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Supplemental Information

Relocation of Collapsed Forks to the Nuclear Pore Complex Depends on Sumoylation of DNA Repair Proteins and Permits Rad51 Association

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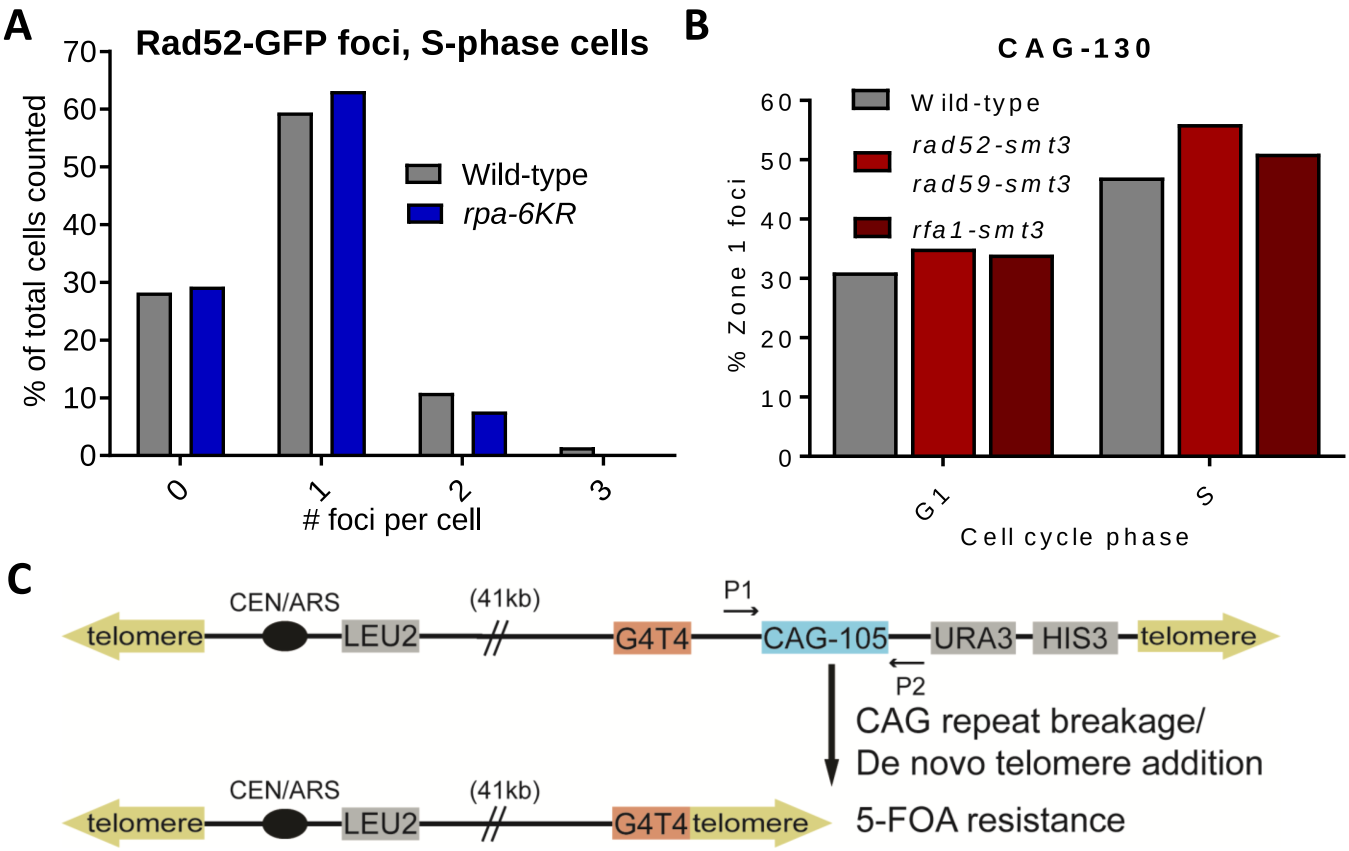


Figure S1 Related to Figures 1 and 2: A) Percentage of S-phase cells with the indicated number of MMS induced Rad52-GFP foci in wild-type and *rpa-6KR* strains**.** 138 cells were analyzed for wild-type and 236 cells were analyzed for *rpa-6KR*; raw values in Table S3. B) Percent of Zone 1 foci for CAG-130 G1- and S-phase cells in wild-type, and *rad52-smt3 rad59-smt3*, and *rfa1-smt3* strains. Smt3 is at the C-terminus of each. The number of cells analyzed per strain ranges from 150-283. See Table S1 for the exact number of cells analyzed, percentages, and P-values. See Table S1 and Figure S7B for zoning data for individual strains. C) Schematic of the CAG fragility assay. P1and P2 arrows indicate primers used to confirm CAG tract length prior to each experiment. The G_4T_4 sequence proximal to the CAG repeat facilitates recovery of end loss events by providing a seed for telomere addition by telomerase.

