Supplementary information for

LncRNA CTBP1-DT-encoded microprotein DDUP sustains DNA damage response signalling to trigger dual DNA repair mechanisms

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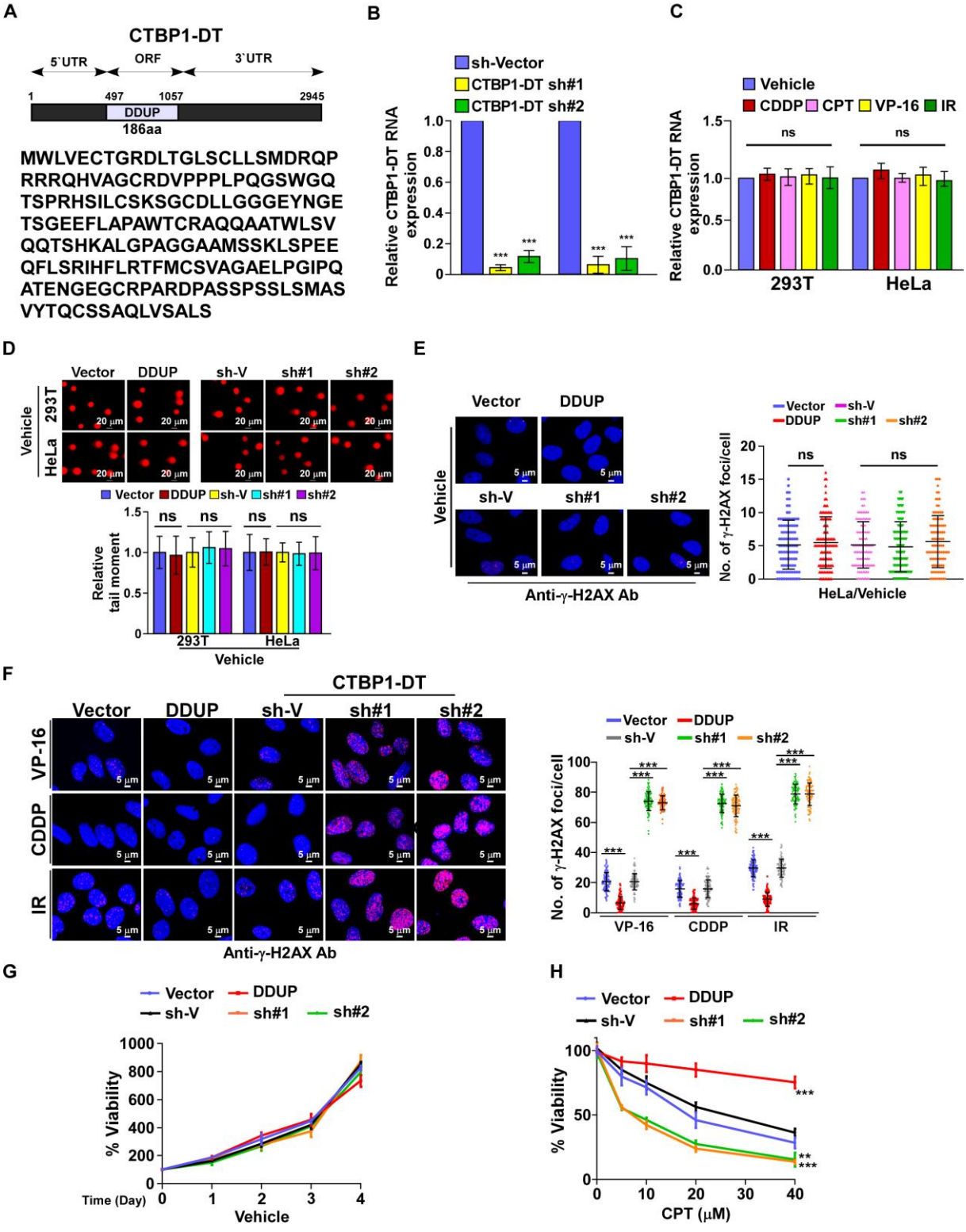
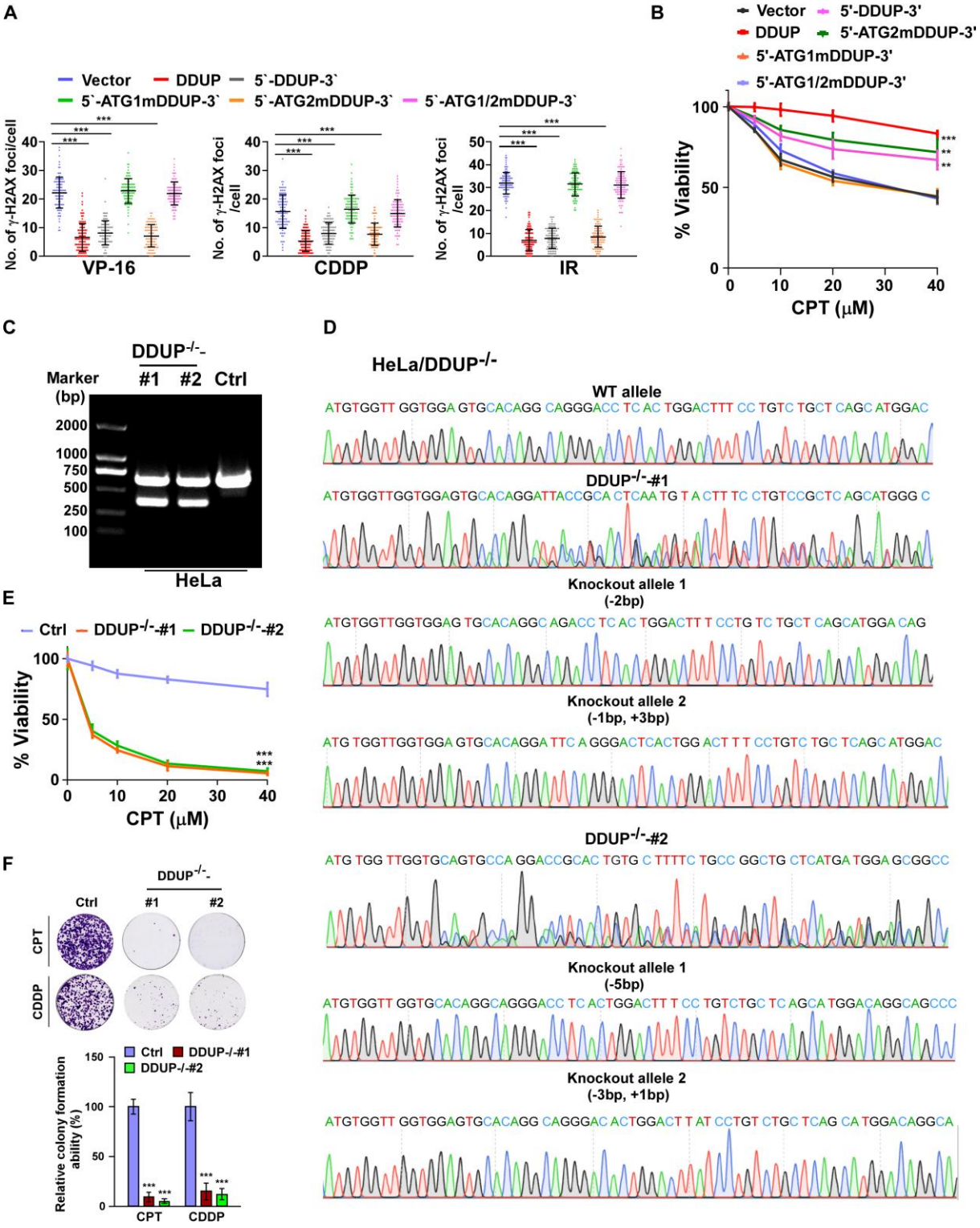
Supplementary Figure S1



