Toxicity analysis of PbS QDs using nano-sized vesicles (exosome) secreted from HEK293 cells

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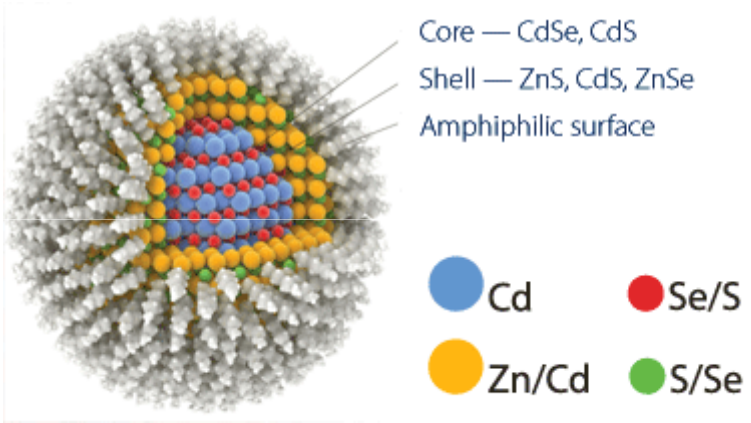
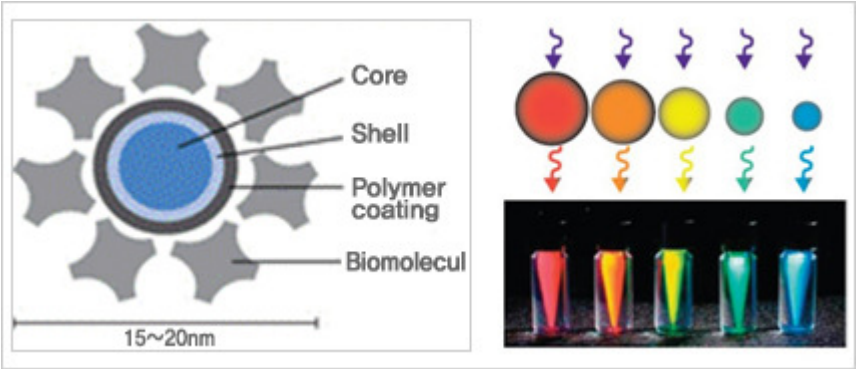
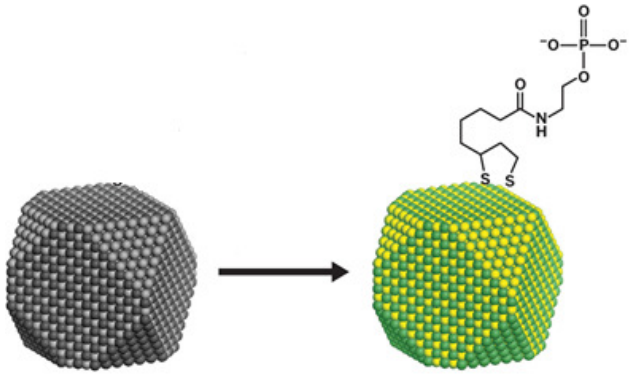
Quantum dots



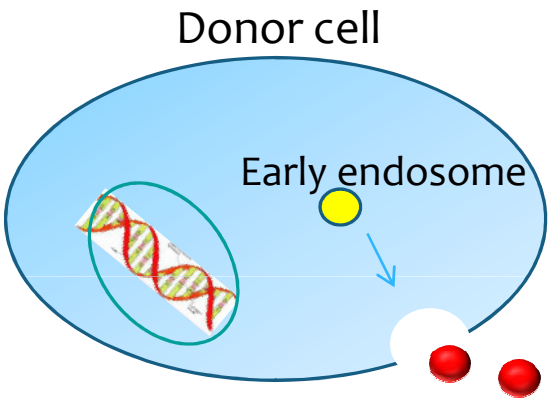
RUSNANO Corporation, http://en.rusnano.com/

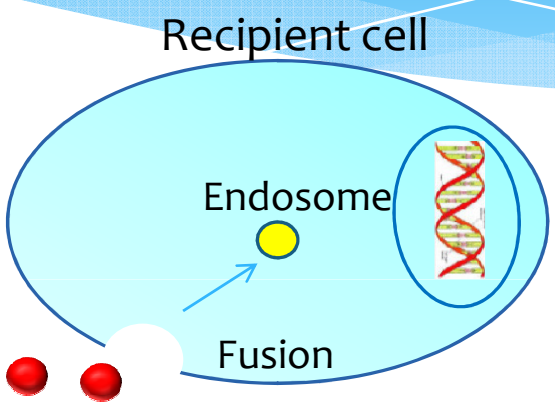
* Nanocrystals
* 2-10 nm diameter, small enough to exhibit quantum mechanical properties
* Fluorophore
* Easily modified by polymers or chemicals with such as –SH
* Semiconductors, agents for medical imaging

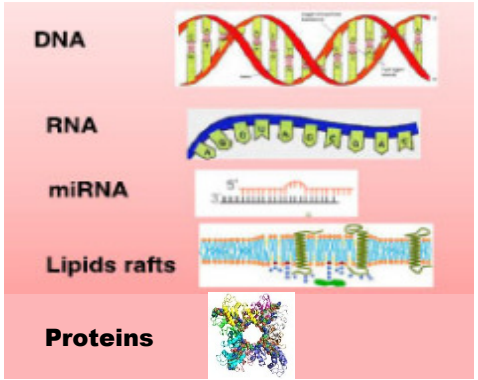




Exosomes







Roles of exosome contents

- Cell-to-cell signaling
- Membrane trafficking
- Representing the disease status and pathological condition of cellular origin

Properties of exosomes in biological signaling

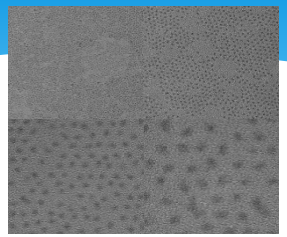
* Released from most types of mammalian cells
* Different cells release different exosomes.
* Potential source of biomarkers for tissue conditions
* The encapsulated molecules by exosomes can be delivered over long distance.
* They play a key role in cell-cell communication during disease progress such as cancer and neurodegeneration.
* Isolated from circulating fluids: serum, urine, CSF and cell culture media

Toxicology with exosomes

* provides new circulating biomarkers for in vivo and in vitro
* makes possible to predict the cellular and molecular mechanism of toxicity efficiently
* is an emerging field for high-throughput screening of disease biomarkers
* is applied to human toxicology

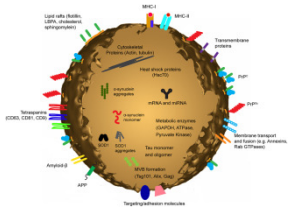
Aims of this study are..

