EXOSOMAL TRANSFER FROM HUMAN RENAL PROXIMAL TUBULE CELLS TO DISTAL TUBULE AND COLLECTING DUCT CELLS

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SUPPLEMENTAL METHODS

Distal Tubule and Collecting Duct Cell Isolation, Culture and Characterization

The DT cell lines isolated using DBA were characterized as staining positively for distal tubule markers DBA, Na-Cl- cotransporter NCC (Millipore), epithelial membrane antigen MUC1 (Dako), Calbindin 1 (Novacastra), and E-cadherin (Abcam). The DT cells isolated using THP were characterized as staining positively for THP and Na-K-2Cl-co-transporter NKCC2 (Sigma, HPA018107), using immunofluorescent and lectin affinity-fluorescent confocal microscopy.

Both types of DT cells (DBA or THP-isolated) had further characterization by their lack of staining for human proximal tubule markers *lotus tetragonolobus lectin* LTA (Vector Labs), CD13 (clone 452) and N-cadherin (DSHB, MNCD2 rat monoclonal clone, found to work on human), or collecting duct marker L1CAM (Abcam).

The CD cell lines were characterized using confocal immunofluorescent microscopy, which confirmed the expression of the collecting duct markers L1CAM and the epithelial sodium channel ENaC (Alomone, ASC-030). The cells showed no staining with proximal tubule markers LTA, CD13 and N-cadherin, or distal tubule markers DBA, THP, and NCC.

Exosome purification

We used the well-accepted ultracentrifugation protocol to isolate exosomes according to Gonzales et al (1). Two modifications of this protocol were used: *1)* no reducing agent was added due to lack of Tamm-Horsfall protein in our proximal tubule cell culture supernatants, and *2)* the first centrifugation step was performed for 30 min rather than 10 min to ensure maximum purity. In brief, 240 mL of cell culture supernatant was subjected to a 17,000 x *g* centrifugal field for 30 min at 4°C to remove whole cells and debris. Supernatants were collected and then subjected to a 200,000 x *g* centrifugal field for 30 min at 4°C. The pellets were then resuspended in PBS.

We verified this ultracentrifugation preparation using an immunomagnetic isolation technique with an anti-CD63 antibody (DSHB) coupled to magnetic microparticles (Dynal). Purified fluorescent exosomes could be depleted by Immunomagnetic capture, and these captured fluorescent exosomes could be released from the magnetic beads and transfer to RPTC, DT, and CD cells. We also used directly immunomagnetic purified exosomes for all experimental models in the paper except for the direct transfer between microcarrier co-cultured cells, and our results were the same as for the ultracentrifugation method. All of the data presented in this paper was using exosomes purified by the ultracentrifugation method.

We use the term exosomes to describe the particles released from the multivesicular endosome. Our ultracentrifugation preparation would not include cell surface-shed microvesicles that are larger (100-1000 microns) and would pellet in the first 17,000 g spin. Also excluded from this preparation would be apoptotic bodies, extruded lysosomes, and endosomes from lysed cells due to differences in both size and marker epitope orientation. Ectosomes are a subcategory of microvesicles that do have an overlapping size distribution with exosomes and could be present in the ultracentrifugation preparation. However, ectosomes have been characterized from immune cells as CD63 minus and phosphatidylserine positive (2). Since we used CD63 depletion and immunomagnetic isolation in our verifying preparation, the particles we are isolating would not be considered ectosomes.

Proteomic Analysis by Mass Spectrometry

Preparation: Exosomes were purified from immortalized proximal tubule cell culture supernatant as described above. The pellet that resulted from the ultracentrifugation protocol was resuspended in PBS and the protein concentration was quantitated by BCA (Pierce). 20 ug of pellet protein were loaded per lane and separated by SDS polyacrylamide gel electrophoresis until the protein front had progressed 5 cm into the gel. The gel was stained with SimplyBlue (Life Technologies) and each lane had approximately 3mm bands excised. The excised bands were then subjected to in-gel tryptic digestion, and processed for mass spectrometry.

Mass spectrometry: Each trypsinized sample was pressure-loaded onto a self-prepared 100 µm i.d. fused-silica column (Polymicro Technologies, Phoenix, AZ) packed with irregular (5-15 µm, 120 Ǻ) reverse-phase phenyl resin (YMC, Kyoto, Japan) and then connected to a 75 µm i.d. PicoFrit® fused-silica column (New Objective, Woburn, MA) that had a pre-fritted 10 µm tip and had been self-packed with regular (5µm, 120 Ǻ) reverse-phase phenyl resin (YMC, Kyoto, Japan). Nano-flow electrospray ionization was performed in the positive ion mode with a 2.0 kV spray voltage applied to peptides that were eluted with a flow rate of about 200 nL/min from an HPLC gradient of 0-70% Solvent B in 105 minutes, where Solvent A was 0.1 M acetic acid and Solvent B was 80% acetonitrile in 0.1 M acetic acid.

Briefly, the Thermo LTQ-XL ion trap mass spectrometer (Thermo, San Jose, CA) was operated in the data-dependent mode with an Agilent 1100 HPLC system split to nano-flow. The acquisition duty cycle consisted of an initial MS^1 centroid scan with a mass range of 300-1800 m/z for all experiments. The five most abundant ions were sequentially selected for a Zoom MS^1 scan acquired in profile with a width of 10 m/z centered on the precursor ion. Each Zoom MS^1 scan was followed by a MS^2 CID spectrum of that same precursor. After repeating for each of the top five precursor ions, the cycle repeated. The duty cycle for this data acquisition cycle of eleven mass spectral scans was about 2.9 s on average. Further details are provided in Lyons, et al (3).

Mass Spectral Data analysis: Data sets were first processed using a custom Perl script, dubbed MAZIE, which accurately determines the charge and monoisotopic mass for each MS^2 scan precursor ion by analyzing the preceding Zoom MS^1 scan (4). The MAZIE script then generates a concatenated DTA file used for searching with the OMSSA engine (5). MAZIE is distributed under the Creative Commons License and is available, together with its dependencies, at http://faculty.virginia.edu/templeton. The human protein database was obtained from NCBI (ftp://ftp.ncbi.nih.gov/blast/db/, version downloaded on November 4, 2010). Reversed sequences of all the proteins were generated by an in-house Perl script and appended to the database so that a decoy search strategy could be employed. Using this composite (forward and reverse sequences) database, the MS^2 data was searched as a tryptic digest using parameters that were optimized, as described previously (3,4); briefly, the mass of both the precursor and fragment ions were treated as monoisotopic with an m/z tolerance of 0.3 Da and 0.5 Da, respectively; two missed cleavages were allowed; and variable modifications of carbamidomethylation [+57] of cysteine, oxidation [+16] and sulphonation [+32] of methionine, deamidation [+1] of asparagine and glutamine, and phosphorylation [+80] of serine, threonine and tyrosine were permitted. A false discovery rate (FDR) was calculated by tabulating the OMSSA search results that identified natural (forward) protein sequences, representing potential real sequence matches, together with those that identified reversed protein sequences that are, by definition, false matches (6). All of the mass spectral data was compiled and stored in a MySQL database that is accessed and presented via a PHP Web-based user interface, WiMS-AMP, which will be presented in a future publication.

