

Supplemental Material to:

**Large oncosomes mediate intercellular transfer of
functional microRNA**

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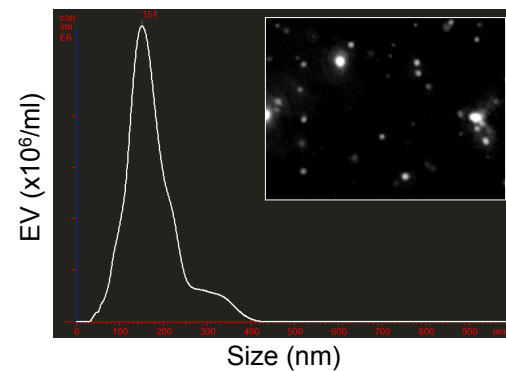
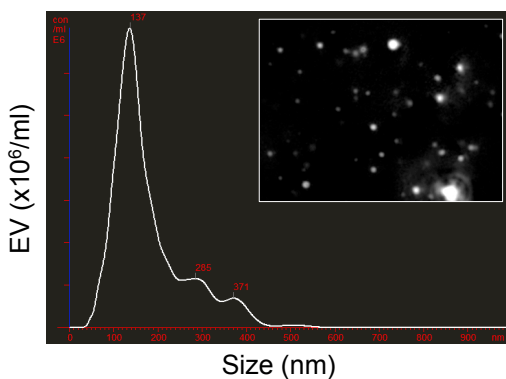
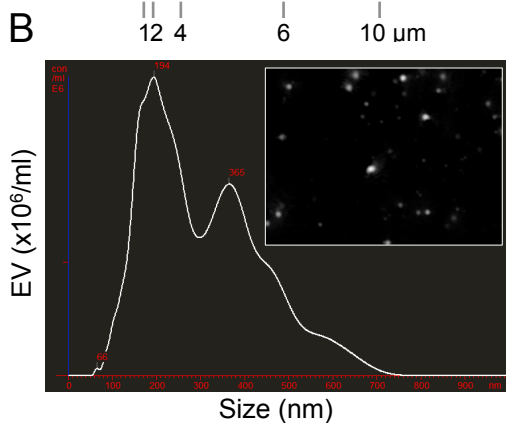
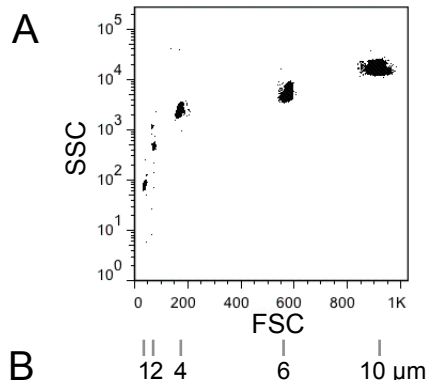


Figure S1. (A) One, 2, 4, 6 and 10 μm size beads were used to set size gates for flow cytometry analysis. **(B)** Representative NTA images of size distribution of the particles obtained by centrifugation (10,000 g and 100,000g, top and bottom panel, respectively), and filtration (middle panel).

Pearson's correlation coefficient for EV and cell samples

	A1: EV1	B1: EV2	A2: EV1	B2: EV2
A1: EV1	1	0.850	0.910	0.807
B1: EV2	0.850	1	0.849	0.801
A2: EV1	0.910	0.849	1	0.810
B2: EV2	0.807	0.801	0.810	1

	A1: RWPE-1	B1: RWPE-2	A2: RWPE-1	B2: RWPE-2
A1: RWPE-1	1	0.896	0.912	0.889
B1: RWPE-2	0.896	1	0.914	0.893
A2: RWPE-1	0.912	0.914	1	0.893
B2: RWPE-2	0.889	0.893	0.893	1

