

## **SI Appendix**

### **Supplementary Methods:**

#### **Antibodies**

For Western blot and immunohistochemical analysis the following primary antibodies were used: mouse monoclonal anti-amyloid precursor protein (APP, clone 22C11, Millipore), mouse monoclonal anti- $\alpha$ -Synuclein (Syn-1; clone 42, BD Biosciences), rabbit monoclonal anti-phospho-Ser129- $\alpha$ -Synuclein (clone EP1536Y, Abcam), rabbit polyclonal anti-Iba1 (019-19741, Wako), mouse monoclonal anti-ubiquitin (clone P4D1, Cell Signaling Technology), mouse monoclonal anti-phospho-Ser202/Thr205-tau (clone AT8; Pierce Biotech), mouse monoclonal anti-tau (clone TAU5, MAB361, Millipore), anti-rabbit polyclonal anti-glial fibrillary acidic protein (GFAP; E18320, Spring Bioscience).

#### **Gallyas silver stain**

For Gallyas silver staining, sections were pre-incubated in 4% PFA (in 0.1 M PB) for  $\geq 2$  days at 4°C before processing with the FD NeuroSilver™ kit II (FD Neurotechnologies, Inc.) according to the manufacturer's protocol. Sections were mounted on Superfrost plus slides (Fisher Scientific), dehydrated with increasing ethanol concentrations and xylene, and coverslipped using Entellan (Merck). Images were captured using a light microscope (Axio Imager M2, Zeiss) equipped with a color CCD camera (AxioCam, Zeiss).

#### **Image analysis**

Images were captured using an Axio Scan.Z1 slide scanning microscope at 20x (Carl Zeiss, Germany), and were analyzed with HALO software v3.0.311.356 (Indica labs, USA). Quantification was performed using different algorithms: "Area quantification v2.1.3" for analysis of APP-positive inclusions, and "Microglial activation v1.4" for analysis of Iba1-positive microglia. One representative image from the dorsal ipsilateral striatum was used per animal. For quantifying APP-positive inclusions, the 1-stain method was used: one APP inclusion was chosen as the reference and the minimum optical density of APP signal was adjusted to the reference inclusion until all background was eliminated. For quantifying microglial activation, the activation process thickness was set at 2000  $\mu\text{m}$ . For silver analysis, images from the dorsal ipsilateral striatum were subjected to color deconvolution and separation using NIH

ImageJ with a custom macro to isolate black neurites and particles, as previously described (1). The total silver-positive signal was calculated for each image, sampled across 3 consecutive sections with 15 images per section taken at random (total of 45 images per animal).

### ***In vivo* immunoprecipitation (IP)**

FLAG-tagged human LRRK2 proteins were enriched from primary neurons or injected rat striatum using Protein G-Dynabeads (50  $\mu$ l; Invitrogen) pre-coupled with mouse anti-FLAG-M2 antibody (5  $\mu$ g; Sigma). Triton-soluble neuronal extracts (30  $\mu$ g protein) or brain lysates (1.5 mg protein) were incubated with Dynabead-antibody complexes in 1 ml of IP buffer (1X PBS pH 7.4, 1% Triton X100) with rotation for 24 h at 4°C. Dynabead-antibody complexes were washed 3x with 1 ml IP buffer and 3x with 1X PBS and proteins were eluted at 70°C for 10 min in 2X Laemmli sample buffer (50  $\mu$ l; Bio-Rad) containing 5% 2-mercaptoethanol. Immunoprecipitates or input lysates were resolved by SDS-PAGE (7.5% Tris-Glycine gels) and subjected to Western blotting with antibodies to pSer1292-LRRK2 (Abcam), pSer935-LRRK2 (Abcam) and FLAG-M2 (Sigma).

### **References**

1. Tsika E, *et al.* (2014) Parkinson's disease-linked mutations in VPS35 induce dopaminergic neurodegeneration. *Hum Mol Genet* 23(17):4621-4638.

## **Supplementary Figures:**

**Figure S1. Pilot study evaluating role of kinase activity using new Ad5-LRRK2-G2019S vectors at low titer.** **A)** Validation of Ad5 vectors in SH-SY5Y cells. Cells were transduced with increasing viral particles (vp) of each Ad5-eGFP or Ad5-LRRK2 vector ( $0.5, 1$  or  $1.5 \times 10^{10}$  vp/well). Western blot analysis of cell extracts with GFP or FLAG antibody to detect a titer-dependent increase in human LRRK2 levels. Ponceau S stain was used as a protein loading control. Molecular mass markers are indicated in kilodaltons. **B)** Ad5-eGFP and Ad5-LRRK2 (G2019S, G2019S/D1994N or G2019S/K1906M) vectors were delivered at a single injection site ( $4.5 \times 10^9$  vp/site, in  $2.5 \mu\text{l}$ ) in the ipsilateral substantia nigra pars compacta of rats, as indicated (red arrow). Immunohistochemistry for eGFP (anti-GFP antibody) or human LRRK2 variants (anti-FLAG antibody) in the ipsilateral substantia nigra at 10 days post-injection. Images indicate low and high magnification for each vector. Scale bar:  $500 \mu\text{m}$ . **C)** Immunohistochemistry revealing dopaminergic neurons (anti-TH antibody) in contralateral and ipsilateral substantia nigra at 21 days post-delivery of Ad5 vectors. All sections were counterstained with Cresyl violet. **D)** Analysis of neurodegeneration in rat substantia nigra at 21 days. TH-positive dopaminergic neuron number in the substantia nigra assessed by unbiased stereology. Bars represent % neuronal loss in the injected ipsilateral nigra relative to the contralateral nigra (mean  $\pm$  SEM,  $n = 4$  animals/vector),  $*P < 0.05$  by one-way ANOVA with Bonferroni's multiple comparisons test. *n.s.*, non-significant.

