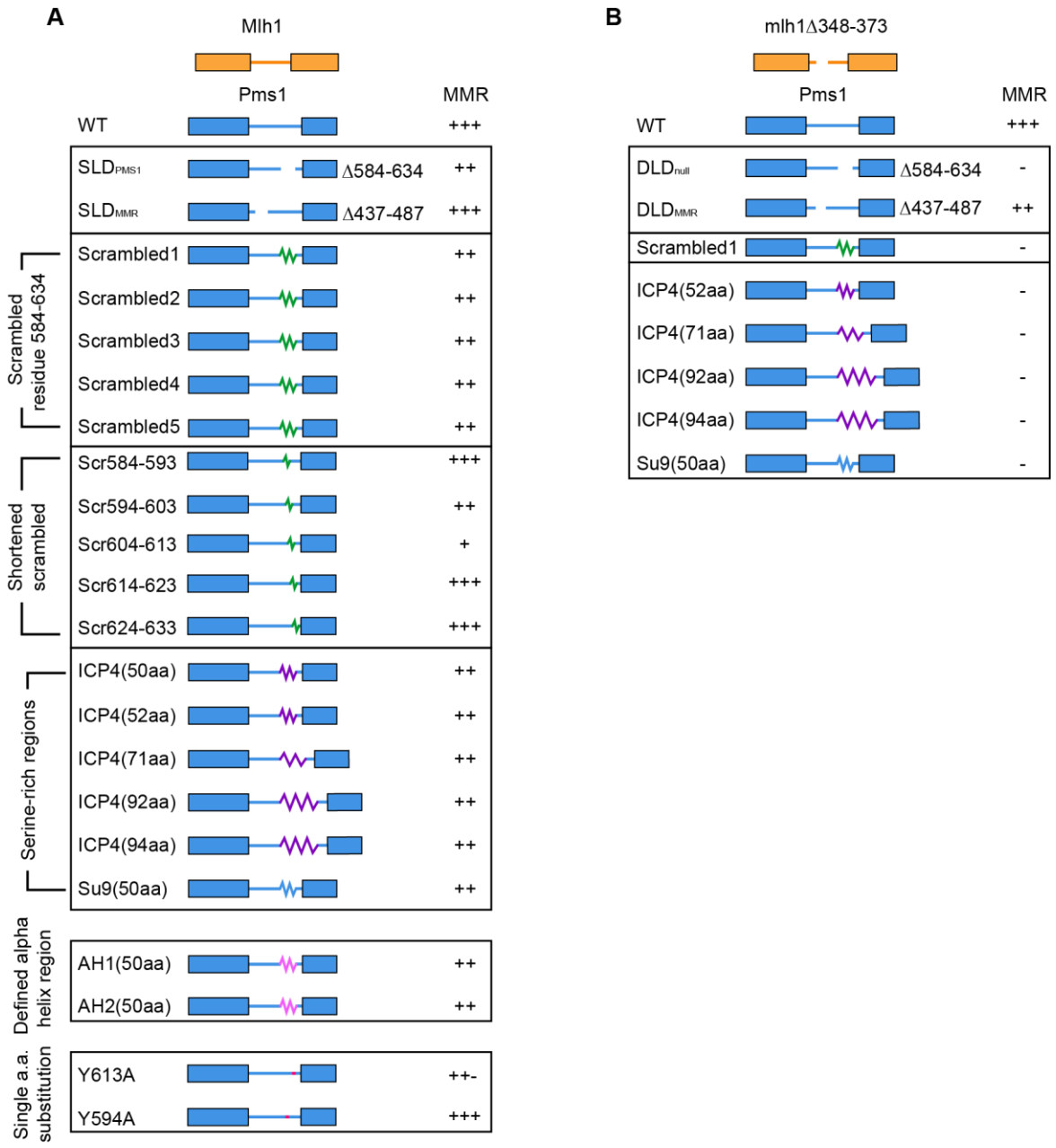

Figure S1. (A) Schematic of specific sequences that replace the IDRs in Pms1, followed by the mutator phenotype conferred by the indicated alleles in the *MLH1* strain background. +++ indicates a wild-type mutation rate, ++, ++- and + indicate hypomorph phenotypes, and – indicates a null phenotype. See text and ***Supplementary Table S1*** and ***Supplementary Table S4*** for quantitative data and detailed description of the specific sequences. (B) Analysis of linker alleles presented in panel A in the *mlh1-Δ348-373* background.

