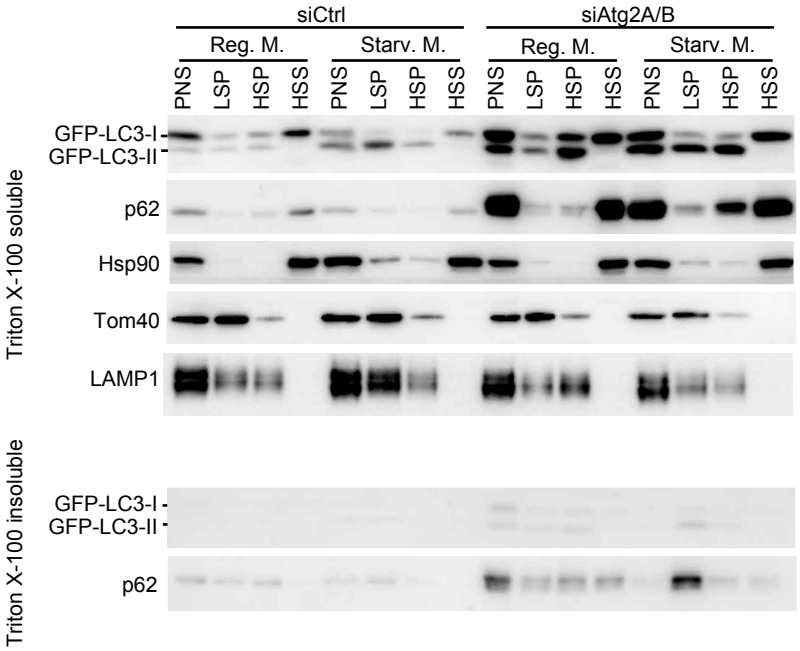
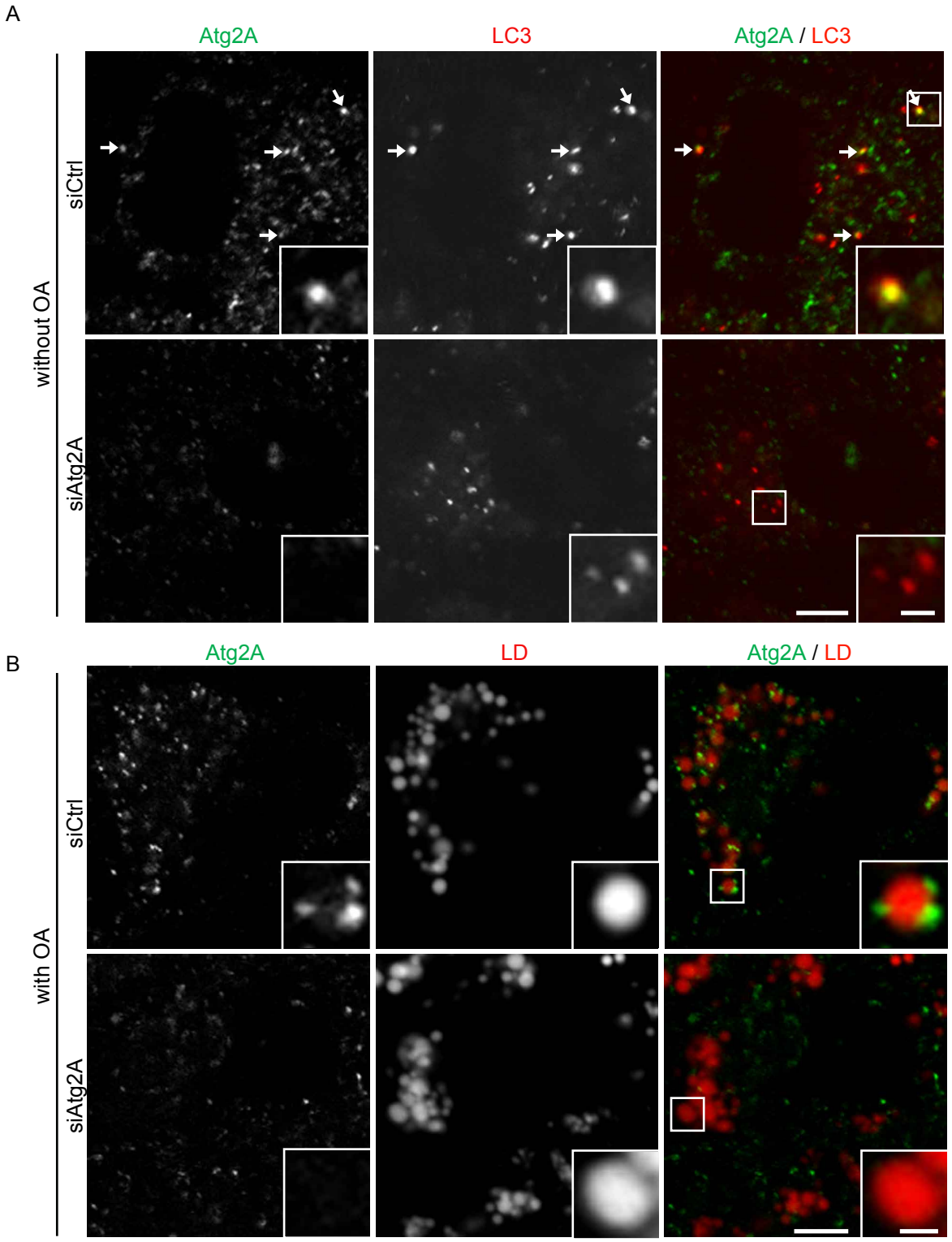


