Label-free identification and chemical characterization of single EVs and lipoproteins by synchronous Rayleigh and Raman scattering

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Nanoparticle tracking analysis characterization

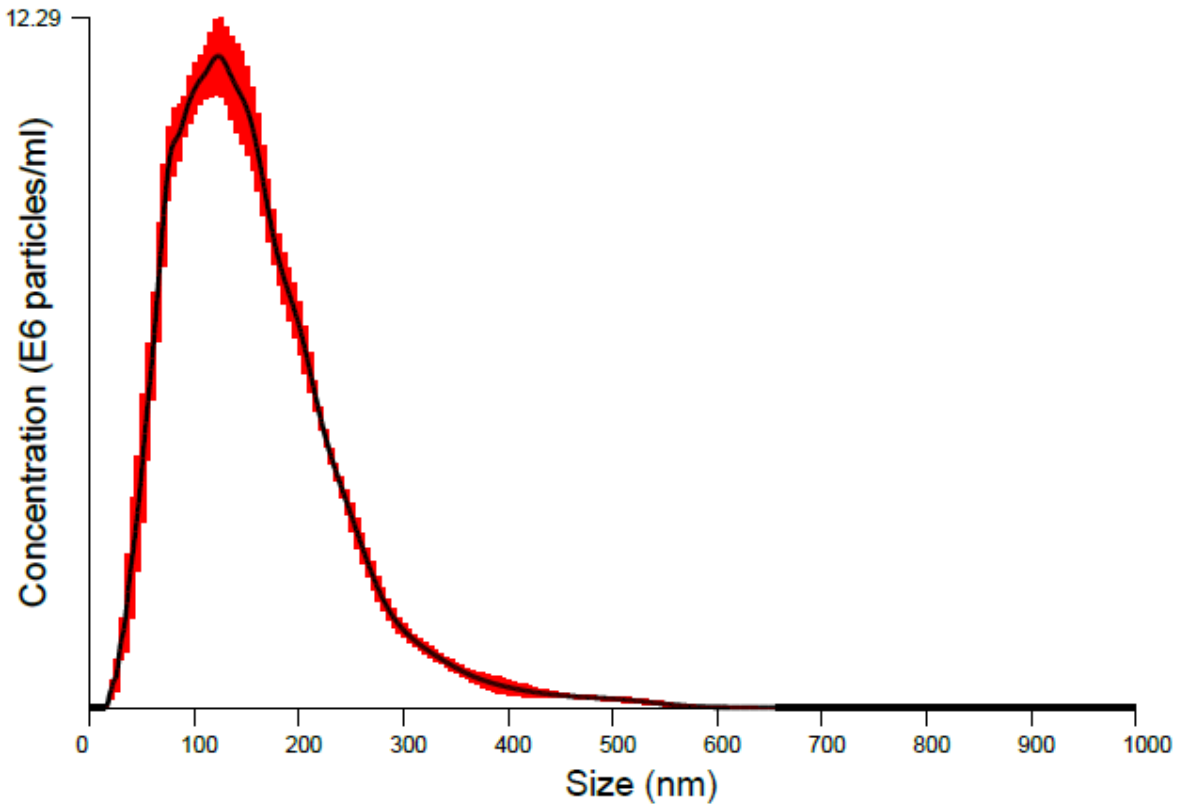

Figure S1. PC-3 EV diameter distribution was determined using nanoparticle tracking analysis (NanoSight NS500). An EV concentration of 1.9×10^9 particles/mL with a mean diameter (± standard deviation) of 157 (±79) nm was found. Black line indicates the average size distribution and red error bars indicate ±1 standard error of the mean. Measurements were done with a sCMOS camera, shutter length of 8.9 ms, gain of 250 and detection threshold of 6. Five videos of 60 s were captured at 22.0 °C and analyzed by NTA v2.3 (NanoSight), assuming a medium viscosity of 0.95 cP.

