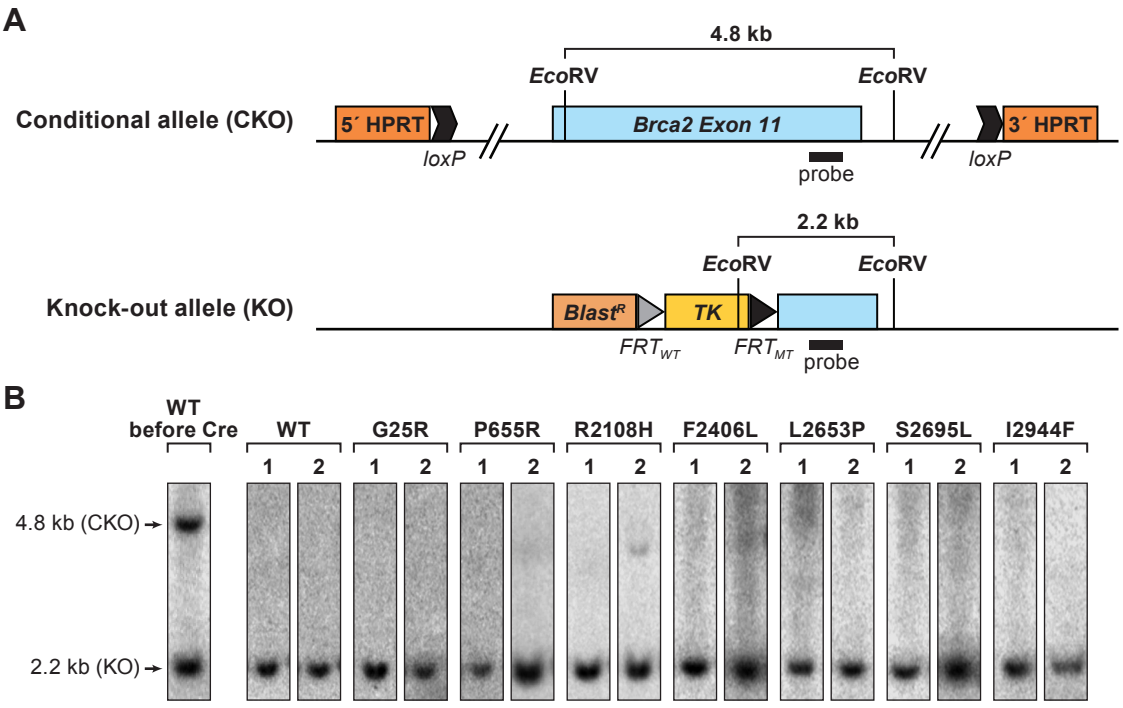
SUPPLEMENTARY FIGURES

Supplementary Figure S1: Genotyping of HAT^r colonies obtained after Cre expression in the mouse ES cells expressing different variants of BRCA2. (A) Scheme showing the targeting of *Brca2* exon11. *Eco*RV was used to digest the genomic DNA. Position of the probe is marked by thick line. (B) Southern blot analysis of HAT^r ES cell colonies. Upper band corresponds to the conditional allele (CKO) and lower band corresponds mutant allele (KO). Sizes of the band are marked on the right. The clones used for further analysis are shown. "WT before Cre" is a control ES cell line showing the presence of the conditional allele.

