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SUPPLEMENTARY INFORMATION

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Ribose-seq: global mapping of ribonucleotides embedded in genomic DNA

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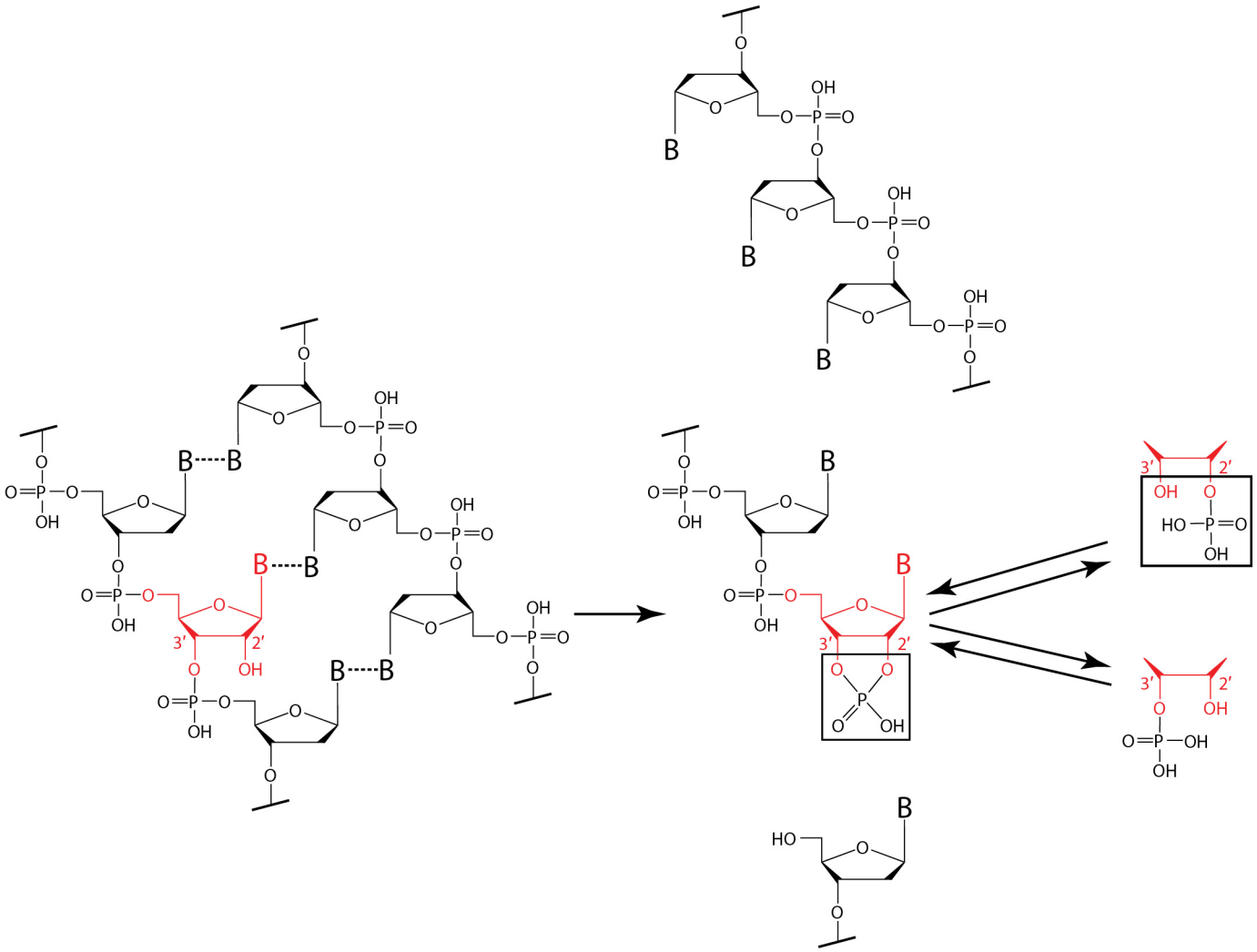
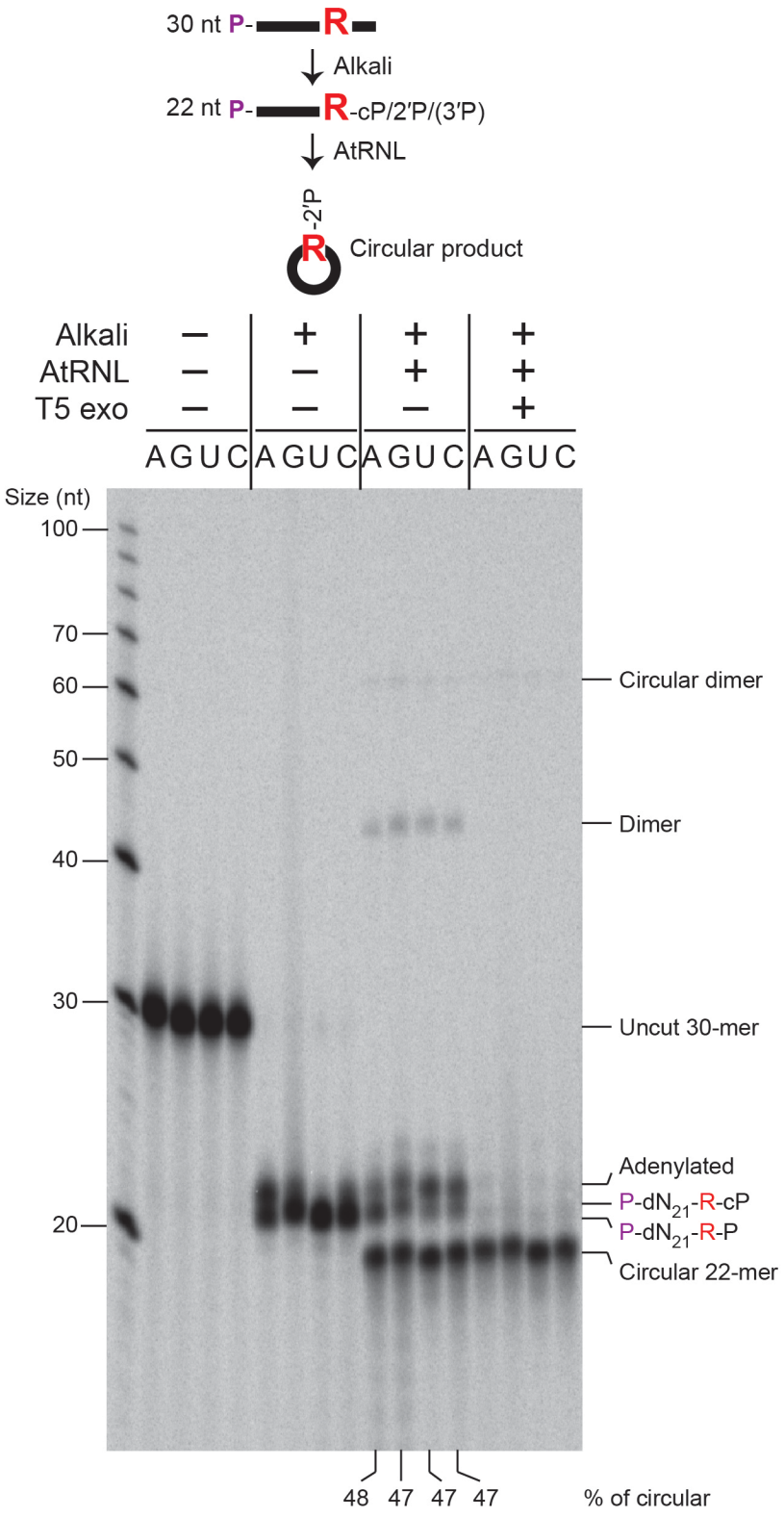


