FIGURE S1. Expression of GFP-SYP31 and vacuolar morphology
Confocal images of BY-2 cells expressing GFP-SYP31 (*cis*, green) and mRFP-VAM3 (vacuole, magenta). Scale bars = 20 μm.
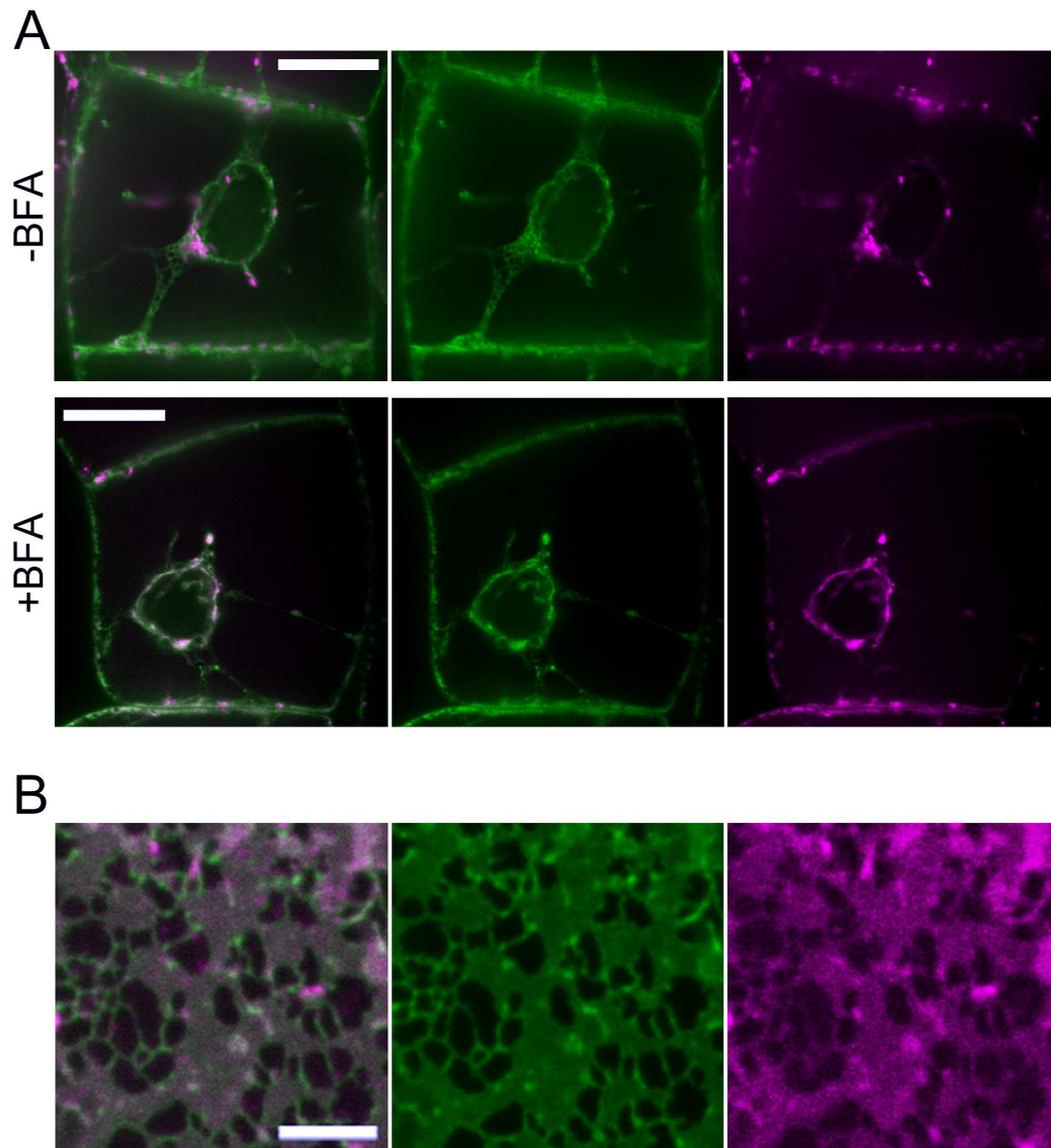
FIGURE S2. Relocalization of ST-mRFP to the ER
BY-2 cells expressing SP-GFP-HDEL (ER lumen, green) and ST-mRFP (*trans*, magenta). (A)
Without BFA treatment (-BFA) and with 50 μM BFA treatment for 1.5 h (+BFA). (B) Confocal
image of a BFA treated cell at cell periphery. Scale bars = 20 μm (A); 5 μm (B).
