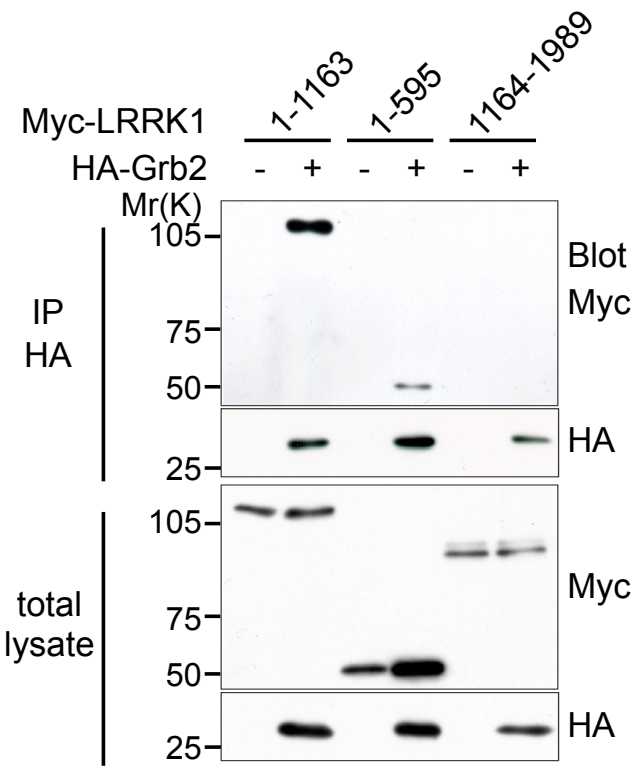
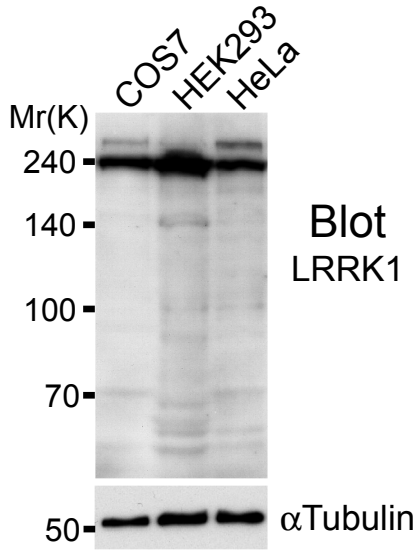
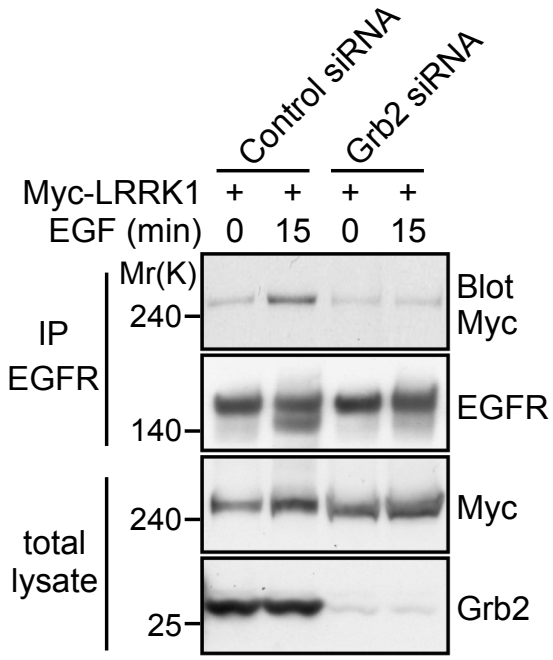


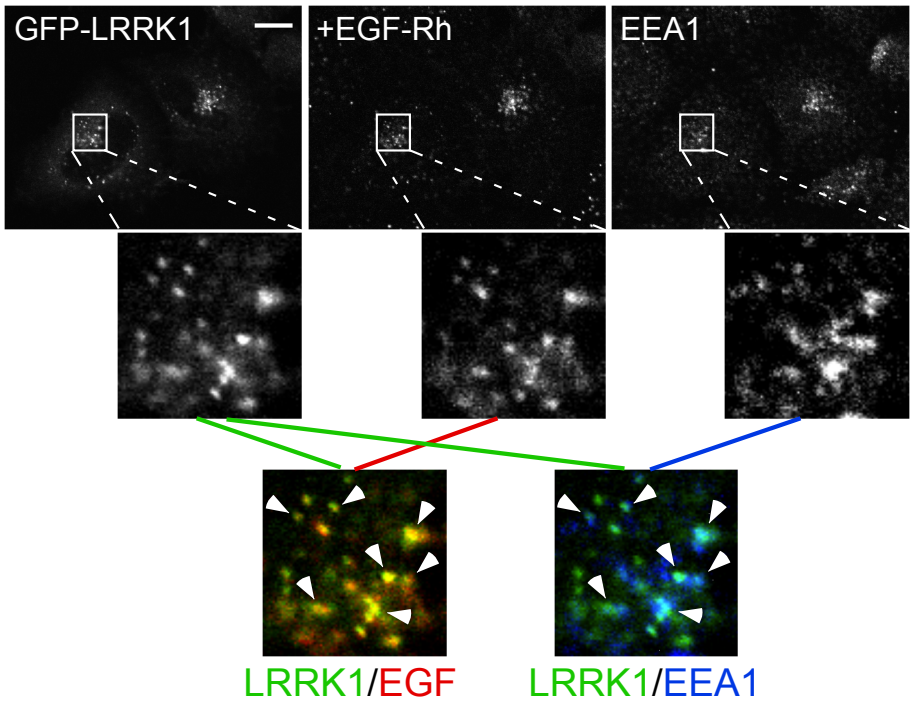
Supplementary Figure S1. N-terminal fragments of LRRK1 bind to Grb2.
COS7 cells were cotransfected with Myc-LRRK1(1-1163, 1-595 and 1164-1989) and HA-Grb2, as indicated. Complex formation was detected by immunoprecipitation (IP) with anti-HA antibodies, followed by immunoblotting (Blot) with anti-Myc antibodies.