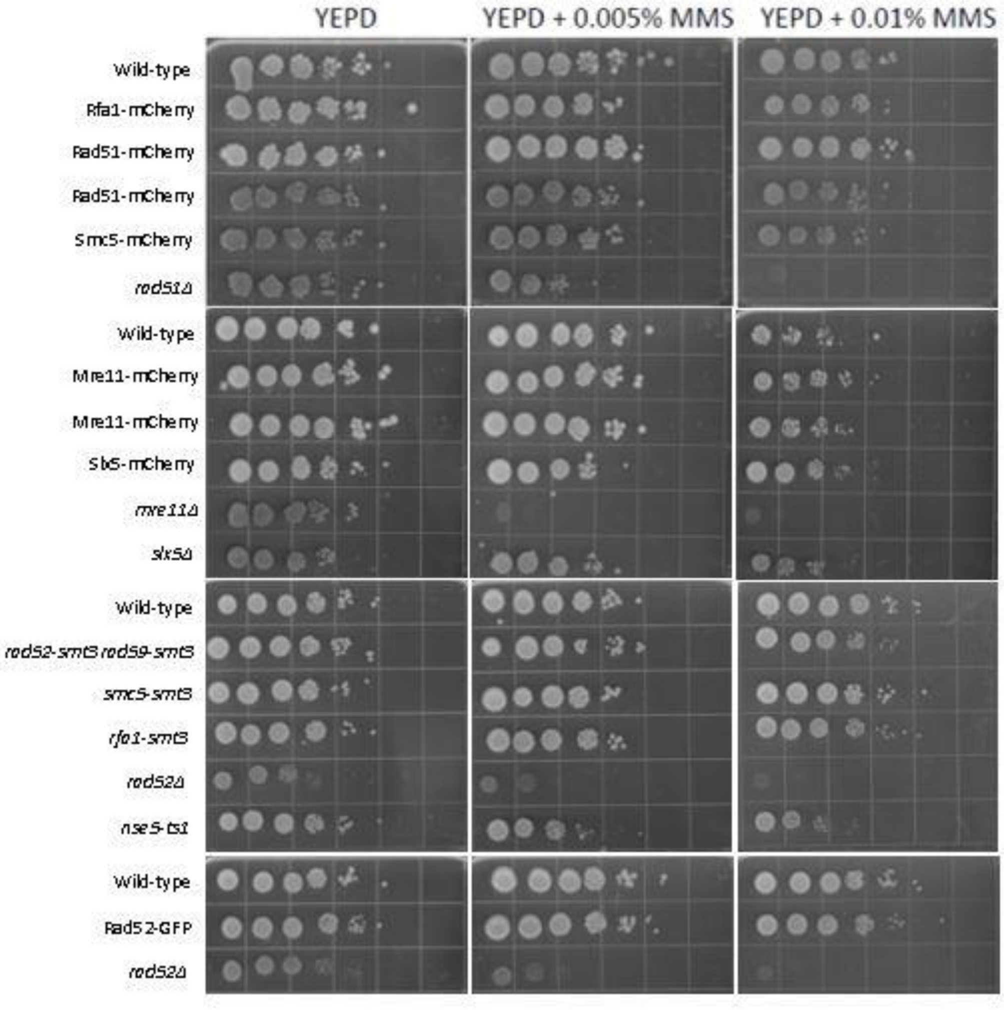
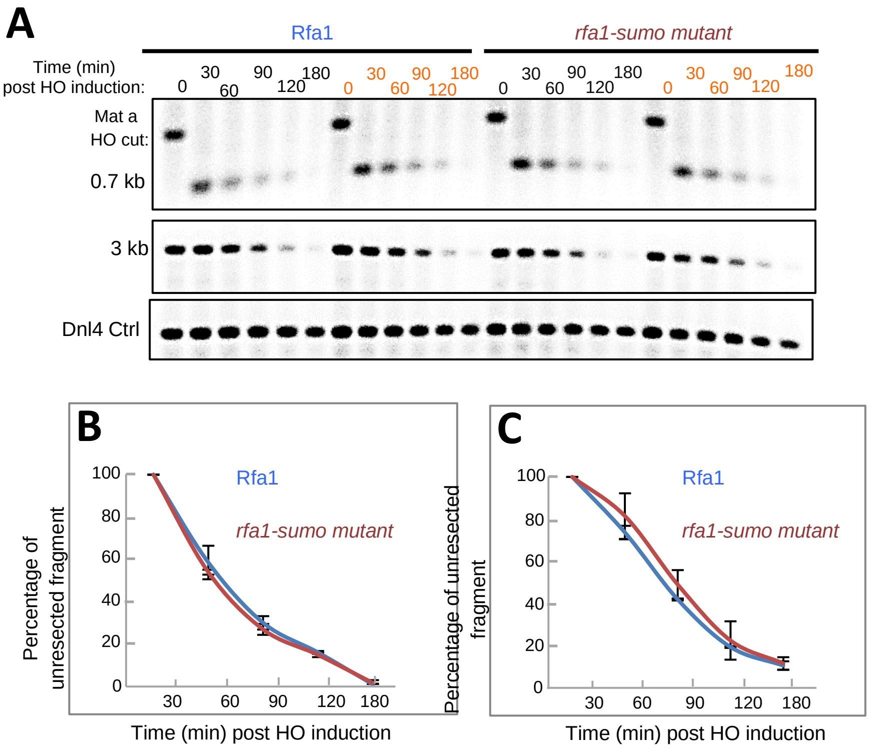


