Supplementary Information

Zhang et al. MORC2 regulates DNA damage response through a PARP1-dependent pathway

Supplementary Figures and Figure legends

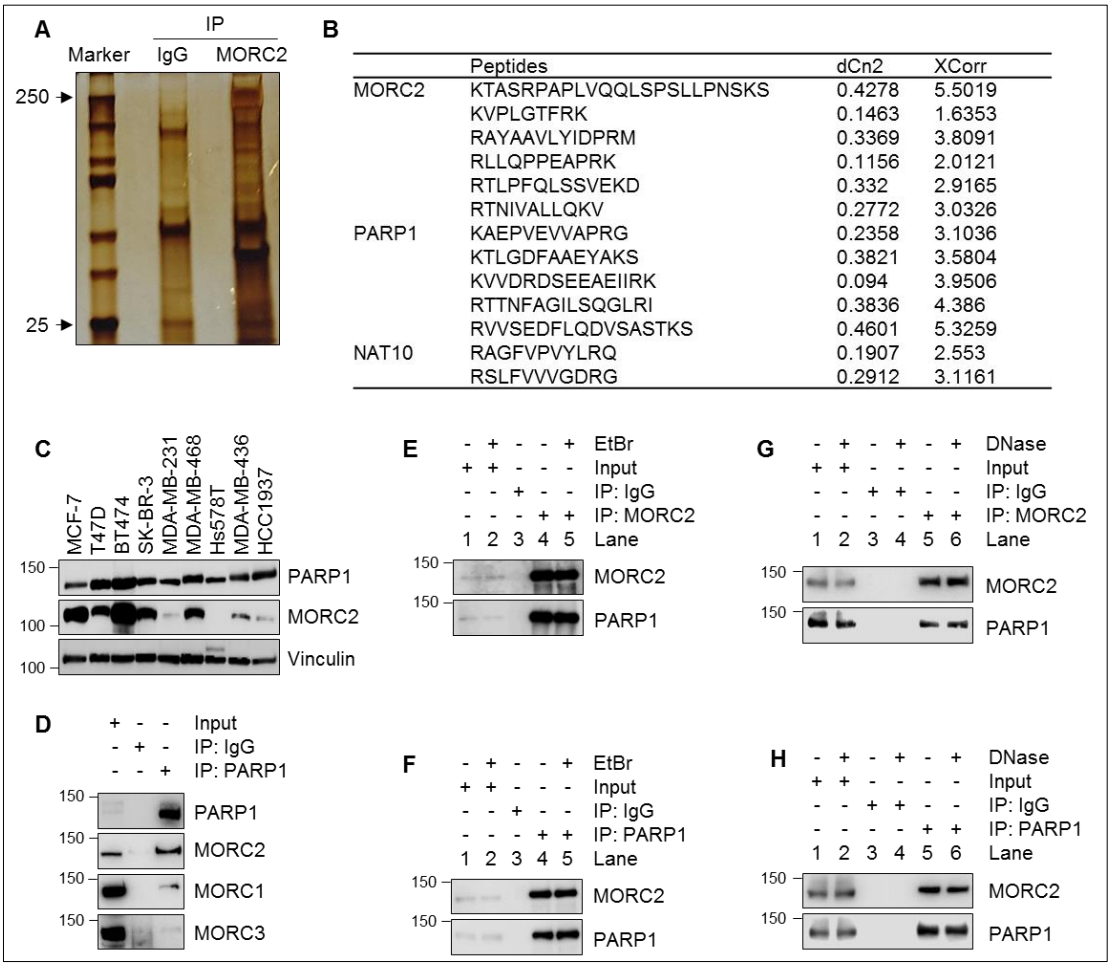

Figure S1. MORC2 interacts with PARP1

(A-B) Nuclear extracts of HeLa cells were immunoprecipitated with an anti-MORC2 antibody or control IgG, and the precipitated protein complexes were subjected to sliver staining and liquid chromatography-tandem mass spectrometry (A). The identified peptides for MORC2, PARP1, and NAT10 are showed in B. Protein identification results were filtered with the correlation factor (Xcorr) (>1.9) and delta correlation factor (dCn) (>0.1) as a conservative criteria set developed by Yates and coworkers (1). (C) Immunoblotting analysis of the expression levels of PARP1 and MORC2 in 9 breast cancer cell lines. (D) Lysates from MCF-7 cells were subjected to IP and immunoblotting analysis with the indicated antibodies. (E-F) Lysates from MCF-7 cells were immunoprecipitated with control IgG or an anti-MORC2 (E) or an

anti-PARP1 (F) antibody in the presence or absence of 50 μg/ml ethidium bromide (EtBr), followed by immunoblotting analysis with the indicated antibodies. (G-H) Lysates from MCF-7 cells were immunoprecipitated with control IgG or an anti-MORC2 (G) or an anti-PARP1 (H) antibody in the presence or absence of 0.35 U/μl DNase, followed by immunoblotting analysis with the indicated antibodies.

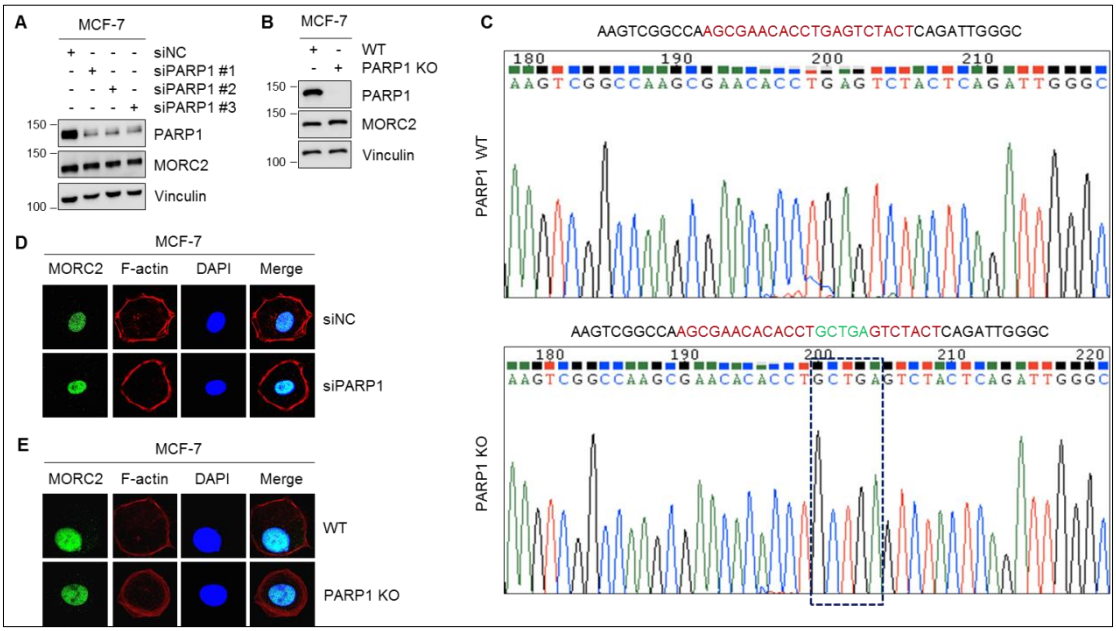

Figure S2. Depletion of PARP1 does not affect the protein expression levels and nuclear localization of MORC2

(A) MCF-7 cells were transfected with siNC or three siRNAs targeting PARP1. After 48 h of transfection, cells were harvested for immunoblotting analysis with the indicated antibodies. (B) Lysates from WT and PARP1 KO MCF-7 cells were subjected to immunoblotting analysis with the indicated antibodies. (C) Validation of PARP1 KO cells by PCR and sequencing across the CRISPR target sites. Sequences in red indicate CRISPR target sites, while the sequences in green represent the mutations generated. (D) MCF-7 cells were transfected with siNC or siPARP1. After 48 h of transfection, cells were immunostained with the indicated antibodies. Actin filaments (F-actin) were labeled CytoPainter Phalloidin-iFluor 680 reagent (red). Nuclei were stained with DAPI. (E) WT and PARP1 KO MCF-7 cells were immunostained with the indicated antibodies. Actin filaments (F-actin) were labeled CytoPainter Phalloidin-iFluor 680 reagent (red). Nuclei were stained with DAPI.

