Supporting Information for Publication

Assessment of extracellular vesicles purity using proteomic standards

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Table S1. Transitions used for quantification.

Protein	Peptide		Precursor (m/z)	Product Ions (m/z)		
				1	2	3
albumin	LVNEVTEFAK	L	573.1	694.4 (y6)	823.4 (y7)	937.5 (y8)
		H	581.3	701.4 (y6)	831.4 (y7)	947.4 (y8)
	VFDEFKPLVEEPQNLIK	L	682.4	777.9 (y13)	842.5 (y14)	900.0 (y15)
		H	689.3	786.4 (y13)	851.4 (y14)	909.5 (y15)
	QTALVELVK	L	500.8	587.4 (y5)	700.5 (y6)	771.5 (y7)
		H	506.3	593.4 (y5)	707.4 (y6)	779.5 (y7)
α-2-macroglobulin	LVHVEEPHTETVR	L	773.4	839.4 (y7)	968.5 (y8)	1097.52 (y9)
		H	783.4	851.4 (y7)	981.4 (y8)	1111.5 (y9)
	LPPNVVEESAR	L	605.8	691.3 (y6)	789.4 (y7)	1000.5 (y9)
		H	613.3	699.3 (y6)	799.4 (y7)	1013.5 (y9)
Transferrin	DGAGDVAFVK	L	489.7	563.4 (y5)	678.4 (y6)	806.4 (y8)
		H	495.2	569.3 (y5)	685.4 (y6)	815.4 (y8)
apoE	LGPLVEQGR	L	484.8	489.2 (y4)	588.3 (y5)	701.4 (y6)
		H	491.2	497.2 (y4)	597.2 (y5)	711.4 (y6)
HSPA8	FEELNADLFR	L	627.3	735.3 (y6)	848.5 (y7)	977.5 (y8)
		H	634.3	745.3 (y6)	859.4 (y7)	989.5 (y8)
moesin	IGFPWSEIR	L	552.8	690.4 (y5)	787.4 (y6)	991.5 (y8)
		H	559.3	699.3 (y5)	797.4 (y6)	1003.5 (y8)
HSP90AA1	ADLINNLGTIAK	L	621.9	716.4 (y7)	830.5 (y8)	943.6 (y9)
		H	629.3	725.4 (y7)	841.4 (y8)	955.5 (y9)
EHD4	SISVIDSPGILSGEK	L	751.4	800.5 (y8)	887.5 (y9)	1002.5 (y10)
		H	759.4	809.4 (y8)	897.5 (y9)	1013.5 (y10)
flotillin-1	VTGEVLDILTR	L	608.4	617.4 (y5)	730.4 (y6)	829.5 (y7)
		H	615.3	625.3 (y5)	739.4 (y6)	839.5 (y7)
HDAC1,2	YGEYFPGTGDLR	L	687.8	715.4 (y7)	862.4 (y8)	1025.5 (y9)
		H	695.3	725.3 (y7)	873.4 (y8)	1037.5 (y9)
HSP90AB1	YESLTDPSK	L	520.3	547.3 (y5)	660.4 (y6)	747.4 (y7)
		H	525.2	553.3 (y5)	667.3 (y6)	755.4 (y7)
TSG101	ASLISAVSDK	L	495.8	519.3 (y5)	606.3 (y6)	719.4 (y7)
		H	501.3	525.3 (y5)	613.3 (y6)	727.4 (y7)
DNAJB1	DGSDVIYPAR	L	546.8	619.4 (y5)	718.4 (y6)	833.5 (y7)
		H	553.3	627.3 (y5)	727.4 (y6)	843.4 (y7)
L1CAM	DATQITQGPR	L	543.8	558.3 (y5)	671.4 (y6)	799.4 (y7)
		H	551.3	567.3 (y5)	681.4 (y6)	811.4 (y7)

Transitions are listed for both unlabeled, light (L) and fully ^{15}N-labeled, heavy (H) peptides. All precursors were +2 charge and product ions were +1 charge. Additionally, y-ion information is included for reference.

Table S2. ^{15}N-incorporation in EXO1 and EXO2 QconCATs.

