



**Fig. S1. Analysis of BIR kinetics and repair products.** A, BIR kinetics revealed by pulsed-field gel electrophoresis (PFGE) for indicated cells. The BIR product is indicated by the arrow. B, Southern blot analysis of BIR kinetics for the WT and mutant cells. *ADE1* was used as probe. C-D, PFGE and Southern blot analysis of recombination products from Ade<sup>+</sup>, NAT<sup>R</sup> Leu<sup>-</sup> colonies of *rtt105* $\Delta$  cells. \* denotes recombination events associated with the rearrangement of the recipient chromosome.

Fig. S2



**Fig. S2. Analysis of BIR repair.** A, Model showing the H-0 BIR system(intra chromosome BIR). B, Southern blot analysis of BIR kinetics for the WT and mutant cells in the H-0 system. Samples were collected 0, 1, and 6 hr after DSB induction. *MATa* was used as probe. C, Plot showing the quantification of BIR repair efficiency in B. Repair efficiency at 6 hr after DSB induction was calculated as the percentage of normalized pixel intensity of the BIR product band at 6 hrs compared to the normalized parental bands at 0 hr. D, Table showing repair outcomes for the WT or *rtt105-EL2A* cells in the allelic BIR strains (AM1003 background). GC: gene conversion, HCO: half-crossover. Loss: chromosome loss. Cells cultured in the pre-induction liquid media were plated on YEP-Galactose media to induce DSBs. Colonies formed were replica plated on Leu<sup>-</sup> or Ade<sup>-</sup> dropout media. The frequency for each category of repair outcome was calculated based on the percentage of colonies carrying markers specific for each repair outcome.



**Fig. S3. Rtt105 promotes the DNA damage response and recovery.** A, Analysis of chromosome integrity by PFGE. Cells were collected at different time points during the recovery from short MMS treatment (0.03%, 1hr). B, DNA damage sensitivity test for indicated strains at indicated drug concentrations.

## Mutation spectrum for CanR colonies derived from WT cells

1 1	-A(2) G>T C>T G>T -A ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGA <mark>A</mark> GCATATGTACAATGAGCGGGCCACAACCCTCTTTCACGAGGCGTTGAAGCTTCA M T N S K E D A D I E E K H M Y N E P V T T L F H D V E A S
91 31	+C +C $r^{-G}$ c>a(2) T>a (4) CAAACACACCACAGGCGTGGGCAATACCATTAAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT Q T H H R R G S I P L K D E K S K E L Y P L R S F P T R V N
181 61	GGCGAGGATACGTTCTCTATGGAGGATGGCATAGGTGATGAAGATGAAGGAGAGTACAGAACGCTGAAGTGAAGAGAGAG
271 91	$\begin{array}{cccc} c_{>x} & c_$
361 121	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
451 151	GA TCCTCTTTTCACAGTGTTCTCACAAAGATTCCTTTCTCCAGCATTGGCGGCCAATGGTTACATGTATTGCTTTTCTTGGCAATCACT S S F T V F S Q R F L S P A F G A A N G Y M Y W F S W A I T
541 181	CSA CSA CST -T CSA(2) TTTGCCCTGGAACTTAGTGTGTGTGTGTGTGTTTTTTTTT
631 211	G>C(2) G>A ATTATCACAATAATGAACTTGTTCCCTGTCAAATATTACGGTGAATTCGAGTTCTGGGGTCGCTTCCAAAGTTTTAGCCATTATCGGG I I T I M N L F P V K Y Y G E F E F W V A S I K V L A I I G G>A
721 241	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
811 271	$\begin{array}{cccc} & & & & & & & & & & & & & & & & & $
	T>G C>T(4)
901 301	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
901 301 991 331	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
901 301 991 331 1081 361	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
901 301 991 331 1081 361 1171 391	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
901 301 991 331 1081 361 1171 391 1261 421	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
901 301 991 331 1081 361 1171 391 1261 421 1351 451	CPT(4)         -A (2)       CPT (25 T GSA         -A (2)       TO GSA         GAACTAGTIGGTACACTGCAAGCTGCAAGACTCCCAGAAAAATCCGTTCCAAGAGGCCATCAAAAAAGGTTGCGTTCCGGTATCTTAAGAC         CAACTAGTIGGTACACTGCAAGCTGCAAGACTCCCAGAAAAATCCGTTCCAAGAGGCCATCAAAAAAGGTTGCGTTCCGTATCTTAAGAC         CAACTAGTIGGCTCCCATATTATTCATTGGACTTTTAGTCCAAGAAAATCCAACTAACT
901 301 991 331 1081 361 1171 391 1261 421 1351 451	CPT (4)         CACTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGT
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481	TC CONTRACTOR CONTRACT
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	TYG         CYT (4)           GAACTAGT TGGTATCACTGGTGGAAGCGCGAAACCCCCAGAAAATCGGTCCAAGAGGCCATCAAAAAAGGTTTTCCGTAAGTGGCCATCAAAAAAGGCCATGAGGCCACAAAAAAGGCCATGAGGACCCCAAAAAAAGGCCATGAGGACCCCAAAAAAAGGCCATGAGGACGCCAAAAAAAGGCCATGAGGACGCCAAAAAAAGGCCAAAAAAAGGCCAAGGAGGACGCCAAAAAA

## Mutation spectrum for CanR colonies derived from *rtt105*∆ cells

	-A(2) -A(2)
1	ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAAGCATATGTACAATGAGCCCGGTCACAACCCTCTTTCACGACGTTGAAGCTTCA
Ţ	M T N S K E D A D I E E K H M I N E P V T T L F H D V E A S
	24 24 24 (E)A<2
91	CAAACACACCACAGACGTGGGTCAATACCATTAAAAGATGAGAAAGATTAAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT
31	Q T H H R R G S I P L K D E K S K E L Y P L R S F P T R V N
	duralization (213-229 hn)
1.0.1	
61	
	281-307(-27bp) duplication(281-307bp)
	A>G T T>C T>G G>A(2) G>A G>T
271	AGAC <mark>A</mark> TATTG <mark>CTATGATTGCCCTTGGTGGTACTATTG</mark> GTACAG <mark>G</mark> TCTTTTCATTGGTTTATCCACACCCTCTGACCAACGCC <u>G</u> GCCCA <i>G</i> TG
91	R H I G M I A L G G T I G T G L F I G L S T P L T N A G P V
	435-513 (-740p) 445-508 (-540p)(2)
361	GCGCCTCTTATATCATATTTATTATGGGTTCTTTGGCATATTCTGTCACGCGGCGGTGAAATGGCTACATTCATCCCTCTGTTACA
121	G A L I Š Y L F M G S L A Y S V T Q S L G E M A T F I P V T
	516-610(-96bp) 533-628 (-96bp) 537-632 (-96bp)
	G>C T G>A T
451	TCCTCTTCACAGGTGTTCTCACAAAATTCCTTTCTCCAGCATTGGTGCGCCAATGGTTACATGTTTTCTTGGGCAATCACT
101	SSEIVESURELSEAEGAANGIMIWESWAII G>A
	÷
541	TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCACTGGCGGCATGGATTAGTATTTTT <u>TCGGTA</u>
181	F A L E L S V V G Q V I Q F W T Y K V P L A A W I S I F W V
	G>A C-A 720-858 (-139bp)
631	ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο ο
211	I I T I M N L F P V K Y G E F E F W V A S I K V L A I I G
	752-799 (-49bp)(2) 760-808 (-49bp) duplication(761-809bp) -G
	T>G CC C>G G>A
721	TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTCCGTTATTGGAGAAACCCAGGTGCCTGGGGT
241	F L I Y C F C M V C G A G V T G P V G F R Y W R N P G A W G
811	
271	P G I I S K D K N E G R F L G W V S S L I N A A F T F O G T
	G>C G>A C>G -A
901	<u>G</u> AACTAGTTGGTATCACT <u>G</u> CTGGTGAAG <u>C</u> TGCAAACCCCCAGAAAATCCGTTCCAAAGAGCCATCAAAAAA <mark>AC</mark> TTGTTTTCCGTATCTTAACC
301	E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T
991	G>T T>C ▷A TTCTACATTGGCTCTCTATTATTCATT <b>G</b> GACT <b>T</b> TTAGTTCCATA <b>C</b> AATGACCCTAAACTAACACAATCTACTTCCTACGTTTCTACTTCT
991 331	67 7% CA TTCTACATTGGCTCTCTATTATTGATTGGGACTTTTAGCTTCCATAGCACTGAACCTAACACAATCTACTTCCTACGTTTCTACTTCT F Y I G S L L F I G L L V P Y N D P K L T Q S T S Y V S T S
991 331	G>T T>C C>A TTCTACATTGGCTCTCTATTATTCATTGGCACTTTTAGTTCCATACACCAAACTAACACAAATCTACTTCCTACGTTTCTACTTCT F Y I G S L L F I G L L V P Y N D P K L T Q S T S Y V S T S 1134-1148 (-15bp)
991 331	G>T TC CA TTCTACATTGGCTCTCTATTATTCATTGGCCTTTTAGTTCCATACAACTAACT
991 331 1081	G>T TC CA TTCTACATTGGCTCTCTATTATTCATTGGCACT F Y I G S L L F I G L L V P Y N D P K L T Q S T S Y V S T S G>T 1134-1148 (-15bp) G>T CCCTTTATTATGCTATTGGCACAAAGGTTTTGCCACATATCTTCAACGGCGGTTATCTTAACAACCATTATTTCTGCCGCA
991 331 1081 361	G>T T>C CA TTCTACATTGGCTCTCTATTATTCATTGGCCTTTTAGTTCCATAGAATGACCCTAAACTAACAACAATCTACTTCCTACGTTTCTACTTCT F Y I G S L L F I G L L V P Y N D P K L T Q S T S Y V S T S 1134-1148 (-15bp) G>T CCCTTTATTATTGCTATTGGAGAACTCTGGTACAAAGGTTTTGCCACATATCTTCAACGGCTGTTATCTTAACAACCATTATTTCTGCCGCA P F I I A I E N S G T K V L P H I F N A V I L T T I I S A A (195-1232 (-38bp)
991 331 1081 361	G-T TC CA TTCTACATTGGCTCTCTATTATTCATTGGCACTTTTAGCTACCAATGACCCAAACCAAACCAAACCAAACTAACACAATCTACTTCCTACGTTTCTACTTCT F Y I G S L L F I G L V P Y N D P K L T Q S T S Y V S T S 1134-1148 (-15bp) G-T CCCTTTATTATTGCTATTGAGAACCTCTGGTACAAAGGTTTTGCCACATATCTTCAACGCTGTTATCTTACAACAACCATTATTTCTGCCGCA P F I I A I E N S G T K V L P H I F N A V I L T T I I S A A 1194-1232(-33bp) 1194-1232(-33bp) 1194-1232(-33bp) CCC
991 331 1081 361 1171	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451	67       FX       FA         CTCTACACTEGGCCTCTATTATTEGGCCTTTTGGCCCTATACACACACTACACACACACTCACCTACACTACCTCCCCCC
991 331 1081 361 1171 391 1261 421 1351 451 1441	6-7 TCC CTTATTATTCCTTCGTGCTCTGCGCCCTTGCCTTG
991 331 1081 361 1171 391 1261 421 1351 451 1441 481	647       TC       CA         TTCTACATTEGGCCTCTATTATTEGGCCTTTTATGGCCCTAAAGGACCCAAAGCAACCAACAACCAAC
991 331 1081 361 1171 391 1261 421 1351 451 1441 481	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451 1441 481	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	67       64         TTCTACATTGGCCTCCTATATTACTATTAGGCCCTTAGCAGCCCAAACCAACC
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621	67       7.6       CA         TCTCACATTGGCTCTCATATATCATTGGACATTGGCCATAGAATGACCCCTAAACTAACCAATCTACTTCACGTTCTAACTACTTCACTTCT       A       V       <
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	67       7.6       7.6         7       7.6       7.8       7.8       8.8       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8       8.9       7.8<
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	67       10       CA         CATCATATATATATATATATATATATATATATATATATA
991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	$134 \ 148 \ 150 \ 160 \$

