Supporting Information

Multiplexed Immunophenotyping of Circulating Exosomes on Nano-engineered ExoProfile Chip towards Early Diagnosis of Cancer

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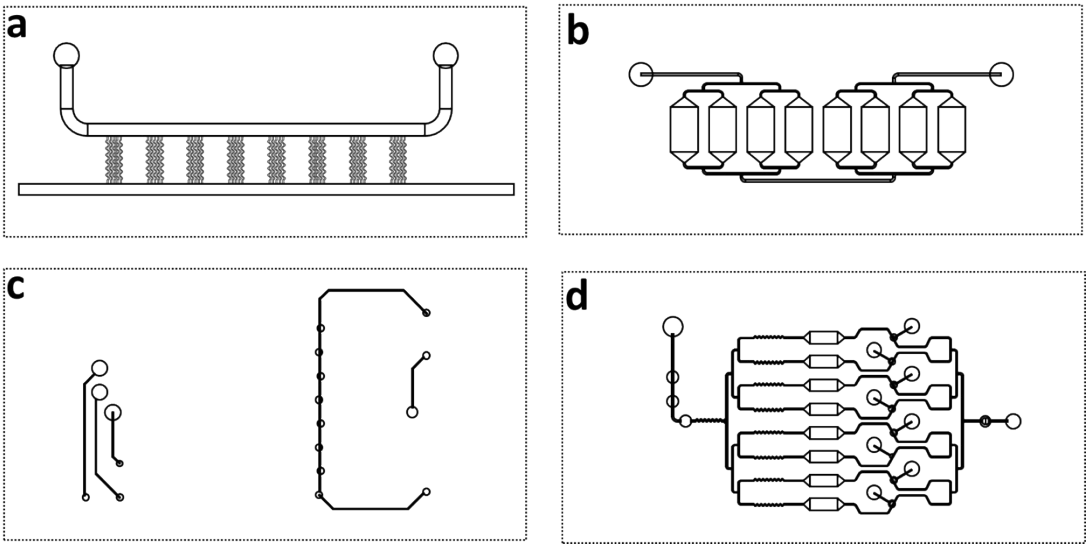

Fig. S1. Designed patterns for each PDMS layer of ExoProfile chip, (a) beads pattern layer, (b) antibodies immobilization layer, (c) pneumatic control layer and (d) fluidic layer.

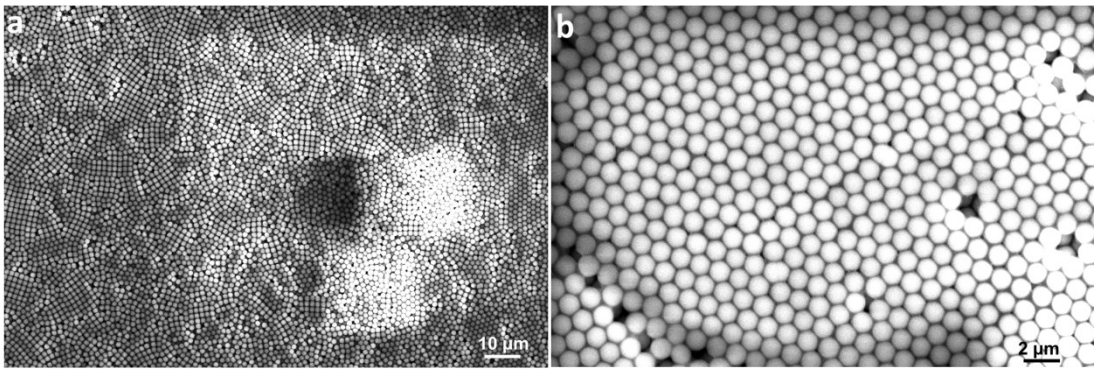

Fig. S2. SEM images of 3D self-assembled serpentine nanostructures at different magnification showing highly ordered crystalline structure.

