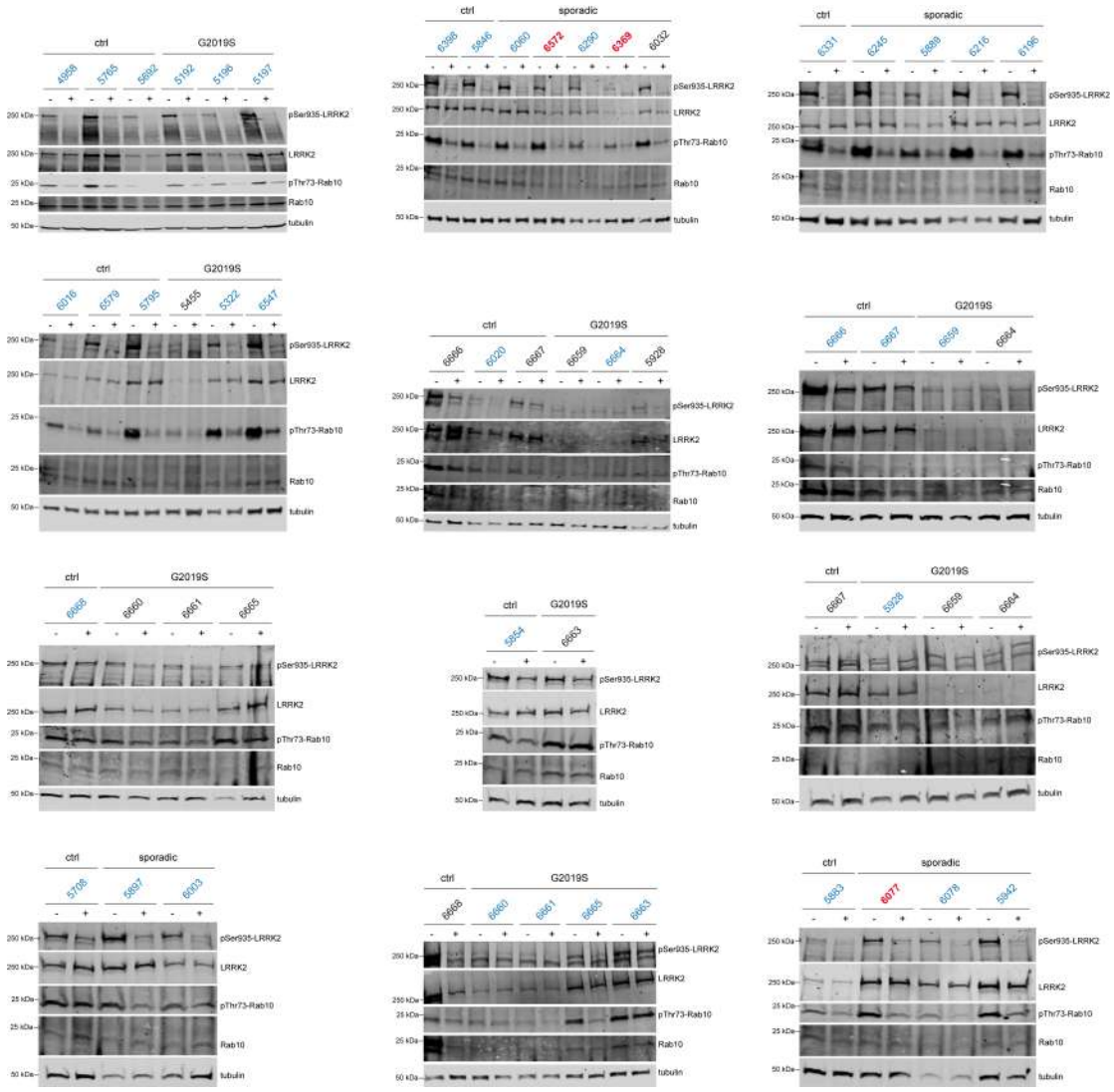
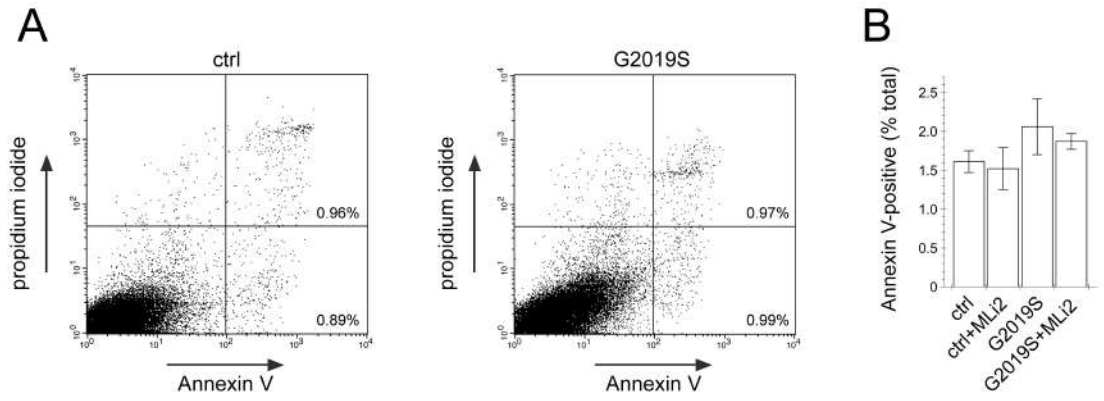
Supplementary Figures

