

Hypermethylation of gene body in *SRCIN1* involved in breast cancer cell proliferation with potential as a blood-based biomarker for early detection and poor prognosis

Hsieh-Tsung Shen^{1,2}, Clilia Davis³, Chih-Ming Su^{4,5}, Li-Min Liao⁵, Hsiu-Ming Shih¹,
Kuan-Der Lee¹, Chin-Sheng Hung^{2,4,5,6*}, Muhamad Ansar^{7*}, and Ruo-Kai Lin^{1,2,3,7,8,9*}

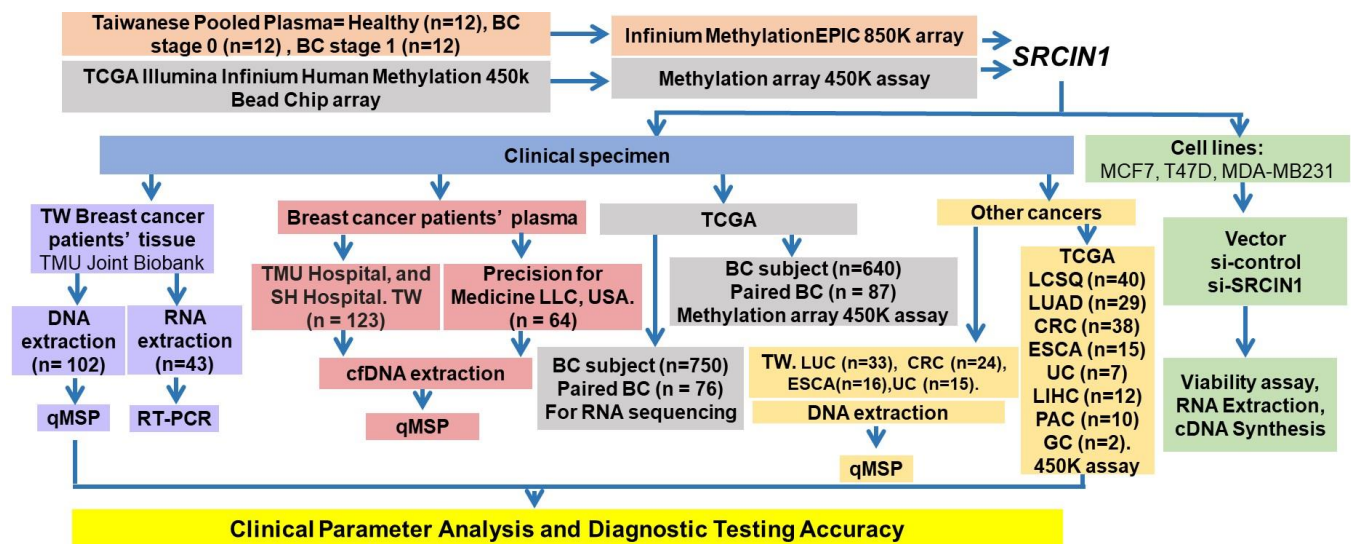


Figure S1. Flowchart of the study design, datasets, and specimens used. For each step, the sample types and number of samples used for the analyses are indicated. TW, Taiwan; TCGA, The Cancer Genome Atlas; BC, Breast Cancer; AN, adjacent normal; CRC, colorectal cancer; LCSQ, lung squamous cell carcinoma; lung adenocarcinoma; ESCA, esophageal cancer; UC, Uterine Cancer; LIHC, Liver Cancer; PAC, Pancreatic Cancer, cfDNA, circulating cell-free DNA; QMSP, quantitative methylation-specific PCR; qRT-PCR, quantitative reverse-transcription PCR, methylation450K array, Illumina Infinium HumanMethylation450 BeadChip array. methylation480K array, Illumina Infinium HumanMethylation850 BeadChip array.

