

EpCAM levels across cancers (TCGA)

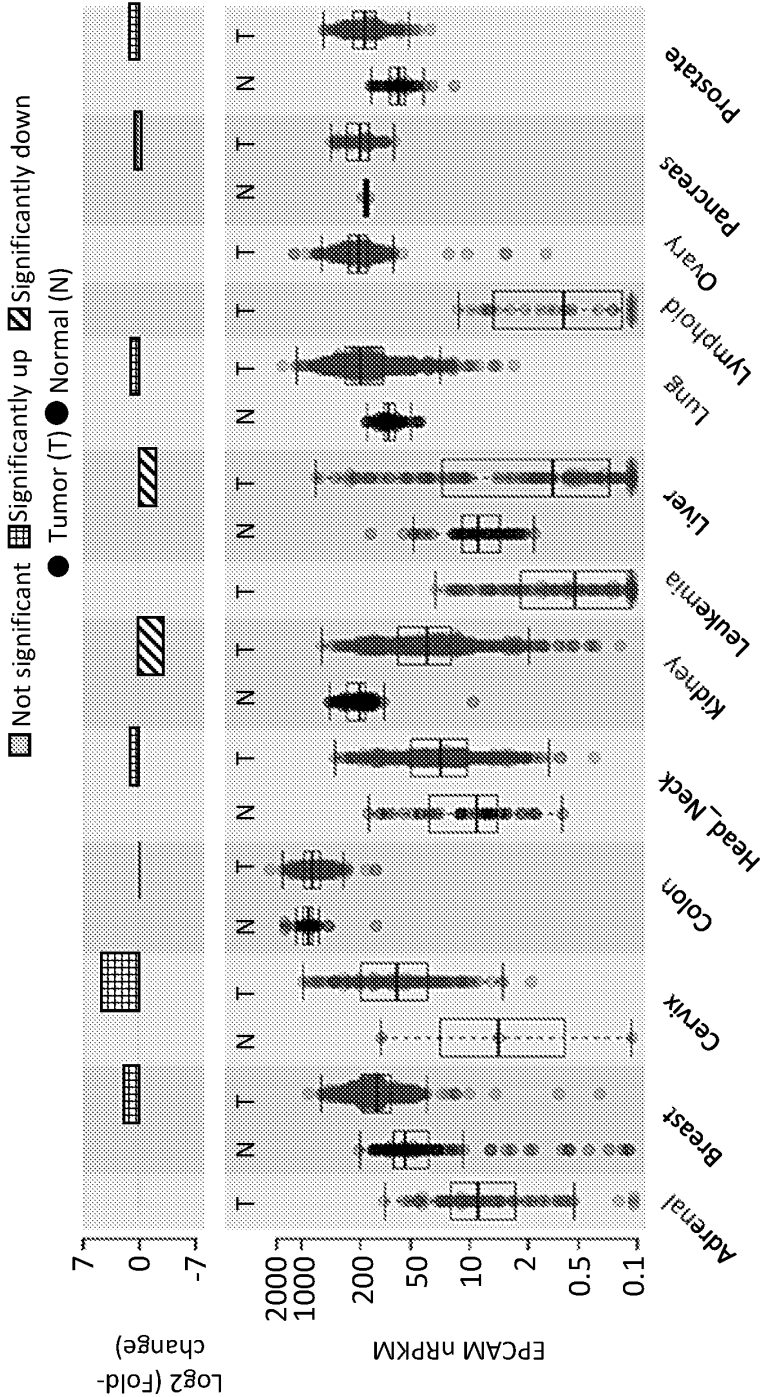


Fig. 1A

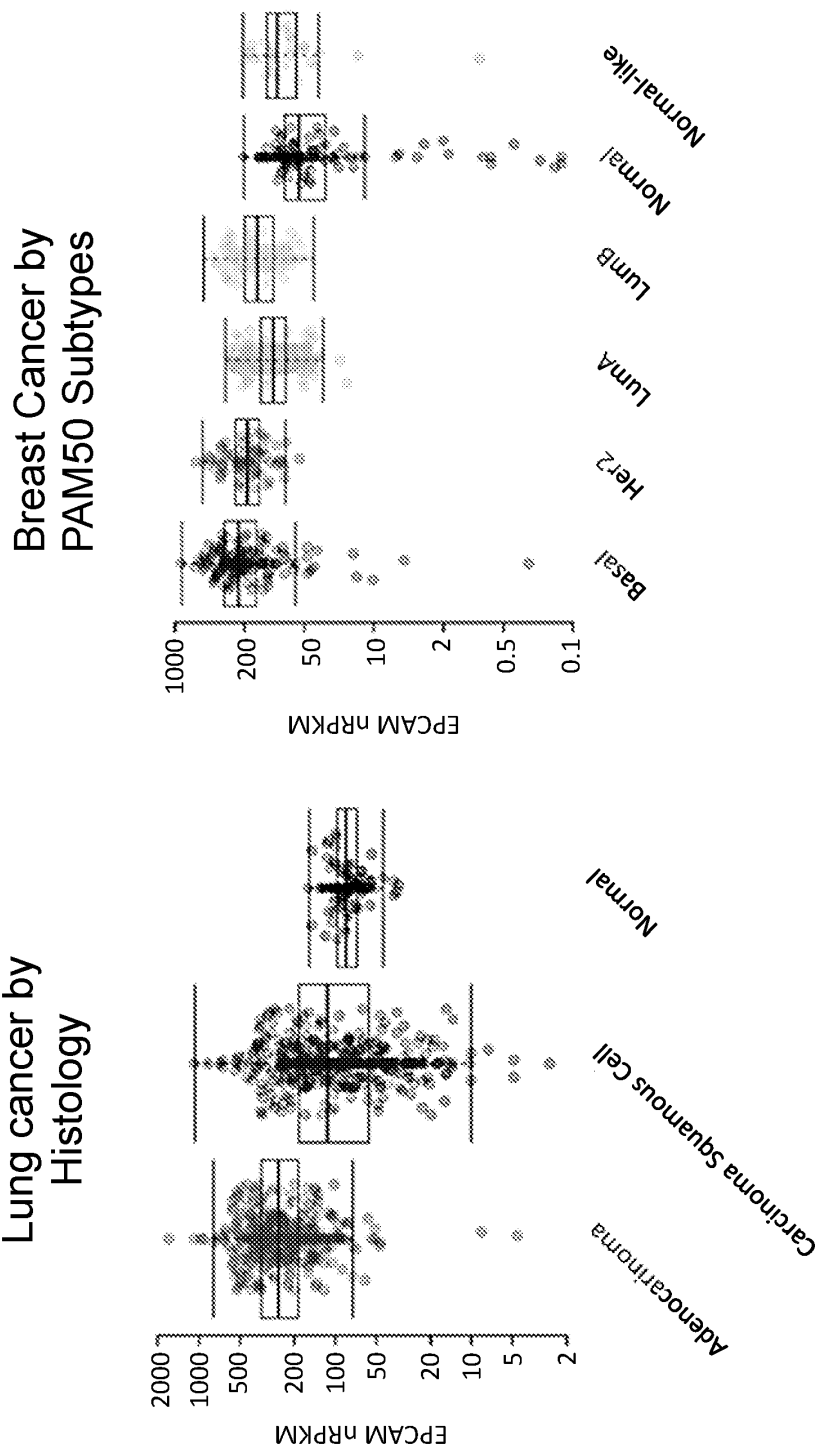


Fig. 1B

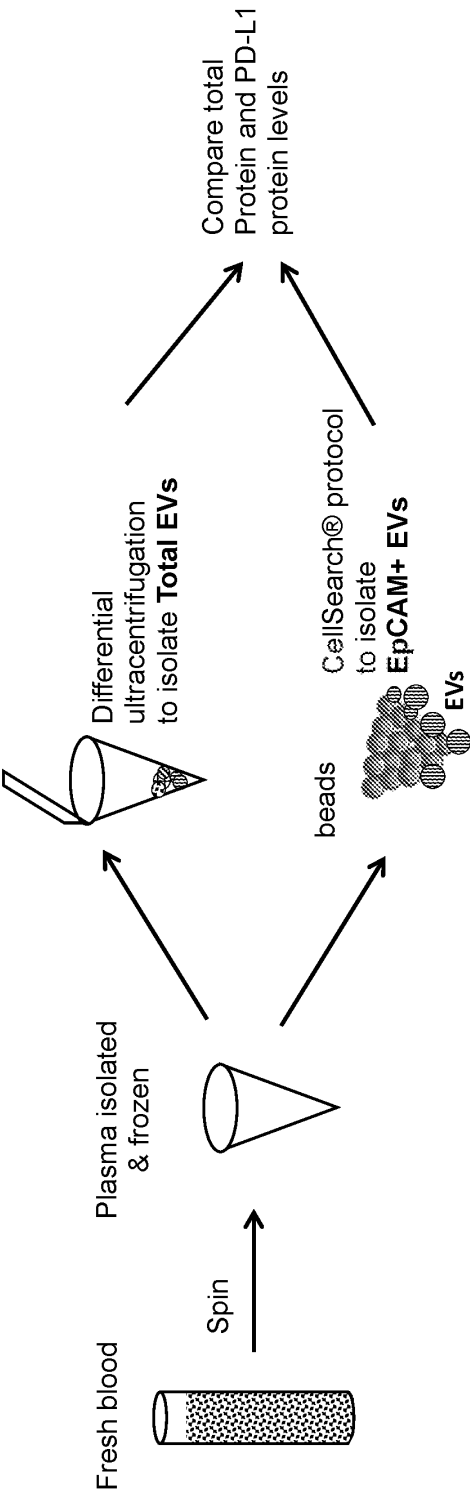


Fig. 1C

EpCAM expression from a set of solid