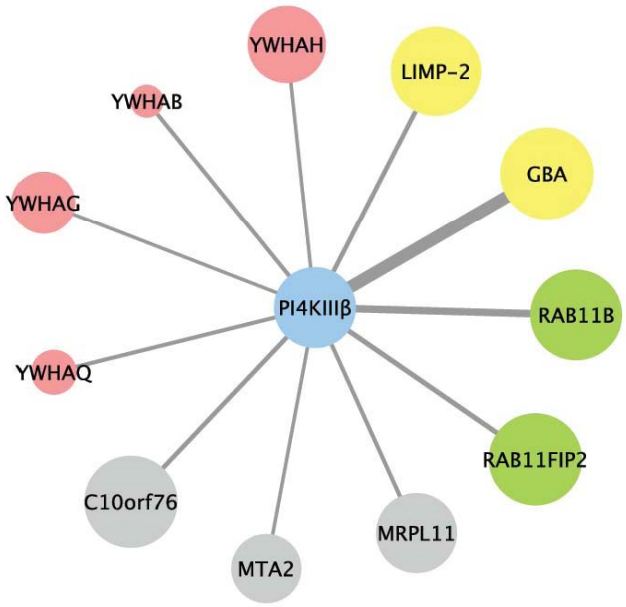
Supplementary Table 1. Detailed AP-MS data for FLAG-PI4KIIIβ (Hugo gene name PI4KB) replicate. Complete mass spectrometry information for interaction partners of each biological replicate of FLAG-PI4KIIIβ is listed below. "ProHits ID" is the unique identifier for the sample in our internal interaction database. “Bait Name” is the HUGO gene name of the bait (referred throughout the paper as PI4KIIIβ**)**. “Hit Name” is the HUGO gene name of each filtered interactor. “Hit Gene ID” is the NCBI Gene ID for each hit. “gi number” is the NCBI protein ID which can be used to refer to the protein sequence. "Mascot" is the score from the Mascot search engine. "n specs" is the total number of peptides, or spectral counts. "n peps" is the number of unique peptides (as defined by the Mascot search engine). "% cover" is the proportion of the protein sequence that has been detected by mass spectrometry.

