Cdc25A is involved in ciclopirox-induced inhibition of cancer cell proliferation

Tao Shen[1,2], Chaowei Shang[1,2], Hongyu Zhou[1], Yan Luo[1,3], Mansoureh Barzegar[1], Yoshinobu Odaka[1,2], Yang Wu[1,3], Shile Huang[1,2,*]

Supplementary figures

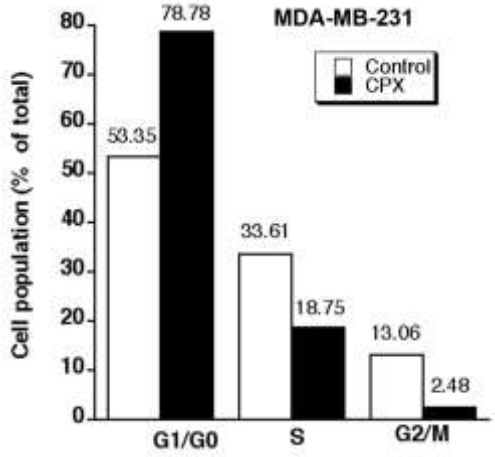

Figure S1: CPX induces accumulation of MDA-MB-231 cells at G_1 phase of the cell cycle. MDA-MB-231 cells were exposed to CPX (0 and 5 μM) for 24 h, followed by cell cycle analysis.

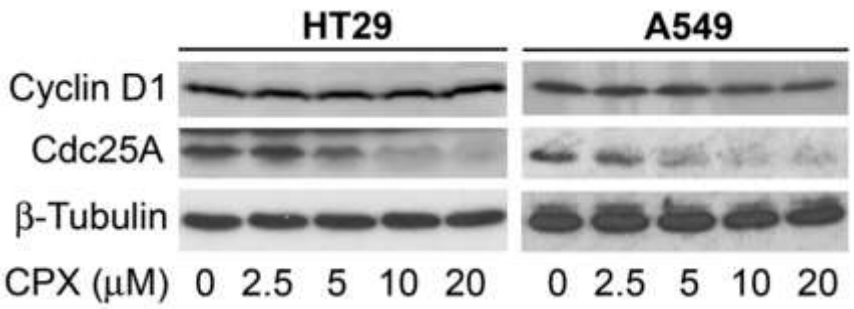

Figure S2: CPX downregulates the cellular protein level of Cdc25A in tumor cells in a concentration-dependent manner. HT29 and A549 cells were treated with CPX (0-20 μM) for 24 h, followed by Western blotting with indicated antibodies.