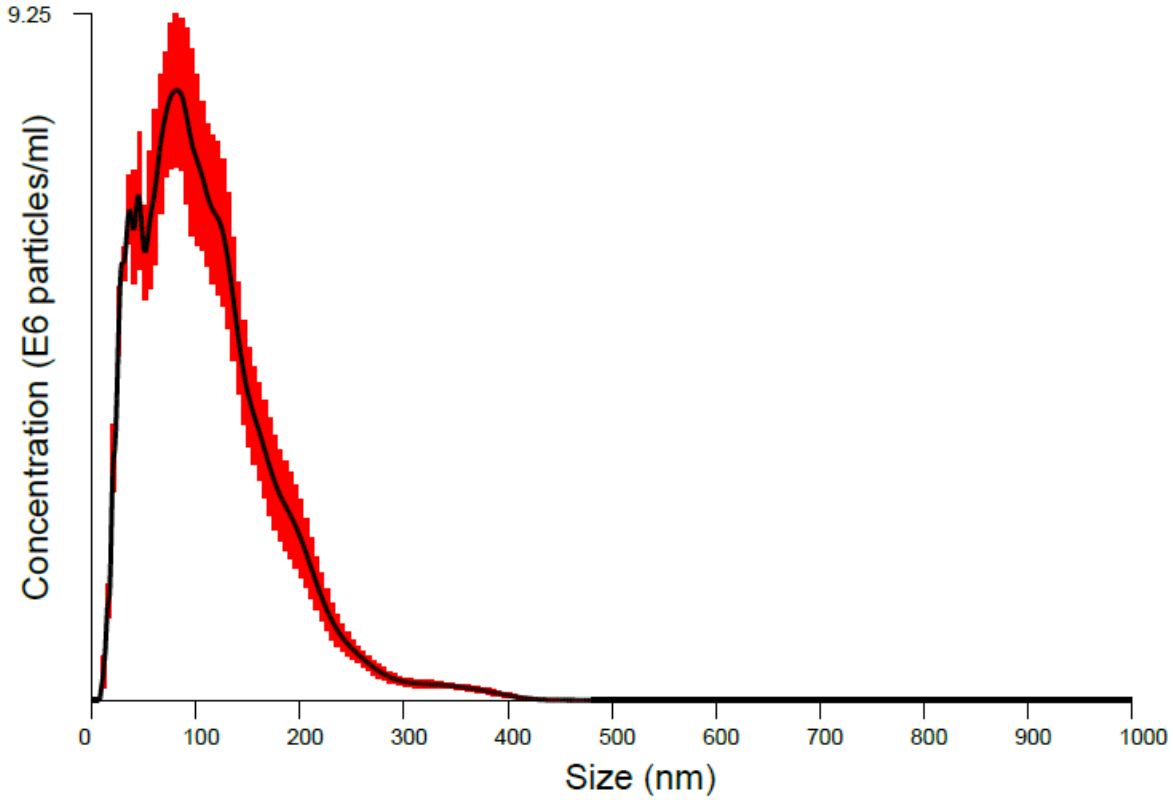

Figure S2. LNCaP EV diameter distribution was determined using nanoparticle tracking analysis (NanoSight NS500). An EV concentration of $1.1x10^9$ particles/mL with a mean diameter (± standard deviation) of 109 (±64) nm was found. Black line indicates the average size distribution and red error bars indicate ±1 standard error of the mean. Measurements were done with a sCMOS camera, shutter length of 5 ms, gain of 250 and detection threshold of 7. Five videos of 60 s were captured at 22.0 °C and analyzed by NTA v2.3 (NanoSight), assuming a medium viscosity of 0.95 cP.

