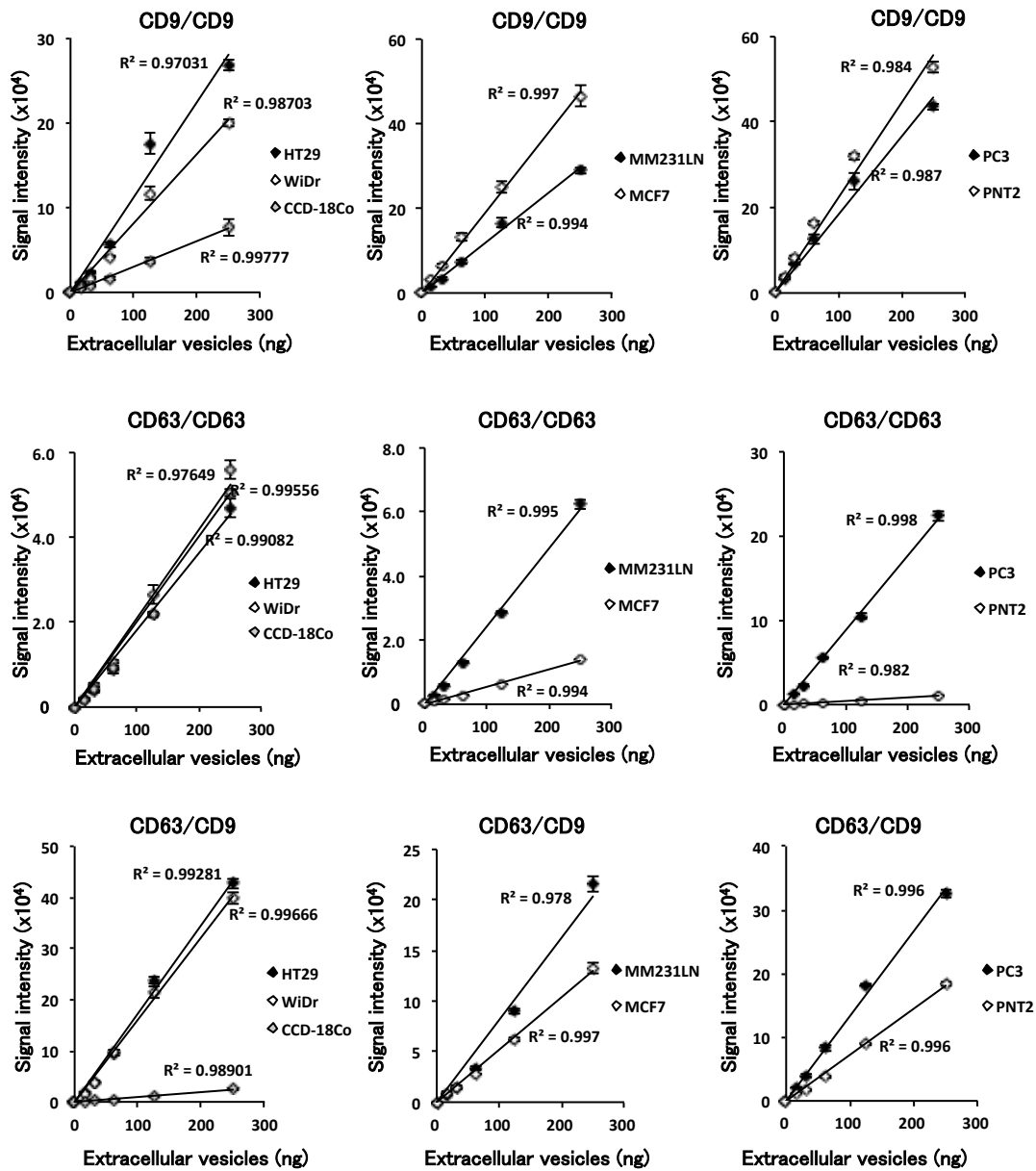
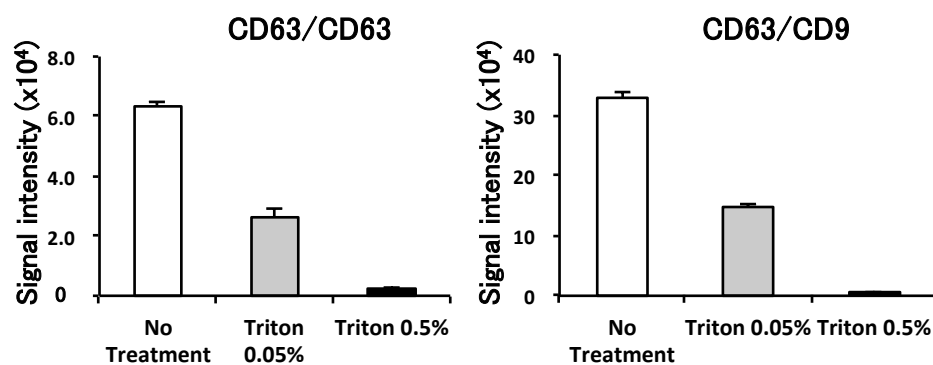


