nature portfolio

Corresponding author(s): DAPR, NCOMMS-26-039971

Last updated by author(s): May 27, 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	Flow cytometry data were collected using a Beckman Coulter Cytoflex flow cytometer, a BD Symphony S6 flow cytometer, and an In Vivo Imaging System (IVIS) with Living Image software 4.8.2 (Revvity). Mass spectrometry raw data were generated on an liquid chromatography-mass spectrometer (LC-MS/MS Orbitrap Fusion, FSN10518)
Data analysis	Statistical calculations, graphs, and survival curves were generated using GraphPad Prism (version 9) and R (version 4.4.2) statistical software. Flow cytometry analysis was performed via FlowJo V10 and CytExpert (version 2.3). Confocal colocalization analysis was performed using coloc2 plugin from ImageJ (version 1.54). For immunopeptidomics, mass spectrometry RAW files were processed using MS-GF+ (version 2024.03.26) and visualized with PeptideShaker (version 3.0.11)

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

T cell epitope prediction data and its analysis along with custom code were deposited into GitHub database and are available at the following URL: https://github.com/raj1693/flu_tcell_vaccine, Proteomics data along with raw files and result files has been uploaded on PRIDE-PRoteomics IDEntifications Database (ref id: 1-20260527-134106-1000007345)

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	Sex and Gender were not considered in the study design. Sex and gender information obtained were self reported.
Reporting on race, ethnicity, or other socially relevant groupings	Self reported information of race are included in the manuscript in supplementary section
Population characteristics	The study included healthy volunteer participants aged 17 and older.
Recruitment	Donor that wished to participate and donate blood according to the criteria for blood donation as set forth by the Food and Drug Administration (FDA) were included.
Ethics oversight	The study was conducted as per the protocol approved by the Emory University Institutional Review Board (IRB) (#IRB00045947) and Centers for Disease Control and Prevention-Reliance IRB (#1652) in compliance with all applicable Federal regulations governing the protection of human subjects.

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 sizes (n = 4 to 8 per group for animal challenge/immunogenicity cohorts; n = 5 for human PBMC validation assays) were chosen based on standard field precedents for statistical validation and to achieve optimal power based on expected historical variations in lethal viral challenges and cellular activation assays.
Data exclusions	No data points or experimental animals were excluded from any analyses.
Replication	All experimental procedures, PLGA particle formulations characterization, and in-vitro assays were independently replicated at least three times. Robust viral challenge confirmations were validated across distinct sub-strains and subtypes (H1N1 maCal/09, H1N1 X31, H3N2 HK/19, and H5N1 HALO). All attempts at replication were fully successful.
Randomization	Mice within age-matched and sex-matched cohorts were randomly assigned to different experimental treatment groups (unvaccinated, free peptide pool + adjuvant, or PLGA-encapsulated microparticles) prior to immunization schedules.
Blinding	Histopathological evaluation of pulmonary tissue sections (perivascular infiltration, bronchial damage, and alveolar pneumonia scores) was performed under strict blinded conditions by a qualified veterinary pathologist

