Supplementary Materials for

Tracing brain genotoxic stress in Parkinson’s disease with a novel single-cell genetic sensor

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Supplementary Methods

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Supplemental Table 1: Demographic, clinical and *post-mortem* characteristics of human brain donor samples

| Sample ID | Analysis* | Neuropath. grouping | Sex | Age  | Race          | PMI  | Cause of death                                      |
|-----------|-----------|----------------------|-----|------|---------------|------|-----------------------------------------------------|
| 6168      | 1, 2, 3   | Cont. aged           | M   | 68   | Caucasian     | 28   | HTN** Atherosclerotic Disease                       |
| 15HABA003 | 1, 2, 3   | Cont. aged           | M   | 67   | Caucasian     | 22.5 | Unaffected                                          |
| 5919      | 1, 2      | Cont. aged           | M   | 63   | Caucasian     | 24   | Drowning                                            |
| 5890      |           | Cont. aged           | M   | 60   | Caucasian     | 37   | ASCVD***                                            |
| 5824      | 1, 2      | Cont. aged           | M   | 74   | African American | 18  | HTN** Calcific Atherosclerotic Disease              |
| 16HDLA007 | 1, 2, 3   | Cont. aged           | M   | 71   | Caucasian     | 19.4 | Unaffected                                          |
| 17HETA003 | 3         | Cont. aged           | M   | 61   | Caucasian     | 27.1 | Unaffected                                          |
| 6274      | 3         | Cont. young          | M   | 31   | Caucasian     | 23   | Fentanyl and Morphine Intoxication                  |
| 6230      | 3         | Cont. young          | M   | 33   | Caucasian     | 27   | Cardiomegaly w/ LVH****                            |
| 6169      | 3         | Cont. young          | M   | 25   | Caucasian     | 12   | HTN Atherosclerotic Disease                        |
| 6061      | 3         | Cont. young          | M   | 24   | Caucasian     | 33   | Acute and Chronic Lung Disease                     |
| 6259      | 1, 2      | PD                   | M   | 72   | Caucasian     | 24   | Complications of disorder                           |
| 6248      | 1, 2      | PD                   | M   | 71   | Caucasian     | 22   | Complications of disorder                           |
| 6126      | 1, 2      | PD                   | M   | 77   | Caucasian     | 8    | Complications of disorder                           |
| 6257      | 1, 2      | PD                   | M   | 63   | Caucasian     | 6    | Complications of disorder                           |
| 18006A003 | 1, 2      | PD                   | M   | 83   | Caucasian     | 9.21 | Complications of disorder                           |
| 18029A0003| 1, 2      | PD                   | M   | 69   | Caucasian     | 12.8 | Complications of disorder                           |
| HBRRRA002 | 1, 2      | PD                   | M   | 91   | Caucasian     | 20.9 | Complications of disorder                           |
| 18071A003 | 1, 2      | PD                   | M   | 69   | Caucasian     | 22.3 | Complications of disorders                           |

*Analysis 1 refers to Fig. 6 O; Analysis 2 to Fig. 6 P, and Analysis 3 refers to Supp. Fig. 8 C.*

**HTN: Hypertensive

***ASCVD: Atherosclerotic Cardiovascular Disease

****LVH: Left Ventricular Hypertrophy

PMI: postmortem interval.
Supplemental Table 2. Confocal optical settings for post-mortem tissue immunostaining.
Confocal optical parameters are shown for a subset of experiments shown in Fig. 6L-N.

| Test group | ID      | DAPI HV | DAPI offset | DAPI laser | FITC HV | FITC offset | FITC laser | TRITC HV | TRITC offset | TRITC laser |
|------------|---------|---------|-------------|------------|---------|-------------|------------|----------|--------------|-------------|
| Aged       | 5919    | 141     | -90         | 2.56       | 34      | 8           | 0.7        | 38       | 11           | 1.96        |
| Aged       | 15HABA003 | 142    | -75         | 2.56       | 53      | 5           | 0.7        | 43       | 8            | 1.96        |
| Aged       | 5890    | 135     | -73         | 2.56       | 30      | 1           | 0.7        | 40       | 9            | 1.96        |
| Aged       | 5824    | 128     | -82         | 2.56       | 27      | 9           | 0.7        | 38       | 10           | 1.96        |
| PD         | 6257    | 136     | -90         | 2.56       | 41      | 8           | 0.7        | 38       | 11           | 1.96        |
| PD         | 6248    | 143     | -90         | 2.56       | 35      | 8           | 0.7        | 38       | 11           | 1.96        |
| PD         | 6259    | 135     | -80         | 2.56       | 40      | 9           | 0.7        | 40       | 11           | 1.96        |
| PD         | 6126    | 133     | -74         | 2.56       | 53      | 6           | 0.7        | 44       | 8            | 1.96        |
| PD         | HBRRA002 | 136  | -78         | 2.56       | 43      | 9           | 0.7        | 42       | 10           | 1.96        |
| PD         | 16HDLA007 | 138  | -75         | 2.56       | 41      | 9           | 0.7        | 42       | 10           | 1.96        |
| PD         | 18006A003 | 136 | -75         | 2.56       | 42      | 9           | 0.7        | 42       | 10           | 1.96        |
| PD         | 18029A0003 | 140 | -75         | 2.56       | 43      | 9           | 0.7        | 42       | 10           | 1.96        |
| Young      | 6061    | 132     | -63         | 2.56       | 52      | 7           | 0.7        | 44       | 7            | 1.96        |
| Young      | 6274    | 145     | -75         | 2.56       | 41      | 9           | 0.7        | 42       | 10           | 1.96        |
| Young      | 6230    | 136     | -75         | 2.56       | 42      | 9           | 0.7        | 42       | 10           | 1.96        |
| Young      | 6169    | 146     | -78         | 2.56       | 43      | 9           | 0.7        | 42       | 10           | 1.96        |
Supplemental Table 3. Confocal laser settings to image PRISM γH2AX co-expression.

