NUCLEOSOME DYNAMICS REGULATE DNA PROCESSING

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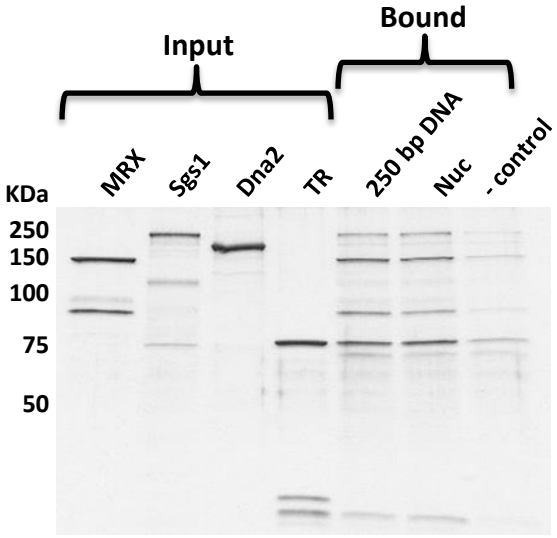

Input
Bound
MRX
Sgs1
Dna2
TR
250 bp DNA
Nuc
- control
KDa
250
150
100
75
50

Fig S1. Resection enzyme recruitment detected by silver staining. The Sgs1/Dna2 machinery was bound to biotinylated DNA (250 bp naked, linear) or the 601-250 mononucleosome (50 bp free end DNA), and complexes were captured on streptavidin-magnetic beads. Silver stained PAGE of the input and bound fractions are shown.

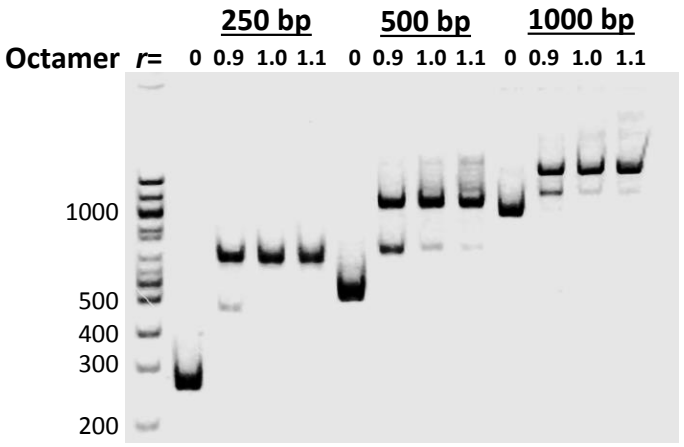
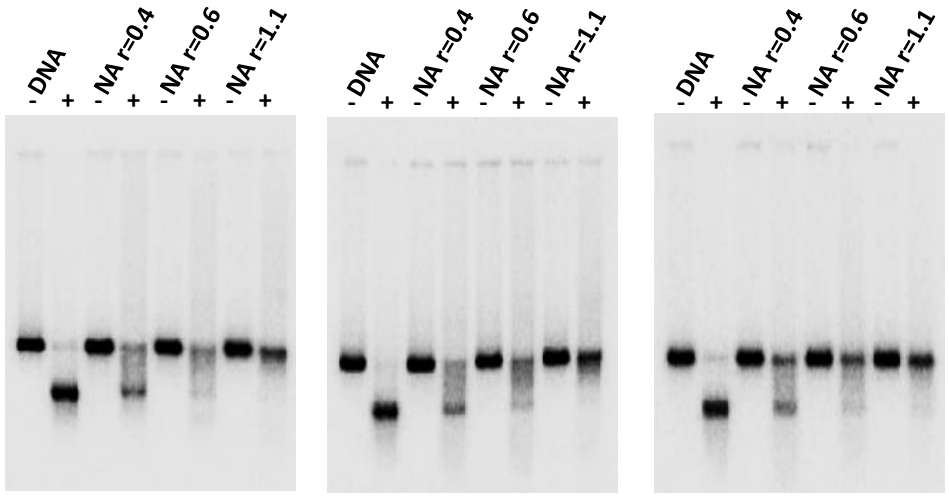

250 bp
500 bp
1000 bp
Octamer r= 0 0.9 1.0 1.1 0 0.9 1.0 1.1 0 0.9 1.0 1.1
1000
500
400
300
200

Fig S2. Chromatin substrate reconstitution. Native 4% PAGE of mononucleosomes with increasing free, adjacent DNA as described in **Fig. 3a**. Nucleosome saturation levels were assessed as the amount of free DNA at increasing octamer to 601 sequence ratios (r).

a.


