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Supplemental Information

RNase H and Postreplication Repair Protect Cells from Ribonucleotides Incorporated in DNA

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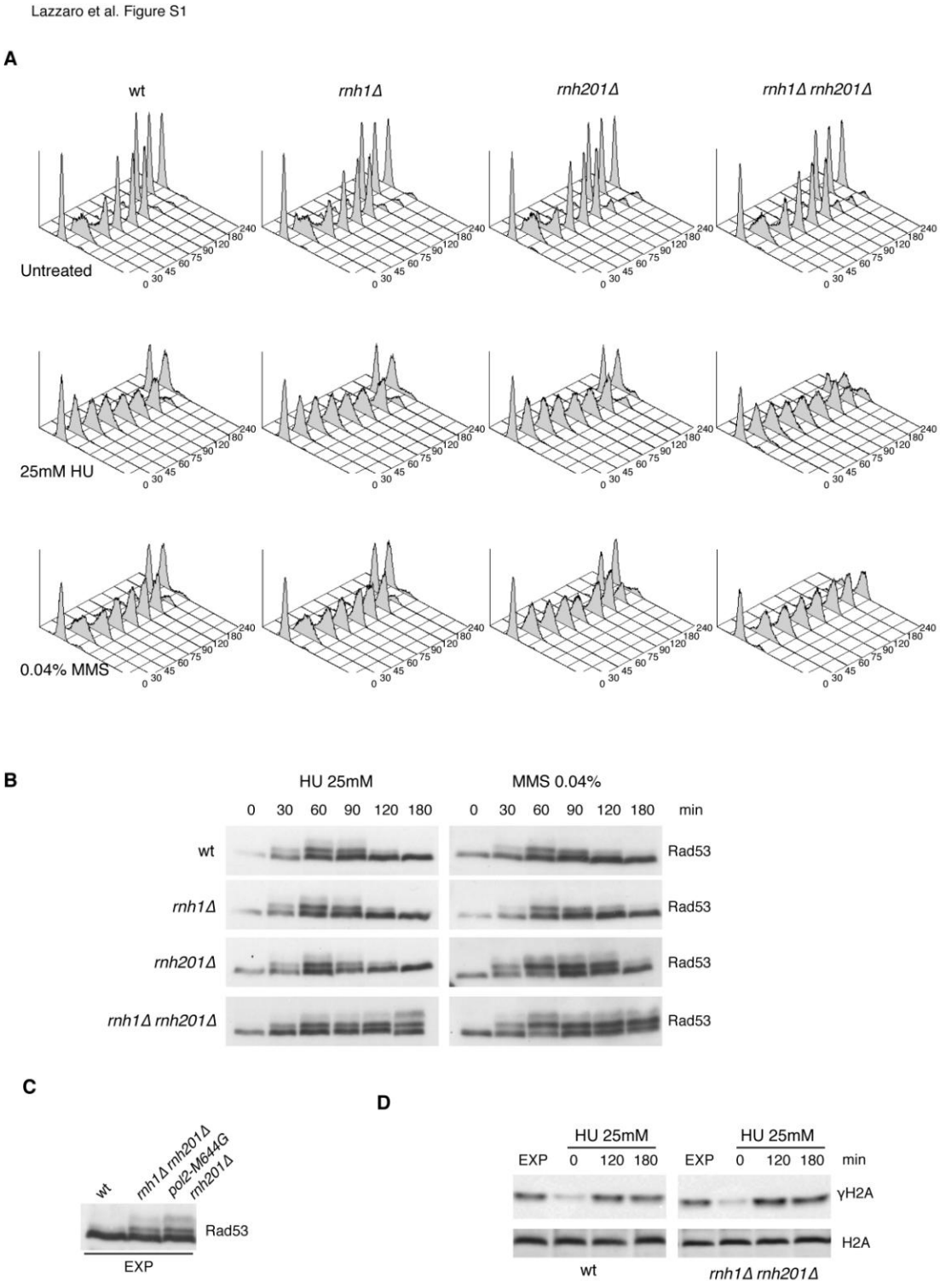

Figure S1, Related to Figure 1. Lack of RNase H Causes Replication Stress and a G2-M Delay

(A) Exponentially growing yeast cultures were arrested in G1 phase with α-factor. Cells were released in the presence of 25 mM HU, 0.04% MMS or fresh medium, and cell cycle progression was analyzed by FACS. The figure shows the number of cells in relation to the DNA content.

