Donovan and Bretscher, http://www.jcb.org/cgi/content/full/jcb.201501118/DC1

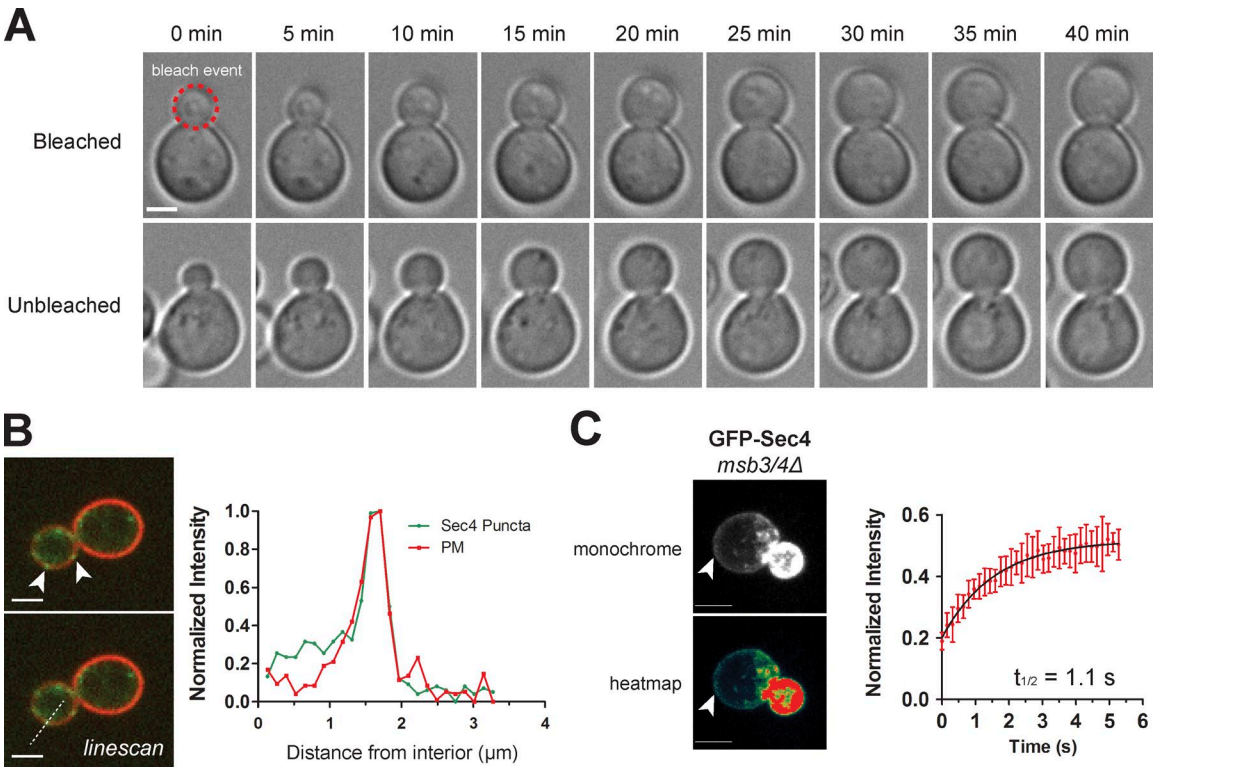


Figure S1. **Bleaching the bud does not interrupt or inhibit exocytosis, and secretory vesicles marked by GFP-Sec4 tether to the plasma membrane before fusion.** (A) Small-budded cells were bleached with the same settings used in vesicle tracking assays. There was no difference in the growth of the bud of bleached or unbleached samples, indicating that the bleach event does not cause defects in exocytosis. Differential interference contrast images were adjusted for presentation. The red circle indicates the bleach area. Bar, 2 µm. (B) Still frame of medium budded cell showing vesicle marker GFP-Sec4 and plasma membrane marker pRS415-pTOM3-mCherry-(2X Ist2$^{928\text{-}946}$) after the bleach event. Vesicles marked by GFP-Sec4 clearly contact the plasma membrane (arrowheads), as indicated by the normalized line scan graph. The image and line scan are representative of $n = 25$ cells analyzed. Bars, 2 µm. (C) The GAP-null *msb3Δ msb4Δ* strain contains cortex-localized GFP-Sec4 throughout the mother cell, likely due to reduced rates of GTP hydrolysis. Free GFP-Sec4 diffuses quickly throughout the plasma membrane as determined by a FRAP experiment bleaching a small area on the mother cell. Arrowheads point to cortex-localized GFP-Sec4 pool in this mutant. Bars, 2 µm. Error bars indicate standard deviation.

