Supporting Information

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SI Materials and Methods

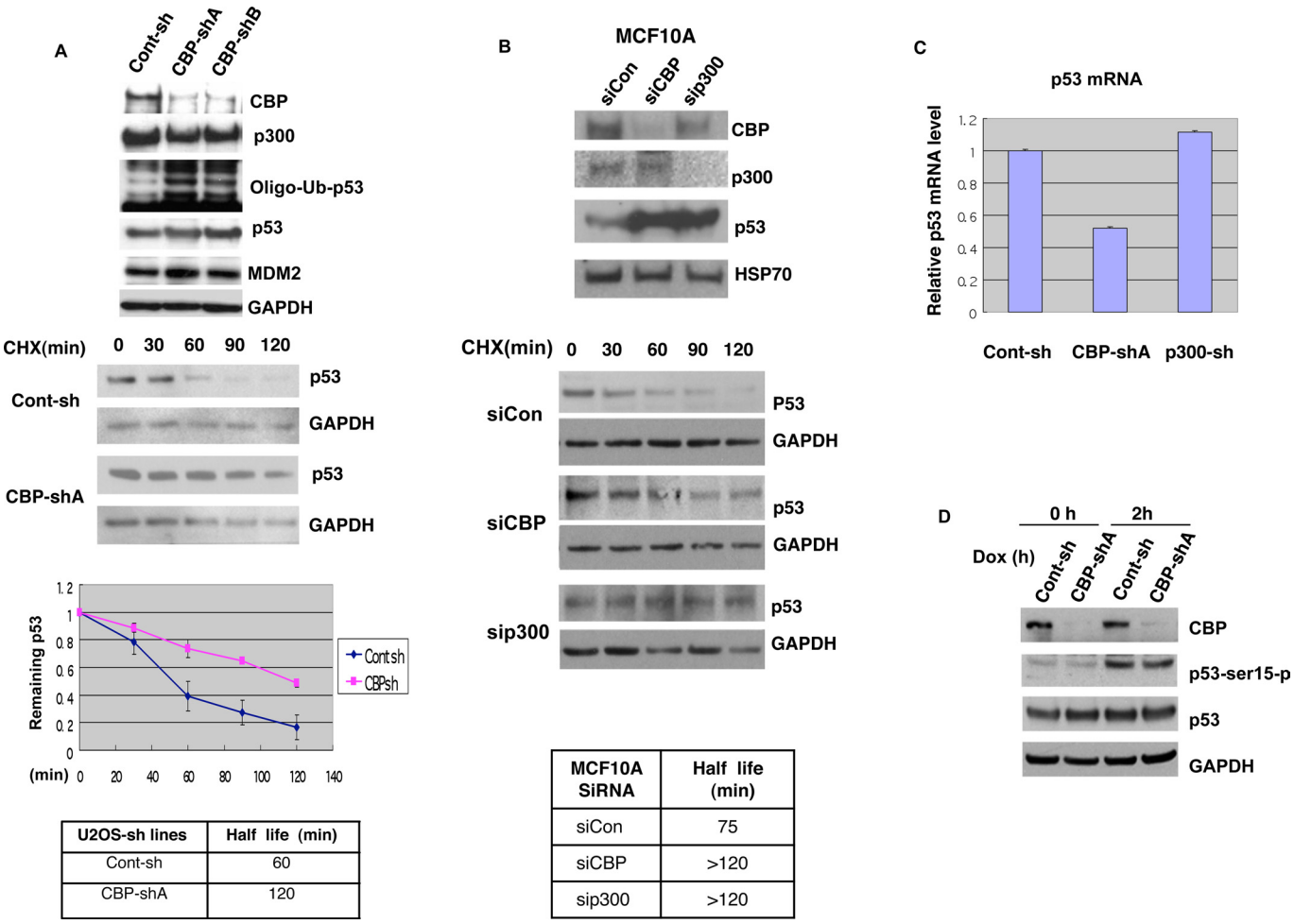
Generation of Knockdown Cell Lines. Stable hairpin RNA (shRNA)-expressing cell lines were generated using the following shRNA target sequences: CATAAACAACTGTCGGAGC (CBP-shA), TAGTAACTCTGGCCATAGC (CBP-shB), TCATTTCACACTGGAAGAA (p300-sh). The CBP short hairpin oligonucleotides were cloned into pSuperior.puro (Oligoengine) using *Bgl*II/*Xho*I cloning sites. After transfection of the hairpin constructs, the cells were subjected to selection with 1 μg/mL puromycin (AG Scientific). Independent clones were selected and evaluated for silencing by immunoblot.

