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Supplementary Materials for

A conserved SUMO pathway repairs topoisomerase DNA-protein cross-links by engaging ubiquitin-mediated proteasomal degradation

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SUPPLEMENTARY FIGURE LEGENDS

Fig. S1: Inhibiting SUMOylation Prevents the Repair of Human TOP-DPCs.
(A) MG132, TAK-243 or ML-792 alone does not induce TOP-DPCs. HEK293 cells were treated with CPT (1 μM), ETP (10 μM), MG132 (10 μM), TAK-243 (10 μM) or SAE inhibitor ML-792 (10 μM) for 1 h. Cells were subjected to ICE assay for detection of TOP-DPCs using anti-TOP1, anti-TOP2α and anti-TOP2β antibodies. Samples were probed with anti-dsDNA antibody in parallel.
(B) Viability curves for 72 h CPT treatments in HCT116 cells (mean ± SD, n = 3). 25 nM ML-792 and 25 nM TAK243 were administered 4 h before the CPT treatments.
(C) Viability curves for 72 h ETP treatments in HCT116 cells (mean ± SD, n = 3). 25 nM ML-792 and 25 nM TAK243 were administered 4 h before the ETP treatments.

Fig. S2: SUMO and Ub Modifications of Human TOP-DPCs.
(A) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub-TOP1 signals generated from triplicate experiments including blots shown in Fig. 2D. Density of the PTM-TOP1/density of tubulin of each group was normalized to that of DMSO-treated WT His6-TOP1 cells.
(B) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP1-DPC signals generated from triplicate experiments including blots shown in Fig. 2G. Density of the PTM-TOP1-DPCs or total TOP1-DPCs/density of DNA of each group was normalized to that of cells treated with CPT for 10 min.
(C) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP2-DPC signals generated from triplicate experiments including blots shown in Fig. 2H. Density of the PTM-TOP2-DPCs or total TOP2-DPCs/density of DNA of each group was normalized to that of cells treated with ETP for 10 min.
(D) HEK293 cells were exposed to increasing concentrations of CPT for 30 min, followed by DUST assay for detection of SUMO-2/3-TOP1-DPCs, SUMO-1-TOP1-DPCs, Ub-TOP1-DPCs and total TOP1-DPCs.
(E) HEK293 cells were exposed to increasing concentrations of ETP for 30 min, followed by DUST assay for detection of SUMO-2/3-TOP1-DPCs, SUMO-1-TOP2-DPCs, Ub-TOP2-DPCs and total TOP2-DPCs.
(F) HEK293 cells were pretreated with MG132 (10 μM, 1 h) or DMSO then exposed to 20 μM CPT for 30, 120 and 360 min, followed by DUST assay for detection of SUMO-2/3-, SUMO-1-, Ub- and total TOP1-DPCs.
(G) HEK293 cells were pretreated with MG132 (10 μM, 1 h) or DMSO then exposed to 20 μM ETP for 30, 120 and 360 min, followed by DUST assay for detection of SUMO-2/3-, SUMO-1-, Ub- and total TOP2-DPCs.
(H) HeLa WT and TOP2B CRISPR KO cells transfected with control siRNA or TOP2A siRNA were treated with ETP (200 μM, 30 min) before performing DUST assay for detection of SUMO-1-TOP2-DPCs, SUMO-2/3-TOP2-DPCs, Ub-TOP2-DPCs and total TOP2-DPCs.

Fig. S3: SUMOylation and Ubiquitylation Linkages of Human TOP-DPCs

(A) HEK293 cells were transfected with indicated plasmids to overexpress HA-tagged WT and mutant ubiquitin proteins, followed by CPT treatment (20 μM, 1 h) and DUST assay for ubiquitylated TOP1-DPC detection using HA antibody.

(B) HEK293 cells were transfected with indicated plasmids to overexpress HA-tagged WT and mutant ubiquitin proteins, followed by ETP treatment (200 μM, 1 h) and DUST assay for ubiquitylated TOP2-DPC detection using HA antibody.

(C) HEK293 cells were transfected with indicated plasmids and siRNAs, followed by CPT treatment (20 μM, 1 h) and DUST assay for detection of SUMO-1 and SUMO-2 modified TOP1-DPCs using HA antibody.

(D) HEK293 cells were transfected with indicated plasmids and siRNAs, followed by ETP treatment (200 μM, 1 h) and DUST assay for detection of SUMO-1 and SUMO-2 modified TOP2-DPCs using HA antibody.

Fig. S4: PIAS4 Physically Interacts with TOP1, TOP2α and TOP2 β in the Absence or Presence of Topoisomerase Inhibitors.

(A) Cells were transfected with the 6×His tagged TOP1 expression plasmid, followed by treatments with DMSO or 20 μM CPT in the absence or presence of 10 μM MG132 for 30 min. Native His-pull down was performed and His-pull down and input samples (same input samples shown in Fig. 2A) were subjected to WB using indicated antibodies.

(B) Cells were transfected with the FLAG tagged TOP2α expression plasmid, followed by treatments with DMSO or 200 μM ETP in the absence or presence of MG132 for 30 min. Native FLAG-IP was performed and IP and input samples (same input samples shown in Fig. 2B left panel) were subjected to WB using indicated antibodies.

(C) Cells were transfected with the FLAG tagged TOP2β expression plasmid, followed by treatments with DMSO or ETP in the absence or presence of MG132 for 30 min. Native FLAG-IP was performed and IP and input samples (same input samples shown in Fig. 2B right panel) were subjected to WB using indicated antibodies.

(D) U2OS cells were transfected with empty vector (EV), FLAG-PIAS4 or RNF4-FLAG expression plasmids before DMSO or CPT treatment (20 μM, 30 min), followed by proximity ligation assay (PLA) using anti-TOP1 and anti-FLAG antibodies. Scale bar represents 10 μm.

(E) U2OS cells were transfected with empty vector, FLAG-PIAS4 or RNF4-FLAG expression plasmids before DMSO or ETP treatment (200 μM, 30 min), followed by PLA using anti-TOP2α and anti-FLAG antibodies. Scale bar represents 10 μm.

(F) Cells were transfected with indicated siRNAs, followed by WB of the whole cellular lysates to validate downregulation of each protein.

(G) PIAS4 does not auto-SUMOylate. SUMO-1 and SUMO-2 conjugation assay incubated with SUMO E1, SUMO E2, SUMO proteins and increasing concentrations of PIAS4 in the absence of topoisomerases. Reaction products were separated by SDS-PAGE and monitored by WB using anti-PIAS4 antibody, anti-SUMO-1 antibody and anti-SUMO-2/3 antibody.

(H) RNF4 does not auto-ubiquitylate. Ub conjugation assay incubated with Ub E1, Ub E2, SUMO proteins and increasing concentrations of RNF4 in the absence of topoisomerases. Reaction products were separated by SDS-PAGE and monitored by WB using anti-RNF4 antibody and anti-Ub antibody.

Fig. S5: Coordination of PIAS4 and RNF4 for TOP-DPC repair.

(A) WB with anti-PIAS4 antibody to validate the depletion of PIAS4 in HCT116 PIAS4 CRISPR KO cells.

(B) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP1-DPC signals generated from triplicate experiments including blots shown in Fig. 4A. Density of the PTM-TOP1-DPCs or total TOP1-DPCs/density of DNA of each group was normalized to that of WT cells treated with CPT for 30 min. NS, not significant.

