Supplemental Video Legends

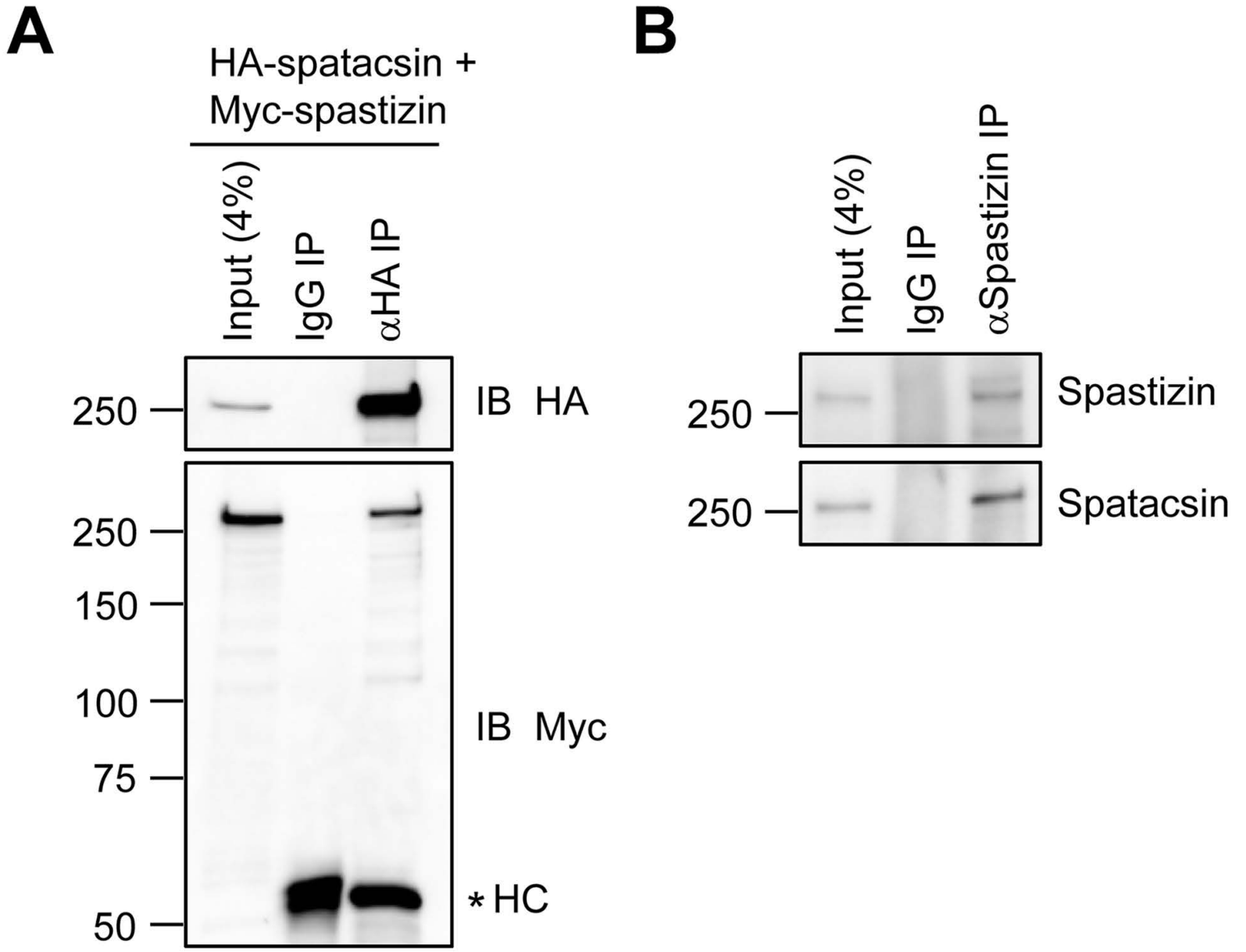
Video 1 LAMP1-GFP, EBSS 0 h. HeLa cells stably expressing LAMP1-GFP were starved with EBSS for 0 h, and images were acquired at 1 s intervals for 40 s.

Video 2 LAMP1-GFP, EBSS 8 h. HeLa cells stably expressing LAMP1-GFP were starved with EBSS for 8 h, and images were acquired at 1 s intervals for 40 s.

Video 3 LAMP1-GFP in siCTL, EBSS 8 h. HeLa cells stably expressing LAMP1-GFP were transfected with control siRNA and starved with EBSS for 8 h. Images were acquired at 1 s intervals for 20 s.