- DNA
+
- NA r=0.4
+
- NA r=0.6
+
- NA r=1.1
+
Sgs1 + MRX + TR + RPA
Sgs1 + TR + RPA
Sgs1 + MRX + RPA

b.

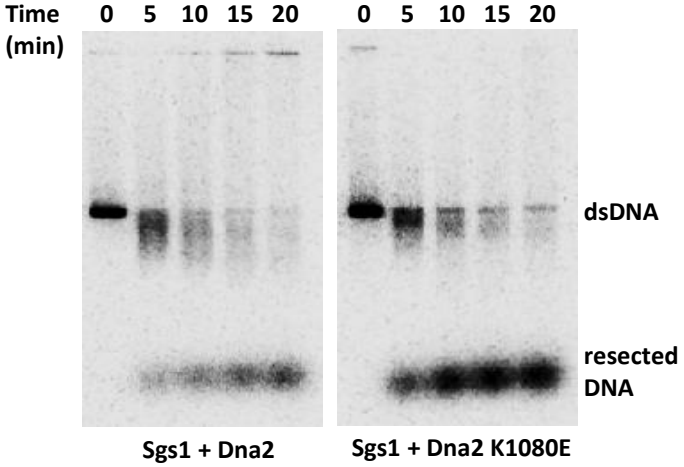

Time (min)
0 5 10 15 20
0 5 10 15 20
dsDNA
resected DNA
Sgs1 + Dna2
Sgs1 + Dna2 K1080E

Fig S3. Individual contributions of the Sgs1/Dna2 pathway on resection activity. (**a**) Helicase assay to determine the effects of MRX (10 nM) and Top3-Rmi1 (TR) (10 nM) on Sgs1 (10 nM) helicase activity. (**b**) Resection assays on nucleosomal array at r=0.6 with MRX (10 nM), Top3-Rmi1 (10 nM), RPA (100 nM), Sgs1 (10 nM) and either Dna2 (20 nM) or the helicase deficient Dna2 K1080E (20 nM) to assess Dna2's helicase effect on the efficiency of Sgs1/Dna2 resection.