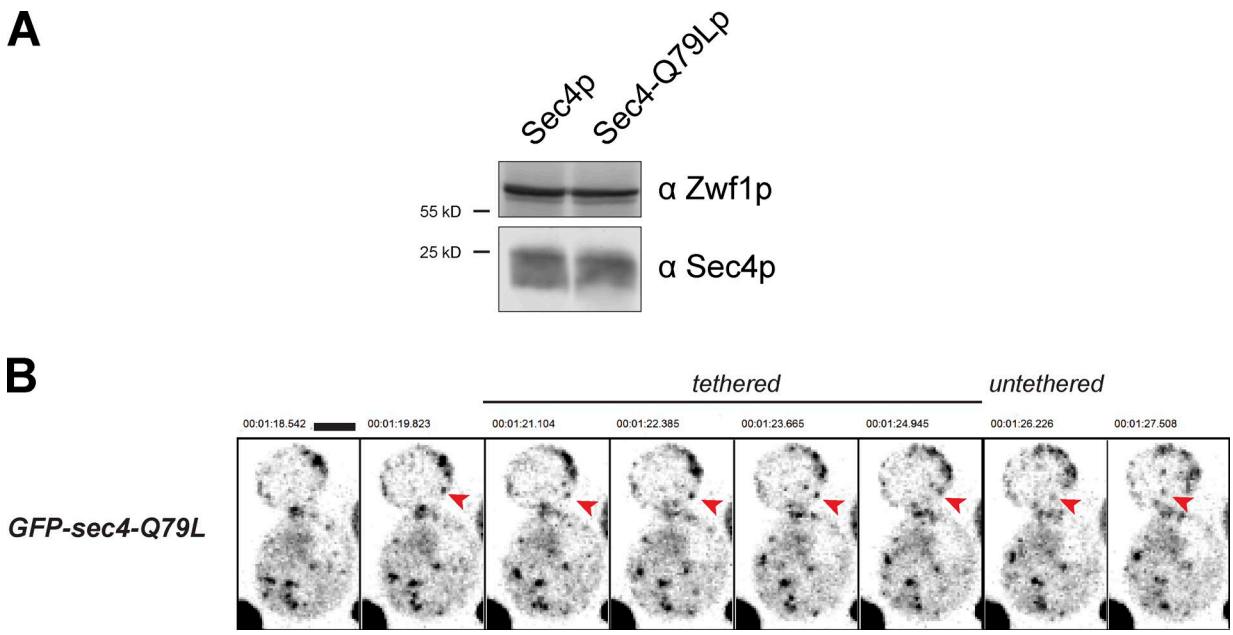


Figure S2. **Constitutively active Sec4-Q79Lp expresses similarly to wild type, and an example of an aborted tethering event in a GFP-Sec4-Q79L cell.** (A) Expression of Sec4p and Sec4-Q79Lp, as probed with an Sec4p antibody generated in a rabbit. The glucose-6-hydrogenase enzyme Zwf1p was used as a loading control. (B) Still frame micrographs of an abortive tethering event in the GFP-Sec4-Q79L strain. Arrowheads point to a vesicle that docked for ~5 s before untethering and reentering the bud cytoplasm. Bar, 2 µm.

