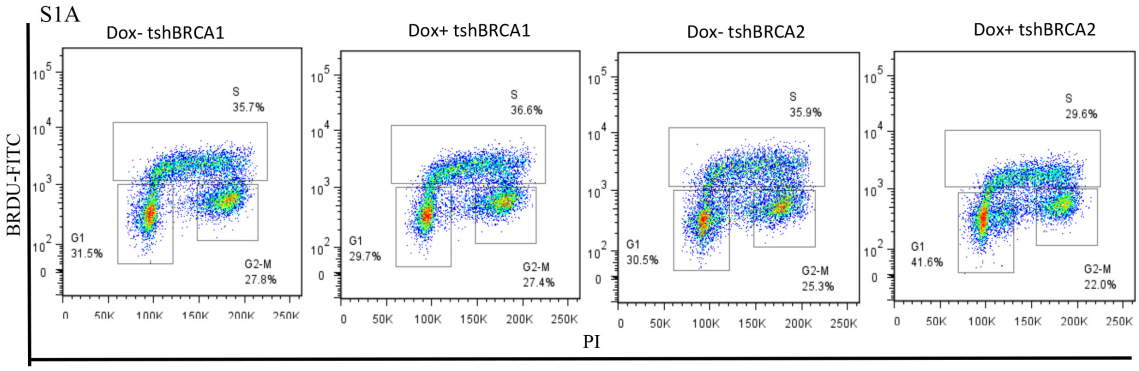
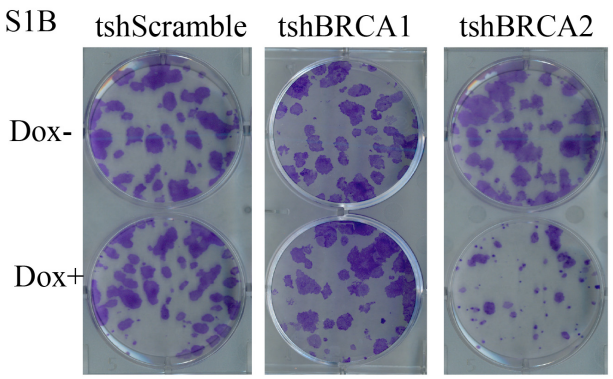
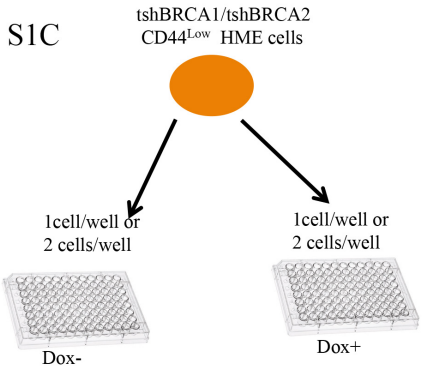






S1D

	Number of wells containing ≥ 1 clone (Add 1 cell/well)	P-Value	Number of wells containing ≥ 1 clone (Add 2 cells/well)	P-value
hScramble Dox-	36/96		56/96	
hScramble Dox+	27/96	0.22	57/96	1.0
shBRCA1 Dox-	27/96		50/96	
shBRCA1 Dox+	26/96	1.0	40/96	0.20
shBRCA2 Dox-	35/96		57/96	
shBRCA2 Dox+	10/96	<0.0001	20/96	<0.0001

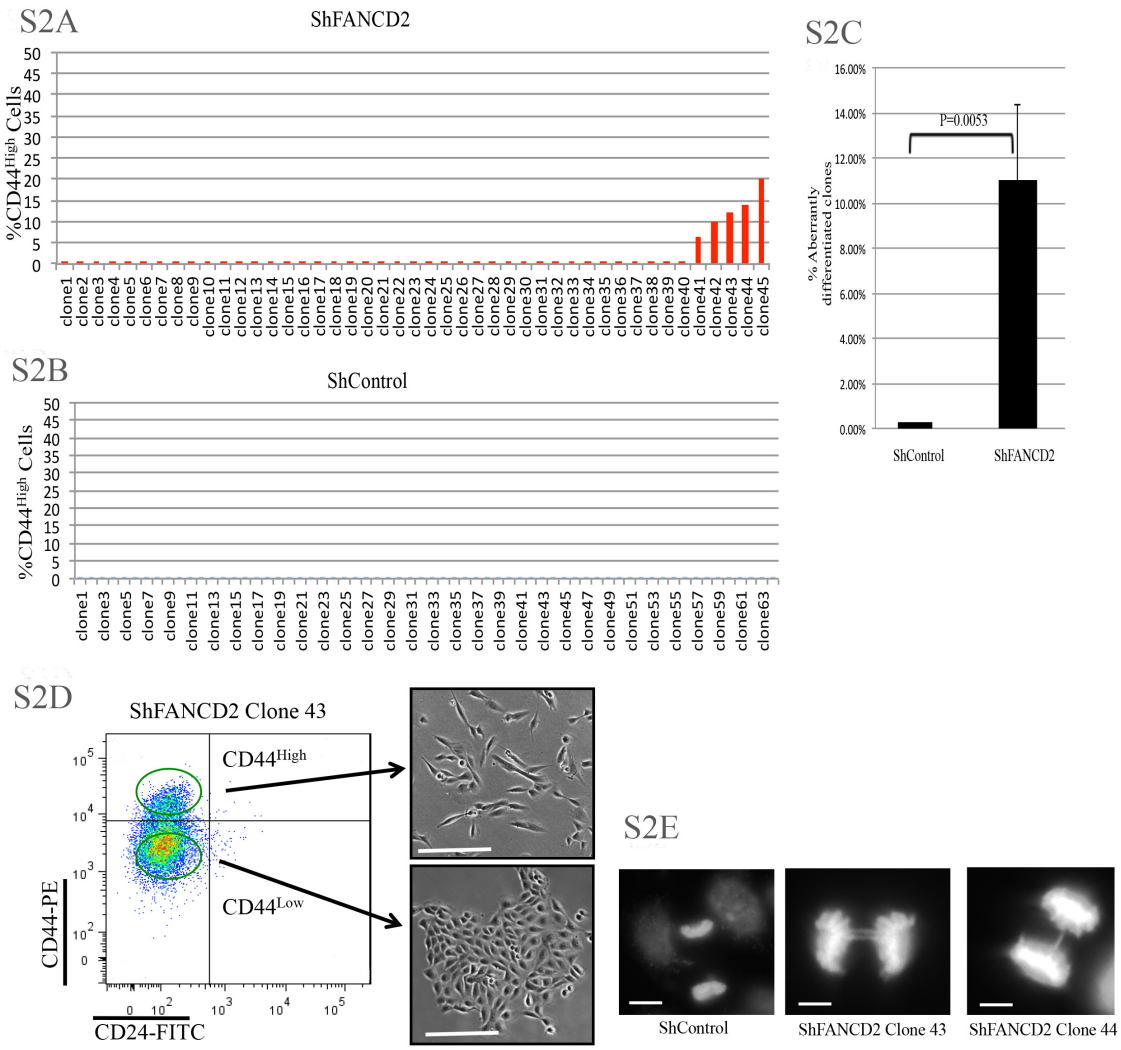
Supplemental Figure 1 (related to Figure 1)- Effect of BRCA1 or BRCA2 depletion on cell cycling, colony formation, and state of differentiation of CD44^{low} HME cells.

