Supplementary Information

A quantitative *ex vivo* study of the interactions between reconstituted high-density lipoproteins and human leukocytes

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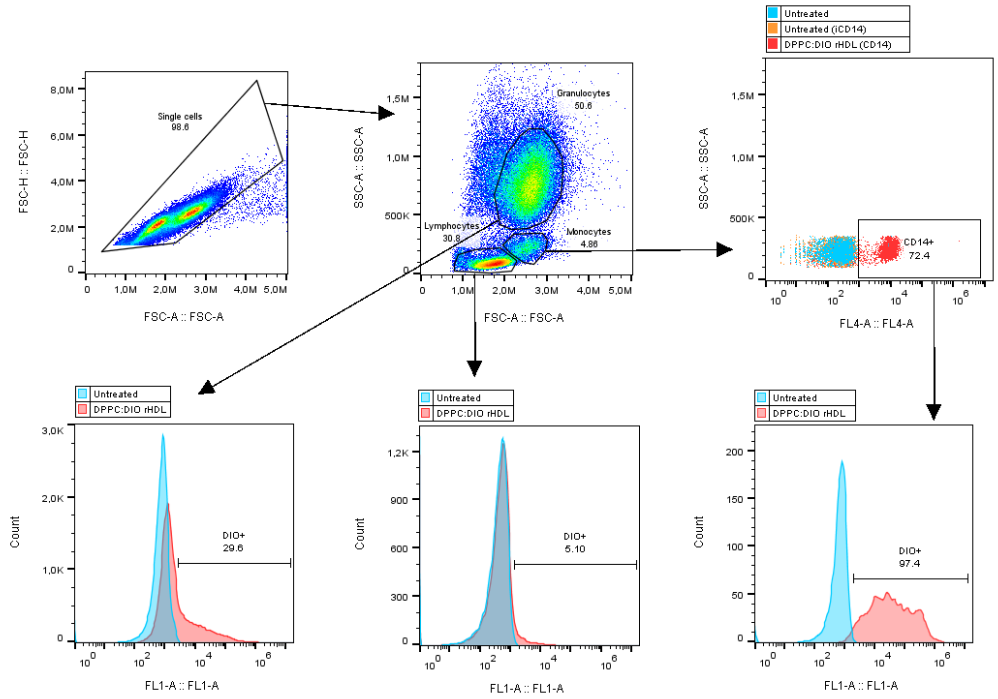

Figure S1: The gating strategy used for the flow cytometry analysis (Figure 4 – 6), here illustrated for a single sample (DPPC:DiO 99:1 rHDL). Similar gating was used for all samples.

