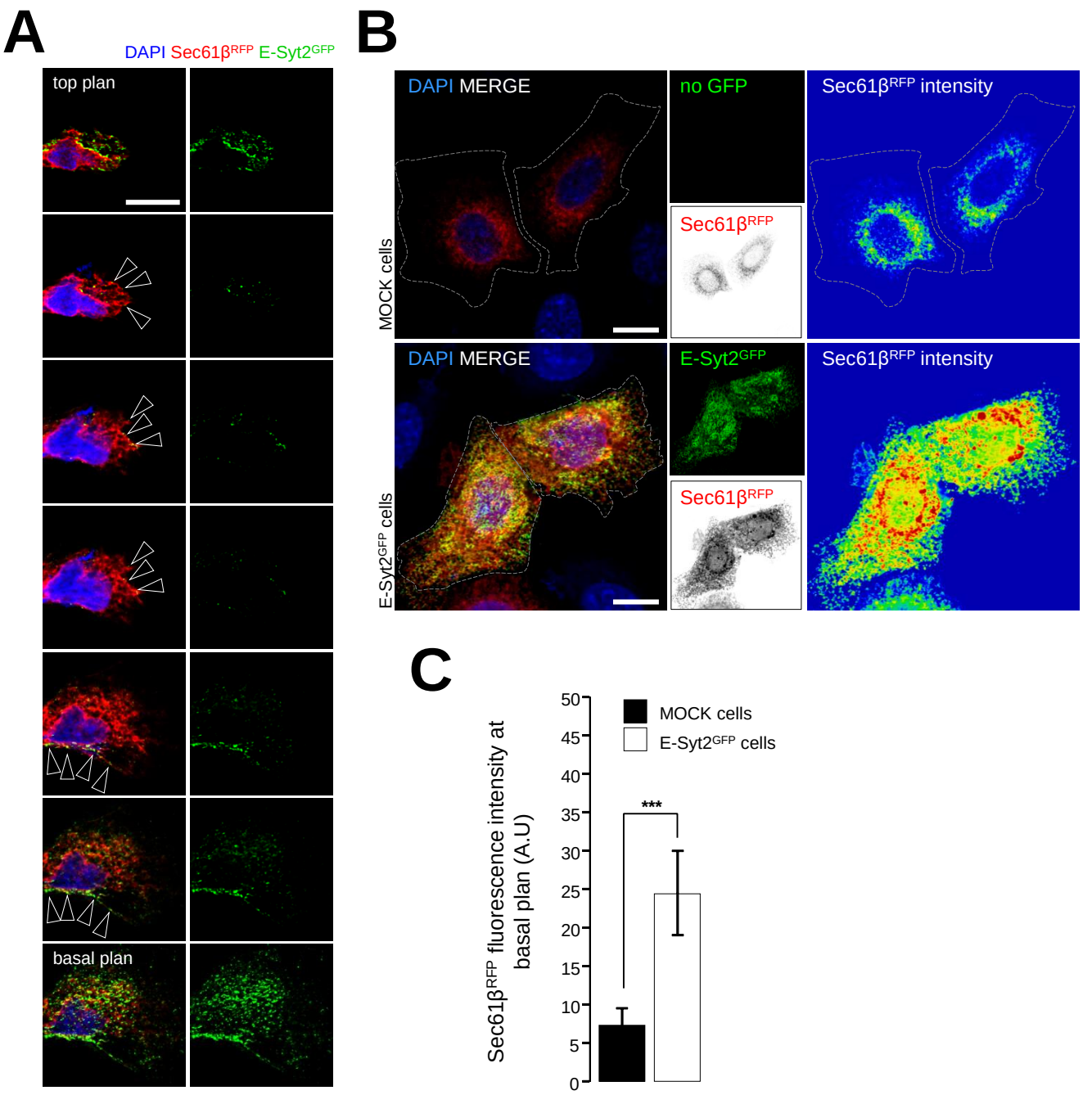
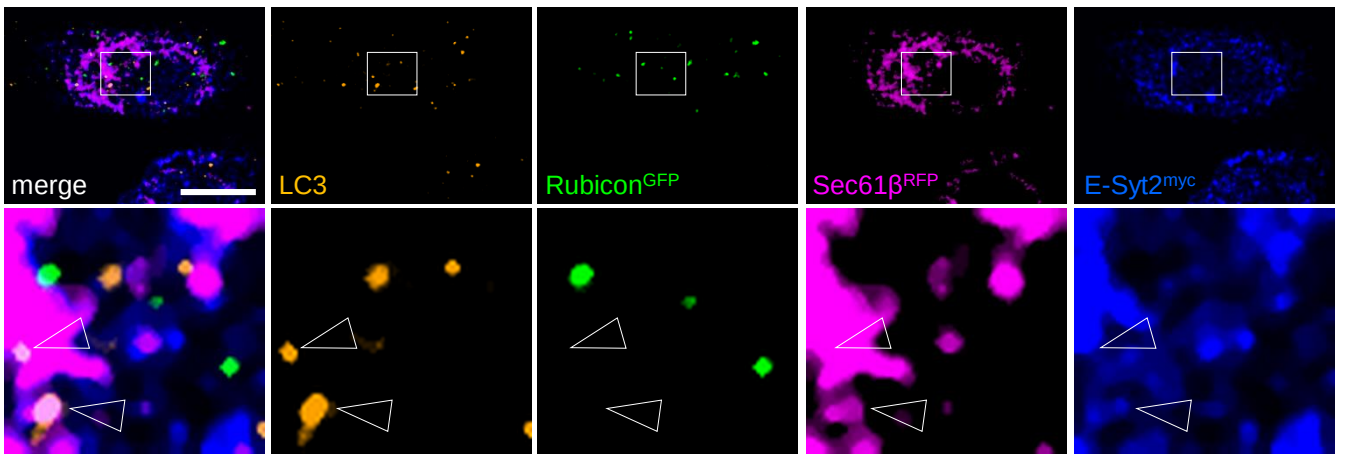
Nascimbeni et al.

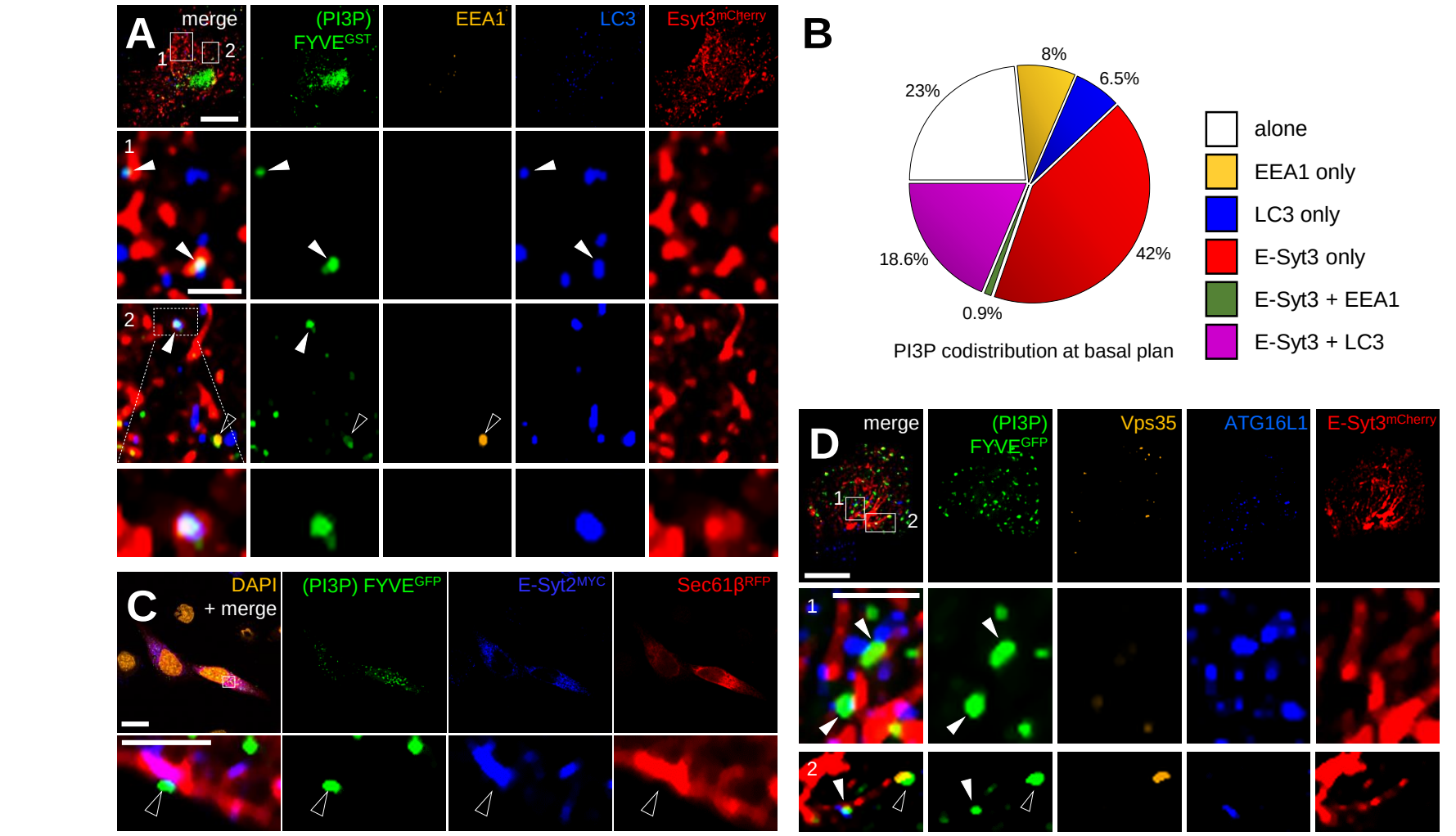
Table of content:



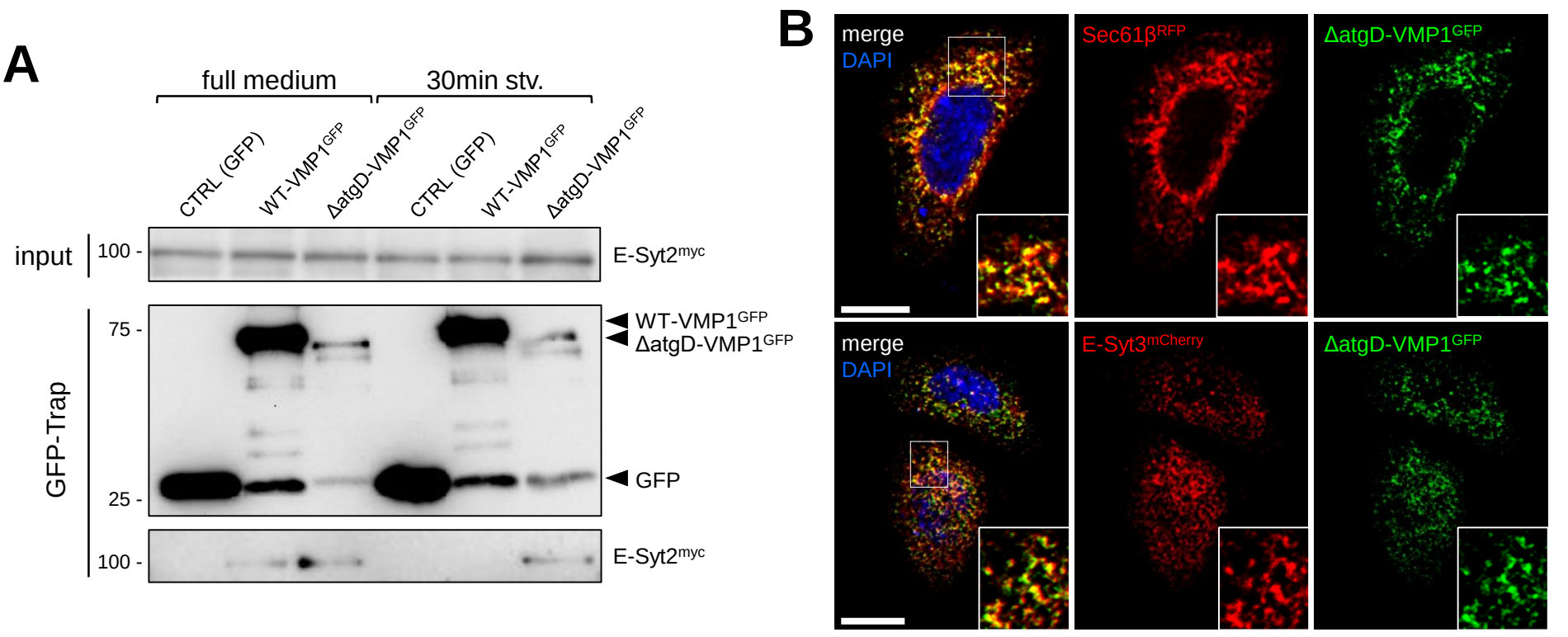
Appendix figure S1: E-Syt2 localizes at the cell periphery and its overexpression increases the cortical ER (the number of ER-PM contacts). Confocal microscope images of HeLa cells co-transfected with RFP-Sec61β and GFP-E-Syt2 or with mock vector. Nuclei are marked with DAPI. **A,** Sequential acquisitions going from top to basal plans of RFP-Sec61β and GFP-E-Syt2-transfected HeLa cells, showing the co-localization of E-Syt2 with the ER marker at the periphery of the cell. **B**, Fluorescence intensity of the ER marker Sec61β. Scale bars, 10 