

Supplementary Figures

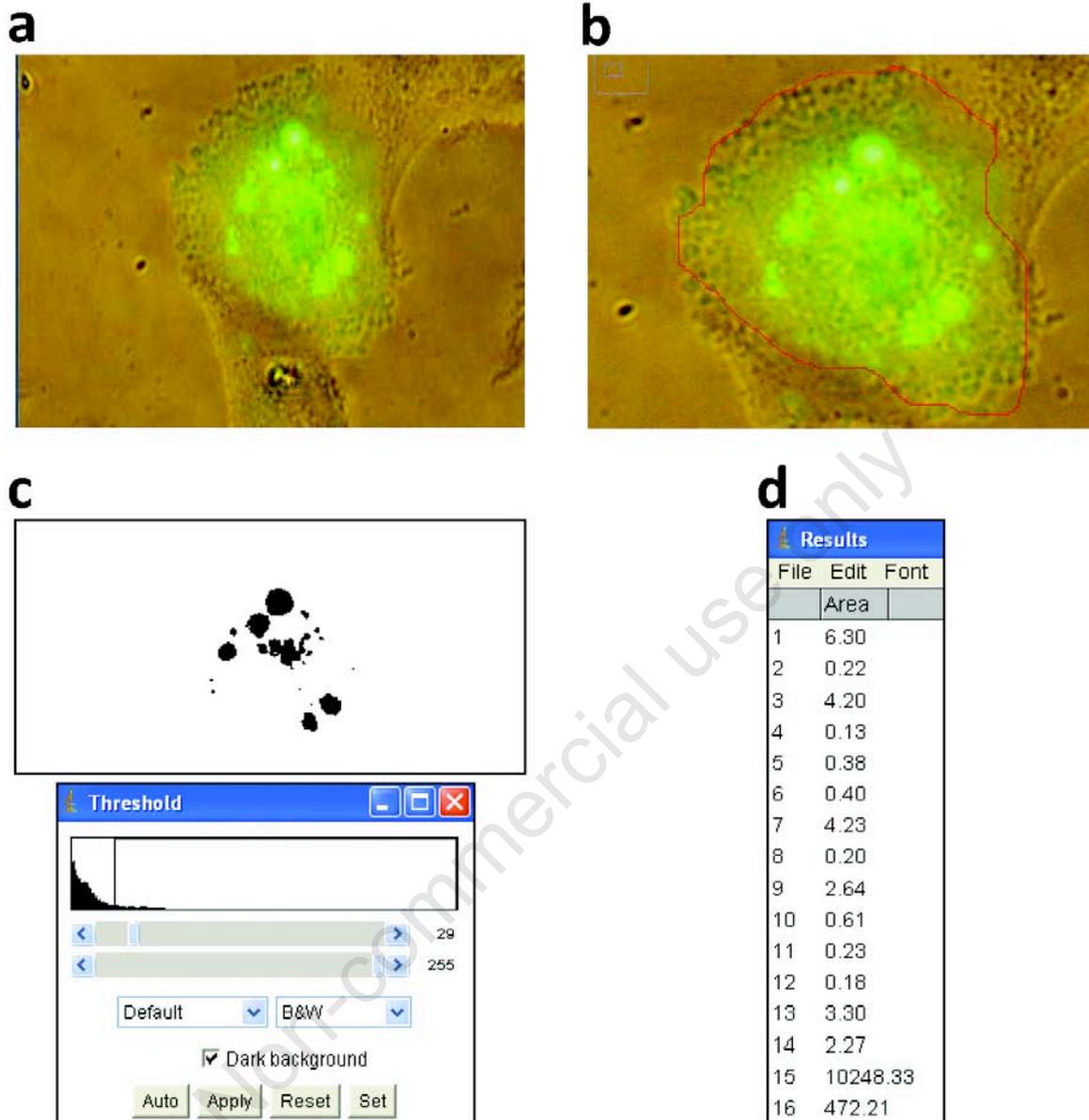


Figure 1. AUTOCOUNTER exemplified operating scheme. The program required an ImageJ installation with the custom-made AUTOCOUNTER JavaScript (*i.e.* a list of ImageJ commands written in Java programming language). The former is available at <http://rbsweb.nih.gov/ij/>, the latter upon request to the corresponding author. a) ImageJ opened a high-resolution light-fluorescent-microscope image. b) The cell contour was then highlighted by the operator using the ImageJ *Freehand Selection Tool*. c) Window with the detected vesicles (black particles) with the corresponding ImageJ *Threshold Tool* to enhance the contrast between detected black particles and white background. d) Finally, AUTOCOUNTER results in terms of vesicle numeric label (left column) and vesicle area in μm^2 (right column). The two last rows of the list reported the total area of the original microscope image (panel a) and the area of the highlighted cell (panel b), respectively. The measurements were calibrated through an ImageJ command that combined a known micrometric bar with its corresponding length in pixel.

