Supplemental Material

A macro-histone variant links dynamic chromatin compaction to BRCA1-dependent genome maintenance.

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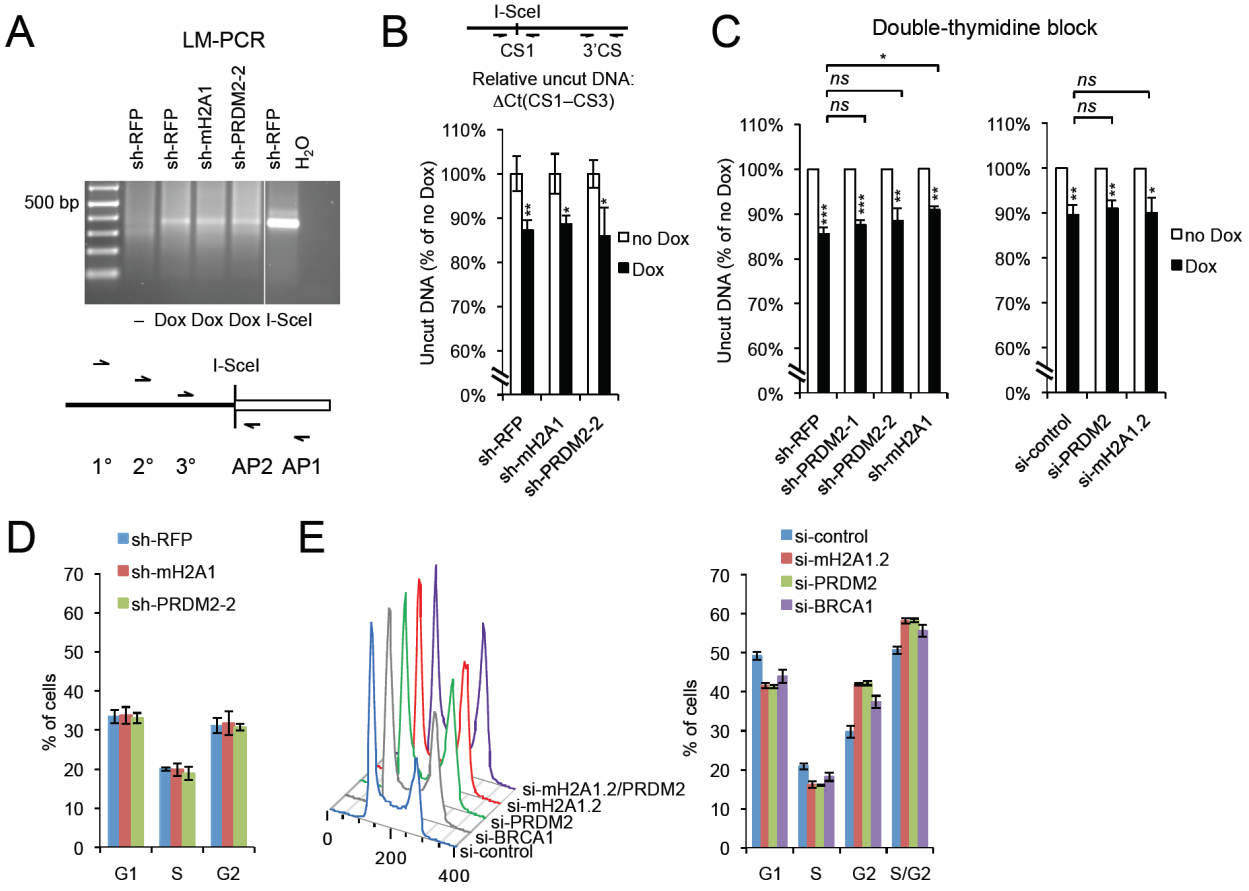

Figure S1, related to Figures 1 and 2. Efficiency of DSB induction and cell cycle profiles upon macroH2A1 or PRDM2 depletion. (A) LM-PCR analysis of Dox-induced I-SceI-mediated DSB induction in asynchronous cells. DNA was isolated 12 h post Dox treatment (Dox) and samples were subjected to three rounds of semi-nested PCR following linker ligation (see schematic for primers, linker primer AP2 was used for 2nd and 3rd round PCRs). Genomic DNA digested with I-SceI served as positive control (I-SceI). (B) qPCR analysis of I-SceI-mediated DSB cutting in asynchronous cells treated as in (A). Genomic DNA from the indicated knockdown cell lines was analyzed using a DSB spanning primer (CS1) and normalized to a primer pair downstream of I-SceI (CS3, see schematic). The amount of uncut DNA relative to no Dox (no I-SceI) is shown, samples were analyzed in triplicate, values are expressed as mean and SD. (C) qPCR for cutting efficiency analyzed as in (B), genomic DNA was isolated 8 h after release from double-thymidine block in the presence or absence of Dox. Values are expressed as mean and SEM (sh-PRDM2-2, n = 2; all others, n = 3). (D) Cell cycle distribution following shRNA-mediated depletion of the HR modulators macroH2A1 and PRDM2. Propidium Iodide (PI) staining was used to determine DNA content. Samples were analyzed by FACS in triplicate, values are expressed as mean and SD. (E) Cell cycle analysis following siRNA-induced knockdown of the indicated mRNAs, samples were analyzed as in (D), a representative experiment is shown.

