

Supplemental Table I

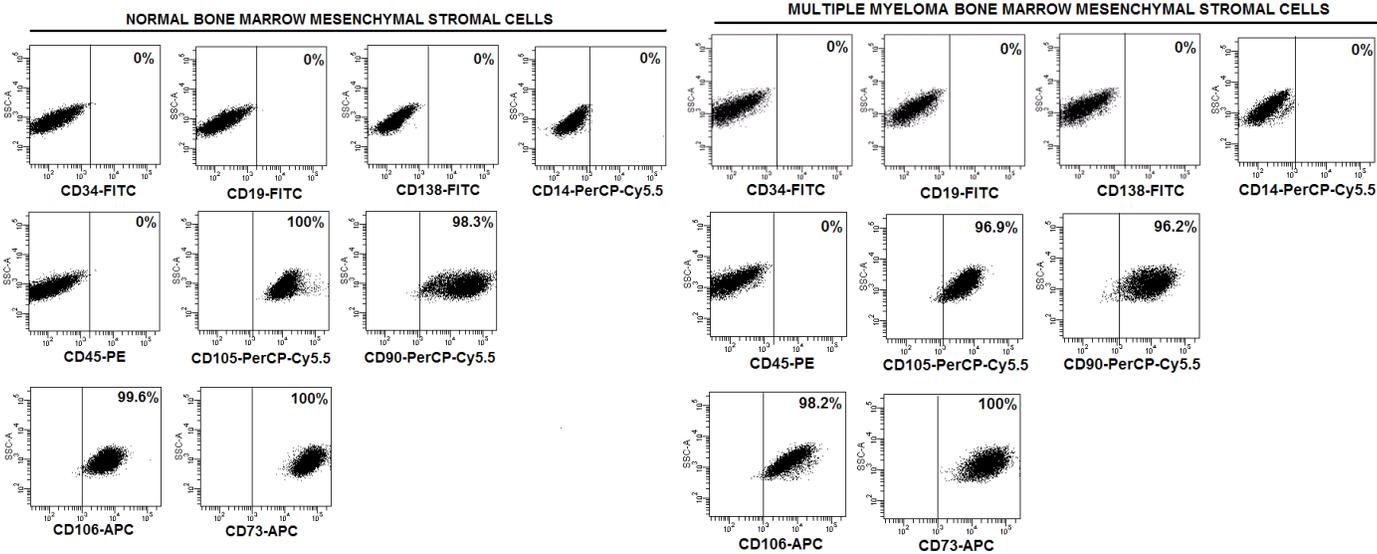
**Phenotype of Bone Marrow Mesenchymal Stromal Cells (BM-MSCs)  
(%)**

	<b>NBM1</b>	<b>NBM2</b>	<b>NBM3</b>	<b>MM1</b>	<b>MM2</b>	<b>MM3</b>
<b>CD14</b>	0.5	0	0.2	0	0.1	0.4
<b>CD34</b>	0	0	0	0	0	0
<b>CD45</b>	0	0	0	0	0	0
<b>CD138</b>	0	0	0	0	0	0
<b>CD73</b>	95.3	100	97.5	100	96.2	100
<b>CD90</b>	96.2	98.3	96.9	96.2	97.2	100
<b>CD105</b>	97.9	100	98.1	96.9	98.6	100
<b>CD106</b>	98.4	99.6	98.3	98.2	95.7	97.8

**Supplemental Table 1. Phenotype of bone marrow mesenchymal stromal cells (BM-MSCs)**

Phenotype of normal (n=3) and MM (n=3) BM-MSCs was evaluated by flow cytometry using anti-CD14, -34, -45, -138, -73, -90, -105, -106 antibodies. Appropriate isotype controls were also included. Both normal and MM MSCs were devoid of hematopoietic cells (CD34-, CD138-, CD45-, CD14-), and positive for the remaining markers, indicating their multipotent mesenchymal stromal cell phenotype.

