SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Analysis of Distribution of HMR Topoisomer— Topological analyses of HMR were performed as described previously and as outlined below and in Supplementary Fig. 3 (1,2). Yeast were grown logarithmically to approximately 0.5 OD_{600}/ml in minimal medium containing adenine, leucine, uracil and tryptophan plus 2% raffinose to express Gal4-Sir1p or analogous media also containing methionine to repress Gal4-Sir1p expression. To induce FLP recombinase to excise *HMR* from the chromosome, galactose was added to the medium to a final concentration of 2% and cells were incubated for one and one-half hours at 30°C. Then, 0.5 M EDTA, pH 8.0, was added to the medium to a final concentration of 40 mM, and an equal volume of iced growth termination cocktail (95:3:2 ratio by volume of ethanol:toluene: 1 M Tris-HCl, pH 8.0) was added to the culture. Cells were pelleted by centrifugation, resuspended in 500 µl spheroplasting solution (0.95 M Sorbitol, 25 mM EDTA, pH 8.0, 4.8 mM β-mercaptoethanol, 0.4 mg/ml Zymolyase) per 25 OD_{600} of cells and incubated at 30°C for 30 minutes. Spheroplasts were pelleted by centrifugation and resuspended in 360 µl TE (10 mM Tris-HCl, pH 8.0, 10 mM EDTA, pH 8.0). Then, 40 µl of 20% SDS and 100 µl of 5 M potassium acetate were sequentially added to lysates and lysates were incubated for 15 minutes on ice. Lysates were separated by centrifugation, the supernatants were collected and precipitated by adding 2.5 volumes of 100% ethanol. Samples were resuspended in TE, RNAse A was added to a final concentration of 0.15 mg/ml, and samples were incubated at 37°C for 20 minutes. Samples were extracted with phenol:chloroform twice, ethanol precipitated and resuspended in dH_2O.

DNA samples were separated by electrophoresis in 0.8% agarose gels containing 0.65 µg/ml chloroquine and TBE (40 mM Tris-HCl, pH 8.0, 1% boric acid, 10 mM EDTA, pH 8.0) in TBE buffer containing 0.65% µg/ml chloroquine at 1.4 V/cm for 20 hours. More negatively supercoiled DNA will migrate more rapidly through the gel under these conditions. DNA blotting was performed as previously described (3), and blots were probed with a *Nru*I-*Pst*I fragment of pJR1619 (4) containing a synthetic silencer with four Gal4p binding sites, a Rap1p binding site and an Abf1p binding site in place of the *HMR-E* silencer and the genes encoding **a2** and **a1** but lacking the *HMR-I* silencer. The probe was labeled with ^{32}P α-dCTP using the Rediprime II Random Prime Labeling System (Amersham Biosciences) or ^{32}P α-dATP using the Strip-EZ Kit (Ambion) as per manufacturers' instructions. Blots were analyzed using a PhosphorImager (Molecular Dynamics) to generate histograms of the topoisomers of each sample. As the topoisomers exhibited a non-Gaussian distribution in these analyses, the area under each isoform peak was determined, expressed as a fraction of the total area and multiplied by the rank order of each isoform. The sum of this ranked order was divided by 100 to calculate the center of distribution of topoisomers for each sample.

SUPPLEMENTARY RESULTS

Effects of pol30 Mutants on the Topology of HMR— The topology of circular double-stranded DNA molecules is affected by several factors including nucleosome density, transcription, Sir proteins and histone acetylation (5-18). Therefore, to examine *pol30*-dependent effects on chromatin independent of transcription, we tested whether *pol30* mutants affected the topology of an *HMR* locus that lacked Sir proteins and that lacked internal promoter sequences. To do so, we used a modified *HMR, HMRaΔp266,* differing from the *HMR* locus used in the previous experiment only in that the ***a1***-***a2*** promoter region was replaced with an equivalent amount of heterologous DNA, which disrupted transcription of the ***a1*** and ***a2*** genes (Supplementary Fig. 3A). We confirmed that transcription of **a1** mRNA from *HMRa*Δp266 was defective by quantitative real-time PCR. In the absence of Gal4-Sir1p, the amount of **a1** detected in *POL30* cells containing *HMRaΔp266* averaged less than 2% (1.2, 2.5%, n = 2) of that observed in *POL30* cells containing *HMR-GalSS*. Thus, any alterations in supercoiling of *HMRaΔp266* in *pol30* mutants relative to *POL30* cells would reflect transcription-independent

