Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental Materials

S1: Secondary confirmation of mExo surface PEGylation

As a secondary confirmation of surface PEGylation, exosomes were loaded with DSPE-PEG-azide (DPA) tagged with DBCO-Cy5 dye as described in *Methods 2.8* at a 2,000:1 molar excess of DPA. Non-inserted DPA molecules were separated from PEG-Cy5-mExo by size exclusion chromatography (SEC) as described in *Methods 2.2,* which enabled qualitative confirmation of loading by measuring Cy5 fluorescence in each SEC fraction. Two separate peaks of Cy5 fluorescence were observed (**Fig. S1, A**), the earlier of which coincides with the mExo particle elution profile (see *Results 3.1*), confirming association of DPA-Cy5 molecules with the mExo surface. The later Cy5 peak in fractions 20-25 represents free DPA-Cy5 and DBCO-Cy5 molecules.

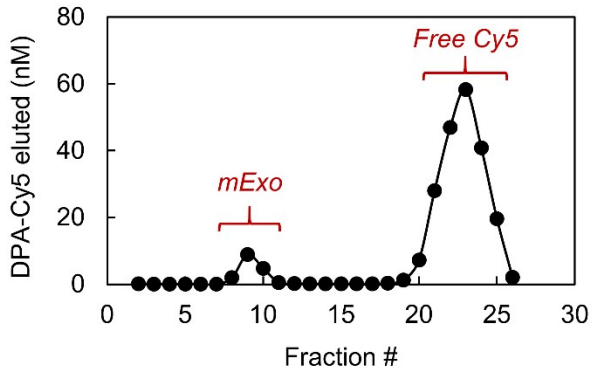

Figure S1: Separation of free DPA-Cy5 from PEGylated mExo size exclusion chromatography

S2: Fluorescently-tagged mExo standard curve and determination of optimal mExo concentration for cell uptake

See Methods section *2.12* for a description of the ExoGlow fluorescent labelling process and Methods section *2.16* for a description of cell uptake studies.

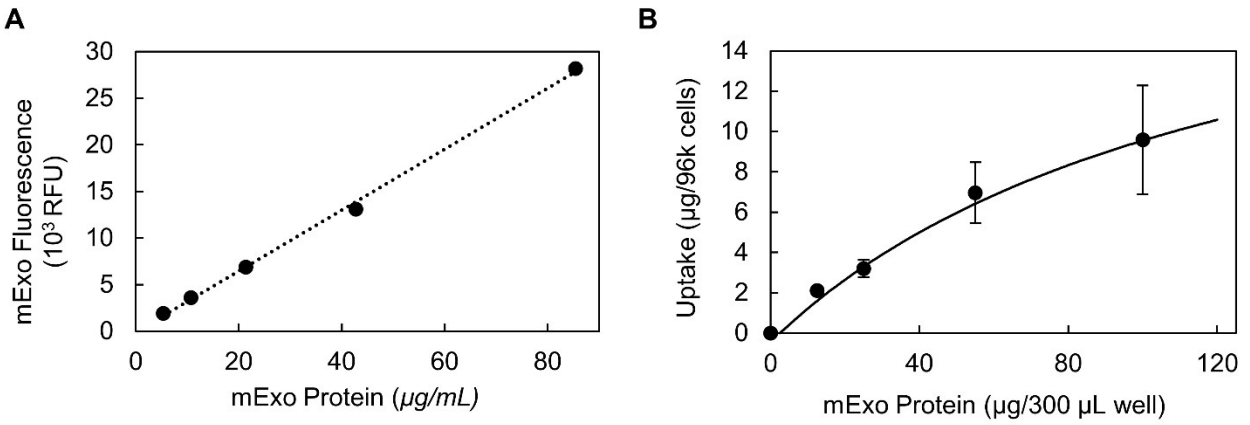

Figure S2: (A) Standard curve of green-fluorescently labelled mExo showing linear fluorescent signal above 80 µg/mL mExo protein. **(B)** Quantitative uptake of labelled mExo into Caco-2 cells, used to optimize the treatment concentration for imaging studies. Fitting to Michaelis-Menten model indicated K_m = 129.1 µg and V_{max} = 22.21 µg/96k cells.

