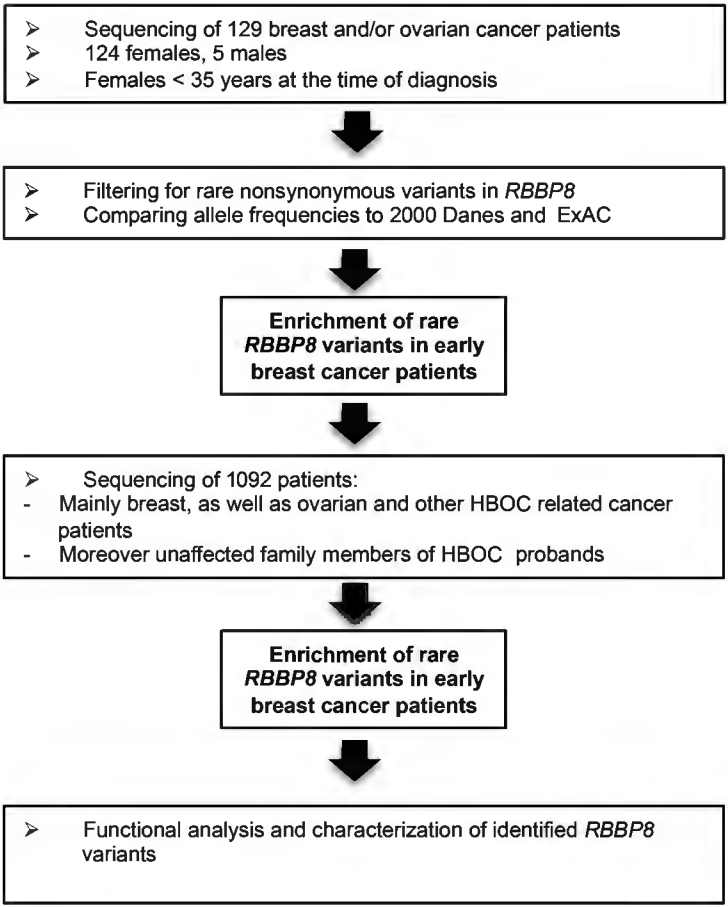
