Supporting Information

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SI Materials and Methods

Plasmids. Epitope-tagged PI4KIIα cDNAs, myc-PIP5Kβ, GFP-OSBP-PH, mRFP-GFP-LC3, and GFP-LC3 were described previously in our publications. GFP-GABARAP, mCherry-GATE-16, and DSRED-LAMP1 plasmids were gifts of Zvulun Elazar.

siRNAs. siRNAs were transfected with RNAiMAX, and cells were analyzed after 48–72 h. In rescue experiments, cells were transfected with plasmids using RNAiMAX 48 h after the initial siRNA transfection and used 24 h later.

Immunofluorescence Microscopy and Image Analysis. Colocalization in serial Z stacks of optical sections was analyzed by using the Imaris Autoquant software, version 7.6.5 (Bitplane). Colocalization in a single confocal plane was determined using the Zeiss LSM510 software. ImageJ (NIH) was used to quantify fluorescence intensity (corrected integrated density function) and particle size. Endogenous LC3 was stained after methanol fixation for 5 min at −20 °C and permeabilization in cold acetone. This procedure improves signal-to-noise ratio and reports endogenous LC3 localization more reliably than the conventional triton permeabilization procedure. PI4P was stained using a fixation protocol optimized for preserving PI4P in internal membranes.

Time-Lapse Microscopy. HeLa/GFP-LC3 cells were transfected with siCtrl or si*PI4KIIα* for 48 h, and then with pDSRED-LAMP1. After 24 h, cells were treated with EBSS for 1 h, placed in an environmental chamber with 5% (vol/vol) CO_2, and held at 37 °C. Cells were imaged on a Zeiss LSM 510 confocal microscope, using a ×63 1.4 NA plan Apochromat oil immersion lens at 37 °C. Two hundred images (488- and 543-nm laser lines) were collected with a 5-s cycle delay and analyzed with the Zeiss LSM Image Browser software. The Imaris version 7.7.5 particle tracking algorithm (Bitplane) was used to analyze particle trajectory.

Electron Microscopy. For correlative light and electron microscopy, HeLa/GFP-LC3 cells were plated in 35-mm dishes containing a glass coverslip with grids (MatTek Corp), fixed and imaged by fluorescence microscopy, and subsequently processed for electron microscopy by the University of Texas Southwestern Electron Microscopy Core Facility.

Statistical Analyses. Data were expressed as mean ± SEM, and $P < 0.05$ is considered statistically significant. A two-tailed unpaired test was performed to compare two groups, and a one-way ANOVA analysis of variance was used to compare values among multiple groups, using the Sigma Plot software.

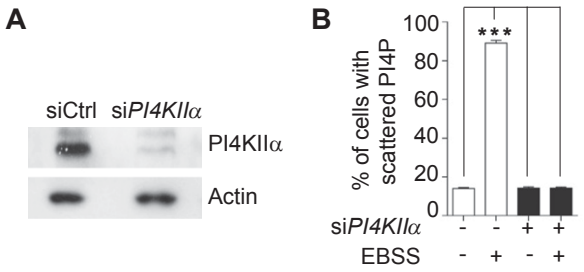


Fig. S1. PI4KIIα depletion blocks appearance of extra-Golgi PI4P puncta after EBSS treatment. (*A*) Western blot with anti-PI4KIIα and anti-actin. (*B*) Quantitation of percentage of cells with abundant scattered PI4P puncta (mean ± SEM; $n = 3$; ***$P < 0.001$), similar to those shown in Fig. 1. Fifty cells per condition were analyzed per experiment in a blinded fashion.

