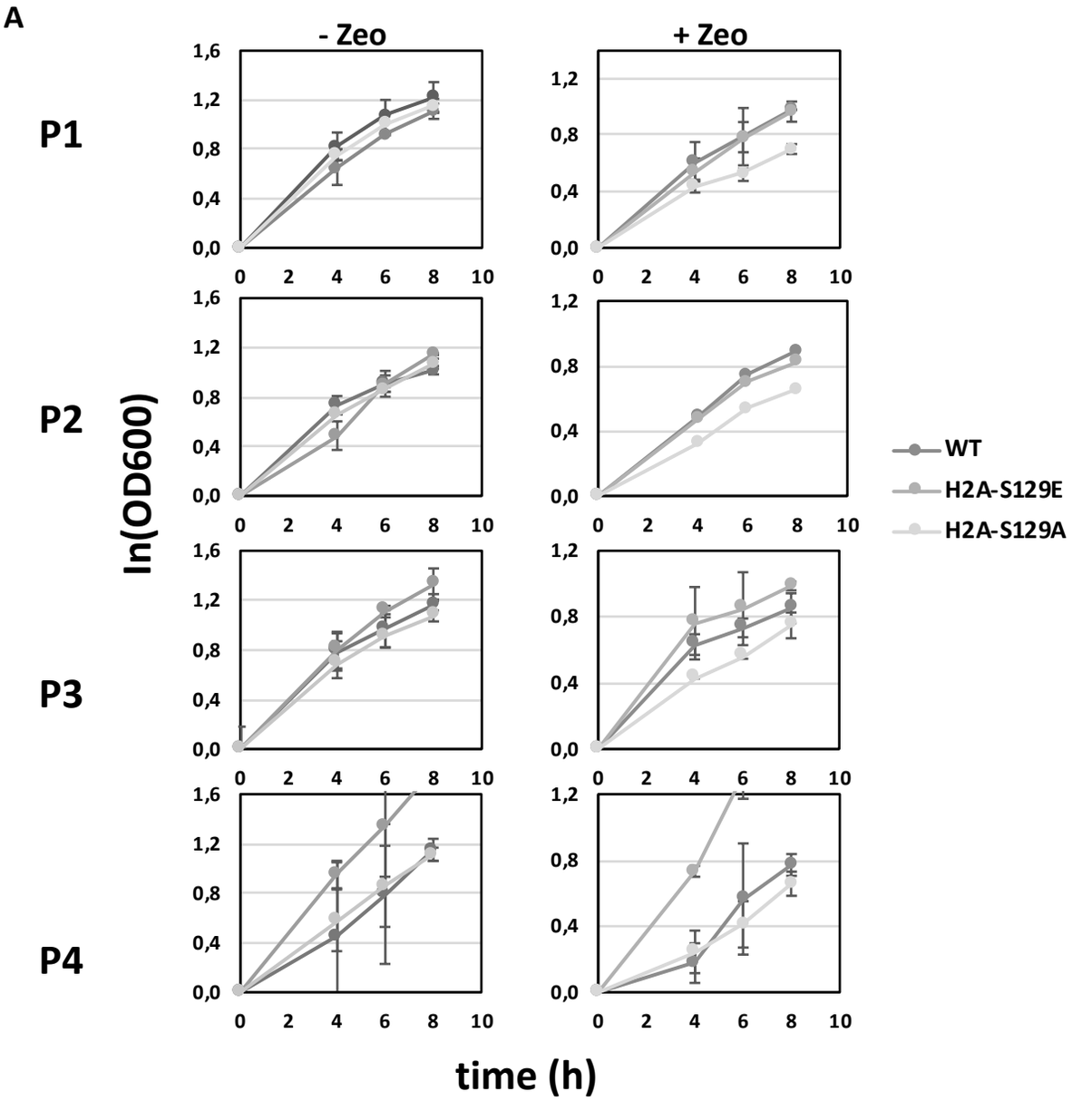