S1A) Cell cycle analysis before and after BRCA1 or BRCA2 depletion in CD44^{low} HME cells.

S1B) HME cell colony formation before and after BRCA1or BRCA2 depletion. ~300 cells were plated in each dish with or without doxycycline, and cells were fixed and analyzed at 10 days.

S1C) Experimental scheme used to measure colony-forming efficiency after BRCA1 or BRCA2 depletion. Each well of a 96 well plate received 1 or 2 cells based upon the cell concentration of the relevant loading sample. At day 8, the number of wells containing 1 or more clones was then assessed.

S1D) Statistical analysis of colony formation efficiency after BRCA1 or BRCA2 depletion.



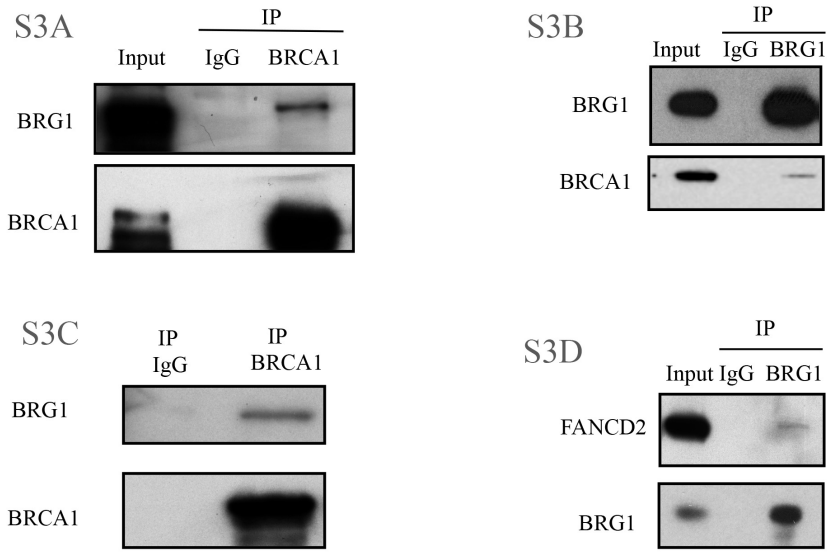
Supplemental Figure 2 (related to Figure 3) FANCD2 is essential for the maintenance of the differentiation state of CD44^{low} HME cells.

S2A-S2B) Histograms depicting the percentage of CD44^{high} cells in individual clones after ShFANCD2 or ShControl virus infection of CD44^{low} HME cells.

S2C) Analysis of the effect of FANCD2 depletion on the frequency at which clones containing CD44^{high} cells appeared. The clones were analyzed in three, randomly selected, non- overlapping groups of 15, and a students t-test was used for the statistical analysis of the results.

S2D) Phase-contrast images of CD44^{high} and CD44^{low} HME cells derived from an aberrantly differentiated cell containing clone (clone 43) isolated after FANCD2 depletion. Scale bar: 100μm

S2E) Representative analysis of anaphase bridge formation in ShControl CD44^{low} cells and two, independent ShFANCD2-infected CD44^{high} cell- containing clones. Scale Bar: 10μm.