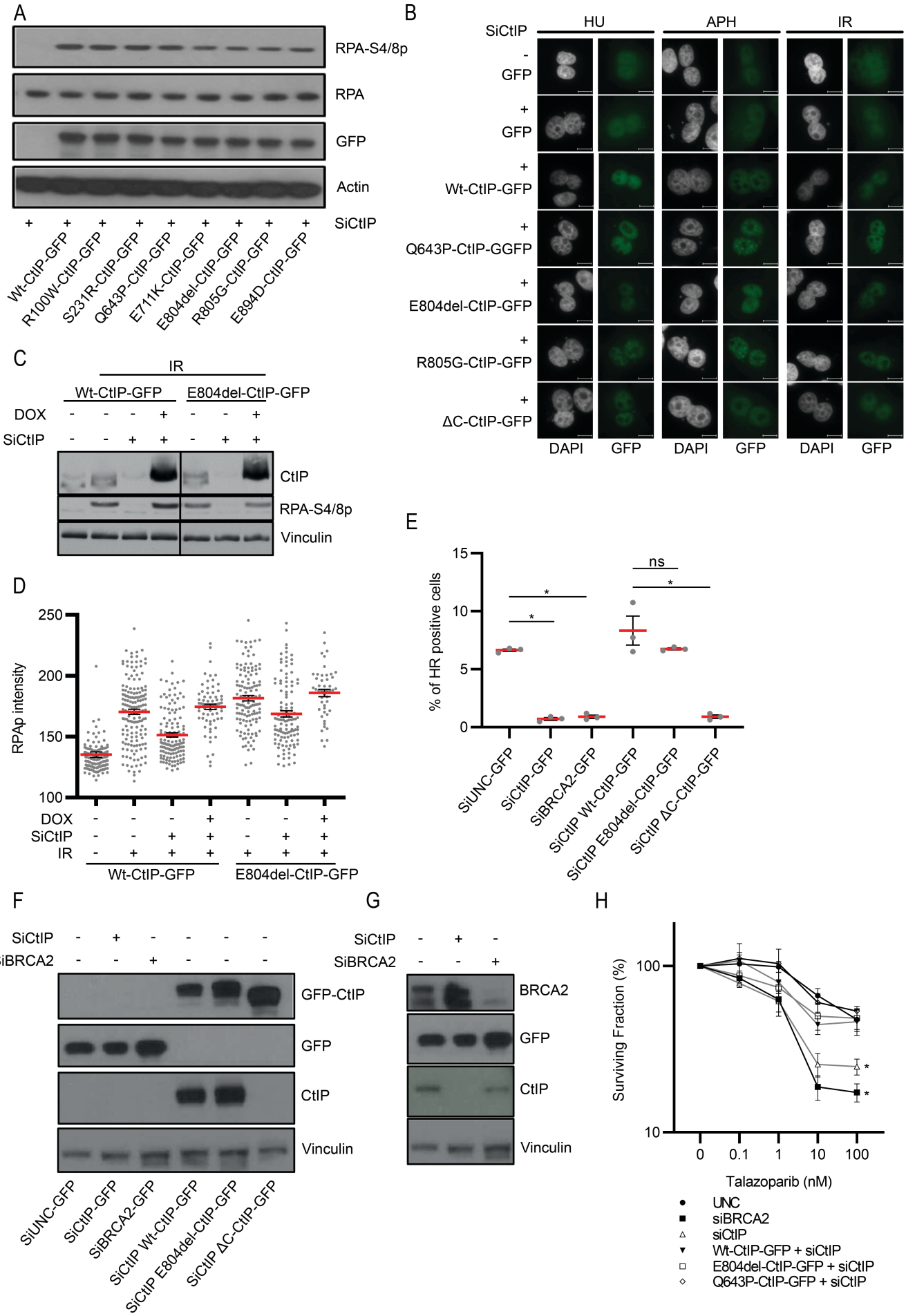
Supplementary Figure 1.