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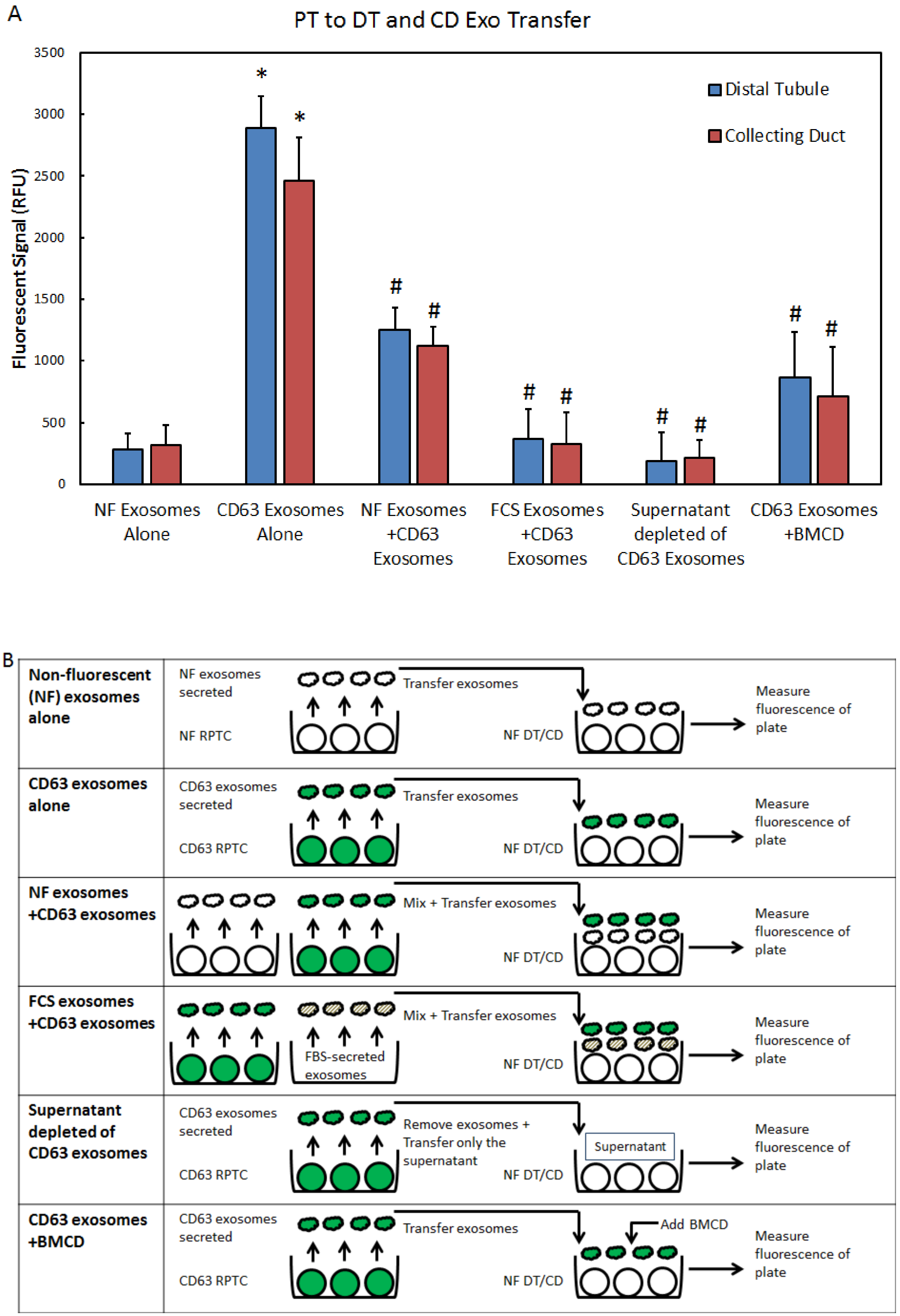
(4) Victor, K. G., M. Murgai, C. E. Lyons, T. A. Templeton, S. A. Moshnikov, and D. J. Templeton. 2010. MAZIE: A Mass and Charge Inference Engine to Enhance Database Searching of Tandem Mass Spectra. *Journal of the American Society for Mass Spectrometry* 21:80-87.

(5) Geer, L. Y., S. P. Markey, J. A. Kowalak, L. Wagner, M. Xu, D. M. Maynard, X. Y. Yang, W. Y. Shi, and S. H. Bryant. 2004. Open mass spectrometry search algorithm. *Journal of Proteome Research* 3:958-964.

(6) Kall, L., J. D. Storey, M. J. MacCoss, and W. S. Noble. 2008. Assigning significance to peptides identified by tandem mass spectrometry using decoy databases. *Journal of Proteome Research* 7**:**29-34.


A
PT to DT and CD Exo Transfer
Fluorescent Signal (RFU)
3500
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FCS Exosomes +CD63 Exosomes
Supernatant depleted of CD63 Exosomes
CD63 Exosomes +BMCD
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Non-fluorescent (NF) exosomes alone
NF exosomes secreted
NF RPTC
Transfer exosomes
NF DT/CD
Measure fluorescence of plate
CD63 exosomes alone
CD63 exosomes secreted
CD63 RPTC
Transfer exosomes
NF DT/CD
Measure fluorescence of plate
NF exosomes +CD63 exosomes
Mix + Transfer exosomes
NF DT/CD
Measure fluorescence of plate
FCS exosomes +CD63 exosomes
FBS-secreted exosomes
Mix + Transfer exosomes
NF DT/CD
Measure fluorescence of plate
Supernatant depleted of CD63 exosomes
CD63 exosomes secreted
CD63 RPTC
Remove exosomes + Transfer only the supernatant
Supernatant
NF DT/CD
Measure fluorescence of plate
CD63 exosomes +BMCD
CD63 exosomes secreted
CD63 RPTC
Transfer exosomes
Add BMCD
NF DT/CD
Measure fluorescence of plate

Figure S1. Transfer of fluorescent exosomes from donor human renal proximal tubule cells (RPTC) to distal tubule (DT) or collecting duct (CD) cells (see corresponding experimental model in Panel B to match each bar set in Panel A). When purified exosomes from non-fluorescent (NF) RPTC are transferred for 24 hours to DT or CD cells, little fluorescent signal was measured in the recipient cells. However, there was a significant increase in signal in the recipient DT and CD cells when CD63-GFP purified exosomes were transferred (N=12, *P<0.05 vs NF Exosomes). Addition of NF exosomes to the same concentration of CD63 exosomes produced a significant decrease in signal in DT and CD cells, suggesting exosome-specific binding and uptake by the NF exosomes. When NF exosomes that were derived from FCS were added to CD63-purified exosomes, this also caused decreased signal, indicating that the binding and uptake of proximal tubule-derived exosome particles into DT and CD cells are not unique interactions between these cell types. Depletion of CD63-containing exosomes by use of magnetic beads conjugated with anti-CD63 monoclonal antibody completely blocked the fluorescent signal. Addition of 2 mmol/L beta-methyl-cyclodextrin (BMCD), a lipid raft disrupting and cholesterol binding molecule, produced a significant decrease in fluorescent signal in both DT and CD cells. NF exosome competition with proximal tubule or FCS-derived exosomes, antibody depletion, or lipid raft disruption significantly inhibited the fluorescent signal by DT and CD cells (N=12 per group, #P<0.05 vs CD63 Exosomes).

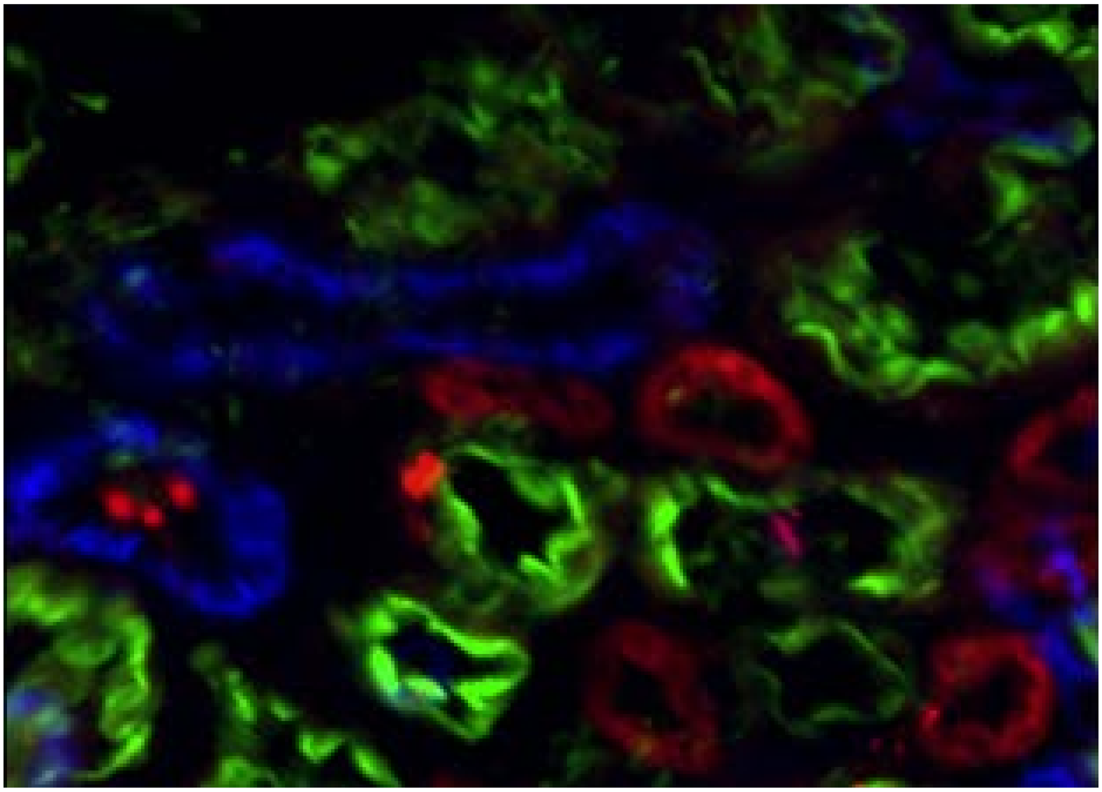

Figure S2. Proximal, distal and collecting duct tubule segment specific labeling in human renal tissue. Fresh frozen human kidney section at the cortical medullary interface was simultaneously probed with FITC labelled LTA lectin (GREEN), TRITC labelled DBA lectin (RED), and Alexa 647 labelled L1CAM (Pseudocolored BLUE). LTA (GREEN) labels the apical membrane of the proximal tubule. DBA (RED) labels the apical, cytoplasmic and basolateral membranes of the distal tubule cells. L1CAM (BLUE) primarily labels the basolateral membrane of the collecting duct. These affinity reagents show no crossreactivity between tubule segments, suggesting optimal concentrations for separation of tubule segments. Image was taken at 400x magnification.

