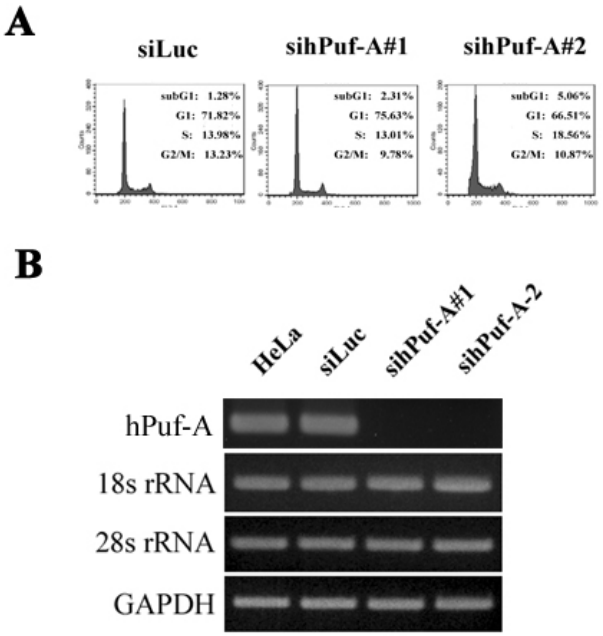
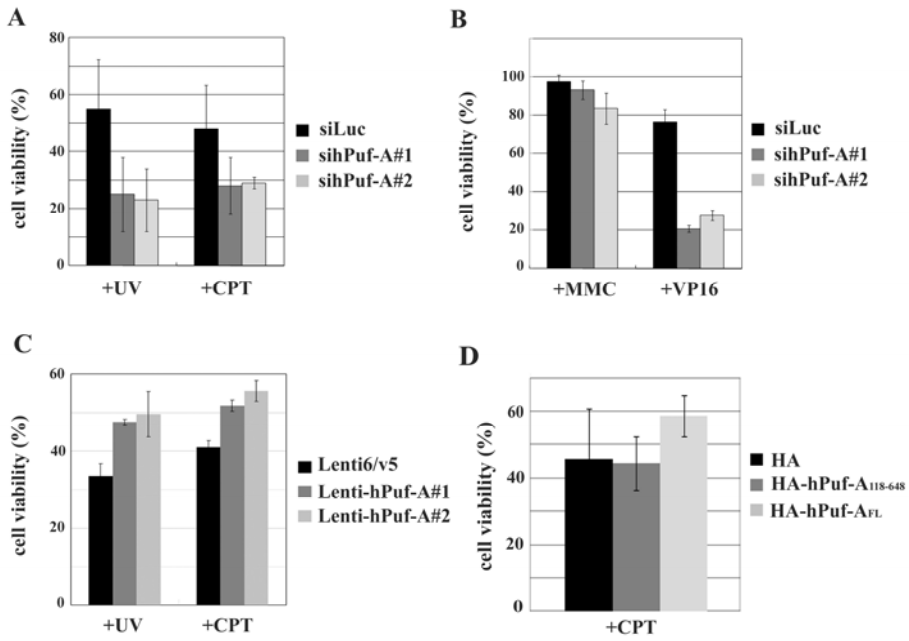
Drug	Function	Dose	Nucleolus → nucleoplasm
Actinomycin D (ActD)	RNA pol I inhibitor	1 μg/ml	Yes (+++)
5,6-dichlorobenzimidazole riboside (DRB)	RNA pol II inhibitor	2 μg/ml	Yes (++)
Camptothecin (CPT)	Topoisomerase I inhibitor	10 nM	Yes (++)
Topotecan hydrochloride	Topoisomerase I inhibitor	10 μg/ml	Yes (++)
Etoposide (VP16)	Topoisomerase II inhibitor	125 μM	Yes (++)
Amsacrine hydrochloride	Topoisomerase II inhibitor	10 μg/ml	Yes (++)
UV light	T-T dimer, 6-4 photoproducts	30 J/m2	Yes (+)
4-Nitroquinoline N-oxide (4-NQO)	PARP-1 activating agent	3 μg/ml	Yes (+)
Mitomycin C (MMC)	DNA intrastrand crosslinker	1 mg/ml	No
Wortmannin	PI3K inhibitor	0.3 μg/ml	No
X- ray	Double strand break	10 Gy	No
MG132	Proteasome inhibitor	10 μM	No