tumors and normal tissue (microarray)

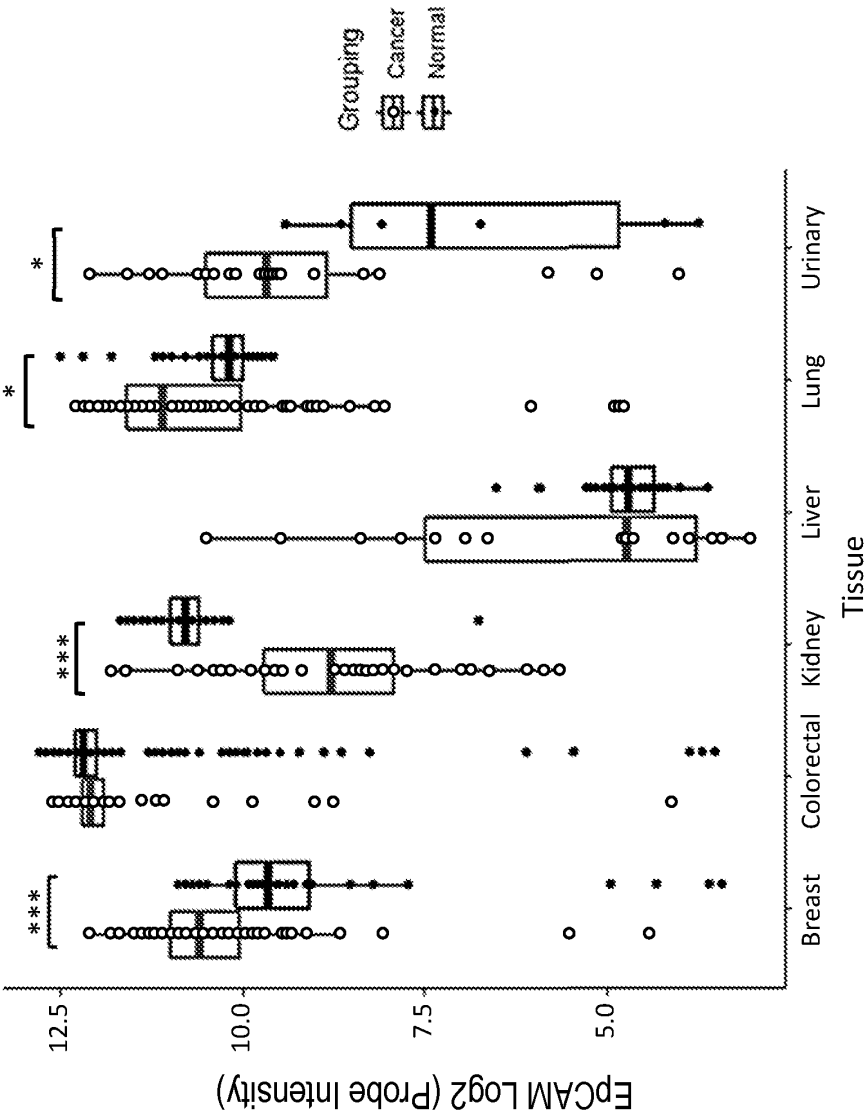


Fig. 2

Transmission electron microscopy analysis of NSCLC, TNBC and healthy donor plasma demonstrates EVs of various sizes in respective fractions

