

SUPPLEMENTAL FIGURES

Separation and Isolation of CD9-Positive Extracellular Vesicles from Plasma Using Flow Cytometry

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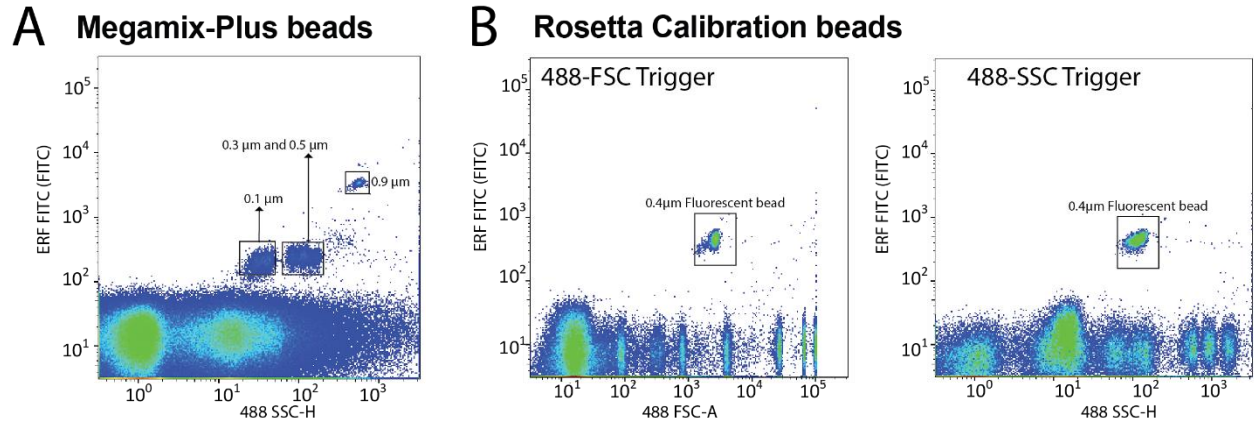


Figure S1: (A) Megamix-Plus polystyrene beads triggered using 488 SSC-H analyzed on the FITC channel. (B) Rosetta calibration beads analysed on the Astrios EQ using different triggering strategies. Left plot, resolution of beads when triggered using 488-FSC. Right plot, resolution of beads when triggered using 488-SSC.

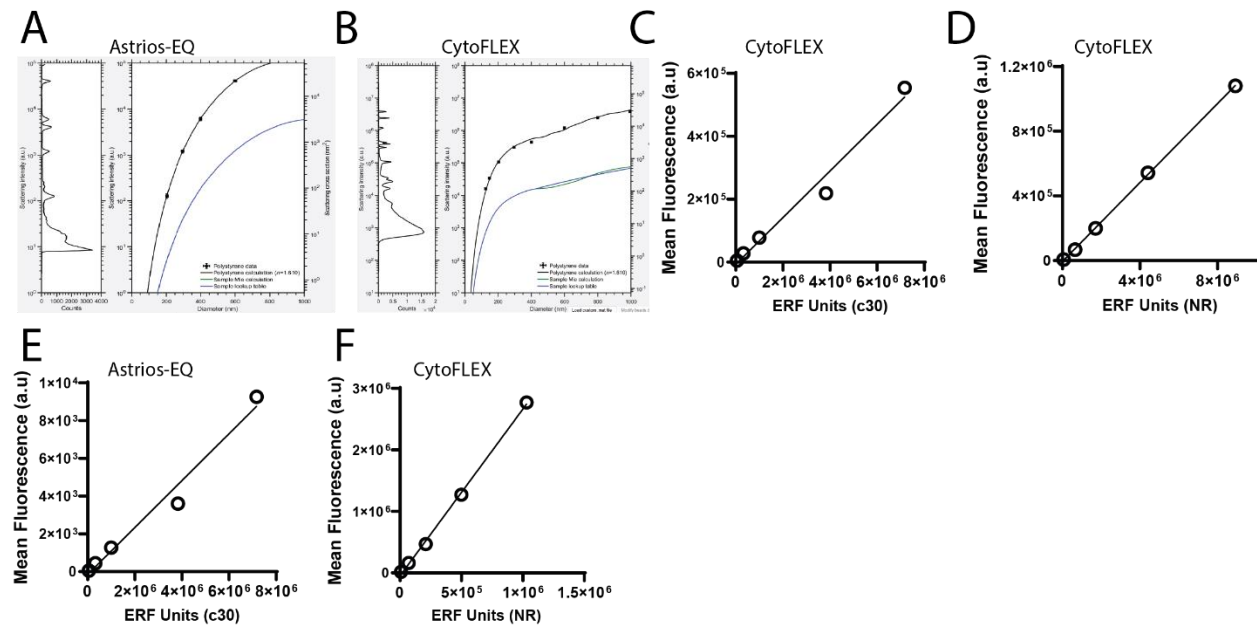


Figure S2: Calibration plots for the Astrios-EQ (A) and CytoFLEX (B) using Rosetta beads and calibration software. (C-F) Calibration plot using SPHERO™ Rainbow Calibration Particles with known Equivalent Number of Reference Fluorophores (ERF) value for each bead plotted against their fluorescence intensity (arbitrary units).

