*pharmaceutics*

MDPI

# Supplementary Materials: Clinically expired platelet concentrates as a source of extracellular vesicles for targeted anti-cancer drug delivery

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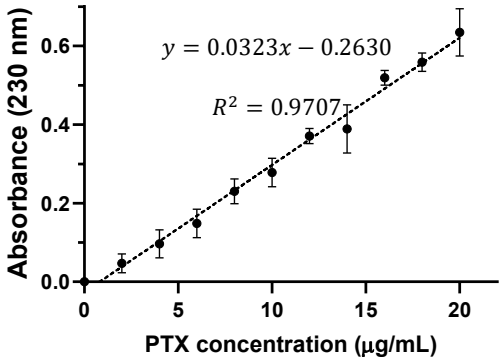



**Figure S1.** Paclitaxel calibration curve used to estimate PTX concentration into pEV. *n*=3, *n* represents independent experiments carried out inter-days.

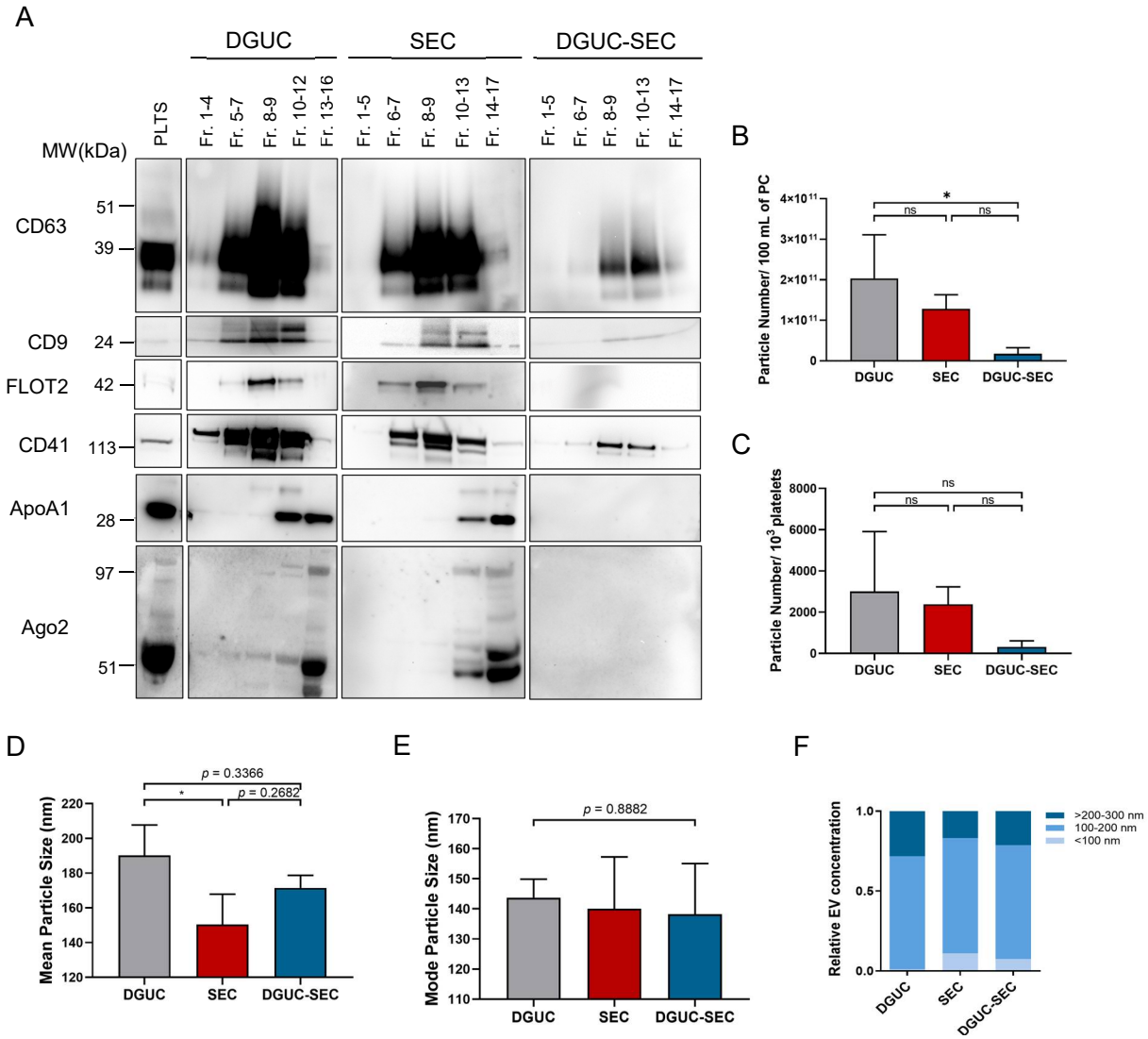
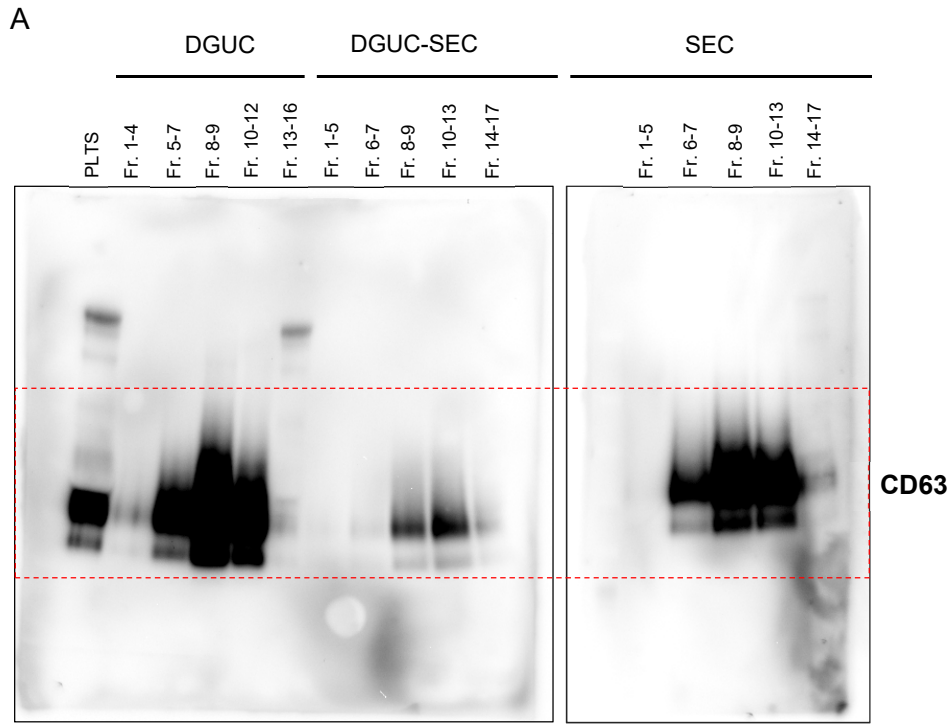



