

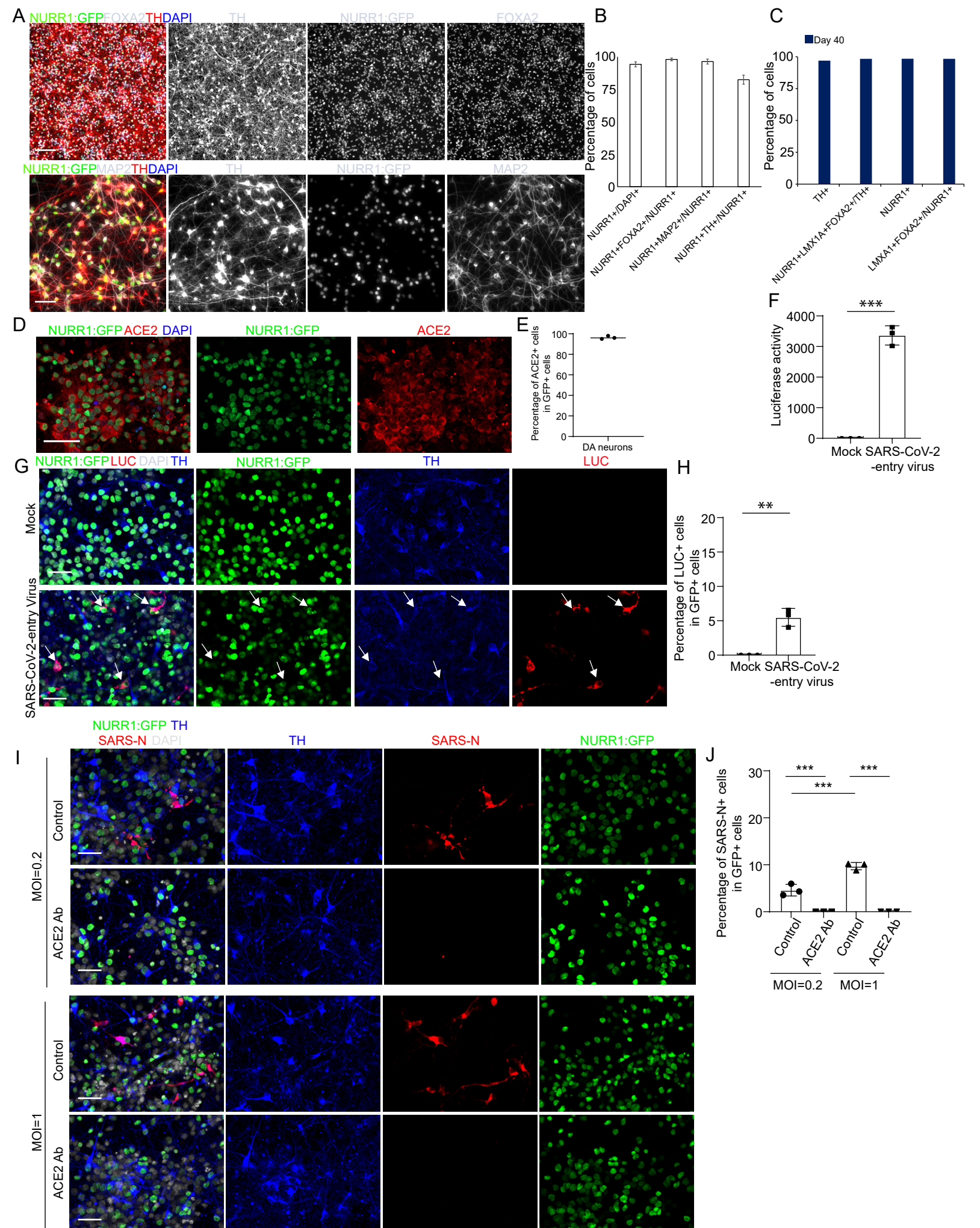
Supplemental Information

SARS-CoV-2 infection causes dopaminergic

neuron senescence

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Figure S1



SUPPLEMENTAL INFORMATION

Figure S1. Characterization of hPSC-derived DA neurons. Related to Figure 1.

(A and B) Representative confocal images (A) and quantification (B) of NURR1: GFP H9-derived DA neurons stained with antibodies recognizing NURR1-GFP, TH, MAP2 or FOXA2. Scale bar=100 μm (upper panel) and 50 μm (lower panel). n=3 independently biological replicates.

(C) Single cell RT-qPCR analysis of DA neuron marker genes from FACS based NURR1-GFP⁺ purified cells at day40 (67 cells) from previous study ¹.

(D and E) Representative confocal images (D) and quantification of ACE2⁺ cells in NURR1-GFP⁺ cells (E) of purified NURR1: GFP-derived DA neurons stained with ACE2 antibody. Scale bar=50 μm . n=3 independent biological replicates.

(F) Luciferase activity in lysates from purified NURR1: GFP-derived DA neurons at 24 hpi following exposure to SARS-CoV-2-entry virus at MOI=0.01. The Y-axis indicates the absolute luciferase activity between mock and infect groups. n=3 independent biological replicates.

(G and H) Representative confocal images (G) and quantification (H) of purified NURR1: GFP-derived DA neurons infected with SARS-CoV-2-entry virus (MOI=0.01) at 24 hpi using antibodies against luciferase and DA neuron markers. Scale bar=50 μm . n=3 independent biological replicates.

(I and J) Representative confocal images (I) and quantification (J) of purified NURR1: GFP-derived DA neurons infected with SARS-CoV-2 virus at different MOI (MOI=0.2 or MOI=1) and treated with ACE2 blocking antibody at 72 hpi using antibodies against SARS-N and DA neuron markers. Scale bar=50 μm . n=3 independent biological replicates.

Data was presented as mean \pm STDEV. *P* values were calculated by unpaired two-tailed Student's *t* test. ***P* < 0.01, ****P* < 0.001.

