Supporting information

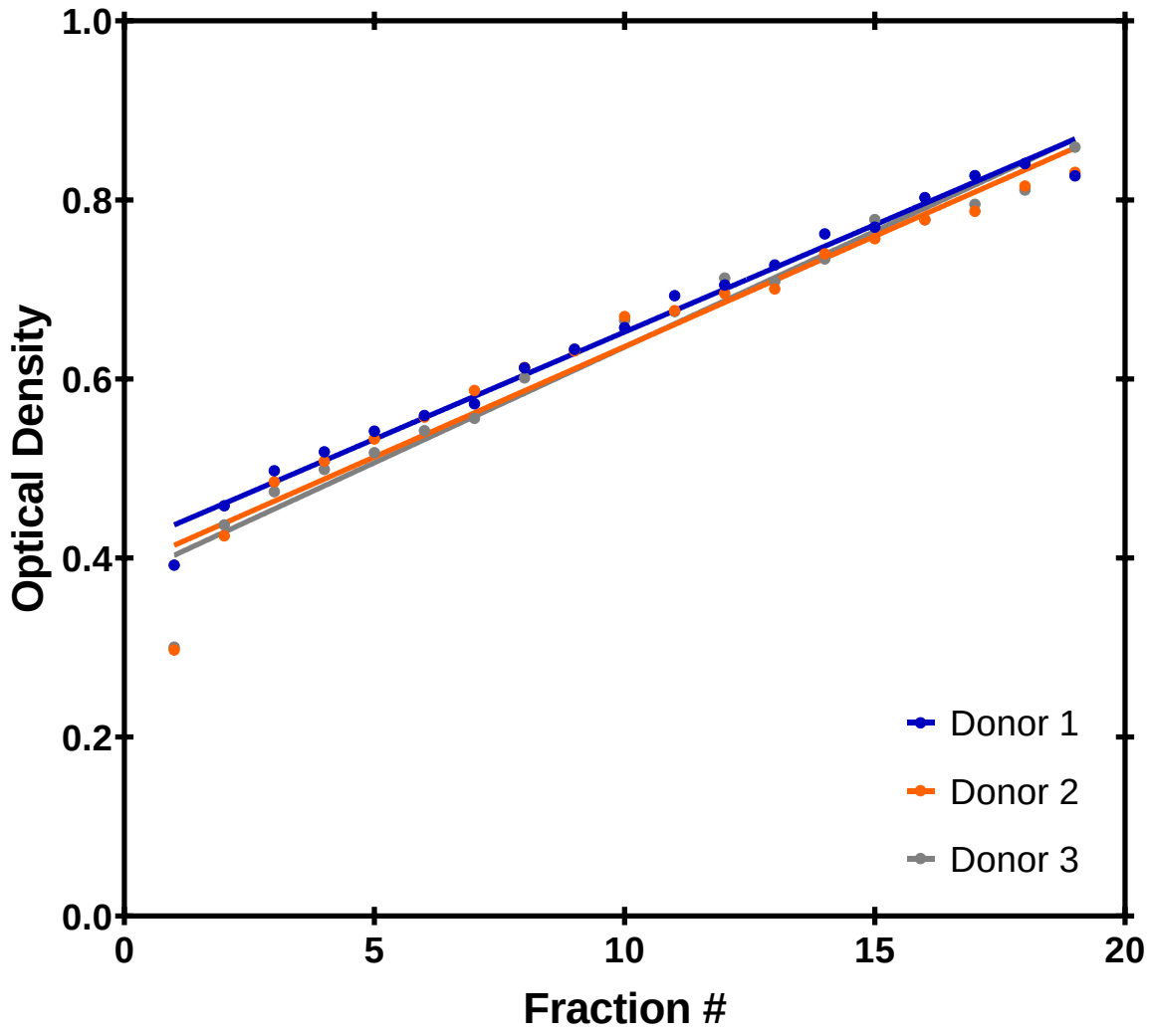
