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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 *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 *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	Accuri (BD Biosciences); Cytoflex (Beckman Coulter); Biacore Control T100 (Cytiva); Miseq (illumina); SSRL Beamline BL12
Data analysis	Graphpad Prism 8.4 and 9.0; FlowJo 10; Biacore T100 Evaluation Software 2.0.4; CyteExpert 2.0.0.153; CCP4 package v7.0; PHASERv2.5.5; PHENIX v1.12; COOT v0.9.6; Custom code for deep-sequencing data processing is available: https://github.com/jlmendozabio/NGSpeptideprepandpred

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 protein sequences used for target prediction were obtained from Uniprot (http://www.uniprot.org/). The proteome ID is UP000005640. The processed deep-

sequencing data is deposited into GEO database under accession code GSE215018. The TCR-pMHC complexes structures were deposited in PDB with accession code: 7N2N, 7N2O, 7N2P, 7N2Q, 7N2R, 7N2S, and 8CX4. There is no restriction on data availability.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	For gender information on patients with Axial Spondyloarthritis/Ankylosing Spondylitis: AS1311 Male AS1541 Male AS1802 Female AS1455 Male AS1567 Male AS1661 Female AS1803 Male Patient S Male Patient K Male Patient P Male Patient N Male For gender information on patients with Acute Anterior Uveitis: UV019 Female UV027 Male UV122 Female UV180 Female
Population characteristics	Patients with Axial Spondyloarthritis/Ankylosing Spondylitis fulfilling ASAS/Modified New York criteria were recruited from outpatient clinics at the Nuffield Orthopaedic Centre and Pirogov Russian National Research Medical University. Patients with HLA-B*27-associated Acute Anterior Uveitis met the Standardization of Uveitis Nomenclature (SUN) Working Group classification criteria. As this is a tertiary medical center, it is possible (although unlikely) that it is not reflective of the general population.
Recruitment	Patients with Axial Spondyloarthritis/Ankylosing Spondylitis fulfilling ASAS/Modified New York criteria were recruited from secondary care specialized outpatient clinics at the Nuffield Orthopaedic Centre and Pirogov Russian National Research Medical University. All patients provided full informed consent. Patients were recruited from the Washington University Scchool of Medicine Ophthalmology practice if they had an active flare of HLA-B*27+ AAU.
Ethics oversight	For Patients attending the Nuffield Orthopaedic Centre ethical approval was obtained from the South Central COREC Oxford C committee (CORC 06/Q1606/139) IRAS 209484. For patients attending clinic of Faculty Therapy Department of Pirogov Russian National Research Medical University ethical approval was obtained from Local Ethical Committee of Pirogov Russian National Research Medical University (#170 18 Dec 2017). For patients in the Washington University School of Medicine clinics, ethical approval was obtained from Washington University School of Medicine Institutional Review Board (#201704141).

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	Sample sizes were chosen based on prior experience with similar experiments carried out in the lab e.g. Birnbaum et al. Cell 2014; Gee et al. Cell 2018. For in-vitro peptide dose response curve generation, 3 biological replicates were included. For the DSF measurement, technical triplicates were performed.
Data exclusions	No data was excluded
Replication	All peptides selected for dose response curve generation were replicated three independent biological replicates. All attempts at replication were successful. All reported data is summarizing or representative of all the replicates made. Individual repeats and sample sizes as well as significance levels are described in Figures or Figure legends.
Randomization	Randomization is needed for interventional trials. This study was an observational study without a therapeutic intervention and not an

Randomization	interventional study.
Blinding	Blinding is needed to control for a placebo effect in interventional studies or for interpretation bias for subjective outcomes. Neither is applicable 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 and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Dilutions Used:
FACS:
Antibodies for flow cytometry were used at a concentration of 1:100 unless recommended otherwise by the manufacturer.

Antibody List:
Flow Cytometry:
Alexa (R) 488 anti-c-Myc clone D84C12 Cell Signaling 2279S
Alexa (R) 488 anti-HA clone C29F4 Cell Signaling 2350S
Alexa Fluro 647 anti-αβ TCR clone IP26 BioLegend 306708
PE anti-CD69 clone FN50 BioLegend 310906
APC anti-CD69 clone FN50 BioLegend 310910
APC anti-human HLA-A, B, C clone W6/32 BioLegend 311409
APC anti-CD3 clone UCHT1 BioLegend 300439
APC anti-CD3 clone OKT3 BioLegend 317318
PE anti-CD3 clone OKT3 BioLegend 313708
FITC anti-CD3 clone HIT3a BD Pharmingen 555339
PE anti-CD8 clone HIT8a BD Pharmingen 555635
PerCP/Cy5.5 anti-human TCR 'Vbeta1' (IMGT: Vbeta9) clone BL37.2 BioLegend 360104
Anti-HLA-B27 clone ME1 (in house)

Validation

All primary antibodies used throughout this study were validated by the vendors and/or are from well-known and characterized clones. Further details in validation can be found in Biolegend Reproducibility and Validation webpage (https://www.biolegend.com/de-at/reproducibility/committed-to-functional-quality), Cell Signaling Technology (https://www.cellsignal.com/about-us/our-approach-process/antibody-validation-flow-cytometry), BD Pharmingen (https://www.bdbiosciences.com/en-us/search-results?searchKey=antibody%20validation). Anti-HLA-B27 clone ME1 is validated in Ellis et al. 1982

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Jurkat, 293T, K562 cells were purchased from ATCC. SKW-3 were purchased from DSMZ. LentiX cells were purchased from Clontech. Expi293 and GNTI- expi cells were purchased from Thermo Fisher Scientific.
Authentication	Cell lines were periodically authenticated (at least once per year) using short tandem repeat analysis.
Mycoplasma contamination	Cell lines were periodically tested for Mycoplasma contamination (at least once per year) using mycoplasma detection kit (Biotool).
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed in the ICLAC database

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	All yeast cell populations were washed with MACS buffer before staining and washed twice with MACS buffer prior for flow cytometry analysis. All cell lines were washed with MACS buffer before staining and washed twice with MACS buffer prior for flow cytometry analysis.
Instrument	CytoFLEX; Accuri C6
Software	FlowJo 10; BD Accuri C6 Software; CyteExpert 2.0.0.153
Cell population abundance	All cell populations were highly abundant. For yeast staining, at least 100000 cells are stained. For T cell activation assay, 10000 of SKW-3 T cells were incubated with 10000 of K562 cells per well.
Gating strategy	All samples were gated on SSC and FSC-H, followed by FSC-W and FSC-H, followed by gating on the Propidium Iodide negative population. From here, when working with primary cells these were further gated on CD3+CD4+ or CD3+CD8+ for further analysis. Fluorophores were chosen to minimize spectral overlap. Compensation beads labeled with appropriate antibodies were used prior to all data collection (UltraComp eBeads 01-2222-42 eBiosciences). The yeast was gated on Cmyc+ or HA+ through rounds of selections.

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