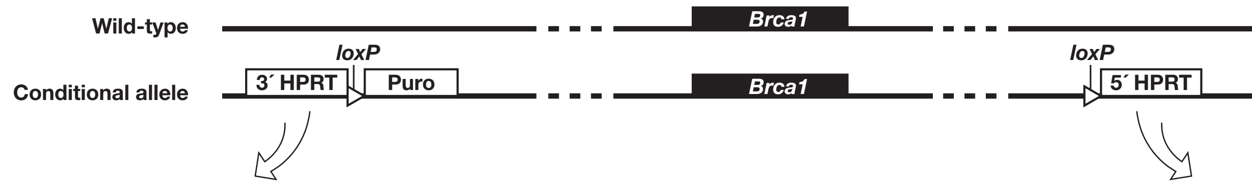
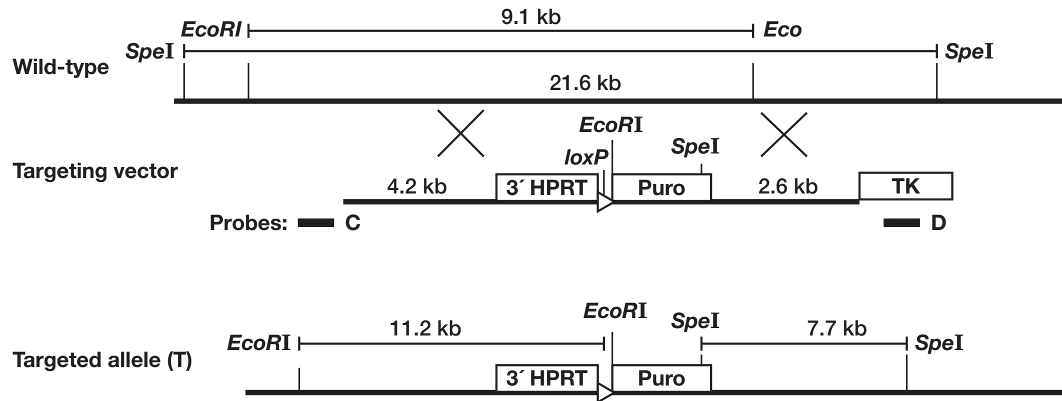
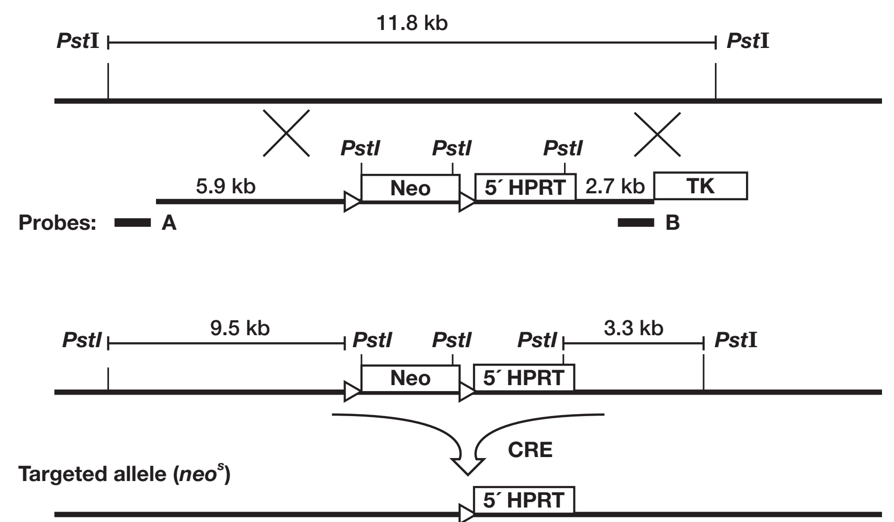
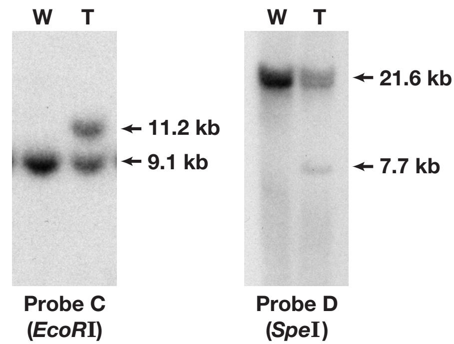
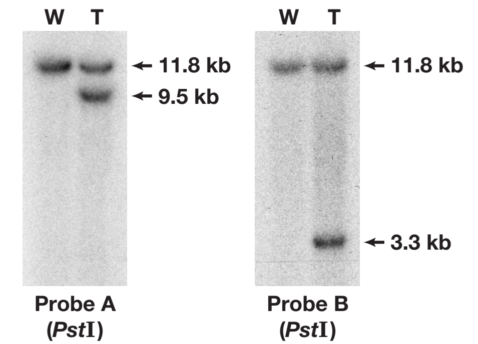
Supplementary Figure 1











