SUPPLEMENTARY INFORMATION

METHODS

Mouse embryonic fibroblasts (MEF)

MEFs were generated from E13.5 embryos as described previously (1). Briefly, after removal of neuronal and hematopoietic organs, the embryos were disintegrated and digested in trypsin and cultured in DMEM (1X) media supplemented with 10% FBS and 0.5mg/ml GPS, at low O_2 (3%) levels.

Generation of *Brca2*$^{\Delta ex4-7}$ knock-in mice with a targeted deletion of exons4-7

To generate the ∆ex4-7 knock-in, exons 4-7 of the mouse Brca2 gene were first replaced with *loxP*-Neo-*loxP* cassette. The cassette was amplified from plasmid PL452 and targeted into the mouse *Brca2* gene, carried on a BAC, to delete1873 bp of genomic region from the middle of intron 3 to the middle of intron 7. The retrieval vector was constructed in pLMJ235 by cloning 229 bp of homology from 5´ region of *Brca2* into *Eco*RI/*Bam*HI sites and 207 bp from 3' region into *Bam*HI/*Not*I sites. Targeting the Neo cassette and retrieval of targeting vector was conducted using the recombineering technology (2). The retrieved targeting vector had a 5412bp homology arm upstream of the first *loxP* site and 4320bp homology arm downstream of the last *loxP* site.

The targeting vector was linearized with *Not*I and electroporated into 129XB6 F1 hybrid V6.4 ES cells derived from a 129/B6 mouse line and clones were selected for Neomycin and FIAU resistance. Resistant clones were screened by Southern analysis following digestion with *Bam*HI using the 5´ external probe yielding a

9.5kb wild-type band and 7.6kb mutant band, and *EcoR*1 digestion using the 3´ internal probe yielding a 9kb wild-type and 7.5kb mutant band. Correctly targeted ES cell clones carrying the *Brca2*$^{\Delta ex4\text{-}7\text{-}Neo}$ allele were injected into C57BL/6 blastocysts to generate chimeras. Chimeras were crossed to C57BL/6 wild type mice and progeny were genotyped to examine germline transmission of the targeted allele. *Brca2*$^{\Delta ex4\text{-}7\text{-}Neo/+}$ mice were crossed to β-actin-Cre mice to excise the Neo cassette in order to generate the *Brca2*$^{\Delta ex4\text{-}7/+}$ knock-in mice. Loss of the Neomycin insert was confirmed via Southern using the 3' internal probe following *Eco*RI digestion, which yields a 5.6kb band. *Brca2*$^{\Delta ex4\text{-}7/+}$ mice were also crossed to *Brca2*$^{Ko/+}$ mice to generate *Brca2*$^{\Delta ex4\text{-}7/Ko}$ offspring. Primer sequences for targeting, Southern analysis and routine genotyping are listed in Supplementary Table S3.

Flow cytometry analysis of the bone marrow compartment

Combination of antibodies for FACS analysis of bone marrow mononuclear cells: to identify hematopoietic stem and progenitors (HSC) – biotinylated antibodies against lineage markers (Mac-1 (CD11b, clone M1/70), Gr-1 (Ly-6G, clone RB6-8C5), Ter119 (clone TER-119), B220 (clone RA3-6B2), CD4 (clone GK1.5), CD8 (clone 53-6.7), ILR7Rα (clone A7R34), cKit (clone 2B8), Sca1 (D7), CD34 (clone RAM34), CD48 (clone HM48-1), CD150 (clone mShad150), Flk2 (clone A2F10); to assess DNA damage and proliferation – γH2AX (Ser139, clone 20E3) and Ki67 (BD, 556027); to assess status of committed progenitors – Gr-1, Mac-1, Ter119, CD71 (clone R17217), B220 and IgM (clone 11/41). To sort cells as long-term (LT) and short-term (ST) stem cells and multipotent progenitors (MPP),

mononuclear cells were bead (Dynabeads M-280 streptavidin) depleted of committed progenitors using Lineage antibodies and then stained with cKit, Sca1 CD150, CD48, Flk2 for flow cytometric analysis. All antibodies were either from eBioscience or Biolegend. FACS sorting and analysis was performed using BD LSRII, BD Canto II and BD FACSAria II.

RT-PCR and sequencing

RNA was extracted using RNA-BEE (Tel-Test, Inc.) according to the manufacturer's protocol. To detect an alternatively spliced form of *Brca2*, RT-PCR analysis was performed using Titan one-step RT-PCR kit (Roche) according to the manufacturer's protocol. The ∆ex4-7 splice variant band was excised from the gel, purified and used as template for sequencing using BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems)

Immunofluorescense

To detect RAD51 foci, 300,000 MEFs of each genotype were plated/well in a 6-well gelatinized plate containing 20mm round coverslips. Adhered cells were exposed to 6Gy radiation and, 4h later, were fixed with 4% paraformaldehyde for 10 min and permeabilized with PBS buffered 0.25% TritonX-100 for 10 min. Cells were washed with PBS and blocked in 5% BSA/PBS for 1 hour. DNA damage and foci formation was detected using antibodies against γH2AX (Upstate, 07-164) and RAD51 (Calbiochem, PC130), respectively, and nuclei were stained with 2-(4-amidinophenyl)-1H -indole-6-carboxamidine (DAPI). To visualize the Flemming body, embryoid bodies generated by differentiating mouse ES cells

were seeded on gelatinized cover slips to allow outgrowth of differentiated cells. Cells were then fixed and stained as above and the Flemming body was detected using antibody against PRC1 (Abcam, 119338).

