Supplementary Material

Western blots

Log phase cultures of JSY1505 (*msh3Δ* derivative of EAY33[26]) carrying wild-type *MSH2* (pMMR8[79]) and either *MSH3* (pMMR20[79]) or *msh3* allele overexpression plasmids were grown up in the presence of 2% lactate and 2% glycerol (strains used are listed in Table S6). Protein expression was induced by the addition of 2% galactose. Cells were harvested and lysed as described previously.[26] JSY1505 was used as negative control and partially purified Msh3 protein was used as a positive control. Cleared lysates were separated by SDS-PAGE and transferred to Bio-Rad nitrocellulose membranes (0.45μM) using a semi-dry transfer cell (Bio-Rad). The membrane was blocked for an hour in 10% milk and then incubated with the 1:1000 diluted primary α-Msh3 antibody (Santa Cruz Biotechnology) overnight at 4^{o}C. This was followed by incubation with 1:10,000 diluted horseradish peroxidase conjugated rabbit anti-goat antibody (Thermo Scientific) for 20 minutes. The blot was then treated, as directed, with Western Bright^{TM} Quantum (Advansta). The membrane was developed on Chemi-doc MP (Bio-Rad) and the Msh3 bands were quantified using ImageLab (Bio-Rad). The amount of Msh3 or msh3 mutant protein in each lane was normalized to the same non-specific band that appears in all lanes, including the strain that does not carry an overexpression plasmid.

Figure S1: Western blot of msh3 proteins. Wild-type *MSH3* and the *msh3* alleles were overexpressed (strains are provided in Table S6). Cleared lysate was obtained, separated by SDS PAGE, transferred onto nitrocellulose membrane and Msh3 was probed with goat α-Msh3 antibody. Partially purified Msh3 was used as positive control (arrow) and the strain with no *MSH3* plasmid (empty) was used as negative control. **(a)** Alleles carrying R744A, P774A, G795A, G796A and Y942A mutations were detected. **(b)** P745A, R761A, N762A and F940A msh3 alleles were detected. **(c)** R744L, P745L, R761L, N762L, P774L, G795D, G796D and Y925A msh3 mutations were detected. The upper row in each panel shows Msh3 and msh3

protein levels. The lower row in each panel is a non-specific band present in all lanes that was used as a loading control. All of the sections in each panel are from the same blot and exposure. Numbers below the blot represent fold differences in level of expression relative to the wild-type control on the same blot.

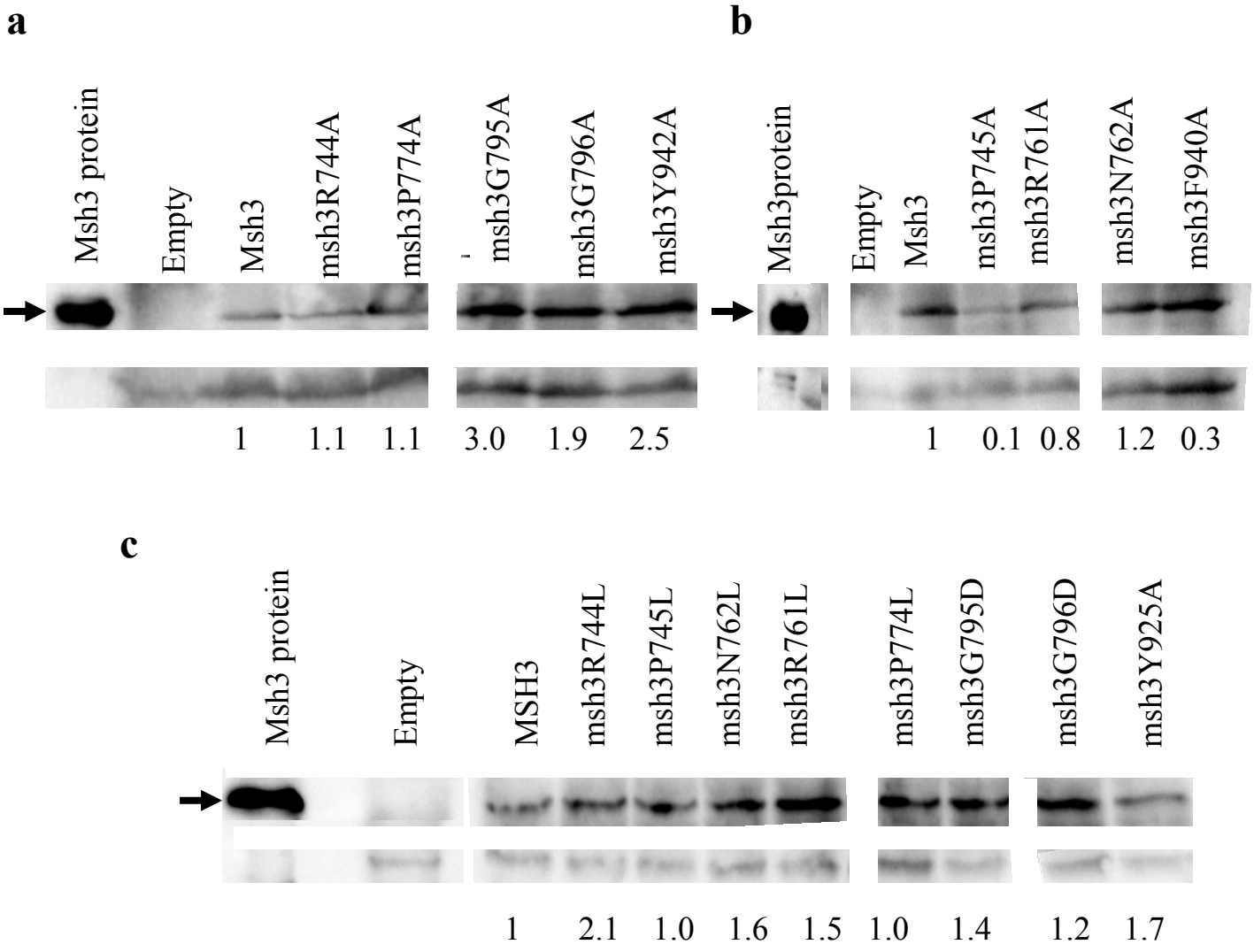
a
Msh3 protein
Empty
Msh3
msh3R744A
msh3P774A
msh3G795A
msh3G796A
msh3Y942A
1 1.1 1.1 3.0 1.9 2.5
b
Msh3protein
Empty
Msh3
msh3P745A
msh3R761A
msh3N762A
msh3F940A
1 0.1 0.8 1.2 0.3
c
Msh3 protein
Empty
MSH3
msh3R744L
msh3P745L
msh3N762L
msh3R761L
msh3P774L
msh3G795D
msh3G796D
msh3Y925A
1 2.1 1.0 1.6 1.5 1.0 1.4 1.2 1.7