(C) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP2-DPC signals generated from triplicate experiments including blots shown in Fig. 4B. Density of the PTM-TOP2-DPCs or total TOP2-DPCs/density of DNA of each group was normalized to that of WT cells treated with ETP for 30 min.

(D) WB with anti-RNF4 antibody to validate the depletion of RNF4 in MCF7 RNF4 CRISPR KO cells.

(E) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP1-DPC signals generated from triplicate experiments including blots shown in Fig. 4C. Density of the PTM-TOP1-DPCs or total TOP1-DPCs/density of DNA of each group was normalized to that of WT cells treated with CPT for 30 min.

(F) Densitometric analyses comparing SUMO-2/3-, SUMO-1-, Ub- and total TOP2-DPC signals generated from triplicate experiments including blots shown in Fig. 4D. Density of the PTM-TOP2-DPCs or total TOP2-DPCs/density of DNA of each group was normalized to that of WT cells treated with ETP for 30 min.

(G) WB with anti-FLAG antibody in HCT116 cells transfected with empty vector, FLAG-PIAS4 WT, FLAG-PIAS4, FLAG-PIAS4 SAPΔ or FLAG-PIAS4 C342A expression plasmids for 48 h.

(H) WB with anti-FLAG antibody in MCF7 cells transfected with empty vector or RNF4-FLAG WT, RNF4-FLAG SIMΔ or RNF4-FLAG H156A expression plasmids for 48 h.

(I) Left: U2OS cells were transfected with indicated plasmids (FLAG-tagged) before CPT treatment (20 μM, 30 min), followed by PLA using anti-TOP1 and anti-FLAG antibodies. Right: U2OS cells were transfected with indicated plasmids (FLAG-tagged) before ETP treatment (200 μM, 30 min), followed by PLA using anti-TOP2α and anti-FLAG antibodies. Scale bars represent 10 μm.

(J) Densitometric quantitation of foci per cells of each treatment group as shown in panel I.

Fig. S6: SUMOylation and SUMO-dependent ubiquitylation of TOP-DPCs are induced independently of replication, transcription and DDRs in Human Cells.

(A) HEK293 cells were pre-treated with the DNA-PK inhibitor VX984 (10 μM, 6 h), the ATM inhibitor KU55399 (10 μM, 6 h), the ATR inhibitor AZD6738 (10 μM, 6 h), the replication inhibitor aphidicolin (APH, 10 μM, 2 h), the transcription inhibitor DRB (100 μM, 2 h) before treatment with CPT (20 μM, 1 h). DUST assay was performed to detect SUMO-2/3-, SUMO-1-, Ub-, and total TOP1-DPCs.

(B) HEK293 cells were pre-treated with the DNA-PK inhibitor VX984 (10 μM, 6 h), the ATM inhibitor KU55399 (10 μM, 6 h), the ATR inhibitor AZD6738 (10 μM, 6 h), the replication inhibitor aphidicolin (APH, 10 μM, 2 h), the transcription inhibitor DRB (100 μM, 2 h) before treatment with ETP (200 μM, 1 h). DUST assay was performed to detect SUMO-2/3-, SUMO-1-, Ub-, and total TOP2-DPCs.

(C) HEK293 cells were transfected with or without the RNF4 overexpression plasmid as indicated. Following pre-treatment with APH, DRB or ML-792, 20 μM CPT was added for an additional 1 h. DUST assay was performed to detect Ub- and total TOP1-DPCs.
(D) HEK293 cells were transfected with or without the RNF4 overexpression plasmid as indicated. Following pre-treatment with APH, DRB or ML-792, 200 μM ETP was added for an additional 1 h. DUST assay was performed to detect Ub- and total TOP2-DPCs.

Fig. S7: RNF4 Drives Proteasomal Degradation of TOP-DPCs and Responses to Topoisomerase-Mediated DNA damage.
(A-C) Densitometric analyses comparing TOP1-, TOP2α- or TOP2β-DPC signals generated from triplicate experiments including blots shown in Fig. 5A-C. Density of TOP-DPCs/density of DNA of each group was normalized to MCF WT cells treated with CPT or ETP alone.
(D) HEK293 cells were transfected with empty vector, HA-Ub K48 or HA-Ub K63, followed by transfection with RNF4 as indicated. Following CPT treatment (20 μM, 1 h), cells were subjected to DUST assay for detecting ubiquitylated TOP1-DPCs using anti-HA antibody.
(E) HEK293 cells were transfected with empty vector, HA-Ub K48 or HA-Ub K63, followed by transfection with RNF4 as indicated. Following ETP treatment (200 μM, 1 h), cells were subjected to DUST assay for detecting ubiquitylated TOP2-DPCs using anti-HA antibody.
(F) U2OS cells were transfected with indicated siRNAs, followed by 1 h CPT (1 μM) treatment and IF for detection of γH2AX. Scale-bar represents 20 μm.
(G) Densitometric quantitation of foci per cells for each treatment group from experiments performed as shown in panel F.
(H) U2OS cells were transfected with indicated siRNAs, followed by 1 h ETP (5 μM) treatment and IF for detection of γH2AX. Scale-bar represents 20 μm.
(I) Densitometric quantitation of foci per cells of each treatment group from experiments performed as shown in panel H.
(J) HEK293 cells were transfected with indicated siRNAs, followed by CPT (20 μM) treatment. Cells were collected at indicated time points for WB for detection of γH2AX. Density of γH2AX/density of tubulin of each group was normalized to that of control cells at time = 0.
(K) HEK293 cells were transfected with indicated siRNAs, followed by ETP (200 μM) treatment. Cells were collected at indicated time points for WB for detection of γH2AX. Density of γH2AX/density of tubulin of each group was normalized to that of control cells at time = 0.

Fig. S8: The SUMO-Ub-Proteasome Pathway Repairs Topoisomerase-Mediated DNA Damage in Yeast.
(A) TOP1 levels in YMM10 cells carrying pYX112 HA-TOP1 plasmid after treatments with DMSO, 10 μM MG132, 10 μg/ml CPT or CPT + MG132 for a total of 4 h. Lysates were analyzed by WB with anti-HA antibody.
(B) TOP2 levels in YMM10 cells carrying pDED1 TOP2-3×HA plasmid following treatments with DMSO, MG132, 100 μg/ml ETP or ETP + MG132 for a total of 4 h. Lysates were analyzed by WB with anti-HA antibody.
(C) YMM10 cells expressing HA-TOP1 were exposed to DMSO, 10 μM MG132, 5 μg/ml CPT or CPT + MG132 at 30°C for different times (2, 4, 6, 24 h). Aliquots were removed, diluted, and plated to SD-ura plates for clonogenic assay. Cell survival is expressed as the percentage of

surviving cells at the time points relative to the viable titer at the time drug was added (t = 0). Error bars represent the standard deviation of three independent experiments. Data points without error bars have error bars that are smaller than the symbol drawn on the graph.
(D) YMM10 cells expressing TOP2-HA were exposed to DMSO, 10 μM MG132, 50 μg/ml ETP or ETP + MG132 at 30°C for different times (2, 4, 6, 24 h) for clonogenic assay.
(E) WB showing TOP1 levels in YMM10 strains carrying the HA-TOP1 WT or 3KR plasmid.
(F) WB showing TOP2 levels in YMM10 strains carrying the TOP2-HA WT or *SNM* plasmid.
(G) WB showing TOP1 levels in indicated BY4741 strains carrying the HA-TOP1 plasmid.
(H) WB showing TOP2 levels in indicated BY4741 strains carrying the TOP2-HA plasmid.
(I) WB showing TOP1 levels in indicated BY4741 strains carrying the HA-TOP1 plasmid.
(J) WB showing TOP2 levels in indicated BY4741 strains carrying the TOP2-HA plasmid.
(K) Densitometric analyses comparing TOP1-DPC signals generated from triplicate experiments including blots shown in Fig. 6I. Density of TOP1-DPCs/density of DNA of each group was normalized to that of WT cells.
(L) Densitometric analyses comparing TOP2-DPC signals generated from triplicate experiments including blots shown in Fig. 6J. Density of TOP2-DPC/density of DNA of each group was normalized to that of WT cells.

