Supplemental Materials to:

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V-ATPase and osmotic imbalances activate endolysosomal LC3 lipidation

Inventory of Supplemental Materials

Supplemental Figures 1 to 5

Figure S1, related to Figure 1. Effect of lysosome inhibitors on lysosomal pH and canonical autophagy deficiency in *atg13*$^{-/-}$ MEFs.

Figure S2, related to Figure 1. Repeat western blots and quantification of LC3 lipidation.

Figure S3, related to Figure 2. Quantification of GFP-LC3 recruitment to phagosomes and entotic corpse vacuoles and effects of V-ATPase inhibitors Baf and ConA; measurement of GFP-LC3 dynamics during chloroquine-mediated GFP-LC3 recruitment to entotic corpse vacuole.

Figure S4, related to Figure 3. Inhibition of canonical autophagy and disruption of 2xFYVE-mCherry localization by PtdIns 3-kinase inhibitors.

Figure S5, related to Figure 5. GFP-LC3 localization following osmotic swelling of entotic vacuoles containing live and dead cells.

Supplemental Movies 1 to 4

Movie S1. GFP-LC3 and LAMP1-RFP expressing MCF10A cell containing an entotic corpse treated with chloroquine (100 μM).

Movie S2. LAMP1-GFP expressing MCF10A cell treated with chloroquine (100 μM).

Movie S3. GFP-LC3 and LAMP1-RFP expressing MCF10A cell containing an entotic corpse treated with hypotonic media.

Movie S4. GFP-LC3 and LAMP1-RFP expressing MCF10A treated with hypotonic media.

