Supplementary Table 1. Primer sequences for qRT-PCR

mRNA	number of nucleotides	direction	sequence (5' -> 3')	source
hMSH3	22	sense	ATGCAGAGATTGCAGCCCGAGA	this study
	23	antisense	ACCAGGCGGCGTACATGAACAAA	
HIF-1α	24	sense	AGCCAGACGATCATGCAGCTACTA	Takara Bio Co
	25	antisense	TGTGGTAATCCACTTTCATCCATTG	
ribosomal 18S	20	sense	CGGCTACCACATCCAAGGAA	[1]
	18	antisense	GCTGGAATTACCGCGGCT	

Supplementary Table 2. Primer sequences of inserts and site-directed mutagenesis for reporter plasmids

initial position	number of nucleotides	direction	sequence (5' -> 3')
-3102	32	sense	ATATGG**GGTACC**CATGGGCTACACCTTGACCT
-2658	32	sense	ATATGG**GGTACC**GCGGTGGCTGGCACGAACTT
-2480	32	sense	ATATGG**GGTACC**GACTCACCGGGGCAGGGATG
-2275	32	sense	ATATGG**GGTACC**AGTAAGGTGGGCAGGGAGTT
-1267	32	sense	ATATGG**GGTACC**TTGGGATTTTTGAGACTGGC
-681	32	sense	ATATGG**GGTACC**CCCATGCCTGATAATTTGCT
+20	33	antisense	GCATCCC**AAGCTT**CTGTCATGGTTGGTTCGCTA
+50	33	antisense	GCATCCC**AAGCTT**TAACGAGCGGGCTCGGAGGT
-2530	28	sense	TTGCTGC<u>A</u>T<u>A</u>CTTGATACTTGTTCCTTT
-2516	28	antisense	TCAAG<u>T</u>A<u>T</u>GCAGCAACGCAGCTCAAAAC
-1644	28	sense	ACAAA<u>TTT</u>TGTTAACCCCTTGTGGTTTA
-1630	28	antisense	GTTAACA<u>AAA</u>TTTGTCTTGTGGGTGACA

Restriction enzyme (Kpn I or Hind III) recognition sites are in bold.
Mutated nucleotides are underlined.

Supplementary Table 3 . Oligonucleotide sequences of HRE1 and HRE2 in hMSH3 promoter for EMSA and super-shift assay

name	initial position	number of nucleotides	direction	sequence (5’ -> 3’)
WT-HRE1	-2520	24	sense	TTGCT GCGTG CTTGA TACTT GTTC
MT1-HRE1	-2520	24	sense	TTGCT TTTTG CTTGA TACTT GTTC
MT2-HRE1	-2520	24	sense	TTGCT GCATA CTTGA TACTT GTTC
WT-HRE2	-1634	24	sense	ACAAA ACGTG TTAAC CCCTT GTGG
MT-HRE2	-1634	24	sense	ACAAA TTTTG TTAAC CCCTT GTGG

HRE in wild type (WT) and corresponding mutated (MT) sequence is underlined. For assays, a double stranded oligomer generated with a complementary oligomer was used.

Supplementary Table 4.

