Supplementary materials for the article:

Zhong F. et al. Significance of *Fusobacterium nucleatum* Combined with SFRP2 and SDC2 Gene Methylation Detection in Early Screening of Colorectal Cancer.
Pol J Microbiol. 2025, Vol. 74, No 2, 218-231

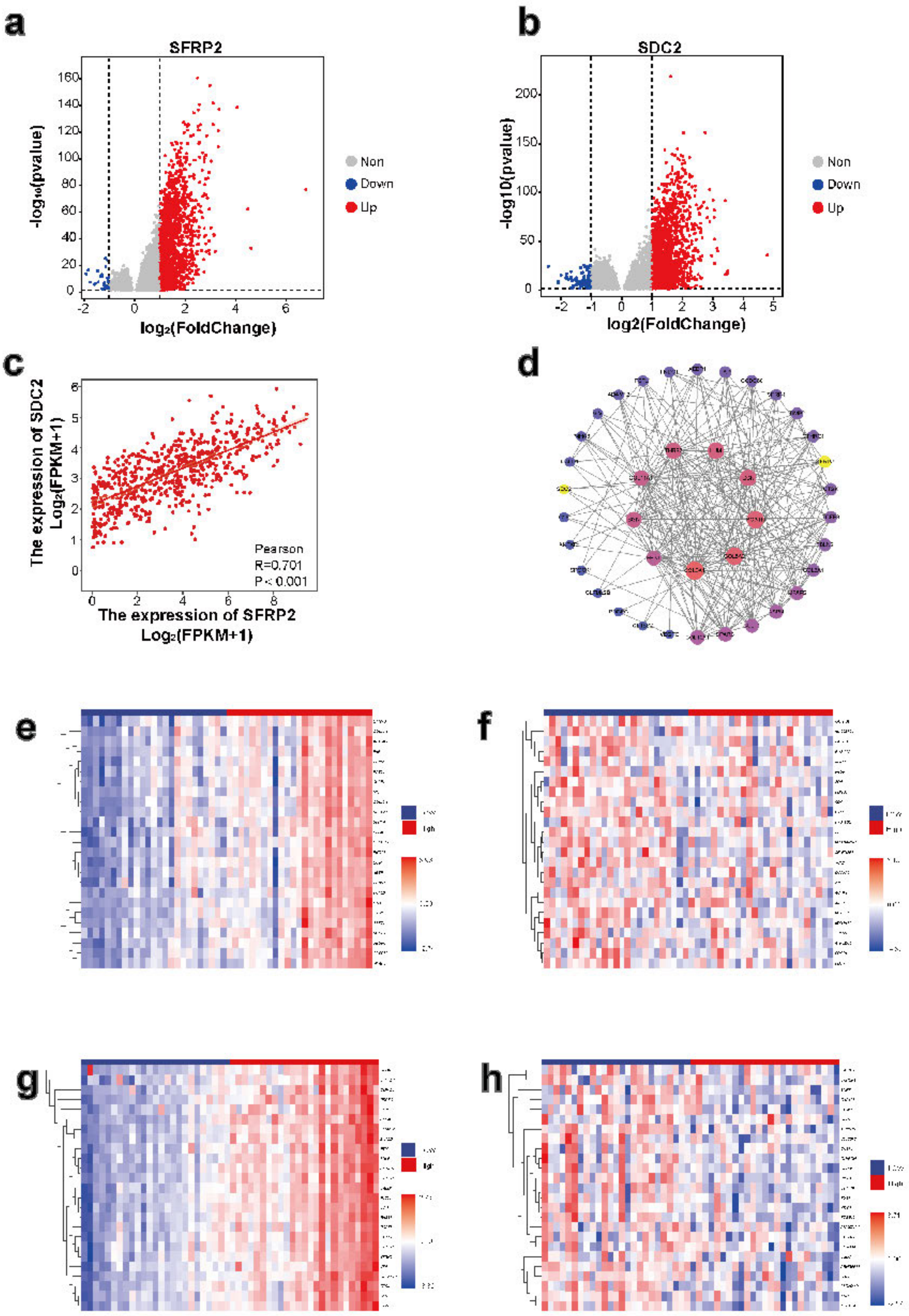

Fig.S1. Analysis of biological functions associated with SFRP2 and SDC2.