| Exp. | Test Group | DAPI HV | DAPI offset | DAPI laser | FITC HV | FITC offset | FITC laser | TRITC HV | TRITC offset | TRITC laser |
|------|------------|---------|-------------|------------|---------|-------------|------------|---------|--------------|-------------|
| 1*   | DOX        | 126     | -99         | 4.84       | 17      | -102        | 0.41       | 15      | -94          | 0.64        |
| 1    | DOX        | 129     | -99         | 4.84       | 17      | -102        | 0.41       | 15      | -94          | 0.64        |
| 1    | DOX        | 133     | -93         | 4.84       | 17      | -102        | 0.41       | 15      | -94          | 0.64        |
| 1    | Saline     | 108     | -99         | 4.84       | 23      | -102        | 0.41       | 37      | -94          | 1.44        |
| 1    | Saline     | 126     | -99         | 4.84       | 17      | -102        | 0.41       | 28      | -94          | 0.64        |
| 1    | Saline     | 128     | -99         | 4.84       | 17      | -102        | 0.41       | 17      | -94          | 0.64        |
| 2**  | PQ         | 125     | -101        | 4.84       | 35      | -78         | 0.41       | 49      | -94          | 0.64        |
| 2    | PQ         | 120     | -101        | 4.84       | 55      | -78         | 0.41       | 55      | -94          | 0.64        |
| 2    | PQ         | 128     | -88         | 4.84       | 43      | -78         | 0.41       | 49      | -94          | 0.64        |
| 2    | Saline     | 123     | -68         | 4.84       | 51      | -78         | 0.41       | 47      | -94          | 0.64        |
| 2    | Saline     | 110     | -68         | 4.84       | 58      | -78         | 0.41       | 48      | -94          | 0.64        |
| 2    | Saline     | 120     | -68         | 4.84       | 42      | -78         | 0.41       | 48      | -94          | 0.64        |
| 3**  | PQ         | 128     | -94         | 4.84       | 58      | -78         | 0.41       | 35      | -94          | 0.64        |
| 3    | PQ         | 134     | -94         | 4.84       | 58      | -78         | 0.41       | 35      | -94          | 0.64        |
| 3    | PQ         | 134     | -94         | 4.84       | 58      | -78         | 0.41       | 35      | -94          | 0.64        |
| 3    | Saline     | 123     | -94         | 4.84       | 58      | -78         | 0.41       | 34      | -94          | 0.64        |
| 3    | Saline     | 121     | -77         | 4.84       | 58      | -78         | 0.41       | 34      | -94          | 0.64        |
| 3    | Saline     | 125     | -121        | 4.84       | 58      | -78         | 0.41       | 35      | -94          | 0.64        |
| 4****| TG+PFF     | 136     | -78         | 2.56       | 37      | 12          | 0.3        | 50      | -10          | 0.36        |
| 4    | TG+PFF     | 136     | -78         | 2.56       | 37      | 12          | 0.3        | 50      | -10          | 0.36        |
| 4    | TG+PFF     | 136     | -78         | 2.56       | 35      | 14          | 0.3        | 50      | -10          | 0.36        |
| 4    | WT+Sal     | 136     | -78         | 2.56       | 35      | 13          | 0.3        | 50      | -10          | 0.36        |
| 4    | WT+Sal     | 136     | -78         | 2.56       | 37      | 12          | 0.3        | 50      | -10          | 0.36        |
| 4    | WT+Sal     | 136     | -78         | 2.56       | 37      | 12          | 0.3        | 50      | -10          | 0.36        |

Confocal optical parameters are shown for a subset of multiple experiments. The corresponding experiment is indicated in column 1. Each row represents a unique individual subject.

1*Data shown in Fig 3. (PRISM γH2AX co-expression)
2**Data shown in Fig 4. (PRISM labelling frequency in Striatum of PQ group)
3**Data shown in Fig 4. (PRISM labelling frequency in Striatum of PQ group)
4****Data shown in Fig 5. (PRISM labelling frequency in SN of PFF group)
### Supplemental Table 4. Antibodies