## Mutation spectrum for CanR colonies derived from *rtt105-EL2A* cells

	-C A>T	T>C(2)
1	- +G -A(4) -A(12) CA ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGGAGGAGGAGGAGGACGATATGTACAATGAGCCGGTCACAACCCTCTTTCACGACGTTG	-A(4) AAGC <b>T</b> TC <mark>A</mark>
1	M T N S K E D A D I E E K H M Y N E P V T T L F H D V	EAS
	-G T>G G>1	
91	$\texttt{CAAACACCACACAGACGTGG} {\tt G} {\tt G} {\tt T} \texttt{CAATACCAT} {\tt T} {\tt AAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGAC} {\tt G} {\tt A} {\tt G} {\tt G} {\tt G} {\tt A} {\tt G} {\tt G$	GAGTAAAT
31	Q T H H R R G S I P L K D E K S K E L Y P L R S F P T	R V N
	257-273 (-17bp)	ination (257 272 hp)
181	oup <b>7 3 4 5 5</b> 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	
61	G E D T F S M E D G I G D E D E G E V O N A E V K R E	LKO
	duplication(281-307bp)(2)	-
271	AGACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGCCTTTTCATTGGTTTATCCACACCTCTGACCAACGCC	GCCCAGTG
91	R H I G M I A L G G T I G T G L F I G L S T P L T N A	G P V
	C>A	
361	$GGGGGCTCTTATAT\underline{\mathbf{C}}ATATTTATTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCTTGGGT\underline{\mathbf{C}}AAATGGCTACATTCATCC$	CTGTTACA
121	G A L I S Y L F M G S L A Y S V T Q S L G E M A T F I	2 V T
	C>A G>C G>A G>A T>AG>	r
451	$\texttt{TCCTCTTTCA} \underline{\mathbf{C}} \texttt{AGTGTTCTCACAAAGATTCCTTTCTCCAGCATTT} \underline{\mathbf{G}} \texttt{GTGCGGCCAATG} \underline{\mathbf{G}} \texttt{TTACATGTATTG} \underline{\mathbf{G}} \texttt{TTTTCT} \underline{\mathbf{T}} \texttt{G} \underline{\mathbf{G}} \texttt{G}$	CAATCACT
151	SSFTVFSQRFLSPAFGAANGYMYWFSW.	A I T
	+T	+T(3)
5.4.5		<b>–</b>
541	TTTGCCCTGGAACTTAGTGTAGTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCACTGGCGGCATGGATTAGTATTT	ft <mark>tg</mark> ggta
181	FALELSVVGQVIQFWTIKVPLAAWISI	± w ∨
		6~^
631	ATTATCACAATAATGAACTTGTTCCCTGTCAAATATATACGGTGGAATTCGGGTCGGGTCGCTTCCATCAAAGTTTTAGCCA	TTATCGGG
211	I I T I M N L F P V K Y Y G E F E F W V A S I K V L A	IIG
	-4	<b>C</b> 1
	duplication +G G>A(2)	-G
721	$\mathtt{TTTCTAATATACTGTTT}{\underline{\mathbf{TT}}}\mathtt{GTATGG}{\underline{\mathbf{TT}}}\mathtt{TGTGGTGCTGGGGTTACCGGCCCAGTTGGATTCCGTTATTG}{\underline{\mathbf{GA}}}\mathtt{GAAACCCAGGTG}$	CCTG <mark>GG</mark> GT
241	FLIYCFCMVCGAGVTGPVGFRYWRNPG	A W G
	+G +T	G>A
011		A G>A
271		AAGGIACI
2/1	r G I I S K D K N E G K F E G W V S S E I N A A F I F	2 G 1
	$(\Sigma A/2)$ by $(\Sigma - \Sigma C)$ $(\Sigma - \Sigma C)$	TNA
901	GAACTAGTTGGTATCACTGCTGGTGGAGCTGCAAAACCCCCAGAAAAATCGTTCCGTAGAAGAGCCATCAAAAAAGTGTTTTCCGTA	TCTTAACC
301	E L V G I T A G E A A N P R K S V P R A I K K V V F R	ILT
	G>A T>G C>A	
991	$\texttt{TTCTACATTGGCTCTCTATTATTCATT}\underline{\mathbf{G}} \texttt{GACTTTTAGTTCCATACAATGACCCTAAAC}\underline{\mathbf{T}} \texttt{AACACAATCTACTTCCTA}\underline{\mathbf{C}} \texttt{GTTT} \texttt{C} \texttt{C} \texttt{T} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} \texttt{C} C$	CTACTTCT
331	FYIGSLLFIGLLVPYNDPKLTQSTSYV	S T S
	,1134-1148 (-15bp)	
	-AT	
1081	CCCTTTATTATTGCTATTGGGAACCCTGGTACAAAGGTTTTGCCACATATCTCAACGCTGTTATCTTAACAACCATTATTT	CTGCCGCA
361	PFILALEN SGTKVLPHIFNAVILTTII:	5 A A
	CSA 1195-1232(-38bp)(2) -C	
1171	I>A(2) БАС В СБС ГОТАНТИСКАТАТИТСКАТАТИТСКАТАТИТСКАТАТСА В СВАСТА СТИТАСТИ В АТИТСКАТА В СВАСТА В АТИТСКАТА В ССВОСА. В АТИТСА В АТИТСАТИТСКАТИСТИТИТИТИТСКИТСЯ В СВАСТА В СТИТСКАТА В АТИТСКАТИТСКАТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТИТ	CAAAGGT
391	N S N I Y V G S R I L F G L S K N K L A P K F L S R T '	r K G
	1279-1341(-63bp) 1324-1339 (-16bp)	
	duplication (1279-1296 bp)	
1261	GGTGTTCCATACATTGCA	FTTTCGAA
421	G V P Y I A V F V T A A F G A L A Y M E T S T G G D K Y	/ F E
	1395-1418 (-24bp) 1418-1511(-93bp)	
	G>T G>A(2)	
1351	TGCCTATTAAATATCACTGCTGTGCTGCACGCTTTTTTGCATGGTATTTTATCCAATCTCGCACATCACATTTATCCAAGCTT	IGAAATAC
451	W L L N I T G V A G F F A W L F I S I S H I K F M Q A .	JKI
1441	CGTGGCATCTCTCGTGACGAGTTACCATTTAAAGCTAAATTAATGCCCGGCTTGGCTTATTATGCGGCCCACATTTATGACGA	ICATTATC
481	R G I S R D E L P F K A K L M P G L A Y Y A A T F M T	I I I
-		-
	+T	
1531	$atta\textit{t}tcaaggtttcacggcttttgcaccaaaattcaatggtgttagctt \underline{\mathbf{rg}}ctgccgcctatatctctgttttcctgttct$	FAGCTGTT
511		
	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F :	AV
	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F :	LAV
	I I Q G F T A F A P K F N G V S F A A Y I S V F L F :	LAV
1621	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F : TGGATCTTATTTCAATGCAATGCAGATTTATTTGGAAGATTGGAGATGTCGACATCGATTCCGATAGAAGAGAGACA	L A V
1621 541	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F TGGATCTTATTTCAATGCATATTCAGATGCAGATTATTTGGAAGATTGGAGATGTCGACATCGATCG	L A V TTGAGGCA I E A
1621 541	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTT	L A V ITGAGGCA I E A
1621 541	I I Q G F T A F A P K F N G V S F A A A Y I S V F L F : TGGATCTTATTTCAATGCATATTCAGATGCAGATTTATTT	L A V ITGAGGCA [ E A