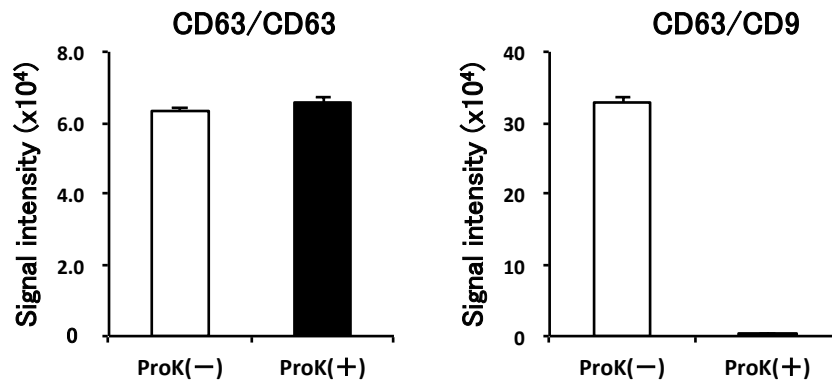
SUPPLEMENTARY INFORMATION



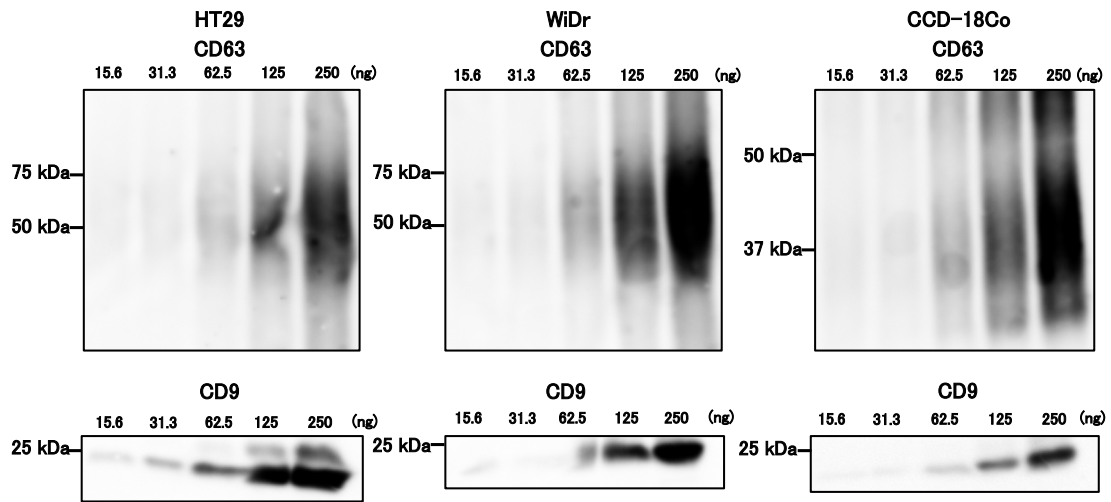
Supplementary Fig. 1. Correlation between ExoScreen measurements for CD9-positive, CD63-positive, or CD63/CD9 double-positive EVs and EV protein concentration in a dilution series. EV protein concentration was measured via the Qubit system, and EVs were purified from conditioned medium of the colorectal cancer cell lines HT29 and WiDr and normal colon fibroblast cell line CCD-18Co, PC3 prostate cancer cell line, PNT2 prostate epithelial cell line, and the breast cancer cell lines MDA-MB-231LN and MCF7. Error bars are s.e.m. (n=3 for each condition). Data are representative of at least three independent experiments each.



