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	Flow cytometry: FACSDiva 9.0 and MACSQuantify 2.13.
Data analysis	Flow Cytometry: FlowJo 10.8.1 and FlowLogic 8.7; Thermal Unfolding Analysis software: PR.Panta Analysis v1.2; HLA typing: Assign ATF 1.5.0.1158 and HLA Explore 2.0.0; DNA sequence analyses: SnapGene 5.3.3 and Sequencher 5.4.6; MD simulation analysis: Amber 22 package (implementing SHAKE and Berendsen algorithms), PyMOL 2.4.0, and Prism 9; Mass spectrometry: Skyline 22.2.0.351 and Proteome Discoverer 2.4; Statistical analysis: Prism 9.

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

Publicly available HLA genetic datasets used in this study include Allele Frequency Net Database (www.allelefrequencies.net), IPD-IMGT/HLA Database

(www.ebi.ac.uk/ipd/imgt/hla), NMDP Registry Haplotype Frequencies (http://frequency.nmdp.org), and the 2014 1000 Genomes HLA data (www.internationalgenome.org/category/hla). The structure of the human CD94/NKG2A complex with HLA-E (3CDG) was downloaded from the Protein Data Bank (www.RCSB.org). Source data are provided with this paper. All other data are available within the article and supplementary materials.

Human research participants

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

Reporting on sex and gender	No sex/gender specified analysis was performed.
Population characteristics	Blood samples were collected from healthy donors. Age and gender were not considered in this study. Donors were typed for HLA and screened for HCMV serostatus.
Recruitment	Healthy blood donors were randomly recruited from the NIH Blood Bank and University Medical Center, Hamburg-Eppendorf. There was no potential self-selection bias or other biases present in the donor sets used in this study.
Ethics oversight	Blood samples were collected at the NIH Blood Bank under an IRB-approved protocol (99-CC-0168) or as byproducts of allogeneic blood donation, and at the University Medical Center Hamburg-Eppendorf under a protocol approved by the ethical committee of the Landesärztekammer Hamburg (PV4780).

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	Blood donor sample size was restricted by the availability of human specimens and was sufficient to detect the effect of the SP variation on NK cell responses. The BLCL samples were selected based on HLA genotypes so that each comparison group contained at least 10 samples. To maximize the statistical power, we chose the maximum number of BLCLs that we were able to process in flow cytometry assay on one day.
Data exclusions	No data/sample was excluded.
Replication	Triplicate measurements were performed in the experiments using cell lines. Due to limited blood volume, a single measurement was performed for each donor. ELISA-based peptide binding and thermal stability assays were repeated six times. SPR measurements were performed in two independent experiments. Mass spectrometry data represent three technical replicates. Replication of experiments was successful and demonstrated consistent results.
Randomization	Blood donors were randomly recruited and were not allocated into groups. BLCLs were grouped based on their HLA genotypes.
Blinding	Blinding was not applicable to the experiment with donor NK cells since there was no grouping of donors. All experimental data collection and analyses were performed objectively and did not require blinding.

