

**Supplementary Fig. S1. Time course of EV-HDL- and EL-HDL- induced eNOS phosphorylation.** EA.hy 926 cells were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for indicated time periods. Ten  $\mu\text{g}$  of cell lysate protein were separated by 10% SDS-PAGE followed by Western blotting analyses of eNOS and eNOS phosphorylated at Ser1177. Protein size annotations refer to protein marker bands on the membrane. The values are mean  $\pm$  SEM of 3 independent experiments, performed in duplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Supplementary Fig. S2. Impact of cell washing with PBS on eNOS phosphorylation.** EA.hy 926 cells plated in 24-well plates were cultured in full medium until confluency. Thereafter, cells were incubated in DMEM without FCS for 16 h followed by removal of the medium and either cell lysis with RIPA buffer (w/o PBS washing) or washing 3 times with warm PBS prior to cell lysis with RIPA buffer (w/ PBS washing). Results show a representative Western blot and the values are expressed as mean  $\pm$  SEM of 3 independent experiments, performed in duplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ .

**Supplementary Fig. S3. Impact of EL-HDL on  $\text{Ca}^{2+}$  ionophore A23187-induced eNOS activity.** EA.hy 926 cells were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 16 h followed by incubation with L- $^3\text{H}$ arginine and 0.5  $\mu\text{mol}/\text{L}$  A23187 for 5 min. L- $^3\text{H}$ citrulline was subsequently quantitated after its separation from L- $^3\text{H}$ arginine by exchange chromatography was done. eNOS activity is presented as % conversion of L- $^3\text{H}$ arginine to L- $^3\text{H}$ citrulline; the values obtained in the cells incubated with EV-HDL were set to 100%. The values are expressed as mean  $\pm$  SEM of 4 independent experiments, performed in triplicate and analyzed by two-tailed unpaired t-test; \*\* $p < 0.01$ .

**Supplementary Fig. S4. Impact of EL-HDL on subcellular localization of eNOS-GFP in EA.hy 926 cells in the presence of A23187.** EA.hy 926 cells overexpressing eNOS-GFP and (A) Golgi-RFP, or (B) stained with MitoTracker<sup>®</sup> Red FM were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 16 h, followed by structured illumination microscopy performed in the presence of 0.25  $\mu\text{mol}/\text{L}$  A23187. Representative images and the Pearson's co-localization coefficients are shown. The values are presented as mean  $\pm$  SEM of 4 independent experiments performed in duplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ .

**Supplementary Fig. S5. Impact of EL-HDL on subcellular localization of eNOS-GFP with Golgi in ECV 304 cells.** ECV 304 cells overexpressing eNOS-GFP and Golgi-RFP were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 16 h, followed by structured illumination microscopy in the absence (A) or presence (B) of 0.25  $\mu\text{mol}/\text{L}$  A23187. Representative images and the Pearson's co-localization coefficients are shown. The values are presented as mean  $\pm$  SEM of 4 independent experiments performed in duplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Supplementary Fig. S6. Impact of EL-HDL on subcellular localization of eNOS-GFP with mitochondria in ECV 304 cells.** ECV 304 cells overexpressing eNOS-GFP were stained with MitoTracker® Red FM and incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 16 h, followed by structured illumination microscopy performed in the (A) or presence (B) of 0.25  $\mu\text{mol}/\text{L}$  A23187. Representative images and the Pearson's co-localization coefficients are shown. The values are presented as mean  $\pm$  SEM of 4 independent experiments performed in duplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ .

**Supplementary Fig. S7. Impact of EL-HDL on A23187-induced eNOS-GFP shuttling into perinuclear region.** ECV 304 cells overexpressing eNOS-GFP were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 16 h. Fluorescence was measured by confocal microscopy after 16 h incubation with EV-HDL or EL-HDL and during subsequent incubation with 0.25  $\mu\text{mol}/\text{L}$  A23187 for 500 seconds. eNOS-GFP abundance in the perinuclear region is presented as the ratio of perinuclear to cytosolic fluorescence intensity. The values are expressed as mean  $\pm$  SEM of 4 independent experiments performed in duplicate and analyzed by multiple t-test with Sidak-Bonferroni correction for multiple comparison; \* $p < 0.05$ .

**Supplementary Fig. S8. Impact of 5 h incubation with EL-HDL on subcellular localization of eNOS-GFP with Golgi.** ECV 304 cells overexpressing eNOS-GFP and Golgi-RFP were incubated with 100  $\mu\text{g}/\text{mL}$  EV-HDL or EL-HDL for 5 h followed by structured illumination microscopy performed in the absence (A) or presence (B) of 0.25  $\mu\text{mol}/\text{L}$  A23187. Representative images and the Pearson's co-localization coefficients are shown. The values are presented as mean  $\pm$  SEM of 3 independent experiments performed in triplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ , \*\* $p < 0.01$ .