* To provide nanotoxicological data for PbS QDs
* To propose a toxicologic pathology using exosome biomarkers

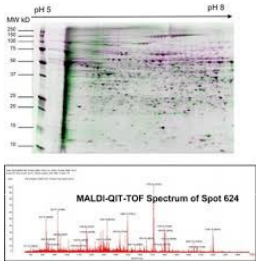


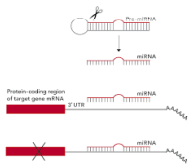
1 Preparation of MPA-coated PbS

2 Exposure of PbS-MPA to HEK293 cells

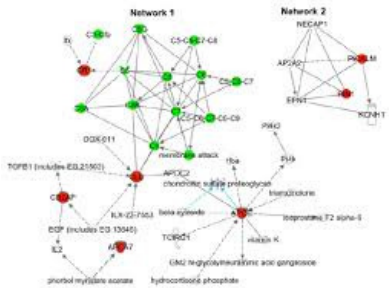


3 Exosome isolation

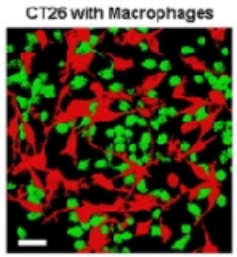




4 Analysis of molecular markers: miRNA & proteins



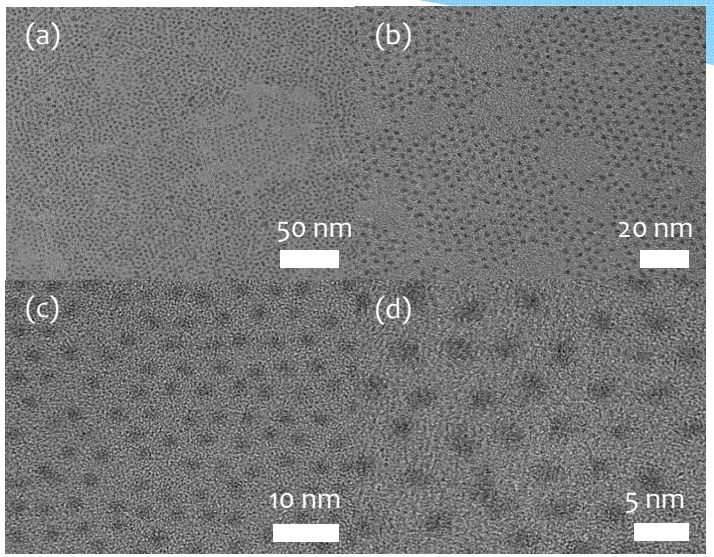
5 Identification of Induced & depressed molecules

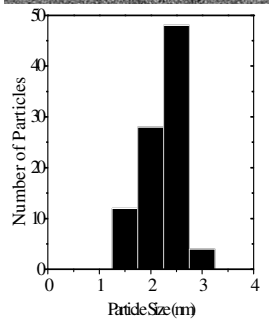


6 Functional analysis

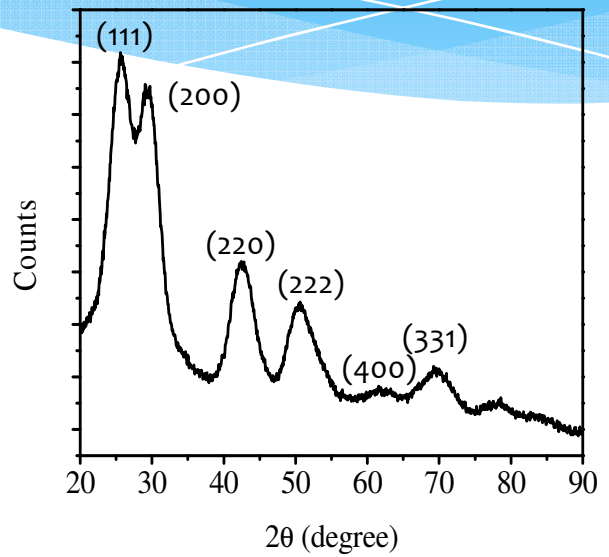
7 Prediction of toxicologic pathology in vitro

Synthesis of PbS particles





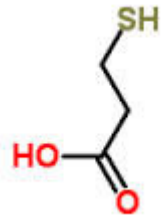
D_{ave}=2.15 nm (n=92)



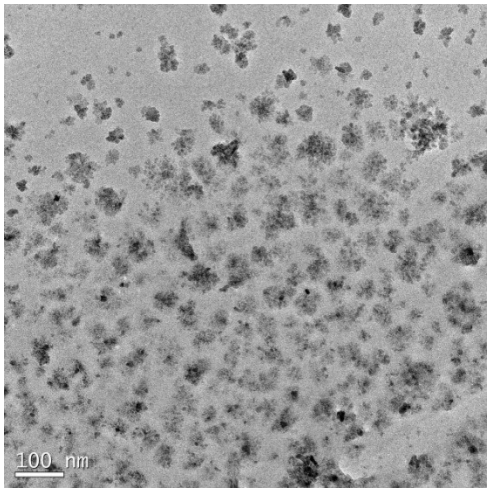
JCPDS: 03-065-0692 Akhtar, et al. J. Mater. Chem., 2010, 20, 2336-2344

PbS quantum dots with < 5 nm particle sizes were prepared by the colloidal chemistry method according to the theory of fast nucleation at high temperature and slow growth at low temperature. Sodium sulfide was used as a sulfur precursor (odorless and less noxious).
Oleic acid was used as a stabilizing agent to control the particle growth and it assisted in the formation of mono-dispersed PbS QDs.

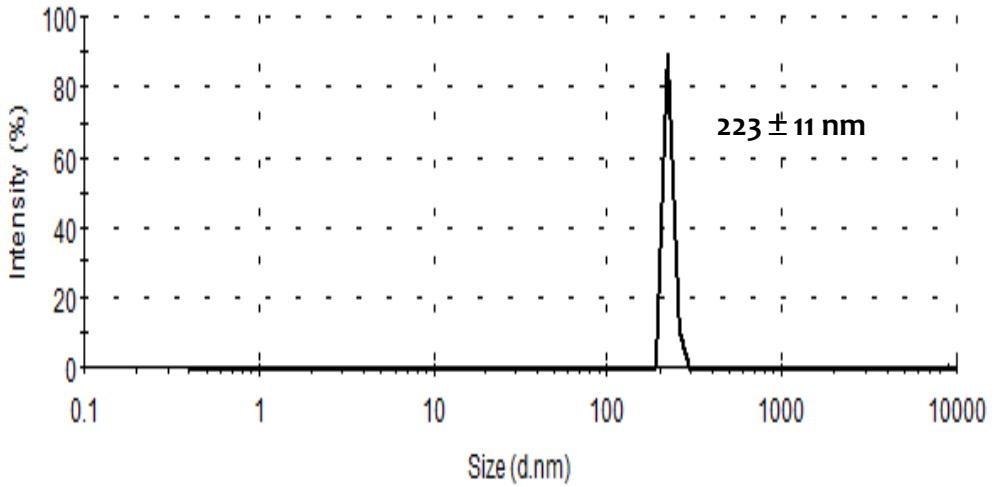
Surface modification PbS by MPA



3-mercaptopropionic acid (MPA)

