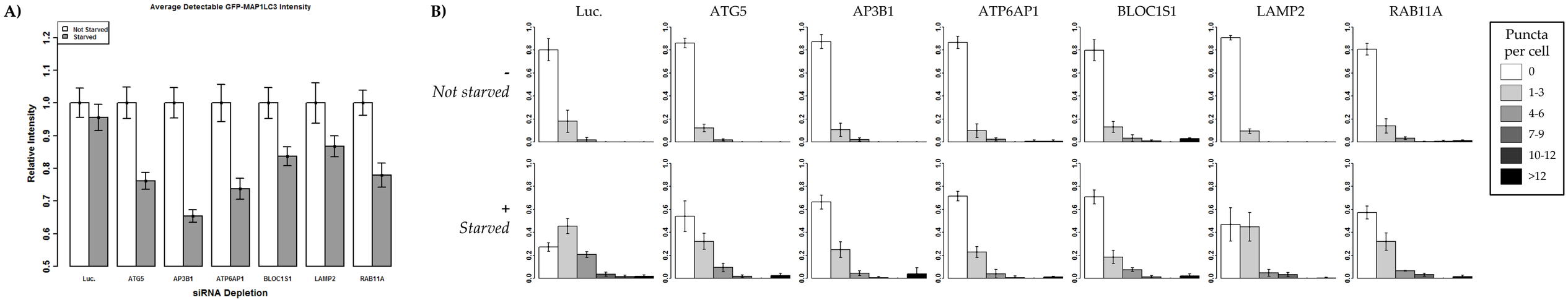

Supplemental Figure 5: Quantification of autophagosome formation in starved cells. MAP1LC3 is typically diffuse throughout the cytoplasm in non-starved cells. Under normal conditions, starved cells will initiate autophagy, process MAP1LC3 to the MAP1LC3-II isoform, and form punctate autophagosomes to which it is localized. We measured the degree to which this was impaired by luciferase (negative control), ATG5 (positive control), AP3B1, ATP6AP1, BLOC1S1, LAMP2, RAB11A, and VAMP7 siRNA depletions using immunoblotting (see Figure 4) and automated and manual inspection of ten images for each condition (totaling 80 images). While VAMP7 knockdowns showed no effect (see Discussion), siRNA knockdowns of the remaining five genes inhibited normal autophagy. A) Automated image analysis detects a significant decrease in fluorescent GFP-tagged MAP1LC3-II under starvation conditions for the positive control (ATG5) and five validated knockdowns. Bars show standard error of average relative intensity as quantified by CellProfiler (Carpenter et al. 2006) over a collection of 10 images per condition (80 total). This decrease in detectable fluorescence indicates that normal MAP1LC3-II processing (and thus autophagy) is impaired when these five protein levels or the ATG5 positive control are reduced. B) Manual inspection of the number of puncta per cell shows decreased autophagosome formation when autophagy is impaired. Error bars indicate standard error over counts by three independent investigators viewing randomized, unlabeled images. The number of puncta increases when cells are starved under the luciferase control condition, but this increase is substantially impaired in ATG5 (positive control), AP3B1, ATP6AP1, BLOC1S1, LAMP2, and RAB11A siRNA conditions.