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Reporting Summary

Nature Research 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 Research 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	Mass spectra recorded on a Q Exactive Orbitrap mass spectrometer and Q Exactive Orbitrap mass spectrometer with Ultra High Mass Range (Thermo Fisher Scientific) were pre-processed using Xcalibur version 4.1. Mass spectra recored on Synapt G2S quadrupole-ion mobility separation-time of flight mass spectrometer (Waters Corporation) were initially processed using MassLynx (version 4.1). Immunofluorescence images taken on: ImageJ version 1.52p Flow cytometry performed on: BD FACSDivaTM software Version 8.0.1
Data analysis	Graphpad Prism 8, FlowJo LLC. Version 9.9.6, SWARM (https://github.com/pkitov/CUPRA-SWARM), GlycoWorkbench software (https://code.google.com/archive/p/glycoworkbench/)

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The authors declare that all data supporting the findings of this study are available within the paper and its supplementary information files. Please contact the corresponding authors, Dr. John S. Klassen (john.klassen@ualberta.ca) or Dr. Matthew S. Macauley (macauley@ualberta.ca) for questions regarding the raw data. Further information will be made available upon reasonable request.

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	For pseudoviral infection studies, the sample size is n=3 biologically independent replicates. These were examined over n=3 independent experiments. No sample size calculation was preformed, a sample size of 3 was chosen to make sure that there was no variance between biological replicates or samples. This was deemed sufficient as there was no significant variation between the replicates. For the authentic virus studies, two biological replicates each containing two technical replicates, at two different MOIs were performed. This was deemed sufficient as there was no significant variation between the replicates.
Data exclusions	No data was excluded from this study.
Replication	Three analytical replicates were done for all pseudoviral infection studies on 3 independent occasions. All attempts at replications were successful. Two biological replicates each containing two technical replicates, at two different MOIs were performed for authentic virus studies.
Randomization	Randomization was not applicable for this study as known immortalized cell lines and tissues were tested. To account for the lack of randomization, all experiments were performed with several controls.
Blinding	Blinding was not applicable to this study as negative controls were always run in parallel.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	For flow cytometry analysis we used: Elderberry Bark Lectin (FITC, Vector Laboratories, cat. no. FL-1301), Peanut Agglutinin (FITC, Vector Laboratories, cat. no. FL-1071), Cholera Toxin B subunit (FITC, Sigma Aldrich, cat. no. C1655), Human/Mouse/Rat/Hamster ACE-2 Antibody (R&D Systems, cat. no. AF933), Donkey anti-goat IgG (H+L) (AF647, ThermoFisher, cat. no. A-21447) For immunofluorescence staining we used: Biotinylated Sambucus Nigra Lectin (Vector Laboratories, cat. no. B1305), Biotinylated Erythrina Cristagalli Lectin (Vector Laboratories, cat. no. B1145), Streptavidin Alexa Fluor 555 conjugate (AF555, ThermoFisher, cat. no. S21381), Anti-ACE3 (Abcam, cat. no. 15348), Goat anti-Rabbit IgG (H+L) (AF488), Invitrogen, A-11008), GmbH StrepMAB (Chromeo 546, IBA Lifesciences. cat. no. 2-1550-050), IgG (H+L) Cross-Adsorbed Goat anti-Mouse (AF555, ThermoFisher, cat. no. A21422)
Validation	All of the antibodies were commercially purchased from the following companies according to the links provided :1) https://vectorlabs.com/, 2) https://www.sigmaaldrich.com/canada-english.html, 3) https://www.rndsystems.com/, 4) https://www.thermofisher.com/ca/en/home.html 4) https://www.abcam.com/ 5) https://www.iba-lifesciences.com/home.html Each of the antibody can be searched by their catalogue numbers on their respective website. In general, all of the antibodies were quality control tested by immunofluorescence staining with flow cytometric analysis by the companies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293, HEK293T, Vero-E6
Authentication	All the parental cell lines were purchased from ATCC, cell lines were not authenticated within the lab.
Mycoplasma contamination	No Mycoplasma contamination detected (ABM Mycoplasma detection kit, Canada).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Ferrets were obtained from the Department of Veterinary Pathobiology, Utrecht University, Utrecht,The All ferrets were femail and between 20 and 24 months old.
Wild animals	The study did not involve wild animals.
Field-collected samples	Study did not involve samples collected from the field.
Ethics oversight	Animals were handled in an ABSL3 biocontainment laboratory. Research was conducted in compliance with the Dutch legislation for the protection of animals used for scientific purposes (2014, implementing EU Directive 2010/63) and other relevant regulations. The licensed establishment where this research was conducted (Erasmus MC) has an approved OLAW Assurance # A5051-01. Research was conducted under a project license from the Dutch competent authority and the study protocol (#17–4312) was approved by the institutional Animal Welfare Body. Animals were housed in groups of 2 animals in Class III isolators allowing social interactions, under controlled conditions of humidity, temperature and light (12-hour light/12-hour dark cycles). Food and water were available ad libitum. Animals were cared for and monitored (pre- and post-infection) by qualified personnel. The animals were sedated/ anesthetized for all invasive procedures

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Primary human nasal epithelial cells were obtained from a commercial supplier (Promo Cell) and cultured according to the manufacturer's recommendations.
Recruitment	Obtained from commercial supplier (Promo Cell).
Ethics oversight	Primary human nasal epithelial cells were cultured in a BSL3 certified by Alberta Health Services.

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	For adherent cell lines studied, cells were removed from the plate using 1 mM EDTA/PBS and centrifuged at 300 xG for 5 minutes before staining. All flow samples were run in flow buffer (HBSS containing Calcium and Magnesium, 0.1% BSA) or FACS buffer (HBSS containing 1% FBS and 500 µM EDTA).
Instrument	BD LSRFortessa TM X-20
Software	BD FACSDivaTM software V8.0.1

Cell population abundance	10,000 cells were collected for staining and infection studies.
Gating strategy	The cells in all experiments are initially gated through SSC-A/FSC-A channels looking at live cells, then are further gated through SSC-W/FSC-A channels looking at singlet populations. For the genetic cell line determination, antibody binding, and protein binding, FITC, AF647, and PE-Cy5 fluorescence were directly investigated. In all infection studies, the % of GFP+ cells were determined. The gating strategy for this is demonstrated in Figure 4c.

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