(a) Volcano maps of differentially expressed genes related to SFRP2; (b) Volcano maps of differentially expressed genes related to SDC2; Orange: up-regulated genes; Blue: down-regulated genes; (c) Intersection-analysis of SFRP2 and SDC2-related genes (Pearson correlation analysis; $p < 0.001$). (d) Protein-protein interaction analysis of SFRP2 and SDC2-related proteins. Analysis of the top 50 differential genes between high and low SFRP2 levels (e, f) and SDC2 levels (g, h) using the Complex Heatmap package.

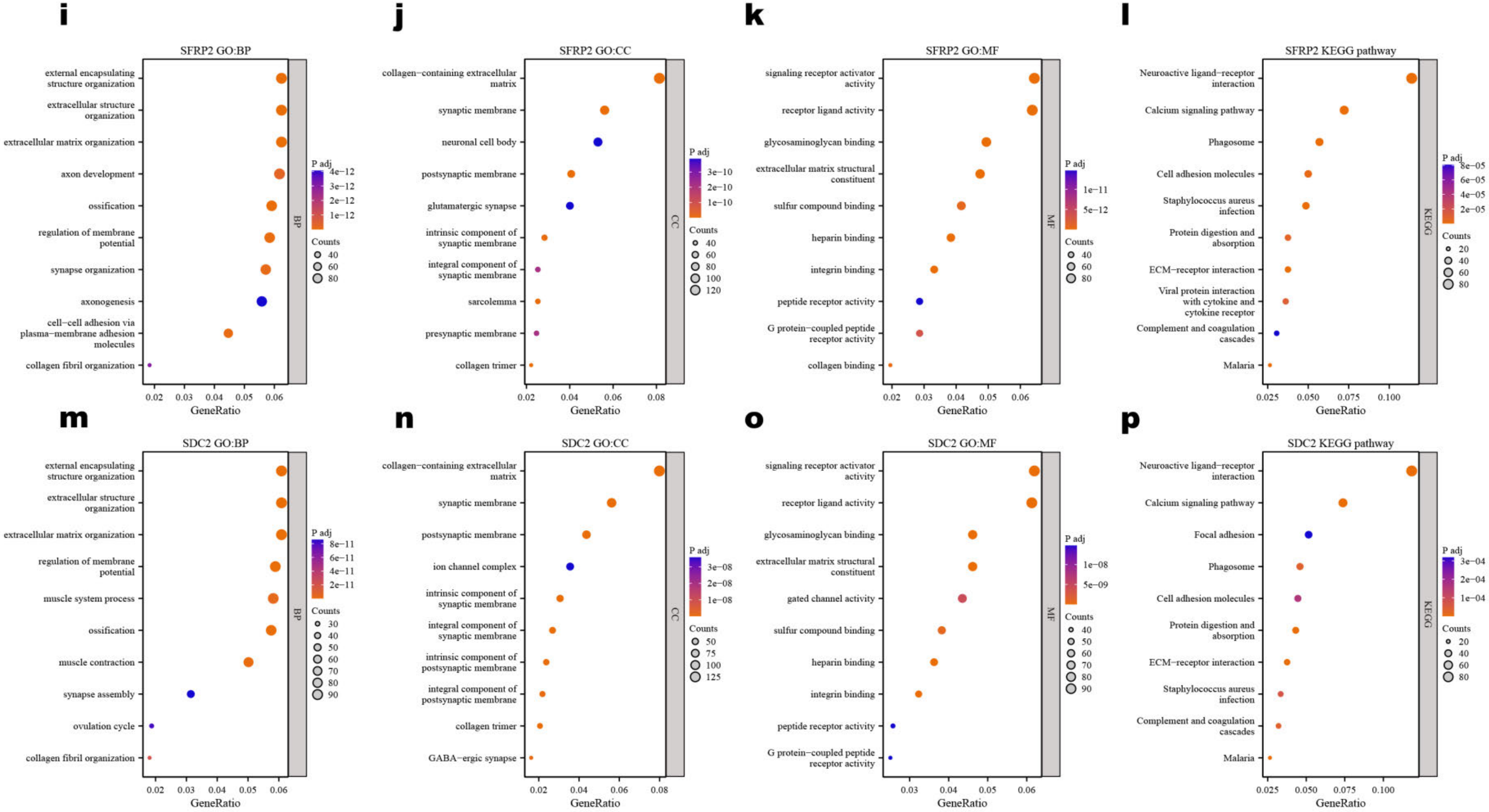

