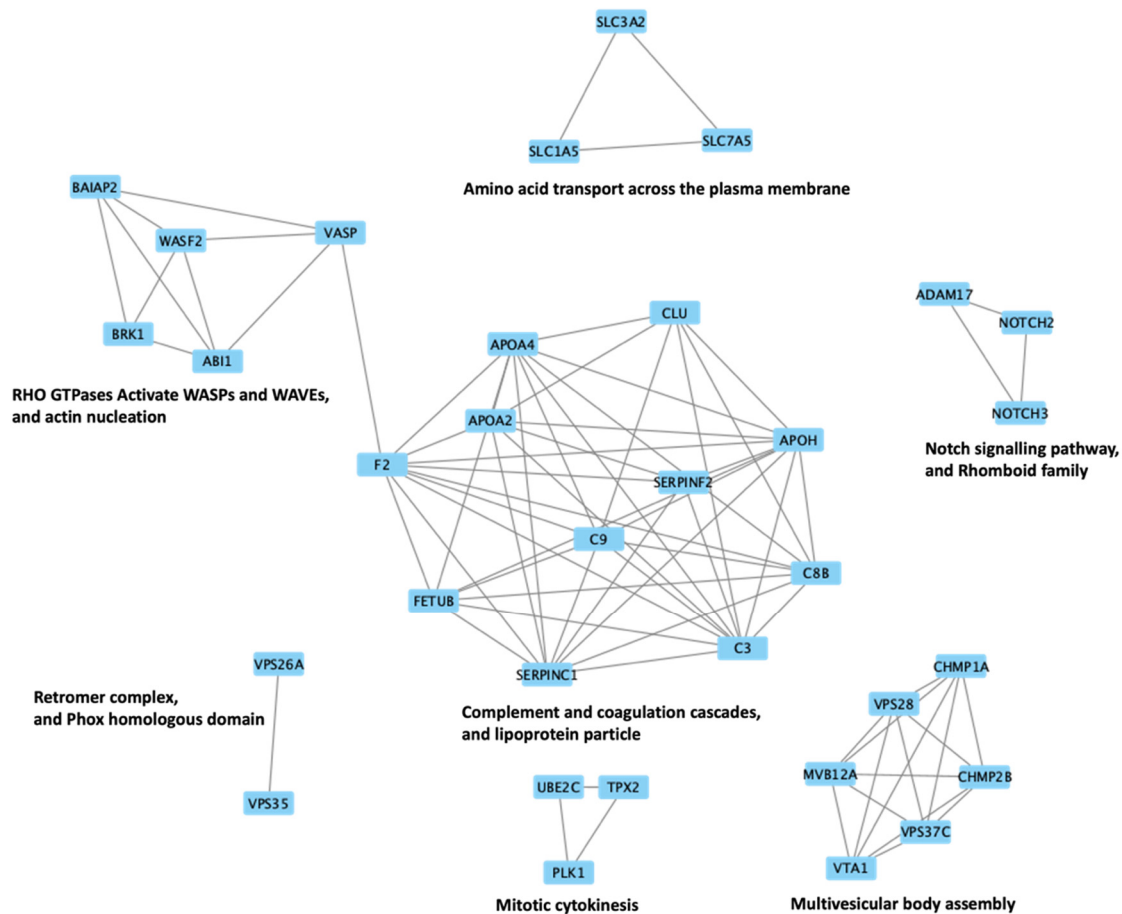
