Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary information

Hyperspectral imaging-based exosome microarray for rapid molecular profiling of extracellular vesicles

Supplementary Note 1

Exosome tracking analysis. After the ultracentrifugation, the extracted EV samples were analyzed using a nanoparticle analysis tool (Nano-Sight LM10, Malvern Instruments, Malvern, UK) and transmission electron microscopy (TEM) to characterize the concentration, size, and morphology of the harvested EVs. The EV samples were resuspended in 500-µL dPBS and analyzed using Nano-Sight. The size distributions of $EV_{naïve}$, EV_{M1}, and EV_{M2} particles are shown in **Fig. S1a-c**, respectively. The $EV_{naïve}$ sample contains a high number of 83-nm and 97-nm particles. In contact, the diameter EV_{M1} sample fell in the 100 nm ±50 nm and the diameters of EV_{M2} particles were between 90 nm to 180 nm. **Fig. S1d** shows the TEM images of an $EV_{naïve}$ particles. The donut morphology of this particle is a treat of common EVs.

a **b**

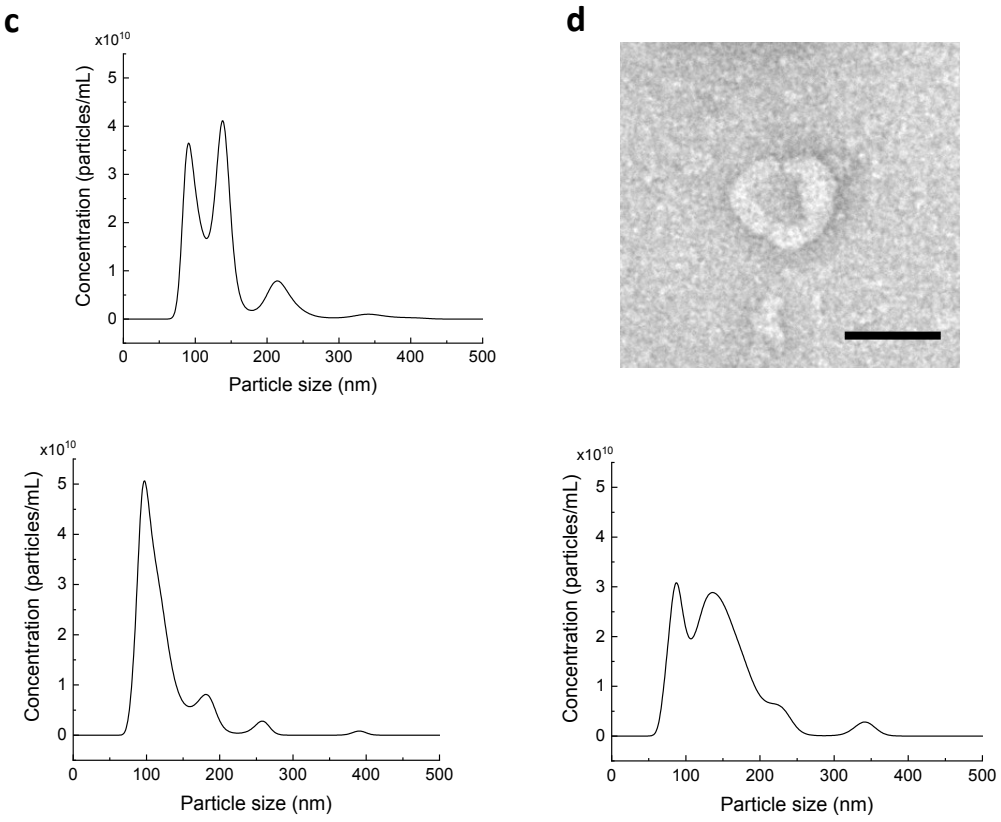

Figure S1: Characterization of harvested EV samples. Measured size distributions of (a) $EV_{naïve}$ (b) EV_{M1} and (c) EV_{M2} samples. (d) TEM image of an $EV_{naïve}$ particle. Scalebar: 100nm.

