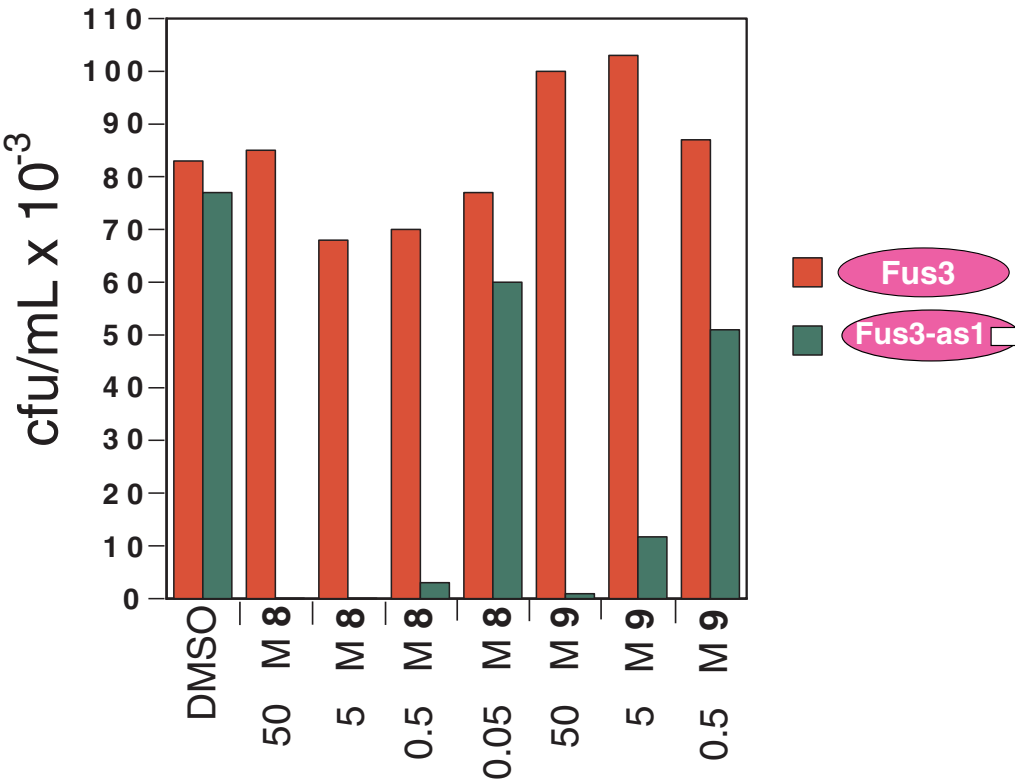
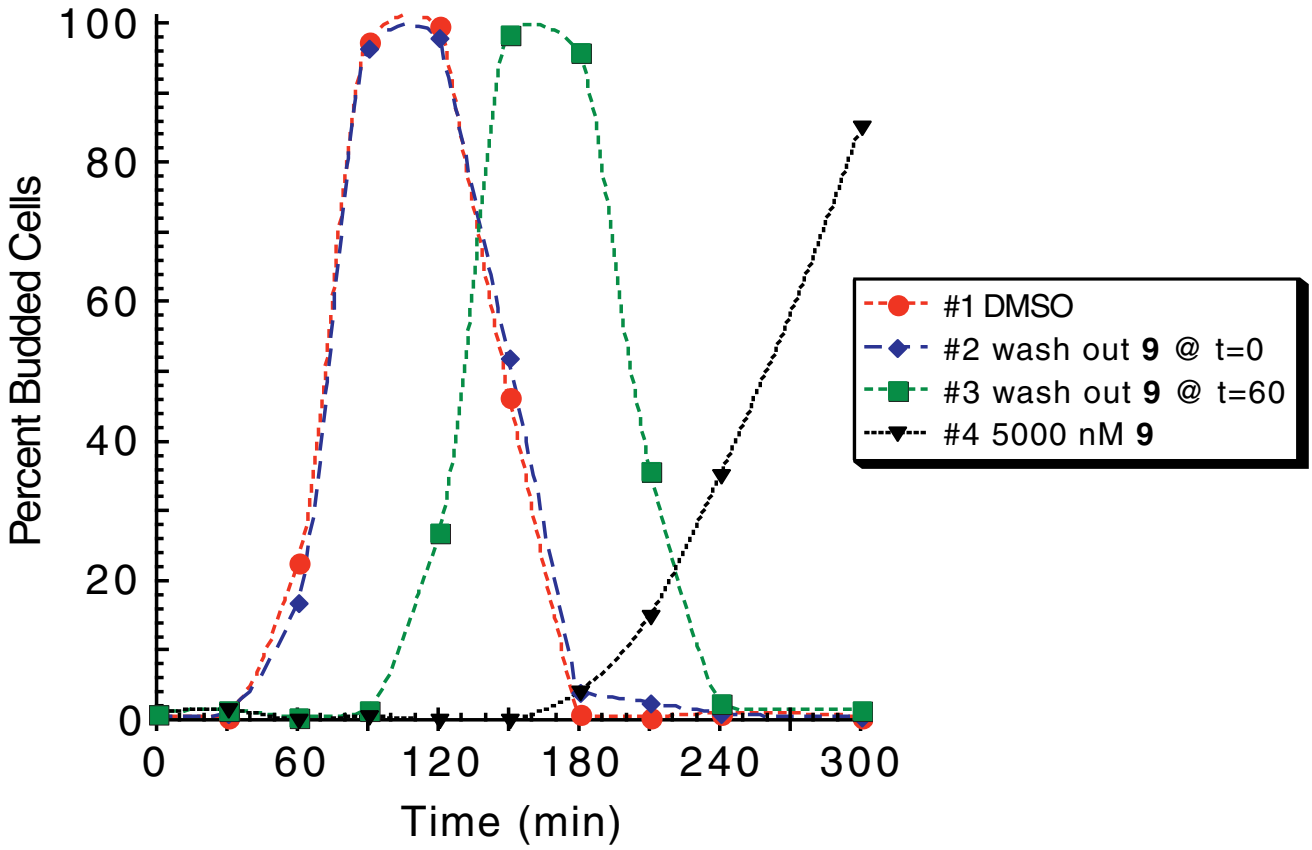
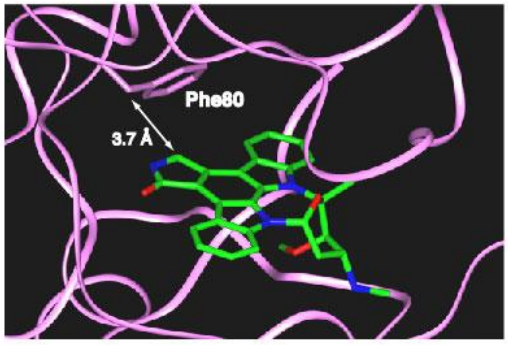


