nature research

Corresponding author(s): Darrell Irvine

Last updated by author(s): Dec 19, 2019

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.

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 data were obtained using BD FACSDiva software. ELISA plates and plate-based fluorescence experiments were measured by using Tecan Infinite M200 Pro absorbance/fluorescence plate reader and software. ELISPOT measurements were taken using CTL Immunospot Analyzer and associated software. Fluorescence measurements in lymph nodes were made using a LI-COR Odyssey reader and LI-COR imaging software. Confocal images were taken on an Olympus X71 microscope and used accompanying software.
Data analysis	FlowJo v10.5 was used for flow cytometry analysis. For scRNA-seq, Tophat v1.4.1, HTSeq-count v0.11.0, DESeq2 v3.1, Seurat v2.3.4, RStudio v1.1453, and Squencher v5.1 were used. GraphPad Prism 8 was used for data analysis and plots. Fiji (ImageJ v2.0.0) was used for image processing of confocal images. IVIS images were analyzed using Living Image v4.5.

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. 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

All requests for raw and analyzed data and materials are promptly reviewed by the MIT Technology Licensing Office to verify if the request is subject to any intellectual property or confidentiality obligations. Any data and materials that can be shared will be released via a Material Transfer Agreement.

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	A sample size of 5 was used to detect a significant difference (p<0.05) between groups with a signal to noise ratio of 2.0 with 80% power.
Data exclusions	In Figure 6g, two data points from the control group were excluded from the area under the curve analysis, since these two mice that had MD39-binding titers that were at background levels in the absence of the base-binding antibody. This exclusion criteria was not pre-established.
Replication	All murine experiments report pooled results from multiple experiments or data shown is one representative of at least two experiments. All attempts at replication were successful. Rabbit studies employed 6 animals/group for biological replicates.
Randomization	Randomization was not used for this study. Prior to immunizations, mice were evenly distributed into experimental groups from the same cohort of mice.
Blinding	Investigators were blinded for the neutralization analysis of rabbit sera.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Histology:
anti-mouse B220 AF488 (clone RA3-6B2; Biolegend; 1:100 dilution)
anti-mouse CD35 BV421 (clone 8C12; BD; 1:100 dilution)

Flow Cytometry:
anti-mouse B220 PE-Cy7 (clone RA3-6B2; Biolegend; 1:200 dilution)
anti-mouse B220 BV785 (clone RA3-6B2; Biolegend; 1:200 dilution)
anti-mouse GL7 FITC (clone GL7; Biolegend; 1:100 dilution)
anti-mouse GL7 PerCP-Cy5.5 (clone GL7; Biolegend; 1:100 dilution)
anti-mouse CD3 PerCp-Cy5.5 (clone 17A2; Biolegend; 1:100 dilution)
anti-mouse CD38 PE (clone 90; Biolegend; 1:75 dilution)
anti-mouse CD38 PE-Cy7 (clone 90; Biolegend; 1:75 dilution)
anti-mouse CD38 BV711 (clone 90; Biolegened; 1:100 dilution)
anti-mouse CD83 PE (clone Michel-19; Biolegend; 1:100)
anti-mouse CD86 BV605 (clone GL-1; Biolegend; 1:400 dilution)
anti-mouse MHC II PE-Cy7 (clone M5/114.15.2; Biolegend; 1:400 dilution)
anti-mouse MHC II AF700 (clone M5/114.15.2; Biolegend; 1:400 dilution)
anti-mouse CD62L BV711 (clone MEL-14; Biolegend; 1:200 dilution)
anti-mouse CD62L PerCP-Cy5.5 (clone MEL-14; Biolegend; 1:200 dilution)
anti-mouse CD95 APC-R700 (clone SA367H8; Biolegend; 1:100 dilution)
anti-mouse CD138 BV711 (clone 281-2; Biolegend; 1:100 dilution)

