Appendix

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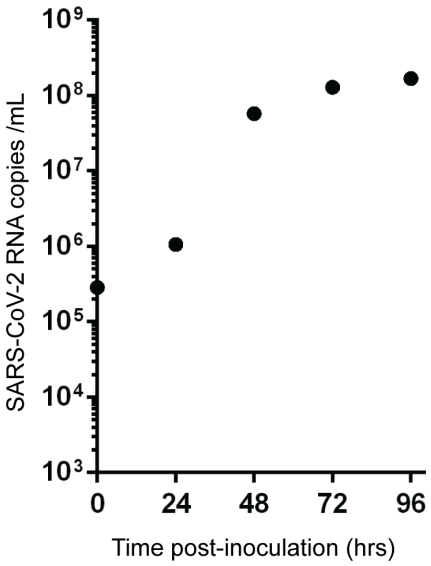
A

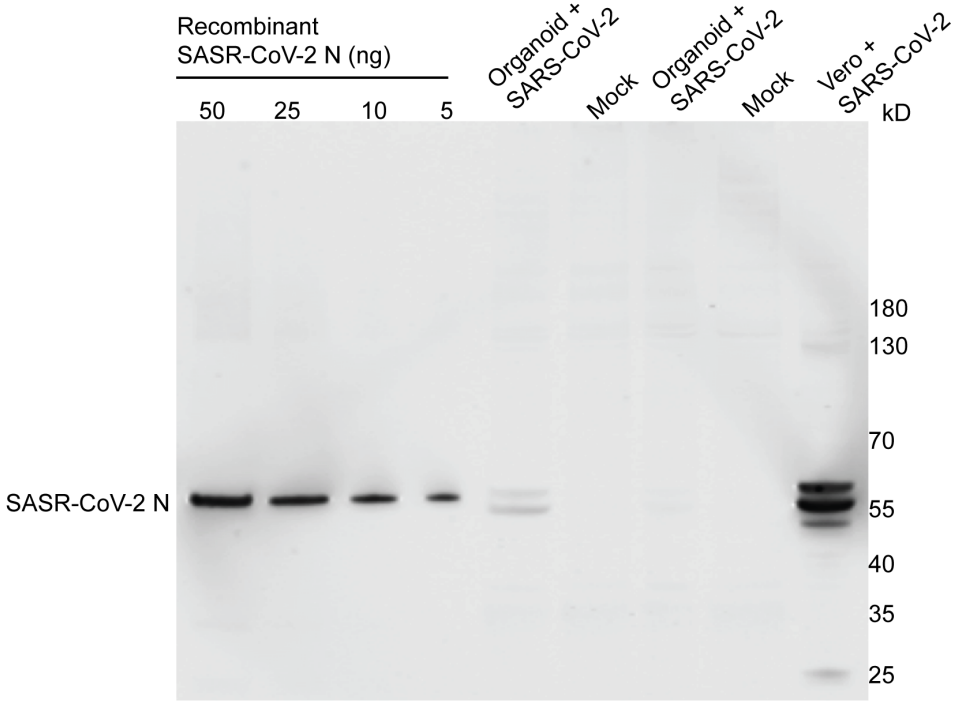


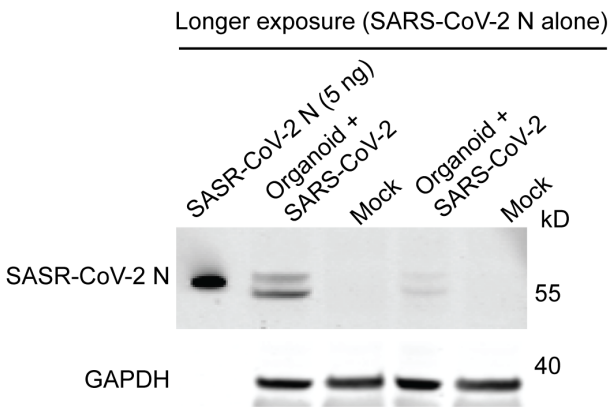
B

ELISA against the spike protein of SARS-CoV-2

Serum Sample	IgA		S1- IgG	
	Ratio	Result	Ratio	Result
AB4	6.63	Positive	4.95	Positive
AB3	9.96	Positive	2.75	Positive
AB1	>7	Positive	9.61	Positive
AB2	>7	Positive	0.09	Negative

