Supplemental Information

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SI Materials and Methods

Plasmids and antibodies. To generate Iduna's mutant plasmids, site-directed mutagenesis were carried out using the QuickChange change site-directed mutagenesis kit (Stratagene) with following primers; C60A Forward 5'-GTT TTC TGT TAT CTG GCT GTA AAG GGT GCT T-3', C60A Reverse 5'-AAG CAC CCT TTA CAG CCA GAT AAC AGA AAA C-3', H54A Forward 5'-AGT CTG CCC TGT AAG GCT GTT TTC TGT TAT CTG-3' and H54A Reverse 5'-CAG ATA ACA GAA AAC AGC CTT ACA GGG CAG ACT-3. All mutation sites were confirmed by DNA sequencing analysis. TAP-Iduna plasmid was constructed by insertion of full-length Iduna cDNA into the *EcoR*I and *Xho*I site on pNTAPB vector. All other plasmids are as previously reported (1). Antibodies used for immunoblot analysis were as follows: anti-Nucleolin from Novus Biologicals; anti-ATP synthase subunit α and anti-PARP1 from BD Biosciences; anti-GFP, anti-importin 7, anti-H1.2 and anti-KU70/86 from Abcam; anti-CBP from, anti-DNA ligase III, anti-H3 and anti-γH2AX from Upstate Biotech; anti-XRCC-1 and anti-actin-HRP from Sigma; Anti-ubiquitin from DAKO; secondary antibody conjugated HRP or fluorescence from Jackson Lab. Anti-Paris (clone N196/16) and anti-Iduna (clone N201/35) from NeuroMab; Anti-GST, anti-PAR and anti-Iduna antibody were previously described (1, 2).

Lentiviral preparations for overexpression. Invitrogen ViraPower lentiviral packaging system was employed for high-titer viral preparations for effective transduction. All lentiviral particles were prepared as previously described (1). Briefly, lentiviral vectors were transfected into HEK 293FT cells along with viral packaging plasmids using calcium phosphate method (1). After 12 h, cells were shocked with 10% DMSO in PBS for 2 minute thereafter cells were further incubated during 18 h with fresh medium. Viral particles were precipitated by centrifugation at 25,000 g for 3 h. Pellets were dissolved with serum free medium and stored at -80°C.

Cell culture and establishment of stably overexperessing and RNAi mediated knock down cell lines. Both HEK 293FT (Human embryonic kidney cell line) and MCF7 (Human breast cancer cell line) cells were purchased from American Type Culture Collection (ATCC), and were cultured in Dulbecco's modified Eagle's medium (DMEM) and Earle's minimal essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 50 units/ml penicillin, and 50 μg/ml streptomycin, respectively. MCF7 stable cells expressing GFP, GFP-Iduna, GFP-Iduna C60A and GFP-Iduna YRAA, were established by infection using each lentiviral particles. All stably overexpressed cell lines were maintained in complete medium. To generate Iduna knockdown MCF7 cells, five RNAi TRC clones were purchased from Open Biosystem.

TRCN0000033979/1E4 (Human) 5'-CCGGCCTGTGAGATGTTTGATATTACTCGAGTAATATCAAACATCTCACAGGTTTTTG-3'

TRCN0000033980/1E5 (Human and Mouse) 5'-
CCGGGCAGGAAGATTAAGCGAGATACTCGAGTATCTCGCTTAATCTTCCTGCT
TTTTG-3'
TRCN0000033981/1E7 (Human) 5'-
CCGGCCTGTTCTAATACTGCACCTTCTCGAGAAGGTGCAGTATTAGAACAGGT
TTTTG-3'

TRCN0000033982/1E8 (Human and Mouse) 5'-
CCGGGCCAGTAGTGATAGTGAGGATCTCGAGATCCTCACTATCACTACTGGC
TTTTTG-3'

TRCN0000033983/1E9 (Human, Mouse and Rat) 5'-
CCGGGCTCATTTACAACTCAGTGGACTCGAGTCCACTGAGTTGTAAATGAGCT
TTTTG-3'

These plasmids were transfected into MCF7 cells and selected by puromycin (2 mg/ml) for 7 days. Knockdown efficiency was analyzed by immunoblotting with anti-Iduna antibody. Two clones showed significant knockdown of endogenous Iduna. These cell lines were maintained in complete medium containing puromycin (200 μg/ml). SK-N-SH cells (Human neuroblastoma cells) was purchased from ATCC and cultured in DMEM with 10% fetal bovine serum (FBS), 50 units/ml penicillin, and 50 μg/ml streptomycin. To establish a TAP-Iduna expressing cell line, pNTAP-Iduna was stably transfected to SK-N-SH cells and treated with a geneticin selection (1 mg/ml) for 3 weeks.

Tandem affinity purification. Iduna's substrates were isolated using the Interplay mammalian TAP system (Stratagene). Briefly, SK-N-SH cells expressing pNTAP or pNTAP-Iduna were harvested and collected at 500 x *g* at 4°C and lysed in lysis buffer (Stratagene). The TAP procedure was then performed by following the manufacturer's instructions, except that the streptavidin and calmodulin-binding reactions were incubated overnight at 4°C. Eluted proteins were boiled in SDS sample buffer and resolved on 8-16% SDS-polyacrylamide gels. The presence of TAP and TAP-Iduna was determined by immunoblot with anti-CBP (Calmodulin binding peptide) and anti-Iduna antibody.

***In vitro* ubiquitination assay**. To measure of autoubiquitination activity of GST free Iduna, E1 (50 nM), UbcHs (50 nM) and Iduna (IP samples or recombinant protein) were incubated with recombinant ubiquitin (200 mM) at 37°C in reaction buffer containing 50 mM Tris-Cl, pH 7.5, 2.5 mM $MgCl_2$, 2 mM DTT, 2 mM ATP. For reducing conditions, samples were treated with SDS sample buffer and boiled supernatant were separated by 8-16% SDS-PAGE. Both polymerized ubiquitin chains and ubiquitinated proteins were detected by immunoblot with anti-ubiquitin antibody. All proteins loaded in SDS-PAGE were separately visualized by coomassie staining. Recombinant E1, UbcHs and ubiquitin were purchased from either Calbiochem or Boston Biochem.

Synthesis of [^{32}P] and biotin-labeled PARP1 and purification of PARP-free PAR polymer. Automodified PARP1 and free PAR polymer were purified as previously

described (1). Briefly, [^{32}P]-labeled (PerkinElmer) or biotin-labeled NAD (Trevigen) was incubated in reaction buffer containing 100 mM Tris-cl, pH 8.0, 10 mM $MgCl_2$, 8 mM DTT, 10% glycerol, 23 ug calf thymus activated DNA, 4 mM biotin-labeled NAD or 75 uCi [^{32}P]-labeled NAD. 100% ethanol was added in drops for 10% concentration by volume. Sequentially, twenty units of recombinant PARP1 (Trevigen) was incubated for 30 min at 30°C. To collect automodified PARP1, 3 M CH_3COONa and isopropanol were added in sample thereafter automodified PARP1 was precipitated by centrifugation at 10,000 g for 10 min. To purify PARP-free biotin-labeled or [^{32}P]-labeled PAR polymer, collected samples were hydrolyzed in reaction buffer containing 1 M KOH and 50 mM EDTA. Hydrolyzed PAR polymers were incubated with AAGE9 buffer (250 mM NH_4Ac, pH 9.0, 6 M guanidine, 10 mM EDTA) and then it was adjusted to pH 9.0 with 4N HCl. The samples were loaded on prepacked Dihydroxyboryl Bio-Rex column (DHBB) and washed with AAGE9 buffer followed by 1 M NH_4Ac solution. Free biotin-labeled or [^{32}P]-labeled PAR polymer was eluted by water at 37°C. Polymer size distribution was analyzed by 20% TBE-PAGE (90 mM Tris-borate pH 8.0, 2 mM EDTA).

