

Table S1 Summary of 63 hMLH1 variants

hMLH1 Variants	Exon	Nucleotide Change	SIFT Analysis	Interaction ^(c)			MMR status ^(d)	Reference ^(e)
				hEXO1	hPMS2	hMRE11		
I25F ^(a)	1	A → T	+	-	-	-	-	Weber et al. 1999
I25I	1	T → C	+	-	-	-	+	Takahashi et al. 2007
P28L ^(a)	1	C → T	+	-	-	-	-	Wehner et al. 1997
I32V ^{(a)(b)}	1	A → G	+	++	++	+	-	Kondo et al. 2003
M35R	1	T → G	+	-	-	-	-	Tannergard et al. 1995
S44F	2	C → T	+	-	-	-	-	Bronner et al. 1994
Q62K	2	C → A	+	-	-	-	-	Wijnen et al. 1997
N64S ^(a)	2	A → G	+	-	-	-	-	Wijnen et al. 1997
G67R	2	G → A	+	-	-	-	-	Tannergard et al. 1995
I68N	2	T → A	+	-	-	-	-	Tannergard et al. 1995
C77Y ^(a)	3	G → A	+	-	-	-	-	Shimodaira et al. 1998
E102K	4	G → A	+	-	-	-	-	Ellison et al. 2001
I107R	4	T → G	+	-	-	-	-	Nystrom-lahti et al. 1996
T117M	4	C → T	+	-	-	-	-	Liu et al. 1996
T117R	4	C → G	+	-	-	-	-	Buerstedde et al. 1995
A128P ^(a)	5	G → C	+	-	-	-	-	Pensotti et al. 1997
V185G ^(a)	7	T → G	+	-	-	-	-	Kohonen-Corish et al. 1996
E199Q	7	G → C	+	-	-	-	+	Wahlberg et al. 1999
V213M ^{(a)(b)}	8	G → A	+	++	++	-	+	Fidalgo et al. 2000
R217C ^(b)	8	C → T	+	++	++	+	+	Miyaki et al. 1995
I219L ^{(a)(b)}	8	A → C	+	++	++	+	+	Kondo et al. 2003
I219V ^{(a)(b)}	8	A → G	+	++	++	-	+	Liu et al. 1995
G244D	9	G → A	+	-	-	-	-	Pensotti et al. 1997
I262 del 3bp	9	Del ATC	+	-	-	-	-	Hunter et al. 1998
R265C	10	C → T	+	-	-	-	-	Wahlberg et al. 1999
R265H ^{(a)(b)}	10	G → A	+	++	++	+	+	Viel et al. 1997
V326A ^(a)	11	T → C	+	+	++	+	-	Liu et al. 1996
S406N ^{(a)(b)}	12	G → A	+	++	++	-	+	Wu et al. 1997
A492I	13	G → A	+	+	++	+	-	Moslein et al. 1996
497 ins 1bp	13	Ins C	+	-	-	-	-	Moslein et al. 1996
497 del 1bp	13	Del G	+	-	-	-	-	Weber et al. 1997
V506A ^(a)	13	T → C	+	-	+	-	-	Liu et al. 1996
519 ins 1bp	13	Ins T	+	-	-	-	-	Kolodner et al. 1995
E523D ^(a)	14	G → T	+	-	-	-	+	Sasaki et al. 1996
541 del 1bp	14	Del C	+	-	-	-	-	Lamberti et al. 1999
Q542L	14	A → T	+	++	+	+	-	Han et al. 1995
L549P ^(a)	14	T → C	+	-	-	-	-	Han et al. 1996
N551I ^(a)	14	A → C	+	-	-	-	-	Wang et al. 1997
L574P ^(a)	15	T → C	+	-	-	-	-	Han et al. 1995
E578G	16	A → G	+	+	++	++	-	Tannergard et al. 1995
L582V ^(a)	16	C → G	+	+	++	+	+	Han et al. 1995
586 ins 1bp	16	Ins C	+	-	-	-	-	Han et al. 1995
590 del 4bp	16	Del TAGA	+	-	-	-	-	Moslein et al. 1996
595 del 2bp	16	Del AG	+	-	-	-	-	Wijnen et al. 1996
K616 del 3bp	16	Del AAG	+	-	+	-	-	Miyaki et al. 1995
K618A	16	AA → GC	+	+	+	-	-	Mauillon et al. 1996
K618T	16	A → C	+	-	++	-	-	Han et al. 1995
632 del 1bp	16	Del G	+	-	-	-	-	Buerstedde et al. 1995
L653R	17	T → G	+	-	-	-	-	Takahashi et al. 2007
P654L ^(a)	17	C → T	+	-	-	-	-	Raevaara et al. 2005
R659P	17	G → C	+	-	-	-	-	Nystrom-lahti et al. 1996
R659X	17	C → T	+	-	-	-	-	Nystrom-lahti et al. 1996
E663G	17	A → G	+	-	-	+	+	Dieumegard et al. 2000
L676R	18	T → G	+	-	-	-	-	Takahashi et al. 2007
A681T	18	G → A	+	-	+	-	-	Froggatt et al. 1996
W712X	19	G → A	+	-	-	-	-	Liu et al. 1996
W714X	19	G → A	+	-	-	-	-	Hunter et al. 1996
V716M ^(a)	19	G → A	+	-	-	-	-	Hunter et al. 1998
H718Y ^(b)	19	C → T	+	++	++	-	-	Weber et al. 1999
726 del 4bp	19	Del CACA	+	-	-	-	-	Papadopoulos et al. 1994
L729V ^{(a)(b)}	19	C → G	+	++	++	+	+	Kondo et al. 2003
733 ins 4bp	19	Ins AAAC	+	-	-	-	-	Risinger et al. 1996
756 ins 4bp	19	Ins TGTT	+	-	-	-	-	Papadopoulos et al. 1994

