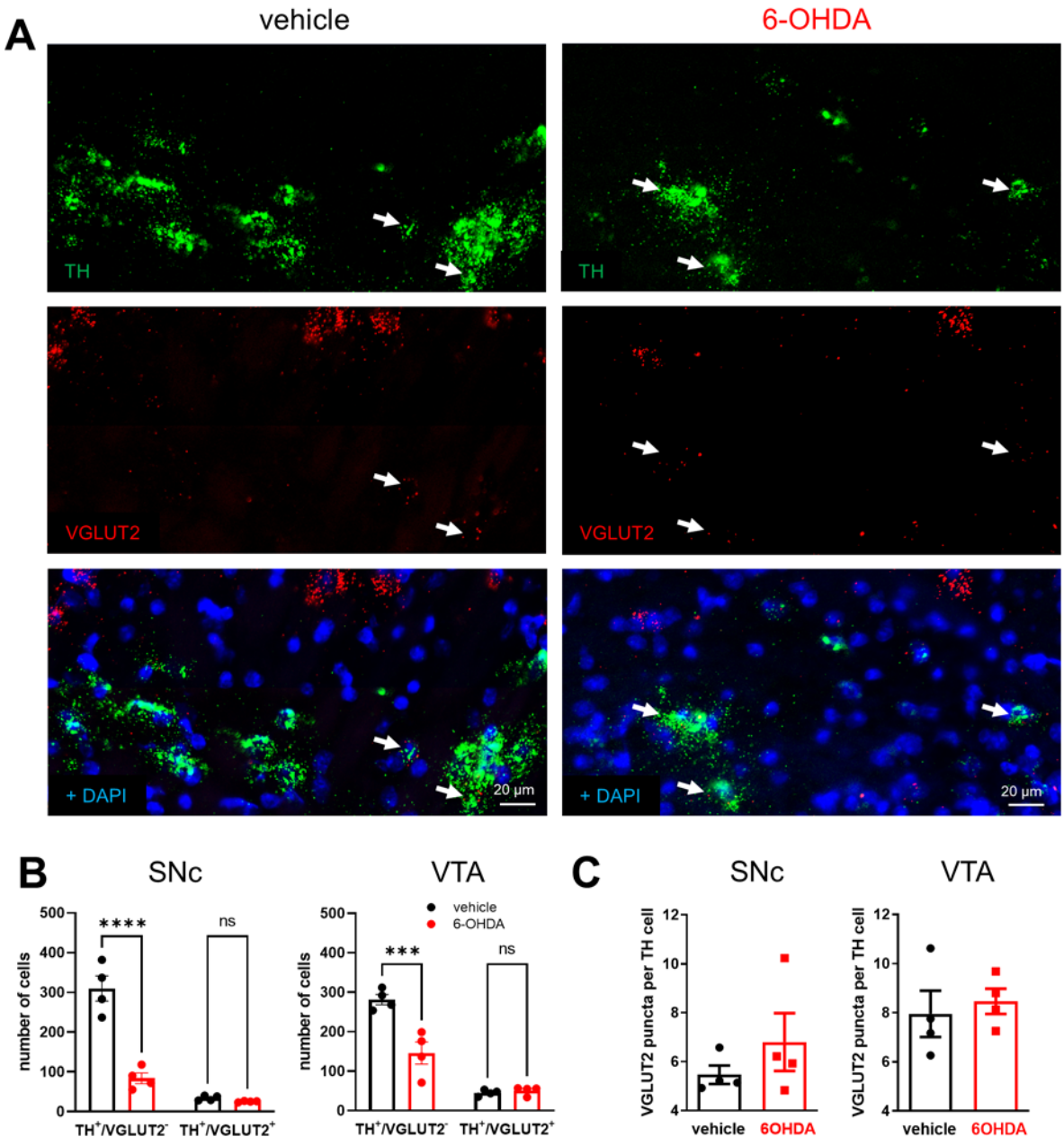
Supplementary Figures

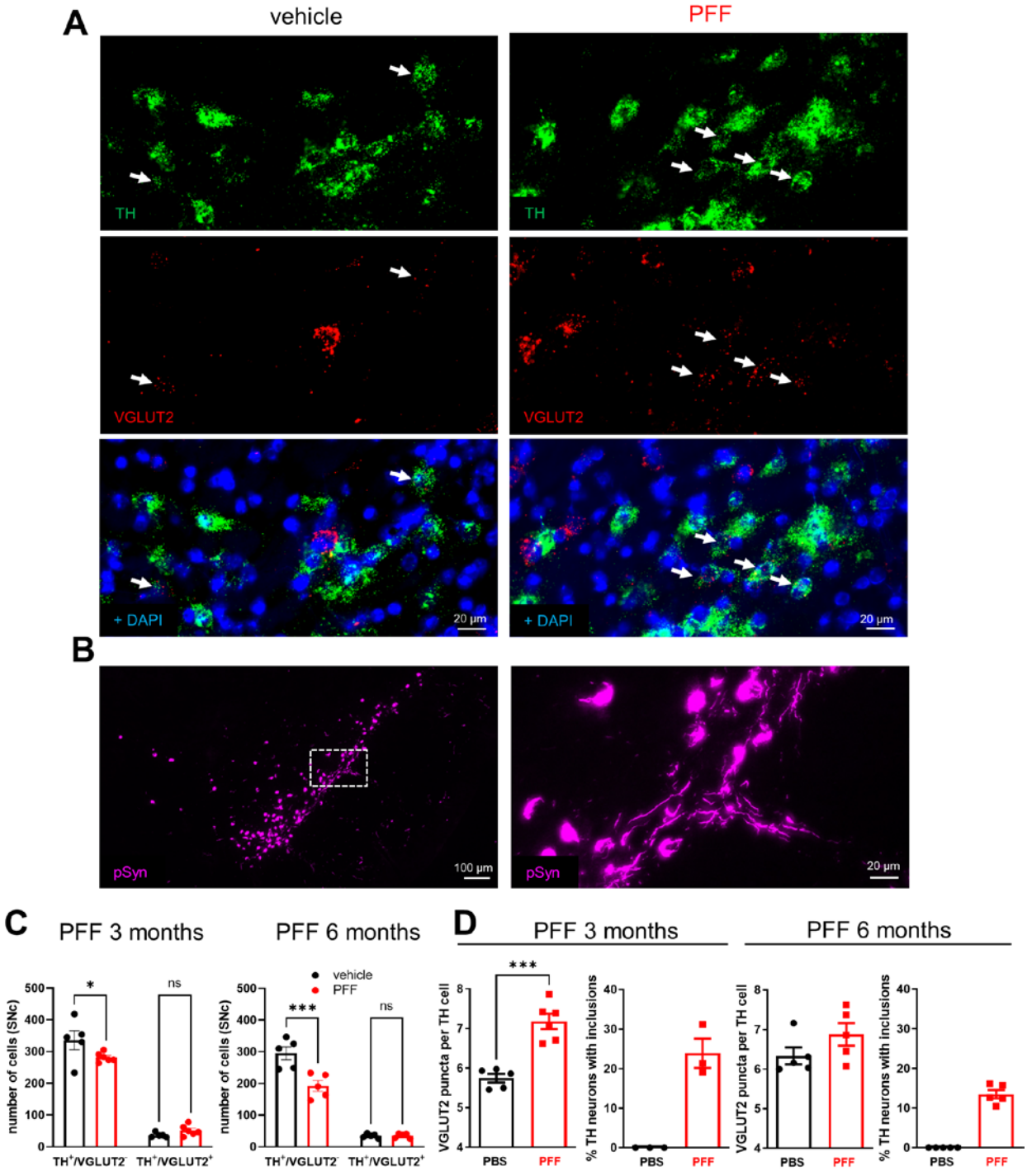
Supplemental Figure 1



Supplemental Figure 1, relates to Figure 1: **VGLUT2 mRNA expression in TH+ neurons after 6-OHDA lesion.** (**A**) Example *in situ* hybridization images in coronal sections through SNc of vehicle- or 6-OHDA-treated animals; mRNA encoding TH (green) and VGLUT2 (red) with DAPI counter-stain (blue). (**B**) Total neuron counts of TH+/VGLUT- and TH+/VGLUT2+ neurons in SNc and VTA after vehicle or 6-OHDA treatment; two-way

