Supporting Information

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SI Materials and Methods

Nucleic Acids, Protein, and Cell Culture. Genomic DNA (gDNA) and RNA extraction, cDNA synthesis, PCR, DNA sequencing, and immunoblotting for p21 and GAPDH were conducted as previously described (1). Cell culture conditions for nontransformed human breast epithelial cell lines MCF-10A, *hTERT*-IMEC (2) and their derivatives have been previously described (1), with the exception that DMEM/F12 (1:1) (Invitrogen) medium containing phenol red was used in the current study.

Knock-In of 185delAG *BRCA1* Mutation. Knock-in of the 185delAG *BRCA1* mutation was performed with an adeno-associated viral vector, as previously described (1, 3). The targeting construct containing a neomycin resistance gene driven by an SV40 early promoter was constructed by PCR, using genomic DNA (gDNA) from an immortalized human mammary epithelial cell line 90PE6E7 (4) as a template for the homology arms. Transduction of the targeting vector into cells, G418 (Invitrogen) selection, PCR-based screening to identify cells that had undergone homologous integration of the targeting vectors, and Cre-loxP recombination were conducted as described previously (1, 5). In evaluating gene-targeted clones, sequencing and capillary electrophoresis of RT-PCR products were conducted at the Johns Hopkins Core Facility. Primer sequences for PCR are provided in Table S5.

In the attempts to create cell clones carrying homozygous *BRCA1* 185delAG mutations, pre-Cre *BRCA1*-het clones were infected with the same AAV targeting vector with shown in Fig. 1*A*, except that a neomycin-resistance gene was replaced with a hygromycin-resistance gene, and concurrently selected with neomycin and hygromycin. In each attempt, at least 5,000 drug-resistant cell clones were screened for homologous recombination (HR) events applying a protocol previously described (5).

Plasmid-Based Homology-Mediated Repair Assay. A 1.0-kb BamHI-HindIII fragment containing a SceGFP and a polyadenylation site was isolated from DR-GFP (6), blunt-ended, and then inserted into the Eco47III site of pEGFP-C1 (BD Clontech). The resulting plasmid carrying a SceGFP fragment in the forward direction, termed pEGFPx2-C1, was linearized with I-SceI and isolated after fractionation on an agarose gel. Linearized pEGFPx2-C1 or a negative control pBluescript II (Agilent Technologies) was then transfected into MCF-10A and *hTERT*-IMEC clones plated in triplicate in 12-well plates using FuGENE 6 (Roche). After 2-d incubation, cells were washed extensively and harvested by Trypsin-EDTA. Half of each cell sample was suspended in PBS, and at least 1×10^4 cells were analyzed in flow cytometric analysis using BD-LSR and CellQuest software (BD Biosciences) to determine the GFP-positive percentage of cell clones. Lack of functional GFP expression from intact pEGFPx2-C1 was confirmed by the transfection of uncut pEGFPx2-C1 into MCF-10A cells, which demonstrated low GFP-positive rates (< 0.3%) in comparison with pEGFP-C1 transfection conducted in parallel (22.4%).

The remaining half of the cell samples were subjected to gDNA extraction and used in quantitative real-time PCR analyses conducted with MyiQ (Bio-Rad) to assay the efficiency of pEGFPx2-C1 transfection, as previously described (3). Oligonucleotide primers amplifying the kanamycin/neomycin resistance gene and *GAPDH* are shown in Table S5. A standard curve was generated for each session using serially diluted samples, and the quantification data in each sample was determined in reference to the standard curve. Transfection efficiency was determined by the amount of plasmid in each sample normalized to *GAPDH* expression. Finally, GFP-positive cell percentages measured by flow cytometric analysis were normalized to the transfection efficiency obtained via quantitative real-time PCR to determine homology-mediated DNA repair efficiency in each clone.

