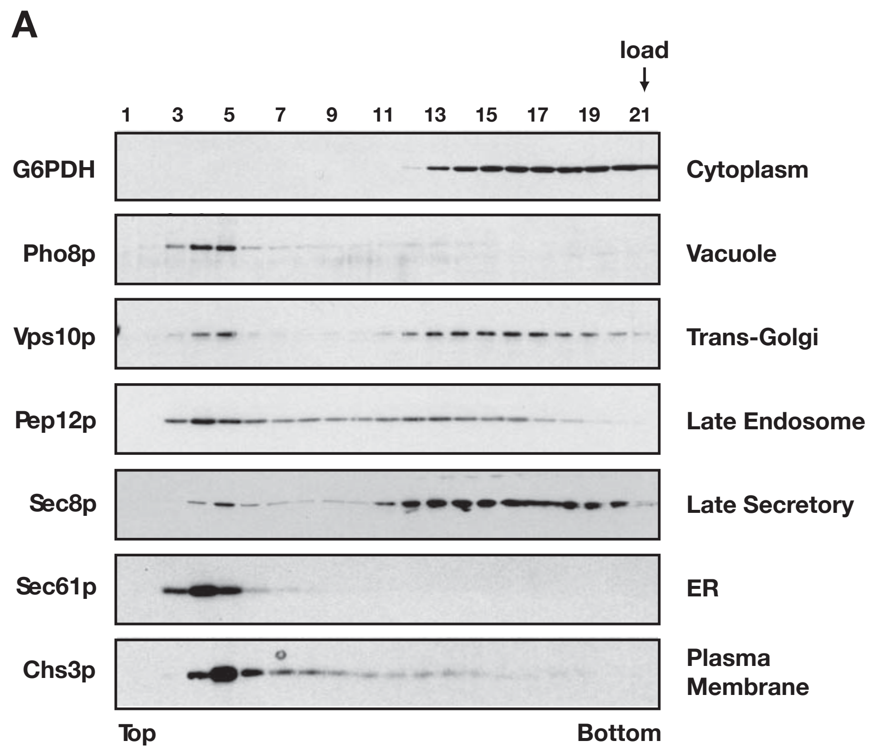

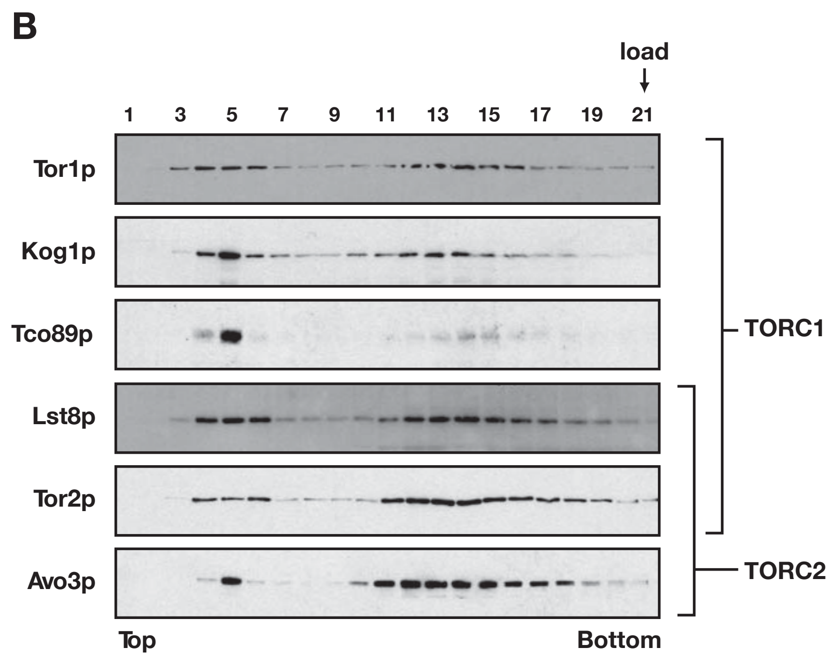

Figure S1
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Legend to Supplemental Figure 1

Cell extracts were prepared from W303a-derived strains and subjected to a percoll-underlay gradient and used for Western blot analysis, probing either for **(A)** representative markers for secretory and endocytic membrane compartments or **(B)** representative components of TORC1 and/or TORC2. Methods: 250 OD cells were converted to spheroplasts and subjected to dounce homogenization in 5 ml yeast extract buffer/sorbitol (YEB/Sorbitol: 50mM HEPES-KOH, pH 7.1, 100 mM β-glycerophosphate, 50 mM NaF, 1 mM EGTA, 10% glycerol, 0.25% Tween-20, 0.8M sorbitol). Extracts were cleared by low speed centrifugation at 1,000 x g for 3 minutes at 4ºC. High speed membranes were then prepared by combining 4.5 ml of cleared extract with an additional 6 ml of YEB/sorbitol and centrifuging through a 200 µl 67% Percoll cushion in 12.5 ml tubes in an SW41 rotor (Beckman Coulter) at 100,000 x g for 60 minutes at 4°C. Following removal of the supernatant, approximately 500 µl of concentrated membrane pellets were placed on ice for 60 minutes to allow easy resuspension. Next, resuspended membranes were adjusted to 58% Percoll, loaded into and SW41 rotor tube, and were overlayed with 1.5 ml steps containing 55, 50, 40, 35, 30 and 25% Percoll. Gradients were spun for 1 hr at 100,000 x g and 0.5 ml fractions were collected from the top. A portion of the material collected from each of the fractions was analyzed by SDS-PAGE and Western blot analysis.