Supporting Information

Chemically edited exosomes with dual ligand purified by microfluidic device for active targeted drug delivery to tumor cells

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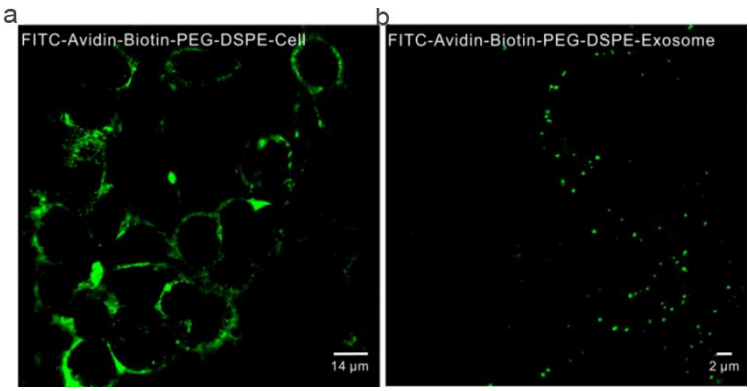

Figure S1. The specificity and efficiency of the cell membrane biotinylation and exosomes membrane biotinylation (a) Fluorescence image of biotinylated cell after labeled with FITC-Avidin. (b) Fluorescence image of biotinylated exosome secreted by biotinylated cell after labeled with FITC-Avidin.

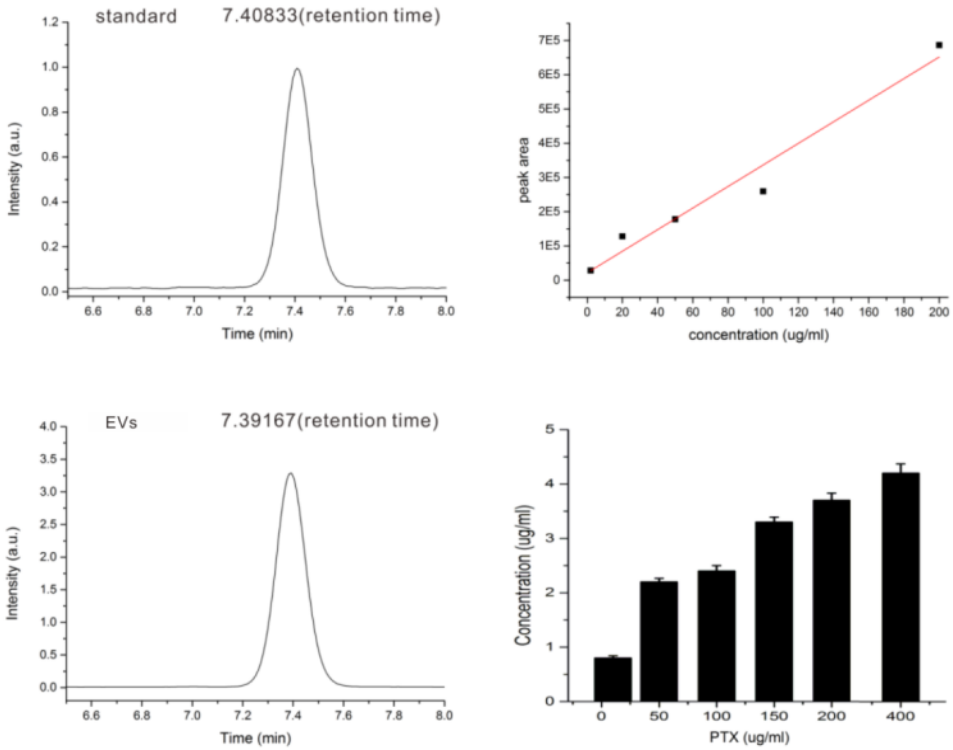

Figure S2. 1 × 10^8 HUVEC tumor cells in 5 mL culture media were treated with different concentrations of PTX. The exosomes were prepared and their exosome content was analyzed by high performance liquid chromatography (HPLC) according to previous methods (Bioconjug Chem. 2006; 17:1411-7). Paclitaxel was quantified by using a Waters 2695 HPLC system on a C18 column. Column temperature was maintained at a constant 40 °C and PTX was detected by UV absorption at 227 nm. The mobile phase consisted of acetonitrile and water (60 : 40, volume : volume), the flow rate of 1 mL/min. According to standard curve, samples concentrations were calculated according to the standard curve. Data shown were the representative of three independent experiments. Bars correspond to mean ± SD. Data were collected from three independent experiments.