Figure S2

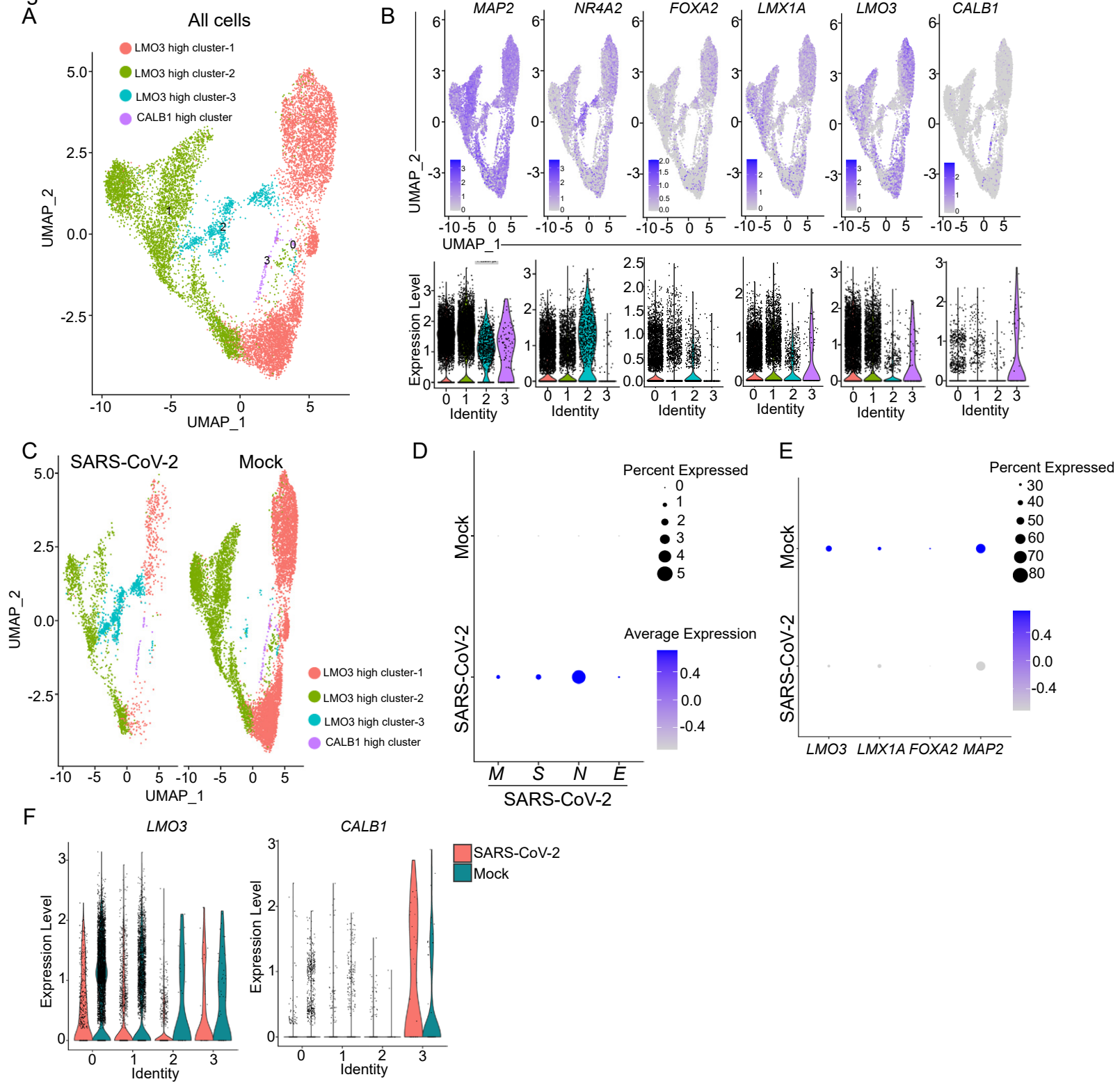


Figure S2. hPSC-derived DA neurons are susceptible and permissive to SARS-CoV-2 infection. Related to Figure 2.

(A) UMAP analysis of the mock and SARS-CoV-2 infected purified NURR1: GFP-DA neurons at 48 hpi (MOI=0.2).

(B) UMAP and violin plot analysis of DA neuron marker genes.

(C) UMAP analysis of the mock and SARS-CoV-2 infected purified NURR1: GFP-DA neurons at 48 hpi (MOI=0.2).

(D) Dot plot analysis of SARS-CoV-2 viral transcripts in the mock and SARS-CoV-2 infected purified NURR1: GFP-DA neurons at 48 hpi (MOI=0.2).

(E) Dot plot analysis of expression levels of DA neuron marker genes in the mock and SARS-CoV-2 infected purified NURR1: GFP-DA neurons at 48 hpi (MOI=0.2).

(F) Violin plot analysis of expression levels of DA neuron marker genes in different populations of the mock and SARS-CoV-2 infected purified NURR1: GFP-DA neurons at 48 hpi (MOI=0.2).

