# Supporting Information

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### SI Materials and Methods

**Cells and Reagents.** Human amniotic WISH cells (CCL 25), mandin darby bovine kidney cells (CCL22), L-929 (CCL-1), HEK 293T cells (CRL-11268), and vesicular stomatitis virus (VSV) (Indiana Strain) were obtained from ATCC. Human FS-11 foreskin fibroblasts (1) were kindly provided by M. Revel (Weizmann Institute, Rehovot, Israel). Human LDL receptor (LDLR)-deficient GM701 fibroblasts isolated from a homozygous familial hypercholesterolemia patient were obtained from the Coriell Institute (Camden, NJ). WISH and MDBK cells were grown in MEM supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (MEM-10) at 37 °C in 5% (vol/vol) $CO_2$ in humid air. HEK 293T, L cells, FS-11, and GM701 cells were grown in DMEM supplemented with 10% (vol/vol) FBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin (DMEM-10) at 37 °C in 8% (vol/vol) $CO_2$ in humid air. Human sLDLR (soluble form of LDLR), corresponding to codons 25–313, encompassing the seven class A cysteine-rich repeats, was produced in CHO cells and purified to homogeneity. It was kindly provided by Inter-Lab, Yavne, Israel. Monoclonal anti-LDLR antibody C7 (catalog #MABS26) was from Millipore. Monoclonal anti LDLR antibodies 29.8 and 28.28 were kindly provided by Inter-Lab. Monoclonal anti-VSV-G (VSV surface glycoprotein) antibody clone P5D4 was from Sigma. Biotinylated goat anti-mouse antiserum was from Chemicon (Millipore, catalog #AP124B). Cy-3-labeled streptavidin (catalog #016-160-084) was from Jackson ImmunoResearch. HRP-rabbit polyclonal anti-mouse antibody conjugate (catalog #ab6728) was from Abcam. Biotinylated anti-human LDLR antibody (catalog #BAF2148) and streptavidin HRP (catalog #89088) were from R&D Systems.

**Assay of sLDLR Antiviral Activity.** Cells (WISH and MDBK, 30,000 cells per 0.1 mL per well; L cells, 20,000 cells per 0.1 mL per well) in 96-well plates were incubated for 15 min with serially twofold-diluted sLDLR, starting at 8 μg/mL (214 nM). VSV [1 multiplicity of infection (MOI)] was then added, and after 17–24 h the cultures were stained with crystal violet [5% (wt/vol) in 66% (vol/vol) aqueous methanol] and photographed. For viability assays, WISH cells were similarly incubated with serially twofold-diluted sLDLR starting at 1 μg/mL and challenged with VSV. Cells remaining after VSV challenge were incubated with Neutral red (70 mg/L in 0.1 mL DMEM-10 per well 37 °C, 30 min), washed three times with PBS, extracted with a lysis buffer [28 mM trisodium citrate, 0.06% 10 N HCl, 48.9% (vol/vol) methanol, 0.1 mL], and $OD_{540}$ was determined.

**Quantitative PCR of Cell-Associated VSV.** WISH cells were plated in six-well plates ($7.5 \times 10^5$ cells per well, in 2 mL of MEM-10, 17 h). The plate was then cooled to 4 °C for 1 h, and sLDLR was then added at the indicated concentrations. After 15 min VSV (10 MOI) was added, and the cultures were kept at 4 °C for 4 h. The cells were then washed three times with cold PBS, and total RNA was prepared using the PerfectPure RNA Cultured Cell Kit from 5 Prime. cDNA was prepared from total RNA (1 μg) using High Capacity cDNA Reverse Transcriptase Kit (catalog #4368814, Applied Biosystems). Unique probes and gene-specific primer pair combinations for TATA box binding protein (TBP) mRNA, which served as a reference transcript, and target gene probes were designed (nucleotides 1146–1207 of VSV-G) using Roche ProbeFinder software version 2.32 (Roche Diagnostics). TBP cDNA samples were diluted 20-fold for use as working templates in quantitative RT-PCR (qRT-PCR). qRT-PCR was performed using TaqMan Universal PCR Master Mix (Applied Biosystems). qRT-PCR was carried out using a Roche LightCycler 480 Real-Time PCR System, and the reactions (10 μL final volume) contained 0.25 μM primers (Sigma) and 0.1 μM probe (Roche Diagnostics). The amplification program was as follows: an initial denaturation step at 95 °C for 15 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 1 min, and 40 °C for 30 s. The gene expression level was normalized to the TBP reference mRNA. The fold change in gene expression compared with mRNA from control cells was calculated using LightCycler software version 4.05. The results are represented as the mean ± SD of triplicate and displayed as histograms.