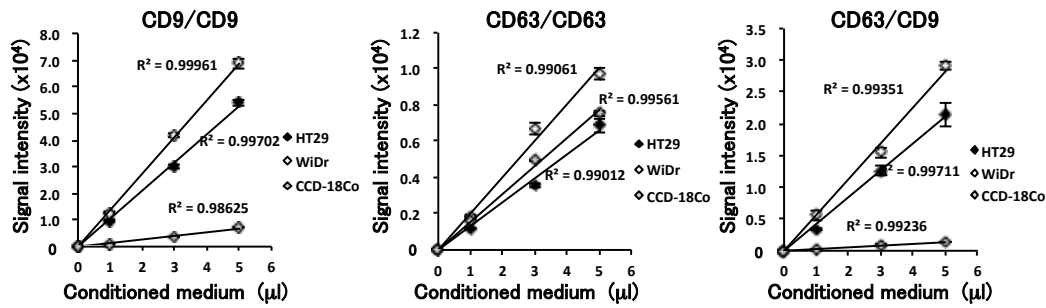
Supplementary Fig. 2. Evaluation of ExoScreen specificity against purified EVs from HCT116 cell treated with or without 0.05% and 0.5% Triton X-100. Two hundred fifty ng of EVs were detected by ExoScreen using CD63 antibodies (left panel) and, CD63 and CD9 antibodies (right panel). Error bars are s.e.m. (n=3 for each condition). Data are representative of at least three independent experiments each.



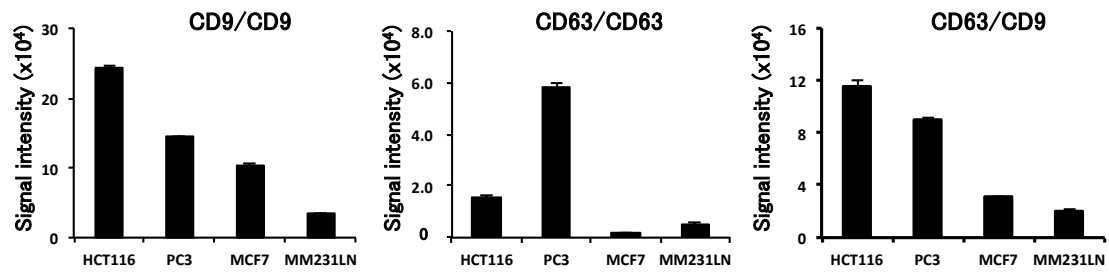
Supplementary Fig. 3. Evaluation of ExoScreen specificity against EVs from HCT116 cells treated with or without Proteinase K. Two hundred fifty ng of EVs were detected by ExoScreen using CD63 antibodies (left panel) and, CD63 and CD9 antibodies (right panel). Error bars are s.e.m. (n=3 for each condition). Data are representative of at least three independent experiments each.



