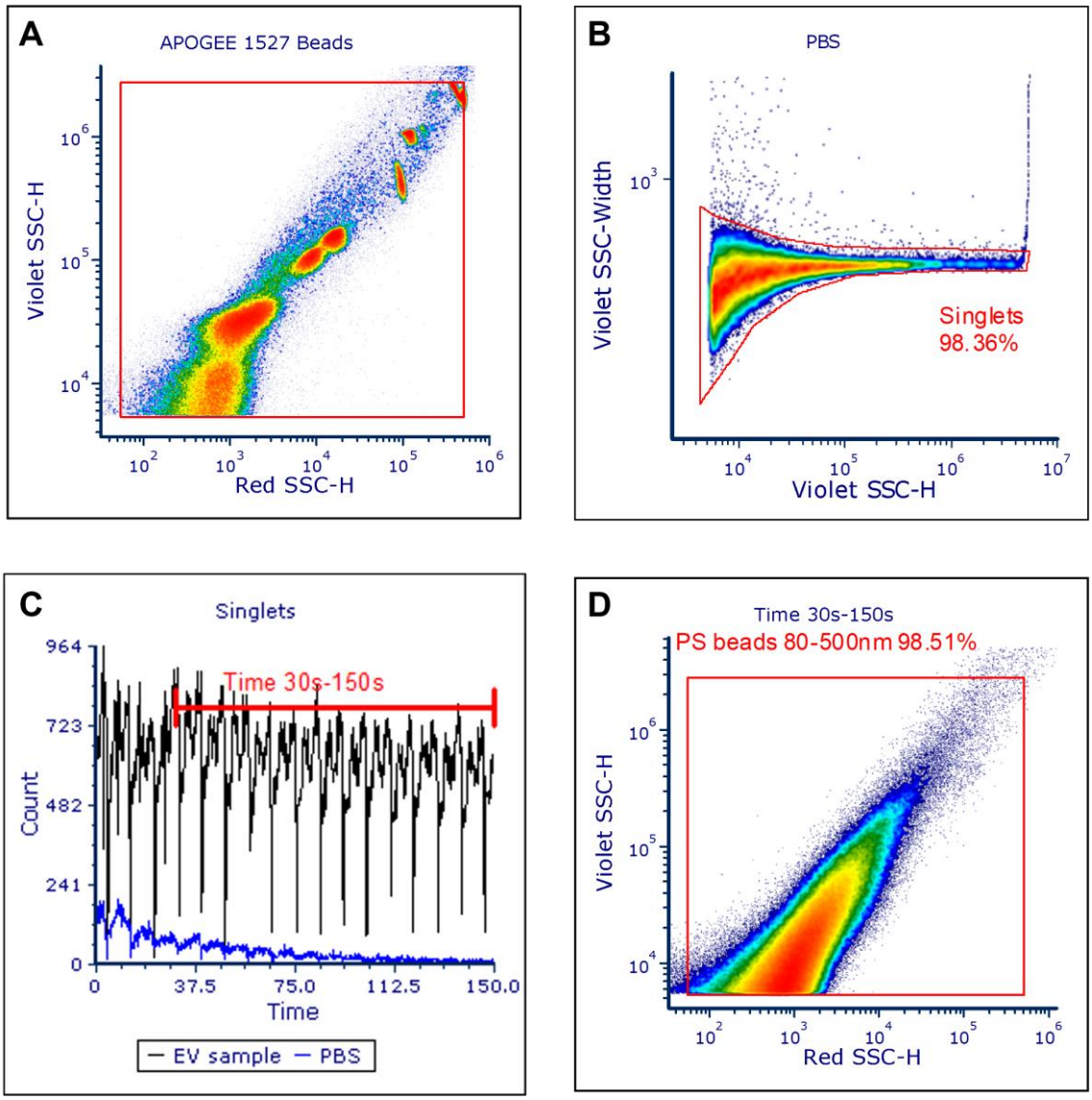

Figure S1. *Gating Strategy for extracellular vesicle(EVs) identification and counting.*

A) Cytoflex flow cytometer setup based on violet SSC using APOGEE 1527 reference beads and a PS 80-500nm region was defined on a violet SSC-H/ red SSC-H dot-plot (.fcs files were transformed by the FCS express 6 system). **B)** Filtered PBS was used to setup a Singlet region was defined on a violet SSC-width/ violet SSC-H dot-plot and plotted on a time histogram. **C)** A 30s and 150s time gate was plotted on the time histogram to record 2mins of stable time without sample carryover. **D)** The 30s and 150s time events were then plotted on a violet SSC-H/ red SSC-H dot-plot and events within the PS 80-500nm region were used for analysis.