Supplementary Figure S1. LncRNA CTBP1-DT-encoded DDUP microprotein promotes DNA damage repair. A. Diagram of the 5'- and 3'-untranslated regions (UTRs) and the open reading frame (ORF) in human lncRNA CTBP1-DT (upper) and the corresponding 186 amino acid (aa) sequence (lower). **B.** Real-time PCR analysis of CTBP1-DT expression in sh-vector- and CTBP1-DT shRNAs-transduced cells. GAPDH served as a control. **C.** Real-time PCR analysis of CTBP1-DT expression in

the indicated cells treated with CPT (10 μM) or VP-16 (10 μM) or CDDP (5 μM) for 1 hour or with IR (10 Gy) and were allowed to recover for six hours. GAPDH served as a loading control. **D.** Representative images (left) and quantification (right) of damaged DNA in the indicated vehicle-treated cells determined by comet assay (n = 100). Scale bar = 20 μm. **E.** Representative images (left) and quantification (right) of γ-H2AX foci in the indicated cells with vehicle treatment. At least 100 cells were counted. Scale bar = 5 μm. **F.** Representative images (left) and quantification (right) of γ-H2AX foci in the indicated cells treated with CPT (10 μM), VP-16 (10 μM), CDDP (5 μM) for 1 hour or IR (10 Gy) (>100 counted cells). Scale bar = 5 μm. **G-H.** MTT assay analysis of indicated cell survival in response to vehicle (G) or CPT(H) treatment at the indicated concentration. Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