effects. In this experiment, *HMRaΔp266* was excised from Chromosome III in logarithmically growing *POL30* and *pol30* cells by inducing Flp1p. DNA was purified, and topoisomers of *HMRaΔp266* were electrophoretically separated on chloroquine gels and monitored via DNA blots using a probe that annealed to the *HMR* locus. The topological patterns of *HMRaΔp266* were nearly identical in *POL30* and *pol30* cells in the absence of Sir protein recruitment (Supplementary Fig. 3B, lane 5 versus lanes 6, 7 and 8 and Supplementary Fig. 3C, lower panel; (ΔLK = -0.04 ± 0.1, 0.04 ± 0.2 and -0.003 ± 0.3 for *pol30-8*, *pol30-6* and *pol30-79*, respectively, relative to *POL30*; n = 3 for each comparison). These results indicated that the DNA at *HMR* was efficiently packaged into nucleosomes in the *pol30* mutants.

To examine the effects of Sir proteins on chromatin structure independent of any topological changes related to the inactivation of transcription, the topology of *HMRaΔp266* in the presence of Sir proteins was also assessed in the *pol30* mutants. Expression of Gal4-Sir1p in these strains caused a Sir protein-dependent increase in negatively supercoiled *HMRaΔp266* DNA (Supplementary Fig. 3B, lanes 5, 6, 7 or 8 versus lanes 1, 2, 3, or 4, respectively, and Supplementary Fig. 3C lower versus upper panels) that also led to a small Sir-dependent shift in the center of distribution of *HMRaΔp266* topoisomers (ΔLK = 0.4 ± 0.4, 0.4 ± 0.3, 0.4 ± 0.3 and 0.5 ± 0.3 for *POL30, pol30-8*, *pol30-6* and *pol30-79*, respectively, in the absence relative to the presence of Gal4-Sir1p; n = 3 for each comparison; p = 0.061 for *POL30* and 0.018 for each mutant) that was of similar magnitude in both *POL30* and *pol30* cells (Supplementary Fig. 3B, lane 1 versus lanes 2, 3 or 4; p = 0.83 for each mutant relative to *POL30*). Notably, this minor shift in topology of *HMRaΔp266* upon Sir protein expression was a function of the Sir proteins themselves and not simply due to the loss of transcription from *HMR*.

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SUPPLEMENTARY TABLE 1. Yeast strains used in this study.