Supplementary Table 1

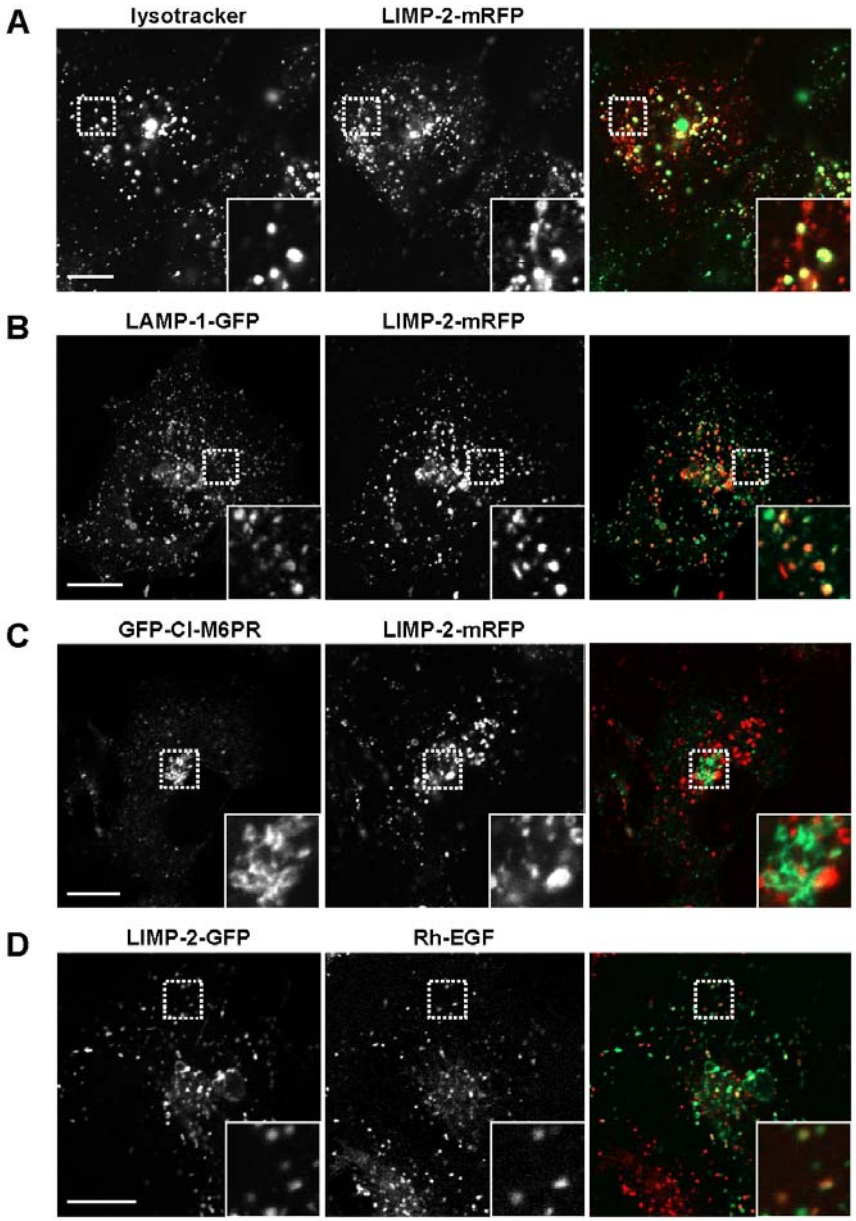
ProHits ID	Bait Name	Hit Name	Hit Gene ID	gi number	Mascot	n specs	n peps	% cover	Freq
1st expt									
1716	PI4KB	GBA	2629	54607043	939	47	19	41.8	0.53
1716	PI4KB	RAB11B	9230	190358517	482	21	9	42.2	1.06
1716	PI4KB	RAB11FIP2	22841	7662394	199	5	4	10.5	0.53
1716	PI4KB	LIMP-2	950	5031631	144	4	3	12.6	1.59
1716	PI4KB	C10orf76	79591	154350213	90	3	3	5.4	0.53
1716	PI4KB	YWHAQ	10971	5803227	245	7	2	23.7	19.05
1716	PI4KB	MRPL11	65003	7705618	88	2	2	15.6	5.29
1716	PI4KB	YWHAB	7529	4507949	84	9	2	6.9	23.81
1716	PI4KB	YWHAG	7532	21464101	205	6	1	15.8	12.17
1716	PI4KB	YWHAH	7533	4507951	160	6	1	14.2	6.35
1716	PI4KB	MTA2	9219	14141170	85	3	1	2.2	9.52
2nd expt									
1747	PI4KB	GBA	2629	54607043	801	73	14	36.2	0.53
1747	PI4KB	YWHAG	7532	21464101	883	74	10	61.5	12.17
1747	PI4KB	YWHAH	7533	4507951	891	70	9	59.3	6.35
1747	PI4KB	YWHAQ	10971	5803227	825	56	8	51.4	19.05
1747	PI4KB	C10orf76	79591	154350213	410	15	8	16.8	0.53
1747	PI4KB	YWHAB	7529	4507949	833	72	7	62.6	23.81
1747	PI4KB	LIMP-2	950	5031631	129	6	3	10.9	1.59
1747	PI4KB	MTA2	9219	14141170	115	4	3	5.2	9.52
1747	PI4KB	RAB11B	9230	190358517	134	5	2	13.8	1.06
1747	PI4KB	RAB11FIP2	22841	7662394	97	2	2	4.9	0.53
1747	PI4KB	MRPL11	65003	25306275	93	2	1	9.9	5.29

Supplementary Figure 1.



