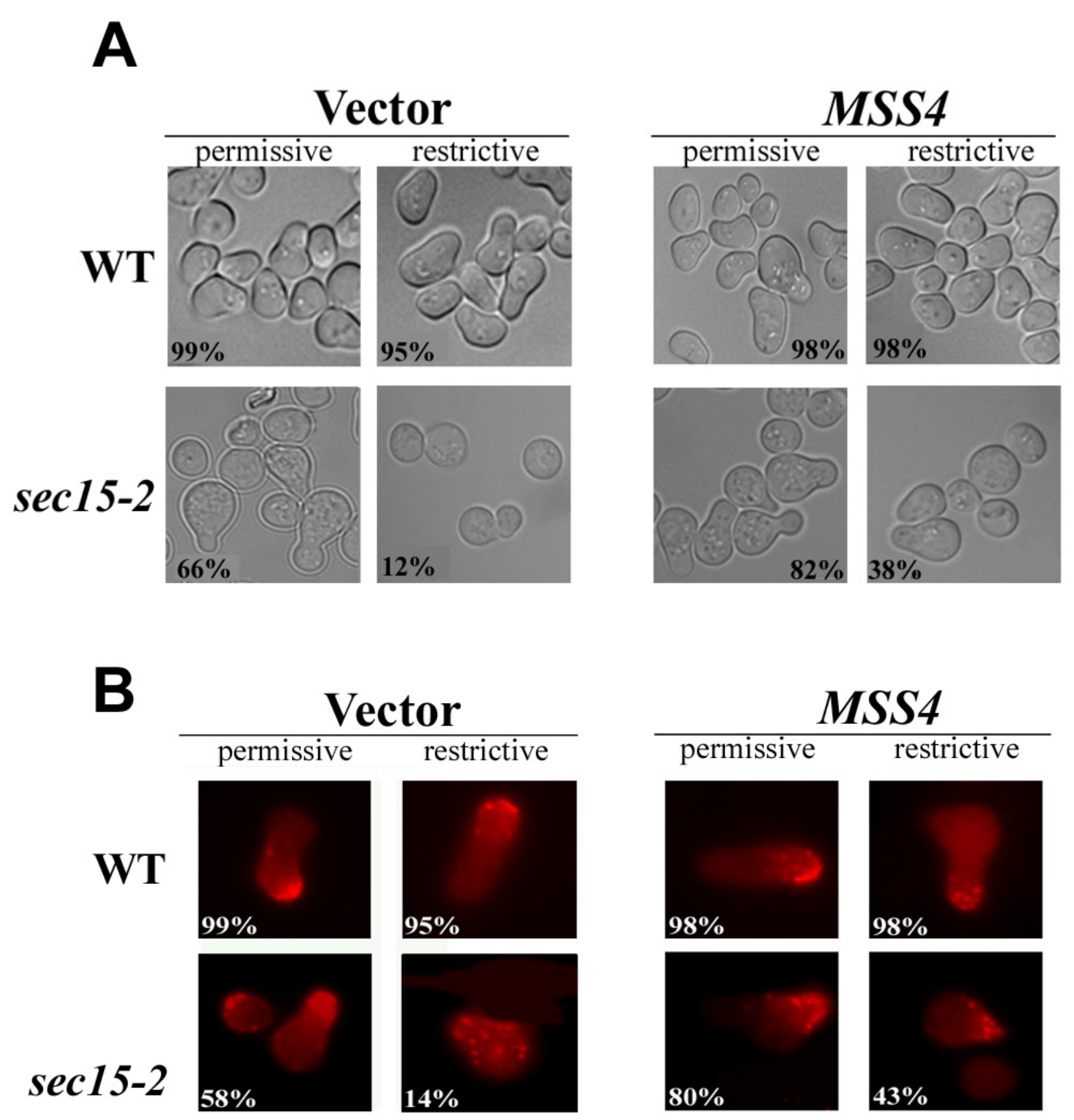
Supplementary Figure Legends

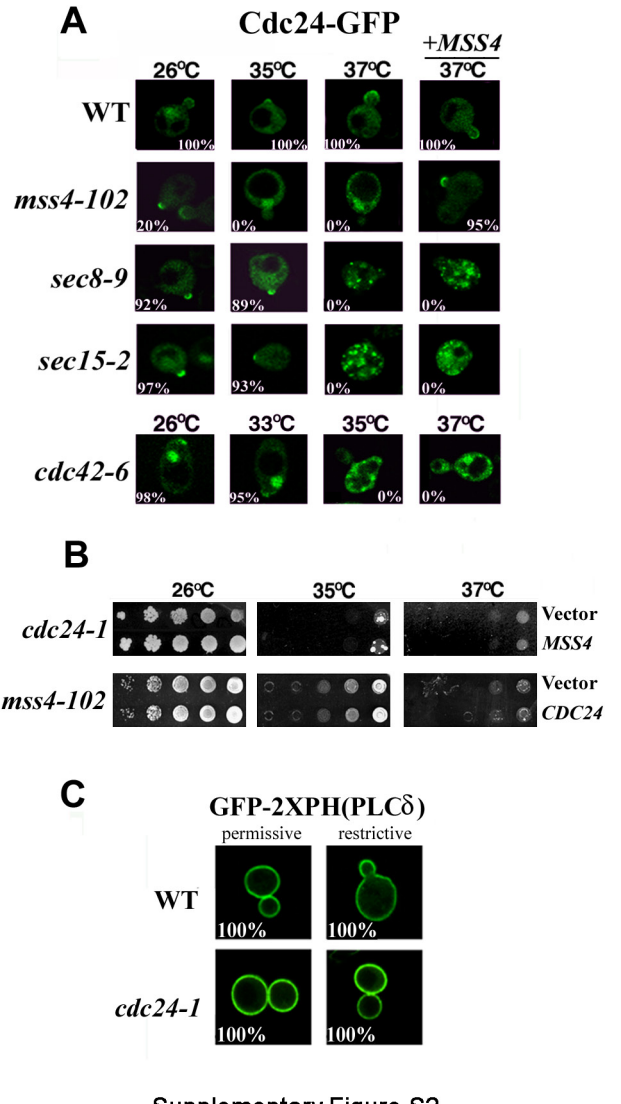
Supplementary Figure S1. *MSS4* over-expression restores shmoo formation and actin polarization in α-factor–treated *sec15-2* cells. (A) *MSS4* over-expression restores shmoo formation in a late *sec* mutant. WT and *sec15-2* cells were transformed either with a control multi-copy vector (pRS426) or a vector over-expressing *MSS4* (pRS426Mss4). Cells were grown to mid-log phase, incubated with 500nM α-factor for 2hr, and either maintained at 26°C (permissive temperature) or shifted to 37°C (restrictive temperature) for 1h. Numbers indicate the percentage of shmooing cells (n=100 cells counted). **(B) *MSS4* over-expression restores actin polarization in α-factor–treated *sec15-2* cells.** Cells from (A) were subjected to fixation and *in situ* labeling with rhodamine-conjugated phalloidin. Numbers indicate the percentage of cells in which polarized actin was observed (n=100 cells counted).

Supplementary Figure S2. Cdc24 does not play a role in transducing the exocytic signal to Cdc42 via Mss4. (A) Cdc24 is mislocalized in late *sec* mutants in an Mss4-independent fashion. The indicated strains were transformed with a single-copy vector expressing Cdc24-GFP (pUG35-Cdc24-GFP) from a *MET* promoter. Cells were induced in medium lacking