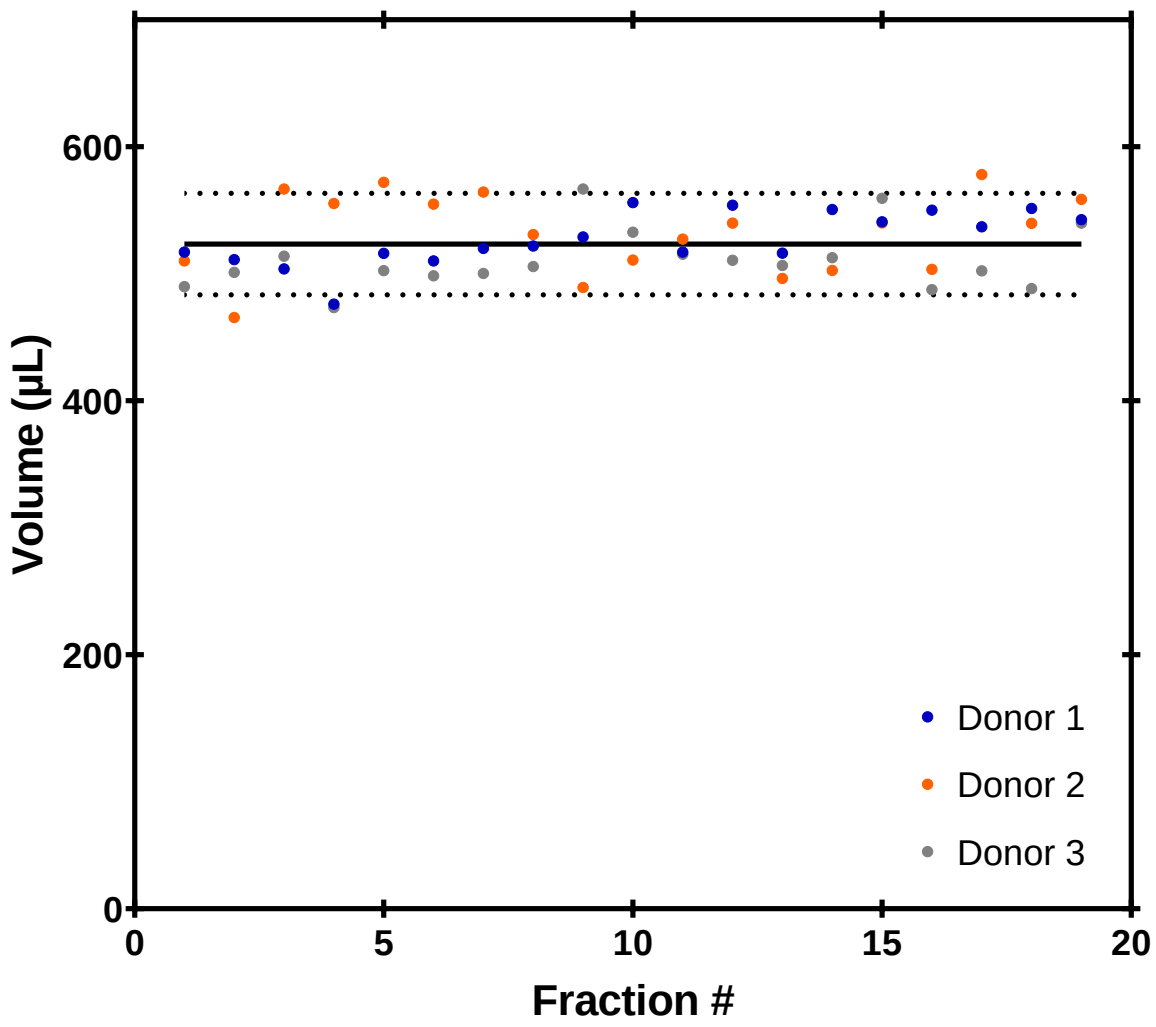