ANOVA followed by posthoc Sidak's multiple comparisons test; ***p<0.001. ****p<0.0001. (**C**) Mean number of VGLUT2 puncta per TH neuron in SNc (left panel) and VTA (right panel) after vehicle or 6-OHDA treatment.

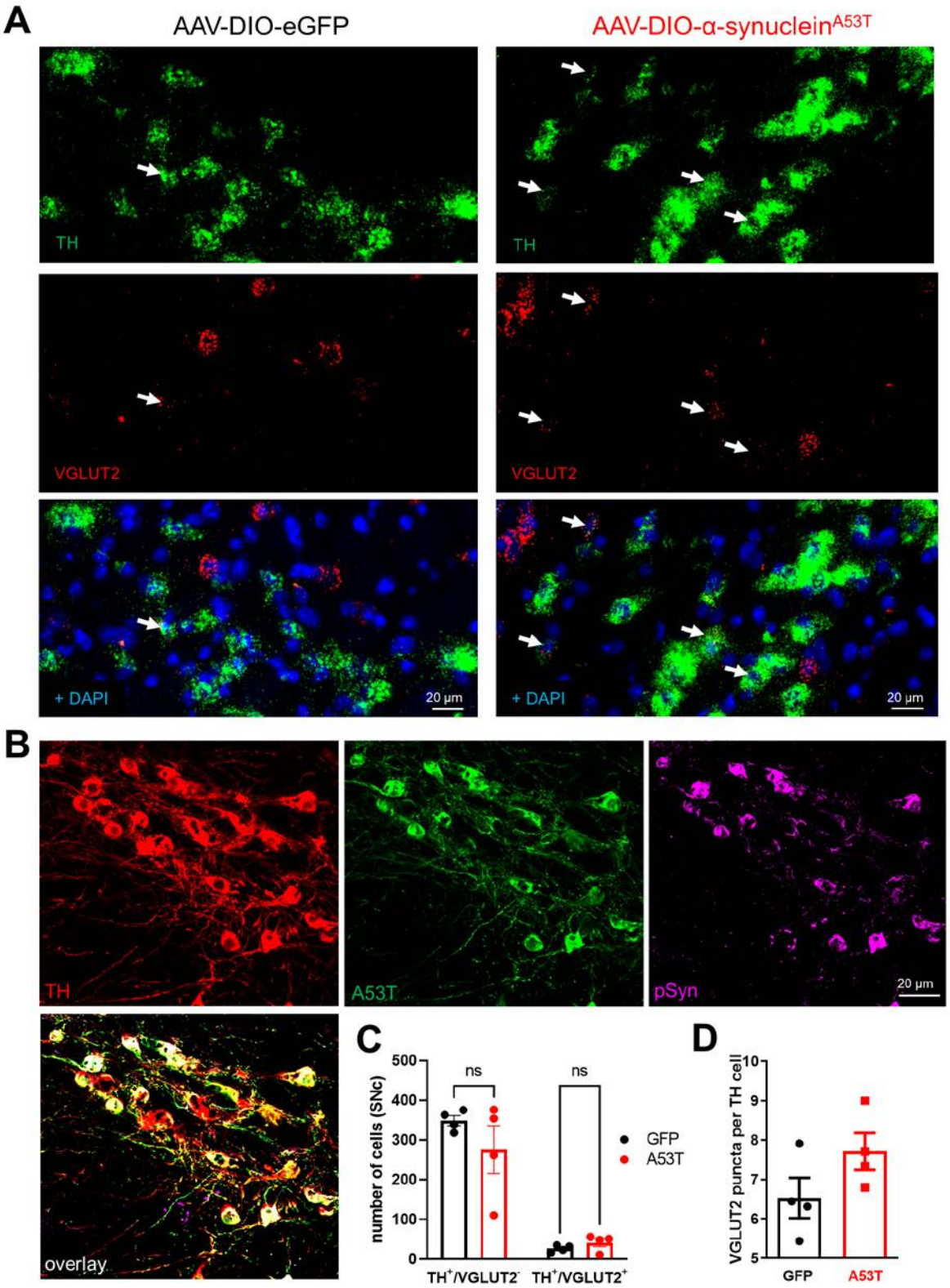
Supplemental Figure 2



Supplemental Figure 2, relates to Figure 2: VGLUT2 mRNA expression in TH+ neurons after PFF exposure. (**A**) Example *in situ* hybridization images in coronal sections through SNc of vehicle- or PFF-treated animals; mRNA encoding TH (green) and VGLUT2 (red) with DAPI counter-stain (blue). (**B**) pSyn-positive (purple) inclusions in the SNc (left) and zoom-in (right) from PFF-treated animal. (**C**) Total neuron counts of TH+/VGLUT2- and TH+/VGLUT2+ neurons in SNc; two-way ANOVA followed by posthoc Sidak's multiple

comparisons test; *p<0.05, ***p<0.001. (**D**) Mean number of VGLUT2 puncta per TH neuron (left panels) and percentage of TH neurons with p-Syn inclusions (right panels) 3 months (left) and 6 months (right) after PFF or vehicle injection; ***p<0.001, two-tailed unpaired t-test.

