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Supplemental Data

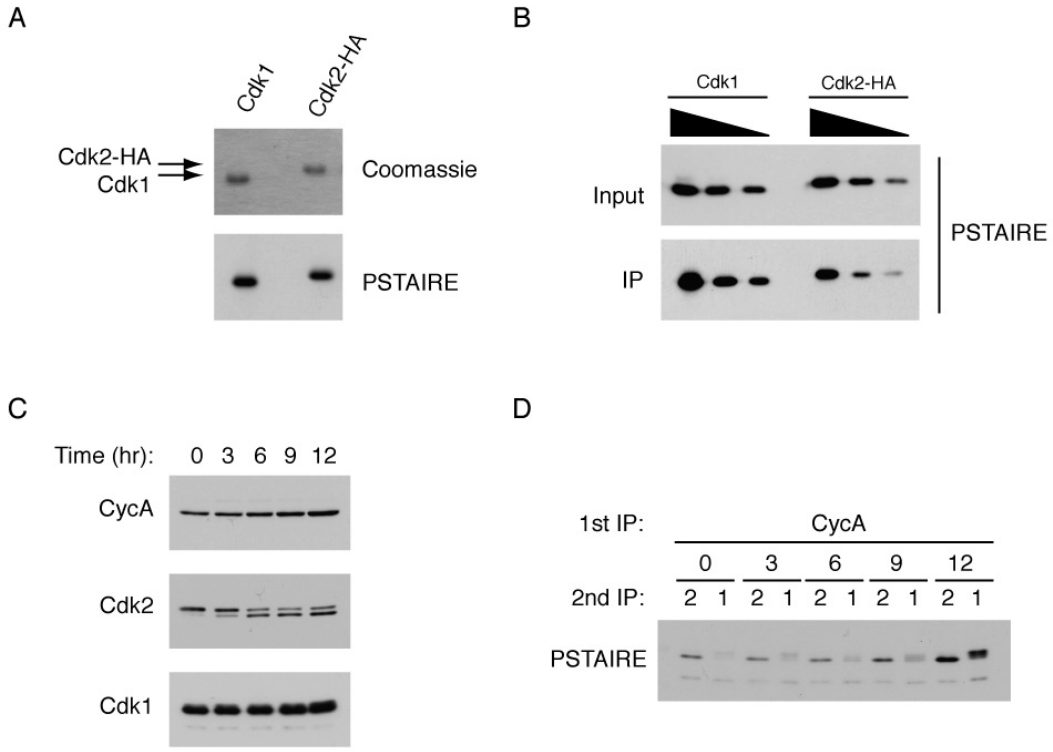
Distinct Activation Pathways Confer Cyclin-Binding Specificity on Cdk1 and Cdk2 in Human Cells

Karl A. Merrick, Stéphane Larochelle, Chao Zhang, Jasmina J. Allen, Kevan M. Shokat, and Robert P. Fisher

Supplemental Table 1. Apparent kinetic parameters of Cdk2 and Cdk2/Cyclin A phosphorylation by Cdk7/Cyclin H/Mat1

Parameter	$Cdk2^{D145N}$	$Cdk2^{D145N}$/CycA
K_M (μM)	5.8 ± 1.4	0.59 ± 0.12
V_{max} (pmol P_i/min)	5.1 ± 1.1	0.32 ± 0.02
k_{cat} (/s)	1.2	0.07
k_{cat}/K_M	0.21	0.13