QconCAT	Peptide	^{15}N-incorporation[a] (%)	Average[b] (%)
EXO1	LPPNVVEESAR	99.92	
EXO1	LVNEVTEFAK	99.13	99.66 ± 0.27
EXO1	VTGEVLDILTR	99.94	
EXO2	DKPHNIFK	99.98	
EXO2	YESLTDPSK	99.96	99.74 ± 0.27
EXO2	FEELNADLFR	99.29	

[a] Mean value based on three transitions per peptide. [b] Average was calculated based on 3 peptides in each QconCAT.

Table S3. Identification of proteins in silver-stained gel bands marked in Figure 1

Band and protein[a]	Nominal Mass (kDa)	Sequence coverage (%)	Score[b]
P1. alpha-2-macroglobulin (224053)	160	7	88(76)
P2. alpha-2-macroglobulin (224053)	160	9	102(76)
P3. alloalbumin Venezia (178345)	69	27	253(76)
P4. immunoglobulin mu heavy chain, partial (390994295)	66	16	147(76)
P5. chain B, Igg1 Fc fragment (401817558)	24	30	285(76)
P6. anti-HBsAg immunoglobulin Fab kappa chain(3721651)	24	14	119(76)

[a] The number in parentless is a NCBI GI number

[b] MOWSE score from Mascot software (http://www.matrixscience.com/search_form_select.html). The number in parentless is the score at which statistical significance (*p*<0.05) occurred for that particular search.

Figure S1. EXO1 and EXO2 QconCATs

EXO1 - Number of amino acids: 592; Molecular weight: 66686.19; Theoretical pI: 9.30
Grand average of hydropathicity (GRAVY): -0.323

MTSTTLKLSPYVHYTFRVTAINPIDKKAPDFVFYAPRLRINKRELISRLPEIYIQLQREYQI
SASVRIRGEAEAFAIGARARAEAEVNTRRAQADLAYQLQVAKTKQQIERQIKKQTALVE
LVKHKPKATIGAAKVTGEVLDILTRLPESVENLANRSHAELETALRDSSRVLFDAHKLDI
SDEFSEAIKAFRGQDRLTPKIGFPWSEIRNISFNDVVESRAQLLVVGSPGPVPRLVLSD
LAFVAARSFVQGLGVASDVVRKVAQVPNQVLKSISVIDSPGILSGEKQRISRGFIQTRG
GTFYVEPAERYIKDRTGRGHARLVHVEEPHTETVRKYFPETGEALRRGVIDLDVFLKH
VRLLSEDTIRASLISAVSDKLRWRIKEELSLKLPPNVVEESARASVSVLHEAYAKVFDEF
KPLVEEPQNLIKQNAELFEDHVKLVNEVTEFAKTAVADEANLKVKDATQITQGPRSTIEK
KVDANGKIYLPYLHEWKHPQSDLHQRAKRAVSHEDQFLRLDFHAHQIYGAKGFSLSDV
PQAEISGEHLRIAPQGYWILADKSSFISVLQTSSSSLRIGAYVFKLAAALEHHHHHH

Peptides (marked red) with 6 a.a. residues flanking sequences on both sides (marked black) from 10 proteins listed below were randomly concatenated in EXO1 sequence. 4 Cys were replaced with Ala (marked green) and 4 Met were replaced with Ile (marked blue) in the flanking sequences. 6 x His tag and linker sequence (marked orange) was from expression vector.

Albumin
1. FEDHVKLVNEVTEFAKTCVADE
2. HECYAKVFDEFKPLVEEPQNLIKQNCELF
3. ERQIKKQTALVELVKHKPKAT

Alpha-2-macroglobulin
1. GRGHARLVHVEEPHTETVRKYFPET
2. EELSLKLPPNVVEESARASVSVL

L1CAM (single-pass)
1. ANLKVKDATQITQGPRSTIEKK
2. DVVESRAQLLVVGSPGPVPRLVLSDL
3. TSTTLKLSPYVHYTFRVTAINK

ADAM10 (single-pass)
1. HQRAKRAVSHEDQFLRLDFHAH
2. GFIQTRGGTFYVEPAERYIKDRT

CD82 (multi-pass)
1. WILADKSSFISVLQTSSSSLRMGAYVF

Glypican-1 (lipid-anchor, GPI-anchor)
1. QIYGAKGFSLSDVPQAEISGEHLRICPQGY
2. ENLANRSHAELETALRDSSRVL
3. AFVAARSFVQGLGVASDVVRKVAQVP

