

Supplementary Table 1. Yeast strains used in this study

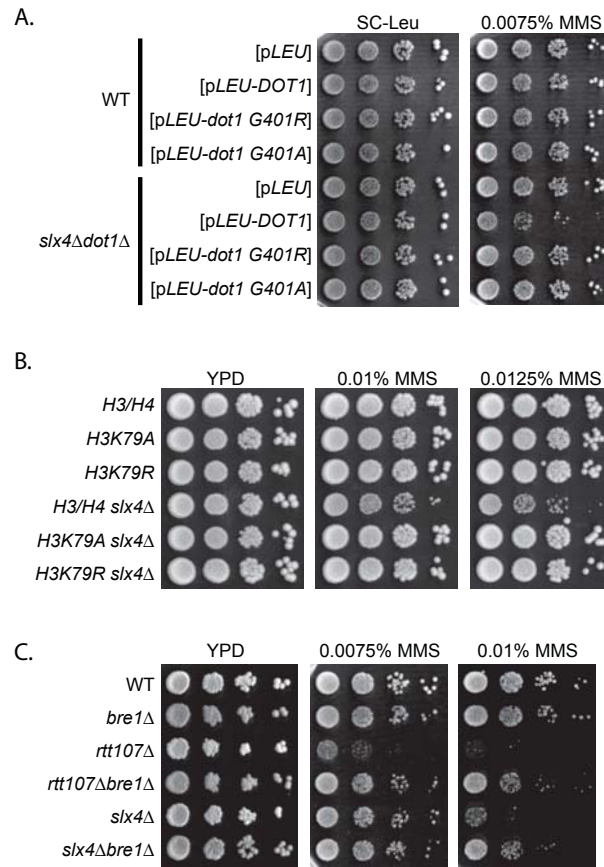
Strain	Relevant Genotype	Source*
MKY5	<i>MATa ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2</i>	
MKY6	<i>MATA ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ</i>	
MKY7	<i>MATA ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 LYS2</i>	
MKY399	<i>MATa ADE2 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ</i>	
MKY10	<i>MATa his4</i>	
MKY11	<i>MATA his4</i>	
MKY921	MKY5, <i>dot1Δ::his5MX6</i>	
MKY922	MKY5, <i>rtt107Δ::KANMX6 lys2Δ</i>	
MKY923	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6</i>	
MKY924	MKY7, <i>slx4Δ::KANMX6</i>	
MKY925	MKY5, <i>slx4Δ::KANMX6 dot1Δ::his5MX6</i>	
MKY927	MKY5, <i>bre1Δ::HYGMX6 lys2Δ</i>	
MKY928	MKY5, <i>rtt107Δ::KANMX6</i>	
MKY929	MKY5, <i>rtt107Δ::KANMX6 bre1Δ::HYGMX6 lys2Δ</i>	
MKY930	MKY5, <i>slx4Δ::KANMX6 lys2Δ</i>	
MKY931	MKY5, <i>slx4Δ::KANMX6 bre1Δ::HYGMX6 lys2Δ</i>	
MKY932	MKY5, <i>[pRS315]</i>	
MKY933	MKY5, <i>[pRS315, DOT1]</i>	
MKY934	MKY5, <i>[pRS315, dot1G401R]</i>	
MKY935	MKY5, <i>[pRS315, dot1G401A]</i>	
MKY936	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315]</i>	
MKY937	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, DOT1]</i>	
MKY938	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1G401R]</i>	
MKY939	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1G401A]</i>	
MKY940	<i>MATA/a ADE2/ade2-1 can1-100/can1-100 his3-11/his3-11 leu2-3,112/leu2-3,112 LYS2/lys2Δ</i>	
MKY941	MKY940, <i>rtt107Δ::KANMX6/rtt107Δ::NATMX6</i>	
MKY942	MKY940, <i>dot1Δ::his5MX6/dot1Δ::his5MX6</i>	
MKY943	MKY940, <i>rtt107Δ::KANMX6/rtt107Δ::KANMX6 dot1Δ::his5MX6/ dot1Δ::his5MX6</i>	
MKY944	MKY7, <i>Rad 52-GFP::NATMX6</i>	
MKY945	MKY7, <i>rtt107Δ::KANMX6 Rad 52-GFP::NATMX6</i>	
MKY946	MKY7, <i>dot1Δ::his5MX6 Rad 52-GFP::NATMX6</i>	
MKY947	MKY7, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 Rad 52-GFP::NATMX6</i>	
MKY949	MKY7, <i>dot1Δ::his5MX6</i>	
MKY950	MKY7, <i>rtt107Δ::KANMX6</i>	
MKY951	MKY399, <i>rtt107Δ::KANMX6</i>	
MKY952	MKY6, <i>dot1Δ::his5MX6</i>	
MKY953	MKY399, <i>rev1Δ::HYGMX6</i>	
MKY954	MKY6, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6</i>	
MKY955	MKY399, <i>dot1Δ::his5MX6 rev1Δ::HYGMX6</i>	
MKY956	MKY399, <i>rtt107Δ::KANMX6 rev1Δ::HYGMX6</i>	
MKY957	MKY6, <i>rtt107Δ::KANMX6 dot1Δ::his5MX6 rev1Δ::HYGMX6</i>	
MKY958	MKY5, <i>rev3Δ::HYGMX6</i>	