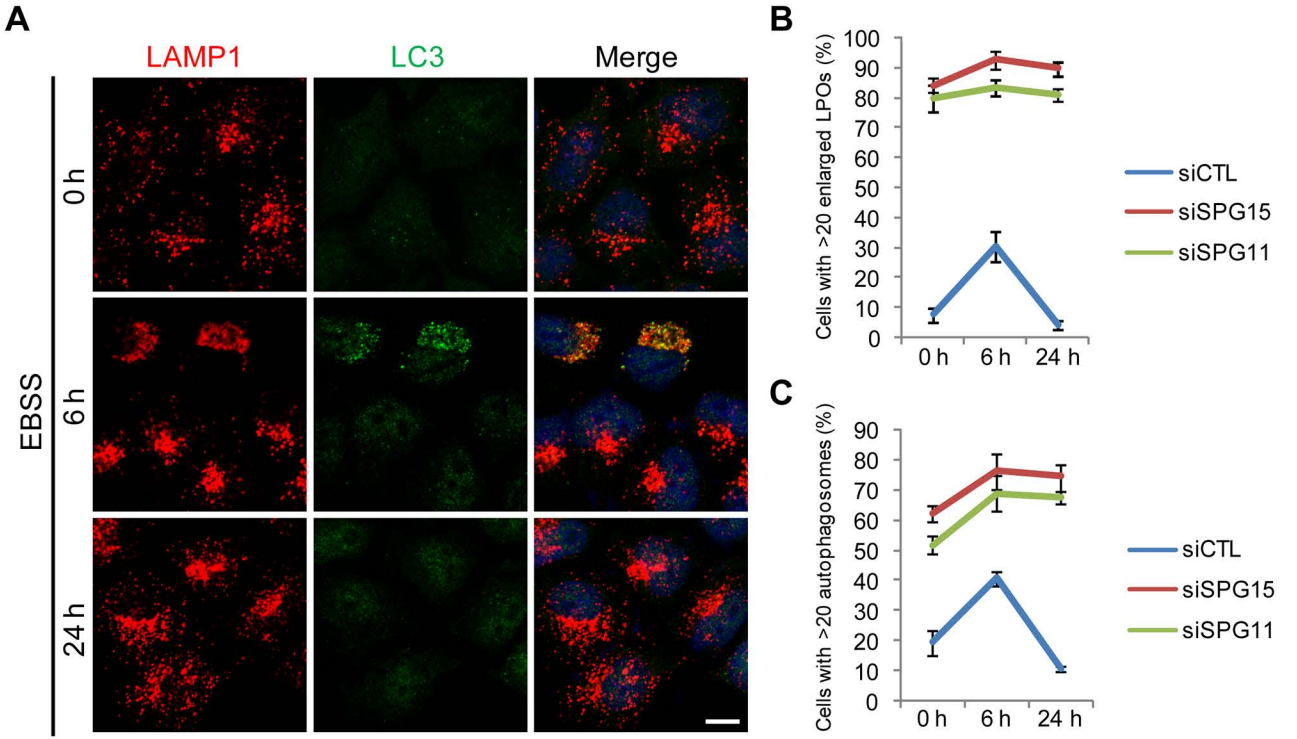
Video 4 LAMP1-GFP in siSPG15, EBSS 8 h. HeLa cells stably expressing LAMP1-GFP were transfected with spastizin siRNA and starved with EBSS for 8 h. Images were acquired at 1 s intervals for 20 s.

Video 5 LAMP1-GFP in siSPG11, EBSS 8 h. HeLa cells stably expressing LAMP1-GFP were transfected with spatacsin siRNA and starved with EBSS for 8 h. Images were acquired at 1 s intervals for 20 s.