Adkins et al., Supplemental Fig. 4

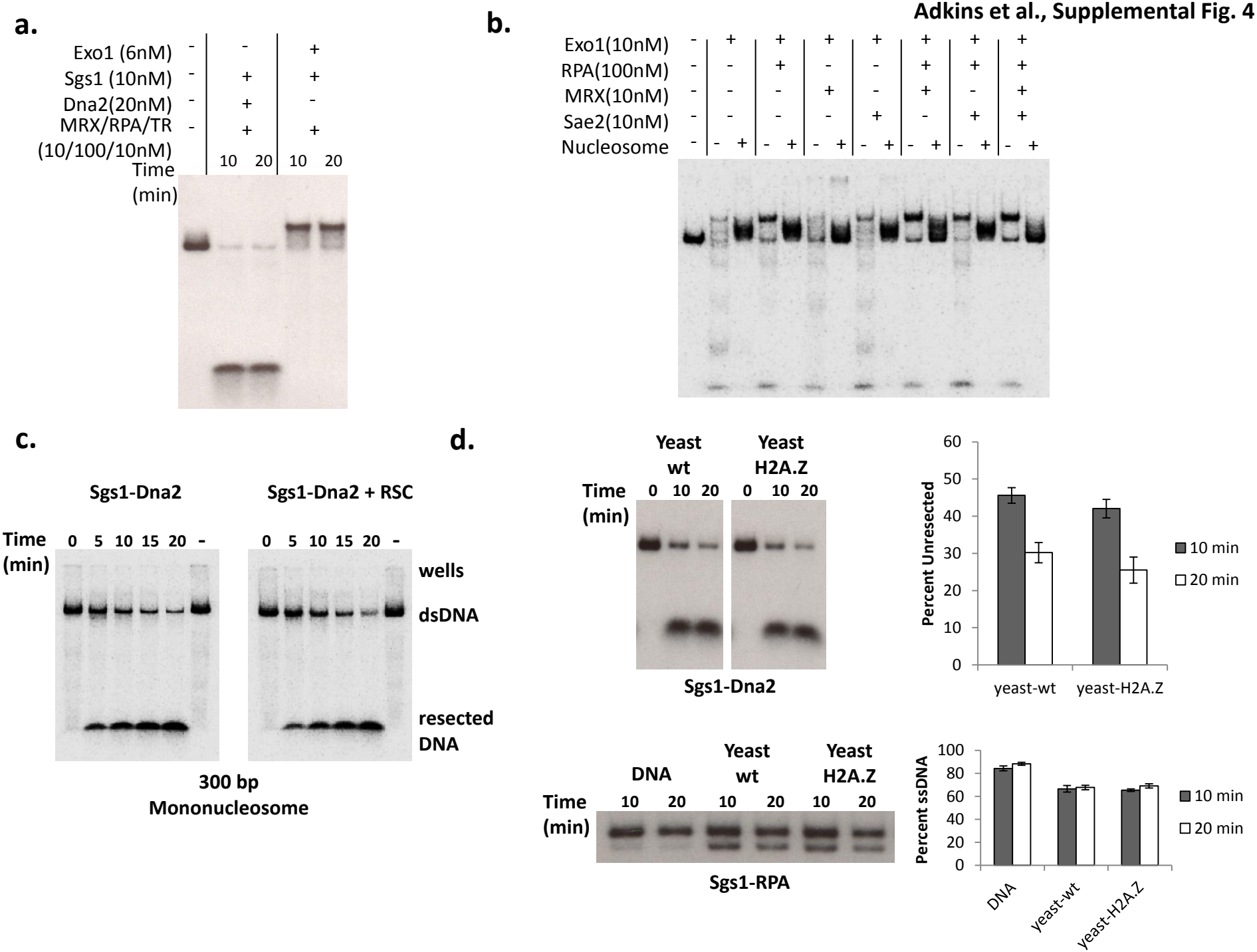
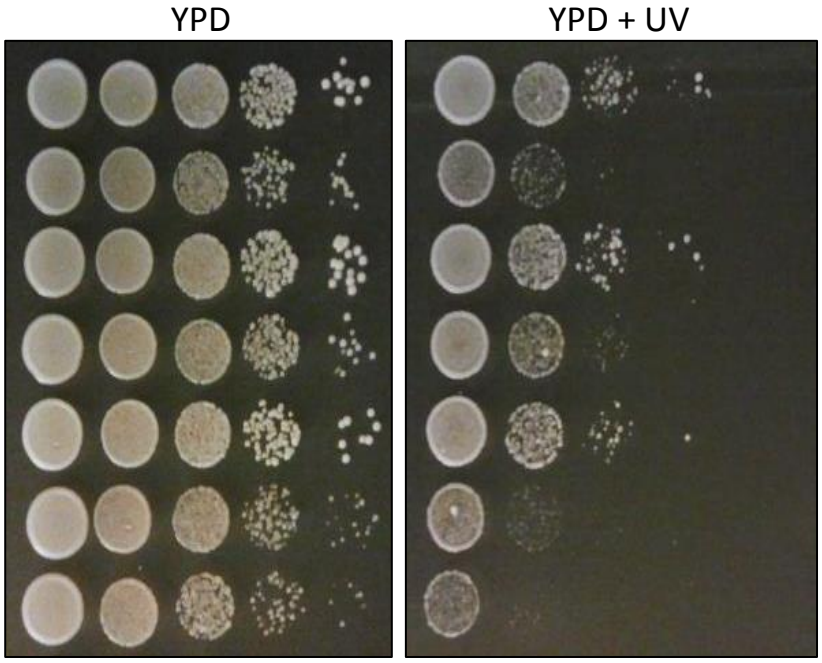


