Supplementary Table S1. Gene validation process using RT-PCR and MSP Analyses in colon cancers

	Analyses	Expression (RT-PCR)	Expression(RT-PCR) and Methylation (MSP)	Methylation (MSP)		
No.	**Genes**	**1) Expression in Normal Colon**	**2) Correlation between gene silencing and DNA methylation in colon cancer cell lines***	**3) Colon Cancer Patient tissues (n=20)**	**4) Normal Colon tissues** (n=20)**	**Others**
1	ABCA1	+	YES	0%		Excluded (no methylation in CRC)
2	ALS2CL	+	YES	0%		Excluded (no methylation in CRC)
3	APC2	+	YES	100%	0%	
4	ARHGEF4	+	YES	100%	55%	Excluded (methylation in NC)
5	CD109	+	YES	33%	0%	
6	CD248	-				
7	CHD5	+	YES	10%	0%	
8	EPHB6	+	YES	0%		Excluded (no methylation in CRC)
9	EVL	+	YES	60%	0%	
10	GPNMB	+	YES	100%	0%	
11	HDAC4	+	YES	0%		Excluded (no methylation in CRC)
12	ICAM5	+	YES	5%	0%	
13	LGR6	+	YES	15%	0%	
14	LMO7	+	YES	100%	100%	Excluded (methylation in NC)
15	MAP2	+	YES	0%		Excluded (no methylation in CRC)
16	MKRN3	+	YES	100%	100%	Excluded (methylation in NC)
17	MMP2	+	YES	95%	0%	
18	NRCAM	+	YES	50%	0%	
19	PCDHB15	+	YES	100%	100%	Excluded (methylation in NC)
20	PTPRD	+	YES	50%	0%	
21	RET	+	YES	11%	0%	
22	SEMA5B	+	NO			
23	RNF182	+	YES	0%		Excluded (no methylation in CRC)
24	SERPINB1	+	YES	0%		
25	SORL1	+	YES	0%		Excluded (no methylation in CRC)
26	STARD8	+	YES	55%	0%	
27	SULF2	+	YES	0%		Excluded (no methylation in CRC)
28	SYNE1	+	YES	95%	0%	
29	ZNF432	+	YES	10%	5%	Excluded (methylation in NC)

30	ZNF442	+	YES	0%		Excluded (no methylation in CRC)
31	AGC11	+	NO			
32	AKAP12	+	NO			
33	APC	+	NO			
34	ARFGEF2	+	NO			
35	BCL11A	+	NO			
36	CACNA2D3	-				
37	CENTG1	-				
38	COL7A1	-				
39	CPAMD8	+	YES	90%	0%	
40	CSPP1	+	NO			
41	EYA4	+	YES	70%	0%	
42	FBN2	+	YES	90%	0%	
43	FBXW7	-				
44	FLNC	+	YES	30%	0%	
45	FN1	+	NO			
46	GAB1	+	NO			
47	GNAS	+	NO			
48	GRID1	+	YES	60%	0%	
49	GUCY1A2	+	YES	50%	0%	
50	HAPLN1	+	YES	10%	0%	
51	IGFBP3	+	YES	25%	0%	
52	JARID1B	+	NO			
53	KALRN	+	NO			
54	KIAA0182	+	NO			
55	KIAA0664	-				
56	KIAA1632	+	NO			
57	KIAA1946	+	NO			
58	KRAS1	+	NO			
59	LAMA1	+	YES	100%	0%	
60	MAPKBP1	-				
61	MYO5C	+	NO			
62	NOTCH1	-				
63	NTNG1	+	YES	70%	0%	

64	PKNOX1	+	NO			
65	PLCG2	-				
66	PPM1E	+	YES	55%	0%	
67	PRKD1	+	YES	20%	0%	
68	SFRS6	+	NO			
69	SLC22A15	+	YES	5%	0%	Excluded (no methylation in CRC)
70	SMAD3	+	NO			
71	TCERG1L	+	YES	100%	0%	
72	TDRD6	+	YES	0%		Excluded (no methylation in CRC)
73	THBS3	-				
74	ZNF569	+	YES	40%	0%	
75	SH3TC1	+	YES	40%	0%	
Total Gene No.		65	45	34	29	

1) '+: indicated that gene is expressed. -: indicates that gene is not expressed,

2) * colon cancer cell lines (HCT116, RKO, SW480, and Colo320) were tested for RT-PCR and MSP analyses

3) **Normal colon tissues are cancer-free normal colon tissues

4) Grey color filled genes are 29 genes that cancer-specifically methylated in colon cancer.