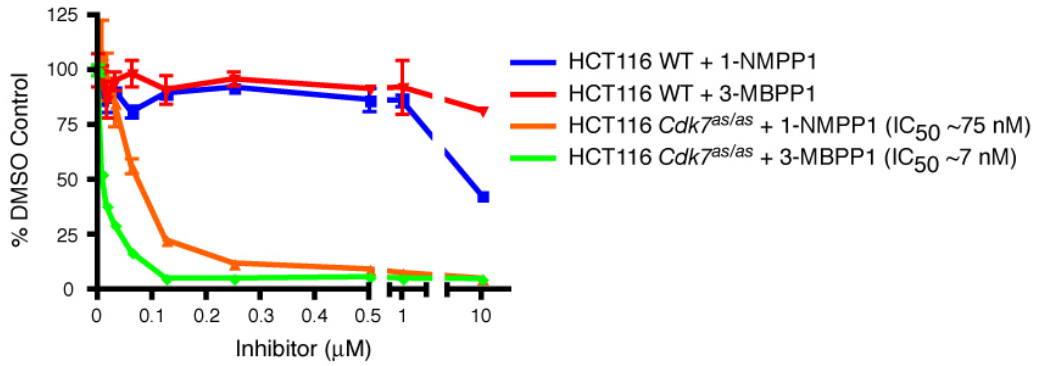
Supplemental Figure 1:



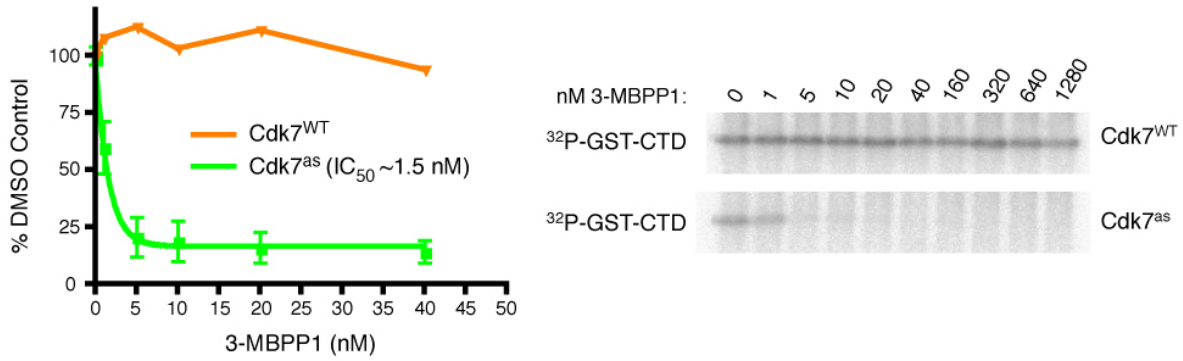
(A) Equal amounts of recombinant Cdk1 and Cdk2-HA, as determined by staining with Coomassie blue (top), were detected by immunoblotting with PSTAIRE antibody (bottom).
(B) Cdk1 and Cdk2 are immunoprecipitated with comparable efficiency. Purified, recombinant Cdk1 and Cdk2-HA were combined in a fixed ratio at three successive 2-fold dilutions in HBS supplemented with BSA (top). The CDK mixes were denatured by boiling in 1% SDS, diluted with HBS + 0.5% Triton-X 100, and Cdk1 and Cdk2 were immunoprecipitated from each. The amount of Cdk1 and Cdk2 recovered at the end of the assay was detected by immunoblot with the PSTAIRE antibody (bottom). Note imbalance in Cdk1 and Cdk2-HA inputs: initial reaction mixtures and corresponding immunoprecipitates both contained approximately twice as much Cdk1 as Cdk2-HA
(C) HCT116 WT cells were starved of serum for 48 hr and then stimulated by serum addition. Time points were taken when indicated, and the levels of cyclin A, Cdk1, and Cdk2 were measured by immunoblotting.
(D) Cyclin A was immunoprecipitated from extracts in (C) and the immune complexes were denatured, diluted, and re-immunoprecipitated with antibodies to Cdk1 and Cdk2. The amount of each CDK recovered was determined by immunoblotting with PSTAIRE antibody.