Fig. S1: Inhibiting SUMOylation Prevents the Repair of TOP1- and TOP2-DPCs in Human Cells.

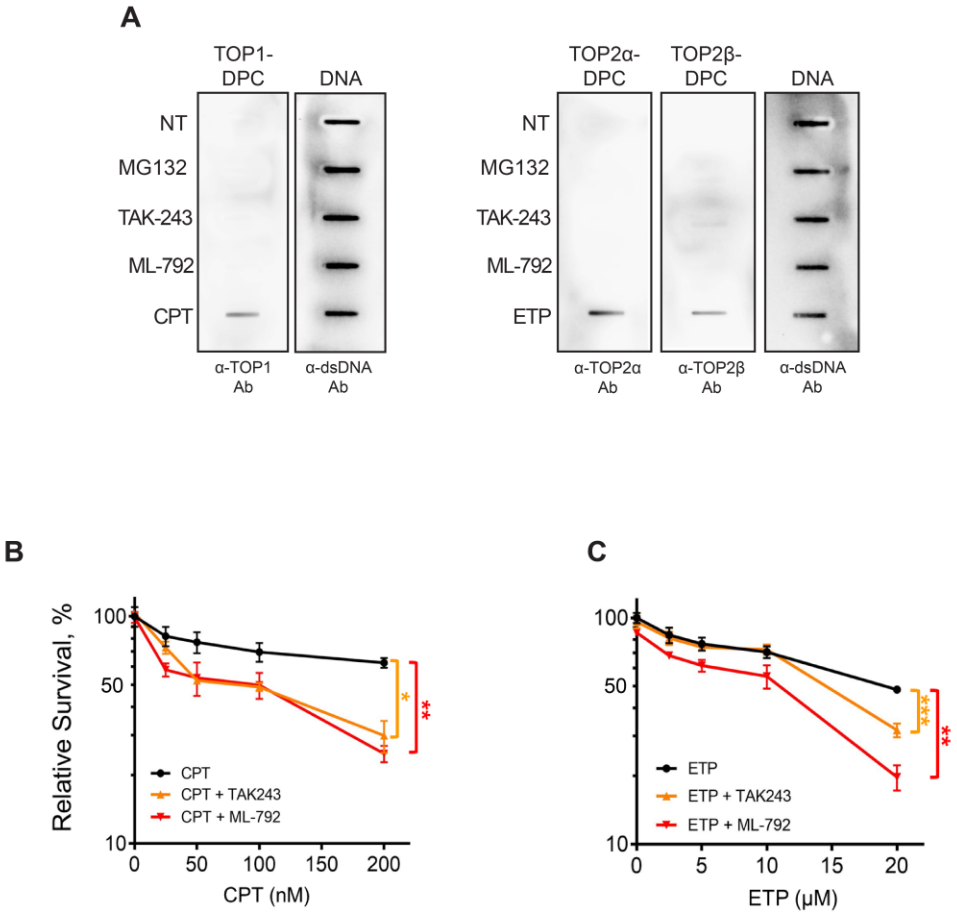

Fig. S2: SUMO and Ub Modifications of TOP1- and TOP2-DPCs in Human Cells

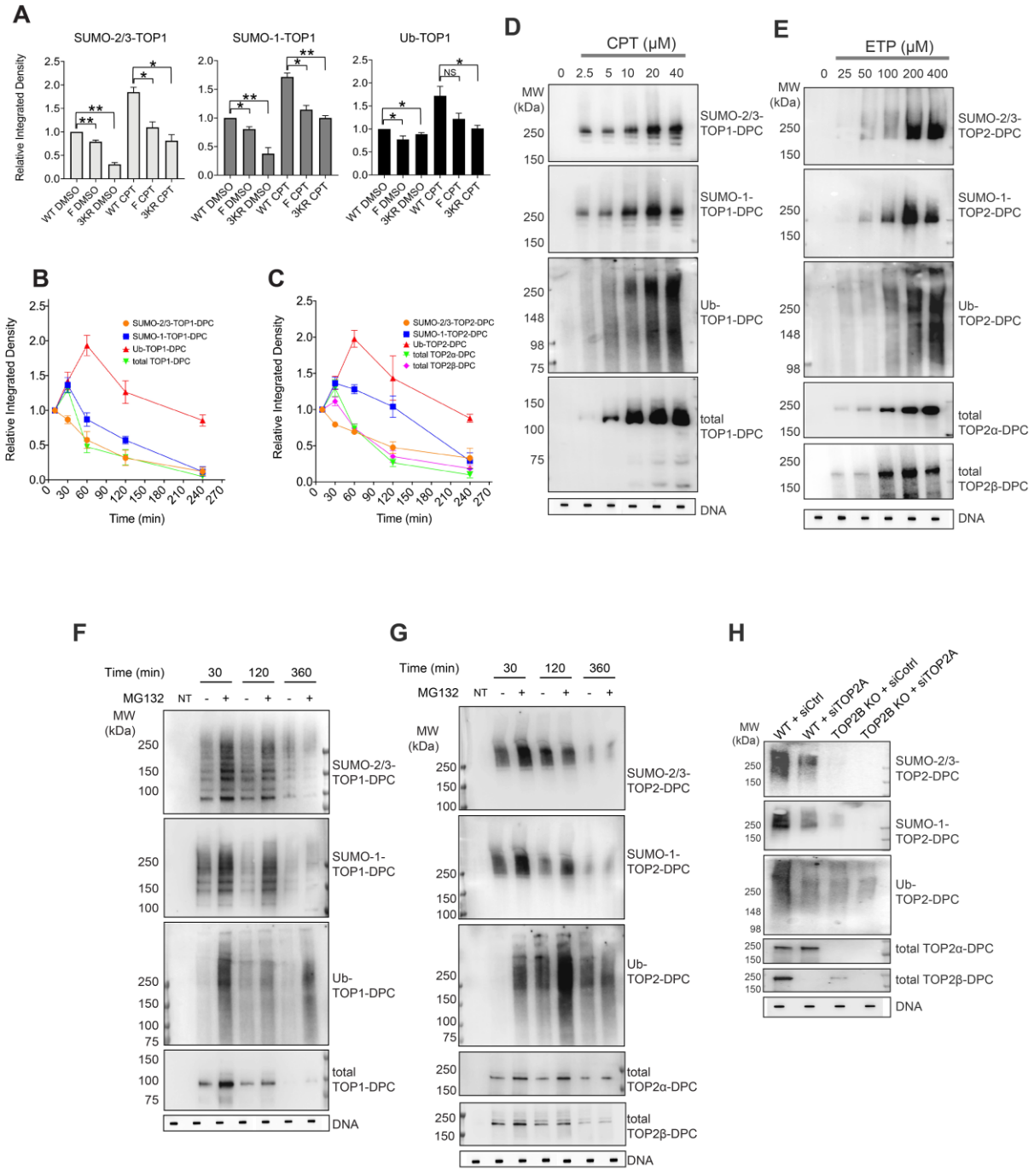

Fig. S3: SUMOylation and Ubiquitylation Linkages of TOP1- and TOP2-DPCs in Human Cells.

