

**Supplementary Figure 1.** Wild-type cells (DLY5) were grown to exponential phase in YEPD at 30°C and treated with the indicated doses of Lat A or Lat B for 2 h. Separate aliquots were processed to visualize F-actin or Cdc42p. A) Representative cells from each sample, photographed using the same exposure times. B) Quantitation of Cdc42p polarization, showing that in this experiment 100  $\mu$ M Lat B and 30  $\mu$ M Lat A promoted similar degrees of Cdc42p dispersal, while higher and lower doses of Lat A allowed greater maintenance of Cdc42p polarization. C) Actin organization in cells treated with 100  $\mu$ M Lat B and 30  $\mu$ M or 100  $\mu$ M Lat A (and untreated controls), photographed using different exposures optimized to detect the remaining actin structures. Only cortical patches (or their remnants) are detected in the treated cells. Surprisingly, there was reproducibly much less total F-actin in cells treated with 30  $\mu$ M Lat A (the dose at which Cdc42p was most dispersed) than in cells treated with 100  $\mu$ M Lat B (where Cdc42p was also dispersed). Both samples lacked detectable cables, but the remaining patches were much brighter in the Lat B-treated cells, suggesting that patch components can compete effectively with Lat B, but not with Lat A, for actin monomers.