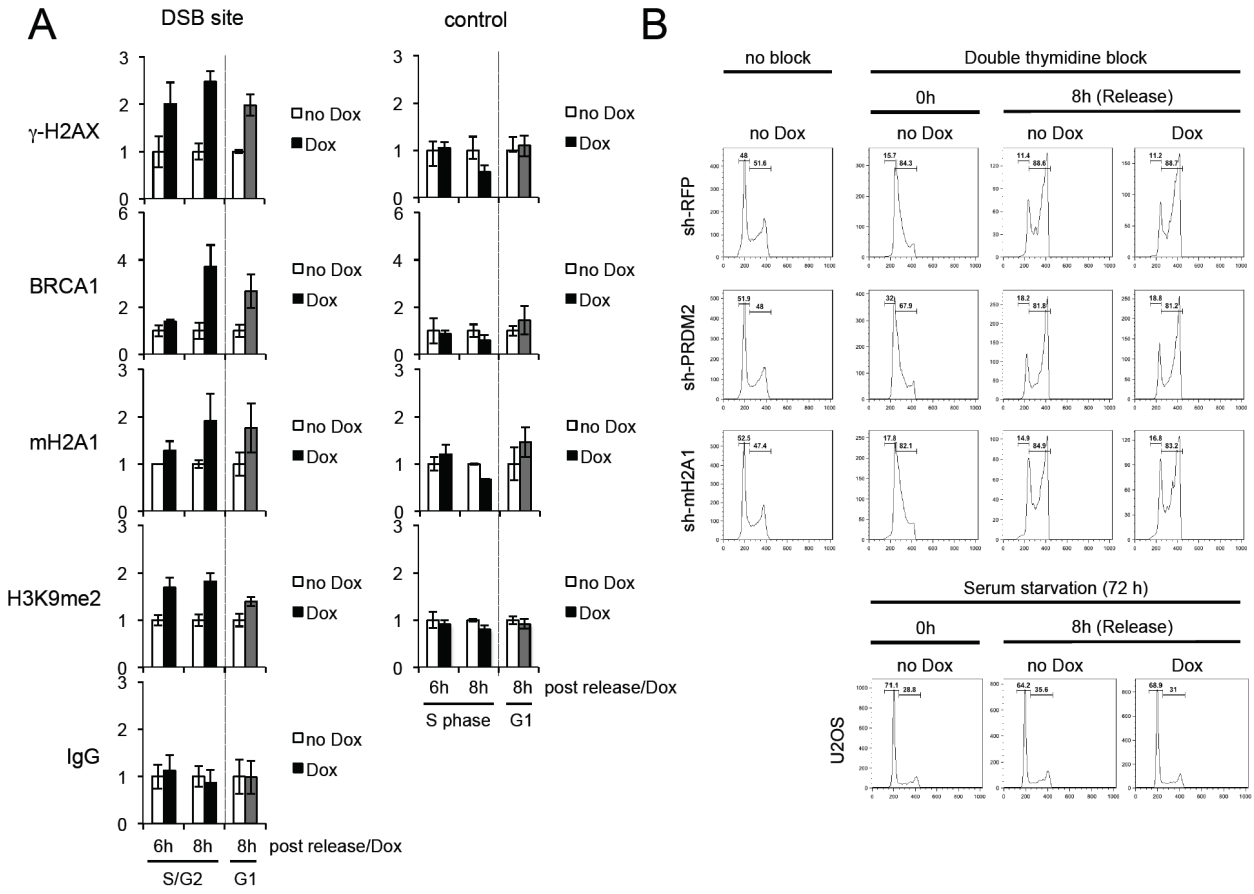

Figure S2, related to Figure 1. ChIP analysis of macroH2A1, H3K9me2 and DSB marks in S/G2 and G1. (A) ChIP analysis for γ–H2AX, BRCA1, macroH2A1, H3K9me2 and IgG following Dox-dependent DSB induction. Cells were released either from double-thymidine block (S/G2) or from serum starvation (G1) for the indicated time frames in the presence or absence of Dox. A non-DSB associated genomic locus was analyzed as control. Enrichment was normalized to pre-DSB induction (no Dox), values are expressed as mean and SEM (n = 3 for G1, n = 2 for S/G2). (B) Cell cycle profiles following double thymidine block or serum starvation 0 h or 8 h after release in control and knockdown cells.

