

Figure S1, related to main Figure 1 and main Table1

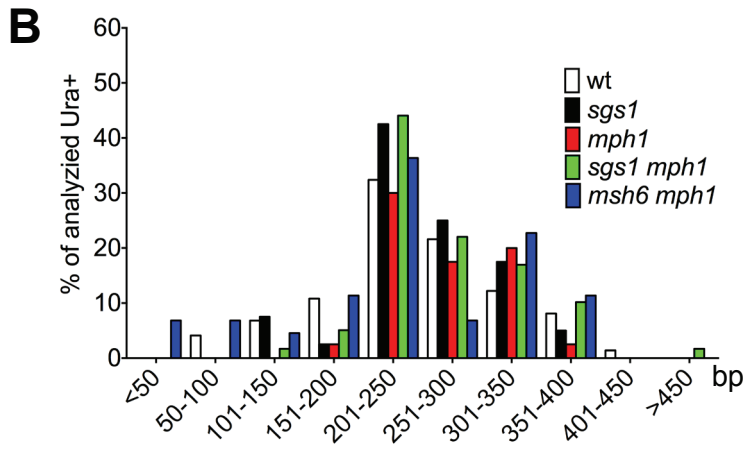
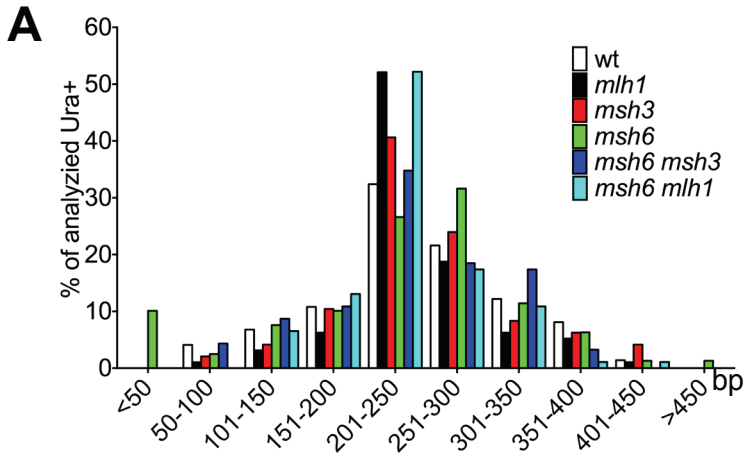


Figure S2, related to main Figure 4

(1) ATGTCCACAAAATCATATACCAGTAGAGCTGAGACTCATGCAAGTCCGGTTGCATCGAACTTTTACGTTTAATGGATGAAAAGAAGACCAATTTGTGTG

(101) CTTCTCTTGACGTTTCGTTGACTGATGAGCTATTGAAACTTGTGAAACGTTGGGTCCATACATTTGCCTTTTGAAAACACACGTTGATATCTTGGATGA

(201) TTTTCAGTTATGAGGGTACTGTCGTTCCATTGAAAGCATTGGCAGAGAAATACAAGTTCCTTGATATTTGAGGACAGAAAATTCGCCGATATC**GGTAA**TACA

(301) GTCAAATTGCAGTACTCTGCGGGTGTATACAGAATAGCAGAATGGTCTGATATCACCAACGCCACGGGGTTACTGGTGCTGGTATTGTTAGCGGTTTGA

(401) AGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAAATGTCATGCAAGGGCTCCCTAGCTACTGCGAGAATATACTAAGGG

(501) TACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATTCGGCTTTATTGCTCAAAGAGACATGGGTGGAAGAGATGAAGGTTAC**GATTGG**CTAATCATG

