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Supplementary Information for

Pathogenic LRRK2 regulates ciliation probability
upstream of Tau Tubulin kinase 2 via Rab10 and RILPL1 proteins

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This PDF file includes:

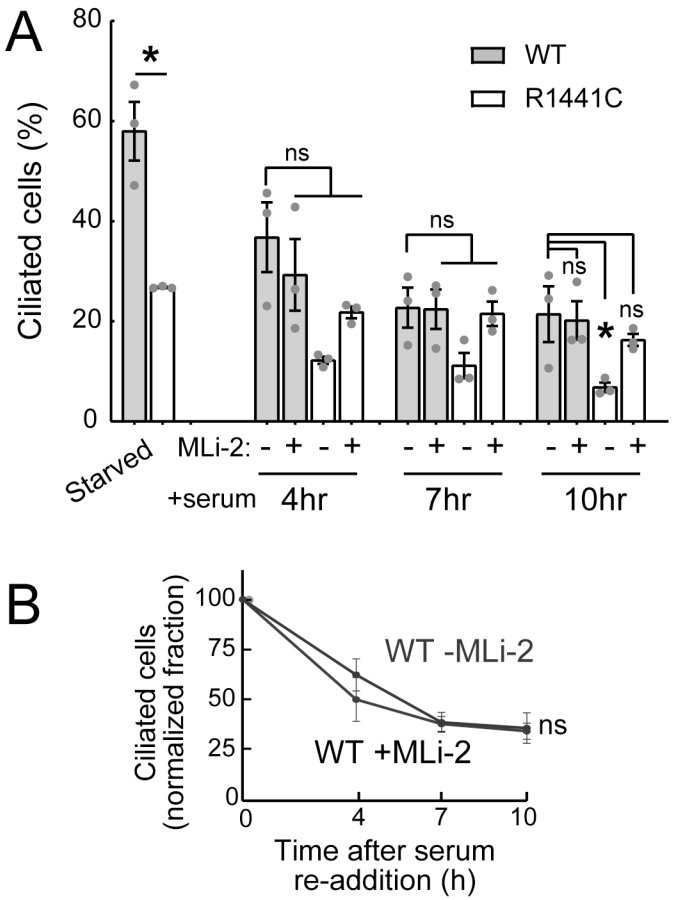
Figures S1 to S7
Legends for Movies S1 to S6

Other supplementary materials for this manuscript include the following:

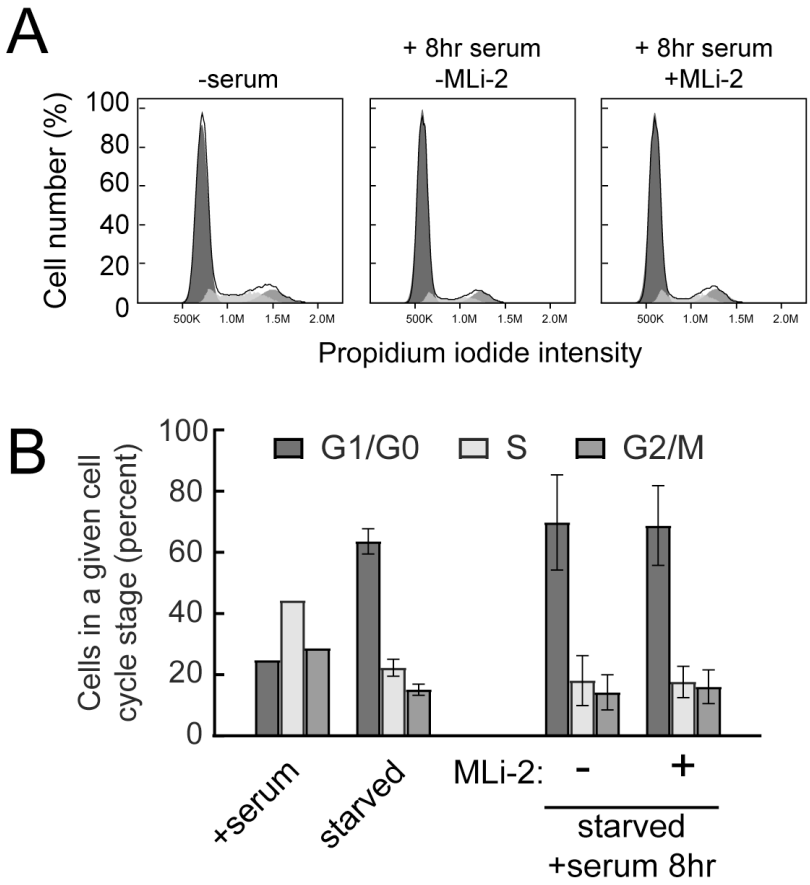
Movies S1 to S6

Supplemental Movie Legends

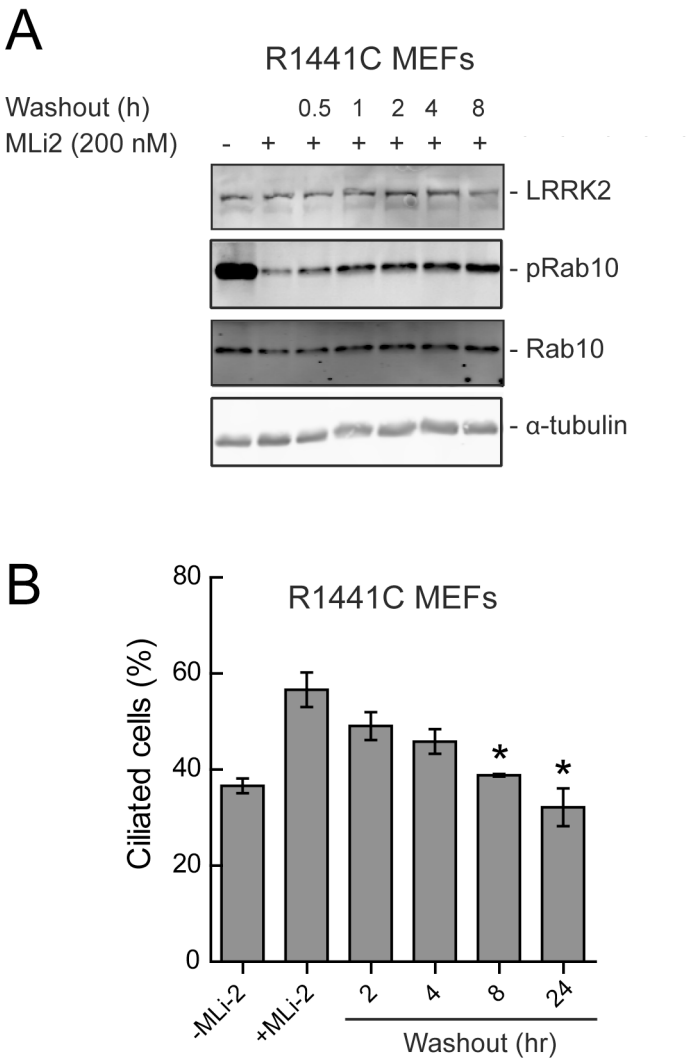
Supplemental Movie 1: Movie of cilium formation. R1441C LRRK2 MEF cells were serum starved at -2h with MLi-2. Green: SSTR3-GFP Red: mKO2-PACT. Arrow indicates newly formed primary cilium. Related to Fig1A.
Supplemental Movie 2: No cilium formation. R1441C LRRK2 MEF cells were serum starved at -2h without MLi-2. Green: SSTR3-GFP Red: mKO2-PACT. Related to Fig1A.
Supplemental Movie 3: Cilium loss after serum re-addition. Serum was re-added at -2h to R1441C LRRK2 MEF cells without MLi-2. Arrows indicate decapitated vesicles and arrowhead indicates disappearing cilium. Related to Fig2B.
Supplemental Movie 4: Cilium in R1441C LRRK2 MEF cells. MLi-2 was present throughout. Related to FigS4B.
Supplemental Movie 5: Cilium in R1441C LRRK2 MEF cells after MLi-2 washout at -15 min. Related to Fig S4B.
Supplemental Movie 6: Cilium formed in the absence of MLi-2 in R1441C LRRK2 MEF cells. Green: SSTR3-GFP Red: mKO2-PACT. Arrow indicates decapitated vesicle. Related to FigS5B.



