Supplementary Materials

Quantitative Capillary Electrophoresis for Analysis of Extracellular Vesicles (EVqCE)

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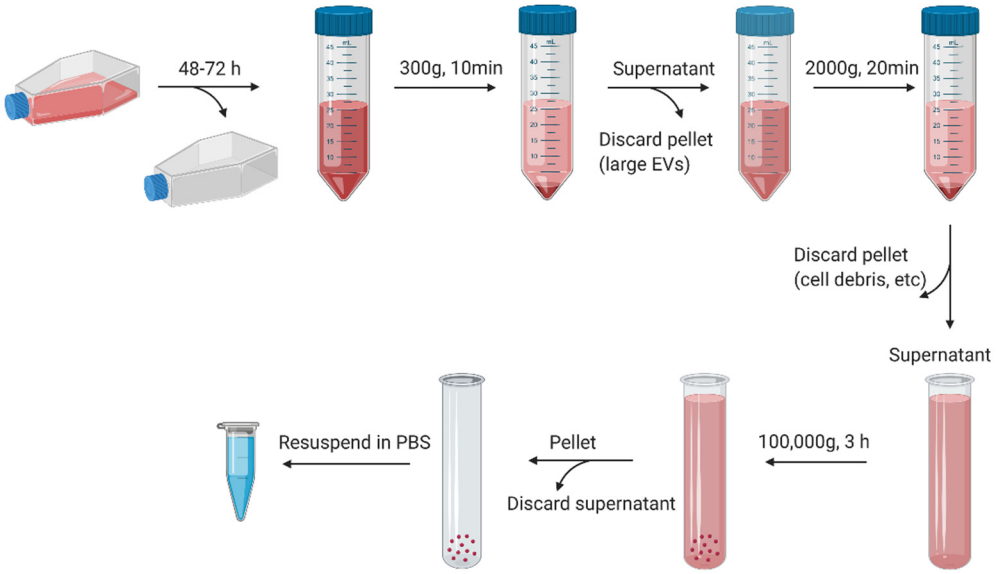

Figure S1. Schematic representations of EV isolation from cancer cells

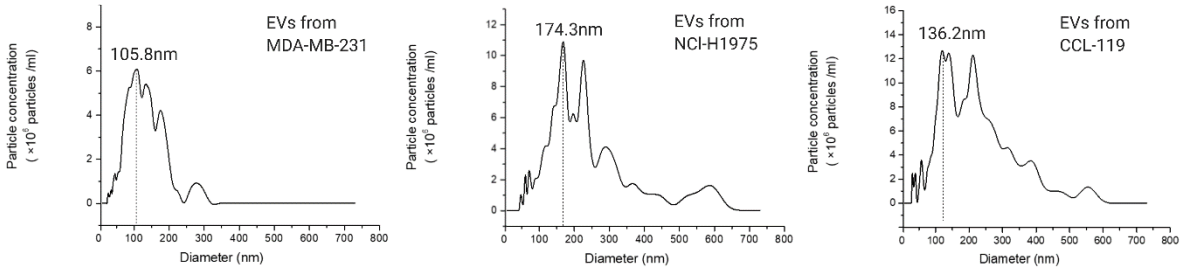

Figure S2. Nanoparticle tracking analysis (NTA) of EVs. The size distribution of EVs derived from cancer cell lines, MDA-MB231, NCl-H1975, CCL-119. The maximum peak of the respective EVs is shown with the dotted line.