Supplementary Table S2. Characteristics and clinical information on the initial cohort of colon cancer patients in this study

Patient #	Sex	Race	Age	Cancer Stage	Grade
CR071	F	White	59.3	Stage 3	Moderately
CR073	F	Black	80.8	Stage 3	Moderately
CR074	F	White	87.4	Stage 3	Moderately
CR075	F	Black	66.8	Stage 3	High
CR076	M	White	69.6	Stage 3	High
CR077	M	Black	45.4	Stage 3	Moderately
CR078	M	White	56.0	Stage 3	High
CR079	M	White	70.6	Stage 3	Moderately
CR080	F	Asian	48.3	Stage 3	High
CR081	M	White	74.8	Stage 3	Moderately
CR082	M	White	53.1	Stage 3	Well
CR083	M	White	47.0	Stage 3	Moderately
CR084	F	White	65.5	Stage 3	High
CR085	F	Black	43.5	Stage 3	Moderately
CR086	F	Black	45.3	Stage 2	Well
CR088	M	Black	62.7	Stage 3	Well
CR089	F	White	74.6	Stage 3	Well
CR091	F	Black	78.7	Stage 3	Well
CR092	F	Black	57.1	Stage 3	Well
CR093	F	White	52.2	Stage 3	High
CR094	F	White	60.5	Stage 3	High

Supplementary Table S3. Methylation pattern of CAN genes and others in ECM pathway in individual colorectal tumor samples

		Colon tumor patient samples (CRC)																				
	CAN genes	71	73	74	75	76	77	78	79	80	81	82	83	84	85	86	88	89	91	92	93	94
1	**CD109**	M	U	U	U	M	M	U	M	M	U	U	U	U	U	U	U	U	U	M	U	M
2	**EVL**	U	M	U	U	M	M	M	M	U	M	U	U	M	M	M	M	M	M	M	M	U
3	**GPNMB**	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
4	**ICAM5**	U	U	U	U	U	U	U	U	U	U	U	U	M	U	U	U	U	M	U	U	M
5	**MMP2**	M	M	M	M	M	M	M	M	M	M	M	U	M	M	M	M	M	M	M	M	M
6	**NRCAM**	M	M	U	U	U	M	U	U	M	U	U	U	M	U	M	M	M	M	M	M	M
7	**CPAMD8**	M	M	M	M	M	M	M	M	M	M	M	U	M	M	M	M	M	M	M	M	M
8	**FBN2**	M	M	M	M	M	M	M	M	M	M	M	M	M	U	M	M	M	M	M	M	M
9	**FLNC**	M	U	U	U	M	U	U	U	M	U	U	U	M	U	U	M	M	U	U	U	M
10	**HAPLN1**	U	U	U	U	U	U	U	U	U	U	U	U	M	U	U	U	U	U	U	U	U
11	**IGFBP3**	U	U	U	U	U	M	U	M	U	U	U	U	M	U	U	M	U	U	M	U	M
12	**LAMA1**	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
13	**NTNG1**	M	U	U	U	M	M	U	M	M	M	M	M	M	U	M	M	M	M	M	M	M

		Colon tumor patient samples (CRC)																				
	ECM genes	71	73	74	75	76	77	78	79	80	81	82	83	84	85	86	88	89	91	92	93	94
1	**PLAU**	U	M	U	M	M	M	M	M	M	M	M	M	U	M	M	M	M	M	M	M	M
2	**TIMP3**	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	U	M	U	U	U
3	**MMP9**	M	M	M	M	M	M	M	M	M	M	M	U	M	M	M	M	M	M	M	M	M
4	**NID1**	M	M	M	M	U	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
5	**TIMP2**	M	M	M	M	U	M	U	U	U	U	U	U	U	M	M	M	M	U	U	U	U
6	**Osteonectin**	U	U	M	M	U	M	M	M	M	M	U	U	M	M	U	M	M	U	M	M	U

Supplementary Table S4. The frequency of aberrant methylation of the genes (*IGFBP3, EVL, CD109,FLNC* and *NRCAM)* and its association with various clinicopathologic findings

	Training Set				Validation Set			
	IGFBP3 U	**IGFBP3 M**	**EVL U**	**EVL M**	**IGFBP3 U**	**IGFBP3 M**	**EVL U**	**EVL M**
N	76	69	37	107	24	48	24	48
Median Age (years)	60.5	70.2	57	69.6	65.7	70.7	68.4	74
Location								
Right Colon	37	41	16	62	22	18	13	27
Left Colon	37	28	21	44	24	8	11	21
Stage								
Stage I	19	17	8	28	10	8	5	13
Stage II	24	24	12	35	16	11	6	20
Stage III	21	15	10	26	16	4	11	9
Stage IV	12	13	7	18	4	3	1	6

