

**Supplementary Figure I.** Transfer of HDL-[<sup>3</sup>H]FC to Serum Lipoproteins. Grey dashed line indicates the peak elution volume for HDL. Experiments were conducted as in **Figure 2** for various incubation times and temperatures as labeled prior to SEC analysis.



Supplementary Figure II. Mouse Plasma LCAT Activity vs. nHDL and HDL. [<sup>3</sup>H]FC was evaporated onto a small piece of filter paper, which was incubated with nHDL or HDL in TBS for three hours at room temperature. nHDL- or HDL-[<sup>3</sup>H]FC (~50 µg; 0.05 µCi) were added to 1 mL mouse plasma on wet ice, mixed and 50  $\mu$ L were transferred to a scintillation vial for  $\beta$ -counting. The remaining mixture was transferred to a 37°C bath and additional 50 µL aliquots were transferred to test tubes containing 100  $\mu$ L methanol at t = 2, 5, 10, 30, and 120 min and the lipids partitioned into 1 mL 2-propanol/hexane (2/3 v/v) with vortexing followed by low speed centrifugation. Aliquots (500 µL) of each organic phase were transferred to conical tubes and the volume reduced under a nitrogen stream. The residues were redissolved in chloroform/methanol (25  $\mu$ L; 1/1; v/v) containing unlabeled CE and FC for visualization by  $I_2$ vapor. Each sample was applied to silica gel thin-layer plates and eluted with hexane/ethyl acetate (80:20). CE- and FC-positive spots were visualized by exposure to  $l_2$  vapor, collected by cutting the  $I_2$ -positive spots, transferred to vials containing scintillation fluid, and  $\beta$ -counted. LCAT activity was calculated as a ratio of CE<sub>DPM</sub>/ (FC<sub>DPM</sub> + CE<sub>DPM</sub>). These data show that the activities of mouse LCAT vs. nHDL and HDL are similar. According to the slopes of the kinetic curves, ~ 5% of FC was esterified in 10 min.



**Supplementary Figure III**. Transfer of nHDL-[<sup>14</sup>C]PL to TLP (total lipoproteins without lipid-free proteins). A) SEC of TLP according to its absorbance; nHDL-[<sup>14</sup>C]PL ( $\circ$ ). B-H) SEC profile of [<sup>14</sup>C]PL after incubation with TLP at 37°C for various times as labeled ( $\bullet$ ). Grey dashed line indicates the peak elution volume for nHDL. Insert to A (left) shows the %[<sup>14</sup>C]PL in LDL as a function of time; Insert to A (right) shows the rPLTP-mediated increase in the transfer of nHDL-[<sup>14</sup>C]PL after 30 min incubation at 37 °C. TLP is obtained by ultracentrifugation of whole plasma for 3 d in buffer made d = 1.21 g/mL (Beckman 50Ti @ 40,000) by the addition of KBr; TLP floats to the top and is collected.



**Supplementary Figure IV**. Transfer of nHDL-[<sup>14</sup>C]PL to Isolated LDL. A) SEC of LDL according to its absorbance, grey fill; nHDL-[<sup>14</sup>C]PL ( $\circ$ ). B-I) SEC profile of [<sup>14</sup>C]PL after incubation with LDL at 37°C for various times as labeled ( $\bullet$ ). Grey dashed line indicates the peak elution volume for nHDL. Insert to A shows the %[<sup>14</sup>C]PL in LDL as a function of time; t<sub>1/2</sub> = 4.1 ± 0.4 h.



**Supplementary Figure V**: Transfer of nHDL-[<sup>14</sup>C]PL to Isolated HDL. A) SEC of HDL according to its absorbance, grey fill; nHDL-[<sup>14</sup>C]PL ( $\circ$ ). B-I) SEC profile of [<sup>14</sup>C]PL after incubation with HDL at 37°C for various times as labeled ( $\bullet$ ). Grey dashed line indicates the peak elution volume for HDL.



**Supplementary Figure VI.** In vivo distribution of <sup>3</sup>H, <sup>14</sup>C, and <sup>125</sup>I at various times after injection of nHDL-[<sup>3</sup>H]FC, -[<sup>14</sup>C]PL, and -[<sup>125</sup>I]apo AI. Curves are fitted to the data using a smoothing function in Sigma Plot (Systat Software, Inc.). Other details are in the legend to **Figure 6** and in the **Materials and Methods**.



**Supplementary Figure VII**. In vivo distribution of <sup>3</sup>H, <sup>14</sup>C, and <sup>125</sup>I 30 min after injection of nHDL-[<sup>3</sup>H]FC, [<sup>14</sup>C]PL, and [<sup>125</sup>I]Apo AI. Details are in the **Materials and Methods**.



**Supplementary Figure VIII**. In Vivo Plasma Kinetics of HDL- and nHDL-[<sup>3</sup>H]FC and -[<sup>14</sup>C]PL. As described in **Materials and Methods**, radiolabeled HDL and nHDL were injected into mice. At various times mice were sacrificed and their plasma collected and  $\beta$ -counted. The plotted data were fitted to a 2-parameter exponential decay curve from which the halftimes were calculated.



Supplementary Figure IX: SEC of nHDL-analytes before and after short term incubations (0 or 10 min) with HDL or serum as labeled. Taken from Figure 2, 4, and Supplementary Figure V.