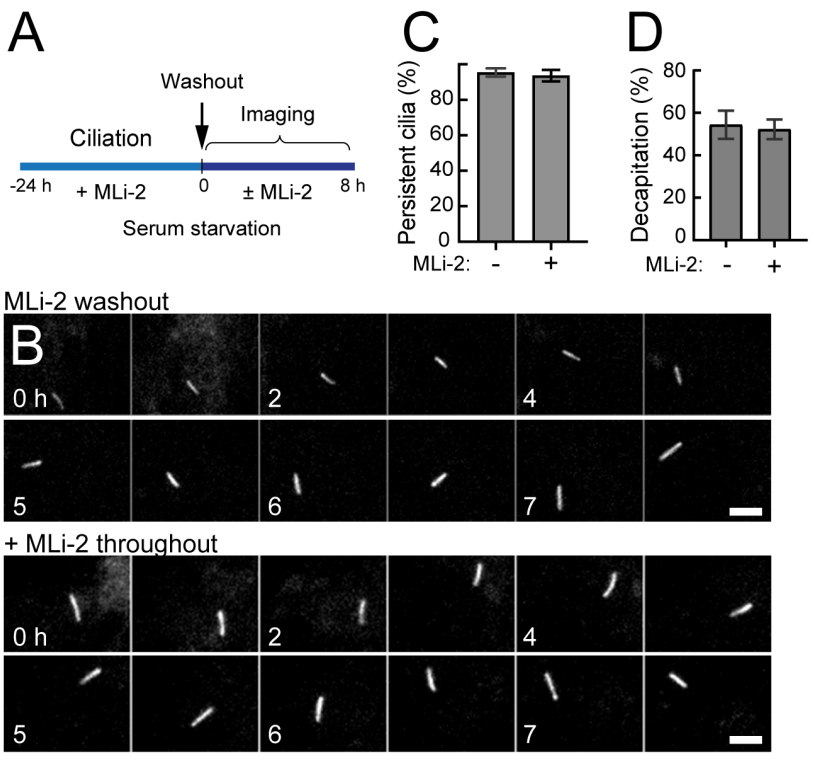
Supplemental Figure S1. Cilia loss in MEF WT cells and shRNA depletion of Rab10 and RILPL1. A. Cilia loss properties of MEF cells harboring WT LRRK2 were examined. Cells were starved for 24 hr and then serum was readded. Cells were fixed at the indicated time points and stained with Arl13B antibody. Values represent the mean ± SEM from 3 independent experiments, each containing > 24 cells. Significance of starved cells was determined by paired t-test (*P=0.034). Significance for the WT-MLi-2 experiment was determined by the repeated measures one-way ANOVA with Dunnett post-hoc test. (4hr: WT +MLi2 : P=0.35, R1441C -MLi2 : P=0.16, R1441C +MLi2 : P=0.34, 7hr: WT +MLi2 : P= 1.0, R1441C -MLi2 : P=0.32, R1441C +MLi2 : P=1.0,10hr: WT +MLi2 : P= 0.99, R1441C -MLi2 : *P=0.043, R1441C +MLi2 : P=0.61). **B**. Percentage of remaining cilia compared with time=0 (100%) after serum re-addition. Significance was determined by unpaired t-test (4hr: P=0.41 7hr: P=0.90 10hr: P=0.87). **C.** Immunoblot of R1441C LRRK2 MEF cell extracts after infection with lentivirus carrying shRNA for Scr (scramble), Rab10 or RILPL1.