**Figure S2.** Characterization of pEV fractions isolated by DGUC, SEC, and DGUC-SEC in terms of purity, yield, and size distribution. **(A)** Western Blot analysis of specific EV markers (tetraspanins CD63 (glycosylated form) and CD9, and cytosolic protein flotillin-2), platelet-specific marker (CD41), and non-EV markers [apolipoprotein (ApoA1) and argonaute 2 (Ago2)] in pooled fractions and platelet lysate (PLTS). Molecular weight markers (MW, KDa) are indicated. **(B)** pEV-enriched fractions (Fr.8-9) were analyzed using nanoparticle tracking analysis. Particle number was normalized to platelet concentrate (PC) volume (100 mL) and **(C)** platelet count ($10^3$ platelets); (*n*=3). **(D)** Mean and **(E)** mode particle size (nm) of pEV samples; (*n*=3). **(F)** Relative pEV distribution ranged into three different size classes: < 100 nm, 100-200 nm and >200-300 nm; (*n*=3). *n* represents biologically independent replicates. Data are represented as mean ± S.D. *p < 0.05, n.s. not significant, determined by one-way ANOVA followed by Tukey's multiple comparison test.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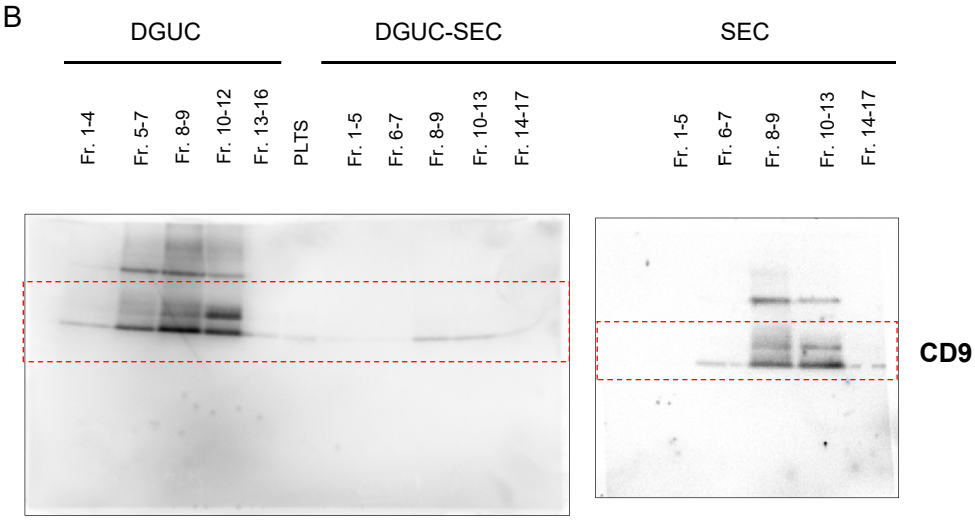
CD63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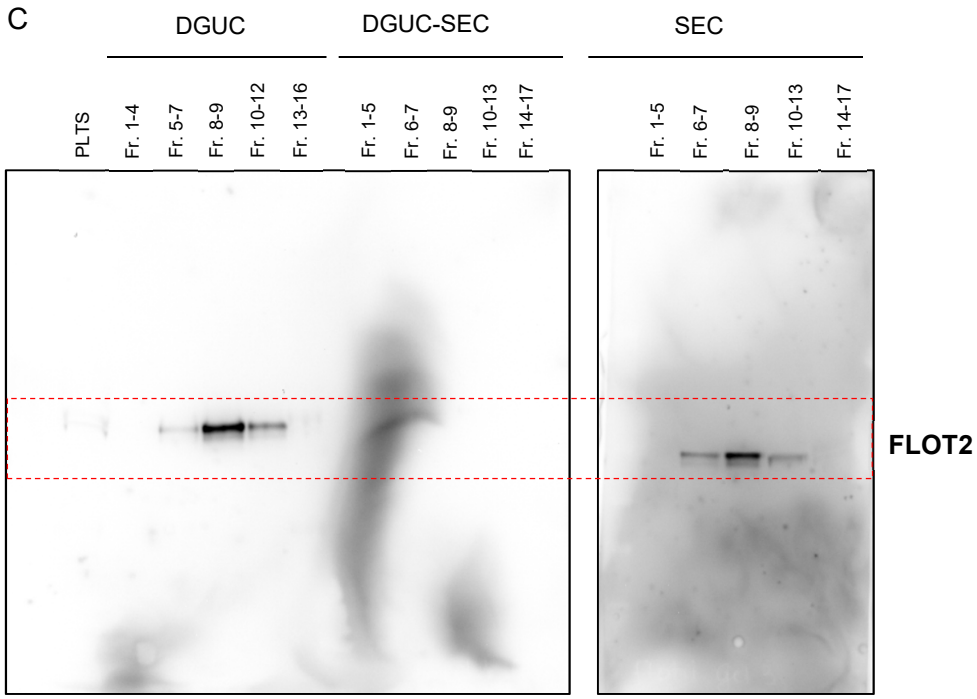
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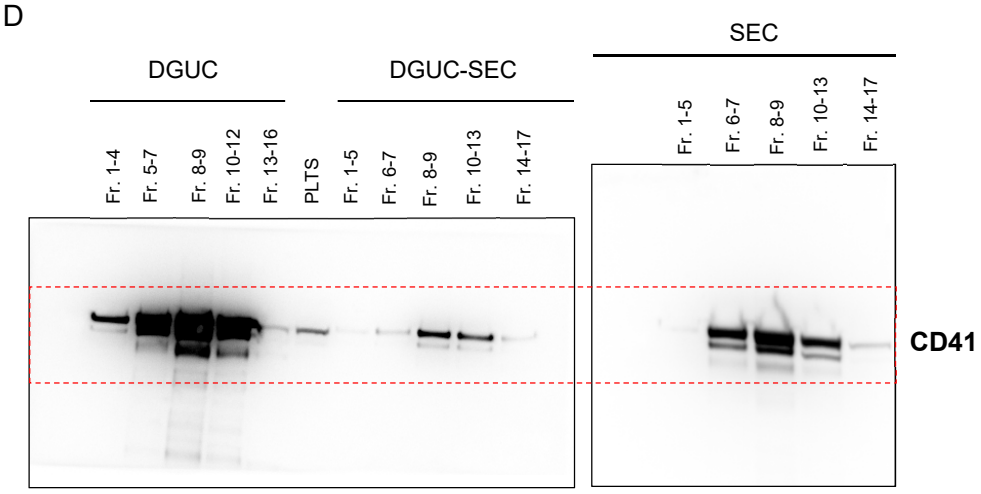
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CD41