anti-mouse CD138 BV650 (clone 281-2; Biolegend; 1:100 dilution)
anti-mouse IgD BV510 (11-26c.2a; Biolegend; 1:100 dilution)
anti-mouse IgD APC (11-26c.2a; Biolegend; 1:100 dilution)
anti-mouse CD4 APC-Cy7 (GK1.5; Biolegend; 1:100 dilution)
anti-mouse CD8-alpha APC-Cy7 (53-5.8; Biolegend; 1:100 dilution)
anti-mouse PD-L2 PE (TY25; Biolegend; 1:50 dilution)
anti-mouse PD-L2 BUV396 (TY25; BD; 1:50 dilution)
anti-mouse CD80 BV421 (16-10A; Biolegend; 1:50 dilution)
anti-mouse CD73 BV605 (TY/11.8; Biolegend; 1:50 dilution)
anti-mouse CD45.1 BV711 (A20; Biolegend; 1:100 dilution)
anti-mouse CD45.1 BV510 (A20; Biolegend; 1:50 dilution)
anti-mouse CD45.2 FITC (104; Biolegend; 1:50 dilution)
anti-mouse IgG1-biotin (RMG1-1; Biolegend; 1:50 dilution)
anti-mouse IgG1 PE/Dazzle 594 (RMG1-1; Biolegend; 1:50 dilution)
anti-mouse IgM APC (II/41; BD; 1:150 dilution)
biotinylated eOD-GT8 coupled to Streptavidin BV711 (Streptavidin purchased from Biolegend; 1:400 dilution)
anti-mouse Ly6C APC-Cy7 (clone HK1.4; Biolegend; 1:100 dilution)
anti-mouse Ly6G BV711 (clone 1A8; Biolegend; 1:100 dilution)
anti-mouse F4/80 PE (clone BM8; Biolegend; 1:50 dilution)
anti-mouse CD11c BV421 (clone N418; Biolegend; 1:100 dilution)
anti-mouse CD169 PE-Cy7 (clone 3D6.112; Biolegend; 1:50 dilution)
anti-mouse CD11b BUV395 (clone M1/70; BD; 1:100 dilution)

ELISAs:
Goat anti-mouse IgG-HRP (Bio-rad, 1:1000 dilution)

Validation

We relied on publications and validation cited by manufacturer. Links for each antibody are given below:

anti-mouse B220 (clone RA3-6B2, Biolegend)
https://www.biolegend.com/en-us/products/brilliant-violet-785-anti-mouse-human-cd45r-b220-antibody-7960
anti-mouse GL7 (clone GL7, Biolegend)
https://www.biolegend.com/en-us/products/fitc-anti-mouse-human-gl7-antigen-t-and-b-cell-activation-marker-antibody-8284
anti-mouse CD3 (clone 17A2, Biolegend)
https://www.biolegend.com/en-us/products/percp-cyanine5-5-anti-mouse-cd3-antibody-5596
anti-mouse CD38 (clone 90, Biolegend)
https://www.biolegend.com/en-us/products/pe-anti-mouse-cd38-antibody-183
anti-mouse CD83 (Michel-19; Biolegend)
https://www.biolegend.com/en-us/products/pe-anti-mouse-cd83-antibody-3580
anti-mouse CD86 (GL-1; Biolegend)
https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd86-antibody-7798
anti-mouse MHC II (M5/114.15.2; Biolegend)
https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413
anti-mouse CD62L (MEL-14; Biolegend)
https://www.biolegend.com/en-us/search-results/brilliant-violet-711-anti-mouse-cd62l-antibody-10317
anti-mouse CD95 (SA367H8; Biolegend)
https://www.biolegend.com/en-us/products/apc-anti-mouse-cd95-fas-antibody-13906
anti-mouse CD138 (281-2; Biolegend)
https://www.biolegend.com/en-us/search-results/brilliant-violet-650-anti-mouse-cd138-syndecan-1-antibody-8800
anti-mouse IgD (11-26c.2a; Biolegend)
https://www.biolegend.com/en-us/products/brilliant-violet-510-anti-mouse-igd-9032
anti-mouse CD4 (GK1.5; Biolegend)
https://www.biolegend.com/en-us/products/apccyanine7-anti-mouse-cd4-antibody-1964
anti-mouse CD8-alpha (53-5.8; Biolegend)
https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd8a-antibody-2269
anti-mouse PD-L2 (TY25; Biolegend)
https://www.biolegend.com/en-us/products/pe-anti-mouse-cd273-b7-dc--pd-l2-antibody-2547
anti-mouse CD80 (16-10A; Biolegend)
https://www.biolegend.com/en-us/products/pe-anti-mouse-cd80-antibody-43
anti-mouse CD73 (TY/11.8; Biolegend)
https://www.biolegend.com/nl-nl/products/brilliant-violet-605-anti-mouse-cd73-antibody-8153
anti-mouse CD45.1 (A20; Biolegend)
https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd45-1-antibody-8925
anti-mouse CD45.2 (104; Biolegend)
https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd45-2-antibody-6
anti-mouse IgG1 (RMG1-1; Biolegend)
https://www.biolegend.com/en-us/search-results/pe-dazzle-594-anti-mouse-igg1-antibody-14778
anti-mouse Ly6C (clone HK1.4, Biolegend)
https://www.biolegend.com/en-us/products/apccyanine7-anti-mouse-ly-6c-antibody-6758
anti-mouse Ly6G (clone 1A8, Biolegend)
https://www.biolegend.com/nl-nl/products/brilliant-violet-711-anti-mouse-ly-6g-antibody-12062
anti-mouse F4/80 (clone BM8, Biolegend)
https://www.biolegend.com/en-us/products/pe-anti-mouse-f4-80-antibody-4068
anti-mouse CD11c (N418, Biolegend)
https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd11c-antibody-7149
anti-mouse CD169 (clone 3D6.112, Biolegend)

