Supporting information

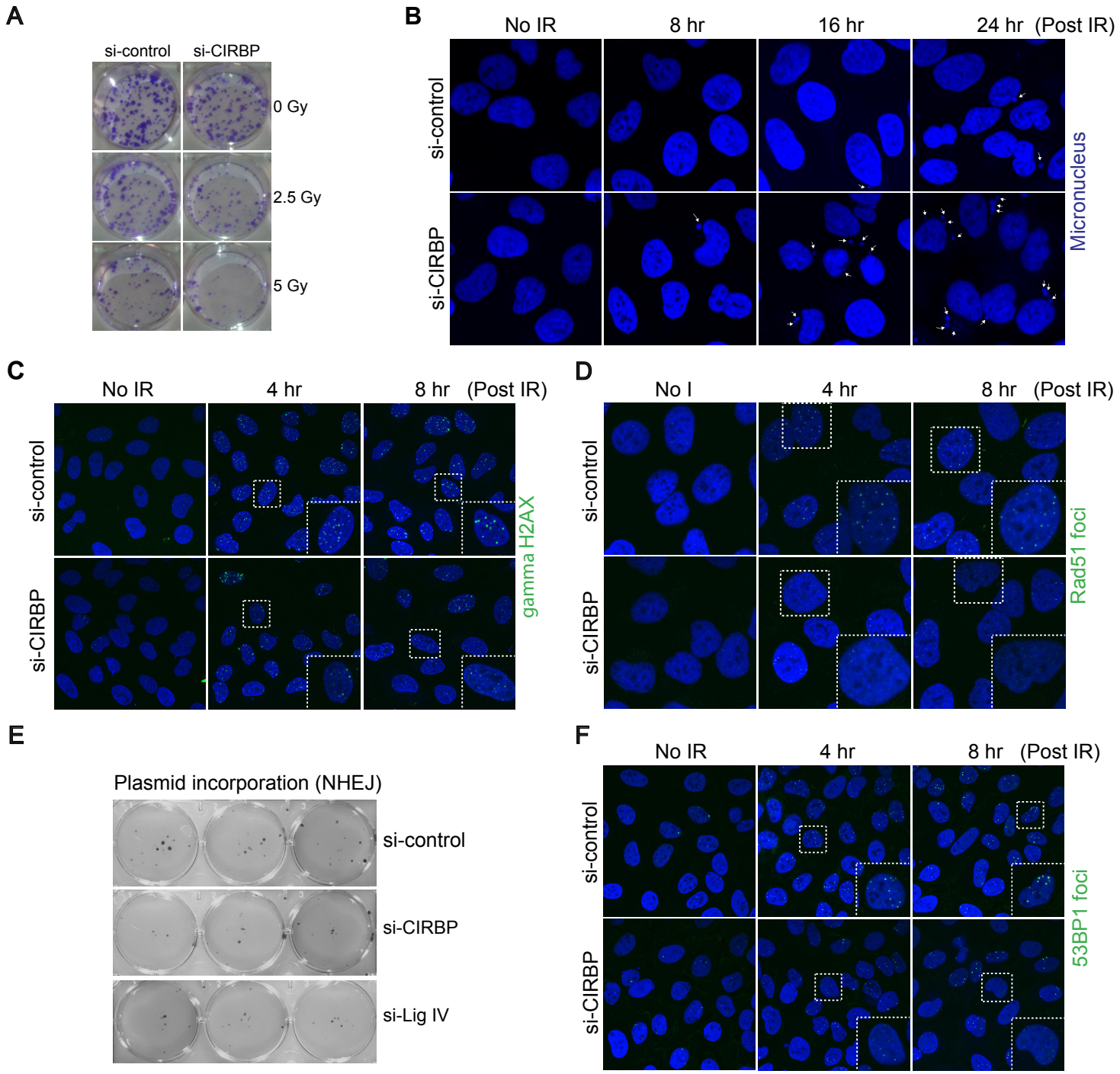

Fig. S1. CIRBP promotes genome stability and double-strand breaks repair**,** data are Fig 1 related. (A) CIRBP depletion increases irradiation (IR) sensitivity. The results show representative images from the experiment shown in Fig. 1A. (B) CIRBP-depletion increases IR-induced micronuclei formation. The results show representative images from the experiment described in Fig. 1B. Associated micronuclei are indicated with white arrows. (C) CIRBP-depletion reduces nuclear γH2AX intensity. The results show representative images from the experiment shown in Fig. 1C. γH2AX is shown in green, counterstain with DAPI in blue. Inserts represent a zoom-in image to highlight γH2AX foci (boxed region) (D) CIRBP-depletion reduces IR-induced Rad51 foci formation. The results show representative images from the experiment described in Fig. 1E. Rad 51 was stained with green and merged with nucleus counterstained with DAPI. Inserts represent a zoom-in image to highlight Rad51 foci (boxed region) (E) CIRBP-knockdown reduces non-homologous end joining (NHEJ). NHEJ efficiency was determined by random plasmid incorporation assay. The results show representative images from the experiment shown in Fig. 