anti-mouse TNF (clone MP6-XT22 dilution 1:40), anti-mouse Ki-67 (clone 16A8 dilution 1:40), anti-CD44 (clone IM7 dilution 1:40), anti-mouse CD62L (clone MEL-14 dilution 1:40), anti-human CD4 (clone RPA-T4 dilution 1:40), anti-human CD8 (clone SK1 dilution 1:40), anti-human CD127 (clone A019D5 dilution 1:40), anti-human CD25 (clone BC96 dilution 1:40), anti-granzyme B (clone GB11 dilution 1:40), anti-human IFNγ (clone 4S.B3 dilution 1:40), anti-human TNFα (clone MAb11 dilution 1:40), anti-human Ki-67 (clone Ki-67 dilution 1:40) were purchased from Biolegend, anti-mouse CD25 (clone PC61 dilution 1:40), anti-human CD45 (clone HI30 dilution 1:40), anti-human CD3 (clone UCHT1 dilution 1:40), anti-human CD4 (clone SK3 dilution 1:40), anti-human PD-1 (clone MIH4 dilution 1:40) were purchased from BD Biosciences.

For immunoblots, anti-PD-1 (clone OTI21F5 dilution 1:1000) was purchased from OriGene, anti-PD-L1 (clone E1L3N dilution 1:1000) from Cell Signaling Technology, anti-GAPDH (clone 1E6D9 dilution 1:2000) from ProteinTech, HRP-conjugated goat anti-rabbit and HRP-conjugated goat anti-mouse from Beyotime Technology. Monoclonal antibodies and polyclonal antibodies against PD-1^28 were generated by Genscript.

For the PD-L1 blockade experiment, anti-PD-L1 (clone 10F.9G2) and IgG (clone LTF-2) antibodies were purchased from BioXCell.

Validation

The WB antibodies (Polyclonal antibody, 13D4, 2E5) and FACS antibody (13D4) for human PD-1^28 were validated. We expressed PDCD1^28 in human HEK293T cells. Polyclonal antibody, 13D4,2E5 and anti-Flag were used to immunoblotted PD-1^28. The target bands blotted by Polyclonal antibody, 13D4,and 2E5 were the same with anti-FLAG, indicating that these antibody successfully immunoblotted PD-1^28 (Supplementary Figure 1H). Similarly, the FACS antibody (13D4) was also validated in HEK293T cells overexpressing PD-1^28 (Supplementary Figure 1J). Other commercial antibodies have been validated by manufactures and the statements can be found on the manufactures' websites.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The human embryonic kidney HEK293T (GNHu 43) and NCI-H1299 (SCSP-589) cells lines were obtained from the Shanghai Cell Bank Type Culture Collection Committee (Shanghai, China). Jurkat cells (TIB-152) were obtained from the American Type Culture Collection. MC38 (337716) and B16F10 (100309) cells were obtained from BNCC (BeNa Culture Collection).

Authentication

We have done authentication for all the cell lines used in this study by STR analysis

Mycoplasma contamination

All cell lines are negative for Mycoplasma

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used in this 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

PDCD1^28flox/flox mice were generated by Cyagen Biosciences (Suzhou, China) on its CRISPR-Cas9 gene targeting platform. The knock-in mice were created at the locus of ROSA26 which is located on mouse chromosome 6 in C57BL/6J mice (#C001089). The CAG promoter-loxP-stop unit-loxP-PDCD1^28-rBG pA cassette was inserted between exon 1 and 2 of ROSA26. Homology arms generated by PCR using BAC clone as template. Cas9 and gRNA co-injected into fertilized eggs with targeting vector for mice production. Correctly targeted mice were determined by PCR and gene sequencing. PDCD1^28flox/flox mice were then crossed with CD4cre transgenic mice (#C001381) to obtain PDCD1^28CKI mice with PD-1^28 knockin in T cells. For animal experiments with PDCD1^28CKI mice, littermate controls with normal PD-1^28 expression (PDCD1^28flox/flox) were used. CD4cre transgenic mice were obtained from Cyagen Biosciences (Suzhou, China). NOD.Cg-PrkdcscidIL 2rgtm1Sug/JicCrl (NOG) mice (#408) were purchased from Beijing VitalRiver Laboratory Animal Technology Co. Ltd.

Mice were housed in individually ventilated cages under 12h light-12h dark cycle with normal food and water.

Wild animals	This study didn't involve any wild animals.
Reporting on sex	The research results can be used for male or female mice
Field-collected samples	This study didn't involve any field-collected samples.
Ethics oversight	All animal experiments were previously approved by the Ethics Committee for the Use of Experimental Animals of the Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences (Suzhou, Jiangsu, China). Monitoring Institute approved all animal protocols used in this study.

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Mouse primary T cells were isolated from spleen by negative selection kit. Jurkat cell line, B16F10 and MC38 cell line were cultured in 1640 medium as described in Methods. Isolation of tumor infiltrating T cells samples were described in Methods.
Instrument	The Flow cytometric data were collected by BD FACS Celesta machine (BD FACS Diva Software V8.0).
Software	Data were analysed with FlowJo software Version 10.6.2;
Cell population abundance	We collected data on at least 10,000 cells per experimental condition.
Gating strategy	For all experiments, cells were first gated by FSC/SSC to exclude debris, followed by gating FSC-A and FSC-H to eliminate nonsinglets. Then, target cell population for further analysis were gated by cell surface marker (e.g. human or mouse CD8). For surface marker, cytokine and transcriptional factor staining, isotype control antibodies were used to define background and non-specific binding signal.

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