Supporting information

Role of phosphorylation of Cdc20 in the regulation of the action of APC/C in mitosis.

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Figures S1 to S3

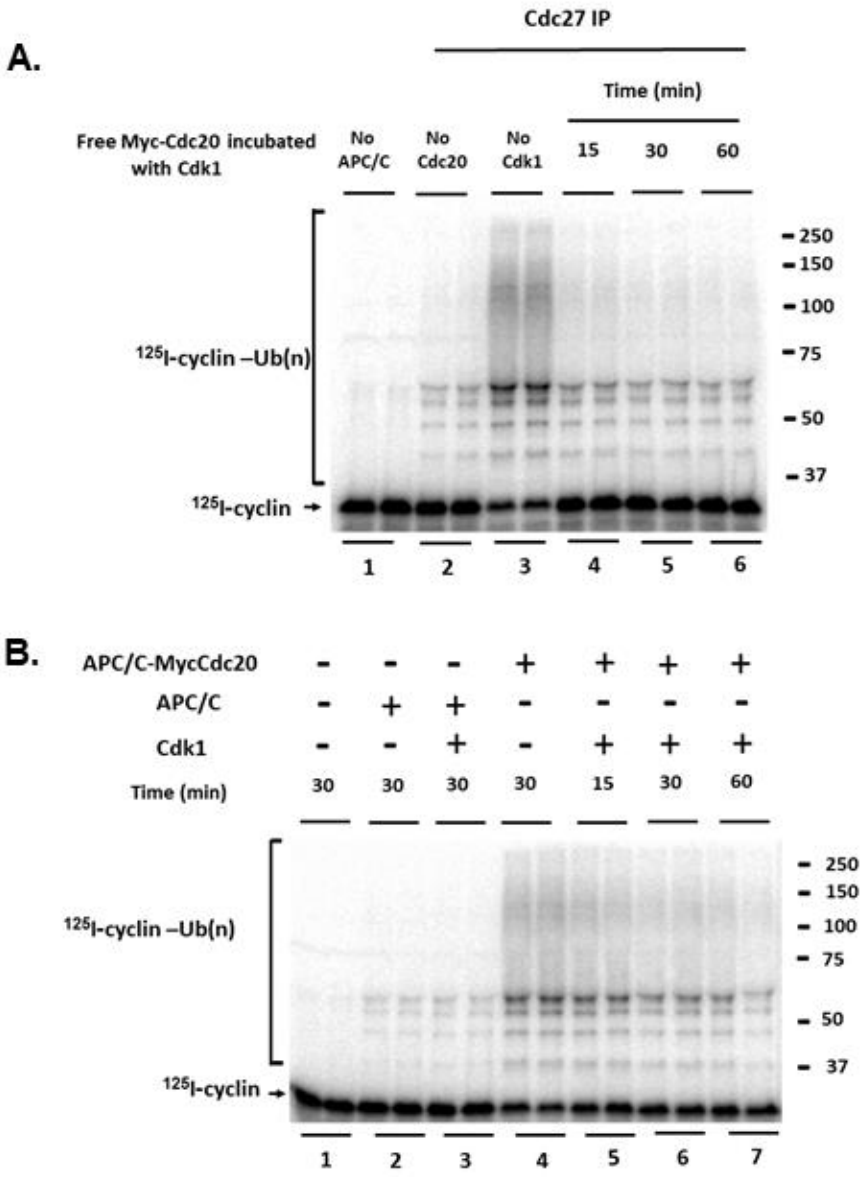

Fig. S1. Effects of phosphorylation of free or APC/C-bound myc-Cdc20 on the activity of APC/C. *(A)* Time-course of the effects of Cdk1-catalyzed phosphorylation of free myc-Cdc20 on its stimulation of APC/C activity. The experiment was similar to that described for Fig. 2A, *upper panel*, except that samples were not subjected to treatment with lambda phosphatase. The activity of APC/C in the ubiquitylation of ^{125}I-cyclin B was determined as described under *Materials and Methods*, in samples

of 1 μl of APC/C beads. “No Cdc20”, activity of endogenous APC/C without added myc-Cdc20. Numbers on the right indicate the position of molecular mass marker proteins. *(B)* Time-course of the effects of Cdk1-catalyzed phosphorylation of APC/C-bound Cdc20 on APC/C activity. The experiment was similar to that described under Fig. 2A, *lower panel*, but without lambda phosphatase treatment of samples. APC/C activity was assayed as described under *Materials and Methods*, in samples of 1 μl of anti-Cdc27 beads containing either APC/C without myc-Cdc20 (lanes 2, 3) or APC/C to which myc-Cdc20 had been bound (lanes 4-7).