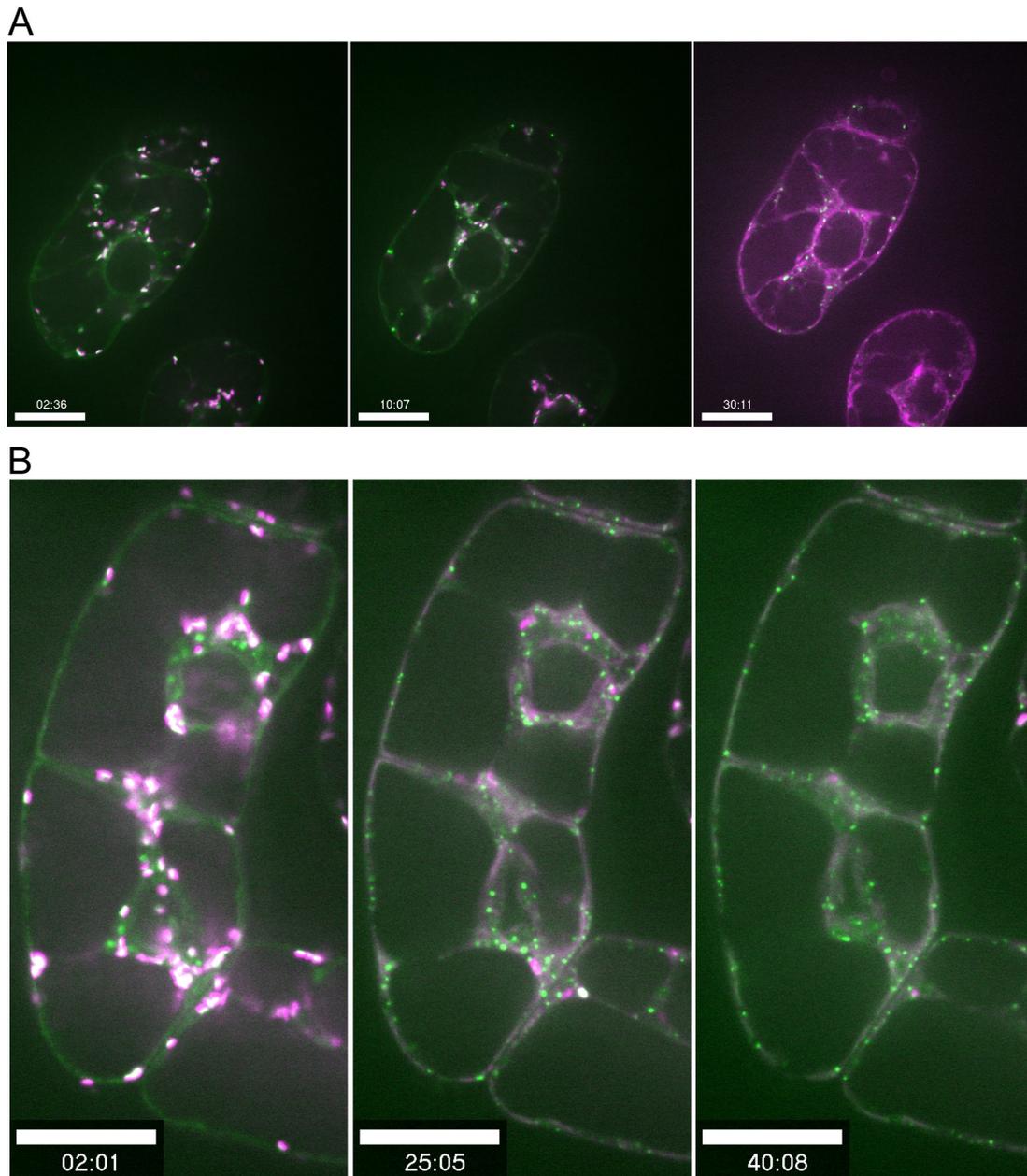
FIGURE S3. Effects of cycloheximide or LatB during BFA treatment
Time-lapse observations of BY-2 cells expressing GFP-SYP31 (*cis*, green) and ST-mRFP (*trans*, magenta) during BFA treatment. (A) The cells were treated with 2 μM LatB for 30 min prior to the addition of BFA at 50 μM. (B) The cells were treated with 100 μM cycloheximide for 30 min prior to the addition of BFA at 50 μM. The indicated times mean the elapsed time after BFA addition. Scale bars = 20 μm.