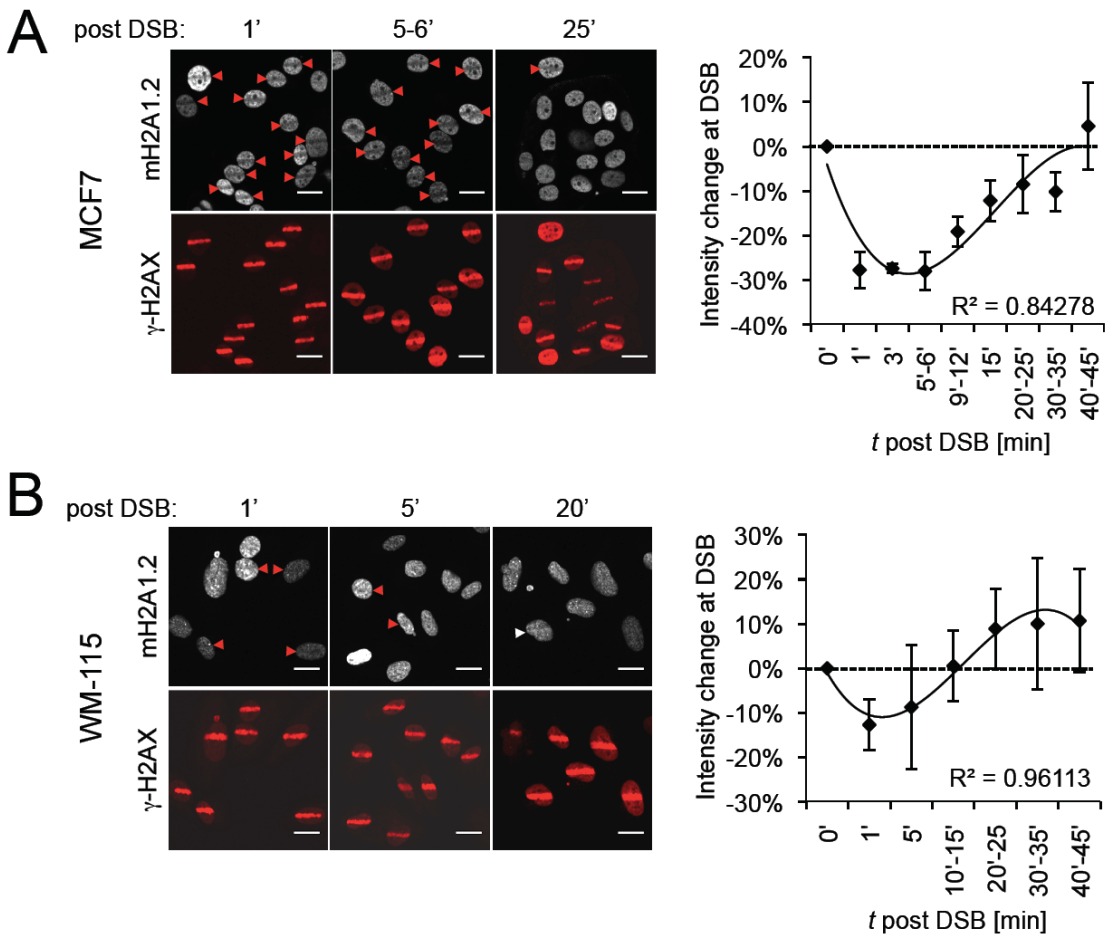

Figure S3, related to Figure 1. MacroH2A1.2 dynamics at DSBs in MCF7 and WM-115 cells. (A, B) Co-immunostaining for macroH2A1.2 and γ–H2AX in MCF7 (A) or WM-115 cell lines (B) at the indicated time points post laser microirradiation, arrow heads depict enrichment (white) or depletion (red), scale bar: 20 μm. DSB-associated macroH2A1.2 intensity changes were measured as the ratio of γ–H2AX^{+} over γ–H2AX^{-} nuclear areas. Values are expressed as mean and SEM (n ≥ 2) (A) or mean and SD (B), 5-17 cells were analyzed per time point. R^2 values are based on a third order polynomial regression.

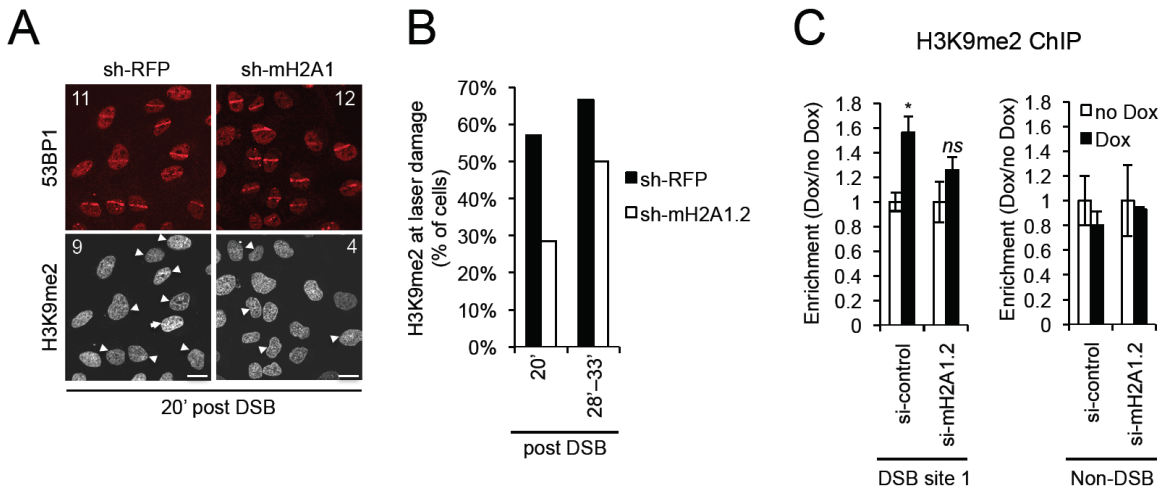

Figure S4, related to Figure 2. H3K9me2 accumulation at laser-induced DSBs depends on macroH2A1.2. (A) One of at least three representative experiments depicting the frequency of H3K9me2 accumulation at sites of laser damage in sh-macroH2A1 or sh-RFP expressing cells, scale bar: 20 μm. (B) Frequency of H3K9me2 accumulation at sites of laser damage in sh-mH2A1.2 or sh-RFP cells. (C) H3K9me2 ChIP in si-macroH2A1.2 and si-control cells 8 h after release from double-thymidine block in the presence or absence of Dox. Enrichment at the DSB site and a non-DSB associated control locus was normalized to no Dox, values are expressed as mean and SEM (n = 3).

