SUPPLEMENTAL DATA

γH2A Binds Brc1 to Maintain Genome Integrity During S-Phase

Jessica S. Williams[1,5,6], R. Scott Williams[1,2,5,6], Claire L. Dovey[1], Grant Guenther[1,2], John A. Tainer[1,2,3,4]*, and Paul Russell[1,3]*

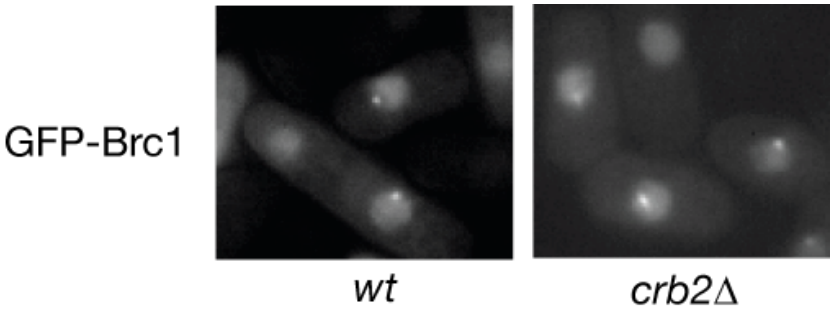

Figure S1. Crb2 is not required for spontaneous Brc1 foci formation. Cells were grown to mid-log phase in selective medium and live cell microscopy was performed of ectopically expressed GFP-Brc1 driven by the *nmt41* promoter in wt and *crb2Δ* cells.

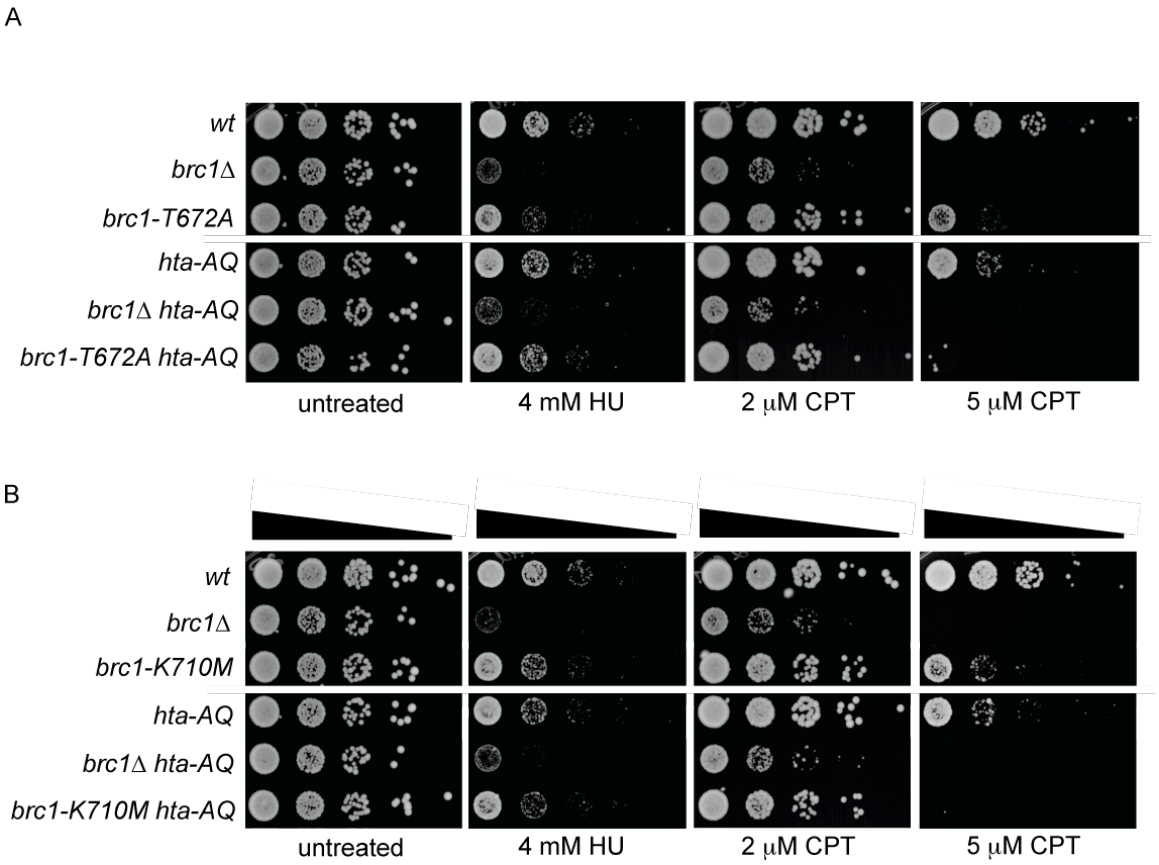

Figure S2. Genetic epistasis analysis of the *brc1-BRCT* and *hta-AQ* mutants. Ten-fold serial dilutions of cells were exposed to the indicated S-phase DNA damaging agent and incubated at 30ºC for 3 days.

