



Figure 3, figure supplement 2. Analysis of stoichiometry of Ser57 phosphorylation of ubiquitin. Isotopically labelled (heavy) standard peptides corresponding to unmodified (bottom left) or Ser57 phosphorylated (bottom right) ubiquitin were spiked into ubiquitin affinity purified from *ppz* mutant yeast cells and multiple reaction monitoring analysis was performed. The top panels show the corresponding signal from the biological samples. Inset legends indicate plots for specific transitions. Based on transition peak areas we calculate 9 pmol of the unmodified peptide in the biological sample. The phosphopeptide in the biological sample is below our limit of quantification but occurs at significantly less than 2 fmol.