Supplemental Table 1A: Summary of the kinetics of the catalytic domains of Cdc25B (Δ25B1 vs. Δ25B2 vs. Δ25B3).

Substrate	Parameter	Δ25B2	Δ25B1	Δ25B3
pNPP	k_{cat} (s^{-1})	0.315 ± 0.012	0.361 ± 0.020	0.329 ± 0.015
pNPP	k_{cat}/K_m (M^{-1}s^{-1})	35.2 ± 2.3	28.4 ± 1.9	35.0 ± 2.2
mFP	k_{cat} (s^{-1})	1.93 ± 0.05	2.17 ± 0.08	2.03 ± 0.05
mFP	k_{cat}/K_m (10^3 M^{-1}s^{-1})	51.4 ± 2.1	32.5 ± 1.1	50.1 ± 2.8
mFP	burst size (equivalents)	0.548 ± 0.004	0.565 ± 0.005	0.553 ± 0.005
mFP	burst rate (s^{-1})	20.3 ± 2.4	12.8 ± 2.7	19.4 ± 1.8
Cdk2-pTpY/CycA	k_{cat}/K_m (10^4 M^{-1}s^{-1})	358 ± 5	35 ± 12	344 ± 9

Supplemental Table 1B: Summary of the kinetics of the various site-directed mutants of Δ25B2 (W550F, W550A, A551E, S555A, R556L, S561A, R562L, and DBL (both R556L and R562L).

Substrate	Parameter	W550F	W550A	A551E	S555A	R556L	S561A	R562L	DBL
pNPP	k_{cat} (s^{-1})	0.410 ± 0.027	0.350 ± 0.019	0.315 ± 0.019	0.256 ± 0.009	0.350 ± 0.017	0.350 ± 0.019	0.347 ± 0.022	0.174 ± 0.010
pNPP	k_{cat}/K_m (M^{-1}s^{-1})	31.2 ± 2.2	33.9 ± 2.5	27.7 ± 1.9	37.5 ± 2.7	25.3 ± 2.7	34.1 ± 2.5	27.7 ± 1.9	19.3 ± 1.2
mFP	k_{cat} (s^{-1})	1.87 ± 0.05	2.33 ± 0.06	1.88 ± 0.07	1.94 ± 0.10	2.15 ± 0.08	2.10 ± 0.07	2.34 ± 0.11	1.83 ± 0.09
mFP	k_{cat}/K_m (10^3 M^{-1}s^{-1})	53.9 ± 2.4	46.0 ± 1.9	57.3 ± 2.3	58.7 ± 3.2	58.5 ± 3.0	41.4 ± 2.5	48.2 ± 2.1	47.8 ± 1.7
mFP	burst size (equivalents)	0.549 ± 0.008	0.572 ± 0.007	0.487 ± 0.006	0.403 ± 0.002	0.481 ± 0.001	0.526 ± 0.002	0.432 ± 0.001	0.382 ± 0.001
mFP	burst rate (s^{-1})	17.2 ± 3.0	14.6 ± 2.2	17.9 ± 1.8	22 ± 5.1	21.9 ± 5.4	19.4 ± 1.4	17.8 ± 1.8	18.8 ± 1.0
Cdk2-pTpY/CycA	k_{cat}/K_m (10^4 M^{-1}s^{-1})	439 ± 51	327 ± 40	390 ± 21	499 ± 69	164 ± 58	437 ± 34	223 ± 35	94 ± 21

Supplemental Table 2: Summary of the kinetics of the catalytic domains of Cdc25C ($\Delta25$C1 vs. $\Delta25$C2, the chimeric Cdc25 protein ($\Delta25$CB) and its site-directed mutant of the two arginine residues ($\Delta25$CB-DBL).

Substrate	Parameter	$\Delta25$C2	$\Delta25$C1	$\Delta25$CB	$\Delta25$CB-DBL
pNPP	k_{cat} (s^{-1})	0.45 ± 0.08	0.62 ± 0.13	0.62 ± 0.07	0.24 ± 0.05
pNPP	k_{cat}/K_m (M^{-1}s^{-1})	38.9 ± 3.9	55.2 ± 9.7	42.6 ± 5.2	24.3 ± 3.9
mFP	k_{cat} (s^{-1})	0.84 ± 0.14	1.32 ± 0.04	1.11 ± 0.08	0.82 ± 0.04
mFP	k_{cat}/K_m (10^3 M^{-1}s^{-1})	48.4 ± 3.4	34.8 ± 4.2	62.4 ± 8.7	35.7 ± 3.0
mFP	burst size (equivalents)	0.477 ± 0.013	0.469 ± 0.019	0.378 ± 0.020	0.302 ± 0.009
mFP	burst rate (s^{-1})	6.02 ± 0.27	5.73 ± 0.71	10.39 ± 0.75	9.93 ± 0.046
Cdk2-pTpY/CycA	k_{cat}/K_m (10^4 M^{-1}s^{-1})	4.15 ± 0.61	2.89 ± 0.46	5.98 ± 0.16	1.35 ± 0.60

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