Supplementary Material for

Unveiling urinary extracellular vesicle mRNA signature for early diagnosis and prognosis of bladder cancer

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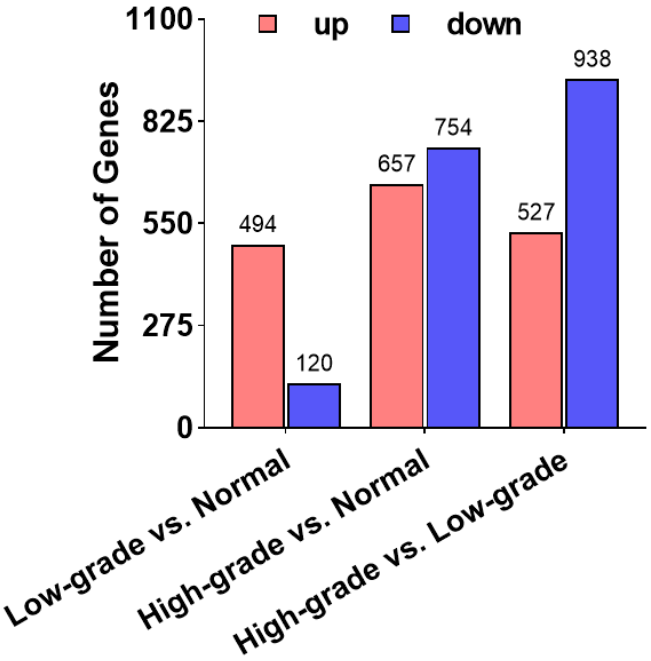

Figure S1. DEG numbers in EVs derived from the cells: low-grade BC cells relative to normal cells, high-grade BC cells relative to normal cells, and high-grade BC cells relative to low-grade cells. P-value < 0.05 and fold change (FC) > 1.5 are used to define DEGs.

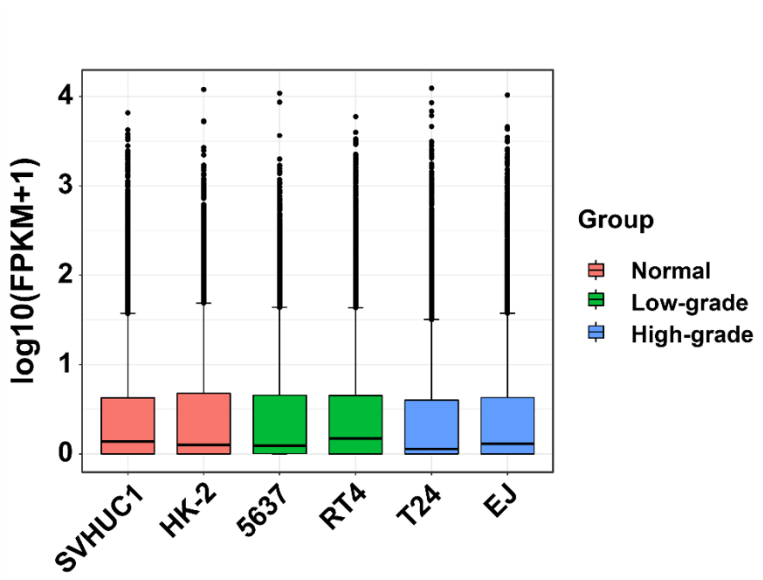

Figure S2. Analysis of gene expression levels for EVs derived from 6 cell lines.

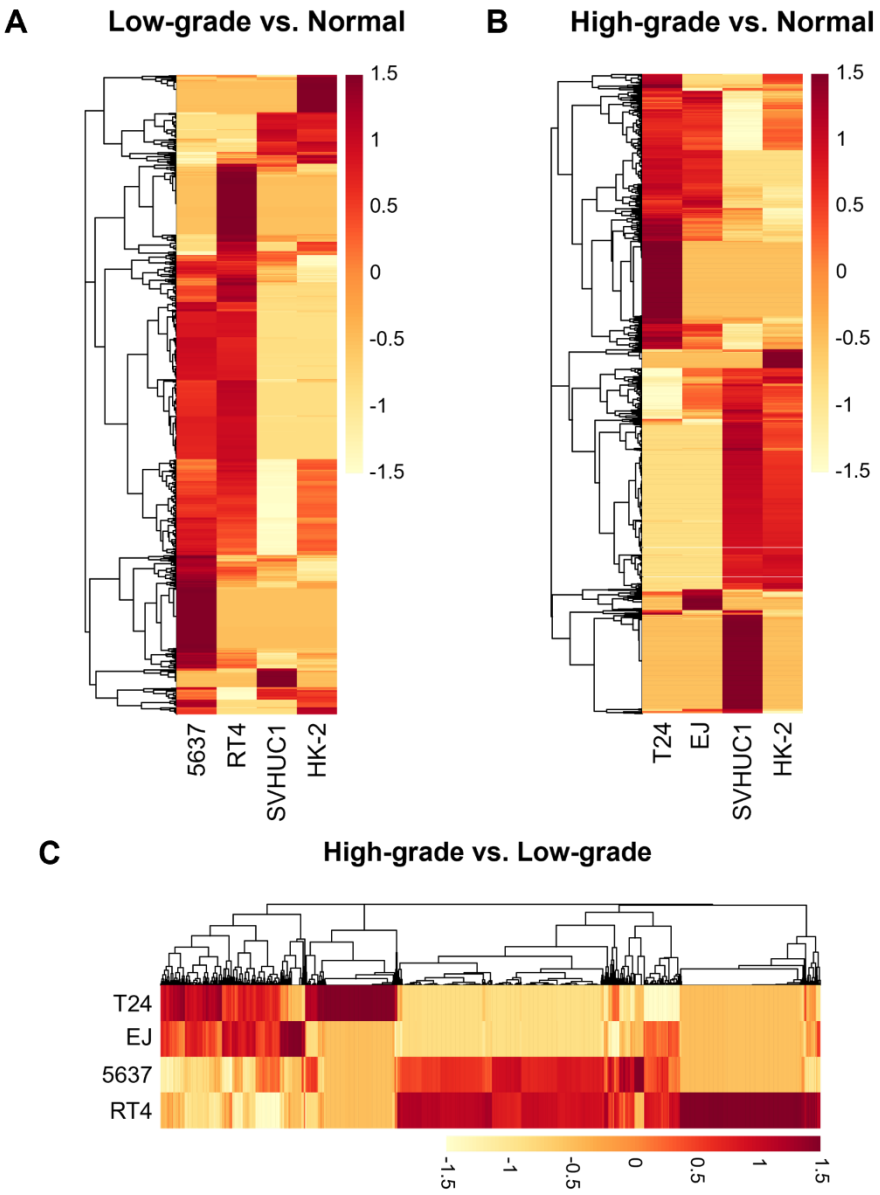

Figure S3. Hierarchical clustering analysis of proteomic data for EVs derived from the cells: (**A**) low-grade BC cells relative to normal cells, (**B**) high-grade BC cells relative to normal cells, and (**C**) high-grade BC cells relative to low-grade BC cells.

