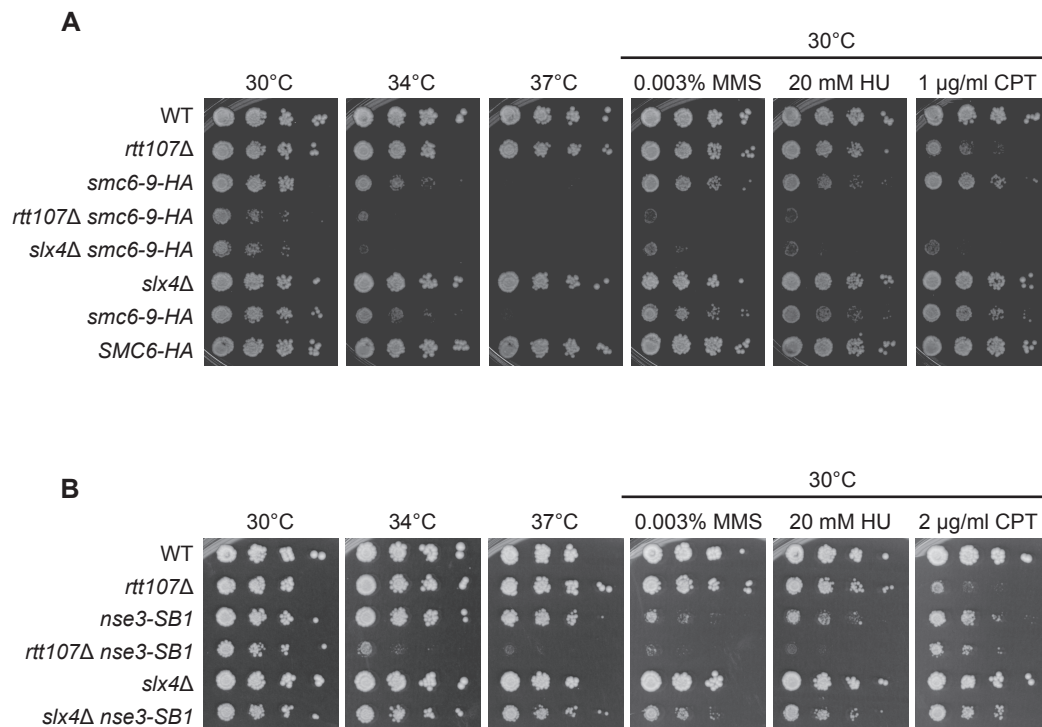


Supplementary Figure 1. Rtt107 interacted with multiple subunits of the SMC5/6 complex. Analytical-scale immunoprecipitations were performed on whole-cell extracts of the indicated strains. Immunoblotting was performed using the indicated antibodies. Pgk1 in the input fraction was used as a loading control. (A) Rtt107-VSV co-purified with Smc6-HA. (B) Nse1-FLAG co-purified with Rtt107-VSV. (C) Mms21-HA co-purified with Rtt107-VSV.



Supplementary Figure 2. *Rtt107* and *Slx4* had functions that were independent of the SMC5/6 complex. Tenfold serial dilutions of the indicated strains were plated onto media containing various DNA damaging agents and incubated at the indicated temperatures. Double mutants containing (A) *smc6-9* or (B) *nse3-SB1* and *rtt107Δ* or *slx4Δ* grew significantly more slowly than the respective single mutants.

