nature portfolio

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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	Viral titers (focus forming units [FFU] per mL) were obtained by serial dilution on VeroE6-TMPRSS2 cells in 96 well plates and counting of GFP-expressing cells using a live cell microscope following 24 hours (Incucyte SX5, Sartorius). Surface SARS-CoV-2 spike levels were analysed using a LSRFortessa flow cytometer (BD Biosciences). Fluorescence and luminiscence was measured in a VictorX multi-plate reader (Perking Elmer, Waltham, MA, USA). Fluorescence imaging was performed with a Zeiss Axiovert 5 fluorescence microscope both with transmitted light and GFP channels with a 10X objective. Detection of immunoreactive proteins was carried out using the enhanced chemiluminescence reaction (Amersham ECL Prime, Cytiva) and detected by the Image Quant LAS 4000 Mini (GE Healthcare).
Data analysis	Flow cytometry analysis was carried out using BDFACS Diva 8.0.2 and FlowJo X softwares. Image analysis was performed using Fiji. Statistics and graph representations were performed using GraphPad Prism (v9). Figures were built with Inkscape 1.3.2. Alignments of coronavirus Spike sequences were performed using T-Coffee. Aligned sequences were then exported and viewed in Jalview.

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 data supporting the findings of this study are available within the paper and its Supplementary Information, including uncropped images of western blots and an example of gating strategy for flow cytometry experiments.

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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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 previous sample-size calculation was needed as all experiments were done with in vitro cell cultures or computational approaches.
Data exclusions	No data was excluded from the analysis.
Replication	All measures were performed in at least three technical replicates and all experiments were replicated independently at least three times (biological replicates, n of at least 3).
Randomization	No randomization was needed as all experiments were done with in vitro cell cultures or computational approaches.
Blinding	No blinding was needed as all experiments were done with in vitro cell cultures or computational approaches.

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

A monoclonal antibody targeting VSV-G (a kind gift of Rafael Sanjuan, University of Valencia, Spain)
anti-SARS-CoV-2 RBD antibody (Invitrogen, PA5-114529)
Goat anti-Rabbit IgG (H+L) AlexaFluor 568 (ThermoFisher, A11011)
SARS-CoV-2 Spike S1 (Invitrogen PA5-81795)
rabbit anti c-Myc (Sigma PLA0001)
mouse anti-GAPDH (Santa Cruz sc-47724)
anti-rabbit or sheep anti-mouse IgG horseradish peroxidase conjugate (Sigma DC02L or GE Healthcare NXA931, respectively)

Validation

All antibodies were obtained from commercial sources except for anti-VSV-G. The use of this antibody is validated here: PMID 38918479. The use of anti-RBD is validated in PMID 35884842. The use of anti-S1 is validated on PMID 34352039. The use of anti-myc was validated on PMID 33247105.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Human embryonic kidney (HEK293T) were from American Type Cell Collection, (Manassas, VA, USA).
VeroE6-TMPRSS2 were obtained from the JCRB Cell Bank (catalog JCRB1819).

Authentication

None of the cell lines were authenticated as there are no KO cell lines used.

Mycoplasma contamination

Cell lines were tested negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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	Two days post-transfection, cells were washed once with PBS and detached by pipetting in 500 µl of FACS buffer (0.1% sodium azide, 0.5% BSA in PBS). Then, 200 µl of cells were collected by centrifugation at 1500 rpm for 5 min at 4ºC and stained with anti-SARS-CoV-2 RBD antibody (Invitrogen, PA5-114529). After centrifugation, cells were incubated with Goat anti-Rabbit IgG (H+L) AlexaFluor 568 (ThermoFisher, A11011). Antibodies were diluted to 1:1000 in FACS buffer and 100 µl per sample were used for resuspending cells, following a 30 min incubation on ice in both cases. After three final washes with FACS buffer, surface SARS-CoV-2 spike levels were analysed.
Instrument	LSRFortessa flow cytometer (BD Biosciences).
Software	Analysis was carried out using BDFACS Diva 8.0.2 and FlowJo X softwares.
Cell population abundance	Typically, 10,000 live cells were selected based on morphology and acquired.
Gating strategy	10.000 cells with values over 50 in the FSC-A and 100 in the SSC-A axis were gated as live cells and selected for next gating steps. Then single cells were selected from the FSC-H vs. FSC-A plot and GFP+ positive cells were gated from the FSC-A vs FITC plot with FITC values >1000. Finally, Spike positive cells were selected from the FSC-A vs PE-A plots with PE values >1000.

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