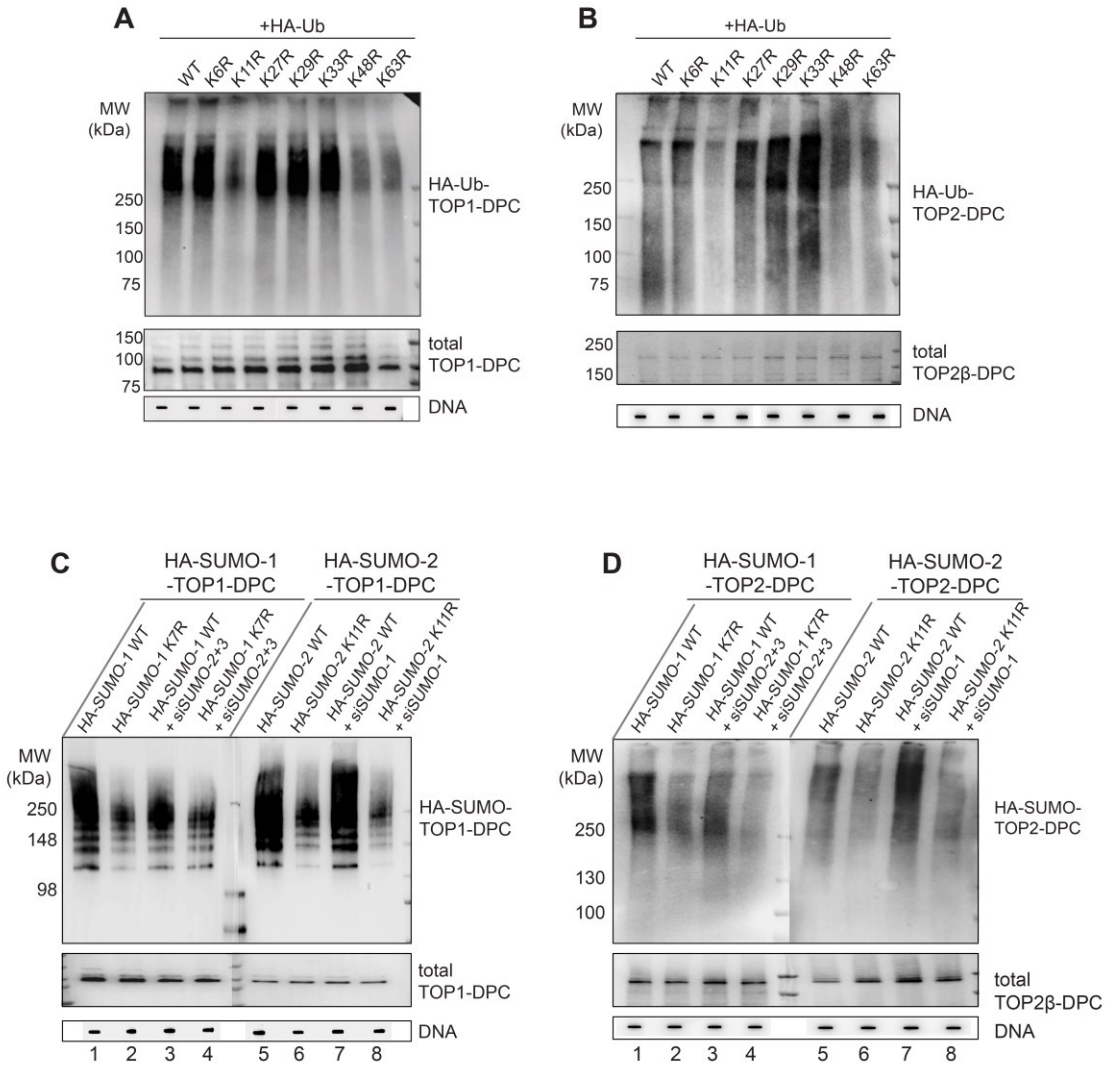

Fig. S4: PIAS4 Physically Interacts with TOP1, TOP2α and TOP2 β in the Absence or Presence of topoisomerase Inhibitors