Figure S3

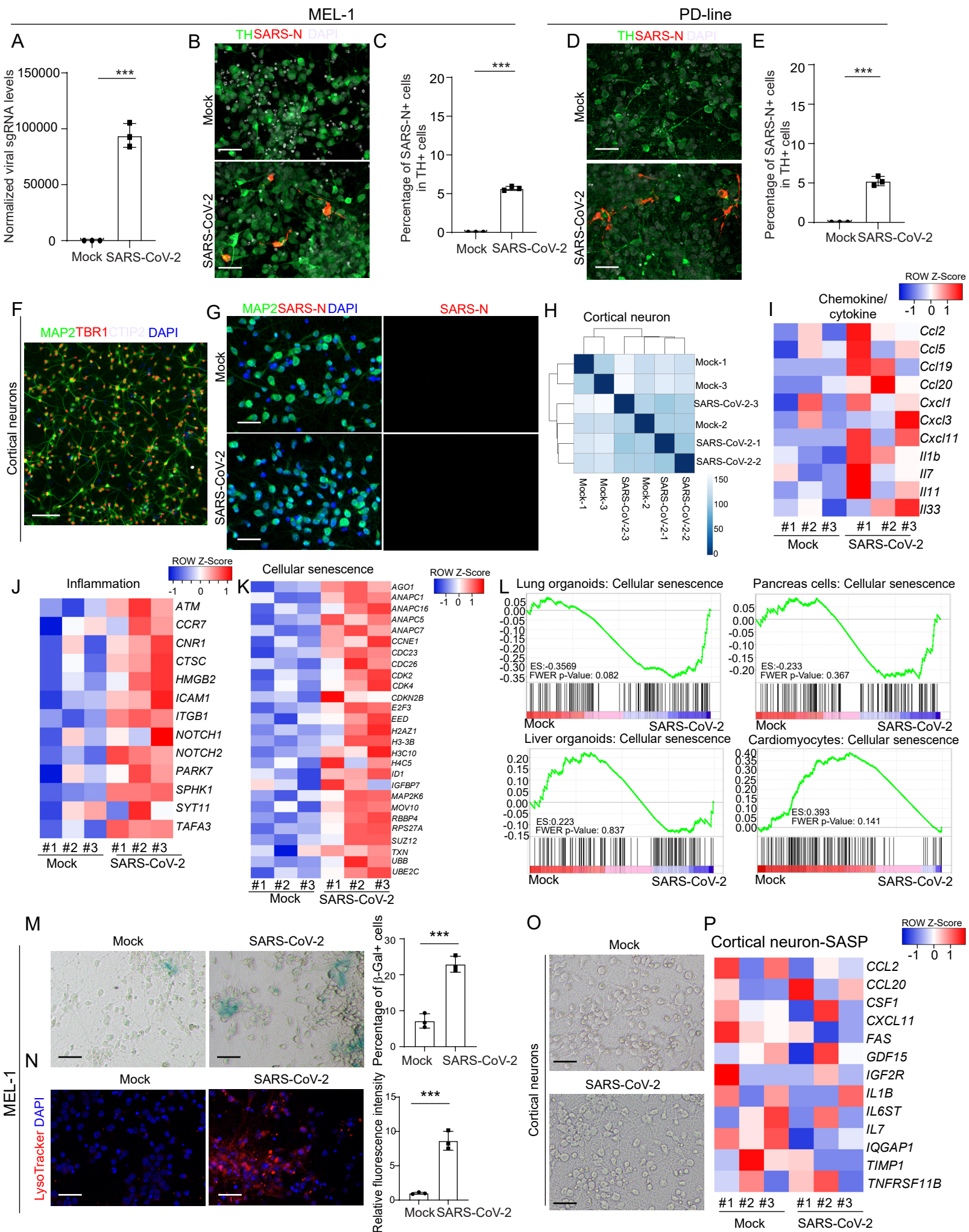


Figure S3. SARS-CoV-2 infection of hPSC-derived DA neurons triggered senescence phenotype. Related to Figure 1 and Figure 3.

(A) RT-qPCR analysis of total RNA extracted from MEL1-derived DA neurons at 48 hpi of SARS-CoV-2 infection (MOI=0.2) for viral N sgRNA. The graph depicts the mean sgRNA level normalized to ACTB. n=3 independent biological replicates.

(B and C) Representative confocal images (B) and quantification of the percentage of SARS-N⁺ cells in TH⁺ cells (C) of MEL1-DA neurons infected with SARS-CoV-2 (MOI=0.1) at 72 hpi using antibodies against SARS-CoV-2 Nucleocapsid protein (SARS-N) and marker for DA neurons. Scale bar=50µm. n=3 independent biological replicates.

(D and E) Representative confocal images (D) and quantification of the percentage of SARS-N⁺ cells in TH⁺ cells (E) of SNCA PD line-2copy-DA neurons infected with SARS-CoV-2 (MOI=0.1) at 72 hpi using antibodies against SARS-CoV-2 Nucleocapsid protein (SARS-N) and marker for DA neurons. Scale bar=50 µm. n=3 independent biological replicates. N=3 independent biological replicates.

(F) Representative confocal images of H9-cortical neurons using antibodies against MAP2, TBR1, and CTIP2. Scale bar=100 µm. n=3 independent biological replicates.

(G) Representative confocal images of H9-cortical neurons infected with SARS-CoV-2 (MOI=0.1) at 72 hpi using antibodies against SARS-CoV-2 Nucleocapsid protein (SARS-N) and MAP2. Scale bar=50µm. n=3 independent biological replicates.

(H) Clustering analysis of the mock or SARS-CoV-2 infected H9-derived cortical neurons at 48 hpi (MOI=0.2).

(I and J) Heatmap of chemokine/cytokines (I) and inflammation associated genes (J) in the mock or SARS-CoV-2 infected NURR1: GFP H9-derived DA neurons at 48 hpi (MOI=0.2).

(K) Heatmap of senescence associated genes in the mock or SARS-CoV-2 infected NURR1: GFP H9-derived DA neurons at 48 hpi (MOI=0.2).

(L) Gene set enrichment analysis (GSEA) of cellular senescence pathway in the mock or SARS-CoV-2 infected hPSC-derived lung organoids (GSE155241), pancreas cells (GSE151803), liver organoids (GSE151803) and cardiomyocytes (GSE151880).

(M) β-Gal staining and quantification of the mock or SARS-CoV-2 infected MEL1-derived DA neurons at 72 hpi (MOI=0.1). Scale bar=75µm. n=3 independent biological replicates.

(N) Representative confocal images and quantification of MEL1-DA neurons infected with the mock or SARS-CoV-2 (MOI=0.1) at 72 hpi using LysoTracker. Scale bar=50µm. n=3 independent biological replicates.

(O) β-Gal staining and quantification of the mock or SARS-CoV-2 infected H9-derived cortical neurons at 72 hpi (MOI=0.1). Scale bar=75µm. n=3 independent biological replicates.

