## Supplementary Figures

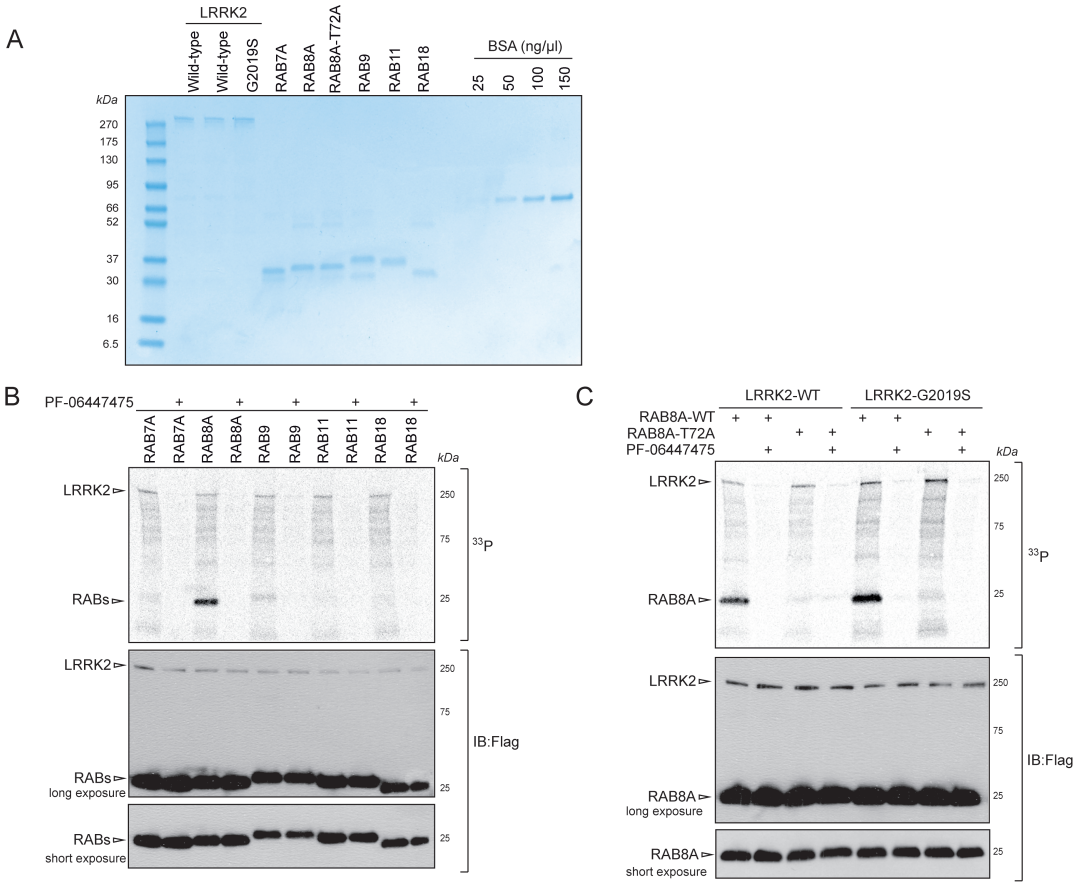


**Fig. S1.** LRRK2 phosphorylates RAB8A but not endolysosomal RAB7A/RAB9 proteins. (**A**) Representative Coomassie blue stained SDS-PAGE of purified 3x-flag-tagged LRRK2 or 3x-flag-tagged RAB proteins after 3xflag peptide elution. Protein concentration was estimated by a calibration curve with BSA standards of known concentrations. (**B**) *In vitro* radioactive kinase assays of 3x-flag-tagged wildtype LRRK2 and 3x-flag-tagged RAB7A, RAB8A, RAB9, RAB11 or RAB18, purified from

HEK293T cells (1:10 ratio of LRRK2:Rab proteins). Incorporated radioactivity was revealed by phosphoscreen (upper panel), and total protein loading by anti-flag (IB:Flag) immunoblotting (lower panel). LRRK2 kinase inhibitor PF 06447475 was used at 1 μM to confirm LRRK2-specific phosphorylation. (**C**) *In vitro* radioactive kinase assays of 3x-flag-tagged wildtype or G2019S LRRK2 in the presence of either 3x-flag-tagged wildtype RAB8A or phosphodeficient RAB8A-T72A as indicated. Incorporated radioactivity was revealed by phosphoscreen (upper panel), and total protein loading by anti-flag (IB:Flag) immunoblotting (lower panel). LRRK2 kinase inhibitor PF 06447475 was used at 1 μM to confirm LRRK2-specific phosphorylation.