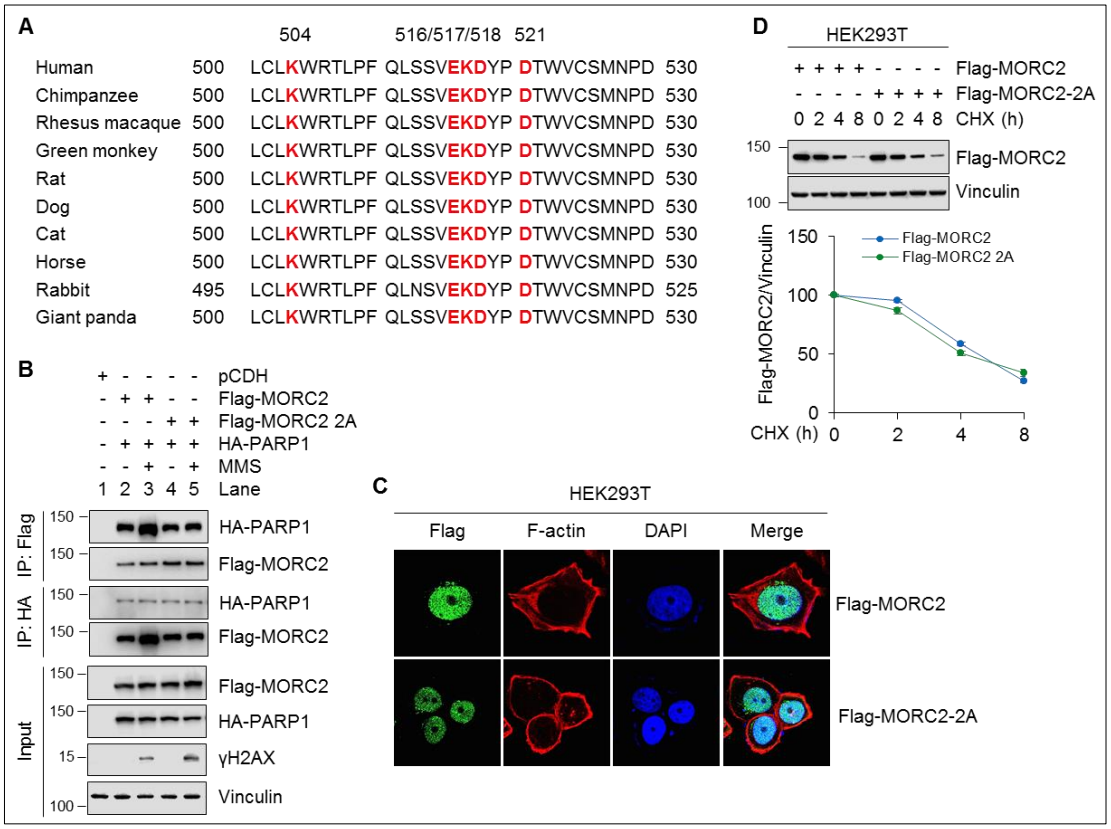

Figure S3. The effect of PARylation on the subcellular localization and stability of MORC2

(A) Sequence alignments of the CW-ZF domain of MORC2 from several species revealed five evolutionary conserved residues (K504, E516, K517, D518, and D521) that can potentially undergo PARylation. (B) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, cells were treated with or without 1 mM MMS for 30 min and lysates were subjected to IP and immunoblotting analyses with the indicated antibodies. (C) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, cells were immunostained with the indicated antibodies. Actin filaments (F-actin) were labeled CytoPainter Phalloidin-iFluor 680 reagent (red). Nuclei were stained with DAPI. (D) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, cells

were treated with 100 μg/ml CHX for the indicated times and analyzed by immunoblotting with the indicated antibodies. Relative Flag-MORC2 expression levels (Flag-MORC2/vinculin) are shown in lower panel. The quantitative results are represented as mean ± s.d. as indicated from three independent experiments.

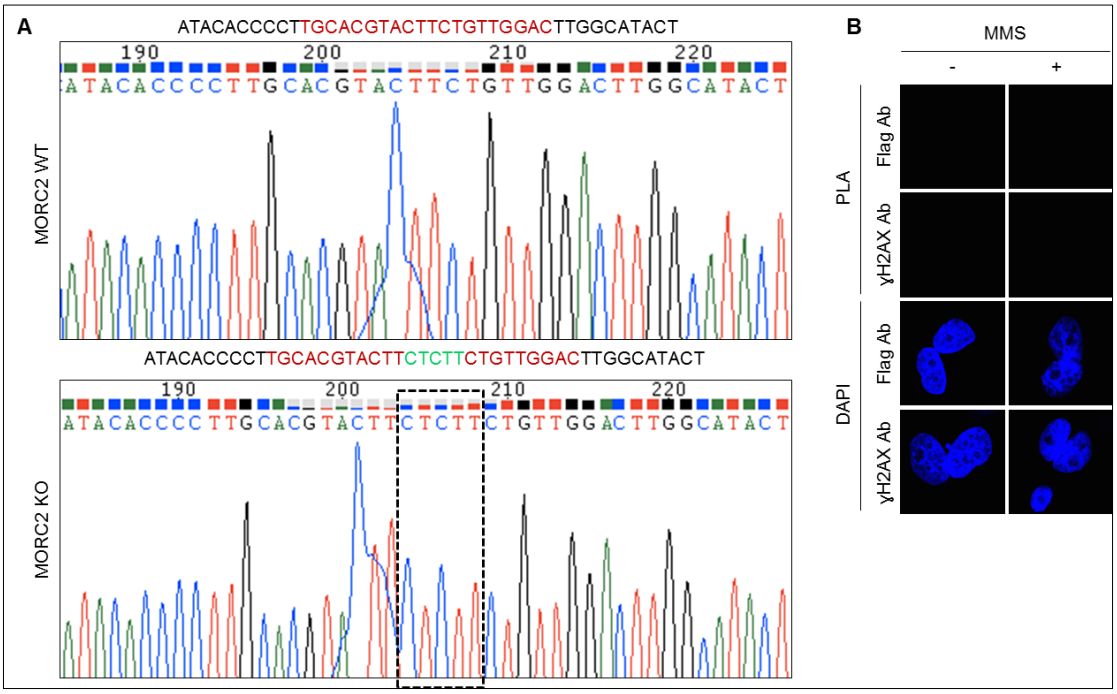

Figure S4. Validation of MORC2 KO cells and negative controls for PLA assays

(A) Validation of MORC2 KO cells by PCR and sequencing across the CRISPR target sites. Sequences in red indicate CRISPR target sites, while the sequences in green represent the mutations generated. (B) MORC2 KO MCF-7 cells stably expressing Flag-MORC2 and Flag-MORC2-2A were treated with or without 1 mM MMS for 30 min. Negative controls for *in situ* PLA assays were performed using a single antibody against Flag or γH2AX. Nuclei were counterstained with DAPI (blue).