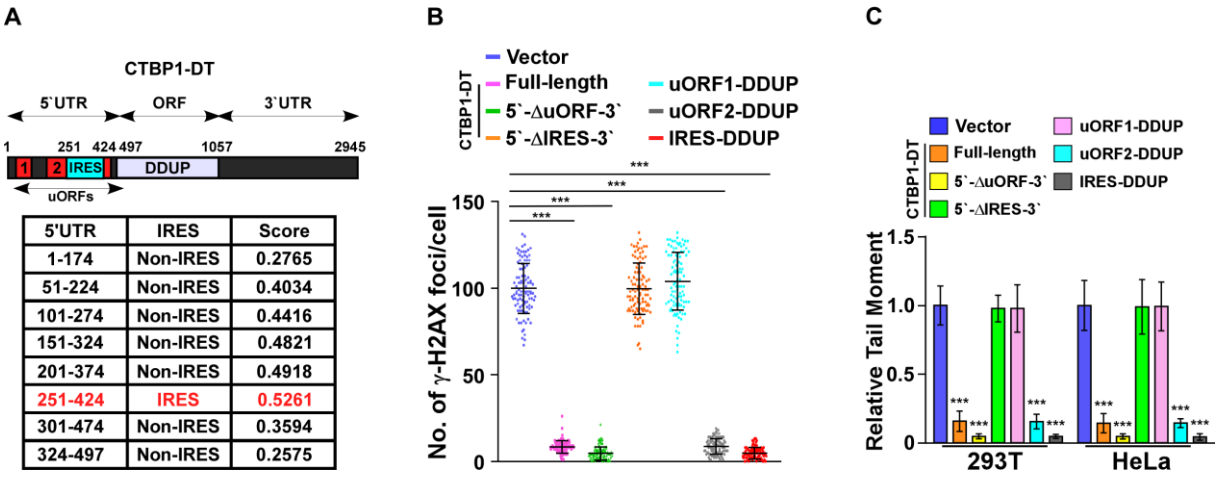
Supplementary Figure S2



Supplementary Figure S2 Microprotein DDUP, but not CTBP1-DT RNA, promotes DNA damage repair. **A.** Quantification of γ-H2AX foci in the indicated cells treated with CPT (10 μM), VP-16 (10 μM), CDDP (5 μM) for 1 hour, or IR (10 Gy) (>100 counted cells). **B.** MTT assay analysis of indicated cell survival in response to CPT treatment at the indicated concentration. **C.** Mutagenesis of target sites revealed by the presence of a PCR product with 300 bp digestion products in T7 endonuclease 1 (T7E1) assays in HeLa/DDUP$^{-/-}$-#1 and DDUP$^{-/-}$-#2 cells. **D**. Genomic DNA sequence analysis showing that

homozygous DDUP deletion occurred downstream of the neighbouring start codon (ATG) in HeLa/DDUP$^{-/-}$-#1 and DDUP$^{-/-}$-#2 cells. **E.** MTT assay analysis of the survival of control and DDUP-KO cells in response to CPT treatment at the indicated concentration. **F**. Representative images (upper) and quantification (lower) of the colony formation ability of the indicated cells treated with CPT (10 μM) or CDDP (5 μM), as determined by a colony-formation assay. Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

