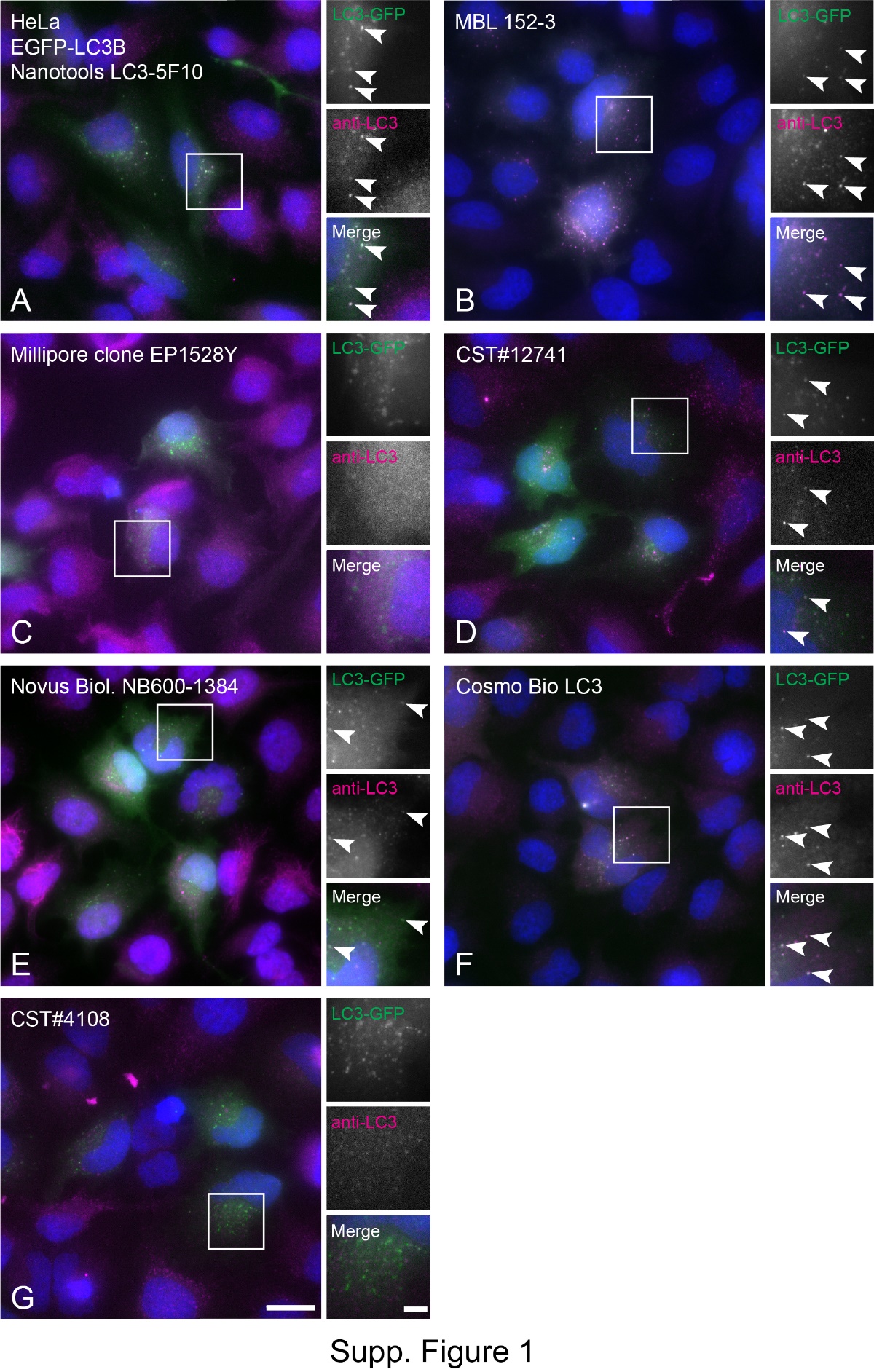
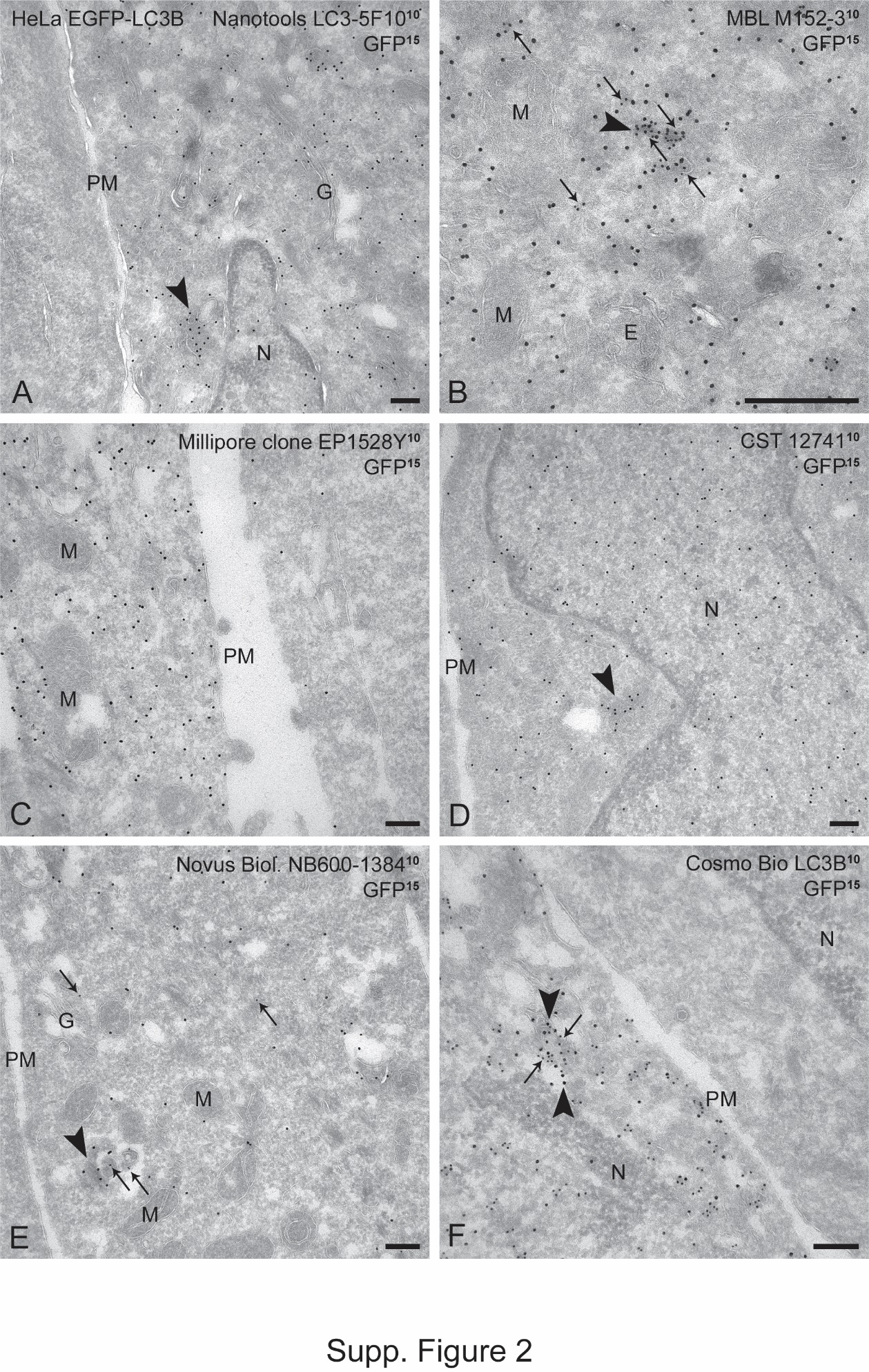