Nikon Eclipse Ti inverted microscope, equipped with a 64 μm pixel CoolSNAP HQ^2 camera (Photometrics) and Intensilight C-HGFIE illuminator, using 100x NA 1.42 Plan Apo objective with 1.5x magnifying tube lens was used to analyze stained cells. ImageJ (National Institute of Health, Bethesda, MD), and NIS-Elements software package was used to assemble acquired images, and to quantify γH2AX stain density of bone marrow cells. Integrated density of DAPI and γH2AX -Alexa488 staining were quantified from maximal projection of immunofluorescent images, and ratio of Alexa488:DAPI was used to measure difference in levels of DNA damage.

Live cell imaging

MEFs growing on the coverslip were assembled in a Rose Chamber and imaged by a Nikon Eclipse Ti inverted microscope equipped with 100x and 60x NA 1.45 or 20x NA 0.75 objectives, Yokogawa spinning disc, 405, 488, 561 and 640 nm laser launch (Agilent technology MCL-400), back-illuminated 16 μm pixel or 13 μm EMCCD camera (Andor, DU897 and DU888, respectively), with 1.5x magnifying tube lens.

Evaluation of chromosomal aberrations

mES cells were given a single dose of 2Gy irradiation and surviving colonies were grown for two passages before treatment with colcemid. Following colcemid treatment, cells were trypsinized, washed and resuspended in hypotonic solution at 37 °C (0.075M KCl) for 15 min and fixed in a methanol–acetic acid mixture (3:1 vol/vol). Metaphase spreads were stained in Giemsa solution (10% Sorensen's buffer and 2% Giemsa, J.T. Baker). 20-25 metaphases were examined blindly, for each genotype, for structural aberrations.

References

1. Kuznetsov SG, Haines DC, Martin BK, Sharan SK. Loss of Rad51c leads to embryonic lethality and modulation of Trp53-dependent tumorigenesis in mice. Cancer Res 2009; 69: 863-872
2. Sharan SK, Thomason LC, Kuznetsov SG, Court DL. Recombineering: a homologous recombination-based method of genetic engineering. Nat Protoc 2009; 4: 206-223.

SUPPLEMENTARY FIGURES

Supplementary Figure S1: Southern strategy and genotyping to generate and maintain *Brca2*$^{\Delta ex4\text{-}7}$ knock-in mice

(A) Schematic diagram of the mouse *Brca2*$^{\Delta ex4\text{-}7}$ targeting strategy including *Wt* genomic locus, targeting vector, targeted allele *(Brca2*$^{\Delta ex4\text{-}7\text{-}Neo}$), after CRE mediated deletion of *Neo* cassette (*Brca2*$^{\Delta ex4\text{-}7}$) and the null allele (*Brca2*^{Ko}). Open boxes represent exons; triangles indicate position and orientation of loxP sites; B and E indicates *Bam*HI and *Eco*RI restriction sites, respectively; Neomycin (*Neo*); Thymidine kinase (*TK*); Hypoxanthine-guanine phosphoribosyltransferase1(*HPRT1*)

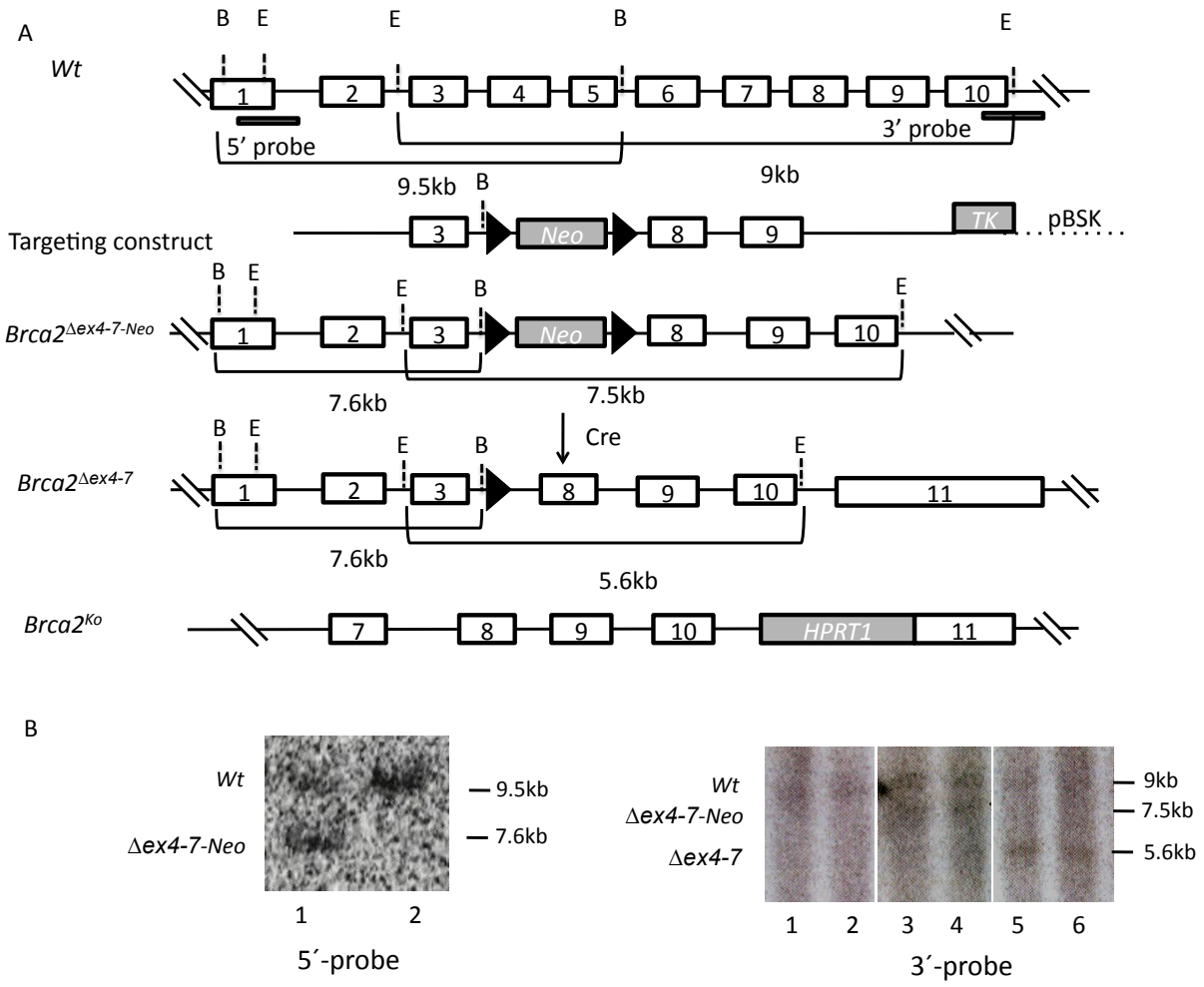
(B) Southern analysis results to detect *Brca2*$^{\Delta ex4\text{-}7\text{-}Neo}$ targeting by *Bam*HI digestion (for 5´ probe, left) and *Eco*RI digestion (3´ probe, right). Deletion of *Neo* cassette confirmed using 3´ probe. Left panel: lane 1 is *Brca2*$^{\Delta ex4\text{-}7\text{-}Neo/+}$ 2 is *Wt*,; Right panel: Lanes 1, 2 are *Wt,* lanes 3, 4 are *Brca2*$^{\Delta ex4\text{-}7\text{-}Neo/+}$ and lanes 5, 6 are *Brca2*$^{\Delta ex4\text{-}7/+}$.