Supplementary Table 1. Yeast strains used in this study

Strain	Relevant Genotype	Source*
MKY6	<i>MATa 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</i>	
MKY399	<i>MATa can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 lys2Δ</i>	
MKY102	MKY7, <i>RTT107-TAP::TRP1</i>	
MKY103	MKY7, <i>SLX4-TAP::TRP1</i>	
MKY107	MKY7, <i>NSE5-3XFLAG::KANMX6</i>	
MKY108	MKY7, <i>RTT107-TAP::TRP1 NSE5-3XFLAG::KANMX6</i>	
MKY109	MKY7, <i>SLX4-TAP::TRP1 NSE5-3XFLAG::KANMX6</i>	
MKY123	MKY7, <i>RTT107-TAP::TRP1 NSE5-3XFLAG::KANMX6 slx4::HIS3</i>	
MKY124	MKY7, <i>NSE5-3XFLAG::KANMX6 rtt107::HIS3 [pRS314, 4XTAP]</i>	
MKY125	MKY7, <i>NSE5-3XFLAG::KANMX6 rtt107::HIS3 [pRS314, 4XTAP-RTT107aa1-1070]</i>	
MKY126	MKY7, <i>NSE5-3XFLAG::KANMX6 rtt107::HIS3 [pRS314, 4XTAP-RTT107aa1-512]</i>	
MKY127	MKY7, <i>NSE5-3XFLAG::KANMX6 rtt107::HIS3 [pRS314, 4XTAP-RTT107aa512-1070]</i>	
MKY139	MKY7, <i>rtt107::HIS3 [pRS314, 4XTAP]</i>	
MKY140	MKY7, <i>rtt107::HIS3 [pRS314, 4XTAP-RTT107aa1-1070]</i>	
MKY141	MKY7, <i>rtt107::HIS3 [pRS314, 4XTAP-RTT107aa1-512]</i>	
MKY142	MKY7, <i>rtt107::HIS3 [pRS314, 4XTAP-RTT107aa512-1070]</i>	
MKY1237	<i>PJ69-4a/α (MATa/α trp1-901/trp1-901 leu2-3,112/leu2-3,112 ura3-52/ura3-52 his3-200/his3-200 ga14A/ga14A ga18OA/ga18OA LYSZ::GALI-HIS3/LYSZ::GALI-HIS3 GAL2-ADE2/GAL2-ADE2 metZ::GAL7-lacZ/metZ::GAL7-lacZ)</i>	(1)
MKY1238	MKY1237, <i>[pGAL4 DBD-Dest] [pGAL4 AD-Dest]</i>	
MKY1239	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-Dest]</i>	
MKY1240	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-RTT107aa1-1070]</i>	
MKY1241	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-SMC5]</i>	
MKY1242	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-SMC6]</i>	
MKY1243	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-NSE1]</i>	
MKY1244	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-MMS21]</i>	
MKY1245	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-NSE3]</i>	
MKY1246	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-NSE4]</i>	
MKY1247	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-NSE5]</i>	
MKY1248	MKY1237, <i>[pGAL4 DBD-RTT107aa1-1070] [pGAL4 AD-NSE6]</i>	
MKY1249	MKY1237, <i>[pGAL4 DBD-RTT107aa1-512] [pGAL4 AD-Dest]</i>	
MKY1250	MKY1237, <i>[pGAL4 DBD-RTT107aa1-512] [pGAL4 AD-NSE6]</i>	
MKY1251	MKY1237, <i>[pGAL4 DBD-RTT107aa512-1070] [pGAL4 AD-Dest]</i>	
MKY1252	MKY1237, <i>[pGAL4 DBD-RTT107aa512-1070] [pGAL4 AD-NSE6]</i>	
MKY1253	MKY1237, <i>[pGAL4 DBD-Dest] [pGAL4 AD-NSE6]</i>	
MKY1254	MKY1237, <i>[pGAL4 DBD-NSE6] [pGAL4 AD-Dest]</i>	
MKY1255	MKY1237, <i>[pGAL4 DBD-NSE6] [pGAL4 AD-SMC5]</i>	
MKY1256	MKY1237, <i>[pGAL4 DBD-NSE6] [pGAL4 AD-NSE5]</i>	

