

Supplementary Table 1: Inclusion and exclusion criteria of cases and controls.

Criteria	Cases	Controls
Inclusion criteria	(1) Women (2) Age 18–75 years (3) Pathologically diagnosed as cervical cancer (4) No treatment (5) Written informed consent	(1) Women (2) Age 18–75 years (3) Clinical confirmation of cervical cancer-free (4) Written informed consent
Exclusion criteria	(1) History of previous or concomitant malignancies (2) Other severe systematic diseases that are considered to be unsuitable for the study (3) History of antitumor therapy (4) Pregnant or lactating women (5) Without informed consent	(1) History of previous or concomitant malignancies (2) Other severe systematic diseases that are considered to be unsuitable for the study (3) Pregnant or lactating women (4) Without informed consent

Supplementary Table 2: Clinical characteristics of the 108 CC patients and 108 healthy controls enrolled in the study.

Variables	Training stage		Testing stage		External validation stage	
	Cases (n = 30)	Controls (n = 30)	Cases (n = 60)	Controls (n = 60)	Cases (n = 18)	Controls (n = 18)
Age (years)						
<60	20 (66.7)	22 (73.3)	40 (66.7)	45 (75.0)	10 (55.6)	12 (66.7)
≥60	10 (33.3)	8 (26.7)	20 (33.3)	15 (25.0)	8 (44.4)	6 (33.3)
TNM stage						
I	10 (33.3)		32 (53.3)		10 (55.6)	
II	8 (26.7)		14 (23.3)		5 (27.8)	
III	7 (23.3)		8 (13.3)		2 (11.1)	
IV	5 (16.7)		6 (10.0)		1 (5.6)	
Pathological subtype						
Squamous carcinoma	27 (90.0)		53 (88.3)		17 (94.4)	
Adenocarcinoma	3 (10.0)		6 (10.0)		1 (5.6)	
Neuroendocrine carcinoma	0		1 (1.7)		0	

Data are presented as n (%). CC: Cervical cancer; TNM: The T(tumor) N(nodes) M(metastases) classification of a malignant tumor.

Supplementary Table 3: Differentially expressed miRNAs in the screening stage.

miRNA	Fold change		
	Pool 1	Pool 2	Mean fold
miR-10b-5p	2.80	4.32	3.56
miR-122-5p	3.49	3.18	3.34
miR-125b-5p	2.12	3.51	2.81
miR-141-3p	2.61	2.04	2.32
miR-151a-3p	3.30	2.34	2.82
miR-190a	3.58	1.65	2.62
miR-192-5p	1.72	6.69	4.21
miR-195-5p	3.77	3.09	3.43
miR-200a-3p	7.78	2.68	5.23
miR-205-5p	1.79	2.65	2.22
miR-208a	3.77	5.41	4.59
miR-29a-5p	3.73	1.61	2.67
miR-320a	1.62	1.80	1.71
miR-320b	1.51	4.26	2.88
miR-326	1.51	1.93	1.72
miR-346	2.76	4.09	3.43
miR-361-3p	3.77	5.41	4.59
miR-376a-3p	1.63	6.73	4.18
miR-485-3p	2.13	2.12	2.13
miR-495-3p	3.77	9.75	6.76
miR-605	3.07	5.41	4.24
miR-92b-3p	3.15	1.94	2.55
miR-99a-5p	2.63	6.36	4.50
let-7b-3p	-29.26	-43.57	-36.42
miR-19b-3p	-19.85	-26.25	-23.05
miR-133a-3p	-10.42	-8.83	-9.63
miR-142-5p	-10.41	-8.81	-9.61
miR-199a-3p	-9.06	-12.76	-10.91
let-7i-5p	-5.73	-10.69	-8.21

Supplementary Table 4: The expression levels of the three serum miRNAs in the training and testing stages.

miRNA	Training stage					Testing stage				
	Cases	Controls	FC	Z	P	Cases	Controls	FC	Z	P
miR-122-5p	-1.694 (-3.154, 1.699)	1.173 (0.026, 3.482)	1.540	-4.929	<0.001	-0.884 (-2.241, 3.225)	1.024 (-2.122, 4.273)	1.504	-4.403	<0.001
miR-20a-5p	-1.633 (-4.382, 0.024)	1.482 (0.172, 2.491)	1.547	-4.836	<0.001	-0.928 (-3.952, 4.343)	2.577 (-0.458, 5.457)	1.511	-4.336	<0.001
miR-133a-3p	2.625 (-0.023, 5.721)	-1.221 (-3.490, 2.347)	0.561	-5.103	<0.001	2.368 (-0.641, 5.293)	-0.570 (-2.482, 3.585)	0.533	-5.425	<0.001

Data are presented as median (P25, P75). ΔCt , relative to miR-16-5p and *cel-miR-39*. FC: Fold change.

Supplementary Table 5: DIANA-miRPath v.3.0 analysis for the target genes of identified miRNAs from the DIANA-TarBase v.7.0 database to decipher the potential function of the miRNAs.

A: Common KEGG pathways involved by the three miRNAs.

