

Supplement for

Mlh1 interacts with both Msh2 and Msh6 for recruitment during mismatch repair

By

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Supplemental Figure 1. Conservation of Msh2, Msh6, and Msh3 residues predicted to interact with Mlh1. **A.** Depiction of residues at the MutS connector domain interaction with MutL with residues involved in the interaction highlighted in red (left). The equivalent *S. cerevisiae* residues (right) are shown above a sequence logo generated by WebLogo [66] depicting the conservation of those sites in 744 fungal Msh2 sequences. Below this is a plot of the Cx interface score (see Methods) for the equivalent *E. coli* MutS residues from the MutS-MutL co-crystal structure [21] broken into the averaged atomic score for side chain (black) and main-chain (grey) atoms; larger Cx interface scores correspond to increased burial upon complex formation (see Methods). **B.** Analysis of the residues at the MutS ATPase/core domains interface and the equivalent *S. cerevisiae* Msh6 and Msh3 residues using alignments involving 747 fungal Msh6 sequences and 652 fungal Msh3 sequences. Analysis displayed as in panel A, except that residues at the *E. coli* MutS-MutL interface are shown in blue (left).

Supplemental Figure 2. Conservation of Mlh1 residues predicted to interact with Msh2, Msh6, and Msh3. **A.** Analysis of the conservation of Mlh1 residues predicted to be at the Msh2 connector domain interaction depicted as in Supplemental Figure 1A. **B.** Analysis of the conservation of Mlh1 residues predicted to be at the Msh6 and Msh3 ATPase/core domain interaction depicted as in Supplemental Figure 1B.

Supplemental Table 1. *S. cerevisiae* strains.

Strain	Genotype
RDKY5964	MATa <i>ura3-52 leu2Δ1 trp1Δ63 hom3-10 his3Δ200 lys2-10A</i>
RDKY9658	RDKY5964 <i>msh2::HIS3</i>
RDKY9670	RDKY5964 <i>mlh1::HIS3</i>
RDKY9792	RDKY5964 <i>mlh1-K54C</i>
RDKY9789	RDKY5964 <i>mlh1-Q57L,T59L</i>
RDKY9673	RDKY5964 <i>pms1::HIS3</i>

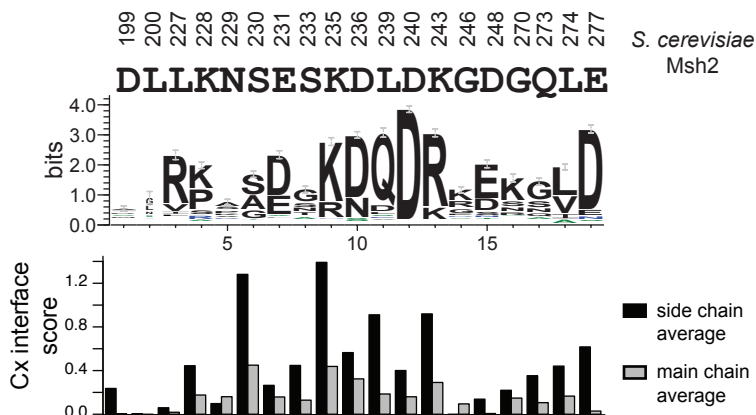
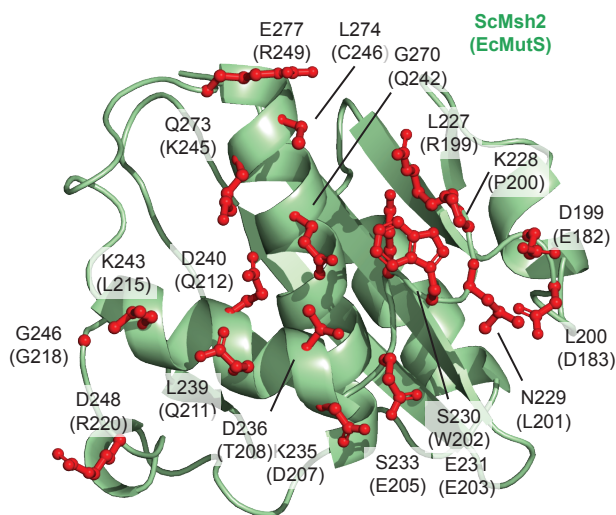
Supplemental Table 2. Plasmids.