Fig.S1. Analysis of biological functions associated with SFRP2 and SDC2.

(i–k, m–o) Gene Ontology analysis of biological processes, cellular components, and molecular functions of the co-expressed genes of SFRP2 and SDC2 in colorectal cancer. (l, p) KEGG pathway analysis of the co-expressed genes of SFRP2 and SDC2 in colorectal cancer.

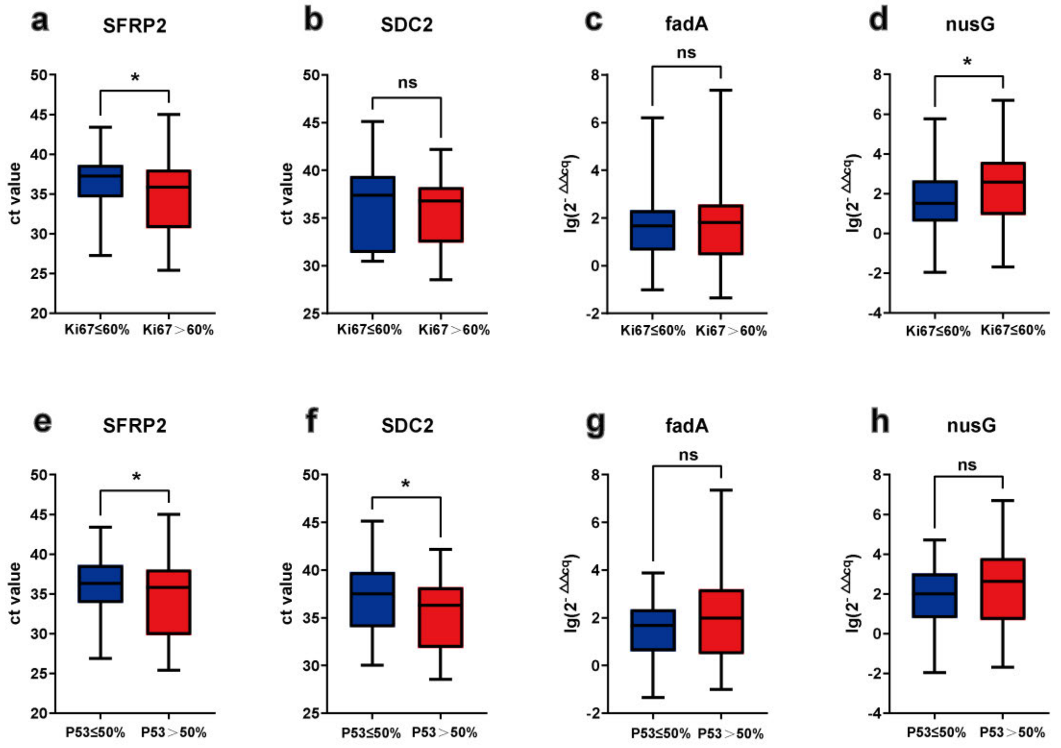

Fig. S2. Correlation of SFRP2, SDC2, fadA and nusG with Ki67 and P53 expression. ns – not significant; * $p < 0.05$

Table SI

Inclusion criteria and exclusion criteria of study population.

