Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/6/47/eabc1204/DC1

Supplementary Materials for

High-throughput single-EV liquid biopsy: Rapid, simultaneous, and multiplexed detection of nucleic acids, proteins, and their combinations

Jian Zhou, Zuoren Wu, Jie Hu, Dawei Yang, Xiaoyan Chen, Qin Wang, Jie Liu, Maosen Dou, Wenjun Peng, Yuanyuan Wu, Wenhao Wang, Chenjian Xie, Ming Wang, Yuanlin Song, Hengshan Zeng*, Chunxue Bai*

*Corresponding author. Email: bai.chunxue@zs-hospital.sh.cn (C.B.); dixion9@163.com (H.Z.)

Published 20 November 2020, *Sci. Adv.* **6**, eabc1204 (2020)
DOI: 10.1126/sciadv.abc1204

This PDF file includes:

Supplemental Material

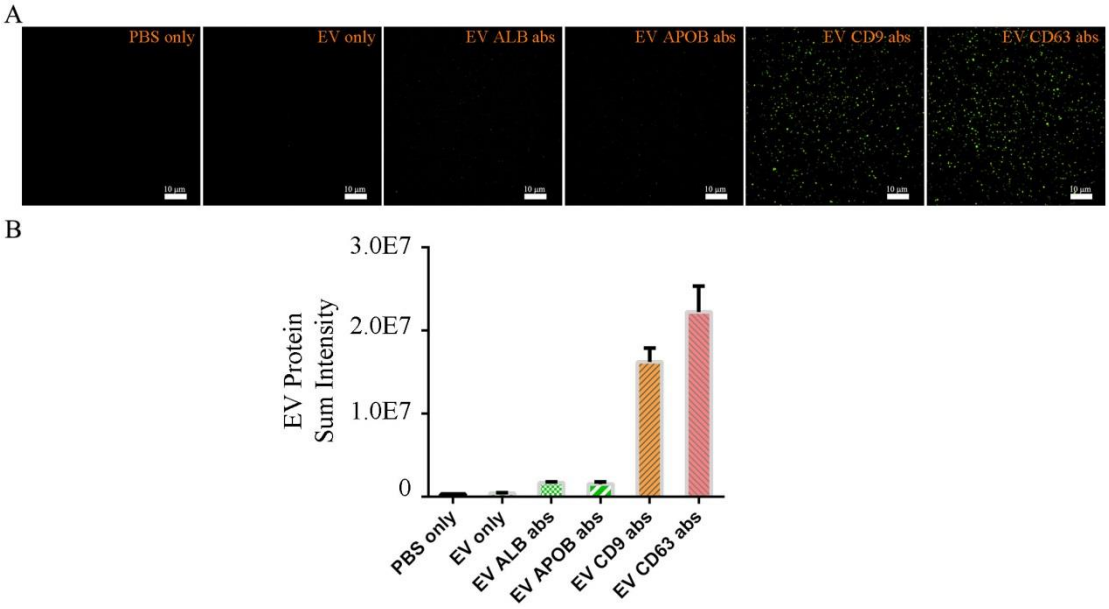

Fig. S1. Measurement of ALB and APOB protein expression on EVs. (**A**) Representative images of PBS only (no EVs and no antibodies), EV only (EVs without staining with any antibodies), EV ALB abs (EVs stained with ALB antibody), EV APOB abs (EVs stained with APOB antibody), EV CD9 abs (EVs stained with CD9 antibody), and EV CD63 abs (EVs stained with CD63 antibody). (**B**) Fluorescence signals of PBS only, EV only, EV ALB abs, EV APOB abs, EV CD9 abs, and EV CD63 abs measured by HNCIB system.

