Preparation of Blood Cells-Derived EVs.

Red blood cell concentrate (150 mL) obtained from Sanquin (Amsterdam, The Netherlands) was diluted 1:1 with filtered phosphatebuffered saline (PBS; 154 mM NaCl, 1.24 mM Na2HPO4·2H2O, 0.2 mM NaH2PO4·2H2O, pH 7.4; supplemented with 0.32% trisodium-citrate; 0.22 mm filter (Merck Chemicals BV, Darmstadt, Germany)) and centrifuged three times for 20 min at 1 560g, 20 °C using a Rotina 46RS centrifuge (Hettich, Tuttlingen, Germany). The EV-containing supernatant was pooled, and aliquots of 50 µL were frozen in liquid nitrogen and stored at −80 °C.

Platelet concentrate (100 mL) obtained from Sanquin (Amsterdam, The Netherlands) was diluted 1:1 with filtered PBS. Next, 40 mL acid of citrate dextrose (ACD; 0.85 M trisodiumcitrate, 0.11 M D-glucose, and 0.071 M citric acid) was added and the suspension was centrifuged for 20 min at 800g, 20 °C. Thereafter, the supernatant was centrifuged (20 min at 1 560g, 20 °C). This centrifugation procedure was repeated twice to ensure removal of platelets. The vesiclecontaining supernatant was pooled, and aliquots of 50 µL were frozen in liquid nitrogen and stored at −80 °C. Samples were thawed on melting ice for 30 min before use.