Strain Background Drives LRRK2 Kinase Activity in the Rat Brain

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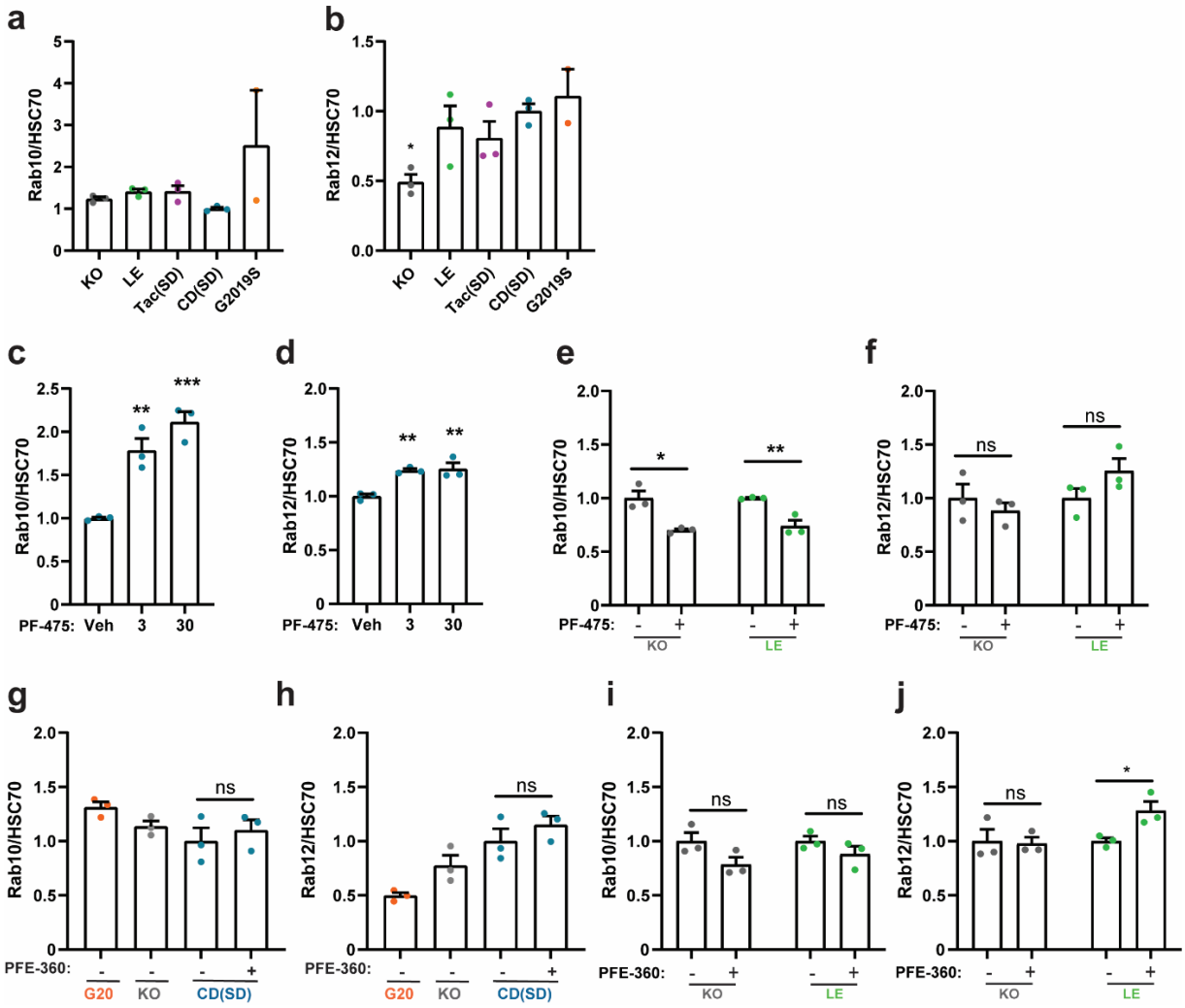
Supplemental Tables and Figures