Retroviral Vector-Based HR Repair Assay. To develop an efficient assay of GFP signaling expressed in breast epithelial cells upon HR, a retroviral vector backbone and an internal ribosome entry site (IRES) were introduced in the HR reporter system as follows. Initially, a 1.0-kb EcoRI-NotI fragment containing SceGFP was excised from DR-GFP (6), and inserted into an EcoRI-NotI site of pIRESneo3 (BD Clontech). The resulting plasmid was digested with XbaI and *Bst*Z17I to remove the polyadenylation site, blunt-ended, and then ligated to a 0.8-kb blunt-ended HindIII-HindIII fragment containing iGFP from DR-GFP. To modify the IRES sequence, the resulting plasmid carrying the iGFP fragment in the forward direction was subsequently digested with PstI, and the largest 5.9-kb fragment was ligated with a 0.8-kb PstI-PstI fragment from pSEPT (7), resulting in the replacement of the IRES. The cloned plasmid which had incorporated a new IRES in the forward direction was digested with EcoRI and AccI to excise the entire HR recognition assembly, and the isolated assembly was transferred to the pBABE retroviral vector backbone by directional subcloning to EcoRI-BspDI site.

The resulting plasmid, pBABE-HR, was used to produce retroviral particles as described previously (1). After integration in the genome, this construct provides a GFP signal when I-SceI sites are cleaved and repaired via HR using iGFP fragments as templates. MCF-10A and its derivatives were then infected with the retroviral reporter BABE-HR, and multiple single-cell clones were isolated after G418 selection. Single-cell clones were seeded in 12-well plates at ~30% confluence, and transfected with an I-SceI expression plasmid pCBASce (8), an empty vector pCAG, or pEGFP-C1 using FuGENE 6 in the absence of G418. After 3-d incubation, cells were harvested with trypsin-EDTA and suspended in PBS. 3×10^4 cells (pCBASce and pCAG transfectants) or 1×10^4 cells (pEGFP-C1 transfectant) were analyzed by flow cytometry to determine the GFP-positive cell ratio in each sample. HR efficiency was determined by the following equation: HR efficiency = ("I-SceI plasmid" – "empty vector")/("pEGFP-C1"), where quotation marks represent GFP-positive ratio upon the transfection of indicated plasmids. Assays included 6 single-cell clones derived from the MCF-10A parental cell line, 9 clones from the Control cell line, and 12 clones each from both *BRCA1*-het #1 and #2 cell lines. BABE-HR copy numbers (CNs) in single-cell clones were determined as relative values by quantitative real-time PCR of the neomycin resistance gene normalized to *GAPDH*, with no detectable differences between *BRCA1* heterozygous clones versus their wild-type counterparts.

Drug Sensitivity Assay. For Doxorubicin assay, MCF-10A derivatives (1×10^3 cells per well) or *hTERT*-IMEC derivatives (3×10^3 cells per well) were seeded in 96-well plates in quintuplicate and exposed to 0 to 100 ng/μL of Doxorubicin (Sigma-Aldrich) for 4 h, washed with HBSS (Invitrogen) three times, and then maintained in their respective complete media. Four days later, the cells were incubated in media containing 0.5 mg/mL of 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma-Aldrich) for 4 h, and the resulting precipitates were dissolved in 99.5% isopropanol containing 0.04 N HCl. Cell numbers as mea-

sured by colorimetric changes were determined by absorbance at wavelength 450 nm with subtraction of 690 nm background absorbance measured by a microplate reader (SpectraMax Plus; Molecular Devices), and expressed relative to mock-treated cells.

NU1025 was purchased from Calbiochem. MCF-10A derivatives were seeded in 24-well plates (4×10^3 cells per well) in triplicate with MCF-10A complete medium, allowed to attach for ~16 h, and exposed to 0 to 400 μM of NU1025. After 4-d exposure, the cells were washed with HBSS, fixed and stained with 3.7% formaldehyde containing 0.2% (wt/vol) Crystal violet at room temperature for 20 min, washed five times with tap water, and then completely dried. The dye was then dissolved in 500 μL of 2N acetic acid by gentle agitation at room temperature for 20 min. Cell numbers were determined as the absorbance measured by SpectraMax Plus at wavelength 540 nm, and expressed relative to mock-treated cells.

