SUPPLEMENTARY INFORMATION

Assessing mechanisms of GPIHBP1 and lipoprotein lipase movement across endothelial cells

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Supplementary Methods

Transport of a GPIHBP1-specific Monoclonal Antibody Across Endothelial Cells *In Vivo*. We examined the transport of a fluorescently labeled monoclonal antibody against mouse GPIHBP1 (11A12) across capillary endothelial cells of live mice. Mice were anesthetized with isoflurane or a ketamine/xylazine cocktail and CF568-labeled 11A12 and 1% Evans Blue (to identify the injection site) were injected directly into interscapular brown adipose tissue. The mice were returned to their cages, and after 120 min, the mice were perfused with PBS and tissues fixed with 4% paraformaldehyde (PFA). The tissues were embedded in OCT and processed for immunohistochemistry.

In some experiments, we noted an absence of antibody transport in mice that had been anesthetized with ketamine/xylazine. To determine if a low body temperature in anesthetized mice inhibited antibody transport, wild-type mice were anesthetized with a ketamine/xylazine cocktail, and the brown adipose tissue was injected with CF568-labeled 11A12. Mice were then either placed on a heating pad or left in the cage at room temperature (25° C). The heating pad kept body temperature at 37° C (as judged by a rectal probe), while the body temperature without the heating pad approached room temperature (25° C).

Quantification of Capillary Endothelial Cell Invaginations. Electron micrographs of capillary endothelial cells from *Cav1*$^{+/+}$ and *Cav1*$^{-/-}$ mice were collected as described in *Materials and Methods*. The length of the basolateral or luminal plasma membrane from each endothelial cells was measured using imageJ (v1.46r). The number of invaginations from each membrane was counted and the density of invaginations was calculated by dividing the total number of invaginations by the total length of the membranes examined.

GPIHBP1 and CD36 localization studies. RHMVECs were transfected with a construct expressing S-protein tagged GPIHBP1 and a construct expressing CD36 (see below) by electroporation with the Nucleofector II apparatus (Lonza) and the HMVEC-L Nucleofector kit (Lonza). The next day cells were incubated with CF568-labeled antibody 11A12 for 1 h at 37º C, fixed with cold methanol for 5 min., and permeablized with 0.2% Triton X-100. CD36 was detected with a goat antibody against mouse CD36 (R&D Systems; 1:1000) followed by an Alexa488-conjugated donkey anti-goat IgG (1:500). For all experiments, cells were stained with DAPI and confocal fluorescence microscopy was performed with a Zeiss LSM700 laser scanning microscope.