| Antibody            | Source, Cat#                  | Dilution          | Corresponding Figure               |
|---------------------|-------------------------------|-------------------|-----------------------------------|
| Anti-RFP            | Rockland, 600-401-379         | IF (1:1,000)      | 1C-F, 4G, Supp Fig 1             |
|                     |                               | IF (1:500)        |                                   |
| Anti-pATM           | Santa Cruz                    | IHC (1:500)       | Supp Fig 4A-B and 7D              |
| Anti-H2AX           | Santa Cruz                    | IHC (1:500)       | 3G, H, K, 4D-F, 6O-Q . Supp Figs 3A-F, 5G-H, 6, 8 |
| Anti-53BP1          | Santa Cruz                    | IHC (1:400)       | Supp Fig 4C-G                     |
| Anti-IBA1           | IHC (1:5,000)                 |                  | 3P-Q, T-U                         |
| Anti-GFAP           | IHC (1:1,000)                 |                  | 3R-S, V                           |
| Anti-8OHdG          | Santa Cruz                    | IHC (1:500)       | 3I-J, L, Supp Fig 3G-L            |
|                     |                               | IF (1:500)        |                                   |
| anti-P-α-Syn (Ser129) | Abcam 51253                 | IHC (1:1,000)     |                                   |
| anti-α-synuclein (211) | Santa Cruz sc-12767.       | IHC (1:1,000)     |                                   |
| Anti-Tyrosine       | Pel Freeze Biologics P40101  | IF (1:1,000)      | 4O-R,6O-Q, S Supp Fig 7 A-C      |
| Hydroxylase         |                               | IF (1:1,000)      |                                   |
| Vectastain Elite    | Vector PK-7100                | Ready to use (R.T.U) |                                   |
| ABC Peroxidase, R.T.U |                               |                  |                                   |
| Alexa goat anti-    | Invitrogen A11008             | IF (1:300)        |                                   |
| rabbit 488          |                               |                  |                                   |
| Streptavidin, Alexa| Invitrogen S11223             | IF (1:300)        |                                   |
| 488 conjugate       |                               |                  |                                   |
| Streptavidin, Alexa| Invitrogen S11227             | IF (1:300)        |                                   |
| 594 conjugate       |                               |                  |                                   |
| Alexa 594 Tyramide  | Invitrogen B40944             | IF (1:300)        |                                   |
| SuperBoost, goat    |                               |                  |                                   |
| anti-rabbit IgG     |                               |                  |                                   |
| Biotinylated Goat   | Vectorlabs BA-1000            | IF (1:200)        |                                   |
| Anti-Rabbit IgG     |                               |                  |                                   |
| Antibody            | Vectorlabs BA-9200            | IF (1:200)        |                                   |
| Biotinylated Goat   |                               |                  |                                   |
| Anti-Mouse IgG      |                               |                  |                                   |
| Antibody            |                               |                  |                                   |
Supplementary Movies, Figures, and Legend:

Supplementary Movie 1

Sparse single-neuron labeling and visualization of dendrites, axons, dendritic spines, and axon buttons of a striatal medium spiny neuron labelled with PRISM.

Supplementary Movie 2

The dendritic spines of a PRISM-labeled striatal medium spiny neuron were 3D-rendered in IMARIS and classified using a MATLAB plugin to differentiate mushroom, stubby, thin, and filopodia spines.

Supplementary Movie 3

Cell function in IMARIS was used to reconstruct and segment the cell body, nucleus, and subcellular pathology (e.g., foci of phosphorylated Histone 2A) in a PRISM-labeled striatal medium spiny neuron.
Supplementary Figure 1. Workflow to facilitate rendering and analysis of acquired images of PRISM-mediated single labeled MSNs. (A) Established custom workflow to facilitate rendering and analysis of acquired images of PRISM-mediated single labeled MSNs. Morphology is indicative of pathology. PRISM vector can be used to visualize gross neuronal morphology, including arbor complexity and detailed dendritic and spine structures in single labeled neurons in vivo. First, 80 µm thick sagittal sections are prepared, and z-stacked confocal images of a single labeled neuron of interest are acquired at 40x magnification in an unbiased manner (confocal settings: 1024x1024 pixel, z-setp 0.3 µm, Numerical Aperture 1.3, 1.2) AU, (scale bar=15 µm). Figure B illuminates dendritic spine architecture, which is highlighted by the yellow box (scale bar=4 µm). The neuronal structure is then separated from the background using Imaris software by creating an encompassing Surface (source channel TRITC) and masking the TRITC channel. A filament structure is automatically generated, which then permits spine reconstruction (C) and type classification if of interest to the researcher. (D) All statistical values can be quickly exported as .csv files, which are cleaned in the open-source software R, and then parsed to extract and analyze those values of interest. Cleaning scripts and data files are provided in supplemental documents.
Supplementary Figure 2

A) Control
B) Mutagen 9-AA

C) 
D) 

E) 
F) 

G) 

Fluorescent intensity (MGV)

Saline  DOX
Supplementary Figure 2. PRISM reports neuronal genomic instability inflicted by frameshift mutagen and DNA double-strand break inducer. (A-B) Representative images of the hippocampus of Ai9 mice transduced with PRISM vector with 8 µm mutagen 9-aminoacrinine (9-AA) or vehicle control (saline) (Red, tdTomato RFP; Blue, DAPI counterstaining, scale bar=200 µm). (C-F) Representative images of the genomic instability reporter labeling of entire mouse striatum after intrastriatal injection of 200 µm DOX (D, F) or saline (C, E) (scale bar = 50 µm). (G) In addition to cell count and soma area, total fluorescence intensity using mean gray value (MGV) was compared. MGV derived from ImageJ v1.43 and followed a similar trend as cell count and soma area. MGV of the DOX (M=21.04, SD=8.36, n=3) group was significantly higher than the saline (M=6.67, SD=1.4, n=3) group (independent sample student t-test, t=2.94, df=4, *p=0.043).
Supplementary Figure 3. PRISM labeling and assay outcome is not a product of DOX-mediated transgene overexpression. Representative images of animals co-infused with AAV-GFP + PRISM and either saline (A) of DOX (B) via intrastriatal infusion (A-C). Intensity profile acquired from a max projection of images captured using identical staining and optical settings. The fluorescent intensity of AAV-mediated GFP and PRISM-mediated RFP expression was compared between saline and DOX treated animals. (D) Relative to the saline (M=605, SD=357.8, N=44) treated group, DOX (M=1156, SD=481, N=263) elicited a significant increase in AAV-mediated GFP expression intensity (independent t-test, t=7.262, df=305, p<0.0001). There was not a significant difference (independent t-test, t=1.281, df=21, p=0.214) in PRISM-mediated genetic expression of RFP between the saline (M=1581, SD=942.7, N=4) and DOX (M=2086, SD=689.7, N=19) treated groups. (E-H) Qualitative assessment of DOX-mediated change to the fluorescent intensity profile of AAV-GFP and PRISM (AAV-G22Cre). The visible change in variability of the green fluorescent intensity profile (green line) between the saline and DOX groups is in contrast to the stable red fluorescent intensity profile (red line) that remains relatively unchanged between treatment conditions - this indicates DOX had a relatively lower influence on the intensity of PRISM labeled neurons in contrast to AAV-GFP labeled neurons. E-F shows GFP intensity reached approximately 375 (X-axis) in the saline condition and over 1,200 in the DOX group, while PRISM-mediated RFP intensity (G-H) was approximately 375 in the saline group and did not increase past 400 in the DOX group. X-axis is intensity and Y-axis is frequency. Scale bar = 20 µm.
Supplementary Figure 4