Fig. S1

Supplementary Table1: Parental strains used in this study.

Strain	Genotype	Reference
EAY1042	*Δho, HMLα MATa::KanMX hmrΔ::ADE1 ade1-100 leu2-3 lys5 trp1::hisG ura3-52 ade3::Gal::HO MSH2-HA$_4$::LEU2*	[1]
EAY1118 [a]	EAY 1042 *msh3Δ::hisG*	[1]
FY23 [b]	*MAT*a *ura3-52 leu2Δ1 trp1Δ63*	[2]
FY86 [b]	*MAT*α *ura3-52 leu2Δ1 his3Δ200*	[2]
EAY420 [c]	*ura3-52 leu2Δ1 trp1Δ63 msh3Δ::hisG*	[3]
JSY1505 [d]	*ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL msh3Δ::hisG*	This study
EAY1125	*ura3-52 trp1 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL pMMR20 pMMR8*[e]	[4]
GCY559	*ade2-101c his3Δ ura3-Nhe lys2ΔRV::hisG leu2-R-(HIS3::intron::cb2-4L IR)-LEU2 Gal+*	[5]
EAY1613	*GCY559 msh2Δ::hisG-URA3::hisG*	[6]
EAY1625 [f]	*GCY559 msh3Δ::hisG-URA3::hisG*	[6]

[a] *msh3Δ* strain used to measure 3'NHTR and MMR of *msh3* alleles in Table 1.

[b] *MAT**a*** and *MAT**α*** strains used to determine mating type switching. FY23 also used for dominant negative tests in Table 3.

[c] *msh3Δ* used to perform mutational analysis in Table 2.

[d] Protease deficient and *msh3Δ* strains used to perform Western blots.

[e] Protease deficient strain overexpressing *MSH2* and *MSH3* used as a positive control in Western blots.

[f] *msh3Δ* strains used to assay heteroduplex rejection in Table 4.

Supplementary Table 2: Oligonucleotides used for site directed mutagenesis.