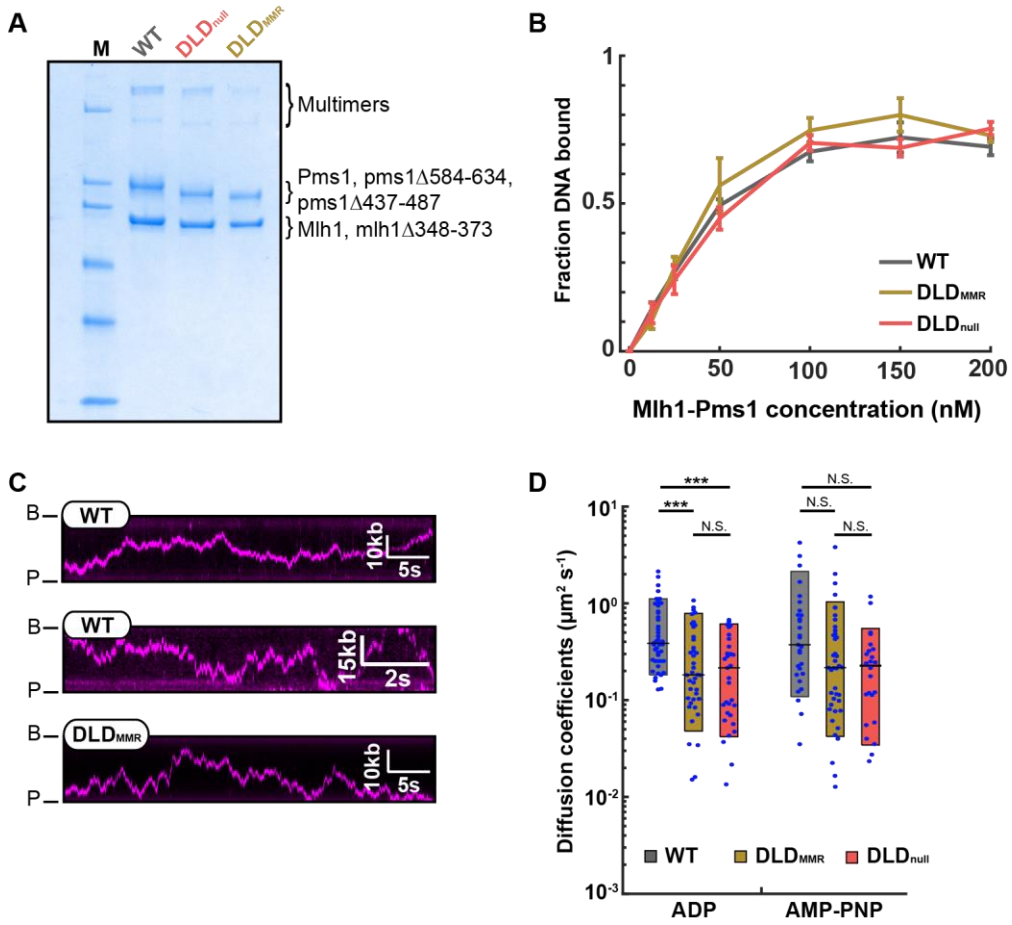

Figure S2. (A) SDS-PAGE analysis (8% Coomassie blue R250 stained gel) of purified wild-type (WT) Mlh1-FLAG-Pms1, DLD_{null} (mlh1Δ348-373-FLAG-pms1Δ584-634), and DLD_{MMR} (mlh1Δ348-373-FLAG-pms1Δ438-487). (B) Analysis of DNA binding in the absence of nucleotide. For each complex, DNA binding was analyzed by filter binding. Mlh1-Pms1 variants were included at final concentrations of 12.5 nM, 25 nM, 50 nM, 100 nM, 150 nM and 200 nM in buffer containing 25 mM NaCl. DNA binding was quantified by scintillation counting. Four replicates were averaged; error bars indicate the SD. (C) Representative kymographs of WT and DLD_{MMR} complexes loaded at 50 mM NaCl and imaged at 150 mM NaCl (top and bottom), and WT Mlh1-Pms1 loaded and imaged at 150 mM NaCl (middle). These images show that the proteins are freely diffusing on DNA. Complexes that were loaded at low or high NaCl concentration were indistinguishable in the single-molecule assays. B and P indicate barrier and pedestals, respectively. (D) Diffusion coefficients of the four Mlh1-Pms1 complexes with ADP or AMP-PNP. The black bar in the box plot represents the median of the distribution. * *P*-values <0.05, ** *P*-value < 0.01, and *** *P* value < 0.005. N.S. indicates p > 0.05.

