Supporting Information: Tracking small extracellular vesicles using a minimally invasive PicoGreen labeling strategy

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SUPPLEMENTARY TABLES

Table S1. List of antibodies used for capillary-based Simple Western Assay (WES)

Antibody details	Vendor	Catalogue number	Dilution for WES
CD9-rabbit monoclonal (D801A)	Cell Signaling	13174S	1:50
CD63-rabbit monoclonal (E1W3T)	Cell Signaling	52090S	1:50
CD81-rabbit monoclonal (D3N2D)	Cell Signaling	56039S	1:50
mCherry-rabbit monoclonal (E5D8F)	Cell Signaling	43590S	1:100

Table S2. Table with components of fallopian tube organoid media.

Media Component	Details, Company, and Catalog Number	For 200 mL Fallopian Tube Organoid Media
ADF+	Advanced DMEM/F12 supplemented with 1x Glutamax, 10 mM HEPES, and 1x Pen/Strep	122 mL
WNT Conditioned Media	Conditioned in ADF+ media from L Wnt-3A cells (ATCC CRL-2647 ™)	50 mL
Noggin	Noggin conditioned media produced via r-PEX protein expression platform at U-Protein Express BV	2 mL
RSPO1 Conditioned media	Conditioned in ADF+ media from Cultrex HA-R-Spondin1-Fc 293T cells (R&D Systems 3710-001-01)	20 mL
B27 Supplement	50x, Invitrogen #17504044	4 mL
N-Acetylcysteine	500 mM, Sigma-Aldrich A9165-5G	0.5 mL
Primocin	Invitrogen ant-pm1	0.4 mL
Nicotinamide	1M, Sigma Aldrich N0636	2 mL
A83-01	5 mM, Bio-Techne (R&D systems 2939)	20 µL
Y27632 Dihydrochloride (Rho-ki)	100 mM, Abmole bioscience #Y-27632	10 µL

SUPPLEMENTARY FIGURES

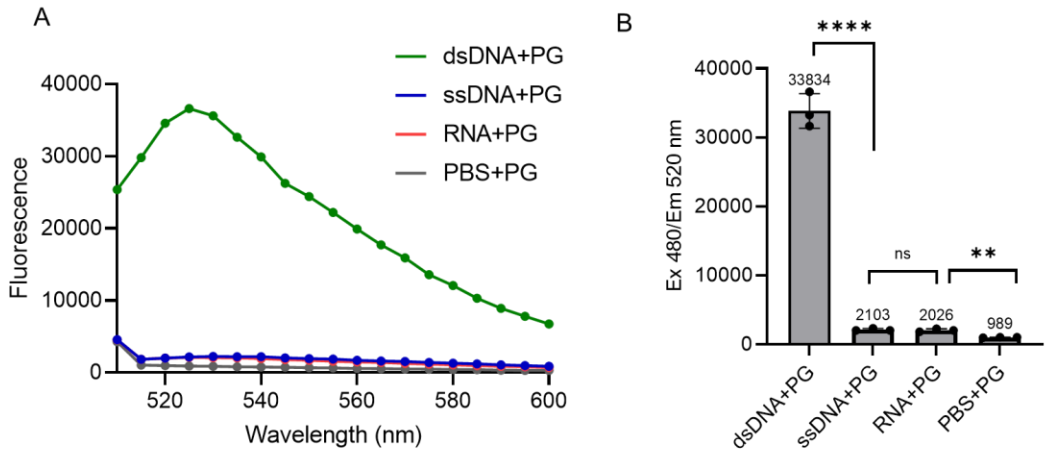

Figure S1. PicoGreen preferentially intercalates with dsDNA to give a fluorescent complex. (**A**) PG is incubated with the equivalent amount (650 ng/mL) of dsDNA (lambda DNA from E. coli), ssDNA (Oligo from IDT), RNA (total RNA from E. coli), and PBS at room temperature for 1 h and emission fluorescence spectra are quantified at the excitation wavelength of 480 nm. PG-dsDNA complex gives an excitation maximum at 525 nm, whereas no significant excitation peak is observed for PG-ssDNA and PG-RNA incubation. PBS was used as a control. (**B**) Quantification of fluorescence of PG-dsDNA, PG-ssDNA, and PG-RNA after 1 h incubation at room temperature. PG-dsDNA shows a dramatic increment in fluorescent confirming PG preferentially intercalates to dsDNA giving fluorescent complex.