(A) yH2AX

(B) yH2AX/RFP/Overlap/DAPI

(C) SALINE

(D) DOX

(F) 8-OHdG survival area (% of control)

(G) PRISM neurons with 8-OHdG

(D) 8-OHdG/RFP/COX IV/DAPI

(E) 8-OHdG/COX IV/DAPI

(F) 8-OHdG/COX IV/DAPI
Supplementary Figure 4.  Representative immunofluorescent staining and stacked confocal image of γH2AX (A-C) or 8OHdG (D-E) co-localized to PRISM labeled MSN following DOX treatment. (C) A YZ 2D slice through each MSN illustrates overlap with γH2AX. (D) Representative immunofluorescent images of PRISM labeled dorsal striatal MSNs immunostained for 8OHdG (green) in saline (D) or DOX (E) treated animals. White box highlights PRISM labeled MSNs in both groups, and a white triangle highlights a MSN from the DOX treated group with 8OHdG staining. (F) 8OHdG staining covered significantly more striatal area in the DOX treated group relative to the saline control group (Student t test, t=2.950, df=6, p=0.0256 N=4 per group). (G) The percentage of PRISM-labeled neurons with 8OHdG staining (Student t-test, t=2.465, df=6, p=0.049; Scale bars: G, H: 20 µm; J, K: 200 µm). (H) A single PRISM-tagged MSN expressing tdT is shown with the nuclear marker DAPI and mitochondria marker, COX IV. Scale bar = 2 µm. (I) Colocalization of 8OHdG with COX IV in a PRISM-labeled neurons in mouse striatum. Scale bar = 1 µm. (J) Nuclear and cytoplasmic distribution 8OHdG, with cytoplasmic 8OHdG colocalized with mitochondria marker, COX IV in SNc of PD patients. Scale bar = 1 µm.
Supplementary Figure 5. **DOX increased 53BP1 immunostaining.** Representative images of striatal (A, B) and cortical (C, D) 53BP1 immunostaining in DOX (B, D) and saline (A, C) treated groups (scale bar=10 um). (E) Striatal immunostaining of 53BP1 was significantly higher in the DOX treated group (M=206.6%, SD=84.69%, n=5) than the saline (M=100%, SD=19.92%, n=4) group (t=2.433, df=7, *p=0.045).
Supplementary Figure 6. Neuroinflammation. (A-D): Representative images of striatal IBA1 (A, C) and striatal GFAP (B, D) immunostaining in DOX (C, D) and control (A, B) groups. (E) The total microglial area was significantly higher in the DOX (n=5) treated group than the saline (n=4) group (unpaired student t-test, **p=0.009). (F) The DOX group (n=10) exhibited a significant shift in the ratio of de-ramified microglial to total microglia when compared to the saline group (n=5), indicating activation of microgliosis (unpaired student t-test, ***P=0.0008). (G) The mean grey value (MGV) of striatal GFAP expression was significantly higher in the DOX (n=4) group than the saline (n=4) control group (*p=0.0275), indicating astrogliosis.
Supplementary Figure 7: PRISM-mediated labeling in hippocampus and cortex after PQ treatment. Representative images of AAV-PRISM-mediated labeling in hippocampus (A-B) and cortex (C-D) from mice treated with a sub-chronic PQ (B, D, E) drug regimen (10 mg/kg PQ, i.p., once/week for three weeks) or saline control (A, C), scale bar=10 µm. (E, F) Pyramidal neuron from dentate gyrus from a mouse subjected to the low dose PQ regimen, showing robust region γH2AX expression (E: scale bar = 30 µm, F: scale bar =10 µm, X: scale bar=5 µm). (G-H) A single isolated 3D PRISM-tagged hippocampal neurons labeled by PRISM (white dash box) is shown in Blend Mode (Imaris v9.2.5) (H) with typical nuclear γH2AX. Scale bar = 5 µm.
Supplementary Figure 8: Immunohistochemistry staining for γH2AX in different brain regions after PQ treatment. (A-D) Representative images of cortical mouse brain after immunohistochemistry staining for γH2AX from paraquat and saline-treated groups. Staining illustrates the higher prevalence of γH2AX-positive cells and elevated intensity of nuclear foci (scale bar = 100 µm, 10 µm). (E) Number of cortical neurons expressing γH2AX foci in paraquat (M=123.4, SD=15.25, n=12) treated mice was significantly higher than in the saline (M=99.40, SD=11.22, N=14) treated group (unpaired student t-test; t=4.62, df=24, ***p=0.0001). H2A can be transiently phosphorylated under normal conditions. Therefore, we measured MGV/neuron of the top 15% most intense neurons. (F) Mean intensity of γH2AX/cortical neuron was significantly (t=4.865, df=352, p<0.0001) higher in the PQ (M=105.4, SD=10.87, N=180) treated group than the control saline (M=100, SD=9.8, N=174) group. (G-H) Representative images of hippocampus of mouse brain after immunohistochemistry staining for γH2AX from paraquat and saline-treated groups. Dentate gyrus (DG) and the polymorph layer of the DG (PoDG) are shown. White dashed lines outline the granule cell layer of DG (GrDG). Staining illustrates elevated intensity of nuclear foci in the PQ group (scale bar = 100 µm). (I) Number of hippocampi neurons expressing γH2AX foci in PQ (M=121.9, SD=38.17, n=4) treated mice was higher but not significantly relative to the saline (M=100, SD=19.18, N=4) treated group (unpaired student t-test; t=1.025, df=6, p=0.35). We also measured MGV/neuron of the top 15% most intense neurons as we did in the cortex. (J) Intensity of γH2AX was significantly (t=3.74, df=111, p=0.0003) higher in the PQ (M=107.1, SD=11.40, N=60) treated group than the control saline (M=100, SD=8.21, N=53) group.
Supplementary Figure 9