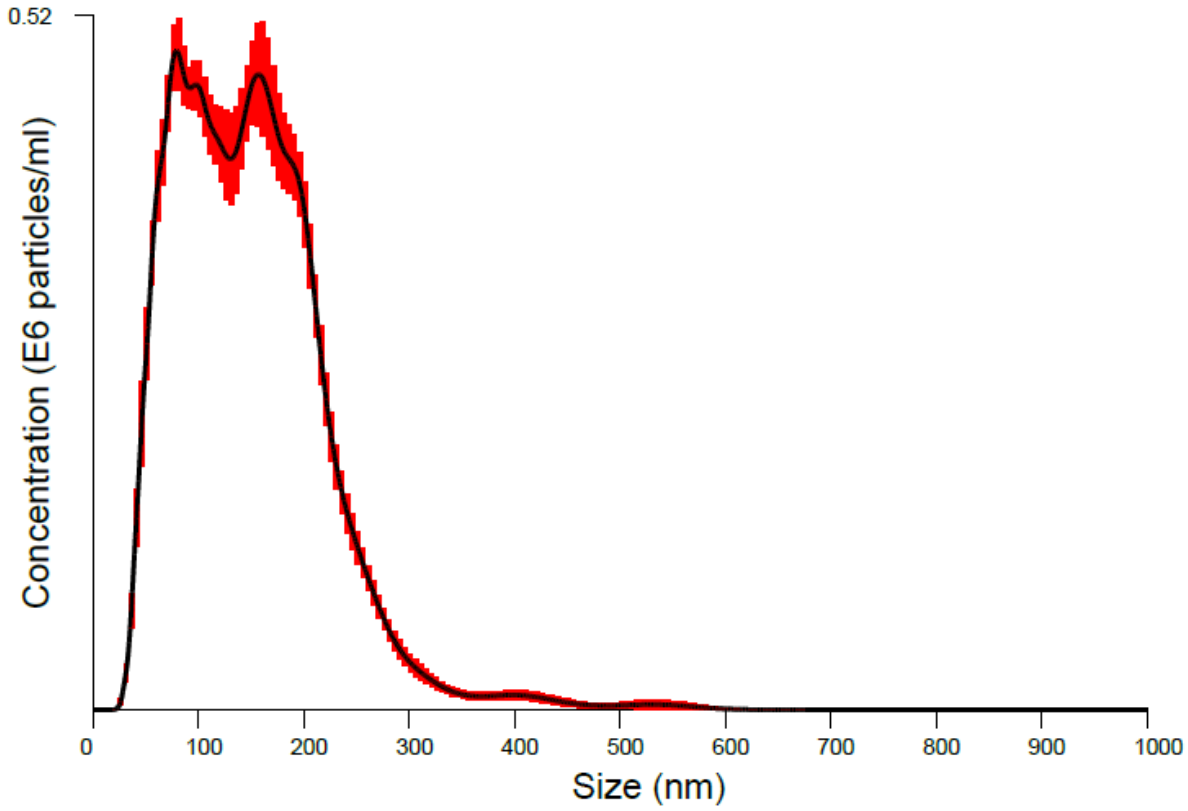

Figure S3. RBC EV diameter distribution was determined using nanoparticle tracking analysis (NTA NS500; Nanosight, Amesbury, UK). An EV concentration of 4.25×10^{10} particles/mL with a mean diameter (± standard deviation) of 148 (±70) nm was found. Black line indicates the average size distribution and red error bars indicate ±1 standard error of the mean. Measurements were done with an electron multiplying charge-coupled device (EMCCD) camera, shutter length of 33 ms, gain of 400 and detection threshold of 10. Ten videos of 30 s were captured at 22.0 °C and analyzed by NTA v2.3 (NanoSight), assuming a medium viscosity of 0.95 cP.

