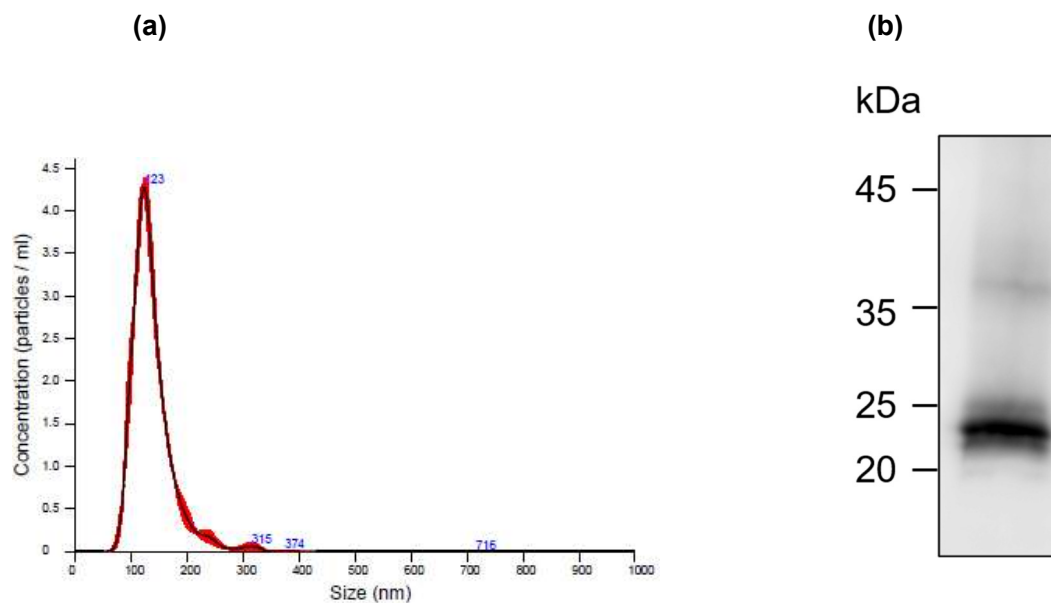


Supplementary Figure 1

Characterization and Validation of Isolated sEVs by nanoparticle tracking assay (NTA) (a) and western blot for CD9 (b).

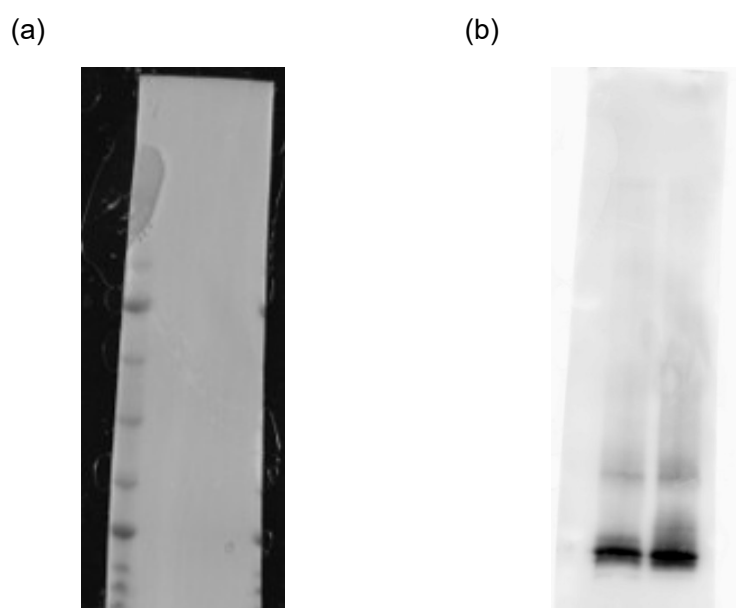


Supplementary Figure 2

Uncropped full-length images for Western blot analysis.

(a) Optical image of the blot membrane displaying the molecular weight ladder. (b) Full-length, uncropped chemiluminescence exposure image (exposure time: 10 seconds).

For both panels, samples loaded in each lane from left to right are: Lane 1, molecular weight marker; Lane 2, MCF7-derived exosome sample; Lane 3, HCT116-derived exosome sample.