Supplementary Figure 1

Mechanism of alkaline cleavage of ribonucleotides in DNA

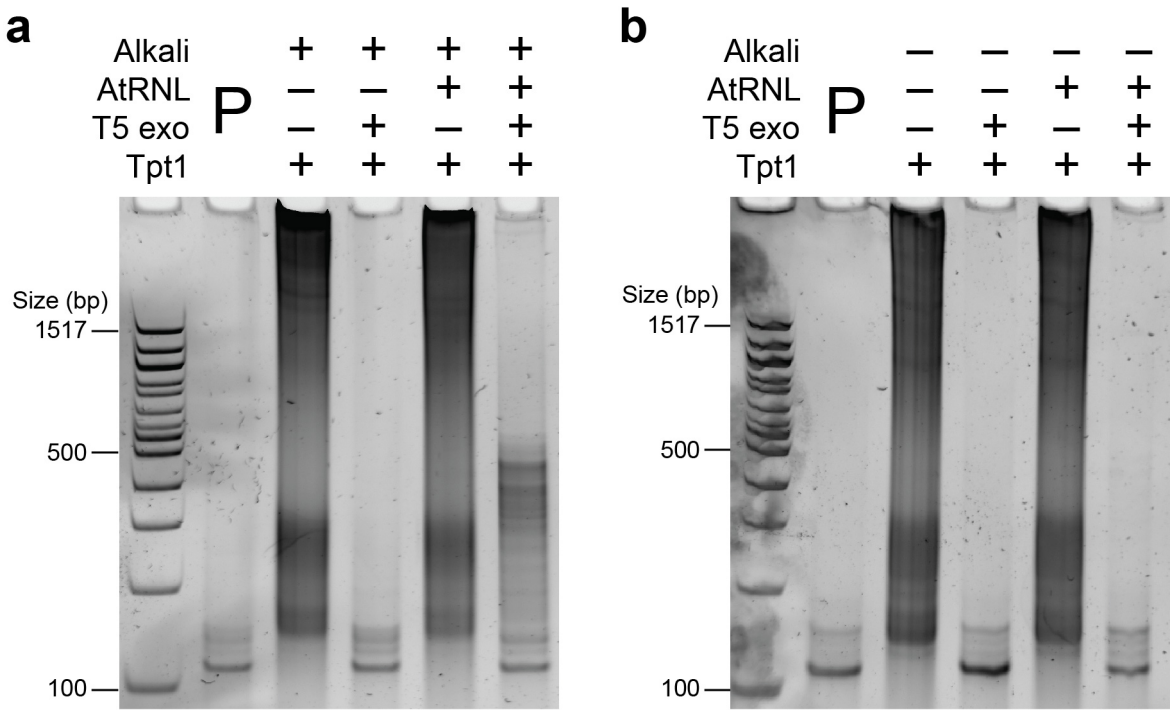
The ribonucleoside embedded in double-stranded DNA is in red. During alkaline treatment, DNA strands are denatured, and cleavage occurs at the rNMP site, generating a 2′,3′-cyclic phosphate end and an opposite 5′-hydroxyl end. The 2′,3′-cyclic phosphate is in equilibrium with 2′-phosphate and 3′-phosphate forms. Boxes in black indicate the 2′,3′-cyclic phosphate and 2′-phosphate DNA termini, which are substrates of AtRNL.