(B–D) The same cultures were used to verify checkpoint activation at the indicated time-points after the release. Total cell extracts were analyzed by western blotting to monitor Rad53, H2A and H2A phosphorylation, using specific antibodies.

Lazzaro et al., Figure S2

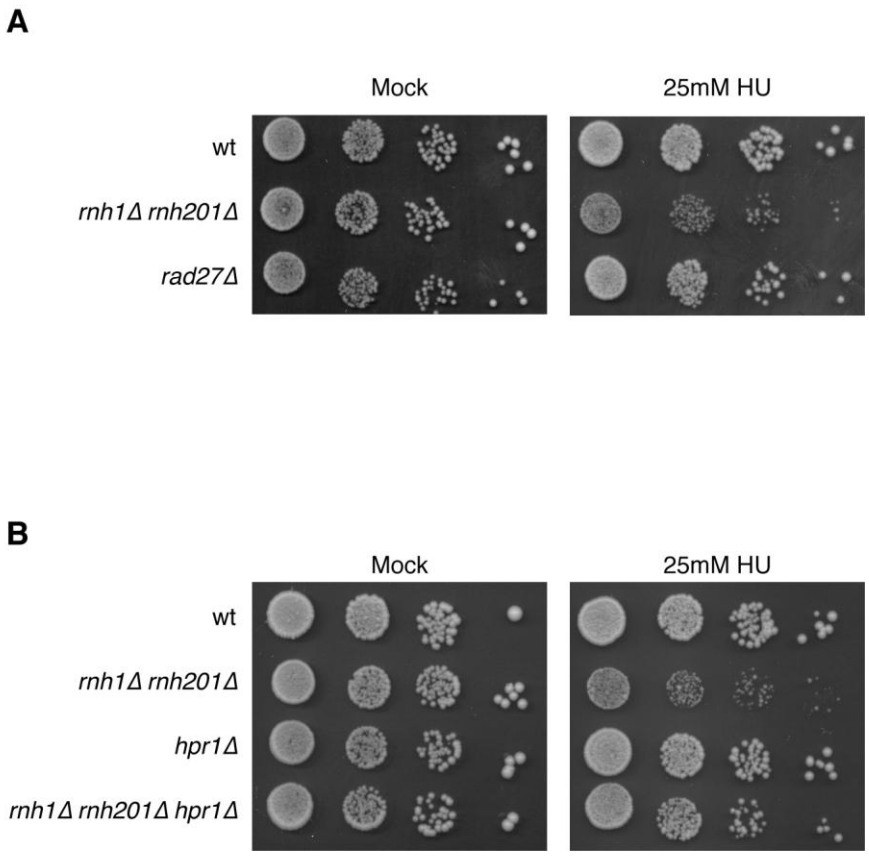

Figure S2, Related to Figure 1. **Sensitivity to Replication Stress in RNase H Mutant Cells Is Unlinked from Defective Okazaki Fragments or R-Loops Processing**

To verify the effect of mutants defective in processing of Okazaki fragments (A) or R-loops (B) on HU sensitivity, ten-fold serial dilutions of yeast overnight cultures were spotted on YPD plates containing a sublethal HU dose or mock. Ability to grow was analyzed after 3 days incubation.

