SUPPLEMENTARY MATERIAL

for

A STRUCTURAL HINGE IN EUKARYOTIC MUTY HOMOLOGUES MEDIATES CATALYTIC ACTIVITY AND RAD9-RAD1-HUS1 CHECKPOINT COMPLEX INTERACTIONS

Paz J. Luncsford[1†], Dau-Yin Chang[1†], Guoli Shi[1], Jade Bernstein[1], Amrita Madabushi[1], Dimeka N. Patterson[1], A-Lien Lu[1,2]* and Eric A. Toth[1,2]*

Table SD1: Strains used in this study

Strain	Genotype	Source
JSP303	**h$^+$ *leu1-32 his3-D1***	**C. Hoffman**
JSP303-Y4 (*myh1Δ*)	**h$^+$ *myh1*::*his3*$^+$ *leu1-32 his3-D1***	**This lab**
Hus1-MYC	**h$^-$ *leu1-32 ura4-D18 ade6-706 hus1*$^+$*-MYC***	**A. Carr**
BM2681	**h$^+$ *leu1-32 ura4-D18 Cds1*$^+$-13*MYC***	**P. Russell**
TMN3309	**h$^-$ *ade6-M216 leu1-32 ura4-D18 chk1*$^+$-9*MYC*-2HA-6His-*ura4*$^+$**	**P. Russell**

Table SD2: Oligonucleotides used

Name	Sequence	Purpose
C4F-B	5′GGCAGCCATATGGCCTCTGTCTCCFTCATACCA3′	5′ primer of hMYH(65-350) in pET19b
R-EC5	5′GACTCGCTCGAGTCACTTGCGGCTGGCCTTTCTGG3′	3′ primer of hMYH(65-350) in pET19b
CHANG219	5′GGAGATATACATATGTCGGATTCAAATCATTC3′	5′ primer of SpMYH in pET11a
CHANG220	5′GCAGCCGGATCCTTAGCACTCTGCTTTCGT3′	3′ primer of SpMYH in pET11a
Sp-IA-E262Q-F	5′ATTAAATATGATGCACAGGATGTACCC3′	Sense, I261A and E262Q mutant
Sp-IA-E262Q-R	5′GGGTACATCCTGTGCATCATATTTAAT3′	Antisense, I261A and E262Q mutant
Sp-5-Bam	5′GCATCGGGATCCATGTCGGATTCAAATCAT3′	5′ primer of SpMYH in pGEX-4T-2
Sp-3-Xho	5′TGCGGTCTCGAGTTAGCACTCTGCTTTCGTTAC3′	3′ primer of SpMYH in pGEX-4T-2
Xho-5-Sp	5′GCATCGCTCGAGATGTCGGATTCAAATCAT3′	5′ primer of SpMYH in pREP41X
Xma-3-Sp	5′TGCGGTCCCGGGTTAGCACTCTGCTTTCGT3′	3′ primer of SpMYH in pREP41X
Hus-5-Bam	5′GATCTGGGATCCATGAGATTTAAAACAAGGATT3′	5′ primer of SpHus1 in pGEX-4T-2
Hus-3-Bam	5′GTCTGAGGATCCTTAGTCCACATAGGTACTAATGTA3′	3′ primer of SpHus1 in pGEX-4T-2
Rad1-5-Bam	5′GATATGGGATCCATGTTTCAAGCAGAAACAGTA3′	5′ primer of SpRad1 in pGEX-4T-2
Rad1-3-Bam	5′GTCTGAGGATCCTTAGCTATCCTCATCCTCGGTCTC3′	3′ primer of SpRad1 in pGEX-4T-2
Rad9-5-Bam	5′GATCTGGGATCCATGGAATTCACTGTTTCAAAT3′	5′ primer of SpRad9 in pGEX-4T-2
Rad9-3-Bam	5′GGAGCTGGATCCCTAGTCTTCCTGAGAGAAAATGCC3′	3′ primer of SpRad9 in pGEX-4T-2
SpMyh245-Xho	5′ATCGGGCTCGAGTACCAAGAACAAAACGTAATA3′	5′ primer of Sp-HIP in p4X-G
SpMyh245-Bam	5′ATCGGGGGATCCTTACTTAGCTGGATGGACTGGATA3′	3′ primer of Sp-HIP in p4X-G
SpMyh245-Xho-ATG	5′ATCGGGCTCGAGATGTACCAAGAACAAAACGTAATA3′	5′ primer of Sp-HIP in pREP41X
SpMyh245-His-Xma	5′GGGCTACCCGGGTTAGTGGTGGTGGTGGTGGTGCTTAGC TGGATGGACTGGATA3′	3′ primer of Sp-HIP in pREP41X
TOTH382/SpMyh-F	5′ATCGTGCAGGTACCGATGTCGGATTCAAATCATTTTT3′	5′ primer of SpMYH in pLM303
TOTH371/SpMyh-R	5′ATCGTGCGGATCCTTAGCACTCTGCTTTCGTTACAATC3′	3′ primer of SpMYH in pLM303
SpMyh-Sal-F	5′GGGTTGTTGCGAGATATCCAGTCGACCCAGCTAAGACAA AACAACG3′	Sense, SalI-mutant of SpMyh1
SpMyh-Sal-R	5′CGTTGTTTTGTCTTAGCTGGGTCGACTGGATATCTCGCA ACAACCC3′	Antisense, SalI-mutant of SpMyh1
SpMyh_NdeI	5′TGCAATCGCATATGTCGGATTCAAATCATTTTT3′	5′ primer of SpMYH in pET11a
SpMyh_SalI	5′ATCGTGCGTCGACCGTCTGTTTCGGTTTTTTGCCCGGAT AAAGCGCCCAGCTATTTGCTTTACAAATTTCAGAGATTG GGC3′	3′ primer, Ec-linker + NTD-SpMyh1[a]
SpCHIM-Sal-to-Nat-F	5′CCGAAACAGACGGTCCATCCAGCTAAGACAAAACAACG3′	Sense, revert SalI-mutant to WT
SpCHIM-Sal-to-Nat-R	5′CGTTGTTTTGTCTTAGCTGGATGGACCGTCTGTTTCGG3′	Antisense, revert SalI-mutant to WT