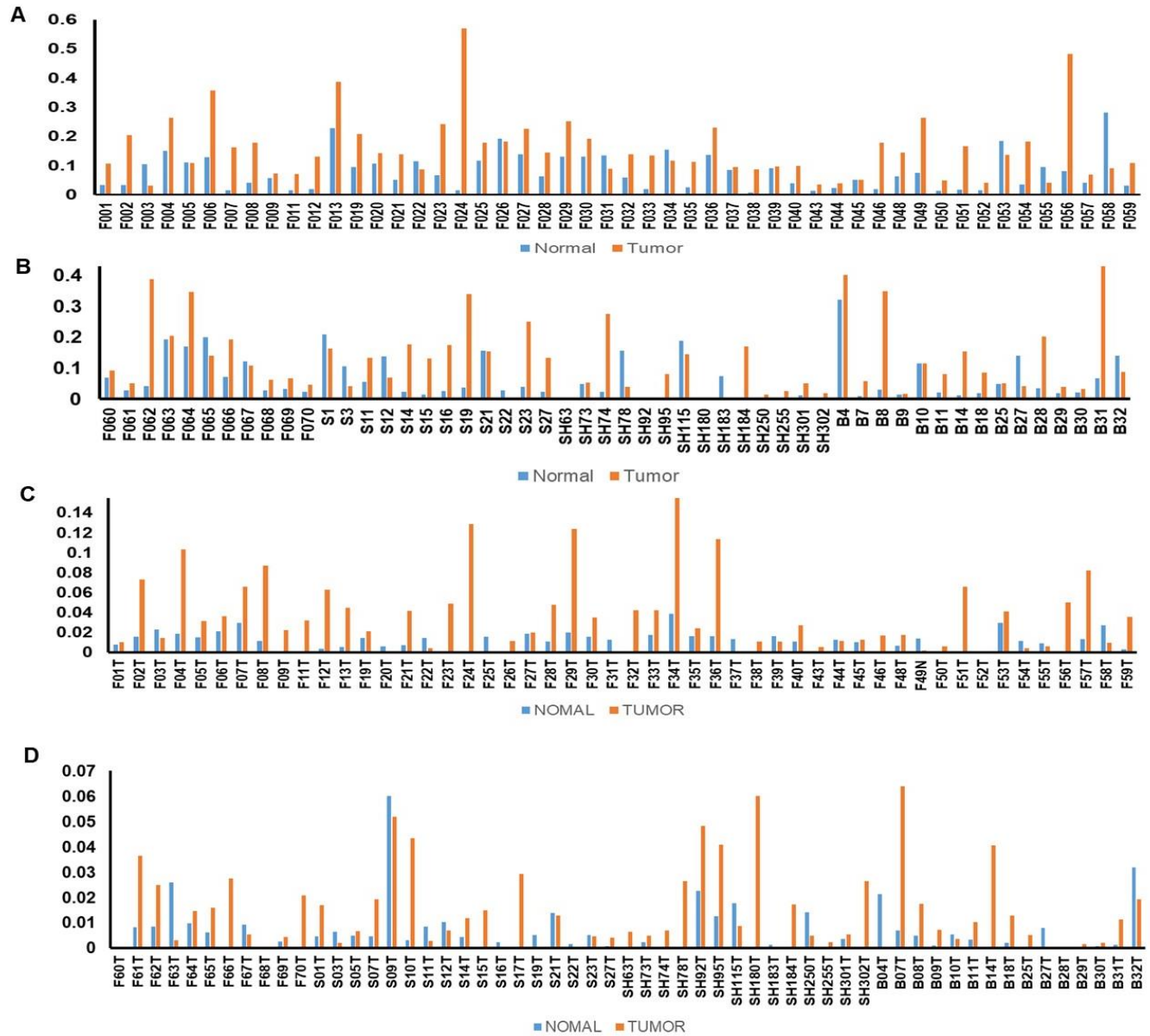


Figure S2. *SRCIN1* methylation level in Taiwanese breast cancer patients: (A)(B)(C)(D) Levels of methylated *SRCIN1* were determined using quantitative methylation-specific polymerase chain reaction (QMSP) in tumor tissue and adjacent normal tissue samples from 102 Taiwanese patients with breast cancer. ACTB was used as the internal control.

