SUPPLEMENTARY METHODS

Cell culture and transfection

ES cells were cultured on mitotically inactivated feeder cells as described earlier[1]. The Human breast cancer cell lines MCF7, HCC1937, MDA-MB-436 (obtained from American Type Culture Collection (ATCC), Rockville, MD), HEK 293 and MDA-MB-468 (generous gift from Dr. Esta Sterneck, NCI) were cultured in DMEM supplemented with 10% FBS and antibiotics. MCF10A cells were cultured in F-12/DMEM (1:1) with supplements as described (ATCC, CRL-10317). Mouse BRCA1 deficient cell line (#69) was cultured in DMEM supplemented with 10% FBS and antibiotics. For transfection of MDA-MB 436 cells, 15μl of Lipofectamine 2000 (Invitrogen) was mixed with 5μg of DNA per one 100mm culture dish with 5ml of Opti-MEM (Invitrogen). For the reporter assay in HCC1937 or MDA-MB-468 cells, the cells in 24 well plates were transfected with 20ng-100ng of DNA mixed with 0.1-0.5 μl of lipofectamine 2000. For stable knockdown of BRCA1 in HEK293 cells, 12 clones of BRCA1 shRNA set (see the supplementary Table 6) was transfected and the cells were selected under 1mg/ml G-418 for 1 week. The G-418 positive clones were further expanded to 6 wells and analyzed for BRCA1 level.

DNA constructs

A bacterial artificial chromosome clone (RPCI 11-812-5O) containing the full-length human *BRCA1* was used to generate R1699Q using recombineering-based "Hit and Fix" method as described previously[2]. M1652I variant was described previously[1]. Sequence of oligonucleotides used to generate the mutations in the BAC is described in

Supplementary Table 6. The reporter plasmid of miR-155 promoter was constructed by cloning 1.5 kb genomic fragment of mouse *BIC* prompter containing the TATA box into pGL3 Enhancer plasmid (Promega). The first mutation on the BIC promoter (Mut1) was generated by designing a 200mer oligonucleotide with 9 TGGT > GCCG change for putative BRCA1 binding sites. The second mutation (Mut2) was generated by QuickChange II site directed mutagenesis kit (Stratagene). The double mutant was generated by introducing Mut2 in Mut1 reporter construct. miRNA-155 luciferase reporter was constructed by cloning the oligonucleotide of the complementary sequence for matured miR-155 into pMIR-REPORT luciferase reporter plasmid (Ambion). The GFP sponge of miR-155 was generated by using the same oligonucleotide for luciferase reporter, cloned into pEGFP-C1 (Clontech). The HA tagged BRCA1 expression plasmid (BRCA1-HA) is a generous gift from Dr. David M. Livingston. The R1699Q mutation (c.5095G>A) was introduced in BRCA1-HA using QuickChange II site directed mutagenesis kit (Stratagene). For the inducible miR-155, two complementary oligonucleotides with the sequence of the miR-155 precursor were cloned into pSUPERIOR.retro.puro plasmid (OligoEngine) into the *Bgl*II and *Hin*dIII sites. pcDNA/TR6 vector (Invitrogen) was used to express Tet repressor. The pCS2+BIC134-283 (Designated as CMV-pri-miR-155, generous gift from Dr. David L. Turner) was used for the over-expression of miR-155. All DNA constructs were confirmed by sequencing.

Embryoid body analysis

To generate embryoid bodies, ES cells were trypsinized and the feeder cells were removed by incubating the cells on gelatinized plate for one hour. The ES cells in supernatant were counted and diluted to $5X10^4$ cells/ml. The cell suspension was cultured in Petri dish, in DMEM-10 media. The media was changed on day 3 and later it was changed every other day. After 14 days, the embryoid bodies were collected.
For histology, the embryoid bodies were fixed in 10% formalin and embedded in paraffin and sectioned. The sections were stained with H&E. To analyze cell death, DeadEnd TUNEL staining kit (Promega) was used as per the manufacture's protocol. For miRNA *in situ* hybridization in embryoid body, DIG labeled LNA-miR-155 probe (Exiqon) was used, followed by the colorimetric detection using REMBRANDT® In Situ Hybridization and Detection kit (Panpath).

Western blots and co-immunoprecipitation (co-IP) analysis

For western blot, cells were lysed on ice in RIPA buffer with protease inhibitor tablet (Roche) for 20minutes. The cleared lysate was added with 2X protein sample buffer and used for western. For co-IP, freshly isolated mammary epithelial cell (10^7 cells) was lysed in modified RIPA buffer (150 mM NaCl, 10 mM Tris, pH 7.2, 0.1% SDS, 0.5% Triton X-100, 0.5% Deoxycholate, 5 mM EDTA) with protease inhibitors. The human specific BRCA1 antibody or rabbit monoclonal HDAC2 antibody was used for IP. The co-immunoprecipitated BRCA1 and HDAC2 were detected by E1 (for BRCA1) or mouse monoclonal HDAC2 antibody.