Supplemental Figure 2:

A



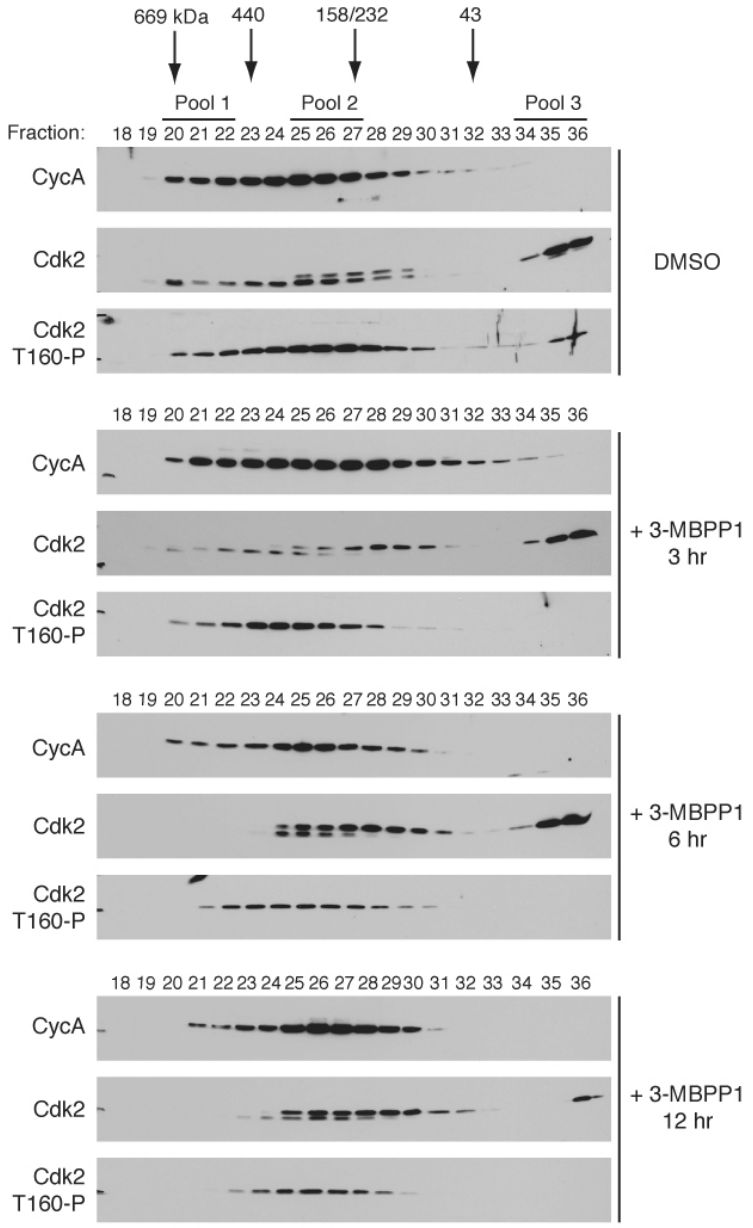
B



(A) 3-MBPP1 is more specific and potent than 1-NMPP1 in inhibiting cell proliferation. Wild-type and *Cdk7*$^{as/as}$ HCT116 cells were treated with indicated concentrations of either drug for 96 hr before measuring viability by MTT assay. Error bars represent the standard deviation of the mean in triplicate samples from a single representative experiment.