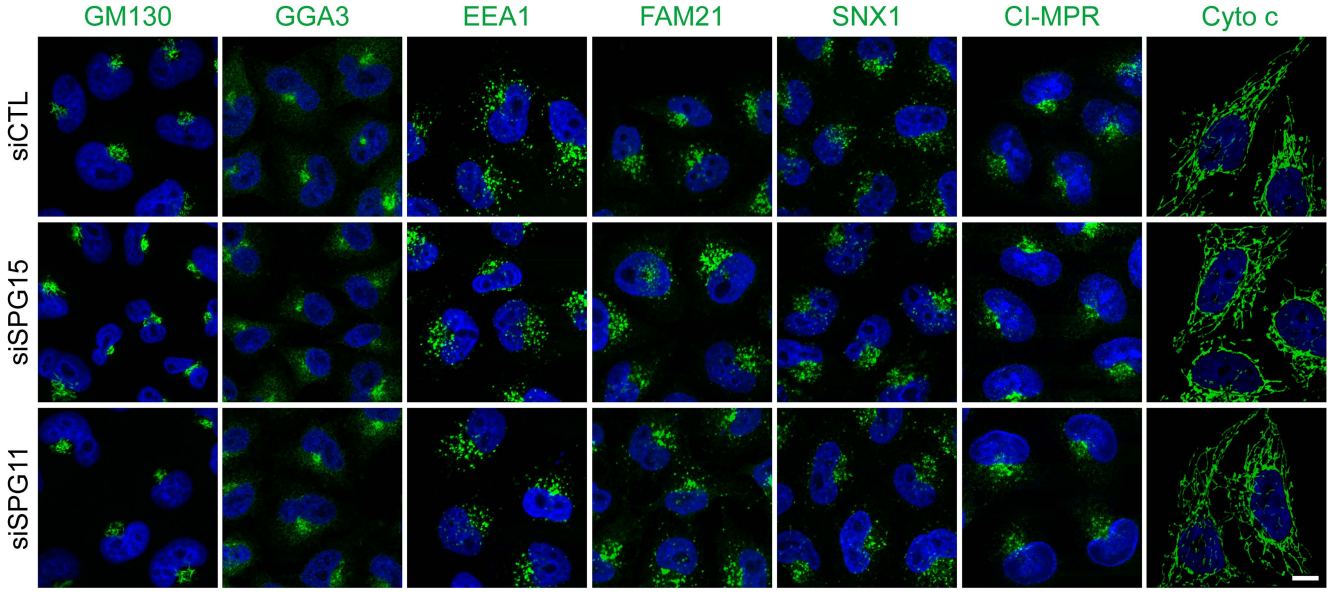
Supplemental Figure 1

Spastizin co-immunoprecipitates with spatacsin. (**A**) HA-spatacsin and Myc-spastizin were co-expressed in HEK293T cells, and lysates were immunoprecipitated (IP) and immunoblotted (IB) with the indicated antibodies. (**B**) Endogenous spastizin in HEK293T cells was immunoprecipitated (IP) with anti-spastizin antibodies or else control IgG, and immunoblotted. An asterisk (*) identifies the IgG heavy chain (HC). Migrations of molecular weight standards (in kDa) are at the left in both panels.