S3: Control images to assess background signal in Caco-2 uptake of single- and dual-labelled mExo/PEG-mExo

To account for potential sources of background signal in Caco-2 uptake images with single- and dual-labelled, cells were incubated with free DBCO-Cy5, DPA-Cy5, and ExoGlow dye at both high and low concentrations, aligning with concentrations of these moieties in mExo samples pre- and post-purification. These cells were imaged as described above to evaluate the extent to which these potential sources of background signal contribute to single- and dual-labelled uptake images.

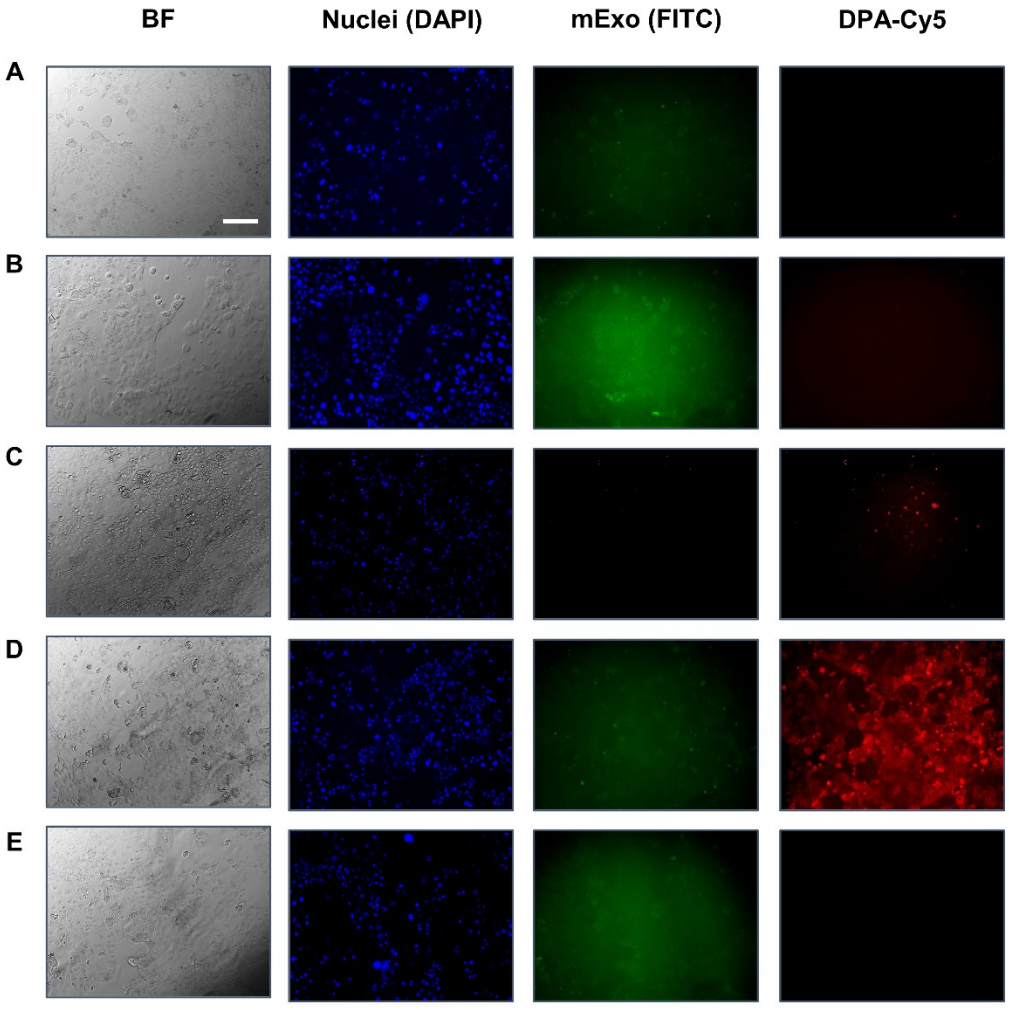

Figure S3: Control images of potential sources of background signal for PEG-mExo uptake in Caco-2 cells. (A) mExo + 0.01 mg/mL DBCO-Cy5 (starting concentration before PEG-mExo purification) **(B)**

