

Supplemental Figure 1. LC-MS/MS spectra of phospopeptides identified in the Cac1p subunit. For each phosphopeptide, the experimental and theoretical masses of the nonfragmented peptides are indicated. The experimental masses indicate the presence of a single phosphate in each peptide.

A) Phosphorylation of S94: The $y_7^+ PO_4^-$, $b_9^+ PO_4^-$ and $b_{10}^+ PO_4^-$ ions are all consistent with phosphorylation of S94. The y_3 , y_4 and y_6 ions are inconsistent with phosphorylation of S98 or T99. *=Cysteine carbamylation caused by *in vitro* alkylation of Cac1p prior to trypsin digestion. B) Phosphorylation of S238: The $y_{10}^+ PO_4^-$, $y_{11}^+ PO_4^-$ and $b_4^+ PO_4^-$ ions are all consistent with phosphorylation of S238. The y_7 , y_8 and y_9 ions are inconsistent with phosphorylation of S240 or T242.

C) Phosphorylation of S501: The y₅, y₇, y₉, y₁₁, y_{15}^{2+} , y_{16} , y_{16}^{2+} , y_{17}^{2+} and y_{18}^{2+} ions are inconsistent with phosphorylation at sites other than S501.

D) Phosphorylation of S503: The y_{17}^{2+} -H₃PO₄ ion corresponds to a 98Da phosphate loss during MS³ fragmentation of the y_{17}^{2+} ion phosphorylated at S503. The y_5 , y_6 , y_8 , y_9 , y_{10} , y_{11}^{2+} , y_{13}^{2+} , y_{14}^{2+} , y_{15}^{2+} and y_{16}^{2+} ions are inconsistent with phosphorylation at sites other than S501 or S503. **E**) Phosphorylation of S515: The y_5 -H₃PO₄ ion corresponds to a 98Da phosphate loss during MS³ fragmentation of the y_5 ion phosphorylated at S515. The b_{14} ion is inconsistent with phosphorylation at sites other than S515.

Details on the Experimental procedures are available upon request.