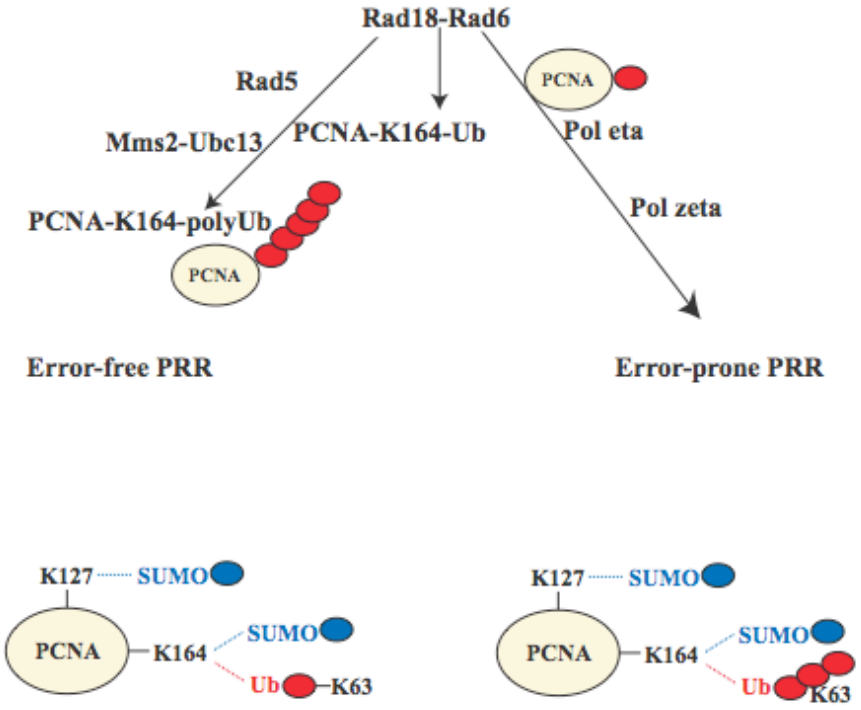
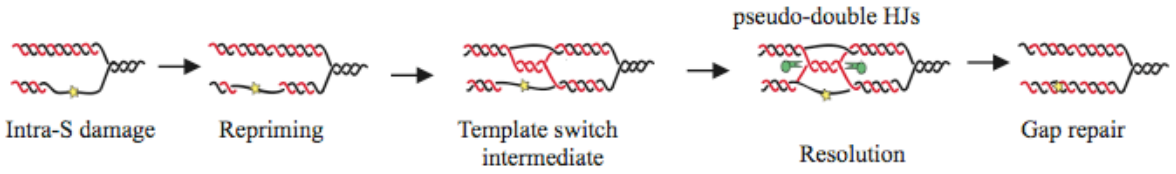
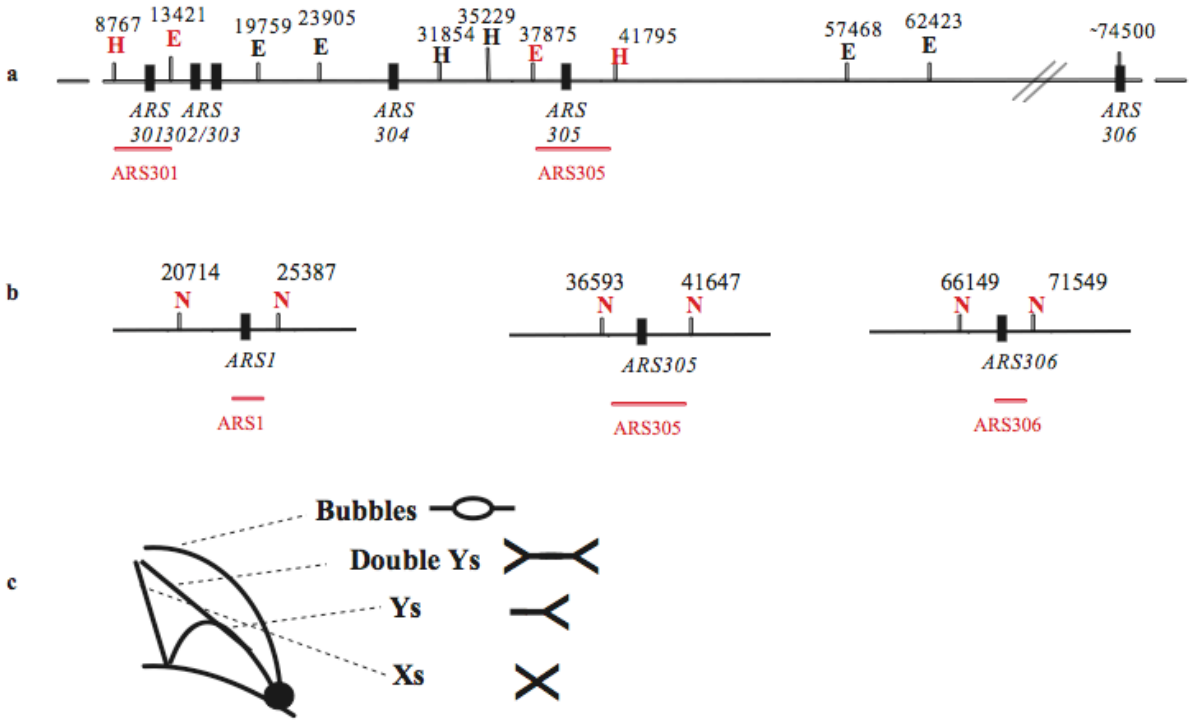
SUPPLEMENTARY INFORMATION



