Table W1. Characteristics of 99 Patients.

Variables	No. Patients	%
Sex		
Male	59	60
Female	40	40
Age, years		
<60	37	37
≥60	62	63
Tumor position		
Colon	57	58
Rectum	42	42
Differentiation		
Nonpoor	95	96
Poor*	4	4
T factor		
Tis	5	5
T1	15	15
T2	7	7
T3	68	69
T4	4	4
N factor		
N0	55	56
N1	26	26
N2	18	18
Distant metastasis		
Present	23	23
Absent	76	77
Peritoneal dissemination		
Present	5	5
Absent	94	95
UICC stage		
I	25	25
II	25	25
III	24	24
IV	25	25
Lymphatic invasion		
Negative	16	16
Positive	83	84
Vascular invasion		
Negative	23	23
Positive	76	77
Preoperative CEA		
Normal (≤2.5 ng/ml)	56	57
Elevated (>2.5 ng/ml)	43	43
Preoperative CA19-9		
Normal (≤37 ng/ml)	71	72
Elevated (>37 ng/ml)	28	28
Q-MSP value		
<2.89	43	43
≥2.89	56	57

*Poor includes poorly differentiated, mucinous, and undifferentiated types.

Table W2. Prosgnostic Analysis of Clinicopathologic Variables in 170 Patients with Stage III CRC.

Variables	No. Patients	%	5-Year DSS*			
			Univariate Analysis		Multivariate Analysis	
			HR (95% CI)	$P^{\dagger}$	HR (95% CI)	$P^{\dagger}$
Sex						
Male	97	57.1	1.12 (0.84-1.47)	.438	n/d	n/d
Female	73	42.9				
Age, years						
<60	74	43.5	0.97 (0.73-1.28)	.818	n/d	n/d
≥60	96	56.5				
Tumor position						
Colon	96	56.5	0.89 (0.67-1.18)	.406	n/d	n/d
Rectum	74	43.5				
Differentiation						
Nonpoor	149	87.6	1.38 (0.93-1.93)	.103	n/d	n/d
Poor[‡]	21	12.4				
T factor						
T1, 2	20	11.8	1.56 (0.95-3.17)	.087	1.36 (0.81-2.79)	.275
T3, 4	150	88.2				
N factor						
N1	119	70.0	1.98 (1.50-2.63)	<.001	1.92 (1.42-2.59)	<.001
N2	51	30.0				
Intramural lymphatic involvement						
Negative	0	0.0	n/a	n/a	n/a	n/a
Positive	170	100.0				
Intramural vascular involvement						
Negative	7	4.1	693.7	.023	n/d	n/d
Positive	163	95.9				
Preoperative CEA						
Normal (≤2.5 ng/ml)	103	60.6	1.81 (1.36-2.43)	<.001	1.51 (1.12-2.07)	.008
Elevated (>2.5 ng/ml)	67	39.4				
Preoperative CA19-9						
Normal (≤37 ng/ml)	150	88.2	1.67 (1.16-2.29)	.007	1.12 (0.74-1.63)	.582
Elevated (>37 ng/ml)	20	11.8				
Q-MSP value						
<2.89	85	50.0	1.35 (1.02-1.81)	.037	1.40 (1.02-1.92)	.035
≥2.89	85	50.0				

CI indicates confident interval; HR, hazard ratio; n/a, not applicable; n/d, not determined.

There was no event in intramural invasion-negative cases, so that this variable was excluded from multivariate analysis.

*End point: date of death or March 31, 2007.

[†]Cox proportional hazard model.

[‡]Poor includes poorly differentiated, mucinous, and undifferentiated types.

Table W3. Sequences of Primers and Fluorescent Probes.