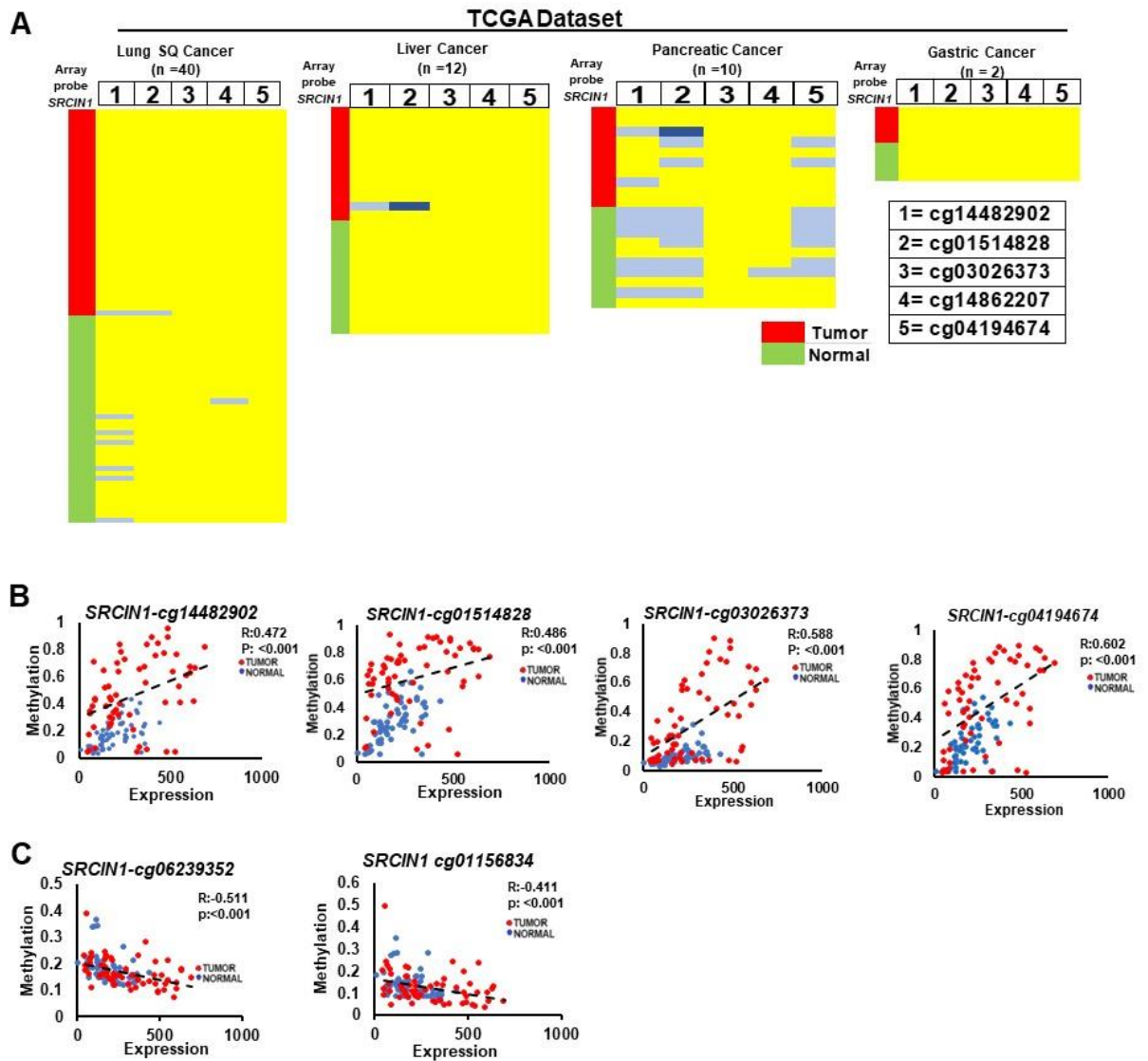


Figure S3. *SRCIN1* DNA methylation in different cancers. Differentially methylated CpG heatmap of *SRCIN1* (A) From TCGA dataset lung, liver cancer, pancreatic, and gastric cancer. Pearson correlation analysis of tissues between DNA methylation and RNA sequencing in 87 paired adjacent normal tissue samples and 87 paired breast cancer tissues from patients (B) Gene body (C) Promoter sites.

Table S1. List primer sequences and their reaction conditions used in the present study.