Supplementary Figure 1

Work flow used to screen for identification of rare *RBBP8*-variants.

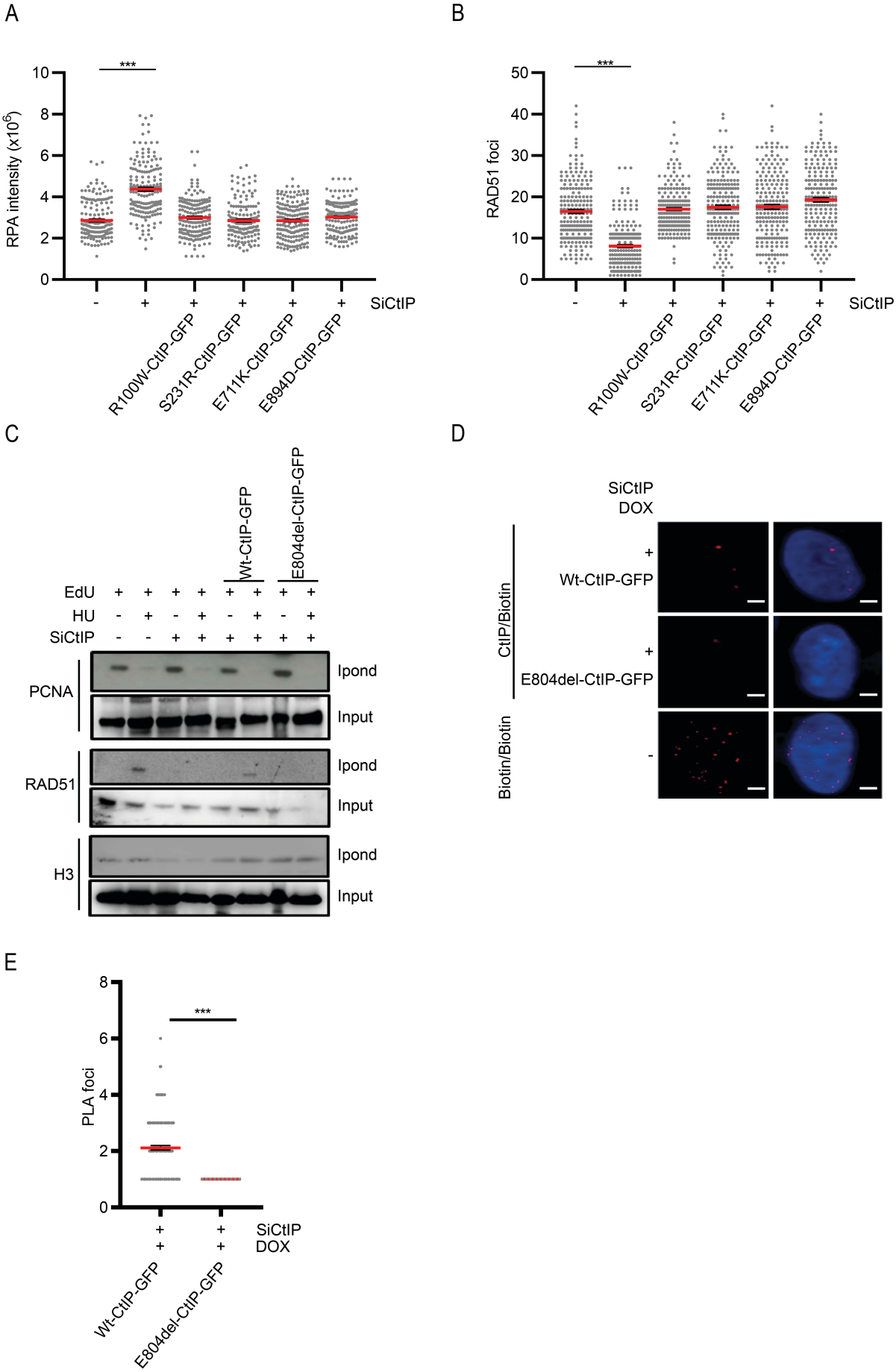
Supplementary Figure 2.



Supplementary Figure 2

(A) MCF7 cells were transfected with the indicated siRNA followed by transfection of Wt or mutant CtIP. Cells were harvested 3 h post-irradiation with 15 Gy and western blots were performed with the indicated antibodies. (B) Representative images displaying micronuclei. MCF7 cells were transfected with the indicated siRNA followed by transfection of Wt or mutant CtIP. Cells were treated with IR, APH or HU and Cytochalasin B. (C) DOX-inducible U-2-OS cell lines were transfected with the indicated siRNA and 24 h later, DOX was added for 24 h, cells were harvested 3 h post-irradiation with 15 Gy. Western blots were performed with the indicated antibodies. Samples were run on the same gel, but were non-contiguous. (D) The relative intensity of pRPA (S4/8) was examined in Wt and E804del expressing cell lines treated as in (C). Cells were imaged with a 20x objective on a Scan^R workstation (Olympus); mean relative pRPA (S4/8) intensity was calculated from background subtracted images using the Scan^R analysis software. Per sample n≥48 nuclei were analyzed. (E) Homologous recombination activity was analyzed in U-2-OS cells transfected with the indicated siRNA. The next day, a reporter sgRNA targeting *LMNA* and a plasmid with homology arms towards Lamin A and mRuby together with empty vector, Wt, or CtIP variants were transfected. After 48 h cells were fixed and mRuby-tagged LMNA was monitored by microscopy. Three independent biological replicates were performed. Holm-corrected multiple testing was performed of ranked data fitted by a linear mixed model. (F-G) Western blot analysis of samples shown in (E). (H) DOX-inducible U-2-OS cell lines were transfected with the indicated siRNA and treated with DMSO and PARPi. 5 days post transfection, cell viability was measured using CellTiter-Glo. Surviving fractions were calculated relative to DMSO-exposed cells for each PARPi concentration. Data represent three independent biological replicates. Holm-corrected multiple testing was performed of Johnson-transformed data fitted by a linear mixed model.