C



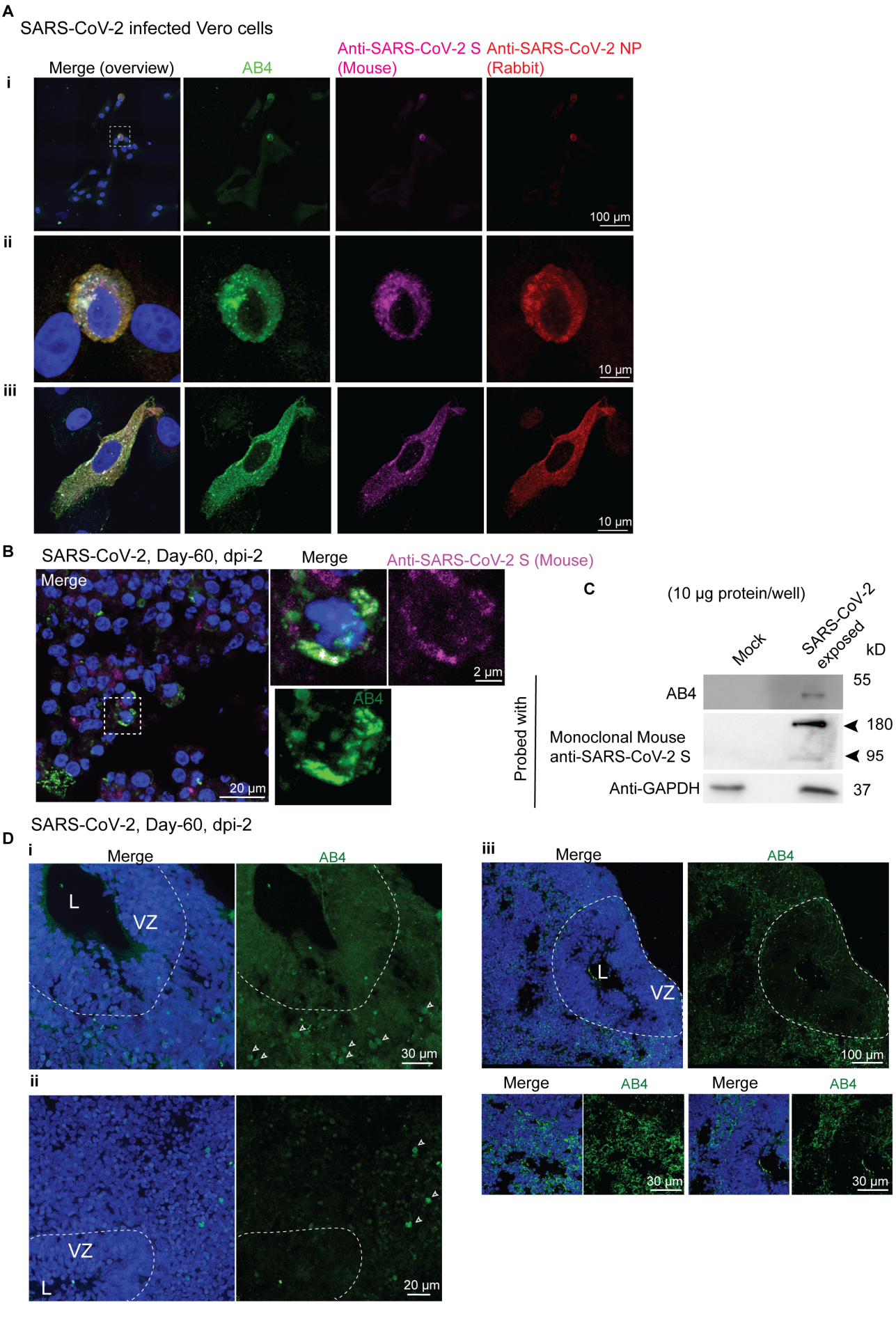


Appendix Figure S1. Assaying of SARS-CoV-2 replication and convalescent serum (Related to Figure 1)

A. SARS-CoV-2 productively replicates in inoculated Vero cells. Real-time quantitative polymerase chain reaction (qPCR) analysis of Vero cell culture supernatant shows that SARS-CoV-2 RNA drastically increases from 0- dpi until 3-dpi.

