Supplementary Information, Furman et al., Handcuffing intrinsically disordered regions in Mlh1-Pms1 disrupts mismatch repair

Supplementary Figure S1. Functional domains of yeast Mlh1 and Pms1.

(A) Cartoon representations of the Mlh1 and Pms1 subunits, with the N-terminal ATP binding and C-terminal endonuclease/MLH interaction domains separated by IDRs. Amino acid locations of the domains in yeast Mlh1 and Pms1 are shown. IDR deletions that do not disrupt or only weakly disrupt Mlh1 or Pms1 functions (black lines) or confer *mlh1* or *pms1* null phenotypes (red lines), are shown (Plys *et al*, 2012). The green triangles indicate insertion sites for FRB and FKBP into the Mlh1 and PMs1 IDRs. FRB and FKBP were inserted immediately after the indicated amino acid position of Mlh1 and Pms1. (B) SDS-PAGE analysis (8% gel stained with Coomassie blue R250) of wild-type, Complex #2 (mlh1-$FKBP_{464}$, pms1-FRB_{627}), Complex# 3 (mlh1-FRB_{464}, pms1-$FKBP_{460}$), Complex #4 (mlh1-$FKBP_{464}$, pms1-FRB_{460}), and Complex #5 (mlh1-FRB_{355}-pms1-$FKBP_{460}$). Complex #1 (mlh1-FRB_{355},$FKBP_{464}$, PMS1) is shown in the right panel with a molecular weight standard and wild-type included for reference. Note the presence of higher molecular weight Mlh1-Pms1 complexes seen in this panel as described previously (Hall et al. 2001).

Supplementary Figure S2. Analysis of Complex #2 (mlh1-$FKBP_{464}$, pms1-FRB_{627}) in DNA binding, ATP binding, and endonuclease assays.

(A) MST analysis of Complex #2 in the presence and absence of 49 bp homoduplex DNA (20 nM), ATP (1 mM), and rapamycin (1 μM). Three independent experiments and were performed (error bars indicate the mean ± standard deviation) using at least two independently purified batches of each protein. F_{norm} was calculated by dividing F_{hot} (average fluorescence value in the heated state) by F_{cold} (average fluorescence value measured in the cold state before the infrared laser is turned on) and plotted as parts per thousand (‰). See the Materials and Methods for details. (B) ATP hydrolysis activity of Complex #2; (0.40 μM) was determined in the presence

and absence of PCNA (0.250 μM), 49-bp homoduplex DNA (0.75 μM), and rapamycin (1 μM). Error bars indicate ± one standard deviation for three replicates. (C) Endonuclease activity of Complex #2 (50 nM) was determined on a closed circular DNA substrate (cc) in the presence (+) or absence (-) of $MnSO_4$, ATP, rapamycin, and yeast PCNA/RFC (Materials and Methods). $MnSO_4$, ATP, rapamycin, RFC and PCNA were included at 5 mM, 0.5 mM, 1 μM, 125 nM and 250 nM, respectively. n= nicked product. (D) Endonuclease activities of Complex #2 were determined at the indicated concentrations. Assays were performed in the presence of $MnSO_4$, ATP, RFC, PCNA. Rapamycin was included as indicated. Error-bars indicate the standard deviation of three replicates (Supplementary Figure S3). In panels B, C, and D, the analysis of Mlh1-Pms1 presented in Fig 3 is shown again for comparison purposes.

Supplementary Figure S3. Gel images of protein titration endonuclease activity assays for wild-type, Complex #2 and Complex #5. The assays shown in this figure were used to make the graphs presented in Figure 3D and Supplementary Figure S2D. Endonuclease assays (Materials and Methods) were performed on closed circular plasmid DNA in the presence of $MnSO_4$, ATP, RFC, PCNA without (A) or with (B) rapamycin. cc = closed circular, n= nicked, l = linear.

Supplementary Figure S4. Gel images of protein titration endonuclease activity assays for wild-type and Complex #1. (A) Additional replicates of the assay shown in Figure 4C. Note that Assays 1 and 2 were performed with pUC18 DNA substrate and 3 and 4 with pUC19 DNA substrate. The "½" notation refers to conditions in which Mlh1-Pms1 and Complex #1 were present at 25 nM. (B) Individual titrations used to make the graph presented in Figure 4D. Endonuclease assays (Materials and Methods) were performed on closed circular plasmid DNA in the presence of Mlh1-Pms1, Complex #1, RFC, $MnSO_4$, ATP at the concentrations presented in Figure 4C, and increasing concentrations of PCNA (12.5, 25, 50, 100, 200 nM) in the absence of rapamycin.

Supplementary Table S1. Strains used in this study.

Strains used in this study were derived from the S288c background.

Supplementary Table S2. Plasmids used in this study.

Full plasmid descriptions can be found in the Materials and Methods. *mlh1-FRB$_{XXX}$, mlh1-FKBP$_{XXX}$*, *pms1-FRB$_{XXX}$,* and *pms1-FKBP$_{XXX}$* refer to the amino acids (XXX) in Mlh1 or Pms1 immediately after which the FRB or FKBP domains were inserted (Figure 1; Materials and Methods).

Supplementary Table S3. XL-MS lysine crosslinks for Mlh1-Pms1 (A), Complex 5 (B) and summary of intermolecular crosslinks for Mlh1-Pms1 and Complex #5 (C)

Lower case k indicates site of cross-link

Uniprot: https://www.uniprot.org

Score: MaXLinker score (Yugandhar et al. 2020)

#CSMs: Number of cross-link spectrum matches

XL MH+: Deconvoluted mass of the cross-link. It is a standard practice to present the deconvoluted mass along with the mass of an additional H+

Z: Charge of the cross-link

XL m/z: Cross-link mass divided by its charge

Supplementary Table S4. Phenotypic analysis of *mlh1* and *pms1* alleles that map within or near Mlh1-Pms1 crosslinking sites as determined by XL-MS

+ indicates phenotype similar to wild-type, - indicates similar to MMR null, +/- intermediate phenotype.

REFERENCES

Arana ME, Holmes SF, Fortune JM, Moon AF, Pedersen LC, Kunkel TA (2010) Functional residues on the surface of the N-terminal domain of yeast Pms1. *DNA Repair* **9**, 448-457.

Argueso JL, Kijas AW, Sarin S, Heck J, Waase M, Alani E (2003) Systematic mutagenesis of the *Saccharomyces cerevisiae MLH1* gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. *Mol. Cell. Biol.* **23**, 873-886.

Hall MC, and Kunkel TA (2001) Purification of eukaryotic MutL homologs from *Saccharomyces cerevisiae* using self-cleaving affinity technology. *Prot. Exp. Purif.,* **21**, 333-342.

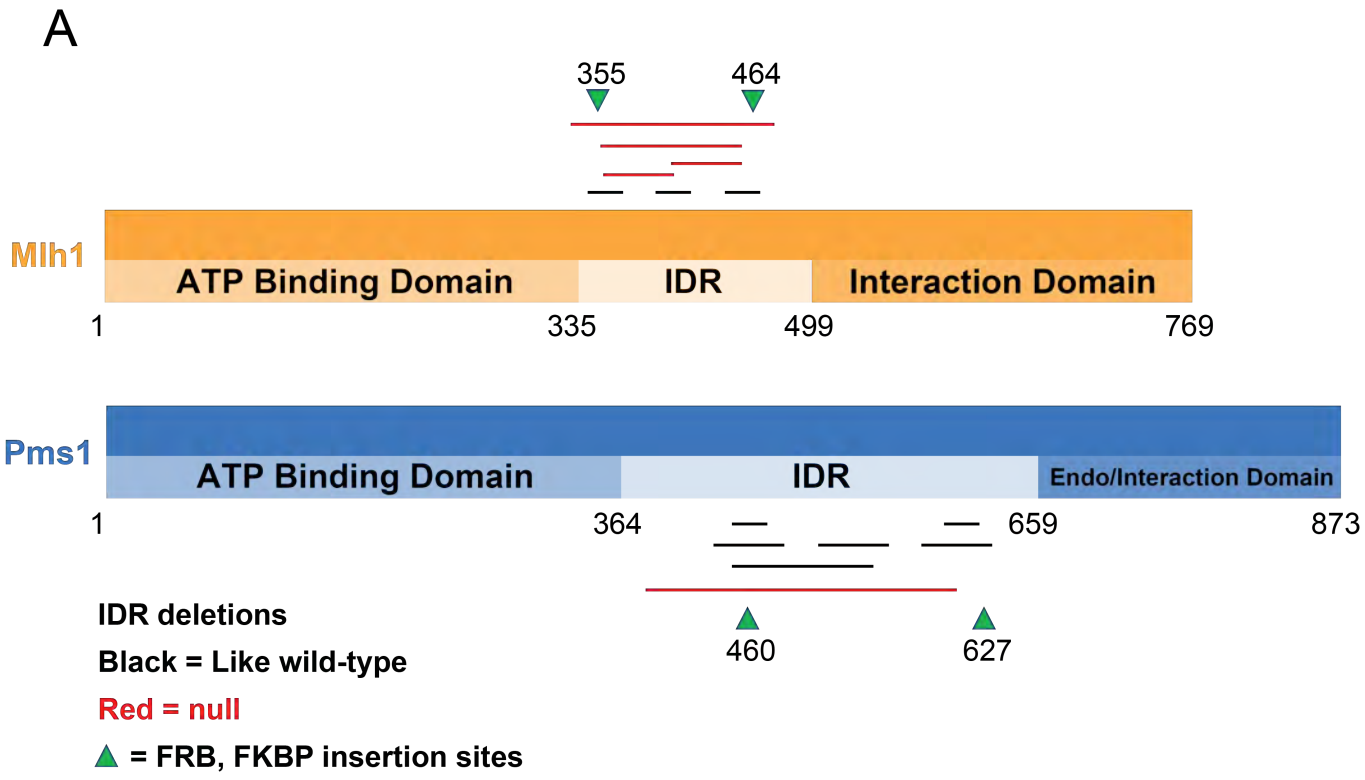
Hall MC, Shcherbakova PV, Fortune JM, Borchers CH, Dial JM, Tomer KB, Kunkel TA (2003) DNA binding by yeast Mlh1 and Pms1: implications for DNA mismatch repair. *Nucleic Acids Res.* **31**, 2025-2034.

