Hollow organosilica beads as reference particles for optical detection of extracellular vesicles

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Supplementary Information

NTA and TEM characterization of cell-depleted plasma

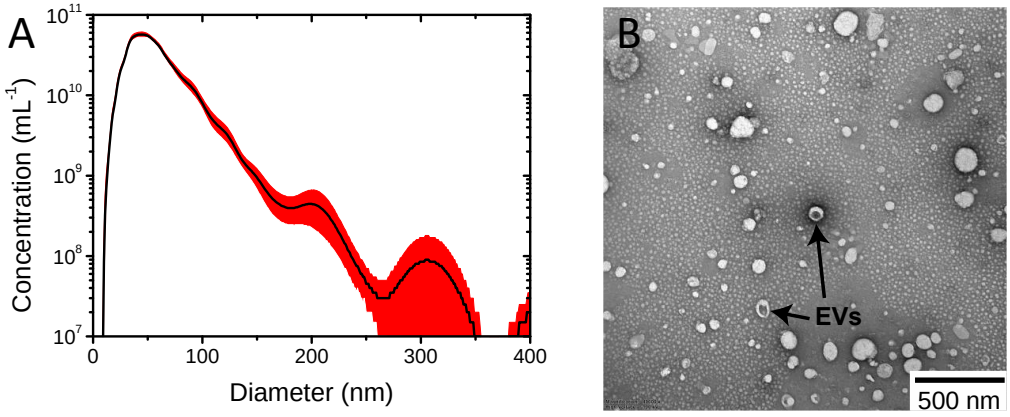


Figure S1 Size distribution of all particles in the cell-depleted plasma by (A) nanoparticle tracking analysis, and (B) transmission electron microscopy.

Protocol for detection of EVs by transmission electron microscopy (TEM)

To confirm the presence of EVs, 100 µL of cell-depleted plasma was thawed at 37 °C and loaded on a Sepharose (CL-2B, GE Healthcare, Sweden) column of 3.64 mL. To collect the EV fraction, 1 mL was discarded and the next 0.5 mL was collected. To increase the EV concentration, the EV fraction (0.5 mL) was ultra-centrifuged (60 minutes, 150, 000 · g, 4 ◦C). The supernatant (475 µL) was discarded and the pellet (25 µL) was vortexed and used for TEM. Next, 10 µL of the pellet was applied to a formfar-carbon coated 300 mesh grid (Electron Microscopy Sciences, USA) for 2 minutes, followed by staining with 2.00 % (w/v) uranyl acetate for 2 minutes. Excess of sample and uranyl acetate was removed with filtration paper. The sample was allowed to dry at room temperature for 2 hours and imaged with a transmission electron microscope (Technai-12, FEI, The Netherlands).

Protocol for detection of EVs by nanoparticle tracking analysis (NTA)

Cell-depleted plasma was diluted 50,000-fold in 50 nm filtered (Whatman) PBS and measured by a dark-field microscope (NS500; Nanosight, Amesbury, UK). Per sample, 10 videos of 5s were captured at 22.0 °C using camera level 14. Data were analysed by NTA 2.3 Build 0025 (Nanosight), assuming a medium viscosity of 0.95 cP and using a threshold of 12 gray values.

Summary of size distribution parameters of HOBs

Table S1 Mean diameters and the full-width-at-half-maximum values (FWHMs) of the size distributions of HOBs obtained by transmission electron microscopy (TEM), dynamic light scattering (DLS), particle tracking analysis (PTA), tunable and microfluidic resistive pulse sensing (RPS), and small-angle X-ray scattering (SAXS). In case of SAXS, the estimated uncertainty is given for the mean diameter value.