B. Isolation of COVID-19 convalescent serum and their analysis in an enzyme-linked immunosorbent assay (ELISA). Except for AB2, the rest of the convalescent serums contained IgG.

C. Affinity purified antibodies against SARS-CoV-2 N derived from convalescent serum were used at a concentration of 2 µg/mL to detected the SARS-CoV-2 nucleoprotein in lysates from virus-exposed organoids. Vero cells infected with SARS-CoV-2 and bacterially expressed and purified recombinant His6-tagged SARS-CoV-2 N at different concentrations served as positive controls. GAPDH was used as loading control to ensure equivalent protein loading of the lysates. Below a longer exposure of the same blot is shown in order to enhance the signal of the SARS-CoV-2-exposed organoids.


A
SARS-CoV-2 infected Vero cells
Merge (overview)
AB4
Anti-SARS-CoV-2 S (Mouse)
Anti-SARS-CoV-2 NP (Rabbit)
i
ii
iii
100 µm
10 µm
10 µm
B
SARS-CoV-2, Day-60, dpi-2
Merge
Anti-SARS-CoV-2 S (Mouse)
Merge
AB4
20 µm
2 µm
C
(10 µg protein/well)
Mock
SARS-CoV-2 exposed
kD
AB4
55
Probed with
Monoclonal Mouse anti-SARS-CoV-2 S
180
95
Anti-GAPDH
37
D
SARS-CoV-2, Day-60, dpi-2
i
Merge
AB4
L
VZ
30 µm
ii
VZ
L
20 µm
iii
Merge
AB4
L
VZ
100 µm
Merge
AB4
Merge
AB4
30 µm
30 µm

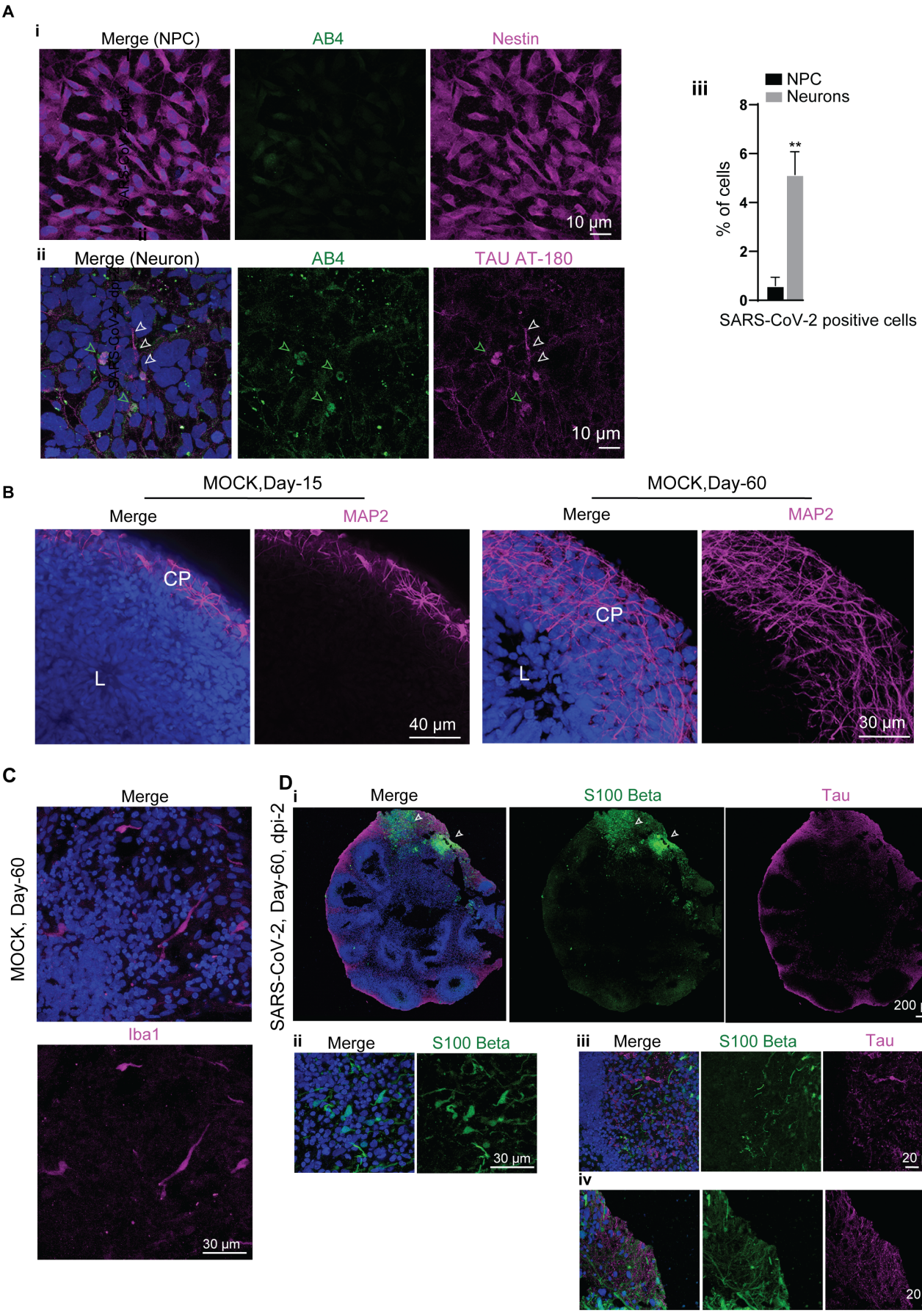
Appendix Figure S2. The convalescent serum AB4 detects SARS-CoV-2-positive cells (Related to Figure 1)