Antibodies. αp53-DO1 (blotting), αp53-FL393R (IP), αactin C-2, αUb P4D1, αCBP A22, αHSP70 W27, αMDM2 N-20 (blotting, Fig. S1*A*), αMDM2 D-7 (IP) (all Santa Cruz Biotechnology), αMDM2 Ab-5 (blotting, Fig. S4*B*) (Calbiochem), αGAPDH 6C5 (Advanced Immunochemical), αmyc-tag 4A6, αp300 RW128 (Upstate), αRb (BD PharMingen), and αphospho-p53 (Ser-15; Cell Signaling).

siRNA Transfection. siRNA duplex corresponding to CBP (forward: 5′-AAUCCACAGUACCGAGAAAUGUU-3′; reverse: 5′-CAUUUCUCGGUACUGUGGAUUUU-3′), p300 (forward: 5′-CAGAGCAGUCCUGGAUUAGtt-3′; reverse: 5′-CUAAUCCAGGACUGCUCUGtt-3′), and Control (siGFP) were synthesized by Dharmacon. U2OS cells (1×10^7) were transfected with 0.2 pmol siRNA using Oligofectamine (Invitrogen). Seventy-two hours after transfection, cells were harvested and analyzed for the expression level of CBP, p300, GAPDH, and p53 by western blotting.

Ni-NTA Pulldown of His-Tagged Ub Conjugates. Cells were washed with ice-cold PBS and lysed in 7 mL guanidinium lysis buffer (6 M guanidinium-HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, 0.01 M Tris-HCl, pH 8.0, 5 mM imidazole, 10 mM β-mercaptoethanol). HisLink Protein Purification Resin (75 μL; Promega) was added to the lysate, and the mixture was incubated by end-over-end rotation at 4 °C for 16 h. The beads were successively washed with guanidinium buffer (6 M guanidinium-HCl, 0.1 M Na_2HPO_4/NaH_2PO_4, 10 mM Tris-HCl, pH 8.0, 10 mM β-mercaptoethanol), buffer A (8 M urea, 0.1 M Na_2HPO_4/NaH_2PO_4, 10 mM Tris-HCl, pH 6.3, 10 mM β-mercaptoethanol), buffer A plus 0.2% Triton X-100, and buffer A plus 0.1% Triton X-100. Ubiquitinated proteins were eluted with 200 mM imidazole in Ubiquitin Elution Buffer (5% SDS, 30% glycerol, 0.72 M β-mercaptoethanol, 0.15 M Tris-HCl, pH 6.7). The eluate was supplemented with NuPAGE LDS sample buffer and subjected to SDS-PAGE. The proteins were transferred to a nitrocellulose membrane and probed with the anti-p53 antibody DO-1 to detect ubiquitinated p53.