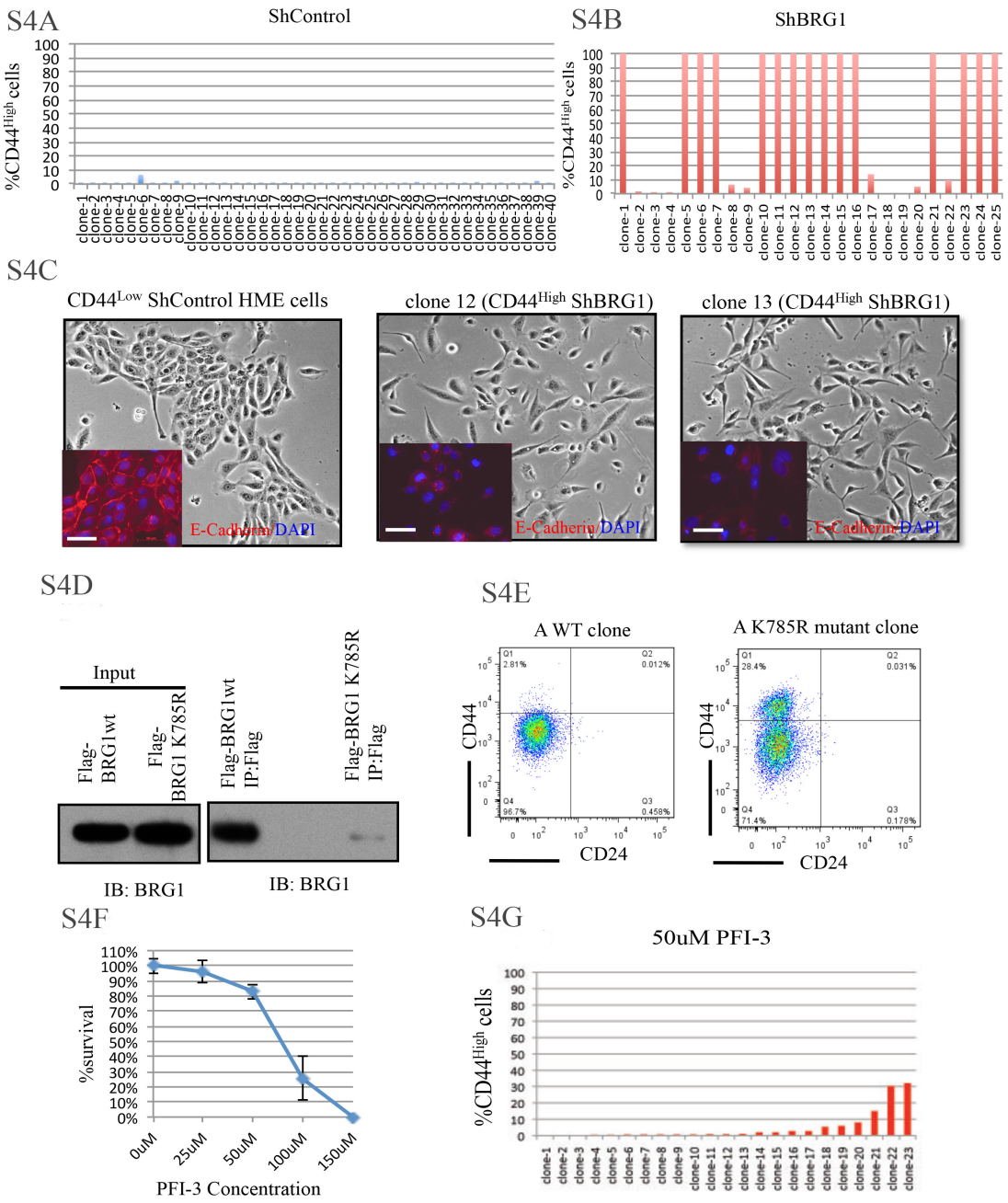
Supplemental Figure 3 (related to Figure 4)- Endogenous BRG1 co-immunoprecipitates with endogenous BRCA1 and FANCD2.

S3A) BRCA1 antibody (D9, Santa Cruz) co-immunoprecipitated endogenous BRCA1 and BRG1 present in a 293T cell extract.

S3B) Anti-BRG1 (Bethyl labs) co-immunoprecipitated endogenous BRCA1 and endogenous BRG1 in a 293T cell extract.

S3C) Anti-BRCA1 (D9) co-immunoprecipitated endogenous BRCA1 and endogenous BRG1 in an MCF7 cell extract.

S3D) Anti-BRG1 (Bethyl labs) co-immunoprecipitated endogenous BRG1 and endogenous FANCD2 in a 293T cell extract.



Supplemental Figure 4 (related to Figure 4) BRG1 suppresses aberrant differentiation in CD44^{low} HME cells.

S4A-S4B) A histogram reflecting the percentage of CD44^{high} cells in individual clones derived from CD44^{low} HME cells after transduction of an ShControl vector and after depletion of BRG1.

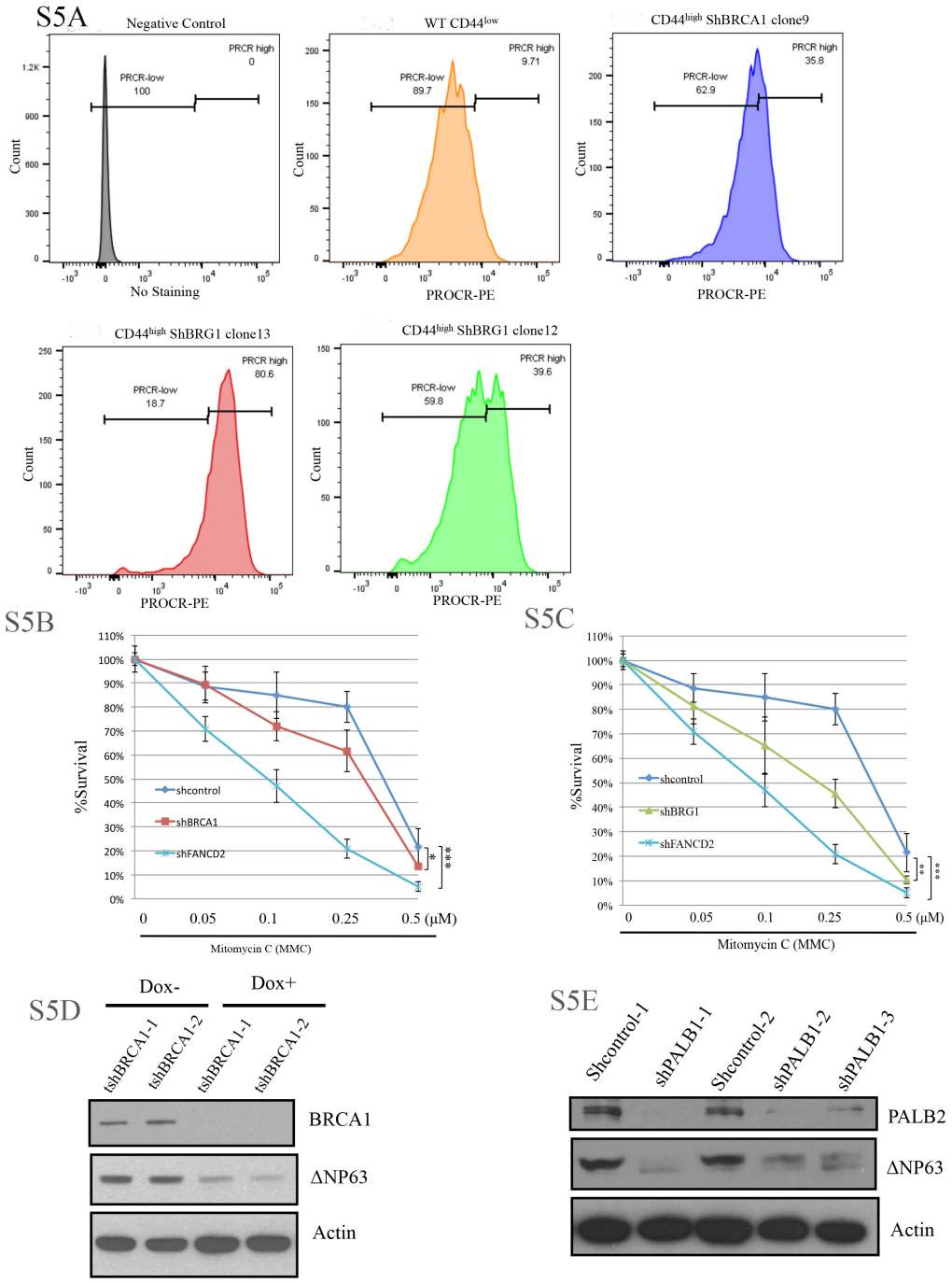
S4C) Phase-contrast images of CD44^{low} Shluc cells and CD44^{high} cells (clone12 and clone13) isolated after BRG1 depletion. The inserts depict E-cadherin(red) and DAPI (blue) staining in CD44^{low} and CD44^{high} cells, respectively. Scale Bar:50μm.