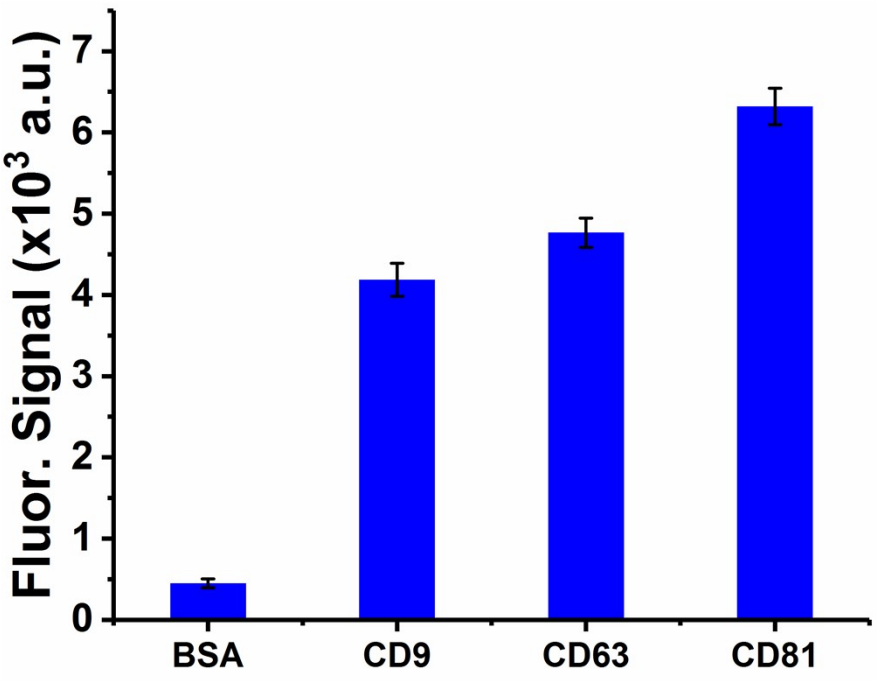

Fig. S3. Optimization of capture antibodies for efficient capture of SKOV3 exosomes. UC purified exosomes (10^5 μL^{-1}) were captured on the BSA, anti-CD9, anti-CD63, and anti-CD81 mAb immobilized ExoProfile chip, respectively, and detected with a mixture of biotinylated CD9, CD63 and CD81 antibodies. Error bar: S.D. (n = 3).

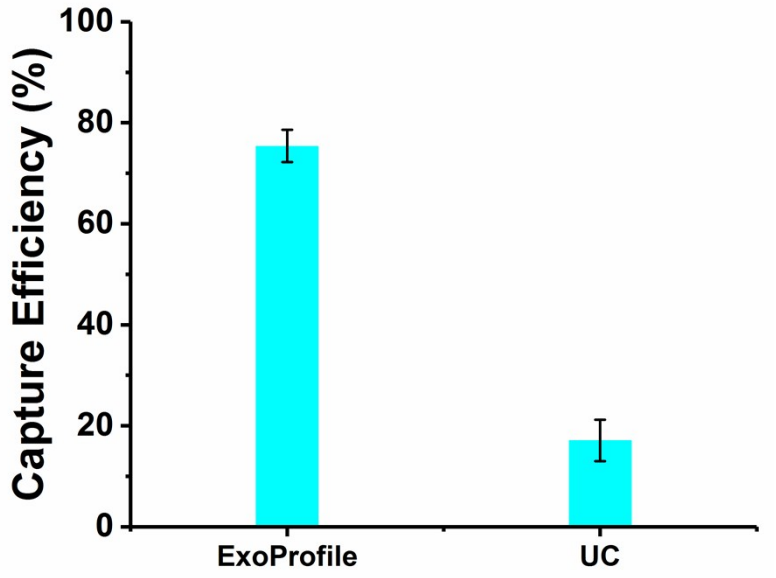

Fig. S4. Capture efficiency of SKOV3 exosomes on CD81 modified ExoProfile chip and conventional ultracentrifugation, respectively. The SKOV3 standard exosomes (10^6 μL^{-1}) were stained with DiO dye and then captured on ExoProfile chip. The fluorescent signal of exosomes solution before and after capture was measured. The capture efficiency of SKOV3 exosomes on CD81 modified chip was calculated to 75.4% in average. As a comparison, the capture efficiency of conventional ultracentrifugation was determined to be 17.3%. Error bar: S.D. (n = 3).