Supplementary Figure 2

3′ base bias for AtRNL ligation

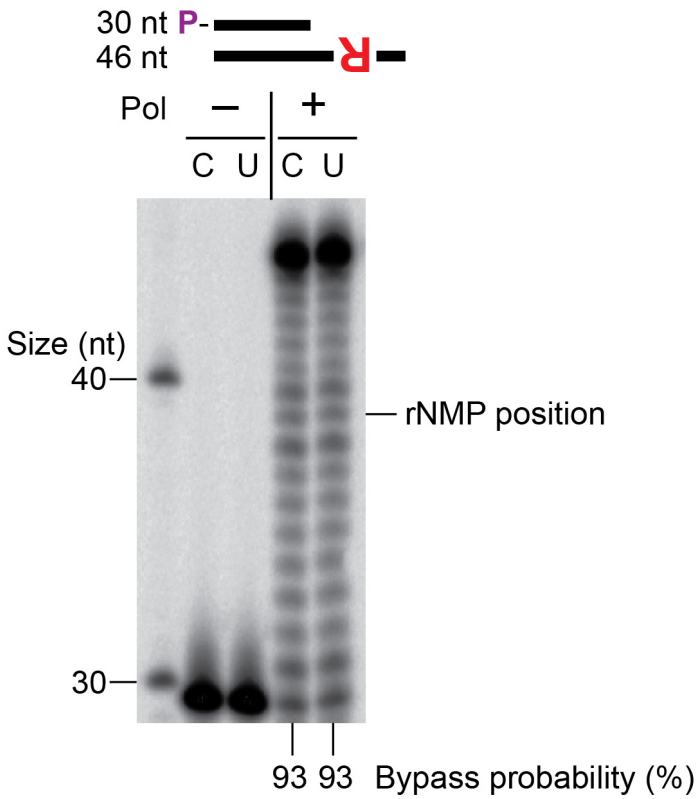
Hot 5′-radiolabeled 30-nt DNA oligo with a single rNMP (either A, G, U, or C) in the 22nd position was mixed with cold equimolar 30-nt DNA oligos with rNMPs of 3 other bases in the 22nd positions. 5′-radiolabel is indicated by 'P' in purple. The mixture was treated with 0.3M NaOH for 2 hr at 55 °C and neutralized. 100 nM of alkali-cleaved products (25 nM of each base) were then incubated with 1 μM AtRNL in appropriate buffer (see Methods) for 1 hr at 30 °C. The resulting products were treated with T5 exonuclease for 2 hr at 37 °C. Aliquots were withdrawn after appropriate steps and quenched. The products were analyzed by urea-PAGE. The circular 22-mer migrates faster than the unligated, linear 22-mer. Only circular products were resistant to T5 exonuclease while all linear substrates/products were degraded. Median percentages of circular 22-mer formation from four independent reactions are displayed. See Supplementary Table 1 for more statistics. First left lane, ss DNA ladder. No 3′ base bias was observed for AtRNL ligation (see Supplementary Table 1). Self-ligation was preferred to dimerization with a shorter 22-nt substrate; however, with the shorter substrate, lower levels of linear dimers, which are not resistant to T5 exonuclease, and circular dimers were observed. Increasing the length of the ss DNA substrate from 22 nt to 32 nt eliminated dimerization (Fig. 1a).



Supplementary Figure 3

Ribose-seq library from genomic DNA of *S. cerevisiae rnh201*Δ (KK-100) cells

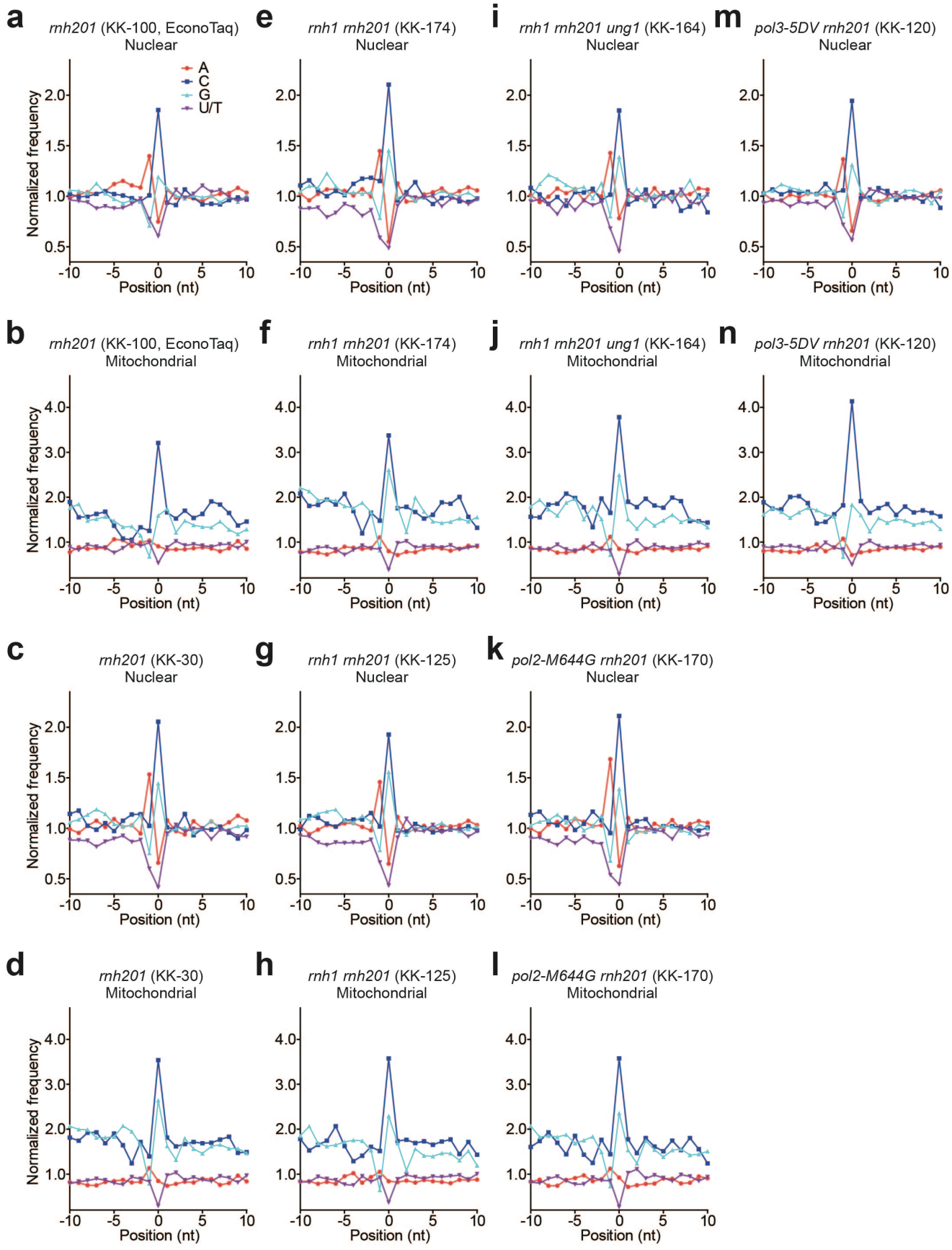
Appropriate PCR products were analyzed by PAGE. 'P' indicates primers-only. No amplification product was observed when either (a) AtRNL ligation step or (b) alkali treatment was omitted. Tpt1 denotes the step of 2'-phosphate removal at the ligation junction in Figure 1a. First left lane, ds DNA ladder.



