Supplementary Table 1 Primer sequences of colorectal cancer 14-gene panel

Oligonucleotide	Sequence 5′↔3′	T_m
CHST11		
Forward primer	GTTTCGCGTTTTTCGGGTTTC	
Reverse primer	AACTTTATCCTAACTACGAAAACCG	
Probe	FAM-CGCGGCGGGGTTTTTGTTTTTGCG-BHQ1	
PCO	CGCAAAATTAAAAATCCTGCTGCG-PHO	49.00
ZNF671		
Forward primer	GTTCGTTAGTTTCGTTATAGGATC	
Reverse primer	AATACCTACGTCCCCGATCG	
Probe	HEX-CTACCGCTCCCGACAACTATCCGC-BHQ1	
PCO	GCGATTAGTTGTTGGGAGTGATAG-PHO	48.70
VAV3-AS1		
Forward primer	GATCGAGGGAGTAGGAGTC	
Reverse primer	CTACAACGACGACCGAATCG	
Probe	ROX-CGACGCCCGCGACCCGTCAAC-BHQ2	
PCO	GTTAATGGGACGCGGGTGTCG-PHO	49.60
ZEB2		
Forward primer	CGATTTTATCGGATTTCGCGC	
Reverse primer	ATAACGATCCGCGAACAACG	
Probe	FAM-CCCATCGCGCCCAAACCCCGAAA-BHQ1	
PCO	TTTCGAAGTTTGGATGTGAAGGG-PHO	35.91
IKZF1		
Forward primer	TTTCGTTTTTTGTATCGGAGTAGC	
Reverse primer	TACCCCGCAACCGACTCG	
Probe	HEX-CGCGCACCTCTCGACCGCCTC-BHQ1	
PCO	GAGGTGGATGAGAAGTATGCG-PHO	33.96
NDRG4		
Forward primer	ATTTAGTATAGTTCGCGCGGC	
Reverse primer	ACCTAAAAAACACCCCGATCG	
Probe	HEX-CCCCCGCCGACTTCTCACCCG-BHQ1	

PCO	CGGGAGAGAAGTCGGTGAGGG-PHO	57.29
ITGA4		
Forward primer	GGTTTCGTTTTTGTTTACGCGC	
Reverse primer	CTAAAAAACCCGAACCAAAACGC	
Probe	FAM-CAACCGAAATTCCCCAACGCCCCCT-BQH1	
PCO	AGGGAGTGTTGGGGAATTATGGTTG-PHO	55.56
C9orf50		
Forward primer	GATTTTTTAGTTTTCGGAGTTTCGC	
Reverse primer	CCTAACGACGAAAACTTCCG	
Probe	HEX-CGCCCGCGCTCCGAACGACTC-BHQ1	
PCO	GAGACGTTTGGAGTGTGGGCG-PHO	55.73
BCAN		
Forward primer	TACGTTCGCGTAGTTTTCGTC	
Reverse primer	CGAAATTTATCGCCTCCTCCG	
Probe	ROX-CTCCTCCGAATACACGACTCAATCCTACAA	
PCO	TTGAAGGATTGAGTTGTGTATTTGGAAGAG-PHO	55.33
BMP3		
Forward primer	CAACTAATTTAAAATTCAACCCTCG	
Reverse primer	GCGTCGTTGCGGGATATTTC	
Probe	ROX-AGGAGTCGGCGGCGGAGTCG-BHQ2	
PCO	CGATTCCGTTGCCGATACCT-PHO	41.07
SDC2		
Forward primer	CCCGAACCCCGAACCCG	
Reverse primer	TTCGGCGTAGTTCGCGTATAC	
Probe	FAM-CCCGAACCTAAACCGCAATCGCTACGA-BH	
PCO	TCGAAGCGATTGTAGTTTAGGTTTGGG-PHO	55.16
SEPT9-1		
Forward primer	TGGATGGGATTATTTCGGATTTC	
Reverse primer	CGCAAAATCCTCTCCAACACG	
Probe	HEX-CGACCGCAACAACCAACCCAACAC-BHQ1	
PCO	GTGTTAAGTTGATTGTAGTGGTTG-PHO	37.87

SEPT9-2		
Forward primer	AGGGCGCGTAGGGTTTAC	
Reverse primer	TAATCCAAACGCCCTCCCG	
Probe	FAM-CGCCGCGCGCCCCCGAC-BHQ1	
PCO	GTCAGGGGTGTGTGGCG-PHO	45.09
SFMBT2		
Forward primer	TAGAAGGGCGGGAGCGC	
Reverse primer	CCACCTTCCCTAAAACGCG	
Probe	ROX-CGTAGGGTCGAGGCGAGGGGTTG-BHQ2	
PCO	CAATTCCTCGTTTCGACCTGACG-PHO	44.80
ACTB		
Forward primer	GTGATGGAGGAGGTTTAGTAAGTT	
Reverse primer	CCAATAAAACCTACTCCTCCCTTAA	
Probe	CY5-ACCACCACCCAACACACAATAACAAACAC	

Supplementary Table 2 Primer sequences used in cancerous and paracancerous tissues

Oligonucleotide	**Sequence 5′↔3′**
CHST11	
Forward primer	GTTTCGCGTTTTTCGGGTTTC
Reverse primer	AACTTTATCCTAACTACGAAAACCG
Probe	FAM-CGCGGCGGGGTTTTTGTTTTTGCG-BHQ1
VAV3-AS1	
Forward primer	GATCGAGGGAGTAGGAGTC
Reverse primer	CTACAACGACGACCGAATCG
Probe	ROX-CGACGCCCGCGACCCGTCAAC-BHQ2
ZEB2	
Forward primer	CGATTTTATCGGATTTCGCGC
Reverse primer	ATAACGATCCGCGAACAACG
Probe	FAM-CCCATCGCGCCCAAACCCCGAAA-BHQ1
IKZF1	

