Supporting Information

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SI Materials and Methods

Animals. All experiments were approved by the local Institutional Animal Care and Use Committee. Long–Evans outbred rats in this study originated from the Charles Rivers collection and were obtained directly from SAGE Laboratories. Zinc-finger nuclease technology was used to generate a 10 bp deletion in leucine-rich repeat kinase 2 (LRRK2) exon 30 that resulted in a frame shift and a stop codon within the coding region of exon 30 (Fig. S1*A*). Genotyping was accomplished with forward primers 5′-CAC TTC ATG ACC CAG AGA GCC CTG-3′ and 5′-TCA AAG CTG TTG TGA GAA GAC TGT TTA TTG-3′, together with reverse primer 5′-GCA AAA CAA AAC AAA ACC AGA AGC ATA G-3′. WT rat DNA produces a ∼180 bp band, whereas a LRRK2 knockout allele generates a ∼310 bp band (Fig. S1*A*). Rats in this study were derived from three generations of LRRK2 knockout (KO) and wild-type (WT) rats that were bred and housed separately throughout the study. In animals from the last (third) generation, eight rats were randomly selected (four each of WT and KO) and analyzed with a panel of informative microsatellite markers developed by Charles Rivers to estimate genetic heterozygosity in outbred rat strains. The average heterozygosity between the two groups was 17.53% and was not significantly different between the WT and KO groups ($P > 0.05$, Student t test). A control DNA from in-bred rats (Lewis) did not show any allelic heterozygosity with the analysis (i.e., 0% heterozygosity). For reference, the manufacturer (Charles Rivers) reports that its Sprague–Dawley line is generally maintained at 30% heterozygosity in the marker panel. All rats were maintained on an ad libitum diet during the experiments, with all surgeries occurring in 10–12-wk-old animals.

Virus Production and Intracranial Surgery. Recombinant adeno-associated virus 2/1 (rAAV2/1)–α-synuclein and rAAV2/1–EGFP viruses were obtained from the Virus Core of the University of North Carolina. Intracranial injections were conducted under isoflurane anesthesia using a stereotaxic frame (David Kopf frames), and animals received a single unilateral 4 μL injection of either rAAV2/1–α-synuclein ($n = 60$ rats), rAAV2/1–EGFP ($n = 40$ rats), ultrapure LPS (50,000 endotoxin units, $n = 25$ rats), or PBS ($n = 15$ rats). Both virus and LPS stocks were diluted into PBS, pH 7.4, before injection. Reagents were drawn into a 32-gauge custom needle (Hamilton) with a 110° bevel fitted to a gas-tight syringe. Solution was injected into the right substantia nigra pars compacta (SNpc) at the following empirically derived coordinates: 4.65 mm posterior and 2.25 mm lateral to bregma, and 7.45 mm ventral relative to skull, with the needle bevel facing laterally, and a flow rate of 0.3 μL/min. Scalp incisions were closed by suture, and animals were monitored for successful recovery, with food and water consumption expected in the first few hours postsurgery.