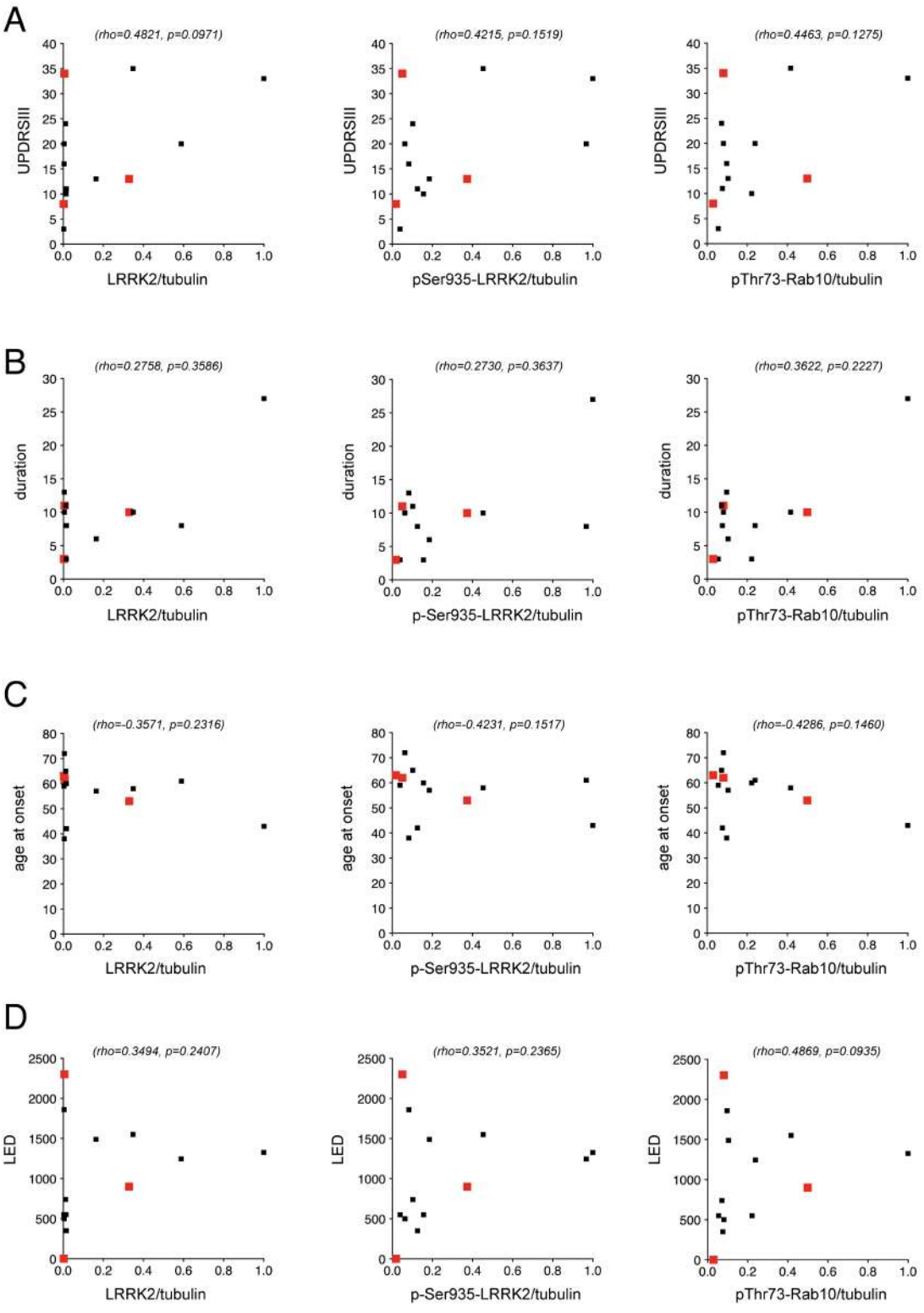


Supplementary Figure 1. Western blots detecting LRRK2, LRRK2 Ser935, Rab10 and Rab10 Thr73 phosphorylation in LCLs from control, G2019S LRRK2 PD and sporadic PD patients. All Western blots of extracts upon treating cells in presence (+) or absence (-) of 10 nM MLi2 for 2 h from control, G2019S LRRK2 and sporadic PD LCLs analyzed in the