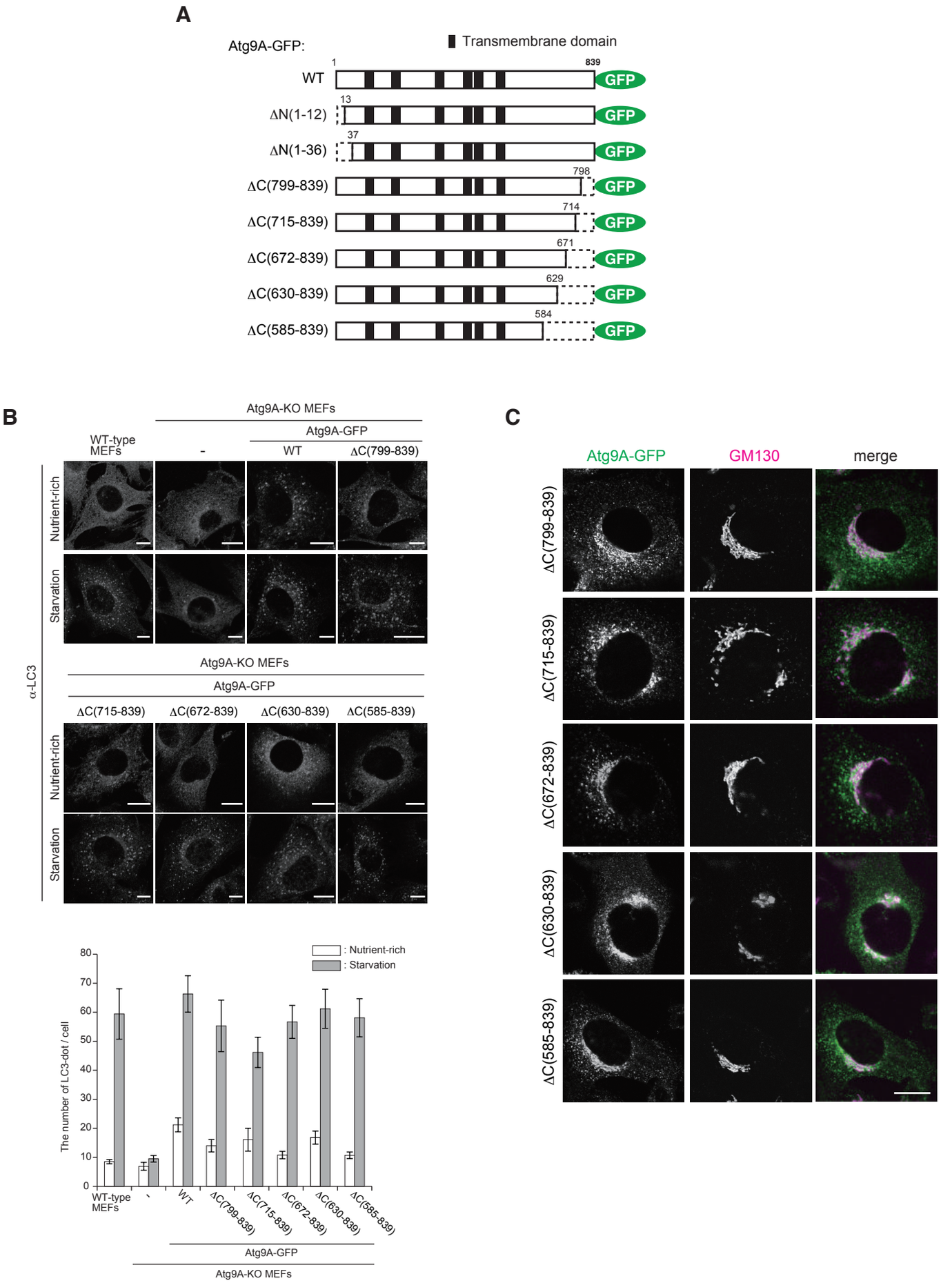