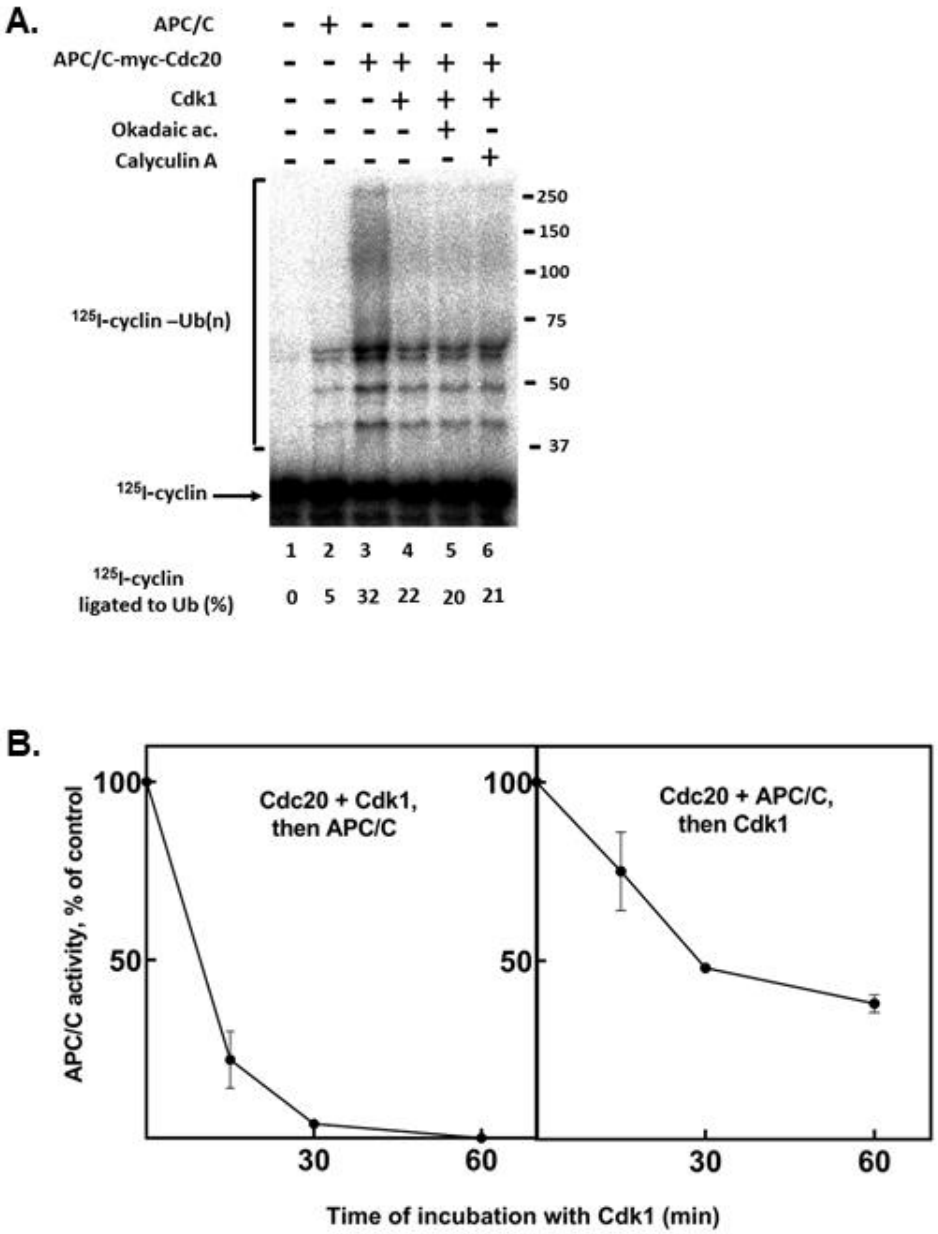

Fig. S2. (*A*) Effects of okadaic acid and calyculin A on the activity of APC/C-mycCdc20 incubated with Cdk1. Immunopurified APC/C to which myc-Cdc20 has been bound ("APC/C-myc-Cdc20") was incubated under conditions as in Fig. 2A, with additions as indicated. Okadaic acid and calyculin A were supplemented at 0.5 μM. Following incubation at 23ºC for 30 min, beads were washed and APC/C activity was

determined as described under *Materials and Methods*. *(B)* Preincubation of APC/C from HeLa cells with Cdc20 reduces the extent of the inhibition of its action by Cdk1. Phosphorylated APC/C was immunopurified from extracts of mitotic HeLa cells as described under *Materials and Methods*. *Left panel*, “Cdc20 + Cdk1, then APC/C” : Free myc-Cdc20 was first incubated with Cdk1 and ATP, under conditions similar to those described in Fig. 2A, except that the reaction volume was 10 μl, and incubations were carried out at 18 ºC. Cdk1 action was terminated by at the times indicated by the addition of 3 μM $p27^{kip1}$, and then samples were mixed with HeLa cell APC/C (on 1 μl of anti-Cdc27 beads), at 1,400 rpm for 15 min. Finally, ubiquitylation mixture containing ^{125}I-cyclin B (*see Materials