SUPPLEMENTARY DATA

RNase H2 roles in genome integrity revealed by unlinking its activities

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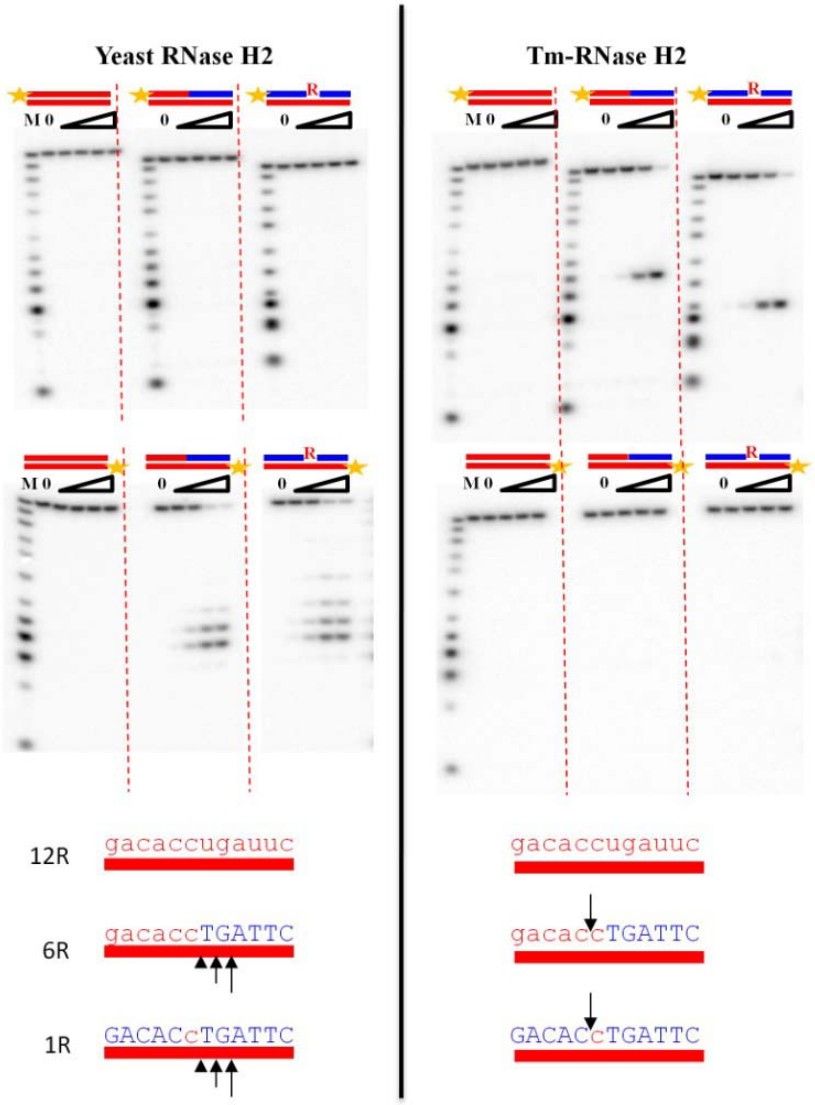
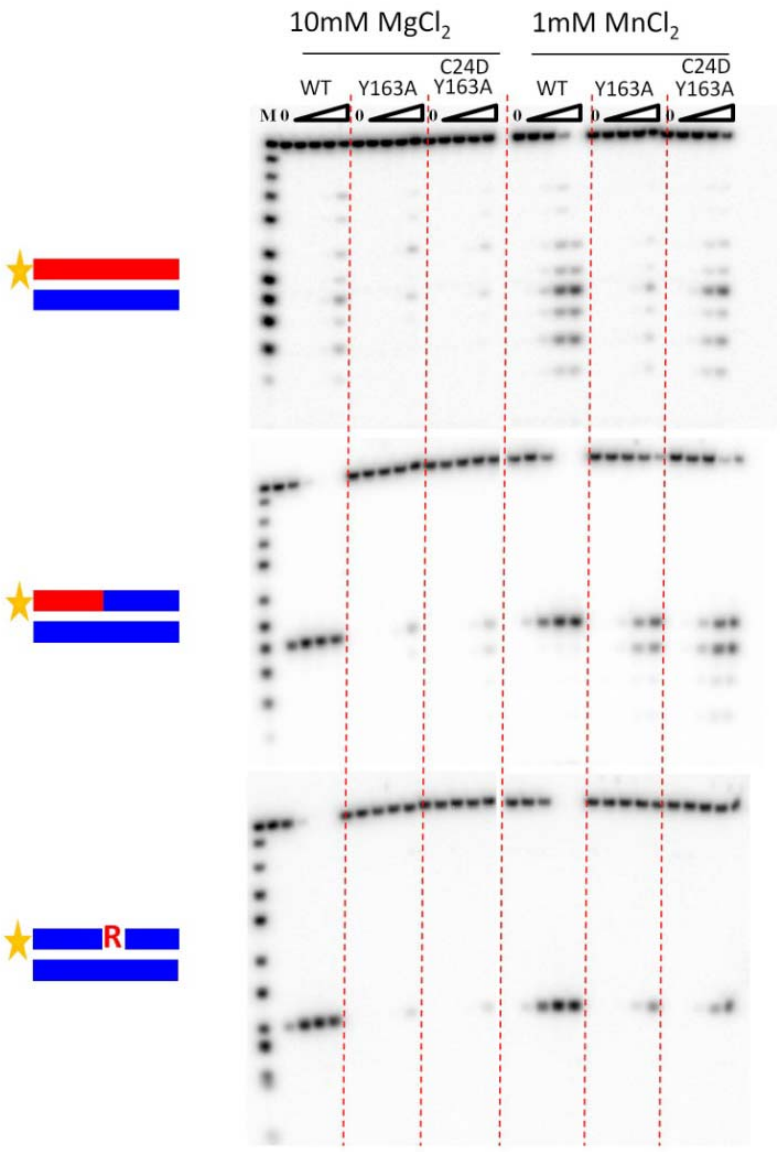