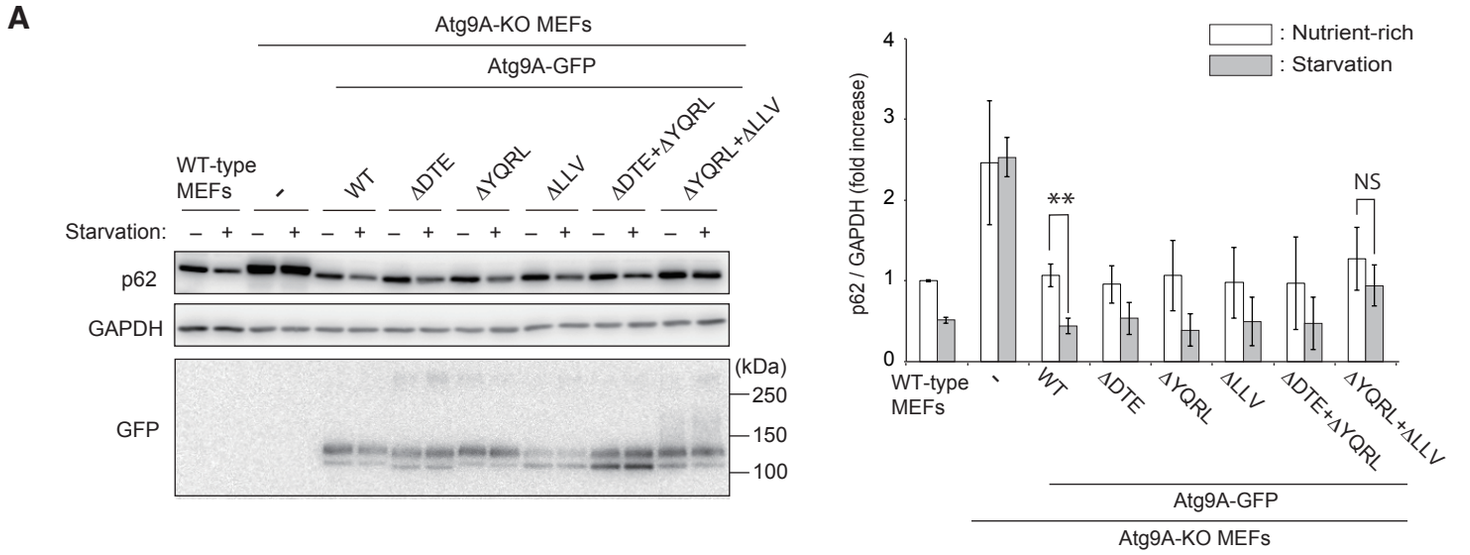