Inclusion criteria	Exclusion criteria
1) Untreated patients with newly diagnosed CRC or adenoma by pathological diagnostic criteria (with complete clinical data)	1) Patients with a history of other cancers or who had received any form of anti-tumor treatment
2) Healthy volunteers without signs of intestinal disease	2) Patients with colonic lymphoma, inflammatory bowel disease and other colorectal diseases
3) Patients who had not undergone bowel preparation in the week before stool collection	3) Patients or volunteers who regularly take probiotics or yogurt over a long period of time
4) Patients with clear consciousness, normal mind and good compliance	4) Patients or volunteers who had used antibiotics in the 4 weeks before sample collection
	5) Patients with severe organ dysfunction such as heart, liver, spleen, lung and kidney, coagulopathy or immune function diseases

Table SII

The primers and probes for the target genes.

		probe	Fluorescent channel	Fluorophore
SDC2-meth-1-F	TTTGAGTTTTGAGTTTGAGTTTTT	TTGTAATTGTTGTGGTATT	HEX-MGB	Cy5
SDC2-meth-1-R	TCCTACCCAACGCTCGACG			
SFRP2-meth-1-F	GGTTGTTGAACGGTGGTTGG	ACGAAGTTCGTCGAGGCGGT	HEX-MGB	ROX
SFRP2-meth-1-R	AAAACCCGAAACCTACCCGC			
Sep9-meth-1-F	AGTTTGAAATGATTTTATTTAGTTGTGT	CGCTACCCACCAACCATCATA	FAM-MGB	FAM-MGB
Sep9-meth-1-R	CCTCACCACTACCCTCCG			
MLH1-meth-1-F	CGATAGATTAGGTATAGGGTTTTATCGTTTTTC	ACGTTGGGTTTATTCGGGTCGGAA	HEX-MGB	Cy5
MLH1-meth-1-R	GCCCAAAAAAAACAAAATAAAAATCGACG			
FadA-meth-1-F	GCTTCAGCATTTGCAGCAAA	CAAGCATTAGATGCTGAATACCAA	FAM-MGB	
FadA-meth-1-R	CTAGTGCTTGTTTAGCGGCAT			
nusG-meth-1-F	CAACCATTACTTTAACTCTACCATGTTCA	TCAGCAACTTGTCCTTCTTGATCTTTAAATGAACC	FAM-MGB	
nusG-meth-1-R	ATTGACTTTACTGAGGGAGATTATGTAAAAATC			
ACTB-meth-F	GTGACGTGGACATCCGCAAA	TACGCCAACACAGTGCTGTCTGGCGG	HEX-BHQ2	VIC-MGB
ACTB-meth-R	GGAAAGACACCCACCTTGATCTTC			
QACTB-meth-F	AGTATAATGAAGATTAAGGTGGGTGTT	TTGTTTGAGTTGATTTGGGTAGGTTGG?	HEX-BHQ2	VIC-MGB
QACTB-meth-R	CCCCACACACCACAAAACCC			
16S-F	ACGGTCCAGACTCCTACGG	TCTTCCGCAATGGGCGAAAGC	HEX-MGB	
16S-R	CGACCTTCTTCATTCACGCA			

Table SIII

The relationship between methylation rates of SFRP2 and SDC2 and different clinicopathological features.

