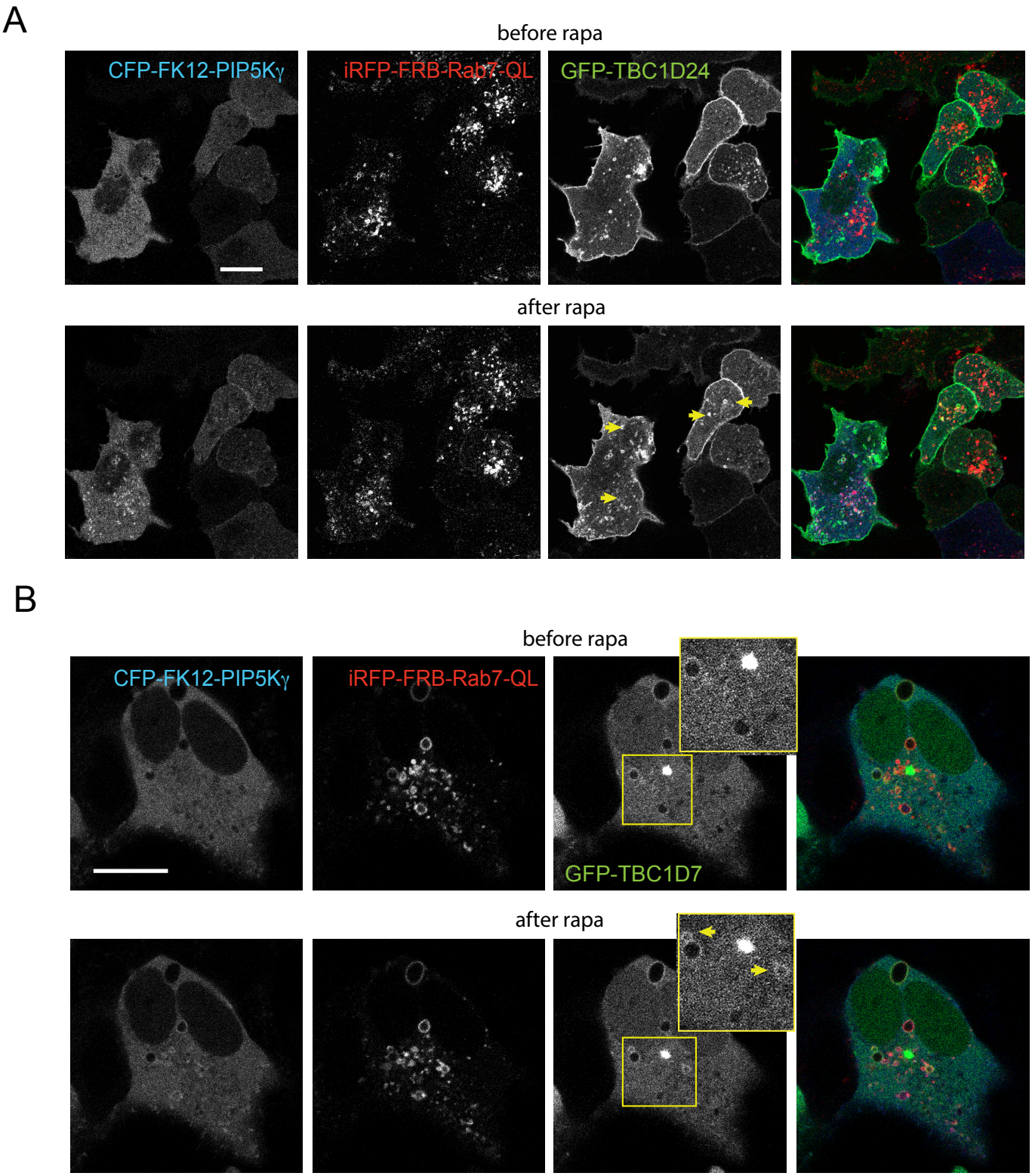