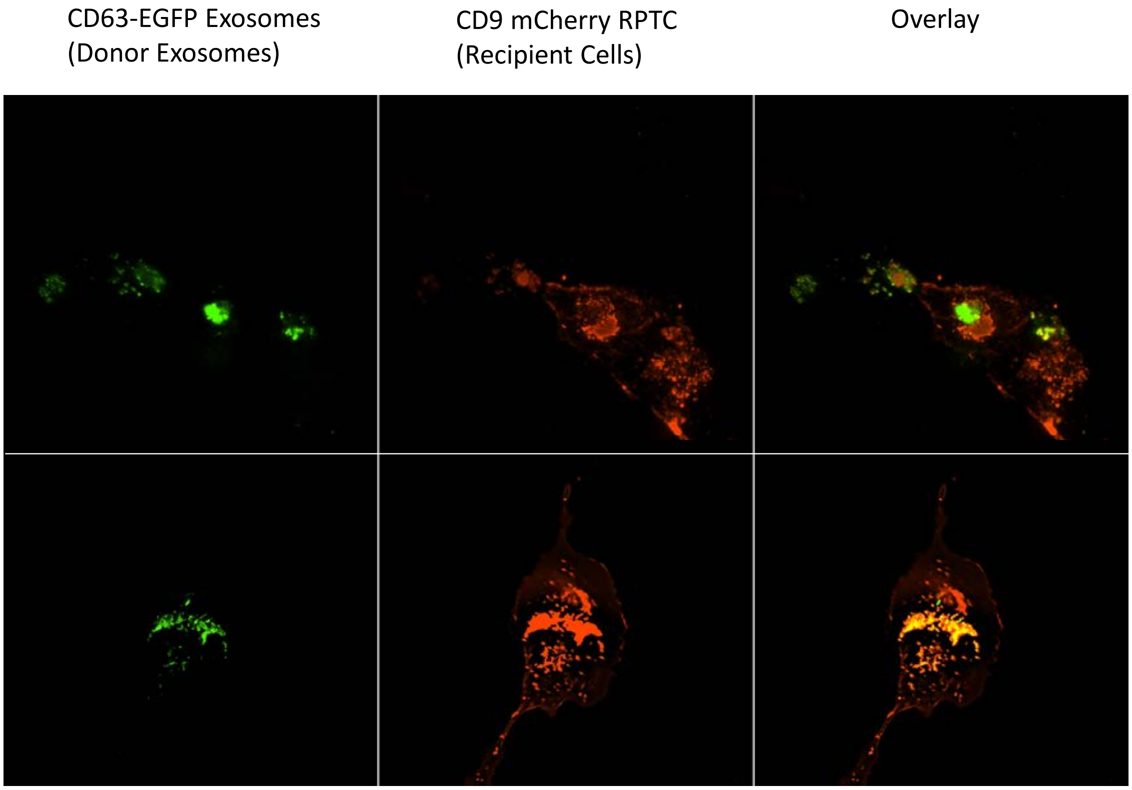

Figure S3. Purified CD63-EGFP exosomes (GREEN) isolated from renal proximal tubule cells (RPTCs) transfer to CD9-RFP stably infected RPTCs. In the upper set of panels is an example of transferred exosomes incorporating into the recipient cells but displaying very little overlap in signal. The stably infected recipient cells (RED) mark the multi-vesicular body as well as the plasma membrane. In the lower set of panels is an example of transferred exosomes (GREEN) that show a high degree of overlap (YELLOW) in the recipient cells. Image was taken at 600x magnification.