Disruption of Fus3-dependent mating with **8** and **9** is selective and dose-dependent. Red bars indicate the cfu/mL x 10^{-3} for wild-type yeast at the indicated inhibitor concentrations. Green bars indicate the cfu/mL x 10^{-3} for *fus3-as1* yeast at the indicated inhibitor concentrations. Mating assays were performed as described in the Figure 2 legend.



Sup. Fig. # The G1 arrest induced in synchronous *cdc28-as1* cells by 5000 nM **9** is reversible. *cdc28-as1* cells were arrested in G1 with 1 µg/ml α-factor for 3 hours. The culture was split in four; #1 was treated with DMSO and #2-4 with 5000 nM **9** for 20 minutes. Cells were released from G1 by washing and resuspension in media containing the following: #1 – DMSO, #2 – DMSO, #3 and #4 – 5000 nM **9**. Samples were removed every 30 minutes and fixed for analysis of budding index. After 60 minutes, **9** was removed from #3 by washing and resuspension in media containing DMSO. Cells in #4 eventually budded after 180 to 240 minutes of inhibitor treatment, and arrested with large hyperpolarized buds. 1 µg/ml α-factor was added back to cultures #1-3 at 90 minutes, 90 minutes, and 150 minutes respectively.

a



b

Compound / Kinase	1	3	6
v-Src	0.020	>33	12
c-Fyn	0.17	>33	>33
c-Abl	2.2	>33	>33
CDK2	0.020	22	3.7
CAMK II	0.0054	1.7	0.54
v-Src-as1		0.00023	0.0023
c-Fyn-as1		0.00055	0.0043
c-Abl-as2		3.1	9.6
CDK2-as1		0.070	0.037
CAMKII-as1		0.95	0.0040

a, Crystal structure of (+)-Staurosporine bound to CDK2[11]. CDK2 is shown in magenta with the peptide backbone illustrated as a ribbon and the F80 side chain as sticks. Staurosporine is colored by atom type as follows: carbon (green), nitrogen (blue), oxygen (red). Hydrogens are not shown. **b**, 50% inhibitory concentrations (μM) for K252a (**1**) and selected C(7)-derivatized K252a analogs (**3**, **6**) against a panel of wild-type and rationally engineered protein kinases. Each wild-type protein kinase is shown as an oval. The corresponding engineered kinase is shown as an equivalently colored oval with a notch cut out. IC_{50} values for the best K252a derivative/engineered kinase pair are shown in blue.