MKY1101	JKM139 (<i>MATa hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO</i>)	(2)
MKY1102	JKM179 (<i>MATa hoΔ hmlΔ::ADE1 hmrΔ::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisG ura3-52 ade3::GAL::HO</i>)	(2)
MKY1257	MKY1102, <i>RTT107-3XFLAG::NATMX6</i>	
MKY1258	MKY1101, <i>SMC5-3XFLAG::NATMX6</i>	
MKY1259	MKY1101, <i>SMC5-3XFLAG::NATMX6 rtt107::KANMX6</i>	
MKY1260	<i>ade2-1 trp1-1 his3-11 his3-15 ura3-1 leu2-3 leu2-112 Cir0 LEU2::GAL10-Flp(H305L)::leu2Δ1 fob1::HIS</i>	Parent from (3)
MKY1261	MKY1260, <i>RTT107-3XFLAG::NATMX6</i>	
MKY1262	MKY1260, <i>3XRFB-G418-FRT RTT107-3XFLAG::NATMX6</i>	
MKY1263	MKY1260, <i>SMC5-3XFLAG::NATMX6</i>	
MKY1264	MKY1260, <i>3XRFB-G418-FRT SMC5-3XFLAG::NATMX6</i>	
MKY1265	MKY1260, <i>SMC5-3XFLAG::NATMX6 rtt107::KANMX6</i>	
MKY1266	MKY1260, <i>3XRFB-G418-FRT SMC5-3XFLAG::NATMX6 rtt107::KANMX6</i>	
MKY1267	MKY6, <i>RTT107-3XFLAG::KANMX6</i>	
MKY1268	MKY6, <i>RTT107-3XFLAG::KANMX6 nse3-SB1::URA3</i>	Parent from (4)
MKY1269	MKY6, <i>RTT107-3XFLAG::KANMX6 nse5-R103G::NATMX6</i>	
MKY1270	MKY6, <i>RTT107-3XFLAG::KANMX6 smc6-9-3XHA::HIS3</i>	Parent from (5)
MKY419	MKY6, <i>RAD52-GFP::NATMX6</i>	
MKY1271	MKY6, <i>RAD52-GFP::NATMX6 rtt107::KANMX6</i>	
MKY1272	MKY6, <i>RAD52-GFP::NATMX6 smc6-9-3XHA::HIS3</i>	Parent from (5)
MKY1273	MKY6, <i>RAD52-GFP::NATMX6 smc6-9-3XHA::HIS3 rtt107::KANMX6</i>	
MKY1274	MKY6, <i>RAD52-GFP::NATMX6 nse3-SB1::URA3</i>	Parent from (4)
MKY1275	MKY6, <i>RAD52-GFP::NATMX6 nse3-SB1::URA3 rtt107::KANMX6</i>	
MKY1276	MKY6, <i>RAD52-GFP::NATMX6 nse5-R103G::HYGMX</i>	
MKY1277	MKY6, <i>RAD52-GFP::NATMX6 nse5-R103G::HYGMX rtt107::KANMX6</i>	
MKY317	MKY6, <i>rtt107::KANMX6</i>	
MKY426	MKY6, <i>rtt107::KANMX6 nse5-R103G::NATMX6</i>	
MKY541	MKY6, <i>smc6-9-3XHA::HIS3</i>	Parent from (5)
MKY542	MKY6, <i>smc6-9-3XHA::HIS3 rtt107::KANMX6</i>	
MKY543	MKY399, <i>SMC6-3XHA::HIS3</i>	
MKY544	MKY399, <i>smc6-9-3XHA::HIS3 slx4::KANMX6</i>	
MKY547	MKY6, <i>nse5-R103G::NATMX6 slx4::KANMX6</i>	
MKY1278	MKY6, <i>nse5-R103G::NATMX6</i>	
MKY1279	MKY6, <i>slx4::KANMX6</i>	
MKY1280	MKY6, <i>nse3-SB1::URA3</i>	Parent from (4)
MKY1281	MKY6, <i>nse3-SB1::URA3 rtt107::KANMX6</i>	
MKY1282	MKY6, <i>nse3-SB1::URA3 slx4::KANMX6</i>	
MKY414	MKY6, <i>SMC6-3XHA::HIS3</i>	