Plys AJ, Rogacheva MV, Greene EC, Alani E (2012) The unstructured linker arms of Mlh1-Pms1 are important for interactions with DNA during mismatch repair. *J Mol Biol* **422**, 192-203.

Tran PT, Liskay RM (2000) Functional studies on the candidate ATPase domains of *Saccharomyces cerevisiae* MutLalpha. *Mol. Cell. Biol.* **20**, 6390-6398.

Smith CE, Mendillo ML, Bowen N, Hombauer H, Campbell CS, Desai A, Putnam CD, Kolodner RD (2013) Dominant mutations in *S. cerevisiae PMS1* identify the Mlh1-Pms1 endonuclease active site and an exonuclease 1-independent mismatch repair pathway*. PLoS Genet.* **9**, e1003869.

Yugandhar K, Wang TY, Leung AKY, Lanz MC, Motorykin I, Liang J, Shayhidin EE, Smolka MB, Zhang S, Yu H (2020) MaXLinker: proteome-wide cross-link identifications with high specificity and sensitivity. *Mol. Cell. Proteomics* **19**, 554-568.


A
355
464
Mlh1
ATP Binding Domain
IDR
Interaction Domain
1
335
499
769
Pms1
ATP Binding Domain
IDR
Endo/Interaction Domain
1
364
659
873
460
627
IDR deletions
Black = Like wild-type
Red = null
= FRB, FKBP insertion sites

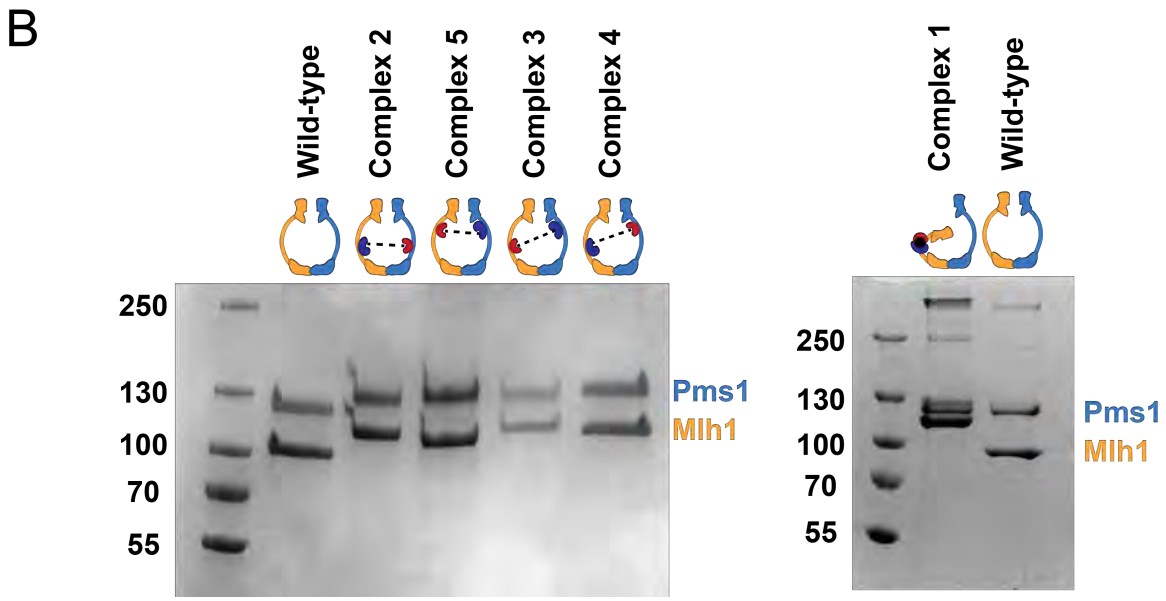

B
Wild-type
Complex 2
Complex 5
Complex 3
Complex 4
250
130
100
70
55
Pms1
Mlh1
Complex 1
Wild-type
250
130
100
70
55
Pms1
Mlh1

Figure S1

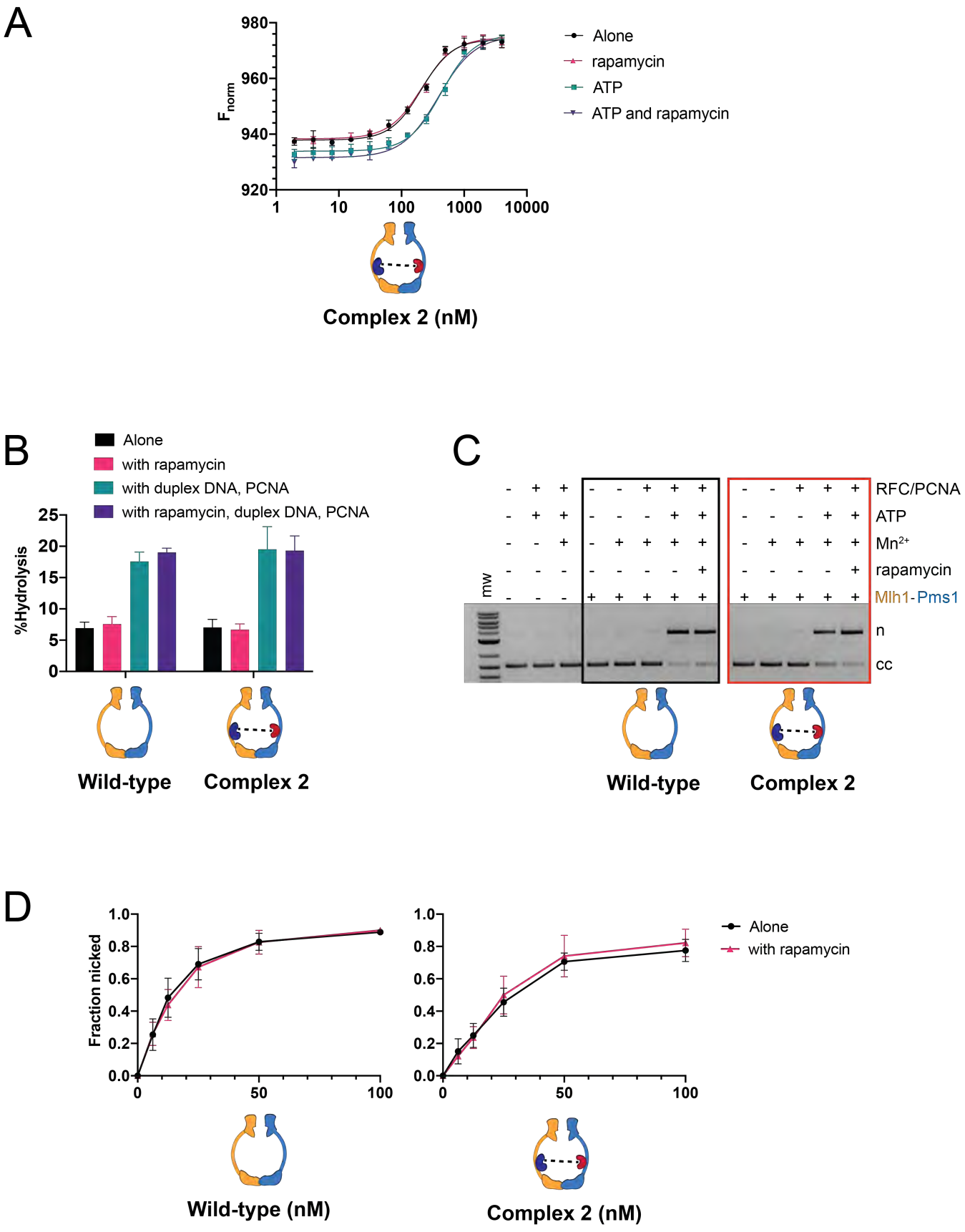

A
980
960
940
920
F_{norm}
1
10
100
1000
10000
Alone
rapamycin
ATP
ATP and rapamycin
Complex 2 (nM)
B
Alone
with rapamycin
with duplex DNA, PCNA
with rapamycin, duplex DNA, PCNA
%Hydrolysis
25
20
15
10
5
0
Wild-type
Complex 2
C
RFC/PCNA
ATP
Mn^{2+}
rapamycin
Mlh1-Pms1
mw
n
cc
Wild-type
Complex 2
D
Fraction nicked
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
Alone
with rapamycin
Wild-type (nM)
Complex 2 (nM)

