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	No software was used for data collection.
Data analysis	Mass analysis: Waters MassLynx Mass Spectrometry Software 4.1 FACS: FlowJo vX.0.7 SDS-PAGE: ImageJ 1.54f Western Blot: ImageJ 1.54f ELISA: Microsoft Excel Office 2019 & GraphPad Prism v8.0.2 Statistics: GraphPad Prism v8.0.2 Figure visualization: Adobe Illustrator 2024

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 main data supporting the results of this study are available within the paper and its supplementary information. Source data for the figures are provided with this paper. The raw data can be made available to interested researchers on reasonable request to the corresponding authors, and provided that a data-transfer agreement is approved by the legal department of the institution of the requesting researcher and by all legal departments of the institutions that provided data for the study.

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	Sex or gender were not considered for the human research samples used in this study (buffy coats).
Reporting on race, ethnicity, or other socially relevant groupings	Researchers were blinded for race, ethnicity or other socially relevant groupings for the human research samples used in this study.
Population characteristics	Healthy volunteers, 18–65 years old, in the area of Sanquin Nijmegen.
Recruitment	Healthy volunteers provided blood at Sanquin Nijmegen and gave informed consent to participate in the study before taking part.
Ethics oversight	CMO Arnhem Nijmegen approved the use of human samples in this study.

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 calculations were performed using Piface (Lenth, R. V. (2006). Java Applets for Power and Sample Size [Computer software]). Sigma and effect size were determined by pilot experiments and/or based on the literature. The minimal power was set at 0.80.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were reproduced in technical and/or biological replicates, as indicated in the figure captions.
Randomization	The animals were divided randomly in cages upon arrival and tattooed for identification. Subsequently, the animals were randomly divided in different groups using random.org.
Blinding	Animal caretakers were blinded during the experiment to avoid bias in animal handling. During analysis, the researchers were not blinded, as all analyses were performed in bulk, applying the exact same conditions to all samples and thereby avoiding bias.

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

mouse CD8α (1:100 dil., PerCP, clone 53-6.7, Biolegend, cat#100732)
mouse CD8α (1:100 dil., FITC, clone 53-6.7, Biolegend, cat#100706)
mouse CD25 (1:100 dil., FITC, clone PC61, Biolegend, cat#102006)
mouse CD25 (1:100 dil., PerCP-Cy5.5, clone PC61, Biolegend, cat#102030)
mouse CD44 (1:50 dil., PE/Cy7, clone IM7, Biolegend, cat#103030)
mouse 4-1BB (1:100 dil., APC, clone 17B5, ThermoFisher, cat#17-1371-82)
mouse DEC205 (1:1000 dil., PE, clone NLDC-145, Biolegend, cat#138214)
human CD8 (1:20 dil., APC, clone RPA-T8, BD Biosciences, cat#555369)
human CD25 (1:50 dil., PE/Cy7, clone BC96, BioLegend, cat#302612)
human CD69 (1:20 dil., PerCP, clone L78, BD Biosciences, cat#340548)
human 4-1BB (1:20 dil., PE, clone 4B4-1, BD Biosciences, cat#555956)

Validation

All antibodies used are commercially available and were validated for use in flow cytometry. Data are available on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The following hybridoma cell lines were modified for the stable expression of ubi-tagged antibodies or antibody fragments: KT3 (kindly provided by Ramon Arens (LUMC, The Netherlands)), NLDC-145 (ATCC HB-290), and TA99 (ATCC HB-8704). Other cell lines used in this study were: EL4 (kindly provided by dr. Jacques Neefjes (LUMC, The Netherlands)), OP9-DL1 cells (kindly provided by dr. M. Dalod, Centre National de la Recherche Scientifique (CNRS), France), Jurkat T cells (kindly provided by Gosse Adema (Radboud UMC, The Netherlands)), KPC3 and KPC3-TRP1 (obtained in house as described in DOI: 10.1158/1535-7163.MCT-18-0679)

Authentication

The cell lines were authenticated by morphology analysis.

Mycoplasma contamination

All cell lines were routinely tested for mycoplasma contamination using a MycoAlert® Mycoplasma Detection Assay.

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

All mice were purchased from Charles River Laboratories, France. Female C57BL/6 WT and OT-I (Tg(TcraTcrb)1100Mjb/Crl) 8–12 weeks of age and 18–25 g in body weight were used for the in vitro and in vivo experiments. Mice were sacrificed by cervical dislocation.

Wild animals

The study did not involve wild animals.

Reporting on sex

All studies were performed on female mice.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

All animal studies were approved by the local authority for the Ethical Evaluation of Animal Experiments and Animal Welfare (Instantie voor Dierenwelzijn Radboudumc). All mice were kept in accordance with federal and state policies on animal research and with Annex III of the EU Directive (Directive 2010-63-EU).

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	For FACS analysis, cells were washed with PBS, followed by life/death staining (20 min., rt) in 50 µL eBioscience™ Fixable Viability Dye eFluor™ 780 (1:2000, ThermoFisher). Cells were washed once with PBA and antibody mixes were added (30 min., 4 °C). Cells were washed twice with PBA, taken up in 100 µL PBA and FACS analyses were performed on a FACSLyric™ (BD Biosciences) or a FACSVerse™ (BD Biosciences).
Instrument	FACSVerse™ (BD Biosciences) or FACSLyric™ (BD Biosciences)
Software	FlowJo vX.0.7 was used for FACS analysis.
Cell population abundance	Cells were not sorted for any of the experiments.
Gating strategy	All relevant gating strategies are depicted in supplementary Fig. 21.

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