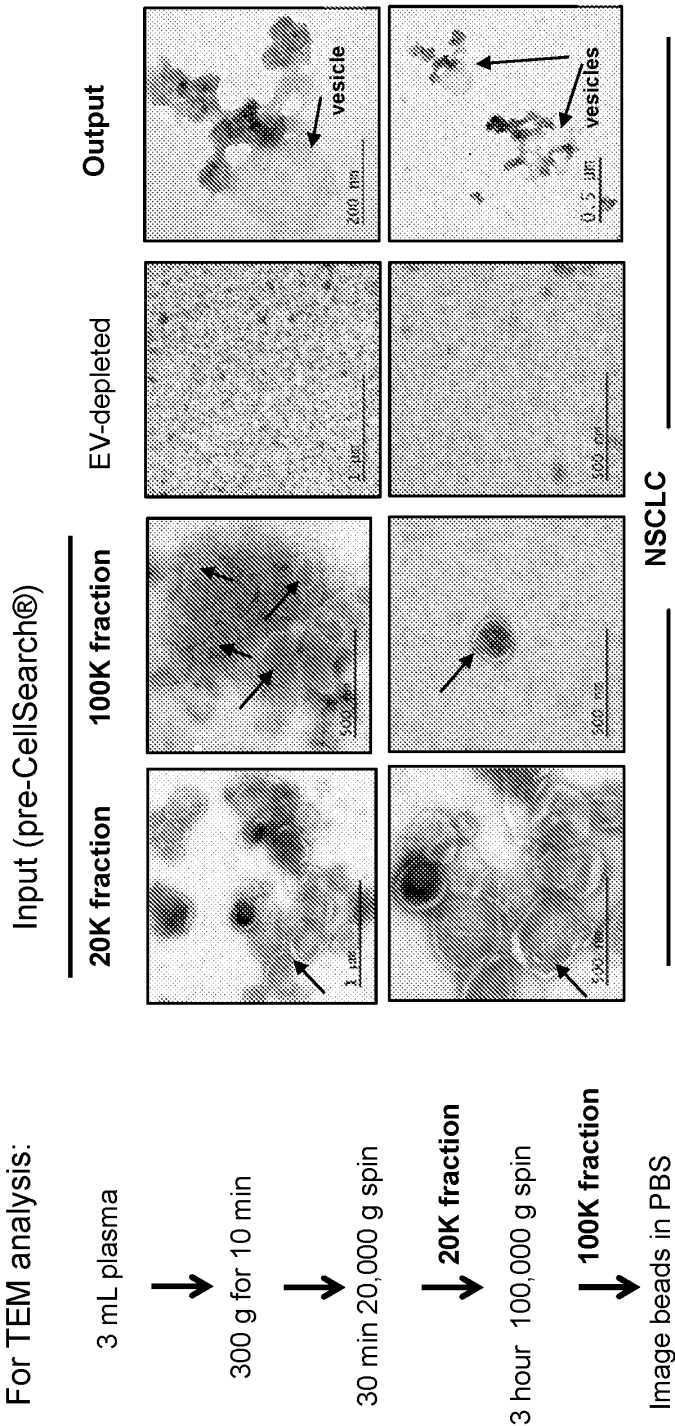


Fig. 3A

Fig. 3B

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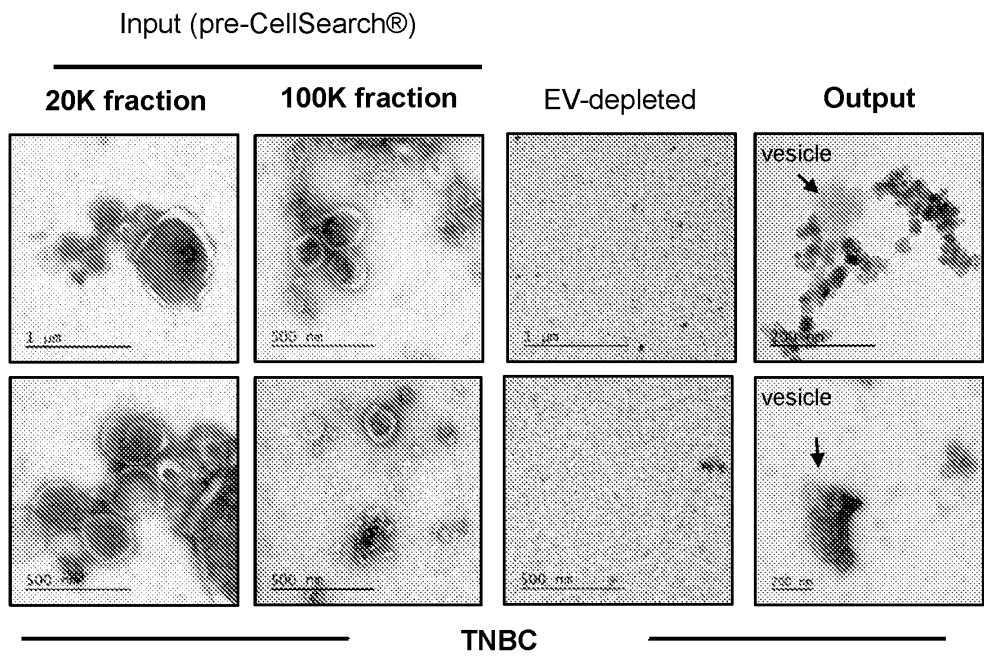


Fig. 3C

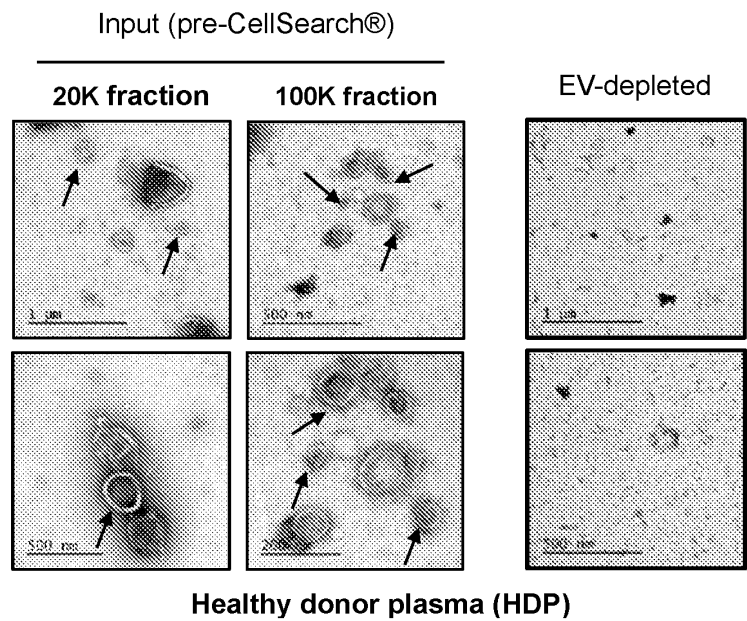


Fig. 3D

Nanosight Tracking Analysis confirms the presence of vesicles within the expected size range of EVs in cancer patient plasma