Supplementary Figure 4

Bypass of a single rNMP by Phusion DNA polymerase

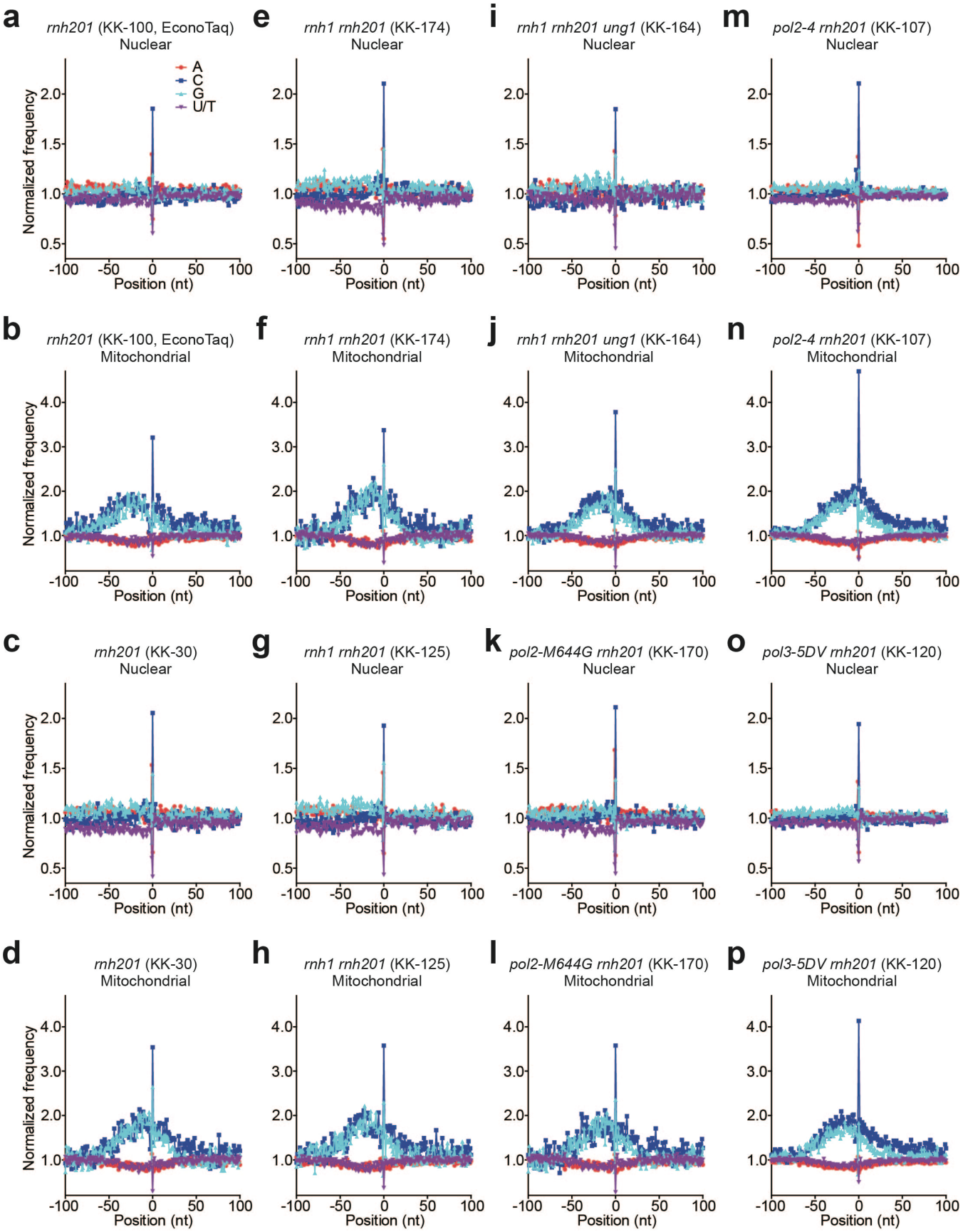
5′-radiolabeled 30-nt primer, ByPrim (Supplementary Table 8), was annealed to the 46-nt template oligo containing either rCMP (ByTemp.rC) or rUMP (ByTemp.rU) in the 8th position. 100 nM of annealed substrate was incubated with 0.2 units of Phusion High-Fidelity DNA Polymerase (NEB) and 2 mM dNTPs in appropriate buffer (see Methods) for 30 sec at 72 °C. The reactions were quenched and analyzed by urea-PAGE. Median bypass probabilities from four independent reactions are shown. See Supplementary Table 5 for more statistics. First left lane, ss DNA ladder. The primer extension assay showed no significant difference between bypass efficiency over rUMP and rCMP by Phusion DNA polymerase (Supplementary Table 5).


a
rnh201 (KK-100, EconoTaq)
Nuclear
A
C
G
U/T
Normalized frequency
Position (nt)
b
rnh201 (KK-100, EconoTaq)
Mitochondrial
c
rnh201 (KK-30)
Nuclear
d
rnh201 (KK-30)
Mitochondrial
e
rnh1 rnh201 (KK-174)
Nuclear
f
rnh1 rnh201 (KK-174)
Mitochondrial
g
rnh1 rnh201 (KK-125)
Nuclear
h
rnh1 rnh201 (KK-125)
Mitochondrial
i
rnh1 rnh201 ung1 (KK-164)
Nuclear
j
rnh1 rnh201 ung1 (KK-164)
Mitochondrial
k
pol2-M644G rnh201 (KK-170)
Nuclear
l
pol2-M644G rnh201 (KK-170)
Mitochondrial
m
pol3-5DV rnh201 (KK-120)
Nuclear
n
pol3-5DV rnh201 (KK-120)
Mitochondrial