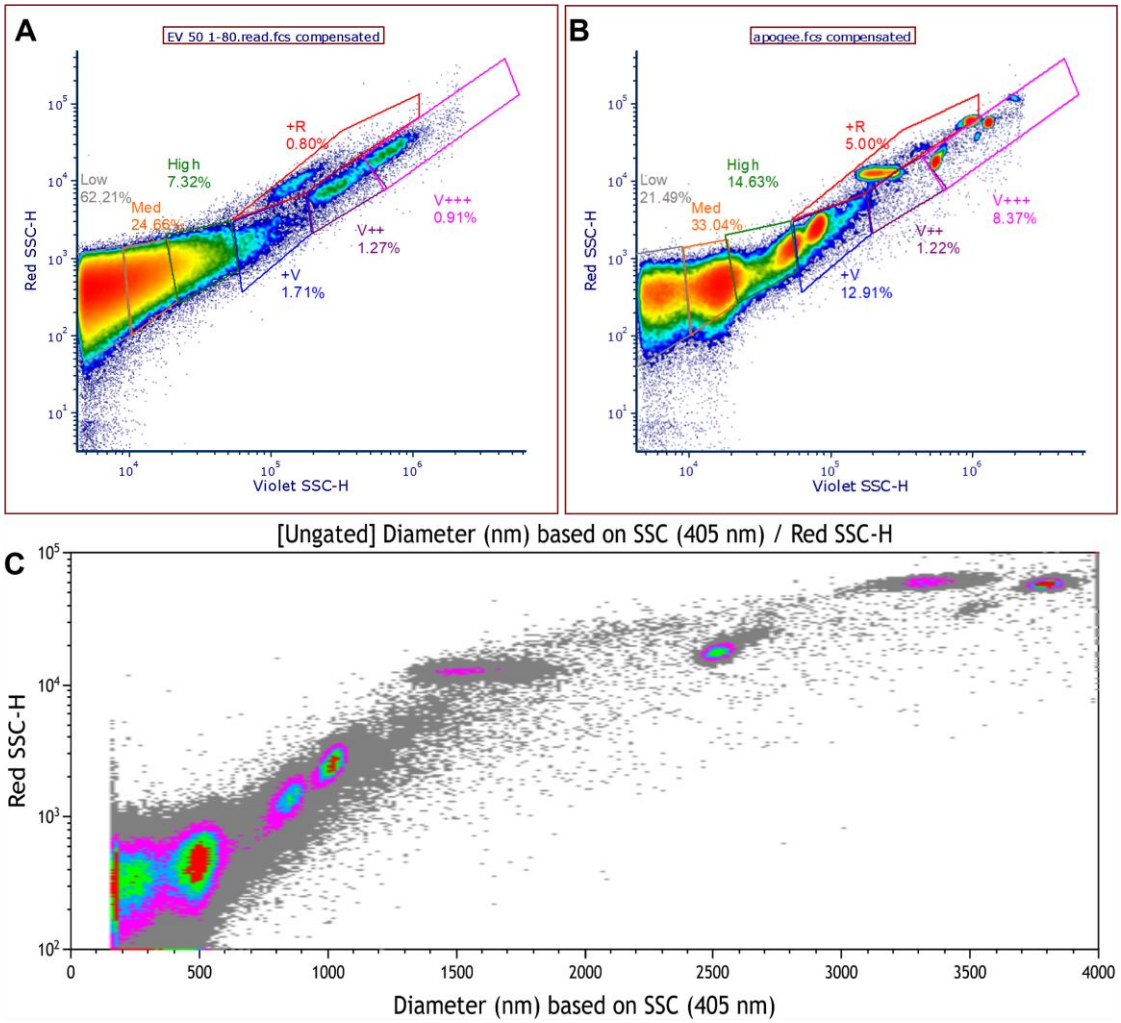

Figure S2. *Gating Strategy for extracellular vesicle (EVs) subpopulation analysis.*

A) A violet SSC-H/ red SSC-H dot-plot with a representative AT-MSC-derived EV sample was gated into low, med, high, +V, V++, V+++, and +R regions, which were created based differences between biological samples and their replicates. (Data was reanalysed using the Beckman Coulter CytExpert v. 2.5.0.77 software and the DeNovo Software FCSExpress Plus v7.). **B)** A violet SSC-H/ red SSC-H dot-plot showing the position of the different APOGEE 1527 reference beads within the established gates. **C)** Conversion of the APOGEE 1527 reference beads violet SCC (405nm) to equivalent EV diameter using Rosetta calibration.