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	Mouse BMDC Phenotyping: Anti-CD11c (BD Biosciences, Cat# 564079), Anti-F4/80 (BD Biosciences, Cat# 565411), Anti-CD80 (Cat# 17080182), Anti-CD86 (Invitrogen, Cat# 47086282), and Anti-MHCII (Invitrogen, Cat# 1453282) Mouse Intracellular Cytokine Staining (ICS): Anti-CD3-APC-eFluor 780 (Invitrogen, Cat# 47038182), Anti-CD4-FITC (Invitrogen, Cat# 11-0042-82), Anti-CD8-Super Bright 780 (Invitrogen, Cat# 78-0081-82), Anti-TNF-PE-Cy7 (Invitrogen, Cat# 25-7321-82), Anti-IFN-gamma-PE (Invitrogen, Cat# 12-7311-82), and Anti-IL-2-APC (Invitrogen, Cat# 177021-82). Human PBMC Assays & AIM: Antibodies for CD8-BV570, CD3-BUV805, CD4-BV480, IFN-gamma-PE-Cy7, TNFalpha-BB700, IL-2-BV650, CXCR5, CCR7, CXCR3, and markers CD40L, CD69, OX-40, 4-1BB, PD-1/PD-L1 as listed in full in internal Manuscript Tables S5 Immunoprecipitation / Microscopy: Anti-MHC I antibody (clone IVA26, Cat# MA5-44043) and Anti-LAMP-1 lysosomal antibody (Abcam, Ca#ab24170)
Validation	All commercially acquired regulatory, surface marker, and neutralizing antibodies were fully validated by their respective manufacturers for specificity in human or murine cross-reactive flow cytometry and imaging applications, and were verified internally using negative controls (empty PLGA/unstimulated control and media controls)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Madin-Darby Canine Kidney (MDCK) cells were sourced from the National Centre for Cell Science (NCCS), Pune, India. Human monocytic THP-1 cells were obtained from the American Type Culture Collection (ATCC, TIB-202)
Authentication	Cell lines were obtained directly from authorized national and repository storage centers (ATCC/NCCS) and phenotypic validation was confirmed internally through continuous monitoring of baseline morphology and growth profiles
Mycoplasma contamination	All cell lines systematically tested negative for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No cell lines listed on the ICLAC register were utilized in this research.

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	Female C57BL/6 mice (aged 6–8 weeks) were sourced from the Central Animal Facility, Indian Institute of Science (IISc), Bengaluru, India
Wild animals	This study did not utilize wild animals
Reporting on sex	Tightly age-matched and weight-matched female mice were prioritized uniformly to optimize cluster consistency across structural mucosal delivery and lethal virion challenge models
Field-collected samples	No field-collected specimens were used.
Ethics oversight	All murine challenge and vaccination procedures were performed within the accredited Viral Biosafety Level-3 (BSL-3) facility with approval from the Institutional Animal Ethics Committee (IAEC/IISC/ST/748/2020). Protocols strictly adhered to experimental parameters defined by the Indian Council of Medical Research (ICMR), the Department of Biotechnology (DBT), and CPCSEA guidelines

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

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable

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	Spleens and lung tissues were collected on 8 days post-virus infection. Lungs underwent enzymatic digestion with collagenase type 1 and DNase I. Single-cell suspensions were subjected to red blood cell (RBC) lysis, washed, and checked for viability prior to flu peptidepool stimulation (10 microgram/mL) in the presence of Brefeldin A. Human PBMCs were thawed, rested, blocked with anti-CD40, and similarly pulsed
Instrument	Beckman Coulter Cytoflex and BD Symphony S6 flow cytometers
Software	FlowJo V10 and CytExpert version 2.3 software
Cell population abundance	Target populations (CD4+ and CD8+ T lymphocytes, and surface-activated cross-presenting BMDCs) were explicitly sorted or gated using initial FSC/SSC profiles to eliminate cellular debris, followed by single-cell and live/dead discrimination staining
Gating strategy	Preliminary boundaries identified total leukocyte populations using forward-scatter (FSC) vs. side-scatter (SSC) areas. Doublet exclusion was conducted via FSC-Area vs. FSC-Height tracking. Live cell subsets were isolated using fixable organic validation dyes. Specific cellular distributions were gated via surface canonical lineage profiles (CD3+ expression, followed by explicit CD4+ vs. CD8+ segregation). Frequencies of cytosine-producing subsets (IFN-gamma+, TNFalpha+, and IL-2+) were strictly evaluated using unstimulated media controls and empty polymer vehicles to benchmark baseline gating metrics. Similar gating strategies have been used for identifying mature BMDCs and proliferating T cells, the respective gating stragies have been mentioned in the supplementary figure S12

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