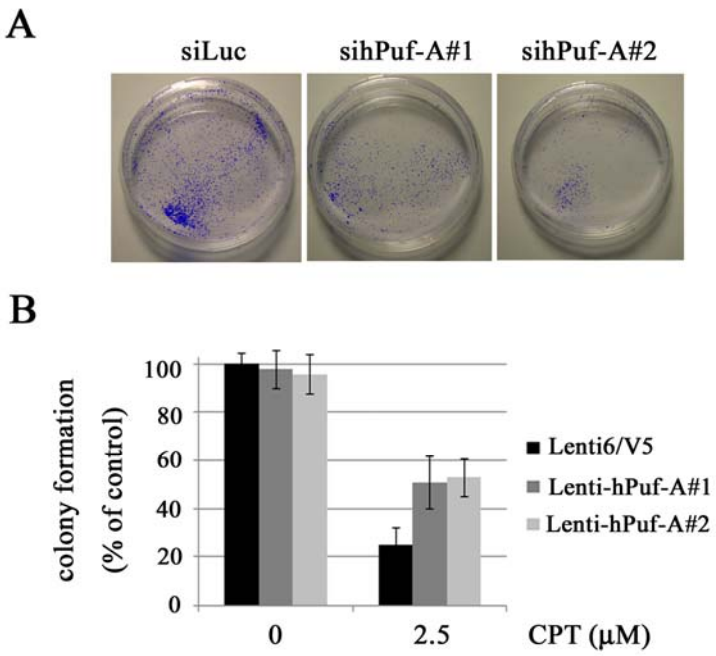
Supplementary Table S1. Nucleolar loss of hPuf-A at 2 h post-drug treatment. "+++" indicates over 95% of HeLa cells with hPuf-A signals appeared in the nucleoplasm. "++" indicates approximate 60% of HeLa cells with hPuf-A signals appeared in the nucleoplasm. "+" indicates less than 30% of HeLa cells with hPuf-A signals appeared in the nucleoplasm.



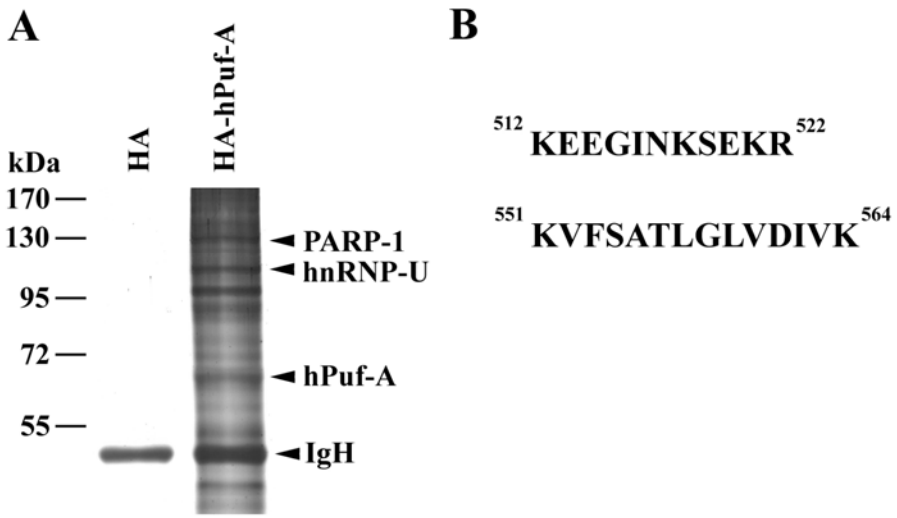
Supplementary Fig. S1. (A) HeLa cells were transfected with siRNA duplexes and exposed to CPT (5 μM) for 24 h. DNA content was determined by flow cytometry. (B) HeLa cells were transfected with siRNA duplexes and total RNAs were isolated for the first strand cDNA synthesis using random-primers. RT-PCR was carried out with the indicated primers.