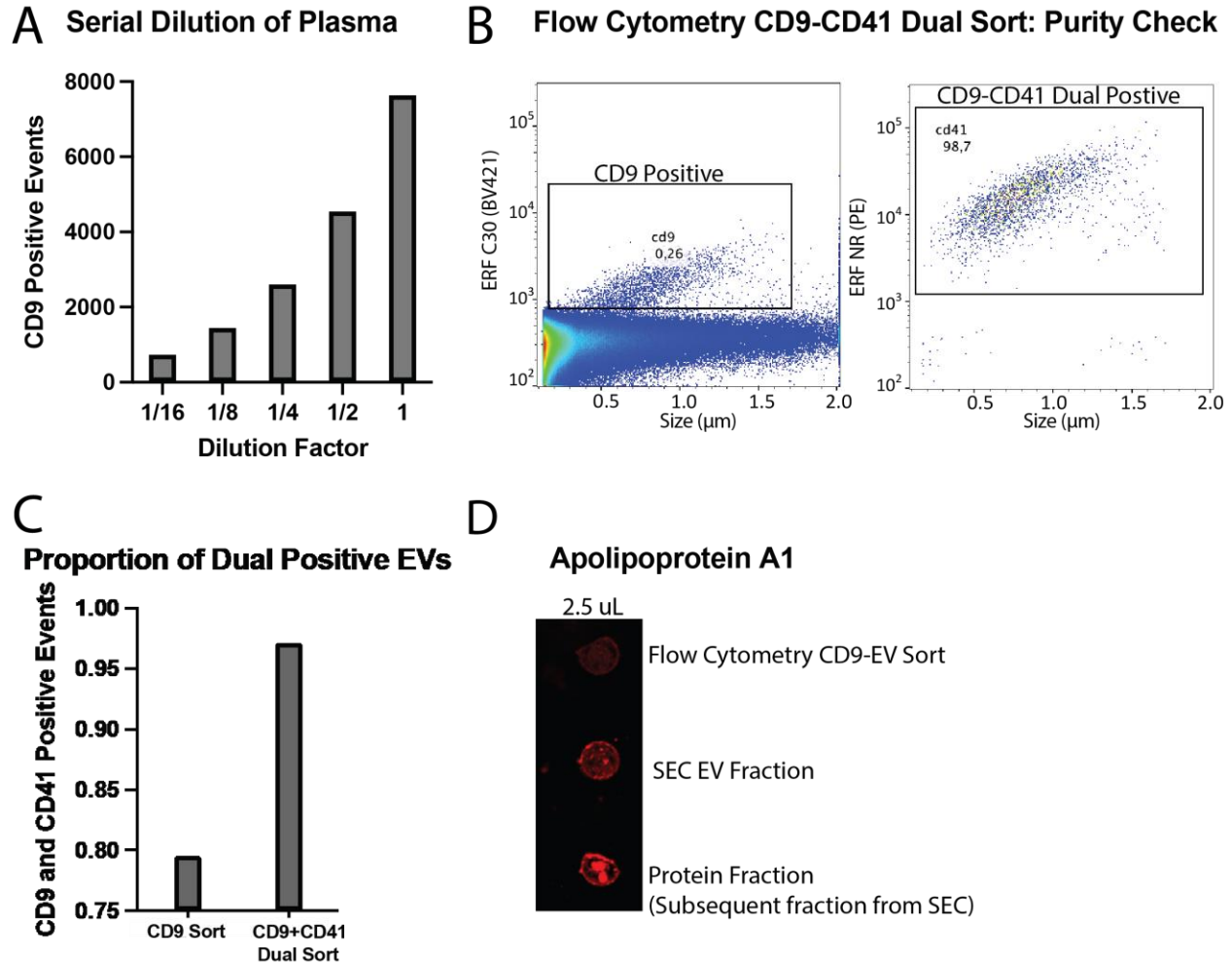


Figure S3: CD9 extracellular vesicles sorting by flow cytometry. **(A)** The presence of swarming as assessed by plasma serial dilution and quantification of events by flow cytometry. **(B)** Flow cytometry CD9-CD41 dual positive EVs run on a flow cytometry analyzer. Left plot shows CD9-positive events and right plot shows the number of those events that are dual positive by CD41 immunostaining. **(C)** The proportion of dual positive CD9-CD41 EVs from a CD9-positive sort and a CD9-CD41 dual positive sort (n=2). **(D)** The presence of a common protein contaminant, Apolipoprotein A1, probed on a dot blot (n=2) using CD9-positive flow cytometry isolated EVs (top row), SEC isolated EVs (middle row), and the SEC proteinaceous fraction, collected after the EV fraction, (bottom).