

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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | 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

X-ray crystallography, softwares for data collection and processing were provided by Australian synchrotron:
- qeGUI: graphic user interface control for crystal mounting and diffracting.
- XDS: package for data reduction, indexing, integration, correction

Surface plasmon resonance, softwares provided by Cytiva: Biacore T200 Control

Flow cytometry: FlowJo v10

Data analysis

X-ray crystallography:
- Pointless (ccp4 package v4.9): Laue group and space group determination
- Aimless (ccp4 package v4.9): data scaling and validation
- Matthews_coef (ccp4 package v4.9): Matthew coefficient determination and molecular weight estimation
- Phaser-MR (Phenix suit 1.21.2-5419-000): molecular replacement for structure solution
- Coot v0.9.8.96 (ccp4 package v4.9): structure building and manual refinement
- phenix.refine (Phenix suit 1.21.2-5419-000): structure refinement
- eLBOW (Phenix suit 1.21.2-5419-000): ligand restraint generation
- ReadySet (Phenix suit 1.21.2-5419-000): ligand restraint incorporation
- Comprehensive validation (Phenix suit 1.21.2-5419-000): final structure validation

Surface plasmon resonance:

- Softwares provided by Cytiva: BIAevaluation
- Graphpad Prism, licensed via Monash University.

PyMol v2.6.2: structural visualization and figure rendering.

Flow cytometry:

- Graphpad Prism 10, licensed via Monash University.

Lipid elution analysis:

- MassHunter
- R package (ver3.4.2) XCMS
- In house designed software method described by Layre et al, Chemistry & Biology 2011

Statistical analysis:

- Graphpad Prism 10, licensed via Monash University.

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 crystallographic datasets were deposited to the Protein Data Bank (PDB) under following codes: 9OHX (CD1c presenting endogenous lipids), 9OHY (CD1c presenting phosphomycoketide in its open conformation), 9OHV (CD1c presenting dual lipids MPM and GD3 ganglioside), 9OHW (CD1c presenting GM1 ganglioside), 9OHU (CD1c presenting GM3 ganglioside), 9OHT (CD1c presenting GD3 ganglioside), 9OHZ (Crystal structure of CD1a presenting ganglioside GD3), and 9OI0 (Crystal structure of CD1b presenting ganglioside GD3).

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

Healthy PBMC donors (n=20): 11 male, 8 female, and 1 unknown.

Tonsil samples: All three tonsil donors are female. Sex and gender based analyses were not considered in the study design.

Reporting on race, ethnicity, or other socially relevant groupings

The ethnicity is not captured in both PBMC and tonsil samples.. Race and ethnicity are not relevant in this study.

Population characteristics

Healthy PBMC donors (n = 20): Age available for 19 donors: median 46 years (range 24–71; mean \pm SD 44.4 \pm 12.8). While demographic data beyond age and gender are not provided according to our agreement with the Australian Red Cross, they are notionally reflective of demographics of those donating blood to the Australian Red Cross in Melbourne, Australia

Tonsil samples:

Tonsil donor 1, 25 years old, with recurrent severe tonsillitis

Tonsil donor 2, 21 years old, with Grade 4 tonsils, recurrent tonsillitis

Tonsil donor 3, 28 years old, with recurrent tonsillitis

Recruitment

Peripheral blood samples of healthy donors were obtained from healthy human donors from the Australian Red Cross Blood Service.

Tonsils were collected from recurrent tonsillitis patients who consented to the use of their tissue for research at the John Radcliffe Hospital, Oxford, United Kingdom

Ethics oversight

Use of PBMC samples for analysis was approved by the University of Melbourne Human Ethics Committee (1035100). This study was reviewed and approved by the Oxford Radcliffe Biobank (ORB) Tissue Access Committee to obtain pseudonymised tissue samples and associated clinical data from patients recruited under ORB