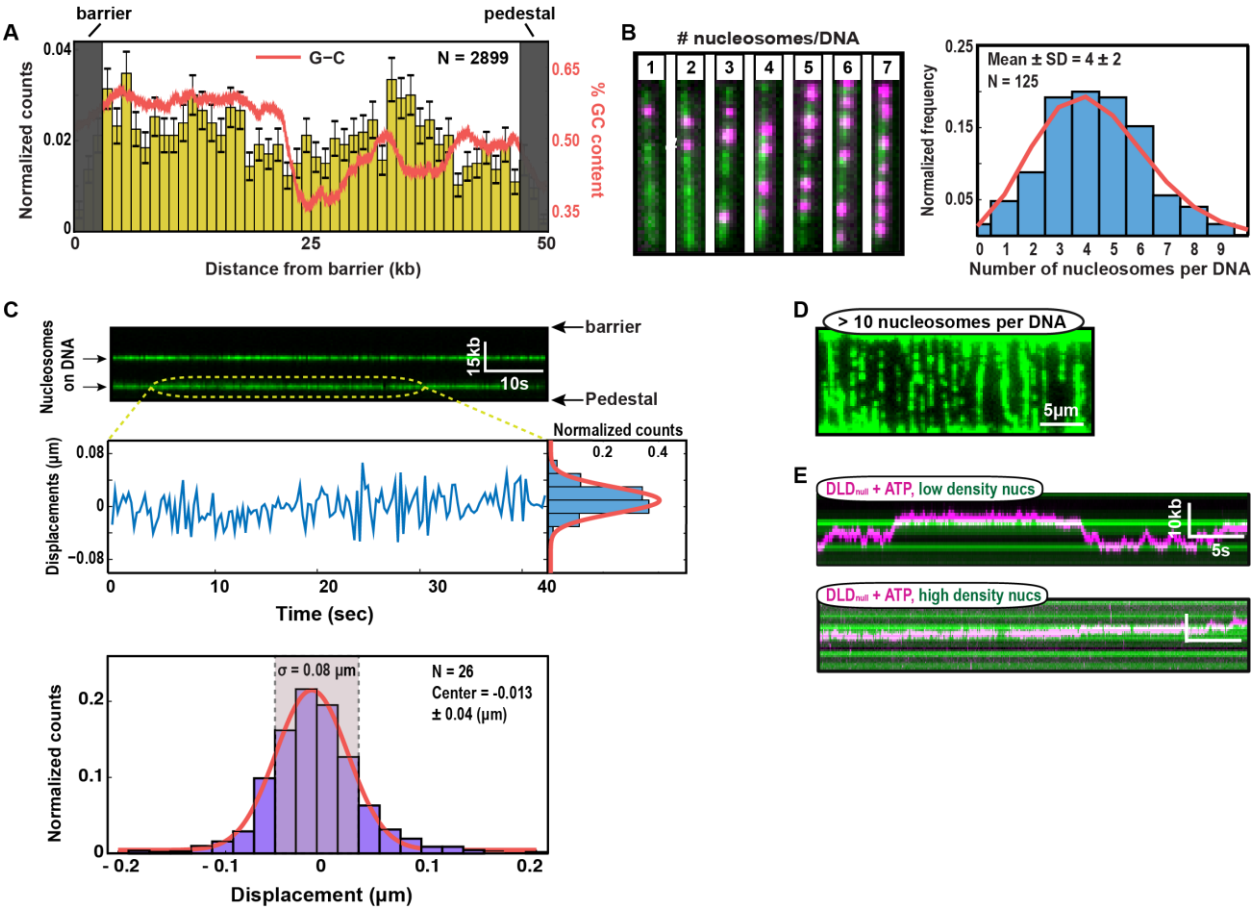

Figure S3. (A) Distribution of human nucleosomes on DNA curtains indicates a weak preference for GC-rich sequences. Error bars were generated by bootstrap analysis. The % GC content for a 2 kbp sliding window is shown in the red line. (B) Representative images of various numbers of nucleosomes per DNA (left), and a histogram of frequency of nucleosome deposition on DNA fitted to a Poisson distribution (red line) with the mean of the data (right). (C) Definition of 'nucleosome zone' for bypass analysis. To determine the spatial resolution, a distribution of the net displacement of single nucleosomes was fit to a Gaussian distribution (red line). For analyzing single nucleosome bypass frequencies, the nucleosome zone was defined as a three-sigma region surrounding the mean nucleosome position (bottom). (D) A fluorescent image of double-tethered DNA curtain with > 10 nucleosomes per DNA. Nucleosomes were labeled with anti-HA antibody conjugated QDs (green). The position of each nucleosome cannot be determined due to overlapping fluorescent nucleosome signals. (E) Representative kymograph of DLD_{null} on different nucleosome density substrate in the presence of ATP.