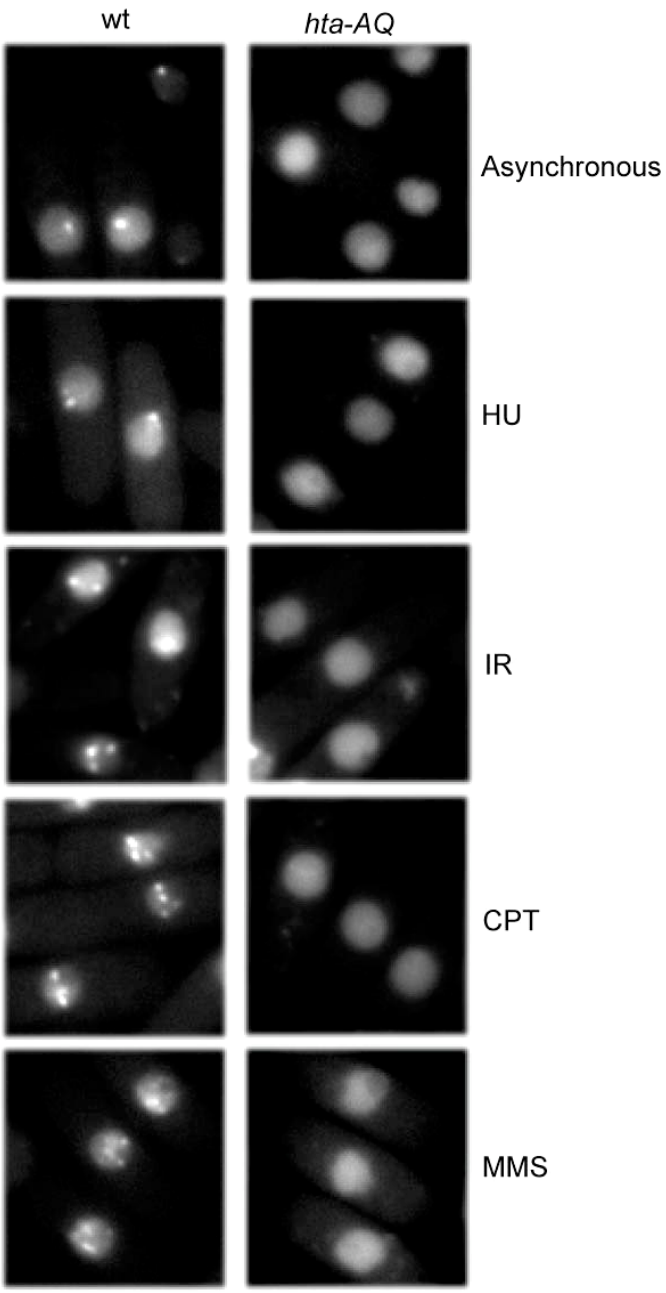

Figure S3. γH2A is required for Brc1 foci formation in response to DNA damaging agents. Cells were grown to mid-log phase in selective medium and live cell microscopy was performed of ectopically expressed GFP-Brc1 driven by the *nmt41* promoter in wt and *hta-AQ* cells. Treatment was with 12 mM HU for 4h, 30 μM CPT for 4h, 0.03% MMS for 5h, or 120 Gy IR plus 2h recovery.

