nature portfolio

Corresponding author(s): Yoshihiro Kawaoka

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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	No software was used for data collection.
Data analysis	GraphPad Prism software, version 9.5.1 IRMA v1.0.2 snakemake workflow v1.0.1 iVar v 1.4.2 Samtools v1.19.2 bwa v0.7.17-r1188

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 underlying animal and receptor binding experiments described herein are available in the online version of the 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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	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	Animal study sample size was based on previously published work and limited by space and cost. No statistical methods were used to predetermine sample sizes. In general, group sizes were large enough (n=4-8 animals per group) to enable statistical testing if desired. Small numbers of biological replicates (n=1-3) were used in receptor binding assays and minireplicon assays; however, three independent experiments were performed, and the results were reproducible across the experiments. Replication in human epithelial cells had small numbers of biological replicates (n=2-3).
Data exclusions	No data was excluded from the study.
Replication	All experiments with animals were performed once. Growth curve analyses in primary human epithelial cells were performed one time with 2-3 replicates per condition. Three independent minireplicon experiments were performed (two with three biological replicates and one with two biological replicates) and the data from all three were combined for analysis and presentation. Three independent receptor binding experiments were performed, each with a single biological replicate for all conditions, and all three experiments are presented. In vitro antiviral susceptibility experiments were performed once. All attempts at replication were successful. All experimental data are included in the figures and/or underlying data files.
Randomization	Primary cell cultures were allocated into infection groups at random for growth curve analyses. Allocation of animals was completed at random. Randomization was not employed for minireplicon assays, receptor binding assays, or in vitro antiviral assays since locations of different conditions need to be known to interpret the data. In addition, experiments other than the minireplicon assays were performed under select agent regulations which require separation of agents and clear identification.
Blinding	Blinding was not possible as the experiments were performed under select agent regulations which require separation of agents and clear identification.

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	Broadly Neutralizing Antibodies Against Influenza A And B Viruses, Fully Human, CR9114, HumImmu (catalog no. A90001; 1:1000 dilution); Goat Anti-Human IgG H&L, HRP conjugated, Abcam (catalog no. ab6858; 1:4000).
Validation	CR9114 was characterized by Dreyfus C, et al. (2012). Highly conserved protective epitopes on influenza B viruses.Science. 2012 Sep 14;337(6100):1343-8. Abcam validated ab6858 for ICC/IF, Dot blot, ELISA, IHC-P, IHC-Fr, Immunomicroscopy, WB. Additional validation can be found here: https://www.citeab.com/antibodies/2361176-ab6858-goat-anti-human-igg-h-l-hrp.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Madin-Darby canine kidney (MDCK) cells, human embryonic kidney (293T) cells and chicken fibroblast (DF-1) cells, were originally sourced from the ATCC. Mature primary human epithelial cells were sourced from MatTek.
Authentication	None of the cell lines were authenticated.
Mycoplasma contamination	MDCK, 293T and DF-1 cell lines tested negative for mycoplasma contamination. Human epithelial cells were not tested.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the 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	Mus musculus (Mouse), Balb/cJ, 7-week old females. Mustela furo (Ferret), 6- to 8-month old females 10-day-old embryonated chicken eggs
Wild animals	The study did not involve wild animals.
Reporting on sex	No sex-based analysis was performed as studies were limited due to cost.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	Institutional Care and Use Committees of the University of Wisconsin (UW)-Madison School of Veterinary Medicine.

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