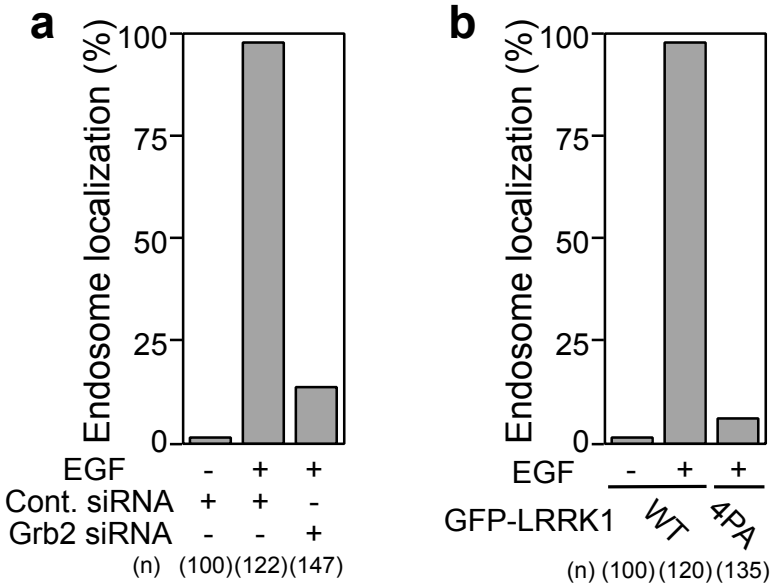
Supplementary Figure S2. Detection of LRRK1 proteins by anti-LRRK1 antibody.
Cell lysates were immunoblotted with anti-LRRK1 or anti-αTubulin antibodies. The affinity-purified antibody against hLRRK1 specifically recognizes endogenous LRRK1 in extracts from COS7, HEK293, and HeLa cells, respectively. αTubulin serves as a loading control.



Supplementary Figure S3. Inteeraction of LRRK1 with EGFR.
HEK293 cells treated with control or Grb2 siRNA were transfected with Myc-LRRK1, and stimulated with 100 ng/ml EGF. Complex formation was detected by immunoprecipitation (IP) with anti-EGFR antibodies, followed by immunoblotting (Blot) with anti-Myc antibodies.



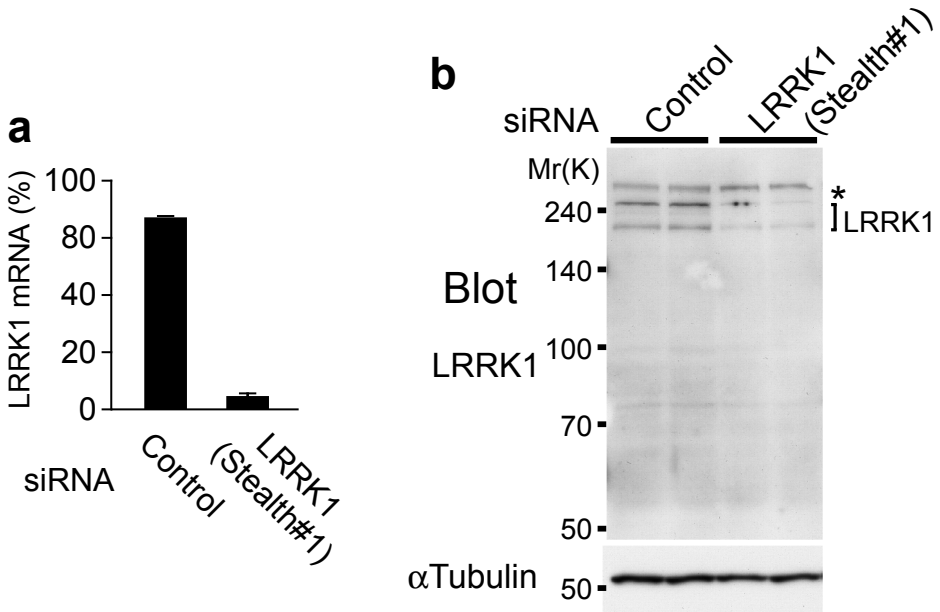
Supplementary Figure S4. Colocalization of LRRK1 with EGF and EEA1.
HeLa cells were transfected with GFP-LRRK1. After 16 hr of serum starvation, cells were incubated with 50 ng/ml EGF-Rh for 45 min at 4°C, followed by warming for 15 min at 37°C, and stained with anti-EEA1 antibodies. Cells were imaged by confocal microscopy. The boxed regions in Fig. 2b show magnified images. Colocalization of GFP-LRRK1 (green) with EGF-Rh (red) and EEA1 (blue) are indicated. Examples of colocalization of GFP-LRRK1, EGF-Rh and EEA1 are indicated with arrowheads. The scale bars represent 10 μm.