***In vitro* PARP1 ubiquitination assay.** PARP1 or biotin-labelled PARP1 were incubated with Glutathione Sepharose 4B (GE-healthcare) linked-GST-Iduna for 2 h at 4°C. After Sepharose 4B pull-down, beads were washed three times, and GST was cleaved from recombinant GST-Iduna by using PreScission Protease (GE Healthcare). Elute proteins were dialyzed with binding buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, and then incubated with E1, ubcH5a and ubiquitin at 37°C in reaction buffer containing 50

mM Tris-Cl, pH 7.5, 2.5 mM $MgCl_2$, 2 mM DTT, 2 mM ATP. Final samples were treated with SDS sample buffer and boiled supernatant were separated by 8-16% SDS-PAGE. The ubiquitination of PARP1 and Iduna was analyzed by western blot with anti-ubiquitin, anti-PARP1, anti-Iduna and anti-PAR antibodies.

***In vitro* PAR pull down and EMSA analysis.** To PAR pull down analysis, [^{32}P]-labeled PAR polymer (10,000 cpm/μl) was incubated for 1 h with recombinant Histone H3 (Novus Biologicals), Iduna and various mutants. After washing with buffer containing 0.1 % Triton X-100, 0.1 % NP-40, protease inhibitors cocktail, and 1 mM PMSF, it was incubated with anti-histone H3 or anti-Iduna antibody (2 μg) linked to protein-G agarose slurries for 2 h at 4°C. The complex between [^{32}P]-labeled PAR polymer and those proteins was collected by centrifugation at 1000 x g for 1 min and then each collected sample was hydrolyzed by 1 M KOH and 50 mM EDTA. To measure the PAR-binding activity, 10 μl of each sample was analyzed by LS 6500 Liquid Scintillation Counting System. For the EMSA analysis, protein free [^{32}P]-labeled PAR polymer from residual samples were purified by a DHBB column and then samples resolved in 20% TBE-PAGE (90 mM Tris-borate pH 8.0, 2 mM EDTA). The gel was dried and developed by autoradiography using a Typhoon 9400 Imager (GE Health Care).

Two-dimensional gel electrophoresis-western blot (2DE-WB*).* Each sample from *in vitro* ubiquitination assay was suspended in 1.5 mL of sample buffer containing 40 mM Tris, pH 7.4, 7 M urea (Sigma), 2 M thiourea (Sigma), 4% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propane-sulfonate) (Sigma), 65 mM DTT (Bio-

Rad Laboratories), 1 mM EDTA, protease inhibitors cocktail (Roche) and 1 mM PMSF (Phenylmethylsulfonyl chloride). Samples were desalted and concentrated by ultracentrifugal filter (Millipore). Samples were applied on immobilized pH 3-10 non-linear gradient strips (13 cm). Focusing started at 200 V and the voltage was gradually increased to 8000 V at 3 V/min (approximately 151,358 Vhr total). After the first dimension, strips were equilibrated for 15 min in the equilibration buffer containing 6 M urea, 20% glycerol, 2% SDS, 2% DTT and then for 15 min in the same equilibration buffer containing 2.5% iodoacetamide instead of DTT. After equilibration, strips were loaded on 9-16% gradient SDS gels for second-dimensional separation. The gels (180 x 200 x 1.5 mm) were run at 40 mA per gel. Immediately after the second dimension running, the gels were fixed for 18 h in 50% methanol, 10% acetic acid solution. The gels were stained with either Colloidal Coomassie Blue (Invitrogen) or SilverQuest (Invitrogen). Molecular masses were determined by standard protein markers (Bio-Rad) covering a range of 10-250 kDa. p*I* value was used as given by the supplier of the immobilized pH gradient strips (GE Healthcare). The Gels were destained with water and scanned with UMAX Scanner. For 2DE-western, gels were soaked in transfer buffer for 15 min, transferred onto nitrocellulose membrane and analyzed by immunoblot.

***In vivo* PARP1 stability assay**. GFP, GFP-Iduna, GFP-Iduna C60A, GFP-Iduna YRAA, shRNA-Iduna and shRNA-Iduna/GFP-Iduna stably overexpressing cells were treated with 500 μM MNNG for 15 min, and replaced with fresh growth media for posttreatment 0 or 1 h in presence of DMSO or of MG132 (Sigma). The cells were harvested and then lysed with immunoprecipitation (IP) lysis buffer containing 25 mM HEPES, pH 7.4, 1

mM EDTA, 10 mM NaCl, 0.5% Triton X-100, protease inhibitors cocktail (Roche) and 1 mM PMSF (Phenylmethylsulfonyl chloride). Equal amount of proteins from cell lysates was incubated overnight at 4°C with protein-G and anti-PARP1 antibody in IP lysis buffer. After protein-G pull-down, beads were washed five times with IP wash buffer (25 mM HEPES, pH 7.4, 1 mM EDTA, 100 mM NaCl, 0.5% Triton X-100), boiled in SDS sample buffer (Bio-Rad), and proteins were separated by 8-16% SDS-PAGE and analyzed by immunoblot with anti-PARP1 and anti-PAR antibody.

Cell death assay. GFP, GFP-Iduna, GFP-Iduna C60A, GFP-Iduna YRAA, shRNA-Iduna and shRNA-Iduna/GFP-Iduna stably transfected cells were treated with 500 μM MNNG for 15 min, and replaced with fresh growth media. After 24 h, the cells were stained with 5 mM Hoechst 33342 (Invitrogen) and 2 mM propidium iodide (PI) (Invitrogen) and counted by automated computer-assisted program (Carl Zeiss). The percentage of cell death was determined as the ratio of live to dead cells compared with the percentage of cell death in control as described previously (1, 3).

Immunoprecipitation of endogenous Iduna. The cells were pretreated with DMSO, DPQ or AG14361 for 1 h. Following washes with PBS, cells were lysed using an immunoprecipitation (IP) lysis buffer containing 25 mM HEPES, pH 7.4, 1 mM EDTA, 10 mM NaCl, 0.5% Triton X-100, protease inhibitors cocktail and 1 mM PMSF. Equal amount of protein was incubated overnight with protein-G sepharose beads (Amersham and mouse IgG or specific antibody in IP lysis buffer. After pull-down, protein-G beads were washed five times with IP washing buffer (25 mM HEPES, pH 7.4, 1 mM EDTA,

100 mM NaCl, 0.5% Triton X-100) and boiled with SDS sample buffer (Bio-Rad) containing 5% β-mercaptoethanol (Sigma). Proteins were separated by 8-16% SDS PAGE and transferred to nitrocellulose membrane (0.45 μm). 5% dried milk in PBST or TBST (phosphate or Tris buffered saline with 0.1% Tween 20) was incubated for blocking, and the membranes were applied with specific antibodies as described on previous material section. After washing with PBST or TBST and incubation with horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG (Amersham Bioscience), the antigen-antibody was detected by chemiluminescence (ECL) (Pierce) and X-ray film (RPI).

Comet assay. Single cell gel electrophoresis assay was performed by following the manufacturer's instructions. Briefly established stable cell lines were irradiated (2 Gy) with a Gammacell irradiator and then further incubated for 15 min at 37°C. Cells were collected at 500 x *g* at 4°C and rinsed twice with ice cold PBS (Ca^{++} and Mg^{++} free). A total of 5 x10^5 cells were counted and resuspended into PBS (Ca^{++} and Mg^{++} free) and then combined with LMAgarose (low melting agarose) at a ratio 1:10 (v/v). Each samples were spotted into CometSlide^{TM} and lysed with buffer (supplied with CometAssay Kit, Trevigen) for 1 h at 4°C. After draining the excess lysis buffer, slides were immersed with alkaline unwinding solution (200 mM NaOH, pH >13, 1 mM EDTA) for 1 h at RT. To the single cell electrophoresis, each comet slide was placed in electrophoresis slide tray with 1 L of alkaline unwinding solution and applied to 21 Volts for 30 min for electrophoresis. After draining the excess electrophoresis buffer, slides were rinsed twice with dH_2O and then fixed with 70 % ethanol for 5 min. To facilitate single cell

observation, slides were dried at 40°C and stained with SYBR Green I (supplied with CometAssay Kit) for 5 min at 4°C. Cell images were captured using a Zeiss epi-fluorescent microscope (Axiovert 200M) and image analysis was performed with a CASP software (version 1.2.2) (4). Fifty cells per slide were monitored and the DNA damage was calculated using comet tail length and head diameter parameters.