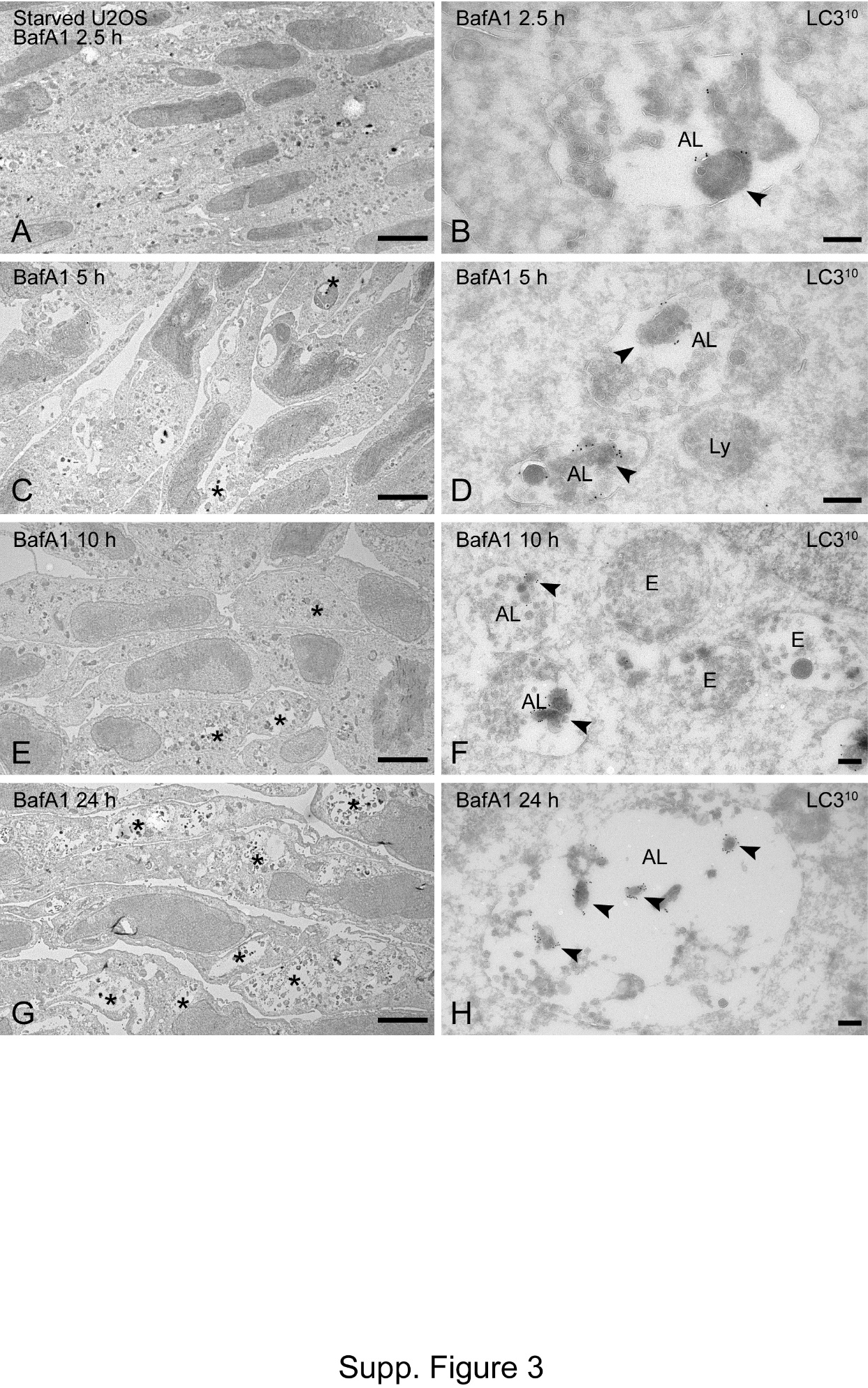
**Supplemental figures**