µm. **C,** Quantification of the Sec61β fluorescence intensity at the basal plan in E-Syt2 transfected cells, compared to mock. Means ± s.e.m. are plotted. ***P < 0.001, unpaired two-tailed *t*-test.



Appendix figure S2: LC3-associated phagocytosis (LAP) phagosomes do not co-localise with ER-PM contact sites. Confocal microscope images of HeLa cells co-transfected with GFP-Rubicon, RFP-Sec61β, and myc-E-Syt2 and immunostained for LC3. Empty arrowheads denote co-distribution of LC3 with Sec61β and E-Syt2. Scale bar, 10 µm.



Appendix figure S3: The autophagosome-associated (but not the endosome-associated) PI3P pool co-distributes with E-Syt2 and E-Syt3. A, HeLa cells expressing mCherry-E-Syt3 were immunostained for PI3P (via a FYVE-GST peptide and anti-GST fluorescent antibody), EEA1 (an endosomal marker), and LC3. White arrowheads indicate co-distributing PI3P/LC3/E-Syt3, while empty arrowheads indicate co-distribution of PI3P/EEA1 only. **B**, Quantification of PI3P co-distribution with EEA1, E-Syt3 and LC3, at basal plan of the cell (as in a). n=80 cells. **C,** Confocal images of HeLa cells co-transfected with GFP-2xFYVE (a PI3P marker), myc-E-Syt2, and RFP-Sec61β show PI3P at E-Syt2-positive ER (Sec61β- and E-Syt2-positive) sites (empty arrowheads). **D,** Confocal images of HeLa cells co-transfected with GFP-2xFYVE, VPS35 (an endosome marker), and mCherry-E-Syt3 and immunostained for ATG16L1 (an early autophagic marker) show the co-distribution of PI3P and ATG16L1 at ER-PM contact sites (E-Syt3-positive sites, white arrowheads), but not at endosomes (VPS35-positive sites, empty arrowheads). Scale bars, 10 µm and 3 µm (magnified areas).



Appendix figure S4: Unlike WT-VMP1, the autophagy incompetent VMP1 mutant (ΔatgD-VMP1) interacts permanently with E-Syt2 even upon starvation. A, HeLa cells transiently co-transfected with myc-E-Syt2 and GFP-VMP1 (WT) and GFP-ΔatgD-VMP1 or empty vector were grown in complete medium or starved (30 min). Cells were immunoprecipitated by GFP-trap beads and immunostained by antibodies indicated on the right. **B,** Confocal images of HeLa cells, showing the co-distribution of the co-transfected GFP-ΔatgD-VMP1 and the ER marker RFP-Sec61β or the ER-PM contacts marker mCherry-E-Syt3, upon starvation (3 h). Scale bars, 10 µm.