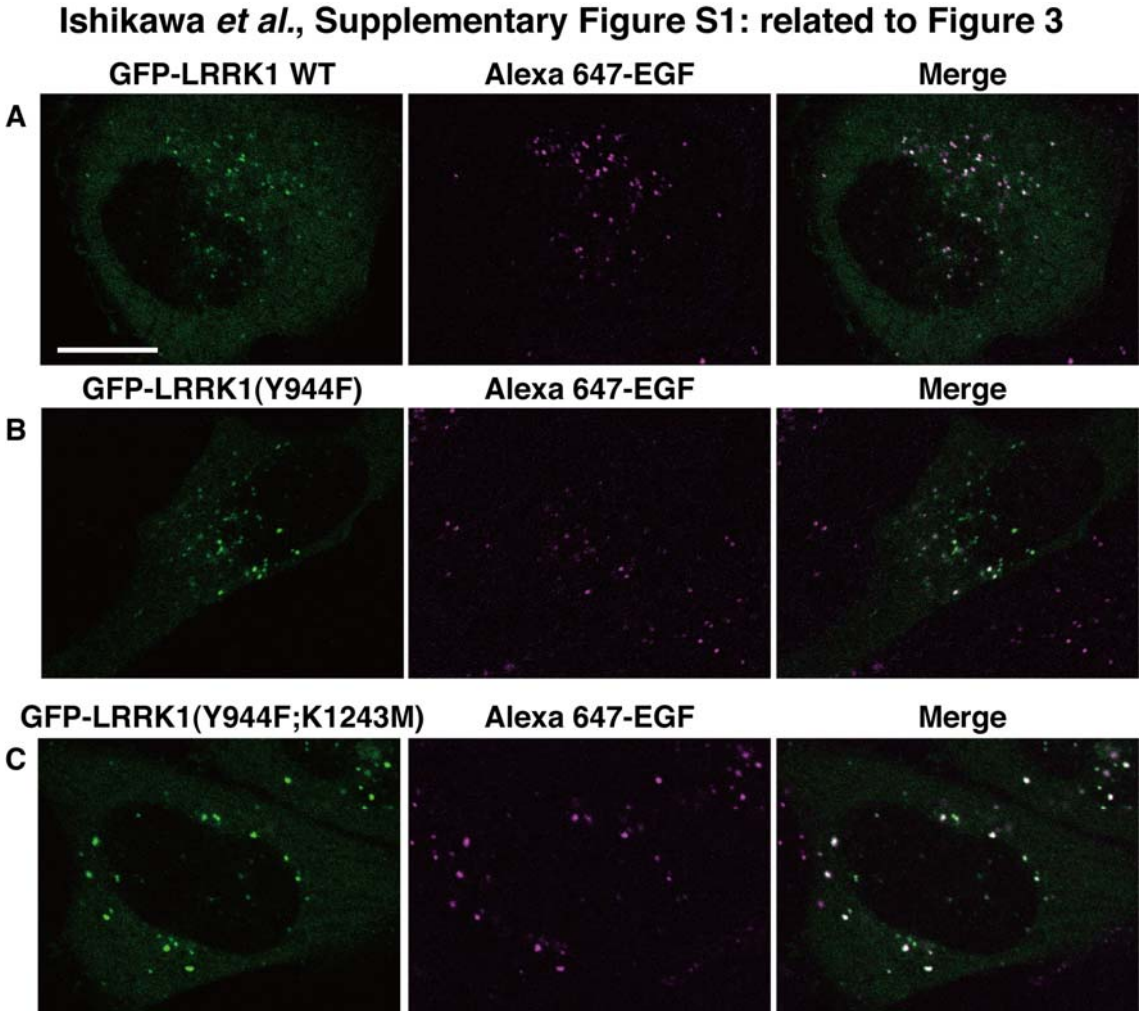