Immunohistochemistry and Immunofluorescence. Rats were deeply anesthetized with isoflurane and transcardially perfused with room temperature PBS supplemented with 0.025% heparin, followed by ice-cold 4% PFA (wt/vol) and PBS solution. Brains were dissected and postfixed in 4% PFA and transferred to 30% sucrose in PBS for cryopreservation. Once saturated in sucrose, brains were fresh frozen in isopentane cooled to −55 °C and stored at −80 °C until sectioning at 40 μm widths on a freezing sliding microtome (Leica). In experiments requiring LRRK2 detection, fresh sections were cut the day before each experiment, as the signal for LRRK2 greatly diminishes with time, with no detectable LRRK2 protein apparent after several weeks of section storage at −20 °C or warmer. Free-floating sections were quenched in 0.3% hydrogen peroxide in 100% methanol for 30 min followed by incubation with 10 mM sodium citrate pH 6.0 containing 0.05% Tween-20 for 30 min at 37 °C with agitation. Sections were treated with 10% normal serum (matched to secondary antibody species) in 0.3% Triton X-100 in PBS for 60 min, and incubated in primary antibody in 5% normal serum/PBS for 48 h at 4 °C with mild agitation. The following primary antibodies were used: human α-synuclein syn208 and nitrosylated-specific α-synuclein syn514 (mouse monoclonals, 1:250, courtesy of the Virginia Lee laboratory), tyrosine hydroxylase (TH; rabbit polyclonal, 1:1,000, Sigma and Santa Cruz), CD68 (mouse monoclonal, 1:250, AdSerotec), EGFP (goat polyclonal, 1:1,000, Santa Cruz), GFAP (mouse monoclonal, 1:1,000, Sigma), LRRK2 (mouse monoclonal, 1:200, N241, NeuroMAB), and Iba-1 (rabbit polyclonal, 1:1,000, Wako). For immunohistochemistry, the ABC (Avidin-Biotin Complex reagent, Vector Labs) kit was used with ImmPACT–DAB (3, 3′-diaminobenzidine) substrate (Vector Labs) for less than 1 min. Most sections were counterstained using a standard Nissl staining protocol. For immunofluorescence and confocal analysis, sections were mounted on superfrost slides (Fisher) in Prolong Gold (Invitrogen). Iba1 cell morphological analyses were performed with Axiovision v4.8 (Carl Zeiss) automatic measurement program using perimeter calculations.

Immunoblotting. Midbrain tissue surrounding the substantia nigra was rapidly dissected from rapidly euthanized rats and lysed immediately into a buffered solution containing Triton X-100 (0.5%, for soluble fractions) and insoluble material then pelleted and resuspended into SDS buffer (1%) to generate an insoluble fraction of protein. Lysates were quantified for protein content by BCA analysis (Pierce), and ∼5 μg per lane of total protein were analyzed on Tris-Glycine-eXtended (TGX) SDS/PAGE (Bio-Rad) and transferred to PVDF. Antibodies used were the same as above, with secondary antibodies provided by LiCOR and analyzed and protein quantified on an Odyssey system.

Unbiased Estimations and Statistical Analysis. Unbiased stereological estimation of the total number of TH+/Nissl+/CD68+ cells in the SNpc was performed using an optical fractionator (MicroBrightField) by an investigator blinded to the experiment identity. A low-power objective was used to delineate the borders of the SNpc, identified through Nissl contrast stain, at all levels. Sections used for counting covered the entire SNpc and were equally spaced 200 μm apart, with the counting frame placed randomly on the first counting area and systematically moved through all counting areas until the entire delineated region was sampled. Counting frame density was adjusted so that at least 100 objects were counted for each observation, with most estimation based on ∼200 objects counted per observation. Statistical analysis and graphs were generated with Graphpad software.