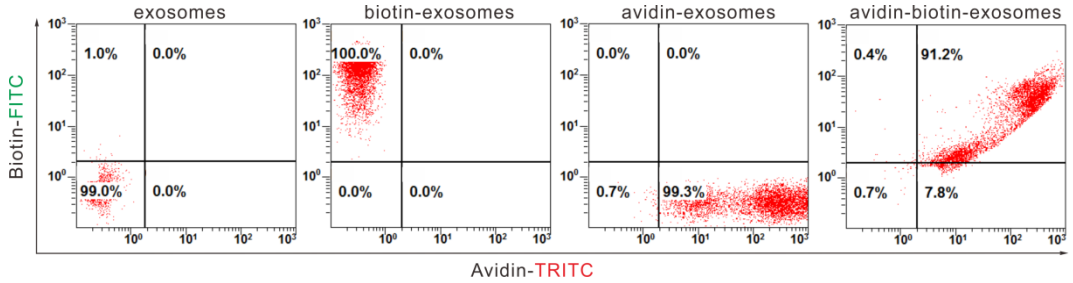

Figure S3. Quantification of biotin and avidin on the membrane of unmodified exosomes, DSPE-PEG-biotin modified exosomes (biotin-exosomes), DSPE-PEG-avidin modified exosomes (avidin-exosomes), and dual ligand labeled exosomes (avidin-biotin-exosomes) was performed by flow cytometry.

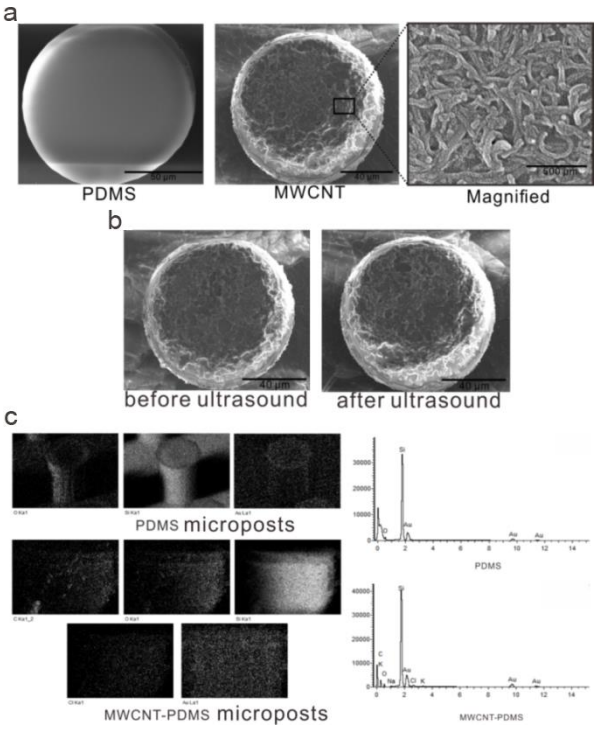

Figure S4. (a) Scanning electron microscopic images of the MWCNTs modified PDMS, magnified images showed the modification is three dimensional, thus demonstrated the possibility of high capturing efficiency of exosomes. (b) The modification was stable, even after ultrasonic. (c) Scanning electron microscopy and energy dispersive spectrum analysis of the microfluidic pillars.