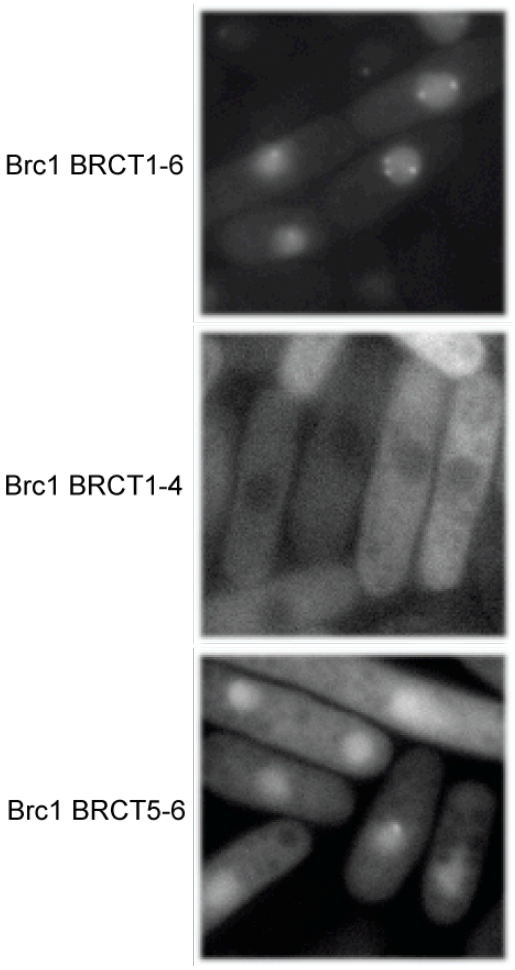

Figure S4. The Brc1 $BRCT_5$-$BRCT_6$ domain is necessary and sufficient for spontaneous foci formation. Cells were grown to mid-log phase in selective medium and live cell microscopy was performed of ectopically expressed Brc1 full-length (BRCT1-6), BRCT1-4 and BRCT5-6 versions driven by the *nmt41* promoter in wt cells.

Table S1. The genotypes of strains used in this study.

Strain	Genotype	Source
CD4101	*h- rad22-D2::LEU2*	Lab stock
LLD3260	*h- ura4-D18 crb2::ura4$^+$ leu1-32::2xYFP-crb2-wt-leu1$^+$*	Lab stock
LLD4116	*h- ura4-D18 crb2::ura4$^+$ leu1-32::2xCFP-crb2-wt-leu1$^+$*	Lab stock
1598	*h- leu1-32*	Lab stock
3259	*h- leu1-32 ura4-D18 crb2::ura4$^+$*	Lab stock
3333	*h+ ura4-D18 leu1-32 his3-D1 rad22-YFP-KanMx*	Lab stock
JW29	*h- ura4-D18 leu1-32 pRep41-N-GFP*	This study
JW59	*h- leu1-32 brc1::KanMx*	This study
JW60	*h+ leu1-32 brc1::KanMx*	This study
JW98	*h? ura4-D18 leu1-32 pRep41-N-GFP-brc1$^+$hta1-S129A::ura4$^+$ hta2-S128A::his3$^+$*	This study
JW107	*h- ura4-D18 leu1-32 pRep41-N-GFP-brc1$^+$*	This study
JW109	*h- ura4-D18 leu1-32 pRep41-N-GFP-brc1-T672A*	This study
JW113	*h- ura4-D18 leu1-32 brc1::KanMx pRep41-N-GFP-brc1$^+$*	This study
JW184	*h- ura4-D18 leu1-32 crb2::ura4$^+$ brc1::KanMx*	This study
JW219	*h? ura4-D18 leu1-32 ade6-M210 his3-D1 rad3::ura4$^+$ tel1::KanMx pRep41-N-GFP-brc1$^+$*	This study
JW223	*h+ ura4-D18 leu1-32 his3-D1 ade6-M216 rad3::ura4$^+$ pRep41-N-GFP-brc1$^+$*	This study
JW228	*h+ ura4-D18 leu1-32 tel1::KanMx his3-D1 ade6-M216 pRep41-N-GFP-brc1$^+$*	This study
JW265	*h+ leu1-32 brc1-wt-2xGFP:HphMx*	This study
JW270	*h? ura4-D18 leu1-32 crb2::ura4$^+$ pRep41-N-GFP-brc1$^+$*	This study
JW271	*h- ura4-D18 leu1-32 pRep41-N-GFP-brc1-K710M*	This study
JW307	*h+ leu1-32 brc1-T672A-2xGFP:HphMx*	This study
JW309	*h+ leu1-32 brc1-K710M-2xGFP:HphMx*	This study

