Supplementary information

Materials and Methods Table S1-S7, Figure legends S1-S6

Materials and Methods

Table S1: Introduction of point mutations in EGFPMBP-BRCA2, 2xMBP-BRCA2$_{1-250}$ and GST-BRCA2$_{190-283}$ constructs

Mutation	Oligo name	Sequence (5'-3')
S193A	Fw : oAC543	CCC ACC CTT AGT TCT GCT GTG CTC ATA GTC
	Rv : oAC544	GAC TAT GAG CAC AGC AGA ACT AAG GGT GGG
M192T	Fw : oAC283	GTGGATCCTGATACGTCTTGGTCAAGTTC
	Rv : oAC284	GA ACT TGA CCA AGA CGT ATC AGG ATC CAC
S196N	Fw : oAC026	CCTGATATGTCTTGGTCAAATTCTTTAGCTACACCACC
	Rv : oAC027	GGTGGTGTAGCTAAAGAATTTGACCAAGACATATCAGG
S206C	Fw : oAC028	CCACCCACCCTTAGTTGTACTGTGCTCATAGTCAG
	Rv : oAC029	CTGACTATGAGCACAGTACAACTAAGGGTGGGTGG
T200K	Fw : oAC285	CAA GTT CTT TAG CTA AAC CAC CCA CCC TTA G
	Rv : oAC286	CTA AGG GTG GGT GGT TTA GCT AAA GAA CTT G
T207A	Fw : oAC545	GGA TCC TGA TAT GGC TTG GTC AAG TTC TTT AGC
	Rv : oAC546	GCT AAA GAA CTT GAC CAA GCC ATA TCA GGA TCC

Table S2: Sequencing primers

Construct	Oligo name	Binding site	Sequence (5'-3')
GFPMBP-BRCA2, GST-BRCA2$_{190-283}$	Rv : oAC131	aa 273 BRCA2	TTAGTTCGACTTATCCAATGTGGTCTTT
2xMBP-BRCA2$_{1-250}$	Fw : oAC149	aa 1-6 BRCA2	TTATTTGCTAGCCCTATTGGATCCAAAGAG

Table S3: Primers used to subclone $BRCA2_{192-283}$ into the pGEX-6P-1 vector

Construct	**Oligo name**	**Sequence (5'-3')**
Amplifying $BRCA2_{192-283}$	Fw: oAC130	TTAGGATCCATGTCTTGGTCAAGTTCT
	Rv: oAC131	TTAGTTCGACTTATCCAATGTGGTCTTT
L191D in NTshort	Fw: oAC593	CAG GGG CCC GAT ATG TCT TGG TCA AGT TCT
	Rv: oAC594	AGA ACT TGA CCA AGA CAT ATC GGG CCC CTG

Table S4: Primers used to subclone *PLK1* cDNA into pFastBac HT

Primer name	**Sequence (5'-3')**
GA_pFBtev_R	GCCCTGAAAATACAGGTTTTCGGTCGTTGGGAT
GA_pFB_UTR_F	TTGTCGAGAAGTACTAGAGGATCATAATCA
GA_hPLK_F	ATCCCAACGACCGAAAACCTGTATTTTCAGGGCATGAGTGCTGCAG TGACTGCA
GA_hPLK_R	TGATTATGATCCTCTAGTACTTCTCGACAATTAGGAGGCCTTGAGAC GGTT

Table S5: Primers used to clone $PLK1_{PBD}$ (aa 326-603) into pT7-His6-SUMO

Primer name	**Sequence (5'-3')**
GA_PLKPDBwt_F	ATTGAGGCTCACCGCGAACAGATTGGTGGCTCGATTGCTCCCA GCAGCCT
GA_PLKPDBwt_R	TTCCTTTCGGGCTTTGTTAGCAGCCGGTCATTAGGAGGCCTTGA GACGGT

Table S6: Peptide sequences for Isothermal Titration Calorimetry (ITC)

Peptide	Sequence
pS197	DMSWSS{pS}LAT
T207	WSSSLATPPTLSSTVLI
pT207	WSSSLATPPTLSS{pT}VLI
T207A	WSSSLATPPTLSSAVLI
CpT207	WSSSLATPPTLSC{pT}VLI

Table S7: Primers for amplifying BRCA2 (aa 1-267) from genomic DNA,

Primer name	Sequence (5'-3')
Fw : OAC035	GGTCGTCAGACTGTCGATGAAGCC
Rv : OAC056	CAAAGAGAAGCTGCAAGTCATGGATTTGAAAAAACATCAGGG

Figure Legends

Figure S1. Related to Figure 1. **PLK1 phosphorylation kinetics of BRCA2$_{190-283}$ and conservation of PLK1 phosphosites**

(a) Phosphorylation kinetics resulting from a duplicate experiment performed with a different PLK1 kinase aliquot on ^{15}N labelled BRCA2$_{190-283}$ (283K, 600 MHz). Error bars correspond to SD (n=2). **(b)** Alignment of the region 190-283 of BRCA2 in 30 different species. Amino acids conserved in more than 66 % of the species are highlighted with coloured background. The percentage of identity to human BRCA2 sequence is added after the species name. Arrows show the amino acids identified as phosphorylated by PLK1 in the NMR experiments. **(c)** Bar graph showing the evolutionary conservation of the amino acids phosphorylated by PLK1 detected by NMR.