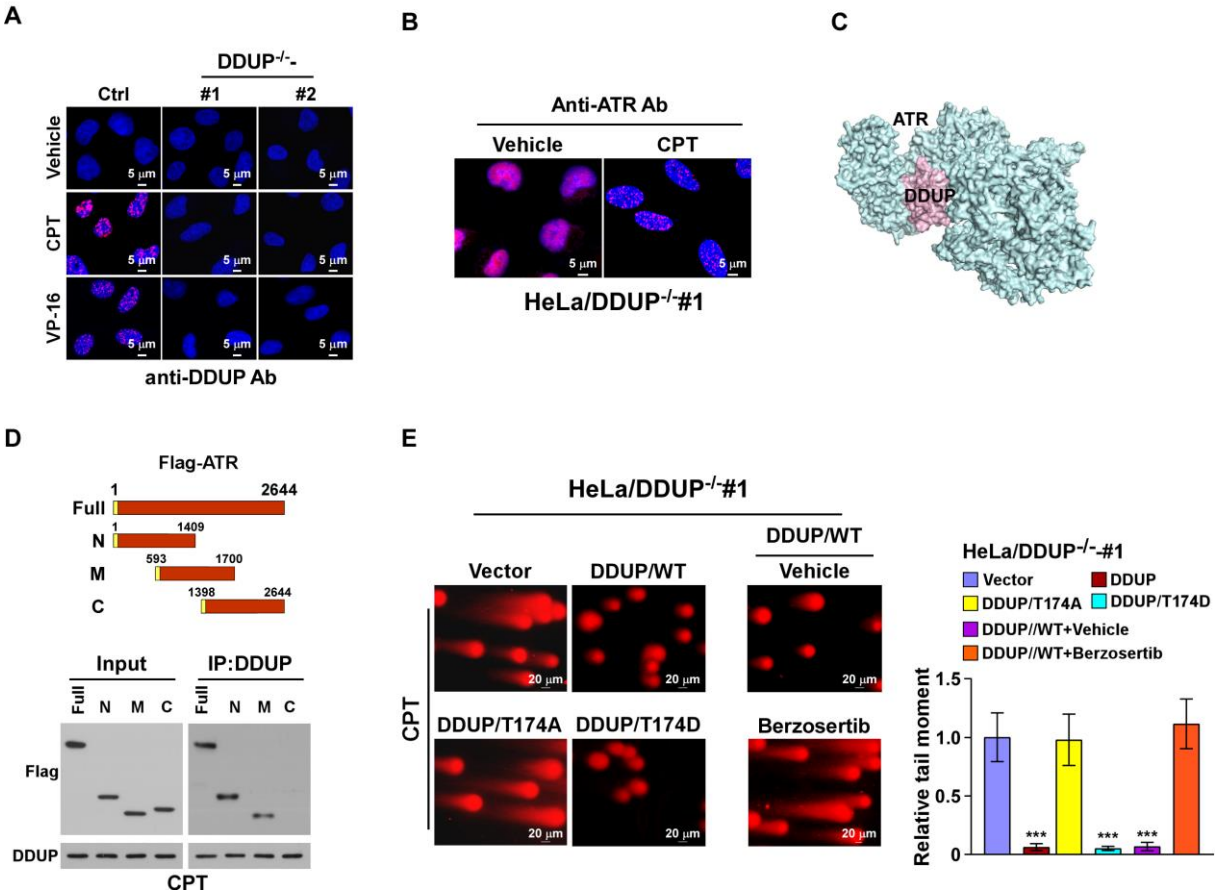
Supplementary Figure S3



5'UTR	IRES	Score
1-174	Non-IRES	0.2765
51-224	Non-IRES	0.4034
101-274	Non-IRES	0.4416
151-324	Non-IRES	0.4821
201-374	Non-IRES	0.4918
251-424	IRES	0.5261
301-474	Non-IRES	0.3594
324-497	Non-IRES	0.2575

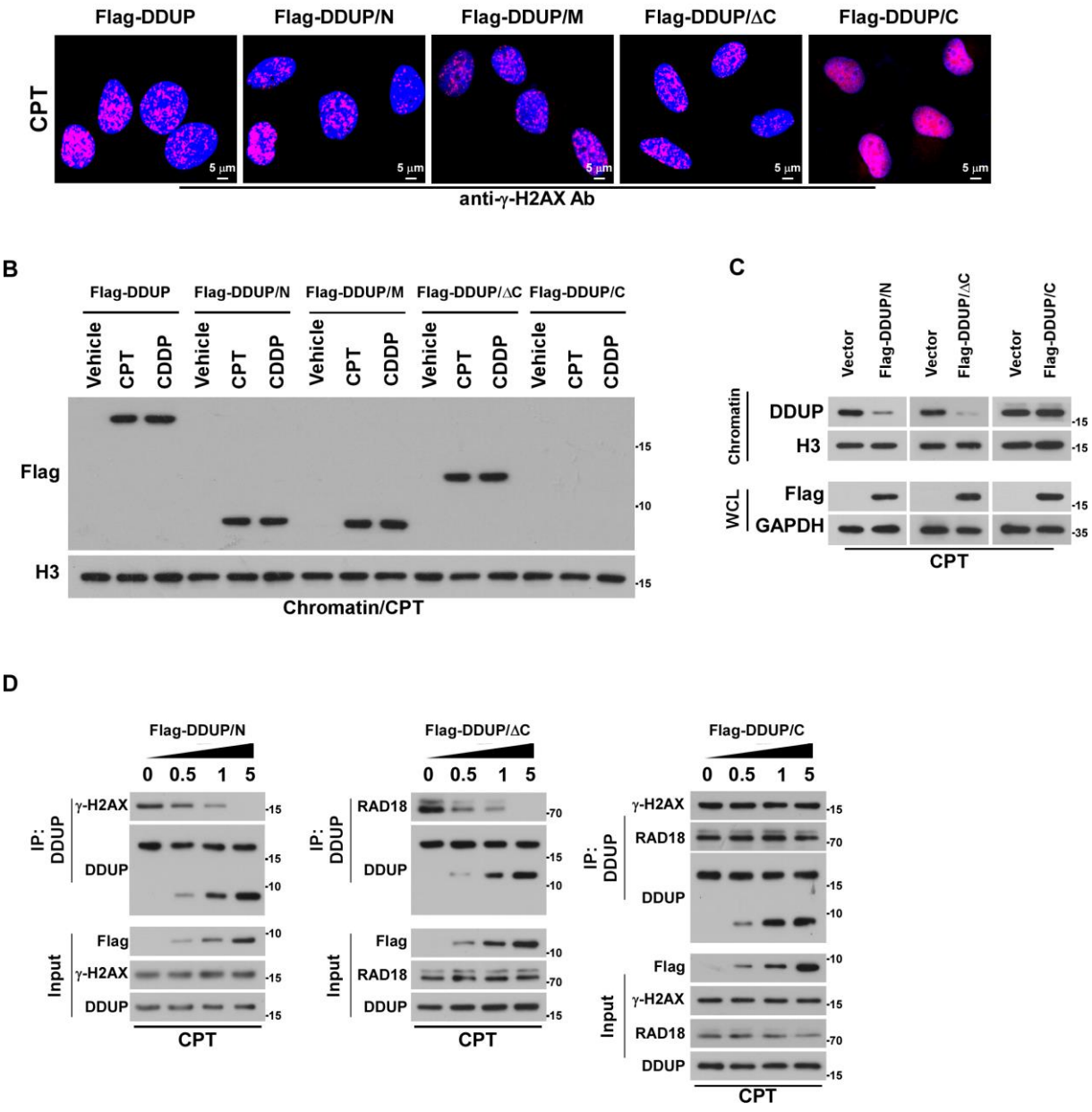
Supplementary Figure S3. The IRES in the 5'UTR of CTBP1-DT is essential for DNA damage-induced DDUP translation. A. Upper: Diagram of uORFs, putative IRES and DDUP-coding regions in CTBP1-DT RNA. Lower: IRESfinder was used to predict the potential IRES region in the 5'UTR of CTBP1-DT RNA. **B.** Quantification of γ-H2AX foci in HeLa cells with CPT (10 μM, 1h) (>100 counted cells). **C.** Quantification of damaged DNA in the indicated cells with CPT (10 μM, 1h) analyzed by comet assay (n = 100). Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Figure S4



