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Corresponding author(s): Wan, Xiu-Feng

Last updated by author(s): 3/17/2026

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	1) ELISpot plates were scanned and visualized using an ImmunoSpot reader (Cellular Technology Ltd., Cleveland, OH, USA). 2) Stained histological slides were scanned at 40× magnification using an Aperio ScanScope CS system operated by the MU Pathology Informatics Whole-Slide Imaging Analytics Lab.
Data analysis	1) Statistical analyses were performed using GraphPad Prism (10.1.2; GraphPad Software, La Jolla, CA, USA). 2) Ingenuity Pathway Analysis (IPA) was conducted using the IPA platform (QIAGEN Inc.; https://digitalinsights.qiagen.com/products-overview/discovery-insights-portfolio/analysis-and-visualization/qiagen-ipa). 3) Single-cell RNA and TCR/BCR sequencing data were processed using Cell Ranger (v8.0.0; 10x Genomics). Clustering analysis was conducted using the Seurat package (v4.4.0), and Scrublet (v0.2.3) was used for doublet detection. Trajectory inference was performed using the PAGA method via the scanpy.tl.paga function in Scanpy (v1.9.1). Differentially expressed genes (DEGs) were visualized with the EnhancedVolcano package (v1.14.0; https://github.com/kevinblighe/EnhancedVolcano). Cell-cell communication was predicted using CellChat (v2.1.1). Single-cell TCR and BCR sequences were processed with the Cell Ranger VDJ pipeline (10x Genomics) using the GRCm38 mouse VDJ reference genome and analyzed with the Scirpy package (v0.10.1). Clonotypes were defined based on CDR3 nucleotide sequence identity, and clonal diversity metrics were calculated using the alakazam R package (v1.2.1). 4) Influenza NP-positive cells were quantified using the Positive Cell Detection function in QuPath (v0.4.0). Code availability. The raw count matrices and analysis code used in this study have been deposited in Zenodo (https://doi.org/10.5281/zenodo.17781231) and are publicly available.

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

The single-cell RNA sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1285482 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1285482). These data are publicly available. Source data are provided with this paper.

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	YES
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	Adults aged 18–89 years who received a documented influenza vaccination were eligible for inclusion. Individuals with obstructive sleep apnea (OSA) were identified using ICD-10-CM codes G47.3 and G47.33, while controls were required to have no sleep-apnea–related codes. To ensure engagement with the healthcare system, the cohort logic required documentation of an ambulatory visit within six months of vaccination. Influenza vaccination events were identified using CPT/HCPCS codes including 90630, 90653–90662, 90672–90674, 90682, and 90685–90689. Patients with any influenza diagnosis prior to the index event were excluded.
Recruitment	N/A
Ethics oversight	The human cohort analyses were performed using de-identified electronic health records from the TriNetX Global Collaborative Network. The study was deemed exempt from review by the Institutional Review Board of The Hadassah Medical Center. TriNetX operates under its own governance framework to ensure HIPAA-compliant handling of de-identified data, and no protected health information was accessed or transferred outside the secure platform. The participating healthcare organizations contributing to TriNetX have their own IRB approvals or waivers permitting inclusion of de-identified patient data for research use.

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	1) Sample sizes were determined based on pilot data and power calculations. In total, 105 male mice were assigned into four groups: sleep fragmentation (SF), sleep control (SC), mock (infection without vaccination), and a negative control group. The SF, SC, and negative control groups each included 30 mice, while the mock group included 15 mice. Within the 30 mice per group, 10 were allocated for survival analysis, and 5 mice per group were euthanized at each of four time points (post-vaccination/pre-challenge, and at 3, 5, and 7 days post-infection) for blood and tissue collection. In the mock group, 10 mice were used for survival analysis and 5 were euthanized at 3 days post-infection, as prior experience indicated that all mice in this group would succumb by 4–5 days post-infection. Power calculations were performed based on data from an initial experiment, which showed 100% survival in vaccinated controls compared to approximately 50% survival in SF-vaccinated mice following virus challenge. For survival outcomes, power analysis indicated that 7 animals per group would achieve 80% power using a two-sided test at $\alpha = 0.05$ (Cohen's $d \approx 1.57$); therefore, 10 mice per group were used to ensure adequate power. For lung viral titers, power analysis based on an observed effect size (Cohen's $d \approx 1.76$) indicated that 5 mice per group would provide approximately 70% power ($\alpha = 0.05$) to detect biologically meaningful differences between groups. In the study, we used n=10 per group for survival analyses and n=5 per group per necropsy time point for virological analyses, aligned with our housing configuration of five mice per cage.Minor variations in sample size at certain time points (e.g., n = 4) occurred due to occasional animal loss during the acclimation period.