1F. (F) CIRBP-depletion reduces IR-induced 53BP1 foci formation. The results show representative images from the experiment described in Fig. 1G. 53BP1 is shown in green, counterstained with DAPI in blue. Inserts represent a zoom-in image to highlight 53BP1 foci (boxed region)

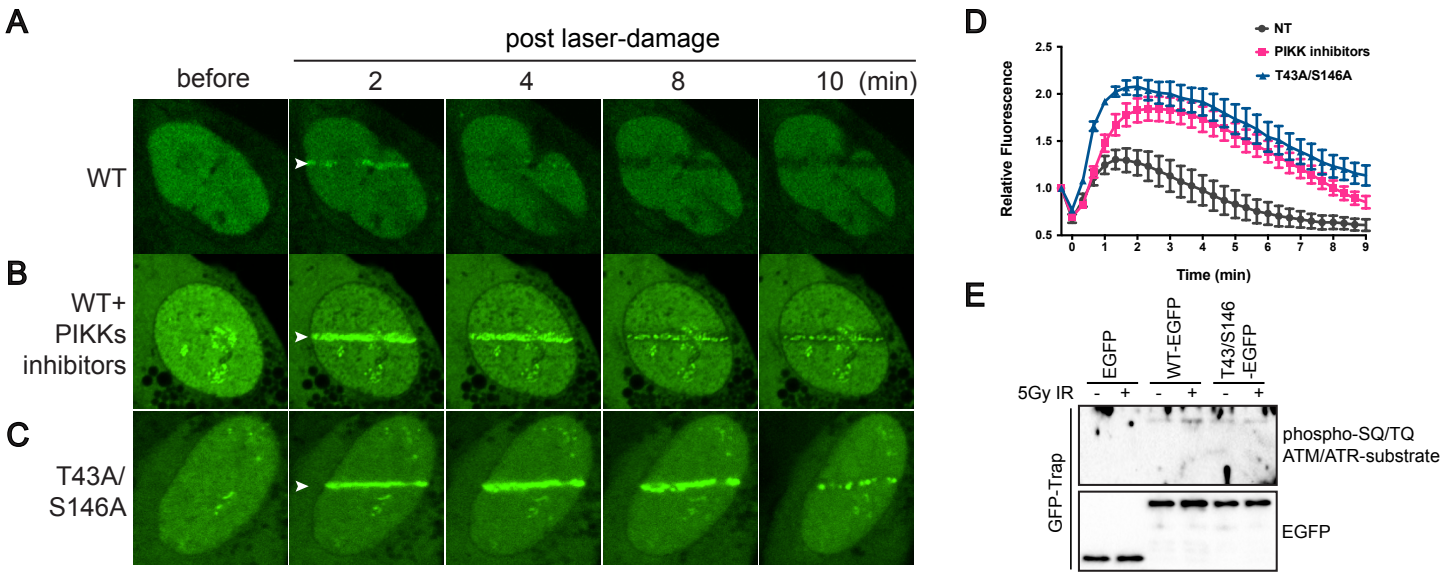

Fig. S2. PIKKs mediate CIRBP exclusion from laser-damaged regions. (A-C) The accumulation of CIRBP at laser-damaged regions lasts longer upon PIKK inhibition or mutation at potential PIKKs phosphorylation sites. The WT or T43A/S146A CIRBP was overexpressed in U2OS cells. Forty-eight hours after transfection, cells were treated with PIKK inhibitors or DMSO. The fluorescent signals at laser microirradiated regions from ~10 cells were recorded and normalized to the signal level from the same regions prior to irradiation. (D) The quantification results of Fig. S2A-C are presented as mean ± s.e.m. (E) EGFP vector, CIRBP WT-EGFP and CIRBP T43A/S146A-EGFP were overexpressed in U2OS cells. Forty-eight hours after transfection, cells were exposed to either 5 Gy irradiation or none, and lyzed in RIPA buffer. EGFP-tagged CIRBP protein was pulled down by GFP-trap beads and subjected to immunoblotting against phospho-SQ/TQ ATM/ATR substrate and GFP antibodies.