Supplemental Figure 1

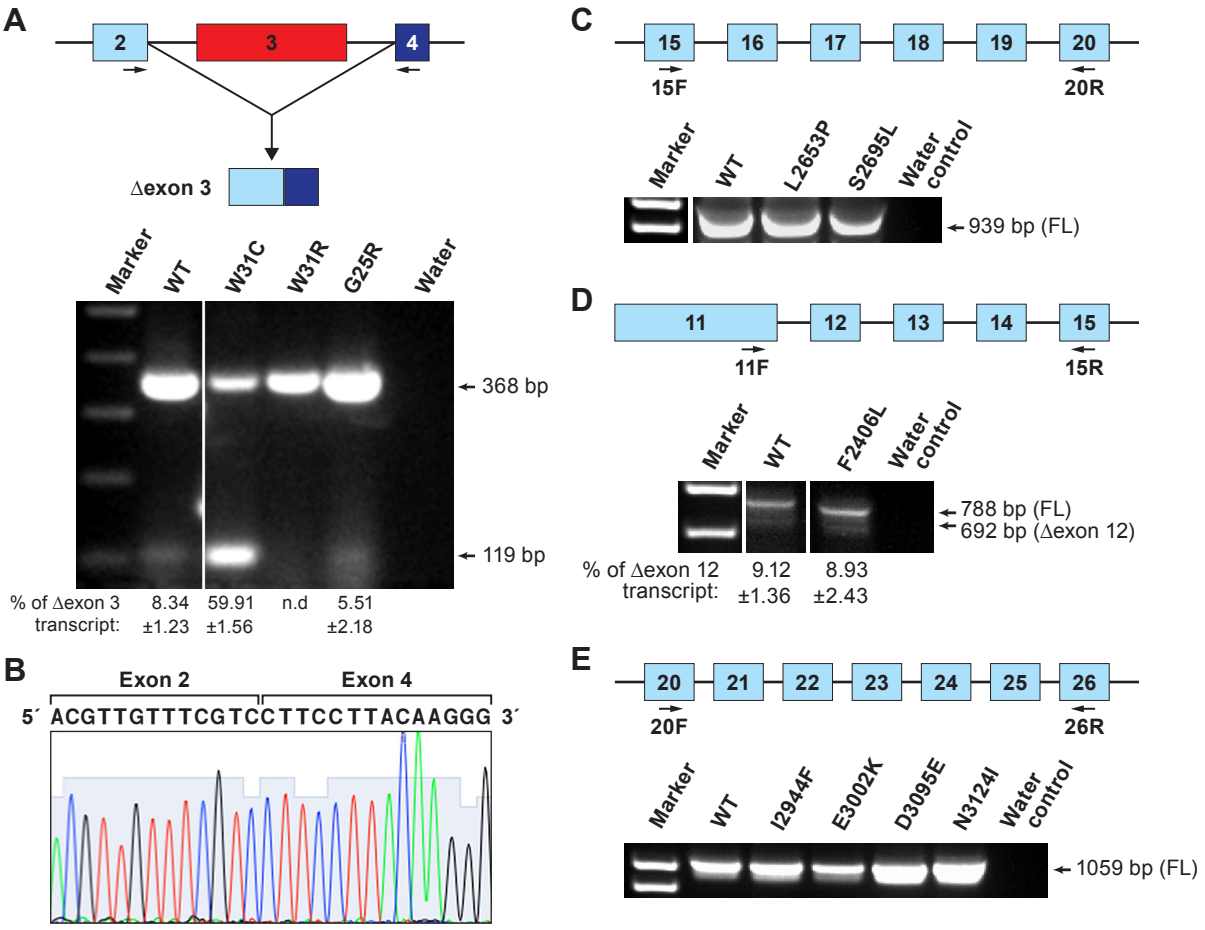
Biswas et al.



Supplementary Figure S2: Splicing pattern of the ES cells expressing different BRCA2 variants. (A) RT-PCR analysis of the ES cells expressing c.321G>T (p.W31C), c.319T>C (p.W31R) and c.301G>A (p.G25R), variants. FL: Full length transcript, Δexon3: transcript with deletion of exon 3. Sizes of the band are marked on right side. Top panel shows the genomic region used for RT-PCR analysis. Arrows mark location of primers. Sequences of the oligonucleotides are listed in Supplementary Table 1 (B) Chromatogram showing the deletion of exon 3. (C-E) RT-PCR analysis of ES cells expressing different variants. (C) c.8186T>C (p.L2653P), c.8312C>T (p.S2695L), (D) c.7446T>G (p.F2406L) and c.7444-7446 TTT>GAC (p.F2406D), (E) c.9058A>T (p.I2944F), c.9232G>A (p.E3002K), c.9513C>G (p.D3095E) and c.9599A>T (p.N3124I). Top panel in each case of C-E shows the genomic region subjected to RT-PCR analysis. Arrows represent the primers used for RT-PCR and the primer sequences are listed in table S2. FL: Full length transcript, Δexon12: transcript with deletion of exon 12 [this is a natural, alternatively spliced form of BRCA2 (Li, et al., 2009)]. Numbers in the boxes indicate the exon number. Ratios of exon inclusion/exclusion were quantified using ImageQuant TLv2005 software (Amersham Biosciences).