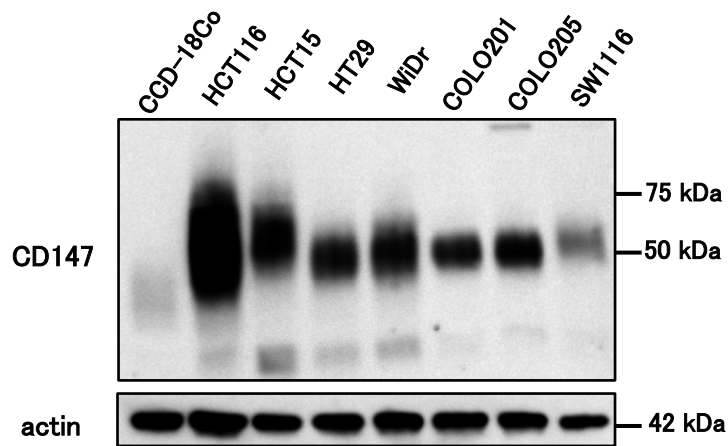
Supplementary Fig. 4. Immunoblotting analysis of CD63 (upper panels) or CD9 (lower panels) against the EVs isolated from HT29, WiDr and CCD-18Co cells. EV protein concentrations were measured via the Qubit system. EVs were purified from HT29, WiDr and CCD-18Co cell conditioned medium. Data are representative of at least three independent experiments each.



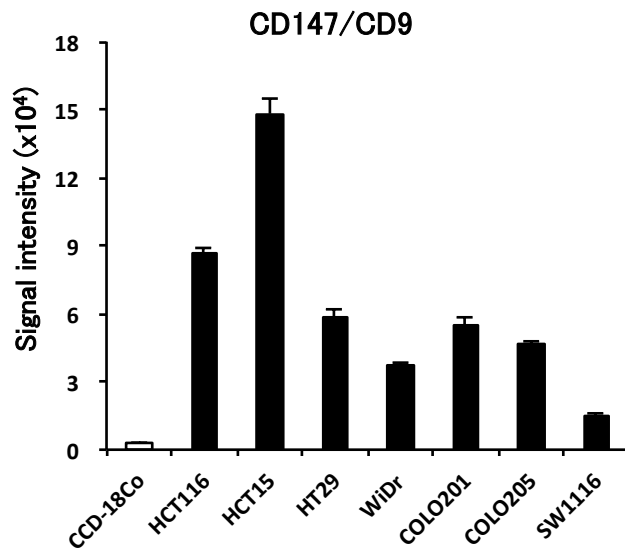
Supplementary Fig. 5. Correlation of ExoScreen measurements for CD9-positive, CD63-positive or CD63/CD9 double-positive EVs and HT29, WiDr and CCD-18Co conditioned medium in a dilution series. Conditioned medium was prepared in a 5 μl volume and diluted as indicated. EVs in conditioned medium were detected by ExoScreen using CD9 antibodies (left panel), CD63 antibodies (middle panel) and, CD63 and CD9 antibodies (right panel). Error bars are s.e.m. ($n=3$ for each condition). Data are representative of at least three independent experiments each.