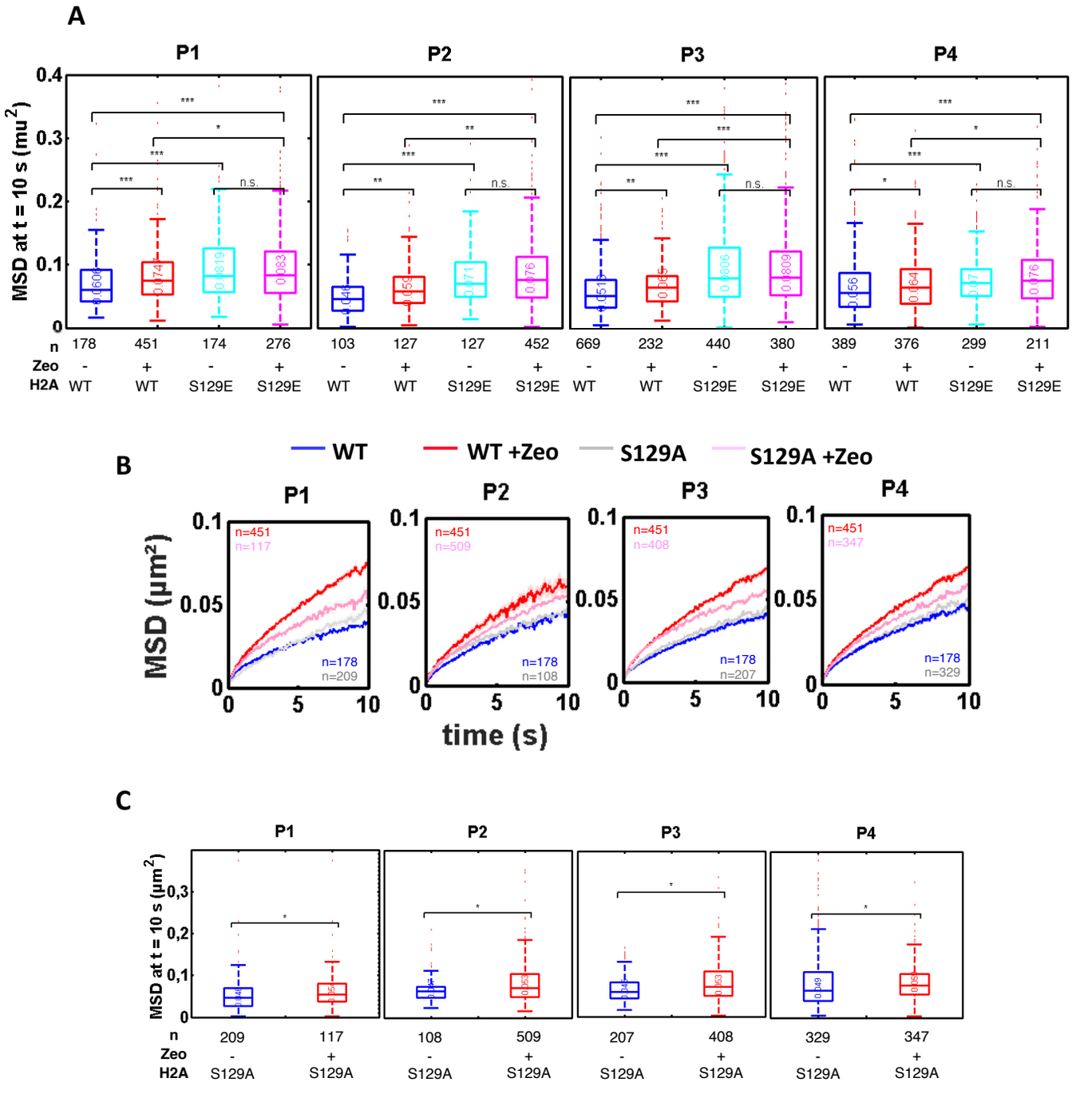
Supplementary Figure 1

Figure S1.