		SDC2			SFRP2		
		Methylation results (%)			Methylation results (%)		
Diagnosis	n	Positive	Negative	*p*	Positive	Negative	*p*
NC	42	6 (14.29)	36 (85.71%)		7 (16.67%)	35 (83.33%)	
AD	22	7 (31.82%)	15 (68.18%)		9 (40.91%)	13 (59.09%)	
CRC	132	87 (65.91%)	45 (34.09%)		99 (75%)	33 (25%)	
Age							
≤ 50 y	40	27 (67.50%)	13 (32.50%)	0.7993	30 (75%)	10 (25%)	> 0.9999
> 50 y	92	60 (65.22%)	32 (34.78%)		69 (75%)	23 (25%)	
Gender							
Male	78	53 (67.95%)	25 (32.05%)	0.5524	60 (76.92%)	18 (23.08%)	0.5397
Female	54	34 (62.96%)	20 (37.04%)		39 (72.22%)	15 (27.78%)	
Location							
Left	96	65 (67.71%)	31 (32.29%)	0.4764	74 (77.08%)	22 (22.92%)	0.3667
Right	36	22 (61.11%)	14 (38.89%)		25 (69.44%)	11 (30.56%)	
TNM stage							
I–II	70	40 (57.14%)	30 (42.86%)	**0.024**	53 (75.71%)	17 (24.29%)	0.8404
III–IV	62	47 (75.81%)	15 (24.19%)		46 (74.19%)	16 (25.81%)	
Lymph node metastasis							
Positive	68	55 (80.88%)	13 (19.12%)	**0.0002**	56 (82.35%)	12 (17.65%)	**0.0443**
Negative	64	32 (50%)	32 (50%)		43 (67.19%)	21 (32.81%)	
S100							
Positive	65	47 (72.31%)	18 (27.69%)	0.1266	53 (81.54%)	12 (18.46%)	0.0875
Negative	67	40 (59.70%)	27 (40.30%)		46 (68.66%)	21 (31.34%)	
Differentiation							
well	19	9 (47.37%)	10 (52.63%)	0.1579	12 (63.16%)	7 (36.84%)	0.3111
Moderate	97	66 (68.04%)	31 (31.96%)		76 (78.35%)	21 (21.65%)	
poor	16	12 (75%)	4 (25%)		11 (68.75%)	5 (31.25%)	

Table SIV

Relationship between positive rates of fadA and nusG and different clinicopathological features.

Diagnosis	n	fadA		*p*	nusG		*p*
		Positive	Negative		Positive	Negative	
AD	22	7 (31.82%)	15 (68.18%)		8 (36.36%)	14 (63.63%)	
CRC	132	78 (59.09%)	54 (40.91%)		91 (68.94%)	41 (31.06%)	
Age							
≤ 50 y	40	22 (55%)	18 (45%)	0.5285	27 (67.50%)	13 (32.50%)	0.8137
> 50 y	92	56 (60.87%)	36 (39.13%)		64 (69.57%)	28 (30.43%)	
Gender							
Male	78	48 (61.54%)	30 (38.46%)	0.7922	59 (75.64%)	19 (24.36%)	0.0455
Female	54	32 (59.26%)	22 (40.74%)		32 (59.26%)	22 (40.74%)	
Location							
Left	96	55 (57.29%)	41 (42.71%)	0.2031	64 (66.67%)	32 (33.33%)	0.3568
Right	36	25 (69.44%)	11 (30.56%)		27 (75%)	9 (25%)	
TNM stage							
I–II	70	40 (57.14%)	30 (42.86%)	0.3869	47 (67.14%)	23 (32.86%)	0.6355
III–IV	62	40 (64.52%)	22 (35.48%)		44 (70.97%)	18 (29.03%)	
Lymph node metastasis							
Positive	68	41 (60.29%)	27 (39.71%)	0.0756	46 (67.65%)	22 (32.35%)	0.7408
Negative	64	39 (60.94%)	25 (39.06%)		45 (70.31%)	19 (29.69%)	
S100							
Positive	65	41 (63.08%)	24 (36.92%)	0.5672	44 (67.69%)	21 (32.31%)	0.7604
Negative	67	39 (58.21%)	28 (41.79%)		47 (70.15%)	20 (29.85%)	
Differentiation							
Well	19	14 (73.68%)	5 (26.32%)	0.3427	14 (73.68%)	5 (26.32%)	0.7251
Moderate	97	58 (59.79%)	39 (40.21%)		65 (67.01%)	32 (32.99%)	
Poor	16	8 (50%)	8 (50%)		12 (75%)	4 (25%)	

Table SV

The relationship between the positive rate of blood tumor marker CEA and different clinicopathological features.

