ADVANCED SCIENCE
Open Access

# Supporting Information

for *Adv. Sci.*, DOI 10.1002/advs.202304926

Ultrasensitive and High-Resolution Protein Spatially Decoding Framework for Tumor Extracellular Vesicles

*Chi-An Cheng*, Kuan-Chu Hou, Chen-Wei Hsu and Li-Chiao Chiang*

# Supporting Information

## Ultrasensitive and High-Resolution Protein Spatially Decoding Framework for Tumor Extracellular Vesicles

*Chi-An Cheng,*[a, ‡,*] *Kuan-Chu Hou,* [b,‡] *Chen-Wei Hsu,* [a,‡] *Li-Chiao Chiang* [a]

[a] School of Pharmacy, College of Medicine, National Taiwan University, Taipei, 10050, Taiwan

[b] Department of Medicine, College of Medicine, National Taiwan University, Taipei, 10050, Taiwan

* Corresponding author: cacheng123@ntu.edu.tw

# Table of Contents

## Supporting Figures

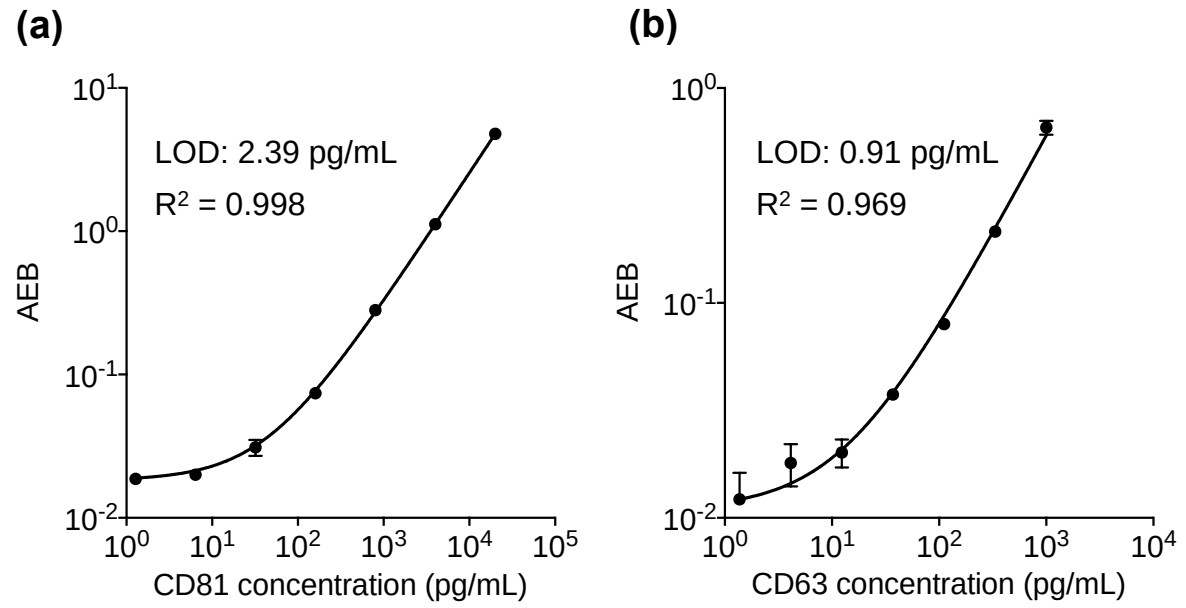


**Figure S1.** (a) The standard curve of the CD81 Simoa assay. The LOD value of the assay was 2.39 pg/mL. (b) The standard curve of the CD63 Simoa assay. The LOD value of the assay was 0.91 pg/mL. All measurements were performed in duplicate.