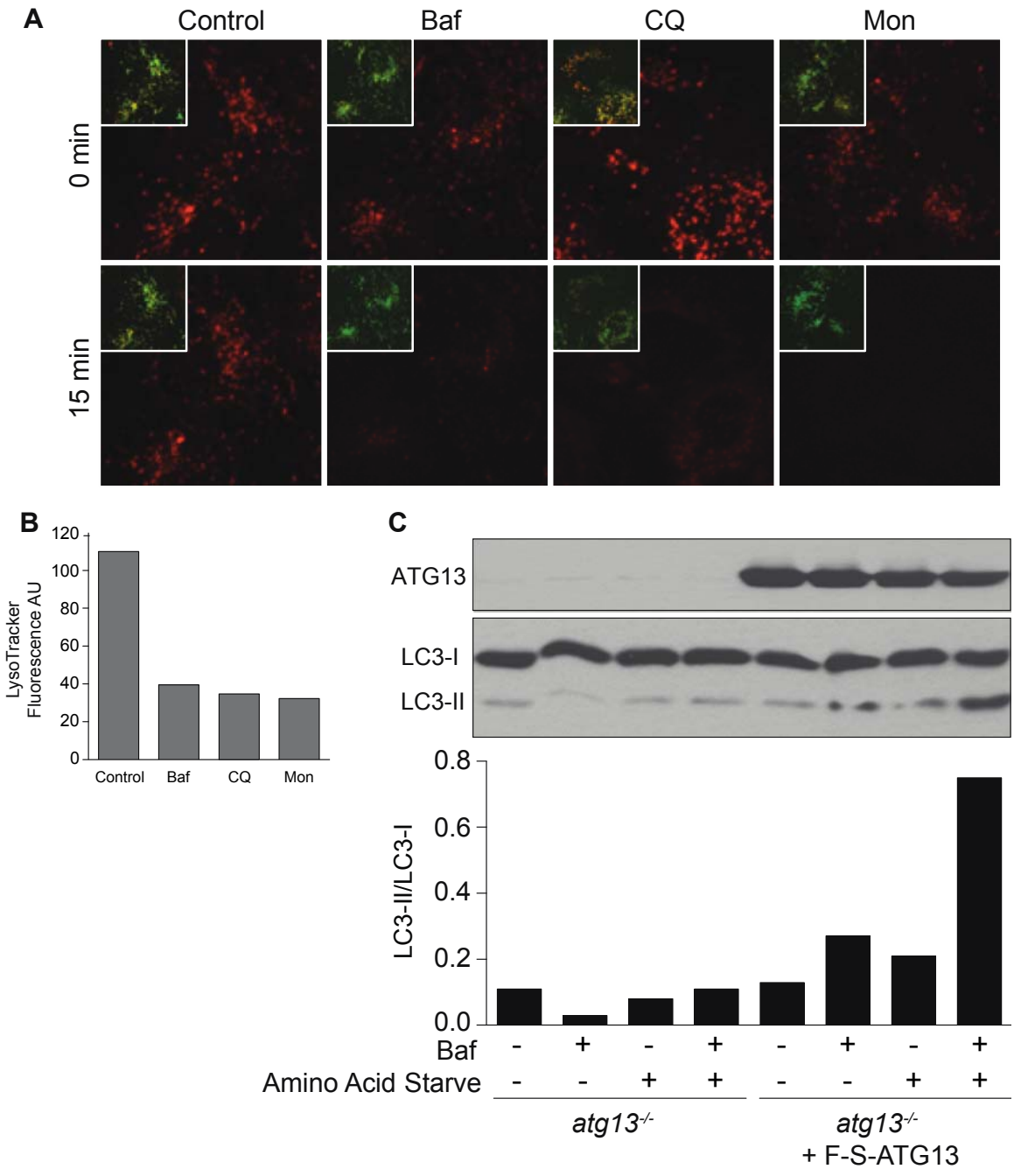

Figure S1, related to Figure 1. Effect of lysosome inhibitors on lysosomal pH and canonical autophagy deficiency in *atg13*$^{-/-}$ MEFs. (**A**) Confocal images of MCF10A cells expressing LAMP1-GFP and stained with LysoTracker Red at time 0 or 15 min after treatment with Baf (100 nM), CQ (100 μM) or Mon (100 μM). Main panels show LysoTracker Red signal; insets show LAMP1-GFP and LysoTracker Red merge. (**B**) Quantification of LysoTracker Red intensity in LAMP1-GFP compartments from experiment described in (**A**). (**C**) Western blot analysis of ATG13 and LC3 in *atg13*$^{-/-}$ MEFs and *atg13*$^{-/-}$ MEFs rescued with wild-type ATG13, following treatment with Baf (100 nM), 30 min amino acid starvation or both.

