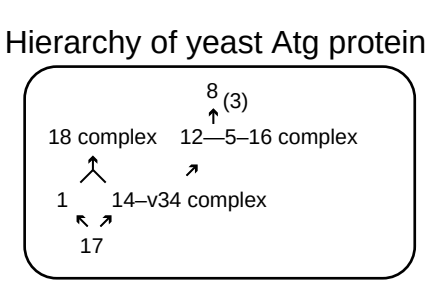
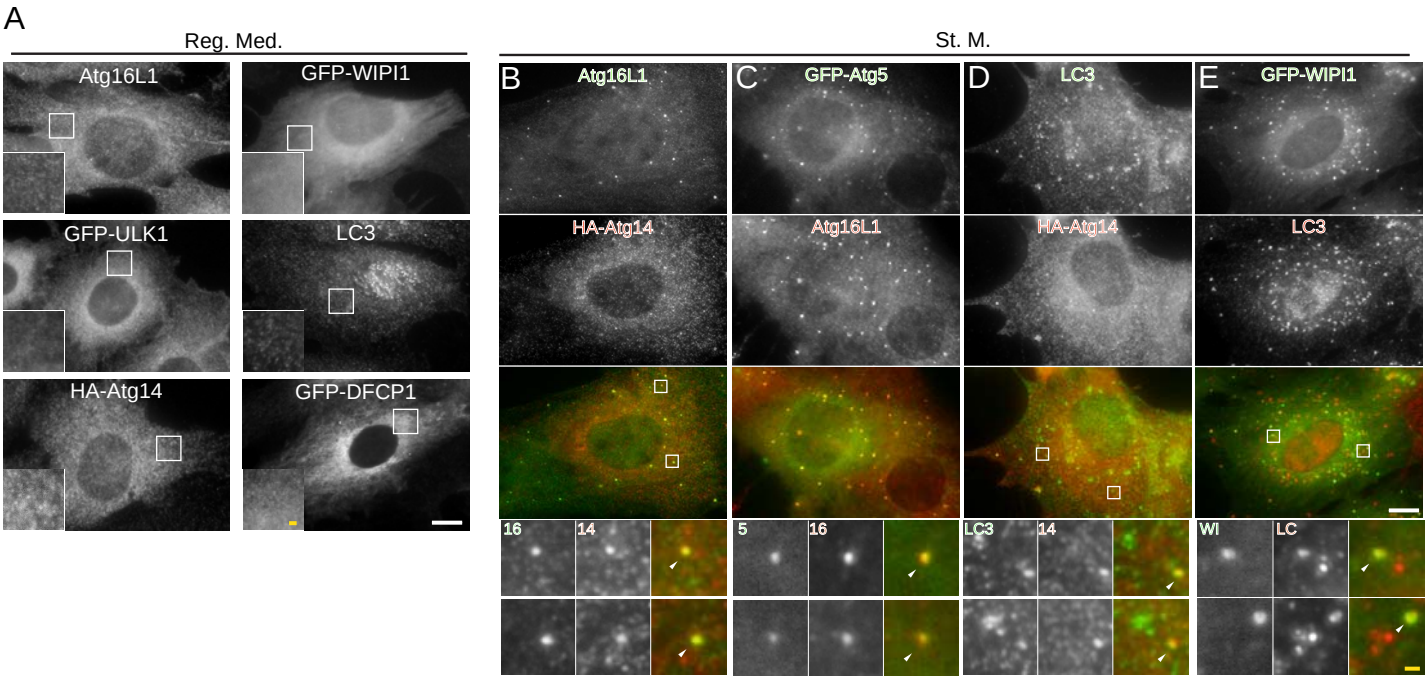
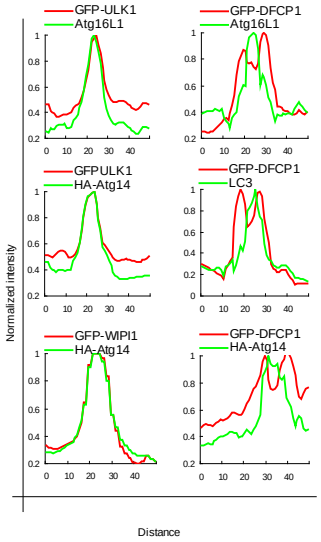


Yeast		Mammal
Atg1	=	ULK1
Atg3	=	Atg3
Atg5	=	Atg5
Atg8	=	LC3
Atg12	=	Atg12
Atg14	=	Atg14
Atg16	=	Atg16L1
Atg17	=	FIP200?
Atg18	=	WIPIs
Vps34	=	Vps34

