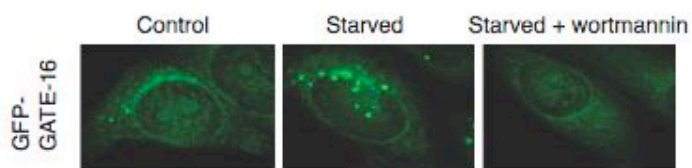
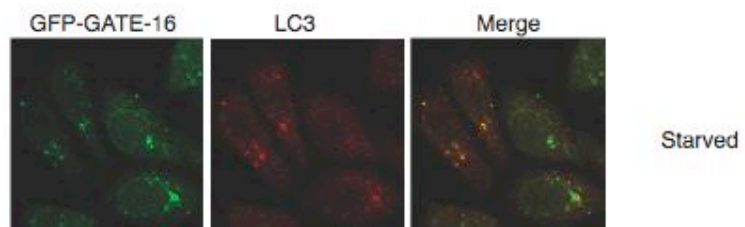


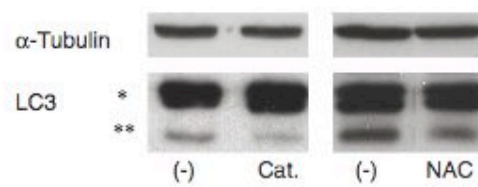
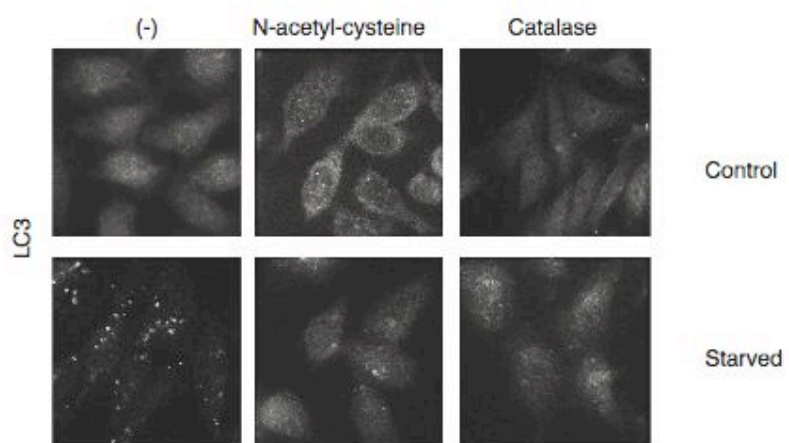
A



B



C



Supplementary Figure 3. GATE-16 and LC3 colocalize to the same autophagosomes during starvation. (A) CHO cells stably transfected with GFP-GATE-16 were starved for 2.5 h in EBSS in the presence or absence of 100 nM wortmannin, or kept in a control medium (α -MEM, 10% FCS). The cells were then fixed in methanol and visualized by a confocal microscope. (B) CHO cells stably transfected with GFP-GATE-16 were starved for 2.5 h in EBSS, fixed in methanol, permeabilized in acetone and stained with anti-LC3 antibodies. (C) **ROS accumulation is essential for formation of LC3-labeled autophagosomes.** CHO cells were pre-treated with 10 mM NAC or 1000 u/ml catalase for 10 min before starvation in EBSS for 2 h in the presence or absence of NAC or catalase, or grown in a control medium (α -MEM, 10% FCS) containing NAC or catalase for 2 h. The cells were then fixed in methanol, permeabilized in acetone and stained with anti-LC3 antibodies, or lysed in RIPA buffer and analyzed by western blot with the indicated antibodies.