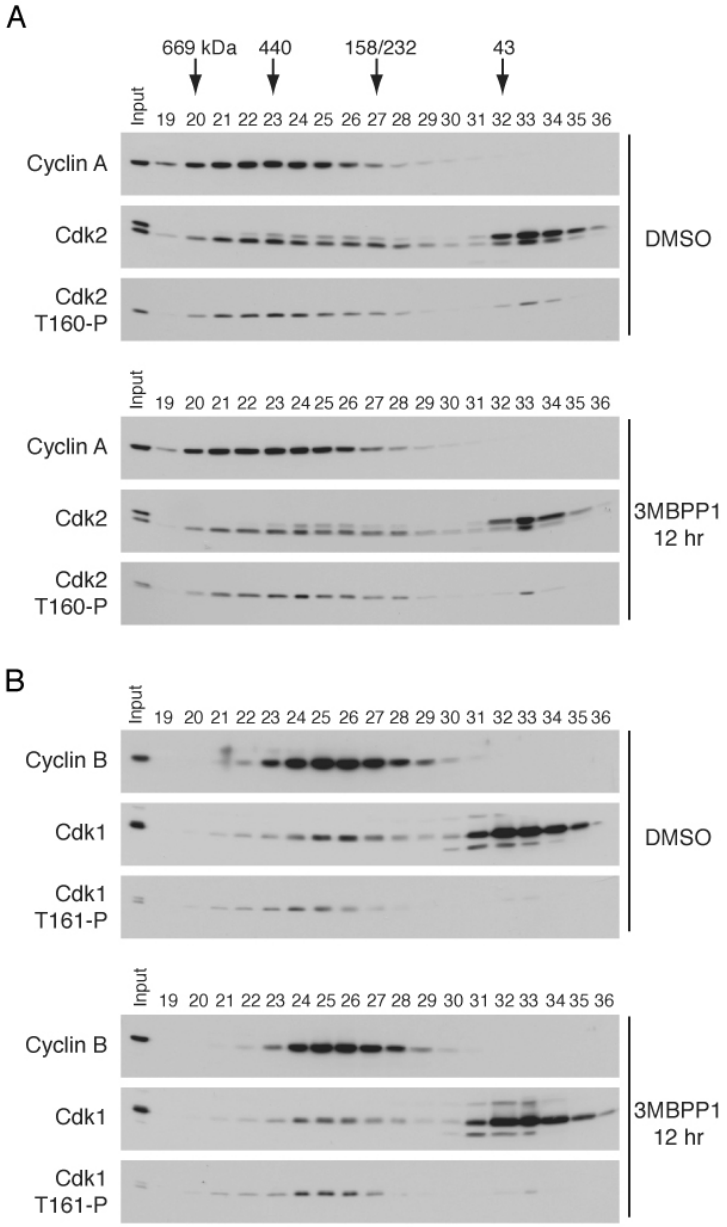
(B) 3-MBPP1 is a potent inhibitor of Cdk7^{as} but not Cdk7^{WT} in vitro. Cdk7/cyclin H/Mat1 was incubated with varying concentrations of inhibitor prior to testing kinase activity towards a fusion protein containing the Pol II CTD (GST-CTD). Error bars represent the standard deviation of the mean of triplicate samples.

Supplemental Figure 3:



HCT116 *Cdk7*$^{as/as}$ cells were treated with DMSO (control) for 12 hr or 5 μM 3-MBPP1 for 3, 6, or 12 hr, as indicated. Extracts were separated by gel exclusion chromatography, and fractions were immunoblotted and probed with cyclin A, Cdk2, and Cdk2 T160-P antibodies. The fractions were pooled as indicated for the direct comparisons shown in Figure 2B, C, and D.

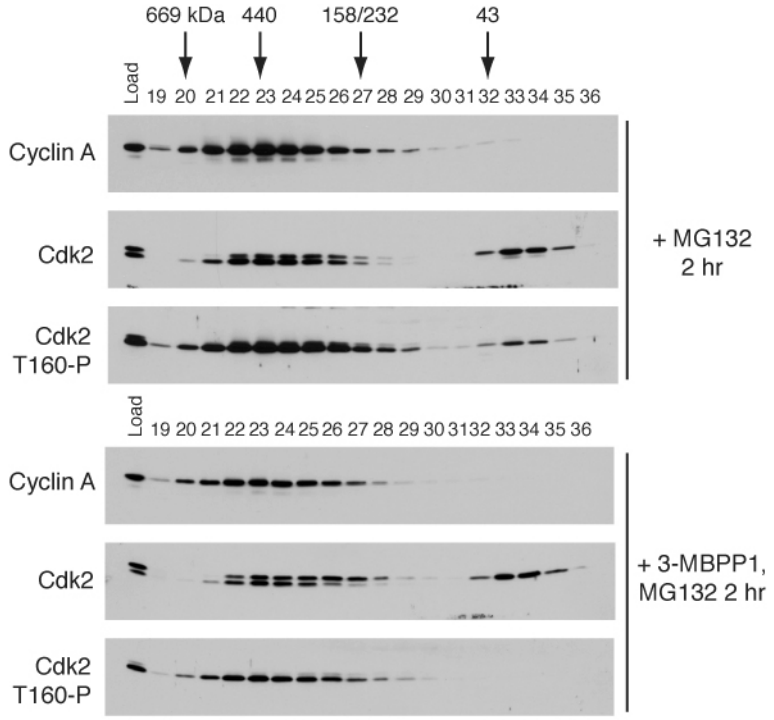
Supplemental Figure 4:



(A) Wild-type HCT116 cells were treated with DMSO (control) or 5 μM 3-MBPP1 for 12 hr. Extracts from these cells were separated by gel exclusion chromatography. Fractions were immunoblotted with cyclin A, Cdk2, and Cdk2 T160-P antibodies.

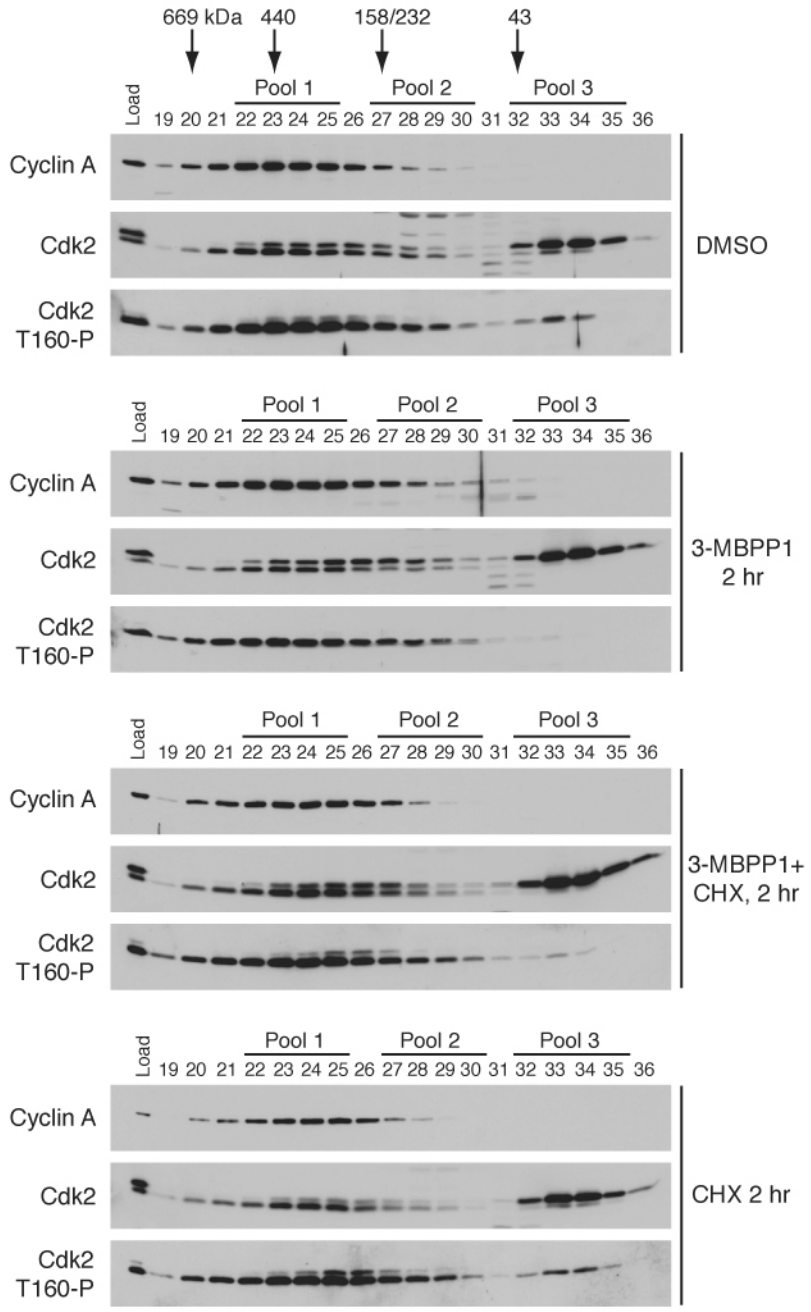
(B) Fractions from (A) were probed with cyclin B, Cdk1, and Cdk1 T161-P antibodies.