Strain	Genotype	Source
JRY2726	*MAT**a** his4*	P. Schatz
W303	*MAT**a*** or α *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*	R. Rothstein
AKY1785	W303 *MAT**a** HMR::ADE2 POL30*Δ*::hisG* plus PK169	(19)[1]
AKY1692	W303 *MAT**a** HMR::ADE2 hht1-hhf1*Δ*::LEU2 hht2 hhf2*Δ*::HIS3* plus PK189	(19)[1]
AKY1721	AKY1692 *POL30*Δ*::hisG* plus PK169	(19)[1]
AKY2809	AKY1692 *cac1*Δ*::kanMX*	(19)[1]
JRY7130	W303 *mat*Δ*::ADE2* FRT-4xGal4-Rap1-Abfl *HMR**a*** Δ*I*-FRT *leu2-3,112*::*LEU2 FLP1* (pFV17) [cir^o] plus pJR1811	(20)
AKY762	JRY7130 *POL30*Δ*::hisG* plus pPK169	This Study[1]
AKY2401	AKY762 *bar1*Δ::KanMX	This Study[1]
AKY1776	AKY762 FRT-4xGal4-Rap1-Abfl *HMRa*Δ*p*266 Δ*I*-FRT	This Study[1]
AKY743	W303 *mat*Δ*::ADE2* FRT-3xGal4-Rap1-Abfl *HMR**a*** Δ*I*-FRT *leu2-3,112*::*LEU2 FLP1* (pFV17) [cir^o] *cac1*Δ*::hisG* plus pJR1811	This Study
AKY843	W303 *mat*Δ*::ADE2* FRT-4xGal4-Rap1-Abfl *HMR**a*** Δ*I*-FRT *leu2-3,112*::*LEU2 FLP1* (pFV17) [cir^o] *asf1*Δ*::TRP1* plus pJR1811	This Study
AKY863	AK743 *asf1*Δ*::KanMX*	This Study
AKY1677	W303 *MAT*α *HMR**a****	This Study
AKY1872	W303 *MAT*α *HMR**a**** *POL30*Δ*::hisG* plus PK169	This Study[1]
AKY2399	AKY1677 *cac1Δ::KanMX*	This Study
AEY23633	W303 *MAT*α *HMR**a**** *asf1Δ::KanMX*	(21)
JRY5475	*MAT*α *HMR**a**** *cdc44-5::URA3 ade2 trp1 leu2 ura3*	(22)
AEY474	W303 *MAT*α *HMR**a**** *sas2*Δ*::TRP1*	(21)
AKY1744	W303 *MAT*α *HMR**a**** *hht1-hhf1*Δ*::LEU2 hht2 hhf2*Δ*::HIS3* plus PK189	(19)[1]
BY4741	*MATa his3Δ1 leu2Δ200 met15Δ0 ura3Δ0*	(23)
AKY5104	BY4741 *RTT109-link-yEmCitrine-SpHIS5*	This Study
AKY5110	BY4741*POL30-link-yEmCFP-CaURA3*	This Study
AKY5152	AKY5110 *RTT109-link-yEmCitrine-SpHIS5*	This Study
AKY5214	AKY5110 *SAS4-link-yEmCitrine-SpHIS5*	This Study
AKY5216	AKY5110 *SAS5-link-yEmCitrine-SpHIS5*	This Study
AKY5230	AKY5104 *TRX3-link-yEmCFP-CaURA3*	This Study
AKY5234	AKY5110 *SPC29-link-yEmCitrine-SpHIS5*	This Study
AKY5324	W303 *MAT**a** POL30*Δ*::hisG RTT109-link-yEmCitrine-SpHIS5* plus pAK1105	This Study[1]
AKY5326	W303 *MAT**α** POL30*Δ*::hisG SAS5-link-yEmCitrine-SpHIS5* plus pAK1105	This Study[1]

[1]Parental Strains used for this study. See Supplementary Table 2 for description of plasmids that were introduced into the above strains during strain construction or for experiments described in text.

SUPPLEMENTARY TABLE 2. Plasmids used in this study.

Plasmid	Description	Source
pPK169	*POL30/ARS/CEN/URA3*	P. Kaufman
pBL243	*POL30Δ::hisGURA3hisG*	(24)
pBL230	*POL30/ARS/CEN/TRP1*	(24)
pBL230-8	*pol30-8/ARS/CEN/TRP1*	(24)
pBL230-6	*pol30-6/ARS/CEN/TRP1*	(24)
pBL230-79	*pol30-79/ARS/CEN/TRP1*	(25)
pBL245-42	*pol30-42/ARS/CEN/TRP1*	(24)
pBL231	*pol30-51/ARS/CEN/TRP1*	(24)
pBL230-3	*pol30-3/ARS/CEN/TRP1*	(24)
pBL230-16	*pol30-16/ARS/CEN/TRP1*	(24)
pPK189	*HHT2 HHF2 ARS/CEN/URA3*	P. Kaufman
pMP3	*HHT2 HHF2 ARS/CEN/TRP1*	(26)
pAK873	H3 K9R H4 *ARS/CEN/TRP1*	S.D. Briggs
pAK995	H3 K9Q H4 *ARS/CEN/TRP1*	This Study
pAK965	H3 K56R H4 *ARS/CEN/TRP1*	(19)
pAK973	H3 K56Q H4 *ARS/CEN/TRP1*	(19)
pAK1004	H3 K9R K56Q *ARS/CEN/TRP1*	This Study
pAK1005	H3 K9R K56R *ARS/CEN/TRP1*	This Study
pAK1008	H3 K9Q K56R *ARS/CEN/TRP1*	This Study
pAK928	H3 K14R H4 *ARS/CEN/TRP1*	This Study
pWZ-414-F24	H3 H4 K16R *ARS/CEN/TRP1*	(27)
pMP72/AK283	H3 K9,14R H4 *ARS/CEN/TRP1*	(26)
pAK929	H3 K14R H4 K16R *ARS/CEN/TRP1*	This Study
pAK923	H3 K9,14R H4 K16R *ARS/CEN/TRP1*	(28)
pJR1811	*MET3p GAL4-SIR1/ARS/CEN/HIS3*	(4)
pFV17	*GAL10p FLP1-LEU2*	(29)
pAK876	FRT-4xGal4-Rap1-Abf1 *HMRaΔp*266 Δ*I*-FRT in *EcoR1-HindIII* fragment of *HMR* locus in pUC18	This Study
pFA6::kanMX4		(30)
pFA6::natMX4		(31)
pKT212	pFA6a-link-yEmCFP-*CaURA3*	(32)
pKT211	pFA6a-link-yEmCitrine-*SpHIS5*	(32)
pRS415	*CEN/ARS/LEU2*	(33)
pRS416	*CEN/ARS/URA3*	(33)
pAK1105	*POL30-link-yEmCFP CEN/ARS/LEU2*	This Study
pAK1106	*POL30-link-yEmCFP CEN/ARS/URA3*	This Study
pAK1107	*pol30-6-link-yEmCFP CEN/ARS/URA3*	This Study
pAK1108	*pol30-79-link-yEmCFP CEN/ARS/URA3*	This Study
pAK1126	*pol30-8-link-yEmCFP CEN/ARS/URA3*	This Study