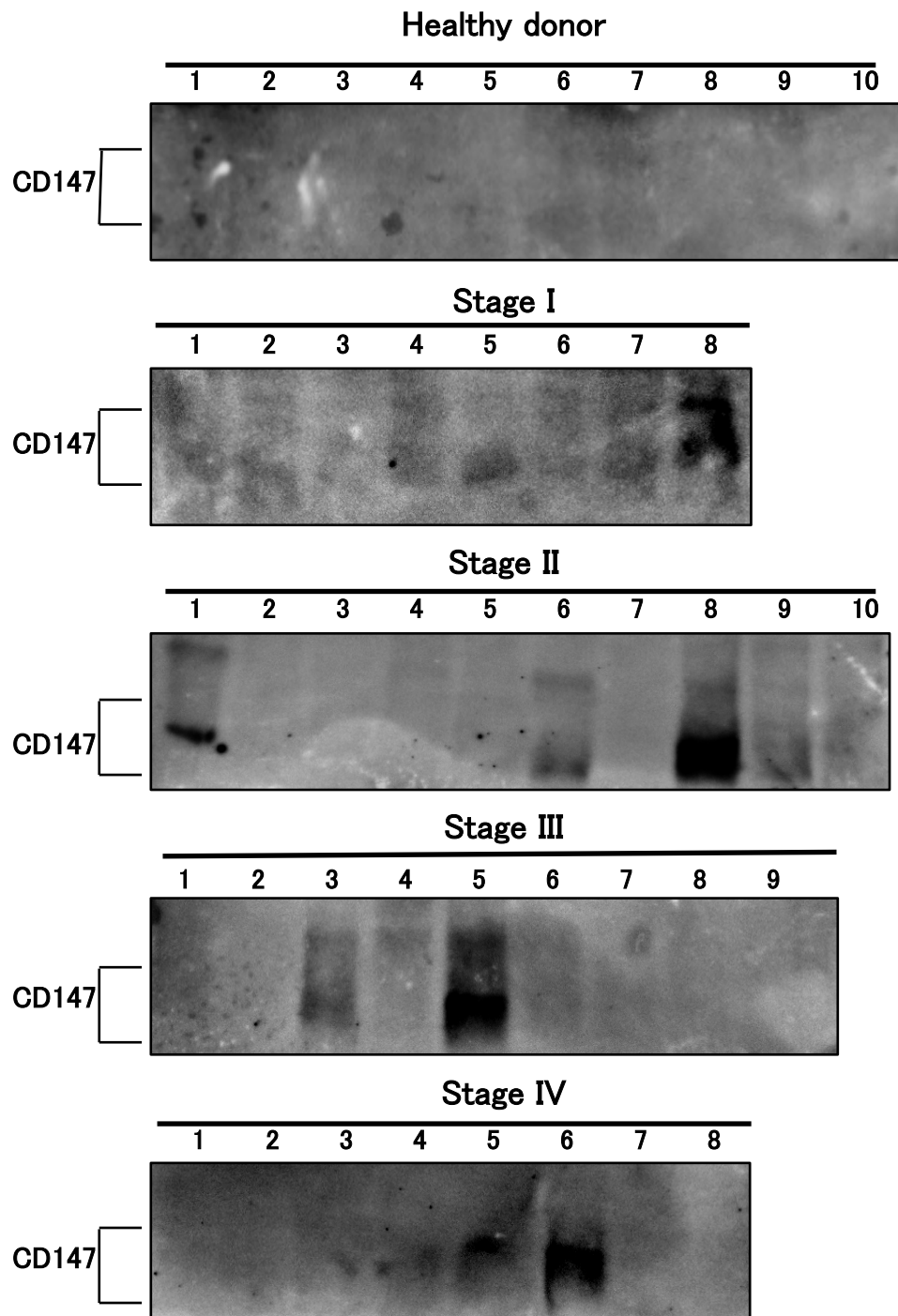
Supplementary Fig. 6. Detection of EVs in 5 μ l of conditioned medium from indicated cancer cell lines without purification of EVs. EVs in conditioned medium were detected by ExoScreen using CD9 antibodies (left panel), CD63 antibodies (middle panel) and, CD63 and CD9 antibodies (right panel). Error bars are s.e.m. ($n=3$ for each condition). Data are representative of at least three independent experiments each.



Supplementary Fig. 7. Immunoblotting analysis of CD147 or actin against cell lysates from CCD-18Co cells, HCT116 cells, HCT15 cells, HT29 cells, WiDr cells, COLO201 cells, COLO205 cells and SW1116 cells. Cell lysate (1 μ g per lane) was used for the detection of actin. CD147 was detected using 5 μ g of whole cell lysates. Data are representative of at least three independent experiments each.

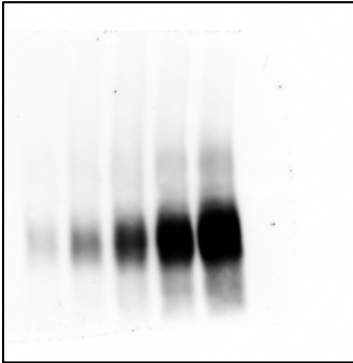


Supplementary Fig. 8. Purified EVs from indicated cell lines was used for the detection of CD147/CD9 double-positive EVs. EVs (62.5 ng) were detected by ExoScreen using CD147 and CD9 antibodies. Error bars are s.e.m. (n=3 for each condition). Data are representative of at least three independent experiments each.

