nature research

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Last updated by author(s): Apr 25, 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	Olymphus cellSens Standard 1.18 (for microscopic images), BD FACSDiva 8.0.1 10X chromium 5'-mRNA library and BCR library kit HISEQ4000 (illumina sequencing)
Data analysis	Graphpad Prism 7.0a & 8.0, FlowJo 10.4.2, Cell Ranger 2.2.0 (10X chromium), Rv3.4.2, Python 3.5.4, IgBlast v1.7.0, Seurat v2, Chage-O v0.4.2, Alakazam v0.2.11, PHYLIP 3.696

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

RNA-seq data will be deposited and made public in the BioProject under accession number PRJNA524497.

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	No sample-size calculation was performed. We aimed to get at least 3 sample sizes in each group in each independent experiment based on our previous studies and/or pilot studies.
Data exclusions	No data were excluded.
Replication	Experiments were repeated with at least two biologically independent for all results presented in the manuscript. If the group size was small (due to limited availability of reagents or mouse strains), data from replicate experiments were pooled for graphical representation. All replicates are biological replicates obtained from biologically independent experiments.
Randomization	Allocation was not random. Since we used littermate animals in each experimental and control groups, randomization was not relevant to our study.
Blinding	All animal studies were not blinded since treatment and experimental analysis could not be separated, blinding of the investigators was not possible.

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

antibody name/ supplier name /catalog number /clone name/ lot number /dilution /validation (website)
CD16/32 BioXCell BE0307 2.4G2 1:500 https://bxcell.com/product/invivomab-anti-mouse-cd16-cd32/
CD45.2 BioLegend 109824 104 B240422 1:300 https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd45-2-antibody-3906
CD45.1 BioLegend 110708 A20 B146359 1:300 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd45-1-antibody-199
CD45 BioLegend 103116 30-F11 B266564 1:300 https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd45-antibody-2530
CD3e BioLegend 100308 145-2C11 B253780 1:300 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd3epsilon-antibody-25
CD4 BioLegend 100451 GK1.5 B223832 1:300 https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd4-antibody-10708
CD4 BioLegend 100540 RM4-5 B161056 1:300 https://www.biolegend.com/en-us/products/percpcyeanine5-5-anti-mouse-cd4-antibody-4230
CD8a BioLegend 100744 53-6.7 B266536 1:300 https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd8a-antibody-7636
CD8a BioLegend 100747 53-6.7 B259952 1:300 https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd8a-antibody-7926
CD19 BioLegend 115540 6D5 B264622 1:200 https://www.biolegend.com/en-us/products/brilliant-violet-605-anti-mouse-cd19-antibody-7645
CD19 BD Biosciences 564296 1D3 8039779 1:200 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunology-reagents/anti-mouse-antibodies/cell-surface-antigens/buv737-rat-anti-mouse-cd19-1d3/p/564296

