Supplementary Materials: Genome-Wide Methylation Approach Identifies a New Hypermethylated Gene Panel in Ulcerative Colitis

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Gene	Accession No.	Gene Description	Methylation Fold Changes (UC/N)
ACAT2	NM_005891	Acetyl-CoA acetyltransferase 2	2.0
ACSS2	NM_018677	Acyl-CoA synthetase short-chain family member 2	1.7
CALN1	NM_031468	Calneuron 1	2.2
CDRT15	NM_001007530	CMT1A duplicated region transcript 15	1.8
CDX2	NM_001265	Caudal type homeobox 2	1.8
DNAJC17	NM_018163	DnaJ (Hsp40) homolog, subfamily C, member 17	2.3
ESYT3	NM_031913	Extended synaptotagmin-like protein 3	1.8
EXT1	NM_000127	Exostosin glycosyltransferase 1	1.9
FAM19A5	NM_015381	Family with sequence similarity 19 (chemokine (C-C motif)-like), member A5	2.6
FAM217B	NM_022106	Family with sequence similarity 217, member B	2.3
FAM46A	NM_017633	Family with sequence similarity 46, member A	1.8
FSTL1	NM_007085	Follistatin-like 1	1.7
FXYD3	NM_005971	FXYD domain containing ion transport regulator 3	1.9
GAS1	NM 002048	Growth arrest-specific 1	1.8
H3F3AP4	NR_002315	H3 histone, family 3A, pseudogene 4	1.7
HNMT	NM_006895	Histamine N-methyltransferase	1.9
HNRNPF	NM_004966	Heterogeneous nuclear ribonucleoprotein F	1.9
HOXA9	NM_152739	Homeobox A9	1.8
IFITM1	NM 003641	Interferon induced transmembrane protein 1	1.8
KBTBD11	NM_014867	Kelch repeat and BTB (POZ) domain containing 11	2.0
KIAA1614	NM_020950	KIAA1614	1.9
LHPP	NM_022126	Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	1.7
LHX1	NM 005568	LIM homeobox 1	1.7
MBNL2	NM 144778	Muscleblind-like splicing regulator 2	1.7
MED26	NM_004831	Mediator complex subunit 26	1.7
MIB2	NM 080875	Mindbomb E3 ubiquitin protein ligase 2	1.8
		Microtubule associated monooxygenase,	
MICALI	NM_022765	calponin and LIM domain containing 1	1.7
МҮОЗА	NM_017433	Myosin IIIA	2.1
NADK	NM_023018	NAD kinase	1.7
PAX5	NM_016734	Paired box 5	1.9
PITPNC1	NM_012417	Phosphatidylinositol transfer protein, cytoplasmic 1	1.9
PLEC	NM_000445	Plectin	2.4
PLLP	NM_015993	Plasmolipin	1.8
PRICKLE1	NM_153026	Prickle homolog 1 (Drosophila)	1.7
PTGER2	NM_000956	Prostaglandin E receptor 2 (subtype EP2), 53kDa	2.0
RAB3B	NM_002867	RAB3B, member RAS oncogene family	1.7
RIBC2	NM_015653	RIB43A domain with coiled-coils 2	2.1
RPL31	NM_000993	Ribosomal protein L31	1.8
SCAMP1	NM_004866	Secretory carrier membrane protein 1	1.9
SETD1B	NM 015048	SET domain containing 1B	2.0

Table S1. List of candidate genes.

Gene	Accession No.	Gene Description	Methylation Fold Changes (UC/N)
SLC38A2	NM_018976	Solute carrier family 38, member 2	1.8
SLC38A4	NM_018018	Solute carrier family 38, member 4	1.8
TFAP2E	NM_178548	Transcription factor AP-2 epsilon (activating enhancer binding protein 2 epsilon)	2.4
THSD7A	NM_015204	Thrombospondin, type I, domain containing 7A	1.7
TSPYL1	NM_003309	TSPY-like 1	1.8

Table S1. Cont.