S4D) Immunoprecipitation(IP)-western blotting of flag-tagged WT BRG1 and of the BRG1 K785R mutant.

S4E) CD24 and CD44 profiles of clones ectopically expressing wt BRG1 (clone16) or a BRG1 mutant allele (K758R; clone8).

S4F) A clonal cell survival assay was used to measure the sensitivity of CD44^{low} HME cells to the BRG1 inhibitor, PFI-3. Cells were plated at low density and exposed to PFI-3 for 8 days at the indicated concentrations and then fixed to count colonies.

S4G) Percentage of CD44^{high} cells in individual clones isolated after PFI-3 exposure (50uM) as measured by flow cytometry.

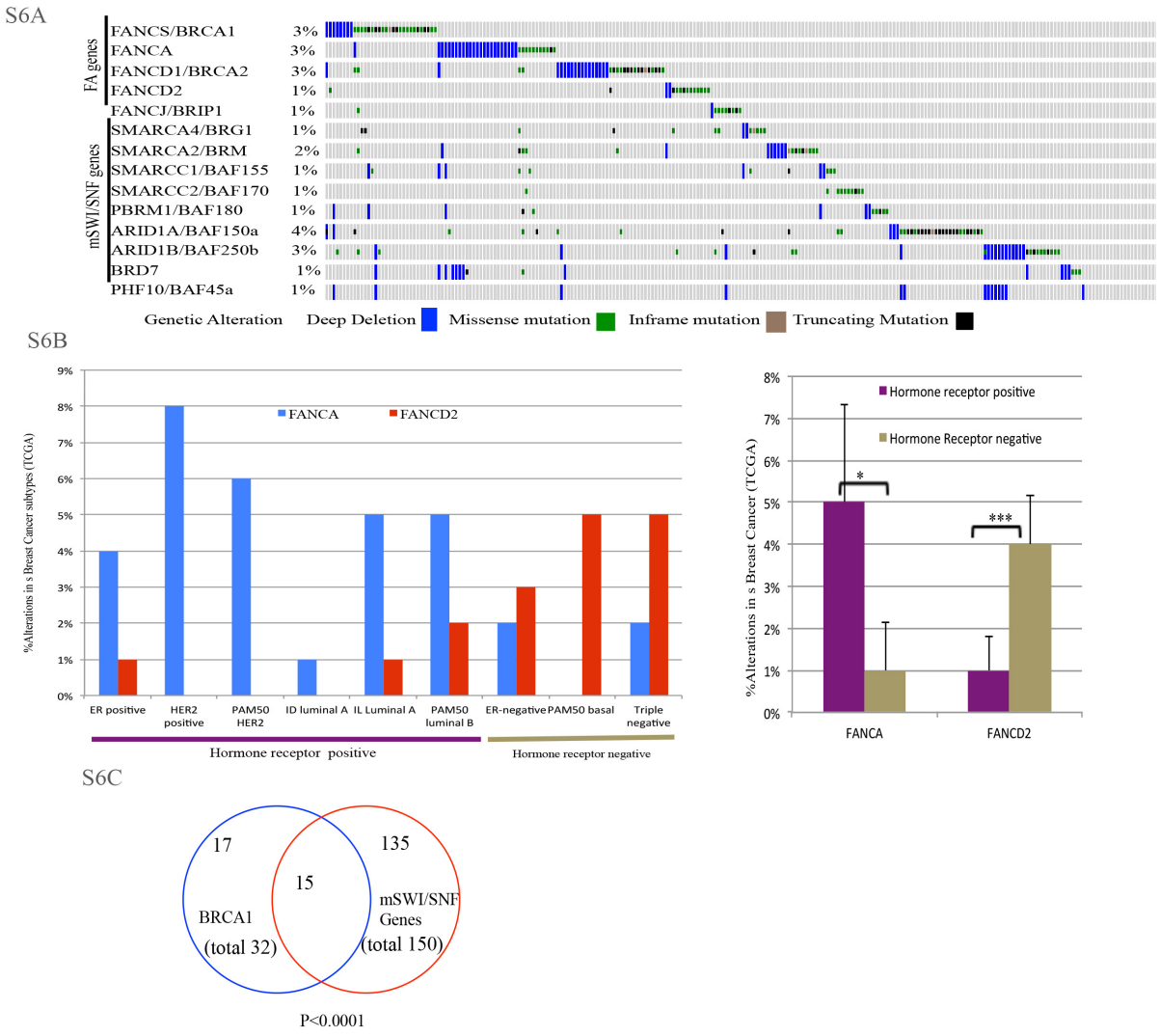


Supplemental Figure 5 (related to Figure 5) BRCA1 and BRG1 contribute to DNA-damage-dependent differentiation maintenance in human mammary epithelial cells.

S5A) Representative Protein C Receptor (PROCR) profiles in CD44^{high} cells isolated after BRCA1 or BRG1 depletion. Various freshly trypsinized HME cells were analyzed. Some were left antibody-free (negative control). Others were incubated with Protein C receptor antibody and analyzed by flow cytometry. The nature of the cells being analyzed is noted atop each panel.

