Table S1: qPCR primers used in this study

Gene	primer name	sequence
RAD6	C1205	TGCCCCACCGGGTGTA
	C1166	GCGTTCCATACCATGACGTTATC
RAD18	C1167	GTTCAGGTTCAGTGGGACATTCT
	C1168	GGTTCCCTGGTCACTTTGAACT
UBC13	C1171	TGACGTCGCTGAAGATTGGA
	C1172	CATTCGCGAGCCTTAGCTTT
REV3	C1177	CCATCGCACGGTGAAAGC
	C1178	AAGCACCAAACACCCTGATGT
RNR2	C1197	CCATTCACACCATCCCAGAAA
	C1198	CTTGAATCCATCTTAAAGCCCATT
ALG9	C1215	CTGTGGAATTATTGCCTTC
	C1216	GCCTAGTATACTAGCCAGG

Table S2: Strains used in supplemental material

Strain	***Genotype***	***Source***
JC2257	W303 *MAT***a** *rev1*Δ*::HIS3*	Xiao lab (WXY29-36)
JC2283	W303 *MAT***a** *yng2*Δ*::URA3, mms2*Δ*::HIS3*	this study
JC2290	W303 *MAT***a** *mms2*Δ*::HIS3*	this study
JC2619	W303 *MAT***a** *yng2*Δ*::URA3, rev1*Δ*::HIS3*	this study
JC2769	W303 *MAT***a** *esa1-L254P::NatRMX4, rev1*Δ*::HIS3*	this study
JC2773	W303 *MAT***a** *esa1-L254P::NatRMX4, mms2*Δ*::HIS3*	this study
JC3119	W303 *MAT***a** *eaf1*Δ*::URA3, ubc13*Δ*::HIS3*	this study
JC3220	W303 *MAT***a** *eaf1*Δ*::URA3, rev3*Δ*::LEU2*	this study
JC3430	W303 *MAT***a** *eaf1*Δ*::URA3*	this study