SUPPLEMENTARY TABLE 3. Relative level of Sir3p at *HMR-GalSS* in PCNA mutants: Data for individual replicates of anti-Sir3p chromatin immuoprecipitations in Fig. 2A.

Genotype	Replicate 1			Replicate 2		
	MAT	*GalSS*	***a1***	*MAT*	*GalSS*	***a1***
POL30	1	28	11	1	38	12
pol30-6	1	22	6.6	1	26	7.4
pol30-8	1	23	9.4	1	22	11
pol30-79	1	26	10	1	36	14

SUPPLEMENTARY TABLE 4. Relative level of Sir2p at *HMR-GalSS* in PCNA mutants: Data for individual replicates of anti-Sir2p chromatin immuoprecipitations in Fig 2B.

Genotype	Replicate 1			Replicate 2		
	MAT	*GalSS*	***a1***	*MAT*	*GalSS*	***a1***
POL30	1	25	3.8	1	33	4.5
pol30-6	1	25	3.7	1	31	3.8
pol30-8	1	18	2.6	1	20	3.1
pol30-79	1	26	4.9	1	29	6.4

SUPPLEMENTARY TABLE 5. PCNA interacts with SAS-I by FLIM-FRET analysis.

Genotype	Lifetime (ns)	FRET Efficiency	Distance (nm)
POL30-CFP	1.406 ± 0.101	-	-
POL30-CFP SAS5-YFP	1.120 ± 0.0600	0.231	6.356
TRX3-CFP RTT109-YFP	1.412 ± 0.0950	-	-
POL30-CFP SPC29-YFP	1.386 ± 0.210	-	-

SUPPLEMENTARY TABLE 6. *pol30* mutants with defects in Asf1p- and CAF-I-dependent pathways rescue silencing at *HMR**a**e***

Genotype	Relative Efficiency of Mating[1]
POL30	1
pol30-6	10 ± 4.5
pol30-8	3.5 ± 1.3
pol30-42	18.4 ± 7.0
pol30-51	1.0 ± 0.97
pol30-3	0.56 ± 0.26
pol30-16	0.92 ± 0.58

[1]The efficiency of mating of *MATα HMR**a**e*** *POL30* cells to tester strain JRY2726 (*MAT**a***) was determined relative to their plating efficiency on minimal (YM) medium containing supplements (2.6 ± 0.14%, n = 3), and was set to 1. The mating efficiency of each *pol30* mutant relative to *MATα HMR**a**e*** *POL30* cells is shown. Avg. ± St. Dev., n = 3.

