nature portfolio

Corresponding author(s): Jeffrey A. Hubbell, Stephan Kontos and D. Scott Wilson

Last updated by author(s): Jul 11, 2023

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	Image Lab Software (v 5.2.1) (BioRAD), BD FACSDIVA software v8.0.1, Gen5 Data Analysis Software (BioTek), Agilent 4200 Tapestation controller software A.02.02 (SR1), Biacore x100 Control Software (v2.0.2).
Data analysis	Graph Pad Prism (v9.3.1) and Graph Pad online tools were used for analysis, alongside FlowJo (v10.8.1), Image Lab Software (v 5.2.1) (BioRAD), Microsoft Excel for Mac v16.64, and BIAevaluation software (Cytiva, version 2.0.2). The RNA-seq analysis was performed under the R programming and software environment for statistical computing and graphics version 3.6 (R Core Team, 2019), FastQC tool (version 0.11.5), Spliced Transcripts Alignment to a Reference (STAR) aligner (version 2.7.2a) 1-pass algorithm, FeatureCounts tool from the subread package (version 1.5.2), Picard tools (version 2.18.7), Alignment-free transcriptome quantification method kallisto (v0.46.1), R package tximport, Ingenuity Pathway Analysis Fall 2020 release (Qiagen), Agilent Tapestation Analysis Software A.02.02 (SR1), and Simplified Presentation of Incredibly Complex Evaluation (SPICE) software (version 6, Vaccine Research Center NIH).

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

Gene-expression data obtained by RNA-seq are available from the NCBI Gene Expression Omnibus, via the accession number GSE192671. Source data for the figures are provided with this paper. All other data needed to evaluate the conclusions of the study are available within the paper and its Supplementary Information.

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	The study did not involve human research participants.
Reporting on race, ethnicity, or other socially relevant groupings	—
Population characteristics	—
Recruitment	—
Ethics oversight	—

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 size was calculated using StatMate, with a significance level of 5% and a statistical power of 80%. The effect size and standard deviation of the outcome were taken from similar experiments performed in our lab.
Data exclusions	Mice exhibiting outliers for percent OTI or OTII recovery in the dLN calculated as 1.5-fold outside the upper or lower quartiles were removed from the analyses. One NHP subject was excluded from the study after being diagnosed with diabetes before initiation of the first glycopolymer treatment.
Replication	Each experiment was repeated at least twice, unless otherwise stated. Replicates were reproducible.
Randomization	Mice were purchased form vendors, numbered, and then selected at random.
Blinding	The investigators were blinded to group allocation during data collection and analysis.

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

1 Biolegend 100233 CD3 17A2 BV510
2 Biolegend 101915 CD25 3C7 PECy7
3 Biolegend 103005 CD44 IM7 FITC
4 Biolegend 103032 CD44 IM7 PercpCy55
5 Biolegend 103116 CD45 30-F11 APCCy7
6 Biolegend 103235 B220 RA3-6B2 PercpCy55
7 Biolegend 103255 B220 RA3-6B2 BV711
8 Biolegend 103504 CD49b HM92 Af488
9 Biolegend 104406 CD62L MEL-14 FITC
10 Biolegend 104512 CD69 H1.2F3 PECy7
11 Biolegend 106305 CTLA4 UC10-4B9 PE
12 Biolegend 106314 CTLA4 UC10-4B9 PECy7
13 Biolegend 106315 CTLA4 UC10-4B9 PercpCy55
14 Biolegend 110720 CD45.1 A20 AF647
15 Biolegend 115539 CD19 6D5 BV605
16 Biolegend 123147 F4/80 BM8 BV711
17 Biolegend 125207 Lag3 C9B7W PE
18 Biolegend 126419 FOXP3 MF-14 BV421
19 Biolegend 127215 CD73 TY/11.8 BV605
20 Biolegend 127219 CD73 TY/11.8 FITC
21 Biolegend 135039 CD127 A7R34 APCCy7
22 Biolegend 135219 PD1 29F.1A12 BV605
23 Biolegend 135231 PD1 29F.1A12 BV711
24 Biolegend 505026 IL10 JES5-16E3 PECy7
25 Biolegend 505810 IFNg XMG1.2 APC
26 Biolegend 505813 IFNg XMG1.2 FITC
27 Biolegend 505829 IFNg XMG1.2 BV421
28 Biolegend 506304 TNFa MP6-XT22 FITC
29 Biolegend 506939 IL17 TC11-18H10.1 APCCy7
30 Biolegend 506941 IL17a TC11-18H10.1 BV711
31 BD 560403 FoxP3 MF23 AF488
32 BD 560578 CD451 A20 PECy7
33 BD 561872 CD45.1 A20 PE
34 BD Bioscience 562683 RORgt Q31-378 Percpcy55
35 BD Bioscience 563058 CD44 IM7 BV605
36 BD 563565 CD3 145-2C11 BUV395
37 BD 563892 B220 RA3-6B2 BV711
38 BD 564081 IL10 JES5-16E3 BV711
39 BD Bioscience 564217 TCF1 S33-966 PE
40 BD 564225 CD45 30-F11 BV786
41 BD Bioscience 564673 Lag3 C9B7w PercpCy55
42 BD 564747 GMCSF MP1-22E9 BV421
43 BD 565213 CD62L MEL-14 BUV737
44 BD 566346 Tim3 5D12 PE
45 BD 612759 CD8 53-6.7 BUV737
46 BD 612950 B220 RA3-6B2 BUV495
47 eBioscience 25544580 FR4 eBio12A5 PECy7
48 eBioscience 501124422 TOX (e660) TXRX10 APC
49 Invitrogen 12-6502-80 TOX TXRX10 PE
50 Invitrogen 12-7321-82 TNFa MP6-XT22 PE
51 invitrogen 17-7222-80 IL22 IL22JOP APC
52 Invitrogen 47-0291-82 CD29 ebioHMb1-1 APC
53 Invitrogen 47-5812-80 TCRva2 B20.1 APCCy7
54 BD BDB563058 CD44 IM7 BV605
55 BD BDB563898 CD8 53-6.7 BV421
56 BD BDB612952 CD4 GK1.5 BUV495
57 Invitrogen L34966 LD BV510

