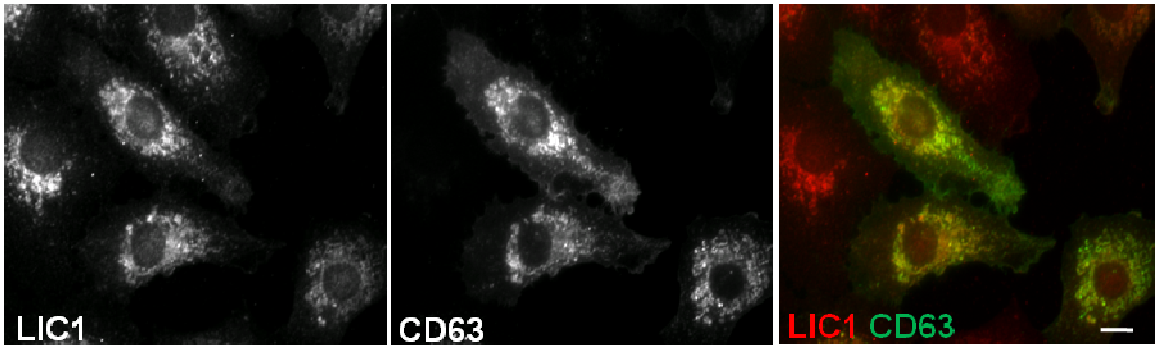


Figure S1. LIC1 Colocalizes with Over-expressed Late Endocytic Marker GFP-CD63. Immunocytochemistry of Rat2 cells over-expressing a GFP-CD63 (green) and stained for LIC1 (red). Scale bars, 5µm.