Absence of growth defects and intrinsic DNA damages in H2A-S129E mutant.

A. Representative growth curves for the WT, H2A-S129A, H2AS129E strains in the absence (left) or presence (right) of 250µg/ml Zeocin treatment. Mean values for two independent experiments are plotted for each time point, with error bars showing standard error of the mean (SE).

B. No intrinsic DNA damage in H2A-S129E in absence of Zeocin treatment but prolonged exposure to Zeocin increases DNA damage. Rad52-GFP foci are shown (arrowheads) in representative images of yeast cells that were either untreated or exposed to 250µg/ml of the genotoxic drug Zeocin for 4 and 6 h in WT (black) and H2A-S129E strain (grey). Bar graphs show mean ± s.e.m. P values are calculated after a non-parametric t test (n.s, not significant P>0.05).



Supplementary Figure 2

Figure S2.

Values of MSDs at 10 sec. in WT, H2A-S129E and H2A-S19E mutated strains

A. Values of MSD at 10 sec for WT, H2A-S129E and H2A-S129A. Boxplots show the distribution of MSD at 10 s in absence of Zeocin (WT, blue; H2A-S129E, light blue) or after 3 h Zeocin exposure (WT, red; H2A-S129E, pink), for the four loci Gr1–Gr4. The horizontal line at the center of each box indicates the median value, the bottom and top limits indicate the lower and upper quartiles, respectively. The whiskers indicate the full range of measured values, except for outliers, which are shown as small red dots. Brackets indicate the result of a Wilcoxon rank-sum test between distributions, with “n.s.” for “not significant” ($P > 0.05$), * for $P < 0.05$, ** for $P < 10^{-2}$ and *** for $P < 10^{-3}$.

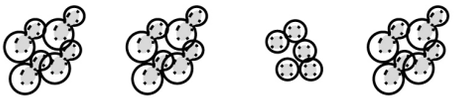
B. MSDs for WT and H2A-S129A. Mean square displacement measured as in figure 1D as function of time interval of the four Gr1 to Gr4 green loci in H2A-S129A background. Blue and red curves are for untreated and treated Wild-Type cells (WT); grey and pink curves are for untreated and treated H2A-S129A mutant, respectively. The numbers of cells used to compute each curve (n) are indicated.

C. Values of MSD at 10 sec for H2A-S129A in the presence or absence of treatment. Boxplots show the distribution of MSD at 10 s as in B, in absence of Zeocin (blue) or after 3 h Zeocin exposure (red), for the four loci Gr1–Gr4 in H2A-S129A mutated background.

A

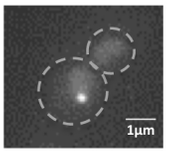
% of G2/M arrested cells

	WT		H2A-S129E		Δ*rad9*		Δ*rad9* H2A-S129E	
ZEO	-	+	-	+	-	+	-	+
%	10	92	9	89	3	4	7	94