Fig S4. Additive impact of enzyme combinations on nucleosome resection. (**a)** 500 bp mononucleosome with a centered position 601 sequence was incubated with MRX, Top3-Rmi1, RPA, Sgs1 with either Dna2 or Exo1 for the indicated times at 30^{o} C. (**b**) 250 bp of DNA with and without a nucleosome incubated with Exo1 individually and combinatorially with RPA, MRX, and Sae2 for 20 min at 30^{o} C. (**c**) 300 bp mononucleosome with a centered position 601 sequence incubated with MRX (10 nM), Top3-Rmi1 (10 nM), RPA (100 nM), Sgs1 (10 nM) and Dna2 (20 nM) with and without RSC (1 nM). (**d**) Top Left: 250 bp mononucleosome containing either wt yeast octamers or yeast H2A.Z incubated with MRX (10 nM), Top3-Rmi1 (10 nM), RPA (100 nM), Sgs1 (10 nM) and Dna2 (20 nM) at 30^{o} C for indicated times. Top Right: Graph of quantified values of dsDNA signal with +/- SEM. Bottom Left: Helicase assay of DNA, yeast wt mononucleosome, and H2A.Z mononucleosome of Sgs1 (10 nM) with RPA (100 nM) at 30^{o} C for indicated times. Bottom Right: Graph of ssDNA quantified as percent of ssDNA compared to total ssDNA and dsDNA signal with +/- SEM (n=3).

a.


YPD
YPD + UV
WT
sgs1Δ
exo1Δ
swr1Δ
exo1Δ swr1Δ
sgs1Δ swr1Δ
sgs1Δ exo1Δ

b.

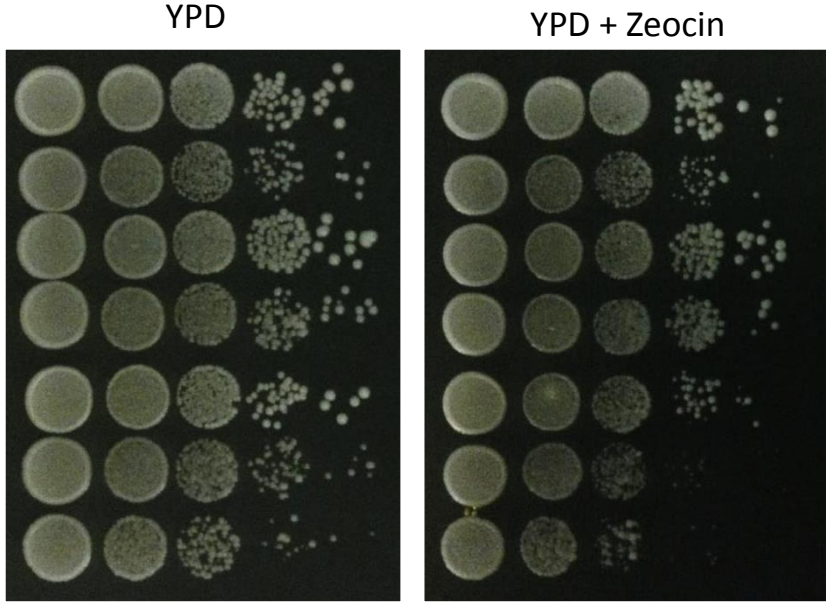
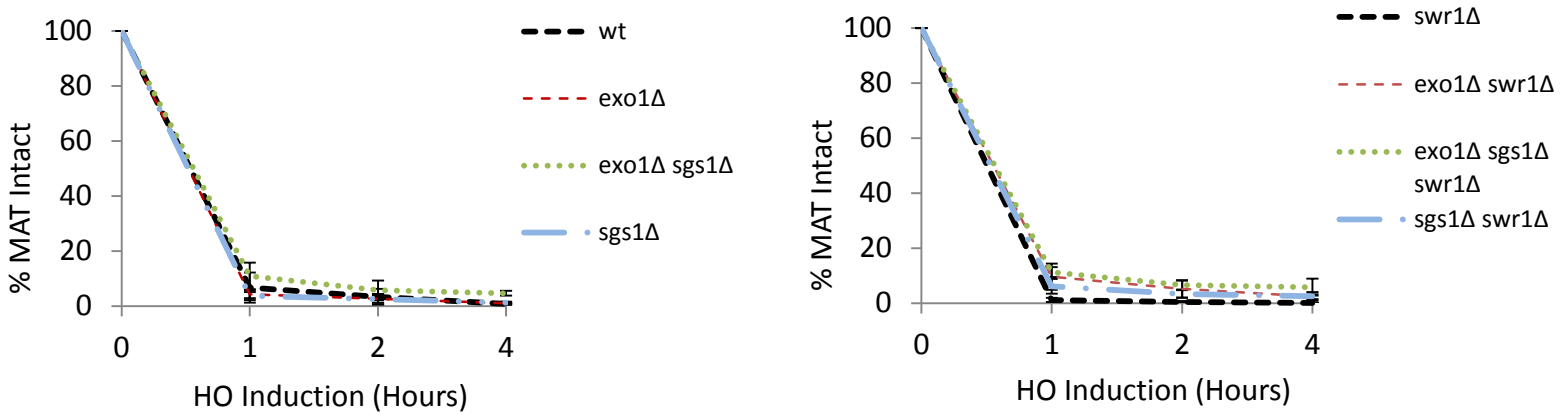
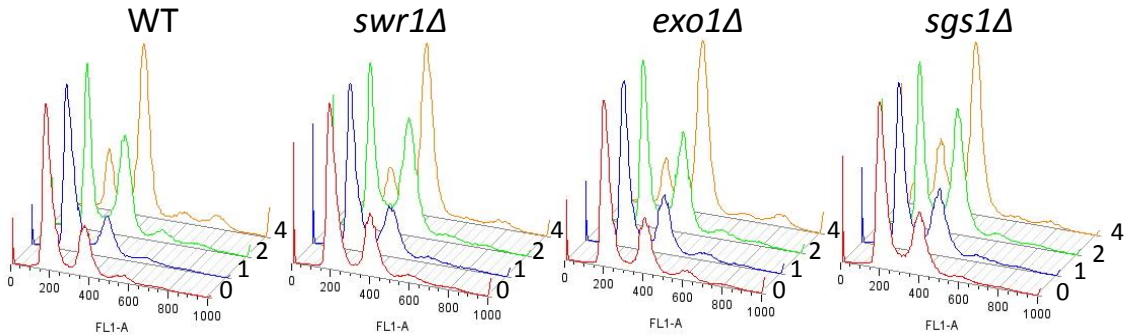