TSG101 (peripheral)
1. VDANGKIYLPYLHEWKHPQSDL
2. SEDTIRASLISAVSDKLRWRMK
3. GEALRRGVIDLDVFLKHVRLLS

Flotillin-1 (peripheral)
1. EVNTRRAQADLAYQLQVAKTKQQIE
2. ASVRMRGEAEAFAIGARARAEAE
3. TMGAAKVTGEVLDILTRLPESVE

EHD4 (peripheral)
1. PNQVLKSISVIDSPGILSGEKQRISRG
2. LFDAHKLDISDEFSEAIKAFRGQD
3. RELISRLPEIYIQLQREYQISA

Moesin (peripheral)
1. DRLTPKIGFPWSEIRNISFND
3. KPIDKKAPDFVFYAPRLRINKR

EXO2 - Number of amino acids: 493; Molecular weight: 55528.5; Theoretical pI: 5.52
Grand average of hydropathicity (GRAVY): -0.537

MSTLDNRTIVITSHPGQIVKHGDIKALIIEFKVNFPENGFLSPDKLSLLEKITFPKEGDQTS
NNIPADIVFVLKDKPHNIFKRDGSDVIYPARISLREAKTPEKRGDLIIEFEVIFPERIPQTS
RYGEFYKSLTNDWEDHLAVKHFSVEGIGITKADLINNLGTIAKSGTKAFEKERDKEVSD
DEAEEKEDKEEEYLNFIRGVVDSEDLPLNISREILQQSITRARFEELNADLFRGTLDPVIP
KIQKLLQDFFNGKELNKSITGETKDQVANSAFVERLRKHGLNDQGNRTTPSYVAFTDT
ERLIGDAAQRQATKDAGTIAGLNVLRIINEPTAGIFEVKSTAGDTHLGGEDFDNRIVNHFI
KEIFLRELISNASDALDKIRYESLTDPSKLDSGKELKIDIIPNPQERTLTLVDGIGITKADLI
NNLGTIAKSGTKAFLAALRKGESGQSWPRLTKERAKLNWLSVDFNNWKDWEDDSKLA
AALEHHHHHH

Peptides (marked red) with 6 a.a. residues flanking sequences on both sides (marked black) from 6 proteins listed below were randomly concatenated in EXO2 sequence. 3 Cys were replaced with Ala (marked green) and 4 Met were replaced with Ile (marked blue) in the flanking sequences. 6 x His tag and linker sequence (marked orange) was from expression vector.

DNAJA1 (hDj-2)
1. STLDNRTIVITSHPGQIVKHGDIKC
2. LIIEFKVNFPENGFLSPDKLSLLEK

DNAJB1 (Hsp40)
1. KITFPKEGDQTSNNIPADIVFVLKDKPHNIFKRDGSDVIYPARISLREA
2. KTPEKRGDLIIEFEVIFPERIPQTSR

HSP90AA1
1. GIGMTKADLINNLGTIAKSGTKAF
2. EKERDKEVSDDEAEEKEDKEEE
3. YGEFYKSLTNDWEDHLAVKHFSVEG
4. YLNFIRGVVDSEDLPLNISREMLQQS
5. ITGETKDQVANSAFVERLRKHGL

HSP90AB1

KEIFLRELISNASDALDKIRYESLTDPSKLDSGKELKIDIIPNPQERTLTLVDGIGMTKADLINNLGTIAKSGTKAF

HSPA8 (Hsc70)

1. NDQGNRTTPSYVAFTDTERLIGDAA
2. QRQATKDAGTIAGLNVLRIINEPTA
3. GIFEVKSTAGDTHLGGEDFDNRMVNHFI
4. SITRARFEELNADLFRGTLDPV
5. IPKIQKLLQDFFNGKELNKSI

PTGES3 (p23)

1. LCCLRKGESGQSWPRLTKERAKLNWLSVDFNNWKDWEDDS

Figure S2. A 9 % SDS-PAGE of purified EXO1 (1) and EXO2 (2). The molecular mass standards in kDa are shown on the *left*.

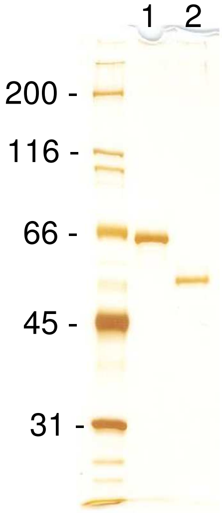

Figure S3. Scheme depicting basic and precipitation-based protocols.