Gene	Primer	5'→3'sequences	Application
ACTB	Forward primer	TGGTGATGGAGGAGGTTTAGTAAGT	MSP-M
	Reverse primer	AACCAATAAAACCTACTCCTCCCTTAA	
	Probe	ACCACCACCCAACACACAATAACAAACACA	
SRCIN1	Forward primer	GTATGTTTATGGTGAGTTTTTGCGG	
	Reverse primer	CAAAAATACTATTCTTACAATTTCGAA	
	Probe	TGTTTAGGTTGTTGATTTCGTGCGT	
GAPDH	Forward primer	AGCCACATCGCTCAGACAC	RT-PCR Real-time
	Reverse primer	GCCAATACGACCAAATCC	
SRCIN1	Forward primer	AGCCCCGACAAAAGCAAA	
	Reverse primer	CCAAAGGAAGTCAATACAGGGATAG	
CKS	Forward primer	TGTTGTCGTGGATTTGGTGC	
	Reverse primer	TGCACCTCTCAGTGCCATT	
CDK2	Forward primer	GGCACGTACGGAGTTGTGTA	
	Reverse primer	CATCCAGCAGCGTGTCCA	
CDK6	Forward primer	TCTGATTACCTGCTCCGCGA	
	Reverse primer	CAGAATCATTGCACCTGAGGG	
CYCLIN A	Forward primer	CGGTCCTGGAAGTGGTTCA	
	Reverse primer	TGGGGCGATGTTTCTTCTCG	
CYCLIN A2	Forward primer	GGCGGTACTGAAGTCCGGG	
	Reverse primer	TTAAGGGGTGCAACCCGTCTC	
SKP2	Forward primer	TGGCTGAACATTTCACTACTCTC	
	Reverse primer	AGCAAAGTCTGCAGGGCAAA	
CRM1	Forward primer	TGCCGATCTGAAAGCCACTT	
	Reverse primer	TGGGCTCATTTTCGGTAGCAG	
NCOA3	Forward primer	GTGAGCTCCCAGGGTTTTCT	
	Reverse primer	CCACCCTCTGTTGTCGGAAG	
P27KIP1	Forward primer	ACCTGCAACCGACGATTCTT	
	Reverse primer	TTGGGGAACCGTCTGAAACA	

Table S2. *SRCIN1* methylation and expression level versus clinical parameters breast cancer patients; data were generated from TCGA datasets^a.

Characteristics	N ^b (%)	<i>SRCIN1</i> Methylation ^c			<i>p</i> -value	N (%)	<i>SRCIN1</i> mRNA Expression ^d			<i>p</i> -value
		Low (%)	Normal (%)	High (%)			Low (%)	Normal (%)	High (%)	
Overall	640					755				
Tumor Stage					0.673					0.048 ^c
I and II	309 (70.5)	62 (20.1)	52 (16.8)	195 (63.1)		373 (71.3)	54 (14.5)	150 (40.2)	169 (45.3)	
III and IV	129 (29.5)	26 (20.2)	27 (20.9)	76 (58.9)		150 (28.7)	10 (6.7)	66 (44.0)	74 (49.3)	
Tumor size										0.004 ^c
<65	449 (72.6)	91 (20.3)	83 (18.5)	275 (61.2)	0.293	542 (72.4)	66 (12.2)	245 (45.2)	231 (42.6)	
≥65	169 (27.4)	25 (14.8)	31 (18.3)	113 (66.9)		207 (27.6)	24 (11.6)	68 (32.9)	115 (55.6)	
Histological Type										0.000 ^c
IDC	397 (71.3)	77 (19.4)	64 (16.1)	256 (64.5)	0.020 ^b	170 (24.7)	4 (2.4)	53 (31.2)	113 (66.5)	
ILC	152 (27.3)	27 (17.8)	34 (22.4)	91 (59.9)		492 (71.6)	73 (14.8)	219 (44.5)	200 (40.7)	
Mixed type	8 (1.4)	5 (62.5)	0 (0.0)	3 (37.5)		25 (3.6)	1 (4.0)	17 (68.0)	7 (28.0)	
Tumor size					0.321					0.473
T0–T1	114 (25.9)	19 (16.7)	19 (16.7)	76 (66.7)		135 (25.6)	13 (9.6)	60 (44.4)	62 (45.9)	
T2–T4	327 (24.1)	69 (21.1)	61 (18.7)	197 (60.2)		392 (74.4)	52 (13.3)	158 (40.3)	182 (46.4)	
Lymph nodes					0.990					0.535
N0	198 (45.1)	40 (20.2)	35 (17.7)	123 (62.1)		237 (45.2)	25 (10.5)	99 (41.8)	113 (47.7)	
N1–N3	241 (54.9)	48 (19.9)	44 (18.3)	149 (61.8)		287 (54.8)	39 (13.6)	120 (41.8)	128 (44.6)	
Race					0.074					0.429
White	338 (78.4)	75 (86.2)	61 (18.0)	202 (59.8)		413 (79.9)	53 (12.8)	165 (40.0)	195 (47.2)	
American	30 (7.0)	5 (16.7)	9 (30.0)	16 (53.3)		72 (13.9)	7 (9.7)	32 (44.4)	33 (45.8)	
Asian	63 (14.6)	7 (11.1)	9 (14.3)	47 (74.6)		32 (6.2)	3 (9.4)	18 (56.3)	11 (34.4)	
Menopause State					0.695					0.124
Premenopaus e	7 (14.9)	1 (14.3)	3 (42.9)	3 (42.9)		121 (25.7)	18 (14.9)	55 (45.5)	48 (39.7)	
Perimenopau se	5 (10.6)	2 (40.0)	0 (0.0)	3 (60.0)		16 (3.4)	3 (18.8)	9 (56.3)	4 (25.0)	
Postmenopa use	35 (74.5)	6 (17.1)	11 (31.4)	18 (51.4)		333 (70.9)	37 (11.1)	129 (38.7)	167 (50.2)	