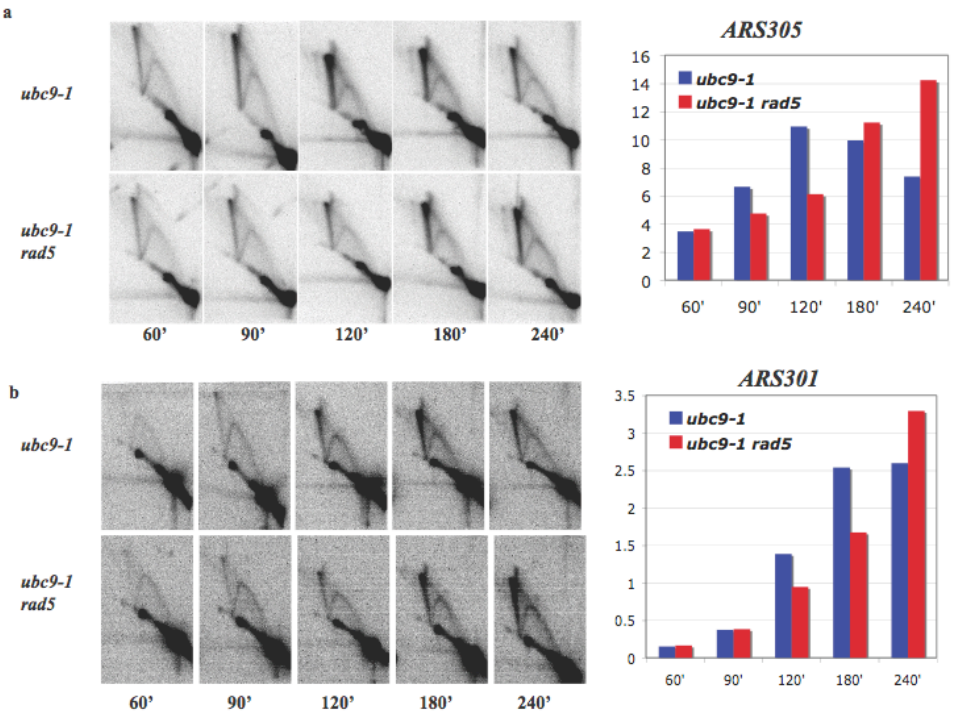
Supplementary Figure 1. Schematic representation of the post-replication repair pathway (PRR) and PCNA modifications. a, Rad18 and Rad6 control error-free and error-prone modes of PRR. Monoubiquitilation of PCNA at K164 promotes its interaction with Pol eta, which together with Pol zeta, inserts correct or incorrect nucleotides across the damaged site. This pathway mediated by translesion synthesis polymerases is also called error-prone PRR. Interaction of Rad18-Rad6 with Rad5 promotes recruitment of Ubc13-Mms2 to sites of damage and further polyubiquitilation of PCNA through K63-linked ubiquitin chains. This pathway, referred to as error-free PRR, is required for gap-filling repair. **b,** In addition to being mono- or polyubiquitilated (by means of K63 linked polyubiquitin chains) at K164,PCNA is also sumoylated at K164 by Ubc9 and the Siz1 SUMO ligase, and sumoylated at a secondary site, K127, by Ubc9.



