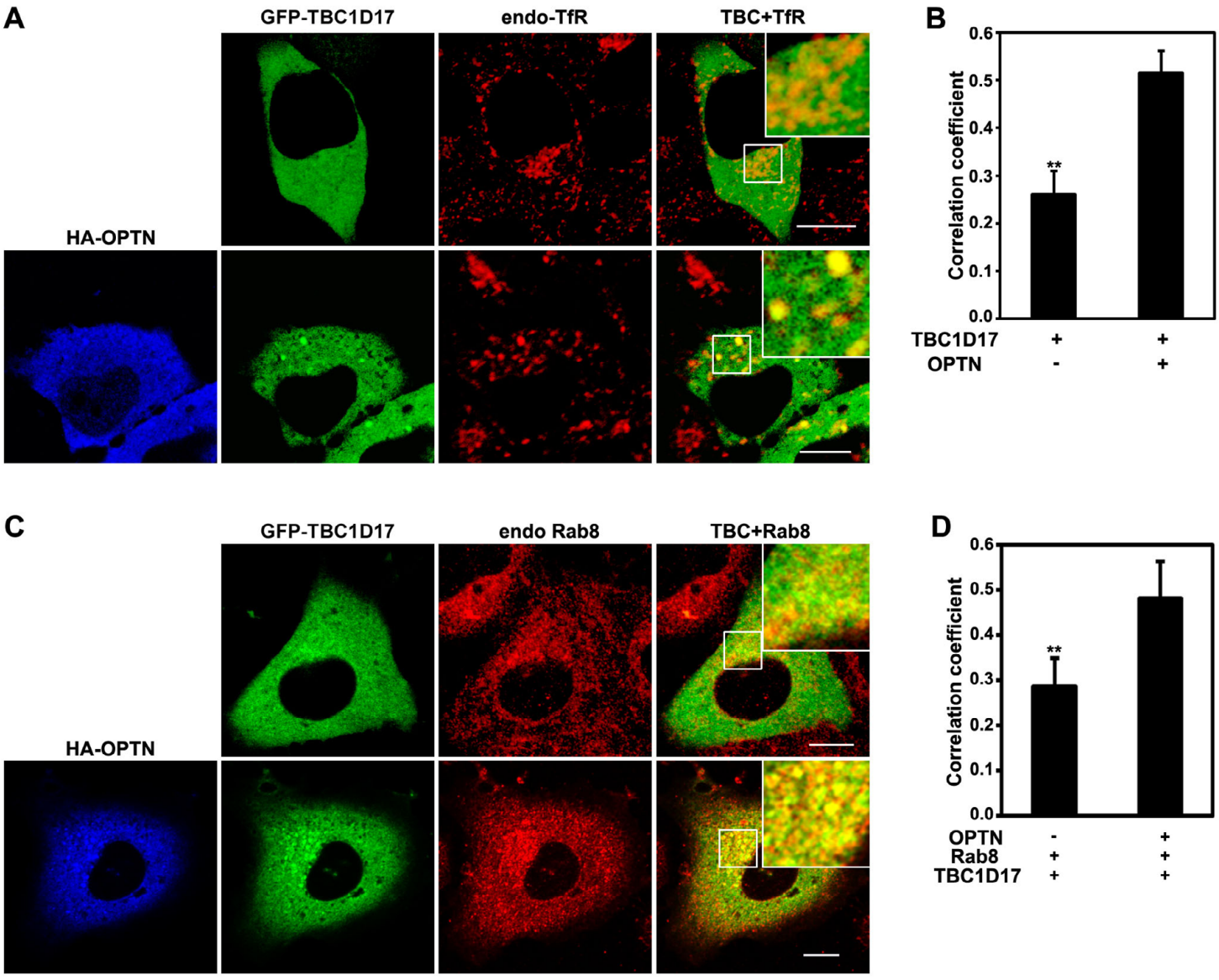
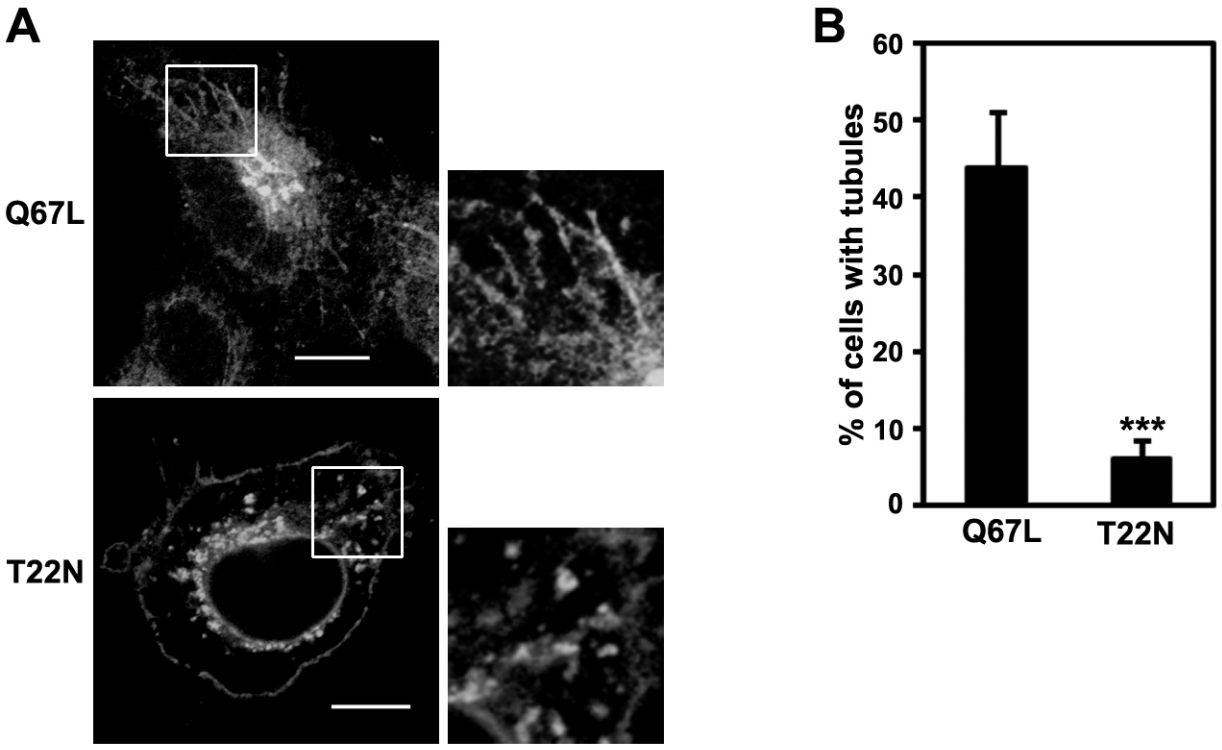
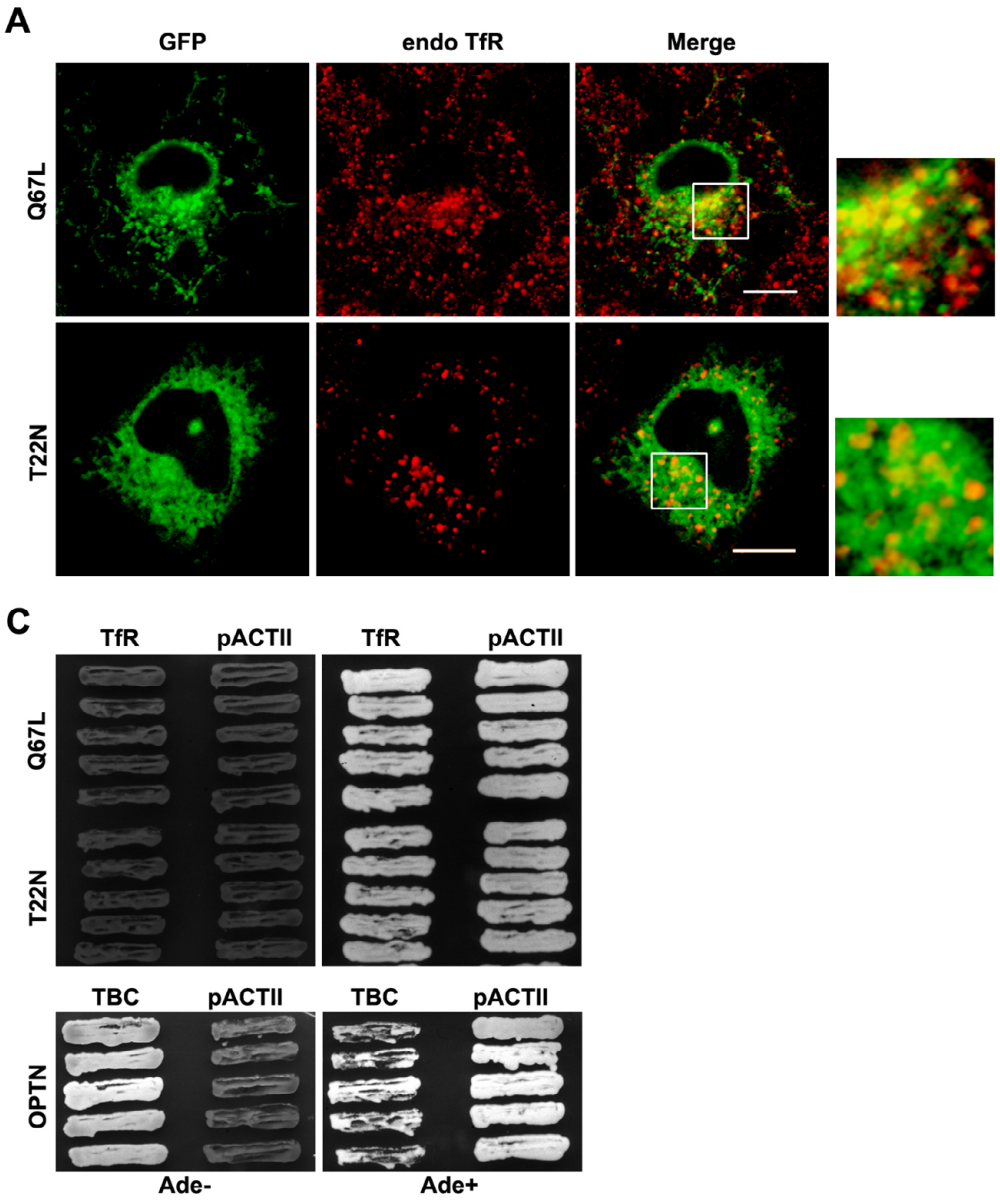


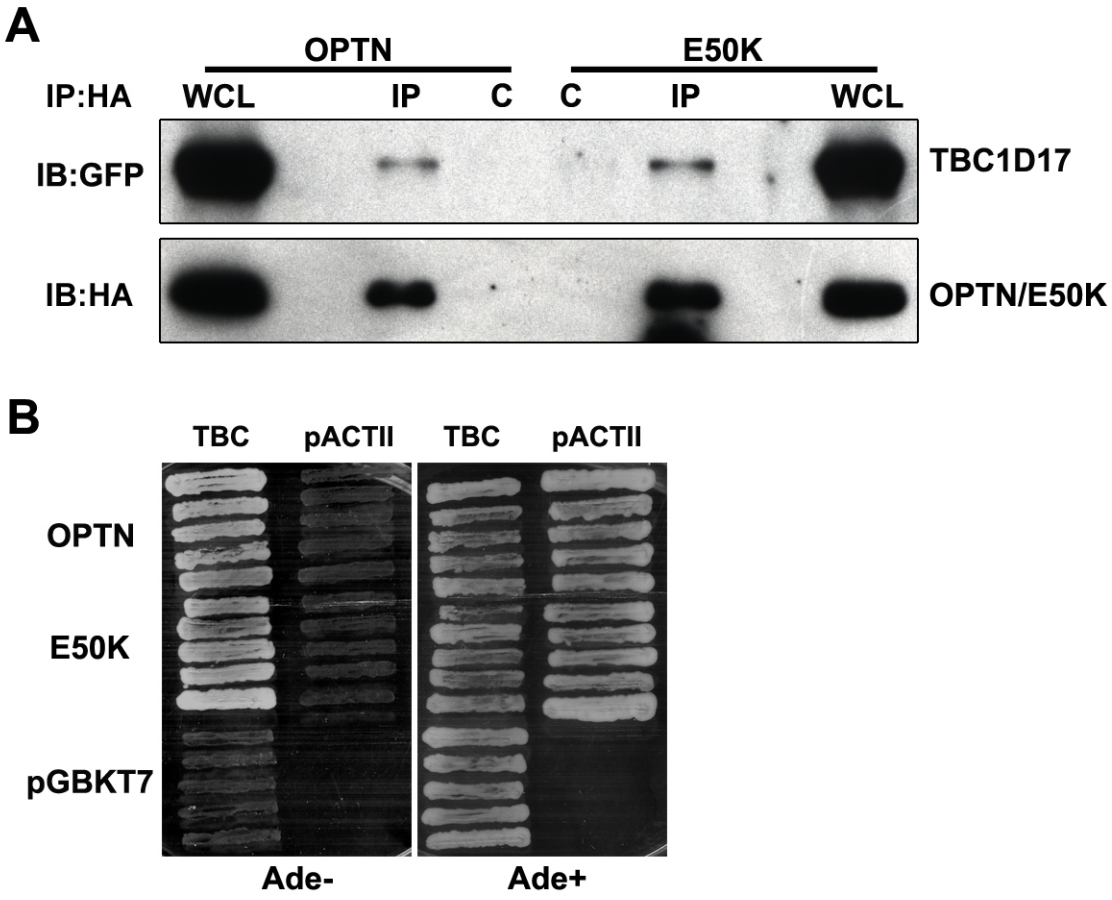
Supplementary figure S1. Optineurin enhances colocalisation of TBC1D17 with TfR and Rab8. A. Hela cells grown on coverslips were transfected with GFP-tagged TBC1D17 alone or alongwith HA-tagged optineurin. After 24 h, these cells were stained with anti-TfR and anti-HA antibodies and observed by confocal microscopy. Scale bar, 10μm. B. The graph shows correlation coefficient of colocalisation between TBC1D17 and TfR in absence and presence of optineurin. C. Hela cells grown on coverslips were transfected with GFP-TBC1D17 alone or alongwith HA-optineurin. After 24 h, these cells were stained with anti-Rab8 and anti-HA antibodies and observed by confocal microscopy. Scale bar, 10μm. D. The graph shows correlation coefficient of colocalisation between TBC1D17 and endogenous Rab8 in absence and presence of optineurin. **P<0.01.



