**F) Supplemental Text and Figures**

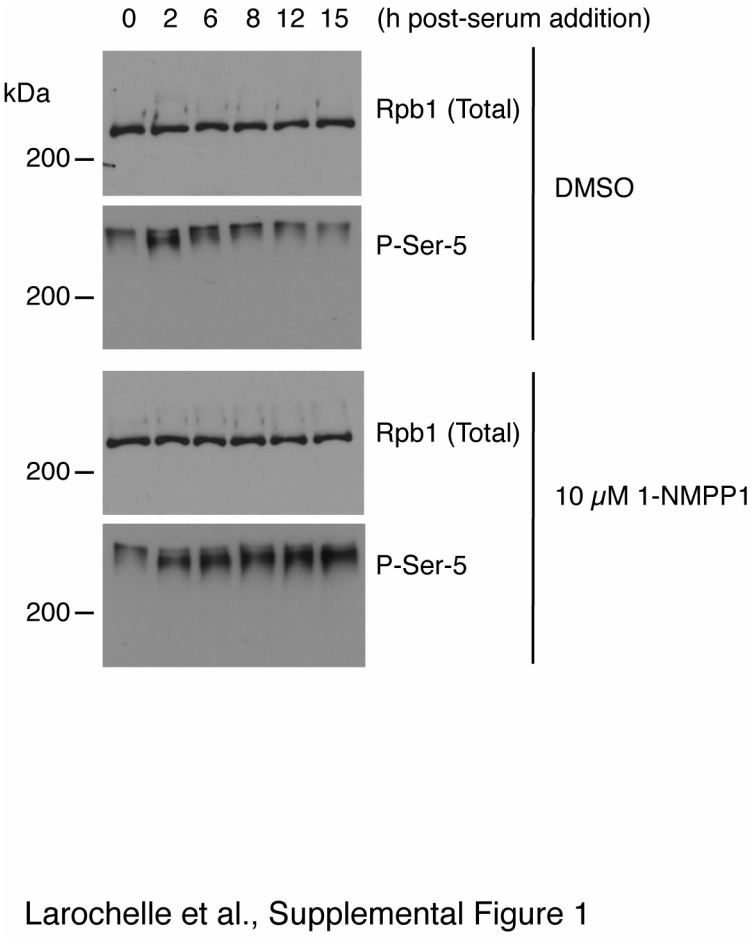
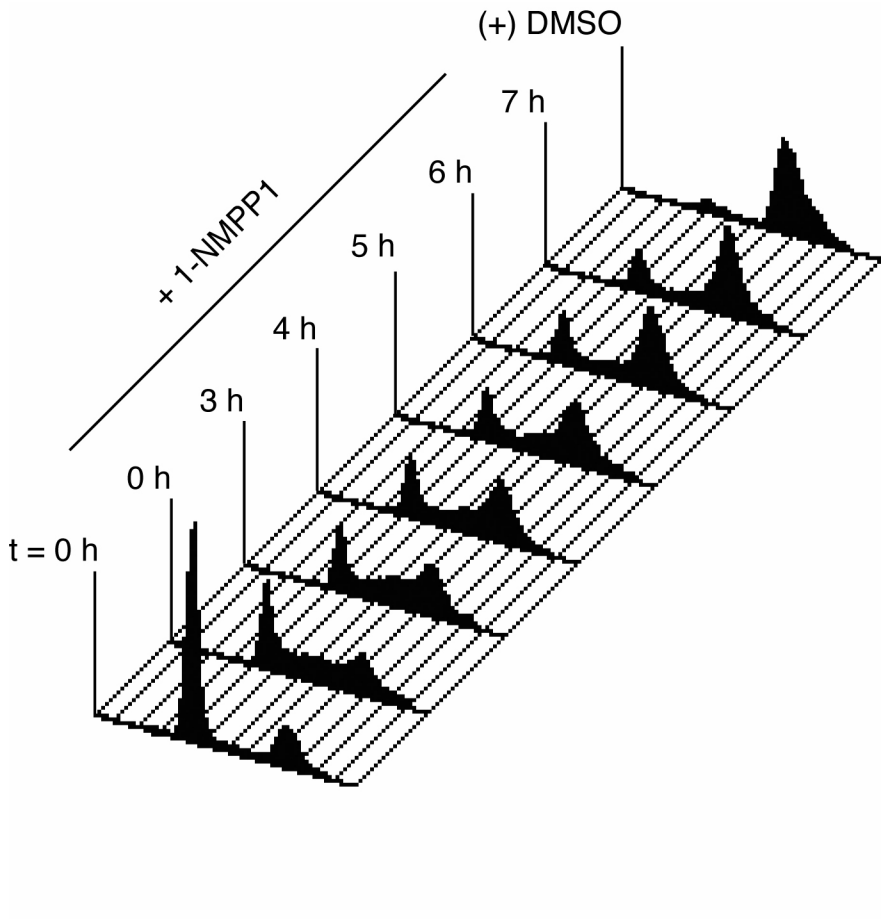