Supplementary Figure 5

Normalized frequency of nucleotides surrounding the rNMP sites

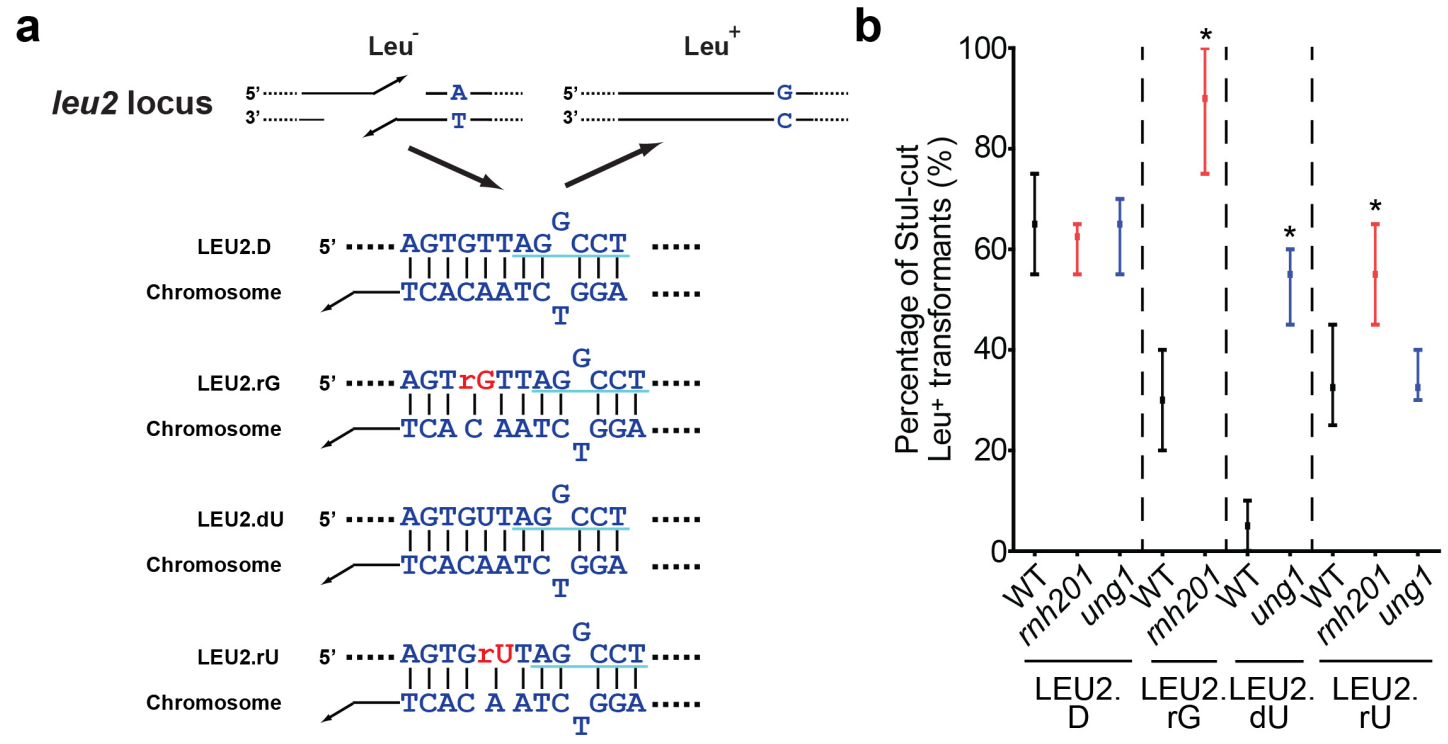
Normalized frequency of nucleotides relative to (a) nuclear and (b) mitochondrial mapped positions of sequences from ribose-seq library, PCR-amplified with EconoTaq DNA Polymerase (Lucigen), of genomic DNA from *S. cerevisiae rnh201*Δ (KK-100) cells. Position 0 corresponds to the rNMP. Negative and positive numbers (from -10 to -1 and 1 to 10) correspond to upstream and downstream positions from the rNMP, respectively. Frequencies were normalized to either nuclear or mitochondrial genomic mononucleotide frequencies. Normalized frequency of nucleotides relative to (c) nuclear and (d) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh201*Δ (KK-30) cells. Normalized frequency of nucleotides relative to (e) nuclear and (f) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ (KK-174) cells. Normalized frequency of nucleotides relative to (g) nuclear and (h) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ (KK-125) cells. Normalized frequency of nucleotides relative to (i) nuclear and (j) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ *ung1*Δ (KK-164) cells. Normalized frequency of nucleotides relative to (k) nuclear and (l) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae pol2-M644G rnh201*Δ (KK-170) cells. Normalized frequency of nucleotides relative to (m) nuclear and (n) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae pol3-5DV rnh201*Δ (KK-120) cells.


a
rnh201 (KK-100, EconoTaq)
Nuclear
A
C
G
U/T
Normalized frequency
Position (nt)
b
rnh201 (KK-100, EconoTaq)
Mitochondrial
c
rnh201 (KK-30)
Nuclear
d
rnh201 (KK-30)
Mitochondrial
e
rnh1 rnh201 (KK-174)
Nuclear
f
rnh1 rnh201 (KK-174)
Mitochondrial
g
rnh1 rnh201 (KK-125)
Nuclear
h
rnh1 rnh201 (KK-125)
Mitochondrial
i
rnh1 rnh201 ung1 (KK-164)
Nuclear
j
rnh1 rnh201 ung1 (KK-164)
Mitochondrial
k
pol2-M644G rnh201 (KK-170)
Nuclear
l
pol2-M644G rnh201 (KK-170)
Mitochondrial
m
pol2-4 rnh201 (KK-107)
Nuclear
n
pol2-4 rnh201 (KK-107)
Mitochondrial
o
pol3-5DV rnh201 (KK-120)
Nuclear
p
pol3-5DV rnh201 (KK-120)
Mitochondrial