MKY1283	MKY6, <i>MMS21-3XHA::HIS3</i>	
MKY1284	MKY6, <i>NSE1-3XFLAG::NATMX6</i>	
MKY1285	MKY6, <i>RTT107-3XVSV::KANMX6</i>	
MKY1286	MKY6, <i>NSE1-3XFLAG::NATMX6 RTT107-3XVSV::KANMX6</i>	
MKY1287	MKY6, <i>SMC6-3XHA::HIS3 RTT107-3XVSV::KANMX6</i>	
MKY1288	MKY399, <i>MMS21-3XHA::HIS3 RTT107-3XVSV::KANMX6</i>	
MKY1320	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX</i>	
MKY1321	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX mec1::HIS3</i>	
MKY1322	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX nse3-SB1::URA3</i>	
MKY1323	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX nse3-SB1::URA3 mec1::HIS3</i>	
MKY1324	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX nse5-R103G::NATMX6</i>	
MKY1325	MKY399, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX nse5-R103G::NATMX6 mec1::HIS3</i>	
MKY1326	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX smc6-9-3XHA::HIS3</i>	
MKY1327	MKY6, <i>RTT107-3XFLAG::KANMX6 sml1::HYGMX smc6-9-3XHA::HIS3 mec1::NATMX6</i>	
MKY1334	MKY6, <i>SLX4-3XFLAG::NATMX6 SMC5-3XHA::HIS3</i>	
MKY1335	MKY6, <i>RTT107-TAP::TRP1 SLX4-3XFLAG::NATMX6 SMC5-3XHA::HIS3</i>	

* 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 for strains MKY1237-MKY1256.

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Supplementary Table 2. Primers used in this study for qPCR

	Forward	Reverse	Location
MAT	CCCATCGTCTTGCTCTTGTT	ATCCGTCCCGTATAGCCAAT	0.2 kb from HO site
TAF2	CAAGGATGCCCTTGTTTTGT	TTTTGACGGCCAATCTTTTC	1 kb from HO site
YCR043C*	CCAAGGAACTAATGATCTAAGCACA	CATGTTGGTACTCTAAATCACCTCC	5 kb from HO site
IMG1*	TGGATCATGGACAAGGTCCTAC	GGCGAAAACAATGGCACTCT	10 kb from HO site
PWP2*	GACACACTTTACTTTGGCTTGGTT	GACTTCCAAAGACTTAAGCGCA	20 kb from HO site
FRT†	AAGTTCGACATGGGCTTCAG	TGATCTGCATGGGTTCGTTA	0.1 kb from FRT site
ROG3†	ATCTTTGCCAAATTGCTTCG	TTTGTCGGGCCATGAGTTAT	0.85 kb from FRT site
ATG18†	GAAACTTCCCGTTGAAACCA	TATCCATCCGAGGAAACGAC	2.7 kb from FRT site
LSB3†	AAGAACGAGCCCTTGACTGA	TCGACGATGATGATGACGAT	5.8 kb from FRT site
CDC14	TATTCGCCGTAGAAAGGTTGG	TTGGCGGCTTATATCCCTTA	12 kb from FRT site
PRP8	GGATGTATCCAGAGGCCAAT	AACCCGCGTATTAAGCCATA	Reference locus

* Primer sequences from Kim, J. A., and Haber, J. E. (2009) *Proc. Natl. Acad. Sci. U. S. A.* **106**, 1151-1156

† Primer sequences from Nielsen, I., Bentsen, I. B., Lisby, M., Hansen, S., Mundbjerg, K., Andersen, A. H., and Bjergbaek, L. (2009) *Nat. Methods* **6**, 753-757