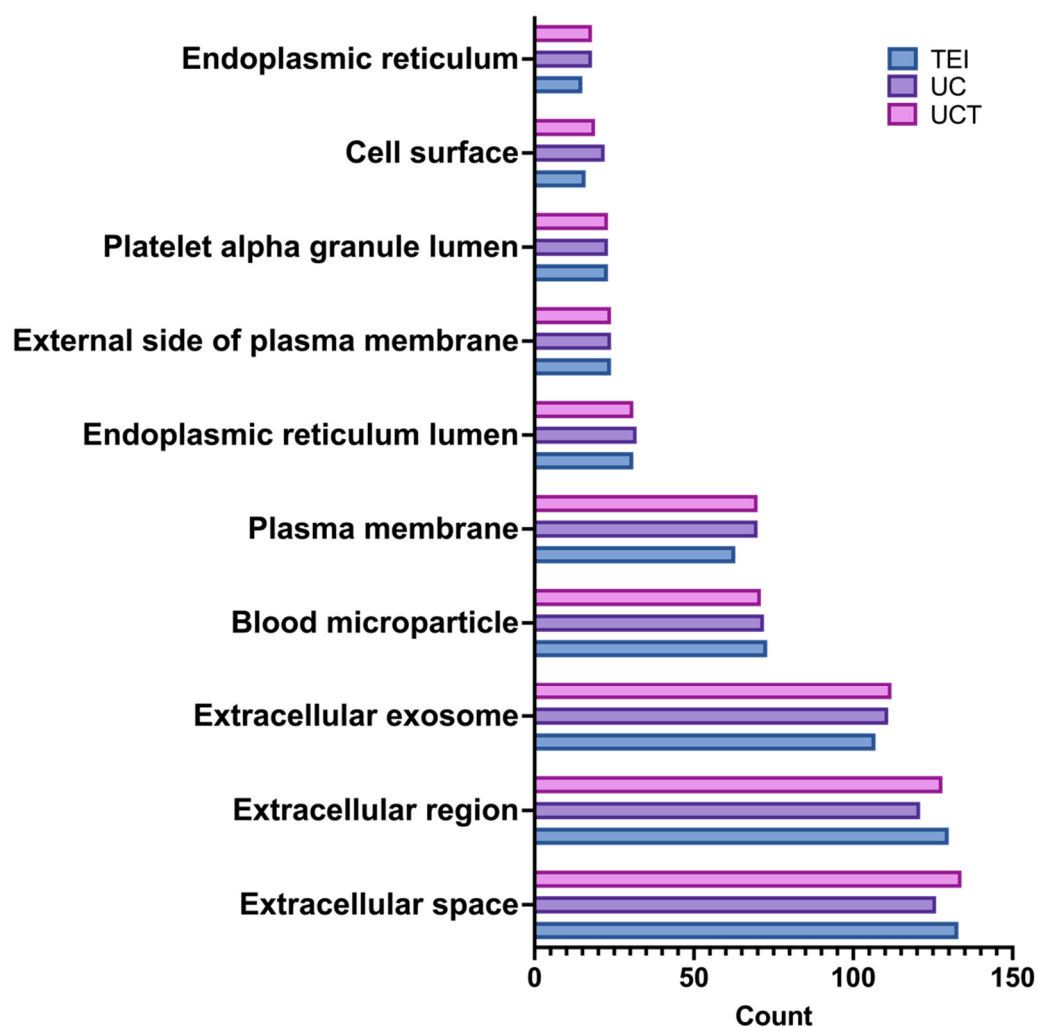