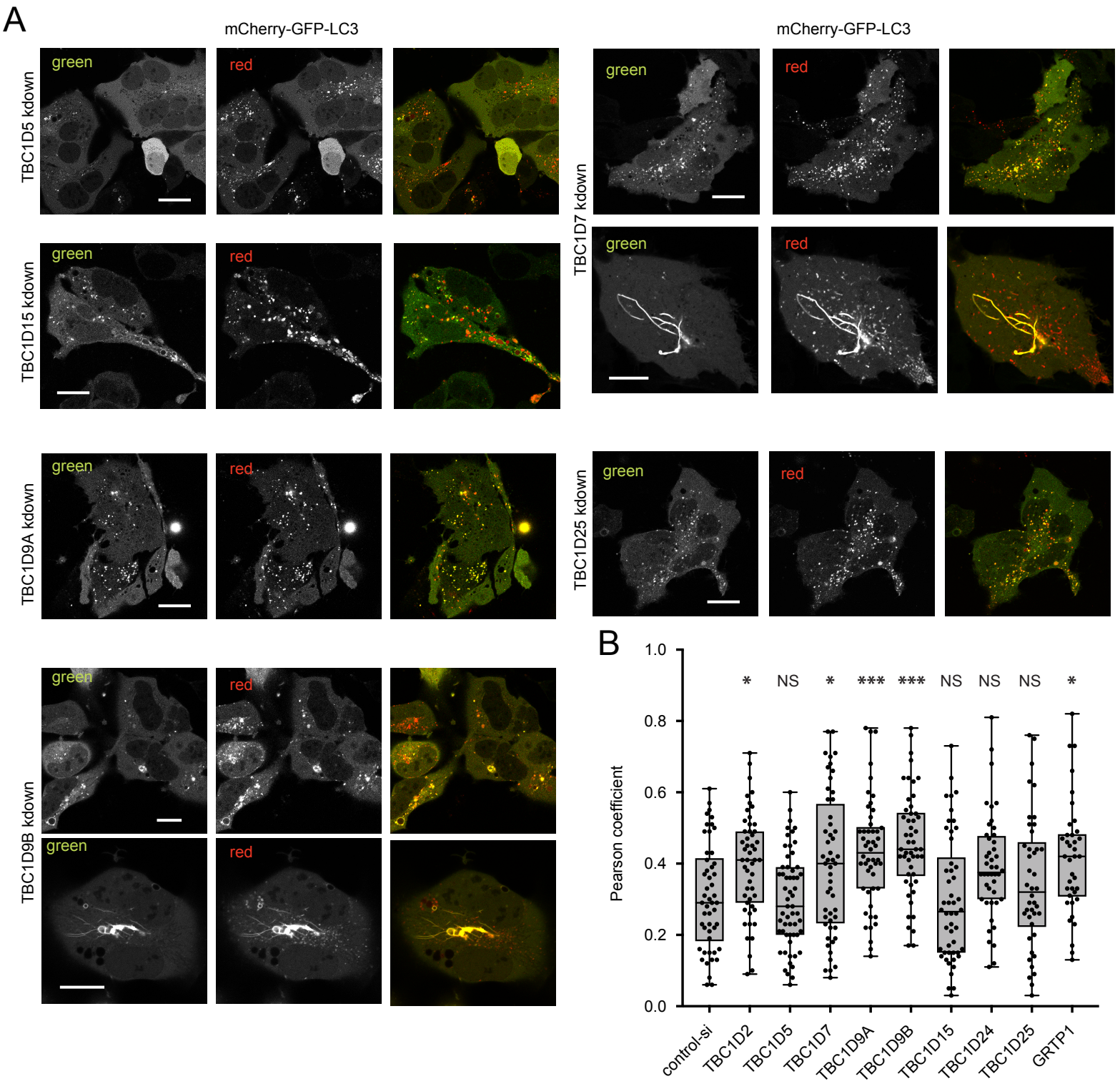
Appendix

