Supplemental Materials

FIGURE LEGENDS

Figure S1. S85 phosphorylation by Cdk and requirement of ERK-dependent S85 phosphorylation for MEK-CA-induced Xe-Cdc25A degradation

(A) Activated eggs were injected with either buffer (Control) or 18 ng of $p21^{Cip1}$ mRNA, reinjected 40 min later with 2 ng of Myc-Xe-Cdc25A D231A mRNA, further injected 2.5 h later with 9 ng of MEK-CA mRNA, collected at the indicated times, and analyzed for Myc-Xe-Cdc25A (D231A), phospho-S85, and phospho-ERK by immunoblotting. Note that, even in the presence of $p21^{Cip1}$, the stable D231A mutant of Xe-Cdc25A undergoes continuous S85 phosphorylation after (but not before) ERK activation, indicating that this phosphorylation is ERK-dependent. (B) Activated eggs were injected with 18 ng of $p21^{Cip1}$ mRNA, reinjected 40 min later with 2 ng of either WT or S85A Myc-Xe-Cdc25A mRNA, further injected 2.5 h later with 9 ng of MEK-CA mRNA, and analyzed for Myc-Xe-Cdc25A (WT or S85A) and phospho-ERK by immunoblotting. Note that, even in the presence of $p21^{Cip1}$, the S85A mutant of Xe-Cdc25A is significantly more stable than the WT after ERK activation, indicating that ERK-dependent S85 phosphorylation alone is sufficient for the S85 phosphorylation-dependent degradation of Xe-Cdc25A.

Figure S2. Contribution of both p90rsk and ERK phosphorylations to the MEK-CA-induced degradatiaon of human Cdc25A

Activated eggs were injected with 2 ng of mRNA encoding Myc-human Cdc25A constructs, reinjected 2.5 h later with 9 ng of MEK-CA mRNA, and analyzed by immunoblotting with anti-Myc antibody. In the figure, RXXS:4A (S107/124/178/293A; Busino *et al.*, 2003) is a RXXS motif mutant (of human Cdc25A), corresponding to Xe-Cdc25A RXXS:4A; SP:3A (S40/88/116A; see Figure 3A) is an SP motif mutant corresponding to Xe-Cdc25A SP:4A; 7A is a double mutant of RXXS:4A and SP:3A, corresponding to Xe-Cdc25A 8A; DS/DDG is a double mutant of the DSG motif (S82A; Busino *et al.*, 2003) and the DDG motif (D220A; Kanemori *et al.*, 2005), corresponding to Xe-Cdc25A D231A. Note that both the RXXS:4A and SP:3A mutants are significantly more stable than the WT but not the 7A or DS/DDG

mutants after MEK-CA expression, suggesting that ERK activation can target human Cdc25A for SCF$^{\beta\text{-TrCP}}$-dependent degradation and that this degradation requires both p90rsk and ERK phosphorylations of the phosphatase.

Figure S3. Induction of Xe-Cdc25A degradation by the p38 pathway

Activated eggs were injected with 2 ng of Myc-Xe-Cdc25A mRNA together with or without 9 ng of Flag-tagged *Xenopus* MK2 mRNA, reinjected 2.5 h later with 9 ng of MKK6-CA mRNA, and analyzed for Myc-Xe-Cdc25A, Flag-MK2, and phospho-p38 by immunoblotting. Note that coexpression of MK2 and MKK6-CA can induce more efficient degradtion of Xe-Cdc25A than expression of MKK6-CA alone, indicating that the p38 pathway can target Xe-Cdc25A for degradation via MK2. The mobility upshift of MK2 after MKK6-CA expression is probably due to its phosphorylation (or activation; Reinhardt *et al*., 2007) by p38.

Figure S4. ERK-dependent degradation of Cdc25A in TPA-treated human cells

(A) HeLa cells synchronized at G1/S by double thymidine block were released, treated with 100 nM 12-O-tetradecanoyl-phorbol-13-acetete (TPA) or its solvent DMSO 5 h after the release, and, at the indicated times (after TPA treatment), subjected to immunoblotting for Cdc25A, phospho-ERK, and β-actin (as control). TPA treatment activates the ras/ERK pathway and thereby causes cell-cycle arrest at G2 in mammalian cells (Alblas *et al*., 1998; Dangi *et al*., 2006). Note that Cdc25A is rapidly degraded coincidently with ERK phosphorylation (or activation) in TPA-treated but not control cells. (B) HeLa cells synchronized at G1/S by double thymidine block were released, treated with DMSO or 20 μM U0126 (MEK inhibitor) 4 h after the release, retreated with DMSO or 100 nM TPA 1 h after the U0126 treatment, and 30 min later subjected to immunoblotting. Note that inhibiting ERK activation by the MEK inhibitor U0126 significantly prevented Cdc25A degradation in TPA-treated cells, indicating that ERK activation can induce Cdc25A degradation in human cells.