**Supplementary Fig. S9. Impact of 5 h-incubation with EL-HDL on subcellular localization of eNOS-GFP with mitochondria.** ECV 304 cells overexpressing eNOS-GFP were stained with MitoTracker® Red FM and incubated with 100 µg/mL EV-HDL or EL-HDL for 5 h followed by structured illumination microscopy performed in the absence (A) or presence (B) of 0.25 µmol/L A23187. Representative images and the Pearson's co-localization coefficient are shown. The values are expressed as mean ± SEM of 3 independent experiments performed in triplicate and analyzed by two-tailed unpaired t-test; \* $p < 0.05$ .

**Supplementary Fig. S10. TLC of four independent experiments used for densitometry, statistical analyses, and generation of the results shown in Fig. 4B.** EA.hy 926 cells plated in 6-well plates were incubated with 100 µg/mL of EV-HDL protein or EL-HDL protein for 16 h. Thereafter, the cells were washed with PBS and lipids were extracted with hexane/isopropanol (3:2, v:v). Extracts were evaporated and dissolved in chloroform before thin layer chromatography using hexane-diethyl ether-glacial acetic acid (70:29:1, v:v:v) as a mobile phase. The signals corresponding to phospholipids (PL), free cholesterol (FC), triacylglycerols (TAG), fatty acids (FA), and cholesterol ester (CE) were visualized by primulin and the signal intensity determined by densitometry. Annotations of the lipid species refer to the lipid standards on the plate.

**Supplementary Fig. S11. Role of SR-BI in EL-HDL -induced eNOS activity.** EA.hy 926 cells were transduced with SR-BI-Ad or EV-Ad. After 24 h (A) cells were lysed and the levels of SR-BI overexpression were analyzed by Western blotting (protein size annotations refer to protein marker bands on the membrane) or (B) were incubated with 100 µg/mL EV-HDL or EL-HDL for 16 h followed by incubation with L-[<sup>3</sup>H]arginine and subsequent quantification of L-[<sup>3</sup>H]citrulline after its separation from L-[<sup>3</sup>H]arginine by exchange chromatography. eNOS activity is presented as % conversion of L-[<sup>3</sup>H]arginine to L-[<sup>3</sup>H]citrulline. The values for EV-HDL incubated with cells transduced with EV-Ad were set to 100%. Results in (C) show the fold-increase in EV-HDL- and EL-HDL-induced eNOS activity triggered by SR-BI overexpression. The values are mean ± SEM of 3 independent experiments performed in triplicate and analyzed by two-tailed unpaired t-test; \*\* $p < 0.01$ .

**Supplementary Fig. S12. Incorporation of [<sup>3</sup>H]oleic acid into [<sup>3</sup>H]CE and [<sup>3</sup>H]TAG.**

EA.hy 926 cells were incubated with 100 µg/mL EV-HDL protein or EL-HDL protein for 5 or 16 h, washed with PBS and pulsed with [<sup>3</sup>H]oleic acid/BSA for 30 min. Cells were then washed

twice with ice-cold PBS and cellular lipids were extracted with hexane/isopropanol (3:2, v/v) and analysed by TLC using a solvent system for the separation of neutral lipids (hexane/diethyl ether/ acetic acid, 70:29:1, vol/vol). After primuline staining lipids were visualized using an UV lamp (366 nm) and [<sup>3</sup>H]CE and [<sup>3</sup>H]TAG spots were cut out for scintillation counting. The values for [<sup>3</sup>H]CE (A) and [<sup>3</sup>H]TAG (B) are presented as cpm/min/mg protein and are mean ± SEM of 3 independent experiments performed in triplicate and analyzed by two-tailed unpaired t-test.

**Supplementary Fig. S13. Size of ATTO-HDL vesicles.** EA.hy 926 cells overexpressing a cytosolic marker geNOP-GFP were incubated with 100 µg/mL ATTO-EV-HDL or ATTO-EL-HDL for (A) 1 and (B) 16 h followed by 3D confocal spinning disk microscopy to analyze the radius and volume of ATTO-HDL containing vesicles. The values are mean ± SEM of 3 independent experiments performed in triplicate and analyzed by two-tailed unpaired t-test.

**Supplementary Fig. S14. Levels of *Abca1*, *Abcg1* and *Scarb1* mRNA in EA.hy 926 cells.** Cells were plated in 12 well dishes followed by isolation of RNA 24 h after plating. Total RNA was isolated using TriFast™ reagent according to the manufacturer's protocol. One µg of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit. Quantitative real-time PCR was performed using the GoTaq® qPCR MasterMix (Promega, Madison, WI) on a Bio-Rad CFX96 Touch™ Real-Time PCR. Samples were analyzed in duplicate. Expression profiles and associated statistical parameters were determined using the 2<sup>-ΔΔCT</sup> method. The values are mean ± SEM of 3 independent experiments performed in duplicate and analyzed by one-way ANOVA. \*\**p*<0.01, \*\*\**p*<0.001.