Figure S2

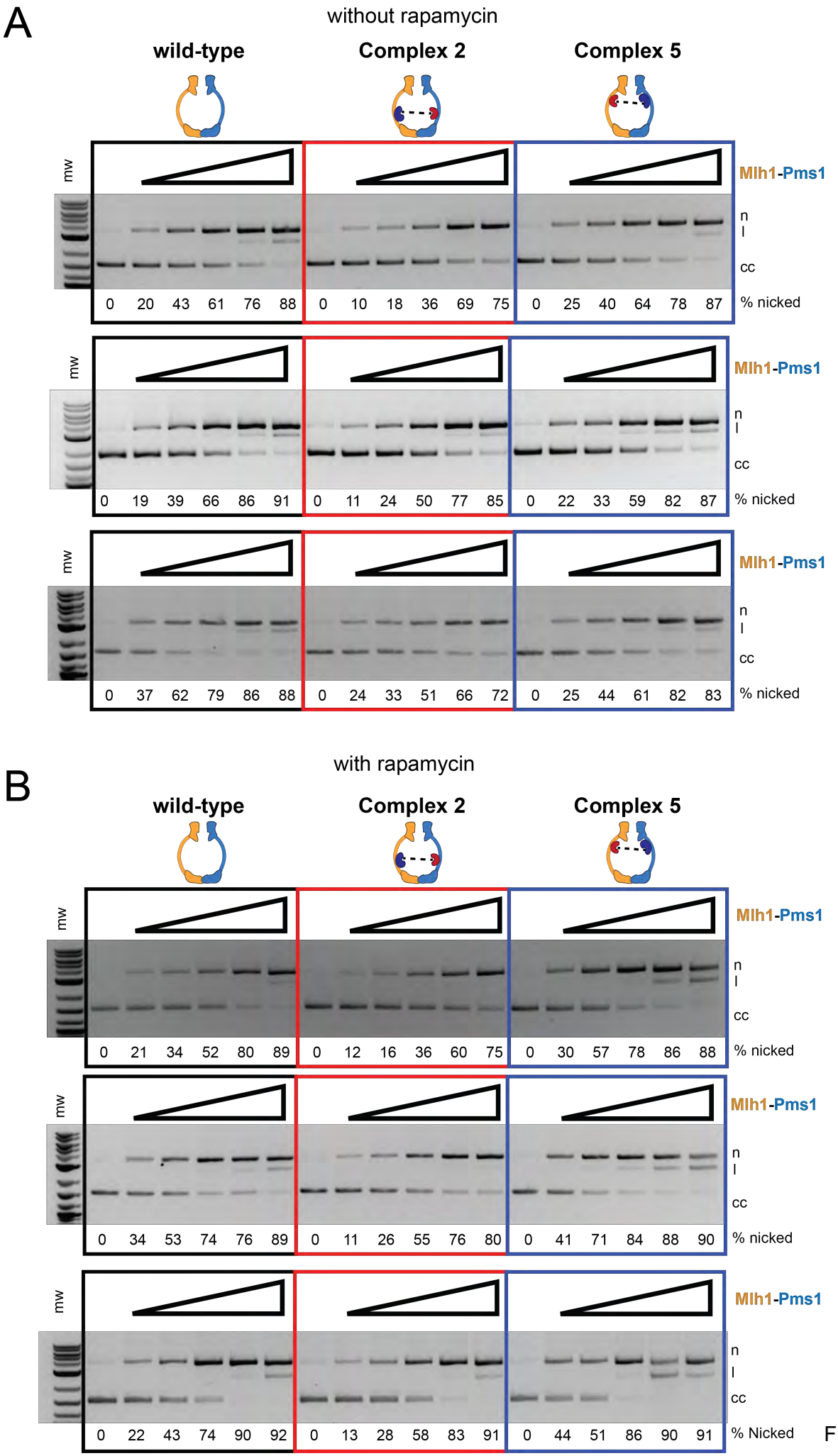
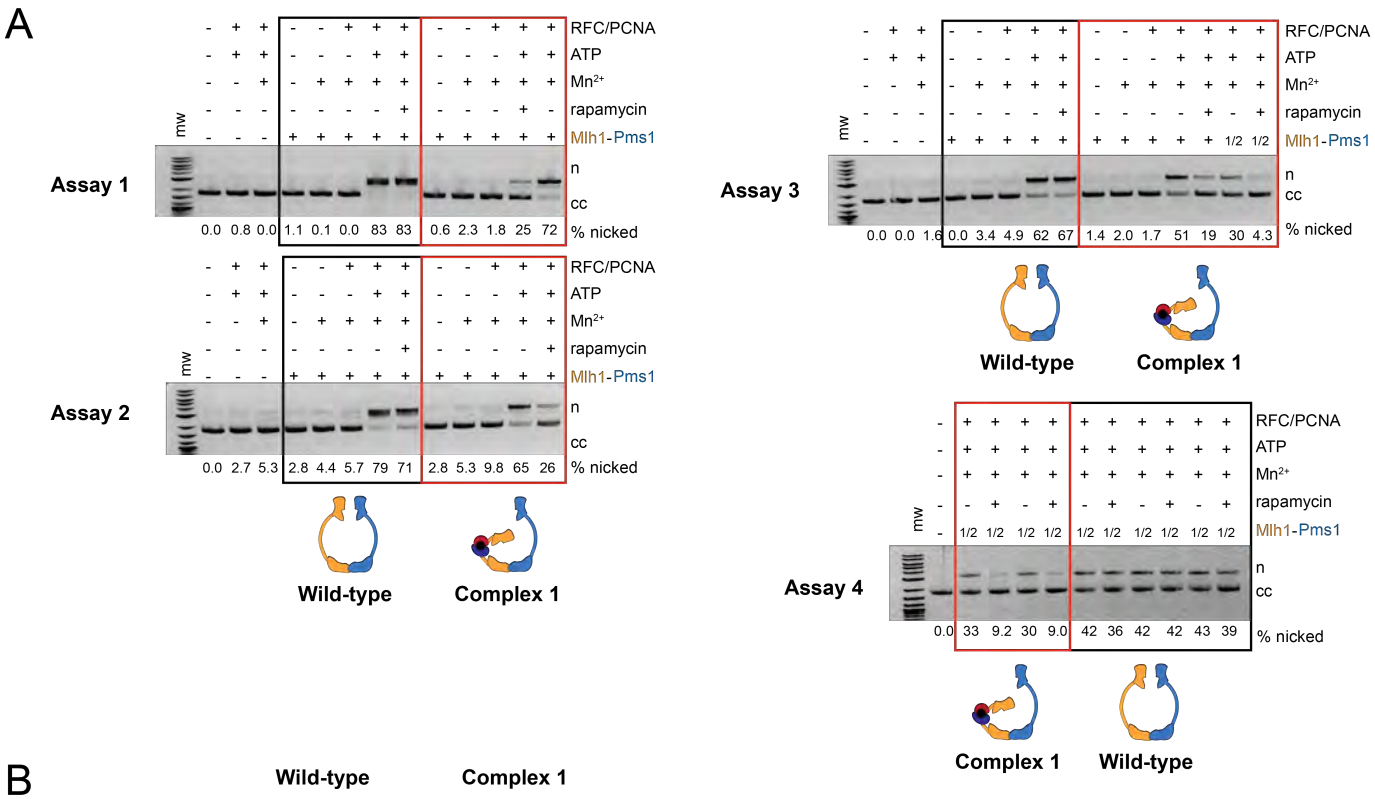

A
without rapamycin
wild-type
Complex 2
Complex 5
mw
Mlh1-Pms1
n
l
cc
0 20 43 61 76 88 0 10 18 36 69 75 0 25 40 64 78 87 % nicked
mw
Mlh1-Pms1
n
l
cc
0 19 39 66 86 91 0 11 24 50 77 85 0 22 33 59 82 87 % nicked
mw
Mlh1-Pms1
n
l
cc
0 37 62 79 86 88 0 24 33 51 66 72 0 25 44 61 82 83 % nicked
B
with rapamycin
wild-type
Complex 2
Complex 5
mw
Mlh1-Pms1
n
l
cc
0 21 34 52 80 89 0 12 16 36 60 75 0 30 57 78 86 88 % nicked
mw
Mlh1-Pms1
n
l
cc
0 34 53 74 76 89 0 11 26 55 76 80 0 41 71 84 88 90 % nicked
mw
Mlh1-Pms1
n
l
cc
0 22 43 74 90 92 0 13 28 58 83 91 0 44 51 86 90 91 % Nicked

Fig. S3


A
Assay 1
Assay 2
Assay 3
Assay 4
RFC/PCNA
ATP
Mn2+
rapamycin
Mlh1-Pms1
mw
n
cc
% nicked
Wild-type
Complex 1

