Fujita et al., http://www.jcb.org/cgi/content/full/jcb.201304188/DC1

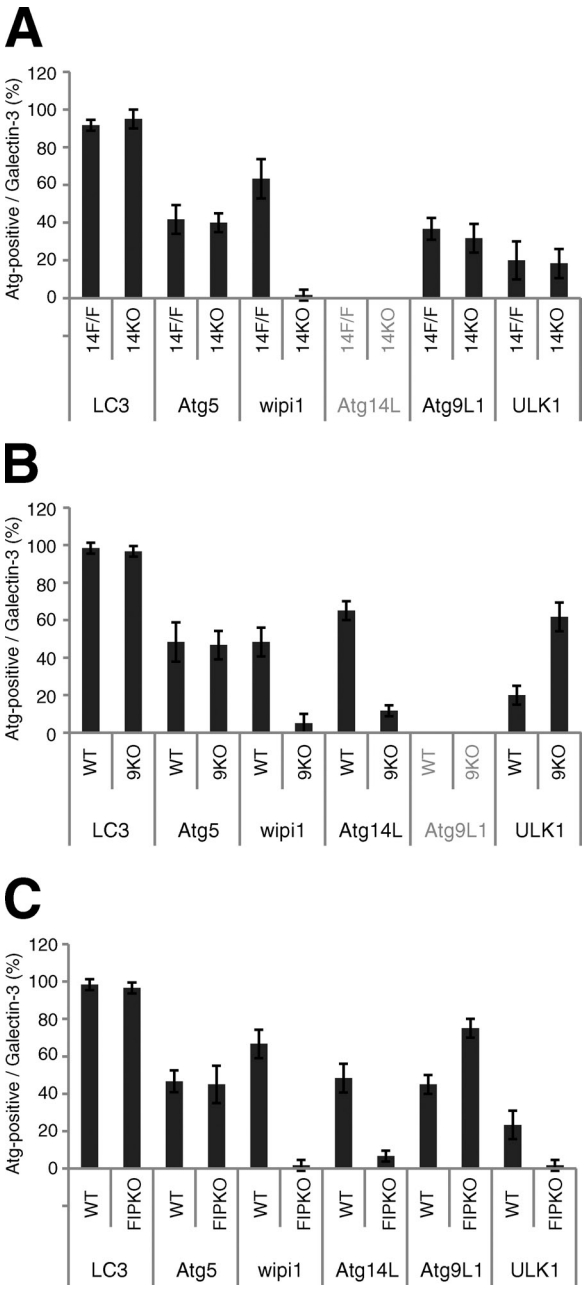


Figure S1. **Hierarchal analysis of Atg proteins in bead-transfected cells.** (A–C) Wild-type and autophagy-deficient cells (Atg14L-KO [A], Atg9L1-KO [B], and FIP200-KO [C]) stably expressing GFP-tagged LC3, Atg5, WIPI-1, Atg14L1, Atg9L1, or ULK1 were transfected with Effectene-coated latex beads for 3 h and subjected to immunocytochemistry for galectin3. The percentages of Atg-positive beads per galectin3-positive beads were enumerated. At least 30 beads were counted (n = 3). The values indicate the mean ± SD.