A. Control
B. PQ

C. TH striatal optical density (mm²)

D. TH nigral soma area (mm²)

E. TH nigral cell count

Saline  PQ  Saline  PQ  Saline  PQ

** ns  ns

Scale bars: 0.5 mm
Supplementary Figure 9: DA neuron and terminal pathology in the mice that received intranigral infusion of low dose PQ. (A-B) Representative images of immunostaining of TH terminal density in the striatum of mice that received intranigral infusion of saline or 3nmol PQ (scale bar = 500 µm). (C) TH terminal optical density was significantly lower in the PQ treated group (M=88.31, SD=6.94, n=4) than the saline (M=115.1 SD=4.94, n=3) group (t=5.651, df=5, p=0.0024). (D) There was not a significant difference between the number of TH nigral neurons between the saline (M=78.25, SD=14.99, N=4) and PQ (M=72.88, SD=21.54, N=4) treated groups; counted all TH neurons within 120 µm of tissue at 1.56mm lateral of midline. (E) Nor was there a significant or detectable difference between the cumulative soma area of TH nigral neurons between the saline (M=11826 µm², SD=3132 µm², N=4) or PQ (M=10415 µm², SD=3838 µm², N=4) treated groups. (I) TH-immunoreactive neurons were estimated using design-based stereology. Stereological estimation of TH cell counts in the SNpc did not reveal a statistically significant loss of DA neurons in TH+PFF group in comparison to WT+Saline group (Student t test, t=0.07103, df=8, p=0.945, n=5 mice per treatment group).
Supplementary Figure 10: Insoluble proteinase-K-resistant α-synuclein immunostaining.
Representative images of insoluble proteinase-K-resistant α-synuclein immunostaining in TG SNCA-OVX mice infused with saline (A, C) or human α-synuclein PFF (B, G) into the striatum. Proteinase-K resistant α-syn immunostaining in the striatum (A-B) and nigral (C-D). Scale bar = 20 μm.
Supplementary Figure 11: Behavioral and pathology validation of humanized PD mouse model infused with human α-synuclein PFF. A battery of behavioral assays was performed on the WT+saline and TG+PFF groups. (A) Spontaneous rearing activity: control group reared significantly (student unpaired t-test; t=2.427, df=13, *p=0.0305) more times than the TG+PFF group. (B) Adhesive removal test: control group made contact with adhesive significantly (t=2.263, df=13, p=0.0414) faster than the TG+PFF group. (C) Beam transversal challenge: for errors per step, there was not a significant (t=2.031, df=12, p=0.065) difference between the groups at the widest portion of the beam, but the TG+PFF group made significantly (t=2.216, df=12, p=0.047) more errors/step at the narrowest portion of the beam. (D-F) Open field: No difference detected for forwarding movement velocity (p=0.6674), total distance traveled (p=0.6315), or time spent in the center of the chamber (p=0.75). (G) Accelerating rotarod test did not detect a significant difference in motor function between the groups (Repeated-measures ANOVA; F(8, 88) = 1.147, p=0.341 for interaction between trial and genotype; F(8,88) = 0.785, p=0.294 for interaction between trial and gender). (H) There was not a significant difference in animal weight at the time of surgery or sacrifice (p>0.05). Unpaired student t-tests were used unless noted otherwise.
**Supplementary Figure 12**: (A) TH immunostaining of SN in human postmortem brain tissue of PD patients reveals LB inclusion (white arrow). (B-C) Representative images of TH IF staining in the SN of PD patients and healthy age-matched controls (Scale bar: 30 μm). (D-E) Dopaminergic neurons and glial were significantly decreased and increased, respectively, in PD brains relative to healthy controls (student t-test, p=0.0042; p=0.0452).
Supplementary Figure 13

Cell area = 581.35 μm²

Cell channel 3 intensity mean = 313

Potential foci automatically identified (grey dots), diameter = 0.4μm

Typical foci are more than 6x brighter than background signal

```R
data%>%
  filter(cha3mean_foci >
        (cha3mean_cell(*6)))
```
Supplementary Figure 13: Using Imaris Surface and Cell function to determine H2AX foci

(A) DAPI stained nucleus with typical H2AX foci stain with Alexa Fluro 594 (red). (B-C) Using Imaris (Bitplane) Surface and Cell function to mask the nuclear region, and Spot function to label nuclear foci in an unbiased automatic manner. Figure D illustrates potential foci Imaris labeled. Values indicate TRITC channel intensity in the center of foci (grey spheres). To improve accuracy, foci statistics were exported and were parsed in R. (E-F) A selection criteria was determined by examining characteristics of typical H2AX foci from positive controls stained and imaged using the same protocol as data to be analyzed. Selection criteria selected only those foci 6x brighter than the Cell’s background signal. Additional criteria were applied (not illustrated): eliminated foci located closer than 0.4um to the cell surface, and to rule out FITC bleed over, TRITC channel intensity had to be greater than FITC intensity.
Supplementary Figure 14