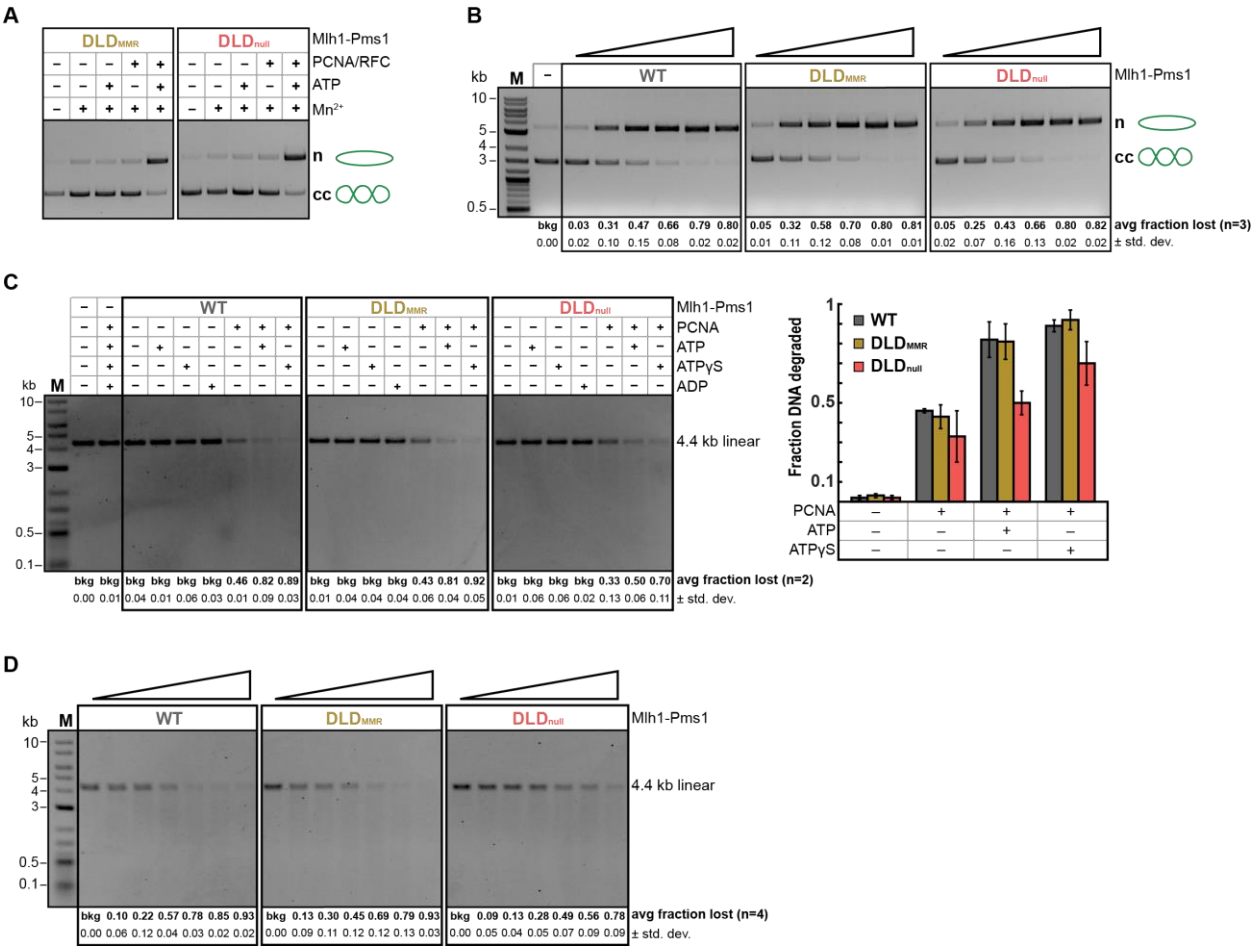

Figure S4. (A) Endonuclease activity on a closed circular DNA in the presence (+) or absence (-) of $MnSO_4$, ATP, and yeast RFC/PCNA. Where + is indicated, the concentration of $MnSO_4$ was 2.5 mM, ATP was 0.5 mM, RFC and PCNA were each 500 nM. The final concentration of Mlh1-Pms1 variants was 100 nM. All variants were analyzed side-by-side with WT as a control (***Figure 4A***). (B) Gel for data in ***Figure 4A*** right panel. Mlh1-Pms1 variants were titrated from 0-200 nM in the presence of 2.5 mM $MnSO_4$, 0.5 mM, ATP, 500 nM RFC, and 500 nM PCNA. All variants were analyzed side-by-side with WT as a control. Final image is arranged for simplicity. Quantification from three separate experiments is shown below the gel. (C) Left panel, where indicated, the final concentration of nucleotide is 0.5 mM. The amount of substrate lost was quantified and expressed as a fraction by comparing to the average of lanes 2-3. The average fraction lost was calculated from duplicate experiments. The standard deviation between two independent experiments is expressed below the gel. All variants were analyzed side-by-side with WT as a control. Final image is arranged for simplicity. Right panel, analysis of endonuclease activity of wild-type and mutant Mlh1-Pms1 complexes (100 nM) on linear DNA. Reactions contain 500 nM PCNA and 0.5 mM ATP or ATPγS as indicated. 5 mM $MnSO_4$ is included in all reactions. (D) Gel for data reported in ***Figure 4B***. The amount of substrate lost was quantified and expressed as a fraction by comparing to lane 2, 9, or 16 for each variant. The average fraction lost and SD were calculated from four replicates (SD expressed below the gel). All variants were analyzed in a single gel. Final image is arranged for simplicity.