Supplementary Table S5. Primers used for MSP and RT-PCR analyses

Gene List	primers	Sense (5'-3')	Antisense (5'-3')
APC	Unmethy(U)	GTGTTTTATTGTGGAGTGTGGGTT	CCAATCAACAAACTCCCAACAA
	Methy(M)	TATTGCGGAGTGCGGGTC	TCGACGAACTCCCGACGA
	RT1	GAGACAGAATGGAGGTGCTGC	GTAAGATGATTGGAATTATCTTCT
CD109	Unmethy(U)	GTAGTGGATTGTAGTTTAGGTAGATGTTGTTG	CACAACAATACACACACAAAAAAAATAAACAACA
	Methy(M)	GATTGTAGTTTAGGTAGACGTCGTC	CGATACACACGCAAAAAAAATAAACGACG
	RT1	TTGAATTCCCAATCCTGGAG	TTGTTGCCACTAACCACCAA
CHD5	Unmethy(U)	GGGAGGAGTGTTTGGGTTTTGTG	CAACAAACAAAACAACCTCAACAAAAAAATAACA
	Methy(M)	GAGCGTTCGGGTTTTGC	CGACCTCGACGAAAAAATAACG
	RT1	GTGGTGCGCGAGGAGGACG	CTCGATGTTGCCACCAACTCG
CPAMD8	Unmethy(U)	AATGAGTGGTGTTTTGTTTTGGTTGTTGTTTTTG	CAACCTCCTCTATCACCAAAAAATACTCACA
	Methy(M)	GCGTTTTGTTTTGGTCGTTGTTTTC	CTCTATCGCCGAAAAATACTCACG
	RT1	CATGGATCTCCACCAGCAAG	AACCTGGATAAGGCCACCAC
	RT2	CACTCAGTGTGAAGGTCCCG	GAAGGTCTTCAGCAGGGAGG
EVL	Unmethy(U)	GTGTGTTTTTTTTTTGAGGATTTGGAGTTGTTTG	ACCACCAAAAAATAAAAAAACAAAAAACAAACCA
	Methy(M)	GAGGATTCGGAGTCGTTC	CCGAAAAATAAAAAAACGAAAAACGAACCG
	RT1	TCGTTGGAGTCAAGTTGCAG	GAGAACGTGGTTGCCTCTTC
EYA4	Unmethy(U)-2	TTTTTTTGAGGTTTTTGGTGGTTTTATGAGTTTG	AAATTTTACACAAAAACCAAAAACAACCAAAACA
	Methy(M)-2	TTTTCGGCGGTTTTACGAGTTC	AAAACCGAAAACGACCGAAACG
	RT1	GAGCCCTTGAACAGCAGTGA	TGTGTGGATAGGGCTTGGAA
	RT2	CTTTCTTTTGTTGGGGAGGG	CAGTTTCTTGCTGATGCGGT
FBN2	Unmethy(U)-2	GTTTTGTTGGGTTTTTAAAATTTTTGTGTTGTG	AAATAACAACTACAAAACCAAACAAAAATACA
	Methy(M)-2	GGGTTTTTAAAATTTTCGCGTCGC	CTACGAAACCGAACGAAAATACG
	RT1	AGGGATCCTTCATTTGCCAC	GCCCGGGGTATTTACACACT
	RT2	CTCAACCTTCGCTTTGTGGA	ACTCCCCAGGGTGTTGTAGC
FLNC	Unmethy(U)-1	AGTGAGTTTTGTGGTTGTGTTTTAATAGTGTTTG	CTAAATAACCACTATTATTCATCATACTAACACA
	Methy(M)-1	TGGTCGCGTTTTAATAGCGTTC	TAAATAACCGCTATTATTCATCATACTAACGCG
	RT1	GTCATTGCCCCTGAGGAGAT	TGTCATTGTTGGGAACCACC
	RT2	AAGCTGGATGTGCAGTTTGC	CCACCTTGCTATTAAGGCCC
GPNMB	Unmethy(U)-1	AGGTTTGAGATGTGGGTTGTGTTTTG	CCAAAAACATAAACATTTTCCCAAATCACAATCA
	Methy(M)-1	ACGTGGGTCGCGTTTC	TAAACGTTTTCCCGAATCGCAATCG
	RT1	CCAGAAATTGGGACGATGTT	GGGGAGATCTTTGAGGAAGG
GRID1	Unmethy(U)-1	TAGTTGTGGGAAGTGTTGTTGTTATAGTTGTG	CCAACCCAACCCAACACAATCA
	Methy(M)-1	GAAGCGTCGTTGTTATAGTCGC	CCAACCCAACGCGATCG
	RT1	ATAACACCTCCTGGAACGGG	TACCGCTTGCTGAAGTCCAC
	RT2	GAGTACTTCCGAGCCAAGGG	GGCGTAGTTCCCCTTCTTTG
GUCY1A2	Unmethy(U)-2	TTTGATGTGTTTAATGTTGTTGTTTGTTGGTTTTG	TCCTTCAAAACATACTACCAACAAAACTACAACA
	Methy(M)-2	TTTAACGTTGTCGTTTGTCGGTTTC	AAACATACTACCGACGAAACTACAACG
	RT1	CACAGGGAACCTCCCAAGTT	GGTAGACAGTCGCAGCAGGA
	RT2	TGGTGATGTAGCCCAGCAAT	CTTCAAGGCCATCAGAGCAA