**Figure S1.** Cdk7 inhibition perturbs normal Pol II CTD phosphorylation patterns during G1. Cells were synchronized by serum withdrawal and re-addition, as in the experiments in Figure 2. DMSO or 10 μM 1-NMPP1, as indicated, was added 1 h after serum. Cells were harvested for lysate preparation at the times indicated above each lane, and whole-cell extracts were subjected to immunoblotting for total RNA Pol II (Rpb1), with an antibody that recognizes an amino-terminal epitope, or with the H14 monoclonal antibody, which recognizes the CTD phosphorylated on Ser5 (P-Ser5).




Larochelle et al., Supplemental Figure 2

**Figure S2.** Cells were serum-starved for 48 h and subsequently released into fresh medium containing 50 ng/ ml nocodazole. DMSO or 1-NMPP1 was added at the time indicated and cells were collected when all the cells in the DMSO control were rounded (~24 h). No 1-NMPP1-treated plates contained significant numbers of rounded cells, so the 4N peak represents G2 cells.

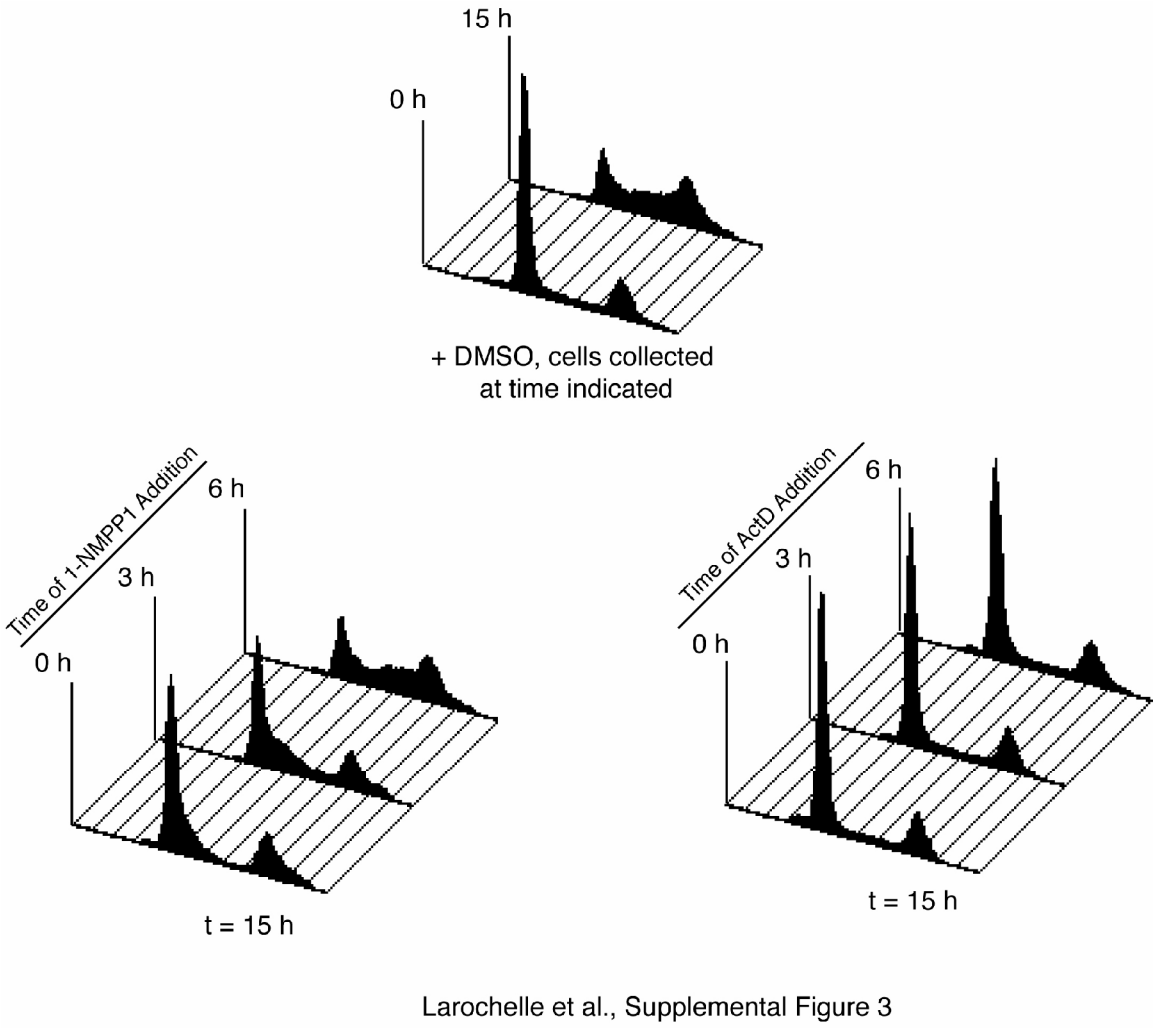
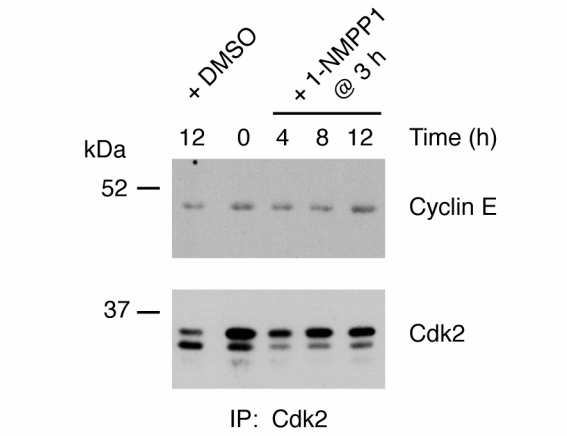
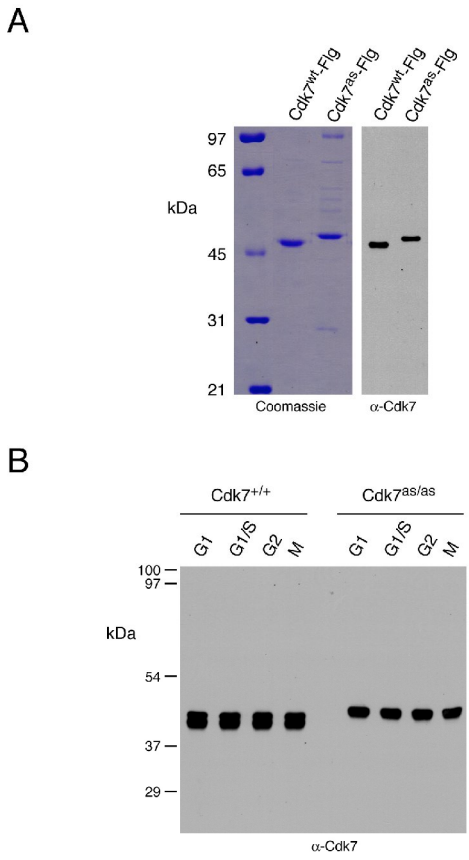



