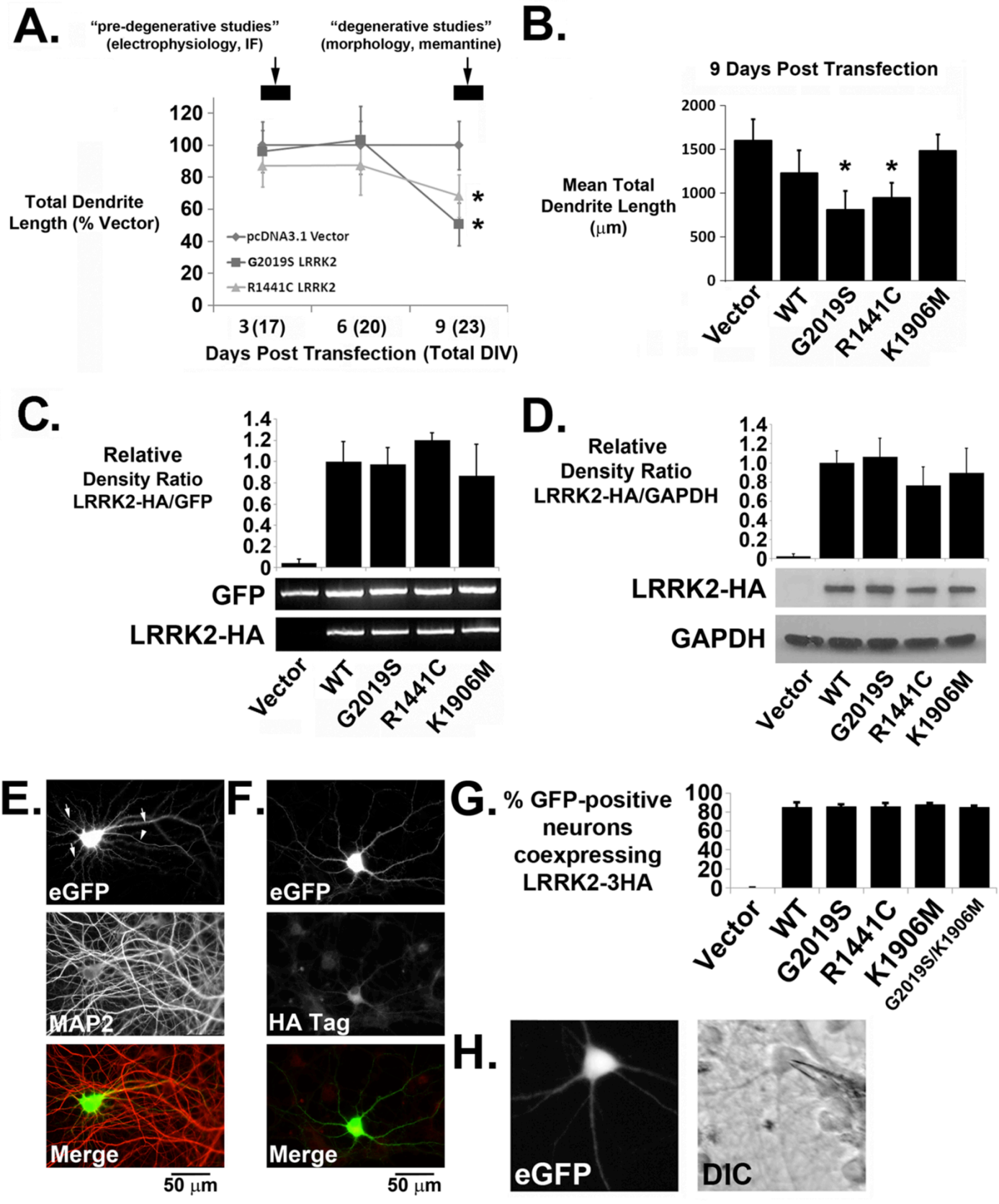
