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Supplemental Data

A Coupled Chemical-Genetic and Bioinformatic Approach to Polo-like Kinase Pathway Exploration

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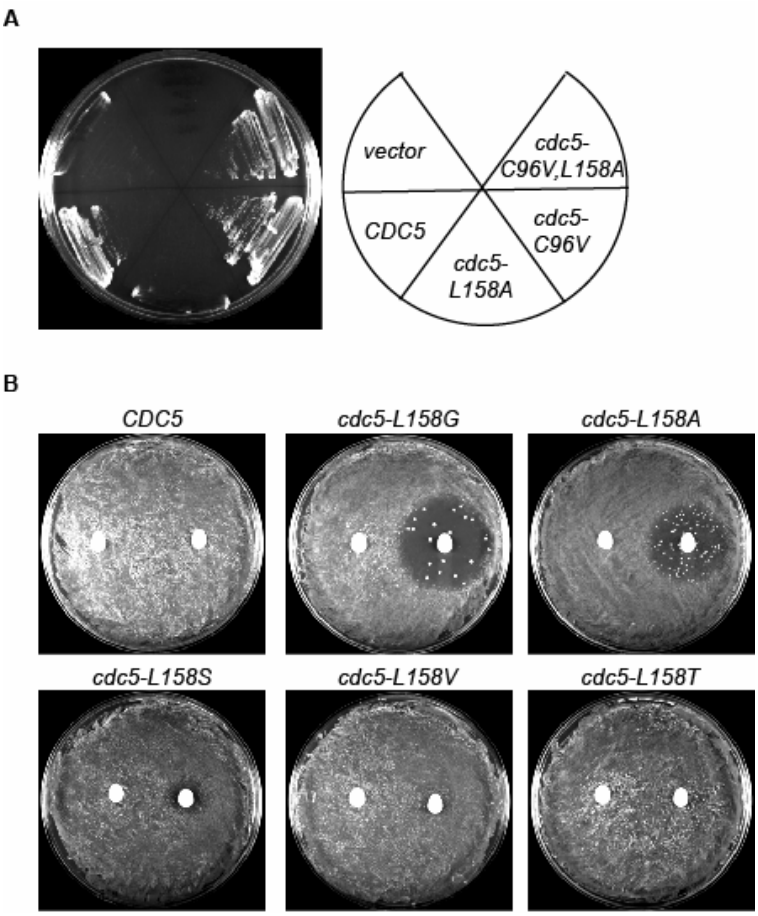

Figure S1. Additional Analysis of Cdc5 Sequence Requirements for Inhibition by CMK

(A) Cell viability of strains grown on 10 μM CMK. Growth of strains requiring the indicated plasmid-borne *CDC5* alleles for survival, showing that both the space creating L158A mutation and a cysteine at position 96 are required in combination for inhibition by CMK.
(B) CMK inhibition of Cdc5 alleles. *cdc5-1 ts* strains bearing a plasmid expressing Cdc5 or the mutant indicated was plated and 10 nmol scaffold molecule (left) or CMK (right) applied to the filter disc. CMK dependent growth inhibition of only the *cdc5-L158G* and *cdc5-L158A* cells was apparent after 3 days at restrictive temperature (37 degrees).

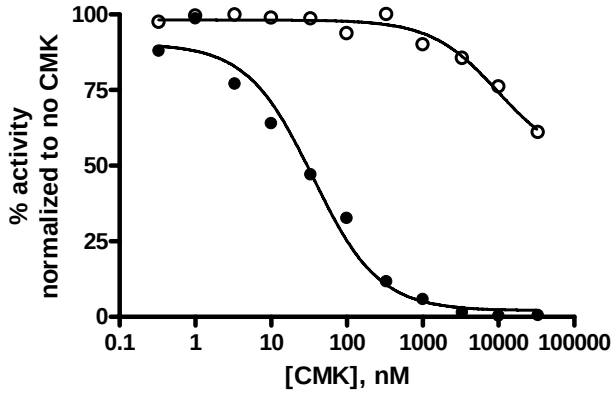

Figure S2. IC_{50} for CMK Inhibition of Cdc5(L158G)

10 ng purified recombinant His6-Cdc5 (open circles) or His6-Cdc5(L158G) (closed circles) was pre-incubated at room temperature for 5 minutes with 10 µg alpha-casein (dephosphorylated) in 25 mM HEPES, pH 8.0, 60 mM KCl, 15 mM $MnCl_2$, and 100 ug/ml BSA in the presence or absence of the indicated concentrations of CMK. After 5 minutes, [γ-^{32}P] ATP (to 10 µM, 100 µCi / ml) and DTT (to 1 mM) were added to begin the kinase reaction. After 30 minutes at room temperature, 10 µl of each reaction solution was spotted on a nitrocellulose disk, washed 4 times (5 min each) in 1 M NaCl, 1% phosphoric acid, dried under a heat lamp for 15 min, and counted with a scintillation counter. Data was analyzed using GraphPad Prism software. The IC_{50} for CMK inhibition of Cdc5(L158G) was determined by non-linear regression (R2 = 0.98) to be 36 nM (95% confidence interval, 20 nM to 62 nM).