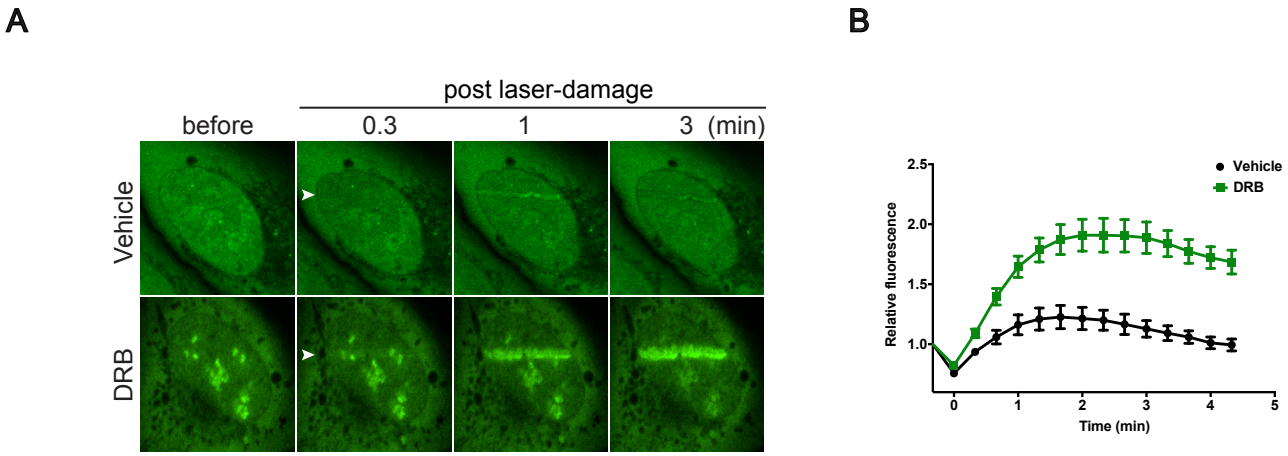

Fig. S3. Transcription inhibition enhances CIRBP recruitment. (A) EGFP-CIRBP overexpressed U2OS cells were treated with either transcription inhibitor (DRB) or DMSO for 2 h and then subjected to laser microirradiation assay. (B) The quantification data are presented as mean ± SD.