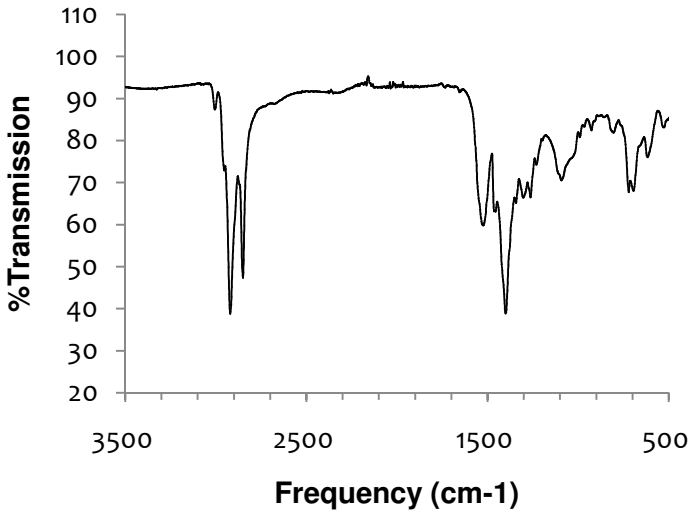


TEM image

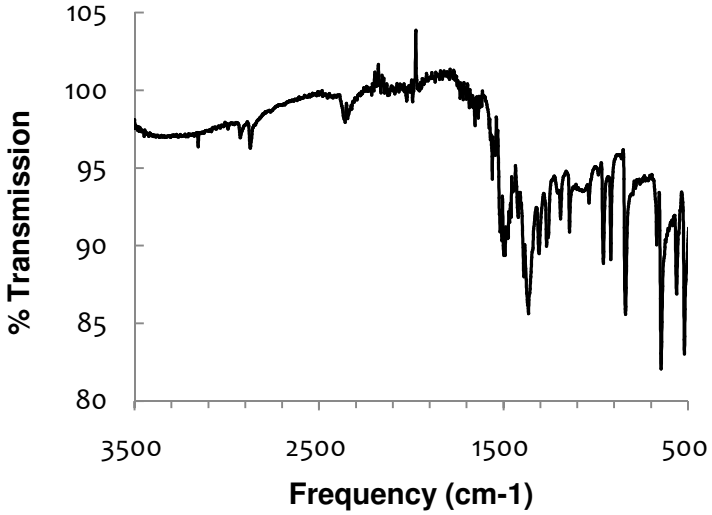


DLS analysis

FT-IR analysis

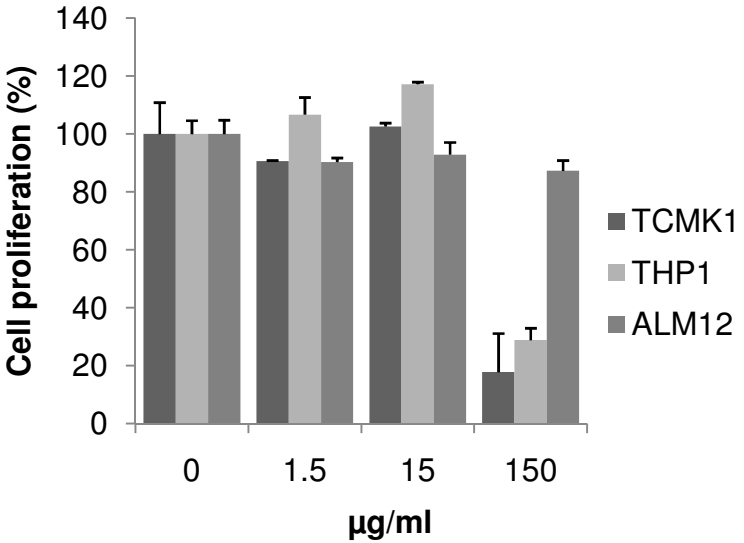


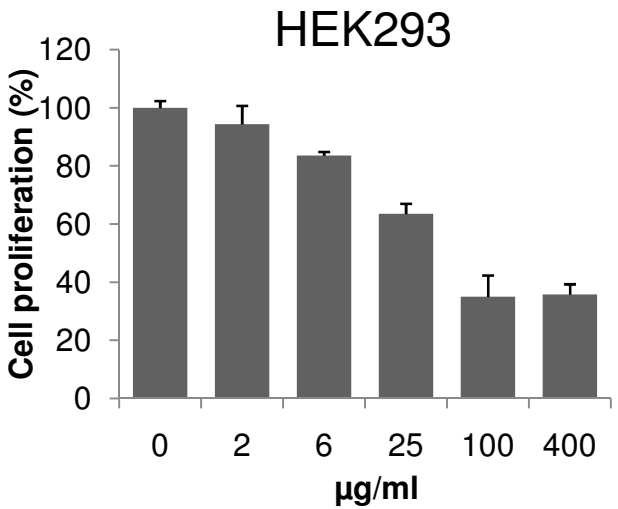
PbS



PbS-MPA

Cytotoxicity of PbS-MPA

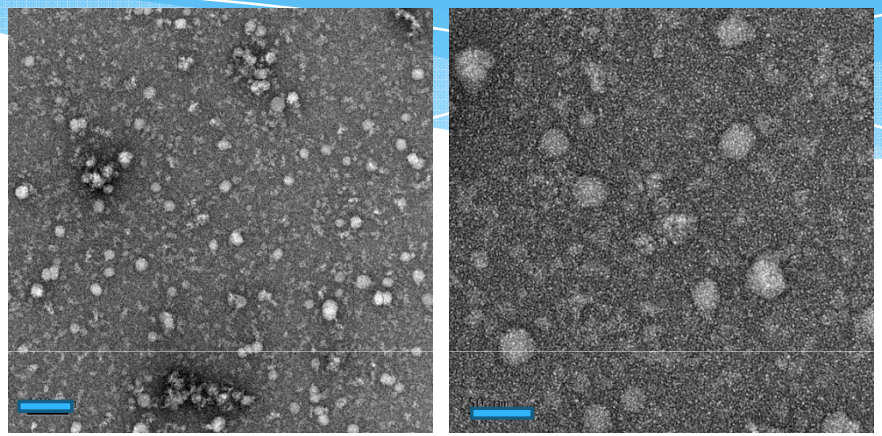




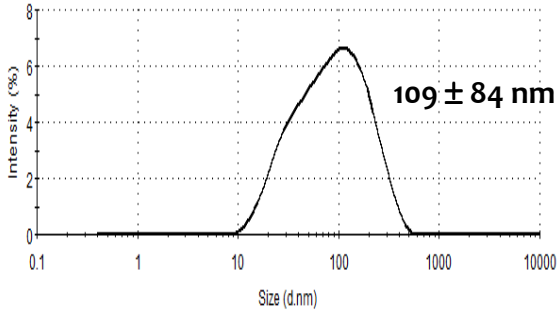
Isolation and characterization of exosomes

