

Supplementary Information

Figure S1. Targeted ES cells by TALEN method. **(A)** Sequence alignment of BRCA1 for human, cow, rat and mouse. The marked fragment in mouse sequence is the deleted fragment, 421-701aa, **(B)** TALEN target sequences. An arrow shows the targeted site. **(C)** Donor BRCA1 targeting vector. **(D)** RT-PCR of gene-targeted BRCA1. WT: 3529bp, Δ DBR: 2713bp, Δ 11:214bp **(E)** Western blot analysis of targeted mouse ES cells using antibodies for Flag and BRCA1. **(F)** MBP-tagged DBR of mouse BRCA1 (422-692aa) Left: Commassie staining of purified MBP-422-692aa, Right: EMSA using dsDNA. The concentration of labelled dsDNA and proteins are 40nM and 100nM, respectively.

A

Human GESESNAKVADVLDVLENEVDYSGSSEKIDLLASDPHEALICKSERVHSHKSVESNIEDKIFGKTYRKKASLPNLSHVTEENLIGAFVTEPQIIQERPLTN 500
 Cow GGSESNEVAGAVEIPNKVDGYSGSEKINLMASDPHGTLIH--ERVHSHKSVESNIEDKIFGKTYRKKSSLPNFSHIAEDLILGAFTVEPQITQEQLPLTN 498
 Rat RRPASNAEAAVLEVSNEVDGCFSSSKIDLVA PDPDNA VMCTSGRDFSKPVENIINDKIFGKTYQRKGRPHLNHVTE--IIGFTTTEPQIIQEPPFTN 496
 Mouse RRHESNAEAAVLEVSNEVDGGFSSSRKTDLVTDPHHTLMCKSGRDFSKPVEDNISDKIFGKSYQRKGRPHLNHVTE--IIGTFITEPQITQEPPFTN 495

Human KLRKRRTSGLHPDEFIKKADLAVQ-KTPEMINQGTNQTQEQNGVQVMNITNSGHENKTKGDSIQNEKNPNPIESLEKESAFKTKAEPISSSISNMELELN 599
 Cow KLRKRRTSGLHPDEFIKKVDLVTIVPKTEKMTGETDQTEQKCHGMNITSDGHENKTKRDYVQKEQNANPAESLEKESVFRTEAEPISISISNMELELN 598
 Rat KLRKR--STCLHPDEFIKKADLTVVQRISENLNQGTDQMEPNDAQMSITNSNGENRATGNDLQRGRNAHPIESLRKEPAFTAKAKSISNSISDLEVELN 594
 Mouse KLRKR--STSLQPEDEFIKKADSAGVQRTPDNINQGTDLMEPNQAVSTTNSCQENQIAGSNLQKEKSAHPTESLRKEPASTAGAKSISNSVSDLEVELN 593

Human IHNSKAPKKNRLRRKSSSTRHIAHALELVVSRNLSPPNCTELQIDSCSSSEIEKKKKYNQMPVHRSLRNLQLMEGKEPATGAKKSNKPNEQTSKRHSDTFPE 699
 Cow IHRSKAPK-NRLRRKSSSTRKIPELELVVSRNLSPLNHTLEPIDSSSSNEEMKKKSSQMPVRSQKQLQIGDKELTAGAK-NNKTYEQINKRLASDAFPE 696
 Rat VHSSKAPKKNRLRRK-TRCVLPLEPIS-RNPSPTCAELQIESCGSSEETKKNNSNQTPAGHIREPQLIEDTEPAADAK-KNEPNEHIRKRSASDAFPE 691
 Mouse VHSSKAPKKNRLRRKSSIRCALPLEPIS-RNPSPTCAELQIDSCGSSSEETKKNHNSNQPAHGLRREPQLIEDTEPAADAK-KNEPNEHIRKRRASDAFPE 691

Human LKLTNAPGSFTKCSNTSELKEFVNPSLPREEKEEKLETVKVSNNAEDPKD 749
 Cow LKLTNTPGYFTNCS--SKPEEFVHPSLQREEN--LGTIQVSNSTKDPKD 741
 Rat EKL MNKAGLLTSCSSPRKPGVPVNSPERKGIEQ-LEMCQMPDNNKELGD 740
 Mouse EKL MNKAGLLTSCSSPRKSQGPVNSPQRTGTGEQ-LETRQMSDSAKELGD 740

B

5' TALE binding target
 5' TCAAAACGAAGTGGATGGGGGTTTTAGTTCCTCAAGGAAAACAGACTTAGT 3'
 3' AGTTTGCTTCAOCTACCCCCAAAATCAAGAAGTTCCTTTTCAGTGAATCA 5'
 3' TALE binding target