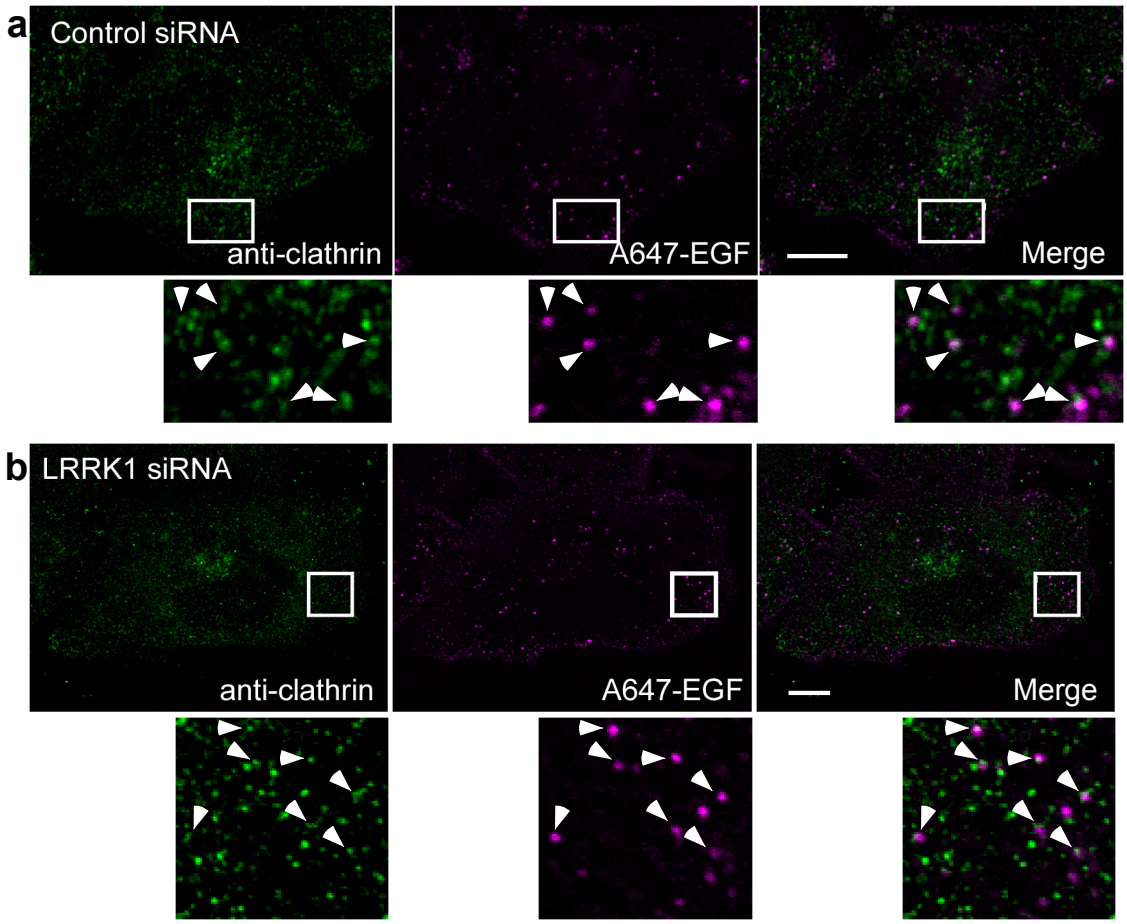
Supplementary Figure S5. Quantification of LRRK1 localization to early endosomes.

(a) Effect of Grb2 depletion on LRRK1 localization. HeLa S3 cells treated with control or Grb2 siRNA were transfected with GFP-LRRK1. After 16 hr of serum starvation, cells were stimulated with 100 ng/ml EGF for 15 min and stained with anti-EEA1 antibodies. Data are presented as the percentage of cells that have five or more EEA1-labeled vesicles that also contain GFP-LRRK1. Numbers (n) indicate the counted cells. Experiments were performed three times with similar results.