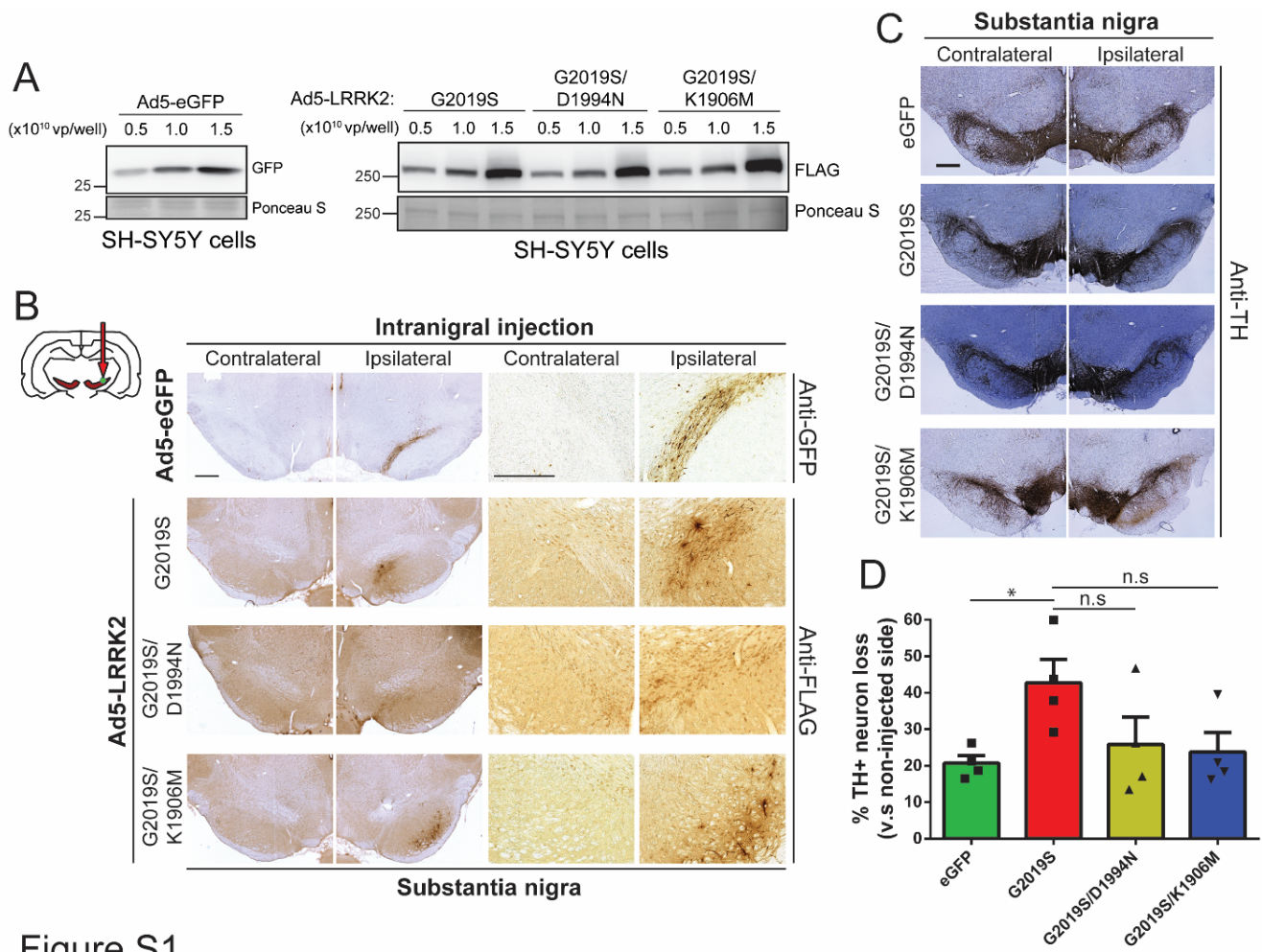


Figure S1

**Figure S2. Efficient retrograde transport of Ad5-GFP vector from striatum to nigral dopaminergic neurons in rat brain.** Ad5-eGFP vector was unilaterally delivered at six injection sites ( $1.5 \times 10^{10}$  vp/site, in  $2.5 \mu\text{l}$ ) to the rat striatum. Immunofluorescence co-localization of GFP-positive neurons (anti-GFP, green) and TH-positive dopaminergic neurons (anti-TH, red) in the ipsilateral substantia nigra at 10 days post-injection. For the ipsilateral nigra, representative images from a rostro-caudal series are shown with each section separated by  $80 \mu\text{m}$  from each other. Scale bars:  $500 \mu\text{m}$ .

