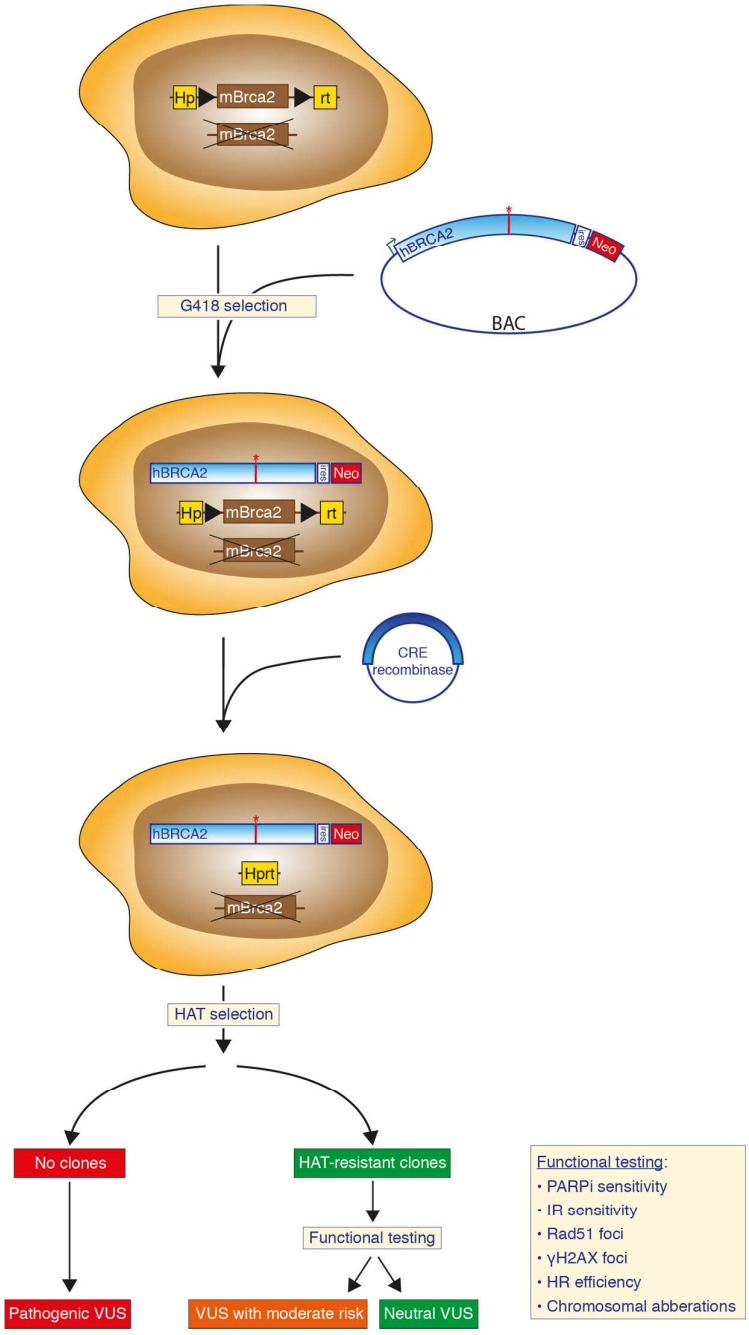
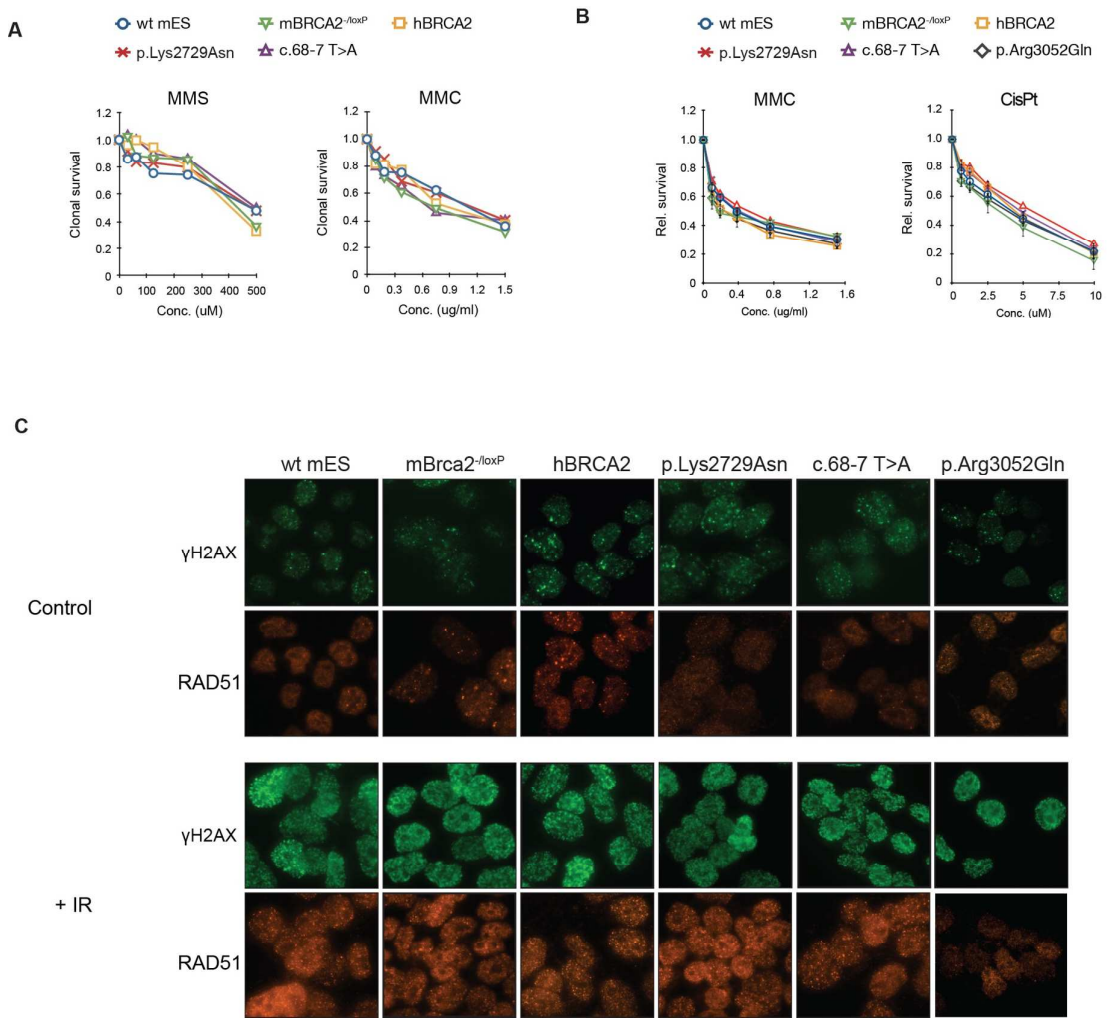


Supplementary Figure 1: Outline for functional testing of VUS by complementation of *BRCA2*-deficient mES cells. m*Brca2*$^{-/loxP}$ conditional knock out mES cells were transfected with a *hBRCA2* BAC containing a specific VUS. The IRES Neomycin selection cassette greatly increases the frequency of mES clones that contain the complete *hBRCA2* gene. The conditional *mBrca2* gene is removed upon transient expression of the Cre recombinase, resulting in functional restoration of an *HPRT* minigene and subsequent HAT resistance. Transfection of *hBRCA2* variants that can complement the lethal phenotype of *mBrca2* deficient mES cells will result in large numbers of HAT resistant clones. VUS that do not give complementation are considered pathogenic. HAT resistant mES cell clones are subjected to various functional tests to determine whether a VUS does not affect *hBRCA2* function and can be considered neutral or whether *hBRCA2* functionality is partially impaired.