**Figure S1. Top 10 most abundant GO analysis term of identified proteins in MDA-MB-231 cell derived sEV isolates from three different isolation methods.** Go enrichment analysis of total identified protein isolated by TEI, UC, and UCT, isolation methods were carried out using DAVID. The graph shows 10 most abundant GO cellular component term and its count (P value<0.05, FDR<0.01). **Abbreviations:** TEI: total exosome isolation kit; UC: ultracentrifugation; UCT: ultracentrifugation followed by total exosome isolation kit.

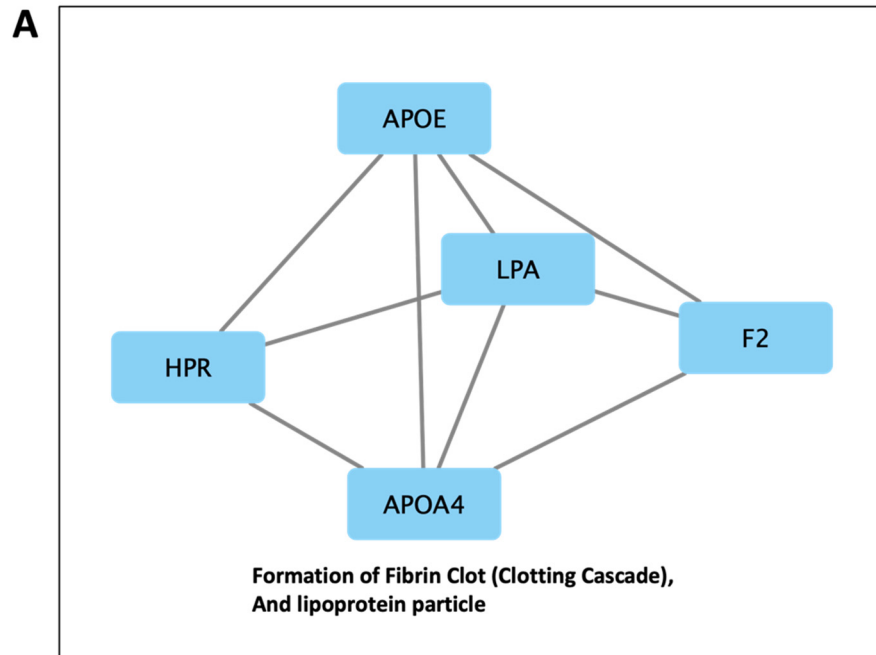


**Figure S2. STRING network analysis of upregulated and downregulated sEV proteins in BC cell lines by UC.** The figure shows the predicted sEV protein-protein interaction in BC cell lines and each cluster demonstrates its biological function (FDR=0.01).

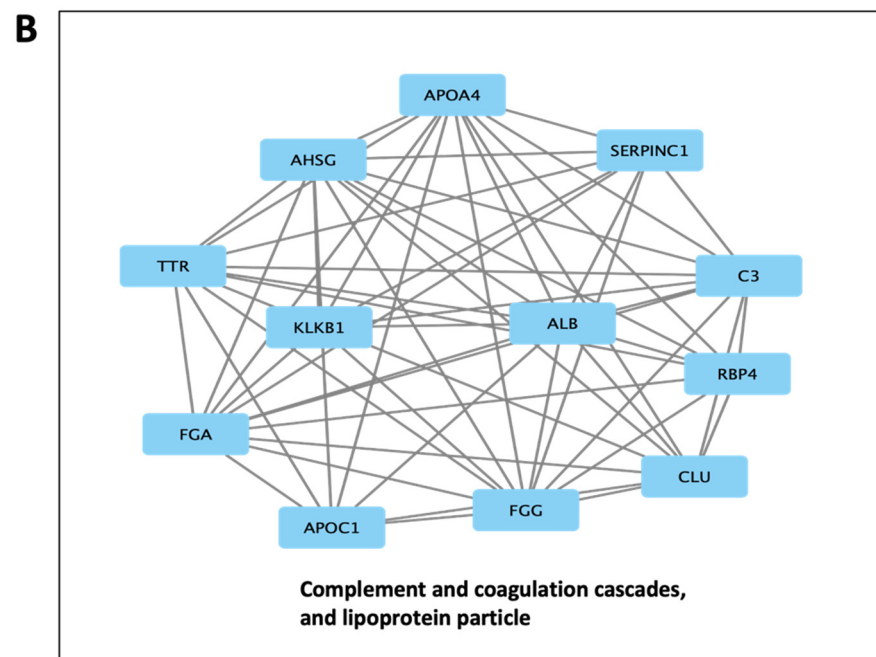


**Figure S3. Top 9 most abundant GO analysis term of identified proteins in BC plasma derived sEV isolates from three different isolation methods.** Go enrichment analysis of total identified protein isolated by UC, UCT, and TEI isolation methods were carried out using David. The graph shows 9 most abundant GO cellular component term and its count ( $P < 0.05$ ,  $FDR < 0.01$ ). **Abbreviations:** TEI: total exosome isolation kit; UC: ultracentrifugation; UCT: ultracentrifugation followed by total exosome isolation kit.





**UC**



**UCT**

**Figure S5. STRING network analysis of upregulated and downregulated sEV proteins in BC plasma isolated by UC and UCT.** The figures represent the predicted sEV protein-protein interaction in BC plasma and each cluster demonstrates its biological function (FDR=0.01).