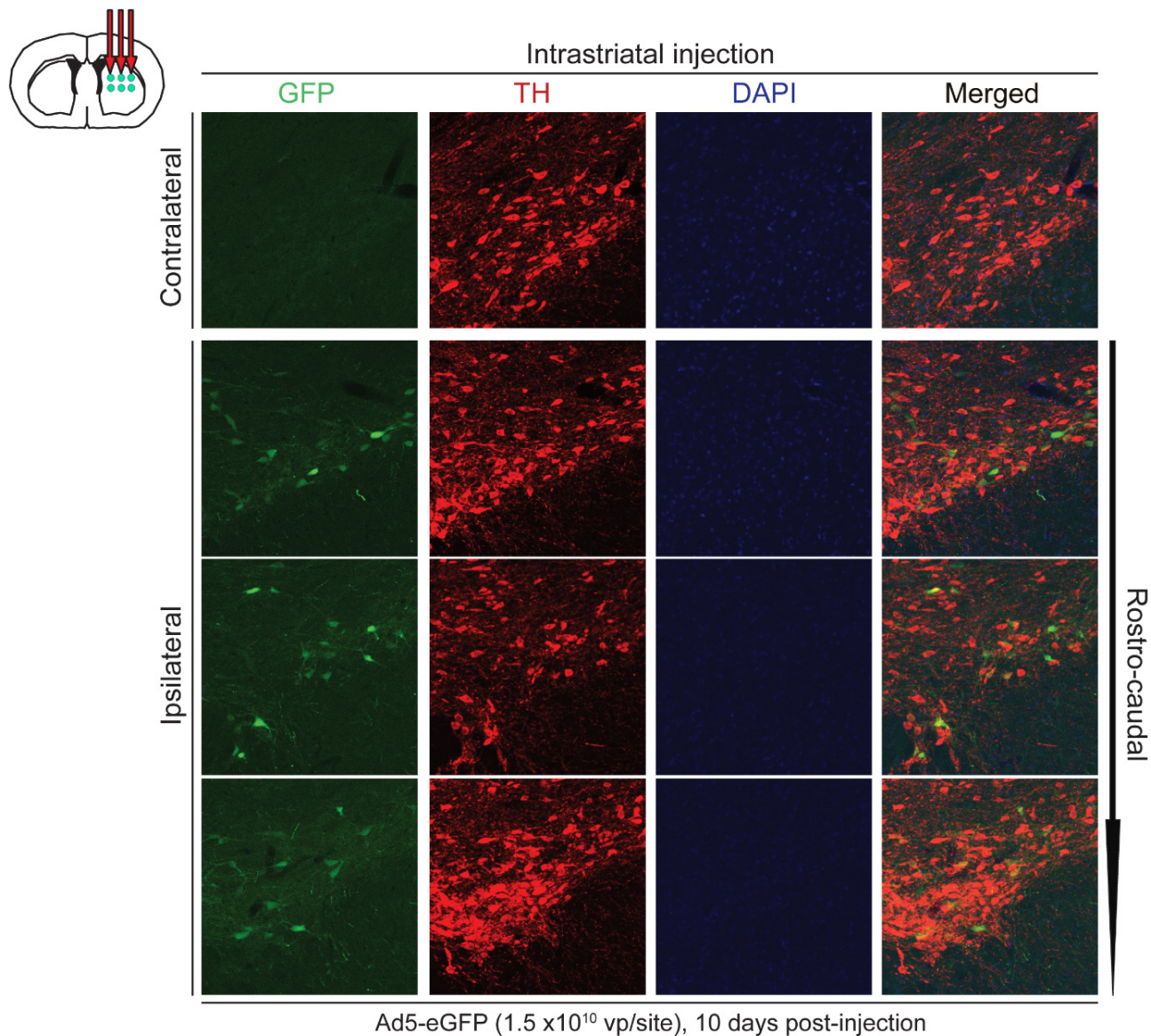


Figure S2

**Figure S3. Evaluation of human LRRK2 kinase activity following intrastriatal delivery of Ad5-LRRK2 vectors in rats.** **A)** Human LRRK2 levels and Rab10 phosphorylation in striatum. Ad5-LRRK2 (WT, G2019S or G2019S/K1906M) vectors were unilaterally delivered at six injection sites ( $1.5 \times 10^{10}$  vp/site, in 2.5  $\mu$ l) to the rat striatum. Ipsilateral striatum was harvested at 10 days post-delivery and subjected to sequential detergent extraction in 1% Triton X100 and 2% SDS. Striatal fractions were analyzed by Western blot analysis ( $n = 3$  animals/vector) indicating the levels of human LRRK2 variants (FLAG) or pT73-Rab10 and total Rab10. GAPDH was used as a loading control. NI, non-injected striatum. Graphs indicate densitometric analysis of human LRRK2 variant levels (FLAG) normalized to GAPDH levels in each detergent fraction. Bars represent the mean  $\pm$  SEM ( $n = 3$  animals/vector). *n.s.*, non-significant by one-way ANOVA with Bonferroni's multiple comparisons. **B)** Immunoprecipitation (IP) of human LRRK2 from brain and neurons. Triton-soluble extracts of ipsilateral striatum from **(A)** ( $n = 4$  animals/vector), or soluble extracts from primary cortical neurons infected with Ad5-LRRK2 or Ad5-GFP vectors, were subjected to IP with anti-FLAG antibody to immunopurify human LRRK2 variants. Western blot analysis of IP and input fractions indicates levels of pS1292-LRRK2, pS935-LRRK2 and human LRRK2 (FLAG). **C)** Human LRRK2 kinase activity in dopaminergic neurons. Ad5-LRRK2 (G2019S and G2019S/K1906M) vectors were unilaterally delivered at six injection sites ( $1.5 \times 10^{10}$  vp/site, in 2.5  $\mu$ l) to the rat striatum. Immunofluorescence co-localization of human LRRK2 (anti-FLAG antibody) and pSer1292-LRRK2 in ipsilateral striatum and nigra at 42 days post-injection. Arrowheads indicate FLAG-positive dopaminergic neurons (green), their corresponding pSer1292-LRRK2 signal (red), and merged images. Scale bars: 500  $\mu$ m.

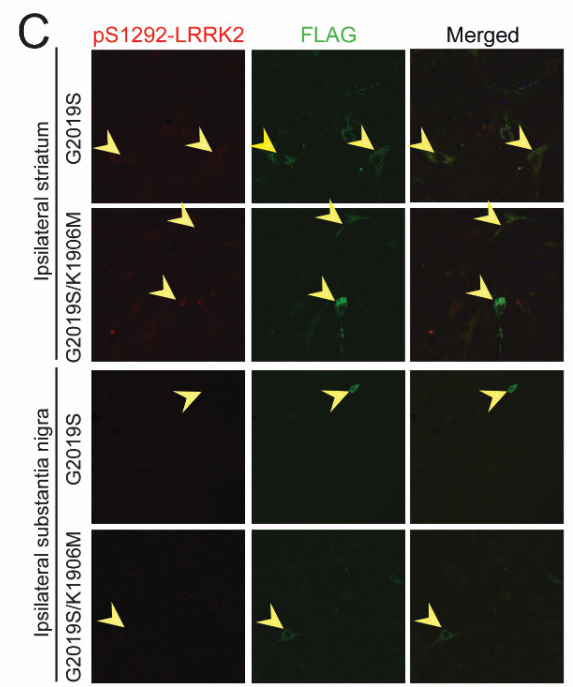
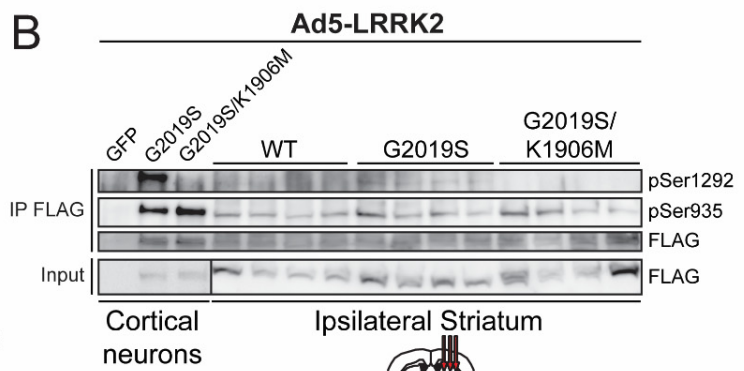
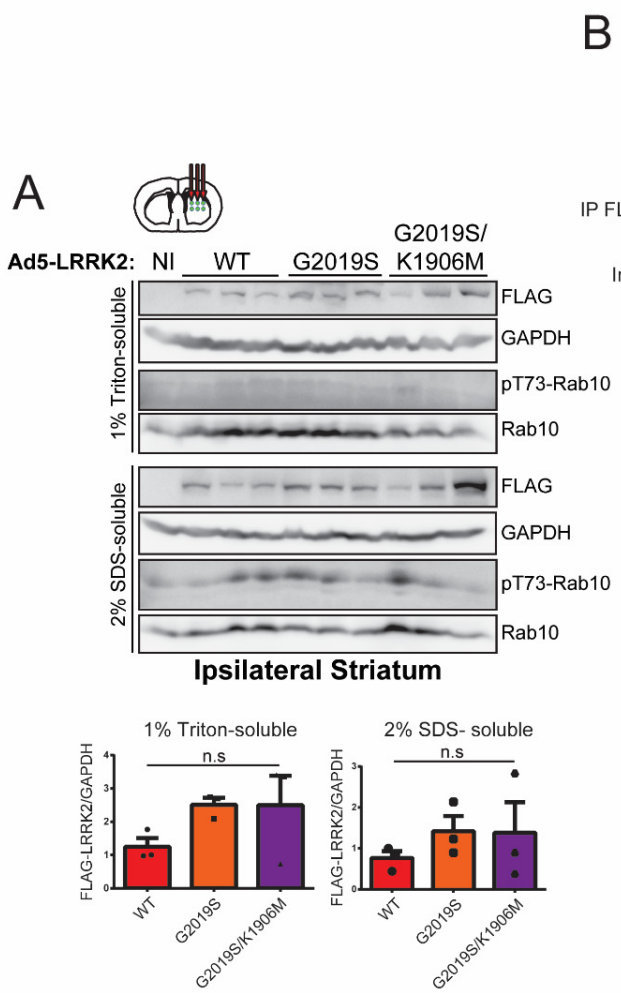