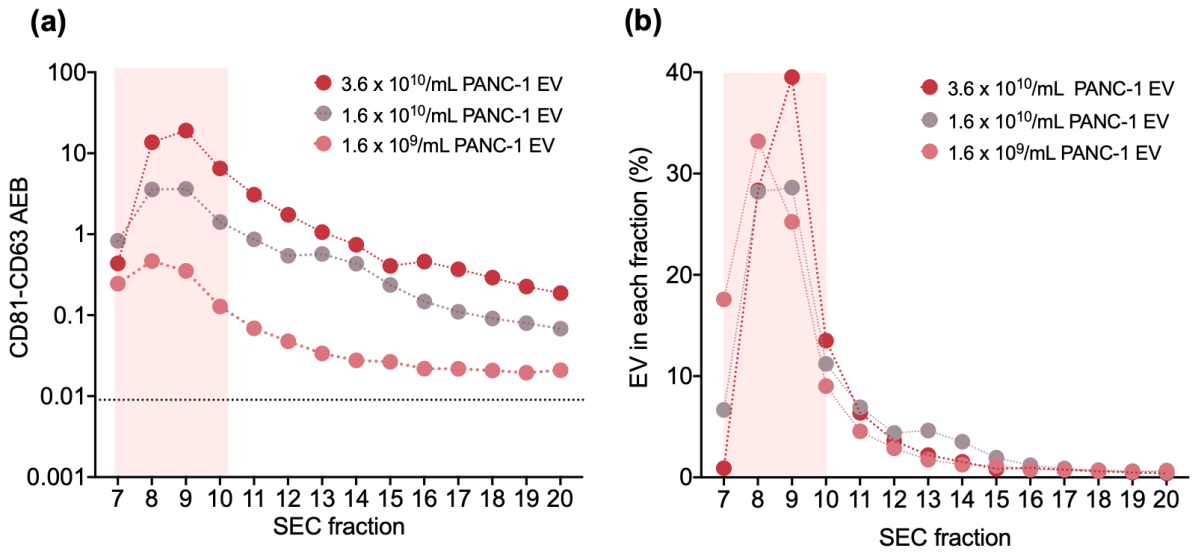


**Figure S2.** (a) *eSimoa* quantification of CD81-CD63 levels in SEC fractions of PANC-1 EV sample at various EV concentrations. (b) The percentages of EVs in each fraction were determined based on the CD81-CD63 signal. The pinky region (fractions 7–10) was defined as “EV fractions”. Fractions 17–20 were defined as “soluble protein fractions”. All measurements were performed in duplicate. The dotted lines locate the LOD of the assay.

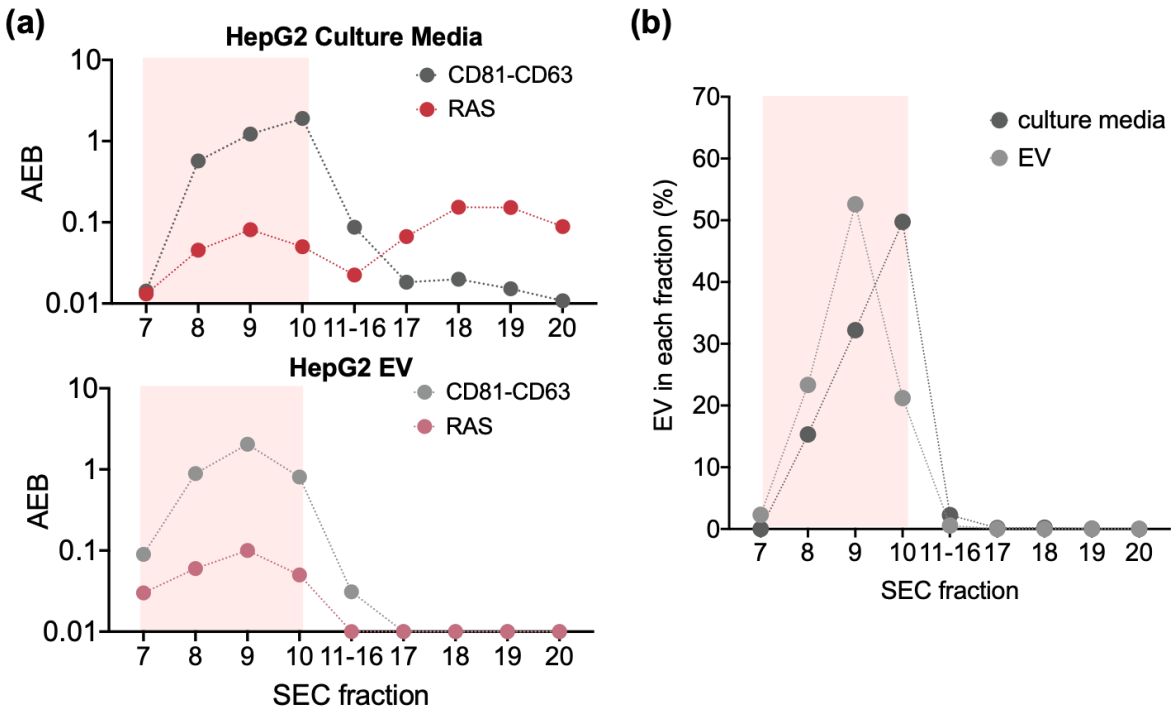


**Figure S3.** (a) *eSimoa* quantification of CD81-CD63 and RAS levels in SEC fractions of HepG2 culture media (top) and the purified HepG2 EV sample (bottom). (b) The percentages of EVs in each fraction were determined based on the CD81-CD63 signal. The pinky region (fractions 7–10) was defined as “EV fractions”. Fractions 17–20 were defined as “soluble protein fractions”. All measurements were performed in duplicate. The dotted lines locate the LOD of the assay.