**Semiquantitative RT-PCR of Cell-Associated VSV.** WISH cells were plated in six-well plates as before. sLDLR (5 μg/mL, 133.5 nM) was added to the cells for 15 min before infection with VSV (0.2 MOI) for 10 min at 4 °C or at 37 °C. The cells were then washed 10 times with cold PBS to remove any unbound virus, cells were trypsin-digested, and viral RNA was extracted using the High Pure Viral Nucleic Acid Kit (catalog #11 858 874 001, Roche Diagnostics). RT-PCR was performed (M-MLV, catalog #M170A, Promega). For first-strand cDNA synthesis, 1 μg of total RNA was reverse-transcribed using random primers and 200 U of M-MLV reverse transcriptase in a Tris·HCl buffer (pH 8.3, 50 mM, 25 μL) containing 75 mM KCl, 3 mM $MgCl_2$, 500 μM dNTP, and 10 mM DTT. PCR was performed with the Red Load Taq master mix (Larova) with the designed primer pairs of VSV sequences (amplifying nucleotides 6096–6223 of the VSV genome). The amplification program was as follows: initial denaturation step at 95 °C, 15 min, followed by 31 cycles of 94 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min. The PCR products were analyzed by gel electrophoresis.

**Surface Plasmon Resonance.** Analyses were performed using a BIAcore 3000 instrument (GE Healthcare). sLDLR (0.02 mg/mL) in sodium acetate buffer (15 mM, pH 3.8, 40 μL) was immobilized on a CM5 sensor chip according to the manufacturer's instructions. VSV (molecular mass $2.66 \times 10^8$ Da) was suspended at $10^7$ pfu/mL in PBS with or without $CaCl_2$ (1 mM) and passed over the immobilized sLDLR in the sensor chip. For measuring VSV avidity to sLDLR, the VSV suspension was passed on the sensor chip at the indicated concentrations. For measuring VSV-G affinity to sLDLR, a preparation of VSV-G-pseudotyped lentiviral vectors (VSV-G-LV) (15 μg/mL) was immobilized on a sensor chip, and sLDLR in PBS plus $CaCl_2$ was passed on the chip at the indicated concentrations. The dissociation constants were calculated using BIAevaluation 4.1 software according to the manufacturer's instructions.

**Coimmunoprecipitation of VSV-G and sLDLR.** Purified VSV (50 ng) and sLDLR (6 μg/mL) were incubated (2 h at room temperature). Protein-G Sepharose beads (100 μL) were incubated with 10 μg of anti-LDLR mAb 28.28 (2), anti-LDLR mAb C7, an isotype-matched control mAb, or no antibody (1 h at 4 °C). Then the antibodies-conjugated beads were washed, mixed with the VSV–sLDLR complex, and incubated for 2 h at 4 °C. Next the beads were washed with 0.1% BSA and 1 M NaCl in PBS to overcome nonspecific interactions. The bound proteins were eluted with a sample buffer and subjected to SDS/PAGE [7.5% (wt/vol) acrylamide]. Solubilized VSV was run in parallel to provide a VSV-G marker. Proteins were then transferred to a nitrocellulose membrane, and the membrane was immunoblotted with anti

VSV-G (Sigma, 9.8 mg/mL diluted 1:1,000) or the anti-LDLR mAb 29.8 followed by HRP-rabbit anti-mouse conjugate (Abcam, 2 mg/mL, 1:20,000) as a second antibody.