S5B-S5C) Clonogenic survival assay after BRCA1, BRG1, or FANCD2 depleting CP29-HME cells exposed to increasing concentrations of mitomycin C (MMC). ShFANCD2 is from the same experiment in both Figure S5B and S5C. The data represented are mean±S.D.; and a two-way ANOVA analysis was used for statistical analysis. *P<0.05, **P<0.01 and ***P<0.001.

S5D-S5E) Immunoblotting for ΔP63 before and after BRCA1 or PALB2 depletion in HME cells, which remain CD44^{low} during the assay.



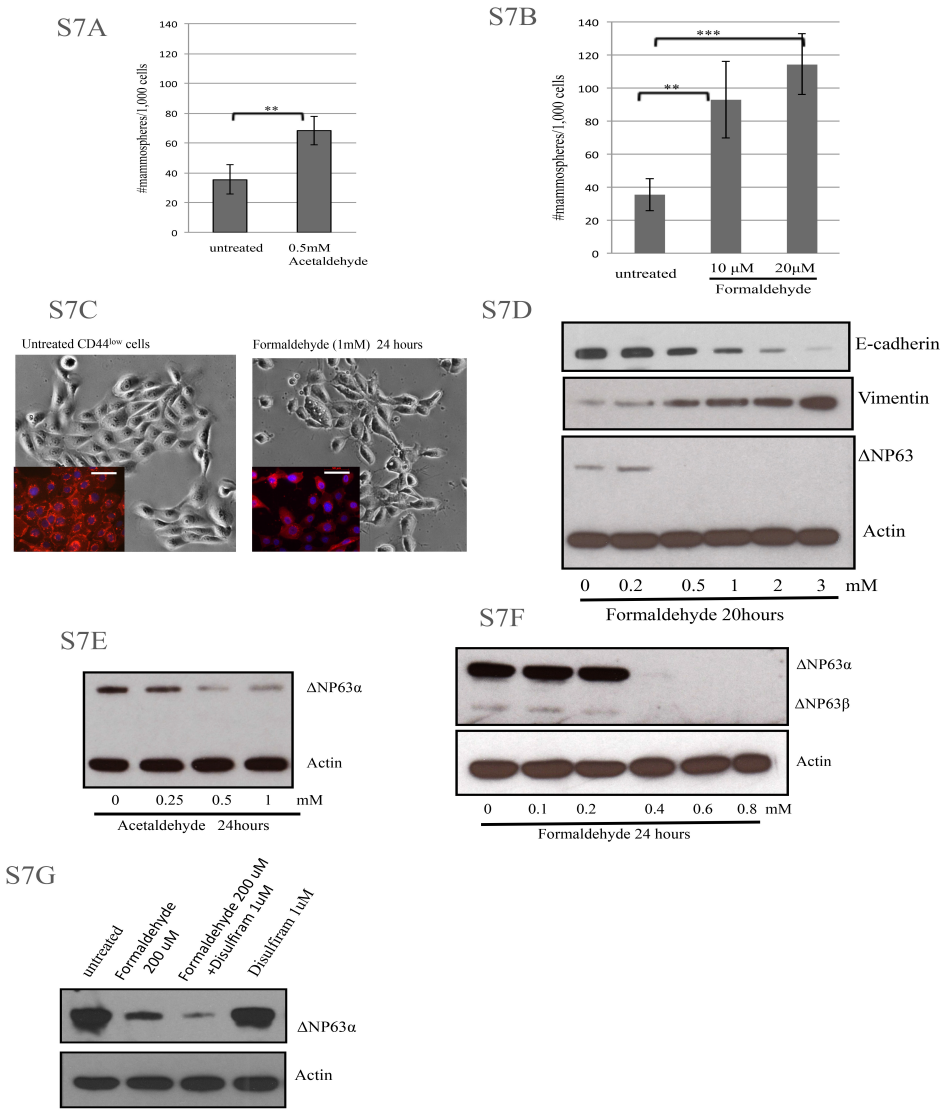
Supplemental Figure 6 (related to Figure 5) Mutation spectrum of BRCA1/Fanconi anemia (FA) and mSWI/SNF genes in breast cancer.

S6A) In interrogating the TCGA breast cancer data base, mutations in all BRCA1/FA genes and in 17 core mSWI/SNF genes were detected in 28% (264/974) of the fully analyzed breast cancer collection, cf **http://bit.ly/1LQL130.** The most abundant, mutated BRCA1/FA and core mSWI/SNF family genes (mutations present in ≥1% of tumors) are shown here. Collectively, these most abundant mutations were detected in 23% (217/974) of the TCGA breast cancer collection.