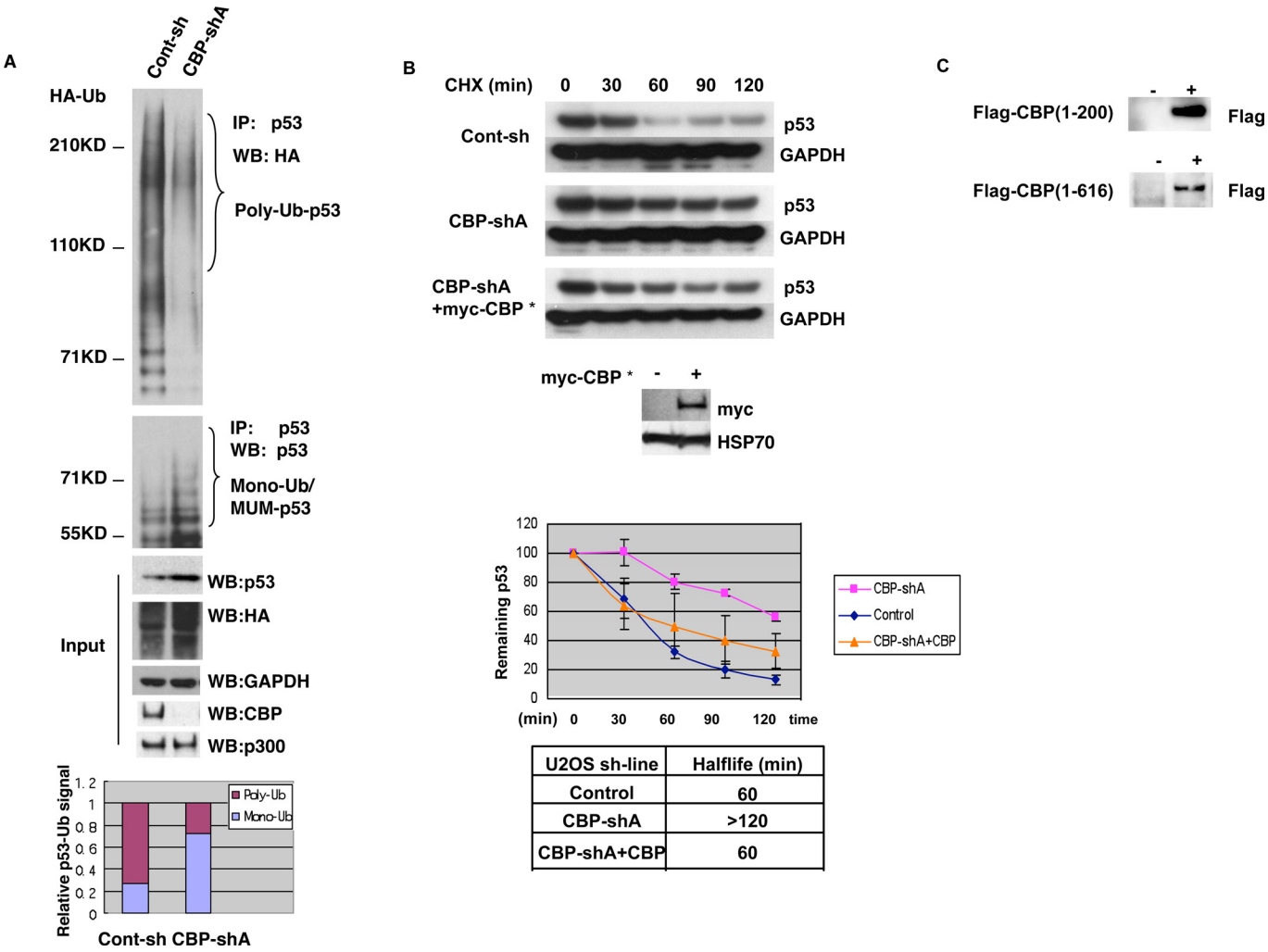
Subcellular Fractionation. Cytoplasmic extracts were prepared by Dounce homogenization of cells (Type A pestle, 40 strokes) using low salt buffer (10 mM HEPES, pH 7.5, 10 mM NaCl, 1 mM $MgCl_2$, 15% glycerol supplemented with fresh 1 mM DTT, 5 mM NEM, and protease inhibitors), followed by low speed pelleting of nuclei (1,000 RPM for 5 min). The supernatant of the low speed spin was centrifuged at high speed (14,000 RPM for 10 min) to remove membranes, and the supernatant was removed for use as cytoplasm. Nuclei pelleted from the low speed spin were washed three times in low salt buffer and then extracted with high salt buffer (10 mM HEPES, pH 7.5, 150 mM NaCl, 150 mM KCl, 1 mM $MgCl_2$, 0.5% Triton X-100 supplemented with fresh 5 mM NEM and protease inhibitors) for 30 min at 4 °C. The nuclear extract was then clarified by centrifugation (14,000 RPM for 15 min).



U2OS-sh lines	Half life (min)
Cont-sh	60
CBP-shA	120

MCF10A SiRNA	Half life (min)
siCon	75
siCBP	>120
sip300	>120

Fig. S1. (*A*) (Top) p53 abundance in CBP stably deficient U2OS cells. Lysates of drug-selected clones of U2OS cells harboring the indicated control (empty vector) or CBP shRNAs were analyzed by immunoblotting with anti-CBP, anti-p53, anti-MDM2, and anti-GAPDH antibodies. (Bottom three panels) The indicated shRNA-expressing cell lines were treated with cycloheximide and lysates harvested at the indicated times and analyzed by p53 immunoblot. p53 levels were quantitated by densitometry, and half-life calculated based on decay of normalized (to GAPDH loading control) p53 levels to 50% of their original level. Values are an average of three independent experiments. Error bars, ±1 S.D. (*B*) Effect of CBP or p300 siRNA on p53 stability in MCF10A breast epithelial cells. MCF10A cells transiently transfected with the indicated siRNAs for 72 h were treated with cycloheximide and lysates harvested at the indicated times. (Top) The lysates from the time 0 min of the cycloheximide treatment were blotted with CBP, p300, p53, and HSP70 antibodies. (Middle) Lysates from the cycloheximide time course were analyzed by p53 immunoblot. (Bottom) p53 half-life was calculated as in Fig. S1*A*. (*C*) p53 mRNA level in CBP and p300-deficient U2OS cells. p53 mRNA abundance in control, CBP-shA, and p300-sh U2OS cells was assessed by QRT-PCR. Each sample was internally standardized to GAPDH. Relative expression levels (with standard errors of the mean) are presented. (*D*) p53-ser 15 phosphorylation in CBP-shA cells. Mock or Dox (2 μM) treated control-sh or CBP-shA cells were immunoblotted for p53 phosphoserine 15, total p53, CBP, and HSP70 (loading control) levels.