**Preparation of VSV-G-LV and Lymphocytic Choriomeningitis Virus-Pseudotyped Lentiviral Vector.** EGFP expressing VSV-G pseudotyped lentiviral vectors were produced as described (3) but with the following modifications: HEK 293T cells in DMEM-10 (20 mL) were plated in a 15-cm dish precoated with 0.01% poly-D-lysine. The culture was transfected by a mixture of four expression vectors (30 μg DNA in 2 mL PBS in total, encoding VSV-G:Gag-Pol:Rev: TurboGFP at a 1:1:1:2 ratio, respectively (Invitrogen, except TurboGFP, which was from Sigma), using jetPEI (60 μg, Polyplus Transfection) as a transfection reagent. After 24 h, media were changed to DMEM-2 (15 mL). Culture medium containing the resulting viral vectors was collected at 24 and at 48 h and was concentrated 75-fold by ultrafiltration on a 10-kDa cutoff membrane (Centricon 10, Millipore). A plasmid encoding the lymphocytic choriomeningitis virus (LCMV) coat protein was kindly provided by A. Panet (Hebrew University, Jerusalem, Israel) and was used as above instead of the VSV-G plasmid for production of LCMV-pseudotyped lentiviral vector (LCMV-LV).

**Transduction of Cells.** Cells (20,000 per 0.3 mL DMEM-10 per well or 20,000 per 1 mL per well) were seeded in either eight-well μ-slides (ibidi) or in 24-well plates, respectively. After 17–24 h, indicated reagents (sLDLR, receptor-associated protein, etc.) were added, and after 30 min the cultures were transduced with VSV-G pseudotyped or LCMV-LV encoding EGFP. After 72 h nuclei were counterstained with Hoechst 33258, and the extent of EGFP expression was evaluated by fluorescence microscopy.

**Fluorescence Microscopy: EGFP and Hoechst 33258.** Cells (20,000 cells per 0.3 mL per well) were cultured in eight-well μ-slides or in 24-well plates for 2–4 d. Nuclei were stained with Hoechst 33258 (2 μg/mL in DMEM-10, 15 min). The cultures were observed by fluorescence microscopy (Olympus IX71) using excitation at wavelength <485 nm and emission of wavelength >530 nm for EGFP, wavelength <380 nm and >420 nm for Hoechst 33258.

**Image Analysis.** Images of Hoechst 33258-stained nuclei were selected by the Magic Wand tool of Adobe Photoshop, copied into a separate image, and counted by the analyze particle tool of the ImageJ program (www.imagej.nih.gov/ij/). The extent of EGFP expression (the green channel) in three to four fields was determined using the analyze-histogram tool of the ImageJ program. The total fluorescence (sum of pixel intensity on a scale of 0–255 times the pixel counts) was then normalized to the number of nuclei. Means ± SD of three to four separate measurements in fields containing at least 30 nuclei are represented.

**Trypsin Digestion of the Cell Surface VSV Receptor.** Human epithelial WISH cells were grown to confluence in T75 flasks, and the cultures were washed three times with PBS and then treated either with Trypsin/EDTA or EDTA alone for 30 min at 37 °C. The resulting cell suspensions were then washed three times with MEM-10 and suspended in MEM-10. VSV (MOI = 0.015) was then added for 15 min at 37 °C, and the cultures were then washed three times by MEM 10, suspended in MEM-10, and then plated at $5*10^5$ cells/mL in 96-well plates (0.1 mL per well). The cultures were incubated for 17 h at 37 °C, culture supernatants were collected for plaque assay, and the cell cultures were stained with crystal violet and photomicrographed.

**Plaque Assay of VSV-G.** Human WISH cells (900,000 per well, 0.2 mL) were grown to confluence in six-well plates. Serially 10 times diluted samples containing VSV (0.1 mL) were added to the plates, and incubation continued for 30 min at room temperature. MEM-10 (2 mL) containing 0.6% agarose, preheated to 37 °C, was added to each well. The cultures were then incubated for 17 h at 37 °C or until plaques were microscopically visible. The agarose layer was then carefully removed, and the cultures were stained with crystal violet and photographed. Plaques were counted using the ImageJ program. Data are averages ± SD of three to four replicates.

**Competition Between VSV and Fluorescently Labeled LDL.** FS-11 cells (5,000 cells per 0.3 mL DMEM per well) in eight-well μ-slides (ibidi) were cooled to 4 °C, and VSV was added at the indicated MOI for 5 min on ice. Fluorescently labeled LDL (Dil-LDL, 1.67 μg/mL) was added and incubation continued at 4 °C for 4 h. The cells were then washed three times with cold PBS and incubated at 37 °C for 1 h in DMEM. Nuclei were then stained with Hoechst 33258 as before, and the cultures were evaluated by fluorescence microscopy. Dil-LDL was visualized by excitation at <530 nm and emission at >600 nm.