Figure S2: MMS sensitivity spot assay for mutants. Related to Figures 2, 3, 5, and 6.
Growth of mutants on YEPD media containing 0.005% and 0.01% methyl methanesulfonate (MMS) as compared to YEPD media without MMS. Cells were 10-fold serially diluted six times and grown at 30C for two days.



0.7 kb Fragment

3 kb Fragment

Figure S3: Loss of RPA1 sumoylation does not reduce DSB resection efficiency. Related to Figure2. Southern blot (A) and quantification (B-C) showing the kinetics of the disappearance of two fragments with end points located 0.7 kb (top panel) or 3 kb (middle panel) away from the HO cut site for the wt (Rfa1) or *rfa1-4KR* strains. Dnl4 signal (bottom panel) indicates equal loading. Two independent isolates are shown for each genotype. Quantification shows the ratios of 0.7 kb (B) and 3 kb (C) fragment signals to the Dnl4 signal, with the ratio at 30 mins set to 100%.

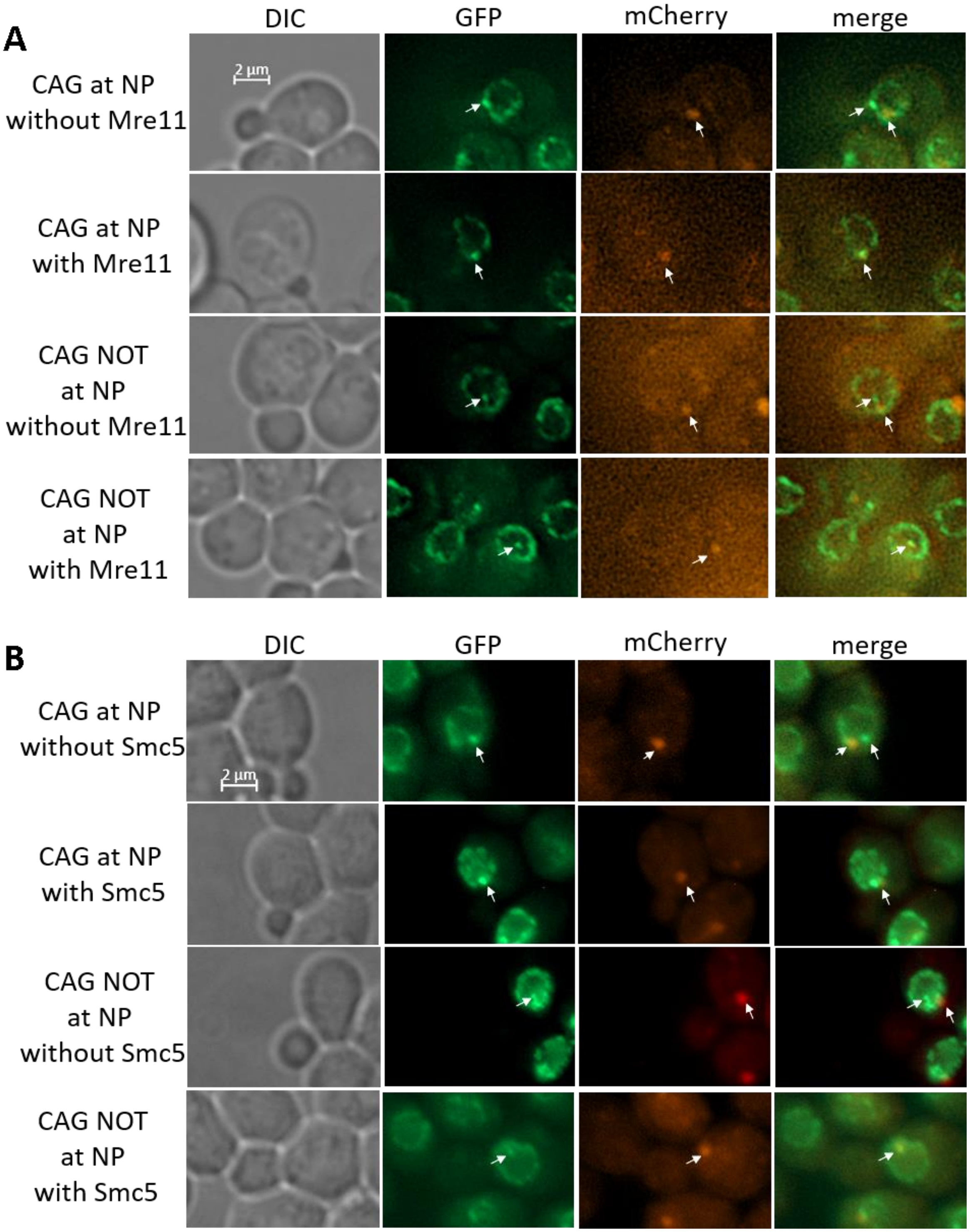


Figure S4: Co-localization example images in S-phase cells. Related to Figure 5. Example images of the CAG locus (GFP) and tagged protein foci (mCherry) co-localization for data in Figure 5. A) Mre11-mCherry B) Smc5-mCherry.

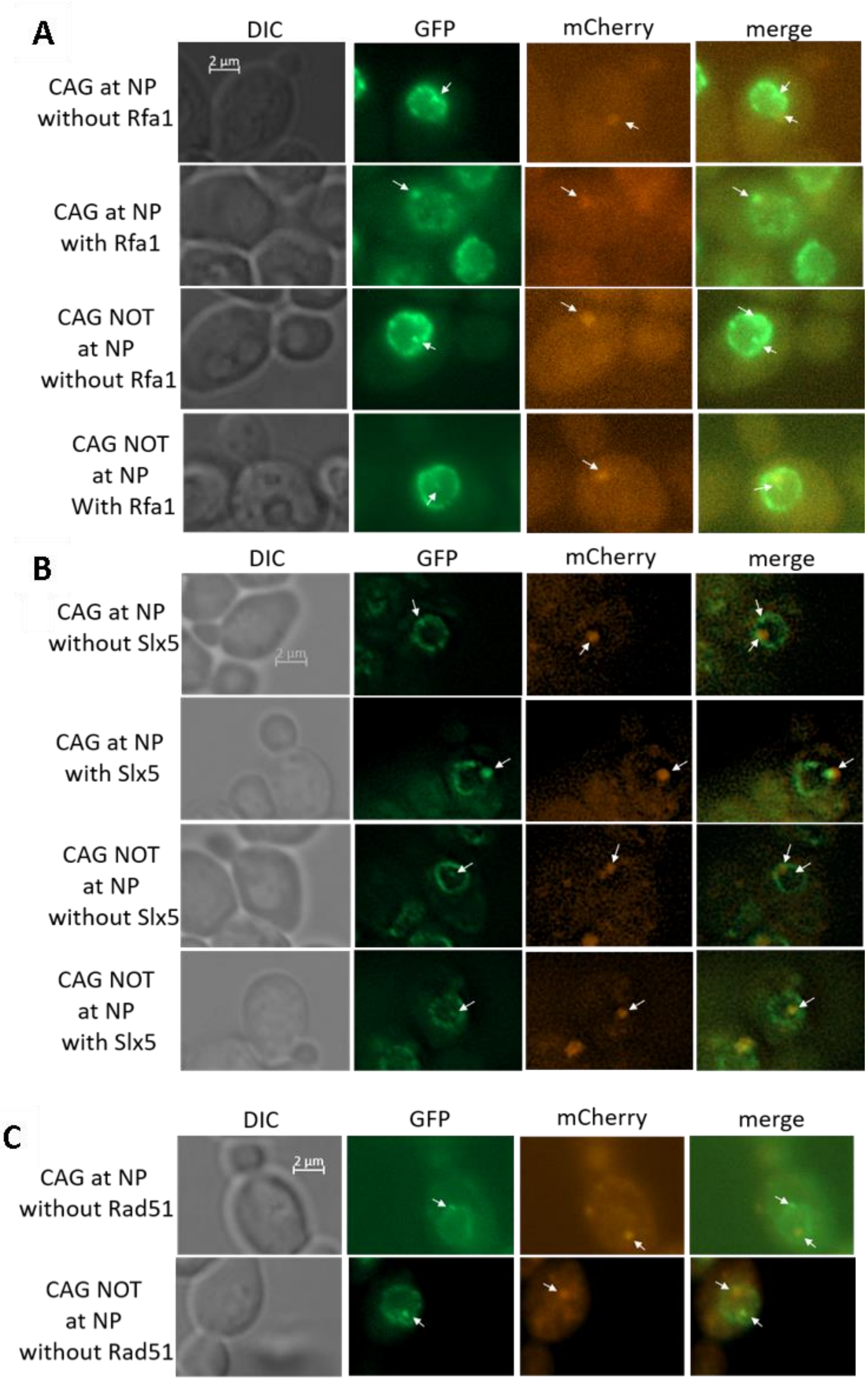


Figure S5: Co-localization example images in S-phase cells. Related to Figures 5 and 6. Example images of the CAG locus (GFP) and tagged protein foci (mCherry) co-localization for data in Figure 5 and 6. A) Rfa1-mCherry B) Slx5-mCherry and C) additional Rad51-mCherry examples.