	2) Body weight loss and survival rates from three independent experiments using different vaccine–challenge dose combinations: 0.1μg HA – 500LD_{50}, 0.1μg HA – 500LD_{50} (repeat), and 0.3μg HA – 5,000LD_{50}. Experiment 1: 0.1μg HA – 500LD_{50}; gSF (n=9), gSC (n=10), Mock (n=7), Negative (n=9); Experiment 2: 0.1μg HA – 500LD_{50}; gSF (n=5), gSC (n=5), Mock (n=5), Negative (n=5); Experiment 3: 0.3μg HA – 5000LD_{50}; gSF (n=5), gSC (n=5), Mock (n=5), Negative (n=4). Sample sizes varied slightly between groups due to animal loss during the acclimation period prior to the start of the experiments.
Data exclusions	No data were excluded from this study.
Replication	There was four independent experiments.
Randomization	Mice were stratified by weight, then randomly assigned to treatment groups using block randomization to ensure balanced distribution across groups. Each treatment block (e.g., body weight measurement, necropsy, sample collection) was designed to include five mice, with a minimum of four mice required per group to be included in statistical analyses.
Blinding	Blinding was not performed due to logistical constraints and the nature of the procedures, which required knowledge of group assignments during treatment administration and data collection.

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	Antibodies and ELISpot kits. The following antibodies and kits were used in this study. Mouse IgG (HRP) ELISpot kit (Cat. 3825-2H, Mabtech, Cincinnati, OH, USA) and mouse IFN-γ (HRP) ELISpot kit (Cat. 3321-2H, Mabtech, Cincinnati, OH, USA) were used according to the manufacturer's instructions. For IFN-γ detection, anti-mouse IFN-γ capture antibody (clone AN18, Cat. 3321-3-250, Mabtech) and biotinylated anti-mouse IFN-γ detection antibody (clone R4-6A2, Cat. 3321-6-250, Mabtech; 1:1000 dilution in PBS with 0.5% FBS) were used. For immunohistochemistry, NP rabbit monoclonal antibody (Cat. MA5-42363, Invitrogen, Waltham, MA, USA) and VR Horse Anti-Rabbit IgG Polymer Detection Kit (Cat. MP-6401-15, Vector Laboratories, Newark, CA, USA) were used. For ELISA, the following secondary antibodies were used: Goat anti-Mouse IgM (Cat. 31440, Invitrogen), Goat anti-Mouse IgG Fc (Cat. 31437, Invitrogen), Goat anti-Mouse IgG3 (Cat. 75952S, Cell Signaling Technology), Goat anti-Mouse IgG2b (Cat. 43593S, Cell Signaling Technology), Goat anti-Mouse IgG1 (Cat. 96714S, Cell Signaling Technology), Goat anti-Mouse IgG2c (Cat. 56970S, Cell Signaling Technology), and peroxidase-conjugated goat anti-rabbit IgG (Cat. A16110, Thermo Fisher Scientific, Waltham, MA, USA). Flow cytometry antibodies. The following antibodies were used for flow cytometry: anti-B220 (clone RA3-6B2, APC, Cat. 553092, BD Biosciences; 1:700), GL7 (clone GL7, PE, Cat. 561530, BD Biosciences; 1:20), CD138 (clone 281-2, BV750, Cat. 747070, BD Biosciences; 1:20), CD86 (clone GL1, BV650, Cat. 564200, BD Biosciences; 1:20), CD11c (clone HL3, AF700, Cat. 560583, BD Biosciences; 1:20), MHC class II (clone 10-3.6, FITC, Cat. 562014, BD Biosciences), CD20 (clone SA275A11, BV421, Cat. 150405, BioLegend), and IgM (clone R6-60.2, PE-Cy7, Cat. 552867, BD Biosciences; 1:50). T cell markers included anti-CD3 (clone 17A2, PE, Cat. 100206, BioLegend; 1:400), CD4 (clone RM4-5, AF700, Cat. 557956, BD Biosciences; 1:1000), CD8α (clone 53-6.7, Pacific Blue, Cat. 100725, BioLegend; 1:750), CD62L (clone MEL-14, APC, Cat. 104412, BioLegend; 1:20), CD44 (clone IM7, FITC, Cat. 103005, BioLegend; 1:40), CD40 (clone 3/23, BV510, Cat. 745041, BD Biosciences; 1:40), CXCR5 (clone 2G8, RB613, Cat. 759651, BD Biosciences; 1:40), and ICOS (clone C398.4A, BV650, Cat. 568041, BD Biosciences; 1:40).
Validation	All antibodies were commercially sourced and used according to the manufacturers' instructions. Specificity was supported by manufacturer validation and further confirmed in our assays using appropriate positive and negative controls.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Madin-Darby canine kidney (MDCK; CCL-34) cells were obtained from American Type Culture Collection (Manassas, VA, USA).
Authentication	We maintain frozen stocks of the cell lines in liquid nitrogen tanks. Cell lines are replenished about every 20 passages. Cell cultures are inspected daily for sterility, maintenance of adherence, and distinctive morphology. Cell growth media and fetal bovine serum are tested for quality control and support of cell viability before large-scale purchases are made.
Mycoplasma contamination	We confirm that all cell lines used in this study tested negative for mycoplasma contamination. Cell lines are routinely screened for mycoplasma contamination every six months and additionally whenever contamination is suspected.
Commonly misidentified lines (See ICLAC register)	N/A