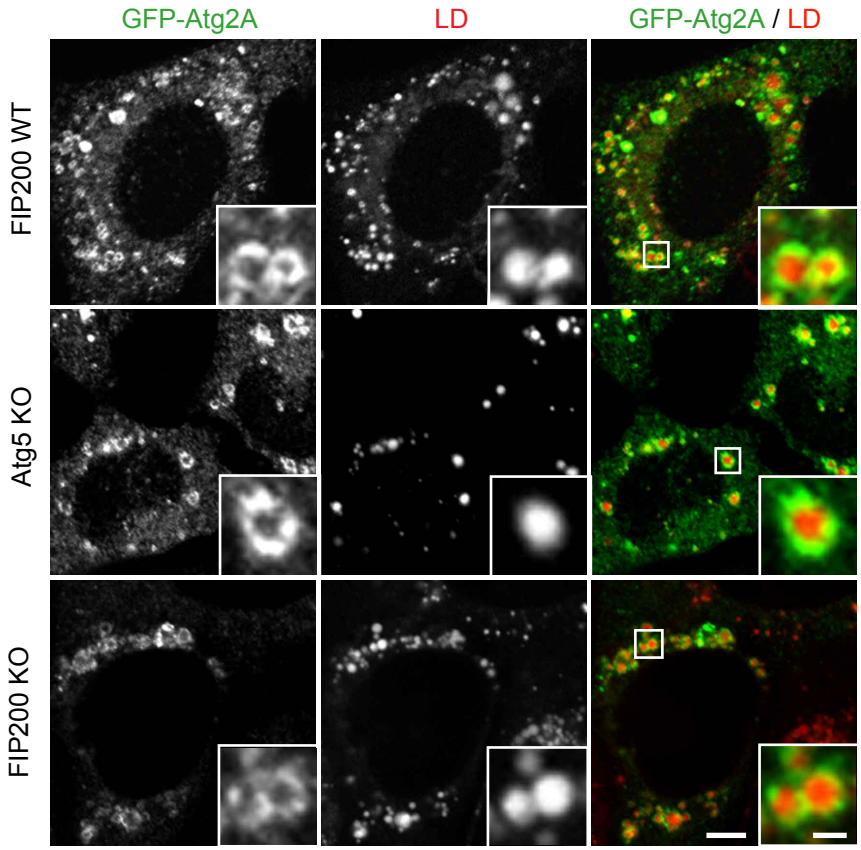
Supplemental Figure S1. Separation of autophagy-related membranes in wild-type and Atg2A/B-depleted cells.

HeLa cells expressing GFP-LC3 were treated with control siRNA or a mixture of siRNAs against Atg2A and Atg2B, and cultured in regular DMEM or starvation medium for 2 h. The PNS was separated into LSP, HSP, and HSS fractions. These samples were treated with 1% Triton X-100, separated into soluble (supernatant) and insoluble (pellet) fractions, and then analyzed by SDS-PAGE and immunoblotting using anti-GFP, anti-p62, anti-Lamp1, anti-Hsp90, and anti-Tom40 antibodies.



Supplemental Figure S2. Silencing of Atg2A abolishes Atg2A-LC3 colocalization and Atg2A recruitment to lipid droplets.