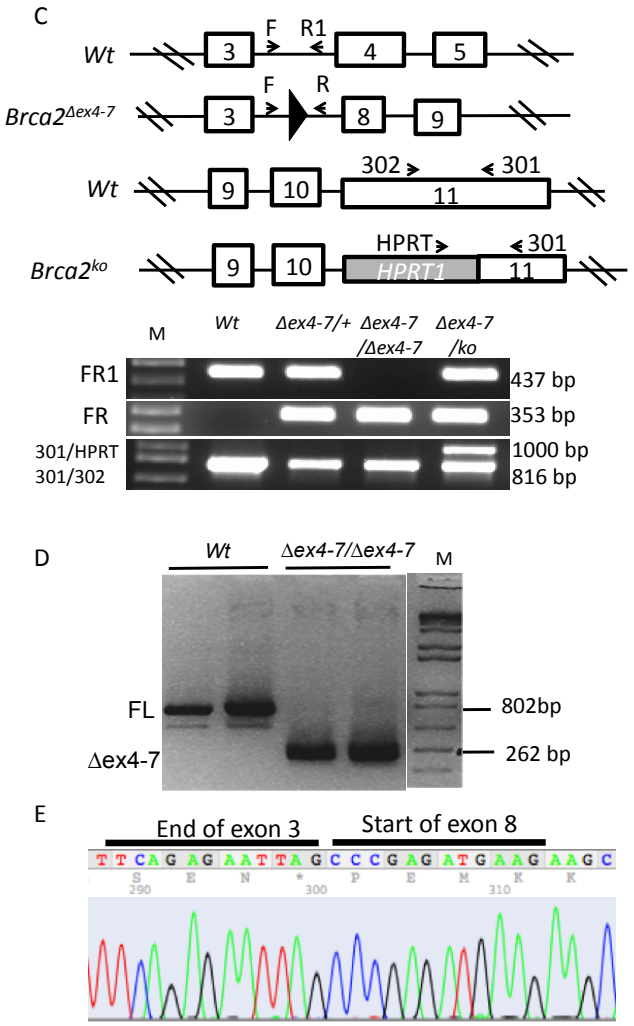
(C) Representative gel pictures showing PCR-based genotyping results of mice using tail genomic DNA using primers indicated at the top. Arrows represent PCR primers, M indicates DNA size marker.

(D) RT-PCR results showing expression of *Brca2*$^{\Delta ex4\text{-}7}$ transcript in *Wt* and *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ MEFs. RT-PCR was performed using primers to exon 2 and exon 9 to detect the full length (FL) and the ∆ex4-7 splice variant. M indicates DNA size marker

(E) Sequence analysis of *Brca2*$^{\Delta ex4\text{-}7}$ transcript lacking exons 4-7