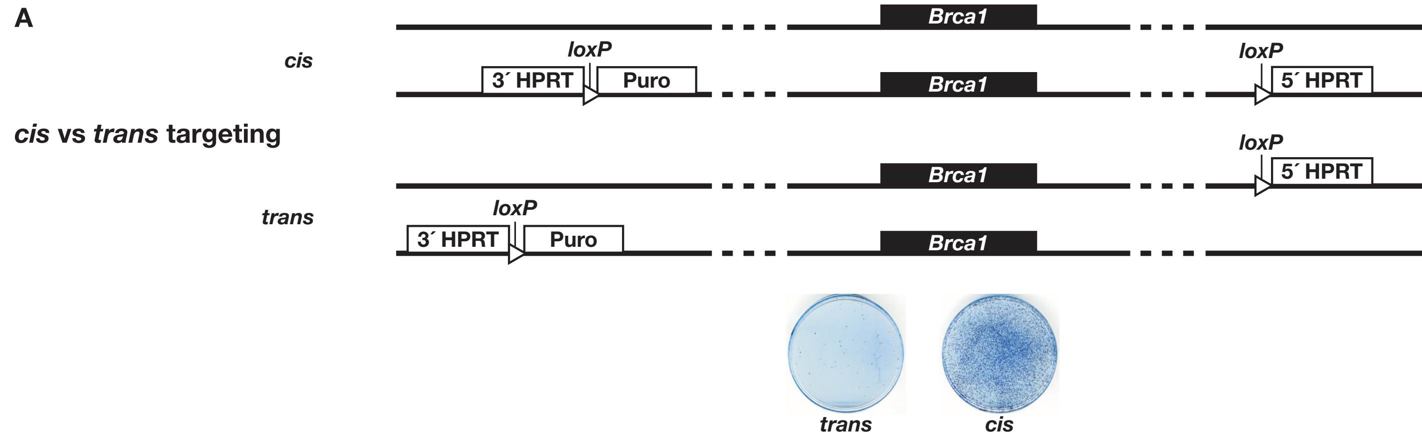
Supplementary Figure 1. Generation of a conditional allele of *Brca1* in mouse AB2.2 ES cells.

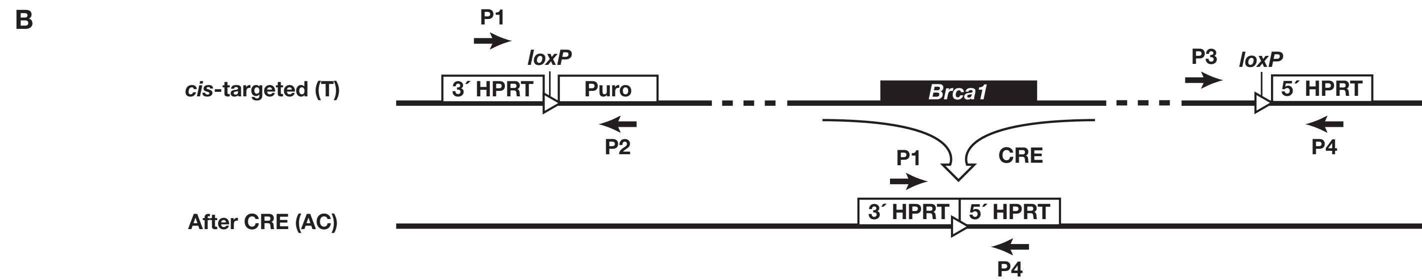
The conditional allele of *Brca1* was generated by inserting two *loxP* sites at the 5′ and 3′ ends of *Brca1* gene in mouse AB2.2 ES cells that are *Hprt*-deficient. The 5′and the 3′ends were marked by the SP6 ends of BACs RP22 33-10I (in short, referred to as BAC 33) and RP22 496-3D (in short, referred to as BAC 496), respectively. These two ends that are 303 kb apart, were sequentially targeted to insert the two *loxP* sites.