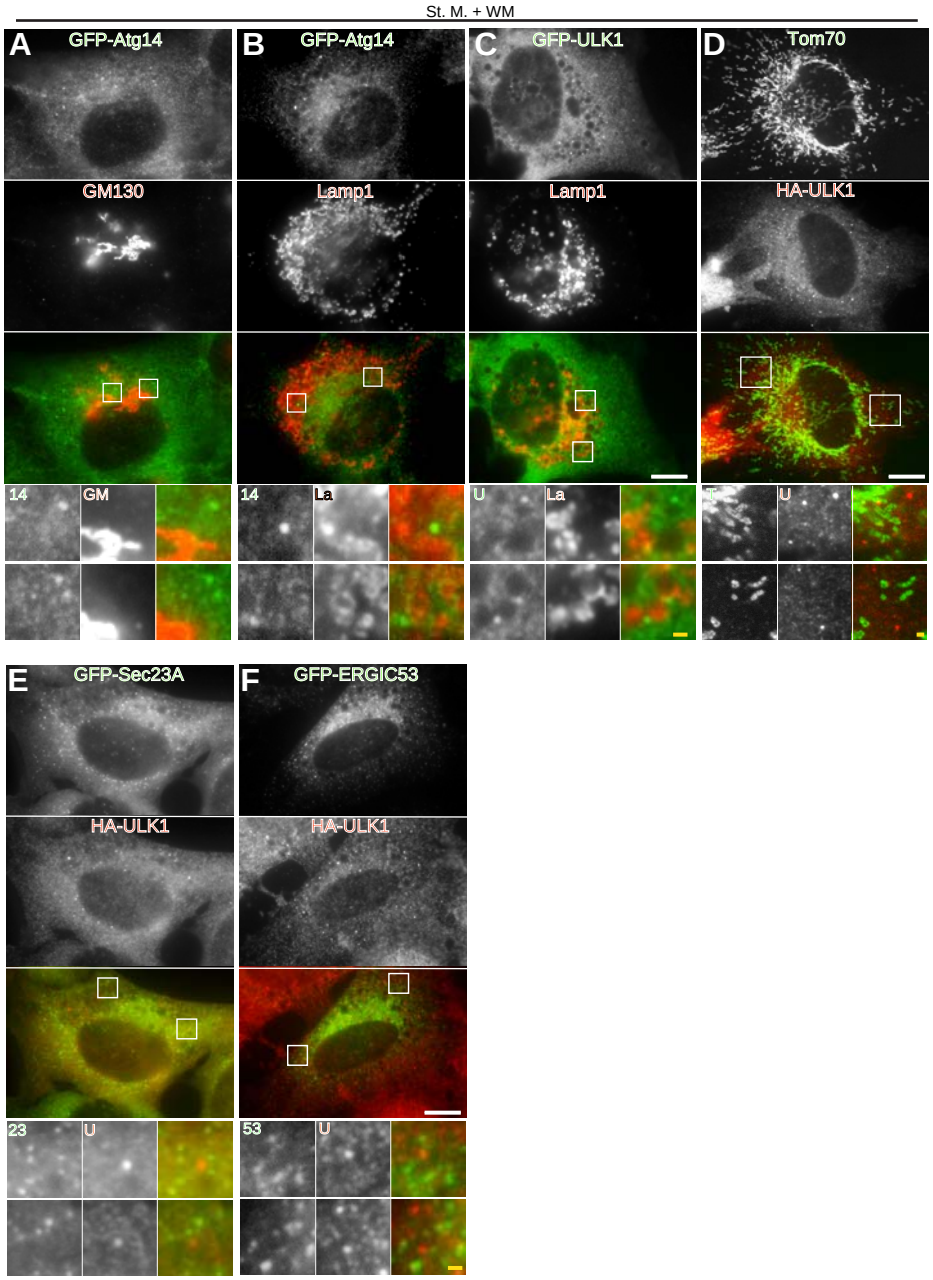
Supplementary Figure 1. Hierarchical relationship of yeast Atg proteins.
A current model of the hierarchical relationship of yeast Atg proteins is shown in the left panel (modified from reference 10 to include factors used in this study). Their mammalian counterparts are shown in the right panel.