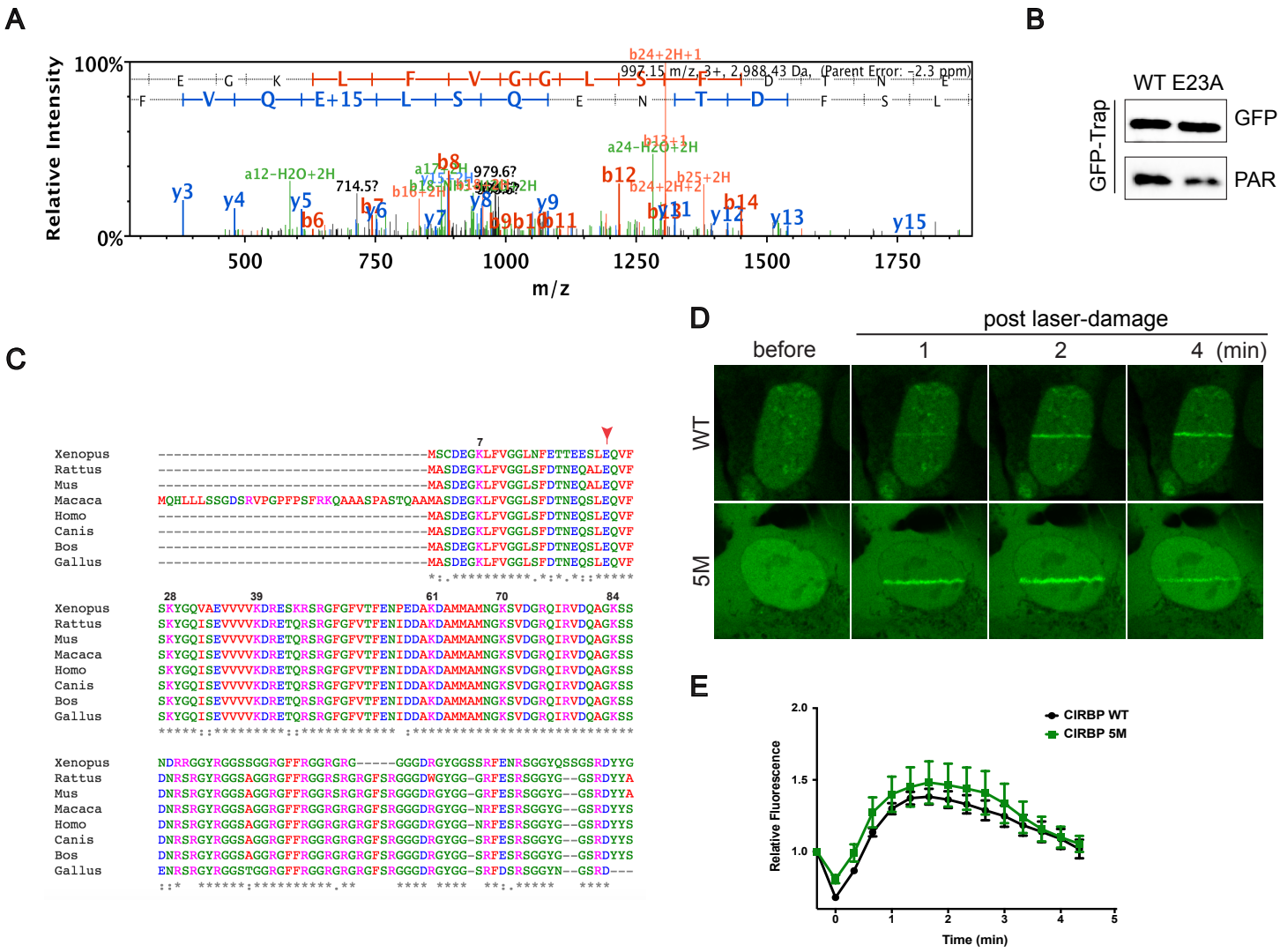

Fig. S4. Characterization of the PARylation sites on CIRBP. (A) E23 is identified as PARylation site by LC-MSMS. EGFP tagged-CIRBP was overexpressed in 293T cells. After exposure to 5 Gy irradiation, the EGFP-tagged CIRBP were pulled down by GFP-trap beads and incubated with 0.5 M hydroxylamine overnight. LC-MSMS results were acquired and searched for additional 15 Da on PARylated Asp and Glu residues. (B) E23 is not the only PARylation site. EGFP-tagged CIRBP WT and E23A mutant were overexpressed in 293T cells and exposed to either 5 Gy irradiation or none, and lyzed in RIPA buffer for 10 min post IR. EGFP-tagged CIRBPs were pulled down by GFP-trap beads and subjected to immunoblotting against PAR and GFP antibodies. (C) T-COFFEE multiple sequence alignment is carried out to identify highly conserved lysine residues as potential PARylation sites. (D) Poly(ADP-ribosyl)ation is not required for CIRBP recruitment. (E) Quantification results from the experiments described in Fig. S4D. The data are presented as mean ± SD.

