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	ddPCR raw data was collected using QuantaSoft version 1.7.4.0917 (Bio-Rad).
Data analysis	ddPCR raw data was analyzed using QuantaSoft Analysis Pro version 1.0.596 (Bio-Rad). HT-SGS data analysis is described in detail in PMID 33831133; the bioinformatic pipeline for HT-SGS data analysis has been deposited (https://github.com/niaid/UMI-pacbio-pipeline) Raw sequence reads from the P38 thalamus isolated virus were trimmed of Illumina adapter sequence using Cutadapt version 1.1241 and then trimmed and filtered for quality using the fastq_quality_trimmer and fastq_quality_filter tools from the FASTX-Toolkit 0.0.14 (Hannon Lab, CSHL);reads were mapped to the SARS-CoV-2 2019-nCoV/USA-WA1/2020 genome (MN985325.1) using Bowtie2 version 2.2.942 with parameters --local --no-mixed -X 1500; PCR duplicates were removed using picard MarkDuplicates, version 2.26.10 (Broad Institute). Digitalized ISH images were automatically analyzed using Visiopharm software v2021.09.02 (Visiopharm, Hørsholm, Denmark). All statistical analysis were performed using SAS version 9.4. The SAS code for statistical analysis has been deposited (https://github.com/niaid/COVID-19-Autopsy-SAS-Code).

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 datasets that support the findings of this study are available in Supplementary Data 1, 2 and 3. Positive and negative controls for ISH, IF, and IHC are available in Supplementary Data 3. The sequencing of SARS-CoV-2 isolated from Vero E6-TMPRSS2-T2A-ACE2 cell culture of P38 thalamus has been deposited to GenBank (OP125352).

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	The sample size of autopsy cases (n=44) was not predetermined as this was an observational study. Tissue and fluids samples were collected from autopsy each case as able and exact numbers were not predetermined. Cases and tissues selected for sequencing via HT-SGS were determined by gRNA and sgRNA levels within the tissues as determined by ddPCR and qPCR, respectively. n values reported in Extended Data Fig. 4 refer to the depth of sequencing reads recovered for the haplotype(s) detected within the individual tissue.
Data exclusions	No data were excluded from analyses.
Replication	ddPCR results were generated from technical replicates with all samples required to meet manufacturer internal control standard levels. All tissues that underwent ISH, chromogenic IHC, and IF staining had concurrent internal and external positive and negative controls; for ISH images displayed in Fig 2 and Fig. 3 replicates of the listed samples or another sample from the same anatomic location in a different patient were performed; all samples were orthogonally confirmed by ddPCR for SARS-CoV-2 and the ISH assay was validated across a 4-log dynamic range against ddPCR. In Extended Data Fig. 5 all H&E (and Masson trichrome) photomicrographs are exemplars of histopathology observed across a diversity of patients within the cohort, see extended data table 4 and supplemental data 2. The histopathologic observations were validated by a minimum of two board certified anatomic pathologist.
Randomization	This was an observational study.
Blinding	This was an observational 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	1) Rabbit anti-SARS_CoV_2 Nucleocapsid protein 1 (Wuhan) 1:250, GenScript #U864YFA140-4/CB2093

Antibodies used	2) Mouse anti-NeuN clone A60 1:200, Millipore Sigma #MAB377, lot# 060101159 3) Donkey anti-Rabbit IgG (H+L) Highly cross-Adsorbed Secondary Antibody, Alexa Fluor 488 conjugate, ThermoFisher #A-21206, lot# 2289872 4) Donkey anti-Mouse IgG (H+L) Highly cross-Adsorbed Secondary Antibody, Alexa Fluor 594 conjugate, ThermoFisher #A-21203, lot# 10608644 5) Human TMEM119 Antibody, R&D Systems #MAB10313, lot#CNBT0120051 6) Rabbit IgG, Control Antibody, Vector Laboratories, Inc. #I-1000-5, lot#ZH1201
Validation	1) Validation by Munster et al. described here: https://doi.org/10.1038/s41586-020-2324-7. Antibody was additionally validated by C. Winkler using mock and SARS-CoV-2 infected Vero cells and human cerebral organoids. 2) Validation details can be found here: https://www.emdmillipore.com/US/en/product/Anti-NeuN-Antibody-clone-A60,MM_NF-MAB377, specificity: MILLIPORE's exclusive monoclonal antibody to vertebrate neuron-specific nuclear protein called NeuN (or Neuronal Nuclei) reacts with most neuronal cell types throughout the nervous system of mice including cerebellum, cerebral cortex, hippocampus, thalamus, spinal cord and neurons in the peripheral nervous system including dorsal root ganglia, sympathetic chain ganglia and enteric ganglia. Developmentally, immunoreactivity is first observed shortly after neurons have become postmitotic, no staining has been observed in proliferative zones. The immunohistochemical staining is primarily localized in the nucleus of the neurons with lighter staining in the cytoplasm. The few cell types not reactive with MAB377 include Purkinje, mitral and photoreceptor cells. The antibody is an excellent marker for neurons in primary cultures and in retinoic acid-stimulated P19 cells. It is also useful for identifying neurons in transplants." 3) Details from Thermo Fisher can be found here: https://www.thermofisher.com/antibody/product/Donkey-anti-Rabbit-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21206 4) Details from Thermo Fisher can be found here: https://www.thermofisher.com/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21203, validation: These donkey anti-mouse IgG (H+L) whole secondary antibodies have been affinity-purified and show minimum cross-reactivity to bovine, chicken, goat, guinea pig, hamster, horse, human, rabbit, rat, and sheep serum proteins. Cross-adsorption or pre-adsorption is a purification step to increase specificity of the antibody resulting in higher sensitivity and less background staining. The secondary antibody solution is passed through a column matrix containing immobilized serum proteins from potentially cross-reactive species. Only the nonspecific-binding secondary antibodies are captured in the column, and the highly specific secondaries flow through. The benefits of this extra step are apparent in multiplexing/multicolor-staining experiments (e.g., flow cytometry) where there is potential cross-reactivity with other primary antibodies or in tissue/cell fluorescent staining experiments where there may be the presence of endogenous immunoglobulins. 5) Details from R&D Systems can be found here: https://www.rndsystems.com/products/human-tmem119-antibody-1023426_mab10313. 6) Details from Vector Laboratories, Inc. can be found here: https://vectorlabs.com/products/antibodies/rabbit-igg#product.info.overview.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 cells (organism: Cercopithecus aethiops/tissue: kidney/disease: none) were obtained from Ralph Baric at the University of North Carolina, Chapel Hill and Vero E6-TMPRSS2-T2A-ACE2 cells (organism: Cercopithecus aethiops/tissue: kidney/disease: none) were newly procured from BEI Resources (NR-54970; https://www.beiresources.org/Catalog/cellBanks/NR-54970.aspx).
Authentication	Vero E6 cells and Vero E6-TMPRSS2-T2A-ACE2 cells not authenticated in house.
Mycoplasma contamination	All cells are tested monthly to confirm the absence of Mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cells were used.