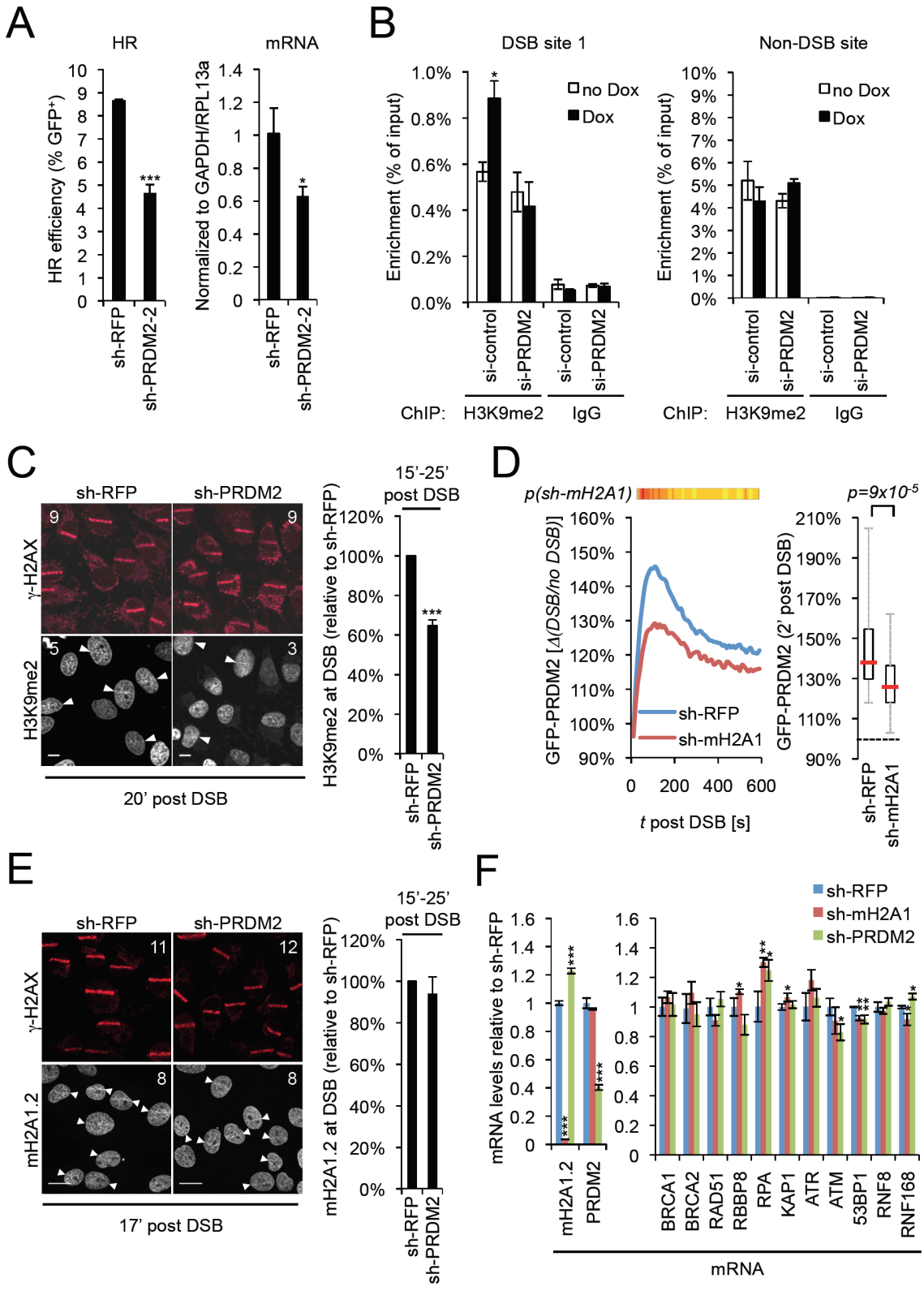

Figure S5, related to Figure 2. MacroH2A1 and PRDM2 promote HR and H3K9 dimethylation at DSBs. (A) HR efficiency and PRDM2 mRNA levels in sh-PRDM2-