HAPLN1	Unmethy(U)-2	GTGGTGGTGGTTTTAAGAAGTTTGTAGTTTTG	AATTACAACAAACAAAACCCAATATCACAAAACA
	Methy(M)-2	GGCGGTTTTAAGAAGTTCGTAGTTTC	CAAAACCCGATATCGCGAAACG
	RT1	CGGTGCAAGCTTGTCTCAAT	GCACTGCAGCCTCAGTAGGA
	RT2	GGCTCAGTGATGGCTCTGTG	ATTGAGACAAGCTTGCACCG
	RT3	GCAACAGGTTCCACAAGCAC	TTTTGGTCAGAAAGGCAGGA
ICAM5	Unmethy(U)-1	TTTAGTTTTGTGTTTTGGTTTTGTGTTTTTTATTG	TCCTAACAAAATACCAAAATACAAAAAAAATACA
	Methy(M)-1	CGTTTCGGTTTCGTGTTTTTTATC	CTAACAAAATACCGAAATACGAAAAAAATACG
	RT1	GGCGGCCAATGATCAAGGCG	CGAGTCCTCCAGAGCGCACG
IGFBP3	Unmethy(U)	GTGTTTGGGTTGTTTAGATGTGAGTATTGTG	CTACTTAACAAACTAAACACACAAAAATAAAACA
	Methy(M)	GGTCGTTTAGATGCGAGTATTGC	TACTTAACAAACTAAACGCGCAAAAATAAAACG
	RT1	CAGGGGTGTACACATTCCCA	TCCTTGGCAGTCTTTTGTGC
	RT2	AAAGACAGCCAGCGCTACAA	TGGGAATGTGTACACCCCTG
LAMA1	Unmethy(U)	TGTTTTGGTTATGTGGGTTTTTTGTGTTTGTTG	AAACCTAAACCTACAAAAAACAAACAACATCCA
	Methy(M)	CGTGGGTTTTTCGCGTTTGTC	TAAACCTACAAAAAACGAACAACGTCCG
	RT1	AGGAACTGGCCAGAGATGCT	TCTTTGGTTTTGGCTCCCTT
	RT2	ACTTTCCCGACTGTGTTCCC	CACACGCAGAAGAGGCTGAT
LGR6	Unmethy(U)	TGGGTAGGGGTATGGTTAGGTG	CCCTAACTACACACACATACCCAAAAACTAAACA
	Methy(M)	GTAGGGGTACGGTTAGGC	GCACGTACCCGAAAACTAAACG
	RT1	TGCCTTATGCCTACCAGTGC	TGCCTTATGCCTACCAGTGC
MMP2	Unmethy(U)	GTGGTTATATGTATTGAGTTAGTGATTTTTGGGTG	AAAAAACAAAACACCCTCAAAAAACCCATAAACA
	Methy(M)	TATCGAGTTAGCGATTTTCGGGC	CGCCCTCAAAAAACCCGTAAACG
	RT1	GAAAATGGATCCTGGCTTCC	TGATGCTTCCAAACTTCACG
	RT2	ACATCAAGGGCATTCAGGAG	TGAACCGGTCCTTGAAGAAG
NRCAM	Unmethy(U)	TTTGTTTGGGTTTTTGTGGATATGTTTGGGTG	CCAAACAACAACTAAAAAAACAAAATCAACCTCA
	Methy(M)	GTTTTTGCGGATACGTTTGGGC	CGACTAAAAAAACGAAATCGACCTCG
	RT1	GACGAAAGCCTCAGAACCAG	ACATTTGCCACAACCACAGA
NTNG1	Unmethy(U)	TTTTTGGGTTTGTTTTTTTGTTGTTTTTTTTTTTG	CAAAACCCTACTCCAAACAAACAACA
	Methy(M)	TTTCGGGTTTGTTTTTTTGTCGTTTTTTTTTTC	CCTACTCCGAACGAACAACG
	RT1	CGGAAGAAGGGAAAGTTTGG	GATTGCCCATTGCACAGAAC
	RT2	ACGCAATATGGCTTCCCTCT	TACACTTGCACCTTCCTCGC
PPM1E	Unmethy(U)-2	GTGGTGGTAGTTTTGGGGTATTTG	CCTCCACCTCCTCACAAATAATACA
	Methy(M)-2	GCGGTAGTTTCGGGGTATTC	CCACCTCCTCGCGAATAATACG
	RT1	CCCGTTCTGTCTTCAGCAAA	GATCATGGGGGAACATCTCC
	RT2	GGTGCACCAAAGAAAGCAAA	CTCCCCTGTTGAACCCAAAT
PRKD1	Unmethy(U)-2	TTTGTTGAGGTGGTTTTGAGTTTTGTTATTTG	ACAACCACAATAACCACAAAATACCCAATCA
	Methy(M)-2	GGCGGTTTCGAGTTTCGTTATTC	CAATAACCGCAAAATACCCGATCG
	RT1	GCCTCAGCTGATCCTTTTCC	GATGACCCCAACAGACCACA
	RT2	GACGCCAACAGAACCATCAG	ATGAGGATTGGCCCCATTAG
PTPRD	Unmethy(U)-1	TTTTGTTTTTTGTTGTTTGGTTTTGTGTTTTGTTG	CAATACAACAACCACTACAACAACTACCA
	Methy(M)-1	GTTCGGTTTTGCGTTTCGTC	CGCTACGACAACTACCG
	RT1	TCAGACAGGGGTCTCTGGCG	TCTGATCGTAACTGCTTAATACG