Method	Gene	Forward (5'-3')	Fluorescent Probe (5'-3')	Reverse Primer (5'-3')	Annealing Temperature (°C)
Bisulfite sequencing	HOPX-β	TAGTTTTGTTTTGGAGAGGGTTTTAAAG		AACCTCCCCTAAAAACAAACTTAAC	62
TaqMan-Q-MSP	HOPX-β	TTTGGAGAGGGTTTTAAAGCG	CGGAGATAGAAGGTCGTTTATCGGGGAGGTCG	AACAAACTTAACAAATCGCGAA	60
TaqMan-Q-MSP	β-actin	TGGTGATGGAGGAGGTTTTAGTAAGT	ACCACCACCCAACACACAATAACAAACACA	AACCAATAAAACTTACTCCTCCCCTTAA	60
RT-PCR/qRT-PCR	HOPX-α and γ	CAAACCCAGGGCTTGCGCTT		GCGGAGGAGAGAAACAGAGAT	62
RT-PCR/qRT-PCR	HOPX-β	GGTCCCCCTTTCGGGAGGAA		GCGGAGGAGAGAAACAGAGAT	62
RT-PCR/qRT-PCR	HOPX-core	CAGAGGACCAGGTGGAAATCC		GCGGAGGAGAGAAACAGAGAT	62
qRT-PCR	PRDX2	GCAGTGACACGATTAAGC		TATCCGTTAGCCAGCCTA	60
qRT-PCR	WT1P	AGAACAGTCAGAGGCCACAAG		TTCCCTGGAGAAGAAGGAAAG	55
qRT-PCR	FOS	TTACTACCACTCACCCGCAGA		GAATGAAGTTGGCACTGGAGA	55
qRT-PCR	CYR61	CATGATGATCCAGTCCTG		TTGAACAGCCTGTAGAAG	55
qRT-PCR	EPHA2	CTGAGCGTATCTTCATTG		AATGGTGTCAATCTTGGT	60
qRT-PCR	SLC2A3	TCTATTACTCAACAGGAAT		CAGTGAAGATAGTATTAACC	55
qRT-PCR	EMP1	CTGGCAAGAGCAGATACT		TTTGCTTTGGGTCAGAAG	55
RT-PCR/qRT-PCR	β-actin	GCTCGTCGTCGACAACGGCTC		CAAACATGATCTGGGTCATCTTCT	55/62

Table W5. Downregulated Genes by HOPX in DNA Microarray at a Two-fold Ratio.

