Supplemental Table 1 – List of proteins selectively enriched in exosomes (Exos) and shed microvesicles (sMVs).

	Category	Gene Name [D]	Protein Description[D]
Enriched proteins in Exos [A]	*ESCRTs*	TSG101*	Tumor susceptibility gene 101
		VPS37B	Vacuolar protein sorting-associated protein 37B
		CHMP2A	Charged multivesicular body protein 2a
	ESCRT accessory	PDCD6IP/Alix	Programmed cell death 6 interacting protein
	Tetraspanins	CD63*	CD63 antigen
		CD82	CD82 antigen
		CD151	CD151 antigen
		TSPAN3*	Tetraspanin-3
		TSPNA6*	Tetraspanin-6
	RNA binding proteins	RPL13	60S ribosomal protein L13
		RPL26	60S ribosomal protein L26
		RPS25	40S ribosomal protein S25
		RPS4Y1	40S ribosomal protein S4, Y isoform 1
		RPS4Y2	40S ribosomal protein S4, Y isoform 2
	Cargo selection	NEDD4	E3 ubiquitin-protein ligase NEDD4
		NEDD4L	E3 ubiquitin-protein ligase NEDD4-like
	Trafficking / sorting	SDCBP	Syndecan-binding protein 1
		ARRDC1	Arrestin domain-containing protein 1
		RAB1B	Ras-related protein Rab-1B
		RAB5A	Ras-related protein Rab-5A
		RAB5B	Ras-related protein Rab-5B
		RAB8A	Ras-related protein Rab-8A
		RAB11A	Ras-related protein Rab-11A
		RAB11B	Ras-related protein Rab-11B
	Integral membrane proteins	ITGAV	Integrin alpha-V
		ADAM10*	Disintegrin and metalloproteinase domain-containing protein 10
		IGSF8	Immunoglobulin superfamily member 8 (CD81 partner 3)
		EPHB3	Ephrin type-B receptor 3
	GPI-anchor	GPC1	Glypican-1
Enriched proteins in sMVs [B]	*RNA binding proteins*	HNRNPH1*	Heterogeneous nuclear ribonucleoprotein H
		HNRNPL*	heterogeneous nuclear ribonucleoprotein L
		PABPC1	Polyadenylate-binding protein 1
		CALR	Calreticulin
		CPNE3	CPNE3 protein
	ABC transporters	ABCE1	ATP-binding cassette sub-family E member 1
	Mitochondrial proteins	VDAC1*	voltage-dependent anion channel 1
		VDAC2*	Voltage-dependent anion-selective channel protein 2
		PHB2*	Prohibitin-2
		PDIA4*	Protein disulfide-isomerase A4
		ATP5O*	ATP synthase subunit O, mitochondrial
		SLC25A3*	Phosphate carrier protein, mitochondrial
	Integral membrane proteins	CKAP4	Cytoskeleton-associated protein 4
	Cytoskeleton / microtubule	MYO1D	Unconventional myosin-Id
		MYO6	Unconventional myosin-Id
		FLNB	Filamin-B
		RACGAP1*	Rac GTPase activating protein 1
		KIF23*	Kinesin-like protein KIF23
		TPM3	Tropomyosin alpha-3 chain
	Enzymes	AARS	Alanine--tRNA ligase, cytoplasmic
		ATIC	Bifunctional purine biosynthesis protein PURH
		ATP5A1	ATP synthase subunit alpha
		ERO1L	ERO1-like protein alpha
		GNB2	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2
	Chaperones	CSE1L	Exportin-2
Commonly	*ESCRTs*	VPS28	Vacuolar protein sorting-associated protein 28
	ESCRT accessory	VPS4B	Vacuolar protein sorting-associated protein 4B