(601) ACCCCA**GGTGT**GGGTTTAGATGACAAGGGAGAC**GCATTGGGTCA**GCAGTACAGAACCGTCGACGAAGTTGTAAGTGGTGGATCAGATATCATCATTTGTTG

(701) GCAGAGGACTTTTCGCCAAGGGTAGAGATCCTAAGGTTGAAGGTGAAAGATACAGAAATGCTGGATGGGAAGCGTACCAAAAGAGAATCAGCGCTCCCA

(801) TTAA

Figure S3, related to main Figure 4

(1) ATGTCCACAAAATCATATACCAGTAGAGCTGAGACTCATGCAAGTCCGGTTGCATCGAACTTTTACGTTTAATGGATGAAAAGAAGACCAATTTGTGTG

(101) CTTCTCTTGACGTTTCGTTGACTGATGAGCTATTGAAACTTGTGAAACGTTGGGTCCATACATTTGCCTTTTGAAAACACACGTTGATATCTTGGATGA

(201) TTTTCAGTTATGAGGGTACTGTCGTTCCATTGAAAGCATTGGCAGAGAAATACAAGTTCTTGATATTTGAGGACAGAAAATTCGCCGATATCGGTAACACA

(301) **GTCAAATT**GCAGTACTCTGCGGGTGTATACAGAATA**GCAGAATGG**TCTGATATCACCAACGCCACGGGGTTACTGGTGCT**GGTATTGTT**AGCGGTTTGA

(401) AGCAGGCGGCGGAAGAAGTAACAAAGGAACCTAGAGGCCTTTTGATGTTAGCAGAAATGTCATGCAAGGGCTCCCTAGCTACTCGAGAATATACTAAGGG

(501) TACTGTTGACATTGCGAAGAGCGACAAAGATTTTGTATTCGGCTTTATTGCTCAAAGAGAC**ATGGG**AGGAAGAGAAGAAGGGTTTGATTGGCTAATCATG

(601) ACCCCAGGTGTAGGTTTAGACGACAAAGGCGATGCATTGGGTGAGCAGTACAGAACCGTCGACGAAGTTGTAAGTGGTGGATCAGATATCATCATTTGTTG

(701) GCAGAGGACTTTTCGCCAAGGGTAGAGATCCTAAGGTTGAAGGTGAAAGATACAGAAATGCTGGATGGGAAGCGTACCAAAGAGAATCAGCGCTCCCCA

(801) TTAA

Supplemental Figure Legend.

Figure S1, related to main Figure 1 and main Table 1. Length of sequences copied from the ectopic *ura3-52* donor

A. Lack of effect on the length of the copied ectopic *Sc-ura3-52* donor when several mismatch repair genes are deleted. **B.** Minor effect of deleting the 3' to 5' helicases Sgs1 and Mph1 on the length of copied sequences. Minimums of 48 sequences were analyzed for each genotype.

Figure S2, related to main Figure 4. Example of chimeric *mat::Kl-Sc-Kl-Sc-Kl-URA3* gene from *msh6* Ura⁺ colony with a multiple patches of *Sc-ura3-52* embedded in *mat::Kl-ura3* as a result of short-patch MMR. Red letters represent *Kl-ura3*, blue letters represent *Sc-ura3*, and the strike-through sequence represents $\Delta 32$; sequences highlighted in grey represent shared microhomology.

Figure S3, related to main Figure 4. Example of chimeric *mat::Kl-Sc-Kl-Sc-Kl-URA3* gene from *msh6* Ura⁺ colony with a multiple patches of *Sc-ura3-52* embedded in *mat::Kl-ura3* as a result of multiple ICTS. Red letters represent *Kl-ura3*, blue letters represent *Sc-ura3*, and the strike-through sequence represents $\Delta 32$; sequences highlighted in grey represent shared microhomology.

Table S2, related to main Figure 2 and Figure 5. Rates of template switching in different strains

