



Figure 5, figure supplement 5. Phenotypic analysis of yeast cells expressing L73P ubiquitin. **(A)** SUB280 yeast (expressing ubiquitin from a single pLYS source plasmid) were transformed with a second ubiquitin-expressing plasmid (pURA) and grown in the presence of DL- α -aminoadipic acid to counterselect against the pLYS plasmid ("plasmid shuffle"). **(B)** Wildtype yeast cells (SEY6210.1 background) transformed with either empty vector or plasmids expressing ubiquitin (wildtype, S57A or L73P) from the ADH1 promoter were plated on minimal media with or without canavanine. **(C)** Wildtype yeast cells expressing pHluorin-tagged Mup1 (SEY6210.1 background) transformed with either empty vector or plasmids expressing ubiquitin (wildtype, S57A or L73P) from the ADH1 promoter were monitored using flow cytometry measurements of pHluorin signal in a time course following methionine stimulation.