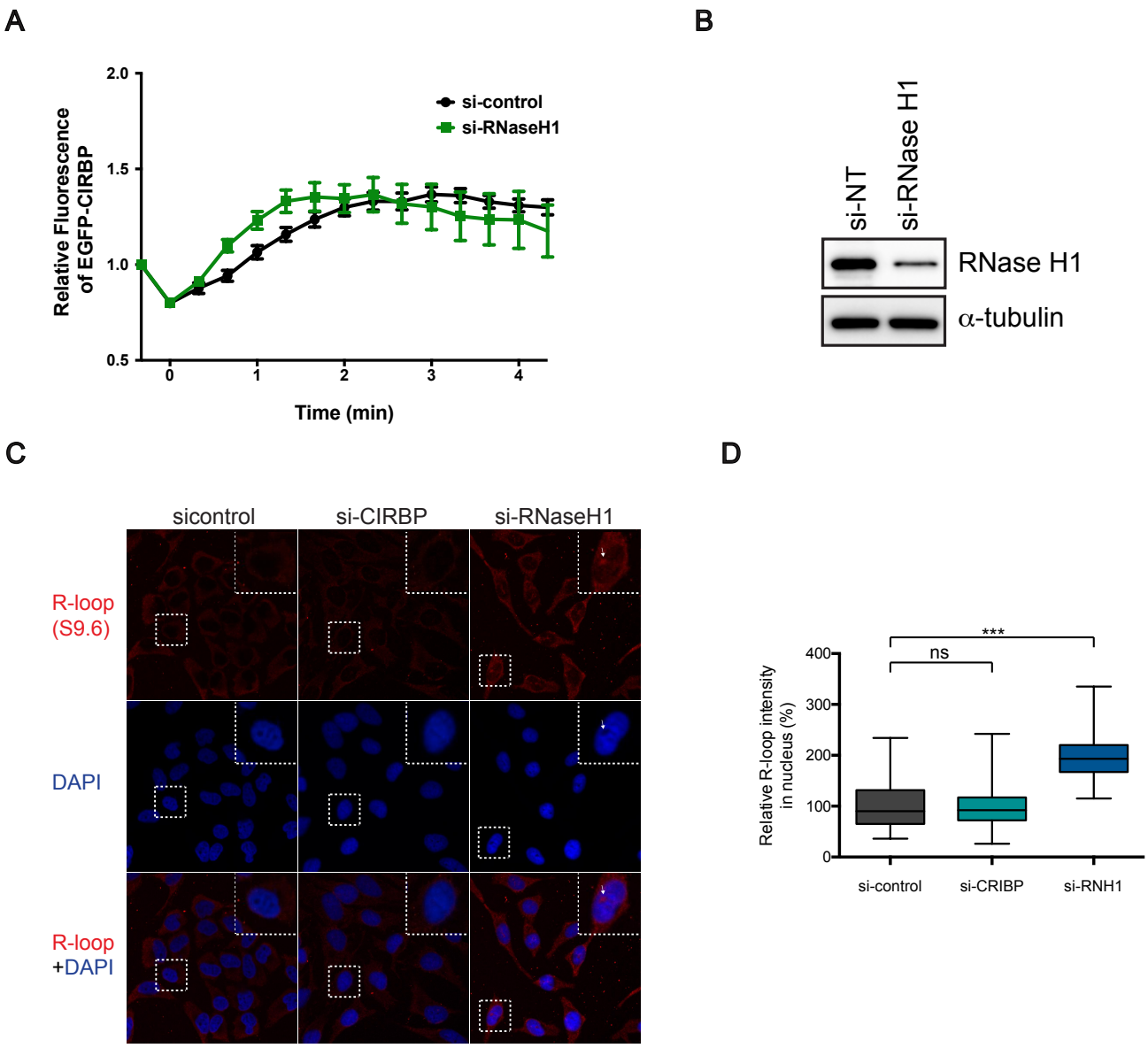

Fig. S5. Relationship between CIRBP and DNA/RNA hybrid (R-loop) (A) R-loop is not responsible for the recruitment of CIRBP to the laser microirradiated regions. EGFP-CIRBP overexpressed U2OS cells were transfected with non-targeting, RNaseH1 siRNA, and then subjected to laser microirradiation assay. (B) RNase H1 knockdown efficiency in U2OS cell line using siRNA. (C) CIRBP knockdown does not affect R-loop level in nucleus. HeLa cells were transfected with non-targeting, CIRBP and RNaseH1 siRNA, fixed and stained with R-loop (S9.6) antibodies. Cells were then counterstained with DAPI and the nuclear R-loop intensity was analyzed by Fiji ImageJ. Inserts represent a zoom-in image to highlight a single cell (boxed region). The white arrow indicates nucleolus. (D) Quantification of results in Fig S7C. The data are presented as median, 25th to 75th centile range (boxes) and minimum to maximum centile range (whiskers) from two independent experiments. ns, no significant difference, ***$P < 0.001$ (Mann-Whitney *U* test).