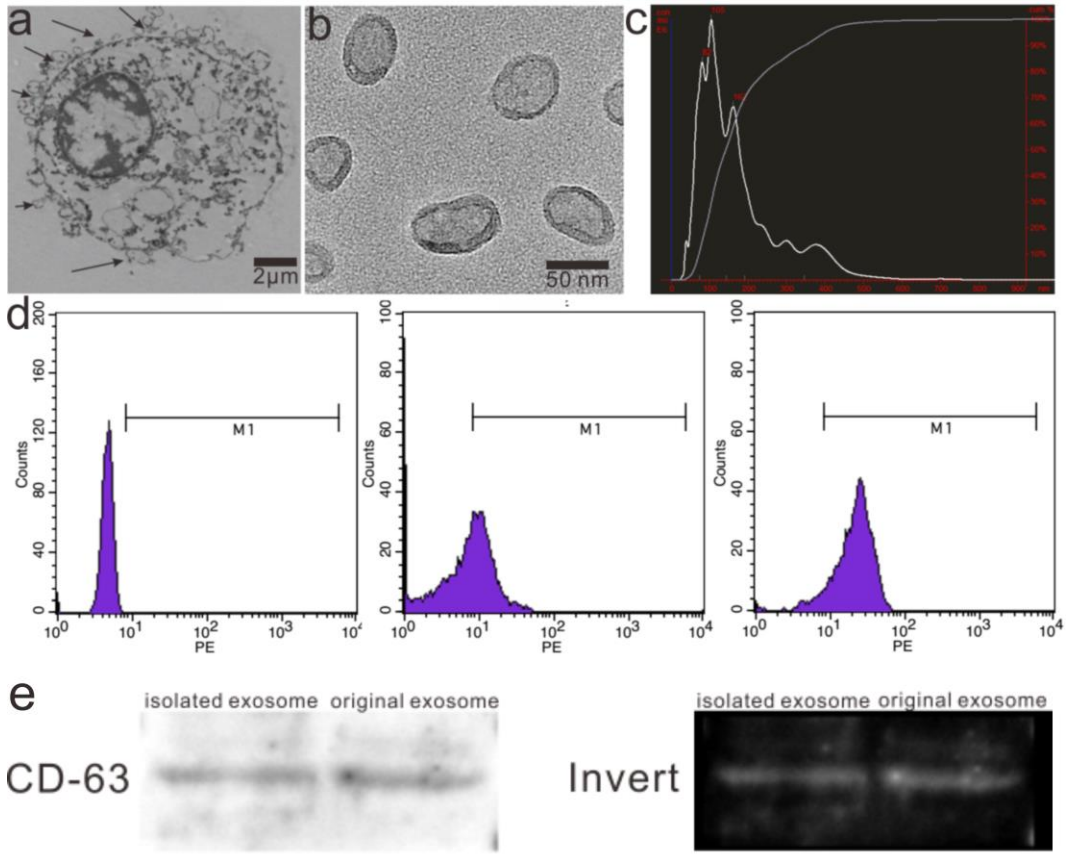

Figure S5. (a) Transmission electron microscope (TEM) was applied to visualize the exosome-releasing process (indicated by arrowheads) of biotinylated HUVEC. (b) TEM micrograph of the isolated exosome enriched by microfluidic chip. (c) Nanosight analysis to test the concentration and size distributions of exosome isolated by microfluidic chip. (d) Exosomes enriched by the microfluidic chip were stained with IgG-PE, CD9-PE, CD63-PE and analyzed by flow cytometry, repectively. IgG-PE served as the control, M1 = 0.08 %; CD9, M1 = 49.91 %; CD63, M1 = 93.25 %. (e) The Western blot analysis of CD63 expression of original exosomes and isolated exosomes.

Table S1 Comparison of exosome capture efficiency of our device with previous reports

exosome isolation method	total time-consuming	total nucleic acid	total protein	sample volume	recovery	reference
ultracentrifugation	4-5 h	187 ng	100 μg	5 mL	5%-23%	25
immuno-chip	1-1.5 h	30 ng	15-18 μg	400 μL	42%-94%	26
sieving chip	1 h	49 ng	100 μg	100μL	2%	27
porous structures trapping	over night	N/A	N/A	N/A	N/A	28
our device	40 min	30 ng	120 μg	400 μL	80.54%	this