Methylation Specific PCR Gene Target Forward Primer (5'-3') **Reverse Primer (5'-3')** Unmehtylation TTTTTGTGATGTTAAGATGTTTTTG AATCCTATACATTTCTCCTAACACC ACAT2 Methylation ATTTTTGTGACGTTAAGACGTTTTC AAAATCCTATACGTTTCTCCTAACG Unmehtylation TTAGGATTGTGTAATTAGATTATGG ΑΤΑΑΑΑΤΑΑΤΑΑΑΑΑΑΤΑΑCCCAAC ACSS2 Methylation TTAGGATTGTGTAATTAGATTACGG Unmehtvlation TTTGGTTATTGGTTTTTTTTTTTTTT AAAAACTACCCACATTAACTCCAAC CALN1 Methylation TTTCGGTTATTGGTTTTTTTATTTC AACTACCCACGTTAACTCCGAC Unmehtylation ATAGAGTTTTGTAAATATTTGTTAATTATG CCTTCCCACTAAACTACAAAAACAA CDX2 Methylation GAGTTTTGTAAATATTCGTTAATTACGG CTTCCCACTAAACTACAAAAACGAA GTTGTAAGATTAAGTTTTTGGATGT Unmehtylation ATCTCAACTCTACAAACCCTACAAT DNAJC17 Methylation GTCGTAAGATTAAGTTTTTGGACGT ATCTCAACTCTACAAACCCTACGAT Unmehtylation GGGTGTTTTATAGATGGTTAAGATG ACTAACCCCTAAAACCTACAACACT ESYT3 Methvlation GGGCGTTTTATAGATGGTTAAGAC AACTAACCCCTAAAACCTACAACG Unmehtylation GTTTAGGAGGTTAGATTTTTTAGGGT ТАСАААСТАССААСАААСТАССААА EXT1 Methvlation TTTAGGAGGTTAGATTTTTTAGGGC AAACTACCGACGAACTACCG Unmehtvlation GGATTAGGTAGTGGTAGAGGTGTTT ATATACCCTATTACCCAAAATCAAA FAM19A5 Methylation GGATTAGGTAGTGGTAGAGGTGTTC ATATACCCTATTACCCAAAATCGAA Unmehtylation GTAGGGATGAGAGATGATTTTTTGA AATAACAAACCAAAAATAAAACACA FAM217B Methylation GTAGGGATGAGAGACGATTTTTC AAAATAACGAACCAAAAATAAAACG Unmehtvlation AGAGAATTTGTAGTAAAGGTATTGG AAACTAAACAAACCAAATAAACACA FAM46A Methylation AGAGAATTCGTAGTAAAGGTATCGG AAAACTAAACGAACCGAATAAACG Unmehtylation TATTTTTTTGTGGTAGGGATTTATG CTCCCCCTTCTACTACAACAAC FSTL1 Methylation TTATTTTTTTGTGGTAGGGATTTAC CTCCCCCTTCTACTACAACGAC Unmehtylation TTTTTAAGAGTAGGAGGAGTTTTGG САААССАСТААААТААААТТАСААА GAS1 Methylation TTTTTTAAGAGTAGGAGGAGTTTCG TACGAACCGCTAAAATAAAATTACG Unmehtylation TATATATTGTTTAGAGGTTGATGTGA ACCAAATACAACCAAATCCTACAAA H3F3AP4 Methylation TTATATATTGTTTAGAGGTCGACGC AAATACGACCGAATCCTACGAA Unmehtylation GAGGTTTGGGGGGTTTTTTATATTAT ССТААСААСААААСТАСАТС HNRNPF Methylation GAGGTTTGGGGGGTTTTTTATATTAC CTAACGACAAAACGAAACTACGTC

Table S2. Primer information in this study.

