

**Supplemental information**

**Anatomical barriers against SARS-CoV-2  
neuroinvasion at vulnerable interfaces  
visualized in deceased COVID-19 patients**

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### Patient characteristics

	COVID-19 (n=45)	Control (n=8)
Age	79 (75–91)	77 (69–85)
Men	31 (69%)	3 (38%)
Medical history		
Body mass index		
BMI < 18.5	3 (7%)	0 (0%)
BMI 18.5 – 25	18 (40%)	4 (50%)
BMI 25 – 30	14 (31%)	2 (25%)
BMI 30 – 40	9 (20%)	1 (13%)
BMI > 40	1 (2%)	1 (13%)
Diabetes mellitus type 2	23 (51%)	1 (13%)
Hypertension	36 (80%)	6 (75%)
Chronic kidney disease	23 (51%)	1 (13%)
Cardiovascular disease	25 (56%)	6 (75%)
Chronic lung disease	19 (42%)	5 (63%)
Neurodegenerative disease	8 (18%)	0 (0%)
Smoking	31 (69%)	5 (63%)
Current	5 (11%)	2 (25%)
Former	26 (58%)	3 (38%)
Active oncological condition	12 (27%)	1 (13%)
Organ transplant	1 (2%)	0 (0%)
Immunosuppressive medication	16 (36%)	0 (0%)
Vaccination status		
Vaccinated	37 (82%)	8 (100%)
Unvaccinated	4 (9%)	0 (0%)
Unknown	4 (12%)	0 (0%)

### Disease characteristics

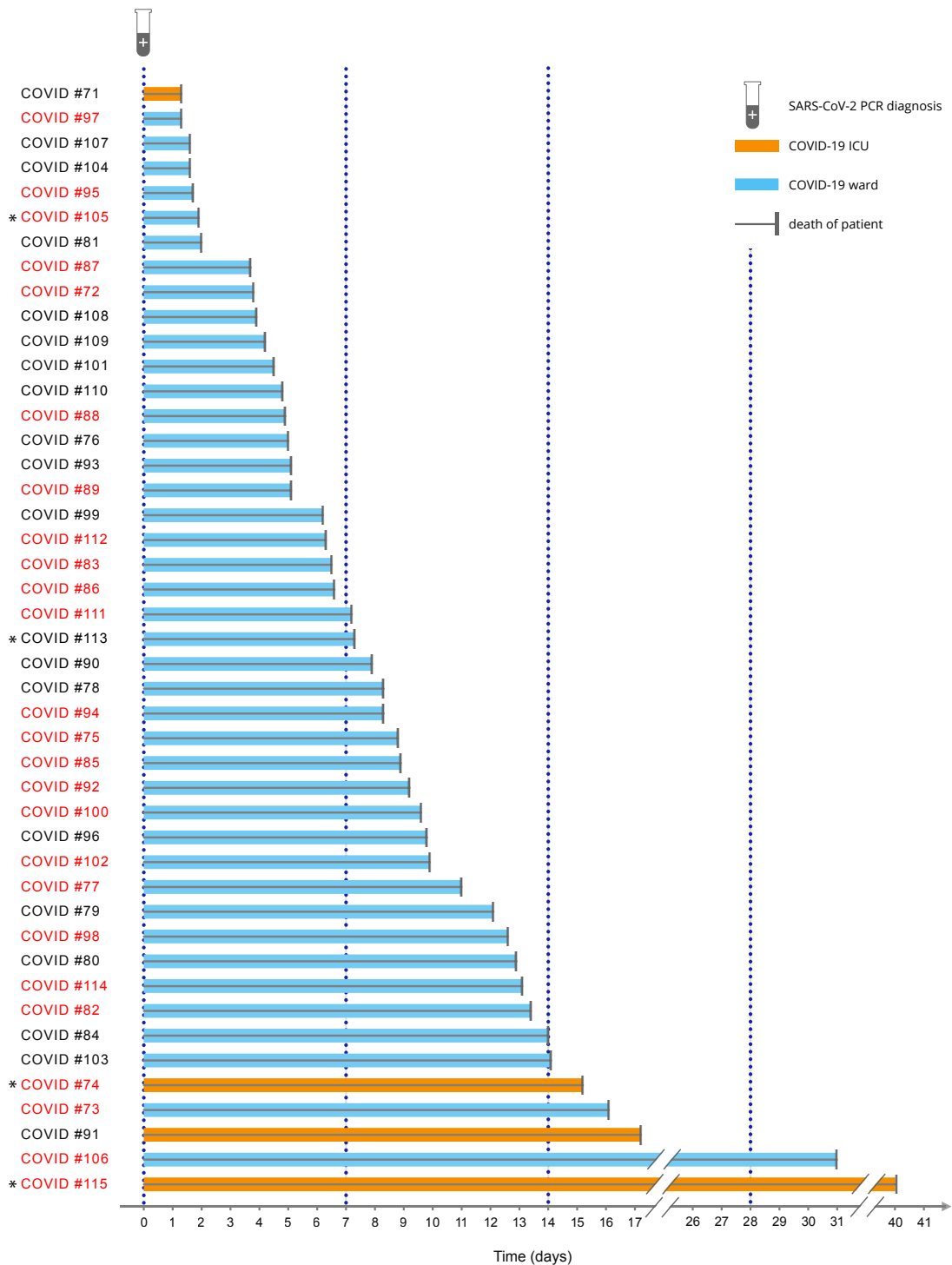
	COVID-19 (n=45)	Control (n=8)
COVID-19 diagnosis by PCR		
≤ 7 days prior to death	21 (47%)	
> 7 and ≤ 14 days	18 (40%)	
> 14 and ≤ 21 days	4 (9%)	
> 21 and ≤ 28 days	0 (0%)	
> 28 days	2 (4%)	
Cause of death		
from COVID-19	30 (67%)	
with COVID-19	15 (33%)	
Fatal breakthrough infection	25 (56%)	
Days hospitalized	6 (4–10)	15 (5–21)
Intensive care	4 (9%)	8 (100%)
Respiratory support		
Non-invasive modalities	43 (96%)	0 (0%)
Conventional oxygen therapy	21 (47%)	0 (0%)
High-flow nasal cannula	22 (49%)	0 (0%)
Invasive mechanical ventilation	2 (4%)	8 (100%)
Prone	2 (4%)	0 (0%)
Extracorporeal membrane oxygenation	0 (0%)	1 (13%)
Pharmacological therapy		
Anticoagulation	37 (82%)	8 (100%)
Steroids	33 (73%)	4 (50%)
Antivirals	2 (4%)	0 (0%)
Antibiotics	32 (71%)	8 (100%)
Interleukin receptor modulators	1 (2%)	0 (0%)
Convalescent plasma	0 (0%)	0 (0%)