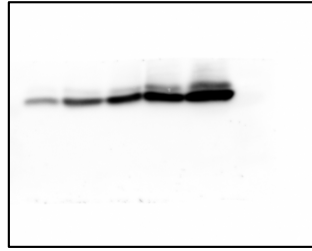


Supplementary Fig. 9. Immunoblotting analysis for CD147 with EVs isolated from colorectal cancer patient serum and healthy control serum. Purified EVs from serum samples that are identical to those in Fig. 6c were employed. Data are representative of at least three independent experiments each.

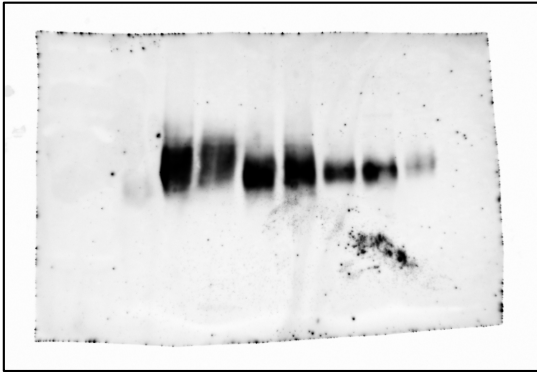
(a)



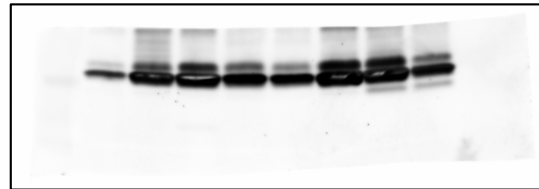
(b)



(c)



(d)



Supplementary Fig. 10. Uncropped scans of blots shown in figures 3a and 5a. (a) Fig.3a, CD63, (b) Fig.3a, CD9, (c) Fig. 5a, CD147, (d) Fig.5a, CD9.

Supplementary Table 1. Membrane proteins selected by proteomic analysis of extracellular vesicles from HCT116 cells and CCD-18Co cells

HCT116 cells		CCD-18Co cells	
1	Annexin A5	1	Lactadherin
2	Prostaglandin F2 receptor negative regulator	2	Annexin A5
3	Integrin beta-4	3	Erythrocyte band 7 integral membrane protein
4	Moesin	4	CD44 antigen
5	Neutral amino acid transporter B(0)	5	Transmembrane protein 176B
6	Heat shock cognate 71 kDa protein	6	CD81 antigen
7	Sodium/potassium-transporting ATPase subunit alpha-1	7	CD9 antigen
8	Epithelial cell adhesion molecule	8	CD63 antigen
9	CD9 antigen	9	Thy-1 membrane glycoprotein
10	4F2 cell-surface antigen heavy chain	10	HLA class I histocompatibility antigen, A-33 alpha chain
11	Sodium-coupled neutral amino acid transporter 2	11	Heat shock cognate 71 kDa protein
12	Integrin beta-1	12	Vesicle-associated membrane protein 3
13	Lactadherin	13	Mas-related G-protein coupled receptor member F
14	CUB domain-containing protein 1	14	Transmembrane protein 176A
15	Integrin alpha-3	15	Integrin alpha-1
16	Heat shock protein HSP 90-beta	16	4F2 cell-surface antigen heavy chain
17	Podocalyxin	17	CD59 glycoprotein
18	Monocarboxylate transporter 4	18	Protein eva-1 homolog B
19	Ephrin type-A receptor 2	19	Neutral amino acid transporter B(0)
20	CD63 antigen	20	Phospholipid scramblase 3
21	CD44 antigen	21	Galectin-1
22	CD81 antigen	22	Lipid phosphate phosphohydrolase 3
23	CD151 antigen	23	Protein Wnt-7a
24	Basigin (CD147)	24	Amyloid beta A4 protein
25	Tetraspanin-6	25	Uncharacterized protein C17orf80
26	Tetraspanin-14		
27	Choline transporter-like protein 1		
28	Solute carrier family 2, facilitated glucose transporter member 1		
29	Disintegrin and metalloproteinase domain-containing protein 10		
30	Equilibrative nucleoside transporter 1		
31	Transferrin receptor protein 1		
32	Lysosome-associated membrane glycoprotein 2		
33	Heat shock protein beta-1		
34	Myoferlin		
35	Erythrocyte band 7 integral membrane protein		
36	Leucine-rich repeat-containing protein 8B		
37	Prominin-1		
38	Synaptic vesicle membrane protein VAT-1 homolog		
39	Plexin-B2		
40	Junctional adhesion molecule A		
41	Synaptotagmin-1		
42	Protein crumbs homolog 1		
43	Integrin alpha-2		
44	p53 apoptosis effector related to PMP-22		