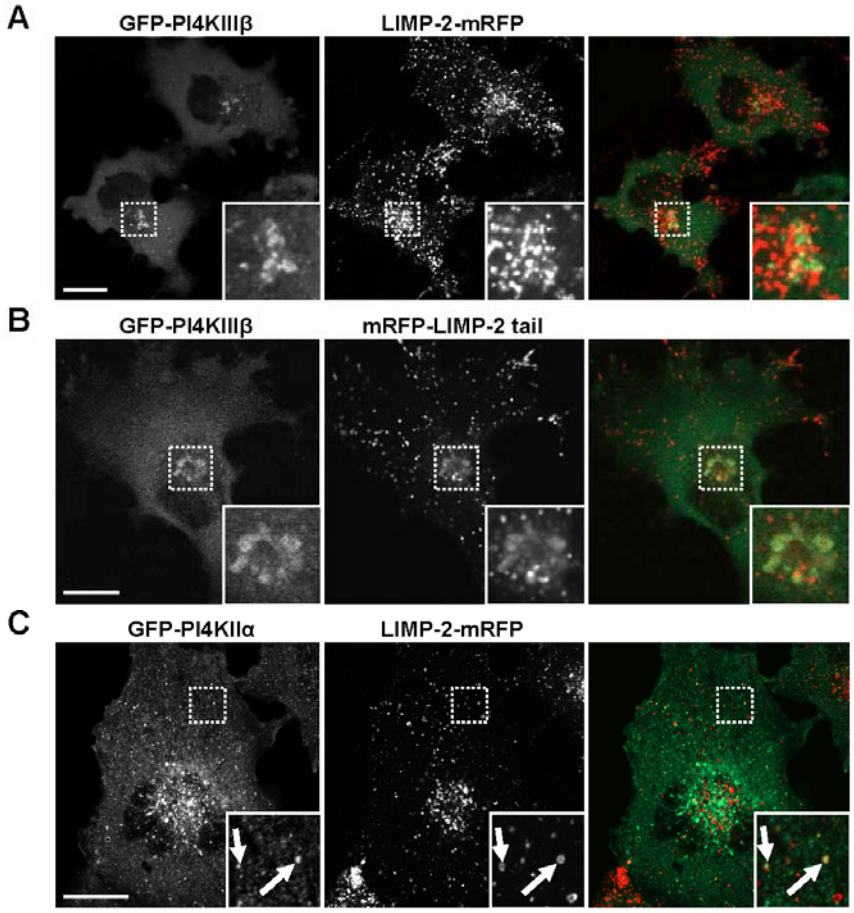
Supplementary Figure 1. PI4KIIIβ interactors identified by mass spectrometry. AP-MS and analysis was performed as described under "Materials and Methods". The *thickness* of the edges is proportional to the number of unique peptides for each interaction partner (prey) and the *size* of the node indicates the frequency of the prey in our database (small being most frequent). The *color* indicates: Bait (*blue*), LIMP-2 and GBA (yellow), Rab11 and related proteins (*green*), 14-3-3 proteins (*pink*), other proteins (*gray*). See Supplementary Table 1 for complete mass spectrometric data. The protein names are listed as they have been used in this manuscript; refer to Hit Gene ID in Supplementary Table 1 for access to the HUGO gene names.

Supplementary Figure 2.