Table S1

Urine Exosome Database												i16 Cell Line						i22 Cell Line			
RefSeq Protein Accession	**Peptide #**	**Gene Symbol**	**Entrez Gene ID**	**Gene Name**	**HPA Kidney Link**	**gi**	**Protein defLine**	**Protein Length**	**Protein Mass**	**Sequence Coverage**	**Protein pValue**	**3**	**4**	**5**	**6**	**7**	**Spectrum Count**	**4**	**5**	**6**	**Spectrum Count**
NP_000468	36	ALB	213	albumin	ENSG00000163631	178344	albumin [Homo sapiens]	609	69.36	0.06	0.00E+000							2 (2)	5 (56)	X	58
NP_002017	9	FN1	2335	fibronectin 1	ENSG00000115414	16933542	fibronectin isoform 3 preproprotein [Homo sapiens]	2355	259.23	0.32	0.00E+000	65 (193)	2 (12)	3 (36)	1 (7)	5 (8)	256	7 (9)	3 (3)	3 (6)	18
NP_009057	2	VCP	7415	valosin containing protein	ENSG00000165280	6005942	transitional endoplasmic reticulum ATPase [Homo sapiens]	806	89.32	0.70	0.00E+000	5 (8)	X	X	X	X	8	53 (119)	X	X	119
		HRNR	388697	hornerin	ENSG00000197915	57864582	hornerin [Homo sapiens]	2850	282.40	0.06	0.00E+000	X	7 (13)	X	1 (3)	5 (13)	29	10 (39)	1 (1)	4 (9)	49
NP_005558	26	LGALS3BP	3959	lectin, galactoside-binding, soluble, 3 binding p	ENSG00000108679	5031863	galectin-3-binding protein [Homo sapiens]	585	65.33	0.48	7.26E-261	5 (8)	5 (9)	X	X	X	17	32 (333)	4 (15)	X	348
NP_001093	1	ACTN1	87	actinin, alpha 1	ENSG00000072110	4501891	alpha-actinin-1 isoform b [Homo sapiens]	892	103.06	0.25	2.15E-122							18 (39)	X	X	39
NP_001952	4	EEF2	1938	eukaryotic translation elongation factor 2	ENSG00000167658	4503483	elongation factor 2 [Homo sapiens]	858	95.34	0.21	1.91E-107							14 (26)	X	X	26
		ACTN4	81	actinin, alpha 4	ENSG00000130402	12025678	alpha-actinin-4 [Homo sapiens]	911	104.85	0.19	1.11E-092							12 (22)	X	X	22
		ACTA1	58	actin, alpha 1, skeletal muscle	ENSG00000143632	4501881	actin, alpha skeletal muscle [Homo sapiens]	377	42.05	0.24	1.09E-085	1 (2)	2 (3)	7 (35)	1 (1)	3 (5)	46	3 (6)	2 (4)	12 (40)	50
		HSP90AB1	3326	heat shock protein 90kDa alpha (cytosolic), cla	ENSG00000096384	20149594	heat shock protein HSP 90-beta [Homo sapiens]	724	83.26	0.22	4.85E-076	1 (1)	X	X	X	X	1	18 (49)	1 (1)	X	50
		FLG2	388698	filaggrin family member 2	ENSG00000143520	62122917	filaggrin-2 [Homo sapiens]	2391	248.08	0.05	1.50E-054	X	5 (8)	X	X	4 (8)	16	5 (17)	X	1 (1)	18
		ENO1	2023	enolase 1, (alpha)	ENSG00000074800	4503571	alpha-enolase isoform 1 [Homo sapiens]	434	47.17	0.14	1.97E-051							X	X	8 (15)	15
		FLG	2312	filaggrin	ENSG00000143631	60097902	filaggrin [Homo sapiens]	4061	435.18	0.03	3.30E-048	X	8 (13)	X	X	1 (2)	15	4 (7)	X	X	7
NP_908930	7	ALDOA	226	aldolase A, fructose-bisphosphate	ENSG00000149925	4557305	fructose-bisphosphate aldolase A [Homo sapiens]	364	39.42	0.13	1.15E-045							X	X	6 (11)	11
NP_001008844	10	DSP	1832	desmoplakin	ENSG00000096696	58530840	desmoplakin isoform I [Homo sapiens]	2871	331.77	0.07	1.08E-042	X	X	X	X	7 (9)	9	15 (20)	X	X	20
NP_872270	17	PKM	5315	pyruvate kinase, muscle	ENSG00000067225	33286418	pyruvate kinase isozymes M1/M2 isoform a [Homo sapiens]	531	57.94	0.18	3.79E-037							X	8 (30)	2 (3)	33
NP_002037	10	GAPDH	2597	glyceraldehyde-3-phosphate dehydrogenase	ENSG00000111640	7669492	glyceraldehyde-3-phosphate dehydrogenase [Homo sapiens]	335	36.05	0.23	9.26E-035	X	X	X	1 (1)	1 (1)	2	2 (3)	X	4 (9)	12
NP_006588	19	HSPA8	3312	heat shock 70kDa protein 8	ENSG00000109971	5729877	heat shock cognate 71 kDa protein isoform 1 [Homo sapiens]	646	70.90	0.14	1.74E-034							X	8 (22)	X	22
		DSG1	1828	desmoglein 1	ENSG00000134760	119703744	desmoglein-1 preproprotein [Homo sapiens]	1049	113.75	0.05	6.32E-034	X	X	X	X	2 (2)	2	3 (5)	X	X	5
		PSMD2	5708	proteasome (prosome, macropain) 26S subuni	ENSG00000175166	25777602	26S proteasome non-ATPase regulatory subunit 2 [Homo sapiens]	908	100.20	0.07	7.04E-032							4 (6)	X	X	6
		TUBA1C	84790	tubulin, alpha 1c	ENSG00000167553	14389309	tubulin alpha-1C chain [Homo sapiens]	449	49.90	0.12	1.14E-031	X	2 (5)	X	X	X	5	1 (1)	1 (2)	5 (13)	16
		HSP90AA1	3320	heat shock protein 90kDa alpha (cytosolic), cla	ENSG00000080824	153792590	heat shock protein HSP 90-alpha isoform 1 [Homo sapiens]	854	98.16	0.09	9.72E-030							7 (9)	X	X	9
		YBX1	4904	Y box binding protein 1	ENSG00000065978	34098946	nuclease-sensitive element-binding protein 1 [Homo sapiens]	324	35.92	0.17	9.85E-030							X	X	4 (6)	6
NP_068831	9	JUP	3728	junction plakoglobin	ENSG00000173801	4504811	junction plakoglobin [Homo sapiens]	745	81.74	0.09	1.92E-024	X	X	X	X	2 (2)	2	6 (7)	X	X	7
		C1R	715	complement component 1, r subcomponent	ENSG00000159403	66347875	complement C1r subcomponent precursor [Homo sapiens]	705	80.20	0.08	1.11E-022							4 (5)	X	X	5
NP_001060	7	TUBB2A	7280	tubulin, beta 2A class IIa	ENSG00000137267	4507729	tubulin beta-2A chain [Homo sapiens]	445	49.91	0.18	1.22E-021							X	1 (1)	7 (15)	16
		RPS3	6188	ribosomal protein S3	ENSG00000149273	15718687	40S ribosomal protein S3 [Homo sapiens]	243	26.69	0.28	2.54E-021	X	X	X	X	6 (7)	7				
		ACTB	60	actin, beta	ENSG00000075624	4501885	actin, cytoplasmic 1 [Homo sapiens]	375	41.74	0.13	2.97E-021	1 (1)	X	2 (9)	X	1 (2)	12	1 (1)	X	2 (6)	7
		TUBB4B	10383	tubulin, beta 4B class IVb	ENSG00000188229	5174735	tubulin beta-2C chain [Homo sapiens]	445	49.83	0.07	3.81E-020							X	1 (1)	3 (9)	10
NP_001613	1	AHSG	197	alpha-2-HS-glycoprotein	ENSG00000145192	156523970	alpha-2-HS-glycoprotein [Homo sapiens]	367	39.34	0.05	2.43E-018							X	3 (11)	1 (1)	12
NP_056991	1	LAP3	51056	leucine aminopeptidase 3	ENSG00000002549	41393561	cytosol aminopeptidase [Homo sapiens]	519	56.17	0.05	1.10E-017							X	X	2 (2)	2
		XRCC5	7520	X-ray repair complementing defective repair ir	ENSG00000079246	10863945	X-ray repair cross-complementing protein 5 [Homo sapiens]	732	82.70	0.08	1.82E-017							3 (5)	2 (3)	X	8
NP_005106	6	MVP	9961	major vault protein	ENSG00000013364	19913410	major vault protein [Homo sapiens]	893	99.33	0.06	1.08E-016							3 (4)	X	X	4
		RPL4	6124	ribosomal protein L4	ENSG00000174444	16579885	60S ribosomal protein L4 [Homo sapiens]	427	47.70	0.07	1.44E-016							X	X	3 (4)	4
NP_002256	2	KPNB1	3837	karyopherin (importin) beta 1	ENSG00000108424	19923142	importin subunit beta-1 [Homo sapiens]	876	97.17	0.05	3.74E-016							3 (4)	X	X	4
NP_006576	4	CCT8	10694	chaperonin containing TCP1, subunit 8 (theta)	ENSG00000156261	48762932	T-complex protein 1 subunit theta [Homo sapiens]	548	59.62	0.04	8.14E-016							X	2 (3)	X	3
NP_003237	9	THBS1	7057	thrombospondin 1	ENSG00000137801	40317626	thrombospondin-1 precursor [Homo sapiens]	1170	129.38	0.07	1.41E-015	6 (8)	X	X	X	X	8				
		RPSA	3921	ribosomal protein SA	ENSG00000168028	9845502	40S ribosomal protein SA [Homo sapiens]	295	32.85	0.11	2.27E-015	X	X	X	X	2 (2)	2	X	1 (2)	3 (6)	8
		HSPA9	3313	heat shock 70kDa protein 9 (mortalin)	ENSG00000113013	24234688	stress-70 protein, mitochondrial precursor [Homo sapiens]	679	73.68	0.04	2.29E-015							X	2 (2)	X	2
NP_444513	1	DCD	117159	dermcidin	ENSG00000161634	16751921	dermcidin preproprotein [Homo sapiens]	110	11.28	0.23	1.11E-014	X	1 (1)	1 (1)	X	1 (2)	4	2 (2)	1 (1)	X	3
NP_001017992	2	ACTBL2	345651	actin, beta-like 2	ENSG00000169067	63055057	beta-actin-like protein 2 [Homo sapiens]	376	42.00	0.05	8.68E-014	X	X	1 (3)	X	X	3	1 (2)	X	1 (2)	4
		EEF1G	1937	eukaryotic translation elongation factor 1 gam	ENSG00000254772	4503481	elongation factor 1-gamma [Homo sapiens]	437	50.12	0.03	2.24E-013							X	X	1 (2)	2
		RPS4X	6191	ribosomal protein S4, X-linked	ENSG00000198034	4506725	40S ribosomal protein S4, X isoform X isoform [Homo sapiens]	263	29.60	0.14	3.07E-013	X	X	X	X	5 (6)	6				
		RPL8	6132	ribosomal protein L8	ENSG00000161016	4506663	60S ribosomal protein L8 [Homo sapiens]	257	28.02	0.13	6.40E-013	X	X	X	X	5 (7)	7				
		TUBB	203068	tubulin, beta class I	ENSG00000196230	29788785	tubulin beta chain [Homo sapiens]	444	49.67	0.06	1.59E-012							X	1 (2)	2 (5)	7
NP_001447	1	FLNA	2316	filamin A, alpha	ENSG00000196924	116063573	filamin-A isoform 1 [Homo sapiens]	2639	280.01	0.01	2.75E-012							X	1 (3)	1 (2)	5
NP_059830	10	WDR1	9948	WD repeat domain 1	ENSG00000071127	9257257	WD repeat-containing protein 1 isoform 1 [Homo sapiens]	606	66.19	0.03	3.48E-012							X	2 (3)	X	3
NP_001393	6	EEF1A1	1915	eukaryotic translation elongation factor 1 alph	ENSG00000156508	4503471	elongation factor 1-alpha 1 [Homo sapiens]	462	50.14	0.06	3.84E-012	1 (1)	1 (1)	X	X	1 (1)	3	1 (1)	1 (1)	3 (8)	10
		LOXL2	4017	lysyl oxidase-like 2	ENSG00000134013	4505011	lysyl oxidase homolog 2 precursor [Homo sapiens]	774	86.72	0.03	6.94E-012							1 (2)	X	X	2
		RUVBL1	8607	RuvB-like 1 (E. coli)	ENSG00000175792	4506753	ruvB-like 1 [Homo sapiens]	456	50.23	0.03	8.02E-012							X	X	1 (1)	1
NP_002517	1	NT5E	4907	5'-nucleotidase, ecto (CD73)	ENSG00000135318	4505467	5'-nucleotidase isoform 1 preproprotein [Homo sapiens]	574	63.37	0.02	9.18E-012							X	1 (3)	X	3
NP_005712	6	ACTR3	10096	ARP3 actin-related protein 3 homolog (yeast)	ENSG00000115091	5031573	actin-related protein 3 [Homo sapiens]	418	47.37	0.03	9.64E-012							X	X	1 (1)	1