## Mutation spectrum for CanR colonies derived from rfa1-V106A cells

	-A(4) -A(5)	
1	ATGACAAATTCAAAAGAAGACGCCGACATAGAGGAGAGAGA	
T	M T N S K E D A D I E E K H M I N E P V T T L F H D V E A S	
91	CAAACACCACACAGACGTGGGT C AATACCATTAAAAGATGAGAAAAGTAAAGAATTGTATCCATTGCGCTCTTTCCCGACGAGAGTAAAT	
31	Q T H H R R G S I P L K D E K S K E L Y P L R S F P T R V N	
	+T	
	G>T duplication(224-255bp) duplication (257-273)	bp)
181	Geograficated TTCTCTATEGAGGATEGCATAGETGATEGAAGGAGAAGTACAGGACGCTGAAGGTGAACAGGAGGCTTAAGGAA	
01	G E D I E S M E D G I G D E D E G E V Q N A E V K K E L K Q	
	281-307 (-27bp)(2) C1 G-C	
271	AGACATATTG TATGATTGCCCTTGGTGGTACTATTGGTACTAGGTCTTTTCATTGGTTATCCACACCCCTGGCCAACGCCGGCCCCAGTG	
91	R H I G M I A L G G T I G T G L F I G L S T P L T N A G P V	
	duplication(389-422 bp)	
361	GGCGCTCTTATATCATATTATTATGGCTTCTTGGCATATTCTGCCACGCCGCCCTCGGCGCAAATGGCTACATTCATCCCTGTTACA	
121	GALISILFMGSLAYSVTQSLGEMATFIPVT 506.692(106bb)	(hn)
	30-900(-1030) 30-902 (300	up)
451		
151	SSFTVFSQRFLSPAFGAANGYMYWFSWAIT	
	588-1021(-434bp)	
	A>G T>G duplication(516-610bp)	
541	<u>TTTGCCCTGGAACTTAGTGTAGTTGGCCAAGTCATTCAATTTTGGACGTACAAAGTTCCACTGGCGGCATGGATTAGTATTTTT</u> TG <mark>GGTA</mark>	
181	FALELSVVGQVIQFWTYKVPLAAWISIFWV	
	G>C(2) C>T	
631	Col         ASI         ASI         Col         GSZ         GSZ <td></td>	
211	T T T T M N I. F P V K Y Y G E F E F W V A S T K V I. A T T G	
	G>A(2)	
721	TTTCTAATATACTGTTTTTGTATGGTTTGTGGTGCTGGGGTTACCGGCCCAGTTGGATTCCGTTATTGGAGAAACCCAGGTGCCTGGGGT	
241	F L I Y C F C M V C G A G V T G P V G F R Y W R N P G A W G	
	+A	
811	CCAGGTATATATCTAAGGATAAAAGGAGGGGGGGTTCTTAGGTTGGGTTTCCTCTTTTATTACCCTGCCTTCACATTCAAGGACG	
2/1	EGIISKDKWEGKELGGWVSSLINAAEIEQGI	
	$\frac{+A}{2}$	
	174 0.1	
901	GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAACCCCAAGAAAATCCGTTCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC	
901 301	GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAAACCCGAGAAAAACCGTTCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T	
901 301	GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAAAACCCGCAGAAAAACCGTTCCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T	
901 301	GAACTAGTTGGTATCACTGCTGGTGAAGCTGCAAAACCCGCAGAAAAACCGTTCCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T	
901 301	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAAACCCCCAGAAAAACCGTTCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T CA(3) 6>C 6>T	
901 301 991	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAAACCCCAGAAAAACCGTTCCAAGAGGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T CA(3) 6>C 6>T TTCTACATTGGCTCTCTATTATTCATTGGACCTTTAGTTCCATACAATGACCCTAAACTAACT	
901 301 991 331	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAAACCCCAGAAAAACCCGTTCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC         E       L       V       G       I       T       A       G       E       A       N       P       R       S       V       P       R       I       K       K       V       V       F       R       I       L       T         CA(3)       G-C       G>T       G       G       G>T       G       G       TTTTGGCTCTCTATTATTCGTTGGGC       TTTTAGTTCCATACAATGACCCTAAACTAACAAATCTACTACTACTACGTTTCTACTTCT         F       Y       I       G       S       L       L       V       P       Y       N       D       P       K       L       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S       T       S       Y       S <td></td>	
901 301 991 331	GAACTAGTTGGTATCACTGCTGGTGAAGCCGCAAGAAAACCCGTTCCAAGAAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC         E L V G I T A G E A A N P R K S V P R A I K K V V F R I L T         CA(3) G>C         GAT         TTCTACATTGGCTCCTATTTTCCGTAGCCCTAAACTAACAACTAACCAAATCTACTTCCTACGTTTCTACTTCT         F Y I G S L L F I G L L V P Y N D P K L T Q S T S Y V S T S	
901 301 991 331	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
901 301 991 331 1081 361	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
901 301 991 331 1081 361	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAAACCCCAGAAAAACCGTTCCAAGAGCCATCAAAAAGTTGTTTTCCGTATCTTAACC         E       L       V       G       I       T       A       G       P       R       S       V       P       R       I       K       K       V       V       F       R       I       L       T         CA(3)       G>C       G>T       G       G       G       T       T       I       L       T         CA(3)       G>C       G>T       G       I       I       V       P       R       I       K       K       V       V       F       R       I       L       T         CA(3)       G>C       G>T       G       I       L       V       P       N       D       P       K       I       V       V       S       T       S	
901 301 991 331 1081 361	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
901 301 991 331 1081 361 1171	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
901 301 991 331 1081 361 1171 391	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGCCATCAAAAAAGTTGTTTTCCGTATCTTAACCC         E       L       V       G       I       T       A       G       E       A       A       N       P       R       K       S       V       P       R       A       I       K       K       V       V       F       R       I       L       T         CA(3) 6-C       6>T         TTTCTACATTGGCTCTCTATTATTCATTGGAC         F       I       G       C       L       L       V       P       R       A       I       K       K       V       V       F       R       I       L       T         CA(3) 6-C         F       I       G       L       L       V       P       Y       N       D       P       K       L       T       S       Y       V       S       T       S       Y       V       S       T       S       S       T       S       S       T       S       S       T       S       S       T       S       S       T       S       S       T       S       S       S	
901 301 991 331 1081 361 1171 391	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	
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901 301 991 331 1081 361 1171 391 1261 421 1351	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAACCCCAGAAAATCCGTTCCAAGAGGCCATCAAAAAAGTTGTTTTCCGTATCTTAACC         E       L       V       G       I       T       A       G       E       A       A       N       P       R       S       V       P       R       A       I       K       K       V       V       F       R       I       L       T         CA(3)       G>C       G       S       L       L       F       I       G       S       I       L       T	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481	GAACTAGTTGGTATCACTGCTGGTGGAAGCTGCAAAACCCCCAGAAAAACCCGTTCCAAGAGGCCATCAAAAAAGTTGTTTTCCGTATCTTAACCC       F       N       N       P       R       K       S       V       V       F       R       I       L       T         CA(3)       6>C       6>T       6>T       T       K       K       V       V       F       R       I       L       T         F       Y       I       G       S       L       L       F       I       G       L       V       P       N       D       P       K       L       T       V       V       V       S       T       S       Y       V       S       T       S       Y       V       S       T       S       Y       V       S       T       S       Y       V       S       T       S       S       Y       V       S       T       S       S       S       T       S       V       V       P       Y       N       D       P       K       L       T       T       I       I       S       A       A       A       A       A       A       A       A       A       A       A       A       A<	A
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901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
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901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511	CALCTAGTTGGTATCALCTGCTGGTGTGAGCCGCCAAAACCCCAAAGAGCCATCAAAAAACTTGTTTTCCGTTTCTTAACCC         E       L       V       G       I       T       A       G       E       A       N       P       R       S       V       P       R       I       K       K       V       V       F       R       I       L       T         CA(3)       G       G       G       G       I       I       N       P       R       S       V       P       R       I       K       K       V       P       R       I       L       T       T       I       I       T       I       I       I       T       I       I       I       T       I <td>A</td>	A
901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	А
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901 301 991 331 1081 361 1171 391 1261 421 1351 451 1441 481 1531 511 1621 541	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	A

**Fig. S4. Mutation spectrum of the** *CAN1* **gene for indicated yeast strains.** CanR isolates were randomly picked and sequenced for the *CAN1* gene. Base substitution, deletion, insertion and duplication events are marked. Arrows mark the positions where large deletion events occurred. The exact positions and lengths of deleted or duplicated sequences are indicated. The deleted sequences are marked in red, while the duplicated sequences are marked in blue and underlined. The numbers in brackets represent the frequency of observation of the same event.

Strain	Mutation type	Sequence	GC %	Position	Size (bp)	Micro- homology (bp)
		ACATATTGGTATGATTGCCCTTGGTGGTACTATTG	40.6	281-307	27	5
		TTCTTGGGCAATCACTTTTTTGGGTAATTAT	43.0	533-628	96	5
		GCAGTTTTCGTTACTCAAAGTTTTCGAATGGCT	45.7	1279-1341	63	7
		CCT <mark>GTTACATCCTCTTCAATGGTTACAT</mark> GTATTGG	43.7	445-508	64	7
		CCT <mark>GTTACATCCTCTTCCAATGGTTACAT</mark> GTATTGG	43.7	445-508	64	7
		GTGCTGGGGTTACCGGGTGCCTGGGGTCCAGGTA	58.9	760-808	49	7
		GCTTTGGCTTACATGGCCCGGCTTGGCTTATTATGCG	38.0	1314-1500	187	8
		TCTACTGGTGGTGACAATATCACTGGTGTTGCAGG	39.1	1331-1369	39	7
		TCTACTGGTGGTGACAATATCACTGGTGTTGCAGG	39.1	1331-1369	39	7
		TGCAGTTTTCGTTACTGACAAAGTTTTCGAATGGCT	45.1	1286-1348	63	8
	Deletion	ATC <mark>GGGTTTC</mark> TAATATTAGGTT <mark>GG</mark> GTTTCCTCTTTGA	50.0	720-858	139	7
	(22/89)		0.0	1629-1632	4	2
		TGT <mark>GGTGC</mark> TGGGGAACCCA <mark>GGTGC</mark> CTGGGG	58.5	752-799	48	5
rtt105		TGT <mark>GGTGC</mark> TGGGGAACCCA <mark>GGTGC</mark> CTGGGG	58.5	752-799	48	5
11105		GGCTACATTCATCAATGGTTACATGTATTGGT	42.9	435-513	79	5
		TTACATGTATTGGCTGGCGGCATGGATTAGT	44.4	516-610	95	4
		TTACATGTATTGGCTGGCGGCATGGATTAGT	44.4	516-610	95	4
		GT <mark>TCC</mark> CGTATTTGGCTCCTAAATTC	39.0	1194-1232	39	3
		GTTATTTATCTCAAATCAGATTTATGCAAGCT	30.0	1395-1418	24	6
		TGC <mark>AGATTTATTTGGA<mark>AGATT</mark>GGAGA</mark>	22.2	1656-1668	13	5
		GGT <mark>TCC</mark> CGTATTGTTGGC <mark>TCC</mark> TAAATTC	39.0	1195-1232	38	3
		GGCAATCACTTTTGCCCTTTTTGGGTAAT	40.4	537-632	96	3
		GCAT <mark>AGG</mark> TGATGAAGATGAAGGAGAAGT	44.4	213-229	17	5
		CAGGCTTTTTTGCATGCAAGCTTTGAAATAC	34.5	1380-1429	49	7
	Duplication	GTGCTGGGGTTACCGGCCCCCCAGGTGCCTGGGGTCCAG	58.9	761-809	49	7
	(6/89)	GTGTTAG <mark>CT</mark> TTGCTGCCCTGTTC <mark>TTAG</mark> CTGTTTGGAT	43.2	1578-1615	38	6
		ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGTC	40.0	1550-1577	28	8
		ACGGCTTTTGCACCAAAATTCAATGGTGTTAGCTTTGCTGCCG	37.5	280-307	27	5
		TGGTTCCCGTATTTTCTCCTAAATTCCTGTCAAGG	36.0	1195-1232	38	4
		GTG <mark>AAGAGAGAGCTTAAGCAAAGA</mark> CATATTGGTATG	38.1	257-273	17	4
		GTT <mark>ATTTAT</mark> CTCAATCTCGCACATCAGATTTATGCAAGC	30.0	1395-1418	24	6
EL2A	Deletion	TGCAGTTTTCGTTACTGCGACAAAGTTTTCGAATGGCT	44.1	1279-1341	63	8
	(8/101)	ACATATCTTCAACGCTGTTATCTTAACAACCATTATTTCTGC	33.3	1134-1148	15	6
		CAG <mark>ATTTATGCAAGCTTGGCCAC</mark> ATTTATGACGATCATT	41.0	1419-1511	93	7
		GGA <mark>GACATCTACTGGTGGTGACA</mark> AAGTTTTCGAATGGCT	50.0	1324-1339	16	4
		TGGTTCCCGTATTTTTGGCTCCTAAATTCCTGTCAAGGA	36.0	1195-1232	38	4
		GTG <mark>AAGAGAGAGCTTAAGCAAAGA</mark> CATATTGGTATGATTG	38.1	257-273	17	4
	Duplication	ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGTC	40.0	281-307	28	7
	(5/101)	CATTGCAGTTTTCGTTACTGCTGCATTTGGCGCTTTGGC	45.5	1279-1296	18	4
		ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGTC	40.0	281-307	28	8
		TGTTTTGTATGGTTTGTGGTGCTGGG	0.0	738-739	2	2