Supplementary Figure 2: Functional analysis of hBRCA2 variants. (A) Sensitivity of mES cells expressing the *hBRCA2* variants p.Lys2729Asn and c.68 7T>A or wild type *hBRCA* for MMS and MMC. Wild type mES cells and *mBrca2*$^{-/loxP}$ conditional knockout mES cells were used as control. Sensitivity was determined in a clonal survival assay. (B) Sensitivity of mES cells expressing the *hBRCA2* variants p.Lys2729Asn, c.68 7T>A, p.Arg3052Gln or wild type *hBRCA* for MMC and Cisplatin. Wild type mES cells and *mBrca2*$^{-/loxP}$ conditional knockout mES cells were used as control. Sensitivity was determined in a cell count assay. (C) RAD51 foci formation in response to 10Gy ionizing radiation in mES cells expressing the non pathogenic *hBRCA2* variants p.Lys2729Asn and c.68 7T>A, the intermediate variant p.Arg3052Gln and wild type *hBRCA2*. Phosphorylation of histone H2AX was used to confirm the induction of double strand breaks ionizing radiationIR.

Supporting Table 1: primer sequences for hBRCA2 variant generation and sequence conformation.

Primername	Purpose	Primer sequence 5'-3'
Integration of IRES-Neo		
hBrca2-Cterm-IRES-F	IRES-Neo with hBRCA2 homology arms	AATGTGAGAAAAATAAGCAGGACACAATTACAACTAAAAAATATACTAATGATCTAGAGTCGAGTTAATTAAG
hBrca2-Cterm-Neo-R	IRES-Neo with hBRCA2 homology arms	TAAACTGGAAAGGTTAAGCGTCAATAATTTATTGTCGCCTTTGCAAATGCTCAGAAGAACTCGTCAAGAAG
hBrca2-CGFP-F2	Check proper BAC integration	GTCAGTGAATCCACTAGGAC
hBrca2-CGFP-R2	Check proper BAC integration	TGCCCGATACACAAACGCTG
Removal of loxP sites		
LoxP1-rpsl-amp-F	rpsL-Amp with hBRCA2 homology arms	CTTATCGATGATAAGCTGTCAAACATGAGAATTGATCCGGAACCCTTAATCGGTTTTATGGACAGCAAGCGAAC
LoxP1-rpsl-amp-R	rpsL-Amp with hBRCA2 homology arms	CCGATGCAAGTGTGTCGCTGTCGACGGTGACCCTATAGTCGAGGGACCTATTACCAATGCTTAATCAGTGAG
LoxP1-del-F	dsDNA fragment for Red/ET recombineering	CTTATCGATGATAAGCTGTCAAACATGAGAATTGATCCGGAACCCTTAATTAGGTCCCTC
LoxP1-del-R	dsDNA fragment for Red/ET recombineering	CCGATGCAAGTGTGTCGCTGTCGACGGTGACCCTATAGTCGAGGGACCTAATTAAGGGTT
LoxP1-check-F	Check proper BAC integration	CGCATAGAAATTGCATCAACGC
LoxP1-Check-R	Check proper BAC integration	CAAGGGCAAGTATTGACATGTC
LoxP2-rpsl-amp-F	rpsL-Amp with hBRCA2 homology arms	CGTAAGCGGGGCACATTTCATTACCTCTTTCTCCGCACCCGACATAGATACGGTTTTATGGACAGCAAGCGAAC
LoxP2-rpsl-amp-R	rpsL-Amp with hBRCA2 homology arms	CGGGGCATGACTATTGGCGCGCCGGATCGATCCTTAATTAAGTCTACTAGTTACCAATGCTTAATCAGTGAG
LoxP2-del-F	dsDNA fragment for Red/ET recombineering	CGTAAGCGGGGCACATTTCATTACCTCTTTCTCCGCACCCGACATAGATACTAGTAGACT
LoxP2-del-R	dsDNA fragment for Red/ET recombineering	CGGGGCATGACTATTGGCGCGCCGGATCGATCCTTAATTAAGTCTACTAGTATCTATGTC
