***Supplemental Data***

**e-Methods**

*Tissue preservation and preparation*

Autopsy was performed at the Institute of Medical Genetics and Pathology at the University Hospital of Basel, Switzerland. Brains were removed 20 hours *post mortem* upon opening the skull with a handsaw, avoiding aerosolization of SARS-CoV-2, and *in toto* fixed in 4% (w/v) phosphate-buffered formalin as recently described1. The study was approved by the Ethics Committee of Northwestern and Central Switzerland (ID 2020-00629 and 2020-00969). Formalin-fixed paraffin-embedded (FFPE) tissue was routinely prepared to 5 µm thick slides.

*Immunohistochemistry*

FFPE tissue samples were first deparaffinized with xylene and ethanol solutions of decreasing concentration. Next, slides were heat-treated with citric buffer (pH = 6) following distilled water heat-treatment. Tissues were incubated with H2O2 in 1x phosphate-buffered saline (PBS) solution (1:300) for 5 min and subsequently blocked with 1x PBS/0.01% Triton X-100/ 10% goat sera for 30 minutes at room temperature (RT). Primary antibody binding was carried out overnight at RT using mouse anti-CD45 (1:200, clone HI30, BioLegend), rat anti-CD3 (1:100, clone CD3-12, BioRad), rat anti-MBP (1:200, clone 12, Merck), rat anti-GFAP (1:500, clone 2.2B10, ThermoFisher), and rabbit anti-Iba1 (1:500, Wako). After two washing steps in 1x PBS, samples were incubated with biotinylated secondary antibodies diluted 1:500 in 1x PBS/ 0.01% Triton X-100 for 2 hours at RT (goat anti-rat/mouse/rabbit biotinylated IgG, ThermoFisher). Following another washing step and avidin-biotin complex incubation for one-hour (1:500, Vector), subsequent color revelation with diaminobenzidine according to the manufacturer’s recommendations (DAB, Dako) was performed. The samples were counterstained with 50% Haematoxylin for 15s and dehydrated with increasing concentration ethanol solutions and xylene.

Dysferlin immunohistochemistry2 was performed on an automated staining platform (Dako/Agilent, Autostainer Link 48). Sections were pre-treated with "target retrieval solution low" (citrate buffer pH 6.1, Dako/Agilent) for 20 minutes at 95°C in a pre-treatment module (Dako/Agilent, PT Link). The primary dysferlin antibody (monoclonal mouse, NCL-Hamlet, Novocastra/Leica Biosystems) was diluted 1:10 in antibody diluent (Dako/Agilent) and incubated for 60 minutes at RT. Blockade of the endogenous peroxidase was omitted according to the instructions of the primary antibody's manufacturer. As a secondary antibody, a biotinylated link antibody (Dako/Agilent) was applied for 30 min at RT. Sections were then incubated with streptavidin-conjugated horseradish peroxidase (Dako/Agilent) for 30 minutes at RT. Staining was developed with diaminobenzidine (Dako/Agilent) for 10 minutes at RT. Counterstaining of nuclei was achieved with Mayer's hemalaun solution (Merck Millipore) for 30 seconds at RT. For diagnostic purposes and to characterize general pathology, haematoxylin and eosin (HE) and Luxol fast blue (LFB) staining as well as Bielschowsky silver impregnation were carried out.

*Fluorescent in situ RNA hybridization*

Single molecule *in situ* hybridization (ISH) was performed according to the previously established protocols3,4. Sequences of target probes, amplifier, and label probes are proprietary and commercially available (Biotecne, Advanced Cell Diagnostics). Target probes cover approx. 1000bp and typically contains 20 ZZ probe pairs (approx. 50 bp/pair). These manual RNAscope assay probes were used: *ACE2*, *V-nCoV2019-S*-C3*.* Prior to staining the FFPE tissue was deparaffinized using xylene and pure ethanol. Tissue was stained with TSA Plus Fluorophores (Cyanine 3, Cyanine 5) and DAPI. Channel-specific negative (*DapB*) and positive (*POLR2A*, *PPIB*-C2, *UBC*-C3) ISH control probes were run in parallel for each sample.