Blank media + 1 µL ExoGlow dye (high concentration) and **(C)** residual ExoGlow dye left over after UFC purification of dye in the absence of mExo (low concentration). **(D)** Blank media + 2.5 µM DPA-Cy5 (high concentration) and **(E)** residual DPA-Cy5 left over after UFC purification of dye in the absence of mExo (low concentration). White scale bar represents 200 µm.

S4: siRNA loading into mExo by electroporation

Exosomes were loaded via electroporation using the Invitrogen Neon transfection system (Thermo Fisher). First, mExo were mixed with Cy3-siRNA at a range of molar ratios (1:100 – 1:900 siRNA:exo) and diluted 10x in Resuspension Buffer T. Samples were then electroporated, 100 µL at a time, using a range of voltages (500, 750, 1000 V) and pulse counts (1, 5, 10 pulses) and a 20 ms pulse width. As a control, samples were electroporated with 0 V. Following electroporation, samples were incubated on ice for 30 min to allow for membrane recovery. Loaded mExo were then separated from unloaded siRNA by SEC using the Izon qEV column, and loading was quantified by measuring Cy3 fluorescence in SEC fractions.

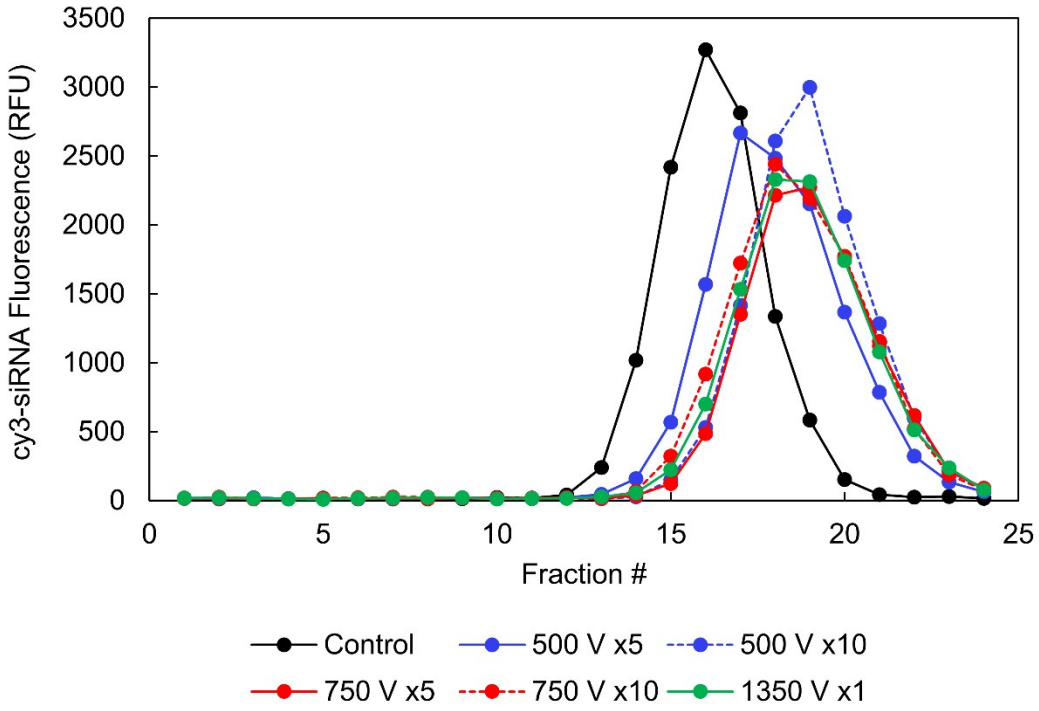

Figure S4: Intra-luminal siRNA loading using electroporation. The Invitrogen Neon (Thermo Fischer) transfection system was used to load milk exosomes with fluorescent siRNA for loading quantification using SEC. An absence of Cy3-siRNA signal in mExo elution fractions 7-11 suggests inefficient intra-luminal siRNA loading.