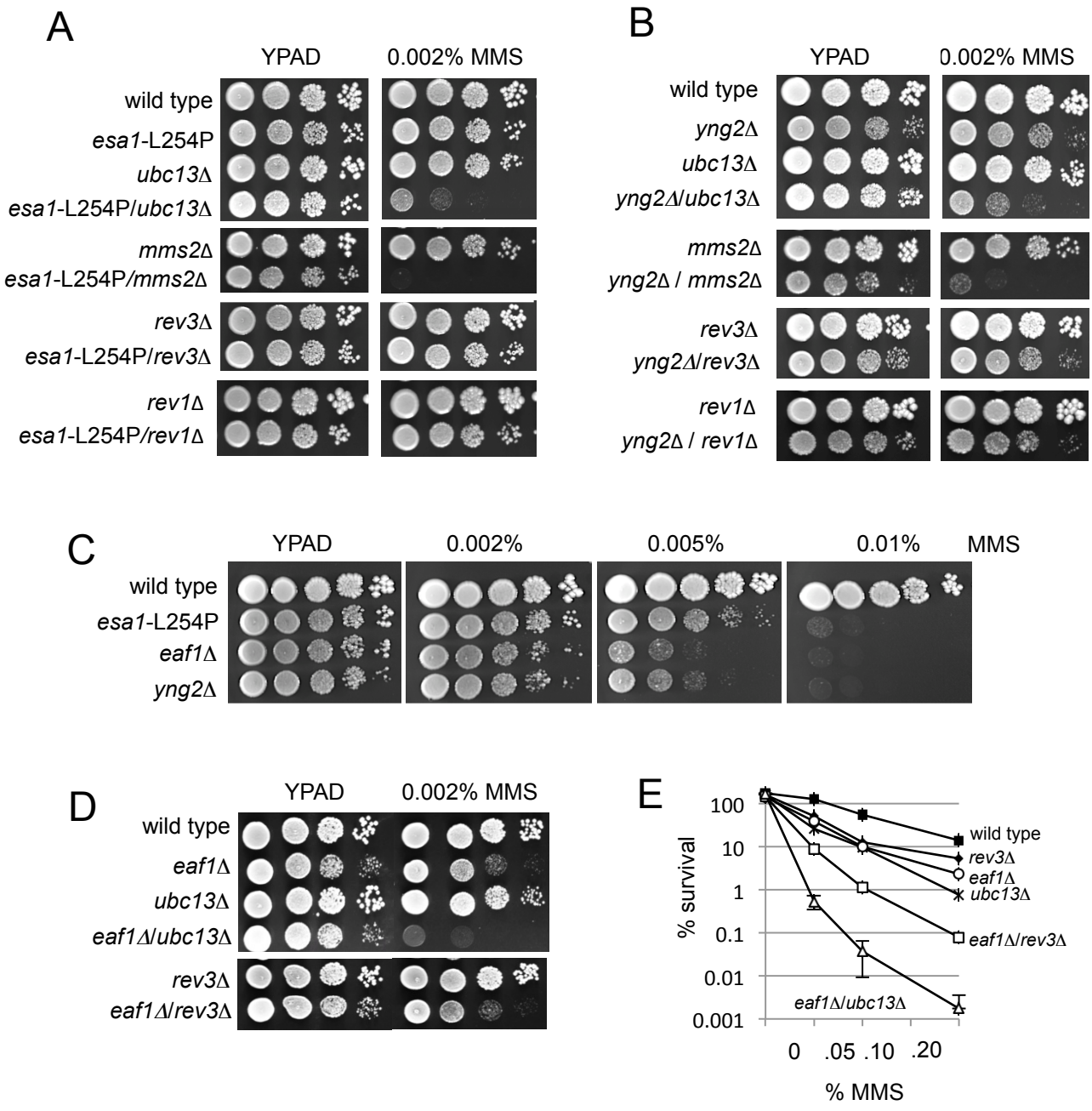

Figure S1 Similar to other NuA4 components, the loss of EAF1 interacts genetically with the DNA damage tolerance (DDT) pathway. (A) Drop assays (1:10 serial dilutions) from exponentially growing cultures were performed on YPAD +/- media containing the indicated concentrations of MMS at 30°C for wild type (JC470), *esa1*-L254P (JC2767), *ubc13*Δ (JC2291), *esa1*-L254P/*ubc13*Δ (JC2775), *mms2*Δ (JC2290), *esa1*-L254P/*mms2*Δ (JC2773), *rev3*Δ (JC2289), and *esa1*-L254P/*rev3*Δ (JC2771), *rev1*Δ (JC2257), and *esa1*-L254P/*rev1*Δ (JC2769). (B) *yng2*Δ (JC2036), *yng2*Δ /*ubc13*Δ (JC2285), *yng2*Δ/*mms2*Δ (JC2283), *yng2*Δ /*rev3*Δ (JC2281) and *yng2*Δ / *rev1*Δ (JC2619). (C) *eaf1*Δ (JC3430), *yng2*Δ (JC2036), and (D) *ubc13*Δ (JC2291), *eaf1*Δ /*ubc13*Δ (JC3219), *rev3*Δ (JC2289), and *eaf1*Δ /*rev3*Δ (JC3220). (E) Cell survival was measured after transient exposure to increasing concentrations of MMS for 1 hr. with the same strains in D.