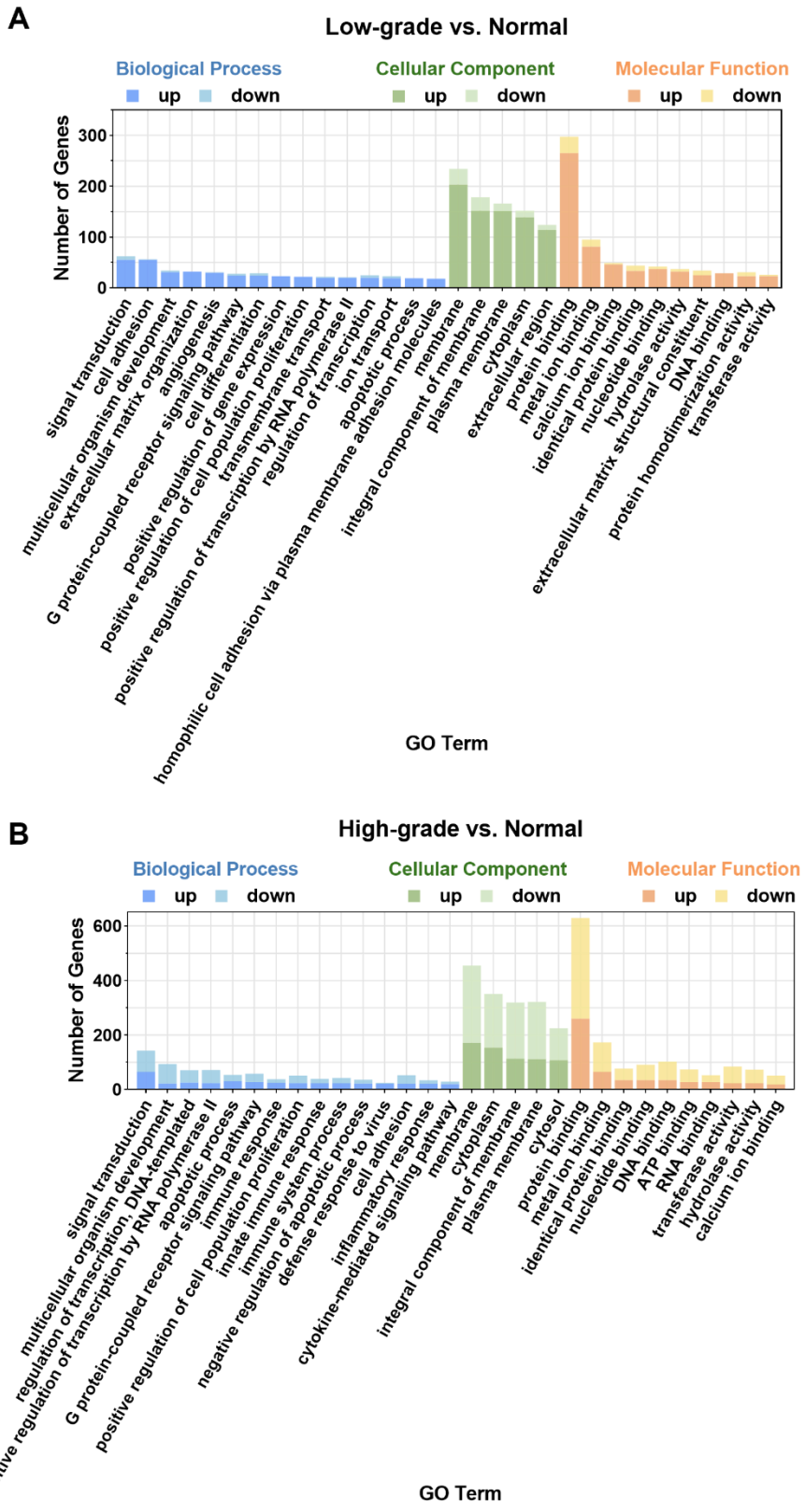

Figure S4. GO analysis based on the identified DEGs in EVs derived from the cells: (**A**) low-grade BC cells relative to normal cells and (**B**) high-grade BC cells relative to normal cells.