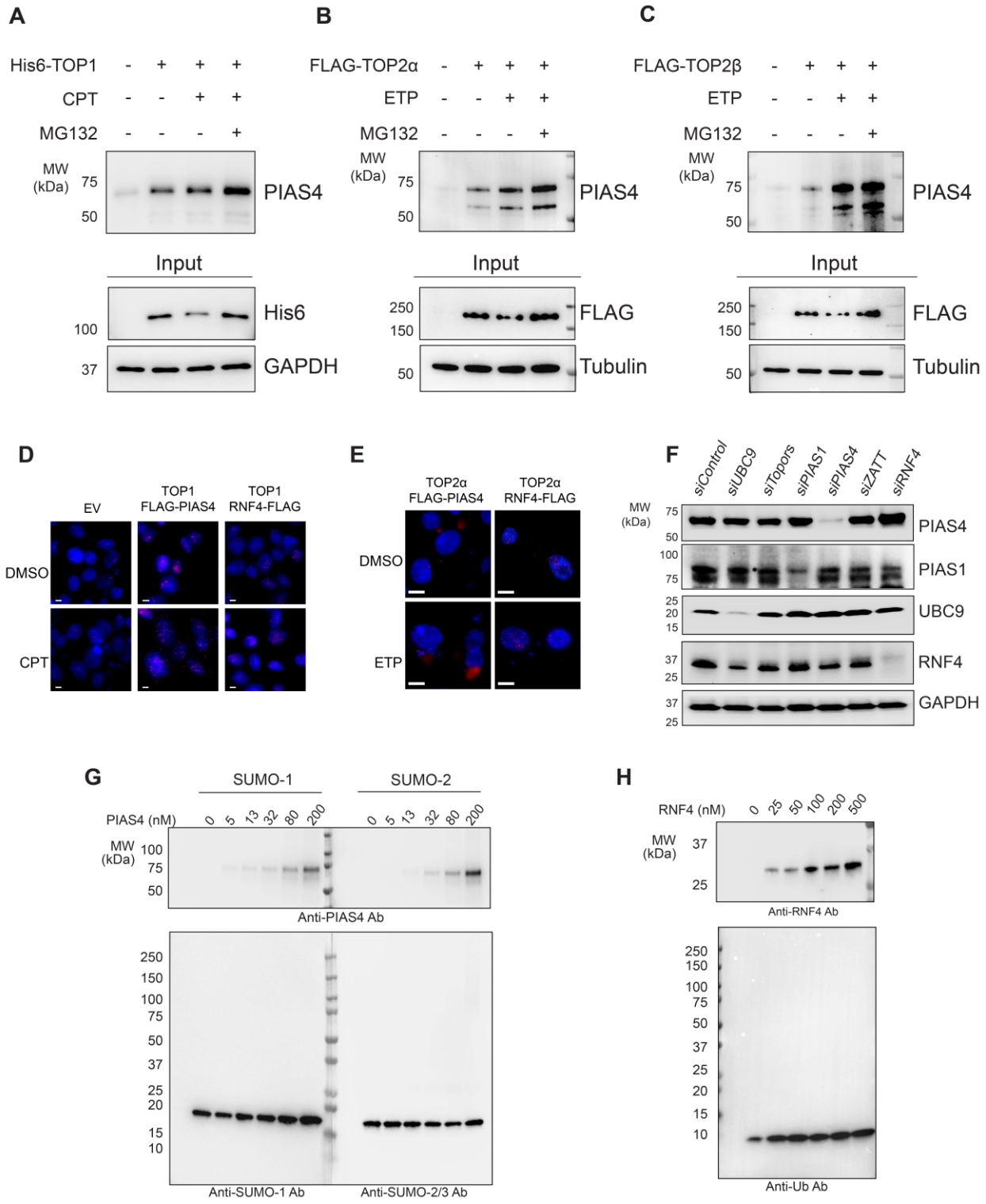

Fig. S5: Coordination of PIAS4 and RNF4 for TOP-DPC repair

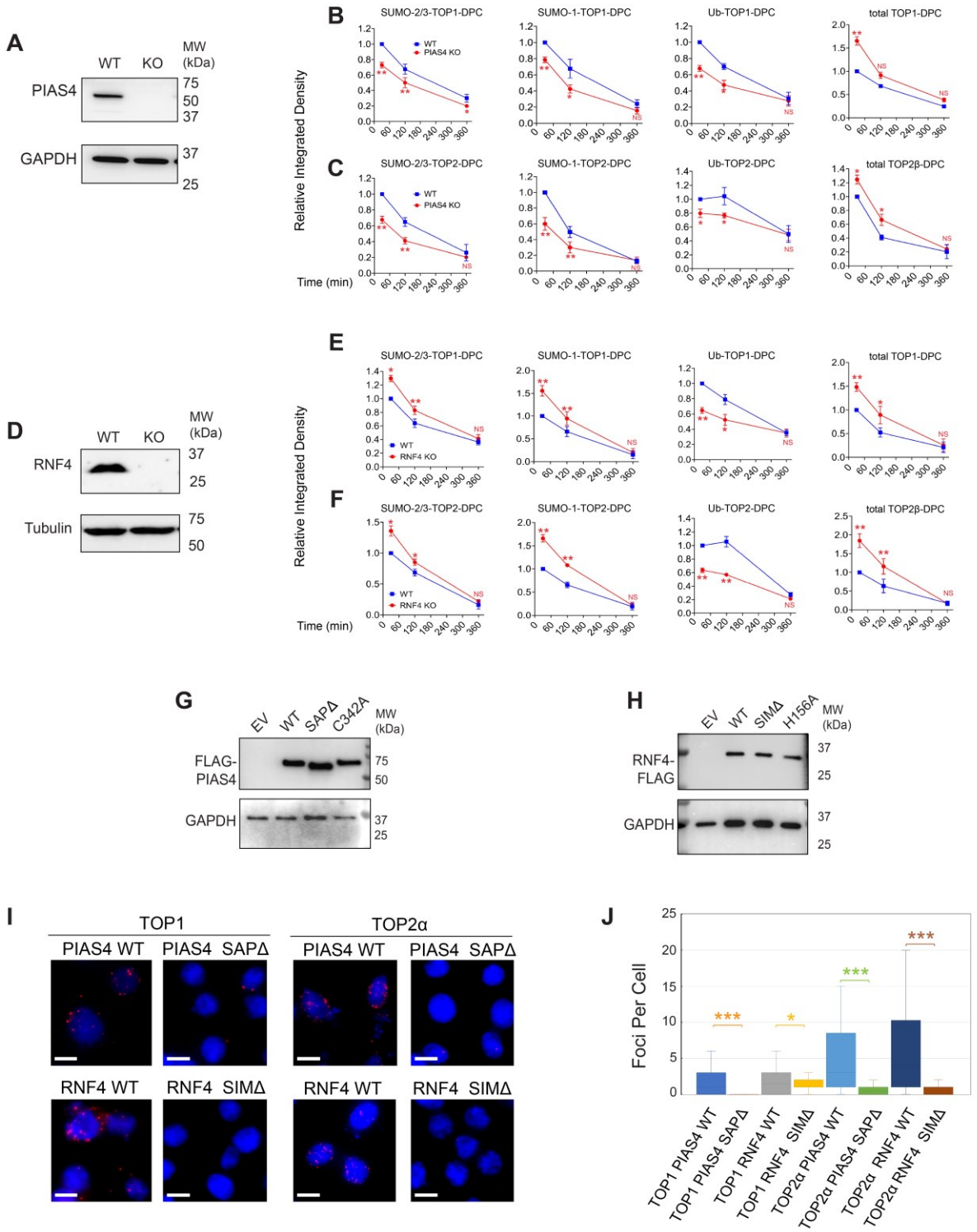

Fig. S6: SUMOylation and SUMO-dependent ubiquitylation of TOP-DPCs are induced independently of replication, transcription and DDRs in Human Cells