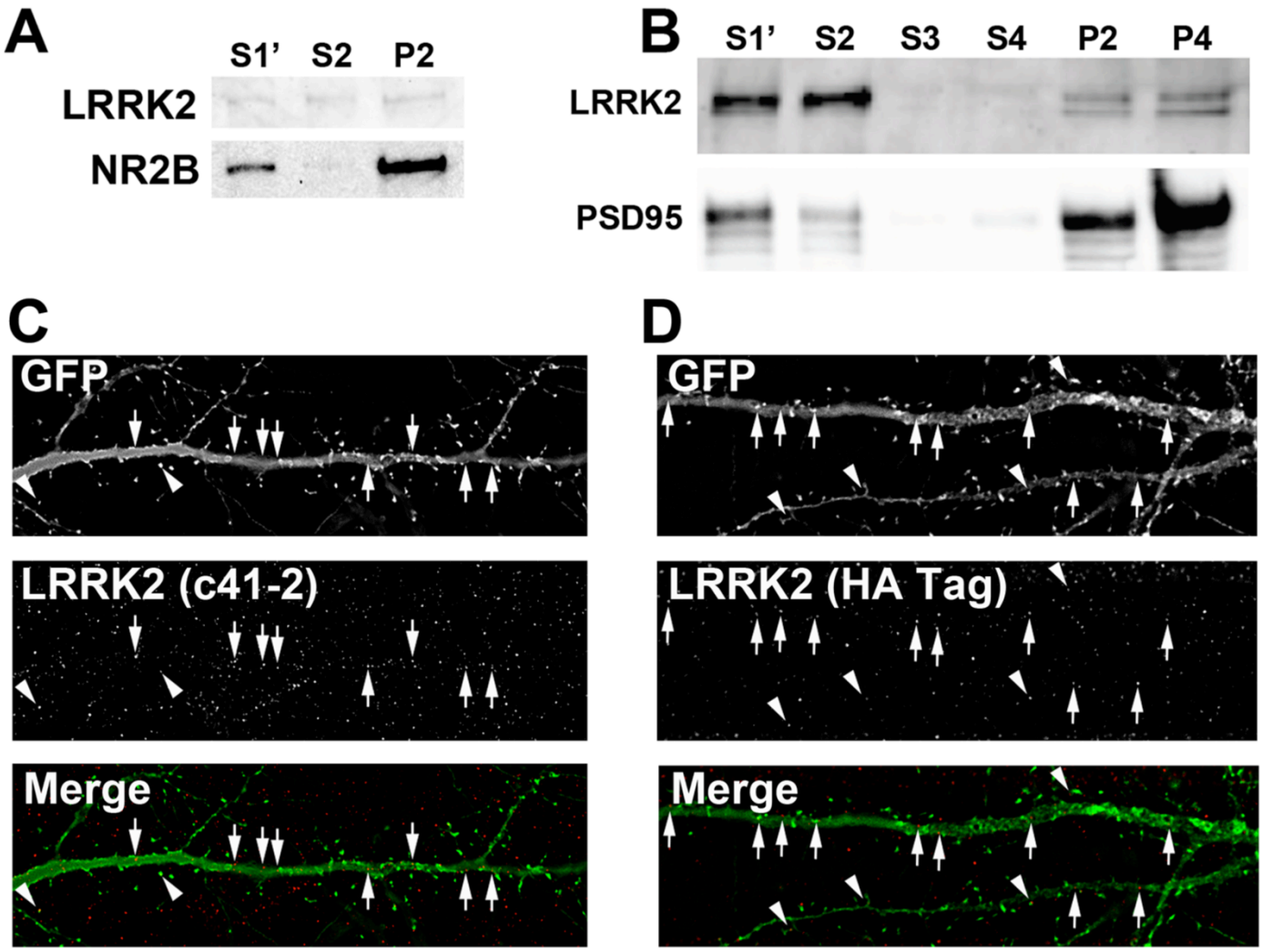
Figure S1.


