Supplementary Figure Legends

Supplementary Figure 1. Phosphorylation sites labeled with $P^{18}O_1$ and $P^{18}O_2$. A. MS1 spectra of phosphorylation sites that incorporate the intermediate labeled phosphate over time. The population of isotopic peaks that should encompass the "light" species is labeled in blue and the "heavy" peak shifted 6 Da away is labeled in red. Also depicted is a phosphorylated peptide with two labeled phosphorylation sites. In this case the MS1 of the species with one heavy phosphorylation site and one light site is in purple and the fully labeled species is in red. B. Signal to noise frequency distribution of peptides with 0 (light) through 9 (three heavy) labeled phosphorylation sites. The signal to noise is the intensity of the species divided by the error from the model fit.

Supplementary Figure 2. Mass spectra of [γ -$^{18}O_4$]ATP. A. Direct infusion of [γ -$^{18}O_4$]ATP. B. [γ -$^{18}O_4$]ATP extracted from a SILAP sample.

Supplementary Figure 3. Percent-wise kinase-substrate relationship distribution. Kinase substrate relationships were predicted using iGPS. Plotted for clarity are the percent of substrates matched to each kinase group.

Supplementary Figure 4. Flow cytometry data showing cell cycle synchronization for the G1/S phase and M phase SILAP samples. Cells were fixed with ethanol and propidium iodide stained. N is the amount of DNA in the cells. The cells synchronized using a double thymidine block were primarily synchronized to G1/S phase (N), while cells synchronized with an additional nocodazole block were primarily synchronized to M phase (2N).

Supplementary Figure 5. Histone H1 N-terminal peptide MS1 spectra. Matching MS1 spectra for the phosphorylation progress curves in Figure 7 from different N-terminal H1 variant phosphorylation sites.