Figure S3

**Figure S4. Ad5-G2019S-LRRK2 induces axonal damage in the rat striatum. A-D)** Ad5-LRRK2 (WT, G2109S and G2019S/K1906M) vectors were unilaterally delivered at six injection sites ( $1.5 \times 10^{10}$  vp/site, in 2.5  $\mu$ l) to the rat striatum. **A)** Immunohistochemistry showing Ad5 capsid expression (anti-Hexon antibody) in the ipsilateral striatum at 42 days post-delivery. **B)** Immunohistochemistry showing increased ubiquitin-positive immunoreactivity (anti-ubiquitin antibody, clone P4D1) induced equivalently by all Ad5-LRRK2 vectors, and **C)** increased amyloid precursor protein-positive inclusions/spheroids (anti-APP antibody, clone 22C11) induced by G2019S LRRK2 in ipsilateral striatum. **C)** Graph indicates the percent (%) of APP-positive inclusions in the ipsilateral striatum for each LRRK2 variant identified by Halo analysis. Bars represent mean  $\pm$  SEM ( $n = 3$  animals/vector). **D)** Gallyas silver staining reveals increased axonal degeneration (black fibers) induced by G2019S LRRK2 in striosomal compartments of the ipsilateral striatum. Graph indicates the total silver-positive signal (black neurites) in the ipsilateral striatum for each LRRK2 variant. Bars represent mean  $\pm$  SEM ( $n = 8$  animals/vector). Images indicate low and high magnification for each vector. Scale bars: 500  $\mu$ m. \*\*\* $P < 0.001$  by one-way ANOVA with Dunnett's multiple comparison's test. *n.s.*, non-significant.

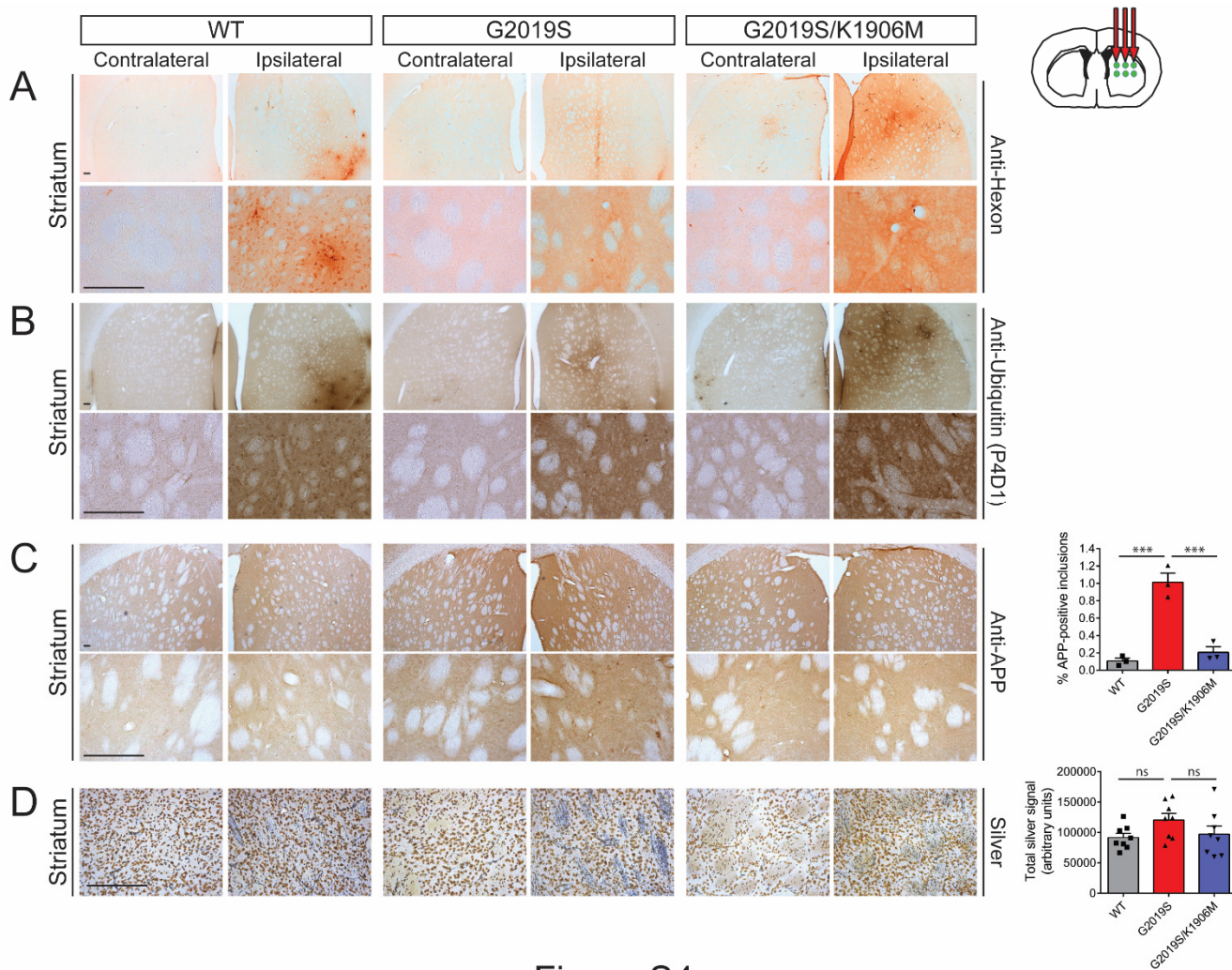


Figure S4



**Figure S5. Normal  $\alpha$ -synuclein solubility, levels and Ser129 phosphorylation following intrastriatal delivery of Ad5-LRRK2 vectors in rats. A)** Western blot analysis of  $\alpha$ -synuclein levels (Syn-1 antibody) or Ser129 phosphorylation (anti-pSer129- $\alpha$ Syn antibody) in 1% Triton-soluble or 2% SDS-soluble fractions from ipsilateral striatum and ventral midbrain of rats at 10 days after intrastriatal delivery of Ad5-LRRK2 vectors (WT, G2019S and G2019S/K1906M). Monomeric and high-molecular weight  $\alpha$ -synuclein species are shown. GAPDH was used as a loading control. Molecular mass markers are indicated in kilodaltons. **B)** Densitometric analysis of pSer129- $\alpha$ -synuclein levels normalized to total  $\alpha$ -synuclein, and total  $\alpha$ -synuclein normalized to GAPDH for each detergent fraction. Bars represent the mean  $\pm$  SEM ( $n = 3$  animals/vector). **C)** Immunohistochemistry indicating a lack of Ser129 phosphorylated  $\alpha$ -synuclein in substantia nigra at 42 days post-injection. Images indicate low and high magnification for each vector. Scale bars: 500  $\mu$ m.

