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	All mass spectrometry data collection was performed using Xcalibur 4.1 (Thermo Scientific)
Data analysis	Peptide identifications were performed using Byonic 3.6.0 (Protein Metrics Inc.) Peptide deuterium uptake was calculated using HDExaminer 3.2 (Sierra Analytics). Bimodal peptide analysis and plotting were performed in Jupyter notebooks using Python.

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

Figures using protein structures were generated using either PDB accession no. 6M0J or a full-length structural model of the spike protein created by the Amaro lab and accessible at amarolab.ucsd.edu/covid19.php (PSF-PDB_spike_open_prot_glyc_memb_wat_ions_amarolab.tar.gz). Centroid values for continuous labeling hydrogen exchange experiments are included as supplementary csv files. Select peptide spectra used for bimodal analysis are available in raw and extracted form as supplementary csv files. Raw mass spectrometry data is 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	Continuous labeling hydrogen exchange experiments were performed in technical triplicate. Pulsed labeling time courses were single replicates except where noted in table 1.
Data exclusions	During manual curation of data in HDExaminer, low quality peptides (peptides with low signal intensity or peptides that have inconsistent monoisotopic masses) are removed. To be included in the manuscript analysis of continuous labeling experiments, peptides must have triplicate data for each time point, and the deuterium uptake at each time point must have a standard deviation below 1 deuteron.
Replication	Continuous labeling hydrogen exchange experiments were performed in technical triplicate with standard deviations reported in the supplementary data and included as error bars where applicable. Pulsed labeling time courses were single replicates except where noted in table 1. The agreement seen between the replicates indicates in table 1 was used as evidence of technical reproducibility for temperature incubation time courses, allowing us to forgo replicates for the remaining time courses and conserve sample. Additionally, for pulsed-labeling experiments the data for two bimodal peptides that were non-overlapping in sequence were combined and the time course was fit to a single exponential, resulting in the rate constants reported in table 1. The agreement between peptides from different regions provided additional confidence that additional time-course replicates were not necessary.
Randomization	For every condition (e.g. S-2P in the presence of ACE2) all time points (i.e. 15 s, 60 s, 180 s, 600 s, 1800 s, 5400 s, and 14400 s of deuteration exposure for continuous labeling experiments, and various incubation times for pulsed labeling experiments) were injected in random order to ensure that observations were not the result of instrument variation.
Blinding	No blinding was performed for this study.

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	3A3 (non-commercial Antibody, produced by Maynard Lab)
Validation	Described in detail in Y. Huang, A. W. Nguyen, C.-L. Hsieh, R. Silva, O. S. Olaluwoye, R. E. Wilen, T. S. Kaoud, L. R. Azouz, A. N. Qerqez, K. C. Le, A. L. Bohanon, A. M. DiVenere, Y. Liu, A. G. Lee, D. Amengor, K. N. Dalby, S. D'Arcy, J. S. McLellan, J. A. Maynard, Identification of a conserved neutralizing epitope present on spike proteins from all highly pathogenic coronaviruses. bioRxiv (2021), p. 2021.01.31.428824, , doi:10.1101/2021.01.31.428824.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293F (ThermoFisher cat. A14527) FreeStyle-293F cells (Invitrogen)
Authentication	Cell lines were purchased commercially and were not further validated.
Mycoplasma contamination	Expi293F cells were not tested. FreeStyle-293F cells (Invitrogen) have tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*