

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 Andor SOLIS 4.31.30024.0; NIS Elements software (v5.42.06); Image Studio 6.0; Azure Biosystems 300

Data analysis ImageJ 1.54p; ThunderStorm dev-2016-09-10-b1; Matlab R2023b; PYME 23.06.15; SharpVISu v2.0.3

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 Atlas described in this paper is provided as high-resolution fluorescence SR images of each infected cell at the Stanford Data Repository, which can be freely accessed at this DOI: <https://doi.org/10.25740/my248kj2080>. This also includes SR images of the accessory proteins ORF3a, ORF6, ORF7a, ORF8 and ORF9b which are provided as an additional resource. Source Data for the plots in the figures are provided with this paper.

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](https://www.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	Each sample preparation resulted in approximately 9 wells of fixed cells with different stained targets. For each prepared sample well, typically 5-10 cells were imaged using SR microscopy and 50-100 cells were imaged using confocal microscopy. Because different targets appear in many pairs or combinations of labels, each target was imaged multiple times (2-500 cells) using SR microscopy. The detailed numbers of cells imaged using SR microscopy for each target were listed in Supplementary Table 1.
Data exclusions	No data exclusion.
Replication	The experimental measurements were replicated at least 3 times, starting with cell growth and infection (independent biological replicates). All biological replicates were successful.
Randomization	No randomization was used in this study. Due to the small samples, randomization was not relevant for this study.
Blinding	Blinding was not possible in this study, because due to intrinsically low throughput of SR microscopy, only cells with a positive signal of SARS-CoV-2 markers were selected to be imaged and analyzed in the infected cell groups.

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 used

Primary antibodies and the dilutions and concentrations used are as follows:

mouse monoclonal anti-dsRNA (J2 clone, SCICONS, 10010200, lot J2-2017, 1:200, 5 µg/mL),
 rabbit polyclonal anti-nsp1 (ProteinTech, 29287-1-AP, lot 00095243, 1:200, 5 µg/mL),
 sheep polyclonal anti-nsp1 (MRC PPU, DA103, 1:70, 5 µg/mL),
 sheep polyclonal anti-nsp2 (MRC PPU, DA105, 1:52, 5 µg/mL),
 rabbit polyclonal anti-nsp3 (Thermo Fisher, PA5-116947, lot YA3808034B, 1:134, 5 µg/mL),
 sheep polyclonal anti-nsp3 (MRC PPU, DA126, 1:24, 5 µg/mL),
 sheep polyclonal anti-nsp4 (MRC PPU, DA239, 1:36, 5 µg/mL),
 rabbit polyclonal anti-nsp4 (Novus, NBP3-15991-20uL, lot 5500003317, 1:100, 3.1 µg/mL),
 sheep polyclonal anti-nsp5 (MRC PPU, DA118, 1:68, 5 µg/mL),
 rabbit polyclonal anti-nsp5 (ProteinTech, 29286-1-AP, lot 104362, 1:90, 5 µg/mL),
 rabbit monoclonal anti-nsp6 (Abcam, ab284633, lot 1062329-1, 1:200, 5 µg/mL),
 rabbit monoclonal anti-nsp7 (GeneTex, GTX636719, lot 44620, 1:200, 5 µg/mL),
 sheep polyclonal anti-nsp7 (MRC PPU, DA093, 1:26, 5 µg/mL),
 sheep polyclonal anti-nsp8 (MRC PPU, DA110, 1:54, 5 µg/mL),
 sheep polyclonal anti-nsp9 (MRC PPU, DA094, 1:40, 5 µg/mL),
 sheep polyclonal anti-nsp10 (MRC PPU, DA091, 1:44, 5 µg/mL),
 sheep polyclonal anti-nsp12 (MRC PPU, DA119, 1:25, 10 µg/mL),
 rabbit polyclonal anti-nsp12 (Sigma-Aldrich, SAB3501287-100UG, lot 926721011, 1:500, 2 µg/mL),
 sheep polyclonal anti-nsp13 (MRC PPU, DA111, 1:36, 5 µg/mL),
 sheep polyclonal anti-nsp14 (MRC PPU, DA112, 1:26, 10 µg/mL),
 rabbit polyclonal anti-nsp15 (ProteinTech, 29301-1-AP, 1:200, 5 µg/mL),
 sheep polyclonal anti-nsp16 (MRC PPU, DA113, 1:32, 5 µg/mL),
 rabbit polyclonal anti-Spike S1 (GeneTex, GTX135356, lot 43957, 1:40, 5 µg/mL),
 rabbit polyclonal anti-Spike S1 (GeneTex, GTX135385, lot 43978, 1:40, 5 µg/mL),
 rabbit monoclonal anti-Spike S1 (Sino Biological, 40150-R007, lot MA14MY2901-A, 1:200, 5 µg/mL),
 goat polyclonal anti-Spike S2 (R&D System, AF10774, 1:20, 10 µg/mL),
 sheep polyclonal anti-Membrane protein (MRC PPU, DA 107, 1:26, 5 µg/mL),
 mouse monoclonal anti-Nucleocapsid (Thermo Fisher, MA5-29981, lot XF3619701, 1:500, 2.48 µg/mL),
 sheep polyclonal anti-Nucleocapsid (MRC PPU, DA114, 1:52, 5 µg/mL),
 sheep polyclonal anti-ORF3a (MRC PPU, DA102, 1:70, 5 µg/mL),
 sheep polyclonal anti-ORF3a (125-275) (MRC PPU, DA100, 1:38, 5 µg/mL),
 rabbit monoclonal anti-ORF6 (Abcam, ab286911-1006, lot 1101778-2, 1:200, 5 µg/mL),
 rabbit monoclonal anti-ORF7a (Abcam, ab283962, lot 1096595-1, 1:100, 5 µg/mL),
 rabbit monoclonal anti-ORF8 (Abcam, ab283914-1006, lot 1077625-3, 1:200, 5 µg/mL),
 rabbit monoclonal anti-ORF9b (GeneTex, GTX637667, lot 44914, 1:200, 5 µg/mL),
 sheep polyclonal anti-ORF9b (MRC PPU, DA089, 1:50, 5 µg/mL),
 rabbit polyclonal anti-Giantin (GOLGB1) (Sigma-Aldrich, HPA011555-100UL, 1:42, 5 µg/mL),
 rabbit polyclonal anti-TGN46 (ProteinTech, 13573-1-AP, 1:116, 5 µg/mL),
 rabbit monoclonal anti-LAMP1 (Cell Signaling, 9091S, 1:500)
 rabbit polyclonal anti-GFP (Novus, NB600-308SS, 1:163, 4 µg/mL).

Secondary antibodies and the optimal dilutions and concentrations used are as follows:

AF647-conjugated donkey anti-rabbit IgG (Thermo Fisher, A-31573, lot 2359136, 1:500, 4 µg/mL),
 AF647-conjugated donkey anti-mouse IgG (Thermo Fisher, A-31571, lot 2555690, 1:500, 4 µg/mL),
 AF647-conjugated donkey anti-sheep IgG (Thermo Fisher, A-21448, lot 2454134, 1:500, 4 µg/mL),
 CF583R-conjugated donkey anti-rabbit IgG (Biotium, CUSTOM-CFDYE, lot 23C0811, 1:250, 4 µg/mL),
 CF583R-conjugated donkey anti-mouse IgG (Biotium, Custom CF Dye, lot 23C1122, 1:250, 4 µg/mL),
 CF583R-conjugated donkey anti-sheep IgG (Biotium, 20897-500uL, lot 24C0222, 1:500, 4 µg/mL),
 CF583R-conjugated donkey anti-goat IgG (Biotium, Custom CF Dye, lot 23C1128, 1:250, 4 µg/mL),
 CF680-conjugated donkey anti-rabbit IgG (Biotium, 20418-250UL, 1:500, 4 µg/mL),
 CF680-conjugated donkey anti-sheep IgG (Biotium, 20062-1, 1:500, 4 µg/mL),
 CF680-conjugated donkey anti-goat IgG (Sigma-Aldrich, SAB4600196-50UL, 1:500, 4 µg/mL),
 CF568-conjugated donkey anti-mouse IgG (Sigma-Aldrich, SAB4600075-50UL, lot 17C1116, 1:500, 4 µg/mL),
 CF568-conjugated donkey anti-goat IgG (Sigma-Aldrich, SAB4600074-50UL, lot 18C0723, 1:500, 4 µg/mL).
 IRDye® 800CW donkey anti-rabbit IgG (Licorbio, 926-32213, 1:5,000, 0.2 µg/mL).

