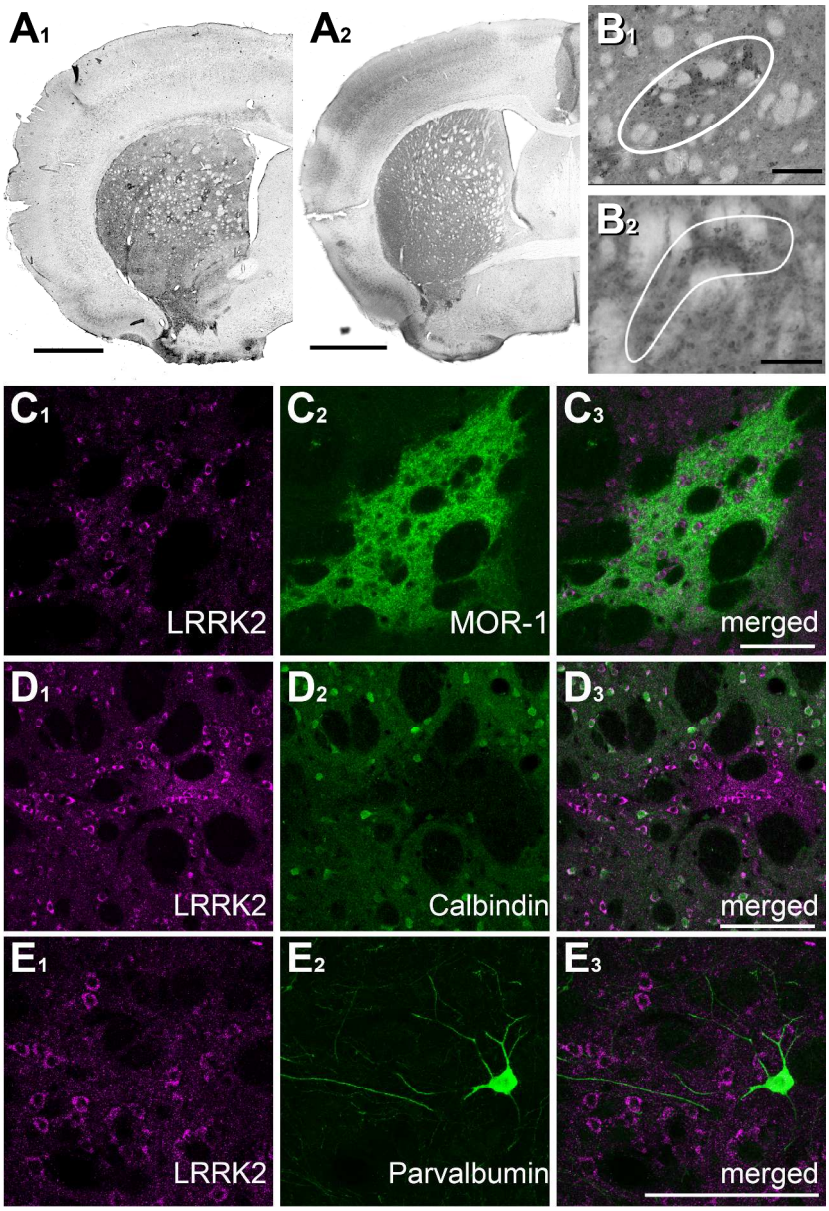
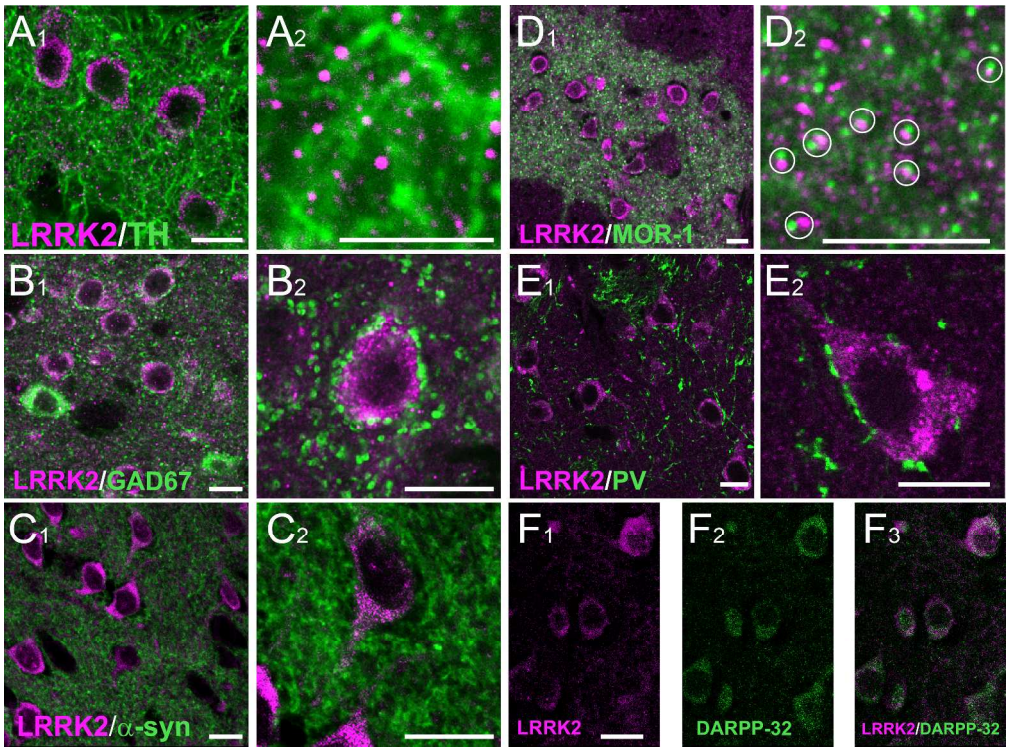


Supplemental Figure 1. LRRK2 in adult rodent striatum. A) Monoclonal antibody N241A/34 (rat brain, panel A1) and c41-2 (mouse brain, panel A2) detection of LRRK2 in rodent sections using DAB immunohistochemistry. An anterior section of striatum is shown at comparable levels between mouse and rat. Comparable results were also obtained in serial sections using monoclonal antibody c41-2 in both species. Results are representative of at least pairs (WT with a KO control) of mice and rats processed. Scale bars are 1 mm. B) Higher magnification reveals intense LRRK2 signal enrichment (highlighted with a white surround) in striosomes, in rat coronal striatum sections (panel B1) and mouse coronal striatum sections (panel B2). The striosome enrichment in mouse is typically not as pronounced as in rat (relative to neighboring matrix LRRK2 staining). C) Double labeling immunofluorescence reveals enrichment of LRRK2 in MOR-1 positive striosomes in rat striatal sections. D) Calbindin staining shows inverse striosome (i.e., matrix) distribution. LRRK2 shows reduced signal (compared with striosome signal) in calbindin matrix staining in rat striatum. E) Shown is a parvalbumin positive interneuron within a striosome from rat striatum. LRRK2 does not show any expression in parvalbumin interneurons. Scale bars for panels B-E are

all 0.1 mm.
168x244mm (500 x 500 DPI)



Supplemental Figure 2. LRRK2 in wild-type adult rodent striatal neurons. In the anterior striatum of rats, A) no LRRK2 expression could be detected along TH positive fibers, but LRRK2 puncta were often directly juxtaposed to TH fibers, suggestive of LRRK2 being post-synaptic to dopaminergic input. B) LRRK2 signal was excluded from GAD67 positive interneurons, although GAD67 could be observed adjacent to LRRK2, suggesting GAD67 synapses on many LRRK2 positive soma. C) LRRK2 shows no overlap with the presynaptic marker, α-synuclein, and similar to GAD67, α-synuclein could be observed adjacent to LRRK2 positive neurons. D) LRRK2 signal outside of neuronal perikarya did not overlap with MOR-1, but rather formed pairs with MOR-1 puncta (prominent pairs highlighted with white circles). E) An 86 nm thick confocal slice showing intense parvalbumin (PV)-positive terminals adjacent to LRRK2-positive neurons. F) An 86 nm thick confocal slice showing overlap between LRRK2 and DARPP-32 in striatal neurons. Scale bars are all 10 μm.