Sample No.	NCI[a]	EMAST Status[b]	MSH3 IHC % negative[c]	GLUT1 IHC[d]	LOH[e]				*TP53* LOH[f]
					TP53Alu	p*53-1*	*p53-3*	*mfd15*	
CR026	L	E	48	++	NI	NI	+	-	+
CR053	L	E	34	+++	-	NI	-	NI	-
CR074	L	E	50	++	+	+	+	-	+
CR338	L	E	28	+++	NI	-	-	NI	-
CR007	S	E	37	+++	NI	NI	+	-	+
CR040	S	E	39	++	NI	NI	-	-	-
CR057	S	E	31	+++	NI	+	+	-	+
CR069	S	E	37	+++	+	+	+	NI	+
CR212	S	E	30	+++	NI	NI	+	-	+
CR213	S	E	34	++	NI	NI	-	-	-
CR335	S	E	24	+++	+	+	+	-	+
CR341	S	E	29	+++	NI	NI	+	-	+
CR013	S	E	29	++	NI	+	+	NI	+
CR087	S	E	23	+++	-	NI	-	-	-
CR006	S	E	35	++	+	+	-	NI	+
CR041	S	E	39	+++	+	NI	+	-	+
CR045	S	E	27	+++	+	+	+	NI	+
CR054	S	E	26	+/-	NI	NI	-	-	-
CR088	S	E	35	+/-	NI	NI	+	-	+
CR061	S	E	22	+/-	-	NI	-	-	-
CR033	S	non-E	7	++	-	NI	-	-	-
CR067	S	non-E	2	+/-	NI	-	-	NI	-
CR342	S	non-E	5	++	-	NI	-	-	-
CR073	S	non-E	6	+/-	NI	NI	+	-	+
CR058	S	non-E	13	+/-	-	-	NI	-	-
CR337	S	non-E	10	+/-	+	NI	+	-	+
73	S	non-E	8	+/-	NT	NT	NT	NT	NT
124	S	non-E	11	+/-	NT	NT	NT	NT	NT

[a]MSI status determined by NCI markers; L represents MSI-L, S represents MSS [2].
[b]EMAST status determined by EMAST markers; E represents EMAST-positive, non-EMAST represents EMAST-negative [2].
[c]The percentage of hMSH3-negative cells in a tumor tissue determined by MSH3 IHC staining [2].
[d]GLUT1 expression determined by GLUT1 IHC; +++ represents more than 50% of tumor area showed positive staining, ++ represents less than 50% of tumor area showed positive staining , +/- represents sporadic staining or negative staining.
[e]LOH status at chromosome 17, *TP53Alu* (17p13.1) and *mfd15* (17q11.2-12), was determined as described previously [3]. Two additional polymorphic markers present within the *TP53* locus, *TP53-1* and *TP53-3,* were also used. When at least one of three markers exhibited LOH in a tumor sample, we defined the tumor as positive for *TP53* LOH. PCR primers for *TP53-1*: sense (5'-TTTGAACCCAGGAGATGGAG-3'), antisense (5'-CACTTGCCTCAGTCTGGCTA-3'); and for *TP53-3*: sense (5'-CTCCCAAAGTGCTGGGATTA-3'), antisense (5'-TCGTCAACATAGCCAGACCTC-3'). NI; not infomative, +; LOH, -; heterozygote, NT; not tested.
[f]P value of EMAST vs non-EMAST determined by χ^2-test was 0.169.

Supplementary Table 5.

GLUT1 expression[a]	No. of cases with		P value[d]
	EMAST[b]	non-EMAST[c]	
+++, ++	17	2	0.002
+/-	3	6	

[a] +++,++ represents over expression. +/- represents sporadical or negative expression.
[b] EMAST status determined by EMAST markers; EMAST represents EMAST-positive [2].
[c] non-EMAST represents EMAST-negative [2].
[d] P value was determined by χ^2-test.

References

[1] J.A. Kalish, D.J. Willis, C. Li, J.J. Link, E.R. Deutsch, M.A. Contreras, W.C. Quist, F.W. Logerfo, Temporal genomics of vein bypass grafting through oligonucleotide microarray analysis, J Vasc Surg 39 (2004) 645-654.

[2] A.C. Haugen, A. Goel, K. Yamada, G. Marra, T.P. Nguyen, T. Nagasaka, S. Kanazawa, J. Koike, Y. Kikuchi, X. Zhong, M. Arita, K. Shibuya, M. Oshimura, H. Hemmi, C.R. Boland, M. Koi, Genetic instability caused by loss of MutS homologue 3 in human colorectal cancer, Cancer Res 68 (2008) 8465-8472.

[3] K. Yamada, S. Kanazawa, J. Koike, H. Sugiyama, C. Xu, K. Funahashi, C.R. Boland, M. Koi, H. Hemmi, Microsatellite instability at tetranucleotide repeats in sporadic colorectal cancer in Japan, Oncol Rep 23 (2010) 551-561.

