Electronic Supplementary Information for

Breast cancer plasma biopsy by in situ determination of exosomal microRNA-1246 with a molecular beacon

Yun-Chen,[a†] Ling-Yan Zhai,[b†] Li-Min Zhang,[a] Xiao-Shan Ma,[a] Zheng Liu,[a] Min-Min Li,[c] Jin-Xiang Chen[a]* and Wen-Jun Duan[a]*

† These authors contributed equally

[a] Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Sciences, Southern Medical University, Guangzhou 510515, China

[b] Shenzhen Baoan Shiyan People's Hospital, Shenzhen 518108, China

[c] Center of Clinical Laboratory, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China

* Corresponding author: Jin-Xiang Chen (E-mail: jxchen@smu.edu.cn)

*Corresponding author: Wen-Jun Duan (E-mail: wjduan@smu.edu.cn)

Exosomes characterization

Nanoparticle Tracking Analysis (NTA)

Separated exosomes (1 μL) were diluted about 1000 X in PBS and were injected into the Nanosight NS300 System. The nanoparticles in the injected sample were visualized by their scattering of a 405 nm laser beam and the Brownian motions were captured 3 times for 60 seconds each. The videos were then analyzed to derive the size distribution and concentration profiles of the exosomes by the NTA 3.2 Software.

Transmission Electron Microscopy (TEM)

The morphology of the exosomes and the rupture effect of Triton X-100 to the exosomes membrane were observed by TEM. Exosomes (10 μL) were added on a plastic screen to form a liquid drop for a copper grid to adsorb the suspending vesicles for 1 min. Then, the copper grid was sequentially stained negatively by a drop of 3% phosphotungstic acid (pH 7.0) for 2 min, washed with a drop of double distilled water, sucked by filtrate paper, and dried at room temperature for 30 min. The final prepared sample was imaged under a JM-1010 electron microscope operated at 80 kV. Exosomes treated by 1% Triton X-100 for 30 min were sampled under the similar procedure and performed TEM imaging under a JEM-2100 F electron microscope.

Western Blotting Analysis

One culture dish (10 cm) of MCF-7 cells was washed 3 times by PBS after the culture medium was discarded. The cells were then dissociated by 1 mL of trypsin solution for 2 min and was centrifuged at 800 rpm for 5 min. The cell sediment was washed by 3 more centrifuging, and was lysed afterward on ice for 20 min in 1 mL of RIPA lysis buffer (containing 1 mM PMSF). The lysate was clarified by spinning for 5 min at 12, 000 × g at 4 °C. The supernatant was transferred to a fresh tube and stored at -20 °C. The total protein content in the extract was measured by a BCA protein assay kit according to the user manual. For the preparation of exosomes proteins, 20 μL of MCF-7 exosomes (about 10^9 particles/μL) was lysed in 10 μL of RIPA lysis buffer (containing 1 mM PMSF) for 20 min on ice.

A certain volume of aforementioned cell total protein extract containing 100 μg proteins and 20 μL of the exosomes lysate were mixed with 5 μL of 5 × loading buffer respectively in EP tubes, and were heated at 100 °C in the metal bath for 15 min. Those two denatured protein samples and marker protein were loaded to the 10% SDS-PAGE to perform electrophoresis, running the stacking gel with 80V for 15 min and separating gel with 120V for 80 min. When finished, the bands on the gel was transferred to a PVDF membrane on ice in transfer buffer at 200 mA for 60 min. The membrane was cut according to the maker and the molecular weight of the proteins of interest. Then they were washed with TBST buffer, and blocked with 5% skim milk for 1.5 h. Subsequently, the membranes were incubated respectively with primary antibodies against CD63, TGS101, and Calnexin (all in 1:1000 dilution) overnight at 4 °C on a shaker. After that, the membranes were washed by TBST and incubated

with anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h. Finally, the membranes were successively washed by TBST, covered by chemiluminescence detection reagents, and exposed to a multi-functional imaging analysis system to visualize the protein bands.

Table S1. Sequences of miR-1246, miR-21, miR-122, and Random DNA1-10

Oligonucleotide	**Sequence (5'-3')**
miR-1246	AAU GGA UUU UUG GAG CAG G
miR-21	UAG CUU AUC AGA CUG AUG UUG A
miR-122	UGG AGU GUG ACA AUG GUG GUG UUU G
Random DNA1	AAT GGA TTT TTG GAG CAG T
Random DNA2	AAT GGA TTC TTG GAG CAG G
Random DNA3	AAT GGA TTT TTG GAG CAA T
Random DNA4	AAT GGA TTC TTG GTG CAG G
Random DNA5	AAT GTC TTT TTG CTG CAG G
Random DNA6	AAT GGA TTT TTG TCA AGA T
Random DNA7	GCG CCT CCG ACA GAG CAG G
Random DNA8	GCG CCT CCG ACA TCA AGA TAA ATG GAT TTT TGG AGC AGG GCG CCT CCG ACA TCA AGA TA
Random DNA9	GCG CCT CCG ACA TCA AGA TCA CAT CAA GAT AAT GGA TTT TTG GAG CAG GGC GCC TCC GAC ATC AAG ATC ACA TCA AGA T
Random DNA10	GGA GGT CGC GTA TGC CTC CGA CAT CAA GAT CAC ATC AAG AT AAT GGA TTT TTG GAG CAG G GCG CCT CCG ACA TCA AGA TCA CAA ATA ACT AAA AAA AAA AAA AAA AAA AA

Note: Bases underlined with "___" are completely or partially same with bases in *hsa*-miR-1246 from 5' to 3' end ("T" in DNA equals to "U" in RNA).