YPD
YPD + Zeocin
WT
sgs1Δ
exo1Δ
swr1Δ
exo1Δ swr1Δ
sgs1Δ swr1Δ
sgs1Δ exo1Δ

Fig S5. Phenotypic plate assay of yeast deletion mutants. **(a)** UV phenotypic plate assay of *swr1Δ* mutations. Indicated strains were plated in ten-fold serial dilutions on YPD plates with UV treated plate subjected to 90 J/m^2 UV. (**b**) Zeocin phenotypic plate assay of *swr1Δ* mutations. Indicated strains were plated in ten-fold serial dilutions on YPD plates with 1 μg/ml zeocin.

a.



b.

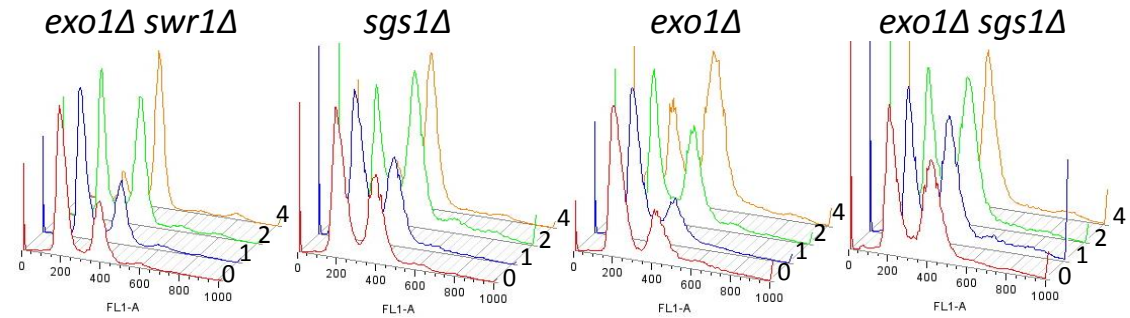


Time (hrs)	G1	G2/M
0	62.30%	26.30%
1	66.90%	22.90%
2	41.20%	43.80%
4	18.70%	62.70%

Time (hrs)	G1	G2/M
0	55.70%	28.80%
1	57.50%	30.00%
2	40.90%	46.50%
4	16.30%	69.40%

Time (hrs)	G1	G2/M
0	58.70%	28.30%
1	57.80%	29.90%
2	43.70%	42.60%
4	16.40%	67.40%

Time (hrs)	G1	G2/M
0	57.40%	32.20%
1	55.00%	33.10%
2	40.10%	48.50%
4	22.70%	60.20%