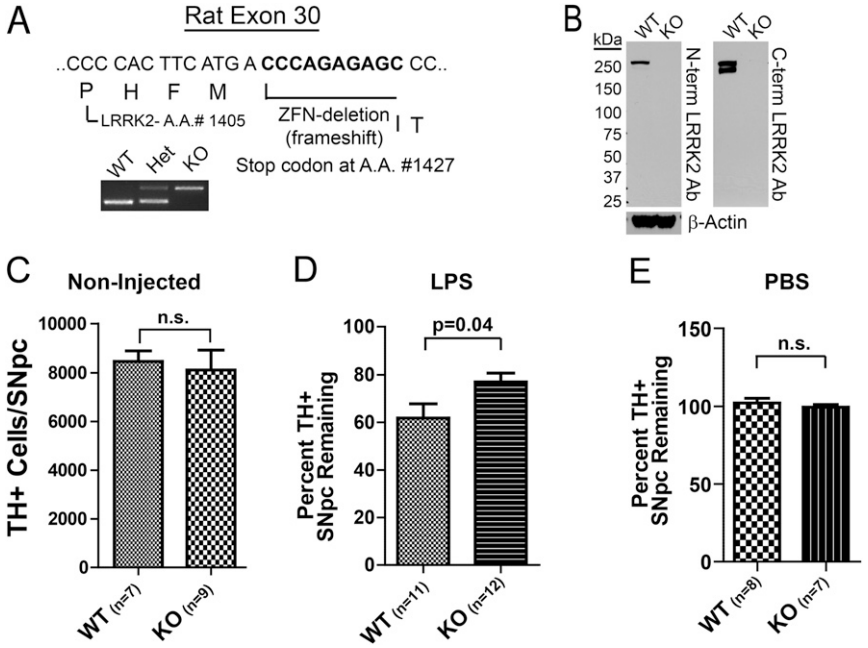

Fig. S1. Characterization of LRRK2 KO rats and protection from LPS-induced neurodegeneration. (*A*) A zinc-finger nuclease generates a 10 bp deletion in exon 30 of the rat *LRRK2* gene, resulting in a premature stop codon also located in exon 30 (amino acid 1427). Genotyping with primers that stretch across the deletion reveals heterozygous KO animals. (*B*) The small in-del localized to exon 30 results in complete ablation of protein, as shown with an antibody targeting the N terminus of LRRK2 (antibody N231B/34) and an antibody targeting the C terminus of LRRK2 (antibody N241A/34). (*C*) Unbiased stereology estimated the total number of TH-positive SNpc neurons in LRRK2 WT and KO rat midbrain sections. (*D*) Stereological data for TH-positive cells in the SNpc expressed as a percentage loss calculated by remaining ipsilateral cells divided by contralateral cells for LPS or (*E*) PBS (control) unilaterally injected rats. *P* values were calculated by Student's two-tailed *t* tests, with error bars representing SEM. n.s. is $P > 0.05$.