Strain	Description	Rate	SEM
TOY79	<i>intrachromosomal</i> <i>homeologous</i>	1.0×10^{-6}	$\pm 0.7 \times 10^{-6}$
TOY47	<i>interchromosomal</i> <i>homologous</i>	3.0×10^{-3}	$\pm 0.1 \times 10^{-3}$
TOY74	<i>intrachromosomal</i> <i>homologous</i>	8.0×10^{-3}	$\pm 1.0 \times 10^{-8}$
TOY62	<i>rdh54</i> Δ	3.3×10^{-9}	$\pm 0.8 \times 10^{-9}$
TOY83	<i>rdh54</i> Δ <i>intrachromosomal</i> <i>homeologous</i>	5.0×10^{-8}	$\pm 0.2 \times 10^{-8}$
TOY64	<i>rdh54</i> Δ <i>interchromosomal</i> <i>homologous</i>	3.4×10^{-4}	$\pm 0.5 \times 10^{-4}$
TOY77	<i>rdh54</i> Δ <i>intrachromosomal</i> <i>homologous</i>	3.0×10^{-3}	$\pm 0.1 \times 10^{-3}$
TOY89	<i>uls1</i> Δ	1.3×10^{-7}	$\pm 0.3 \times 10^{-7}$
TOY88	<i>dmc1</i> Δ	2.2×10^{-7}	$\pm 0.2 \times 10^{-7}$
TOY85	<i>rad59</i> Δ	2.6×10^{-8}	$\pm 0.7 \times 10^{-8}$
TOY90	<i>rad59</i> Δ <i>interchromosomal</i> <i>homologous</i>	3.7×10^{-4}	$\pm 0.1 \times 10^{-4}$
TOY91	<i>rad59</i> Δ <i>rdh54</i> Δ <i>interchromosomal</i> <i>homologous</i>	1.2×10^{-4}	$\pm 0.1 \times 10^{-4}$
TOY62	<i>rdh54</i> Δ [<i>CEN-RDH54</i>]	9.0×10^{-8}	$\pm 2.1 \times 10^{-8}$
TOY62	<i>rdh54</i> Δ [<i>CEN-rdh54-Δ75</i>]	1.3×10^{-9}	$\pm 1.2 \times 10^{-9}$
TOY62	<i>rdh54</i> Δ [<i>CEN-rdh54-K352R</i>]	1.5×10^{-9}	$\pm 1.1 \times 10^{-9}$
TOY62	<i>rdh54</i> Δ [<i>pGAL-RAD51</i>]	1.3×10^{-9}	$\pm 0.9 \times 10^{-9}$
TOY43	wt [<i>PGK-RAD51</i>]	1.9×10^{-7}	$\pm 0.5 \times 10^{-7}$
TOY43	wt [<i>YEp13-RDH54</i>]	1.6×10^{-7}	$\pm 0.2 \times 10^{-7}$

Table S3, related to main Figure 2 and Figure 5. Spontaneous recombination in different strains

Strain	Relevant genotype	Recombination frequency \pm SEM, $\times 10^{-7}$
TOY47	wild type <i>ura3-52Δ::Kl-ura3-KANMX</i>	2.8 \pm 1.1
TOY49	<i>msh6Δ ura3-52</i>	0.0007 \pm 0.0001
TOY61	<i>msh6Δ ura3-52Δ::Kl-ura3-KANMX</i>	4.3 \pm 1.4
TOY64	<i>rdh54Δ ura3-52Δ::Kl-ura3-KANMX</i>	3.4 \pm 0.9
TOY90	<i>rad59Δ ura3-52Δ::Kl-ura3-KANMX</i>	0.9 \pm 0.5
TOY91	<i>rad59Δ rdh54Δ ura3-52Δ::Kl-ura3-KANMX</i>	0.9 \pm 0.2

*Recombination was measured as Ura⁺ frequency in a corresponding strain switched to *MATa*-like, i.e. recombination is measured between *Kl-ura3* on chromosome 5 and either *mat::Kl-ura3- Δ 32* or *hmr::Kl-ura3- Δ 32* both lacking HO cut site (see Experimental Procedures).

Table S4, related to Table 1, Figure 2 and Figure 5. Relative survival after DSB induction in different strains

Strain	Description	Average survival compared to wild type, %
TOY43	wt *	na
TOY46	<i>sgs1</i> Δ	98.4 ± 6.9
TOY45	<i>mph1</i> Δ	90.6 ± 4.3
TOY60	<i>sgs1</i> Δ <i>mph1</i> Δ	83.4 ± 2.7
TOY52	<i>mlh1</i> Δ	58.8 ± 5.5
TOY49	<i>msh3</i> Δ	100 ± 4.9
TOY44	<i>msh6</i> Δ	100 ± 4.3
TOY57	<i>msh6</i> Δ <i>msh3</i> Δ	73.0 ± 9.0
TOY58	<i>msh6</i> Δ <i>mlh1</i> Δ	110 ± 15.6
TOY72	<i>rad1</i> Δ	50.9 ± 3.7
TOY84	<i>msh6</i> Δ <i>mph1</i> Δ	100 ± 17.8
TOY86	<i>msh6</i> Δ <i>rad1</i> Δ	31.0 ± 4.4
TOY67	<i>rad54</i> Δ	0
TOY62	<i>rdh54</i> Δ	100 ± 5.8
TOY85	<i>rad59</i> Δ	29.5 ± 2.2
TOY43	wt [<i>PGK-RAD51</i>]	82.9 ± 8.0
TOY62	<i>rdh54</i> Δ [<i>pGAL-RAD51</i>]	47.0 ± 0.8
TOY89	<i>uls1</i> Δ	100 ± 2.8
TOY88	<i>dmc1</i> Δ	97.4 ± 3.1
TOY43	wt [YE _p 13]	91.3 ± 1.8
TOY43	wt [YE _p 13- <i>pRDH54-RDH54</i>]	45.6 ± 0.7

