Supplementary Information

1. **Supplementary Tables 1,2,3 (Table 1 is an Excel file)**

Supplemental Table 1. Frequency and types of DNA insertions (separate Excel file)

Supplemental Table 2. List of strains used in this study.

Strain name	Parental strain	Genotype	Source
JKM139		DELho *hml*::*ADE1 MAT***a** *hmr*::*ADE1 ade1 leu2-3,112 lys5 trp1*::*hisG ura3-52 ade3*::*GAL10*::*HO*	[1]
yYY363	JKM139	*nuc1::klTRP1*	this study
yYY661	JKM139	*nuc1*-H138A	this study
yYY337	JKM139	*sch9::klTRP1*	this study
yWH475	JKM139	*dna2::kanMX pif1*-m2	[2]
yYY387	JKM139	*pol32::natMX*	this study
yYY466	JKM139	*rad51::kanMX*	this study
yYY399	JKM139	*lig4::klTRP1*	this study
yYY400	JKM139	*pol4::klTRP1*	this study
yYY339	JKM139	*spt3::klTRP1*	this study
PTY44		*MATα ura3-52 lys2 leu2-3,112 trp1-Δ1 [ρ+, TRP1]*	[3]
yYY583	PTY44	*nuc1::kanMX*	this study
yYY660	PTY44	*nuc1*-H138A	this study, constructed using pCORE-UH
yYY664	PTY44	*MAT***a** *ura3-52 lys2 leu2-3,112 trp1-Δ1 [ρ+, TRP1]*	this study, constructed using HO plasmid to switch mating type
yYY671	yYY664	*nuc1::pCORE-UH*	this study
PTY44/ YY664	PTY44	*MAT***a**/Matα *ura3-52/ ura3-52 lys2/lys2 leu2-3,112/ leu2-3,112 trp1-Δ1/ trp1-Δ1 [ρ+, TRP1]*	this study, constructed by mating PTY44 and yYY664
yYY671/ YY583	yYY583	*MAT***a**/Matα *ura3-52/ ura3-52 lys2/lys2 leu2-3,112/ leu2-3,112 trp1-Δ1/ trp1-Δ1 [ρ+, TRP1] nuc1::kanMX/ nuc1::pCORE-UH*	this study, constructed by mating yYY671 and yYY583
DG1657		*MAT***a** *ura3-167 his3Δ-200 trp1-hisG leu2-hisG Ty1-270his3-AI Ty1-588neo Ty1-146[tyb1::lacZ]*	[4]
yJL67	DG1657	*nuc1::klTRP1*	this study

Supplemental Table 3. List of DNA oligos used in this study.

Name	Sequence(5' to 3')	Comments
A. Primers for amplification of the *MAT***a** locus for *Break-In* sequencing		
mata-xxx-F1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGxxxGCATAGTCGGGTTTTTCTTTTAGTTTCAGC	To amplify the *MAT***a** locus for Break-In sequencing. 11/18 bp from HO cut site (Fig. 1b). xxx reprentents home index.
mata-xxx-R1	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGxxxCAACCACTCTACAAAACCAAAACCAGGGT	