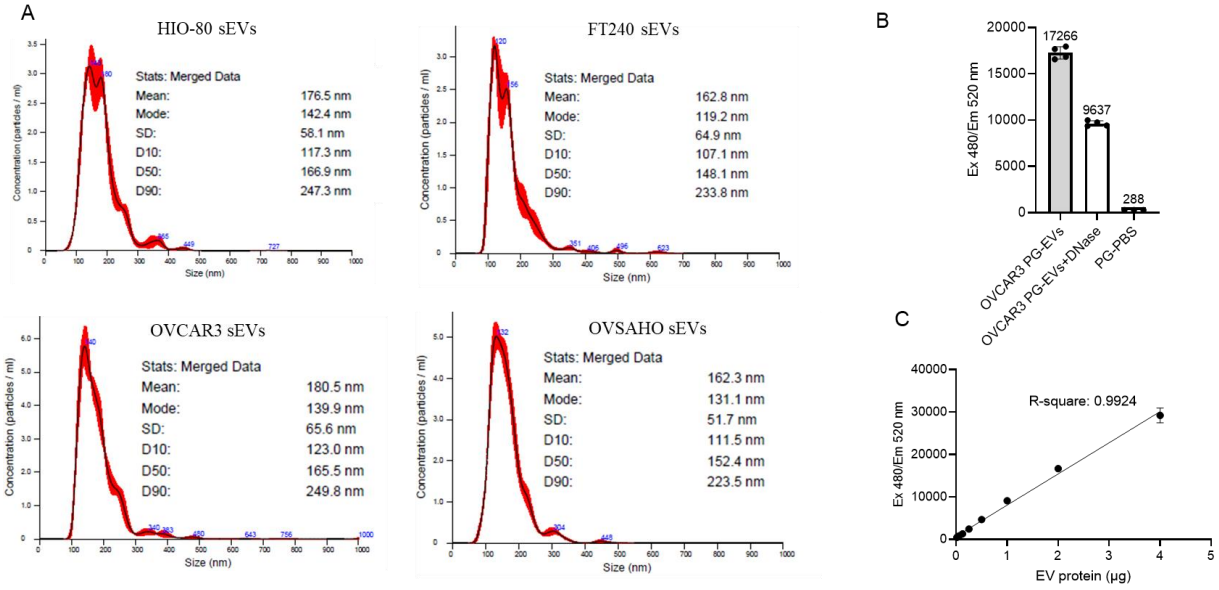

Figure S2. Size distribution profile of sEVs from different cell sources and analysis of PG-EV fluorescence. (**A**) Size distribution profile of sEVs derived from HIO-80, FT240, OVCAR3, and OVSAHO cell lines. The average size of sEVs ranges from 160-177 nm, quantified by nanoparticle tracking analysis. (**B**) Fluorescent measurement of OVCAR3 PG-EVs following DNase treatment. 2 µg OVCAR3 PG in 100 µL PBS is labeled with PG and treated with DNase to cleave non-EV associated DNA. PG-EV fluorescence decreases by 44% following DNase treatment. (**C**) Correlation analysis of OVCAR3 PG-EV fluorescence with respect to sEV protein content. PG-EV fluorescence shows linear increment with increasing sEV protein content.