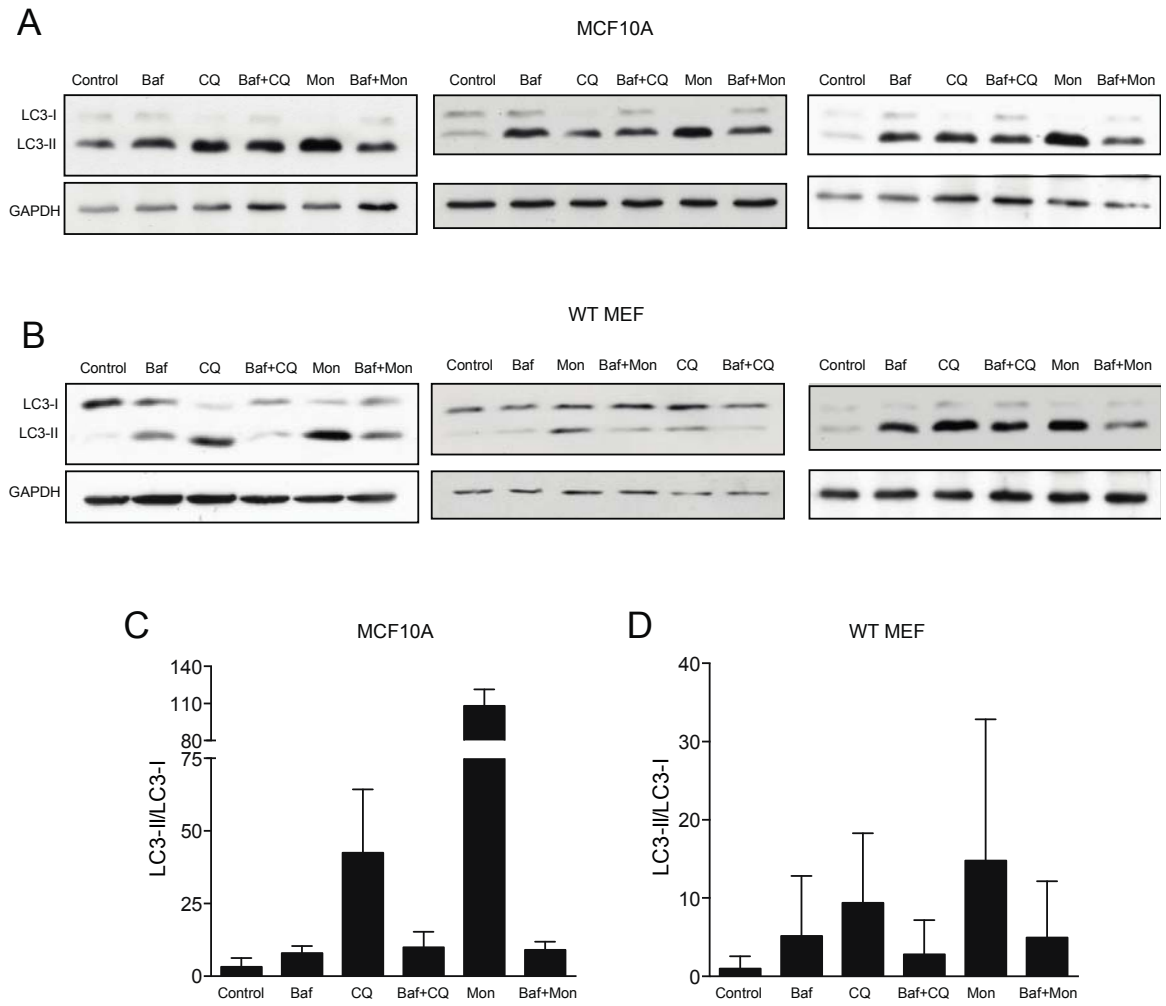

Figure S2, related to Figure 1. Quantification of LC3 western blots. (**A and B**) 3 separate experiments showing western blot images of LC3 and GAPDH following treatment with Baf (100 nM) Chloroquine (100 μM) or Monensin (100 μM) with or without 15 min pretreatment with Baf in (**A**) MCF10A cells and (**B**) wild-type MEFs. (**C and D**) Quantification of LC3-II/LC3-I ratios from blots in (**A and B**). Data represent mean ± standard deviation from 3 experiments.