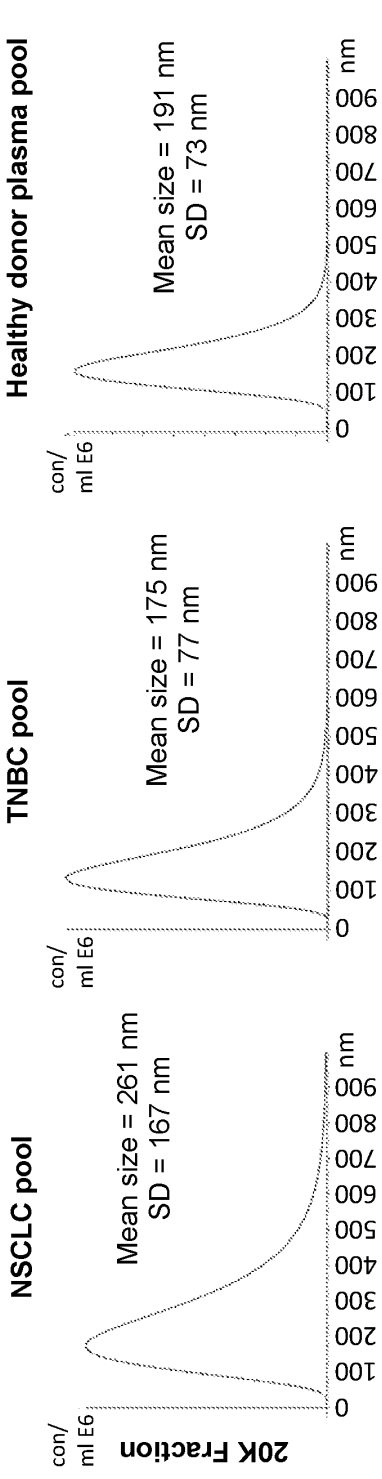


Fig. 4A

Fig. 4B

Fig. 4C

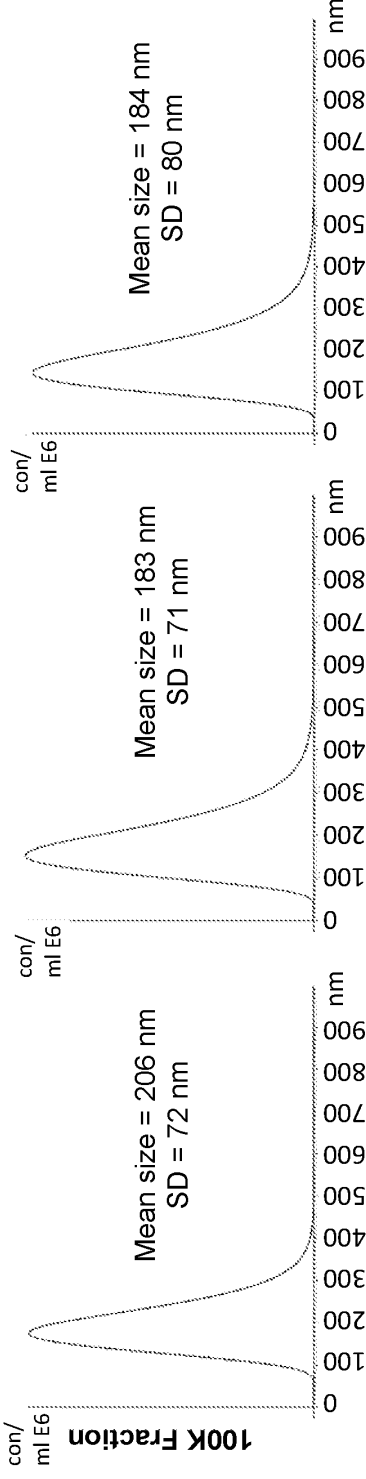


Fig. 4D

Fig. 4E

Fig. 4F

Custom Quanterix PD-L1 assays demonstrate that EpCAM+ EVs are specific to cancer patient plasma

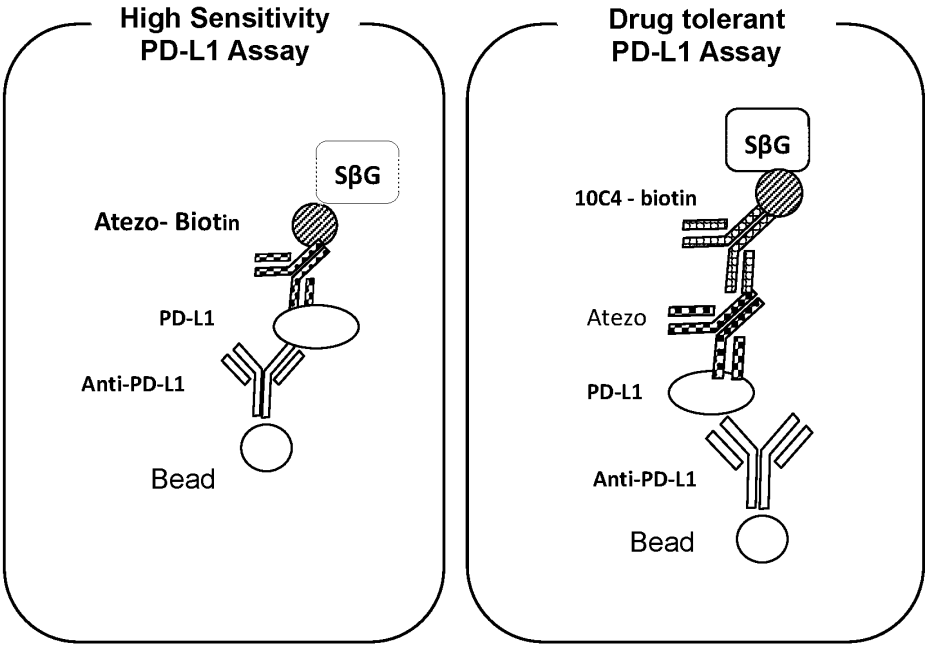


Fig. 5A

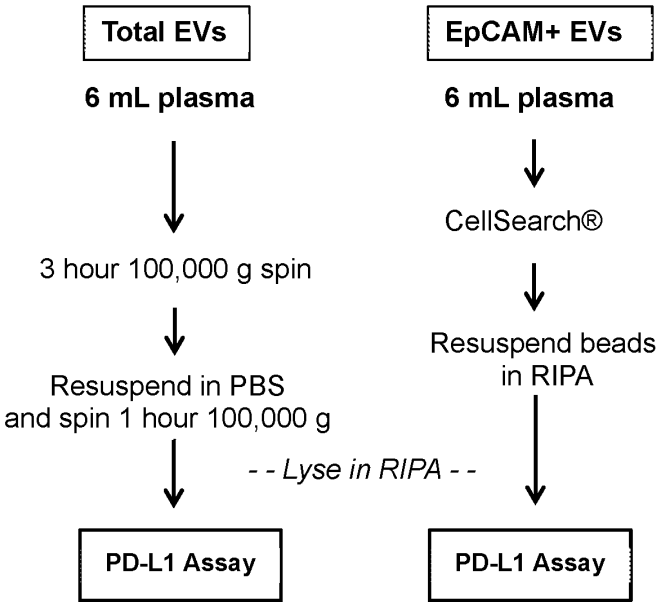
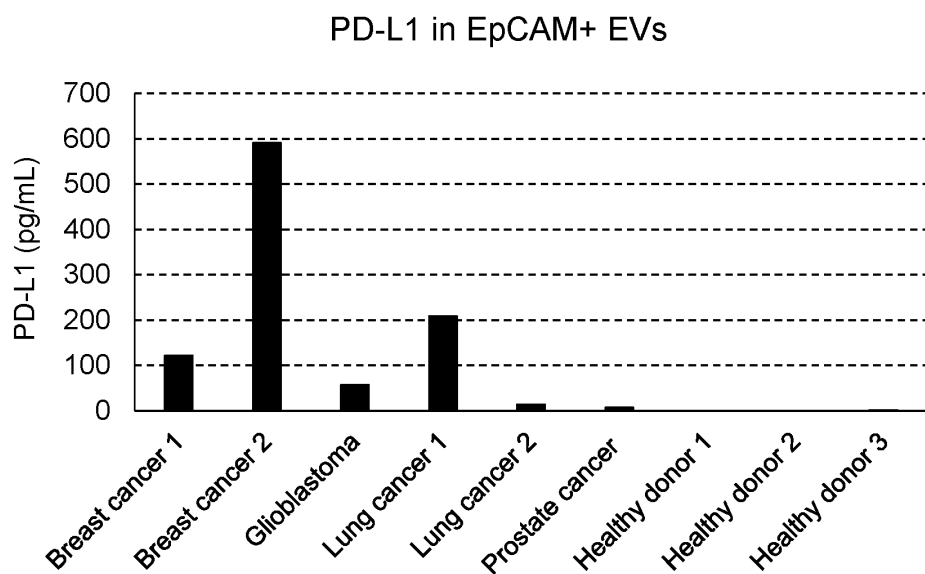