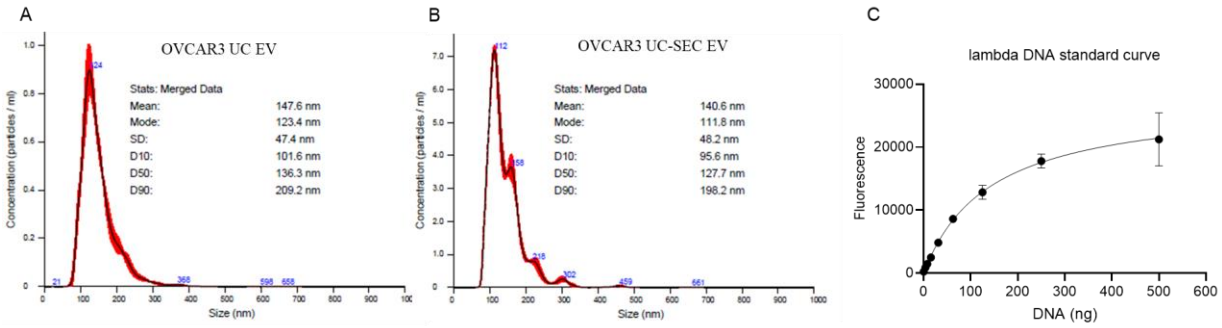

Figure S3. Size distribution profile of sEV before and after SEC purification and quantification of DNA amount using lambda DNA standard curve. (**A**) Size distribution profile of OVCAR3 sEVs isolated from differential ultracentrifugation (UC). (**B**) Size distribution profile of OVCAR3 sEV isolated from differential ultracentrifugation and further purified by size exclusion chromatography (UC-SEC). The size distribution profile was quantified by NTA. (**C**) Calibration curve of lambda dsDNA standard. Different dilutions of lambda dsDNA standard were prepared, labeled with PG, and fluorescent were measured to create a dsDNA standard curve. This curve was used to quantify dsDNA amount in sEVs.

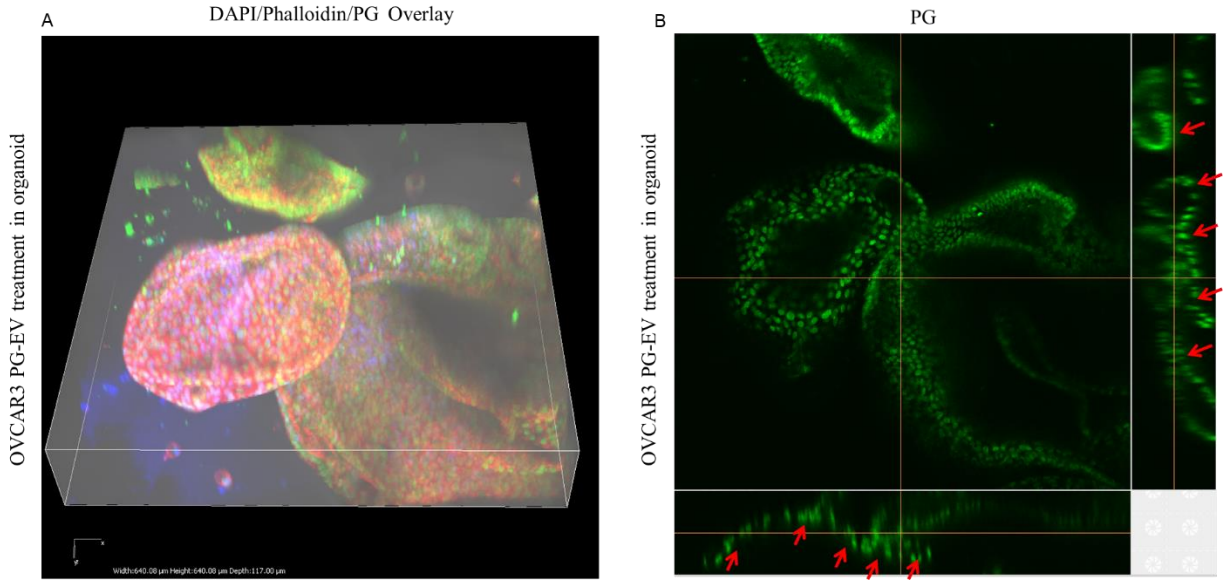

Figure S4. **Three-dimensional view of organoid showing PG-EV traversing to an entire organoid.** (**A**) Volume view of organoid showing 3-dimensional structure. The depth of the image is 117 µm. 79 images were taken across the depth (Z-axis) of ~117 µm and stacked (Z-stacking) using NIS-Element Viewer to represent the 3-dimensional structure of organoid. (**B**) Single plane image of organoid with cross-sectional slice view. Horizontal and vertical slice view shows signal around the middle (red arrow) suggesting PG-EV going inside the 3D organoid structure.