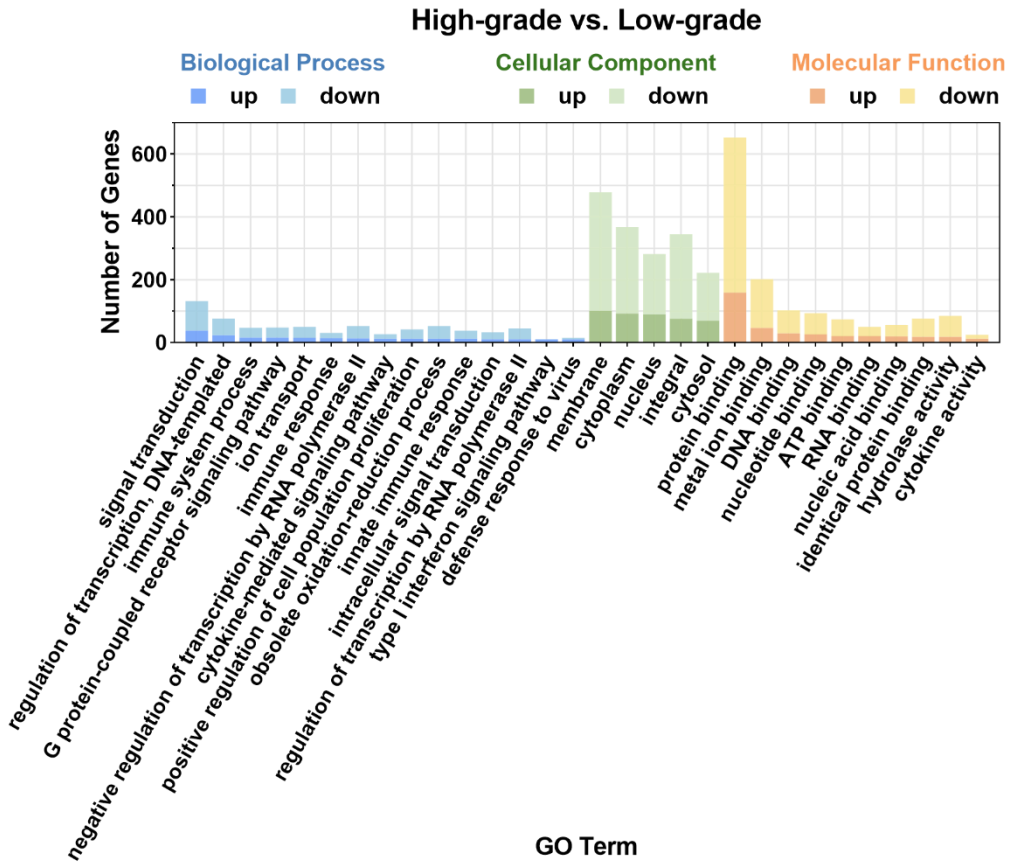

Figure S5. GO analysis based on the identified DEGs in EVs derived from the cells (high-grade BC cells relative to low-grade BC cells).

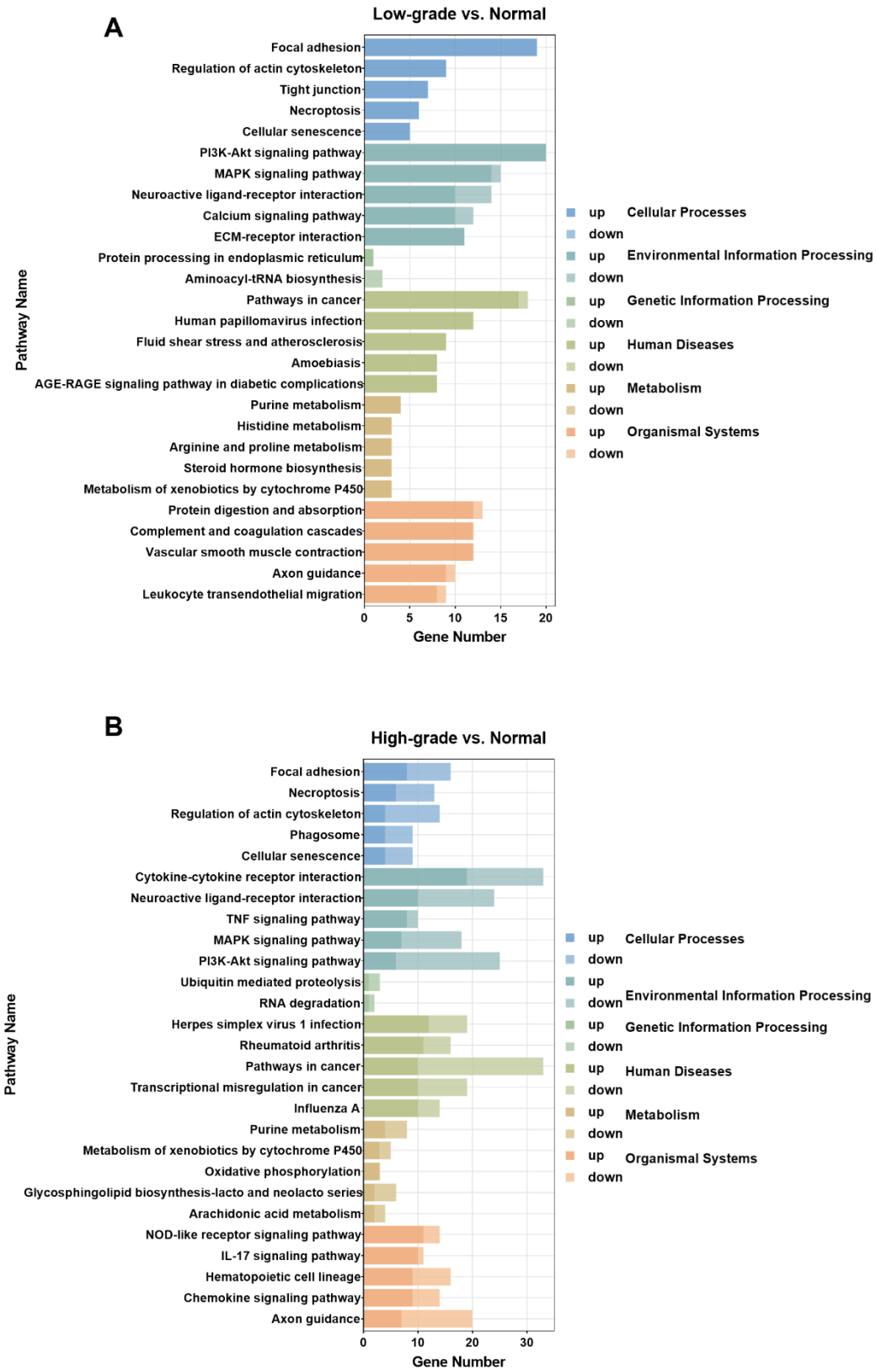

Figure S6. KEGG analysis based on the identified DEGs in EVs derived from the cells: (**A**) low-grade BC cells relative to normal cells and (**B**) high-grade BC cells relative to normal cells.