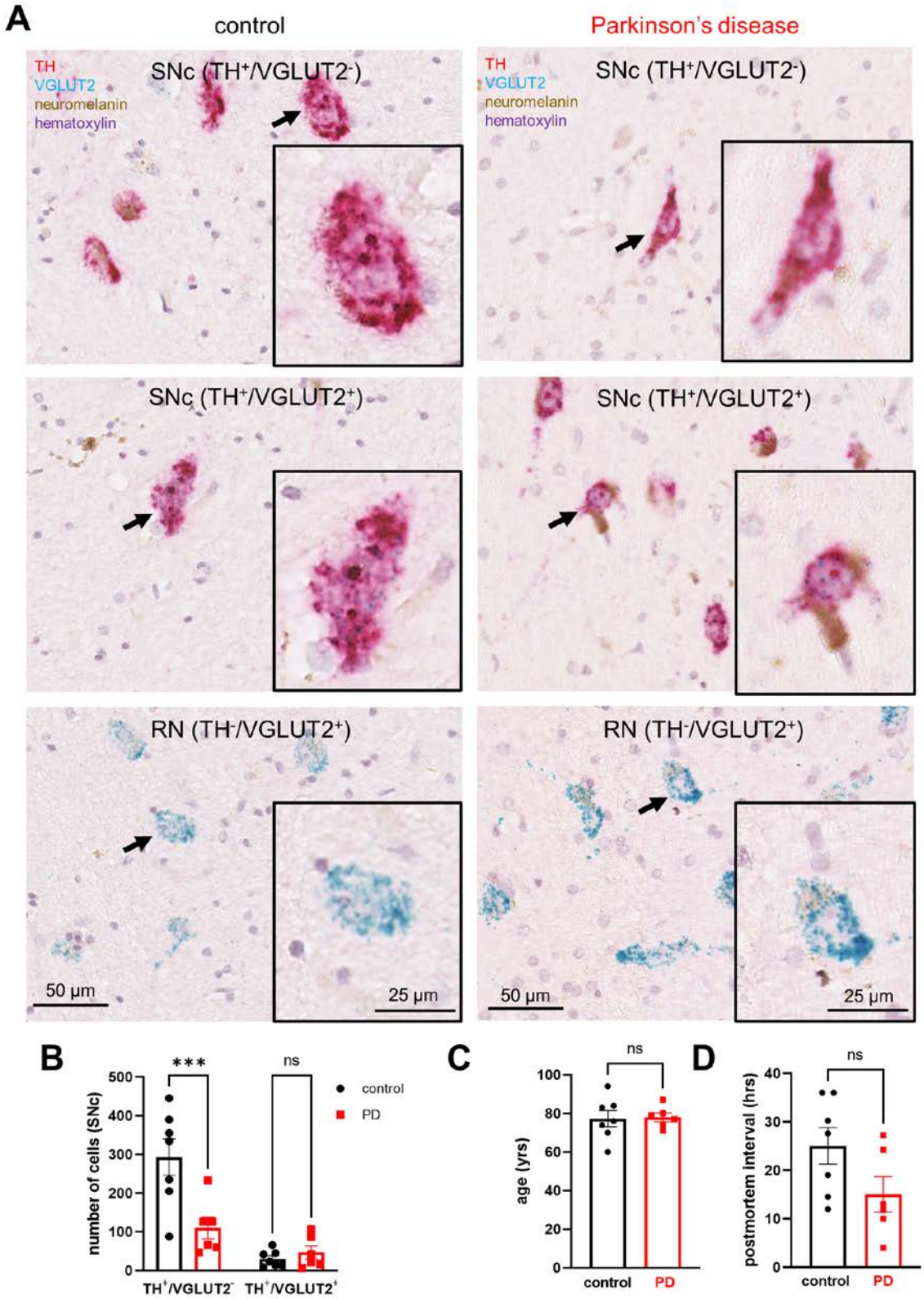
Supplemental Figure 3



Supplemental Figure 3, relates to Figure 3: VGLUT2 mRNA expression in TH+ neurons after α-synuclein overexpression. (**A**) Example *in situ* hybridization images in

coronal sections through SNc of AAV-DIO-GFP or AAV-DIO-α-synuclein^{A53T} injected DAT^{Cre} mice; mRNA encoding TH (green) and VGLUT2 (red) with DAPI counter-stain (blue); scale bar: 20 µm. (**B**) Immunohistochemistry showing staining for human α-synuclein^{A53T} using a human-specific monoclonal antibody (A53T; green) and Ser-129 phosphorylation of α-synuclein (p-Syn; purple) selectively in TH+ DA neurons (red) in the SNc of DAT^{Cre} mouse. (**C**) Total neuron counts of TH+/VGLUT2- and TH+/VGLUT2+ neurons in SNc; two-way ANOVA followed by posthoc Sidak's multiple comparisons test; $p>0.05$. (**D**) Mean number of VGLUT2 puncta per TH neuron in DAT^{Cre} mice overexpressing human α-synuclein^{A53T} or GFP (vehicle).

Supplemental Figure 4



Supplemental Figure 4, relates to Figure 4: Human neuropathology and demographic information. (**A**) Widefield view of transverse section containing SNc from another control (left panels) and PD subject (right panels) with chromogenic labeling for mRNAs encoding