System Bioscience Inc.
http://www.systembio.com/

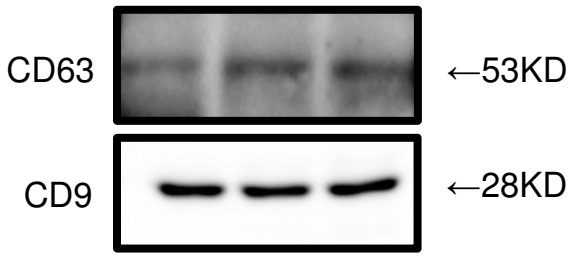
Bio-TEM



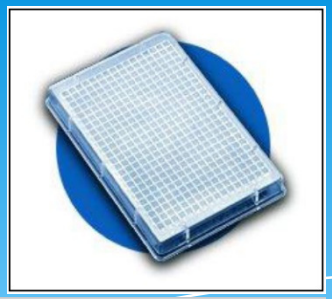
DLS

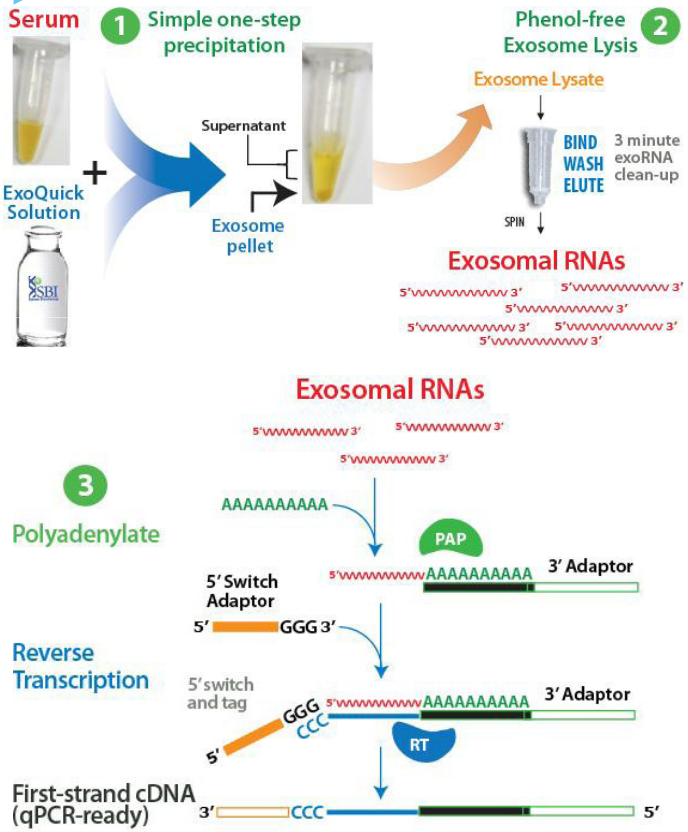


Exosome markers



Profiling of miRNA





System Bioscience Inc., http://www.systembio.com/

A29 | B1

Flag values | Ct values below 10 or above 32 | Cat# RA810A-1

Test Sample #2 | **Control Sample #1** | **Normalized MicroRNA Expression levels**

Copy/paste Ct data below | Copy/paste Ct data below

	Test Sample #2			Control Sample #1						
	V/well	MicroRNA	Ct plate #1	Average Ct value	V/well	MicroRNA	Ct plate #1	Average Ct value	ΔCT controls	MicroRNA	ΔCt	Test sample expression level compared to control
5	A1	hsa-let-7a		#DIV/0!	A1	hsa-let-7a		#DIV/0!	#DIV/0!	hsa-let-7a	#DIV/0!	#DIV/0!
6	A2	hsa-let-7b		#DIV/0!	A2	hsa-let-7b		#DIV/0!		hsa-let-7b	#DIV/0!	#DIV/0!
7	A3	hsa-let-7c		#DIV/0!	A3	hsa-let-7c		#DIV/0!		hsa-let-7c	#DIV/0!	#DIV/0!
8	A4	hsa-let-7d		#DIV/0!	A4	hsa-let-7d		#DIV/0!		hsa-let-7d	#DIV/0!	#DIV/0!
9	A5	hsa-let-7e		#DIV/0!	A5	hsa-let-7e		#DIV/0!		hsa-let-7e	#DIV/0!	#DIV/0!
10	A6	hsa-let-7f		#DIV/0!	A6	hsa-let-7f		#DIV/0!		hsa-let-7f	#DIV/0!	#DIV/0!
11	A7	hsa-let-7g		#DIV/0!	A7	hsa-let-7g		#DIV/0!		hsa-let-7g	#DIV/0!	#DIV/0!
12	A8	hsa-let-7i		#DIV/0!	A8	hsa-let-7i		#DIV/0!		hsa-let-7i	#DIV/0!	#DIV/0!
13	A9	hsa-miR-1		#DIV/0!	A9	hsa-miR-1		#DIV/0!		hsa-miR-1	#DIV/0!	#DIV/0!
14	A10	hsa-miR-7		#DIV/0!	A10	hsa-miR-7		#DIV/0!		hsa-miR-7	#DIV/0!	#DIV/0!
15	A11	hsa-miR-9		#DIV/0!	A11	hsa-miR-9		#DIV/0!		hsa-miR-9	#DIV/0!	#DIV/0!
16	A12	hsa-miR-10a		#DIV/0!	A12	hsa-miR-10a		#DIV/0!		hsa-miR-10a	#DIV/0!	#DIV/0!
17	A13	hsa-miR-10b		#DIV/0!	A13	hsa-miR-10b		#DIV/0!		hsa-miR-10b	#DIV/0!	#DIV/0!
18	A14	hsa-miR-15a		#DIV/0!	A14	hsa-miR-15a		#DIV/0!		hsa-miR-15a	#DIV/0!	#DIV/0!
19	A15	hsa-miR-15b		#DIV/0!	A15	hsa-miR-15b		#DIV/0!		hsa-miR-15b	#DIV/0!	#DIV/0!
20	A16	hsa-miR-16		#DIV/0!	A16	hsa-miR-16		#DIV/0!		hsa-miR-16	#DIV/0!	#DIV/0!
21	A17	hsa-miR-17		#DIV/0!	A17	hsa-miR-17		#DIV/0!		hsa-miR-17	#DIV/0!	#DIV/0!
22	A18	hsa-miR-18a		#DIV/0!	A18	hsa-miR-18a		#DIV/0!		hsa-miR-18a	#DIV/0!	#DIV/0!
23	A19	hsa-miR-18b		#DIV/0!	A19	hsa-miR-18b		#DIV/0!		hsa-miR-18b	#DIV/0!	#DIV/0!
24	A20	hsa-miR-19a		#DIV/0!	A20	hsa-miR-19a		#DIV/0!		hsa-miR-19a	#DIV/0!	#DIV/0!
25	A21	hsa-miR-19b		#DIV/0!	A21	hsa-miR-19b		#DIV/0!		hsa-miR-19b	#DIV/0!	#DIV/0!
26	A22	hsa-miR-20a		#DIV/0!	A22	hsa-miR-20a		#DIV/0!		hsa-miR-20a	#DIV/0!	#DIV/0!
27	A23	hsa-miR-20b		#DIV/0!	A23	hsa-miR-20b		#DIV/0!		hsa-miR-20b	#DIV/0!	#DIV/0!
28	A24	hsa-miR-21		#DIV/0!	A24	hsa-miR-21		#DIV/0!		hsa-miR-21	#DIV/0!	#DIV/0!
165	P1	hsa-miR-526b		#DIV/0!	P1	hsa-miR-526b		#DIV/0!		hsa-miR-526b	#DIV/0!	#DIV/0!
166	P2	hsa-miR-527		#DIV/0!	P2	hsa-miR-527		#DIV/0!		hsa-miR-527	#DIV/0!	#DIV/0!
167	P3	hsa-miR-532-3p		#DIV/0!	P3	hsa-miR-532-3p		#DIV/0!		hsa-miR-532-3p	#DIV/0!	#DIV/0!
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173	P9	hsa-miR-543		#DIV/0!	P9	hsa-miR-543		#DIV/0!		hsa-miR-543	#DIV/0!	#DIV/0!
174	P10	hsa-miR-544		#DIV/0!	P10	hsa-miR-544		#DIV/0!		hsa-miR-544	#DIV/0!	#DIV/0!
175	P11	hsa-miR-545		#DIV/0!	P11	hsa-miR-545		#DIV/0!		hsa-miR-545	#DIV/0!	#DIV/0!
176	P12	hsa-miR-548a-3p		#DIV/0!	P12	hsa-miR-548a-3p		#DIV/0!		hsa-miR-548a-3p	#DIV/0!	#DIV/0!
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180	P16	hsa-miR-548c-3p		#DIV/0!	P16	hsa-miR-548c-3p		#DIV/0!		hsa-miR-548c-3p	#DIV/0!	#DIV/0!
181	P17	hsa-miR-548c-5p		#DIV/0!	P17	hsa-miR-548c-5p		#DIV/0!		hsa-miR-548c-5p	#DIV/0!	#DIV/0!
182	P18	hsa-miR-548d-3p		#DIV/0!	P18	hsa-miR-548d-3p		#DIV/0!		hsa-miR-548d-3p	#DIV/0!	#DIV/0!
183	P19	hsa-miR-548d-5p		#DIV/0!	P19	hsa-miR-548d-5p		#DIV/0!		hsa-miR-548d-5p	#DIV/0!	#DIV/0!
184	P20	hsa-miR-548e		#DIV/0!	P20	hsa-miR-548e		#DIV/0!		hsa-miR-548e	#DIV/0!	#DIV/0!
185	P21	Spike-in Control		#DIV/0!	P21	Spike-in Control		#DIV/0!		Spike-in Control	#DIV/0!	#DIV/0!
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187	P23	Spike-in Control		#DIV/0!	P23	Spike-in Control		#DIV/0!		Spike-in Control	#DIV/0!	#DIV/0!
188	P24	NTC control		#DIV/0!	P24	NTC control		#DIV/0!		NTC control	#DIV/0!	#DIV/0!
189												
190		Geomean	#DIV/0!			Geomean	#DIV/0!					
191												

