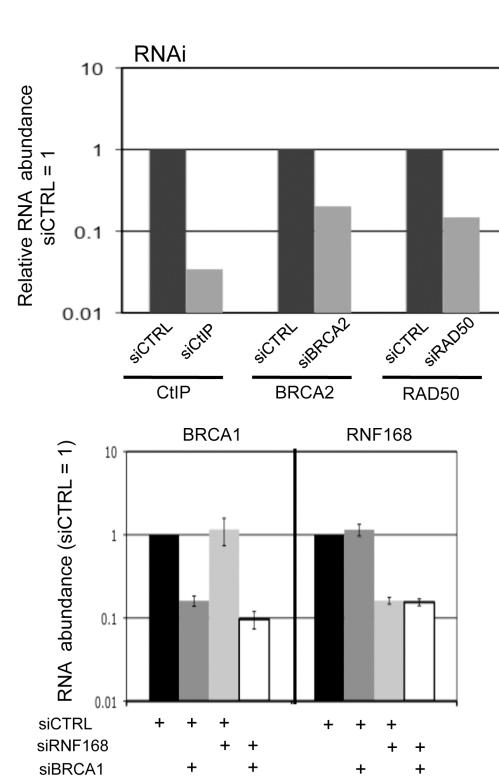


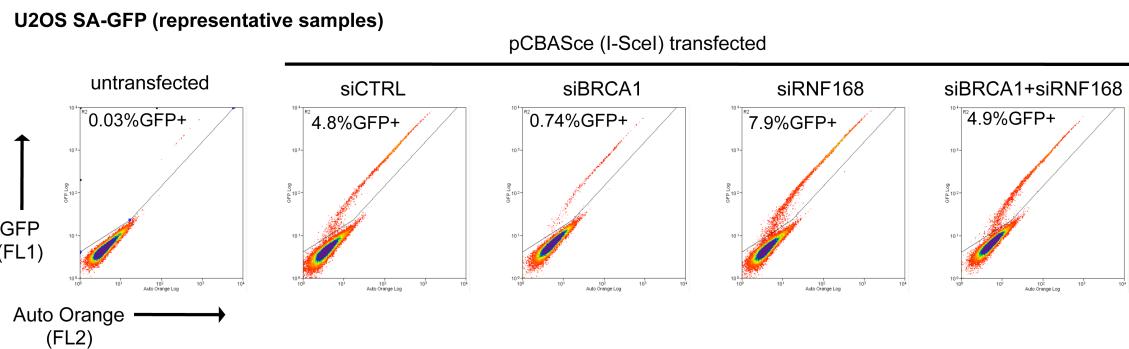
SUPPLEMENTAL MATERIALS

Supplemental Figure S1. RNAi validation by RT-PCR. **A.** Shown are siRNA targeting sequences as provided by the manufacturers, and sequences of RT-PCR primers used in this study. Also shown are primers used to amplify Flag-RNF168 for insertion into the expression vector pcAGGS-BSKX, and for generating silent mutations to resist siRNF168#18. **B.** Shown is evidence of RNA depletion for siRNAs shown in A. Two days after siRNA transfection, RNA was isolated using the RNAeasy kit with the gDNA eliminator column (Qiagen), which was reverse transcribed using M-MLV-RT and random primers (Promega). The RT reactions were amplified using an iQ Cycler with SYBR-green supermix (BioRad). Shown is relative RNA abundance, based on threshold cycle (Ct) values from the PCR amplification, for individual genes normalized to actin, and relative to siCTRL treated cells.