TH (diffuse magenta) and VGLUT2 (blue puncta) counter-stained with hematoxylin; brown signal is neuromelanin; cp, cerebral peduncle; RN, red nucleus; insets show higher magnification of individual neurons (black arrows). (**B**) Total neuron counts of TH+/VGLUT2^{-} and TH+/VGLUT2+ neurons in SNc; two-way ANOVA followed by posthoc Sidak's multiple comparisons test; ***$p<0.001$. (**C**) Comparison statistics for age (left) and PMI (right) of control and PD cases selected for neuropathology studies. Unpaired t-test, $p>0.05$.

Supplemental Table 1

Subject	Sex	Age (yrs)	PMI (h)	diagnosis	source
1	M	94	12	control	UCSD
2	M	84	36	control	UCSD
3	M	76	30	control	NIH
4	M	60	28	control	NIH
5	M	83	15	control	UPenn
6	M	70	36	control	UPenn
7	M	74	19	control	UPenn
8	M	74	4	PD	UCSD
9	M	71	12	PDD	NIH
10	M	87	24	PDD	NIH
11	M	79	27	PDD	NIH
12	M	77	10	PD	UPenn
13	M	80	13	PD	UPenn

PD = Parkinson's disease

PDD = Parkinson's disease dementia

Supplemental Table 1, relates to Figure 4 De-identified demographic information on tissue from human subjects.