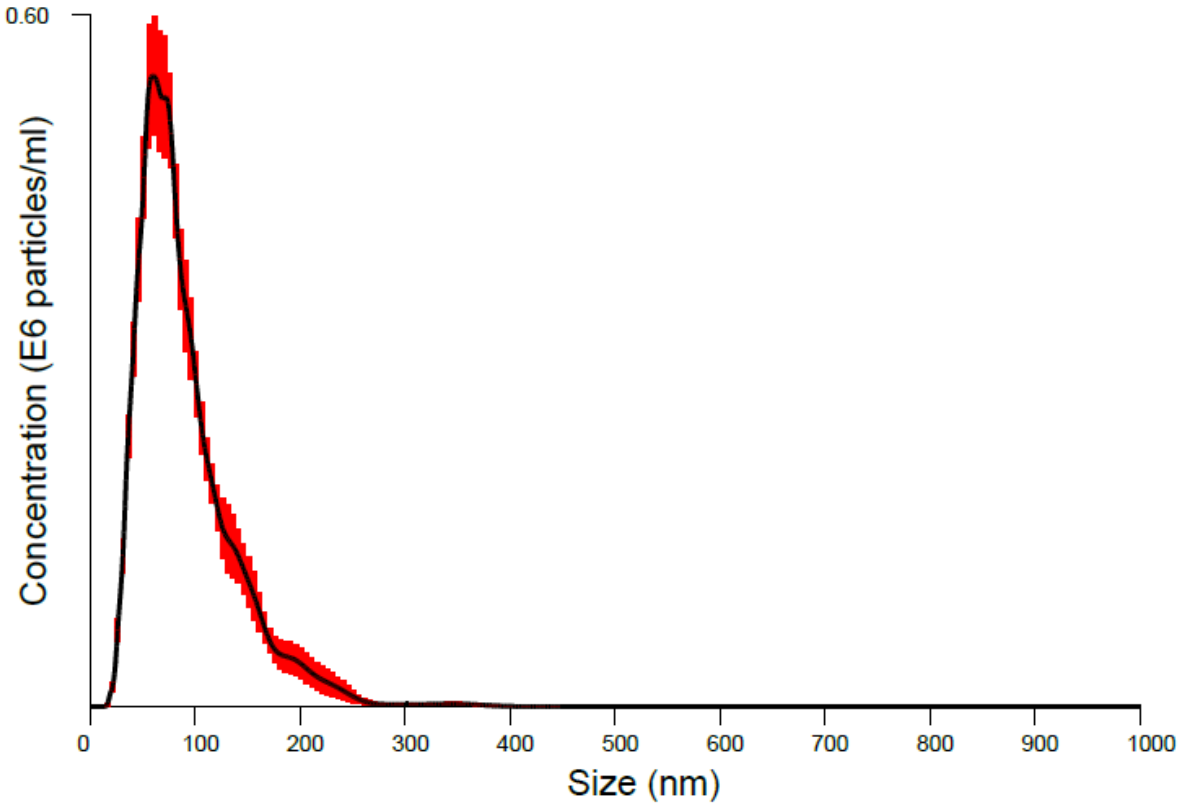

Figure S4. "Platelet particles" diameter distribution was determined using nanoparticle tracking analysis (NTA NS500; Nanosight, Amesbury, UK). An EV concentration of $8.4x10^{10}$ particles/mL with a mean diameter (± standard deviation) of 89 (±43) nm was found. Black line indicates the average size distribution and red error bars indicate ±1 standard error of the mean. Measurements were done with an electron multiplying charge-coupled device (EMCCD) camera, shutter length of 33 ms, gain of 400 and detection threshold of 10. Ten videos of 30 s were captured at 22.0 °C and analyzed by NTA v2.3 (NanoSight), assuming a medium viscosity of 0.95 cP.