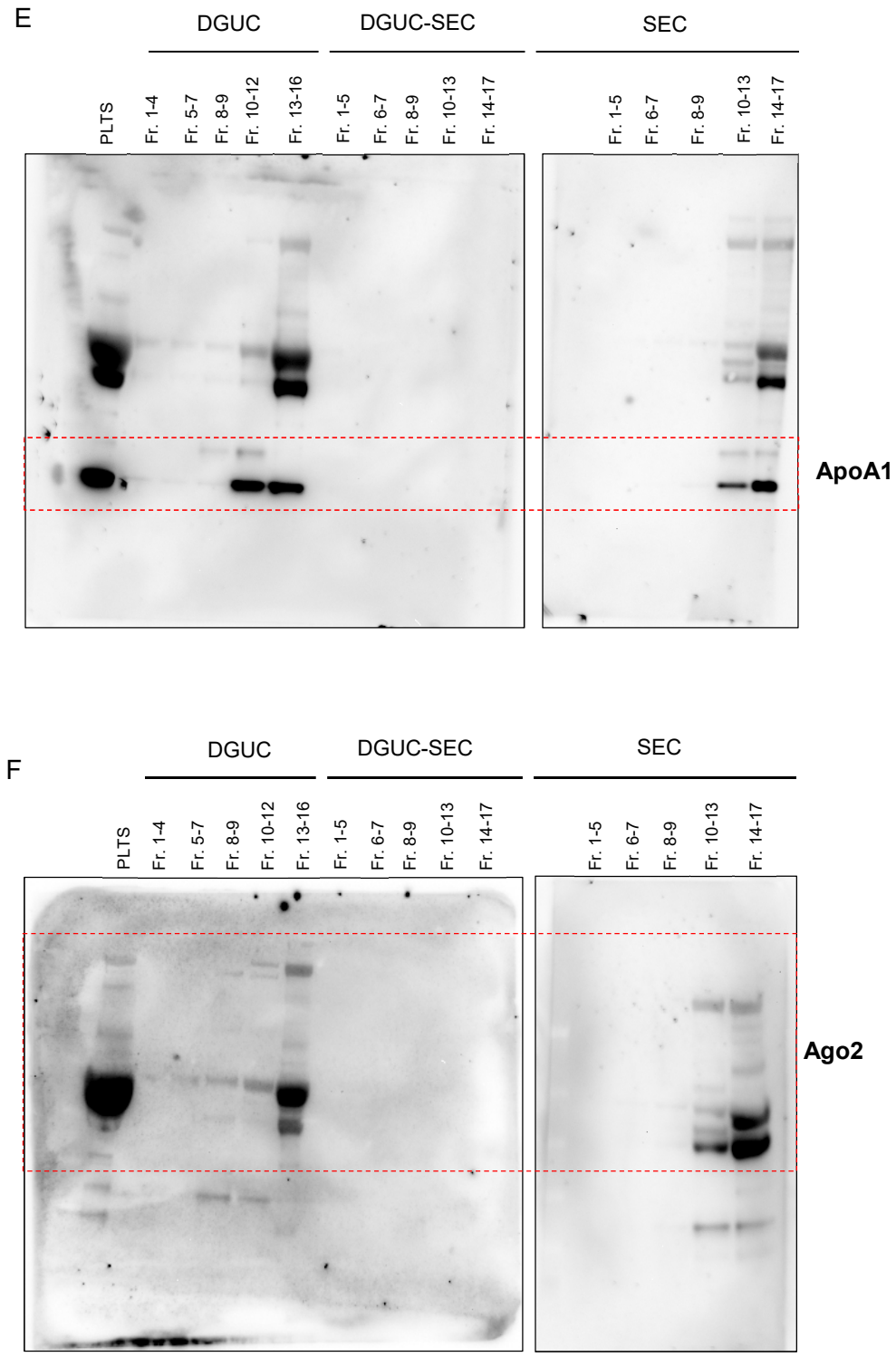


**Figure S3.** Full-length western blotting images for the results shown in main figures and supplementary Figure S2. Insets indicate cropped regions.