methionine for 1hr and either maintained at 26°C (permissive), shifted for 1hr to 35°C (semi-restrictive), or shifted for 1hr to 37°C (restrictive) prior to visualization by fluorescence confocal microscopy. Numbers indicate the percentage of cells in which bud-tip localization of Cdc24-GFP was observed (n=100 cells counted).

(B) *CDC24* and *MSS4* do not interact genetically. *cdc24-1* cells were transformed either with a control vector (pRS426) or vector over-expressing *MSS4* (pRS426Mss4). *mss4-102* cells were transformed either with a control vector (pUG35) or a single-copy vector over-expressing *CDC24* (pUG35Cdc24-GFP) under the control of a methionine-starvation inducible *MET* promoter. Cells were grown overnight in selective medium, diluted serially (5-fold), and spotted onto solid selective medium lacking methionine. Plates were incubated for 48hrs at the indicated temperatures (^{0}C). **(C) PI(4,5)P_2 is normally distributed in *cdc24-1* cells.** The indicated WT and *cdc24-1* cells were transformed with a multi-copy vector expressing a GFP-2XPH(PLCδ) fusion probe (pRS426GFP-2XPH(PLCδ)) and maintained at either 26°C (permissive) or shifted for 1hr to 37°C (restrictive). Numbers indicate the percentage of cells in which GFP-2XPH(PLCδ) localizes to the PM (n=100 cells counted).



Supplementary Figure S1.



Supplementary Figure S2.