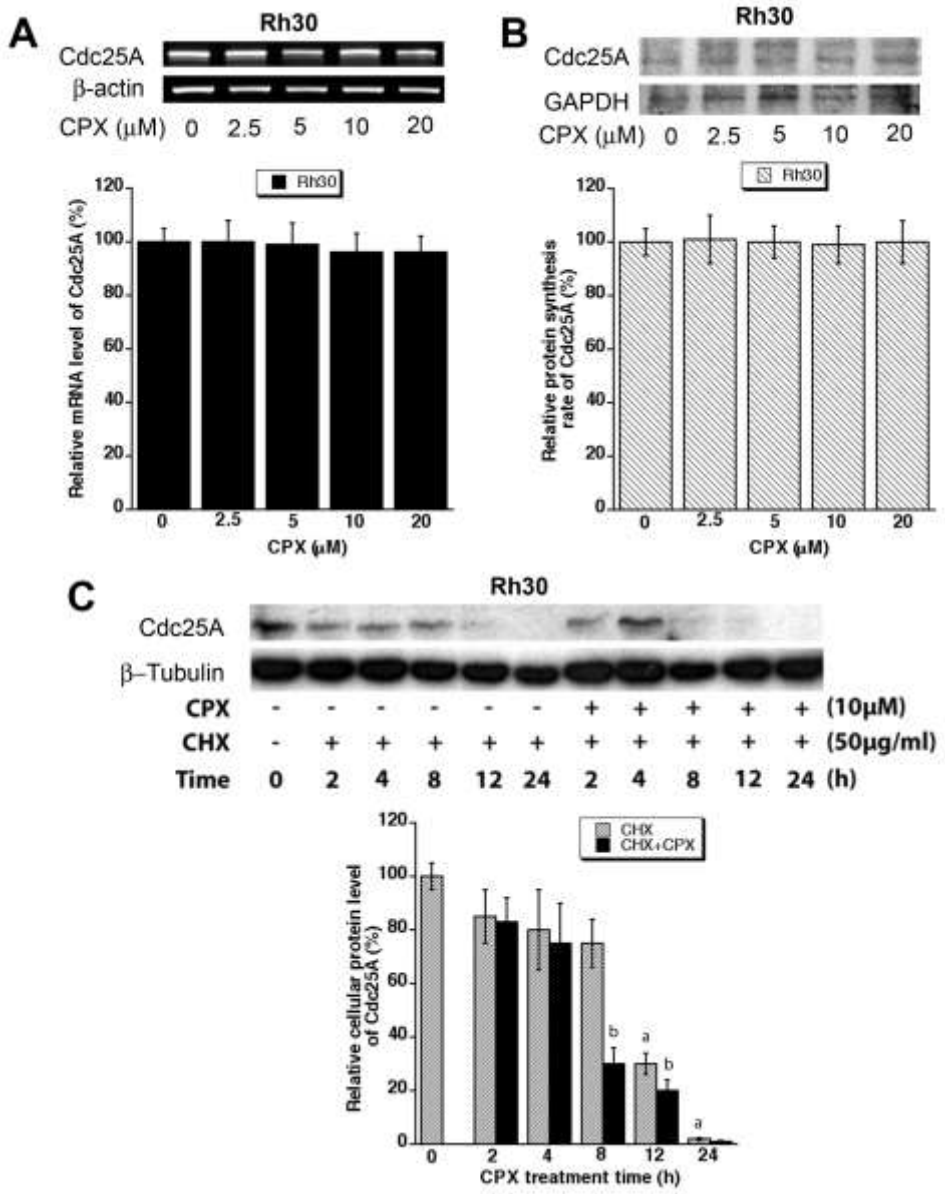

Figure S3: CPX does not reduce Cdc25A mRNA level or protein synthesis significantly, but promotes Cdc25A protein degradation remarkably. (A) Rh30 cells were treated with CPX (0-20 μM) for 24 h. Total mRNA was extracted, followed by RT-PCR. β-actin was used as internal control. (B) Rh30 cells were treated with CPX at 0-20 μM for 30 h, and then pulsed with ^{35}S-Met/Cys for 6 h in the presence of CPX (0-20 μM). The proteins in the cell lysates were subjected to SDS-PAGE and transferred to a PVDF membrane, followed by autoradiography. GAPDH served as a control. (C) Rh30 cells were treated with 50 μg/ml cycloheximide (CHX), in the presence or absence of 10 μM CPX, for 0-24 h, followed by Western blotting with indicated antibodies. β-tubulin was used as a loading control. NIH Image J was used for semi-quantitative analysis of the intensities of the bands. Results are means ± SE and are pooled from three independent experiments. $^{a}P < 0.05$, difference *versus* control group. $^{b}P < 0.05$, difference *versus* CHX group.

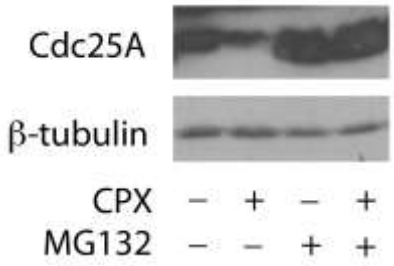

Figure S4: Inhibition of proteasome with MG132 prevents CPX from reducing Cdc25A protein level. MDA-MB-231 cells were treated with or without CPX (10 μM) for 24 h, in the presence or absence of MG132 (10 μM), followed by Western blotting with indicated antibodies.