present study, and blotted with the indicated antibodies as described in Materials and Methods. Most samples were run multiple times, and sample codes in blue indicate blots where protein and phospho-

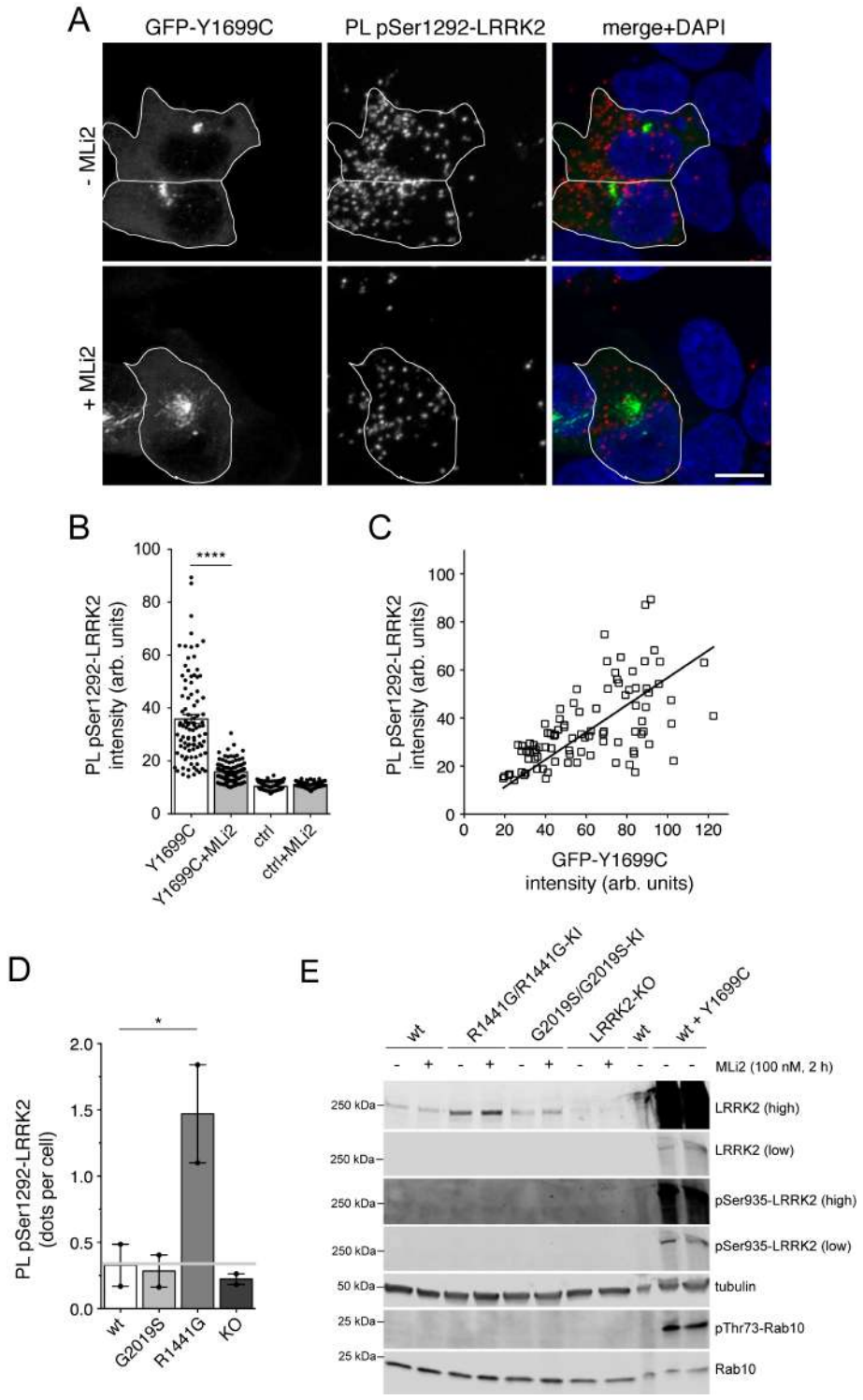
protein quantifications were analyzed from. Red codes indicate the three sporadic PD LCLs which display a centrosomal cohesion deficit.