(P) Heatmap of SASP associated genes in the mock or SARS-CoV-2 infected H9-derived cortical neurons at 48 hpi (MOI=0.2).

Data was presented as mean ± STDEV. *P* values were calculated by unpaired two-tailed Student's *t* test. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Figure S4

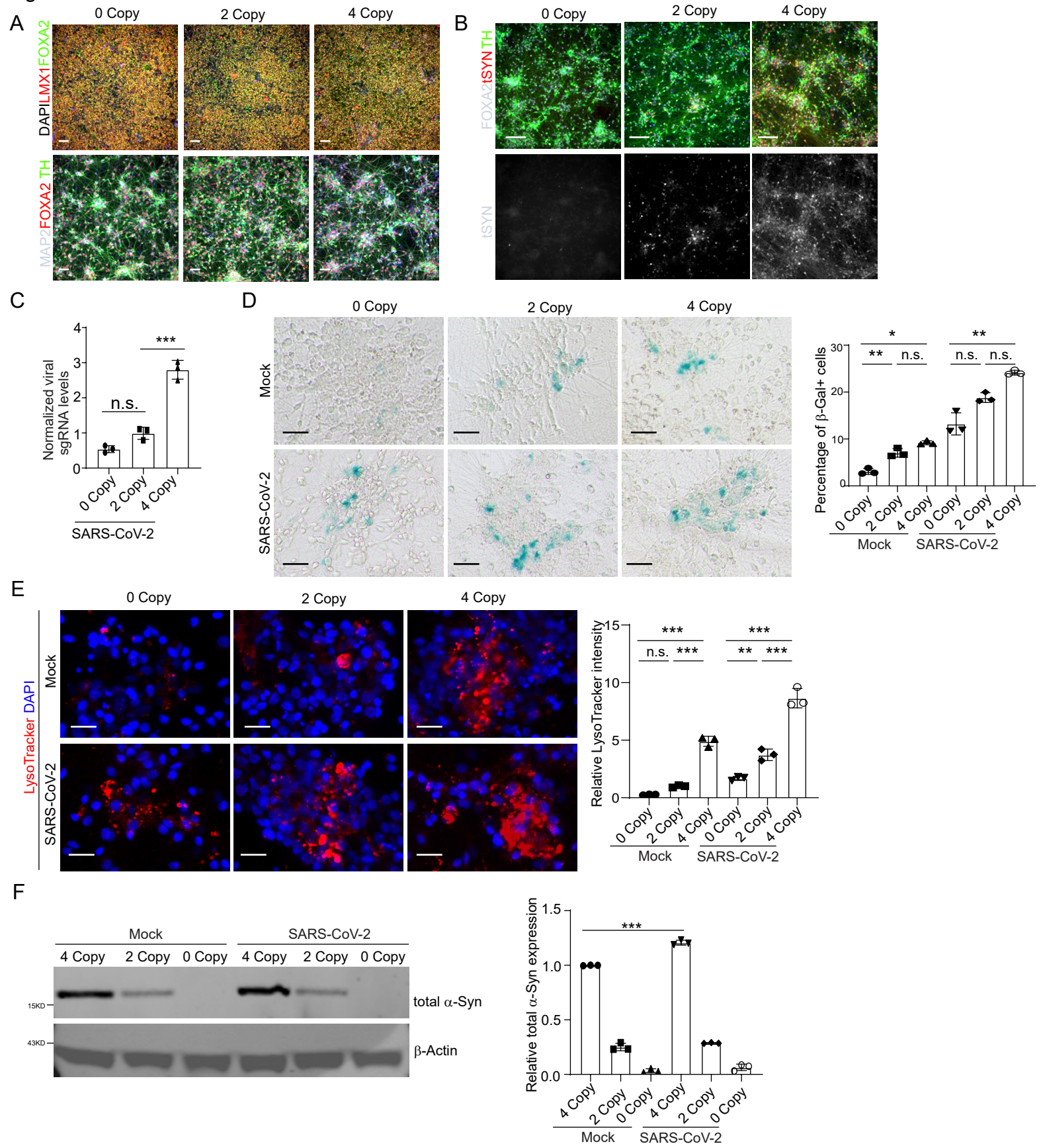


Figure S4. SARS-CoV-2 infection on SNCA 0 copy, 2 copy and 4 copy PD-iPSC-derived DA neurons. Related to Figure 3.

(A) Representative confocal images of *SNCA* lines-derived DA neurons at day 20 (upper) and day 45 (lower) using antibodies against markers for DA neurons. Scale bar=50 μ m. n=3 independent biological replicates.

(B) Representative confocal images of *SNCA* lines-derived DA neurons at day 45 using antibodies against total α -Syn and markers for DA neurons. Scale bar=50 μ m. n=3 independent biological replicates.

(C) qRT-PCR analysis of total RNA extracted from *SNCA* lines-derived DA neurons at 48 hpi of SARS-CoV-2 infection (MOI=0.2) for viral N sgRNA. The graph depicts the mean sgRNA level normalized to *ACTB*. n=3 independent biological replicates.

(D) β -Gal staining and quantification of the mock or SARS-CoV-2 infected *SNCA* lines-derived DA neurons at 72 hpi (MOI=0.1). Scale bar=75 μ m. n=3 independent biological replicates.

(E) Representative confocal images and quantification of *SNCA* lines-derived DA neurons infected with the mock or SARS-CoV-2 (MOI=0.1) at 72 hpi stained with LysoTracker. Scale bar=50 μ m. n=3 independent biological replicates.

(F) Western blot analysis and quantification of senescence related proteins of total α -Syn in mock or SARS-CoV-2 infected *SNCA* lines-derived DA neurons at 48 hpi (MOI=0.2). n=3 independent biological replicates.