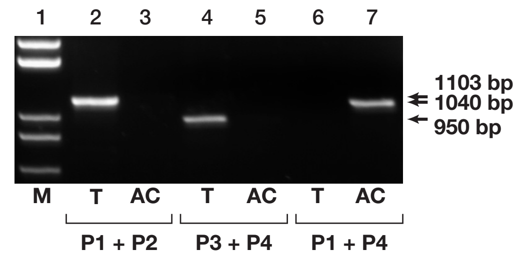
Targeting *loxP* + *5′HPRT1* to a 3′ region of *Brca1* (Right panels). A 8.6 kb *Hind*III/*Sal*I genomic fragment was sub-cloned from the SP6 end of BAC 496 into *pBluescript*. A 4.9 kb cassette (2.5 kb of 5′end of human *HPRT1* minigene containing *PGK* promoter, exons 1-2 and intron 2 with a *loxP* site and 1.4 kb of the *PGK neo* cassette with a second *loxP* site at the 3′end) was cloned into the middle of the insert at a *Eco*RV site by blunt end ligation. A *Thymidine Kinase* (*TK*) gene under the control of the MC1 promoter was cloned into the *Sal*I site. The resulting targeting vector with 5.9 kb and 2.7 kb homology arms was linearized with *Not*I and electroporated into AB2.2. cells, which were then selected for resistance to G418 and FIAU. Correctly targeted clones were identified by Southern blot analysis using probes A and B. Probe A (599 bp) was generated by PCR using BAC496-3D and the primers SKS-529 5′-CCTCTACAGATAAGGCTTCC-3′ and SKS-530 5′-CTGTCACGTACACTCATCCA-3′. Probe B (623 bp) was generated by PCR using BAC496-3D and the primers SKS-531 5′-GTGGGCAAGAGCGTTCATAT-3′ and SKS-532 5′-CTGTCACGTACACTCATCCA-3′ The *neo* cassette was deleted from the targeted clones ($G418^s$) by transient expression of the Cre recombinase and confirmed by

Southern analysis (data not shown). **Targeting *loxP + 3′HPRT1* to a 5′ region of *Brca1* (Left panel)** A 7.0 kb *EcoR*V/*Pst*I genomic fragment was sub-cloned from the SP6 end of BAC 33 into *pBluescript*. A 4.5 kb cassette (3.0 kb of the 3′end of the human *HPRT1* minigene containing intron 2 with a *lox*P site, exons 3-9, SV40polyA signal and 1.5 kb of *PGK puro* cassette) was cloned into the middle of the insert between two *Nhe*I sites (200bp apart) by the blunt end ligation. The *TK* gene under the control of the MC1 promoter was cloned into the *Kpn*I site of the vector. The resulting targeting vector with 4.2 kb and 2.6 kb homology arms was linearized with *Not*I and electroporated into ES cells containing *loxP + 5′HPRT1* in the 3′ region of *Brca1*. Cells were selected for resistance to puromycin and FIAU. Correctly targeted clones were identified by Southern blot analysis using probes C and D. Probe C (709 bp) was generated by PCR using BAC 33-10I and primers SKS-446 5′-TGCCCCAAGTACCTCAGTAA-3′ and SKS-447 5′-CTCTTCCAGCTTTGCTATGC-3′. Probe D (801 bp) was generated by PCR using BAC 33-10I and primers SKS-535 5′- AAGAGCTTGGGGAAGGAAAG-3′ and SKS-536 5′-ACTCAGGAGCTCTAGAAGAG-3′. Representative Southern blots for each targeting event are shown. W, wild-type; T, targeted allele

Supplementary Figure 2







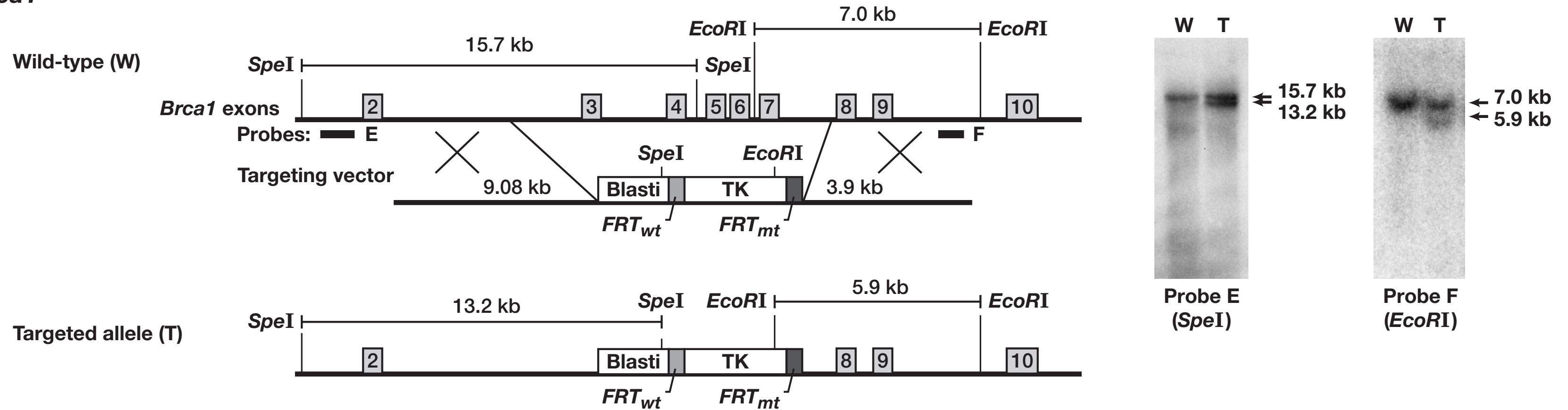
Supplementary Figure 2. Strategy to distinguish between clones in which *loxP* sites were targeted to the same chromosome (*cis*) or different chromosomes (*trans*).