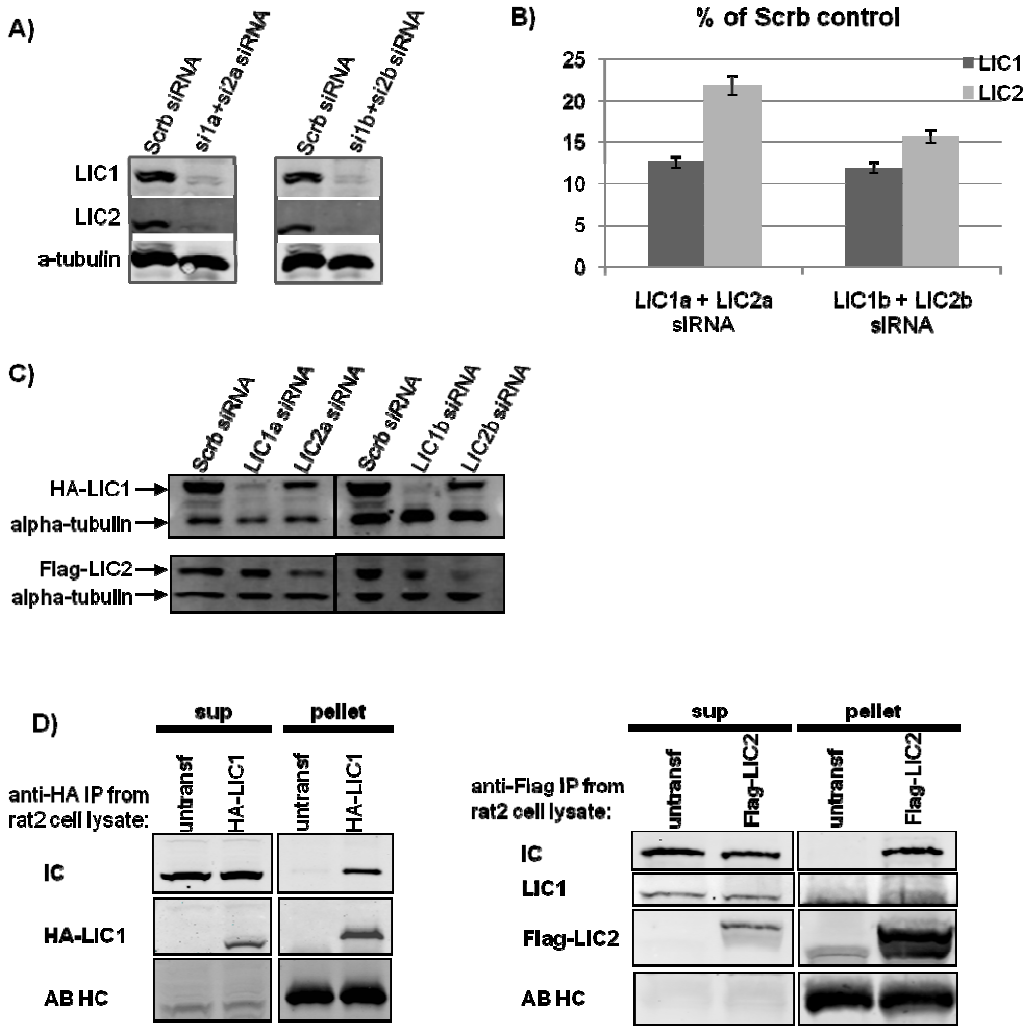


Figure S2. (A) Dual RNAi against LIC1 and LIC2 Leads to Knockdown of Endogenous Levels of both LIC1 and LIC2. siRNAs against both LIC1 and LIC2 as well as scrambled control siRNA were expressed in Rat2 cells. Cells were harvested after 48 hours for Western Blotting and lysates were immunoblotted with anti-LIC1, anti-LIC2, and anti-alpha-tubulin as a loading control. (B) Quantification of dual RNAi treatment against LIC1 and LIC2. LIC1 and LIC2 protein levels are shown as percentages of LIC1 and LIC2 levels in Scrambled Control RNAi conditions. Values shown are averages from immunoblotting of 3 independent RNAi experiments. Quantitation of immunoreactive intensities was performed and analyzed using Image J. (C) LIC1 and LIC2 RNAi specifically knockdown the recombinant LICs, HA-LIC1 and Flag-LIC2. HA-LIC1 and Flag-LIC2 were each co-expressed with Scrambled, LIC1, or LIC2 siRNA in Rat2 cells. Cells were harvested after 48 hours for Western Blotting and lysates were immunoblotted with anti-HA and anti-FLAG antibodies and anti-alpha-tubulin as a loading control. (D) Incorporation of the over-expressed LICs into the dynein complex. Immunoprecipitation from lysate of Rat2 cells over-expressing HA-LIC1 (left panel) or Flag-LIC2 (right panel) with a mAb against HA or Flag and SDS-PAGE and Western Blotting of supernatant and pellet samples indicate incorporation of LICs into the dynein complex according to the IC signal in the pellets. Western Blots were blotted against dynein IC, LIC1, HA, Flag and antibody heavy chain (AB HC). Note the absence of LIC1 in the anti-Flag pellets.