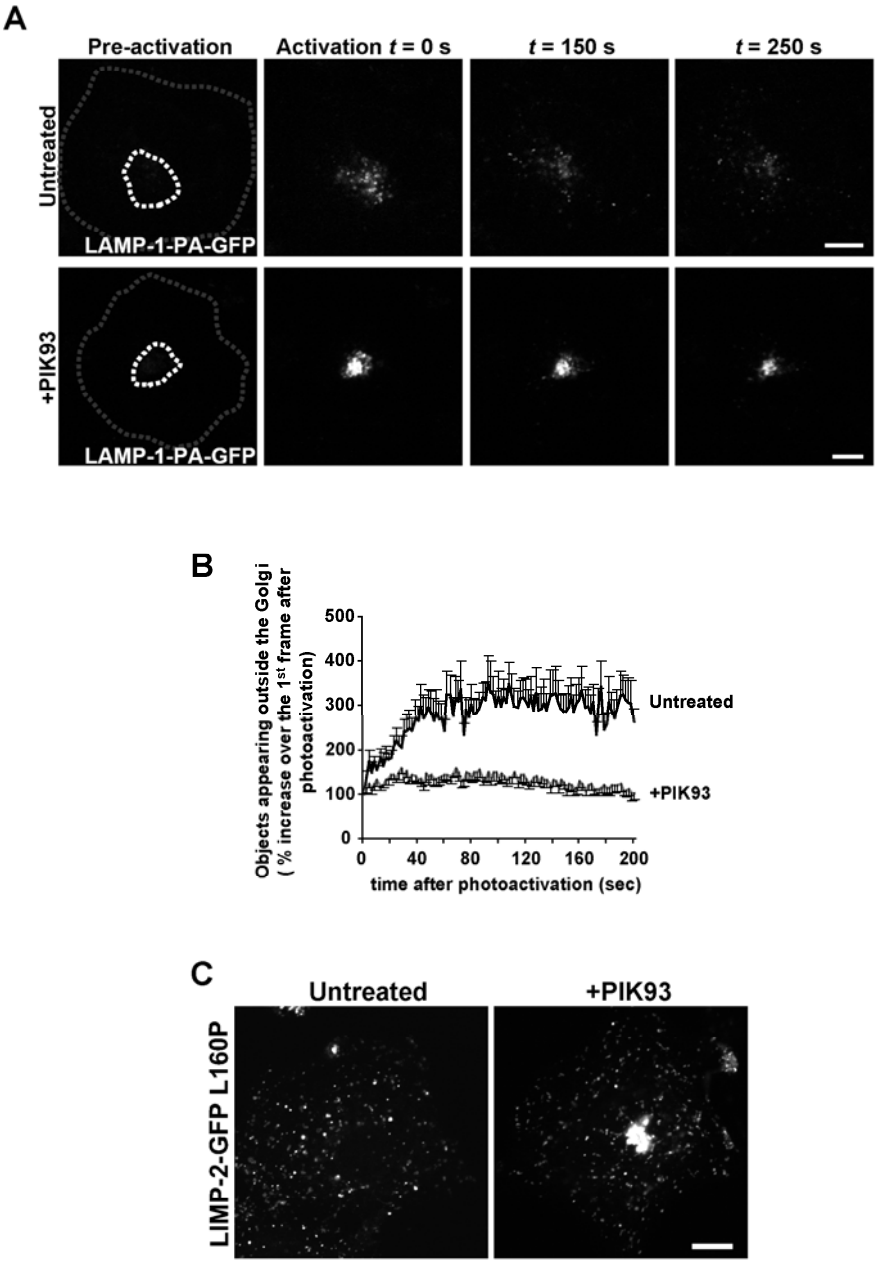
Supplementary Figure 2. Subcellular distribution of LIMP-2. COS-7 cells were transfected either with LIMP-2-mRFP alone (A), or together with LAMP-1-GFP (B) or GFP-CI-M6PR (C). After 24 h cells were either pre-incubated for 10 min with 100 nM lysotracker-green (A), with Rh-EGF (D), or directly mounted and analyzed by live cell confocal microscopy. Bars, 10μm.

Supplementary Figure 3.



Supplementary Figure 3. LIMP-2 transiently resides in PI4K-rich compartments. LIMP-2-mRFP was coexpressed with bovine GFP- PI4KIIIβ (A) or human GFP-PI4KIIα (C) in COS-7 cells, mounted on a heated stage and subjected to live cell microscopy. Only limited number of LIMP-2 molecules exhibited localization to PI4K-enrinched compartments, as they passed through en route to lysosomes (see insets). (B) Cells co-transfected with bovine GFP-PI4KIIIβ and mRFP-LIMP-2-tail were analyzed after 24 h. Delayed sorting of LIMP-2-tail out of the Golgi makes it possible to show the transient co-localization with PI4KIIIβ in the Golgi. Bars, 10μm.