LOH analysis of the tumors

To test the LOH of the tumors from the *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mice, Southern hybridization was performed using freshly isolated tumor DNA digested with PstI. The probe for *Brca1* detection was generated by PCR (See Supplementary Table5 for sequence) and labeled with Prime-It random primer labeling kit (Stratagene). To measure the copy number of BRCA1 in the R1699Q human tumor, two of 10mM sections of tumor and normal sample were dissolved in paraffin dissolver (Clontech) and the genomic DNA was isolated by FFPE genomic DNA isolation kit (Qiagen). The copy number of *BRCA1* in the genomic DNA was quantified by real-time PCR using *CCNB1* as a control.

Teratoma analysis

The R1699Q and WT ES cells were harvested and washed in PBS. Cells were counted and diluted to a concentration of $5X10^7$ cell/ml in PBS. 100 μl of the cell suspension was injected subcutaneously into athymic nude mouse (C3H/HeNCr-nu). The growth of teratoma was measured after one week, and then measured every other day. Tumor volume (in mm^3) was calculated as a product of 2 x length x width. Mice were maintained under limited access conditions at the National Cancer Institute (Frederick) and animal care was provided according to the procedures outlined in the Guide for the Care and Use of Laboratory Animals, under an approved Animal Care and Use Committee (ACUC) protocol.

Inducible miR-155 expression in ES cells

For inducible expression of the miR-155, the pSUPERIOR-puro-miR-155 (see DNA constructs), along with pcDNA/TR6 and pmiRep-miR-155, were co-electroporated into wild-type ES cells and selected with puromycin (300 μg/ml) for 5 days. The puromycin resistant colonies were picked and expanded in a 96-well plate. After splitting the cells into three 96 well plates, one plate was treated with tetracycline to test miR-155 induction. By measuring the ratio of miR-155 reporter luciferase activity from induced and uninduced, the ES clone with Tet induced miR-155 expression was identified and used for further analysis. The induction of miR-155 was confirmed by miRNA Northern hybridization as well as real time PCR (Fig 2d).

Tumor tissue array analysis

For human breast tumor analysis, Breast Tumor Tissue Array (BioChain Institute, Inc.) containing 70 breast tumor tissues was used. To access the BRCA1 level in these tumor samples, two tissue array slides were deparaffinized, hydrated and boiled in citrate buffer for antigen exposure. After blocking, the slides were probed with anti-BRCA1 antibody (Ab-1, Calbiochem) or normal mouse IgG (Oncogene), respectively. The BRCA1 signal was detected by Elite ABC HRP detection kit (Vectastain). For the detection of miR-155, DIG labeled LNA-miR-155 probe (Exiqon) was used, following the manufacturer's protocol. The miR-155 expression was visualized by anti-DIG-AP conjugate antibody and NBT/BCIP substrate (included in RAMBRANDT RISH kit used above). One set of tissue microarray was stained with H&E. The stained tissue array was analyzed under the Axioplan2 upright microscope (Zeiss).

Generation and analysis of the R1699Q BAC transgenic mice

BAC DNA with R1699Q mutation was prepared by Qiagen Maxiprep kit. The purified DNA was diluted in TE buffer to 10ng/ml concentration and used for the microinjection. Mice were genotyped by Southern analysis as described previously[1]. The BAC transgenic founder mice were mated with mice carrying a null allele of *Brca1* (*Brca1*$^{ko/+}$) to obtain BAC transgenic mice on *Brca1* heterozygous background (*Brca1*$^{ko/+}$;*Tg*^{R1699Q}). The expression of R1699Q *BRCA1* was examined in several tissues by RT-PCR. To test the embryo development of *Brca1*$^{ko/ko}$;*Tg*^{R1699Q} transgenic mice, a pair of R1699Q transgenic mouse with Brca1 heterozygous background (*Brca1*$^{ko/+}$;*Tg*^{R1699Q}) were intercrossed. Embryos at days 7.5 of gestation were dissected under the microscope (LEICA MZ8) and photographed. Embryos were genotyped by PCR using primers as listed in Supplementary Table 6. To generate the *cis*-*Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{R1699Q} mouse, *Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$ mice were crossed with *BRCA1;Tg*^{R1699Q} mouse and the co-segregation of *Brca1* and *Trp53* was screened by genotyping PCR. *cis*-*Brca1*$^{ko/+}$;*Trp53*$^{ko/+}$;*Tg*^{M1652I} mouse was generated by the same method.

Mouse Mammary Epithelial Cell (MEC) isolation and culture

To isolate mammary epithelial cells, the protocol from Stem Cell Technology was used (http://www.stemcell.com/en/Products/All-Products/EpiCultB-Mouse-Medium-Kit.aspx). Briefly, freshly dissected mammary gland was digested in digestion media (Epicult-B medium supplemented with 5% FBS and Collagenase/Hyaluronidase) for 8 hrs at 37ºC. The dissociated tissue was centrifuged and red blood cells were removed by treating with 0.8% NH_4Cl with 0.1 mM EDTA. The collected organelles were further digested in

trypsin-EDTA, followed by DNase I and dispase treatment to make a single cell suspension. The resulting cells were plated in 150mm gelatinized culture dish with Epicult-B medium with 5% FBS. One the next day the media was changed to serum free Epicult-B medium supplemented with EGF and FGF and cultured up to near confluency.

REFERENCES

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