Validation

Each antibody was validated by comparing their IF signal in SARS-CoV-2 infected and non-infected cells or in cells expressing Sec61β-GFP and WT cells for the anti-GFP antibodies. All the antibodies are commercialized, and validation statements and references are provided by the vendor's websites:

<https://nordicmubio.com/products/mouse-anti-double-stranded-rna-j2/10010200>
<https://www.ptglab.com/products/SARS-COV-2-NSP1-Antibody-29287-1-AP.htm?srsltid=AfmBOoogVxo8cyTD4EYg3oZVD3QcnTilD6uBI8A7-tbzggsD0u1w-1q>
<https://mrcppu-covid.bio/antibodies/134231>
<https://mrcppu-covid.bio/antibodies/134230>
<https://www.thermofisher.com/antibody/product/SARS-CoV-2-Nonstructural-Protein-3-Antibody-Polyclonal/PA5-116947>
<https://mrcppu-covid.bio/antibodies/134551>
<https://mrcppu-covid.bio/antibodies/134560>
https://www.novusbio.com/products/sars-cov-2-nsp4-antibody_nbp3-15991
<https://mrcppu-covid.bio/antibodies/134216>
https://www.ptglab.com/products/SARS-COV-2-NSP5-Antibody-29286-1-AP.htm?srsltid=AfmBOorvqwrNZI-9gl5yhj8qd-iOpBverV7eZM3-PbMkrJ7Lf1Fb_YfY