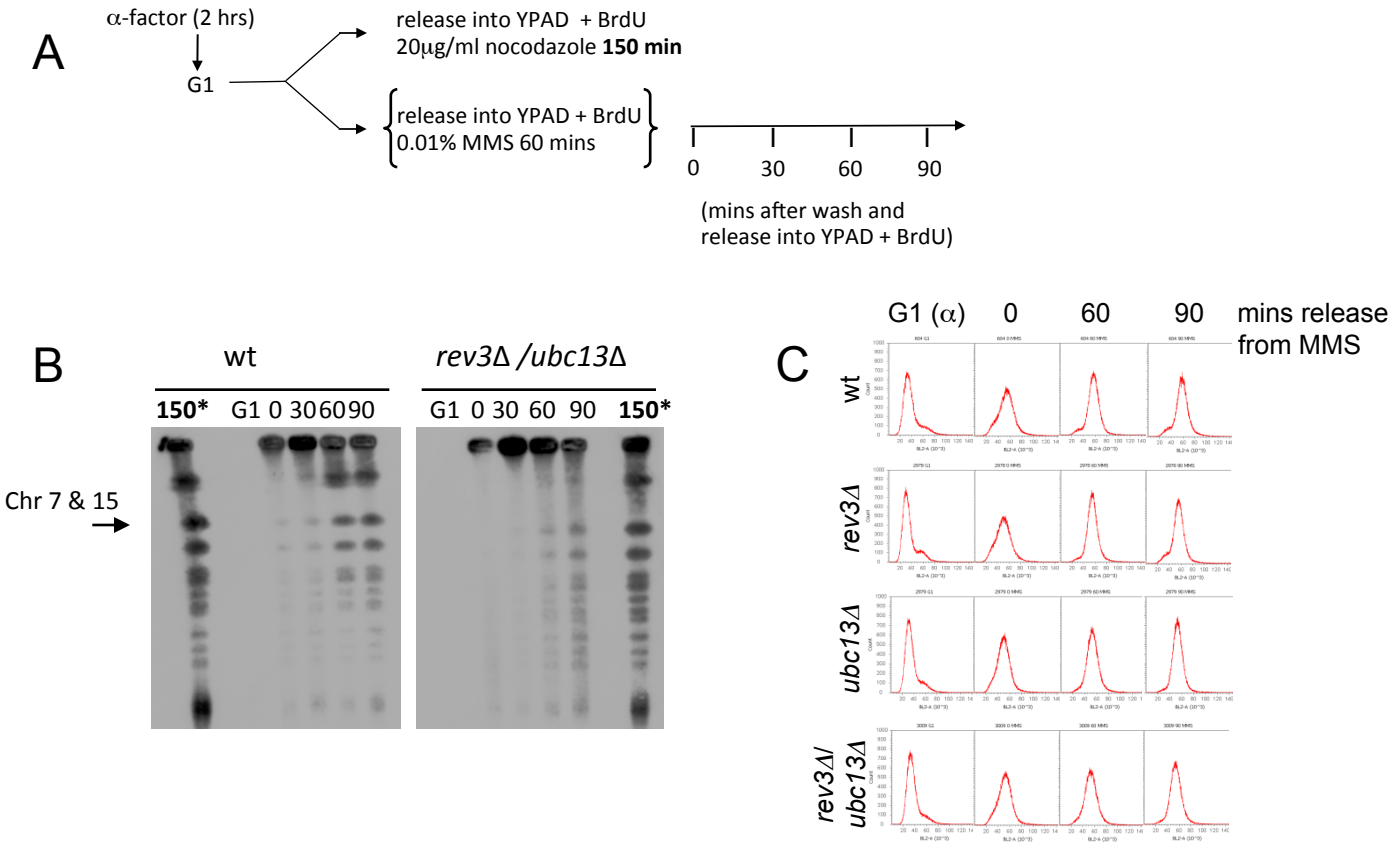

Figure S2 Pulse Field Gel Electrophoresis (PFGE) was performed on cells disrupted for DDT factors. (A) Cells were arrested in α-factor for 2 hours followed by release into YPAD media containing bromodeoxyuridine (BrdU; 400μg/ml) and either nocodazole (7.5μg/ml) for 2.5 hours or 0.01% MMS for 1 hour. Following MMS treatment, cells were released into YPAD and samples were collected at the indicated time points. (B) Pulse Field Gel Electrophoresis (PFGE) was performed, followed by a Southern transfer to nitrocellulose, and blotted with α-BrdU antibodies in wild type (JC604) and *rev3Δ/ubc13Δ* (JC3009), (C) The cell cycle stage was monitored by flow cytometry. All data shown here follows the same protocols as in Figs. 2 and 3.