2 and sh-RFP control cells. Samples were analyzed in triplicate, mRNA levels are relative to sh-RFP and were normalized to GAPDH and RPL13a. (B) ChIP for H3K9me2 and IgG 8 h after release from double-thymidine block in the presence or absence of Dox. Enrichment over input is shown at the I-SceI DSB site and a non-DSB control locus, values are expressed as mean and SEM (n = 3). (C) Frequency of cells with H3K9me2 enrichment at sites of laser-damage in sh-PRDM2-1 or sh-RFP cells, individual experiments were normalized to sh-RFP controls. Values are expressed as mean and SEM (n = 7), $p = 0.0004$, Student's paired *t*-test. A representative experiment is shown, scale bar: 10 μm. (D) Analysis of GFP-PRDM2 recruitment in sh-macroH2A1 (n=38) or sh-RFP control cells (n=42). Cells from at least three independent experiments were pooled to determine average recruitment, *p(sh-mH2A1)* heat map and box-plot were generated as described in Fig. 2E. (E) Frequency of cells with macroH2A1.2 enrichment at sites of laser-damage in sh-PRDM2-1 or sh-RFP cells, individual experiments were normalized to sh-RFP controls. Values are expressed as mean and SEM (n = 7). A representative experiment is shown, scale bar: 20 μm. (F) DSB repair factor expression in sh-macroH2A1, sh-PRDM2-1 and sh-RFP expressing cells in the absence of DNA damage, mRNA levels were normalized to RPL13a, GAPDH and RPS16. Samples were analyzed in triplicate, values are expressed as mean and SD.

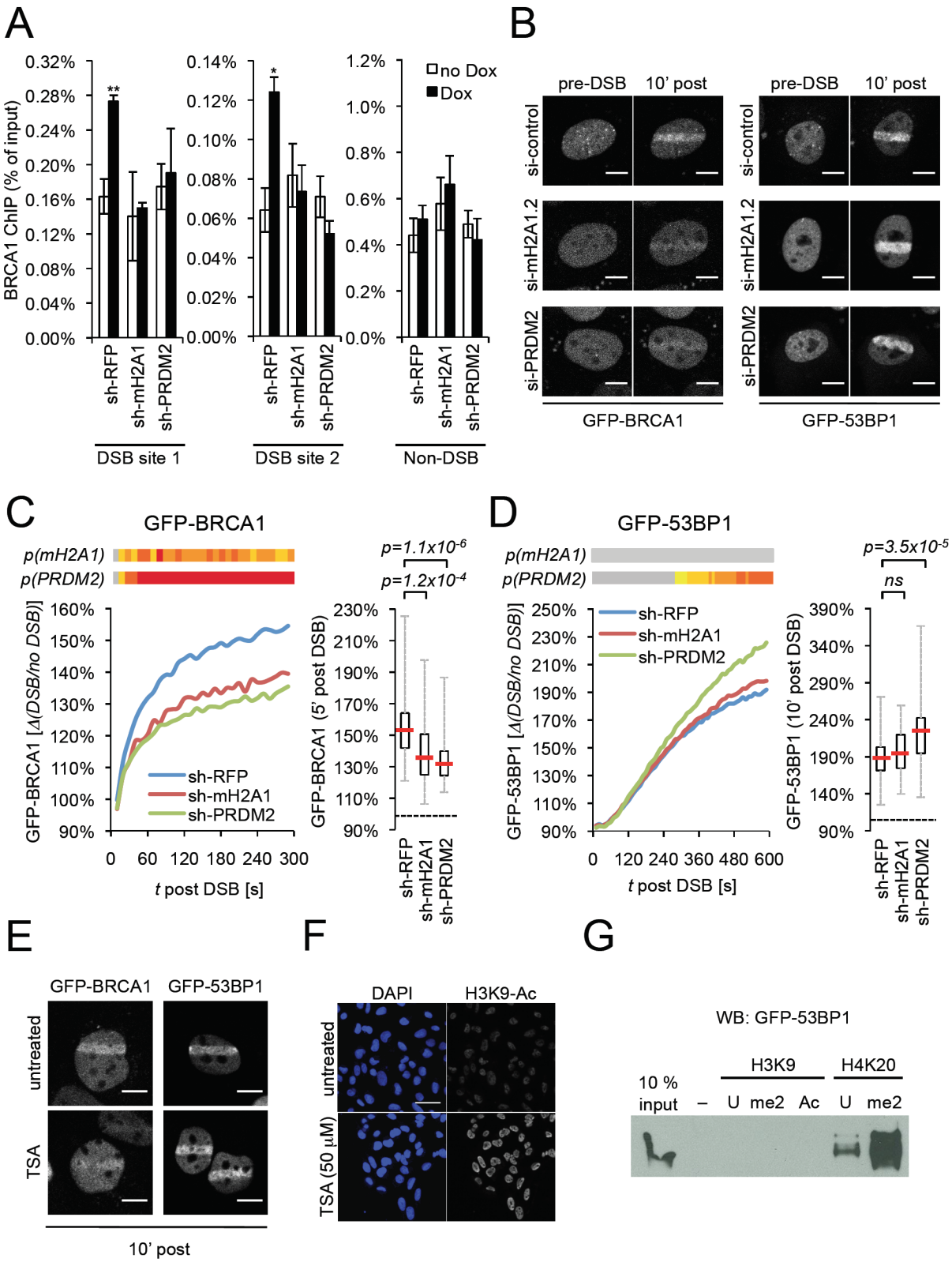

Figure S6, related to Figure 6. Loss of macroH2A1 or PRDM2 impairs BRCA1 but not 53BP1 Recruitment to DSBs. (A) ChIP for BRCA1 and IgG in sh-PRDM2-