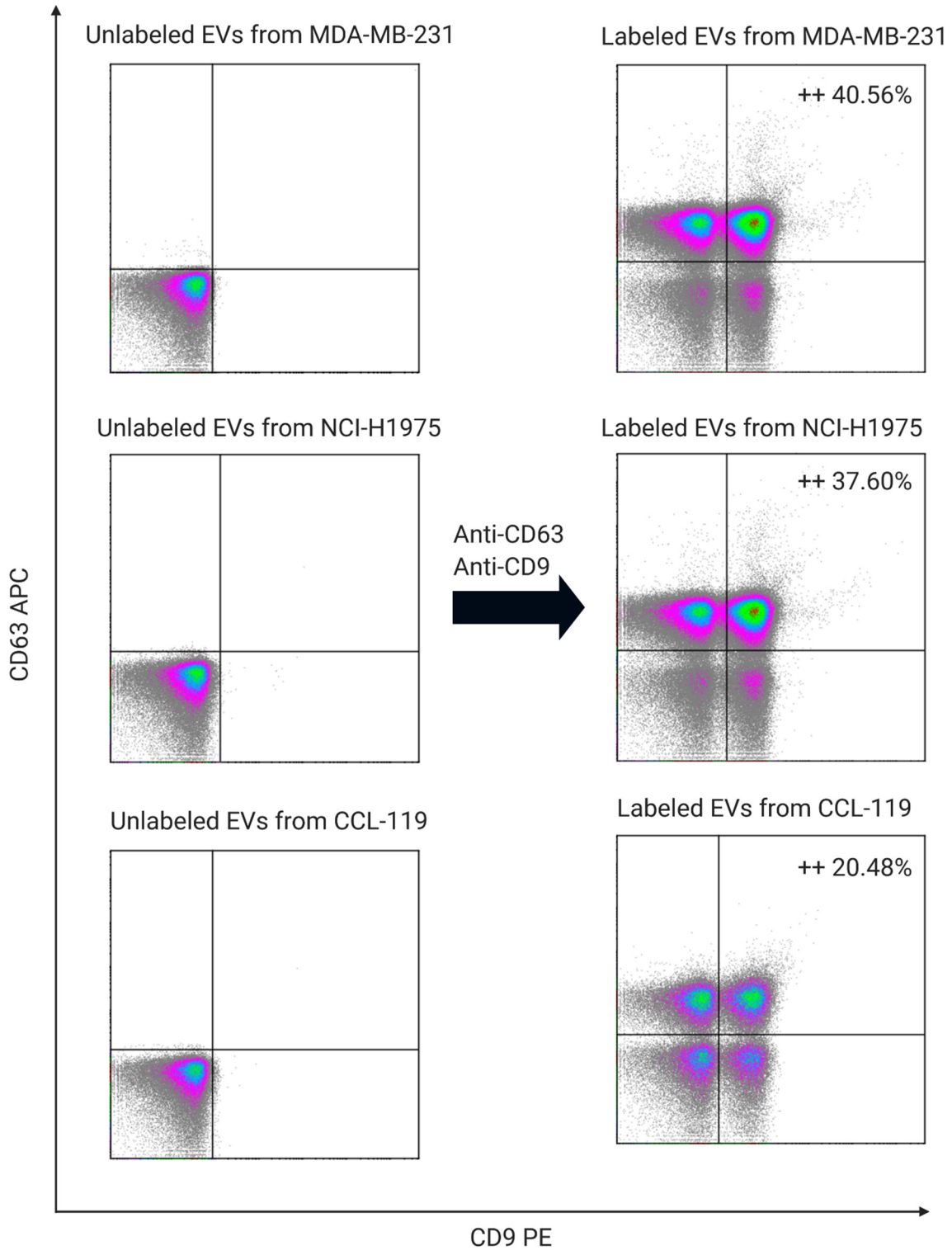

Figure S3. Flow cytometry analysis of EV isolates from cancer cell lines, MDA-MB231, NCl-H1975, CCL-119.