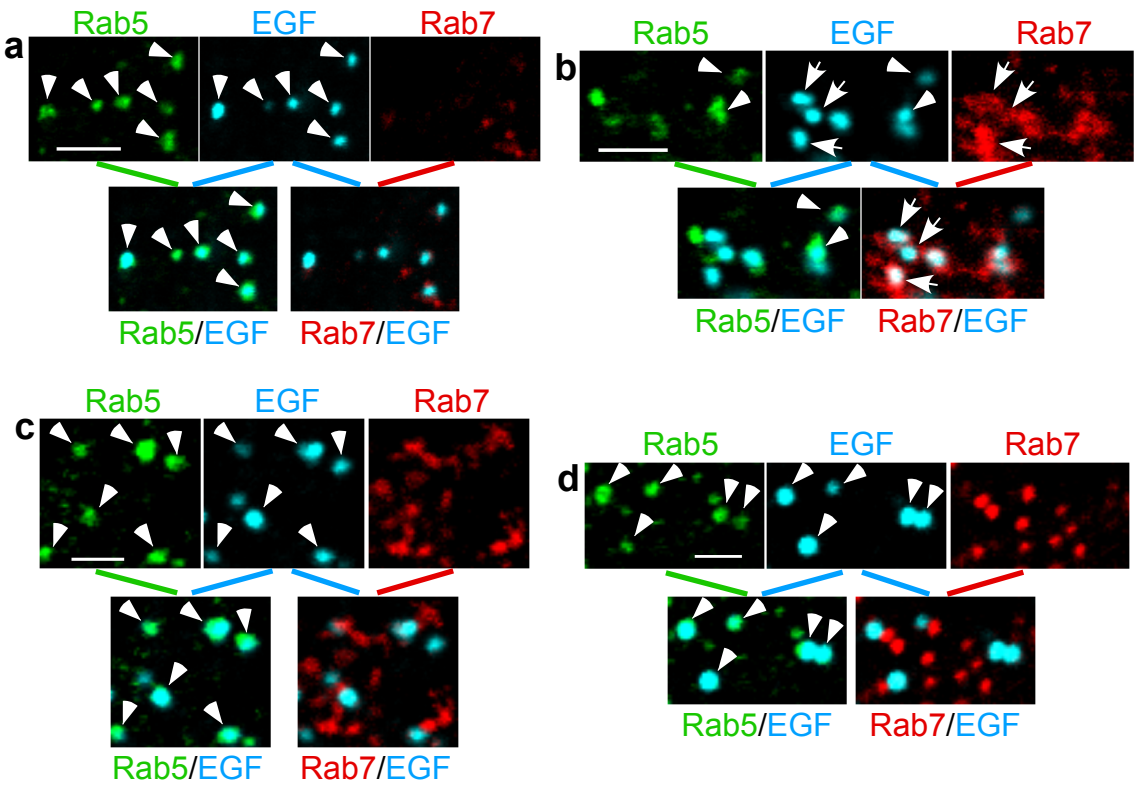
(b) Effect of the 4PA mutation on LRRK1 localization. HeLa cells were transfected with GFP-LRRK1 (wild type and the 4PA mutant). After 16 hr of serum starvation, cells were incubated with or without 50 ng/ml EGF-Rh for 45 min at 4°C, followed by warming for 15 min at 37°C, and stained with anti-EEA1 antibodies. Data are presented as the percentage of cells that have five or more EEA1-labeled vesicles that also contain GFP-LRRK1. Numbers (n) indicate the counted cells. Experiments were performed three times with similar results.



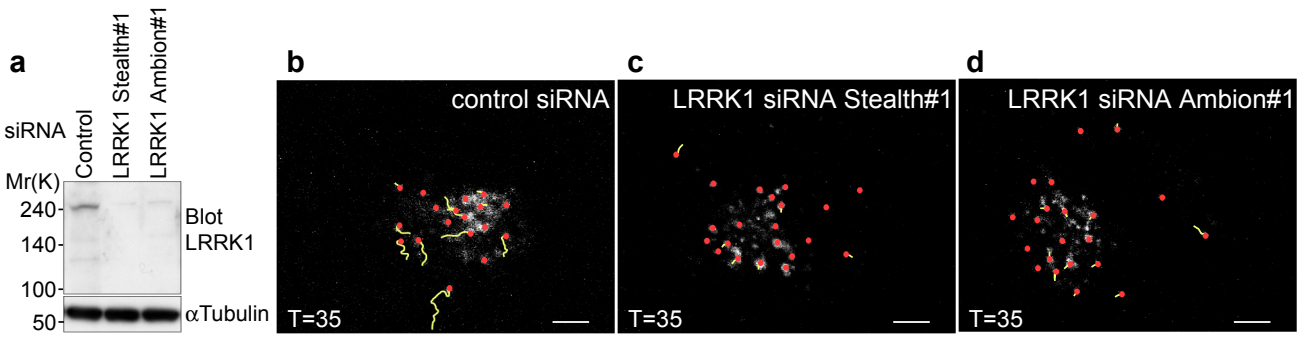
Supplementary Figure S6. Effect of LRRK1 siRNA.
(a) Quantitative real time RT-PCR analysis of LRRK1 transcript. HeLa S3 cells were treated with control or LRRK1 siRNA (Stealth#1). Levels of LRRK1 expression were normalized to those of actin expression. The values shown are average (±SD) of three independent experiments.
(b) Effect of LRRK1 siRNA. HeLa S3 cells were treated with control or LRRK1 siRNA (Stealth#1). Cell lysates were immunoblotted with anti-LRRK1 or anti-αTubulin antibodies. Asterisk indicates a cross-reacting non-specific protein.