Figure S1A) Linearity Optiprep gradient. The linear relation between the optical density measured at 340 nm wavelength of Optiprep concentrations (4%-8%) and each fraction measured with the Spectramax i3 spectrophotometer. A gradient mixer produces a linear gradient for donor 1-3 (R^2=0.99, R^2=0.94, R^2=0.96 respectively).



B) Volume of rate zonal centrifugation fractions. The volume in each fraction is determined by weighing the Eppendorf tube with sample and subtracting the weight of an empty Eppendorf tube (average of 20). 87.7% (50/57) of the fractions is within the range of one drop (40 µL) below and one drop above the average volume fractionated.

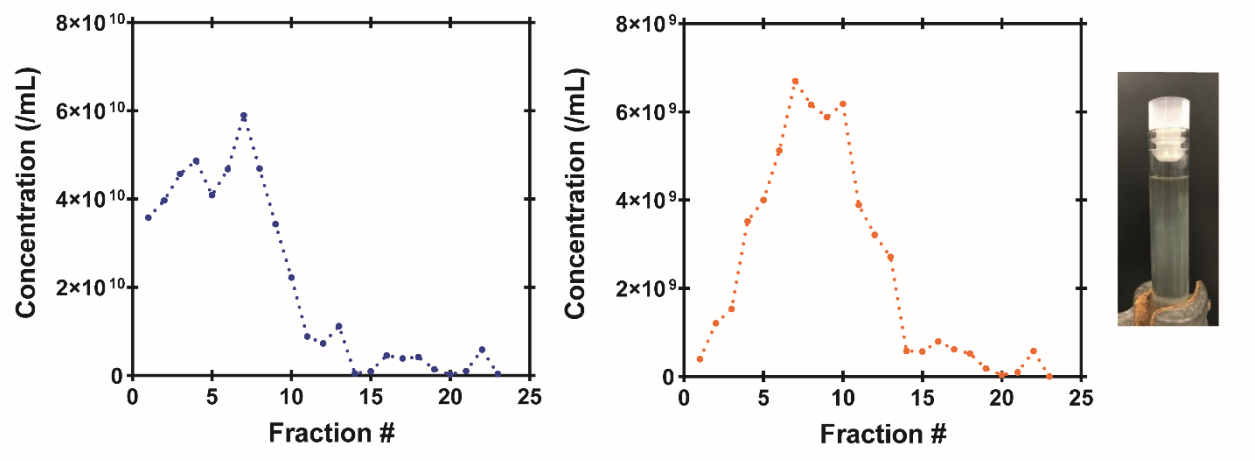
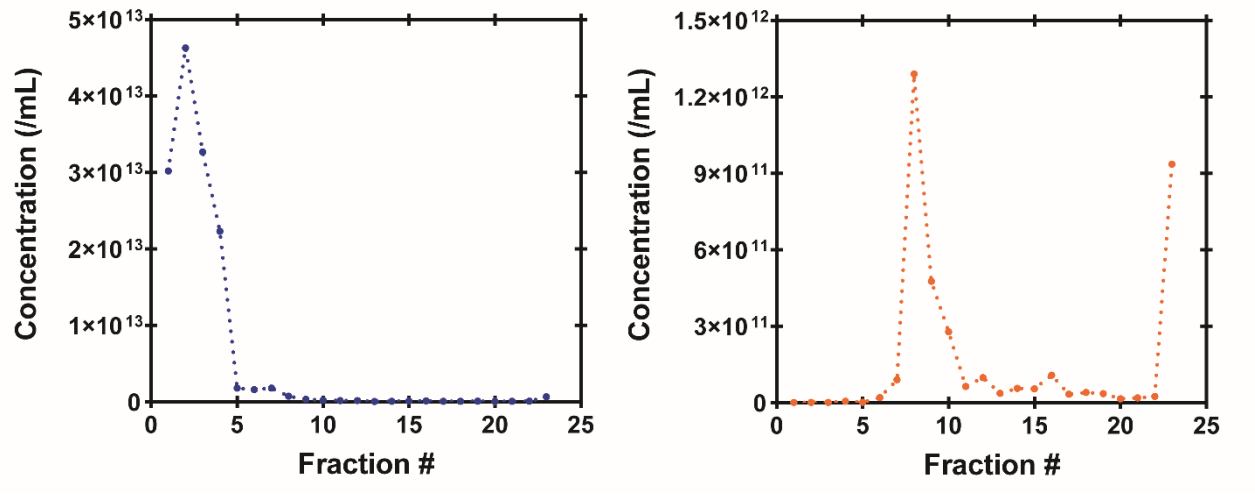
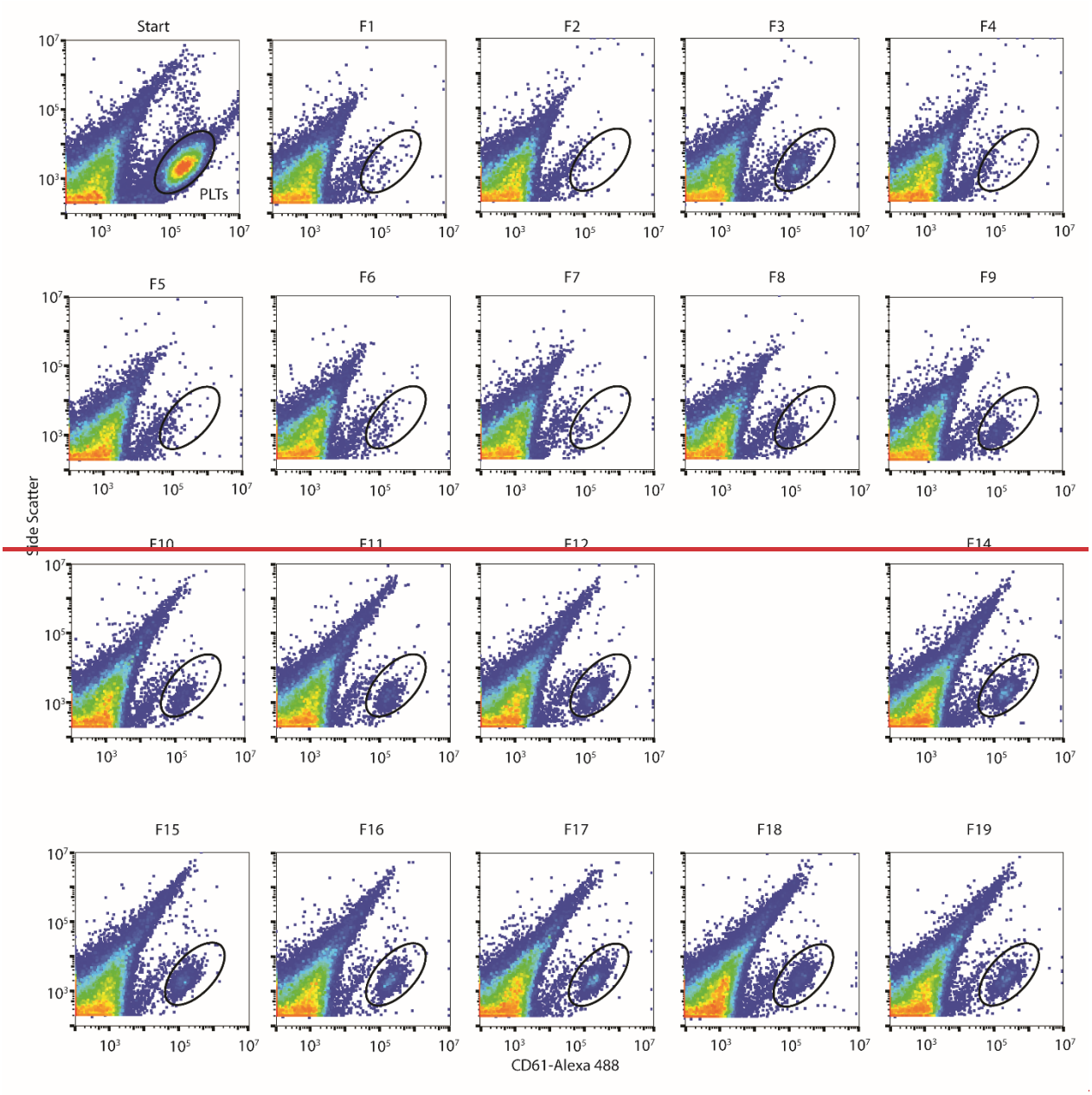