Supplementary Figure 3.

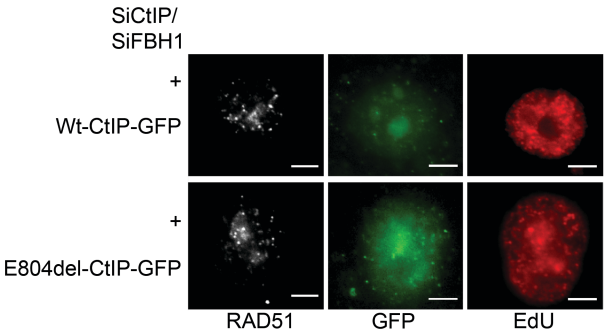


Supplementary Figure 3

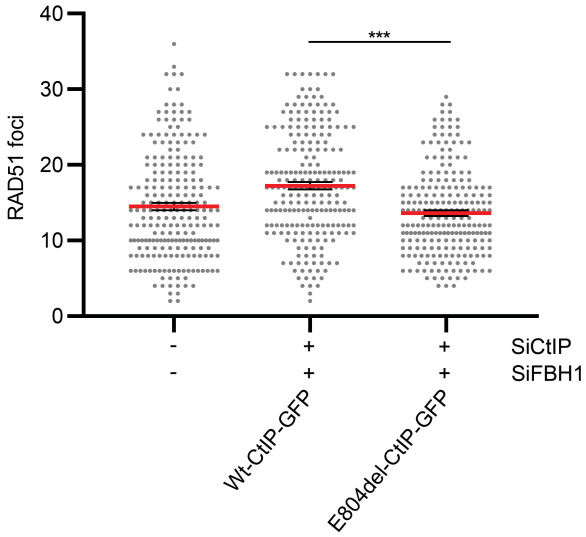
(A) MCF7 cells were transfected with the indicated siRNA and 24 h later, cells were transfected with mutant CtIP variants. Cells were pulsed with EdU and exposed to 4 mM Hu for 5 h. Cells in S phase (EdU+) at the time of HU treatment were Click-IT labeled with an Alexa Fluor 594 azide and RPA intensity in EdU-positive cells were enumerated using Image J/Fiji. Representative data of three independent biological replicates, per sample n≥172 were analyzed. Holm-corrected multiple testing was performed of ranked data fitted by a linear mixed model. (B) MCF7 cells were transfected with the indicated siRNA and 24 h later, cells were transfected with mutant CtIP variants. Afterwards cells were pulsed with 10 μM EdU for 20 min prior to addition of 4 mM HU. Cells in S phase (EdU+) at the time of HU treatment were Click-IT labeled with an Alexa Fluor 594 azide and RAD51 foci in EdU-positive cells were enumerated using Image J/Fiji. Representative data of three independent biological replicates, per sample n≥207 were analyzed. Statistical testing was perform as described in (A). (C) Western blot of iPOND samples in DOX-inducible U-2-OS cell lines. Cells were transfected with both UNC (negative control) or CtIP siRNA and 24 h later, cells were induced with DOX for 24 h. (D) Representative images of PLA foci obtained from HU-treated U-2-OS cell lines. DOX-inducible U-2-OS cells were transfected with the indicated siRNAs and 24 h later, cells were induced with DOX for 24 h. Scale bar= 20 μm. (E) CtIP/Biotin PLA foci per cell in HU-treated DOX-inducible U-2-OS cells. Representative data of three independent biological replicates, per sample n≥10 were analyzed. A linear mixed model of the ranked data was fitted and the p value for differences of least squares means calculated.