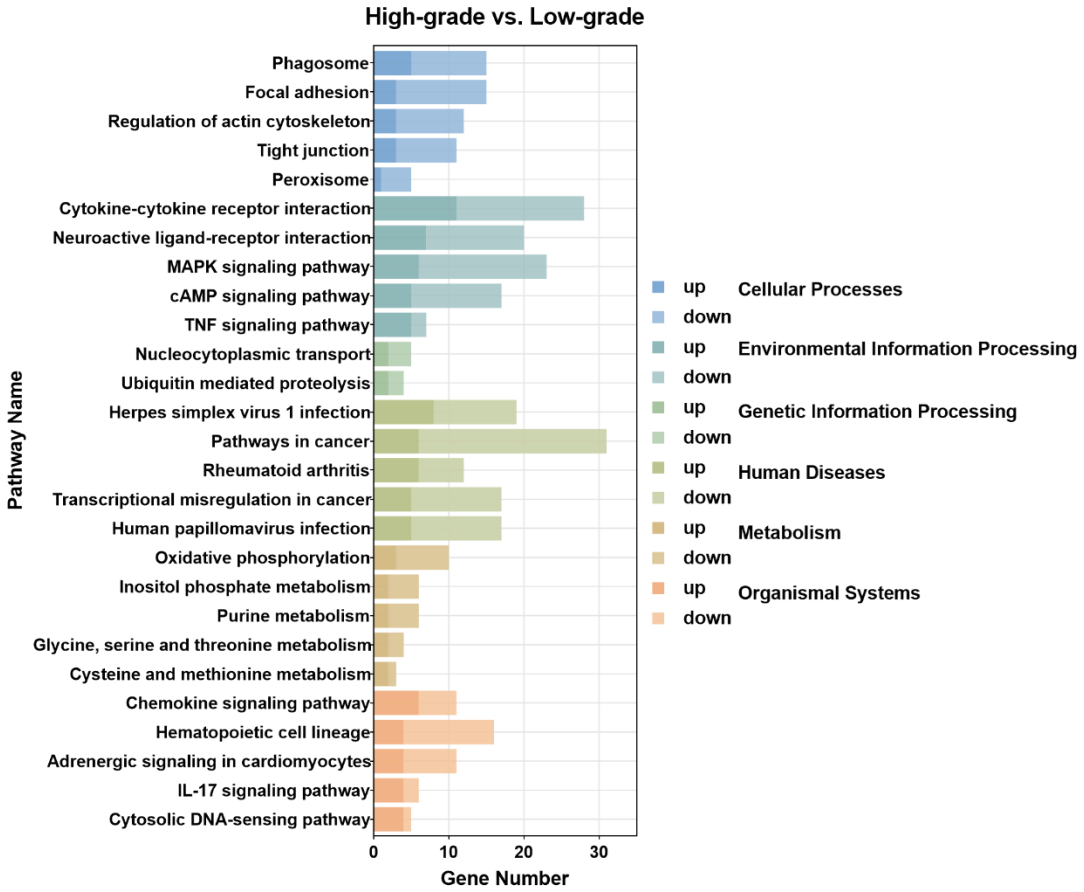

Figure S7. KEGG analysis based on the identified DEGs in EVs derived from the cells (high-grade BC cells relative to low-grade BC cells).

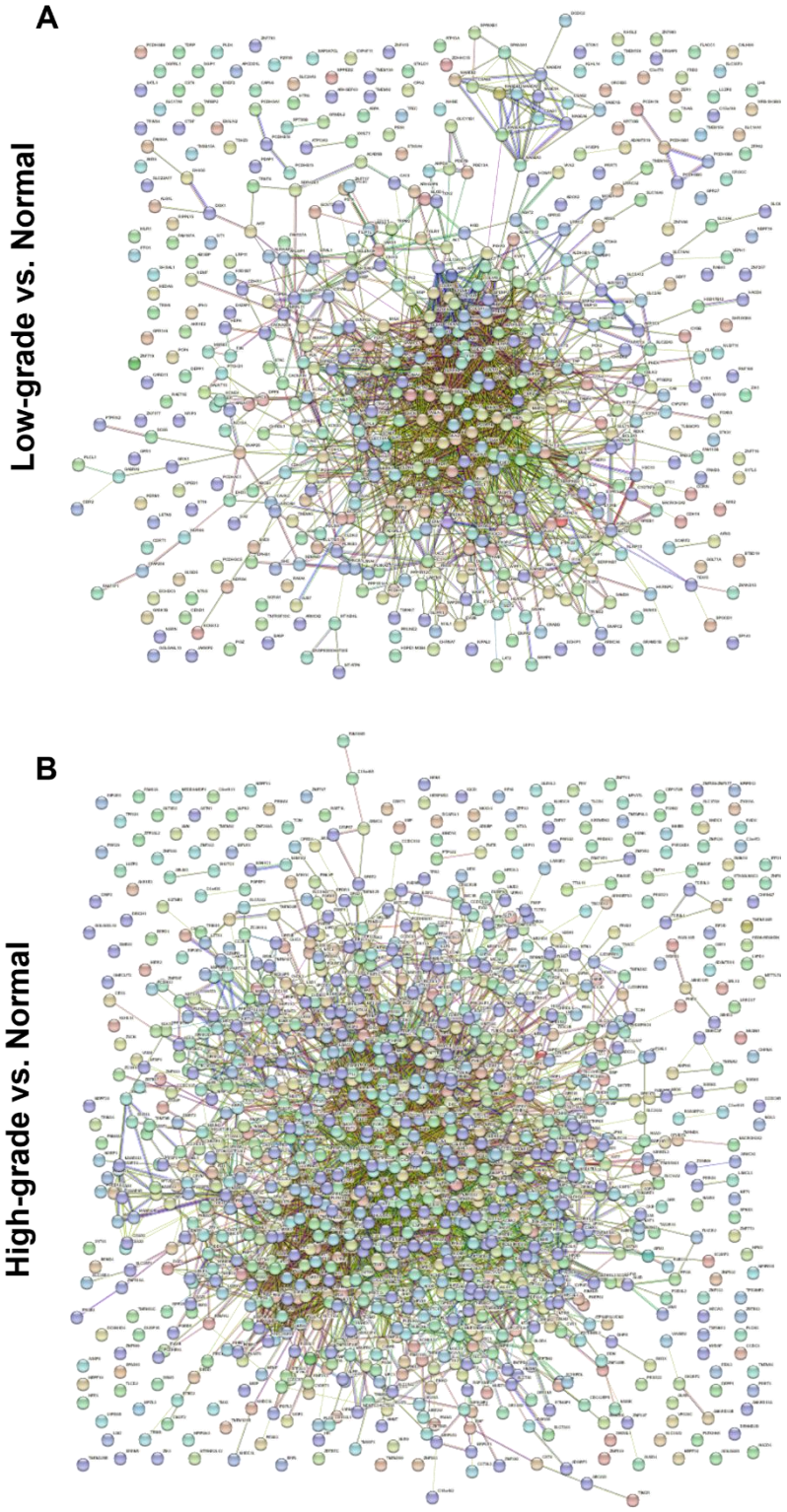

Figure S8. PPI network diagrams of the identified DEGs in EVs derived from the cells: (**A**) low-grade BC cells relative to normal cells and (**B**) high-grade BC cells relative to normal cells.