Supplementary Figure 2. Analysis of cell viability of the three control and three G2019S LRRK2 LCLs depicted in Figure 1. (**A**) FACS-based cell viability analysis from one control and one G2019S LCL line as described in Materials and Methods. (**B**) Quantification of annexin V-positive cells from three control and three G2019S LCLs in the absence or presence of 10 nM MLi2 for 2 h as indicated.


A
(rho=0.4821, p=0.0971)
UPDRSIII
LRRK2/tubulin
(rho=0.4215, p=0.1519)
UPDRSIII
pSer935-LRRK2/tubulin
(rho=0.4463, p=0.1275)
UPDRSIII
pThr73-Rab10/tubulin
B
(rho=0.2758, p=0.3586)
duration
LRRK2/tubulin
(rho=0.2730, p=0.3637)
duration
p-Ser935-LRRK2/tubulin
(rho=0.3622, p=0.2227)
duration
pThr73-Rab10/tubulin
C
(rho=-0.3571, p=0.2316)
age at onset
LRRK2/tubulin
(rho=-0.4231, p=0.1517)
age at onset
p-Ser935-LRRK2/tubulin
(rho=-0.4286, p=0.1460)
age at onset
pThr73-Rab10/tubulin
D
(rho=0.3494, p=0.2407)
LED
LRRK2/tubulin
(rho=0.3521, p=0.2365)
LED
p-Ser935-LRRK2/tubulin
(rho=0.4869, p=0.0935)
LED
pThr73-Rab10/tubulin

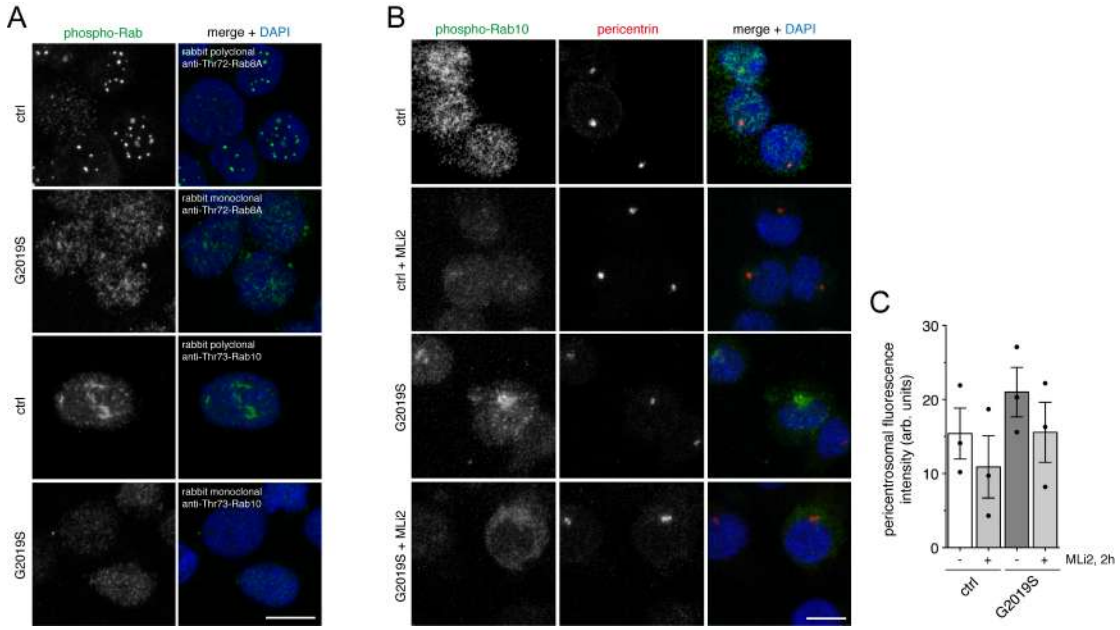
Supplementary Figure 3. Correlation analysis between the levels of LRRK2, LRRK2 Ser935 or Rab10 Thr73 and various PD clinical variables from the sporadic PD patient cohort. (**A**) Spearman correlation analysis between LRRK2 levels, LRRK2 Ser935 levels or Rab10 Thr73 levels and UPDRSIII score. (**B**) Spearman correlation analysis between LRRK2 levels, LRRK2 Ser935 levels or Rab10 Thr73 levels and disease duration. (**C**) Spearman correlation analysis between LRRK2 levels, LRRK2 Ser935 levels or Rab10 Thr73 levels and age at disease onset. (**D**) Spearman correlation analysis between LRRK2 levels, LRRK2 Ser935 levels or Rab10 Thr73 levels and the calculated L-dopa-equivalent dose (LED). The maximal datapoint values for the respective proteins and/or phospho-proteins were normalized to 1.



