nature portfolio

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Last updated by author(s): Apr 26, 2023

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	Software used for data acquisition is described in Methods. Flow-cytometry data was collected on a BD Fortessa LSRII or BD Symphony. SHG imaging was performed on a Leica SP5 X MP Inverted Confocal Microscope. Cryo-SEM was performed on a pre-cooled Zeiss FESEM Ultra55 Scanning Electron Microscope.
Data analysis	Flow-cytometry analyses were performed using FlowJo version 10 or R version 4.0.5. SHG quantification was performed using Imaris and Cryo-SEM was analyzed with ImageJ. scRNA-seq and bulk RNA-seq data were analysed using R or Python, as described in Methods.

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 data supporting the results in this study are available within the paper and its Supplementary information. Single-cell-sequencing data are available via the NCBI

Gene Expression Omnibus repository (GSE230631, subseries GSE230628, GSE230629). The following RNA-sequencing datasets used to validate the in vitro findings were assessed from publicly available GEO repositories: NSCLC (GSE99254), liver cancer (GSE98638), colorectal cancer (GSE108989), breast cancer (GSE114727), liver fibrosis (GSE136103), IPF (GSE135893, GSE159354), bulk-RNA-sequencing dataset (GSE126117). Source data for the figures are provided with this paper. All data acquired during the study are available from the corresponding author on reasonable request.

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	The study did not involve human participants.
Reporting on race, ethnicity, or other socially relevant groupings	—
Population characteristics	—
Recruitment	—
Ethics oversight	—

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	No statistical tests were used to predetermine sample size. Sample sizes were chosen to yield statistical significance on the basis of information from previous studies.
Data exclusions	No data were excluded.
Replication	The experiments were repeated, both with the same donor and with different donors, to demonstrate reproducibility.
Randomization	Randomization was not relevant for the in vitro studies. Mice that had been inoculated with tumors were randomized prior to treatment.
Blinding	Blinding was not performed.

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

Human Antibody Channel Catalog Number Vendor

CD62L BV510 304844 Biolegend
LAG3 APC/Fire 750 369214 Biolegend
CD45RA PE/Cy7 304126 Biolegend
CCR7 FITC 353216 Biolegend
Trail PE 308206 Biolegend
CD25 BV711 356138 Biolegend
Fas/CD95 PE/Dazzle 305634 Biolegend
Annexin V BV421 640924 Biolegend
Trail-R2 APC 307408 Biolegend
CD8 PerCP/Cy5.5 344710 Biolegend
CD3 PerCP/Cy5.5 300328 Biolegend
CD4 BV510 344634 Biolegend
CD8 APC/Cy7 344714 Biolegend
CAR BV711 352920 Biolegend
IL17A PE/Dazzle 512336 Biolegend
IFNG APC 502512 Biolegend
GZMB BV421 515408 Biolegend
TNF PE 502909 Biolegend
IL2 PE/Cy7 500326 Biolegend
CD4 BV711 563033 BD Biosciences
CD62L BV510 304844 Biolegend
CD103 (ITGAE) APC 350216 Biolegend
CD27 PE/Dazzle 356422 Biolegend
CD127 (IL7R) PE 351304 Biolegend
PD1 BV421 621608 Biolegend
CD25 PE/Dazzle 356126 Biolegend
TIM3 BV510 345030 Biolegend
OX40 PE 350004 Biolegend
CD39 (ENTPD1) PE/Cy7 328212 Biolegend
TIGIT APC 372706 Biolegend
CTLA4 BV421 369606 Biolegend
LAG3 FITC 369210 Biolegend
OX40 PE/Dazzle 350020 Biolegend
CD62L APC 304810 Biolegend
pAKT1 (Thr308) PE 13842S Cell Signaling
Jun AF647 sc-74543 AF647 Santa Cruz
pJun (Ser73) AF488 12714S Cell Signaling
pERK1/2 (Thr202/Tyr204) Pac Blue 14196S Cell Signaling
pMAPK14 (Thr180/Tyr182) AF594 8632S Cell Signaling
pMAPK8 (Thr183/Tyr185) PE 5755S Cell Signaling
pAKT1 (Ser473) PE/Cy7 88106S Cell Signaling
pPLCG2 (Tyr759) APC 17-9866-42 ThermoFisher
pPLCG1 ((Tyr783)) AF488 25678S Cell Signaling
pCD3z (Y142) PE 558448 BD Biosciences
pZAP70 (Y319)/Syk (Y352) AF488 557818 BD Biosciences
pPyk2 PE 560255 BD Biosciences
Anti-rabbit IgG (H+L), F(ab')2 Fragment PE 79408S Cell Signaling
JunB Unconjugated 3753S Cell Signaling
c-Jun Unconjugated 9165S Cell Signaling
BATF Unconjugated 8638S Cell Signaling
c-Fos Unconjugated 2250S Cell Signaling
FosB Unconjugated 2251S Cell Signaling
CD45 (Intracellular Domain) Unconjugated 13917S Cell Signaling
Anti-rabbit IgG HRP-linked 7074S Cell Signaling
CD4 (SK3) BUV 805 612887 BD Biosciences
CD127 (IL7R) AF 700 351344 Biolegend
CD25 APC 302610 Biolegend
TIM3 APC Cy7 345026 Biolegend
CD39 BV 711 328228 Biolegend
TIGIT BV 605 372712 Biolegend
LAG3 BV 785 369322 Biolegend
CD45RO BUV 563 748369 BD Biosciences