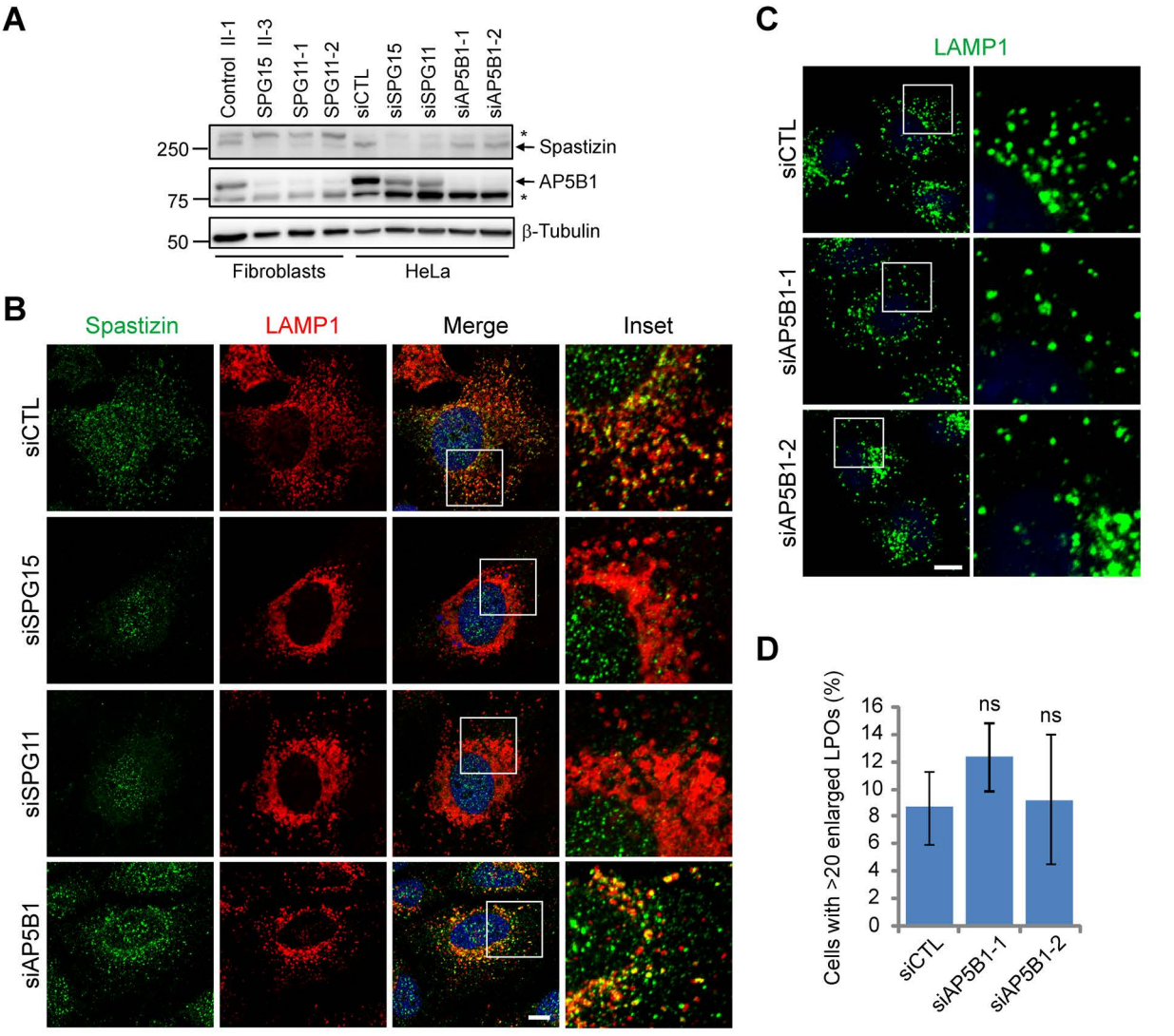
Supplemental Figure 2

Depletion of spastizin or spatacsin impairs EBSS-induced dynamic alterations in LPO size and autophagosome number. (**A**-**C**) HeLa cells transfected with control (siCTL), spastizin (siSPG15) or spatacsin (siSPG11) siRNAs were co-immunostained for LAMP1 (red) and LC3 (green). (**A**) Representative images of control cells starved for the indicated time periods. (**B**) Starved cells with >20 enlarged LPOs from **A** were quantified (n = 3; >200 cells per experiment). (**C**) Starved cells with >20 autophagosomes from **A** were quantified (n = 3; >200 cells per experiment). Scale bar: 10 μm.