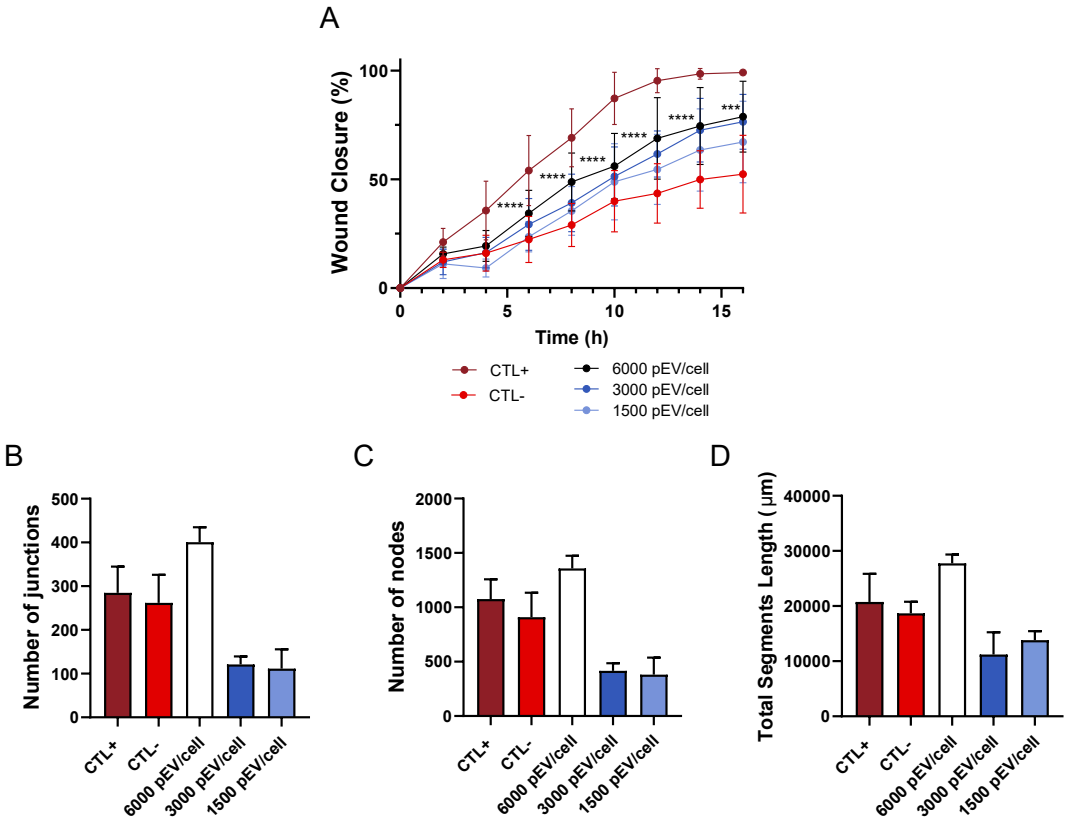


**Figure S4.** Effect of different pEV densities on HUVEC angiogenesis. **(A)** Quantitative analysis of the wound closure percentage throughout culture time. HUVEC were treated with 1500, 3000 and 6000 pEV/cell or with controls, CTL+ (cells cultured in supplemented media) and CTL- (8-9 fractions of DPBS gradient), for 16 h; (*n*=3). **(B)** Number of junctions, **(C)** nodes and **(D)** total segments length (µm) of HUVEC incubated with pEV or with controls, CTL+ and CTL-, for 8 h. Data are represented as mean ± S.D. One-way ANOVA followed by Tukey's multiple comparison test (6000 pEV/cell versus CTL-). ***p < 0.001, ****p < 0.0001.

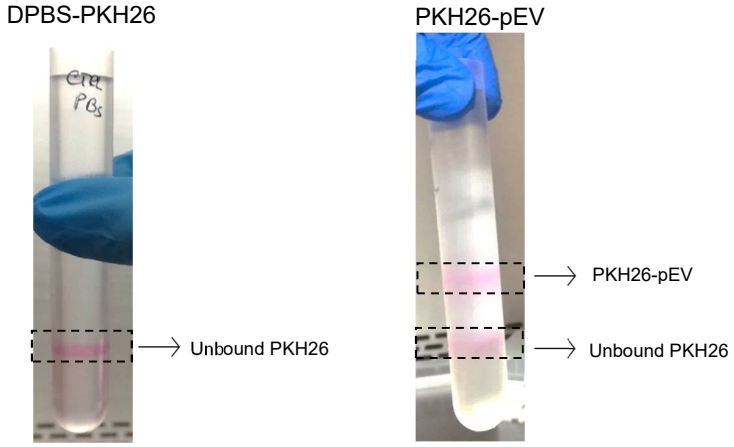


**Figure S5.** Separation of PKH26-pEV by DGUC. The presence of the two bands marked in the figure indicates a clear separation of PKH26-pEV (upper band) at the level of fractions enriched in pEV (fractions 8 and 9) from the unbound dye (lower band) located in denser fractions.