Data was presented as mean \pm STDEV. *P* values were calculated by unpaired two-tailed Student's t test. n.s. no significance, **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Figure S5

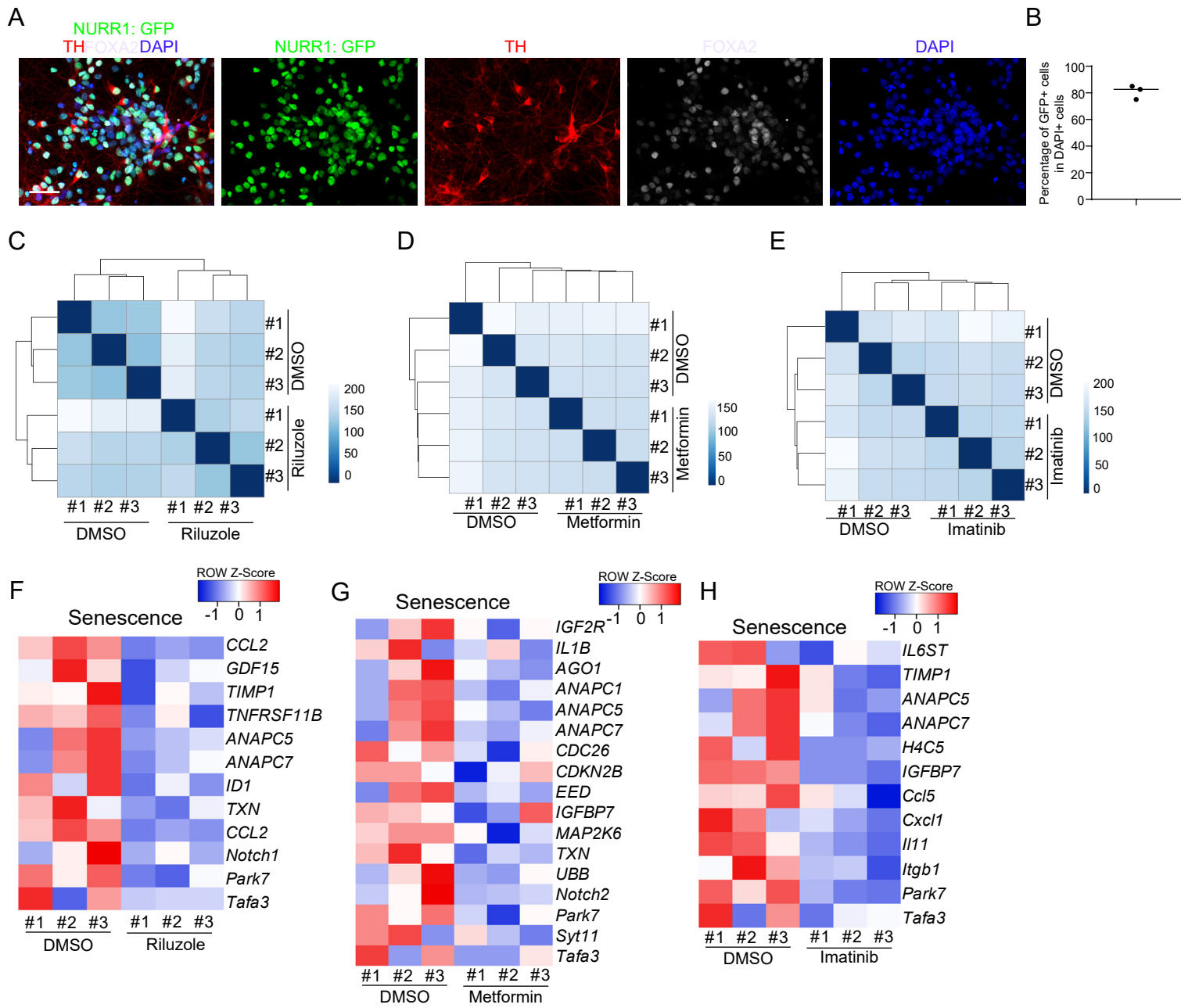


Figure S5. Three drug candidates rescue senescence phenotype of SARS-CoV-2 infected hPSC-derived DA neurons. Related to Figure 4.

(A and B) Representative confocal images (A) and quantification of the percentage of NURR1-GFP⁺ cells (B) of NURR1: GFP H9 lines-derived DA neurons for drug screening. Scale bar=50 μ m. n=3 independent biological replicates. Data was presented as mean \pm STDEV.

(C-E) Clustering analysis of gene expression profiles of DMSO or drug candidate-treated purified hPSC-derived DA neurons at 48 hpi upon SARS-CoV-2 infection (MOI=0.1).

(F-H) Heatmap of SASP associated genes of DMSO or drug candidate-treated purified hPSC-derived DA neurons at 48 hpi upon SARS-CoV-2 infection (MOI=0.1).