Probe Set	Gene Symbol	HCT116 Mock Signal	HCT116 HOPX Signal	DLD1 Mock Signal	DLD1 HOPX Signal	HCT116 Log$_2$ Ratio	DLD1 Log$_2$ Ratio
37028_at	PPP1R15A	136.5	62.2	1092	75.4	−1.1	−3.9
36829_at	PER1	262.1	101.7	403.3	55.8	−1.4	−2.9
242669_at	UFM1	219.3	60.8	161.7	64.7	−1.9	−1.3
239451_at	—	143	50	169.2	50	−1.5	−1.8
230265_at	SEL1L	104.4	50	182.1	84	−1.1	−1.1
229460_at	FAM126B	192.3	90.8	218.4	60.2	−1.1	−1.9
228234_at	TICAM2 /// TMED7	851.6	281	372.5	105.8	−1.6	−1.8
228173_at	GNAS	110.7	53.4	130.6	59.8	−1.1	−1.1
227747_at	MPZL3	148.7	70	261.1	52.4	−1.1	−2.3
227404_s_at	EGR1	339.7	93.6	695.6	154.4	−1.9	−2.2
227345_at	TNFRSF10D	843.5	247.6	693.4	234.1	−1.8	−1.6
227309_at	YOD1	703.4	264	785.5	133.5	−1.4	−2.6
226640_at	DAGLB	132.9	50	133.8	50	−1.4	−1.4
226275_at	MXD1	101.3	50	263.9	97.4	−1.0	−1.4
225832_s_at	DAGLB	111	50	129.6	50	−1.2	−1.4
225799_at	LOC541471 /// NCRNA00152	297.2	122.6	1208.7	263.4	−1.3	−2.2
225090_at	SYVN1	206.4	97.9	176.3	57.2	−1.1	−1.6
224797_at	ARRDC3	251.1	95.2	101.5	50	−1.4	−1.0
222690_s_at	TMEM39A	268.7	104.3	353.1	101.3	−1.4	−1.8
222262_s_at	ETNK1	223.4	91.8	334.6	84.3	−1.3	−2.0
222088_s_at	SLC2A14 /// SLC2A3	1580.1	243	110.9	50	−2.7	−1.1
222018_at	NACA /// NACA2 /// NACAP1	201.5	93.9	267.9	100.8	−1.1	−1.4
217173_s_at	LDLR	146.1	50	102.1	50	−1.5	−1.0
216268_s_at	JAG1	355	114	972.4	370.9	−1.6	−1.4
215222_x_at	MACF1	211.6	64.6	133.2	60	−1.7	−1.2
214752_x_at	FLNA	242.3	100	380	182.4	−1.3	−1.1
214683_s_at	CLK1	338.3	94.8	726.9	249.6	−1.8	−1.5
214016_s_at	SFPQ	936.4	415.3	1675	511.9	−1.2	−1.7
213746_s_at	FLNA	254.6	66.9	313.9	145.4	−1.9	−1.1
212457_at	TFE3	164.6	69.1	355.4	152	−1.3	−1.2
212444_at	—	2573.1	1029.2	1157	466.8	−1.3	−1.3
210764_s_at	CYR61	791.7	141.8	1948	51.7	−2.5	−5.2
210676_x_at	RGPD5 /// RGPD6 /// RGPD8	180	72.7	605.6	187.6	−1.3	−1.7
210664_s_at	TFPI	443.9	176.8	201.4	50.2	−1.3	−2.0
210346_s_at	CLK4	130	57.1	309.9	112.3	−1.2	−1.5
209907_s_at	ITSN2	173.3	84	144.2	50	−1.0	−1.5
209305_s_at	GADD45B	156.8	76.5	1576.8	102.5	−1.0	−3.9
209304_x_at	GADD45B	192.5	79.2	1224.6	101.5	−1.3	−3.6
209270_at	LAMB3	1134.9	218.9	1541.6	646.7	−2.4	−1.3
209189_at	FOS	877.6	76	1499.3	50	−3.5	−4.9
209099_x_at	JAG1	399.1	103.5	1059	445.3	−1.9	−1.2
208744_x_at	HSPH1	123	50	2317.9	115.6	−1.3	−4.3
207574_s_at	GADD45B	312.4	143.1	2184.9	176.2	−1.1	−3.6
206976_s_at	HSPH1	801.6	289.5	7006.9	647	−1.5	−3.4
204733_at	KLK6	115.5	55.1	996.1	427.2	−1.1	−1.2
204363_at	F3	1017.9	394.9	343.8	157.7	−1.4	−1.1
203499_at	EPHA2	1002.5	219.9	1027.2	191.4	−2.2	−2.4
203397_s_at	GALNT3	1004.9	375.3	696.5	235.3	−1.4	−1.6
203108_at	GPRC5A	4713.6	1335.8	1984.9	519.3	−1.8	−1.9
203021_at	SLPI	103.1	50	433.1	106.5	−1.0	−2.0
202912_at	ADM	5707.9	1915.5	2337.8	1041.2	−1.6	−1.2
202842_s_at	DNAJB9	465.9	194.5	779.2	197	−1.3	−2.0
202771_at	FAM38A	672.6	323	566.1	249.3	−1.1	−1.2
202733_at	P4HA2	1168.1	357.6	1595.5	513	−1.7	−1.6
202679_at	NPC1	1602	357	893.5	230.7	−2.2	−2.0
202558_s_at	HSPA13	483.1	127.5	245.3	50	−1.9	−2.3
202499_s_at	SLC2A3	4317.3	628.8	318.1	50	−2.8	−2.7
202497_x_at	SLC2A3	1223.2	159.1	112	50	−2.9	−1.2
202185_at	PLOD3	919.6	339.2	800.5	344.1	−1.4	−1.2
202130_at	RIOK3	1233.7	535.7	1550.9	665.4	−1.2	−1.2
202067_s_at	LDLR	219.3	50	205.3	86.2	−2.1	−1.3
201939_at	PLK2	806.2	350.8	693.3	323.3	−1.2	−1.1
201694_s_at	EGR1	1635.6	416.5	4085.5	527.7	−2.0	−3.0
201693_s_at	EGR1	393.5	114.7	1281.7	104	−1.8	−3.6
201531_at	ZFP36	555.6	233.7	917.6	164.5	−1.2	−2.5
201465_s_at	JUN	115.7	50	661.4	114.8	−1.2	−2.5
201464_x_at	JUN	730.5	298.1	3342.3	857.3	−1.3	−2.0
201325_s_at	EMP1	878.7	118.1	250.7	50	−2.9	−2.3
201324_at	EMP1	2645.2	498	797.3	58.1	−2.4	−3.8
201289_at	CYR61	1482.8	284.7	3062.1	131.6	−2.4	−4.5
201041_s_at	DUSP1	618.7	280.2	1524.8	181.1	−1.1	−3.1
200924_s_at	LOC442497 /// SLC3A2	488.1	242.8	2518.9	589.5	−1.0	−2.1