SUPPLEMENTARY FIGURE LEGENDS

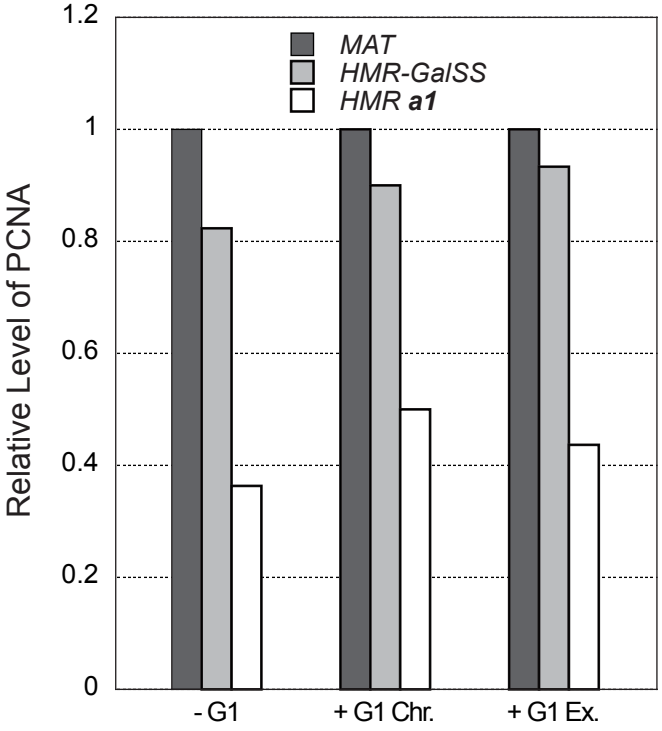
SUPPLEMENTARY FIGURE 1. Pol30p does not preferentially associate with *HMR* during G1. Yeast containing the modified *HMR* shown in Fig. 1A were grown in the absence of Gal4-Sir1p and arrested in G1 with α factor. The culture was divided into two, *HMR* was left in the chromosome in one culture and Flp1p was induced with 2% galactose resulting in the excision of *HMR* in the other culture. Expression of Gal4-Sir1p was then induced in both G1-arrested cultures, and FLP recombinase was repressed by incubating yeast in medium lacking methionine and containing 2% raffinose. DNA co-precipitating with antibodies against Pol30p were analyzed using real time PCR by amplification of DNA within the synthetic silencer (*HMR-GalSS*), the *HMR**a1*** open reading frame and sequences adjacent to the *MAT* locus. The efficiency of co-precipitation of each locus at each time point is expressed relative to *MAT* at that same time point; $2^{(MAT\ C_T\ -\ Locus\ C_T)}$. Average of n = 2 is shown. (-) or (+), absence or presence of Gal4-Sir1p, respectively; G1, α factor-arrested cells; Chr., *HMR* within the chromosome; Ex., *HMR* excised from the chromosome.

SUPPLEMENTARY FIGURE 2. Expression of PCNA in *POL30* and *pol30* cells. Immunoblot analyses of whole cell extracts from *POL30* and *pol30* cells using anti-PCNA and anti-H3 (loading control) antibodies (see Experimental Procedures).

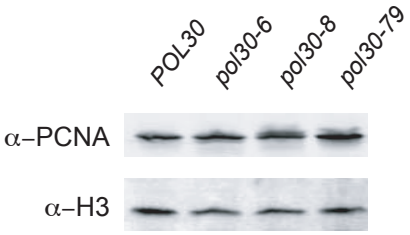
SUPPLEMENTARY FIGURE 3. Topological patterns of *HMR* were similar in *POL30* and *pol30* cells in the absence of transcription. A. A regulatable *HMR* locus lacking the ***a2-a1*** promoters. *HMRa*Δ*p266*, is analogous to the modified *HMR* shown in Fig. 1A except the ***a2-a1*** promoter region was deleted and replaced with 266 bp of heterologous DNA, thereby disrupting transcription from *HMR*. B. Sir proteins altered topology at *HMRa*Δ*p266*. *HMRa*Δ*p266* was excised from logarithmically growing *POL30* and *pol30* cells by inducing Flp1 recombinase, and topoisomers were monitored by DNA blot analyses as described in Experimental Procedures. Arrow and - indicates that more negatively supercoiled DNA migrates more rapidly through the gel under these assay conditions. * indicates nicked DNA, +, - indicates Gal4-Sir1p expression was on or off, respectively; arrowheads indicate centers of distribution of topoisomers. C. Traces of topoisomers of *HMRa*Δ*p266* observed in strains analyzed in B. Arbitrary units corresponding to distance of migration through gel are noted below traces to facilitate comparison of migration patterns between each panel.