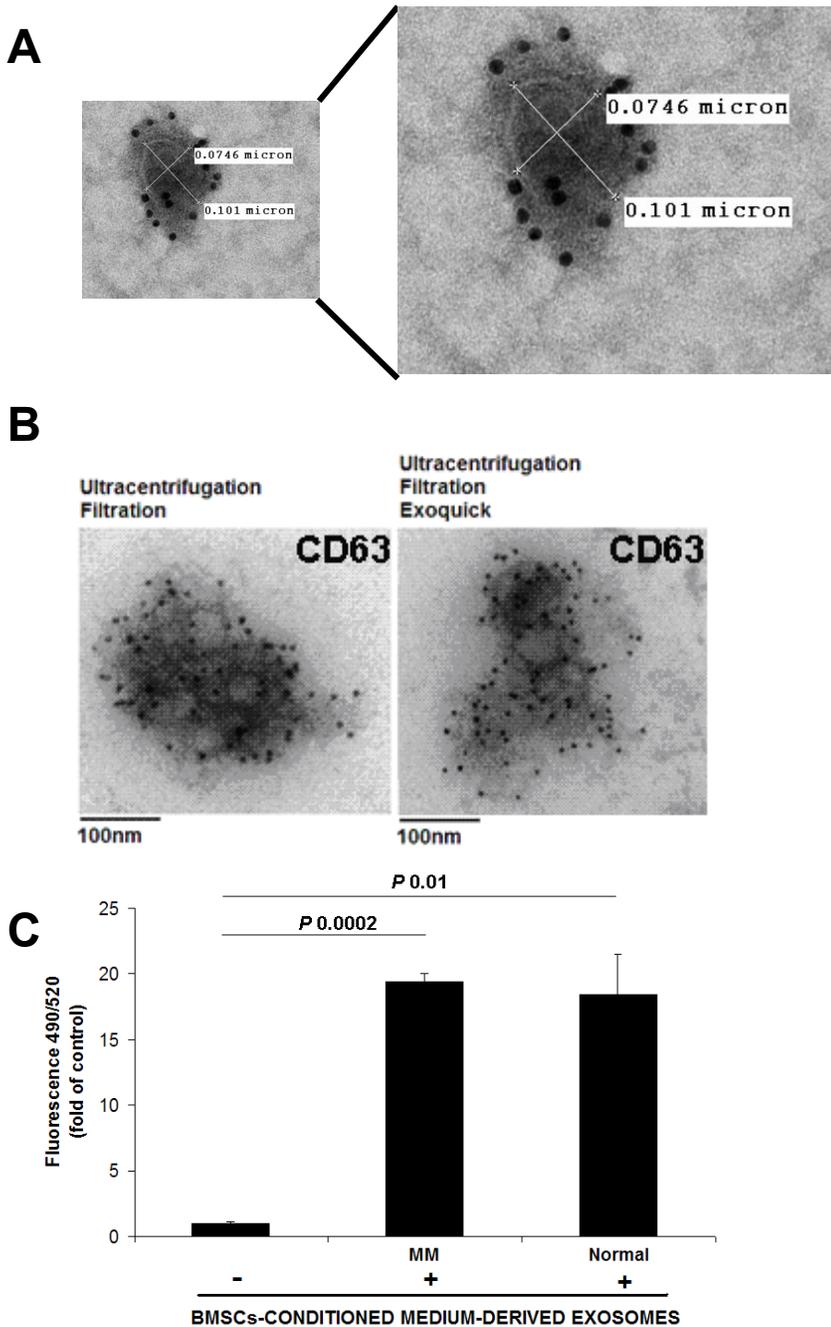
# Supplemental Figure 1



## Supplemental Figure 1. Primary human BM-MSCs: phenotype.

Phenotype of primary multipotent mesenchymal stromal cells have been evaluated by flow cytometry using anti-CD34-FITC, CD19-FITC, CD-138-FITC, CD105-PerCPCy5.5, CD90- PerCPCy5.5, CD14-PerCPCy5.5, CD106-APC, CD73-APC, and CD45-PE antibodies.

## Supplemental Figure 2

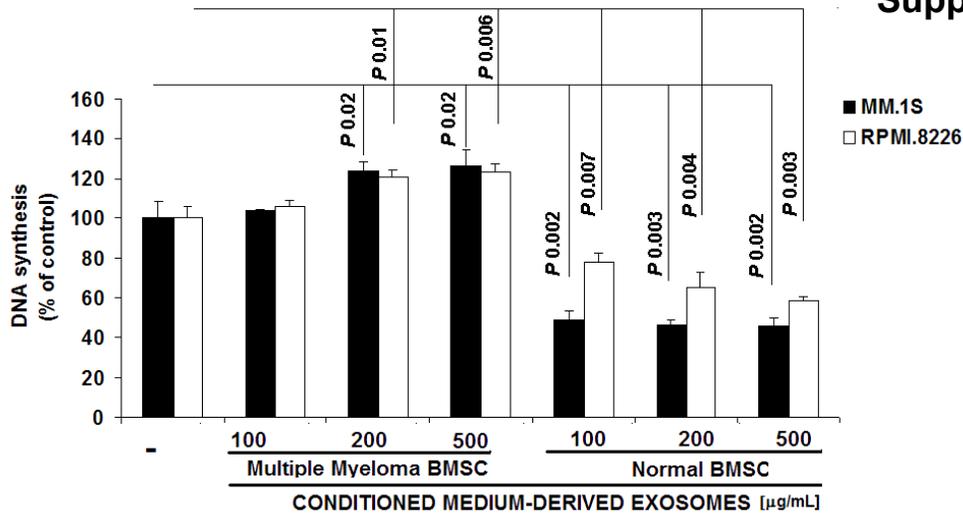


### Supplemental Figure 2. Characterization of bone marrow mesenchymal stromal cells (BM-MSCs)-derived exosomes and their ability to be transferred to MM cells.

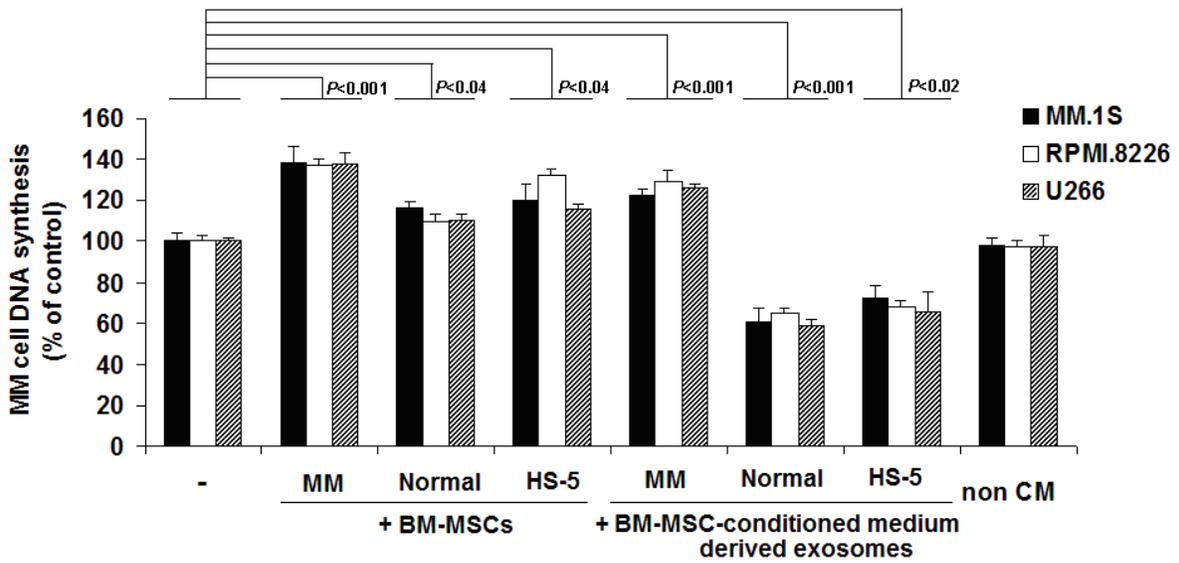
(A) Electron microscopy of MM-BM-MSC-derived exosomes, showing small vesicles of 74.6x100.1nm. Arrows indicate a cell membrane-like bi-layer. (B) Exosomes have been isolated from the conditioned medium of MM-BM-MSCs, using ultracentrifugation, with and without using ExoQuik solution. Exosomes have been immunogold labeled with anti-CD63, showing similar results between the two procedures adopted. The scale bar indicates 100nm. (C) MM.1S cells were cultured in absence (I) or presence of normal (II) or MM-BM-MSCs (III)-derived PKH67-labelled exosomes, for 30 minutes. Uptake of fluorescently labeled exosomes by MM cells was also detected by using a fluorescence plate reader (excitation wavelength 490nm; emission wavelength 520nm). All data represent mean ( $\bar{x}$  S.D.) of triplicate experiments.

