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## Supplemental Information

# Cellular Senescence Is Induced by the Environmental Neurotoxin Paraquat and Contributes to Neuropathology Linked to Parkinson's Disease

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**Supplementary Information**

**Supplementary Experimental Procedures:**

**IL-6 effects on viability of human DAergic neurons:** Primary human DAergic neurons were sourced and maintained as described in Online Methods: *Viability of human DAergic primary cultures*. 24 hrs after plating, DAergic neurons were cultured for 72 hrs in the recommended complete medium containing either recombinant human IL-6 (Perkin Elmer; 1.5 µM) or vehicle (water). The MTT assay was performed as described in Online Methods. The MTT experiment was run 3 times and each condition was run in quadruplicate; statistics were calculated with n = 3 experiments. We also conducted a LIVE/DEAD fluorescent-based assay (CyQuant Direct Cell Cytotoxicity Assay; Invitrogen; C35011) in which a fluorescence signal is directly correlated to the number of viable cells. We normalized fluorescent signals from individual treated wells to the mean fluorescent signal in vehicle treated wells and expressed the data as % of vehicle treatment. The LIVE/DEAD experiment was run 3 times and each condition was run in quadruplicate; statistics were calculated with n = 3 experiments.

***In vivo* Ki67 immunostaining**

Tissue preparation and immunostaining were carried out as described in On Line Methods. Horizontal sections containing SNpc and hippocampi were stained with antibodies against Ki67 (1:200; ab15580; Abcam), GFAP or tyrosine hydroxylase (TH). Primary and secondary antibody sources and dilutions are those listed in the main text. For dentate gyrus (DG) staining, sections containing the interior hippocampus (midway along rostral-caudal axis) were selected. Confocal imaging was carried out as described in On Line Methods. SN resident astrocytes ($GFAP^+$) were selected as described; $TH^+$ neurons the SN were chosen for analysis of Ki67. Cells in the inner DG that expressed high-levels of Ki67 by eye were selected to calculate mean expression of Ki67 in actively dividing to cells to determine whether any astrocytic Ki67 expression fell within these values. Image data were analyzed using ImageJ.

**Supplementary Figures:**

# S1

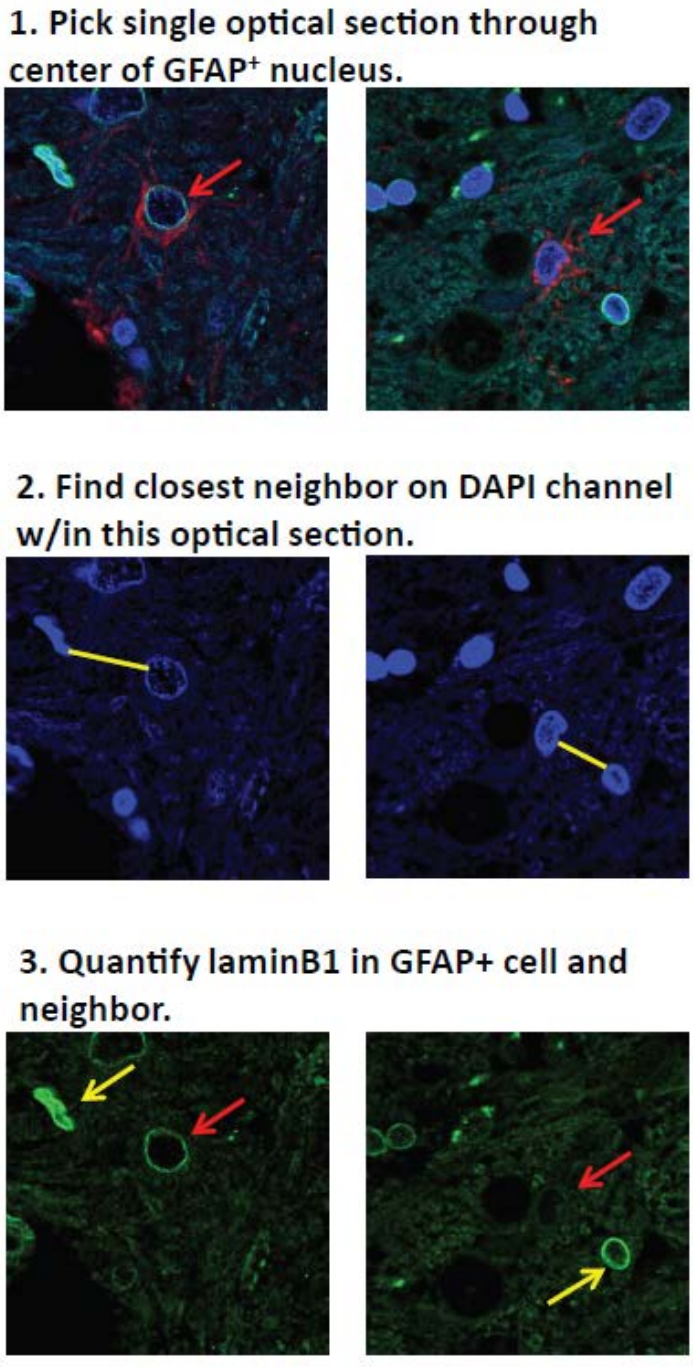
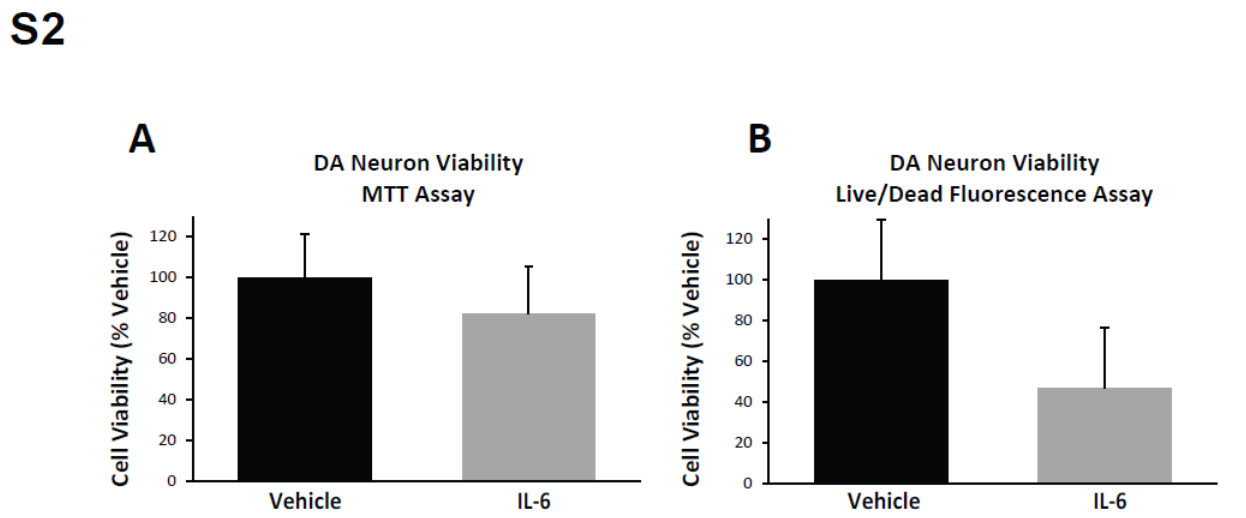