CD147 is highlighted in yellow and CD9, CD63 and CD81 which are frequently used as EV marker are highlighted in aqua.

After proteomic analysis of EVs from HCT116 cells and CCD-18Co cells, membrane proteins were selected by the descriptions of Gene Ontology which are “integral to plasma membrane”, “integral to membrane”, “external side of plasma membrane” and “cell surface”. The proteins are listed in the order of protein amount in EVs.

Supplementary Table 2. CEA and CA19-9 in preoperative patient serum (stage 0 and I)

Patient #	signal intensity of CD147/CD9	CEA (ng ml ⁻¹)	CA19-9 (U ml ⁻¹)
1	5467	1	6
2	5798	5	6
3	6487	3	5
4	7799	1	13
5	7854	2	13
6	8854	4	26
7	10935	1	25
8	11430	2	21
9	13604	3	6
10	17502	2	18
11	18502	1	13
12	19672	1	11
13	30618	2	5
14	32269	1	5
15	33081	3	9
16	35710	1	26
17	119708	3	12

CEA: Carcinoembryonic Antigen, normal value range is 0–5 ng ml⁻¹.

CA19-9: Carbohydrate Antigen 19-9, normal value range is lower than 37 U ml⁻¹.

An average of the signal intensity of CD147/CD9 in healthy donor serum is 1847.

An average of the signal intensity of CD147/CD9 in cancer patient serum is 6038.

Patients #3, #12 and #17 are stage 0. The other are stage I.