- **A**: Bar graph showing the frequency of TH neurons with γH2AX foci for Control and PD groups. Pearson's correlation coefficient (r) is 0.521 with a p-value of 0.196.
- **B**: Bar graph showing the frequency of TH neurons with γH2AX foci across different age groups (Aged, PD, Young). Pearson's r is 0.403 with a p-value of 0.29.
- **C**: Scatter plot for the PD group showing frequency of TH neurons with γH2AX foci vs. age. Pearson's r is 0.3 with a p-value of 0.48.
- **D**: Scatter plot for the PD group showing frequency of TH neurons with γH2AX foci vs. PMI. Pearson's r is 0.135 with a p-value of 0.75.
- **E**: Scatter plot for the Control (Age-matched) group showing frequency of TH neurons with γH2AX foci vs. age. Pearson's r is -0.187 with a p-value of 0.76.
- **F**: Scatter plot for the Control (Age-matched) group showing frequency of TH neurons with γH2AX foci vs. PMI. Pearson's r is 0.162 with a p-value of 0.77.
- **G**: Scatter plot for the Control (Age-matched) group showing frequency of gialls with γH2AX foci vs. age. Pearson's r is 0.3 with a p-value of 0.63.
- **H**: Scatter plot for the Control (Age-matched) group showing frequency of gialls with γH2AX foci vs. PMI. Pearson's r is -0.406 with a p-value of 0.5.
**Supplementary Figure 14: PMI nor age influenced statistical analysis.** (A-B) There is not a significant difference in age or PMI between the PD and control groups (Age, t=1.337, df=11, p=0.21). There is not a significant difference in PMI between the three groups (PD, Age-matched control group or Young control group); PD and Age-matched group, t=1.855, df=11, p=0.091; Young and Aged groups, t=0.314, df, p=0.76. (C-J) The potential influence of PMI or age were further probed at the within-group level. (C-D) Within the PD group (red dots), there was not a correlation between age and frequency of dopaminergic neurons with yH2AX foci (Pearson(r)=0.521, p=0.19). There was not a correlation between PMI and frequency of dopaminergic neurons with yH2AX foci (Pearson(r)=0.403, p=0.29). (E-F) Within the PD group, there was not a correlation between age and frequency of glial with yH2AX foci (Pearson(r)=0.3, p=0.48). There was not a correlation between PMI and frequency of glial with yH2AX foci (Pearson(r)=-0.135, p=0.75). (G-H) Within the age-matched group (blue dots), there was not a correlation between age and frequency of dopaminergic neurons with yH2AX foci in (Pearson(r)=-0.187, p=0.76). There was not a correlation between PMI and frequency of dopaminergic neurons with yH2AX foci (Pearson(r)=0.182, p=0.77). (I-J) Within the age-matched group, there was not a correlation between age and frequency of glial with yH2AX foci (Pearson(r)=0.3, p=0.63). There was not a correlation between PMI and frequency of glial with yH2AX foci (Pearson(r)=0.406, p=0.5).

**Supplementary Methods: Animal care**

Mouse care in the current study was in accordance with the United States Public Health Service Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Institutional Animal Care and Use Committee at LSUHSC. Animals were housed in a standard barrier facility at LSUHSC, up to four mice per cage with food and water available ad libitum. They were housed in a temperature-controlled environment with 12 hours light/dark cycle.
Mouse embryo fibroblast derivation and cell culture conditions

Ai9 homozygous breeding was established, and gestation was monitored daily. Day 18 post coitum mouse embryos were isolated and manually dissociated and then trypsinized to produce single-cell suspensions following a previously described protocol. A portion of tissue from each embryo was separated and used to confirm the genotype. Aseptic technique was maintained once embryos were isolated. Cell culture media and supplements were obtained from Mediatech. The cell suspension was plated onto a six-well plate, and cell culture was maintained in Dulbecco's modified Eagle’s medium (DMEM) with 10% fetal bovine serum (Atlanta Biologicals), L-glutamine, G418 (400 μg/ml), and penicillin-streptomycin, and incubated at 37°C, 5% CO2 in a humidified chamber.

MEF in-vitro experiment:  A sterile poly-lysine coated coverslip was placed in each well of a 6 well-plate before plating 2.4x10^6 MEF cells/well in DMEM 10% FBS 1X pen/strep. Cells were grown to 70% confluency. Prior to transfection, media was replaced with 50% complete media + 50% reduced serum Opti-MEM (Thermo Fisher, 11058021). An equal amount of transfection reagents and CMV-GFP-Cre or CMV-PRISM were used to transfect six wells each. An additional six wells were transfected with twice the amount of CMV-G22-Cre. Captured images using an inverted fluorescent microscope at 24-hour intervals for four days. At the end of the experiment, coverslips were carefully removed and fixed in 4% PFA and then stained using anti-RFP antibody conjugated to fluorophore 594 and counterstained with DAPI. A 10x objective was used to image each quadrant of all remaining coverslips. Total cell count (DAPI) and the number of cells expressing Cre-mediated tdT were quantified.

Immunofluorescence

Cryosections were washed three times for 10 minutes in PBS and blocked with 1% BSA and 5% NGS in PBST for 1 hour at room temperature before starting primary antibody incubation.
Sections were incubated at 4 °C overnight with respective primary antibodies diluted in 1%BSA + 1%NGS in PBST (all antibodies and dilution factors provided in Supplemental Table 4).

Sections were then washed seven times for 5-7 minutes in 0.1M PBS and then incubated with secondary antibodies. Sections incubated with anti-TH primary were incubated in Alexa Fluro 488 Goat Anti-Rabbit (1:300) for two hours; sections incubated with anti-H2AX were incubated in Alexa Fluro 488 Goat Anti-Mouse (1:300) for two hours. Double immunofluorescent staining was performed using anti-TH and anti-H2AX as well, and incubation sets were performed simultaneously. Lastly, the sections were washed four times for 5 min with PBST and rinsed for 10 minutes in PBS before mounting in DAPI hard mounting medium (VECTOR).