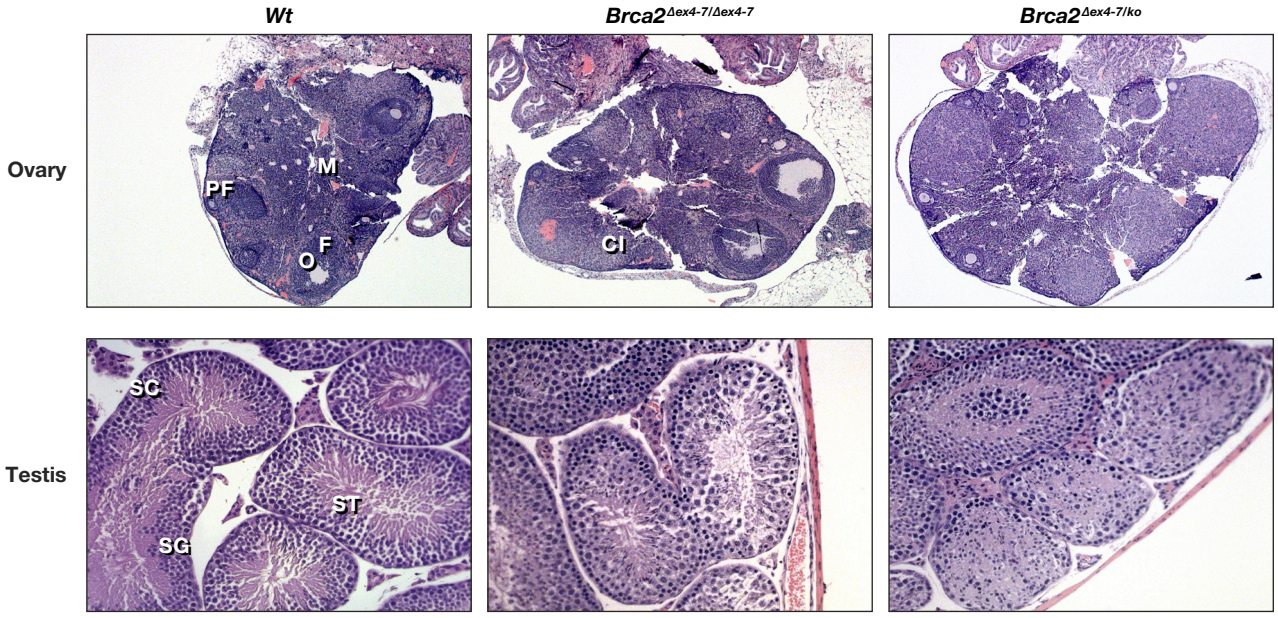
Supplementary Fig. S1





Supplementary Figure S2: Histology of ovaries and testes. H&E stained sections of ovaries (top) and testes (bottom) of mice of various genotypes at 7-11 months of age.

THIRTHAGIRI—Supplementary Figure S2



PF=Primary Follicle
F=Folicle
O=Oocyte
M=Medulla
CI=Corpus luteum

SC=Spermatocyte
SG=Spermatogonia
ST=Spermatids

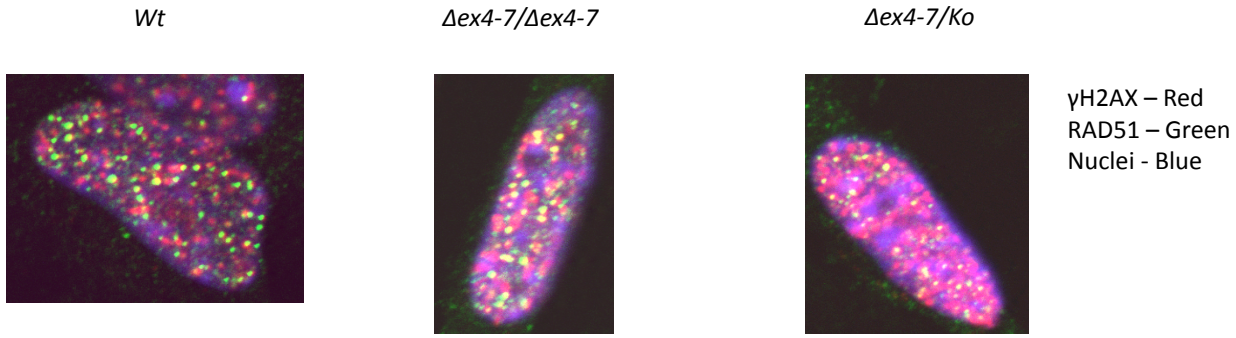
Supplementary Figure S3: Loss of exons 4-7 of BRCA2 does not affect RAD51 foci formation in primary MEFs

(A) Representative images of RAD51 (green) and γH2AX (red) co-localized foci in MEFs derived from *Wt, Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ mice. DNA is stained in blue with DAPI.

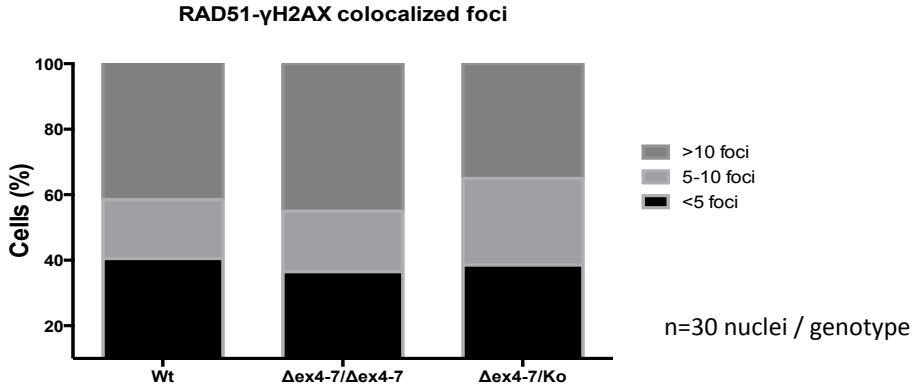
(B) Quantification of RAD51 foci co-localizing with γH2AX foci. Foci in a total of 30 MEFs were quantified per genotype.