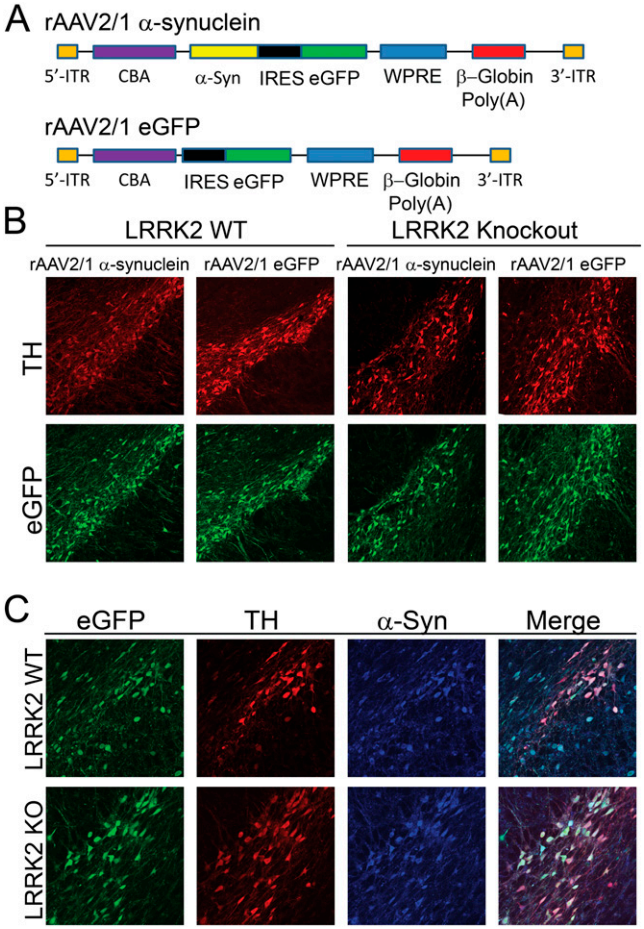

Fig. S2. Efficient rAAV2/1 transduction in the SNpc in both LRRK2 WT and KO rats. (*A*) Diagram showing the components of the two rAAV2/1 viruses used in this study. α-Syn, human WT α-synuclein open-reading frame; CBA, chicken-beta-actin promoter; eGFP, enhanced green fluorescent protein; IRES, internal ribosomal entry site; ITR, inverted terminal repeats; WPRE, woodchuck hepatitis virus posttranscriptional regulatory element. (*B*) Representative coronal confocal images of LRRK2 WT and KO rat midbrain sections showing the entirety of the SNpc posttransduction with either eGFP or α-synuclein rAAV2/1 viruses. eGFP epifluorescence was enhanced using eGFP primary/Cy2 secondary (green), combined with TH primary/Cy3 secondary fluorescence (red). No eGFP signal could be visualized in the noninjected midbrain sections under evaluation. (*C*) Confocal images of LRRK2 WT and KO rat midbrain sections transduced with α-synuclein rAAV2/1 virus. Green signal shows eGFP enhanced with Cy2 secondary antibodies, red signal is TH (Cy3), and blue signal is from a human-specific α-synuclein antibody (Cy5). All animals included in this study showed a robust (>90%) SNpc transduction in the ipsilateral (remaining) TH-positive neurons.

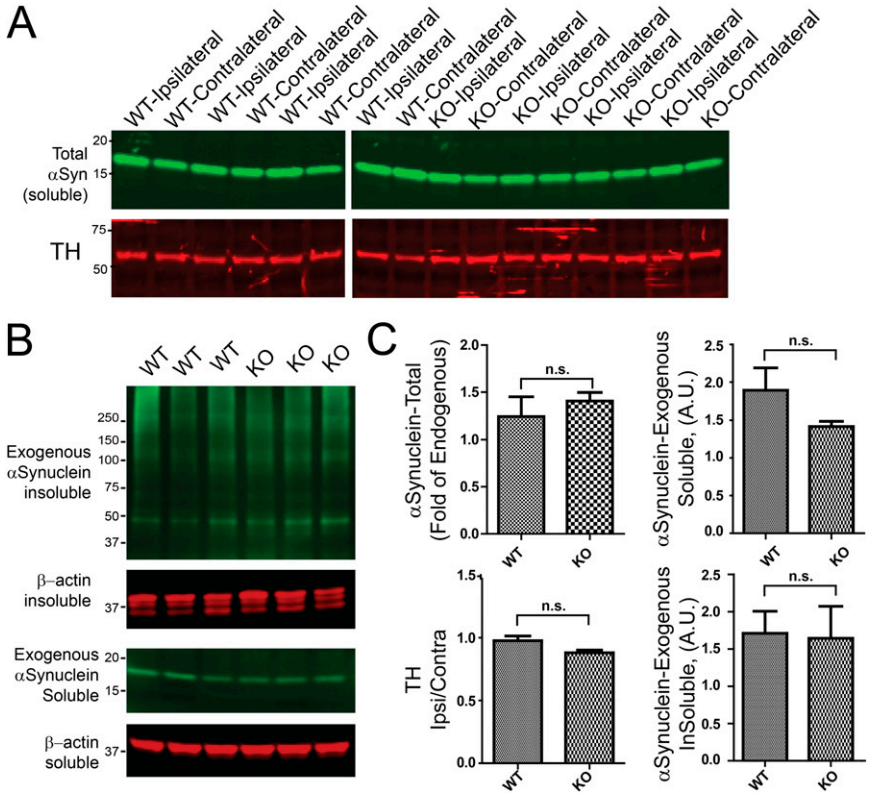

Fig. S3. Viral-induced α-synuclein expression and solubility is similar in WT and LRRK2 KO rats. Analysis of 10–12-wk-old LRRK2 WT and KO rats unilaterally injected with rAAV2/1–α-synuclein viral particles and midbrain tissue dissected 2 wk postinjection. (*A*) Tissue samples lysed into Triton X-100 containing buffer (i.e., soluble) were analyzed by immunoblot for total α-synuclein levels with a pan anti–α-synuclein antibody or (*B*) further processed into SDS (i.e., insoluble) lysis buffers and analyzed for human (from rAAV2 transduction) α-synuclein expression with a human isoform-specific antibody. (*C*) In the dissected midbrain, a modest overall increase in total α-synuclein levels was observed (group mean ∼1.3-fold induction), whereas no differences were observed between LRRK2 WT and KO groups in soluble or insoluble α-synuclein levels using a human isoform-specific antibody (i.e., exogenous), or TH levels, at the 2-wk time-point posttransduction. n.s., nonsignificant ($P > 0.05$, Student's two-tailed *t* test); all error bars represent SEM.