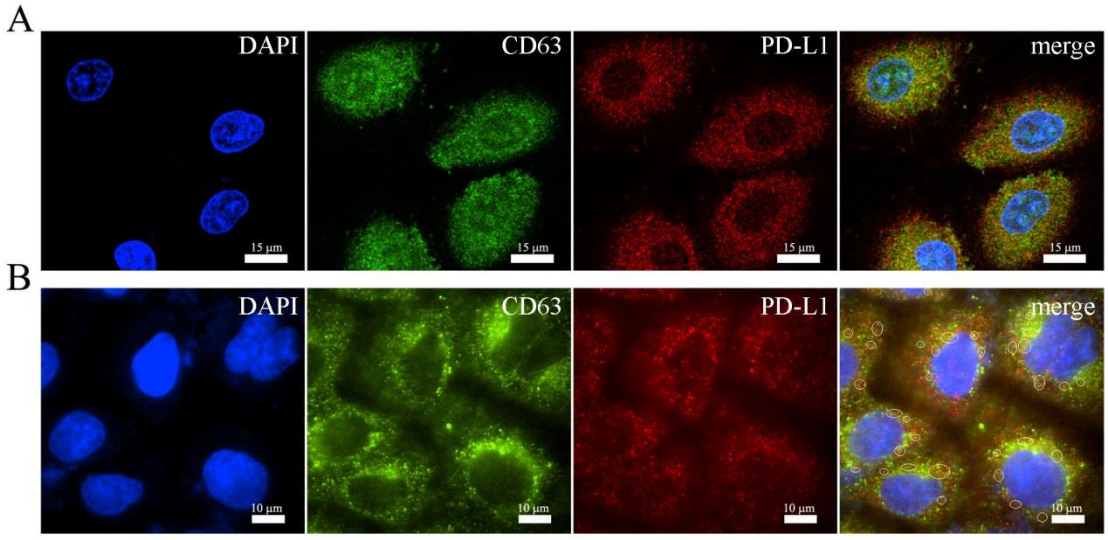

Fig. S2. Double immunofluorescence staining for membrane protein of cells. (**A**) A549 cells staining with DAPI, CD63 and PD-L1 antibodies, and imaging with a confocal microscopy (Ti2E microscope with Yokogawa CSU-W1 Sora confocal scanner unit, Nikon, Japan). (**B**) A549 cells staining with DAPI, CD63 and PD-L1 antibodies, and imaging with a TIRFM (Eclipse Ti-E, Nikon, Japan). White cycles containing areas expressed both CD63 and PD-L1 protein.

Table S1. Signal-to-noise ratio and specificity.

Fig. 2A	**Mean value**	**Ratio**	
PBS	2.83E+05	64	CD63 abs/PBS (Signal-to-noise ratio)
Fig.2A CD63 plasma (EV only)	4.08E+05	44	CD63 abs/EV only (Sensitivity)
Fig.2A CD63 plasma (EV CD63 isotype)	2.02E+06	9	CD63 abs/CD63 isotype (Specificity)
Fig.2A CD63 plasma (EV CD63 abs)	1.81E+07		
Fig. 2B	**Mean value**	**Ratio**	
PBS	3.19E+05	41	PD-L1 abs/PBS (Signal-to-noise ratio)
Fig.2B PD-L1 plasma (EV only)	3.62E+05	36	PD-L1 abs/EV only (Sensitivity)
Fig.2B PD-L1 plasma (EV PD-L1 isotype)	1.93E+06	7	PD-L1 abs/CD63 isotype (Specificity)
Fig.2B PD-L1 plasma (EV PD-L1 abs)	1.30E+07		
Fig. S1	**Mean value**	**Ratio**	
Fig. S1 EV ALB abs	1.65E+06	10	CD9 abs/ALB abs
Fig. S1 EV APOB abs	1.52E+06	15	CD63 abs/APOB abs
Fig. S1 EV CD9 abs	1.62 E+07	11	CD9 abs/ APOB abs
Fig. S1 EV CD63 abs	2.22E+07	14	CD63 abs/ALB abs

Table S2. Demographic characteristic of patients and healthy donors.

	Healthy Group (N)	Adenocarcinoma Group (N)	*P* value
Number	35	34	
Age (years)			0.067
<65	33	27	
⩾65	2	7	
Gender			0.549
Male	16	18	
Female	19	16	
Smoker	9	6	0.494
Nonsmoker	26	26	
Clinical stage			
1/2		5	
3/4		29	

N, number of cases; *P* value represents the probability from a Chi-square test.
$^{*}P<0.05$.

Table S3. Antibodies used.

Antibody	Company	Catalog Number	Species
CD63	Novus Biologicals	NBP2-42225AF488	Mouse IgG1, K
Isotype control	R&D Systems	IC002G	Mouse IgG1
PD-L1	Abcam	ab209960	Rabbit IgG
PD-L1	Novus Biologicals	NBP1-76769	Rabbit IgG
Isotype control	Novus Biologicals	NBP2-24891	Rabbit IgG
Secondary Ab	Thermo Fisher Scientific	A-11011	Rabbit IgG
CD9	Novus Biologicals	NB500-327AF488	Mouse IgG1
ALB	Novus Biologicals	IC1455G	Mouse IgG2A
APOB	Abcam	ab27637	Goat IgG

Biomarker detection by HNCIB

The nano-biochip was fabricated using a cleanroom-free process. An ultra-low auto-fluorescent glass was used as the substrate. After vigorous and thorough cleaning, the substrate was functionalized with a series of agents to achieve high surface grafting density of targeting functional moiety for nanoparticle immobilization. 96 or 384 wells were glued onto the functionalized substrate to form the final device.

A typical experiment using HNCIB was as follows. Cationic nanoparticles with molecular beacon was prepared in phosphate buffered saline (PBS, 14190144, Thermo Fisher Scientific, Waltham, MA, USA). To each well 50 μL nanoparticle suspension was added and incubated at room temperature for 30 minutes followed by washing with ultrapure water (>18 MΩ) for 5 minutes. 50 μL EV samples were added to each well and incubated for 60 minutes at 37 ºC. Following incubation samples were washed with ultrapure water for 5 minutes. This is followed by TIRFM image acquisition. For simultaneous detection of RNA and membrane protein, the samples were then further washed with ultrapure water for 5 minutes, followed by incubation with 2% bovine albumin serum (BSA, A1933, Sigma-Aldrich, St. Louis., MO, USA) in PBS at room temperature for 30 minutes. Samples were again washed with ultrapure water for 5 minutes. 50 μL antibody was added to the well and incubated at 37 ºC for 60 minutes and when applicable the secondary antibody may be added and incubated for an additional 30 minutes. After washing with ultrapure water for 5 minutes, the samples were ready for membrane protein observation by TIRFM.