Method	*Nominal 200 nm HOB*		*Nominal 400 nm HOB*	
	Diameter (nm)	*Distribution FWHM (nm)*	*Diameter (nm)*	*Distribution FWHM (nm)*
TEM	184	25	381	55
DLS	188	52	356	118
PTA	178	31	339	59
Tunable RPS	208	34	336	62
Microfluidic RPS	201	49	364	101
SAXS	189±2	27	374±10	53

SAXS details

Data S1: SAXS model

SAXS measurements were performed with a photon energy of $E = (6000.0 \pm 0.6)$ eV. The samples were filled into vacuum-proof glass capillaries and inserted into a high vacuum chamber. A vacuum-compatible Pilatus 1M hybrid pixel detector (Dectris Ltd, Baden, Switzerland) with a pixel size of $d = (172.1 \pm 0.2)$ μm [24] collected the scattered X-ray photons during 20 minutes for each sample at a distance $L = (4544 \pm 1)$ mm from the capillaries containing the investigated samples. The scattering intensity is measured as a function of the momentum transfer q, which is related to the half of the scattering angle θ

between the direction of the incident beam and the scattered light according to $q = 4\pi E/(hc)\sin(\theta)$, where h is the Planck constant and c is the speed of light in vacuum. The obtained scattering curves were normalized to the exposure time, the solid angle, the sample transmittance and the incident photon flux. The scattering curves were interpreted in terms of least-squares fitting of a model function describing core-shell spherical particles with a Gaussian size distribution. The main parameters obtained from the fitting procedure were the size distribution of the particles and the thickness of the organosilica shell.

A model equation describing core-shell spherical particles with a Gaussian size distribution was fitted to the data using least-squares adjustment. Due to the hollow structure of the samples, the fitting parameter describing the density of the core was restricted to values close to that of water. Besides, an additive two-component background comprising a constant intensity and a term proportional to q^{-4} as well as a second population of smaller spheres with a Lognormal size distribution were assumed. The main parameters obtained from the fitting procedure were the size distribution of the particles and the thickness of the organosilica shell.

SAXS results

The model fits to the experimental scattering curves were performed on the momentum transfer region between $q = 0.025$ nm^{-1} and $q = 0.82$ nm^{-1}. The most relevant fitting parameters are summarized in the following table:

	HOB200	**HOB400**
Diameter (nm)	189 ± 2	374 ± 10
Shell thickness (nm)	8.1 ± 0.5	6.4 ± 0.7
Polydispersity degree (%)*	14	14
Second Population Mean Diameter (nm)	6	15
Second Population LogNormal Sigma	0.5	0.4
Maximum Core Electron Density (nm^{-3})	344	346
Normalized Goodness of Fit (χ^2)	102	67