Table S2. Differently expressed hsa-miR-1246 in MCF-7 and MCF-10a secreted exosomes (Next-generation sequencing by BGISEQ- 500 platform)

miRNA id	Read count /MCF10a	Read count /MCF7	Expression /MCF10a	Expression /MCF7	log_2Ratio MCF7/MCF10a	Up down regulatio	P value	Q value
hsa-miR-1246	134	7385	6.04	259.38	4.44	up	0	0

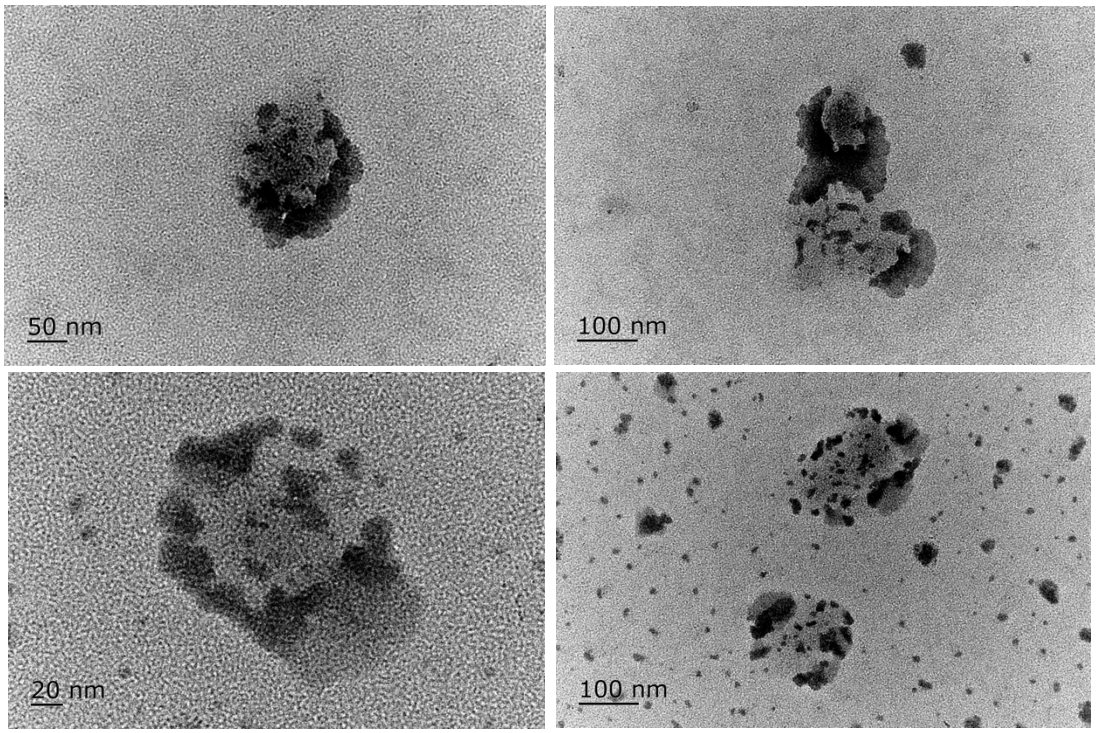

Fig. S1. TEM images of MCF-7 exosomes incubated with 1% Triton X-100.

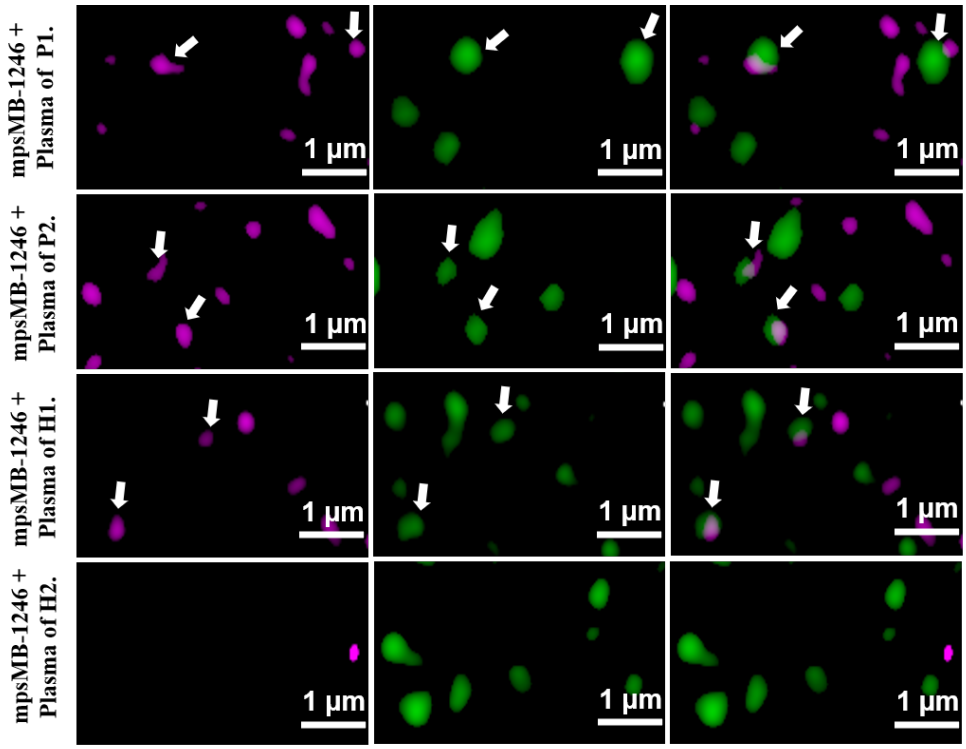

Fig. S2. Representative images of super-resolution optical microscopy (in SIM mode) of plasma samples of two breast cancer patients (P1, P2) and two healthy donors (H1, H2) after incubation with mpsMB-1246.