SUPPLEMENTARY MATERIAL

A BI-FUNCTIONAL POLYMERIC COATING FOR THE CO-IMMOBILIZATION OF PROTEINS AND PEPTIDES ON MICROARRAY SUBSTRATES

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Western Blot Analysis

Protein content was determined using protein assay kit (BioRad, CA, USA). Bovine serum albumin (BSA) was used as standard. For tetraspanin detection, sample was lysed in non reducing sample buffer (62.5 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, and 0.04% bromophenol blue) and boiled for 5 min at 95°C. Then, 15 µg of proteins were loaded on 12% SDS-PAGE gel. For the detection of other proteins, sample was lysed in reducing sample buffer (62.5 mM Tris–HCl pH 6.8, 10% glycerol, 2% SDS, 1.25% 2-mercaptoethanol and 0.01% bromophenol blue) and boiled for 5 min at 95°C. Then, 24 µg of proteins were loaded on 12% SDS-PAGE gel. After protein separation, gels were electro-transferred onto a nitrocellulose membrane. Nonspecific binding sites were blocked with 5% (w/v) skimmed milk in T-TBS (150mM NaCl, 20mM Tris-HCl pH 7.4, and 0.5% Tween 20). Membranes were incubated overnight at 4°C with the following antibodies: anti-CD63 (1:1000, #556019, BD Pharmingen, CA, USA), anti-CD81 (1:5000, #555675; BD Pharmingen), anti-Alix (1:500, #sc-271975, Santa Cruz, CA, USA) and anti-Calnexin (1:1000, #C7617, Sigma-Aldrich, MO, USA). After several washes in T-TBS, membranes were incubated with goat anti-mouse IgG conjugated to horse-radish peroxidase (1:5000, #170-6516, BioRad Laboratories Inc., CA, USA) for 45 min. Positive immunoreactive bands were detected by the enhanced chemiluminescence method (Immobilon^{TM} HRP substrate, #WBKLS0500, Millipore Corp., MA, USA).

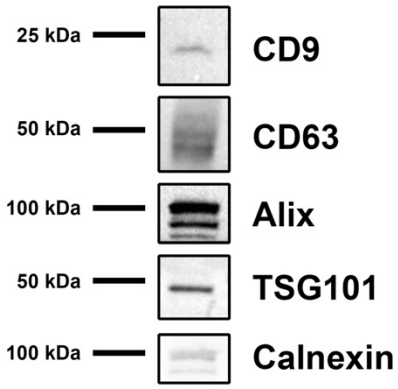

Figure S 1 Western Blot analysis of EVs obtained from HEK-293 cell culture supernatants by ultracentrifugation. EVs related markers, both intraluminal (Alix and TSG101) and trans membrane (CD9 and CD63) were detected. Calnexin was analyzed as negative control. Results show high levels of EV proteins with low degree of contaminants.

TEM Microscopy

Transmission electron microscopy (TEM) was performed on isolated EVs, resuspended in PBS, to analyze their ultrastructural morphology. According to proper dilution, the sample was adsorbed to 300 mesh carbon-coated copper grids (Electron Microscopy Sciences, Hatfield, PA, USA) for 5 min in a humidified chamber at room temperature. EVs on grids were then fixed in 2% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) in PBS for 10 min and then briefly rinsed in milli-Q water. Grids with adhered sEVs were examined with a Philips CM 100 transmission electron microscope TEM at 80kV, after negative staining with 2% phosphotungstic acid, brought to pH 7.0 with NaOH. The images were captured by a Kodak digital camera.

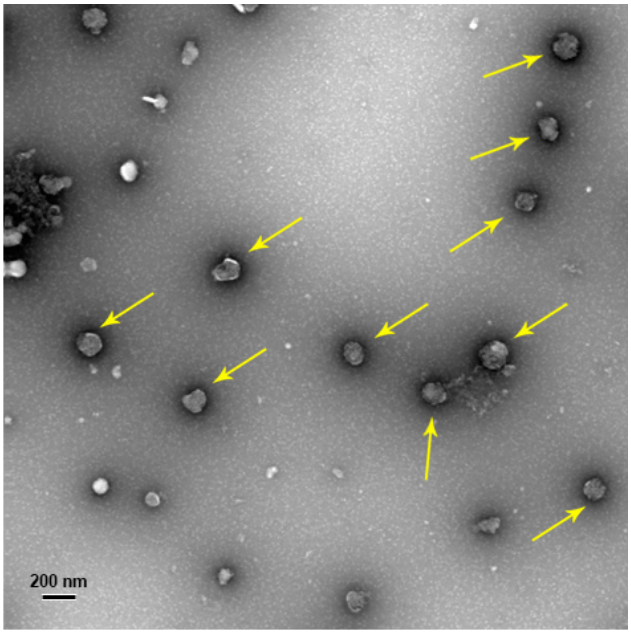

Figure S 2 TEM microscopy of EVs obtained from HEK-293 cell culture supernatants by ultracentrifugation. Scale bar indicates 200 nm. Yellow arrows highlight small extracellular vesicles.