KEGG pathways	FDR	Gene count
Proteoglycans in cancer	<0.001	58
Chronic myeloid leukemia	<0.001	32
Cell cycle	<0.001	44
TGF-beta signaling pathway	<0.001	30
Glioma	<0.001	25
Pathways in cancer	<0.001	104
Prostate cancer	<0.001	35
Pancreatic cancer	<0.001	28
Small cell lung cancer	<0.001	33
Insulin signaling pathway	<0.001	48
Protein processing in the endoplasmic reticulum	<0.001	52
FoxO signaling pathway	<0.001	46
Hepatitis B	<0.001	44
Non-small cell lung cancer	<0.001	22
Bladder cancer	<0.001	19
Sphingolipid signaling pathway	<0.001	37
Viral carcinogenesis	<0.001	47
Melanoma	<0.001	24
Long-term depression	<0.001	17
Circadian rhythm	<0.001	15
p53 signaling pathway	0.001	26
Focal adhesion	0.002	59
Axon guidance	0.002	36
ECM-receptor interaction	0.002	14
Colorectal cancer	0.003	23
AMPK signaling pathway	0.003	38
Signaling pathways regulating pluripotency of stem cells	0.003	39
Adherens junction	0.003	26
Other types of O-glycan biosynthesis	0.004	9
Transcriptional misregulation in cancer	0.005	42
PI3K-Akt signaling pathway	0.005	82
Thyroid hormone signaling pathway	0.005	35
mTOR signaling pathway	0.006	21
Neurotrophin signaling pathway	0.006	36
Lysine degradation	0.006	12
Renal cell carcinoma	0.007	23
MAPK signaling pathway	0.007	65
Spliceosome	0.007	40

mRNA surveillance pathway	0.009	27
Regulation of actin cytoskeleton	0.009	55
RNA degradation	0.009	26
RNA transport	0.012	45
Acute myeloid leukemia	0.015	19
ErbB signaling pathway	0.016	27
Central carbon metabolism in cancer	0.017	20
Apoptosis	0.020	27
Hepatitis C	0.020	37
Hippo signaling pathway	0.027	40
Prolactin signaling pathway	0.028	21
Endometrial cancer	0.039	17
Ubiquitin-mediated proteolysis	0.044	38
Endocytosis	0.045	49
Glycosaminoglycan biosynthesis — keratan sulfate	0.048	4

FDR: False discovery rate; KEGG: Kyoto Encyclopedia of Genes and Genomes; TGF: Transforming growth factor.

B: Common biological processes involved by the three miRNAs according to GO annotation.

GO terms	FDR	Gene count
Organelle	<0.001	1994
Cellular nitrogen compound metabolic process	<0.001	1015
Biosynthetic process	<0.001	832
Ion binding	<0.001	1115
Cellular protein modification process	<0.001	544
Gene expression	<0.001	190
molecular_function	<0.001	2784
Nucleoplasm	<0.001	308
Enzyme binding	<0.001	321
Symbiosis, encompassing mutualism through parasitism	<0.001	149
cellular_component	<0.001	2773
Protein complex	<0.001	729
Viral process	<0.001	135
Catabolic process	<0.001	406
Mitotic cell cycle	<0.001	122
Cytosol	<0.001	556
RNA binding	<0.001	402
biological_process	<0.001	2675
Neurotrophin TRK receptor signaling pathway	<0.001	77
Protein binding transcription factor activity	<0.001	130
Nucleic acid-binding transcription factor activity	<0.001	216
Cellular component assembly	<0.001	266
Response to stress	<0.001	424

Small molecule metabolic process	<0.001	405
Cellular protein metabolic process	<0.001	104
Nucleobase-containing compound catabolic process	<0.001	187
Cell death	<0.001	195
Macromolecular complex assembly	<0.001	183
Fc-epsilon receptor signaling pathway	<0.001	45
Microtubule organizing center	<0.001	115
Blood coagulation	<0.001	96
Membrane organization	<0.001	121
G2/M transition of mitotic cell cycle	<0.001	49
Transcription, DNA-templated	<0.001	458
mRNA metabolic process	<0.001	54
DNA metabolic process	<0.001	158
Enzyme regulator activity	<0.001	163
RNA metabolic process	<0.001	58
mRNA processing	<0.001	124
Cytoskeletal protein binding	<0.001	147
Protein complex assembly	<0.001	145
Post-translational protein modification	<0.001	39
Transcription initiation from RNA polymerase II promoter	<0.001	56
RNA splicing	<0.001	81
Epidermal growth factor receptor signaling pathway	<0.001	49
Apoptotic signaling pathway	<0.001	39
Fc-gamma receptor signaling pathway involved in phagocytosis	<0.001	21
G1/S transition of mitotic cell cycle	<0.001	48
Intrinsic apoptotic signaling pathway	<0.001	24
Extracellular matrix disassembly	<0.001	29
Small conjugating protein binding	<0.001	29
Termination of RNA polymerase II transcription	<0.001	17
Activation of signaling protein activity involved in unfolded protein response	<0.001	20
Fibroblast growth factor receptor signaling pathway	<0.001	44
Phosphatidylinositol-mediated signaling	<0.001	34
Cell cycle	<0.001	175
Generation of precursor metabolites and energy	<0.001	65
Nucleocytoplasmic transport	<0.001	75
Positive regulation of protein insertion into mitochondrial membrane involved in an apoptotic signaling pathway	<0.001	12
Glycosaminoglycan metabolic process	<0.001	25
mRNA 3'-end processing	<0.001	14