**Flow Cytometry.** FS-11 cells (50,000 cells per 1 mL DMEM per well) in 12-well plates were cooled to 4 °C, and VSV suspended in DMEM or medium alone was added (5 min, 4 °C), followed by Dil-LDL (4.8 μg/mL). Incubation continued at 4 °C for 4 h; the cells were then washed three times with cold PBS and then incubated in DMEM-10 at 37 °C for 1 h. The cultures were trypsin-digested, and Dil-LDL uptake was determined using flow cytometry (BD FACSAria III). Analysis was performed on a window that excluded aggregates, and 10,000 cells were measured. Dil-LDL excitation was at <530 nm and emission was at >600 nm.

**Immunoblotting.** Cells in six-well plates were lysed with 0.1 mL per well of radio-immunoprecipitation assay (RIPA) buffer [150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris (pH 8.0), supplemented with Sigma protease inhibitors]. The lysates were centrifuged (10,000 × *g*, 10 min), and the supernatants containing the cellular proteins were collected. Protein concentration was determined using a BCA Protein assay reagent kit (Pierce) using BSA as a standard. Protein samples were boiled for 3 min in SDS/PAGE sample buffer containing 25 mM DTT, and the supernatants were resolved by gradient SDS/PAGE [7.5% (wt/vol) acrylamide]. Proteins were then transferred onto a nitrocellulose membrane and then incubated with the indicated antibodies. Second antibody conjugates were visualized by the Super Signal Detection Kit (Pierce). Human LDLR was detected by anti-LDLR mAb 29.8 (7 μg /mL) (2) or by biotinylated anti-human LDLR antibody (0.2 μg/mL).

**Blocking of VSV Infection with mAbs to LDLR.** Cells (30,000 cells per well in MEM-10) were plated in 96-well plates 17 h before the assay. Medium was replaced with MEM-1; the plates were then cooled to 4 °C, and antibodies were added to the cultures at the indicated concentrations for 30 min. VSV (MOI = 0.05) was then added and incubation continued at 4 °C for 1 h. The plates were then washed two times with antibody-containing MEM-1 and cultured at 37 °C for 17–24 h in the presence of the antibodies. The cultures were then stained with crystal violet as above, and the extent of protection from the cytopathic effect was determined microscopically.

**Measuring VSV Binding and Internalization.** FS-11 fibroblasts or GM701 fibroblasts (50,000 cells per well in DMEM-10) were seeded and grown to confluency on coverslips in 24-well plates. The cells were then preincubated with various combinations of receptor-associated protein (200 nM) and anti-LDLR mAbs 29.8 or 28.28 (50 μg/mL each) for 30 min at 37 °C. VSV (MOI = 500) was then added for 4 min at 37 °C; the cultures were washed three times with PBS and immediately fixed at room temperature with freshly prepared 3% (wt/vol) paraformaldehyde and 0.02% Triton X-100 (2 min) followed by 3% paraformaldehyde alone (20 min) and

washed three times with PBS. The paraformaldehyde-generated Schiff bases were reduced with $NaBH_4$ (1 mg/mL, 20 min), washed, blocked with 0.1% BSA, incubated with anti-VSV-G (9.8 μg/mL, 1 h), biotinylated goat anti-mouse antibody (stock diluted 1:500, 1 h, room temperature), and Cy-3-labeled streptavidin (3 μg/mL, 30 min, room temperature). Nuclei were then stained with Hoechst 33258 (2 μg/mL in DMEM-10, 15 min). The cultures were observed by fluorescence microscopy (Olympus IX71) using excitation at wavelength <560 nm and emission of wavelength >580 nm for Cy-3, wavelength <380 nm and >420 nm for Hoechst 33258.

To calculate bound and internalized VSV foci, images were processed using Photoshop as follows: background was selected using the Magic Wand tool (tolerance = 40), the selection was inverted, and the resulting foci copied to a new image and saved as black and white images. Foci were then counted using ImageJ in triplicate fields, each having approximately 40 nuclei. Data are presented as foci/cell.