* survival of wt strain is 92% ± 5.4%

Table S5, related to Experimental Procedures. Yeast strains used in this study

Strain	Genotype	Reference
JKM153	<i>hoΔ hml::ADE1 MATα HMRa ade1 leu2 lys5 trp1::hisG ura3-52 ade3::Gal-HO</i>	Hicks, 2010
TOY19	<i>hmra::Kl-ura3-Δ32</i>	This study
TOY43	<i>hmra::Kl-ura3-Δ32 sir3Δ::HPHMX</i>	This study
TOY44	<i>hmra::Kl-ura3-Δ32 msh6Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY45	<i>hmra::Kl-ura3-Δ32 mph1Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY46	<i>hmra::Kl-ura3-Δ32 sgs1Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY47	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::Kl-ura3-KANMX6 sir3Δ::HPHMX</i>	This study
TOY49	<i>hmra::Kl-ura3-Δ32 msh3Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY52	<i>hmra::Kl-ura3-Δ32 mlh1Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY57	<i>hmra::Kl-ura3-Δ32 msh6Δ::KANMX6 msh3Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY58	<i>hmra::Kl-ura3-Δ32 msh6Δ::KANMX6 mlh13Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY60	<i>hmra::Kl-ura3-Δ32 sgs1Δ::KANMX6 mph1::TRP1 sir3Δ::HPHMX</i>	This study
TOY61	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::Kl-ura3-KANMX msh6Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY62	<i>hmra::Kl-ura3-Δ32 rdh54Δ::KANMX6 sir3Δ::HPHMX</i>	This study

TOY64	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::Kl-ura3-KANMX6 rdh54Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY67	<i>hmra::Kl-ura3-Δ32 rad54Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY68	<i>hmra::Kl-ura3-Δ32 rdh54Δ::KANMX chk1Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY72	<i>hmra::Kl-ura3-Δ32 rad1Δ::KANMX sir3Δ::HPHMX</i>	This study
TOY74	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::NATMX thr4Δ::Kl-ura3-TRP1 sir3Δ::HPHMX</i>	This study
TOY77	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::NATMX thr4Δ::Kl-ura3-TRP1 rdh54Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY79	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::NATMX thr4Δ::Sc-ura3-KANMX6 sir3Δ::HPHMX</i>	This study
TOY83	<i>hmra::Kl-ura3-Δ32 ura3-52Δ::NATMX thr4Δ::Sc-ura3-KANMX6 rdh54Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY84	<i>hmra::Kl-ura3-Δ32 msh6Δ::KANMX6 mph1Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY85	<i>hmra::Kl-ura3-Δ32 rad59Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY86	<i>hmra::Kl-ura3-Δ32 rad1Δ::KANMX msh6Δ::TRP1 sir3Δ::HPHMX</i>	This study
TOY88	<i>hmra::Kl-ura3-Δ32 dmc1Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY89	<i>hmra::Kl-ura3-Δ32 uls1Δ::KANMX6 sir3Δ::HPHMX</i>	This study
TOY90	<i>hmra::Kl-ura3-Δ32 rad59Δ::TRP1 ura3-52Δ::Kl-ura3-KANMX sir3Δ::HPHMX</i>	This study
TOY91	<i>hmra::Kl-ura3-Δ32 rad59Δ::TRP1 rdh54Δ::NATMX ura3-52Δ::Kl-ura3-KANMX sir3Δ::HPHMX</i>	This study