Fig. 5B

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	Protein in Total EVs ($\mu\text{g/mL}$)	Protein in EpCAM+ EVs ($\mu\text{g/mL}$)	% Protein in EpCAM+/Total EVs
Breast cancer 1	5445.4	30.7	0.5
Breast cancer 2	2038.2	16.4	0.8
Glioblastoma	1093.9	27.7	2.5
Lung cancer 1	6751.8	23.3	0.3
Lung cancer 2	424.4	4.7	11
Prostate cancer	3100.4	12.6	0.4
Healthy donor 1	580.5	0	0
Healthy donor 2	2311.9	0	0
Healthy donor 3	3095.1	1.4	0.04

Fig. 5C*Fig. 5D*

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PD-L1 is detectable in Total EVs from ccancer and healthy donors

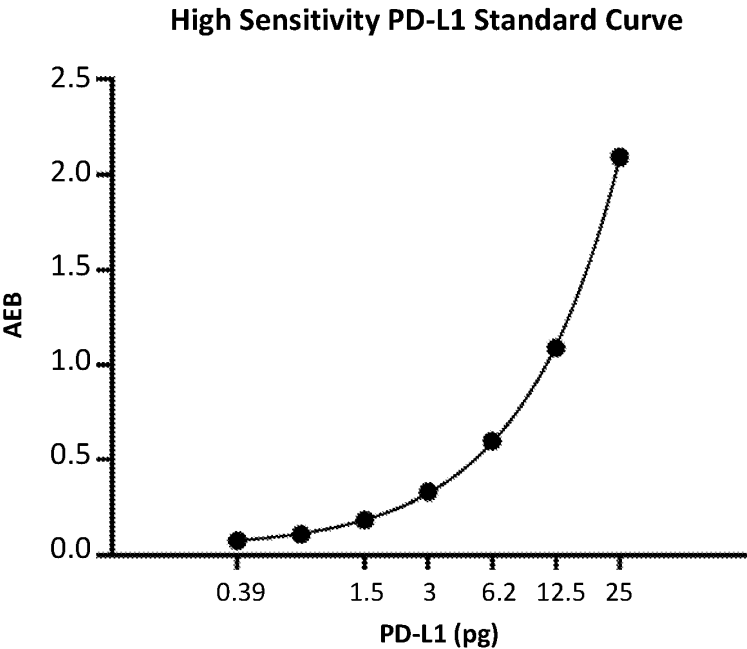


Fig. 6A

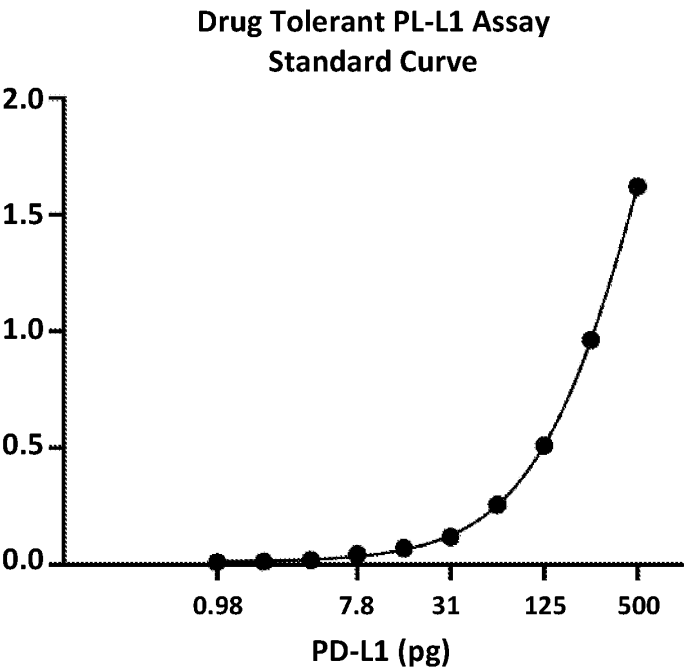


Fig. 6B

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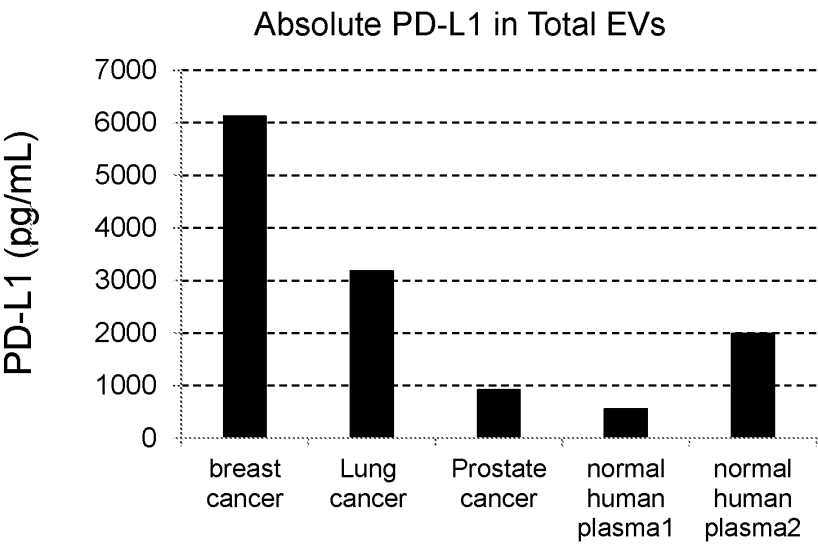