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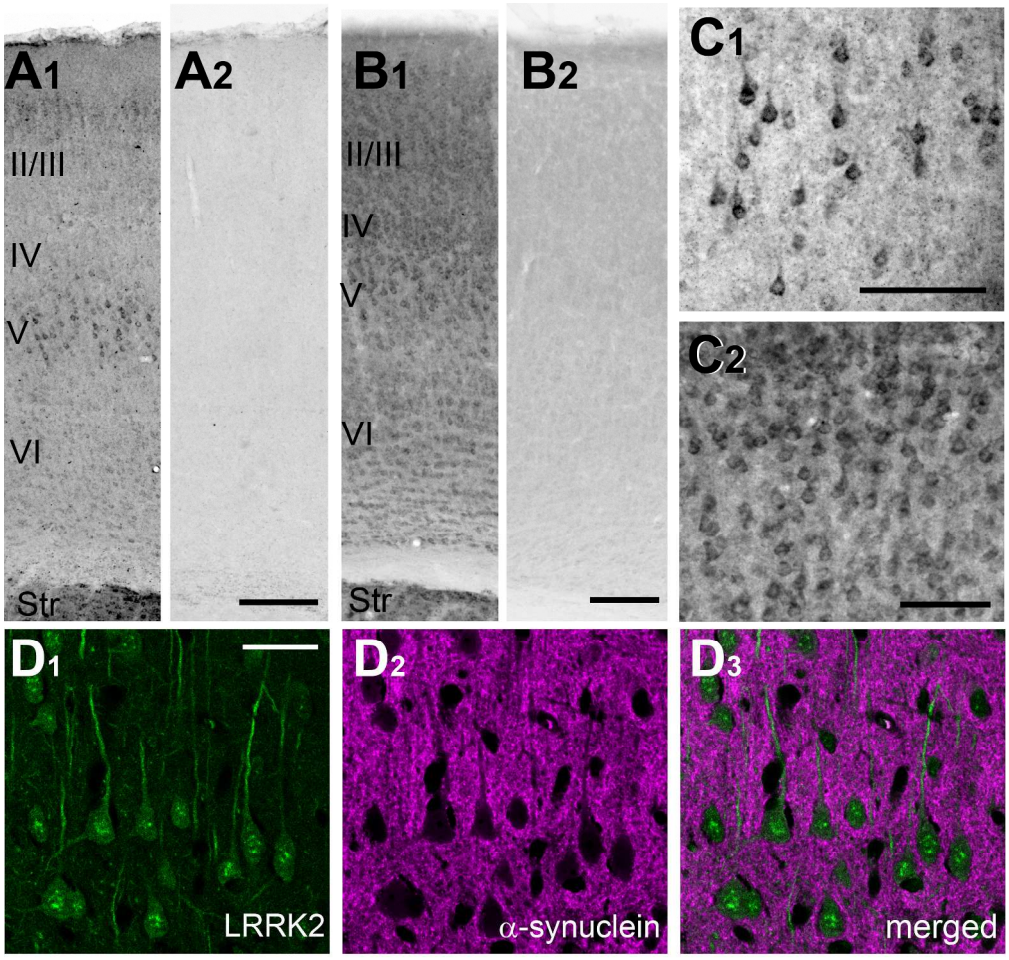

Figure 3. LRRK2 in adult rodent cortex. A) LRRK2 distribution using DAB-immunohistochemistry in wild type rat motor cortex (panel A1) and LRRK2 KO rat motor cortex (panel A2) with monoclonal antibody N241A/34. Scale bars are 0.3 mm. B) Wild-type mouse motor cortex (panel B1) and LRRK2 KO mouse motor cortex (panel B2) with monoclonal antibody c41-2. Scale bars are 0.15 mm for mouse. Cortical layering was defined by cresyl violet staining (not shown). C) Higher magnification of cortical Layer V in rat (panel C1) and mouse (panel C2) demonstrate a more restricted expression pattern of LRRK2 in rat compared to mouse. Scale bar is 0.15 mm for rat (panel C1) and 75 μm for mouse (panel C2). D) Immunofluorescence with antibody N241A/34 in rat cortex layer V using confocal microscopy demonstrate a prominent somatodendritic localization of LRRK2 in Layer V of the cortex, although possible LRRK2 expression in non-branching, non-tapering axons cannot be ruled out through these images. LRRK2 also localizes to perinuclear puncta. LRRK2 does not co-localize with the presynaptic marker, α –synuclein, which demarcates LRRK2 positive apical dendrites. Scale bar is 25 μm for all panels in D.