Supplemental Figure 5:



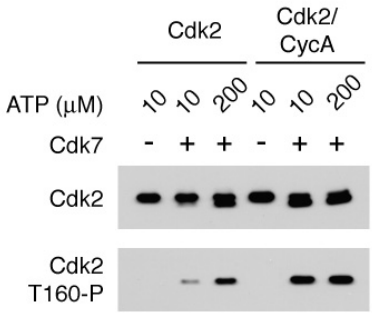
HCT116 *Cdk7*$^{as/as}$ cells were treated with 10 μM MG132 without or with 5 μM 3-MBPP1, as indicated, for 2 hr. Extracts from each treatment were separated by gel exclusion chromatography, immunoblotted and probed with indicated antibodies.

Supplemental Figure 6:



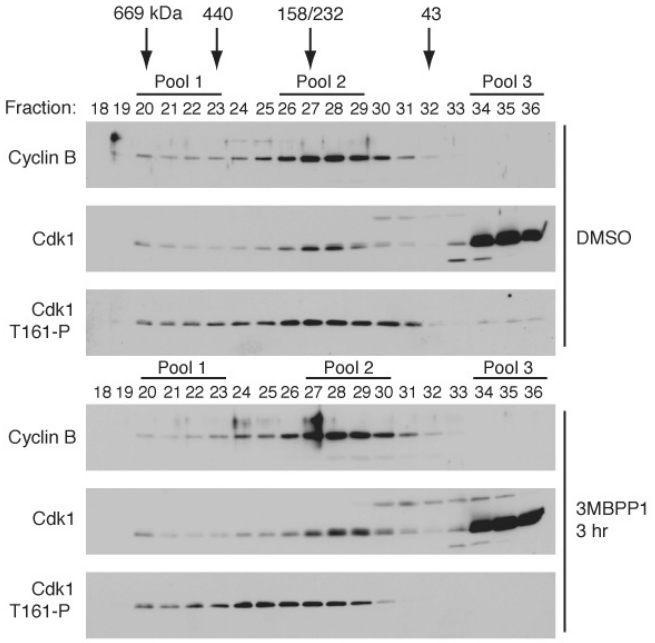
HCT116 *Cdk7*$^{as/as}$ cells were treated with DMSO (control), 5 μM 3-MBPP1, 100 μg/mL cycloheximide (CHX), or 3-MBPP1 and CHX for 2 hr, as indicated. Extracts from each treatment were separated by gel exclusion chromatography, immunoblotted and probed with the indicated antibodies. Fractions were pooled as indicated for the direct comparisons shown in Figure 3B.

Supplemental Figure 7:



Direct CAK assays were performed as described in Kaldis et al., 1998. Briefly, 0.2 μM Cdk2 or pre-formed Cdk2/cyclin A complex (0.2 μM Cdk2 mixed with 0.3 μM cyclin A) was incubated with 0.06 μM T-loop phosphorylated Cdk7/cyclin H/Mat1 in HBS + 10 mM $MgCl_2$ supplemented with 1 mg/ml BSA in a final reaction volume of 16 μl. The assay was performed with 10 μM ATP, as in Kaldis et al, 1998, or with 200 μM ATP as in the kinetic experiments shown in Figure 5, as indicated. The reaction was allowed to proceed for 30 min before it was stopped by addition of SDS sample buffer. Phosphorylation of Cdk2 was detected by immunoblotting with a Cdk2 T160-P specific antibody.

Supplemental Figure 8:



Sizing column fractions (same as in Supplemental Figure 3) were probed for cyclin B, Cdk1, and Cdk1 T161-P to determine effects of CAK inhibition on different Cdk1 populations.