Figure S1. The C-terminal deletions of Atg9A dose not affect autophagosome formation and its localization.

(A) Schematic diagram of wild-type Atg9A and its deletion mutants tagged with GFP. The transmembrane domain is indicated as black boxes. (B) Wild-type or Atg9A-KO MEFs stably expressing the indicated constructs were cultured in growth medium (Nutrient-rich) or EBSS (Starvation) for 1 h, then analyzed by immunocytochemistry for LC3. The number of LC3 puncta in each cell was counted for more than 30 cells. The average ± SD is shown for three independent experiments. Bar, 20 µm. (C) Atg9A-KO MEFs stably expressing the indicated constructs were analyzed by immunocytochemistry for anti-GM130. Bar, 20 µm.



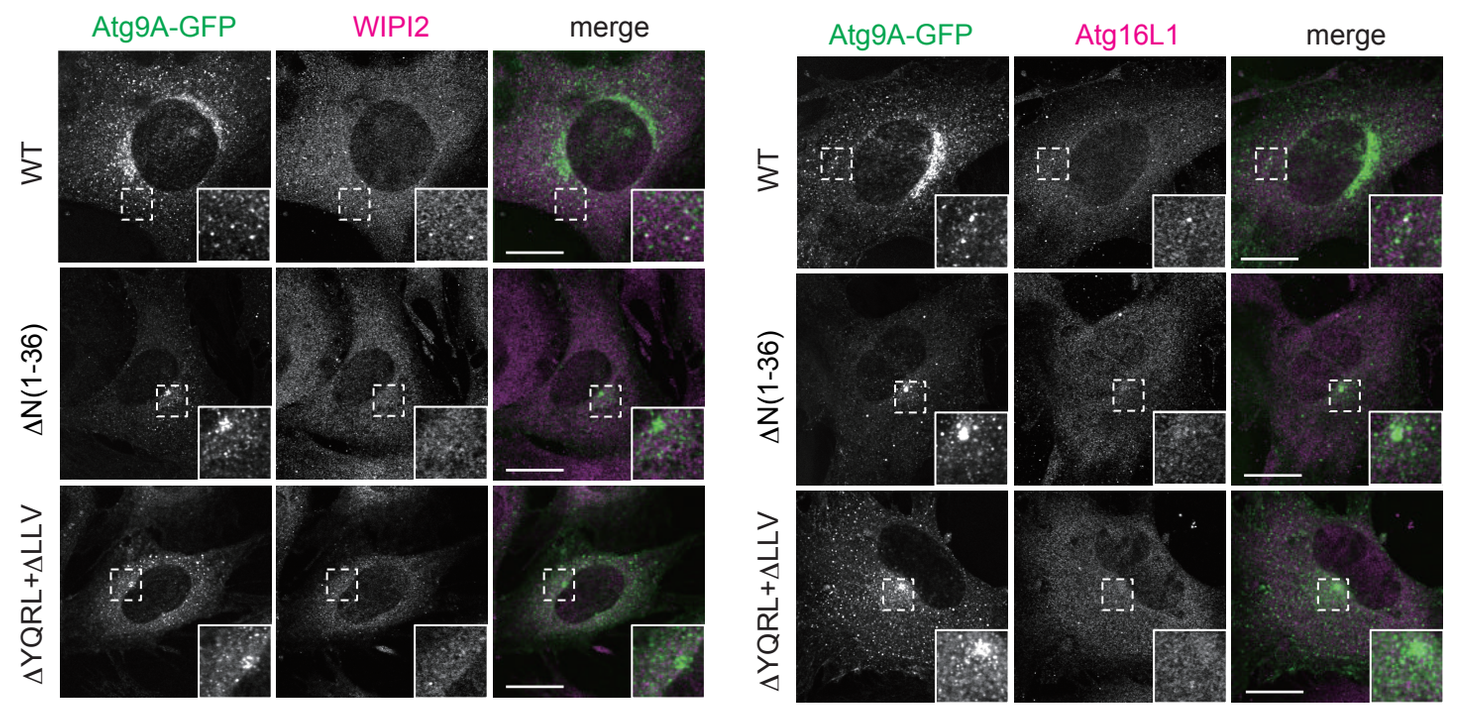


Figure S2. The effects of alanine substitutions in sorting motifs on autophagy.

(A) Wild-type MEFs or Atg9A-KO MEFs stably expressing the indicated constructs were cultured in growth medium (Nutrient-rich) or EBSS (Starvation) for 2 h, and subjected to western blotting using indicated antibodies. The graph indicates the p62 signal relative to GAPDH. The average ±SD is shown for three independent experiments. Statistical analysis was performed by two-tailed paired Student' s t-test: **, $P < 0.01$; NS, not significant. (B) Atg9A-KO MEFs stably expressing the indicated constructs were cultured in EBSS (Starvation) for 1 h, fixed, and then analyzed by immunocytochemistry for anti-WIPI2 or anti-Atg16L1. Bar, 20 μm.