Forward primer	TTTCGTTTTTTGTATCGGAGTAGC
Reverse primer	TACCCCGCAACCGACTCG
Probe	HEX-CGCGCACCTCTCGACCGCCTC-BHQ1
ITGA4	
Forward primer	GGTTTCGTTTTTGTTTACGCGC
Reverse primer	CTAAAAAACCCGAACCAAAACGC
Probe	FAM-CAACCGAAATTCCCCAACGCCCCCT-BQH1
C9orf50	
Forward primer	GATTTTTTAGTTTTCGGAGTTTCGC
Reverse primer	CCTAACGACGAAAACTTCCG
Probe	HEX-CGCCCGCGCTCCGAACGACTC-BHQ1
SEPT9-2	
Forward primer	AGGGCGCGTAGGGTTTAC
Reverse primer	TAATCCAAACGCCCTCCCG
Probe	ROX-CGCCGCGCGCCCCCGAC-BHQ2
SFMBT2	
Forward primer	TAGAAGGGCGGGAGCGC
Reverse primer	CCACCTTCCCTAAAACGCG
Probe	ROX-CGTAGGGTCGAGGCGAGGGGTTG-BHQ2
ACTB	
Forward primer	GTGATGGAGGAGGTTTAGTAAGTT
Reverse primer	CCAATAAAACCTACTCCTCCCTTAA
Probe	CY5-ACCACCACCCAACACACAATAACAAACACA-BHQ2

Supplementary Table 3 Primer sequences for colorectal cancer 8-gene outer and inner panel

Oligonucleotide	**Sequence 5′↔3′**
CHST11	
Outer forward primer	TTGTTTAGTTTTGTTTCGCGTTTTTC
Outer reverse primer	AACTTTATCCTAACTACGAAAACCG

Inner forward primer	GTTTCGCGTTTTTCGGGTTTC
Inner reverse primer	TAACTACGAAAACCGAAATATCCG
Probe	FAM-CGCGGCGGGGTTTTTGTTTTTGCG-BHQ1
VAV3-AS1	
Outer forward primer	TTTTTCGCGCGGGATCGAG
Outer reverse primer	CTACAACGACGACCGAATCG
Inner forward primer	GATCGAGGGAGTAGGAGTC
Inner reverse primer	GACGACCGAATCGCGACG
Probe	ROX-CGACGCCCGCGACCCGTCAAC-BHQ2
ZEB2	
Outer forward primer	GTTTTCGAGTTATTATCGATTTTATC
Outer reverse primer	ATAACGATCCGCGAACAACG
Inner forward primer	CGATTTTATCGGATTTCGCGC
Inner reverse primer	CCGCGAACAACGAAACCCG
Probe	FAM-CCCATCGCGCCCAAACCCCGAAA-BHQ1
IKZF1	
Outer forward primer	TTTGCGCGTTTCGTTTTTTGTATC
Outer reverse primer	TACCCCGCAACCGACTCG
Inner forward primer	TTTCGTTTTTTGTATCGGAGTAGC
Inner reverse primer	CAACCGACTCGACCCCG
Probe	HEX-CGCGCACCTCTCGACCGCCTC-BHQ1
ITGA4	
Outer forward primer	GGTCGGGTTTCGTTTTTGTTTAC
Outer reverse primer	CTAAAAAACCCGAACCAAAACGC
Inner forward primer	GGTTTCGTTTTTGTTTACGCGC
Inner reverse primer	GAACCAAAACGCGAATCCTAC
Probe	FAM-CAACCGAAATTCCCCAACGCCCCCT-BHQ1
C9orf50	
Outer forward primer	TCGTTTTTCGGGATTTTTTAGTTTTC
Outer reverse primer	CCTAACGACGAAAACTTCCG

Inner forward primer	GATTTTTTAGTTTTCGGAGTTTCGC
Inner reverse primer	CGACGCAACAACGACCCG
Probe	HEX-CGCCCGCGCTCCGAACGACTC-BHQ1
SEPT9-2	
Outer forward primer	AGGGCGCGTAGGGTTTAC
Outer reverse primer	TAATCCAAACGCCCTCCCG
Inner forward primer	TTTTTACGTAGGCGGCGCG
Inner reverse primer	CAAACGCCCTCCCGCTAAA
Probe	ROX-CGCCGCGCGCCCCCGAC-BHQ2
SFMBT2	
Outer forward primer	GTGGGGTAGAAGGGCGG
Outer reverse primer	CCACCTTCCCTAAAACGCG
Inner forward primer	TAGAAGGGCGGGAGCGC
Inner reverse primer	TTCCCTAAAACGCGAAACCCAAA
Probe	ROX-CGTAGGGTCGAGGCGAGGGGTTG-BHQ2
ACTB	
Forward primer	GTGATGGAGGAGGTTTAGTAAGTT
Reverse primer	CCAATAAAACCTACTCCTCCCTTAA
Probe	CY5-ACCACCACCCAACACACAATAACAAACACA-BHQ2

Supplementary Table 4 2 × 2 contingency tables for the three-marker methylation panel across different subgroups

Subgroup	TP	FN	FP	TN
Overall cohort (117 CRC *vs* 128 combined controls: 68 healthy + 60 benign)	98	19	6	122
CRC *vs* healthy-only controls (117 CRC *vs* 68 healthy individuals)	98	19	0	68
CRC *vs* benign-only controls (117 CRC *vs* 60 benign gastrointestinal patients)	98	19	6	54

TP: True positive; FN: False negative; FP: False positive; TN: True negative; CRC: Colorectal cancer.