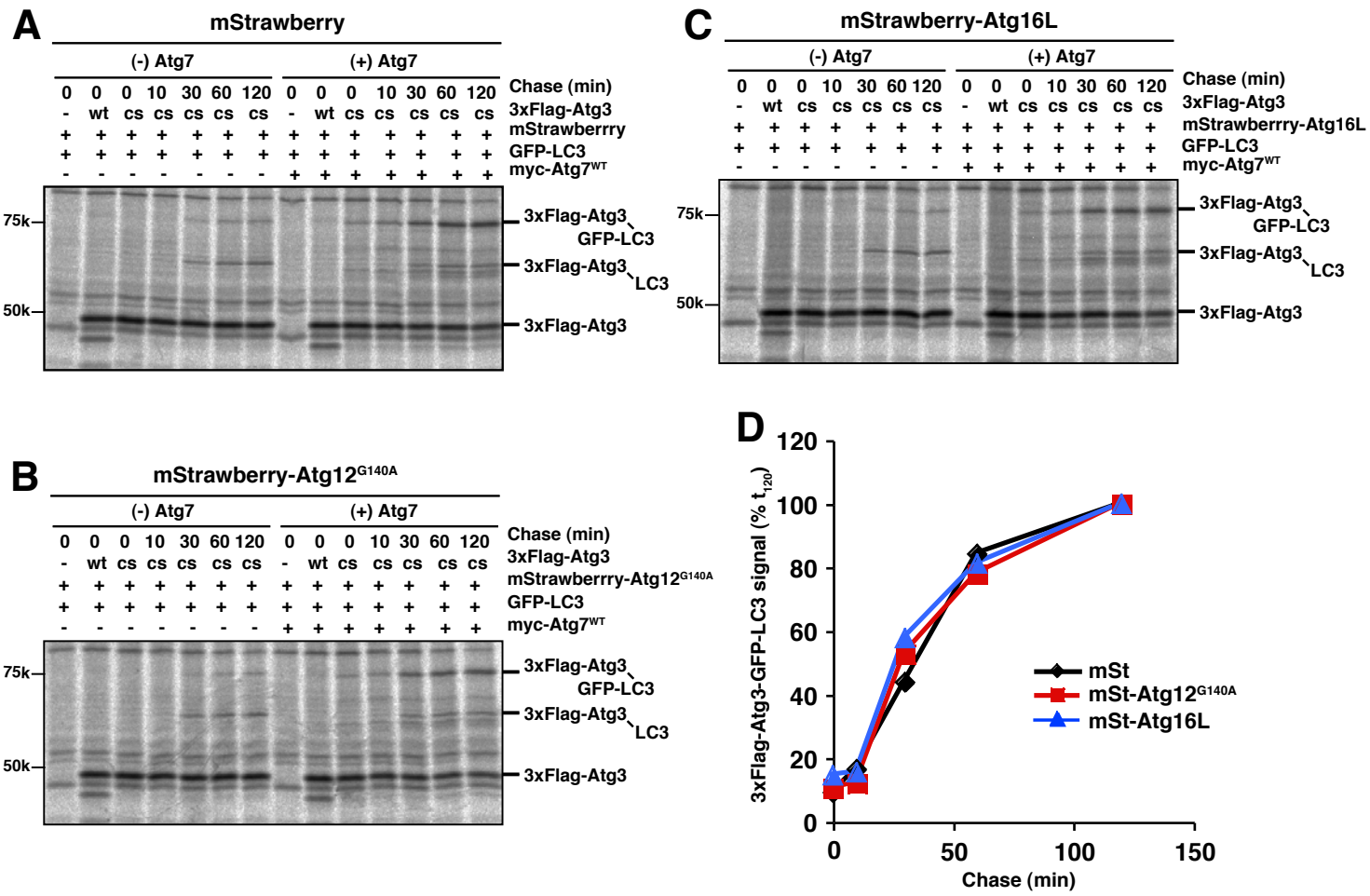