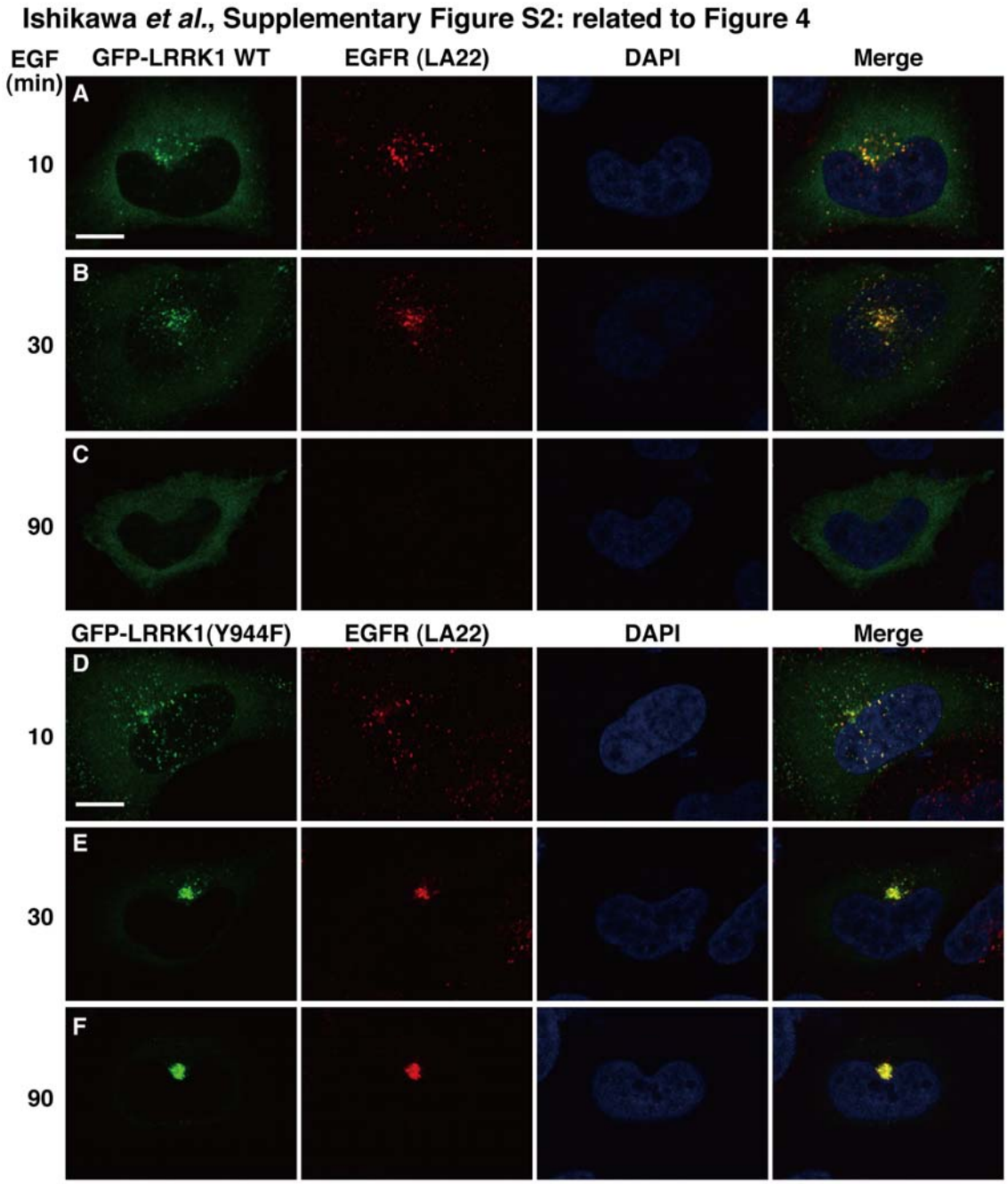
Supplemental Figure Legends