msh3 allele	Oligo name	Sequence
msh4	SO234	5’-GTAAATTATGTTGCACCAACTTTTGTGAAT-3’
R744A	SO235	5’-ATTCACAAAAGTTGGTGCAACATAATTTAC-3’
msh3	SO480	5’-TGCAACGTAAATTATGTTCTACCAACTTTTGTGAAT-3’
R744L	SO481	5’-ATTCACAAAAGTTGGTAGAACATAATTTACGTTGCA-3’
msh3	SO236	5’-GTAAATTATGTTAGAGCAACTTTTGTGAAT-3’
P745A	SO237	5’-ATTCACAAAAGTTGCTCTAACATAATTTAC-3’
msh3	SO482	5’-TGCAACGTAAATTATGTTAGACTAACTTTTGTGAAT-3’
P745L	SO483	5’-ATTCACAAAAGTTAGTCTAACATAATTTACGTTGCA-3’
msh3	SO222	5’-GCAAAAAATGCAGCAAATCCAATTATC-3’
R761A	SO223	5’-GATAATTGGATTTGCTGCATTTTTTGC-3’
msh3	SO484	5’-ATCGCAAAAAATGCACTAAATCCAATTATCGAGTCG-3’
R761L	SO485	5’-CGACTCGATAATTGGATTTAGTGCATTTTTTGCGAT-3’
msh3	SO389	5’-GCAAAAAATGCAAGAGCTCCAATTATCGAGTCGCTG-3’
N762A	SO340	5’-CAGCGACTCGATAATTGGAGCTCTTGCATTTTTTGC-3’
msh3	SO486	5’-ATCGCAAAAAATGCAAGACTTCCAATTATCGAGTCG-3’
N762L	SO487	5’-CGACTCGATAATTGGAAGTCTTGCATTTTTTGCGAT-3’
msh3	SO220	5’-GTTCATTATGTAGCAAATGATATCATG-3’
P774A	SO221	5’CATGATATCATTTGCTACATAATGAAC-3’
msh3	SO488	5’-GATGTTCATTATGTACTAAATGATATCATGATGTCC-3’
P774L	SO489	5’-GGACATCATGATATCATTTAGTACATAATGAACATC-3’
msh3	SO383	5’GAGGACTGGATGAGTGTAATTGCTCTATATAAGTTAAAAAAGGGATTG-3’
F940A	SO384	5’-CAATCCCTTTTTTAACTTATATAGAGCAATTACACTCATCCAGTCCTC-3’
msh3	SO385	5’-TGGATGAGTGTAATTTTTCTAGCTAAGTTAAAAAAGGGATTGACTTAT-3’
Y942A	SO386	5’-ATAAGTCAATCCCTTTTTTAACTTAGCTAGAAAAATTACACTCATCCA-3’

msh3	SO494	5’-AGGAATTATCATATGGATGCCGTGGAAGAACAAAAA-3’
Y925A	SO495	5’-TTTTTGTTCTTCCACGGCATCCATATGATAATTCCT-3’
msh3	SO286	5’-GGGCCGAATATGGCTGGGAAATCATCT-3’
G795A	SO287	5’-AGATGATTTCCCAGCCATATTCGGCCC-3’
msh3	SO490	5’-ACGGGGCCGAATATGGATGGGAAATCATCTTATATT-3’
G795D	SO491	5’-AATATAAGATGATTTCCCATCCATATTCGGCCCCGT-3’
msh3	SO387	5’-CGGGGCCGAATATGGGTGCGAAATCATCTTATATTA-3’
G796A	SO388	5’-CTAATATCCGATGATTTCGCACCCATATTCGGCCCC-3’
msh3	SO492	5’-ACGGGGCCGAATATGGGTGATAAATCATCTTATATT-3’
G796D	SO493	5’-AATATAAGATGATTTATCACCCATATTCGGCCCCGT-3’

Supplementary Table 3: Plasmids used in this study.

msh3 allele	Plasmid	Overexpression Plasmid
MSH3	pGW2	pMMR20
msh3R744A	pCK17	pBC2
msh3R744L	pCK70	pCK95
msh3P745A	pCK18	pCK96
msh3P745L	pCK71	pCK88
msh3R761A	pCK21	pCK97
msh3R761L	pCK80	pCK89
msh3N762A	pCK40	pCK41
msh3N762L	pCK81	pCK90
msh3P774A	pCK20	pBC1
msh3P774L	pCK72	pCK91
msh3G795A	pCK35	pBC4
msh3G795D	pCK82	pCK92
msh3G796A	pCK38	pCK42
msh3G796D	pCK83	pCK93
msh3F940A	PCK36	pCK44
msh3Y942A	pCK37	pCK43
msh3Y925A	pCK84	pCK94
Empty Vector	pEAA378	

Supplementary Table 4: Strains used for double strand break repair.

msh3 allele in EAY1118 background	Strains for DSBR assay	Strains for slippage assay
MSH3	JSY1443-45	JSY2402-04
msh3R744A	JSY1455-57	JSY2408-10
msh3R744L	JSY2478-80	-
msh3P745A	JSY1458-60	JSY2411-13
msh3P745L	JSY2481-83	-
msh3R761A	JSY1660-62	JSY2381-83
msh3R761L	JSY2487-89	JSY2487-89
msh3N762A	JSY2299-2301	JSY2393-95
msh3N762L	JSY2490-92	-
msh3P774A	JSY1657-59	JSY2375-77
msh3P774L	JSY2484-86	-
msh3G795A	JSY2216-18	JSY2384-86
msh3G795D	JSY2493-95	-
msh3G796A	JSY2296-98	JSY2390-92
msh3G796D	JSY2496-98	-
msh3F940A	JSY2317-19	JSY2396-98
msh3Y942A	JSY2293-95	JSY2387-89
msh3Y925A	JSY2499-2501	-
Empty Vector	JSY1452-44	JSY2405-07