A. Identification of *cis* and *trans* targeted *Brca1* alleles. *loxP* sites on the same chromosome recombine 100-1000-fold more efficiently than those on different chromosomes. Representative plates with HAT^r ES-cell colonies obtained after expression of the Cre-recombinase are shown below. Note that the *cis* clone has many more colonies compared to the *trans* clone.

B. Genotyping scheme and representative PCR analysis results to confirm the targeting of the two *loxP* sites in *cis*. Correct targeting was confirmed in HAT^r clones by PCR analysis using PCR primers P1, P2, P3, P4. Primers P1 (5´-GGTACTCTGATATAACATCAGG-3´) and P2 (5´-GGTCATGGTAAGCTTGGGCTGC-3´) amplify a 1040bp fragment (lane 2) and P3 (5´-GAACACATTGCTGTCTGTCC-3´) and P4 (5´-ACCTAATCATTATGCTGAGG-3´) amplify a 950 bp fragment (lane 4) in ES cell clones containing the targeted (T) conditional allele of *Brca1*. After Cre mediated recombination (AC), the fragments are not amplified (lanes 3 and 5) due to loss of the region corresponding to P2 and P3. Note that the P1 and P4 primers that are 300 kb apart in the targeted clone fail to amplify any product (lane 6), but result in a 1103 bp amplicon (lane 7) after *Cre*-mediated (AC) deletion of the intervening region. M, marker (lane 1)

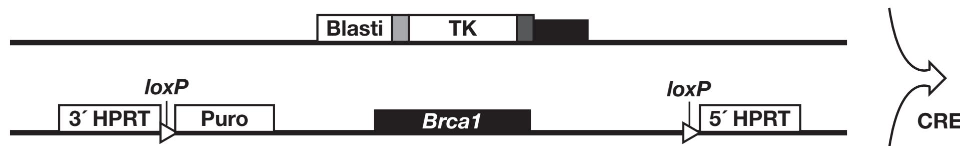
Supplementary Figure 3

A **Targeting *Brca1***



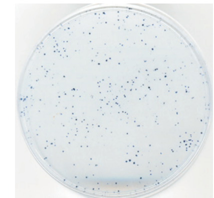
B **Distinguishing between wild-type (*trans*) vs. condition allele (*cis*) targeting**

Targeted wild-type allele (*trans*)

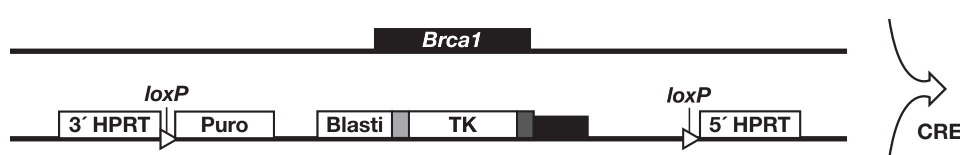


CRE

HAT^R colonies will NOT be obtained after CRE due to loss of both copies of *Brca1*

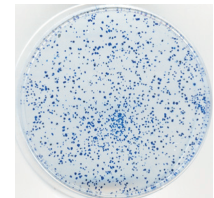


Targeted conditional allele (*cis*)



CRE

HAT^R colonies will be obtained after CRE due to the presence of one wild-type allele of *Brca1*

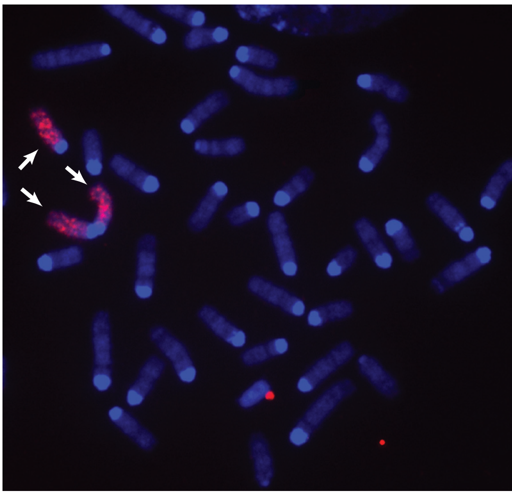


Supplementary Figure 3. Disruption of the second copy of *Brca1*.