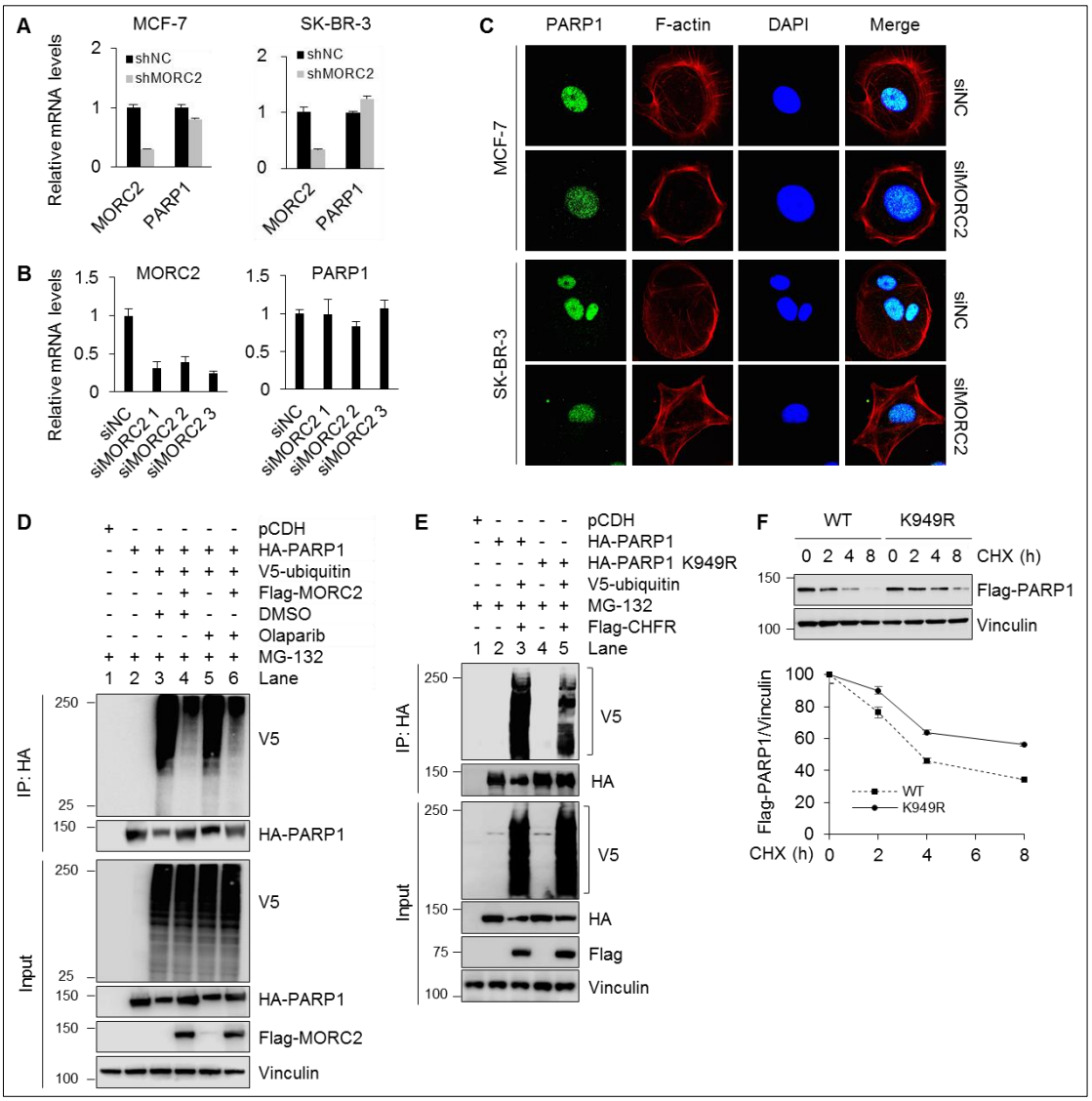

Figure S5. MORC2 does not affect the mRNA levels and subcellular localization of PARP1

(A) qPCR analysis of MORC2 and PARP1 mRNA levels in MCF-7 and SK-BR-3 cells stably expressing shNC and shMORC2. (B) MCF-7 and SK-BR-3 cells were transfected with siNC or three different siRNAs targeting MORC2 (siMORC2 #1-3). After 48 h of transfection, RNA was isolated and then subjected to qPCR analysis of MORC2 and PARP1 mRNA levels. (C) MCF-7 and SK-BR-3 cells were transfected with siNC or siMORC2. After 48 h of transfection, cells were immunostained with the indicated antibodies. Actin filaments (F-actin) were labeled CytoPainter

Phalloidin-iFluor 680 reagent (red). Nuclei were stained with DAPI. (D) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, cells were treated with 10 μM MG-132 for 5 h and then incubated with or without 5 μM Olaparib for another 1 h. IP and immunoblotting analyses were performed with the indicated antibodies. Lysis buffer was also supplemented with 10 μM MG-132 in the subsequent assays. (E) HEK293T cells were transfected with the indicated plasmid DNAs. After 48 h of transfection, cells were treated with 10 μM MG-132 for 6 h, and sequential IP and immunoblotting analyses were performed with the indicated antibodies. Lysis buffer was also supplemented with 10 μM MG-132 in the subsequent assays. (F) HEK293T cells were transfected with HA-PARP1 or HA-PARP1 K949R. After 48 h of transfection, cells were treated with 100 μg/ml CHX for the indicated times and analyzed by immunoblotting with the indicated antibodies. Relative HA-PARP1 expression levels (HA-PARP1/Vinculin) are shown in lower panel. The quantitative results are represented as mean ± s.d. as indicated from three independent experiments.