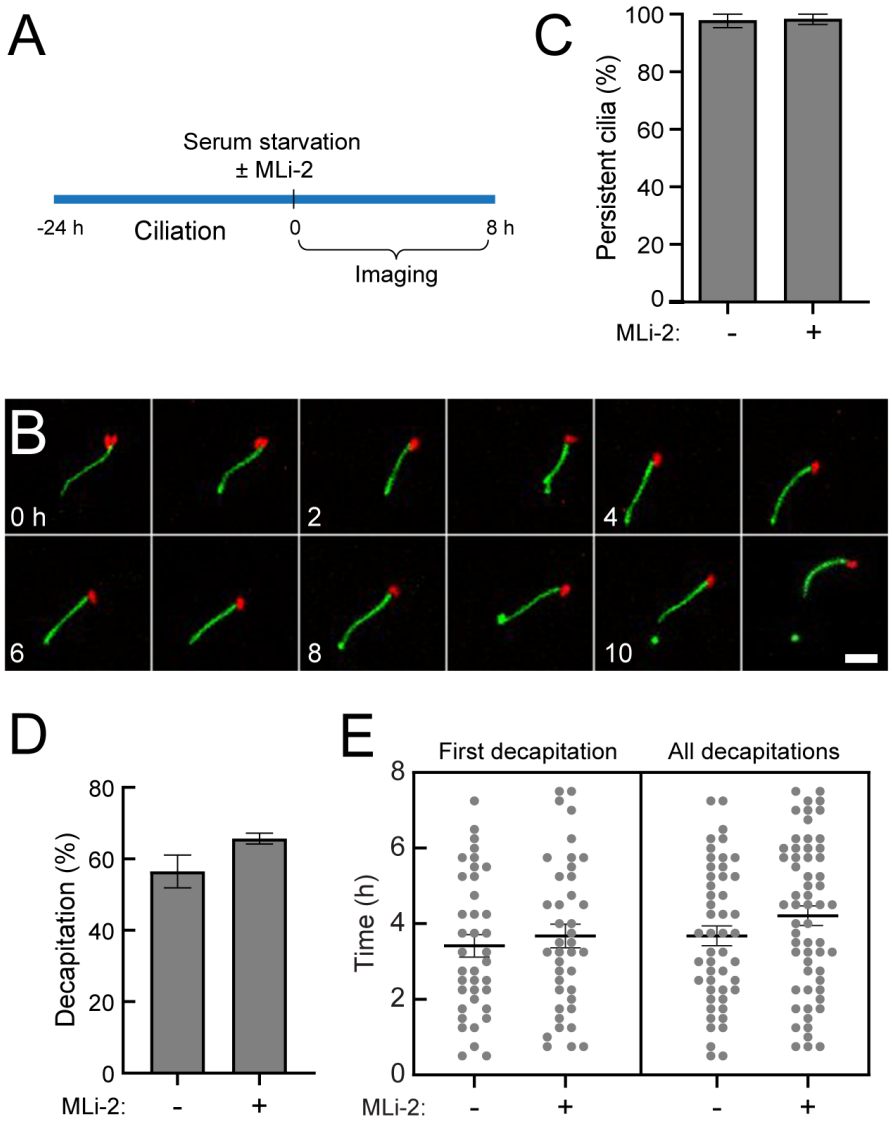
Supplemental Figure S2. Cell cycle status of MEF R1441C LRRK2 cells after serum stimulation. A. Cell cycle analysis after serum re-addition in R1441C LRRK2 MEF cells. Cells were starved for 24hr, then serum was added ± MLi-2. Cells were fixed 8hr after serum re-addition and stained with propidium iodide (PI). G0/G1, S, G2/M phases were fitted to a model provided by flowjo v.10 software. **B.** Summary of cell cycle populations in A. Values represent the mean ± SEM from 2 independent experiments, at least 10,000 cells in each condition. Significance was determined by paired t-test (8hr -MLi-2 vs 8hr +MLi-2 (G0/G1: P=0.76, S: P=0.91, G2/M: P=0.084).



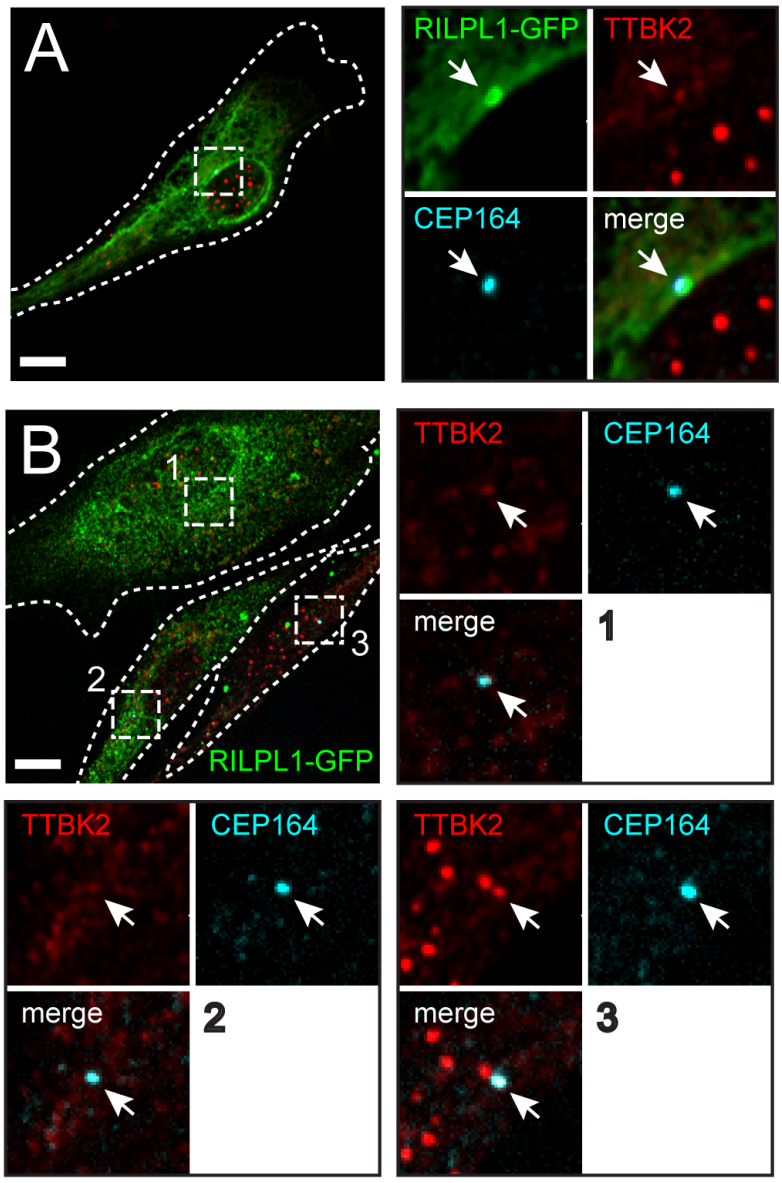
Supplemental Figure S3. MLi-2 washout in MEF R1441C LRRK2 cells.
A. R1441C MEF cells were serum starved ±200 nM MLi-2 for 24 hours. MLi-2 was removed by washing twice with PBS and cells were cultured for the specified time points in complete DMEM. Cells were lysed and 40 µg analyzed by immunoblotting with the indicated antibodies. **B**. To monitor ciliation, cells were fixed in 3.5% PFA and stained for Arl13b and DAPI. The percentage of ciliated cells was quantified by dividing the number of ciliated cells by the total number of nuclei counted. Error bars represent SEM from two independent experiments. Significance was determined by the Repeated measures one-way ANOVA with Dunnett post-hoc test (8 h (P=0.040), 24 h (P=0.013)).