γ-Irradiation Sensitivity Assay. MCF-10A or *hTERT*-IMEC derivatives were seeded sparsely in 75-cm^2 cell culture flasks in triplicate and subjected to 0 to 6 Gy γ-irradiation at a dose of 0.7 Gy/min with a Gammacell 40 Exactor (MDS Nordion), and maintained until colonies were readily visible (~10 d). For colony counting, cells were fixed and stained with 3.7% formaldehyde containing 0.2% (wt/vol) Crystal violet.

Cell-Cycle Profiling and Proliferation Assay. For cell-cycle profiling, cells were harvested with trypsin-EDTA and washed, resuspended in PBS containing 3.7% formaldehyde and 0.5% Nonidet P-40 (Sigma-Aldrich), and stained with 2 μg/mL Hoechst 33258 (Sigma-Aldrich). 1×10^4 cells were then analyzed for each sample by flow cytometric analysis. The G0/G1 fraction was determined by ModFit LT ver. 2.0 (Verity).

For cell proliferation assays, exponentially growing MCF-10A derivatives (5×10^4 cells per flask) or *hTERT*-IMEC derivatives (1×10^5 cells per flask) were propagated in 25-cm^2 cell-culture flasks. Cell numbers were counted with a Z1 Cell and Particle Counter (Beckman Coulter) every three days, and 5×10^4 cells (MCF-10A) or 1×10^5 cells (*hTERT*-IMEC) were transferred to new 25-cm^2 flasks each time.

G2 Checkpoint Analysis. Immunostaining of phospho-histone H3 and flow cytometric detection of mitotic cells were carried out as previously described with modification (9). Briefly, exponentially growing cells were plated in their respective growth media at a density of 8×10^5 cells per 25-cm^2 cell-culture flask, and γ-irradiation at various doses was applied the next day. Cells were harvested by trypsinization at predetermined time points, washed, and fixed with 3.7% formaldehyde with 0.5% Nonidet P-40. Rabbit antiphospho-histone H3 antibody (Upstate Biotechnology) and FITC-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories) were used to label mitotic cells, as described previously (9). At least 1×10^4 cells were analyzed by flow cytometry after stained with 2 μg/mL Hoechst 33258.

SNP Array. *BRCA1* heterozygous MCF-10A clones and their wild-type counterparts were subjected to single-cell cloning by a limiting dilution protocol immediately after 6 Gy or mock irradiation. Single-cell clones were processed for gDNA extraction after 4 wk in culture, and subjected to a focused SNP array analysis, using the Cancer SNP Panel (Illumina) conducted at the Johns Hopkins University SNP Center. The Cancer SNP Panel contains 1,421 markers located within 408 cancer-related genes distributed across all autosomal and X chromosomes. SNP genotyping was performed according to the manufacturer's protocol. Briefly, 2.5 μg of gDNA was amplified ~1,000-fold, fragmented into 300- to 500-bp fragments, and used for hybridization. The array carries SNP specific probes of ~50-bp long designed directly adjacent to the SNP site. After hybridization, the SNP-specific probes were subjected to the single-base extension reaction using a single hapten-labeled dideoxynucleotide. The fluorescently stained product was then visualized and quantified by scanning each beadchip into the Illumina BeadArray Reader.

CN and loss of heterozygosity (LOH) analyses were performed using controls acquired from the Johns Hopkins University SNP Center as well as a set of mock-irradiated MCF-10A parental single-cell clones as baseline samples. Normalized intensity values were acquired via the Illumina Beadstudio software. Raw and inferred CNs and LOH values were obtained by implementing the Hidden Markov Model function in dChip program. The CN scores of individual SNP markers within a gene were averaged and used as the CN score of the corresponding gene. Genes showing CN changes were counted in each of 6 Gy or mock-irradiated single-cell clones. The gains and losses of gene copy were defined as scores more than 2.5 and less than 1.5, respectively. If multiple genes subject to CN changes were contiguous or uninterruptedly connected by a region of milder CN change (> 2.25 or < 1.75), those genes were considered as a single event of CN change.