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

Field-specific reporting

Life sciences study design

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

Sample size	No sample size calculation was performed for both PBMC and tonsillar MNCs. For PBMC, the sample size of 20 was chosen to maximise the chance of isolating CD1c reactive TCR toward gangliosides. For tonsillar MNCs, the sample size is limited due to the available tonsil samples. Sample sizes for activation assay and tetramer staining experiments are chosen to ensure reproducibility.
Data exclusions	In Figure 5a, Right: Data from 20 donors are presented for each CD1c tetramer, except for the CD1c-endo tetramer, which includes data from 19 donors. Due to an unknown computer issue during acquisition, one data point could not be retrieved; however, this missing data did not affect the overall findings.
Replication	<ul style="list-style-type: none">- TCR trap and mass spectrometry: size-exclusion chromatography experiment to separate CD1c-lipid-TCR complex and identify the lipid content was done twice with two different batches of protein production.- Surface plasmon resonance: binding analyses was replicated with different batches of protein production and batches of lipid loading onto CD1c.- Flow cytometry and activation assay: replications were done with different batches of cells and batches of lipid loading onto CD1c tetramer.
Randomization	All the experiments in this study did not require randomization.
Blinding	All the experiments in this study did not require blinding.

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	Methods
<div><div>n/a</div><div>Involvement in the study</div><div><div><input type="checkbox"/></div><div><input checked="" type="checkbox"/></div>Antibodies</div><div><div><input type="checkbox"/></div><div><input checked="" type="checkbox"/></div>Eukaryotic cell lines</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>Palaeontology and archaeology</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>Animals and other organisms</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>Clinical data</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>Dual use research of concern</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>Plants</div></div>	<div><div>n/a</div><div>Involvement in the study</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>ChIP-seq</div><div><div><input type="checkbox"/></div><div><input checked="" type="checkbox"/></div>Flow cytometry</div><div><div><input checked="" type="checkbox"/></div><div><input type="checkbox"/></div>MRI-based neuroimaging</div></div>

Antibodies

Antibodies used	APC-Cy7 mouse anti-human CD14 (clone MφP9, cat # 557831, BD Biosciences); APC-Cy7 mouse anti-human CD19 (clone SJ25C1, cat # 561743, BD Biosciences); Alexa Fluor 700 mouse anti-human CD3 (clone UCHT1, cat # 557943, BD Biosciences); BV421 mouse anti-human CD4 (clone SK3, cat # 566907, BD Biosciences); BV650 mouse anti-human CD8 (clone RPA-T8, cat # 563822, BD Biosciences); FITC mouse anti-human γδTCR (clone 11F2, cat # 347903, BD Biosciences); Mouse anti-human CD36 (clone 5-271, cat # 336202, BioLegend); BV421 mouse anti-human CD19 (clone SJ25C1, cat # 363018, BioLegend); BV786 mouse anti-human CD3 (clone UCHT1, cat # 300472, BioLegend); BUV805 mouse anti-human CD4 (clone RPA-T4, cat # 569196, BD Biosciences); BUV395 mouse anti-human CD8 (clone RPA-T8, cat # 563795, BD Biosciences); PE-Cy7 mouse anti-human CD69 (clone FN50, cat # 561928, BD Biosciences); APC-Cy7 mouse anti-human CD69 (clone FN50, cat # 310914, BD BioLegend); Mouse anti-human CD1c (clone L161, cat # 331502, BioLegend); LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (cat # L10119, Invitrogen); Zombie Green Fixable Viability Kit (cat # 423112, BD BioLegend).
Validation	<ul style="list-style-type: none">•APC-Cy7 mouse anti-human CD14 (clone MφP9, cat # 557831, BD Biosciences): PMID: 34852226; Zebley C et al. CD19-CAR T cells undergo exhaustion DNA methylation programming in patients with acute lymphoblastic leukemia. Cell Rep 2021 Nov 30;37(9):110079.•APC-Cy7 mouse anti-human CD19 (clone SJ25C1, cat # 561743, BD Biosciences): PMID: 34242577; Li D et al. In vitro and in vivo functions of SARS-CoV-2 infection-enhancing and neutralizing antibodies. Cell. 2021 Aug 5;184(16):4203-4219.e32.•Alexa Fluor 700 mouse anti-human CD3 (clone UCHT1, cat # 557943, BD Biosciences): PMID: 36130603; Chappert P et al. Human anti-smallpox long-lived memory B cells are defined by dynamic interactions in the splenic niche and long-lasting germinal center

imprinting. Immunity. 2022 Oct 11;55(10):1872-1890.e9.

