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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	Cell counts for lung samples were obtained on a Guava easyCyte (Luminex, Austin, TX), and cells were run on a CytoFLEX flow cytometer (Beckton Dickson) and analyzed with FlowJo v.10 (BD Biosciences).
Data analysis	All statistical analyses were conducted using Prism v.10 (GraphPad Software, San Diego, CA).

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 datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request and all 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	This study did not involve human research participants.
Reporting on race, ethnicity, or other socially relevant groupings	This study did not involve human research participants.
Population characteristics	This study did not involve human research participants.
Recruitment	This study did not involve human research participants.
Ethics oversight	This study did not involve human research participants.

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	Group sizes, listed for each study, were based off power calculations that allowed for differentiation of dosing and formulation effects in immunogenicity assays.
Data exclusions	Of note, flow cytometry data in Figure 4 excluded IL-2 (i.e., bifunctional instead of polyfunctional) due to poor data quality based on in-run standards. Samples with insufficient threshold events (<50,000) collected per well and low viability (<40% Zombie Green negative) were excluded from analysis.
Replication	Assays were run with technical replicates and internal controls to control for intra- and inter-assay variability. Key experimental groups were repeated across experiments to verify reproducibility of experimental findings.
Randomization	Mice and ferrets were randomized between groups within sex.
Blinding	Full blinding at study onset did not occur, but technicians were blinded as to the identity of individual samples during assays.

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	The following antibodies were used: Flow Cytometry (all verified mouse reactive by vendor): CD16/CD32 (Invitrogen #14-0161-82; 1:200), CD4 (APC-Cy7, BD Bioscience #552051; 1:250), CD8 (BV510, BD Biosciences #563068; 1:50), CD44 (PE-CF594, BD Biosciences #562464; 1:500), CD154 (BV605, BD Bioscience #745242; 1:50), CD69 (PE, BD Biosciences #561932; 1:33), CD103 (BV711, Biolegend #121435; 1:20), TNFα (BV421,

BioLegend #506328; 1:100), IL-2 (PE-Cy5, BioLegend #503824; 1:2000), IFNγ (PE-Cy7, BD Biosciences #557649; 1:400), IL-5 (APC, Biolegend #504306; 1:286), and IL-17A (AF700, BD Biosciences #560820; 1:100).

ELISA: anti-HA A/Vietnam/1203/04 influenza virus (VN04-8) antibody (Rockland Immunochemicals #200-301-976; 1:4 or 1:40 serially diluted 1:2), Anti-H7N9 Hemagglutinin/HA Antibody (Sino Biological #11082-MM04; 1:4 or 1:40 serially diluted 1:2), Anti-Mouse IgG (Fc specific)-Alkaline Phosphatase antibody (Sigma-Aldrich #A2429; 1:4000), anti-ferret HRP-conjugated antibody (Abcam #ab112770; 1:4000), and Anti-Mouse IgA (α-chain specific)-Alkaline Phosphatase antibody (Sigma-Aldrich #A4937; 1:1000).

Validation

Each commercial antibody was validated by the manufacturer for the intended use, followed by an in-house AAHI validation for the intended purposes. Antibody concentrations appropriate for each assay were determined using experimental titration curves of each individual antibody in each assay.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK-293 cells (American Type Culture Collection [ATCC], Manassas, VA; #CRL-3216) and Madin-Darby canine kidney (MDCK) cells were used in this study.
Authentication	HEK-293 and MDCK cell seed stocks were recently (within the last two years) obtained directly from ATCC (HEK-293) or BEI Resources (MDCK), expanded and cryopreserved at low passage (<p6), and used only at low passage number (<p20). Regular cell culture visualization was conducted to confirm cell morphology and size as expected. Additional authentication was not conducted.
Mycoplasma contamination	Cell lines were obtained mycoplasma-free directly from ATCC. Cultures were only used at low passage number to minimize risk of mycoplasma contamination. No further mycoplasma testing was routinely conducted.
Commonly misidentified lines (See ICLAC register)	The cell lines used in this study are not listed on the list of known misidentified cell lines