Strain	Mutation type	Sequence	GC%	Position	Size (bp)	Micro- homology (bp)	
		TGG <mark>TTCC</mark> CGTATTTTATGGCTCCTAAATTCCTGTCAAGGA	36.0	1195-1232	38	4	
		GCA <mark>ATCAC</mark> TTTTGCCCTTTGGGTAATTATCACAATAATGAA	40.0	537-632	96	4	
		GGTGCGGCCAATGGTTAGTTCCACTGGCGGCATGGATTAG	43.0	504-608	105	6	
	Deletion	ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGTC	40.0	281-307	28	8	
	(9/95)	GGAC <mark>GTACAAAGTTCAAAACGAAGG</mark> GAGGTTCTTAGGT	38.0	588-1021	434	6	
		CAG <mark>GCTTTTTTGCATGGATTTATGCAAG</mark> CTTTGAAATACCGT	34.5	1380-1429	50	7	
V106A		AGGTGGTGTTCCATACATTAAATATCACTGGTGTTGCAGGCTT	43.0	1270-1377	108	4	
		ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGTCTT	40.0	281-307	28	4	
		TGATGAAGATGAAGGAACGCTGAAGTGAAGAGAGAGAGCTTA	42.1	224-255	32	4	
	Duplication	TTACATGTATTGGTTTTCTGGCGG <mark>CAT</mark> GGATTAGTATTTTTTG	44.4	516-610	95	7	
	(4/95)	GTGAAGAGAGAGCTTAAGCAAAGACATATTGGTATGATTGCCCT	38.1	257-273	17	4	
		TTATGGGTTCTTTGGCATATTCTGTCACGCAGTCCT <mark>TGGGT</mark> GAAA	50.0	389-422	34	8	
		TTACGTTGGTTCCCGTATTTTATTTGGTCTATCAAAGAACAAGTTG GCTCCTAAATTCCTGTCA	34.2	1195-1232	38	3	
		ATGGGTTCTTTGGCATATTGGGAGGTTCTTAGGTTGGG	42.9	395-851	457	7	
		AGATGAAGGAGAAGTACAGAACGCTGAAG <mark>TGAAG</mark> AGAGAG	46.2	230-255	26	5	
		ATTCATCCCTGTTACATCC	45.5	443-453	11	5	
		GGA <mark>GACATCTACTGGTGGTGACA</mark> AAGTTTTCGAATGGCTATTAA	50.0	1324-1339	16	4	
		AAA <mark>GGTGGTGTTCCATACATCTACT<mark>GGTGGTG</mark>ACAAAGTT</mark>	59.7	1265-1336	72	7	
		ACATATTGGTATGATTGCCCTTGGTGGTACTATTGGTACAGGT	44.4	284-310	27	8	
20/22	Deletion (16/72)	TT <mark>CATCCCTGTTACATCC</mark> TCTTTCACAGTGTTCTCACA	45.5	443-453	11	5	
<i>p</i> 0/32		TTCCTACGTTTCTACCTTCTCCCCTTTATTATTGCTA	37.5	1069-1076	8	4	
		GTTCCCTGTCAAATATTACGGTGAATTC	0	662-663	2	1	
		TACTATTGGTACAGGTCTTTTCATTGGTTTATCCA	38.9	308-325	18	5	
		TTG <mark>GCTTATTATGCCCTGTTCTTAGCT</mark> GTTTGGATCT	39.2	1496-1615	120	3	
		ACATATTG <mark>GTA</mark> TGATTGCCCTTGGTGGTACTATTGGTACAGGTCTT	44.4	281-307	27	8	
		ATCTTATTTCAATGCATATTCA <mark>GATGCAGATTTATTTGGA</mark> AGATTGG AGATGTCGACAT	33.3	1648-1662	15	2	
		GGA <mark>GACATCTACTGGTGGTGACA</mark> AAGTTTTCGAATGGCT	50.0	1324-1339	16	4	
		TGGTGGTACTAT TGGTACAGGTCTTTTCATTGGTTTA	33.3	303-311	9	6	
		GGGTTCTTTGGCATATTCTGTCACGCAGTCCTTGATTCATCC TGTTACATCCTCTTTCACAGTGTTCTCAC	35.9	411-449	39	4	
			TAAGCAAAGACATATGGGTTCTTTG <mark>GCA</mark> TATTCTGTCACG	43.8	270-399	130	3
		TGCAGTTTTCGTTACTGCTGACAAAGTTTTCGAATGGCTA	46.0	1286-1348	63	8	
		AAAGGTGGTGTTCCATACATCTACTGGTGGTGACAAAGT	59.7	1265-1336	72	7	
		CAGGCTTTTTGCATGGTTTTATGCAAGCTTTGAAATACC	34.0	1384-1433	50	5	
pol32	Deletion	GGTTCCCGTATTTTAGTTGGCTCCTAAATTCCTGTCAAGG	34.2	1195-1232	38	4	
rtt105	(35/74)	ATTGCGCTCTTTCCCGACGACCAGTGGGCGCTCTTATATCAT	48.1	164-369	206	8	
		AAA <mark>GGTGGTG</mark> TTCCATACGACATCTACT <mark>G</mark> GTGGTGACAAAG	45.8	1259-1330	72	7	
		AAAGGTGGTGTTCCATACGACATCTACTGGTGGTGACAAAG	45.8	1259-1330	72	7	
		AAA <mark>GGTGGTG</mark> TTCCATACGACATCTACT <mark>GGTGGTG</mark> ACAAAG	45.8	1259-1330	72	7	
		AAA <mark>GGTGGTG</mark> TTCCATACGACATCTACT <mark>G</mark> GTGGTGACAAAG	45.8	1259-1330	72	7	
		GGCCCAGTTGGATTCCGTGCCTGGGGTCCAGGTATAATATCT	54.8	773-814	42	4	

