Analyst

Electronic Supplementary Information (ESI)

Exosomes: Improved methods to characterize their morphology, RNA content, and surface protein biomarkers

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The supporting information includes the following additional figures:

Figure S1: SEM images of frozen exosomes from mouse melanoma cells B16F0 and human breast cancer cells SKBR3.

Figure S2: Calibration of HER1 and HER2 expression on SKBR3 cells by flow cytometry.

Figure S3: Quantification of the forward scatter properties of fluorescent nanoparticles that bracketed the expected size range of exosomes.

Figure S4: Flow cytometric analysis of B16F0 exosomes stained using APC conjugated IL12RB2 mAb and the lipophilic dye DiI.

Table S1: Primers for semi-quantitative PCR.

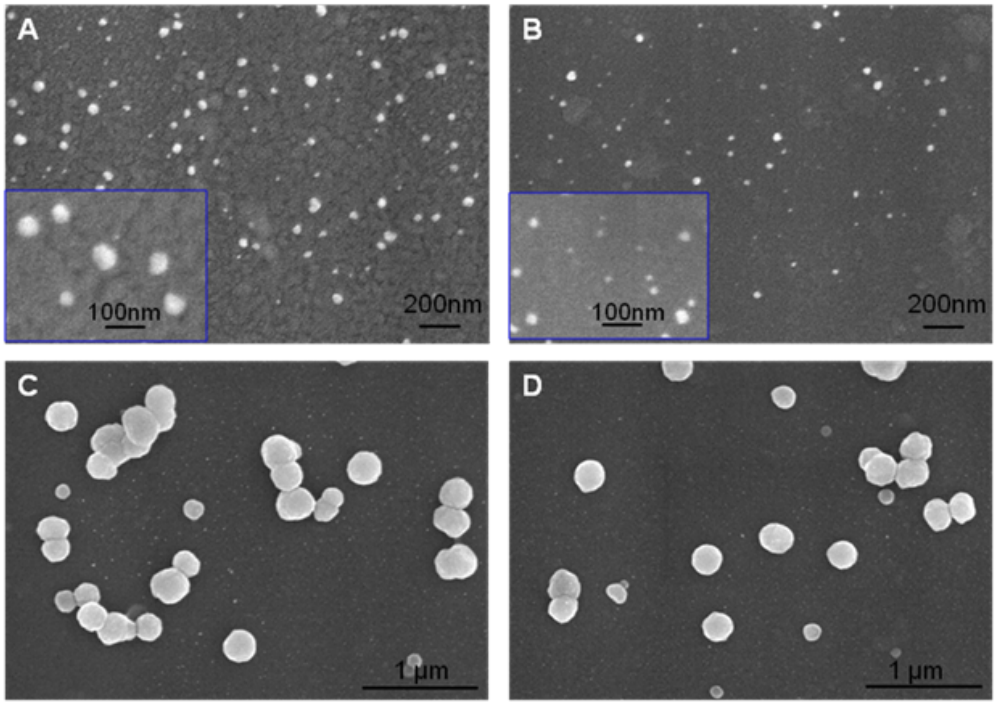

Figure S1. SEM images of frozen exosomes from mouse melanoma cells B16F0 (A, C and D) and human breast cancer cells SKBR3 (B). (A and B) Exosomes were stored at -80°C in serum free medium for 1-2 weeks, thawed on ice, fixed by paraformaldehyde and examined by SEM. (C and D) Exosomes from B16F0 were frozen at -80°C in serum free medium with 5-10% DMSO as a cryoprotectant and thawed on ice (C) or thawed quickly at 37°C (D).

