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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data.
Data analysis	The following software/code was used to analyze data: FlowJo v10 for analyzing flow cytometry data. GraphPad Prism v6 for statistical analyses. Custom code for analyzing sequencing data was used. The code is deposited in GitHub (https://github.com/Baltimore-Lab/nat-methods-SABR-trogo). Sequence Alignment was done using Burrows-Wheeler Alignment: Li, H. & Durbin, R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25, 1754-1760, doi:10.1093/bioinformatics/btp324 (2009).

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 upon request. 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

The data that support the findings of this study are available from the corresponding author upon request.The list of epitopes in the antigen libraries can be found in supplementary tables 1 and 2.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	We used minimal sample size for statistical comparisons.
Data exclusions	No data exclusions
Replication	All attempt at replication were successful.
Randomization	Randomization is not relevant to this study because no comparisons between experimental groups were made.
Blinding	Blinding was not possible because the target epitopes for each TCR tested are known.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	The antigen libraries are available from the corresponding author upon request.

Antibodies

Antibodies used	PE anti-human CD3 Antibody (BioLegend, 317308, Clone OKT3, lot .B225591). Used at 1:200. Brilliant Violet 510™ anti-human CD8 Antibody (BioLegend, 344731, Clone SK1, lot. B236260). Used at 1:200. Pacific Blue™ anti-human TCR α/β Antibody (BioLegend, 306715, Clone IP26, lot. B210800). Used at 1:100. PE/Cy7 anti-human TCR α/β Antibody (BioLegend, 306720, Clone IP26, lot. B247926). Used at 1:100. PE/Cy7 anti-mouse TCR β chain Antibody (BioLegend, 109222, Clone H57-597, lot. B241527). Used at 1:200. PE anti-human CD271 (NGFR) Antibody (BioLegend, 345106, Clone ME20.4, lot. B215084). Used at 1:1000.

APC anti-human CD271 (NGFR) Antibody (BioLegend, 345108, Clone ME20.4, lot. B236644). Used at 1:1000.
Brilliant Violet 510™ anti-human HLA-A2 Antibody (BioLegend, 343319, Clone BB7.2, lot. B212450). Used at 1:200.
Pacific Blue™ anti-human HLA-A2 Antibody (BioLegend, 343312, Clone BB7.2, lot. B231554). Used at 1:200.

Validation

Antibodies were validated by the company; refer to the company website for detailed validation analysis.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Jurkat Cells, Clone E6-1 purchased from ATCC, Manassas, VA; Cat # TIB¬-152
K562 Cells purchased from ATCC, Manassas, VA; Cat # CCL-243
Primary T Cells purchased from UCLA, CFAR Virology Core
HEK-293T Cells purchased from ATCC, Manassas, VA Cat # CRL-3216

Authentication

None of the cell lines were authenticated

Mycoplasma contamination

Cell lines were not tested for mycoplasma

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines 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

Jurkat and K562 cells

Instrument

MACSQuant® Analyzer 10

Software

TreeStar FlowJo® Flow Cytometric Data Analysis Software v10.1

Cell population abundance

Jurkat and K562 cells in a 5:1 or 2:1 ratio

Gating strategy

detailed in the supplementary information

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