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	C57BL/6J mice sex balanced and between 6 and 8 weeks of age at study onset were obtained from The Jackson Laboratory (Harbor, ME) were used for mouse studies in this work. For housing, The Guide was followed as closely as possible: mice are on a 12 hour light cycle, with light hours being from 7am-7pm, rooms are kept between 68-73 degrees, and the humidity between 30-70%. Fitch ferrets (Mustela putorius fero) sex balanced and 3 months of age at purchase were purchased from Triple F Farms (Gillett, PA).
Wild animals	The study did not involve wild animals.
Reporting on sex	All animal studies contained equal numbers of male and female animals in all groups to account for sex-related variability in vaccine responses. Sex-based analyses were not performed because disaggregating by sex would reduce power to unacceptable levels, but there were not significant differences in response by sex.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Mouse work was done under the oversight of the Bloodworks Northwest Research Institute's Institutional Animal Care and Use Committee (IACUC; Seattle, WA). Ferret work was done under the oversight of the Colorado State University IACUC.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Briefly, spleens were individually homogenized by manual maceration through a 70 µm cell strainer. Red blood cells were lysed for 1 minute with ACK Lysing Buffer (ThermoFisher) and quenched with RPMI medium. Samples were filtered through a 2 mL Acroprep filter plate and resuspended in RPMI medium with 10% FBS, and 1-2 x 106 cells/well were seeded in 96-well round-bottom plates in preparation for peptide stimulation. Briefly, lung tissue was dissociated via enzymatic digestion using Hanks' Balanced Salt Solution supplemented with 10% Liberase (MilliporeSigma), 10% aminoguanidine, 0.1% KN-62, and 1.25% DNase. Lungs and enzymatic mix were added to a gentleMACS M tube (Miltenyi Biotec), run on a lung dissociation program in a gentleMACS Dissociator, incubated at 37°C and 5% CO2 for 30 minutes, then run again on the gentleMACS Dissociator. The resulting slurry was washed with RPMI and then filtered through a 70 µm MACS SmartStrainer (Miltenyi Biotec). Cells were counted using a Guava easyCyte cytometer before plating in 96-well round-bottom plates at 1 x 106 cells/well in RPMI medium with 10% FBS in preparation for peptide stimulation. H5 peptides (JPT Peptide Technologies #PM-INFA-HAIndo) used for stimulation comprised a pool of 140 peptides (15mers, 11 amino acid overlaps) derived from the full-length HA of influenza virus A/Indonesia/CDC835/2006(H5N1), which has a 96.64% sequence homology to the H5 HA from A/Vietnam/1203/2004 used in these studies. H7 peptides (custom ordered from GenScript) comprised a pool of 138 peptides (15mers, 11 amino acid overlaps) derived from the full-length HA sequence from A/Anhui/PA-1/2013. Stimulation mixes consisted of RPMI media, 10% FBS, 50 µM 2-mercaptoethanol, α-CD28 (BD), brefeldin A (BioLegend), and of three stimulation treatments: 0.26% dimethyl sulfoxide (DMSO) as a negative stimulation control, 0.2 µg/well (1 µg/mL) per peptide of H5 or H7 peptide pools in 0.26% DMSO, or phorbol myristate acetate-ionomycin positive stimulation solution. Spleen and lung lymphocytes were stained for viability with Zombie Green (BioLegend #423112), then Fc receptors were blocked with CD16/CD32 antibody (Invitrogen #14-0161-82). Cells were surface stained for mouse CD4 (APC-Cy7, BD Bioscience #552051), CD8 (BV510, BD Biosciences #563068), CD44 (PE-CF594, BD Biosciences #562464), and CD154 (BV605, BD Bioscience #745242). Lung cells were additionally stained for mouse CD69 (PE, BD Biosciences #561932) and CD103 (BV711, Biolegend #121435). After extracellular staining, cells were fixed and permeabilized using BD Cytofix/Cytoperm (BD Biosciences #554714) and stained for intracellular cytokines with mouse TNFα (BV421, BioLegend #506328), IL-2 (PE-Cy5, BioLegend #503824), IFNγ (PE-Cy7, BD Biosciences #557649), IL-5 (APC, Biolegend # 504306), and IL-17A (AF700, BD Biosciences #560820).
Instrument	Cells were run on a CytoFLEX flow cytometer (Beckton Dickson).
Software	FlowJo v.10 (BD Biosciences)
Cell population abundance	Cells were not sorted, purity is not applicable.
Gating strategy	Populations were gated using the following strategies after first gating on lymphocytes (FSC-A by SSC-A), singlets (FSC-A by FSC-H or -W), live (viability dye negative): - Spleen Polyfunctional CD4+ T cells: CD44+ CD4+ CD8a- IL-5- IL-17A- IFNγ+ TNFα+ IL-2+ - Spleen Polyfunctional CD8+ T cells: CD44+ CD4- CD8a+ IL-5- IL-17A- IFNγ+ TNFα+ IL-2+ - Spleen IFNγ+ CD8+ T cells: CD44+ CD4- CD8a+ IL-5- IL-17A- IFNγ+ - Lung Polyfunctional CD4+ T cells: CD44+ CD69+ CD4+ CD8a- IL-5- IL-17A- IFNγ+ TNFα+ IL-2+ - Lung Polyfunctional CD8+ T cells: CD44+ CD69+ CD103+ CD4- CD8a+ IL-5- IL-17A- IFNγ+ TNFα+ IL-2+ - Lung IFNγ CD8+ T cells: CD44+ CD69+ CD103+ CD4- CD8a+ IL-5- IL-17A- IFNγ+

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