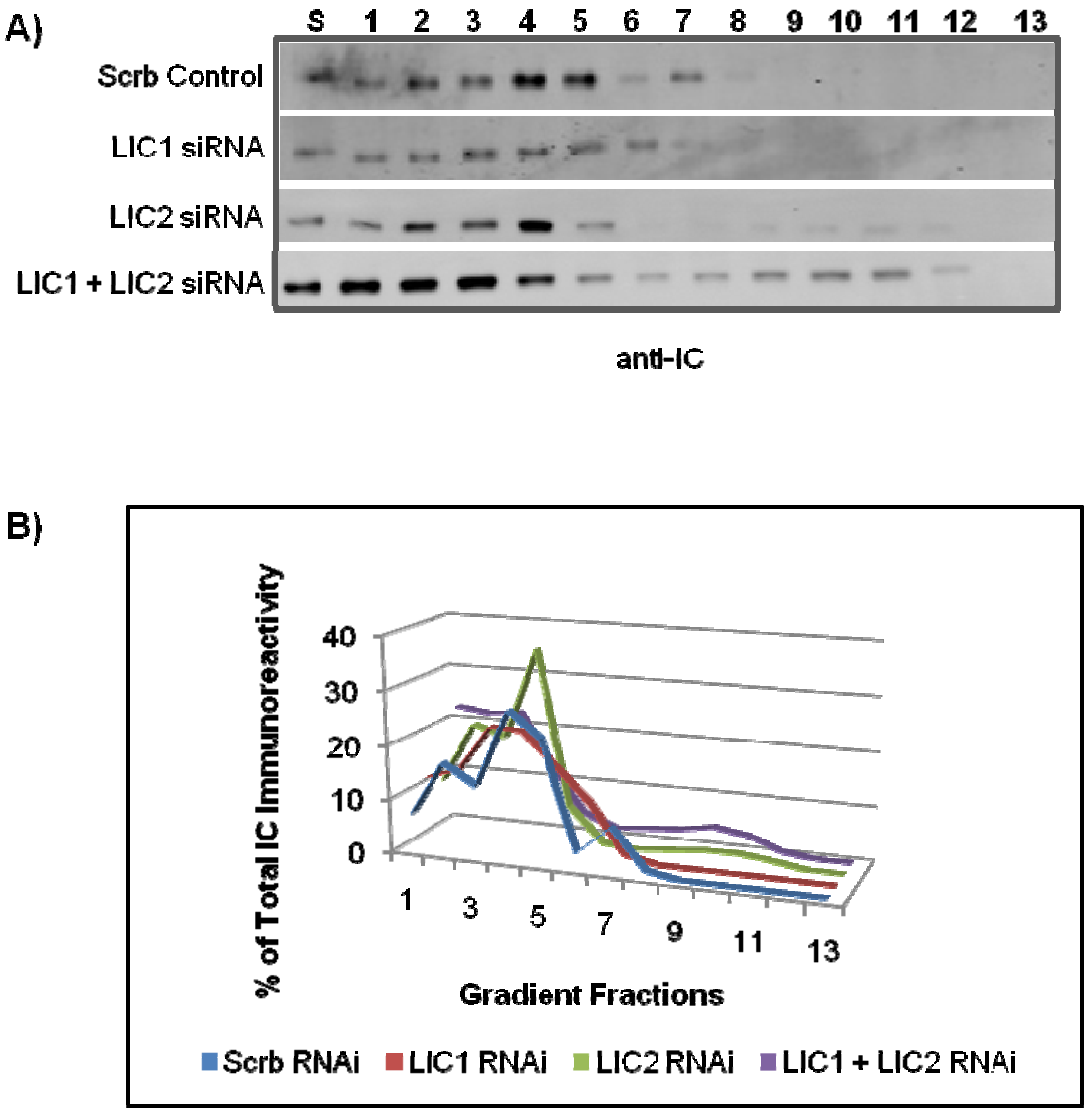


Figure S3. Effect of LIC RNAi Does Not Affect Dynein Integrity in 20%-5% Sucrose Gradients. RNAi against either LIC1 or LIC2 alone has no effect on the integrity of the dynein complex while simultaneous RNAi treatment against both LICs induces a shift of the dynein IC peak to slightly denser fractions. (A) Sucrose density gradient fractions from Scrambled siRNA and LIC1 siRNA treated cells were immunoblotted with antibody against dynein intermediate chain. The starting material for each condition is found in the first lane, denoted "S". Fractions from the bottom (20% sucrose) to the top (5% sucrose) of the gradient are loaded as fractions 1-13. (B) Quantification of dynein intermediate chain immunoreactivity throughout gradient fractions as assayed by Western blotting. Quantitation of immunoreactive intensities from 3 replicate experiments was performed and analyzed using Image J.

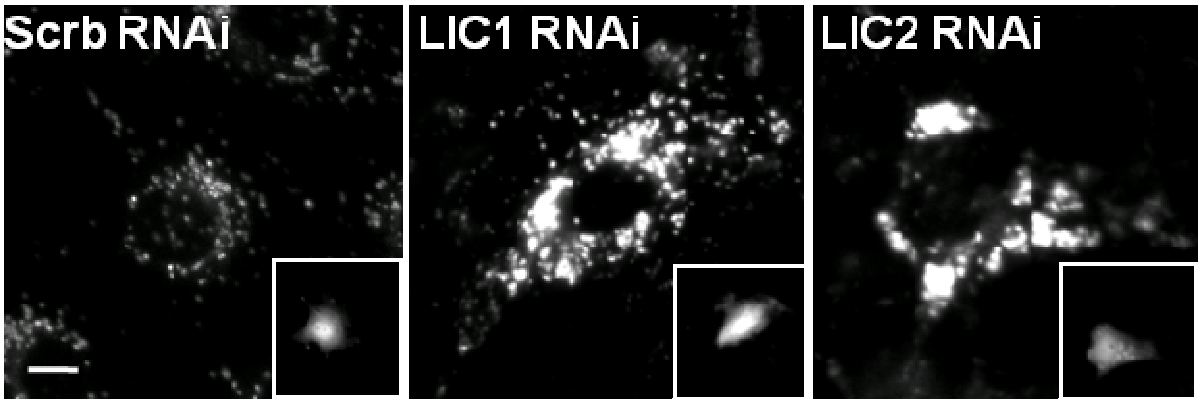


Figure S4. LIC1 and LIC2 RNAi Lead to the Enlargement and Enhancement of Late Endocytic Organelles in Live Cells as Seen with the Live Cell, Fluid-phase Lysosomal Marker, Lysotracker-Red. Cells co-transfected with LIC RNAi and GFP. Insets show co-transfection of GFP. Scale bar 5µm.