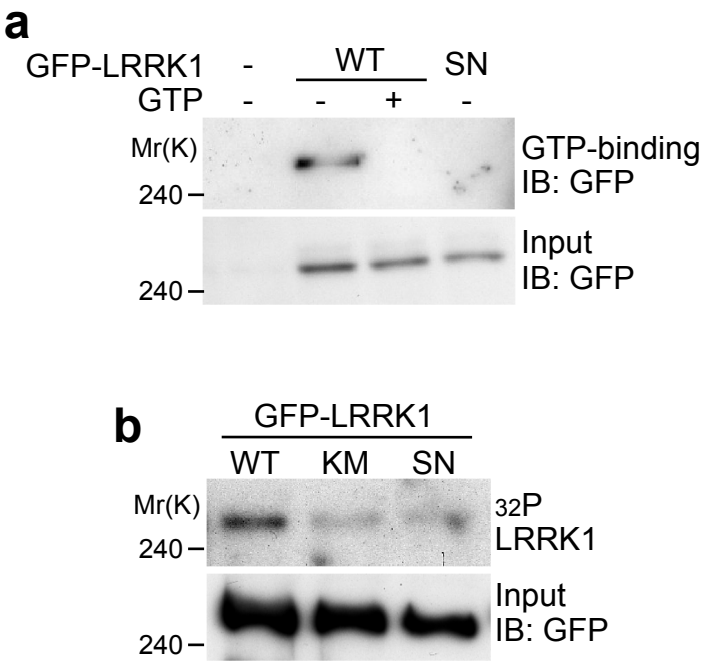
Supplementary Figure S7. Colocalization of EGF with clathrin.
HeLa S3 cells treated with control **(a)** or LRRK1 siRNA (Stealth#1) **(b)** were stimulated with 100 ng/ml Alexa 647-EGF for 5 min, and stained with anti-clathrin antibodies. The boxed regions are magnified. Examples of colocalization of Alexa 647-EGF and clathrin are indicated with arrowheads. The scale bars represent 10 μm.



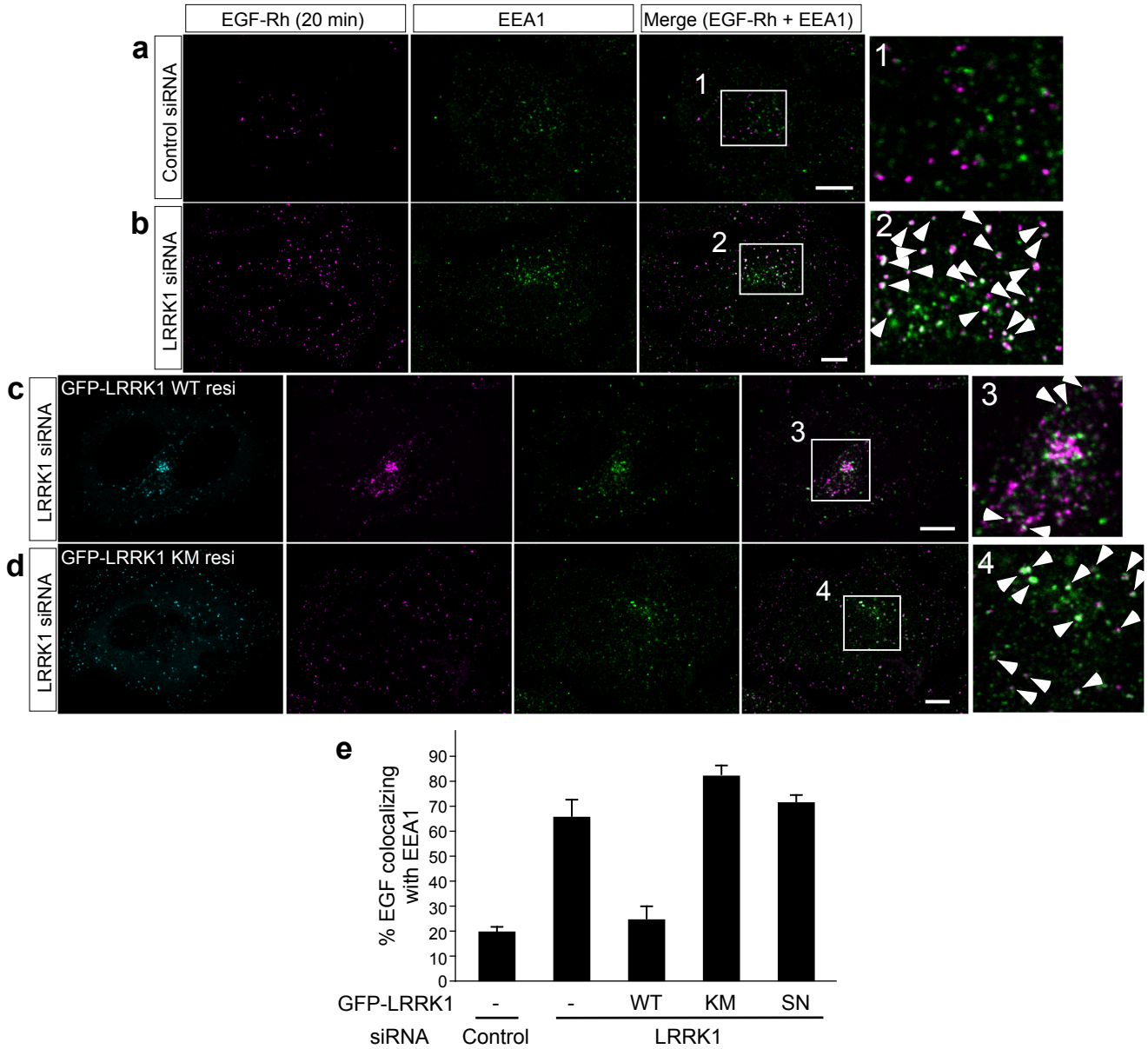
Supplementary Figure S8. Colocalization of EGF with Rab5 and Rab7. HeLa S3 cells treated with control **(a,b)** or LRRK1 siRNA (Stealth#1) **(c,d)** were cotransfected with GFP-Rab5 and DsRed-Rab7. After 16 hr of serum starvation, cells were stimulated with 100 ng/ml Alexa 647-EGF for 3 min at 37°C, followed by washing to remove labeled EGF from the medium. The cells were fixed at 10 min **(a,c)** and 15 min **(b,d)** after the initial exposure to Alexa 647-EGF and imaged by confocal microscopy. The a-d correspond to the a-d in Fig. 3. Arrowheads and arrows indicate colocalization of Alexa 647-EGF with GFP-Rab5 and DsRed-Rab7, respectively. The scale bars represent 10 μm.