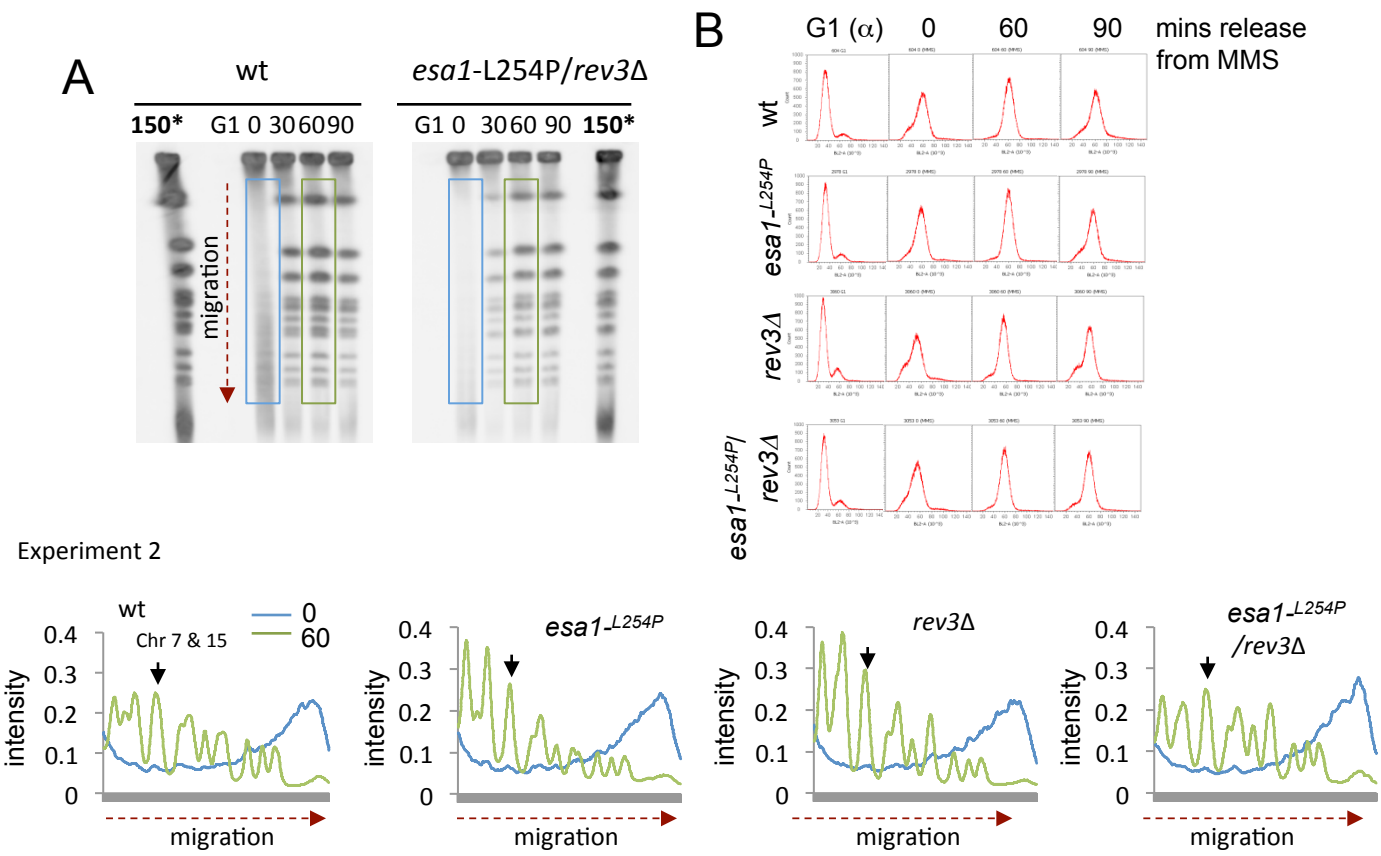

Figure S3 Pulse Field Gel Electrophoresis (PFGE) was performed on cells disrupted for the error prone bypass factor *rev3*Δ in combination with *esa1*-L254P. Cells were arrested in α-factor for 2 hours followed by release into YPAD media containing bromodeoxyuridine (BrdU; 400µg/ml) and either nocodazole (7.5µg/ml) for 2.5 hours or 0.01% MMS for 1 hour. Following MMS treatment, cells were released into YPAD + BrdU and samples were collected at the indicated time points. Pulse Field Gel Electrophoresis (PFGE) was performed, followed by a Southern transfer to nitrocellulose, and blotted with α-BrdU antibodies in wild type (JC604), *esa1*-L254P (JC3060), *rev3*Δ (JC2978) and *esa1*-L254P / *rev3*Δ (JC3053) - (A) pictures of one representative gel for wild type and the double mutant demonstrates that in the absence of MMS, replication and chromosome integrity is indistinguishable between wild type and the double mutant (150* on gel). (B) The cell cycle stage was monitored by flow cytometry. (C) The BrdU signal at the 0 min (blue) and 60 min (green) time points were quantified by Image J with the migration distance of chromosomes vs the intensity of BrdU plotted, giving a measure of newly synthesized chromosomes during one round of DNA replication. All data shown here follows the same protocols as in Figs. 2 and 3.