Both ER+ and ER- cancers were present among these 217 cases. There were 10 ER- and 14 ER+ tumors in the BRCA1 mutant subgroup. 8 BRCA1 mutant cases were not sufficiently characterized to know their ER status. In this regard, a recent study has shown that the incidence of BRCA1 ER+ tumors increased significantly with age at diagnosis (Mavaddat et al, 2012). Of the 217 cases, 147 were ER+ and 65 were ER-. ER status is not known for the remaining cases.

S6B) Mutations/alterations in FANCD2 and FANCA in different sporadic breast cancer subtypes from the TCGA database. A student t-test was used for statistic analysis.

S6C) Coincidence among BRCA1 and all core mSWI/SNF breast cancer mutations is displayed in Euler diagrams. The coexistence of BRCA1 and the core mSWI/SNF genes is reflected in the results of the relevant statistical analysis. The data in each circle represent the number of mutations affecting each gene or gene family. A two-sided Fisher's exact test was used in the analysis.



Supplemental Figure 7 (Related to Figure 6) Small aldehydes elicit aberrant differentiation in HME cells.

S7A-S7B**)** Mammosphere assays performed on wt CP29-HME cell that were either unexposed or exposed for 10 days to acetaldehyde or formaldehyde at indicated concentrations. The data represented are mean values ±S.D. A student's t-test was performed on the results. *P<0.05, **P<0.01 and ***P<0.001. The experiments in S7A and S7B were performed in parallel and employed a common population of untreated cells.

S7C) Phase-contrast images of untreated and formaldehyde (1mM/20hours)- treated $CD44^{low}$ HME cells. The inserts depict E-cadherin (red) and DAPI (blue) staining.

S7D) Immunoblotting for E-cadherin and Vimentin after formaldehyde treatment at the indicated concentrations for 20 hours in $CD44^{low}$ HME cells.

S7E-S7F) Immunoblotting of ΔNP63 after purified, $CD44^{low}$ HME cells were exposed to acetaldehyde or formaldehyde at the indicated concentrations for 24 hours.

S7G) Immunoblotting for ΔNP63 in $CD44^{low}$ cells after exposure to formaldehyde (0.2mM) or Disulfiram (1uM) or both for 30 hours.

Supplemental Tables

Table S1 Summary of the effects of BRCA1/BRCA2 depletion or BRCA1 reconstitution on the differentiation maintenance of CD44^{low} HME cells (Related to Figure1)

		Dox- (total)	Dox+ (total)	p-value
tshScramble	CD44^{high} clones	7 (91)	8 (89)	Not significant
	CD44^{low} clones	84(91)	81(89)	
tshBRCA1	CD44^{high} clones	11 (96)	31 (101)	P<0.01
	CD44^{low} clones	85 (96)	70 (101)	
tshBRCA2	CD44^{high} clones	5 (75)	1 (48)	Not significant
	CD44^{low} clones	70 (75)	47 (48)	
Empty vector +tshBRCA1	CD44^{high} clones	4 (40)	15 (51)	P<0.01
	CD44^{low} clones	36 (40)	36 (51)	
HA-BRCA1 +tshBRCA1	CD44^{high} clones	1 (41)	3 (35)	Not significant
	CD44^{low} clones	40 (41)	32 (35)	

Table S2 Summary of the effects of DNA damage drugs on differentiation maintenance of CD44^{low} HME cells (Related to Figure 3)

	CD44^{high} clones (total)	CD44^{low} clones (total)	P-value
Etoposide(1μM)	0 (35)	35 (35)	Not significant
Cisplatin (0.5μM)	4 (38)	34 (38)	P<0.05
Cisplatin(1μM)	8 (50)	42 (50)	P<0.01
Cisplatin(2μM)	8 (28)	20 (28)	P<0.001
Untreated	0 (53)	53 (53)	Not significant
PBS	0 (41)	41 (41)	

Supplemental Experimental Procedures

Cell culture

One strain of telomerase-immortalized human mammary epithelial cells (also known as HME) was provided by Dr. William Hahn (Dana-Farber Cancer Institute). These cells were cultivated in MEGM medium (Lonza). CP29-HME cells and CP37-HME cells, gifts of Drs. Shailja Pathania and Jean Feunteun, are primary human mammary epithelial cells obtained from the reduction mammoplasty tissue of two, individual BRCA1+/+ donors. These cells, too, were immortalized with hTERT and maintained in MEGM medium as reported previously(Pathania et al., 2014). 293T and MCF7 cells were cultivated in DMEM containing 10% FBS. All cells were cultivated at 37 ^{0}C in a 10% CO_2-containing atmosphere.

Single mammary epithelial cell clone generation and characterization by flow cytometry

In each experiment, ~500 single, tetracycline-inducible shScramble or shbrca1-transduced CD44^{low} HME cells were seeded in a 10cm plate (BD) with or without Doxcycline(Dox) (400ng/ml). After 8-10 days, individual clones were retrieved, and each was expanded in a single well of a 24 well plate for FACS analysis.

To test for the effects of DNA damage upon cell differentiation, 500 or 1,000 BRCA1+/+ CD44^{low} HME cells were seeded in a 10cm plate and exposed to cisplatin for 8 days (0.5μM or 1μM) or 48 hours (2μM) or Etoposide for 48 hours (1μM). After 8-15 days, viable clones were retrieved, and each was expanded in a single well of a 24-well plate.

To analyze the CD24 and CD44 expression of individual clones, cells were trypsinized (0.05% trypsin- Invitrogen) and washed once with PBS. These cells were then incubated with anti-CD24-FITC (BDB555427) and anti-CD44-PE (BDB555479) for 45 minutes at room temperature and washed once with PBS. Live cells were gated (~90%) and analyzed by flow cytometry for CD24 and CD44 expression in a BD LSRII instrument.