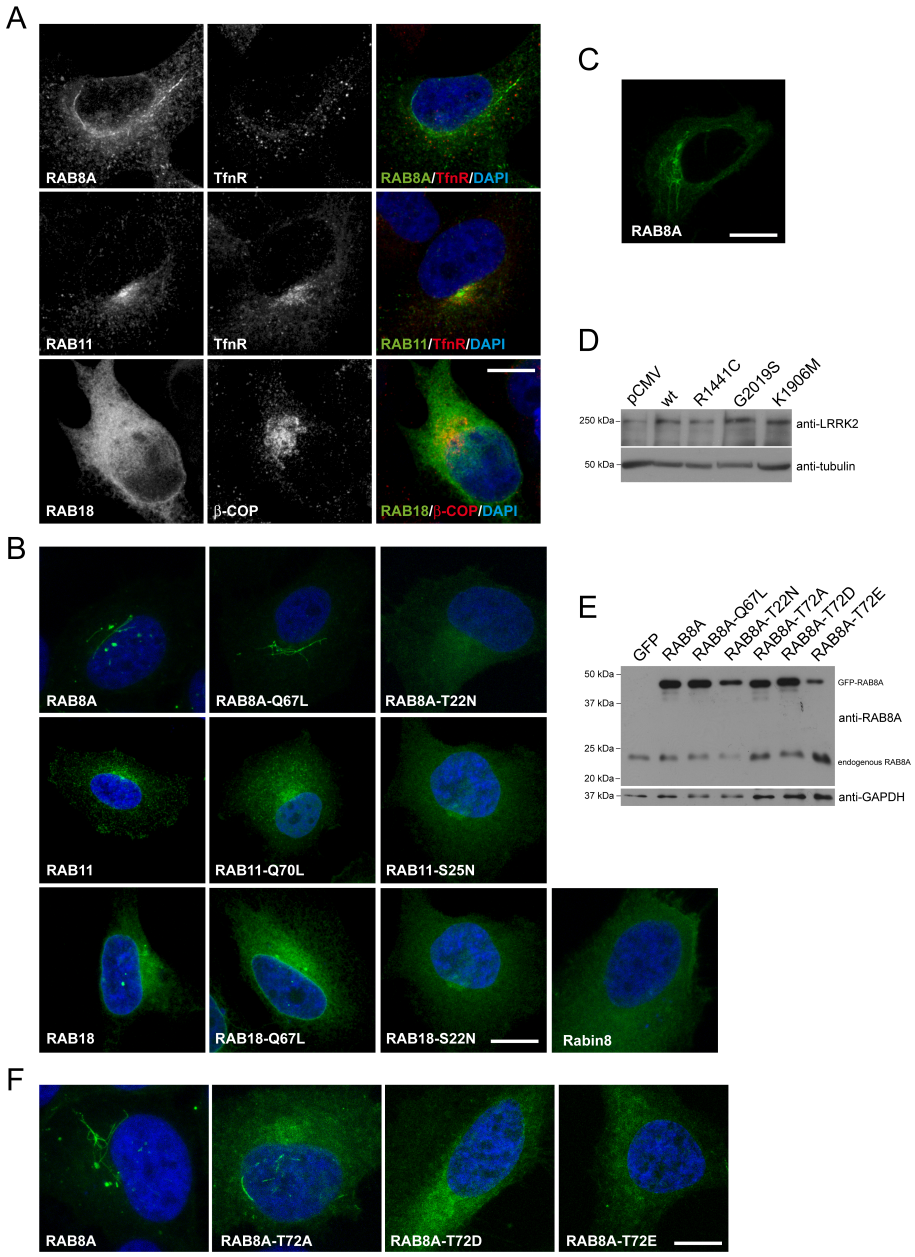


**Fig. S2.** Subcellular localization and expression levels of different RAB proteins. (**A**) HeLa cells were transfected with GFP-tagged RAB constructs as indicated, and stained with antibodies against a marker for endolysosomes (LAMP1), endocytic recycling compartment (TfnR) or trans-Golgi (β-COP). Scale bar, 10 μm. (**B**) HeLa cells were transfected with GFP-tagged RAB constructs as indicated. To maintain proper membrane

association of the different RAB proteins, cells were fixed but only briefly permeabilized before mounting with DAPI. Scale bar, 10 μm. (**C**) Individual z-stack of live HeLa cell transfected with GFP-RAB8A. Scale bar, 10 μm. (**D**) HeLa cells were transfected with either empty vector (pCMV) or the indicated GFP-tagged LRRK2 constructs, and cell extracts (30 μg) analyzed by Western blotting for LRRK2 protein levels, and for tubulin as loading control. (**E**) HeLa cells were transfected with either GFP, or GFP-tagged RAB8A constructs as indicated, and cell extracts (30 μg) analyzed by Western blotting for RAB8A, and for GAPDH as loading control. (**F**) Example of HeLa cells transfected with the indicated GFP-tagged RAB8A variants, fixed and mounted with DAPI (blue). Scale bar, 10 μm.