**Table S1. Top 10 significant GO terms for cellular component of identified proteins from MDA-MB-231 cell line**

TEI				
Cellular component	Count	Percentage of genes (%)	<i>P</i> value	FDR
Cytosol	465	54.4	1.60E-69	3.60E-67
Extracellular exosome	433	50.7	4.60E-200	3.10E-197
Cytoplasm	383	44.8	5.30E-30	4.40E-28
Plasma membrane	318	37.2	2.80E-14	8.80E-13
Membrane	317	37.1	1.10E-41	1.50E-39
Nucleus	312	36.5	5.40E-07	7.00E-06
Nucleoplasm	232	27.2	3.70E-09	7.50E-08
Extracellular region	222	26	4.40E-41	4.90E-39
Extracellular space	182	21.3	7.00E-27	5.20E-25
Focal adhesion	129	15.1	2.50E-75	8.20E-73
UC				
Cellular component	Count	Percentage of genes (%)	<i>P</i> value	FDR
Cytosol	1305	51.7	9.80E-185	4.70E-182
Cytoplasm	1094	43.3	6.90E-80	1.30E-77
Extracellular exosome	973	38.5	0.00E+00	0.00E+00
Nucleus	931	36.9	1.20E-21	5.00E-20
Plasma membrane	860	34.1	1.40E-24	6.90E-23
Membrane	849	33.6	1.60E-92	3.70E-90
Nucleoplasm	754	29.9	2.30E-45	3.10E-43

Extracellular region	455	18	3.60E-38	3.80E-36
Extracellular space	372	14.7	4.80E-21	1.80E-19
Focal adhesion	253	10	7.10E-122	2.20E-119
UCT				
Cellular component	Count	Percentage of genes (%)	<i>P</i> value	FDR
Cytosol	498	55.1	2.30E-76	5.30E-74
Extracellular exosome	456	50.5	1.30E-209	9.10E-207
Cytoplasm	432	47.8	1.30E-41	1.50E-39
Membrane	353	39.1	4.20E-52	7.10E-50
Nucleus	337	37.3	2.40E-08	3.80E-07
Plasma membrane	326	36.1	2.20E-12	5.90E-11
nucleoplasm	248	27.5	5.00E-10	8.50E-09
Extracellular region	219	24.3	2.50E-35	2.20E-33
Extracellular space	182	20.2	9.60E-24	5.50E-22
Focal adhesion	150	16.6	5.40E-95	1.90E-92

**Table S2. Lipoprotein related cellular component in MDA-MB-231 cell line derived sEV proteins**

Isolation method	Cellular component related to Lipoproteins	Count	Percentage of genes (%)	<i>P</i> value	FDR
TEI	Chylomicron	7	0.8	1.10E-05	1.10E-04
	Very-low-density lipoprotein	7	0.8	1.50E-04	1.30E-03

	Spherical high-density lipoprotein particle	4	0.5	3.30E-03	1.90E-02
	High-density lipoprotein particle	5	0.6	2.30E-02	9.00E-02
<i>UC</i>	Very-low-density lipoprotein	13	0.5	1.00E-06	9.80E-06
	Chylomicron	10	0.4	6.30E-06	5.30E-05
	High-density lipoprotein particle	12	0.5	1.60E-04	1.00E-03
	Intermediate-density lipoprotein particle	5	0.2	9.70E-04	4.90E-03
	Spherical high-density lipoprotein particle	6	0.2	1.10E-03	5.20E-03
	Low-density lipoprotein particle	6	0.2	1.40E-02	5.00E-02
<i>UCT</i>	Chylomicron	7	0.8	1.50E-05	1.40E-04
	Very-low-density lipoprotein particle	7	0.8	2.10E-04	1.60E-03
	High-density lipoprotein particle	6	0.7	5.70E-03	2.90E-02
	Low-density lipoprotein particle	4	0.4	1.70E-02	7.10E-02