Supplementary Table S1. *pms1* linker arm insertions are unable to rescue *pms1Δ584-634* MMR defects

relevant genotype	mutation rate (x 10^{-7}) (95% CI)	*n*	relative to wild type
*pms1*Δ (EAY3097)	25,700 (15,400-36,700)	45	7,930
PMS1	3.24 (2.75-4.32)	40	1.0
$pms1\Delta_{584\text{-}634}$	308 (230-452)	24	95
$pms1_{50\text{-}scramble1}$	332 (213-414)	20	103
$pms1_{50\text{-}scramble2}$	238 (184-338)	20	74
$pms1_{50\text{-}scramble3}$	335 (303-405)	20	104
$pms1_{50\text{-}scramble4}$	413 (339-637)	20	127
$pms1_{50\text{-}scramble5}$	520 (357-615)	20	161
$pms1_{40\text{-}alpha\text{-}helix1}$	378 (214-505)	20	117
$pms1_{40\text{-}alpha\text{-}helix2}$	465 (335-645)	20	144
$pms1_{50\text{-}SRR\text{-}1}$	436 (391-575)	20	135
$pms1_{52\text{-}SRR}$	315 (210-444)	20	97
$pms1_{71\text{-}SRR}$	413 (318-592)	20	128
$pms1_{92\text{-}SRR}$	421 (314-639)	20	130
$pms1_{94\text{-}SRR}$	514 (414-745)	20	159
$pms1_{50\text{-}SRR\text{-}2}$	229 (120-340)	20	71
$pms1_{scramble584\text{-}593}$	33.4(12.1-58.3)	15	10
$pms1_{scramble594\text{-}603}$	286(157-428)	15	88
$pms1_{scramble604\text{-}613}$	1,180(870-1,360)	15	365
$pms1_{scramble614\text{-}623}$	28.6(13.8-42.6)	15	9
$pms1_{scramble624\text{-}633}$	26.3(16.7-30.8)	15	8
$pms1_{Y613A}$	89(55-128)	20	27
$pms1_{Y594A}$	22.7(8.8-32.8)	15	7
*mlh1*Δ, *pms1*Δ (EAY1365)	13,400 (9,330-15,400)	30	4,570
MLH1, PMS1	2.94 (2.2-4.1)	20	1.0
$mlh1\Delta_{348\text{-}373}$, *PMS1*	206 (118-256)	20	70
$mlh1\Delta_{348\text{-}373}$, $pms1\Delta_{584\text{-}634}$	8,420 (4,460-10,300)	20	2,860