Supplementary Materials and Methods

Animals

We used adult C57BL/6J wildtype mice (10-12 weeks old) or mice expressing Cre recombinase under the control of the DAT (DAT^{cre}) (*Slc6a3*^{IRESCre}, Jackson stock 006660, The Jackson Laboratory, Bar Harbor, ME; 8-12 weeks of age) which were bred on a C57BL/6J genetic background and backcrossed to C57BL/6J for >12 generations. Mice were group-housed, and maintained on a 12:12-hour light:dark cycle with food and water available *ad libitum*. All mice were used in accordance with protocols approved by the University of California, San Diego (UCSD) Institutional Animal Care and Use Committee.

Human brains

De-identified human formalin-fixed paraffin-embedded (FFPE) tissue blocks were obtained from NIH NeuroBioBank (through the University of Miami, Miller School of Medicine, Brain Endowment Bank), the University of Pennsylvania, and UCSD neuropathology cores. Patient material was selected based on the availability of brain regions (SNc, red nucleus, cerebral peduncle). For every PD case, a matched control sample was selected according to the following criteria: age, sex, post-mortem interval and race. Also see **Supplemental Table 1** and **Supplemental Figure 4C-D** for more information. All subjects were males and Caucasian (non-Hispanics) based on availability. All labeling and counting were done by blinded experimenters.

Stereotaxic injections

Mice were anaesthetized with isoflurane (2-5%) and placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA). 2.5 µl of sonicated preformed fibrils of α-synuclein (PFF) or PBS (vehicle) was infused at 100 nl/min into the dorsal striatum at the following coordinates: +0.4 anterior-posterior (AP), -1.8 medial-lateral (ML), -3.5 dorsal-ventral (DV); in mm from *Bregma*. For viral injections, serotype DJ, replication-incompetent AAV were used to drive expression of eGFP or human α-synuclein^{A53T} under the control of the human synapsin I promoter: AAV_{DJ}-hSyn1-DIO-eGFP (3.6×10^{13} genome copies per ml [gc/ml])or AAV_{DJ}-hSyn1-DIO-hASYN^{A53T} ($1.4x10^{13}$ gc/ml). The GFP virus was packaged at the Salk GT3 vector core (La Jolla, CA) and the synuclein virus was packaged by Vigene Biosciences

(Rockville, MD). The respective viruses were microinfused (300 nl) into the left SNc (-3.4 AP, -1.25 ML, -4.25 DV) using custom-made 30G stainless steel injectors at 100 nl/min.

Preformed fibrils (PFF) of α-synuclein

Mouse α-synuclein PFF (5 mg/ml) were prepared as described previously [30]. Recombinant mouse α-synuclein was stored in aliquots at -80°C until use. To induce fibrillization, aliquots were thawed and diluted in PBS (2 mg/ml) before sonication using a Sonics Vibra Cell Model CV18 at 20 % power (ca. 26 W), 1 sec on, 1 sec off, for a total of 30 sec. Sonicated PFF solutions were kept at room temperature and injected within six hours.

6-OHDA lesions

6-OHDA-HBr (Sigma-Aldrich; 3.2 µg/µl of the free base) was dissolved in 0.2% (wt/vol) ascorbate in saline and injected unilaterally into the medial forebrain bundle (AP -1.2, ML -1.2, DV -4.65) using a 5-µl Hamilton syringe. Control mice received 0.2% ascorbate in saline (vehicle). The total infusion was 1 µl at a speed of 0.5 µl/min. After surgeries, body weights of mice were monitored daily, and mice received subcutaneous 5% glucose (wt/vol) in saline injections once daily for the next 7 days to prevent dehydration and promote feeding.