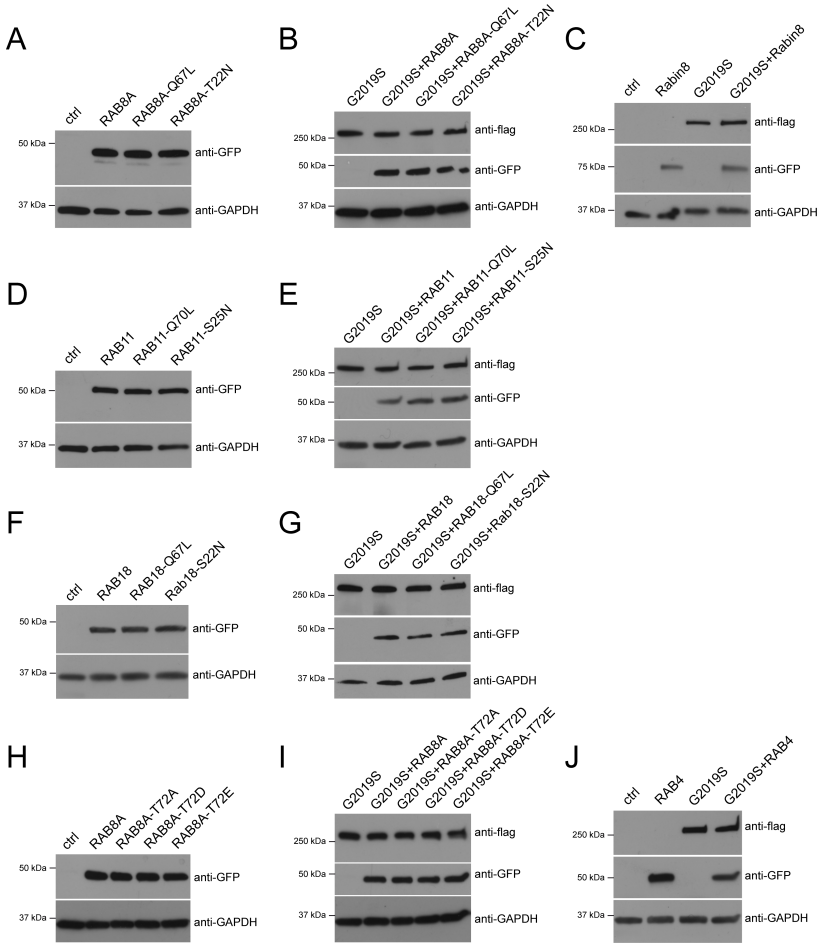


**Fig. S3.** Expression and co-expression levels of various RAB proteins with G2019S LRRK2. (**A**) HeLa cells were transfected with GFP-tagged RAB8A constructs as indicated, and cell extracts (30 μg) analyzed by Western blotting for GFP, and for GAPDH as loading control. (**B**) HeLa cells were transfected with flag-G2019S along with the indicated GFP-tagged RAB8A constructs as indicated, and cell extracts (30 μg) analyzed by Western blotting for flag, GFP, and for GAPDH as loading control. (**C**) HeLa cells were transfected with GFP-tagged Rabin8, flag-G2019S, or co-transfected as indicated, and cell extracts (30 μg) analyzed by Western blotting for flag, GFP, and for GAPDH as loading control. (**D**) Same as (A), but cells transfected with GFP-tagged

RAB11 constructs as indicated. (**E**) Same as (B), but cells co-transfected with flag-G2019S and the GFP-tagged RAB11 constructs as indicated. (**F**) Same as (A), but cells transfected with GFP-tagged RAB18 constructs as indicated. (**G**) Same as (B), but cells co-transfected with flag-G2019S and GFP-tagged RAB18 constructs as indicated. (**H**) Same as (A), but cells transfected with GFP-tagged RAB8A constructs as indicated. (**I**) Same as (B), but cells co-transfected with flag-G2019S and GFP-tagged RAB8A constructs as indicated. (**J**) Cells were transfected with GFP-tagged RAB4, flag-G2019S, or co-transfected as indicated, and cell extracts (30 μg) analyzed by Western blotting for flag, GFP, and for GAPDH as loading control.