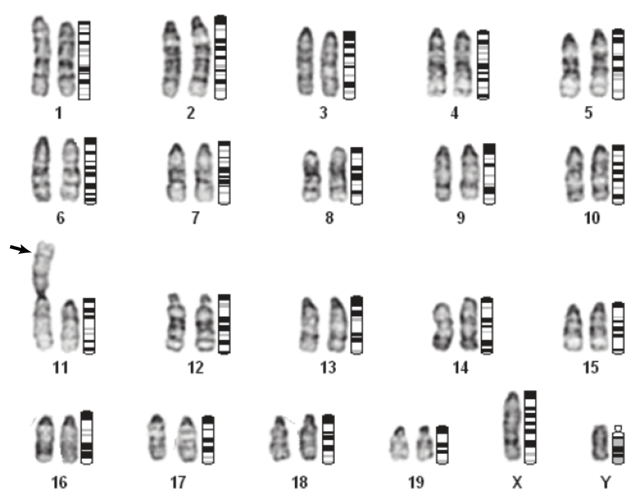
A. To disrupt the second allele of *Brca1* in ES cells already containing the conditional allele, a targeting vector was generated to delete 8.09 kb of genomic region containing exons 3-7. 5´ and 3´ homology arms of the targeting vector were 9.08 kb and 3.9 kb in length, respectively. A *Blasticidin* resistance gene was used for positive selection. In addition, a *TK* gene flanked by two heterologous *FRT* sites was inserted 3´ of the *Blasticidin* resistance gene. The *TK* cassette was included to facilitate insertion of a cDNA or a genomic clone into the *Brca1* locus using *FRT*-Flp-mediated recombination in future experiments. Targeted clones were identified by Southern blot analysis using probes E and F. Probe E (673 bp) was generated by PCR using BAC 493-19G as template and primers SKS-545 5´-CCTGAATGCTGGGTAGTTGA-3´ and SKS- 5465´-CAGAACAGCCAGGGCTATAT-3´. Probe F (853 bp) was generated by PCR using BAC 493-19G as template and primers SKS-509 5´-TAGAAGAGGCTATCAGAGCC-3´ and SKS-510 5´-GCCAGCTTGAGGATTGAACAC-3´.

B. Targeted clones were screened to identify those in which the wild-type allele of *Brca1* was targeted and not the conditional allele. This was achieved by selecting for HAT resistant clones after Cre-mediated recombination.

A



B

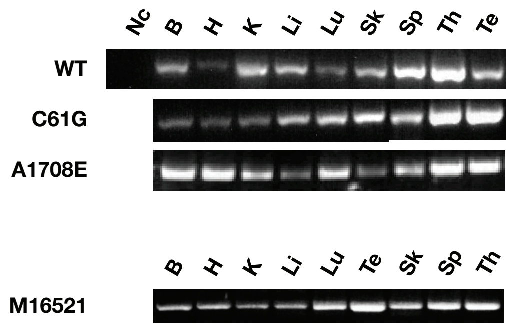


Supplementary Figure 4

Supplementary Figure 4. Analysis of chromosome 11 of HAT resistant ES cells.

A. The metaphase spread of a HAT resistant ES cell line was hybridized with a chromosome 11 specific probe and counterstained with DAPI. The three arrows indicate the presence of three copies of chromosome 11. This observation supports the hypothesis that the HAT resistant PL2F8 cells (background colonies) may retain a conditional allele of *Brca1* in addition to a knocked-out allele and a recombined conditional allele that results in a functional HPRT1 minigene**.**

B. Karyotype of the ES cell shown in A revealing the presence of three copies of chromosome 11 (arrow).



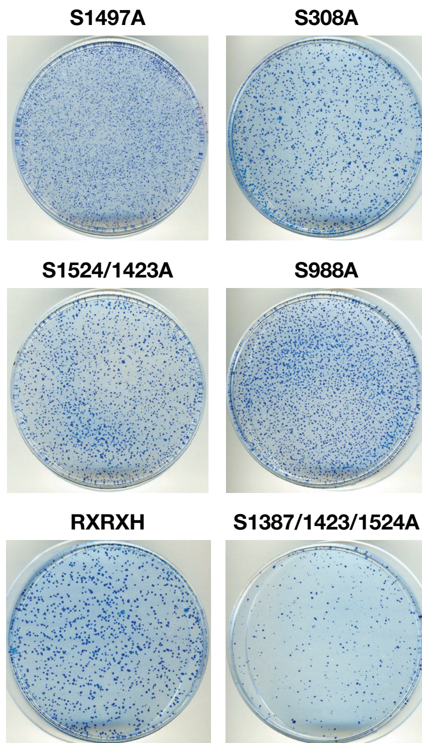
Supplementary Figure 5

Supplementary Figure 5. Expression of the BRCA1 transgene in multiple mouse tissues.