Figure S6

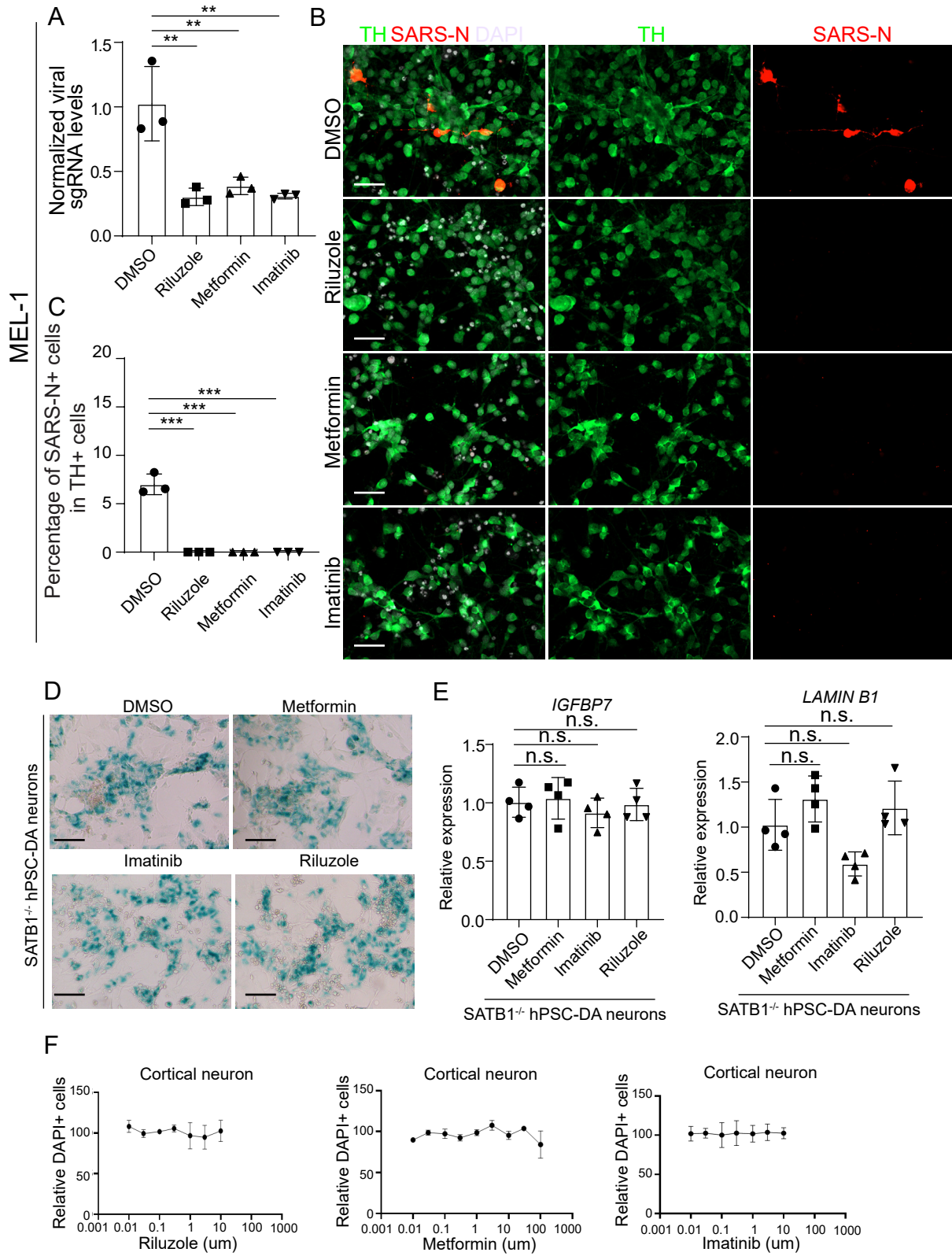


Figure S6. Three drug candidates block SARS-CoV-2 infection of hPSC-derived DA neurons. Related to Figure 5 and Figure 6.

(A) RT-qPCR analysis of total RNA extracted from DMSO or drug candidates-pre-treated MEL1-derived DA neurons following SARS-CoV-2 infection (MOI=0.2) for viral N sgRNA at 48 hpi. The graph depicts the mean sgRNA level normalized to *ACTB*. n=3 independent biological replicates.

(B and C) Representative confocal images (B) and quantification of the percentage of SARS-N⁺ cells in TH⁺ cells (C) of DMSO or drug candidates-pre-treated MEL1-DA neurons following SARS-CoV-2 infection (MOI=0.2) at 72 hpi using antibodies against SARS-CoV-2 Nucleocapsid protein (SARS-N) and marker for DA neurons. Scale bar=50 μm. n=3 independent biological replicates.

(D) β-Gal staining and quantification of DMSO or drug candidates-treated *SATBI*^{-/-} hPSC-derived DA neurons at 72 hpi (MOI=0.1). Scale bar=75μm. n=3 independent biological replicates.

(E) RT-qPCR analysis of senescence related genes of *IGFBP7* and *LAMIN B1* in DMSO or drug candidates-treated *SATBI*^{-/-} hPSC-derived DA neurons at 48 hpi (MOI=0.2). n=3 independent biological replicates.

(F) Cytotoxicity curves of riluzole, metformin and imatinib in H9-derived cortical neurons. n=3 independent biological replicates.

Data was presented as mean ± STDEV. *P* values were calculated by unpaired two-tailed Student's t test. n.s. no significance, ***P* < 0.01, and ****P* < 0.001.