Supplemental Figure 3

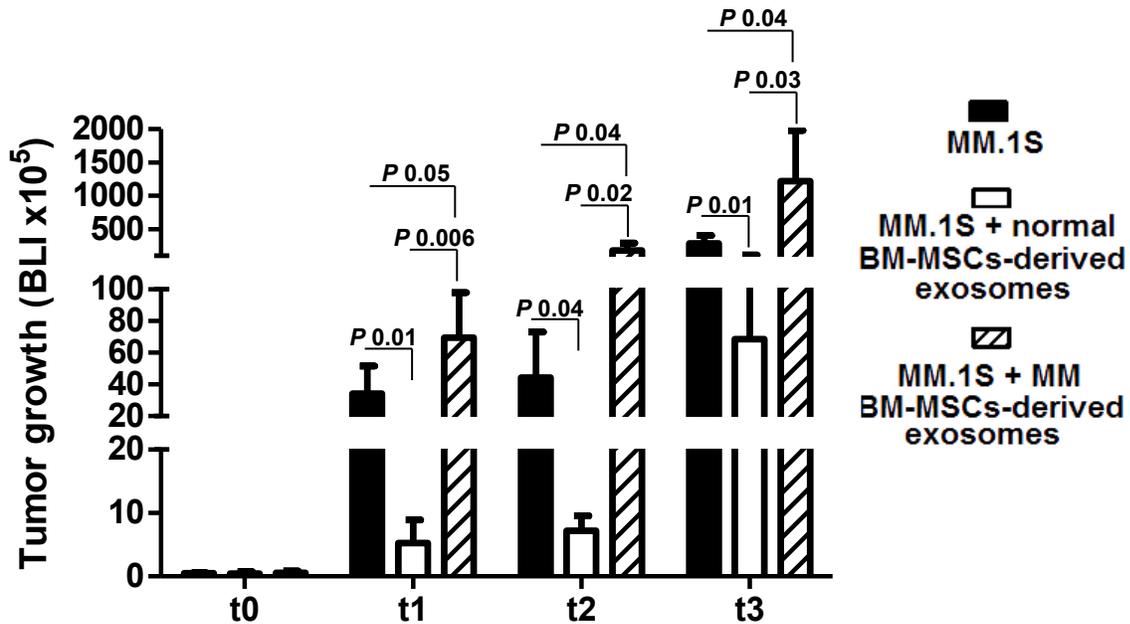
**A**



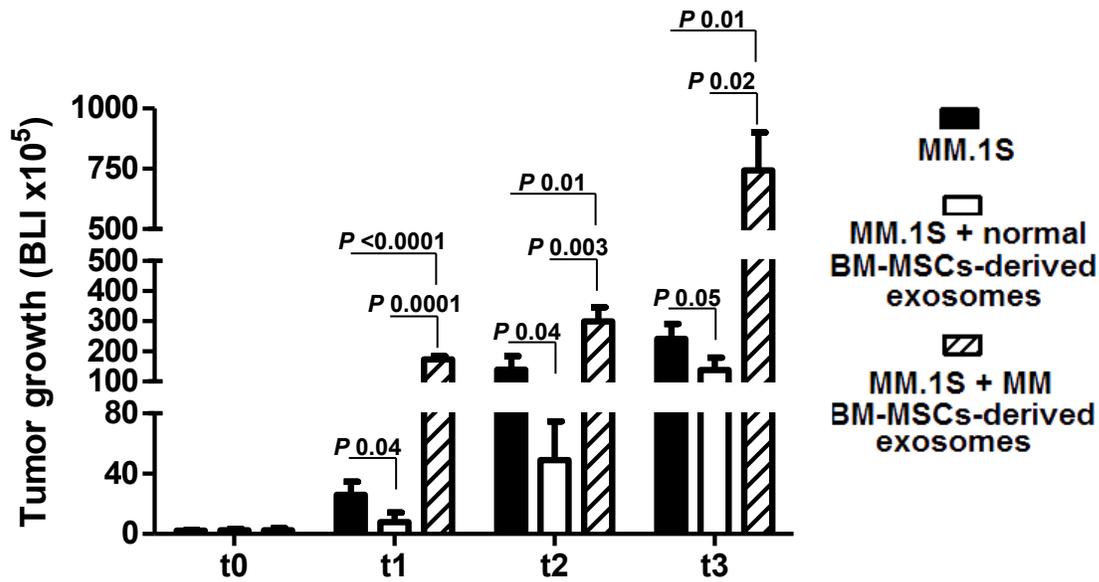
**B**



**C**

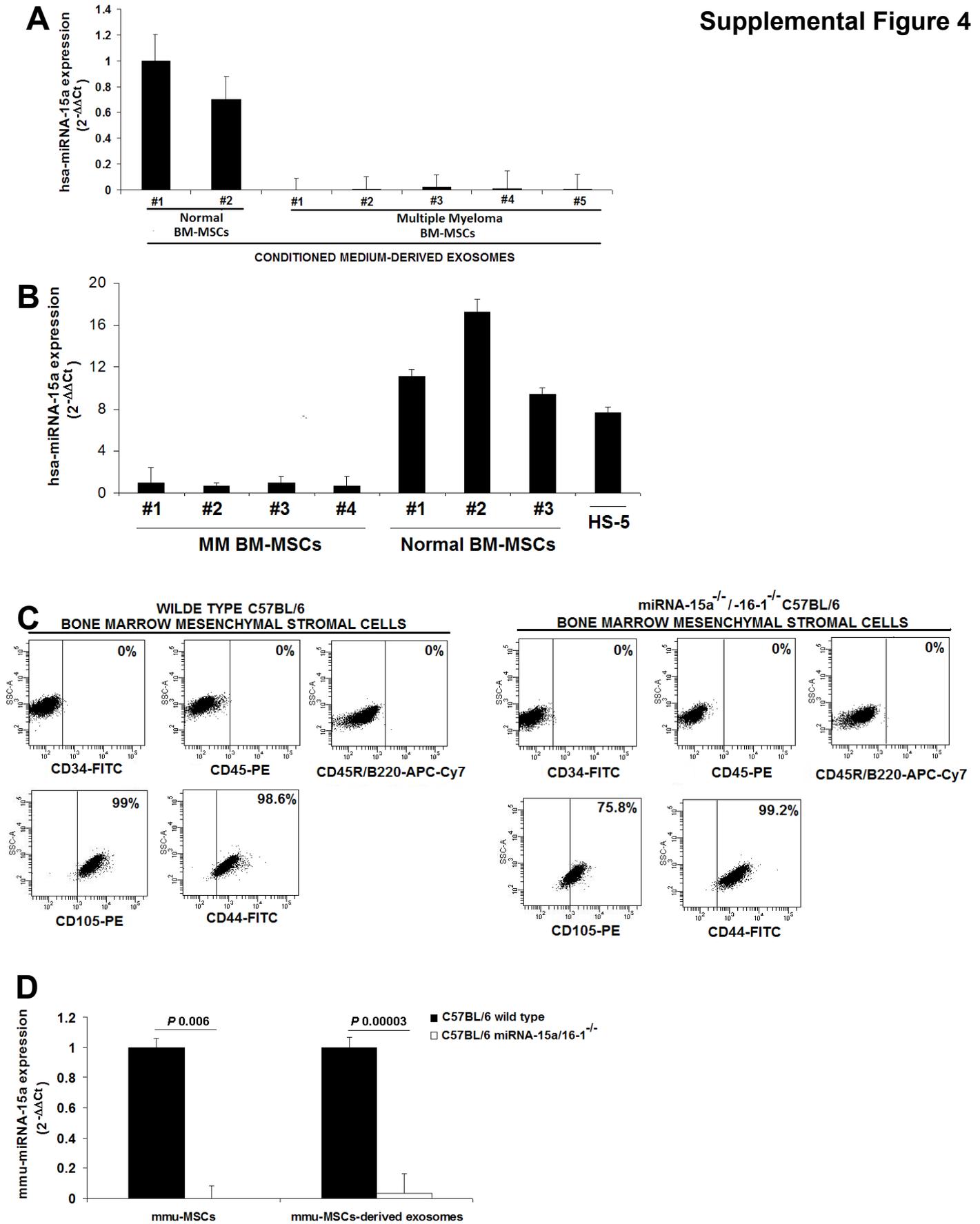


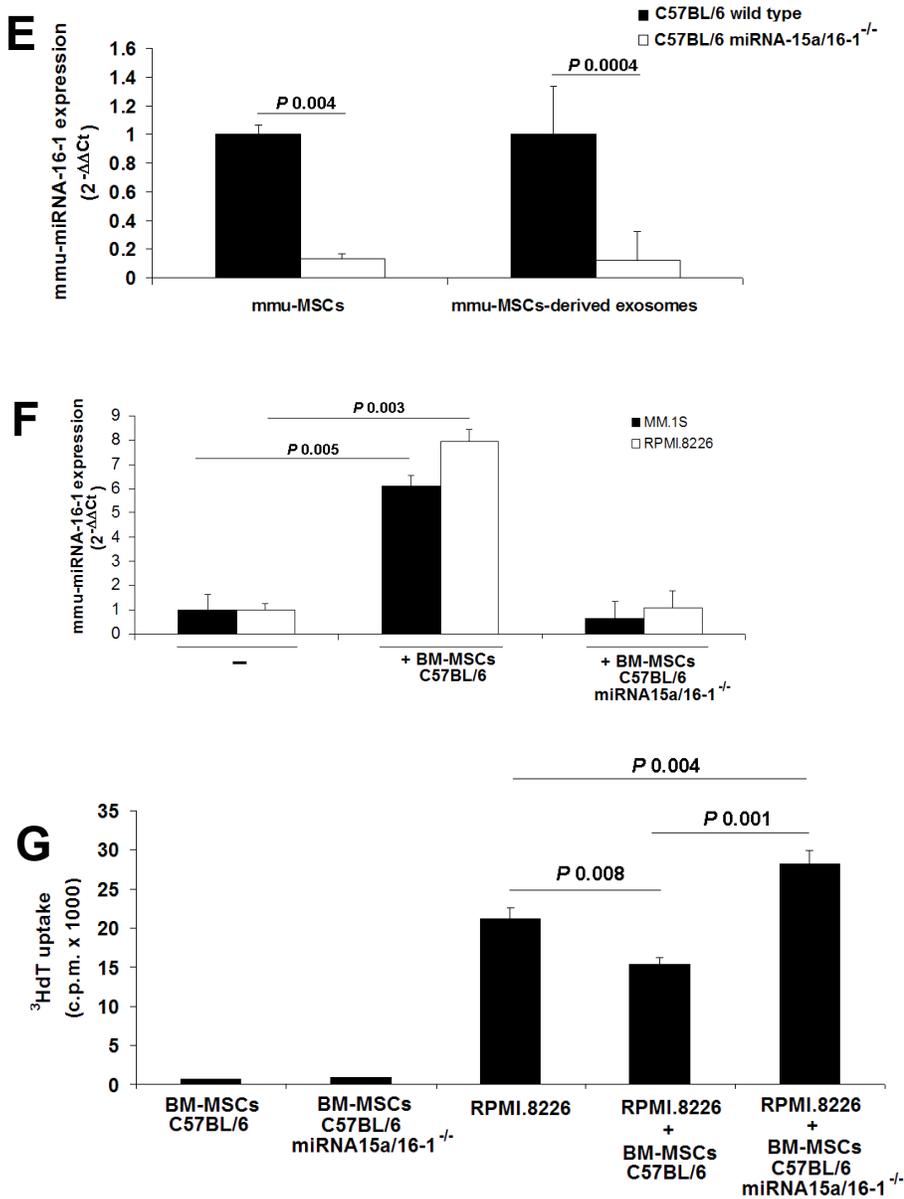
D



**Supplemental Figure 3. Normal and MM BM-MSC-derived exosomes differentially target MM cells in vitro and in vivo.**

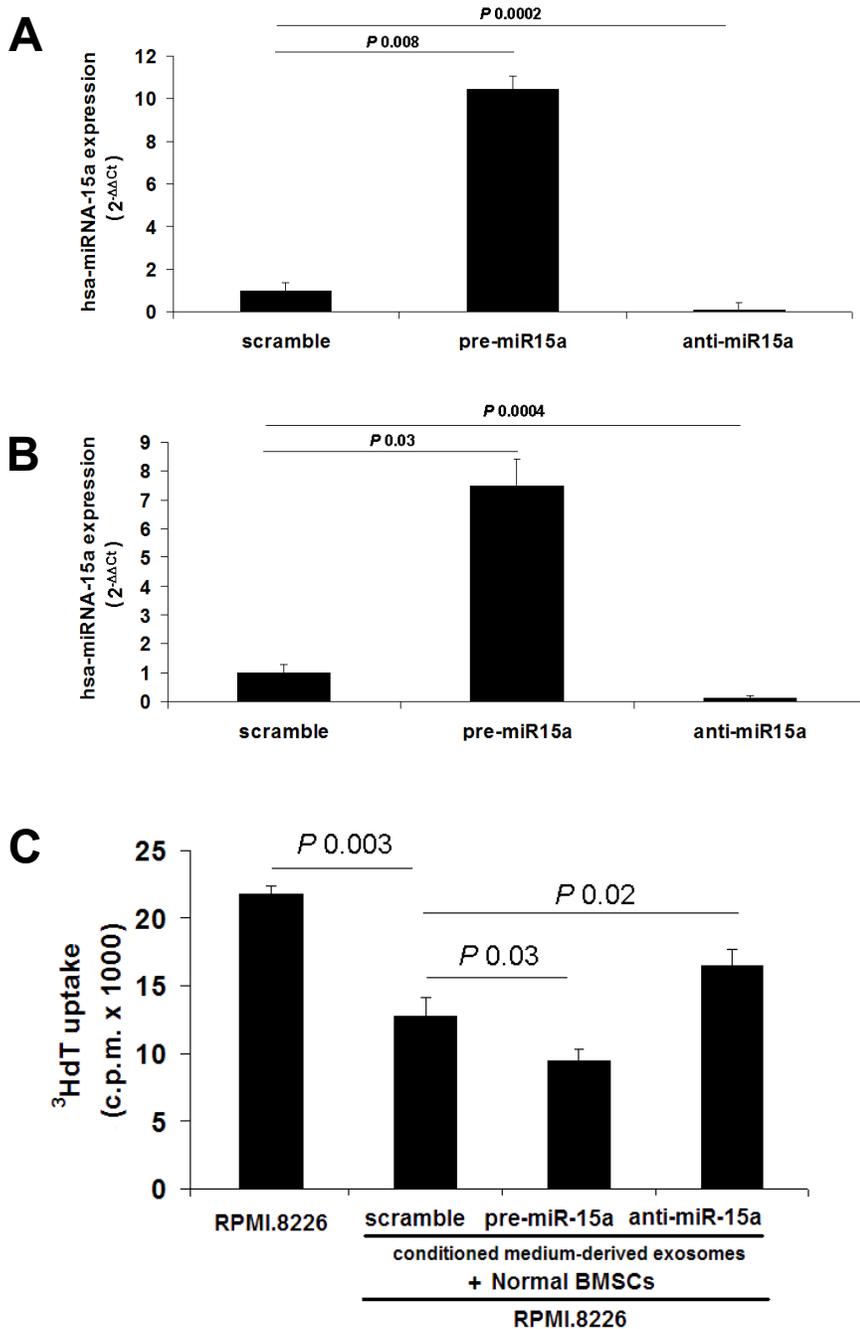
(A) MM cells (MM.1S; RPMI.8226) were cultured in absence (-) or presence of MM or normal BM-MSC-derived exosomes (100-500mg/mL; 48h). Cell proliferation was assessed using [<sup>3</sup>H]-thymidine uptake). (B) MM cells (MM.1S; RPMI.8226; U266) were cultured in absence (-) or presence of MM, normal-BM-MSCs, HS-5 cells; or in presence of MM or normal BM-MSC-derived exosomes (200mg/mL) for 48h. Cell proliferation was assessed using [<sup>3</sup>H]-thymidine uptake. (C; D) Tissue-engineered bones (T.E.B.) were loaded with either MM.1S-GFP+/Luc+ cells alone or with primary MM or normal-BM-MSC-derived exosomes (3x10<sup>6</sup>cells/T.E.B.; 1mg exosomes), and implanted subcutaneously (S.C.) in SCID mice. Detection of tumor growth, as performed by measuring bioluminescence imaging (BLI) intensity, at baseline (t0), day 7 (t1), 10 (t2), and 14 (t3) (in panel C: n=5/each group; in panel D: n=3/each group).





