Yeast high mobility group protein HMO1 stabilizes chromatin and is evicted during repair of DNA double strand breaks

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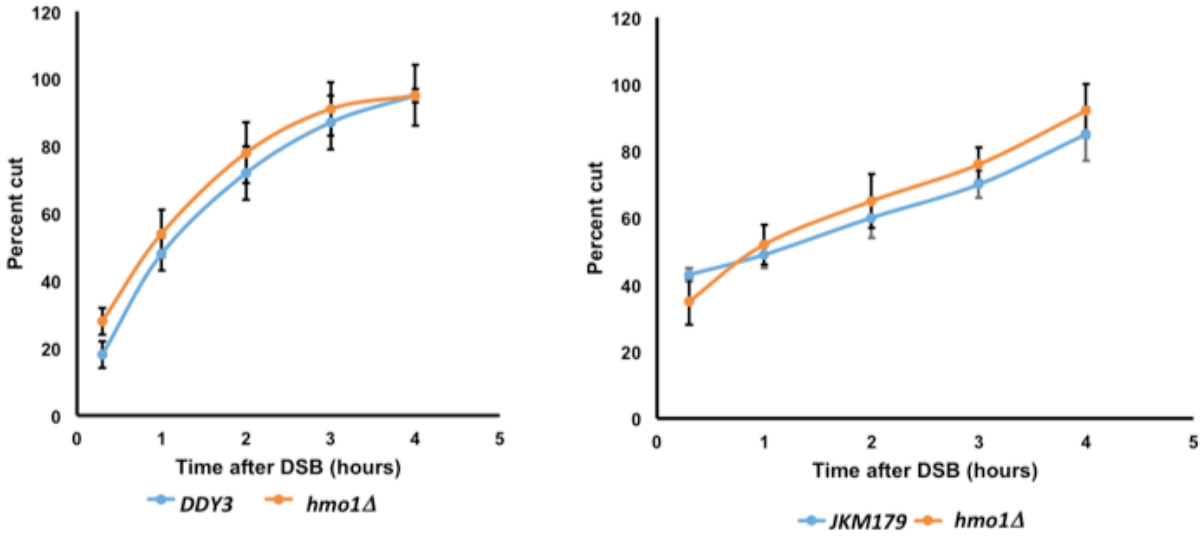
Supplementary Table S1

Strain	Genotype
DDY3	MAT**a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100*
DDY3hmo1FLAG	MAT**a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100 hmo1-FLAG(KANMX4)*
DDY1299	MAT**a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100 hmo1Δ::HIS3*
DDY3-AB	*MAT***a** ADE2 *his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100 hmo1*-tail::URA3
DDY3kuΔ	MAT**a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100 kuΔ::URA3*
DDY3hmo1ΔkuΔ	MAT**a** *ADE2 his3-11 leu2-3,112 lys2Δ trp1-1 ura3-1 can1-100 hmo1Δ::HIS3 kuΔ::URA3*
JKM179	MAT*α hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO*
JKM179hmo1Δ	MAT*α hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO hmo1Δ::URA3*