Validation	All antibodies were purchased from the vendors mentioned above. These antibodies are routinely used in our laboratory without additional validation.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK-Blue TLR4 were purchased from Invitrogen.
Authentication	HEK-Blue TLR4 cells were treated with LPS, and found to activate in response to the TLR4 ligand.
Mycoplasma contamination	Cells were tested for mycoplasma contamination, and all cell lines tested negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Unless otherwise specified, mice were maintained in a specific pathogen-free facility at the University of Chicago. 9-to-10-week-old female C57BL/6 mice were purchased from The Jackson Laboratory and used as wild-type mice in all studies unless otherwise indicated. OT-I (stock no: 003831) and OT-II (stock no: 004194) were crossed to CD45.1 mice (stock no: 002014) to yield congenically labelled OT-I and OT-II. All in vivo portions of the chronic EAE model were performed at the Hooke Laboratories using donor B6.SJL mice and C57BL/6 recipient mice. SJL/JCrHsd mice from Envigo were used for the relapsing-remitting EAE model. NHP studies used Cynomolgus macaques (Macaca fascicularis) originating from Mauritian AALAC certified breeding centers. All NHP were housed in IDMIT infrastructure facilities (CEA, Fontenay-aux-roses, France), under BSL-2 and BSL-3 containment when necessary (Animal facility authorization #D92-032-02, Prefecture des Hauts de Seine, France).
Wild animals	The study did not involve wild animals.
Reporting on sex	All mouse experiments were done using female mice to enable co-housing of groups without inflammation due to aggression, for consistent adoptive cell transfer across groups, and because of the increased prevalence of autoimmune disease in females. Therefore, sex-based analysis was not performed. All NHP studies were done in males.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All mouse studies were carried out in accordance with procedures approved by the Institutional Animal Care and Use Committee at the University of Chicago. NHP studies were conducted in compliance with European Directive 2010/63/EU, the French regulations and the Standards for Human Care and Use of Laboratory Animals of the Office for Laboratory Animal Welfare (OLAW, assurance number #A5826-01, US). The protocols were approved by the institutional ethical committee “Comité d'Ethique en Expérimentation Animale du Commissariat à l’Energie Atomique et aux Energies Alternatives” (CEtEA #44) under statement numbers A16-010 and A17-042. These studies were authorized by the “Research, Innovation and Education Ministry” under registration numbers APAFIS#6018-2016070811509491 v2 and v4.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Draining lymph nodes (axillary, brachial, inguinal, and popliteal), spleen, or spinal cord were isolated from mice. Lymph nodes were mechanically disrupted and digested at 37 deg for 45 min in collagenase D. Digested lymph nodes, spleens, or spinal cords were processed into single-cell suspensions via mechanical disruption and passage through a sterile 70-µm strainer. Red blood cells in splenocyte suspensions were lysed in ACK lysing buffer (Gibco) for 5 min before quenching with 15 mL DMEM + 10% FBS. The single-cell suspensions were then washed once with DMEM and resuspended in IMDM + 10% FBS + 1% pen/strep. For ex vivo stimulation experiments cells from spleen or lymph node were restimulated in vitro with the addition of peptide epitopes as described: PLP-SH (HCLGKWLGHPDKF-GGSGCRG) 10.0 µg/mL, OVA MHC class I (SIINFEKL) and MHC class II (ISQAVHAAHAEINEAGR) 2.0 µg/mL (Genscript). For phenotypic analysis of NHP cells, whole blood was collected

	into EDTA tubes and frozen until analysis. On the day of analysis, whole blood was thawed; stained with surface antibodies; and fixed, permeabilized and stained intranuclearly for FoxP3.
Instrument	Sample collection was done on a BD LSR flow cytometer.
Software	FlowJo (v10) was used to analyse flow-cytometry data.
Cell population abundance	The population abundance is given in the main text and the Supplementary Information.
Gating strategy	The gating strategy is outlined in the Supplementary Information.

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