U2OS sh-line	Halflife (min)
Control	60
CBP-shA	>120
CBP-shA+CBP	60

Fig. S2. (*A*) CBP depletion modulates the pattern of p53 ubiquitination. Lysates of control-sh or CBP-shA cells expressing HA-Ub were immunoprecipitated with anti-p53 antibody, and the IPs and input lysates immunoblotted with the indicated antibodies. The anti-HA (Ub) blot is preferentially sensitive for polyubiquitinated species due to epitope density, and the anti-p53 blot is preferentially sensitive for mono-Ub/MUM species. The abundance of polyubiquitinated and mono-Ub/MUM species were quantitated by densitometry and plotted as a percentage of total ubiquitinated species from each cell line. (*B*) Rescue of p53 instability by CBP in CBP-sh cells. (Top) The indicated shRNA-expressing cell lines were treated with cycloheximide, and lysates harvested at the indicated times and analyzed by p53 immunoblot. CBP* indicates that a silently mutated shRNA-resistant CBP cDNA was transfected into CBP-shA cells 48 h before cycloheximide treatment. (Middle) The expression of myc-tagged CBP* was evaluated by anti-myc immunoblot of the time = 0 lysate. (Bottom) p53 abundance was quantitated at each time point, and t1/2 calculated after normalization for loading. (*C*) Expression of the indicated truncated FLAG-tagged CBP alleles used in Fig. 2*A* was confirmed by anti-FLAG immunoblot.