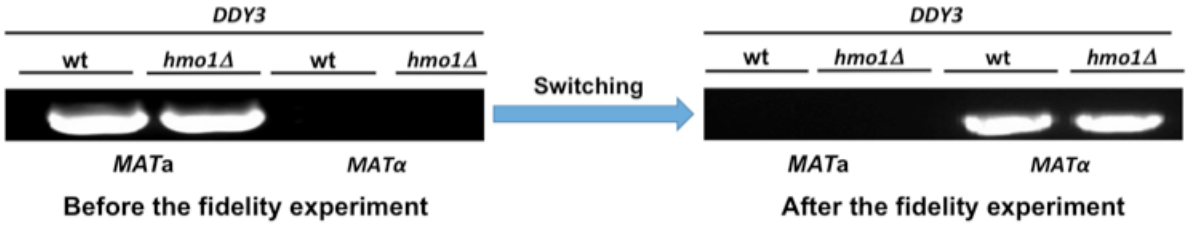
Supplementary Table S2

MAT p1	TCCCCATCGTCTTGCTCT
MAT p2	GCATGGGCAGTTTACCTTTAC
0.2 kb upstream p1	AAAGAAGAAGTTGCAAAGAAATGTGG
0.2 kb upstream p2	TGTTGCGGAAAGCTGAAACTAAAAG
1.6 kb upstream p1	ATGTCCTGACTTCTTTTGACGAGG
1.6 kb upstream p2	ACGACCTATTTGTAACCGCACG
29.8 kb upstream p1	TCGTCGTCGCCATCATTTTC
29.8 kb upstream p2	GCCCAAGTTTGAGAGAGGTTGC
3.1 kb downstream p1	CTAATGCTGCAAAATCCATATGCT
3.1 kb downstream p2	CTCTATGGTGTTTTTACCTACCGC
9.5 kb downstream p1	TGGATCATGGACAAGGTCCTAC
9.5 kb downstream p2	GGCGAAAACAATGGCACTCT
MAT**a** p1	GTGGCATTACTCCACTTCAAGTAAG
MAT**a** p2	AACTAGCAAACAAAGGAAAGTC
MATα p1	AATGGCACGCGGACAAAATGC
MATα p2	AACTAGCAAACAAAGGAAAGTC
HO cut site p1	ATGTGAACCGCATGGGCAGT
HO cut site p2	TGTTGTCTCACTATCTTGCC
POL5 p1	TCCTTGTTCACCTTTGGTGGA
POL5 p2	GTGTTCCCATAGTCTACCCATCG
q MAT**a** p1	GGCGGAAAACATAAACAGAACTCTG
q MAT**a** p2	CCGTGCTTGGGGTGATATTGATG
IPP1 Fw	CCCAATCATCCAAGACACCAAGAAGG
IPP1 Re	AGCAATAGTTTCACCAATTTCCAACACATC

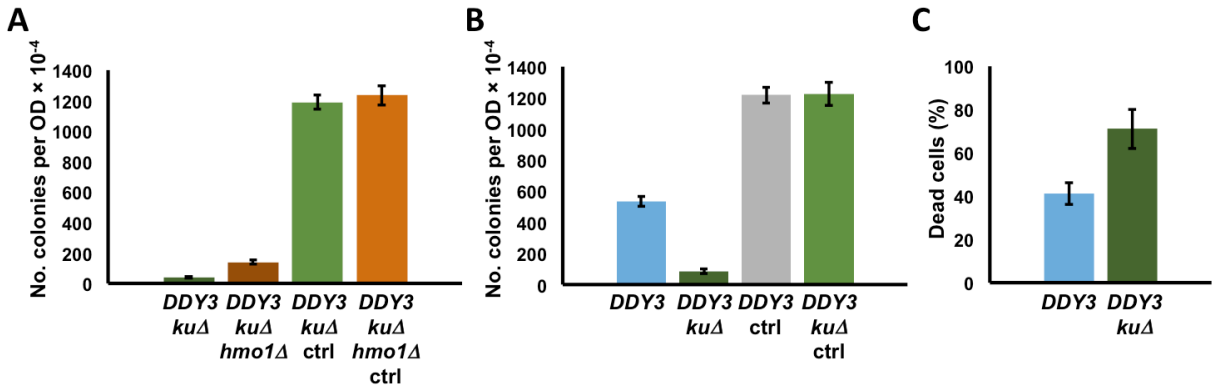
Sequences of primers used for ChIP, DNA resection, and gene expression.