[a]NTD is abbreviated for “N-terminal domain”

Figure SD1. hMYH(65-350) is catalytically active as an adenine glycosylase. Lane 1 of the gel is the DNA substrate containing a centrally located A/8-oxoG mispair. In lanes 2-8, the DNA substrate (0.18 nM) was incubated with increasing concentrations of recombinant hMYH(65-350) (1, 2, 4, 8, 16, 32, and 64 nM, respectively). Reactions were carried out at 37 °C for 30 minutes and the products were separated on a 14% DNA sequencing gel. Arrows mark the intact DNA substrate (I) and the nicked product (N). The gel image was viewed on a PhosphorImager.

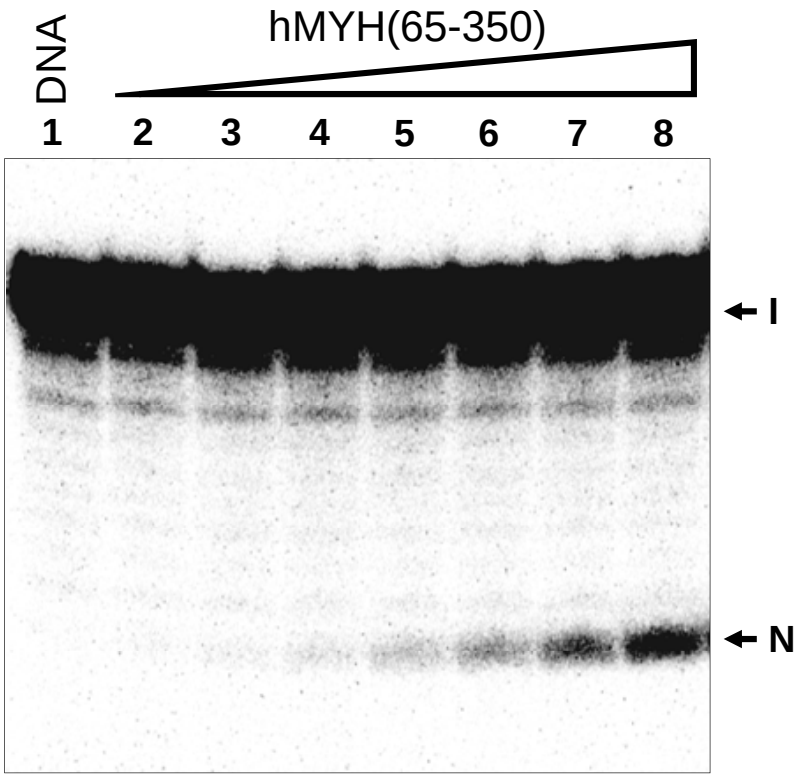

Figure SD2. Stereo diagram of a (2Fo-Fc) electron density map of the hMYH(65-350) IDC. The map includes residues 292-301 of hMYH(65-350) and is contoured at 1σ.