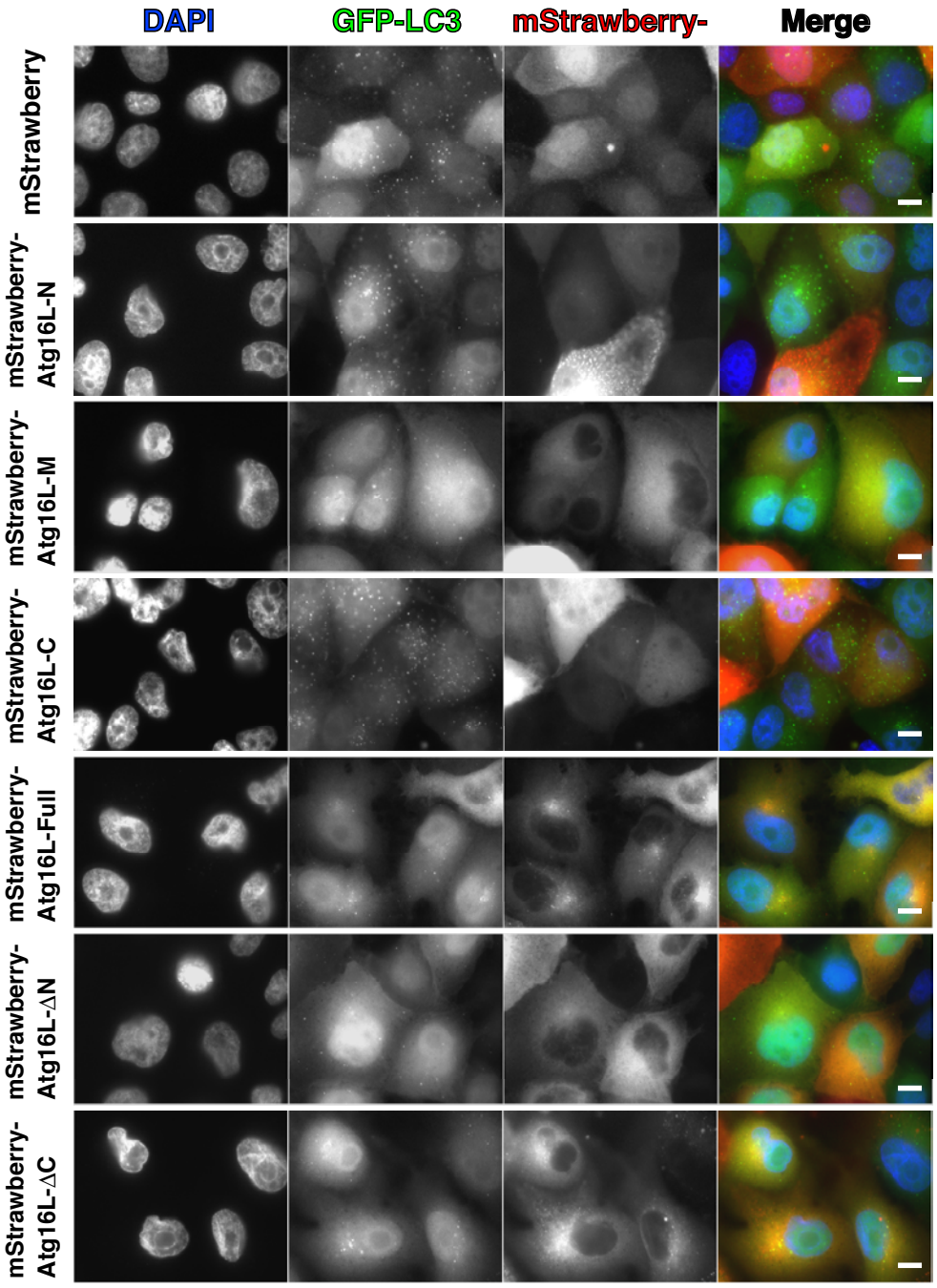