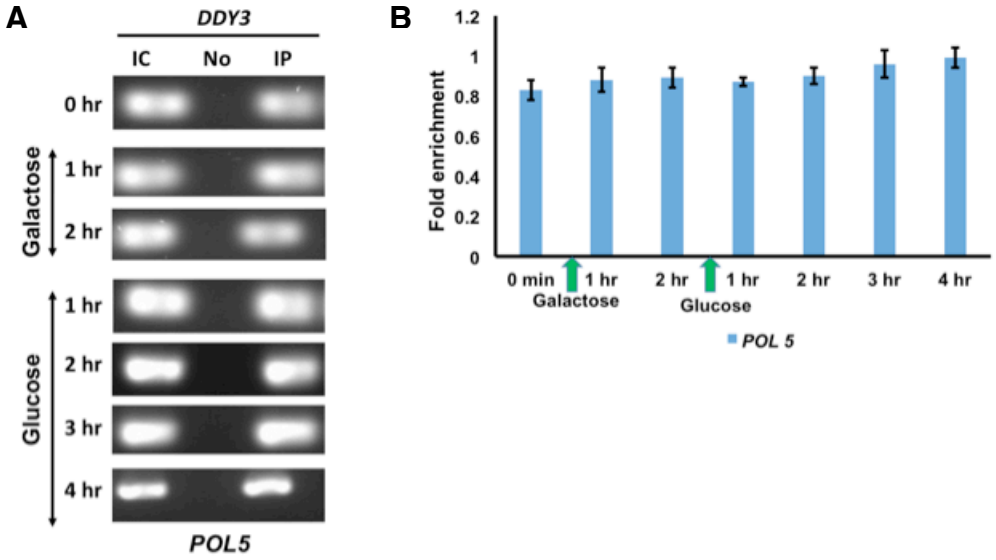
Supplementary Figure S1. Efficiency of DSB induction. Kinetics of HO cleavage at *MAT* was monitored as a reduction in qPCR signals amplified with primer pairs flanking the *MAT* locus using genomic DNA isolated from *DDY3* and corresponding *hmo1Δ* strain (left) and *JKM179* and corresponding *hmo1Δ* strain (right). qPCR signal of no damage control sample was used to calculate percent cut. Experiment was repeated three times. Error bars represent standard deviation.



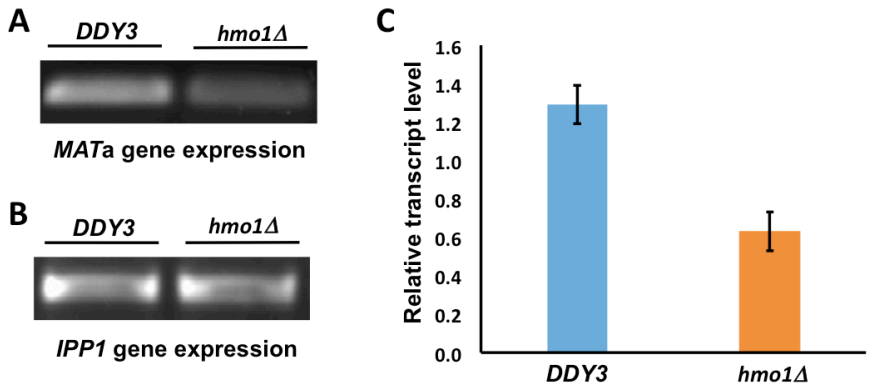
Supplementary Figure S2. Fidelity assay. PCR product obtained using genomic DNA isolated from *DDY3* WT and corresponding *hmo1Δ* strains using primer specific for *MAT***a** and *MATα* showing gene conversion from *MAT***a** to *MATα* following DNA repair.



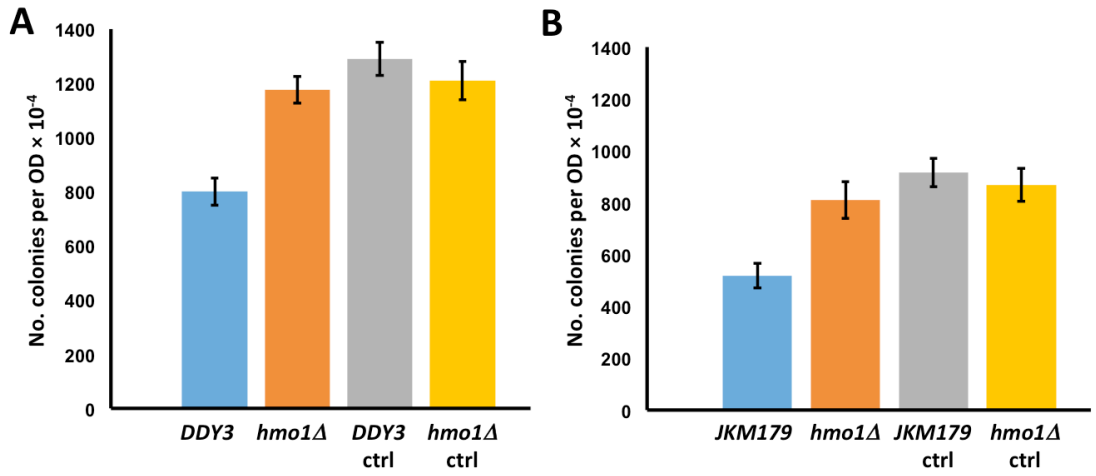
Supplementary Figure S3. Survival of *DDY3kuΔ* and *DDY3kuΔhmo1Δ* strains following induction of DNA double strand breaks. **(A)** Survival of *DDY3kuΔ* and corresponding *hmo1Δ* strain. After DSB induction, cells were diluted 10^4-fold and plated and colonies counted. Survival is represented as colonies per OD. Cells not induced to express *HO* were plated as control (ctrl). **(B)** Survival of *DDY3* and corresponding *kuΔ* strain. After DSB induction, cells were diluted 10^4-fold and plated and colonies counted. Survival is represented as colonies per OD. Cells not induced to express *HO* were plated as control (ctrl). **(C)** Viability of *DDY3* and corresponding *kuΔ* strain as determined by trypan blue exclusion. Three independent experiments were performed. Error bars represent standard deviation.