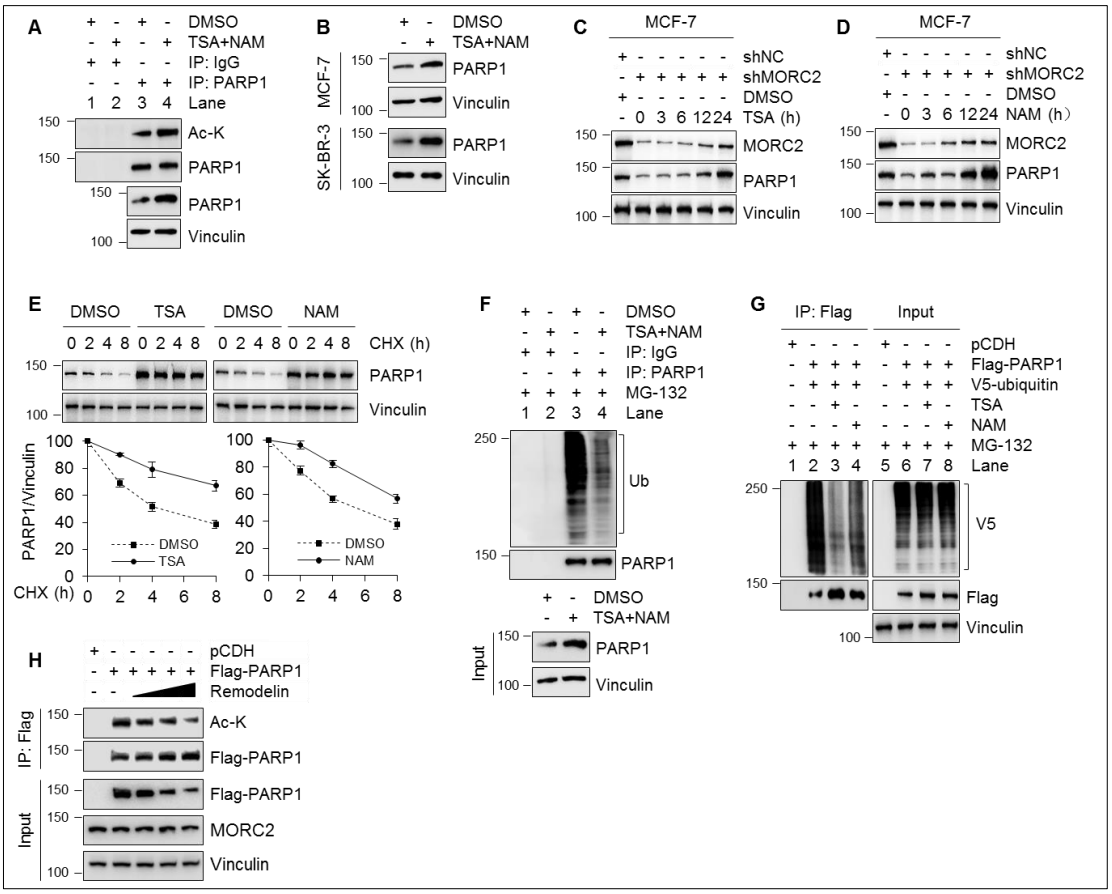

Figure S6. MORC2 stabilizes PARP1 in an acetylation-dependent manner

(A) MCF-7 cells were treated with DMSO or 5 μM TSA plus 5 mM NAM for 12 h. Lysates were immunoprecipitated with control IgG or an anti-PARP1 antibody, followed by immunoblotting analysis with the indicated antibodies. (B) MCF-7 and SK-BR-3 cells were DMSO or 5 μM TSA plus 5 mM NAM for 12 h. Lysates were analyzed by immunoblotting with the indicated antibodies. (C-D) MCF-7 cells stably expressing shNC and shMORC2 were treated with DMSO or 5 μM TSA (C) or 5 mM NAM (D) for the indicated times. Lysates were analyzed by immunoblotting with the indicated antibodies. (E) MCF-7 cells were pretreated with DMSO and 5 μM TSA (left) or 5 mM NAM (right) for 12 h. Then, cells were treated with 100 μg/ml CHX for the indicated times and analyzed by immunoblotting with the indicated antibodies.

Relative PARP1 expression levels (PARP1/Vinculin) are shown in lower panels. The quantitative results are represented as mean ± s.d. as indicated from three independent experiments. (F) MCF-7 cells were treated with DMSO or 5 μM TSA plus 5 mM NAM for 6 h, and then incubated with 10 μM MG-132 for another 6 h. Lysates were immunoprecipitated with control IgG or an anti-PARP1 antibody, followed by immunoblotting analysis with the indicated antibodies. Lysis buffer was also supplemented with 10 μM MG-132 in the subsequent assays. (G) HEK293T cells were transfected with the indicated plasmid DNAs. After 48 h of transfection, MCF-7 cells were treated with DMSO or 5 μM TSA or 5 mM NAM for 6 h, and then incubated with 10 μM MG-132 for another 6 h. Lysates were subjected to sequential IP and immunoblotting analyses were performed with the indicated antibodies. Lysis buffer was also supplemented with 10 μM MG-132 in the subsequent assays. (H) HEK293T cells were transfected with pCDH or Flag-PARP1. After 48 h of transfection, cells were treated with the increasing doses of NAT10 inhibitor remodelin (0, 1, 5 or 10 μM) for 12 h and then subjected to IP and immunoblotting analyses with the indicated antibodies. The immunoprecipitated PARP1 has been adjusted to be equal to make the levels of acetylated PARP1 comparable to those of immunoprecipitated PARP1.