*mlh1*Δ$_{348\text{-}373}$, *pms1*Δ$_{437\text{-}487}$	429 (393-857)	20	146
*mlh1*Δ$_{348\text{-}373}$, *pms1*$_{50\text{-}scramble1}$	13,100 (9,280-17,400)	20	4,450
*mlh1*Δ$_{348\text{-}373}$, *pms1*$_{52\text{-}SRR}$	3,000 (2,150-7,470)	20	1,020
*mlh1*Δ$_{348\text{-}373}$, *pms1*$_{71\text{-}SRR}$	3,858 (3,260-7,150)	20	1,320
*mlh1*Δ$_{348\text{-}373}$, *pms1*$_{92\text{-}SRR}$	5,870 (5,100-7,330)	20	2,000
*mlh1*Δ$_{348\text{-}373}$, *pms1*$_{50\text{-}SRR\text{-}2}$	3,430 (2,890-6,180)	20	1,170
Mlh1, *pms1*$_{Mlh1\ linker}$	13,700 (9,580-19,700)	15	4,670
mlh1$_{Pms1\ linker}$, *Pms1*	7,680 (4,170-11,700)	15	2,610
mlh1$_{Pms1\ linker}$, *pms1*$_{Mlh1\ linker}$	8,330 (3,130-20,400)	15	2.830

The indicated *mlh1* and *pms1* alleles were tested in the *lys2::insE-A*$_{14}$ reversion assay, and Lys$^+$ reversion rates (CI, confidence interval) were calculated as described in the *Materials and Methods*. n, number of independent measurements. The *PMS1* and *pms1* alleles were expressed from the native *PMS1* promoter in pRS413 derived *ARS-CEN HIS3* plasmids. The *MLH1* and *mlh1* alleles were expressed from the native *MLH1* promoter in pRS415 derived *ARS-CEN LEU2* plasmids. These plasmids (***Supplementary Table S4***) were transformed into EAY3097 (*pms1*Δ) and EAY1365 (*mlh1*Δ, *pms1*Δ) strains.

Supplementary Table S2. Nucleotide-dependent Mlh1-Pms1 diffusion coefficients

Nucleotide type	Protein type	1D diffusion ± S.E.M. ($\mu m^2 s^{-1}$)	Number of molecules	p-value (relative to WT)	
				t-test	K-S test
None	WT	0.507 ± 0.07	56	N/A	
	DLD_{MMR}	0.223 ± 0.04	41	0.1	0.01
	DLD_{null}	0.078 ± 0.01	39	$1.5x10^{-6}$	$6.7x10^{-9}$
ADP	WT	0.584 ± 0.07	46	N/A	
	DLD_{MMR}	0.303 ± 0.04	40	0.001	0.0007
	DLD_{null}	0.376 ± 0.14	34	0.15	0.001
AMP-PNP	WT	0.753 ± 0.18	30	N/A	
	DLD_{MMR}	0.451 ± 0.11	41	0.13	0.99
	DLD_{null}	0.275 ± 0.05	26	0.02	0.07
ATP	WT	0.918 ± 0.10	59	N/A	
	DLD_{MMR}	0.646 ± 0.10	50	0.069	0.007
	DLD_{null}	0.171 ± 0.02	42	$8.2x10^{-8}$	$9.3x10^{-13}$

All data points were acquired in imaging buffer containing 150 mM NaCl.