identified proteins in Exos and sMVs [C]	*Tetraspanins*	CD9	CD9 antigen
		CD81	CD81 antigen
		TSPAN8	Tetraspanin-8
	RNA binding proteins	HNRNPC	Heterogeneous nuclear ribonucleoproteins C
		HNRNPE1	Heterogeneous nuclear ribonucleoprotein E1
		HNRNPE2	Heterogeneous nuclear ribonucleoprotein E2
		HNRNPD	Heterogeneous nuclear ribonucleoprotein D0
		HNRNPK	Heterogeneous nuclear ribonucleoprotein K
		HNRNPA2B1	Heterogeneous nuclear ribonucleoproteins A2/B1
		ANXA2	Annexin A2
		EEF2	Elongation factor 2
	ABC transporters	ABCB1	Multidrug resistance protein 1
		ABCG2	ATP-binding cassette sub-family G member 2
	Mitochondrial proteins	GLUD1	Glutamate dehydrogenase 1
	Trafficking / sorting	RAB21	Ras-related protein Rab-21
		RAB14	Ras-related protein Rab-14
		RAB15	Ras-related protein Rab-15
		RAB1A	Ras-related protein Rab-1A
		RAB35	Ras-related protein Rab-35
		RAB43	Ras-related protein Rab-43
		RAB13	Ras-related protein Rab-13
		RAB8B	Ras-related protein Rab-8B
		FLOT2	Flotillin-2
		CD44	CD44 antigen
	Integral membrane proteins	FASN	Fatty acid synthase
		EMMPRIN	Extracellular matrix metalloproteinase inducer
		ITGA2	Integrin alpha-2
		EPHB1	Ephrin type-B receptor 1
		EPHA2	Ephrin type-A receptor 2
		EPHB2	Ephrin type-B receptor 2
		EPHB4	Ephrin type-B receptor 4
		EPHA6	Ephrin type-A receptor 6
		EPHA7	Ephrin type-A receptor 7
	Cytoskeleton / microtubule	MYH9	Myosin-9
		MYH14	Myosin-14
		ACTB	Actin, cytoplasmic 1
		TUBB2C	Tubulin beta-2C chain
		TUBB	Tubulin, beta
		TUBA1B	Tubulin alpha-1B chain
		EZR	Ezrin
		IQGAP1	Ras GTPase-activating-like protein
	Heat shock proteins	HSP90AA1	Heat shock protein HSP 90-alpha
		HSP90AB1	Heat shock protein HSP 90-beta
		HSPA8	Heat shock 70 kDa protein 8
	Enzymes	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
		ACLY	ATP-citrate synthase
		ENO1	Alpha-enolase
	Chaperones	YWHAE	14-3-3 protein epsilon
		YWHAZ	14-3-3 protein zeta

A *Exos enriched: protein selection category based on references: [1-8]. Raw mass spectrometry data is deposited in the PeptideAtlas and can be accessed at http://www.peptideatlas.org/PASS/PASS00749*

B *sMV enriched: protein selection category based on references [1,6]. Raw mass spectrometry data is deposited in the PeptideAtlas and can be accessed at http://www.peptideatlas.org/PASS/PASS00749*

C *Co-Exos and sMV enriched: protein selection category based on references: [1-8]*

D *Identified gene and protein information can be obtained from http://www.ncbi.nlm.nih.gov/gene and www.uniprot.com*

* *Proteins that we consider specific markers for Exos and sMVs (based on unique/predominant expression in purified EV types)*