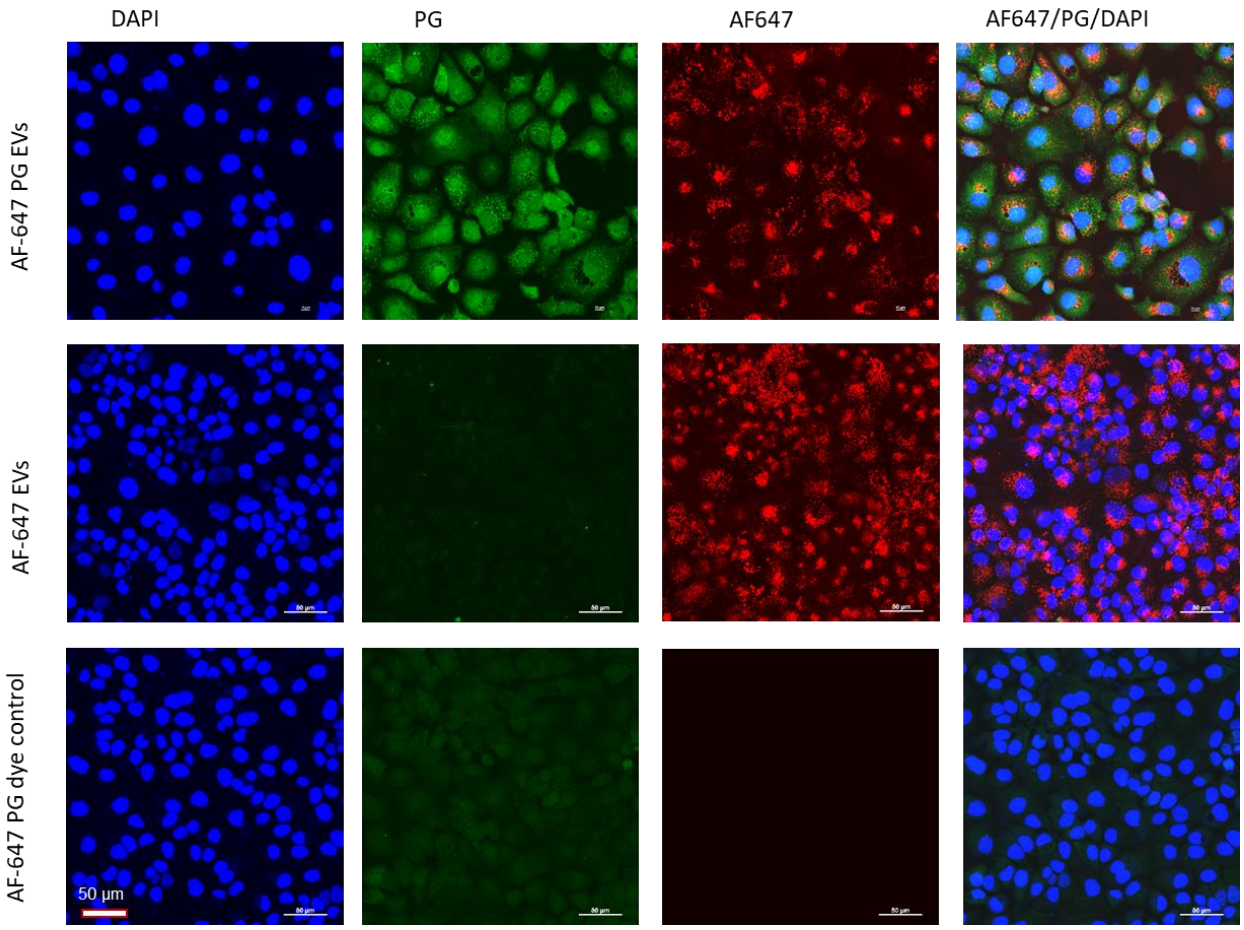

Figure S5. OVCAR3 EV tracking in OVCAR3 cells using different labeling approaches. Shown are images for AF647-PG dual labeled EVs, AF647 EVs, and AF647 dye controls. 2 µg of sEVs were treated to cells for 24 h. The scale bar represents 50 µm. Imaged were obtained using confocal microscopy (40x with oil, maximum intensity projection).