CD45R/B220 BioLegend 103236 RA3-6B2 B253647 1:200 https://www.biolegend.com/en-us/products/percp-cy5-5-anti-mouse-human-cd45r-b220-antibody-4267
CD138 BioLegend 142519 281-2 B248101 1:200 https://www.biolegend.com/en-us/products/brilliant-violet-711-anti-mouse-cd138-syndecan-1-antibody-8909
CD138 BioLegend 142507 281-2 B253736 1:200 https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd138-syndecan-1-antibody-7852
CD38 BioLegend 102717 90 B242687 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd38-antibody-3926
CD38 BioLegend 102719 90 B139813 1:200 https://www.biolegend.com/en-us/products/pacific-blue-anti-mouse-cd38-antibody-6652
GL7 BD Biosciences 561530 GL7 4330708 1:200 http://www.bdbiosciences.com/ds/pm/tds/561530.pdf
MHCII (I-A/I-E) BioLegend 107622 M5/114.15.2 B264454 1:800 https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-i-a-i-e-antibody-3413
CD11c BioLegend 117329 N418 B258584 1:200 https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd11c-antibody-7149
IgD BD Biosciences 564274 11-26c.2a 8046964 1:200 http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/second-step-reagents/anti-ig/buv395-rat-anti-mouse-igd-11-26c2a/p/564274
CD44 BioLegend 103026 IM7 B244378 1:200 https://www.biolegend.com/en-us/products/alexa-fluor-700-anti-mouse-human-cd44-antibody-3406
PD-L2 BioLegend 107210 TY25 B245340 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd273-b7-dc--pd-l2-antibody-12353
CD80 BioLegend 104734 16-10A1 B249923 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd80-antibody-9320
CD69 BioLegend 104512 H1.2F3 B253212 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd69-antibody-3168
CD86 BioLegend 105011 GL1 B245161 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd86-antibody-2896
AF488 goat anti-mouse IgG (H+L) Invitrogen A11029 polyclonal 1829910 1:800
AF647 donkey anti-mouse IgG (H+L) Fab Jackson ImmunoResearch 715-607-003 polyclonal 128907 1:600
CCR1 R&D Systems FAB5986P 643854 ABQH0314071 10ul/test https://www.rndsystems.com/products/mouse-ccr1-pe-conjugated-antibody-643854_fab5986p
CCR2 R&D Systems FAB5538F 475301 ABPZ0212011 10ul/test https://www.rndsystems.com/products/mouse-ccr2-fluorescein-conjugated-antibody-475301_fab5538f
CCR3 BD Biosciences 557974 83103 1:200 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-2-cells/surface-markers/mouse/alexa-fluor-647-rat-anti-mouse-cd193-83103/p/557974
CCR4 BioLegend 131214 2G12 B201546 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd194-ccr4-antibody-6333
CCR5 BioLegend 107012 HM-CCR5 B186964 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd195-ccr5-antibody-3939
CCR6 BioLegend 129815 29-2L17 B187956 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd196-ccr6-antibody-6152
CCR7 BioLegend 120105 4B12 B254167 1:200 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd197-ccr7-antibody-2799
CCR8 BioLegend 150311 SA214G2 B224699 1:200 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd198-ccr8-antibody-13428
CCR9 BioLegend 128705 CW-1.2 B120356 1:200 https://www.biolegend.com/en-us/products/fitc-anti-mouse-cd199-ccr9-antibody-5123
CCR10 R&D Systems FAB2815P 248918 LXL0207011 10ul/test https://www.rndsystems.com/products/mouse-ccr10-pe-conjugated-antibody-248918_fab2815p
CXCR1 R&D Systems FAB8628P 1122A AENE0115111 10ul/test https://www.rndsystems.com/products/mouse-cxcr1-il-8-ra-pe-conjugated-antibody-1122a_fab8628p
CXCR2 BioLegend 129101 TG11/CXCR2 B119951 1:200
CXCR3 BioLegend 126506 CXCR3-173 B137934 1:200 https://www.biolegend.com/en-us/products/pe-anti-mouse-cd183-cxcr3-antibody-4592
CXCR3 BioLegend 126512 CXCR3-173 B233225 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd183-cxcr3-antibody-4683
CXCR4 BD Biosciences 551966 2B11/CXCR4 1:200 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/regulatory-t-cells/surface-markers/mouse/pe-rat-anti-mouse-cd184-2b11cxcr4/p/551966
CXCR5 BioLegend 145515 L138D7 B170134 1:200 https://www.biolegend.com/en-us/products/pe-cy7-anti-mouse-cd185-cxcr5-antibody-8617
CXCR6 BioLegend 151105 SA051D1 B224895 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-cd186-cxcr6-antibody-13065
IFN-g BioLegend 505810 XMG1.2 B221722 1:200 https://www.biolegend.com/en-us/products/apc-anti-mouse-ifn-gamma-antibody-993
CD4 blocking Ab BioXCell BE0003-1 GK1.5 699918M2 https://bxcell.com/product/m-cd4/
IFN-g blocking Ab BioXCell BE0054 R4-6A2 601516A2 https://bxcell.com/product/m-inf-gamma/
FITC mouse IgG Jackson ImmunoResearch 211-095-109 polyclonal 131785
mIgG1 anti-HSV gD MAb Absolute Antibody Ab00442-1.1 E317 T1832B15 https://absoluteantibody.com/product/anti-glycoprotein-d-of-hsv-e317/Ab00442-1.1_Mouse_IgG1/
CD4 BD Biosciences 553649 H129.19 1:320 http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/mouse/biotin-rat-anti-mouse-cd4-h12919/p/553649
CD45R/B220 BioLegend 103208 RA3-6B2 B224683 1:200 https://www.biolegend.com/en-us/products/pe-anti-mouse-human-cd45r-b220-antibody-447
CD138 BioLegend 142511 281-2 B183667 1:100 https://www.biolegend.com/en-us/products/biotin-anti-mouse-cd138-syndecan-1-antibody-8449
CD31 eBioscience 13-0311-81 390 1:100 https://www.thermofisher.com/antibody/product/CD31-PECAM-1-Antibody-clone-390-Monoclonal/13-0311-81