The information of hMLH1 variants was compiled from various databases (www.insight-group.org) and studies referenced above. hMLH1 HNPCC variants: (a) no change of charge or polarity; (b) polymorphism; (c) Kondo et al., 2003 (relative protein interaction strength categorized into three groups: “-”, <25%; “+”, 25%-75%; “++”, > 75%); (d) Takahashi et al., 2007 (“+”, variants show dominant mutator effect by three reporter genes and possessed > 60% MMR activity as suggested by the authors); (e) see supplemental references. SIFT prediction: “+” sign indicates that the amino acid change could affect protein function (blocks.fhcrc.org; Ng and Henikoff, 2003). The relative hMRE11-hMLH1 interactions were analyzed in the present study.

Supplementary Fig. S1

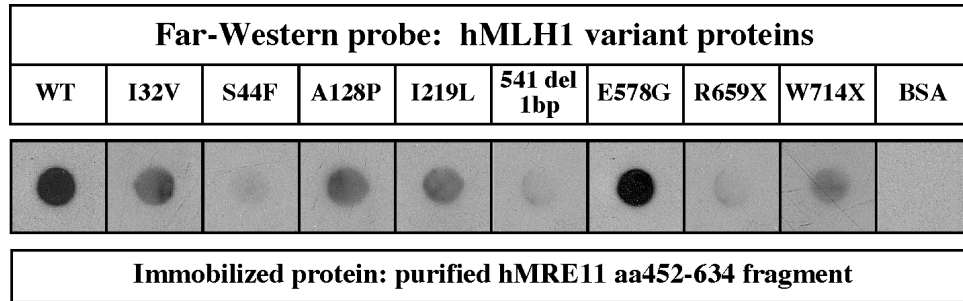


Figure S1. Far-Western analysis of the effects of hMLH1 Lynch syndrome mutations on the interaction between hMRE11 and hMLH1. Membranes that were immobilized with purified hMRE11⁴⁵²⁻⁶³⁴ polypeptide and BSA were probed independently with lysates containing wildtype and various hMLH1 mutant proteins. Captured hMLH1 proteins were detected by conventional Western blot analysis.