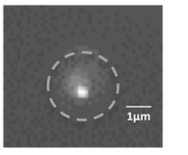


+ Zeo

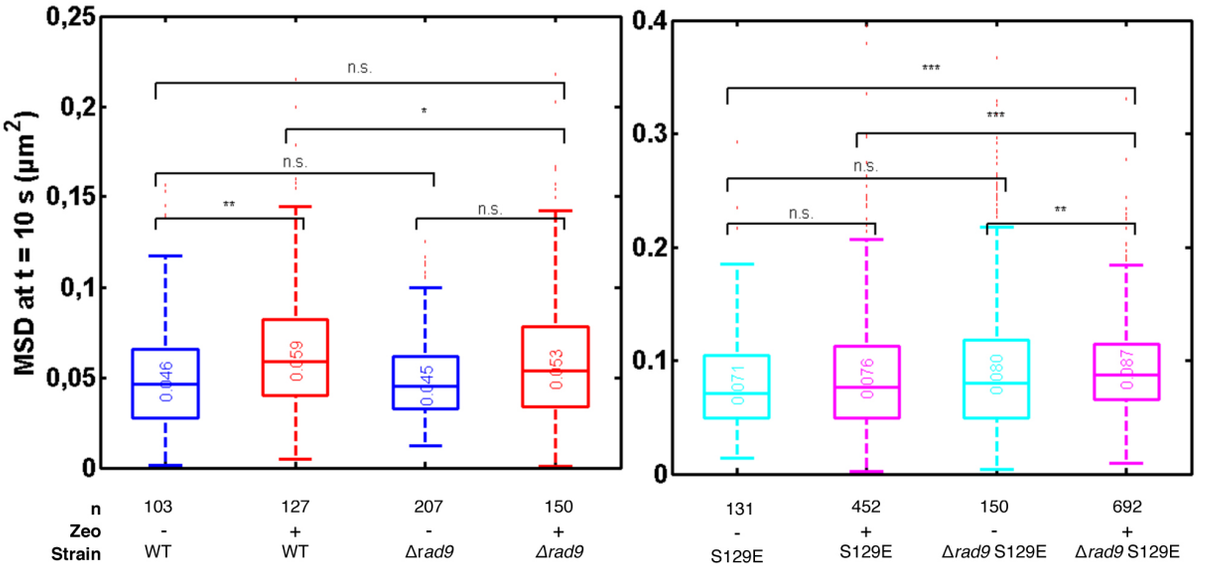
Δ *rad9* S129E



Δ *rad9*



B



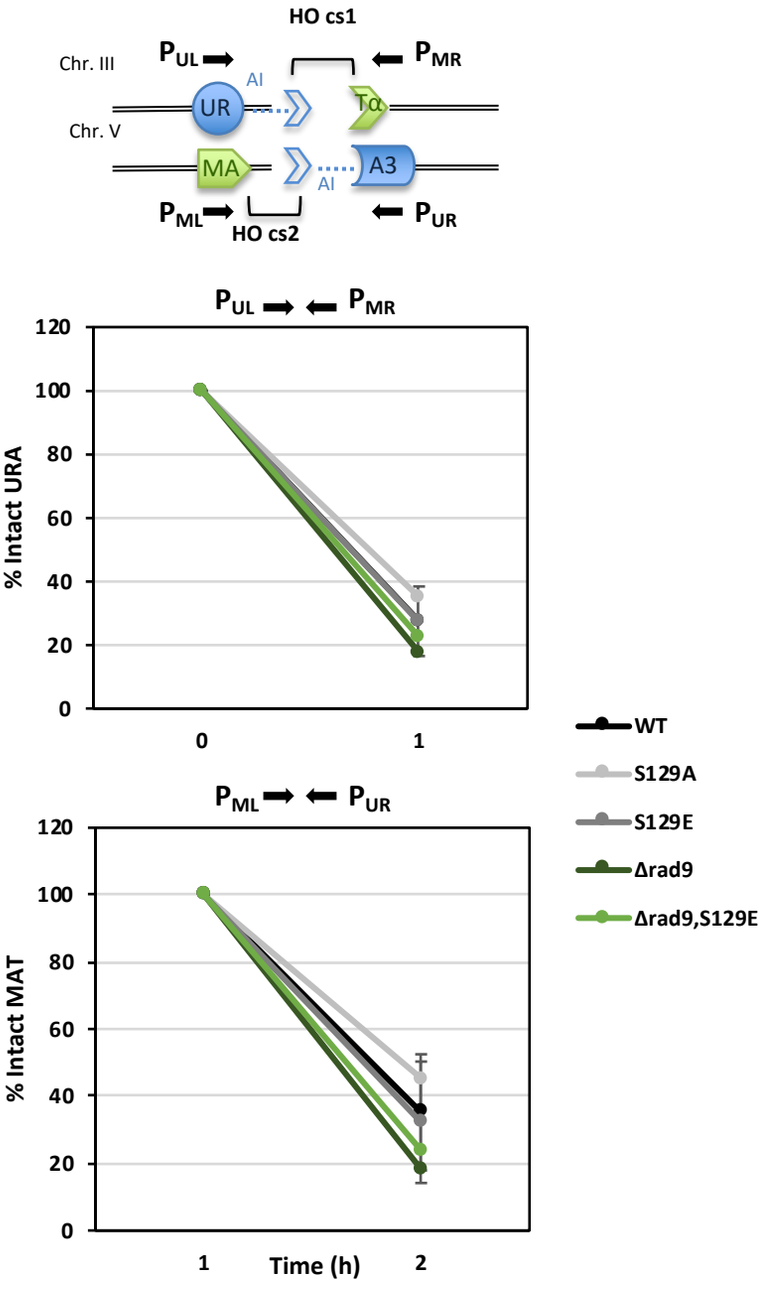
Supplementary Figure 3

Figure S3.

Percent of G2/M arrested cells and values of MSD at 10 sec for WT, H2A-S129E, Δ*rad9* and Δ*rad9* H2A-S129E mutated cells in the absence or the presence of Zeocin for.

A. Cells expressing fluorescent LacI-GFP protein to lacO-array were inspected after image acquisition. Unbound LacI-GFP was used as a nuclear staining. G2/M arrested cells show nuclear masses separated between mother and daughter cells, but no septum formed. Examples are shown on the right.

B. Boxplots show the distribution of MSD at 10 s of WT compared to Δ*rad9* strains (left) and H2A-S129E compared to Δ*rad9* H2A-S129E double mutant (right) in absence of Zeocin (bleu and cyan, respectively) or after 6 h Zeocin exposure (red and magenta, respectively). The horizontal line at the center of each box indicates the median value, the bottom and top limits indicate the lower and upper quartiles, respectively. The whiskers indicate the full range of measured values, except for outliers, which are shown as small red dots. Brackets indicate the result of a Wilcoxon rank-sum test between distributions, with “n.s.” for “not significant” ($P > 0.05$), * for $P < 0.05$, ** for $P < 10^{-2}$ and *** for $P < 10^{-3}$. Analyzed cells range from (n) ~100 - ~1000.



Supplementary Figure 4

Figure S4

Efficiency of the two HO-cleavages in translocation assay strains by Q-PCR.

Efficiency of HO cleavage in WT, H2A-S129A, H2A-S129E, *Δrad9* and *Δrad9*, H2A-S129E strains carrying the two HO cleavage sites at the MATα and *URA3* locus was determined by quantitative PCR using primers flanking HO recognition sites before (t0h) and after (t1h) galactose induction, normalized by the amount of *ACT1* sequence.