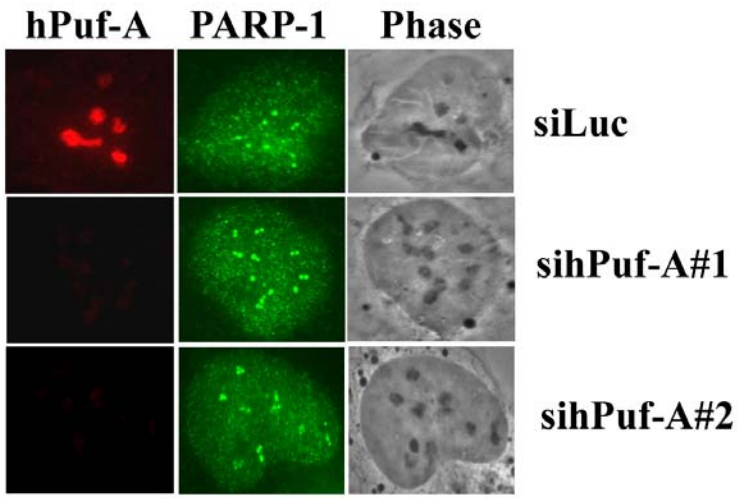
Supplementary Fig. S2. (A) HeLa cells were transfected with siRNA duplexes for 48 h and exposed to UV (20J/m^2) and CPT (5 μM) for another 24 h. Cell viability was determined by MTT assay. (B) HeLa cells were transfected with siRNA duplexes for 48 h and exposed to MMC (1 mg/ml) and VP16 (100 μM) for another 24 h. Cell viability was determined by MTT assay. (C) Control and hPuf-A stably expressing U2OS cells were either irradiated with UV or treated with CPT for 24 h and cell viability was determined by MTT assay. These data were carried out by three independent experiments. (D) U2OS cells were transfected with HA vector, HA-hPuf-$A_{119-648}$, and HA-hPuf-A_{FL} for 24 h and treated with CPT for another 24 h. Cell viability was determined by MTT assay.



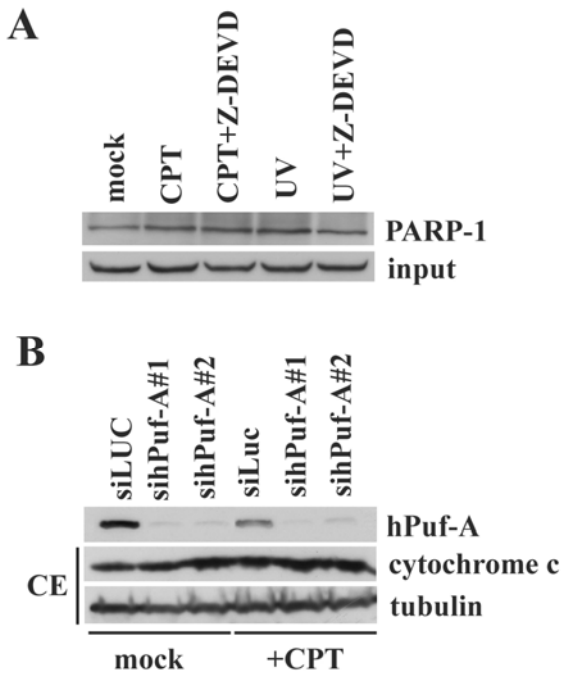
Supplementary Fig. S3. (A) HeLa cells were transfected with siRNA duplexes for 48 h and exposed to CPT (5 μM) for another 24 h. Cells were stained with 0.25% crystal violet at 48 h post CPT treatment. (B) Control and hPuf-A overexpressing U2OS cells were treated with CPT for 24 h and colonies were stained with 0.25% crystal violet at 10 days post CPT treatment. Survival colonies were counted and expressed as a percentage of the untreated control cells. Bar: standard error.



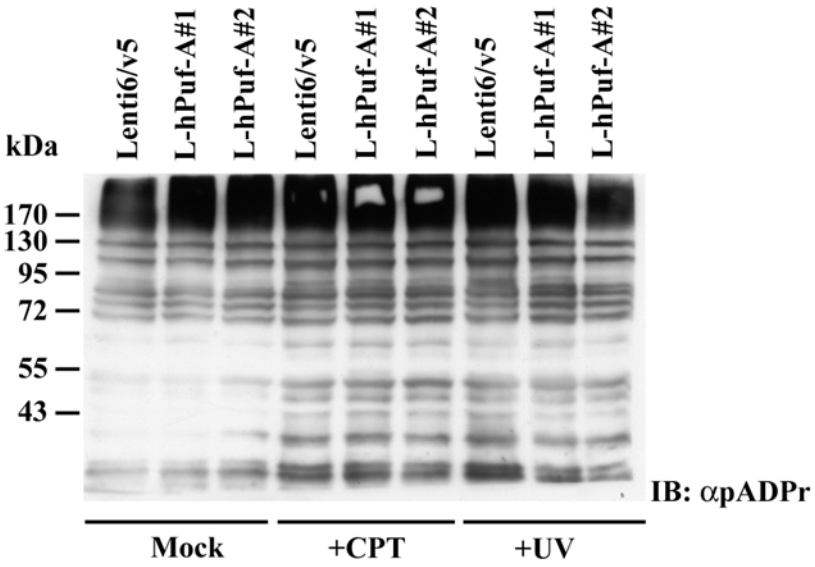
Supplementary Fig. S4. (A) HA-hPuf-A was transfected into HEK293T cells for 48 h and cell extracts were immunoprecipitated by anti-HA agarose. Silver staining was carried out to show potential hPuf-A associated proteins. (B) Two tryptic peptides from Mass spectrometry analysis were corresponding to fragments of human poly(ADP-ribose) polymerase 1.