RET	Unmethy(U)-1	TTGGTTTTGTTTGGTTTATTTTTGGATTGTTTTTG	CTACACACCCTACTTCAATCACAAAACTAAAACA
	Methy(M)-1	GGTTTCGTTTGGTTTATTTTTGGATCGTTTTC	CTACTTCGATCGCGAAACTAAAACG
	RT1	AAGGCGACGTCCGGTGCCG	CAGGAGCCTGTAGGCCACG
SLC22A15	Unmethy(U)-1	GTGTTTTTTGTTTTTTGTTTGGTGTTTATTTTTTG	TAAACCCACCAAAACAAAAATAAAAACACTAACA
	Methy(M)-1	GTTTTTCGTTCGGCGTTTATTTTTC	CCGAAACGAAAATAAAAACGCTAACG
	RT1	AGCCATTCCTTGTCCTTGCT	CAAGCCCAACATTCCTGATG
	RT2	TCCAAGCAAATCTGACCCAA	TGCCCAACCACCATTTAAGA
TCERG1L	Unmethy(U)-1	TTTTTTTGGTTGGTTGGTTTTGAGTGAGTTG	ACAAAACAAAAACACATTACCTACCCATAAAACA
	Methy(M)-1	GGTCGGTTTCGAGCGAGTC	AAAACACGTTACCTACCCGTAAAACG
	RT1	TGCTCTTGCAACCCCTATTG	TGAGGTGCTAACACCACCGT
	RT2	CACTTCCGAGACATGCTGCT	TTGAGCAGGAGATAGCGTGG
TDRD6	Unmethy(U)-3	TTTTTAATTTTTGGAAGTGGGGGTTGTGTTG	CACAACTACACCAAAATCACATCAAAATACAC
	Methy(M)-3	TGGGGGTCGCGTC	CTACACCGAAATCACATCGAAATACACG
	RT1	TAGTTGACCGAGGCAATTCG	AAGTTTTTCCCAAAGGCCAA
ZNF569	Unmethy(U)	GTTTGTGTAAAATTTAGGTTGAGTTTTTTTGTGTG	CACCCAAACAAAACTAACTTCACTATTTACACA
	Methy(M)	TTCGTGTAAAATTTAGGTTGAGTTTTTTTGCGC	CGAACGAAACTAACTTCACTATTTACGCG
	RT1	GCCTTCTCCCAAAGCTCATC	ATGGCCTTGCCATATTCACA
BGN	Unmethy(U)-1	TTAGTTTTTAGTTTTTTGTTTGTTGT	TAAAATAATAAACCATCCACACATC
	Methy(M)-1	TAGTTTTTAGTTTTTCGTTCGTCGT	ATAATAAACCGTCCGCACGT
	RT1	TGCCCAGGAGTGAGTAGCTG	CGAAGCTTCCTCATCGTTCA
	RT2	AACCTGTACTGTCCCGGAGG	TGGACCAGAAGGATAAGGGG
SH3TC1	Unmethy(U)-2	TTGTTTAGGAAAGAGTTAATGATTAATTGGTTGTG	TACTTCCTCCCTACACCTAAAAACCAATAACA
	Methy(M)-2	CGTTTAGGAAAGAGTTAATGATTAATCGGTTGC	CCTACGCCTAAAAACCGATAACG
	RT1	ACCTCATCGAGATCCTTGGG	GGCATCCTCCTCAGAAAAGC
	RT2	TGCTCTCGGCTTCACTCATC	GGTGGTCAGGACACAGGACA

