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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<br>*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<br>*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 | As described in the methods: CryoEM data collected using EPU 2.12. No custom code used. |
| Data analysis | As described in the methods: CryoEM data analyzed using cryoSPARC v4.3, TOPAZ v0.2.5, Coot 0.9.8.94 EL, Phenix 1.21.1 (including MolProbity), Chimera 1.16, ChimeraX1.6.1, and PyMOL 2.5.4. Flow cytometry data were analyzed with FlowJo V10 and GraphPad Prism (v9.5.1). No custom code used. |

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

Regeneron materials described here may be made available to qualified, academic, noncommercial researchers through a material transfer agreement upon request at https://regeneron.envisionpharma.com/vt_regeneron/. For questions about how Regeneron shares materials, use the email address

preclinical.collaborations@regeneron.com. The cryoEM maps and coordinates of the G115-OKT3, G115-Fab1, 9C2-Fab2, and 9C2-Fab3 are deposited and available at PDB and EMDB with access codes 9CQ4, EMD-45808; 9CQ7, EMD-45810; 9CQ8, EMD-45811; 9CQL and EMD-45814, respectively.

## 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 | Human PBMCs were obtained from consenting volunteers via a commercial vendor, AllCells. More detailed information on AllCells' donor procurement process and range of donor characteristics/criteria can be found on AllCells website: https://allcells.com/. Sex or gender analysis was not carried out since the PBMCS were used in in vitro studies and are isolated from their original biological context. Thus, sex and/or gender does not apply to the fundamental biological process studied in this case. |
| Reporting on race, ethnicity, or other socially relevant groupings | Human PBMCs were purchased from a commercial vendor, thus we do not report on race, ethnicity, or other socially relevant groupings. |
| Population characteristics | N/A |
| Recruitment | Human PBMCs were obtained from consenting volunteers via a commercial vendor, AllCells. |
| Ethics oversight | N/A |

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 | Numbers of particles in the cryoEM samples are described in the methods. For flow cytometry experiments a sample size of 1 was used, as Antibody 3 has been characterized extensively in the original patent application (see ref. 34) and our results are in line with those reported in the patent. |
| Data exclusions | CryoEM data processing, including which particles are included or excluded from the final map, is described in the methods. |
| Replication | CryoEM structure determinations were not repeated, as is standard in the field due to the large amount of time and effort involved in each structure determination. |
| Randomization | CryoEM particles were randomly assigned to two half-sets, following the "gold standard" FSC protocol. |
| Blinding | Blinding is not relevant to the cryoEM. Investigators were not blinded to group allocation during flow cytometry data collection as the chances of subject bias is not as critical in non-human/animal studies. |

# 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

CryoEM experiments used antibodies which were generated from publicly available sequences (three TCR delta chain binders, Fabs 1-3; and the OKT3 antibody) and a CD3e/d binding antibody that was developed and generated internally at Regeneron.

Specific antibody clone/conjugates used for flow cytometry experiments are described below. Fluorophore conjugates are also indicated on figures.

Notation: Target [clone] (dilution): (Conjugate, Company, Catalog Number, URL).

Human CD45 [HI30] (1:400): (Brilliant Violet 711™, Biolegend, 304050, https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-human-cd45-antibody-9736?GroupID=BLG5926)

Human CD3e [UCHT1] (1:200): (BUV395, BD Biosciences, 563546, https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/buv395-mouse-anti-human-cd3.563546)

Human Vδ1 [REA173] (1:200): (VioBlue, Miltenyi Biotec, 130-120-443, https://www.miltenyibiotec.com/US-en/products/tcr-vd1-antibody-anti-human-reafinity-rea173.html#conjugate=vioblue:size=100-tests-in-200-ul)

Validation

Regeneron antibodies are validated by DNA sequencing of the expression plasmids, and intact mass spectrometry of the purified proteins. No additional validation was done by the authors of this manuscript. Antibodies used in flow cytometry experiments are commercially available and are validated by the manufacturer (which can found via specific URLs provided above).

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293F (ThermoFisher catalog number R79007) and ExpiSf9 (ThermoFisher catalog number A35243) cells for cryoEM protein production came from Thermo Fisher. Human PBMCs were purchased from AllCells (allcells.com).

Authentication

Human PBMCs, HEK293, and ExpiSf9 cells were not authenticated by the authors of this manuscript.

Mycoplasma contamination

Human PBMCs, HEK293, and ExpiSf9 cells were not tested for mycoplasma.

Commonly misidentified lines (See ICLAC register)

No commonly misidentified cell lines were used in this study.

# 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 | Sample preparation of human Vδ1+ γδ T cells is described in methods in the "Expansion of Vδ1 expressing γδ T-cells" section. Single-cell suspensions of T cells were incubated in PBS containing 2% FBS and 1 mM EDTA with Human TruStain FcX (Fc Receptor Blocking Solution, Biolegend Cat. # 422302), Near-IR Viability Dye (Thermo Fisher Cat. # L34975 used at 1:1000 dilution) and indicated detection antibodies (listed below). Fc-Block was added 10 minutes prior to staining with detection antibodies according to the manufacturer's protocol. Cells were stained with detection antibodies for 30 minutes, followed by fixation (eBioscience™ Fixation/Permeabilization Cat. # 00-5521-00) according to the manufacturer's protocol. |
| Instrument | All flow cytometry experiments were performed using a LSR Fortessa flow cytometer. |
| Software | Flow cytometry data were collected via Diva (BD Biosciences), analyzed by FlowJo V10 (TreeStar), and plotted using GraphPad Prism (v 9.5.1). |
| Cell population abundance | The purities and abundance of cells were determined by flow cytometry and are reported in the figures as % of Live, CD45+ cells. |
| Gating strategy | Dead cells were first excluded by Near-IR staining. Subsequently, singlets were gated according to the pattern of FSC-H vs. FSC-A, followed by FSC-H vs. FSC-W. Live immune cells were gated on the expression of CD45. Positive populations were determined by the specific antibodies, which were distinct from negative populations. |

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