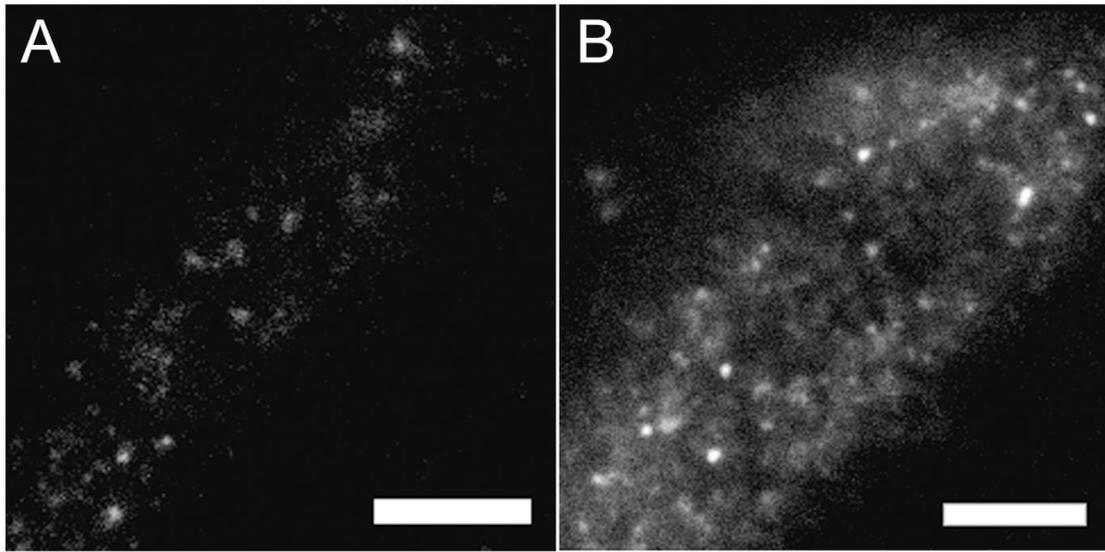
FIGURE S4. TIRFM observation of GFP-SYP31

GFP signals in BY-2 cells expressing GFP-SYP31 (*cis*) and ST-mRFP (*trans*) were observed by TIRFM. (A) Without BFA treatment. (B) 50 µM BFA treatment for 2 h. Scale bars = 10 µm.
