McLelland et al., http://www.jcb.org/cgi/content/full/jcb.201603105/DC1

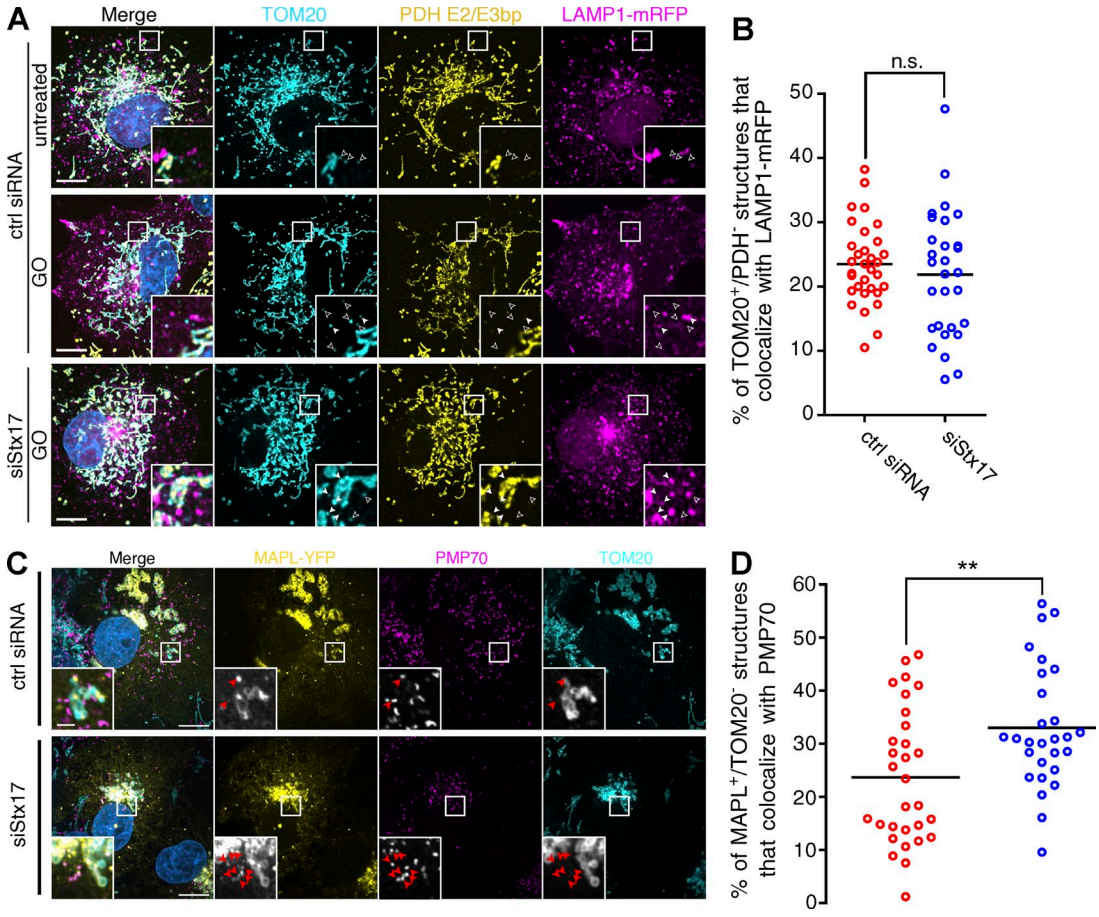

Figure S1. **Silencing of Stx17 does not perturb other MDV pathways.** (A) Representative confocal images of cells expressing LAMP1-mRFP (magenta) treated with glucose oxidase (GO), then fixed and immunostained for PDH E2/E3bp (yellow) and TOM20 (cyan; Hoescht, blue). TOM20$^+$/PDH E2/E3bp$^-$ structures that are positive (white arrowheads) or negative (empty arrowheads) for LAMP1 are indicated. Bars: (main) 10 µm; (inset) 2 µm. (B) Quantification of the percent of TOM20$^+$/PDH E2/E3bp$^-$ structures that colocalize with LAMP1-mRFP in Stx17-silenced COS7 cells treated with 50 mU/ml glucose oxidase or left untreated. Bars represent mean; n = 32–39 cells per condition; n.s., not significant. (C) COS7 cells transfected with MAPL-YFP (yellow) and the indicated siRNA were fixed and immunostained for TOM20 (cyan) and PMP70 (magenta, a peroxisomal marker; Hoescht, blue). Red arrowheads indicate PMP70-labeled peroxisomes that also contain MAPL-YFP. Bars: (main) 10 µm; (inset) 2 µm. (D) Quantification of the percentage of PMP70-labeled peroxisomes that also contain MAPL-YFP per cell in control siRNA (red) and siStx17 (blue) cells from C. Bars represent the mean; n = 30 and 28 cells for ctrl siRNA and siStx17, respectively; **, $P < 0.01$.

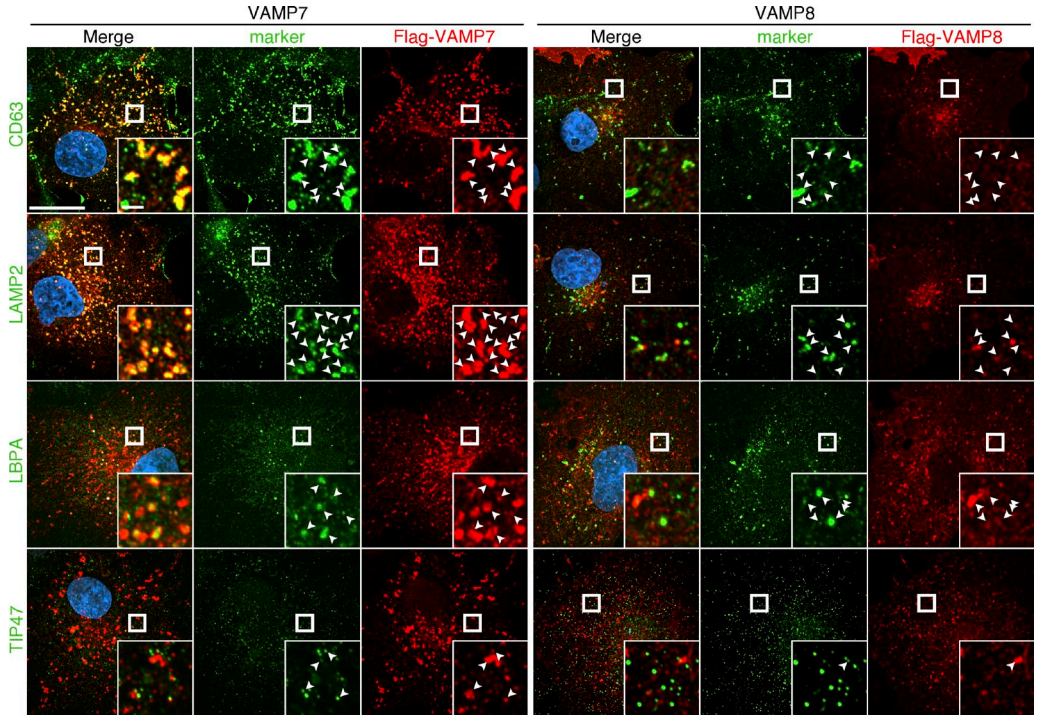

Figure S2. **VAMP7 and VAMP8 localize to late endosomes and lysosomes.** COS7 cells expressing Flag-VAMP7 (left) or Flag-VAMP8 (right) were fixed and immunostained for the Flag tag (red) and the indicated late endosomal/lysosomal (CD63, LAMP2) or strictly late endosomal (TIP47, LBPA) marker (green; Hoescht, blue). Arrowheads indicate structures that are positive for both the Flag-VAMP and the corresponding marker. Bars: (main) 20 µm; (inset) 2 µm.