SUPPLEMENTARY FIGURE 4. Acetylation of K16 on chromatin-associated histone H4. Data reflects one replicate of the experiment shown in Table 4 (see Experimental Procedures).

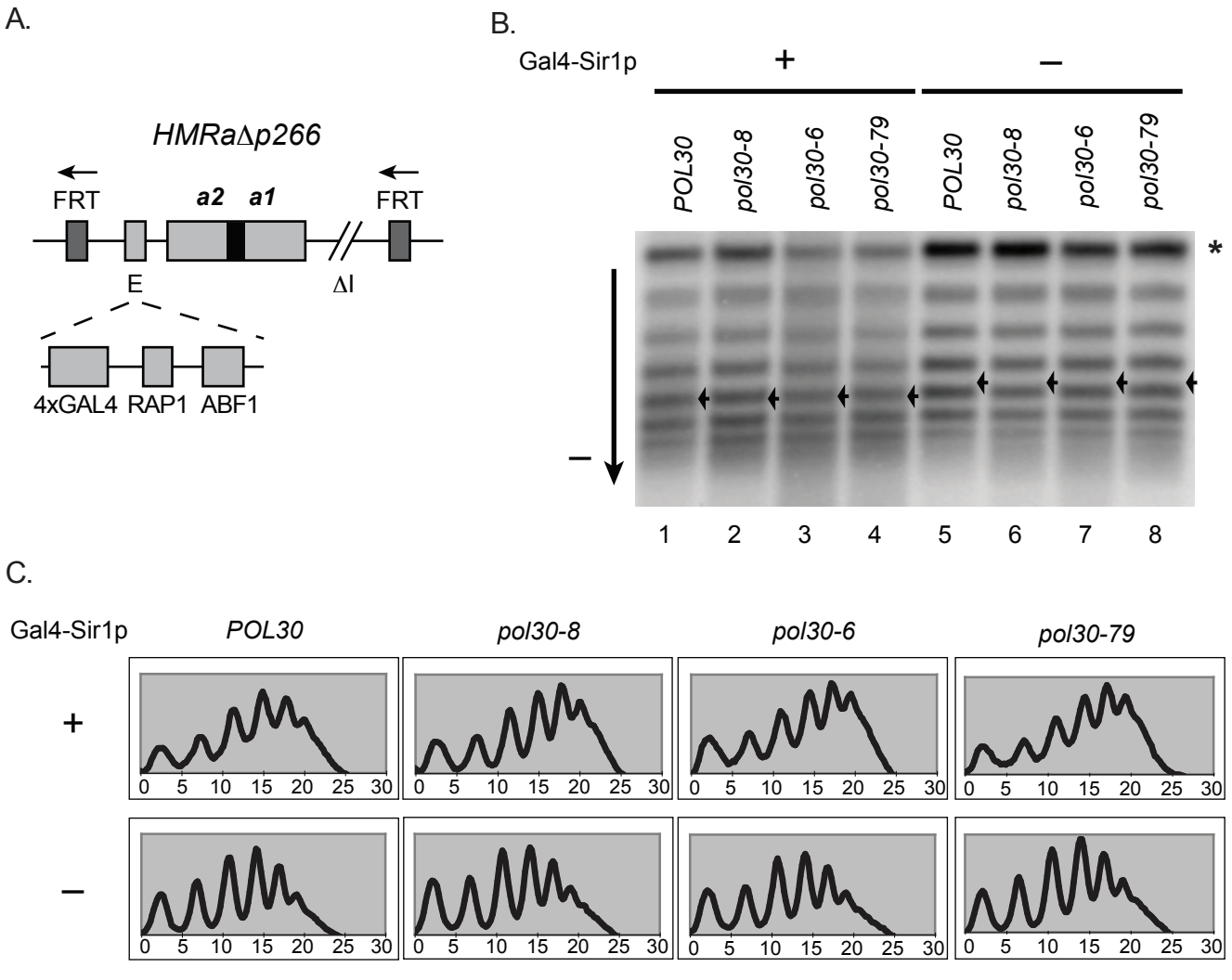
SUPPLEMENTARY FIGURE 5. PCNA interacts with Sas5p. Confocal fluorescence lifetime image of CFP (A) and Fluorescence Lifetime distribution (B) in yeast expressing Pol30-CFPp (top panel), Pol30-CFPp plus Sas5-YFPp (second panel), Trx3-CFPp plus Rtt109-YFPp (third panel) or Pol30-CFPp plus Spc29-YFPp (bottom panel). Scale bar: 15 μm.