Supplementary Figure 4.

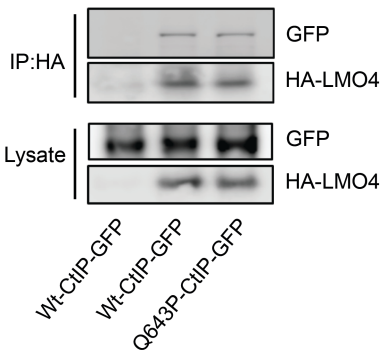
A



B



C



Supplementary Figure 4

(A) Representative images displaying RAD51 in HU-treated EdU-positive cells. Scale bar= 20 μm. (B) MCF7 cells were transfected with the indicated siRNA and 24 h later, cells were transfected with Wt of mutant E804del variant. Cells were pulsed with EdU and exposed to HU as in (A). Cells in S phase (EdU+) at the time of HU treatment were Click-IT labeled with an Alexa Fluor 594 azide and RAD51 foci in EdU positive cells were enumerated using Image J/Fiji. The data represents three independent biological replicates, per sample n≥223 were analyzed. Holm-corrected multiple testing was performed of ranked data fitted by a linear mixed model. (C) MCF7 cells, transfected with HA-tagged LMO4, Wt or mutated Q643P plasmids, were harvested for immunoprecipitation 24 h post-transfection, using HA antibody. Immunoprecipitation of HA in total cell lysates from MCF7 cells transfected with Wt and Q643P-CtIP-GFP, followed by a Western blot, using HA or GFP.

Supplementary Table 1. Cohort description.

Diagnose	Gender (M/F)	Age (time of diagnosis)	WHO Diagnosis	Surgical procedure	Positive lymph nodes	Tumor Size (mm)	IHC Grading	Family history (Any 1. or 2. degree relative with BC or OC)
BC	F	32	IDC	Lumpectomy	8	56	3	Y
BC	F	32	IDC	Mastectomy	1	25	3	N
BC	F	28	N/A	N/A	N/A	N/A	N/A	Y
BC	F	30	IDC	Mastectomy	0	N/A	3	Y
BC	F	23	IDC	Lumpectomy	0	15	2	N
BC	F	22	IDC	Lumpectomy	0	16	2	N
BC	F	26	IDC	Mastectomy	1	13	3	N
BC	F	28	IDC	Lumpectomy	0	11	3	**Y**
BC	F	31	IDC	Mastectomy	4	20	3	N
BC	F	34	IDC	Mastectomy	0	N/A	Unfit	N
BC	F	24	IDC	Mastectomy	5	31	3	Y
BC	F	35	IDC	Mastectomy	4	75	1	Y
BC	F	30	IDC	Mastectomy	0	15	2	Y
BC	F	35	IDC	Mastectomy	7	16	3	N
BC	F	35	IDC	Mastectomy	1	38	2	N
BC	F	34	IDC	Lumpectomy	0	11	3	N
BC	F	30	IDC	Lumpectomy	0	15	3	N
BC	F	33	IDC	Lumpectomy	0	11	3	N
BC	F	28	IDC	Mastectomy	7	18	3	Y
BC	F	30	IDC	Lumpectomy	4	35	2	Y
BC	F	35	IDC	Mastectomy	0	20	3	N
BC	F	22	IDC	Mastectomy	0	20	1	N
OC	F	28	N/A	N/A	N/A	N/A	N/A	**Y**
BC	F	34	IDC	Lumpectomy	1	13	2	Y
BC	F	28	IDC	Mastectomy	1	17	1	Y
BC	F	30	IDC	Mastectomy	11	19	2	N
BC	F	31	IDC	Lumpectomy	0	25	2	Y
BC	F	34	IDC	Mastectomy	N/A	N/A	N/A	N
BC	F	30	IDC	Mastectomy	4	60	2	N/A
BC	F	34	IDC	Lumpectomy	3	19	2	N
BC/OC	F	32	N/A	N/A	N/A	N/A	N/A	Y
BC	F	28	N/A	N/A	N/A	N/A	N/A	Y
OC	F	30	N/A	N/A	N/A	N/A	N/A	N
BC	F	29	IDC	Lumpectomy	0	12	3	N
BC	F	32	IDC	Mastectomy	11	40	3	Y
BC	F	24	IDC	Lumpectomy	0	15	3	Y
BC	F	26	IDC	Mastectomy	4	40	3	N
BC	F	30	IDC	Mastectomy	2	55	N/A	Y
BC	F	31	IDC	Mastectomy	18	40	2	N
BC	F	29	IDC	Mastectomy	0	9	3	Y
BC	F	33	ILC	Mastectomy	2	37	N/A	Y
BC	F	34	IDC	Lumpectomy	1	9	1	N
BC	F	32	IDC	Lumpectomy	1	N/A	2	N