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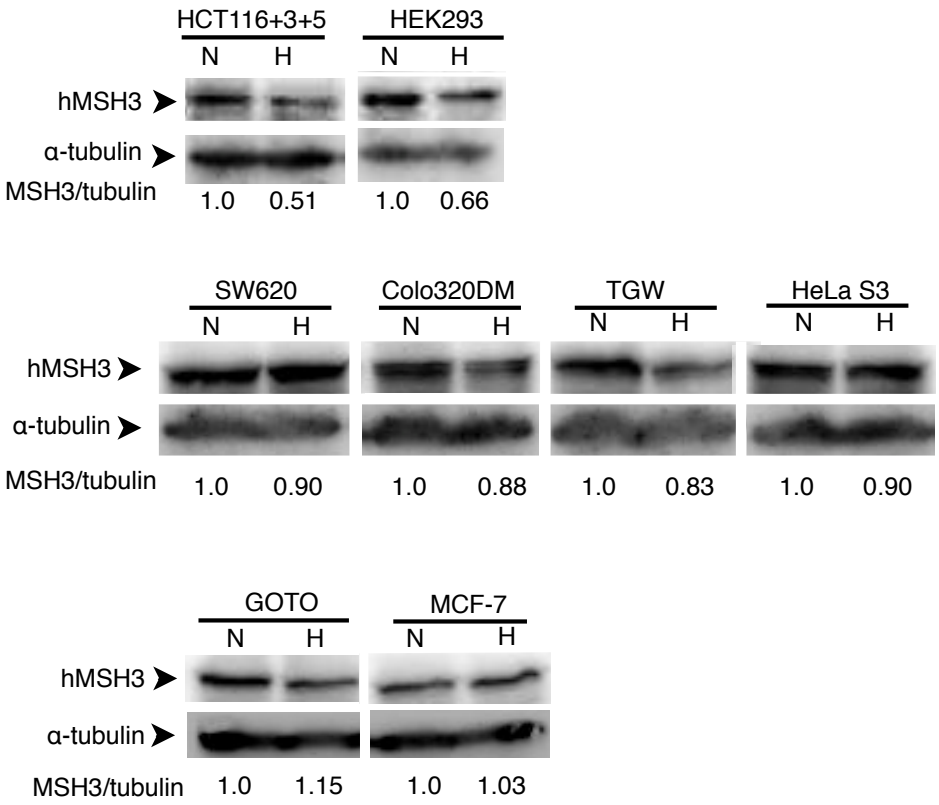
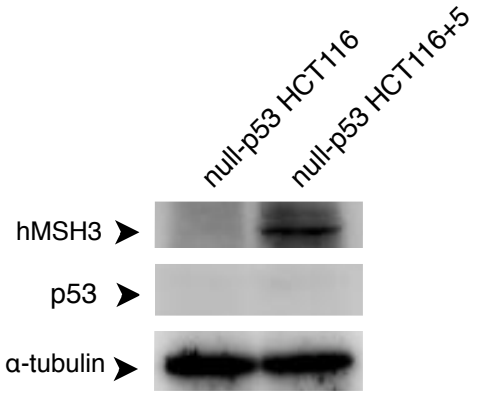


sFig. 1. hMSH3 down-regulation pattern in hypoxia at protein level in 8 human cell lines

Cells were cultured in normoxia (N) and hypoxia for 3 days (H). Cell lysate was prepared and subjected to Western blot analysis with antibody to hMSH3. Anti α-tubulin antibody was used as a loading control.

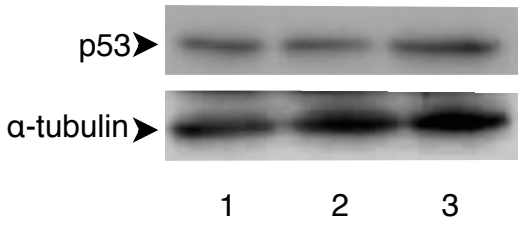
Li et al. Supplementary Figure 2



sFig. 2. Expression of hMSH3 and p53 proteins in HCT116 sublines, null-p53 HCT116 (*hMSH3*$^{-/-}$, p53$^{-/-}$), null-p53 HCT116+5 (*hMSH3*$^{+/+}$, p53 $^{-/-}$).