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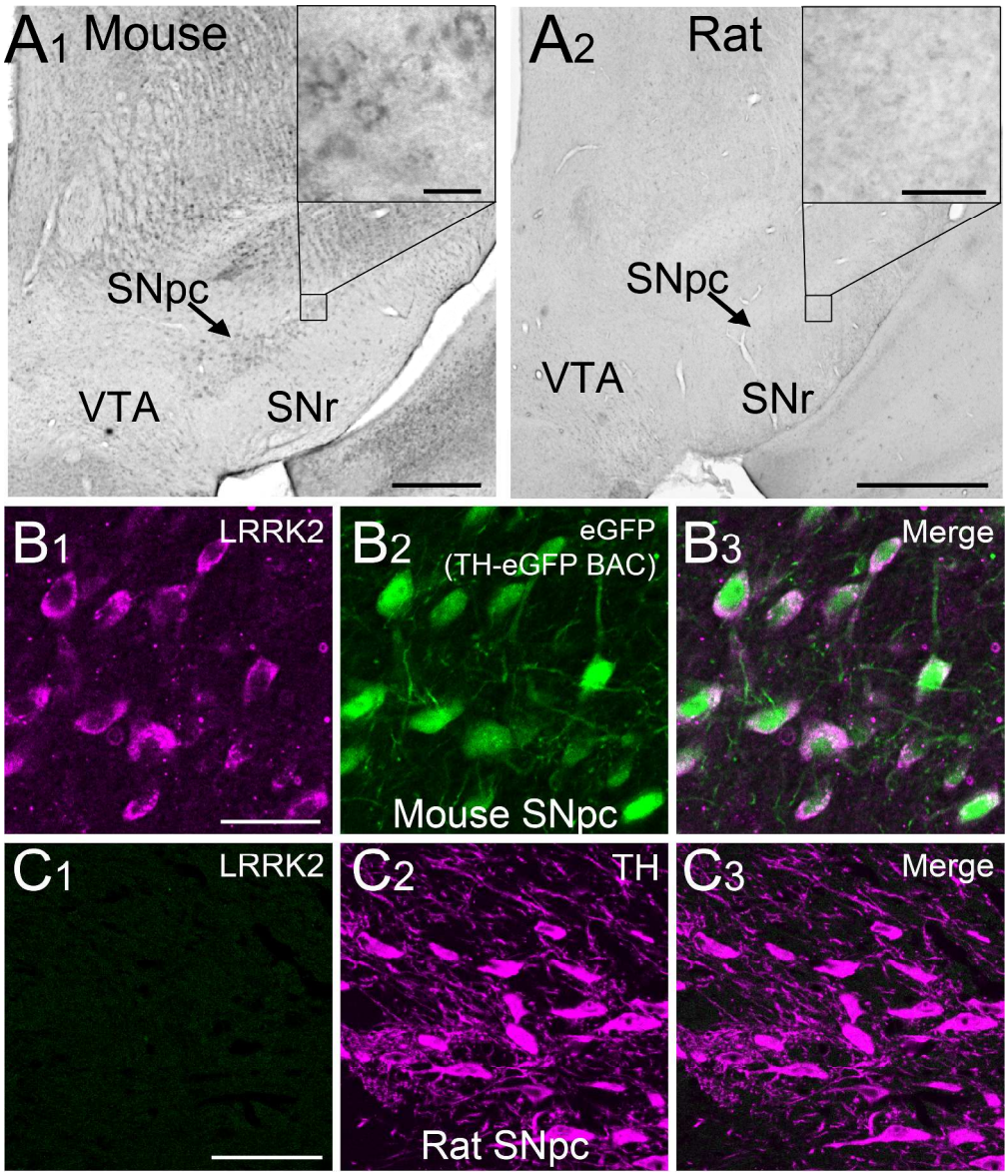
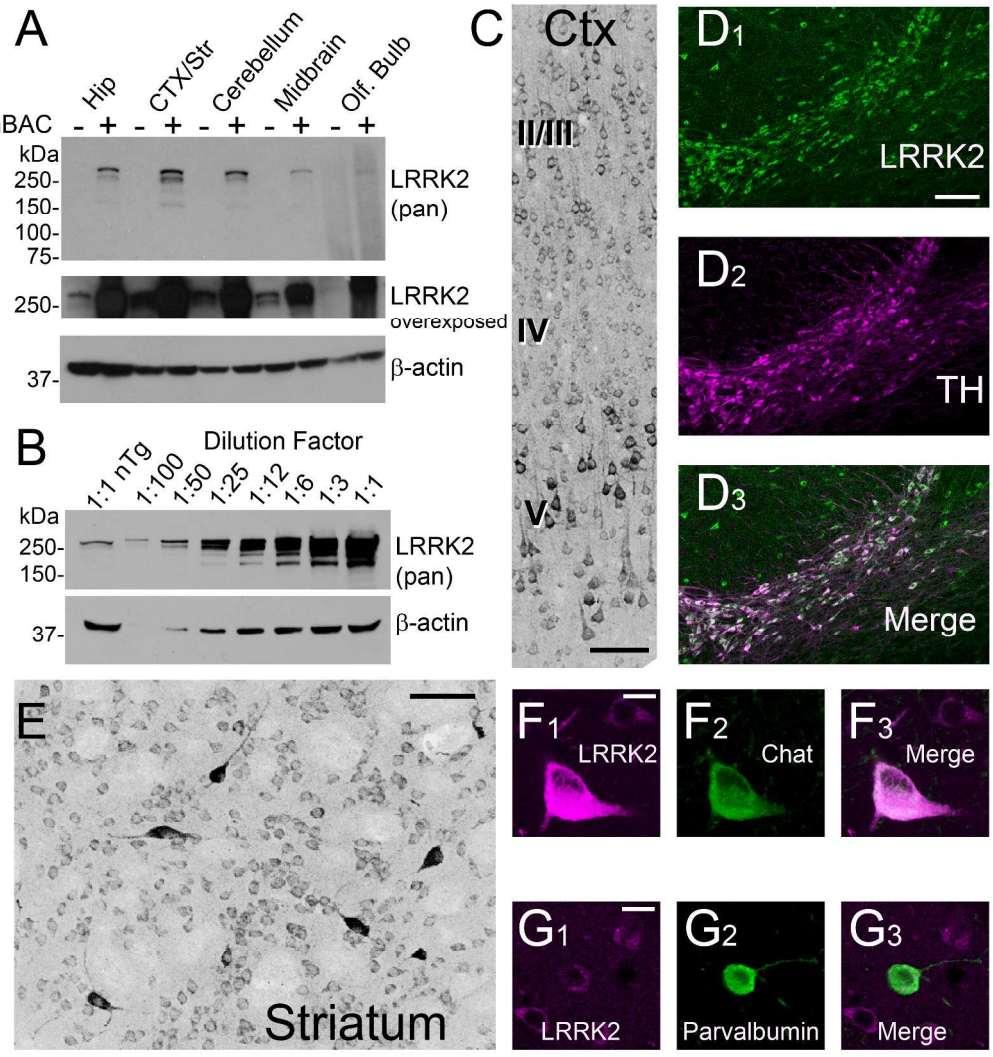


Supplemental Figure 4. LRRK2 in rodent SNpc A) LRRK2 using DAB immunohistochemistry in adult male rats or mice with the substantia nigra pars compacta (SNpc), substantia nigra pars reticulata (SNr), and Ventral Tegmental Area (VTA) indicated. No specific signal was obtained in simultaneously processed mouse LRRK2 KO tissue (Supplemental Figure 5). Results are shown using antibody c41-2. LRRK2 immunoreactivity could be seen in mouse sections, but not in rat using either c41-2 antibody or N241A/34 monoclonal antibody(shown). Scale bars are 0.5 mm, inset scale bars are 50 µm. B) A strain of mouse produced by the GeneSat project that expresses eGFP under the endogenous TH promoter from a BAC construct (see Methods) was used to evaluate LRRK2 in the mouse midbrain. eGFP epifluorescence could be visualized in the SNpc, and these cells were positive for LRRK2 protein. Scale bar is 30 µm. C) At a comparable level of the SNpc in rats, robust TH expression could be observed but none of these cells were LRRK2 positive using antibodies N241A/34 (shown) or c41-2. Scale bar is 50 µm.
193x225mm (500 x 500 DPI)