Fig. 6C

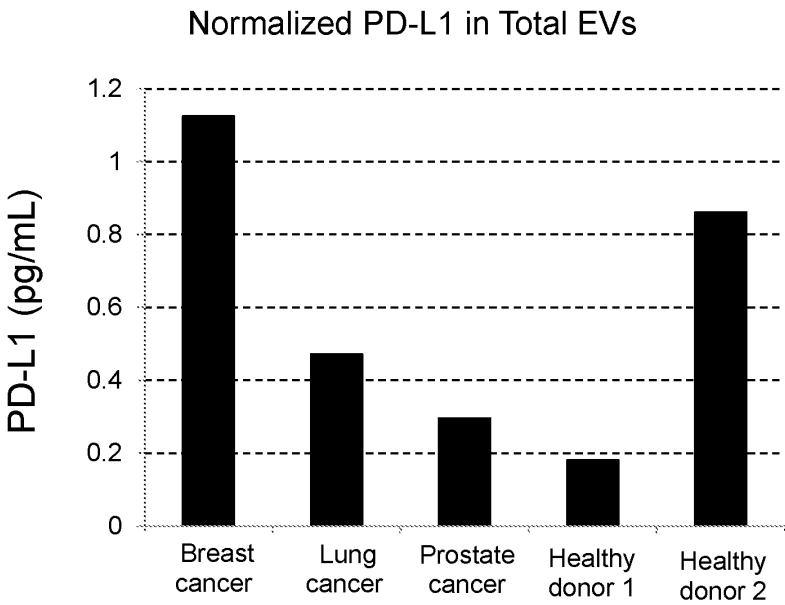


Fig. 6D

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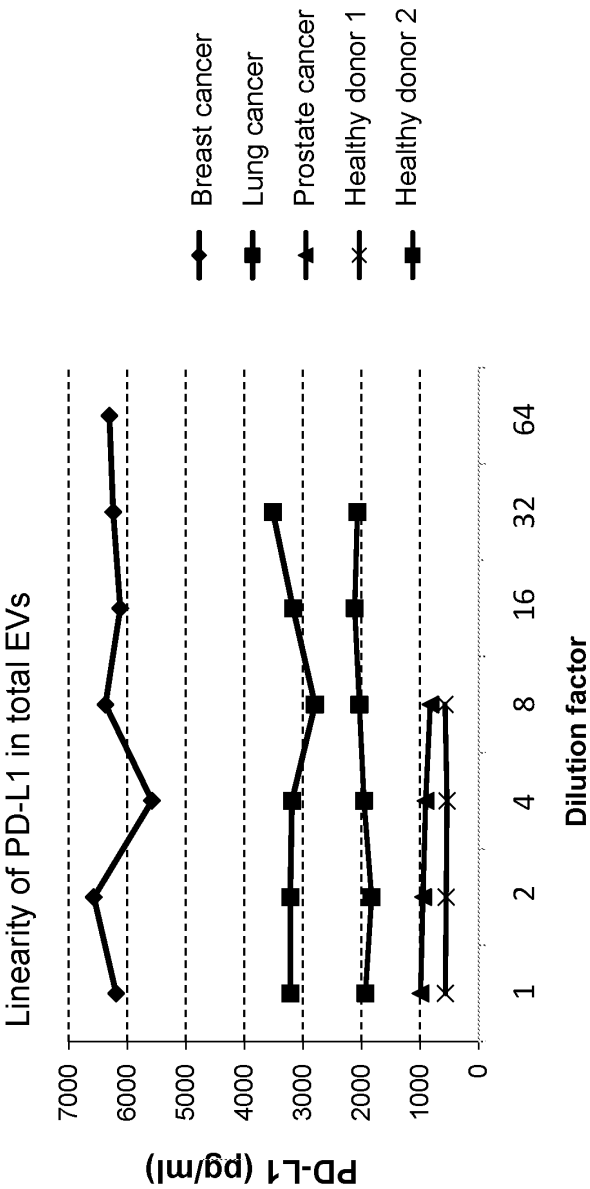


Fig. 6E

Isolation of EpCAM+ EVs from cancer cell lines is reproducible and specific

Cell Line	EpCAM mRNA:	PD-L1 mRNA:
H1975	248	16.3
BT474	114	0.017
A549	3.2	0.36

Fig. 7A

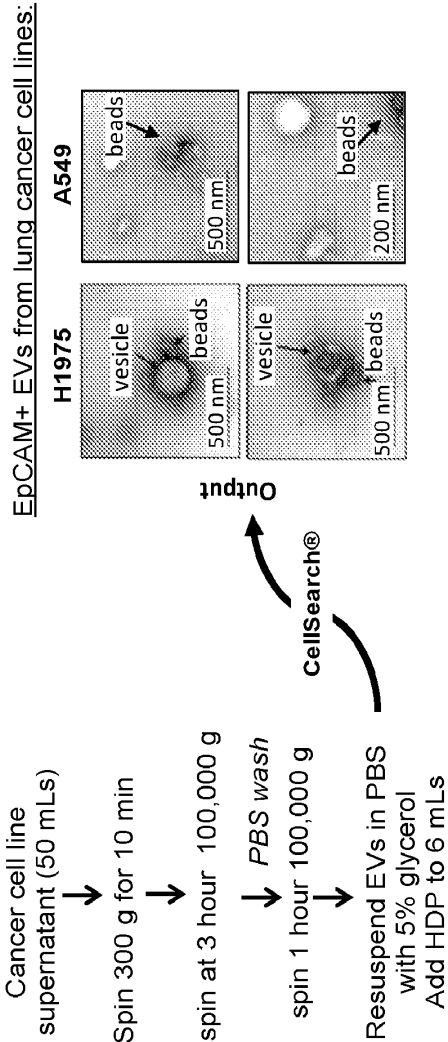


Fig. 7B

EpCAM+ EVs from TNBC line:

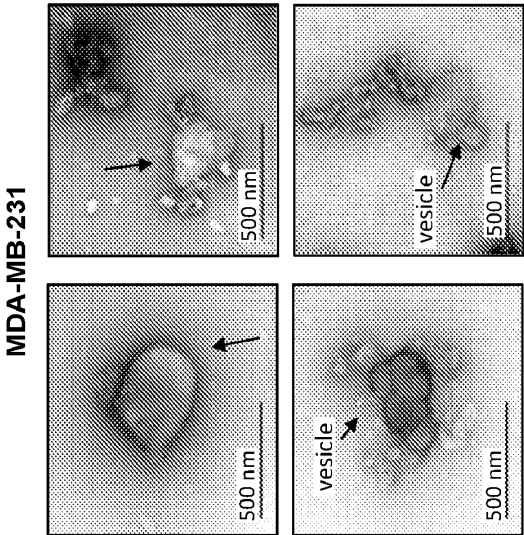


Fig. 7C

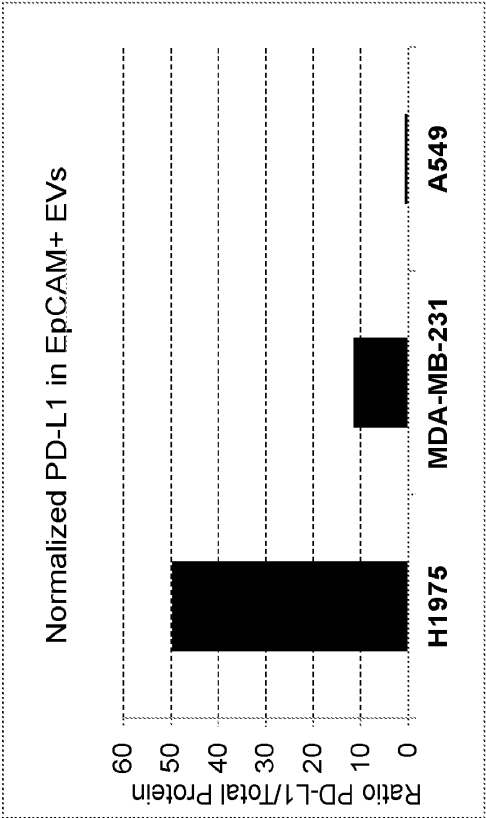


Fig. 7D

9 ml (1:1 diluted) of HDP spiked w/ 250 uL of EVs from H1975 line

↓

3 separate vials with 3 mLs each were run on CellSearch

↓

Sample	Total Protein Conc. (ng/mL)	%CV	PD-L1 Conc. (pg/mL)	%CV	Ratio PD-L1 (pg):Total Protein (ng)	%CV
H1975 #1	320	35%	2404	23%	7.52	12%
H1975 #2	449		2794		6.23	
H1975 #3	219		1747		7.97	

Fig. 7E

Comparable PD-L1 levels which commensurate with total protein

Sample	Total Protein Conc. (ng/mL)	PD-L1 Conc. (pg/mL)	PD-L1 (pg):Total Protein (ng) Ratio
50:50	179	1588	8.88
H1975:Jurkat			