Care	Tamad	Methylation	1 Specific PCR
Gene	Target	Forward Primer (5'–3')	Reverse Primer (5'-3')
	Unmehtylation	TAAATTTTATTGTAGAGTGGTATGA	AATCACCTAATAAATTACCAACACC
НОХА9	Methylation	ATAAATTTTATCGTAGAGCGGTACG	ACAATCACCTAATAAATTACCGACG
VDTDD11	Unmehtylation	TTTGGTATTTATGGAAATTTTATTGT	ATATAACCTCTACCTCCACTCAAA
KBIBDII	Methylation	TTTCGGTATTTATGGAAATTTTATC	AATATAACCTCTACCTCCACTCG
VIA A1C1A	Unmehtylation	AGGTTTGGTTTTTTAGTTTTTTT	TAACATCAACTATCCATTCACAAC
KIAA1614	Methylation	AGGTTCGGTTTTTTAGTTTTTTC	AATAACATCAACTATCCGTTCACG
	Unmehtylation	AGTAGTTGGGATTATAGGTATGTGT	AATCACTTAAAATCAAAAATTCAAA
LHPP	Methylation	AGTAGTTGGGATTATAGGTATGCGT	TAAATCGCTTAAAATCAAAAATTCG
LUV1	Unmehtylation	GTTGTGATTGGAGTTATGATGTATG	CCTCAACCCTTAACCTACCTAACA
LHXI	Methylation	AGTTGTGATTGGAGTTACGATGTAC	TCGACCCTTAACCTACCTAACG
MIDO	Unmehtylation	AGTTTGGGAGTTAATAGGATTATGT	CCATTTTAACCAAACTAATTTCAAA
IVIIB2	Methylation	AGTTTGGGAGTTAATAGGATTACGT	ACCATTTTAACCAAACTAATTTCGA
MICALI	Unmehtylation	TAAATTTTTTGAGGAGTTTGAGTGT	CATACAAAACTAACTCTTTCCCCAC
MICALI	Methylation	TTTAAATTTTTTGAGGAGTTTGAGC	GTACAAAACTAACTCTTTCCCCG
MED26	Unmehtylation	TTTTTAATTATGGGGTTTTTTGTTG	CCTCAACACCACTAACATTTAAACA
MED26	Methylation	TTTTTAATTACGGGGTTTTTTGTC	CTCGACACCACTAACATTTAAACG
	Unmehtylation	AATTTATTATATTTTTTGGGTTTGA	ACAAAACCCTTAACTAACTACCAAA
MYO3A	Methylation	AATTTATTATATTTTTCGGGTTCGA	GCAAAACCCTTAACTAACTACCGA
NADK	Unmehtylation	TAGTTTGTGGGGTGTGTAAAATATG	ТАТССАТСААААСТААААААААААС
NADK	Methylation	TTTGTGGGGTGCGTAAAATAC	TATCCGTCGAAACTAAAAAACG
DAVE	Unmehtylation	TTTTTTAAAAGTATTTGTTTGGTTGA	AATCACAACCCTCTACACTATACAC
PAAS	Methylation	TTTTTAAAAGTATTTGTTTGGTCGA	GCGACCCTCTACGCTATACG
DITENICI	Unmehtylation	GTATTTAGGGTATAATGGAGAGTGG	AAACAACATATCTCTACCAAACAACAA
PIIPNCI	Methylation	AGTATTTAGGGTATAATGGAGAGCG	AACATATCTCTACCAAACGACGAA
	Unmehtylation	TTGTTTTTAGGTTTTTAGTTTTGT	CTAACCCCTAATAATAAAATCAAC
PLEC	Methylation	TTGTTTTTAGGTTTTTAGTTTCGT	ACTAACCCCTAATAATAAAATCGAC
מזזת	Unmehtylation	GTGTTTGGAGTTATATAGGGATTTG	TACTAACTTTCAACAAAAACTCAAC
PLLP	Methylation	GCGTTTGGAGTTATATAGGGATTC	TACTAACTTTCGACGAAAACTCGA

Care	T	Methylatio	on Specific PCR
Gene	Target	Forward Primer (5'–3')	Reverse Primer (5'-3')
	Unmehtylation	TAGATTAGTTTGTGGGATTATATGA	CTATTTAAATTCCTTCTCCTTCAAA
PRICKLEI	Methylation	TAGATTAGTTTGTGGGATTATACGA	CTATTTAAATTCCTTCTCCTTCGAA
DTCTDO	Unmehtylation	TTTTGATTTTTGTGTATTTTGTGT	CAATCTAACCTCAACTAAAAAACATT
PIGERZ	Methylation	GTTTCGATTTTTGTGTATTTTGC	AATCTAACCTCGACTAAAAAACGTT
	Unmehtylation	TTTTTTGGGTTTTTGTGAAGAT	CCCCCATATCTAAATAACTCTCATA
KADOD	Methylation	TTTTTTGGGTTTTTGTGAAGAC	CCCCCATATCTAAATAACTCTCGTA
DIRCO	Unmehtylation	TAGTTATTAGAGTTGTTGGGGTTGA	CACTTAATAACACAAATAAAACATA
KIDC2	Methylation	TTTAGTTATTAGAGTCGTTGGGGTC	GCTTAATAACGCGAATAAAACGTA
21 מת	Unmehtylation	TTATTTATGTATAGGGATGATGTGA	CCAACACTAACTAAACCTACATCAAA
KPL51	Methylation	TTATTTACGTATAGGGACGACGC	CAACACTAACTAAACCTACGTCGAA
	Unmehtylation	GGGTTTTATATTTTTTGAGGATTTTG	CCACACTCCAAACTTTAACTACAAC
SCAMPT	Methylation	GGGTTTTATATTTTTCGAGGATTTC	ACGCTCCGAACTTTAACTACG
CETD1P	Unmehtylation	ATTTTTTGGAAGGGTTATTTTTT	TAAAAACCAACAAATTATTATCATC
SEIDID	Methylation	TTTTTCGGAAGGGTTATTTTTC	TAAAAACCAACAAATTATTATCGTC
	Methylation	TAAGGGAGTGAGTTTTGTTTTTTT	ACAAATCTAAAACACTTTCTACAAA
SLCSØAZ	Unmehtylation	TAAGGGAGTGAGTTTTGTTTTTTC	ATACGAATCTAAAACGCTTTCTACG
CI C 20 A A	Methylation	TTTAATAATATTGGGTATATTTGTGG	CCAACATTACAAACAAACAACAAC
SLC30A4	Unmehtylation	TTTAATAATATTGGGTATATTCGCG	CAACATTACAAACAAACAACGAC
	Methylation	TTTAGTTTAGGAAGTTTGAGAGTGA	CATACACACACAATATCAATACATA
IFAP2E	Methylation	TTTTTAGTTTAGGAAGTTTGAGAGC	CATACGCACACAATATCAATACGTA
	Unmehtylation	TTTTTTTGTTTTTTGGGTTTATGT	ACCTCTAACAAAAATACTACTACCACT
INSD/A	Methylation	GTTTTTTTGTTTTTTGGGTTTAC	GCCTCTAACGAAAATACTACTACCG
ΤΩΡΛΙ 1	Unmehtylation	TATTGGAGAGTAGGTATTGTTATGG	TACTAAATCTAAAAACACAAACAAC
15111	Methylation	TATTGGAGAGTAGGTATTGTTACGG	TACTAAATCTAAAAACACGAACGAC
LOVE1	Unmehtylation	TCGGTTTTTGAATTTTTCGGGTTTTAGTCGATC	GATAAAAACCCCACAACGTCAAAACG
FUXEI	Methylation	GTTGGTTTTTGAATTTTTTGGGTTTTAGTTGATTG	AACAACACAATAAAAACCCCACAACATCAAAACA
CVNIF1	Unmehtylation	GTGGTTGGGTTTTTGTAGTTTTGTAGATTGTG	CAACTCTCTACACCCAAACTCAACA
SINEI	Methylation	GTTGGGTTTTCGTAGTTTTGTAGATCGC	CTACGCCCAAACTCGACG
Alu	Methylation	ATTAGTCGGGCGTGGTGG	CCCGAATTCAAACGATTCTCC

