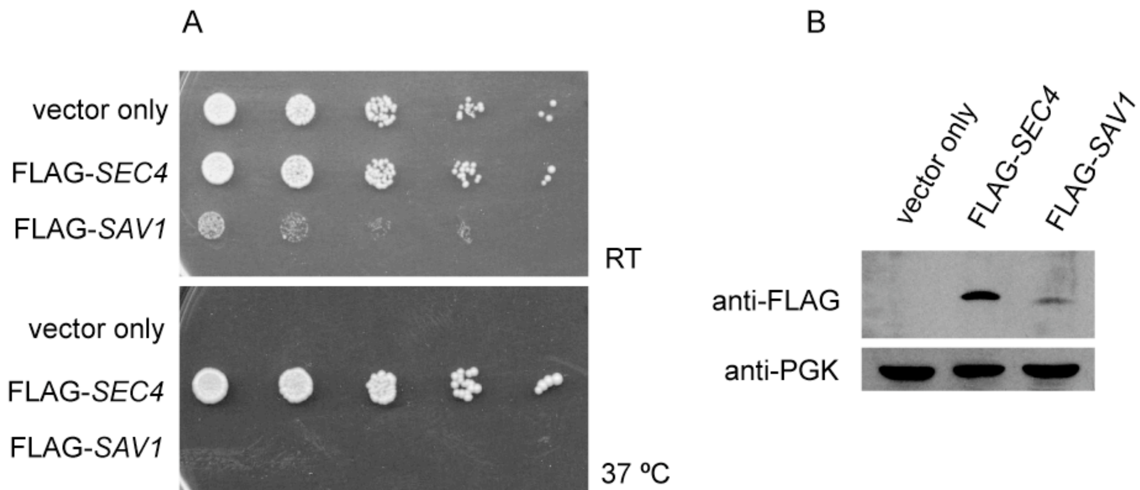
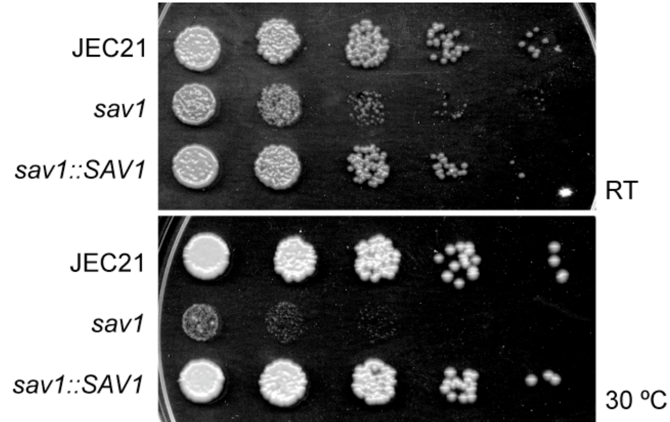


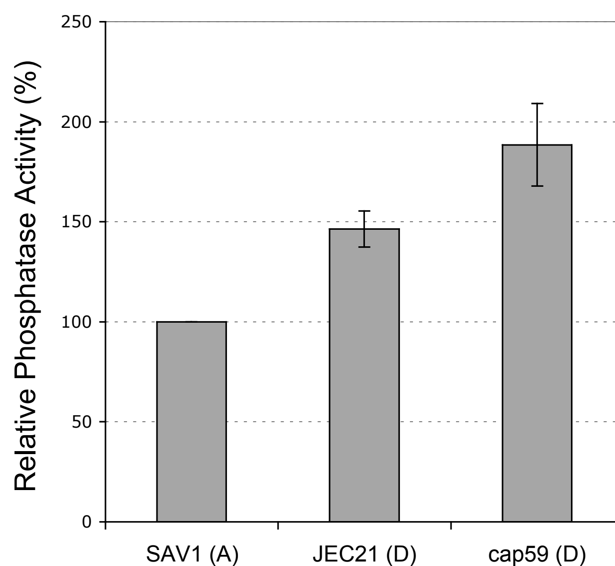
Supplemental Figure 1. *SAV1* does not rescue temperature sensitivity of the *S. cerevisiae sec4-8* mutant.