S5: Flow cytometry quantification of mExo-mediated GFP silencing in HEK293 cells

See Methods section *2.19* for a description of flow cytometry measurements.

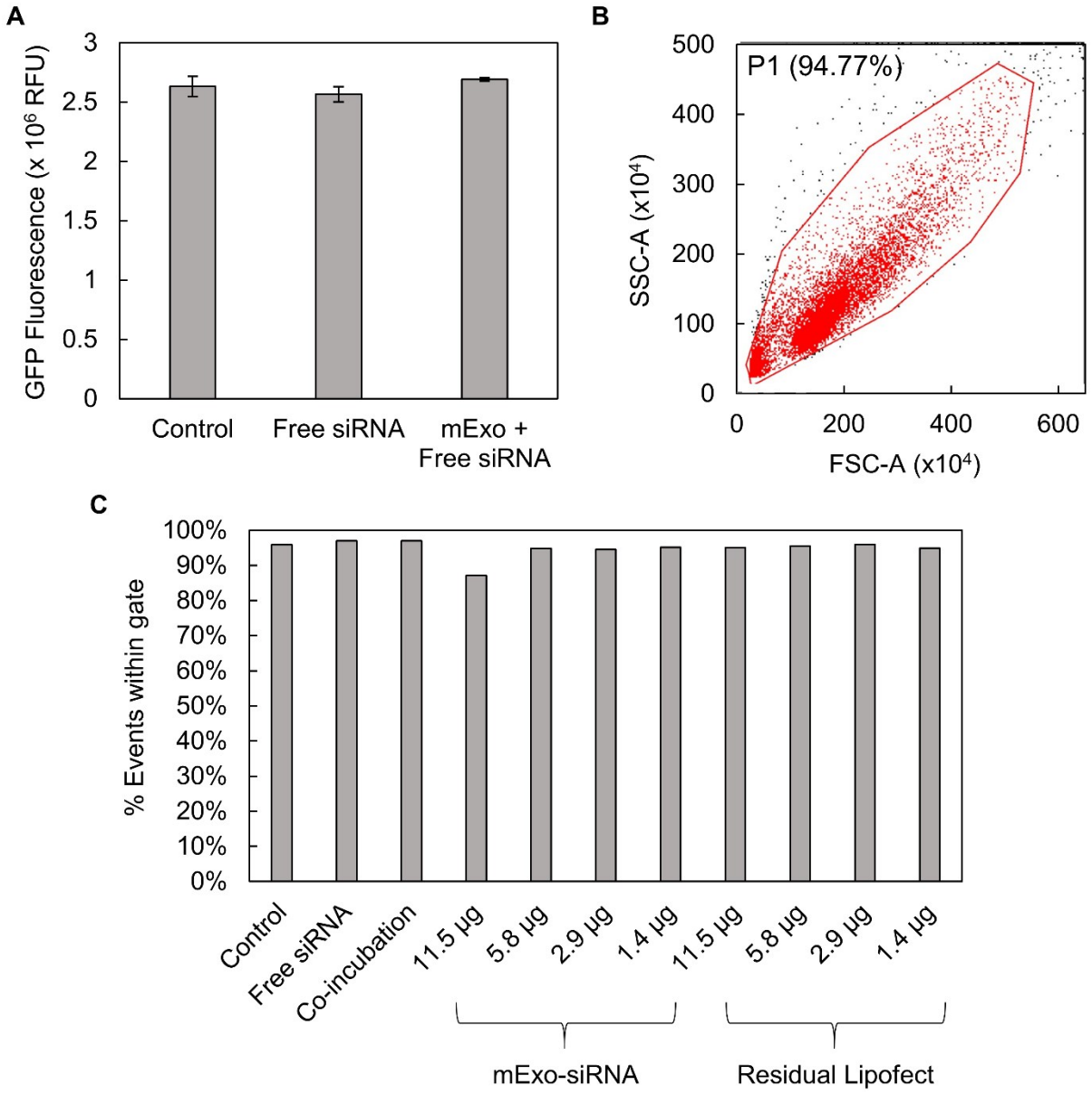

Figure S5: Analysis of mExo-mediated GFP silencing in HEK293 cells using flow cytometry. (A) Relative FITC signal in cells treated with un-loaded GFP-targeting siRNA (negative controls), compared to untreated control cells. **(B)** Custom SSC/FSC gate used in flow cytometry analysis, prepared using untreated control cells. **(C)** % of cells detected within the pre-set gate in every treatment condition in GFP silencing studies.

S6: Uptake of PEG-mExo-siRNA in HEK293 cells

PEGylated siRNA was formulated by first preparing mExo-siRNA via Lipofectamine fusion as described in *Methods 2.18,* followed by purification with ExoQuick-TC. To confirm that PEGylated mExo-siRNA gets taken up by HEK293 cells, cells were incubated with 140 µg of PEG-mExo-siRNA loaded with DPA-Cy5 to allow for