- In SBI miRNA analysis kit, 380 primers found in exosomes were provided

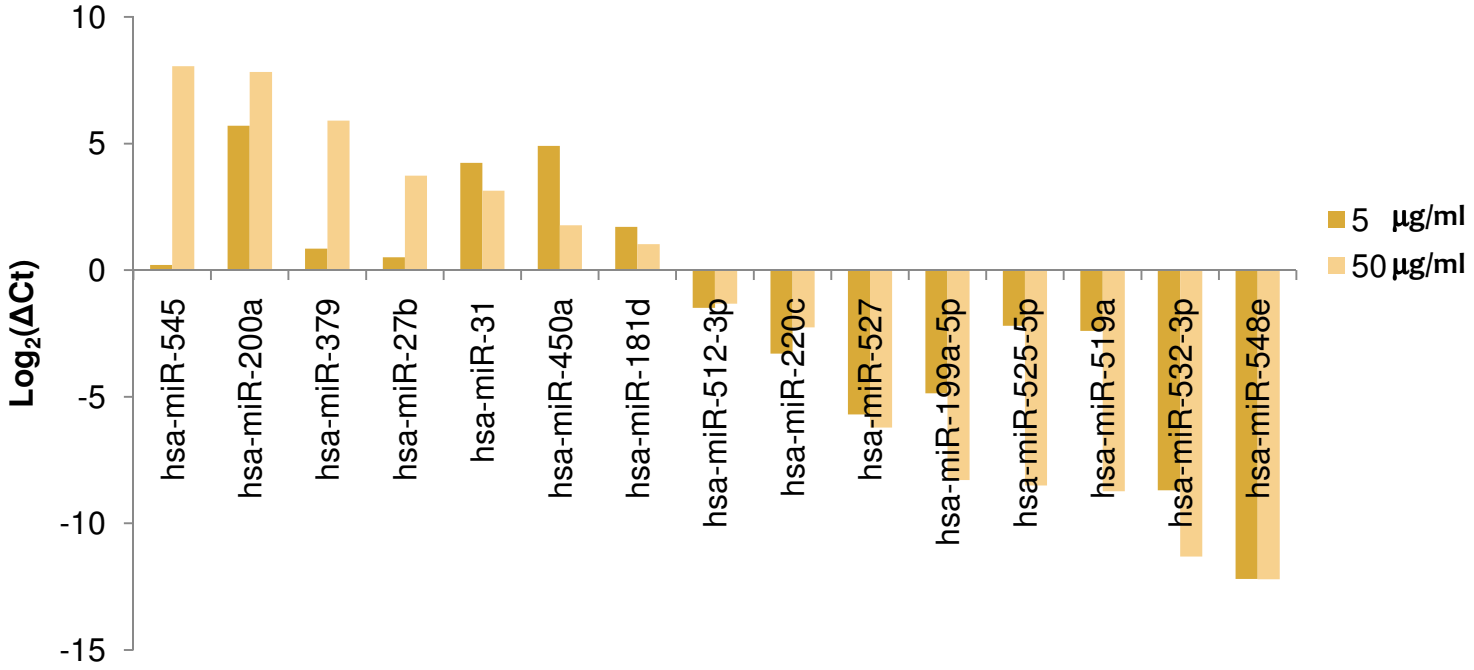
Differentially expressed exosome miRNAs (DEGs)

Selection criteria:

1) Increment or decrement simultaneously in both of 5 and 50 μg/ml

2) Cut-off for 2-fold changes in 5 or 50 μg/ml

***3) p*<0.05**



Top functional networks

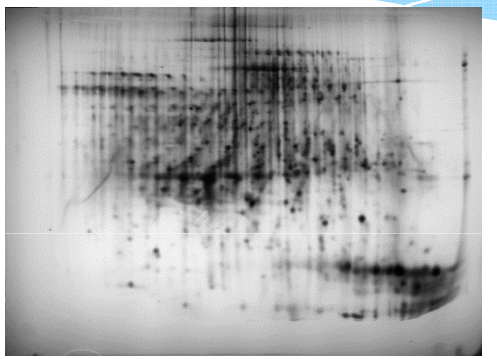
Diseases and disorders	p-value	# Molecules
Cancer	6.52E-04 - 4.38E-02	11
Organismal Injury and Abnormalities	6.52E-04 - 2.51E-02	8
Reproductive System Disease	2.85E-03 - 4.95E-02	4
Inflammatory Response	2.18E-03 - 2.79E-02	4
Associated Network Functions	**Score**	
Cancer, Organismal Injury and Abnormalities, Reproductive System Disease	20	

By Ingenuity Pathway Analysis (IPA)

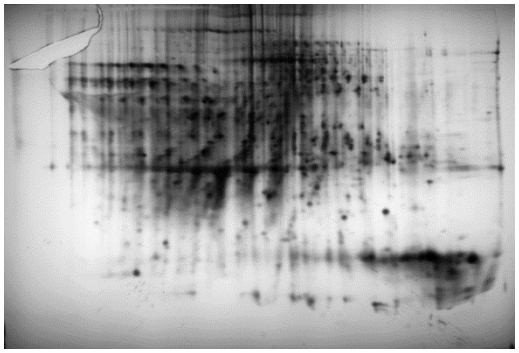
2D gel electrophoresis of exosome proteins