LoxP2-check-F	Check proper BAC integration	CCTTAAACGCCTGGTTGCTAC
LoxP2-Check-R	Check proper BAC integration	CCTATAGTGAGTCGTATTAGC
hBRCA2 variants		
Brca2-7878-rpsl-F	rpsL-Amp with hBRCA2 homology arms	GTGGATCCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGCGGTTTTATGGACAGCAAGCGAAC
Brca2-7879-amp-R	rpsL-Amp with hBRCA2 homology arms	AGCAAATTCCTTAGGAAAGGCACATTCCATAGCTGCCAGTTTCCATATGATTACCAATGCTTAATCAGTGAGGC
Brca2-7878-G>C-F	dsDNA fragment for Red/ET recombineering	GTGGATCCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGCATCATATGGAA
Brca2-7878-G>C-R	dsDNA fragment for Red/ET recombineering	AGCAAATTCCTTAGGAAAGGCACATTCCATAGCTGCCAGTTTCCATATGATGCATCTATAGT
Brca2-7879-A>T-F	dsDNA fragment for Red/ET recombineering	GTGGATCCAAAGCTTATTTCTAGAATTTGGGTTTATAATCACTATAGATGGTTCATATGGAA
Brca2-7879-A>T-R	dsDNA fragment for Red/ET recombineering	AGCAAATTCCTTAGGAAAGGCACATTCCATAGCTGCCAGTTTCCATATGAACCATCTATAGT
Brca2-7878-7879-F2	Check proper BAC integration	GCCACCATGCTCAGCAATGA
Brca2-7878-7879-R2	Check proper BAC integration	CACTGACAACTGGCTTGTGC
Brca2-8187-rpsl-F	rpsL-Amp with hBRCA2 homology arms	ACCCAAAAAGTGGCCATTATTGAACTTACAGATGGGTGGTATGCTGTTAACGGTTTTATGGACAGCAAGCGAAC
Brca2-8187-amp-R	rpsL-Amp with hBRCA2 homology arms	GTCAGTCTGCCATTCTTTAAGACAGCTAAGAGGGGAGGATCTAACTGGGCTTACCAATGCTTAATCAGTGAGGC
Brca2-8187-G>T-F	dsDNA fragment for Red/ET recombineering	ACCCAAAAAGTGGCCATTATTGAACTTACAGATGGGTGGTATGCTGTTAATGCCCAGTTAG
Brca2-8187-G>T-R	dsDNA fragment for Red/ET recombineering	GTCAGTCTGCCATTCTTTAAGACAGCTAAGAGGGGAGGATCTAACTGGGCATTAACAGCAT
Brca2-8187-F2	Check proper BAC integration	GATACGGAAATTGATAGAAGCAG
Brca2-8187-R2	Check proper BAC integration	GCTTCAAGAGGTGTACAGGC
Brca2-9154-Rpsl-F	rpsL-Amp with hBRCA2 homology arms	TTTGTTTTCTGTAGGTTTCAGATGAAATTTTATTTCAGATTTACCAGCCACGGTTTTATGGACAGCAAGCGAAC
Brca2-9155-Amp-R	rpsL-Amp with hBRCA2 homology arms	AAGATGGCTGAAAGTCTGGATCTAAAAATTTGCTGAAGTGAAGGGGCTCCCCAATGCTTAATCAGTGAGGCAC
Brca2-9154-C>T-F	dsDNA fragment for Red/ET recombineering	TTTGTTTTCTGTAGGTTTCAGATGAAATTTTATTTCAGATTTACCAGCCATGGGAGCCCCTT
Brca2-9154-C>T-R	dsDNA fragment for Red/ET recombineering	AAGATGGCTGAAAGTCTGGATCTAAAAATTTGCTGAAGTGAAGGGGCTCCCATGGCTGGTAA
Brca2-9155-G>A-F	dsDNA fragment for Red/ET recombineering	TTTGTTTTCTGTAGGTTTCAGATGAAATTTTATTTCAGATTTACCAGCCACAGGAGCCCCTT
Brca2-9155-G>A-R	dsDNA fragment for Red/ET recombineering	AAGATGGCTGAAAGTCTGGATCTAAAAATTTGCTGAAGTGAAGGGGCTCCTGTGGCTGGTAA
Brca2-9154-9155-wt-F	dsDNA fragment for Red/ET recombineering	TTTGTTTTCTGTAGGTTTCAGATGAAATTTTATTTCAGATTTACCAGCCACGGGAGCCCCTT
Brca2-9154-9155-wt-R	dsDNA fragment for Red/ET recombineering	AAGATGGCTGAAAGTCTGGATCTAAAAATTTGCTGAAGTGAAGGGGCTCCCGTGGCTGGTAA