and Methods)* was added and incubation was continued at 18 ºC for 30 min, with shaking at 1,400 rpm. *Right panel*, “Cdc20 + APC/C, then Cdk1”: Experimental conditions were similar to those described for left panel, except that the order of incubations was reversed, so that myc-Cdc20 was first mixed with APC/C from HeLa cells for 15 min, then Cdk1 was added for the time periods indicated, followed by ^{125}I-cyclin B ubiquitylation mixture. Results were expressed as in Fig. 2C.

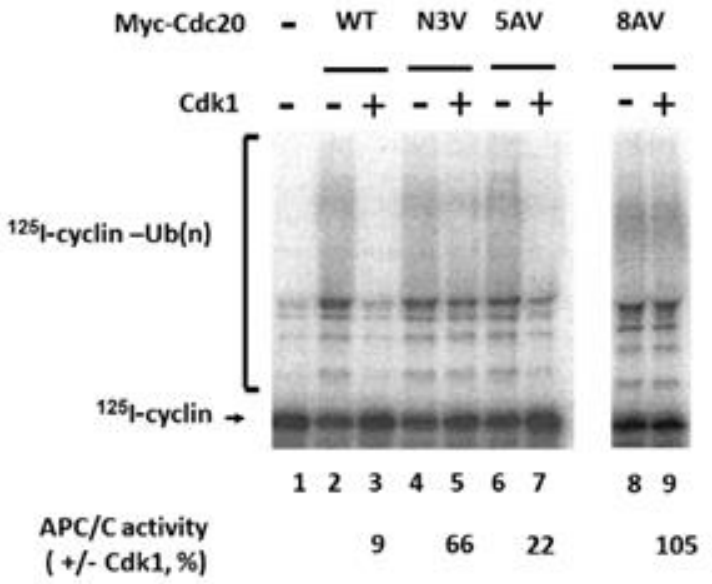

Fig. S3. Effects of Cdk1-catalyzed phosphorylation of different free phosphorylation site mutants of Cdc20 on their action to stimulate the activity of APC/C. Wild type ("WT") myc-Cdc20 or its mutants as indicated, were incubated (23 ºC,30 min) with or without Cdk1, under conditions similar to those described in Fig. 2A, and then were added to immunopurified APC/C on anti-Cdc27 beads, as described under *Materials and Methods*. The activity of APC/C on ubiquitylation of ^{125}I-cyclin B (*see Materials and Methods)* was estimated in samples of 1 μl APC/C beads. The activity of APC/C following treatment of Cdc20 constructs with Cdk1 was expressed as the percentage of respective controls incubated without Cdk1.