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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	cryoET data were collected using serialEM (v3.8) with PACE-tomo script, AutoTEM (v2.4.2). The software and script have cited in the main text.
Data analysis	All the software used in this study are publicly available. cryoET data processing: emClarity (v1.6.2), IMOD (v.4.11.24), RELION (v4), Aretomo(v1.3.4); Topaz (v0.2.5a); Model building: AlphaFold2, Coot(0.9.8), Chimera (v1.17.3), ChimeraX (v1.6.1), Phenix (v1.21), ISOLDE(1.6.0); Structure validation: checkMySequence/1.5.3Pore radius analysis: HOLE (https://www.holeprogram.org/); Sequence alignment and analysis: Snapgene viewer (v7.1.1), WebLogo 3; FACS data analysis: BD FACSDiva/9.4.

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

The cryo-EM maps and corresponding atomic coordinates of models have been deposited in the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB) under the following accession codes: DMV nsp3-4 full-pore complex (C6 symmetry): EMD-39107 and 8YAX; DMV nsp3-4 consensus-pore complex (C6 symmetry): EMD-39109 and 8YB5; DMV nsp3-4 consensus-pore complex (C3 symmetry): EMD-39112 and 8YB7; full-length-pore complex (C6 symmetry): EMD-39159; extended-pore complex (C6 symmetry): EMD-39111; mini-pore complex (C6 symmetry): EMD-39113. The motion-corrected tilt-series have been deposited to EMPIAR with the accession code: EMPIAR-12038. The structures and maps from other studies were used in this work under the following accession codes: PDB 4RNA, PDB 6WRH, PDB 3GZF, EMD-11514, EMD-27245, EMD-29218, EMD-15963, EMD-15964, EMD-15965.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	For cryoET subtomogram averaging, sample sizes were those required for the resolution. The details of datasets, including sample sizes, are listed in Extended Data Table 1. For Co-IP and western blot experiments, at least two independent biological replicates were performed for each.
Data exclusions	The low-intensity tilt images from the high-angle tilt were removed prior to tilt-series alignment. The non-pore complex particles were removed based 3D classification, following the standard procedure in RELION.
Replication	For cryoET subtomogram averaging, two randomly divided half datasets were processed independently and combined to give rise to the final structure. The resolution of the final map is assessed by comparing the two independent maps.
Randomization	The subtomograms were randonly divided into ODD and EVEN datasets, as standard approach implemented in emClarity and RELION.
Blinding	The data analysis is not blinded.

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

The following antibodies were used for western blots: rabbit polyclonal anti-SARS-CoV-2 nsp3 (Thermo Fisher Science, PA5-116947, dilution 1:5000), rabbit polyclonal anti-SARS-CoV-2 nsp4 (ABclonal, A20281, dilution 1:1000 for Western Blot) and mouse monoclonal anti-GAPDH (0411, Santa Cruz, sc-47724, dilution 1:5000 for Western Blot) were used as the primary antibodies, respectively. Anti-rabbit IgG-HRP antibody (Cell Signaling, 7074S, dilution 1:2000 for Western Blot) and anti-mouse IgG-HRP antibody (Cell Signaling, 7076S, dilution 1:2000 for Western Blot) were used as secondary antibodies. The following antibody was used for Immunofluorescence assay: SARS-CoV-2 N polyclonal antibody (Made in house (Riva, L. et al. Nature 586, 113–119, 2020), 1:4000) and Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (Thermo scientific, A-11064, 1:1000).

Validation

The antibodies were validated using blank controls from this study, as shown in Figure 3i and Extended Data Fig. 10b. The cells transfected with empty vector were used as the negative control to validate the specificity of primary antibodies. SARS-CoV-2 N polyclonal antibody has been validated in this paper: Riva, L. et al. Nature 586, 113–119, 2020.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK 293F (Expi293™ Expression System Kit, A14635), HEK 293T cells (ATCC, CRL-11268), VeroE6 cells (RRID, CVCL_0574). BHK21-ACE2 and VeroE6-TMPRSS2 were used in the previous study, referenced in this manuscript (Ref 44: Qin, B. et al. Identification of the SARS-unique domain of SARS-CoV-2 as an antiviral target. Nat Commun 14, 3999 (2023))

Authentication

The cell lines were not authentificated.

Mycoplasma contamination

Mycoplasma contamination were experimentally checked and not present.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

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	VeroE6 cells were transfected with the wild type or mutant pcDNA3.1-TwinStrep-EGFP-nsp3-nsp4 plasmids. 24 h post-transfection, VeroE6 cells were washed with 1 × PBS and treated with 0.25% trypsin/EDTA solution at 37 °C until cells were detached from the bottom of the 10 cm culture dish. Cells were pelleted at 200 × g for 3 mins and washed with 1 × PBS, then re-suspended in 500 µl FACS buffer (1 × PBS, 25 mM HEPES (pH 7.5), 2% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin). The cell suspensions were filtered through 40 µm Falcon™ Cell Strainers (BD, 352340) before acquisition on a flow cytometer. Single cell sorting and FACS analysis were performed by BD FACSAriaTM SORP Cell Sorter with a 488 nm laser.
Instrument	BD FACSAriaTM SORP Cell Sorter
Software	BD FACSDiva/9.4
Cell population abundance	8%-10%
Gating strategy	Cells were considered positive when the fluorescence intensity was above a threshold value which was determined by the maximum intensity of the non-transfected control cells.

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