LOH genes were identified when LOH was detected at one or more of the markers within the genes. Genes harboring at least one retained marker but no LOH markers were regarded as retained genes. Genes were omitted from analysis when all of the markers located on the genes were noninformative. In counting LOH genes for individual single-cell clones, multiple neighboring genes demonstrating a sequential LOH were regarded as a single LOH event similar to the evaluation of CN changes. When CN changes or LOH were present at a gene or a region ubiquitously in all of the single-cell clones originating from one of the isogenic cell clones, such genetic aberration was excluded from counting because this is likely to have emerged before the corresponding cell clone was established.

FISH. To prepare cell-culture samples for FISH, cells were propagated in exponential growth condition, dissociated with trypsin, incubated in 40 mM KCl for 15 min at 37 °C, fixed with 3:1 mixture of methanol and glacial acetic acid, and then dropped onto glass slides. For probes, DNA was extracted from BAC clones listed in Table S3 and labeled by nick translation with Green dUTP or Red dUTP (Abbott Laboratories) using DNA polymerase/DNase I (Invitrogen). Slides and probes were denatured and hybridized over night at 37 °C in a humid chamber. Slides were counterstained with DAPI before allele counting. For each cell line, 200 interphase cells were counted for each probe under fluorescence microscopy.

For FISH analysis in breast tissues, noncancerous breast tissues from nine *BRCA* carriers were sampled at the time of either prophylactic mastectomy or mastectomy for breast cancer, and nine control breast tissues were obtained at the time of reduction mammoplasty. In cancer cases, tissues sufficiently distant from tumor margins (at least 1 cm) were sampled. All samples were processed into formalin-fixed paraffin-embedded specimens, and then used to construct a tissue microarray (TMA). A series of TMA slides bearing the same sections (i.e., sequential slices of the same specimens) were created, and one of these slides was initially stained with H&E to verify the presence of noncancerous human breast epithelial cells and absence of contaminating neoplastic cells by two independent pathologists (E.G. and P.A). Before hybridization, TMA slides were deparaffinized with xylene, rehydrated through a graded ethanol series, and pretreated with protease (Pretreatment Kit I; Abbott Laboratories). Fluorescently labeled *TP53* and *MYC* probes (Table S3) were purchased from Abbott Laboratories and used as per the manufacturer's directions. Slides were codenatured with probes at 94 °C for 5 min, hybridized overnight at 37 °C in a humid chamber, washed, and counterstained with DAPI before imaging. Cells were examined under microscopy gradually

changing focus depth from the top to the bottom of individual nuclei. For each specimen, at least 100 cells for each probe were examined to count alleles. This was performed in a blinded fashion, as the evaluator for this portion of the study (S.C.) did not have prior knowledge of *BRCA* carrier status of the individual specimens on the TMA. To determine the reproducibility of counting alleles, we initially analyzed each area of a section two times in a sequential fashion and demonstrated high reproducibility (> 97%) and this was confirmed by independent review (B.H.P.). Study pathologists verified that the counted cells were breast epithelial cells by rereview of the FISH slides along with the corresponding H&E-stained sections. Digital images were obtained with Eclipse 50i epifluorescence microscope equipped with appropriate fluorescence filter sets (Nikon), a CoolsnapEZ digital monochrome camera (Photometrics), and Elements software (Nikon), and processed for pseudocoloring and merging for presentation. The Johns Hopkins University School of Medicine and School of Public Health Institutional Review Boards' approval and informed consent had been obtained for the use of clinical samples in this study.