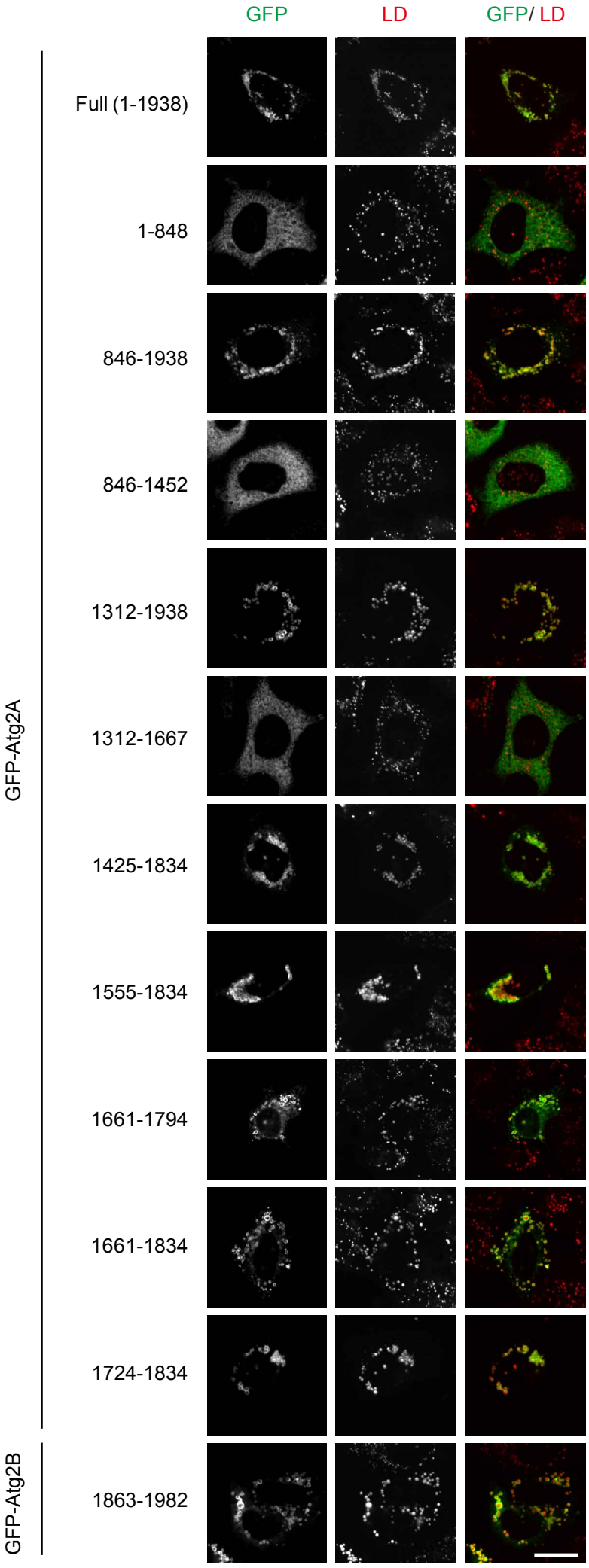
(A and B) HeLa cells were treated with control or Atg2A siRNA for 5 days, then cultured in the absence (A) or presence (B) of oleic acid–BSA conjugate for 24 h. Cells were then starved for 2 h (A, B), and stained with BODIPY® 558/568-C12 as in Figure 5A (B). Endogenous Atg2A and LC3 were detected with anti-Atg2A and anti-LC3 antibodies, and subjected to confocal microscopy. Signal color is indicated by color of typeface. Scale bars, 5 μm, and1 μm in inset.



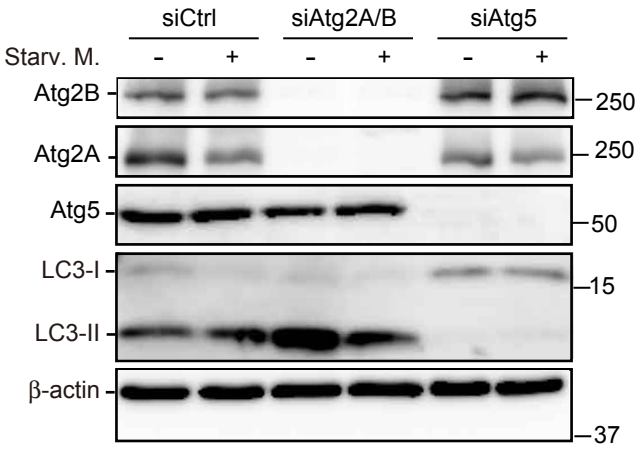
Supplemental Figure S3. Localization of Atg2A to lipid droplets is independent of FIP200 and Atg5.

Wild-type MEFs, FIP200 KO MEFs, and Atg5 KO MEFs stably expressing GFP-Atg2A were cultured in regular DMEM containing oleic acid–BSA conjugate for 36 h. After 1 h of culture in starvation medium containing BODIPY® 558/568-C12, cells were fixed, stained with anti-GFP antibody, and subjected to confocal microscopy. Signal color is indicated by color of typeface. Scale bars, 5 μm, and 1 μm in inset.

Figure S4



Supplemental Figure S4. Localization of GFP-Atg2A and GFP-Atg2B deletion mutants to lipid droplets.
HeLa cells transfected with expression plasmids encoding the indicated GFP-fused Atg2A and Atg2B fragments were cultured with oleic acid-BSA for 24 h, and then with BODIPY® 558/568-C12 for additional 1 h. Cells were subjected to immunofluoresence microscopy using anti-GFP antibody. Signal color is indicated by color of typeface. Scale bar, 20 μm.



Supplemental Figure S5. Silencing efficiency of Atg2A/B and Atg5 in HeLa cells.
HeLa cells were treated with control siRNA, a mixture of siRNAs against Atg2A and Atg2B, or siRNA against Atg5. Cells were cultured in regular or starvation medium for 2 h, and then subjected to immunoblot analysis using the indicated antibodies.

Supplementary Movies S1 and S2. Lipid droplet distribution and movement in control siRNA- and Atg2A/B siRNA-treated HeLa cells.

Hela cells were treated with control siRNA (Movie S1) or a mixture of siRNAs against Atg2A and Atg2B (Movie S2) for 5 days and cultured with oleic acid for 16 h. After 1 h of culture in the presence of BODIPY® 558/568 C12, the BODIPY signals were imaged at 1 frame per 2.2 sec. The playback rate is 6 frames per sec.