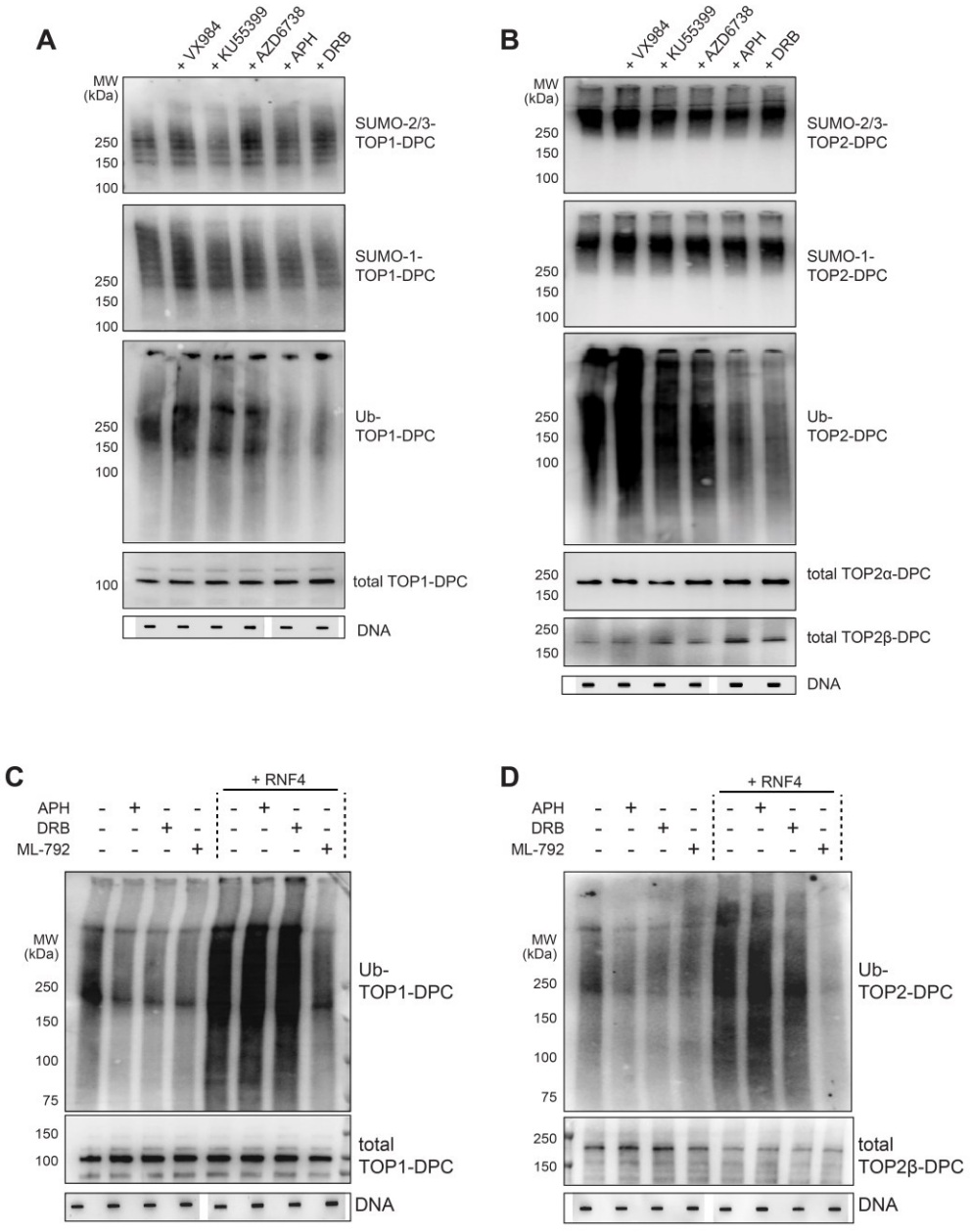

Fig. S7: RNF4 Drives Proteasomal Degradation of TOP-DPCs and Responses to Topoisomerase-mediated DNA damage

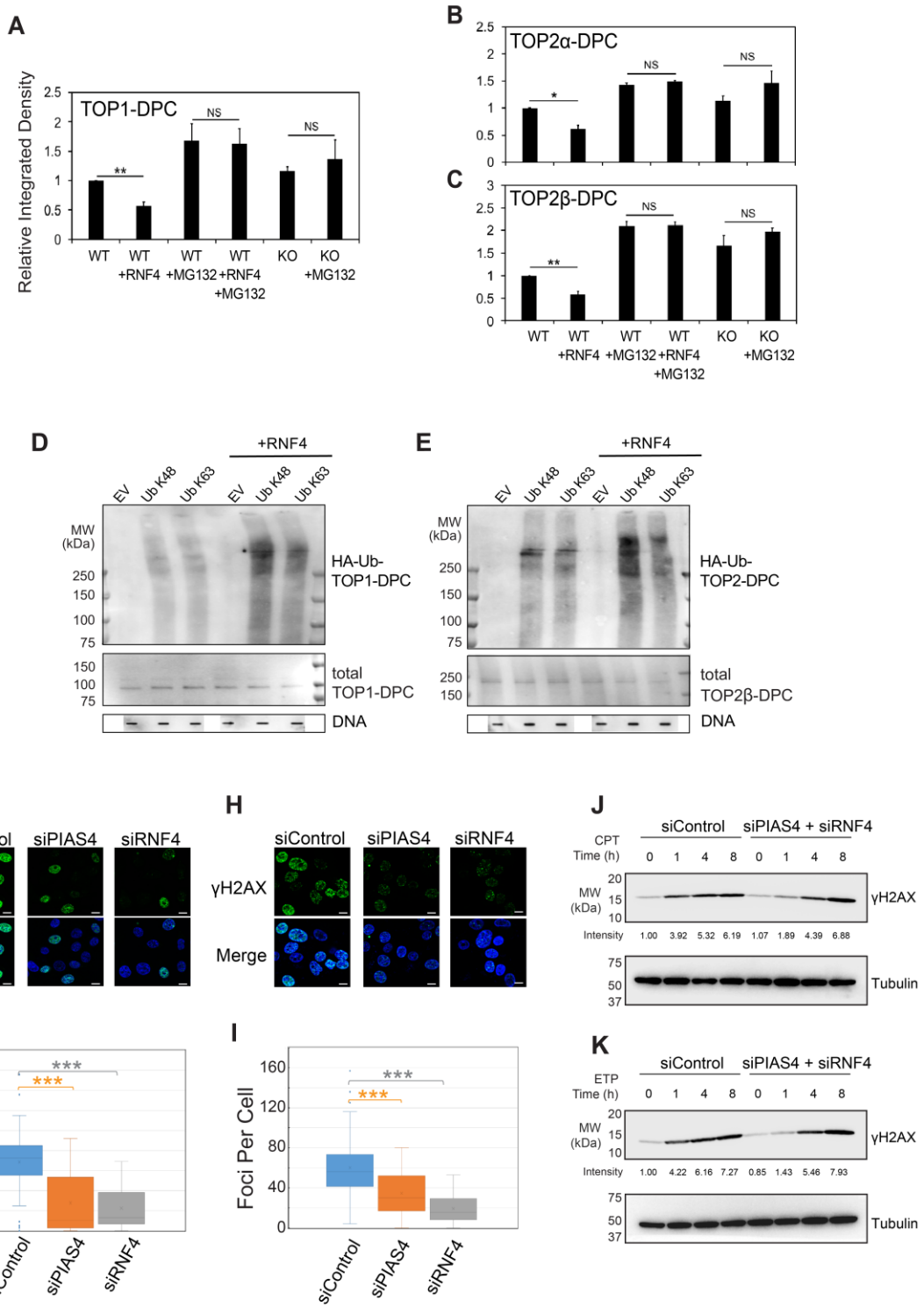

Fig. S8: The SUMO-Ub-Proteasome Pathway Repairs Topoisomerase-Mediated DNA Damage in Yeast