Statistical Analyses. Statistical analyses were carried out with Intercooled Stata (Stata) to evaluate the difference between *BRCA1* heterozygous clones (*BRCA1*-het #1 and #2) and their wild-type counterparts (parent and control) in each of MCF-10A and *hTERT*-IMEC cell lines. Two-tailed Mann–Whitney *U* test was applied for the plasmid-based and retroviral vector-based DNA repair assays, the densitometric analysis of p21 immunoblotting, the comparison of gene CN and LOH conducted separately in 6-Gy and mock-irradiated cell clones in Cancer SNP Panel analysis, and FISH allele counting with cell lines and TMA samples. Two-way factorial ANOVA was carried out for Doxorubicin and γ-irradiation sensitivity assays, G0/G1 fraction analysis, cell proliferation assays, and gene CN analysis of the entire Cancer SNP Panel data. *P* values less than 0.05 were considered statistically significant.

1. Konishi H, et al. (2007) Knock-in of mutant K-ras in nontumorigenic human epithelial cells as a new model for studying K-ras mediated transformation. *Cancer Res* 67:8460–8467.
2. DiRenzo J, et al. (2002) Growth factor requirements and basal phenotype of an immortalized mammary epithelial cell line. *Cancer Res* 62:89–98.
3. Abukhdeir AM, et al. (2008) Tamoxifen-stimulated growth of breast cancer due to p21 loss. *Proc Natl Acad Sci USA* 105:288–293.
4. Annab LA, et al. (2000) Establishment and characterization of a breast cell strain containing a BRCA1 185delAG mutation. *Gynecol Oncol* 77:121–128.
5. Konishi H, et al. (2007) A PCR-based high-throughput screen with multiround sample pooling: Application to somatic cell gene targeting. *Nat Protoc* 2:2865–2874.
6. Pierce AJ, Johnson RD, Thompson LH, Jasin M (1999) XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. *Genes Dev* 13:2633–2638.
7. Topaloglu O, Hurley PJ, Yildirim O, Civin CI, Bunz F (2005) Improved methods for the generation of human gene knockout and knockin cell lines. *Nucleic Acids Res* 33: e158.
8. Richardson C, Moynahan ME, Jasin M (1998) Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. *Genes Dev* 12:3831–3842.
9. Xu B, Kim St, Kastan MB (2001) Involvement of Brca1 in S-phase and G(2)-phase checkpoints after ionizing irradiation. *Mol Cell Biol* 21:3445–3450.

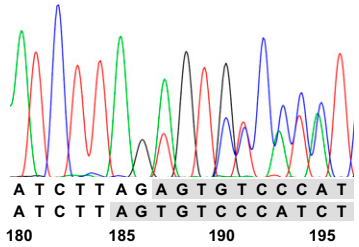

Fig. S1. A representative chromatograph of the direct sequencing of RT-PCR products at the targeted locus in *BRCA1* knock-in clones. DNA sequences downstream of the 185delAG mutation site are highlighted with gray boxes in both intact and targeted alleles. Primer sequences are provided in Table S5.

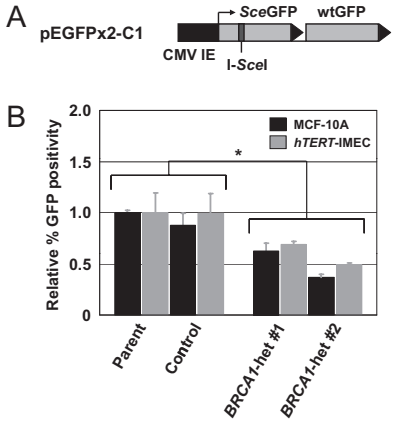

Fig. S2. (*A*) A plasmid-based GFP reporter construct pEGFPx2-C1 measuring homologous recombination and single strand annealing efficiency. CMV-IE, a human cytomegalovirus immediate early promoter; wtGFP, wild-type GFP; SceGFP, inactive GFP in which an 18-bp sequence is substituted with an I-SceI recognition site; filled triangles, polyadenylation sites; arrows, transcription start sites. (*B*) Homology-mediated repair efficiency determined by the transfection of I-SceI–cleaved pEGFPx2-C1. Data are shown relative to those in parental cell lines (mean ± SD; $n = 3$). *$P = 0.004$ for both MCF-10A clones and *hTERT*-IMEC clones.