Supplementary Note 2

Fabrication and characterization of photonic crystal substrate. The major steps of the PC fabrication are summarized in **Fig. S2a**. The silicon replication mold stamp was purchased from LightSmyth Technologies (SNS-C18-2009). This silicon stamp carriers a linear grating with the period, depth, and grating width of 555.5 nm, 140 nm, and 340 nm, respectively. To transfer the grating pattern, a drop of liquid UV curable epoxy (NOA-88, Norland Products, Inc.) was injected between the mold and a 0.18 mm-thick glass coverslip. Subsequently, the UV curable epoxy was crosslinked using UV light at room temperature. As a result, the epoxy was polymerized and the grating was formed on the surface of polymer. After curing, the silicon mold was carefully separated from the silicon stamp. The separation was facilitated by pretreatment of the mold with an anti-adhesion saline (Trichloro(1H,1H,2H,2H-perfluorooctyl), Sigma-Aldrich). Next, the TiO_2 layer was deposited on the polymer grating using an electron beam evaporation (BJD-1800, Temescal). The thickness and refractive index of the TiO_2 layer were measured using a spectral reflectometer (F20, Filmetrics). The transmission spectra of the PC sensor were measured and shown in **Fig. S2b**. **Fig. S2b** shows the transmission spectrum before and after the functionalization with CD63 antibody (0.5 mg/mL).

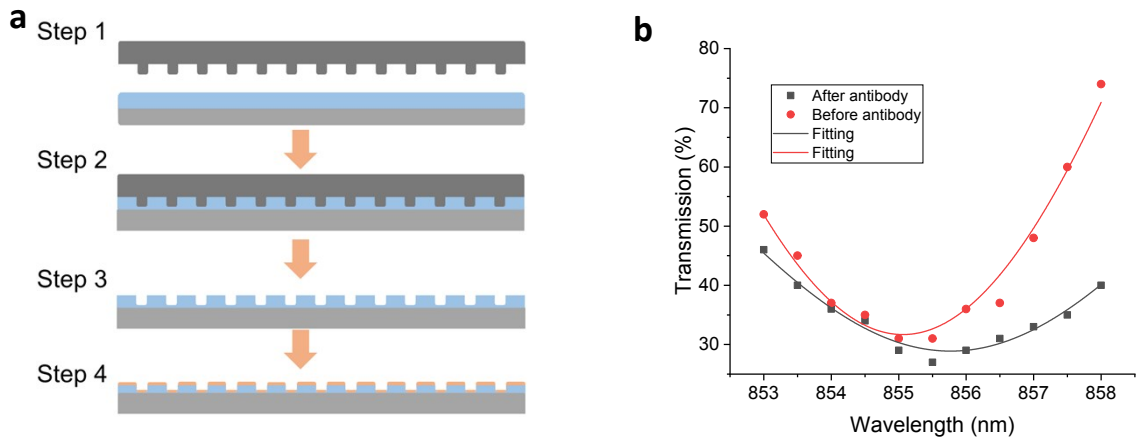

Figure S2: (a) Fabrication flowchart for the photonic crystal sensor. Step 1: Deposition of liquid UV epoxy polymer thin layer between glass substrate and silicon wafer stamp. Step 2: Liquid UV epoxy polymer is converted to solid after the exposure of UV light. Step 3: The silicon wafer stamp is carefully separated from cured epoxy. Step 4: TiO_2 thin layer is applied on the cured epoxy polymer grating. **(b)** Measured transmission spectra before and after the printing of CD63 antibody.

Supplementary Note 3

Fabrication process for the exosome microarray. The microarray pattern was created using a negative photoresist (SU-8 2000.5, MicroChem). The photoresist was spun on the photonic crystal and patterned using conventional UV photolithography following the Steps 1-3 in **Fig. S3**. After the photolithography, the 500-nm-thick SU-8 layer formed the array of microwells, whose diameter is 60 µm. The PC-based microarray was treated using oxygen plasm for two minutes to remove the residues of photoresist and developer and increase its hydrophilicity. Then, the microarray was coated using PVAm and glutaraldehyde to immobilize the antibodies. The antibodies were printed into the microwell as shown by the Step 4 in **Fig. S3**.