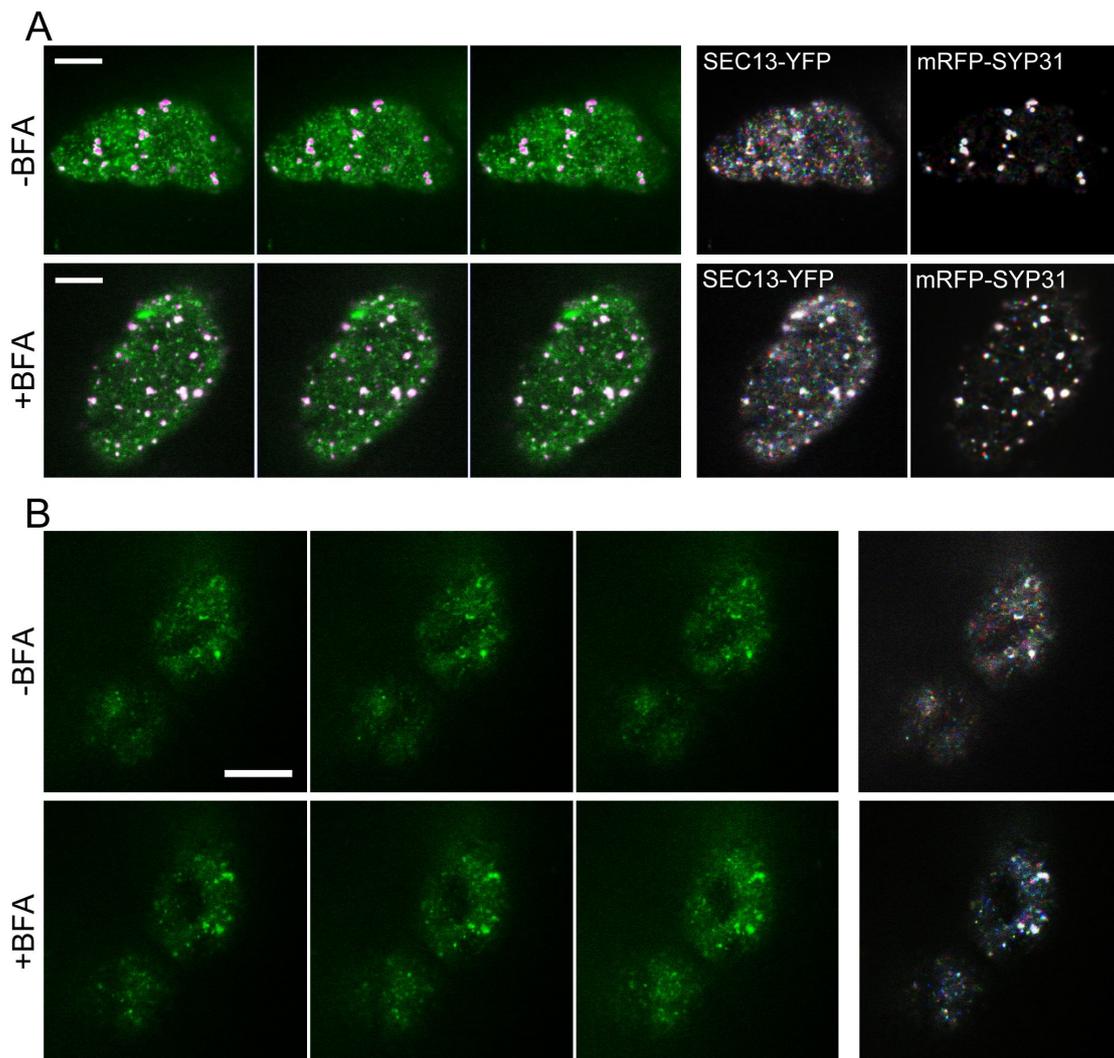
FIGURE S5. Relationship between SYP31 and SEC13 signals

(A) BY-2 cells expressing SEC13-YFP (ERES, green) and mRFP-SYP31 (*cis*, magenta) were treated with 2 μM LatB for 30 min (-BFA) and 50 μM BFA for 1.5 h additionally (+BFA). Left three panels are time-lapse images with 3 sec intervals. Right two panels are maximum intensity projection (MIP) images of each marker, with pseudo-colors associated with time (0 sec: red, 3 sec: green, 6 sec: blue.) (B) Time-lapse observation of BY-2 cells expressing SEC13-YFP alone. The cells were treated with 2 μM LatB for 30 min prior to the addition of BFA at 50 μM. Just after BFA addition (-BFA) and 1.5 h after BFA addition (+BFA). Left three panels are time-lapse images with 3 sec intervals. Rightmost panels are MIP images with the pseudo-colors same as (A). Scale bars = 10 μm.