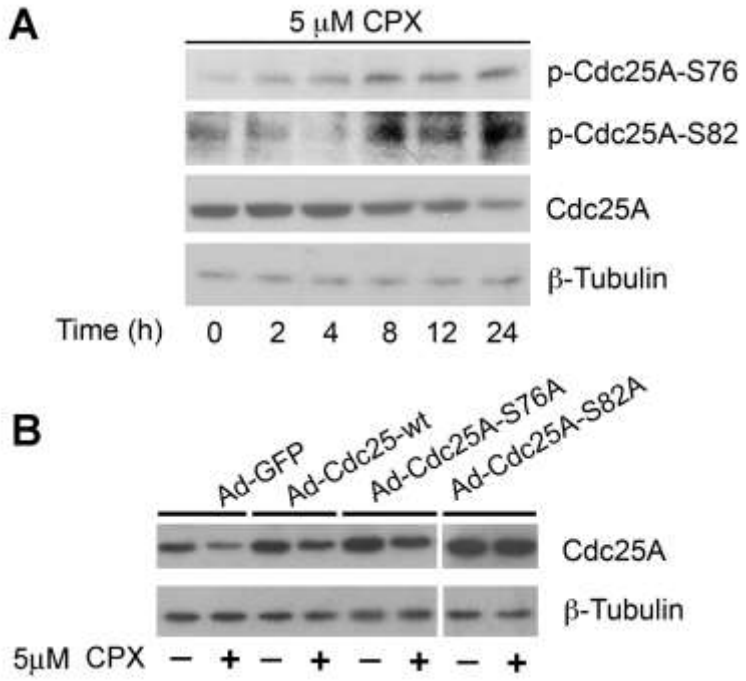

Figure S5: CPX-induced Cdc25A phosphorylation contributes to its degradation. (A) MDA-MB-231 cells were treated with CPX (5 μM) for 0-24 h, followed by Western blotting with indicated antibodies. (B) MDA-MB-231 cells, infected with indicated recombinant adenoviruses, were treated with or without CPX (5 μM) for 24 h, followed by Western blotting with indicated antibodies. β-tubulin was used as a loading control.

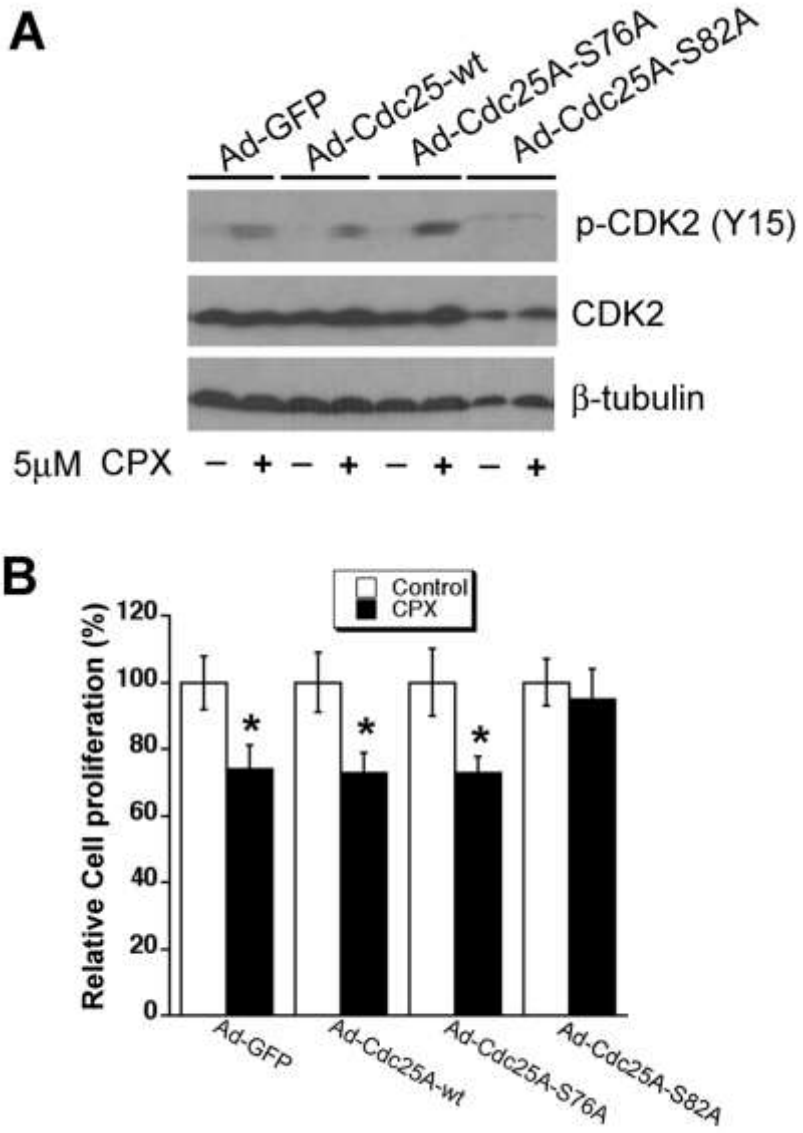

Figure S6: Ectopic expression of Cdc25A mutant (S82A) renders resistance to CPX inhibition of cell proliferation. (A, B) MDA-MB-231 cells, infected with indicated recombinant adenoviruses, were treated with 5 μM of CPX for 24 h, followed by Western blotting with indicated antibodies (A), and MTS assay (B). All data represent the means ± SE (n=3). **P*< 0.05.