References

1. Xu R, Greening DW, Rai A*, et al.* Highly-purified exosomes and shed microvesicles isolated from the human colon cancer cell line LIM1863 by sequential centrifugal ultrafiltration are biochemically and functionally distinct. *Methods*. 87(11-21 (2015).
2. Tauro BJ, Greening DW, Mathias RA*, et al.* Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. *Methods*. 56(2), 293-304 (2012).
3. Ji H, Greening DW, Barnes TW*, et al.* Proteome profiling of exosomes derived from human primary and metastatic colorectal cancer cells reveal differential expression of key metastatic factors and signal transduction components. *Proteomics*. 13(10-11), 1672-1686 (2013).
4. Tauro BJ, Mathias RA, Greening DW*, et al.* Oncogenic H-ras reprograms Madin-Darby canine kidney (MDCK) cell-derived exosomal proteins following epithelial-mesenchymal transition. *Mol Cell Proteomics*. 12(8), 2148-2159 (2013).
5. Mathivanan S, Lim JW, Tauro BJ*, et al.* Proteomics analysis of A33 immunoaffinity-purified exosomes released from the human colon tumor cell line LIM1215 reveals a tissue-specific protein signature. *Mol Cell Proteomics*. 9(2), 197-208 (2010).
6. Tauro BJ, Greening DW, Mathias RA*, et al.* Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. *Mol Cell Proteomics*. 12(3), 587-598 (2013).
7. Lim JW, Mathias RA, Kapp EA*, et al.* Restoration of full-length APC protein in SW480 colon cancer cells induces exosome-mediated secretion of DKK-4. *Electrophoresis*. 33(12), 1873-1880 (2012).
8. Ji H, Erfani N, Tauro BJ*, et al.* Difference gel electrophoresis analysis of Ras-transformed fibroblast cell-derived exosomes. *Electrophoresis*. 29(12), 2660-2671 (2008).

Supplemental Table 2 – Selected list of EV studies on biofluids.

Biofluid type	Disease *(and patient cohort)*	EV isolation method[A]	Biofluid volume/ EV particle concentration	EV validation	EV type *(reported in study)*	EV selection marker	Reference
Plasma	Pancreatic cancer (n=8)	DC	NT µl, Patient ~0.035 pg MIF /10^8 Control~ 0.005 pg MIF / 10^8	Size: 100 nm (TEM/NTA) MS: Alix, TSG101, CD63, CD81	Exos	MIF	[1]
	Breast cancer (n=50)	DC	NT µl Patient 750 / µl Control 300/ µl	WB: CD9, Flotilin-2, MHC-I	sMVs	FAK, EGFR	[2]
	Melanoma (n= 36)	1.2 µm, DC, 40% sucrose cushion (DGC)	NT µl Patient 0.0018 µg/ 10^8 Control 0.0004 µg/ 10^8	Size : 100 nm (TEM) WB: GAPDH	Exos	TYRP2, VLA-4, HSP70, MET	[3]
	Glioblastomas (n=30)	0.8 µm, 0.20 µm, DC, µNMR		Size: 100 nm (TEM) CD63	Exos	EGFR, EGFR vIII,	[4]
	Ovarian cancer (n=22)	0.22 µm, DC	300 µl	Size: 100 nm (SEM) WB: LAMP-1	Exos	MAGE 3/6, TGF-β1	[5]
	Ovarian cancer (n=63)	DC	200 µl	Size: 100 nm (TEM) Density: 1.18 g/mL	Exos	CLDN4	[6]
	Lung cancer (n=10)	ExoQuick	500 µl		Exos	miR-378a, miR-379, miR-139-5p, miR-200b-5p	[7]
	Gastric cancer (n= 37)	DC		Size: 10-800 nm (TEM, AFM, DLS) FACS: Her-2/neu$^+$ MV	sMVs	Her-2/neu$^+$ MV mRNA MAGE-1 and Her-2	[8]
	Gastric cancer (n= 10)	Total Exosomes isolation reagent	300 µl	Size: 100 nm (TEM)	Exos	LINC00152	[9]
	Gastric cancer	ExoQuick	400 µl	qRT-PCR, FISH	Exos	miR-185, miR-20a,	[10]