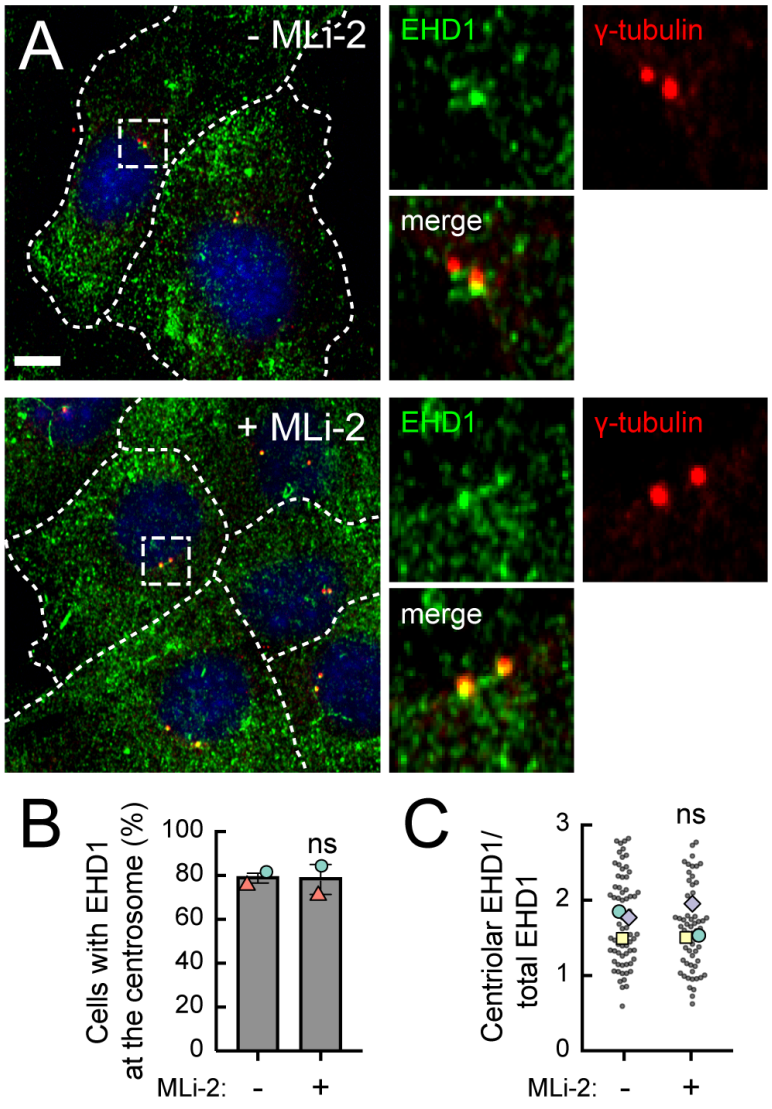
Supplemental Figure S4. Pathogenic LRRK2 activity does not alter cilia stability under serum starvation conditions. A. Timeline of this imaging experiment. **B.** Time-lapse images of R1441C LRRK2 MEF cells stably expressing SSTR3-GFP. Cells were serum starved in the presence of MLi-2 for 24hr to generate "wild type" cilia. After drug washout, cells were imaged every 15 minutes under continued serum starvation ± MLi-2. Scale bar, 5μm. Times are indicated in hours. **C**. Probability of persistent cilia (P=0.68) and **D**. Percentage of cilia that displayed decapitation events (P=0.80) during 8 hr of imaging. Values represent the mean ± SEM from 3 independent experiments, each containing >10 cells. Significance was determined by unpaired t-test. **E.** Relative growth of cilia after MLi-2 washout. Values represent the mean ± SEM from 39 cells +MLi-2 and 50 cells -MLi-2. **F.** Timing of first decapitation of each cilium (P=0.91) and all decapitations (P=0.96) are shown. Values represent the mean ± SEM. Significance was determined by unpaired t-test (ns). **G, H.** Cells were ciliated with MLi-2 for 24 h then serum were added ± MLi-2. **G.** Probability of persistent cilia (ciliated without MLi-2: *P=0.012; ciliated with MLi-2: *P=0.037). **H.** Timing of first decapitation of each cilium (ciliated without MLi-2: P=0.42, ciliated with MLi-2: P=0.25), all decapitations (ciliated without MLi-2: P=0.88, ciliated with MLi-2: P=0.44) and timing of cilia loss (ciliated without MLi-2: P=0.62, ciliated with MLi-2: P= 0.52) are shown. Values represent the mean ± SEM from 3 independent experiments, each containing 10-18 cilia. Significance was determined by paired (G) or unpaired (H) t-test.



