Impact of Long-Term Refrigeration, Freezing, and Repeated Freeze-Thaw Processes on the Physicochemical Characteristics of Extracellular Vesicles

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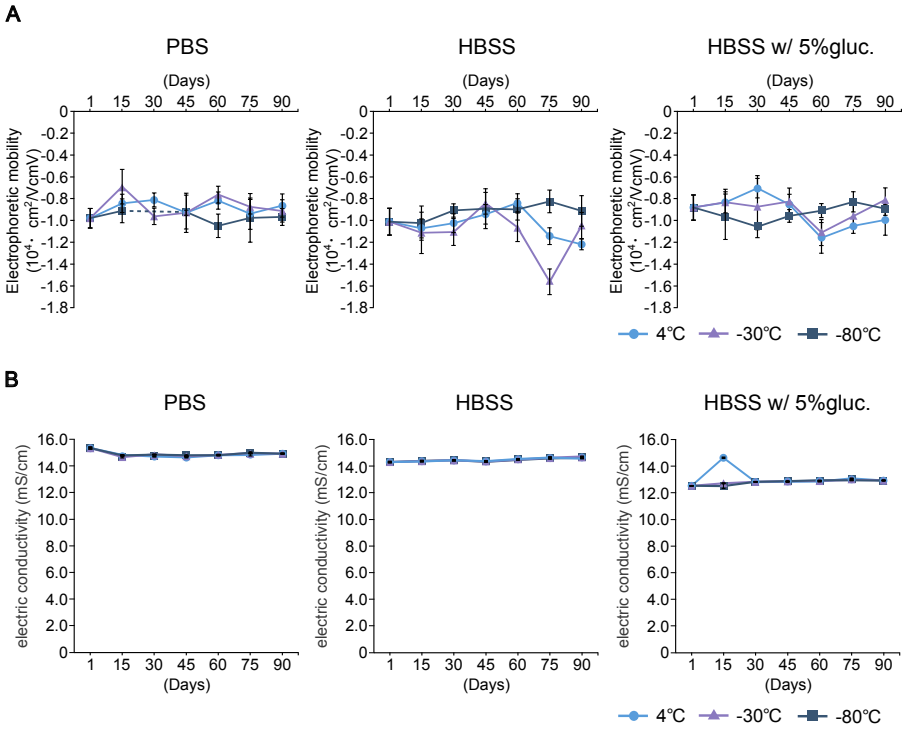
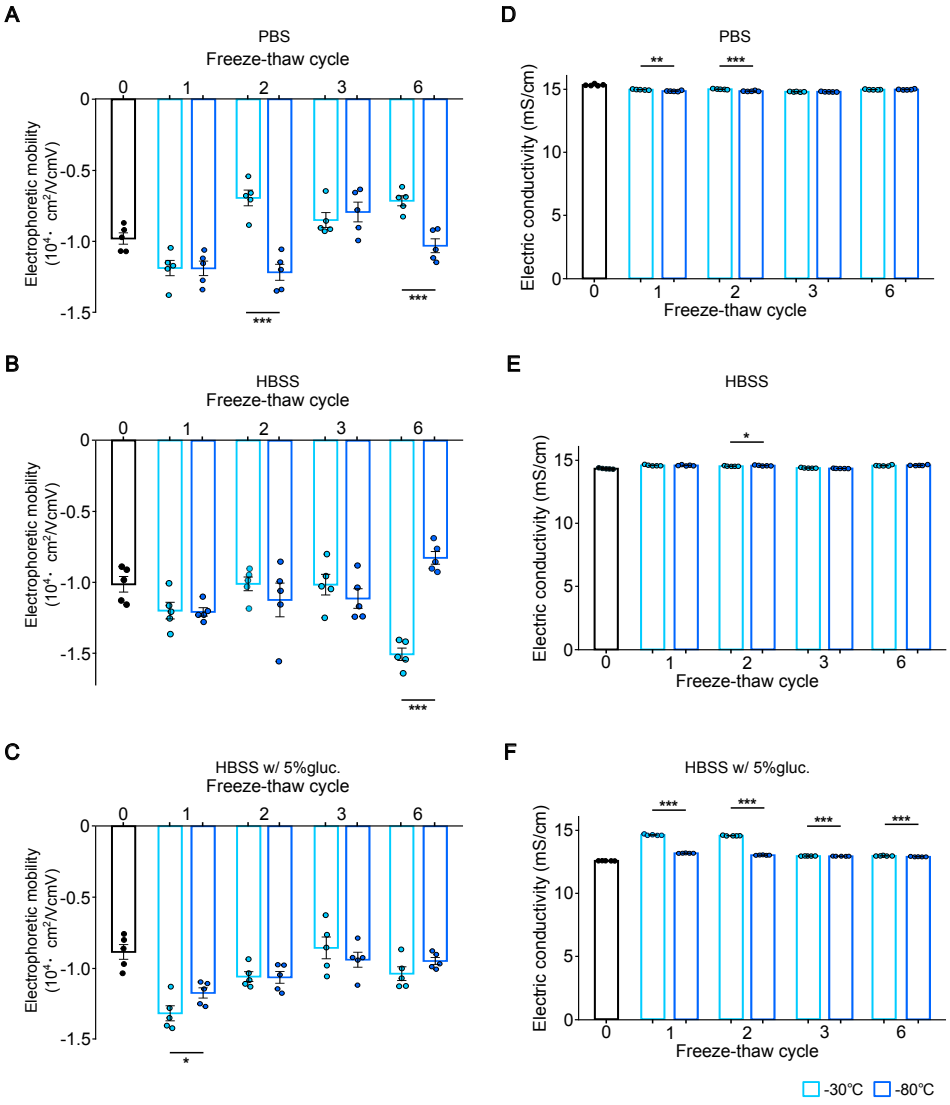