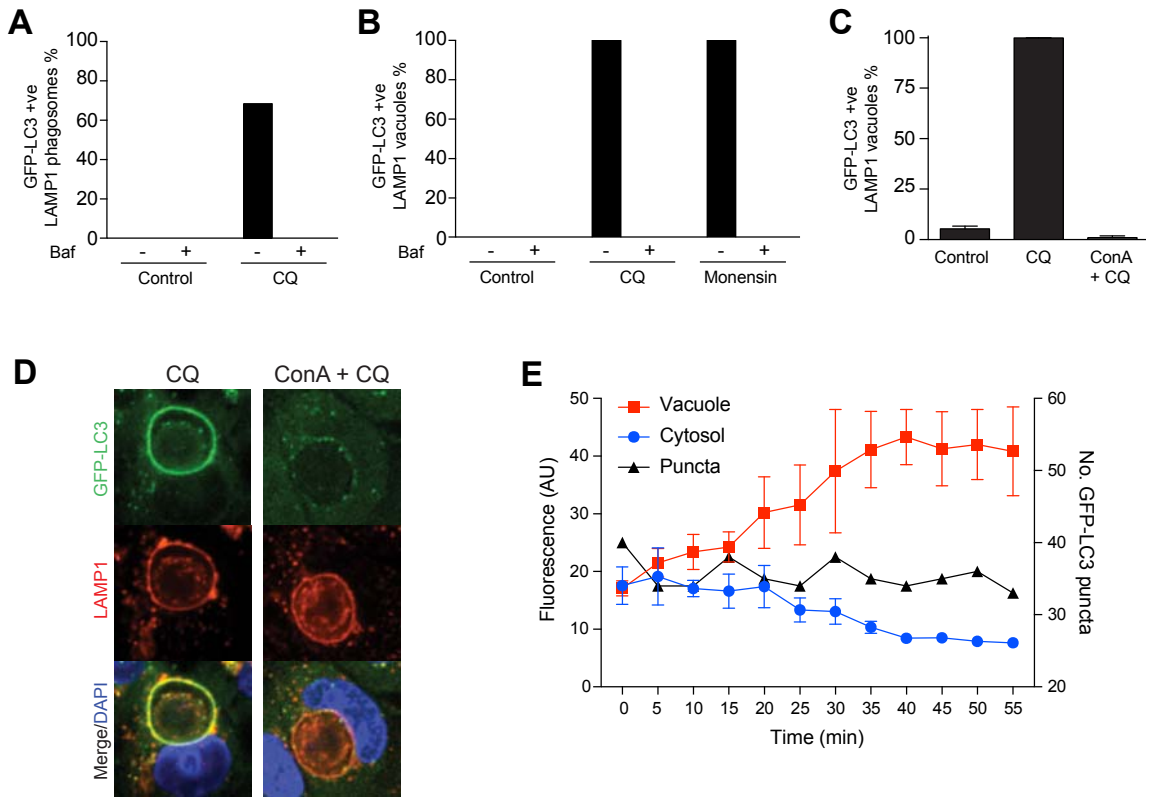

Figure S3, related to Figure 2. Quantification of GFP-LC3 recruitment to phagosomes and entotic corpse vacuoles and effects of V-ATPase inhibitors Baf and ConA; measurement of GFP-LC3 dynamics during chloroquine-mediated GFP-LC3 recruitment to entotic corpse vacuole. (**A**) Quantification of GFP-LC3 recruitment to uncoated-latex bead LAMP1-positive phagosomes in J774 cells following treatment with CQ (100 μM) with or without Baf (100 nM). (**B**) Quantification of GFP-LC3 recruitment to LAMP1-positive entotic corpse vacuoles in MCF10A cells following treatment with CQ (100 μM), Mon (100 μM) with or without Baf (100 nM) or ConA (100 nM). Data represent mean ± SEM from 3 separate experiments. (**C**) Quantification of GFP-LC3 recruitment to LAMP1-positive entotic corpse vacuoles in MCF10A cells following treatment with CQ (100 μM) with or without ConA (100 nM). Data represent mean ± SEM from 3 separate experiments. (**D**) Confocal mages of GFP-LC3 and LAMP1 on entotic corpse vacuoles in MCF10A cells treated with CQ or ConA (100 nM) + CQ for 1 h. (**E**) Analysis of GFP-LC3 intensity, left y-axis, through time in the cytosol and on the membrane of an entotic corpse vacuole. Error bars represent the SD of 3 separate measurements at each time point. Number of GFP-LC3 puncta (autophagosomes) measured and plotted on the right y-axis at each time point.