FACS

CD44^{high} and CD44^{low} HME cells and CP29-HME cells (BRCA1+/+) were stained with CD24-FITC and CD44-PE antibodies and sorted in a BD FACSAria II instrument.

Also CP37-HME cells, another freshly isolated BRCA1+/+ HME cell strain, were incubated with CD24-FITC and CD44-PE antibodies. CD24^{high}, CD24^{medium}cells were then purified by FACS.

Mammosphere analysis

Mammosphere culture was performed as described(Dontu et al., 2003; Mani et al., 2008) with a slight modification. Briefly, 1,000 cell aliquots were seeded in each well of a 24-well ultra-low attachment plate (Corning, 3473). Cells were cultivated in MEGM supplemented with B27 (Invitrogen), 20 ng/mL EGF, and 20 ng/mL bFGF, 4 μg/mL heparin, and 0.8% methyl cellulose (4000cP, Sigma). After 10-14 days, mammosphere numbers were counted under a light microscope.

Western blotting

Cells were lysed for 2 hours on ice with occasional pipetting in NETN-250 lysis buffer [250mM NaCl, 0.5 mM EDTA, 20 mM Tris-Cl pH 8.0, 0.5% [v/v] Nonidet P-40 [NP-40]) supplemented with protease inhibitors (Roche). Total lysates were centrifuged at 13,200g at 4^{o}C. The soluble fraction was diluted into Laemmli SDS loading buffer (Bio-Rad) and separated electrophoretically in a 4-12% Bis-Tris SDS Gel (Invitrogen), which was then blotted onto a Nitrocellulose membrane. The membrane was blocked with 3% non-fat milk for 30 minutes at room temperature and incubated with primary antibody at 4^{o}C overnight with continuous rocking. HRP-conjugated secondary antibody (GE) and Western Lightning ECL (Pierce) were used to visualize the signal.

To separate the soluble and chromatin fractions, cells were lysed for 30 minutes on ice in NETN-250 lysis buffer. Soluble and chromatin fractions were separated by centrifugation at 13,200g. Each fraction was dissolved in Laemmli SDS loading buffer (Bio-Rad) and boiled for 10 minutes at 95°C. The soluble and chromatin fractions were then subjected to western blotting analysis.

Immunoprecipation

Immunoprecipitation was performed by incubation of NTEN 250-based soluble extract with mouse anti-BRCA1 (D9, Santa-Cruz) for 8 hours or Rabbit anti-BRG1 (Bethyl labs) overnight at 4°C following the addition of protein A-coupled Sepharose beads (GE). The beads were washed in NTEN100 buffer (NaCl 100mM) four times, boiled for 5 min at 95°C in Laemmli buffer (Bio-Rad), and centrifuged. The supernatants were subjected to immunoblotting analysis.

Antibodies

Antibodies used for western blotting and immunoprecipitation were BRCA1(D9, Santa Cruz Biotechnologies), BRCA1(MS110, Calbiochem), BRG1(H-10, Santa Cruz Biotechnologies), BRG1 (Bethyl lab A300-813A), FANCD2(FI17, Santa Cruz Biotechnologies), FANCD2(NB100-182, Novus), E-cadherin(610181,BD Bioscience), E-cadherin(24E10, Cell signaling), Vimentin(H-84, Santa Cruz Biotechnologies), N-cadherin(H-63, Santa Cruz Biotechnologies), Zeb1(H-102, Santa Cruz Biotechnologies), HA(Covance, MMS-101P), P63 (A4A and H-129, Santa Cruz Biotechnologies). Antibodies for immunofluorescence were directed at E-cadherin (610181, BD Bioscience), HA(Covance, MMS-101P), BRCA1 (Millipore, 07-434). Antibodies for flow cytometry are CD24-FITC(BDB555427), CD44-PE(BDB555479) and protein C Receptor (PROCR-PE) (Biolegend, 351904)

Clonogenic and cell growth assays

Cells were seeded at a density of ~ 300 cells/well in 6-well plastic plates, cultivated overnight, and then exposed to the indicated cisplatin concentrations for 8 hr. Cisplatin was then washed away, and, after one week's further cultivation, cells were fixed. Colonies containing more than 50 cells were identified and counted.

To measure cell growth after BRCA1 depletion, ~300 cells were plated in each well of a 6-well plate, and doxycycline was added to deplete BRCA1 through shBRCA1 expression. After 10 days, cells were fixed and visualized with crystal violet.

To measure colony-forming efficiency of each mammary epithelial cell line before vs after BRCA1 depletion, one or two cells from that line were seeded in each well of a 96-well plate with or without doxycycline. After 8 days, cells were fixed and stained with crystal violet to visualize individual clones.

ShRNAs and SiRNA

A tet (Dox)-on inducible shBRCA1 (tshBRCA1) allele in a PLKO backbone was generated by a method, described previously(Sheng et al., 2010; Wiederschain et al., 2009). Dox-inducible shScramble or constitutive shLuciferase vectors were previously reported(Hu et al., 2014; Sheng et al., 2010). The tshBRCA1 species used here targets the BRCA1 3'UTR. ShFANCD2 (Cat.SC-35356-V) and shcontrol (Cat.SC-108080) lentiviruses were purchased from Santa Cruz Biotechnology. shRNA for BRG1 (TRCN0000015550) was obtained from the RNAi facility at Dana-Farber Cancer Institute and was sequenced to confirm its validity. Both tshBRCA1 and shBRG1 sequences are listed in supplemental item 3.

The siP63 species (Dharmacon) used here targets the 5'UTRs of both ΔNP63α and ΔNP63β, as previously reported(Lindsay et al., 2011). A Dharmcon non-targeting siRNA pool was used as a control. Cells in 12 well plates were transfected twice with siRNAs at a final concentration of 80nM using Lipofectamine RNAiMax (Invitrogen) according to the manufacturers' instructions. All hairpin sequences are listed in supplement item 3.