A. The convalescent serum AB4 (green) specifically recognizes SARS-CoV-2 infected Vero cells **(i** to **iii).** Mouse monoclonal Anti-SARS-CoV-2 S (magenta) and a polyclonal Anti-SARS-CoV-2 NP (red) validate the specificity of the AB4. Dapi is used to stain the nuceus. At least 100 cells were examined. Figures display scale bars. At least two exemplary images of cells **(ii** and **iii)** are shown.

B. Immunostaining detection of SARS-CoV-2 in organoids exposed to SARS-CoV-2. Mouse monoclonal Anti-SARS-CoV-2 S (magenta) detects SARS-CoV-2-positive cells labeled by AB4 (green). Figures display scale bars.

C. Detection of SARS-CoV-2 in organoid extracts exposed to SARS-CoV-2 in Western blots. AB4 recognizes a ~55 kD protein band that is similar to the size of the nucleoprotein of the virus. The monoclonal Anti-SARS-CoV-2 S recognizes cleaved spike protein in addition to ~180 kD corresponding to the sizes of un-cleaved S protein (S0, ~180 kD). Arrowheads mark un-cleaved spike protein (S0, ~180 kD) and the cleaved spike protein (~75 kD). Representative blots from three independent experiments are given.

D. Representative overview images **(i-iii)** of SARS-CoV-2-positive cells specified by AB4. Dashed lines mark elongated nuclei-containing compact ventricular zone (VZ). Arrow heads point SARS-CoV-2-positive cells, which are mostly away from VZ. Figure panels have scale bars.