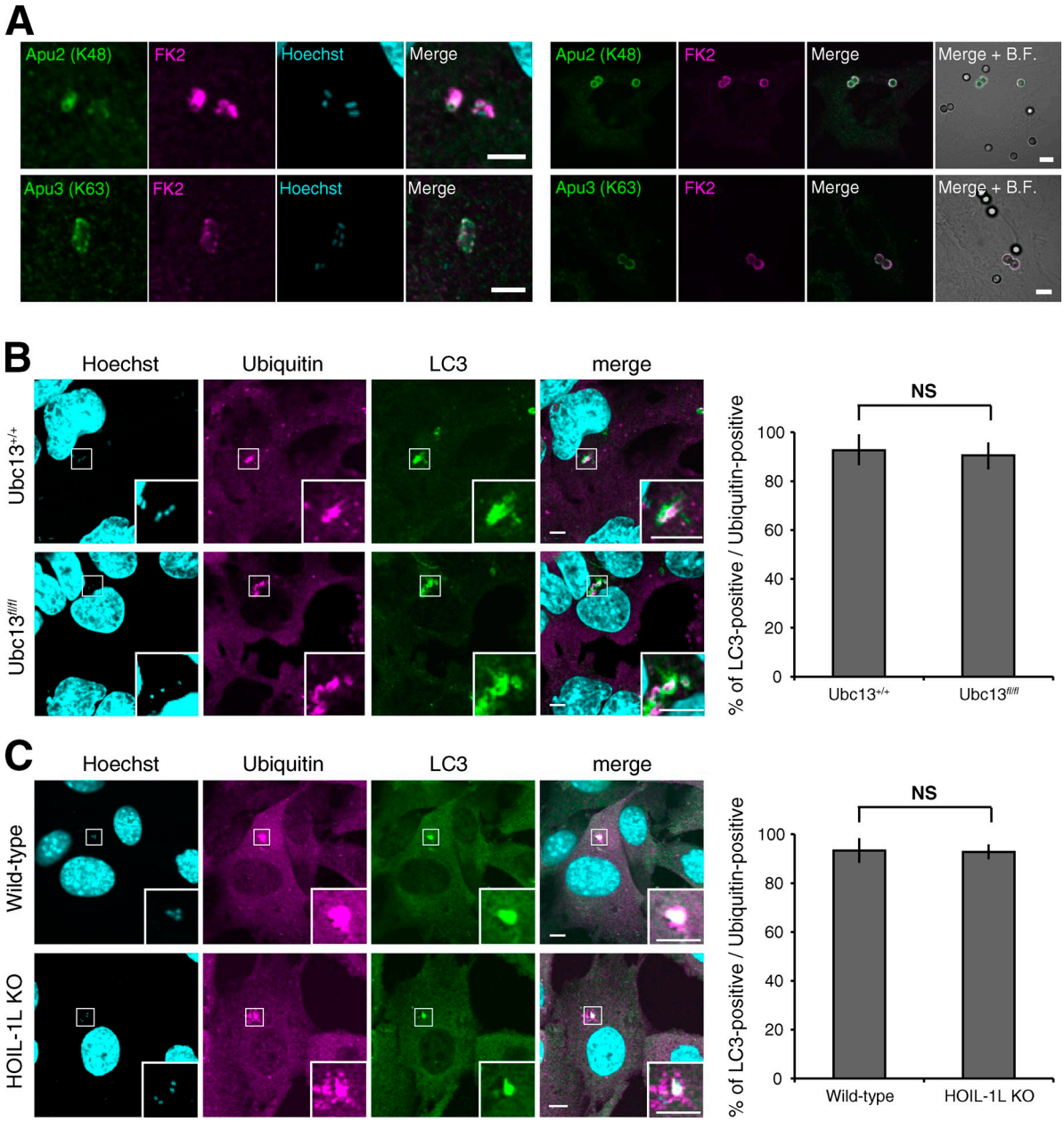


Figure S2. **Ub linkages in selective autophagy against invading *Salmonella* or transfected beads.** (A) HeLa cells were infected with *Salmonella* for 1 h (left) or transfected with Effectene-coated latex beads for 3 h (right), and then subjected to immunocytochemistry for poly-Ub (FK2) and K48-linked Ub (Apu2) or K63-linked Ub (Apu3). Bar, 5 µm. (B) Ubc13$^{+/+}$ or Ubc13$^{fl/fl}$ MEFs were infected with *Salmonella* for 1 h and then subjected to immunocytochemistry for poly-Ub (FK2) and LC3. Bar, 10 µm. The percentages of LC3-positive beads per Ub-positive *Salmonella* were enumerated. At least 30 Ub-positive *Salmonella* were counted (n = 3). The values are the mean ± SD. Statistical analysis was performed using Student's t test: NS, not significant. (C) Wild-type or HOIL 1L-KO MEFs were infected with *Salmonella* for 1 h and then subjected to immunocytochemistry for poly-Ub (FK2) and LC3. Bar, 10 µm. The percentages of