Supplementary Table 3. CEA and CA19-9 in healthy donor serum

Donor #	signal intensity of CD147/CD9	CEA (ng ml ⁻¹)	CA19-9 (U ml ⁻¹)	Donor #	signal intensity of CD147/CD9	CEA (ng ml ⁻¹)	CA19-9 (U ml ⁻¹)	Donor #	signal intensity of CD147/CD9	CEA (ng ml ⁻¹)	CA19-9 (U ml ⁻¹)
1	484	2	10	65	1019	<0.5	<1.0	129	1533	3	11
2	495	<0.5	8	66	1020	<0.5	7	130	1538	<0.5	12
3	496	2	9	67	1021	<0.5	4	131	1556	6	21
4	525	<0.5	17	68	1022	3	12	132	1563	4	25
5	547	3	<1.0	69	1023	<0.5	12	133	1569	<0.5	5
6	549	3	10	70	1028	2	13	134	1583	5	23
7	557	2	<1.0	71	1031	14	25	135	1583	<0.5	4
8	570	3	25	72	1036	3	<1.0	136	1586	2	5
9	573	<0.5	<1.0	73	1039	2	6	137	1593	4	17
10	583	<0.5	6	74	1053	5	9	138	1612	2	<1.0
11	595	5	18	75	1055	2	17	139	1614	2	11
12	607	4	12	76	1057	3	12	140	1628	3	57
13	620	5	24	77	1058	8	21	141	1643	11	5
14	623	2	9	78	1073	<0.5	8	142	1653	2	7
15	635	2	14	79	1074	<0.5	22	143	1713	2	9
16	652	12	41	80	1076	5	55	144	1718	3	11
17	656	2	40	81	1084	3	69	145	1720	2	13
18	692	<0.5	<1.0	82	1085	5	6	146	1746	3	8
19	692	3	7	83	1092	5	11	147	1749	<0.5	8
20	721	8	<1.0	84	1096	8	<1.0	148	1754	<0.5	5
21	742	148	<1.0	85	1097	2	211	149	1758	2	7
22	749	2	8	86	1116	7	<1.0	150	1766	<0.5	4
23	757	<0.5	4	87	1118	2	4	151	1774	3	14
24	765	2	<1.0	88	1129	4	5	152	1776	2	8
25	773	<0.5	<1.0	89	1155	<0.5	11	153	1816	3	<1.0
26	773	<0.5	48	90	1178	2	<1.0	154	1816	5	32
27	774	3	9	91	1190	<0.5	5	155	1828	2	8
28	774	<0.5	<1.0	92	1199	3	5	156	1839	3	<1.0
29	778	2	14	93	1212	8	12	157	1846	4	9
30	785	3	40	94	1213	<0.5	5	158	1860	<0.5	9
31	785	2	<1.0	95	1230	<0.5	10	159	1867	2	<1.0
32	793	5	9	96	1231	<0.5	6	160	1878	3	3
33	807	5	15	97	1235	<0.5	6	161	1897	2	9
34	809	3	13	98	1239	<0.5	52	162	1968	<0.5	6
35	811	<0.5	9	99	1252	5	21	163	2002	3	<1.0
36	814	3	<1.0	100	1263	3	<1.0	164	2003	3	22
37	828	<0.5	<1.0	101	1275	3	49	165	2036	<0.5	13
38	829	8	12	102	1295	2	35	166	2063	<0.5	6
39	833	5	17	103	1296	<0.5	16	167	2073	3	15
40	833	4	28	104	1306	3	26	168	2075	2	24
41	836	2	6	105	1311	<0.5	7	169	2147	2	23
42	851	3	24	106	1316	2	20	170	2193	4	16
43	872	2	<1.0	107	1352	<0.5	12	171	2215	2	16
44	881	3	15	108	1358	<0.5	7	172	2380	<0.5	27
45	887	7	33	109	1364	<0.5	5	173	2383	3	21
46	890	5	20	110	1366	3	<1.0	174	2399	<0.5	33
47	891	3	10	111	1371	4	7	175	2512	3	23
48	913	<0.5	<1.0	112	1397	3	10	176	2525	<0.5	5
49	932	7	29	113	1398	2	20	177	2563	<0.5	5
50	933	<0.5	5	114	1411	<0.5	5	178	2685	2	28
51	936	3	23	115	1427	<0.5	10	179	2783	6	3
52	943	5	28	116	1435	<0.5	11	180	2870	<0.5	15
53	953	2	<1.0	117	1437	2	15	181	2986	<0.5	5
54	967	<0.5	10	118	1451	2	13	182	3005	<0.5	29
55	970	5	53	119	1455	<0.5	5	183	3124	2	16
56	971	<0.5	8	120	1457	2	63	184	3367	<0.5	7
57	974	5	60	121	1459	<0.5	<1.0	185	4145	3	5
58	986	2	8	122	1462	4	39	186	4243	<0.5	6
59	994	4	9	123	1493	2	81	187	4309	<0.5	4
60	998	<0.5	7	124	1512	<0.5	9	188	4322	<0.5	6
61	999	<0.5	9	125	1512	17	13	189	4906	2	20
62	1005	3	6	126	1513	<0.5	7	190	11760	2	10
63	1008	5	68	127	1522	<0.5	12	191	76476	2	<1.0
64	1016	2	54	128	1530	2	12				

CEA: Carcinoembryonic Antigen, normal value range is 0–5 ng ml⁻¹.

CA19-9: Carbohydrate Antigen 19-9, normal value range is lower than 37 U ml⁻¹.

Exceeding the normal value range are presented in red text.