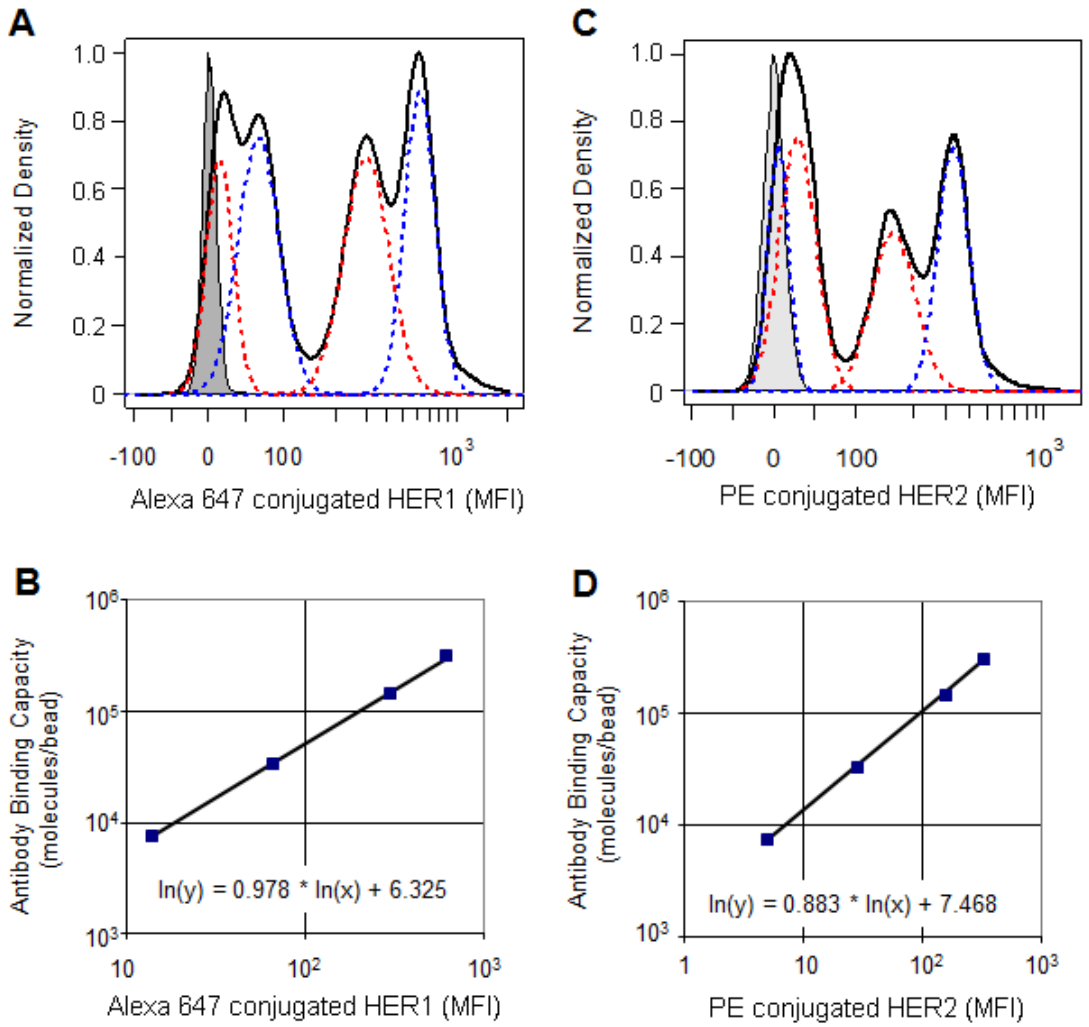

Figure S2. Calibration of HER1 and HER2 expression on SKBR3 cells to equivalent copy numbers per cell by flow cytometry using quantum calibration beads. Copy number expression was obtained using five quantum simply cellular microsphere populations, one blank and four labeled with increasing amounts of anti-mouse IgG antibody, that exhibit a defined antibody binding capacity. Probability density functions for the five microsphere populations stained using Alexa 647-conjugated anti-HER1 mAb (Panel A) and uing PE-conjugated mouse anti-HER2 mAb (Panel C). Plots of the median MFI for the four antibodystained microspheres versus the corresponding antibody binding capacity, expressed in molecules per bead (HER1 - panel B, HER2 - panel D).