1) 1<2<3 or 1>2>3 2) 1 vs. 2 or 1 vs. 3, 2-fold cut off

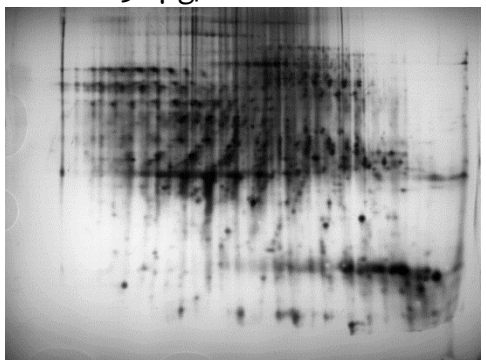
1 Control



2 5 µg/ml PbS-MPA

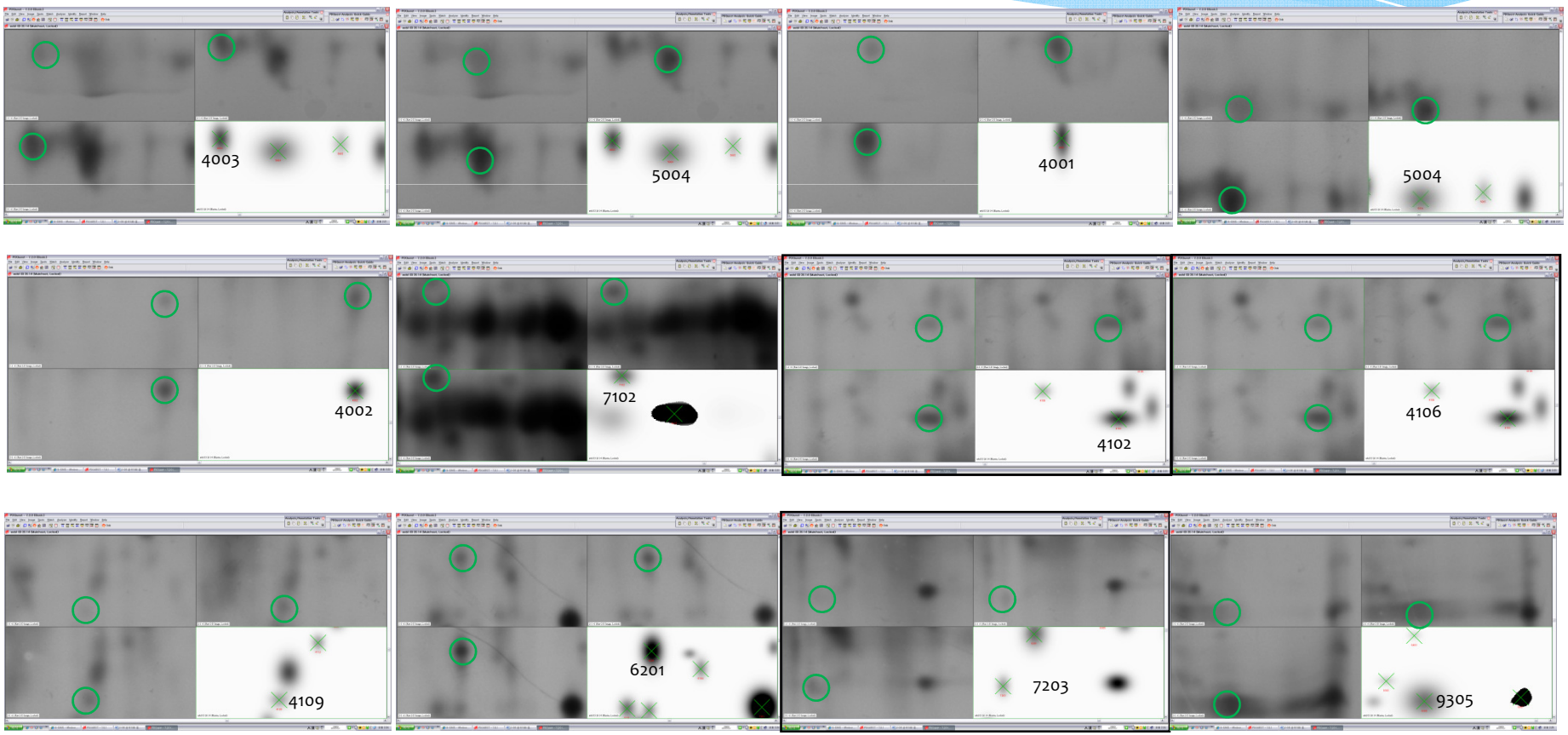


3 50 µg/ml PbS-MPA

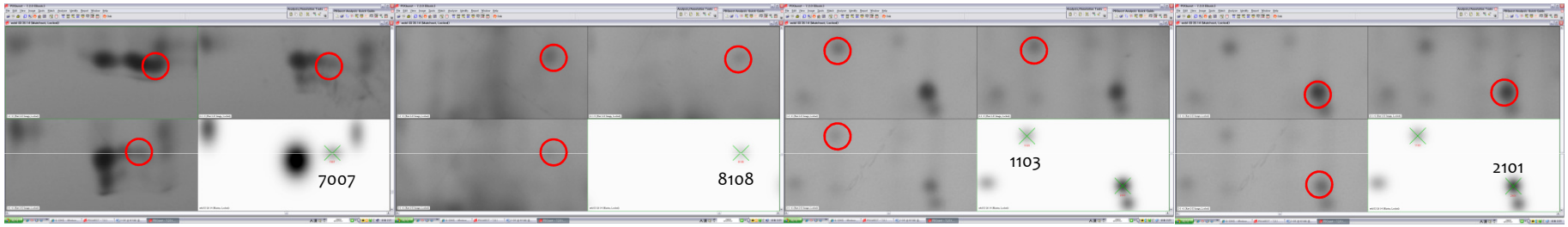


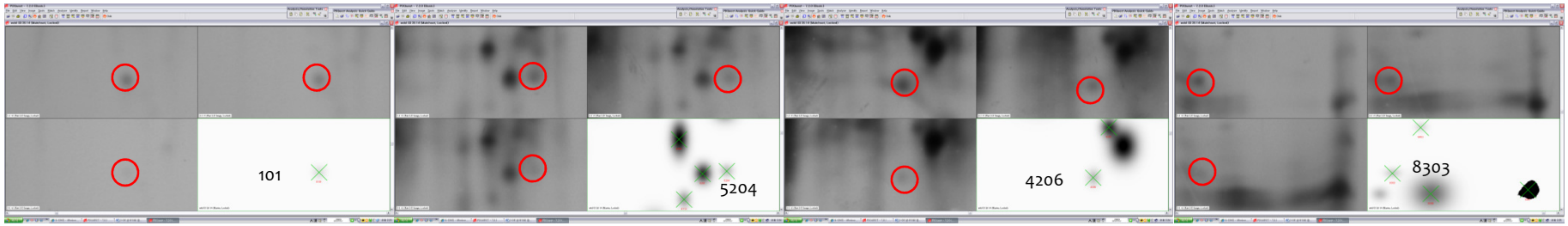
Spot selection (29 spots)

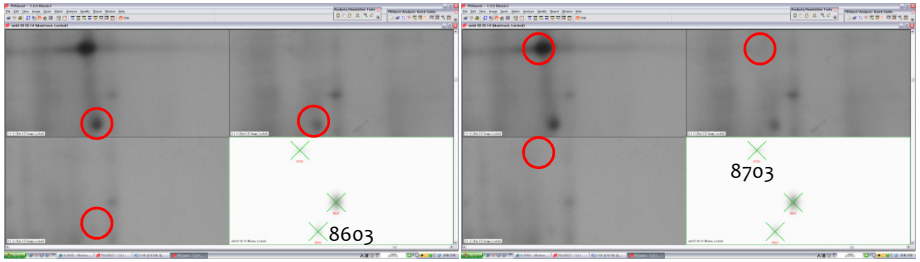
A: Increased changes (19)