Supplemental Figure S1: (related Figure 3) Co-localization of LRRK1 with EGF.

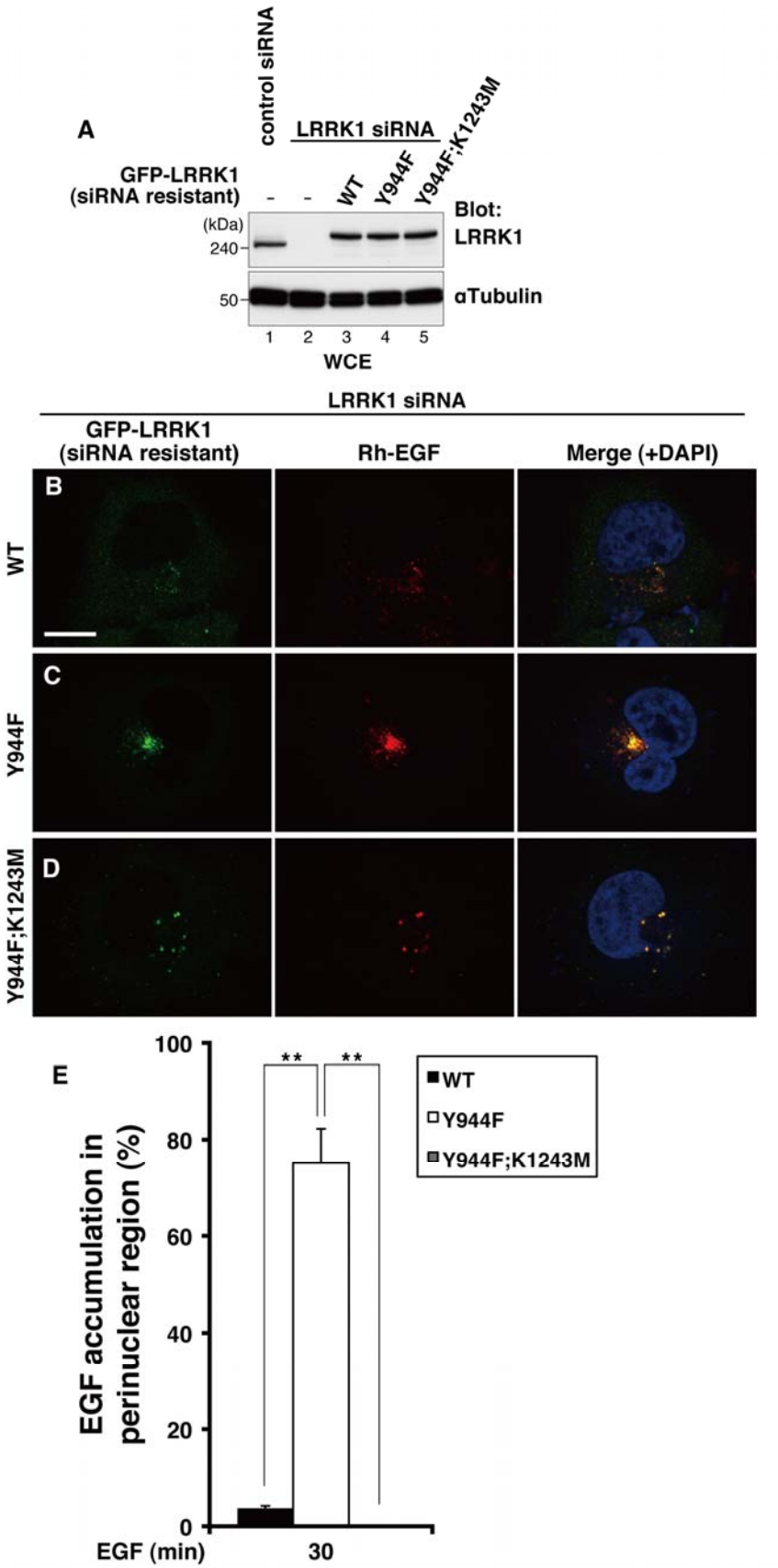
HeLa S3 cells were transfected with wild-type (WT) GFP-LRRK1 (**A**), GFP-LRRK1(Y944F) (**B**) and GFP-LRRK1(Y944F; K1243M) (**C**), and pulse-labeled with Alexa 647-EGF for 3 min. Cells were imaged by confocal microscopy. Each image shows the first frame (0 sec) of time-lapse confocal fluorescence microscopy. Scale bar, 10 μm.



Supplemental Figure S2: (related to Figure 4) LRRK1(Y944F) promotes EGFR accumulation in perinuclear endosomes.