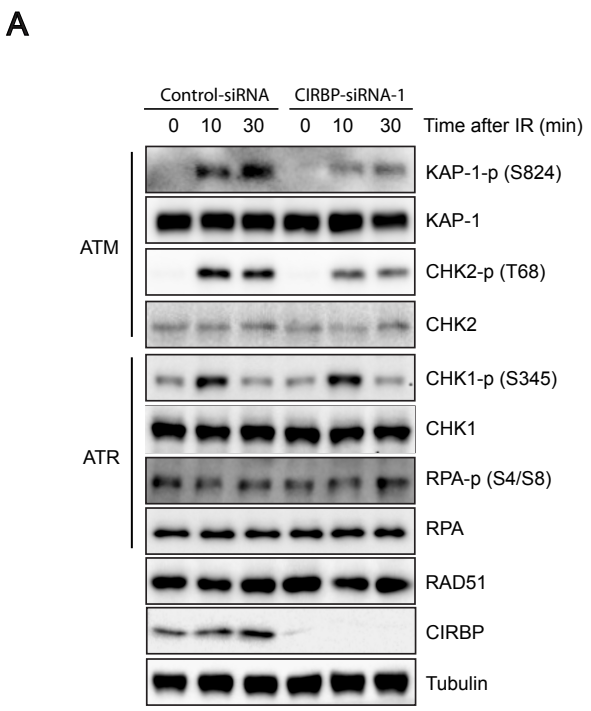

Fig. S6. CIRBP-depletion specifically affects ATM signalling. (A) Control or CIRBP siRNA treated U2OS cells were exposed to either 5 Gy IR or none. The total cell lysates were prepared using Laemmli buffer and subjected to immunoblotting against indicated antibodies.

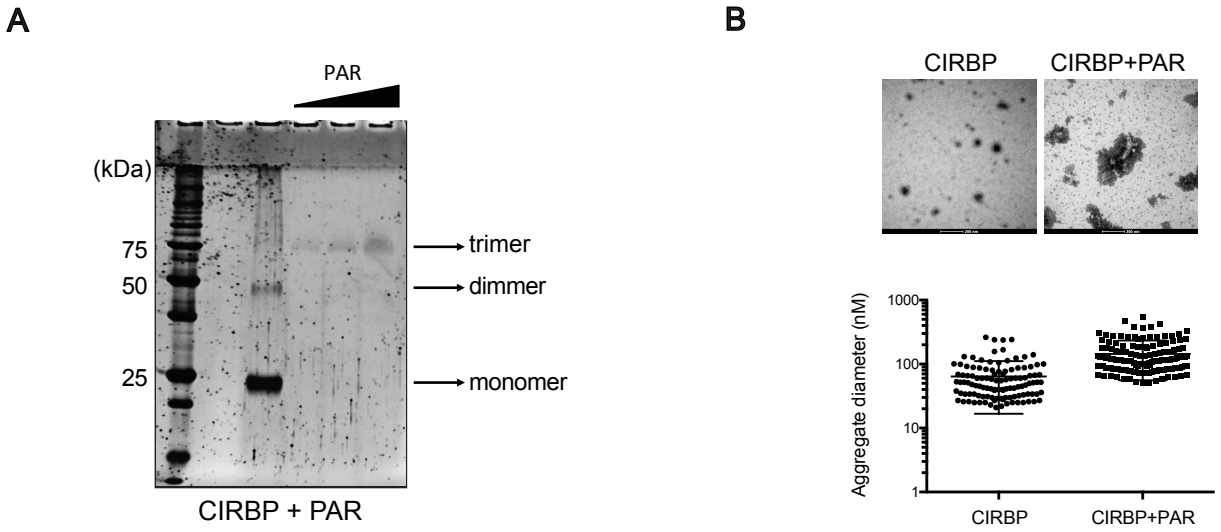

Fig. S7. PAR polymer facilitates CIRBP aggregation *in vitro.* (A) Poly(ADP-ribose) triggers CIRBP protein to form aggregates. Recombinant His-tagged CIRBP was incubated with or without purified PAR polymer and crosslinked in 0.4% formaldehyde for 15 min. The reaction mixture was analyzed by SDS electrophoresis and stained with SYPRO Ruby solution. (B) CIRBP aggregates detected by transmission electron microscopy (TEM). Recombinant full-length CIRBP was incubated with or without sub-stoichiometric amount of purified PAR polymer and the CIRBP aggregates were analyzed by TEM. The data are presented as mean ± SD.