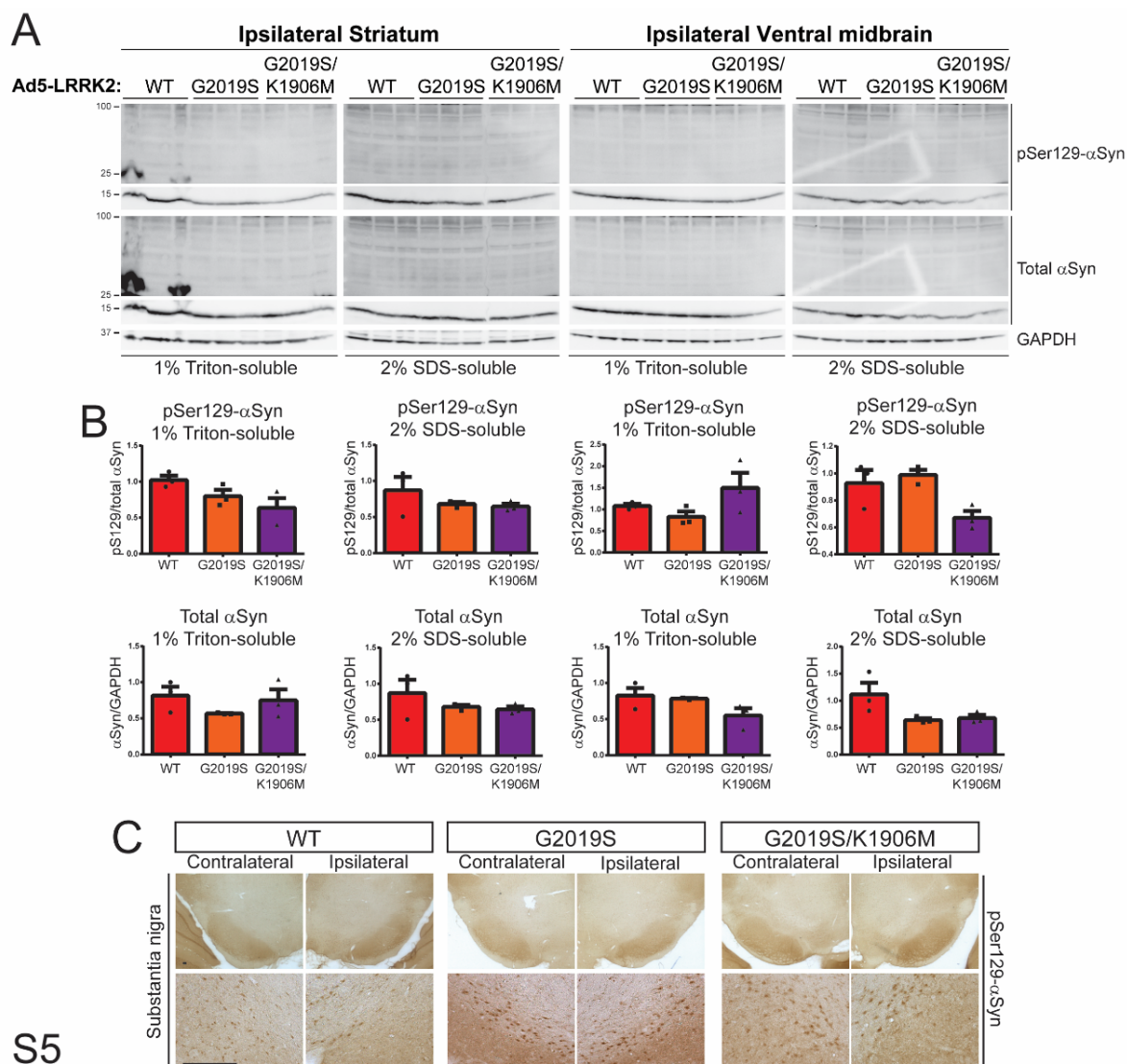


Figure S5

**Figure S6. LRRK2 variants do not alter tau solubility, levels or Ser202/Thr205 phosphorylation (AT8) following intrastratial delivery of Ad5-LRRK2 vectors in rats.** **A)** Western blot analysis of tau levels (TAU5 antibody) or Ser202/Thr205 phosphorylation (AT8 antibody) in 1% Triton-soluble or 2% SDS-soluble fractions from ipsilateral striatum and ventral midbrain of rats at 10 days after intrastratial delivery of Ad5-LRRK2 vectors (WT, G2019S and G2019S/K1906M). GAPDH was used as a loading control. Molecular mass markers are indicated in kilodaltons. **B)** Densitometric analysis of pSer202/Thr205-tau levels normalized to total tau, and total tau normalized to GAPDH for each detergent fraction. Bars represent the mean  $\pm$  SEM ( $n = 3$  animals/vector). **C)** Immunohistochemistry indicating increased Ser202/Thr205 phosphorylated tau (AT8-positive) in the ipsilateral substantia nigra at 42 days post-injection with each Ad5-LRRK2 vector. Images indicate low and high magnification for each vector. Scale bars: 500  $\mu$ m.