Validation

All antibodies are routinely tested by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Raji was purchased from American Type Culture Collection (ATCC). B16F10 was purchased from American Type Culture Collection (ATCC). HEK 293T was purchased from American Type Culture Collection (ATCC).
Authentication	Cell lines had been authenticated by ATCC.
Mycoplasma contamination	Mycoplasma-contamination tests had been performed by ATCC on the cell lines. We did not run additional tests.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	NSG mice, 5–6 weeks old, from The Jackson laboratory, catalogue number #005557.
Wild animals	The study did not involve wild animals.
Reporting on sex	Female mice were used for all animal studies, and sex was not considered in the studies.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The animal studies were performed in accordance with the National Institutes of Health and the Harvard University Faculty of Arts and Sciences' Institutional Animal Care and Use Committee (IACUC) guidelines.

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	To isolate T cells from collagen gels, collagen matrices were mechanically chopped into small pieces and digested in 100U/ml collagenase type IV (ThermoFisher Scientific #17104019) at 37ºC for 1 hour in digestion media (RPMI 1640 + 10% FBS), with intermittent mechanical disruption through 1-ml pipette tips. Collagenase was then quenched with MACS buffer (PBS, 0.5% BSA, 2mM EDTA), after which cells were then passed through 30-µM strainers (Miltenyi #130-098-458), washed twice in PBS, and resuspended for downstream analysis. Cells isolated from Col-Nb cells were kept at 4 ºC throughout immunostaining. Cells were stained with dead cell stain (ThermoFisher Scientific #L23105) according to the manufacturer's protocol. Cells were then blocked with FcX Fc receptor blocking solution (BioLegend #101319, #422301) for 20 min and stained with surface protein antibodies for 20 min. Brilliant violet staining buffer (BD Horizon #563794) and flow cytometry staining buffer (Invitrogen #00-4222-26) were used during staining. For phosphorylated protein staining, T cells were fixed/permeabilized while in collagen matrices with cold methanol for two hours to preserve the phosphorylation profile of the T cells. The gels were then digested, cells blocked with FcX Fc receptor blocking solution, and stained for phosphorylated proteins with prechilled True-Phos Perm Buffer (BioLegend #425401) according to manufacturer's protocol. Flow cytometry was then performed on a BD Fortessa LSRII. Gating was performed based on fluorescence-minus-one controls.
Instrument	BD Fortessa LSRII, BD Symphony
Software	Facs DIVA was used to acquire flow-cytometry data. FlowJo and R were used for the analyses.
Cell population abundance	No sorting was performed.

Gating strategy	Lymphocytes were gated using FSC-A and SSC-A. Doublets were gated out using FSC-A and FSC-H. Live CD3+ T cells were gated as CD3+, fixable live/dead blue negative (Thermo #L23105). Subsequent gatings were performed using fluorescence minus 1 (FMO) controls.

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