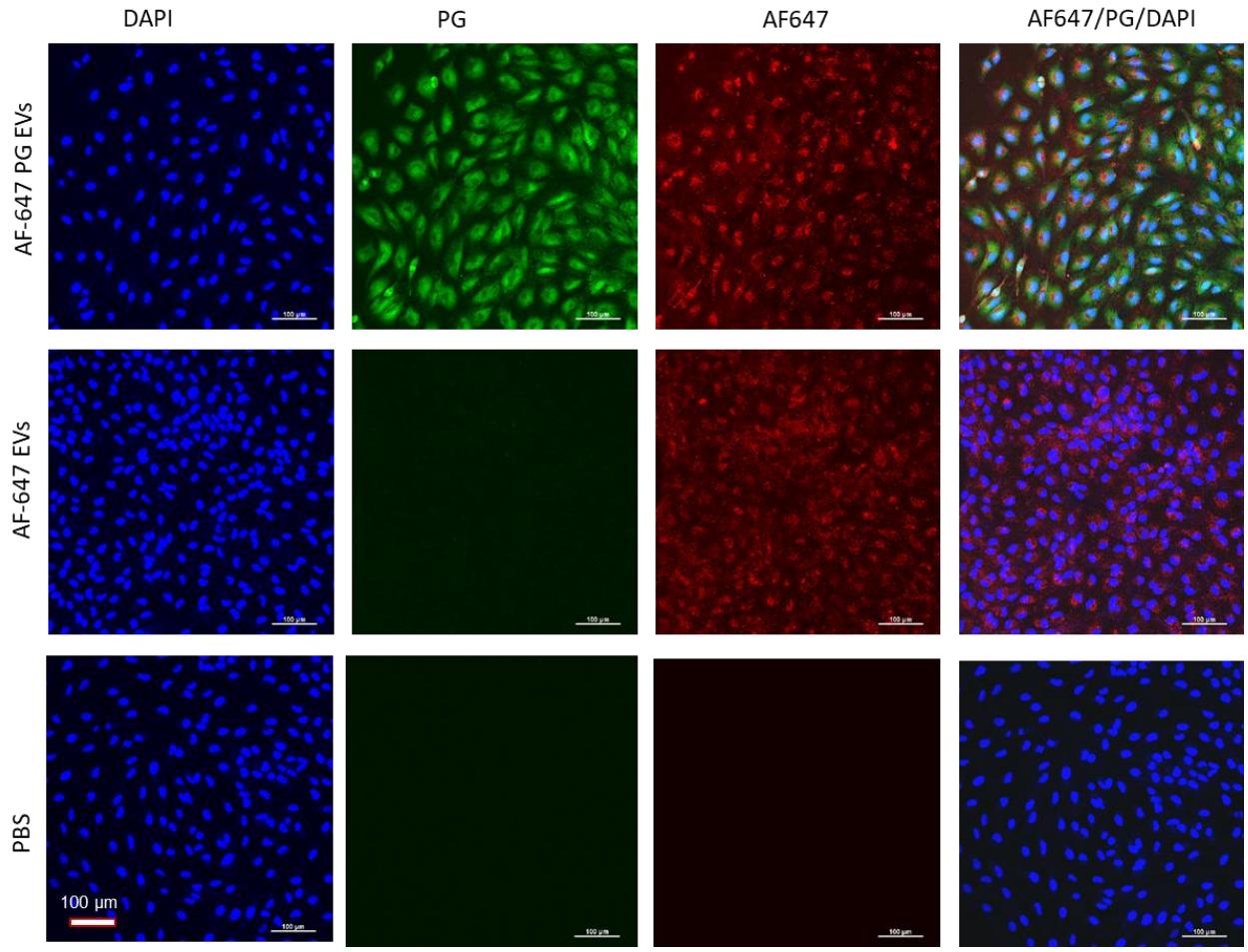

Figure S6. OVCAR3 EV tracking in FT240 cells using different labeling approaches. Shown are images for AF647-PG dual labeled EVs, AF647 EVs, and AF647 dye controls. 2 µg of sEVs were treated to cells for 24 h. The scale bar represents 100 µm. Imaged were obtained using confocal microscopy (20x, maximum intensity projection).

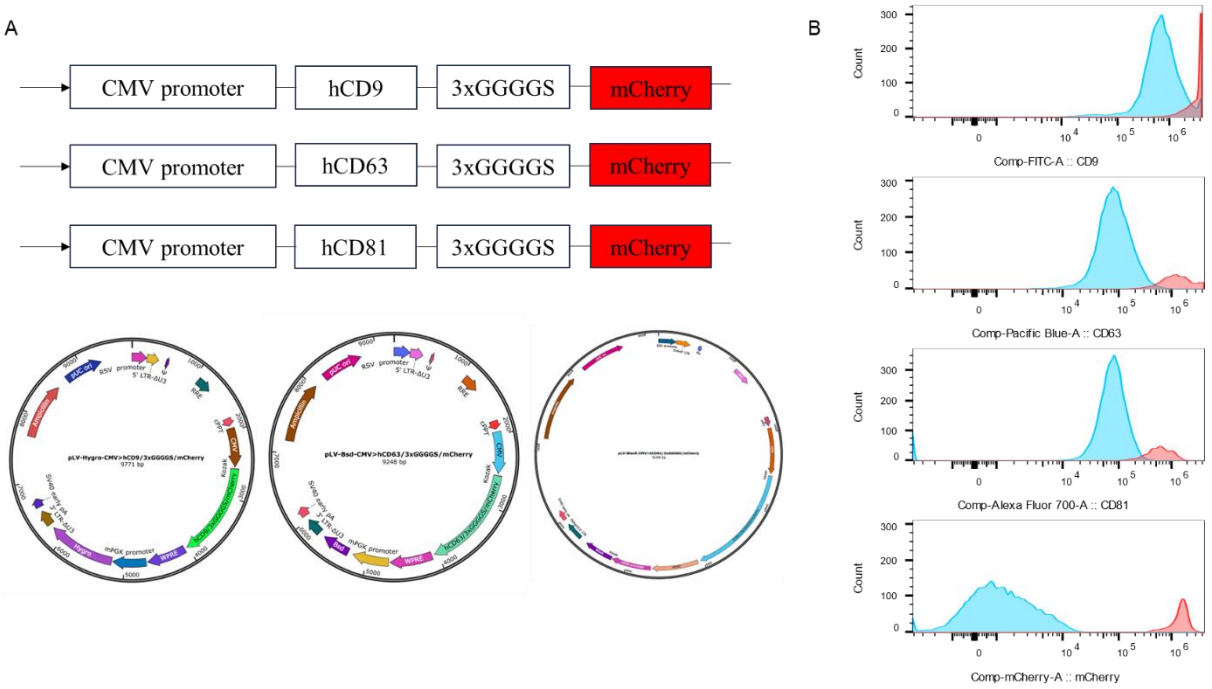

Figure S7. Transfer plasmid construct for mCherry expression and selection of monoclonal cell lineage of FT240 CD9/CD63/CD81 mCherry cell line. (**A**) Transfer Plasmids for mCherry-tagged tetraspanins: Three different transfer plasmids were designed and purchased from Vectorbuilder.com. The lentiviruses were designed to express codon-optimized C-terminus mCherry-tagged CD9, CD63, or CD81 driven by a CMV promoter. Selection markers were chosen because FT240 cells are already resistant to puromycin, hygromycin, and neomycin. (**B**) CD9, CD63, CD81, and mCherry expression profiles in FT240 CD9/CD63/Cd81 mCherry cells were analyzed by flow cytometry to identify a colony of cells that uniformly expressed all three markers. The monoclonal cell lineage (red on histograms) showed uniform overexpression of CD9, CD63, CD81 and mCherry relative to the parental FT240 cell line (blue on histogram).