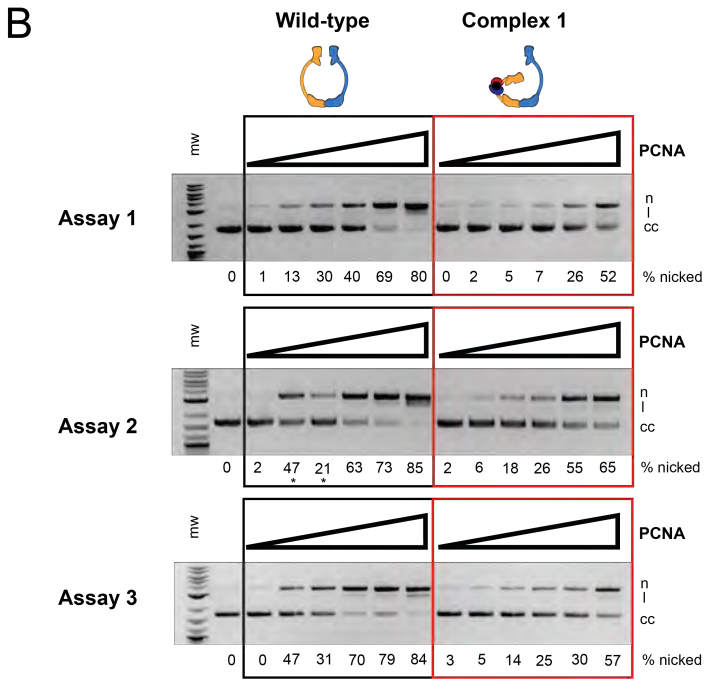

B
Wild-type
Complex 1
Assay 1
Assay 2
Assay 3
PCNA
mw
n
I
cc
% nicked

Figure S4

Table S1. Strains used in this study

EAY1269	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$*
EAY4209-4211	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A*
EAY4212-4215, 4449	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::KANMX*
EAY4450	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX*
EAY4488-4490	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1Δ::KANMX*
JJY70	*MATa, fpr1Δ::NAT, tor1-1, leu2-3,112, ura3-52, his3-Δ200, trp1-Δ901, suc2-Δ9 lys2-801; GAL*
BJ2168	*MATa, ura3–52, leu2–3, 112, trp1–289, prb1–1122, prc1–407, pep4–3*
***mlh1* integrations**	
EAY4470-4471	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FKBP$_{464}$::KANMX*
EAY4472-4473	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{355}$::KANMX*
EAY4474-4475,4491	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{464}$::KANMX*
EAY4598-4600	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{355}$,FKBP$_{464}$::KANMX*
***pms1* integrations**	
EAY4492-4494, 4504	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, pms1-FRB$_{460}$::LEU2*
EAY4495-4496, 4505	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, pms1-FKBP$_{460}$::LEU2*
EAY4497-4498, 4506	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, pms1-FRB$_{627}$::LEU2*
EAY4499-4500, 4507	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, pms1-FKBP$_{627}$::LEU2*
***mlh1, pms1* integrations**	
EAY4451-4452, 4486	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FKBP$_{464}$::KANMX, pms1-FRB$_{627}$::LEU2*
EAY4453-4454, 4508	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{464}$::KANMX, pms1-FKBP$_{460}$::LEU2*
EAY4455-4456, 4509	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FKBP$_{464}$::KANMX, pms1-FRB$_{460}$::LEU2*
EAY4457-4458, 4487	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{355}$::KANMX, pms1-FKBP$_{460}$::LEU2*
EAY4501-4503	*MATa, ura3-52, leu2Δ1, trp1Δ63, lys2::insE-A$_{14}$, tor1-1-S1972A, fpr1Δ::NATMX, mlh1-FRB$_{355}$::KANMX, pms1-FKBP$_{627}$::LEU2*

Strains used in this study were derived from the S288c background.

Table S2. Plasmids used in this study

Plasmid	Relevant genotype	Vector type	Source
pRS415	empty vector	*ARS-CEN, LEU2*	Christianson et al., 1992
pRS416	empty vector	*ARS-CEN, URA3*	Christianson et al., 1992
pEAA213	*MLH1::KANMX*	*ARS-CEN, LEU2*	Alani lab
pEAA238	*PMS1*	*ARS-CEN, HIS3*	Alani lab
pEAA248	*MLH1*	*ARS-CEN, URA3*	Alani lab
pEAA67	*MLH1, PMS1, MSH2*	*ARS-CEN, URA3*	Alani lab
pEAA672	*mlh1-FRB$_{355}$::KANMX*	*ARS-CEN, LEU2*	Alani lab; this study
pEAA674	*mlh1-FKBP$_{464}$::KANMX*	*ARS-CEN, LEU2*	Alani lab; this study
pEAA675	*mlh1-FRB$_{464}$::KANMX*	*ARS-CEN, LEU2*	Alani lab; this study
pEAA713	*mlh1-FRB$_{355}$,FKBP$_{464}$::KANMX*	*ARS-CEN, LEU2*	Alani lab; this study
pEAA676	*pms1-FRB$_{460}$*	*ARS-CEN, HIS3*	Alani lab; this study
pEAA677	*pms1-FKBP$_{460}$*	*ARS-CEN, HIS3*	Alani lab; this study
pEAA678	*pms1-FRB$_{627}$*	*ARS-CEN, HIS3*	Alani lab; this study
pEAA671	*pms1-FKBP$_{627}$*	*ARS-CEN, HIS3*	Alani lab; this study
pEAE448	*GAL1-mlh1-FRB$_{355}$(FLAG$_{499}$)-VMA1-CBD*	*2μ, TRP1*	Alani lab; this study
pEAE447	*GAL1-mlh1-FKBP$_{464}$(FLAG$_{499}$)-VMA1-CBD*	*2μ, TRP1*	Alani lab; this study
pEAE446	*GAL1-mlh1-FRB$_{464}$(FLAG$_{499}$)-VMA1-CBD*	*2μ, TRP1*	Alani lab; this study
pEAE460	*GAL1- mlh1-FRB$_{355}$,FKBP$_{464}$-VMA1-CBD*	*2μ, TRP1*	Alani lab; this study
pEAE435	*GAL10-pms1-FRB$_{460}$*	*2μ, LEU2*	Alani lab; this study
pEAE433	*GAL10-pms1-FKBP$_{460}$*	*2μ, LEU2*	Alani lab; this study
pEAE431	*GAL10-pms1-FRB$_{627}$*	*2μ, LEU2*	Alani lab; this study
pEAI453	*pms1-FRB$_{460}$::LEU2*	*ARS-CEN, LEU2, HIS3*	Alani lab; this study
pEAI454	*pms1-FKBP$_{460}$::LEU2*	*ARS-CEN, LEU2, HIS3*	Alani lab; this study
pEAI455	*pms1-FRB$_{627}$::LEU2*	*ARS-CEN, LEU2, HIS3*	Alani lab; this study
pEAI468	*pms1-FKBP$_{627}$::LEU2*	*ARS-CEN, LEU2, HIS3*	Alani lab; this study
pEAI160	*mlh1Δ::KANMX*	*Integration*	Alani lab
pEAE269	*GAL1-MLH1(FLAG$_{499}$)-VMA1-CBD*	*2μ, TRP1*	Alani lab
pMH8	*GAL10-PMS1*	*2μ, LEU2*	Hall and Kunkel, 2001

Full plasmid descriptions can be found in the Materials and Methods. *mlh1-FRB$_{XXX}$, mlh1-FKBP$_{XXX}$, pms1-FRB$_{XXX}$,* and *pms1-FKBP$_{XXX}$* refer to the amino acids (XXX) in Mlh1 or Pms1 immediately after which the FRB or FKBP domains were inserted (Figure 1; Materials and Methods).