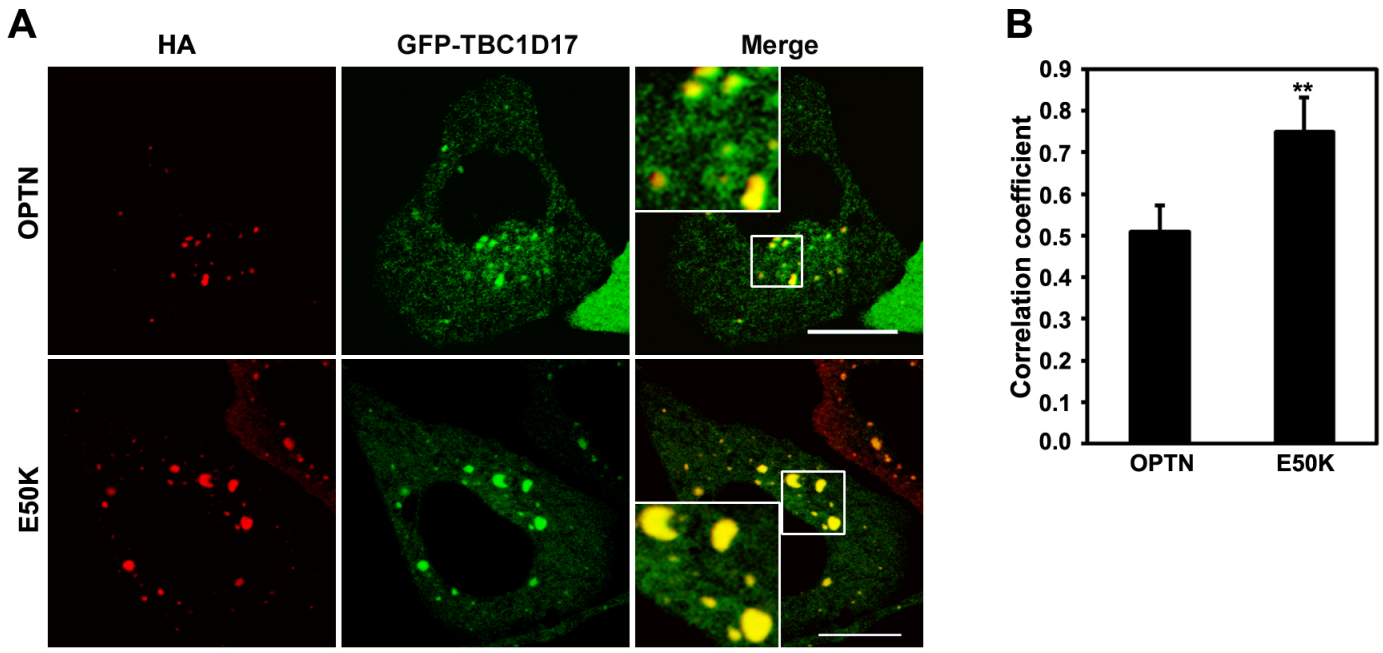
Supplementary figure S2. The activated form of Rab8 (Q67L) is selectively recruited to the tubules. A. Hela cells grown on coverslips were transfected with GFP-Q67L-Rab8 or GFP-T22N-Rab8. After 24 h of transfection, these cells were fixed and observed by confocal microscopy. Scale bar, 10μm. B. The graph shows percentage of cells with Rab8-positive tubules in Q67L and T22N transfected cells. ***P<0.001.