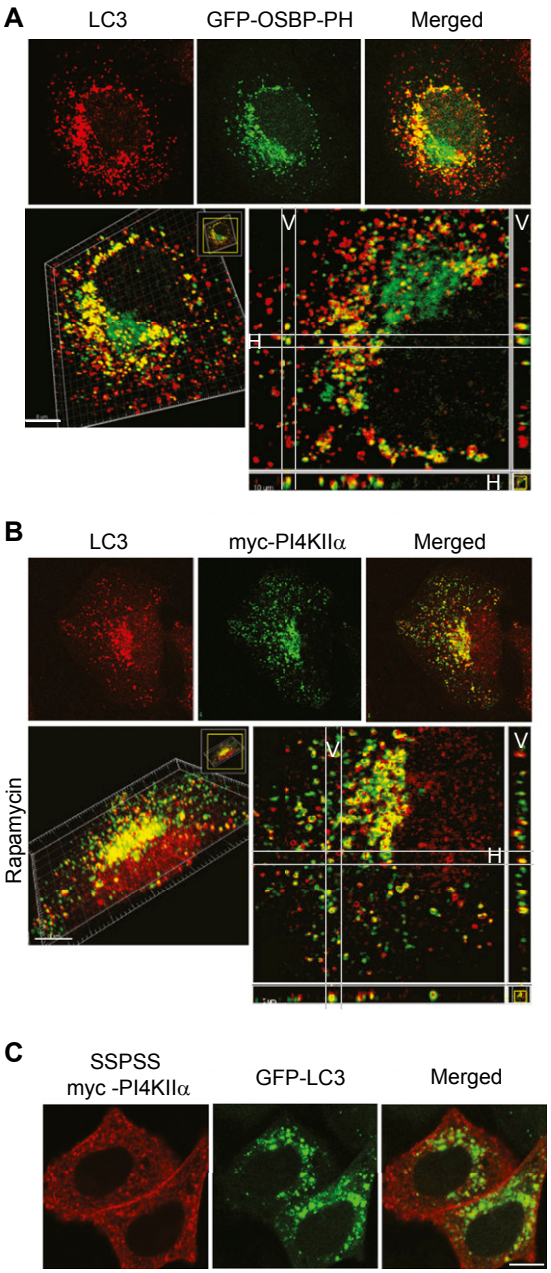


Fig. S2. LC3 localization. (*A* and *B*) Z stacks from EBSS- or Rap-treated HeLa cells showing LC3 localization relative to GFP-OSBP-PH and myc-PI4KIIα. Related to Fig. 2 *C* and *D*. (*C*) SSPSS myc-PI4KIIα was expressed in HeLa/GFP-LC3 cells. Related to Fig. 2*E*.

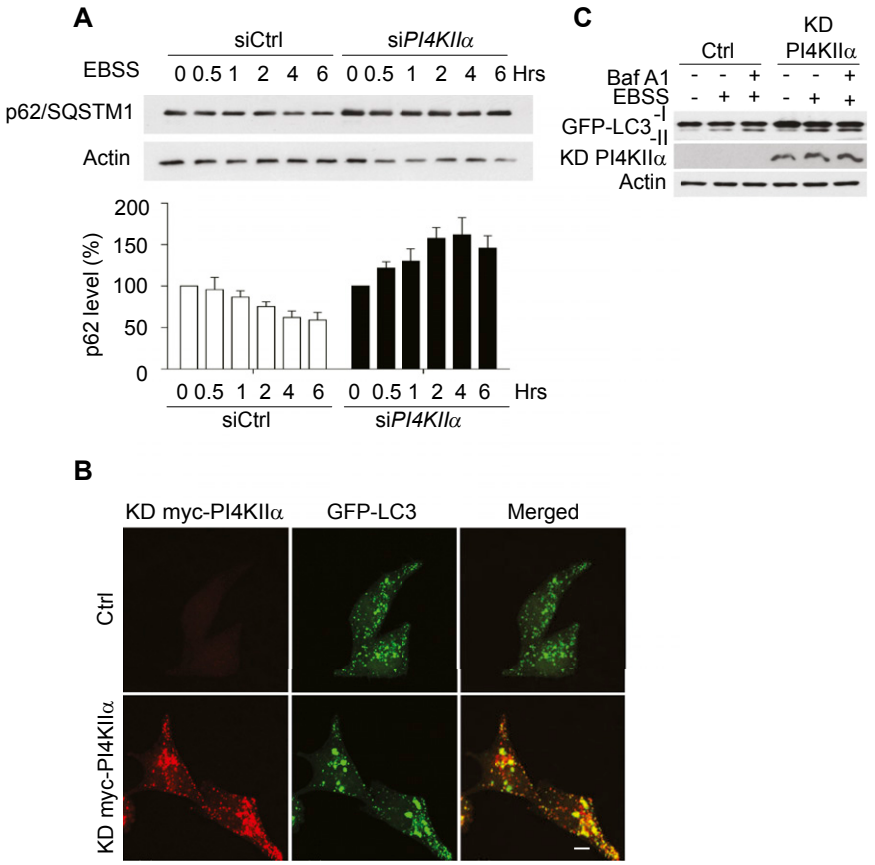


Fig. S3. Autophagy flux. (*A*) Effects of si*PI4KIIα* on p62/SQSTM1 degradation. (*B*) Effects of KD PI4KIIα overexpression on autophagosomal size and autophagic flux. HeLa/GFP-LC3 cells with or without overexpressed KD myc-PI4KIIα were starved in EBSS and stained with anti-myc. (Scale bar, 5 μm.) (*C*) GFP-LC3 autophagic flux determined by Western blotting. Densitometry results are shown in Fig. 3*C*.