Supplementary Fig. S3

A



B

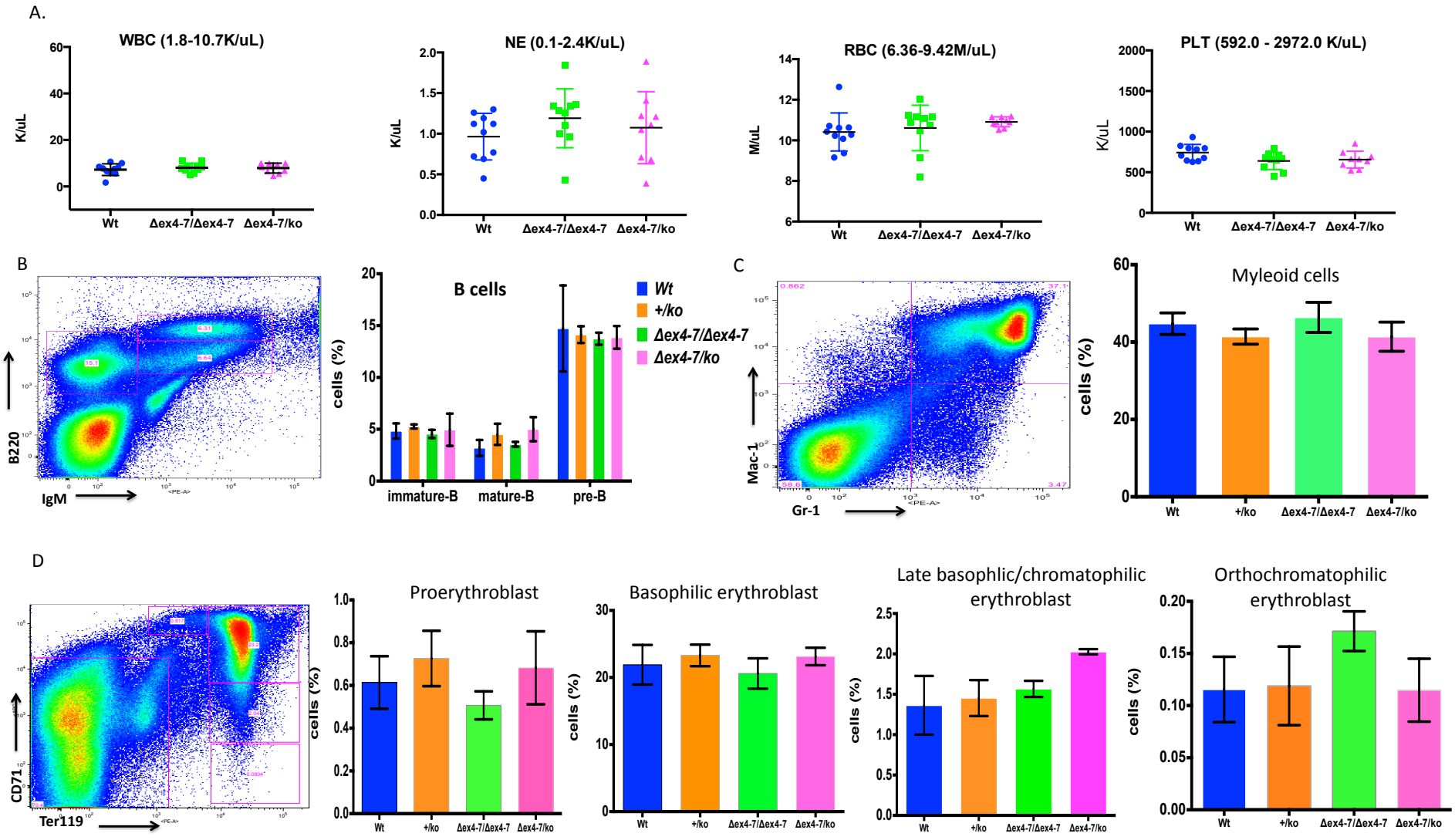


Supplementary Figure S4: Characterization of peripheral blood and bone marrow compartment of *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ mice.

(A) Eight week old mice were bled for complete blood analysis. Data shown is for white blood cells (WBC), neutrophils (NE), red blood cells (RBC) and platelet (PLT) with normal range indicated in bracket. (n=10 mice / genotype).

(B-D) Flow cytometric analysis of differentiated cell compartment of femur bone marrow of unchallenged 8 weeks old mice. Cells were stained with antibodies against IgM/B220 to detect B cells, Gr-1/Mac-1 to detect myeloid cells, and Ter119/CD71 to detect various maturation stages of the erythroid cell population. (n=3 mice / genotype).

Supplementary Fig. S4



Supplementary Figure S5: Assessment of bone marrow cells and survival of *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and Brca2$^{\Delta ex4\text{-}7/Ko}$ mice in response to IR and 5FU treatment.