Table S3A. XL-MS lysine crosslinks for Mlh1-Pms1

Score	#CSMs	XL MH+	z	XL m/z	Peptide A	Protein A Gene Name-Uniprot ID	Location	Peptide B	Protein B Gene Name-Uniprot ID	Location
87	8	3205.696	5	641.9455	IkALDASVVNK	Mlh1-P38920	N-Term-6	VSYAEGkMLESPKPVAGK	Mlh1-P38920	N-Term-131
85	6	2760.253	4	690.8192	LkTEVFDDR	Pms1-P14242	Linker-380	McSQSEQQAQkR	Pms1-P14242	Linker-377
83	4	2822.453	4	706.3692	VNVNLTSIkK	Mlh1-P38920	C-Term-515	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
83	4	3000.543	5	600.9149	VSYAEGkMLESPKPVAGK	Mlh1-P38920	N-Term-131	FTTSkLQK	Mlh1-P38920	N-Term-81
83	4	3221.687	4	806.1777	IkALDASVVNK	Mlh1-P38920	N-Term-6	VSYAEGkmLESPKPVAGK	Mlh1-P38920	N-Term-131
82	3	4354.043	4	1089.267	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
82	3	2776.243	4	694.8166	LkTEVFDDR	Pms1-P14242	Linker-380	mcSQSEQQAQkR	Pms1-P14242	Linker-377
81.91	3	2200.121	4	550.7862	QkEFSK	Pms1-P14242	N-Term-159	FSVWNITPkGK	Pms1-P14242	N-Term-196
81	2	2973.482	4	744.1263	VNVNLTSIkK	Mlh1-P38920	C-Term-515	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
81	2	4419.106	5	884.6275	ITSFLSSSQQFNFEGSSTkR	Mlh1-P38920	Linker-427	QLSEPkVTNVSHSQEAEK	Mlh1-P38920	Linker-434
81	2	3283.683	4	821.6766	QLSEPkVTNVSHSQEAEK	Mlh1-P38920	Linker-434	VNVNLTSIkK	Mlh1-P38920	C-Term-515
81	2	3663.776	4	916.6999	DANTINDNDLKDQPkKK	Mlh1-P38920	Linker-471	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
81	2	3407.579	4	852.6506	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
81	2	4131.011	5	827.0085	LGDYkVPSIADDEKNALPISK	Mlh1-P38920	Linker-480	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
81	2	2705.374	4	677.0993	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	QkLGDYK	Mlh1-P38920	Linker-475
81	2	2969.591	4	743.1536	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	VNVNLTSIkK	Mlh1-P38920	C-Term-515
81	2	2961.346	4	741.0924	McSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
81	2	2852.33	4	713.8383	LkTEVFDDR	Pms1-P14242	Linker-380	VkGYISQNSFGcGR	Pms1-P14242	N-Term-263
81	2	2619.256	4	655.5698	LkTEVFDDR	Pms1-P14242	Linker-380	kNISSVFGAGGmR	Pms1-P14242	N-Term-213
81	2	1954.962	4	489.4965	TPLkNSR	Pms1-P14242	Linker-583	SISkDNYR	Pms1-P14242	Linker-591
81	2	2088.15	4	522.7935	TPLkNSR	Pms1-P14242	Linker-583	VNVNLTSIkK	Mlh1-P38920	C-Term-515
81	2	2897.342	4	725.0914	kSEAQENIIK	Pms1-P14242	Linker-638	NkDELEDFEQGEK	Pms1-P14242	Linker-649
81	2	2334.269	4	584.3231	IAkFQDVAK	Pms1-P14242	N-Term-83	IkALDASVVNK	Mlh1-P38920	N-Term-6
80.98	2	2176.149	4	544.793	TPLkNSR	Pms1-P14242	Linker-583	SSImIGkPLNK	Pms1-P14242	C-Term-825
80.96	2	1901.051	4	476.0187	VPkER	Mlh1-P38920	C-Term-504	VNVNLTSIkK	Mlh1-P38920	C-Term-515
80.96	2	3293.614	4	824.1593	kSISPIFFINNR	Mlh1-P38920	N-Term-254	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
80.93	2	3053.439	4	764.1157	VkGYISQNSFGcGR	Pms1-P14242	N-Term-263	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
80.92	2	2977.342	4	745.0913	mcSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
80	1	3851.886	4	963.7274	QENkLVR	Mlh1-P38920	Linker-398	IDASQAkITSFLSSSQQFNFEGSSTK	Mlh1-P38920	Linker-408
80	1	3682.816	4	921.4599	DANTINDNDLKDQPkK	Mlh1-P38920	Linker-471	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489
80	1	3663.744	5	733.5551	QkLGDYKVPSIADDEK	Mlh1-P38920	Linker-475	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
80	1	2929.445	4	733.117	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480	VcNLNFISkK	Mlh1-P38920	N-Term-253
80	1	3058.571	4	765.3986	LGDYKVPSIADDEkNALPISK	Mlh1-P38920	Linker-489	VPkER	Mlh1-P38920	C-Term-504
80	1	3755.916	4	939.7348	mLESPkPVAGK	Mlh1-P38920	N-Term-137	VLQITDNGSGINkADLPILcER	Mlh1-P38920	N-Term-67
80	1	2266.126	4	567.2875	VcNLNFISkK	Mlh1-P38920	N-Term-253	QENkLVR	Mlh1-P38920	Linker-398
80	1	3739.915	4	935.7347	VLQITDNGSGINkADLPILcER	Mlh1-P38920	N-Term-67	MLESPkPVAGK	Mlh1-P38920	N-Term-137
80	1	2694.394	4	674.3543	VkLLSLPTSK	Pms1-P14242	C-Term-768	LkIDEEEEFGSR	Pms1-P14242	C-Term-756
80	1	3210.427	4	803.3626	McSQSEQQAQkR	Pms1-P14242	Linker-377	VkGYISQNSFGcGR	Pms1-P14242	N-Term-263
80	1	2603.262	4	651.5714	LkTEVFDDR	Pms1-P14242	Linker-380	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
80	1	2453.136	4	614.0399	TPLkNSR	Pms1-P14242	Linker-583	McSQSEQQAQkR	Pms1-P14242	Linker-377
80	1	2669.391	4	668.1037	TPLkNSR	Pms1-P14242	Linker-583	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489
80	1	2803.277	4	701.5751	NkDELEDFEQGEK	Pms1-P14242	Linker-649	QmSSIISkR	Pms1-P14242	Linker-636
79.98	1	1810.087	4	453.2776	VPkER	Mlh1-P38920	C-Term-504	LkSLPLLLK	Mlh1-P38920	C-Term-650
79.98	1	3539.748	5	708.7558	QENkLVR	Mlh1-P38920	Linker-398	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446
79.98	1	2435.256	4	609.5698	QELALPkR	Pms1-P14242	Linker-365	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
79.98	1	1858.982	4	465.5014	TPLkNSR	Pms1-P14242	Linker-583	QENkLVR	Mlh1-P38920	Linker-398
79.98	1	2160.151	4	540.7936	TPLkNSR	Pms1-P14242	Linker-583	SSIMIGkPLNK	Pms1-P14242	C-Term-825
79.98	1	3143.54	4	786.6409	TPLkNSR	Pms1-P14242	Linker-583	VVEEPVYFDIDGEkFQEK	Pms1-P14242	Linker-520
79.96	1	1586.767	4	397.4477	cSkIR	Pms1-P14242	C-Term-807	QkEFSK	Pms1-P14242	N-Term-159
79.96	1	1992.059	4	498.7706	TPLkNSR	Pms1-P14242	Linker-583	IAkFQDVAK	Pms1-P14242	N-Term-83
79.94	1	2595.388	4	649.6029	SSIMIGkPLNKK	Pms1-P14242	C-Term-825	LkTEVFDDR	Pms1-P14242	Linker-380
79.92	1	3294.622	4	824.4113	ITSFLSSSQQFNFEGSSTkR	Mlh1-P38920	Linker-427	QENkLVR	Mlh1-P38920	Linker-398
79.88	1	2948.408	4	737.858	FGDSNYSLSVkPSYTVQDR	Mlh1-P38920	N-Term-204	VPkER	Mlh1-P38920	C-Term-504
79.83	1	2132.102	4	533.7814	TPLkNSR	Pms1-P14242	Linker-583	kSEAQENIIK	Pms1-P14242	Linker-638
79.81	1	3001.548	4	751.1428	VNVNLTSIkK	Mlh1-P38920	C-Term-515	kFEDEILEYNLSTK	Pms1-P14242	Linker-605
79.8	1	2382.199	4	596.3057	kSEAQENIIK	Pms1-P14242	Linker-638	QmSSIISkR	Pms1-P14242	Linker-636
79.78	1	1835.032	4	459.5138	kSISK	Pms1-P14242	Linker-589	VNVNLTSIkK	Mlh1-P38920	C-Term-515
79.75	1	2490.25	4	623.3183	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	kTMTR	Pms1-P14242	C-Term-830
62.96	4	1981.026	4	496.0124	kTMTR	Pms1-P14242	C-Term-830	SSIMIGkPLNK	Pms1-P14242	C-Term-825
62	3	2482.279	4	621.3256	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	VPkER	Mlh1-P38920	C-Term-504
61.96	3	3274.503	4	819.3816	FGDSNYSLSVkPSYTVQDR	Mlh1-P38920	N-Term-204	DIGFSckK	Mlh1-P38920	N-Term-192
61.81	3	2918.353	4	730.3442	VDTSDASLSEDEkAQFINR	Mlh1-P38920	C-Term-717	kTMTR	Pms1-P14242	C-Term-830
61	2	1996.051	4	499.7686	QENkLVR	Mlh1-P38920	Linker-398	FTTSkLQK	Mlh1-P38920	N-Term-81
61	2	2124.14	4	531.7908	QkLGDYK	Mlh1-P38920	Linker-475	VNVNLTSIkK	Mlh1-P38920	C-Term-515
61	2	2267.237	4	567.5651	FTTSkLQK	Mlh1-P38920	N-Term-81	IkALDASVVNK	Mlh1-P38920	N-Term-6
60.96	2	2274.012	4	569.2589	McSQSEQQAQkR	Pms1-P14242	Linker-377	kTMTR	Pms1-P14242	C-Term-830
60.92	2	2323.123	4	581.5366	kLPSIK	Pms1-P14242	Linker-468	McSQSEQQAQkR	Pms1-P14242	Linker-377
60	1	2708.484	4	677.8769	VNVNLTSIkK	Mlh1-P38920	C-Term-515	kSISPIFFINNR	Mlh1-P38920	N-Term-254
60	1	2933.35	4	734.0933	VDTSDASLSEDEkAQFINR	Mlh1-P38920	C-Term-717	NDFkK	Pms1-P14242	C-Term-672
60	1	2159.186	4	540.5525	QENkLVR	Mlh1-P38920	Linker-398	VNVNLTSIkK	Mlh1-P38920	C-Term-515
60	1	3504.696	4	876.9299	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446	QkLGDYK	Mlh1-P38920	Linker-475
60	1	2593.283	4	649.0767	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480	QENkLVR	Mlh1-P38920	Linker-398
60	1	2836.404	4	709.8569	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	SISkDNYR	Pms1-P14242	Linker-591
60	1	2740.425	4	685.8621	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	QENkLVR	Mlh1-P38920	Linker-398
60	1	1755.893	4	439.7291	VkEDR	Mlh1-P38920	N-Term-117	FTTSkLQK	Mlh1-P38920	N-Term-81
60	1	2977.348	4	745.0929	McSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGmR	Pms1-P14242	N-Term-213
60	1	2608.225	4	652.812	mcSQSEQQAQkR	Pms1-P14242	Linker-377	QELALPkR	Pms1-P14242	Linker-365
60	1	2234.155	4	559.2946	LkTEVFDDR	Pms1-P14242	Linker-380	QELALPkR	Pms1-P14242	Linker-365
60	1	2701.342	4	676.0913	TPLkNSR	Pms1-P14242	Linker-583	kFEDEILEYNLSTK	Pms1-P14242	Linker-605
59.98	1	3768.913	5	754.5889	VNVNLTSIkK	Mlh1-P38920	C-Term-515	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446
59.98	1	2026	4	507.2559	QENkLVR	Mlh1-P38920	Linker-398	SISkDNYR	Pms1-P14242	Linker-591
59.98	1	2451.247	4	613.5676	QELALPkR	Pms1-P14242	Linker-365	kNISSVFGAGGmR	Pms1-P14242	N-Term-213
59.98	1	2553.182	4	639.0515	TPLkNSR	Pms1-P14242	Linker-583	NkDELEDFEQGEK	Pms1-P14242	Linker-649
59.96	1	2514.244	4	629.3168	VPkER	Mlh1-P38920	C-Term-504	kFEDEILEYNLSTK	Pms1-P14242	Linker-605
59.95	1	2335.152	4	584.5438	VPkER	Mlh1-P38920	C-Term-504	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
59.9	1	3094.462	4	774.3714	VVEEPVYFDIDGEkFQEK	Pms1-P14242	Linker-520	QkEFSK	Pms1-P14242	N-Term-159
59.87	1	2166.142	4	542.2913	kLPSIK	Pms1-P14242	Linker-468	kNISSVFGAGGMR	Pms1-P14242	N-Term-213