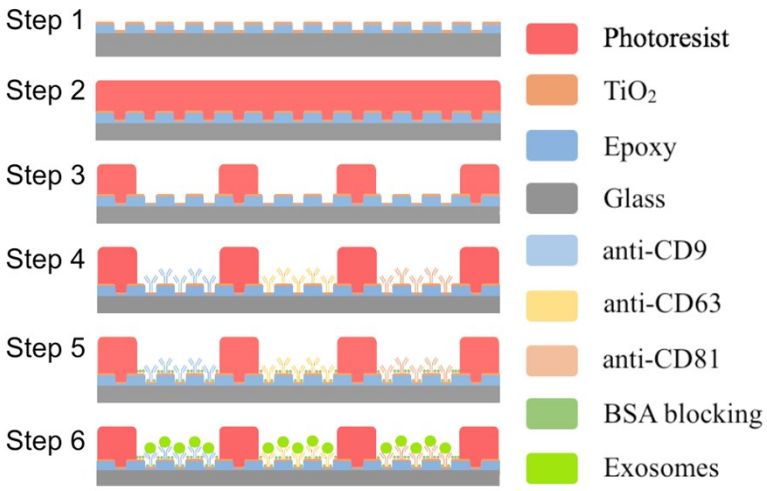

Figure S3: Flowchart for the fabrication of exosome microarray on the surface of a photonic crystal sensor. Step 1: Photonic crystal structure. Step 2: Photoresist is spin coated on the photonic crystal surface. Step 3: Photoresist is patterned using UV photolithography. Step 4: Antibodies are printed on the photonic crystal. Step 5: Non-specific bindings is prevented by bovine serum albumin (BSA). Step 6: PC sensor surface is covered with exosomes, None-Bonded exosomes are washed away. Bonded exosomes with corresponding antibodies remain on the PC sensor surface.

Supplementary Note 4

Label-free images of the EVs from M1 and M2 macrophages. Fig. S4a and **fig. S4b** present the measured label-free images of the EV microarray for the EV_{M1} and EV_{M2} samples. The concentration and volume of the EV_{M1}, and EV_{M2} samples were 2×10^{13} particles mL^{-1} and 5 µL, respectively. The sensor output ($\Delta\lambda_r$) for each antibody-EV binding was calculated and summarized in **Fig. 6**.

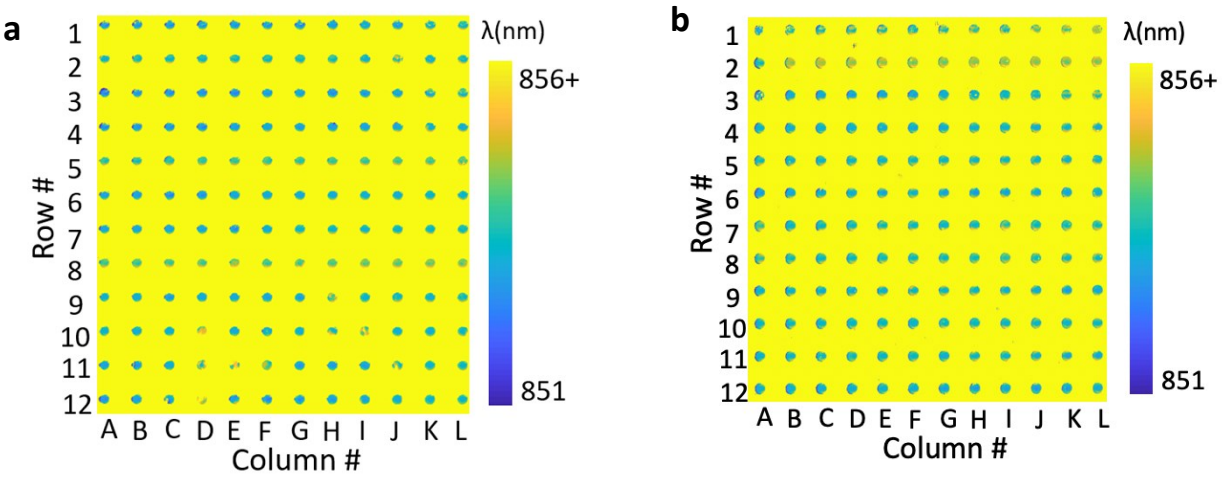

Figure S4: (a) Label-free image of the bindings of M1 macrophage-derived vesicles on the EV microarray. (b) Label-free image of the bindings of EVs collected from the M2 macrophages.