HeLa S3 cells were transfected with wild-type (WT) GFP-LRRK1 (**A**-**C**) and GFP-LRRK1(Y944F) (**D**-**F**), as indicated. After serum starvation, cells were incubated with anti-EGFR antibodies (LA-22) for 2 hr at 37°C, followed by Alexa 555-goat anti-mouse IgG antibodies for 1 hr at 37°C. After washing to remove antibodies from the medium, the cells were stimulated with EGF for the indicated times and then fixed. Yellow colors in the merged images indicate co-localization of GFP-LRRK1 and EGFR. Scale bars, 10 μm.

Ishikawa *et al.*, Supplementary Figure S3: related to Figure 4

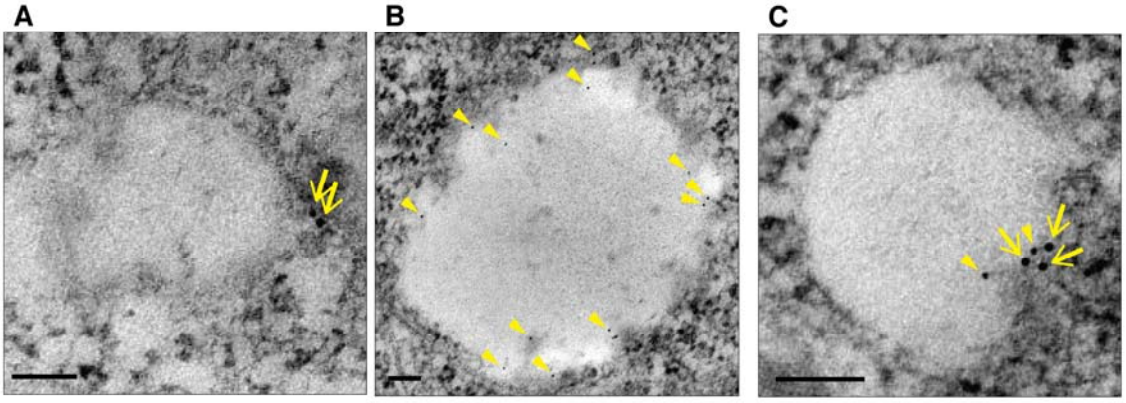


Supplemental Figure S3: (related to Figure 4) LRRK1(Y944F) expression leads to EGF accumulation in endosomal compartments in the perinuclear

region.