Supplementary Fig. S2

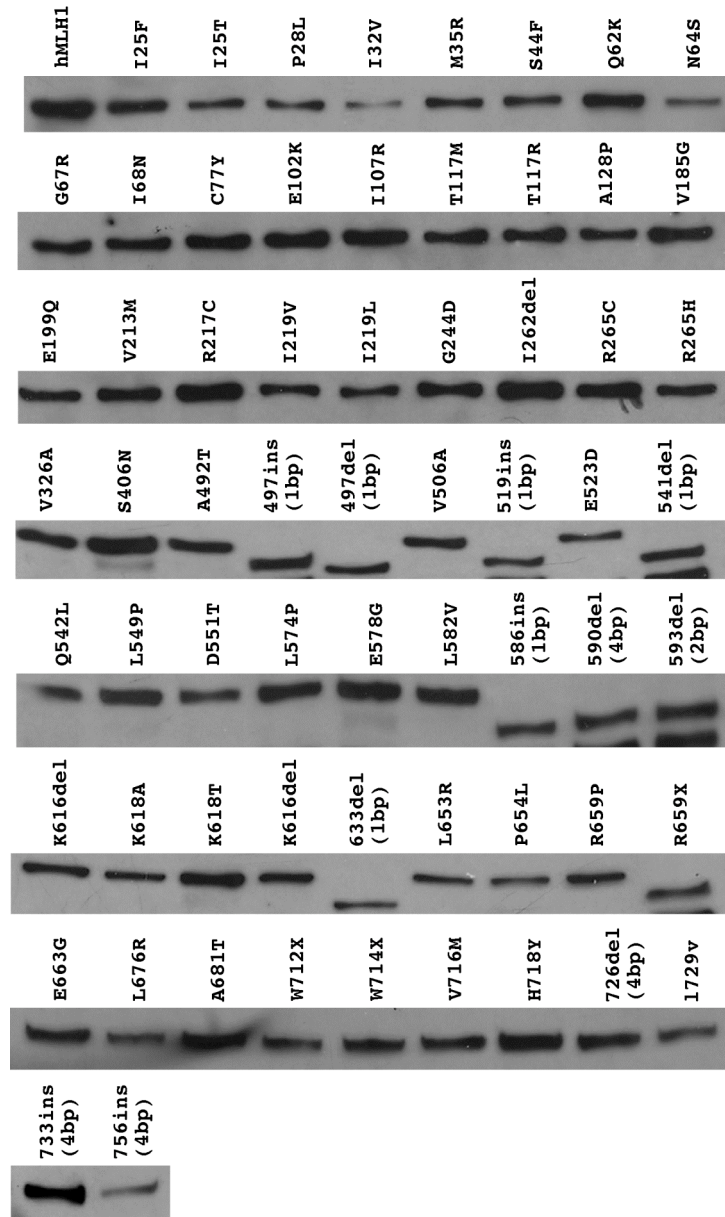


Figure S2. Analysis of the expression of hMLH1 variants in BL21 (DE3) RIL cells by immunoblots. Wildtype and mutant hMLH1 proteins were expressed from pET-28a bacterial expression constructs that harbored full-length hMLH1 cDNA sequences encoding relevant hMLH1 mutants.

Additional references:

1. Bronner C.E., Baker S.M., Morrison P.T., Warren G., Smith L.G., Lescoe M.K., Kane M., Earabino C., Lipford J., Lindblom A., et al. (1994) *Nature* 368, 258-261
2. Buerstedde J. M., Alday P., Torhorst J., Weber W., Muller H., and Scott R. (1995) *J Med Genet.* 32, 909-912
3. Dieumegard B., Grandjouan S., Sabourin J. C., Le Bihan M. L., Lefrere I., Bellefqih, Pignon J. P., Rougier P., Lasser P., Benard J., Couturier D., and Bressac-de Paillerets B. (2000) *Br J Cancer.* 82, 871-880
4. Ellison A. R., Lofing J., and Bitter G. A. (2001) *Hum Mol Genet.* 10, 1889-1900
5. Fidalgo P., Almeida M. R., West S., Gaspar C., Maia L., Wijnen J., Albuquerque C., Curtis A., Cravo M., Fodde R., Leitao C. N., and Burn J. (2000) *Eur J Hum Genet.* 8, 49-53
6. Froggatt N. J., Brassett C., Koch D. J., Evans D. G., Hodgson S. V., Ponder B. A., and Maher E. R. (1996) *J Med Genet.* 33, 726-730
7. Han H. J., Yuan Y., Ku J. L., Oh J. H., Won Y. J., Kang K. J., Kim K. Y., Kim S., Kim C. Y., Kim J. P., Oh NG, Lee K. H., Choe K. J., Nakamura Y., and Park J. G. (1996) *J Natl Cancer Inst.* 88, 1317-1319
8. Hutter P., Couturier A., Membrez V., Joris F., Sappino A. P., Chappuis P. O. (1998) *Int J Cancer.* 78, 680-684
9. Hutter P., Couturier A., Scott R. J., Alday P., Delozier-Blanchet C., Cachat F., Antonarakis S. E., Joris F., Gaudin M., D'Amato L., and Buerstedde J. M. (1996) *J Med Genet.* 33, 636-640
10. Kohonen-Corish M., Ross V. L., Doe W. F., Kool D. A., Edkins E., Faragher I., Wijnen J., Khan P. M., Macrae F., and St John D. J. (1996) *Am J Hum Genet.* 59, 818-824
11. Kolodner R. D., Hall N. R., Lipford J., Kane M. F., Morrison P. T., Finan P. J., Burn J., Chapman P., Earabino C., and Merchant E. (1995) *Cancer Res.* 55, 242-248
12. Kolodner R. D., Hall N. R., Lipford J., Kane M. F., Rao M. R., Morrison P., Wirth L., Finan P.J., Burn J., and Chapman P. (1994) *Genomics.* 24, 516-526
13. Han H. J., Maruyama M., Baba S., Park J. G., and Nakamura Y. (1995) *Hum Mol Genet.* 4, 237-242

14. Lamberti C., Kruse R., Ruelfs C., Caspari R., Wang Y., Jungck M., Mathiak M., Malayeri H. R., Friedl W., Sauerbruch T., and Propping P. (1999) *Gut*. 44, 839-843
15. Liu B., Nicolaides N. C., Markowitz S., Willson J. K., Parsons R. E., Jen J., Papadopoulos N., Peltomäki P., de la Chapelle A., and Hamilton S. R. (1995) *Nat Genet*. 9, 48-55
16. Liu B., Parsons R., Papadopoulos N., Nicolaides N. C., Lynch H. T., Watson P., Jass J. R., Dunlop M., Wyllie A., Peltomäki P., de la Chapelle A., Hamilton S. R., Vogelstein B., and Kinzler K. W. (1996) *Nat Med*. 2, 169-174
17. Miyaki M., Konishi M., Muraoka M., Kikuchi-Yanoshita R., Tanaka K., Iwama T., Mori T., Koike M., Ushio K., and Chiba M. (1995) *J Mol Med*. 73, 515-520
18. Moslein G., Tester D. J., Lindor N. M., Honchel R., Cunningham J. M., French A. J., Halling KC, Schwab M., Goretzki P., and Thibodeau S. N. (1996) *Hum Mol Genet*. 5, 1245-1252
19. Nyström-Lahti M., Wu Y., Moisio A. L., Hofstra R. M., Osinga J., Mecklin J. P., Järvinen H. J., Leisti J., Buys C.H., de la Chapelle A., and Peltomäki P. (1996) *Hum Mol Genet*. 5, 763-769
20. Papadopoulos N., Nicolaides N. C., Wei Y. F., Ruben S. M., Carter K. C., Rosen C. A., Haseltine W. A., Fleischmann R. D., Fraser C. M., and Adams M. D. (1994) *Science* 263, 1625-1629
21. Pensotti V., Radice P., Presciuttini S., Calistri D., Gazzoli I., Grimalt Perez A., Mondini P., Buonsanti G., Sala P., Rossetti C., Ranzani G. N., Bertario L., and Pierotti M. A. (1997) *Gene Chromosome Canc*. 19, 135-142
22. Rævaara T. E., Korhonen M. K., Lohi H., Hampel H., Lynch E., Lonnqvist K. E., Holinski-Feder E., Sutter C., McKinnon W., Duraisamy S., Gerdes A. M., Peltomäki P., Kohonen-Ccorish M., Mangold E., Macrae F., Greenblatt M., de la Chapelle A., and Nystrom M. (2005) *Gastroenterology*. 129, 537-549
23. Risinger J. I., Barrett J. C., Watson P., and Lynch H. T. Boyd J. (1996) *Cancer*. 77, 1836-1843
24. Sasaki S., Horii A., Shimada M., Han H. J., Yanagisawa A., Muto T., and Nakamura Y. (1996) *Hum Mutat*. 7, 275-278
25. Tannergard P., Lipford J. R., Kolodner R., Frodin J. E., Nordenskjold M., and Lindblom A. (1995) *Cancer Res*. 55, 6092-6096
26. Viel A., Genuardi M., Capozzi E., Leonardi F., Bellacosa A., Paravatou-Petsotas M.,