LC3-positive beads per ubiquitin-positive salmonella were enumerated. At least 50 Ub-positive *Salmonella* were counted (n = 3). The values are the mean ± SD. Statistical analysis was performed using Student's t test. NS, not significant.

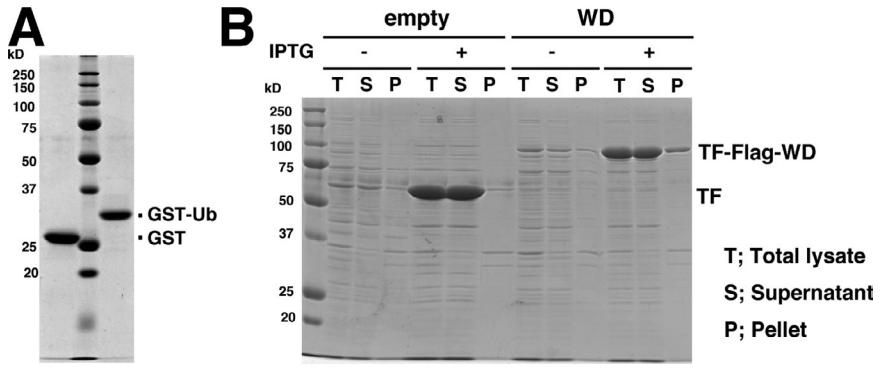


Figure S3. **Bacterially expressed recombinant proteins.** (A) GST or GST-ubiquitin were purified from *E. coli* lysates over glutathione Sepharose 4B and dialyzed with TBS. The recombinant proteins were subjected to SDS-PAGE followed by Coomassie brilliant blue staining. (B) *E. coli* BL21 (DE3) cells were transformed with the pCold-TF control (Empty) or pCold-TF encoding the WD40 β-propellers of Atg16L1 (WD). To induce the expression of the recombinant proteins, the bacteria were incubated at 16°C for 12 h in the presence or absence of 100 μM IPTG. Total cell lysates (T) were centrifuged at 15,000 rpm for 20 min and then separated into the soluble supernatant (S) and insoluble pellet (P).