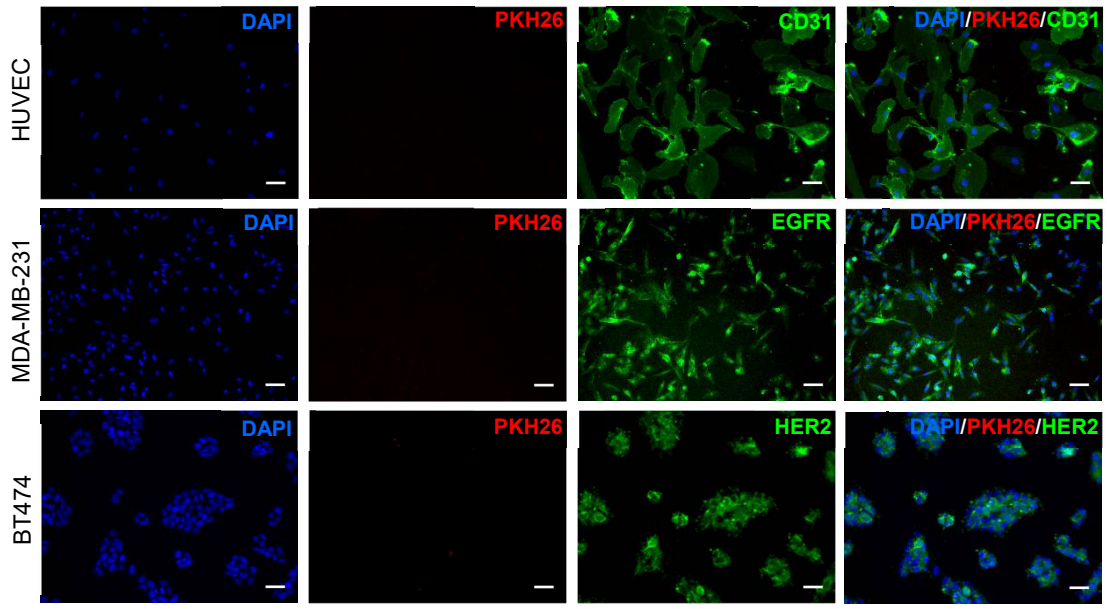


**Figure S6.** Cellular uptake of DPBS-PKH26 (negative control) by HUVEC, MDA-MB-231, and BT474 cells after 24 h of incubation. Representative immunofluorescence images of DPBS-PKH26 treatment. HUVEC, MDA-MB-231, and BT474 cells were stained for CD31, EGFR, and HER2 (green), respectively, and nuclei (DAPI, blue). Scale bars: 50 μm.

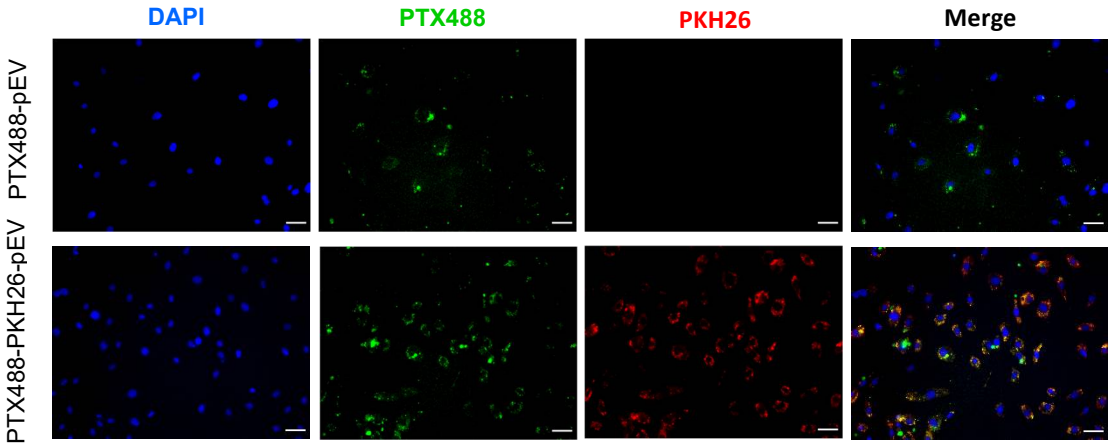


**Figure S7.** Intracellular distribution of PTX-loaded pEV in HUVEC cells after 24 h of incubation. HUVEC were treated with pEV loaded with tagged PTX (PTX488, green) and PTX488-pEV labeled with PKH26 (red) (PTX488-PKH26-pEV). Cell nuclei were stained with DAPI (blue), and cells were analyzed by fluorescence microscopy. Scale bars: 50 μm.