Table S6, related to Experimental Procedures. Primers used in this study*

Name	5' → 3' sequence	Comments
KlURA3p1	ATGTCCACAAAATCATATACCAGTAGAGCTG	
MATdist2R	AGGCACCCAAGAAGGCGAATAAGA	
MATDp8	CGCATGGGCAGTTTACCTTT	
MATX F	CTTTTCTTCTTCTATTTCGAAACCCA	
MATXp1	TGTTACACTCTCTGGTAACTTAGG	
Slx1p1	CCGACTAGATCGTCATATGAG	
Slx1R2	CGAGAATTTGACAGGCATTCA	
OT09	GCATTACGAAGATTCTCGAT	
OT10	CTATGTTGATCATAAGTCTCTTC	
OT12	GATTATTGATGCTTGCGTGAATATACTAAGGGTACCGTTG	
OT13	GTACCCTTAGTATATTCACGCAAGCATCAATAATCCCCTTG	
OT18	TTTACATTTAGCAATATATATATATATATTTCAAGGATATACCATTCTAATGTCTGCCCTATGTCCACAAAATCATATAC	
OT19	CGGCGGGGACGAGGCAAGCTAAACAGATCTGGCGGCCTTAATTAACCCGGGGATCCGCTTAAATGGGGAGCGCTGATTC	
OT20	GACGGATCCCCGGGTTAATTAAGGCGCGCCAGATCTGTTAGCTTGCCCTCGTCCCCGCCG	used with OT18 and OT19 to create pRS314- <i>pLEU2-Kl-URA3-KanMX6</i>
OT21	TCTATTATGAATTTCAATTTATAAAGTTTATGTACAAATATCATAAAAAAAGAGAATCTTTGCCGATTCATTAATGCAGGT	
OT22	CGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATAAACGA	used with OT18 and OT19 to create pRS314- <i>pLEU2-Kl-URA3-TRP1</i>
OT23	TCTATTATGAATTTCAATTTATAAAGTTTATGTACAAATATCATAAAAAAAGAGAATCTTTCTATTTCTTAGCATTTTGA	
OT28	TTTACATTTAGCAATATATATATATATATTTCAAGGATATACCATTCTAATGTCTGCCCTATGTGCGAAAGCTACATATAA	used with OT20 and OT21 to create pRS314- <i>pLEU2-Sc-URA3-KanMX6</i>
OT29	CGGCGGGGACGAGGCAAGCTAAACAGATCTGGCGGCCTTAATTAACCCGGGGATCCGCTTAGTTTGGCTGGCCGCATC	
OT31	TTAAATGAAGCTCTAATTTGTGAGTTTAGTATACATGCATTTACTTATAATACAGTTTTGAATTCGAGCTCGTTTAAAC	
OT40	TGCGAGGCATATTTATGGTGAAGGATAAGTTTTGACCATCAAAGAAGGTTAATGTGGCTGCAGTAGAGCTGAGACTCATG	to delete <i>ura3-52</i> and its promoter with <i>Kl-ura3-KanMX6</i>

OT51	TTATAATTCATTTTAGCAAGTTCTTCTTCAATAGCGTTTTTGACCAATTCAACGTCAGCGAATTCGAGCTCGTTTAAAC	to delete <i>THR4</i> with <i>Sc-ura3-KanMX6</i>
OT55	GTTCTTAGACGGCCACCATCCCTCACACCAATGCCAAGCTTCCCAAGTTTACAGATCTCAGTAGAGCTGAGACTCATG	to delete <i>THR4</i> with <i>Kl-ura3-TRP1</i>
OT70	GAATTGTCTTCCAAGGGTCTCTAG	
OT71	CTAGAGAACCCTTGGAAGACAATTC	
OT79	TTATAATTCATTTTAGCAAGTTCTTCTTCAATAGCGTTTTTGACCAATTCAACGTCAGCCTATTCTTAGCATTTTGA	
OT88	CGCCAAGGGTAGAGATCCTAAG	
OT90	GTTCTTAGACGGCCACCATCCCTCACACCAATGCCAAGCTTCCCAAGTTTACAGATCTGGAACGTGCTGCTACTCATC	

* Primer sequences for other gene deletions are available upon request. All gene disruptions/alteration were confirmed by PCR