Supplementary Table S3. Probability of single nucleosome bypass by Mlh1-Pms1 complexes

Nucleotide type	Protein-Condition	Number of trajectories	Number of collisions	Probability ± std. dev.	P-value (relative to WT)	P-value (relative to DLD_{MMR})	Average number of collisions per trajectory ± std. dev.
Minus	WT	31	1361	0.30 ± 0.003	N/A		44 ± 44
	DLD_{MMR}	29	1166	0.18 ± 0.005	$2.8x10^{-11}$		40 ± 29
	DLD_{null}	27	1033	0.19 ± 0.002	$6.1x10^{-9}$	0.54	38 ± 24
ATP	WT	34	1223	0.21 ± 0.007	N/A		35 ± 35
	DLD_{MMR}	30	1572	0.063 ± 0.004	$4.8x10^{-28}$		56 ± 60
	DLD_{null}	30	1111	0.070 ± 0.002	$1.9x10^{-19}$	0.08	37 ± 30

P-values are determined from fitting binary logistic regression relative to WT with same nucleotide condition.

Supplementary Table S4. Plasmids used in this study

Plasmid	Relevant genotype	Vector type	Source
pRS413		*ARS-CEN, HIS3*	(Christianson et al., 1992)
pRS415		*ARS-CEN, LEU2*	(Christianson et al., 1992)
pEAA213	*MLH1*	*ARS-CEN, LEU2*	(Plys et al., 2012)
pEAA526	$mlh1\Delta_{348–373}$ $(FLAG_{499})$	*ARS-CEN, LEU2*	(Plys et al., 2012)
pEAA238	*PMS1*	*ARS-CEN, HIS3*	(Plys et al., 2012)
pEAA544	$pms1\Delta_{437-487}$ (HA_{565})	*ARS-CEN, HIS3*	(Plys et al., 2012)
pEAA548	$pms1\Delta_{584–634}$ (HA_{565})	*ARS-CEN, HIS3*	(Plys et al., 2012)
pEAA644	$pms1_{50\text{-}scramble1}$	*ARS-CEN, HIS3*	This study
pEAA645	$pms1_{50\text{-}scramble2}$	*ARS-CEN, HIS3*	This study
pEAA646	$pms1_{50\text{-}scramble3}$	*ARS-CEN, HIS3*	This study
pEAA647	$pms1_{50\text{-}scramble4}$	*ARS-CEN, HIS3*	This study
pEAA648	$pms1_{50\text{-}scramble5}$	*ARS-CEN, HIS3*	This study
pEAA649	$pms1_{40\text{-}alpha\text{-}helix1}$	*ARS-CEN, HIS3*	This study
pEAA650	$pms1_{40\text{-}alpha\text{-}helix2}$	*ARS-CEN, HIS3*	This study
pEAA651	$pms1_{50\text{-}SRR\text{-}1}$	*ARS-CEN, HIS3*	This study
pEAA652	$pms1_{52\text{-}SRR}$	*ARS-CEN, HIS3*	This study
pEAA653	$pms1_{71\text{-}SRR}$	*ARS-CEN, HIS3*	This study
pEAA654	$pms1_{92\text{-}SRR}$	*ARS-CEN, HIS3*	This study
pEAA655	$pms1_{94\text{-}SRR}$	*ARS-CEN, HIS3*	This study
pEAA656	$pms1_{50\text{-}SRR\text{-}2}$	*ARS-CEN, HIS3*	This study
pEAA657	$pms1_{full\ mlh1\ Linker}$	*ARS-CEN, HIS3*	This study
pEAA658	$mlh1_{full\ pms1\ Linker}$	*ARS-CEN, LEU2*	This study
pEAA659	$pms1_{Y613A}$	*ARS-CEN, HIS3*	This study