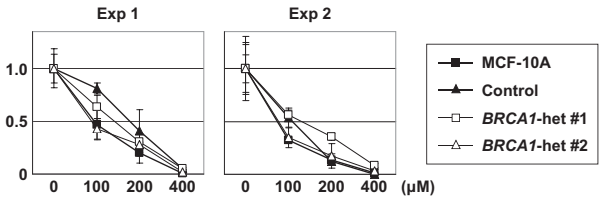

Fig. S3. Sensitivity of MCF-10A derivative clones to a PARP inhibitor NU1025 in two independent experiments (*Left* and *Center*). Data are shown relative to those of nontreated cells (n = 3; mean ± SD).

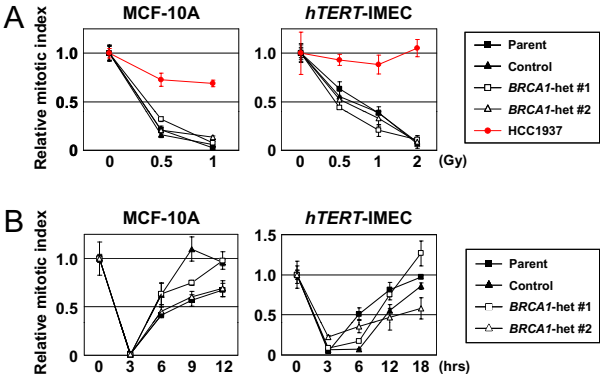

Fig. S4. G2 checkpoint response is not impaired upon γ-irradiation in *BRCA1* heterozygous clones. (*A*) Flow cytometric analyses assessing phosphorylated histone H3 1 h after 0- to 2-Gy γ-irradiation. Mitotic indices represented by phospho-histone H3-positive cell ratios are shown relative to mock-irradiated cells in each clone (mean ± SD; n = 3). HCC1937, a breast cancer cell line harboring a homozygous *BRCA1* mutation, has been shown to have a defective G2 checkpoint response (9), and therefore was used in this study as a positive control. (*B*) Mitotic indices over time after 6 Gy γ-irradiation determined as in *A* (mean ± SD; n = 3).

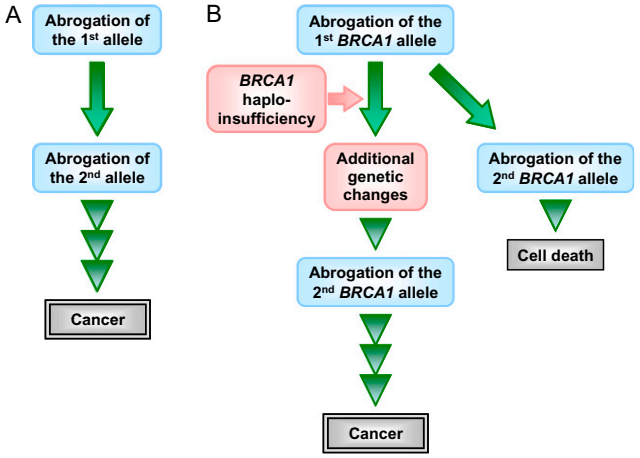

Fig. S5. A schematic overview of the concept in this study. (*A*) Original two-hit theory for the incidence of familial cancers proposed by Knudson (1). (*B*) A modified two-hit theory for *BRCA1*-mediated breast carcinogenesis suggested by the present study.