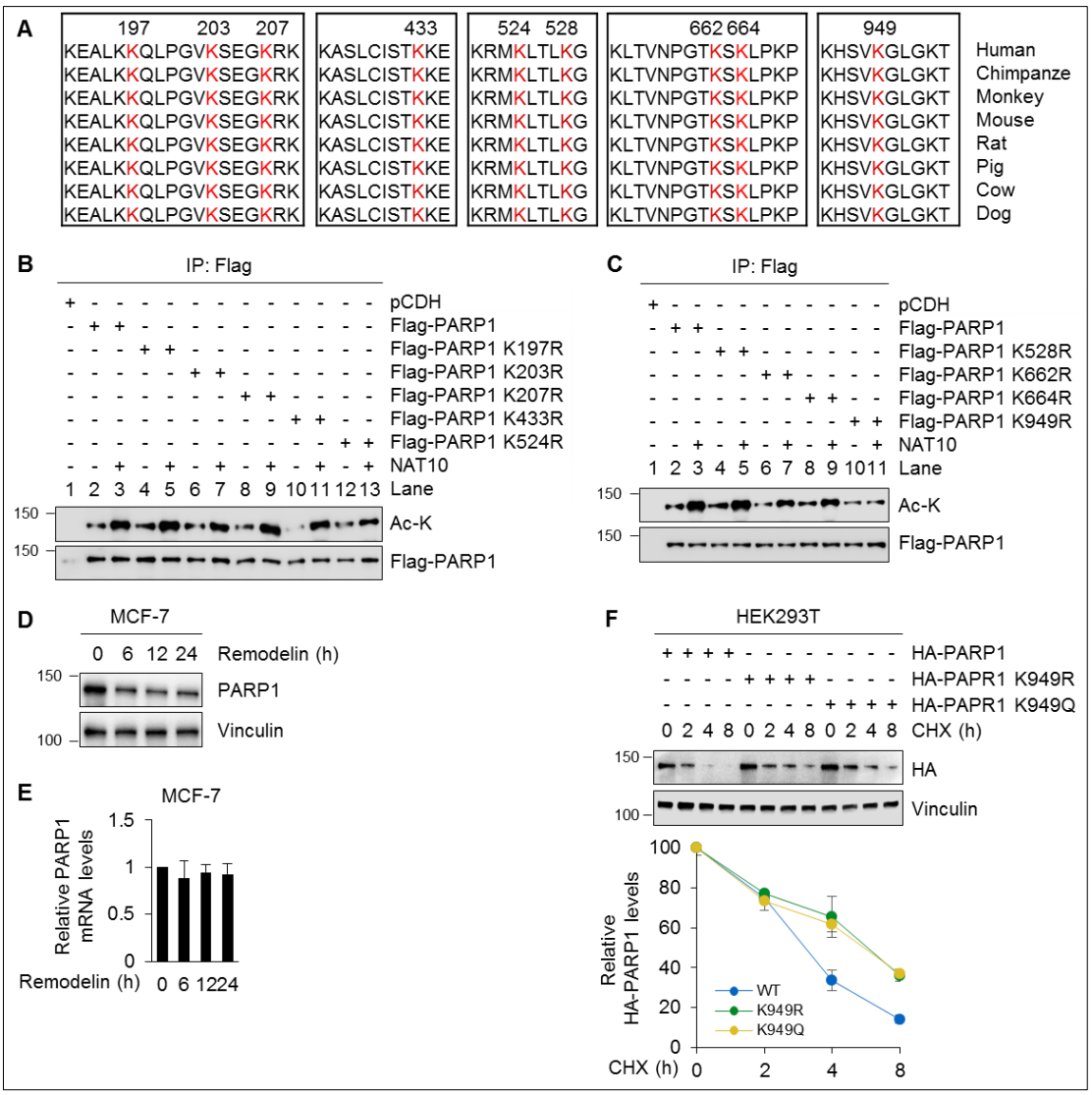

Figure S7. NAT10 acetylates PARP1 at lysine 949

(A) Potential acetylation sites of PARP1 identified by proteomic analyses. Sequence alignments of PARP1 from several species showed that these potential acetylation sites are evolutionary conserved. (B-C) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, lysates were subjected to IP and immunoblotting analyses with the indicated antibodies. (D-E) MCF-7 cells were treated with 5 μM remodelin for the indicated times. Cells were harvested and subjected to immunoblotting (D) or qPCR (E) analysis of PARP1 protein or mRNA

levels, respectively. (F) HEK293T cells were transfected with HA-PARP1, HA-PARP1 K949R, or HA-PARP1 K949Q. After 48 h of transfection, cells were treated with 100 μg/ml CHX for the indicated times and analyzed by immunoblotting with the indicated antibodies. Relative HA-PARP1 expression levels (HA-PARP1/Vinculin) are shown in lower panel. The quantitative results are represented as mean ± s.d. as indicated from three independent experiments.

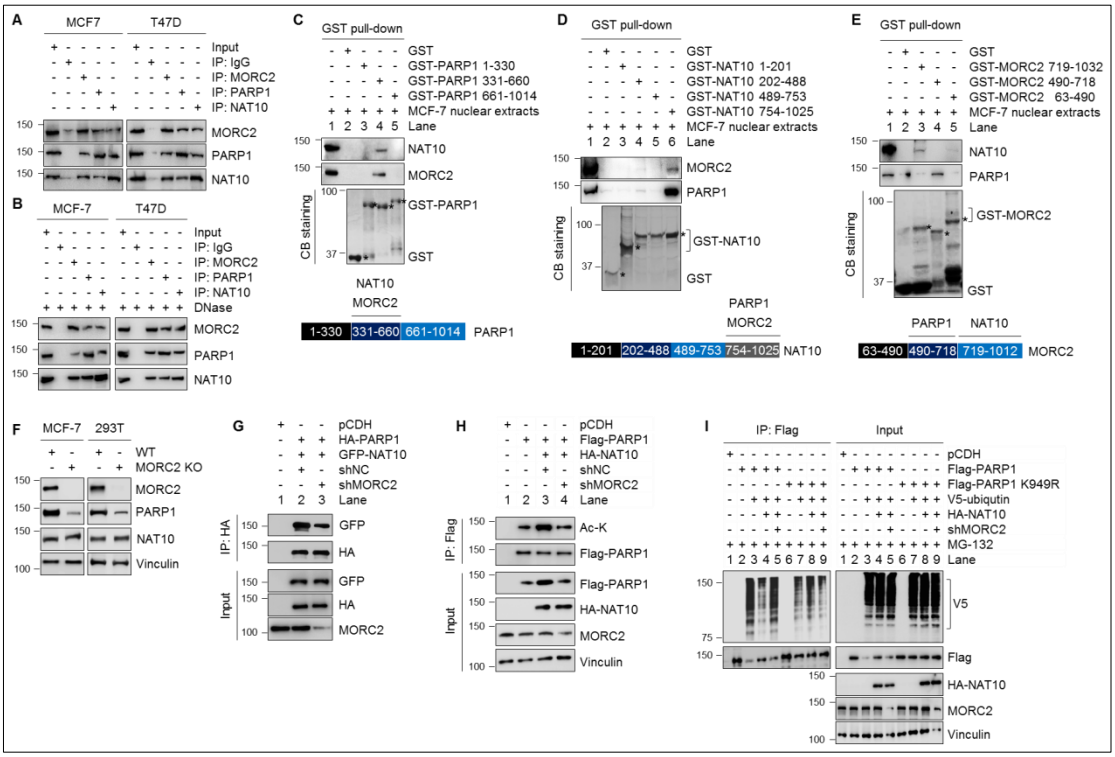

Figure S8. MORC2, PARP1, and NAT10 form a ternary complex

(A-B) Lysates from MCF-7 and T47D cells were immunoprecipitated with control IgG or antibodies against MORC2, PARP1, or NAT10 in the absence (A) or the presence (B) of 0.35 U/μl DNase, followed by immunoblotting analysis with the indicated antibodies. (C) GST or GST-PARP1 deletion fragments were incubated with MCF-7 nuclear extracts. The pull-down protein complex was subjected to immunoblotting analysis with an anti-MORC2 or anti-NAT10 antibody. GST or GST-PARP1 proteins were stained by Coomassie brilliant blue (CB) solution as loading controls. The domain of PARP1 for MORC2 and NAT10 binding is indicated in bottom panel. (D) GST or GST- NAT10 deletion fragments were incubated with MCF-7 nuclear extracts. The pull-down protein complex was subjected to immunoblotting analysis with an anti-MORC2 or anti-PARP1 antibody. GST or GST-NAT10 proteins were stained by

CB solution as loading controls. The region of NAT10 for MORC2 and PARP1 binding is shown in bottom panel. (E) GST or GST-MORC2 deletion fragments were incubated with MCF-7 nuclear extracts. The pull-down protein complex was subjected to immunoblotting analysis with an anti-NAT10 or anti-PARP1 antibody. GST or GST-MORC2 proteins were stained by CB solution as loading controls. The region of MORC2 for NAT10 and PARP1 binding is shown in bottom panel. (F) WT and MORC2 KO cells (MCF-7 and HEK293T) were harvested for immunoblotting analysis with indicated antibodies. (G-H) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, lysates were subjected to sequential IP and immunoblotting analysis with the indicated antibodies. (I) HEK293T cells were transfected with the indicated expression vectors. After 48 h of transfection, cells were treated with 10 μM MG-132 for 6 h, and lysates were subjected to sequential IP and immunoblotting analysis with the indicated antibodies. Lysis buffer was also supplemented with 10 μM MG-132 in the subsequent assays.