Supplementary Figure S4. Phosphorylation of DDUP is essential for DDUP-mediated damage repair. A. IF staining using anti-DDUP antibody in vehicle-, or CPT (10 μM)-, or VP-16 (10 μM)-treated control and DDUP-KO HeLa cells. **B.** IF staining of ATR foci in vehicle- and CPT (10 μM, 1h)-treated DDUP-KO HeLa cells. **C.** Molecular docking between ATR and DDUP performed using the Cluspro 2.0 web server (https://cluspro.org/help.php) shown in cartoon representation. **D.** Co-IP assay analysis of the DDUP-interacting region in ATR using anti-ATR antibody in CPT-treated (10 μM,1h) 293T cells transfected with full-length or truncated ATR fragments (right). **E.** Representative images (left) and quantification (right) of damaged DNA in the indicated cells analysed by comet assay (n = 100). The indicated cells were pre-treated with or without berzosertib (80 nM) for 1 hour, and then treated with CPT (10 μM) for 1 hour. Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

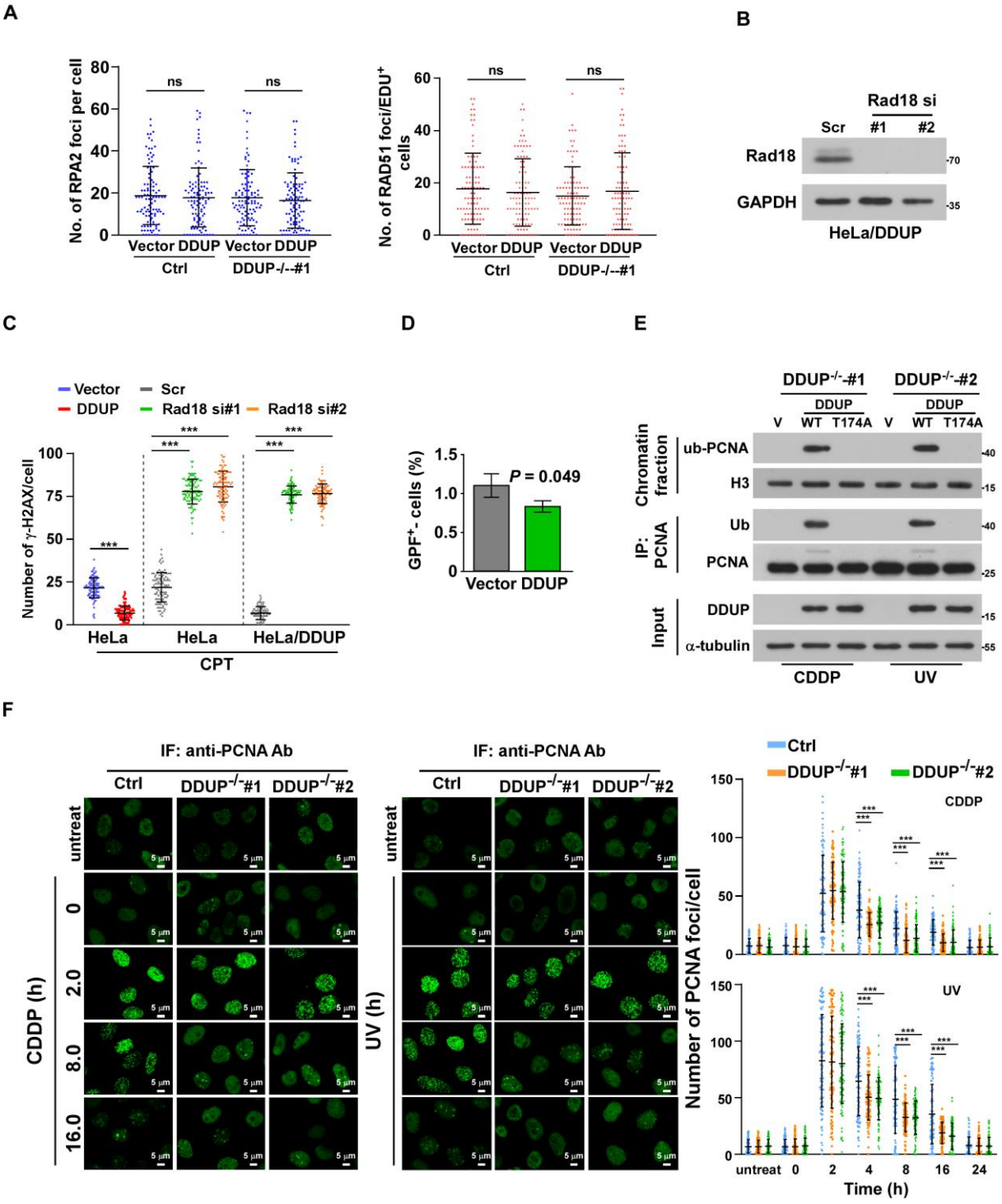
Supplementary Figure S5



Supplementary Figure S5. Phosphorylated DDUP forms a complex with γ-H2AX and RAD18. A. IF staining analysis of the expression of the indicated truncated DDUP protein in CPT (10 μM, 1h)-treated HeLa cells. **B.** Chromatin fraction and IB analysis of expression of DDUP/WT, DDUP/N, DDUP/M, DDUP/ΔC, and DDUP/C in chromatin in CPT (10 μM, 1h)-treated 293T cells. H3 served as a loading control. **C.** IB analysis of DNA-bound endogenous DDUP expression in chromatin fraction (upper) and whole cell lysates (lower) extracted from CPT (10 μM, 1h)-treated 293T cells transfected with DDUP/N, DDUP/ΔC or DDUP/C. H3 served as a loading control for chromatin fractions and GAPDH served as a loading control for whole cell lysates. **D.** Co-IP assay analyses of the DDUP/γ-H2AX interaction in CPT (10 μM, 1h)-treated 293T cells transfected with Flag-tagged DDUP/N (0, 0.5, 1.0, 5.0 μg), the

DDUP/RAD18 interaction in CPT (10 μM, 1h)-treated 293T cells transfected with Flag-tagged DDUP/ΔC (0, 0.5, 1.0, 5.0 μg), and formation of the DDUP/γ-H2AX/RAD18 complex in CPT (10 μM, 1h)-treated 293T cells transfected with Flag-tagged DDUP/C (0, 0.5, 1.0, 5.0 μg). The detailed technical information was described in the Material and Methods section.