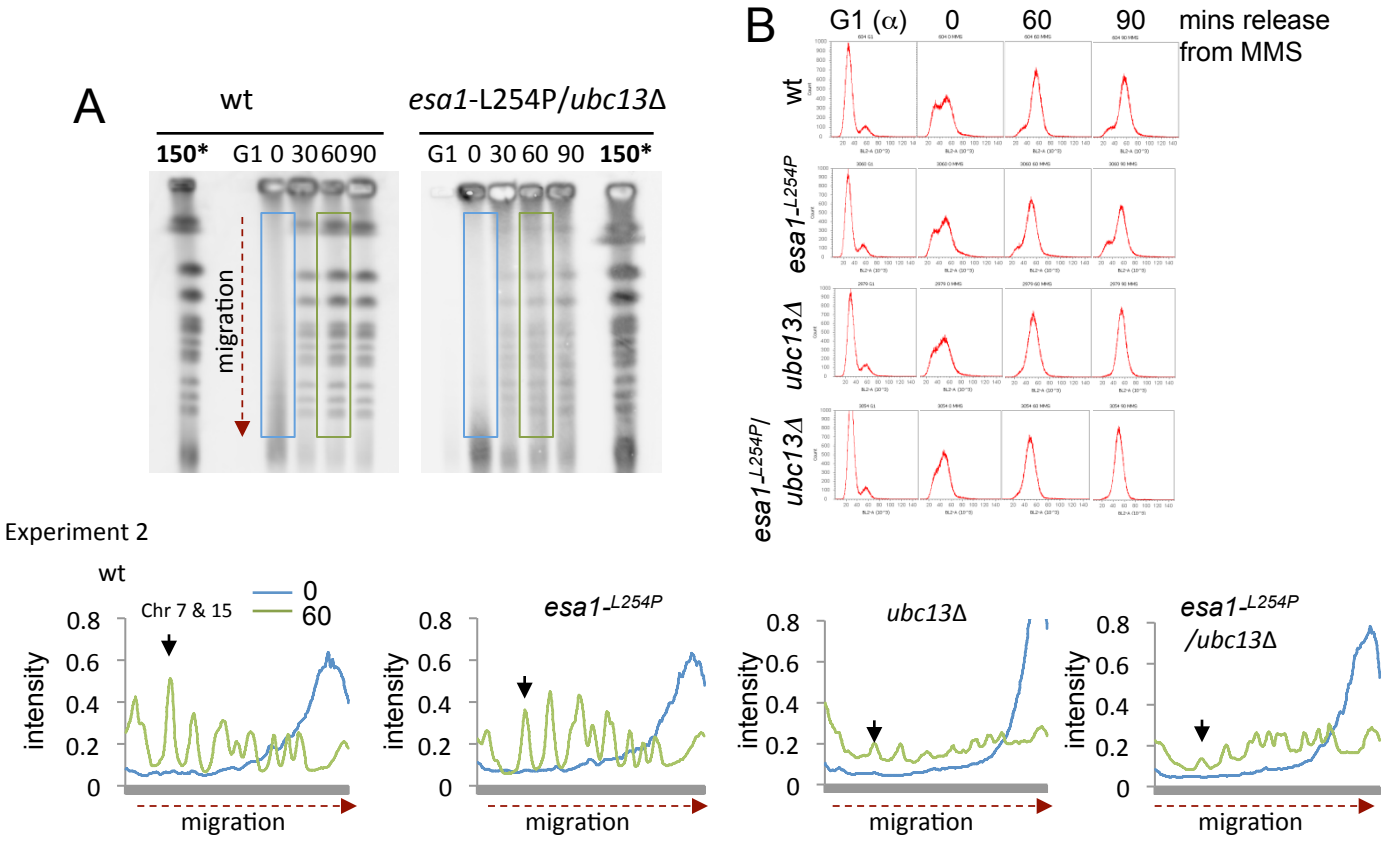

Figure S4 Pulse Field Gel Electrophoresis (PFGE) was performed on cells disrupted for the error free bypass factor *ubc13*Δ in combination with *esa1*-L254P. Cells were arrested in α-factor for 2 hours followed by release into YPAD media containing bromodeoxyuridine (BrdU; 400µg/ml) and either nocodazole (7.5µg/ml) for 2.5 hours or 0.01% MMS for 1 hour. Following MMS treatment, cells were released into YPAD + BrdU and samples were collected at the indicated time points. Pulse Field Gel Electrophoresis (PFGE) was performed, followed by a Southern transfer to nitrocellulose, and blotted with α-BrdU antibodies in wild type (JC604), *esa1*-L254P (JC3060), *ubc13*Δ (JC2979) and *esa1*-L254P / *ubc13*Δ (JC3054) - (A) pictures of one representative gel for wild type and the double mutant demonstrates that in the absence of MMS, replication and chromosome integrity is indistinguishable between wild type and the double mutant (150* on gel). (B) The cell cycle stage was monitored by flow cytometry. (C) The BrdU signal at the 0 min (blue) and 60 min (green) time points were quantified by Image J with the migration distance of chromosomes vs the intensity of BrdU plotted, giving a measure of newly synthesized chromosomes during one round of DNA replication. All data shown here follows the same protocols as in Figs. 2 and 3.