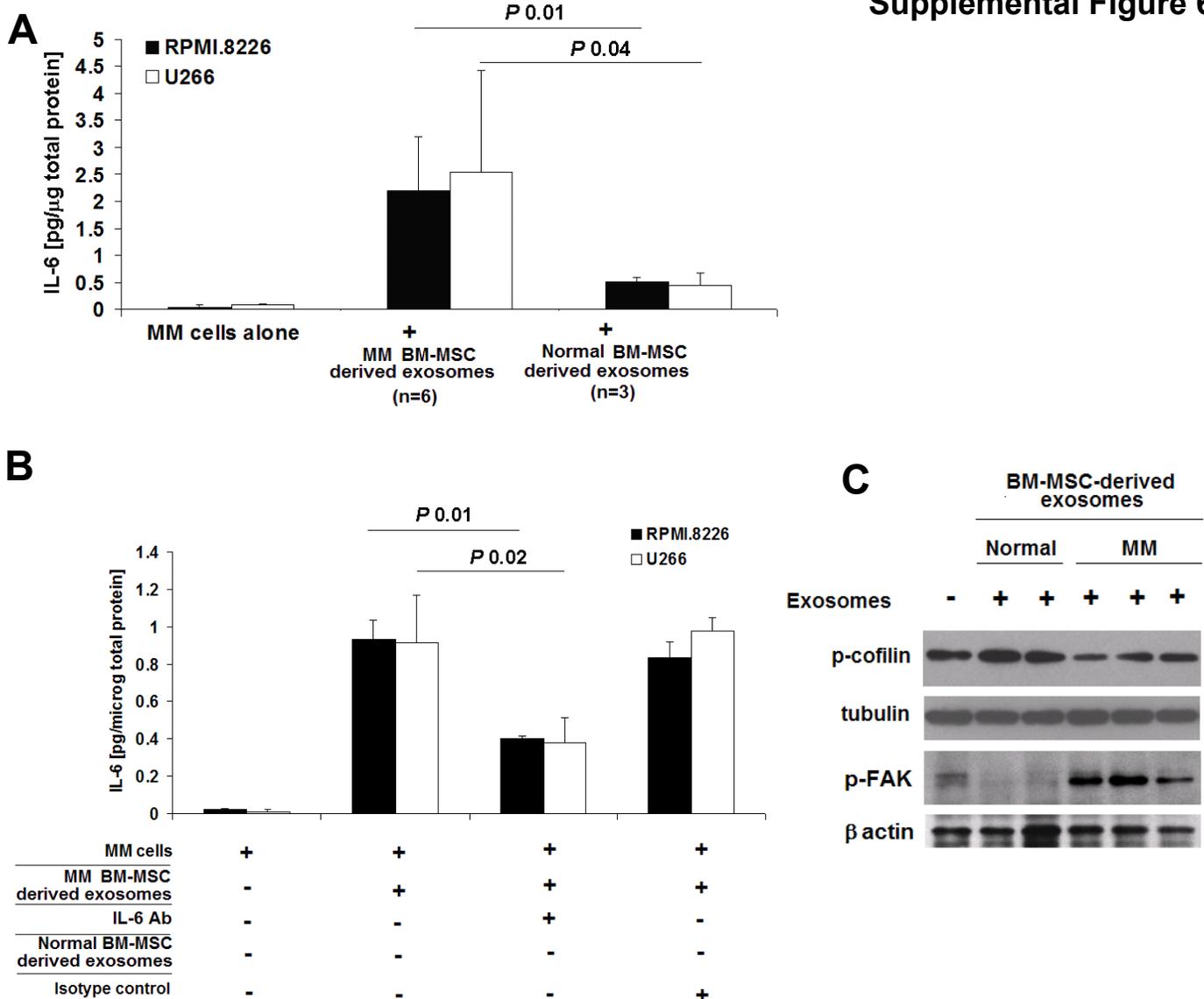
#### Supplemental Figure 4. Detection of miRNA-15a and -16-1 expression.

(A) miRNA-15q levels have been evaluated in primary normal (n=2) and MM (n=5) BM-MSC-derived exosomes, by qRT-PCR using the DDcT method, with normalization to the reference *C. Elegans* miRNA-39, used as spiked control. (B) miRNA-15a expression level has been evaluated by qRT-PCR in primary MM (n=4), normal (n=3) BM-MSCs, and the HS-5 cell line, using the DDcT method, with normalization to the reference RNU6B miRNA. Bars represent S.D. (C) Murine BM-MSCs were isolated from C57BL/6-15a/16-1<sup>-/-</sup> or wild type mice. Phenotype has been evaluated by flow cytometry using anti-CD34-FITC, CD45-PE, CD45R-APC-Cy7, CD105- and CD44-FITC antibodies. (E; F) miRNA-15a and -16-1 expression levels have been evaluated on both BM-MSCs (C57BL/6-15a/16-1<sup>-/-</sup> or wild type) and BM-MSC-derived exosomes (C57BL/6-15a/16-1<sup>-/-</sup> or wild type), by qRT-PCR using the DDcT method with normalization to the reference of *C. Elegans* miRNA-39 or RNU6B, respectively. Bars indicate S.D. (E) Murine exosomes have been isolated from the BM of either C57BL/6 or C57BL/6 miRNA-15a/16-1<sup>-/-</sup> mice; and subsequently added to MM cells (MM.1S; RPMI.8226) for 48 hours. mmu-miRNA-16-1 levels have been evaluated in human MM cells by qRT-PCR using the DDcT method, with normalization to the reference *C. Elegans* miRNA-39, used as spiked control. Bars represent S.D. (F) RPMI.8226 cells were cultured in presence or absence of murine C57BL/6 BM-MSCs (either wild type or miRNA-15a/16-1<sup>-/-</sup>) for 48 hours. Cell proliferation was assessed using [<sup>3</sup>H]-thymidine uptake. Bars indicate S.D.



### Supplemental Figure 5. Functional effect of miRNA-15a on MM cell behavior.

HS-5 cells (**A**) and primary normal BM-MSCs (**B**) have been transfected with either precursor(pre), anti-miRNA-15a, or scrambled probe. Hsa-miRNA-15a expression levels have been evaluated in transfected cells by qRT-PCR, using the DDCT method with normalization to RNU6B. (**C**) Primary normal BM-MSCs were transfected with either scramble, pre-miR-15a, or anti-miR-15a-probe. RPMI.8226 cells were exposed to conditioned medium-derived exosomes isolated from BM-MSC-transfected BM-MSCs for 48 hours. Cell proliferation was assessed using [ $^3\text{H}$ ]-thymidine uptake assay. Bars indicate S.D.



