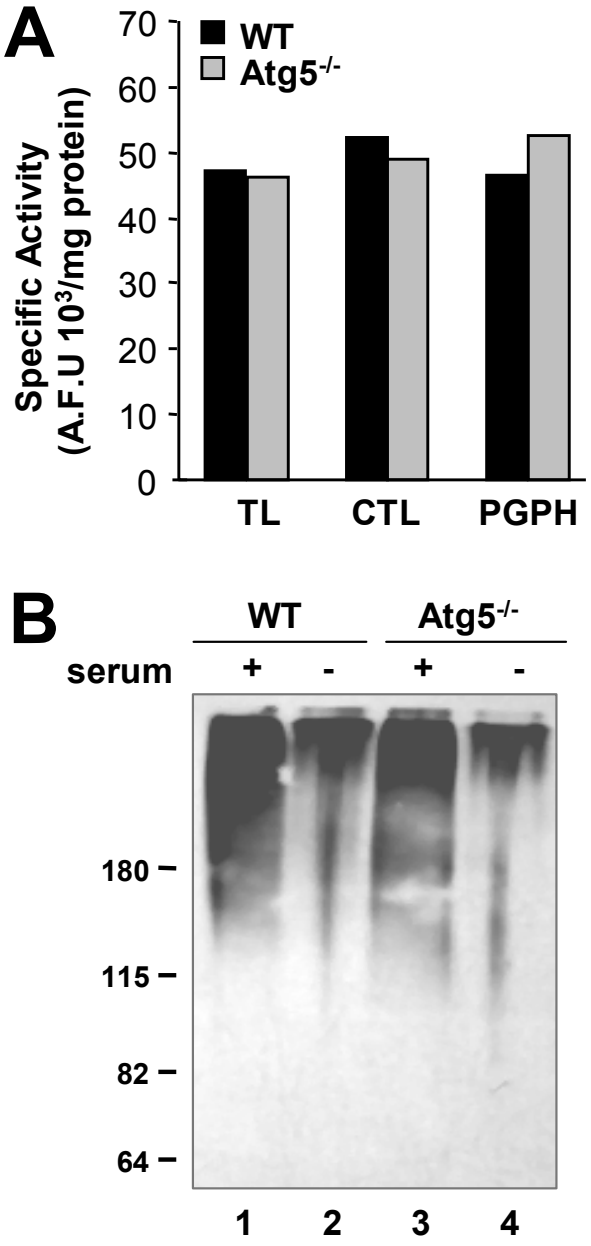
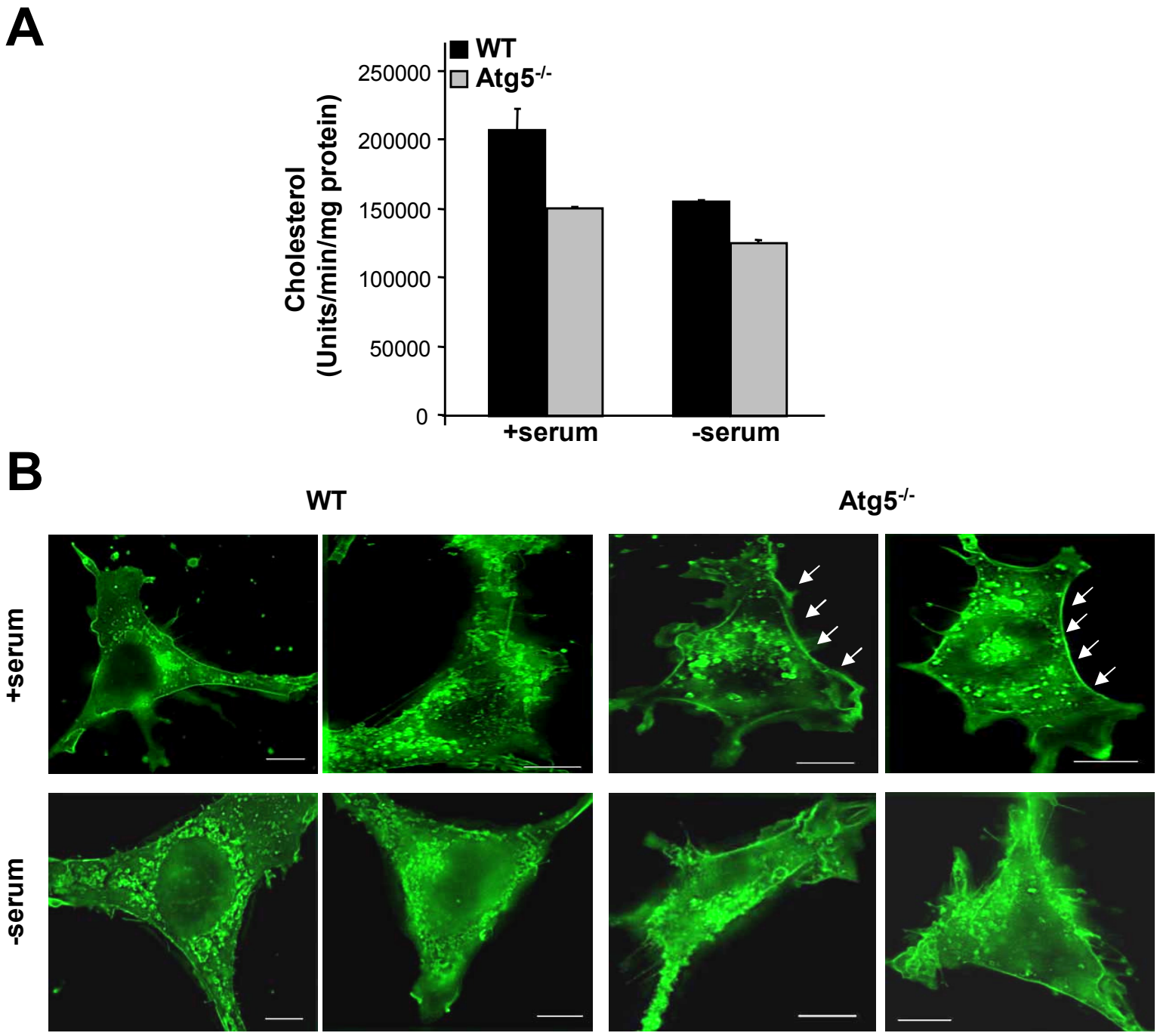
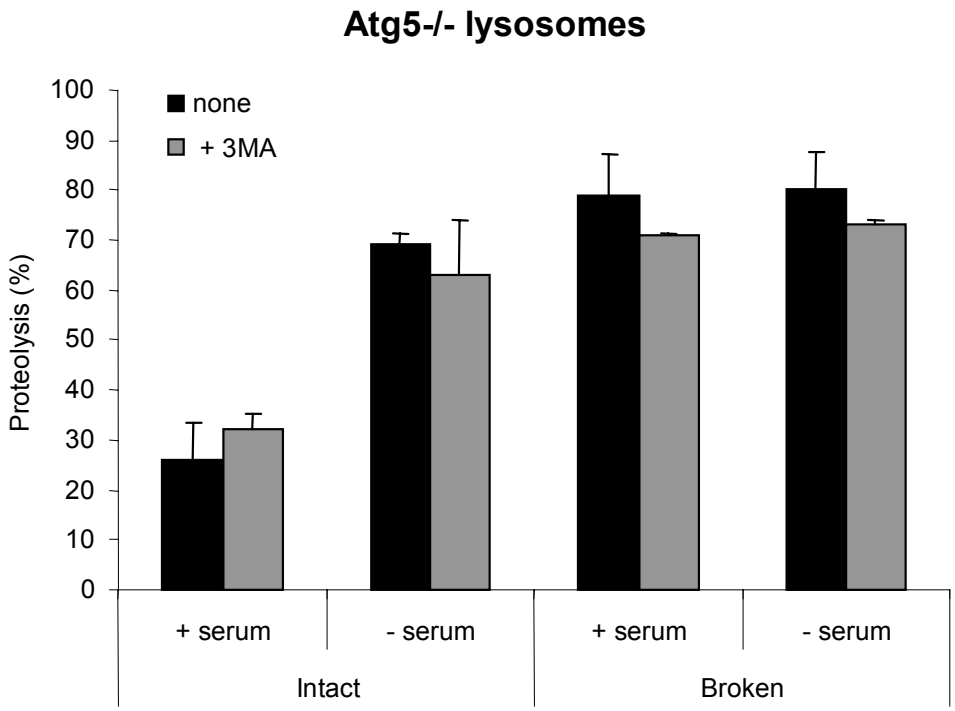


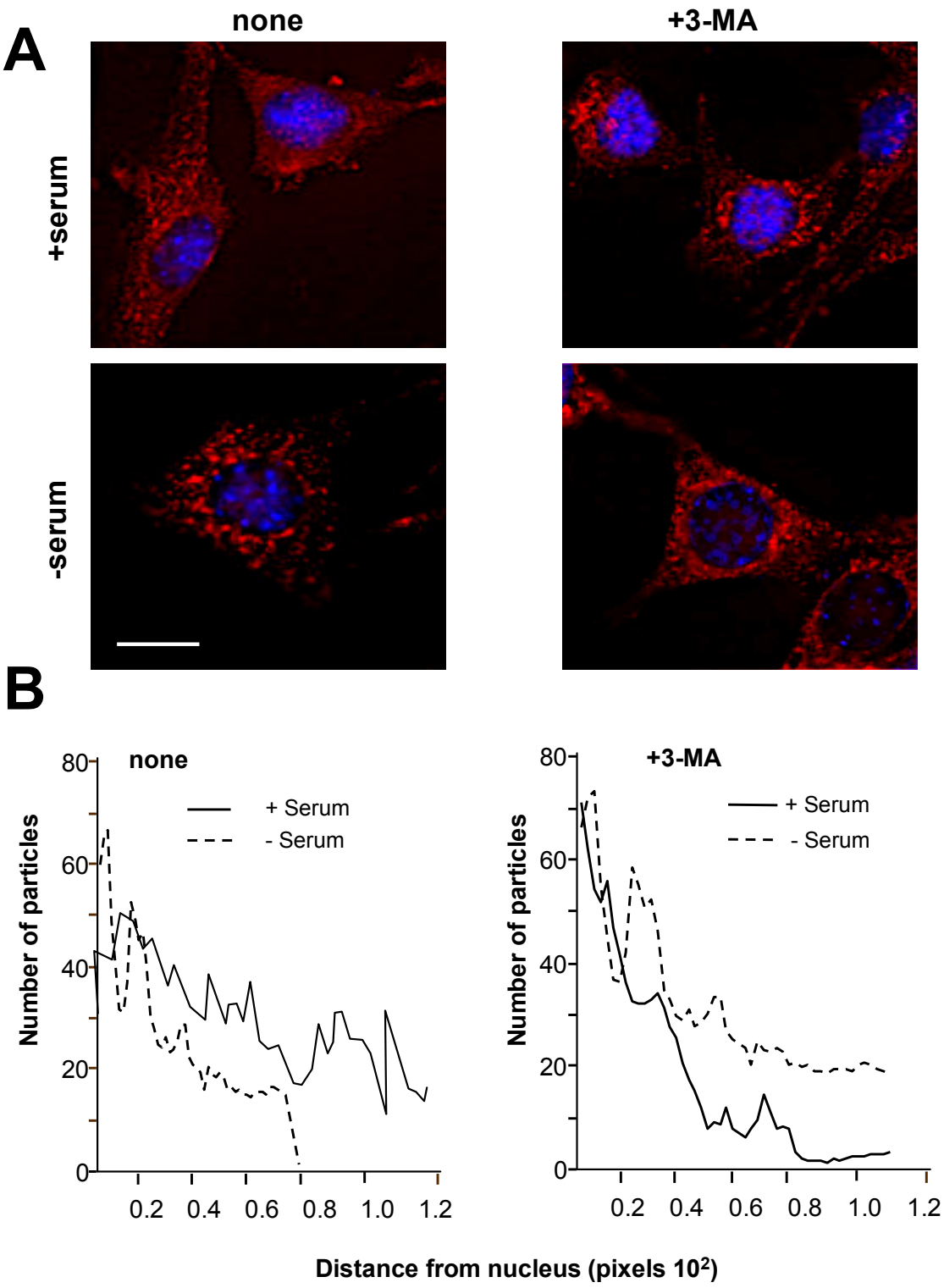
Supplemental Figure 1. The Ubiquitin/Proteasome system in Atg5-/- cells. A. Three different proteasome activities (trypsin-like (TL); chymotrypsin-like (CTL) and peptidylglutamyl peptide hydrolase (PGPH)) were measured in total cellular extracts from wild-type and two different clones of Atg5-/- MEFs, as described under Material and Methods. Values are expressed as specific activity (arbitrary fluorescence units per mg of protein) and are mean of 5 different experiments with triplicate samples. The S.E. of the samples was less than 10% of the total value. **B.** Total cell lysates (150μg each) from wild-type and Atg5-/- cells maintained in the presence or absence of serum for 16h were subjected to SDS-PAGE followed by immunoblot for ubiquitin.