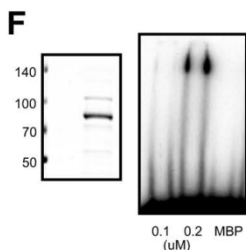
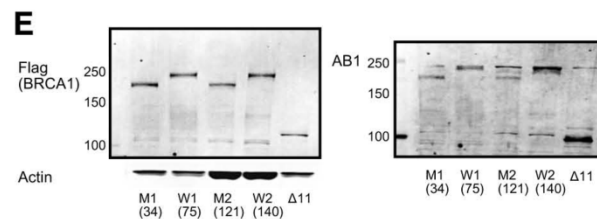
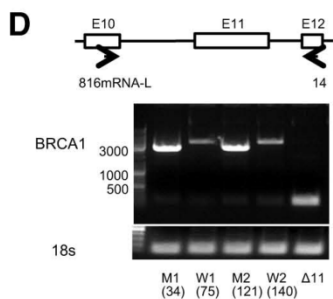
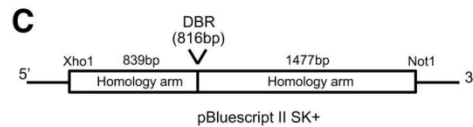


Figure S2. HR assay using HeLa cells in which BRCA1 is stably knocked down by shBRCA1 or the control. **(A)** Western blot analysis of HeLa cells for BRCA1 expression. **(B)** HR assay. * $P < 0.05$, $N = 3$ ** $N.S.$, $N = 3$.

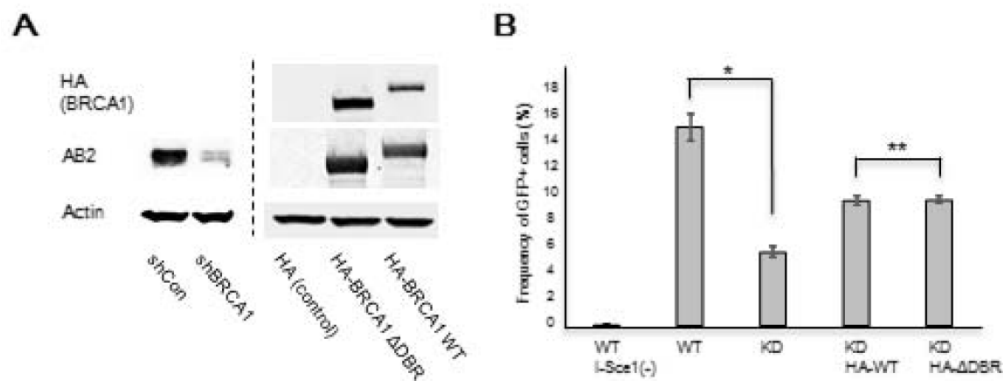


Figure S3. Purified BRCA1 proteins stained with Coomassie. **(A)** MBP, MBP-full-length BRCA1, MBP-BRCA1 deficient in DBR. **(B)** GST-BRCA1 fragments. **(C)** MBP-BRCA1 fragments.