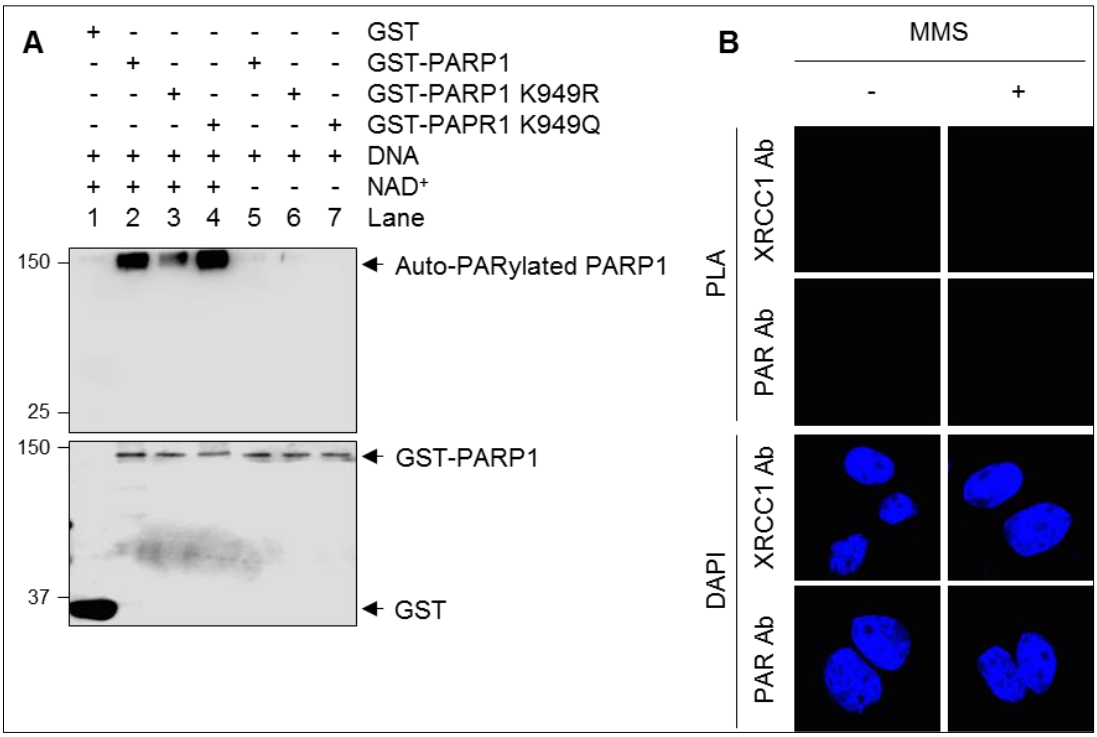

Figure S9. Acetylation of PARP1 at K949 affects its PAR activity

(A) *In vitro* PARylation assay was performed using purified WT, K949R, or K949Q mutant of PARP1 in the presence or absence of NAD+ and sonicated salmon sperm DNA as indicated. PARylated PARP1 was detected with an anti-PAR antibody. (B) MCF-7 cells were treated with or without 1 mM MMS for 30 min. Negative controls for *in situ* PLA assays were performed using a single antibody against XRCC1 or PAR. Nuclei were counterstained with DAPI (blue).

Supplementary Tables

Table S1. Information for the expression vectors used in this study

Plasmids	Sources	Vectors
Myc-DDK-MORC2	Origene	pCMV6-Entry
Flag-MORC2-WT	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-K504A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-E516A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-K517A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-D518A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-D521A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-2A	Subcloned	pCDH-CMV-MCS-EF1-Puro
Flag-MORC2-5A	Subcloned	pCDH-CMV-MCS-EF1-Puro
GST-MORC2	Subcloned	pGEX-6P-1
GST-MORC2 63-490	Subcloned	pGEX-6P-1
GST-MORC2 491-718	Subcloned	pGEX-6P-1
GST-MORC2 491-718 5A	Subcloned	pGEX-6P-1
GST-MORC2 719-1032	Subcloned	pGEX-6P-1
GST-MORC2-K504A	Subcloned	pGEX-6P-1
GST-MORC2-E516A	Subcloned	pGEX-6P-1
GST-MORC2-K517A	Subcloned	pGEX-6P-1
GST-MORC2-D518A	Subcloned	pGEX-6P-1
GST-MORC2-D521A	Subcloned	pGEX-6P-1
GST-MORC2-2A	Subcloned	pGEX-6P-1
Myc-DDK-PARP1	Origene	pCMV6-Entry
Myc-DDK-PARP1 K197R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K203R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K207R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K433R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K524R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K528R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K662R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K664R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 K949R	Subcloned	pCMV6-Entry
Myc-DDK-PARP1 E988K	Subcloned	pCMV6-Entry
HA-PARP1	Subcloned	pCDH-CMV-MCS-EF1-Puro
HA-PARP1 E988K	Subcloned	pCDH-CMV-MCS-EF1-Puro
HA-PARP1 E988Q	Subclone	pCDH-CMV-MCS-EF1-Puro
His-PARP1	Subcloned	pET28a(+)
GST-PARP1 1-331	Subcloned	pGEX-6P-1
GST-PARP1 332-661	Subcloned	pGEX-6P-1
GST-PAPR1 662-1014	Subcloned	pGEX-6P-1
HA-NAT10	Subcloned	pCDH-CMV-MCS-EF1-Puro
HA-NAT10 G641E	Subcloned	pCDH-CMV-MCS-EF1-Puro
GFP-NAT10	Origene	pCMV6-AC-GFP
GST-NAT10 1-201	Subcloned	pGEX-6P-1
GST-NAT10 201-488	Subcloned	pGEX-6P-1
GST-NAT10 489-753	Subcloned	pGEX-6P-1
GST-NAT10 754-1025	Subcloned	pGEX-6P-1
Flag-CHFR	Vivogene	pENTER
GST-CHFR	Subcloned	pGEX-6P-1
shMORC2	Origene	pGFP-C-shLenti
LentiCas9	Addgene	LentiCas9-Blast
LentiGuide	Addgene	LentiGuide-Puro

