LEGENDS FOR SUPPLEMENTAL MOVIES

Movie S1. Movie showing a dual-axis electron microscopy tomogram of a capillary from a wild-type mouse injected with triglyceride-rich lipoproteins and stained with Alcian blue. Related to figure 2.

Movie S2. Movie showing a dual-axis electron microscopy tomogram of a capillary from a wild-type mouse injected with triglyceride-rich lipoproteins and stained with Alcian blue. Related to figure 2.

Movie S3. Movie showing a dual-axis electron microscopy tomogram of a venule from a wild-type mouse injected with triglyceride-rich lipoproteins and stained with Alcian blue. Related to figure 2.

Movie S4. Movie showing a dual-axis electron microscopy tomogram of a heart from a wild-type mouse injected with triglyceride-rich lipoproteins. Related to figures 3 and S2.

Movie S5. Movie showing a close-up view of a triglyceride-rich lipoprotein particle attached to a nanovillus on the surface of a heart capillary endothelial cell. Related to figures 3 and S2.

Movie S6. Movie showing a close-up view of multiple nanovilli within invaginations and vesicles of a heart capillary endothelial cell. Related to figures 3 and S2.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animal models. *Gpihbp1*$^{-/-}$ (Beigneux et al., 2007), *Angptl4*$^{-/-}$ (Desai et al., 2007), *Ndst1*$^{fl/fl}$*Tek-Cre*$^{+}$ (Wang et al., 2005), L0-MCK mice (*Lpl*$^{-/-}$ mice harboring a muscle-specific human *LPL* transgene) (Levak-Frank et al., 1997), and EC-hLpLH mice (mice expressing a human *LPL* minigene driven by the Tie2 promoter) (Takahashi et al., 2008), have been described previously. Mice were fed a chow diet and housed in a barrier facility with a 12-h light-dark cycle. Triglyceride levels were measured on plasma samples with the Serum Triglyceride Determination Kit (Sigma). All studies were approved by UCLA's Animal Research Committee.

Isolation and labeling of lipoproteins. Triglyceride-rich lipoproteins (TRLs) were isolated from the plasma of *Gpihbp1*$^{-/-}$ mice by ultracentrifugation. Plasma (0.4 ml) was overlaid with PBS and centrifuged at 100,000 rpm (424,000 $\times$ *g*) at 10° C for 2 h in a Beckman TLA-100.3 rotor. The *d* < 1.006 g/dl lipoproteins were placed into a new tube, overlaid with PBS, and subjected to a second round of ultracentrifugation. The lipoproteins were removed and stored at 4° C. Cholesterol-rich very low density lipoproteins (β-VLDL) were isolated from the plasma of *Apoe*$^{-/-}$ mice after an overnight fast by ultracentrifugation. Human VLDL was purchased from Athens Research, and mouse HDL was isolated by ultracentrifugation (Havel et al., 1955). The apolipoproteins of TRLs as well as antibodies were labeled with infrared (IR) dyes (DyLight IR680-dye or DyLight IR800-dye) (Thermo Scientific). The proteins were diluted in PBS (2 mg/ml) containing 50 mM borate buffer (pH 8.5) and then incubated with the IR-dye for 1.5 h at RT with constant rotation. To separate labeled TRLs and antibodies from unincorporated dye, samples were applied to an Econo-Pac 10DG gel-filtration

column (Bio-Rad). Lipoproteins and antibodies were also labeled with fluorescent dyes (Alexa488, Alexa555, Alexa647) (Invitrogen). The proteins were diluted in PBS (2 mg/ml) containing 100 mM bicarbonate buffer (pH 8.3) and incubated with the fluorescent dye for 1.5 h at RT with constant rotation. Again, the labeled proteins were separated from unincorporated dye by gel filtration. TRLs and Intralipid were also labeled with the fluorescent lipid dye 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Invitrogen) for cell culture studies (the DiI-label was not suitable for *in vivo* studies). The dye was dissolved in DMSO (3 mg/ml), and 10 μl of this solution was added to TRLs or Intralipid along with lipoprotein-deficient human serum. DiI-labeled particles were isolated by density gradient ultracentrifugation.

Fluorescence microscopy. Tissues were perfusion fixed *in situ* with freshly prepared 3% paraformaldehyde (PFA) in PBS, and tissue samples were frozen in O.C.T. Tissue sections (8–10-μm thick) were processed for immunohistochemistry as described previously (Davies et al., 2010). GPIHBP1 was detected with the mouse GPIHBP1–specific rat monoclonal antibody 11A12 (mAb11A12) followed by Alexa488-labeled anti–rat IgG (1:200) or with Alexa555-labeled mAb11A12 (3 μg/ml). Endothelial cells were identified with a hamster anti-CD31 monoclonal antibody (1:200) and Alexa647-labeled anti–hamster IgG (1:200), or by injecting mice with FITC-labeled tomato lectin (100 μg). LPL was detected with a goat antibody against bovine LPL (Davies et al., 2010) and Alexa647-labeled anti–goat IgG. Nuclei were stained with DAPI. Images were obtained with an Axiovert 200 MOT microscope with Apotome correction or by confocal fluorescence microscopy on an Axiovert 200 MOT microscope with an LSM 700 confocal scanning module, and processed with the Zen 2010 software (all from Zeiss).

Measurement of mouse and human LPL levels. Mouse LPL was measured by ELISA as described previously (Weinstein et al., 2008). Human LPL protein was measured by a modification of a sandwich ELISA described by Peterson *et al*. (Peterson et al., 1992). Monoclonal antibody clone 5F9 was used as the trapping antibody and biotinylated monoclonal antibody 5D2 was used as the detecting antibody. Both antibodies were raised against bovine LPL. Plasma samples were denatured in 1.2 M guanidinium hydrochloride at 4° C for 1 h and diluted to 0.24 M guanidium hydrochloride before assay. Purified bovine LPL was used as a standard.

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