MKY959 MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6*
 MKY960 MKY5, *dot1Δ::his5MX6 rev3Δ::HYGMX6*
 MKY961 MKY5, *rtt107Δ::KANMX6 rev3Δ::HYGMX6*
 MKY962 MKY5, *rtt107Δ::KANMX6 dot1Δ:: his5MX6 rev3Δ::HYGMX6*
 MKY963 MKY399, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS316, HHT2-HHF2]* (1)
 MKY964 MKY6, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS316, HHT2-HHF2]*
 MKY965 MKY399, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
 MKY966 MKY6, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
 MKY992 MKY5, *SLX4-3XFLAG::NATMX6*
 MKY993 MKY5, *rtt107Δ::KANMX6 SLX4-3XFLAG::NATMX6*
 MKY994 MKY5, *dot1Δ::his5MX6 SLX4-3XFLAG::NATMX6*
 MKY995 MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 SLX4-3XFLAG::NATMX6*
 MKY996 MKY5, *RTT107-3XFLAG::NATMX6 lys2Δ*
 MKY997 MKY5, *slx4Δ::KANMX6 RTT107-3XFLAG::NATMX6*
 MKY998 MKY7, *dot1Δ::his5MX6 RTT107-3XFLAG::NATMX6 lys2Δ*
 MKY999 MKY7, *slx4Δ::KANMX6 dot1Δ::his5MX6 RTT107-3XFLAG::NATMX6 lys2Δ*
 MKY1000 MKY6, *hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
 MKY1001 MKY399, *rtt107Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
 MKY1040 *MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
 MKY1041 MKY1040, *rtt107Δ::his5MX6*
 MKY1042 MKY1040, *dot1Δ::HYGMX6*
 MKY1043 MKY1040, *rtt107Δ::his5MX6 dot1Δ::HYGMX6*
 MKY1055 MKY5, *rtt107Δ::KANMX6 [pRS315, RTT107]*
 MKY1056 MKY5, *rtt107Δ::KANMX6 [pRS315, rtt107-4AQ]*
 MKY1057 MKY5, *rtt107Δ::KANMX6 [pRS315]*
 MKY1058 MKY5, *rtt107Δ::KANMX6 dot1Δ: his5MX6 [pRS315, RTT107]*
 MKY1059 MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315, rtt107-4AQ]*
 MKY1060 MKY5, *rtt107Δ::KANMX6 dot1Δ::his5MX6 [pRS315]*
 MKY1082 MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315]*
 MKY1083 MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, DOT1]*
 MKY1084 MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1 G401A]*
 MKY1085 MKY5, *slx4Δ::KANMX6 dot1Δ::his5MX6 [pRS315, dot1 G401R]*
 MKY1086 MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, HHT2-HHF2]*
 MKY1087 MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79A-HHF2]*
 MKY1088 MKY6, *slx4Δ::KANMX6 hht1Δ-hhf1Δ::LEU2 hht2Δ-hhf2Δ::his5MX6 [pRS314, hht2K79R-HHF2]*
 MKY1094 MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc* (2)
 MKY1095 MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc rtt107Δ::KANMX6*
 MKY1096 MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc dot1Δ::HYGMX6*
 MKY1097 MKY6, *ars603.5Δ::URA3 ars608Δ::HIS3 ars609Δ::TRP1 LEU2::BrdU-Inc rtt107Δ::KANMX6 dot1Δ::HYGMX6*