NP_115914	1	TUBB6	84617	tubulin, beta 6 class V	ENSG00000176014	14210536	tubulin beta-6 chain [Homo sapiens]	446	49.86	0.03	1.42E-011							X	X	1 (1)	1
		RPL7	6129	ribosomal protein L7	ENSG00000147604	15431301	60S ribosomal protein L7 [Homo sapiens]	248	29.22	0.04	3.63E-011	X	X	X	X	1 (2)	2				
		PYGB	5834	phosphorylase, glycogen; brain	ENSG00000100994	21361370	glycogen phosphorylase, brain form [Homo sapiens]	843	96.69	0.04	4.48E-011							3 (7)	X	X	7
NP_002796	1	PSMC5	5705	proteasome (prosome, macropain) 26S subuni	ENSG00000087191	24497435	26S protease regulatory subunit 8 isoform 1 [Homo sapiens]	406	45.63	0.03	4.60E-011							X	X	1 (1)	1
		ATP1A1	476	ATPase, Na+/K+ transporting, alpha 1 polypep	ENSG00000163399	21361181	sodium/potassium-transporting ATPase subunit alpha-1 isoform a [Homo sapiens]	1023	112.89	0.03	5.14E-011							2 (2)	X	X	2
		HTRA1	5654	HtrA serine peptidase 1	ENSG00000166033	4506141	serine protease HTRA1 precursor [Homo sapiens]	480	51.29	0.03	1.18E-010							X	X	1 (1)	1
		GFAP	2670	glial fibrillary acidic protein	ENSG00000131095	4503979	glial fibrillary acidic protein isoform 1 [Homo sapiens]	432	49.88	0.03	1.22E-010	X	X	X	X	1 (2)	2	1 (1)	X	X	1
NP_006614	2	PHGDH	26227	phosphoglycerate dehydrogenase	ENSG00000092621	23308577	D-3-phosphoglycerate dehydrogenase [Homo sapiens]	533	56.65	0.05	1.42E-010							X	X	2 (2)	2
NP_001055	1	TKT	7086	transketolase	ENSG00000163931	4507521	transketolase [Homo sapiens]	623	67.88	0.03	1.44E-010							X	2 (3)	X	3
		C1S	716	complement component 1, s subcomponent	ENSG00000182326	4502495	complement C1s subcomponent precursor [Homo sapiens]	688	76.68	0.03	1.62E-010	X	X	X	1 (1)	X	1	2 (3)	X	X	3
		CSTA	1475	cystatin A (stefin A)	ENSG00000121552	4885165	cystatin-A [Homo sapiens]	98	11.01	0.21	2.70E-010							1 (1)	X	X	1
NP_004850	12	CLTC	1213	clathrin, heavy chain (Hc)	ENSG00000141367	4758012	clathrin heavy chain 1 [Homo sapiens]	1675	191.61	0.02	3.06E-010	3 (3)	X	X	X	X	3				
NP_000230	1	LYZ	4069	lysozyme	ENSG00000090382	4557894	lysozyme C precursor [Homo sapiens]	148	16.54	0.19	3.70E-010	X	1 (1)	X	X	2 (2)	3	1 (1)	X	X	1
		CYR61	3491	cysteine-rich, angiogenic inducer, 61	ENSG00000142871	31542331	protein CYR61 precursor [Homo sapiens]	381	42.03	0.06	5.07E-010							X	X	2 (3)	3
		TNC	3371	tenascin C	ENSG00000041982	153946395	tenascin precursor [Homo sapiens]	2201	240.85	0.01	6.60E-010	2 (3)	X	X	X	X	3				
NP_036205	3	CCT5	22948	chaperonin containing TCP1, subunit 5 (epsilo	ENSG00000150753	24307939	T-complex protein 1 subunit epsilon [Homo sapiens]	541	59.67	0.02	8.96E-010							X	1 (2)	X	2
		C3	718	complement component 3	ENSG00000125730	115298678	complement C3 precursor [Homo sapiens]	1663	187.15	0.01	1.29E-009							X	1 (3)	X	3
		A2M	2	alpha-2-macroglobulin	ENSG00000175899	66932947	alpha-2-macroglobulin precursor [Homo sapiens]	1474	163.29	0.03	3.03E-009							3 (4)	1 (1)	X	5
NP_945189	1	TGM2	7052	transglutaminase 2 (C polypeptide, protein-glu	ENSG00000198959	39777597	protein-glutamine gamma-glutamyltransferase 2 isoform a [Homo sapiens]	687	77.33	0.03	4.65E-009							X	X	2 (2)	2
		TUBA8	51807	tubulin, alpha 8	ENSG00000183785	9507215	tubulin alpha-8 chain isoform 1 [Homo sapiens]	449	50.09	0.04	1.42E-008	X	1 (1)	X	X	X	1	X	X	1 (2)	2
		RPLP0	6175	ribosomal protein, large, P0	ENSG00000089157	4506667	60S acidic ribosomal protein P0 [Homo sapiens]	317	34.27	0.04	1.61E-008	X	X	X	X	1 (1)	1				
		HNRNPA1	3178	heterogeneous nuclear ribonucleoprotein A1	ENSG00000135486	4504445	heterogeneous nuclear ribonucleoprotein A1 isoform a [Homo sapiens]	320	34.20	0.06	5.45E-008	1 (1)	X	X	X	X	1				
NP_001822	6	CLU	1191	clusterin	ENSG00000120885	355594753	clusterin preproprotein [Homo sapiens]	501	57.83	0.03	5.51E-008							X	X	1 (1)	1
		MX1	4599	myxovirus (influenza virus) resistance 1, interf	ENSG00000157601	222136617	interferon-induced GTP-binding protein Mx1 [Homo sapiens]	662	75.52	0.02	9.50E-008							X	1 (2)	X	2
		RPS6	6194	ribosomal protein S6	ENSG00000137154	17158044	40S ribosomal protein S6 [Homo sapiens]	249	28.68	0.04	1.26E-007	X	X	X	X	2 (3)	3				
		HLA-C	3107	major histocompatibility complex, class I, C	ENSG00000204525	339882741	HLA class I histocompatibility antigen, Cw-1 alpha chain precursor [Homo sapiens]	368	41.47	0.06	1.26E-007							X	X	2 (2)	2
NP_000678	10	AHCY	191	adenosylhomocysteinase	ENSG00000101444	9951915	adenosylhomocysteinase isoform 1 [Homo sapiens]	432	47.72	0.03	1.73E-007							X	X	1 (2)	2
		RUVBL2	10856	RuvB-like 2 (E. coli)	ENSG00000183207	5730023	ruvB-like 2 [Homo sapiens]	463	51.16	0.05	2.10E-007							X	X	2 (3)	3
		HIST1H1A	3024	histone cluster 1, H1a	ENSG00000124610	4885373	histone H1.1 [Homo sapiens]	215	21.84	0.07	3.79E-007	X	X	X	X	2 (2)	2				
		FBLN1	2192	fibulin 1	ENSG00000077942	34734062	fibulin-1 isoform C precursor [Homo sapiens]	683	74.46	0.05	5.15E-007							3 (3)	X	X	3
		TGFBI	7045	transforming growth factor, beta-induced, 68k	ENSG00000120708	4507467	transforming growth factor-beta-induced protein ig-h3 precursor [Homo sapiens]	683	74.68	0.02	2.46E-006							X	1 (3)	X	3
NP_005505	1	HLA-B	3106	major histocompatibility complex, class I, B	ENSG00000234745	17986001	major histocompatibility complex, class I, B precursor [Homo sapiens]	362	40.46	0.06	2.64E-006	X	X	2 (2)	X	X	2	X	X	1 (1)	1
NP_005337	4	HSPA1B	3304	heat shock 70kDa protein 1B	ENSG00000204388	167466173	heat shock 70 kDa protein 1A/1B [Homo sapiens]	641	70.05	0.02	2.68E-006							X	1 (2)	X	2
		RPL7A	6130	ribosomal protein L7a	ENSG00000148303	4506661	60S ribosomal protein L7a [Homo sapiens]	266	29.99	0.14	3.51E-006	X	X	X	X	3 (6)	6				
		FNBP1L	54874	formin binding protein 1-like	ENSG00000137942	41581463	formin-binding protein 1-like isoform 2 [Homo sapiens]	547	63.57	0.02	3.83E-006							1 (1)	X	X	1
		TUBAL3	79861	tubulin, alpha-like 3	ENSG00000178462	13376181	tubulin alpha chain-like 3 isoform 1 [Homo sapiens]	446	49.91	0.02	3.85E-006							X	X	1 (2)	2
		COPB1	1315	coatomer protein complex, subunit beta 1	ENSG00000129083	7705369	coatomer subunit beta [Homo sapiens]	953	107.14	0.03	5.50E-006							1 (1)	X	X	1
NP_005403	1	SHMT2	6472	serine hydroxymethyltransferase 2 (mitochon	ENSG00000182199	19923315	serine hydroxymethyltransferase, mitochondrial isoform 1 precursor [Homo sapiens]	504	55.99	0.04	7.76E-006							X	X	2 (3)	3
		SRSF1	6426	serine/arginine-rich splicing factor 1	ENSG00000136450	5902076	serine/arginine-rich splicing factor 1 isoform 1 [Homo sapiens]	248	27.74	0.09	8.56E-006	X	1 (1)	X	X	1 (1)	2				
		CCT7	10574	chaperonin containing TCP1, subunit 7 (eta)	ENSG00000135624	5453607	T-complex protein 1 subunit eta isoform a [Homo sapiens]	543	59.37	0.04	9.08E-006							X	2 (2)	X	2
		COPB2	9276	coatomer protein complex, subunit beta 2 (be	ENSG00000184432	4758032	coatomer subunit beta' [Homo sapiens]	906	102.49	0.01	9.86E-006							1 (2)	X	X	2
		DHX9	1660	DEAH (Asp-Glu-Ala-His) box polypeptide 9	ENSG00000135829	100913206	ATP-dependent RNA helicase A [Homo sapiens]	1270	140.96	0.01	1.27E-005	1 (1)	X	X	X	X	1				
		HLA-A	3105	major histocompatibility complex, class I, A	ENSG00000206503	337752170	HLA class I histocompatibility antigen, A-1 alpha chain precursor [Homo sapiens]	374	42.09	0.03	1.81E-005							X	X	1 (1)	1
NP_006089	1	GNB2L1	10399	guanine nucleotide binding protein (G protein	ENSG00000204628	5174447	guanine nucleotide-binding protein subunit beta-2-like 1 [Homo sapiens]	317	35.08	0.03	1.96E-005	X	X	X	X	1 (1)	1				
NP_005727	1	ACTR1A	10121	ARP1 actin-related protein 1 homolog A, centr	ENSG00000138107	5031569	alpha-centractin [Homo sapiens]	376	42.61	0.03	3.29E-005							X	X	1 (2)	2
		PSMD7	5713	proteasome (prosome, macropain) 26S subuni	ENSG00000103035	25777615	26S proteasome non-ATPase regulatory subunit 7 [Homo sapiens]	324	37.02	0.04	3.95E-005							X	X	1 (1)	1
NP_004108	1	FKBP5	2289	FK506 binding protein 5	ENSG00000096060	4758384	peptidyl-prolyl cis-trans isomerase FKBP5 isoform 1 [Homo sapiens]	457	51.21	0.04	4.31E-005							X	X	2 (2)	2
		HSP90B1	7184	heat shock protein 90kDa beta (Grp94), memb	ENSG00000166598	4507677	endoplasmin precursor [Homo sapiens]	803	92.47	0.01	5.75E-005							1 (2)	X	X	2
		BSG	682	basigin (Ok blood group)	ENSG00000172270	38372919	basigin isoform 1 precursor [Homo sapiens]	385	42.20	0.02	7.55E-005							X	X	1 (1)	1
		FARSA	2193	phenylalanyl-tRNA synthetase, alpha subunit	ENSG00000179115	4758340	phenylalanyl-tRNA synthetase alpha chain [Homo sapiens]	508	57.56	0.03	7.61E-005							X	1 (1)	X	1
		DSC1	1823	desmocollin 1	ENSG00000134765	4826702	desmocollin-1 isoform Dsc1b preproprotein [Homo sapiens]	840	93.83	0.02	1.12E-004	X	X	X	X	1 (1)	1				
		MARS	4141	methionyl-tRNA synthetase	ENSG00000166986	14043022	methionyl-tRNA synthetase, cytoplasmic [Homo sapiens]	900	101.12	0.02	1.14E-004							1 (1)	X	X	1
NP_000192	1	ICAM1	3383	intercellular adhesion molecule 1	ENSG00000090339	167466198	intercellular adhesion molecule 1 precursor [Homo sapiens]	532	57.83	0.02	1.60E-004							1 (2)	X	X	2
		TGM3	7053	transglutaminase 3 (E polypeptide, protein-glu	ENSG00000125780	189458821	protein-glutamine gamma-glutamyltransferase E precursor [Homo sapiens]	693	76.63	0.05	1.81E-004	X	1 (1)	X	1 (1)	X	2	1 (1)	X	X	1
		VIM	7431	vimentin	ENSG00000026025	62414289	vimentin [Homo sapiens]	466	53.65	0.03	2.51E-004							X	X	1 (1)	1

