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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	Sequences for isolates were retrieved manually from NCBI. Flow cytometry data were collected with Attune NXT software.
Data analysis	Alignments were performed using Protein Blast with default settings (NCBI). Graphs and statistics were generated using GraphPad Prism 9 for mac. Flow cytometry data were analyzed using FlowJo v10.8. Calculations were performed using Excel 365. WB membranes were processed using Image Studio Lite v5.2.5. Sequences were aligned using MAFFT (v.7.467) and the alignment was checked for accuracy using BioEdit v7.2.5. The ModelFinder application, as implemented in IQ-TREE v2.0.6 (PMID: 25371430), was used as described in the methods. The phylogenies were visualized using the auspice module from Nextstrain61. Representations of HKU1 spike alignments and of TMPRSS2 were generated using the seqvisr v0.2.7 package (Github: https://doi.org/10.5281/zenodo.6583981) in R, v4.2.2.

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

Data generated or analyzed during this study are available in the published article or on the supplementary information files and methods. Raw sequence data (human reads removed) for the HKU1 virus were deposited in the European Nucleotide Archive (ENA) portal (https://www.ebi.ac.uk/ena/) under bioproject accession number PRJEB64017. The raw data of the main figures are available within the supplementary information. The raw data of the extended data are available from the corresponding author upon reasonable request.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	The sample size is indicated in the figure legends and method section. The sample size was determined to ensure enough replicates for statistical analysis.
Data exclusions	No data were excluded.
Replication	Experiments were replicated as indicated in the legends, in order to allow statistical analysis of the results.
Randomization	Not applicable.
Blinding	Biological analysis did not require any subjective analysis, so no blinding was necessary, all samples were processed and analyzed the same way.

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

Rabbit anti-TMPRSS2 (Atlas HPA035787), rabbit anti-human actin (Abcam, 60008-1-Ig), rabbit anti-HKU1 S1 polyclonal antibody (Thermofisher Scientific, PA5-120768), rabbit anti-HKU1 S2 polyclonal antibody (Thermofischer Scientific, PA5-120769)
Goat anti-Rabbit antibody (Thermo Fisher Scientific, A-21245), Goat anti-human antibody AF647 (Thermo Fisher Scientific, A-21445) Coupled anti-His antibody (R&D Systems, IC0501R), mab10 (Planchais, C. et al., J. Exp. Med. 2022), anti-TMPRSS2-VHH-nanobodies (home-made, described in this study), mouse anti-c-myc antibody, clone 9E10 (Thermo - M4439), goat anti-Mouse antibody (Thermo Fisher Scientific, A-21242, 1:500), CD13-PE (130-120-312, Miltenyi Biotec, 1:50) and CD26-PE (130-126-41, Miltenyi Biotec, 1:50).

Validation

All antibodies used in this study are commercially available, except the anti-TMPRSS2 VHH and anti-coronavirus mab10, and have been validated by the manufacturers and used by other publications. Rabbit anti-TMPRSS2 (Atlas HPA035787) was validated for WB and IHC by the manufacters by transfecting HEK-293T with TMPRSS2, rabbit anti-human actin (Abcam, 60008-1-Ig) was validated by manufacturer in WB in multi-cells/tissue, rabbit anti-HKU1 S1 polyclonal antibody (Thermofisher Scientific, PA5-120768) and rabbit anti-HKU1 S2 polyclonal antibody (Thermofischer Scientific, PA5-120769) were raised using antigen sequence deriving from isolate N1 of coronavirus Spike (YP_173238.1), Swiss-Prot: Q5MQD0 and validated using a recombinant protein comprising S1+ S2 from N5. In our hands, they recognized soluble spike and cell lysates expressing spike from isolate HKU1A N1 and not from isolate HKU1B N5. The anti-TMPRSS2 VHH were validated as described in this article. CD13-PE (130-120-312, Miltenyi Biotec, 1:50) and CD26-PE (130-126-41, Miltenyi Biotec, 1:50) were validated by the manufacturer for FC by using respectively Human peripheral blood cells after erythrocyte lysis or Splenocytes of C57BL/6 cells. Mouse anti-c-myc antibody, clone 9E10 (Thermo - M4439) was validated by the manufacturer for FC, by using HEL 11.4 induced IPS cells.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Vero E6, HEK293T (293T) and Caco2/TC7 were purchased from from ATCC

Authentication

Human cell lines were authenticated by Eurofins using the Applied Biosystems Cell Line Authentication (CLA) IdentiFiler Plus and Direct kits, and CLA GlobalFiler PCR Amplification Kit. VeroE6 were authenticated as African Green Monkey cells by Eurofins.

Mycoplasma contamination

Mycoplasma detection was performed every other week, cells were negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

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

One young adult (6 months old) male alpaca (lama pacos) was used to generate anti-TMPRSS2 VHHs.

Wild animals

Not applicable.

Reporting on sex

Not applicabble.

Field-collected samples

No field samples.

Ethics oversight

Animal procedures were performed according to the French legislation and in compliance with the European Communities Council Directives (2010/63/UE, French Law 2013-118, February 6, 2013). The Animal Experimentation Ethics Committee of Pasteur Institute (CETEA 89) approved this study (2020-27412).

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	Sample preparation was described in the methods. Cells were detached using PBS/EDTA, stained and fixed for surface staining, fixed, permeabilized and stained for intracellular staining.
Instrument	Attune NxT Acoustic Focusing Cytometer, blue/red/violet/yellow (catalog number : 15360667)
Software	AttuneNxT Software v3.2.1 for acquisition, FlowJo v10.8 for analysis
Cell population abundance	At least 10,000 cells were acquired for each condition.
Gating strategy	Live cells were gated using the FSC-A/SSC-A, single cells were then selected using the FSC-A/FSC-H. Gates for positive cells were then set using control cells (no spike or TMPRSS2 expression). Gating Strategy is described in the Supplementary Information S4.

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