A multiplex flow cytometric approach to define molecularly distinct extracellular vesicle subsets

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Supplementary Material

Figure S1:

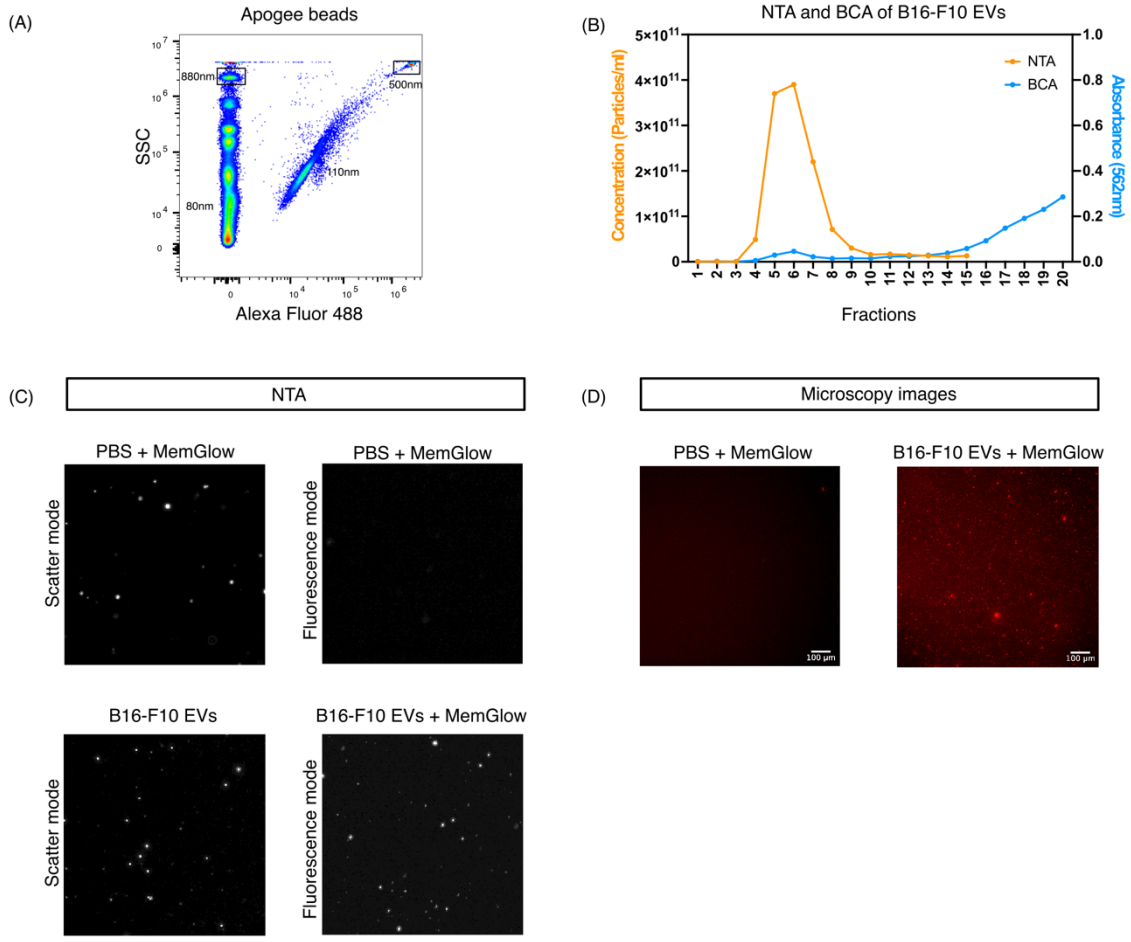

Figure S1. Characterization of B16-F10-derived EVs. (A) FACS plot of Apogee beads indicating the efficacy of the instrument in resolving beads of various sizes starting from 80nm. (B) Representative nanoparticle tracking analysis (NTA) and bicinchoninic acid (BCA) assay demonstrating EV concentration distribution and protein content across different fractions isolated by SEC. (C) Representative NTA image of PBS vs. B16-F10 EVs labeled with MemGlow, showing particle detection in scatter mode (left) and fluorescence (MemGlow) signal (right). (D) Fluorescence microscopy of PBS with MemGlow as a negative control and B16-F10 EVs labeled with MemGlow.

Figure S2:

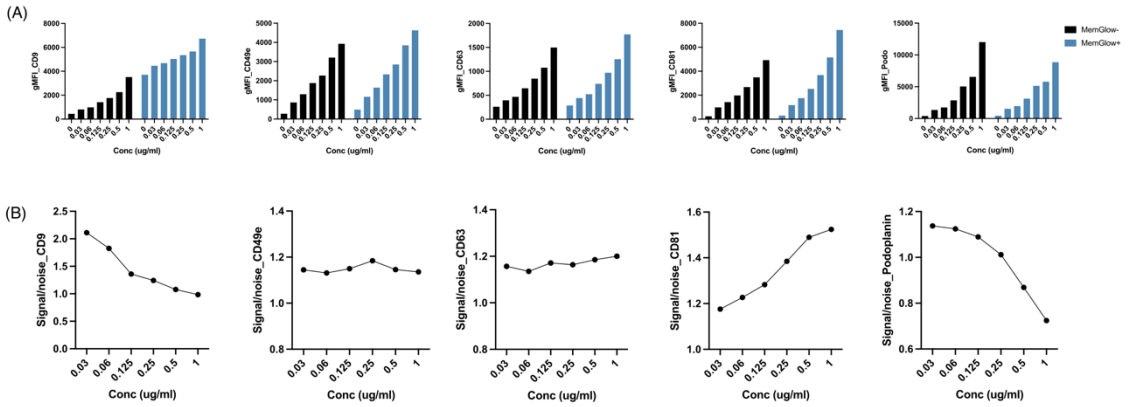

Figure S2. Optimization of antibody staining for B16-F10 EVs. (A) Raw signal intensities measured for individual antibody titration series in combination with MemGlow staining in B16-F10-derived EV samples, gated for MemGlow- events (black) and MemGlow+ EVs (blue). (B) Signal-to-noise ratios calculated as (MFI of MemGlow+ EVs / MFI of MemGlow- events) for each of the antibodies.

Table S1:

Antibodies	Fluorophore	Company / Cat no	Final Conc.
CD63	Alexa fluor 700	Biolegend / 143924	0.5 µg/ml
CD9	APC fire 750	Biolegend / 124814	0.03 µg/ml
CD81	PE/Cy7	Biolegend / 104914	0.5 µg/ml
CD49e	BV605	BD biosciences / 743111	0.125 µg/ml
Podoplanin	Superbright 436	Invitrogen / 62-5381-82	0.03 µg/ml