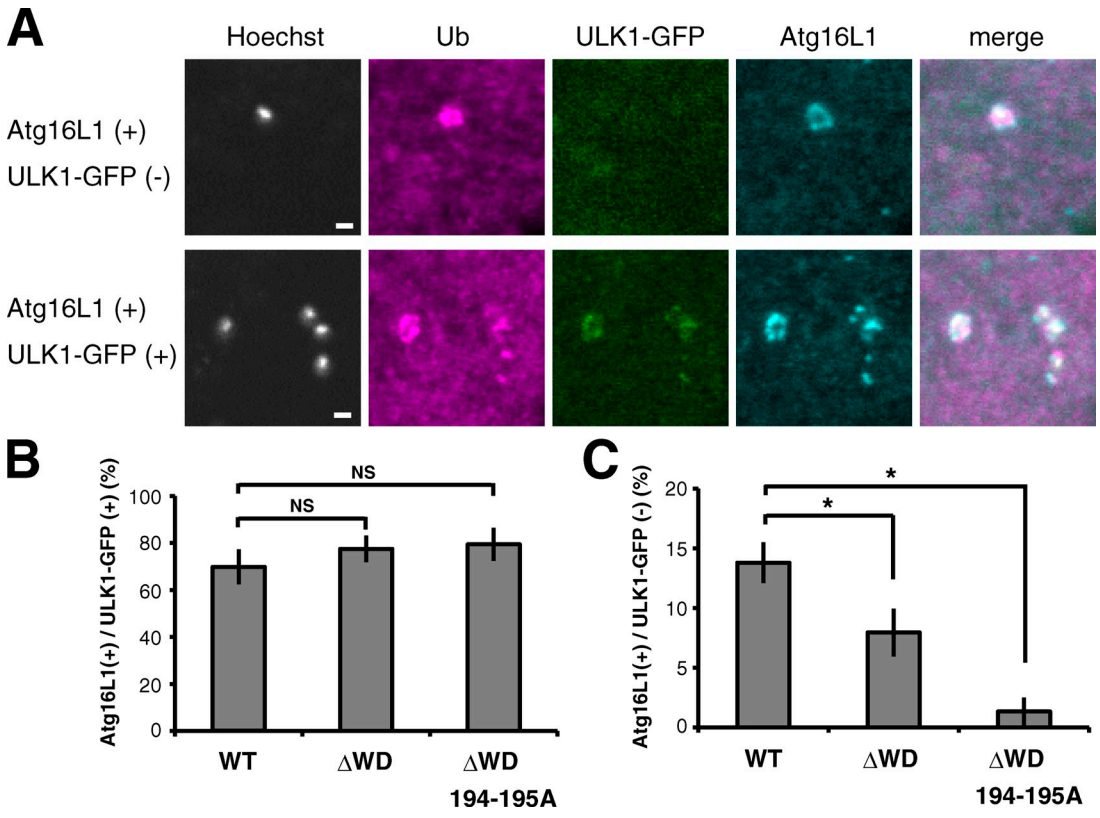


Figure S4. **Effect of deletion of WDR in Atg16L1 on the order of the Atg16L1 and ULK1-FIP200 complex recruitment to Ub-positive *Salmonella*.** Atg16L1-Δ/Δ MEFs stably expressing both ULK1-GFP and Atg16L1 full-length, ΔWD, or ΔWD + 194–195A mutant were infected with *Salmonella* (MOI = 100) for 10 min, chased for another 10 min, and then fixed. The samples were analyzed by immunocytochemistry for Atg16L1 and Ub. Bar: (A) 1 μm. The percentage of Atg16L1- and ULK1-GFP–positive bacteria per Ub-positive bacteria was enumerated by fluorescence microscopy (B and C). At least 50 bacteria were counted. The average ± SD is shown for three independent experiments. Statistical analysis was performed using Student's *t* test. *, $P < 0.05$; NS, not significant.