poletion         TetAtGetTrateCoefGetTAACCEGCCASTGetTeg         46.1         761-925         165         3           AAGTITIAGCCATTACGEGETTCCAAACCCAGAAAA         44.3         720-739         70         2           TCTATEGEGETGCAACCCAGGGGTC         44.3         720-739         70         2           TCTATEGEGEGTGCAAACCCAGGTGCCTGGGGGGTC         39.1         1331-1369         39         7           GEGTAATTCATCCTGTTACCAGTGTTCCAACAGTGTTCCAACAA         40.0         437-461         15         5           GEGTAATTCATCCTGTTACAGTCTCTTTCCAACAGTGTTCCAACAA         400.0         437-461         15         5           TTTGGGCTGTAAATTCCGTGTAACGCCTTTTCCCACAA         40.0         437-461         15         5           GGTGCCGTATTCTA_TATTCGGGTGTAAGGCAACCAACAA         40.0         437-461         15         5           GGTGCCGTATTCTA_TATTCGGGTGTAAGTCGGGAATCACTTTGCCACAA         35.1         1196-1232         37         3           ACCTGTGGTAAGAGGTTTCCAAGTGTGTAAGTCGGTGTAAGTCCCACAA         35.1         1196-1232         38         3           GGTTCCCGTATTTATAAGTTGGCTGCTAAGTCCGTCAAGT         38.1         1128-1170         42         2           CCTGTGTGAACAAGGTTTGCAAGTGGTGCTAAGTCCGTCAAGT         38.1         1196-1232         38         3         3           gG	Strain	Mutation type	Sequence	GC%	Position	Size (bp)	Micro- homology (bp)
AACTITTAGCCATTATCOGGCITTCAATAACTGTTGATCCGTT 44.3 720-789 70 2 ATTGGAGAAAACCCACTATCGGGGTC CAAGGGGCC 39.1 1331-1369 39 7 CICACTGGTGGTGCACAAATTAATAACCCTGGTGTTGCAGGCC 39.1 1331-1369 39 7 GCACTGGTGCGACATAATTAATACCCTGGTGTTGCAGGCC ATATGGTACTGCACTGTTCCCCTTTACACGCGTGTTGCCAGCAC 40.0 437-451 15 5 ACTGGCGTAATTCGTTATGGTTTGCTGGCGAATCACTTTGC 44.4 400-534 135 4 CCACTGGCGATATTCGTTATGGTTGTCCGGGAATCACTTTGC 44.4 400-534 135 4 CCACTGGCGATATTCGTTATGGTTGTCCGGGAATCACTTTGC 44.4 400-534 135 4 CCACTGGCGATATTCAATTTAGGTGGTGCCGCGATTGTTGCC 44.4 400-534 135 4 CCACTGGCGGAATTCCAATTTAGGTGGTGCCGCGATTGTTGCC 44.4 400-534 135 4 CCACTGGCGGAATTCCAATTTAGGTGGTGCCGCGATTGTTGCC 44.4 400-534 135 4 CCACTGGCGGAATTCCAATTTAGGTGGTGCCGCGATTGTTGCC 44.4 400-534 135 4 CCCGGTGCCGTAATTTCGCTGTAATGCGTGGTGCCGCGAATCACTTGC ACT TGGGTGCCGTAATTCAGGTGGTGCCGCAATCACTTAGC 44.2 196-1232 38 3 GGTGCCCGTAATTCAATCCGTGGTGCCGCAATCACTTCCGGCAA GGTGCCCGTATTTATACAGGCTCTTCAATGCTGCTAAG 34.2 1195-1232 38 3 GGTGCCCGTATTTATAGTGGCGCTAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTATAGTGGCTGCTAAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTGCCCGTATTTATAGTGGCGCTAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTATAGTGGCGCTAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTATAGGTGGCGTAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTATAGGTGGCCAATGGTAATTGCGTGCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTATAGGTGGCCAATGGTAATTGCGTGCAAG 34.2 1195-1232 38 3 GGTGCCGTATTTGCGCCGTCCAATGGTAATTGCGTGCAA 45.3 521-615 95 3 CCCGGTGTGCGTATTTGCGCCGTCCAATGGGCAATTGCGTAA 45.3 521-615 95 3 CTCACAGAGGTTGCCAATGGCCAATGGCTAATTCCAGGGGGGGG			TGTATGGTTTGTGGGGCCCGGCTACCGGCCCAGTTGGTTGGT ATCACTGCTGGTGAAGCTGCAAACCCCAGAAA	46.1	761-925	165	3
polection         TCTACTGCTGCTGACAAATTAATTACACTGGTGTTGCAGGCT         39.1         1331-1369         39         7           GCTACATTCATCCCTGTTACATCCCTGTTACATCGGTGTTGCAGGAA         40.0         437-451         15         5           GCTACATTCGTTATGGTTTGTGGGGAATCACTTTGC         44.4         400-534         135         4           TTTGGGATATTCGTTATGGTTTGTGGGGAATCACTTTGC         44.4         400-534         135         4           CAATTCAANTTTACGTGGTGGTAAATCCACTTGGC         44.4         400-534         135         4           CAATTCAANTTTACGTGGTGGTAAATCCACTTGGCACCACAAA         35.1         1196-1232         37         3           ACTCTGGTACAAAGGTTTGCCTGTCAAAGGACCACCACAAA         35.1         1128-1170         42         2           CATCTGGTACAAAGGTTTGCCTGTCAAAGTACGTTTACCACAC         42.2         281-325         45         6           GGTTGCCGTAATTTATAAGTTGGCTGCTAAATTCCAATCCAGGAG         34.2         1195-1232         38         3           GGTTGCCGTATTTATAAGTTGGCTGCTAAATTCCATGCAGG         34.2         1195-1232         38         3           GGTTGCCGTATTTATAAGTTGGCTGCTAAATTCCGTGCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTATAAGTTGGCTGCTAAATTCCGTGCAAG         34.2         1195-1232         38         3			AAGTTTTAGCCATTATCGGGTTTCTAATATACTGTTGATTCCGTT ATTGGAGAAACCCAGGTGCCTGGGGTC	44.3	720-789	70	2
Point         GGCTACATTCATCCCTCTTACATCCTCTTCACAGTGTTCTCACAA AGATTCC         40.0         437-451         15         5           AGATTCC         AGATTCC         ATTGGCATATCTGTTATTGGTTTCTCGGCAATCACTTTGC         44.4         400-534         135         4           TTTGGCATATCTGTTATTGGTTTCTCGGCAATCACTTTGC         44.4         400-534         135         4           CAATTCAAATATTACGTTGGTCGCAAAGGTCCCGCAATCAAT			TCT <mark>ACTGGTGGTGACAAATTAAATATCACTGGTG</mark> TTGCAGGCT TTTTTG	39.1	1331-1369	39	7
Poist         TITGEGATATICTGTTATIGGTITICTGEGGATCACTITIGC         44.4         400-534         135         4           CAATTCAATATICTGTTATIGGTITICTGEGGATCACTITIGC         44.4         400-534         135         4           CAATTCAATATITAGGTIGGTCCGGATCACATTITGC         44.4         400-534         135         4           CAATTCAATATITAGGTIGGTCCGGAATCCACTITIGC         44.4         400-534         135         4           CAATTCAATATITAGGTIGGTCCGGAATCCACATACAC         35.1         1195-1232         37         3           ACTCTGGTACAAAGGTTTITATCAGGTCCTTAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTITAT			GGCTACATTCATCCCTGTTACAT AGATTCC	40.0	437-451	15	5
poli2         TITGECATATICTGTTATGGTTICTGGECATCACTITIGC         44.4         400-534         135         4           CAANTCAANATATICCTGGTECCGATITIGTGGECCAACACTITIGGT         35.1         1196-1232         37         3           ACTCTGGTACAACAGTITIGCCCGATATCTCAACGCTTATT         38.1         1128-1170         42         2           CANTTCAATGTTACAATGTTTACCCGTGATTCCCCG         38.1         1128-1170         42         2           CATATGCAATGATTACAGGTCTTTCATGGTTATCCCCG         42.2         281-325         45         6           GGTTGCCGTATTTATAAGTGGCTCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTTATAAGTGGCTCGTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTTAT			TTT <mark>GGCATATTCTGTTATTGGTTTTCTTGGGCA</mark> ATCACTTTTGC	44.4	400-534	135	4
Poiss         CAMATICAAATATTIACGTIGGTIECCOTATTIATIGGTCATCAA AGAACAAGGTIGGCCOCAAAGGTITTCCAAGGACCACCAAA AGAACAAGGTIGGCCCCAAAGGTITTCCAAGGACCACCCAAA CICCIGGTCAAAGGTITTCCACGCCCCCTATTI CICCIGGCCAAATICAAATATTIACGTIGGTIATCCACC GGTICCCGTAATTCAAATATTIACGTIGGTIATCCACC GGTICCCGTATTITATACAGGTCTITTCATTGGTIATCCACC GGTICCCGTATTITATAACTGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAACTGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAACTGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATAAGTIGGCTCCTAAATICCTGTCAAG GGTICCCGTATTITATGGGGCCAATGGTIACGAATGGCTAA GGTICCCGTATTITATGGGGCCAATGGTICCACGGGGCATG GGTICCCGTATTITATGGGGCCAATGGTICCACTGGCGGCATG GGTICCCGTATTITATGGGGCCAATGGTICCACTGGCGGCATG GGTICCCGTATTITATGGGGCCAATGGTICCACTGGCGGCATG GGTICCCGTATTITATGGGGCCAATGGTICCACTGGCGGCATG GATTAGTAAT TTACAAATATCAA. CCTGTACAAAGATCTCCTCCTACGGTICCACTGGCGGCATG GATTAGTAAT TATCGAGATTCCTCCTACGGTICTATTCCAATGCAATTTCAGAA GGTACCACTGGCCCCTGAACTCCCTCCACGGGGCATG GATTAGTAAT TATCGGGCTTCCAATGTACTCCACCGGCGGCATG GATTAGTAAT TATCGGGCTTCCAATGCCCCCCGCTCCCACGAAGGTICCACCAA GTACAAAGTTCCACTGCCCCCGCTCCCACGAAGGTCCACCAA GTACAAAGTTCCACTGCCCCCGCTCCCATCAAAGTTCCCCTCCACGAA GCCGCGCGTGAAGCTCCCCCTTATTCCAATGCCAATGCCCCCAGACGGGGGCCAA GCCGCGCGTGAAGCTTCCCCCTTATTCCAATGGCAATTCCCCGCGCGGGCCAA GCCGCGCGTGAAGCTTCCCCCTTATTGGCAATGGCTATT GCCAGCTTGACCAAA. CCCGTCTGACCAAA GCCGCGCGTGAAGCTTCCCCCTTATTGGCAATGGCTATT GCCAGCTTGACCAAA GCCGCGCGTGAAGCTCCCCCTTATTGGCTATTGGGCGCGGTCAA GCCGCGCGTGAAGCTCCCCCTTATTGGCTATTGGCAATGGCTATT GCCAGCTTGACCAAA GCCGCGCGCGCGCGCGCGCGCGCAAGGTCCCCGGGGCCAA GCCCGCGCGGCGTGACCAAAGGTT GCCAGCTTGACCCCCCCCCC			TTTGGCATATTCTGTTATTGGTTTTCTTGGGCAATCACTTTTGC	44.4	400-534	135	4
ACTCTGGTACAAAGGTTTTGCCACAATATCTTCAACGCTTATTT CTGCCGUAATTCGATTCAATTCAATTCAGTTGGTTCACGG CATATTGGTATGATACGGGTCTTTTCATTGGTTATCCACCC 42.2 281-325 45 6 GGTTGCCCGTATTTATACGGGCTCTTTATCGTCAAG 34.2 1195-1232 38 3 GGTTGCCGTATTTATAAGTGGCTGCTAAATTCCTGTCAAG 34.2 1195-1232 38 3 GGTTGCCGTATTTATAGTGGCGCCAAGGTTTGGAAG 34.2 1195-1232 38 3 GGTTGCCGTATTTATAGTGGCGCCAAGGTTTGGAAG 46.0 1279-1341 63 8 CCCGGTTGCGGTTTCTGGGGGCCAAGGTTAGGATTATGGG 45.3 521-615 95 3 GTTGCAGAAGATTCCTGGGGGCAAGGTTAGGATTATGGG 45.3 521-615 95 3 CTCAGAAGAATCTCTGGGGGCCAAGGATTAGGATTATTGGG 42.9 477-595 119 6 GTTGCGAAGTATCCTGGGCGCAAGGATTAGGATATTCGAG 42.9 477-595 119 6 GTTGCGAAGTATCCATCGGCCCAAGGATTGGAAGTTTCGAGA 42.9 477-595 119 6 GTTGCGAGTTGCAATAT 41CGGGGTTTCTAATTGCCCCCCGCGCATG 42.9 477-595 119 6 GTTGCGAGTTGCAATTCC 42.2 277-321 45 6 CCCGGAGCTTGAACGCCCCCAGGTTGCAAGGAGGGGGGAA 45.4 79-106 28 5 CCCGGAGCTTGAACTGCCCCGGTCCCAAGGAGGGGGGGAA 45.4 79-106 28 5 CCCGGAGCTTGAACTGCCC			CAAATTCAAATATTTACGTTGGTTCCCGTATTTTATTTGGTCTATCAA AGAACAAGTTGGCTCC	35.1	1196-1232	37	3
polaz         CATATIGGTATGATACAGGTCTITICATIGGTITATCCACAC         42.2         281-325         45         6           Deletion (3574)         GGTTGCCGTATITTATAGTTGGCTGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATITTATAGTTGGCTGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATITTATAGTTGGCTGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATITTATAGGTGGCTGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATITTATAGGTGGCTGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTTATAGGTGGCGCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTTATAGGTGGCAAAGGTTTCGAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTGCCGTATTTTATAGGTGGTGAAATGCTTCAAATTCCTGTCAAG         34.2         1195-1232         38         3           TGCAGTTTTCATTGCGGTGCAAAGGTTTCGAATGGGTA         46.0         1279-1341         63         8           CTGCAGAAGATTCCTGGCGGCAATGGTTAGTATTGCGGCGGCATG         42.9         477-595         119         6           TACGAAGGTTCACATGCGCGGGTGCACAAGGTTTAGCTCCATGGGGAA			ACTCTGGTACAAAGGTTTTGCCA <mark>CATATCTTCAACGCTTATTT</mark> CTGCCG <mark>CA</mark> AATTCAAATATTTACGTTGGTTCCCG	38.1	1128-1170	42	2
pc/32 rrt105         GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           pc/32 rrt105         GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTCCCGTATTTTATAGGTGGCCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTCCCGTATTTTATAGGTGGCCCTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTCCCGTATTTAT			CAT <mark>ATTGGT</mark> ATGATTACAGGTCTTTTC <mark>ATTGGT</mark> TTATCCACAC CTCTGACCAAC	42.2	281-325	45	6
Deletion (3574)GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG34.21195-1232383gGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG34.21195-1232383GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG34.21195-1232383GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG34.21195-1232383TCCAGTTTCGTTACTGGTGGTGACAAAGTTTCGGAAAGGCTA46.01276-1341638TTAAATATCACCCTGTTACATCCTCTTTTGCGGCCAATGGTTACATGATTGG45.3521-615953CCTGACAAAGATTCCTGACGTACAAAGTTCCACTGGCGGCATG42.9477-5951196ACTAACAATATCACTACTTCCTACCAATGGTTACAGAAGTTTCAGAA39.61075-1620546-CTCACAAAGATCCACTGGCCTCGCTTCCATCAAAGTTTAGCAATATCAGA39.8595-7021087CTACAATGGCTTCTATTATTCAATAT39.8595-7021087CTACAATGGCTTCTATTATTCAATGGAATTATCGGAACATCTTT44.4400-5341354CTGTTGGAATCTTATTCAATGCACTTAGGCAACACACACA			GGT <mark>TCC</mark> CGTATTTTATAAGTTGGC <mark>TCC</mark> TAAATTCCTGTCAAG	34.2	1195-1232	38	3