Figure S7

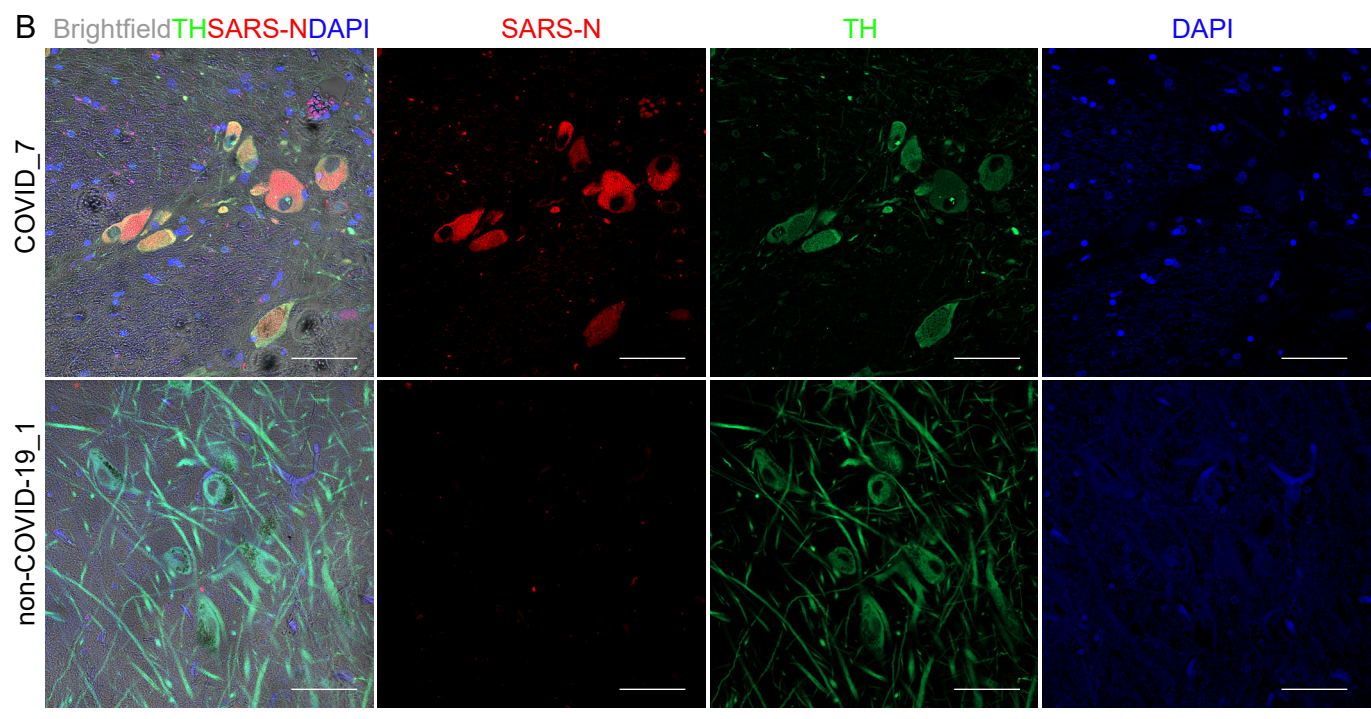
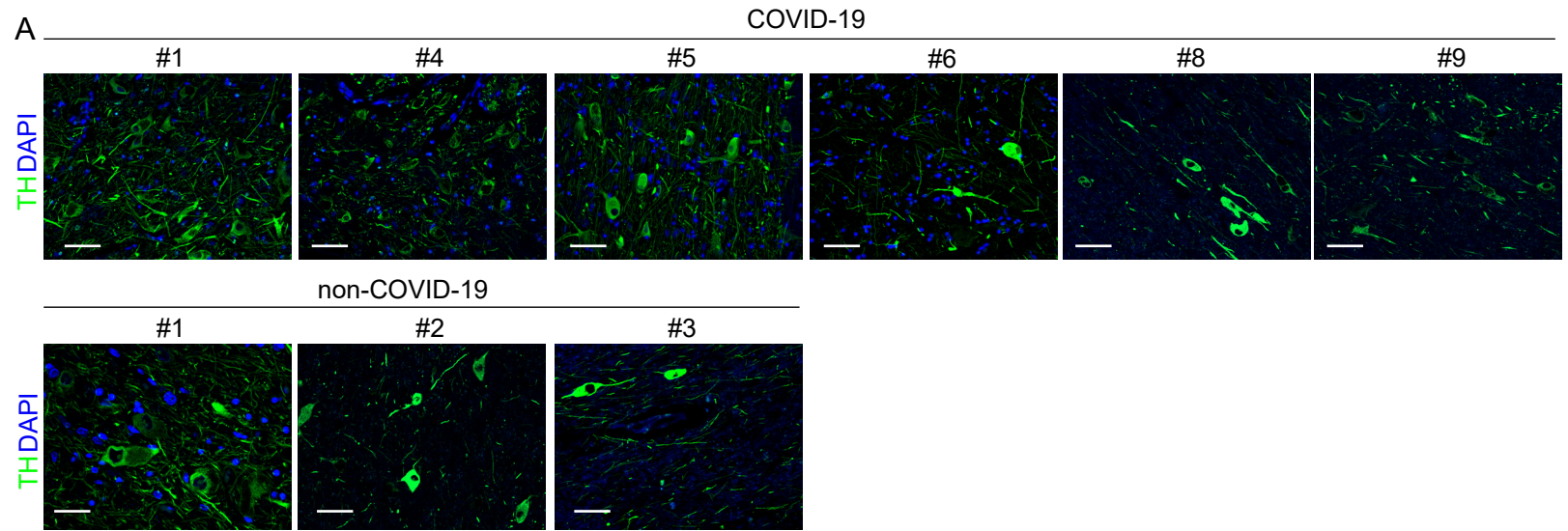


Figure S7. Inflammatory and senescence phenotypes were detected in autopsy substantia nigra samples of COVID-19 patients. Related to Figure 7.

(A) Representative confocal images of TH staining in the autopsy substantia nigra sections of COVID-19 patients versus non-COVID-19 patients. (Cohort 1: N=6 COVID-19 patients; N=3 non-COVID-19 patients). Scale bar=50 μ m.

(B) Representative confocal images of SARS-N and TH staining in the autopsy substantia nigra sections of COVID-19 patient versus non-COVID-19 patient. (Cohort 2: N=1 COVID-19 patients; N=1 non-COVID-19 patients). Scale bar=50 μ m.

Table S1. Demographics and pathological characterization of control autopsy cohort. Related to Figure 7 and Figure S7.

Table S2. Demographics and pathological characterization of COVID-19 autopsy cohort. Related to Figure 7 and Figure S7.

Table S3. Demographics and pathological characterization of PD(D) autopsy cohort. Related to Figure 7 and Figure S7.

Table S4. Patient information. Related to Figure 7 and Figure S7.