Lazzaro et al., Figure S3

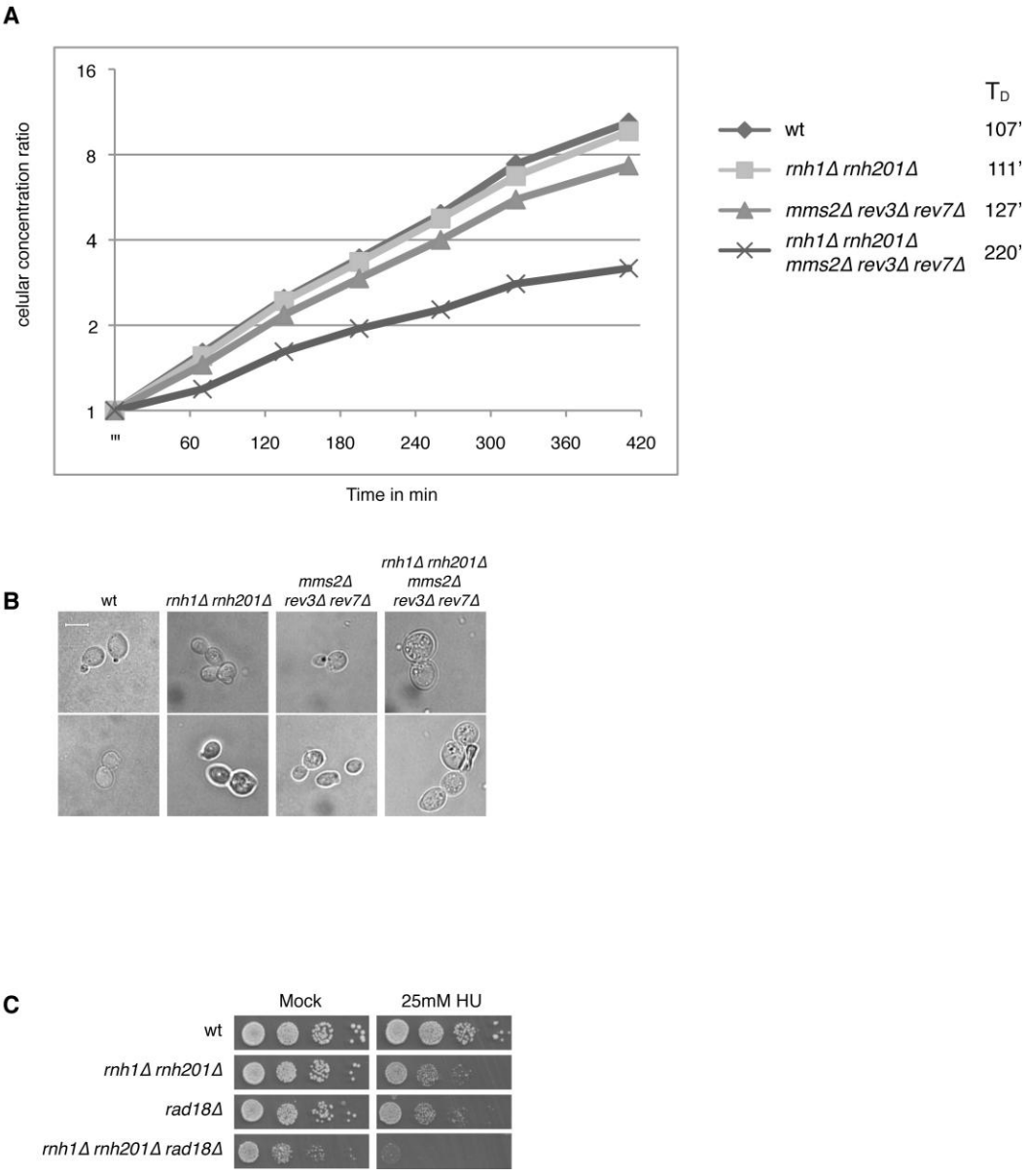

Figure S3, Related to Figure 5. PRR Pathways Play a Crucial Role in the Survival of RNase H-Defective Cells

(A) Growth curves and duplication times (T_D) for exponentially growing cells of the indicated strains were obtained by measuring cell concentrations at different time-points.

(B) The shape and size of wt and mutant cells form exponentially growing cultures were visualized by microscopic analysis. The white bar represents 5µm.

(C) To test the effect on HU sensitivity of a mutation eliminating Rad18, the enzyme responsible for conjugation of ubiquitin to PCNA, ten-fold serial dilutions of yeast overnight cultures were spotted on YPD plates containing a sublethal HU dose or mock. Ability to grow was analyzed after 3 days incubation.