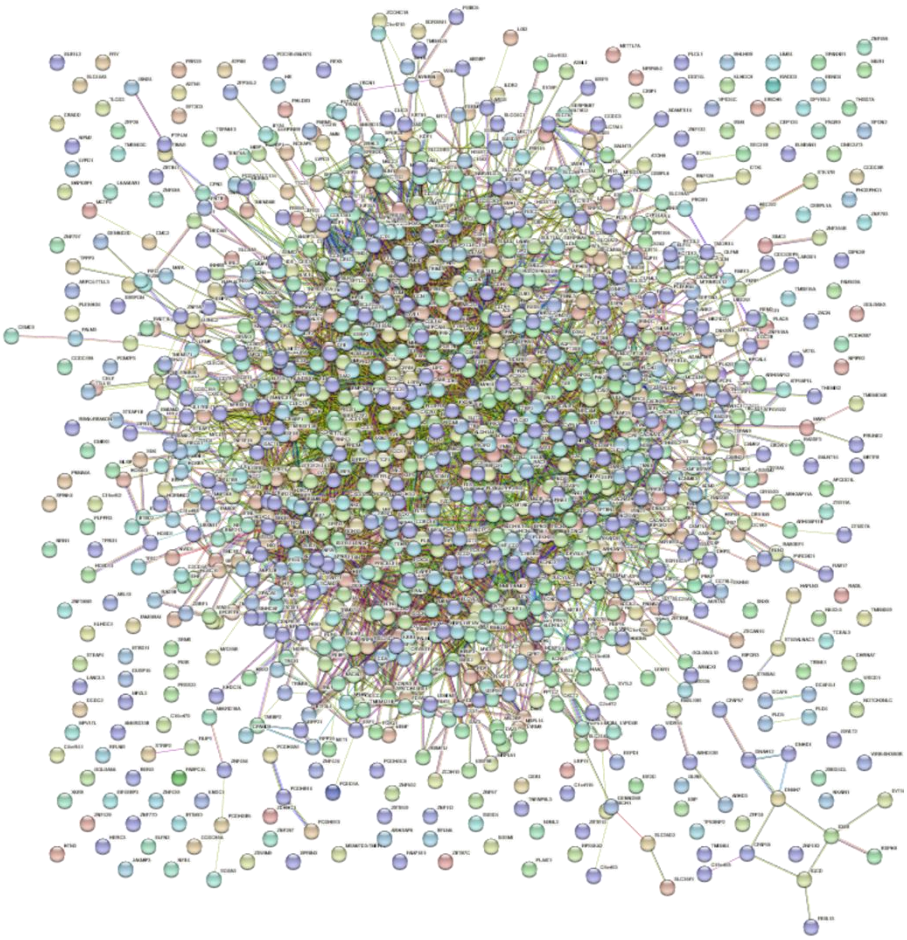

Figure S9. PPI network diagrams of the identified DEGs in EVs derived from the cells (high-grade BC cells relative to low-grade BC cells).

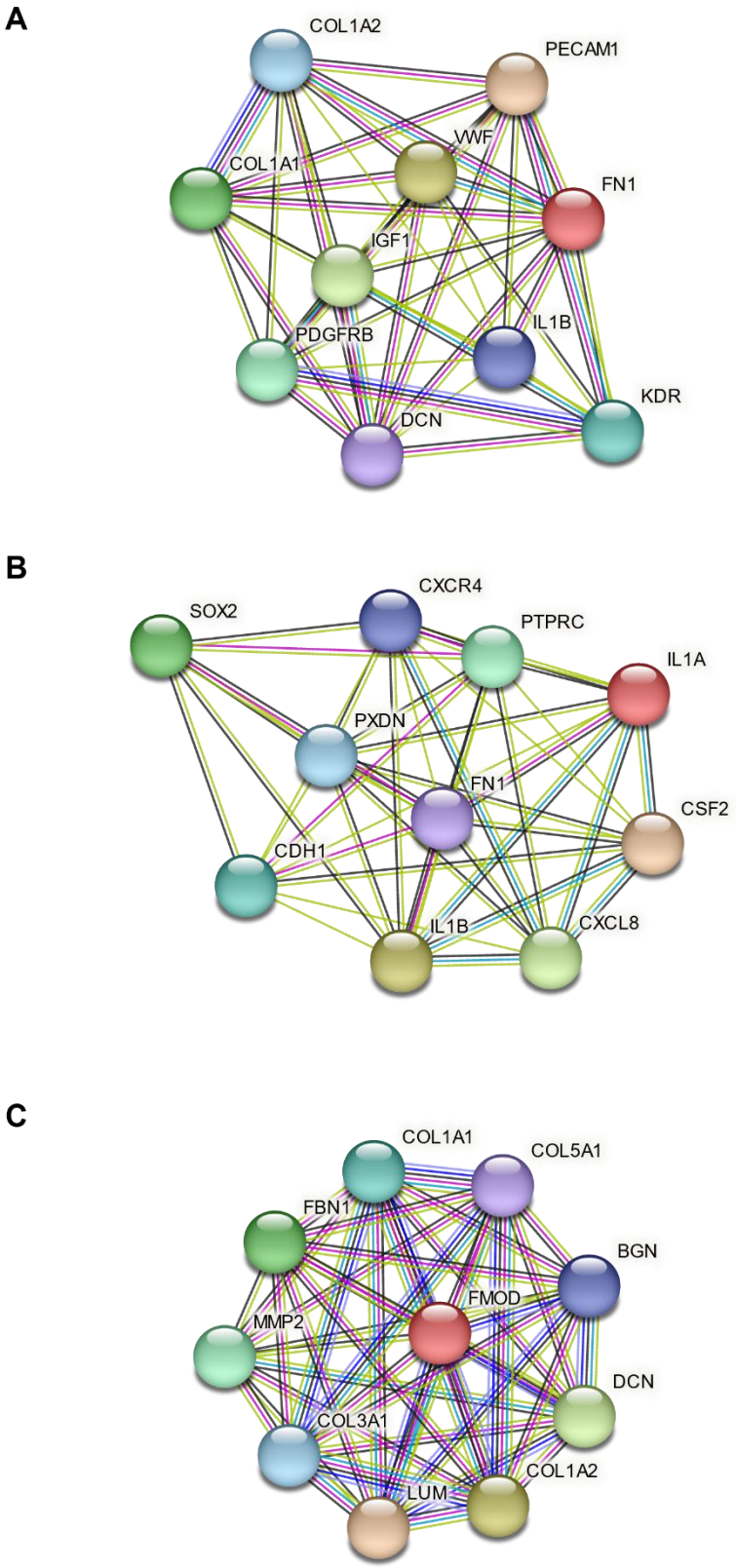

Figure S10. Hub genes generated from PPI network in EVs derived from the cells: (**A**) low-grade BC cells relative to normal cells, (**B**) high-grade BC cells relative to normal cells, and (**C**) high-grade BC cells relative to low-grade cells.