Risulfite Seguencing			

Cano Logue		Bisulfite Sequencing		
Gene		Forward Primer (5'-3')	Reverse Primer (5'-3')	
FAM217B	chr20 (58,515,279-58,515,559)	GGTTCGCGGTTTTAGGGTTA	AACGAACCAAAAATAAAACGCAC	
KIA1614	chr1 (180,881,647-180,882,085)	GAGGTTYGGTTTTTTAGTTTTTT	AAAAAAAATAACATCAACTATCC	
RIBC2	chr22 (45,809,169-45,809,433)	TAGGGGTTGGGTTAGGGTTA	CTACTACTTATAAAAAATTTCAAATC	
Gene		Quantitative Real-Time RT-PCR		
		Forward Primer (5'-3')	Reverse Primer (5'-3')	
	FAM217B	TGGACGATGTTAGTTAATGATAAATC	TTAAACTCCAACTCTAAAAACCGTA	
	KIA1614	AGTGTTAAGGGGTTAGGATTAATTC	AACGACCCGAAACTTTTATACG	
	RIBC2	GTTAATTTTTAGGGATCGGGTAGAC	GAACTTTAAATCCTAATTCCTCGCT	
	ACTB	AGCGAGCATCCCCCAAAGTT	GGGCACGAAGGCTCATCATT	

Table S2. Cont.



Figure S1. The best candidate genes are frequently hypermethylated in most of colon cancer cell lines. Summary of the methylation patterns for FAM217B, KIA1614, and RIBC2 gene in 10 colon cancer cell lines by MSP analysis. M and U indicates methylation and ummethylation, respectively. DKO and IVD were used for unmethylation and methylation controls in MSP analysis.



Figure S2. Methylation frequency of *SYNE1* and *FOXE1* in UC patients. MSP was performed in 71 UC samples and 8 normal colon tissues. SYNE1 and FOXE1 showed 14.1% (10 out of 71) and 53.5% (38 out of 71) methylation frequencies, respectively. Primer set for MSP analysis was used by Papadia et al. (2014).



Figure S3. DNA methylation profile is associated to the inflammatory activity. (**A**) Multidimensional scaling (MDS) was used to estimate the relationship between samples. The MDS plot shows the similarity between individual samples according to methylation status of the 48 probes. The x and y axis represent arbitrary numbers; (**B**) Clinical implication of the hypermethylated CpG sites. Unsupervised hierarchical clustering was performed with β -values of the CpG sites. Samples were matched with the severity of inflammatory activity.