Plasmid	Description
pRDK1807	pRS316/ <i>MLH1</i>
pRDK1891	pRS316/ <i>mlh1-K54C</i>
pRDK1892	pRS316/ <i>mlh1-Q57A,T59A</i>
pRDK1893	pRS316/ <i>mlh1-Q57L,T59L</i>
pRDK1894	pRS316/ <i>mlh1-A140E,G141A</i>
pRDK1667	pRS316/ <i>PMS1</i>
pRDK1895	pRS316/ <i>pms1-S138E,R139A</i>
pRDK1896	pRS316/ <i>pms1-E53C</i>
pRDK1897	pRS316/ <i>pms1-E56L,S58L</i>
pRDK1898	pRS316/ <i>pms1-E56A,S58A</i>
pRDK2039	pRCC-K/ <i>mlh1-Q57,T59</i>
pRDK2043	pRCC-K/ <i>mlh1-K54</i>
pRDK573	pRS424/p <i>GAL10-MLH1</i>
pRDK1968	pRS424/p <i>GAL10-mlh1-Q57L,T59L</i>
pRDK1973	pRS424/p <i>GAL10-mlh1-K54C</i>

Supplemental Figure 1

A

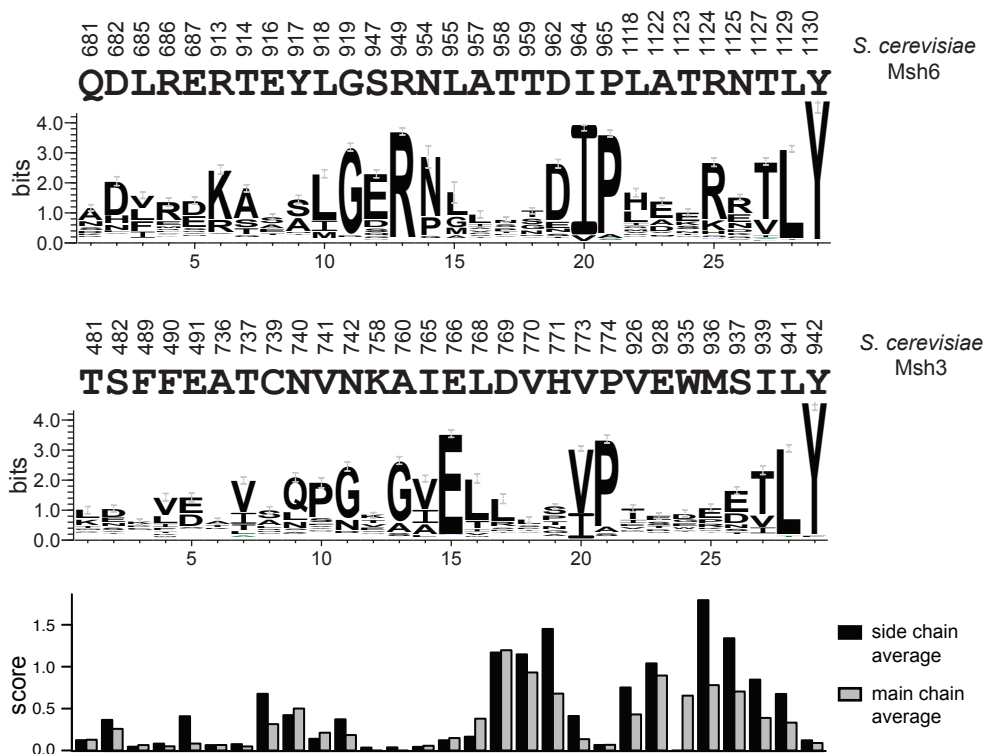
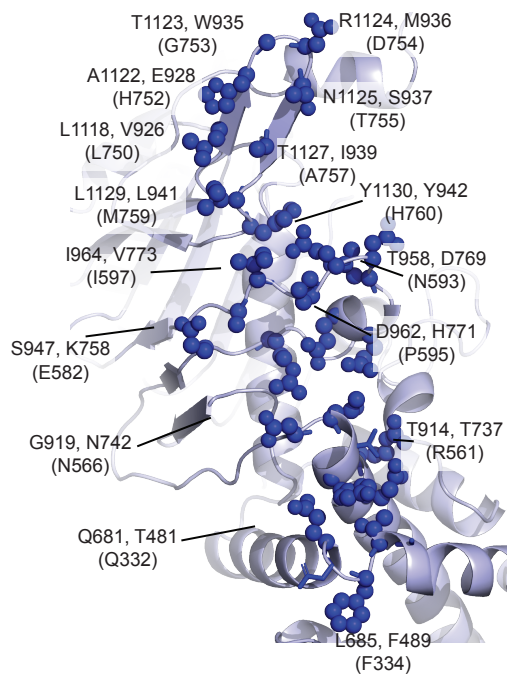
Msh2 connector domain interface



B

Msh6/Msh3 ATPase domain interface

ScMsh6, ScMsh3 (EcMutS)



Supplemental Figure 2