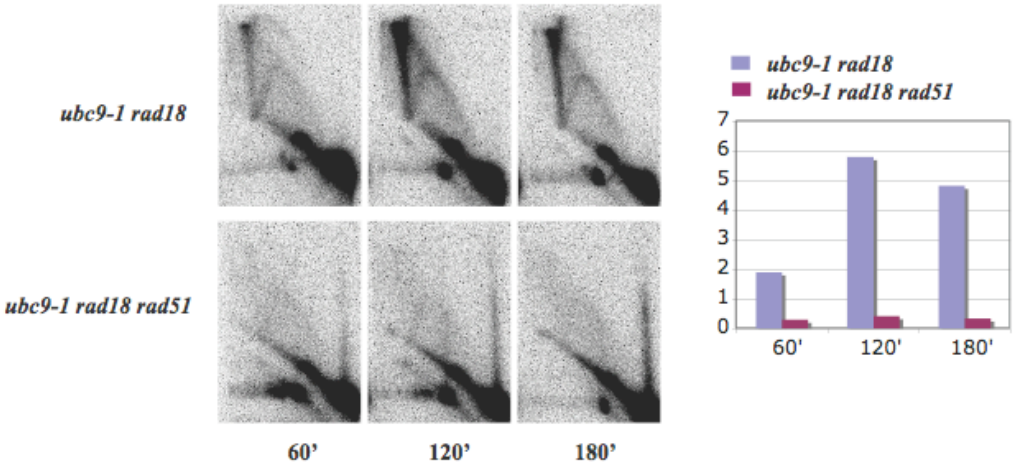
Supplementary Figure 2. Formation of hemicatenane-like intermediates during damage-bypass processes and their resolution by Sgs1-Top3 (green) and Ubc9-Mms21 activities [1,2]. Replication forks encountering DNA damage can reprime downstream the DNA lesion, generating a single stranded (ss) DNA gap. This gap can be filled in using the newly synthesized DNA strand as a template, in a process referred to as template switch. The hemicatenane-like intermediate generated in this process is resolved by the concerted action of Sgs1-Top3 and Ubc9-Mms21-dependent sumoylation[1,2].



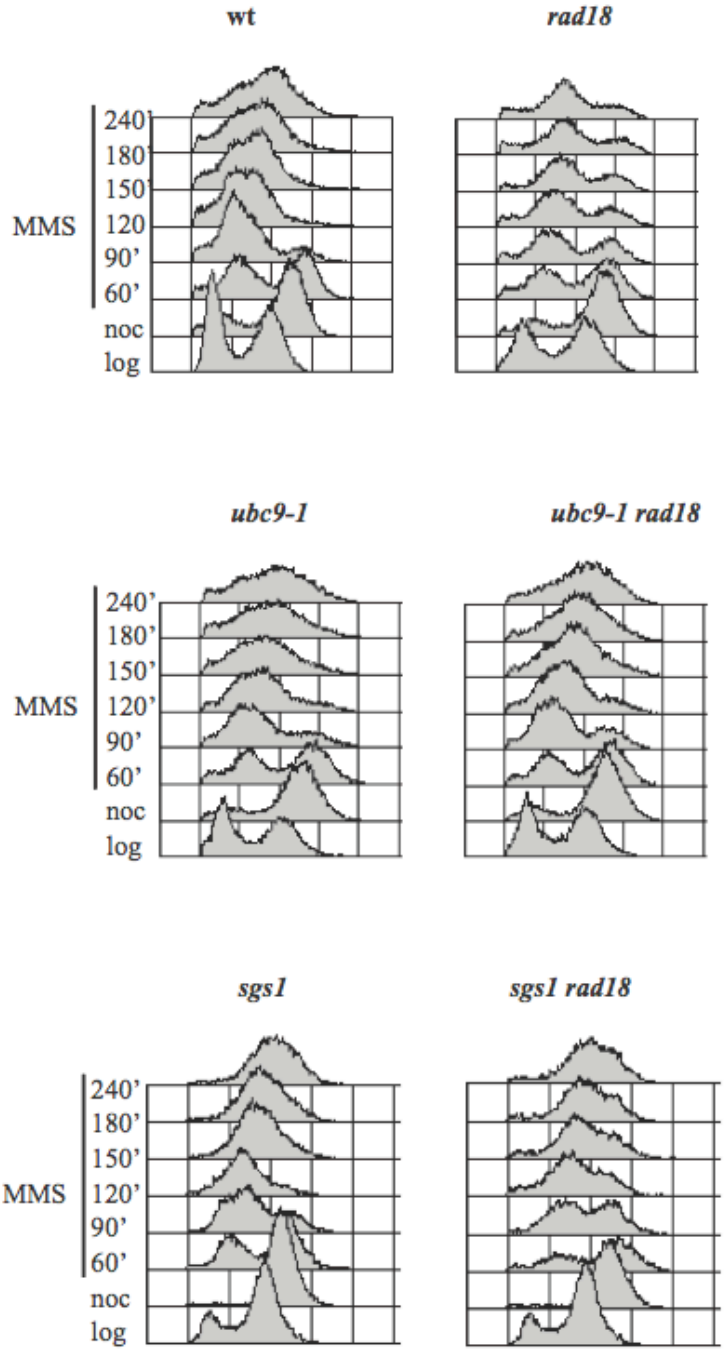
Supplementary Figure 3. Schematic representation of 2D gel replication intermediates and genomic maps. a, The genomic region containing the *ARS305* origin and the flanking regions on chromosome III. E and H stand for *Eco*RV and *Hind*III, respectively. **b,** The genomic regions containing the *ARS1* origin on chromosome IV, and *ARS305*, *ARS306* origins on chromosome III. N stands for *NcoI*. Schematic representation of the replication intermediates visualized by 2D gel electrophoresis. For further details see [1,2].