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	We purchased 6 to 8-week-old male C57BL/6J mice from The Jackson Laboratory (Bar Harbor, ME, USA).
Wild animals	N/A
Reporting on sex	The current study was conducted exclusively in young adult male mice to minimize variability from hormonal cycling and because male mice tend to be more active, which better models sleep fragmentation induced by environmental disruption.
Field-collected samples	N/A
Ethics oversight	The animal experiments were conducted under protocol #38742, approved by the University of Missouri's Care and Use of Laboratory Animals in accordance with USDA Animal Welfare Regulations. All experiments were performed in a biosafety level 2 facility at the University of Missouri-Columbia under protocol #10071, in compliance with the Institutional Biosafety Committee of the University of Missouri-Columbia.

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

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	SSplenocytes were collected at 35 days post-vaccination (dpv) and processed for multiparameter flow cytometry to quantify four splenic B cell subsets, including plasma cells (B220^{-}CD138+), plasmablast (B220+CD138+), GC B cells (GL7+B220 +CD11c^{-}). The expression levels of MHC II and CD86 on B cells were also assessed. In addition, 10 T cell subsets (total CD3^{+} T

	cells; CD4 and CD8 T cells; CD4 naïve, central memory, and effector memory; CD8 naïve, central memory, and effector memory; and Tfh-like cells) and one non–T cell subset ($CD3^-CD40^+$ activated non-T lymphocytes) were assessed.
Instrument	Cytek Aurora cytometer
Software	FlowJo v10.10.0
Cell population abundance	Two million cells were washed in PBS and stained with LIVE/DEAD Fixable Near-IR viability dye (Thermo Fisher, Cat# L10119) according to the manufacturer's instructions.
Gating strategy	Following doublet exclusion and viability gating (LIVE/DEAD™ Fixable Near-IR, ThermoFisher L34975), $CD3^+$ T cells were identified and separated into $CD4^+$ and $CD8^+$ compartments, which were further subdivided into naïve ($CD62L^+CD44^-$), central memory ($CD62L^+CD44^+$), and effector memory ($CD62L^-CD44^+$) subsets. Within the $CD4^+$ population, T follicular helper–like (Tfh-like) cells were defined as $CXCR5^+ICOS^+$. A non–T cell comparator population was delineated as $CD3^-CD40^+$ activated non-T lymphocytes. In parallel, B cell analyses began by gating on B220 and CD138 to distinguish plasma cells ($B220^-CD138^+$) and plasmablasts ($B220^+CD138^+$), after which $B220^+CD138^-$ cells were gated to exclude $CD11c^+$ myeloid contaminants and define conventional B cells. Germinal center (GC) B cells were defined as $GL7^+B220^+CD11c$- within the conventional B cell compartment; among non-GC conventional B cells, expression of MHC class II and CD86 was assessed to evaluate B cell activation status. The gating strategy figures have been provided in the Source Data file (Supplementary Fig. S4).

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