Supplementary Figure S9. Depletion of LRRK1 inhibits the EGF motility.
(a) Effect of LRRK1 siRNA. HeLa cells were treated with control or LRRK1 siRNA (Stealth#1 and Ambion#1). Cell lysates were immunoblotted with anti-LRRK1 or anti-αTubulin antibodies.
(b-d) Effect of LRRK1 depletion on the motility of EGF in HeLa cells. HeLa cells were treated with control **(b)**, LRRK1 Stealth#1 **(c)** or LRRK1 Ambion#1 **(d)**. After 16 hr of serum starvation, cells were stimulated with 100 ng/ml Alexa 647-EGF for 3 min at 37 °C, followed by washing to remove labeled EGF from the medium. Movement of Alexa 647-EGF was observed and analyzed. Imaging started at 15 min after the initial exposure to Alexa 647-EGF, with frames captured at 1.165 sec intervals for 35 sec. The scale bars represent 10 μm.



Supplementary Figure S10. GTP-binding and kinase activities of LRRK1.
(a) GTP-binding activity of LRRK1. COS7 cells were transfected with an empty vector, GFP-LRRK1 (WT) or GFP-LRRK1(S625N) (SN), as indicated. Using GTP-sepharose, GFP-LRRK1 or GFP-LRRK1(S625N) was affinity-purified from cytosolic extracts of transfected COS7 cells in the presence or absence of 2 mM GTP (upper panel). Equal protein input was confirmed by immunoblotting with anti-GFP antibodies (lower panel). **(b)** Kinase activity of LRRK1. COS7 cells were transfected with GFP-LRRK1, GFP-LRRK1(K1243M) (KM) or GFP-LRRK1(S625N), as indicated. Immunoprecipitated samples were incubated with [γ-^{32}P] ATP for 20 min at 30°C. Autophosphorylated LRRK1 was resolved by SDS-polyacrylamide gel electrophoresis and visualized by autoradiography.

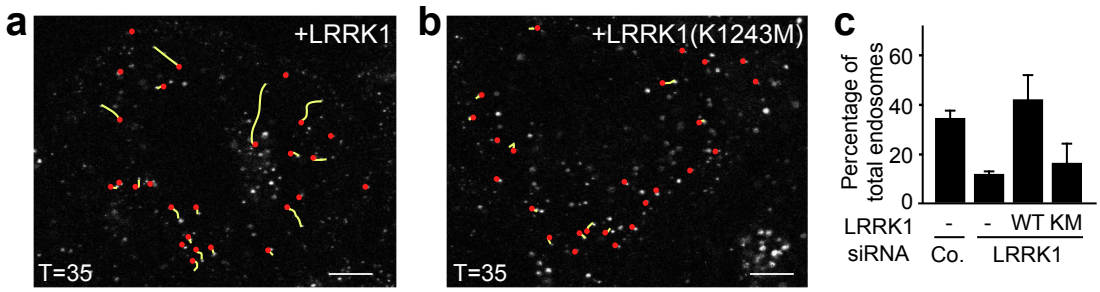


Supplementary Figure S11. Rescue of the defect in EGFR progression from early endosomes caused by LRRK1 depletion.

(a,b) HeLa S3 cells were treated with control **(a)** or LRRK1 siRNA (Stealth#1) **(b)**. After 16 hr of serum starvation, cells were stimulated with 50 ng/ml EGF-Rh for 3 min at 37°C, followed by washing to remove labeled EGF from the medium. The cells were fixed at 20 min after the initial exposure to EGF-Rh, stained with anti-EEA1 antibodies, and imaged by confocal microscopy. The boxed regions are magnified. Arrowheads indicate colocalization of EGF-Rh with EEA1. The scale bars represent 10 μm.