Fig. 7F

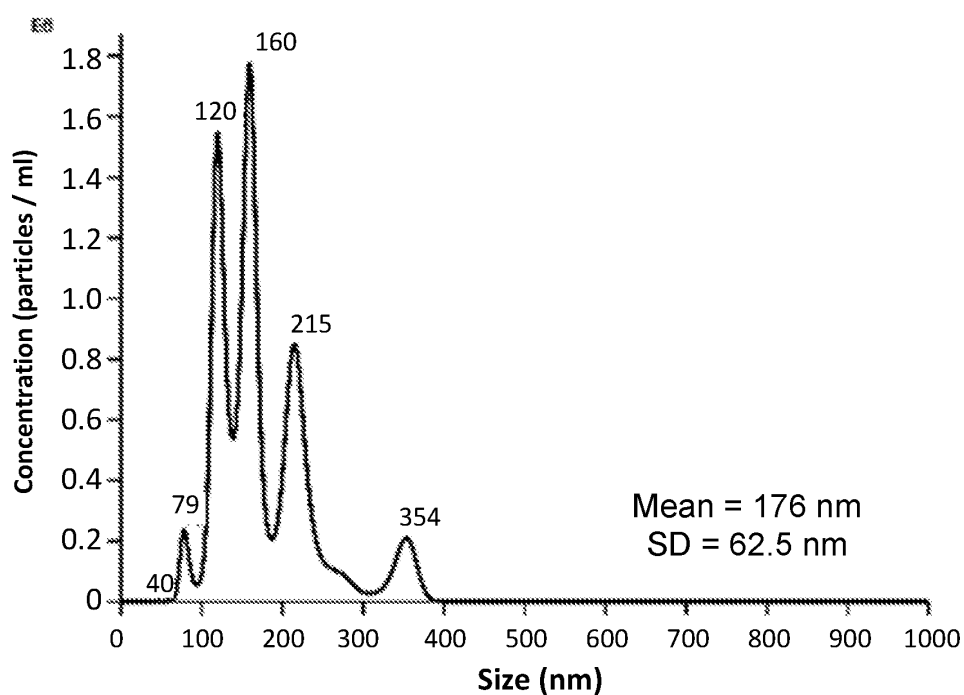
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Precision of Quanterix PD-L1 assay and NTA of total EVs from cancer cell lines

Sample	PD-L1 (pg/mL)	Mean	%CV
Plasma Spike 1	1044	1088	4%
Plasma Spike 2	1116		
Plasma Spike 3	1105		

