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Reporting Summary

Nature Research 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 Research 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	Fluorescence micrographs were collected using Zeiss LSM800, LSM880 or LSM710 confocal system (Zeiss, Germany) equipped with an Electronically Switchable Illumination and Detection (ESID) module and an AiryScan module (for LSM880) and controlled by Zen blue software for LSM800 and LSM880 (v.2.6) and Zen 2012 for LSM 710. EM images were acquired using a FEI Tecnai-12 electron microscope (FEI, Eindhoven, Netherlands) equipped with a VELETTA CCD digital camera (Soft Imaging Systems GmbH, Munster, Germany). Morphometric analysis was performed using iTEM software (Olympus SYS, Germany, v.5.2). For electron tomography a Tecnai G2 Spirit BioTwin electron microscope (FEI) was used. For CLEM experiments, cells and structures of interest obtained by confocal microscopy were identified on EM images using Zen Connect software (Zeiss v.3.0).
Data analysis	Fluorescence images were processed with Fiji (ImageJ v.1.51j8). Brightness and contrast were adjusted with Adobe Photoshop (v.25.4), figure panels were assembled with Adobe Illustrator (v.25.4). 3D reconstructions were rendered using IMOD software (v.4.7.15). FLIM data analysis used SymPhoTime 64 (Picoquant v.2.1.3764). Statistical analyses were performed using GraphPad Prism7 (GraphPad Software Inc v.7.0a) or R software environment for statistical computing (rstatix R package v.4.1.1).

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Full scans for all western blots and autoradiographs are provided in Supplementary Fig. 1. The nucleotide sequence of synthetic IBV NSP6 and NSP6/NSP7 used in this study are in Supplementary Table 1. The oligonucleotides, siRNAs and primers used in this study are in Supplementary Table 2. Source data for each figure are provided in the corresponding "Source Data" files. Raw data supporting the findings of this study are deposited in Zenodo and will be publicly available at 10.5281/zenodo.5929088 (upon publication). Raw EM data, including tilt series and reconstructed 3D tomograms were deposited in EMDB and EMPIAR public databases with EMD-14179 and EMPIAR-10935 accession codes respectively.
SARS-CoV-2 genome data was retrieved from https://www.gisaid.org/; In detail:
SARS-CoV-2 early lineage (SARS-CoV-2/human/BRA/RJ01/2020, GenBank accession no. MT710714); SARS-CoV-2 early lineage (hCoV-19/Brazil/AM-L70-71-CD1739/2020); SARS-CoV-2 gamma variant (GISAID ID: EPI_ISL_1060902); SARS-CoV-2 early lineage B.1 (hCoV-19/Italy/CAM-INMI-32803-66/2020, GISAID ID: EPI_ISL_493333); SARS-CoV-2 gamma variant (hCoV-19/Italy/CAM-IZSM-RD020483D54/2021, GISAID ID: EPI_ISL_2933105).
Phylogenetic analysis was performed using Nextstrain (https://nextstrain.org/ncov/global).
NSP6 topology modelling was performed using the Constrained Consensus TOPology prediction server (CCTOP, Institute of Enzymology, Budapest, Hungary). The amphipathic features of the alpha helix were determined using HELIQUEST (http://heliquest.ipmc.cnrs.fr). Images and cartoons were created with BioRender.com.

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 sample size calculation was done. Experiments were repeated at least three times with similar results and sample size was chosen based on the consistency and significance of measured differences between groups and or conditions. More information are provided in the section “Statistics and Reproducibility” in the Methods section.
Data exclusions	No data exclusions.
Replication	Each experiment in the manuscript was repeated at least three times (unless otherwise stated) under standard and clearly defined conditions; all attempts at replication were successful. Detailed information are provided in “Statistics and Reproducibility” in the Methods section.
Randomization	Images were selected randomly and analyzed equally, no sub-sampling so no randomization was necessary.
Blinding	Blinding was not relevant for the experiments done given the nature of the reagents (chemicals, plasmids, siRNAs).