* Defined as p_d = 100*Full-width half-maximum / mean of size distribution

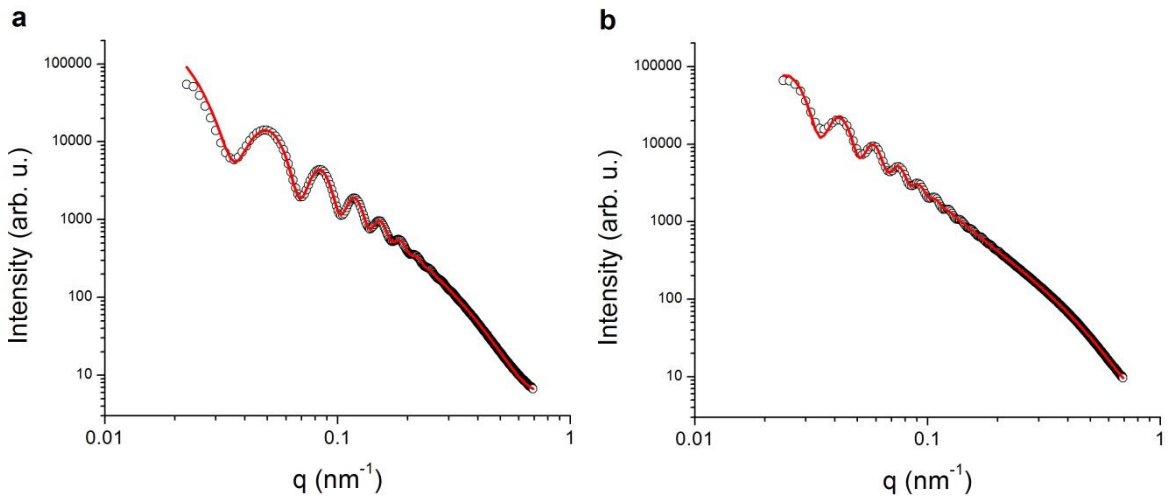


Figure S2: Experimental scattering curves (black circles) of the (a) HOB200 and (b) HOB400 samples and their corresponding fit displayed as a red line.

Flow cytometry details

MESF calibration of Phycoerythrin (PE) channel

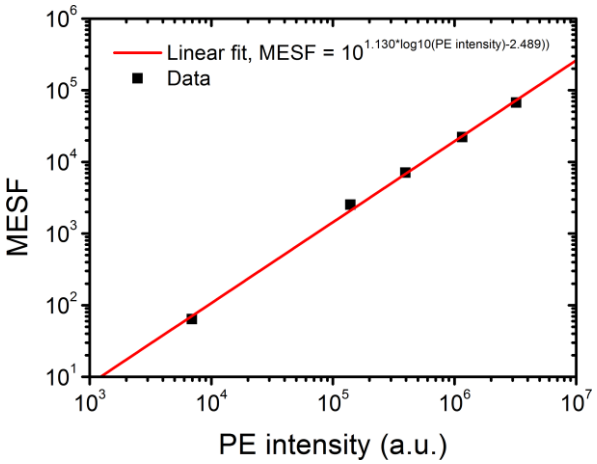
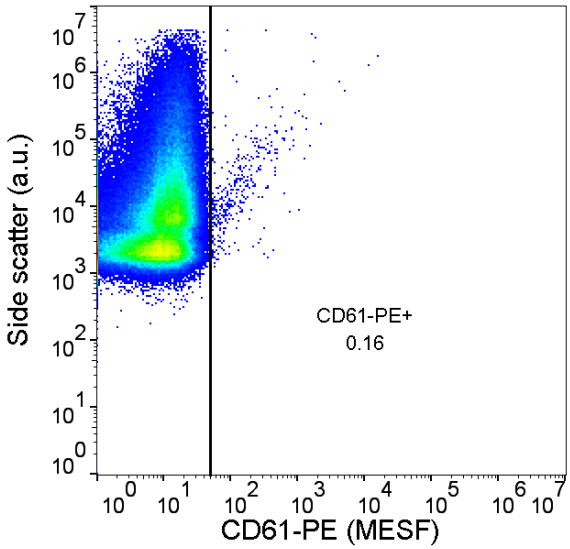


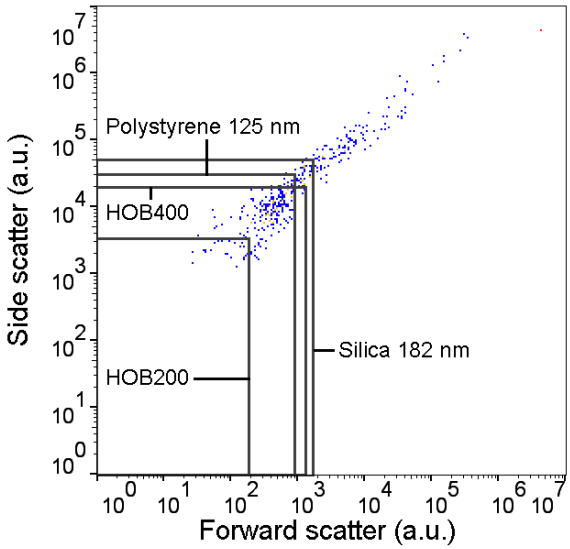
Figure S3: Molecules of equivalent soluble fluorochrome (MESF) versus median fluorescence intensity of the phycoerythin (PE) channel.

