Supplementary Information

Affinity purification and tandem mass spectrometry
We transiently expressed Flag-HA-Cyclin F or empty vector (as negative control) in cells from the U2OS human osteosarcoma cell line. 24 h post-transfection, we synchronized cells in prometaphase using a nocodazole (100 ng/ml) block. Cells were then released from the block into fresh medium, collected at 8 and 16 h time points. Whole-cell extracts were prepared by lysing cells for 45 min in immunoprecipitation buffer (50 mM HEPES, pH 7.2, 150 mM NaCl, 1.0% nonyl phenoxypolyethoxylethanol-40 (NP-40), 1 mM ethylenediamine tetraacetic acid, 1 mM ethylene glycol tetraacetic acid, 1:100 dilution of protease inhibitor cocktail set III, 1:100 dilution of phosphatase inhibitor cocktail set I, 1 mM orthovanadate, 1 mM NaF, 0.5 mM dithiothreitol). Cleared lysates were incubated with anti-HA antibody-conjugated resin (Sigma-Aldrich, St Louis, MO, USA) directed against the HA-tag, for 16 h. Immune complexes were washed 5 x with immunoprecipitation buffer. The immunoprecipitates were eluted from the beads with 100 µg/ml of HA-peptide (Sigma-Aldrich).

The eluate was processed using the expertise of the proteomics core facility at the Moffitt Cancer Center & Research Institute, USA. Briefly, the eluate were then resolved on a 10% SDS-PAGE gel and visualized by Coomassie Brilliant Blue staining. Each lane was cut into 10 equal slices. To reduce and alkylate proteins, destained gel slices were treated with TCEP (Tris (2-carboxyethyl) phosphine and iodoacetamide. Following in-gel tryptic digestion, peptides were extracted and concentrated under vacuum centrifugation. A nanoflow ultra high performance liquid chromatograph (RSLC, Dionex, Sunnyvale, CA, USA) coupled to an electrospray ion trap mass spectrometer (LTQ-Orbitrap, Thermo, San Jose, CA, USA) was used for tandem mass spectrometry peptide sequencing experiments. The sample was first loaded onto a pre-column (2 cm x 75 µm ID packed with C18 reversed-phase resin, 5 µm, 100 Å) and washed for 8 minutes with aqueous 2% acetonitrile and 0.04% trifluoroacetic acid. The trapped peptides were eluted onto the analytical column, (C18, 75 µm ID x 50 cm, Pepmap 100, Dionex, Sunnyvale, CA, USA). The 120-minute gradient was programmed as: 95% solvent A (2% acetonitrile + 0.1% formic acid) for 8 minutes, solvent B (90% acetonitrile + 0.1% formic acid) from 5% to 15% in 5 minutes, 15% to 40% in 85 minutes, then solvent B from 50% to 90% B in 7 minutes and held at 90% for 5 minutes, followed by solvent B from 90% to 5% in 1 minute and re-equilibrate for 10 minutes. The flow rate on analytical column was 300 nl/min. Five tandem mass spectra were collected in a data-dependent manner following each survey scan. The MS scans were performed in Orbitrap to obtain accurate peptide mass measurement and the MS/MS scans were performed in linear ion trap using 60 second exclusion for previously sampled peptide peaks. Mascot searches were performed against the Swiss-Prot human database downloaded on June 11, 2014. Two trypsin missed cleavages were allowed, the precursor mass tolerance was 1.08 Da. MS/MS mass tolerance was 0.8 Da. Dynamic modifications included carbamidomethylation (Cys), oxidation (Met), and phosphorylation (Ser/Thr/Tyr). Results from Mascot were compiled in Scaffold, which was used for manual inspection of peptide assignments and protein identifications. Using these, we have further compiled a list of putative cyclin F-associated proteins (provided as an Excel file; see Supplementary File 1) that were identified based on the presence of at least two unique peptides with a minimum peptide probability threshold of 95% and minimum protein probability threshold of 95% (Supplementary File 1).