

1 **Supplementary Material**

2 **Supplementary Figure S1: No SARS-CoV-2 colocalization of antibody signals is**
3 **observable in tissue from mock-infected control animals.** Volumetric projections
4 of nasal turbinate (A), lung (B), and trachea (C) tissue from mock-infected control
5 animals. No colocalization of the polyclonal serum (#1, green) and the monoclonal mix
6 (#2, magenta) can be detected. Cyan/grayscale = autofluorescence. Edge length of
7 grid squares = 1.000 μm . Total magnification = 1.6x (A and B), 2x (C).

8 **Supplementary Figure S2: No SARS-CoV-2 infection foci were detected in ferret**
9 **tracheal tissue.** Volumetric projection of a large ferret trachea section. Only unspecific
10 background staining is detectable for the polyclonal serum (#1, green) and the
11 monoclonal antibody mix (#2, magenta). No signal overlap of both antibody signals
12 was observable. Cyan/grayscale = autofluorescence. Edge length of grid squares =
13 800 μm . Total magnification = 2x.

14 **Supplementary Table S1: List of chemicals and reagents.**

15 **Supplementary Movie S1: Volumetric 3D projection of an LSFM-acquired, > 200**
16 **mm^3 -sized nasal turbinate section from a SARS-CoV-2-infected ferret.** The tissue
17 morphology was reconstructed using non-specific tissue autofluorescence (cyan).
18 Edge length of grid squares = 2 mm. Total magnification = 1.26x.

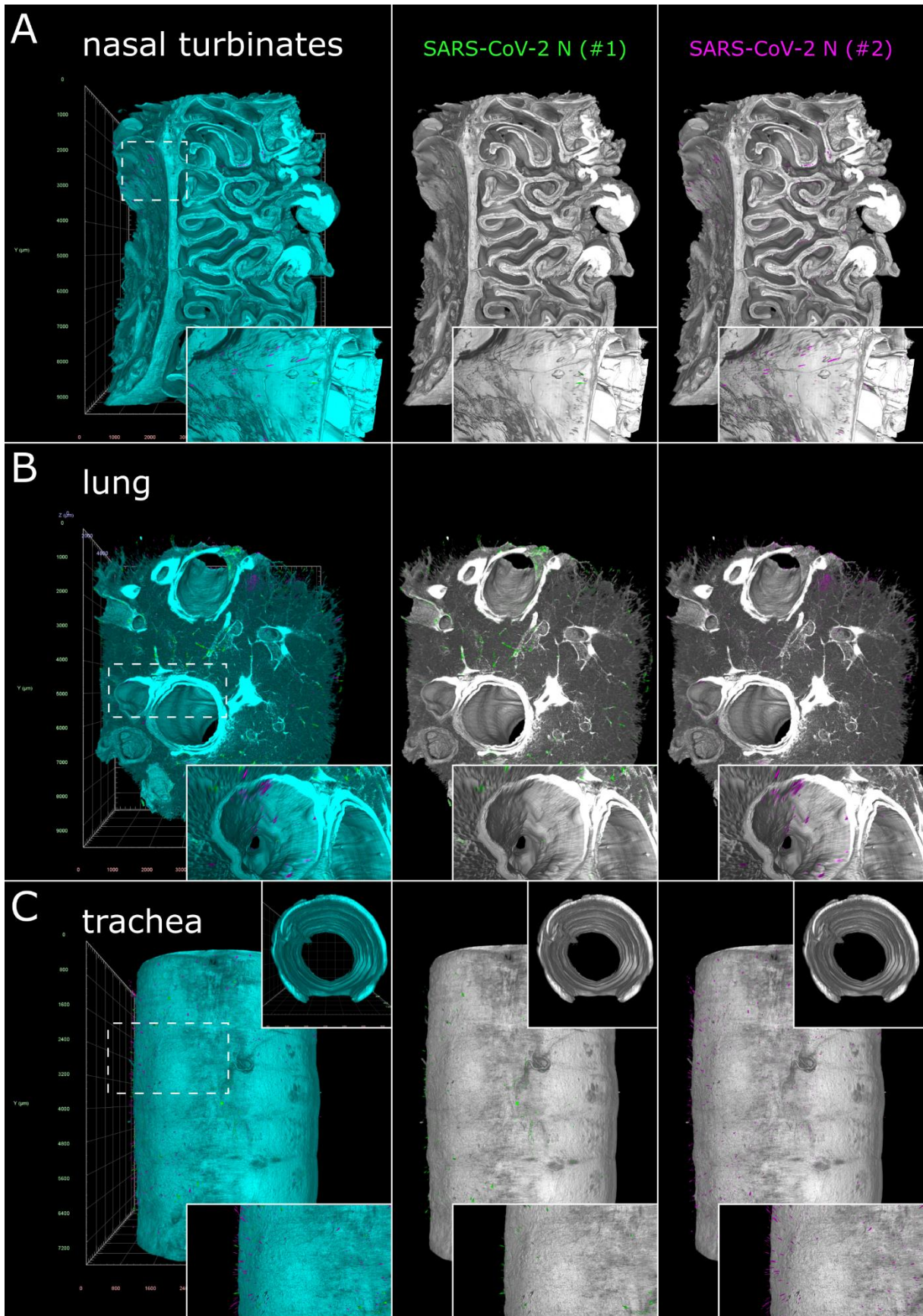
19 **Supplementary Movie S2: Fly-through animation of individual SARS-CoV-2**
20 **infection foci in ferret nasal turbinates at 4 days post-infection.** The three distinct
21 SARS-CoV-2 infection foci from Figures 3 and 4 are highlighted at timestamps (mm:ss)
22 00:09 [A1], 00:14 [A5], and 00:25 [A7]. Cyan = autofluorescence; green = SARS-CoV-
23 2 N #1; magenta = SARS-CoV-2 N #2. Edge length of grid squares = 300 μm . Total
24 magnification = 8x.