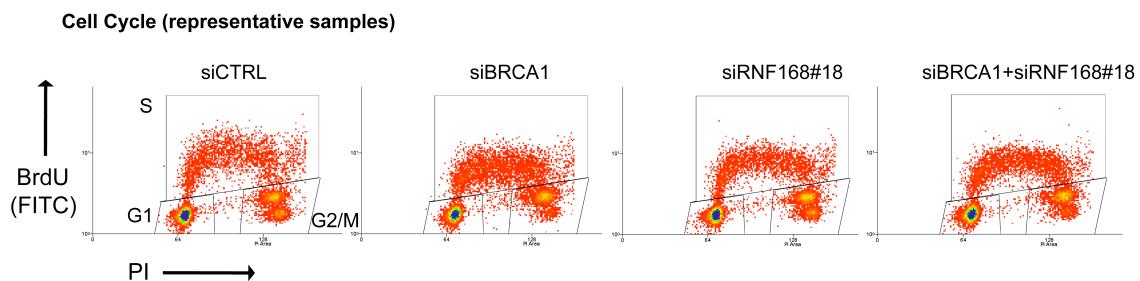
A	RNAi target sequences	RTPCR primers
	siBRCA1	ACTIN
#6	5' GGGAUACCAUGCAACAUAA	5' ACTGGGACGACATGGAGAAG
#7	5' GAAGGAGCUUCAUCAUUC	5' AGGAAGGAAGGCTTGAAGAG
siRNF168 pool		BRCA1
5'	GAAAUCUCUCUGCUAACGU	5' CAAATGCCACAGATCAA
5'	AGAACACAGGACAGGUUA	5' CCAAGGGAGACTTCAGCAG
5'	CAAGUAAGGCCUUGGUAAA	RNF168
5'	GACACUUUCUCCACAGAUA	5' CGAGGCACCTAAACAGAG
siRNF168 #18		5' GGTGTTGGATGCTTGTG
5'	GAGUAUCACUUACGCGCUA	CtIP
siRAD50 pool		5' CCTCAGAAAGTGCTCGCTTC
5'	GAACAAACACUGCAGAAUGU	5' TTCTGCTGCCGGATTTTC
5'	GAACAAGGAUCUGGAJAUU	BRCA2
5'	GCUCAGAGAUUGUGAAAUG	5' ACACCTAGCCAAAAGGCAGA
5'	UAACCUCACUGUUGGGUA	5' CTTAGCTTCGCACTTCC
siBRCA2 pool		RAD50
5'	CCAAGGAUGUUCUGUCAAA	5' GGCGCACAGAAAGGTTATGA
5'	CAAGCUACAUUJUGCAGAA	5' CATTGAATCCCAGGGAGCTA
5'	GAAACGGACUUGCUAUUUA	
siCtIP		
5'	GCUAAAACAGGAACGAUC	
siRNF168#18 resistance mutagenesis primer		
5'	aggatccccatgagtagatcaTCtaAgAgctacatcccccctcca	



Supplemental Figure S2. Representative FACS profiles showing the frequency of HR (% GFP+) for I-SceI transfections of U2OS SA-GFP cells treated with siCTRL, siBRCA1, siRNF168, and siBRCA1+siRNF168.



Supplemental Figure S3. Shown are representative cell cycle profiles for cells treated with siCTRL, siBRCA1, siRNF168#18, and siBRCA1+siRNF168#18.



SUPPLEMENTAL TABLE S1.

Shown are GFP frequencies for the repair assay experiments, determined as described in the Experimental Procedures & Figure Legends.