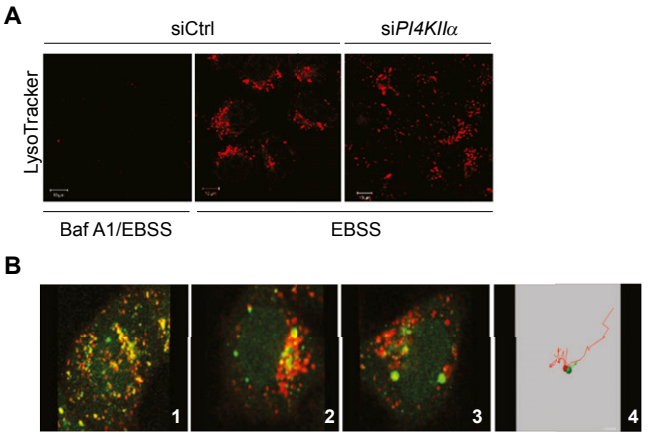


Fig. S4. Effect of si*PI4KIIα* on acidification and A:L fusion. (*A*) LysoTracker labeling. Starved siCtrl and si*PI4KIIα* HeLa cells were starved and incubated with LysoTracker Red DND-99 to label acidic organelles. Some cells were pretreated with Baf A1 to inhibit acidification. Related to Fig. 4*A*. (*B*) Time-lapse microscopy showing movement of GFP-LC3 particles (autophagosomes) and RFP-LAMP1 particles (lysosomes). HeLa/GFP-LC3 cells without or with si*PI4KIIα* transfection were retransfected with pDSRED-LAMP1, starved, and subjected to time-lapse microscopy. Videos generated from time lapse images are shown in Movies S1–S4. Related to Fig. 4*B*.

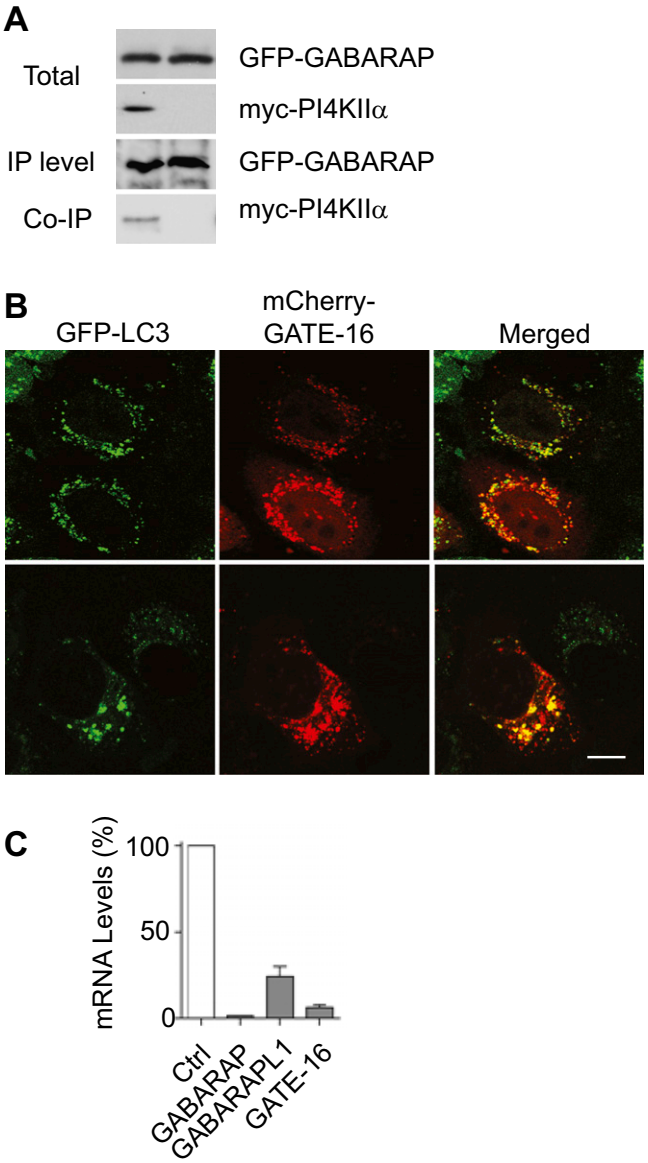


Fig. S5. Relation between GABARAPs and PI4KIIα. (*A*) GFP-GABARAP pull-down of myc-PI4KIIα. Reciprocal of Fig. 5*A*. (*B*) Effects of si*PI4KIIα* on GFP-LC3 and mCherry-GATE-16 colocalization. (*Top*) siCtrl. (*Bottom*) si*PI4KIIα*. Related to Fig. 5 *B* and *C*. (*C*) Quantitation of GABARAP depletion. mRNA levels of GABARAP, GABARAPAPL1, and GATE-16 from cells transfected with a mixed pool of *GABARAP* siRNA was determined by quantitative PCR and expressed relative to Ctrl (mean ± SEM; $n = 3$).