Supplementary Table 5: Strains used to determine mutation rates.

msh3 allele in EAY420 background	Slippage assay strains Tetranucleotide repeat	Slippage assay strains Dinucleotide repeat
MSH3	JSY1624-26	JSY2320-22
msh3R744A	JSY1630-32	JSY2323-35
msh3R744L	JSY2454-56	-
msh3P745A	JSY1633-35	-
msh3P745L	JSY2457-59	-
msh3R761A	JSY1639-41	-
msh3R761L	JSY2463-65	-
msh3N762A	JSY2314-16	-
msh3N762L	JSY2466-68	-
msh3P774A	JSY1657-59	-
msh3P774L	JSY2460-62	-
msh3G795A	JSY1648-53	JSY2429-31
msh3G795D	JSY2469-71	-
msh3G796A	JSY2311-13	JSY2372-74
msh3G796D	JSY2472-74	-
msh3F940A	JSY2317-19	JSY2366-68
msh3Y942A	JSY2308-10	JSY2369-71
msh3Y925A	JSY2475-77	-
Empty Vector	JSY1627-29	JSY2426-28

Supplementary Table 6: Strains used for Western blots.

msh3 allele in JSY1505 background	Overexpression strain
msh3R744A	JSY2117
msh3R744L	JSY2523
msh3P745A	JSY2526
msh3P745L	JSY2502
msh3R761A	JSY2529
msh3R761L	JSY2505
msh3N762A	JSY2532
msh3N762L	JSY2508
msh3P774A	JSY1799
msh3P774L	JSY2511
msh3G795A	JSY2109
msh3G795D	JSY2514
msh3G796A	JSY2329
msh3G796D	JSY2517
msh3F940A	JSY2535
msh3Y942A	JSY2332
msh3Y925A	JSY2520

Supplementary Table 7: Strains used to determine dominant negative phenotypes.

msh3 allele in FY23 background	Low copy plasmid	Overexpression plasmid
MSH3	JSY1699-1701	JSY2432-33
msh3R744A	JSY1676-78	JSY2434-36
msh3P745A	JSY1679-84	-
msh3 R744L	-	JSY2599-2601
msh3P745L	-	JSY2602-04
msh3R761A	JSY1685-87	-
msh3P774A	JSY1688-1700	-
msh3G795A	JSY1702-04	-
msh3G796A	JSY2399-2401	JSY2437-39
msh3Y925A	-	JSY2605-07
msh3F940A	-	JSY2441-43

Supplementary Table 8: Strains used to determine rates of homeologous recombination.

msh3 allele in EAY1625 background	Strains
MSH3	JSY2575-77
Empty Vector	JSY2560-62
msh3R744L	JSY2563-65
msh3Y942A	JSY2572-74
msh3F940A	JSY2596-98
msh3Y925A	JSY2566-68
msh3G795D	JSY2578-80

Supplementary References

1. Lyndaker, A. M., Goldfarb, T. & Alani, E. (2008). Mutants Defective in Rad1-Rad10-Slx4 Exhibit a Unique Pattern of Viability During Mating-Type Switching in Saccharomyces cerevisiae. *Genetics* 179, 1807-1821.
2. Winston, F., Dollard, C. & SL, R.-H. (1995). Construction of a set of convenient Saccharomyces cerevisiae strains that are isogenic to S288C. *Yeast* 11, 53-55.
3. Studamire, B., Quach, T. & Alani, E. (1998). Saccharomyces cerevisiae Msh2p and Msh6p ATPase Activities Are Both Required during Mismatch Repair. *Mol. Cell. Biol.* 18, 7590-7601.
4. Surtees, J. A. & Alani, E. (2006). Mismatch Repair Factor MSH2-MSH3 Binds and Alters the Conformation of Branched DNA Structures Predicted to form During Genetic Recombination. *Journal of Molecular Biology* 360, 523-536.
5. Nicholson, A., Hendrix, M., Jinks-Robertson, S. & Crouse, G. F. (2000). Regulation of Mitotic Homeologous Recombination in Yeast: Functions of Mismatch Repair and Nucleotide Excision Repair Genes. *Genetics* 154, 133-146.
6. Lee, S. D., Surtees, J. A. & Alani, E. (2007). Saccharomyces cerevisiae MSH2-MSH3 and MSH2-MSH6 Complexes Display Distinct Requirements for DNA Binding Domain I in Mismatch Recognition. *Journal of Molecular Biology* 366, 53-66.