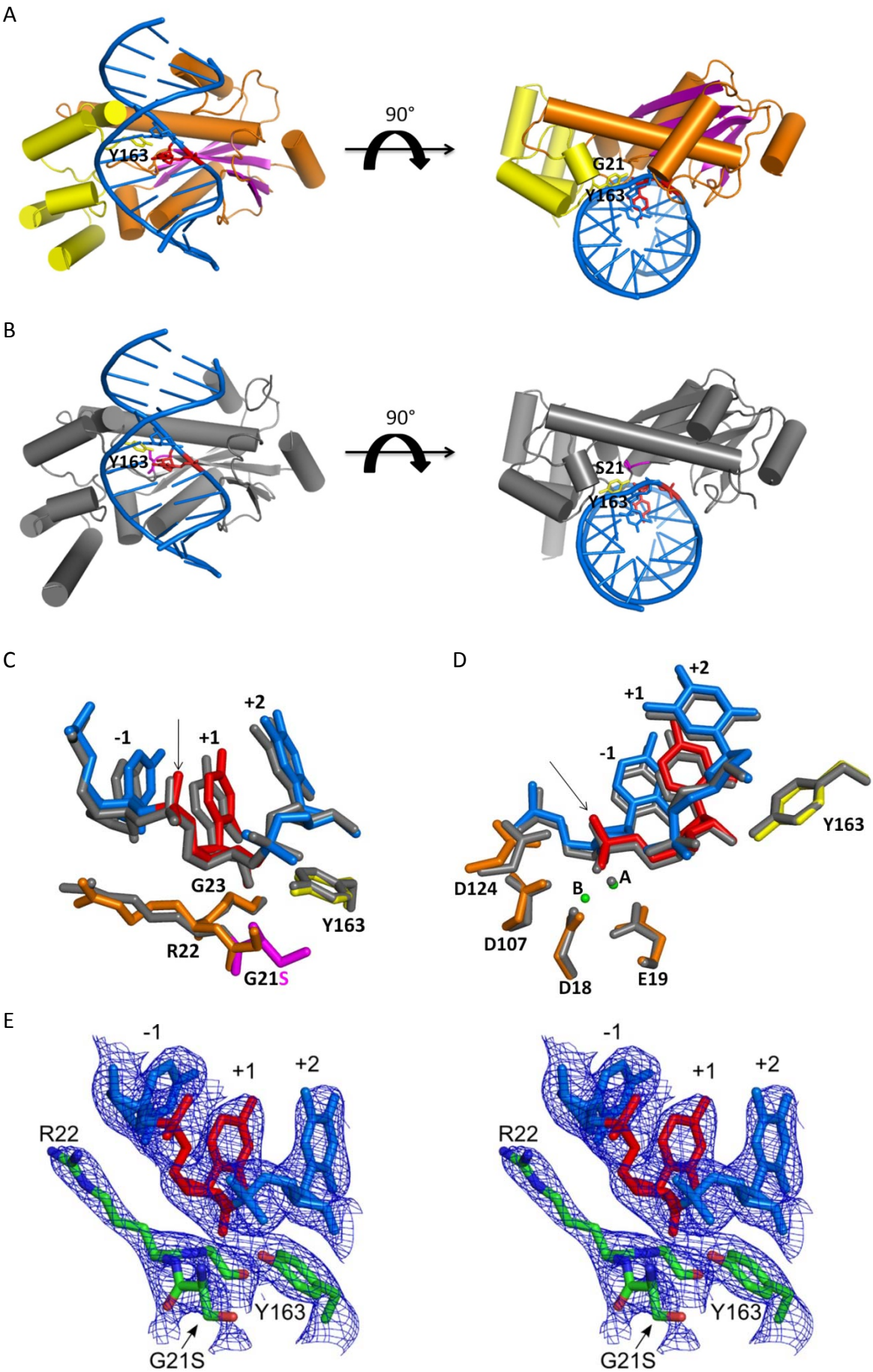
Supplementary Figure S1. Cleavage of short substrates with yeast and Tm-RNases H2.