Supplemental Figure 1. Kinetic analysis of Atg3-LC3 intermediate formation.

(A-D) PC12 cells were co-infected using combination of recombinant a denoviruses as indicated. After 40 hours incubation, cells were incubated with methionine-free DMEM for 1 hour before labeling and then pulse labeled for 10 min with [^{35}S] methionine in methionine-free DMEM. The cells were washed with PBS and then chased for indicated time period in DMEM containing 10 mM methionine. After the chase, the cells were washed with ice-cold PBS and lysed using following buffer: 50 mM Tris-HCl, pH7.6, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM PMSF, and protease inhibitor cocktail (Roche). The lysates were subjected to immunoprecipitation with anti-Flag M2-conjugated agarose beads, and washed. The immune complexes were eluted from the beads by boiling in electrophoresis sample buffer. (A-C) The eluted samples were separated by SDS-PAGE using 8% polyacrylamide gel, and visualized using an image analyzer, BAS1500 (FujiFilm). (D) The intensities of the bands corresponding to 3xFlag-Atg3-GFP-LC3 intermediate in myc-Atg7 expressing cells were quantified, and the number is presented as a percentage of the signal at time 120 min.

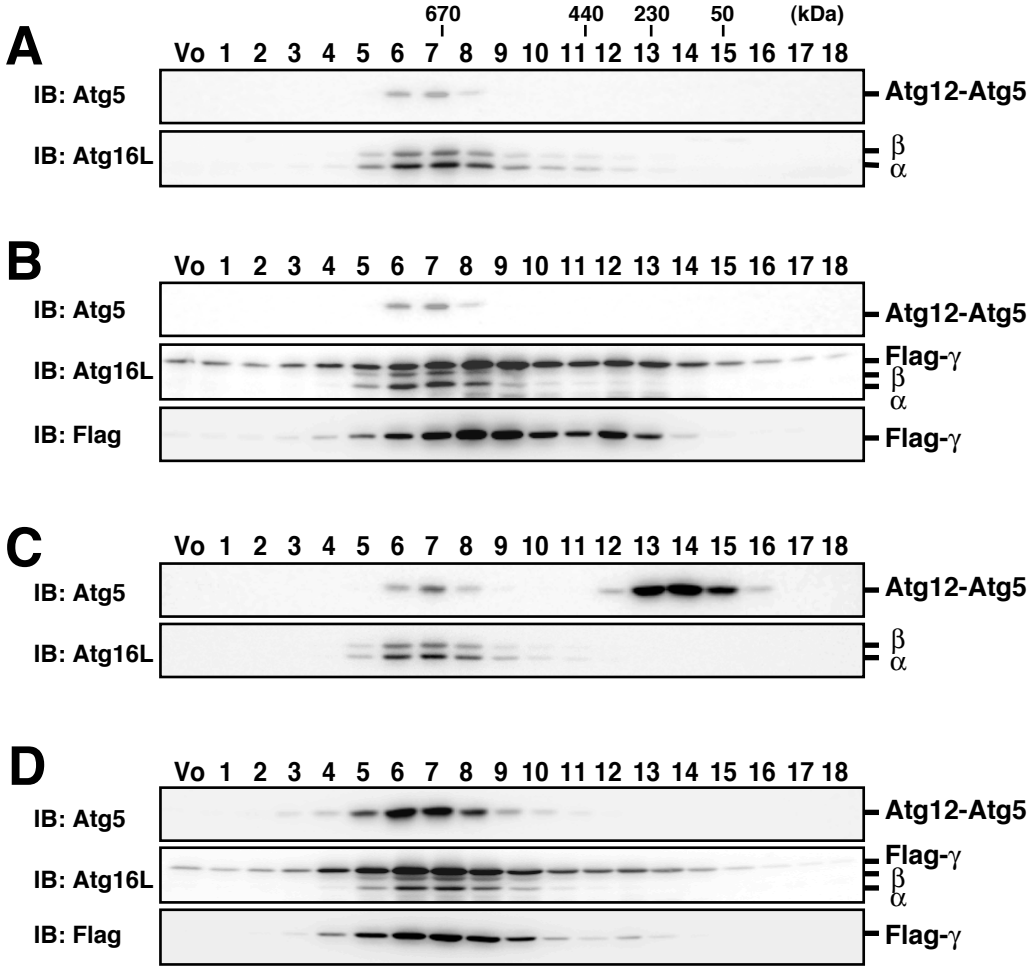
Figure S1



Supplementary Figure 2. Effect of overexpression of mStrawberry-Atg16L constructs on GFP-LC3 dots in MDCK cells.

MDCK cells stably expressing GFP-LC3 were infected with adenovirus bearing mStrawberry (Mock) or mStraberry-fused Atg16L deletion constructs, and incubated for 40 hours. The cells were then cultured in HBSS (Starved) for 2 hours, fixed, and observed using fluorescence microscopy. Bar indicates 10 μm.

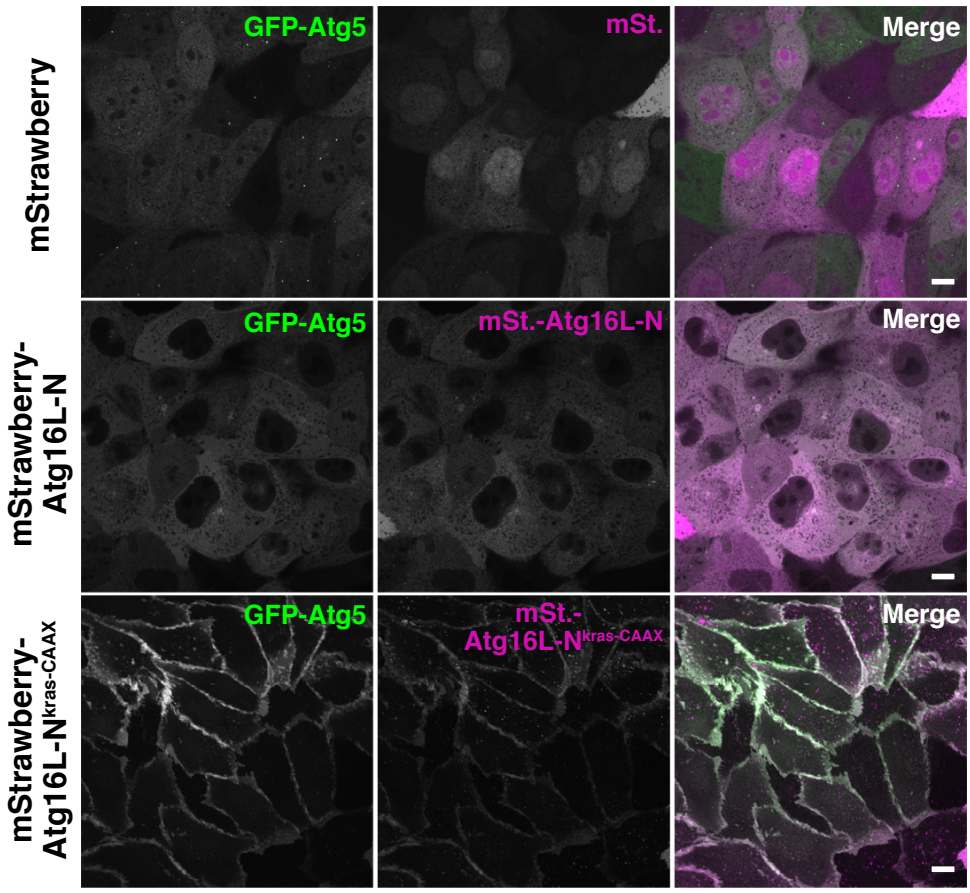
Figure S2



Supplementary Figure 3. Transiently co-expressed Atg12, Atg5, and Flag-Atg16L form a ~800-kDa multimeric complex.