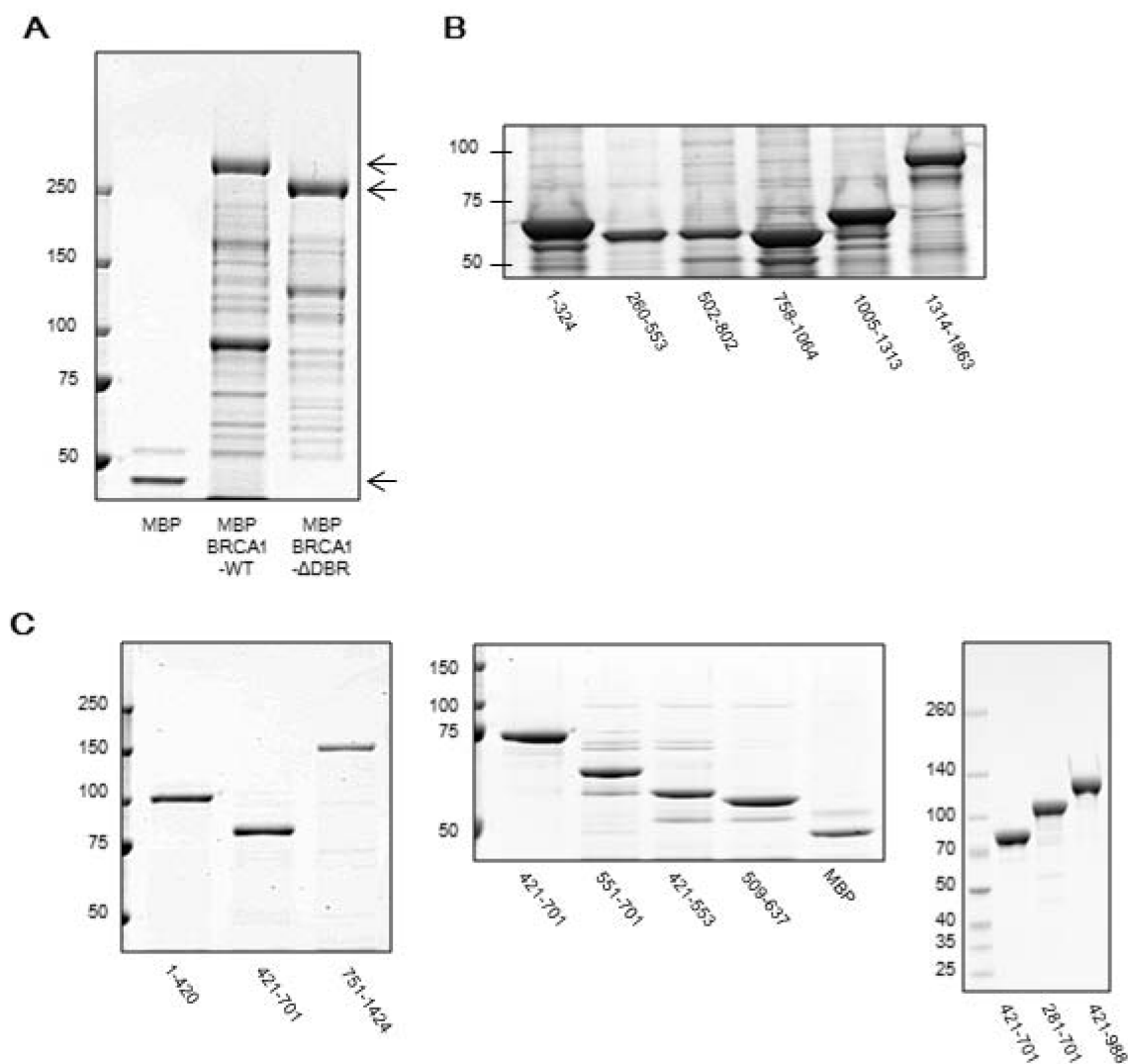
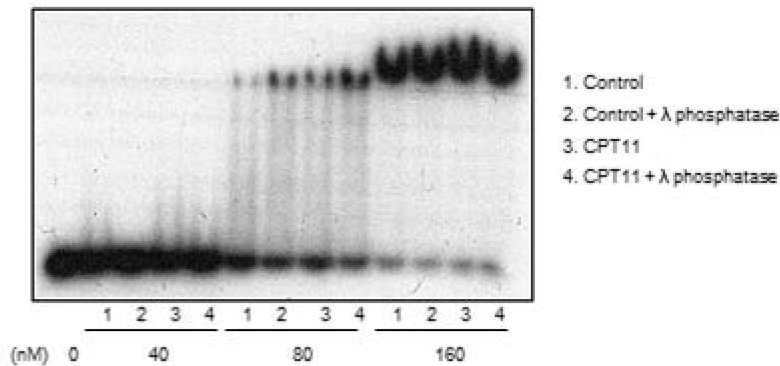
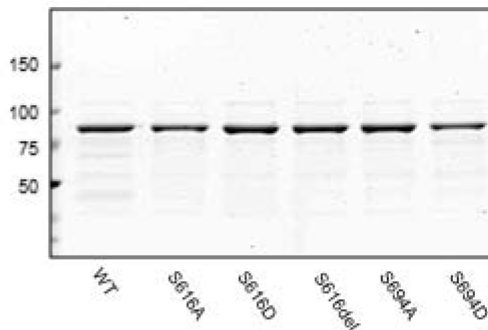


Figure S4. Phosphorylation status of BRCA1-DBR affects the binding affinity to dsDNA *in vitro*. **(A)** EMSA using CPT11-treated BRCA1-DBR or the control with or without incubation with λ protein phosphatase *in vitro*. The concentration of labelled dsDNA is 40nM. **(B)** Purified BRCA1 mimics with Coommasie staining. Mimics of phosphorylation (from serine (S) to aspartic acid (D)) and dephosphorylation (from serine (S) to alanine (A)) in S616 and S694, which are reported to be phosphorylation sites or mutations in breast cancer patients within DBR [31,32]. **(C)** EMSA using the dephosphorylation mimic. They bind to dsDNA better than the wild type or the phosphorylation mimic. The concentration of labelled dsDNA and proteins are 40nM and 100nM, respectively.

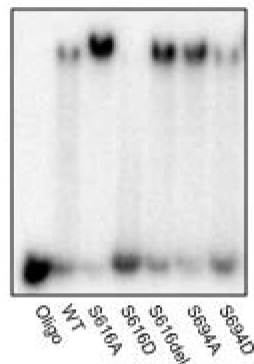
A



B



C



Legend for Illustration: The role of DBR in BRCA1 for genomic integrity.