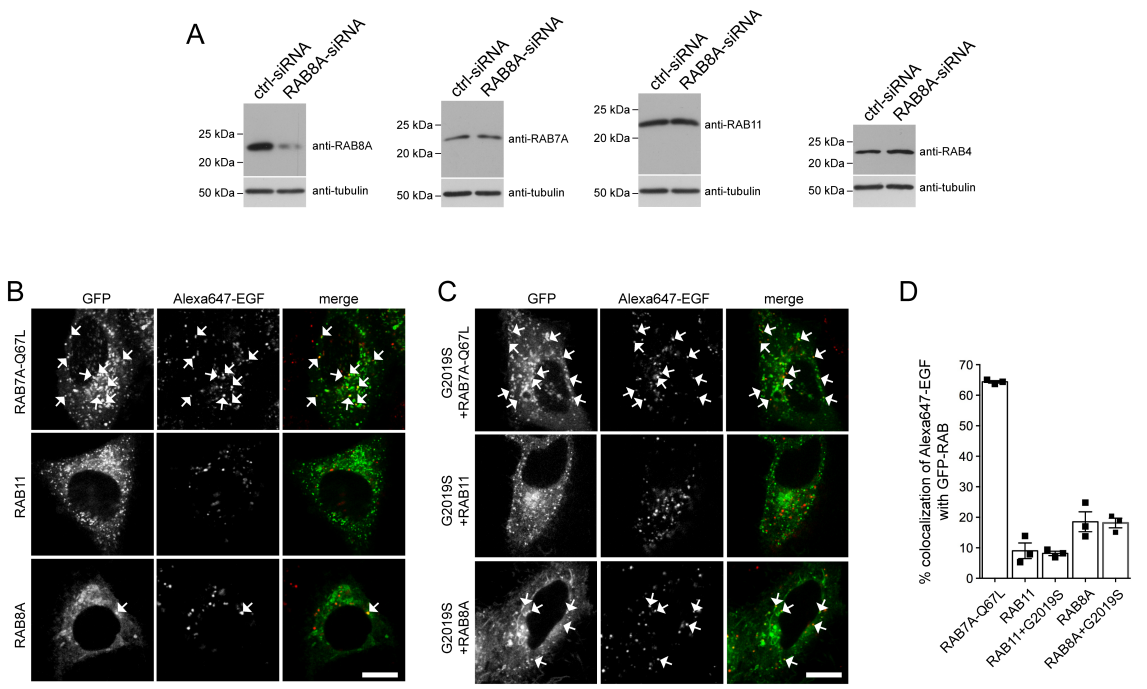


**Fig. S4.** Specificity of RAB8A knockdown, and lack of Alexa647-EGF accumulation in RAB8A- or RAB11-positive compartments. (**A**) HeLa cells were treated with ctrl-siRNA or RAB8A-siRNA, and extracts subjected to Western blotting against various RAB proteins as indicated. (**B**) Cells were transfected with GFP-tagged RAB proteins as indicated, loaded with Alexa647-EGF for 20 min and followed by live imaging. Arrows indicate colocalization. Scale bar, 10 μm. (**C**) Cells were transfected with GFP-tagged RAB proteins and G2019S LRRK2 as indicated, loaded with Alexa647-EGF for 20 min followed by live imaging. Arrows indicate colocalization. Scale bar, 10 μm. (**D**) Quantification of colocalization of Alexa647-EGF with GFP-tagged RAB proteins in the absence or presence of G2019S LRRK2 as indicated (Manders coefficient 1 x 100). N=3 independent experiments.