25 **Supplementary Movie S3: 360° rotation of a trachea section from a SARS-CoV-**
26 **2-infected ferret at 4 days post-infection.** No SARS-CoV-2-associated infection
27 spots were detected. Cyan = autofluorescence; green = SARS-CoV-2 N #1; magenta
28 = SARS-CoV-2 N #2. Edge length of grid squares = 800 μm . Total magnification = 2x.

29 **Supplementary Movie S4: Fly-through animation of likely debris-associated**
30 **SARS-CoV-2 infection in ferret lung tissue.** The colocalization of either SARS-CoV-
31 2 N antibody signal (#1, green; #2, magenta) inside a lung airway can be observed at

32 timestamp (mm:ss) 00:16. Cyan = autofluorescence. Edge length of grid squares =
33 200 μm . Total magnification = 6.4x.

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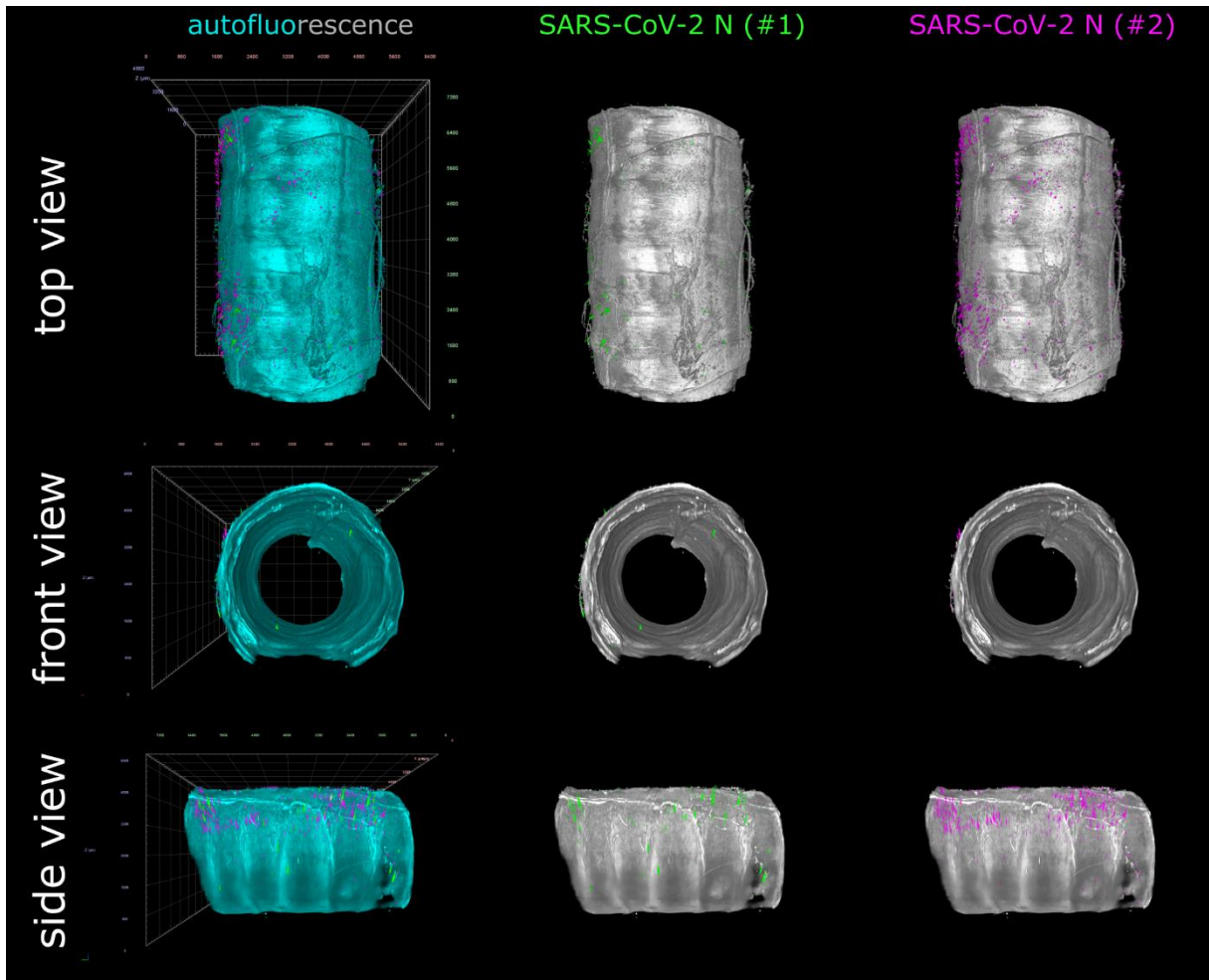


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36 **Supplementary Figure S1: No SARS-CoV-2 colocalization of antibody signals is**
 37 **observable in tissue from mock-infected control animals. Volumetric projections**

38 of nasal turbinate **(A)**, lung **(B)**, and trachea **(C)** tissue from mock-infected control
39 animals. No colocalization of the polyclonal serum (#1, green) and the monoclonal mix
40 (#2, magenta) can be detected. Cyan/grayscale = autofluorescence. Edge length of