JW397	*h+ leu1-32 brc1::HphMx*	This study
JW414	*h- ura4-D18 leu1-32 his3-D1 brc1::HphMx rad22-YFP-KanMx*	This study
JW451	*h- ura4-D18 leu1-32 his3-D1 rad22-RFP:NatMx pRep41-N-GFP*	This study
JW452	*h- ura4-D18 leu1-32 his3-D1 rad22-RFP:NatMx pRep41-N-GFP-brc1^{+}*	This study
JW479	*h- ura4-D18 leu1-32 gar1-RFP:KanMx pRep41-N-GFP*	This study
JW480	*h- ura4-D18 leu1-32 gar1-RFP:KanMx pRep41-N-GFP-brc1^{+}*	This study
JW485	*h- ura4-D18 leu1-32 brc1::HphMx pREP41-N-GFP-brc1^{+}*	This study
JW487	*h- ura4-D18 leu1-32 brc1::HphMx pREP41-N-GFP-brc1-BRCT$_{5-6}$*	This study
JW488	*h- ura4-D18 leu1-32 brc1::HphMx pREP41-N-GFP-brc1-BRCT$_{1-4}$*	This study
JW523	*h? ura4-D18 leu1-32 brc1::HphMx pRep41-N-GFP-brc1-R707E*	This study
JW535	*h-? ura4-D18 leu1-32 brc1::HphMx pRep41-N-GFP-brc1-R704E*	This study
JW702	*h? ura4-D18 crb2::NatMx leu1-32::2xCFP-crb2-wt-leu1^{+} pRep42-N-GFP brc1::HphMx*	This study
JW704	*h? ura4-D18 crb2::NatMx leu1-32::2xCFP-crb2-wt-leu1^{+} pRep42-N-GFP-brc1^{+} brc1::HphMx*	This study

Table S2: X-ray diffraction data collection, phasing, and refinement statistics

Data Collection

Data set		BRCT5/6-MAD			BRCT5/6-Native	BRCT5/6:γH2A complex
Space group		$P2_1$ (β =101.91)			$P2_12_12_1$	$P2_12_12_1$
Cell dimensions	a (Å)	51.29			60.47	41.58
	b (Å)	84.47			83.43	71.12
	c (Å)	50.88			87.25	72.01
		λ1	λ2	λ3		
Wavelength (Å)		0.9796	0.9797	1.0087	1.111	1.111
Resolution range (Å)		50-1.80	50-1.80	50-1.80	50-1.55	50-1.45
Observations[1]		284722	284297	293611	420116	137096
Unique reflections[1]		39433	39454	38791	61324	36848
Data coverage: total/final shell[2] (%)		97.6/90.7	97.5/89.6	98.3/97.3	94.8/67.0	95.6/67.7
<I/σI> total/final shell		23.7/2.8	24.4/2.75	26.1/4.17	38.6/2.2	21.7/2.6
R_{sym} total/final shell (%)[3]		6.1/31.7	5.6/32.2	5.3/32.5	4.5/50.2	5.2/23.9

Phasing Statistics

Resolution range (Å)	50-1.80
# of Selenium Sites	4/4 for 440 aa
<FOM> from Solve	0.37
<FOM> from Resolve	0.59

Refinement Statistics

Resolution range(Å)		50-1.55	50-1.45
R_{work}/R_{free} (%)[4]		17.9/22.5	13.1/18.8
Refined atoms	Protein	3368	1696
	Solvent	407	451
	Peptide	-	49
R.m.s. deviations	Bonds (Å)	0.020	0.021
	Angles (°)	1.79	1.84
Average B-factors(Å^2)	Protein	27.1	11.3
	Solvent	35.4	31.5
	Peptide	-	13.2
Ramachandran[5]	Core	94.6	93.4
	Allowed	5.4	6.6
	Generous	0.0	0.0
	Disallowed	0.0	0.0

[1] For MAD datasets, Bijvoets (I+ and I-) were kept separate during scaling and for calculation of statistics.
[2] Final shell: 1.85-1.80 Å (MAD); 1.61-1.55 (Brc1-Native); 1.5-1.45 (Brc1-γH2A complex)
[3] $R_{sym}=\Sigma |(I_{hkl}) - <I>| / \Sigma(I_{hkl})$ where I_{hkl} is the integrated intensity of a given reflection.
[4] $R_{work} = \Sigma_h|F_o(h) - F_c(h)| / \Sigma_h | F_o(h)|$, where $F_o(h)$ and $F_c(h)$ are observed and calculated structure factors. R_{free} calculated with 5% of all reflections excluded from refinement stages using the native data set. No I/σI cutoff was used in the refinement.
[5] PROCHECK Ramachandran plot statistics.