1, sh-macroH2A1 or sh-RFP expressing cells 8 h after release from double-thymidine block in the presence or absence of Dox. Enrichment over input is shown. Values are expressed as mean and SEM (n = 3). (B) Representative images for recruitment of GFP-BRCA1 (see Fig. 6B) and GFP-53BP1 (see Fig. 6C) in cells transfected with the indicated siRNAs. (C) GFP-BRCA1 recruitment to laser-induced DSBs in sh-macroH2A1 (n=41), sh-PRDM2-1 (n=44) and sh-RFP control cells (n=68). Cells from at least three independent experiments were pooled, *p(mH2A1)* and *p(PRDM2)* heat maps and box plot were generated as described in Figure 2, knockdown with sh-PRDM2-2 gave a similar result (not shown). (D) GFP-53BP1 recruitment to laser-induced DSBs in sh-mH2A1 (n=47), sh-PRDM2-1 (n=39) and sh-RFP control cells (n=48). Cells from three independent experiments were pooled and analyzed as in (B). (E) Representative images for recruitment of GFP-BRCA1 and GFP-53BP1 the presence or absence of TSA (see Figs. 6D, E). (F) IF analysis for H3K9-acetylation in U2OS cells that were left untreated or treated with 50 μM TSA for 30 min. Nuclei were stained with DAPI, scale bar: 50 μm. (G) Interaction of GFP-53BP1 with modified or unmodified histone H3 or H4 N-terminal peptides. Peptide pull-down was followed by α–GFP immunoblot, beads alone (–) served as negative control. One of two representative experiments is shown. U: unmodified peptide.