Supplementary Table S6. Multivarate cox proportional regression of methylation status of 5 genes adjusted for age in CRC stage 2 (n=76).

Gene	Stage II CRC (Training + Validation Cohorts)		
	HR	95%CI	p value
IGFBP3	3.02	1.42-6.40	0.004
EVL	1.45	0.55-3.78	0.453
CD109	2.41	1.14-5.10	0.021
NRCAM	1.29	0.61-2.75	0.505
FLNC	0.90	0.43-1.86	0.774
IGFBP3 + EVL	2.30	0.70-7.61	0.172
IGFBP3 + CD109	5.85	2.03-16.83	0.001
EVL + CD109	2.50	0.71-8.83	0.153
IGFBP3 + EVL + CD109	4.82	0.90-25.68	0.066

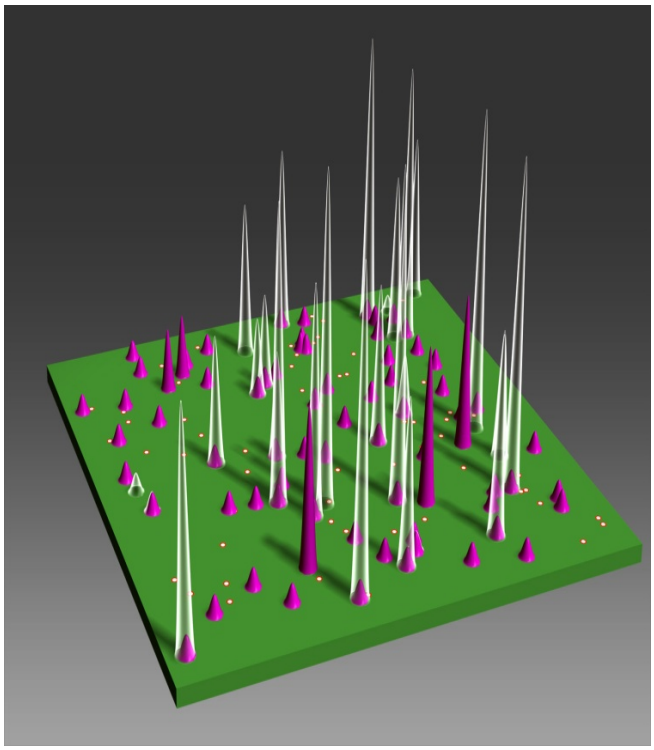
Supplementary Materials and Methods

Quantitative Methylation-Specific PCR (MSP) using real-time PCR

Sodium bisulfite modification, which converts unmethylated cytosine residues to uracil residues, was carried out on 1ug genomic DNA isolated from the paraffin-embedded tissue sections with the use of an EZ DNA methylation kit (Zymo Research Co) according to the manufacturer's instructions. NDRG4 methylation-specific PCR analysis was performed on bisulfite-modified DNA as described in detail elsewhere (20,21).

For quantitative real-time analyses, the Power SYBR Green PCR kit (Applied Biosystems) was used and the amplification conditions consisted of an initial 10-min denaturation step at 95 °C, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing and extension for 30 s and 60 s, respectively. A ABI StepOnePlusReal-Time PCR System was used (Applied Biosystems), and for quantitation the comparative cycle threshold (Ct) method was used, normalizing the Ct values for the indicated gene to the Ct values of Unmethylated reaction relative to a methylated reaction sample.