pEAA660	*pms1*$_{Y594A}$	*ARS-CEN, HIS3*	This study
pEAA661	*pms1*$_{scramble584-593}$	*ARS-CEN, HIS3*	This study
pEAA662	*pms1*$_{scramble594-603}$	*ARS-CEN, HIS3*	This study
pEAA663	*pms1*$_{scramble604-613}$	*ARS-CEN, HIS3*	This study
pEAA664	*pms1*$_{scramble614-623}$	*ARS-CEN, HIS3*	This study
pEAA665	*pms1*$_{scramble624-633}$	*ARS-CEN, HIS3*	This study
pEAE269	*GAL1-MLH1(FLAG*$_{499}$*)-VMA1-CBD*	*2μ, TRP1*	(Plys et al., 2012)
pEAE308	*GAL1-mlh1*$\Delta_{348–373}$*(FLAG*$_{499}$*)-VMA1-CBD*	*2μ, TRP1*	(Plys et al., 2012)
pMH8	*GAL10-PMS1*	*2μ, LEU2*	(Hall and Kunkel, 2001)
pEAE388	*GAL10-pms1Δ*$_{437-487}$	*2μ, LEU2*	This study
pEAE419	*GAL10-pms1Δ*$_{584–634}$	*2μ, LEU2*	This study

Plasmid constructs expressing pms1 proteins in which the specified amino acid sequences replace those deleted in *pms1Δ*$_{584–634}$.

Plasmid	insert	*amino acid sequence replacement*
pEAA644	*pms1*$_{50\text{-}scramble1}$	*SSKSNKFGINSNKSLIDGKRNERFLMLDKL KNSEQIISTRESDSKYYETHI*
pEAA645	*pms1*$_{50\text{-}scramble2}$	*ISKMSKILKKNIESSSGEYNKSFLNSEQRNS YSGTLINLKDDTEDIKFRHR*
pEAA646	*pms1*$_{50\text{-}scramble3}$	*DTIERNGESIKLDFSESFKKSISTKSKLKNM ESSIRYKIRGLLNDNYQSNH*
pEAA647	*pms1*$_{50\text{-}scramble4}$	*NKSSQSKIGLRSRLYIGTMTENSSKSFEDIK KHSDSNILLYKNFRDEKINE*
pEAA648	*pms1*$_{50\text{-}scramble5}$	*GGRNIKEINFKLIQIKKEDSLLSKSIDMRH TKSNFTNYESYSDKESRSSNL*
pEAA649	*pms1*$_{40\text{-}alpha\text{-}helix1}$	*NSRKSEAAAKEAAAKEAAAKEAAAKEAAA KEAAAKEAAAKEAAAKQMSSII*
pEAA650	*pms1*$_{40\text{-}alpha\text{-}helix2}$	*SMISQEAAAKEAAAKEAAAKEAAAKEAAA KEAAAKEAAAKEAAAKNKISSR*
pEAA651	*pms1*$_{50\text{-}SRR\text{-}1}$	*SSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSS SSSASSSSGSGTMKHGT*
pEAA652	*pms1*$_{52\text{-}SRR}$	*RRSSSTSSDSGSSSSSSASSSSGSSSTSSDSGS SSSSSASSSSGSGTMKHGT*

pEAA653	*pms1*$_{71\text{-}SRR}$	*SSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSS SSSASSSSGSSSTSSDSGSSSSSSASSSSGSGT MKHGT*
pEAA654	*pms1*$_{92\text{-}SRR}$	*SSSTSSDSGSSSSSSASSSSGSSSTSSDSGSSS SSSASSSSGSSSTSSDSGSSSSSSASSSSGSSST SSDSGSSSSSSASSSSGSGTMKHGT*
pEAA655	*pms1*$_{94\text{-}SRR}$	*RRSSSTSSDSGSSSSSSASSSSGSSSTSSDSGS SSSSSASSSSGSSSTSSDSGSSSSSSASSSSGSS STSSDSGSSSSSSASSSSGSGTMKHGT*
pEAA656	*pms1*$_{50\text{-}SRR\text{-}2}$	*MASTRVLASRLASQMAASAKVARPAVRVA XVSKRTIQTGSPLQTRAYSS*

Plasmid constructs expressing pms1 proteins in which the specified amino acid sequences replace those deleted in the designated region

pEAA661	*pms1*$_{scramble584\text{-}593}$	*ISKMSKILKK*
pEAA662	*pms1*$_{scramble594\text{-}603}$	*NIESSSGEYN*
pEAA663	*pms1*$_{scramble604\text{-}613}$	*KSFLNSEQRN*
pEAA664	*pms1*$_{scramble614\text{-}623}$	*SYSGTLINLK*
pEAA665	*pms1*$_{scramble624\text{-}633}$	*DDTEDIKFRH*

Full plasmid descriptions can be found in the Materials and Methods.