pol32 rtt105         (307.4)         GGTTECCGTATITTATAAGTTGGCTECTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTECCGTATITTATAAGTTGGCTECTAAATTCCTGTCAAG         34.2         1195-1232         38         3           GGTTECCGTATITTATAAGTTGGCTECTAAATTCCTGTCAAG         34.2         1195-1232         38         3           TGCAGTTTEGTACTGGTGGTGCCAAAGTTTEGGATGGCTA         46.0         1279-1341         63         8           CCTGTTACATCCTCTTTTGCGGCCAATGGTTACATGTATTGG         45.3         521-615         95         3           TGTATGGATTTGCTGGCGTCACAAGGTTCCACTGGGGGCAA         45.3         521-615         95         3           CTCACAAAGATTCCTGGCGTCACAAGGTTCCACTGGCGGCATG         42.9         477-595         119         6           ACTAACACAAAGTTCCCTGCGCTTCATCACTGCCCTTTAT         39.6         1075-1620         546         -           TCCTGACTACTACTTGCTACCTGCCCTTGGCTTCCATCAAAGTTTCCACAA         33.3         1002-1019         18         6           GTTGGGATCTATATT         GGTTGCAGCATATCGTGCCAA         33.3         1002-1019         18         6           CTACATGGCTCTATTATTCCAAACCAACCACCACACAAGCATGGGTGGATCAA         45.3         55         5         5           Duplication         CATATTGCTACCT <td< td=""><td></td><td>Deletion</td><td>GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG</td><td>34.2</td><td>1195-1232</td><td>38</td><td>3</td></td<>		Deletion	GGTTCCCGTATTTTATAAGTTGGCTCCTAAATTCCTGTCAAG	34.2	1195-1232	38	3
Influst       GGTTCCCCGTATTITATAAGTTGGCTCCTAAATTCCTGTCAAG       34.2       1195-1232       38       3         TGCAGTTTCCGTTACTGGTGGTGACAAAGTTTCGAAAGGCTA       46.0       1279-1341       63       8         CCTGTTACATCCTCTTTTGCGGCCAATGGTTACATGTATTGG       45.3       445-508       64       7         TGTATTGGTTTCTTGCGGCCAATGGTTACATGTATTGG       45.3       521-615       95       3         CTCACAAAGATTCCTGACGTACAAAGTTCCACTGGCGGCATG       42.9       477-595       119       6         ACTAACACAATAT       GGTACCAATAT       39.6       1075-1620       546       -         TACCACATGGCTCTACCTGCCCCCGCTCCATCAAAGTTTAGGCAATTCAGA       33.3       1002-1019       18       6         GTACAAAGTTCCACTGCCCGCGTCCATCAAAGTTTTAGTCCATACAA       33.3       1002-1019       18       6         CTACACATGGCTCTCATATTGGACTTTATTCAAGGAATTGGA       28.9       1625-1662       38       6         CTGTTGGATCTTATTCCAACACACACACACACACACACAC	po/32	(33/74)	GGT <mark>TCC</mark> CGTATTTTATAAGTTGGC <mark>TCC</mark> TAAATTCCTGTCAAG	34.2	1195-1232	38	3
Image: construction of the con	111105		GGT <mark>TCC</mark> CGTATTTTATAAGTTGGC <mark>TCC</mark> TAAATTCCTGTCAAG	34.2	1195-1232	38	3
Image: constraint of the second sec			TGC <mark>AGTTITCG</mark> TTACTGGTGGTGACAA <mark>a</mark> gttttCGAATGGCTA TTAAATATCAC	46.0	1279-1341	63	8
Image: construct con			CCT <mark>GTTACATCCTCTTTTGCGGCCAATGGTTACAT</mark> GTATTGG	45.3	445-508	64	7
rad59DeletionCATATIGGTATGACTACTACTTCCCCTTTATTTCCATGGCGGCAACG42.9477-5951196rad59DeletionCATATGGTATTCCCTTCCCTCACGTTTCCACCGCGCAACG39.61075-1620546-rad59CACTACACACATCCCCTTATTTCCATGGACTTTTCGTCCCTTATTTCCAACGACAACGTTCCCACCAACGACAACGTTTCGAACACAACTCTGGTAC42.9477-5951196rad59DeletionTACCGTTTCCACTCCCTTTATTTCCATGCAACAACGTTTCGAACAACACCACCGAACGTTGGAACTCTGGTAC45.71279-1341638rad59CACGACGTTCGCAACACCCCCTTTTTATTCGTACGCAACGTTTGGAACGTTTTGGAACTCTGGTAC43.0533-628965rad59DeletionTACGTTTCCACTCCCCTTTATTTGGAACAACGCCGCGAACAACGTATT45.71279-1341638rad59CACGACGTTCGCAACACCCCCTTTTTATTCGCAATGCCAACA43.0533-628965rad59CACGACGTTCGCCATCACCTTTTCATTGCCCTTTATTTCGCAATGCCCAACA43.0533-628965rad59CACGACTTCACCACCACCCCCTTTATTATTGCCAACACACCACCAACACCCTGGTAC43.0533-628965rad59CACGACCACTTCTCCCCTTTATTATTGCCAACACACCACCAACAACGCTTGGAACAACACCCGGTACCAACA43.0533-628965rad59CACGACTTCACCACCACCACCACAACGTTTTCGACAACACCCGGTACCAACA43.0533-628965rad59CACGACTTCACCACCACCACCACCACCACAACGTTTCGCCCTTATTATTCGACAACACACCACCACAACGCTACCACAACACACCACCACAACACCACCACACACA			TGT <mark>ATTGGTTTTC</mark> TTGGCGGCATGG <mark>ATT</mark> AGTATTTTTTGGGTAA TTATCACAATA	45.3	521-615	95	3
ACTAACACAATCTACTTCCTACGTTCTACCTTCTCCCTTTAT			CTC <mark>ACAAAGATTCCTGACGT<u>ACAAAG</u>TTCCACTGGCGGCATG GATTAGTAT</mark>	42.9	477-595	119	6
rad59       Deletion (1/74)       TACGGTTTCTACTTCCCCTTATTATTGCTATCGAAGGTTTTGGGCAATCCACAA AAAGGTT       33.3       595-702       108       7         rad59       Deletion (1/74)       CATACTTGGCACCACCCTTTATTGCACAAAGTTTTCGAAGGAATCGGAACACCTTGGAAGCTTTCACAAAGGTTTCCACAAAAGTTTCGGCAATCACTTT       45.7         rad59       Deletion (1/74)       TACGTTTCGTACCGGGTGACAAAGTTTCGAAGGACTCTGGTAC AAAGGTT       45.7       1279-1341       63       8         rad59       TGCAGTTTCGACGTTCACTTTTLCACTCGGTGACAAAGTTTCGAATGGCTATT       45.7       1279-1341       63       8         rad59       TGCAGTTTCGTACCGTTCCCTTTATTATTGCTATTGGAAGGCTATT       45.7       1279-1341       63       8         TCCTGGGCCAATCACTTTT       45.7       1279-1341       63       8       4         AAAGGTTTCTACTTCCCCTTTATTATTGCTATTGGAAGACTCTGGTAC       43.0       533-628       96       5			ACTAACACAATCTACTTCCTACCTTTCTACTTCTCCCTTTAT	39.6	1075-1620	546	-
rad59Deletion (1/74)TACGTITCGTTACTGCTCCCTTTATTGCGGCAACCTGGGAC CACGTTCGACCAA33.31002-1019186rad59Deletion (1/74)CACGTTTCGTACCGGGTGTTCGTGGGACATCGGAC CACGTTCGACCAA45.71279-1341638rad59Deletion (1/74)TACGTTTCGTACTGGGTGACCAAGGTTTCGAAGGCTATT CACCTCTGACCAA45.71279-1341638rad59TACGTTTCGTACTGGGTGACCAAAGTTTTCGAATGGCTATT (1/48)45.71279-1341638TCCTGGGCAATCACTTTT TCCTACGTTTCTCCCTTTATTATTGCTATTGGTATTGCAA43.0533-628965TCCTGGGCCAACCACCACCACCACCACCACCACCACCACCACCA			GTA <mark>CAAAGTT</mark> CCACTGGCTCGCTTCCAT <mark>CAAAGTT</mark> TTAGCCAT TATCGGGTTTCTAATAT	39.8	595-702	108	7
Integer and the			CTACATTGGCTCTCTATTATTCATTGGACTTTTAGTTCCATACAA	33.3	1002-1019	18	6
rad59       Deletion (1/48)       CACGATTICATTCCCCTTTATTGCGACCACCACAGACGTGGGTACA AAAGGTT       45.7       1625-1662       38       6         rad59       Deletion (1/48)       CACGATTICACTACTCCCCTACAGGTCTTTCCATGGTTATCCA AAAGGTT       42.2       277-321       45       6         rad59       Deletion (1/48)       TACGTTTCCTACTTCTCCCTTTATTATTGCTATTGAGAACTCTGGTAC AAAGGTT       0       1090-1092       3       5         TGCAGTTTTCGTTACTGGGTGACAAAGTTTTCGAATGGCTATT       45.7       1279-1341       63       8         TTCTTGGGCAATCACTTTTTAGTATTTTTGCTATTGCTATTGAGAAACC       43.0       533-628       96       5         TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA       37.5       1069-1076       8       4			TTT <mark>GGCATATTCTGTCACGGGTTTTCTTG<mark>GGCA</mark>ATCACTTTT</mark>	44.4	400-534	135	4
CACGACGTTGAAGCTTCACAAACACACCACCAGACGTGGGTCAA       46.4       79-106       28       5         Duplication (1/74)       CATATTGGTATGATGCCCTACAGGTCTTTTCATTGGTTTATCCA CACCTCTGACCAA       42.2       277-321       45       6         rad59       Deletion (1/48)       TACGTTTCTACTTCCCCTTTATTATTGCTATTGGAAACTCTGGTAC AAAGGTT       0       1090-1092       3       5         rad59       Deletion (1/48)       TACGTTTCTACTTCTCCCCTTTATTATTGCTATTGCGAATGGCTATT       45.7       1279-1341       63       8         TTCTTGGGCAATCACTTTTTAGTATTTTTGGGTAATTATCACA       43.0       533-628       96       5         TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA       37.5       1069-1076       8       4			CTGTTTGGATCTTATTTCAATCAGATTTATTTGGAAGATTGGA	28.9	1625-1662	38	6
Duplication (1/74)         CATATTGGTATGATTGCCCTACAGGTCTTTTCATTGGTTTATCCA CACCTCTGACCAA         42.2         277-321         45         6           rad59         Deletion (1/48)         TACGTTTCTACTTCTCCCTTTATTATTGCTATTGAGAACTCTGGTAC AAAGGTT         0         1090-1092         3         5           rad59         Deletion (1/48)         TACGTTTCTACTTCTCCCTTTATTATTGCTATTGAGAACTCTGGTAC AAAGGTT         0         1090-1092         3         5           TGCAGTTTTCGTTACTGGGTGACAAAGTTTTCGAATGGCTATT         45.7         1279-1341         63         8           TTCTTGGGCAATCACTTTTTAGTATTTTTGGGTAATTATCACA         43.0         533-628         96         5           TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA         37.5         1069-1076         8         4			CACGACGTTGAAGCTTCACAAACACACCACAGACGTGGGTCAA	46.4	79-106	28	5
rad59       Deletion (1/48)       TACGTTTCTACTTCTCCCTTTATTATTGCTATTGAGAACTCTGGTAC AAAGGTT       0       1090-1092       3       5         TGCAGTTTTCGTTACTGGGTGACAAAGTTTTCGAATGGCTATT       45.7       1279-1341       63       8         TTCTTGGGCAATCACTTTTTAGTATTTTTGGGTAATTATCACA       43.0       533-628       96       5         TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA       37.5       1069-1076       8       4		Duplication (1/74)	CAT <mark>ATTGGT</mark> ATGATTGCCCTACAGGTCTTTTC <mark>ATTGGT</mark> TTATCCA CACCTCTGACCAA	42.2	277-321	45	6
TGCAGTTITCGTTACTGGGTGACAAAGTTTTCGAATGGCTATT       45.7       1279-1341       63       8         TTCTTGGGCAATCACTTTTTAGTATTTTTTGGGTAATTATCACA       43.0       533-628       96       5         TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA       37.5       1069-1076       8       4         AAACCTCCTTCTCCCCTTATTATCTCCTCCCTCACAAAAC       45.8       1259 1320       73       7	rad59	Deletion (1/48)	TACGTTTCTACTTCTCCCT <mark>TTATTATT</mark> GCTATTGAGAACTCTGGTAC AAAGGTT	0	1090-1092	3	5
TTCTTGGGCAATCACTTTTTAGTATTTTTGGGTAATTATCACA       43.0       533-628       96       5         TTCCTACGTTTCTACTTCCCCTTTATTATTGCTATTGA       37.5       1069-1076       8       4         AAACCTCCTTTCCCCTTATTACTCCCTCCCCCCACAAAC       45.8       1250-1220       73       7			TGCAGTTTTCGTTACTGGGTGACAAAGTTTTCGAATGGCTATT	45.7	1279-1341	63	8
TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA 37.5 1069-1076 8 4			TTCTTGGGCAATCACTTTTTAGTATTTTTTGGGTAATTATCACA	43.0	533-628	96	5
			TTCCTACGTTTCTACTTCTCCCTTTATTATTGCTATTGA	37.5	1069-1076	8	4
AAA <mark>BBTBBTB</mark> TTCCATACBACATCTACTBBTBGTBACAAAB 45.0 1259-1550 12 1			AAA <mark>GGTGGTG</mark> TTCCATACGACATCTACT <mark>G</mark> GTGGTGACAAAG	45.8	1259-1330	72	7
rad59 Deletion TGCATTTGGCGCTTTGGCTTAATGCAGATTTATTTGGAAGATT 39.3 1302-1662 361 6	rad59	Deletion	TGCATTTGGCGCTTTGGCTTAATGCAGATTTATTTGGAAGATT	39.3	1302-1662	361	6
rtt105 (10/103) ACCACCAAAGGTGGTGTTCCACTACTGGTGGTGACAA 45.8 1259-1330 72 7	rtt105	(10/103)	ACCACCAAA <mark>GGTGGTG</mark> TTCCACTACT <mark>G</mark> GTGGTGACAA	45.8	1259-1330	72	7
GGCCAATGGTTACATGTCAATTTTGGACGTACAAAGTTCCA 40.2 507-593 87 3			GGCCAATGGTTACATGTCAATTTTGGACGTACAAAGTTCCA	40.2	507-593	87	3
GTGGTGCTGGGGTTACCGGCTGCCTGGGGTCCAGGTATAA 58.0 760-809 50 7			GTGGTGCTGGGGTTACCGGCTGCCTGGGGTCCAGGTATAA	58.0	760-809	50	7
CAAAGGTGGTGTTCCATACATTG 33.3 1264-1266 3 2			CAAAGGTGGTGTTCCATACATTG	33.3	1264-1266	3	2
ACATTGCAGTTTTCGTTACTTCACTGGTGTTGCAGGCTTTTTT 42.4 1280-1378 99 6			ACATTGCAGTTTTCGTTACTTCACTGGTGTTGCAGGCTTTTTT	42.4	1280-1378	99	6
Duplication TCTTTGGCATATTCTGTCACGCAGTCCTTGGGTGAAATGGCTA 50 398-421 24 4		Duplication	TCTTTGGCATATTCTGTCACGCAGTCCTTGGGTGAAATGGCTA	50	398-421	24	4