Supplementary Figure 4. Detection of active LRRK2 by proximity ligation assays in HEK293 cells. (**A**) Example of proximity ligation signal (PL pSer1292-LRRK2) as a readout for LRRK2 kinase activity in HEK293 cells transfected with GFP-tagged pathogenic Y1699C LRRK2, either in the absence or presence of MLi2 (100 nM, 2 h)

as indicated, and stained for DAPI. Scale bar, 10 μm. (**B**) Quantification of the proximity ligation signal intensity (PL-pSer1292-LRRK2) was performed over non-processed images acquired on the same day and with the same laser intensities with Leica Applied Systems (LAS AF6000) image acquisition software, and around 100 cells quantified per condition. ****, $p < 0.001$. (**C**) Correlation between the level of GFP-tagged pathogenic Y1699C LRRK2 expression and the proximity ligation assay signal intensity from around 100 transfected cells. Spearman correlation analysis indicated a significant association between LRRK2 expression levels and proximity ligation assay signal intensity (rho = 0.66, $p < 0.0001$). (**D**) Quantification of the proximity ligation assay signal (PL-pSer1292-LRRK2) from wildtype (wt), G2019S/G2019S-KI, R1441G/R1441G-KI or LRRK2-KO HEK293 cells. Proximity ligation assay signal was quantified as the number of fluorescent dots per cell from around 300 individual cells per condition and experiment. The non-specific proximity ligation signal obtained when omitting the pSer1292 antibody is indicated as grey line. Bars represent mean ± s.e.m. (n=2 independent experiments); *, $p < 0.05$. (**E**) Immunoblots of wildtype (wt), R1441G/R1441G-KI, G2019S/G2019S-KI, LRRK2-KO, or wildtype HEK293 cells in the absence or presence of transient transfection with GFP-tagged Y1699C mutant LRRK2 (wt + Y1699C), and in the absence or presence of MLi2 (100 nM, 2 h) as indicated. Cells were lysed and extracts subjected to quantitative immunoblot analysis with the indicated antibodies, and membranes developed using Odyssey CLx scan Western Blot imaging system. For total LRRK2 and pSer935-LRRK2 blots, both a high exposure (to detect endogenous LRRK2) and a low exposure (to detect GFP-tagged Y1699C pathogenic LRRK2) of the same membranes are depicted. Note that phospho-Ser935-LRRK2 and pThr73-Rab10

levels were only detectable upon transient overexpression of pathogenic LRRK2 in this cell type.



Supplementary Figure 5. Staining of control and G2019S LCLs with antibodies against phospho-Rab8A or phospho-Rab10. (**A**) Staining of LCLs with a rabbit polyclonal phospho-Rab8a antibody (1:250; generous gift of D. Alessi, University of Dundee, UK), a rabbit monoclonal phospho-Rab8a antibody (1:1000; Abcam, ab230260), a rabbit polyclonal phospho-Rab10 antibody (1:1000; generous gift of D. Alessi, University of Dundee, UK), or a rabbit monoclonal phospho-Rab10 antibody (1:1000; Abcam, ab230261), respectively, along with DAPI. Cells were fixed and permeabilized with Triton-X100 as described in Materials and Methods. Scale bar, 10 μm. None of these phospho-Rab antibodies were able to specifically detect the pericentrosomal/centrosomal accumulation of endogenous phospho-Rabs by immunocytochemistry techniques in either control or G2019S LRRK2 LCLs, similar to what we have previously described for the currently available phospho-Rab8a antibodies in some cell types, such as SH-SY5Y cells [34]. (**B**) Staining of LCLs with a rabbit monoclonal phospho-Rab10 antibody (1:100, Abcam, ab237703), as well as

pericentrin and DAPI. Cells were treated with or without 100 nM MLi2 for 2 hrs as indicated, fixed with PFA, and permeabilized with saponin as described in Materials and Methods. Scale bar, 10 μm. The phospho-Rab10 antibody detects pericentrosomal accumulation in some cells, but the signal is not gone in the presence of MLi2. (**C**) Quantification of phospho-Rab10 fluorescence intensity in a 3 μm circle positioned over the centrosome as detected using pericentrin staining from 100 cells per condition. The experiment was repeated twice with similar results, and the mean value of fluorescence intensity from the two experiments is plotted for each control and G2019S line. Bars represent mean ± s.e.m. (from three control and three G2019S LRRK2 lines).