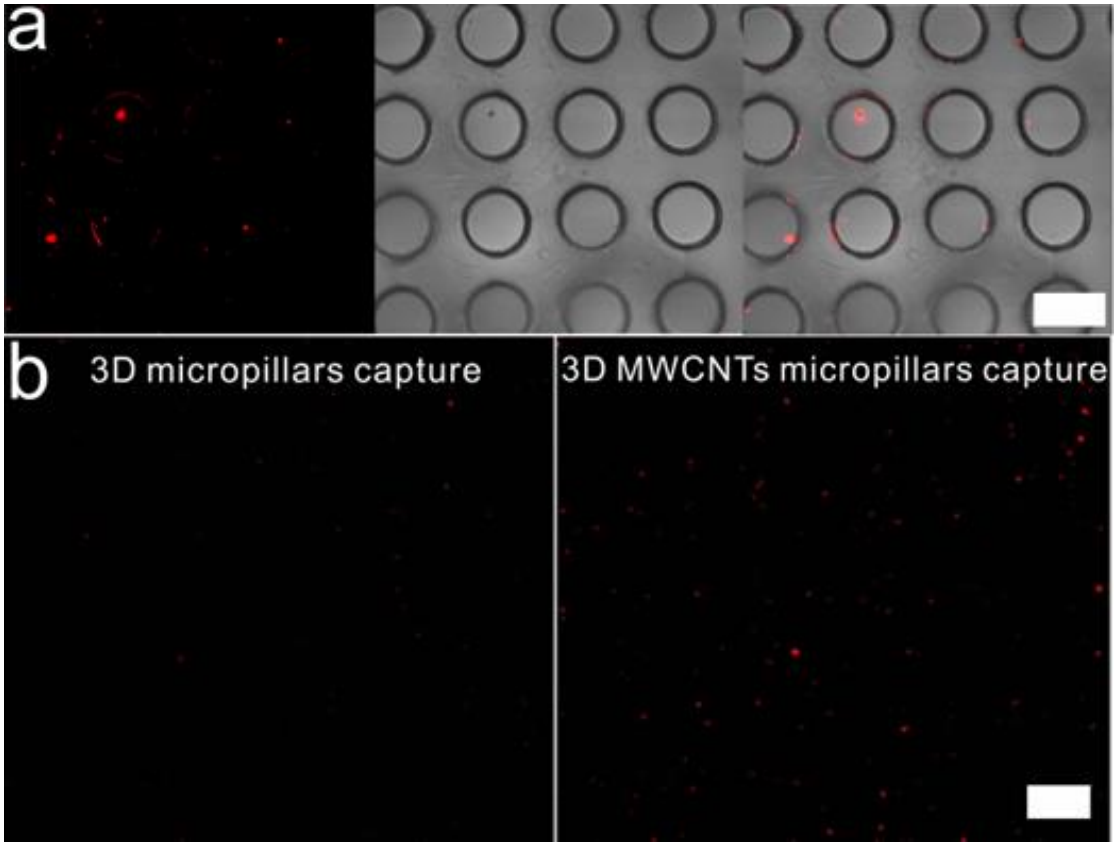

Figure S6. (a) The fluorescent image, bright image and merge image of exosomes captured on the chip surface. (b) The comparison of capture efficiency between the MWCNT-micropillars and micropillars. Scale bar, 60 μm.

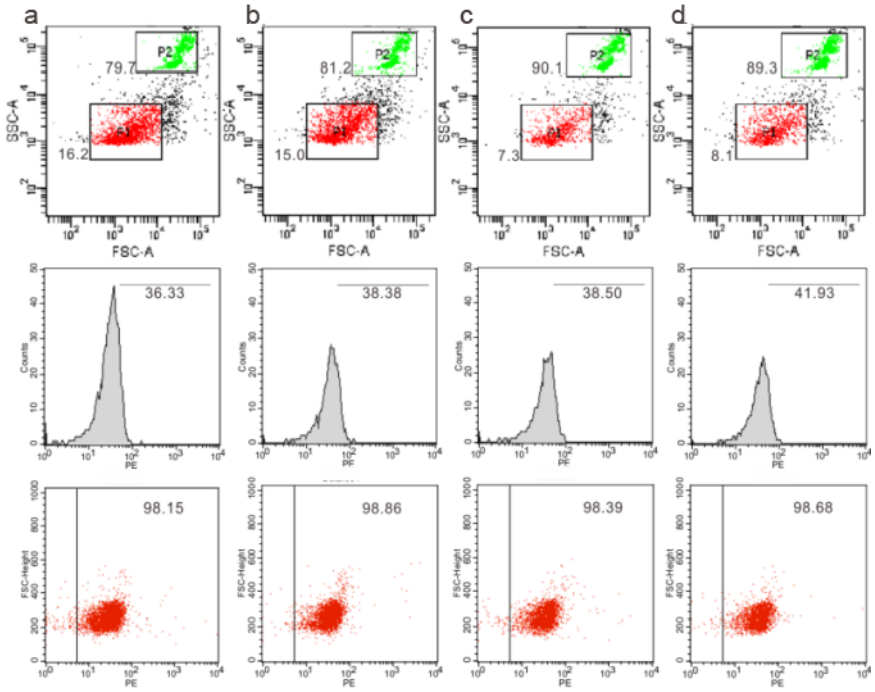

Figure S7. HUVEC cell-derived exosomes enriched from microfluidic chip were suspended in 0.1 μm filtered PBS and treated with different conditions, including put in 4 °C for a day (a) and a week (b), 4 °C (c) or 37 °C (d) for a week.