Supplementary Figures

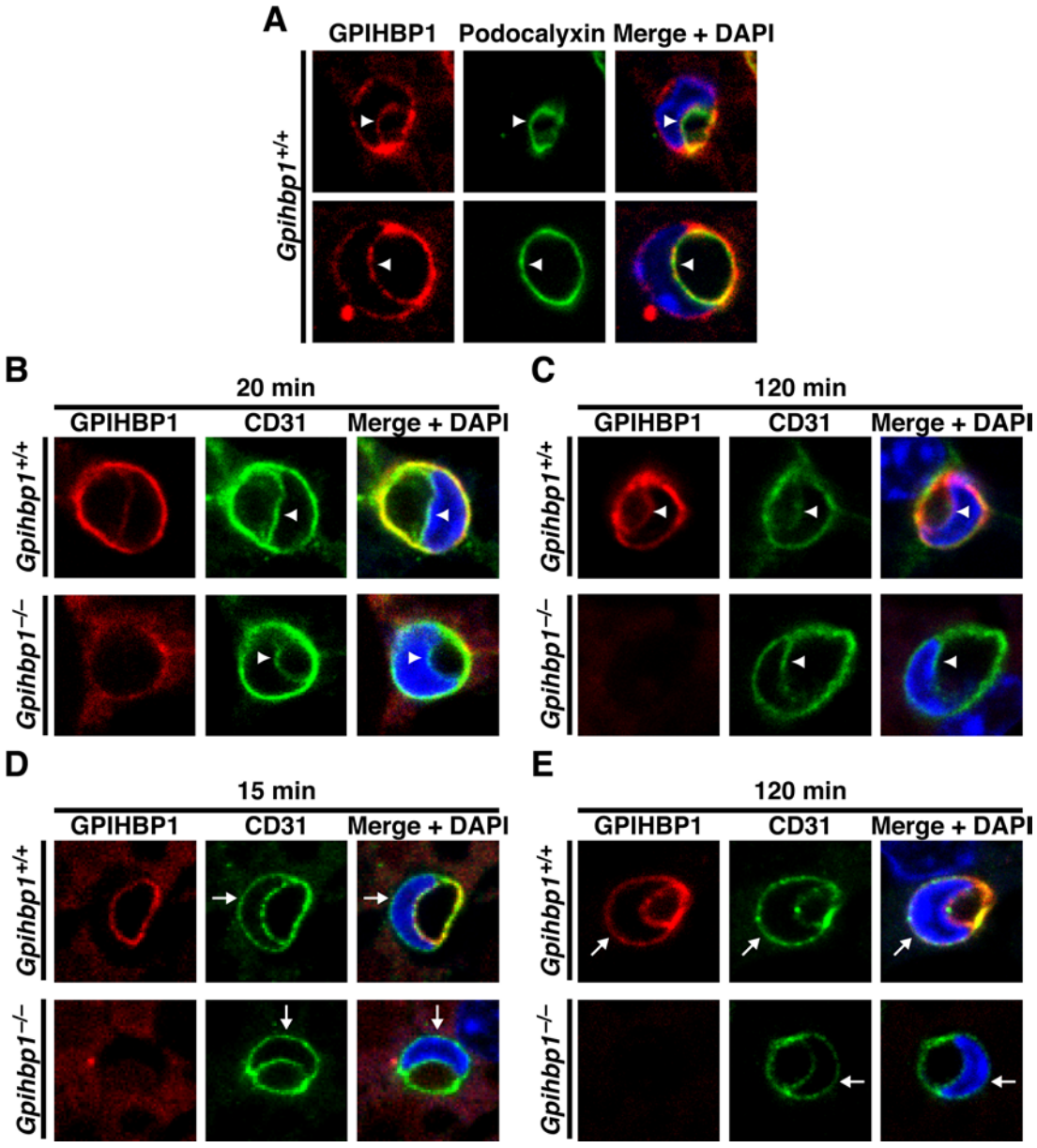

Figure S1. Bidirectional movement of GPIHBP1 across capillaries. (A) Confocal micrographs showing the luminal and basolateral surfaces of capillaries. Brown adipose tissue sections from wild-type mice were stained with antibodies against GPIHBP1 and podocalyxin and with DAPI. Luminal and basolateral surfaces of endothelial cells can be resolved in capillary cross sections that contain endothelial cell nuclei. Podocalyxin (green), a protein of the glycocalyx, is found only on the luminal face of endothelial cells (arrowheads), whereas GPIHBP1 (red) is located on both the basolateral and luminal surfaces. (B, C) GPIHBP1 transport from the basolateral to the luminal face of capillary endothelial cells. An Alexa555-labeled rat monoclonal antibody against GPIHBP1 (11A12) (red) was injected into the interstitial spaces of brown adipose tissue (BAT) of a

wild-type or *Gpihbp1*$^{-/-}$ mouse with a 31-gauge needle. After either 20 min (B) or 120 min (C), mice were sacrificed, and BAT sections were stained with an antibody against CD31 (green) to identify endothelial cells and DAPI (blue) to identify nuclei. The luminal face of endothelial cells is marked by arrowheads. (D, E) GPIHBP1 transport from the luminal to the basolateral face of capillary endothelial cells. CF568-labeled antibody 11A12 was injected intravenously into a wild-type or *Gpihbp1*$^{-/-}$ mouse. After either 15 min (D) or 120 min (E), the mice were sacrificed, and BAT sections were stained with an antibody against CD31 (green) and DAPI (blue). The basolateral face of endothelial cells is marked by arrows.