1. Knudson AG, Jr. (1971) Mutation and cancer: Statistical study of retinoblastoma. *Proc Natl Acad Sci USA* 68:820–823.

Table S1. Gene loci undergoing CN changes in SNP array analysis

Clones	γ-Irradiation (Gy)	No. of single-cell subclones*	Gene loci with CN changes		Gene loci with CN changes in multiple single-cell clones	
			Gain	Loss	Gain	Loss
MCF-10A	0	4	10	3	*BHMT* (5q14.1) *IRF1* (5q31.1) *DRD1* (5q35.2) *TGFB1* (19q13.2)	*OGG1* (3p25.3)
	6	4	11	5	*BHMT* (5q14.1) *DRD1* (5q35.2) *SLC4A2* (7q36.1) *ABCC4* (13q32.1) *MPO* (17q22) *TGFB1* (19q13.2)	—
Control	0	3	10	4	*GPX1-RHOA* (3p21.31) *BHMT* (5q14.1) *LCAT* (16q22.1)	*OGG1* (3p25.3) *ANKK1* (11q23.1)
	6	4	10	4	*GPX1-RHOA* (3p21.31) *BHMT* (5q14.1) *TGFB1* (19q13.2) *LIG1* (19q13.32) *MAOA* (Xp13.3)	—
BRCA1-het #1	0	4	9	9	*BHMT* (5q14.1) *IRF1* (5q31.1) *LCAT* (16q22.1) *MAOA* (Xp13.3)	*OGG1* (3p25.3) *CYP2C19* (10q23.33) *METTL1* (12q14.1)
	6	4	13	9	*MYNN* (3q26.2) *DRD1* (5q35.2)	*OGG1* (3p25.3) *MLH1* (3p22.2) *PTGS1* (9q33.2) *ANKK1* (11q23.1) *METTL1* (12q14.1)
BRCA1-het #2	0	4	13	12	*BCL6* (3q27.3) *BHMT* (5q14.1) *DRD1* (5q35.2) *ABCC4* (13q32.1) *RPA4* (Xq21.33)	*RGS5* (1q23.3) *OGG1* (3p25.3) *ESR1-IGF2R* (6q25.1-q25.3) *VDR* (12q13.11) *METTL1* (12q14.1) *PDGFB* (22q13.1)
	6	3	23	23	*ABCC4* (13q32.1)	*ESR1-SOD2* (6q25.1-q25.3) *VDR* (12q13.11) *PDGFB* (22q13.1)

Dashes indicate that no gene loci were lost in the denoted cell clones and conditions.
*Single-cell subclones isolated after 6 Gy or mock irradiation and used in SNP array analysis.

Table S2. Gene loci undergoing LOH in SNP array analysis

Clones	γ-Irradiation (Gy)	No. of single-cell subclones	LOH occurrence*	Gene loci undergoing LOH
MCF-10A	0	4	0.50 ± 0.87	*BRIP1-AXIN2* (17q23.2-q24.1) *TYMS* (18p11.32)
	6	4	0.50 ± 0.87	*EDN1-OPRM1* (6p24.1–6q25.2) *TYMS* (18p11.32)
Control	0	3	0.00 ± 0.00	—
	6	4	0.00 ± 0.00	—
BRCA1-het #1	0	4	0.00 ± 0.00	—
	6	4	1.00 ± 0.71	***TP63* (3q28)** *CCNH* (5q14.3)
BRCA1-het #2	0	4	1.00 ± 1.00	***TP63* (3q28)** ***ESR1-OPRM1* (6q25.1-q25.2)**
	6	3	1.67 ± 1.25	***TP63* (3q28)** ***ESR1-OPRM1* (6q25.1-q25.2)**

Boldface indicates gene loci undergoing LOH in multiple single-cell clones. Dashes indicate that no gene loci underwent LOH in the denoted cell clones and conditions.
*Mean ± SD $P = 0.04$ and not statistically significant in 6-Gy and mock-irradiated cell clones, respectively. Statistics compare *BRCA1* heterozygous clones (*BRCA1*-het #1 and #2) with wild-type counterparts (MCF-10A and control).