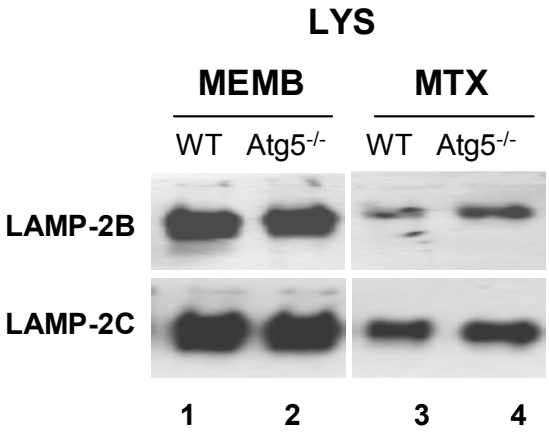
Supplemental Figure 2. Cholesterol in Atg5-/- cells. A. Levels of intracellular cholesterol in wild-type and Atg5$^{-/-}$ MEFs maintained in the presence and absence serum for 16h. **B.** Intracellular distribution of unesterifed cholesterol visualized through Filipin staining in wild-type and Atg5$^{-/-}$ MEFs maintained in the presence and absence of serum for 16h. Bar: 5 μm.



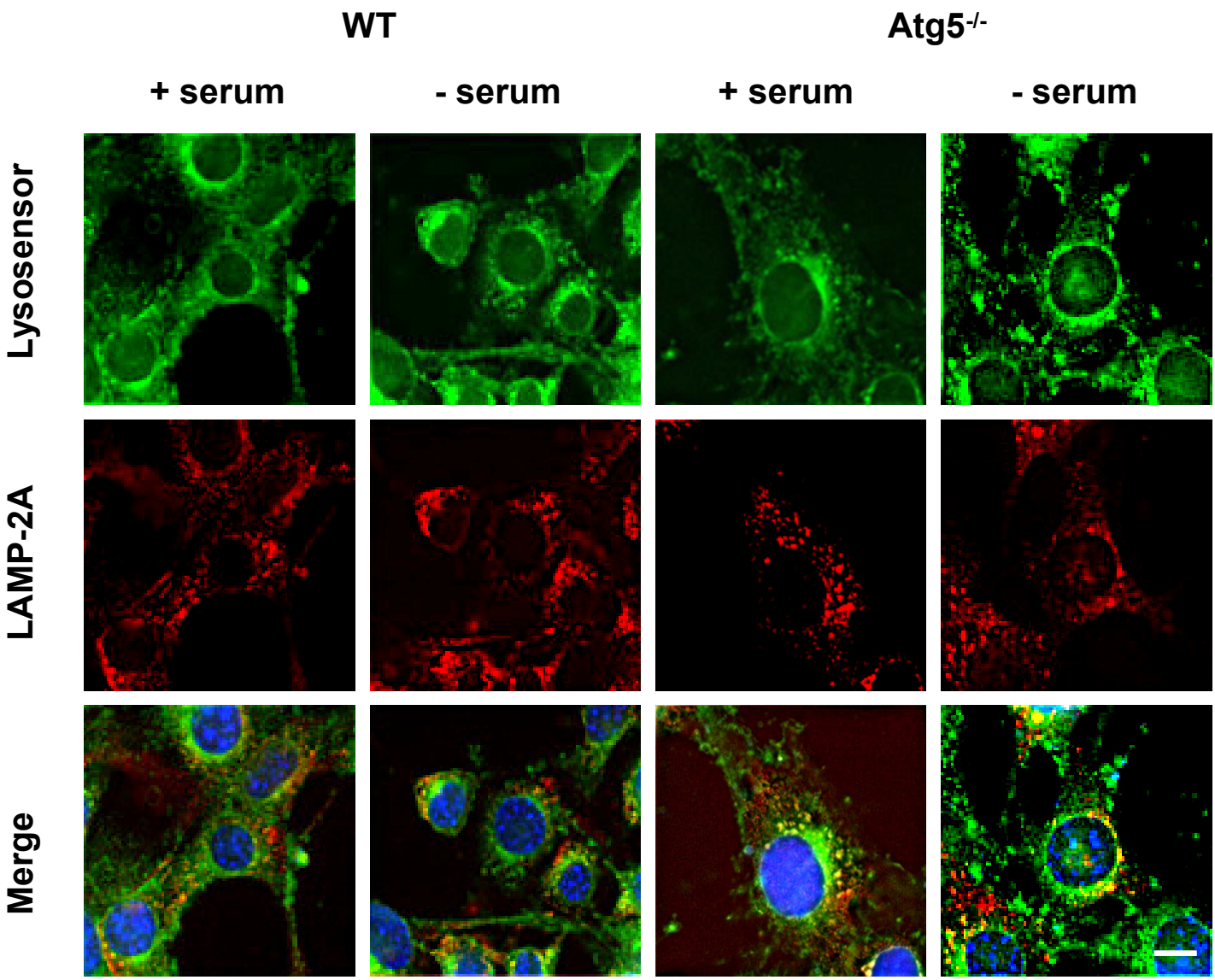
Supplemental Figure 3. Effect of 3MA treatment in Atg5-/- lysosomes. Intact isolated lysosomes (20 μg protein) (left) or lysosomes disrupted by a hypotonic shock (5 μg protein) (right) were incubated in an isotonic medium or in water, respectively, Degradation of a [^{14}C] labeled pool of cytosolic proteins by intact or broken lysosomes (disrupted by a hypotonic shock) isolated from Atg5-/- cells maintained in the presence of absence of serum after the indicated treatments. Values are mean + S.E. of triplicate samples from two different experiments.



Supplemental Figure 4. Relocation of CMA-active lysosomes toward the perinuclear region during pharmacological blockage of macroautophagy. NIH3T3 cells treated or not with 10 mM 3-metyladenine (3-MA) were maintained in the presence and absence of serum. After 6 hours, cells were fixed and subjected to immunofluorescence for LAMP-2A. Mounting media contains DAPI to highlight the cell nuclei. **A.** Representative cells for each condition Bar: 5 μm. **B.** Quantification of the distribution of lysosomes (LAMP-2A-positive puncta) with respect to the nucleus in untreated (left) and 3-MA-treated (right) cells under basal conditions and after serum removal. Values are mean of the quantification of 20 cells per condition in two independent experiments. No change in mean cell size was observed after treatment.



Supplemental Figure 5. Lysosomal components in Atg5$^{-/-}$ cells during nutritional stress. Lysosomal membranes and matrices isolated from lysosomes from wild-type and Atg5$^{-/-}$ cells after hypotonic shock and centrifugation were subjected to SDS-PAGE and immunoblotted for LAMP-2B and LAMP-C.



Supplemental Figure 6. Characteristics of the acidic compartment in Atg5-/- cells. Wild-type and Atg5-/- MEFs maintained in the presence or absence serum for 16h, as indicated, were incubated with lysosensor. After 15 min, cells were extensively washed, fixed, blocked and processed for immunofluorescence with an antibody against LAMP-2A. Lysosensor (green; top) and LAMP-2A (red; middle) staining and the merged image of both channels (bottom) is shown. Bar: 5 mm.