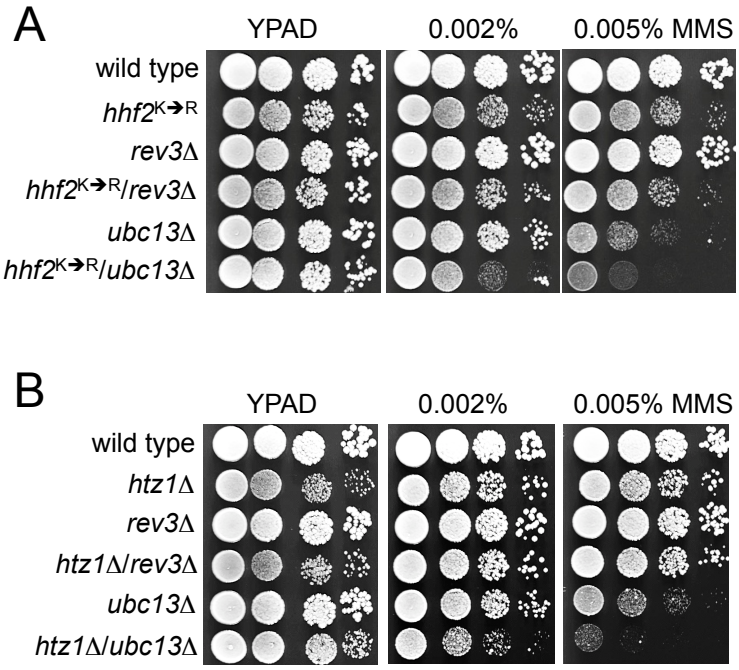

Figure S5 NuA4 target histone H4 shows genetic interactions with the DDT pathway (A) Drop assays (1:10 serial dilutions) from exponentially growing cultures were performed on YPAD +/- media containing the indicated concentrations of MMS at 30°C for wild type (JC470), *hhf2*$^{K\rightarrow R}$ (JC3178), *rev3*Δ (JC2289), *hhf2*$^{K\rightarrow R}$/*rev3*Δ (JC3195) *ubc13*Δ (JC2291), *hhf2*$^{K\rightarrow R}$/*ubc13*Δ (JC3179), and (B) *htz1*Δ (JC2090), *htz1*Δ/*rev3*Δ (JC2762) and *htz1*Δ/*ubc13*Δ (JC2764)

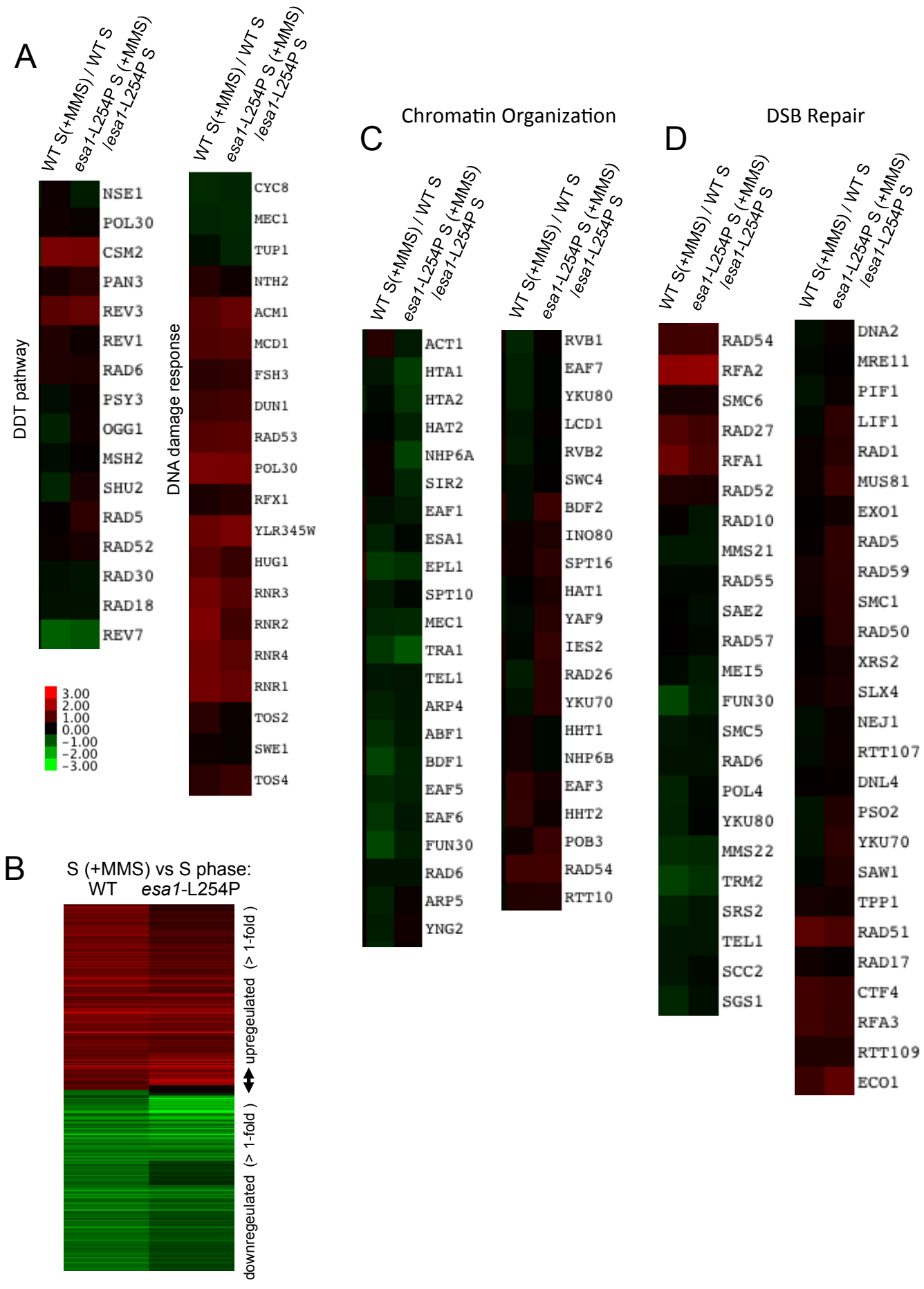
A
WT S(+MMS) / WT S
esa1-L254P S (+MMS) /esa1-L254P S
DDT pathway
NSE1
POL30
CSM2
PAN3
REV3
REV1
RAD6
PSY3
OGG1
MSH2
SHU2
RAD5
RAD52
RAD30
RAD18
REV7
3.00
2.00
1.00
0.00
-1.00
-2.00
-3.00
DNA damage response
CYC8
MEC1
TUP1
NTH2
ACM1
MCD1
FSH3
DUN1
RAD53
POL30
RFX1
YLR345W
HUG1
RNR3
RNR2
RNR4
RNR1
TOS2
SWE1
TOS4
B
S (+MMS) vs S phase:
WT esa1-L254P
upregeulated (> 1-fold)
downregeulated (> 1-fold)
C
Chromatin Organization
ACT1
HTA1
HTA2
HAT2
NHP6A
SIR2
EAF1
ESA1
EPL1
SPT10
MEC1
TRA1
TEL1
ARP4
ABF1
BDF1
EAF5
EAF6
FUN30
RAD6
ARP5
YNG2
RVB1
EAF7
YKU80
LCD1
RVB2
SWC4
BDF2
INO80
SPT16
HAT1
YAF9
IES2
RAD26
YKU70
HHT1
NHP6B
EAF3
HHT2
POB3
RAD54
RTT10
D
DSB Repair
RAD54
RFA2
SMC6
RAD27
RFA1
RAD52
RAD10
MMS21
RAD55
SAE2
RAD57
MEI5
FUN30
SMC5
RAD6
POL4
YKU80
MMS22
TRM2
SRS2
TEL1
SCC2
SGS1
DNA2
MRE11
PIF1
LIF1
RAD1
MUS81
EXO1
RAD5
RAD59
SMC1
RAD50
XRS2
SLX4
NEJ1
RTT107
DNL4
PSO2
YKU70
SAW1
TPP1
RAD51
RAD17
CTF4
RFA3
RTT109
ECO1