The 5'-^{32}P labeled 12 mer substrates indicated above the gels were digested by Yeast and Tm-RNase H2 in the presence of 10 mM $MgCl_2$. The lanes marked with 0 contained no enzyme, and lanes marked with triangle contained increasing amount of the proteins (0.16, 1.6, 16 and 160 nM in the case of Tm-RNase H2, 0.011, 0.11, 1.1 and 11 nM in the case of yeast RNase H2). Products of the hydrolysis were analyzed by 20% TBE-urea gels. The sizes of products were measured based on molecular size markers indicated as M (products of digestion of ^{32}P-labeled strands without complementary strand by phosphodiesterase I). Major cleavage sites are summarized on the bottom.



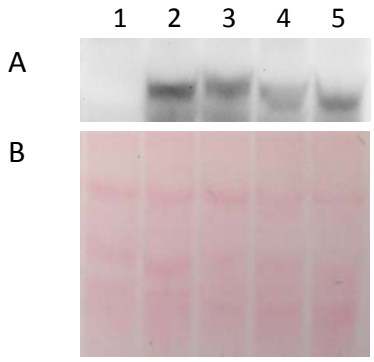
Supplementary Figure S2. Effect of RNase H3-like double mutation in Tm-RNase H2.

The 5'-^{32}P labeled 12 mer substrates schematically shown on the left of the gels were digested by Tm-RNase H2 WT, Y163A and C24D-Y163A mutants in the presence of 10 mM $MgCl_2$ and 1 mM $MnCl_2$. The lanes marked with 0 contained no enzyme, and lanes marked with triangle contained increasing amount of the proteins (1.6, 16, 160 and 1600 nM). Products of the hydrolysis were analyzed by 20% TBE-urea gels. The sizes of products were measured based on molecular size markers indicated as M (products of digestion of ^{32}P-labeled strands without complementary strand by phosphodiesterase I).


A
Y163
90°
G21
Y163
B
Y163
90°
S21
Y163
C
-1
+1
+2
G23
Y163
R22
G21S
D
+2
+1
-1
Y163
D124
B
A
D107
D18
E19
E
-1
+1
+2
R22
Y163
G21S
-1
+1
+2
R22
Y163
G21S