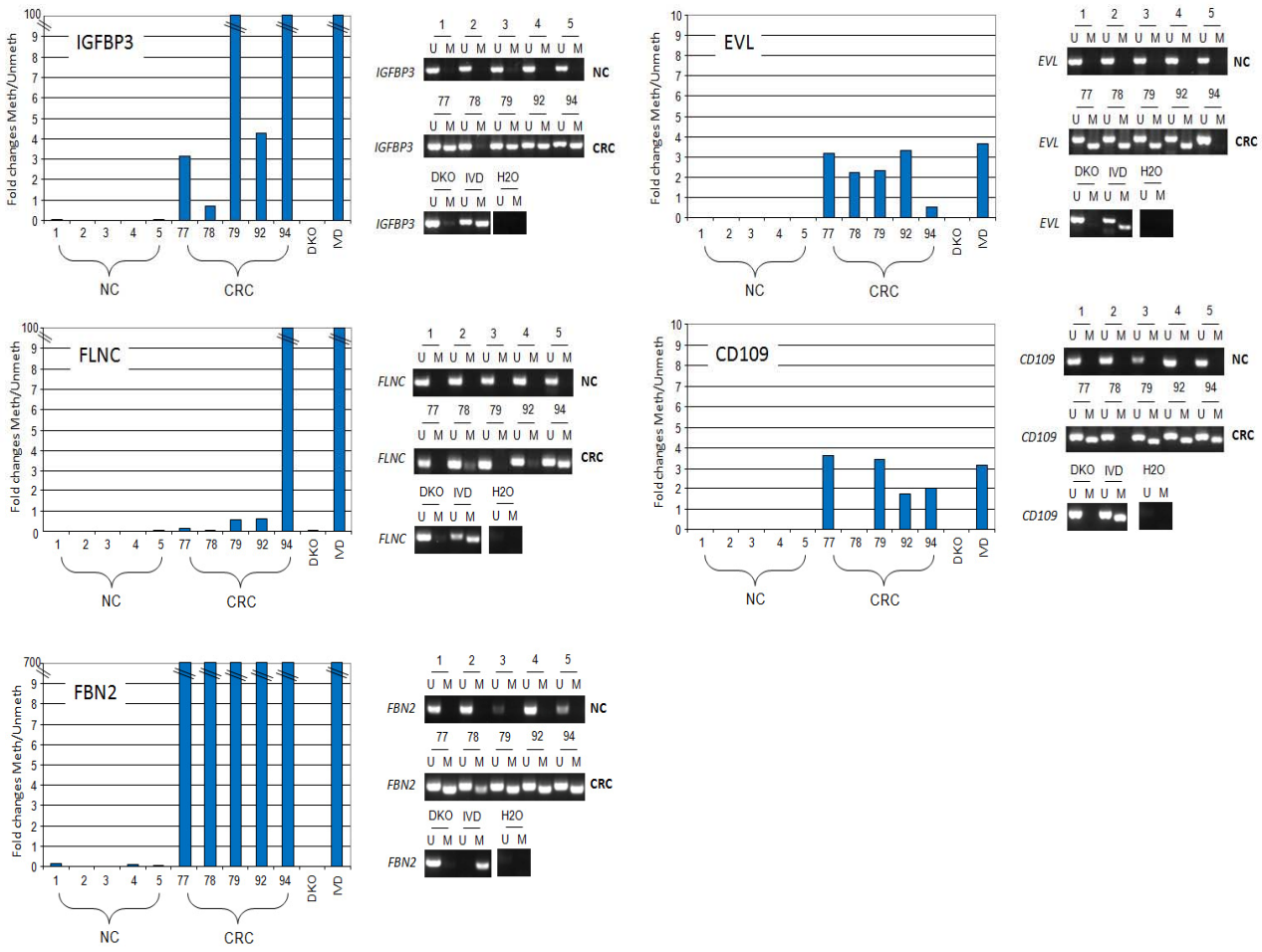
Supplementary Figure S1. Yi *et al.*



No.	Genes	Colon Cancer	
		Mutation*	Methylation***
1	*HAPLN1*	5.7%	10.0%
2	*SLC22A15*	5.7%	5.0%
3	*ICAM5*	< 5% *	5.0%
4	*CHD5*	< 5%	10.0%
5	*RET*	8.6%	10.5%
6	*LGR6*	7.1%	15.0%
7	*PRKD1*	5.7%	20.0%
8	*IGFBP3*	5.7%	25.0%
9	*FLNC*	5.7%	30.0%
10	*CD109*	5.7%	33.3%
11	*ZNF569*	< 5%	40.0%
12	*SH3TC1*	5.7%	40.0%
13	*GUCY1A2*	8.6%	50.0%
14	*NRCAM*	< 5%	50.0%
15	*PTPRD*	7.1%	50.0%
16	*PPM1E*	< 5%	55.0%
17	*STARD8*	< 5%	55.0%
18	*GRID1*	5.7%	60.0%
19	*EVL*	5.7%	60.0%
20	*EYA4*	5.7%	70.0%
21	*NTNG1*	5.7%	70.0%
22	*CPAMD8*	5.7%	90.0%
23	*FBN2*	11.4%	90.0%
24	*MMP2*	8.6%	95.0%
25	*SYNE1*	14.3%	95.0%
26	*LAMA1*	11.4%	100.0%
27	*TCERG1L*	5.7%	100.0%
28	*APC2*	< 5%	100.0%
29	*GPNMB*	< 5%	100.0%

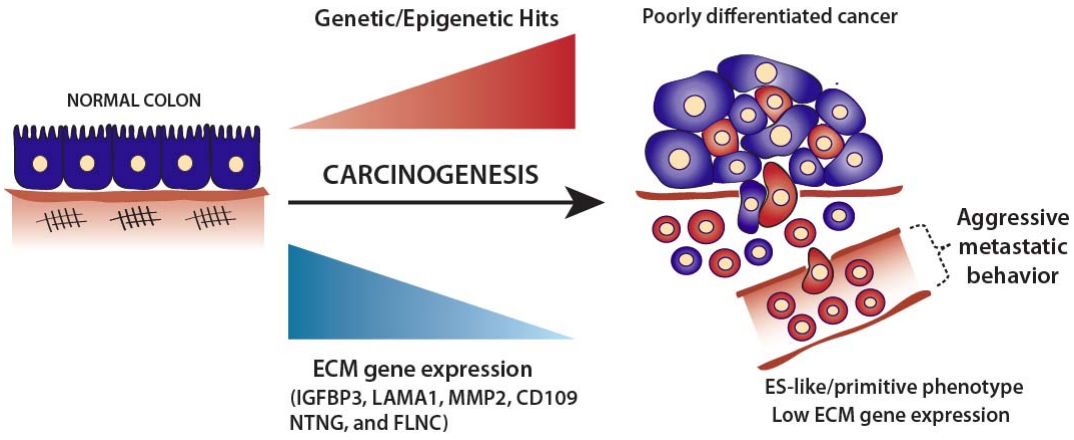
Supplementary Figure S1. (Left) Mutated genes are plotted in two-dimensional space representing chromosomal positions of CAN genes (purple). 29 hypermethylated genes are plotted with CAN genes (Wood et al., 2007). Transparent peaks correspond to hypermethylated genes and peak heights reflect DNA methylation frequency in colon primary cancers. (Right) Table display the mutation and methylation rates. Mutation rate was adopted from Wood et al., 2007. * and ** indicate mutation score and mutation score was less than 5% rate, respectively. *** indicates methylation frequency of genes in CRC patient samples (n=20)

Supplementary Figure S2. Yi *et al.*