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

Antibodies used: The following antibodies were used: mouse monoclonal anti-HA (BioLegend, 901503, clone 16B12- dilution 1:600 for IF and 1:1500 for WB), rabbit polyclonal anti-HA (Sigma-Aldrich, H6908- dilution 1:200 for IF), goat polyclonal anti-HA (Bethyl, A190-138A- dilution 1:600 for IF), rabbit polyclonal anti-actin (Sigma-Aldrich, A2066- dilution 1:10000 for WB), rabbit polyclonal anti-NSP6 (ProSci Inc, 9177- dilution 1:200 for IF and 1:1000 for WB), sheep anti-NSP3 (The University of Dundee, DA126- dilution 1:100 for IF and 1:1000 for WB), rabbit polyclonal ADRP/Perilipin 2 (Proteintech, 15294-1-AP- dilution 1:200), rabbit monoclonal anti-DFCP1 (Cell Signaling, 38419, clone E9Q1S- dilution 1:1000 for WB), mouse monoclonal anti-FLAG (Sigma-Aldrich, F1804, clone M2- dilution 1:400 for IF and 1:1500 for IF), goat polyclonal anti-FLAG (Bethyl, A190-101A - dilution 1:200 for IF), mouse monoclonal anti-c-Myc (Santa Cruz, sc-40, clone 9E10- dilution 1:200 for IF), mouse monoclonal anti-GAPDH (Santa Cruz, sc-32233, clone 6C5- dilution 1:1000 for WB), mouse monoclonal anti-LAMP1 (Hybridoma Bank, H4A3, clone H4A3-, dilution 1:200 for IF), rabbit monoclonal anti-EEA1 (BD Biosciences, 610456, clone 14- dilution 1:1000 for IF), sheep anti human anti-TGN46 (BioRad, AHP500GT- dilution 1:750 for IF), rabbit polyclonal anti-GFP (Abcam, ab6556- dilution 1:250 for IF), mouse monoclonal anti-GFP (Santa Cruz, sc-9996, clone B-2- dilution 1:2000 for WB), mouse monoclonal anti-mCherry (Abcam, ab125096, clone 1C51- dilution 1:2000 for WB), mouse monoclonal anti-V5 (ThermoFisher R960-25- dilution 1:200 for IF and 1:1000 for WB), rabbit polyclonal anti-LC3 (Novus Biologicals, NB100-2220- dilution 1:200 for IF), mouse monoclonal anti-dsRNA (Scicons, 10010500, clone J2- dilution 1:10 for IF), DAPI (Sigma-Aldrich, D9542- dilution 1:10000 for IF), rabbit 1.4 nm gold-conjugated Fab' fragment (Nanoprobes, 2004- dilution 1:50), mouse 1.4 nm gold-conjugated Fab' fragment (Nanoprobes, 2002- dilution 1:50) and Alexa Fluor®-546 FluoroNanogold™-anti-mouse Fab' (7402- dilution 1:50). Anti-GM130 (dilution 1:1000) and anti-VAPA (dilution 1:300) were produced in our laboratory as previously described Ref.34,35.

Validation

Most of the antibodies used in the study were bought from commercial vendors and were validated by the manufacturers and/ or other studies. Some of the antibodies were further validated using KO/knocked-down cell lines. See individual antibody's web page (link shown below) on the manufacture's website for validation and relevant citations:

-mouse monoclonal anti-HA (BioLegend, 901503, clone 16B12): https://www.biolegend.com/en-us/products/purified-anti-ha-11-epitope-tag-antibody-11374?Clone=16B12
-rabbit polyclonal anti-HA (Sigma-Aldrich, H6908): https://www.sigmaaldrich.com/IT/it/product/sigma/h6908
-goat polyclonal anti-HA (Bethyl, A190-138A): https://www.fortislife.com/products/primary-antibodies/goat-anti-ha-tag-antibody-fitc-conjugated/A190-138F
-rabbit polyclonal anti-actin (Sigma-Aldrich, A2066): https://www.sigmaaldrich.com/IT/it/product/sigma/a2066?gclid=CjwKCAiA3L6PBhBvEiwAINlJ9NJSu74wv3ABV-kmOZ5qMc9bU2LV-J_Cja5GC8JDjpF6-pexA_9cUBoCyHkQAvD_BwE
-rabbit polyclonal ADRP/Perilipin 2 (Proteintech, 15294-1-AP):
https://www.ptglab.com/products/ADRP-Antibody-15294-1-AP.htm
-rabbit monoclonal anti-DFCP1 (Cell Signaling, 38419, clone E9Q1S):
https://www.cellsignal.com/products/primary-antibodies/dfcp1-e9q1s-rabbit-mab/38419
-mouse monoclonal anti-FLAG (Sigma-Aldrich, F1804, clone M2): https://www.sigmaaldrich.com/IT/it/product/sigma/f1804
-goat polyclonal anti-FLAG (Bethyl, A190-101A): https://www.thermofisher.com/antibody/product/ECS-DYKDDDDK-Tag-Antibody-Polyclonal/A190-101A
-mouse monoclonal anti-c-Myc (Santa Cruz, sc-40, clone 9E10): https://www.scbt.com/p/c-myc-antibody-9e10?gclid=Cj0KCQiA_8OPBhDtARIsAKQu0gZwuTcI4rrpilEY2ea3C5cjM1b3Vx--CDVU7-BMVWEPA2IxzXfdIiwaApamEALw_wcB
-mouse monoclonal anti-GAPDH (Santa Cruz, sc-32233, clone 6C5): https://www.scbt.com/it/p/gapdh-antibody-6c5
-mouse monoclonal anti-LAMP1 (Hybridoma Bank, H4A3, clone H4A3): https://dshb.biology.uiowa.edu/H4A3
- rabbit monoclonal anti-EEA1 (BD Biosciences, 610456, clone 14): https://www.bdbiosciences.com/en-us/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-eea1.610456
-sheep anti human anti-TGN46 (BioRad, AHP500GT):
https://www.bio-rad-antibodies.com/polyclonal/human-tgn46-antibody-ahp500.html?f=purified
-rabbit polyclonal anti-GFP (Abcam, ab6556):
https://www.abcam.com/gfp-antibody-ab6556.html
-mouse monoclonal anti-GFP (Santa Cruz, sc-9996, clone B-2):
https://www.scbt.com/it/p/gfp-antibody-b-2
-mouse monoclonal anti-mCherry (Abcam, ab125096, clone 1C51):
https://www.abcam.com/mcherry-antibody-1c51-ab125096.html
-mouse monoclonal anti-V5 (ThermoFisher R960-25):
https://www.thermofisher.com/antibody/product/V5-Tag-Antibody-Monoclonal/R960-25
-rabbit polyclonal anti-LC3 (Novus Biologicals, NB100-2220):
https://www.novusbio.com/products/lc3b-antibody_nb100-2220
-mouse monoclonal anti-dsRNA (Scicons, 10010500, clone J2):
https://www.labome.com/product/SCICONS/10010500.html
-DAPI (Sigma-Aldrich, D9542):
https://www.sigmaaldrich.com/IT/it/search/d9542?focus=products&page=1&perPage=30&sort=relevance&term=D9542&type=product_name
-rabbit polyclonal anti-NSP6 (ProSci Inc, 9177): https://www.prosci-inc.com/sars-cov-2-covid-19-nsp6-antibody-9177.html, this antibody was validated in this study through western blot and immunofluorescence experiment. (Extended Data Fig. 1c for WB, and Figure 3b for IF)
-sheep anti-NSP3 (The University of Dundee, DA126): https://mrcppureagents.dundee.ac.uk/reagents-view-antibodies/703270, this antibody was validated in this study through western blot and immunofluorescence (Extended Data Fig. 6c for WB, and Figure 3b for IF).
Anti-GM130 and anti-VAPA were validated in Marra et al. see ref 34, and Jansen et al. see ref 35.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell line sources: HeLa cells were obtained from ATCC; Calu-3 cells (human lung adenocarcinoma) were a kind gift from Louis J. Galietta (TIGEM, Naples), originally purchased from ATCC. HeLa stably expressing inducible HA-NSP6/FLAG-NSP6/HA-NSP6ΔSGF/FLAG-NSP6ΔSGF were generated in this study.
Authentication	All stable cell lines were authenticated by WB or IF. Commercial cell lines were purchased recently from ATCC and validated by morphological analysis.
Mycoplasma contamination	Mycoplasma contamination: Cell lines were routinely tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.