

**Figure 4, figure supplement 1.** Hyper-active endocytic trafficking in the presence of Ser57Asp phosphomimetic ubiquitin requires conjugation. In this experiment, the indicated ubiquitin variants (wildtype, Ser57Asp, and Ser57Asp combined with mutations that prevent ubiquitin conjugation) were expressed exogenously from the pRS416 plasmid under the control of the *ADH1* promoter in the SEY6210 strain background. Canavanine hypersensitivity is indicative of an endocytic defect, while canavanine resistance is indicative of hyper-active endocytic trafficking.