mata-xxx-F3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGxxxTGAGATCTAAATAAATTCGTTTTCAATGAT	To amplify the *MAT***a** locus for Break-In sequencing. 48/48 from HO cut site (Extended Data Figure 2b). xxx reprentents home index.
mata-xxx-R3	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGxxxCGTCACCACGTACTTCAGCATAATTATTCG	
B. Primers for amplification of DNA probes used for Southern blot		
TRP1-Fw	GACTGACGCCAGAAAATGTTGG	Extended Data Figure 5
TRP1-Rw	CAAGAATCGGGTCATTGTAGCG	Extended Data Figure 5
Typeak fw	CGCAGTATCCATCATCAGTTG	Fig. 4b
Typeak rv	AAGTGTTATACAAGAAGGTGAGTTC	Fig. 4b
mtDNA F1	CACCACTAATTGAAAACCTGTCTG	Fig. 3e
mtDNA R2	AACCGTACGTGCGACTTTCATC	Fig. 3e
ACT1-fw	TCTTCCCATCTATCGTCGGTAGAC	Fig. 3e, 4b loading control
ACT1 rv	GGTCAATACCGGCAGATTCC	Fig. 3e, 4b loading control
TRA1 fw	GTCCTAATACGACTTTTCAAATTGTCCTTTATGTCCGTCA	Fig. 3e, 4b loading control
TRA1 rv	ATACTTGTAAGCACTCTTCCTGTAGTGAATATCACTTTTG	Fig. 3e, 4b loading control
TOM1 fw	CTTCAAAATTGAAGATCATG	Fig. 3e, 4b loading control
TOM1 rv	CGATTGATTGAGCGATGATG	Fig. 3e, 4b loading control
C. Primers for making point mutants		
Nuc1-pCORE-Fw	CTTATCCACCTACCCAGAAACCTAATAGTAATATTCAATCTCACTCTTTCTTCGTACGCTGCAGGTCGAC	
Nuc1-pCORE-Rw	GCTGTTGGTGCTTCTGCAACAATCAATTTAAAAAAGTGCGTTGGAACAGCCCGCGCGTTGGCCGATTCAT	
Nuc1-F5	ATGTGCAGTAGGATACTCTTGT	
Nuc1-R5	AAGTTCTAGCCCAGTACTTCTC	
D. Primers for amplification of the 84-1000 bp fragments for transformation/insertion analysis (Fig. 3f)		
Lambda-84-Fw	AACACGGTGGGCTCAGAGAATCC	
Lambda-84-Rw	TGTTAGGATGACACTGTACTGACCGT	
Lambda-100-Fw	AACATGGGCACGCTGGAGAC	
Lambda-100-Rw	TGTTCGCGGTATCAGGACGACCAATA	
Lambda-150-Fw	AACATGCTGATTAAGGCAGAGGCTGC	
Lambda-150-Rw	TGTTCGGGCGGCTTCAAGCGCAA	
Lambda-200-Fw	AACAGGCGTTTCCGTTCTTCTTC	
Lambda-200-Rw	TGTTTACGGATACTCGCACCGAA	
Lambda-300-Fw	AACAGGACAAAAATGCGCAGCA	
Lambda-300-Rw	TGTTCGTCAGCAGGGCAGCATGAG	
Lambda-400-Fw2	AACAGTGCTACCCGAACACGGC	
Lambda-400-Rw2	TGTTCGCTGAGCACATCCCACGCC	
Lambda-500-Fw2	AACAACGACGGCACGAACGG	
Lambda-500-Rw2	TGTTGCATTCATTCAGTGTTTCCTGCC	
Lambda-1k-Fw	AACAGGCTTCGCTCACTGTTCAGG	
Lambda-1k-Rw	TGTTCCCTTTCGTTTTCATCCAGTC	
E. Primers for amplification of the 24-84 bp fragments for transformation/insertion analysis (Fig. 3f)		
M13-X-24-Fw	AACACTGGTAAACAAGGGTTAACA	
M13-X-24-Rw	TGTTAACCCTTGTTTACCAGTGTT	
M13-X-34-Fw	AACATTATCGAACACTGGTAAACAAGGGTTAACA	
M13-X-34-Rw	TGTTAACCCTTGTTTACCAGTGTTCGATAATGTT	
M13-X-44-Fw	AACAATTTTGAACATTATCGAACACTGGTAAACAAGGGTTAACA	
M13-X-44-Rw	TGTTAACCCTTGTTTACCAGTGTTCGATAATGTTCAAAATTGTT	
M13-X-54-Fw	AACAGCCTCTAACAATTTTGAACATTATCGAACACTGGTAAACAAGGGTTAACA	
M13-X-54-Rw	TGTTAACCCTTGTTTACCAGTGTTCGATAATGTTCAAAATTGTTAGAGGCTGTT	
M13-X-64-Fw	AACATTTTGCAACAGCCTCTAACAATTTTGAACATTATCGAACACTGGTAAACAAGGGTTAACA	
M13-X-64-Rw	TGTTAACCCTTGTTTACCAGTGTTCGATAATGTTCAAAATTGTTAGAGGCTGTTGCAAAATGTT	
M13-X-74-Fw	AACAGCAAAAAACATTTTGCAACAGCCTCTAACAATTTTGAACATTATCGAACACTGGTAAACAAGGGTTAACA	
M13-X-74-Rw	TGTTAACCCTTGTTTACCAGTGTTCGATAATGTTCAAAATTGTTAGAGGCTGTTGCAAAATGTTTTTTGCTGTT	

1. Moore, J.K., and Haber, J.E. (1996). Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in Saccharomyces cerevisiae. Mol Cell Biol *16*, 2164-2173.
2. Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. (2008). Sgs1 helicase and two nucleases dna2 and exo1 resect DNA double-strand break ends. Cell *134*, 981-994.
3. Thorsness, P.E., and Fox, T.D. (1993). Nuclear mutations in Saccharomyces cerevisiae that affect the escape of DNA from mitochondria to the nucleus. Genetics *134*, 21-28. 10.1093/genetics/134.1.21.
4. Sundararajan, A., Lee, B.S., and Garfinkel, D.J. (2003). The Rad27 (Fen-1) nuclease inhibits Ty1 mobility in Saccharomyces cerevisiae. Genetics *163*, 55-67.