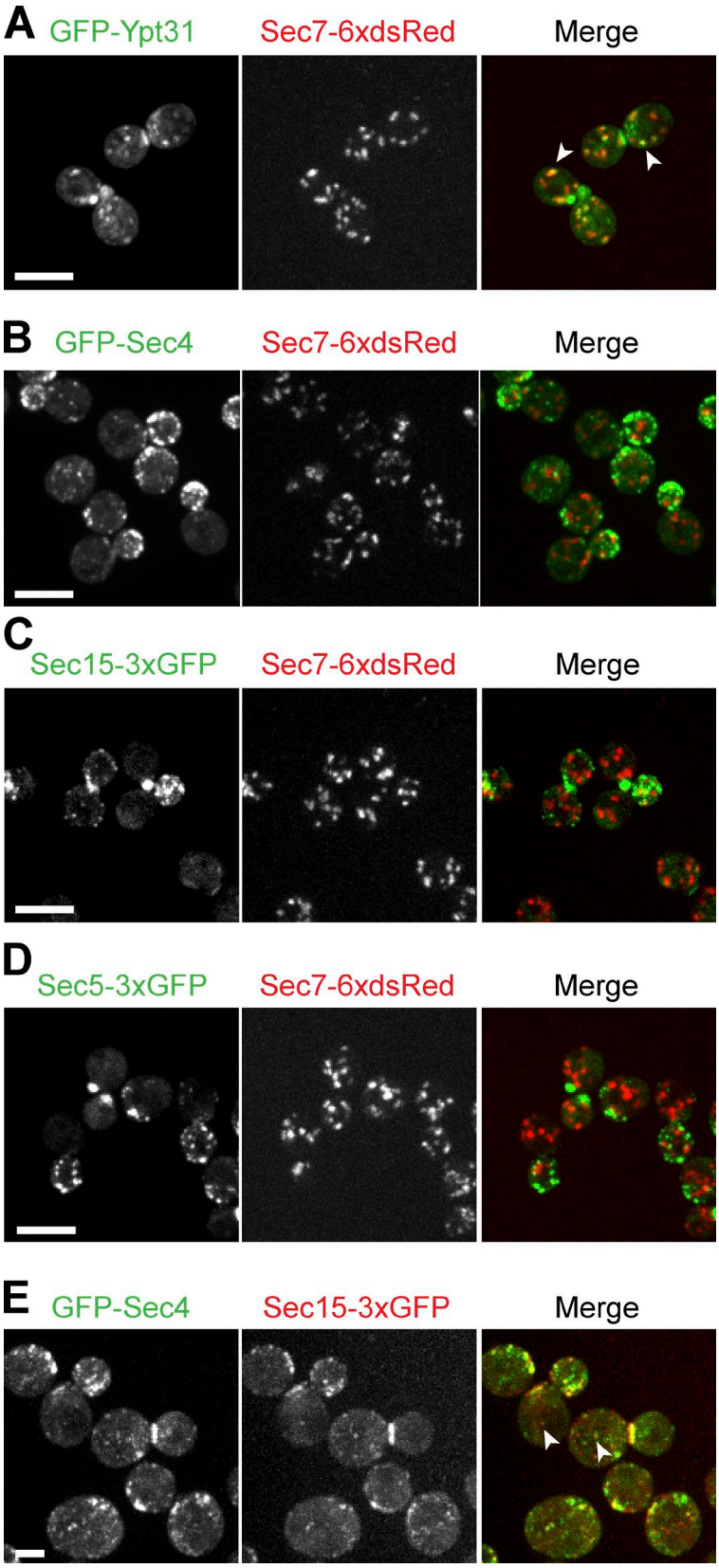
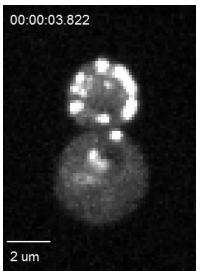


Figure S3. **Colocalization of exocyst complex subunits, Rabs, and trans-Golgi network markers.** (A) Still frame micrographs showing colocalization of the Rab GFP-Ypt31 and the trans-Golgi marker Sec7-6×dsRed. Arrowheads show examples of overlap. Bar, 5 µm. (B) Still frame micrographs of the Rab GFP-Sec4 and the trans-Golgi marker Sec7-6×dsRed. There was no significant overlap. Bar, 5 µm. (C) Still frame micrographs of the exocyst component Sec15-3×GFP and the trans-Golgi marker Sec7-6×dsRed. There was no significant overlap. Bar, 5 µm. (D) Still frame micrographs of the exocyst component Sec5-3×GFP and the trans-Golgi marker Sec7-6×dsRed. There was no significant overlap. Bar, 5 µm. (E) Still frame micrographs of the Rab GFP-Sec4 and the exocyst component Sec15-3×mCherry. Some GFP-Sec4 puncta in the mother cell were positive for Sec15-3×mCherry. Bar, 2 µm.

