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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	NovoExpress v1.5.6, Nis-Elements v4.6
Data analysis	GraphPad Prism v10.4.1, FlowJo v10.10.0, ImageJ v1.54p, Fiji v2.16.0/1.54p, Custal Omega V1.2.2

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 source data relevant for this study is available within the manuscript and has been provided as a source data file and supplementary information.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	No statistical method were used to determine sample size. Sample size was determined base on previous studies in the field using similar methods (https://doi.org/10.1038/s41564-024-01771-1)
Data exclusions	No data was excluded form the analysis.
Replication	All experiments presented in this study were performed at least three independent times and results were consistent and reproducible.
Randomization	Allocation of samples was randomized.
Blinding	Researchers were not blind to group allocations.

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

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	-Alexa 488-conjugated anti-human: ThermoFisher, catalog number: A-11013 -APC-conjugated anti-mice: ThermoFisher, catalog number: 17-4015-82 -HLA-DR monoclonal antibody clone LN3: ThermoFisher, catalog number: 14-9956-82, lot 2781646 -SLA class II DR antibody clone 2E9/13: BioRad, catalog number: MCA2314GA, lot 159559 -anti-Multi Hemagglutinin (H3N2), eEnzyme, catalog number: IA-PAN4-0100, lot 0519YQ
Validation	All antibodies used in this study are commercial and validated by the seller. Additionally, anti-MHCII antibodies (anti-HLA-DR and anti-

Validation	SLA-DR) were evaluated on HEK-293T cells transfected with empty plasmids or the corresponding target. The anti-HA antibody was validated on mock or HEK-293T and A549 infected cells.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Madin-Darby canine kidney (MDCK) were a kind gift from Dr. Robert Webster (St. Jude Children's Research Hospital). Human lung carcinoma (A549, cat# CCL-185) and HEK-293T (cat# CRL-1573) cells were purchased from the American Type Culture Collection (ATCC).
Authentication	Short tandem repeat profiling by Idexx BioResearch is done periodically on commercial cells.
Mycoplasma contamination	Cells tested negative for mycoplasma by PCR
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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	3-weeks-old cross-bred pigs were obtained from Midwest Research Swine Inc (Glencoe, MN, USA) and housed in animal biosafety level 2 (ABSL2) facilities at the University of Georgia.
Wild animals	No wild animals were used in this study.
Reporting on sex	Sex was not a variable considered in this study, but both sexes were included.
Field-collected samples	No field-collected samples were used in this study.
Ethics oversight	All animal studies were reviewed and approved by the Institutional Care and Use Committee (IACUC) at the University of Georgia (protocol A2019 03-031-Y3-A9)

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	24 hours after infection, supernatants from A549- and HEK-293T-infected cells were collected, and cells were trypsinized and centrifuged at 1,500 rpm for 5 minutes. Cells were resuspended in PBS supplemented with 5% FBS and stained with the LIVE/DEAD Fixable Violet Dead Cell Stain Kit (ThermoFisher Scientific, Waltham, MA) according to the manufacturer's instructions. Cells were then fixed with 4% paraformaldehyde for 15 minutes at room temperature, followed by a 15-minute permeabilization with 0.3% Triton-X (Sigma-Aldrich, St Louis, MO). Afterwards, cells were washed three times and incubated for 1 hour with the following primary antibodies: Alexa 647-conjugated anti-SLA-DR clone 2E9/13 (Bio-Rad, Hercules, CA), anti-HLA-DR clone LN3 (ThermoFisher Scientific, Waltham, MA) at a 1:200 dilution in PBS and an anti-H3 HA antibody (stem specific antibody) clone CR8020 at a 1:500 dilution. Following incubation, cells were washed three times with PBS. For simultaneous detection of FLUAV and MHCII, samples were incubated for 1 hour with an Alexa 488-conjugated anti-human secondary antibody and an APC-conjugated anti-mice secondary antibody respectively (ThermoFisher Scientific, Waltham, MA), both in a 1:1,000 dilution. Finally, cells were washed three times with PBS.
Instrument	NovoCyte Quanteon.
Software	FlowJo v10.10.0.
Cell population abundance	100,000 events were collected for each experiment.
Gating strategy	Initially cells were selected based on SSC/FSC and single cells were gated on FSC-A/FSC-H plots. From the single cell population, live cells were selected as LIVE/DEAD negative cells. Selection of MHCII and HA positive cells was selected based

on control cells. These controls are described within the manuscript and comprised non-transfected, and transfected cells with an empty pCAGGS plasmid (MHC negative). FLUAV positive cells were determined based on HA expression among both MHCII positive and negative cells. This was determined using mock-infected and cells infected with the mentioned viruses in presence of sialic acid.

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