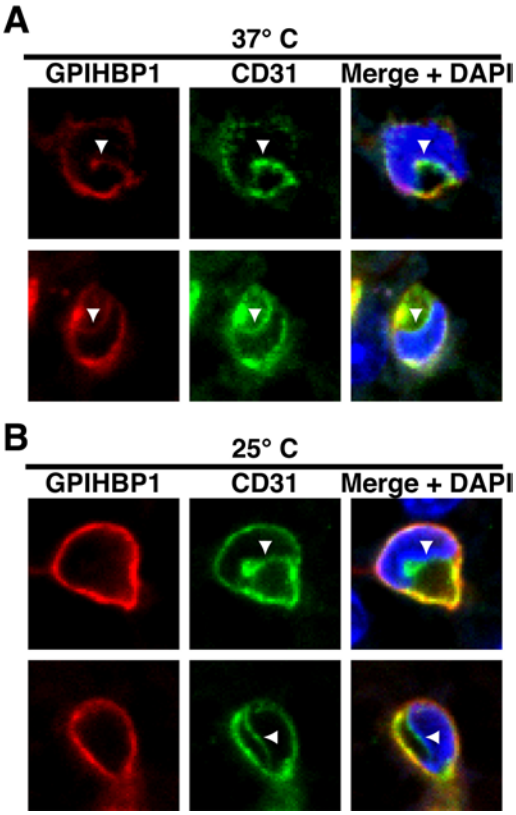

Figure S2. Effect of low body temperature on GPIHBP1 transport across capillaries. A CF568-labeled rat monoclonal antibody against GPIHBP1 (11A12) (red) was injected into the interstitial spaces of brown adipose tissue (BAT) of wild-type mice. Before the injection, mice were anesthetized with a ketamine/xylazine cocktail and were kept anesthetized during the course of the experiment. One mouse was returned to his cage at ambient temperature (25° C), allowing its core body temperature to drop (B), while the other was kept at 37° C with a heating pad (A). After 2 h, the mice were sacrificed and BAT sections were stained with an antibody against CD31 (green) to identify endothelial cells and DAPI (blue) to identify nuclei. The luminal face of endothelial cells is marked by arrowheads.

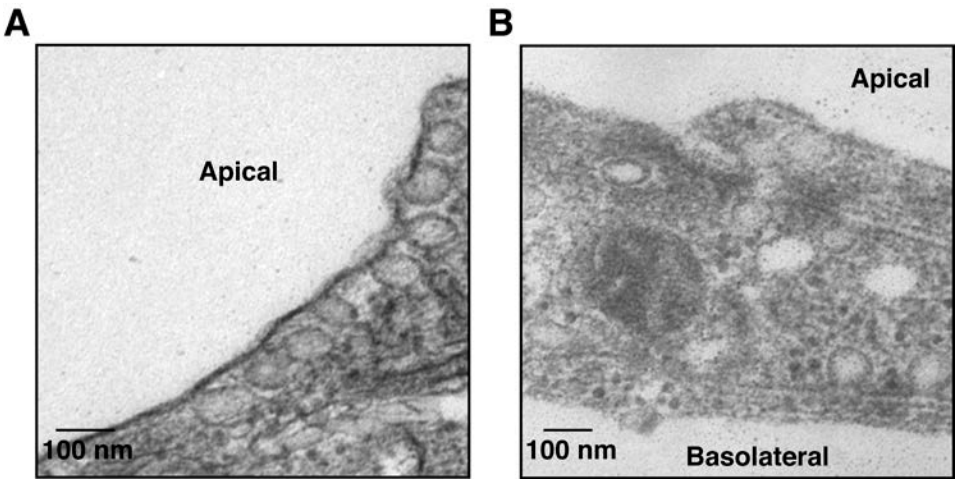

Figure S3. Electron micrographs showing binding of anti-GPIHBP1 antibodies and LPL to control endothelial cells (*i.e.*, expressing CD59 rather than GPIHBP1). (A) Gold-labeled anti-GPIHBP1 monoclonal antibody does not bind to control endothelial cells expressing CD59 rather than GPIHBP1. CD59-expressing endothelial cells were grown on filters and incubated for 2 h at 37° C with GPIHBP1-specific monoclonal antibody 11A12 conjugated to 6-nm gold beads. Cells were then fixed and examined by electron microscopy. (B) LPL does not bind to control endothelial cells expressing CD59. CD59-expressing endothelial cells were grown on filters and incubated with V5-tagged human LPL followed by a V5-specific monoclonal antibody conjugated to 6-nm gold beads. Cells were fixed and examined by electron microscopy. These experimental controls were performed at the same time as the studies shown in Figure 3.