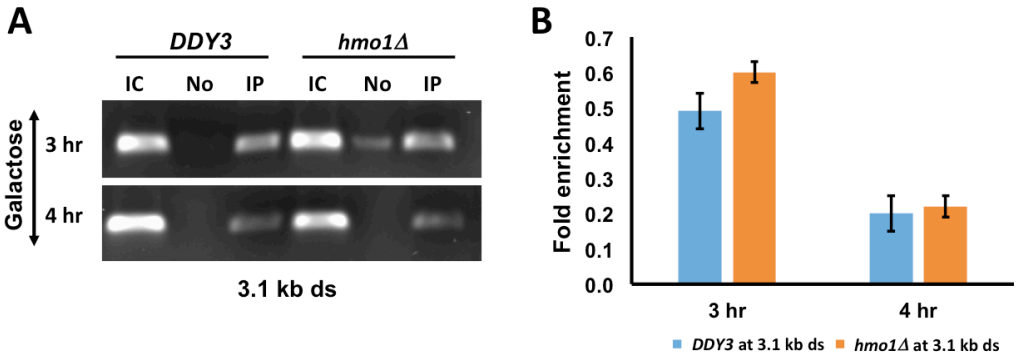
Supplementary Figure S4. HMO1 localization at *POL5*. **(A)** ChIP with *DDY3* using antibody to FLAG-tagged HMO1 showing HMO1 localization at *POL5* during DNA damage (galactose) and repair (glucose). IC, input control; No, no antibody; IP, immunoprecipitation with antibody to FLAG-tagged HMO1. **(B)** Densitometric analysis of ChIP data shown in (a), obtained with ImageJ software. Fold enrichment = ChIP/Input DNA. Three independent experiments were performed. Error bars represent standard deviation.



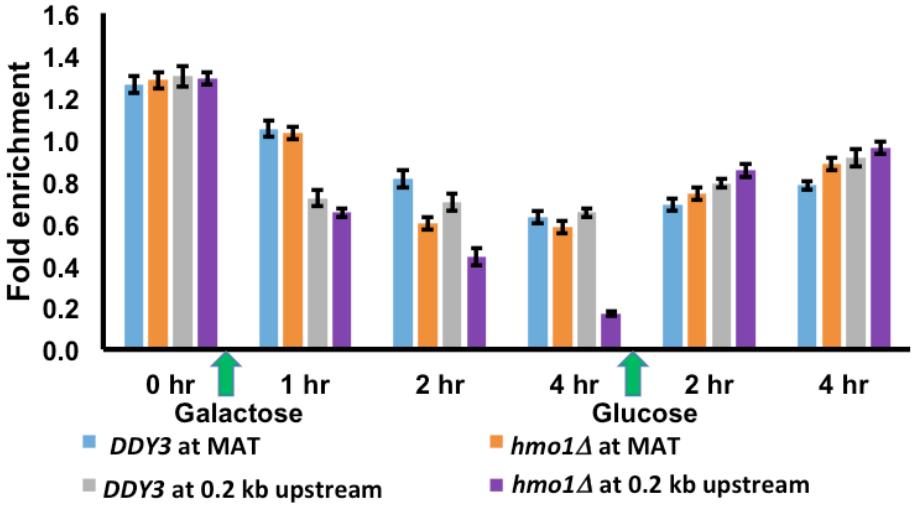
Supplementary Figure S5. Role of HMO1 in *MAT***a** transcription. **(A)-(B)** PCR product amplified using cDNA generated from total RNA extracted from *DDY3* and corresponding *hmo1Δ* strain. **(A)** *MAT***a**. **(B)** *IPP1* (inorganic pyrophosphatase). **(C)** Densitometric analysis of gene expression. Relative transcript level = *MAT***a** gene expression/*IPP1* gene expression. Three independent experiments were performed. Error bars represent standard deviation.