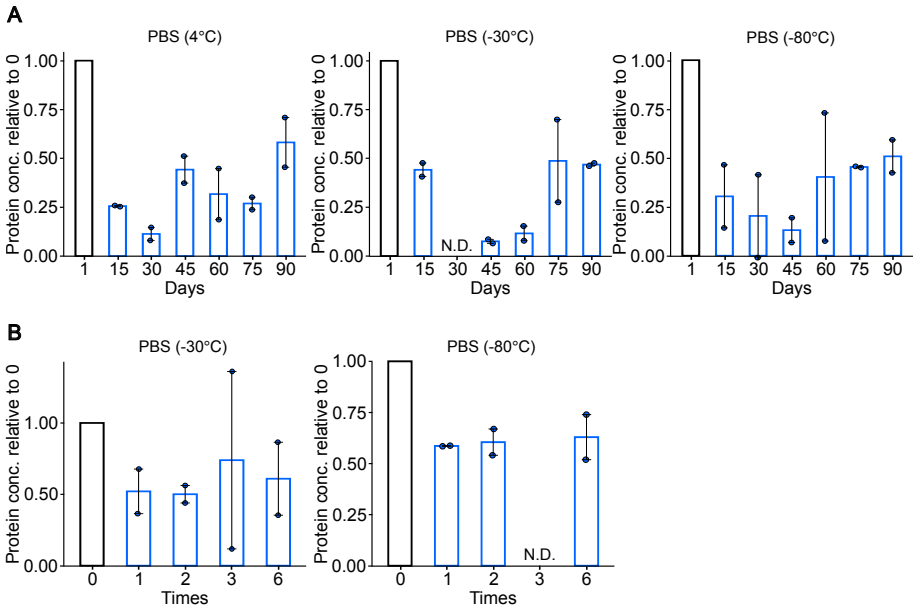
Supplementary Figure 1. Stability of PC3-derived EVs under various storage conditions over 90 days, examining electrophoretic mobility and electric conductivity.

Time-course analysis of (A) electrophoretic mobility (10^4 · cm^2/VcmV) and (B) electric conductivity (mS/cm) of EVs. EVs were suspended in PBS (left column), HBSS (middle column), or HBSS with 5% glucose (right column) and stored at 4 ℃ (light blue line with circles), −30 ℃ (purple line with triangles), or −80 ℃ (dark blue line with squares) for up to 90 days. Data are presented as mean ± SD.



Supplementary Figure 2. Stability of PC3-derived EVs against repeated freeze-thaw cycles, examining electrophoretic mobility and electric conductivity.

EVs were suspended in PBS (A, D), HBSS (B, E), or HBSS with 5% glucose (C, F) and subjected to 0, 1, 2, 3, or 6 freeze-thaw (FT) cycles. Freeze-thaw cycles were performed by freezing at either −30 ℃ (light blue bars) or −80 ∘C (dark blue bars) and thawing at room temperature. (A-C) Electrophoretic mobility (10^4·cm^2/VcmV). (D-F) Electric conductivity (mS/cm). Data are presented as mean ± SD (n=5-6 per group). Statistical significance was determined by Student's t-test. $p < 0.05$, $p < 0.01$, $p < 0.001$.



Supplementary Figure 3. Protein concentration of PC3-derived EVs in PBS under various storage and freeze-thaw conditions.

(A, C, E) Protein concentration of EVs suspended in PBS and stored at (A) 4 ℃, (C) −30 ℃, or (E) −80 ℃ for up to 90 days. Protein concentration is shown relative to Day 1 (0 days of storage). (B, D) Protein concentration of EVs suspended in PBS and subjected to 0, 1, 2, 3, or 6 freeze-thaw (FT) cycles at (B) −30 ℃ or (D) −80 ℃. Protein concentration is shown relative to the non-frozen control (0 FT cycles). Data are presented as mean ± SD (n=2 per group). N.D. = Not Detected