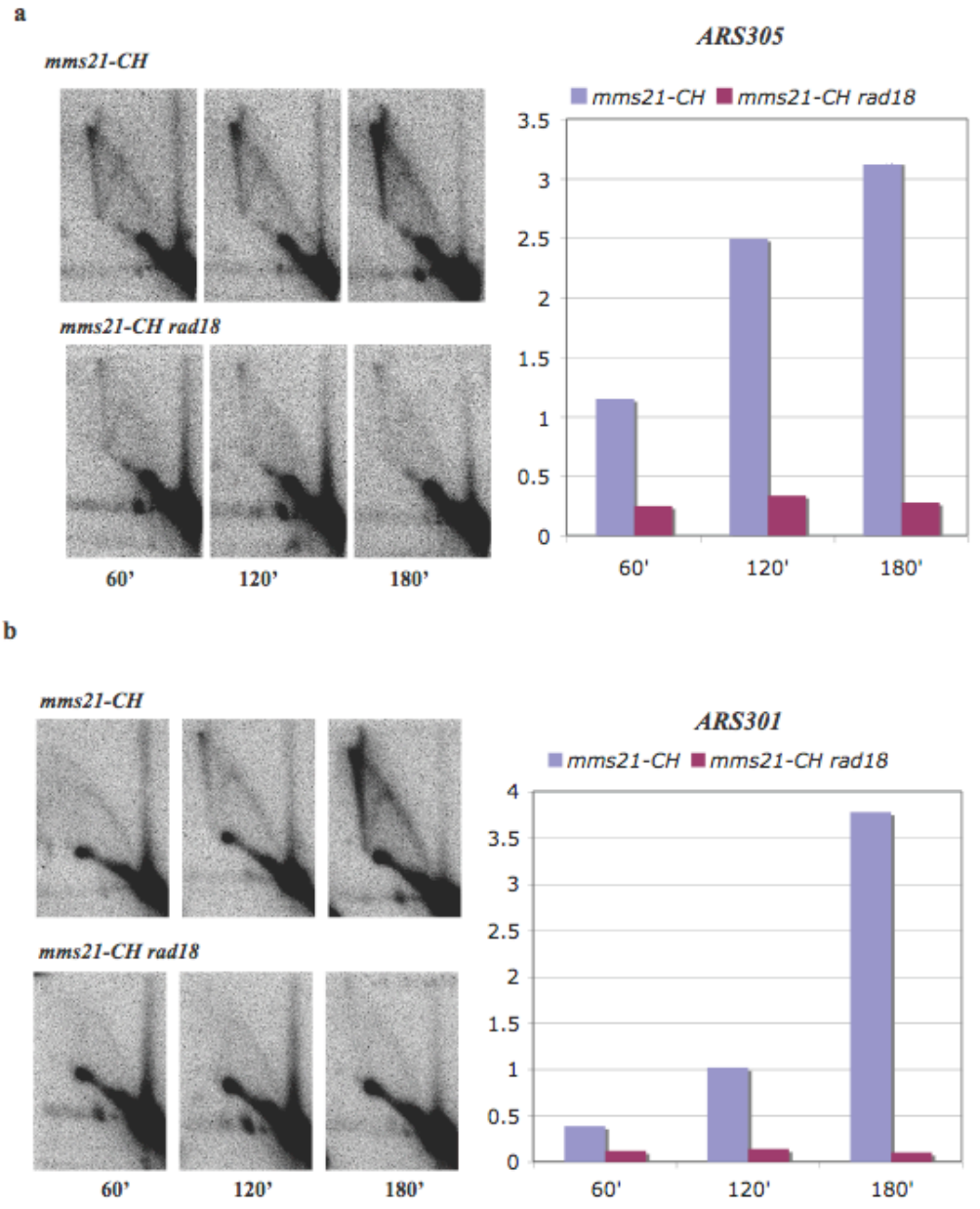
Supplementary Figure 4. Rad5 does not contribute to the X molecules accumulating in ubc9-1 mutants during replication of damaged templates. The profile of replication intermediates from *ubc9-1* (Y0174) and *ubc9-1 rad5* (HY0517) at **a,** *ARS305* and **b,** *ARS301*, with quantifications. The relative amount of X versus other intermediates is represented on the Y-axes of the quantification graphs for each time point analyzed, indicated on the X-axes in minutes (').