SAMPLE	mean %GFP+	SDEV	SAMPLE	mean %GFP+	SDEV
Figure 1B/C			Figure 5B		
<i>DRGFP</i> (<i>n</i> =9)			<i>DRGFP</i> (<i>n</i> ≥3)		
siCTRL	8.09	1.8	siCTRL	7.66	2.65
siRNF168	12.63	2.8	siRNF168	13.11	2.92
siBRCA1	1.00	0.58	siCtIP	2.46	0.52
siRNF168/siBRCA1	3.52	2.1	siCtIP/siRNF168	3.10	0.35
<i>SAGFP</i> (<i>n</i> =6)			siRAD50	3.87	1.20
siCTRL	4.63	0.17	siRAD50/siRNF168	6.51	2
siRNF168	7.63	0.35	<i>SAGFP</i> (<i>n</i> ≥3)		
siBRCA1	0.61	0.26	siCTRL	3.36	0.17
siRNF168/siBRCA1	4.04	1.2	siRNF168	5.60	0.80
<i>EJ2GFP</i> (<i>n</i> =3)			siCtIP	0.80	0.07
siCTRL	0.61	0.06	siCtIP/siRNF168	1.05	0.25
siRNF168	0.60	0.02	siRAD50	1.42	0.32
siBRCA1	0.25	0.02	siRAD50/siRNF168	1.66	0.44
siRNF168/siBRCA1	0.17	0.03	Figure 5C		
Figure 2B			<i>DRGFP</i> (<i>n</i> ≥3)		
<i>DRGFP</i> (<i>n</i> =3)			siCTRL/EV	4.24	0.07
siCTRL/EV	6.17	0.28	siRNF168/EV	7.00	1.47
siBRCA1/EV	0.15	0.01	siCTRL/BRC3	0.82	0.12
siBRCA1/siRNF168#18/EV	6.91	0.90	siRNF168/BRC3	0.93	0.17
siBRCA1/siRNF168#18/WT RNF168	2.18	0.20	siCTRL/RAD51K133R	1.14	0.02
siBRCA1/siRNF168#18/C19S RNF168	5.11	0.47	siRNF168/RAD51K133R	0.57	0.07
<i>SAGFP</i> (<i>n</i> =3)			siBRCA2	0.14	0.05
siCTRL/EV	3.07	0.28	siBRCA2/siRNF168	0.36	0.04
siBRCA1/EV	0.18	0.05	<i>SAGFP</i> (<i>n</i> ≥3)		
siBRCA1/siRNF168#18/EV	3.52	0.29	siCTRL/EV	1.98	0.41
siBRCA1/siRNF168#18/WT RNF168	1.32	0.30	siRNF168/EV	3.03	0.56
siBRCA1/siRNF168#18/C19S RNF168	2.62	0.60	siCTRL/BRC3	9.18	2.55
Figure 2C			siRNF168/BRC3	8.8	1.55
<i>DRGFP</i> (<i>n</i> =6)			siCTRL/RAD51K133R	7.13	0.26
siCTRL	9.47	0.83	siRNF168/RAD51K133R	7.24	0.53
siRNF168#18	13.74	0.89	siBRCA2	6.38	0.92
siBRCA1#6	0.17	0.06	siBRCA2/siRNF168	8.93	1.9
siRNF168#18/siBRCA1#6	2.57	0.60	Figure 6B (53BP1-/-)		
<i>SAGFP</i> (<i>n</i> =6)			<i>DRGFP</i> (<i>n</i> =9)		
siCTRL	3.70	0.40	EV	4.39	0.70
siRNF168#18	4.65	0.34	53BP1	2.37	0.47
siBRCA1#6	0.57	0.14	<i>SAGFP</i> (<i>n</i> =6)		
siRNF168#18/siBRCA1#6	4.66	0.48	EV	1.61	0.32
Figure 5A			53BP1	0.61	0.14
<i>DRGFP</i> (<i>n</i> =3)			<i>EJ2GFP</i> (<i>n</i> =6)		
siCTRL/EV	4.24	0.07	EV	1.95	0.21
siRNF168/EV	7.00	1.47	53BP1	1.48	0.17
siCTRL/BARD1-hb202	2.62	0.18	Figure 6D		
siRNF168/hB202	7.18	0.44	<i>DRGFP</i> (<i>n</i> ≥6)		
<i>SAGFP</i> (<i>n</i> =6)			siCTRL/EV	5.56	0.57
siCTRL/EV	1.98	0.41	siCTRL/dn53BP1	11.79	0.79
siRNF168/EV	3.03	0.56	siRNF168	9.43	0.98
siCTRL/BARD1-hb202	1.25	0.24	siRNF168/dn53BP1	10.59	0.94
siRNF168/hB202	3.19	0.71	<i>SAGFP</i> (<i>n</i> =6)		
			siCTRL/EV	2.28	0.54
			siCTRL/dn53BP1	4.50	0.68
			siRNF168	3.54	0.25
			siRNF168/dn53BP1	3.80	0.34