•BV421 mouse anti-human CD4 (clone SK3, cat # 566907, BD Biosciences):

PMID: 38195752; Pant S et al. Lymph-node-targeted, mKRAS-specific amphiphile vaccine in pancreatic and colorectal cancer: the phase 1 AMPLIFY-201 trial. Nat Med. 2024 Feb;30(2):531-542.

•BV650 mouse anti-human CD8 (clone RPA-T8, cat # 563822, BD Biosciences):

PMID: 33020644; Deng Q et al. Characteristics of anti-CD19 CAR T cell infusion products associated with efficacy and toxicity in patients with large B cell lymphomas. Nat Med. 2020 Dec;26(12):1878-1887.

•FITC mouse anti-human $\gamma\delta$ TCR (clone 11F2, cat # 347903, BD Biosciences):

PMID: 37938576; Lee D et al. Unlocking the potential of allogeneic V δ 2 T cells for ovarian cancer therapy through CD16 biomarker selection and CAR/IL-15 engineering. Nat Commun. 2023 Nov 8;14(1):6942.

•Mouse anti-human CD36 (clone 5-271, cat # 336202, BioLegend):

PMID: 33521697; Chevrier S et al. A distinct innate immune signature marks progression from mild to severe COVID-19. Cell Rep Med. 2020 Dec 26;2(1):100166.

•BV421 mouse anti-human CD19 (clone SJ25C1, cat # 363018, BioLegend):

PMID: 33945505; Hohmann MS; Antibody-mediated depletion of CCR10+EphA3+ cells ameliorates fibrosis in IPF. JCI Insight. 2021 Jun 8;6(11):e141061.

•BV786 mouse anti-human CD3 (clone UCHT1, cat # 300472, BioLegend):

PMID: 18490743; Thakral D et al. Differential expression of the human CD8beta splice variants and regulation of the M-2 isoform by ubiquitination. J Immunol. 2008 Jun 1;180(11):7431-42.

•BUV805 mouse anti-human CD4 (clone RPA-T4, cat # 569196, BD Biosciences):

PMID: 35094878; Ho TH et al. Identification of a CD4+ T cell line with Treg-like activity. Hum Immunol. 2022 Apr;83(4):281-294.

•BUV395 mouse anti-human CD8 (clone RPA-T8, cat # 563795, BD Biosciences):

PMID: 37919903; Lee IK et al. A genetically encoded protein tag for control and quantitative imaging of CAR T cell therapy. Mol Ther. 2023 Dec 6;31(12):3564-3578.

•PE-Cy7 mouse anti-human CD69 (clone FN50, cat # 561928, BD Biosciences):

PMID: 35982798; Marzan-Rivera et al. Infection order outweighs the role of CD4+ T cells in tertiary flavivirus exposure. iScience. 2022 Jul 16;25(8):104764.

•APC-Cy7 mouse anti-human CD69 (clone FN50, cat # 310914, BD BioLegend):

PMID: 36715448; Lin C et al. TSC2 regulates tumor susceptibility to TRAIL-mediated T-cell killing by orchestrating mTOR signaling. EMBO J. 2023 Mar 1;42(5):e111614.

•Mouse anti-human CD1c (clone L161, cat # 331502, BioLegend):

PMID: 33376221; Radtke AJ et al. IBEX: A versatile multiplex optical imaging approach for deep phenotyping and spatial analysis of cells in complex tissues. Proc Natl Acad Sci U S A. 2020 Dec 29;117(52):33455-33465.

•LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (cat # L10119, Invitrogen):

PMID: 23650439; Gasteiger G et al. IL-2-dependent adaptive control of NK cell homeostasis. J Exp Med. 2013 Jun 3;210(6):1179-87.