	Patient ID	Gender	Age	Experiments	Source
Cohort 1	COVID_1	Male	66	Fig. 7A-7E and Fig. S7A	Columbia University
	COVID_4	Female	78	Fig. 7A-7E and Fig. S7A	Columbia University
	COVID_5	Female	80	Fig. 7A-7E and Fig. S7A	Columbia University
	COVID_6	Male	80	Fig. 7A-7E and Fig. S7A	Columbia University
	COVID_7	Male	74	Fig. S7B	The Netherlands
	COVID_8	Male	67	Fig. 7A-7E, Fig. 7F and Fig. S7A	The Netherlands
	COVID_9	Male	59	Fig. 7A-7E, Fig. 7F and Fig. S7A	The Netherlands
	non-COVID_1	Male	72	Fig. 7A-7E, Fig. 7F and Fig. S7A	The Netherlands
	non-COVID_2	Male	61	Fig. 7A-7E, Fig. 7F and Fig. S7A	The Netherlands
	non-COVID_3	Female	61	Fig. 7A-7E and Fig. S7A	Columbia University
non-COVID_4	Male	82	Fig. 7F	The Netherlands	
Cohort 2	-	-	-	Fig. 7G-7H and Table S1-S3	The Netherlands

Table S5. Antibodies used for immunocytochemistry, intracellular flow cytometry analysis and western blotting analysis. Related to STAR Methods.

Usage	Antibody	Clone #	Host	Catalog #	Vendor	Dilution
Immunocytochemistry	Anti-SARS-CoV/SARS-CoV-2 Nucleocapsid	001	Rabbit	40143-R001	Sino Biological	1:500
Immunocytochemistry	ACE2	Polyclonal	Rabbit	ab15348	Abcam	1:500
Immunocytochemistry	Firefly luciferase Monoclonal Antibody (CS 17)	CS 17	Mouse	35-6700	Thermo Fisher Scientific	1:200
Immunocytochemistry and western blot	Anti-Alpha Synuclein	Syn211	Mouse	S5566-100UL	Millipore	1:250
Immunocytochemistry	Anti-MAP2 antibody	Polyclonal	Chicken	ab5392	Abcam	1:5000
Western blot	p21 Waf1/Cip1 (12D1) Rabbit mAb	12D1	Rabbit	2947	Cell Signaling	1:500
Western blot	Human Lamin B1 Antibody	919007	Mouse	MAB8525	R&D Systems	1:250
Immunocytochemistry	anti- α -Synuclein Phospho	P-syn/81A	Mouse	MMS-5091	Biolegend	1:1000
Immunocytochemistry	Goat polyclonal anti-FOXA2	Polyclonal	Goat	AF2400	R&D Systems	1:250
Immunocytochemistry	Recombinant Anti-TBR1 antibody	EPR8138 (2)	Rabbit	ab183032	Abcam	1:100
Immunocytochemistry	Anti-Ctip2 antibody	25B6	Rat	ab18465	Abcam	1:500
Immunocytochemistry	Anti-Tyrosine Hydroxylase antibody - Neuronal Marker	Polyclonal	Rabbit	ab112	Abcam	1:500

Immunocytochemistry	Human/Mouse Tyrosine Hydroxylase Antibody	779427	Mouse	MAB7566	R&D Systems	1:200
Immunocytochemistry	Anti-FOXA2 Antibody	M-20	Goat	sc-6554	Santa Cruz	1:150
Immunocytochemistry	Anti-GFP antibody	Polyclonal	Chicken	ab13970	Abcam	1:1000
Immunocytochemistry	Rabbit polyclonal anti-LMX-1	Polyclonal	Rabbit	AB10533	Millipore	1:1000
Immunocytochemistry	Donkey anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Polyclonal	Donkey	A-21202	Thermo Fisher Scientific	1:500
Immunocytochemistry	Donkey anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor 594	Polyclonal	Donkey	A-21207	Thermo Fisher Scientific	1:500
Immunocytochemistry	Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 594 conjugate	Polyclonal	Donkey	A-21203	Thermo Fisher Scientific	1:500
Immunocytochemistry	Donkey anti-Mouse IgG (H+L) Secondary Antibody, Alexa Fluor 647	Polyclonal	Donkey	A-31571	Thermo Fisher Scientific	1:500
Immunocytochemistry	Alexa Fluor 488 AffiniPure Donkey Anti-Chicken IgY (IgG) (H+L)	Polyclonal	Donkey	703-545-155	Jackson ImmunoResearch Labs	1:500
Immunocytochemistry	Donkey anti-Goat IgG (H+L) Cross-Adsorbed	Polyclonal	Donkey	A-21447	Thermo Fisher	1:500

	Secondary Antibody, Alexa Fluor 647				Scientific	
Immunocytochemistry	Donkey anti-Goat IgG Secondary Antibody, Alexa Fluor 555	Polyclonal	Donkey	A32816	Thermo Fisher	1:500
Immunocytochemistry	Donkey anti-Rabbit IgG Secondary Antibody, Alexa Fluor 647	Polyclonal	Donkey	A32795	Thermo Fisher	1:500

Table S6. Primers used for RT-qPCR. Related to STAR Methods.

Primer name	Sequence
<i>ACTB-Forward</i>	<i>CGTCACCAACTGGGACGACA</i>
<i>ACTB-Reverse</i>	<i>CTTCTCGCGGTTGGCCTTGG</i>
<i>SARS-CoV-2-TRS-L</i>	<i>CTCTTG TAGATCTGTTCTCTAAACGAAC</i>
<i>SARS-CoV-2-TRS-N</i>	<i>GGTCCACCAAACGTAATGCG</i>
<i>LaminB1-F</i>	<i>AAGCATGAAACGCGCTTGG</i>
<i>LaminB1-R</i>	<i>AGTTTGGCATGGTAAGTCTGC</i>
<i>IGFBP7-F</i>	<i>ATCCCGACACCTGTCCTCAT</i>
<i>IGFBP7-R</i>	<i>CCCAGCCAGT TACTTCATGCT</i>

Reference

1. Riessland, M., Kolisnyk, B., Kim, T.W., Cheng, J., Ni, J., Pearson, J.A., Park, E.J., Dam, K., Acehan, D., Ramos-Espiritu, L.S., et al. (2019). Loss of SATB1 Induces p21-Dependent Cellular Senescence in Post-mitotic Dopaminergic Neurons. *Cell Stem Cell* 25, 514-530 e518. 10.1016/j.stem.2019.08.013.