**Figure S3.** Cells were serum-starved for 48 h to synchronize them in G0/G1. Upon serum addition, media was supplemented with DMSO at time 0 (top), or with 1-NMPP1 (bottom left) or Actinomycin D (ActD, bottom right) at the time indicated. Cells were collected at 15 h and analyzed by flow cytometry to measure DNA content.




Larochelle et al., Supplemental Figure 4

**Figure S4.** HCT116 *Cdk7*$^{as/as}$ cells were starved of serum for 48 h, and released into serum-containing medium at time 0. DMSO was added at time 0, or 10 μM 1-NMPP1 was added 3 h after serum, and cells were incubated for the times indicated before harvest and whole-cell extract preparation. Cdk2 was immunoprecipitated with monoclonal antibody D-12 from 200 μg total protein, and immune complexes were immunoblotted for cyclin E, with polyclonal antibody M20, and for Cdk2, with polyclonal antibody M2.




Larochelle et al., Supplemental Figure 5

**Figure S5.** (A) The F91G mutation retards electrophoretic mobility of Cdk7 without affecting immunoreactivity. Approximately equal amounts of purified, monomeric, His-Flag-tagged Cdk7—wild-type (Cdk7$^{wt}$-Flg) or analog-sensitive (Cdk7$^{as}$-Flg)—were electrophoresed in 10% SDS-PAGE gels and visualized by staining with Coomassie brilliant blue (left) or immunoblotting with monoclonal anti-Cdk7 antibodies (right). (Note: protein loaded for immunoblot detection was ~1,000-fold less than for Coomassie-staining.) (B) Abundance of Cdk7 does not fluctuate during the cell cycle in *Cdk7*$^{+/+}$ (left four lanes) or *Cdk7*$^{as/as}$ (right four lanes) HCT116 cells. Cells were synchronized as follows: in G1, by release from nocodazole block into drug-free medium for ~5 h; at G1/S, by double-thymidine block; in G2, by release from double-thymidine block into

drug-free medium for 7 h; and in M phase by release from thymidine block into nocodazole-containing medium for 12-16 h. Whole-cell extracts were prepared from each population and subjected to immunoblotting with anti-Cdk7 monoclonal antibodies.