Flow cytometry

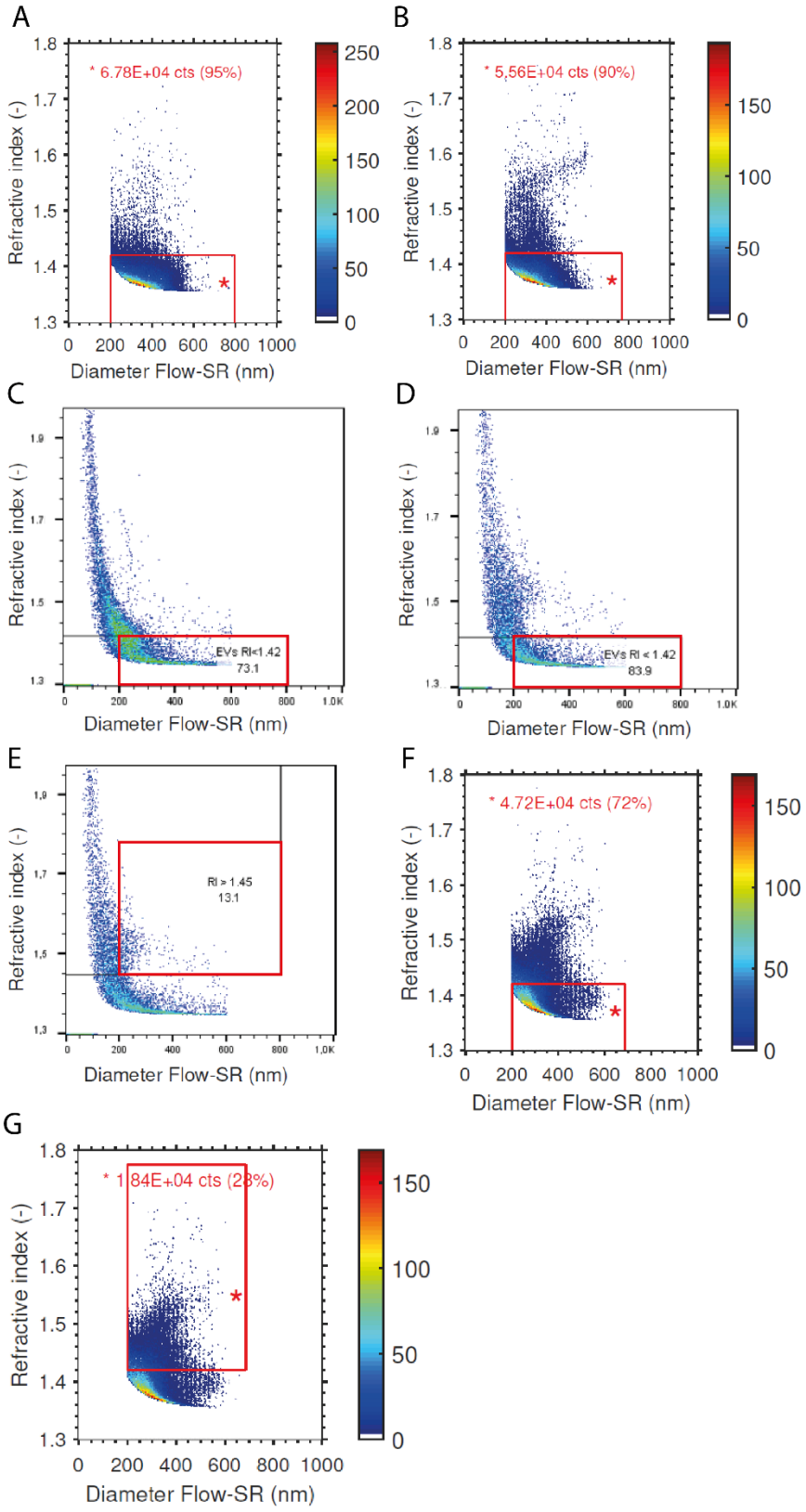

Figure S5. Flow cytometry scatter ratio (Flow-SR). (**A**) PC-3 EVs (RI<1.42). (**B**) LNCaP EVs (RI<1.42). (**C**) RBC EVs (RI<1.42). (**D**) "Platelet particles" (RI<1.42). (**E**) "Platelet particles" (RI>1.45). (**F**) "Plasma particles" (RI<1.42). (**G**) "Plasma particles" (RI>1.45)

Rayleigh-Raman spectrometer

This setup is based on a home-built confocal Raman microscope. Laser light is generated by a Coherent Innova 70C laser (λ_{exc}=647.089) and expanded by a beam expander. The laser light is reflected by a dichroic beam splitter, again expanded (2x) by the 4F-system used for beam steering. The expanded laser beam passes through the microscope objective (Olympus, 40x, NA: 0.95) and illuminates the sample. The Rayleigh and Raman scattered light are epi-detected and propagate together until the dichroic beam splitter is reached. This component reduces the Rayleigh scattering, which is further reduced after passing through an edge filter. The light is guided to the entrance pinhole of the spectrograph by mirrors and an image of the focal plane underneath the objective is made on the pinhole plane by lens. The spatially filtered light is dispersed in a home-built spectrograph such that the wavelength region of 646-849 nm (-30 cm^{-1} to 3670 cm^{-1}) illuminates the CCD camera (Andor Newton DU970N-BV, 1600 × 200 pixels)