Diagnosis	n	CEA		*p*
		Positive	Negative	
NC	42	8 (19.05%)	34 (80.95%)	
AD	22	8 (36.36%)	14 (63.63%)	
CRC	132	53 (40.15%)	79 (59.85%)	
Age				
≤50 y	40	27 (67.50%)	13 (32.50%)	0.237
>50 y	92	52 (56.52%)	40 (43.48%)	
Gender				
Male	78	33 (42.31%)	45 (57.69%)	0.5436
Female	54	20 (37.04%)	34 (62.96%)	
Location				
Left	96	42 (43.75%)	54 (56.25%)	0.1684
Right	36	11 (30.56%)	25 (69.44%)	
TNM stage				
I–II	70	23 (32.86%)	47 (67.14%)	0.0693
III–IV	62	30 (48.39%)	32 (51.61%)	
Lymph node metastasis				
Positive	68	31 (45.59%)	37 (54.41%)	0.189
Negative	64	22 (34.38%)	42 (65.62%)	
S100				
Positive	65	30 (46.15%)	35 (53.85%)	0.1659
Negative	67	23 (34.33%)	44 (65.67%)	
Differentiation				
Well	19	7 (36.84%)	12 (63.16%)	0.9135
Moderate	97	40 (41.24%)	57 (58.76%)	
Poor	16	6 (37.50%)	10 (62.50%)	

Table SVI

Sensitivity and specificity of the stool DNA test, CEA and FOBT.

Inditor	Group	Biomarker	Sensitivity (%)	Specificity (%)	Cut off	AUC	95%CI
	CRC vs. NC	SFRP2	100.00	23.58	0.2358	0.5976	0.4357 to 0.7596
		SDC2	70.00	88.78	0.5878	0.8446	0.7636 to 0.9257
		Fn	86.67	66.07	0.5274	0.7759	0.6945 to 0.8573
		Combined	88.89	88.61	0.7750	0.9339	0.8707 to 0.9971
	CRC vs. AD	SFRP2	78.26	85.85	0.6411	0.8811	0.7941 to 0.9680
		SDC2	100.00	36.73	0.3673	0.6565	0.5112 to 0.8017
		Fn	68.75	58.93	0.2768	0.6127	0.4877 to 0.7377
		Combined	77.22	71.43	0.4865	0.7649	0.5961 to 0.9337
	AD vs. NC	SFRP2	92.31	65.22	0.5735	0.8261	0.6899 to 0.9623
		SDC2	100.00	65.00	0.6500	0.8111	0.6572 to 0.9650
		Fn	56.25	90.00	0.4625	0.7146	0.5415 to 0.8877
		Combined	100.00	57.14	0.5714	0.8254	0.6162 to 1.000
CEA	CRC vs. NC		100.00	23.48	0.2348	0.6197	0.5312 to 0.7082
	CRC vs. AD		100.00	19.70	0.1970	0.5114	0.3956 to 0.6271

Inditor	Group	Biomarker	Sensitivity (%)	Specificity (%)	Cut off	AUC	95%CI
	AD vs. NC		68.18	59.52	0.2770	0.6407	0.4935 to 0.7879
FPBT	CRC vs. NC		69.05	65.91	0.3496	0.6748	0.5812 to 0.7684
	CRC vs. AD		45.45	65.91	0.1136	0.5568	0.4248 to 0.6888
	AD vs. NC		54.55	69.05	0.2360	0.6180	0.4705 to 0.7654