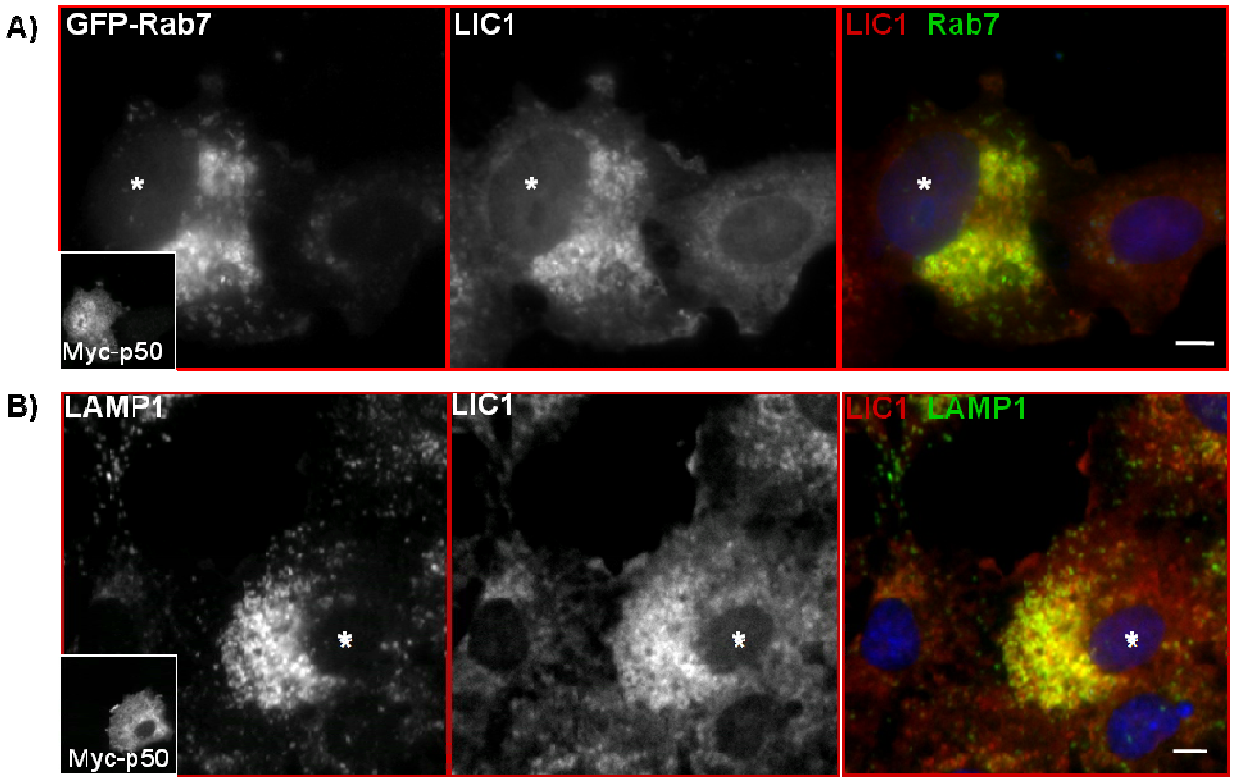


Figure S5. LIC1 Remains Localized to Rab7- and LAMP1-positive Lysosomes after p50 Over-expression. Rat2 cells co-transfected with myc-tagged p50 dynamitin and GFP-Rab7 (A), transfected with dynamitin alone (B), were co-stained with anti-LIC1 and anti-myc (A) or anti-LAMP1 and anti-myc (B). Myc-p50 transfected cells are marked with asterisks and Myc-p50 staining is shown in insets. All merges show LIC1 in red and GFP-Rab7 or LAMP1 in green. (A) LIC1 is not displaced from GFP-Rab7-positive lysosomes in cells co-expressing p50 dynamitin (B) p50 over-expression alone enhances staining of late endocytic and lysosomal vesicles as seen with LAMP1 but does not displace LIC1 from these structures. Scale bars, 5µm.