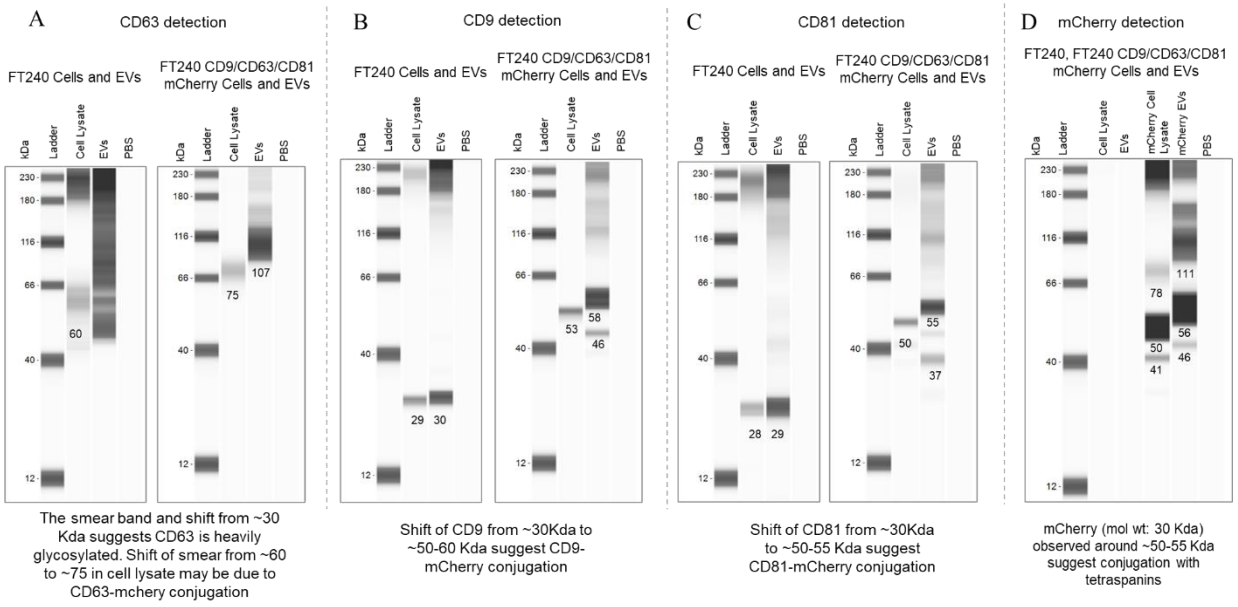

Figure S8. Capillary western blot of cell lysates and EVs showing CD9/CD63/CD81-mCherry expression. Western blot of FT240 cell lysates/EVs and FT240 CD9/CD63/CD81-mCherry cell lysates/EVs with (**A**) CD63 detection, (**B**) CD9 detection, (**C**) CD81 detection, and (**D**) mCherry detection. The shift of band in CD9 and CD81 from ~30 kDa to ~50-60 kDa in FT240 CD9/CD63/CD81-mCherry cells/EVs suggest expression of CD9-mCherry and CD81-mCherry fusion constructs. In the case of CD63, a smear band was observed around 60 kDa, possibly due to heavy glycosylation. The shift of CD63 band to 75 kDda in cell lysates and 107 kDa in EVs suggests expression of mCherry-CD63 fusion construct. The deviation of band to higher molecular weight in the case of CD63 could be due to glycosylation. Expression of mCherry-CD9/CD63/CD81 fusion construct is further supported by the detection of the major band around 50-56 kDa with anti-mCherry antibodies in both mCherry cell lysate and mCherry EVs.