Time (hrs)	G1	G2/M
0	60.20%	30.70%
1	57.50%	31.20%
2	39.50%	48.20%
4	14.30%	73.50%

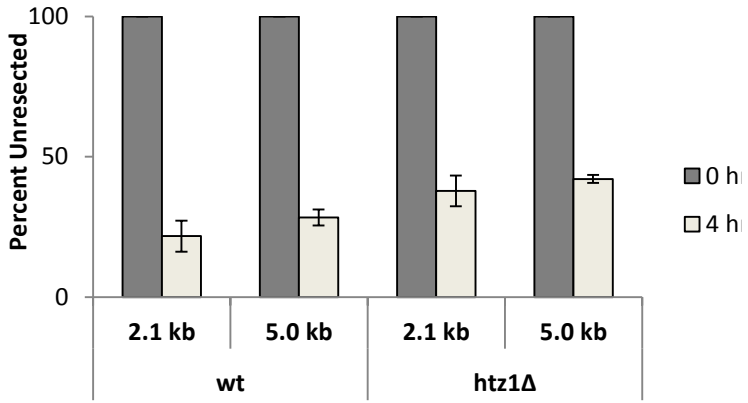
Time (hrs)	G1	G2/M
0	51.50%	38.20%
1	50.40%	37.10%
2	32.30%	49.90%
4	20.50%	64.80%

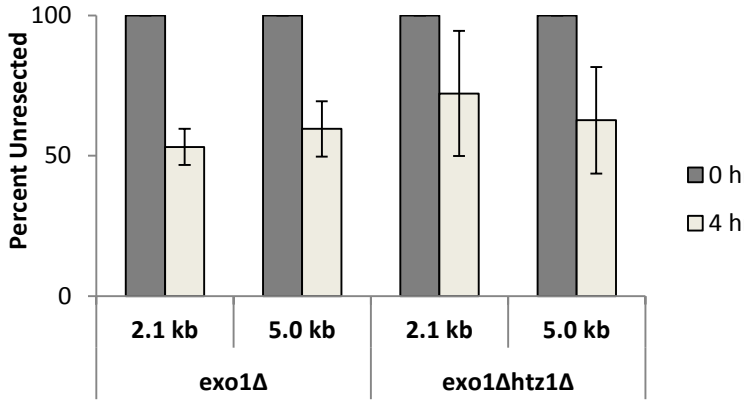
Time (hrs)	G1	G2/M
0	59.80%	27.30%
1	60.20%	27.50%
2	42.80%	42.90%
4	28.30%	59.10%

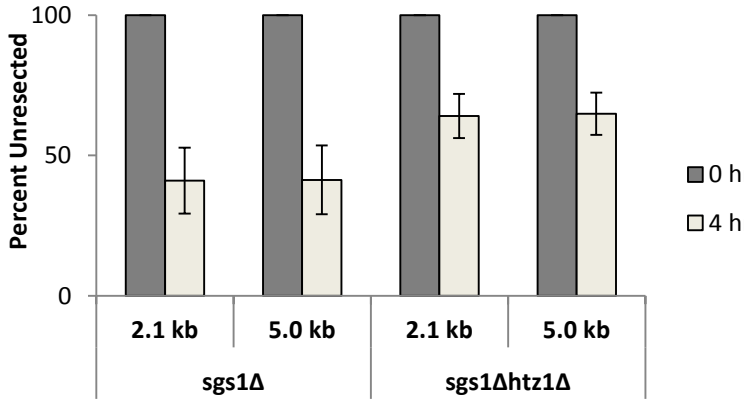
Time (hrs)	G1	G2/M
0	40.40%	41.50%
1	31.10%	44.10%
2	34.20%	49.90%
4	15.90%	64.00%

Fig S6. MAT break induction and cell cycle in asynchronous cell cultures. **(a)** DNA DSB induction by HO expression. Time-course of HO-induced DSB in all strains used for RPA ChIP in Fig. 4. DSBs were measured by quantitative PCR with primers spanning the HO cut site and normalized to actin signal. Chart data was subsequently calculated as % MAT of 0 hour signal +/- SEM (n=3). (**b**) Cell cycle progression during HO induction is unaffected by *swr1Δ*. Cell samples of all strains were collected at indicated hours of DSB induction and the DNA contents were analyzed by flow cytometry. Percent of cells in G1 and G2/M were quantified with FlowJo software.