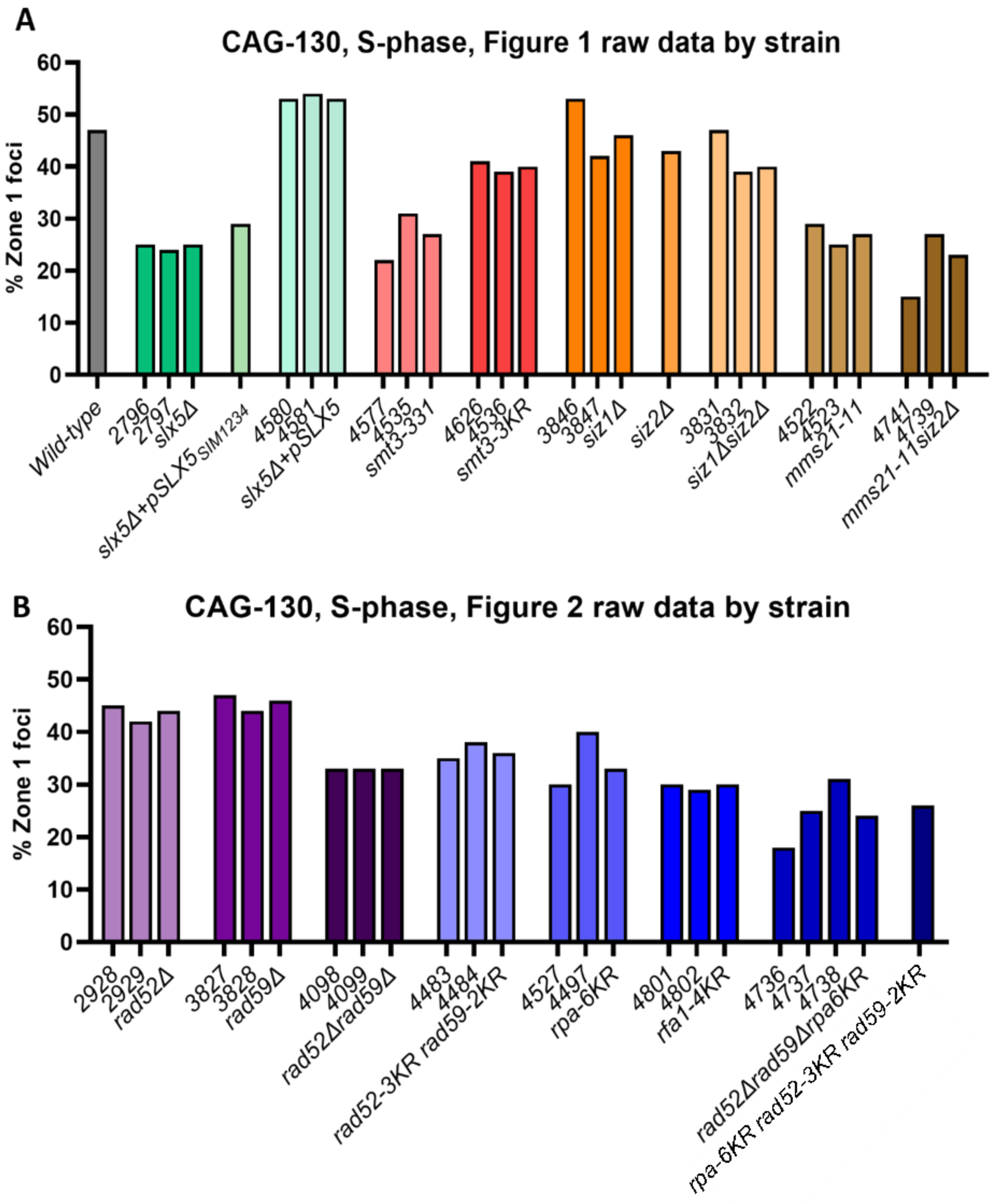


Figure S6: Raw zoning assay data separated by strain. Related to Figures 1and 2. Percentage of zone 1 foci for individual strains from A) Figure 1 and B) Figure 2. Each mutant has a unique color and the data for individual strain numbers is shown followed by the combined data labeled with the mutant name. See Table S1 for the exact number of cells analyzed, percentages, and P-values.