Supplementary Figure 2. Localization of Atg14, WIPI-1, Atg5, Atg16L1 and LC3.
MEFs stably expressing either GFP-ULK1, GFP-WIPI1, HA-Atg14, or GFP-DFCP1 (A), HA-Atg14 (B and D), GFP-Atg5 (C), and GFP-WIPI1 (E) were cultured in regular (A) or starvation medium (B-E) for 1 hour. Cells were then fixed, permeabilized, and subjected to immunofluorescence microscopy using anti-GFP (for GFP-ULK1 and GFP-DFCP1), anti-HA, anti-Atg16L1, anti-LC3 antibodies. Nearly complete co-localization is indicated by arrowheads. Signal color is indicated by color of typeface. Scale bars, 10 μm (white) and 1 μm (yellow).

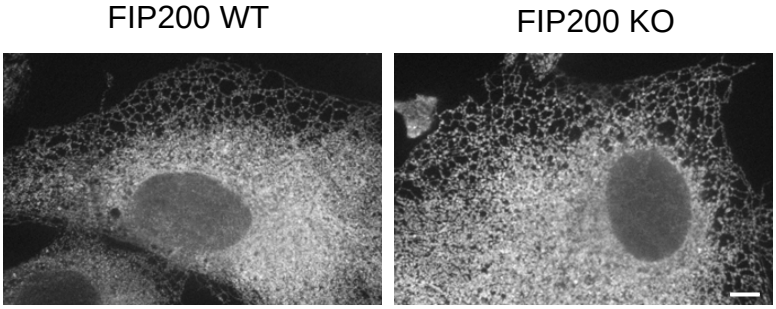


Supplementary Figure 3. Linescan plot analysis of autophagy-related punctate structures.
Linescans were obtained from representative punctate structures showing co-localization of the indicated protein combination. The original structures are shown in Fig. 1 and Fig. S1 (indicated by dashed lines).

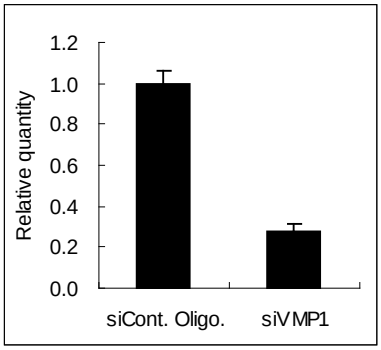


Supplementary Figure 4. Atg14 and ULK1 are not co-localized with cis-Golgi, late endosome, mitochondria, ER exit site and ER–Golgi intermediate compartment markers.
NIH3T3 cells stably expressing GFP-Atg14 (A and B) or GFP-ULK1 (C), and MEFs stably expressing HA-ULK1 (D), GFP-Sec23A/HA-ULK1 (E) or GFP-ERGIC-53/HA-ULK1 (F) were cultured in starvation medium containing 0.2 μM wortmannin for 1 hour. Cells were then subjected to immunofluorescence microscopy using anti-GFP (A-E), anti-GM130 (A), anti-Lamp1 (B, C), anti-Tom70 (D) and anti-HA antibodies (D–F). St. M., starvation medium; WM, wortmannin. Signal color is indicated by color of typeface. Scale bars, 10 μm (white) and 1 μm (yellow).

A



B



Supplementary Figure 5. Knockout of FIP200 does not alter the ER structure.
(A) MEFs stably expressing GFP-ER were subjected to immunofluorecence microscopy using anti-GFP antibodies.
(B) HeLa cells were treated with VMP1 or control siRNA oligos, and mRNA level was measured by real-time PCR. Data are expressed as mean ± SE of three PCR reactions.

Video 1

Double imaging of GFP-ULK1 and mRFP-ER during starvation.

GFP-ULK1 puncta is closely associated with the ER. MEFs stably expressing GFP-ULK1 and mRFP-ER imaged in starvation medium at 1 frame per 12.9 seconds. GFP-ULK1 (upper left), mRFP-ER (upper right) and the merged movies are shown. See Fig. 6D for single frames. The playback rate is 4.3 frames per second.

Video 2

Double imaging of GFP-ULK1 and mRFP-ER during starvation with wortmannin treatment.

GFP-ULK1 puncta is closely associated with the ER. MEFs stably expressing GFP-ULK1 and mRFP-ER imaged in starvation medium with wortmannin at 1 frame per 17 seconds. GFP-ULK1 (upper left), mRFP-ER (upper right) and the merged movies are shown. See Fig. 6E for single frames. The playback rate is 4.3 frames per second.