# SUPPLEMENTARY INFORMATION

**Figure S1: FACS analyses of the synchronized HeLa cells used for the experiments.** The time points at which the cells were harvested (after thymidine release) are indicated above each FACS. The corresponding tentative cell phases are also indicated. (Page 4)

**Figure S2: Text analysis results.** A list of the most frequent words in the descriptions of the proteins found in the 1st percentile of each reference cluster. Listed are the number of total occurrences, and the rank of each word when all six clusters were analyzed. (Page 5)

**Figure S3. Additional panels for the *in vitro* APC/C-dependent degradation assays on candidate APC/C substrates and controls.** $^{35}$S-labeled KIF4A, KIF20A and KIF23 were incubated in G1 HeLa S3 cell extracts in the absence/presence of the APC/C inhibitors EMI1 and SECURIN, and their time dependent degradation was assayed by SDS-PAGE and autoradiography. KIF20A was identified as a 2nd non-APC/C substrates in our study and thus used as a negative control. The densitometry-based quantifications are based on three independent degradation assays for KIF4A, and KIF20A, and a single result for KIF23A. (B) Frog extract-based degradation assays for WT-KIFC1 with interphase or interphase supplemented with CDH1 (active APC/C). The D-box mutant (DM) was used as a control. (Pages 6-7)

**Figure S4. Cell cycle dependent Ser6 phosphorylation.** (A) Annotated $MS^2$ spectrum of N-terminally acetylated, methionine-oxidized and S6 phosphorylated KIFC1 peptide ac(Mox)DPQR(pS)PLLEVK. (B) Multiple sequence alignment of KIFC1 orthologues, with

emphasis on the N terminus. The D-box motif encompasses the conserved serine 6 (bold red). The other N-terminal serines known to be phosphorylated (S26, S31 and S33) are also highlighted. (C) $MS^2$ spectrum for KIFC1 pS26: AP(pS)QLPLSGSR. (D) $MS^2$ spectrum for KIFC1 pS31: APSQLPL(pS)GSR. (Pages 8-10)

**Figure S5. *In vitro* phosphorylation of KIFC1.** (A) Biological repeats of degradation assays of $^{35}$S-labeled WT-KIFC1, DM-KIFC1, and various serine-to-alanine and serine-to-aspartic acid mutants as a function of incubation with APC/C-active/G1 HeLa S3 cell extract in the absence/presence of an APC/C inhibitors. The red arrows indicate mutations rendering KIFC1 resistant to APC/C-dependent degradation (B) Extracted ion chromatograms (upper panel) and $MS^1$ spectra (lower panels) for the unmodified or CDK1-phosphorylated KIFC1 N-terminal peptide. (C) Monitoring $^{35}$S-labeled KIFC1 ubiquitination by APC/C as a function of CDK/CYCLIN B activity. The high molecular weight smearing indicates ubiquitinated $^{35}$S-KIFC1 (the hashed box indicates the quantified area). The upper panel is the relative abundance of total higher molecular weight signal when normalized to the 0 time point of either $^{35}$S-KIFC1 co-incubated with APC/C alone, or with CDK1/CYCLIN B, or with CDK1/CYCLIN B and the CDK1 inhibitor purvalanol A. (Pages 11-13)

**Table S1:** Peptide-centric information of the quantitative proteomics data including “Gene identifier”, “IPI accession number”, “Protein description”, “Peptide sequence”, “Peptide modifications”, “TMT126 intensity”, “TMT127 intensity”, “TMT128 intensity “, “TMT129 intensity”, “TMT130 intensity”, “TMT131 intensity”, “MASCOT score”, “retention time”, “expected m/z”, “mass difference”, “missed cleavage sites”, “charge state”, “normalized TMT126 intensity”,

“normalized TMT127 intensity”, “normalized TMT128 intensity”, “normalized TMT129 intensity”, “normalized TMT130 intensity”, “normalized TMT131 intensity”. (Separate Excel file)

**Table S2:** Results of the analysis of the co-regulation proteomics study. Each tab lists “GeneName”, “IPI Accession #”, “Protein Description”, and the mean intensities for the six different samples (i.e. 0 hrs to 15 hrs post release from thymidine block). Tab specific is the information about the distance to the respective reference clusters as shown in Figure 2B. The smaller the value, the more similar the observed profile and the reference cluster. (Separate Excel file)