The DBR in BRCA1 modulates genetic stability through intra-S-phase checkpoint by phosphorylation of Chk1 resulting from replication stress, although its role in HR repair is dispensable.

Table S1. Primer sequences for BRCA1 expression constructs.

Construct	PCR primers (5'-3')
human	
full length	ACCGGTCGCCTCCCTCGGATCCATGGA GGGCCCTCTAGATGCATGCTCGAGCC
full length del 421-701	GATTCCCAGGACCCACACTG CTGAGGGCTGGGATTGACAG
1-420	ACCGGTCATGGATTTATCTGCTCTTC CTCGAGTCAATCTACCTCATTTAGAACG
421-701	ACCGGTCGAATATTCTGGTTCTTCAGA CTCGAGTCACTTCAGCTCTGGGAAAGTA
751-1424	ACCGGTCATGTAAAGTGGAGAAAGGGT CTCGAGTCACTGGCTCCCATGCTGTTCT
421-553	ACCGGTCGAATATTCTGGTTCTTCAGA CTCGAGTCAATGACCACTATTAGTAATATTC
509-637	ACCGGTCACATCAGGCCTTCATCCTGAG CTCGAGTCAAGTACAATTAGGTGGGCTTAG
551-701	ACCGGTCGGTCATGAGAATAAAACAAAAGG CTCGAGTCACTTCAGCTCTGGGAAAGTA
281-701	ACCGGTCAGCTCATTACAGCATGAGAA CTCGAGTCACTTCAGCTCTGGGAAAGTA
421-988	ACCGGTCGAATATTCTGGTTCTTCAGA CTCGAGTCATGACTTGATGGGAAAAG
421-701 S616A	AGGAGGAAGTCTGCTACCAGGCATATTC GAATATGCCTGGTAGCAGACTTCCTCCT
421-701 S616D	GAGGAGGAAGTCTGATAACCAGGCATATTC GAATATGCCTGGTATCAGACTTCCTCCTC
421-701 S616del	ACCAGGCATATTCATGCGCTTG AGACTTCCTCCTCAGCCTATTC
421-701 S694A	ACCGGTCGAATATTCTGGTTCTTCAGA CTCGAGTCACTTCAGCTCTGGGAAAGTATCGGCGTCATG
421-701 S694D	ACCGGTCGAATATTCTGGTTCTTCAGA CTCGAGTCACTTCAGCTCTGGGAAAGTATCGTCGTCATG
mouse	
422-693	ACCGGTCCTTCAAGGAAAACAGACTT CTCGAGTATTTCTTCTGGGAAAGC

Table S2. Oligonucleotides used in EMSA.

Substrate	No	Sequence (5'-3')
Double strand	1	GAACCGCCGCTCCCGCGACATCACAGGAAGGCTGAGAGA TCTCTCAGCCTTCTGTGATGTCGCGGGAGCGGCCGGTTC
	2	CTGATTTGCATAGCCCAATGGCCAAGCTGCATGCAAATGAG CTCATTGTCATGCAGCTTGGCCATTGGGCTATGCAAATCAG
	3	GCTCCCGCGACATCACAGGAAGGCT AGCCTTCTGTGATGTCGCGGGAGC
	4	TCTTTGACACGTTTATGGATTACAG CTGTAATCCATAAACGTGTCAAAGA
	5	GAAGTGGTGTATACGACAGAGACCG CGGTCTCTGTCGTATAACCACTTC
	6	CTCCCGCGACATCACAGGAA TTCCTGTGATGTCGCGGGAG
	7	GGCCGCTCCCGCGACATCACAGGAAGGCTG CAGCCTTCTGTGATGTCGCGGGAGCGGCC
	8	GCGCCTGCGCGAACCGGCCGCTCCCGCGACATCACAGGAAGGCTGAGAGAAAGGGCAGGT ACCTGCCCTTCTCTCAGCCTTCTGTGATGTCGCGGGAGCGGCCGGTTCGCGCAGGCGC
Single strand	9	GAACCGCCGCTCCCGCGACATCACAGGAAGGCTGAGAGA
Splayed-arm	10	GAACCGCCGCTCCCGCGACTAGTGTCTTCCGACTCTCT TCTCTCAGCCTTCTGTGATGTCGCGGGAGCGGCCGGTTC
5' overhang	11	GAACCGCCGCTCCCGCGAC TCTCTCAGCCTTCTGTGATGTCGCGGGAGCGGCCGGTTC
3' overhang	12	ATCACAGGAAGGCTGAGAGA TCTCTCAGCCTTCTGTGATGTCGCGGGAGCGGCCGGTTC