Table S1. Antibodies

Names	Sources	Catalog #	Clone #	Applications
PARP-1	Santa Cruz	sc-8007	F2	WB
CIRBP	Proteintech	10209-2-AP		WB, IF
CIRBP	Abcam	ab191885	EPR18783	WB, IF
ATM	Santa Cruz	sc-135663	1B10	WB
ATM (phospho S1981)	R&D	AF1655		WB, ChIP
ATM (phospho S1981)	Abcam	ab81292	EP1890Y	WB
ATR	Bethyl	A300-137A		WB
DNA-PK	Cell Signalling	4602		WB
Histone 3	Abcam	ab1791		WB
β-Tubulin	Abcam	ab6046		WB
GFP	Invittrogen	A11122		WB, IF
Flag	Sigma	F1804	M2	WB
H2AX	Cell Signalling	2595		WB
γH2AX (phospho S139)	Novus Biologicals	NB100-384		WB, IF
PAR	Trevigen	4335-MC-100-AC	10H	WB
Mre11	Genetex	GTX70212	12D7	WB
Rad50	Genetex	GTX70228	13B3	WB
NBS1	Genetex	GTX70222	1C3	WB, ChIP
KAP1	Cell Signalling	4124T	C42G12	WB
KAP1 (phospho S824)	Cell Signalling	4127T		WB
phospho-(S/T)ATM/ATR substrate	Cell Signalling	2851		WB
CHK2	Cell Signalling	3440T	C12	WB
CHK2 (phospho T68)	Cell Signalling	2197T	C13C1	WB
RPA32	Abcam	ab2175	9H8	WB
RPA32 (phospho S4/8)	Bethyl	A300-245A		WB
CHK1	Santa Cruz	sc-8408	G-4	WB
CHK1 (phospho S345)	Cell Signalling	2348	133D3	WB
RNase H1	Proteintech	15606-1-AP		WB
DNA/RNA hybrid [S9.6]	kerafast	ENH001		IF
Anti-mouse IgG-HRP	Sigma	A4416		WB
Anti-Rabbit IgG-HRP	Cell Signalling	70743		WB
Alexa Fluor 488 goat anti-mouse IgG	Invitrogen	A11029		IF
Alexa Fluor 647 goat anti-mouse IgG	Invitrogen	A21236		IF
Alexa Fluor 488 goat anti-rabbit IgG	Invitrogen	A11034		IF
Alexa Fluor 647 goat anti-rabbit IgG	Invitrogen	A21245		IF

Table S2. Plasmid constructs

Construct	Plasmid digestion	Insert
pcDNA3-CIRBP-WT-EGFP	pcDNA3-EGFP, BamHI+XhoI	PCR amplified WT CIRBP (516 bp)
pcDNA3-CIRBP-3F/A-EGFP	pcDNA3-EGFP, BamHI+XhoI	F15, F49, F51 mutated to A
pcDNA3-4R/A-EGFP	pcDNA3-EGFP, BamHI+XhoI	R91, R94, R116, R121 mutated to A
pcDNA3-9R/A-EGFP	pcDNA3-EGFP, BamHI+XhoI	R91, R94, R101, R105, R108, R110 R112, R116, R121 mutated to A
pcDNA3-5M-EGFP	pcDNA3-EGFP, BamHI+XhoI	E23, K7A, K39, K70, K84 mutated to A
pFastBac-CIRBP-WT-His6	pFastBac-HT-B, BamHI+XhoI	WT CIRBP
pFastBac-CIRBP-3F/A-His6	pFastBac-HT-B, BamHI+XhoI	3F/A CIRBP
pFastBac-CIRBP-9R/A-His6	pFastBac-HT-B, BamHI+XhoI	9R/A CIRBP
pFastBac-CIRBP-5M-His6	pFastBac-HT-B, BamHI+XhoI	E23, K7A, K39, K70, K84 mutated to A
siRNA resistant CIRBP-WT-TAA	pcDNA3-mRFP, BamHI+XhoI	WT CIRBP with gggctgagttttgacaccaat to ggATTATCAttCgaTacAaaC mutations
siRNA resistant CIRBP-9R/A-TAA	pcDNA3-mRFP, BamHI+XhoI	9R/A CIRBP with gggctgagttttgacaccaat to ggATTATCAttCgaTacAaaC mutations
siRNA resistant CIRBP-5M-TAA	pcDNA3-mRFP, BamHI+XhoI	4mt CIRBP with gggctgagttttgacaccaat to ggATTATCAttCgaTacAaaC mutations
pcDNA3-CIRBP-T43A-EGFP	pcDNA3-EGFP, BamHI+XhoI	T43A CIRBP
pcDNA3-CIRBP-S146A-EGFP	pcDNA3-EGFP, BamHI+XhoI	S146A CIRBP
pcDNA3-CIRBP-T43A-S146A-EGFP	pcDNA3-EGFP, BamHI+XhoI	T43A+S146A CIRBP