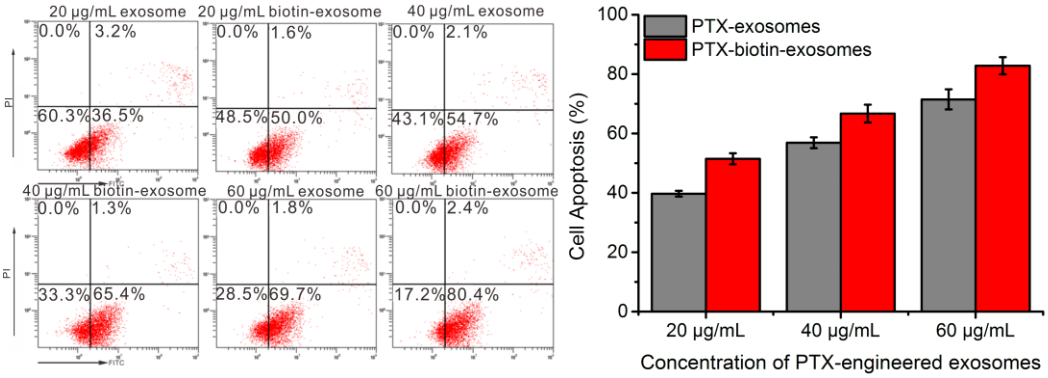

Figure S8. Inhibitory rates against HepG2 cells of PTX loaded exosomes and biotin-exosomes at different concentrations. The apoptosis of cells were analyzed by flow cytometry. 10000 events were collected for this analysis. Bars correspond to mean ± SD. Data (right) were collected from three independent experiments.