Table S3. Probeset used for FISH analyses

Chromosomal localization	BAC clones/probes	Genes assigned
BAC probes used for cell line FISH		
3p14.2	RP11-48E21	*FHIT*
3q26.32	RP11-245C23	*PIK3CA*
6q23.3	RP1-32B1	*MYB*
7p11.2	RP11-792I5*	*EGFR*
8q24.21	RP1-80K22	*MYC*
10q23.31	RP11-380G5	*PTEN*
11q13.3	RP11-300I6	*CCND1*
16q22.1	RP11-410N2*	*CDH1*
16q23.1	RP11-264L1	*WWOX*
17p13.1	RP11-199F11	*TP53*
17q12	RP11-686D22*	*ERBB2*
17q21.31	RP11-242D8	*BRCA1*
20q13.12	RP1-73E16*	*NCOA3*
Probes used for tissue FISH		
8q24.21	Vysis LSI *MYC*†	*MYC*
17p13.1	Vysis LSI *TP53*†	*TP53*

*BAC clones are located adjacent to but not exactly on the indicated genes.
†Purchased from Abbott Laboratories.

Table S4. FISH allele counting in isogenic cell clones

	Parent					Control					*BRCA1*-het #1				
Genes	0	1	2	3	>4	0	1	2	3	>4	0	1	2	3	>4
MCF-10A															
FHIT			200					200					198	1	1
PIK3CA			200					200					200		
MYB			200					200					198	2	
EGFR			200					200					199	1	
MYC				200					200					200	
PTEN			200					200					200		
CCND1			200					200					200		
WWOX			200					200					200		
CDH1			200					200				4	194	2	
TP53			200					200					200		
BRCA1			200					200				1	199		
ERBB2			200					200				1	195	4	
NCOA3			200					200				9	189	2	
hTERT-IMEC															
FHIT			200					200					200		
PIK3CA			194					200				5	194	1	
MYB			200					200					200		
EGFR			200					200					200		
MYC			200					200					200		
PTEN			200					200				11	188	1	
CCND1			200					200					200		
WWOX			200					200			4	48	144	4	
CDH1			200					200					200		
TP53			193	1				200				4	195	1	
BRCA1			200					200				1	199		
ERBB2			200					200					200		
NCOA3			200					200					200		

For each probe and each cell clone, the numbers of cells carrying 0, 1, 2, 3, and >4 alleles are shown from left to right.

Table S5. Primer sequences

Primer	Sequence
PCR screening of BRCA1 185delAG knock-in	
BRCA1 intron 1 forward #1	5′-CTGGTATGTATGAGAGGATG
Targeting vector reverse	5′-GTAAACTCCTCTTCAGACCTA
PCR screening of Cre recombination events	
BRCA1 intron 1 forward #2	5′-ACGTTGTCATTAGTTCTTTGG
BRCA1 intron 2 reverse	5′-AGTGGATGGAGAACAAGGAA
PCR of targeted *BRCA1* gene locus (see Fig. 1 *A* and *B*)	
BRCA1 intron 1 forward #2	5′-ACGTTGTCATTAGTTCTTTGG
BRCA1 intron 2 reverse	5′-AGTGGATGGAGAACAAGGAA
RT-PCR for capillary electrophoresis	
BRCA1 exon 2 forward -FAM	5′-TGGATTTATCTGCTCTTCGC
BRCA1 exon 3 reverse	5′-TAAAGGACACTGTGAAGGCC
RT-PCR and the direct sequencing of 2-bp deletion site	
BRCA1 exon 1 forward	5′-AGGCTGTGGGGTTTCTCAGA
BRCA1 exon 3 reverse	5′-TAAAGGACACTGTGAAGGCC
BRCA1 exon 2 for seq	5′-TTCATTGGAACAGAAAGAAA
Quantitative real-time PCR in the homology-mediated repair assays	
Neo/Kan^R forward	5′-TCGGCTATGACTGGGCACAA
Neo/Kan^R reverse	5′-AGTGACAACGTCGAGCACAG
GAPDH exon 8 forward	5′-ACATCATCCCTGCCTCTAC
GAPDH exon 8 reverse	5′-AGGTCCACCACTGACACGTT