	(n= 133)					miR-210, miR-25, miR-92b	
	Leukaemia (n= 16)	0.22 µm, DC, SEC	9 mL Patient: 5×10^{13} particles/mL Patient: ~55 µg/mL Control: ~20 µg/mL	WB: CD81, TGF-β1 Density: ~1.18 g/mL Size: 100 nm (TEM, NTA) ELISA: TGF-β1	Exos	Exosomal TGF-β1	[11]
	Healthy volunteers (n=57)	Filtration	0.5 mL	FACS (PS, CD63) TEM (sMVs 100-1000 nm; Exos ~100 nm) ELISA (TGF-β)	Exos/sMVs	CD63 are enriched in exos compared with sMVs	[12]
Serum	Pancreatic cancer (n=190)	0.2 µm filter, DC	250 µl, Patient ~2.5×10^{9} Control ~2.1×10^{9}	FACS: GPC1+ Exos TEM: GPC1+ Exos/ CD9+ Exos WB Flotilin 1 Size: 100 nm Density: 1.19 g/mL	Exos	GPC1	[13]
	CHB (n=20), LC (n=20), HCC (n=20)	Exoquick (120 µl)	500 µl Serum	WB: CD63, CD9	Exos	High in HCC: miR-18a, miR-221, miR-222 and miR-224 Low in HCC: miR-101, miR-106b, miR-122 and miR-195	[14]
	Breast cancer (n=96)	DC, DGC	3-6 mL serum Patient ~20 µg/mL Control ~5 µg/mL	WB: ADAM10, CD9, CD24 Size: 100 nm (TEM)	Exos	CD24	[15]
	Pancreatic cancer (n=131)	DC, DGC	1-1.5 mL	FACS: CD9, CD63	Exos	CD44v6,Tspan8, EpCAM, MET, CD104, miR-1246, miR-4644, miR3976, miR-4306	[16]
	Pancreatic cancer (n=4)	0.2 µm filter, DC	500 µl	Size: 100 nm (NTA and TEM) FACS: CD9, TSG101, CD63 WB: TSG101, CD63	Exos	Mutated *KRAS* and *p53* DNA	[17]
	Pancreatic cancer (n=5)	Microfluidic (ExoChip)	400 µl	WB: CD63, Rab5 Size:30-300 nm (TEM)	Exos	CD63, Rab5, miRNAs profiles	[18]
	Breast cancer (n=11)	0.2 µm filter, DC	500 µl Patient ~0.5×10^{9}	Size: 100 nm (TEM, AFM, LLS, NTA)	Exos	Dicer,miR	[19]

			Control ~0.2×10^9	WB: TSG101, CD9, CD63			
	Breast cancer (n=168)	ExoQuick	400 µl	WB: CD63, Mucin1, GAPDH	Exos	miR-101, miR-372, miR-373	[20]
	Melanoma (n=21)	ExoQuick	200 µl	WB: CD63	Exos	miR-125b	[21]
	Hepatocellular carcinoma (n=30)	Total Exosomes isolation reagent		Size: 100 nm (TEM) WB: CD63, TSG101	Exos	miR-21	[22]
	Ovarian cancer (n=30)	DC		Size: 264-410 nm (TEM, NTA) WB: VAMP3/ARF6	sMVs	VAMP3 ARF6 MT1-MMP	[23]
	Glioblastomas (n=30)	0.22 µm + DC	Serum	Size: 50-500 nm (TEM)	sMVs	mRNA EGFRvIII, miR-21	[24]
Ascites	Ovarian cancer (n=20), non-cancer (n=10)	0.22 µm + nPLEX	Ascites (> 10^9 exosomes per mL)	Size: ~ 100 nm (TEM)	Exos	EPCAM, CD24	[25]
	Breast cancer Ovarian cancer Lung cancer	DC + sucrose cushion		WB: ADAM 10, CD9 Size: 100 nm (TEM)	Exos	CD24/EPCAM in Ovarian carcinoma	[15]
Malignant pleural effusion	Various cancers (Breast cancer, lung cancer, Ovarian)	DC, DGC		Size: 100 nm (TEM)	Exos	50 proteins identified including MHC-I, actin, G protein, HSP90, BTG1, Bamacan, PEDF, BTG-1, TSG14 and TSP2	[26]
	Various cancers (lung cancer, breast cancer, ovarian, melanoma, mesothelioma)	DC, DGC		Size: 100 nm (TEM), TEM (MHC-I and -II, TRP, gp100 and CD81), WB: (MHC-I and -II ,MART-1, HER2 and HSC70)	Exos	MART-1, TRP, gp100 and HER2	[27]
Urine	Healthy individuals (n=4)	DC; 0.22 µm + DC; DC + sucrose cushion;	250 mL ~200×10^{-6} mL $CD9^+$ Exos	1D-SDS-PAGE: THP WB: Alix, TSG101, RT-PCR: (miR-1207-5p, miR-192, mRNA AQP2, TSG101 and Alix)	Exos	mRNA AQP2, miR-1207-5p, miR-192	[28]