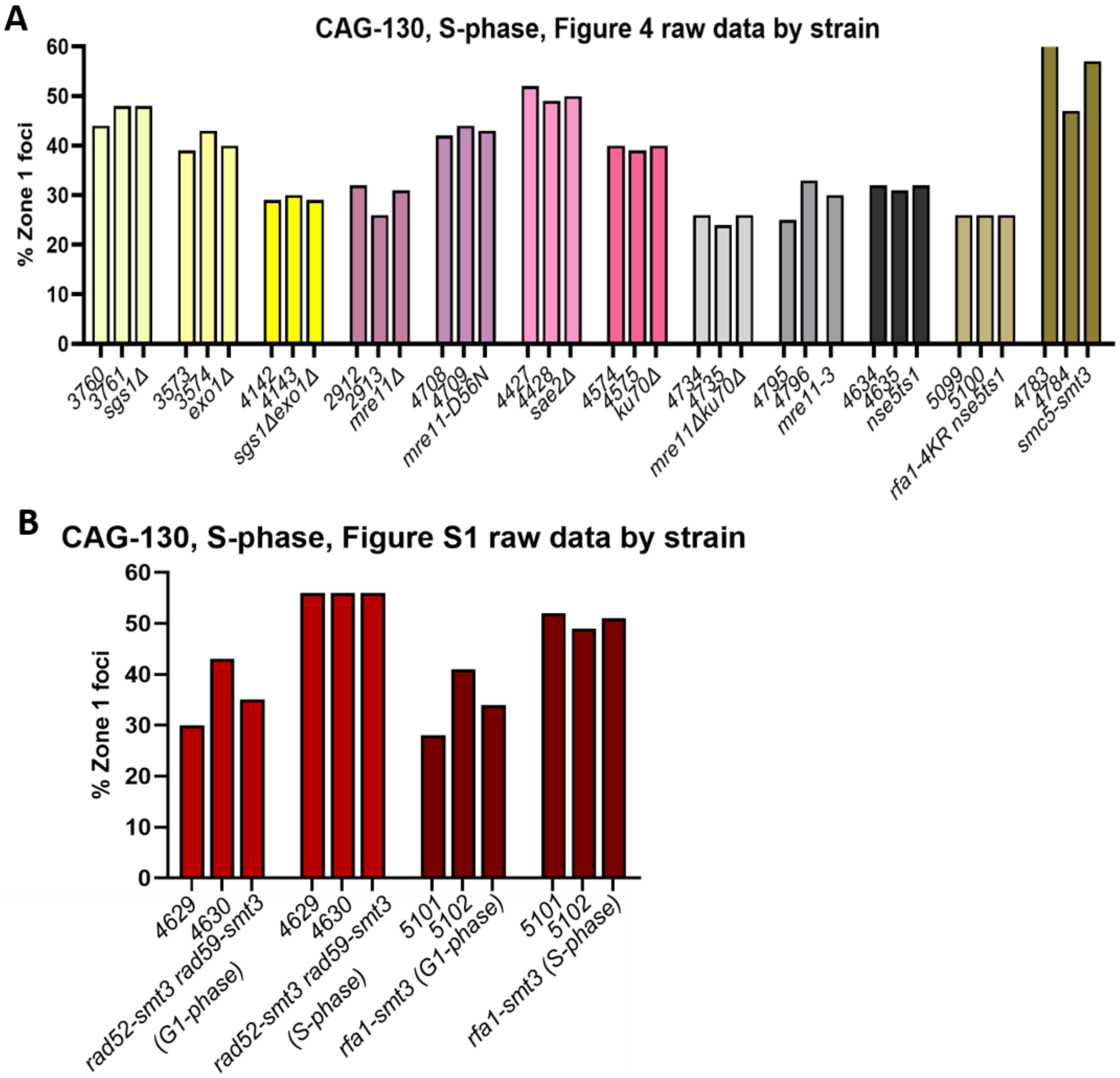


Figure S7: Raw zoning assay data separated by strain. Related to Figures 4 and S1. Percentage of zone 1 foci for individual strains from A) Figure 4 and B) Figure S1. Each mutant has a unique color and the data for individual strain numbers is shown followed by the combined data labeled with the mutant name. See Table S1 for the exact number of cells analyzed, percentages, and P-values.

Supplementary Table 2: Fragility Analysis of CAG-105 repeats on the LEU2-URA3-HIS3 YAC. Related to Figure 1.