Supplementary Fig. S5. U2OS cells were transfected with siRNA duplexes for 72 h. and labeled with a mixture of anti-hPuf-A mAb (red) and anti-PARP-1 polyclonal antibody (green). Phase contrast micrograph shows the same field as the fluorescently labeled cell.



Supplementary Fig. S6. (A) HeLa cells were treated with CPT or UV in addition of Z-DEVD-fmk, a caspase-3 inhibitor, for 4 h and cell extracts were immunoprecipitated with anti-hPuf-A antibody. Immunoblotting was carried out to detect PARP-1. (B) HeLa cells were transfected with siRNA duplexes for 72 h and then treated with CPT for another 4 h. Cytosolic fraction (CE) was isolated and immunoblotted with anti-cytochrome c antibody. α-tubulin was used as a loading control.



Supplementary Fig. S7. Control and hPuf-A overexpressing cells were treated with UV or CPT for 2h. Total cell extracts were collected and immunoblotted with anti-pADPr antibody.

Supplemental Materials and Methods

Lentivirus production. The packaging vector pCMVdeltaR8.91, the vesicular stomatitis virus G protein (VSV-G) envelope glycoprotein–expressing vector pMD.G, and the full-length of hPuf-A in the pLenti6/V5-D-TOPO vector (Invitrogen) were co-transfected into HEK 293T cells. After 48 h incubation, the supernatant medium was centrifuged at 25,000xg for 2 h to collect viral particles. Polybrene (8 g/ml) was added to facilitate viral infection in U2OS cells. Stable clones were selected using blasticidine (Invitrogen) for 21 days.

Immunofluorescence staining. Cells grown on coverslips were fixed with 4% paraformaldehyde (PFA) in PBS (pH 7.2) for 15 min at room temperature and permeabilized in PBS containing 0.5% Triton X-100 for 3 min. Immunostained images were visualized and recorded using a Leica confocal laser scanning microscope (Leica, Germany).

Immunoprecipitation. Cells grown on 6 cm plates were lysed in 300 µl of lysis buffer (1% Triton X-100, 1% NP-40, 150 mM NaCl, 25 mM Tris-HCl pH 8.0, and protease inhibitors), sonicated for 5 min, and spun down at 13,000 rpm for 15 min. Immunoprecipitation was conducted at 4℃ for 6 h and extensively washed with 500 µl of lysis buffer. Protein A beads was incubated with 100 µl of 2x SDS sample buffer and boiled at 95℃ for 5 min.

siRNA transfection. hPuf-A siRNA duplex (final concentration 20 nM) in Opti-MEM medium was incubated with RNAiMax lipofectamine (Invitrogen) at room temp for 20 min. Cells were washed with PBS once and incubated in serum-free culture medium. a siRNA-RNAiMax complex was dropped into cells and mix gently by rocking the plate back and forth. Fetal bovine serum was added at 8 h post-transfection.

Isolation of chromatin-associated proteins. Nuclei from $1x10^8$ cells were resuspended in buffer A (10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM $MgCl_2$, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 0.1% Triton X-100, and protease inhibitors plus 0.2 units of micrococcal nuclease (Sigma-Aldrich). After incubation at 37^0C for 1 minute, nuclei were collected and lysed in 1 mL of buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, and protease inhibitors). Insoluble chromatin was collected by

centrifugation (5 minutes, 2,500 rpm, 4^0C) and resuspended in 0.5 mL Laemmli buffer, sonicated for 5 minutes, and spun down to collect chromatin-associated proteins.