B: Decreased changes (10)

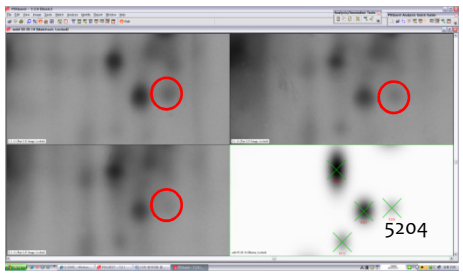


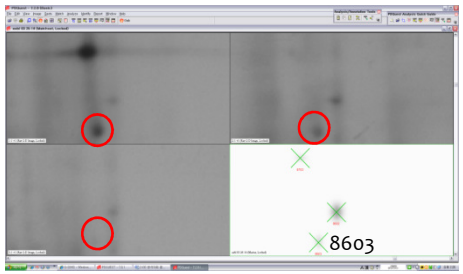


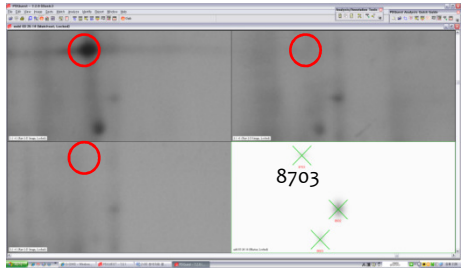


Protein spots identified by Maldi-TOF and PMF analysis

Spot No.	Protein	Expression	Function	Response in cancer
9305	Leucine-rich repeat-containing protein 23 isoform b (IRR23b)	Increase	Exosome protein	Increase
5204	Keratin 5 (KRT5)	Decrease	Breast cancer biomarker (negative relationship)	Decrease
8603	Unnamed protein product	Decrease	-	
8703	Albumin-like	Decrease	-	