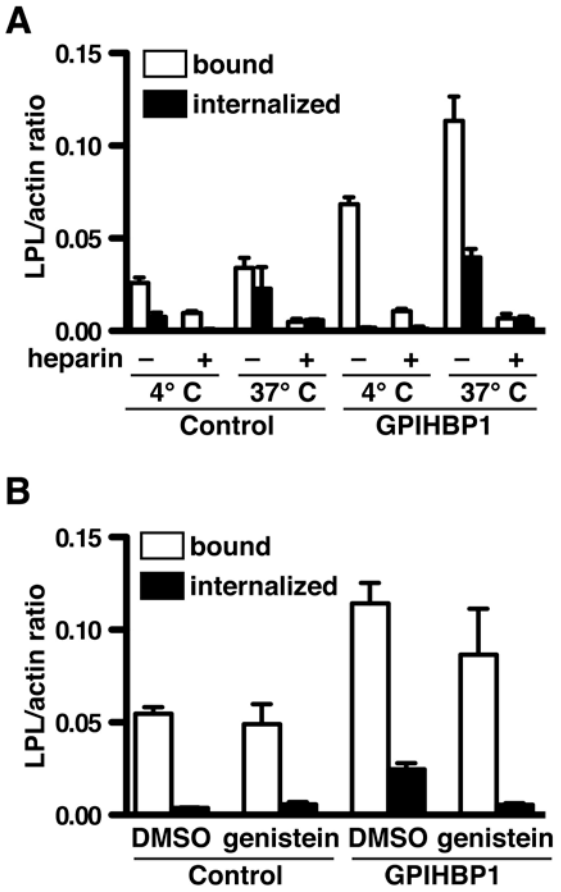

Figure S4. LPL internalization by cultured endothelial cells. GPIHBP1-expressing and control RHMVECs were incubated with V5-tagged LPL for 2 h at 37° C, then treated with sulfo-NHS-SS-biotin at 4° C to biotinylate surface proteins. After quenching the biotinylation reaction with 100 mM glycine and collecting cell extracts, biotinylated proteins were removed with NeutrAvidin agarose beads. LPL internalization was quantified by comparing the amount of LPL (as quantified by western blot) in whole cell extracts to the LPL remaining after the removal of biotinylated cell surface proteins. (A) LPL internalization does not occur at 4° C or in the presence of heparin. LPL incubation was carried out in the presence or absence of heparin (500 U/ml) at 4 or 37° C. Bar graphs show the amount of both bound and internalized LPL relative to actin (mean ± SEM). (B) LPL internalization is blocked by genistein. LPL incubation was carried out at 37° C in the presence of genistein (100 μM) or DMSO alone (0.2%). Bar graphs show the amount of both bound and internalized LPL relative to actin (mean ± SEM).

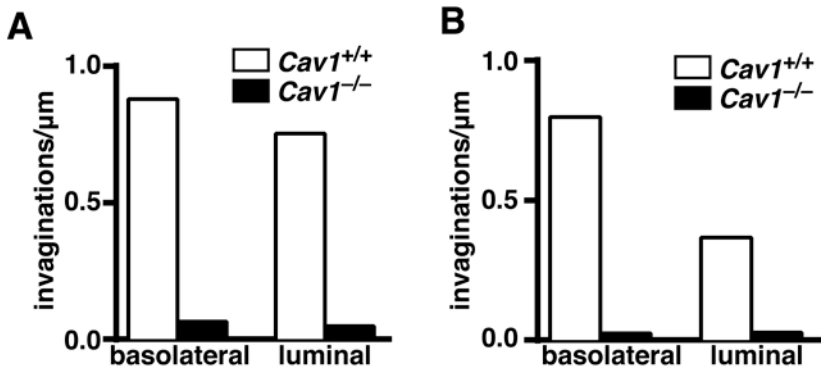

Figure S5. Invaginations in the capillary endothelial cells of *Cav1*$^{+/+}$ and *Cav1*$^{-/-}$ mice. The numbers of invaginations per µm plasma membrane on both basolateral and luminal surfaces were counted using electron micrographs of heart (A) and skeletal muscle (B) from *Cav1*$^{+/+}$ and *Cav1*$^{-/-}$ mice.