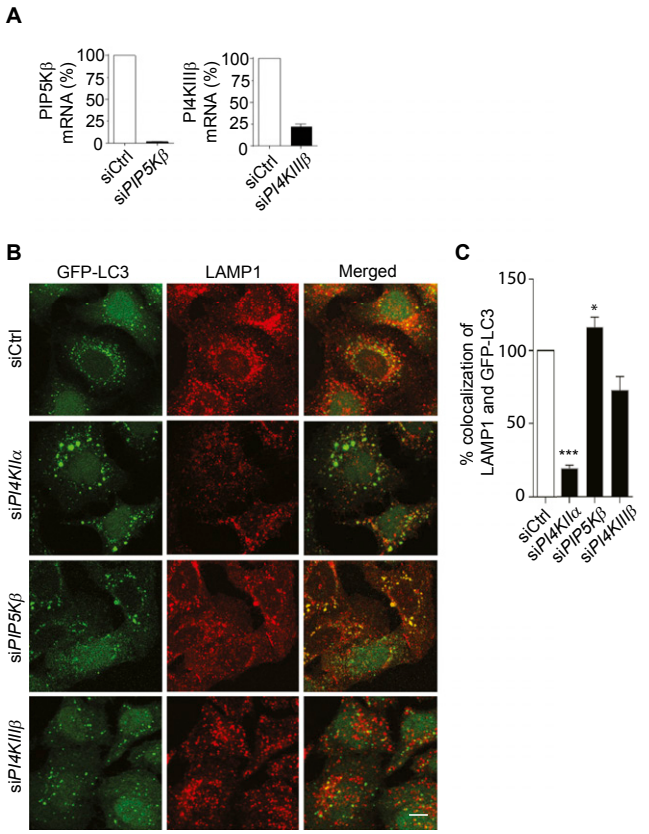
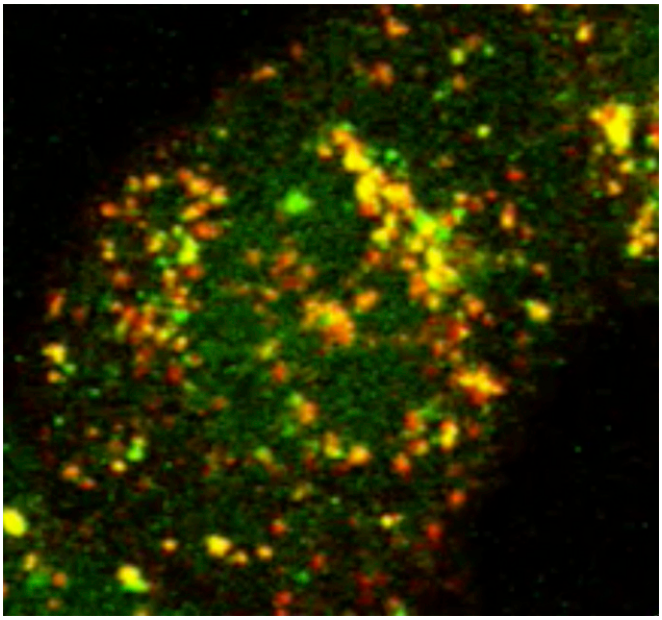
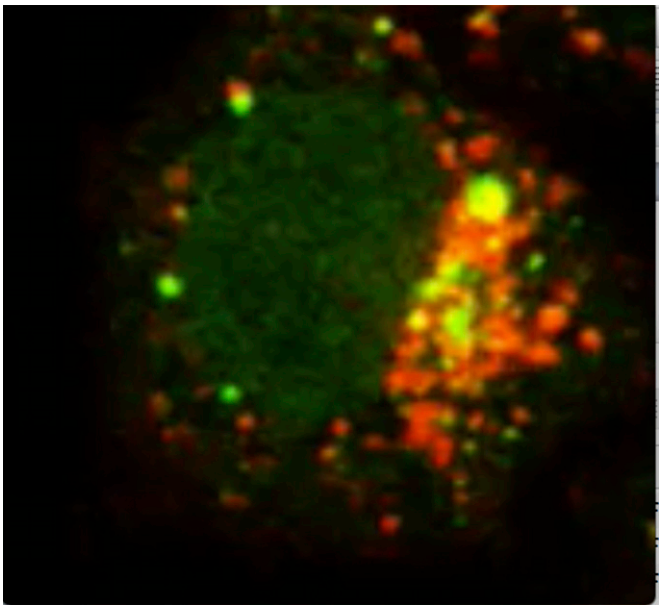