**Table S3. The most unique expressed sEV proteins in BC cell lines compared to normal breast cell.**

		MDA-MB-231/ Normal breast		MCF-7/ Normal breast		SK-BR-3/ Normal breast	
		Abundance Ratio (log2)	P value	Abundance Ratio (log2)	P value	Abundance Ratio (log2)	P value
CEBPZ	Up	9.77	<0.000001	9.97	6.03E-08	9.46	<0.000001
CHMP1A	Up	3.49	0.000565	4.49	2.23E-05	2.73	0.001111
CLTA	Up	7	0.000264	6.99	0.00024545	3.7	0.007280
IFITM1	Up	9.97	0.000020	9.97	2.37E-06	9.97	0.000016
SEC13	Up	7.69	0.000006	8.05	4.87E-06	7.4	0.000013
VTA1	Up	7.87	0.000603	7.96	0.00052427	7.89	0.000703
BDH2	Down	-8.11	0.000717	-8.35	0.00029472	-8.28	0.000312
INS	Down	-9.97	<0.000001	-9.97	4.6098E-07	-9.97	<0.000001
LAMA3	Down	-8.04	0.000318	-9.41	0.00012396	-9.39	0.000095

TPX2	Down	-8.03	0.008227	-9.97	0.0004124 4	-8.54	0.001329
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**Table S4. Top 9 significant GO terms for cellular component of identified proteins from BC patients' plasma.**

TEI				
Cellular component	Count	Percentage of genes (%)	P value	FDR
Extracellular space	133	81.1	1.20E-105	1.10E-103
Extracellular region	130	79.3	4.90E-96	3.00E-94
Extracellular exosome	107	65.2	2.50E-62	1.20E-60
Blood microparticle	73	44.5	9.40E-118	1.70E-115
Plasma membrane	63	38.4	4.30E-04	2.40E-03
Endoplasmic reticulum lumen	31	18.9	2.90E-24	8.90E-23
External side of plasma membrane	24	14.6	4.80E-12	6.80E-11
Platelet alpha granule lumen	23	14	2.70E-30	1.00E-28
Cell surface	16	9.8	1.70E-04	1.10E-03
Endoplasmic reticulum	15	9.1	7.00E-02	2.10E-01
UC				
Cellular component	Count	Percentage of genes (%)	P value	FDR
Extracellular space	126	71.6	4.00E-88	4.60E-86
Extracellular region	121	68.8	6.70E-77	5.10E-75
Extracellular exosome	111	63.1	2.40E-62	1.30E-60
Plasma membrane	72	40.9	1.50E-05	1.10E-04

Blood microparticle	70	39.8	4.30E-108	9.70E-106
Endoplasmic reticulum Lumen	32	18.2	1.60E-24	6.10E-23
External side of plasma Membrane	24	13.6	2.10E-11	5.40E-10
Platelet alpha granule lumen	23	13.1	1.40E-29	6.30E-28
Cell surface	22	12.5	1.20E-07	1.40E-06
Endoplasmic reticulum	18	10.2	1.80E-02	7.40E-02
UCT				
Cellular component	Count	Percentage of genes (%)	P value	FDR
Extracellular space	134	75.7	3.40E-99	3.60E-97
Extracellular region	128	72.3	8.70E-86	6.10E-84
Extracellular exosome	112	63.3	3.90E-63	2.10E-61
Plasma membrane	71	40.1	3.70E-05	2.70E-04
Blood microparticle	70	39.5	7.00E-108	1.50E-105
Endoplasmic reticulum lumen	31	17.5	3.00E-23	1.10E-21
External side of plasma membrane	24	13.6	2.40E-11	5.60E-10
Platelet alpha granule lumen	23	13	1.60E-29	6.70E-28
Cell surface	19	10.7	9.40E-06	7.90E-05
Immunoglobulin complex	18	10.2	6.40E-18	1.90E-16