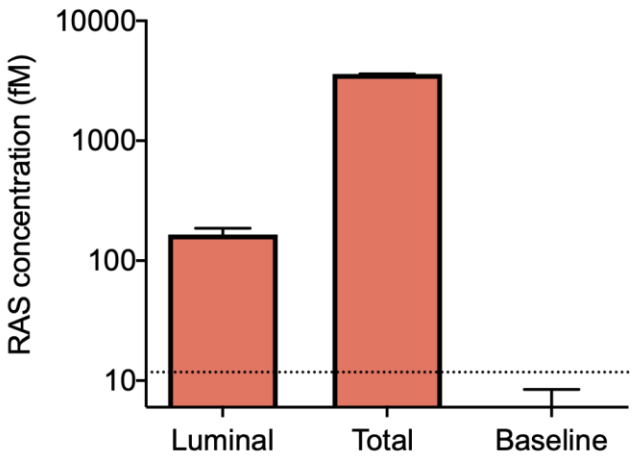


**Figure S4.** The proteinase method decodes sublocalization of RAS proteins within the PANC-1 EVs, which underwent additional SEC purification, using the pan-RAS *eSimoa* assay. Luminal: EVs were treated with proteinase K, followed by lysis of the EVs using Tween after proteinase inactivation. Total: the positive control group consists of EVs without proteinase treatment. Baseline: the negative control group in which EVs were treated with proteinase without instant proteinase inactivation.

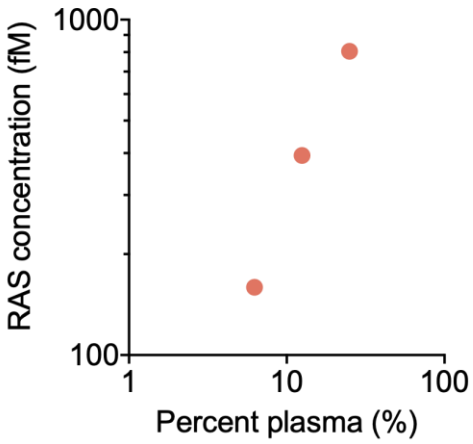


**Figure S5.** The good linearity of pan-RAS *eSimoa* assay in the range of 100 to 1000 fM.

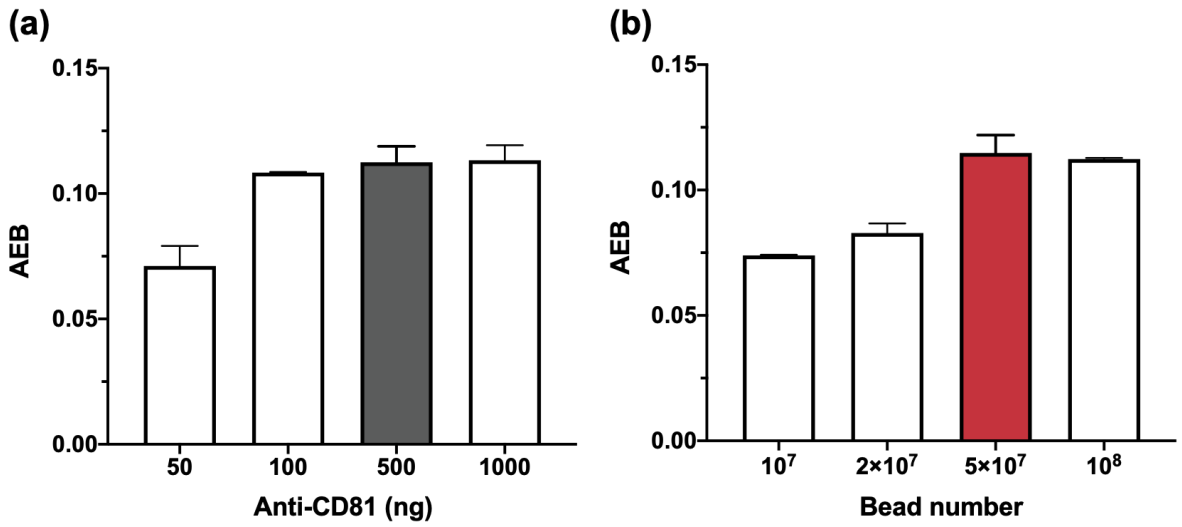


**Figure S6.** The optimization of the capture efficiency for the pulldown *eSimoa* pipeline.

## Supporting Table

| Reagent | Manufacturer |
|---|---|
| CD81 capture antibody | Abcam ab79559 |
| CD81 detector antibody | Biolegend 349502 |
| CD81 protein standard | Origene TP317508 |
| CD63 capture antibody | R&D MAB5048 |
| CD63 detector antibody | BD 556019 |
| CD63 protein standard | Origene TP301733 |
| RAS/ $KRAS^{G12D}$ detector antibody | Abcam ab209974 |
| RAS capture antibody | Abcam ab55391 |
| RAS protein standard | Abcam ab61239 |
| $KRAS^{G12D}$ capture antibody | GeneTex GTX635362 |
| $KRAS^{G12D}$ protein standard | Abcam ab268712 |

**Table S1.** Antibodies and recombinant protein standards used in *eSimoa* assays.