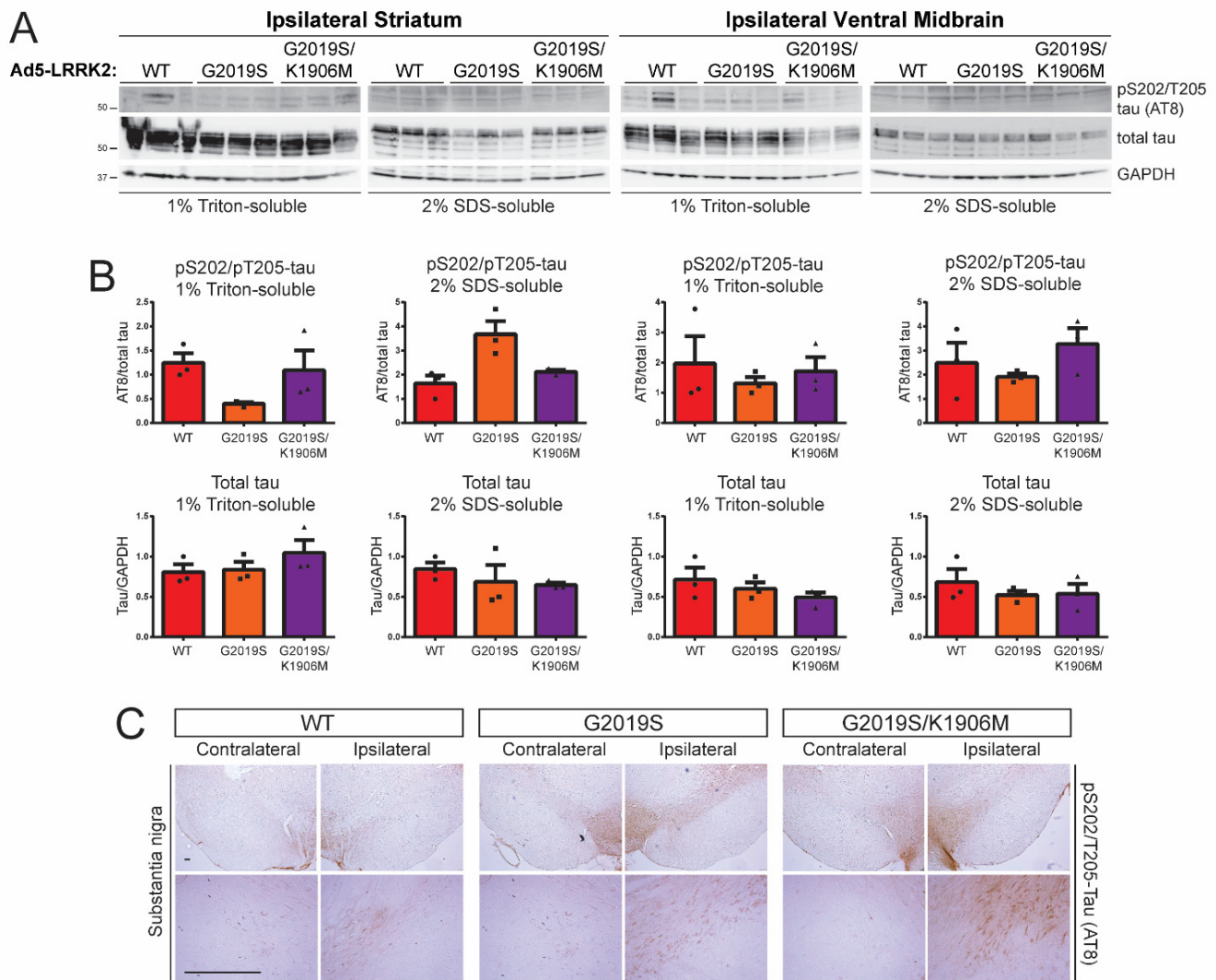


Figure S6

**Figure S7. Ad5-LRRK2 vector delivery induces microgliosis in rat brain. A-B)** Ad5-LRRK2 (WT, G2019S and G2019S/K1906M) vectors were unilaterally delivered at six sites ( $1.5 \times 10^{10}$  vp/site, in  $2.5 \mu\text{l}$ ) in the rat striatum. Immunohistochemistry indicates a modest increase in Iba1-positive microglial staining in the ipsilateral **A)** striatum and **B)** substantia nigra at 42 days post-injection. **C)** Ad5-LRRK2 (WT, G2019S and G2019S/K1906M) vectors were unilaterally delivered at one site ( $1.5 \times 10^{10}$  vp/site, in  $2.5 \mu\text{l}$ ) in the rat substantia nigra. Immunohistochemistry reveals a marked increase of Iba1-positive microglia in the ipsilateral substantia nigra at 21 days post-injection. Images indicate low and high magnification for each vector. Scale bars:  $500 \mu\text{m}$ . **D)** Immunohistochemistry showing Iba1-positive staining in the ipsilateral striatum following Ad5-LRRK2 vector delivery (original images). Analyzed images highlight Iba1-positive “activated” (red) or “non-activated” resting (green) microglia identified by Halo analysis. Graphs indicate the percent (%) of “activated” microglia or the total number of analyzed Iba1-positive microglia for each LRRK2 variant. Bars represent mean  $\pm$  SEM ( $n = 3$  animals/vector). *n.s.*, non-significant ( $P > 0.05$ ) by one-way ANOVA with Dunnett’s multiple comparison’s test.

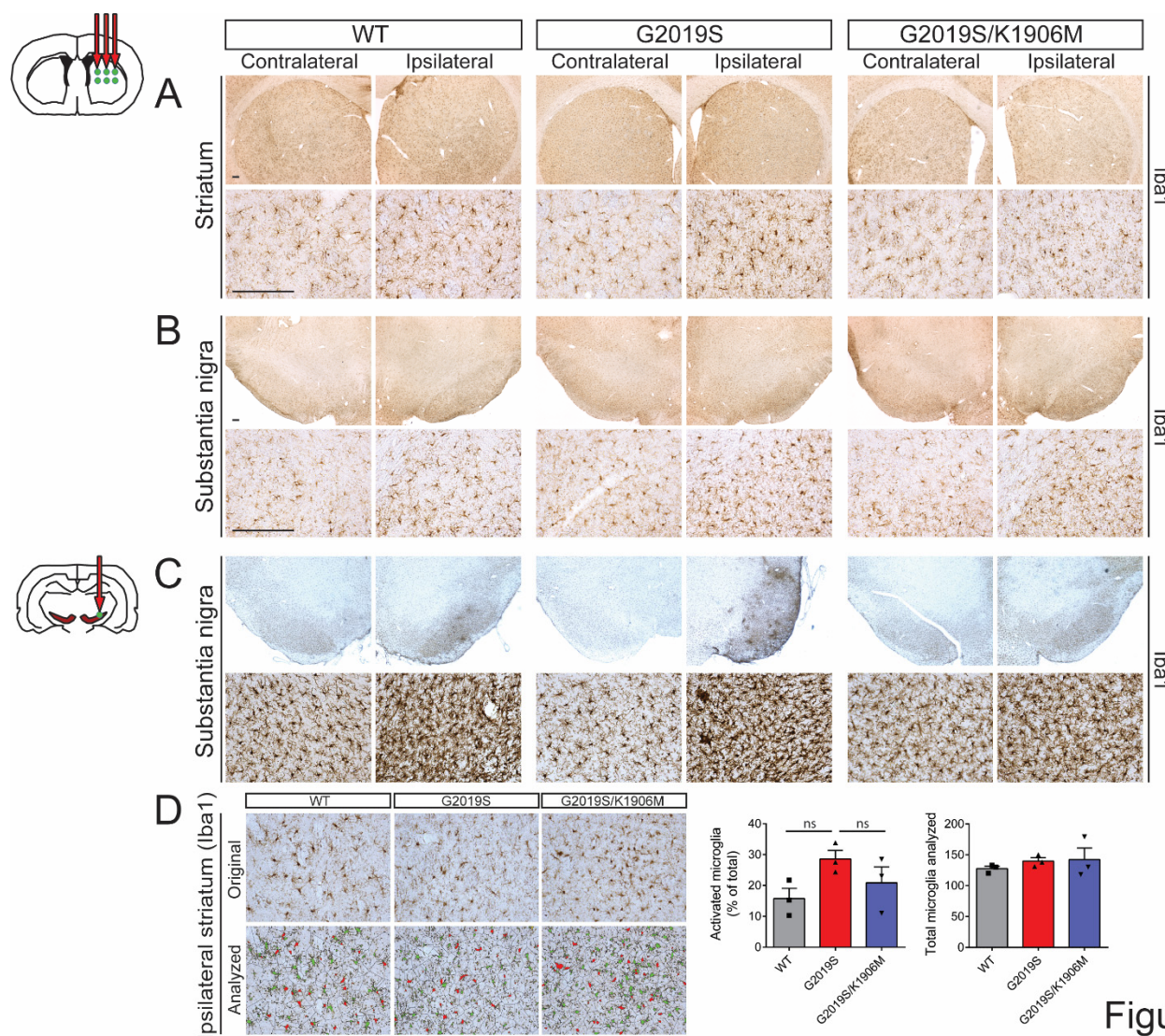


Figure S7

**Figure S8. Endogenous LRRK2 is not required for efficient Ad5 vector infectivity in rodent brain.** Ad5-eGFP vectors were unilaterally delivered at four distinct sites ( $4.2 \times 10^9$  vp/site, in 1  $\mu$ l) to the striatum of adult wild-type (WT) and homozygous *LRRK2* knockout (KO) littermate mice. Immunohistochemistry indicates equivalent eGFP expression (anti-GFP antibody) in the ipsilateral striatum and substantia nigra (SNpc) of WT and KO mice at 10 days post-injection. Scale bars: 125  $\mu$ m.