Fig. S6. PI4KIIα has a unique role in autophagy. HeLa/GFP-LC3 cells were used. (*A*) Quantitation of mRNA level after siRNA targeting. mRNA levels were determined by quantitative PCR and expressed relative to Ctrl (mean ± SEM; $n = 3$). (*B*) Differential effects of si*PI4KIIα*, si*PIP5Kβ*, and si*PI4KIIIβ* on LAMP1 colocalization with GFP-LC3. HeLa/GFP-LC3 cells transfected with siRNAs were starved and stained with anti-LAMP1. (*C*) Comparison of effects of siRNA on percentage colocalization of LAMP-1 with GFP-LC3. Values were normalized against Ctrl value set at 100. Forty-five cells from three experiments were quantified per condition. $*P < 0.05$; $***P < 0.001$.



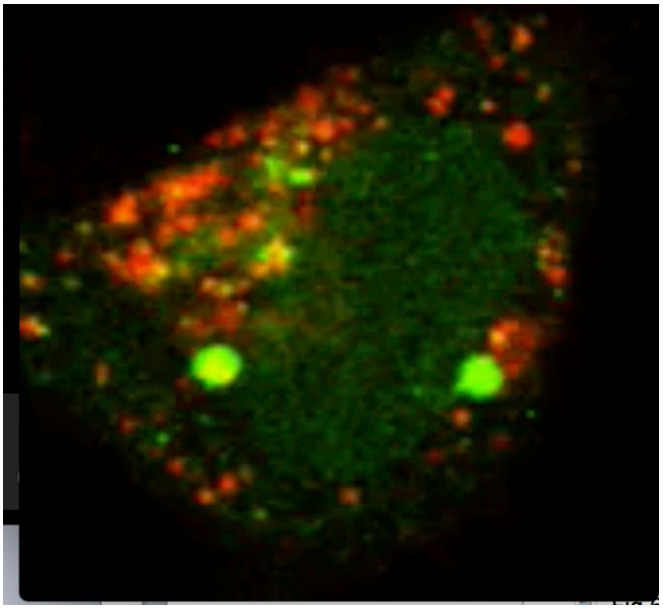
Movie S1. Starved siCtrl cells, showing multiple A:L fusions.

Movie S1



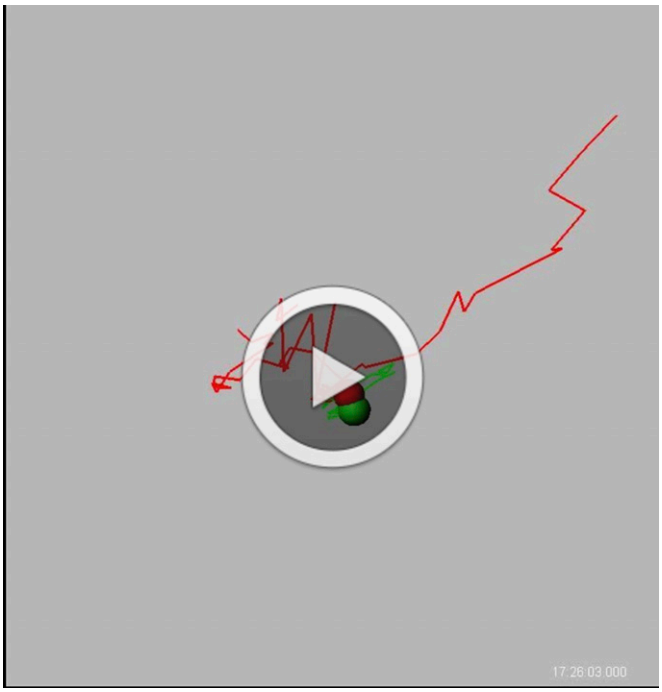
Movie S2. Starved si*PI4KIIα* cell, showing enlarged autophagosomes and fewer productive A:L fusions.

Movie S2



Movie S3. Starved si*PI4KIIα* cell, showing an abnormally enlarged autophagosome extending tubules to contact lysosomes, but failing to fuse.

Movie S3



Movie S4. Particle tracking analysis in a starved si*PI4KIIα* cell, showing that an autophagosome and lysosome pair engage in successive rounds of "engagement and disengagement" without fusion in the time frame analyzed.

Movie S4