*Image acquisition and analysis*

Chromogenic images were taken using the Leica DMI8 microscope with 5x, 10x, 20x or 40x objectives. Fluorescent images were taken with Leica TCS SP8 laser confocal (405/488/552/638 nm) microscope using 10x, 20x, 40x or 63x objectives; fluorescent confocal pictures are Z-stack images, unless stated otherwise. Images were processed using Fiji ImageJ (v2.0)5 and exported to vector-based software (Adobe Illustrator and Affinity Designer) for figure generation.

**e-References**

1. Menter T, Haslbauer JD, Nienhold R, et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. Histopathology 2020;77:198-209.

2. Hochmeister S, Grundtner R, Bauer J, et al. Dysferlin is a new marker for leaky brain blood vessels in multiple sclerosis. J Neuropathol Exp Neurol 2006;65:855-865.

3. Schirmer L, Velmeshev D, Holmqvist S, et al. Neuronal vulnerability and multilineage diversity in multiple sclerosis. Nature 2019;573:75-82.

4. Pröbstel A, Zhou X, Baumann R, et al. Sci Immunol 2020.

5. Schindelin J, Arganda-Carreras I, Frise E, et al. Fiji: an open-source platform for biological-image analysis. Nat Methods 2012;9:676-682.

**Fig. e-1: Histopathological assessment of MS and non-MS COVID-19 affected brain and lung tissue**

(A) Immunohistopathological assessment against GFAP (astrocyte marker) and CD45 (pan-leukocyte marker) within the macroscopically visible lesions. The lack of CD45+ cell infiltrates in lesion areas supports our previous observations of chronic lesion inactivity. WM = white matter. Scale bar indicates 100 µm. (B) Macroscopically visible lesions of the cortex (subpial demyelination) and the brainstem (medulla oblongata) were assessed by Hematoxylin and Eosin (HE), and Luxol Fast Blue (LFB) staining, as well as MBP (Myelin basic protein), Iba1, and dysferlin immunohistochemical evaluation. Given the lack of ongoing myelin phagocytosis and foam cells, all examined lesions were staged as chronic inactive. Scale bar indicates 100 µm. (C) Brain tissue stained with hematoxylin & eosin of cerebellar vermis (left) and hippocampal CA1 region (right) shows neither sign of hypoxic brain damage, i.e. no acute neuronal necrosis or loss of neurons, nor marked inflammation. Scale bar indicates 100 µm. (D) Lung tissue from MS and non-MS COVID-19 patients: tissue was examined by immunohistochemistry staining for CD45 and CD3. Note, presence of CD45+ and CD3+ cells suggesting ongoing inflammation. Scale bar indicates 100 µm. (E) Scheme illustrating the proposed infection route of SARS-CoV-2 and highlighting the choroid plexus epithelial cells and adjacent ependyma as key CNS barrier for viral CNS entry.

**Fig. e-2: Spatial transcriptomic assessment of MS and non-MS COVID-19 affected brain and lung tissue**

(A-B) Positive (+ CTRL) and negative (- CTRL) control probes for the assessment of (A) choroid plexus, lesion area, and ependyma of MS COVID-19 as well as (B) choroid plexus and lung tissue from non-MS COVID-19 including multiplex assessment with *SARS-CoV-2* and *ACE2* of the lung. Scale bar indicates 20 µm.

**Table e-1:** **Patient characteristics**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Patient | Diagnosis | Sex | Age (yrs) | First COVID-19  symptoms to death (days) | Post-mortem  delay (h) | Cause of death |
| 1 | MS & COVID-19 | F | 67 | 13 | 20 | Respiratory failure |
| 2 | COVID-19 | M | 86 | 12 | 26 | Respiratory failure |

**Table e-2:** **qRT-PCR results for SARS-CoV-2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patient | Brainstem | Cerebellum | Olfactory bulb | Lung | Nasopharyngeal swab |
| 1 | negative | negative | positive | negative | positive |
| 2 | negative | negative | positive | positive | positive |