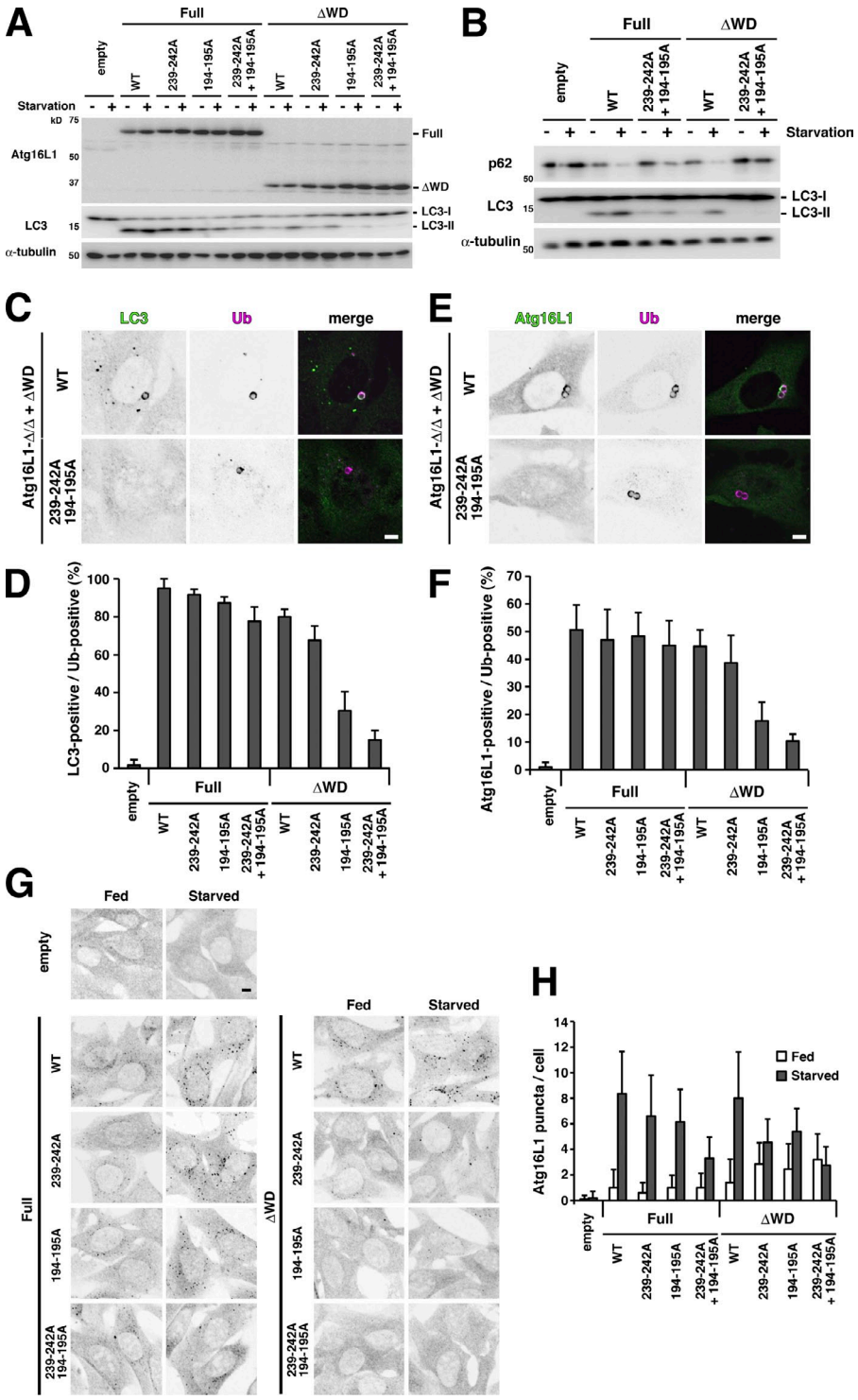
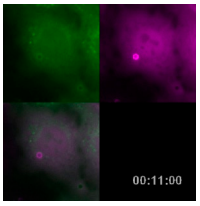


Figure S5. **Effect of Atg16L1 mutations on canonical and selective autophagy against transfected beads.** (A and B) Atg16L1-Δ/Δ MEFs stably expressing the indicated constructs were cultured in growth medium (−) or EBSS (+) for 1 h (A) or 2 h (B) and then harvested. Total cell lysates were examined by Western blot analysis using the indicated antibodies. (C–F) Atg16L1-Δ/Δ MEFs stably expressing the indicated constructs were transfected with beads for 3 h, and then analyzed by immunocytochemistry for LC3 (C) or Atg16L1 (E). Bar, 5 µm. The percentage of LC3- or Atg16L1-positive beads per Ub-positive beads was enumerated by fluorescence microscopy (D and F). At least 50 beads were counted. The average ± SD is shown for three independent experiments. (G) Atg16L1-Δ/Δ MEFs stably expressing the indicated constructs were cultured in growth medium (Fed) or EBSS (Starved) for 1 h and subjected to immunocytochemistry using an anti-LC3 antibody. Bar, 5 µm. (H) Atg16L1-Δ/Δ MEFs stably expressing the indicated constructs were cultured in growth medium (Fed) or EBSS (Starved) for 1 h, and subjected to immunocytochemistry using an anti-Atg16L1 antibody. The number of Atg16L1 puncta in each cell was counted for more than 50 cells. The average ± SD is shown for three independent experiments.