Lazzaro et al. Figure S4

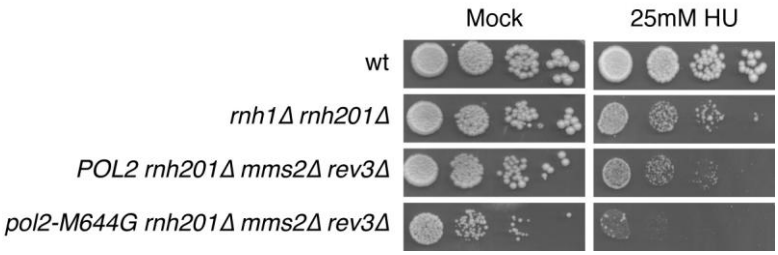

Figure S4, Related to Figure 5. PRR Is Crucial for Cells that Accumulate rNMP in Their Chromosomes

To verify the effect of PRR dysfunction in strains where a mutated DNA polymerase ε incorporates elevated levels of rNMPs in genomic DNA, ten-fold serial dilutions of yeast overnight cultures were spotted on YPD plates containing a sublethal HU dose or mock. Ability to grow was analyzed after 3 days incubation.

Lazzaro et al. Figure S5

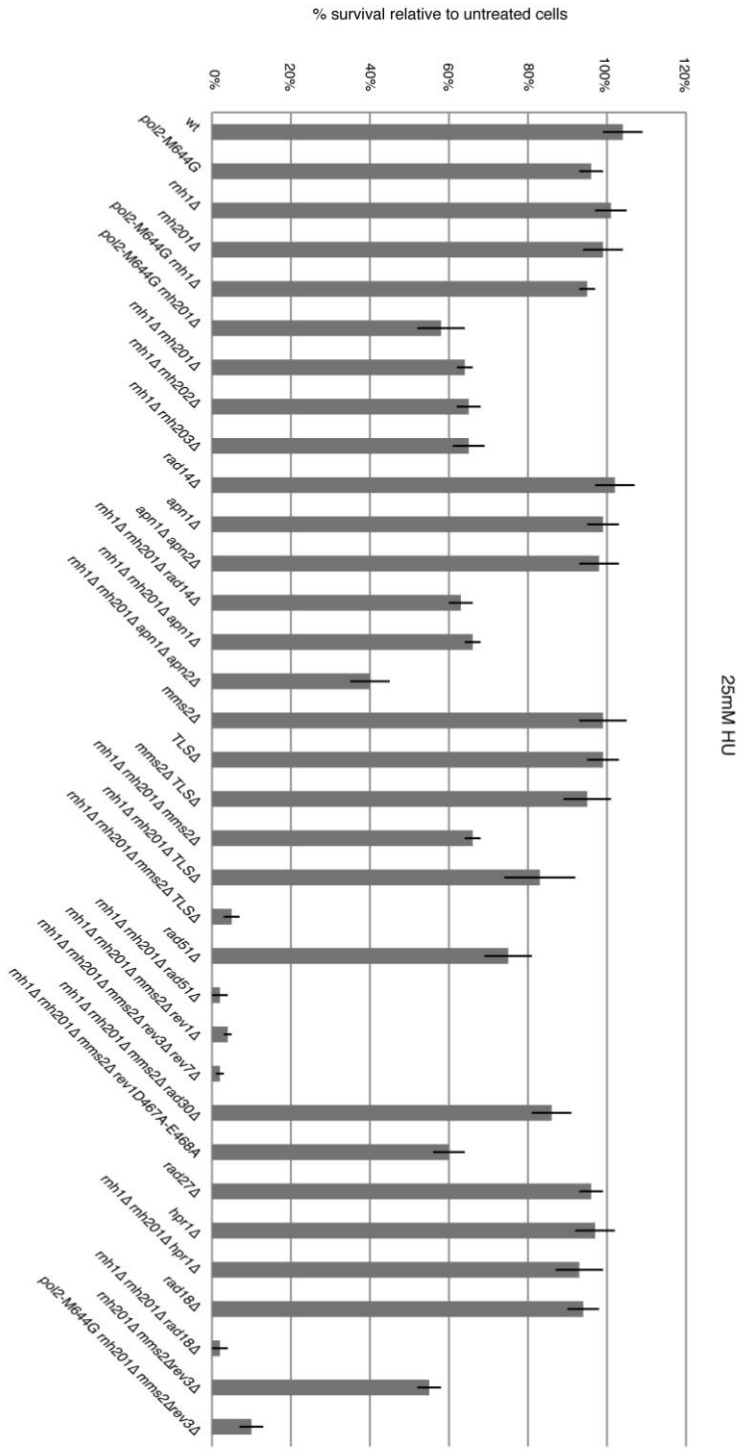

Figure S5, Related to Figures 1–3. Quantitative Survival Assays

To obtain quantitative data on survival upon treatment with 25 mM HU of the yeast strains used throughout this work, survival assays were performed as described in the legend to Figure 1H.

