Expanded View Figures

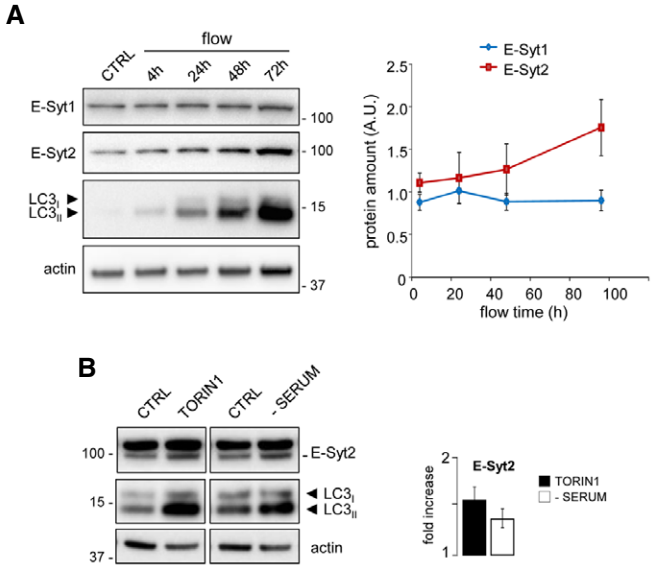

Figure EV1. Multiple autophagy stimuli induce E-Syt2 expression.

A Mechanical stress: Western blots and protein quantifications of protein lysates from KEC cells under basal (ctrl) and mechanical stress (flow) conditions for 4–72 h (n = 3). Plotted are mean ± s.d.

B mTOR inhibition and serum starvation: Western blots and protein quantifications (compared to ctrl) of protein lysates from HeLa cells grown under basal (ctrl) and autophagy-inducing conditions. Cells were cultured with 1.5 μM Torin1, an mTOR inhibitor, for 2 h or in a medium without serum for 1 h, respectively (n = 3). Plotted are mean ± s.d.

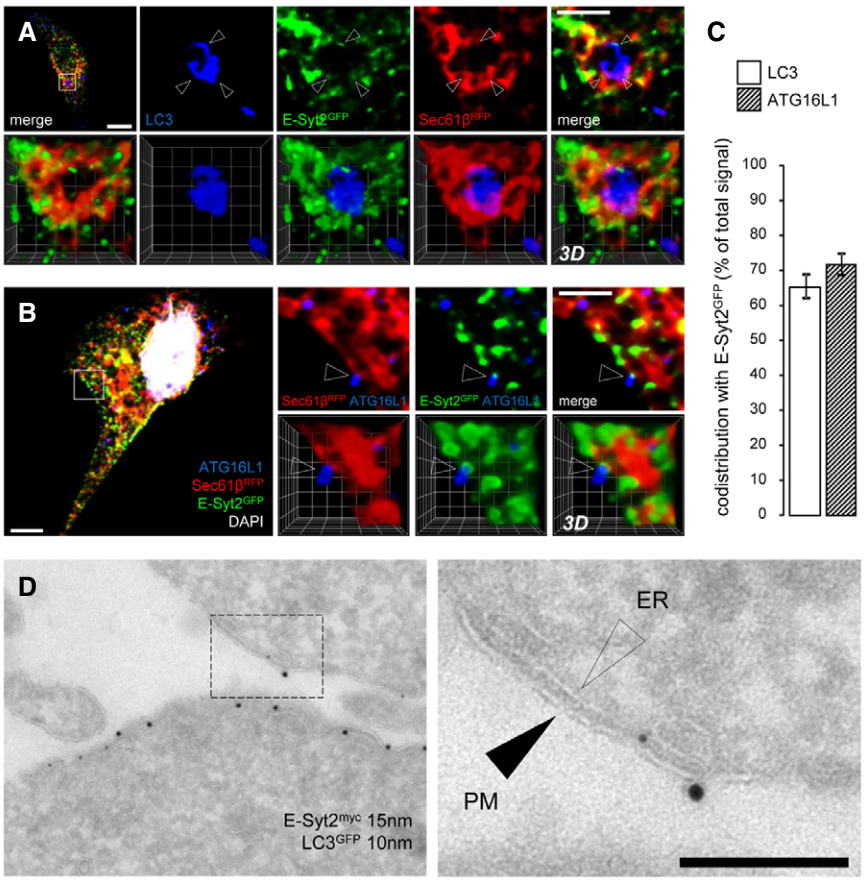

Figure EV2. LC3 and ATG16L1 reside at ER-PM contact sites under starvation conditions.