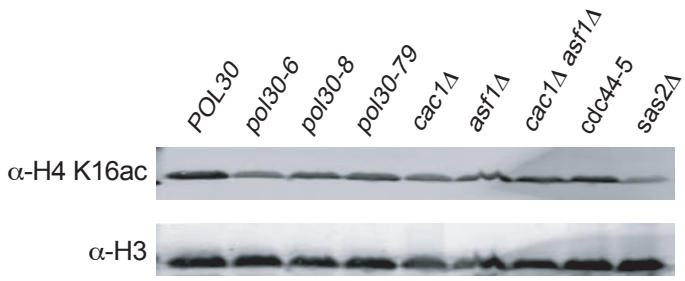
Miller et al., Supplementary Fig. 1



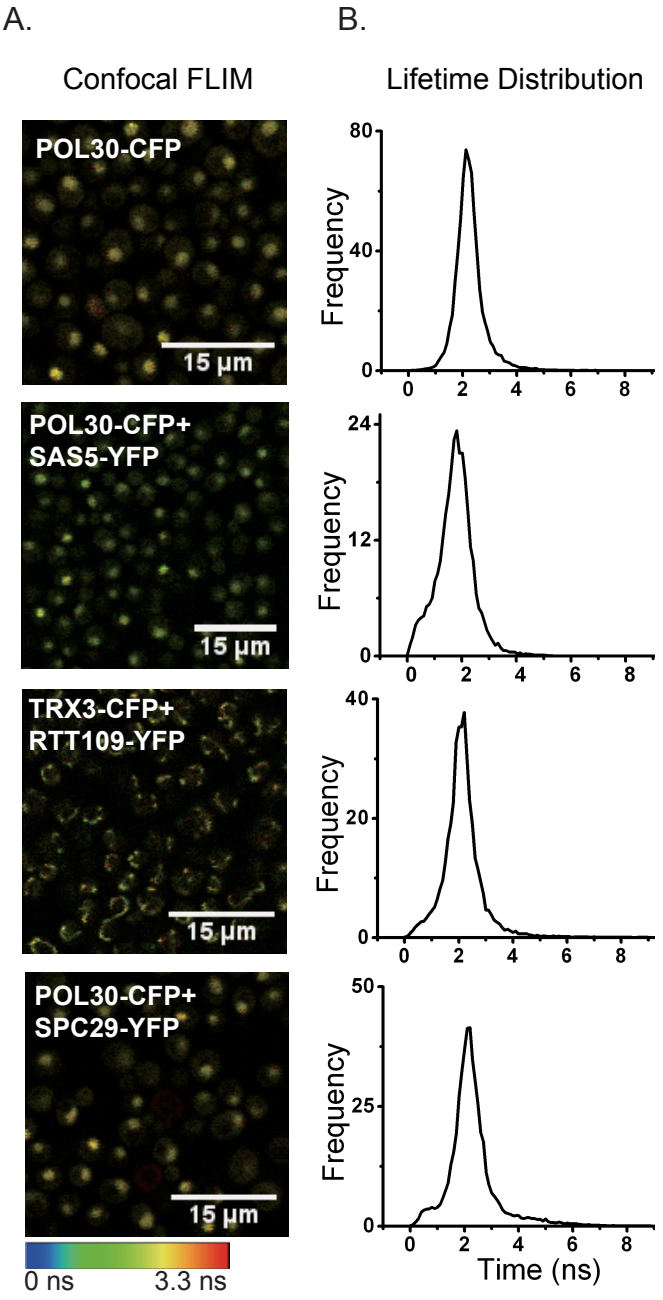
Miller et al., Supplementary Fig. 2



Miller et al., Supplementary Fig. 3



Miller et al., Supplementary Fig. 4



Miller et al., Supplementary Fig. 5