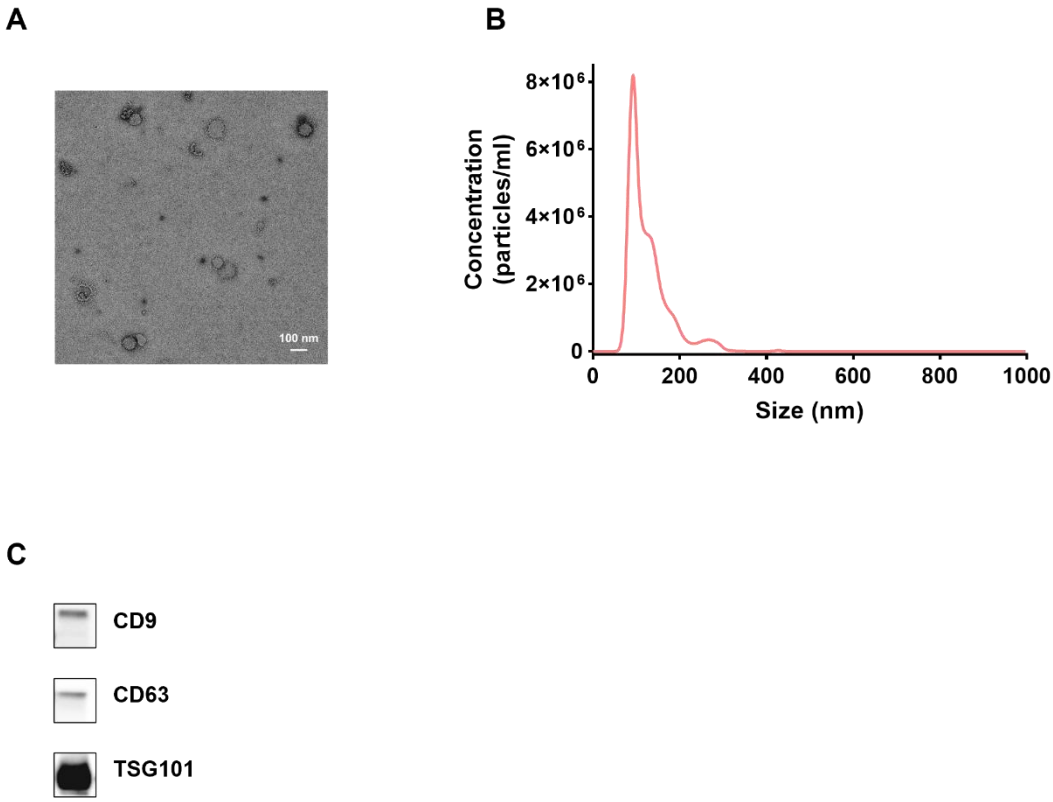

Figure S11. Characterization of uEVs derived from clinical urine sample by (**A**) cryo-TEM, (**B**) NTA, and (**C**) WB.

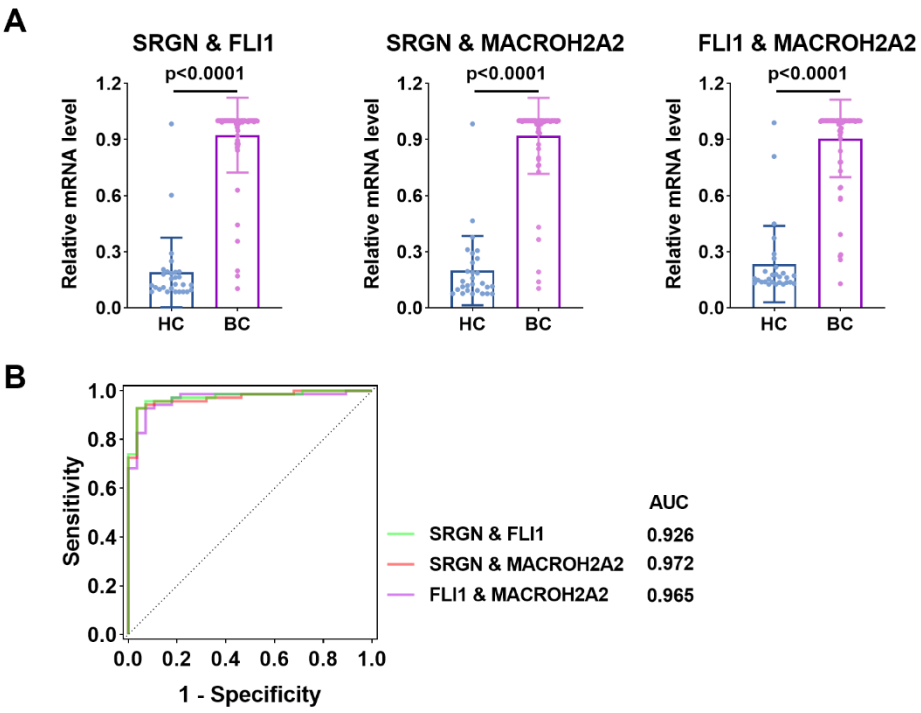

Figure S12. Combining two biomarkers to distinguish HC and BC. (**A**) Scattering plots of the mRNA expression levels of the weighted sum of the biomarkers two by two, SRGN and FLI1, SRGN and MACROH2A2, and FLI1 and MACROH2A2, respectively, in uEVs collected from HC and BC patients. (**B**) ROC curves of the weighted sum of the biomarkers two by two for BC diagnosis. The weighted sum of the three markers was calculated by logistic regression algorithm.