40.74	2	1608.863	4	402.9717	QkEFSK	Pms1-P14242	N-Term-159	kLPSIK	Pms1-P14242	Linker-468
40	1	2965.459	4	742.1207	DANTINDNDLKDQPkKK	Mlh1-P38920	Linker-471	QkLGDYK	Mlh1-P38920	Linker-475
40	1	2993.34	4	749.0908	mcSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGmR	Pms1-P14242	N-Term-213
40	1	2868.362	4	717.8463	SISkDNYR	Pms1-P14242	Linker-591	kFEDEILEYNLSTK	Pms1-P14242	Linker-605
40	1	2081.095	4	521.0297	QkEFSK	Pms1-P14242	N-Term-159	IkALDASVVNK	Mlh1-P38920	N-Term-6
39.98	1	3058.582	5	612.5226	VPkER	Mlh1-P38920	C-Term-504	LGDYkVPSIADDEKNALPISK	Mlh1-P38920	Linker-480
39.96	1	1891.99	4	473.7535	NSkDR	Pms1-P14242	N-Term-278	VNVNLTSIkK	Mlh1-P38920	C-Term-515
39.91	1	2930.352	4	733.3438	NGkQMSSIISK	Pms1-P14242	Linker-628	NkDELEDFEQGEK	Pms1-P14242	Linker-649
39.87	1	2008.037	4	502.7651	VPkER	Mlh1-P38920	C-Term-504	VcNLNFISkK	Mlh1-P38920	N-Term-253
39.86	1	1559.759	4	390.6956	kTMTR	Pms1-P14242	C-Term-830	QkEFSK	Pms1-P14242	N-Term-159
39.56	1	2343.127	4	586.5375	kTMTR	Pms1-P14242	C-Term-830	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
29.82	1	3287.701	4	822.681	IkALDASVVNK	Mlh1-P38920	N-Term-6	LQkFEDLSQIQTYGFR	Mlh1-P38920	N-Term-84
29.78	1	1751.9	4	438.7308	DQPkKK	Mlh1-P38920	Linker-471	QkLGDYK	Mlh1-P38920	Linker-475
29.52	1	2934.349	4	734.3432	kTmTR	Pms1-P14242	C-Term-830	VDTSDASLSEDEkAQFINR	Mlh1-P38920	C-Term-717
19.78	1	1584.828	4	396.9629	kSISK	Pms1-P14242	Linker-589	NFkEISK	Pms1-P14242	C-Term-621

Definitions

lower case k indicates site of cross-link

Uniprot: https://www.uniprot.org

Score: MaXLinker score; see Yugandhar et al. (2020) Mol. Cell Proteomics 19, 554-568 (2020).

#CSMs: Number of cross-link spectrum matches

XL MH+: Deconvoluted mass of the cross-link. It is a standard practice to present the deconvoluted mass along with the mass of an additional H+