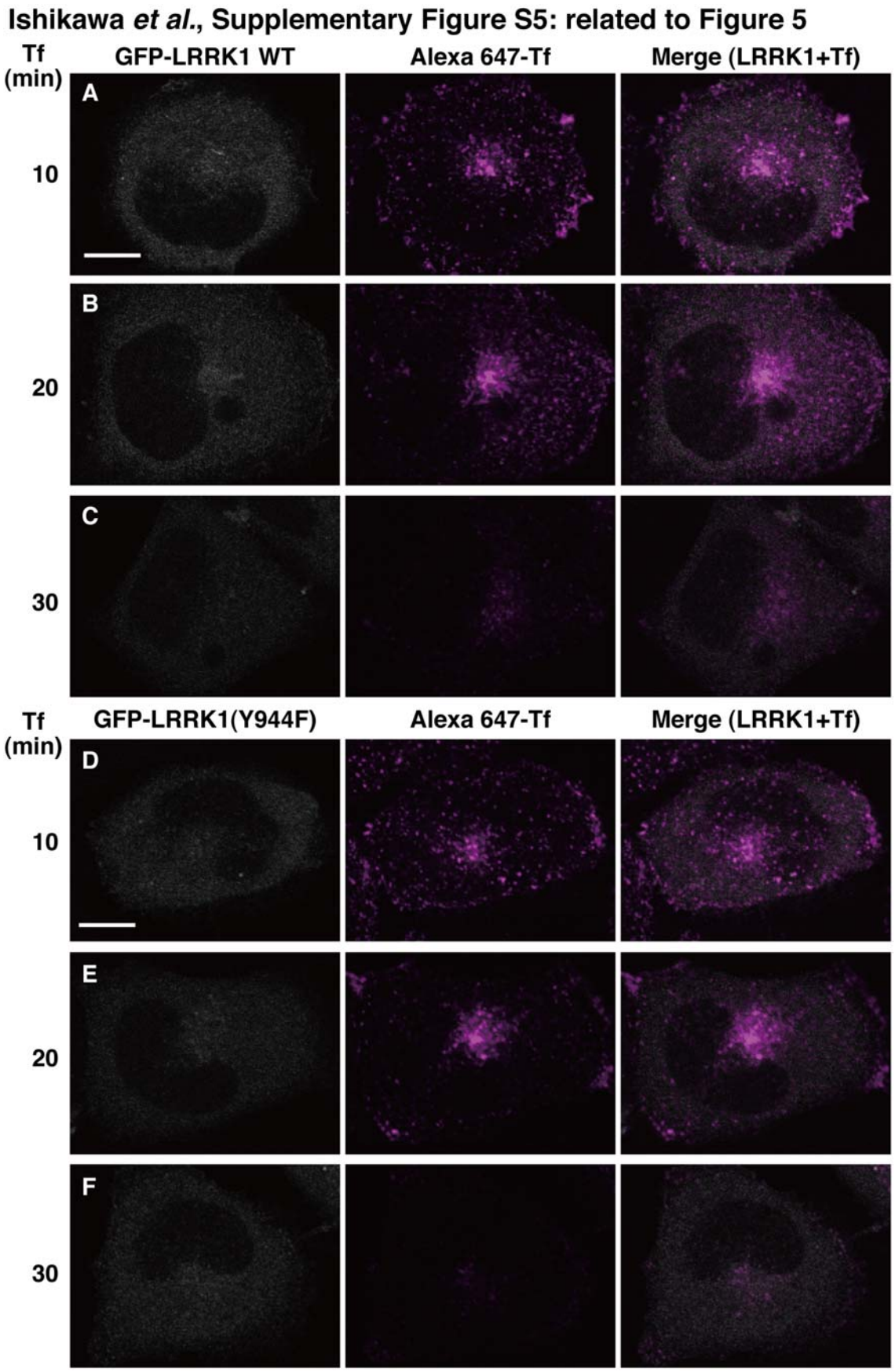
(**A**) HeLa S3 cells treated with control siRNA or LRRK1 siRNA were transfected with siRNA-resistant wild-type (WT) GFP-LRRK1, GFP-LRRK1(Y944F) and GFP-LRRK1(Y944F; K1243M), as indicated. Whole-cell extracts (WCE) were immunoblotted with the indicated antibodies. (**B-D**) HeLa S3 cells treated with LRRK1 siRNA were transfected with siRNA-resistant wild-type (WT) GFP-LRRK1 (**B**), GFP-LRRK1(Y944F) (**C**) and GFP-LRRK1(Y944F; K1243M) (**D**), as indicated. After 16 hr of serum starvation, cells were briefly stimulated with Rh-EGF (40 ng/ml), followed by washing to remove labeled EGF from the medium. Cells were incubated for 30 min after the initial exposure to Rh-EGF and then fixed and stained with DAPI. Yellow colours in the merged images indicate the co-localization of GFP-LRRK1 and Rh-EGF. Scale bar, 10 μm. (**E**) Quantification of EGF accumulation in the perinuclear region. Histogram indicates the percentage of cells that have endosomes (> 2.0 μm diameter) containing Rh-EGF in the perinuclear region. Values reflect the mean standard deviation of three independent experiments, with an average of 25 cells scored per samples. Data are compared using a two-tailed unpaired Student's t test. *, $P < 0.05$; **, $P < 0.01$.