Flow cytometry gates of extracellular vesicles from cell-depleted plasma

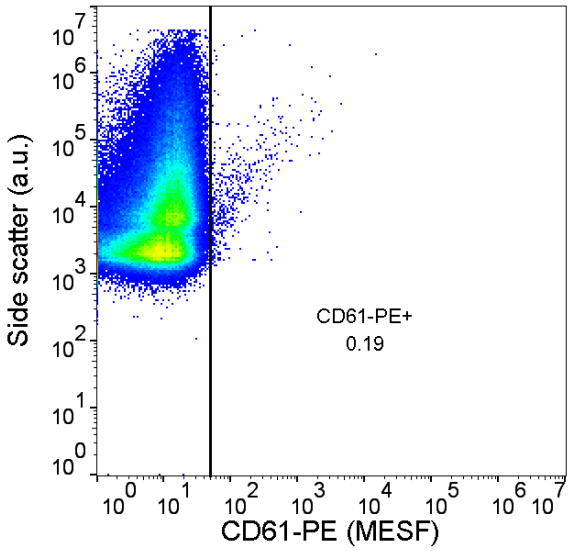
A. Cell-depleted plasma #1, SSC vs. PE



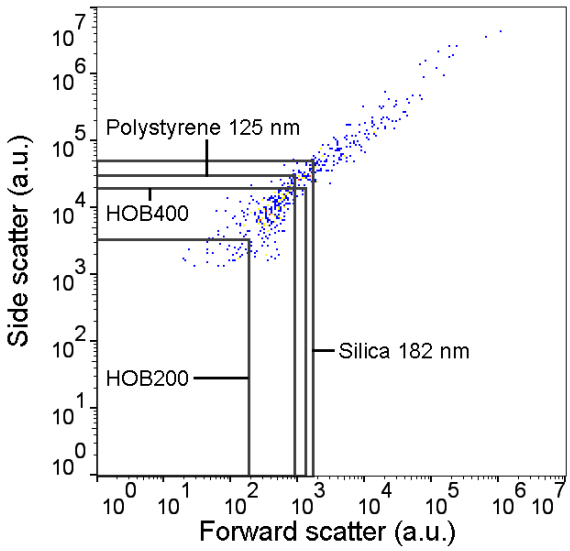
B. Cell-depleted plasma #2, SSC vs. FSC



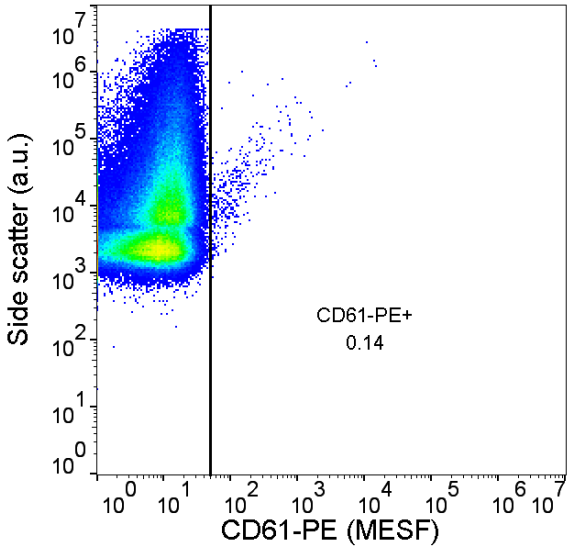
C. Cell-depleted plasma #2, SSC vs. PE



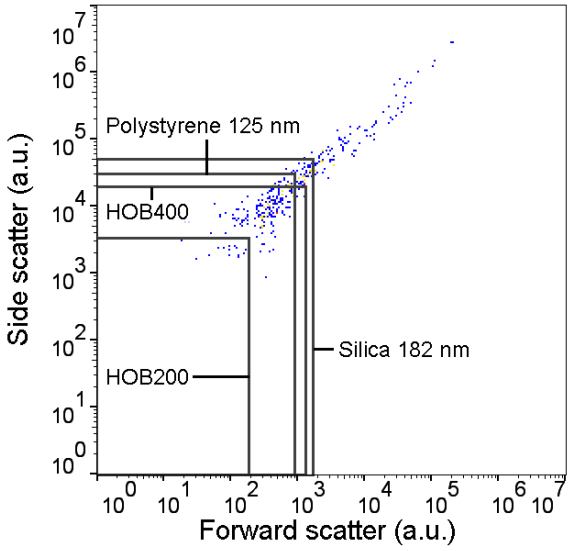
D. Cell-depleted plasma #2, SSC vs. FSC



E. Cell-depleted plasma #3, SSC vs. PE