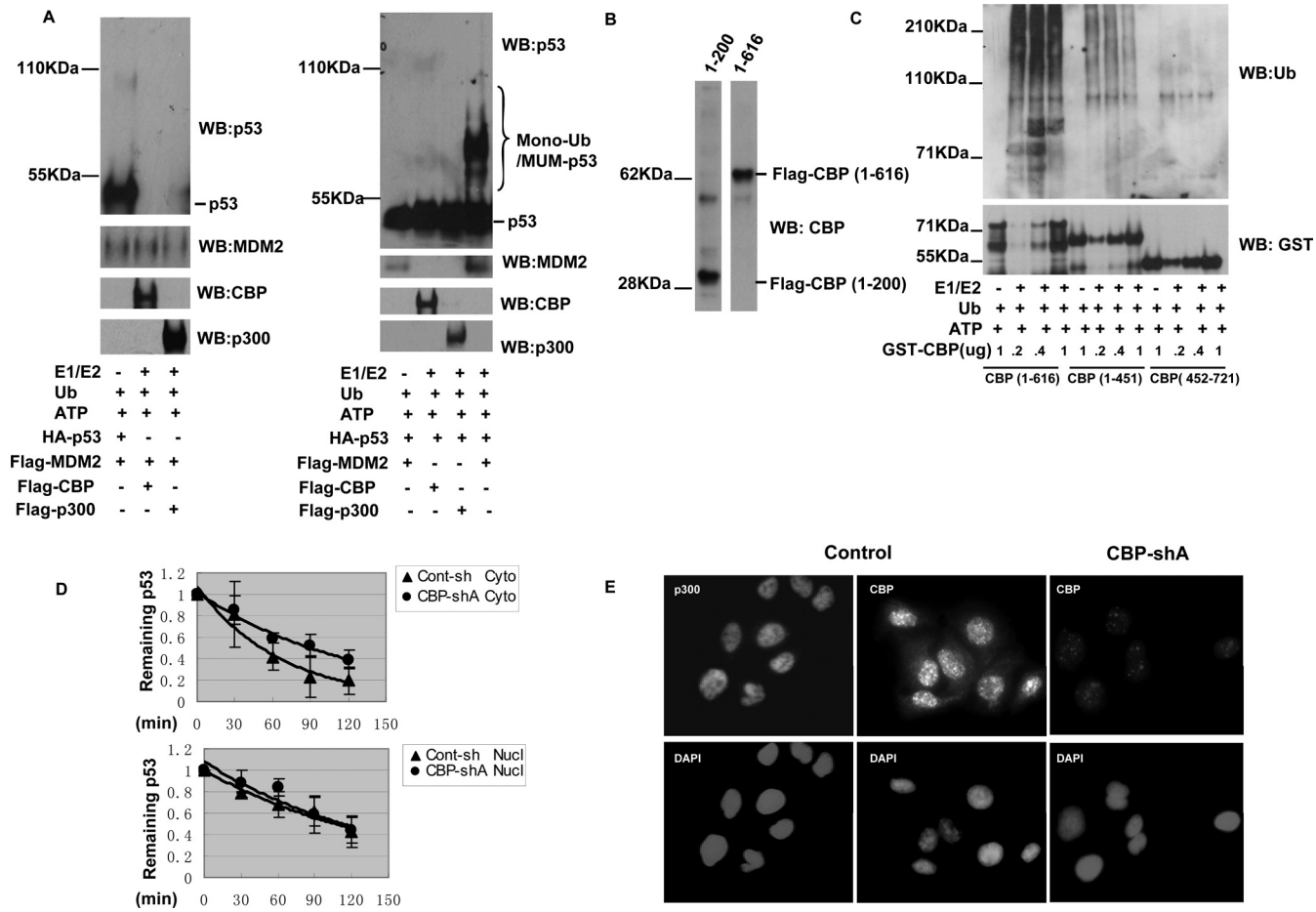


Fig. S3. (*A*) Control experiments for p53 ubiquitination (E4) assay in Fig. 2*B*. (Left) Purified HA-p53, FLAG-MDM2, FLAG-CBP, or FLAG-p300 as indicated were incubated in ubiquitination reactions containing the indicated components, and products were analyzed by western blot with p53 antibody. (Right) Purified HA-p53, FLAG-MDM2, FLAG-CBP, or FLAG-p300 were added as indicated to the indicated components, followed by additional incubation and western blot of the reaction with anti-p53 antibody. (*B*) Immunoblot of purified CBP polypeptides used in the two-step E4 assay in Fig. 2*D*. pN8.FLAG-CBP (1–616) and (1–200) were transfected into U2OS cells, and FLAG-CBP (1–616) and (1–200) polypeptides were purified from cell lysates by FLAG affinity chromatography, followed by immunoblotting of the purified eluted proteins with anti-CBP antibody. (*C*) The CBP N terminus harbors intrinsic E3 activity. (Upper) Purified GST-CBP fusion polypeptides were incubated with E1, ubch5a, ATP, and Ub for 1 h at 37 °C followed by immunoblotting of the reaction products for Ub. (Lower) The immunoblot was reprobed with anti-GST antibody to reveal the abundance of the GST-CBP fusion proteins added to each reaction. (*D*) Regulation of cytoplasmic vs. nuclear p53 half-life by CBP. Decay curves of p53 abundance for the CHX-decay assay of nuclear vs. cytoplasmic p53 in control-sh vs. CBP-shA cells described in Fig. 3*B*. Error bars, ±1 S.D. (*E*) Immunofluorescence analysis of p300 and CBP subcellular localization. Control or CBP-shA cells were stained with DAPI or antibodies against CBP or p300, followed by FITC-conjugated anti-IgG. Photomicrographs of p300 and CBP staining in all panels were taken at the same magnification (600×) and exposure.