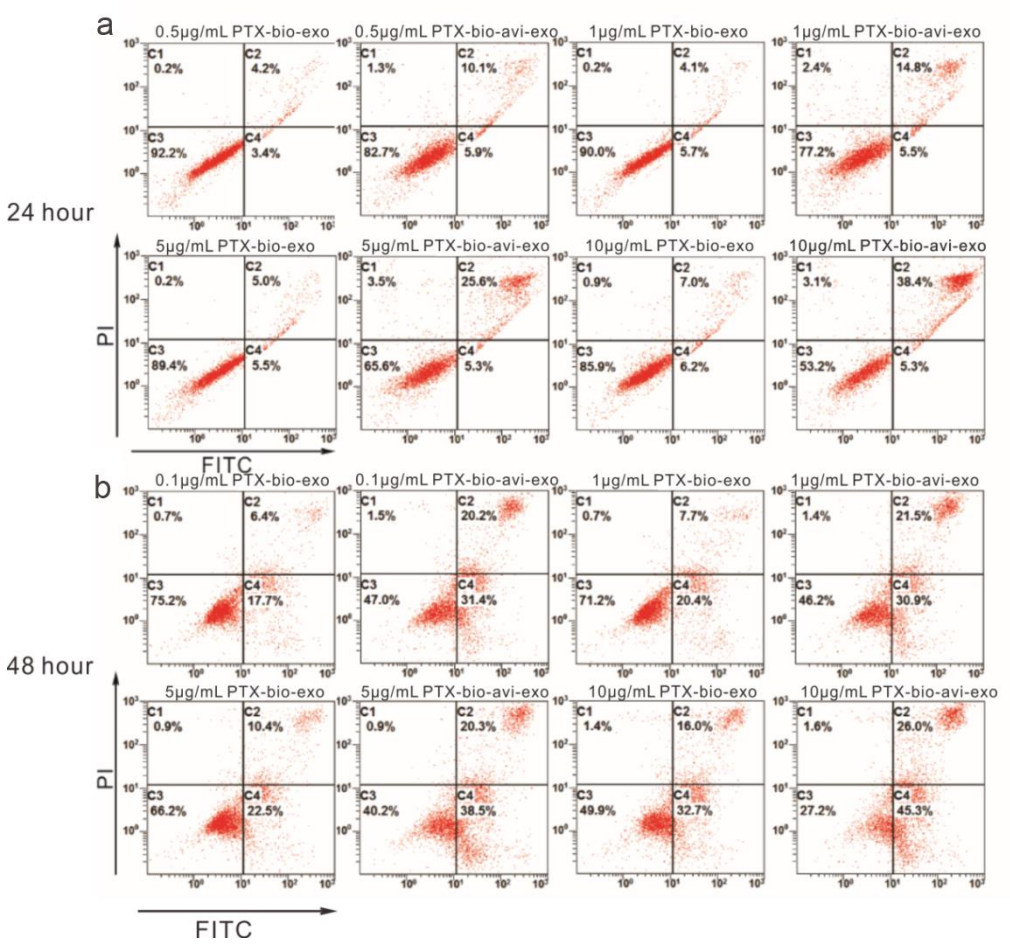

Figure S9. Inhibitory rates against HepG2 cells of PTX loaded biotin-exosomes and avidin-biotin-exosomes at different low concentrations after being incubated with HepG2 cells for 24h (a) and 48h (b). The apoptosis of cells were analyzed by flow cytometry. 10000 events were collected for this analysis.