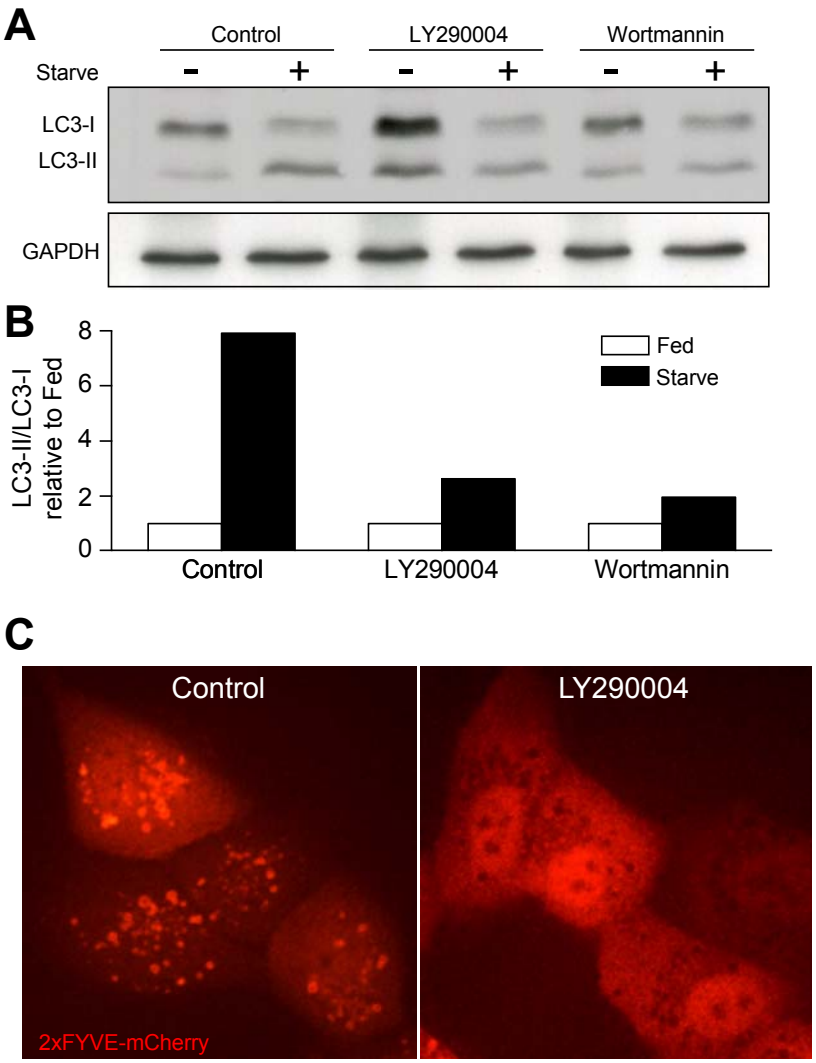

Figure S4, related to Figure 3. Inhibition of canonical autophagy and disruption of 2xFYVE-mCherry localization by PtdIns 3-kinase inhibitors. (**A**) Western blot analysis of LC3 in MCF10 cells in full media or amino acid starvation for 3 h with or without LY290004 (25 μM) or wortmannin (200 nM). (**B**) Quantification of LC3-II/LC3-I fold changes between fed and starved conditions. (**C**) Confocal images of MCF10A cells expressing a 2xFYVE-mCherry construct that labels PtdIns3P-positive membranes in the presence and absence of LY290004 (25 μM).

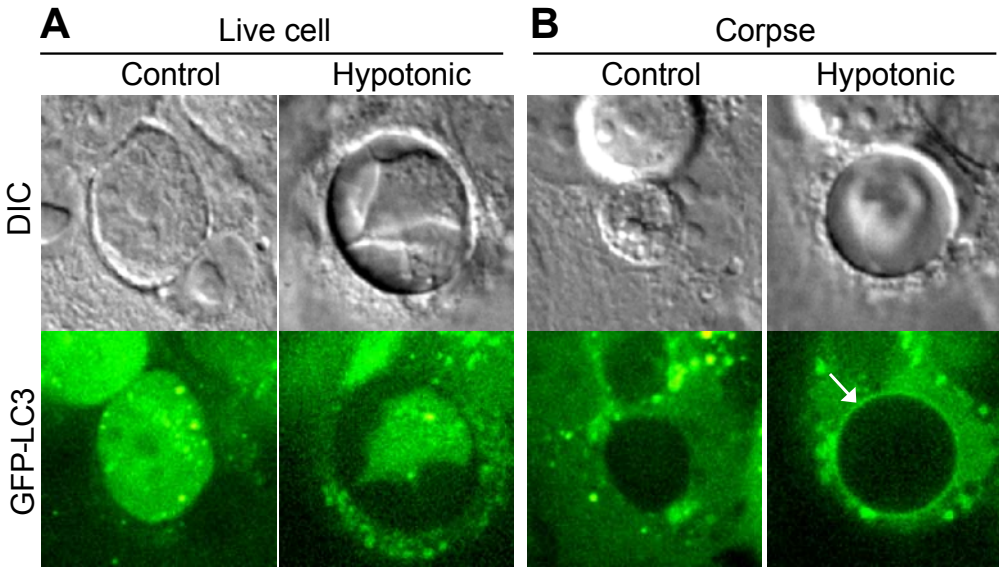

Figure S5, related to Figure 5. GFP-LC3 localization following osmotic swelling of entotic vacuoles containing live and dead cells. Confocal images of DIC and GFP-LC3 in MCF10A cell-in-cell structures containing (**A**) a live cell or (**B**) a corpse, before and after treatment with hypotonic media. Note both entotic vacuole swells, but GFP-LC3 only recruits to the corpse containing vacuole (arrow).