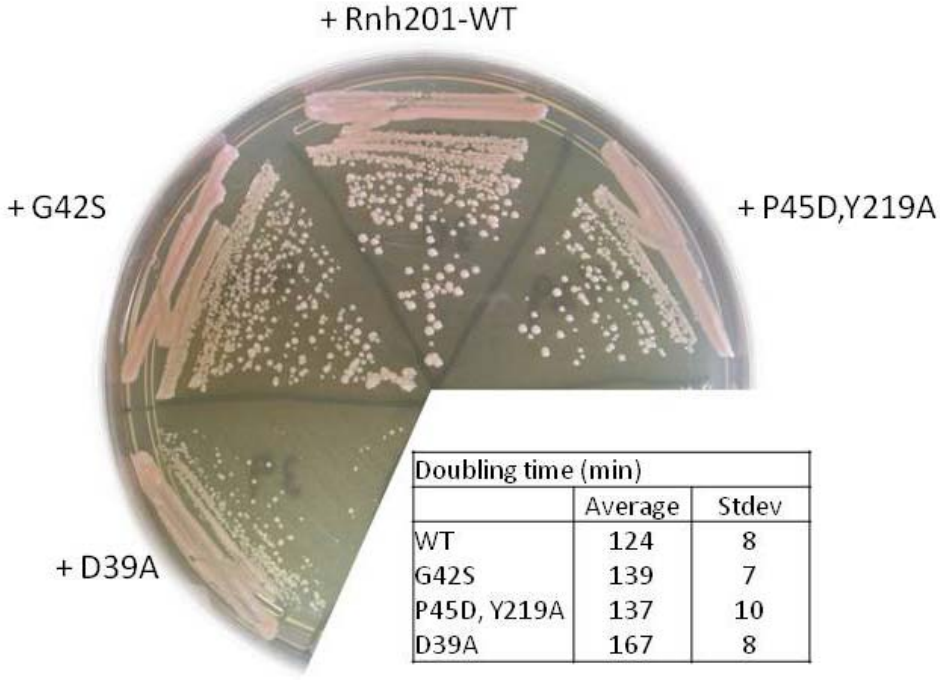
Supplementary Figure S3. Crystal structure of Tm-RNase H2 G21S mutant in complex with DNA containing single ribonucleotide.

Overall structures of wild-type and G21S mutant are shown. (A) N-terminal domain is colored with purple for beta-sheet and orange for loop and alpha-helix. C-terminal domain is in yellow. Residues (G21, K47 and Y163) and RpD junctions are shown in sticks. Cartoon model of the substrate is shown in blue for DNA and red for single ribonucleotide. (B) Protein structure except S21 (in purple), Y163 (in yellow) is shown in gray. (C) Close-up view for the recognition of RpD junction by Tm-RNase H2 WT and G21S mutant. G21S mutant is shown in gray except the G21S mutation (in magenta). The scissile phosphate is indicated with an arrow. (D) Close-up view for the correlation between the recognition of RpD junction by Y163 and catalysis. Structure of G21S mutant is shown in gray. Calcium ions of WT complex is shown in green. The scissile phosphate is indicated with an arrow. (E) Sample of electron density of the GRG(S) motif, Y163 and the RNA-DNA junction. The RNA is shown in red and DNA in blue. A 2Fo-Fc map contoured at 1.0 sigma is overlaid on the structure.



Supplementary Figure S4. Expression of C-terminally FLAG-tagged RNase H2A from the plasmid, ycNPH2-FL2 and its variants

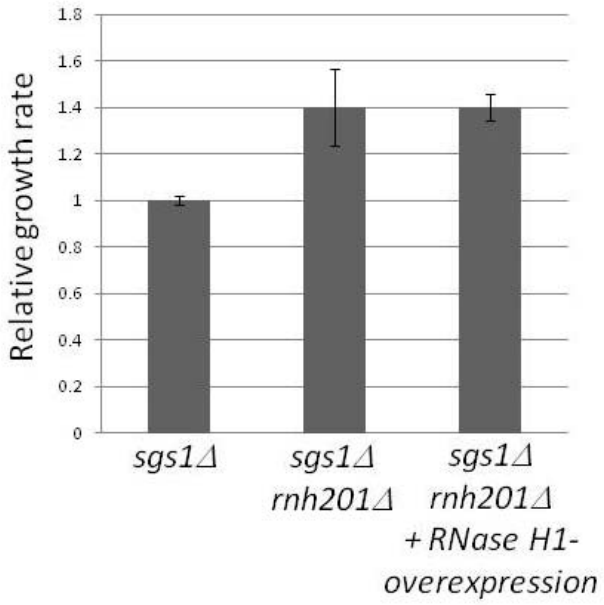
(A) Yeast cells expressing C-terminally FLAG-tagged Rnh201 WT (lane 2), D39A (lane 3), G42S (lane 4), P45D-Y219A (lane 5) or no protein (lane 1) from the plasmids with Rnh201 gene flanked by its own native promoter were analyzed with western analysis using anti-FLAG M2 antibody. (B) The PVDF membrane for Figure S4A was stained with Ponceau-S.



Doubling time (min)		
	Average	Stdev
WT	124	8
G42S	139	7
P45D, Y219A	137	10
D39A	167	8

Supplementary Figure S5. Complementation of slow growth phenotype of *sgs1Δ rnh201Δ* strain with plasmids.