Z: Charge of the cross-link

XL m/z: Cross-link mass divided by its charge

Table S3B. XL-MS lysine crosslinks for Complex #5

Score	#CSMs	XL MH+	z	XL m/z	Peptide A	Protein A Gene Name-Uniprot ID	Peptide A	Peptide B	Protein B Gene Name-Uniprot ID	Peptide B
87	8	3205.692	5	641.9446	VSYAEGkMLESPKPVAGK	Mlh1-P38920	N-Term-131	lkALDASVVNK	Mlh1-P38920	N-Term-6
86	7	3069.565	4	768.1472	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46	VNVNLTSIkK	Mlh1-P38920	C-Term-515
85	6	3827.93	4	957.7385	QFIYVNkRPVEYSTLLK	Pms1-P14242	N-Term-287	VkGYISQNSFGcGR	Pms1-P14242	N-Term-263
84.94	6	2063.137	4	516.5402	kLPSIK	Pms1-P14242	Linker-468	FMLGkQEVIR	Pms1-P14242	FKBP-52
84.8	6	2054.02	4	514.2609	LEGSNSSTPTkK	Pms1-P14242	Linker-467	TFPkR	Pms1-P14242	FKBP-17
83	4	3389.699	4	848.1806	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46	kSISPIFFINNR	Mlh1-P38920	N-Term-254
82	3	3000.538	5	600.9138	VSYAEGkMLESPKPVAGK	Mlh1-P38920	N-Term-131	FTTSkLQK	Mlh1-P38920	N-Term-81
82	3	3287.701	4	822.6812	lkALDASVVNK	Mlh1-P38920	N-Term-6	LQkFEDLSQIQTYGFR	Mlh1-P38920	N-Term-84
82	3	3739.909	4	935.7332	VLQITDNGSGINkADLPILcER	Mlh1-P38920	N-Term-67	MLESPkPVAGK	Mlh1-P38920	N-Term-137
82	3	2603.261	4	651.5712	LkTEVFDDR	Pms1-P14242	Linker-380	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
82	3	2613.349	4	654.093	HYTSkIAK	Pms1-P14242	N-Term-80	FQDVAkVQTLGFR	Pms1-P14242	N-Term-89
81	2	2840.397	4	710.8552	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46	QENkLVR	Mlh1-P38920	Linker-398
81	2	3176.563	4	794.8966	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46	VcNLNFISkK	Mlh1-P38920	N-Term-253
81	2	3202.534	4	801.3895	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46	LEGSNSSTPTkK	Pms1-P14242	Linker-467
81	2	4904.344	5	981.675	ITSFLSSSQQFNFEGSSTkR	Mlh1-P38920	Linker-427	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446
81	2	3663.765	4	916.6971	DANTINDNDLKDQPkKK	Mlh1-P38920	Linker-471	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
81	2	2267.23	4	567.5633	lkALDASVVNK	Mlh1-P38920	N-Term-6	FTTSkLQK	Mlh1-P38920	N-Term-81
81	2	2961.35	4	741.0933	McSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
81	2	3210.418	4	803.3604	McSQSEQQAQkR	Pms1-P14242	Linker-377	VkGYISQNSFGcGR	Pms1-P14242	N-Term-263
81	2	2886.303	4	722.3317	LEGSNSSTPTkK	Pms1-P14242	Linker-467	McSQSEQQAQkR	Pms1-P14242	Linker-377
81	2	2977.338	4	745.0905	mcSQSEQQAQkR	Pms1-P14242	Linker-477	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
81	2	2088.151	4	522.7936	TPLkNSR	Pms1-P14242	Linker-583	VNVNLTSIkK	Mlh1-P38920	C-Term-515
81	2	2769.367	4	693.0976	TPLkNSR	Pms1-P14242	Linker-583	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46
81	2	2130.156	4	533.2949	TPLkNSR	Pms1-P14242	Linker-583	lkALDASVVNK	Mlh1-P38920	N-Term-6
80.99	2	2071.024	4	518.5118	TPLkNSR	Pms1-P14242	Linker-583	EkVDDSIHR	Mlh1-P38920	C-Term-520
80.99	2	2221.11	4	556.0334	TPLkNSR	Pms1-P14242	Linker-583	LEGSNSSTPTkK	Pms1-P14242	Linker-467
80.99	2	2701.34	4	676.0908	TPLkNSR	Pms1-P14242	Linker-583	kFEDEILEYNLSTK	Pms1-P14242	Linker-605
80.99	2	2897.312	4	725.0839	kSEAQENIIK	Pms1-P14242	Linker-638	NkDELEDFEQGEK	Pms1-P14242	Linker-649
80.99	2	2200.112	4	550.7839	QkEFSK	Pms1-P14242	N-Term-159	FSVVWNITPkGK	Pms1-P14242	N-Term-196
80.97	2	2705.366	4	677.0973	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	QkLGDYK	Mlh1-P38920	Linker-475
80.91	2	2272.978	4	569.0004	mcSQSEQQAQkR	Pms1-P14242	Linker-477	NSkDR	Pms1-P14242	N-Term-278
80.83	2	2285.135	4	572.0396	FQDVAkVQTLGFR	Pms1-P14242	N-Term-89	NSkDR	Pms1-P14242	N-Term-278
80.81	2	1569.757	4	393.195	VkEDR	Mlh1-P38920	N-Term-117	QkEFSK	Pms1-P14242	N-Term-159
80.78	2	2918.355	4	730.3447	VDTSDASLSEDEkAQFINR	Mlh1-P38920	C-Term-717	kTMTR	Pms1-P14242	C-Term-830
80	1	2564.138	4	641.7905	YMkSGNVK	Mlh1-P38920	FRB-70	McSQSEQQAQkR	Pms1-P14242	Linker-377
80	1	2159.184	4	540.5518	QENkLVR	Mlh1-P38920	Linker-398	VNVNLTSIkK	Mlh1-P38920	C-Term-515
80	1	1996.052	4	499.769	QENkLVR	Mlh1-P38920	Linker-398	FTTSkLQK	Mlh1-P38920	N-Term-81
80	1	3960.018	5	792.8099	TVFNkSVASNLITFHISK	Mlh1-P38920	N-Term-219	GPQTLkETSFNQAYGR	Mlh1-P38920	FRB-46
80	1	2592.234	4	648.8143	McSQSEQQAQkR	Pms1-P14242	Linker-377	QELALPkR	Pms1-P14242	Linker-365
80	1	2760.225	4	690.8121	LkTEVFDDR	Pms1-P14242	Linker-380	McSQSEQQAQkR	Pms1-P14242	Linker-377
80	1	2528.218	4	632.8104	LkTEVFDDR	Pms1-P14242	Linker-380	LEGSNSSTPTkK	Pms1-P14242	Linker-467
80	1	2642.32	4	661.3359	LEGSNSSTPTkK	Pms1-P14242	Linker-467	FmLGkQEVIR	Pms1-P14242	FKBP-52
80	1	4996.394	4	1249.854	LEGSNSSTPTkK	Pms1-P14242	Linker-467	LPSIkTDSQNLSDLNLNNFSNPEFQNITSPDK	Pms1-P14242	Linker-473
80	1	2776.241	4	694.816	mcSQSEQQAQkR	Pms1-P14242	Linker-477	LkTEVFDDR	Pms1-P14242	Linker-380
80	1	2015.083	4	504.5266	TPLkNSR	Pms1-P14242	Linker-583	RQENkLVR	Mlh1-P38920	Linker-398
80	1	3468.698	5	694.5458	TPLkNSR	Pms1-P14242	Linker-583	VTNVSHSQEAEkLTLNESEQPR	Mlh1-P38920	Linker-446
80	1	2083.056	4	521.52	TPLkNSR	Pms1-P14242	Linker-583	KSISkDNYR	Pms1-P14242	Linker-591
80	1	1954.964	4	489.4968	TPLkNSR	Pms1-P14242	Linker-583	SISkDNYR	Pms1-P14242	Linker-591
80	1	1920.002	4	480.7563	TPLkNSR	Pms1-P14242	Linker-583	HYTSkIAK	Pms1-P14242	N-Term-80
79.99	1	3515.94	4	879.741	FTTSkLQK	Mlh1-P38920	N-Term-81	ALDASVVNklAAGEIIISPVNALK	Mlh1-P38920	N-Term-15
79.99	1	2929.47	5	586.7003	TPLkNSR	Pms1-P14242	Linker-583	DANTINDNDLKDQPKkK	Mlh1-P38920	Linker-472
79.99	1	2669.384	4	668.1019	TPLkNSR	Pms1-P14242	Linker-583	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489
79.99	1	2327.173	4	582.5491	SISkDNYR	Pms1-P14242	Linker-591	SSIMIGkPLNK	Pms1-P14242	C-Term-825
79.99	1	2366.206	4	592.3074	kSEAQENIIK	Pms1-P14242	Linker-638	QMSSIISkR	Pms1-P14242	Linker-636
79.98	1	3221.674	5	645.1411	VSYAEGkmLESPKPVAGK	Mlh1-P38920	N-Term-131	lkALDASVVNK	Mlh1-P38920	N-Term-6
79.98	1	2377.033	4	595.0141	kFDSSR	Pms1-P14242	FKBP-35	McSQSEQQAQkR	Pms1-P14242	Linker-377
79.98	1	2129.106	4	533.0325	TPLkNSR	Pms1-P14242	Linker-583	MLESPkPVAGK	Mlh1-P38920	N-Term-137
79.98	1	2172.049	4	543.7681	QkEFSK	Pms1-P14242	N-Term-159	LEGSNSSTPTkK	Pms1-P14242	Linker-467
79.97	1	2124.136	4	531.79	QkLGDYK	Mlh1-P38920	Linker-475	VNVNLTSIkK	Mlh1-P38920	C-Term-515
79.97	1	1898.943	4	475.4915	TPLkNSR	Pms1-P14242	Linker-583	YMkSGNVK	Mlh1-P38920	FRB-70
79.97	1	2382.2	4	596.3058	kSEAQENIIK	Pms1-P14242	Linker-638	QmSSIISkR	Pms1-P14242	Linker-636
79.96	1	2562.282	4	641.3265	LEGSNSSTPTkK	Pms1-P14242	Linker-467	MLESPkPVAGK	Mlh1-P38920	N-Term-137
79.95	1	2095.016	4	524.5099	TPLkNSR	Pms1-P14242	Linker-583	LkTEVFDDR	Pms1-P14242	Linker-380
79.94	1	2453.132	4	614.0389	TPLkNSR	Pms1-P14242	Linker-583	McSQSEQQAQkR	Pms1-P14242	Linker-377
79.94	1	2780.531	4	695.8886	SISkDNYR	Pms1-P14242	Linker-591	SLPLLLkGYIPSLVK	Mlh1-P38920	C-Term-657
79.93	1	2051.987	4	513.7526	VkEDR	Mlh1-P38920	N-Term-117	LEGSNSSTPTkK	Pms1-P14242	Linker-467
79.92	1	2266.04	4	567.266	VPkER	Mlh1-P38920	C-Term-504	McSQSEQQAQkR	Pms1-P14242	Linker-377
79.92	1	1702.789	4	426.4532	YMkSGNVK	Mlh1-P38920	FRB-70	NSkDR	Pms1-P14242	N-Term-278
79.92	1	2482.28	4	621.3259	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	VPkER	Mlh1-P38920	C-Term-504
79.92	1	2256.973	4	564.9992	McSQSEQQAQkR	Pms1-P14242	Linker-377	NSkDR	Pms1-P14242	N-Term-278
79.91	1	1849.882	4	463.2264	YMkSGNVK	Mlh1-P38920	FRB-70	QkEFSK	Pms1-P14242	N-Term-159
79.91	1	1605.86	4	402.2209	kSISK	Pms1-P14242	Linker-589	QENkLVR	Mlh1-P38920	Linker-398
79.9	1	2024.957	4	506.9951	LEGSNSSTPTkK	Pms1-P14242	Linker-467	NSkDR	Pms1-P14242	N-Term-278
79.87	1	1515.687	4	379.6777	NSkDR	Pms1-P14242	N-Term-278	kFDSSR	Pms1-P14242	FKBP-35
79.81	1	1719.823	4	430.7116	kTMTR	Pms1-P14242	C-Term-830	YMkSGNVK	Mlh1-P38920	FRB-70
79.8	1	2332.117	4	583.7852	YMkSGNVK	Mlh1-P38920	FRB-70	LEGSNSSTPTkK	Pms1-P14242	Linker-467
79.79	1	1536.788	4	384.9529	DQPkKK	Mlh1-P38920	Linker-471	kTMTR	Pms1-P14242	C-Term-830
79.79	1	2274.011	4	569.2587	kTMTR	Pms1-P14242	C-Term-830	McSQSEQQAQkR	Pms1-P14242	Linker-377
79.68	1	1645.815	4	412.2096	kSISK	Pms1-P14242	Linker-589	YMkSGNVK	Mlh1-P38920	FRB-70
67	8	3274.501	4	819.3812	FGDSNYSLSVkPSYTVQDR	Mlh1-P38920	N-Term-204	DIGFSckK	Mlh1-P38920	N-Term-192
65.97	7	2026.06	4	507.2708	FMLGkQEVIR	Pms1-P14242	FKBP-52	TFPkR	Pms1-P14242	FKBP-17
63	4	4075.958	4	1019.745	YAIHSkDIGFScK	Mlh1-P38920	N-Term-185	VEDLNLESVDGkVcNLNFISK	Mlh1-P38920	N-Term-244
62	3	1901.046	4	476.0175	VPkER	Mlh1-P38920	C-Term-504	VNVNLTSIkK	Mlh1-P38920	C-Term-515
61.97	3	3851.873	4	963.7242	IDASQAkITSFLSSSQQFNFEGSSTK	Mlh1-P38920	Linker-408	QENkLVR	Mlh1-P38920	Linker-398
61	2	1927.044	4	482.5169	TPLkNSR	Pms1-P14242	Linker-583	QELALPkR	Pms1-P14242	Linker-365
60.99	2	2977.344	4	745.0918	McSQSEQQAQkR	Pms1-P14242	Linker-377	kNISSVFGAGGmR	Pms1-P14242	N-Term-213
60.96	2	3210.635	5	642.9332	RQENkLVR	Mlh1-P38920	Linker-398	QLSEPkVTNVSHSQEAEK	Mlh1-P38920	Linker-434
60.96	2	1835.029	4	459.5132	kSISK	Pms1-P14242	Linker-589	VNVNLTSIkK	Mlh1-P38920	C-Term-515
60.88	2	2079.136	4	520.5399	kLPSIK	Pms1-P14242	Linker-468	FmLGkQEVIR	Pms1-P14242	FKBP-52
60.83	2	2042.062	4	511.2715	TFPkR	Pms1-P14242	FKBP-17	FmLGkQEVIR	Pms1-P14242	FKBP-52
60	1	3054.518	5	611.7098	QENkLVR	Mlh1-P38920	Linker-398	QLSEPkVTNVSHSQEAEK	Mlh1-P38920	Linker-434
60	1	4419.104	5	884.627	ITSFLSSSQQFNFEGSSTkR	Mlh1-P38920	Linker-427	QLSEPkVTNVSHSQEAEK	Mlh1-P38920	Linker-434
60	1	2234.146	4	559.2924	LkTEVFDDR	Pms1-P14242	Linker-380	QELALPkR	Pms1-P14242	Linker-365
60	1	1925.015	4	482.0097	TPLkNSR	Pms1-P14242	Linker-583	FTTSkLQK	Mlh1-P38920	N-Term-81
59.99	1	2701.365	4	676.0971	FMLGkQEVIR	Pms1-P14242	FKBP-52	kNISSVFGAGGMR	Pms1-P14242	N-Term-213
59.98	1	1729.822	4	433.2113	VkEDR	Mlh1-P38920	N-Term-117	YMkSGNVK	Mlh1-P38920	FRB-70
59.97	1	2965.461	5	593.8984	QkLGDYK	Mlh1-P38920	Linker-475	DANTINDNDLkDQPKKK	Mlh1-P38920	Linker-467
59.96	1	2597.347	4	650.0927	VPSIADDEkNALPISK	Mlh1-P38920	Linker-489	DQPkKK	Mlh1-P38920	Linker-471
59.95	1	2426.408	4	607.3579	VPkER	Mlh1-P38920	C-Term-504	SLPLLLkGYIPSLVK	Mlh1-P38920	C-Term-657