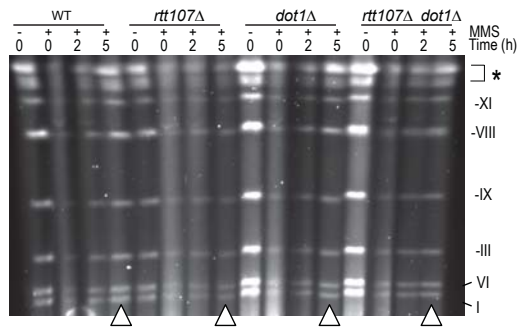
* unless otherwise indicated, all strains were constructed for this work (details available upon request) or were from the laboratory collections. All strains are in the W303 background, except strains MKY1040-MKY1043, which are in the S288C background.

References

1. Yang, B., and Kirchmaier, A. L. (2006) *Mol. Biol. Cell* **17**, 5287-5297
2. Viggiani, C. J., and Aparicio, O. M. (2006) *Yeast* **23**, 1045-1051



Supplementary Figure 1. Abrogation of H3 K79 trimethylation suppressed the MMS sensitivity of strains lacking Slx4. Tenfold serial dilutions of the indicated strains were plated onto media containing 0.0075% , 0.01% , or 0.0125% MMS. (A) Loss of Dot1's catalytic activity, (B) H3 K79A, K79R, or (C) loss of Bre1 suppressed MMS sensitivity of *slx4Δ* mutants.



Supplementary Figure 2. Requirement of Rtt107 for resumption of replication after DNA damage was partially suppressed by deletion of *DOT1*. PFGE analysis indicated *rtt107Δdot1Δ* mutants had more efficient DNA replication after DNA damage than *rtt107Δ* mutants.
 * : unresolved chromosomes.

SUPPLEMENTARY METHODS

MMS Sensitivity Measurements – Overnight cultures grown in YPD or SC-Leu at 30°C were diluted to 0.3 O.D₆₀₀. The cells were tenfold serially diluted and spotted onto solid YPD plates or SC-Leu plates with MMS (Sigma) at various concentrations. The plates were then incubated at 30°C for 2-3 days.

Pulsed-Field Gel Electrophoresis – Briefly, cells were arrested in G1 by addition of 2 µg/ml of α -factor for 2 h at 30°C in YPD, then washed with sterile 1X PBS and resuspended in YPD containing 0.03% MMS for 1 h. MMS was removed by treating with 2% sodium thiosulfate and washing with sterile 1X PBS. The cells were resuspended in YPD and incubated at 30°C during the recovery phase. Aliquots were removed at the indicated times and processed further.

Cells were treated using the CHEF Yeast Genomic DNA Plug Kit (Bio-Rad, CA). As described by the manufacturer, cells were washed with cold 50 mM EDTA and resuspended in Cell Suspension Buffer containing 0.75% agarose and Lyticase, and incubated for 2 h at 37°C. The agarose plugs were incubated with Proteinase K overnight at 50°C, then washed and loaded onto a 1.0% agarose gel in 0.5X Tris-borate EDTA. Pulsed-field gel electrophoresis was carried out as described by the manufacturer (GE Healthcare, UK). Briefly, the gel was subjected to electrophoresis at 100 V with a switch time of 60–120 s for 24 h with constant recirculation of the buffer at 8.5°C. Following electrophoresis, the gels were stained with ethidium bromide (0.5 µg/ml) and photographed.