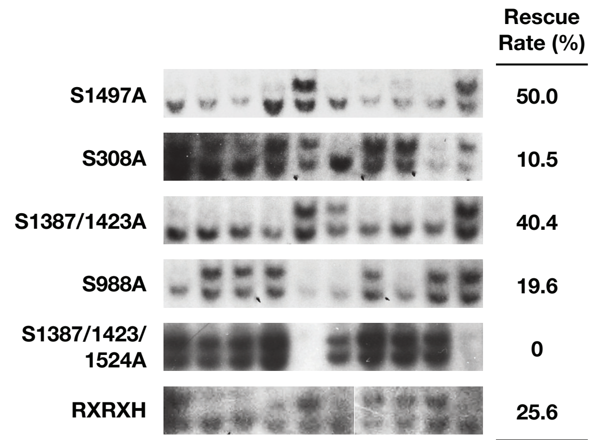
The tissues from the each of the BAC transgenic lines were collected. Total RNA was extracted by Trizol reagent and 3′ end of *Brca1* coding sequence was amplified using Titan one step RT-PCR system (Roche). The PCR products were analyzed on 1.5% agarose gel.

(Nc: Negative Control, B: Brain, H: Heart, K: Kidney, Li : Liver, Lu: Lung, Ov : Ovary, Sk: Skeletal Muscle, Sp: Spleen, Th: Thymus, Te: Testis)

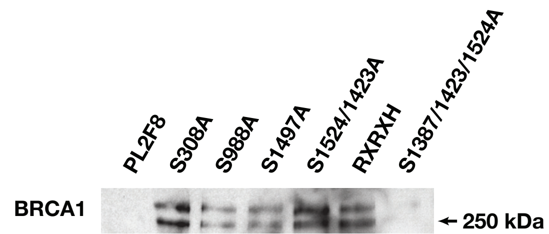
A



B



C



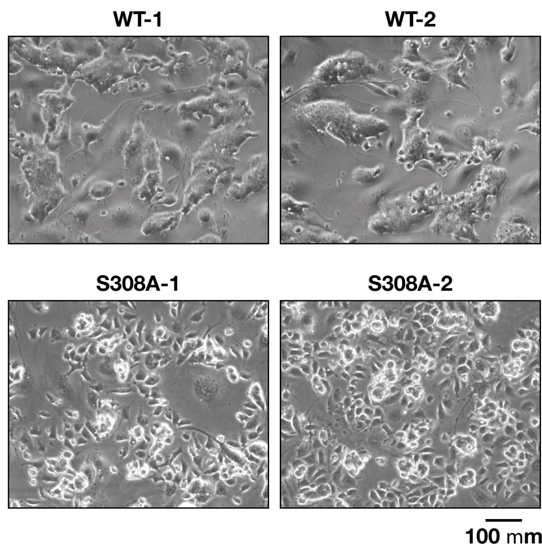
Supplementary Figure 6

Supplementary Figure 6. Analysis of BRCA1 mutations of known phosphorylation sites and Rb-binding motif (LXCXE)

A. Rescue of *Brca1*-null ES cells by BRCA1 carrying mutations in phosphorylation residues or the putative RB interacting domain. Each variant was expressed in conditional PL2F8 cells and Cre was introduced to delete the conditional allele. The recombinants were selected in HAT media and the resulting plates were stained with methylene blue.

B. Genotyping of the HAT resistant ES clones by Southern blot analysis. Colonies on the plates shown B were picked and cultured in 96-well plates. The DNA from the cells was subsequently analyzed by Southern blot analysis. With the exception of the S1387/1423/1524A triple mutant, all other mutations resulted in a number of *Brca1*$^{ko/ko}$ ES cell clones. Upper band: conditional allele, lower band: mutant allele.

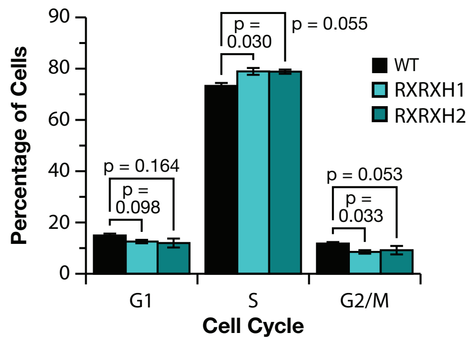
C. Expression of BRCA1 variants in PL2F8 cells. The protein was detected by immunoprecipitation using human BRCA1 antibody E1 and Western analysis by Ab-1.



Supplementary Figure 7

Supplementary Figure 7. Morphological difference of S308A mutant ES cells cultured in the absence of feeder.