Table S2. Primers used for molecular cloning of expression vectors

Plasmids	Primers	Sequences
pCDH-Flag-MORC2	Forward	ACCTCCATAGAAGATTCTAGAGCCACC ATGCTTTGCTTTTTGGATGATGGAG
	Reverse	GATCCATTTAAATTCGAATTCTTACTTA TCGTCGTCATCCTTGTAATC
pCDH-Flag-MORC2-K504A	Forward	ACCACCATCCAGTGCGATTTGTGTCT GGCTTGGAGAACCCTC
	Reverse	ACTCAGCTGGAAGGGGAGGGTTCTC CAAGCCAGACACAAATC
Flag-MORC2-E516A	Forward	CTCCCCTTCCAGCTGAGTTCTGTGGC TAAAGATTACCCT
	Reverse	AACCCAGGTGTCAGGGTAATCTTTAG CCACAGAACTCAG
Flag-MORC2-K517A	Forward	CCCTTCCAGCTGAGTTCTGTGGAAGC TGATTACCCTGAC
	Reverse	GCAAACCCAGGTGTCAGGGTAATCAG CTTCCACAGAACT
Flag-MORC2-D518A	Forward	TTCCAGCTGAGTTCTGTGGAAAAAGC TTACCCTGACACC
	Reverse	GGAGCAAACCCAGGTGTCAGGGTAA GCTTTTTCCACAGA
Flag-MORC2-D521A	Forward	AGTTCTGTGGAAAAAGATTACCCTGCT ACCTGGGTTTGCTCCATGAACCCT
	Reverse	AGGGTTCATGGAGCAAACCCAGGTAG CAGGGTAATCTTTT
Flag-MORC2-2A	Forward	CCCTTCCAGCTGAGTTCTGTGGCTGC TGATTACCCTGAC
	Reverse	GCAAACCCAGGTGTCAGGGTAATCAG CAGCCACAGAACT
pCDH-Flag-MORC2-E516/K517/D518/D521A	Forward	ACCCTCCCCTTCCAGCTGAGTTCTGT GGCTGCTGCTTACCCTGCTACCTGGG TTTGC
	Reverse	AGGGTTCATGGAGCAAACCCAGGTAG CAGGGTAAGCAGCAGCCACAGAACTC AG
GST-MORC2	Forward	AAGTGTCGACATGCTTTGCTTTTTGGA TGAT
	Reverse	AAATGCGGCCGCGTCCCCCTTGGTGA TGAG
GST-MORC2 K504A	Forward	ACCACCATCCAGTGCGATTTGTGTCT GGCTTGGAGAACCCTC
	Reverse	CAGCTGGAAGGGGAGGGTTCTCCAA GCCAGACACAAAT
GST-MORC2 E516A	Forward	CTCCCCTTCCAGCTGAGTTCTGTGGC TAAAGATTACCCT
	Reverse	AACCCAGGTGTCAGGGTAATCTTTAG CCACAGAACTCAG
GST-MORC2 K517A	Forward	CCCTTCCAGCTGAGTTCTGTGGAAGC TGATTACCCTGAC
	Reverse	GCAAACCCAGGTGTCAGGGTAATCAG CTTCCACAGAACT
GST-MORC2 D518A	Forward	TTCCAGCTGAGTTCTGTGGAAAAAGC TTACCCTGACACC

	Reverse	GGAGCAAACCCAGGTGTCAGGGTAA GCTTTTTCCACAGA
GST-MORC2 D521A	Forward	AGTTCTGTGGAAAAAGATTACCCTGCT ACCTGGGTTTGCTCCATGAACCCT
	Reverse	AGGGTTCATGGAGCAAACCCAGGTAG CAGGGTAATCTTTT
GST-MORC2 63-490	Forward	CTGGGATCCCCGGAATTCATGCTTTG CTTTTTGGATGATGGAG
	Reverse	GCGGCCGCTCGAGTCGACTTAGACC AGGTAGGGCACATCAACAAC
GST-MORC2 491-718	Forward	CTGGGATCCCCGGAATTCATGGTCCT GGAGCCTACACACAACAAACA
	Reverse	GCGGCCGCTCGAGTCGACTTATGGA GTCTTGATGACTTTGGGAGA
GST-MORC2 491-718 2A	Forward	CCCTTCCAGCTGAGTTCTGTGGCTGC TGATTACCCTGAC
	Reverse	GCAAACCCAGGTGTCAGGGTAATCAG CAGCCACAGAACT
GST-MORC2 491-718 5A	Forward	CTGGGATCCCCGGAATTCATGGTCCT GGAGCCTACACACAACAAACA
	Reverse	GCGGCCGCTCGAGTCGACTTATGGA GTCTTGATGACTTTGGGAGA
GST-MORC2 719-1032	Forward	CTGGGATCCCCGGAATTCATGGTGGT GAAGAAGACAGAGTCACCC
	Reverse	GCGGCCGCTCGAGTCGACTTAGTCC CCCTTGGTGATGAGGTC
Myc-DDK-PARP1 K197R	Forward	GCTACAGAGGATAAAGAAGCCCTGAA GCGACAGCTCCCAGGA
	Reverse	TCCTTCACTCTTGACTCCTGGGAGCT GTCGCTTCAGGGCTTC
Myc-DDK-PARP1 K203R	Forward	GCCCTGAAGAAGCAGCTCCCAGGAG TCCGAAGTGAAGGAAAG
	Reverse	CTCATCGCCTTTTCTCTTTCCTTCACT TCGGACTCCTGGGAG
Myc-DDK-PARP1 K207R	Forward	CAGCTCCCAGGAGTCAAGAGTGAAG GACGAAGAAAAGGCGAT
	Reverse	CACTCCATCCACCTCATCGCCTTTTCT TCGTCCTTCACTCTT
Myc-DDK-PARP1 K433R	Forward	AACAAGGCTTCCCTGTGCATCAGCAC CCGAAAGGAGGTGGAA
	Reverse	CTTCTTATTCATCTTTTCCACCTCCTTT CGGGTGCTGATGCA
Myc-DDK-PARP1 K524R	Forward	GGTATCAACAAATCTGAAAAGAGAATG CGATTAACTCTTAAA
	Reverse	CACAGCTGCTCCTCCTTTAAGAGTTAA TCGCATTCTCTTTTC
Myc-DDK-PARP1 K528R	Forward	TCTGAAAAGAGAATGAAATTAACTCTT CGAGGAGGAGCAGCT
	Reverse	AGAATCAGGATCCACAGCTGCTCCTC CTCGAAGAGTTAATTT
Myc-DDK-PARP1 K662R	Forward	AAGAAGCTGACAGTAAATCCTGGCAC CCGATCCAAGCTCCCC
	Reverse	GTCCTGAACTGGCTTGGGGAGCTTG GATCGGGTGCCAGGATT
Myc-DDK-PARP1 K664R	Forward	CTGACAGTAAATCCTGGCACCAAGTC