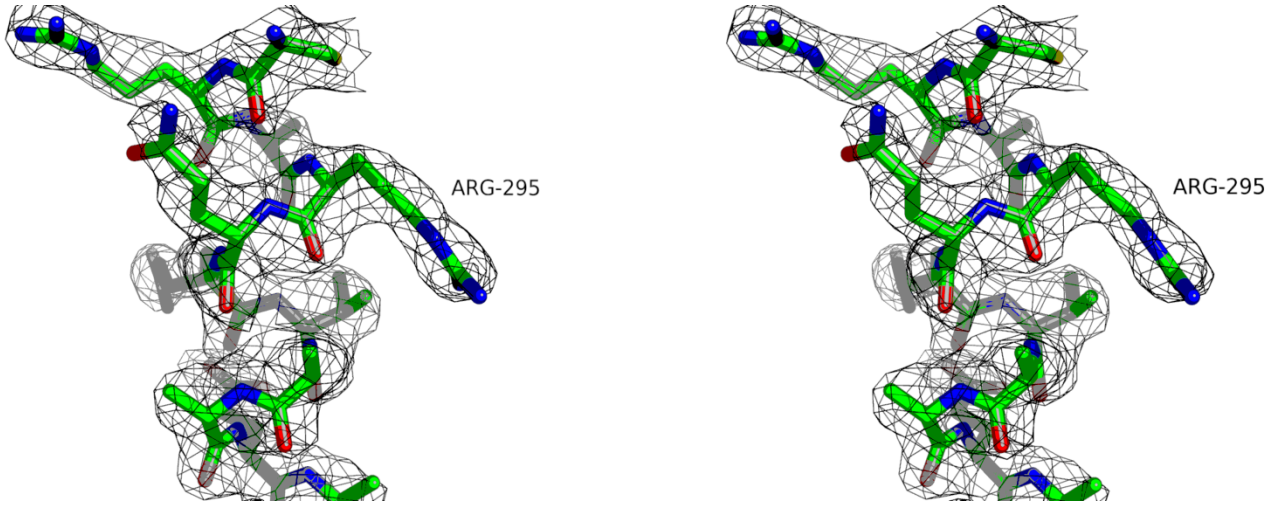

Figure SD3. Homology model of the SpMyh1-Chimera. Prior to constructing the SpMyh1-Chimera, we used SWISS-MODEL (http://swissmodel.expasy.org/) to determine whether the EcMutY linker would be long enough to accommodate the bulkier N- and C-terminal domains of SpMyh1-WT without creation of unfavorable steric interactions. The N- and C-terminal domains of SpMyh1-WT (residues 1-244 and 289-461 of SpMyh1) are depicted in blue on the right and left, respectively. The EcMutY linker (residues 214-227 of EcMutY) is shown in red.