(c,d) HeLa S3 cells treated with LRRK1 siRNA (Stealth#1) were transfected with siRNA-resistant GFP-LRRK1 **(c)** or siRNA-resistant GFP-LRRK1(K1243M) **(d)**. After 16 hr of serum starvation,cells were stimulated with 50 ng/ml EGF-Rh for 3 min at 37°C, followed by washing to remove labeled EGF from the medium. The cells were fixed at 20 min after the initial exposure to EGF-Rh, stained with anti-EEA1 antibodies, and imaged by confocal microscopy. The boxed regions are magnified. Arrowheads indicate colocalization of EGF-Rh with EEA1. The scale bars represent 10 μm.

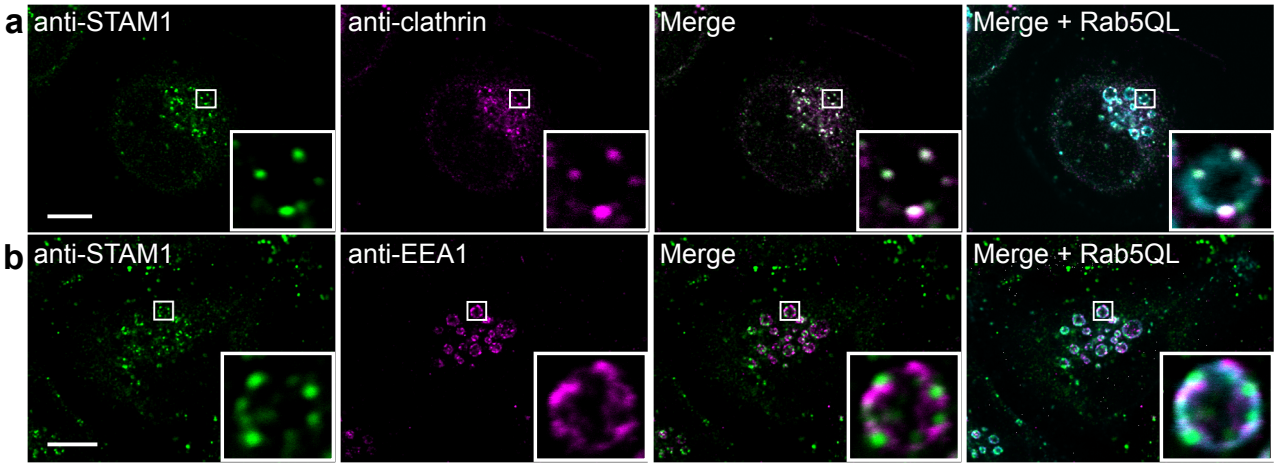
(e) Quantification of EGFR dissociated from early endosomes. Data are presented as percentages of EEA1-labeled EGF-Rh-containing vesicles out of total number of EGF-Rh-containing vesicles per cell. Values reflect the mean standard deviation of three independent experiments, with an average of 15 cells scored per experiment.



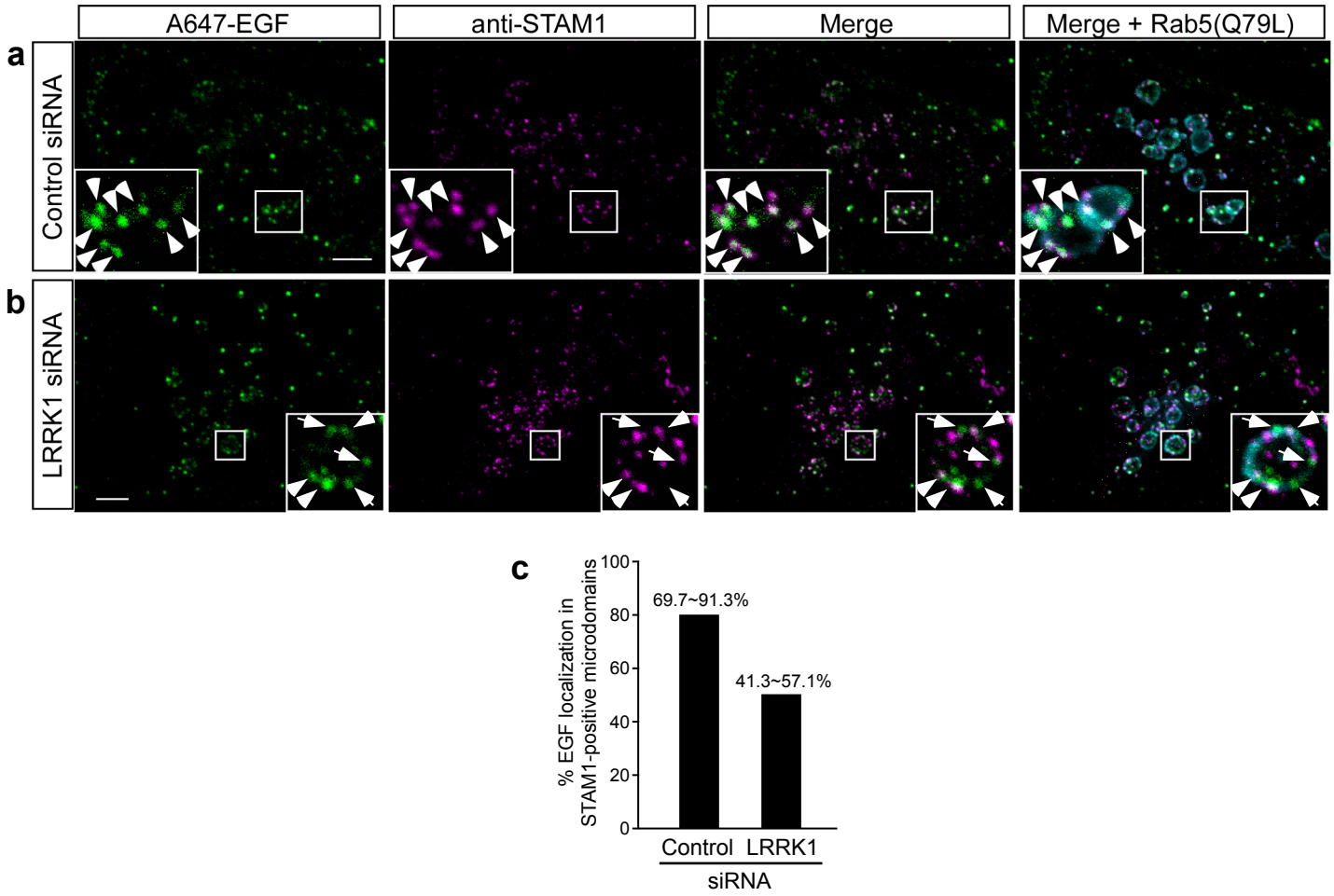
Supplementary Figure S12. Rescue of the defect in EGF motility caused by LRRK1 depletion.