### Procedure characteristics

	COVID-19 (n=45)	Control (n=8)
Postmortem interval in minutes	109 (92–153)	63 (54–74)
< 50 min	1 (2%)	2 (25%)
50 – 100 min	16 (36%)	6 (75%)
100 – 200 min	23 (51%)	0 (0%)
> 200 min	5 (11%)	0 (0%)
Duration of procedure in minutes	100 (74–124)	86 (73–96)
Rapid antigen test on nasopharyngeal swab		
Positive	41 (91%)	0 (0%)
Negative	4 (9%)	8 (100%)

**Figure S1. Patient, disease, and procedure characteristics of cohort II of 45 COVID-19 cases and 8 control cases, related to Figure 1**

Time variables and body mass index are expressed as median (interquartile range: Q1–Q3). Count variables are expressed as percentages.



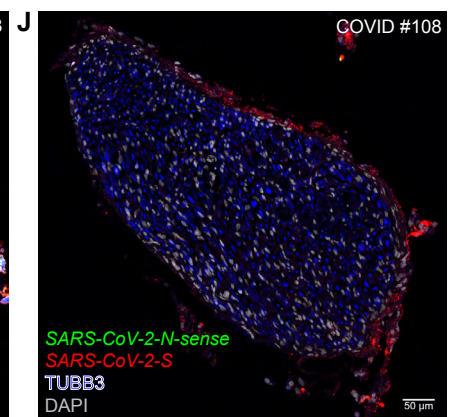
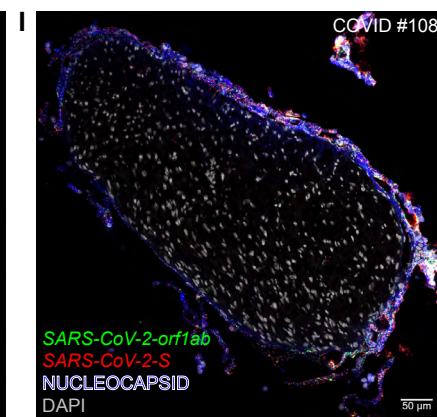
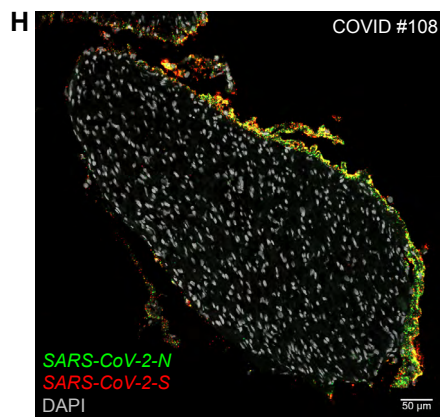
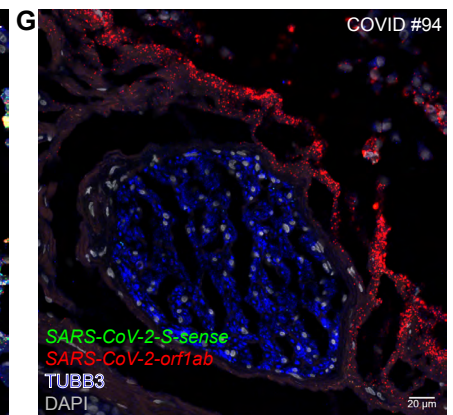
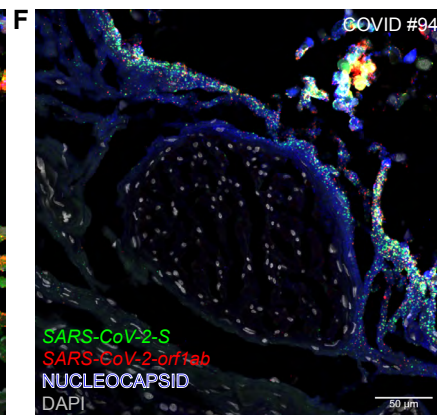
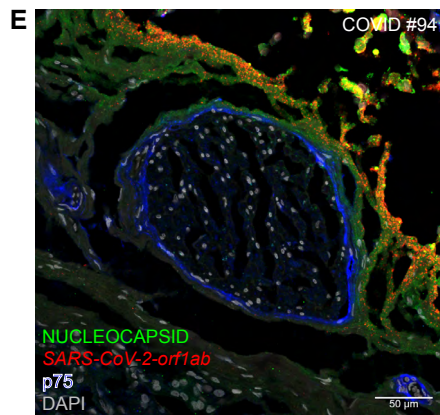
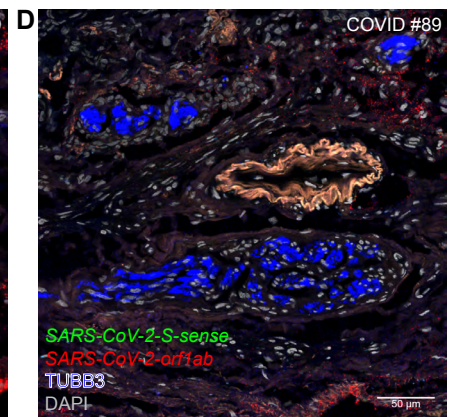
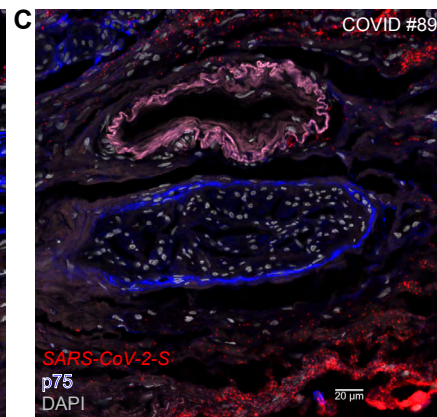
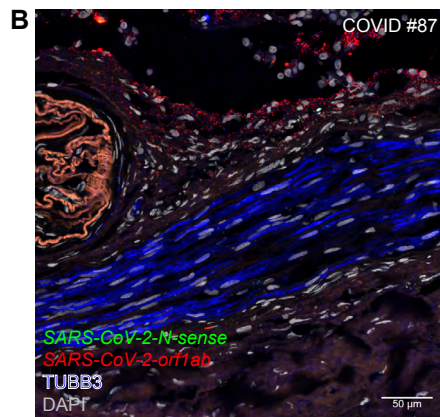
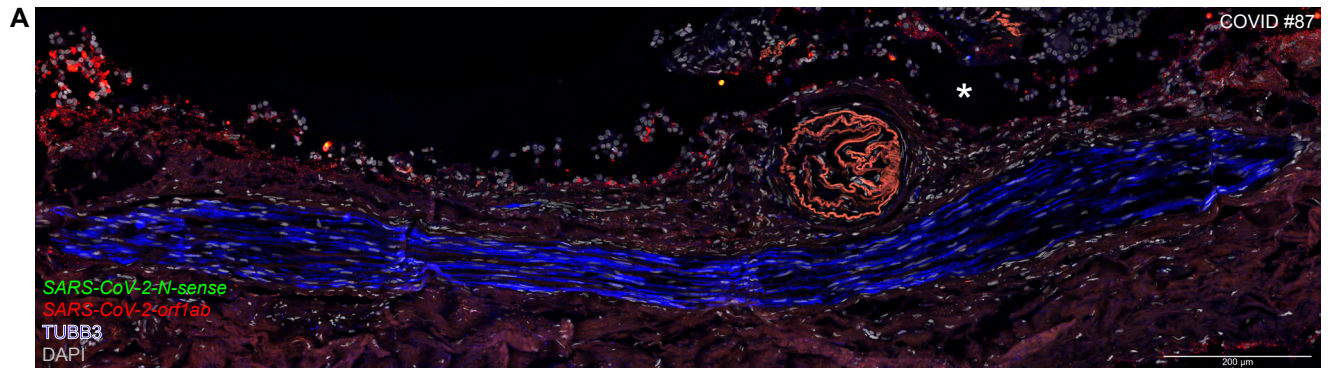
**Figure S2. Swimmer plot of cohort-II of 45 COVID-19 cases, related to Figure 1**