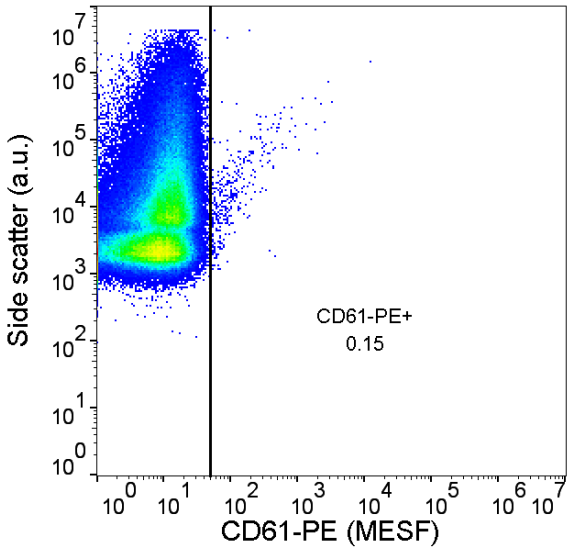


F. Cell-depleted plasma #3, SSC vs. FSC

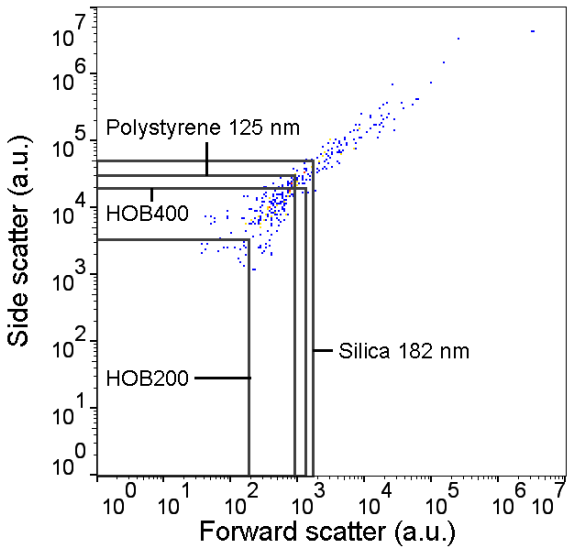


G. Cell-depleted plasma #4, SSC vs. PE

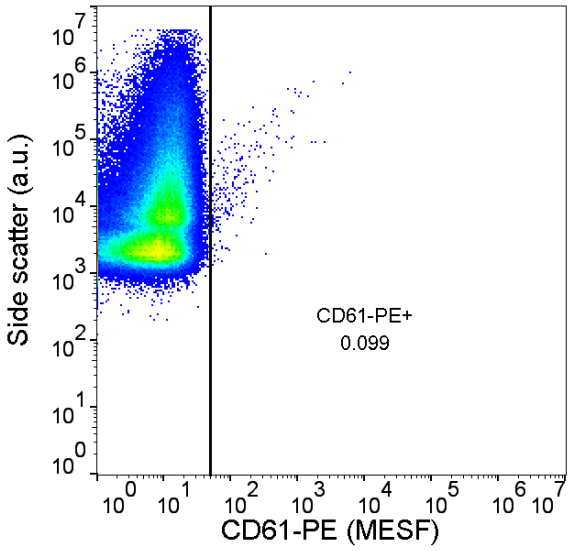
H. Cell-depleted plasma #4, SSC vs. FSC

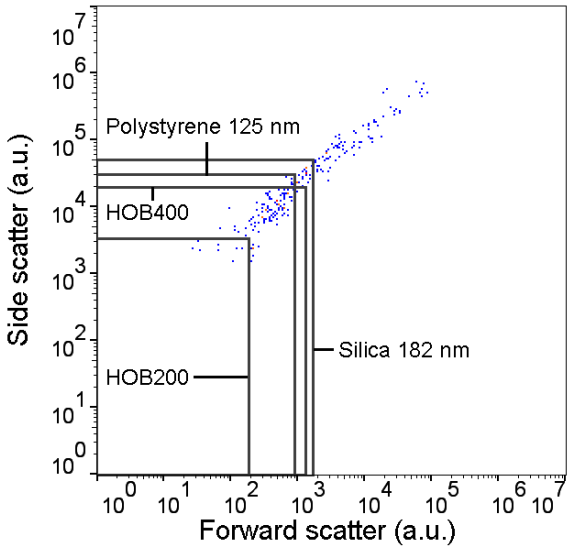


I. Cell-depleted plasma #5, SSC vs. PE

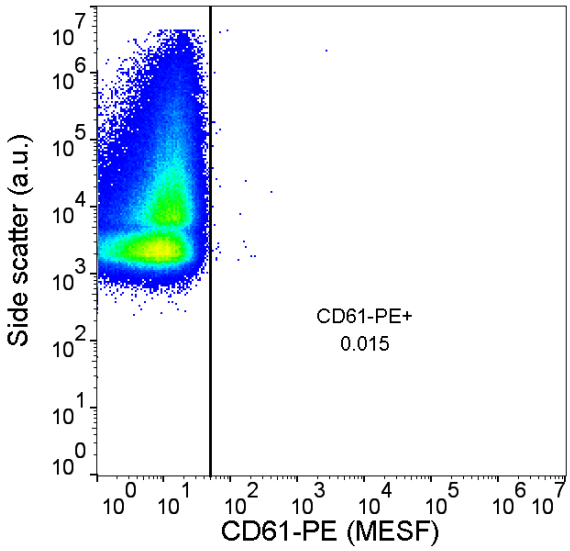


J. Cell-depleted plasma #5, SSC vs. FSC