Table S3. Chemicals

Names	Sources	Catalog #	Target	Stock	Treatment
Olaparib	Selleckchem	AZD2281	PAR systhesis	2 mM / DMSO	1 μM / 1 hr
Etopoxide	Sigma	E1383	Topoisomerase II	40 mM / DMSO	40 μM / 1 hr
Tannic acid	Sigma	403040	PARG	10 mM / water	10 μM / 1 hr
KU55933	Tocris	3544	ATM	20 mM / DMSO	10 μM / 2hr
VE-821	Selleckchem	S8007	ATR	10 mM / DMSO	10 μM / 2hr
NU7441	Tocris	3712	DNA-PK	10 mM / DMSO	10 μM / 2hr
DRB	Sigma	D1916	RNA polymerase II	100 mM / DMSO	100 μM / 2hr
Shield-1	Clontech	632189	Dsstabilized domain	500 mM / ethanol	1 mM / 4 hr
4-OHT	Sigma	T176	Estrogen receptor	1 mM / ethanol	1 μM / 4hr

Table S4. The sequences of siRNAs and qPCR primers

siRNA	Sequences (with 3'-UU overhang)	qPCR primer	Sequences
CIRBP	#1: GGCUCCAGAGACUACUAUA	PARP-1	F: GATGGGTTCTCTGAGCTTCG
	#2: GGCUGAGUUUUGACACCAA		R: TCTGCCTTGCTACCAATTCC
	#3: CAGAGACAGTTACGACAGT		
		PARP-2	F: TGGGGAAGGCTCATGTGTAT
PARP-1	#1: CCAUCGAUGUCAACUAUGA		R: TCCTCTGGGCATCATCTTCT
PARP-2	#1: CCAUUGGACCAACACUAUA	PARG	F: TACACGACCCCAAAACTTGA
			R: TCCCAGAAATCGATCAAAGC
PARG	#1: GAAGAAUGCAGAAACUGUU		
		265-P1	F: GGAAGATGTCCCTTGTATCACCAT
Non-targeting	#1: UGGUUUACAUGUCGACUAA		R: TGGTTGTCAACAGAGTAGAAAGTGAA
	#2: UGGUUUACAUGUUGUGUGA		
	#3: UGGUUUACAUGUUUUCUGA	265-P2	F: GCTGGTGTGGCCAATGC
	#4: UGGUUUACAUGUUUUCCUA		R: TGGCAGAGGGAAAAAGATCTCA
Ligase IV	#1: AGGAAGUAUUCUCAGGAAUUA	265-P3	F: GGCATTTCAGTCAGTTGCTCAA
			R: TTGGCCGATTCATTAATGCA
CtIP	#1: GGUAAAACAGGAACGAAUC		
		265-P4	F: CCACCTGACGTCTAAGAAACCAT
Rnase H1	#1: AAGUUUGCCACAGAGGAUGAG		R: GATCCCTCGAGGACGAAAGG
		GAPDH	F: GCTTGCCCTGTCCAGTTAAT
			R: TAGCTCAGCTGCACCCTTTA

Table S5. Abbreviations

Abbreviation	Full name
CIRBP	Cold-inducible RNA-binding protein
PARP-1	Poly(ADP-ribose) polymerase 1
PARG	Poly(ADP-ribose) glycohydrolase
ATM	Ataxia Telangiectasia Mutated
ATR	ATM- and Rad3-related protein
CtIP	CtBP-interacting protein
PIKKs	Phosphatidylinositol 3-kinase-related kinases
MRN	Mre11-Rad50-NBS1
Mre11	Meiotic recombination 11 homolog A
Rad50	RAD50 homolog
NBS1	Nijmegen Breakage Syndrome 1 (Nibrin)
H2AX	H2A histone family, member X
CHK1	Checkpoint kinase 1
CHK2	Checkpoint kinase 2
Rad51	RAD51 homolog
53BP1	Tumor Protein P53-Binding Protein 1
KAP-1	KRAB [Kruppel-Associated Box Domain]-Associated Protein 1
RPA	Replication protein A2
DNA-PK	DNA protein kinase catalytic subunit
Ku80	X-ray repair cross-complementing protein 5
PAR	Poly(ADP-ribose)
RBP	RNA-binding protein
RRM	RNA-recognition motif
DSB	Double-strand break
HR	Homologous recombination
NHEJ	Non-homologous end joining
DDR	DNA damage response
IR	Ionizing radiation
DR-GFP	Direct repeat green fluorescence protein
ChIP	Chromatin immunoprecipitation