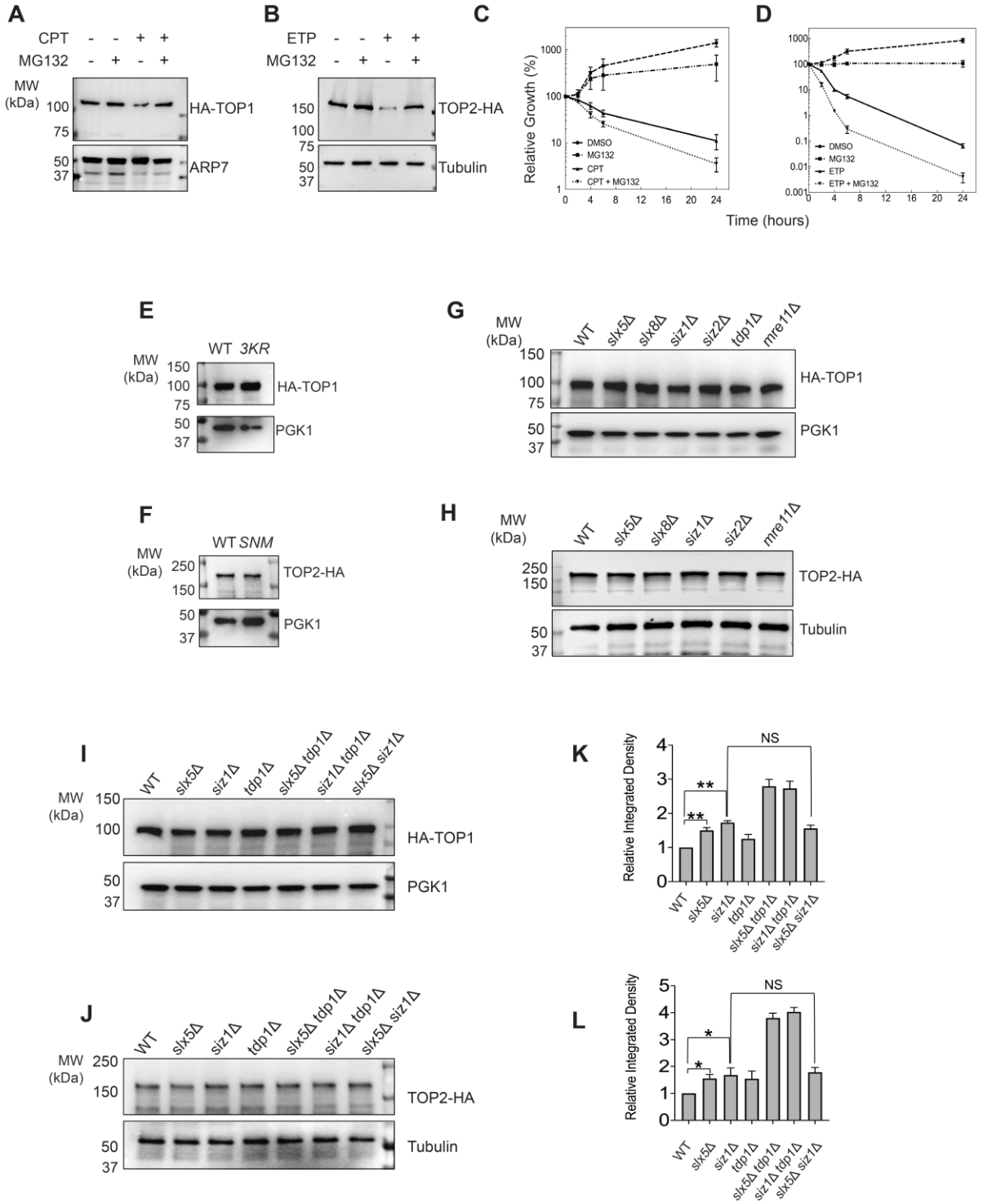

Table S1

Chemicals	Source	Identifier
Camptothecin (CPT)	Sigma	Cat# C9911
Etoposide (ETP)	Sigma	Cat# E1383
Amsacrine	Sigma	Cat# A9809
MG132	Selleckchem	Cat# S2619
TAK-243	Chemietek	Cat# CT-M7243
ML-792	MedKoo	Cat# 407886
VX-984	MedKoo	Cat# 206853
KU-55399	Selleckchem	Cat# S1092
AZD6738	Selleckchem	Cat# S7693
Aphidicolin	Sigma	Cat# A0781
5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside (DRB)	Sigma	Cat# D1916

Table S2

Human Cell Lines	Source
HEK293 cells	ATCC
HCT116 cells	ATCC
HCT116 PIAS4 KO cells	This study
MCF7 cells	ATCC
MCF7 RNF4 KO cells	This study
U2OS cells	ATCC
HeLa cells	ATCC
HeLa TOP2B KO cells	This study

Table S3

Oligonucleotides	Sequences
TOP2B gRNA	5’-CACCGGATTTGGCTGGTTCGTGTAG-3’ and 5’-ACCGTAAATTTGGACAGATCTGGT-3’
PIAS4 gRNA	5’-CACCGCATGCACTCCACCTACGACC-3’ and 5’-CACCGAGAACGGCAGCTTCACCAGG-3’
RNF4 gRNA	5’-CACCGAGGCAAAGAAAATCGAGACC-3’ and 5’-CACCGCATACACTCTCGTCCACGGC-3’
His6-human TOP1	5’-GCATATCCTCGAGCGCCACCATGGCCCATCATCACCATCACCACAGTGGGGACCACCTCCACAAC -3’ and 5’-GCTAGAGCGGCCGCCTAAAACTCATAGTCTTCATCAGC -3’
FLAG-human TOP2α	5’-GCATATCCTCGAGCGCCACCATGGACTACAAGGACGACGATGACAAGGAAGTGTCACCATTGCAGCCTGTAAAT-3’ and 5’-GCTAGAGCGGCCGCTTAAAACAGATCATCTTCATCTGACTC-3’
FLAG-human TOP2β	5’-GCATATCCTCGAGCGCCACCATGGACTACAAGGACGACGATGACAAGGCCAAGTCGGGTGGCTGCGGCGCGGGA-3’ and 5’-GCATATCCTCGAGCGCCACCATGGCCCATCATCACCATCACCACGCCAAGTCGGGTGGCTGCGGCGCGGGA-3’
TOP1 Y723F	5’-CTAGGGTCCAGAAAATTGAGTTTGGAGGTTCCCAGG-3’
TOP1 K117, K153, K103R (CPT3KR)	5’-TCTTCAGGTTCATCTCTAATTTGTGGTGGACTAGAGAAGC-3’ (K117R), 5’-TCTTGGTATCTTCTGTTCTAATTTTCTTAGGTTTATAATCAGCATCATCC-3’ (K153R), 5’-TTCCTTCTCCTTCCTTATTTTTGCATCCCCAGAGGCT-3’ (K103R)
Ubiquitin K6R	5’-ATCTTCGTGAGGACCCTGACTGG-3’
Ubiquitin K11R	5’-CTGACTGGTAGGACCATCACTC-3’
Ubiquitin K27R	5’-GAGAATGTCAGGGCAAAGATCC-3’
Ubiquitin K29R	5’ GTCAAGGCAAGGATCCAAGAC-3’
Ubiquitin K33R	5’-ATCCAAGACAGGGAAGGCATC-3’
Ubiquitin K48R	5’-TTTGCTGGGAGACAGCTGGAA-3’
Ubiquitin K63R	5’-AACATCCAGAGAGAGTCCACCC-3’
SUMO-1 K7R	5’-CAGGAGGCAAGACCTTCAACTG-3’
SUMO-2 K11R	5’-GAAGGAGTCAGGACTGAGAACAAC-3’
PIAS4 C342A	5’-GGCAGAGACCGCCGCCCACCTGCAG-3’
PIAS4 SAPΔ	5’-CAGTTTGACTGTAGCCCTG-3’
RNF4 SIM2, 3, 4Δ	5’-GACGCCACTTGTGAATCTTTAGAGC-3’ (SIM2Δ), 5’-GATGCGACTCACAATGACTCTG-3’ (SIM3Δ), 5’-GCTGCAGACGAAAGAAGAAGACCAAG-3’ (SIM4Δ)
RNF4 H156A	5’-AGAATGCGGCGCTGTCTTCTGTAGC-3’
HA-yeast TOP1	5’-GCATATCCATGGCCTATCCGTATGATGTGCCTGACTACGCAACTATTGCTGATGCTTCCAAAGTT-3’ and 5’-GCTAGACTCGAGTTAAAACCTCCAATTTTCATCTAC-3’
Yeast TOP1 K65, 91, 92R	5’- AGATTTCCAAGAAAAAGACTAAGAAAATAAGGACCGAACCAGTGCAA-3′(K65R), 5′-G GCGCGACATCAAAGCCTAAAAAAATCAGGAGAGAAGATGGTGATG-3′(K91,92R)
Yeast TOP2 SNM	5’-GCTAGAAAGGGCAAAAAAATTAGGGTCGAGGATAAGAATTTTG-3′(K1220R), 5′-GCAAGGCGCCTACAAAGATTAGAAGAGAGAAAACGCCTTCTGTTTC-3′(K1246R, K1247R) and 5′-CTTCTTCTATTTTCGACATAAgGAgAGAAGATAAAGATGAGGGCG-3′(K1277R, K1278R)