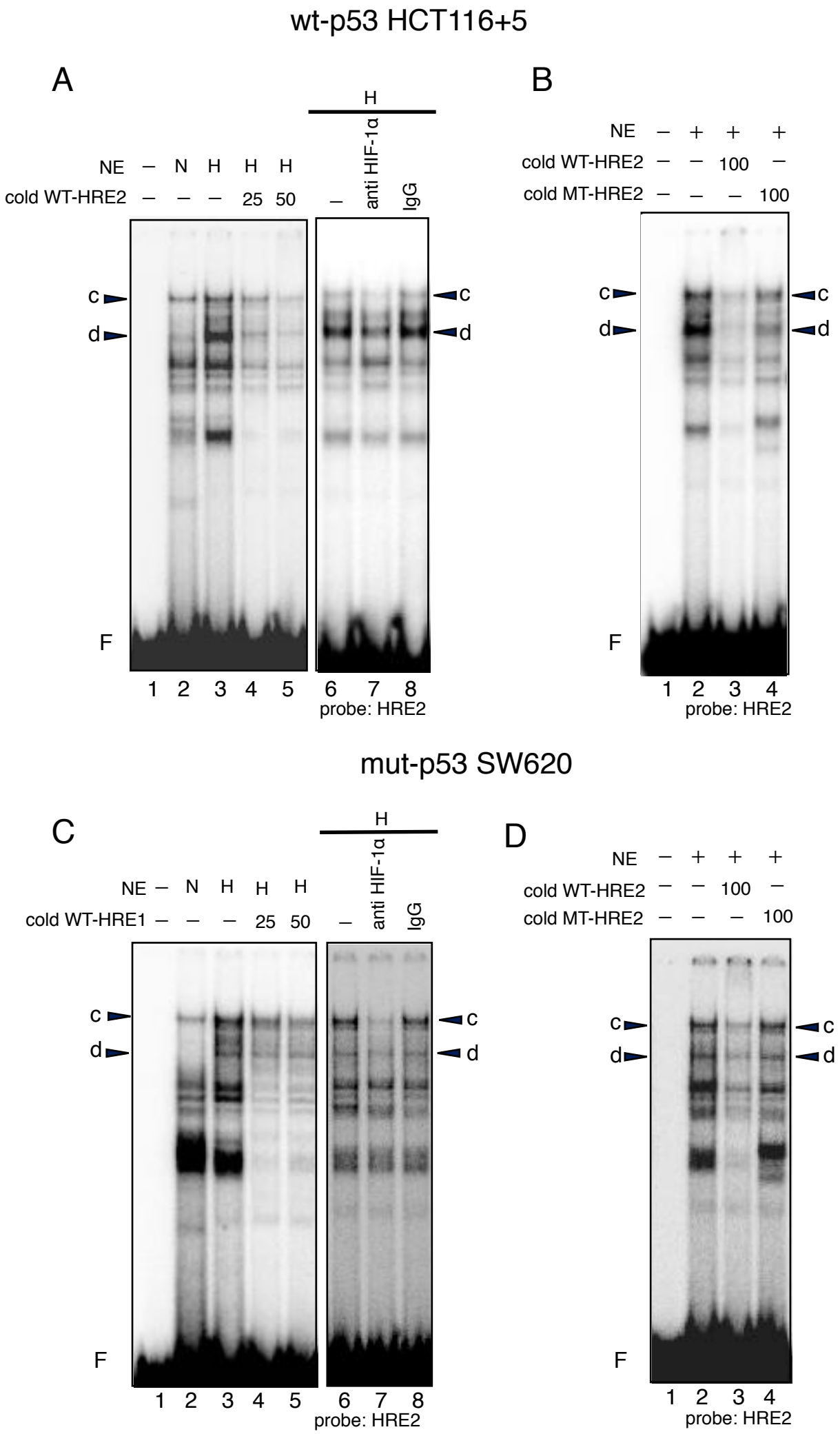
Cell lysate was prepared and subjected to Western blot analysis with antibodies to hMSH3 and p53 (DO-I). An anti α-tubulin antibody was used as a loading control.