Brca2-9154/9155-5'-F2	Check proper BAC integration	GAGCTAACATACAGTTAGCAG
Brca2-9154/9155-3'-R2	Check proper BAC integration	CACCAATATAACATCACAATGGG
Brca2-ivs2-7-rpsl-F	rpsL-Amp with hBRCA2 homology arms	GGTCACAAATTTGTCTGTCACTGGTTAAAACTAAGGTGGGATTTTTTTTTCGGTTTTATGGACAGCAAGCGAAC
Brca2-ivs2-7-amp-R	rpsL-Amp with hBRCA2 homology arms	TGAAGAAAGTTCTTCAAACCAATTAAGACTTATTGGTCCTAAATCTATTTTTACCAATGCTTAATCAGTGAGGC
Brca2-ivs2-7-T>A-F	dsDNA fragment for Red/ET recombineering	GGTCACAAATTTGTCTGTCACTGGTTAAAACTAAGGTGGGATTTTTTTTTAAAATAGATTT
Brca2-ivs2-7-T>A-R	dsDNA fragment for Red/ET recombineering	TGAAGAAAGTTCTTCAAACCAATTAAGACTTATTGGTCCTAAATCTATTTTAAAAAAAAT
Brca2-ivs2-7-F2	Check proper BAC integration	CTATAGATTCGCAAGAGAATGG
Brca2-ivs2-7-R2	Check proper BAC integration	CAGAGTCAGCCCTTGCTCTT
Brca2-ivs15+2-rpsl-F	rpsL-Amp with hBRCA2 homology arms	GAAAGCAGCAGTAGGAGGCCAAGTTCCCTCTGCGTGTTCTCATAAACAGGCGGTTTTATGGACAGCAAGCGAAC
Brca2-ivs15+2-amp-R	rpsL-Amp with hBRCA2 homology arms	ATAAAATTACACTCTGTCATAAAAGCCATCAGTATTGTAGACAAACACATTTACCAATGCTTAATCAGTGAGGC
Brca2-ivs15+2-T>G-F	dsDNA fragment for Red/ET recombineering	GAAAGCAGCAGTAGGAGGCCAAGTTCCCTCTGCGTGTTCTCATAAACAGGGATGTGTTTGT
Brca2-ivs15+2-T>G-R	dsDNA fragment for Red/ET recombineering	ATAAAATTACACTCTGTCATAAAAGCCATCAGTATTGTAGACAAACACATCCCTGTTTATG
Brca2-ivs15+2-F2	Check proper BAC integration	GATTACAGGCGTGAGCCACT
Brca2-ivs15+2-R2	Check proper BAC integration	TGCCACTGCAGGCTAATTAG
Brca2-ivs21+5-rpsl-F	rpsL-Amp with hBRCA2 homology arms	GCTTTATGAAGCAGTGAAGAATGCAGCAGACCCAGCTTACCTTGAGGTGACGGTTTTATGGACAGCAAGCGAAC
Brca2-ivs21+5-amp-R	rpsL-Amp with hBRCA2 homology arms	GCTTCTCACCTTGAATAATCATCAAGCCTCATTATATGTCCTCTTACTCTTTACCAATGCTTAATCAGTGAGGC
Brca2-ivs21+5-G>A-F	dsDNA fragment for Red/ET recombineering	GCTTTATGAAGCAGTGAAGAATGCAGCAGACCCAGCTTACCTTGAGGTGAAAGAGTAAGAG
Brca2-ivs21+5-G>A-R	dsDNA fragment for Red/ET recombineering	GCTTCTCACCTTGAATAATCATCAAGCCTCATTATATGTCCTCTTACTCTTTCACCTCAAG
Brca2-ivs21+5-F2	Check proper BAC integration	GCACTCCCAGATTGGGTGAC
Brca2-ivs21+5-R2	Check proper BAC integration	CATACCACCACACTCGTCTG
cDNA analysis		
RBR2EX2F2M13	Brca2-ivs2-7: Exon2	TGTAAAACGACGGCCAGTTTTTAAGACACGCTGCAACAAAG
RBR2EX10R-M13	Brca2-ivs2-7: Exon10	CAGGAAACAGCTATGACCCAATGTGGTCTTTGCAGCTATTTACT
RBR2EX14F	Brca2-ivs15+2: Exon14	ACAACTAAGGAACGTCAAG
RBR2EX16R	Brca2-ivs15+2: Exon16	TATGCAATGTTTAGAAACG
RBRCA2EX19F	Brca2-ivs21+5: Exon19	ACTTGGATTCTTTCCTGAC
RBRCA2EX23R	Brca2-ivs21+5: Exon23	TAAATCTGATGATGGACGC
RBR2EX22F-M13	Expression level hBRCA2	TGTAAAACGACGGCCAGTTGTTGAATGATAAGAAACAAGCTCA
RBR2EX27R	Expression level hBRCA2	CTCTTTCTCCCCTTTACAAGACTTT
Sus3	mouse Aprt gene	GTCTTCCCCGACTTCCCAAT