Supplemental Figure 2

Biswas et al.

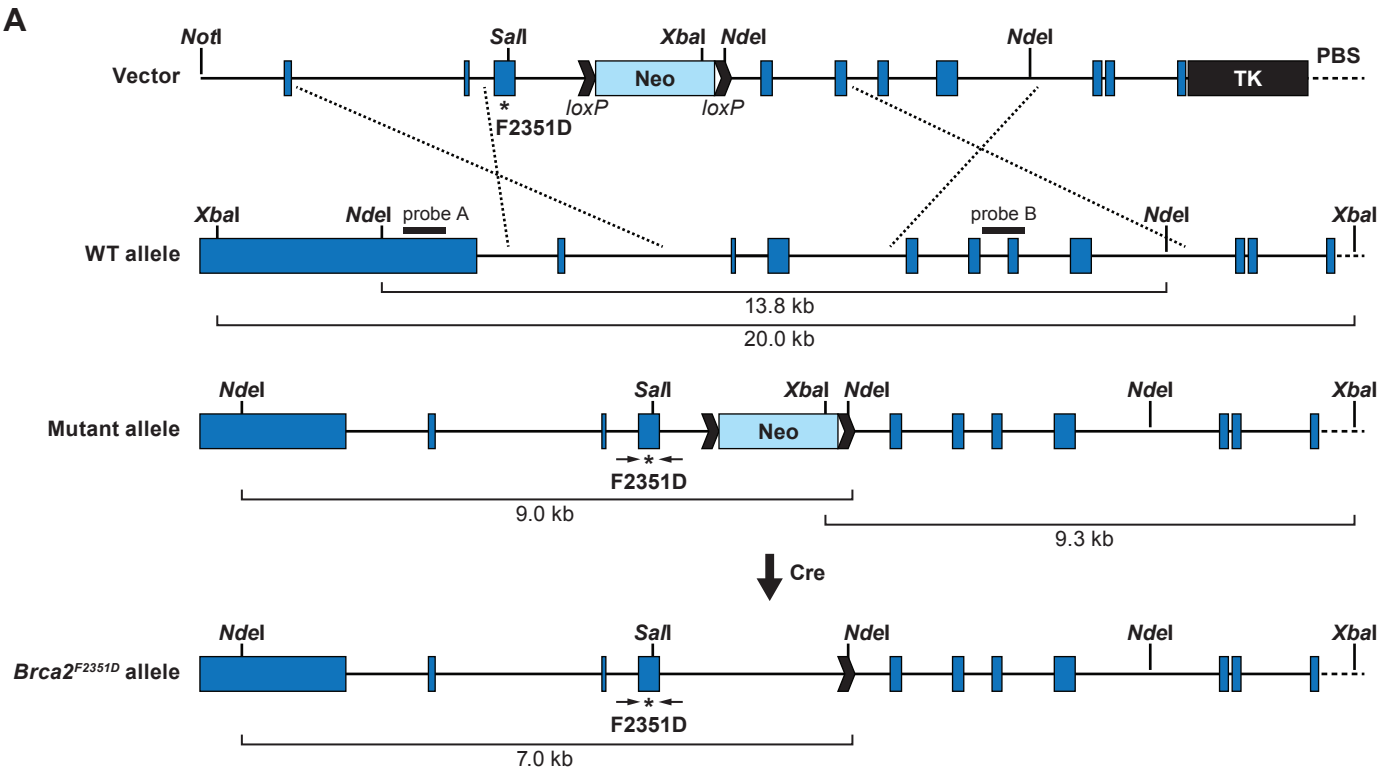


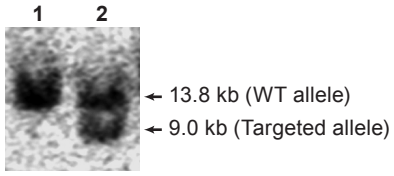
Supplementary Figure S3: Generation of *Brca2*^{F2351D} allele (A) Targeting vector for generating *Brca2*^{F2351D} allele. Filled arrow represents the *loxP* sites inserted into the intron 14. Filled boxes represent exons and the thin lines represent introns. A *Thymidine kinase* (*TK*) gene was used for negative selection during targeting into ES cells. *Not*I site was used to linearize the vector. Asterisk marks the position of F2351D residue and this change generates *Sal*I site. Probes used are marked by thick lines and the arrows represent the primers used to amplify for genotyping. (B) Southern blot analysis of *Nde*I digested DNA to identify correctly targeted clones. Probe A (696 bp) was used to detect the band. Probe A was generated by PCR using the primers *Brca2*-Probe-1 (5′-AACAAAAGAGATAGTCACAG-3′) and *Brca2*-probe-2 (5′-GATACGTCTTCAGGTATAGG-3′) using the BAC421 as a template. (C) Southern blot analysis of *Xba*I digested DNA using the probe B (685 bp). Probe B was generated by amplifying a fragment using the primers *Brca2*-probe-3 (5′-CTCATTGTAGCACCACAAGC-3′) and *Brca2*-probe-4 (5′-CTCTGCCTCCCCTAACCAGC-3′) using BAC421 as template DNA. (D) Agarose gel electrophoresis of *Sal*I restriction digested product of the PCR amplified