Ishikawa *et al.*, Supplementary Figure S4: related to Figure 5



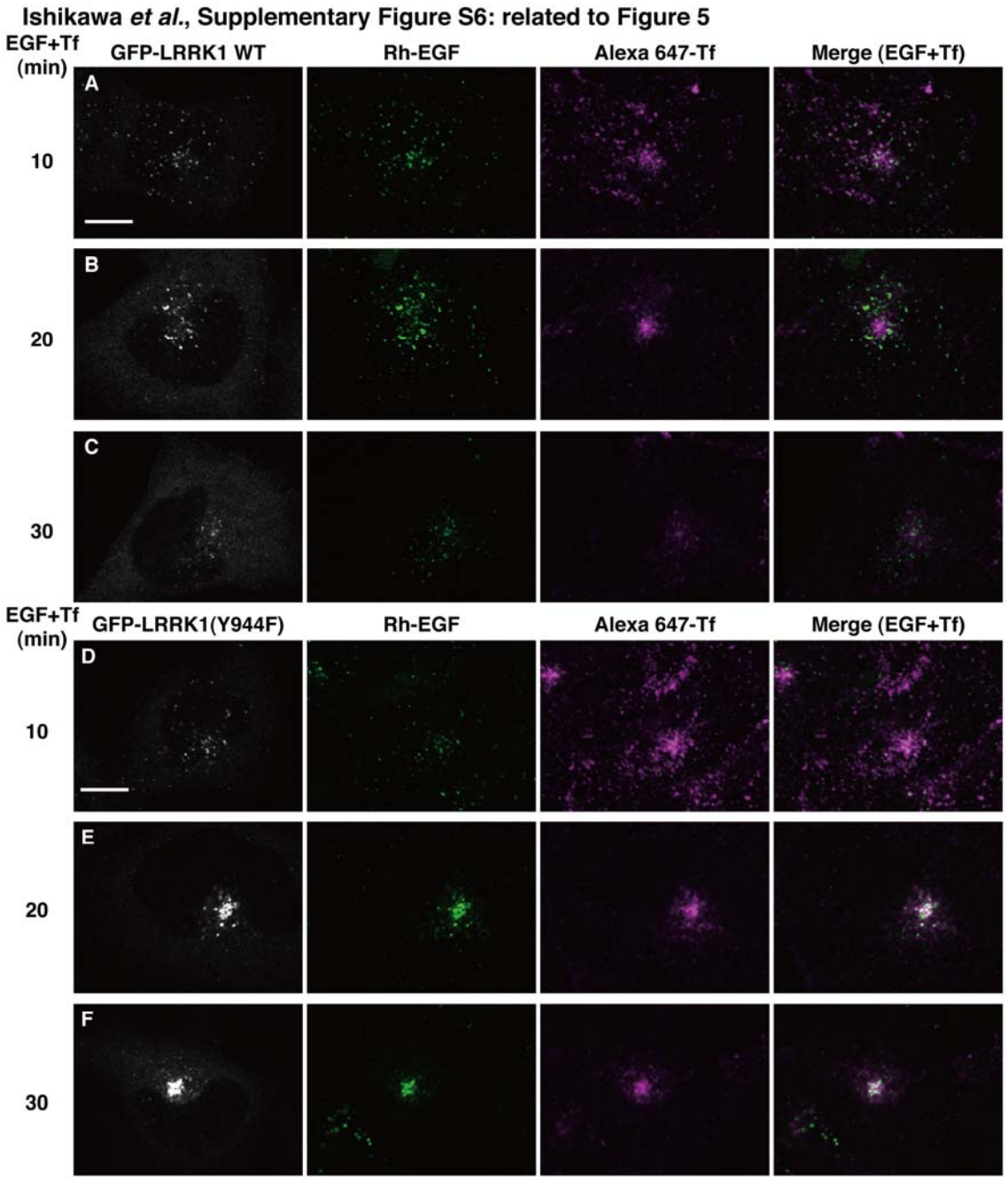
Supplemental Figure S4: (related to Figure 5) LRRK1(Y944F) induces co-localization of EEA1 and CI-MPR to the same compartment.

We generated HeLa S3 cells stably expressing wild-type GFP-LRRK1 (**A**, **B**) and GFP-LRRK1(Y944F) (**C**). After 16 hr of serum starvation, cells were stimulated with EGF (50 ng/ml) for 30 min and then fixed. The localization of EEA1 and CI-MPR was examined by immunoelectron microscopy and endosomes containing gold particles were counted. In wild-type GFP-LRRK1- and GFP-LRRK1(Y944F)-expressing cells, 3 of 24 and 19 of 25 endosomes, respectively, contained both EEA1 and CI-MPR. Arrows and arrowheads indicate gold particles labeling EEA1 (10 nm) and CI-MPR (5 nm), respectively. Scale bars, 100 nm.