		membrane filtration; ExoQuick					
	BPH (n=5) PCa (n=5) Metastatic PCa (n=3)	DC	5 mL	WB: Alix, ITGA3, ITGB1	Exos	ITGA3	[29]

A DC; differential ultracentrifugation, DGC; density-gradient fractionation, SEC; size exclusion chromatography, Exos; exosome, sMVs; shed microvesicles, TEM; transmission electron microscopy, SEM: scanning electron microscopy, WB: western blotting, NTA; nanoparticle trafficking analysis, AFM; atomic force microscopy, MS; mass spectrometry, qRT-PCR; quantitative real-time polymerase chain reaction, FISH; fluorescence in situ hybridization, DLS; dynamic light scatter, ELISA; enzyme-linked immunosorbent assay, FACS; fluorescence-activated cell sorting, THP; tamm-horsfall protein, BPH; benign prostate hyperplasia, PCa; prostate cancer, CHB; chronic hepatitis B, LC; liver cirrhosis, HCC; hepatocellular carcinoma, nPLEX, nanoplasmonic exosomes sensor.

References

1. Costa-Silva B, Aiello NM, Ocean AJ*, et al.* Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver. *Nat Cell Biol*. 17(6), 816-826 (2015).
2. Galindo-Hernandez O, Villegas-Comonfort S, Candanedo F*, et al.* Elevated concentration of microvesicles isolated from peripheral blood in breast cancer patients. *Arch Med Res*. 44(3), 208-214 (2013).
3. Peinado H, Aleckovic M, Lavotshkin S*, et al.* Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med*. 18(6), 883-891 (2012).
4. Shao H, Min C, Issadore D*, et al.* Magnetic Nanoparticles and microNMR for Diagnostic Applications. *Theranostics*. 2(1), 55-65 (2012).
5. Szajnik M, Derbis M, Lach M*, et al.* Exosomes in Plasma of Patients with Ovarian Carcinoma: Potential Biomarkers of Tumor Progression and Response to Therapy. *Gynecol Obstet (Sunnyvale)*. Suppl 4(3 (2013).
6. Li J, Sherman-Baust CA, Tsai-Turton M*, et al.* Claudin-containing exosomes in the peripheral circulation of women with ovarian cancer. *BMC Cancer*. 9(244 (2009).
7. Cazzoli R, Buttitta F, Di Nicola M*, et al.* microRNAs derived from circulating exosomes as noninvasive biomarkers for screening and diagnosing lung cancer. *J Thorac Oncol*. 8(9), 1156-1162 (2013).
8. Baran J, Baj-Krzyworzeka M, Weglarczyk K*, et al.* Circulating tumour-derived microvesicles in plasma of gastric cancer patients. *Cancer Immunol Immunother*. 59(6), 841-850 (2010).
9. Li Q, Shao Y, Zhang X*, et al.* Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. *Tumour Biol*. 36(3), 2007-2012 (2015).
10. Zhou X, Zhu W, Li H*, et al.* Diagnostic value of a plasma microRNA signature in gastric cancer: a microRNA expression analysis. *Sci Rep*. 5(11251 (2015).
11. Hong CS, Muller L, Whiteside TL*, et al.* Plasma exosomes as markers of therapeutic response in patients with acute myeloid leukemia. *Front Immunol*. 5(160 (2014).
12. Grant R, Ansa-Addo E, Stratton D*, et al.* A filtration-based protocol to isolate human plasma membrane-derived vesicles and exosomes from blood plasma. *J Immunol Methods*. 371(1-2), 143-151 (2011).