Table of content:

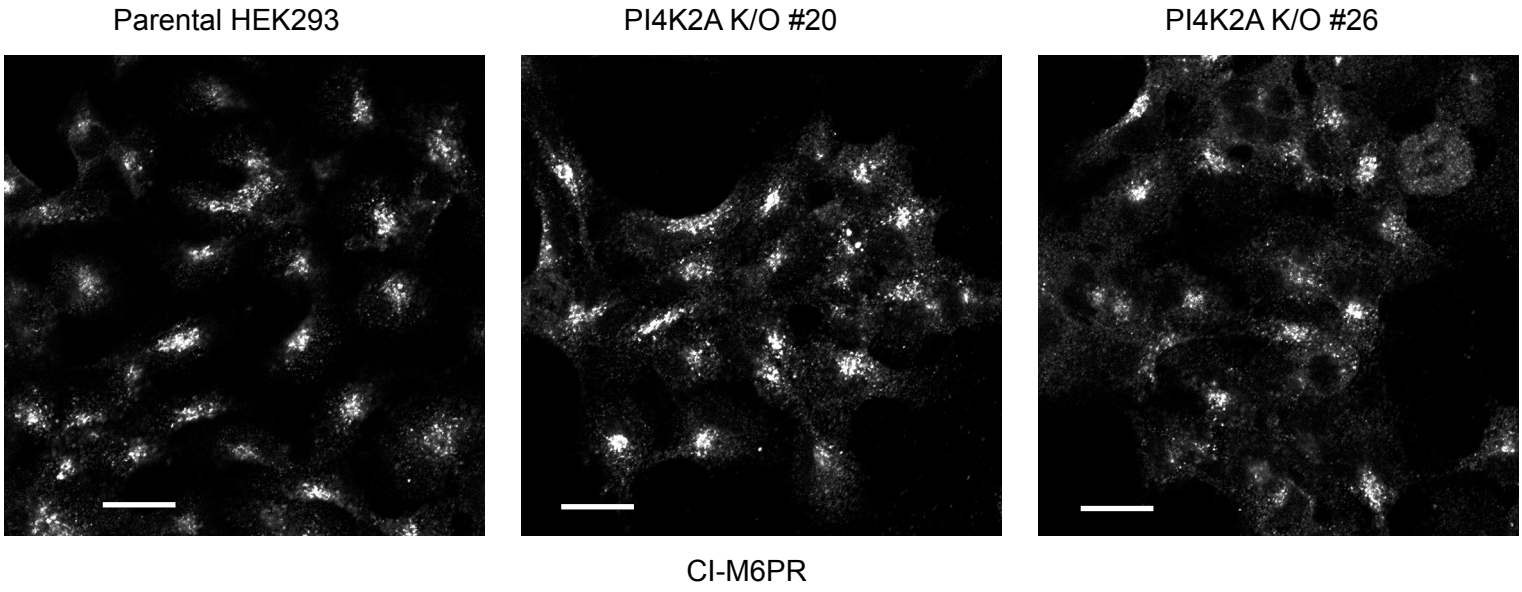


Appendix Figure S1. Association of TBC1D24 and TBC1D7 with Rab7 positive endosomes after PI(4,5)P2 generation. (A,B) HEK293-AT1 cells were transfected with CFP-FKBP12-PIP5Kγ and iRFP-Rab7-Q67L and GFP-TBC1D24 (A) or GFP-TBC1D7 (B). TBC1D24 associates with the PI(4,5)P2-rich plasma membrane but it also gets recruited to the Rab7 endosomes when PI(4,5)P2 is made in that compartment (yellow arrows). It is not clear where the TBC1D24 protein exerts its actions since the majority of the protein is not co-localized with Rab7 under control conditions. (B) PI(4,5)P2 generation causes the recruitment of a small fraction of TBC1D7 (yellow arrows in the zoomed areas). Scale bar: 20 μm.



Appendix Figure S2. Effect of TBC1D protein knock-down on the acidification of LC3 positive structures. (A) HEK293-AT1 cells were treated with the siRNAs targeting the indicated TBC1D proteins for three days and transfected with the mCherry-GFP-LC3 for 24 h. Cells were imaged live with confocal microscopy and saved images were analyzed off-line to calculate Pearson coefficients. Note that knock-down of several TBC1D proteins causes acidification defects and, in some cases, tubulation of the LC3-positive endosomes. Scale bars: 10 μm. (B) Pearson coefficients were compared using Dunnett's multiple comparisons in one-way ANOVA (P=0.0269; 0.9995; 0.0319; 0.0009; P<0.0001; 0.9996; 0.0654; 0.8254; 0.0142; compared to controls from n = 50, 51, 58, 52, 50, 50, 50, 44, 42 and 38 cells for the different groups from left to right, respectively).