	Average Rate of FOA^R (x10^{-6}) ± SEM	**Rate of FOA^R (x10^{-6}) for each assay**	**Fold over WT**	**No. of assays**	**% End loss**	**p-value to WT**
Wild-type	10.1±0.52	11.1	---	5	96	---
		10.2				
		11.2				
		9.6				
		8.4				
slx5Δ	24.53 ±1.99	26.3	2.4	4	92	0.0001
		29.3				
		20.9				
		21.6				
smt3-331	38.1 ±8.53	55.1	3.8	5	98	0.0113
		21.6				
		60.4				
		35				
		18.4				
mms21-11	19.45 ±2.49	16.4	1.9	6	97	0.0087
		29.8				
		13.4				
		23.3				
		18.7				
		15.1				

Supplementary Table 3: Rad52-GFP foci analysis in wild-type and *rpa-6KR* strains. Related to Figure S1.

Strain	**No. of Rad52-GFP foci per cell**								**Total No. of cells**
	0		**1**		**2**		**3**		
	No. of cells	**%**	**No. of cells**	**%**	**No. of cells**	**%**	**No. of cells**	**%**	
Wild-type	39	28.2	82	59.4	15	11	2	1.4	138
rpa-6KR	69	29	149	63	18	8	0	0	236

Supplementary Table 4: Zoning Assay time course analysis of *rad52-smt3rad59-smt3* and *smc5-smt3*. Related to Figures 2 and 4.

			Strain			
			Wild-type	*rad52-smt3 rad59-smt3*	*smc5-smt3*	*rfa1-smt3*
Time after α-factor release (minutes)	**20**	**No. Zone 1 foci**	44	37	44	45
		%	21.8	24.7	27	29
		Total No. cells	202	150	161	154
		p-value to WT*	---	0.5254	0.2672	0.1106
	30	**No. Zone 1 foci**	57	57	43	53
		%	32	36.5	28.2	33

		Total No. cells	179	156	152	159
		p-value to WT*	---	0.4186	0.5484	0.8164
	40	**No. Zone 1 foci**	47	88	72	58
		%	26.9	38.2	38.3	38.4
		Total No. cells	175	230	188	151
		p-value to WT*	---	0.02	0.025	0.0323
	50	**No. Zone 1 foci**	71	93	75	83
		%	34	46.5	38.7	45
		Total No. cells	206	200	194	185
		p-value to WT*	---	0.0153	0.4068	0.0386
	60	**No. Zone 1 foci**	89	75	77	69
		%	48.9	46	42.8	46
		Total No. cells	182	163	180	151
		p-value to WT*	---	0.6659	0.2481	0.5828

*using the Fisher's exact test

Supplementary Table 5: Co-localization of mCherry tagged proteins with the CAG repeat in S-phase cells. Related to Figure 5.

Tagged Protein	Total # of cells counted	A	B	C	D	E	F	G	H	% (#) of CAG at NP with tagged protein (B/(G+B))	I	J	% (#) of CAG NOT at NP with tagged protein (D/(I+D))	K
		% (#) CAG at NP without tagged protein	% (#) CAG at NP with tagged protein	% (#) CAG NOT at NP without tagged protein	% (#) CAG NOT at NP with tagged protein	% (#) CAG and tagged protein at NP, but not co-loc	% (#) CAG NOT at NP, tagged protein at NP	% (#) CAG at NP without tagged protein (A+E)	% (#) CAG at NP with tagged protein (B)		% (#) CAG NOT at NP without tagged protein (C+F)	% (#) CAG NOT at NP with tagged protein (D)		% (#) of tagged protein at the NP ((B+E+F) /Total)
Mre11	75	1.3 (1)	20 (15)	0 (0)	10.7 (8)	48 (36)	20 (15)	49 (37)	20 (15)	29 (15)	20 (15)	11 (8)	35 (8)	88 (66)
Smc5	95	4 (4)	36 (34)	6 (6)	14 (13)	22 (21)	18 (17)	26.3 (25)	35.8 (34)	58 (34)	24.2 (23)	13.7 (13)	36 (13)	76 (72)
Rfa1	90	1 (1)	27 (24)	1 (1)	10 (9)	35.5 (32)	25.5 (23)	36 (33)	27 (24)	42 (24)	27 (24)	10 (9)	27 (9)	88 (79)
Slx5	105	3 (3)	44 (46)	3 (3)	13 (14)	10 (11)	27 (28)	13 (14)	44 (46)	73 (46)	30 (31)	13 (14)	31 (14)	81 (85)
Rad51	94	5 (5)	25 (23)	18 (17)	3 (3)	13 (12)	36 (34)	18 (17)	25 (23)	58 (23)	54 (51)	3 (3)	5.6 (3)**	73 (69)

For before relocation (**) p=0.002 compared with Mre11-mCherry by Fisher's exact test. Comparisons were not done for after relocation data since it wasn't normalized to Mre11 occupancy.

Supplementary Table 6: Timecourse of Rad51 co-localization with the CAG repeat. Related to Figure 6.