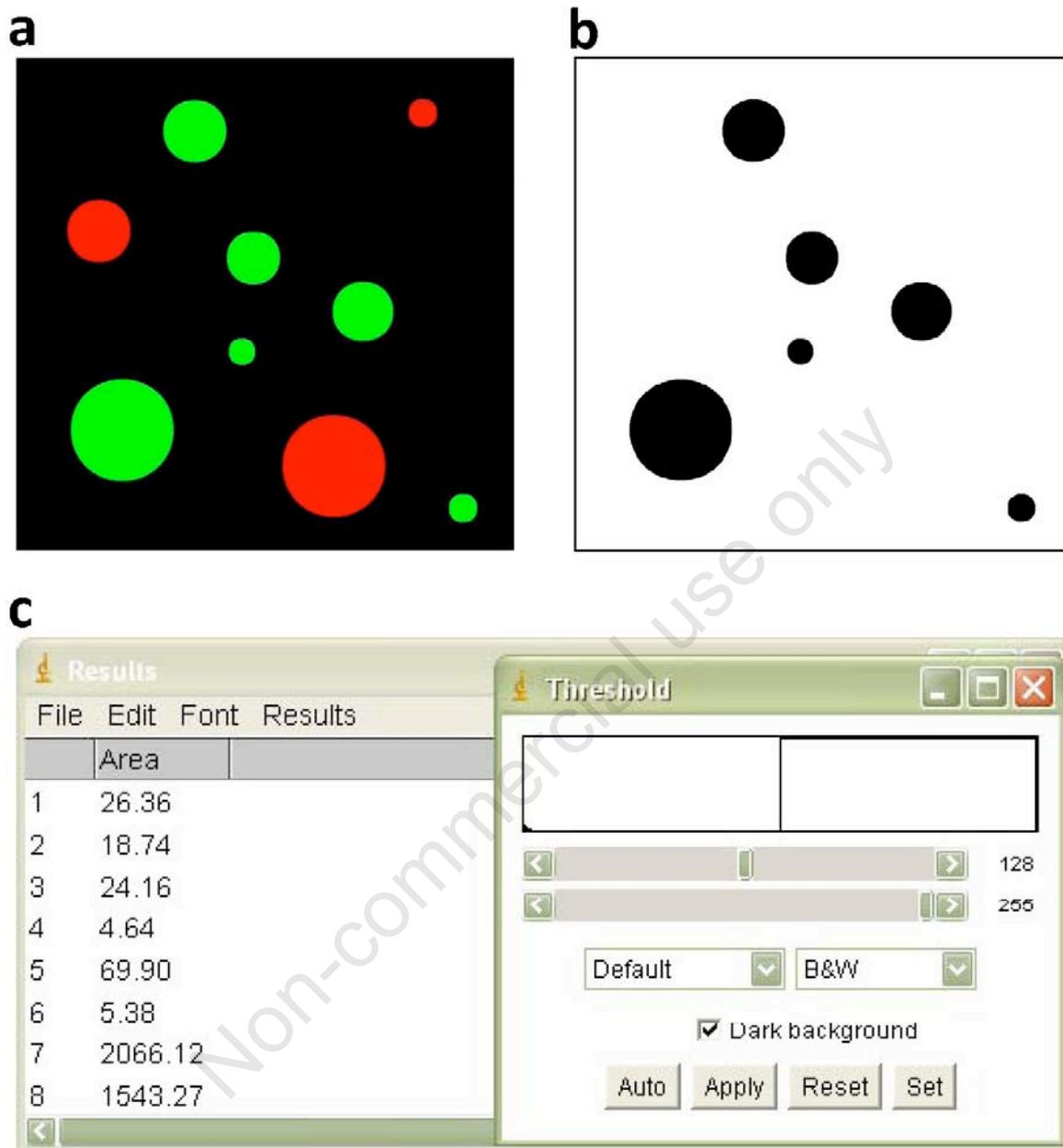


Figure 2. Analysis of a simulated RGB image with different amounts of green and red elements simulating GFP and RFP puncta, respectively. As shown, AUTOCOUNTER was able to correctly extract the information related to the GFP puncta. a) Original image. b) Identification of the GFP puncta. c) Results via AUTOCOUNTER analysis (see the Supplementary Figure 1 for the result structure).