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

Mouse anti human Anti-HLA-E APC-conjugated, Clone 3D12; BioLegend; Cat# 342606; 1:20
Rat Anti-FLAG APC-conjugated, Clone L5; BioLegend; Cat# 637308; 1:50
Rat Anti-FLAG BV421-conjugated, Clone L5; BioLegend; Cat# 637322; 1:50
Mouse anti human Anti-CD3 PerCP/Cy5.5-conjugated, Clone UCHT1; BioLegend; Cat# 300430; 1:50
Mouse anti human Anti-CD3 AF488-conjugated, Clone UCHT1; BioLegend; Cat# 300415; 1:50
Mouse anti human Anti-CD3 AF700-conjugated, Clone SK7; BioLegend; Cat# 344822; 1:100
Mouse anti human Anti-CD4 BV711-conjugated, Clone PRA-T4; BioLegend; Cat# 300558; 1:100
Mouse anti human Anti-CD4 PE/Cy7-conjugated, Clone PRA-T4; BioLegend; Cat# 300512; 1:100
Mouse anti human Anti-CD8 AF700-conjugated, Clone SK1; BioLegend; Cat# 344724; 1:50
Mouse anti human Anti-CD14 BV421-conjugated, Clone HCD14; BioLegend; Cat# 325628; 1:50
Mouse anti human Anti-CD19 FITC-conjugated, Clone HIB19; BioLegend; Cat# 302206; 1:50
Mouse anti human Anti-CD56 PerCP/Cy5.5-conjugated, Clone HCD56; BioLegend; Cat# 318322; 1:50
Mouse anti human Anti-CD56 BV711-conjugated, Clone HCD56; BioLegend; Cat# 318336; 1:50
Mouse anti human Anti-CD69 BV421-conjugated, Clone FN50; BioLegend; Cat# 310930; 1:50
Mouse anti human Anti-CD94 PE-conjugated, Clone DX22; BioLegend; Cat# 305506; 1:100
Mouse anti human Anti-CD107a BV421-conjugated, Clone H4A3; BioLegend; Cat# 328626; 1:20
Mouse anti human Anti-HLA-DR BV785-conjugated, Clone L243; BioLegend; Cat# 307642; 1:50
Mouse anti human Anti-NKG2A APC/Fire750-conjugated, Clone S19004C; BioLegend; Cat# 375116; 1:50
Mouse anti human Anti-NKG2C PE-conjugated, Clone S19005E; BioLegend; Cat# 375004; 1:50
Human Anti-NKG2A PE-Vio® 770-conjugated, Clone REA110; Miltenyi; Cat# 130-114-093; 1:50
Human Anti-NKG2A PE-conjugated, Clone REA110; Miltenyi; Cat# 130-114-093; 1:50
Human IgG1 isotype control PE-conjugated, Clone REA293; Miltenyi; Cat# 130-113-450; 1:50
Human Anti-NKG2C PE-conjugated, Clone REA205; Miltenyi; Cat# 130-119-776; 1:50
Human Anti-NKG2C Vio® Bright FITC-conjugated, Clone REA205; Miltenyi; Cat# 130-117-707; 1:50
Mouse anti human Anti-CD14 BD Horizon™ BUV395-conjugated, Clone MϕP9; BD; Cat# 563561; 1:50
Mouse anti human Anti-NKG2A PC7-conjugated, Clone Z199; Beckman Coulter; Cat# B10246; 1:25
Rabbit andti huaman Anti-beta-2 Microglobulin HRP-conjugated, polyclonal; Thermo Fisher; Cat# PA1-29662; 1:1000
Mouse anti human Anti-HLA-E Purified-conjugated, Clone 3D12; BioLegend; Cat# 342602; 1:500

Validation

All antibodies were tested with proper negative controls including isotype control staining and/or cells that are negative for the corresponding antigen, and titrated using cells expressing the corresponding antigen. Validation statements for all antibodies can be found on vendors' websites using Cat# or in the Antibody Registry database (https://antibodyregistry.org) using AB ID.