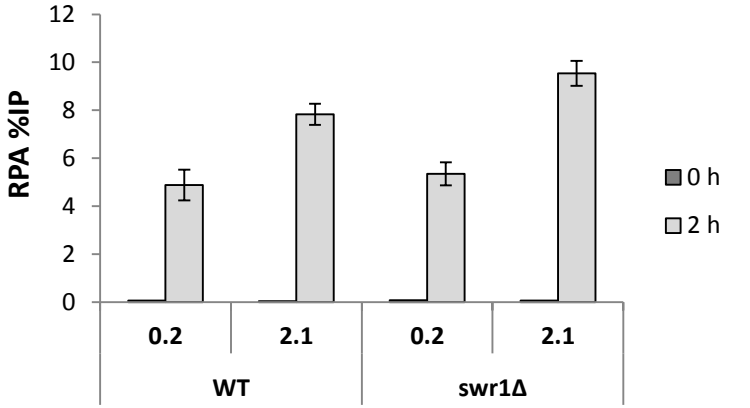
a.

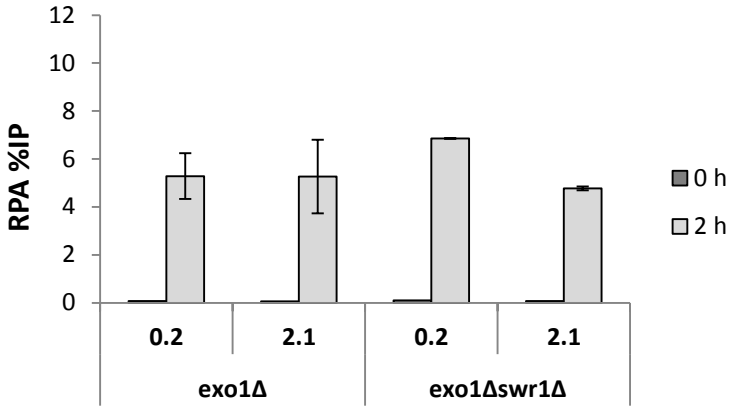

Percent Unresected
100
50
0
2.1 kb
5.0 kb
2.1 kb
5.0 kb
wt
htz1Δ
0 h
4 h


Percent Unresected
100
50
0
2.1 kb
5.0 kb
2.1 kb
5.0 kb
exo1Δ
exo1Δhtz1Δ
0 h
4 h


Percent Unresected
100
50
0
2.1 kb
5.0 kb
2.1 kb
5.0 kb
sgs1Δ
sgs1Δhtz1Δ
0 h
4 h

b.


RPA %IP
12
10
8
6
4
2
0
0.2
2.1
0.2
2.1
WT
swr1Δ
0 h
2 h


RPA %IP
12
10
8
6
4
2
0
0.2
2.1
0.2
2.1
exo1Δ
exo1Δswr1Δ
0 h
2 h

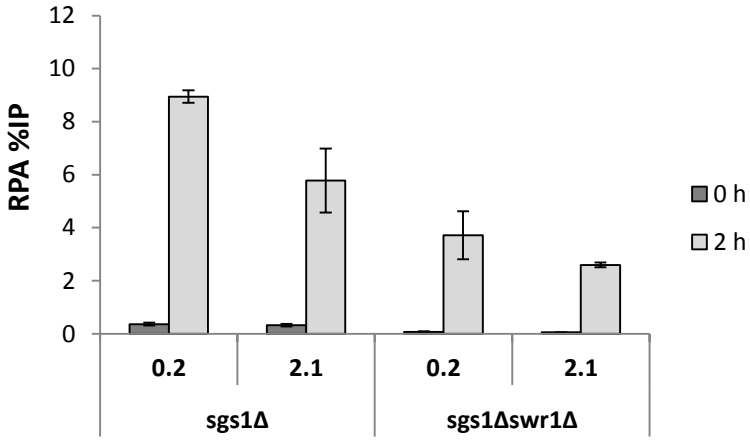

RPA %IP
12
10
8
6
4
2
0
0.2
2.1
0.2
2.1
sgs1Δ
sgs1Δswr1Δ
0 h
2 h

Fig S7. Resection defect in absence of Swr1 and Htz1 **(a)** DNA levels at indicated distances (2.1 and 5.0 kb) to the right of a galactose-inducible DSB were monitored by quantitative real-time PCR in *htz1Δ* strains. DNA levels were normalized to actin and percentage of DSB with the 0 hour time point signals set to 100% with signal at 4 hours indicated as percent with +/- SEM (n=3). Data from **Fig. 6c** plotted for comparison. **(b)** ChIP analysis of RPA occupancy in indicated nocodazole arrested yeast strains. RPA levels were measured at increasing distances from the DSB (0.2 and 2.1 kb) and normalized to the percentage of DSB in each strain. Graph values reflect the percent of precipitated DNA relative to the input at each region +/- SEM (n=3).