(a,b) HeLa S3 cells treated with LRRK1 siRNA (Stealth#1) were transfected with siRNA-resistant GFP-LRRK1 **(a)** or siRNA-resistant GFP-LRRK1(K1243M) **(b)**. After 16 hr of serum starvation, cells were stimulated with 100 ng/ml Alexa 647-EGF for 3 min at 37°C, followed by washing to remove labeled EGF from the medium. Movement of Alexa 647-EGF was observed and analyzed. Imaging started at 15 min after the initial exposure to Alexa 647-EGF, with frames captured at 1.165 sec intervals for 35 sec.The scale bars represent 10 μm.

(c) Quantification of the EGF motility. The movement of EGF (>1 μm) during the 35 sec observation period was quantified using the 'Manual Tracking' Image J plug-in. Values reflect the mean standard deviation of three independent experiments, with an average of 25 EGF-containing endosomes scored per experiment.



Supplementary Figure S13. Localization of STAM1 on microdomains of early endosomes.
HeLa S3 cells were transfected with GFP-Rab5(Q79L), and stained with anti-STAM1 and anti-clathrin antibodies **(a)** or anti-EEA1 antibodies **(b)**, respectively. The boxed regions indicate the Rab5(Q79L)-induced enlarged endosomes and are magnified in the inset. White color in the merged images indicates colocalization. Experiments were performed three times with similar results. The scale bars represent 10 μm.



Supplementary Figure S14. Effect of LRRK1 depletion on EGF localization to STAM-positive microdomains of early endosomes.
(a,b) HeLa S3 cells treated with control **(a)** or LRRK1 siRNA (Stealth#1) **(b)** were transfected with GFP-Rab5(Q79L), and stimulated with 100ng/ml Alexa 647-EGF for 10 min. The cells were fixed, stained with anti-STAM1 antibodies, and imaged by confocal microscopy. Images are three-dimensional reconstructions from a series of confocal Z-stack images (0.3 μm-thick sections). The boxed regions are magnified in insets. Arrowheads and arrows indicate Alexa 647-EGF localization in STAM1-positive and -negative microdomains, respectively. The scale bars represent 5 μm.
(c) Quantification of EGF localization to STAM-positive microdomains of early endosomes. Data show the percentages of STAM1-labeled EGF microdomains per endosomes. Experiments were performed two times with an average of 15 cells (total 80 endosomes) scored per experiment.

Protein ID	Detection frequency[a]	Protein name
Grb2_HUMAN	3	Growth factor receptor-bound protein 2
CC37_HUMAN	3	Hsp90 co-chaperone Cdc37 (p50Cdc37)
IEFS_HUMAN	3	Stress-induced-phosphoprotein (STI1)
NM_024604_FLJ21908	3	hypothetical protein FLJ21908
NM_014412_SIP	3	calcyclin binding protein
NM_006704_SUGT1	3	suppressor of G2 allele of SKP1
NM_006600_NUDC	3	nuclear distribution gene C (A.nidulans) homolog
SOS1_HUMAN	2	Son of sevenless protein homolog 1 (SOS1)
FKB5_HUMAN	2	51 kDa FK506-binding protein (FKBP51)
NM_003707_RUVBL1	2	TATA binding protein interacting protein 49 kDa (RuvB-like 1)
REST_HUMAN	1	Cytoplasmic linker protein-170 alpha-2 (CLIP-170)

The same analysis was performed three times. The bait protein LRRK1 was excluded.
[a]Number indicates times the protein was detected among three trials.

Supplementary Table S1. Identification of LRRK1-interacting proteins by LC-MS/MS.