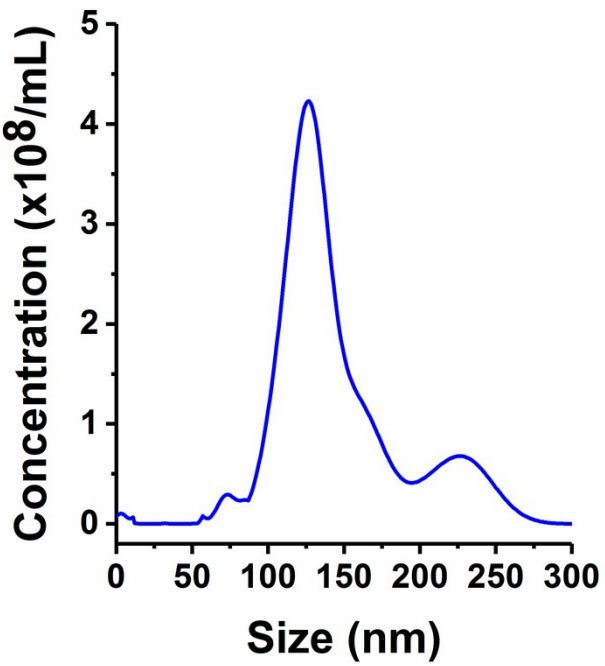

Fig. S5. NTA test of EVs isolated from SKOV3 cell culture media with ultracentrifugation.

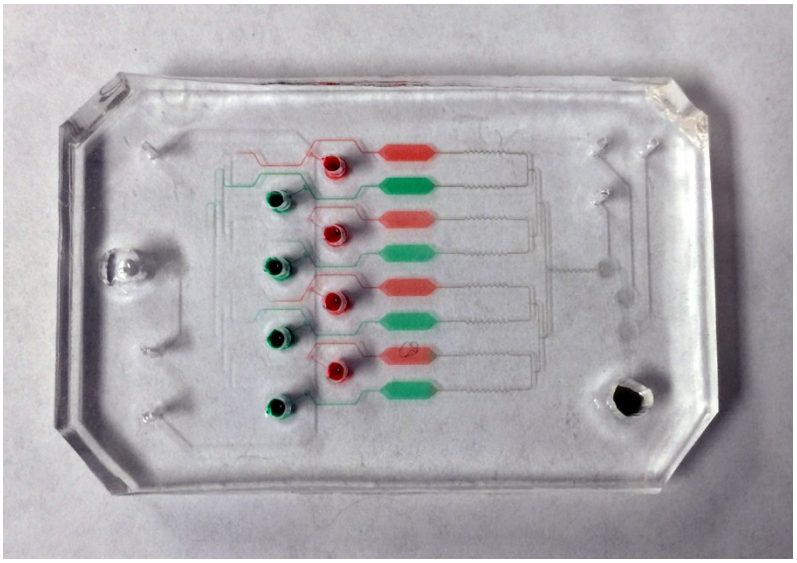

Fig. S6. Green and red dye were injected into eight parallel chambers of ExoProfile chip. After 0.5h, no obvious color mixing was observed between adjacent chambers.

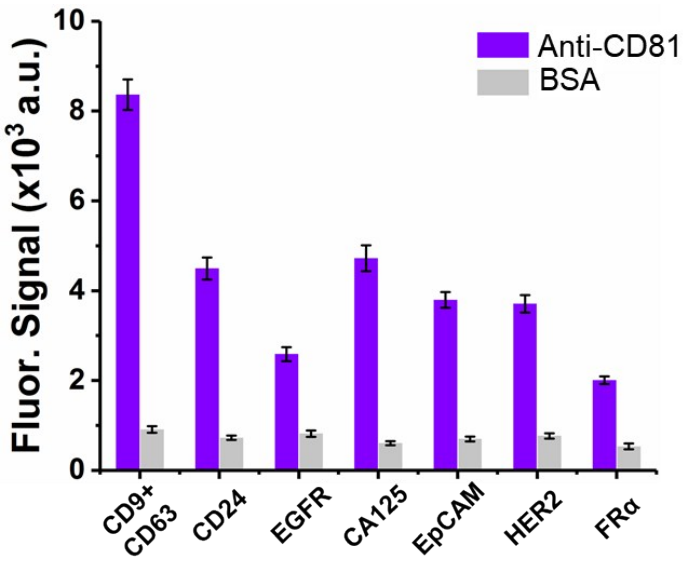

Fig. S7. Seven exosomal markers of ovarian cancer patient plasma measured with an anti-CD81 mAb-coated and only BSA-blocked ExoProfile chip, respectively. The low background signal indicates minimized non-specific adsorption of free proteins in plasma samples and high specificity of our assay. Error bar: S.D. (n = 3).