BC	F	24	CIS	Mastectomy	N/A	N/A	N/A	Y
BC	F	31	IDC	Lumpectomy	1	25	2	N
BC	F	28	IDC	Lumpectomy	0	15	2	N
BC	F	28	IDC	Mastectomy	0	30	2	N
BilatBC	F	32	ILC	Mastectomy	12	40	2	Y
BC	F	32	IDC	Mastectomy	2	18	2	N
BC	F	26	N/A	N/A	N/A	N/A	N/A	Y
BC	F	31	Unclassified	Lumpectomy	2	N/A	N/A	Y
BC	F	32	IDC	Lumpectomy	0	12	2	Y
BC	F	30	CIS	Mastectomy	0	30	N/A	Y
BC	F	35	IDC	Mastectomy	0	20	3	Y
BC	F	31	IDC	Mastectomy	1	15	3	N
BC	F	30	N/A	N/A	N/A	N/A	N/A	N
BC	F	34	IDC	Lumpectomy	0	30	3	N
BC	F	32	IDC	Mastectomy	12	28	3	Y
BC	F	32	IDC	Lumpectomy	1	24	2	N
BC	F	34	N/A	N/A	N/A	N/A	N/A	Y
BC	F	30	IDC	Lumpectomy	0	18	N/A	Y
OC	F	30	N/A	N/A	N/A	N/A	N/A	Y
BC	F	32	IDC	Lumpectomy	0	14	3	Y
BC	F	27	IDC	Mastectomy	2	50	3	N
BC	F	32	Medullary	Lumpectomy	0	15	N/A	Y
BC	F	28	IDC	Mastectomy	7	40	3	N
BC	F	35	IDC	Mastectomy	10	25	3	Y
BC	F	30	IDC	Mastectomy	0	20	3	Y
BC	F	31	IDC	Lumpectomy	0	20	3	N
BC	F	29	N/A	Biopsy	N/A	N/A	N/A	Y
BC	F	31	IDC	Lumpectomy	0	6	2	N
BC	F	35	IDC	Lumpectomy	0	7	1	Y
BC	F	31	IDC	Mastectomy	0	8	2	N
BC	F	31	IDC	Lumpectomy	0	10	3	N
BC	F	30	IDC	Lumpectomy	0	18	3	Y
BC	F	28	IDC	Mastectomy	1	26	3	N
BC	F	27	IDC	Mastectomy	0	75	3	Y
BC	F	28	N/A	N/A	N/A	N/A	N/A	N
BC	F	28	IDC	Lumpectomy	3	30	3	N
BC	F	30	IDC	Mastectomy	5	12	3	Y
BC	F	28	N/A	N/A	N/A	N/A	N/A	N
BC	F	34	IDC	Mastectomy	0	14	3	N
BC	F	28	IDC	Mastectomy	0	10	2	Y
BC	F	27	IDC	Lumpectomy	0	30	3	Y
BC	F	31	IDC	Mastectomy	23	15	2	N
BC	F	31	IDC	Mastectomy	2	40	2	Y
OC	F	27	N/A	N/A	N/A	N/A	N/A	Y
BC	F	29	IDC	Mastectomy	0	5	3	N
BC	F	33	IDC	Lumpectomy	0	15	1	Y
BC	F	34	IDC	Lumpectomy	0	16	2	N
BC	F	32	IDC	Mastectomy	11	25	2	N
BC	F	31	IDC	Lumpectomy	2	24	3	Y
BC	F	32	IDC	Biopsy	1	N/A	Unfit	Y
BC	F	33	IDC	Mastectomy	0	8	2	Y
BC	F	30	IDC	Lumpectomy	0	8	1	Y