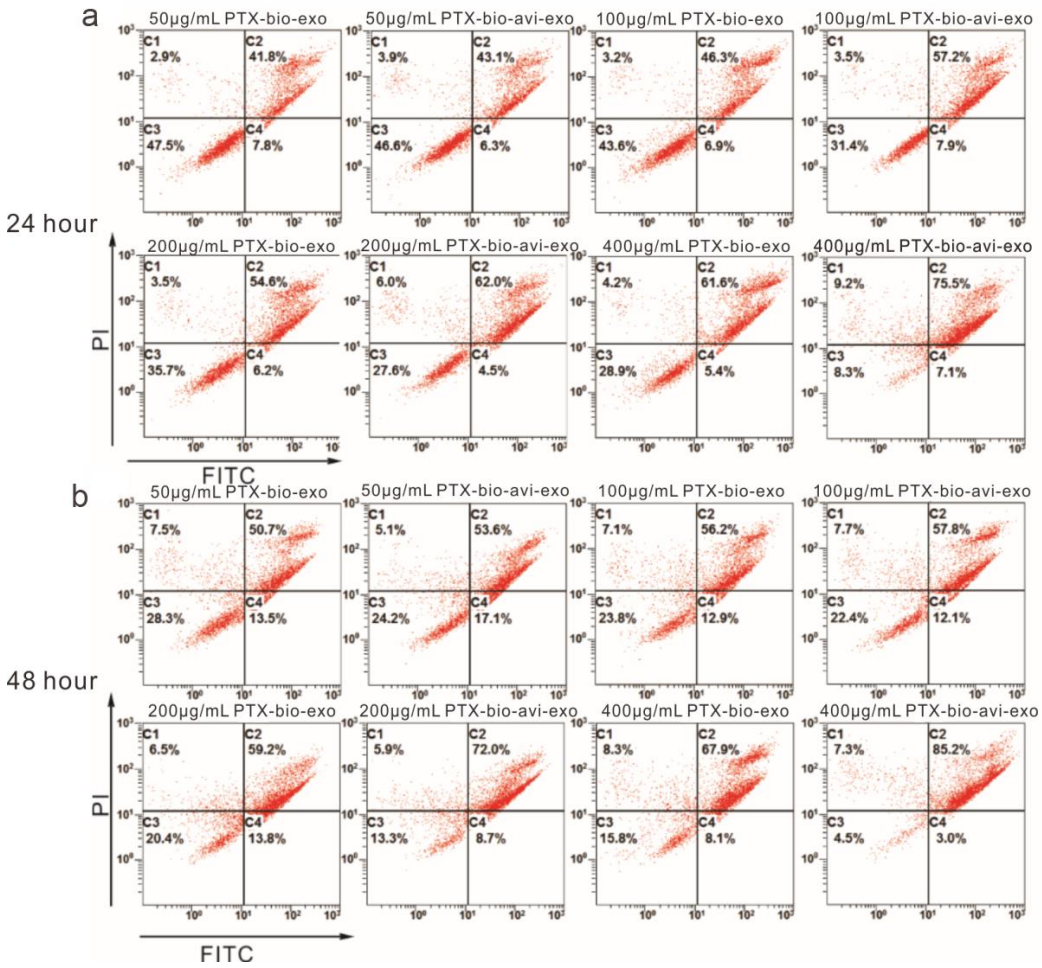

Figure S10. Inhibitory rates against HepG2 cells of PTX loaded biotin-exosomes and avidin-biotin-exosomes at different high concentrations after being incubated with HepG2 cells for 24h (a) and 48h (b). The apoptosis of cells were analyzed by flow cytometry. 10000 events were collected for this analysis.

