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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	flow cytometry data was done by Agilent NovoCyte Quanteon analyzer ELISA data was done by Varioskan LUX Multimode Microplate Reader (ThemoFisher) RT-qPCR data was done by The LightCycler® 480 Instrument II (Roche)
Data analysis	Statistical analysis was done GraphPad Prism version 10. Flow cytometry data was analyzed by FlowJo_v10

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

All data associated with this study are presented within the paper or in the Supplementary materials. For the genetic stability test of DelNS1-cH5N1 and DelNS1-

mH5N1 viruses, whole genome sequences were summited to GenBank (GenBank accession numbers: DelNS1-mH5N1-p1: PV124973-PV124980, DelNS1-mH5N1-p10: PV124993-PV125000). Raw data underling the results are provided with this paper. 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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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	All experiments were repeated at least three times, resulting in a n number of 3 or more. A n number of 3 is considered the standard for biological experiments. No sample size calculation was performed; the sample size was chosen based on the standards of the corresponding field(PMID:37045873).
Data exclusions	No data was excluded.
Replication	Most experiments were repeated once, and similar findings were obtained across all repeats.
Randomization	For all experiments, randomization was applied to the grouping of the animals. Animals were randomly allocated to each group.
Blinding	No blinding was done. Blinding was not relevant to the study because the results are quantitative and objective, and does not require a subjective judgment.

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

Zombie Aqua™ Fixable Viability Kit (Biolegend #423102, Lot -B432360), 1:200 for flow cytometry staining.
Rat Anti-mouse CD45-PE-Cy5 (Biolegend #103110, Clone-30-F11, Lot-B392814), 1:100 for IV labelling.
Rat Anti-mouse CD8a-AF700 (Biolegend #100730, Clone-53-6.7, Lot-B433204), 1: 100 for flow cytometry staining.
Rat Anti-mouse CD4-FITC (Biolegend #100406, clone-GK1.5, Lot-B374032), 1: 100 for flow cytometry staining.
Armenian Hamster Anti-Mouse CD69-BV711(Biolegend #104537, Clone-H1.2F3, Lot-B406939), 1:100 for flow cytometry staining.
Armenian Hamster Anti-Mouse CD103-BV421(Biolegend #121422, Clone-2E7, Lot-B425334), 1:100 for flow cytometry staining.
Rat Anti-mouse IFNg-APC(Biolegend #505810, Clone-XMG1.2, Lot-B335090), 1: 100 for flow cytometry staining.
H-2Kd Influenza NP Tetramer-TYQRTRALV-APC(MBL International-TB-M534-2, Lot-T2405016), 1: 100 for flow cytometry staining.
Rat Anti-mouse CD8a-BV785(Biolegend #100750, Clone-53-6.7, Lot-B420449), 1: 100 for flow cytometry staining.
Goat anti-Mouse IgG (H+L) Secondary Antibody-HRP(Invitrogen, #31430, Lot-2E394436), 1: 10000 for ELISA
Goat anti-Mouse IgA Cross-Adsorbed Secondary Antibody-HRP(Invitrogen, #62-6720, Lot-UK290800), 1: 3000 for ELISA
Influenza A virus Nucleoprotein antibody (GeneTex, Cat. No. GTX125989, Lot-44902), 1:2000 for IHC
Goat anti-Hamster IgG (H+L) Cross-Adsorbed Secondary Antibody, HRP(Invitrogen, #HA6007, Lot-XH356395), 1: 5000 for ELISA

Validation

The commercial antibodies were validated by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	MDCK (CCL34), A549 (CCL-185), and 293T (CRL-3216) was obtained from ATCC.
Authentication	ATCC cell lines were authenticated by ATCC.
Mycoplasma contamination	All cell lines were tested negative by PCR for mycoplasma.
Commonly misidentified lines (See ICLAC register)	Nil

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	6-8 weeks old BALB/c mice and 6-8 weeks old syrian hamsters were used in this study.
Wild animals	Nil
Reporting on sex	BALB/c mice: female; syrian hamsters: male
Field-collected samples	Nil
Ethics oversight	All animal studies were approved by the Committee on the Use of live animals in Teaching and Research at HKU.

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	After intravascular labeling by using an anti-CD45-PE-Cy5 (Biolegend-103110) at the dose of 2 µg per mouse, lungs and spleens of mice were collected. Splenocytes were isolated and homogenized through cell strainers (BD) and resuspended in RPMI medium (10% FBS and P/S). Lung tissue was chopped and digested in RPMI solution with Collagenase D (1 mg/ml) (Roche) for 1 h at 37°C. Red blood cells were lysed by addition of Lysing solution (BD). After washing with RPMI, cells were counted and resuspended in RPMI.
Instrument	flow cytometry data was done by Agilent NovoCyte Quanteon analyzer
Software	FlowJo_v10
Cell population abundance	IFN-γ+ CD69+CD103+ abundance in CD8 T cells is around 2%.
Gating strategy	For ICS, flow cytometry performed with gating with FSC-A vs SSC-A to exclude debris, then FSC-H vs FSC-A to select single cells, and then gating with Zombie vs FSC-A for live cells, IV-PE-Cy5 vs FSC-A for extravascular cells. CD4-FITC vs CD8-BV785 for T cell subsets. CD69 and CD103 for tissue resident markers, IFN-γ-APC vs CD4-FITC or CD8-BV785 for IFN-γ producing CD4 or CD8 T cell subsets. For NP147 tetramer stainning, FSC-A vs SSC-A to exclude debris, then FSC-H vs FSC-A to select single cells, and then gating with Zombie vs FSC-A for live cells, CD45 IV-PE-Cy5 vs FSC-A for extravascular cells, CD8-BV785 vs FSC-A for CD8 T cell subset, then CD8-BV785 vs tetramer-APC for the NP147 Tetramer + CD8 T cell subset.

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