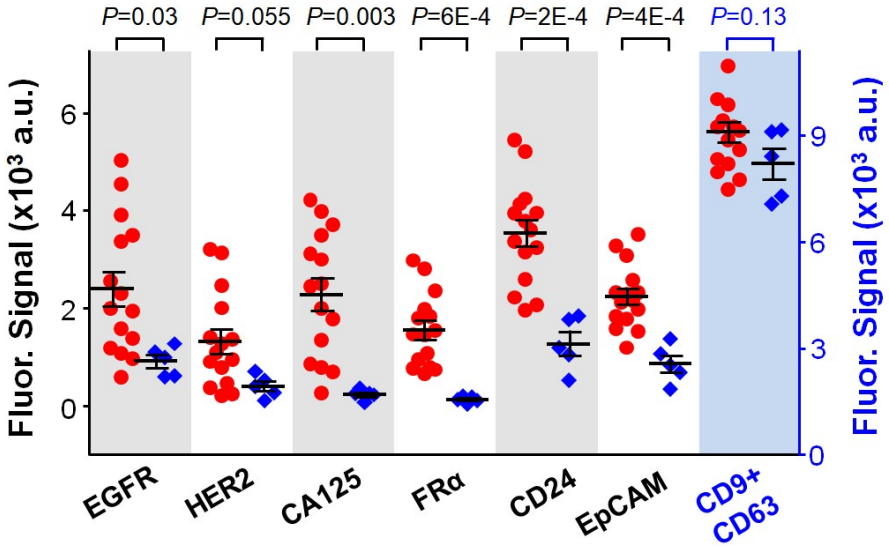

Fig S8. Scattering plots of the seven exosomal markers detected in the controls (n = 5, blue diamonds) and ovarian cancer patients (n = 15, red dots). Two-tailed Student's t-test was used for comparison with the significance level set at $p < 0.05$. Error bars indicate the mean value and standard deviation of the mean (s.e.m.).