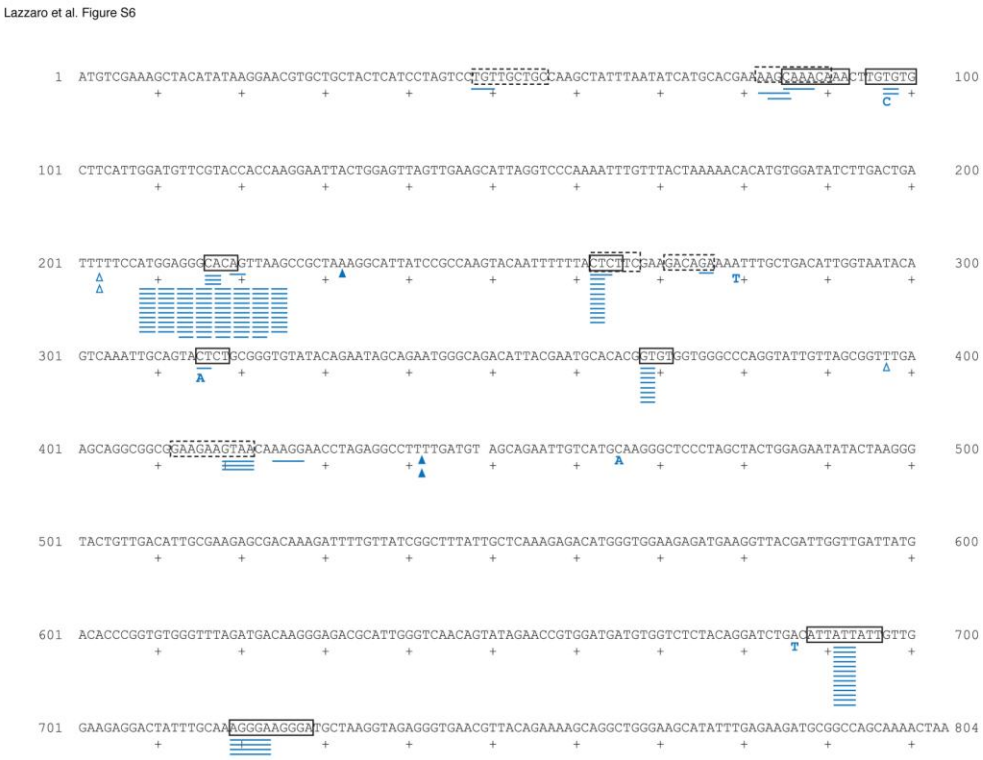

Figure S6, Related to Figure 4. Mutational Spectrum in *pol2-M644G rnh201*Δ *rev3*Δ Strain

The coding strand of the 804-base-pair *URA3* open reading frame is shown. The sequence changes observed in independent 5-FOA resistant mutants are depicted in blue below coding sequence, for *URA3* in orientation 2 as described in (Nick McElhinny, 2010a]. Letters indicate single-base substitutions, closed triangles indicate single-base additions, open triangles indicate single-base deletions and short lines below the coding sequence indicate 2–5-base deletions. Solid boxes enclose perfect direct repeat sequences, and dashed boxes enclose imperfect direct repeat sequences. Among 163 total mutants sequenced, a few contained other sequences changes not depicted here.