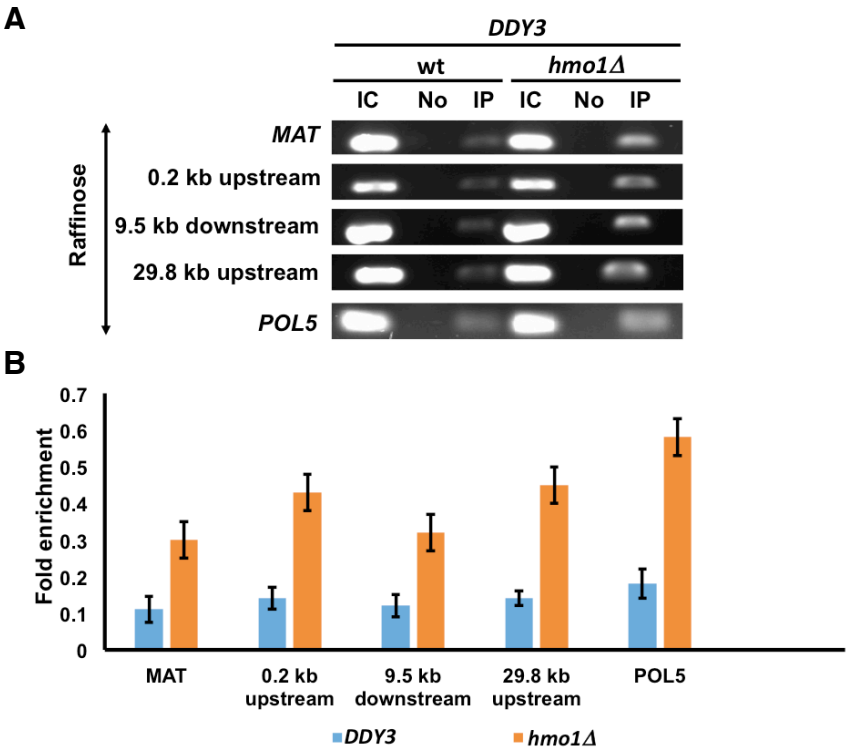
Supplementary Figure S6. Survival after exposure to hydroxyurea (HU). **(A)** *DDY3* and corresponding *hmo1Δ* strain. **(B)** *JKM179* and corresponding *hmo1Δ* strain. After exposure to HU for 8 h, cells were washed, diluted 10^4-fold and plated and colonies counted. Survival is represented as colonies per OD. Each experiment was repeated three times and data reported as mean with standard deviations.



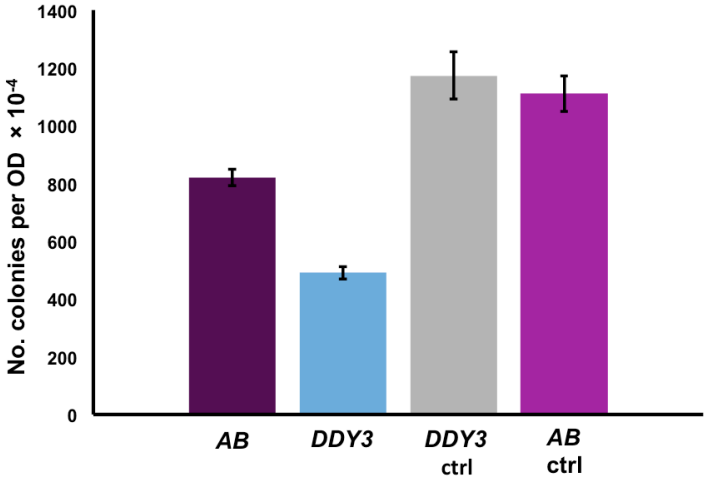
Supplementary Figure S7. Detection of γ-H2A 3.1 kb downstream of DSB. **(A)** ChIP with *DDY3* WT and corresponding *hmo1Δ* strain using antibody to phosphorylated H2A. IC, input control; No, no antibody; IP, immunoprecipitation with antibody to γ-H2AX. **(B)** Densitometric analysis of ChIP data. Fold enrichment = ChIP/Input DNA. Three independent experiments were performed. Error bars represent standard deviation.