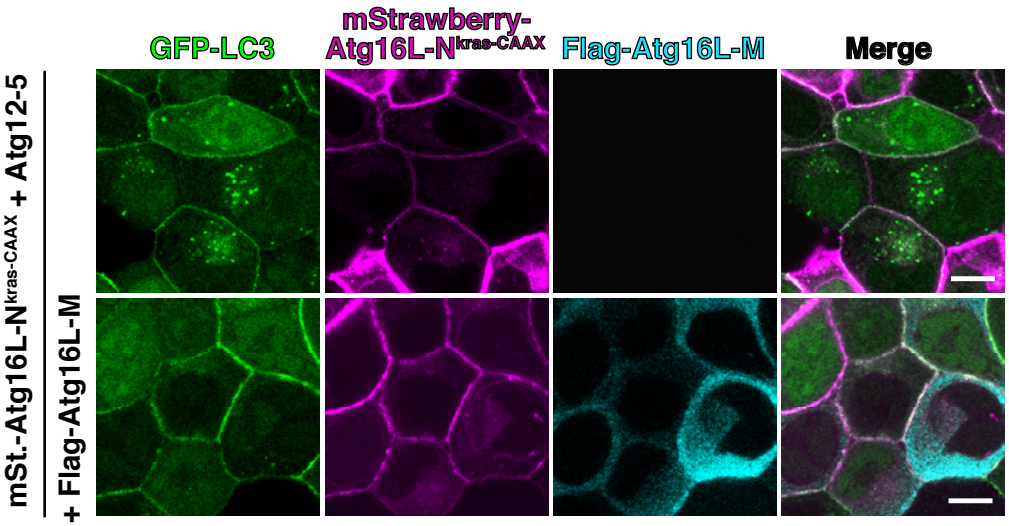
PC12 cells were co-infected using a combination of adenoviral expression vector as follows: (A) Mock, (B) Flag-Atg16L, (C) Atg12 and Atg5, (D) Flag-Atg16L, Atg12, and Atg5. Cytosolic fractions of cell homogenates were separated by size exclusion chromatography. Each fraction was subjected to Western blotting using each antibody. From top, anti-Atg5, anti-Atg16L, anti-Flag. The positions of the molecular mass standards are shown. Vo, void fraction.

Figure S3



Supplementary Figure 4. GFP-Atg5 is recruited to the plasma membrane by an mStrawberry-Atg16L-N chimera with the C-terminal 17 amino acids of K-ras. MDCK cells stably expressing GFP-Atg5 were infected with adenovirus bearing mStrawberry, mStrawberry-Atg16L-N, or mStrawberry-Atg16L-N$^{Kras\text{-}CAAX}$ and incubated for 40 hours. Cells were fixed and observed with a confocal laser scanning microscopy (FV1000, Olympus). Bar indicates 10 μm.

Figure S4



Supplementary Figure 5. The effect of Flag-Atg16L-M overexpression on the localization of GFP-LC3.

MCF7 cells stably expressing GFP-LC3 were infected with adenoviruses as indicated (Upper panel; mStrawberry-Atg16L-N$^{Kras-CAAX}$, Atg12, and Atg5. Lower panel; mStrawberry-Atg16L-N$^{Kras-CAAX}$, Atg12, Atg5, and Flag-Atg16L-M). After 40 hours incubation, the cells were cultured in HBSS for 2 hours, and fixed. Samples were incubated with anti-Flag monoclonal antibody and subsequently anti-mouse antibody conjugated to Alexa647. The cells were observed with a confocal laser scanning microscopy (FV1000, Olympus). Bar indicates 10 μm.

Figure S5