

### **Supplemental Figure S1. Secretory delays are associated with the m11 mutation of Sec24p.**

- A.** Gas1p maturation was monitored at the permissive temperature of 30°C and showed a significant delay in a strain expressing Sec24p-m11 as the sole copy of Sec24p, as compared with the isogenic wild type strain.
- B.** Gas1p (top panel) and CPY (bottom panel) maturation were monitored by pulse-chase analysis at 37°C in strains where the sole copy of Sec24p was the version indicated. The m11 mutant showed similar delays in Gas1p and CPY processing to that observed for the B-site mutant, which has defects in packaging of the v-SNARE, Bet1p. The A-site mutant was largely indistinguishable from wild type.
- C.** Live cell imaging of A-site mutant, *sec24-A4* (left panel), or B-site mutant, *sec24-B3* (right panel), strains expressing the ER marker, GFP-Cyb5p, demonstrates relatively normal internal membrane structures. Scale bar is 5µm.

### **Supplemental Figure S2. Summary of selected validated hits from SDL screen.**

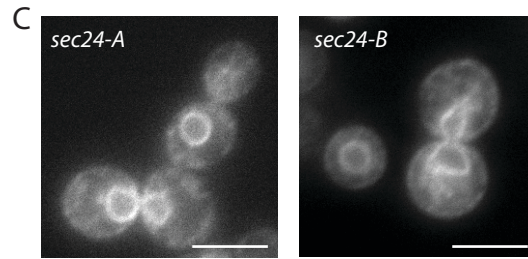
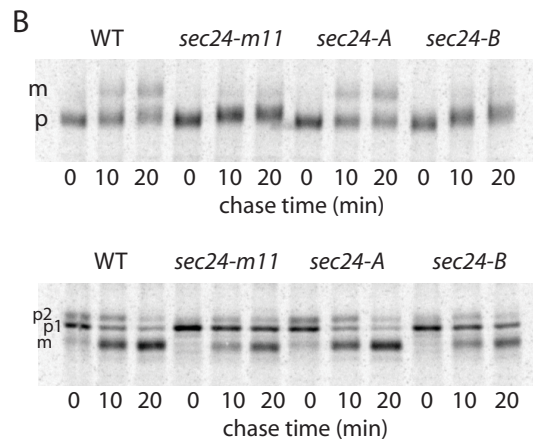
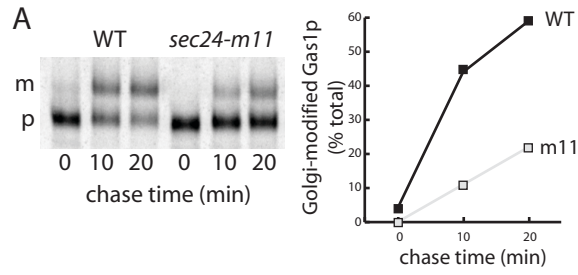
Fifty hits that scored as significantly impaired for growth upon over-expression of *sec24-m11* in either of two independent SDL screens were targeted for validation based on their known functions, localizations, or poorly characterized nature. Forty of these selected hits were confirmed by independent transformation and growth on Cu<sup>2+</sup>-containing media. These validated hits cluster functionally in a number of relevant processes, in particular Golgi trafficking and vacuole trafficking/assembly. In a number of cases, multiple components of known physical complexes (denoted by blue shading) were identified.

### **Supplemental Figure S3. Different fragments of Sec16p show distinct GTPase inhibitory effects.**

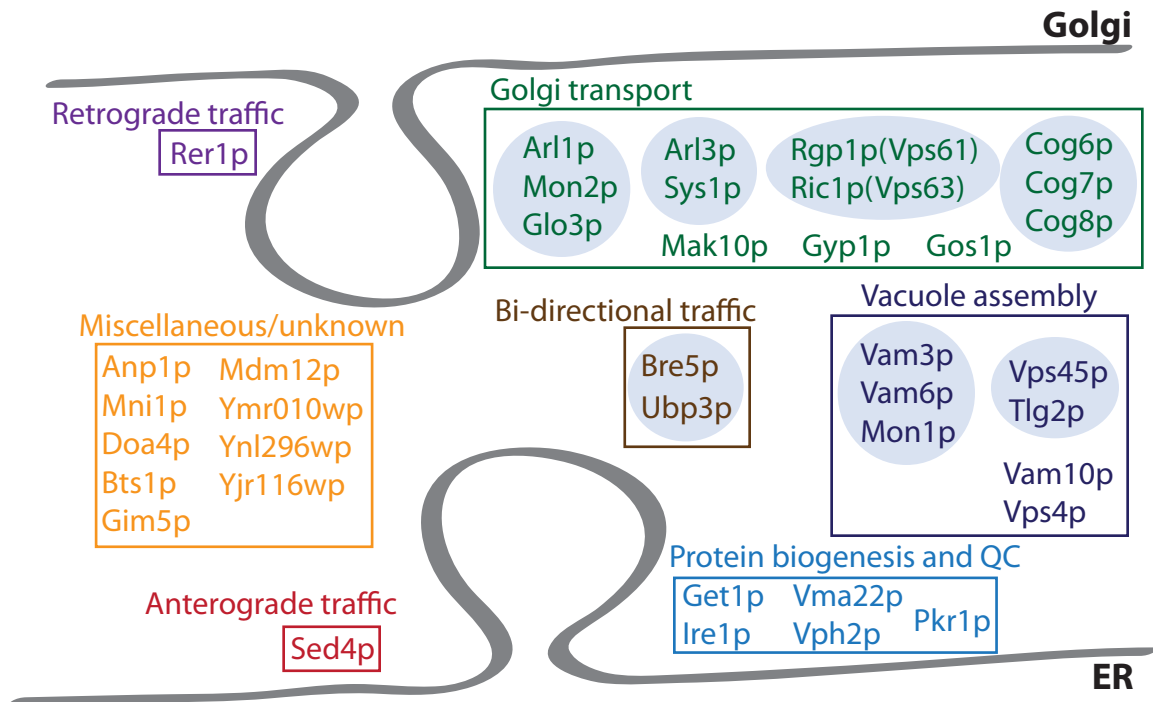
- A.** Full-length Sec16p was included in a tryptophan fluorescence experiment to monitor the GTP-bound state of Sar1p in real time. Increasing concentrations of Sec16p (in the presence of Sec13/31) showed no effect in delaying the conversion of Sar1p from the GTP- (high Trp fluorescence) to GDP-bound state (low Trp fluorescence).
- B.** The C-terminal fragment of Sec16p, encompassing residues 1645-2194 that binds Sec23p, was tested for GTPase inhibitory activity and only partial effect at the highest concentrations.

C. GTPase activity by Sar1p was monitored by radioactive  $^{32}\text{P}$  assay in the presence or absence of Sec13/31p and various amounts of Sec16p- $\Delta\text{N}$ . Increasing the concentration of Sec16p- $\Delta\text{N}$  dramatically inhibited the GTPase activity only in the presence of Sec13/31p.

Supplemental Figure S1.



Supplemental Figure S2



Supplemental Figure S3.