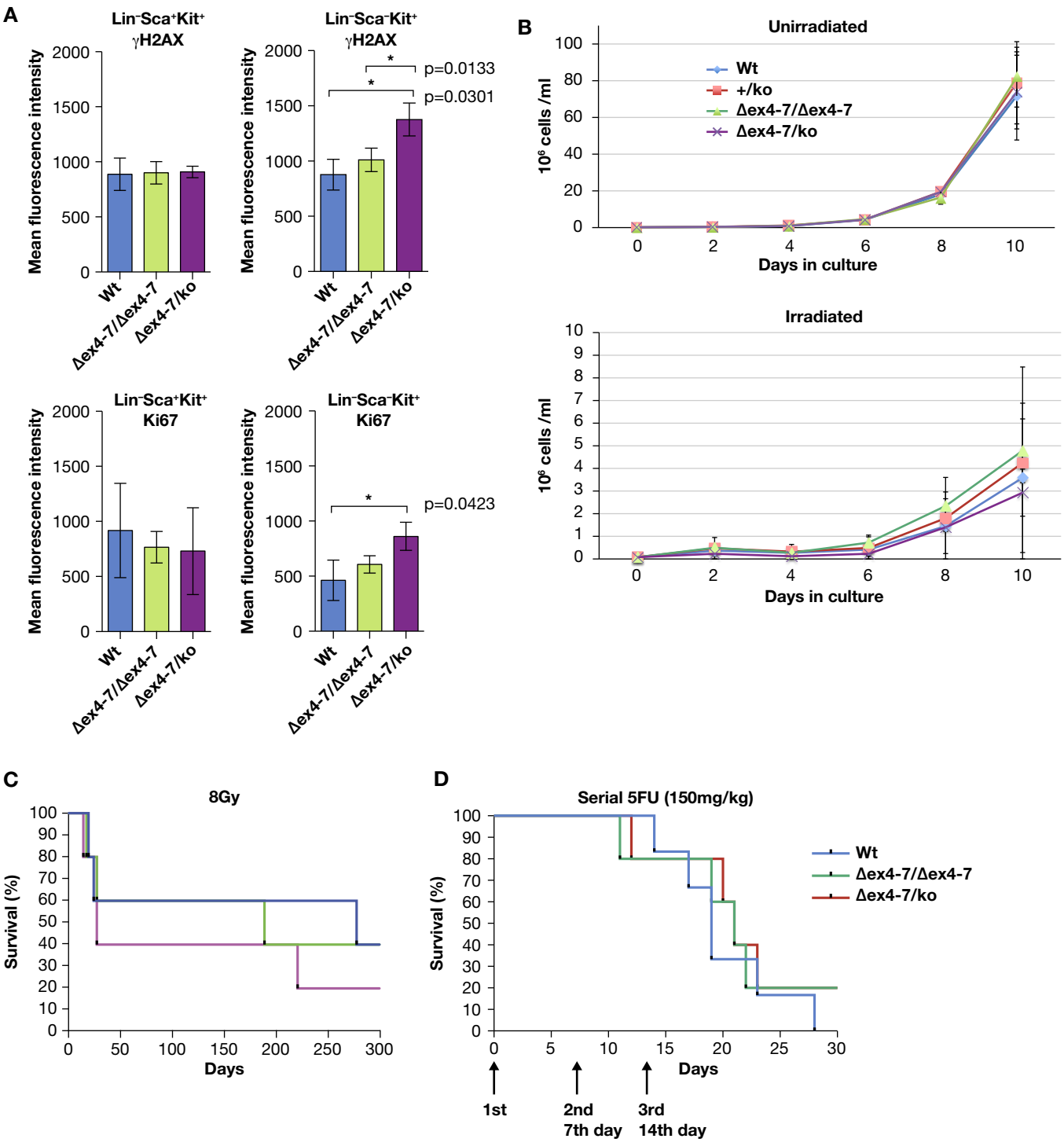
(A) γH2AX and Ki67 staining of stem ($Lin^-Sca^+Kit^+$) and progenitor ($Lin^-Sca^-Kit^+$) population isolated from *Wt, Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ bone marrow, 4 days after 6Gy irradiation. (N=3 mice/genotype).

(B) *In vitro* culture and cell count of mononuclear cells isolated from unirradiated and 6Gy irradiated *Wt, Brca2*$^{Ko/+}$, *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ mice. (N=3 mice/genotype). P = 0.4912

(C) Survival of *Wt, Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ mice following 8Gy irradiation. (N=5 mice/genotype). Over all: $\chi^2 = 0.4021$, p = 0.8179

(D) Survival of *Wt, Brca2*$^{Ko/+}$, *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ mice following serial 5-FU treatments. Three doses of 150mg/kg of 5-FU were administered on day 0, 7 and 14 to successively eliminate cycling cells and force the quiescent stem cells to repopulate the bone marrow. (N=5 mice/genotype). $\chi^2 = 0.4415$, p = 0.8019