The *S. cerevisiae SEC4* gene was PCR amplified from genomic DNA so as to incorporate a 5′-terminal FLAG tag, and *C. neoformans SAV1* was similarly amplified from JEC21 cDNA. Both genes were cloned into p416ADH, and the resulting plasmids and the empty vector were used to transform the *S. cerevisiae sec4-8* mutant. (A) Five-fold dilutions of the strains bearing the indicated plasmid were spotted onto YPD plates and incubated at the indicated temperatures. An untagged set of strains gave the same results (not shown). Expression of *SAV1* in *sec4-8* reduced growth, perhaps by acing in a dominant negative manner. (B) Western blot of whole cell lysates from the strains in panel A shows that FLAG-Sav1p is expressed in the *sec4-8* mutant, although at a low level. Anti-FLAG antibody (F-3165, Sigma) was used to detect the tagged Sec4p or Sav1p, and anti-PGK antibody (A-6457, Invitrogen) was used to detect PGK as a loading control. Immunoblot detection was performed by standard methods using appropriate HRP-conjugated secondary antibodies.



Supplemental Figure 2. The *sav1* mutant generated in a serotype D background is more sensitive to high temperatures than the serotype A *sav1* strain (Figure 3).

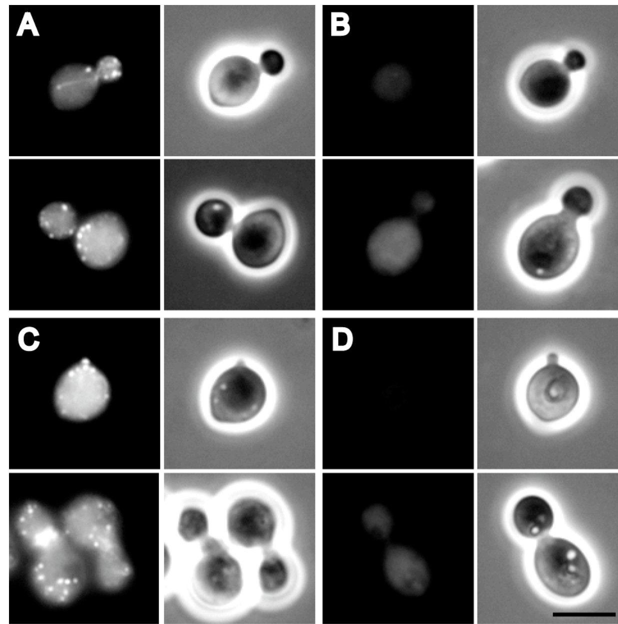
Five-fold dilutions of a cell suspension of JEC21 (the serotype D parent strain), *sav1*, or *sav1::SAV1* were spotted onto YPD plates and incubated at indicated temperatures for 3 days.



Supplemental Figure 3. A strain deleted for *CAP59* exhibits normal acid phosphatase secretion.