Staining with all other primary antibodies followed a similar protocol. After incubating in primary antibody, sections were washed as described above and then incubated with anti-rabbit or anti-mouse biotinylated antibodies (1:200) for two hours at room temperature. Next, sections were washed five times for 5 min in PBS. Sections were then incubated in Strepavidin-Alex Fluro 488 or 594 secondary antibodies (1:300) dissolved in PBS-0.03%Triton (PBST) at room temperature for one hour. Sections were washed and mounted with DAPI.

Confocal images were taken with a Nikon A1R Confocal & Super Resolution System and processed using LSM software (Zeiss), NIS (Elements), and analyzed with either ImageJ Version 1.43 or Imaris Version 9.5. Confocal optical settings were kept consistent between control and variable groups, and are listed in Supplemental Table 3. Epi-fluorescent images were captured with an Amscope Epi Fluorescent microscope equipped with ISCapture camera and software and 10x or 40x objective. Images were analyzed with ImageJ.

Quantifying PRISM vector labeled neurons in vivo
Fig. 3C-D and Supp. Fig. 2G quantification: Four sagittal brain sections from each hemisphere of cohort one animals were selected for analysis. Selected sections were 40 µm thick and straddled the injection track over a 720 µm range. Sections were washed and mounted with DAPI hard mounting medium. An Amscope Epi Fluorescent microscope equipped with IScapture camera and software was used to image the dorsal striatum with a 10x objective using identical exposure settings. Cell count, soma area, and total fluorescent signal was then derived using ImageJ batch processing. Scripts are provided online.

Fig.4A-C quantification: To count PRISM-labeled neurons in cohort two, 80 µm thick sagittal sections were washed and temporarily mounted in glycerol. An epi-fluorescent microscope with a 20x objective was used to explore each section of the entire brain. Sections were examined along an invisible grid pattern, recording neuronal type (MSN, pyramidal, glial, other) and region (striatum, cortex, olfactory bulb, hippocampus, midbrain, other) for every fluorescent-tagged neuron. Sections were then washed and returned to cryoprotectant for later staining and analysis.

Fig. 5E-F, R quantification of striatal and nigra PRISM labeling frequency following local PQ exposure: To count PRISM-labeled striatal neurons in cohort three, 40 µm thick sagittal sections were washed and mounted. To count nigral labeled neurons, 60 µm sections were washed and stained with anti-TH and a secondary antibody conjugated to Alexa fluor 488 (described above). For confocal imaging, a Nikon Confocal Microscope with a 20x objective was used to image sections along the length of the Z-axis. Z-series stack analysis using Imaris batch processing and image J is described below.

To quantify PRISM vector labeling frequency in a cell-type and region specific manner, a manual Surface was created to mask the SNc and DAPI, FITC and TRITC channels were
A second and third Surface was used to mask and segment the soma of PRISM vector-labeled neurons and of dopaminergic neurons, respectively. Dopaminergic neurons were manually counted and PRISM vector-tagged neurons and total cells (DAPI) were automatically counted using the Spots function. The Coloc tool was then used to automatically threshold FITC (TH) and TRITC (RFP) channels before automatically identifying co-localized pixels. A co-localized channel was created, and a researcher manually counted the number of FITC + TRITC co-localized cells. To measure labeling in the striatum, the Spots function was used to automatically count the number of total cells (DAPI) and PRISM-labeled neurons.

Fig. 3H-I (Immunofluorescent γH2AX analysis) quantification of γH2AX and PRISM sensitivity to γH2AX following DOX treatment: Imaris was used to count the number of cells with nuclear γH2AX foci. A Surface was created based on the DAPI channel to segment out the nucleus. The Coloc tool was then used to automatically identify FITC (γH2AX) and DAPI co-localized pixels using default settings. A co-localized channel was created, and the Spots function was ran to count the number of intact cells with co-localized signal. By inspecting the image in 3D, a researcher confirmed that the co-localized signal was not due to bleed over from an adjacent cell. The Spots function was also used to quantify total cell count (DAPI), and the number of PRISM-labeled neurons (TRITC), using default settings. See supplemental files for step-by-step protocol. Results were validated using ImageJ (NIH): the DAPI channel was converted to a binary stacked image, and each slice was denoised. An automatic threshold was applied, and the Particle Analyzer plugin was used to remove small erroneous signals. A mask per each slice was created and applied to the original image stack, segmenting the DAPI channel. An ROI for each nucleus was created for each slice and used to segment the γH2AX channel. A max intensity projection (MIP) was created from the resulting image stack, and the number of cells with overlapping typical γH2AX signal was manually counted using the Cell Counter plugin. The
Particle Analyzer plugin was used to automatically count the number of cells based on the DAPI and TRITC channels. The outcome of each approach was the same.

PRISM sensitivity to was determined using the same data used to quantify mean γH2AX expression following DOX treatment. A Surface was created to represent the soma of PRISM-labeled neurons (TRITC), and was used to clear all co-localized pixels located outside of the Surface. Each PRISM-labeled neuron was inspected and the number of individual neurons with DAPI + FITC co-localized pixels larger than 0.4 μm were counted as expressing nuclear γH2AX.