### Supplemental Figure 6. Normal and MM-BM-MSC-derived exosomes differentially impact IL-6 secretion from MM cells.

(A) MM cells (RPMI.8226; U266) were exposed to exosomes isolated from either MM BM-MSCs (n=6) or normal BM-MSCs (n=3), for 24 hours and IL-6 has been measured in conditioned media, using human IL-6 ELISA. IL-6 concentration has been calculated as pg/mg of total protein. Bars indicate S.D. (B) MM cells (RPMI.8226; U266) were exposed to exosomes isolated from either MM BM-MSCs or normal BM-MSCs, for 24 hours and IL-6 was measured in conditioned media, in the presence or absence of IL-6 blocking antibody (0.2mg/mL) using human IL-6 ELISA. IL-6 concentration has been calculated as pg/mg of total protein. Mouse IgG2B isotype control has been used. Bars indicate S.D. (C) MM.1S cells were cultured in absence (-) or presence of normal or MM BM-MSC-derived exosomes (200mg/mL) for 8 hours. Whole cell lysates were subjected to Western blotting using anti-phospho(p)-cofilin, -p-FAK, -β actin and -tubulin antibodies.

**Supplemental table 2: Analysis of two-channel protein expression microarray experiment.**

rank	protein	Exosomal content			Cellular content		
		Log fold change to the base 2	Standard deviation of the log fold change	p-value	Log fold change to the base 2	Standard deviation of the log fold change	p-value
1	fibronectin 1	0.64027	0.50849	0.00696	-0.17826	0.08325	0.04610
2	junction plakoglobin	0.49493	0.37822	0.01406	0.00881	0.01485	0.80700
3	chemokine (C-C motif) ligand 2	0.30280	0.11573	0.00738	0.06264	0.04945	0.34600
4	mitogen-activated protein kinase kinase 5	0.22301	0.24709	0.15144	0.15800	0.46231	0.43500
5	excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)	0.21293	0.09205	0.02965	-0.03803	0.02577	0.42600
6	ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)	0.20581	0.26500	0.19903	-0.20635	0.09204	0.02950
7	DEAH (Asp-Glu-Ala-His) box polypeptide 38	0.20344	0.09891	0.04338	-0.01924	0.06107	0.79200
8	interleukin 13	0.18181	0.06088	0.02310	-0.17327	0.91401	0.54200
9	cell division cycle 27	0.18140	2.48755	0.70696	0.15235	0.94889	0.59800
10	RAP1, GTPase activating protein 1	0.16593	0.24770	0.28143	-0.17462	0.26251	0.25600
11	non-POU domain containing, octamer-binding	0.16317	0.21599	0.25734	0.16096	0.21214	0.24500
12	interleukin 6 (interferon, beta 2)	0.15085	0.04921	0.03454	-0.01480	0.03152	0.77800
13	ribosomal protein S6 kinase, 90kDa, polypeptide 1	0.15061	0.29210	0.36607	0.01729	0.01997	0.68000
14	protein kinase C, beta 1	0.14945	0.21418	0.29642	0.18858	0.03026	0.00122
15	B-cell linker	0.13891	0.10303	0.16578	-0.07027	0.02750	0.16100
16	early endosome antigen 1, 162kD	0.13469	0.05740	0.07592	-0.03913	0.03138	0.45800

		Exosomal content			Cellular content		
rank	protein	Log fold change to the base 2	Standard deviation of the log fold change	p-value	Log fold change to the base 2	Standard deviation of the log fold change	p-value
17	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	0.13156	0.07975	0.13704	0.07113	0.27361	0.64600
18	nucleoporin 88kDa	0.12876	0.64740	0.60146	0.46419	0.41224	0.02160
19	cholinergic receptor, muscarinic 1	0.12863	0.04813	0.06497	-0.06453	0.02217	0.15200
20	eukaryotic translation initiation factor 4E	0.12816	0.09171	0.17493	0.09579	0.04243	0.12600
21	gelsolin (amyloidosis, Finnish type)	0.12550	0.14656	0.28933	-0.19905	0.04927	0.00561
22	myogenic factor 3	0.12050	0.06909	0.14312	0.06016	0.04793	0.35700
23	protein kinase, cAMP-dependent, catalytic, alpha	0.11398	0.11500	0.27763	-0.03979	0.04790	0.54000
24	sortilin-related receptor, L(DLR class) A repeats-containing	0.11221	0.03889	0.07270	0.01641	0.02050	0.69900
25	antigen identified by monoclonal antibody Ki-67	0.11098	0.05966	0.14652	0.05964	0.03756	0.30400
26	centrosomal protein 2	0.11000	0.15842	0.36997	-0.15400	0.06636	0.05290
27	stromal interaction molecule 1	0.10934	0.09616	0.25538	-0.08721	0.02430	0.06870
28	small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	0.10927	0.04068	0.08644	-0.23426	0.07656	0.00835
29	tumor necrosis factor (TNF superfamily, member 2)	0.10653	0.03856	0.08604	0.11632	0.14001	0.29900
30	farnesyl-diphosphate farnesyltransferase 1	0.10279	0.11789	0.33223	0.15551	0.25355	0.30200
31	RAB5A, member RAS oncogene family	0.10240	0.03077	0.06609	0.04411	0.03292	0.41500
32	O-6-methylguanine-DNA methyltransferase	0.10134	0.03893	0.10296	-0.00992	0.02451	0.83000