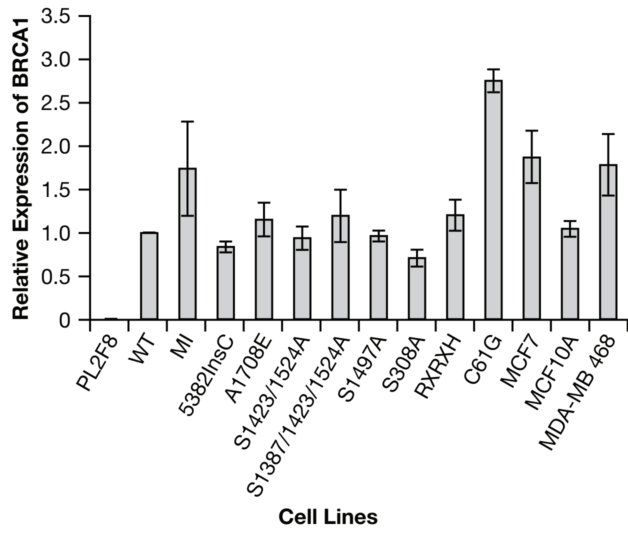
Morphology of ES cells expressing wild-type human BRCA1 (WT) and S308A in the absence of LIF and feeder cells. ES cells in 60 mm plates were trypsinized and plated onto 100 mm plates without feeder. After 1 hr, the unattached cells were removed for embryoid body formation. The remaining cells were cultured 24 hours and then photographed. WT ES cells show very few differentiated cells whereas many differentiated cells are present in S308A ES cells.



Supplementary Figure 8

Supplementary Figure 8. Analysis of RXRXH mutant ES cells

Cell cycle analysis of RXRXH mutant ES cells. ES cells expressing wild-type (WT) and RXRXH BRCA1 were labeled with BrdU/PI. The cell cycle distribution of each clones were analyzed by flow cytometry using CELLQuest software. Compared to WT cells, statistically significant numbers of more S phase and less G2/M phase cells were detected in both of the RXRXH mutant ES cells.



Supplementary Figure 9

Supplementary Figure 9. Comparative analysis of BRCA1 expression level in ES cell clones and human breast cancer cell lines

The expression of human BRCA1 BACs in ES cells was compared with the level of BRCA1 in threer human breast cancer cell lines (MCF7, MCF10A, MDA-MB468). The real time PCR analysis shows that the expression level of BRCA1 BACs is within the range of variability of BRCA1 level in human cancer cell lines. RPL13a was used as an internal control for both ES cells and human cancer cell lines. Primer sequences are provided in Supplementary Table 5.

Supplementary Table 1

Analysis of DNA damage response of BRCA1 mutant ES cells

Mutants	Cisplatin	HU	MMC	MNNG	MMS	IR	UV
NO BAC	No[A]	No	No	No	No	No	No
WT	No	No	No	No	No	No	No
S308A	MR[B]	MR	No	NT[C]	No	No	No
S988A	No	No	No	No	No	No	No
S1497A	No	No	No	No	No	S[D]	MS[E]
S1497/1524A	No	No	No	No	No	S	S
RXRXH	MR	No	No	NT	No	No	No
M1652I	No	No	No	No	No	No	No

[A]No, No hypersensitivity; [B]MR, Marginally resistant; [C]NT, Not Tested; [D]S, Hypersensitive; [E]MS, Marginally hypersensitive.

Supplementary Table 2

Cell cycle analysis of mutant ES cells

Mutants	**G1**	**S**	**G2/M**
NO BAC	10.7	69.5	14.2
WT	10.5	69.5	13.2
S1497A	12.4	69.4	12.6
S308A	12.5	71.7	11.2
S988A	11.9	68.6	13.0
S1423/1524A	12.6	68.8	12.7
RXRXH	9.5	76.6	8.7

Supplementary Table 3

Measurement of homologous recombination efficiency by Rosa26 locus targeting

Cell Line	Targeted/Total[A]	Ratio(%)
WT	24/76	31.6
S308A	10/58	17.2
S988A	25/71	35.2
S1497A	17/84	20.2
S1423/1524	11/49	22.4
RXRXH	21/71	29.6

[A]Targeted/Total: numbers of clones with rosa26 targeting, determined by southern / Total number of clones analyzed.

