

Supplementary information

Urine-based liquid biopsy: non-invasive and sensitive AR-V7 detection in urinary EVs from patients with prostate cancer

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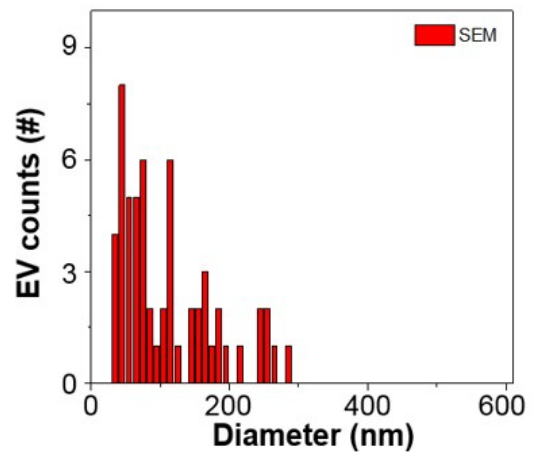
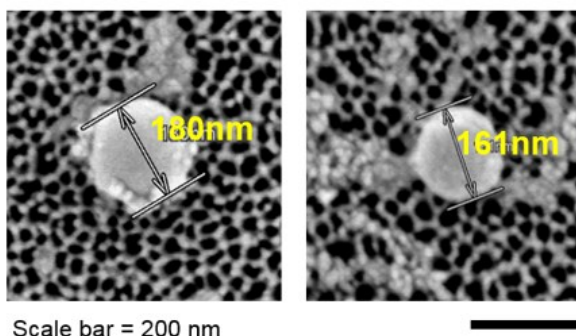
Supplementary Movie

Movie S1: Visualization of the extracellular vesicles (EVs) isolation process on a spinning disc.

Movie S2: Washing efficiency of Exo-Hexa depending upon the height of the chamber underneath the filter.

Supplementary Figure

A SEM



B NTA

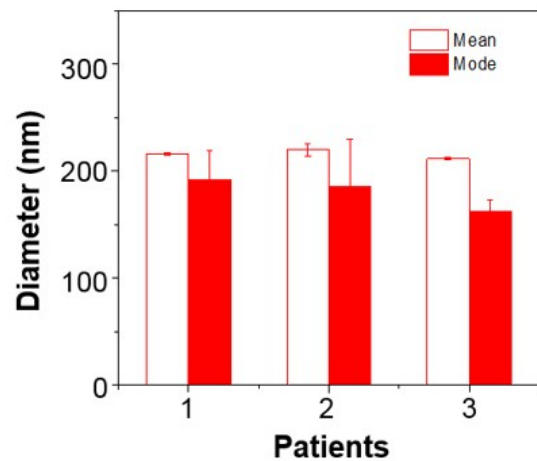
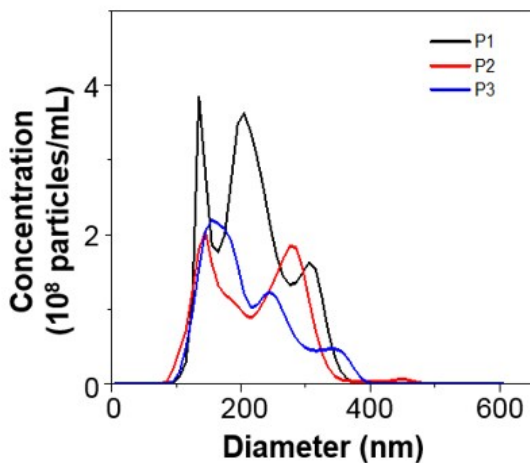
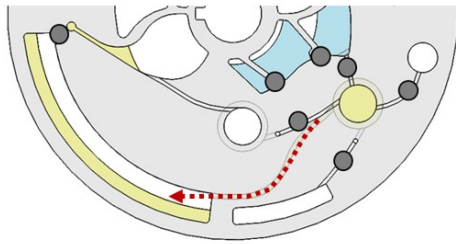
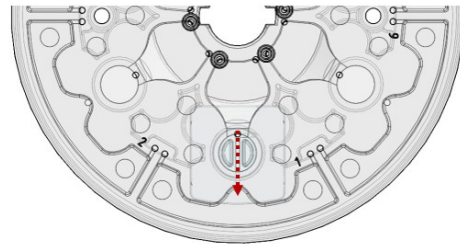
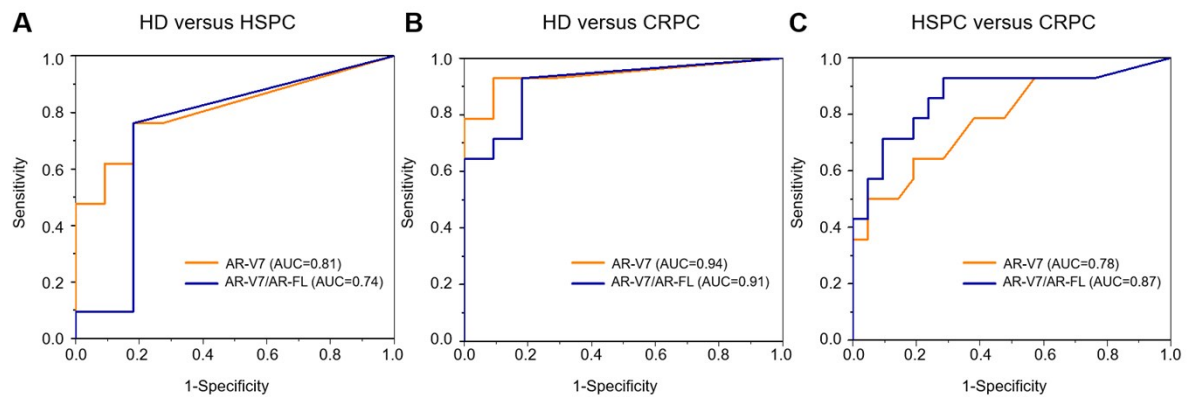


