Supplementary Figure 1. Immunoprecipitation (IP) efficiency of light and heavy CDC27 proteins. **(a)** An immunoblot analysis of the heavy (from wheat germ extract) and light (from HeLa cell lysate) inputs was initially done to establish the appropriate mixing ratio, 1:10 WGE:HeLa, for the IP step such that similar sister peak intensities would result **(b)**. The relative abundance of the representative peptide in **(b)**, $[LAEGEQILSGGVFNK]^{2+}$, is not altered during the nocodazole time course experiment. The two IP experiments were completed months apart and with separate WGE and HeLa lysate sources, but were prepared based on the initial immunoblot analysis. Based on the relative input **(a)** and output data **(b)**, the IP efficiencies for the light and heavy proteins were determined to be the same.

Supplementary Figure 2. Peak profiles of a sample set of stable isotope labeled CDC27 **(a–c)** and APC5 **(d-f)** peptides. *In vitro* synthesis of the labeled protein standards in the wheat germ extract results in 2 – 2.5% of unlabeled peptides. The black and blue arrows indicate the monoisotopic peaks for the light and heavy peptides, respectively.

Supplementary Figure 3. Statistical analysis of the CDC27 and APC5 data sets. To determine the expected error in measuring a peptide we calculated the standard deviation (STD) of the replicate measurements for all replicate measurements. The peptides are organized by rank order of STD for visualization purposes - most peptides have a deviation of under 16 % [2 sigma – see Materials and Methods] for **(a)** CDC27 and **(b)** for APC5. To determine which peptides have been modified during the time course we calculated the STD (arranged by rank order) of each distinct peptide across the four time points for **(c)** CDC27 and **(d)** APC5. Using a STD of less than 16% as a cut-off [2 sigma – see Materials and Methods] the peptides fall into two groups, those that do not change significantly across the time points and those that do. **(e)** As an alternate way to visualize the modified peptides from unmodified peptides we plotted each set of peptides for each time point after normalization. Unmodified peptides are indicated as blue points and modified as red points. Normalization was performed using only the blue peptides. After normalization the expected value of the ratio of light to heavy peptide is 1. The peptides are arranged based on time of measurement with spaces included to show the breaks

between the time points - from left to right: Thymidine phase, 4 hrs, 8 hrs and 10 hrs nocodazole.