Supplemental Table 1

Strain Number	Strains	Description	Source
JKM139	Wild Type	n/a	Zhu et al., 2008
yGI198	*exo1Δ*	*exo1Δ::TRP1*	Zhu et al., 2008
yGI200	*sgs1Δ*	*sgs1Δ::KanMX*	Zhu et al., 2008
yGI199	*exo1Δsgs1Δ*	*exo1Δ::TRP1, sgs1Δ::KanMX*	Zhu et al., 2008
CY1741	*swr1Δ*	*swr1Δ::NATMX6*	This study
CY1742	*swr1Δexo1Δ*	*swr1Δ::NATMX6, exo1Δ::TRP1*	This study
CY1744	*swr1Δsgs1Δ*	*swr1Δ::NATMX6, sgs1Δ::KanMX*	This study
CY1743	*swr1Δexo1Δsgs1Δ*	*swr1Δ::NATMX6, exo1Δ::TRP1,*	This study
		sgs1Δ::KanMX	This study
CY2046	*htz1Δ*	*htz1Δ::NATMX6*	This study
CY2047	*exo1Δhtz1Δ*	*exo1Δ::TRP1, htz1Δ::NATMX6*	This study
CY2048	*sgs1Δhtz1Δ*	*sgs1Δ::KanMX, htz1Δ::HPH*	This study
CY2049	*swr1-degron*	*pMK76::ura3, swr1-IAA17::HPH*	This study
Cy2050	*sgs1Δswr1-degron*	*sgs1Δ::KanMX, pMK76::ura3,*	This study
		swr1-IAA17::HPH	This study

Supplemental Table 2

Primer Name	Sequence
DSB - Forward	5'-GCATAGTCGGGTTTTTCTTTT
DSB - Reverse	5'-ATCCGTCCCGTATAGCCAAT
Actin - Forward	5'-GGCCAAATCGATTCTCAAAA
Actin - Reverse	5'-GCCTTCTACGTTTCCATCCA
0.2 kb - Forward	5'-CCTGGTTTTGGTTTTGTAGAGTGG
0.2 kb - Reverse	5'-GAGCAAGACGATGGGGAGTTTC
0.5 kb - Forward	5'-CATGCGGTTCACATGACTTT
0.5 kb - Reverse	5'-CACCCAAGAAGGCGAATAAG
1.0 kb - Forward	5'-CACAGATTGGACGGAGGACT
1.0 kb - Reverse	5'-CAAGGATGCCCTTGTTTTGT
2.1 kb - Forward	5'-GCCTCTATGTCCCCATCTTGTCTC
2.1 kb - Reverse	5'-GTGTTCCCGATTCAGTTTGACG
5.0 kb - Forward	5'-CCAGCAGTAATAAGTCGTCCTG
5.0 kb - Reverse	5'-CCAGCAGCAAGAAAAGCAAG
YNL092W - Forward	5'- ACAGTTCGTAACAACAGCTG GAAGA
YNL092W - Reverse	5'- TCAGATCCTCACCAATTTTTGCC

Supplemental Note

Flow Cytometry Analysis

FACS analysis was performed on indicated yeast strains as described previously (Papamichos-Chronakis et al. 2006). Briefly, cells were collected at each time point of HO induction, resuspended in 1 ml of 70% ethanol, and fixated with rotation at 4^{o} C overnight. Samples were washed once with water, resuspended in 50 mM Tris, RNase A treated, and incubated with proteinase K. Cells were resuspended in FACS buffer (200 mM Tris-HCl, pH 7.5, 200 mM NaCl, 78 mM $MgCl_2$) with the DNA subsequently stained by Sytox Green. Flow cytometry analysis was carried out with a Becton Dickinson FACSCalibur (BD Biosciences).