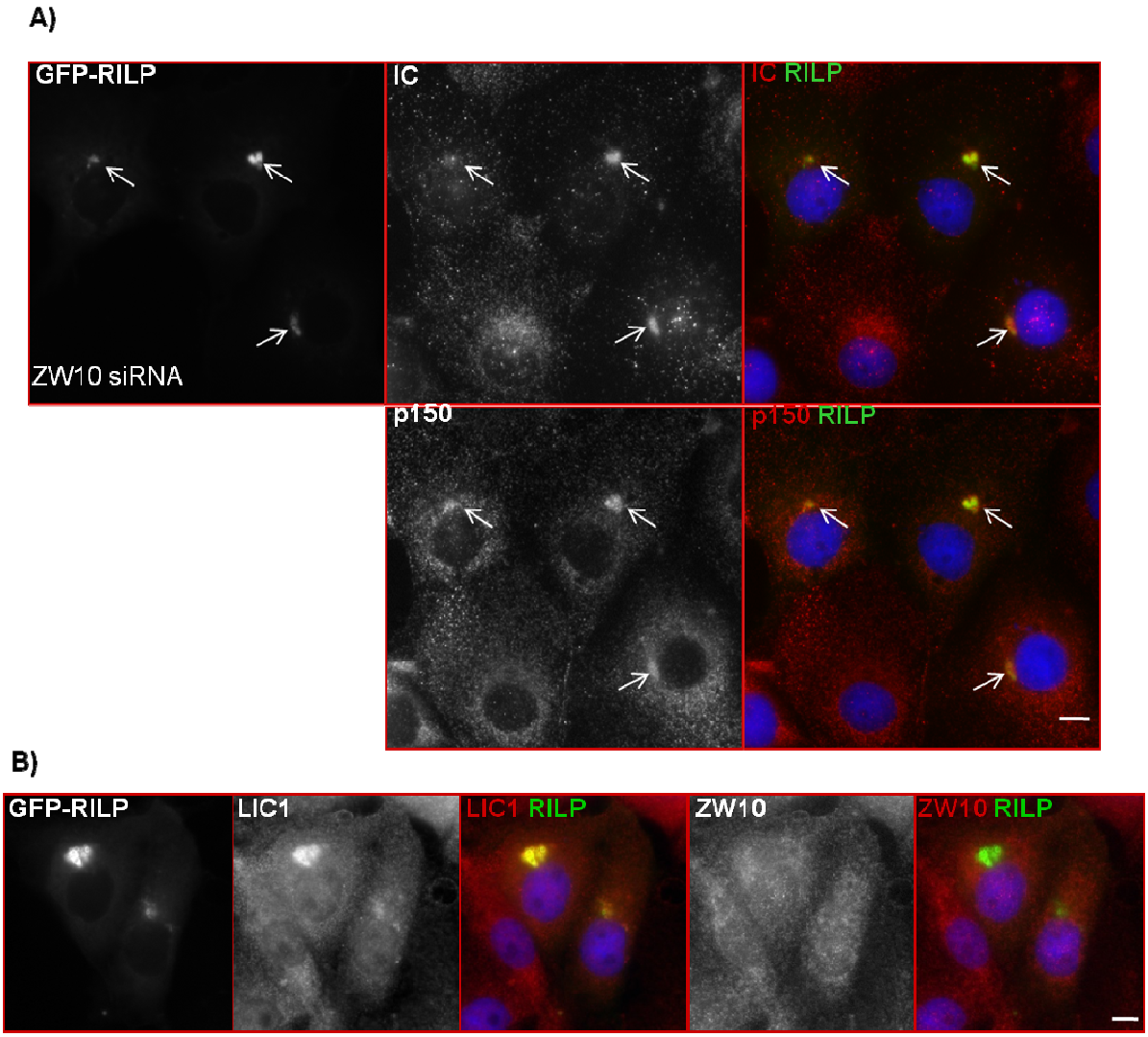


Figure S6. Persistence of Dynein and Dynactin at Lysosomes in ZW10 Knockdown Cells. (A) ZW10 siRNA does not disrupt RILP clustering of lysosomes or displace dynein or dynactin from these clusters (arrows indicate localization of dynein and dynactin to RILP clusters). Cells over-expressing GFP-RILP were treated with a ZW10-specific siRNA oligonucleotide and co-stained for dynein IC and $p150^{Glued}$ (each is shown in red in merged images). Neither dynein IC nor the dynactin subunit $p150^{Glued}$ is lost from RILP clusters. (B) ZW10 is not recruited to RILP clustered lysosomes as seen with LIC1-dynein. Cells over-expressing GFP-RILP were co-stained with anti-LIC1 and anti-ZW10 (shown in red in merged images). Scale bars, 5µm.