13. Melo SA, Luecke LB, Kahlert C*, et al.* Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. *Nature*. 523(7559), 177-182 (2015).
14. Sohn W, Kim J, Kang SH*, et al.* Serum exosomal microRNAs as novel biomarkers for hepatocellular carcinoma. *Exp Mol Med*. 47(e184 (2015).
15. Rupp AK, Rupp C, Keller S*, et al.* Loss of EpCAM expression in breast cancer derived serum exosomes: role of proteolytic cleavage. *Gynecol Oncol*. 122(2), 437-446 (2011).
16. Madhavan B, Yue S, Galli U*, et al.* Combined evaluation of a panel of protein and miRNA serum-exosome biomarkers for pancreatic cancer diagnosis increases sensitivity and specificity. *Int J Cancer*. 136(11), 2616-2627 (2015).
17. Kahlert C, Melo SA, Protopopov A*, et al.* Identification of double-stranded genomic DNA spanning all chromosomes with mutated KRAS and p53 DNA in the serum exosomes of patients with pancreatic cancer. *J Biol Chem*. 289(7), 3869-3875 (2014).
18. Kanwar SS, Dunlay CJ, Simeone DM*, et al.* Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. *Lab Chip*. 14(11), 1891-1900 (2014).
19. Melo SA, Sugimoto H, O'Connell JT*, et al.* Cancer exosomes perform cell-independent microRNA biogenesis and promote tumorigenesis. *Cancer Cell*. 26(5), 707-721 (2014).
20. Eichelser C, Stuckrath I, Muller V*, et al.* Increased serum levels of circulating exosomal microRNA-373 in receptor-negative breast cancer patients. *Oncotarget*. 5(20), 9650-9663 (2014).
21. Alegre E, Sanmamed MF, Rodriguez C*, et al.* Study of circulating microRNA-125b levels in serum exosomes in advanced melanoma. *Arch Pathol Lab Med*. 138(6), 828-832 (2014).
22. Wang H, Hou L, Li A*, et al.* Expression of serum exosomal microRNA-21 in human hepatocellular carcinoma. *Biomed Res Int*. 2014(864894 (2014).
23. Clancy JW, Sedgwick A, Rosse C*, et al.* Regulated delivery of molecular cargo to invasive tumour-derived microvesicles. *Nat Commun*. 6(6919-6930 (2015).
24. Skog J, Wurdinger T, van Rijn S*, et al.* Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 10(12), 1470-1476 (2008).
25. Im H, Shao H, Park YI*, et al.* Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. *Nat Biotechnol*. 32(5), 490-495 (2014).
26. Bard MP, Hegmans JP, Hemmes A*, et al.* Proteomic analysis of exosomes isolated from human malignant pleural effusions. *Am J Respir Cell Mol Biol*. 31(1), 114-121 (2004).
27. Andre F, Schartz NE, Movassagh M*, et al.* Malignant effusions and immunogenic tumour-derived exosomes. *Lancet*. 360(9329), 295-305 (2002).
28. Alvarez ML, Khosroheidari M, Kanchi Ravi R*, et al.* Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. *Kidney Int*. 82(9), 1024-1032 (2012).
29. Bijnsdorp IV, Geldof AA, Lavaei M*, et al.* Exosomal ITGA3 interferes with non-cancerous prostate cell functions and is increased in urine exosomes of metastatic prostate cancer patients. *J Extracell Vesicles*. 2(eCollection 2013 (2013).