Transformants of *sgs1Δ rnh201Δ* strain with plasmids expressing Rnh201-WT, G42S, P45D-Y219A or D39A were streaked on YPD plate and cultured at 30°C. The table shows doubling time of the yeast strains analyzed in YPD medium.



Supplementary Figure S6. Effect of overexpression of RNase H1 on growth rate of *sgs1Δ rnh201Δ* strain.

sgs1Δ rnh201Δ strain was transformed with a plasmid overexpressing Sc-RNase H1 and the doubling time was measured in YPD medium containing G418 at 30°C. The relative growth rate is shown.

Supplementary Table S1. List of yeast strains and plasmids

Strain	Genotype	Source
W303-1A	*MATa ade2-1 can1-100 his3-11,-15 leu2-3,112 trp1-1 ura3-1*	(55)
Cy8338	W303-1A but *sgs1::HIS3*	(56)
SMC306	W303-1A but *sgs1::HIS3 rnh201::HYG-URA3-GAL1-IsceI*	This study
SMC311	W303 genotype but *sgs1::HIS3 Rnh201-D39A*	This study
SMC308	W303 genotype but *sgs1::HIS3 Rnh201-G42S*	This study
SMC310	W303 genotype but *sgs1::HIS3 Rnh201-P45D-Y219A*	This study
SMC307	W303 genotype but *sgs1::HIS3 rnh202::HYG-URA3-GAL1-IsceI*	This study
SMC314	W303 genotype but *sgs1::HIS3 Rnh202-⊿PIP::KanMX*	This study
YAEH275	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 P_{GAL1}-3HA-TOP1 ::KanMx6 rnh201::NatMx6 rnh1::HphMx6*	(40)
Δ|(-2)|-7B-YUNI300	*MATa CAN1 his7-2 leu2-Δ::kanMX ura3-Δ trip1-289 ade2-1 lys2-ΔGG28899-2900 pol2-M644G rnh201Δ::HYG-R*	(5)

Plasmid	Description	Source
ycplac111	LEU2, ARS/CEN	Novagen
ycNPH2-FL2	C-terminally FLAG-tagged Rnh201 flanked by its own native promoter in ycplac111	This study
pYX242-Kan	LEU2, 2micro, KanMX4 at SfoI site	Novagen
pYX242-YH1-Kan	Sc-Rnh1 was cloned under TPI promoter of pYX242, KanMX4 at SfoI site	This study

Supplementary Table S2. Crystallographic data collection and refinement statistics for the Tm-RNase H2 G21S-substrate complex.	
Data collection	
Space group	*C* 2
Cell dimensions	
a, *b*, *c* (Å)	106.95, 47.63, 77.45
a, b, g (°)	90, 131.26, 90
Wavelength (Å)	0.91841
Resolution (Å)*	50.00 – 3.10 (3.15-3.10)
R_{merge} (%)	14.6 (37.3)
I / s(*I*)	9.8 (2.2)
Completeness (%)	96.9 (76.3)
Redundancy	3.3 (2.2)
Refinement	
Resolution (Å)	3.1
No. reflections	5022
R_{work} / R_{free} (%)	17.8/25.4
No. atoms	2189
Protein	1676
Nucleic acids	487
Ligands/ions	2
Water	24
Avg. *B*-factor	40.1
Protein	38.7
Nucleic acids	45.3
Ligands/ions	35.9
Water	30.3
Root-mean-square deviation	
Bond lengths (Å)	0.009
Bond angles (°)	1.255

* Values in parentheses are for highest-resolution shell.

Supplementary Table S3. MolProbity.

All-Atom Contacts	Clashscore, all atoms:	28.21	88th percentile* (N=59, 2.85Å – 9999Å)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.		
Protein Geometry	Poor rotamers	8.67%	Goal: <1%
	Ramachandran outliers	0.46%	Goal: <0.2%
	Ramachandran favored	93.52%	Goal: >98%
	Cβ deviations >0.25Å	0	Goal: 0
	MolProbity score^	3.07	77th percentile* (N=2114, 3.100Å ± 0.25Å)
	Residues with bad bonds:	0.00%	Goal: 0%
	Residues with bad angles:	0.00%	Goal: <0.1%
Nucleic Acid Geometry	Bad backbone conformations#:	0	Goal: 0
	Residues with bad bonds:	0.00%	Goal: 0%
	Residues with bad angles:	0.00%	Goal: <0.1%

All-atom structure was validated for macromolecular crystallography (5).

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