Supplementary Table 1

Summary of analytical signal intensities (mean $\pm$ SD) for different antibody labels and loading protocols across various sEV concentrations.

sEVs (particles)	AuSP one-step (mABS)	AuSP two-step (mABS)	AuPL one-step (mABS)	AuPL two-step (mABS)	ALP (a.u.)	HRP (a.u.)
0	0.8 $\pm$ 1.4	4.6 $\pm$ 6.4	24.6 $\pm$ 1.7	3.8 $\pm$ 1.4	0.3 $\pm$ 0.5	0.4 $\pm$ 0.4
10⁶	N.T.	N.T.	N.T.	N.T.	1.0 $\pm$ 0.8	1.2 $\pm$ 0.8
10⁷	N.T.	5.1 $\pm$ 5.4	N.T.	5.7 $\pm$ 1.4	3.42 $\pm$ 1.1	20.4 $\pm$ 4.2
10⁸	4.6 $\pm$ 6.4	30.8 $\pm$ 2.1	N.D.	22.9 $\pm$ 2.5	21.2 $\pm$ 2.4	173.8 $\pm$ 2.6
10⁹	22.1 $\pm$ 9.8	183.5 $\pm$ 5.6	4.9 $\pm$ 8.4	87.1 $\pm$ 8.1	70.9 $\pm$ 61.4	11.9 $\pm$ 15.9
10¹⁰	416.7 $\pm$ 43.7	521.0 $\pm$ 64.4	105.4 $\pm$ 8.5	170.0 $\pm$ 48.4	129.2 $\pm$ 30.9	2.6 $\pm$ 3.1

N.D.: not detected

N.T.: not tested

Supplementary Table 2. Detailed measurement data (technical replicates, mean, and standard deviation) for each labeling modality.

Labeling Modality	sEVs (particles)	Rep. 1	Rep. 2	Rep. 3	Mean	SD
AuSP one-step (mABS)	0	0.0	2.5	0.0	0.8	1.4
	10⁶	N.T.	N.T.	N.T.	N.D.	N. D
	10⁷	N.T.	N.T.	N.T.	N.D.	N. D
	10⁸	0.0	12.0	1.9	4.6	6.4
	10⁹	10.9	29.3	26.1	22.1	9.8
	10¹⁰	413.8	461.8	374.5	416.7	43.7
AuSP two-step (mABS)	0	11.9	0.0	2.0	4.6	6.4
	10⁶	N.T.	N.T.	N.T.	N.T.	N. D
	10⁷	4.7	0.0	10.7	5.1	5.4
	10⁸	33.2	29.9	29.4	30.8	2.1
	10⁹	177.3	185.2	188.1	183.5	5.6
	10¹⁰	446.6	555.7	560.6	521.0	64.4
AuPL one-step (mABS)	0	23.4	23.8	26.5	24.6	1.7
	10⁶	N.T.	N.T.	N.T.	N.T.	N.D.
	10⁷	N.T.	N.T.	N.T.	N.T.	N.D.
	10⁸	N.D.	N.D.	N.D.	N.D.	N.D.
	10⁹	14.6	0.0	0.0	4.9	8.4
	10¹⁰	111.6	95.8	109.0	105.4	8.5
AuPL two-step (mABS)	0	2.8	5.4	3.2	3.8	1.4
	10⁶	N.T.	N.T.	N.T.	N.T.	N.T.
	10⁷	5.3	7.2	4.5	5.7	1.4
	10⁸	21.5	21.5	25.8	22.9	2.5
	10⁹	81.4	96.3	83.6	87.1	8.1
	10¹⁰	118.9	176.0	215.1	170.0	48.4
ALP (a.u.)	0	-0.3	0.7	0.6	0.3	0.5
	10⁶	1.3	1.7	0.1	1.0	0.8
	10⁷	3.2	2.5	4.6	3.4	1.1

	10⁸	19.5	23.9	20.3	21.2	2.4
	10⁹	54.0	106.6	106.2	70.9	61.4
	10¹⁰	97.3	159.0	131.2	129.2	30.9
HRP (a.u.)	0	0.1	0.8	0.4	0.4	0.4
	10⁶	1.9	1.3	0.4	1.2	0.8
	10⁷	25.2	17.9	18.2	20.4	4.2
	10⁸	174.2	171.0	176.1	173.8	2.6
	10⁹	14.4	29.9	5.7	11.9	15.9
	10¹⁰	0.2	6.0	1.4	2.6	3.1

N.D.: not detected

N.T.: not tested