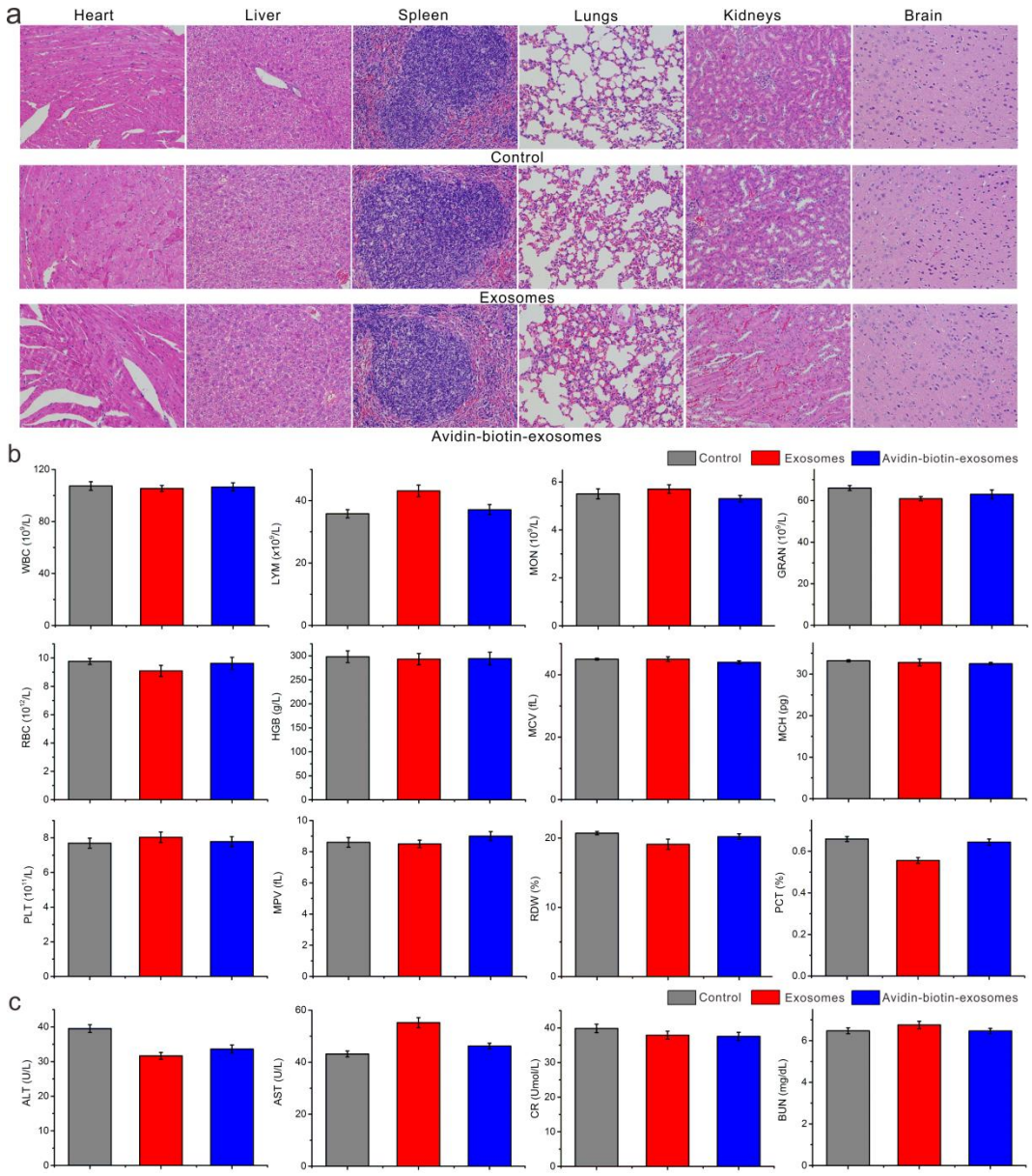

Figure S11. (a) The HE staining images of normal organs from mice treated with Saline, Exosomes and Avidin-biotin-exosomes. (b) Whole blood cell analysis of mice after injection of Saline, Exosomes and Avidin-biotin-exosomes; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; LYM, lymphocyte; MPV, mean platelet volume; MON, monocyte; PDW, platelet distribution width; GRAN, granulocyte; MCH, mean corpuscular hemoglobin; PLT, platelets; MCV, mean corpuscular volume; PCT, plateletcrit. (c) The hepatic (ALT, alanine aminotransferase; AST, aspartate aminotransferase) and renal (CRE, creatinine; BUN, blood urea nitrogen) induces of mice after injection of Saline, Exosomes and Avidin-biotin-exosomes.

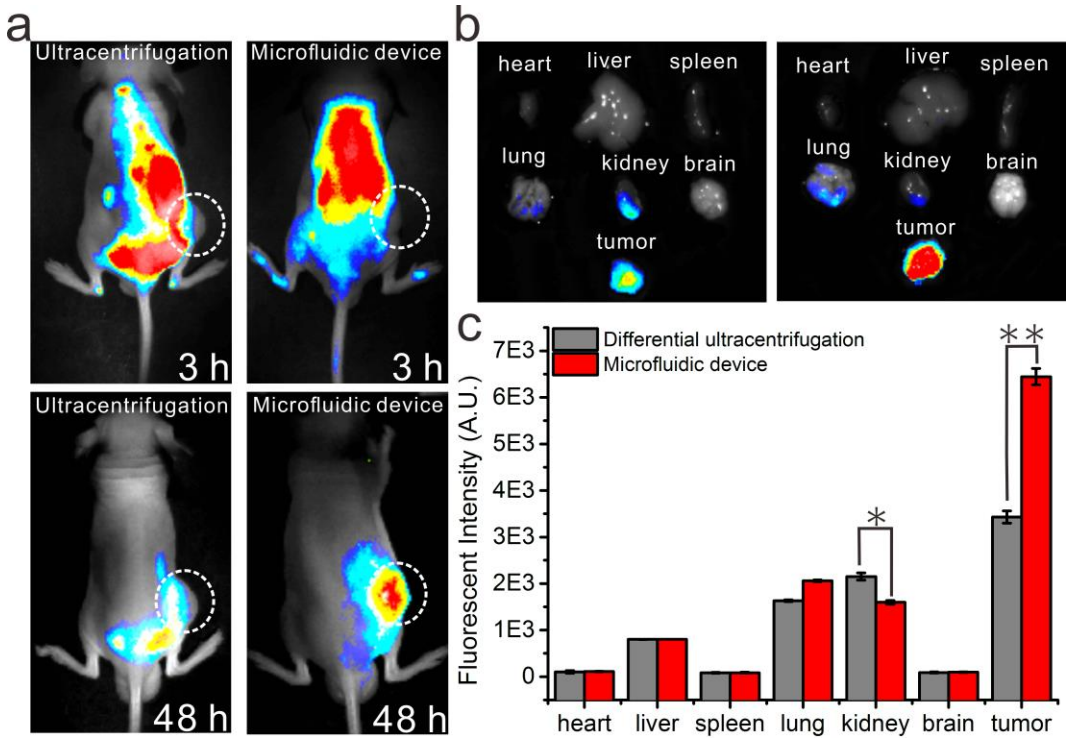

Figure S12. (a) Overall fluorescence imaging of HepG2 xenograft nude mice after the injection of the engineered exosomes (biotin-avidin-exosomes) isolated by the conventional ultracentrifugation and the engineered exosomes isolated by our microfluidic device. In vivo NIR fluorescence images were taken at 3h and 48 h after injection, respectively. (b) Ex vivo fluorescence images of tumor and other major organs after 48 h post-injection. (c) The fluorescent intensity of tumor and normal organs.