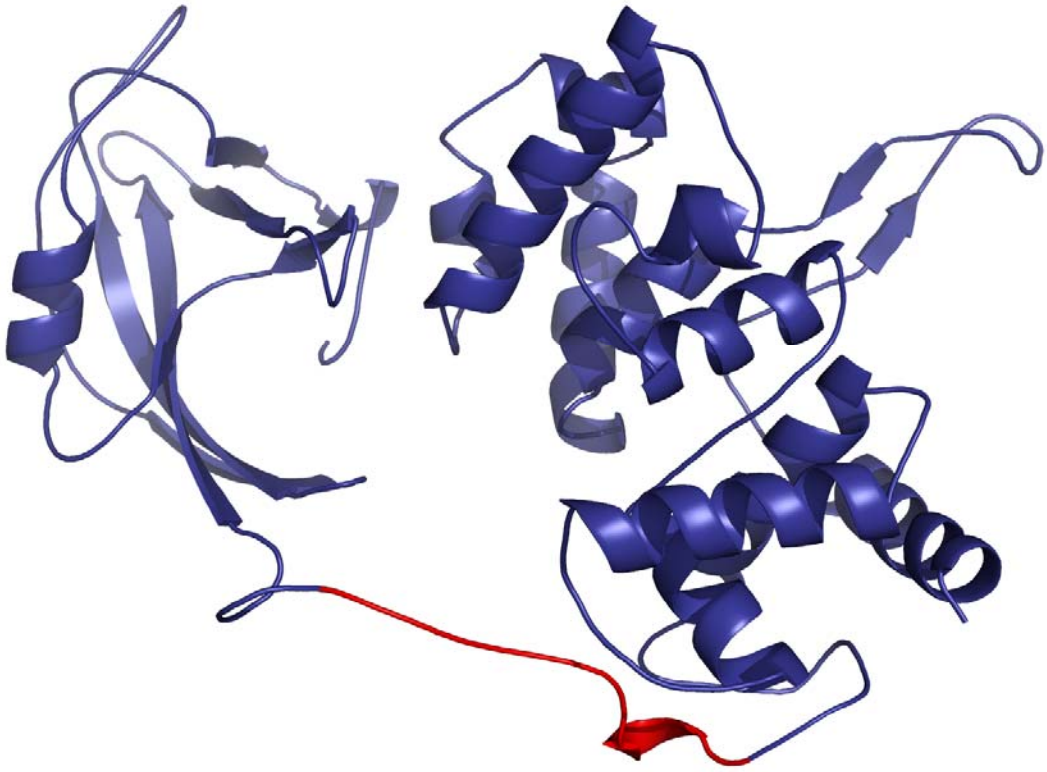

Figure SD4. DNA-binding affinities of SpMyh1-WT and SpMyh1-Chimera for a C:G substrate. A 19 base-pair duplex DNA substrate, with a centrally located C:G pair, was used with one base overhanging at the 5' end of the DNA strand containing guanine. The strand containing the cytosine base was 5'-labeled with fluorescein. The C:G substrate was incubated with either SpMyh1-WT or SpMyh1-Chimera over a range of protein concentrations. Each of the experiments was completed in triplicate. For SpMyh1-WT, the anisotropy values did not increase with increasing protein concentrations indicating that SpMyh1-WT does not recognize DNA containing C:G base pairs as a specific substrate. Alternatively, SpMyh1-Chimera appeared to bind the C:G substrate with modest affinity. Using a binary binding model in GraphPad Prism 3.03, a binding isotherm was fit for the SpMyh1-Chimera data. For three experiments, the average K_d was 98 ± 11 nM.

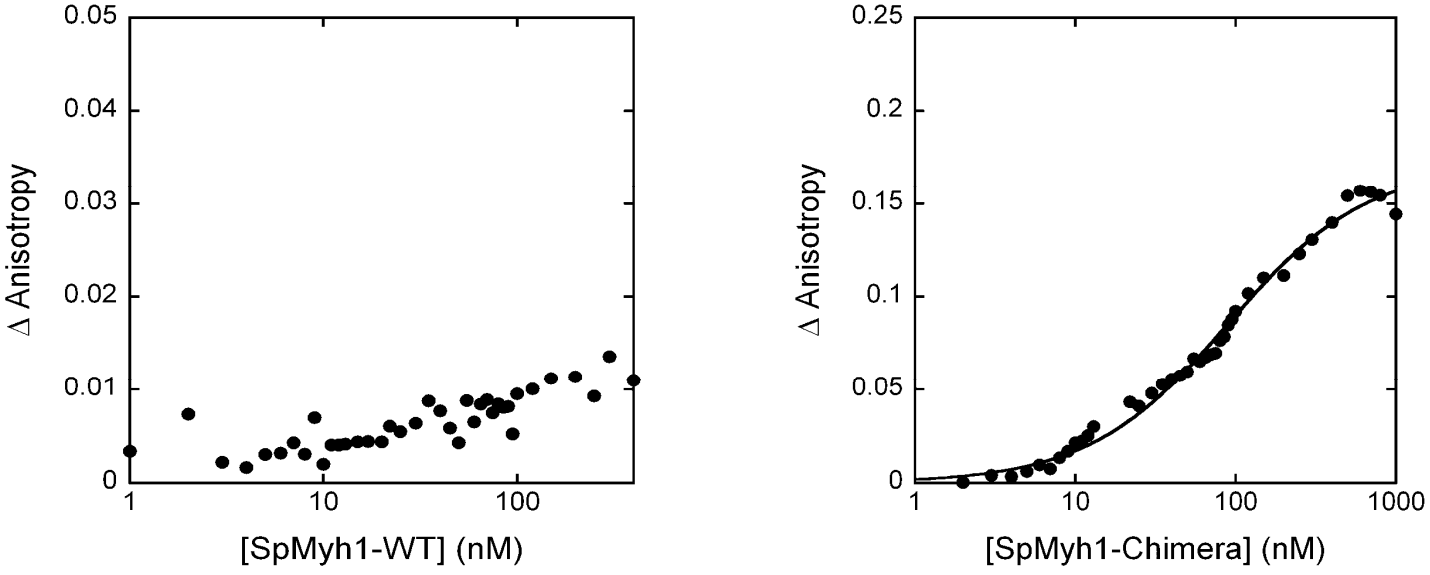

Figure SD5. Superposition of the hMYH(65-350) and BsMutY-DNA Complex structures. The model of the hMYH(65-350) structure (blue) was superposed with the model of BsMutY (red and cyan) bound to DNA (light gray) containing a substrate adenine (PDB ID 1RRQ). The BsMutY linker (cyan) traverses directly away from its N-terminal catalytic domain (red, right side of panel) to its C-terminal 8-oxoG recognition domain (red, left side of panel). Conversely, a helical extension projects the hMYH IDC away from its catalytic domain.