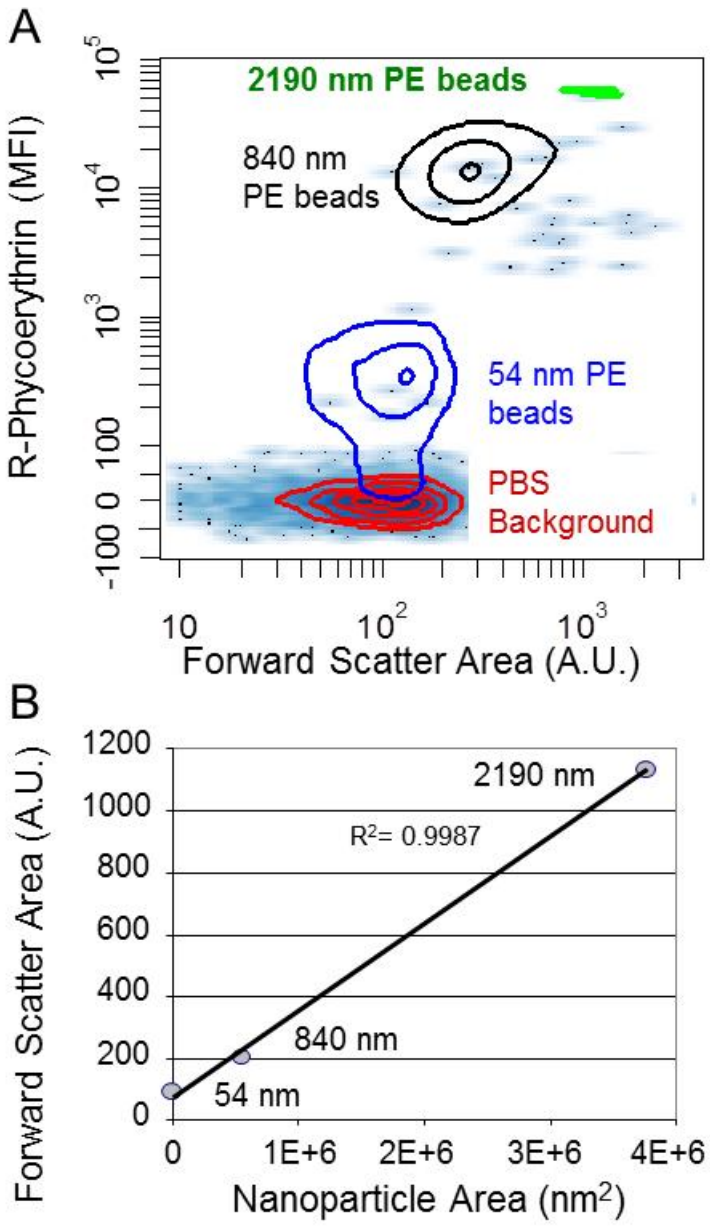

Figure S3. Forward scatter area is proportional to particle size. Forward scatter area versus the fluorescence levels of 3 different sized beads that are fluorescently labeled (A) and the standard curve of particle sizes versus their respective forward scatter area (B). The three sets of beads have nominal diameters of 2.19, 0.84 and 0.054 μm, respectively.

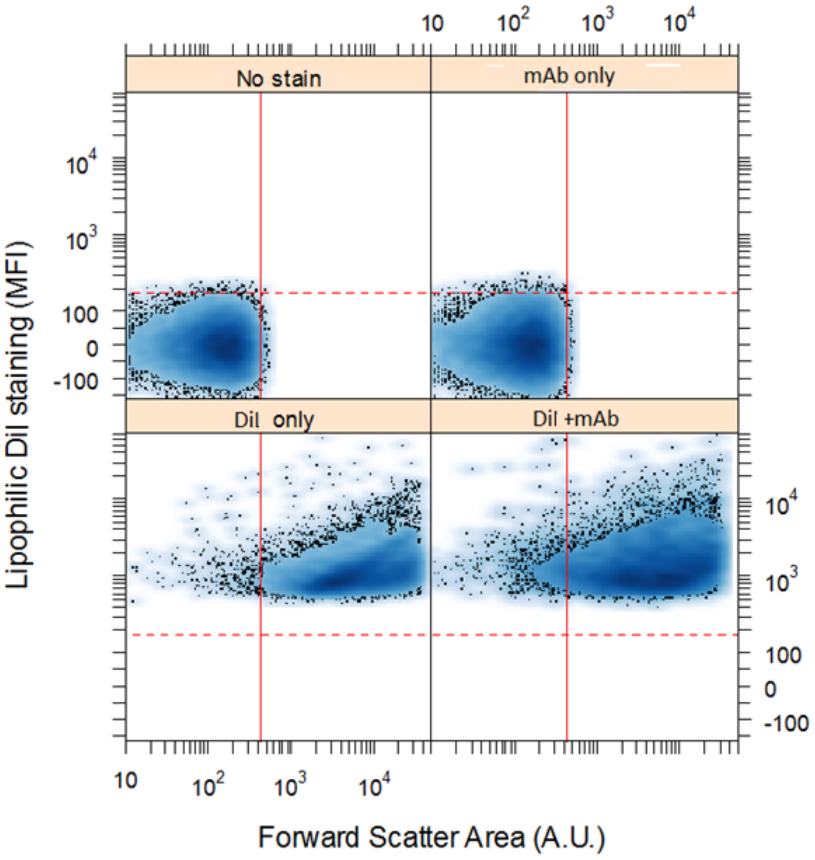

Figure S4. Flow cytometric analysis of B16F0 exosomes stained using APC conjugated IL12RB2 mAb and the lipophilic dye DiI.

Table S1. Primers used for semi-quantitative PCR.

Genes (mouse)	Amplicon	Primers	Sequences
Eif4ebp2	ORF	Forward	ATGTCCGCGTCGGCCGGTG
		Reverse	TCAGATGTCCATCTCAAACTGAG
	Intron # 1	Forward	GTAGAGGCGCTGTCAGGTTT
		Reverse	CCCGACTAGTGTGTGCTGTT
Wsb2	ORF	Forward	ATGGAGGCCGGAGAGGAG
		Reverse	CTAGAAAGTCCTGTATGTGAGG
	Intron # 1	Forward	GTAGGTCCCGACACCCTAGT
		Reverse	CCATCCCTAGCATCTCTGCG
Rnd2	ORF	Forward	ATGGAGGGGCAGAGTGGC
		Reverse	TCACATGAGGTTACAGCTCTTG
	Intron # 1	Forward	GCATCAGAGGATCCGGAAGG
		Reverse	GGTCCAGCTCAGTCCCTAGA