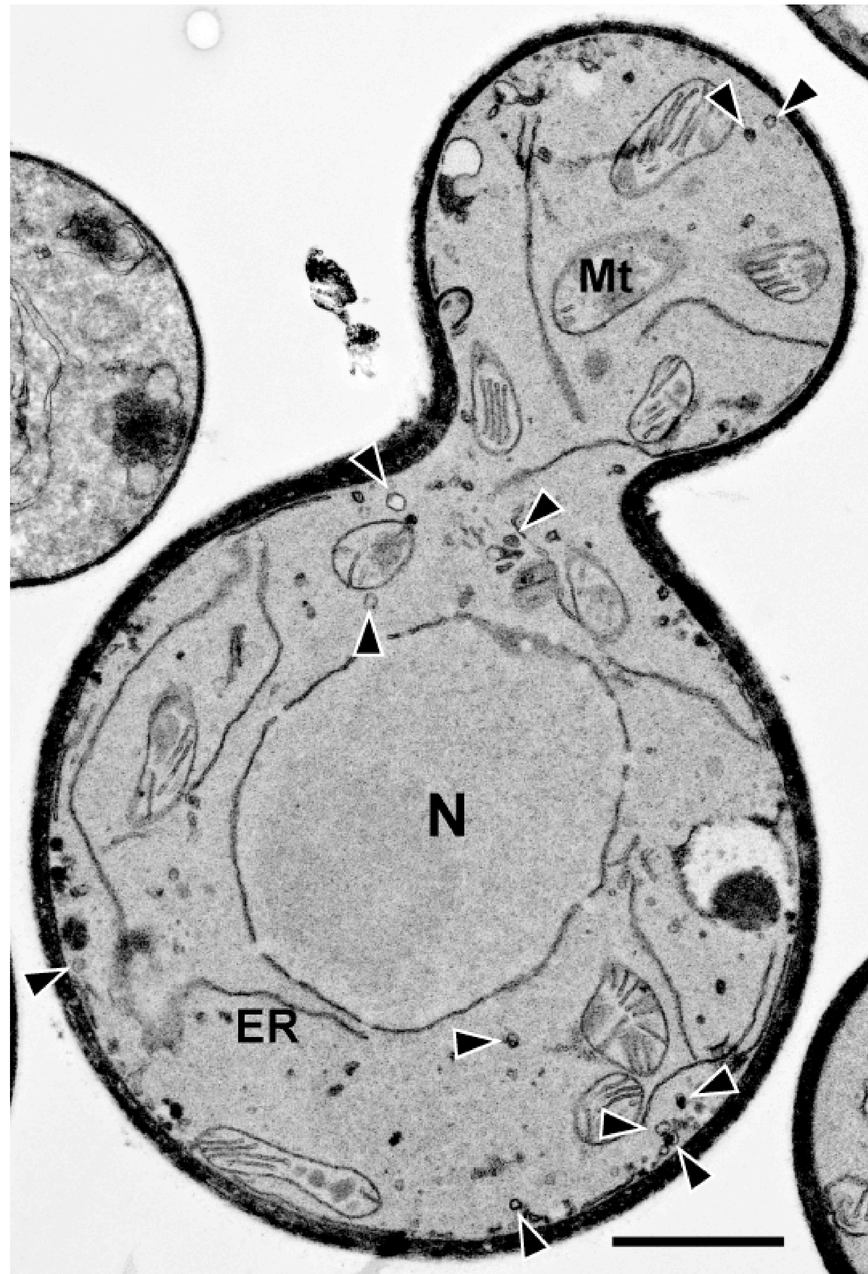
Data were compiled from two independent experiments performed in triplicate; mean and standard error are plotted. Statistical analysis showed no significant difference between secretion in the serotype D *cap59* strain and a wild type serotype D strain JEC21 ($p = 0.12$). Both D strains did secrete significantly more than a wild type serotype A strain ($p < 0.001$ in each case). Assays were performed as described in Materials and Methods with following modifications: All strains were grown at 30 ºC; media were supplemented with uracil because the *cap59* strain is an uracil auxotroph; and A_{420} was corrected to cell number by hemocytometer counts, because *cap59* grows as large clumps, affecting cell density reading.

Strains: *SAV1*, serotype A wild type control strain containing NAT; JEC21, a serotype D wild type strain; *cap59*, a serotype D *CAP59* deletion strain.



Supplemental Figure 4. Latrunculin B (LatB) treatment abolishes F-actin patches, cables and rings in *C. neoformans* strains *SAV1* and *sav1*. A, Ethanol control-treated *SAV1*; B, LatB-treated *SAV1*; C, Ethanol-treated *sav1*; D, LatB-treated *sav1*.

Serotype A *SAV1* and *sav1* strains were grown in minimal medium overnight at RT to reach mid-log phase, and 1.5 ml of culture were incubated at 30 ºC for 1 h with either 400 μM LatB in ethanol (from a 40 mM stock) or 1% ethanol. Cells were then fixed with 5% paraformaldehyde in 100 mM PIPES supplemented with 0.1 M sorbitol, 1 mM $CaCl_2$ and 1 mM $MgCl_2$ for 1 h at RT, washed twice in PBS, permeabilized in 1 ml of 1% Triton-X 100 in PBS for 10 min, washed three times in PBS, and resuspended in 500 μl PBS. 5 μl of 14 μM rhodamine-phalloidin (Cytoskeleton Inc, Denver, CO) was added to 50 μl of cell suspension and incubated at RT for 1 h. Cells were washed in 100 μl PBS 4 times and the cell pellet was resuspended in 15 μl PBS. 3 μl of the stained cell suspension was mixed with 3 μl of Vectashield (Vector Labs, Burlingame, CA) and observed by epifluorescent microscopy (Axioskop 2, Carl Zeiss). Actin images were photographed using AxioCam MRm and Axiovision 4.5 with a Texas red filter (exposure 399 msec).



Supplemental Figure 5. LatB-treated *sav1* cells have only scattered vesicles, with no apparent polarization.

sav1 cells grown in the presence of 400 μM LatB at 30 ºC for 3 h were fixed and processed as described in Materials and Methods. Arrowheads indicate 67-120 nm vesicles. ER, endoplasmic reticulum; N, nucleus; Mt, mitochondria. Scale bar, 1 μm