**Table S5. Lipoprotein related cellular component of sEV proteins derived from BC patients' plasma.**

Isolation method	Cellular component related to Lipoproteins	Count	Percentage of genes (%)	<i>P</i> value	FDR
TEI	Very-low-density lipoprotein particle	9	5.5	2.40E-12	4.10E-11
	High-density lipoprotein particle	9	5.5	2.50E-11	3.10E-10
	Spherical high-density lipoprotein particle	7	4.3	6.20E-12	8.20E-11
	Intermediate-density lipoprotein particle	3	1.8	6.10E-04	3.40E-03
	Low-density lipoprotein particle	3	1.8	4.60E-03	2.20E-02
	Discoidal high-density lipoprotein particle	2	1.2	1.60E-02	6.20E-02
	Chylomicron	7	4.3	6.40E-10	7.00E-09
UC	Very-low-density lipoprotein particle	8	4.5	3.00E-10	5.20E-09
	High-density lipoprotein particle	8	4.5	2.20E-09	3.10E-08
	Spherical high-density lipoprotein particle	6	3.4	2.30E-09	3.10E-08
	Intermediate-density lipoprotein particle	3	1.7	7.10E-04	4.10E-03
	Chylomicron	7	4	9.80E-10	1.50E-08

UCT	Very-low-density lipoprotein particle	8	4.5	3.10E-10	5.50E-09
	High-density lipoprotein particle	8	4.5	2.30E-09	2.90E-08
	Spherical high-density lipoprotein particle	6	3.4	2.40E-09	2.90E-08
	Intermediate-density lipoprotein particle	3	1.7	7.10E-04	4.10E-03
	Chylomicron	7	4	1.00E-09	1.40E-08

**Table S6. GO analysis of distinctively expressed sEV proteins in BC plasma samples using UCT**

CATEGORY	TERM	COUNT	%	PVALUE	GENES	FDR
KEGG_PATHWAY	Complement and coagulation cascades	4	57.1428571	4.41E-06	VTN, CFI, F13A1, C4BPA	5.74E-05
REACTOME_PATHWAY	Regulation of Complement cascade	3	42.8571429	1.79E-04	VTN, CFI, C4BPA	0.00423363
REACTOME_PATHWAY	Complement cascade	3	42.8571429	2.73E-04	VTN, CFI, C4BPA	0.00423363
REACTOME_PATHWAY	Immune System	5	71.4285714	0.0049698	VTN, CFI, F13A1, C4BPA, PRSS3	0.05135459
REACTOME_PATHWAY	Innate Immune System	4	57.1428571	0.00798631	VTN, CFI, C4BPA, PRSS3	0.06189393

**Table S7. GO analysis of distinctively expressed sEV proteins in BC plasma samples using UC.**

CATEGORY	TERM	COUNT	%	PVALUE	GENES	FDR
REACTOME_PATHWAY	Platelet degranulation	8	24.2424242	1.08E-08	TF, VWF, IGF2, FN1, FLNA, TAGLN2, A2M, THBS1	2.02E-06
REACTOME_PATHWAY	Response to elevated platelet cytosolic Ca2+	8	24.2424242	1.41E-08	TF, VWF, IGF2, FN1, FLNA, TAGLN2, A2M, THBS1	2.02E-06
REACTOME_PATHWAY	Hemostasis	11	33.3333333	4.83E-07	SPN, TF, VWF, IGF2, FN1, FLNA, TAGLN2, A2M, THBS1, KLKB1, PF4V1	4.62E-05
REACTOME_PATHWAY	Platelet activation, signalling and aggregation	8	24.2424242	1.42E-06	TF, VWF, IGF2, FN1, FLNA, TAGLN2, A2M, THBS1	1.02E-04
REACTOME_PATHWAY	Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	6	18.1818182	7.93E-06	C3, TF, IGF2, FN1, APOL1, KTN1	4.55E-04
KEGG_PATHWAY	Complement and	5	15.1515152	5.88E-05	C3, VWF, C9, A2M, KLKB1	0.00370256