Supplemental Figure S5. Cilia formed with or without pathogenic LRRK2 activity are stable in the absence of serum. A. Timeline of this imaging experiment. **B.** Time-lapse images of R1441C LRRK2 MEF cells stably expressing SSTR3-GFP (green) and mKO2-PACT (red). Cells were serum starved ± MLi-2 for 24hr then imaged every 15 minutes. Scale bar, 5μm. **C, D.** Probability of cilia persistence (P=0.86) and percentage of cilia showing any decapitation events (P=0.13) during 7hr imaging ± MLi-2. Values represent the mean ± SEM from 3 independent experiments, each containing >19 cells. Significance was determined by unpaired t-test. **E.** Timing of first decapitation of each cilium (P=0.54) and all decapitation events (P=0.15) are shown. Values represent the mean ± SEM. Significance was determined by unpaired t-test.



Supplemental Figure S6. TTBK2 localization in serum starved RPE cells over-expressing RILPL1-GFP as in Figure 6D. Individual cells are outlined in A and B; enlarged regions are boxed and presented at right. In B, #1 and #2 cells express more RILPL1-GFP than #3; the surrounding enlargements are labeled to indicate cell #1, 2 or 3. RILPL1-GFP was detected with chicken anti-GFP (green); CEP164 was detected using mouse anti-CEP164 (turquoise); rabbit anti-TTBK2 staining is shown in red. Scale bar, 10μm. Arrows indicate the location of CEP164 at the mother centriole. Note that centriolar RILPL1 staining is often lost in methanol fixed cells compared with paraformaldehyde fixation, thus merges in B are 2-color, to highlight TTBK2 and CEP164.



Supplemental Figure S7. LRRK2 activity does not change EHD1 concentration at the centrosome. A. R1441C LRRK2 MEF cells stably expressing GFP-TTBK2 were starved for 24h ± MLi-2. Cells were then fixed with cold methanol and stained with rabbit anti-EHD1 (green) and mouse anti-γ-tubulin (red) antibodies. At right are enlarged regions boxed in the larger images at left. Scale bars, 10μm. **B.** Percent of cells with EHD1 at the centrosome, marked by γ-tubulin staining. Values represent the mean and SEM of two independent experiments (n > 30 in each experiment). Significance was determined by the paired t-test; P=0.9429. **C.** Each dot represents the ratio of mean EHD1 signal intensity at the centrosome divided by the mean signal intensity throughout the cell (n > 40). Colored shapes represent the mean of each experiment