Figure S6 Transcriptional comparison of Gene Upregulation in MMS vs. Untreated S phase samples . Cells arrested in α-factor were released into normal YPAD or YPAD/0.05% MMS for 1hr. RNA was extracted followed by microarray. (A) Transcriptome analysis was performed as described in the Materials and Methods. Heat maps of the genome-wide expression profile of 0.05% MMS vs. untreated samples of wild type and *esa1*-L254P in S phase. Sample genes from the DNA damage tolerance pathway and genes upregulated as part of the transcriptional DNA damage response from derepression of Crt1/Rfx1 and Nrm1. (B) Heat map of the genome-wide expression profile of 0.05% MMS vs. untreated samples indicated that the NuA4 allele, *esa1*-L254P, had a similar transcriptional profile compared to wild type cells. Data looking at specific subsets of genes associated with (C) Chromatin Organization and (D) Double-Strand Break Repair shows few differences between strains.

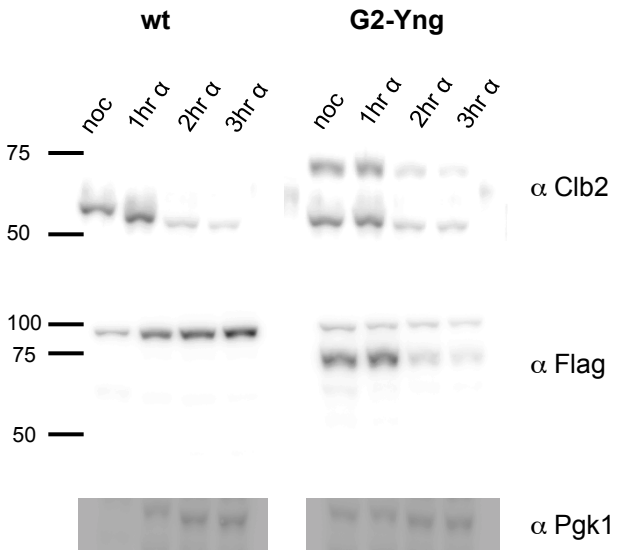

Figure S7 G2-Yng2 expression after Nocodazole release. Degradation of G2-Yng2 after S-phase follows the same pattern as Clb2. During a normal cell cycle Clb2 is rapidly degraded at mitosis. Wild type (JC470) and G2-Yng2 (JC3387) were arrested in nocodazole for 3 hours following release into YPAD media with α-factor with samples taken at indicated times. Immunblots were performed with antibodies α-Clb2 and α-Flag (to visualize G2-Yng2-Flag, and α-Pgk1 used as a loading control. Degradation of G2-Yng2 follows the same kinetics as degradation of Clb2 in both G2-Yng2 and wild type cells.