Fig. 8A

NCI-H1975

*Fig. 8B*

A549

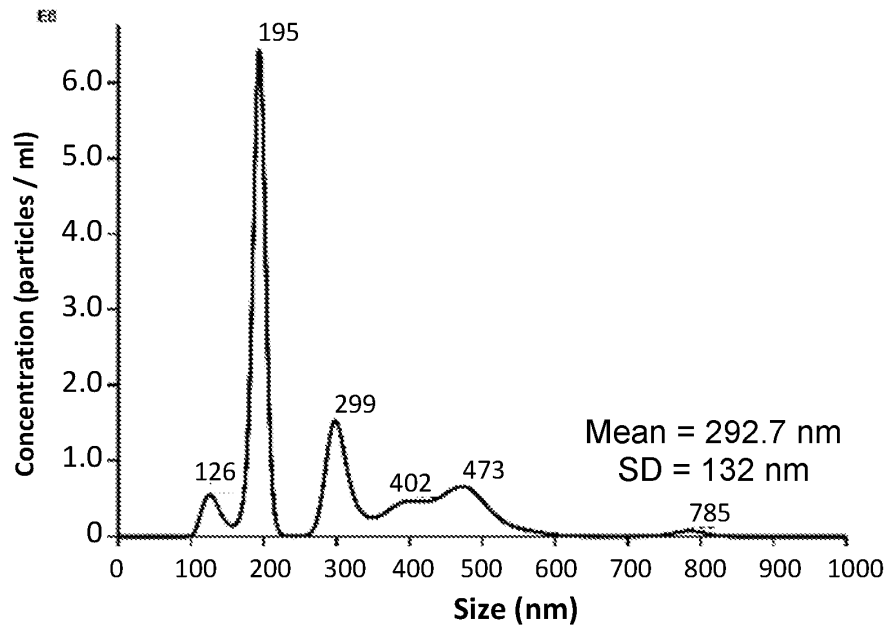


Fig. 8C

MDA-MB-231

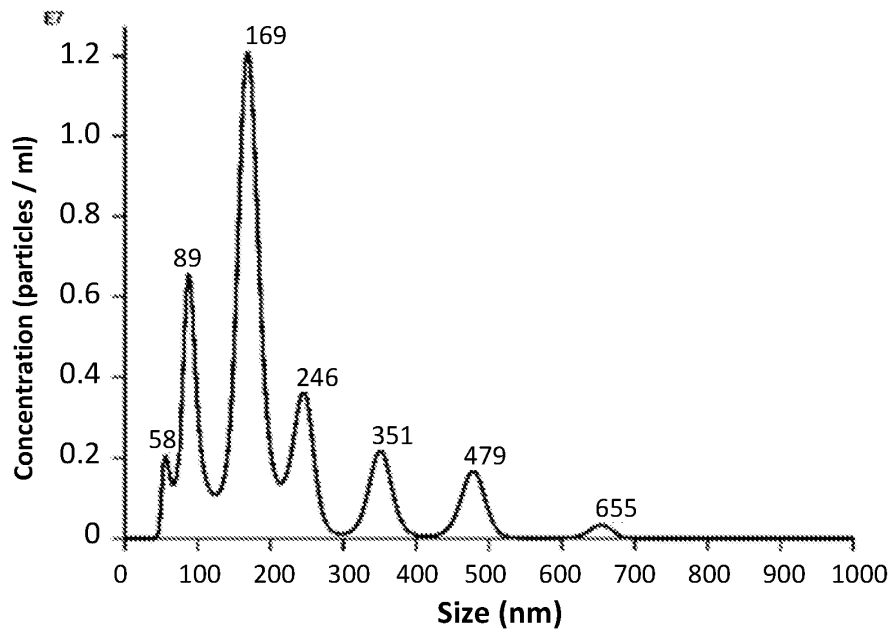


Fig. 8D

PD-L1 assay can be run with different % of RIPA
and various starting volumes

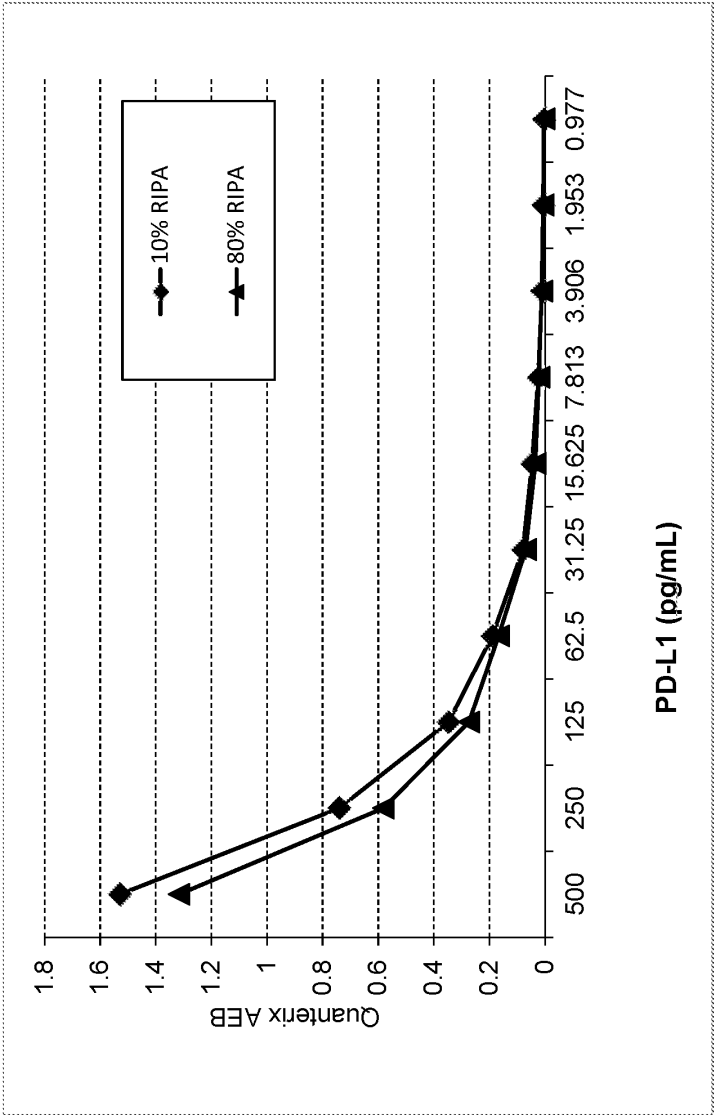


Fig. 9A

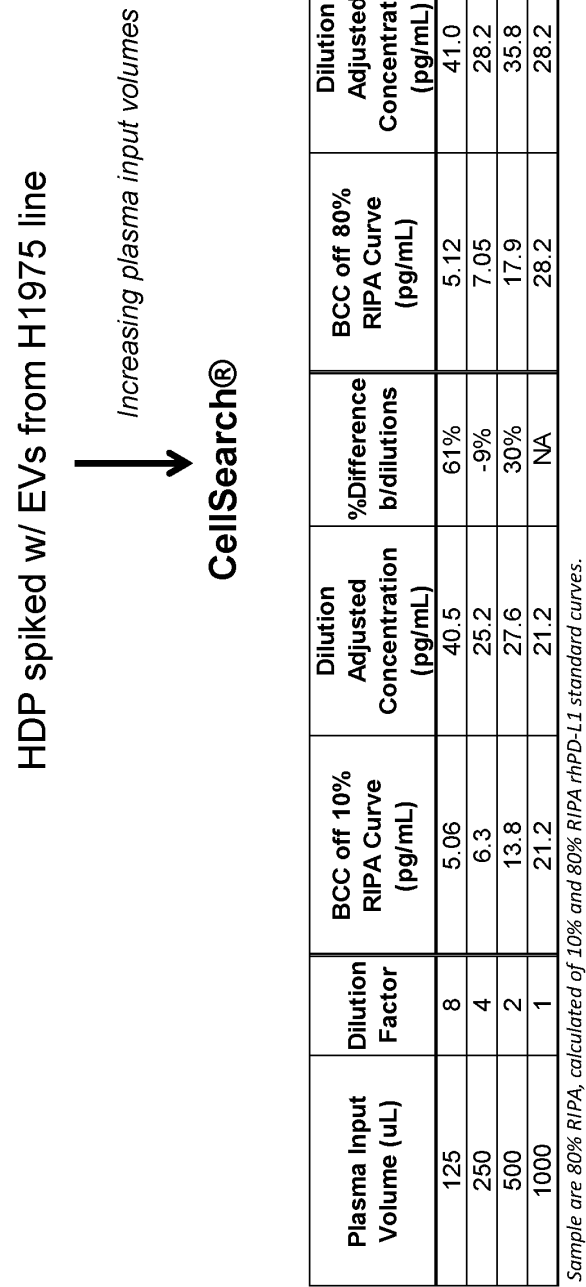


Fig. 9B

250 uL HDP spiked w/ BT474 EVs or NSCLC plasma

↓

CellSearch®

Sample (80% RIPA)	Dilution factor of EVs in plasma	PD-L1 Concentration(pg/mL)
BT474	1:3	1.85
	1:6	LTR
	1:12	LTR
	1:24	LTR
BT474 w 5D1	1:3	LTR [1.19]
NSCLC1	1	4.42
NSCLC2	1	2.47
NSCLC3	1	4.88

Fig. 9C

250 uL HDP spiked w/ NSCLC EVs (from cell lines) mixed with BT474 EVs

*Sample	PDL1 (pg/mL)	% Recovery from 100% H1975	% Difference from Expected
H1975:BT474 100:0	409	N/A	0
H1975:BT474 75:25	272	67%	-11%
H1975:BT474 50:50	171	42%	-16%
H1975:BT474 25:75	79.5	19%	-22%

Since initial conc of EVs is unknown, 100% H1975 is used as “Expected” conc

Fig. 9D

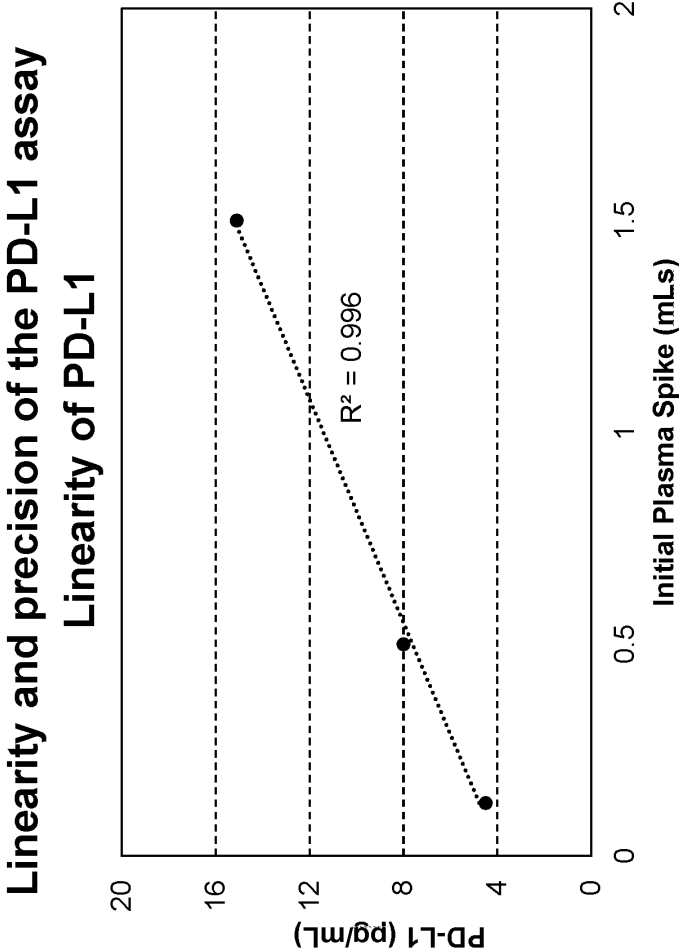


Fig. 10A

PD-L1 (pg/mL)

	Day 1	Day 2	% Difference
H1975	409	501	19%

Fig. 10B