https://www.abcam.com/en-us/products/primary-antibodies/sars-cov-2-nsp6-antibody-epr24845-90-ab284633?srsltid=AfmBOorFOkYHn4tzKkIHfS2duk8Vs6Gdhoc0QlrIJHapcTz_DykDjh-
https://www.genetex.com/Product/Detail/SARS-CoV-2-COVID-19-nsp7-antibody-HL1301/GTX636719?srsltid=AfmBOopld5-A95jQXHXgJELLxGVI_lx29htCiQw-R1TKjL7CT1EV2JH
<https://mrcppu-covid.bio/antibodies/134238>
<https://mrcppu-covid.bio/antibodies/134229>
<https://mrcppu-covid.bio/antibodies/134237>
<https://mrcppu-covid.bio/antibodies/134240>
<https://mrcppu-covid.bio/antibodies/134439>
https://www.sigmaaldrich.com/US/en/product/sigma/sab3501287?srsltid=AfmBOor6nVe8Vn3Z9Kt9T6ak4WBjIRWbTyEHOuDYINOXnpejdp_XpU
<https://mrcppu-covid.bio/antibodies/134228>
<https://mrcppu-covid.bio/antibodies/134227>
<https://www.ptglab.com/products/SARS-COV-2-NSP15-Antibody-29301-1-AP.htm?srsltid=AfmBOorfzumIMHNvVaLtlOHhK-IO95aaKahV1E7IP5VKEWlNa9AKMGZ9>
<https://mrcppu-covid.bio/antibodies/134226>
https://www.genetex.com/Product/Detail/SARS-CoV-2-COVID-19-Spike-antibody/GTX135356?srsltid=AfmBOopwfDGZNG_kdLwZ2LnZU0kYo_3cum86AdbK6EfsVWPQB4yLuOAh
<https://www.genetex.com/Product/Detail/SARS-CoV-2-COVID-19-Spike-RBD-antibody/GTX135385?srsltid=AfmBOorb3hKUtHDFBuMktLwAgRNHwQeVIXPc0e4IP5Usf1rorfD2w0e4>
<https://www.sinobiological.com/antibodies/cov-spike-40150-r007>
https://www.rndsystems.com/products/sars-cov-2-spike-s2-subunit-antibody_af10774
<https://mrcppu-covid.bio/antibodies/134225>
<https://www.thermofisher.com/antibody/product/SARS-SARS-CoV-2-Nucleocapsid-Antibody-clone-05-Monoclonal/MA5-29981>
<https://mrcppu-covid.bio/antibodies/134473>
<https://mrcppu-covid.bio/antibodies/134232>
<https://mrcppu-covid.bio/antibodies/134234>
https://www.abcam.com/en-us/products/primary-antibodies/sars2-cov-2-orf6-antibody-epr24849-21-ab286911?srsltid=AfmBOopEzJv6F_QwAa7ZWUzmH8y6hg6bdvY1EsS6cDyNhV-BXCTlwr
<https://www.abcam.com/en-us/products/primary-antibodies/sars-cov-2-orf7a-antibody-epr24850-55-ab283962>
<https://www.abcam.com/en-us/products/primary-antibodies/sars-cov2-orf8-antibody-epr24851-65-ab283914>
<https://www.genetex.com/Product/Detail/SARS-CoV-2-COVID-19-ORF9b-antibody-HL1918/GTX637667?srsltid=AfmBOooZTtMv21gEPEJC3eM7LJMBslQaHw044AHcUFlosd7SYG1GnEnb>
<https://mrcppu-covid.bio/antibodies/134242>
<https://www.sigmaaldrich.com/US/en/product/sigma/hpa011555?srsltid=AfmBOoonqJZdAV2tcpH7vR3sgZ3QgXCueR1lajNMsKsemldFeW6GRCAJ>
<https://www.ptglab.com/products/TGOLN2,TGN46-Antibody-13573-1-AP.htm?srsltid=AfmBOoollht-uYD8nq3KrUuJucLiaYdb0Dj9OHjISlamWqdvAY0MyXD>
<https://www.cellsignal.com/products/primary-antibodies/lamp1-d2d11-xp-rabbit-mab/9091?srsltid=AfmBOor0B3TB0GGrelwuktmZhTBcyFzRgFHvGUZd9IVlyDNNb2Si5cw>
https://www.novusbio.com/products/gfp-antibody_nb600-308?keywords=NB600-308SS
<https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31573>
<https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-31571>
<https://www.thermofisher.com/antibody/product/Donkey-anti-Sheep-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21448>
https://www.sigmaaldrich.com/US/en/product/sigma/sab4600196?srsltid=AfmBOopiz8SX4dGYUfnDaZ2mGRiSVcDuoqmO0-PMn9d4mGVEkyBZu_te
<https://www.sigmaaldrich.com/US/en/product/sigma/sab4600075>
<https://www.sigmaaldrich.com/US/en/product/sigma/sab4600074>
<https://shop.licorbio.com/irdye-secondary-antibodies/irdye-800cw-donkey-anti-rabbit-igg-secondary-antibody/>

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	A549-ACE2 cells (BEI Resources, NR-53821, sex: male); Vero E6 cells (ATCC, CRL-1586, sex: female); Vero E6-TMPRSS2 cells (BPS Bioscience, 78081, sex: female)
Authentication	Cell lines were authenticated by the suppliers. We did not perform any additional authentication upon reception. We made bulk stocks for each cell line after recovering from the original frozen vials. We discard the cells after passage for 30 days and thaw new cells from liquid nitrogen stocks. Cell morphology was monitored at each passage by microscope.
Mycoplasma contamination	Cells were routinely tested (PCR based test) and were mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this manuscript.

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A