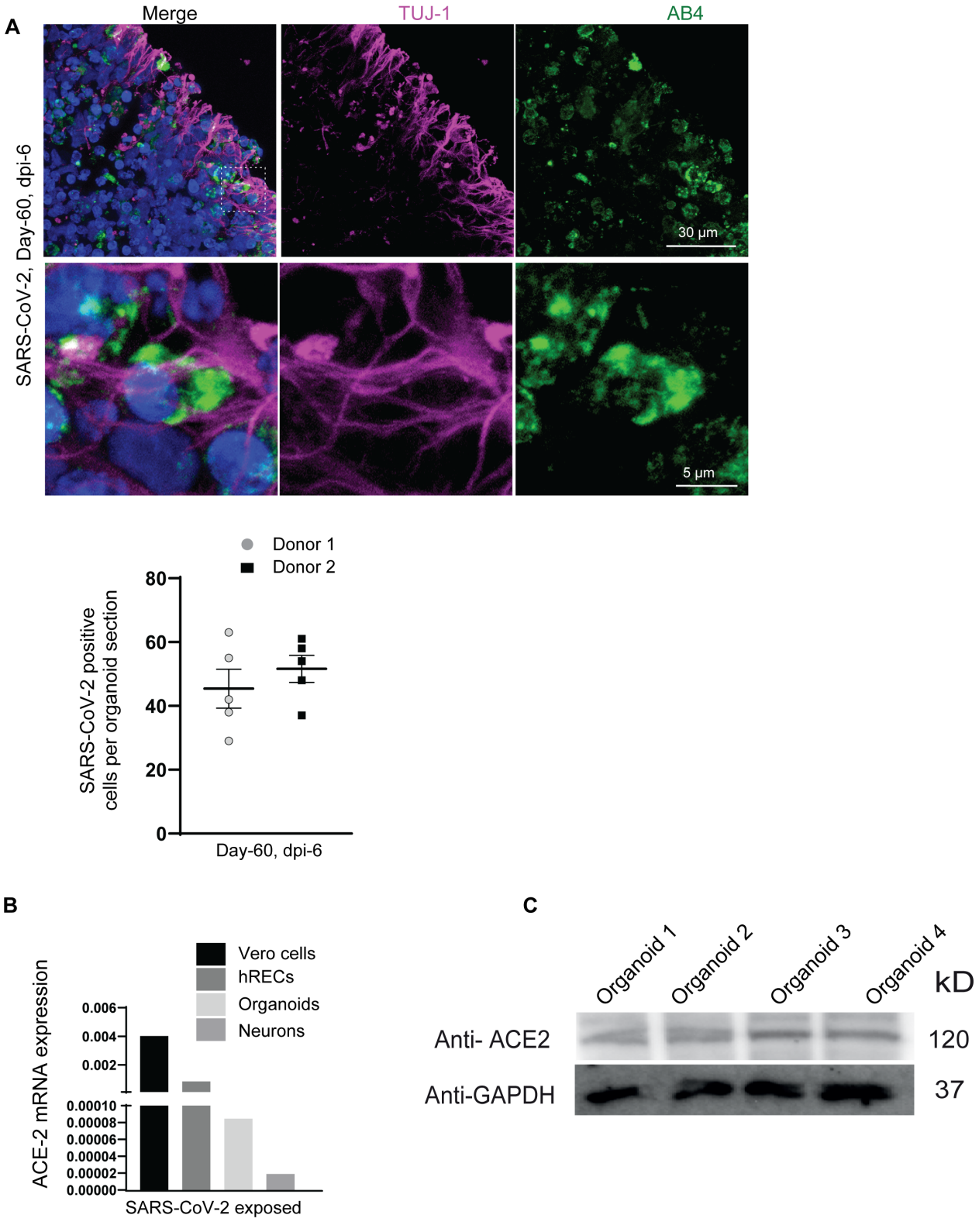

A
i
Merge (NPC)
AB4
Nestin
SARS-CoV-2, dpi-2
10 µm
ii
Merge (Neuron)
AB4
TAU AT-180
10 µm
iii
NPC
Neurons
% of cells
SARS-CoV-2 positive cells
**
B
MOCK,Day-15
MOCK,Day-60
Merge
MAP2
CP
L
40 µm
30 µm
C
Merge
MOCK, Day-60
Iba1
30 µm
D
SARS-CoV-2, Day-60, dpi-2
Merge
S100 Beta
Tau
200 µm
30 µm
20 µm
iv

Appendix Figure S3. 2D experiments, determination of Iba-1-positive and S100β-positive cells in Day-60 organoids (Related to Figure 1)

A. SARS-CoV-2 preferably targets neurons. In contrast to iPSC-derived NPCs (Nestin, Magenta) (**i),** SARS-CoV-2 (green) targets and localizes at the soma of neurons (Tau, Magenta). Arrowheads label Tau containing axons of neurons that are positive for the virus. Dapi is used to stain the nuceus. At least 500 cells in each varieties were examined from two independent (n=2) experiments. Figures display scale bars. Bar diagram at right **(iii)** quantifies the relative proportions of SARS-CoV-2 in NPCs and neurons. At least 300 cells across three independent (n=3) experiments were analyzed. Unpaired t test with Welch's correction, **P<0.01. Error bars represent mean ± SEM.