Supplementary Figure 6

Zoom-out of normalized frequency of nucleotides surrounding the rNMP sites

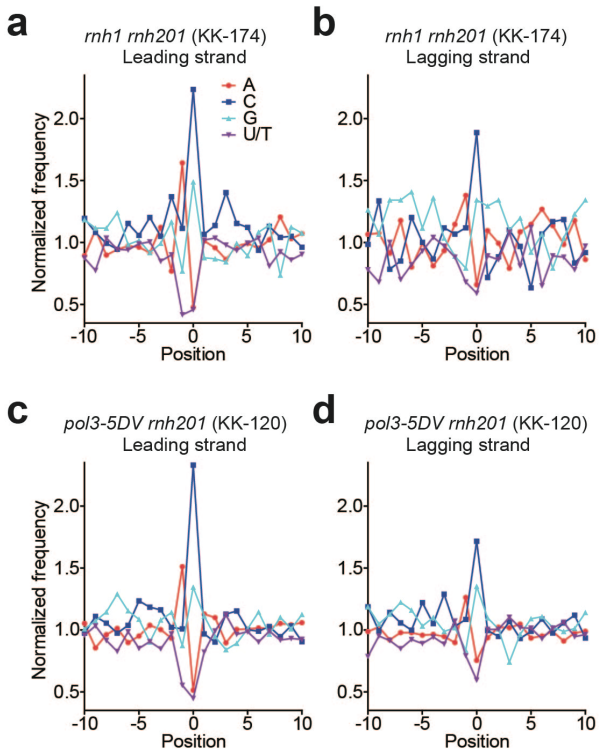
Normalized frequency of nucleotides relative to (a) nuclear and (b) mitochondrial mapped positions of sequences from ribose-seq library, PCR-amplified with EconoTaq DNA Polymerase (Lucigen), of genomic DNA from *S. cerevisiae rnh201*Δ (KK-100) cells. Position 0 corresponds to the rNMP. Negative and positive numbers (from -100 to -1 and 1 to 100) correspond to upstream and downstream positions from the rNMP, respectively. Frequencies were normalized to either nuclear or mitochondrial genomic mononucleotide frequencies. Normalized frequency of nucleotides relative to (c) nuclear and (d) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh201*Δ (KK-30) cells. Normalized frequency of nucleotides relative to (e) nuclear and (f) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ (KK-174) cells. Normalized frequency of nucleotides relative to (g) nuclear and (h) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ (KK-125) cells. Normalized frequency of nucleotides relative to (i) nuclear and (j) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ *ung1*Δ (KK-164) cells. Normalized frequency of nucleotides relative to (k) nuclear and (l) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae pol2-M644G rnh201*Δ (KK-170) cells. Normalized frequency of nucleotides relative to (m) nuclear and (n) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae pol2-4 rnh201*Δ (KK-107) cells. Normalized frequency of nucleotides relative to (o) nuclear and (p) mitochondrial mapped positions of sequences from ribose-seq library of genomic DNA from *S. cerevisiae pol3-5DV rnh201*Δ (KK-120) cells.



Supplementary Figure 7

Targeting of rGMP and rUMP by RNase H2 and uracil DNA N-glycosylase during DSB repair in *S. cerevisiae* cells

a, Diagram and sequence of the chromosomal *leu2* region targeted by DNA-control LEU2.D, rGMP-containing LEU2.rG, dUMP-containing LEU2.dU, and rUMP-containing LEU2.rU oligos (Supplementary Table 8). StuI recognition sequence is underlined in turquoise. Position of either rGMP, dUMP, or rUMP was selected so that it is about 4-5 nt upstream of the G-T mispair. Both RNase H2-initiated excision repair (RER) and base excision repair (BER) remove a short ss DNA region downstream of the damage during the repair[13,27]. b, The oligos were transformed to either RNase H2- and uracil DNA N-glycosylase-proficient wild-type (WT; FRO-767,768), RNase H2-deficient (*rnh201*; FRO-984,985), or DNA N-glycosylase-deficient (*ung1*; KK-158,159) *S. cerevisiae* cells (see Supplementary Table 2). Median percentages of StuI-cut Leu$^+$ transformants from four independent transformations are shown with ranges as bars. For each transformation, 20 Leu$^+$ transformants were selected for analysis. Mann-Whitney *U*-test was implemented for statistical analysis against the WT. *P* values of less than 0.05 are marked as asterisk. See Supplementary Table 6 for more statistics.



Supplementary Figure 8

Normalized frequency of nucleotides surrounding the rNMP sites on leading and lagging strands

Normalized frequency of nucleotides relative to mapped positions of sequences in (a) leading and (b) lagging strands from ribose-seq library of genomic DNA from *S. cerevisiae rnh1*Δ *rnh201*Δ (KK-174) cells. Position 0 corresponds to the rNMP. Negative and positive numbers (from -10 to -1 and 1 to 10) correspond to upstream and downstream positions from the rNMP, respectively. ARSs with T_{rep} of no longer than 25 min were selected with flanking size of 10 kb. Frequencies were normalized to genomic mononucleotide frequencies of either leading or lagging strand of the selected ARSs and flanking size. Normalized frequency of nucleotides relative to mapped positions of sequences in (c) leading and (d) lagging strands from ribose-seq library of genomic DNA from *S. cerevisiae pol3-5DV rnh201*Δ (KK-120) cells.

Supplementary Table 1. Results of 3′ base bias for AtRNL ligation.

a

Base	Circular	Dimer	Circular dimer
A	48% (44–49)	4.3% (1.8–7.1)	1.5% (0.71–2.1)
G	47% (44–48)	4.0% (2.6–5.8)	1.5% (0.88–2.3)
U	47% (45–49)	4.4% (2.1–5.1)	1.5% (0.73–2.0)
C	47% (44–49)	4.5% (1.9–5.0)	1.4% (0.59–1.8)

b

P value	G	U	C
A	0.4857	0.8857	1.0000
G	–	0.8857	0.6857
U	–	–	1.0000

c

Base	Circular	Dimer	Circular dimer
A	27% (23–31)	1.7% (1.5–2.3)	0.53% (0.45–0.74)
G	27% (24–28)	1.9% (1.5–2.5)	0.54% (0.39–1.1)
U	29% (24–30)	2.3% (1.3–2.7)	0.64% (0.48–1.2)
C	29% (25–32)	1.8% (1.7–2.0)	0.53% (0.47–0.80)

d

P value	G	U	C
A	1.0000	1.0000	0.6857
G	–	0.3429	0.2000
U	–	–	0.8857

a, Levels of AtRNL ligation in reaction conditions described in Supplementary Figure 1 are expressed as median percentage and range (in parentheses) from four independent reactions. **b,** Mann-Whitney *U*-test was performed for statistical analysis, and *P* values are displayed, all greater than 0.05. **c,** AtRNL ligation was performed with reduced 200 nM AtRNL, instead of 1 μM, to compare the levels of ligation when the reactions were incomplete. Median percentages and ranges (in parentheses) from four independent reactions are displayed. **d,** Mann-Whitney *U*-test was performed for statistical analysis, and *P* values are displayed, all greater than 0.05. No 3′ base bias was observed for AtRNL ligation.