A



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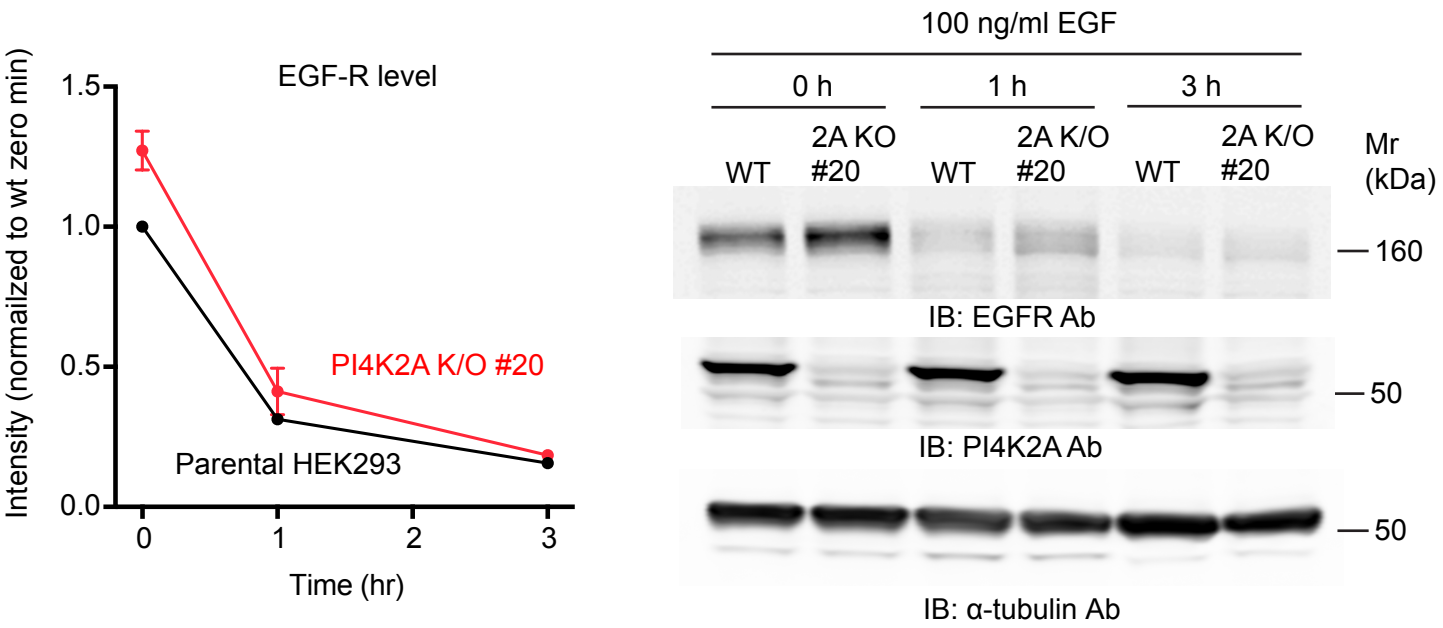
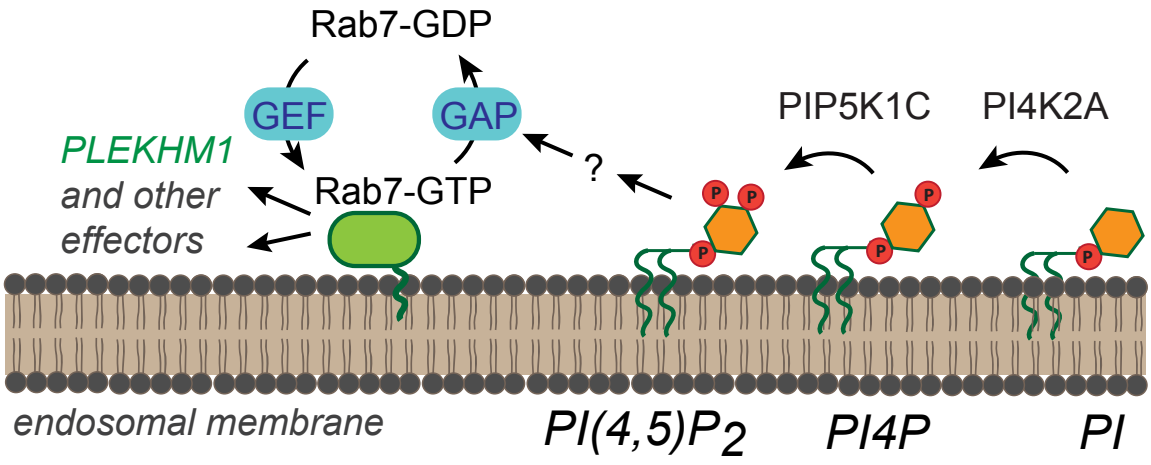