**Figure legend. Quantification method for Lamin B1 in nuclear lamina of astrocytes and neighboring cells.**

Steps 1-3 describe how immunofluorescence of Lamin B1 in the laminae of astrocytes ($GFAP^+$) and non-astrocytic

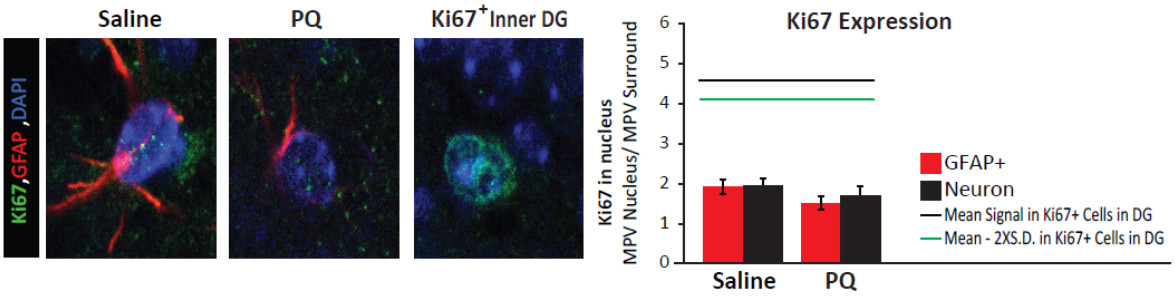
neighbors (GFAP$^-$) was quantified. Although human midbrain tissue is shown here, the same methodology was used when quantifying Lamin B1 in mouse SNpc tissues.

**Supplementary Figure 2. IL-6 alone does not significantly reduce DAergic cell viability.**




**(A)** Human DAergic neurons were cultured in complete DA media containing recombinant human IL-6 (1.5 □M) or water (Vehicle). After 72 hrs, cell viability was assessed using the MTT assay as in Fig. 3 (left) or the Live/Dead fluorescence assay (right). 1.5 □M is the concentration of IL-6 measured in CM from senescent astrocytes which reduced DAergic viability (Fig. 3A); $p>0.05$; unpaired t-test. This experiment was conducted in quadruplicate two times.

**Supplementary Figure 3. Astrocytes in the adult SNpc do not readily divide regardless of PQ exposure.**



Astrocytes (GFAP+; red) in the SNpc of either saline (left panel) or PQ (middle panel) injected animals expressed minimal levels of Ki67 (green). As a positive control, some inner-most cells in the dentate gyrus (DG) were shown to express higher levels of Ki67 (right panel). Quantification, using mean pixel value (MPV), revealed that Ki67 expression in astrocytes (red bars) was in line with levels expressed in post-mitotic neurons (black bars) and well below the mean expression level (black line) and mean - 2X the standard deviation (green line) in presumed Ki67+ granule cell progenitors. None of the bars are significantly different from each other; n = 4 saline animals and 3 PQ animals; $p>0.05$; unpaired t-test. All bars are significantly different from mean Ki67 expression in dividing cells in the inner DG; n = 3 saline animals; $p<0.005$; unpaired t-test.