Rayleigh time traces

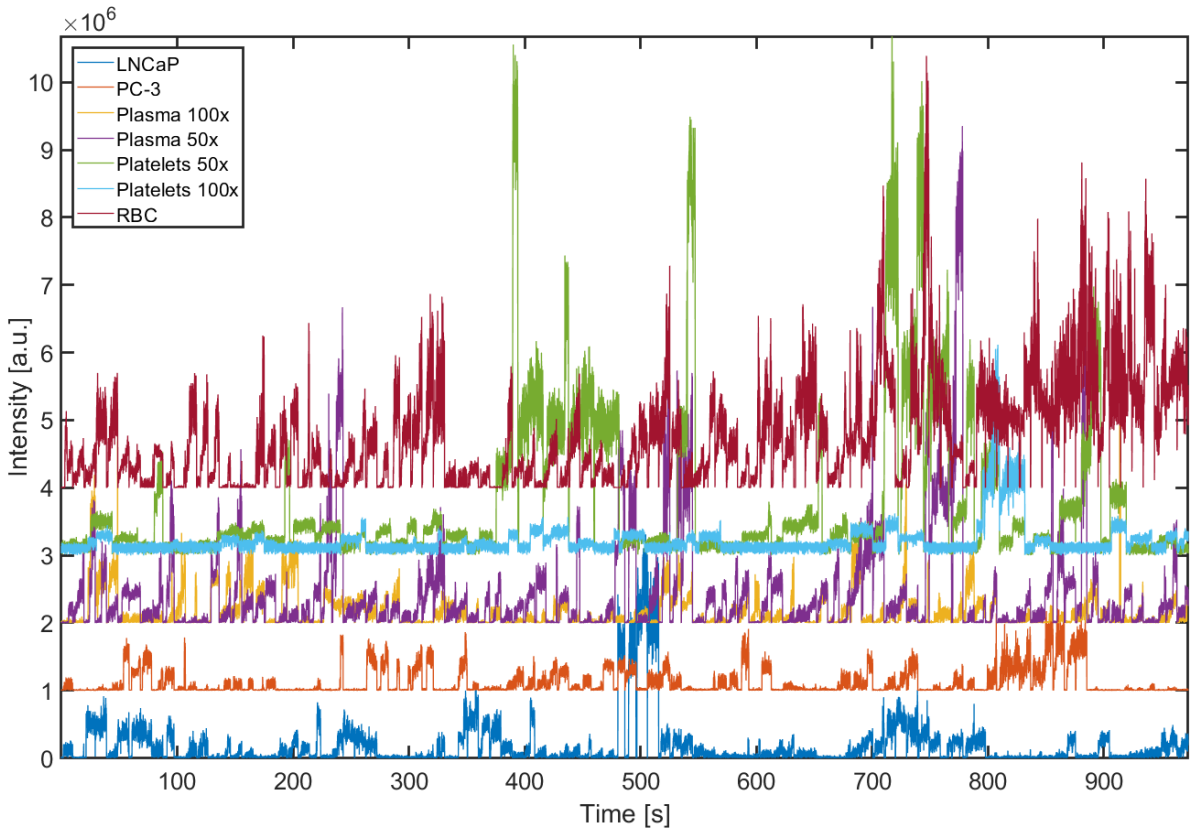

Figure S6. Rayleigh scattering signals of LNCaP sample, PC-3 sample, plasma sample (50x and 100x), platelet sample (50x and 100x) and RBC sample, resulting from peak integration of the Rayleigh peak located between -33 and 58 cm^{-1}.

Rayleigh-Raman scattering of phosphate-buffered saline

50 µL of phosphate-buffered saline 1x (PBS) was placed on a well glass slide (BMS Microscopes; 1.0-1.2 mm thick, Cat. no.: 12290). The sample was covered with a glass cover slip (VWR Ltd, thickness No. 1, diameter: 22mm, Cat. no.: 631-0158) and sealed with glued (EVO-STIK, Impact) onto the well glass slide. The glue was then cured at room temperature for ~30 minutes. The closed well glass slide was placed under the microscope objective of the Rayleigh-Raman spectrometer. The laser focal spot was focused inside the solution, ~37 µm below the cover slip. Rayleigh and Raman scattering spectra were typically acquired 256 times with an acquisition time of 38 ms over a period of ~9.7 seconds. The laser with a power of 70 mW, was then blocked for ~1 second. This measurement cycle was repeated 100 times, which resulted in 25600 Rayleigh-Raman spectra per measurement. Figure S9 shows the time Rayleigh scattering time trace with no visible trapping events.

