Figure S1. The effect on localization of Atg14L with nonconventional autophagy inducers and deletion mutations. (a) HEK293 cells expressing both GFP-DFCP1 and Flag-Atg14L were treated with etoposide for 18 h or LiCl for 24 h. The number of GFP-DFCP1 and Flag-Atg14L puncta per cell was counted, and mean ± SD values are presented. (b and c) HEK293 cells were transiently transfected with plasmid vector of the GFP-Atg14L deletion construct I-II_III (b) or ΔI-II_III (c). Cells were observed before or after starvation. (d) GFP-Atg14LCAAX or GFP-Atg14L4C4ACAAX was transfected. The ratio of signal intensity of GFP-Atg14L, GFP-Atg14LCAAX, or GFP-Atg14L4C4ACAAX in the plasma membrane is indicated, and mean ± SD values are presented. Bars, 10 µm.
Figure S2. **PI3P-positive puncta were increased by overexpression of Atg14L but not by the Atg14L4C4A mutant.** A549 cells were transiently transfected with adenovirus harboring GFP-Atg14L or GFP-Atg14L4C4A. (a and b) The cells before starvation (a) or after starvation (b) were fixed and incubated with the GST-2xFYVE probe and immunostained with anti-GST antibody. Bars, 10 µm.
Figure S3. GFP-ER-Atg14L4C4A shows an ER localization pattern in HEK293 cells. (a) HEK293 cells expressing GFP-ER-Atg14L4C4A were subjected to pull-down by GFP antibody and immunoblotted with the indicated antibodies. (b) HEK293 cells were transiently transfected with GFP-ER-Atg14L4C4A plasmids. The cells were fixed and immunostained with anticalnexin antibody. (c) HEK293 cells were cotransfected with tandem fluorescent-tagged LC3. The dots with only a red signal represent matured autolysosomes and are indicated by an arrowhead. The number of GFP-positive or GFP- and mRFP-positive puncta per cell was counted, and mean ± SD values are presented. (d) HEK293 cells were transiently transfected with GFP, GFP-Atg14L, GFP-Atg14L4C4A, or GFP-ER-Atg14L4C4A plasmids. The cells were fixed and immunostained with anti-LC3 antibodies. Bars, 10 µm.