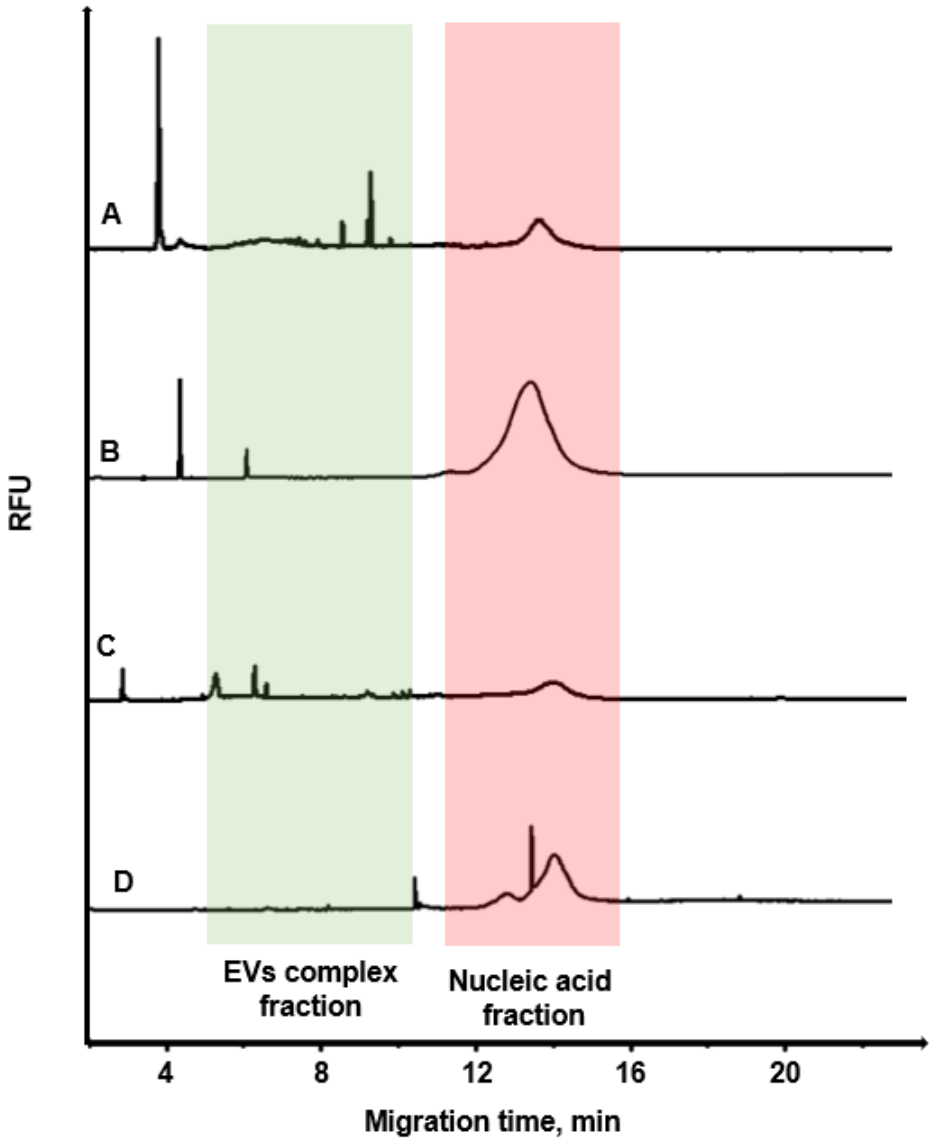

Figure S4. Experimental EVqCE electropherograms of EVs from NCI-H1975 and CCL-119 cell lines. (A) A sample of enriched EVs from NCI-H1975 cell line. (B) Sample A after lysis by 0.1% SDS (C) A sample of enriched EVs from CCL-119 cell line. (D) Sample B after lysis by 0.1% SDS.