Cell cycle analysis. Studies were performed to evaluate the cell cycle response of each stable cell lines. Cells were plated onto 150 mm culture dishes and cultured for 24 h at 37 °C with fresh growth medium. Following replacement of growth medium, cells were irradiated at 2 Gy with a Gammacell irradiator. After 12 h, cells were washed once with PBS and incubated with PBS containing 0.2 % EDTA for 5 min at 37°C, thereafter scraped with fresh growth medium and collected at 500 x *g* at 4°C. Collected cells were washed once with ice cold PBS and then fixed with 70% ethanol. Cells were washed once with PBS containing 1% FBS and resuspended into propidium iodide (PI) staining buffer (PBS, pH 7.4, 50 μg/ml PI, 1% FBS, 100 μg/ml RNase) for 30 min at 37°C. Cells were monitored for DNA content by a flow cytometry (BD Biosciences) and then data were analyzed with FlowJo using Dean-Jett-Fox model for the quantification of each cell cycle phase.

Determination of apurinic/apyrimidinic (AP) sites. The amount of AP sites in genomic DNA was monitored by a DNA damage quantification kit (BioVision). Briefly, cells were treated with either DMSO or 500 μM MNNG for 15 min, and then replaced with normal growth media. After 1 h, cells were scraped and harvested at 500 x *g* for 5 min

and washed once with PBS. Genomic DNA was isolated using a Biovision Genomic DNA Isolation Kit (BioVision) and then AP sites on the 0.5 μg of genomic DNA was labeled with biotin by the Aldehyde Reactive Probe (ARP) reagent (supplied with the DNA damage quantification kit) for 1 h at 37°C. To precipitate the biotin-tagged DNA, sample was mixed with TE buffer containing 2% glycogen and then sequentially incubated with ice cold 70% ethanol for 30 min at -20°C. AP-site tagged DNA was precipitated at 12,000 x *g* for 10 min and washed twice with 70% ethanol. To determine of the number of AP sites in DNA, samples were dissolved in TE buffer and then transferred into 96 well plate with DNA binding buffer (supplied with the DNA damage quantification kit). After 12 h, samples were washed five times with DNA washing buffer (supplied with the DNA damage quantification kit) and then biotin labeled AP sites were quantified using an avidin–biotin assay. Each sample and standards (supplied with a DNA damage quantification kit) were run in triplicate and the OD was measured at 650 nm using a 96-well plate reader (SpectraMax Plus384 Microplate Reader).

Live-cell imaging and Laser micro-irradiation-induced DNA damage: For induction of localized DNA damage, MCF 7 cells stably expressing GFP-Iduna or GFP-Iduna-YRAA were plated onto 25 mm glass bottom culture dishes for 48 h. Cells were presensitized with 10 μM 5-bromo-2'-deoxyuridine (BrdU, Sigma) for 24 h. Cells were incubated with 2 μM Hoechst (Invitrogen) for 5 min and mounted on a preheated ($37^{o}C$) stage on a Zeiss LSM 710 confocal microscope equipped with 405 nm laser source. A laser microbeam was focused on a small rectangular strip of nucleus through 63 X oil objective to induce localized DNA damage. The laser setting was set to 100% power

output with a scanning speed of 1 and 6-10 laser iterations. The kinetics were calculated using Zeiss Zen 2010 software. Time point before irradiation represents -1 min and the time point just after irradiation represent 0 min. Each data series were normalized with respect to base line data intensity values.

References

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Supplemental Figure Legends

Fig. S1. Iduna has ubiquitin E3 ligase activity. The E2s, UbcH5a, UbcH5b, UbcH5c are involved in polyubiquitination by Iduna, whereas UbcH6 is involved with monoubiquitination by Iduna. *(A)* Screening of UbcHE2 enzymes of Iduna. Either immunoprecipitated GFP-Iduna (left panel) or endogenous Iduna (right panel) was subjected into an *in vitro* ubiquitination assay (IVUA) along with recombinant E1, ubiquitin and UbcHE2 enzymes as indicated. Samples were resolved in 8-16% SDS-PAGE and then immunoblotted by anti-Iduna or anti-ubiquitin antibody. Protein samples were visualized by coomassie staining, separately. (*) indicates unmodified GFP-Iduna or Iduna. *(B)* GFP interacting proteins do not have ubiquitin E3 ligase activity. Immunoprecipitated GFP was used as a negative control for the *in vitro* ubiquitination assay. All experiments were repeated three times.

Fig. S2. Polyubiquitination of PARP1 by Iduna. *(A)* Immunoprecipitated GFP and GFP-Iduna were subjected to 2D analysis and then samples were visualized by silver staining and 2D western blot with anti-GFP. An *in vitro* ubiquitination assay was performed using an immunoprecipitated GFP-Iduna as indicated, and self-ubiquitination activity of Iduna was measured by 2D western blot with anti-GFP. *(B)* Polyubiquitination of PARP1 was analyzed by 2D western blot with anti-PAPP-1 antibody. White and black arrows indicate the GFP-Iduna and polyubiquitinated PARP1, respectively. White (*), indicates polyubiquitinated GFP-Iduna. All experiments were repeated two times.

Fig. S3. PAR-binding and RING domains of Iduna are essential for its PAR-dependent ubiquitin E3 ligase activity. *(A)* The RING domain is critical for Iduna's activity. Immnuoprecipitated GFP-Iduna, GFP-Iduna C60A and GFP-Iduna H54A were subjected to *in vitro* ubiquitination assay. Its activity was analyzed by immunoblot with anti-GFP and anti-ubiquitin antibodies. White or black arrows head indicate the immunoglobulin heavy or light chains, respectively. (*) indicates unmodified GFP-Iduna. *(B)* Recombinant GST-Iduna, GST-Iduna C60A and GST-Iduna H54A were purified from IPTG-induced *Ecoli* and visualized by coomassie staining (upper panel). *In vitro* ubiquitination assay was performed as indicated in lower panel. Self-ubiquitination activity was confirmed by anti-ubiquitin and -Iduna antibodies. (*) indicates unmodified GFP-Iduna. *(C)* PAR binding activity of Iduna mutants. Immunoprecipitated GFP-Iduna, GFP-Iduna YRAA and GFP-Iduna C60A were analyzed for PAR binding activity by immunoblot with anti-PAR and anti-GFP antibody. White or black arrows head indicate the immunoglobulin heavy or light chains, respectively. (*) indicates unmodified GFP-Iduna. All experiments were repeated three times.

Fig. S4. Iduna has strong PAR binding activity. *(A)* Pull-down assay of Iduna or Iduna mutants with [^{32}P] labeled PAR. H3 was used as a positive control and PARG enzyme was used for the degradation of PAR. n=3, * $P < 0.05$ by ANOVA with Tukey-Kramer's *post hoc* test. *(B)* PARG activity was measured by incubation with PARsylated PARP1 as indicated. The level of PARsylated PARP1 (Rb-PARP1) was monitored by immunoblot

with anti-PAR antibody. There is a slight difference in the sensitivity of assays used to detect PAR, which accounts for the ability of 1 unit of PARG to eliminate PAR in B, but 1.2 units of PARG is required in A. *(C)* EMSA of Iduna and Iduna mutants with [^{32}P] labeled PAR. Histone H3 was used as a positive control. n = 3 independent experiments. *(D)* [^{32}P]-PAR bound to Iduna or Iduna mutants was analyzed in 20% TBE-PAGE. Values represent ADP-ribose units. H3 was used as a positive control. All experiments were repeated three times.