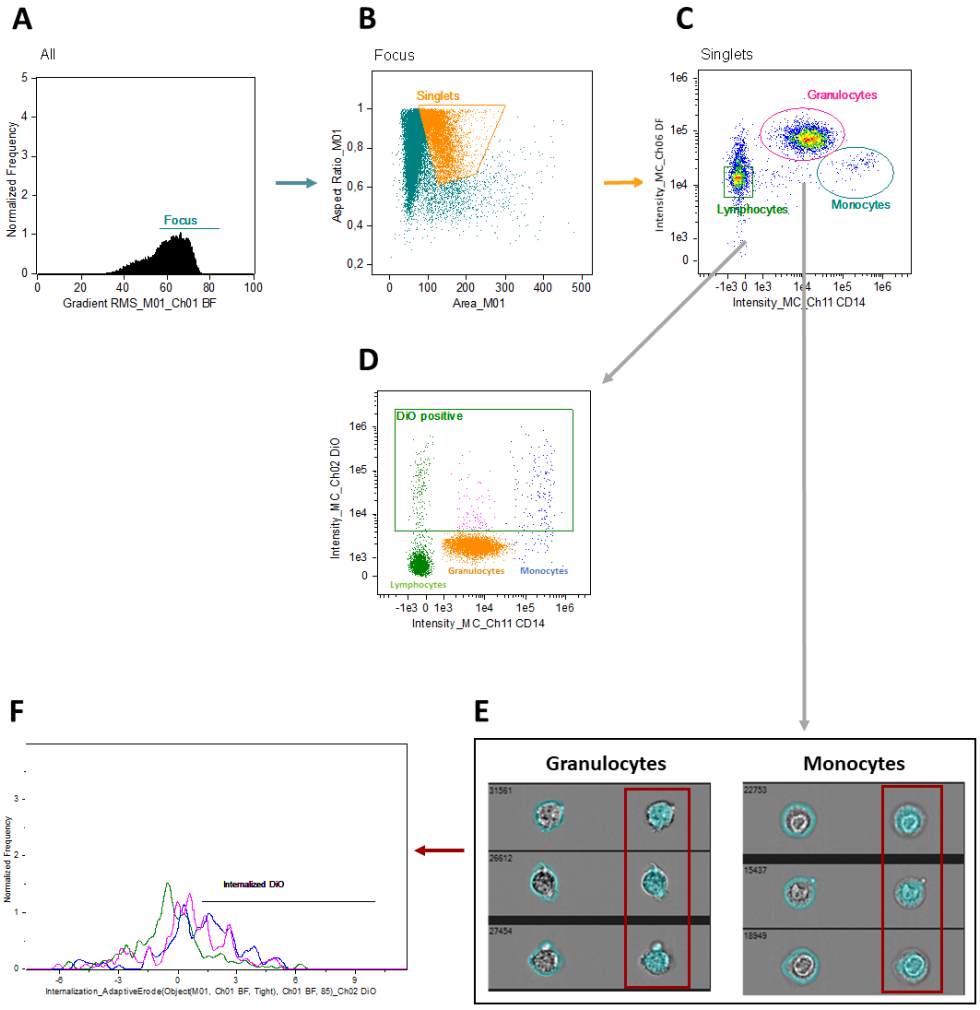

Figure S2: Illustration of the gating strategy used for imaging flow cytometry (Figure 7). Initially images in focus (**A**) and single events (**B**) were chosen. The three cell populations were gated based on intensity in Ch06 (side-scatter/darkfield) and intensity of CD14 (Ch11); (**C**). We determined the DiO positive granulocytes (pink), monocytes (blue), and lymphocytes (green); (**D**). Furthermore, we used masks to define the intracellular part (right) of the cells (**E**), which allowed us to determined the degree of rHDL cargo internalization in granulocytes (pink), monocytes (blue), and lymphocytes (green); (**F**). Similar gating was used for all samples.

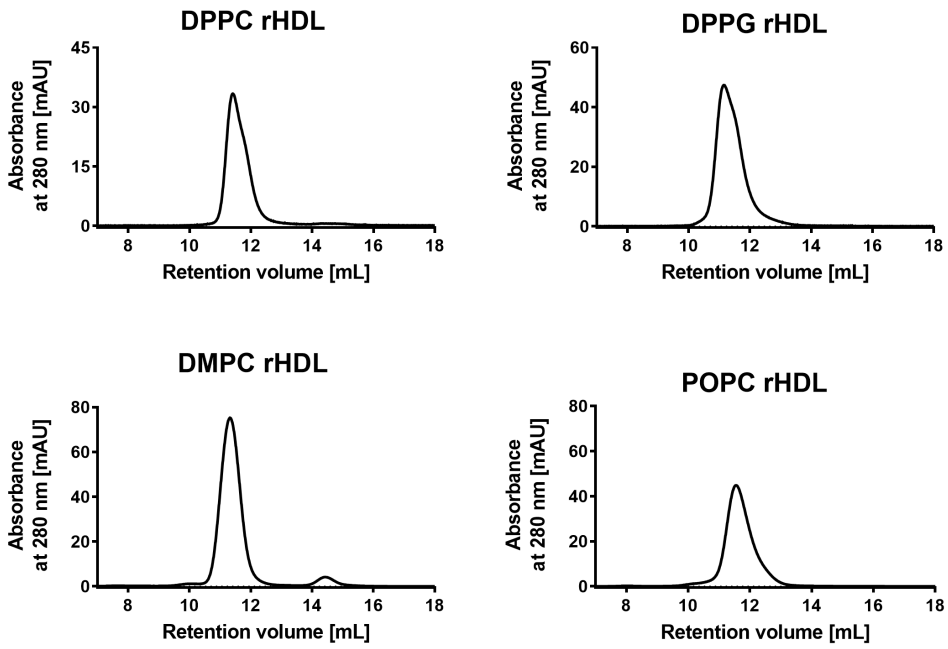

Figure S3: Additional characterization by size exclusion chromatography (SEC) of the rHDL with different lipid composition.