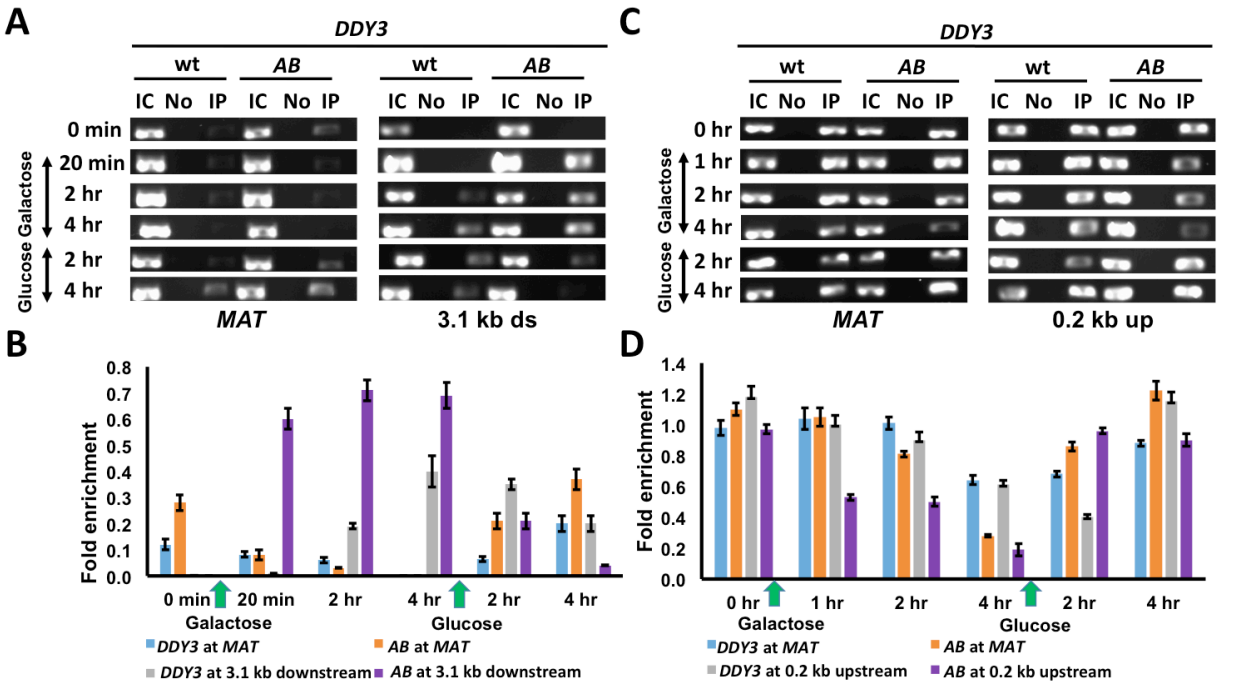
Supplementary Figure S8. Quantitative analysis of H3 localization determined by ChIP followed by qRT-PCR. ChIP with *DDY3* WT and corresponding *hmo1Δ* strain using antibody to H3 at *MAT* and 0.2 kb upstream of DSB during DNA damage (galactose) and repair (glucose). Data is normalized by using corresponding input at each time point. Error bars represent standard deviation.



Supplementary Figure S9. Rad51 localization. **(A)** ChIP with *DDY3* WT and corresponding *hmo1Δ* strain using antibody to Rad51 at *MAT*, 0.2 kb upstream, 9.5 kb downstream, 29.8 kb upstream from DSB and at *POL5* during growth in raffinose (no DSB induction). IC, input control; No, no antibody; IP, immunoprecipitation with antibody to Rad51. **(B)** Densitometric analysis of ChIP data shown in (A), obtained with ImageJ software. Fold enrichment = ChIP/Input DNA. Three independent experiments were performed. Error bars represent standard deviation.