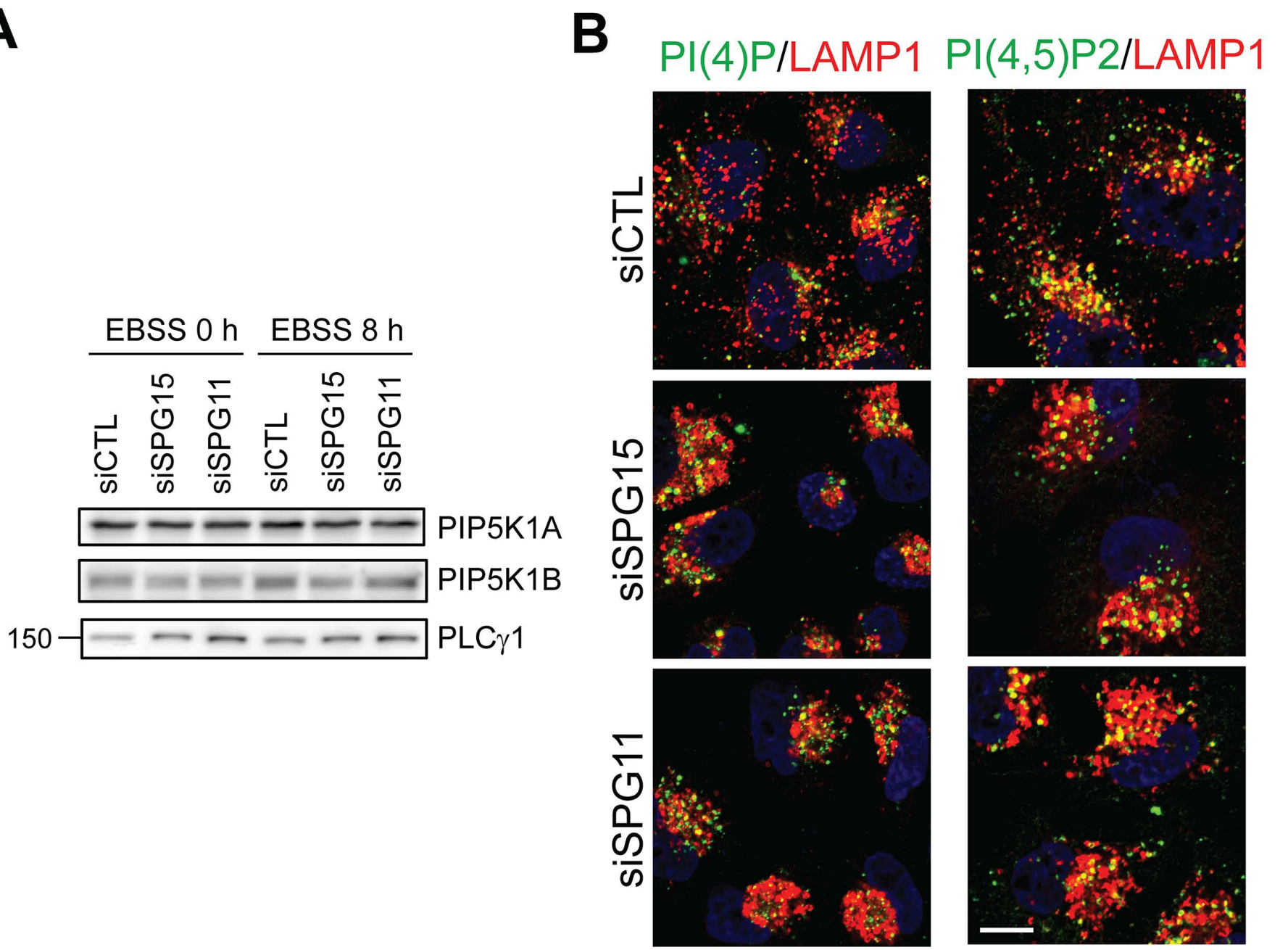
Supplemental Figure 3

Depletion of spastizin or spatacsin does not alter endosomes, mitochondria or Golgi apparatus morphology. HeLa cells were transfected with control (siCTL), spastizin (siSPG15) or spatacsin (siSPG11) siRNAs and immunostained for the indicated organelle markers (green). Hoechst 33342 (blue) stains the nuclei. Cyto *c*, cytochrome *c*. Scale bar: 10 μm.



Supplemental Figure 4

Effects of AP-5 depletion on spastizin and spatacsin levels and sizes of LPOs. (**A**) Lysates were prepared from wild-type (II-1), SPG15 (II-3) or SPG11 (SPG11-1 and SPG11-2) fibroblasts. HeLa cell lysates were prepared from cells transfected with control (siCTL), spastizin (siSPG15), spatacsin (siSPG11) or AP5B1 (siAP5B1-1 and siAP5B1-2) siRNAs and immunoblotted. β-tubulin levels were monitored as a control for protein loading. Asterisks (*) denote cross-reacting bands, and migrations of molecular weight standards (in kDa) are indicated at the left. (**B**) HeLa cells were transfected with siCTL, siSPG15, siSPG11 or siAP5B1 siRNAs, and then co-immunostained for endogenous spastizin (green) and LAMP1 (red). (**C**) HeLa cells were transfected with siCTL, siAP5B1-1 or siAP5B1-2 siRNAs, and then immunostained for LAMP1. (**D**) Cells with >20 enlarged LPOs from C were quantified (n = 3; >200 cells per experiment). Means ± SD are shown. One-way ANOVA followed by Tukey's multiple comparison test; ns, not significant. Insets in the images are enlarged to the right in **B** (3X) and **C** (3.4X). Scale bars: 10 μm.



Supplemental Figure 5

Lysosomal localization of PI(4)P and PI(4,5)P2 is not impaired in spastizin- or spatacsin-depleted cells. (**A**) HeLa cells transfected with control siRNA (siCTL) or else siRNAs specific for spastizin (siSPG15) or spatacsin (siSPG11) were starved with EBSS for 0 or 8 h. Cell lysates were immunoblotted with PIP5K1A (56 kDa) and PIP5K1B (61 kDa) antibodies. PLCγ1 levels were monitored as a control for protein loading. Migrations of molecular weight standards (in kDa) are to the left. (**B**) HeLa cells were transfected with siCTL, siSPG15 or siSPG11 siRNAs, and then co-immunostained for LAMP1 (red) and either PI(4)P or PI(4,5)P2 (green). Scale bar: 10 μm.