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# Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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 | FlowJo (10.5.3), MiSeq 3.1, BaseSpace |
| Data analysis | usearch (v11), ublast (v11), R (3.4.2), UpSetR (1.3.3), ggplot2 (3.1.0), igraph (1.2.4), GraphPad Prism (v8) |

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Paired TCRab repertoire data (Figure 2) has been deposited in the NCBI short read archive under BioProject ID PRJNA541985. Complete TCRab sequences for functionally characterized TCRab clones (Table 2) will be deposited in Genbank prior to publication.

# 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

# 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 |
| Data exclusions | No data were excluded |
| Replication | All attempts at replication were successful |
| Randomization | This was not relevant to this study |
| Blinding | This was not relevant to this study |

# 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 |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☐ | ☒ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

## Antibodies

| | |
|---|---|
| Antibodies used | All antibodies were obtained from BioLegend unless indicated: anti-human CD3 [clone: UCHT1, cat#s 300439 & 300440], anti-human TCRalpha/beta [clone: IP26, cat# 306706], anti-human CD69 [clone: FN50, cat# 310906], anti-human CD62L [clone: DREG-56, cat# 304810], anti-human HLA-A2 [clone: BB7.2, cat# 343304], anti-human CD8B [clone: REA715, Miltenyi Biotec cat# 130-110-509], anti-human CD8A [clone: SK1, BD Pharmingen cat# 560179], anti-human CD4 [clone: SK3, BD Biosciences cat# 566320], anti-human CD107a [clone: REA792, Miltenyi Biotec cat# 130-111-621], and anti-mouse TCRb [clone: H57-597, cat# 109212]<br><br>Dextramers were obtained from Immudex and are listed in Supplementary Table 1. |
| Validation | Antibodies were validated by commercial vendors and this information is available on their websites. |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | Wild type Jurkat cells [clone: E6-1, from ATCC TIB-152]<br>TCRbeta deficient Jurkat cells [clone: J.RT3-T3.5, from ATCC TIB-153]<br>T2 cells [174 x CEM.T2, from ATCC CRL-1992]<br>Lenti-Pac 293Ta (HEK-293) cells (from GeneCopoeia, cat# LT008) |
| Authentication | Product data sheets and certificates of analysis were provided by vendors.<br>We validated the TCRbeta deficient Jurkat cell line by staining for surface CD3 and TCRalpha/beta expression and confirmed loss of expression by flow cytometry. Cell lines have also been authenticated by morphology checking and through use in other experiments. |
| Mycoplasma contamination | Tested negative for mycoplasma contamination. |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |

# 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 | Tissue culture samples were resuspended to single cells for staining. PBMCs were obtained from Leuko Paks collected by AllCells, cryopreserved, and thawed fresh for use. |
| Instrument | Data were acquired using a BD FACSMelody (analysis and sorting) and Beckman Coulter CytoFLEX LX (analysis). |
| Software | Manual gating was used in FlowJo (10.5.3) for analysis. |
| Cell population abundance | The sorted cell populations were limiting and prevented us from re-analyzing their sort purity. However, we routinely measured sorting purity using control samples and obtained >95% purity upon re-running the sorted samples when using the Purity sort precision mode on the FACSMelody. |
| Gating strategy | The gating strategies used are provided in Supplementary Fig 41 and all applicable figures. In brief, our gating strategy was: FSC-A x SSC-A to capture the cell population of interest -> FSC-A x FSC-H to identify single cells -> DAPI x SSC-A to gate live cells. |

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