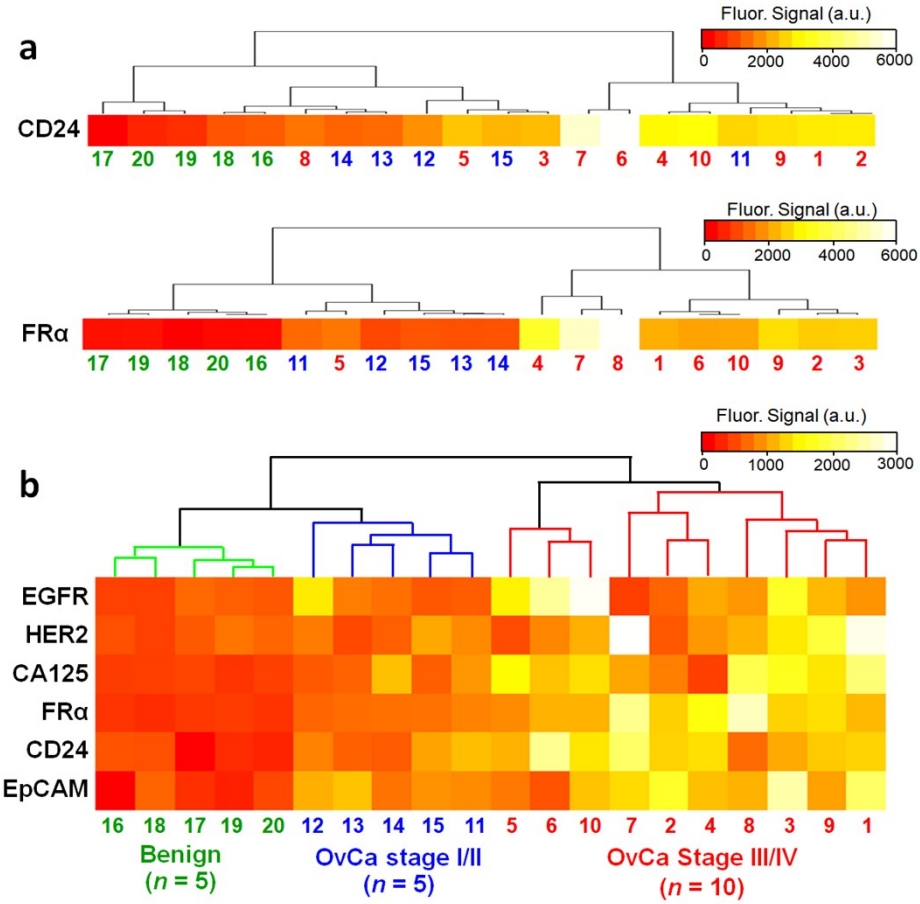

Fig. S9. Non-supervised hierarchical clustering analysis of (a) individual exosomal markers and (b) a panel of six markers in human plasma samples measured in Figure 5a. Samples 1-10 were advanced-stage ovarian cancer, 11-15 early-stage ovarian cancer, and 16-20 benign controls. Clustering analysis was performed with Ward linkage and Euclidean distance.

Table S1. The antibodies used in this research.

Target	Vendor	Catalog No.	Clone
CD9 (biotin)	Ancell	156-030/mono mouse	C3-3A2
CD63 (biotin)	Biolegend	353018/mono mouse	H5C6
CD81 (biotin)	Ancell	302-030/mono mouse	1.3.3.22
CD81	Ancell	302-820/mono mouse	1.3.3.22
EpCAM (biotin)	Abcam	ab187270/mono mouse	MOC-31
CD24 (biotin)	eBioscience	13-0247-80/mono mouse	SN3 A5-2H10
FRα (biotin)	R&D Systems	BAF5646/poly goat	poly
CA125 (biotin)	GeneTex	GTX44293/mono mouse	X306
HER-2 (biotin)	eBioscience	BMS120BT/mono mouse	2G11
EGFR (biotin)	Abcam	ab24293/mono mouse	EGFR1
EGFR	BD Bioscience	555996/mono mouse	EGFR1
IgG (FITC)	Life Technologies	34-152-110413/ poly goat	poly

Table S2. Parameters for the patterning chips and the ExoProfile chip.

PDMS layer	Thickness	Photoresist	PDMS base/curing ratio
Patterning chip	25 μm	SU8 2025	10:1
Antibody coating chip	50 μm	SU8 2050	10:1
Pneumatic control layer	50 μm	SU8 2025	7:1
Fluidic channel layer	35 μm	SU8 2025	15:1

Table S3. Information of clinical plasma samples.

Sample ID	Age	Stage	Histology	Pathology
1	72	IIIC	High grade serous adenocarcinoma	Malignant
2	70	IIIC	High grade serous carcinoma	Malignant
3	80	IIIC	Metastatic high grade papillary serous carcinoma	Malignant
4	66	IV	Metastatic high grade serous carcinoma	Malignant
5	65	IIIB	High grade serous carcinoma	Malignant
6	66	IIIC	High grade serous carcinoma	Malignant
7	64	IIIC	High-grade serous adenocarcinoma	Malignant
8	61	IIIC	Metastatic high grade papillary serous carcinoma	Malignant
9	53	IIIC	High grade papillary serous adenocarcinoma	Malignant
10	55	IIIA	Metastatic ovarian carcinoma	Malignant
11	67	IIA	High grade papillary serous carcinoma	Malignant
12	74	IIA	High grade serous carcinoma	Malignant
13	65	IIA	Mucinous adenocarcinoma	Malignant
14	51	IIA	Low grade serous carcinoma	Malignant
15	58	IA	Mucinous cystadenocarcinoma	Malignant
16	51	n/a	No history of cancer	Control
17	53	n/a	No history of cancer	Control
18	50	n/a	No history of cancer	Control
19	52	n/a	No history of cancer	Control
20	53	n/a	No history of cancer	Control