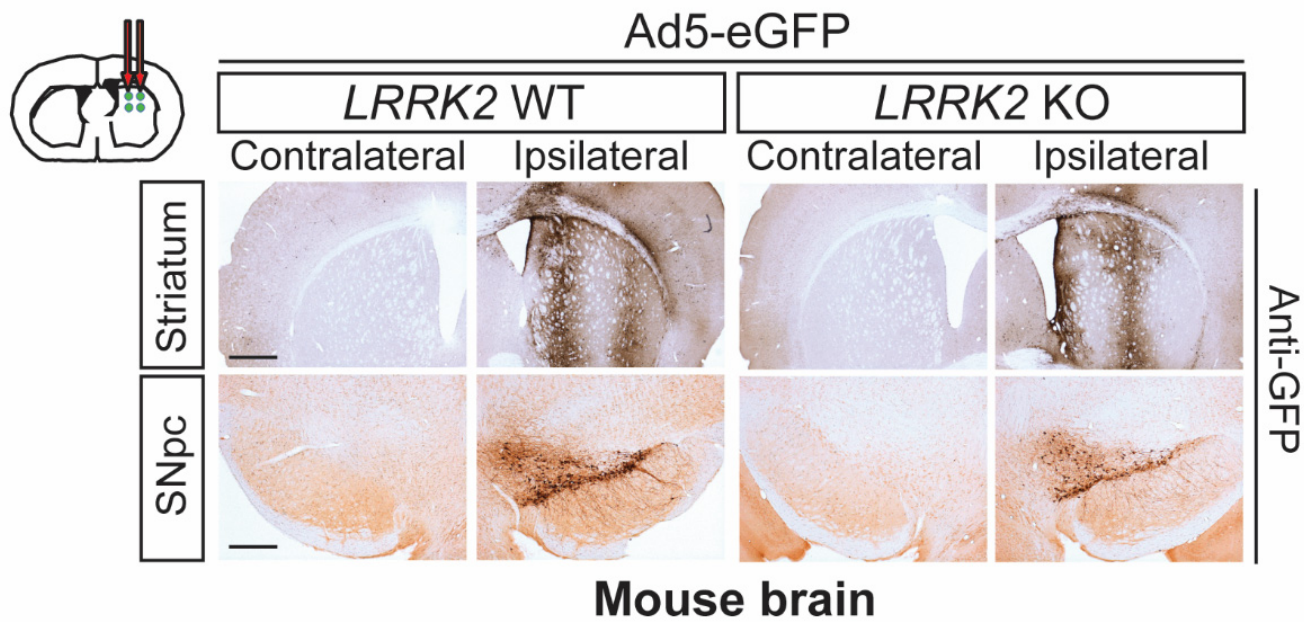


Figure S8

**Figure S9. In-diet pharmacological kinase inhibition (PF-360) markedly destabilizes human WT or G2019S-LRRK2 but not G2019S/K1906M-LRRK2 protein in rat brain.** **A)** Ad5-LRRK2 (WT, G2019S, G2019S/K1906M) vectors were unilaterally delivered at six distinct locations ( $1.5 \times 10^{10}$  vp/site, in  $2.5 \mu\text{l}$ ) in the rat striatum. Rats were fed with control (vehicle) or PF-360 (175 mg/kg) chow at day 7 post-injection for 7 consecutive days. Brain tissues were collected at 14 days post-injection and subjected to extraction in buffer containing 1% Triton X100 (Triton-soluble fraction). **B, D, F)** Western blot analysis of ipsilateral striatum extracts indicating human LRRK2 levels (FLAG), or contralateral striatum extracts indicating endogenous pSer935-LRRK2 and total LRRK2 (c41-2) levels in rats fed with vehicle or PF-360 chow. Shown are the levels of human **B-C)** WT, **D-E)** G2019S, and **F-G)** G2019S/K1906M LRRK2. Dynamin I or  $\beta$ -tubulin were used as loading controls for normalization. NI, non-injected striatum. **C, E, G)** Densitometric quantitation is shown for human LRRK2 (FLAG) levels in ipsilateral striatum, and endogenous pSer935-LRRK2 levels in contralateral striatum, treated with vehicle or PF-360. Bars represent the mean  $\pm$  SEM ( $n = 4-5$  animals/group), \*\* $P < 0.01$ , \*\*\* $P < 0.001$  or \*\*\*\* $P < 0.0001$  by unpaired Student  $t$ -test. n.s. non-significant.