Flow cytometry:
Mouse anti human Anti-HLA-E APC-conjugated, Clone 3D12; BioLegend; Cat# 342606; AB_2565261
Rat Anti-FLAG APC-conjugated, Clone L5; BioLegend; Cat# 637308; AB_2561497
Rat Anti-FLAG BV421-conjugated, Clone L5; BioLegend; Cat# 637322; AB_2750061
Mouse anti human Anti-CD3 PerCP/Cy5.5-conjugated, Clone UCHT1; BioLegend; Cat# 300430; AB_893299
Mouse anti human Anti-CD3 AF488-conjugated, Clone UCHT1; BioLegend; Cat# 300415; AB_389310
Mouse anti human Anti-CD3 AF700-conjugated, Clone SK7; BioLegend; Cat# 344822; AB_2563420
Mouse anti human Anti-CD4 BV711-conjugated, Clone PRA-T4; BioLegend; Cat# 300558; AB_2564393
Mouse anti human Anti-CD4 PE/Cy7-conjugated, Clone PRA-T4; BioLegend; Cat# 300512; AB_314080
Mouse anti human Anti-CD8 AF700-conjugated, Clone SK1; BioLegend; Cat# 344724; AB_2562790
Mouse anti human Anti-CD14 BV421-conjugated, Clone HCD14; BioLegend; Cat# 325628; AB_2563296
Mouse anti human Anti-CD19 FITC-conjugated, Clone HIB19; BioLegend; Cat# 302206; AB_314236
Mouse anti human Anti-CD56 PerCP/Cy5.5-conjugated, Clone HCD56; BioLegend; Cat# 318322; AB_893389
Mouse anti human Anti-CD56 BV711-conjugated, Clone HCD56; BioLegend; Cat# 318336; AB_2562417
Mouse anti human Anti-CD69 BV421-conjugated, Clone FN50; BioLegend; Cat# 310930; AB_2561909
Mouse anti human Anti-CD94 PE-conjugated, Clone DX22; BioLegend; Cat# 305506; AB_314536
Mouse anti human Anti-CD107a BV421-conjugated, Clone H4A3; BioLegend; Cat# 328626; AB_11203537
Mouse anti human Anti-HLA-DR BV785-conjugated, Clone L243; BioLegend; Cat# 307642; AB_2563461
Mouse anti human Anti-NKG2A APC/Fire750-conjugated, Clone S19004C; BioLegend; Cat# 375116; AB_2888866
Mouse anti human Anti-NKG2C PE-conjugated, Clone S19005E; BioLegend; Cat# 375004; AB_2888871
Human Anti-NKG2A PE-Vio® 770-conjugated, Clone REA110; Miltenyi; Cat# 130-114-093; AB_2726449
Human Anti-NKG2A PE-conjugated, Clone REA110; Miltenyi; Cat# 130-114-093; AB_2726171
Human IgG1 isotype control, PE-conjugated, Clone REA293; Miltenyi; Cat# 130-113-450; AB_2733892
Human Anti-NKG2C PE-conjugated, Clone REA205; Miltenyi; Cat# 130-119-776; AB_2751835
Human Anti-NKG2C Vio® Bright FITC-conjugated, Clone REA205; Miltenyi; Cat# 130-117-707; AB_2728023
Mouse anti human Anti-CD14 BD Horizon™ BUV395-conjugated, Clone MϕP9; BD; Cat# 563561; AB_2744288

Mouse anti human Anti-NKG2A PC7-conjugated, Clone Z199; Beckman Coulter; Cat# B10246; AB_2687887

ELISA-based peptide binding assay:
Rabbit andti huaman Anti-beta-2 Microglobulin HRP-conjugated, polyclonal; Thermo Fisher; Cat# PA1-29662; AB_1956329
Mouse anti human Anti-HLA-E Purified-conjugated, Clone 3D12; BioLegend; Cat# 342602; AB_1659247

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	721.221: Sigma-Aldrich, Cat# SCC275; NKL: gift from Dr. Daniel Geraghty; Jurkat cells: ATCC, Cat# TIB-152; HEK293T cells: ATCC, Cat# CRL-3216.
Authentication	Cell lines used were not authenticated.
Mycoplasma contamination	Cell lines were not tested for micoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	Cells were washed two times in PBS/2% FBS and stained with appropriate antibodies and/or 7-AAD in the dark at 4 °C for 30 min. Following staining, samples were washed two times in PBS/2% FBS and resuspended in the same buffer for flow cytometry analysis.
Instrument	BD LSRFortessa™ Cell Analyzer and MACSQuant Analyzer 16.
Software	Data collection: FACSDiva 9.0 and MACSQuantify 2.13. Data analysis: FlowJo 10.8.1 and FlowLogic 8.7.
Cell population abundance	No cell sorting was performed.
Gating strategy	Gating on FSC-A/SSC-A was performed to exclude debris, and singlets were subsequently gated using FSC-A/FSC-H. Live cells were gated using 7-AAD. Blood cell subsets were gated from live singlets based on expression of specific markers: CD14+ (monocytes), CD19+ (B cells), CD14-CD19-CD3-CD56+ (NK cells), CD14-CD19-CD3+CD4+ (CD4 T cells) and CD14-CD19-CD3+CD8+ (CD8 T cells). Jurkat reporter cells were gated from live singlets as CD3+NKG2A+ or CD3+NKG2C+ cells. Purified NK cell subsets were gated from live singlets as CD3-CD56+NKG2A+NKG2C- or CD3-CD56+NKG2A-NKG2C+ cells.

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