nature portfolio

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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	Commercially available software for data acquisition - Bruker Compass Hystar (Bruker Daltonics, Germany)
Data analysis	Peaks Online software was used for MS/MS data analysis (available commercially from Bioinformatics Solutions Inc, Canada) The agrep code is available at https://github.com/PurcellLab/agrep_for_crossreactivity, DOI 10.5281/zenodo.12792072 .

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

Source data are provided with this paper. The mass spectrometry data generated in this study has been deposited in the Massive repository (https://massive.ucsd.edu/) under data set identifier MSV000093193.
All source data for figures is available online

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	Relevant donor information is presented in Supp Data S5
Reporting on race, ethnicity, or other socially relevant groupings	n/a - HLA allotype only reported and their prevalence in the human population discussed
Population characteristics	n/a
Recruitment	As reported in methods section: All human experimental work was conducted according to the Declaration of Helsinki principles and the Australian NHMRC Code of Practice. All blood donors provided written informed consent.
Ethics oversight	Ethics approval was granted from the Human Research Ethics Committee of Melbourne Health (HREC/66341/MH-2020) and The University of Melbourne (13344 and 20782).

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	n/a
Data exclusions	n/a
Replication	n/a
Randomization	n/a
Blinding	n/a

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	W6/32 a pan HLA class I antibody (produced in house from a hybridoma)

Antibodies used

The anti-HLA-DQ SPV-L3 : anti-HLA-DP B7/21 : anti-HLA-DR LB3.1 antibodies were all produced in house from respective hybridomas
Anti-CD107a-AF488 antibody (purchased from Invitrogen, Cat#2423749, eBioH4A3, used at 1:200 dilution)
Anti-CD3-BV510 (purchased from Biolegend, Cat#317332, OKT3, used at 1:200 dilution)
Anti-CD4-BV650 (purchased from BD Biosciences, Cat#563875, SK3, used at 1:200 dilution)
Anti-CD8-PerCPCy5.5 (purchased from BD Biosciences, Cat#565310, SK1, used at 1:100 dilution)
Anti-TNF-AF700 (purchased from BD Biosciences, Cat#557996, MAb11, used at 1:50 dilution)
Anti-IFN-γ-V450 (purchased from BD Biosciences, Cat#560371, B27, used at 1:100 dilution)

Validation

W6/32 was validated by flow cytometry and affinity purification mass spectrometry
The anti-HLA-DQ SPV-L3 : anti-HLA-DP B7/21 : anti-HLA-DR LB3.1 antibodies were validated by flow cytometry and affinity purification mass spectrometry
Anti-CD107a-AF488 antibody (purchased from Invitrogen, Cat#2423749, eBioH4A3, used at 1:200 dilution) was validated by flow cytometry of control cell lines.
Anti-CD3-BV510 (purchased from Biolegend, Cat#317332, OKT3, used at 1:200 dilution) was validated by flow cytometry of control cell lines.
Anti-CD4-BV650 (purchased from BD Biosciences, Cat#563875, SK3, used at 1:200 dilution) was validated by flow cytometry of control cell lines.
Anti-CD8-PerCPCy5.5 (purchased from BD Biosciences, Cat#565310, SK1, used at 1:100 dilution was validated by flow cytometry of control cell lines.
Anti-TNF-AF700 (purchased from BD Biosciences, Cat#557996, MAb11, used at 1:50 dilution) and anti-IFN-γ-V450 (BD Biosciences, Cat#560371, B27, used at 1:100 dilution) were validated by flow cytometry of control cell lines following antigenic stimulation.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

The EBV transformed human B lymphoblastoid cell lines (BLCL) IHW09004 (A*02:01:01:01, B*27:05:02, C*01:02:01, DRA*01:01, DRB1*01:01:01, DRB6*01:01, DQA1*01:01:01, DQB1*05:01:01:03, DPA1*01:03:01:02, DPB1*04:01:01:01) and IHW09087 (A*01:01:01:01, B*08:01:01:01, C*07:01:01:01, DRA*01:02, DRB1*03:01:01, DRB3*01:01:02, DQA1*05:01:01:02, DQB1*02:01:01, DPA1*01:03:01, DPB1*03:01:01, DPB1*04:01:01) were obtained from the Victorian Transplantation and Immunogenetics Service.

Authentication

The cells HLA typing was confirmed by next generation genomic sequencing and the immunopeptidomics consistent wiht the expected HLA expression

Mycoplasma contamination

All cell lines and hybridomas were tested negative

Commonly misidentified lines
(See ICLAC register)

n/a

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.