Appendix Figure S3. PI4K2A K/O cells didn't show obvious defects in CI-M6PR distribution and EGF-R degradation. (A)Wild-type or PI4K2A K/O cells were cultured and fixed with PFA as described in Materials and Methods. Cells were stained with anti-CI-M6PR antibody to visualize endogenous CI-M6PR. (B) EGF-R degradation assay was performed as described in Materials and Methods. Wild-type or PI4K2A K/O cells were serum-starved overnight. Cells were then treated with 100 ng/ml EGF for the indicated times. Cells were lysed and subjected to Western blot analysis using anti-EGFR, PI4K2A and -α-tubulin antibodies. Quantitative data are shown from three experiments (Means ± S.E.M) on the left and a representative blotting result is shown on the right.



Appendix Figure S4. Regulation of Rab7 cycling by phosphoinositides in endosomal membranes. The majority of PI4P in Rab7 positive endosomes is generated by PI4K2A and some of this lipid serves as precursor for the PIP5K1C enzyme converting it to PI(4,5)P_2. PI(4,5)P_2 promotes the inactivation of Rab7 presumably by activating one or more of the numerous Rab7 GAP proteins, a proposition that still needs experimental confirmation. Accelerated Rab7 cycling may affect several Rab7 effectors including PLEKHM1, an important adaptor protein promoting autophagosome-lysosome fusion.

Appendix Table S1. Source of plasmids used in this study

CFP-FKBP-PIP5Kg wild-type and CFP-FKBP-PIP5Kg kinase-dead	Kind gift from Takanari Inoue (Johns Hopkins University School of Medicine, Baltimore, MD)
GFP-PI4K2A; HA-PI4K2A and their kinase dead versions	Balla et al., 2002
PLCd1-PH-GFP	Varnai and Balla, 2008
iRFP-FRB-Rab7	Hammond et al., 2014
GFP-FYVE-EEA1, GFP-Rab4,-5,-7, -11	Hunyady et al., 2002
pGEX-4T-3-mR7BD	Aimee Edinger (Addgene plasmid # 79149), Romero Rosales et al., 2009
pTRE2-Bla(HA–RILP-FLAG)	Steven Weinman (Addgene plasmid # 102424) Wozniak et al., 2016
Gq-QL	kind gift from Sue Goo Rhee (NHLBI, NIH)
GFP-LC3	kind gift from Silvio Gutkind (NINDR, NIH)
mCherry-GFP-LC3	kind gift from Jennifer Lippincott-Schwartz (NICHD, NIH)
mRFP-PIP5K1B	Szentpetery et al. 2010
myc-PIP5K1B	Kind gift from Helen Yin, UT, Southwestern
plentiCRISPR v2	Kind gift from Dr. Choi Uimook (NIAAD, NIH)
pcDNA3.1-TBC1D7	kind gift from Dr. Dr. Francis Barr (Fuchs, E. et al. (2007) JCB., 177: 1133-1143)
Human PIP5Kγ	gift from William Hahn & David Root (Addgene plasmid # 23848)
TBC1D2A	GE Dharmacon, Clone ID: 9030533
TBC1D5	GE Dharmacon, Clone ID: 4510019
TBC1D9A	GE Dharmacon, Clone ID: ccsbBroadEn_07845
TBC1D9B	GE Dharmacon, Clone ID: 6826791
TBC1D15	GE Dharmacon, Clone ID: 4827246
TBC1D24	GE Dharmacon, Clone ID: 40118227

Appendix Table S2: List of siRNAs (from GE Dharmacon)

Gene (Gene ID)	Catalog number
PI4K2B (55300)	L-006769-00-0005
PIP5K1B (8395)	L-004058-00-0005
PIP5K1C (23396)	L-004782-00-0005
TBC1D2A (55357)	L-020463-01-0005
TBC1D5 (9779)	L-020775-01-0005
TBC1D7 (51256)	L-021140-00-0005
TBC1D9A (23158)	L-024048-00-0005
TBC1D9B (23061)	L-031872-02-0005
TBC1D15 (64786)	L-016209-02-0005
TBC1D24 (57465)	L-022880-01-0005
TBC1D25 (4943)	L-024659-01-0005
GRTP1 (79774)	L-014422-02-0005