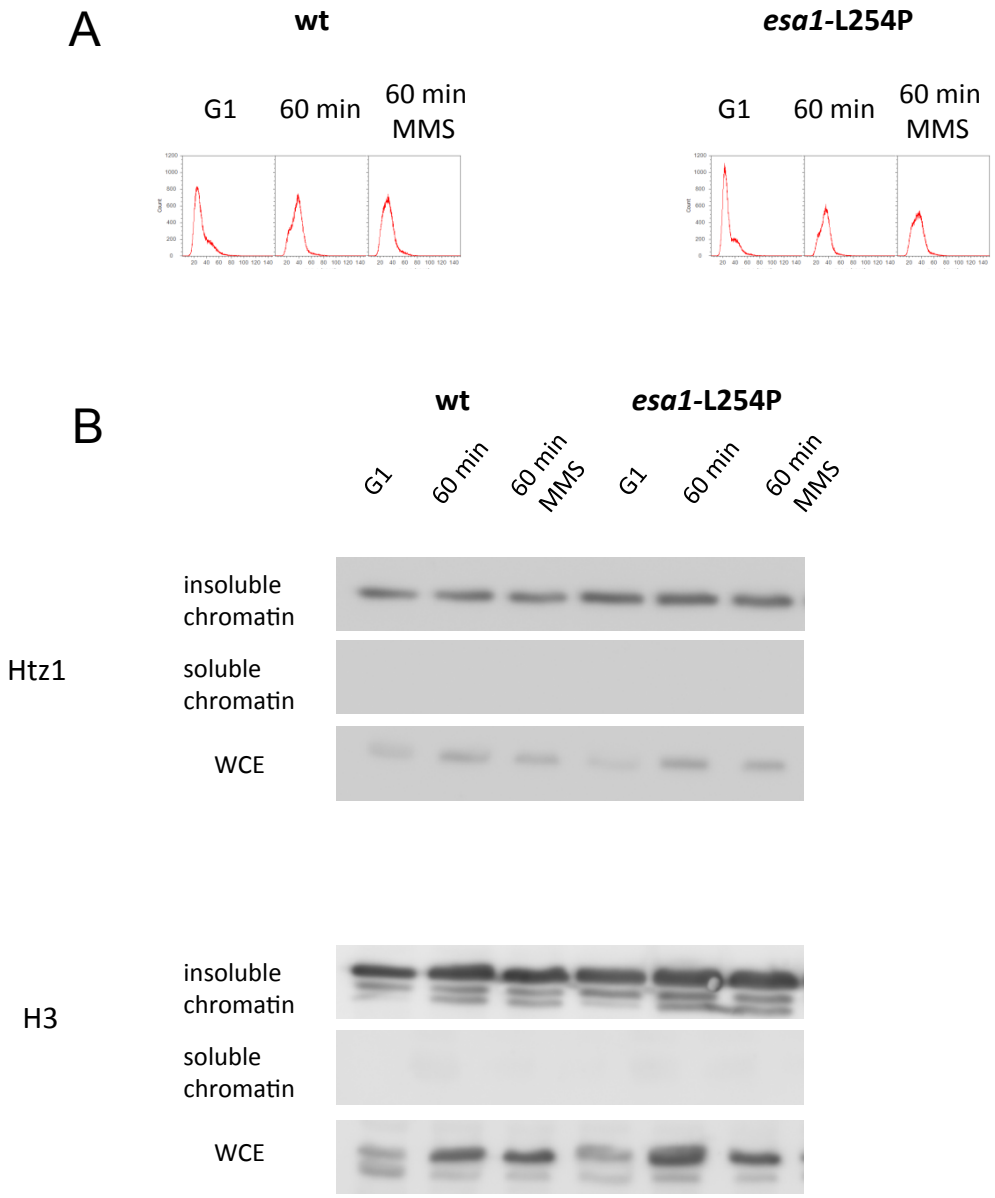

Figure S8 Chromatin association of H2A.Z. H2A.Z (Htz1) levels are the same between wild type (JC470) and *esa1*-L254P (JC2767), independent of cell cycle phase and treatment with MMS. Chromatin association experiment where cells arrested in α-factor are released into normal YPAD or YPAD/0.01% MMS. Cell cycle progression was monitored using flow cytometry (A). Samples were subjected to chromatin association fractionation followed by 18% gel SDS-PAGE and immunoblot with anti-H2A.Z (B). Histone H3 immunoblot was used as both a loading control and control for fractionation.