



Figure 5, figure supplement 1. Ser57Asp phosphomimetic ubiquitin does not alter the rate of Rsp5-mediated conjugation *in vitro*. In this experiment, recombinant yeast proteins GST-Uba1 (E1), 6xHIS-Ubc1 (E2), 6xHIS-Rsp5 (E3), Art1-FLAG (substrate), and ubiquitin or Ser57Asp phosphomimetic ubiquitin were incubated at room temperature in the presence of ATP. Each conjugation time course was analyzed by α -FLAG immunoblotting and quantified over three independent experiments (n=3).