



FIG S2 Mimicking lysine acetylation in cluster three reduces Ume6p binding to URS1<sup>SPO13</sup> in vitro. (A) Biacore analysis was performed (see Materials and Methods) with the Ume6p peptides indicated injected over a bound mutant URS1 probe (URS1<sup>ΔGC</sup>) sequences lacking the consensus Ume6p binding site. Under identical conditions, Ume6p-URS1<sup>SPO13</sup> interactions generated an RU value of >250 (see Fig. 2B). The sensorgrams with URS1<sup>ΔGC</sup> exhibited an interaction value of ~5 RU indicating that stable protein/DNA complexes did not form. (B) The root mean square differences between the computer generated optimal two- ( $k_{d1}$ ,  $k_{d2}$ ) or single- ( $k_{dS}$ ) dissociation curves and the raw data acquired in Fig. 2B. For the separate dissociation constants,  $k_{d1}$  included the first 100s of buffer wash, while  $k_{d2}$  calculated dissociation for the next 180s. Note the increase in

residual values observed in the single  $k_dS$  model for Ume6p and Ume6p<sup>K3R</sup> relative to those observed when the dissociation is separated suggesting the two-component curve better fits the data. The elevated differences for Ume6p<sup>K3Q</sup> compared to wild type reflect the overall instability of this complex.