Table S1. Strains Used in This Study

Strain name	Genotype	Source/ Reference
SY2080	*MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 RAD5*	M. Foiani
YFL1208	(SY2080) *rnh1::HIS3*	This study
YFL1191	(SY2080) *rnh201::KanMX6*	This study
YFL1193	(SY2080) *rnh202::KanMX6*	This study
YFL1196	(SY2080) *rnh203::KanMX6*	This study
YFL1213	(SY2080) *rnh1::HIS3 rnh201::KanMX6*	This study
YFL1216	(SY2080) *rnh1::HIS3 rnh202::KanMX6*	This study
YFL1218	(SY2080) *rnh1::HIS3 rnh203::KanMX6*	This study
YMIC5A3	*MATa ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1 can1-100 rad5-535 mec1-1 sml1*	Sabbioneda, 2007
YFL1449	(SY2080) p*ol2M644G*	This study
YFL1474	(SY2080) p*ol2M644G rnh201::KanMX6*	This study
YMG1146	(SY2080) *mms2::HPH*	This study
YMG1082	(SY2080) *rad30::KanMX6 rev1::KanMX6 rev3::TRP1 rev7::HIS3*	Giannattasio, 2010
YMG1149	(SY2080) *rad30::KanMX6 rev1::KanMX6 rev3::TRP1 rev7::HIS3 mms2::HPH*	This study
YFL1265	(SY2080) *rnh1::HIS3 rnh201::KanMX6 mms2::HPH*	This study
YFL1271	(SY2080) *rad30::KanMX6 rev1::KanMX6 rev3::TRP1 rev7::HIS3 rnh1::HIS3 rnh201::KanMX6*	This study
YFL1294	(SY2080) *rad30::KanMX6 rev1::KanMX6 rev3::TRP1 rev7::HIS3 rnh1::HIS3 rnh201::KanMX6 mms2::HPH*	This study
YFL1331	(SY2080) *rev1::KanMX6 rnh1::HIS3 rnh201::KanMX6 mms2::HPH*	This study
YFL1330	(SY2080) *rev3::TRP1 rev7::HIS3 rnh1::HIS3 rnh201::KanMX6 mms2::HPH*	This study
YFL1341	(SY2080) *rad30::KanMX6 rnh1::HIS3 rnh201::KanMX6 mms2::HPH*	This study
YFL1574	(SY2080) *rev1::KanMX6 rnh1::HIS3 rnh201::KanMX6 mms2::HPH ura3:REV1:URA3*	This study
YFL1575	(SY2080) *rev1::KanMX6 rnh1::HIS3 rnh201::KanMX6 mms2::HPH ura3:rev1-D467A-D468A:URA3*	This study
YFL1376	(SY2080) *leu2::^{6xHIS}POL30:LEU2*	This study
YFL1377	(SY2080) *leu2::^{6xHIS}POL30:LEU2 rnh1::HIS3 rnh201::KanMX6*	This study
YMG649	(SY2080) *rad27::KanMX6*	This study
YNOV59	(SY2080) *hpr1::HIS3*	This study
YNOV61	(SY2080) *rnh1::HIS3 rnh201::KanMX6 hpr1::HIS3*	This study
YFL1671	(SY2080) *rnh201::KanMX6 mms2::HPH rev3::TRP1*	This study

YFL1687	(SY2080) *rnh201::KanMX6 mms2::HPH rev3::TRP1 pol2-M644G*	This study
YFL1580	(SY2080) *rad18::KanMX6*	This study
YFL1629	(SY2080) *rad18::KanMX6 rnh1::HIS3 rnh201::KanMX6*	This study
(Δ|(-2) |-7B-YUNI300)	MATa CAN1 his7-2 leu2::kanMX ura3-Δ trp1-289 ade2-1 lys2-ΔGG2899-2900	Nick McElhinny, 2010a
SNM77	(Δ|(-2) |-7B-YUNI300) URA3-OR2 *pol2-M644G*	Nick McElhinny, 2010a
SNM127	(Δ|(-2) |-7B-YUNI300) URA3-OR2 *pol2-M644G rnh201::HPH*	Nick McElhinny, 2010a
JES184	(Δ|(-2) |-7B-YUNI300) URA3-OR2 *pol2-M644G rnh201::HPH rev3::LEU2*	This study
YFL1541	(SY2080) *rad14::NATr*	This study
YFL1545	(SY2080) *rad14::NATr rnh1::HIS3 rnh201::KanMX6*	This study
YFL1511	(SY2080) *apn1::HPH*	This study
YFL1537	(SY2080) *apn1::HPH apn2::TRP1*	This study
YFL1531	(SY2080) *apn1::HPH apn2::TRP1 rnh1::HIS3 rnh201::KanMX6*	This study
YNOV162	(SY2080) *rad51::HPH*	This study
YFL1451	(SY2080) *pol2-M644G rnh1::HIS3*	This study
YFL1677	(SY2080) *rad51::HPH rnh1::HIS3 rnh201::KanMX6*	This study