		CCGACTCCCCAAGCCA
	Reverse	GATGAGGTCCTGAACTGGCTTGGGGA GTCGGGACTTGGTGCC
Myc-DDK-PARP1 K949R	Forward	AAGTTACCCAAGGGCAAGCACAGTGT CCGAGGTTTGGGCAAA
	Reverse	AGGATCAGGGGTAGTTTTGCCCAAAC CTCGGACACTGTGCTT
Myc-DDK-PARP1 K949Q	Forward	GTGAATGACACCTCTCTACTATATAAC CAGTACATTGTCTAT
	Reverse	ATAGACAATGTACTGGTTATATAGTAGA GAGGTGTCATTCAC
Myc-DDK-PARP1 E988K	Forward	GTGAATGACACCTCTCTACTATATAAC AAGTACATTGTCTAT
	Reverse	TACCTGAGCAATATCATAGACAATGTA CTTGTTATATAGTAG
HA-PARP1	Forward	AAGGTCTAGAATGGCGGAGTCTTCGG ATAA
	Reverse	AAGGGCGGCCGCTTAAGCGTAGTCTG GGACGTCGTATGGGTACCACAGGGA GGTCTTAAAATT
HA-PARP1 E988K	Forward	GTGAATGACACCTCTCTACTATATAAC AAGTACATTGTCTAT
	Reverse	TACCTGAGCAATATCATAGACAATGTA CTTGTTATATAGTAG
HA-PARP1 E988Q	Forward	GTGAATGACACCTCTCTACTATATAAC CAGTACATTGTCTAT
	Reverse	ATAGACAATGTACTGGTTATATAGTAGA GAGGTGTCATTCAC
His-PARP1	Forward	AAGGGTCGACATGGCGGAGTCTTCG GATAA
	Reverse	AAGGCTCGAGTTACCACAGGGAGGTC TTAAAATT
GST-PARP1 1-331	Forward	CTGGGATGGGGGGAATTCATGGCGG AGTCTTCGGATAA
	Reverse	GCGGCCGCTCGAGTCGACTTACATAC ACTTGGTCCAGGCAGT
GST-PARP1 332-661	Forward	CTGGGATGGGGGGAATTCGTCAAGAC ACAGACACCCAAC
	Reverse	GCGGCCGCTCGAGTCGACTTAGGTG CCAGGATTTACTGTCAG
GST-PAPR1 662-1014	Forward	CTGGGATGGGGGGAATTCAAGTCCAA GCTCCCCAAGCCA
	Reverse	GCGGCCGCTCGAGTCGACTTACCACA GGGAGGTCTTAAAATTG
HA-NAT10	Forward	AAGGGAATTCATGCATCGGAAAAAGG TGGAT
	Reverse	AAGGGGATCCTTAAGCGTAGTCTGGG ACGTCGTATGGGTATTTCTTCCGCTTC AGTTTCATA
HA-NAT10 G641E	Forward	GCTGTTCACCCAGATTATCAAGGGAT
	Reverse	GGGCTATGAAAGCCGTGCTCTGCAGC TG
		TTCATAGTACATCTGCAGCAGCTGCAG AGCACGGCTTTCATAGCCCATCCCTT

		GATA
GST-NAT10 1-201	Forward	AACCGGATCCATGCATCGGAAAAAGG TGGAT
	Reverse	AACCGCGGCCGCCTAATCAATGACGA GACACTTCTT
GST-NAT10 201-488	Forward	AACCGGATCCGACCAGCTCAACATCC TGC
	Reverse	AACCGCGGCCGCCTAATCCAGGCACA GCAAGTCAT
GST-NAT10 489-753	Forward	AACCGGATCCTGCCTCAACATCACTC GGAT
	Reverse	AACCGCGGCCGCCTAATCATGCTGAA GACGCTCAC
GST-NAT10 754-1025	Forward	AACCGGATCCGATGAGGATGAGGCTG ACC
	Reverse	AACCGCGGCCGCCTATTTCTTCCGCT TCAGTTTCAT
GST-CHFR	Forward	AATGAATTCATGGAGCGGCCCGAGGA AG
	Reverse	AATCTCGAGTTAGTTTTTGAACCTTGT CTGTTCA

Table S3. siRNA target sequences

	Primers	Sequences
siPARP1#1	Forward	UUCCUGCGUCUUCAUGUCCTT
	Reverse	UUCCUGCGUCUUCAUGUCCTT
siPARP1#2	Forward	GAGGAAGGUAUCAACAAAUTT
	Reverse	AUUUGUUGAUACCUUCCUCTT
siPARP1#3	Forward	GAGACCCAAUAGGCUUAAUTT
	Reverse	AUUAAGCCUAUUGGGUCUCTT
siMORC2#1	Forward	GCAGUACGGGAAUGGGUUATT
	Reverse	UAACCCAUUCCCGUACUGCTT
siMORC2#2	Forward	GCAGAGAAGCCGAUGUCAATT
	Reverse	UUGACAUCGGCUUCUCUGCTT
siMORC2#3	Forward	GGACAUGAAGACGCAGGAATT
	Reverse	UUCCUGCGUCUUCAUGUCCTT
siPARG#1	Forward	GGAAUCAAGACAGCGGAAUTT
	Reverse	AUUCCGCUGUCUUGAUUCCTT
siPARG#2	Forward	GCGGUGAAGUUAGAUUACATT
	Reverse	CCACGACGAAAUGCUAAGATT
siPARG#3	Forward	CCACGACGAAAUGCUAAGATT
	Reverse	UCUUAGCAUUUCGUCGUGGTT
siMacroD1#1	Forward	CCACCGGCGUGUUUGGCUATT
	Reverse	UAGCCAAACACGCCGGUGGTT
siMacroD1#2	Forward	GCUGCUACCUGAGCAGUCUTT
	Reverse	AGACUGCUCAGGUAGCAGCTT
siMacroD1#3	Forward	GGCUGAUCAUCUGCGUGUUTT
	Reverse	AACACGCAGAUGAUCAGCCTT

Table S4. Primers used for the PCR reaction

	Primers	Sequences
MORC2	Forward	CGTTGAGCTAGCACCCTAAAG
	Reverse	GGGCTCCTCTGAGCTGCATT
PARP1	Forward	CCCCTGCTTGGATTTAACAAG
	Reverse	ATTATAGTCAATAGAGGGAC

Table S5. Primary antibodies used in this study

Antibodies	Vendors	Cat#	Species	WB	IHC	IP	IF
MORC2	Bethyl	A300-149	Rabbit pAb	√		√	
Flag	Sigma	F3165	Mouse mAb	√		√	
Vinculin	Sigma	V9131	Mouse mAb	√			
HA	CST	C29F4	Rabbit pAb	√		√	
PARP1	Santa Cruz	SC8007	Mouse mAb	√	√	√	√
NAT10	Abcam	ab194297	Rabbit mAb	√	√	√	√
GFP	Santa Cruz	sc-9996	Mouse mAb	√		√	√
MYC	GNI	GNI4110-MC	Mouse mAb	√		√	
CHFR	CST	4297	Rabbit mAb	√		√	
PAR	Trevigen	4335-MC-100	Mouse mAb	√		√	√
PARG	CST	66564	Rabbit mAb	√		√	
γH2AX	CST	7631s	Rabbit mAb	√			
γH2AX	Abcam	ab26350	Mouse mAb	√	√	√	√
V5	CST	13202S	Rabbit pAb	√		√	√
GST	CST	2624	Mouse mAb	√			
Acetylated-Lysine	CST	9441s	Rabbit mAb	√	√	√	√
Ubiquitin	CST	3936s	Rabbit mAb	√		√	
XRCC1	Abcam	134056	Rabbit pAb	√	√	√	√
Rad51	Abcam	ab133534	Rabbit pAb	√	√	√	√
53BP1	Abcam	ab175933	Rabbit pAb	√	√	√	√
BRCA1	Santa Cruz	sc-6954	Mouse mAb	√		√	√
MacroD1	Origene	A331983	Rabbit pAb	√			

mAb, monoclonal antibody; pAb, polyclonal antibody.

Table S6. Primers for qPCR analysis

Genes	qPCR Primers	Sequences
MORC2	Forward	AAGCCGTTTCAAGACC
	Reverse	GTCGCAACATCACCCT
PARP1	Forward	CTGGGGAGTCGGCGATCTT
	Reverse	GGTTACCCACTCCTTCCGGT
GAPDH	Forward	CGAGATCCCTCCAAAATCAA
	Reverse	TTCACACCCATGACGAACAT

References

1. Washburn, M.P., Wolters, D. and Yates, J.R., 3rd. (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat Biotech*, **19**, 242-247.