product using the primers Mouse-F2351DC2 (5'-CGTCAAAATGTTACATAGTACC-3') and Mouse-F2351DC3 (5'-GCACGCACACACACAGTACG- 3') to identify the *Brca2*^{F2351D} allele. Sizes of the band are marked on the right side. (E) Chromatogram showing the sequence of the PCR products using the primers Mouse-F2351DC2 and Mouse-F2351DC3 from correctly targeted clones. (F) Agarose gel electrophoresis of *Sal*I digestion of PCR amplified product using the primers Mouse-F2351DC2 and Mouse-F2351DC3 for genotyping the mouse having *Brca2*^{F2351D} allele. PCR product from homozygous mice

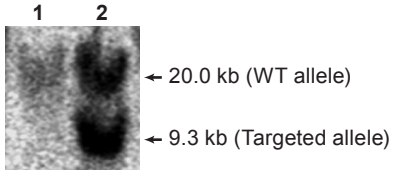
does not show any undigested band (484 bp). Size of the bands are marked on the right side.

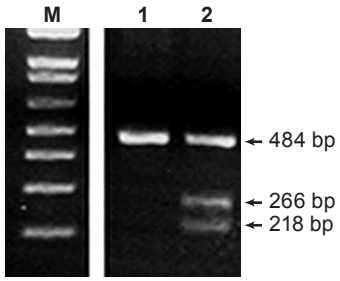
Supplemental Figure 3

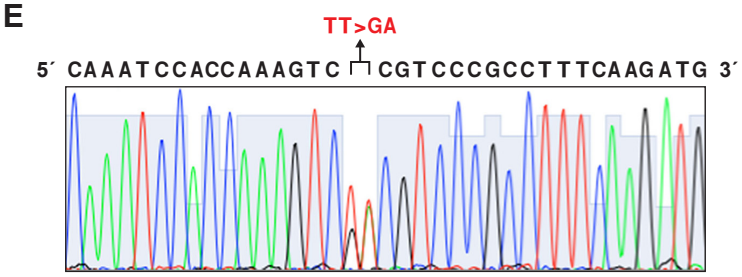
Biswas et al.

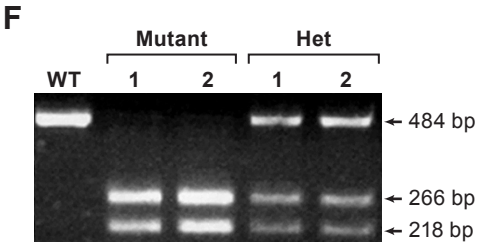












Supplementary Figure S4: Effect of BRCA2^{F2351D} on fertility (A) Histology of the 4 week-old testes and 3 week-old ovaries from WT and homozygous mutant mouse. (B) DMC1 foci in WT and mutant spermatocyte chromosome spreads. Zygotene stage nuclei are shown with SYCP3 staining in red and the DMC1 foci in green. (C) Quantification of DMC1 foci at the zygotene stage; average counts for 10 nuclei are shown. M/M represents the homozygous *Brca2*^{F2351D} mutants.

Supplemental Figure 4

Biswas et al.