mRNA splicing, via spliceosome	<0.001	57
Cell cycle arrest	<0.001	46
Immune system process	<0.001	250
Insulin receptor signaling pathway	<0.001	39
Cell junction organization	<0.001	34
Transforming growth factor-beta receptor signaling pathway	<0.001	44
Hexose transport	<0.001	12
Toll-like receptor 10 signaling pathway	<0.001	15
Ribonucleoprotein complex assembly	<0.001	34
Inositol phosphate metabolic process	<0.001	13
Protein N-linked glycosylation via asparagine	<0.001	23
Toll-like receptor 9 signaling pathway	<0.001	17
Mitotic nuclear envelope disassembly	<0.001	11
chromatin organization	<0.001	29
Nuclear-transcribed mRNA catabolic process, deadenylation-dependent decay	<0.001	17
Stress-activated MAPK cascade	<0.001	15
TRIF-dependent toll-like receptor signaling pathway	<0.001	16
Innate immune response	<0.001	120
Toll-like receptor TLR1:TLR2 signaling pathway	<0.001	15
Toll-like receptor TLR6:TLR2 signaling pathway	<0.001	15
Axon guidance	<0.001	85
Transcription factor binding	<0.001	116
Cellular component disassembly involved in the execution phase of apoptosis	<0.001	12
Cellular lipid metabolic process	<0.001	27
<i>In utero</i> embryonic development	<0.001	72
Regulation of glucose transport	<0.001	10
Toll-like receptor 5 signaling pathway	<0.001	15
Platelet activation	<0.001	36
Vacuole	<0.001	58
Extracellular matrix organization	<0.001	67
JAK-STAT cascade involved in growth hormone signaling pathway	<0.001	9
Transcription from RNA polymerase II promoter	<0.001	114
DNA damage response, signal transduction by p53 class mediator resulting in cell cycle arrest	0.001	18
Regulation of cell cycle	0.001	56
MyD88-independent toll-like receptor signaling pathway	0.001	16
Protein maturation	0.002	40
Toll-like receptor 3 signaling pathway	0.002	17

Nuclear-transcribed mRNA poly	0.002	12
Phosphatidylinositol biosynthetic process	0.002	16
Cytoskeleton organization	0.002	112
Transcription corepressor activity	0.002	54
Chondroitin sulfate metabolic process	0.003	12
Regulation of small GTPase-mediated signal transduction	0.003	42
Toll-like receptor signaling pathway	0.003	22
Endosome	0.003	115
Protein ubiquitination	0.003	104
Toll-like receptor 2 signaling pathway	0.003	16
Cell junction assembly	0.003	14
Negative regulation of transcription from RNA polymerase II promoter	0.004	183
Cytokine-mediated signaling pathway	0.004	57
Regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	0.005	15
Anatomical structure morphogenesis	0.010	22
Ubiquitin-specific protease activity	0.010	31
Negative regulation of transforming growth factor-beta receptor signaling pathway	0.011	25
Plasma membrane organization	0.012	27
Positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle	0.012	14
Cellular component movement	0.013	22
Endoplasmic reticulum unfolded protein response	0.013	27
Leukocyte migration	0.013	22
Viral life cycle	0.015	19
Keratan sulfate metabolic process	0.015	8
Cell motility	0.015	89
Phospholipid metabolic process	0.015	30
Regulation of transcription involved in G1/S transition of mitotic cell cycle	0.017	9
Anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process	0.017	17
Carbohydrate metabolic process	0.020	155
DNA strand elongation involved in DNA replication	0.022	8
Energy reserve metabolic process	0.022	18
Activation of MAPKK activity	0.024	20
Positive regulation of viral transcription	0.024	11
Negative regulation of cell proliferation	0.024	103
Keratan sulfate biosynthetic process	0.026	7
Regulation of defense response to virus by virus	0.026	7

O-glycan processing	0.026	11
Toll-like receptor 4 signaling pathway	0.027	17
Type I interferon signaling pathway	0.030	15
Protein polyubiquitination	0.032	32
Antigen processing and presentation of exogenous peptide antigen via MHC class II	0.033	22
Nuclear body	0.036	18
Cell proliferation	0.036	105
SMAD binding	0.037	20
Ubiquitin protein ligase binding	0.037	60
Proteasome-mediated ubiquitin-dependent protein catabolic process	0.042	31
Vitamin D receptor binding	0.043	7
Ligase activity	0.045	102
Regulation of Ras GTPase activity	0.045	6
Phosphatidylinositol 3-kinase activity	0.046	4
Nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.048	24

FDR: False discovery rate; GO: Gene Ontology.