- Pomponi M. G., Fornasarig M., Percesepe A., Roncucci L., Tamassia M. G., Benatti P., Ponz de Leon M., Valenti A., Covino M., Anti M., Foletto M., Boiocchi M., and Neri G. (1997) *Gene Chromosome Canc.* 18, 8-18
27. Wahlberg S., Liu T., Lindblom P., and Lindblom A. (1999) *Genet Test.* 3, 259-264
 28. Wang Q., Desseigne F., Lasset C., Saurin J. C., Navarro C., Yagci T., Keser I., Bagci H., Luleci G., Gelen T., Chayvialle J. A., Puisieux A., and Ozturk M. (1997) *Int J Cancer.* 73, 831-836
 29. Weber T.K., Conlon W., Petrelli N.J., Rodriguez-Bigas M., Keitz B., Pazik J., Farrell C., O'Malley L., Osholim M., Abdo M., Anderson G., Stoler D., Yandell D. (1997) *Cancer Res.* 57, 3798-3803.
 30. Weber T. K., Chin H. M., Rodriguez-Bigas M., Keitz B., Gilligan R., O'Malley L., Urf E., Diba N., Pazik J., and Petrelli N. J. (1999) *JAMA.* 281, 2316-2320.
 31. Wehner M., Buschhausen L., Lamberti C., Kruse R., Caspari R., Propping P., and Friedl W. (1997) *Hum Mutat.* 10, 241-244
 32. Wijnen J., Khan P. M., Vasen H., Menko F., van der Klift H., van den Broek M., van Leeuwen-Cornelisse I., Nagengast F., Meijers-Heijboer E. J., Lindhout D., Griffioen G., Cats A., Kleibeuker J., Varesco L., Bertario L., Bisgaard M. L., Mohr J., Kolodner R., and Fodde R. (1996) *Am J Hum Genet.* 58, 300-307
 33. Wijnen J, Khan PM, Vasen H, van der Klift H, Mulder A, van Leeuwen-Cornelisse I, Bakker B, Losekoot M, Moller P, and Fodde R. (1997) *Am J Hum Genet.* 61, 329-335
 34. Wu Y., Nystrom-Lahti M., Osinga J., Looman M.W., Peltomaki P., Aaltonen L.A., de la Chapelle A., Hofstra R.M., Buys C.H. (1997) *Genes Chromosomes Cancer* 18, 269-278.