**Fig. S5. Table listing the duplication or deletion events occurred between short repeats.** The deleted or duplicated sequences are marked in red, while the flanking short homologies are marked in gray shadow. The GC content of the DNA sequences between the repeats are indicated. The sizes for the duplication, deletion or micro-homologies are indicated.





Fig. S6. The deletion of *RTT105* does not affect DSB resection or the protein levels of RPA and **Rad51.** A-B, Southern blot analysis and quantification of resection kinetics at indicated locations for the WT and *rtt105* $\Delta$  cells. Samples were collected at indicated time points after DSB induction. C, Western blot analysis of protein levels for RPA or Rad51 in the WT or *rtt105* $\Delta$  cells. D, ChIP analysis of Rad52-3xFLAG recruitment in WT or *rtt105* $\Delta$  cells. Error bar represents standard deviation from three independent experiments. \*\* *p* <0.01 (*t*-test).

WT



**Fig. S7. Fusion of the NLS to the N-terminal of Rfa1 restores RPA nuclear localization in** *rtt105Δ* cells. The plot shows the percentage of cells with normal RPA nuclear localization in indicated strains.

Fig. S8



**Fig. S8. Rtt105 interacts with Rfa1 and stimulates dynamic ScRPA assembly on ssDNA. A.** GST pull-down assay showing the interaction between GST-Rfa1 and 6xHis-Rtt105 or 6xHis-rtt05-EL2A. The position of residues E171 and L172 that are required to mediate the Rtt105-RPA interaction is indicated. B. Immunoprecipitation showing the interaction between Rfa1-3xHA and WT or mutant Rtt105-3xFLAG proteins in indicated strains. C. EMSA showing the effect of Rtt105 on ScRPA binding on ssDNA. 20nM ssDNA (30nt) and 50 nM of 6xHis-Rtt105 were used for the experiment. D. Quantitation of the RPA-bound ssDNA in C. E. Monitoring the kinetics of RPA assembly on ssDNA in real-time by single-molecule twister analysis. F. Single-molecule twister analysis showing that neither the WT or mutant Rtt105 protein interacts with naked ssDNA. G. Rtt105 stimulates the assembly of ScRPA with ssDNA. The ScRPA-ssDNA filaments were assembled with ScRPA (50nM) with or without WT or mutant Rtt105 proteins (20nM). H. Plot showing the calculated binding curve (dark lines) overlaid to the curves monitored by MT. I. Plot showing the *K*<sub>on</sub> value of ScRPA binding on ssDNA in the absence or presence of Rtt105 or rtt105-EL2A. Error bar means standard deviation from at least three independent experiments. \* *p* <0.05 \*\* *p* <0.01 (*t*-test).



**Fig. S9. Rtt105 interacts with human RPA and** *E.coli* **SSB and stimulates their assemblies on ssDNA**. A. GST pull-down assay showing the interaction between GST-Rtt105 and 6xHis-hRPA70. B. GST pull-down assay indicating the interaction between 6xHis-Rtt105 and GST-ScRfa1 or GST-SSB. GST-tagged proteins were stained by Coomassie blue. C. Rtt105 (20nM) stimulates the assembly of hRPA (100nM) with ssDNA. D. Rtt105 (1nM) stimulates the assembly of SSB(1nM) with ssDNA.

## Supplementary Table 1. Yeast strains

Strain name	Parental strain	Genotype	Source
JKM139		MATa ho hml::ADE1 hmr::ADE1 ade1-100 leu2-3,112 trp1::hisG lys5 ura3- 52 ade3::GAL::HO	1
yLJB067	JKM139	rtt105::KanMX	This study
yXJ023	JKM139	rtt105-E171A L172A-TRP1	This study
yXJ215	JKM139	rfa1-V106A-TRP1	This study
yXJ208	JKM139	pol32::KanMX rtt105::HPHMX	This study
yXJ209	JKM139	pol32::KanMX	This study
yXJ306	JKM139	rad59::KanMX	This study
yXJ307	JKM139	rad59::KanMX rtt105::HPHMX	This study
yCW007	JKM139	RFA1-3xFLAG-KanMX	This study
yLJB108	JKM139	RFA1-3xFLAG-KanMX rtt105::HPHMX	This study
yCW005	JKM139	RAD51-3xFLAG-KanMX	This study
yXJ053	JKM139	RAD51-3xFLAG-KanMX rtt105::HPHMX	This study
yXJ315	JKM139	Nup49-mCherry-TRP1 RFA1-YFP-HphMX	This study
yXJ319	JKM139	Nup49-mCherry-TRP1 rtt105-E171A L172A-KanMX RFA1-YFP-HphMX	This study
yXJ317	JKM139	Nup49-mCherry-TRP1 rfa1-V106A-YFP-KanMX	This study
yXJ316	JKM139	Nup49-mCherry-TRP1 rtt105::HPHMX RFA1-YFP-KanMX	This study
yXJ318	JKM139	Nup49-mCherry-TRP1 rtt105::HPHMX NLS-rfa1-YFP-KanMX	This study
yXJ337	JKM139	RTT105-KanMX NLS-RFA1-3xFLAG-NATMX	This study
yXJ079	JKM139	rtt105::HPHMX NLS-RFA1-3xFLAG-NATMX	This study
yXJ081	JKM139	rtt105::HPHMX NLS-RFA1-TRP1 RAD51-3xFLAG-KanMX	This study
yLJB101	JKM139	RTT105-3xFLAG-KANMX RFA1-3xHA-TRP1	This study
yXJ020	JKM139	RFA1-3xHA-TRP1 rtt105-E171A L172A-3xFLAG-KanMX	This study
yXJ012	JKM139	rtt105-E171A L172A-3xFLAG-KanMX	This study
yLJB076	JKM139	RTT105-3xFLAG-KANMX	This study
yXJ005	JKM139	RTT105-3xFLAG-KanMX mec1::NATMX tel1::LEU2 sml1::TRP1	This study
yXJ006	JKM139	RTT105-3xFLAG-KanMX sgs1::NATMX exo1::TRP1	This study
yXJ007	JKM139	RTT105-3xFLAG-KanMX mre11::NATMX	This study
yXJ038	JKM139	RFA1-3xFLAG-KanMX RTT105-3xHA-TRP1	This study
yXJ236	JKM139	rfa1-V106A-3xFLAG-KanMX RTT105-3xHA-TRP1	This study
yXJ237	JKM139	rfa1-L105AV106A-3xFLAG-KanMX RTT105-3xHA-TRP1	This study
yXJ090	JKM139	RFA1-3xFLAG-NATMX rtt105-E171A L172A-TRP1	This study
yXJ226	JKM139	rfa1-V106A-3xFLAG-KanMX	This study
yXJ010	JKM139	rtt105::KanMX + pRS316	This study
yXJ011	JKM139	rtt105::KanMX+pRS316-RTT105	This study
yCW129	JKM139	RAD52-13Myc-HPHMX	This study
yXJ096	JKM139	RAD52-13Myc-HPHMX rtt105::KanMX	This study
yZSH176	JKM139	yku70::KanMX	This study
yXJ014	JKM139	rtt105::HPHMX yku70::KanMX	This study
yXJ176	JKM139	yku70::NatMX rtt105-E171A L172A-TRP1	This study
yXJ256	JKM139	yku70::KanMX rfa1-L105A V106A-TRP1	This study
yWY020	JKM139	rtt105::HPHMX yku70::KanMX Rfa1-NLS-TRP1	This study
yXJ320	JKM139	Rfa1-NLS-TRP1	This study
yXJ321	JKM139	Rfa1-NLS-TRP1 rtt105::KanMX	This study
yXJ073	JKM139	NLS-RFA1-TRP1 rtt105::HPH	This study
yXJ338	JKM139	NLS-RFA1-TRP1 RTT105-kanMX	This study

Strain name	Parental strain	Genotype	Source
tGI354		MATa-inc arg5,6::MATa-HPH ade3::GAL::HO hmr::ADE1 hml::ADE1 ura3- 52	2
yLJB079	tGI354	rtt105::KanMX	This study
yXJ027	tGI354	rtt105-E171AL172A-TRP1	This study
yXJ322	tGI354	RFA1-NLS-TRP1	This study
yXJ318	tGI354	RFA1-NLS-TRP1 rtt105::KanMX	This study
yXJ213	tGI354	rfa1-V106A-TRP1	This study
yLJB156	tGI354	NLS-RFA1-TRP1	This study
yXJ339	tGI354	rtt105::KanMX NLS-RFA1-TRP1	This study
AM1003		hml∆::ADE1/hml∆::ADE3 MATa-LEU2-tel/MATα-inc hmr∆::HPH FS2∆::NAT/FS2 leu2/leu2-3112 thr4 ura3-52 ade3::GAL::HO ade1 met13	3
yXJ068	AM1003	rtt105::KanMX	This study
yXJ324	AM1003	rtt105-E171AL172A-KanMX	This study
GC1	BY4742	MATα his3Δ200 ura3Δ0 met15Δ0 trp1Δ63/YAC(MFA1pr-HIS3 URA3 MET15 TRP1)	4
yLJB081	GC1	rtt105::KanMX	This study
yWH378	yMV80	rad51::URA3	5
yXJ106	yMV80	rtt105-E171A L172A-TRP1 rad51::URA3	This study
yXJ234	yMV80	rfa1-V106A-TRP1 rad51::URA3	This study
yLJ162	yMV80	rtt105::KanMX rad51::URA3	This study
YAM033		ho∆ade3::GAL-HO HMLα-inc MATa hmr::ADE1 bar1∆::ADE3 nej1∆::KANMX ade1 leu2,3-112 trp1::hisG ura3-52 thr4 lys5	6
NP477	YAM033	WΔ,MATX XΔ::Cg-TRP1	7
JL13	NP477	rtt105::HPH	This study

# SI Appendix, Materials and Methods

## Yeast strains and plasmids

Strains used in this study are derivatives of JKM139 (*ho* MAT**a** *hml::ADE1 hmr::ADE1 ade1-100 leu2-3,112 trp1::hisG' lys5 ura3-52 ade3::GAL::HO*), tGI354 (*MATa-inc arg5,6::MATa-HPH ade3::GAL::HO hmr::ADE1 hml::ADE1 ura3-52*), AM1003 (*hmlΔ::ADE1/hmlΔ::ADE3 MATa-LEU2-tel/MATα-inc hmrΔ::HPH FS2Δ::NAT/FS2 leu2/leu2-3112 thr4 ura3-52 ade3::GAL::HO ade1 met13*) or yMV80 (*ho hml ::ADE1 mata ::hisG hmr ::ADE1 his4::NatMXleu2-(Xhol- to Asp718) leu2::MATa ade3::GAL::HO ade1lys5 ura3-52 trp1*). All mutant strains were generated with standard genetic manipulation. Point mutants were confirmed by sequencing. Yeast strains used in this study are listed in Supplemental Table 1.