A, B Confocal microscope images and 3D reconstructions of HeLa cells co-transfected with GFP-E-Syt2 and RFP-Sec61β and immunostained for LC3 or ATG16L1 and DAPI. Arrowheads denote LC3 or ATG16L1 puncta near the E-Syt2-positive niche of the ER. Scale bars, 5 and 2.5 μm (magnified areas).

C Quantification of LC3 and ATG16L1 co-distribution with E-Syt2 at basal plan of the cell n = 80 cells. Mean ± s.e.m. shown.

D Immunogold electron micrographs of starved HeLa cells, showing co-distribution of the myc-E-Syt2 and anti-LC3 antibody at ER (empty arrowheads) and PM (black arrowheads) juxtaposition sites. Scale bar, 250 nm.

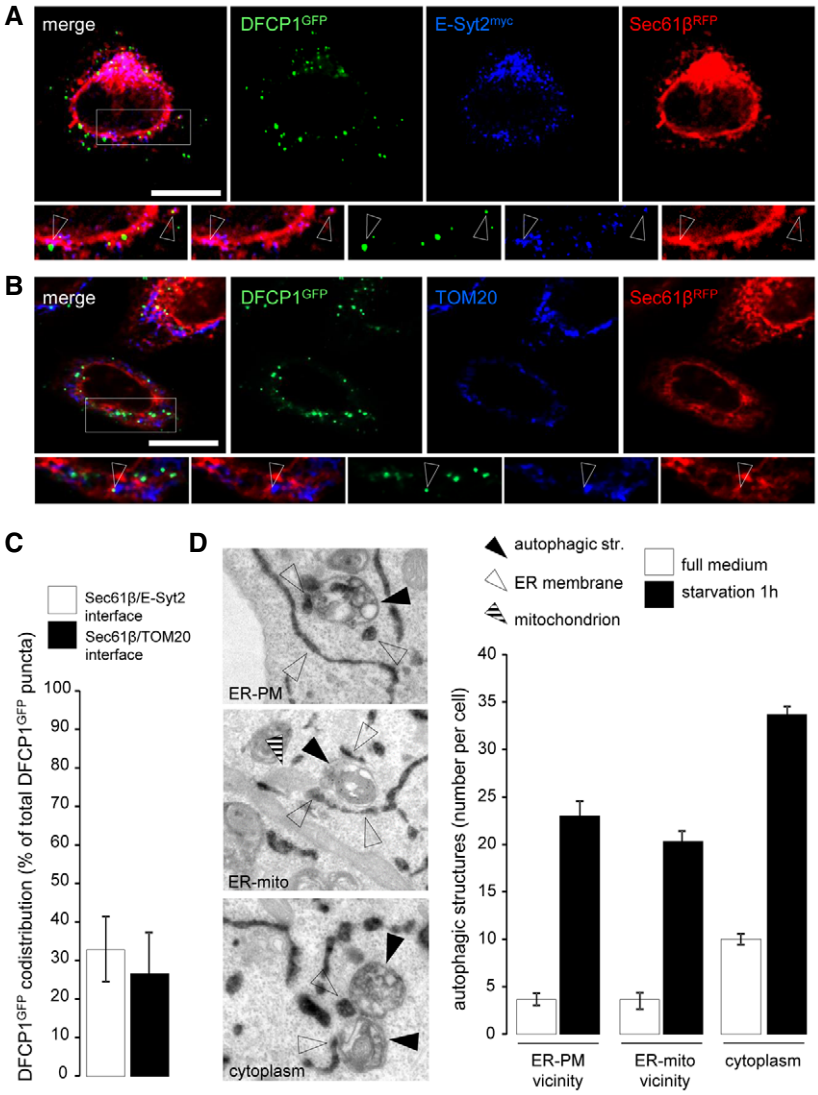

Figure EV3. Early autophagic structures form at ER-PM and ER–mitochondria contact sites.