		ITPKB	3707	inositol-trisphosphate 3-kinase B	ENSG00000143772	194272161	inositol-trisphosphate 3-kinase B [Homo sapiens]	946	102.38	0.01	3.51E-004							1 (1)	X	X	1
		ALDH16A1	126133	aldehyde dehydrogenase 16 family, member A	ENSG00000161618	223972651	aldehyde dehydrogenase family 16 member A1 isoform 1 [Homo sapiens]	802	85.13	0.01	4.83E-004							X	1 (1)	X	1
		PGK1	5230	phosphoglycerate kinase 1	ENSG00000102144	4505763	phosphoglycerate kinase 1 [Homo sapiens]	417	44.61	0.06	5.14E-004							X	X	2 (2)	2
		RPL3	6122	ribosomal protein L3	ENSG00000100316	4506649	60S ribosomal protein L3 isoform a [Homo sapiens]	403	46.11	0.02	8.63E-004							X	X	1 (2)	2
		AP2A1	160	adaptor-related protein complex 2, alpha 1 sul	ENSG00000196961	19913414	AP-2 complex subunit alpha-1 isoform 1 [Homo sapiens]	977	107.54	0.01	1.02E-003							X	1 (1)	X	1
		ARG1	383	arginase, liver	ENSG00000118520	10947139	arginase-1 [Homo sapiens]	322	34.73	0.03	2.05E-003	X	X	X	X	1 (1)	1	1 (1)	X	X	1
NP_944492	1	TSPAN3	10099	tetraspanin 3	ENSG00000140391	5032201	tetraspanin-3 isoform 1 [Homo sapiens]	253	28.02	0.03	2.18E-003							X	X	1 (2)	2
		GC	2638	group-specific component (vitamin D binding p	ENSG00000145321	32483410	vitamin D-binding protein isoform 1 precursor [Homo sapiens]	474	52.92	0.02	2.34E-003							X	X	1 (2)	2
NP_001001521	3	UGP2	7360	UDP-glucose pyrophosphorylase 2	ENSG00000169764	48255966	UTP--glucose-1-phosphate uridylyltransferase isoform a [Homo sapiens]	508	56.94	0.02	2.78E-003							X	X	1 (1)	1
		AP2A2	161	adaptor-related protein complex 2, alpha 2 sul	ENSG00000183020	27477041	AP-2 complex subunit alpha-2 [Homo sapiens]	939	103.96	0.01	2.98E-003							1 (1)	X	X	1
		LTF	4057	lactotransferrin	ENSG00000012223	54607120	lactotransferrin isoform 1 precursor [Homo sapiens]	710	78.18	0.01	3.11E-003							1 (1)	X	X	1
		CATSPER4	378807	cation channel, sperm associated 4	ENSG00000188782	62988334	cation channel sperm-associated protein 4 [Homo sapiens]	472	54.09	0.06	3.90E-003	X	X	1 (1)	X	1 (1)	2				
		CAGE1	285782	cancer antigen 1	ENSG00000164304	45505178	cancer-associated gene 1 protein isoform 3 [Homo sapiens]	641	74.69	0.02	4.01E-003							1 (2)	X	X	2
		CCT4	10575	chaperonin containing TCP1, subunit 4 (delta)	ENSG00000115484	38455427	T-complex protein 1 subunit delta [Homo sapiens]	539	57.92	0.02	4.25E-003							X	1 (2)	X	2
		EIF4A1	1973	eukaryotic translation initiation factor 4A1	ENSG00000161960	4503529	eukaryotic initiation factor 4A-I isoform 1 [Homo sapiens]	406	46.15	0.03	6.19E-003							X	X	1 (1)	1
NP_002955	6	S100A8	6279	S100 calcium binding protein A8	ENSG00000143546	21614544	protein S100-A8 [Homo sapiens]	93	10.83	0.12	7.90E-003							1 (1)	X	X	1
		PAH	5053	phenylalanine hydroxylase	ENSG00000171759	4557819	phenylalanine-4-hydroxylase [Homo sapiens]	452	51.86	0.07	8.52E-003	1 (1)	X	X	X	X	1				
		RPL10A	4736	ribosomal protein L10a	ENSG00000198755	15431288	60S ribosomal protein L10a [Homo sapiens]	217	24.83	0.13	8.85E-003	X	X	X	X	3 (4)	4				
NP_001002857	29	ANXA2	302	annexin A2	ENSG00000182718	4757756	annexin A2 isoform 2 [Homo sapiens]	339	38.60	0.06	9.14E-003							X	X	2 (2)	2
		PAICS	10606	phosphoribosylaminoimidazole carboxylase, p	ENSG00000128050	5453539	multifunctional protein ADE2 isoform 2 [Homo sapiens]	425	47.08	0.03	9.22E-003							X	X	1 (1)	1
NP_001054	12	TF	7018	transferrin	ENSG00000091513	4557871	serotransferrin precursor [Homo sapiens]	698	77.05	0.02	9.60E-003							X	1 (1)	X	1
		RPS27A	6233	ribosomal protein S27a	ENSG00000143947	4506713	ubiquitin-40S ribosomal protein S27a precursor [Homo sapiens]	156	17.96	0.12	9.83E-003	X	X	X	X	1 (1)	1	X	1 (1)	2 (2)	3
		FLT1	2321	fms-related tyrosine kinase 1 (vascular endoth	ENSG00000102755	156104876	vascular endothelial growth factor receptor 1 isoform 1 precursor [Homo sapiens]	1338	150.76	0.01	9.94E-003							1 (1)	X	X	1
		CD99	4267	CD99 molecule	ENSG00000002586	4505183	CD99 antigen isoform a precursor [Homo sapiens]	185	18.85	0.05	1.13E-002	X	X	X	1 (1)	X	1				
NP_000691	19	ANXA1	301	annexin A1	ENSG00000135046	4502101	annexin A1 [Homo sapiens]	346	38.71	0.02	1.17E-002	X	X	X	X	1 (1)	1				
		KIF11	3832	kinesin family member 11	ENSG00000138160	13699824	kinesin-like protein KIF11 [Homo sapiens]	1056	119.16	0.01	1.20E-002							3 (3)	X	X	3
NP_795352	7	INADL	10207	InaD-like (Drosophila)	ENSG00000132849	112382257	inaD-like protein [Homo sapiens]	1801	196.38	0.00	1.41E-002							X	1 (2)	X	2
		FBLN7	129804	fibulin 7	ENSG00000144152	189491645	fibulin-7 isoform 1 [Homo sapiens]	439	47.38	0.02	1.58E-002							X	X	1 (1)	1
		NOTCH3	4854	notch 3	ENSG00000074181	134244285	neurogenic locus notch homolog protein 3 precursor [Homo sapiens]	2321	243.63	0.00	1.61E-002							X	1 (3)	X	3
		TBC1D1	23216	TBC1 (tre-2/USP6, BUB2, cdc16) domain family	ENSG00000065882	50658061	TBC1 domain family member 1 [Homo sapiens]	1168	133.08	0.02	1.80E-002							1 (2)	X	X	2
		TXLNA	200081	taxilin alpha	ENSG00000084652	28460688	alpha-taxilin [Homo sapiens]	546	61.89	0.03	2.07E-002							X	X	1 (1)	1
		ACSS2	55902	acyl-CoA synthetase short-chain family membe	ENSG00000131069	8923896	acetyl-coenzyme A synthetase, cytoplasmic isoform 1 [Homo sapiens]	701	78.58	0.01	2.21E-002							X	1 (2)	X	2
		PLCB4	5332	phospholipase C, beta 4	ENSG00000101333	289547591	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-4 isoform b [Homo sapiens]	1175	134.46	0.01	2.27E-002							1 (1)	X	X	1
		C2orf73	129852	chromosome 2 open reading frame 73	ENSG00000177994	154240728	hypothetical protein LOC129852 [Homo sapiens]	287	32.14	0.04	2.35E-002							1 (1)	X	X	1
		ZNF132	7691	zinc finger protein 132	ENSG00000131849	154800449	zinc finger protein 132 [Homo sapiens]	706	80.62	0.02	2.82E-002	1 (1)	X	X	X	X	1				
NP_037506	42	PDCD6IP	10015	programmed cell death 6 interacting protein	ENSG00000170248	22027538	programmed cell death 6-interacting protein isoform 1 [Homo sapiens]	868	96.02	0.04	3.03E-002	X	X	X	X	1 (1)	1	1 (1)	X	X	1
		RTN4	57142	reticulon 4	ENSG00000115310	24431933	reticulon-4 isoform B [Homo sapiens]	373	40.32	0.04	3.06E-002							X	X	1 (1)	1
		ECSCR	641700	endothelial cell surface expressed chemotaxis	ENSG00000249751	118130809	endothelial cell-specific chemotaxis regulator precursor [Homo sapiens]	205	21.30	0.08	3.21E-002							1 (1)	X	X	1
NP_005989	3	CCT3	7203	chaperonin containing TCP1, subunit 3 (gamm	ENSG00000163468	63162572	T-complex protein 1 subunit gamma isoform a [Homo sapiens]	545	60.53	0.02	3.64E-002							X	1 (1)	X	1
		CPN2	1370	carboxypeptidase N, polypeptide 2	ENSG00000178772	256217721	carboxypeptidase N subunit 2 [Homo sapiens]	545	60.59	0.02	3.76E-002							1 (1)	X	X	1
		ANO2	57101	anoctamin 2	ENSG00000047617	515870434	anoctamin-2 isoform 1 [Homo sapiens]	998	113.49	0.01	4.02E-002							1 (1)	X	X	1
		PRDX2	7001	peroxiredoxin 2	ENSG00000167815	32189392	peroxiredoxin-2 isoform a [Homo sapiens]	198	21.89	0.05	4.07E-002	X	X	X	X	1 (2)	2				
		TNRC6A	27327	trinucleotide repeat containing 6A	ENSG00000090905	116805348	trinucleotide repeat-containing gene 6A protein [Homo sapiens]	1962	210.30	0.00	4.09E-002							X	1 (5)	X	5
		AQR	9716	aquarius homolog (mouse)	ENSG00000021776	38788372	intron-binding protein aquarius [Homo sapiens]	1485	171.29	0.01	4.17E-002							1 (2)	X	X	2
		SERPINB12	89777	serpin peptidase inhibitor, clade B (ovalbumin	ENSG00000166634	17998551	serpin B12 [Homo sapiens]	405	46.28	0.04	4.44E-002							1 (1)	X	X	1
NP_003361	1	VASP	7408	vasodilator-stimulated phosphoprotein	ENSG00000125753	4507869	vasodilator-stimulated phosphoprotein [Homo sapiens]	380	39.83	0.04	4.45E-002							X	X	1 (1)	1
		C6orf222	389384	chromosome 6 open reading frame 222	ENSG00000189325	285002257	hypothetical protein LOC389384 [Homo sapiens]	652	71.93	0.03	4.46E-002	X	1 (1)	X	X	X	1				
		FILIP1	27145	filamin A interacting protein 1	ENSG00000118407	31542634	filamin-A-interacting protein 1 [Homo sapiens]	1213	138.10	0.01	4.81E-002							1 (1)	X	X	1
NP_002435	27	MSN	4478	moesin	ENSG00000147065	4505257	moesin [Homo sapiens]	577	67.82	0.02	7.79E-002							X	1 (1)	X	1
NP_001002236	4	SERPINA1	5265	serpin peptidase inhibitor, clade A (alpha-1 an	ENSG00000197249	50363217	alpha-1-antitrypsin precursor [Homo sapiens]	418	46.74	0.02	1.07E-001							X	1 (1)	X	1
NP_000421	1	PAFAH1B1	5048	platelet-activating factor acetylhydrolase 1b, r	ENSG00000007168	4557741	platelet-activating factor acetylhydrolase IB subunit alpha [Homo sapiens]	410	46.64	0.04	2.25E-001							X	X	1 (1)	1
NP_002797	1	PSMC6	5706	proteasome (prosome, macropain) 26S subuni	ENSG00000100519	195539395	26S protease regulatory subunit 10B [Homo sapiens]	403	45.80	0.05	1.11E+000	1 (1)	X	X	X	X	1				
NP_003352	35	UMOD	7369	uromodulin	ENSG00000169344	56550049	uromodulin precursor [Homo sapiens]	640	69.76	0.01	1.46E+000							1 (1)	X	X	1
NP_001006	1	RPS11	6205	ribosomal protein S11	ENSG00000142534	4506681	40S ribosomal protein S11 [Homo sapiens]	158	18.43	0.17	1.49E+000							1 (1)	X	X	1
NP_002551	2	P2RX4	5025	purinergic receptor P2X, ligand-gated ion chan	ENSG00000135124	28416927	P2X purinoceptor 4 [Homo sapiens]	388	43.37	0.06	1.59E+000							X	1 (1)	X	1