Supplementary Figure S10. Survival assay. Survival of *DDY3* and corresponding *AB* strain expressing HMO1 deleted for its C-terminal tail following induction of DNA double strand breaks. After DSB induction, cells were diluted 10^4-fold and plated and colonies counted. Survival is represented as colonies per OD. Cells not induced to express *HO* were plated as control (ctrl). Three independent experiments were performed. Error bars represent standard deviation.



Supplementary Figure S11. Effect of HMO1 C-terminal tail on Arp5 recruitment and H3 eviction. (**A)** ChIP with *DDY3* WT and *AB* strain expressing HMO1 deleted for its C-terminal tail using antibody to Arp 5 at *MAT* and 3.1 kb downstream of DSB during DNA damage (galactose) and repair (glucose). IC, input control; No, no antibody; IP, immunoprecipitation with antibody to Arp5. **(B)** Densitometric analysis of ChIP data shown in (A), obtained with ImageJ software. (**C)** ChIP with *DDY3* WT and *AB* strain using antibody to H3 at *MAT* and 0.2 kb upstream of DSB during DNA damage (galactose) and repair (glucose). **(D)** Densitometric analysis of ChIP data shown in (C). Fold enrichment = ChIP/Input DNA. Three independent experiments were performed. Error bars represent standard deviation.

MATERIALS AND METHODS

DSB efficiency

To determine DSB efficiency, qPCR was conducted with primer pairs flanking the DSB site. Cells were grown at 30°C to an optical density at 600 nm of 1.0. At this time point, an aliquot of cells was removed and used as no damage control. Galactose was added to the remaining culture to a final concentration of 2% to induce DSB and cells were collected at 20 minutes, 1 hour, 2 hour, 3 hour, and 4 hour. Genomic DNA was isolated and used as template for amplification. qPCR was conducted using an ABI Prism 7000 sequence detection system and SYBR Green for detection. Cleavage was monitored as a decrease in qPCR signals amplified. The qPCR signal of no damage control sample was used to calculate percent cut. Experiments were repeated three times and data reported as mean with standard deviations. All primer sequences are available in Supplementary Table S2.

Fidelity assay

DDY3 and *DDY3hmo1Δ* were transformed with plasmid carrying the galactose-inducible HO endonuclease gene and URA3 as a selectable marker. Transformed cells were grown in raffinose-containing SD drop out media to an optical density at 600 nm of 1.0. To induce DNA damage, 2% galactose was added and *ho* induction and DSB formation continued for 4 hours. Cells were plated at 10^{-3} dilution on glucose-containing SD drop out agar and incubated for 48 hours. Colonies were pooled and cultured in glucose-containing SD drop out media to an optical density at 600 nm of 0.8. Genomic DNA was isolated and PCR was performed using two different primer pairs. One primer pair was specific for *MAT***a** and another for *MATα*. As control, genomic DNA was isolated from *DDY3* and *DDY3hmo1Δ* cells in no damage conditions. PCR products were electrophoresed on 1.4% agarose gel and fidelity assessed by sequencing.

*MAT*a gene expression

Cells were grown at 30°C to OD_{600} of 0.6. Cells were washed with chilled DEPC-treated water and then collected by centrifugation. Spheroplasts were obtained by treating the cells with zymolyase and total RNA was isolated using Illustra RNA spin mini isolation kit. RNA quality was measured by Nanodrop. cDNA was synthesized by incubating RNA with AMV reverse transcriptase and primer in buffer containing 25 mM dNTP and 25 mM $MgCl_2$ for 1 h at 42°C. cDNAs were amplified by PCR using primer specific for the *MAT* and *IPP1* loci. PCR products were loaded on 1.4% agarose gels containing 0.01% ethidium bromide. Signal intensities from PCR data were quantified from the TIFF images by using ImageJ software. Each experiment was repeated three times and average and standard deviations (SD) are reported.

Survival after hydroxyurea exposure

JKM179, DDY3, and the corresponding *hmo1* deletion strains were grown at 30°C to OD_{600} of 1.0, at which point 15 mM hydroxyurea was added and cultures incubated for 8 hours. Cells were washed and plated at 10^{-3} dilution on YPD agar media in replica and incubated at 30°C. After 48 hours colonies were counted. Each

experiment was repeated three times and data reported as mean with standard deviations.