Table S4

Expression Plasmids	Source	Identifier
pT-REx His6-hTOP1	This study	N/A
pT-REx His6-hTOP1 CPT3KR	This study	N/A
pT-REx His6-hTOP1 Y723F	This study	N/A
pT-REx FLAG-hTOP2α	This study	N/A
pT-REx-FLAG-hTOP2β	This study	N/A
pRK5 HA-Ubiquitin WT	Addgene	Cat# 17608
pRK5 HA-Ubiquitin K6R	This study	N/A
pRK5 HA-Ubiquitin K11R	This study	N/A
pRK5 HA-Ubiquitin K27R	This study	N/A
pRK5 HA-Ubiquitin K29R	This study	N/A
pRK5 HA-Ubiquitin K33R	This study	N/A
pRK5 HA-Ubiquitin K48R	This study	N/A
pRK5 HA-Ubiquitin K63R	This study	N/A
pRK5 HA-Ubiquitin K48	This study	N/A
pRK5 HA-Ubiquitin K63	This study	N/A
pcDNA3 HA-SUMO-1 WT	Addgene	Cat# 48966
pcDNA3 HA-SUMO-1 K7R	This study	N/A
pcDNA3 HA-SUMO-2 WT	Addgene	Cat# 48967
pcDNA3 HA-SUMO-2 K11R	This study	N/A
pCMV FLAG-PIAS4	Addgene	Cat# 15208
pCMV FLAG-PIAS4 K360, 370R	This study	N/A
pCMV FLAG-PIAS4 C342A	This study	N/A
pCMV FLAG-PIAS4 SAPΔ	This study	N/A
pCMV RNF4-Myc-FLAG WT	OriGene	Cat# RC207273
pCMV RNF4-Myc-FLAG SIM2, 3, 4Δ	This study	N/A
pCMV RNF4-Myc-FLAG H156A	This study	N/A
pDEST FLAG-HA-PSMD14	Addgene	Cat# 22557
pCMV PSMB5-Myc-FLAG	OriGene	Cat# RC209326
pSpCas9(BB)-2A-GFP (PX458)	Addgene	Cat# 48138
pSpCas9(BB)-2A-Puro (PX459) V2.0	Addgene	Cat# 62988
pYX112 HA-yTOP1	This study	N/A
pYX112 HA-yTOP1 K65, 91, 92R	This study	N/A
pDED1 yTOP2-3×HA	This study	N/A
pDED1 yTOP2-3×HA *SNM*	This study	N/A
pBluescript PDR1-DBD-Cyc8 (pAGS1)	(*60*)	N/A

Table S5

Small interfering RNA	Source	Identifier
Control siRNA	Dharmacon	Cat# D-001206-13-05
TOP2A siRNA	Dharmacon	Cat# M-004239-00-0005
SUMO-1 siRNA	Dharmacon	Cat# M-016005-00-0005
SUMO-2 siRNA	Dharmacon	Cat# L-016450-00
SUMO-3 siRNA	Dharmacon	Cat# L-019730-00
UBC9 siRNA	Dharmacon	Cat# M-004910-00-0005
TOPORS siRNA	Dharmacon	Cat# M-020048-00-0005
PIAS1 siRNA	Dharmacon	Cat# M-008167-00-0005
PIAS4 siRNA	Dharmacon	Cat# M-006445-00-0005
RNF4 siRNA	Dharmacon	Cat# M-006557-00-0005
RNF111 siRNA	Dharmacon	Cat# M-007002-00-0005

Table S6

Antibodies	Source	Identifier
Anti-ubiquitin, mouse monoclonal	Santa Cruz	Cat# sc-8017; RRID: AB 2762364
Anti-SUMO-1, rabbit monoclonal	Cell Signaling	Cat# 4940, RRID: AB_2302825
Anti-SUMO-2/3, rabbit monoclonal	Cell Signaling	Cat# 4971; RRID: AB_2198425
Anti-phospho-Histone H2A.X (Ser139), mouse monoclonal	Millipore	Cat# 05-636-I; RRID: AB_2755003
Anti-TOP1, mouse monoclonal	BD Biosciences	Cat# 556597; RRID: AB_396474
Anti-TOP1cc, mouse monoclonal	Millipore	Cat# MABE1084; RRID: AB_2756354
Anti-TOP2α, mouse monoclonal	Millipore	Cat# MAB4197; RRID: AB_2205862
Anti-TOP2β, mouse monoclonal	BD Biosciences	Cat# 611492; RRID: AB_398952
Anti-His tag, rabbit monoclonal	Cell Signaling	Cat# 12698; RRID: AB_2744546
Anti-FLAG, mouse monoclonal	Sigma	Cat# F1804; RRID: AB_262044
Anti-FLAG, rabbit polyclonal	Sigma	Cat# F7425; RRID: AB_439687
Anti-dsDNA, mouse monoclonal	Abcam	Cat# ab27156; RRID: AB_470907
Anti-UBC9, rabbit monoclonal	Cell Signaling	Cat# 4786; RRID: AB_10559206
Anti-PIAS1, rabbit polyclonal	Abcam	Cat# ab32219; RRID: AB_777265
Anti-PIAS4, rabbit polyclonal	Abcam	Cat# ab58416; RRID: AB_881880
Anti-Topors, mouse monoclonal	Santa Cruz	Cat# sc-101182; RRID: AB_2240782
Anti-RNF4, goat polyclonal	R&D Systems	Cat# AF7964
Anti-RNF111, mouse monoclonal	Novus Biologicals	Cat# H00054778-M05; RRID:AB_2285381)
Anti-HA, rabbit polyclonal	Santa Cruz	Cat# sc-805; RRID: AB_ 631618
Anti-α Tubulin, rat polyclonal	Santa Cruz	Cat# sc-53030; RRID: AB_ 2272440
Anti-ARP7, goat polyclonal	Santa Cruz	Cat# sc-8961; RRID: AB_ 671730
Anti-Smt3, rabbit polyclonal	Abcam	Cat# ab14405; RRID: AB_ 301186
Anti-ubiquitin, rabbit polyclonal	Abcam	Cat# ab19247; RRID: AB_444805

Table S7

Recombinant Proteins	Source	Identifier
Human Recombinant TOP1	This study	N/A
Human Recombinant TOP2α	This study	N/A
Human Recombinant TOP2β	This study	N/A
Human Recombinant Ubiquitin	Boston Biochem	Cat# U-100H
Human Recombinant Ubiquitin Activating Enzyme (UBE1)	Boston Biochem	Cat# E-305
Human Recombinant UbcH5a/UBE2D1	Boston Biochem	Cat# E2-616
Human Recombinant PIAS4	This study	N/A
Human Recombinant RNF4	R&D Systems	Cat# E3-210-050
Human Recombinant SUMO-1	Boston Biochem	Cat# UL-712
Human Recombinant SUMO-2	Boston Biochem	Cat# UL-752
Human Recombinant SUMO E1 (SAE1/UBA2)	Boston Biochem	Cat# E-311
Human Recombinant UBC9/UBE2I	Boston Biochem	Cat# E2-645

Table S8

Yeast Strains	Source
YMM10	(*49*)
BY4741 *slx5*Δ	Dharmacon
BY4741 *slx8*Δ	Dharmacon
BY4741 *siz1*Δ	Dharmacon
BY4741 *siz2*Δ	Dharmacon
BY4741 *tdp1*Δ	Dharmacon
BY4741 *mre11*Δ	Dharmacon
BY4741 *slx5*Δ *tdp1*Δ	This study
BY4741 *siz1*Δ *tdp1*Δ	This study
BY4741 *siz1*Δ *slx5*Δ	This study