B. Compared to Day-15 organoids **(i),** Day-60 organoids **(ii)** exhibit signs of cortical maturation as distinguished by the abundance of MAP2-positive neurons in their cortical plate (CP). Note an increased size and thickness of MAP2-positive cortical plate in Day-60 organoids.

C-D. Day-60 organoids show immunireactivity to Iba-1 which specify microglia (magenta) **(C).** An overview image of whole organoid shows the presence of S100β-positive astrocytes (green) **(Di-iv).** At least two region of interest are shown in high magification **(ii-iv).**

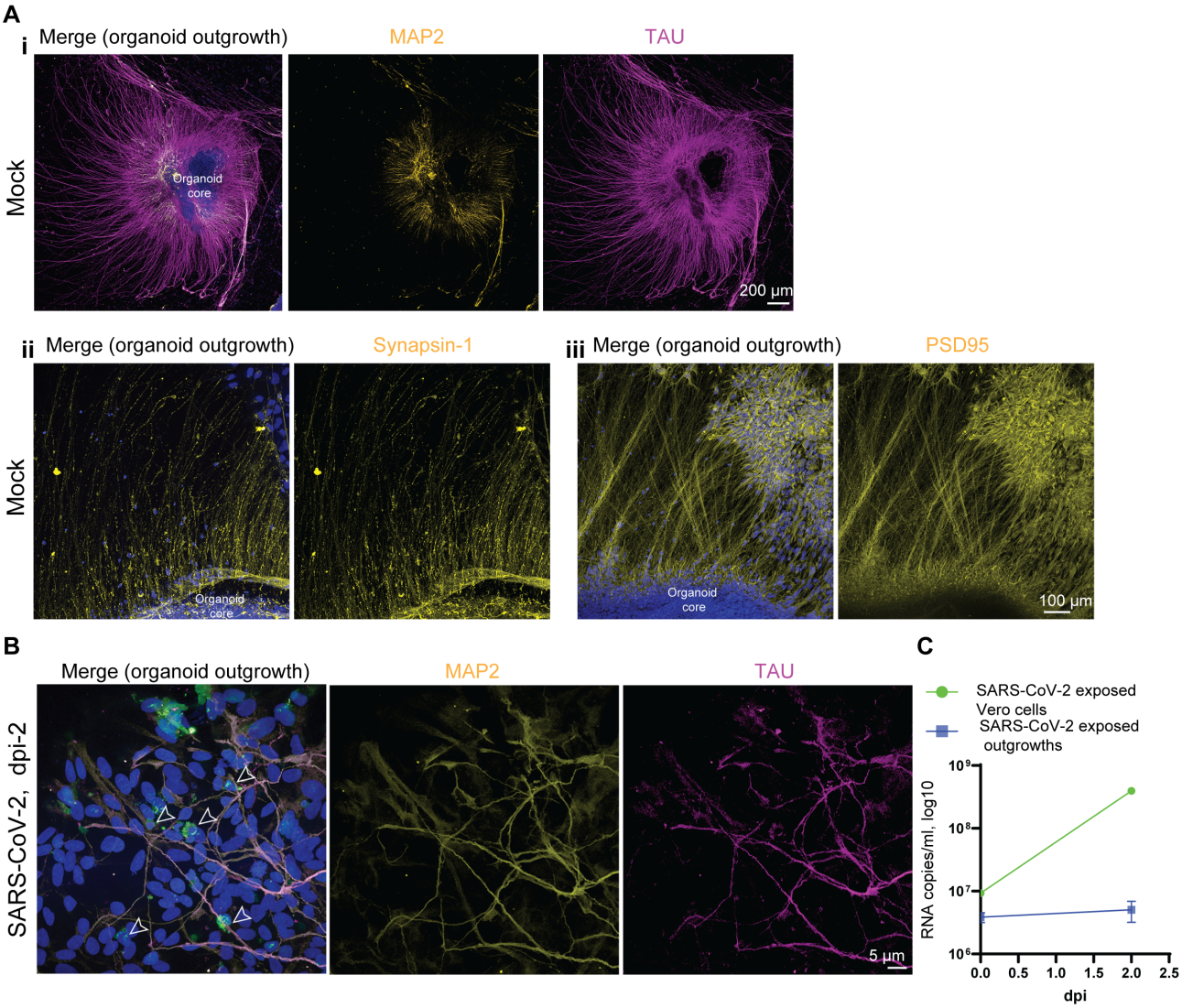


Appendix Figure S4. SARS-CoV-2-positive cells in Day-60 organoids six days after post infection (6-dpi) and determination of ACE2 mRNA and protein levels in organoids (Related to Figure 1)

A. SARS-CoV-2-positive cells in 6-dpi organoids of Day-60. SARS-CoV-2-positive cells (green) in cortical plate of organoids that is specified by TUJ-1-positive neurons (magenta). Magnified region (dotted while box) is given below, showing the peri-nuclear location of SARS-CoV-2 in neurons. Dapi is used to stain the nuceus. At least 5 organoids from two different batches (n=2) are tested. Figures display scale bars. The bar diagram below quantifies frequencies of SARS-CoV-2-positive cells. Each point represents one organoid section. Note, there is no difference between frequencies of SARS-CoV-2-positive cells between dpi-2, dpi-4 and dpi-6 (refer to **Figure 1E**).

B. Relative fold differences of ACE-2 mRNA expression between Vero cells, human respiratory epithelial cells (hRECs), brain organoids, and iPSC-derived neurons. Data are from multiple technical replicates from two (n=4) independent experiments.

C. Western blots for the presence of ACE2 protein in brain organoids. Note, ACE2 antibodies recognize the protein only at higher exposure condition. A representative blot from three independent experiment is shown. At least 4 independent organoids were tested. GAPDH is used as a loading control.