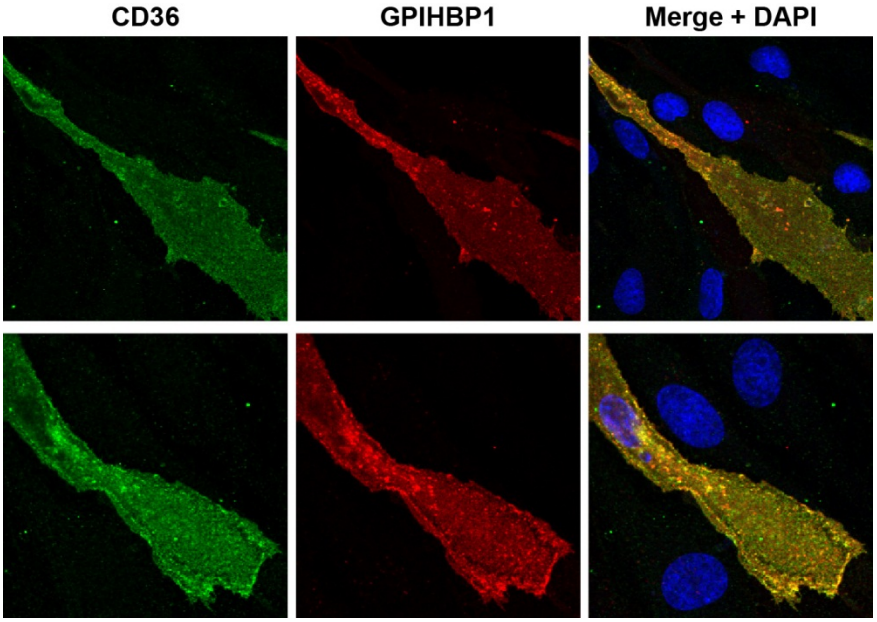

Figure S6. Co-localization of GPIHBP1 and CD36 on the surface of cultured endothelial cells. RHMVECs were co-transfected with GPIHBP1 and CD36. The next day, the cells were stained with antibodies against CD36 (green) and GPIHBP1 (red). DAPI staining (blue) was used to identify cell nuclei (blue). Cells were imaged by confocal microscopy.

Video S1. Movie showing a dual-axis electron tomogram of GPIHBP1-expressing endothelial cells that had been grown on transwell filters and incubated with gold-conjugated antibody 11A12. Gold particles are present in plasma membrane invaginations and vesicles near the apical surface.

Video S2. 3-D model of tomogram shown in Video S1, created by drawing contours around membranes of each section and then reconstructing the contours to generate a 3-D image. The endothelial cell membrane is marked in red, a clathrin coated vesicle in blue and green, and the top of the filter in light blue.

Video S3. Movie showing a dual-axis electron tomogram of GPIHBP1-expressing endothelial cells that had been grown on transwell filters and incubated with gold-conjugated antibody 11A12. Gold particles are present in multi-lobed invaginations and vesicular structures.

Video S4. 3-D model of tomogram shown in Video S3, created by drawing contours around membranes of each section and then reconstructing the contours to generate a 3-D image.

Video S5. Movie showing a dual-axis electron tomogram of GPIHBP1-expressing endothelial cells that had been grown on transwell filters and incubated with gold-conjugated antibody 11A12. Gold particles are present in vesicles near the basolateral surface, including one with no connections to the plasma membrane.

Video S6. 3-D model of tomogram shown in Video S5 created, by drawing contours around membranes of each section and then reconstructing the contours to generate a 3-D image.