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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 Authors & Referees 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<br>*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<br>*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 | Raw luciferase data was exported to Microsoft Excel (v. 16.33). |
| Data analysis | Luciferase data was analyzed in Microsoft Excel (v. 16.33). Graphs of luciferase data were generated in Prism 8. Protein structures were rendered in PyMol (v.2.3.2) using existing, published structural data. |

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/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

Accession numbers for all viral sequences used in this study are provided in extended data figure 1b. Accession numbers for human ACE2, APN, and DPP4 are provided in the methods section, under "plasmids." Unprocessed westernblot images and graphed values are provided as source data. All reagents are freely available upon 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 | Sample-size calculations were not performed. For each pseudotype in our study, we infected cells in triplicate to demonstrate magnitude and consistency of measurable differences. |
| Data exclusions | We did not exclude any data for the experiments presented. |
| Replication | The figures in our manuscript are "final" experiments after many months of preliminary data collection. All viral pseudotypes in our study were generated and tested multiple times before we produced large batches of pseudotypes for the "final" experiments. These results were extremely consistent between different batches of pseudotypes produced at different times and in cells of different passage numbers. |
| Randomization | No specific steps were taken to randomize experimental groups. This is because the generally binary outcome of the assay performed left results to little interpretation. |
| Blinding | All pseudotypes were numbered 1-29 for ease during our experiments. No further steps were taken to blind the investigator, because the data was generally binary - there either was or was not cell entry, leaving little room for investigator bias during data collection. |

# 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 |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Human research participants |
| ☒ | ☐ Clinical data |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | FLAG antibody for westernblot - Sigma - A8592<br>GAPDH antibody for westernblot - Sigma - G8795<br>anti-VSV M - Kerafast Inc., Boston, MA - 23H12 |
| Validation | These FLAG and GAPDH antibodies are well established, commercially available, have been validated by the company and have been used by our group and many others (for example PMID: 30572566, 28031368, 26628364).<br><br>Anti-VSV-m has been used extensively in immunofluorescence labeling and western blot analysis by Andrea Marzi's lab and others (for example PMID: 30038228, 26091335, 12134006). |

## Eukaryotic cell lines

Policy information about cell lines

| | |
|---|---|
| Cell line source(s) | 1. A549 - human lung epithelial - ATCC CCL-185<br>2. Artibeus jamaicensis - primary kidney - obtained from Anthony Schountz, Colorado State University (PMID: 26899616)<br>3. Artibeus jamaicensis - immortalized cells - AJ-primary cells above were immortalized with SV40 T-antigen as described in our maniscript<br>4. BHK - hamster kidney - ATCC CCL-10<br>5. Caco-2 - human colon epithelial cells - ATCC HTB-37 |

6. Huh-7.5 - human liver - obtained from Heinz Feldmann laboratory at Rocky Mountain Labs, Hamilton, MT, 59840
7. HypNi - Hypsignathus monstrosus kidney - obtained from Marcel Müller at Institute of Virology
Charite - Universitätsmedizin Berlin (PMID: 25100832)
8. PK-15 - porcine kidney cells - ATCC CCL-33
9. RaKSM-2.5 - Rousettus aegyptiacus kidney - generated in our lab and previously published (PMID: 31682727)
10. RhiLu - Rhinolophus alcyone lung - obtained from Marcel Müller at Institute of Virology
Charite - Universitätsmedizin Berlin (PMID: 23232719; https://web.expasy.org/cellosaurus/CVCL_RX22)
11. RhiNi - Rhinolophus landeri kidney - obtained from Marcel Müller at Institute of Virology
Charite - Universitätsmedizin Berlin (https://web.expasy.org/cellosaurus/CVCL_RX64)
12. Vero - african green monkey kidney - obtained from Heinz Feldmann laboratory at Rocky Mountain Labs, Hamilton, MT, 59840

Authentication

Cell species was confirmed by Cytochrome B sequencing

Mycoplasma contamination

All cell lines tested negative for mycoplasma by standard PCR prior to experiments

Commonly misidentified lines
(See ICLAC register)

*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*