SUPPLEMENTAL FIGURES

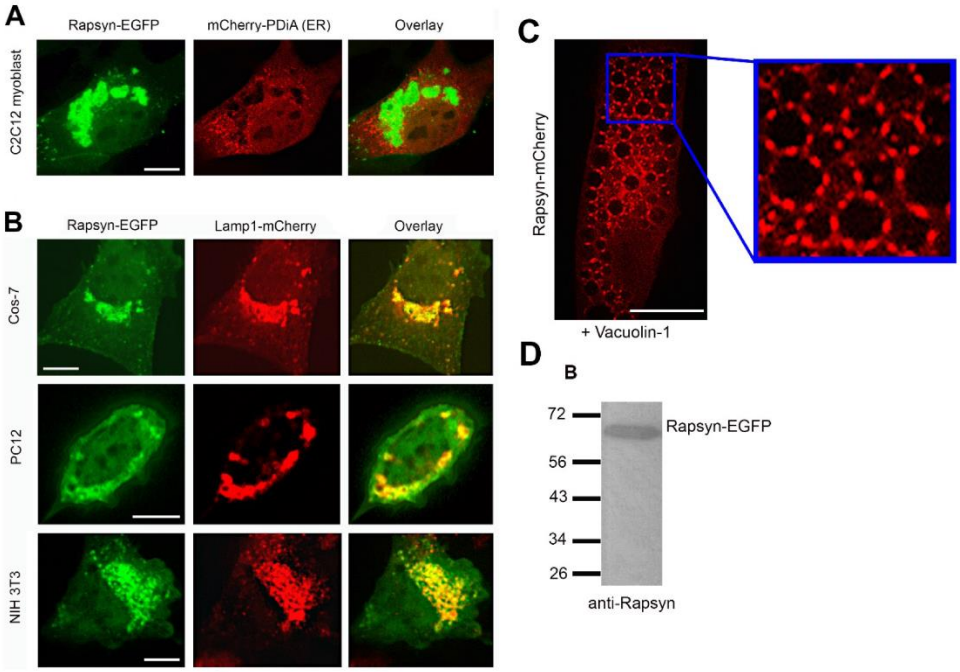


FIGURE S1

A) C2C12 myoblasts were co-transfected with rapsyn-EGFP and endoplasmic reticulum (ER) marker mCherry-PDiA. Transfected cells were imaged live with confocal spinning disk microscope. Representative confocal images showing no overlap between rapsyn-EGFP and mCherry-PDiA, excluding rapsyn targeting to the ER. B) Confocal images of live non-muscle cells co-expressing rapsyn-EGFP and Lamp1-mCherry. As in C2C12 myoblasts, rapsyn-EGFP overlaps with Lamp1-mCherry in COS-7 (top panel), undifferentiated neuronal PC12 (middle panel) and NIH3T3 fibroblasts (lower panel). Scale bar 10μm. C) C2C12 myoblasts were co-transfected with rapsyn-mCherry (mCherry known to resist quenching by the acidic environment of the lysosome lumen) and then treated with vacuolin-1 prior to imaging. Representative confocal image of a live cells showing rapsyn-mCherry concentration at the junctional sites between lysosomal vacuoles and no detectable signal of mCherry inside the lumen. Scale bar 10μm. D) Immunoblot of lysate from C2C12 myoblasts transfected with rapsyn-EGFP and probed with rabbit anti-GFP antibody. No rapsyn proteolytic products were detected.