Supplementary Figure 5. Rad51-dependent accumulation of X molecules in ubc9-1 rad18 mutants during replication of damaged templates. The profile of replication intermediates digested with *Eco*RV and *Hind*III at *ARS305* from *ubc9-1 rad18* (HY0521) and *ubc9-1 rad18 rad51* (HY0870) with quantifications. The relative amount of X versus other intermediates is represented on the Y-axes of the quantification graphs for each time point analyzed, indicated on the X-axes in minutes (').



Supplementary Figure 6. Cell-cycle profiles of cells replicating in the presence of MMS. Exponentially growing cells, were arrested with nocodazole, and released in medium containing MMS. Samples were taken at the indicated time points for 2D gel and cell cycle analysis. The strains represented are wt (Y0002), *rad18* (HY0520), *ubc9-1* (Y0174), *ubc9-1 rad18* (HY0521), *sgs1* (FY1060), and *sgs1 rad18* (HY0701).



Supplementary Figure 7. Rad18 role in promoting SCJ formation during replication of damaged templates does not require Mms21-dependent sumoylation. The profile of replication intermediates from strains *mms21-CH* (FY1003) and *mms21-CH rad18* (HY0746) was analyzed by 2D gel using **a,** the *ARS305* and **b,** the *ARS301* probe (see supplementary information S3). The relative amount of X versus other intermediates is represented on the Y-axes of the quantification graphs for each time point analyzed, indicated on the X-axes in minutes (').