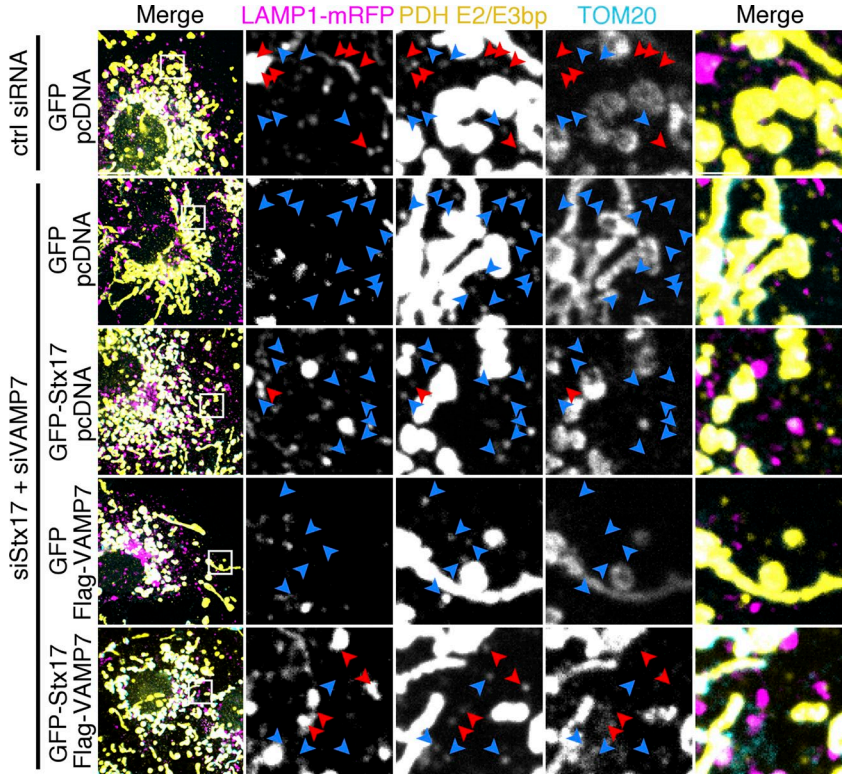

Figure S3. **MDV targeting in cells lacking Stx17-associated SNAREs.** Representative confocal images of COS7 cells from Fig. 5 (I and J) treated with 25 µM antimycin A for 45 min, then fixed and immunostained against PDH E2/E3bp (yellow) and TOM20 (cyan; LAMP1-mRFP, magenta). PDH E2/E3bp^{+}/TOM20^{-} structures that are positive (red arrowheads) or negative (blue arrowheads) for LAMP1 are indicated. Bars: (main) 10 µm; (zoom) 2 µm.