The vertical axis shows the pseudonyms of the cases. Red labels indicate the 25 cases of fatal breakthrough infection. Asterisks indicate the 4 non-informative cases (cases without viral RNA puncta and nucleocapsid-immunoreactive signal in the tissue samples). The horizontal axis shows the period in days starting from the time the nasopharyngeal swab was taken that led to the diagnosis of COVID-19 by PCR (indicated by a test tube at day 0) until the time of death (indicated by a vertical stop line at the end of a bar). Hospitalization in a COVID-19 unit is indicated by the start of the orange bar (ICU, intensive care unit) or the blue bar (ward).

COVID case #	survival (days)	rapid antigen test	Ct NP swab	Ct serum	Ct CSF	Anti-S IgG (AU/mL)	Anti-N IgG	vaccination status	cause of death	type of infection	non-informative	perineurial	lepto-meningeal	frontal lobe
71	1.3	positive	12.6	not available	not available	not available	not available	unvaccinated	from	unvaccinated				
72	3.8	positive	17.0	not available	negative	not available	not available	vaccinated	from	FBI				
73	16.1	positive	10.2	26.6	negative	73.6	negative	vaccinated	from	FBI			✓	
74	15.2	positive	21.6	negative	not available	32298.8	negative	vaccinated	from	FBI	✓			
75	8.8	positive	17.2	negative	negative	78693.4	positive	vaccinated	from	FBI				
76	5	positive	7.9	30.3	negative	144.8	negative	vaccinated	with	breakthrough			✓	
77	11	positive	17.1	32.0	negative	43465	negative	vaccinated	from	FBI				
78	8.3	positive	19.2	negative	negative	96	negative	vaccinated	with	breakthrough			✓	
79	12.1	positive	11.9	30.7	negative	97100.8	negative	vaccinated	with	breakthrough			✓	
80	12.9	positive	17.8	negative	negative	9277	positive	unvaccinated	from	unvaccinated				
81	2	positive	8.7	29.7	negative	72.9	negative	vaccinated	with	breakthrough				
82	13.4	positive	12.3	29.7	negative	248.1	negative	vaccinated	from	FBI				
83	6.5	positive	9.2	31.7	31.1	4092.1	negative	vaccinated	from	FBI			✓	
84	14	positive	15.2	negative	negative	37889	negative	vaccinated	with	breakthrough				
85	8.9	positive	18.0	30.3	negative	515.2	negative	vaccinated	from	FBI				
86	6.6	positive	11.9	negative	negative	84.3	negative	vaccinated	from	FBI				
87	3.7	positive	12.1	31.0	negative	80.2	negative	vaccinated	from	FBI		✓	✓	✓
88	4.9	positive	18.4	33.3	negative	>80000	negative	vaccinated	from	FBI		✓	✓	✓
89	5.1	positive	11.0	22.3	negative	<21	negative	vaccinated	from	FBI		✓	✓	✓
90	7.9	positive	not available	not available	not available	not available	not available	unknown	with	not applicable		✓		
91	17.2	positive	not available	not available	not available	not available	not available	unknown	from	not applicable				
92	9.2	positive	15.1	30.2	negative	>80000	negative	vaccinated	from	FBI				
93	5.1	positive	not available	not available	not available	not available	not available	unvaccinated	from	unvaccinated				
94	8.3	positive	12.8	29.0	34.6	7182.5	negative	vaccinated	from	FBI		✓	✓	
95	1.7	positive	10.3	35.2	negative	812.9	negative	vaccinated	from	FBI				
96	9.8	positive	9.8	negative	negative	38075.6	negative	vaccinated	with	breakthrough				
97	1.3	positive	not available	not available	not available	not available	not available	vaccinated	from	FBI				
98	12.6	positive	13.6	negative	negative	1139.8	negative	vaccinated	from	FBI				