	coagulation cascades					
REACTOME_PATH WAY	Formation of Fibrin Clot (Clotting Cascade)	4	12.12121 21	9.11E-05	VWF, A2M, KLKB1, PF4V1	0.003962 36
REACTOME_PATH WAY	Post- translational protein phosphorylati on	5	15.15151 52	9.66E-05	C3, TF, FN1, APOL1, KTN1	0.003962 36
KEGG_PATHWAY	Focal adhesion	6	18.18181 82	1.24E-04	COMP, VWF, FN1, ZYX, FLNA, THBS1	0.003907 62
REACTOME_PATH WAY	Extracellular matrix organization	6	18.18181 82	5.06E-04	COMP, VWF, FN1, A2M, THBS1, KLKB1	0.018157 43
REACTOME_PATH WAY	Integrin cell surface interactions	4	12.12121 21	9.19E-04	COMP, VWF, FN1, THBS1	0.029296 98
REACTOME_PATH WAY	Intrinsic Pathway of Fibrin Clot Formation	3	9.090909 09	0.001230 35	VWF, A2M, KLKB1	0.035310 94
KEGG_PATHWAY	ECM-receptor interaction	4	12.12121 21	0.001378 8	COMP, VWF, FN1, THBS1	0.028954 86
REACTOME_PATH WAY	Complement cascade	3	9.090909 09	0.007655 19	C3, FCN3, C9	0.199730 96
KEGG_PATHWAY	signalling pathway	5	15.15151 52	0.011381 74	COMP, VWF, IGF2, FN1, THBS1	0.179262 33
KEGG_PATHWAY	Proteoglycans in cancer	4	12.12121 21	0.014668 86	IGF2, FN1, FLNA, THBS1	0.184827 67

REACTOME_PATH WAY	GP1b-IX-V activation signalling	2	6.060606 06	0.027079 04	VWF, FLNA	0.551493 47
REACTOME_PATH WAY	Scavenging of heme from plasma	2	6.060606 06	0.029303 57	HP, APOL1	0.551493 47
REACTOME_PATH WAY	GRB2:SOS provides linkage to MAPK signalling for Integrins	2	6.060606 06	0.033737 99	VWF, FN1	0.551493 47
REACTOME_PATH WAY	p130Cas linkage to MAPK signalling for integrins	2	6.060606 06	0.033737 99	VWF, FN1	0.551493 47
REACTOME_PATH WAY	Diseases of hemostasis	2	6.060606 06	0.035947 9	VWF, KLKB1	0.551493 47
REACTOME_PATH WAY	Defects of contact activation system (CAS) and kallikrein/kinin system (KKS)	2	6.060606 06	0.035947 9	VWF, KLKB1	0.551493 47
REACTOME_PATH WAY	Post- translational protein modification	8	24.24242 42	0.037937 85	C3, TF, PSMA1, DCTN2, FN1, APOL1, THBS1, KTN1	0.551493 47
REACTOME_PATH WAY	Cell surface interactions at the vascular wall	3	9.090909 09	0.038665 2	SPN, FN1, PF4V1	0.551493 47



REACTOME_PATHWAY	Degradation of the extracellular matrix	3	9.09090909	0.04021658	FN1, A2M, KLKB1	0.55149347
REACTOME_PATHWAY	Cell-extracellular matrix interactions	2	6.06060606	0.04035318	FLNA, LIMS1	0.55149347
KEGG_PATHWAY	hsa05165:Human papillomavirus infection	4	12.1212121	0.05051978	COMP, VWF, FN1, THBS1	0.51205955
REACTOME_PATHWAY	Initial triggering of complement	2	6.06060606	0.05128199	C3, FCN3	0.66899686
KEGG_PATHWAY	Phagosome	3	9.09090909	0.05689551	C3, COMP, THBS1	0.51205955
REACTOME_PATHWAY	Syndecan interactions	2	6.06060606	0.05993891	FN1, THBS1	0.71676945
REACTOME_PATHWAY	Integrin signalling	2	6.06060606	0.05993891	VWF, FN1	0.71676945
REACTOME_PATHWAY	Metabolism of proteins	9	27.2727273	0.06580889	C3, TF, PSMA1, DCTN2, IGF2, FN1, APOL1, THBS1, KTN1	0.75548611
REACTOME_PATHWAY	Immune System	9	27.2727273	0.07515349	C3, PI3R, FCN3, PSMA1, DCTN2, C9, HP, FN1, FLNA	0.79195136
REACTOME_PATHWAY	Signalling by high-kinase activity BRAF mutants	2	6.06060606	0.07914058	VWF, FN1	0.79195136