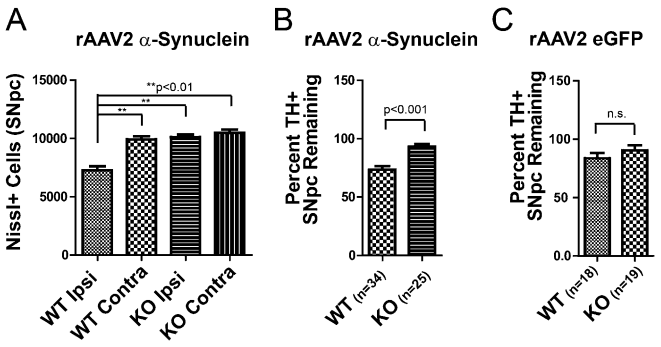

Fig. S4. LRRK2 KO rats are protected from α-synuclein–induced dopaminergic neurodegeneration. (*A*) Analysis of 10–12-wk-old LRRK2 WT ($n = 34$) and KO ($n = 25$) rats unilaterally injected with 6×10^9 rAAV2 α-synuclein viral particles. Raw stereological cell counts of Nissl-positive cells in the SNpc are given, with respect to ipsi (ipsilateral, viral injected) and contra (contralateral, noninjected side). (*B*) Stereological data for TH-positive cells in the SNpc expressed as a percentage loss calculated by remaining ipsilateral cells divided by contralateral cells for rAAV2 α-synuclein or (*C*) equivalent rAAV2 EGFP viral particle-injected rats. *P* values were calculated by one-way ANOVA with Tukey's post hoc test for *A*, and Student's two-tailed *t* test for *B* and *C*, with error bars representing SEM.

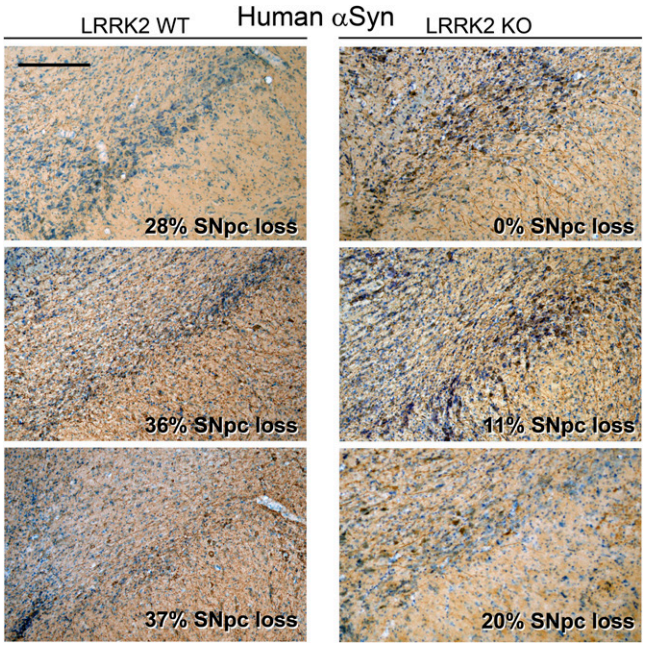

Fig. S5. α-synuclein pathology is correlated with SNpc cell loss. Equivalent viral particles of either rAAV2/1 or α-synuclein were unilaterally injected into the SNpc of 10–12-wk-old LRRK2 WT and KO rats and animals were killed 4 wk postinjection. Representative images across the SNpc (identified by Nissl counterstain, show in blue) of transduced (i.e., exogenous) α-synuclein (shown as brown) in sections approximately the same distance relative to bregma. (Scale bar, 0.2 mm for all panels.)