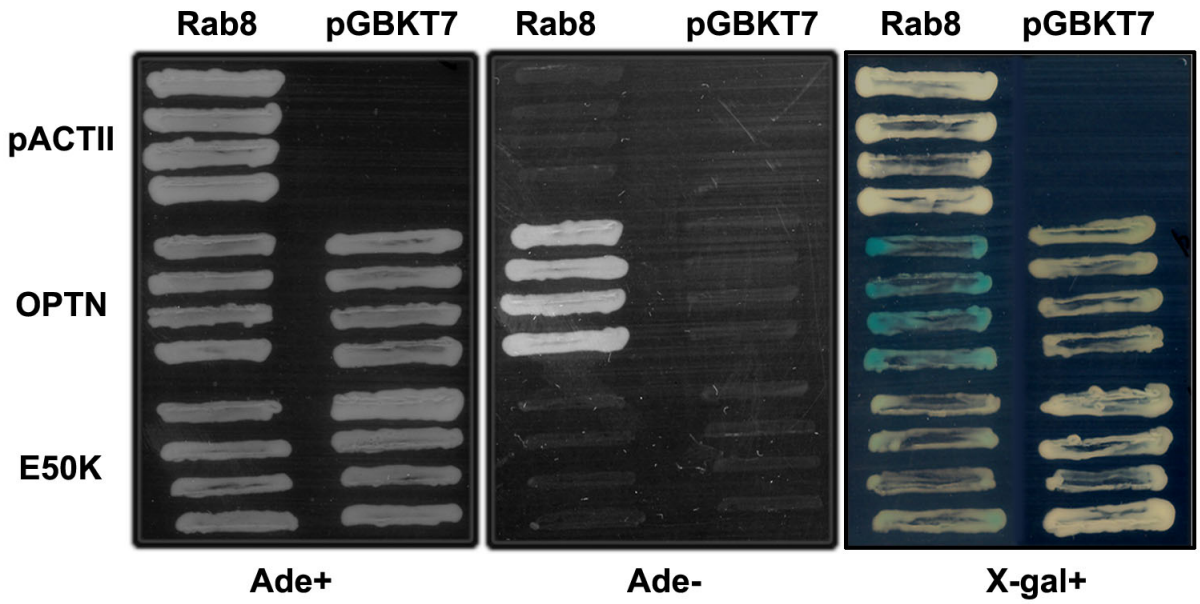
Supplementary figure S3. Colocalization of Rab8 mutants with TfR. A. Hela cells grown on coverslips were transfected with GFP-Q67L or GFP-T22N. After 24 h, cells were stained with anti-TfR antibody and observed by confocal microscopy. Scale bar, 10μm. B. The graph shows correlation coefficient of colocalisation between Q67L or T22N and TfR. **P<0.01. C. Interaction of Rab8 mutants (cloned in pGBKT7 vector) with the cytoplasmic domain of TfR (cloned in pACT2 vector) in yeast two hybrid assay. Transformants were plated on medium without (Ade-) or with (Ade+) adenine. Growth on Ade- plate indicates interaction. As a positive control interaction of TBC1D17 with optineurin is shown.



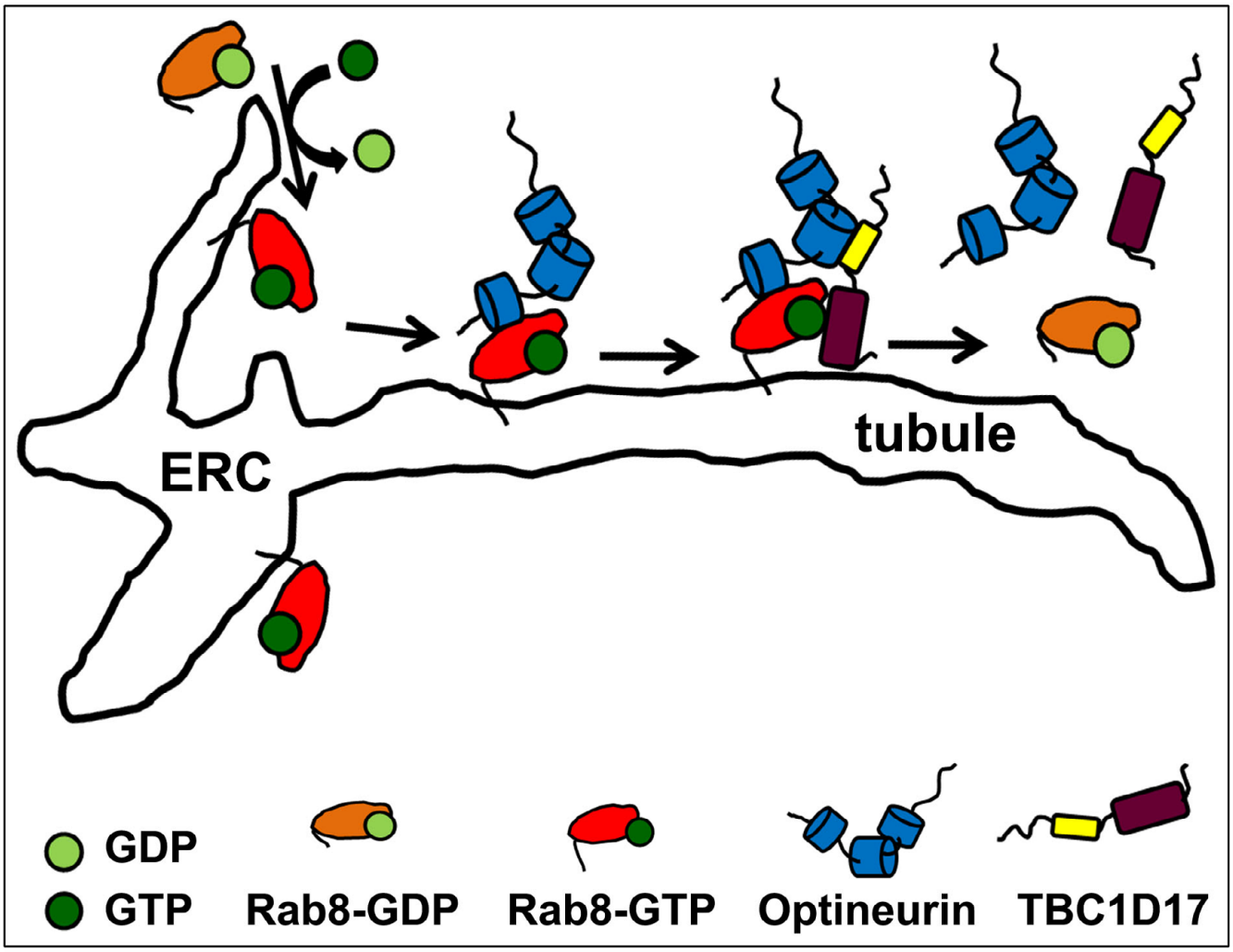
Supplementary figure S4. E50K-optineurin interacts with TBC1D17. A. Hela cells were cotransfected with plasmids expressing GFP-TBC1D17 and HA-optineurin or HA-E50K. After 24 h cell lysates were prepared and immunoprecipitation was carried out using anti-HA antibody or control antibody. The immunoprecipitates were analyzed by western blotting. B. Interaction of TBC1D17 cloned in pACTII with optineurin and its E50K mutant cloned in pGBKT7 was analysed by yeast two hybrid assay.