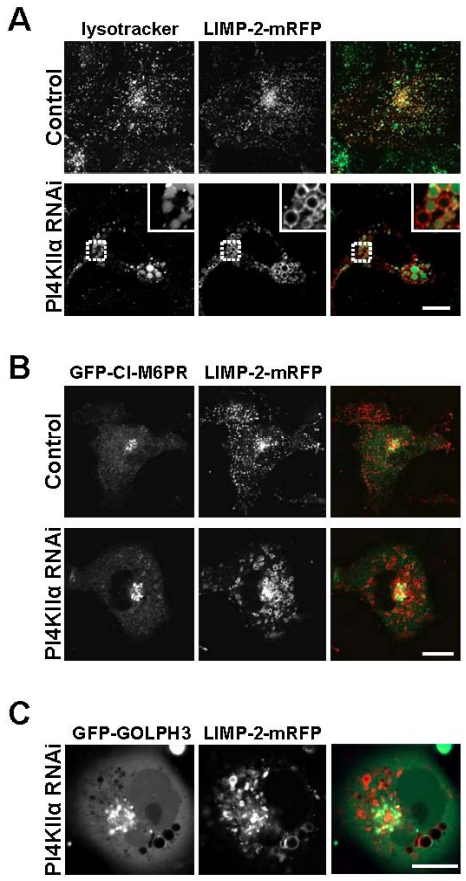
Supplementary Figure 4.



Supplementary Figure 4. COS-7 cells were transfected with LAMP-1-PA-GFP together with mKO-GalT. Golgi area was photoactivated based on the GalT distribution (inner dashed line) using a 405-nm laser line. Emergence of Golgi-derived fluorescent vesicles containing photoactivated LIMP-2 molecules was imaged by time-lapse microscopy in control cells or cells pretreated with PIK93 (1μM) for 20 min. Bars, 10μm. (B) Morphometric analysis of the recordings in (A). The graph shows the number of individual fluorescent vesicles appearing outside the Golgi photoactivation area (dashed line) with a

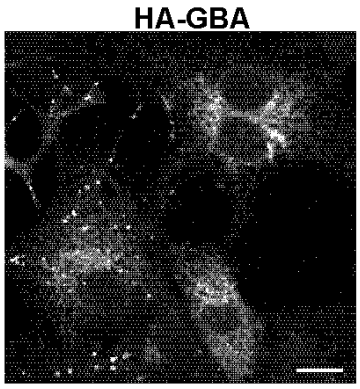
signal above an arbitrary threshold normalized to the value prior to photoactivation. The graph illustrates the first 200 sec of the recordings taken at 1 frame/sec. (Each value represents a mean ± SEM of 11 recordings for each sample - control and PIK93-treated). (C) Following a 24 transfection with LIMP-2-GFP L160P mutant, COS-7 cells that were untreated or treated with PIK93 (1μM) for 20 min were analyzed by live cell confocal microscopy. Bars, 10μm.

Supplementary Figure 5.



Supplementary Figure 5. Effects of PI4KIIα knockdown on the morphology of LE/lysosomes. COS-7 cells treated with control or PI4KIIα siRNA duplexes for 2 days were either incubated with lysotracker-green 10 min prior to analysis by live cell microscopy (A) or transfected with GFP-CI-M6PR (B) or GFP-GOLPH3 (C) one day before. Bars, 10μm.

Supplementary Figure 6.



Supplementary Figure 6. Steady-state subcellular distribution of GBA. Following the transfection with HA-GBA, COS-7 cells were imaged by live cell confocal microscopy. As shown in a representative image, overexpressed HA-GBA is found mostly in the ER, with some minor Golgi and lysosomal localization. Bar, 10μm.