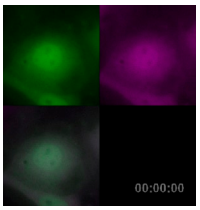
Table S1. **FIP200 peptides detected by affinity purification and mass spectrometry analysis**

Peptide	Mr(expt)	Mr(calc)	Delta	Miss	Score	Expect
CTAVEIR	847.4356	847.4222	0.0135	0	14	2.6
FKVPLGTK	888.5566	888.5433	0.0134	1	17	1.5
EIVLEDLKK	1085.603	1085.6332	−0.0301	1	22	0.35
ELEDTLQVR	1101.5726	1101.5666	0.0061	0	64	2.10E–05
IPLLECLTR	1113.6266	1113.6216	0.0051	0	47	0.00099
LDSELSALER	1131.598	1131.5771	0.0209	0	64	2.30E-05
ENIINDLSDK	1159.579	1159.5721	0.007	0	47	0.0012
VKHLENQIAK	1178.693	1178.6771	0.0159	1	24	0.22
QELANNLHVR	1192.6408	1192.6312	0.0096	0	77	1.10E–06
EAVICLQNEK	1202.6074	1202.5965	0.011	0	61	3.90E-05
ELAQGFLANQK	1217.641	1217.6404	0.0006	0	50	0.00064
DQCISELISR	1219.5934	1219.5867	0.0068	0	30	0.047
EQCDFSNSLK	1226.5378	1226.5237	0.0141	0	61	3.20E–05
TLQLKEEENK	1230.6574	1230.6455	0.0119	1	36	0.014
KENIINDLSDK	1287.6808	1287.667	0.0138	1	47	0.001
FLEQLEEQEK	1291.6422	1291.6296	0.0127	0	32	0.033
LMSQSMSSVSSR	1298.6078	1298.5959	0.012	0	−74	1.80E–06
SLLEQETENLR	1330.6794	1330.6728	0.0066	0	70	5.30E–06
TTNESLLTSFPK	1336.6942	1336.6874	0.0068	0	80	4.00E–07
STELVLSPDMPR	1343.6868	1343.6755	0.0114	0	52	0.0003
GDSSSLVAELQEK	1361.6722	1361.6674	0.0048	0	96	1.20E–08
AELQSLEQSHLK	1381.7353	1381.7201	0.0152	0	14	1.8
TQLALEMYEVAK	1394.7214	1394.7115	0.0099	0	64	1.90E–05
LDSLPEHEDSEK	1397.6436	1397.631	0.0126	0	39	0.0052
ENIINDLSDKLK	1400.7552	1400.7511	0.0042	1	64	1.60E–05
DKDLIESLSEDR	1418.6958	1418.6889	0.007	1	71	3.70E–06
FLEQLEEQEKR	1447.7412	1447.7307	0.0106	1	49	0.00053
KFDCELPDISLK	1463.745	1463.733	0.012	1	17	0.84
ASVSQTSPQSASSPR	1488.7264	1488.7168	0.0096	0	62	3.10E–05
LYALDQMIASCGR	1496.7242	1496.7115	0.0127	0	72	2.70E–06
VTSLHNQAFEIEK	1514.7792	1514.7729	0.0064	0	54	0.00015
GELVCLEEVLQNK	1529.7808	1529.7759	0.0049	0	69	5.50E–06
LKGELVCLEEVLQNK	1770.954	1770.9549	-0.0009	1	46	0.00086
LKGELVCLEEVLQNK	1770.9637	1770.9549	0.0087	1	−33	0.017
IQDNNENYQVGLAELR	1874.92	1874.9122	0.0078	0	93	1.80E–08
TSLIAEQQTNFNTVLTR	1935.0058	1935.0061	−0.0003	0	57	6.50E–05