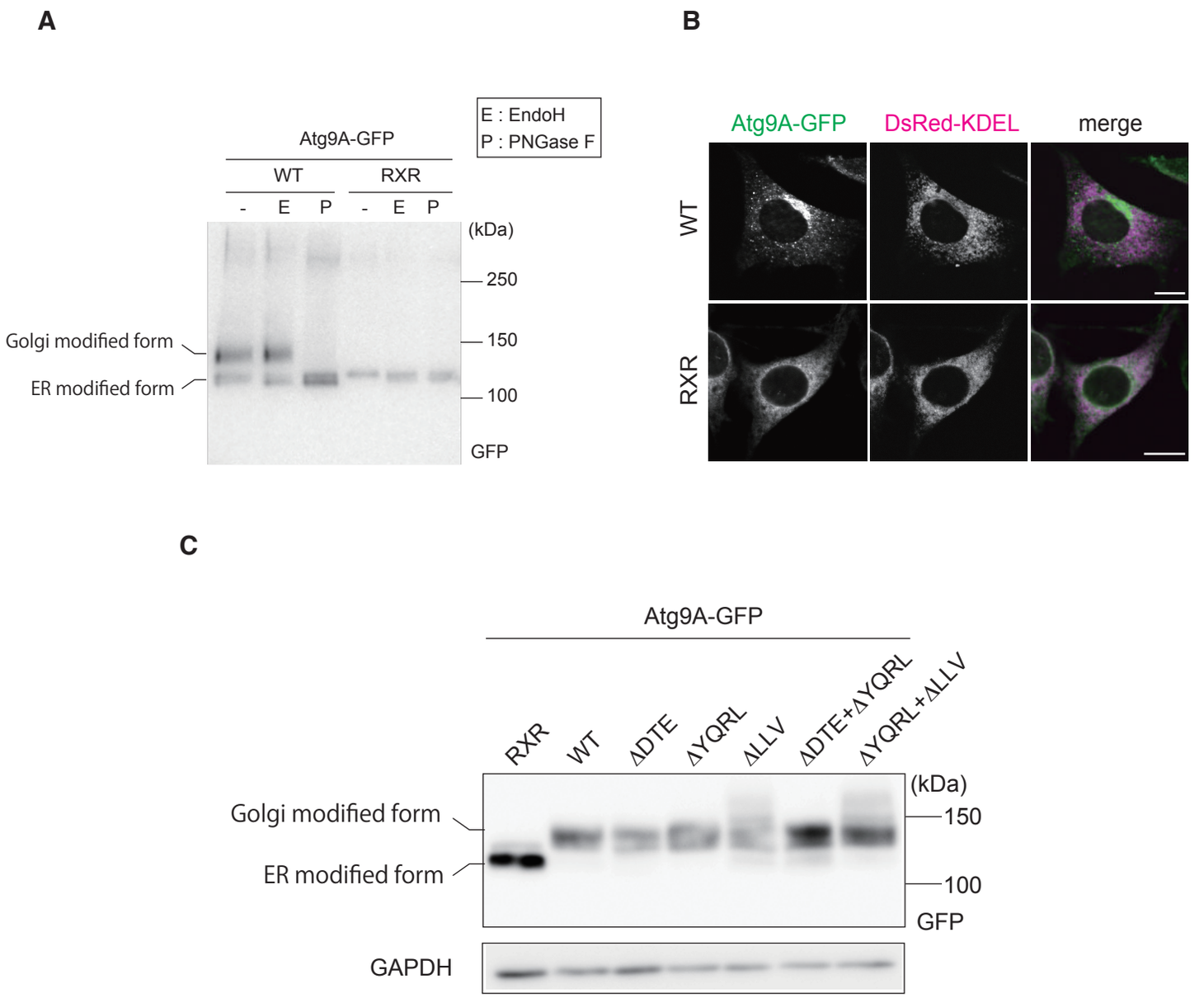


Figure S3. The effects of alanine substitutions in sorting motifs on N-glycan modification of Atg9A.

(A) Atg9A-KO MEFs stably expressing the indicated constructs were lysed and subjected to treatment with EndoH or PNGase F, and analyzed by western blotting with anti-GFP. Atg9A-WT-EGFP was detected as two major bands by western blotting. When the sample was treated with endoglycosidase H (Endo H) which removes only high mannose and some hybrid types of N-glycan from glycoproteins (Maley et al., 1989), the faster migrating band was shifted slightly, whereas the slower migrating band was resistant to EndoH. Treatment with N-Glycosidase F (PNGase F), which removes all N-glycan from glycolproteins (Maley et al., 1989), resulted in mobility shift of both bands to a single band. (B) Atg9A-KO MEFs stably expressing the indicated constructs were transiently transfected with pDsRed-KDEL expression plasmid, fixed, and examined by fluorescence microscopy. When RXR motif, the ER retrieval signal, was added to its C-terminus of Atg9A, the mutant showed good colocalization with an ER marker (DsRed-KDEL) (C) Atg9A-KO MEFs stably expressing the indicated constructs were lysed and analyzed by western blotting with anti-GFP.

Maley, F., Trimble, R. B., Tarentino, A. L. and Plummer, T. H. Jr. (1989). Characterization of glycoproteins and their associated oligosaccharides through the use of endoglycosidases. *Anal. Biochem.* **180**, 195-204.