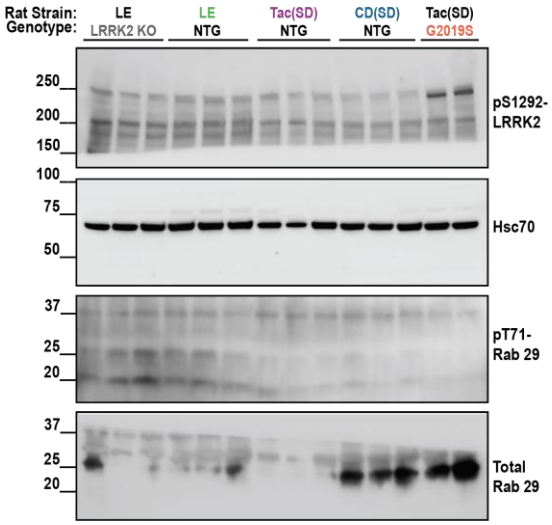
Supplemental Table 1. LRRK2 kinase selectivity profiles for small molecules PF-475 and PFE-360

Kinases >50% Inhibited	
1 µM PF-475	**1 µM PFE-360**
LRRK2	LRRK2
LOK	MAP3K5
MST1	
MST2	
MST3	
MST4	
PIP4K2C	
RSK1	
SLK	
Henderson, 2015 (PMID: 25353650)	Thirstrup, 2017 (PMID: 28860483)



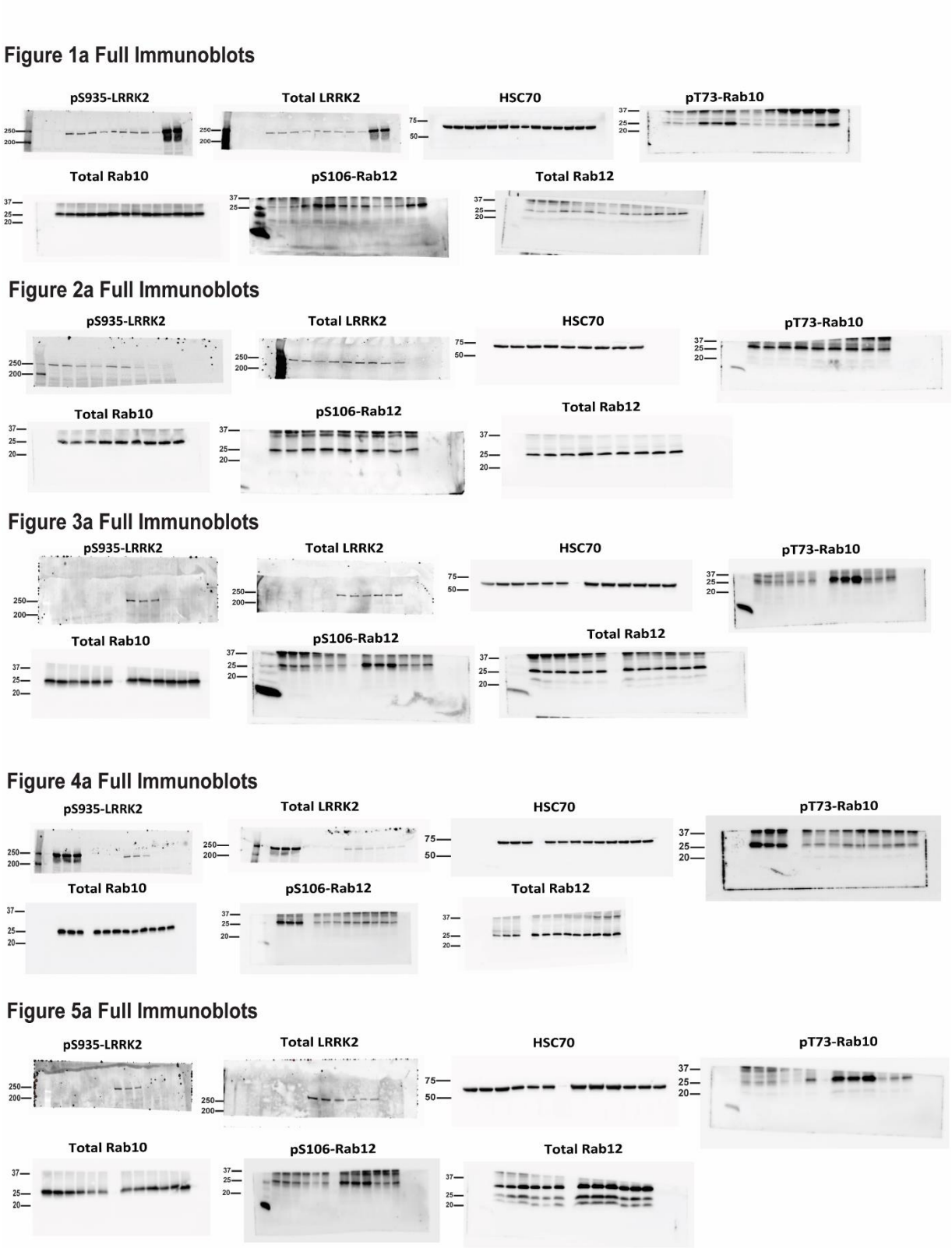
Supplemental Figure 1. Total Rab10 and Rab12 protein levels normalized to HSC70 in forebrain tissue from different rat strains and following oral dosing of small molecules PF-475 or PFE-360. Quantification of **a.** total Rab10 and **b.** total Rab12 protein levels normalized to Hsc70 in forebrain lysates of non-treated LRRK2 knockout (KO, Long-Evans background), NTG Long-Evans (LE), NTG Tac(Sprague Dawley, (Tac(SD)), NTG Charles Rivers(Sprague Dawley, (CD(SD)), and transgenic G2019S-BAC rats (G20, on Tac(SD) background) rats. All data are graphed as average measures from triplicate western blots for biological replicates (N=3 rats per group, except for G2019S N=2)). Bar graphs show group means ± SEM. Quantification of **c.** total Rab10 and **d.** total Rab12 protein levels normalized to Hsc70 in Charles Rivers(Sprague Dawley, (CD(SD)) rats treated with vehicle, 3 $mg \cdot kg^{-1}$, or 30 $mg \cdot kg^{-1}$ of PF-475, corresponding to the in-text Figure 2. Significance between groups was determined by one-way ANOVA with Dunnett's multiple comparison test with respect to the indicated group and the CD(SD) group. Quantification of **e.** total Rab10 and **f.** total Rab12 protein levels normalized to Hsc70 in Lrrk2 KO (KO) and Long-Evans (LE) rats treated with vehicle or PF-475 (30 $mg \cdot kg^{-1}$), corresponding to in-text Figure 3. Quantification of **g.** total Rab10 and **h.** total Rab12 protein levels normalized to Hsc70 in transgenic G2019S-LRRK2, LRRK2 KO (KO), and Charles Rivers(Sprague Dawley, (CD(SD)) rats treated with vehicle or PFE-360 (20 $mg \cdot kg^{-1}$),