Supplementary figure S5. Colocalization of optineurin and its E50K mutant with TBC1D17. A. Hela cells grown on coverslips were transfected with GFP-TBC1D17 alongwith HA-optineurin or HA-E50K. After 24 h, the cells were stained with anti-HA antibody and observed by confocal microscopy. Scale bar, 10μm. B. The graph shows correlation coefficient of colocalisation between TBC1D17 and optineurin or E50K. **P<0.01.



Supplementary figure S6. E50K-optineurin does not interact directly with Rab8. Interaction of optineurin and its E50K mutant cloned in pACTII vector and Rab8, cloned in pGBKT7 vector. Transformants were grown in medium without (Ade–) or with (Ade+) adenine or on X-gal plate (X-gal+). Growth in the absence of adenine or blue colour on X-gal plate indicates the interaction between hybrid proteins.



Supplementary figure S7. A model showing the optineurin mediated regulation of Rab8 by TBC1D17. Activated form of Rab8 (Rab8-GTP) binds to optineurin on the membrane. TBC1D17 is then recruited to this complex of Rab8-GTP and optineurin. This brings TBC1D17 in close proximity to Rab8-GTP in the complex, resulting in the conversion of Rab8-GTP to Rab8-GDP. Inactive Rab8 (Rab8-GDP) then dissociates from the membrane and the molecular complex also dissociates.