Supplementary Table 1. List of Strains Used in This Study

Strain	Genotype	Source
Y0002 (FY0113)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801 trp1-1 (am) ura3-52*	S. Jentsch
Y0174 (FY0114)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801 trp1-1 (am) ura3-52 ubc9Δ::TRP1 leu2::ubc9 Pro-Ser::LEU2*	S. Jentsch
HY0516	**Y0002** genotype but *rad5Δ:: HPHMX4*	[1]
HY0517	**Y0174** genotype but *rad5Δ:: HPHMX4*	[1]
HY0520	**Y0002** genotype but *rad18Δ:: HPHMX4*	[1]
HY0521	**Y0174** genotype but *rad18Δ:: HPHMX4*	[1]
HY0518	**Y0002** genotype but *mms2Δ:: HPHMX4*	[1]
HY0519	**Y0174** genotype but *mms2Δ:: HPHMX4*	[1]
HY0501	**Y0002** genotype but *sgs1::AUR1-C*	[1]
HY0985	**Y0002** genotype but *sgs1Δ::NATMX4*	This study
HY0810	**HY0501** genotype but *mms2Δ:: HPHMX4*	This study
HY1005	**HY0518** genotype *sgs1Δ::NATMX4*	This study
HY0695	**HY0501** genotype but *rad18Δ::HPHMX4*	This study
HY1007	**HY0520** genotype *sgs1Δ::NATMX4*	This study
HY0692	**HY0501** genotype but *rad5Δ:: HPHMX4*	This study
HY1003	**HY0516** genotype *sgs1Δ::NATMX4*	This study
HY0499	**Y0002** genotype but *rad51Δ::loxP-kanMX-loxP*	This study
HY1049	**HY0985** genotype but *rad51Δ::KANMX4*	This study
HY1075	**HY1049** genotype but *rad18Δ:: HPHMX4*	This study
HY1077	**HY1007** genotype but *rad51Δ::KANMX4*	This study
FY1000 (W303)	***MATa*** *ade2-1 can1-100 his3-11,-15 leu2-3,112 trp1-1 ura3-1*	Lab collection
HY0733	**FY1000** genotype but *rad18Δ:: HPHMX4*	This study
FY1060	**FY1000** genotype but *sgs1Δ::HIS3MX6*	Lab collection
HY0701	**FY1000** genotype but *sgs1Δ::HIS3MX6, rad18Δ:: HPHMX4*	This study
FY1012	***MATa*** *ade2-1 can1-100 his3-11,-15 leu2-3,112 trp1-1 ura3-1 mms21-11:LEU2*	[1]
HY0748	**FY1012** genotype but *rad18Δ:: HPHMX4*	This study
FY1003	***MATa*** *ade2-1 can1-100 his3-11,-15 leu2-3,112 trp1-1 ura3-1 mms21-CH::HIS3*	[1]
HY0746	**FY1003** genotype but *rad18Δ:: HPHMX4*	This study
Y1190 (FY0109)	***MATa*** *his3-Δ200 leu2-3, 112 lys2-801 trp1-1 (am) ura3-52 POL30::pol30Δ::KANMX4* locus	S. Jentsch
Y1191 (FY0110)	**Y1190** genotype but *pol30-K127R* at *pol30Δ::KANMX4* locus	S. Jentsch
Y1192 (FY0111)	**Y1190** genotype but *pol30-K164R* at *pol30Δ::KANMX4* locus	S. Jentsch
Y1194 (FY0112)	**Y1190** genotype but *pol30-K127R, K164R* at *pol30Δ::KANMX4* locus	S. Jentsch
HY0766	**Y1190** genotype but *sgs1Δ::NATMX4*	This study
HY0768	**Y1191** genotype but *sgs1Δ::NATMX4*	This study
HY0771	**Y1192** genotype but *sgs1Δ::NATMX4*	This study
HY0776	**Y1194** genotype but *sgs1Δ::NATMX4*	This study
#987 (FY0102)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801, trp1-1 (am), ura3-52, pol30::hisG-URA3-hisG, LEU2::YIP128-P30-POL30*	H. Ulrich
#998 (FY0104)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801, trp1-1 (am), ura3-52, pol30::hisG-URA3-hisG, LEU2::YIP128-P30-pol30-K127R*	H. Ulrich
#990 (FY0103)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801, trp1-1 (am), ura3-52, pol30::hisG-URA3-hisG, LEU2::YIP128-P30-pol30- K164R*	H. Ulrich

#995 (FY0105)	***MATα*** *his3-Δ200 leu2-3, 112 lys2-801, trp1-1 (am), ura3-52, pol30::hisG-URA3-hisG, LEU2::YIP128-P30-pol30-K127R/K164R*	H. Ulrich
HY0718	**FY0102** genotype but *sgs1Δ::NATMX4*	This study
HY0720	**FY0104** genotype but *sgs1Δ::NATMX4*	This study
HY0722	**FY0103** genotype but *sgs1Δ::NATMX4*	This study
HY0724	**FY0105** genotype but *sgs1Δ::NATMX4*	This study
HY0838	**HY0722** genotype but *rad18Δ::HPHMX4*	This study
HY0840	**HY0724** genotype but *rad18Δ::HPHMX4*	This study
HY0872	**HY0695** genotype but *rad51Δ::CaURA3MX4*	This study
HY0874	**HY0724** genotype but *rad51Δ::HPHMX4*	This study
HY0820	**HY0501** genotype but *siz1Δ::KANMX4*	This study
HY0823	**HY0695** genotype but *siz1Δ::KANMX4*	This study
HY0826	**HY0501** genotype but *siz2Δ::KANMX4*	This study
HY0829	**HY0695** genotype but *siz2Δ::KANMX4*	This study
HY0870	**Y0174** genotype but *rad18Δ::hphMX4, rad51Δ::CaURA3MX4*	This study

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