Figure 6Q-U, Supplementary Figure 11I: Stereological quantification of PRISM labeling frequency in SNCA-OVX + PFF mouse model: A subset of animals from the WT + Saline and SNCA-OVX + PFF groups were selected randomly and received intranigral infusion of PRISM. Brains were sectioned at 40 μm and stereological quantification was used to quantify PRISM labeling frequency of SNpc dopaminergic neurons. In total, every 5th section (8 sections total) spanning the SN, from -2.46 mm to -4.04 mm from bregma were selected in a rostral to caudal direction. Sections were stained for TH and visualized using Alexa Fluro 488 (described previously). Using identical confocal optical settings, all sections were imaged simultaneously with 10x objective using confocal scanning mode that moves along XYZ coordinates to cover all 8 sections per slide. The SNpc was outlined using anatomical landmarks previously defined. Batch processing was then applied in Imaris to quantify dopaminergic and PRISM neurons, where all cells from the tissue sample were counted in an unbiased and reproducible way. Counted cells were then used to estimate the total number of TH+ and PRISM neurons in the SNpc. These final amounts were then used to calculate SNpc PRISM labeling frequency per SNpc dopaminergic population. Stats for each subject was exported from Imaris as a .csv file and analyzed using R. Reproducible R script is provided as a supplemental file.
**Immunohistochemistry γH2AX staining analysis**

IHC γH2AX analysis in systemic PQ treated group: Quantitative comparison of immunohistochemical staining of cortical and hippocampal γH2AX foci in mice. For cortical analysis, sections from 4 non-overlapping brain regions were selected from each animal. Four regions selected based on previously documented γH2AX regional and interment cortical expression patterns. Each data point is from a given region, so that for saline n(animals)=4, n(sections)=14; for PQ n(animals)=4, n(sections)=12. H2A can become transiently phosphorylated under normal conditions. Therefore we classified neurons into two groups, either Cell with detectable or elevated γH2AX signal. A custom automated macro then identified oval nuclear ROIs. A point and click plugin were used to quickly add an ROI to those cells with low γH2AX signal that our automated plugin missed. Data was exported to R for cleaning and exploration. The MGV of each ROI was normalized by subtracting background pixel value from its respective image. Each Cell was classified as having a detectable or elevated γH2AX signal. The elevated threshold was determined by averaging the MGV of the 15 highest values from all images (both treatment groups). For hippocampal analysis, only the granule cell layer of the dentate gyrus was imaged and quantified. The same process was used as described above.

Immunohistochemical IBA1 Activation Ratio: Calibrated and set scale of image. Images converted to 8-bit TIFF files. Used ImageJ batch processing to subtract background and denoise images. Used wand tool(legacy=80), to segment each microglial by clicking in the center of the soma. Each selection was measured, and the area was saved. Classified each IBA1+ glial Cell as ramified or deramified based on descriptive morphology of microglial activation states. Calculated percent of total microglial that are deramified.

Immunohistochemical GFAP, 53BP1, and 8OHdG analysis: Images were quantified in a manner similar to IBA1 MGV analysis. Images were calibrated, and the scale was set. Images
were converted to 8-bit TIFF files, and ImageJ batch processing was used to subtract background and denoise images. Obvious artifacts were manually avoided, such as broken tissue. The remaining image was measured, and area, perimeter, MGV, and the number of pixels were saved. Identical optical settings were used between control and variable groups.

**Image Analysis Workflow, human brain**

Immunofluorescent γH2AX analysis: Analysis was performed in Imaris (Bitplane, V9.5) and R (V3.6). Confocal images (.ND2 files) were uploaded to Imaris. Non-dopaminergic and dopaminergic cells were processed separately within Imaris because the nucleus of dopaminergic neurons is significantly larger, and stains lighter, than non-dopaminergic cells. A surface was created to mask DAPI signal for non-dopaminergic cells. The Surface was used to segment the nucleus from the cytoplasm by masking all three channels (DAPI, FITC, TRITC). The Cells and Vesicle functions were then used to identify the nucleus (Cell) and potential γH2AX foci (vesicles) based on the masked DAPI and TRITC channels, respectively. We then repeated the process for dopaminergic neurons. Statistical information for Cells (i.e., nucleus) and Vesicles (i.e., γH2AX foci) were then exported as CSV files. Files were imported into R for cleaning and exploration. The mean intensity of TRITC for each Cell Surface was used as the background. Through manual inspection of typical γH2AX foci within Imaris viewer, we determined that unambiguous γH2AX foci were 3x brighter than the local background, and the TRITC channel intensity was at least twice as bright as FITC intensity. We also excluded spots that were on the edge of the Cell’s membrane because they were often erroneous. We also excluded Spots that co-localized the TRITC and FITC channel as there was sometimes bleed over. R was used to filter foci (i.e., vesicles) meeting this criterion. A step-by-step protocol and R scripts are provided online.

**Neuronal reconstruction and morphometric analysis**
Neuromantic was used to visualize and quantify the gross structure of individual striatal MSNs located within the dorsal striatum. 80 µm thick sagittal sections from cohort two were used. Cohort two was comprised of saline, PQ low, and a PQ high group. Multiple sections within 480 µm of the injections track were searched for intact and fully visible MSNs. Caution was taken to ensure only the most intact and robustly labeled neurons were selected from each animal. Sections were stained with anti-RFP, as described above. After imaging, dendritic arbors were manually traced using Neuromantic. Serial optical sections were taken along the entire Z-axis on the MSN (Z-stacks) in single increments. Images were acquired on an Epi-fluorescent microscope using a 40×/1 NA objective (Zeiss) using the same optical settings. Images were stored at 8-bit image depth at a resolution of 512 × 512 pixels (0.22 × 0.22 × 1 µm). Serial images were batch processed in ImageJ to prepare for reconstruction. The resulting Z-series stacks were imported into Neuromantic, and the dendritic arbors were manually reconstructed. The resulting SWC files were then analyzed in ImageJ using the trace_plugin, and sholl analysis was conducted using 100 µm radius shells.