Figure S1. EVs are isolated from urine by using Exo-Hexa. (A) Scanning electron microscope (SEM) images showing EVs on the filter and the histogram of the size distribution of EVs measured from SEM images ($n=58$). (B) Nanoparticle tracking analysis (NTA) analysis of EVs isolated from urine of three different patients.

A Exodisc**B Exo-Hexa****Figure S2.** Schematic diagram showing Exodisc¹ and Exo-Hexa.**Figure S3.** Receiver operating characteristic (ROC) curves are shown for AR-V7 and the ratio of AR-V7/AR-FL in the classification of (A) healthy donors (HD, n=11) versus hormone-sensitive prostate cancer (HSPC, n=22), (B) HD versus castration-resistant prostate cancer (CRPC, n=14), (C) HSPC versus CRPC, with the corresponding area under the curve (AUC) values.**Table S1.** Comparison of Exodisc¹ and Exo-Hexa

EV characterization		Exodisc ¹	Exo-Hexa (current work)
No. of particles	NTA ($\times 10^9$ particles/mL)	1.62 (± 0.38)	1.60 (± 0.28)
EV protein	Sandwich ELISA CD9/CD81 OD (AU)	1.28 (± 0.00)	1.29 (± 0.03)
Purity	No. of particles/total protein ($\times 10^6$ particles/ μ g)	55.10 (± 12.8)	83.90 (± 14.6)
mRNA	RT-PCR (GAPDH, C _T)	30.49 (± 0.08)	30.07 (± 0.29)
Process time	min/sample	15	5
Throughput	No. of sample/disc	2	6

¹ H.-K. Woo, V. Sunkara, J. Park, T.-H. Kim, J.-R. Han, C.-J. Kim, H.-I. Choi, Y.-K. Kim and Y.-K. Cho, *ACS Nano*, 2017, **11**, 1360-1370.

Table S2. Characteristics of patients

Baseline characteristics	All patients (n = 36)	HSPC patients (n = 22)	CRPC patients (n = 14)
Age, median (range)	71.5 (54–82)	70 (54–81)	71.5 (60–82)
Race, n (%)			
White	1 (3)	0 (0)	1 (7)
Non-white (Asian)	35 (97)	22 (100)	13 (93)
Tumor stage at diagnosis, n (%)			
T1/T2	14 (39)	11 (50)	3 (21.5)
T3/T4	16 (44)	8 (36)	8 (57)
Unknown	6 (17)	3 (14)	3 (21.5)
Gleason sum at diagnosis, n (%)			
≤ 7	16 (44.5)	15 (68)	1 (7)
≥ 8	13 (36)	3 (14)	10 (71.5)
Unknown	7 (19.5)	4 (18)	3 (21.5)
Presence of bone metastases, n (%)			
Yes	7 (19)	1 (5)	6 (43)
No	29 (81)	21 (95)	8 (57)
Presence of lymph node metastases, n (%)			
Yes	5 (14)	1 (5)	4 (29)
No	31 (86)	21 (95)	10 (71)
Prior use of docetaxel, n (%)			
Yes	14 (39)	0 (0)	14 (100)
No	22 (61)	22 (100)	0 (0)
PSA level, median (range) ng/ml	3.21 (0.005–1667)	1.01 (0.005–1667)	19.38 (0.006–646)

HSPC, hormone-sensitive prostate cancer; CRPC, castration-resistant prostate cancer; PSA, prostate-specific antigen