Fig. S5. PAR and PARsylation enhance the Iduna activity. *(A)* Self-ubiquitination activity of Iduna was monitored by incubation with PAR and/or PARG as indicated. *(B)* PAR dependent ubiquitination of PARP1 was analyzed by an *in vitro* ubiquitination with Iduna or Iduna YRAA mutants as indicated. Total protein level was visualized by coomassie brilliant blue staining. Abbreviations: Ub (n), polyubiquitin chains; Ub-PARP1, polyubiquitinated PARP1; Ub-Iduna, poly ubiquitinated Iduna. All experiments were repeated five times.

Fig. S6. PAR-dependent ubiquitination of potential Iduna substrates. PAR-dependent ubiquitination of Iduna substrates was monitored by an *in vitro* ubquitination assay as indicated. Ub (n), polyubiquitin chains. All experiments were repeated two times.

Fig. S7. Knockdown of endogenous Iduna. *(A)* Iduna expression by shRNA-Iduna was detected by immunoblot with anti-Iduna antibody in GFP, GFP-Iduna, shRNA-Iduna or shRNA-Iduna/mIduna stably overexpressing MCF7 cells as described in Figure 3. Loading protein amount was diluted one-fourth for GFP-Iduna and shRNA-Iduna/mIduna. GFP-Iduna expression was detected through short time exposure (30 sec) (top), Endo-Iduna was monitored by immunoblot through long time exposure (5 min) (middle) and actin expression was analyzed as loading control (bottom). All experiments were repeated two times.

Fig. S8. Summary of Iduna mediated cell survival pathway.

Supplemental Table1. Mass spectrometry analysis of the TAP-iduna complex

Protein identity (The number of identified peptides)	Representative Peptide (position)	Protein coverage by amino acid count	Protein coverage by mass
PARP1 (47)	PPSTASAPAAVNSSASADKP LSNMK (369-393)	42.5 %	42.2 %
SMARCA3 (20)	TLTAIAVILTNFHDGRPLPIE R (301-322)	25.5 %	25.1 %
HNRPU(12)	HAAENPGKYNILGTNTIMD K(515-534)	13.2 %	13.4 %
Nucleolin (13)	QKVEGTEPTTAFNLFVGNLN FNK (295-317)	18.8 %	19. 6%
Importin-7(3)	AFAVGVQQVLLK (295-306)	3.1 %	3.1%
Importin-3β (2)	LVLEQVVTSIASVADTAEEK (506-525)	3.1 %	3.0 %
DNA ligase III (19)	ADFTVVAGDEGSSTTGGSSE ENKGPSGSAVSR (749-780)	28.1 %	27.7 %
Ku86 (44)	LGGHGPSFPLKGITEQQKEG LEIVK (184-208)	39.5 %	39.7 %
Ku70 (41)	LGSLVDEFKELVYPPDYNPE GK (517-538)	57.7 %	57.3 %
XRCC1 (3)	SPVTASDPAGPSYAAATLQA SSAASSASPVSR (199-230)	10.0 %	9.2 %
PARP2 (2)	SPLKP ESQLDLR (226-237)	3.8 %	3.8 %
Phospho-RNF146	RQEIPEDFLDKPTLLS#PEEL KA (75-96)	NA	NA
RNF146 (13)	QEIPEDFLDKPTLLSPEELK (76-95)	22.0 %	23.6 %
ATP-synthase subunit α (12)	EVAAFAQFGSDLDAATQQL LSR (442-463)	29.8 %	29.0 %
GRP-78 (23)	VLEDSDLKKSDIDEIVLVGG STR (345-367)	46.2 %	46.4 %
GRP-75 (22)	AMQDAEVSKSDIGEVILVGG MTR (369-391)	40.6 %	40.8 %
β-Tubulin (19)	FWEVISDEHGIDPTGTYHGD SDLQLDR (13-39)	24.9 %	24.8 %
Histone h1.2 (3)	AGGTPRKASGPPVSELITK (28-46)	10.4 %	10.1 %
Histone h1.1 (4)	APAASAAPEK (8-17)	5.7 %	5.6 %
Histone h4 (6)	QGITKPAIRRLARRGGVK (28-45)	16.5 %	16.5 %
Histone h3 (3)	APRKQLATKAARK (16-28)	8.1 %	8.7 %

#, phosphorylation site of Iduna

Supplemental Table 2. Identification of Iduna's ubiquitination sites and ubiquitin linkages

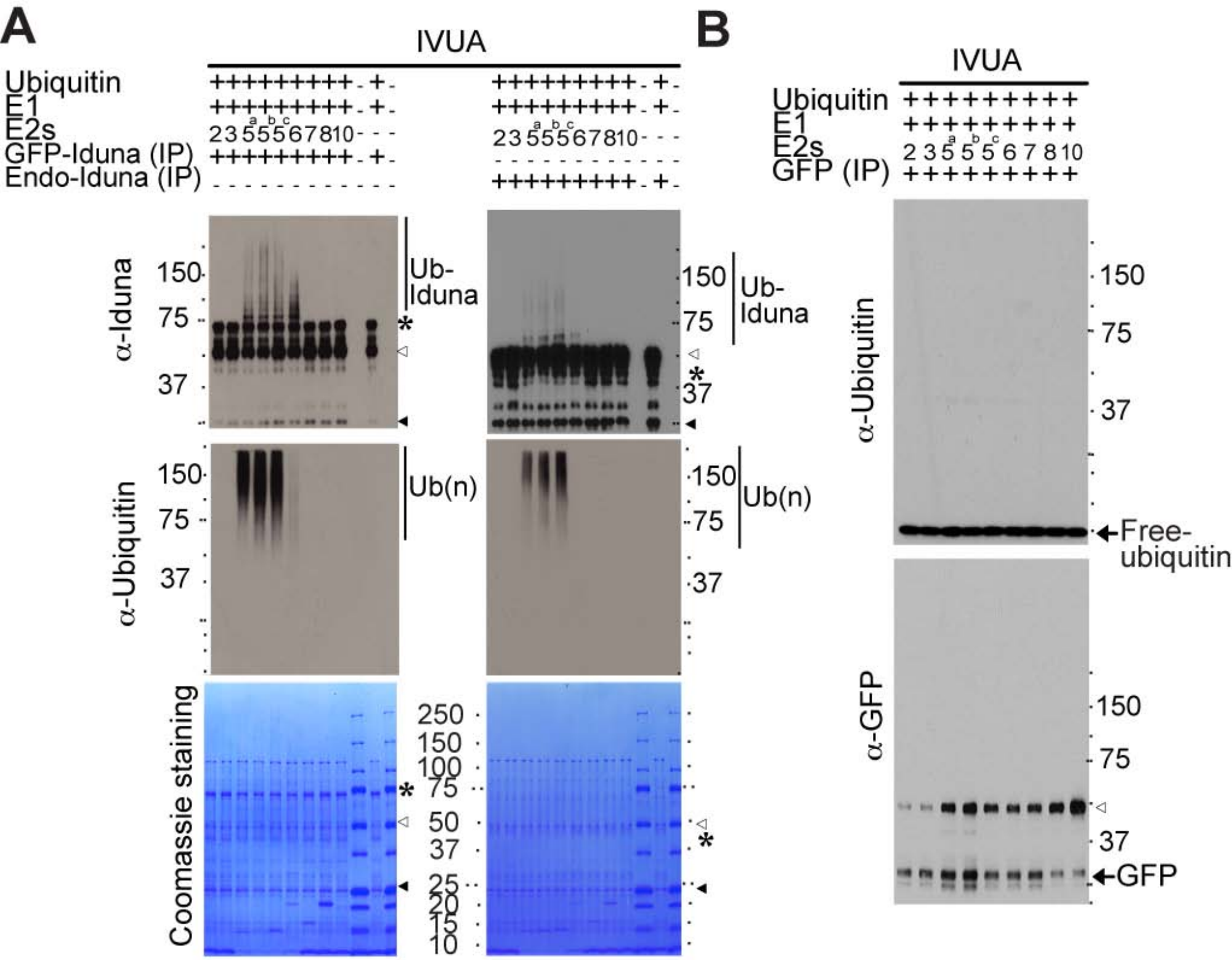
Protein identity (Sample ID)	Ubiquitination sites of Iduna or Ubiquitin (The positions of Ubiquitinated lysine residues)	Protein coverage by amino acid count	Protein coverage by mass
Iduna (1)	MMAGCGEIDH SINMLPTNRK ANESCSNTAP SLTVPECAIC LQTCVHPVSL PCKHVFCYLC VKGASWLGKR CALCRQEIPE DFLD**K**PTLLS PEEL**K**AASRG NGEYAWYYEG RNGWWQYDER TSRELEDAFS KGKKNTEMLI AGFLYVADLE NMVQYRRNEH GRRRKIKRDI IDIPK**K**GVAG LRLDCDANTV NLARESSADG ADSVSAQSGA SVQPLVSSVR PLTSVDGQLT SPATPSPDAS TSLEDSFAHL QLSGDNTAER SHRGEGEEDH ESPSSGRVPA PDTSIEETES DASSDSEDVS AVVAQHSLTQ QRLLVSNANQ TVPDRSDRSG TDRSVAGGGT VSVSVRSRRP DGQCTVTEV (K85, K95, K176)	144/359 = 40.1%	16466/38951 = 42.3%
Iduna (2)	MMAGCGEIDH SINMLPTNRK ANESCSNTAP SLTVPECAIC LQTCVHPVSL PCKHVFCYLC VKGASWLGKR CALCRQEIPE DFLDKPTLLS PEELKAASRG NGEYAWYYEG RNGWWQYDER TSRELEDAFS **K**GKKNTEMLI AGFLYVADLE NMVQYRRNEH GRRRKIKRDI IDIPK**K**GVAG LRLDCDANTV NLARESSADG ADSVSAQSGA SVQPLVSSVR PLTSVDGQLT SPATPSPDAS TSLEDSFAHL QLSGDNTAER SHRGEGEEDH ESPSSGRVPA PDTSIEETES DASSDSEDVS AVVAQHSLTQ QRLLVSNANQ TVPDRSDRSG TDRSVAGGGT VSVSVRSRRP DGQCTVTEV (K131, K176)	126/359 = 35.1%	14609/38951 = 37.5 %
Ubiquitin (1)	MQIFVKTLTG **K**TITLEVEPS DTIENVKAKI QD**K**EGIPPDQ QRLIFAG**K**QL EDGRTLSDYN IQ**K**ESTLHLV LRLRGG (K11, K48)	66/76 = 86.8%	7435/8565 = 86.8%
Ubiquitin (2)	MQIFV**K**TLTG KTITLEVEPS DTIENVKAKI QD**K**EGIPPDQ QRLIFAG**K**QL EDGRTLSDYN IQKESTLHLV LRLRGG (K6, K33, K48)	72/76 = 94.7%	8182/8565 = 95.5%