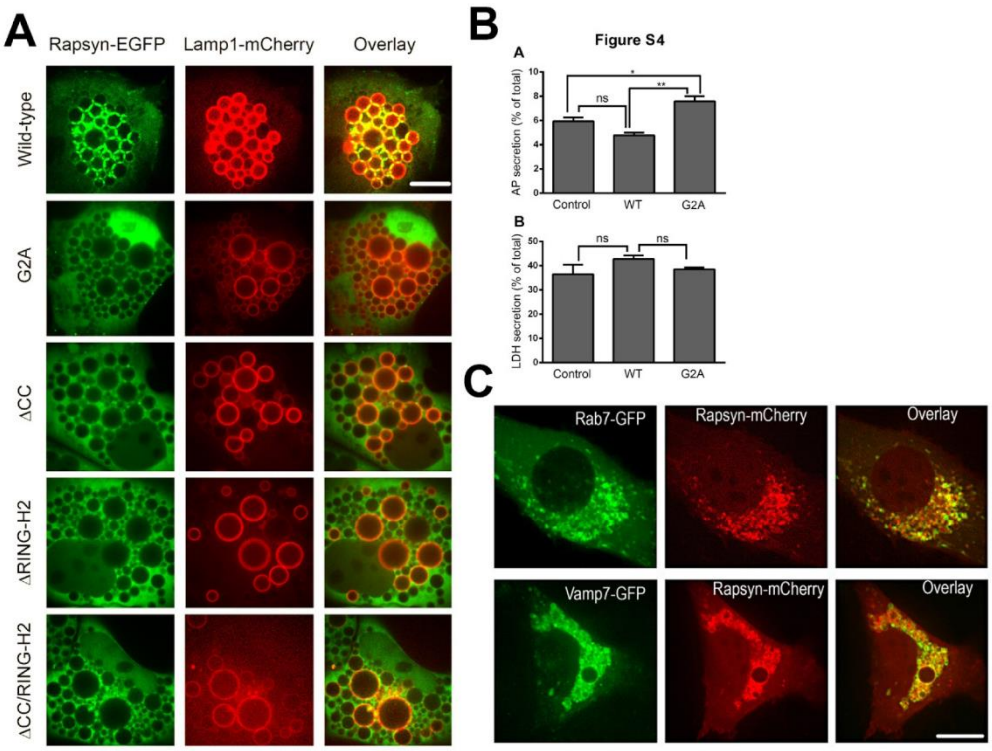
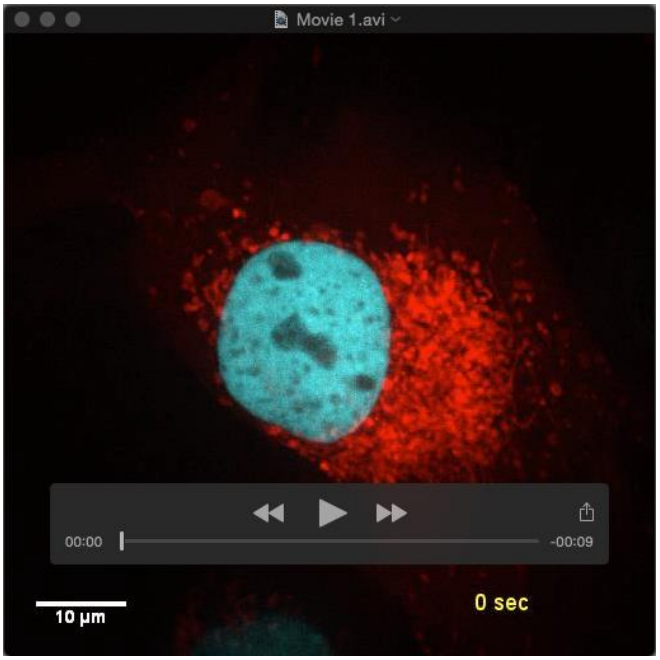