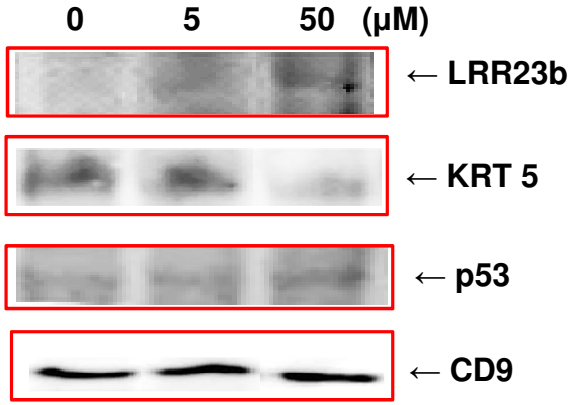


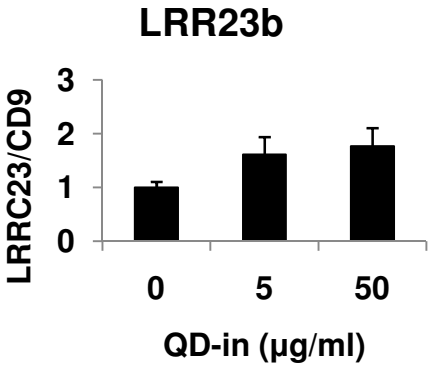


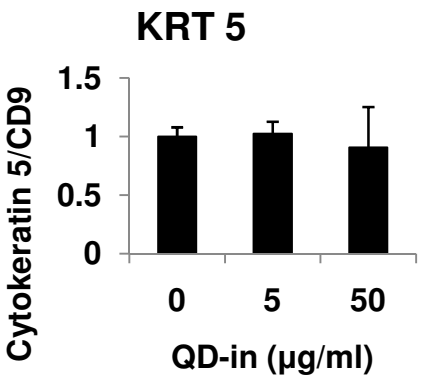


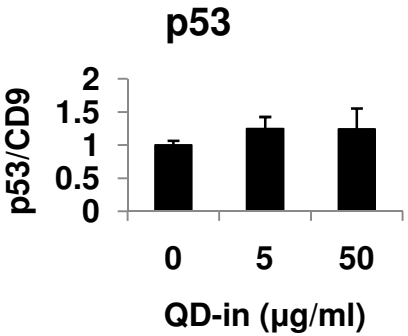
Confirmation of exosome biomarkers

In Exosomes

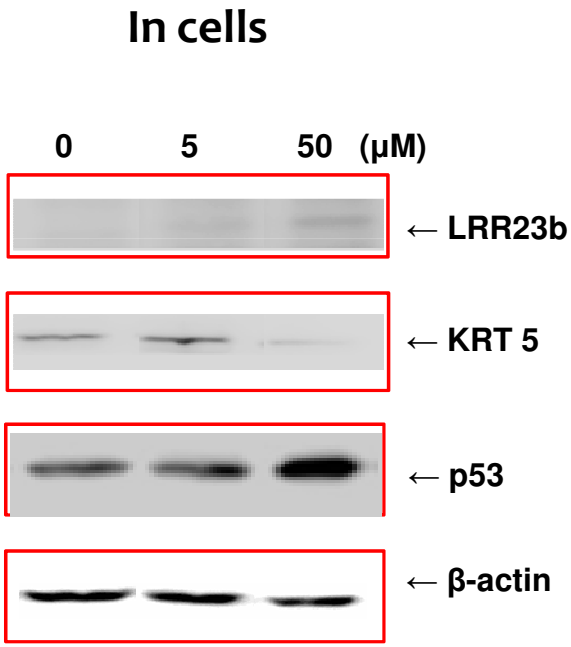


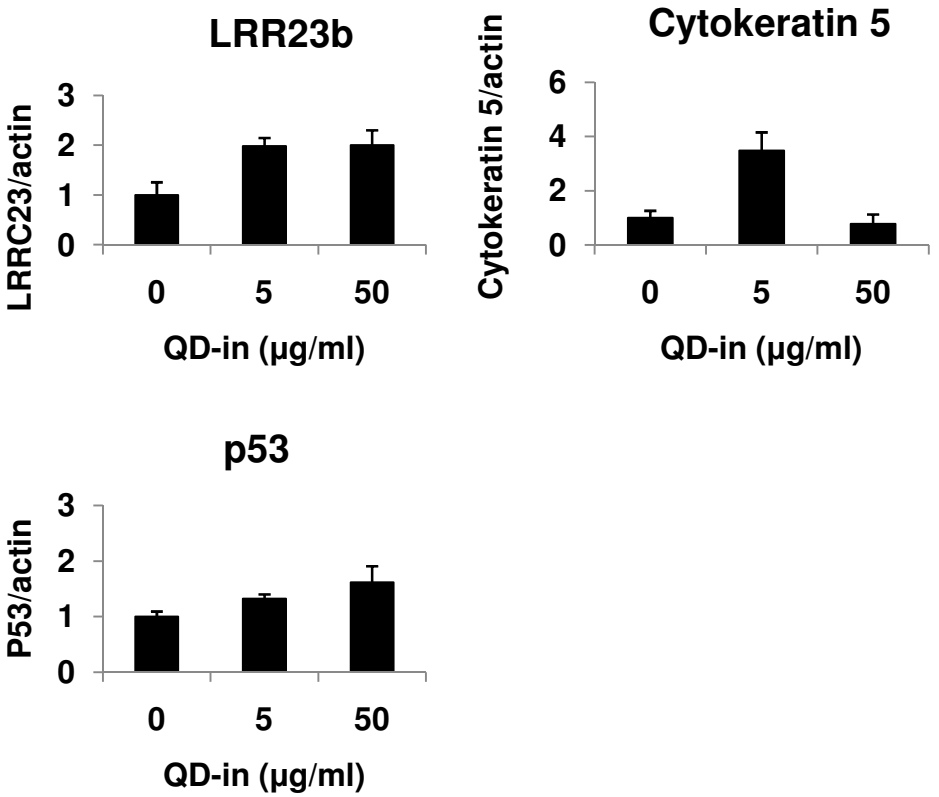




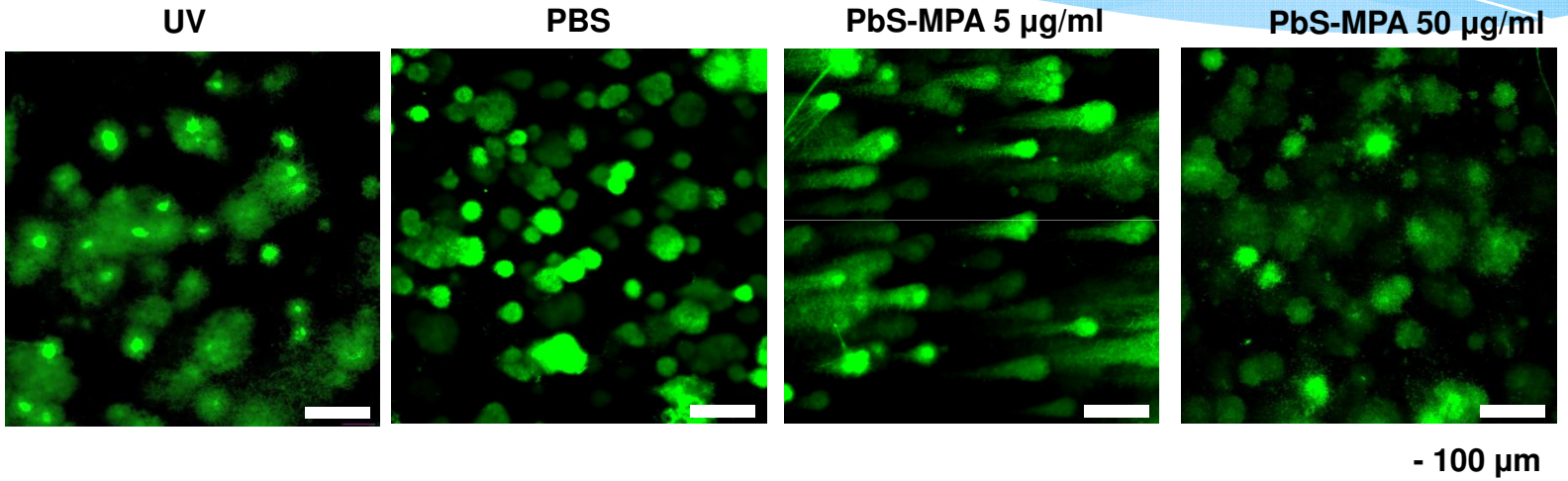


Events in the origin of the exosomes, HEK293 cells: expression of protein biomarkers



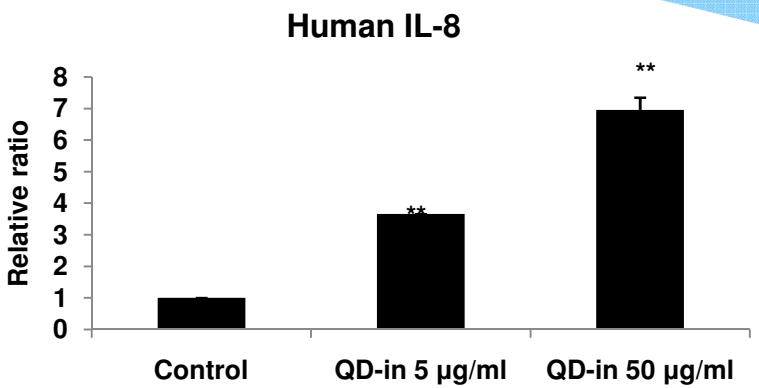


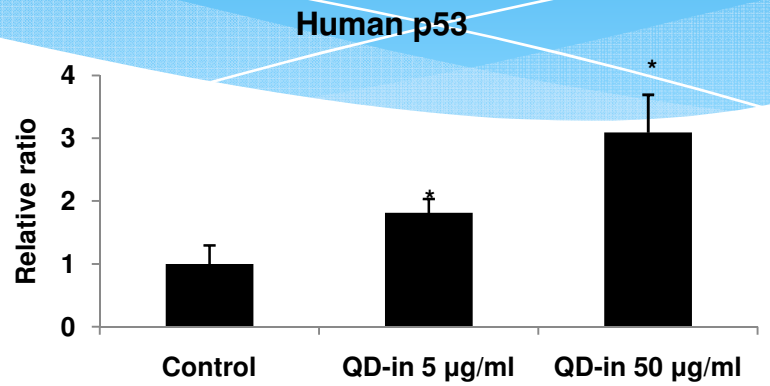
Comet assay for DNA damage

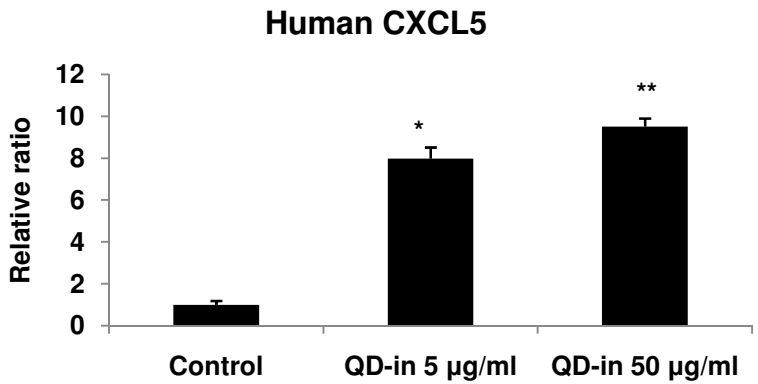


Comet assay responses as indicators of *carcinogen* exposure.

Events in the origin of the exosomes, HEK293 cells: expression of cancer-related biomarkers (mRNA)







*P<0.01,**P<0.001 vs Control

Conclusion

* The exosome miRNA profiles for PbS-MPA to HEK293 cells showed 15 DEGs. These were primarily involved in cancer and organismal injury and abnormalities.
* LRR23b and KRT5 were identified as biomarkers of exosome proteins for PbS-MPA exposure. These proteins are also known as cancer biomarkers.
* Comet assay clearly showed that DNA fragmentation was occurred, and supported the carcinogenic activity of PbS-MPA QDs.
* The exosome derived biomarkers could represent the toxicological response of origin cells.

1. A toxicological response could be identified by genomic and proteomic analysis for secreted exosomes.
2. The eoxsome-based analysis could provide an effective tool for high-throughput screening (HTS) of biomarkers involved in possible toxicology.
3. The HTS of exosome biomarkers will be more efficient than that of the whole cells, because 1) they have less number of molecular contents, 2) are expected to secret critical biomarkers to represent the cellular state and communicate with other cells.

Thank you for your attention