- **Bold type K letters** indicate ubiquitinated Lysine residues on Iduna or ubiquitin.
- Blue type letters with underline indicate Ring domain on Iduna.
- Red type letters with underline indicate WWE domain on Iduna.

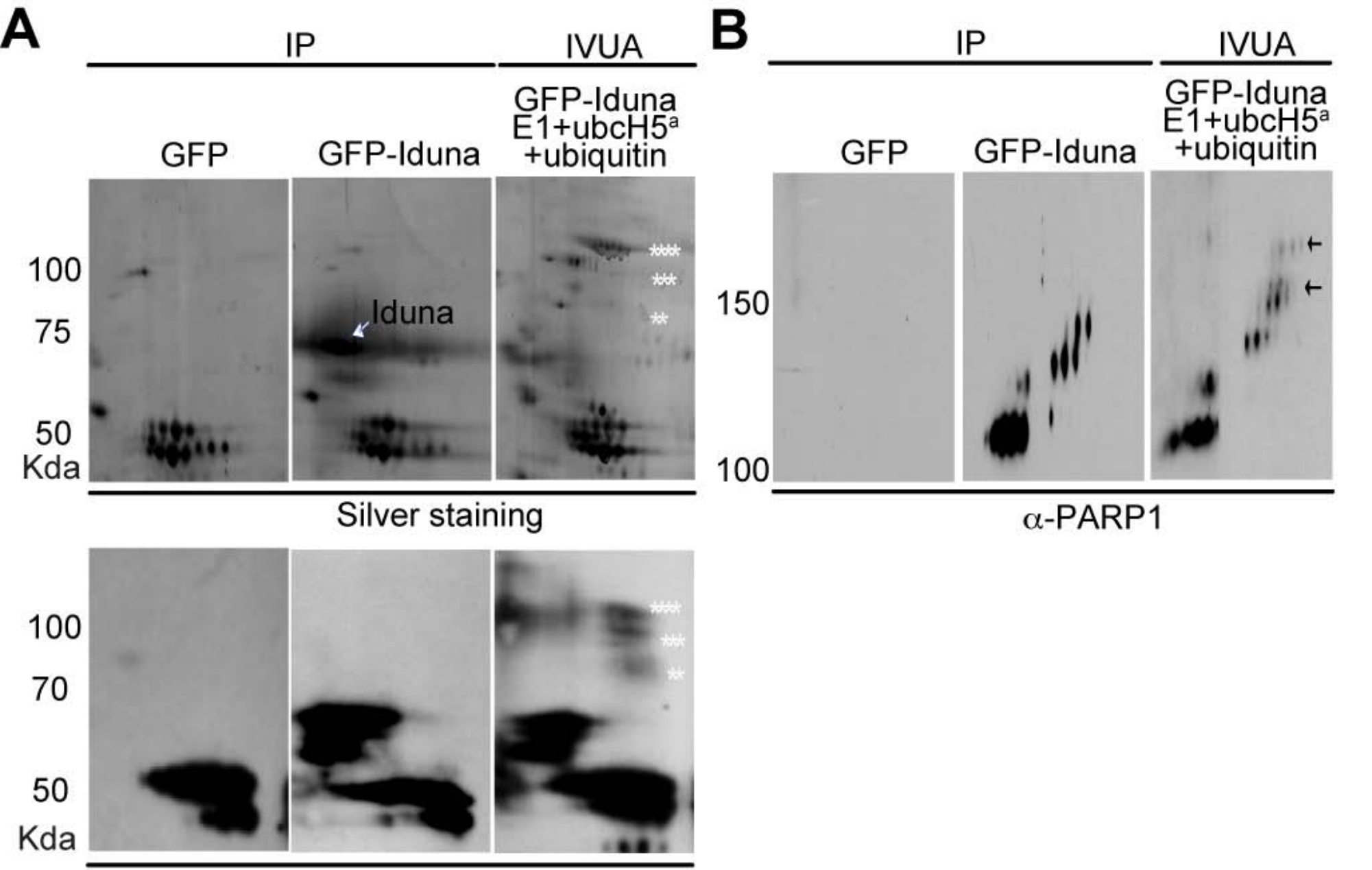
Supplemental Table 3. Identification of PARP1's ubiquitination sites and ubiquitin linkages