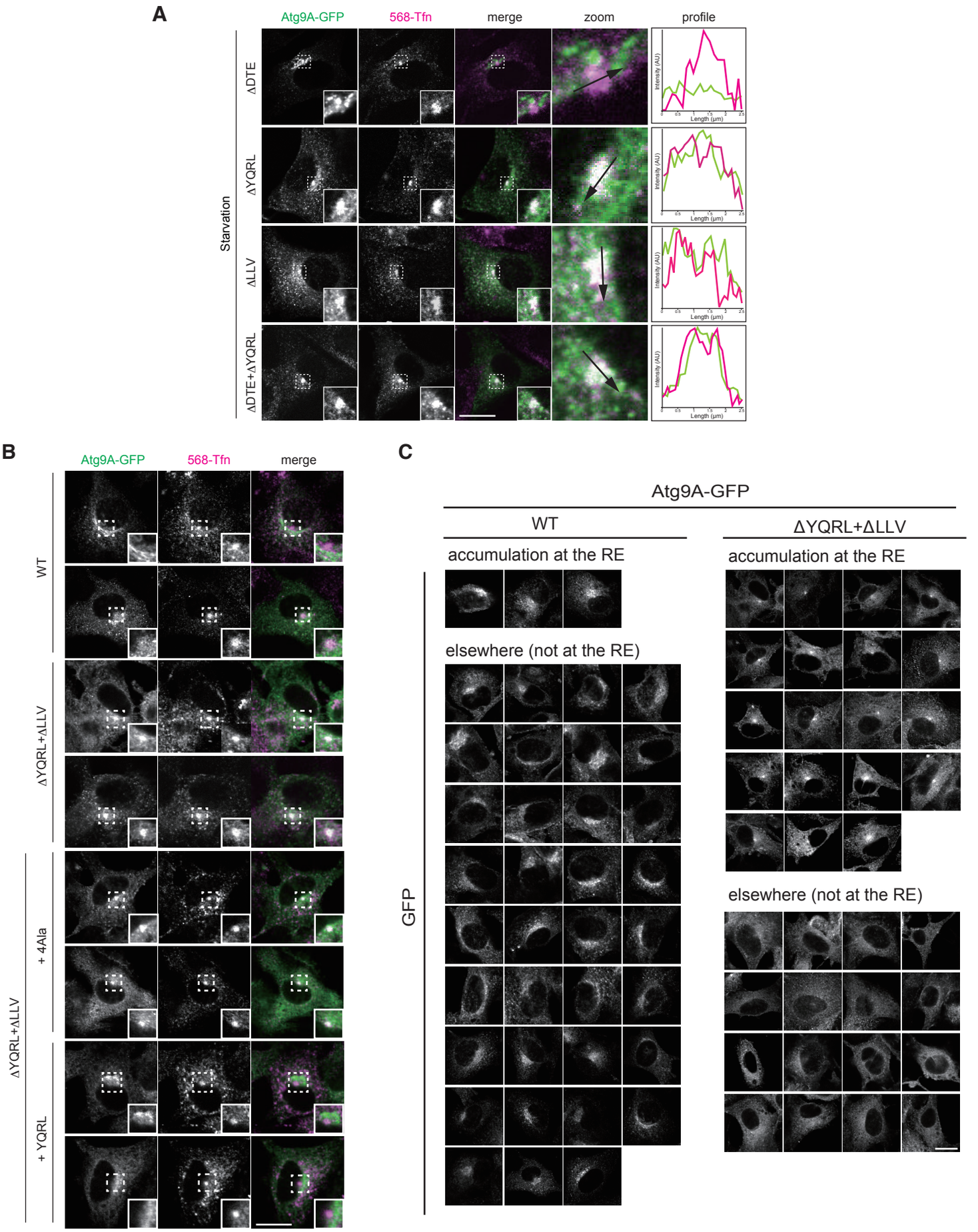


Figure S4. The effects of alanine substitutions in sorting motifs on Atg9A localization.

(A-B) Atg9A-KO MEFs stably expressing the indicated constructs were cultured in EBSS (Starvation) with 10 μg/ml 568-Tfn for 1 h, and then observed by fluorescence microscopy. Bar, 20 μm. (C) Atg9A-KO MEFs stably expressing indicated constructs were cultured in EBSS (Starvation) with 10 μg/ml 568-Tfn for 1 h, fixed, and then observed by fluorescence microscopy. Bar, 20 μm. Examples of Atg9A accumulated at the recycling endosomes (RE) and remained in elsewhere (not at the RE) were shown for both WT and ΔYQRL+ΔLLV mutant cells. Atg9A WT showed the accumulation at the RE phenotype in only 7.9 % cells (3 of 38 total cell counted), while ΔYQRL+ΔLLV mutant showed in 54.3% cells (19 of 35 total cell counted).