59.95	1	1785.842	4	447.2163	VkEDR	Mlh1-P38920	N-Term-117	SISkDNYR	Pms1-P14242	Linker-591
59.95	1	2200.016	4	550.7598	McSQSEQQAQkR	Pms1-P14242	Linker-377	kSISK	Pms1-P14242	Linker-589
59.93	1	2286.03	4	572.2634	McSQSEQQAQkR	Pms1-P14242	Linker-377	TFPkR	Pms1-P14242	FKBP-17
59.93	1	3225.534	4	807.1393	MLGkYTDDPDFLDLDYK	Pms1-P14242	N-Term-246	lAkFQDVAK	Pms1-P14242	N-Term-83
59.92	1	1559.756	4	390.6948	kTMTR	Pms1-P14242	C-Term-830	QkEFSK	Pms1-P14242	N-Term-159
59.9	1	1967.995	4	492.7546	kSISK	Pms1-P14242	Linker-589	LEGSNSSTPTkK	Pms1-P14242	Linker-467
59.88	1	1635.83	4	409.7134	cSklR	Pms1-P14242	C-Term-807	TPLkNSR	Pms1-P14242	Linker-583
59.86	1	1661.828	4	416.2128	kSISK	Pms1-P14242	Linker-589	YmkSGNVK	Mlh1-P38920	FRB-70
59.85	1	3214.525	5	643.7113	YMkSGNVK	Mlh1-P38920	FRB-70	NVkGMFEVLEPLHAMMER	Mlh1-P38920	FRB-25
59.82	1	1755.897	4	439.7301	VkEDR	Mlh1-P38920	N-Term-117	FTTSkLQK	Mlh1-P38920	N-Term-81
59.8	1	1942.012	4	486.2588	kSISK	Pms1-P14242	Linker-589	VcNLNFISkK	Mlh1-P38920	N-Term-253
59.77	1	4007.996	4	1002.755	RQENkLVR	Mlh1-P38920	Linker-398	IDASQAkITSFLSSSQQFNFEGSSTK	Mlh1-P38920	Linker-408
59.77	1	2425.204	4	607.057	lAkFQDVAK	Pms1-P14242	N-Term-83	LEGSNSSTPTkK	Pms1-P14242	Linker-467
59.64	1	1877.041	4	470.0161	kSISK	Pms1-P14242	Linker-589	lkALDASVVNK	Mlh1-P38920	N-Term-6
40	1	2209.136	4	553.04	TPLkNSR	Pms1-P14242	Linker-583	FmLGkQEVIR	Pms1-P14242	FKBP-52
39.99	1	3385.775	4	847.1997	TVFNkSVASNLITFHISK	Mlh1-P38920	N-Term-219	VcNLNFISkK	Mlh1-P38920	N-Term-253
39.99	1	2343.124	4	586.5369	kTMTR	Pms1-P14242	C-Term-830	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
39.97	1	1679.855	4	420.7197	kTMTR	Pms1-P14242	C-Term-830	QENkLVR	Mlh1-P38920	Linker-398
39.96	1	2335.156	4	584.545	VPkER	Mlh1-P38920	C-Term-504	LGDYkVPSIADDEK	Mlh1-P38920	Linker-480
39.96	1	1608.865	4	402.9722	QkEFSK	Pms1-P14242	N-Term-159	kLPSIK	Pms1-P14242	Linker-468
39.95	1	2935.378	5	587.8818	NSkDR	Pms1-P14242	N-Term-278	RPVEYSTLLkccNEVYK	Pms1-P14242	N-Term-297
39.54	1	1773.925	4	444.2372	KYMkSGNVK	Mlh1-P38920	FRB-70	kSISK	Pms1-P14242	Linker-589
30.6	2	2965.451	4	742.1187	DANTINDNDLKDQPkKK	Mlh1-P38920	Linker-471	QkLGDYK	Mlh1-P38920	Linker-475
30.53	2	2016.115	4	504.7847	DQPkKK	Mlh1-P38920	Linker-471	VNVNLTSIkK	Mlh1-P38920	C-Term-515
29.77	1	1551.783	4	388.7015	VPkER	Mlh1-P38920	C-Term-504	QkEFSK	Pms1-P14242	N-Term-159
29.71	1	2613.505	4	654.132	TPLkNSR	Pms1-P14242	Linker-583	SLPLLLkGYIPSLVK	Mlh1-P38920	C-Term-657
29.6	1	2900.422	4	725.8613	RQENkLVR	Mlh1-P38920	Linker-398	DANTINDNDLkDQPK	Mlh1-P38920	Linker-467
29.6	1	2041.99	4	511.2533	LEGSNSSTPTkK	Pms1-P14242	Linker-467	kTMTR	Pms1-P14242	C-Term-830
29.52	1	1943.065	4	486.5222	lkALDASVVNK	Mlh1-P38920	N-Term-6	VPkER	Mlh1-P38920	C-Term-504
29.38	1	1845.964	4	462.2468	RQENkLVR	Mlh1-P38920	Linker-398	VkEDR	Mlh1-P38920	N-Term-117
19.87	1	1528.812	4	382.959	VPkER	Mlh1-P38920	C-Term-504	DQPkKK	Mlh1-P38920	Linker-471
19.76	1	1761.966	4	441.2474	kSISK	Pms1-P14242	Linker-589	RQENkLVR	Mlh1-P38920	Linker-398
19.75	1	1981.026	4	496.0124	kTMTR	Pms1-P14242	C-Term-830	SSIMIGkPLNK	Pms1-P14242	C-Term-825

Definitions

Score: MaXLinker score; see Yugandhar et al. (2020) Mol. Cell Proteomics 19, 554-568 (2020).
#CSMs: Number of cross-link spectrum matches
XL MH+: Deconvoluted mass of the cross-link. It is a standard practice to present the deconvoluted mass along with the mass of an additional H+
Z: Charge of the cross-link
XL m/z: Cross-link mass divided by its charge
Uniprot: https://www.uniprot.org
lower case k indicates site of cross-link

Table S3C. Summary of intermolecular crosslinks for Mlh1-Pms1 and Complex #5.

Mlh1-Pms1 (15 in total, with the amino acid positions crosslinked in each subunit shown)
Mlh1-N-Term-6 + Pms1-N-Term-83
Mlh1-N-Term-6 + Pms1-N-Term-159
Mlh1-Linker-398 + Pms1-Linker-583
Mlh1-Linker-398 + Pms1-Linker-591
Mlh1-Linker-480 + Pms1-C-Term-830
Mlh1-Linker-489 + Pms1-C-Term-830
Mlh1-Linker-489 + Pms1-Linker-583
Mlh1-Linker-489 + Pms1-Linker-591
Mlh1-C-Term-504 + Pms1-Linker-605
Mlh1-C-Term-515 + Pms1-N-Term-278
Mlh1-C-Term-515 + Pms1-Linker-583
Mlh1-C-Term-515 + Pms1-Linker-589
Mlh1-C-Term-515 + Pms1-Linker-605
Mlh1-C-Term-717 + Pms1-C-Term-672
Mlh1-C-Term-717 + Pms1-C-Term-830

Complex #5 (34, with the amino acid positions crosslinked in each subunit shown)
Mlh1-N-Term-6 + Pms1-Linker-583
Mlh1-N-Term-6 + Pms1-Linker-589
Mlh1-N-Term-81 + Pms1-Linker-583
Mlh1-N-Term-117 + Pms1-N-Term-159
Mlh1-N-Term-117 + Pms1-Linker-467
Mlh1-N-Term-117 + Pms1-Linker-591
Mlh1-N-Term-137 + Pms1-Linker-467
Mlh1-N-Term-137 + Pms1-Linker-583
Mlh1-N-Term-253 + Pms1-Linker-589
Mlh1-Linker-398 + Pms1-Linker-583
Mlh1-Linker-398 + Pms1-Linker-589
Mlh1-Linker-398 + Pms1-C-Term-830
Mlh1-Linker-446 + Pms1-Linker-583
Mlh1-Linker-471 + Pms1-C-Term-830
Mlh1-Linker-472 + Pms1-Linker-583
Mlh1-Linker-480 + Pms1-C-Term-830
Mlh1-Linker-489 + Pms1-Linker-583
Mlh1-C-Term-504 + Pms1-N-Term-159
Mlh1-C-Term-504 + Pms1-Linker-377
Mlh1-C-Term-515 + Pms1-Linker-583
Mlh1-C-Term-515 + Pms1-Linker-589
Mlh1-C-Term-520 + Pms1-Linker-583
Mlh1-C-Term-657 + Pms1-Linker-583
Mlh1-C-Term-657 + Pms1-Linker-591
Mlh1-C-Term-717 + Pms1-C-Term-830
Mlh1-FRB-46 + Pms1-Linker-467
Mlh1-FRB-46 + Pms1-Linker-583
Mlh1-FRB-70 + Pms1-N-Term-159
Mlh1-FRB-70 + Pms1-N-Term-278
Mlh1-FRB-70 + Pms1-Linker-377
Mlh1-FRB-70 + Pms1-Linker-467
Mlh1-FRB-70 + Pms1-Linker-583
Mlh1-FRB-70 + Pms1-Linker-589
Mlh1-FRB-70 + Pms1-C-Term-830

Table S4. Phenotypic analysis of *mlh1* and *pms1* alleles that map within or near Mlh1-Pms1 crosslinking sites (Figure 2A) as determined by XL-MS

Crosslinking site (amino acid position) in Mlh1	*mlh1* allele	DNA mismatch repair phenotype	Reference
6	*R4A, K6A*	+/-	Argueso *et al*, 2003
398	*E396A, K398A*	+, but – nearby	Argueso *et al*, 2003
480	*D478A, K480A*	+	Argueso *et al*, 2003
489	*D486A, D487A, E488A, K489A*	+	Argueso *et al*, 2003
504	*K504A, E505A, R506A*	+/-	Argueso *et al*, 2003
515	*K515A, K516A*	+/-	Argueso *et al*, 2003
717	*E714A, D715A, E716A, K717A*	+	Argueso *et al*, 2003
Crosslinking site (amino acid position) in Pms1			
83	*G97A*	-	Tran & Liskay, 2000
159	ATP binding domain		Arana *et al*, 2010
278	Maps to DNA binding region		Hall *et al*, 2003
583-605	*IDR deletion (584-634)*	+/-	Plys *et al*, 2012
672	Maps to endonuclease site		Smith *et al,* 2013
830	*C817R, C848S;* maps to endonuclease site	-	Smith *et al,* 2013

+ indicates phenotype similar to wild-type, - indicates similar to MMR null, +/- intermediate phenotype.