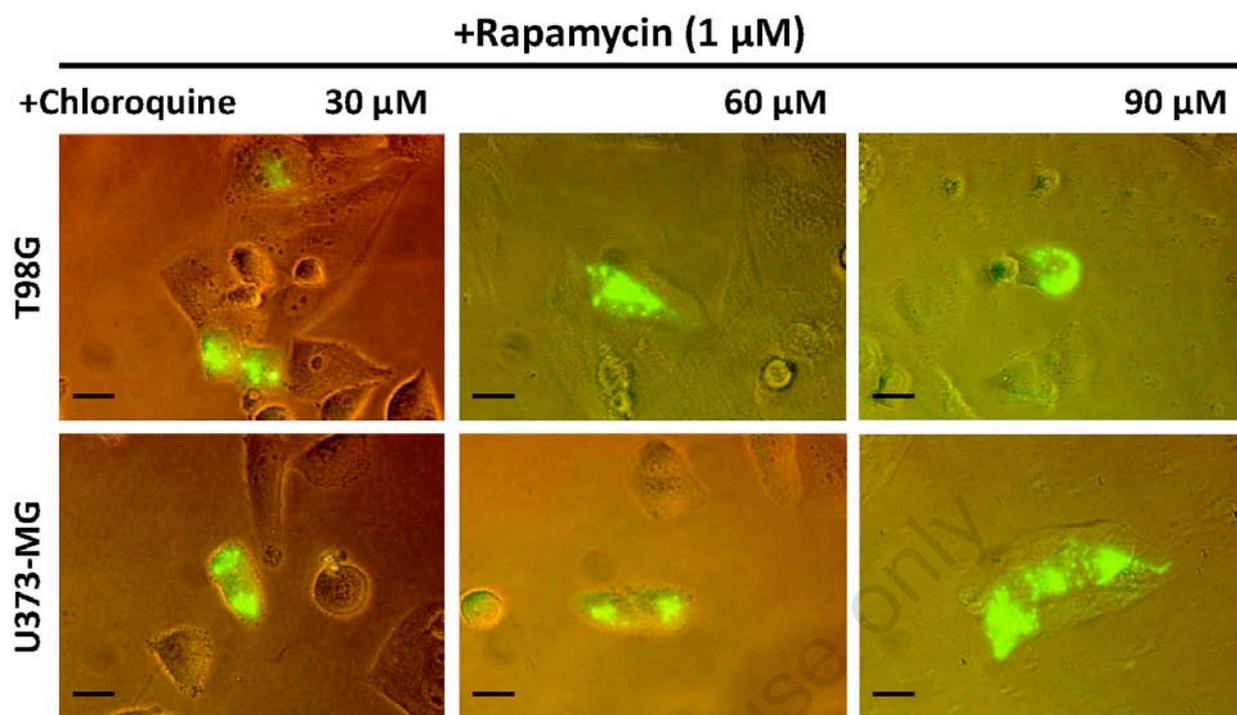


Figure 3. Expression of LC3B-GFP after autophagic flux blockage in T98G and U373-MG cells. Cells were transduced with BacMam LC3B-GFP (MOI=30) and after 12 h treated with Rapamycin (1 μ M) as before; subsequently, the cells were incubated with Chloroquine diphosphate at 30, 60, and 90 μ M for 12 h. Scale bars: 10 μ m.