41 grid squares = 1,000 μm . Total magnification = 1.6x (A and B), 2x (C).

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44 **Supplementary Figure S2: No SARS-CoV-2 infection foci were detected in ferret**
 45 **tracheal tissue at 4 days post-infection.** Volumetric projection of a large ferret
 46 trachea section. Only unspecific background staining is detectable for the polyclonal
 47 serum (#1, green) and the monoclonal antibody mix (#2, magenta). No signal overlap
 48 of both antibody signals was observable. Cyan/grayscale = autofluorescence. Edge
 49 length of grid squares = 800 μm . Total magnification = 2x.

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51 **Supplementary Table 1: List of chemicals and reagents.**

reagents	source	PO number	additional information
DMSO	Carl Roth	4720	dimethyl sulfoxide
ethanol	Carl Roth	9065	dehydrating agent
ethyl cinnamate	Alfa Aesar	A12906	clearing agent
Formical-2000™	Statlab	1314	decalcifier
glycine	Carl Roth	3908	autofluorescence quencher
heparin sodium salt	Carl Roth	7692	reduction of background
hydrogen peroxide	Carl Roth	8070	bleaching agent
n-hexane	Alfa Aesar	43263	delipidating agent
normal donkey serum	Bio-Rad	C06SBZ	blocking agent
Triton X-100	Carl Roth	3051	detergent
Tween-20	AppliChem	A4974	detergent

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