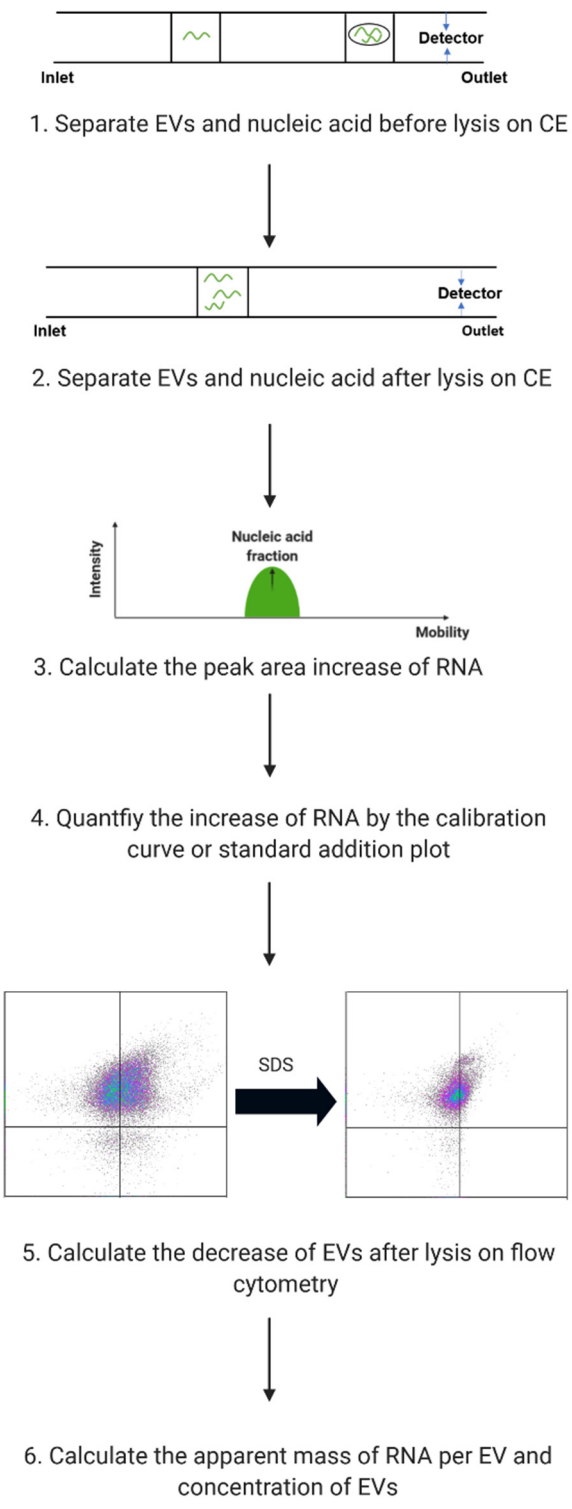

Figure S5. Six steps required for measuring EV concentration.