Figure S2. Related to Figure 2. **PLK1 phosphorylation kinetics of WT vs mutated forms of BRCA2$_{190-283}$**

Phosphorylation kinetics resulting from a duplicate experiment performed with a different PLK1 kinase aliquot on ^{15}N labelled BRCA2$_{190-283}$ (283K, 600 MHz). Comparisons of the WT kinetics with the phosphorylation kinetics of **(a)** T207A and **(b)** T200K are displayed.

Figure S3. Related to Figure 4. **Isothermal Titration Calorimetry (ITC) thermogram showing binding of PLK1$_{PBD}$ to the fragment BRCA2$_{190-283}$ or a 10 aa BRCA2 peptide containing pS197**

Thermogram showing the binding affinity of PLK1$_{PBD}$ to **(a)** phosphorylated or the **(b)** non-phosphorylated BRCA2$_{190-283}$ fragment. **(c)** Thermogram showing the binding affinity of PLK1$_{PBD}$ to a 10 aa BRCA2 peptide comprising pS197.

Figure S4. Related to Figures 5-7**. BRCA2 protein levels of DLD1$^{-/-}$ stable clones expressing the variants as compared to the WT expressing cells utilized in this study. Cell cycle profile of the DLD1$^{-/-}$ stable clones in nocodazole versus untreated conditions.**

(a) Protein levels of EGFP-MBP-BRCA2 WT or the VUS (S206C and T207A) in total protein extracts from respective DLD1$^{-/-}$ stable clones analyzed by western blot using anti-BRCA2 (OP95) and anti-GFP antibodies. **(b, c)** Cell cycle distribution of DLD1$^{-/-}$ stable clones expressing EGFP-MBP-BRCA2 WT or the VUS treated with nocodazole (b) or DMSO (c) as in Figure 5c. Statistical significance of differences was calculated with two-way ANOVA test with Tukey's multiple comparisons test (the

asterisks show differences compared to WT; ns p > 0.05, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

Figure S5. Related to Figure 6**. Protein levels of pT680-BUBR1 in DLD1$^{-/-}$ BRCA2 WT clone upon PLK1 inhibition or phosphatase treatment. PLK1 co-immunoprecipitates with BUBR1 in DLD$^{-/-}$ clones expressing WT BRCA2 or variants S206C and T207A.**

(a) Protein levels of pT680-BUBR1 in DLD1$^{-/-}$ BRCA2 WT stable clone after treatment with PLK1 inhibitors. After 14h culture with media containing nocodazole (100 ng/µl), PLK1 inhibitors (Bi2536 (50 nM) or BTO (50 µM)) were added to the media and the cells were cultured for additional 2h before harvesting and extraction of proteins. The levels of pT680-BUBR1 in total protein lysate was analyzed by western blot. **(b)** Phosphatase (Fast AP phosphatase) treatment of total protein lysate extracted from nocodazole (100 ng/µl) treated DLD1$^{-/-}$ BRCA2 WT stable clone, levels of pT680-BUBR1 was analyzed by western blot. For (a, d and e), stain-free images of the 4-15% SDS-PAGE gel before transfer were used as loading control (cropped images are shown). **(c)** Immunoprecipitation of endogenous BUBR1 in nocodazole (100 ng/ml) treated DLD1$^{-/-}$ BRCA2 WT, S206C and T207A stable clones. Co-immunoprecipitated PLK1 is detected by western blotting.

Figure S6. Purification of PLK1 and PLK1$_{PBD}$ protein

(a) SDS-PAGE showing purified PLK1. Human PLK1 was expressed and purified from sf9 insect cells using Ni-NTA column followed by a second purification step with a cationic exchange Capto S column. The purified protein was loaded on a 4-20% SDS-PAGE (3 µg) and the detected by Coomassie staining. **(b)** SDS-PAGE showing purified PLK1$_{PBD}$. 6His-Sumo-PLK1$_{PBD}$ was expressed and purified from bacteria using a His-TRAP column, the His-tag was cleaved with 6xHis-SUMO Protease and

the cleaved $PLK1_{PBD}$ was further purified using Ni-NTA agarose resin. The purified protein was loaded on a 4-20% SDS-PAGE (1.4 µg) and detected by Coomassie staining. **(c)** *In vitro* kinase assay with the purified PLK1 (0.1 µg) from (a) or PLK1 purchased from Abcam, 0.1 µg PLK1 was used in the kinase reaction with either RAD51 (25 ng) or purified 2xMBP-$BRCA2_{1-250}$ WT (0.5 µg) as substrate in the presence of γ^{32}P-ATP. The samples were resolved by 7.5 % SDS-PAGE and ^{32}P-labeled products were detected by autoradiography.