Supplementary Table 2. *S. cerevisiae* strains used in this study.

a

Strain	Relevant genotype	Source
KK-100	*MATα ade5-1 lys2-14A trp1-289 his7-2 leu2-3,112 ura3-52 rnh201*Δ::*hygMX4*	this study
KK-30	*ho*Δ *hml*Δ::*ADE1 MATa-inc hmr*Δ::*ADE1 ade1 leu2-3,112 lys5 trp1*::*hisG ura3-52 leu2*::*HOcs mata*Δ::*hisG rnh201*Δ::*hygMX4*	this study
KK-174	KK-100 *rnh1*Δ::*kanMX4*	this study
KK-125	KK-30 *rnh1*Δ::*kanMX4*	this study
KK-164	KK-125 *ung1*Δ::*natMX4*	this study
KK-170	KK-30 *pol2-M644G*	this study
KK-107	KK-100 *pol2-4*	this study
KK-120	KK-100 *pol3-5DV*	this study

b

Strain	Relevant genotype	Source
FRO-767,768	*ho*Δ *hml*Δ::*ADE1 MATa-inc hmr*Δ::*ADE1 ade1 leu2-3,112 lys5 trp1*::*hisG ura3-52 ade3*::*GAL*::*HO leu2*::*HOcs mata*Δ::*hisG*	Storici *et al*., 2007[43]
FRO-984,985	FRO-767,768 *rnh201*Δ::*kanMX4*	Storici *et al*., 2007[43]
KK-158,159	FRO-767,768 *ung1*Δ::*hygMX4*	this study

Yeast strains used in (**a**) ribose-seq library construction and (**b**) DSB repair assay with rNMP-containing oligos.

[43]Storici, F., Bebenek, K., Kunkel, T. A., Gordenin, D. A. & Resnick, M. A. RNA-templated DNA repair. *Nature* **447**, 338-341 (2007)

Supplementary Table 3. Ribose-seq coverage for each library in this study.

Ribose-seq library	Coverage (aligned reads/kb)	
	Nuclear	Mitochondrial
rnh201 (KK-100)	0.449	19.5
rnh201 (KK-100, EconoTaq)	0.883	47.8
rnh201 (KK-30)	0.149	8.42
rnh1 rnh201 (KK-174)	0.149	9.92
rnh1 rnh201 (KK-125)	0.239	13.2
rnh1 rnh201 ung1 (KK-164)	0.269	42.2
pol2-M644G rnh201 (KK-170)	0.254	7.89
pol2-4 rnh201 (KK-107)	0.528	34.2
pol3-5DV rnh201 (KK-120)	0.510	33.9

Coverage is expressed as aligned reads per kb and does not reflect the relative abundance of rNMPs among different strains.

Supplementary Table 4. Absolute nucleotide frequencies of rNMPs and 5′ flanking nucleotide.

	Base	Position 0		Position -1	
		Nuclear	Mitochondrial	Nuclear	Mitochondrial
rnh201 (KK-100)	A	15.4%	25.6%	45.0%	45.8%
	C	44.0%	36.8%	22.4%	15.3%
	G	28.1%	19.0%	16.5%	5.8%
	U/T	12.5%	18.7%	16.1%	33.1%
rnh201 (KK-100, EconoTaq)	A	23.2%	38.2%	43.3%	43.5%
	C	35.4%	25.6%	19.3%	10.0%
	G	22.7%	14.5%	13.5%	6.2%
	U/T	18.7%	21.7%	23.9%	40.3%
rnh201 (KK-30)	A	20.4%	35.7%	47.5%	47.8%
	C	39.2%	28.3%	19.6%	11.1%
	G	27.5%	24.1%	14.4%	7.2%
	U/T	12.8%	11.9%	18.5%	33.8%
rnh1 rnh201 (KK-174)	A	17.1%	33.6%	44.9%	46.5%
	C	40.2%	27.0%	22.0%	11.8%
	G	27.7%	23.7%	15.0%	7.4%
	U/T	15.1%	15.7%	18.2%	34.2%
rnh1 rnh201 (KK-125)	A	20.1%	35.4%	45.2%	44.3%
	C	36.8%	28.6%	19.4%	12.1%
	G	29.7%	20.9%	15.0%	5.9%
	U/T	13.4%	15.1%	20.4%	37.7%
rnh1 rnh201 ung1 (KK-164)	A	24.3%	35.8%	44.3%	47.1%
	C	35.3%	30.2%	19.4%	13.2%
	G	26.5%	22.7%	15.3%	6.5%
	U/T	14.0%	11.3%	21.0%	33.2%
pol2-M644G rnh201 (KK-170)	A	19.5%	38.9%	52.2%	47.2%
	C	40.3%	28.6%	18.2%	11.5%
	G	26.5%	21.5%	13.0%	6.6%
	U/T	13.7%	11.0%	16.6%	34.7%
pol2-4 rnh201 (KK-107)	A	14.9%	21.9%	42.5%	46.2%
	C	40.2%	43.1%	22.0%	16.3%
	G	23.6%	16.3%	16.4%	6.4%
	U/T	21.2%	18.6%	19.2%	31.1%
pol3-5DV rnh201 (KK-120)	A	20.4%	30.0%	44.3%	45.3%
	C	37.1%	33.1%	19.4%	14.6%
	G	25.0%	16.7%	15.3%	6.1%
	U/T	17.5%	20.3%	21.0%	34.0%

Absolute nucleotide frequencies of nuclear and mitochondrial rNMPs and the nucleotide immediately upstream (position -1) from each ribose-seq library.

Supplementary Table 5. Results of rNMP bypass by Phusion DNA polymerase.

a

Base	Bypass probability
C	93% (93–93)
U	93% (92–94)

b

P value	U
C	0.6857

a, Bypass probabilities in reaction conditions described in Supplementary Figure 4 are expressed as median percentage and range (in parentheses) from four independent reactions. **b,** Mann-Whitney *U*-test was performed for statistical analysis, and *P* value is displayed.

Supplementary Table 6. Results of DSB repair assay with rNMP-containing oligos.

a

Oligo	WT	*rnh201*	*ung1*
LEU2.D	65% (55–75)	63% (55–65)	65% (55–70)
LEU2.rG	30% (20–40)	90% (75–100)	N/A
LEU2.dU	5.0% (0–10)	N/A	55% (45–60)
LEU2.rU	33% (25–45)	55% (45–65)	33% (30–40)

b

Oligo	*rnh201*	*ung1*
LEU2.D	0.5357	1.0000
LEU2.rG	0.0286	N/A
LEU2.dU	N/A	0.0294
LEU2.rU	0.0421	1.0000

a, Data shown in Supplementary Figure 7 are presented here as median percentages of StuI-cut Leu^+ transformants from four independent transformations and ranges in parentheses. For each transformation, 20 Leu^+ transformants were selected for analysis. **b,** Mann-Whitney *U*-test was implemented for statistical analysis against the WT, and *P* values are displayed. N/A, not applicable because data are not available for comparison.

