



**Figure 4, figure supplement 2.** Hyper-active endocytic trafficking in the presence of Ser57Asp phosphomimetic ubiquitin requires K63-linked poly-ubiquitination. In this experiment, the indicated ubiquitin variants (wildtype, Ser57Asp, and Ser57Asp in combination with mutations that restrict the formation of specific polyubiquitin linkage types) 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.