Figure S2A) Rate zonal centrifugation of 140 nm (blue) and 380 nm (orange) green fluorescent beads in pool plasma at 2,772 *g* for 100 minutes. When the beads are in plasma (ρ = 1.025 g/ml), beads spread throughout the gradient. Therefore, SEC is used to replace plasma by PBS.



B) Rate zonal centrifugation of 140 nm (blue) and 380 nm (orange) green fluorescent beads in pool plasma at 2772 *g* for 100 minutes after size exclusion chromatography. When the beads are in PBS, a clear band of beads is visible**.**


Start
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
F14
F15
F16
F17
F18
F19
PLTs
Side Scatter
CD61-Alexa 488

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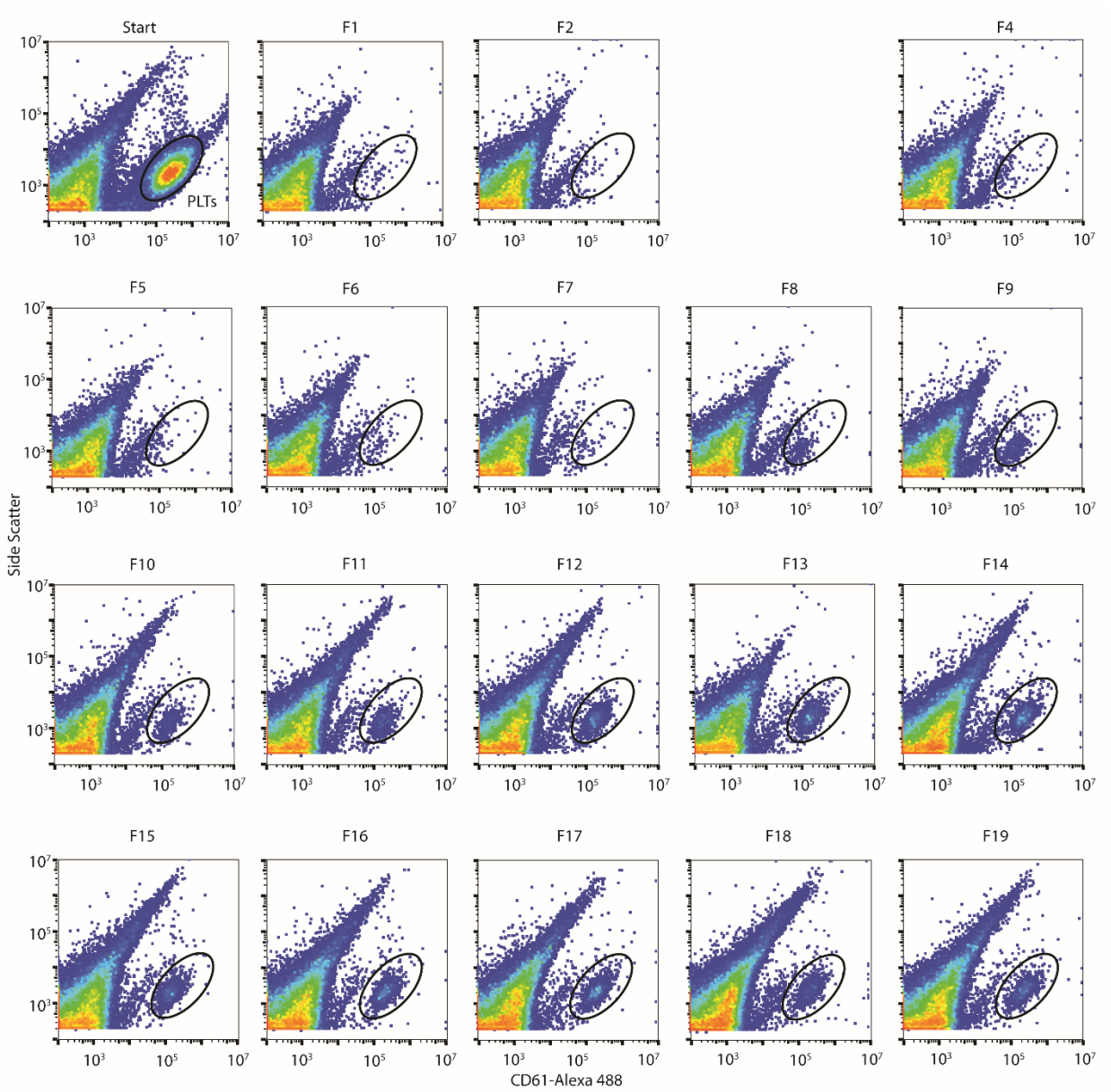

Figure S3. The platelet concentration was also measured by the FACsCalibur (BD, Franklin Lakes, NJ). 5 µL of each fraction was incubated with 5 µL of CD61-Alexa 488 (final concentration 0.01 mg/mL), and 35 µL HEPES buffer (137 mM NaCl, 20 mM Hepes, 5.6 mM D-glucose, 0.1 % BSA, 3.3 mM $NaH_2PO_4.H_2O$, 2.7 mM KCL, 1 mM $MgCl_2.6H_2O$, pH 7.4). After 30 minutes incubation in the dark, the labeling was stopped by adding 2 mL of HEPES buffer to the samples. Samples were measured for 1 minute. The concentration of CD61+ particles in each

sample was measured on the side scatter (15 mW 488 nm laser, flow rate calibrated by weight, SSC 400 V, gain 0, threshold 0). The flow cytometer was calibrated using BD Calibration beads (unlabeled, FITC, APC, PE, PerCp). An unstained sample was used as a control. Starting material which is loaded on top of the gradient contains CD61+ platelets. In fraction 1 the count of these platelets is low compared to fractions further down the gradient. The platelets are mainly present in fraction 8-17 and the scatter signal moves upwards. Fraction ~~13~~ 3 was removed because no CD61+ population was visible.