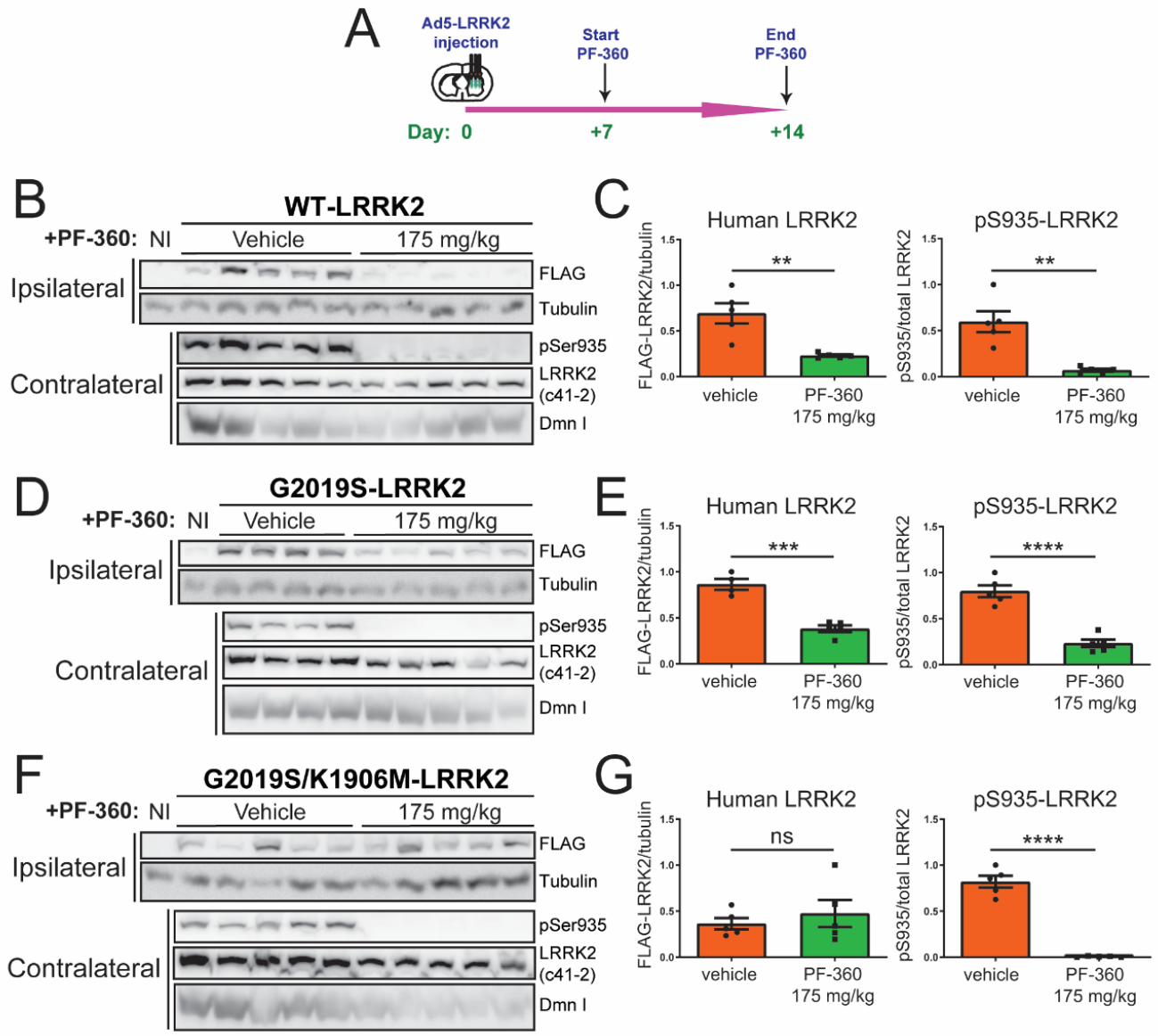


Figure S9

**Figure S10. Chronic in-diet dosing of PF-360 destabilizes human G2019S LRRK2 protein and inhibits endogenous LRRK2 kinase activity in rat brain.** **A)** Ad5-G2019S-LRRK2 vectors were unilaterally delivered at six distinct sites ( $1.5 \times 10^{10}$  vp/site, in 2.5  $\mu$ l) in the rat striatum. Rats were fed continuously with vehicle or PF-360 (175 mg/kg) chow from day 7 to 42 post-injection. Striatal tissues were harvested at 42 days post-injection and subjected to sequential extraction in 1% Triton X100 and 2% SDS. **B-C)** Western blot analysis of **B)** ipsilateral striatum extracts indicating human G2019S-LRRK2 levels (anti-FLAG antibody), or **C)** contralateral striatum extracts indicating endogenous pSer935-LRRK2 and total LRRK2 (c41-2) levels in rats fed with vehicle or PF-360 chow. Dynamin I (DnmI) was used as a loading control for normalization. Densitometric quantitation is shown for **B)** human G2019S-LRRK2 (FLAG) levels in each detergent fraction, and **C)** endogenous pSer935-LRRK2 levels. Bars represent the mean  $\pm$  SEM ( $n = 4$  animals/vector), \* $P < 0.05$  or \*\* $P < 0.01$  by unpaired Student  $t$ -test. **D)** Evaluation of body weight (grams) for individual rats (#1-12) monitored in 7-day periods fed with PF-360 (175 mg/kg) from day 7 to 42 post-injection. **E)** Total consumption of chow (grams) by individual rats (#1-12) monitored in 7-day periods fed with PF-360 (175 mg/kg) from days 7 to 42 post-injection.

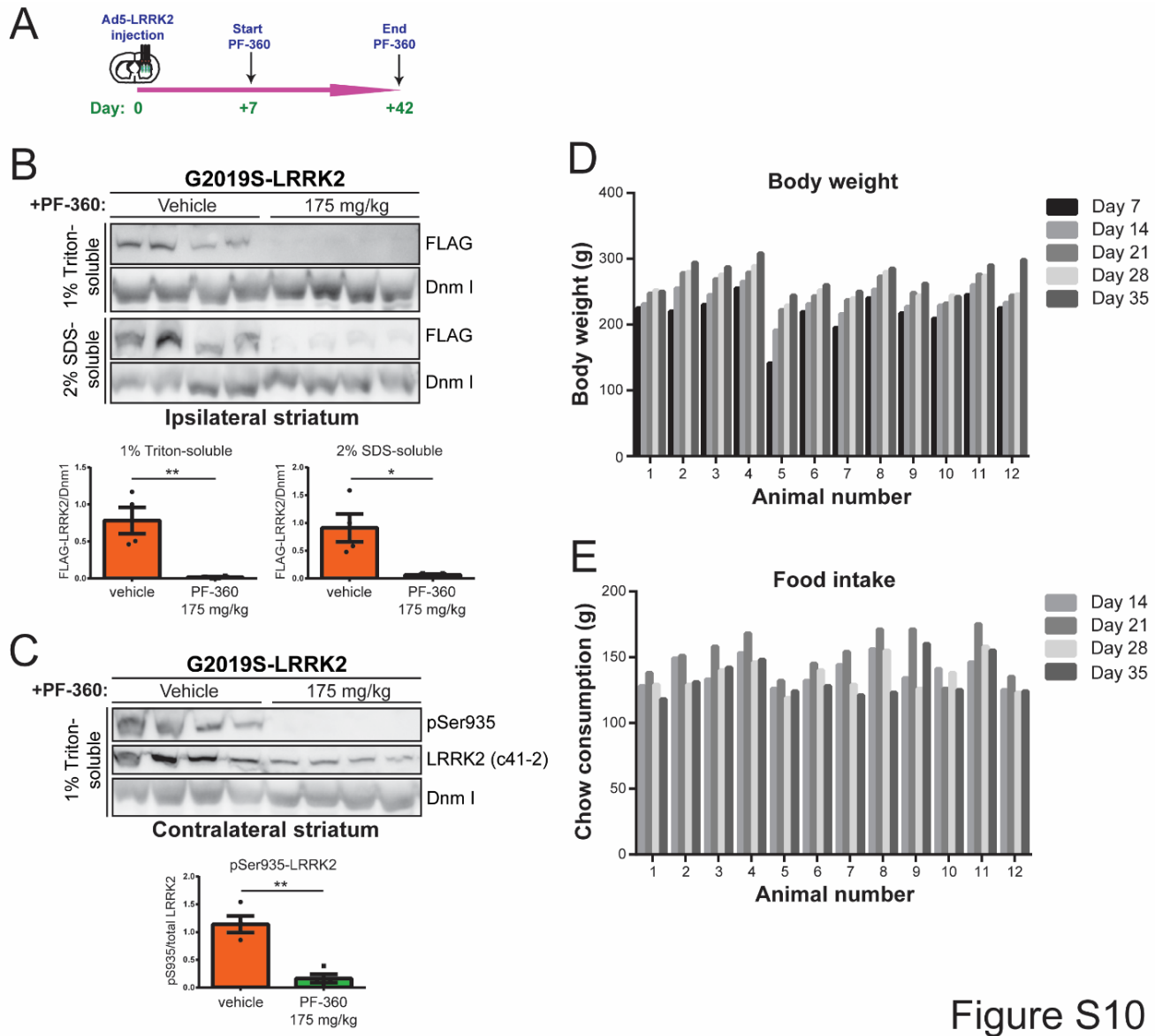


Figure S10