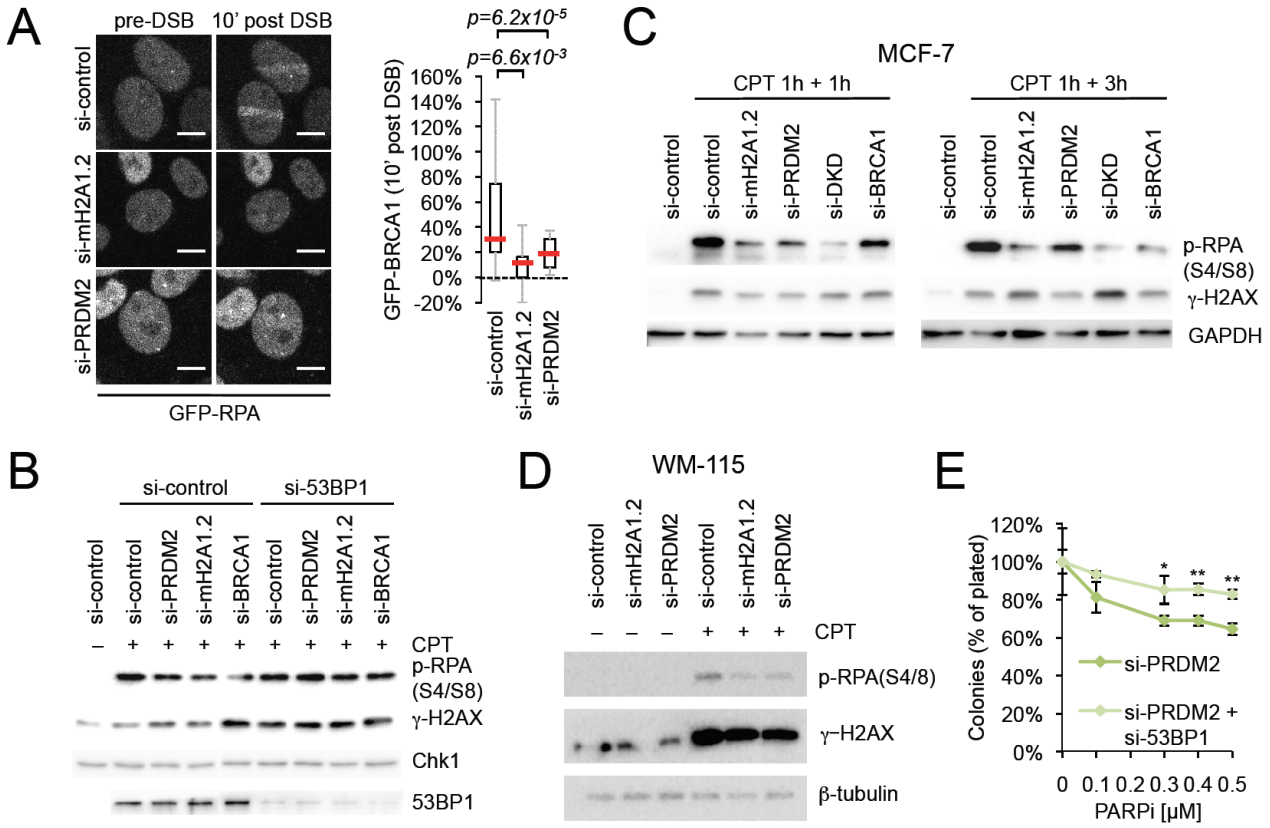

Figure S7, related to Figure 7. Depletion of macroH2A1.2 or PRDM2 affects end resection and PARPi resistance. (A) GFP-RPA recruitment in S phase cells (1-2 h post double-thymidine block). Si-control (n = 23), si-macroH2A1.2 (n = 24) and si-PRDM2 expressing cells (n = 16) were analyzed 10 min post DSB induction, representative images are shown, scale bar: 10 μm. (B) Western blot analysis for p-RPA, γ−H2AX, 53BP1 and Chk1 in U2OS cells expressing the indicated siRNAs together with si-control or si-53BP1. Cells were exposed to CPT for 1h followed by a 3h release. (C) Representative western blot for p-RPA, γ−H2AX and GAPDH in MCF-7 cells expressing the indicated siRNAs. Cells were treated with CPT for 1h followed by a 1h or 3h release, si-DKD: combined knockdown of macroH2A1.2 and PRDM2. (D) Western blot analysis as in (C) using WM-115 cells treated with CPT for 1 h, followed by a 3 h recovery. (E) Clonogenic survival assay following knockdown of PRDM2 in the presence or absence of si-53BP1. Samples were analyzed in triplicate, values are expressed as mean and SD.

Table S1

gene ID	HR down (> 1 SD)	HR down (> 2 SD)	HR up (> 1 SD)	HR up (> 2 SD)	Total hps	KS ranking	2nd best hp ranking
HR down							
TRRAP	**4**	**2**			**5**	**1**	**3**
NPM2	**5**	**1**			**7**	**20**	**12**
H2AFY	**4**	**1**			**5**	**9**	**18**
H2AFY2	**4**				**4**	**2**	**24**
RBBP8	**4**				**5**	**7**	**5**
NPM1	**4**				**5**	**8**	**13**
RNF168	**4**				**5**	**13**	**11**
CENPV	**4**				**5**	**17**	**27**
EPC1	**3**	**2**	**1**		**4**	**22**	**1**
METTL13	**3**	**1**			**4**	**14**	**20**
USP21	**3**	**1**			**5**	**29**	**6**
TRIM16	**3**				**5**	**26**	**30**
WHSC1L1	3				5	39	37
TADA3L	4				5	15	56
FOXP3	3				5	90	16
SUPT4H1	3		1		5	33	45
USP22	3				5	83	17
TAF9	3		1		5	42	41
NASP	3				5	41	58
BPTF	3				4	4	>90
SLBP	2	1			4	53	2
SAFB	2	1			5	47	87
PRDM2	2	1			3	59	75
PRMT8	2	1			4	55	85
HR up							
TADA1L			**3**	**1**	**5**	**9**	**1**
ATXN7L3	**1**		**3**	**1**	**4**	**15**	**3**
ARID5B			**3**	**1**	**3**	**5**	**6**
PDS5B			**4**		**5**	**3**	**19**
ASF1B			**3**		**3**	**2**	**4**
COQ3			**3**		**4**	**7**	**15**
HMGN3			**3**		**4**	**12**	**17**
SIN3B			**2**	**1**	**3**	**11**	**21**
EZH2	1		4	1	7	87	5
SOX2			3		6	38	16
HDAC6			3		3	4	37
TAF6L	1		3		6	58	2
BRDT			3		10	55	36
HMGB2*			3		4		
ASH1L			2	1	4	18	33
MLL2			2	2	5	29	31
PHF11			2	1	4	8	38
CHD8			2	1	4	25	51
HDAC8*			2	1	4		

Table S1, related to Figure 1. List of top-ranking HR modulators. Hits were ranked based on the number of hairpins resulting in a reduction or increase in HR efficiency outside of one or two standard deviations (SD) of control shRNAs, only hits targeted by at least three shRNAs, or two shRNAs with one shRNA resulting in a change greater than 2 SD are listed. Hits that scored in the top 10% of both KS and second-best hairpin rankings are shown in bold, * not included in ranking analysis, see methods for details. Hp: hairpin.

Table S2

siRNAs (ON-TARGET PLUS, Thermo Scientific)	
si-mH2A1.2	GCUUUGAGGUGGAGGCCAUUU
si-PRDM2	GAAAAGGUGUCGACAAUAU
si-53BP1	GCUAUAUCCUUGAAGAUUU
shRNAs (TRC, Open Biosystems)	
sh-mH2A1	GCGTGTGTTGTGGTGCTTTAT
sh-mH2A1.2	CTGAACCTTATTCACAGTGAA
sh-PRDM2-1	CCGTCAATCATGCTTTCAAAT
sh-PRDM2-2	CCTGACTGTAAATGCTCCATT

Table S2, related to Figures 1-7. List of siRNA and shRNA target sequences.