STRAINS

Common name	Strain	Genotype	Reference
Green-Red pairs			
P1	YHB76-4-a	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP- NATMX, yDR003w::tetO-TEF-URA, HIS3::HIS3-LacI-GFP, yDR095c::256lacO-TEF-LEU2	{Herbert:2017it}
P2	YHB103-1-a	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP- NATMX, yDR199w::tetO-TEF-URA3, HIS3::HIS3-LacI-GFP, yDR297w::256lacO-TEF-LEU2	{Herbert:2017it}
P3	YHB215-2-alpha	Mat alpha, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, iyGL117::tetR-mRFP- NATMX, yDR354w::tetO-TEF-URA3, HIS3::HIS3-LacI-GFP, yDR259C::256lacO-TEF-LEU2	{Herbert:2017it}
P4	YHB154-1-alpha	Mat alpha, ura3Δ0, leu2Δ0, his3Δ1, ade2-661,yDR539w::112tetO-TEF-URA3, iyGL117::tetR-mRFP- NATMX, HIS3::HIS3-LacI-GFP, yDR445C::256lacO-TEF-LEU2	{Herbert:2017it}
H2A mutants			
P1 S129E	YEF 1259	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX yDR003W::tetOt-URA3 HIS3::HIS3-LacI-GFP yDR095C::lacO-TEF-LEU2, hta1::HTA1-S129E, hta2::HTA2-S129E	This study
P2 S129E	YEF 1111	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX yDR199W::tetO-URA3 HIS3::HIS3-LacI-GFP yDR297W::lacO-TEF-LEU2, hta1::HTA1-S129E, hta2::HTA2-S129E	This study
P3 S129E	YEF 1261	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, iyGL117::tetR-mRFP NATMX yDR354W::tetO-URA3 HIS3::HIS3-LacI-GFP yDR259C::lacO-TEF-LEU2 , hta1::HTA1-S129E, hta2::HTA2-S129E	This study
P4 S129E	YEF 1163	Mat alpha, ura3Δ0, leu2Δ0, his3Δ1, ade2-661, yDR539w::112tetO-TEF-URA3, iyGL117::tetR-mRFP NATMX yDR354W::tetO-URA3 HIS3::HIS3-LacI-GFP yDR4459C::lacO-TEF-LEU2, hta1::HTA1-S129E, hta2::HTA2-S129E	This study
P1 S129A	YEF 1030	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX yDR003W::tetOt-URA3 HIS3::HIS3-LacI-GFP yDR095C::lacO-TEF-LEU2, hta1::HTA1-S129A-KANMX , hta2::HTA2-S129A-ADE2	{Herbert:2017it}
P2 S129A	YEF 1028	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX yDR199W::tetO-URA3 HIS3::HIS3-LacI-GFP yDR297W::lacO-TEF-LEU2, hta1::HTA1-S129A-TRP1, hta2::HTA2-S129A-ADE2	{Herbert:2017it}
P3 S129A	YEF 1032	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, iyGL117::tetR-mRFP NATMX yDR354W::tetO-URA3 HIS3::HIS3-LacI-GFP yDR259C::lacO-TEF-LEU2, hta1::HTA1-S129A-KANMX , hta2::HTA2-S129A-ADE2	{Herbert:2017it}
P4 S129A	YEF 1034	Mat alpha, ura3Δ0, leu2Δ0, his3Δ1, ade2-661, yDR539w::112tetO-TEF-URA3, iyGL117::tetR-mRFP NATMX YDR354W::tetO-URA3 HIS3::HIS3-LacI-GFP YDR4459C::lacO-TEF-LEU2, hta1::HTA1-S129A-KANMX , hta2::HTA2-S129A-ADE2	{Herbert:2017it}
rad9 mutants			
P2 rad9	YEF 1187	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX YDR199W::tetO-URA3 HIS3::HIS3-LacI-GFP YDR297W::lacO-TEF-LEU2, yDR217C::KANMX	This study
P2 rad9 S129E	YEF 1188	Mat a, ura3Δ0, leu2Δ0, his3Δ1, lys2Δ202, ade2-661, can1::HPHMX, iyGL117::tetR-mRFP NATMX YDR199W::tetO-URA3 HIS3::HIS3-LacI-GFP YDR297W::lacO-TEF-LEU2, hta1::HTA1-S129E, hta2::HTA2-S129E, yDR217C::KANMX	This study
Repair Assay			
Δku70	YEF571	Mat a, ura3Δ0, leu2Δ0, his3Δ1, trp1Δ63, ade2-661, yMR284W::KANMX	Euroscarf
Translocation Assay			
Tr WT	YEF 1375	ho Δ MATΔ3'::intron::ura3Δ5' hmlΔ::ADE1 hmrΔ::ADE1 ura3Δ3'::intron::Hocs ade3::GAL::HO	{Lee:2008it}
Tr S129E	YEF 1377	ho Δ MATΔ3'::intron::ura3Δ5' hmlΔ::ADE1 hmrΔ::ADE1 ura3Δ3'::intron::Hocs ade3::GAL::HO, hta1::HTA1-S129E, hta2::HTA2-S129E	This study
Tr S129A	YEF 1378	ho Δ MATΔ3'::intron::ura3Δ5' hmlΔ::ADE1 hmrΔ::ADE1 ura3Δ3'::intron::Hocs ade3::GAL::HO, hta1::HTA1-S129A, hta2::HTA2-S129A	This study
Tr rad9	YEF 1405	ho Δ MATΔ3'::intron::ura3Δ5' hmlΔ::ADE1 hmrΔ::ADE1 ura3Δ3'::intron::Hocs ade3::GAL::HO, yDR217C::KANMX	This study
Tr rad9 S129E	YEF 1406	ho Δ MATΔ3'::intron::ura3Δ5' hmlΔ::ADE1 hmrΔ::ADE1 ura3Δ3'::intron::Hocs ade3::GAL::HO, hta1::HTA1-S129E, hta2::HTA2-S129E, yDR217C::KANMX	This study
SPB-Cen4 strains			
WT	YEF 1019	CENIV-tetO, tetR-GFP, spc42::SPC42::mCherry-HIS3	This study
H2A S129E	YEF 1364	CENIV-tetO, tetR-GFP, spc42::SPC42::mCherry-HIS3, hta1::HTA1-S129E, hta2::HTA2-S129E	This study
H2A S129A	YEF 1366	CENIV-tetO, tetR-GFP, spc42::SPC42::mCherry-HIS3, hta1::HTA1-S129A, hta2::HTA2-S129A	This study