Protein identity	Ubiquitination sites of PARP1 (The number of Ubiquitinated lysine residues)	Protein coverage by amino acid count	Protein coverage by mass
PARP1	MAESSDKLYR VEYAKSGRAS CKKCSESIPK DSLRMAIMVQ SPMFDGKVPH WYHFSCFWKV GHSIRHPDVE VDGFSELRWD DQQKVK**K**TAE AGGVTG**K**GQD GIGSKAE**K**TL GDFAAEYA**K**S NRSTCKGCME KIE**K**GQVRLS KKMVDPEKPQ LGMIDRWYHP GCFVKNREEL GFRPEYSASQ LKGFSLLATE D**K**EALK**K**QLP GVKSEGKR**K**G DEVDGVDEVA KKKSKKEKDK DSKLE**K**ALKA QNDLIWNIKD ELKKVCSTND LKELLIFN**K**Q QVPSGESAIL DRVADGMVFG ALLPCEECSG QLVFKSDAYY CTGDVTAWTK CMVKTQTPNR KEWVTPKEFR EISYLKKLKV KKQDRIFPPE TSASVAATPP PSTASAPAAV NSSASADKPL SNMKILTLG**K** LSRNKDEVKA MIE**K**LGG**K**LT GTANKASLCI STKKEVEKMN KKMEEV**K**EAN IRVVSEDFLQ DVSASTKSLQ ELFLAHILSP WGAEVKAEPV EVVAPRG**K**SG AALSK**K**SKGQ VKEEGINKSE KRMKLTL**K**GG AAVDPDSGLE HSAHVLEKGG KVFSATLGLV DIVKGTNSYY KLQLLEDD**K**E NRYWIFRSWG RVGTVIGSNK LEQMPSKEDA IEHFMKLYEE KTGNAWHSKN FTKYPKKFYP LEIDYGQDEE AVK**K**LTVNPG TKSKLPKPVQ DLIKMIFDVE SMKKAMVEYE IDLQ**K**MPLGK LSKRQIQAAY SILSEVQQAV SQGSSDSQIL DLSNRFYTLI PHDFGMK**K**PP LLNNADSVQA KVEMLDNLLD IEVAYSLLRG GSDDSSKDPI DVNYE**K**L**K**TD IKVVDRDSEE AEIIRKYVKN THATTHNAYD LEVIDIFKIE REGECQRY**K**P FKQLHNRRLL WHGSRTTNFA GILSQGLRIA PPEAPVTGYM FGKGIYFADM VSKSANYCHT SQGDPIGLIL LGEVALGNMY ELKHASHISK LPKGKHSVKG LGKTTPDPSA NISLDGVDVP LGTGISSGVN DTSLLYNEYI VYDIAQVNLK YLLKLKFNFK TSL (Total 24 ubiquitination sites)	715/1013 = 70.6%	78777/11 2953 = 69.7%
Ubiquitin	MQIFVKTLTG **K**TITLEVEPS DTIENVKAKI QD**K**EGIPPDQ QRLIFAG**K**QL EDGRTLSDYN IQKESTLHLV LRLRGG (K11, K48)	67/76 =88.1%	7681/856 5 = 89.6%

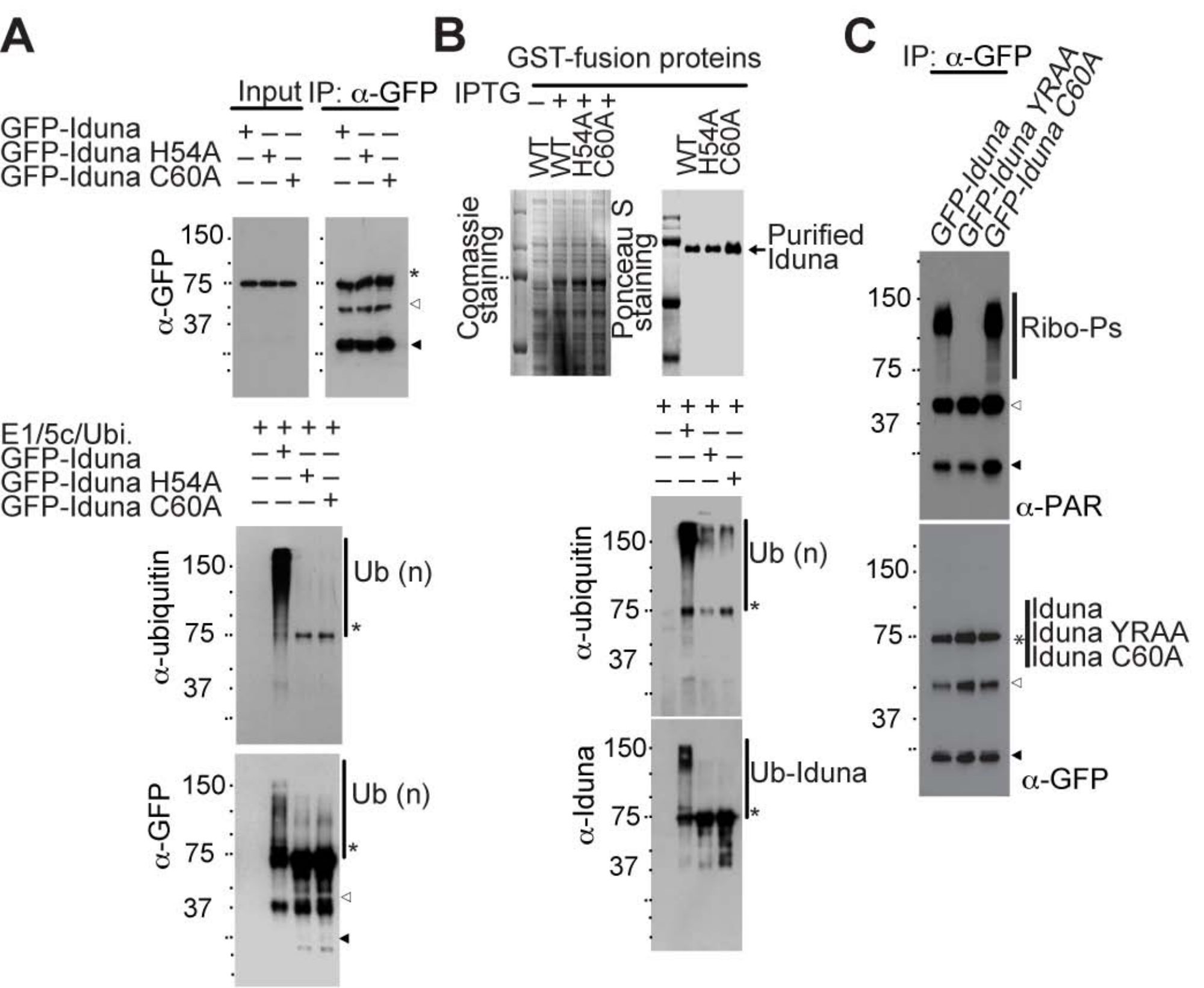
- **Bold type K letters** indicate ubiquitinated Lysine residues on PARP1 or ubiquitin.