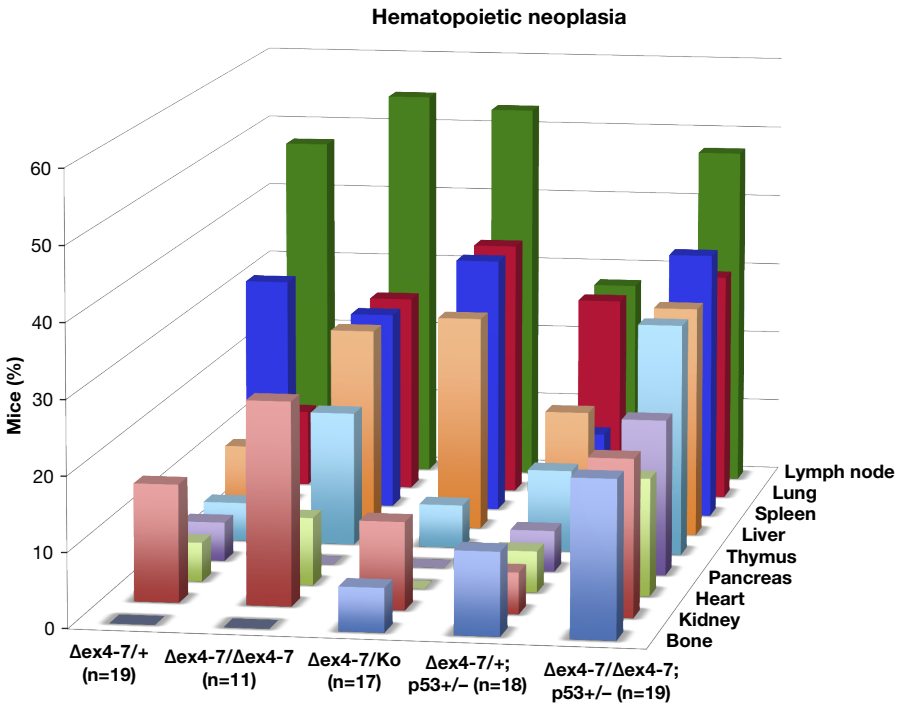
THIRTHAGIRI—Supplementary Figure S5



Supplementary Figure S6: Multiorgan neoplasia in aged mice

Hematopoietic neoplasia in various organs in aged mice of *Brca2*$^{\Delta ex4\text{-}7/+}$, *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ and *Brca2*$^{\Delta ex4\text{-}7/Ko}$ genotypes (10 males and 10 females/genotype) on a *Trp53*$^{+/+}$ background and *Brca2*$^{\Delta ex4\text{-}7/+}$*;Trp53*$^{Ko/+}$ and *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$*;Trp53*$^{Ko/+}$mice. N = confirmed pathology per genotype.

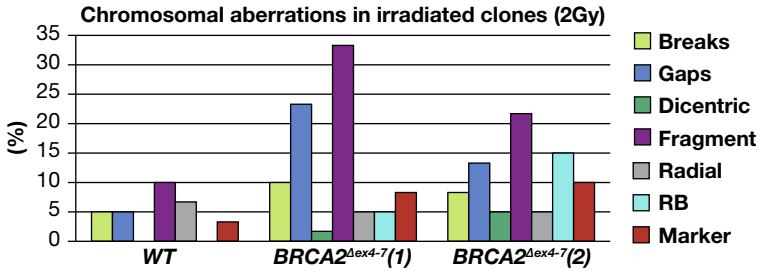
THIRTHAGIRI—Supplementary Figure S6



Supplementary Figure S7: Frequency and types of chromosomal aberrations observed in mES cells, expressing *BRCA2*^{WT} and *BRCA2*$^{\Delta ex4\text{-}7/+}$, following irradiation (2Gy).

N= 60 metaphases were scored per genotype

THIRTHAGIRI—Supplementary Figure S7



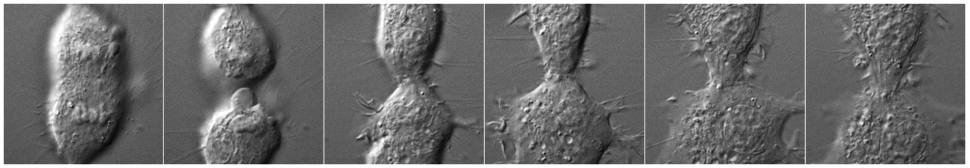
Supplementary Figure S8: Loss of exons 4-7 of BRCA2 does not affect cytokinesis

Time taken from anaphase to abscission, measured using time-lapse imaging of (A) *Wt* and (B) *Brca2*$^{\Delta ex4\text{-}7/\Delta ex4\text{-}}$mouse embryonic fibroblasts.

(C) Midbody was stained with antibody against PRC1 in differentiated mouse ES cells expressing *WT*, *BRCA2*$^{\Delta ex4\text{-}7}$, *BRCA2*^{S193A}.

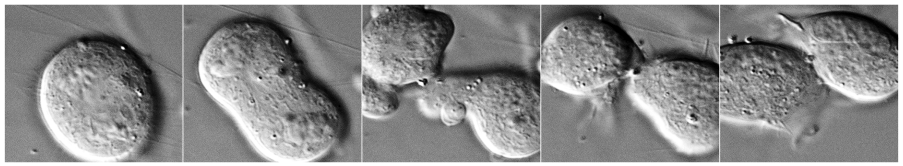
Supplementary Fig. S8

A



Wt MEF

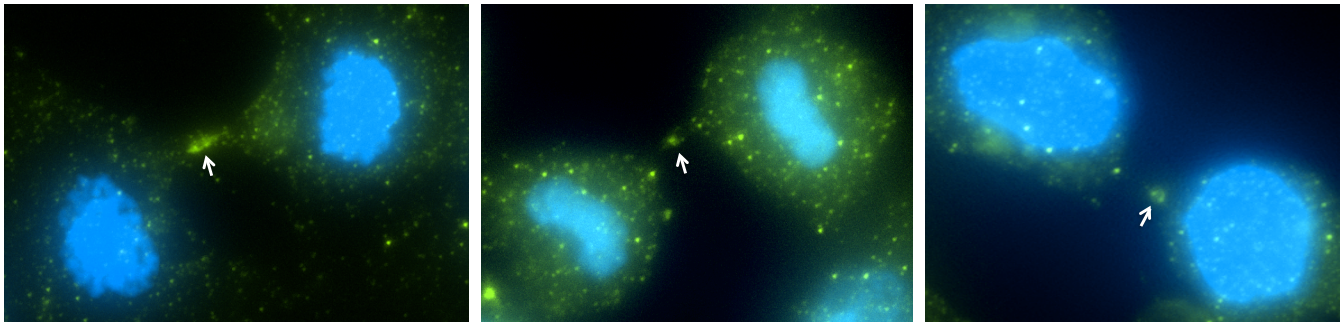
B



Brca2$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$ MEF

C

WT *BRCA2*$^{\Delta ex4\text{-}7}$ *BRCA2*^{S193A}



PRC1 – green
Nuclei - blue

Supplementary Table S1: Observed and expected birth ratio of offspring of various genotypes from *Brca2*$^{\Delta ex4\text{-}7/+}$ intercross.