VCAM-1 BioLegend 105703 429/MVCAM.A 1:100 https://www.biolegend.com/en-us/products/biotin-anti-mouse-cd106-antibody-136
AF647 anti-FITC Ab Jackson ImmunoResearch 200-602-037 135650
AF568 anti-mouse IgG (H+L) Invitrogen A10037 polyclonal
FITC pAbs against HSV1 and 2 Virostat 0196
HRP-conjugated anti-mouse IgG1 SouthernBiotech 1070-05 polyclonal D0812-WC82B 1:1000 https://www.southernbiotech.com/?catno=1070-05&type=Polyclonal#&panel1-1&panel2-1
HRP-conjugated anti-mouse IgG3 SouthernBiotech 1100-05 polyclonal B0308-Z781C 1:1000 https://www.southernbiotech.com/?catno=1100-05&type=Polyclonal#&panel1-1&panel2-1
HRP-conjugated anti-mouse IgM SouthernBiotech 1020-05 polyclonal C0012-P252L 1:1000 https://www.southernbiotech.com/?catno=1020-05&type=Polyclonal#&panel2-1
HRP-conjugated anti-mouse IgA SouthernBiotech 1040-05 polyclonal I5613-R566B 1:1000 https://www.southernbiotech.com/?catno=1040-05&type=Polyclonal#&panel1-1&panel2-1
HRP-conjugated anti-mouse IgG2b SouthernBiotech 1090-05 polyclonal A2513-XA54E 1:1000 https://www.southernbiotech.com/?catno=1090-05&type=Polyclonal#&panel1-1&panel2-1
HRP-conjugated anti-mouse IgG2c SouthernBiotech 1079-05 polyclonal G0313-ZF85D 1:1000 https://www.southernbiotech.com/?catno=1079-05&type=Polyclonal#&panel2-1

Validation

Quality of antibodies was tested by manufacturer (statements on the manufacturer's website) or relevant references were cited on the manufacturer's website. Vendor websites for antibodies were listed above and the validations can be found there.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Vero cells (ATCC CCL-81)

Authentication

Cell lines were authenticated by morphology.

Mycoplasma contamination

Cell lines were routinely tested for mycoplasma contamination, and negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No misidentified cell lines in this study

Animals and other organisms

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

Laboratory animals

Six to eight-week old female C57BL/6 (CD45.2+), congenic C57BL/6 B6.SJL-PtprcaPep3b/BoyJ (B6.Ly5.1) (CD45.1+), B6.129S2-IghtmICgn/J (μMT), B6.FVB-Tg[ITGAM-DTR/EGFP] 34Lan/J (CD11bDTR), B6.Cg-Tg(CAG-DsRed*MST)1Nagy/J (DsRed), and B6.129P2-Cxcr3tm1Dgen/J (CXCR3-/-) mice from the National Cancer Institute and Jackson Laboratory

Wild animals

No wild animals were used in this study

Field-collected samples

No field-collected samples were used in this study

Ethics oversight

All procedures used in this study complied with federal guidelines and institutional policies by the Yale animal care and use committee (Protocol # 2018-10365).

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

The genital tracts of vaginal tissues and upper reproductive tracts including cervix and uterus treated with Depo-Provera were dissected from the urethra and cervix. Tissues were then incubated with 0.5 mg/mL Dispase II (Roche) for 15 min at 37 °C. Thereafter, tissues were digested with 1 mg/mL collagenase D (Roche) and 30 μg/mL DNase I (Sigma-Aldrich) at 37 °C for 25 min. The resulting cells were filtered through a 70-μm filter and single cells were isolated.

Instrument

LSRII flow cytometer

Software	FACS DIVA, FlowJo 10.4.2
Cell population abundance	For B cell-sorted spleen samples, cell numbers were counted and 6,000 cells were prepared. Cells from primary and secondary infected FRT samples were prepared at a cell count proportional to the immune cell infiltration observed by flow cytometry. We separate CD3 and CD19 expressing clusters during scRNA seq analysis.
Gating strategy	live/dead stain (Aqua)- gated -> CD45.2+ or CD45.1+ gated -> single cells (FSC-A/FSC-H) -> lymphocytes gated. We defined positive and negative population at the distinct border or by using isotype control stain.

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