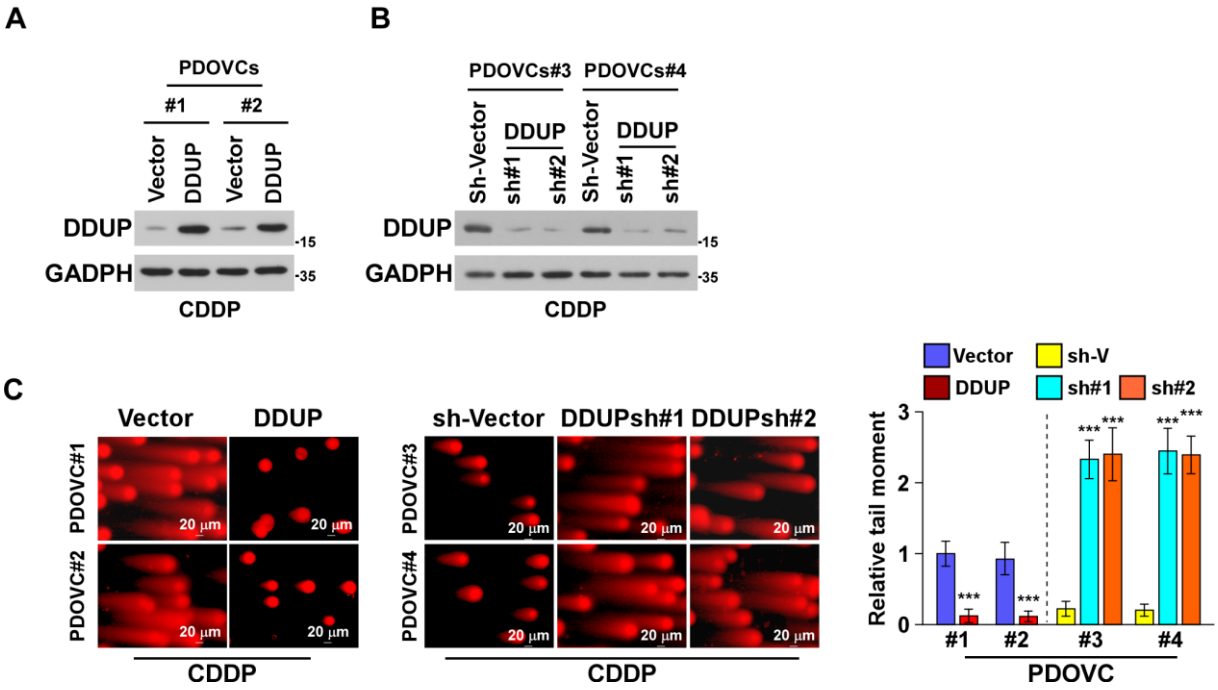
Supplementary Figure S6



Supplementary Figure S6. DDUP enhances the retention of RAD18 at DNA damage sites. A. IF quantification of RPA2-foci using anti-RPA2 antibody (left) and RAD51-foci using anti-RAD51 antibody (right) in the indicated cells treated with CPT (10 μM, 1h). **B.** IB analysis of RAD18 expression in scramble- and RAD18 siRNAs-transfected HeLa/DDUP cells. GAPDH served as a loading control. **C.** Quantification of γ-H2AX foci determined by IF staining using anti-γ-H2AX antibody in the indicated cells treated with CPT (10 μM, 1h). **D.** Percentage of GFP+ and mCherry+ cells, gated on BFP+, of three biological replicates. **E.** IB analysis of expression of DNA-bound and total monoubiquitinated

PCNA and DDUP in the indicated DDUP-KO HeLa cells treated with CDDP (5 μM, 1h) or UV radiation (60 J/m^2). H3 and α-Tubulin served as a loading control. **F.** Representative images (left) and time course (right) of the formation of PCNA foci in untreated or CDDP (5 μM)- or UV radiation (60 J/m^2)-treated control and DDUP-KO HeLa cells, and allowed to recover for the indicated times. Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Figure S7



Supplementary Figure S7. Upregulation of DDUP confers resistance to cisplatin in ovarian cancer cells *in vitro*. **A-B.** IB analysis of DDUP expression in Vector- or DDUP-transduced PDOVCs #1 and #2 (**A**) or sh-vector- or DDUP shRNA(s)-transduced PDOVCs #3 and #4 (**B**). GAPDH served as a loading control. **C.** Representative images (left) and quantification (right) of damaged DNA in the indicated cells analysed by comet assay (n = 100). The indicated cells treated with CDDP(5 μM) for 1 hour. Scale bar = 5 μm. Each error bar represents the mean ± SD of three independent experiments (*p < 0.05, **p < 0.01, ***p < 0.001).

Supplementary Tables

Supplementary Table 1. Label-free quantitative (LFQ) mass spectrometry analysis of the potential DDUP-interacting proteins in CPT- vs. Vehicle-treated cells

Accessions	Gene name	Vehicle _rep1	Vehicle _rep2	CPT_ rep1	CPT_ rep2	Log2 FC	*P* value
sp|Q13535|ATR_ HUMAN	ATR	NA	NA	24.99	24.67	4.81	7.25E-06
sp|Q9NS91|RAD 18_HUMAN	RAD18	NA	NA	24.30	24.51	4.12	7.12E-05
sp|O43502|RAD 51C_HUMAN	RAD51C	NA	NA	24.30	24.50	4.12	7.12E-05
sp|Q00839|HNR PU_HUMAN	HNRNPU	20.26	20.81	24.02	23.95	3.45	8.03E-05
sp|P62805|H4_H UMAN	H4C1	22.25	21.84	24.76	24.96	2.82	1.34E-04
sp|P06748|NPM _HUMAN	NPM1	23.50	23.81	26.41	26.41	2.75	8.17E-05
sp|P23527|H2B1 O_HUMAN	H2BC17	22.67	22.92	25.49	25.49	2.64	8.38E-05
NR_033339.1|D DUP	DDUP	NA	NA	22.89	22.83	2.61	1.16E-04
sp|P16104|H2AX _HUMAN	H2AX	NA	NA	22.04	22.42	2.46	2.09E-04
sp|P78347|GTF2 I_HUMAN	GTF2I	NA	NA	22.61	22.42	2.18	2.75E-04
sp|P11940|PABP 1_HUMAN	PABPC1	23.34	23.20	25.20	25.5	2.08	3.03E-04
sp|Q9Y2X3|NOP 58_HUMAN	NOP58	25.92	25.92	28.11	27.98	2.02	2.42E-04