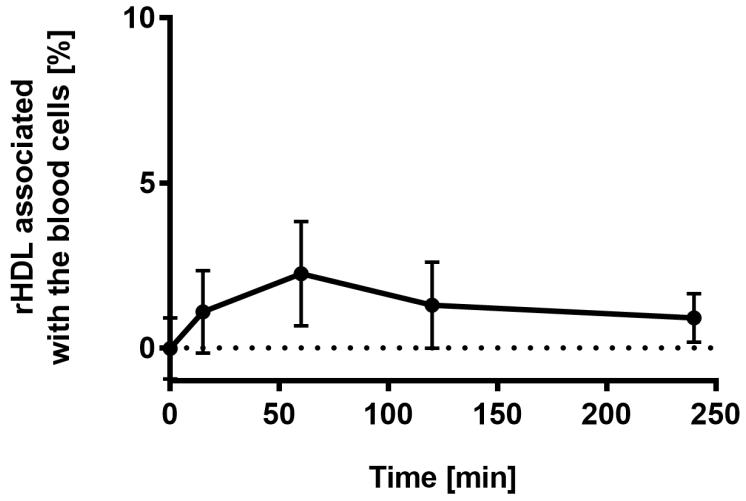

Figure S4: Quantification of the degree of rHDL association with leukocytes and erythrocytes. In these studies DPPC:DiR 99:1 rHDL (50 uL with lipid concentration of 5 mM) was incubated with 450 μL WHB yielding a rHDL lipid concentration of 0.5 mM, which is five fold higher than for the similar study presented in figure 3. After incubation the cells and plasma was separated by centrifugation (2000 g for 15 minutes), and both the cells and plasma was diluted in 900 μL in RIPA and subsequently incubated at 4 °C for 15 min. To remove cell debris the samples were centrifugate (14 000 g for 15 minutes), before measuring the fluorescence using the TECAN Spark microplate reader (TECAN) with excitation wavelength of 710(5) nm and emission wavelength of 800(10) nm. The fluorescence measured in the pellet relative to the fluorescence measured in both pellet and supernatant is considered as the fraction of rHDL associated with the cells. The data represent mean ±SEM from two experiments using WHB from different donors.

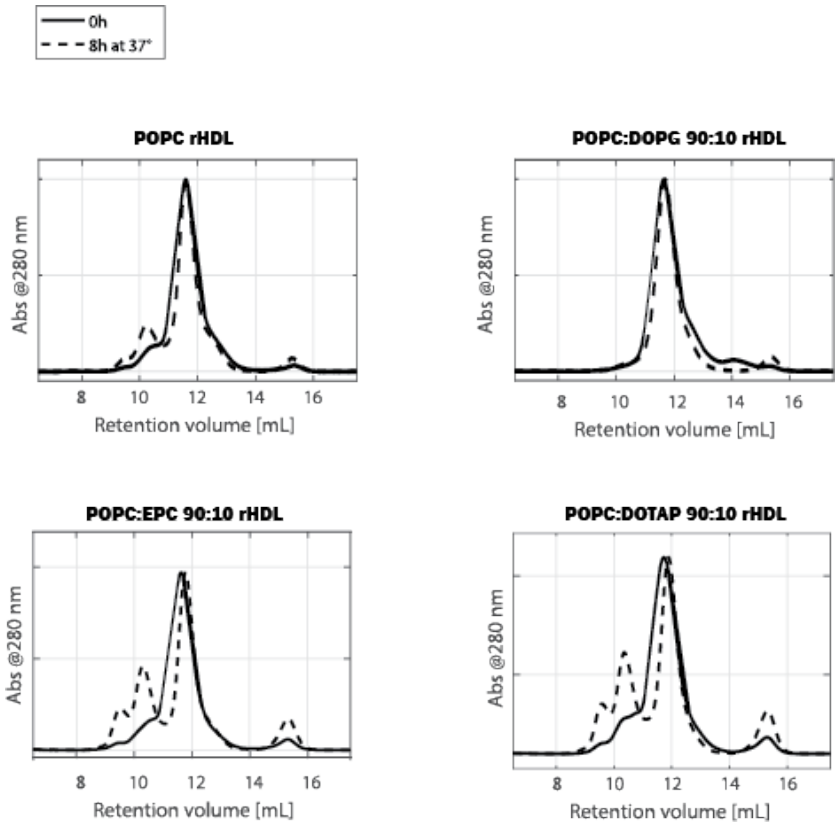

Figure S5: SEC absorbance chromatograms detecting absorbance at 280 nm. SEC analysis of the rHDL was conducted for an non-incubated sample and a sample incubated for 8 hours at 37 °C. Incorporation of 10% cationic lipids (1,2-dioleoyl-*sn*-glycero-3-ethylphosphocholine (EPC) and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)) induced instability of the POPC rHDL as seen by the multiple peaks after incubation at 37 °C. In contrast, the rHDL containing only POPC or POPC and 10 % anionic lipids (1,2-dioleoyl-*sn*- glycero-3-phospho-(1'-*rac*-glycerol) (DOPG)) seem to be stable, as indicated by similar chromatograms before and after incubation at 37 °C.