Immunohistochemistry

Mice were deeply anaesthetized with pentobarbital (200 mg/kg i.p.; Virbac Corp., Westlake, TX) and transcardially perfused with 10–20ml of phosphate-buffered saline (PBS) 3 weeks after viral injection. This was followed by perfusion with 60-70ml of 4% paraformaldehyde (PFA) at a rate of 6 ml/min. Brains were extracted, post-fixed in 4% PFA at 4°C overnight, and cryoprotected in 30% sucrose in PBS for 48–72 hours at 4°C. Brains were snap-frozen in chilled isopentane and stored at -80°C. Coronal sections (30µm) were sectioned using a cryostat (CM3050S, Leica, Wetzlar, Germany) and collected in PBS containing 0.01% sodium azide.

For fluorescent immunostaining, brain sections were blocked with 5% normal donkey serum in PBS containing 0.3% Triton X-100 (blocking buffer) (1-hour, room temperature). Sections were then incubated with one or more of the following primary antibodies in blocking buffer overnight at 4°C (sheep anti-TH, 1:2000, P60101-150, Pel Freeze; rat anti-human α-synuclein, 15G7, Enzo Life Sciences; rabbit anti-phospho-serine-129 α-synuclein, 1:1000,

ab59264, Abcam; mouse anti-phospho-serine-129 α-synuclein, MMS-5091, Biolegends). Sections were rinsed 3×15 min with PBS and incubated in appropriate secondary antibodies (Jackson ImmunoResearch, West Grove, PA) conjugated to Alexa 488, Alexa 594 or Alexa 647 fluorescent dyes (5 μg/ml) (2-hours, room temperature). Sections were washed 3×15 min with PBS, mounted onto glass slides and coverslipped with Fluoromount-G mounting medium (Southern Biotech, Birmingham, AL) supplemented with DAPI (0.5 μg/ml, Roche, Basel, Switzerland). Images were acquired using a Zeiss AxioObserver epifluorescence microscope (Oberkochen, Germany).

Fluorescent mRNA in situ hybridization in mouse brain

Mice were anesthetized with pentobarbital and killed by decapitation. Brains were extracted rapidly, frozen in chilled isopentane and stored at -80°C. Sections were serially cut (20 μm) on a cryostat and mounted directly onto Fisherbrand Superfrost Plus glass slides (Fisher Scientific). Slides were stored at -80°C until starting the multiplex fluorescent RNAscope assay (Advanced Cell Diagnostics). Briefly, sections were fixed with 4% PFA for 15 min at 4°C followed by dehydration in increasing ethanol concentrations and protease IV treatment. RNA hybridization probes included antisense probes against mouse *VGLUT2 (Slc17a6;* #319171-C1) and TH (#317621-C3).

For PFF experiments, we performed immunohistochemistry subsequent to the last wash of the RNAscope assay. Briefly, slides were washed 3 times for 5 min in PBS and incubated in blocking buffer (5% normal donkey serum in PBS containing 0.3% Triton X-100) for 1 hr at room temperature before overnight (4°C) incubation in mouse anti-phospho-serine-129 α-synuclein antibody (Biolegends, MMS-5091; 1:1000). The following day, slides were washed 3 times in PBS followed by incubation with secondary antibody as above. Slides were counterstained with DAPI and coverslipped using Fluoromount-G mounting medium. Images were taken at 20x magnification using a Zeiss Axio Observer epifluorescence microscope.

Chromogenic mRNA in situ hybridization in human brain

FFPE tissue blocks containing unilateral SNc were cut on a rotary microtome at 7 μm and mounted on to Fisherbrand Superfrost Plus glass slides. Slides were dried at room temperature for 1 h and then overnight at 37°C in an oven. RNAscope 2.5 Duplex Assay was used for chromogenic mRNA staining using a modified protocol provided by ACD. Briefly,

slides were baked at 60°C for 1 h and deparaffinized in xylenes followed by washes in 100% ethanol and air dried for 15 min. Next, tissue was pretreated with H_2O_2, boiled in target retrieval solution and incubated protease plus before probe hybridization (Hs-SLC17A6, #415671; C1; Hs-TH-C2, #441651-C2) and amplification. Slides were counterstained with Mayer's hematoxylin and coverslipped using VectaMount media.