•Zombie Green Fixable Viability Kit (cat # 423112, BD BioLegend):

PMID: 33650969; Tereshko L et al. Ciliary neurotrophic signaling dynamically regulates excitatory synapses in postnatal neocortical pyramidal neurons. Elife. 2021 Mar 2;10:e65427.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Expi293F GnTI- cells were sourced from ThermoFisher. SKW-3 cell line (ACC 53) was sourced from German Collection of Microorganisms and Cell Cultures (DSMZ). HEK293T.SCARB1-/- and DN6.SKW-3 cell lines were provided by Dr. Nicholas Gherardin (Peter Doherty Institute for Infection and Immunity, University of Melbourne, Australia)
Authentication	No new cell lines are generated in this study. Cells obtained from collaborators were validated by flow cytometry for expression of genes of interest, without further authentication.
Mycoplasma contamination	Absence of mycoplasma contamination in cell lines was confirmed via PCR.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Plants

Seed stocks	<i>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.</i>
Novel plant genotypes	<i>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.</i>
Authentication	<i>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, mosaicism, off-target gene editing) were examined.</i>

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

CD1c-ganglioside reactive T cells sorted from PBMCs: 50 million frozen PBMCs were thawed and treated with 50 nM dasatinib for 30 minutes at 37 °C, followed by incubation with 50 µg/mL anti-CD36 antibody for 15 minutes at 4 °C. Cells were then stained with PE-labelled CD1c tetramers (1 µg/mL) at room temperature for 30 minutes, washed, and subsequently labelled with an antibody cocktail to identify CD1c-ganglioside reactive T cells (Fixable Near-IR Dead Cell Stain, CD14, CD19, CD3, CD4, CD8, and γδTCR).

CD1c-ganglioside reactive T cells sorted from tonsillar MNCs: 10 frozen tonsillar MNCs were thawed and incubated with 50 µg/mL anti-CD36 antibody for 15 minutes at 4 °C, followed by sequential staining with PE-labelled CD1c tetramers (2–5 µg/mL) at room temperature for 30 minutes. Cells were then washed and stained with an antibody cocktail to identify CD1c-ganglioside-reactive T cells (Zombie Green Fixable Viability Stain, CD19, CD3, CD4, and CD8).

For activation assays and transient TCR expression experiments: cells were harvested by centrifugation and washed with FACS buffer (PBS supplemented with 2% FBS). Cells were then stained with antibodies and/or CD1c tetramers in FACS buffer.

Instrument

BD LSR Fortessa
BD FACSymphon A5
BD FACS Aria III

Software

FlowJo v10

Cell population abundance

For single-cell sorting samples, a 'post-sort' flow cytometry analysis was not possible.
For the tonsillar F10 clone in Figure 5e, post-expansion purity was 94–97% tetramer+ among live, determined by CD1c–GD3 or CD1c–GM3 PE-tetramers.

Gating strategy

TCR transient expression experiments using HEK293.SCARB1-/- were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live cells (Fixable Near-IR Dead Cell Stain, R780 low), tetramers (PE) versus human CD3 high (CD3-AF700).

Tetramer staining experiments using SKW-3 lines were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live cells (Fixable Near-IR Dead Cell Stain, R780 low), tetramers (PE) versus human CD3 high (CD3-AF700).

Tetramer staining experiments using in vitro expanded T cells from PBMCs were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live, CD14-, CD19- cells (Fixable Near-IR Dead Cell Stain, CD14-APC-Cy7, CD19-APC-Cy7, R780 low), CD3+ cells (CD3-AF700), CD1c tetramers (PE).

Plate-bound activation experiments were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); single cells (FSC-A/FSC-H); live cells (Fixable Near-IR Dead Cell Stain, R780 low), GFP high (BV530) versus human CD3 (CD3-AF700), CD69 (CD69-PE-Cy7)

CD1c restricted T cells from PBMCs were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live, CD14-, CD19- cells (Fixable Near-IR Dead Cell Stain, CD14-APC-Cy7, CD19-APC-Cy7, R780 low), CD3+ cells (CD3-AF700), CD1c tetramers (PE).

CD1c restricted T cells from tonsillar MNCs were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live cells (Zombie Green Fixable Viability stain, B530 low), CD19- cells (CD19-BV421), CD3+ cells (CD3-BV786) versus CD1c tetramers (PE).

Tetramer staining experiments using the isolated tonsillar T cell clone: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live cells (Zombie Green Fixable Viability stain, B530 low), CD3+ cells (CD3-BV786) versus CD1c tetramers (PE).

Beads-based activation experiments were gated as follows: cells (FSC-A/SSC-H); single cells (FSC-A/FSC-H); live cells (Zombie Green Fixable Viability stain, B530 low), CD3+ cells (CD3-BV786), CD69+ cells (CD69-APC-Cy7)

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