Sus4	mouse Aprt gene	GCCAGGAGGTCATCCACAAT

Supplementary Table 2. Description of *BRCA2* variants and available data

						Embryonic stem cell based assay							
Exon	**Nucleotide**	**Amino acid**	**IARC Classification**[*1]	**RNA splicing**	**HDR assay**	**Cell Viability**	**Sensitivity to DNA damaging agents**	**HR assay**	**RAD51 foci**	**Chromosomal abberations**	**Centrosome amplification**	**Functional classification**	**References**
2	c.68-7T>A	p.?	Not available	Wildtype and Δ3 transcript from variant allele	ND	ND	ND	ND	ND	ND	ND	Unlikely to affect function	12; 14
15	c.7617+2T>G	p.?	Not available	Skip exon 15	ND	ND	ND	ND	ND	ND	ND	Impairs protein function	14
17	c.7878G>C	p.Trp2626Cys	Class 5	ND	Deficient	Reduced	Yes	Deficient	Absent	Enhanced	ND	Impairs protein function	2; 3; 9
17	c.7879A>T	p.Ile2627Phe	Class 5	ND	Deficient	ND	ND	ND	ND	ND	Increase	Impairs protein function	3; 5; 9
18	c.8165C>G	p.Thr2722Arg	Class 5	Wiltype and Δ18 transcript from variant allele	Deficient	No	NA	NA	NA	NA	Increase	Impairs protein function	3; 4; 5; 8; 9
18	c.8167G>C	p.Asp2723His	Class 5	ND	Deficient	No	NA	NA	NA	NA	Increase	Impairs protein function	5; 6; 8; 9; 16
18	c.8168A>G	p.Asp2723Gly	Class 5	Wildtype transcript and partial deletion of exon 18 transcript from variant allele	Deficient	ND	ND	ND	ND	ND	ND	Impairs protein function	3; 5; 9; 11; 13; 15
18	c.8187G>T	p.Lys2729Asn	Class 1	ND	Normal	Yes	No	Normal	Normal	ND	Wildtype level	Does not affect function	2; 3; 5; 9
18	c.8243G>A	p.Gly2748Asp	Class 5	ND	Deficient	ND	ND	ND	ND	ND	Increase	Impairs protein function	3; 5; 9
18	c.8308G>A	p.Ala2770Thr	Class 2	ND	ND	ND	ND	ND	ND	ND	ND	Does not affect function	9
21	c.8754+5G>A	p.?	Not available	Retention 46 nt from intron 21	ND	ND	ND	ND	ND	ND	ND	Impairs protein function	13
24	c.9154C>T	p.Arg3052Trp	Class 5	ND	Deficient	No	NA	NA	NA	NA	Wildtype level	Impairs protein function	5; 7; 8; 10; 15
24	c.9155G>A	p.Arg3052Gln	Class 2	ND	ND	Yes	Intermediate	ND	ND	ND	ND	Probably affects function	3; 7; 8; 9; 10;

This table describes the results of previous functional studies.
Nucleotide numbering reflects HGVS nomenclature where cDNA numbering +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (BRCA2 NM_000059.3). The initiation codon is codon 1. nt: nucleotides.
[*1] IARC classification (Lindor et al., 2012); Class 1 non-pathogenic; Class 2 likely non-pathogenic; Class 5 pathogenic
ND: Not determined
NA; not applicable since no clones were formed that could be tested
All variants are reported in public database (http://chromium.liacs.nl/LOVD2/cancer/home.php)

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