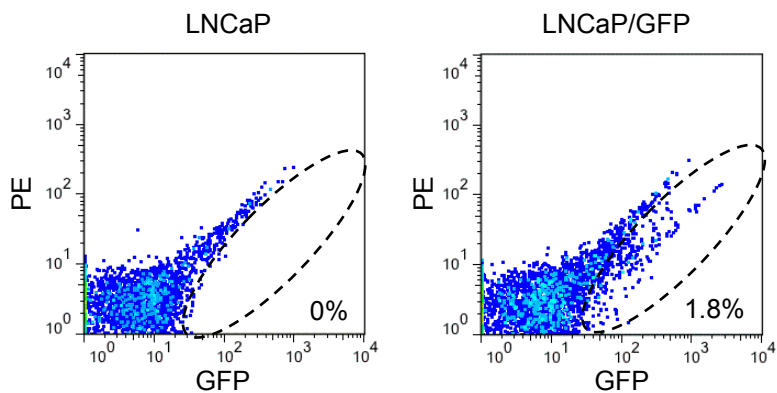
Figure S2. Pearson's correlation coefficients. Technical replicates showed good signal correlation. Overall, EV2 replicates had the lowest correlation values.

miRNA	Fold change	miRNA	Fold change
miR-1227	1.92	let-7f-1*	1.52
miR-615-3p	1.79	miR-448	1.51
miR-541*	1.79	miR-579	1.51
miR-1224-3p	1.77	miR-16-2*	1.50
miR-548c-3p	1.69		
miR-646	1.68		
let-7i*	1.63		
miR-1181	1.62		
miR-1204	1.60		
miR-519c-5p	1.59		
miR-1253	1.59		
miR-506	1.59		
miR-518a-3p	1.58		
miR-34c-5p	1.53		

Figure S3. Tables showing miRNAs differentially expressed in EV2 compared to RWPE-2 cells. The table on the top shows miRNAs significantly (fold change > 1.5) higher in EV2 compared to RWPE-2 cells but non-significantly higher in EV1 compared to RWPE-1 cells; the table on the bottom shows miRNAs significantly (fold change ≤ 1.5) lower in EV2 compared to RWPE-2 cells but non-significantly lower in EV1 compared to RWPE-1 cells. The number of miRNAs lower in EV2 than in RWPE-2 cells is higher than the number of miRNAs higher in EV2 than in RWPE-2 cells.

miRNA	Fold change	miRNA	Fold change	miRNA	Fold change
miR-1827	-5.91	miR-708	-1.76	let-7a*	-1.84
miR-599	-3.20	miR-92a	-1.76	miR-200c	-1.81
miR-548f	-3.14	miR-497*	-1.73	miR-342-5p	-1.80
miR-620	-3.09	miR-130a	-1.71	miR-23b	-1.79
miR-103	-3.02	miR-139-5p	-1.69	miR-632	-1.54
miR-107	-2.25	miR-144*	-1.69	miR-339-5p	-1.53
miR-340*	-2.21	miR-149	-1.67	miR-320c	-1.51
miR-1290	-2.17	miR-578	-1.66	miR-500	-1.50
miR-151-5p	-2.06	miR-193b	-1.65	miR-191	-1.50
miR-22*	-1.93	miR-23a	-1.65		
miR-320d	-1.93	miR-1274a	-1.65		
let-7i	-1.88	miR-182	-1.59		
miR-31*	-1.87	miR-152	-1.58		
miR-603	-1.85	miR-423-3p	-1.54		

A



B

miRNA	Fold change
miR-22*	1.87
miR-220a	1.79
miR-548h	1.76
miR-199b-5p	1.62
miR-301a	1.57
miR-486-3p	1.57
miR-377	1.54
miR-621	1.52
miR-518a-3p	-1.52
miR-132*	-1.52
miR-378*	-1.54
miR-1261	-1.55
miR-302b	-1.62
miR-548c-3p	-1.74

Figure S4. (A) GFP-positive large EV were purified from the conditioned media of LNCaP/GFP cells and analyzed by flow cytometry. (B) Table showing miRNAs differentially expressed in RWPE-2 cells compared to RWPE-1 cells.