Time after alpha factor release	Total # of cells counted	A	B	C	D	E	F	G	H	% (#) of CAG at NP with Rad51 (B/(G+B))	I	J	% (#) of CAG NOT at NP with Rad51 (D/(I+D))	K
		% (#) CAG at NP without Rad51	% (#) CAG at NP with Rad51	% (#) CAG NOT at NP without Rad51	% (#) CAG NOT at NP with Rad51	% (#) CAG and Rad51 at NP, but not co-loc	% (#) CAG NOT at NP, Rad51 at NP	% (#) CAG at NP without Rad51 (A+E)	% (#) CAG at NP with Rad51 (B)		% (#) CAG NOT at NP without Rad51 (C+F)	% (#) CAG NOT at NP with Rad51 (D)		% (#) of Rad51 at the NP ((B+E+F)/Total)
S-phase cells	94	5 (5)	25 (23)	18 (17)	3 (3)	13 (12)	36 (34)	18 (17)	25 (23)	58 (25)	54 (51)	3 (3)	5.6 (3)	73 (69)
50 min	63	2 (1)	16 (10)	9 (6)	0 (0)	25 (16)	48 (30)	27 (17)	15.9 (10)	37 (10)	57.1 (36)	0 (0)	0 (0)	88 (56)
60 min	94	5 (5)	30 (28)	7 (7)	1 (1)	27 (25)	30 (28)	32 (30)	29.8 (28)	48 (28)	37 (35)	1 (1)	2.7 (1)	86 (81)
70 min	95	0 (0)	8 (8)	4 (4)	0 (0)	31 (29)	57 (54)	30.5 (29)	8.4 (8)	22 (8)	61.1 (58)	0 (0)	0 (0)	97 (91)
80 min	96	2 (2)	9 (8)	5 (5)	4 (4)	28 (27)	52 (50)	30.2 (29)	8.3 (8)	22 (8)	57.3 (55)	4.2 (4)	6.8 (4)	89 (85)

Supplementary Table 7: Rad51 co-localization with the CAG repeat in mutant S-phase strains. Related to Figure 6.

Mutant strain	Total # of cells counted	A	B	C	D	E	F	G	H	% (#) of CAG at NP with Rad51 (B/(G+B))	I	J	% (#) of CAG NOT at NP with Rad51 (D/(I+D))	K
		% (#) CAG at NP without Rad51	% (#) CAG at NP with Rad51	% (#) CAG NOT at NP without Rad51	% (#) CAG NOT at NP with Rad51	% (#) CAG and Rad51 at NP, but not co-loc	% (#) CAG NOT at NP, Rad51 at NP	% (#) CAG at NP without Rad51 (A+E)	% (#) CAG at NP with Rad51 (B)		% (#) CAG NOT at NP without Rad51 (C+F)	% (#) CAG NOT at NP with Rad51 (D)		% (#) of Rad51 at the NP ((B+E+F)/Total)
Wild-type	94	5 (5)	25 (23)	18 (17)	3 (3)	13 (12)	36 (34)	18 (17)	25 (23)	58 (23)	54 (51)	3 (3)	5.6 (3)	73 (69)
smt3-331	54	2 (1)	28(15)	(0) 0	7 (4)	39 (21)	24 (13)	41 (22)	28 (15)	41 (15)	24 (13)	7 (4)	24 (4)*	91 (49)
rpa-6KR	79	1.3 (1)	25.3 (20)	1.3 (1)	10.1 (8)	24 (19)	38 (30)	25.3 (20)	25.3 (20)	50 (20)	39 (31)	10.1 (8)	26 (8)*	87 (69)
rfa1-4KR	94	2 (2)	10 (9)	5 (5)	12 (11)	17 (16)	54 (51)	19 (18)	9 (9)	33 (9)	60 (56)	12 (11)	16 (11)	81 (76)
rad52-3KR rad59-2KR	106	3 (3)	25 (27)	2 (2)	0 (0)	16 (17)	54 (57)	19 (20)	25 (27)	57 (27)	56 (59)	0 (0)	0 (0)	95 (101)
slx5Δ	68	3 (2)	16 (11)	3 (2)	4 (3)	34 (23)	40 (27)	37 (25)	16 (11)	30 (11)*	43 (29)	4 (3)	10 (3)	90 (61)

(*)p<0.05 compared with wild-type by Fisher's exact test. Exact p-values listed below in Table S8.

Supplementary Table 8: p-values for Rad51 co-localization with CAG repeat in mutant S-phase strains. Related to Figure 6.

Mutant strain	**p-value to WT (Fisher's exact) for % CAG NOT at NP with Rad51**	**p-value to WT (Fisher 's exact) for % CAG at NP with Rad51**
smt3-331	0.05	0.1733
rpa-6KR	0.046	0.654
rfa1-4KR	0.087	0.0805
rad52-3KR rad59-2KR	0.106	1.0
slx5Δ	0.6664	0.0224