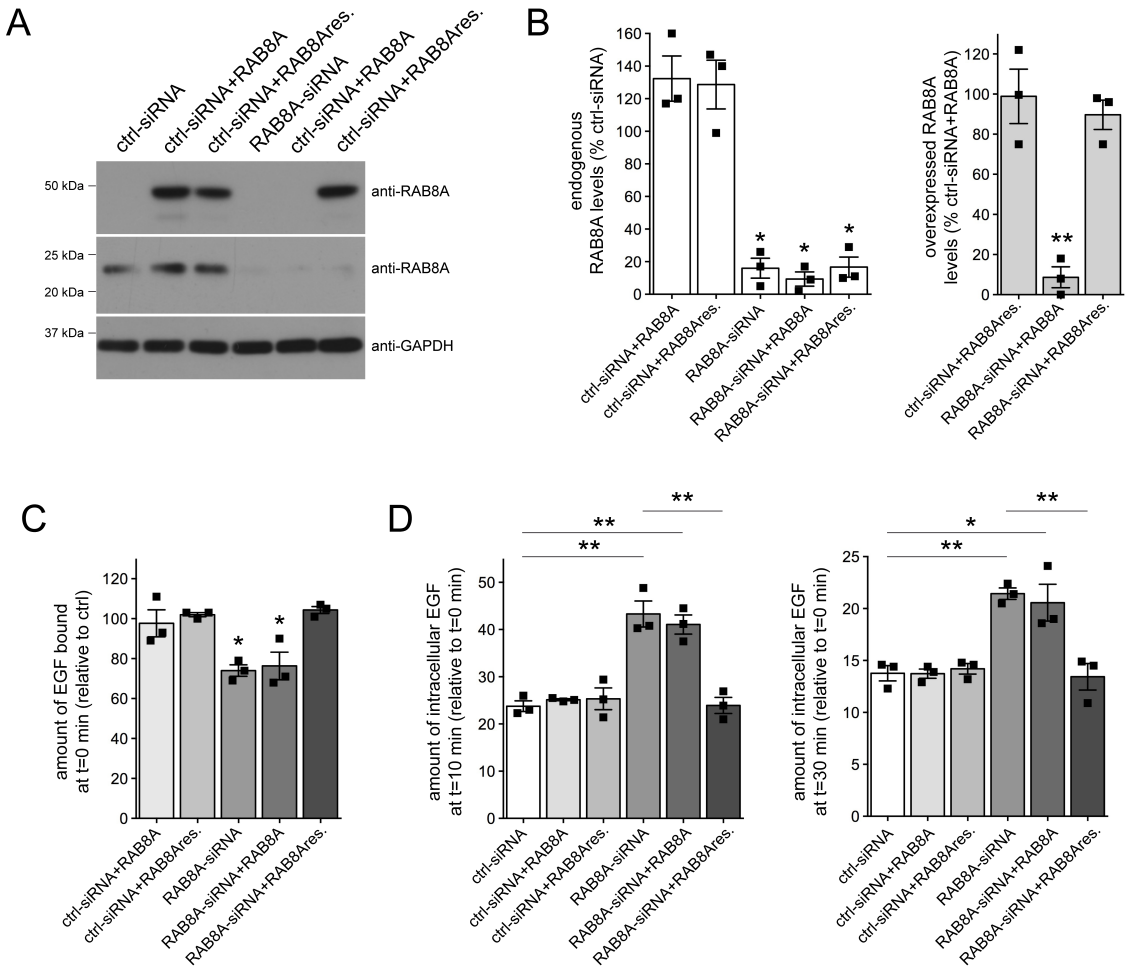


**Fig. S5.** siRNA-resistant RAB8A variant rescues the G2019S LRRK2-mediated endolysosomal trafficking deficits. (**A**) HeLa cells were either transfected with ctrl-siRNA or RAB8A-siRNA, and either with wildtype mRFP-RAB8A (RAB8A) or an siRNA-resistant version of mRFP-RAB8A (RAB8res.), and cell extracts (30 μg) analyzed by Western blotting for mRFP-RAB8A levels, endogenous RAB8A levels and GAPDH as loading control. (**B**) Quantification of the type of experiments depicted in (A). Endogenous RAB8A levels (left) were normalized to levels in the presence of ctrl-siRNA, and overexpressed mRFP-RAB8A levels (right) normalized to levels in the presence of ctrl-siRNA and wildtype RAB8A (RAB8A). N=3 independent experiments.

*P < 0.05; **P < 0.01. (**C**) Cells were either transfected with ctrl-siRNA or RAB8A-siRNA in the presence or absence of wildtype or siRNA-resistant mRFP-RAB8A as indicated, and surface-bound fluorescent Alexa488-EGF quantified. N=3 independent experiments. *P < 0.05. (**D**) Cells were either transfected with ctrl-siRNA or RAB8A-siRNA in the presence or absence of wildtype or siRNA-resistant mRFP-RAB8A as indicated, followed by quantification of internalized fluorescent Alexa488-EGF at 10 min (left) and 30 min (right). N=3 independent experiments. * P < 0.05; ** P < 0.01.