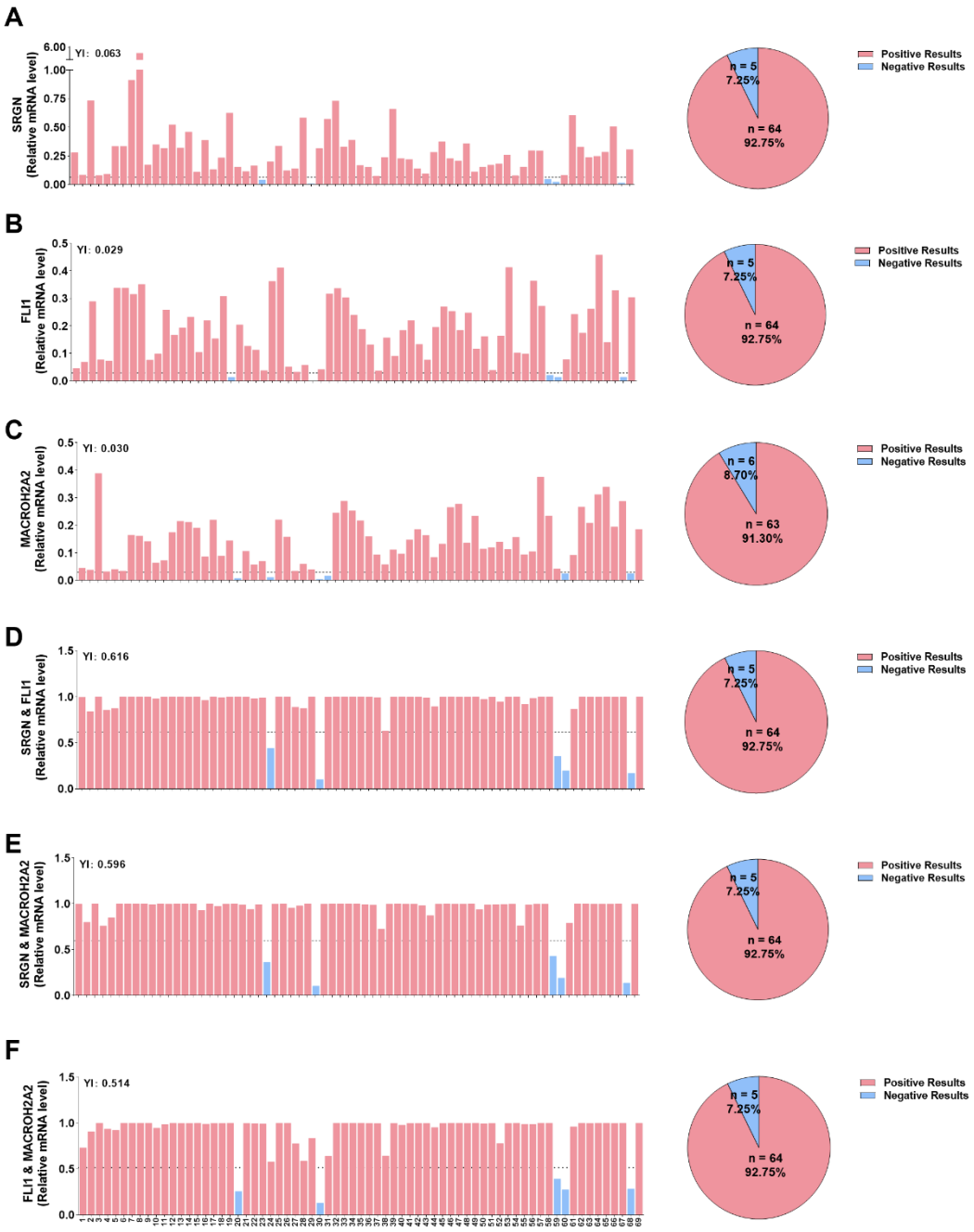

Figure S13. BC subjects were divided into two subgroups of high and low mRNA expression level by the weighted sum of biomarkers. (**A**) SRGN, (**B**) FLI1, (**C**) MACROH2A2, (**D**) SRGN & FLI1, (**E**) SRGN & MACROH2A2, and (**F**) FLI1 & MACROH2A2. Cut-off values were determined using YI.

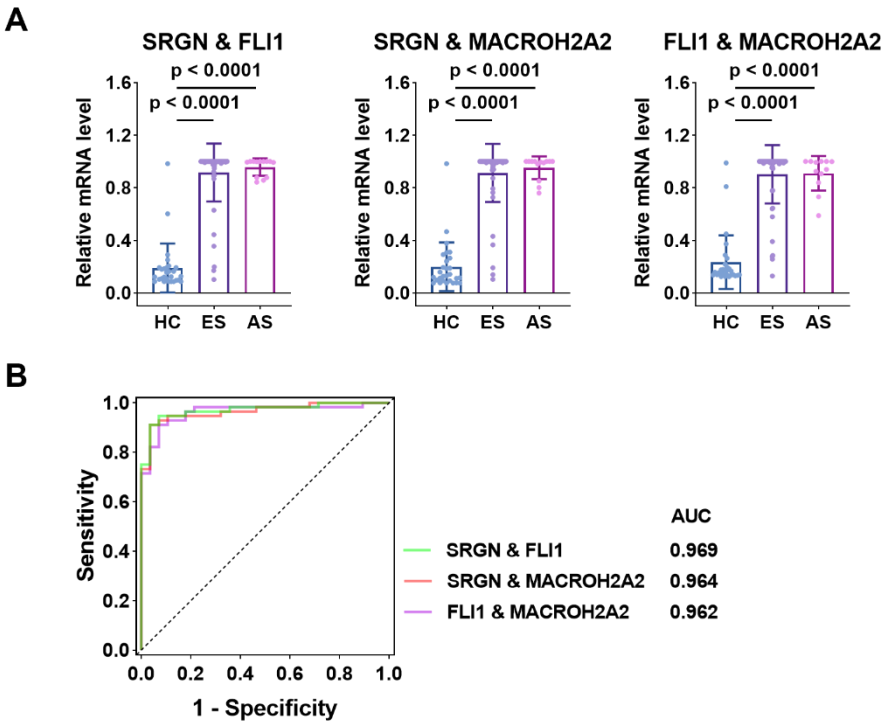

Figure S14. Combining two biomarkers to distinguish HC and early-stage BC. (**A**) Scattering plots of the mRNA expression levels of the weighted sum of the biomarkers two by two, SRGN and FLI1, SRGN and MACROH2A2, and FLI1 and MACROH2A2, respectively, in uEVs collected from HC, early-stage BC patients, and advanced-stage BC patients. (**B**) ROC curves of the weighted sum of the biomarkers two by two for early-stage BC diagnosis. The weighted sum of the three markers was calculated by logistic regression algorithm.