### Fluorescence microscopy

Rfa1-YFP and Nup49-mCherry subcellular localizations in log phase yeast cells were examined using a ZEISS LSM 880 fluorescence confocal microscope carrying an Airyscan with a 63 x oil immersion objective lens and a YFP or RFP filter. Fluorescent images were captured and processed using ZEISS Blue Lite2 software. The percentage of cells with normal RPA nuclear localization were calculated from more than 200 cells.

### Pulsed-field gel electrophoresis (PFGE)

Yeast growing cells (1.2 x 10<sup>7</sup> cells/ml) were treated with 0.03% MMS for 30 mins and then released into fresh YPD media to allow the recovery. Cells were harvested at the indicated time points. Chromosomal DNA plugs were prepared and separated on a 1% agarose gel using the CHEF DRII apparatus (Bio-Rad, parameter settings: initial switch time: 20s, final switch time: 150s, run time: 26-28h, volts/cm: 6V/cm)). Analysis of yeast chromosome integrity by pulsed-field gel electrophoresis was carried out as described by Maringele et al (8).

#### Mutation rate and spectra

The rate of accumulation of CanR mutations was determined as previously described (9). Yeast cells from single fresh colonies were plated on SC arginine- dropout plates containing 60 mg/L canavanine. Mutation rate was determined by fluctuation analysis using the median method. To determine the mutation spectra for each strain, about 100 of fresh single colonies were patched on YPD plates and incubated at  $30^{\circ}$  C overnight followed by multiple replica plating to SC arginine- dropout plates containing 60 mg/L canavanine. This will allow to isolate single mutated colonies. Over 80 of individual colonies were cultured for each strain to extract genomic DNA, which is followed by PCR amplification and sequencing of the *CAN1* gene. The mutation spectra were characterized by analyzing the obtained sequences against the *CAN1* reference sequence.

### Analysis of ectopic recombination, single-strand annealing and alt-EJ

To test the viability of DSB repair by ectopic recombination or SSA, cells were cultured in the pre-induction medium (YEP-Raffinose) overnight to log phase. Cells were then diluted and plated on YEPD or YEP-Gal plates followed by incubating at 30° C for 3 to 5 days. Viability (%)= (the number of colonies grown on YEPD x dilution fold) x 100%. At least three independent experiments were performed for each strain.

The repair kinetics for ectopic recombination were monitored by Southern blot analysis as described (10, 11). The blot was exposed in a Phosphor screen. Signal on the screen was captured by scanning in an OptiQuant Cyclone Plus machine (Perkin Elmer). To measure the repair kinetics for ectopic recombination, we quantified and normalized the pixel intensity of target bands to that of corresponding parental bands on blots. The resulting values were further normalized to that of the control sample (uncut).

### Analysis of 5'-end resection by Southern blot

Yeast cells were grown overnight in YEP raffinose medium (1% yeast extract, 2% peptone, 2% raffinose) to log phase. HO was induced when the cell density was  $\sim 1 \times 10^7$  cells/ml by adding 2% galactose. Samples were collected at 0, 1, 2, 4, 6, 8, 10 and 12 hr after galactose induction. Genomic DNA prepared with a standard phenol extraction method was digested with EcoRI followed by separated on 0.8% agarose gels. The restricted DNA was then transferred onto a Nylon hybridization membrane (GeneScreen). Southern blotting and hybridization with radiolabeled DNA probes was performed as reported(5, 12). Intensities of bands on Southern blots corresponding to probed DNA fragments were analyzed with the OptiQuant software (Perkin Elmer). Quantities of DNA loaded on gels for each time point were normalized using the *TRA1* DNA probe. DSB end resection beyond each EcoRI site for each time point was estimated as a percentage of the signal intensity corresponding to the EcoRI fragment of interest 1 hr after break induction.

### Expression of recombination protein and GST pull-down assay

Protein expression and GST pull-down assay was conducted as described by Li *et al.*(13). 6xHis- or GST-tagged WT or mutated Rtt105 or Rfa1 and 6xHis-hRPA70, 6xHis-hRIP $\alpha$  or GST-tagged hRIP $\alpha$ , hRIP $\beta$  and hRIP $\delta$  recombination proteins were expressed in BL21 (DE3). Protein expression was induced by the addition of 1 mM IPTG at 0.8 OD600. Cells were cultured overnight at 16°C before harvest. After centrifugation at 4000 rpm for 20min, the cell pellets were collected and frozen at -80 °C until use. Cells was then resuspended in lysis buffer (20 mM Tris–HCl, pH 7.4, 50 mM NaCl, 0.5 mM EDTA, 10% glycerol) and lysed by sonication. The lysate was clarified by centrifugation at 12,000 rpm for 30 min at 4°C. For the GST pull-down assay, GST-tagged WT or mutant Rfa1 or Rtt105 was immobilized on 30 µl of bed volume of glutathione agarose beads. After washing with lysis buffer, the resin was then incubated with His-tagged WT or mutant Rtt105 proteins at 4 °C for 4hrs on a rotator. The beads were washed extensively with wash buffer (20 mM Tris–HCl, pH 7.4, 200 mM NaCl, 0.5 mM EDTA, 10% glycerol), and bound proteins were eluted by boiling the samples in 2xSDS loading buffer. The products were detected by Western blot or Coomassie brilliant blue staining of SDS-PAGE gels.

### Protein purification

Purification of yeast RPA complex was performed as described by Binz et al (14). The full-length human RPA composed of three subunits Rfa1, Rfa2, and Rfa3 was expressed and purified according to the protocols described previously(9). For purification of 6xHis-Rtt105 and 6xHis hRIPα protein, cells were lysed by sonication in lysis buffer. Clarified lysate was incubated with Ni-NTA resin (Abclone) for 2 hrs at 4°C on a rotator. The beads were washed extensively with wash buffer containing 20 mM or 50 mM imidazole, followed by elution with wash buffer containing 100 mM imidazole. The eluate for these proteins was collected and dialyzed in 1x PBS overnight. For purification of Rtt105 without any tag, PGEX-6P-1-Rtt105 were transformed into E. coli strain BL21 (DE3). The expression of GST-Rtt105 was induced by adding 1 mM IPTG at 16°C for 16 h in 1 L culture. Cells were lysed and processed as described above. Recombinant GST-Rtt105 was immobilized on 2 mL of bed volume of glutathione agarose beads. After washing with lysis buffer, the resin was then incubated with the prescission protease at 4 °C for 12hrs on a rotator to cut the GST tag. Finally, the flow-through liquid was dialyzed and collected.

### Electrophoretic mobility shift assay (EMSA)

To test the effect of Rtt105 on ScRPA assembly on ssDNA, 50 nM of 5'- biotin labeled ssDNA (30 nt, 5'-CGATAAGCTTGATATCGAATTCCGCAGCC-3') substrate was incubated with various amounts of ScRPA complex for 1hr at 4°C in 1× binding buffer (25 mM Tris-HCl, pH 7.5, 5 mM MgCl2, and 5% glycerol). The reaction mixture (20  $\mu$ l in total) was loaded with 4  $\mu$ l of 6x loading dye. The reaction products were resolved in a 6% native PAGE gel in cold 0.3×TBE buffer. The native PAGE were stained with GelRed. Signals were detected on a G-Box imager (Syngene). To test the effect of hRIPα on hRPA assembly on ssDNA, 20 nM of 5'-Cy5 labeled ssDNA substrate was incubated with various amounts of hRPA complex, and the fluorescent signal on the native PAGE or agarose gel was captured by scanning in a Typhon 9500 scanner. Band intensities were quantified with Image J.

#### Immunoprecipitation (IP)

Yeast cells culture (A600 ~ 1.0) with or without 0.1% MMS treatment (90 min) were collected and lysed on a bead beater in lysis buffer (100 mM HEPES, pH 8.0, 20 mM MgCl<sub>2</sub>, 150 mM NaCl, 10% glycerol, 0.4% Nonidet P-40, 0.1mM EDTA plus protease and phosphatase inhibitors) with benzonase to digest DNA and RNA. The extract was clarified by centrifugation at 12,000 g for 10 min at 4°C, followed by incubating with protein G-agarose beads for 1 hr at 4°C to preclear non-specific binding. After centrifugation, the supernatant was incubated with anti-HA or anti-FLAG antibody at 4°C overnight with agitation. After the addition of protein G-agarose beads, the mixtures were incubated at 4°C for 3 hrs. Subsequently, the beads were washed with lysis buffer for five times (10 min each wash) at 4°C. Immunoprecipitated proteins were eluted by boiling beads in 2xSDS loading buffer for 5 min.

#### Western blotting

Whole-cell extracts were prepared using a trichloroacetic acid (TCA) method as previously described (15). Whole cell extracts, immunoprecipitated protein, or pull down samples were resolved on an 8% or 12% SDS-PAGE gel and transferred onto a PVDF membrane (Immobilon-P; Millipore) using a semi-dry method(Bio-Rad). Anti-HA and anti-FLAG antibodies were purchased from MBL and Sigma, respectively. Anti-mouse and rabbit IgG HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology. Blots were developed using the Western Blotting substrate (Bio-Rad).

#### Streptavidin pull-down assay

The effect of 6xHis-hRIPα on the binding of hRPA on ssDNA was also examined using streptavidin pull-down assay. 5'-biotinylated oligonucleotides (30 nt) (5'- CGATAAGCTTGATAT

CGAATTCCGCAGCC-3') were immobilized on streptavidin MagBeads (GenScript) in TES buffer (10 mM Tris, 1 mM EDTA, 2 M NaCl, pH 7.5) for 30 min at room temperature. After an extensive wash with 1xPBS supplemented with 1 mM EDTA, the biotin-ssDNA-streptavidin beads were incubated with a gradient concentration of purified hRPA complex for 30 min at 4 °C. After wash with the binding buffer (25 mM HEPES, pH 7.5, 15 mM KCl, 150 mM NaCl, 1 mM EDTA, 0.05% TritionX-100, 0.5 DTT, 100 mg/mL BSA), purified 6xHis-hRIPα was added to each sample and incubated for 1 hr at 4 °C. Subsequently, the beads were washed with binding buffer, and the bound protein was eluted and detected by Western blot or Coomassie brilliant blue staining.

#### Single-molecule study

The 12.5 k-nt ssDNA was generated by one-sided PCR, and its two ends were labeled with digoxigenin and biotin groups, respectively. In MT experiments, the digoxigenin-labeled end of a single ssDNA molecule was anchored to the anti-digoxigenin coated glass surface in a flow cell. Then, a superparamagnetic microbead (M-270, Dynal beads) was attached to the biotin-labeled end of the anchored ssDNA molecule. A pair of permanent magnets was used to attract the microbead and thus exert a constant force to the anchored ssDNA molecule. The extension of ssDNA was determined to be the separation between the microbead and glass surface. The assembling buffer contained 100 mM NaAc, 10 mM MgAc<sub>2</sub>, 1 mM ATP and 25 mM Tris-Ac pH 7.5. All experiments were performed at a constant force of 8 pN at 20 °C.

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