References

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Alblas, J., Slager-Davidov, R., Steenbergh, P. H., Sussenbach J. S., and Burg B. (1998). The role of MAP kinase in TPA-mediated cell cycle arrest of human breast cancer cells. Oncogene *16*, 131-139.

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Myc-Xe-Cdc25A (D231A)

Control

p21^{Cip1}

+MEK-CA 0 20 40 60 80 100 120 140 0 20 40 60 80 100 120 140 (min)

IB:Myc

IB:pS85

IB:pERK

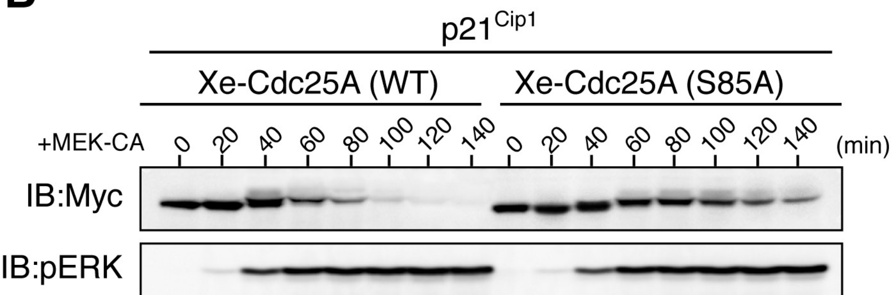


Figure S1

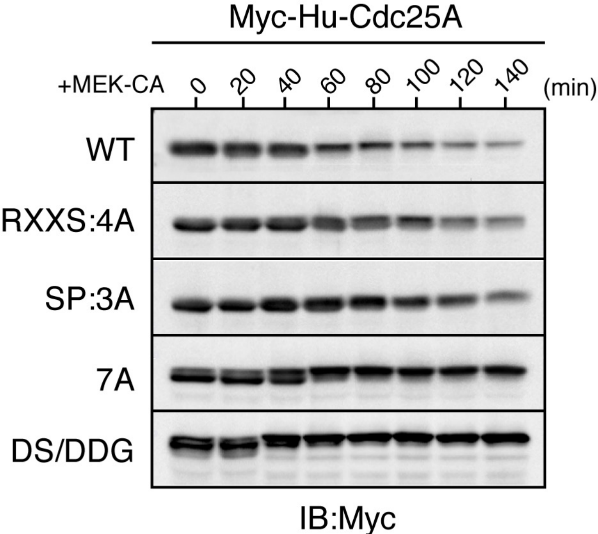


Figure S2

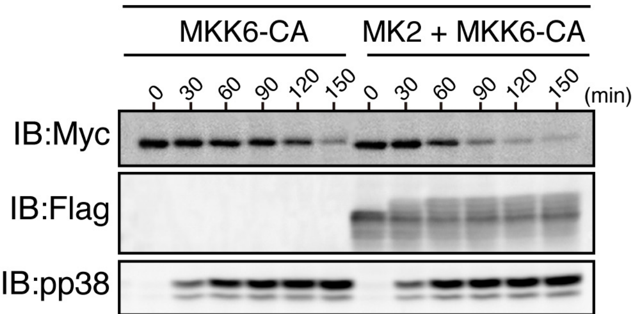
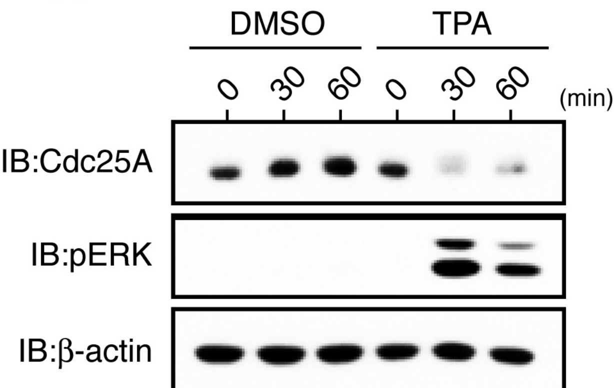


Figure S3



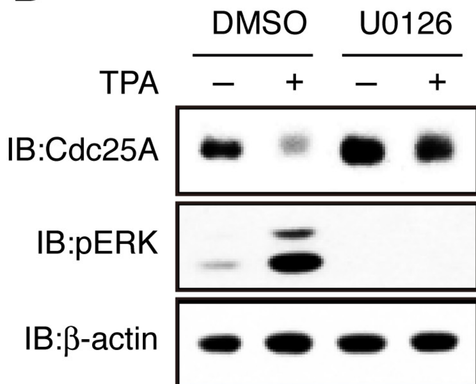


Figure S4