A, B Confocal microscope images of HeLa cells co-transfected with GFP-DFCP1 (an omegasome marker), RFP-Sec61β (an ER marker) and myc-E-Syt2 (an ER-PM contact sites marker) or immunostained for TOM20 (a mitochondria marker) under starved conditions (15 min). Arrowheads denote omegasome formation at (A) ER-PM or (B) ER–mitochondria contact sites. Scale bars, 10 μm.

C Quantification of percent DFCP1-positive structures at ER-PM (Sec61β/E-Syt2 interface) and ER–mitochondria (Sec61β/TOM20 interface) contact sites. Plotted are mean ± s.e.m., $n = 80$.

D Electron microscopy images of HeLa cells transfected with the ER luminal marker ssHRP-myc-KDEL and starved for 1 h showing autophagic structures adjacent (within 1 μm) to ER-PM contact sites (ER-PM), ER-mitochondria contact sites (ER-mito) and to neither organelle (cytoplasm). The quantification of these autophagic structures is shown as well. Plotted are means ± s.e.m., $n = 80$ cells.

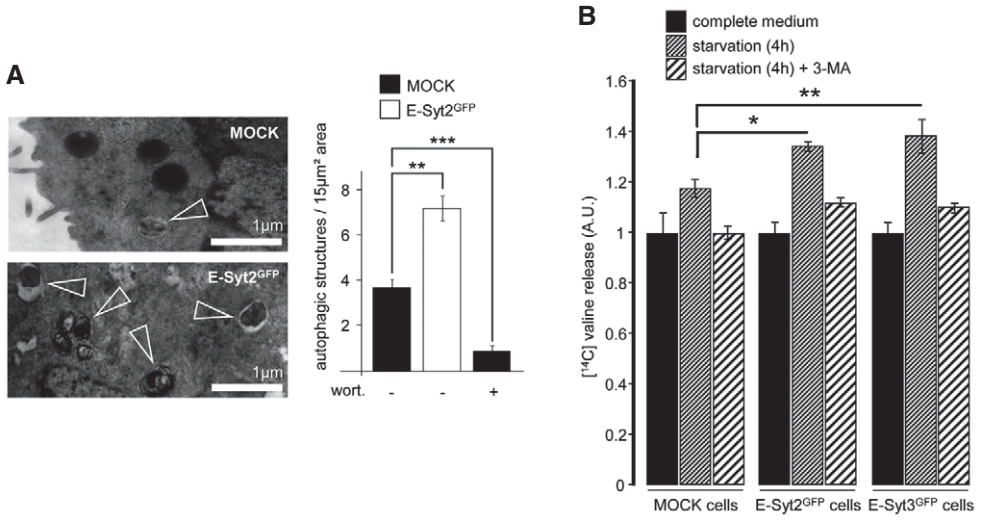

Figure EV4. Overexpression of E-Syt2 and E-Syt3 enhances autophagy.

A An electron micrograph of GFP-E-Syt2-expressing HeLa cells starved for 1 h, showing an increased number of autophagic structures (arrowheads) compared to starved control cells. Scale bar, 1 μm. Wortmannin (wort, 100 nM) was used as a negative control, and autophagic structures were counted in 15 μm^2 areas (n = 10 cells).

B Proteolysis analysis showing an increased protein degradation rate in GFP-E-Syt2- and GFP-E-Syt3-expressing cells (n = 3). 3-methyladenine (3-MA) was used at 10 mM.

Data information: Means ± s.e.m. are plotted. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed t-test.