corresponding to in-text Figure 4. Significance between groups was determined by one-way ANOVA with Dunnet's multiple comparison test with respect to the indicated group and the vehicle treated group mean. Quantification of **i.** total Rab10 and **j.** total Rab12 protein levels normalized to Hsc70 in transgenic LRRK2 KO (KO), and NTG Long-Evans (LE) rats treated with vehicle or PFE-360 (20 $mg \cdot kg^{-1}$), corresponding to in-text Figure 5. Significance between groups were determined by two-tailed unpaired *t*-test. Bar graphs show group means ± SEM.*p< 0.05, **p< 0.01., ***p< 0.001, ns is not significant.



Supplemental Figure 2. Unreliable detection of pS1292-LRRK2, pT71-Rab29, and total Rab29 protein in lysates from rat brains. LRRK2 autophosphorylation site pS1292 is most abundantly detected in G2019S-LRRK2 rats that overexpress LRRK2 protein by ~10-fold, but in all other strains, the signal nearby the 250kDa marker is not different between LRRK2 KOs and other strains. Thus, detection of pS1292-LRRK2 (MJFR-19-7-8, Abcam) in non-transgenic Long-Evans (LE) and Sprague Dawley rats (Taconic (Tac(SD)) and Charles Rivers (CD(SD))) appear similarly to LRRK2 knockout rats (Long-Evans background) and demonstrate a non-specific signal.

Detection of pT71-Rab29 (MJF-R24-17-1, Abcam) and total Rab29 (MJF-R30-124, Abcam) protein was unsuccessful in rat brain tissue, with non-specific background after long exposures evolving at the wrong molecular weights for Rab29 targets. Exact epitopes for the antibodies were not available to compare between rat and mouse Rab29 proteins, as we suspect amino-acid differences between human, mice and rats, that may affect antibody binding and specificity. Alternatively, Rab29 may be lowly expressed in the rat brain compared to other species.



Supplemental Figure 3. Full uncropped immunoblots that correspond to the representative blots presented in main figures.