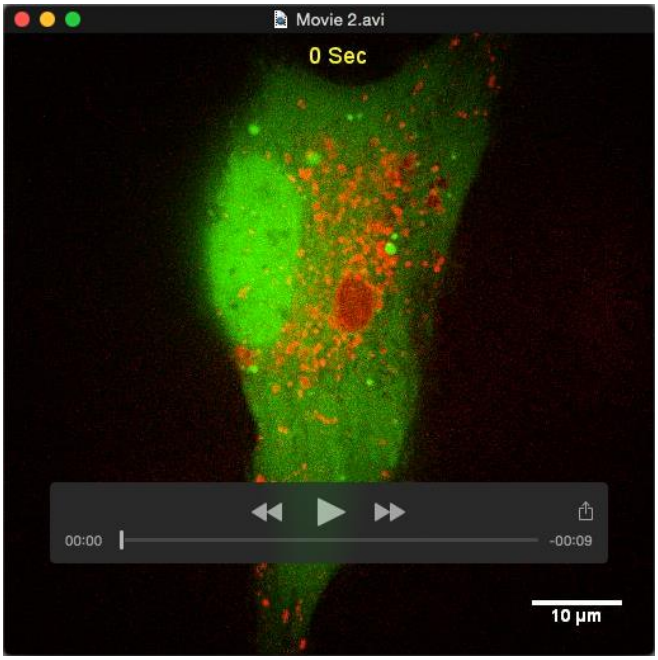
FIGURE S2

A) COS-7 cells were transfected with Lamp1-mCherry and either wild type, G2A, ΔCC, ΔRINGH2 or ΔCC/RINGH2 rapsyn-EGFP constructs. Transfected COS cells were treated with vacuolin-1 prior to live imaging with confocal spinning disk microscopy. Representative confocal images showing that wild type rapsyn-EGFP accumulates in junctional sites between vacuolin-1 enlarged lysosomes in COS cells as was observed in C2C12 myoblasts. G2A mutant however shows prominent accumulation in the nucleus, while ΔCC, ΔRINGH2 and ΔCC/RINGH2 displayed diffuse distribution throughout the cytoplasm. B) Quantification of acid phosphatase (AP) activity released by COS cells either untransfected or transfected with wild-type or G2A rapsyn-EGFP. Released activity is reported as percentage of total activity. B) The corresponding Lactate dehydrogenase (LDH) activities. The data represent mean ±SEM from three independent experiments. C) C2C12 myoblasts were transfected with rapsyn-mCherry and either rab7 (lysosome and late endosome marker) or Vamp7-GFP. Transfected cells were live imaged using spinning disk confocal microscope. Rapsyn-mCherry co-localizes partially with rab7-GFP (top panel) and perfectly with Vamp7-GFP (bottom panel) in the juxtanuclear region of C2C12 myoblasts. Scale bar 10μm.



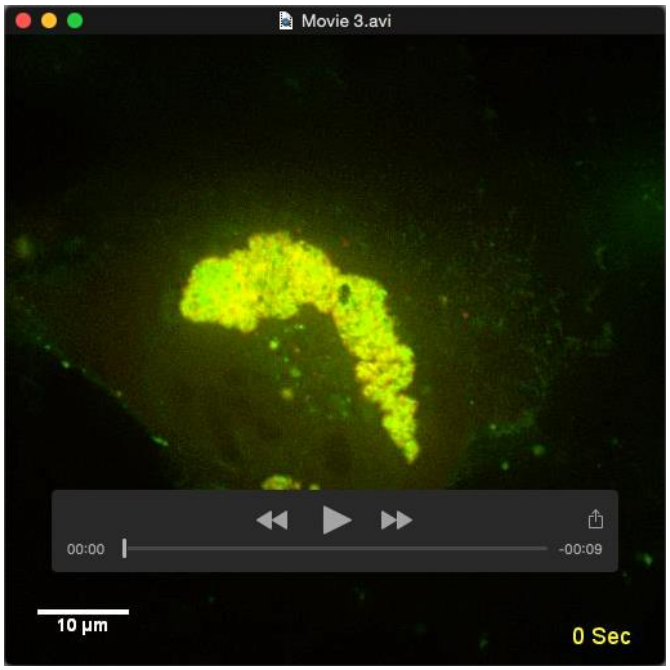
Movie S1

C2C12 control myoblasts were transfected with Lamp1-mCherry and CFP-H2B (to label nuclei) and lysosomal movements were monitored using time lapse imaging. Cells were imaged every 10 seconds. Note that most movements of lysosomes were restricted to the juxtauclear region.



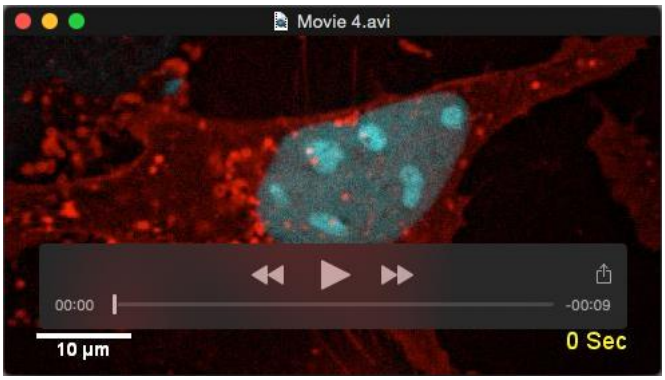
Movie S2

C2C12 myoblasts were co-transfected with Lamp1-mCherry and rapsynG2A-EGFP mutant. Movements of lysosomes in live cells were then monitored by time lapse imaging confocal microscopy. Cells were imaged every 10 seconds. Note that lysosomes are highly mobile in these cells compared to control C2C12 cells.



Movie S3

C2C12 myoblasts were co-transfected with Lamp1-mCherry and wild type rapsyn-EGFP constructs. Note that virtually no movement of lysosomes was seen.



Movie S4

Rapsyn-/- myoblasts were transfected with Lamp1-mCherry and CFP-H2B (to label the nuclei) and movements of lysosomes in live cells were monitored by time lapse imaging. Note that lysosomes are highly mobile in rapsyn-/- compared to control C2C12 cells.