REACTOME_PATH WAY	Platelet Aggregation (Plug Formation)	2	6.060606 06	0.085457 04	VWF, FN1	0.791951 36
REACTOME_PATH WAY	MAP2K and MAPK activation	2	6.060606 06	0.087553 27	VWF, FN1	0.791951 36
REACTOME_PATH WAY	~Innate Immune System	6	18.18181 82	0.090363 5	C3, PIGR, FCN3, PSMA1, C9, HP	0.791951 36
REACTOME_PATH WAY	Binding and Uptake of Ligands by Scavenger Receptors	2	6.060606 06	0.091731 9	HP, APOL1	0.791951 36
REACTOME_PATH WAY	Signalling by RAF1 mutants	2	6.060606 06	0.093814 32	VWF, FN1	0.791951 36

**Table S8. Potential sEV protein biomarker isolated by UC method.**

POTENTIAL SEV BIOMARKERS	EXPRESSION IN BC PLASMA	ABUNDANCE RATIO (LOG2): BC/NON-CANCER	P VALUE
<b>KRT10</b>	Upregulation	1.44	0.046483305
<b>STOM</b>	Upregulation	2.22	0.010063307
<b>F2</b>	Upregulation	2.16	0.012802556
<b>F5</b>	Upregulation	2.11	0.027789899
<b>TUBB1</b>	Upregulation	2.49	0.041497799
<b>HPR</b>	Upregulation	3.87	0.034695762

<b>ITIH1</b>	Upregulation	2.83	0.033002719
<b>GP1BB</b>	Upregulation	1.12	0.021107319
<b>IGHA1</b>	Upregulation	4.65	0.000467739
<b>IGHG4</b>	Upregulation	2.19	0.001960026
<b>LPA</b>	Upregulation	3	0.029432023
<b>APOA4</b>	Downregulation	-3.31	0.004124821
<b>APOE</b>	Downregulation	-1.97	0.005521731
<b>CSN1S1</b>	Downregulation	-0.9	0.005749587

**Table S9. Potential sEV protein biomarker isolated by UCT method.**

<b>POTENTIAL SEV BIOMARKERS</b>	<b>EXPRESSION IN BC PLASMA</b>	<b>ABUNDANCE RATIO (LOG2): BC/NON-CANCER</b>	<b>P VALUE</b>
<b>AHSG</b>	Upregulation	2.34	0.030585
<b>ALB</b>	Upregulation	3.25	0.022753
<b>COL1A1</b>	Upregulation	2.97	0.030104
<b>CSN1S1</b>	Upregulation	2.55	0.031649
		3.06	0.02753
		2.78	0.03789
		3.62	0.033101
<b>EEF1A2</b>	Upregulation	5.32	0.013567
<b>KRT9</b>	Upregulation	1.75	0.031303

<b>RPL3</b>	Upregulation	2.54	0.019181
<b>APOA4</b>	Downregulation	-2.37	0.01328465
<b>APOC1</b>	Downregulation	-2.7	0.04784891
<b>C1S</b>	Downregulation	-4.54	0.00111333
<b>C3</b>	Downregulation	-2.45	0.01899849
<b>C5</b>	Downregulation	-4.47	0.04643546
<b>C7</b>	Downregulation	-2.88	0.00067045
<b>C8G</b>	Downregulation	-1.55	0.00031382
<b>CFHR2</b>	Downregulation	-3.48	0.03053928
<b>CLU</b>	Downregulation	-1.52	0.01986562
<b>FGA</b>	Downregulation	-1.5	0.03166746
<b>FGG</b>	Downregulation	-2.63	0.02426461
<b>ITIH1</b>	Downregulation	-1.98	0.00189802
<b>KLKB1</b>	Downregulation	-2.05	0.03297687
<b>RBP4</b>	Downregulation	-0.94	0.04068649
<b>SERPINC1</b>	Downregulation	-1.82	0.01121957
<b>TTR</b>	Downregulation	-1.37	0.03719059