PLASMIDS

Name	Description	Marker	Reference
pEF562	ycas9- PHU91	KANMX (ARS-CEN)	J. Haber
pEF567	pEF562+guide HTA1	KANMX (ARS-CEN)	This study
pEF568	pEF562+guide HTA2	KANMX (ARS-CEN)	This study
PRS413	PRS413 (SacI site)	HIS3 (ARS-CEN)	Addgene

PRIMERS

Name	Description	Sequence	
pFGF 001	HTA1 guide forward	CTTCTCAAGAATTATAAGATGTTTT	This study
pFGF 002	HTA1 guide reverse	ATCTTATAATTCTTGAGAAGGATCA	This study
pFGF 003	HTA2 guide forward	TTACAGTTCTTGAGAAGCTTTGTTTT	This study
pFGF 004	HTA2 guide reverse	AAGCTTCTCAAGAAACTGTAGATCA	This study
pFGF 005	AmpB reverse	AGCTGAATGAAGCCATACCAAACGA	This study
pFGF 006	80 nt donor sequence for HTA1	GAAGTCTGCCAAGGCTACCAAGGCTGAACAAGAATTATAAGATCGGTTCTGGTATTTTAAAGAAGGCGGAA	This study
pFGF 007	80 nt donor sequence for HTA2	CAAAACTTGTTGCCAAAGAAGTCTGCCAAGACTGCCAAAGCTgaaCAAGAACTGTAAGAACTGAGTTGAAAAGAAACAAA	This study
pFGF 008	HTA1 verification forward	GTTGCCAAAGAAGTCTGCCA	This study
pFGF 009	HTA1 verification reverse	TGGAGAAGCAGTTTAGTTCCTT	This study
pFGF 010	HTA2 verification forward	AATGTTACCATCGCCCAAGG	This study
pFGF 011	HTA2 verification reverse	ACCAGTCTTCCTCATATGACCT	This study
pFGF 012	rad9 integration forward	ACGGCCTTGTTAGCGTTAGA	This study
pFGF 013	rad9 integration reverse	CCATTGGGGTGAATCTCGTT	This study
pFGF 014	rad9 verification forward	TCAAGGGGAAGTGTCAGCAA	This study
pFGF 015	rad9 verification reverse	TGCTGATATGTGTCGTCCCA	This study
pFGF 016	m13 forward	GTAAAACGACGGCCAGT	Addgene
pFGF 017	m13 inverse	GTCATAGCTGTTTCCTG	Addgene

Table S1. Strains, plasmids and primers used in this study.