Genotype	**Expected**	**Observed**
Brca2$^{+/+}$	143.5 (25%)	150 (26%)
Brca2$^{\Delta ex4\text{-}7/+}$	289 (50%)	289 (50%)
Brca2$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$	143.5 (25%)	135 (24%)
Total	574	574

$\chi^2 = 0.8118$, P = 0.67

Supplementary Table S2: Observed and expected birth ratio of offspring of various genotypes from *Brca2*$^{\Delta ex4\text{-}7/+}$ *X Brca2*$^{+/Ko}$ mating.

Genotype	Expected	Observed
Brca2$^{+/+}$	54.75 (25%)	52 (24%)
Brca2$^{\Delta ex4\text{-}7/\Delta ex4\text{-}7}$	54.75 (25%)	51 (23%)
Brca2$^{\Delta ex4\text{-}7/Ko}$	54.75 (25%)	62 (28%)
Brca2$^{Ko/+}$	54.75 (25%)	54 (25%)
Total	219	219

Supplementary Table S3: List of Primers used in the study and their sequence

Primer	Sequence	Purpose	PCR Product size
PL452loxP-F	TCAGTCTCTGTAACATTTATTACTTTCTTGACATGTATGCCCTTTTCTTTAGAGTACTGCTTTGTGATATGCTGGATCCC ATATTCAATAACCCTTAAT	To amplify loxP-Neo-loxP cassette	2030bp
PL452loxP-R	CAGATGGAAGGCATAATTAATGCTAATCACTTCTTATCCTTCAGCCCTTTTATTTTATTATTTCATATAAACAACAAAAAG GATCCCCTCGAGGGACCTA		
mB2-F	ATATCAGTTTCTCTGACCTATGTGC	Screening clones for loxP-Neo-loxP cassette integration and tail genotyping	loxP = 2170bp Tail: ∆105 = 353bp
mB2-R	CAGATGAAAGATCTGTAAAGTTATGTC		
mB2-F	ATATCAGTTTCTCTGACCTATGTGC	Tail genotyping	wt=437bp
mB2-R1	TGCATTCCACCCTTGGTTGT		
SKS302-F	GCAAAAGTAGGACCAAGAGG	Genotype Brca2 knock-out allele	wt=816bp mt = 1000bp
SKS301-R	CCCACTAGCTGTATGAAAAC		
HPRT	AAGTGTTGGATATAAGCCAG		
5'arm-EcoR1-F	AAAAGAATTCCCTATGCCGTGGGTAACAGC	5' mini arm for retrieval vector	229bp
5'arm-BamH1-R	CTTAATAATACTCATGATTCGGATTCAAAA		
3'arm-BamH1-F	AAAAGGATCCTCTTTGTATCCTATGCTGGT	3' mini arm for retrieval vector	207bp
3'arm-Not1-R	AACACCATTTTTAGCTATGTGCGGCCGCAAAA		
5' BH1-F	GATCCGGCGCGTCCAGAGTCCGCGG	Southern blot: 5' external probe	407bp
5' BH1-R	AGTTGGTCTCCTTTTGTATTCAAC		
3' ER1-F	ACCTTACCTCATATTTCTTCTC	Southern blot: 3' internal probe	1073bp
3' ER1-R	ACAATATATGGTGCCAACTGC		
Ex2-F	CCTTACCGAGCATCGGAGAAA	Splice variants	wt = 802bp Δexon4-7= 262bp
Ex9-R	ACCAGCAAGAAGCTTTTAGTCAG		
S193A-hit-F	AAAAAATAAACTATTTTCTTTCCTCCCAGGGTCGTCAGACACCAAAACATATTTCTGAAAGTCTAGGAGCTGAGGTGG AT GGATCCTAGAATTCCTCGAG	Generate hit clone	
S193A-hit-R	TGCTCTTTCTTGTAAATACACATTTGCTATTATTACCTATGAGCACAGTAGAACTAAGGGTGGGTGGTGTAGCTAAAGA ACTCGAGGAATTCTAGGATCC		
S193A-fix-F	AAAAAATAAACTATTTTCTTTCCTCCCAGGGTCGTCAGACACCAAAACATATTTCTGAAAGTCTAGGAGCTGAGGTGG ATCCTGATATGGCTTGGTCAAG	Generate fix clone	
S193A-fix-R	TGCTCTTTCTTGTAAATACACATTTGCTATTATTACCTATGAGCACAGTAGAACTAAGGGTGGGTGGTGTAGCTAAAGA ACTTGACCAAGCCATATCAGG		
S193A seq-F	GTTAAGTGAAATAAAGAGTG	Screening hit and fix clones, and sequencing for mutation confirmation	
S193A seq-R	CTAACACACTTATCAAAGAC		