99	6.2	positive	10.7	negative	negative	18246	negative	vaccinated	with	breakthrough			✓	
100	9.6	positive	11.5	negative	negative	>80000	negative	vaccinated	from	FBI			✓	
101	4.5	positive	not available	not available	not available	not available	not available	vaccinated	with	breakthrough			✓	
102	9.9	positive	16.2	35.4	negative	48.7	negative	vaccinated	from	FBI				
103	14.1	positive	18.3	negative	negative	>80000	positive	vaccinated	with	breakthrough				
104	1.6	positive	12.7	negative	negative	784.7	negative	vaccinated	with	breakthrough				
105	1.9	positive	20.0	negative	negative	12983.4	negative	vaccinated	from	FBI	✓			
106	31	negative	not available	not available	not available	not available	not available	vaccinated	from	FBI				
107	1.6	positive	10.1	negative	negative	6155.4	negative	vaccinated	with	breakthrough				
108	3.9	positive	13.4	34.3	negative	<21	negative	unvaccinated	with	unvaccinated		✓	✓	✓
109	4.2	positive	12.6	negative	negative	2338.3	negative	vaccinated	with	breakthrough				
110	4.8	positive	12.6	32.3	negative	<21	negative	unknown	with	not applicable		✓	✓	✓
111	7.2	positive	not available	not available	not available	not available	not available	vaccinated	from	FBI				
112	6.3	positive	13.9	32.2	not available	459.9	negative	vaccinated	from	FBI				
113	7.3	negative	23.8	negative	negative	11239.6	positive	unknown	from	not applicable	✓			
114	13.1	negative	19.0	negative	negative	15110.9	positive	vaccinated	from	FBI				
115	40	negative	31.0	negative	negative	50.5	positive	vaccinated	from	FBI	✓			

**Figure S3. Summary of the findings of cohort II of 45 COVID-19 cases, related to Figure 2**

Rapid antigen tests carried out on postmortem nasopharyngeal (NP) swabs were scored visually as positive or negative. Ct values are from PCR assays done on a postmortem nasopharyngeal swab, serum sample, and cerebrospinal fluid (CSF) sample. The values of the anti-S IgG titers in AU (Arbitrary Units)/ml are shown graphically in Figure 2D. The type of infection in a vaccinated patient who died *from* COVID-19 is referred to as "FBI", for fatal breakthrough infection. The type of infection in a vaccinated patient who died *with* COVID-19 is referred to as "breakthrough".



**Figure S4. RNAscope with *N*, *S*, *orf1ab*, *N-sense*, and *S-sense* probes and IHC with nucleocapsid antibody on sections of the lamina propria of the olfactory mucosa of perineurial cases, related to [Figure 5](#)**

Confocal images of sections through OM of COVID #87 (Delta), #89 (Delta), #94 (Delta), and #108 (Omicron BA.1). Names of genes (*italics*) and proteins (roman) are at the bottom left, scale bars at the bottom right. DAPI served as nuclear stain. Blood vessels in (A-D) emit autofluorescence in several channels.

TUBB3-IR signal identifies OSNs (A, B, D, G, and J) and p75-IR signal identifies pONFs (C and E). There are no *N-sense* puncta (A, B, and J) and no *S-sense* puncta (D and G), consistent with the absence of ongoing replication at these sites. Extracellular *orf1ab* puncta (A, B, D-G, and I), extracellular *S* puncta (C, F, H-J), and extracellular *N* puncta (H) together with hazy extracellular nucleocapsid-IR signal (E, F, and I) reflect extracellular virions spreading within the interstitium. Virions stay clear of the pONF enwrapment and do not invade olfactory axon fascicles.