The mean Rayleigh intensity of PBS is 6.1202x10^3 counts / 38 ms with a standard deviation (s) of 7.1397x10^3. The coefficient of variation (c_v) is 1.17 and is defined as:

$$c_v = \frac{s}{\bar{x}}$$

being s the sample standard deviation and $\bar{x}$ the sample mean.

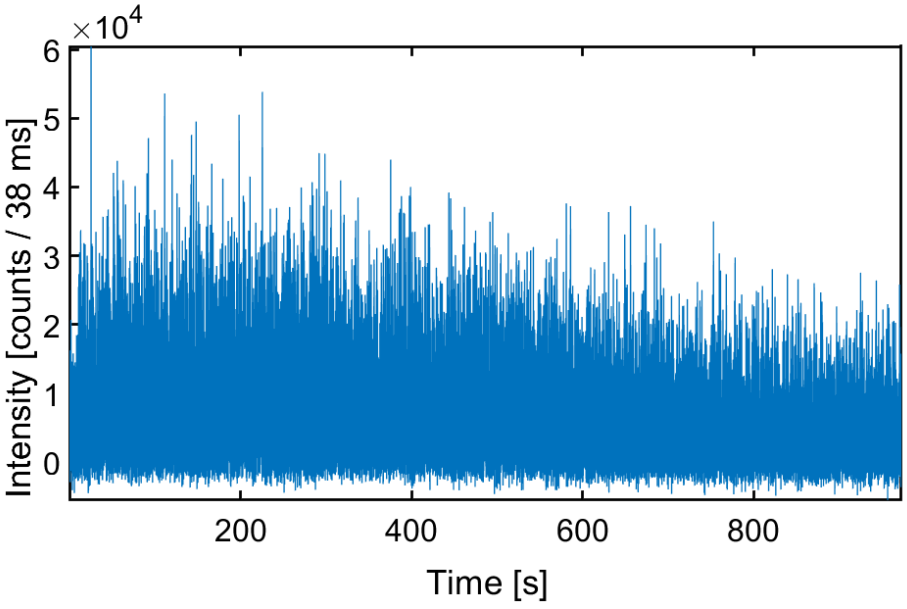

Figure S7. Rayleigh scattering time trace of PBS with a mean intensity of 6.120x10^3 counts / 38 ms and an SD of 7.139x10^3.

The mean Raman spectrum of PBS over the entire time trace is shown in Figure S8.

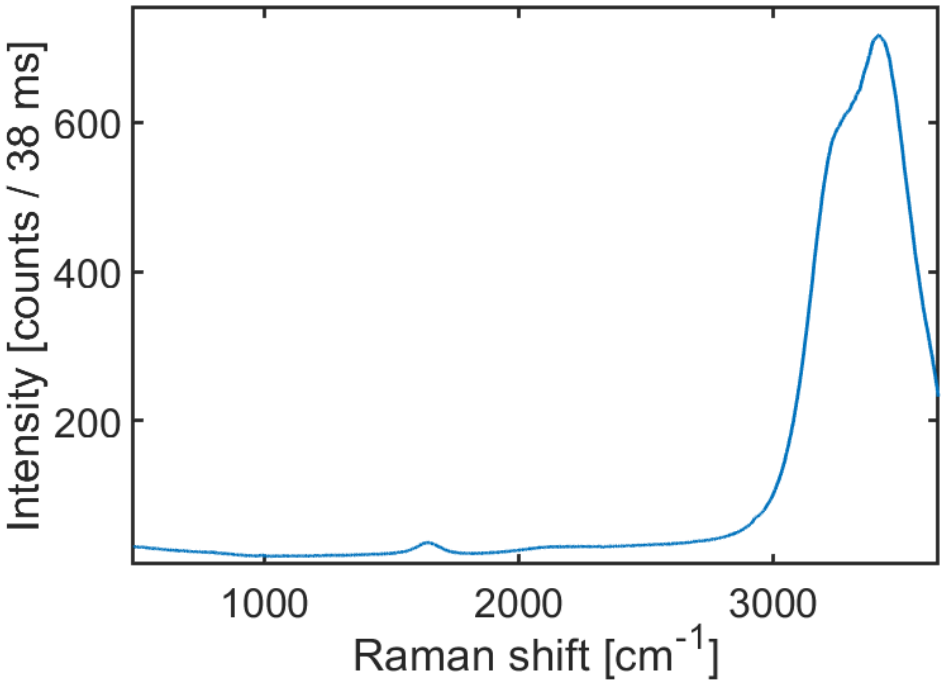

Figure S8. Mean Raman spectrum of PBS from 25600 Raman spectra with an integration time of 38 ms and a laser power of 70 mW.