Supplemental Figure 5. Aberrant LRRK2 Distribution Caused by Expression of Human LRRK2 BAC Constructs in Rats A) Western blot analysis with antibody N241 demonstrating the level of LRRK2 overexpression in the indicated brain region, as dissected from fresh rat brain tissue from either nontransgenic or LRRK2-G2019S human-BAC transgenic rats. Equivalent protein concentrations determined by BCA assay were loaded between lanes (and verified by actin signal), matched by region, between wild-type and transgenic rats. B) Forebrain lysate from nontransgenic rats was loaded in lane 1 and increasing concentrations of LRRK2-G2019S transgenic rat forebrain lysates were loaded in subsequent lanes. LRRK2 is overexpressed between 25 and 50 fold in the transgenic rats. C)The pan-reactive LRRK2 antibody N241A/34 was used to detect LRRK2 in LRRK2-G2019S hBAC positive rats in the motor cortex (scale bar is 50 μm), and D) SNpc, by fluorescent co-label with TH (scale bar is 0.1 mm) . E) A striosome in the anterior striatum of transgenic rats identified by LRRK2, with intensely positive LRRK2 interneurons inside the striosome (scale bar is 50 μm. F,G) Co-localization of LRRK2 with cholinergic interneurons, but weak expression in parvalbumin-positive interneurons (scale bars are 10 μm).
171x180mm (500 x 500 DPI)