**Supplemental table 3: Exosomal and cellular protein contents differ in MM- versus normal BM-MSCs.**

rank	protein	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
		Exosomal Log fold change to the base 2	Cellular Log fold change to the base 2	Exosomal Log fold change to the base 2	Cellular Log fold change to the base 2	Exosomal Log fold change to the base 2	Cellular Log fold change to the base 2	Exosomal Log fold change to the base 2	Cellular Log fold change to the base 2	Exosomal Log fold change to the base 2	Cellular Log fold change to the base 2
1	fibronectin 1	0.35900	0.19095	1.67000	0.31039	-0.49061	0.16656	1.66655	0.01467	-0.00586	0.20874
2	junction plakoglobin	0.37100	0.05285	0.65400	0.00674	0.41327	-0.04109	0.62266	-0.06520	0.41400	0.00266
3	chemokine (C-C motif) ligand 2	0.18900	-0.18363	0.38500	-0.03877	0.11571	0.00376	0.48476	-0.01549	0.33900	-0.07906
4	mitogen-activated protein kinase kinase 5	0.32600	-0.36435	0.18400	-0.10136	-0.04597	-0.23869	0.72870	0.01075	-0.07780	-0.09635
5	excision repair cross-complementing rodent repair deficiency, complementation group 2 (xeroderma pigmentosum D)	-0.01460	0.07869	0.23800	0.10599	0.20842	0.03245	0.22952	-0.09015	0.40300	0.06317
6	ubiquitin-conjugating enzyme E2E 1 (UBC4/5 homolog, yeast)	-0.14600	0.28015	0.34000	0.16874	0.43746	0.33339	-0.37367	0.00906	0.77100	0.24043
7	DEAH (Asp-Glu-Ala-His) box polypeptide 38	0.32700	-0.01864	0.34400	-0.01971	-0.10656	-0.02587	0.53711	0.11110	-0.08390	0.04931
8	interleukin 13	-0.09330	0.40058	0.32600	0.29170	0.28658	0.35945	-0.04807	0.14258	0.43800	-0.32796
9	cell division cycle 27	1.50000	-0.16578	-0.00866	-0.07838	-0.33731	-0.28118	0.47853	0.03660	-0.72700	-0.27299
10	RAP1, GTPase activating protein 1	-0.17500	0.48373	-0.00597	0.28833	0.26670	0.36675	0.18692	-0.15595	0.55700	-0.10976
11	non-POU domain containing, octamer-binding	0.17500	-0.33491	0.28600	-0.20068	-0.22820	-0.17389	0.43278	0.10144	0.15000	-0.19677
12	interleukin 6 (interferon, beta 2)	0.04080	0.10712	0.48400	0.12234	0.02692	0.04404	0.21594	-0.14923	-0.01370	-0.05026
13	ribosomal protein S6 kinase, 90kDa, polypeptide 1	0.22800	-0.02389	-0.05290	0.00517	0.16665	0.00072	0.12922	-0.03355	0.28200	-0.03488
14	protein kinase C, beta 1	0.08790	-0.17616	0.00227	-0.13408	-0.25671	-0.28716	0.04671	-0.12332	0.86700	-0.22220
15	B-cell linker	0.23400	0.10525	0.24100	0.13942	0.06295	0.02853	0.14351	0.00070	0.01230	0.07743

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
		Exosomal	Cellular								
		Log fold									
		change									
rank	protein	to the base 2									
16	early endosome antigen 1, 162kD	0.19400	0.06774	0.52500	0.07767	-0.04353	-0.02409	0.04703	0.00993	-0.04880	0.06440
17	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase; thyroid hormone binding protein p55)	-0.11000	0.01181	0.56700	-0.33091	0.27012	0.10610	-0.10029	0.12952	0.03160	-0.27219
18	nucleoporin 88kDa	0.25900	-0.52738	0.46900	-0.52689	-0.09707	-0.55783	0.24322	-0.15896	-0.23000	-0.54987
19	cholinergic receptor, muscarinic 1	0.24800	0.01519	0.15800	0.12686	0.04865	0.07025	0.07723	0.04479	0.11100	0.06557
20	eukaryotic translation initiation factor 4E	0.18400	-0.13851	0.00732	-0.17014	-0.08794	-0.11377	0.15872	0.15698	0.37900	-0.21350
21	gelsolin (amyloidosis, Finnish type)	0.03400	0.30081	-0.06650	0.14619	0.09676	0.27817	0.38030	-0.03358	0.18300	0.30368
22	myogenic factor 3	0.07130	-0.00748	0.15400	-0.11656	-0.06427	-0.14848	0.44634	0.10645	-0.00502	-0.13470
23	protein kinase, cAMP-dependent, catalytic, alpha	-0.04200	0.07892	0.05950	0.12320	0.23405	0.01507	0.29854	-0.00443	0.01990	-0.01382
24	sortilin-related receptor, L(DLR class) A repeats-containing	0.11400	0.01576	0.23900	-0.01710	-0.02983	0.00340	0.29568	-0.08725	-0.05790	0.00314
25	antigen identified by monoclonal antibody Ki-67	0.00335	0.04259	0.28200	-0.14639	0.13490	-0.02765	-0.10742	-0.08144	0.24200	-0.08533
26	centrosomal protein 2	0.12200	0.20136	0.31500	0.14751	-0.06823	0.19549	0.24541	0.07447	-0.06500	0.15117
27	stromal interaction molecule 1	0.14100	0.12586	0.11800	0.11497	0.09790	0.10009	0.13760	-0.09610	0.05220	0.19124
28	small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)	0.04350	0.41541	0.30200	0.21664	-0.02831	0.23685	0.11422	0.06990	0.11500	0.23252
29	tumor necrosis factor (TNF superfamily, member 2)	0.14000	-0.08282	0.18200	-0.44349	0.10621	-0.02474	0.10761	0.13307	-0.00345	-0.16364
30	farnesyl-diphosphate farnesyltransferase 1	0.25200	-0.28653	0.43000	-0.15096	-0.03307	-0.14350	-0.12425	0.15492	-0.01030	-0.35149
31	RAB5A, member RAS oncogene family	0.11800	0.10700	0.13700	-0.08175	0.11983	-0.09179	0.05372	-0.13730	0.08390	-0.01670

		Sample 1		Sample 2		Sample 3		Sample 4		Sample 5	
rank	protein	Exosomal	Cellular								
		Log fold change to the base 2									
32	O-6-methylguanine-DNA methyltransferase	0.14100	0.00363	0.15600	0.03789	-0.05489	-0.04092	0.22509	0.05180	0.04000	-0.00279