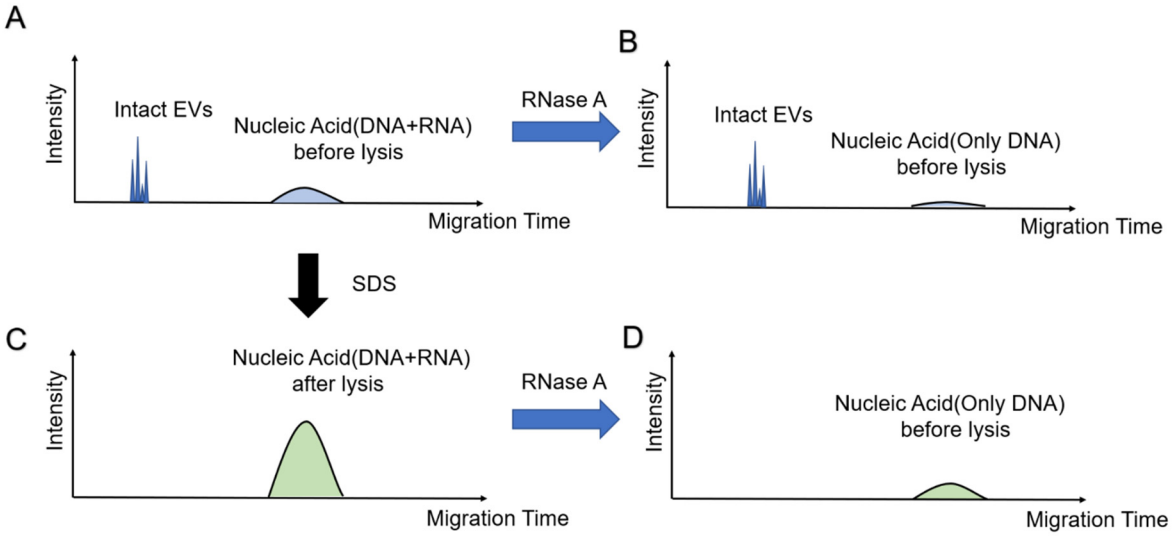

Figure S6. Schematic illustration of RNA quantifications by EVqCE. EVs were stained with YOYO-1 (A) EVs without any treatment. (B) RNAse A treated EVs showing the degradation of RNA. (C) SDS treated EVs showing the degradation of intact EVs and the increase in the intensity of the peak for nucleic acid. (D) SDS and RNase A treated EVs showing the degradation of RNA.

