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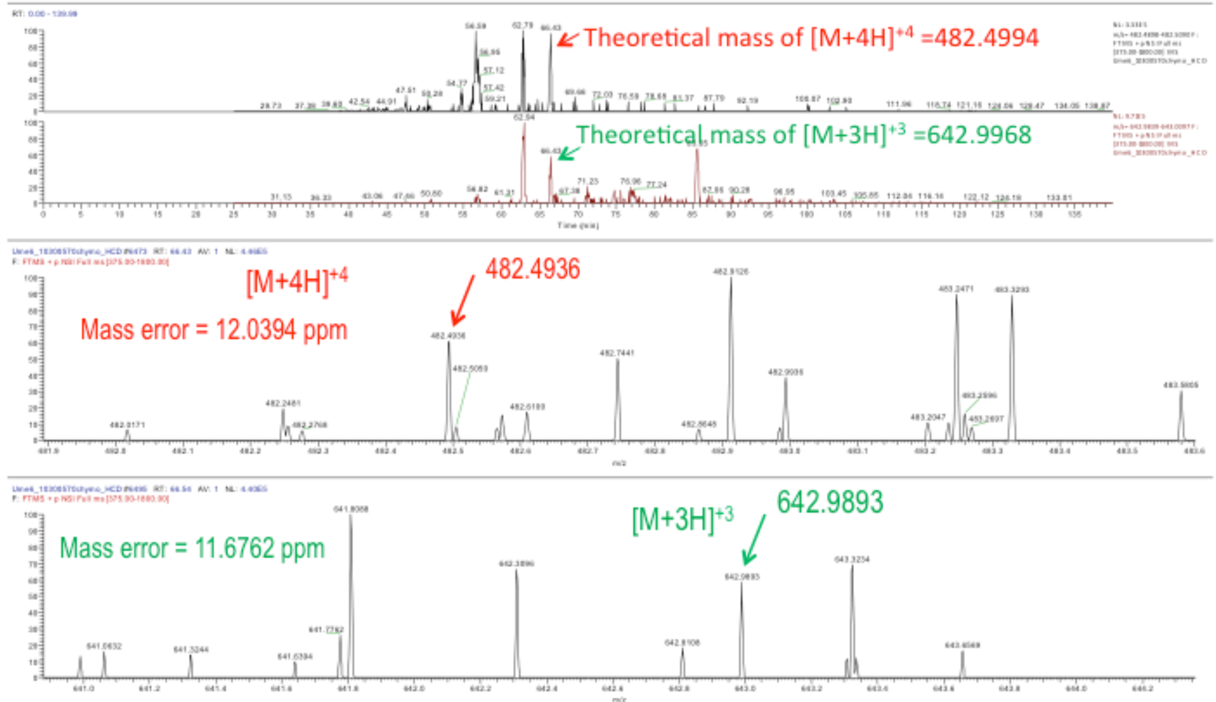

FIG S1 Phosphorylation and acetylation of the lysine cluster three peptide. Mass spectra are shown for a time of flight peaks consistent with double modification of the lysine triplet three peptide. Protein extracts from 6L of mid-logarithmic YPA cultures of RSY1636 (T7-UME6^{KALLR} *rpd3*Δ) were prepared as previously described (18). Protein precipitation using a final concentration of 50% ammonium sulfate was performed and protein pellets were resuspended in binding buffer (40 mM Tris-HCl pH = 8.0, 5% glycerol). The resulting preparation was subjected to ion exchange chromatography using Hi-Trap SP-FF columns (GE Healthcare Life Sciences). Bound protein was washed extensively with binding buffer containing 400 mM NaCl; protein elutions were harvested in

binding buffer with 800 mM NaCl. Eluates were analyzed using Western blots to detect the T7 epitope. Coomassie staining bands corresponding to T7-Ume6p were excised and subjected to mass spectroscopy for detection of post-translational modification. The spectra obtained for both the M+4H and M+3H are shown with potential peaks at those masses indicated by the arrows. The mass accuracy (in PPM) for each peak is indicated.