Table W5. (*continued*)

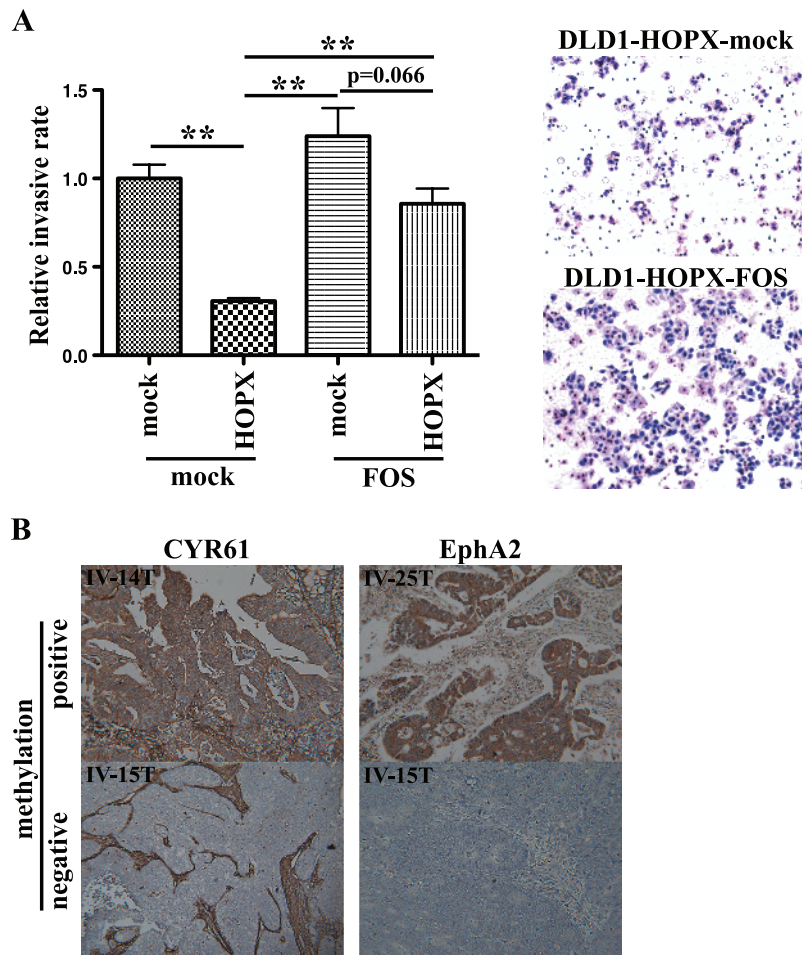| Probe Set	Gene Symbol	HCT116 Mock Signal	HCT116 HOPX Signal	DLD1 Mock Signal	DLD1 HOPX Signal	HCT116 Log$_2$ Ratio	DLD1 Log$_2$ Ratio
200859_x_at	*FLNA*	458.4	103.5	645.2	259	–2.1	–1.3
200825_s_at	*HYOU1*	536.4	189.5	920.8	221.8	–1.5	–2.1
200799_at	*HSPA1A /// HSPA1B*	138.1	50	398	50	–1.5	–3.0
1554462_a_at	*DNAJB9*	118	54.3	144.4	59.3	–1.1	–1.3
1007_s_at	*DDR1*	852.1	320.8	451.6	174.4	–1.4	–1.4