The mean Raman spectrum and standard deviation of particles

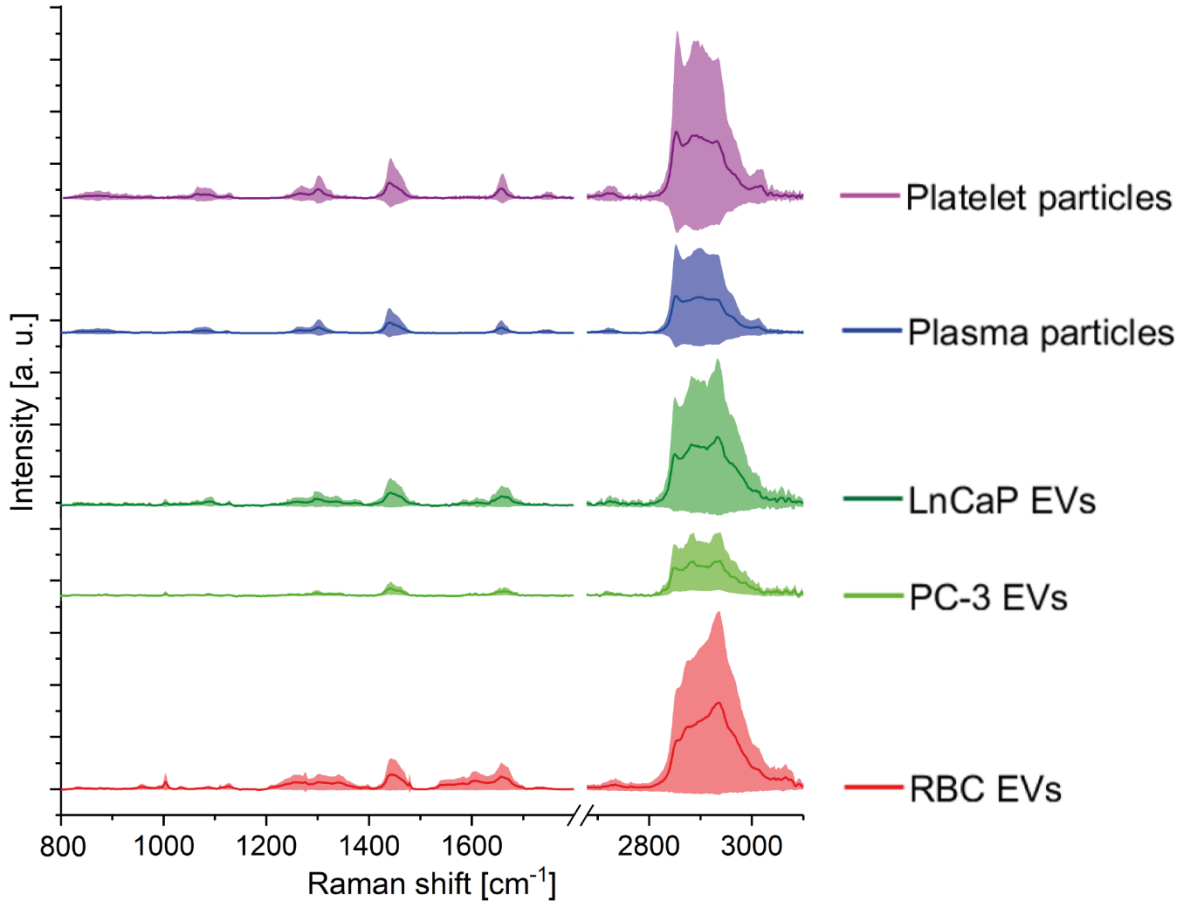

Figure S9. Mean Raman spectra and standard deviation of single RBCs EVs (n=56), PC-3 EVs (n=94), LNCaP EVs (n=75), “plasma particles” (n=153) and “platelet particles” (n=103).