Supplementary Table 4

Sequences of primers used for BAC mutagenesis

Primer Name	Sequences
C61G Hit	aggaagtaaattaaattgttctttctttctttataatttatagattttgcatgctgaaacttctcaaccagaagaaagggggatcctagaattcctcgag
C61G Fix	aggaagtaaattaaattgttctttctttctttataatttatagattttgcatgctgaaacttctcaaccagaagaaagggccttcacagggtcctttatg
S308A Hit	ctcatgccagctcattacagcatgagaacagcagtttattactcactaaagacagaatgaatgtagaaaaggctgaattcggatcctagaattcctcgag
S308A Fix	ctcatgccagctcattacagcatgagaacagcagtttattactcactaaagacagaatgaatgtagaaaaggctgaattctgtaataaagccaaacagcc
RXRXH Hit	ataggcggactcccagcacagaaaaaaaggtagatctgaatgctgatcccctgtgtgagagaaaagaatggaataagcagggatcctagaattcctcgag
RXRXH Fix	ataggcggactcccagcacagaaaaaaaggtagatctgaatgctgatcccctgtgtgagagaaaagaatggaataagcagaaacggccacgctcacacaa
S988A Hit	tcagaggcaacgaaactggactcattactccaaataaacatggacttttacaaaacccatatcgtataccaccactttttggatcctagaattcctcgag
S988A Fix	tcagaggcaacgaaactggactcattactccaaataaacatggacttttacaaaacccatatcgtataccaccactttttcccatcaaggcatttgttaa
S1497A Hit	catttatgctttggctgcccagcaagtatgatttgtcctttcacaattggtggcgatggttttctccttccatttatcttggatcctagaattcctcgag
S1497A Fix	catttatgctttggctgcccagcaagtatgatttgtcctttcacaattggtggcgatggttttctccttccatttatctttctaggtcagccccttctaa
S1387A Hit	tctgaacctctgtttttgttatttaaggtgaagcagcatctgggtgtgagagtgaaacaagcgtctctgaagactgctcaggatcctagaattcctcgag
S1387A Fix	tctgaacctctgtttttgttatttaaggtgaagcagcatctgggtgtgagagtgaaacaagcgtctctgaagactgctcagggctatccgctcagagtga
S1423A Hit	aagcagagggataccatgcaacataacctgataaagctccagcaggaaatggctgaactagaagctgtgttagaacagcaggatcctagaattcctcgag
S1423A Fix	aagcagagggataccatgcaacataacctgataaagctccagcaggaaatggctgaactagaagctgtgttagaacagcatggggcccagcctgctaaca
S1524A Hit	ctaggtcatccccttctaaatgcccatcattagatgataggtggtacatgcacagttgctctgggagtcttcagaatagaggatcctagaattcctcgag
S1524A Fix	ctaggtcatccccttctaaatgcccatcattagatgataggtggtacatgcacagttgctctgggagtcttcagaatagaaactacccagctcaagagga
M1652I Hit	ctgctgggtataatgcaatggaagaaagtgtgagcagggagaagccagaattgacagcttcaacagaaagggtcaacaaaggatcctagaattcctcgag
M1652I Fix	ctgctgggtataatgcaatggaagaaagtgtgagcagggagaagccagaattgacagcttcaacagaaagggtcaacaaaagaatgtccatcgtggtgtc
V1804D Hit	aaaaacaaacaaaaaaaaatgatgaagtgacagttccagtagtcctactttgacactttgaatgctctttccttcctgggggggatcctagaattcctcgag
V1804D Fix	aaaaacaaacaaaaaaaaatgatgaagtgacagttccagtagtcctactttgacactttgaatgctctttccttcctggggatccagggtgaccacccaat
A1708E Hit	ttttcaacttctaatcctttgagtgtttttcattctgcagatgctgagtttgtgtgtgaacggacactgaaatattttggatcctagaattcctcgag
A1708E Fix	tcaacttctaatcctttgagtgtttttcattctgcagatgctgagtttgtgtgtgaacggacactgaaatattttctaggaattgagggaggaaaatggg
R1737X Hit	ctgctccacttccattgaaggaagcttctctttctcttatcctgatgggttgtgtttggtttctttcagcatgattttggatcctagaattcctcgag
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185DelAG Hit	ggaacagaaagaaatggatttatctgctcttcgcgttgaagaagtacaaaatgtcattaatgctatgcagaaaatcttagggatcctagaattcctcgag
185DelAG Fix	ggaacagaaagaaatggatttatctgctcttcgcgttgaagaagtacaaaatgtcattaatgctatgcagaaaatcttagtgtcccatctggtaagtca
5382InsC Hit	gctgaatgagcatgattttgaagtcagaggagatgtggtcaatggaagaaaccaccaaggtccaaagcgagcaagagaatggatcctagaattcctcgag
5382InsC Fix	gctgaatgagcatgattttgaagtcagaggagatgtggtcaatggaagaaaccaccaaggtccaaagcgagcaagagaatccccaggacagaaagatcgt

Supplementary Table 5

Sequences of primers used in this study

Primers

ChIP Primers	**Sequences**
Gadd45a ChIP1	tggaggacaaagggcccgattcc
Gadd45a ChIP2	gctaatttgcataacccaatggc
p21 ChIP1	cggagaccagcagcaaaatcg
p21 ChIP2	tgacacatacacaccccaggcac
Genotyping Primers	**Sequences**
Brca1/PintF	ggacggcagataaatccatttcttcc
Brca1/E2Pr	ggtacaaagccagtgtgggttacatg
Neo/pF	ggaatgtttccacccaatgtcgagc
Neo/pR	catcagagccgattgtctgttg
BRCA1 BAC5'	atggaggaacccacataggc
BRCA1 BAC3'	tatgggatagaggtgagatcc
Human BRCA1 Primers	**Sequences**
Forward	gcttcaacagaaagggtcaac
Backward	tcaagctgtcaattctggcttctc
Real Time PCR Primers	**Sequences**
RPL13a Universal Forward	gaagtaccaggcagtgacag
RPL13a Universal Backward	ggtcttgaggacctctgtg