OC	F	27	MBC	Mastectomy	0	15	N/A	N
BC	F	32	IDC	Biopsy	N/A	N/A	2	Y
BC	F	33	IDC	Lumpectomy	11	15	3	N
BC + uterine cancer	F	33	IDC	Mastectomy	2	25	3	Y
BC	F	29	IDC	Mastectomy	N/A	N/A	N/A	Y
BC	F	27	IDC	Lumpectomy	1	15	3	Y
BC	F	26	IDC	Mastectomy	N/A	N/A	2	N
bilatBC	F	29	N/A	N/A	N/A	N/A	N/A	Y
BC	F	29	N/A	N/A	N/A	N/A	N/A	Y
BC	F	24	IDC	Lumpectomy	2	15	3	N
BC	F	31	IDC	Lumpectomy	0	12	3	Y
BC	F	33	IDC	Mastectomy	1	21	3	Y
BC	F	26	IDC	Mastectomy	3	55	3	Y
BC	F	29	IDC	Lumpectomy	3	19	3	N
BC	F	24	IDC	Lumpectomy	0	20	3	N/A
BC	F	26	IDC	Lumpectomy	0	18	2	N/A
BC	F	30	IDC	Mastectomy	0	15	3	N/A
BC	F	26	IDC	Lumpectomy	4	20	2	N
BC	F	30	IDC	Lumpectomy	0	5	2	Y
BC	F	35	IDC	Lumpectomy	0	12	2	Y
BC	F	35	IDC	Lumpectomy	1	19	2	N
BC	F	35	IDC	Lumpectomy	1	15	2	N
OC	F	20	IDC	Lumpectomy	0	24	2	Y
BC	F	28	IDC	Mastectomy	N/A	N/A	2	N
BC	F	34	IDC	Lumpectomy	0	9	1	N
BC	F	34	IDC	Lumpectomy	0	16	3	Y
BC	F	28	IDC	N/A_M	0	N/A	2	N
BC	F	27	IDC	Mastectomy	4	16	3	Y
BC	F	25	Carcinoma	Lumpectomy	N/A	N/A	N/A	Y
BC/Prostata	M	60/61	IDC	N/A	N/A	14	2	Y
BC	M	68	N/A	N/A	N/A	N/A	N/A	N
BC	M	68?	N/A	N/A	N/A	N/A	N/A	N/A
BC	M	37	N/A	N/A	N/A	N/A	N/A	N/A
BC	M	62	N/A	N/A	N/A	N/A	N/A	N

BC; breast cancer; OC; ovarian cancer; F=female; M=male; N=no; Y=yes; IDC=invasive ductal carcinoma; ILC= invasive lobular carcinoma; MBC= metaplastic breast carcinoma; N/A=non-applicable or missing data

Supplemental Table 2. International cohort data.