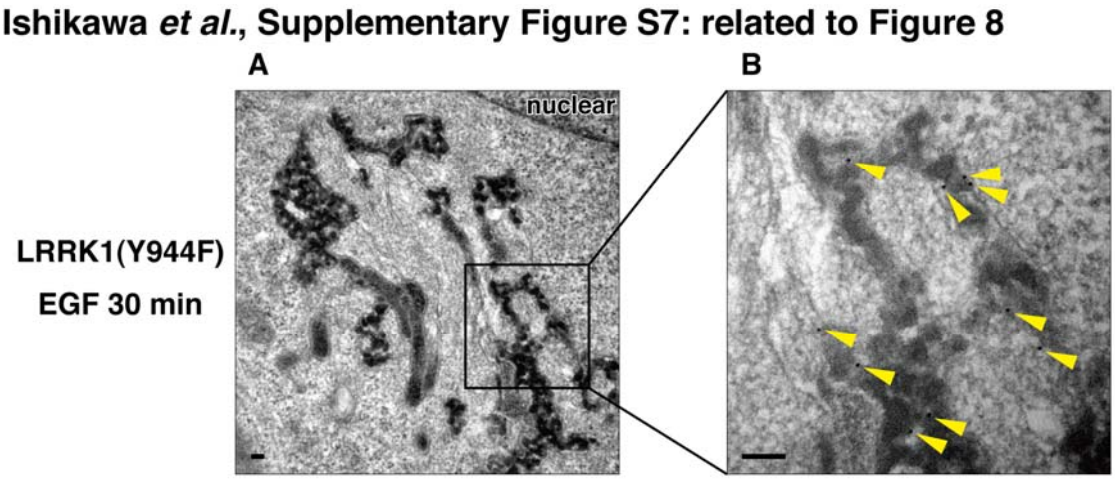
Supplemental Figure S5: (related Figure 5) Effect of LRRK1(Y944F) on Tf recycling without EGF stimulation.

HeLa S3 cells were transfected wild-type (WT) GFP-LRRK1 (**A**-**C**) and GFP-LRRK1(Y944F) (**D**-**F**), as indicated. After 16 hr of serum starvation, cells were briefly treated with Alexa 647-Tf (5 μg/ml), followed by washing to remove labeled Tf from the medium. Then cells were incubated with unlabelled Tf (60 μg/ml) and fixed at the indicated times after the initial exposure to Alexa 647-Tf. Scale bars, 10 μm.



Supplemental Figure S6: (related Figure 5) Effect of LRRK1(Y944F) on Tf recycling with EGF stimulation.

HeLa S3 cells were transfected wild-type (WT) GFP-LRRK1 (**A**-**C**) and GFP-LRRK1(Y944F) (**D**-**F**), as indicated. After 16 hr of serum starvation, cells were briefly treated with Rh-EGF (40 ng/ml) and Alexa647-Tf (5 μg/ml) simultaneously, followed by washing to remove labeled EGF and Tf from the medium. Then cells were incubated with unlabelled Tf (60 μg/ml) and fixed at the indicated times after the initial exposure to Rh-EGF and Alexa 647-Tf. Scale bars, 10 μm.



Supplemental Figure S7: (related Figure 8) LRRK1(Y944F) induces the formation of morphologically abnormal endosomes.

After serum starvation, HeLa S3 cells stably expressing GFP-LRRK1(Y944F) were incubated with anti-EGFR antibodies (LA-22) for 2 hr at 37°C, followed by goat anti-mouse IgG antibodies conjugated to 10 nm gold for 1h at 37°C. After washing to remove antibodies from the medium, the cells were stimulated with EGF for 30 min and then fixed. The localization of EGFR was examined by conventional electron microscopy. The boxed region is magnified. Arrowheads indicate gold particles labeling EGFR. Scale bars, 100 nm.