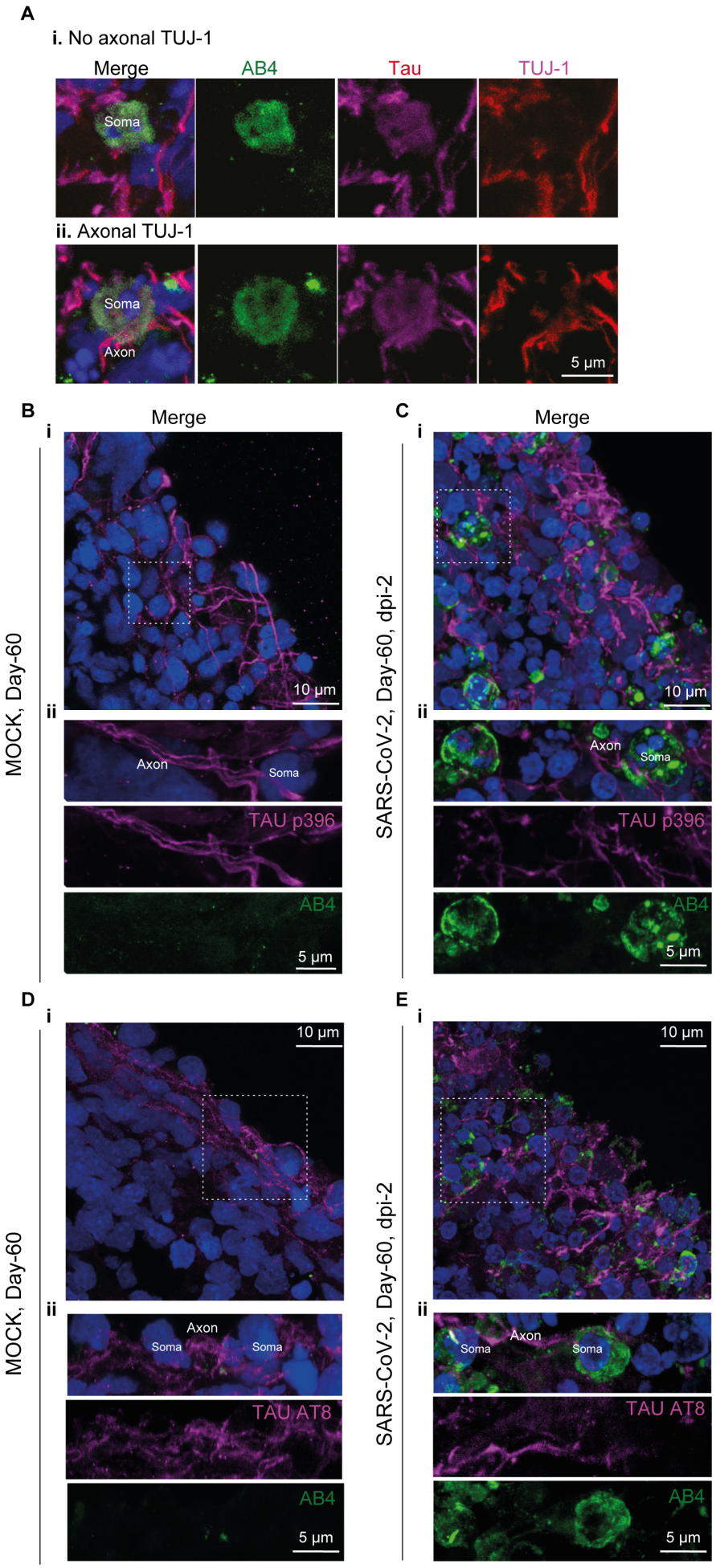


Appendix Figure S5. Organoid out growth experiments (Related to Figure 1)

A. Organoid slices of 60-day old organoids further cultures for two weeks exhibit mature neuronal markers of MAP2, Tau, Synapsin-1 and PSD95 **(i-iii).**

B. Similar to organoids, SARS-CoV-2 (green) localizes at the soma (Arrowheads) of neurons emanating from the organoid slice. Dapi is used to stain the nuceus.

C. Determination of viral progeny. Only a slight and insignifcant increase in viral RNA is measured in the supernatants of organoid slices compared to Vero cells (used as positive control). Data are obtained from five technical replicates from four (n=4) independent batches of organoid outgrowths. Error bars represent mean ± SEM.


A
i. No axonal TUJ-1
Merge
AB4
Tau
TUJ-1
Soma
ii. Axonal TUJ-1
Soma
Axon
5 µm
B
Merge
i
ii
MOCK, Day-60
10 µm
Axon
Soma
TAU p396
AB4
5 µm
C
Merge
i
ii
SARS-CoV-2, Day-60, dpi-2
10 µm
Axon
Soma
TAU p396
AB4
5 µm
D
i
ii
MOCK, Day-60
10 µm
Axon
Soma
Soma
TAU AT8
AB4
5 µm
E
i
ii
SARS-CoV-2, Day-60, dpi-2
10 µm
Axon
Soma
Soma
TAU AT8
AB4
5 µm

Appendix Figure S6. Tau S396 and Tau S202/T205 do not localize into somas of SARS-CoV-2-positive neurons (Related to Figure 2)

A. Fraction of SARS-CoV-2-positive neurons (green) exhibiting Tau- and TUJ-1 positive axons (magenta and red respectively). The example **(i)** shows that there is no intact axon. The second **(ii)** example shows the presence of both Tau and TUJ-1. Note in both examples, Tau but not TUJ-1 localizes to the soma. Dapi is used to stain the nuceus. Refer to **Figure 2Bv** for quantifications.

B. Tau p396 antibody that labels phosphorylated Tau at S396 localizes to axons of Day-60 (magenta, mock and SARS-CoV-2-exposed organoids) and do not localize to soma of SARS-CoV-2-positive neurons (green) **(C).** At least four organoids from two different batches (n=2) are tested. Figures display scale bars.

D. AT8 antibodies that labels phosphorylated Tau at S202 and T205 localizes to axons of Day-60 (magenta, mock and SARS-CoV-2-exposed organoids). This antibody does not recognize Tau in soma of SARS-CoV-2-positive neurons (green) **(E).** At least four organoids from two different batches (n=2) are tested. Figures display scale bars. The insets of panel D and E contain selected stacks compared to the low magnification image for better clarity.