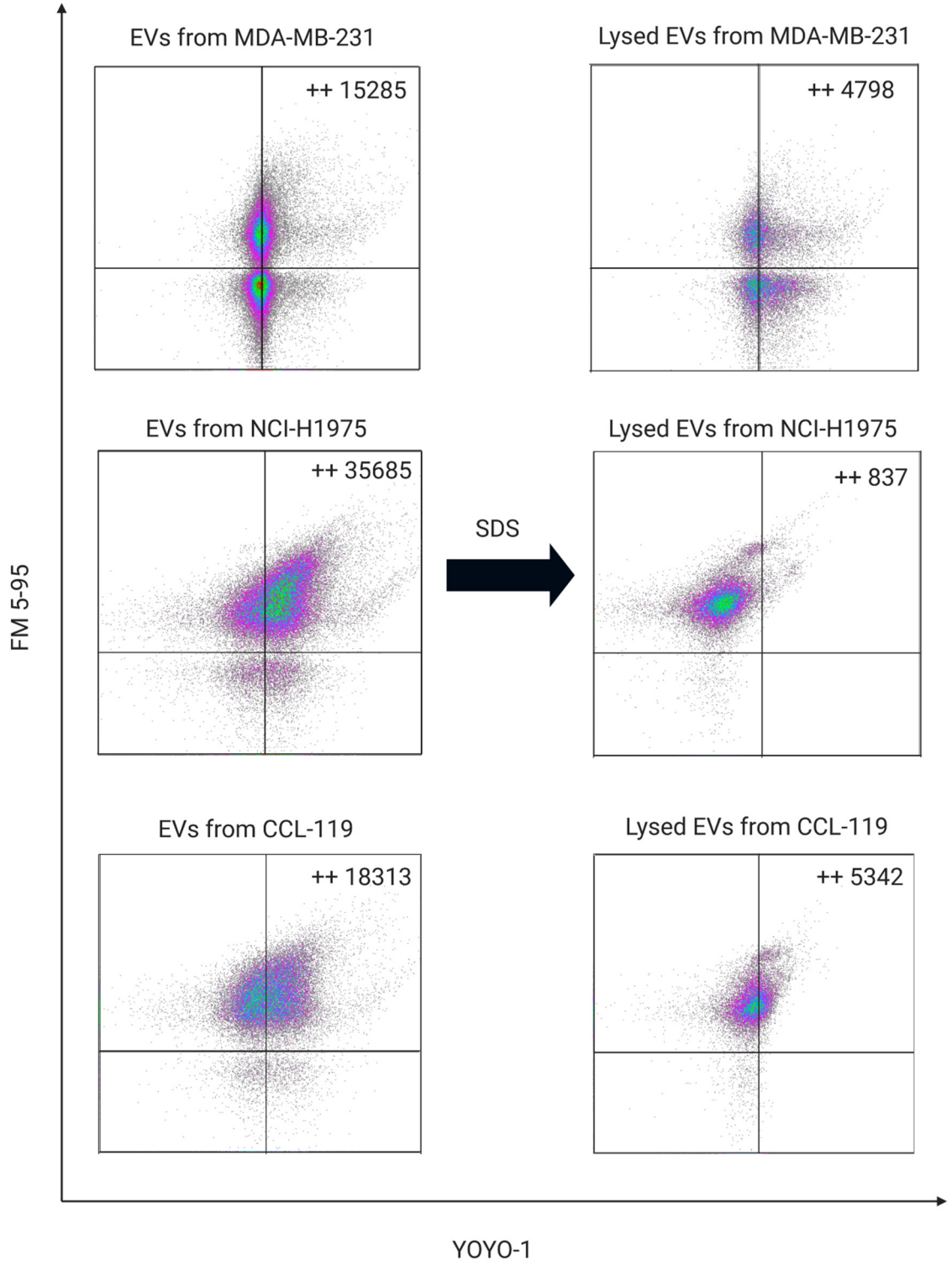

Figure S7. Flow cytometry analysis of EVs before and after the SDS treatment. EVs were stained and gated by anti-CD63 APC and anti-CD9 PE antibodies. The lysis process was performed by 0.1% SDS. The number of double positive (YOYO-1 and FM 5-95) events is shown at the top-right corner.

Method optimization

For EV lysis, 0.1 M n-dodecyl-β-D-maltoside (DDM), Trition-100, SDS were used to lyse EVs. SDS showed a more efficient lysis ability.

For degradation analysis, 1, 5, 10 cycles of freeze-thaw were tried in during the method optimization. 10 cycles was performed in the manuscript because it showed a clear and efficient degradation of EVs. Similar optimizations were tried in vortexing (1, 5, 10 min) and sonication (1, 5, 10 min).

Data on biological and technical replicates.

Technical replicates of EV separations on CE (Figure 2).
Figure 2C, n=3, $\bar{x}$=0.14, RSD=15%
Figure 2D, n=3, $\bar{x}$=4.43, RSD=9%

Technical replicates of EV separations on CE (Figure 4).
Figure S4A, n=3, $\bar{x}$=0.63, RSD=9%
Figure S4B, n=3, $\bar{x}$=4.97, RSD=3%
Figure S4C, n=3, $\bar{x}$=0.39, RSD=8%
Figure S4D, n=3, $\bar{x}$=3.10, RSD=6%

Technical replicates of RNA calibration curve (Figure 3).
RNA 1, n=3, $\bar{x}$=1.93, RSD=23%
RNA 2, n=3, $\bar{x}$=2.00, RSD=20%
RNA 3, n=3, $\bar{x}$=4.07, RSD=11%
RNA 4, n=3, $\bar{x}$=1.46, RSD=17%
RNA 5, n=3, $\bar{x}$=3.33, RSD=5%
RNA 6, n=3, $\bar{x}$=1.03, RSD=6%
RNA 7, n=3, $\bar{x}$=8.67, RSD=2%
RNA 8, n=3, $\bar{x}$=9.73, RSD=3%

Biological replicates of EVs quantification (Table 1).
EVqCE, MDA-MB-231, n=3, $\bar{x}$=5.1, RSD=3%
EVqCE, NCI-H1975, n=3, $\bar{x}$=1.4, RSD=4%
EVqCE, CCL-119, n=3, $\bar{x}$=1.3, RSD=22%

NTA, MDA-MB-231, n=3, $\bar{x}$=4.90, RSD=2%
NTA, NCI-H1975, n=3, $\bar{x}$=2.33, RSD=7%
NTA, CCL-119, n=3, $\bar{x}$=2.6, RSD=10%

Flow cytometry, MDA-MB-231, n=3, $\bar{x}$=1.70, RSD=16%
Flow cytometry, NCI-H1975, n=3, $\bar{x}$=3.60, RSD=17%
Flow cytometry, CCL-119, n=3, $\bar{x}$=5.70, RSD=11%

Technical replicates of EV degradation analysis in (Figure 4).
Figure 4A, n=3, $\bar{x}$=0.21, RSD=31%
Figure 4B, n=3, $\bar{x}$=1.53, RSD=12%
Figure 4C, n=3, $\bar{x}$=0.18, RSD=33%
Figure 4D, n=3, $\bar{x}$=1.78, RSD=16%
Figure 4E, n=3, $\bar{x}$=3.80, RSD=26%