A.
"pre-degenerative studies" (electrophysiology, IF)
"degenerative studies" (morphology, memantine)
Total Dendrite Length (% Vector)
120
100
80
60
40
20
0
pcDNA3.1 Vector
G2019S LRRK2
R1441C LRRK2
*
*
3 (17)
6 (20)
9 (23)
Days Post Transfection (Total DIV)
B.
9 Days Post Transfection
Mean Total Dendrite Length (μm)
2000
1500
1000
500
0
*
*
Vector
WT
G2019S
R1441C
K1906M
C.
Relative Density Ratio LRRK2-HA/GFP
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
GFP
LRRK2-HA
Vector
WT
G2019S
R1441C
K1906M
D.
Relative Density Ratio LRRK2-HA/GAPDH
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
LRRK2-HA
GAPDH
Vector
WT
G2019S
R1441C
K1906M
E.
eGFP
MAP2
Merge
50 μm
F.
eGFP
HA Tag
Merge
50 μm
G.
% GFP-positive neurons coexpressing LRRK2-3HA
100
80
60
40
20
0
Vector
WT
G2019S
R1441C
K1906M
G2019S/K1906M
H.
eGFP
DIC

Supplementary Figure S1. (A) Early, "pre-degenerative" cortical neuron recordings and synapse protein immunofluorescence studies were conducted 3 days following transfection, whereas the dendrite "degenerative" effects of mutant LRRK2 were studied at 9 days post-transfection (*$p<0.05$ compared to vector transfected neurons). (B) Mean total neurite length at 9 days post transfection (23 DIV) was decreased in G2019S LRRK2 and R1441C LRRK2 expressing neurons, but not in K1906M LRRK2 expressing neurons. Wild type overexpressing LRRK2 neurons showed a modest trend towards neurite attenuation at 9 days post transfection. (C) RT-PCR analysis of GFP and LRRK2-3HA transcript levels from rat cortical cultures transfected with LRRK2-3HA cDNA constructs (n=4 for each plasmid) showed no significant difference in WT LRRK2-HA expression relative to LRRK2 mutants (ANOVA: 3df, F-Ratio: 0.132). (D) Western blot analysis of HA Tag immunoreactivity in whole cell extracts from B103 neuroblastoma cultures transfected with LRRK2-3HA cDNA constructs (n=4 for each plasmid) showed no significant difference in WT LRRK2-HA expression relative to the LRRK2 mutants (ANOVA: 3df, F-Ratio: 0.433). (E) GFP expressing transfected neurons demonstrated positive MAP2 immunoreactivity in dendrites (arrow). An arrowhead identifies the axon. (F) Anti-HA Tag immunocytochemistry demonstrated LRRK2 cDNA expression in GFP expressing neurons. (G) Approximately 85% of the GFP-positive neurons demonstrated HA Tag immunoreactivity for the LRRK2 expression constructs employed in these studies at 1 day post-transfection. (H) Transfected neurons were identified via GFP fluorescence and approached with pipette electrodes for patch clamp recordings.

Figure S2



Supplementary Figure S2. (A) A crude synaptosomal preparation from cultured rat cortical neurons probed with a LRRK2 antibody (clone c41-2) demonstrated LRRK2 immunoreactivity, but no LRRK2 enrichment, in P2 fractions. (B) LRRK2 immunoreactivity was also detected, but not enriched, in P4 (PSD-1T) fractions of cultured cortical neurons. S1': whole cell lysate; S2: cytosolic fraction; S3: synaptic vesicle fraction; S4: synaptic membrane; P2: crude synaptosome fraction; P4: PSD-1T fraction. (C, D) Thin-stacked deconvolved images of endogenous rat LRRK2 (C) and ectopic human LRRK2-3HA in GFP expressing dendrites (D) demonstrate LRRK2 immunoreactivity predominantly in dendrites (arrows) and occasionally in dendritic spines (arrowheads).