Immunofluorescence assays

Cells on coverslips were fixed with 4% paraformaldehyde for 15 minutes and permeabilized with cold 0.5% Triton X-100 for 5 minutes. Cells were incubated with primary antibodies for 3 hours and then with secondary antibodies for 1 hour at room temperature. After washing with PBST (PBS containing 0.5% Tween 20), cells were then mounted with medium containing 4',6-diamidino-2-phenylindole (DAPI, Vector lab H1500) and sealed with clear nail polish.

Cell cycle analysis

Cell proliferation was analyzed using a FITC Mouse Anti-BrdU kit (BD, Cat-556028). Cells were plated at 50% confluence, incubated with BrdU for 30 minutes, and fixed with 70% ethanol. Cells were stained with FITC- conjugated BrdU antibody and analyzed according to the manufacturer's protocol.

Anaphase bridges

Proliferating cells were plated at ~ 60% confluence, fixed with 4% paraformaldehyde for 15 minutes, and stained with 4',6-diamidino-2-phenylindole (DAPI; Vector Labs). A total of at least 100 anaphase or early telophase cells were scored in each experiment. To study Anaphase Bridge formation after cisplatin exposure, cells were treated with 1uM cisplatin overnight and fixed with 4% paraformaldehyde.

Comet Assay

Alkaline comet assay were performed on CP37-HME cells before and after BRG1 depleting using the Single-Cell Gel Electrophoresis Assay kit (Trevigen) according to the manufacturer's instructions. About 400 cells were accounted in each experiment.

Quantitative RT-PCR

RNA from relevant cell lines was extracted using an RNeasy Plus Mini Kit (Qiagen). cDNA was prepared using a SuperScript III kit (Invitrogen). cDNA was analyzed by qRT-PCR with iQ SYBR green supermix (Bio-Rad), and the results were analyzed using the delta-delta threshold cycle method. Primer sequences are listed in supplemental item 3.

Vectors and Cloning

ShRNA-resistant HA-BRCA1 was described earlier(Pathania et al., 2011). Flag-tagged BRG1 WT- and K785R- encoding plasmids were gifts of Dr. Robert Kingston (Massachusetts General Hospital). Full-length ΔNP63α and ΔNP63β cDNAs were amplified from BRCA1+/+ $CD44^{low}$ HME cells using specific primers (listed in supplemental item 3). Amplified full length cDNAs were cloned into a POZ-FH-N retro-vector using XhoI and NotI sites as previously described(Nakatani and Ogryzko, 2003).

Virus production and Infection

The DNAs representing the various shRNA species of interest were cloned into lentivirus vectors and packaged in 293T cells, as described(Sheng et al., 2010). Briefly, the shRNA and packaging plasmids (VSV-G and Δ8.9) were cotransfected with Lipfectomine 2000 in 293T cells. Viral particles were collected at 48 hours and 72 hours after transfection. The supernatants were used for infection after filtration through 0.45 μM syringe filter. Infected HME cells were selected with puromycin (2ug/ml) and maintained in puromycin-containing MEGM (Sigma). To produce retroviruses expressing HA/Flag-tagged ΔNP63α, HA/Flag-tagged ΔNP63β, flag-BRG1 wt or flag-BRG1 K785R, viral vectors were contransfected with pMD-MLV and pMD-G (Gifts from Dr. Daniel Silver) using

Lipfectomine 2000 in 293T cells. Supernatants were collected 48 or 72 hours after transfection and centrifuged to concentrate virus for infection.

TCGA Mutational analysis of Fanconi Anemia(FA) genes and mSWI/SNF genes

TCGA-collected mutations in BRCA1/ Fanconi Anemia (FA) and 17 core mSWI/SNF genes were identified at the cbioport website using TCGA 2015 Cell(Cerami et al., 2012; Gao et al., 2013) data (974 tumors with sequencing and CNA data). The analysis results can be retrieved using the link (http://bit.ly/1MpaUnB). Concurrent relationships between mutations in BRCA1/FA, and mSWI/SNF genes were assessed using two-sided Fisher's exact tests and 95% exact binomial confidence intervals.

Sequence for qPCR and P63 cloning and shRNA/siRNA

Primers for qPCR	
Pan ΔNP63 Forward	GGAAAACAATGCCCAGACTC
Pan ΔNP63 Reverse	GTGGAATACGTCCAGGTGGC
TAP63 Forward:	TGTATCCGCATGCAGGACT
TAp63 Reverse	ATATCTCGAGATGAATTTTGAAACTTCACGGTG
ΔNP63α Forward:	AGCGAGGTTGGGCTGTTCATC
ΔNP63α Reverse:	TGGGGTCCGCAGGAGATGAGAA
ΔNP63β Forward	AGCACCAGCACTTACTTCA
ΔNP63β Reverse	CAGACTTGCCAGATCCT
Primers for cloning	
ΔNP63 Forward	ATATCTCGAGATGTTGTACCTGGAAAACAATG
ΔNP63α Reverse	ATATGCGGCCGCCTATCACTCCCCCTCCTCTTTGATGC
ΔNP63β Reverse	ATATGCGGCCGCCTATCAGACTTGCCAGATCCTGACAATG
ShRNA or siRNA	
tshBRCA1-1: (3'-UTR)	CCGGTATAAGACCTCTGGCATGAATCTCGAGATTCATGCCAGAGGTCTTATATTTTTG
tshBRCA1-2: (Exon6)	CCGGGCCTACAAGAAAGTACGAGATCTCGAGATCTCGTACTTTCT TGTAGGCTTTTTG
tshBRCA2:	CCGG TACAATGTACACATGTAACACCTCGAG GTGTTACATGTGTACATTGTATTTTTG
ShBRG1:	CCGGGCCAAGCAAGATGTCGATGATCTCGAGATCATCGACATCTTGCTTGGCTTTTTG
siP63	5' CAUCUGACCUGGCAUCUAAUU 3'