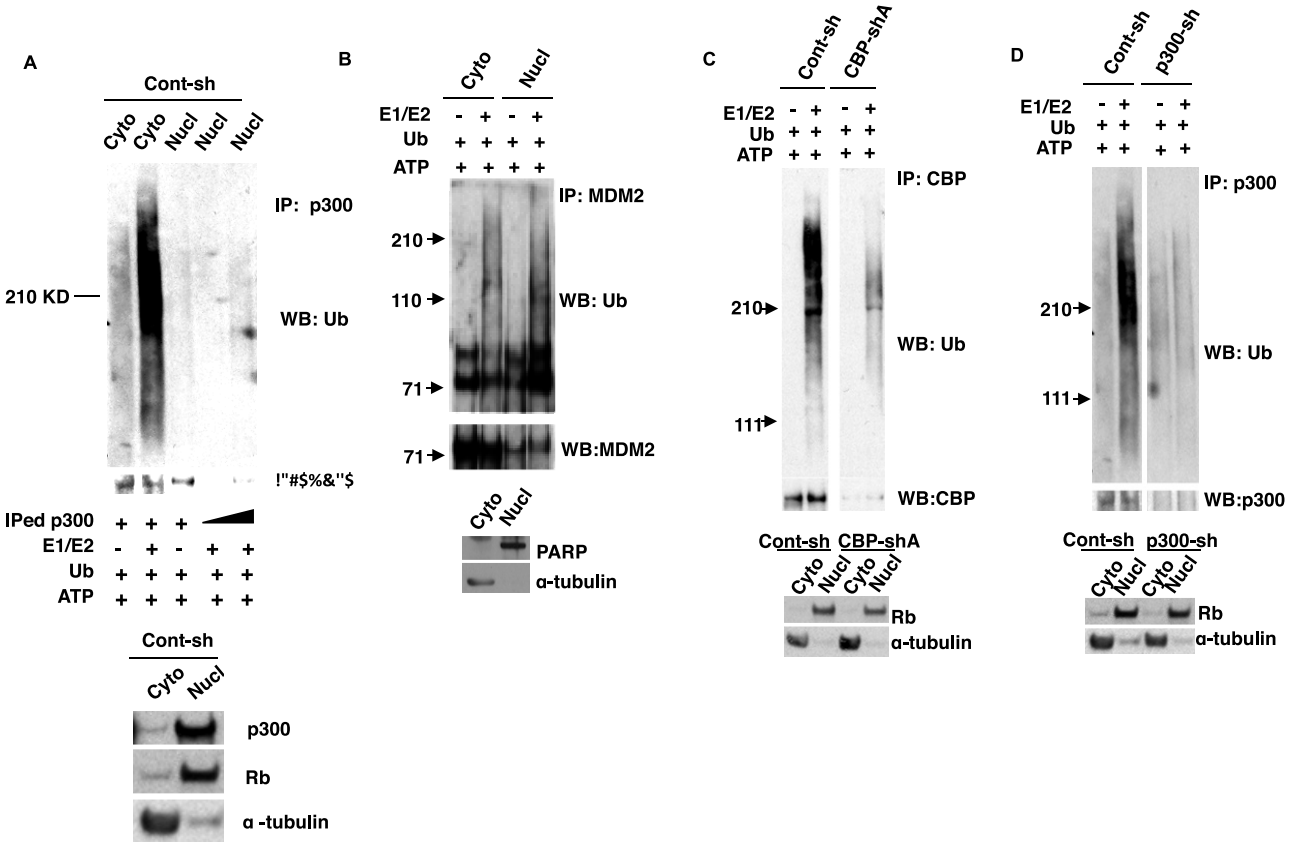


Fig. S4. (*A*) Cytoplasmic p300 is associated with E3 ligase activity. (Upper) Nuclear and cytoplasmic fractions of control-sh U2OS cells were immunoprecipitated with anti-p300 antibody, and the washed IPs were incubated with E1/E2, Ub, and ATP as indicated, followed by Ub and p300 immunoblotting of the reactions. (Lower) Immunoblot of p300, Rb (nuclear marker), and α-tubulin (cytosolic marker) in the nuclear and cytoplamic fractions of control-sh cells. (*B*) Nuclear and cytoplasmic MDM2 E3 activity. Cytoplasmic and nuclear fractions were IP'd with anti-MDM2 antibody, and the IPs incubated with E1/E2, Ub, and ATP, followed by anti-Ub (top) or anti-MDM2 (middle) immunoblotting of the reactions. The subcellular fractions were immunoblotted with anti-tubulin and PARP antibodies (bottom) to confirm the lack of cross-contamination of the fractions. (*C* and *D*) (Top and middle) Cytoplasmic fractions of control, CBP-shA, or p300-sh cells as indicated were immunoprecipitated with anti-p300 or anti-CBP antibodies as indicated. After washing, the IPs were assayed for E3 activity by incubating with E1, E2, ATP, and ubiquitin as indicated, followed by anti-CBP, p300, and ubiquitin immunoblotting. Note: For the purpose of clarity, a duplicate exposure of the 1st 2 lanes of the blot in *A* are provided as the 1st two lanes of *D*, and the first two and last two lanes of *C* and *D* are derived from the same blots with intervening irrelevant lanes removed. (Bottom) Cytoplasmic fractions of the indicated cell lines were immunoblotted with anti-Rb (nuclear marker) and anti-tubulin (cytoplasmic marker) antibodies.