Figure W1. (A) Matrigel invasion assay in double transfectants (mock-mock, HOPX-mock, mock-FOS, and HOPX-FOS). After fixation and staining, invading cells were photographed (right) and counted at 100× magnification. Left: Data are expressed relative to mean invading cells of mock-mock double transfectant. Cell growth for 22 hours determined by the WST-1 assay was similar (data not shown). Two independent experiments were done in triplicate, and values indicate means ± SEM. **$P < .001$. Error bars, SEM. (B) Representative immunohistochemistry of CYR61 and EPHA2 in CRC with low and high Q-MSP value of HOPX-β (original magnification, ×100). Of note, CYR61 is expressed in stroma of all CRC samples but is suppressed in epithelial cells in CRC with low Q-MSP value (left bottom).
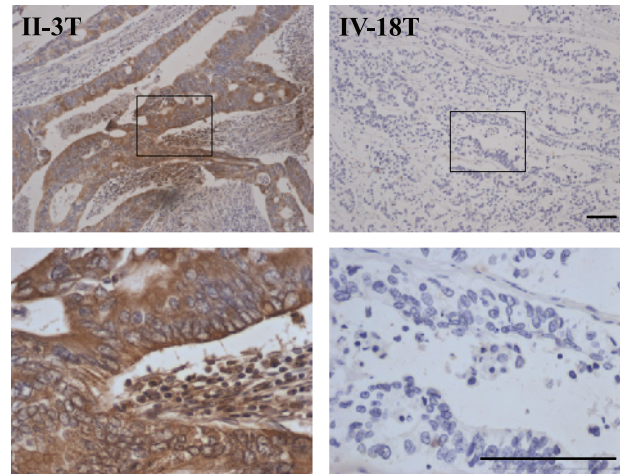
Figure W2. Representative immunohistochemistry of HOPX in well-differentiated CRC with a low Q-MSP value (0.6) of HOPX-β (left) and poor differentiated CRC with a high Q-MSP value (35.1) of HOPX-β (right). Bars, 100 μm.

Table W6. Correlation Analysis of Clinicopathologic Variables in Stage III CRC with Methylation Status of HOPX-β.

Variables	Patient No.	HOPX-β Promoter Hypermethylation				Multivariate Analysis	
		Positive	Negative	Positive Rate (%)	P^*	OR (95% CI)	$P^{\dagger}$
Sex							
Male	97	48	49	49.5	>.999	0.80 (0.41-1.54)	.511
Female	73	37	36	50.7			
Age, years							
<60	74	36	38	48.6	.877	1.13 (0.58-2.18)	.722
≥60	96	49	47	51.0			
Tumor position							
Colon	96	48	48	50.0	>.999	0.99 (0.51-1.91)	.968
Rectum	74	37	37	50.0			
Differentiation							
Nonpoor	149	70	79	47.0	.060	4.05 (1.40-13.34)	.014
Poor	21	15	6	71.4			
T factor							
T1, 2	20	9	11	45.0	.813	1.23 (0.42-3.69)	.710
T3, 4	150	76	74	50.7			
N factor							
N1	119	64	55	53.8	.180	0.47 (0.20-1.11)	.091
N2	51	21	30	41.2			
Intramural lymphatic involvement							
Negative	0	0	0	0.0	n/a	n/a	n/a
Positive	170	85	85	50.0			
Intramural vascular involvement							
Negative	7	4	3	57.1	>.999	0.84 (0.13-5.24)	.853
Positive	163	81	82	49.7			
Preoperative CEA							
Normal (≤2.5 ng/ml)	103	46	57	44.7	.116	1.68 (0.84-3.42)	.147
Elevated (>2.5 ng/ml)	67	39	28	58.2			
Preoperative CA19-9							
Normal (≤37 ng/ml)	150	70	80	46.7	.030	3.32 (1.09-11.68)	.044
Elevated (>37 ng/ml)	20	15	5	75.0			

OR, odds ratio.
*Fischer exact test.
†Multivariate logistic regression analysis.