Supplementary Figure S2. Quantitative MSP analysis with representative genes (*IGFBP3*, *EVL*, *FLNC*, *CD109*, *and FBN2*) using real-time PCR. NC (1, 2, 3, 4, and 5) and CRC (77, 78, 79, 92, and 94) indicate normal colon and colorectal cancer, respectively. Left graphical panel shows MSP using real time PCR and right sided panel shows the gel based showed MSP analysis for the same samples. Real-time MSP is shown as fold change for Methylated signal relative to Unmethylated signal. In MSP analysis (right panel), signals for unmethylated (U) and methylated (M) DNA are shown for each sample.

Supplementary Figure S3. Yi et al.



Supplementary Figure S3. A proposed model showing disruption of the ECM pathway in invasive CRC associated with aggressive behavior. Normal colonic epithelium is depicted on the left as uniform blue cells on an intact basement membrane (pink with track marks). The polarity of the cells and the integrity of the membrane reflect normal ECM regulation by the epithelial and underlying mesenchymal cells. During the process of epithelial cell transformation, there are increasing genetic and epigenetic alterations in the ECM genes as discussed in the text. This loss of function helps generate an invasive cancer (blue and red cells on the right). Cells with the most profound dysregulation of the ECM pathway associated with an epithelial-mesenchymal transition, loss of polarity, loss of cell-cell adhesion properties, and ability to enter the vascular space are shown (red cells on the bottom right). These features characterize CRC with an ESC-like state and the greatest potential for invasion into the lymphovascular space and establishing eventual distant metastases.