Li et al. Supplementary Figure 3



sFig. 3. Western blot analysis of SW480 transfected with p53 expression plasmid.

A p53 expression plasmid, pZX53^{WT}, or mock (pCEP4) was transfected into mut-p53 SW480. Twenty-four hours after transfection, cells were cultured for another 24 hrs in normoxia. Cell lysate were prepared and subjected to Western blot analysis with an antibody to p53 (DO-1), and α-tubulin. Lane 1. no transfection, lane 2, transfected with pCEP4, lane 3, transfected with pZX53^{WT}.


wt-p53 HCT116+5
A
H
NE – N H H H
cold WT-HRE2 – – – 25 50
– anti HIF-1α IgG
c
d
F
1 2 3 4 5 6 7 8
probe: HRE2
B
NE – + + +
cold WT-HRE2 – – 100 –
cold MT-HRE2 – – – 100
c
d
F
1 2 3 4
probe: HRE2
mut-p53 SW620
C
H
NE – N H H H
cold WT-HRE1 – – – 25 50
– anti HIF-1α IgG
c
d
F
1 2 3 4 5 6 7 8
probe: HRE2
D
NE – + + +
cold WT-HRE2 – – 100 –
cold MT-HRE2 – – – 100
c
d
F
1 2 3 4
probe: HRE2

sFig. 4. Specific bindings of protein complexes containing HIF-1α from hypoxic wt- or mut-p53 cells to the HRE2 sites of hMSH3 locus**.**

A : A hot-WT-HRE2 probe was mixed with nuclear extracts (NE) from normoxic wt-p53 HCT116+5 cells (N) or hypoxic cells (H) and subjected to electrophoresis. Five species of protein complexes were induced by hypoxia (lane 3) compared to normoxic cell (lane 2). These binding products were diminished by a 25-fold or 50-fold excess of cold WT-HRE2 probe (lanes 4, 5). Among them bands "c" and "d" were diminished by anti-HIF-1α antibody (lane 7) compared to the control, nothing added (-) (lanes 3 and 6) or mouse IgG added (lane 8). Nothing was added to lane 1 except hot-WT-HRE2 probe. F: free probe.

B: Two binding products, "c" and "d", formed between the hot-WT-HRE2 probe and putative protein complexes A and B respectively from hypoxic wt-p53 HCT116+5 (lane 2) were diminished by a 100-fold excess of cold WT-HRE2 (lane 3) but not by a 100-fold excess of cold MT-HRE2 (lane 4). -: not added, +: added. NE: nuclear extracts, F: free probe. Nothing was added to lane 1except the hot-HRE2 probe.

C: A hot-WT-HRE2 probe was mixed with nuclear extracts (NE) from normoxic mut-p53 SW620 cells (N) or hypoxic cells (H) and subjected to electrophoresis. Five species of protein complexes were induced by hypoxia (lane 3) compared to normoxic cell (lane 2). These binding products were diminished by a 25-fold or 50-fold excess of cold WT-HRE2 probe (lanes 4, 5). Among them bands "c" and "d" were diminished by anti-HIF-1α antibody (lane 7) compared to the control, nothing added (-) (lanes 3 and 6) or mouse IgG added (lane 8). Nothing was added to lane 1except the hot-WT-HRE2 probe. F: free probe.

D: Two binding products, "c" and "d", formed between the hot-WT-HRE2 probe and putative protein complexes A and B respectively from hypoxic mut-p53 SW620 (lane 2) were diminished by a 100-fold excess of cold WT-HRE2 (lane 3) but not by a 100-fold excess of cold MT-HRE2 (lane 4). -: not added, +: added. NE: nuclear extracts, F: free probe. Nothing was added to lane 1 except the hot-WT-HRE2 probe.