Table S1. The primer sequences used for RT-qPCR.

Genes	Forward Primer (5'-3')	Reverse Primer (5'-3')
FAM83A	GGCCCTAAGGGACTGGACT	CACAGTGGCGCTGGATTTTT
SRGN	GATCTGGGAGTGGCTTCCTAACG	CTGAGGGCAGATTCCTGTCAAGAG
FLI1	ATGTGAGGCAATGGCTGGAGTG	CTTCCGTGTTGTAGAGGGTGGTG
THBD	AGTGGGTTACGGGAGACAACAAC	CTCGCAGAGGAAGCCATCGG
PTPRN2	GAAGAGTGTGCTTGTCATCTGC	AATGCCAAACGTGCCTTGTG
ZNF883	TCAGCCGCAGCACACACC	TGGGTTCTCTGATGTCGGATTAGTG
MACROH2A2	GGGAGGCTGATGCGTTATCTGAAG	CCGCTGCCAGGTACTCAATGAC
IL1B	GGACAGGATATGGAGCAACAAGTGG	TCATCTTTCAACACGCAGGACAGG
GAPDH	AAGGAGTAAGACCCCTGGACC	ACTGTGAGGAGGGGAGATTCA

Table S2. Summary of the clinical cohorts.

ID	Sex	Age	Tumor stage	ID	Sex	Age	Tumor stage
1	male	88	T2	**36**	male	68	T1
2	male	84	T3	**37**	male	68	T1
3	female	83	T3	**38**	male	68	T1
4	male	82	T1	**39**	male	68	T1
5	female	82	T1	**40**	male	68	T1
6	female	79	T1	**41**	male	67	T3
7	male	78	Ta	**42**	male	67	Ta
8	male	78	T1	**43**	male	67	Ta
9	male	76	T3	**44**	male	67	Ta
10	female	76	Ta	**45**	male	66	Ta
11	female	75	T2	**46**	male	66	Ta
12	male	75	T1	**47**	male	66	Ta
13	male	74	T1	**48**	male	65	T3
14	male	74	T1	**49**	male	65	T3
15	male	74	Ta	**50**	male	65	T2
16	female	73	Ta	**51**	male	65	T2
17	male	73	T1	**52**	male	64	Ta
18	male	73	T1	**53**	male	64	T1
19	female	73	Ta	**54**	male	64	Ta
20	male	72	T1	**55**	male	63	T1
21	male	72	T2	**56**	male	63	T1

22	male	72	T1	**57**	male	62	T1
23	male	72	Ta	**58**	female	60	Ta
24	male	72	T1	**59**	male	59	Ta
25	male	71	T1	**60**	male	59	T1
26	male	71	Ta	**61**	female	58	T1
27	female	70	T1	**62**	female	58	T1
28	female	70	Ta	**63**	male	55	T1
29	male	70	T1	**64**	male	52	T1
30	male	70	Ta	**65**	female	50	T1
31	male	70	T1	**66**	male	50	Ta
32	male	70	T1	**67**	male	43	Ta
33	female	69	T3	**68**	male	38	Ta
34	female	69	T3	**69**	female	29	Ta
35	male	69	Ta				
1	male	72	n/a	**15**	female	48	n/a
2	female	71	n/a	**16**	male	43	n/a
3	male	69	n/a	**17**	female	41	n/a
4	male	69	n/a	**18**	male	35	n/a
5	male	67	n/a	**19**	male	35	n/a
6	female	66	n/a	**20**	male	32	n/a
7	male	65	n/a	**21**	male	30	n/a
8	male	64	n/a	**22**	female	29	n/a
9	female	60	n/a	**23**	female	27	n/a
10	male	58	n/a	**24**	female	26	n/a
11	male	56	n/a	**25**	female	25	n/a
12	female	54	n/a	**26**	male	25	n/a
13	male	52	n/a	**27**	female	24	n/a
14	male	50	n/a	**28**	female	24	n/a

Table S3. ROC curve analysis results of mRNA combinations for BC diagnosis.

	AUC	95%CI	Sensibility	Specificity
SRGN	0.9695	0.9380-1.000	92.8	96.4
FLI1	0.9658	0.9308-1.000	92.8	92.9
MACROH2A2	0.9260	0.8736-0.9784	91.3	82.1
SRGN & FLI1	0.9731	0.9436-1.000	92.8	96.4
SRGN & MACROH2A2	0.9689	0.9374-1.000	92.8	96.4
FLI1 & MACROH2A2	0.9648	0.9287-1.000	92.8	92.9
SRGN & FLI1 & MACROH2A2	0.9731	0.9436-1.000	92.8	96.4

Table S4. ROC curve analysis results of mRNA combinations for early-stage BC diagnosis.

	AUC	95%CI	Sensibility	Specificity
SRGN	0.9649	0.9289-1.000	94.6	92.9
FLI1	0.9630	0.9248-1.000	91.1	92.9
MACROH2A2	0.9260	0.8702-0.9818	83.9	92.9
SRGN & FLI1	0.9694	0.9358-1.000	94.6	92.9
SRGN & MACROH2A2	0.9643	0.9281-1.000	91.1	96.4
FLI1 & MACROH2A2	0.9617	0.9219-1.000	91.1	92.9
SRGN & FLI1 & MACROH2A2	0.9694	0.9358-1.000	94.6	92.9

Table S5. PCR reaction system.

Reagent	50 mL Reaction system	Final concentration
2 × SGExcel FastSYBR Mixture	25 μL	1 ×
Forward Primer, 10μM	1 μL	0.2 μM
Reverse Primer, 10μM	1 μL	0.2 μM
cDNA	2 μL	-
RNase-Free ddH2O	21 μL	-

Table S6. PCR response procedures.

Procedure	Temperature / ºC	Time / s	Cycle number
Predenaturation	95	180	-
Denaturation	95	5	40
Annealing / Extension	60	20	
	95	10	
Melting analysis	65	60	-
	97	1	