NP_003581	1	CUL3	8452	cullin 3	ENSG00000036257	4503165	cullin-3 [Homo sapiens]	768	88.93	0.02	1.59E+000							X	X	1 (1)	1
NP_055814	2	RAB21	23011	RAB21, member RAS oncogene family	ENSG00000080371	7661922	ras-related protein Rab-21 [Homo sapiens]	225	24.35	0.09	1.81E+000	X	X	X	X	1 (1)	1				
NP_000090	1	CST3	1471	cystatin C	ENSG00000101439	4503107	cystatin-C precursor [Homo sapiens]	146	15.80	0.10	1.85E+000							1 (1)	X	X	1
NP_001072	104	CUBN	8029	cubilin (intrinsic factor-cobalamin receptor)	ENSG00000107611	126091152	cubilin precursor [Homo sapiens]	3623	398.74	0.00	2.21E+000							1 (1)	X	X	1
NP_542416	9	PTPN13	5783	protein tyrosine phosphatase, non-receptor ty	ENSG00000163629	5453992	tyrosine-protein phosphatase non-receptor type 13 isoform 2 [Homo sapiens]	2466	274.93	0.01	2.56E+000							1 (1)	X	X	1
NP_006316	2	RAN	5901	RAN, member RAS oncogene family	ENSG00000132341	5453555	GTP-binding nuclear protein Ran [Homo sapiens]	216	24.42	0.05	3.17E+000	X	X	X	X	1 (1)	1				
NP_775656	1	PDE8A	5151	phosphodiesterase 8A	ENSG00000073417	27734721	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A isoform	829	93.30	0.02	3.62E+000	X	X	X	X	1 (1)	1				
NP_004466	9	FLOT2	2319	flotillin 2	ENSG00000132589	94538362	flotillin-2 [Homo sapiens]	428	47.06	0.03	4.78E+000							X	X	1 (1)	1