Video 1. **Wild-type GFP-Sec4 vesicle tracking example from Fig. 1 (a and b).** Bleach event of the entire bud occurs at 5.1 s. Newly formed vesicles can then be observed entering the bud, docking with the plasma membrane, and disappearing in a frame-to-frame event. Images were analyzed by time-lapse spinning disk confocal microscopy (DMI600B, Leica; CSU-X, Yokogawa Electric Corporation) using Slidebook 5.0 software (Intelligent Imaging Innovations). Each frame is ~1.28 s.



Video 2. **GFP-Sec4-Q79L vesicle tracking example from Fig. 1 d.** Wild-type cells expressing chromosomally integrated GFP-Q79L-Sec4 during a bleach event. A bleach event of the entire bud occurs at 5.1 s. Newly formed vesicles can then be observed entering the bud, docking with the plasma membrane, and disappearing in a frame-to-frame event. Images were analyzed by time-lapse spinning disk confocal microscopy (DMI600B, Leica; CSU-X, Yokogawa Electric Corporation) using Slidebook 5.0 software (Intelligent Imaging Innovations). Each frame is ~1.28 s.

Table S1. **Summary of yeast strains**

Strain	Alias	Genotype	Source
ABY1655	BY4741	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	C. Boone (University of Toronto, Toronto, Ontario, Canada)
ABY1656	BY4742	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	C. Boone
ABY3410	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::URA3*	This study
ABY3146	JGY73	MATa *his3 leu2 lys2 trp1 ura3 s4-Q79L*	E. Bi (University of Pennsylvania, Philadelphia, PA)
ABY3429	NA	MATa *his3 leu2 lys2 trp1 ura3 GFP-sec4-Q79L::URA3*	This study
ABY3547	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::URA3 pRS415*-pTOM3-mCherry-(2X Ist2$^{928\text{-}946}$)	This study
ABY3147	YEF1289	MATa *his3 leu2 lys2 trp1 ura3 msb3Δ::HIS3 msb4Δ::TRP1*	E. Bi
ABY3428	NA	MATa *his3 leu2 lys2 trp1 ura3 msb3Δ::HIS3 msb4Δ::TRP1 GFP-SEC4::URA3*	This study
ABY126	NY17	MATa *ura3-52 s6-4*	P. Novick (University of California, San Diego, La Jolla, CA)
ABY3409	NA	MATa *ura3-52 s6-4 GFP-SEC4::URA3*	This study
ABY123	NY3	MATa *ura3-52 s1-1*	P. Novick
ABY3546	NA	MATa *ura3-52 s1-1 GFP-SEC4::URA3*	This study
ABY2703	NA	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo2-13::HIS3*	This study
ABY3545	NA	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo2-13::HIS3 GFP-SEC4::URA3*	This study
ABY3195	NA	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo2-RAKA::HIS3*	This study
ABY3548	NA	MATa *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 myo2-RAKA::HIS3 GFP-SEC4::URA3*	This study
ABY3587	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3*	This study
ABY3597	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 myo2-3DR::HIS3 GFP-SEC4::URA3*	This study
ABY3719	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::LEU2 SEC2-3xmCHERRY::URA3*	This study
ABY3557	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::LEU2 MYO2-3xmCHERRY::URA3*	This study
ABY3551	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 GFP-SEC4::LEU2 SEC15-3xmCHERRY::URA3*	This study
ABY3571	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC3-3xGFP::HIS3 SEC15-3xmCHERRY::URA3*	This study
ABY3572	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC5-3xGFP::HIS3 SEC15-3xmCHERRY::URA3*	This study
ABY3581	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC3-GFP::HIS3*	This study
ABY3582	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC5-GFP::HIS3*	This study
ABY3414	NA	MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 SEC15-GFP:kanMX6*	This study
ABY3724	NA	Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Sec7-6xdsRed::URA3 pRS415-GFP-Ypt31*	This study
ABY3726	NA	Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Sec7-6xdsRed GFP-SEC4::URA3*	This study
ABY3722	NA	Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Sec7-6xdsRed::URA3 Sec15-3xGFP::HIS3*	This study
ABY3721	NA	Mata *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 Sec7-6xdsRed::URA3 Sec5-3xGFP::HIS3*	This study

NA, not applicable.