tracking of uptake via flow cytometry, using the APC filter to quantify intracellular red fluorescence (see *Methods 2.19* for a description of the flow cytometry process). Uptake was evaluated after 2 days of incubation, an early time point before protein-level silencing of the target gene (GFP) would be expected to be observed. These results indicate that substantial uptake of PEG-mExo-siRNA was observed in HEK293 cells via flow cytometry and fluorescence microscopy, suggesting that the disparity in gene silencing efficacy between mExo-siRNA and PEG-mExo-siRNA (**Fig. 8, B**) may not attributable to cell uptake being compromised by the PEG coating.

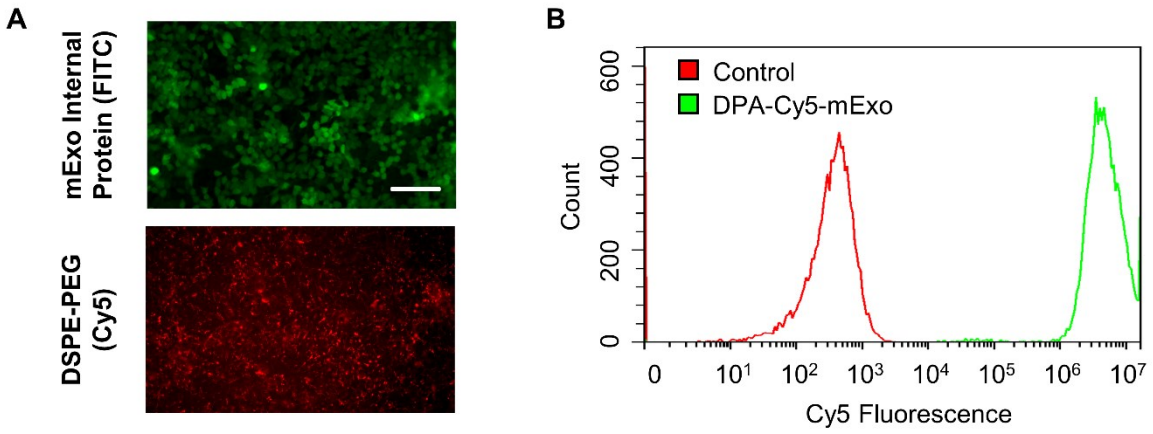

Figure S6: (A) Fluorescence microscopy images depicting HEK293 internal proteins in green and PEG-Cy5-mExo-siRNA in red. **(B)** Distributions of intracellular Cy5 fluorescence between control and PEG-Cy5-mExo-siRNA treated HEK293 cells measured by flow cytometry. White scale bar represents 100 µm.