Table S2

KEGG Pathway Name	Adjusted P Value	#Gene	EntrezGene	Statistics
Ribosome	2.18E-10	12	6130 6122 6191 6233 6129 6132 6194 6124 4736 6188 6175 3921	C=89;O=12;E=0.68;R=17.64;rawP=3.69e-12;adjP=2.18e-10
Phagosome	3.30E-10	14	718 3106 7280 10383 51807 84790 715 79861 60 3107 84617 203068 3105 7057	C=152;O=14;E=1.16;R=12.05;rawP=1.12e-11;adjP=3.30e-10
Pathogenic Escherichia coli infection	1.71E-07	8	7280 10383 51807 60 79861 84790 84617 203068	C=55;O=8;E=0.42;R=19.03;rawP=8.71e-09;adjP=1.71e-07
Gap junction	5.99E-06	8	5332 84790 79861 7280 84617 10383 203068 51807	C=89;O=8;E=0.68;R=11.76;rawP=4.06e-07;adjP=5.99e-06
Antigen processing and presentation	2.21E-05	7	3106 3107 3320 3326 3105 3312 3304	C=76;O=7;E=0.58;R=12.05;rawP=1.87e-06;adjP=2.21e-05
Endocytosis	3.38E-05	10	3106 161 10015 160 1213 2321 3312 3107 3105 3304	C=201;O=10;E=1.54;R=6.51;rawP=3.44e-06;adjP=3.38e-05
Glycolysis / Gluconeogenesis	8.68E-05	6	2597 5315 5230 2023 55902 226	C=65;O=6;E=0.50;R=12.08;rawP=1.03e-05;adjP=8.68e-05
Focal adhesion	2.00E-04	9	7408 2335 2321 81 87 60 2316 3371 7057	C=200;O=9;E=1.53;R=5.89;rawP=2.39e-05;adjP=0.0002
Staphylococcus aureus infection	4.00E-04	5	718 716 715 1828 3383	C=55;O=5;E=0.42;R=11.90;rawP=6.24e-05;adjP=0.0004
Viral myocarditis	1.20E-03	5	3106 60 3107 3105 3383	C=70;O=5;E=0.53;R=9.35;rawP=0.0002;adjP=0.0012

Wiki Pathway Name	Adjusted P Value	#Gene	EntrezGene	Statistics
Parkin-Ubiquitin Proteasomal System pathway	2.49E-13	13	5708 7280 3313 10383 3312 51807 5713 79861 84790 84617 5705 203068 3304	C=70;O=13;E=0.53;R=24.30;rawP=5.93e-15;adjP=2.49e-13
Cytoplasmic Ribosomal Proteins	6.74E-11	12	6191 6122 6130 6233 6194 6132 6129 6124 6188 4736 6175 3921	C=88;O=12;E=0.67;R=17.84;rawP=3.21e-12;adjP=6.74e-11
Pathogenic Escherichia coli infection	4.19E-07	8	7280 10383 51807 84790 79861 60 84617 203068	C=64;O=8;E=0.49;R=16.36;rawP=2.99e-08;adjP=4.19e-07
Proteasome Degradation	9.00E-05	6	5708 3106 5713 3107 5705 3105	C=63;O=6;E=0.48;R=12.46;rawP=8.57e-06;adjP=9.00e-05
Glycolysis and Gluconeogenesis	3.00E-04	5	2597 5230 226 5315 2023	C=50;O=5;E=0.38;R=13.09;rawP=3.92e-05;adjP=0.0003
Focal Adhesion	6.00E-04	8	7408 2335 2321 60 87 2316 3371 7057	C=185;O=8;E=1.41;R=5.66;rawP=9.05e-05;adjP=0.0006
Complement Activation, Classical Pathway	1.80E-03	3	718 715 716	C=17;O=3;E=0.13;R=23.09;rawP=0.0003;adjP=0.0018
Translation Factors	2.80E-03	4	1938 1973 1937 1915	C=51;O=4;E=0.39;R=10.26;rawP=0.0006;adjP=0.0028
Complement and Coagulation Cascades	2.80E-03	4	718 2 715 716	C=51;O=4;E=0.39;R=10.26;rawP=0.0006;adjP=0.0028
Arrhythmogenic right ventricular cardiomyopathy	5.90E-03	5	3728 81 87 60 1832	C=108;O=5;E=0.83;R=6.06;rawP=0.0014;adjP=0.0059

Pathway Commons Name	Adjusted P Value	#Gene	EntrezGene	Statistics
Metabolism of proteins	2.91E-19	26	6122 7280 10383 6132 6129 7203 1973 84617 4736 1937 1915 10694 6175 3921 10575 6191 22948 6130 1938 6233 6194 60 4854 10574 6124 6188	C=260;O=26;E=1.99;R=13.09;rawP=9.98e-22;adjP=2.91e-19
Eukaryotic Translation Elongation	1.77E-14	15	6122 6132 6129 4736 1937 1915 6175 3921 6191 6130 1938 6233 6194 6124 6188	C=86;O=15;E=0.66;R=22.82;rawP=1.21e-16;adjP=1.77e-14
Translation	7.14E-14	16	6122 6132 6129 1973 4736 1937 1915 6175 3921 6191 6130 1938 6233 6194 6124 6188	C=118;O=16;E=0.90;R=17.74;rawP=7.34e-16;adjP=7.14e-14
Peptide chain elongation	1.25E-13	14	6122 6132 6129 4736 1915 6175 3921 6191 6130 1938 6233 6194 6124 6188	C=83;O=14;E=0.63;R=22.07;rawP=2.14e-15;adjP=1.25e-13
Proteoglycan syndecan-mediated signaling events	1.25E-13	42	3178 7184 2335 3728 10096 2 682 383 3371 7018 3383 3105 7057 7408 1938 3491 6194 3320 2023 3837 4907 2597 3312 1973 87 10399 2316 5315 1832 7520 3304 197 8607 5230 160 1213 226 58 81 2289 1191 10856	C=1345;O=42;E=10.28;R=4.09;rawP=1.74e-15;adjP=1.25e-13
Integrin family cell surface interactions	1.96E-13	42	3178 7184 2335 3728 10096 2 383 3371 7018 3383 3105 7057 7408 1938 3491 6194 7052 3320 2023 3837 4907 718 2597 3312 87 1973 7045 5315 1832 3304 7520 197 8607 5230 160 1213 226 58 81 2289 1191 10856	C=1378;O=42;E=10.53;R=3.99;rawP=4.03e-15;adjP=1.96e-13
Influenza Life Cycle	3.06E-13	16	6122 6194 3320 3837 6132 6129 4736 6175 3921 3304 6130 6191 6233 1213 6124 6188	C=136;O=16;E=1.04;R=15.40;rawP=7.33e-15;adjP=3.06e-13
Beta1 integrin cell surface interactions	3.94E-13	41	3178 7184 2335 3728 10096 2 383 3371 7018 3383 3105 7057 7408 1938 3491 6194 7052 3320 2023 3837 4907 2597 3312 1973 87 7045 5315 1832 3304 7520 197 8607 5230 160 1213 226 58 81 2289 1191 10856	C=1351;O=41;E=10.32;R=3.97;rawP=1.08e-14;adjP=3.94e-13
Influenza Infection	4.25E-13	16	6122 6194 3320 3837 6132 6129 4736 6175 3921 3304 6130 6191 6233 1213 6124 6188	C=141;O=16;E=1.08;R=14.85;rawP=1.31e-14;adjP=4.25e-13
Gene Expression	4.61E-13	23	6122 3178 1660 1937 1915 2193 1938 6194 4904 4854 6132 6129 1973 4141 4736 6175 3921 6426 6191 6130 6233 6124 6188	C=379;O=23;E=2.90;R=7.94;rawP=1.58e-14;adjP=4.61e-13

Supplemental Table S1. Exosome Proteomics from Human Proximal Tubule Cell Lines. A list of the 155 distinct proteins identified with high confidence from isolated exosomes. Five gel bands from the “i16” and three gel bands from the “i22” human proximal tubule cell lines were processed as described in Methods. The proteins are sorted in order of a calculated “Protein pValue” that represents the product of the best (smallest) OMSSA E-Value for each of the unique peptides identified for that protein. The “Protein pValue” cutoff for this list represents a 0.8% FDR as determined from the rate of reversed-sequence hits. The “Spectrum Count” tabulates all of the MS^2 scans that had an OMSSA peptide match for the protein across all of the samples for that particular cell line; the listing under each gel band breaks this number down by indicating the number of unique peptides and (in parentheses) the total number of times (scans) these peptides were observed in that sample. The first two columns on the left indicate whether the protein was also observed in the urine exosome database (1) and, if so, how many peptides were identified.

Supplemental Table S2. Gestalt Analysis of Exosome Proteomics from Human Proximal Tubule Cell Lines. The KEGG, Wiki and Pathway analysis results obtained for the 155 distinct “high-confidence” proteins that were identified from isolated exosomes that are listed in the Supplemental File “Exosome Proteomics from Human Proximal Tubule Cell Lines”. The Gene Set Analysis Toolkit (Gestalt) from WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt/) (2, 3) was used with the corresponding Entrez Gene ID for the proteins.

(1) Wang Z, Hill S, Luther JM, Hachey DL, Schey KL. (2012). Proteomic analysis of urine exosomes by multidimensional protein identification technology (MudPIT). Proteomics, 12: 329-338.

(2) Zhang, B., Kirov, S.A., Snoddy, J.R. (2005). WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res, 33(Web Server issue): W741-748.

(3) Wang, J., Duncan, D., Shi, Z., Zhang, B. (2013). WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. Nucleic Acids Res, 41 (Web Server issue): W77-83.