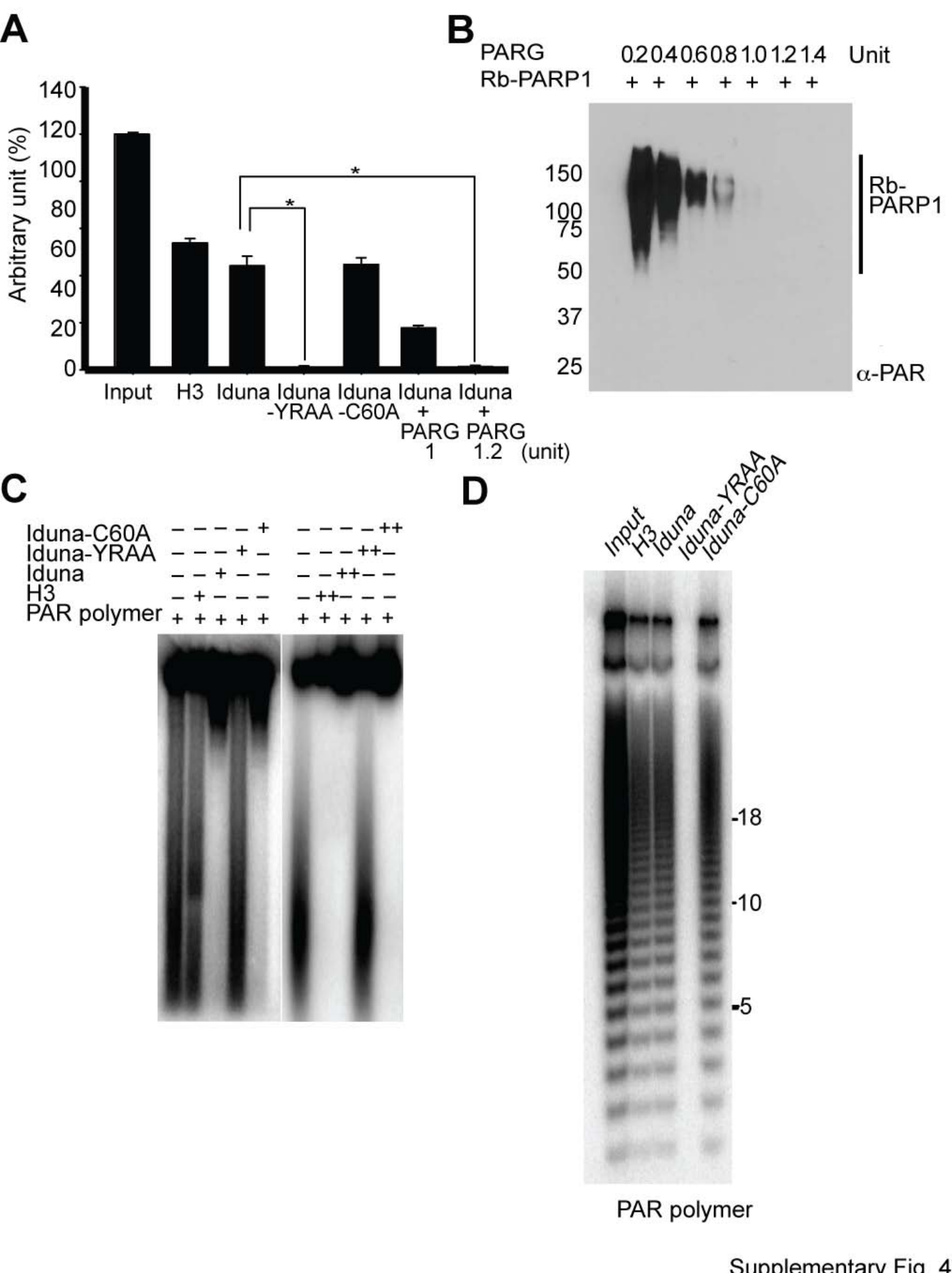
Supplementary Fig. 1



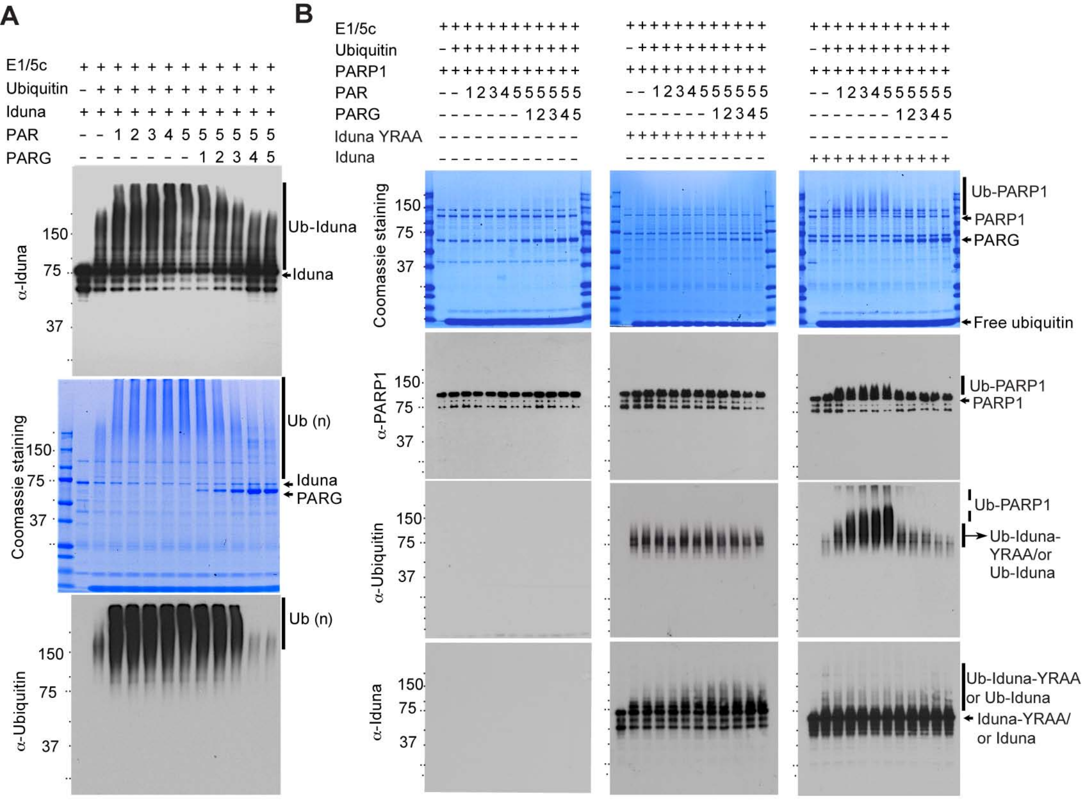
Supplementary Fig. 2



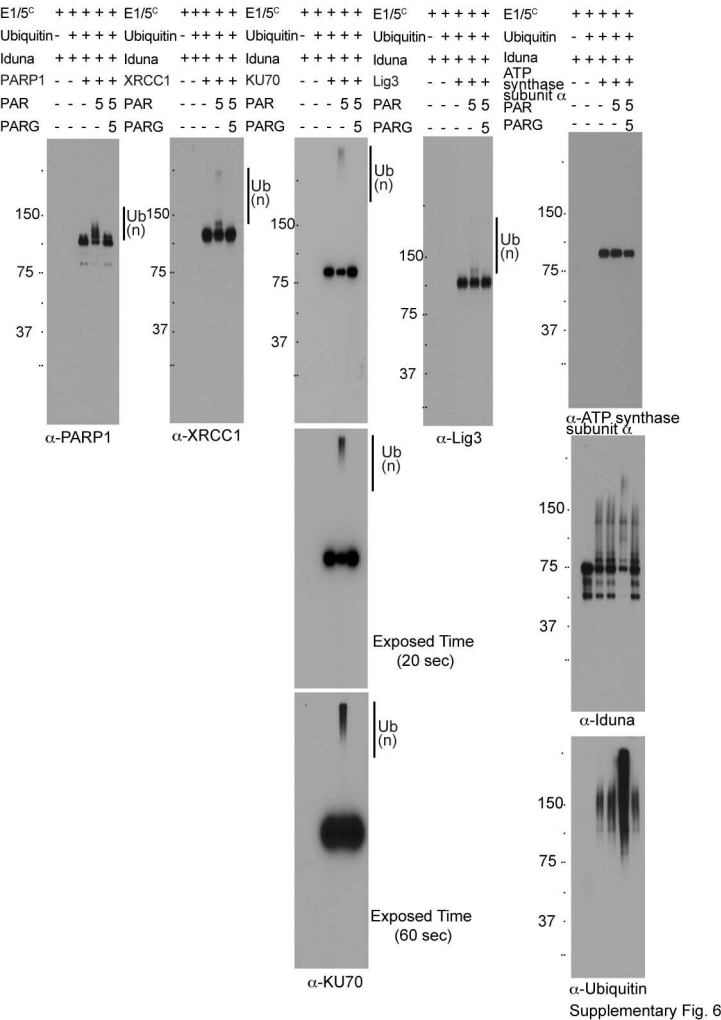
Supplementary Fig. 3



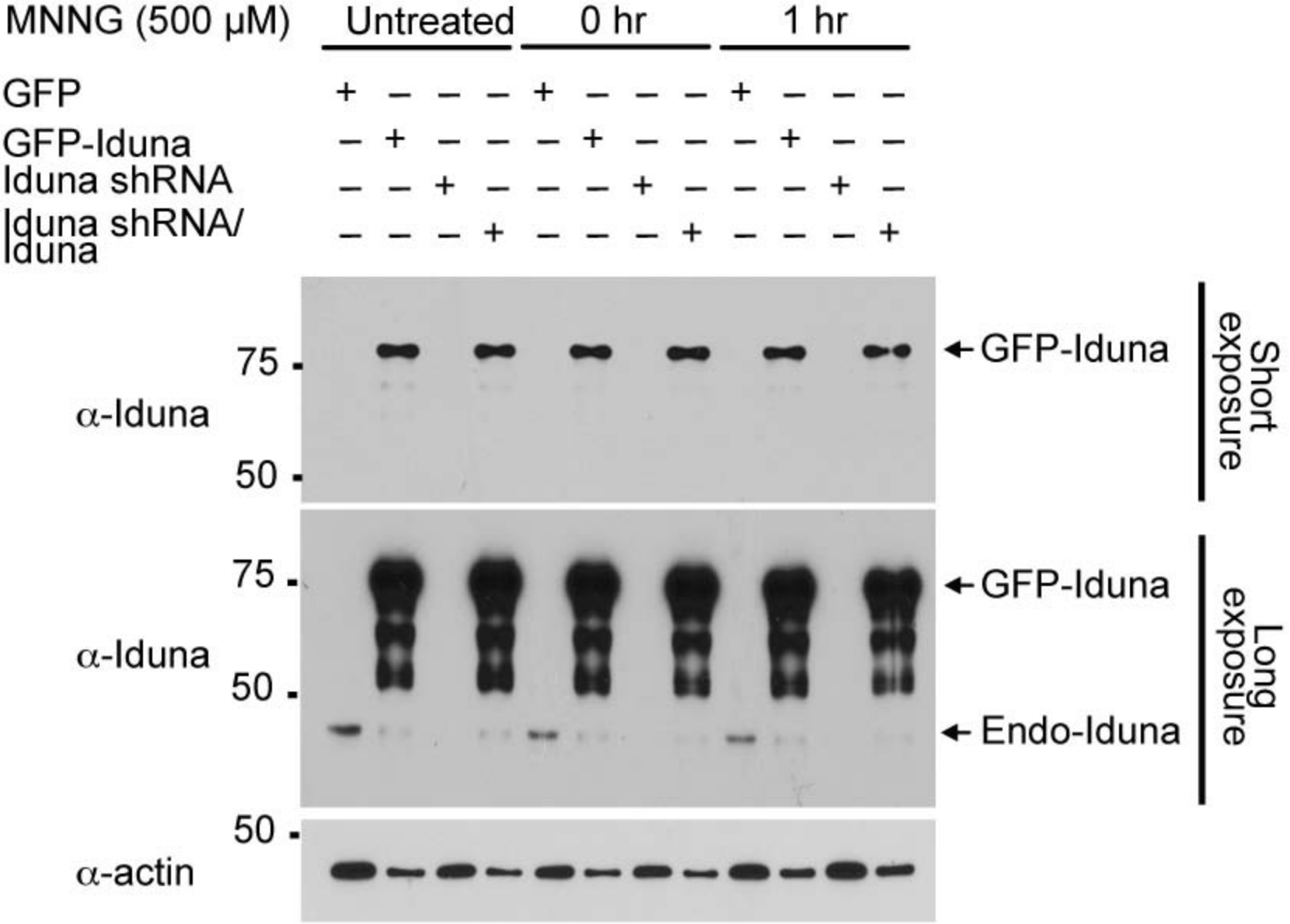
Supplementary Fig. 4



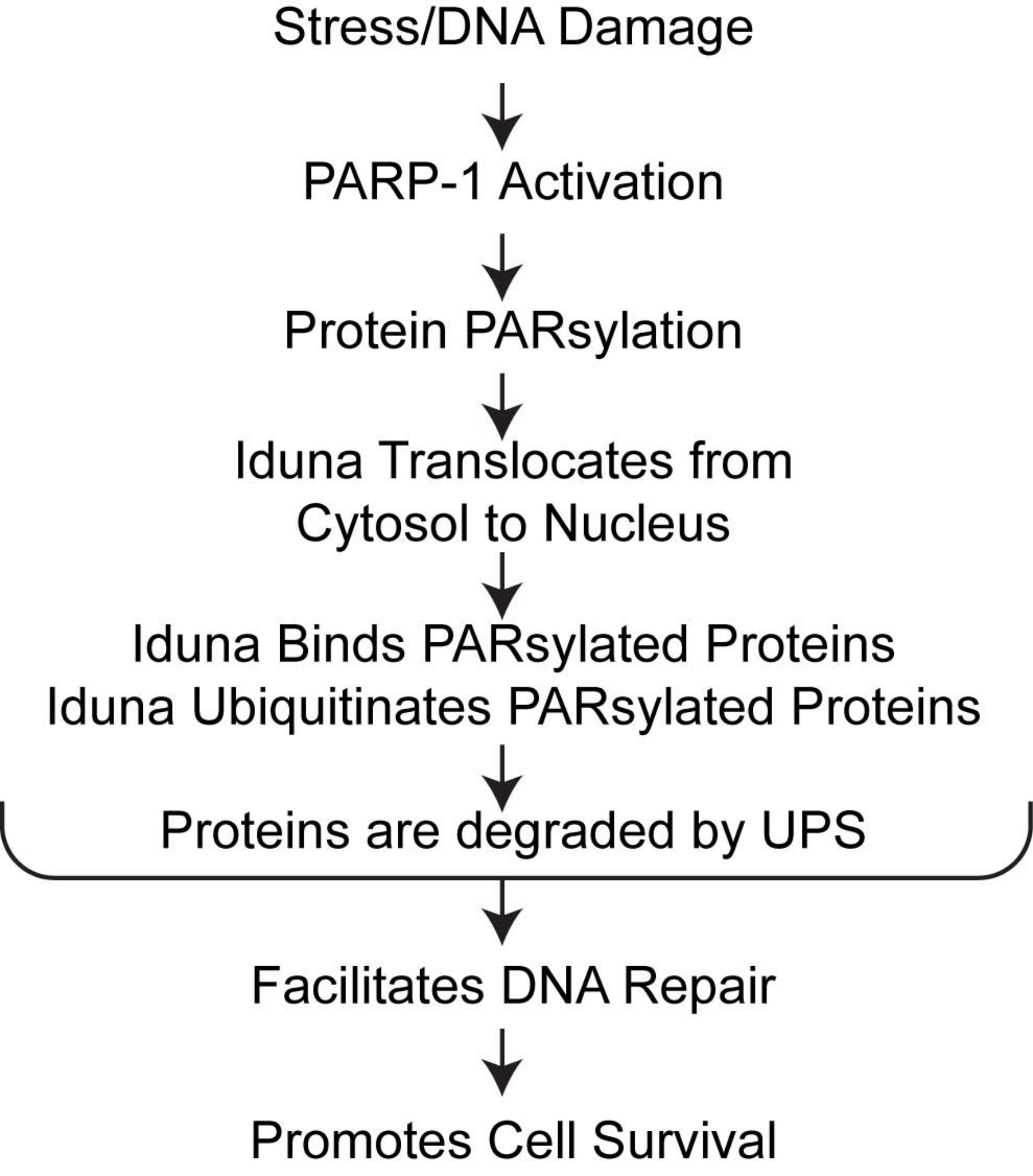
Supplementary Fig.5



Supplementary Fig. 6



Supplementary Fig. 7


Stress/DNA Damage
PARP-1 Activation
Protein PARsylation
Iduna Translocates from
Cytosol to Nucleus
Iduna Binds PARsylated Proteins
Iduna Ubiquitinates PARsylated Proteins
Proteins are degraded by UPS
Facilitates DNA Repair
Promotes Cell Survival