****

**Figure S1.** Testing of commercially available anti-LC3 antibodies in immunofluorescence. HeLa cells were transfected with EGFP-LC3B and fixed in fed conditions. IF was performed with the indicated LC3 primary and an Alexa Fluor 568 secondary antibody. Antibodies used in each subfigure: (**A**) Nanotools 0231-100/LC3-5F10 (1:100); (**B**) MBL M152-3 (1:50); (**C**) Millipore MABC177 clone EP1528Y (1:100); (**D**) CST #12741 (1:100); (**E**) Novus Biologicals NB600-1384 (1:200); (**F**) Cosmo Bio CAC-CTB-LC3-2-IC (1:200); (**G**) CST #4108 (1:200). All tested antibodies display some degree of specific labeling, except Millipore MABC177 (C) and CST 4108 (G). Arrowheads indicate specifically colocalizing puncta. Overall labeling efficacy is summarized in Table 1. Scale bar: 20 µm in overview, 5 µm in inset.

****

**Figure S2.** Performance of commercially available anti-LC3 antibodies in immuno-EM. Examples of cryosections of HeLa cells transiently transfected with EGFP-LC3B, double-labeled for the indicated LC3A/B antibodies (PAG10) and anti-GFP (PAG15). Antibody dilutions and the outcome of this labeling-series are summarized in Table 1. (**A**) Nanotools 0231-100/LC3-5F10 (1:5), 4% PFA ON fixation; (**B**) MBL M152-3 (1:10), 4% PFA ON fixation; (**C**) Millipore MABC177 clone EP1528Y (1:10), 4% PFA ON fixation; (**D**) #CST 12741 (1:5), 2% PFA+0.2% GA fixation; (**E**) Novus Biologicals NB600-1384 (1:10), 2% PFA+0.2% GA fixation; (**F**) Cosmo Bio CAC-CTB-LC3-2-IC (1:5), 4% PFA ON fixation. All cells show GFP label, but (A), (C) and (D) virtually lack LC3 labeling. Arrowheads point to concentrations of GFP label, whereas arrows indicate LC3-representing PAG10. The nuclear localization of overexpressed EGFP-LC3 was previously reported. G, Golgi; E, endosome; M, mitochondrion; N, nucleus; PM, plasma membrane. Scale bars: 200 nm.



**Figure S3.** Extended BafA1 treatment causes enlargement of LC3B-positive compartments. U2OS cells treated with BafA1 for the indicated durations were prepared as in Figure 8. Sections were labeled for LC3B (1:10; PAG10). Overview images are shown on the left (A, C, E, G), higher magnifications of LC3-positive compartments on the right (B, D, F, H). BafA1 treatment for (**A, B**) 2.5 h; (**C, D**) 5 h; (**E, F**) 10 h; (**G, H**) 24 h. After longer incubation with BafA1 cells increasingly display clusters of greatly enlarged autolysosomes (asterisks). Arrowheads, autophagic content. E, endosome; Ly, lysosome. Scale bars: 5 µm (A, C, E, G), 200 nm (B, D, F, H).