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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 v7 and Microsoft Excel 2016 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 raw data can be found in source data file. The list of epitopes in the SABR libraries can be found in supplementary tables 3 and 4. The plasmids for HLA-A*0201-SABR backbone (pCCLc-MND-A0201-SABR-Backbone, ID 119050), HLA-B*2705-SABR backbone (pCCLc-MND-B2705-SABR-Backbone, ID 119051), A2-Mart1-SABR (pCCLc-MND-A0201-Mart1-SABR, ID 119052), and B27-KK10-SABR (pCCLc-MND-B2705-KK10-SABR, ID 119053) are available through Addgene Inc. The sequencing data are deposited in Short Read Archive (SRR8207921: amplicon sequencing of A2-SABR-library co-incubated with F5 TCR, SRR8207922: amplicon sequencing of A2-SABR-library co-incubated with SL9 TCR, SRR8207923: amplicon sequencing of A2-SABR-library co-incubated with No TCR, SRR8207924: amplicon sequencing of A2-NeoAg-library co-incubated with Neo TCR, SRR8207925: amplicon sequencing of A2-NeoAg-library co-incubated with No TCR). The code used to analyze sequences is deposited at GitHub (https://github.com/Baltimore-Lab/nat-methods-SABR-trogo).

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	No particular methods were used to determine sample size. Most experiments rely on measuring the presence of signal above background. The background noise in our assays is minimal. Therefore we reasoned that having n=3 replications will be sufficient to distinguish signal from background noise.
Data exclusions	No data exclusions
Replication	All attempts at replication were successful
Randomization	Randomization is not relevant to our study because we have experimental arms that were constructed independently and not allocated from one pool of samples.
Blinding	Blinding was not possible/relevant. All the experimental arms were based on independently constructed cell lines/vectors. While making these cell lines/vectors, we had to know which arms they belonged to so that we could verify. E.g. while comparing F5 vs Mock TCR, we would verify that the F5 arm actually showed TCR expression before performing the experiment.

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	TThe plasmids for HLA-A*0201-SABR backbone (pCCLc-MND-A0201-SABR-Backbone, ID 119050), HLA-B*2705-SABR backbone (pCCLc-MND-B2705-SABR-Backbone, ID 119051), A2-Mart1-SABR (pCCLc-MND-A0201-Mart1-SABR, ID 119052), and B27-KK10-SABR (pCCLc-MND-B2705-KK10-SABR, ID 119053) are available through Addgene Inc. The epitope libraries are available upon request.

Antibodies

Antibodies used	APC/Cy7 anti-human CD69 Clone: FN50, Biolegend Inc, Cat # 310913, Lot #B261588; PE anti-human CD271 (NGFR) Clone: ME20.4, Biolegend Inc, Cat # 345106, Lot #B227240. Dilution of each antibody is 1:100.
Validation	APC/Cy7 anti-human CD69: Validation profile is on the manufacturer's website (https://www.biolegend.com/nl-nl/products/apc-cy7-anti-human-cd69-antibody-1917#productDocumentation) PE anti-human CD271 (NGFR): Validation done in a previous study (https://www.ncbi.nlm.nih.gov/pubmed/29437954)

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 NFAT-GFP-Jurkat Cells Provided by Arthur Weiss and Yvonne Chen Primary T Cells purchased from UCLA, CFAR Virology Core HEK-293T Cells purchased from ATCC, Manassas, VA Cat # CRL-3216 GXR-B27 cells Provided by Bruce D. Walker HLA-A2.1+ K562 Cells generated in-house by modifying K562 cells purchased from ATCC, Manassas, VA Cat # CCL-243
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	For SABR co-culture assays, cells were harvested, resuspended in MACS buffer (DPBS+2% FBS) and acquired on flow cytometer. Where indicated, cells were harvested, stained with 1:100 dilutions of the appropriate antigbodies in MACS buffer at 4 degC for 20 minutes, washed 2x and acquired on flow cytometer. For cytotoxicity assays, cells were harvested, mixed with half volume of MACS buffer with propidium iodide, and acquired on flow cytometer.
Instrument	Flow cytometry: MACSQuant 10, Miltenyi biotec. FACS: FACS Special Order Research Product, Becton-Dickinson
Software	Flow cytometry plots were analyzed on FlowJo (Treestar)
Cell population abundance	The purity of sorted sample was not determined post-sort.

Gating strategy	For SABR assays: The cells were first gated on FSC/SSC, and subsequently gates as shown in the figures. For cytotoxicity assays: The cells were first gated on FSC/SSC; then gated on PI (negative gate) and then gated on CFSE (positive gate) The gating strategy is shown in Supplementary Fig 9.

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