Appendix Table S3 list of primers used in this study

Mutagenesis

Name	Sequence
CFP-W66A-Rv	TGCGGTCAGGGTGGTCACGAGGGTG
CFP-W66A-Fw	GGCGTGCAGTGCTTCAGCCGCTAC
Rab7 Q67L-Fw	CTCGAGCGGTTCCAGTCCCTT
Rab7 Q67L-Rv	GCCTGCTGTGTCCCAGATCTG
Rab7 N125I-Fw	ATCAAGATTGACCTCGAAAACAGACAAGTGG
Rab7 N125I-Rv	TCCCAACACAACGAAAGGGAAGTTTTCAG

TBC proteins

Name	Sequence
pEGFP-Arumus-Fw	AATGGAGGACACCCCCGAGCGCACT
pEGFP-Arumus-Rv	TCAGCTGTCGCCTTCTCCTTCGTCCTCGCT
GFP-TBC1D5-Fw	GATGTATCATTCCTTATCTGAAACTAGACATCCTCTGCAG
GFP-TBC1D5-Rv	TCAGATGTCCAGGGGACTCACAATG
TBC1D9 full Fw	GATGTGGGTGAACCCGGAGGAGGTGTTG
TBC1D9 full Rv	TCAGCCGGACATGGCCGAGATTTCATAGTC
TBC1D9B full Fw	CTGGATCTTTTCCTGCCCTGGGAGGCTCAAGCACTGCTACAGGAGCAGCAG
TBC1D9B full Rv	ATCTGAGGCCAGAGGAGATGCTTCCGAGGAGCTATCCTCTGTGAAGTAATG
TBC1D9B_Fw	GATGTGGCTCGGGCCCGA
TBC1D9B_Rv	TCAGCCCAAGACCCCAGGG
GFP-TBC1D15-Fw	GATGGCGGCGGCGGGTGTTGT
GFP-TBC1D15-Rv	TCATGCAGGTGTTAATCTGCAGACATCTGAGGACACTGG
GFP-TBC1D24-Fw	GATGGACTCTCCAGGATACAACTGCTTCGTGGAC
GFP-TBC1D24-Rv	TCACTGGGTGTCAGGGTCCTGGAAGC

Others

Name	Sequence
mRFP-Sluc-PLCd1PH-SalI-Fw	ATATGTCGACAGTGAGGGCAGAGGAAGTCTTCTAACATGC
mRFP-Sluc-PLCd1PH-PvuI-Rev	ATATCGATCGTTCTCGAGATCTCTGCTCGTTCTTCAGC
PLCd1-PvuI-Fw	ATATCGATCGGACTCGGGCCGGGACTTCC
PLCd1-SalI-Rv	ATATGTCGACATCCTTCAGGAAGTTCTGCAGCTCC
mRFP-Sluc-PLCd1PH-SalI-Fw	TCGACAGTGAGGGCAGAGGAAGTCTTCTAACATGC
mRFP-Sluc-PLCd1PH-PvuI-Rev2	GATCGTTCTCGAGATCTCTGCTCGTTCTTCAGC
PLEKHM1 for BRET sensor Fw	GATGCTTTCAGTGGTGGAGAATGGACT
PLEKHM1 for BRET sensor Rv	CGGCGAAAATGTTCTGTTCCTGGTACTTG
PLEKHM1 RH for BRET sensor Fw2	GATGCTGAGTGACAGCCACGG
EEA1FYVE-Fw for blunt end	GGTGAAGAAGGAGTGGCAATCT
EEA1FYVE-Rv for blunt end	CTCCTTGCAAGTCATTGAAACATGCATC
VPS35-Fw	GATGCCTACAACACAGCAGTCCCCTCAGGATG
VPS35-Rv	CTAAAGGATGAGACCTTCATAAATTGGCCCCTCGGATTCTG
VPS35-Rv for BRET	CAAGGATGAGACCTTCATAAATTGGCCCCTCGGATTCTG
RILP-FL-Fw	GATGGAGCCCAGGAGGGCGGCGC
RILP-FL-Rv	CTAGGCCTCTGGGGCGGCTGAGGCC
RILP-FL-Rv for BRET	CGGCCTCTGGGGCGGCTGAGGCC
PIP5K1B-Fw	GATGTCTTCTGCTGCTGAAAATGGAGAG
PIP5K1B-Rv	TAATGCTCTGATGTTGCCATCTTGAACCTG