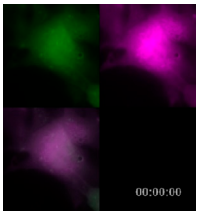
ATG5–ATG12–ATG16L1 protein complexes were affinity purified on Strep-Tactin matrices, and co-purifying proteins were identified by mass spectrometry. In brief, co-purifying proteins were identified after SDS-PAGE separation and band excision. Proteins were digested with trypsin and identified by separating the peptide mixtures by liquid chromatography with online tandem mass spectrometry (LC-MS/MS). Mr(expt), experimental molecular weight (Da); Mr(calc), calculated molecular weight (Da); Delta, mass error (Da); Miss, number of missed cleavages; expect, expectation value.



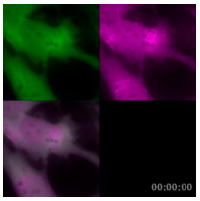
Video 1. **Dynamics of LC3 and galectin3 in autophagy against transfected bead.**



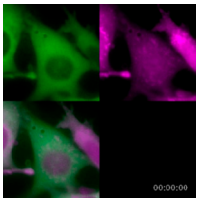
Video 2. **Dynamics of Ub and galectin3 in autophagy against transfected bead.** (S1 and S2) NIH3T3 cells stably expressing mStrawberry (mStr)-Gal3 (magenta) and GFP-LC3 (green, S1) or mStr-Gal3 (magenta) and GFP-Ub (green, S2) were transfected with Effectene-coated latex beads for 30 min and then washed. Live cells were observed at 1-min intervals using a microscope (model IX81; Olympus) equipped with a xenon lamp and cooled charge-coupled device camera (CoolSnap HQ; Roper Scientific) under the control of MetaMorph software. Time is shown in h:min:s.



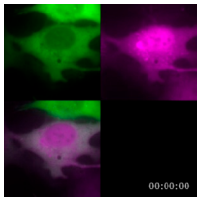
Video 3. **Dynamics of LC3 and Ub in autophagy against transfected bead.**



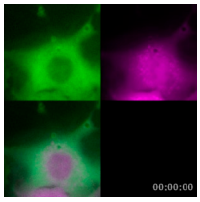
Video 4. **Dynamics of Atg5 and Ub in autophagy against transfected bead.**



Video 5. **Dynamics of WIPI-1 and Ub in autophagy against transfected bead.**



Video 6. **Dynamics of Atg14L and Ub in autophagy against transfected bead.**



Video 7. **Dynamics of ULK1 and Ub in autophagy against transfected bead.** (S3–S7) NIH3T3 cells stably expressing mStr-Ub (magenta) and GFP-tagged (green) LC3 (S3), Atg5 (S4), WIPI-1 (S5), Atg14L1 (S6), or ULK1 (S7) were transfected with Effectene-coated latex beads for 30 min. Live cells were observed at 1-min intervals using a microscope (model IX81; Olympus) equipped with a xenon lamp and cooled charge-coupled device camera (CoolSnap HQ; Roper Scientific) under the control of MetaMorph software. Time is shown in h:min:s. We excluded Atg9L1 in this experiment because Atg9L1 localized to the trans-Golgi network and early endosomes under steady-state conditions, which cannot be distinguished from recruitment to bead-containing endosomes (Young et al., 2006; Kageyama et al., 2011).

References

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Young, A.R., E.Y. Chan, X.W. Hu, R. Köchl, S.G. Crawshaw, S. High, D.W. Hailey, J. Lippincott-Schwartz, and S.A. Tooze. 2006. Starvation and ULK1-dependent cycling of mammalian Atg9 between the TGN and endosomes. *J. Cell Sci.* 119:3888–3900. http://dx.doi.org/10.1242/jcs.03172