Metastasis					0.522				0.091
M0	376 (98.2)	74 (19.7)	70 (18.6)	232 (61.7)		449 (98.0)	59 (13.1)	191 (42.5)	199 (44.3)
M1	7 (1.8)	1 (14.3)	0 (0.0)	6 (85.7)		9 (2.0)	1 (11.1)	7 (77.8)	1 (11.1)
Tumor Markers					0.081				0.000 ^c
Estrogen									
Negative	101 (22.9)	17 (16.8)	15 (14.9)	69 (68.3)		116 (22.1)	40 (34.5)	50 (43.1)	26 (22.4)
Positive	340 (77.1)	71 (20.9)	66 (19.4)	203 (59.7)		408 (77.9)	24 (5.9)	166 (40.7)	218 (53.4)
Progesterone					0.759				0.000 ^c
Negative	141 (32.0)	28 (19.9)	23 (16.3)	90 (63.8)		164 (31.4)	43 (26.2)	69 (42.1)	52 (31.7)
Positive	299 (68.0)	60 (20.1)	58 (19.4)	181 (60.5)		358 (68.6)	21 (5.9)	145 (40.5)	192 (53.6)
HER2					0.609				0.001 ^c
Negative	247 (84.9)	53 (21.5)	44 (17.8)	150 (60.7)		292 (64.6)	47 (16.1)	132 (45.2)	113 (38.7)
Positive	44 (15.1)	7 (15.9)	11 (25.0)	26 (59.1)		56 (12.4)	3 (5.4)	16 (28.6)	37 (66.1)
Equivoc						104 (23.0)	9 (8.7)	40 (38.5)	55 (52.9)
Subtypes					0.374				0.000 ^c
TNBC									
Negative	237 (81.7)	50 (21.1)	48 (20.3)	139 (58.6)		690 (92.0)	64 (9.3)	289 (41.9)	337 (48.8)
Positive	53 (18.3)	10 (18.9)	7 (13.2)	36 (67.9)		60 (8.0)	26 (43.3)	25 (41.7)	9 (15.0)
Luminal A					0.498				0.000 ^c
Negative	97 (33.4)	17 (17.5)	18 (18.6)	62 (63.9)		116 (33.5)	29 (25.0)	41 (35.3)	46 (39.7)
Positive	193 (66.6)	43 (22.3)	37 (19.2)	113 (58.5)		230 (66.5)	21	105 (9.1)	104 (45.7)
Luminal B					0.283				0.448
Negative	230 (79.3)	52 (22.6)	43 (18.7)	135 (58.7)		267 (77.2)	36 (13.5)	117 (43.8)	114 (42.7)
Positive	60 (20.7)	8 (13.3)	12 (20.0)	40 (66.7)		79 (22.8)	14 (17.7)	29 (36.7)	36 (45.6)

^aThese results were analyzed by Pearson's X2 test.

^bFor some categories, the number of samples (n) was lower than the overall number analyzed because clinical data were unavailable for those samples.

^c*SRCIN1* methylation was considered hypermethylation when the *SRCIN1* methylation level was more than two in breast tumors compared to adjacent normal breast tissues.

^d*SRCIN1* was considered high when the *SRCIN1* expression level was more than two in breast tumors compared to adjacent normal breast tissues.