https://www.biolegend.com/ja-jp/products/pe-cy7-anti-mouse-cd169-siglec-1-antibody-9929
anti-mouse CD35 (clone 8C12, BD)
https://www.bdbiosciences.com/eu/applications/research/b-cell-research/surface-markers/mouse/bv421-rat-anti-mouse-cd35-8c12/p/740029
anti-mouse IgM (II/41; BD)
https://www.bdbiosciences.com/us/applications/research/b-cell-research/immunoglobulins/mouse/apc-rat-anti-mouse-igm-ii41/p/550676
anti-mouse CD11b (clone M1/70; BD)
https://www.bdbiosciences.com/us/applications/research/stem-cell-research/mesenchymal-stem-cell-markers-bone-marrow/mouse/negative-markers/buv395-rat-anti-cd11b-m170/p/563553
anti-mouse Goat IgG-HRP (H+L) (Bio-rad, Catalog 170-6516): validated by ELISA
biotinylated eOD-GT8 coupled to Streptavidin BV711 (Biolegend): validated by flow cytometry and previous publication (Abbott, et al., https://doi.org/10.1016/j.immuni.2017.11.023)

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Ramos B cells expressing germline VRC01 were obtained from Daniel Lingwood (Ragon Institute). FreeStyle 293-f was obtained from ThermoFisher. HEK293T were obtained from ATCC. The TZM-bl cell line engineered from CXCR4-positive HeLa cells to express CD4, CCR5, and a firefly luciferase reporter gene (under control of the HIV-1 LTR) was obtained from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (developed by Dr. John C. Kappes, and Dr. Xiaoyun Wu).
Authentication	Ramos B cells expressing germline VRC01 were validated by flow cytometry. TZM-bl cell line from HeLa cells were validated by luciferase assay. FreeStyle 293-f and HEK293T were not validated.
Mycoplasma contamination	All cell lines tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Balb/c, female, 8 week old mice were used for mouse immunizations. C57BL/6, male, 8 week old mice were used for the adoptive transfer experiments. New Zealand white rabbits, female, 2.5-3.0 kg, 3-4 months old, were used for the rabbit immunizations.
Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Experiments and handling of mice were conducted under federal, state, and local guidelines under an IACUC approved protocol through MIT or La Jolla Institute for Immunology.

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	Lymph nodes and spleens were mechanically digested, filtered into single cell suspensions, and stained using antibodies described above.
Instrument	BD Canto and BD Fortessa were used for data collection. BD Aria was used for B cell sorting.
Software	Flow cytometry data was analyzed using FlowJo.
Cell population abundance	1-2x10^5 AF647+ VRC01gHL B cells and 1x10^6 AF647- endogenous B cells were sorted from two mice immunized with pSereOD-GT5/alum or pSer-eOD-GT8/alum for visualization of alum by TEM. For bulk RNA-seq analysis, 0.5x10^4 - 1x10^5

VRC01gHL B cells were sorted from each mouse directly into TRIzol LS for further processing and therefore purity of post-sort fractions were not determined.

Gating strategy

Lymphocytes were gated on the starting cell population in a FCS/SSC plot, followed by FCA/FCH plot to gate single cells. Live cells were gated using either Aqua or Fixable Viability Dye e780. Cells were then gated on B220 positive cells. For adoptive transfer experiments, VRC01 cells were identified using GFP expression and labeling with CTV.

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