Supplementary Table 2. Primers and Oligonucleotides used in this study

Primer used for qPCR	
CTBP1-DT-up	5’-CCATCCTCTGCAGCAAGTCA -3’
CTBP1-DT-dn	5’-CTCCGTTCTCAGTTGCCTGT-3’
GAPDH-up	5’-GGAGCGAGATCCCTCCAAAAT -3’
GAPDH-dn	5’-GGCTGTTGTCATACTTCTCATGG-3’
Primer used for subcloning and plasmid construction	
CTBP1-DT-up	5’-GCCGCCCACGTCAGCGCCTG-3’
CTBP1-DT-dn	5’-TGGCAGTTTTCAGCGATTGT-3’
DDUP-Flag-up	5’-gtgtcgtgaggattgggatccgccATGTGGTTGGTGGAGTGCACA-3’
DDUP-Flag-dn	5’-gaagaattcgtccggggatccTCACTTATCGTCGTCATCCTTGTAATC TGATAACGCGCTGACAAGC-3’
ATG1mDDUP-up	5’-GGGACGATtTGGTTGGTGGAGTGCACAGGCAG-3’
ATG1mDDUP-dn	5’-ACCAACCAaATCGTCCCTTCCCGGCAGCCTCA-3’
ATG2mDDUP-up	5’-CTCAGCATtGACAGGCAGCCCAGGAGAAGGCA-3’
ATG2mDDUP-dn	5’-TGCCTGTCaATGCTGAGCAGACAGGAAAGTCC-3’
DDUP/T174A-up	5’-CGTCTACgCACAGTGTTCGTCTGCACAGCTTG-3’
DDUP/T174A-dn	5’-AACACTGTGcGTAGACGGAGGCCATGGAGAGT-3’
DDUP/T174D-up	5’-CGTCTACgacCAGTGTTCGTCTGCACAGCTTG-3’
DDUP/T174D-dn	5’-AACACTGgtcGTAGACGGAGGCCATGGAGAGT-3’
Flag-DDUP/N-up	5’-gtgtcgtgaggattgggatccgccATGGATTACAAGGATGACGACGATAAG TGGTTGGTGGAGTGCACA-3’
Flag-DDUP/N-dn	5’-gaagaattcgtccggggatccTCAATCACAACCTGACTTGCTGC-3’
Flag-DDUP/ΔC-up	5’-gtgtcgtgaggattgggatccgccATGGATTACAAGGATGACGACGATAAG TGGTTGGTGGAGTGCACA-3’
Flag-DDUP/ΔC-dn	5’-gaagaattcgtccggggatccTCAGGAGAGGAACTGTTCTTCTGG-3’
Flag-DDUP/C-up	5’-gtgtcgtgaggattgggatccgccATGGATTACAAGGATGACGACGATAAG AGGATCCACTTCCTGCGC-3’
Flag-DDUP/C-dn	5’-gaagaattcgtccggggatccTCATGATAACGCGCTGACAAGC-3’

Primer used for CRISPR Cas9 System	
gRNA#1	5’-GGTTGGTGGAGTGCACAGGCAGG-3’
gRNA#2	5’-TGCACAGGCAGGGACCTCACTGG-3’
CTBP1-DT-up	5’-TTATTAGTCGGTGTGTTCAGTATC-3’
CTBP1-DT-dn	5’-AGCAGAGGGTAGAATGGTGTT-3’
shRNA	
ShRNA#1_CTBP1-DT	5’-ACCTCGGAATGATGCAGACTCCTATCTCAAGAGGATAGGAGTCTGCATCATTCCTT-3’
ShRNA#2_CTBP1-DT	5’-ACCTCGTTCGTCTGCACAGCTTGTCATCAAGAGTGACAAGCTGTGCAGACGAACTT-3’
siRNA	
Si RAD18#1	5’-GACCAAAGAGACACGTTCTGT-3’
Si RAD18#2	5’-GCTGTTTATCACGCGAAGAGA-3’
Si DDUP#1	5’-GGAAUGAUGCAGACUCCUAUC-3’
Si DDUP#2	5’-GTTCGTCTGCACAGCTTGTCA-3’
Si H2AX#1	5’-TGGACTAATTTTATTAAAGGATT-3’
Si H2AX#2	5’-GACTAATTTTATTAAAGGATTGT-3’

Supplementary Table 3. Clinicopathological characteristics of clinical samples and expression of DDUP in platinum-treated Ovarian Cancer

Characteristics	No. patients	DDUP expression		*P* value
		Low (n=217)	High (n=150)	
Age (years)				
≤ 62	192	109	83	0.336
> 62	175	108	67	
Histological type				
Serous	270	158	112	
Endomotrioid	43	26	17	0.378
Mucinous	23	11	12	
Undifferentiated	31	22	9	
FIGO stage				
Ⅰ/Ⅱ	89	61	28	0.038
Ⅲ/Ⅳ	278	156	122	
Histologic grade				
1	31	15	16	
2	75	34	41	0.101
3	261	168	93	
Chemo-response status				
Chemoresistance	190	60	130	<0.001
Chemosensitivity	177	157	20	
Recurrence				
Yes	248	111	137	<0.001
No	119	106	13	
Vital status				
Alive	143	112	31	<0.001
Dead	224	105	119	

Supplementary Table 4. Univariate and multivariate analysis of different prognostic parameters in patients with ovarian cancer by Cox-regression analysis.

	Univariate analysis		Multivariate analysis	
	P	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)
Age (years)	0.137	0.819 (0.629-1.065)		
Histological type	0.002	0.773(0.659-0.907)	0.006	0.776 (0.648-0.929)
Histologic grade	< 0.001	0.673（0.547-0.829）	0.007	0.734 (0.585-0.920)
FIGO stage	0.001	1.847（1.267-2.691）	0.013	1.664 (1.111-2.491)
Recurrence	< 0.001	3.068（2.170-4.336）	< 0.001	2.479 (1.545-3.978)
Chemo-response status	< 0.001	2.171（1.642-2.869）	0.009	0.548 (0.349-0.861)
DDUP expression	< 0.001	3.157（2.401-4.152）	< 0.001	2.797 (1.974-3.963)