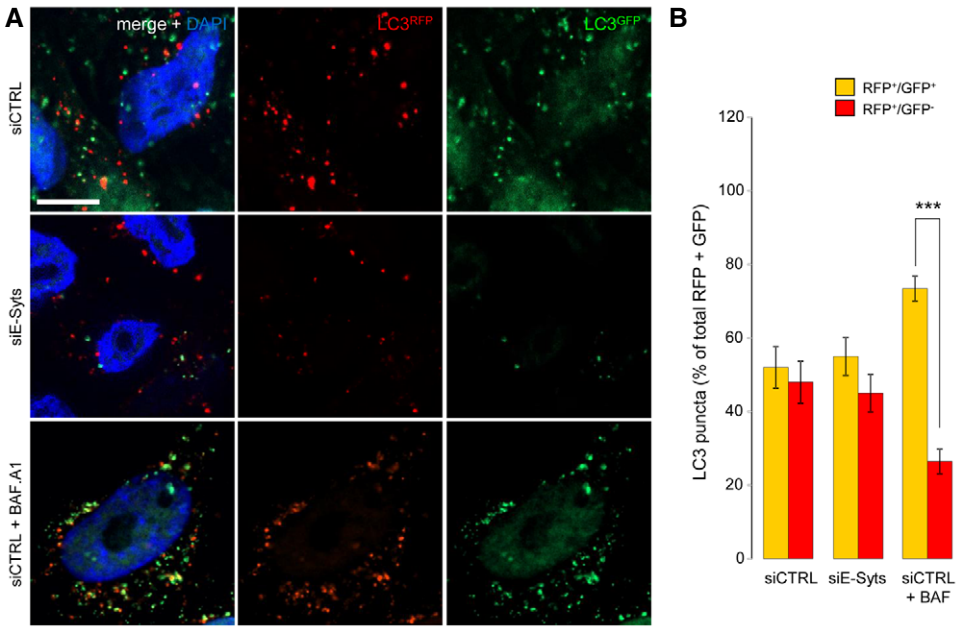

Figure EV5. Autophagosome maturation is not affected in E-Syt-deficient cells.

A, B HeLa cells stably transfected with mRFP-GFP-LC3 and treated with siE-Syts have fewer autophagosomes, but autophagosomes have normal functionality, as evidenced by (A) microscopy and (B) by RFP^+/GFP^+ and RFP^+/GFP^- LC3-puncta counting. Cells treated with Bafilomycin A1 (+BAF. A1) and siCTRL were used as a positive functionality control and showed decreased autophagosome maturation (i.e. reduced RFP^+/GFP^- LC3-puncta compared to siCTRL-treated cells) as expected (n = 3). ***P < 0.001, unpaired two-tailed t-test. Scale bar, 10 μm. Plotted are mean ± s.e.m.

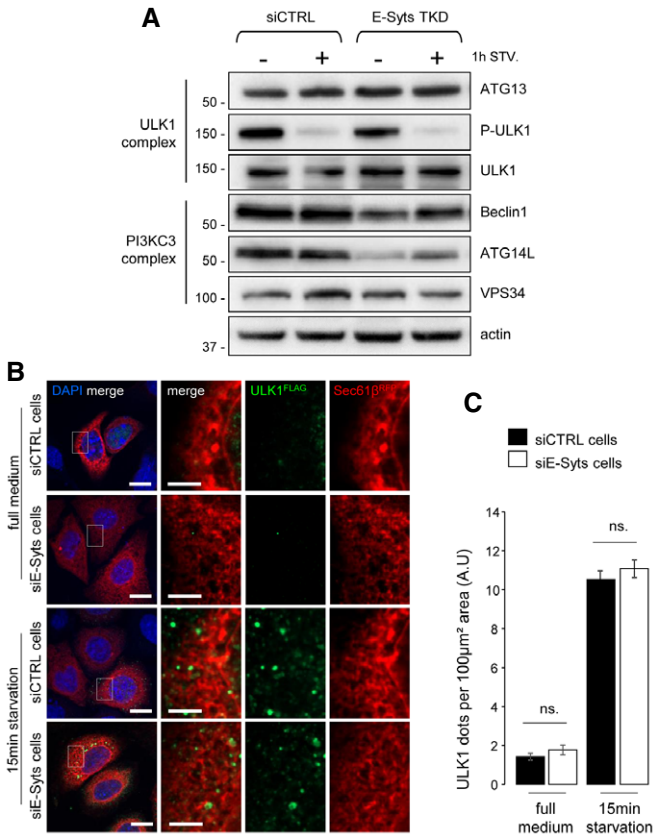

Figure EV6. The ULK1 complex is not affected in E-Syt-deficient cells.

A Western blots of protein lysates from siCTRL- and siE-Syt-treated HeLa cells grown in complete (−) and 1 h of starvation (+1 h STV.) conditions (n = 3). Actin is used as a loading control.

B Confocal microscope images of HeLa cells co-transfected with FLAG-ULK1 and RFP-Sec61β. Nuclei are marked with DAPI. Scale bars, 10 and 4 μm (magnified areas).

C Quantification of ULK1 puncta in images from (B) (n = 3; 20 cells analysed). Mean ± s.e.m. are plotted. NS, non-significant, unpaired two-tailed t-test.