Supplementary Table 7. List of hotspots of rNMP incorporation within *S. cerevisiae* mitochondrial DNA, rDNA repeat, and *Ty1*.

Position	Strand	Gene	Base	Number of rNMP reads								
				rnh201 **(KK-100)**	***rnh201*** **(KK-100, EconoTaq)**	***rnh201*** **(KK-30)**	***rnh1*** ***rnh201*** **(KK-174)**	***rnh1*** ***rnh201*** **(KK-125)**	*rnh1 rnh201 ung1* (KK-164)	*pol2-M644G rnh201* (KK-170)	*pol2-4 rnh201* (KK-107)	*pol3-5DV rnh201* (KK-120)
Chr M 39,224	W	*COB*	A	**30**	**136**	**17**	**24**	**34**	2	20	10	22
Chr XII[a] 453,839	C	*RDN25*	G	**97**	**45**	**18**	**30**	**22**	1	25	12	10
Chr IV[b] 650,383	C[b]	*Ty1*	A	**15**	**42**	**10**	**11**	**19**	46	20	19	110
Chr M 14,688	W	*COX1*	A	**7**	**49**	**5**	**10**	**14**	33	12	8	38
Chr M 14,739	W	*COX1*	A	**8**	**46**	**7**	**14**	**12**	0	5	0	13
Chr M 19,157	W	*COX1*	A	**15**	**73**	**4**	**5**	**22**	28	5	7	27

Hotspots of rNMP incorporation were determined by finding positions of rNMPs within the locus of interest with ribose-seq signal greater than the mean plus three standard deviations for each library from *rnh201*Δ (KK-100), *rnh201*Δ (KK-100, EconoTaq), *rnh201*Δ (KK-30), *rnh1*Δ *rnh201*Δ (KK-174), and *rnh1*Δ *rnh201*Δ (KK-125) cells (in bold). Ribose-seq signal counts found in all other libraries are also shown.

[a]There are two rDNA repeats on Chr XII in the reference genome (sacCer2). Only the first repeat unit is shown as an example.

[b]Because of the presence of multiple copies of *Ty1* in the genome, *YDRCTy1-1* on Chr IV is shown as an example.

Supplementary Table 8. Oligonucleotides used in this study.

Name	Length (nt)	Sequence (5'-3') with end modifications	Purification	Experiment
Lig.47.D	47	CCCGAGTGTGATCATCTGGTCGCTGGGGAATGAGTCAGGCCACGGCG	PAGE	AtRNL ligation assay
Lig.47.R	47	CCCGAGTGTGATCATCTGGTCGCTGGGGAATrGAGTCAGGCCACGGCG	PAGE	AtRNL ligation assay
Lig.30.rA	30	NNNNNNNNNNNNNNNNNNNNrANNNNNNNN	PAGE	AtRNL 3' base bias assay
Lig.30.rG	30	NNNNNNNNNNNNNNNNNNNNrGNNNNNNNN	PAGE	AtRNL 3' base bias assay
Lig.30.rU	30	NNNNNNNNNNNNNNNNNNNNrUNNNNNNNN	PAGE	AtRNL 3' base bias assay
Lig.30.rC	30	NNNNNNNNNNNNNNNNNNNNrCNNNNNNNN	PAGE	AtRNL 3' base bias assay
Adaptor.L	87	P-NNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAG AGGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCAGCGCAGACCGTGA GGT	PAGE	Ribose-seq library construction
Adaptor.S	20	P-CCTCACGGTCTGCGCTGGCT-Am	Desalted	Ribose-seq library construction
PCR.1.Index1	63	CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGT GTGCTCTTCCGATC	Desalted	Ribose-seq library construction
PCR.1.Index2	63	CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGT GTGCTCTTCCGATC	Desalted	Ribose-seq library construction
PCR.1.Index3	63	CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGT GTGCTCTTCCGATC	Desalted	Ribose-seq library construction
PCR.1.Index4	63	CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGT GTGCTCTTCCGATC	Desalted	Ribose-seq library construction
PCR.2	58	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC TTCCGATCT	Desalted	Ribose-seq library construction
ByTemp.rC	46	NNNNNNNrCNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAG	PAGE	Polymerase bypass assay
ByTemp.rU	46	NNNNNNNrUNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAG	PAGE	Polymerase bypass assay
ByPrim	30	CTCTTTCCCTACACGACGCTCTTCCGATCT	PAGE	Polymerase bypass assay
LEU2.D	60	TTAGGTGCTGTGGGTGGTCCTAAATGGGGATCCGGTAGTGTTAGGCCTG AACAAGGTTTA	Desalted	*leu2* DSB repair assay
LEU2.rG	60	TTAGGTGCTGTGGGTGGTCCTAAATGGGGATCCGGTAGTrGTTAGGCCT GAACAAGGTTTA	Desalted	*leu2* DSB repair assay
LEU2.dU	60	TTAGGTGCTGTGGGTGGTCCTAAATGGGGATCCGGTAGTGUTAGGCCT GAACAAGGTTTA	Desalted	*leu2* DSB repair assay
LEU2.rU	60	TTAGGTGCTGTGGGTGGTCCTAAATGGGGATCCGGTAGTGrUTAGGCCT GAACAAGGTTTA	Desalted	*leu2* DSB repair assay
LEU2.3	20	ATGTCTGCCCCTAAGAAGAT	Desalted	*leu2* DSB repair assay
LEU2.6	20	TGCCAAAGAATAAGGTCAAC	Desalted	*leu2* DSB repair assay

Name, length, and sequence of oligos used in this study are described. The purification type and the specific experiments in which the oligos were used are indicated. Ribonucleotides are in red, preceded by 'r'. End modifications of phosphate and amino groups are indicated by 'P' and 'Am', respectively. All PAGE-purified oligos were synthesized by Thermo Scientific Dharmacon with

exceptions for Lig.47.D and Adaptor.L, which were synthesized by Life Technologies and IDT, respectively. All desalted oligos were synthesized by Eurofins Genomics.