Table S3

Primer Name	Sequence (5'-3')	AT
RT-PCR		
mH2A1.1 F	5'-CCCGACAAACACTGACTTCTAC-3'	58
mH2A1.1 R	5'-CAGGACAGCTTCCACAAACT-3'	58
mH2A1.2 F	5'-GCTTTGAGGTGGAGGCCATAATCA-3'	58
mH2A1.2 R	5'-ACT-CCT-TGC-CAC-CTT-TCT-TCT-CCA-3'	58
PRDM2_001 F	5'-AAACAGTAACAGCAACCACCGCAG-3'	58
PRDM2_001 R	5'-TTCACCGAAGCTGCAAACTTGACG-3'	58
RBBP8/CtIP F	5'-CCACTGTCTGGAAGGAAATCA-3'	58
RBBP8/CtIP R	5'-CTTGAGGAGGAGTCTTTGAAGTAG-3'	58
RAP80 F	5'-GTGTGAAGAGGAAGCGTAGAC-3'	58
RAP80 R	5'-GGCCAAACACTTTGCTCTATTC-3'	58
BRCA1 F	5'-GGACAAAGCAGCGGATACA-3'	58
BRCA1 R	5'-TTGATCTCCCACACTGCAATAA-3'	58
BRCA2 F	5'-CAGTGGTATGTGGGAGTTTGT-3'	58
BRCA2 R	5'-ACCTCAGCTCCTAGACTTTCA-3'	58
RPA2 F	5'-CAGGGAACTTTGGTGGGAATAG-3'	58
RPA2 R	5'-CAGGTCTTGGACAAGCCTTAA-3'	58
RAD51 F	5'-TGGCCCACAACCCATTT-3'	58
RAD51 R	5'-GCAACAGCCTCCACAGTAT-3'	58
53BP1 F	5'-GCTATCTCGACACCTTCCTAATC-3'	58
53BP1 R	5'-TACCGTCTCCTCGTTCTTCT-3'	58
RNF8 F	5'-CTGAGGGCCAATGGACAATTA-3'	58
RNF8 R	5'-GTAGTCTCCCTGATGAATGGAATAG-3'	58
RNF168 F	5'-CGAAGAGCGATGGAAGAACA-3'	58
RNF168 R	5'-TCAAGGGAGAAGCCGAGATA-3'	58
KAP1 F	5'-AAGCGTGTGCAAGTGGATGTCAAG-3'	58
KAP1 R	5'-AATGTGCTCCTGGTGCTTCTGGAT-3'	58
ATM F	5'-CTTGTGCCTTGGCTACAGAT-3'	58
ATM R	5'-AGACAGCTCACAGTTAGGTAAAC-3'	58
ATR F	5'-TGTTTGTAAATGTGAGTGGAAGC-3'	58
ATR R	5'-GTCTCGTTATGATCCAATTACTGAA-3'	58
RPS16 F	5'-AAACGCGGCAATGCTCTCATCAAG-3'	58
RPS16 R	5'-TGGAGATGGACTGACGGATAGCAT-3'	58
RPL13a F	5'-GAAGTACCAGGCAGTGACAG-3'	58
RPL13a R	5'-GGTCTTGAGGACCTCTGTG-3'	58
GAPDH F	5'-GGAGTCAACGGATTTGGTCG-3'	58
GAPDH R	5'-GAGGCATTGCTGATGATCTTG-3'	58

ChIP		
DRGFP set 9 F	5'-ATCACATGGTCCTGCTGGAGTT-3'	58
DRGFP set 9 R	5'-TGGCTGATTATGATCTAGAGTCGCGG-3'	58
GRIN2B DHS F	5'-CCCTGAAATGGCTCCTCTAAC-3'	58
GRIN2B DHS R	5'-GGAAGGTTCTGAGCCAAACT-3'	58
RYR-1000 F	5'-ATGTTCCCCAAGCAGTGTTC-3'	56
RYR-1000 R	5'-CAGAAGGAGCCCAGAGATTTT-3'	56
I-SceI cutting efficiency		
I-SceI CS1 F	5'-TCCGGCTAGGGATAACAGGGTAAT-3'	59
I-SceI CS1 R	5'-AAGAAGTCGTGCTGCTTCATGTGG-3'	59
DRGFP set 10 F	5'-GCTGGAGTACAACTACAACAGCCA-3'	59
DRGFP set 10 R	5'-TTGATGCCGTTCTTCTGCTTGTCG-3'	59
I-SceI DHS probe		
I-SceI DHS F	5'-TCAGAACCTTCCCGAACCAGTACA-3'	58
I-SceI DHS R	5'- ACCACACCCAGCGGTTATTACTGT-3'	58
4C contact library primers		
Csp6I-proximal	5'-GACGTTGTGGCTGTTGTAGTTG-3'	60
HindIII-proximal	5'-CGGCATCAAGGTGAACTTC-3'	60
LM-PCR primers		
Upper adaptor:	5'-GTAATACGACTCACTATAGGGCACGCGTGGTCG GGTCGACGGCCCGGGCTGGTTAT-3'	62-58
Lower adaptor	5'-phos-TAACCAGCCCG-3'inverted-dT	62-58
AP1	5'-GTAATACGACTCACTATAGGGC-3'	62-58
AP2	5'-ACTATAGGGCACGCGTGGT-3'	62-58
1st round R	5'-GCAACGTGCTGGTTATTGTGCTGT-3'	62-58
2nd round R	5'-AGAATTCAGATCCGCCGCCAC-3'	62-58
3rd round R	5'-AAGTAGAAGACCCACGAGGCAACA-3'	62-58

Table S3, related to Figures 1-7. List of primers and oligonucleotides. AT: annealing temperature.

Table S4, related to Figure 4. 4C read counts across I-SceI DSB site on chromosome 12. See separate Excel file.