K. IgG1-PE isotype control, SSC vs. PE



L. IgG1-PE isotype control, SSC vs. FSC

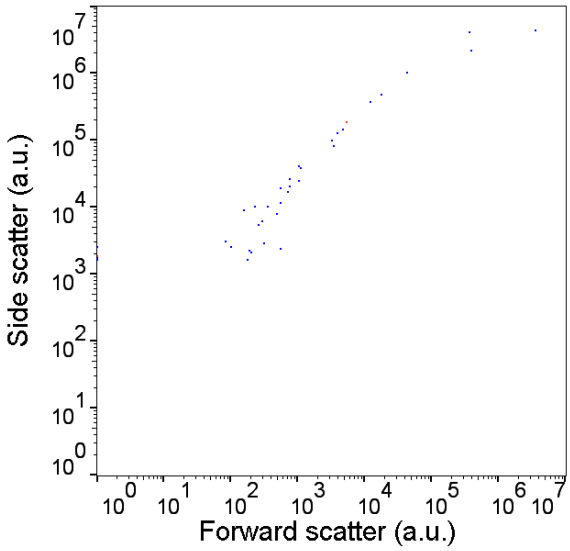


Figure S4: Side scatter versus phycoerythrin (PE) fluorescence (A,C,E,G,I,K) and side scatter versus forward scatter (B,D,F,H,J,L) for plasma extracellular vesicles labeled with CD61 and the isotype control measured by flow cytometry. The vertical line at 51 MESF indicates the gate of the PE channel.