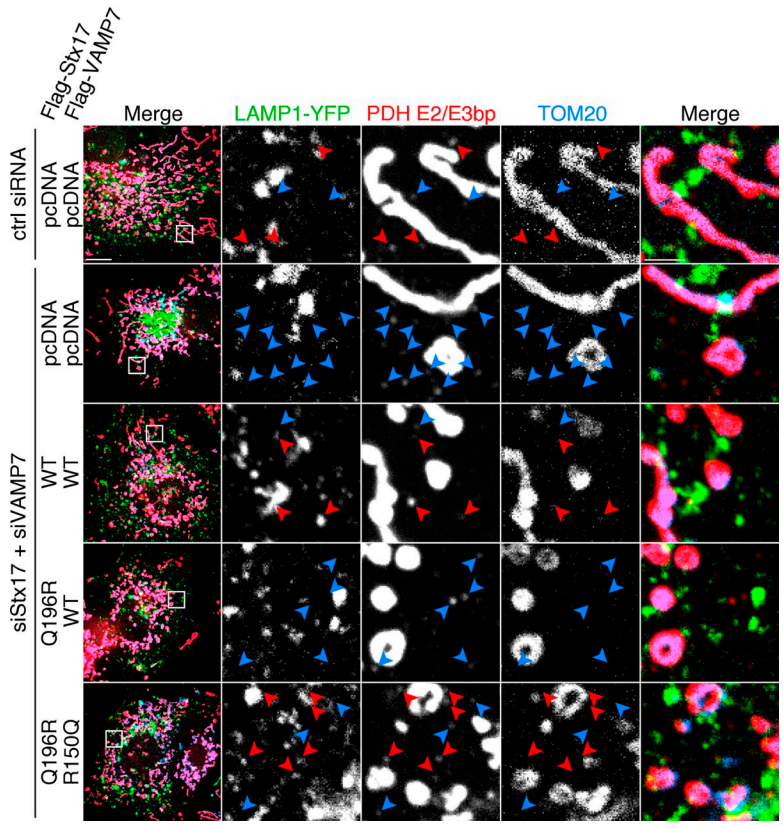

Figure S4. **MDV targeting in cells reconstituted with zero-layer mutants.** Representative confocal images of COS7 cells from Fig. 7 (G and H) treated with 25 µM antimycin A for 45 min, then fixed and immunostained against PDH E2/E3bp (red) and TOM20 (blue; LAMP1-YFP, green). PDH E2/E3bp$^+$/TOM20$^-$ structures that are positive (red arrowheads) or negative (blue arrowheads) for LAMP1 are indicated. Bars: (main) 10 µm; (zoom) 2 µm.

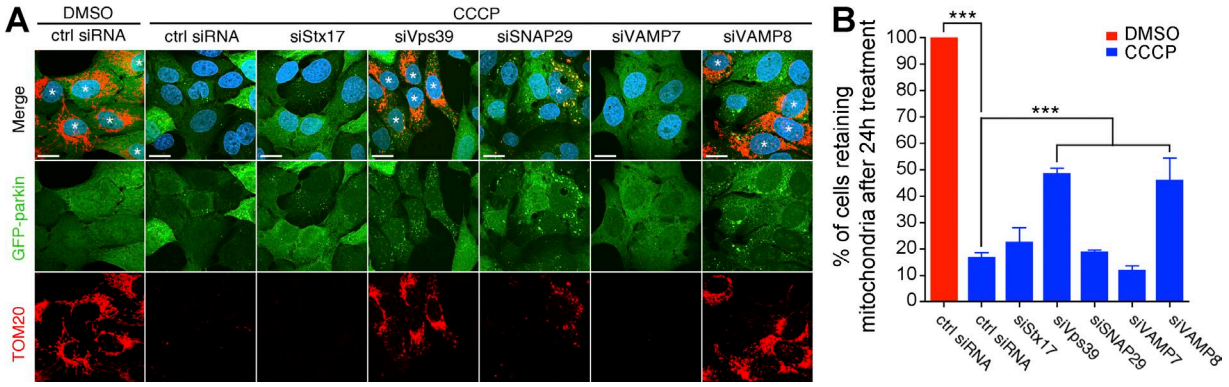

Figure S5. **Stx17 is dispensable for depolarization-induced mitophagy.** (A) Representative confocal images of U2OS:GFP-parkin (green) cells (grown on glucose) treated with 20 µM CCCP or DMSO for 24 h. Mitophagy was monitored by then fixing and immunostaining for TOM20 (red; Hoescht 33342, blue). Cells that have retained their mitochondria are marked by asterisks. Bars, 20 µm. (B) Quantification of the percent of cells retaining mitochondria from cells in A. Bars represent mean ± SEM; n = 3 replicate cells per condition, with >100 cells counted per condition for each replicate; ***, $P < 0.001$.