Nucleotide (HGVS)	Protein (HGVS)	Exon	Complexo group	AF (%)
c.553C>T	p.Arg185*	8	1	0.049
c.592G>A	p.Val198Met	8	1	0.049
c.800A>G	p.Glu267Gly	10	2	0.099
c.814C>G	p.Gln272Glu	11	1	0.049
c.992G>C	p.Gly331Ala	12	1	0.049
c.1055A>C	p.Gln352Pro	12	1	0.049
c.1105A>G	p.Ile369Val	12	1	0.049
c.1115T>G	p.Leu372*	12	1	0.049
c.1242A>T	p.Glu414Asp	12	1	0.049
c.1367A>G	p.His456Arg	12	1	0.049
c.1632G>A	p.Thr544Thr	12	3	0.148
c.1656G>T	p.Glu552Asp	12	1	0.049
c.1928A>C	p.Gln643Pro	13	2	0.099
c.2146G>A	p.Glu716Lys	16	1	0.049
c.2410_2412delGAG	p.Glu804del	18	2	0.099
c.2516G>A	p.Arg839Gln	19	1	0.049
c.2630G>A	p.Arg877His	20	1	0.049

Rare *RBBP8*-variants and allele frequencies (AF) in the international cohort (n=1054).

Supplementary Table 3. *RBBP8* variants in COMPLEXO cohort were analysed for micronuclei formation after irradiation or Hydroxyurea treatment as described before. Variants such as H456R, Q643P, E804del and R839Q were found in both the COMPLEXO and Danish cohort. Damaging variants have significantly increased micronuclei formation in three independent biological repeats using One-way Anova for statistical testing. For each biological repeat 100 binucleated cells were analysed.

Classification of *RBBP8* variants in COMPLEXO cohort based on micronuclei formation after genotoxic stress	**Micronuclei formation after IR**	**Micronuclei formation after HU**
Damaging variants	R185*, L372*	R185*, L372*, Q643P, E804del
Non-damaging variants	V198M, E267G, Q272E, G331A, E352P, I369V, E414D, H456R, E552D, Q643P, E716K, E804del, R839Q, R877H	V198M, E267G, Q272E, G331A, E352P, I369V, E414D, H456R, E552D, E716K, R839Q, R877H

Supplementary Table 4: Primer sequences for verification of identified *RBBP8* variants using Sanger sequencing

Nucleotide (HGVS)	Protein (HGVS)	Exon	Forward primer	Reverse primer
c.298C>T	p.R100W	6	5'-TGGTATAACATGATTTCAGC	5'-CCATCCATTTAATTACAACC
c.329G>A	p.R110Q	6	5'-TTGATTTTCACAGTATTTGC	5'-TATTTCTTACTAAATTAAGC
c.693T>A	p.S231R	9	5'-TGTTTCTTAGTGAAATTAAAGGAGC	5'-GATTAGGGATATTTATATGAATAGG
c.1367A>G	p.H456R	12	5'-TCAAAATCTGAAGATAGTGC	5'-CCTCATAAAGAGTCACTTGC
c.1505G>T	p.R502L	12	5'-CACTGATAAACATTTGGAGC	5'-CAAGGGCTGAAGGATGATGC
c.1928A>C	p.Q643P	13	5'-CATGGAGGATGTGAACTTGCA	5'-ACCGGTAAAATGTGAGAATCGT
c.2024C>T	p.T675I	14	5'-TGTCTATTTAGATCCTTTGC	5'-TACATGTAGGTTTTAAGACG
c.2131G>A	p.E711K	15	5'-TCCTTGGCAGCAGTCCTTCTTTTCC	5'-ACTGAAACTGAGCTTTCCTAAGAGG
c.2410_2412del	p.E804del	18	5'-GGGGATTATTTCTCCTCTGAACTC	5'-TGTTACGCCTGGCTCAAATAAGAG
c.2413A>G	p.R805G	18	5'-GGGGATTACACTGAATTTGC	5'-TAGTAGAGATGGGGTTTCGC
c.2516G>A	p.R839Q	19	5'-CTTCACAGACCAACATCAGC	5'-GCACAATCTTGGCTCACTGC
c.2620C>G	p.P874A	20	5'-AATCATCAGCATCACACAGC	5'-GCGCCTTATTGTTTTAAAGG
c.2682G>C	p.E894D	20	5'-AATCATCAGCATCACACAGC	5'-GCGCCTTATTGTTTTAAAGG

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