**Rescue of LDLR Expression in GM701 Cells.** An LDLR expression vector (product ID A0821, GeneCopoeia) was used for constructing LDLR-encoding VSV-G-LV as described above. GM701 cells (20,000 cells per 1 mL DMEM-10 per well) in 24-well plates were transduced with the LDLR-encoding VSV-G-LV in the presence of polybrene (8 μg/mL). Control GM701 cells were treated with polybrene alone. After 17 h, viral vectors and polybrene were replaced with medium lacking polybrene daily for 3 d. The cultures were then transduced with VSV-G-LV encoding GFP in the absence of polybrene. After 17 h, viral vectors were removed, and after an additional 48 h nuclei were counterstained with Hoechst 33258, and the extent of EGFP expression and nuclei counts were evaluated by fluorescence microscopy.

**Knockdown of LDLR mRNA.** FS-11 cells (200,000 cells per well in six-well plates) in MEM supplemented with 10% (vol/vol) lipoprotein-deficient FBS (2 mL) were transfected either with siRNA pools (ON-TARGETplus, Dharmacon RNAi Technologies) directed against human LDLR mRNA (NM_000527) or nontargeting scrambled control siRNA according to the manufacturer's protocol. After 72 h cells were trypsin-digested, pooled from two wells, and then plated in 24-well plates (20,000 cells per 1 mL DMEM-10 per well) for lentiviral vector transduction as above, or in six-well plates (500,000 cells per 2 mL DMEM-10 per well) for immunoblotting of LDLR.


1. Weissenbach J, et al. (1980) Two interferon mRNAs in human fibroblasts: In vitro translation and *Escherichia coli* cloning studies. *Proc Natl Acad Sci USA* 77(12): 7152–7156.
2. Molina S, et al. (2007) The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol* 46(3):411–419.
3. Segura MM, et al. (2007) Production of lentiviral vectors by large-scale transient transfection of suspension cultures and affinity chromatography purification. *Biotechnol Bioeng* 98(4): 789–799.


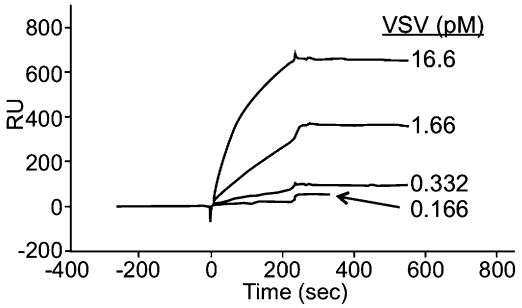


**Fig. S1.** Dose–response of VSV binding to immobilized sLDLR by surface plasmon resonance in PBS containing 1 mM $CaCl_2$. sLDLR (0.02 mg/mL) was immobilized on a CM5 sensor chip, and VSV was passed at the indicated concentrations. The dissociation constant ($K_d$, $10^{-11}$ M) was calculated using the BIAevaluation program. This value reflects the avidity of multiple VSV-G spikes toward the array of immobilized sLDLR molecules.

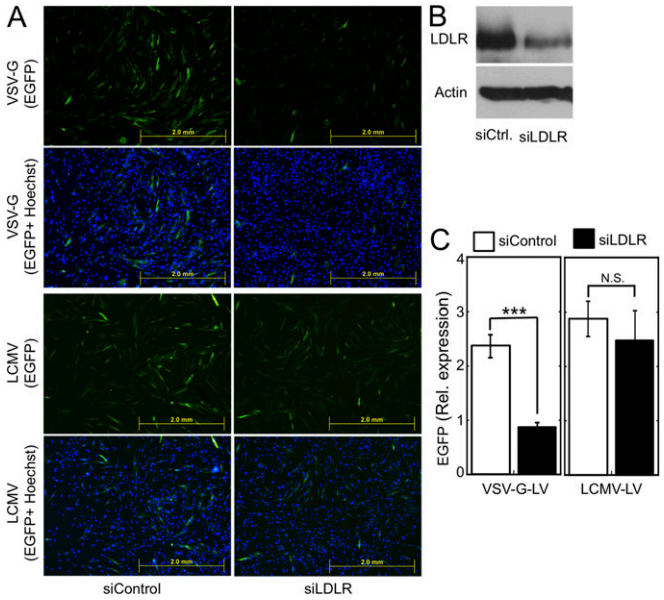


**Fig. S2.** LDLR knockdown diminishes transduction by VSV-G-LV. (*A*) WT FS-11 fibroblasts were transfected with control siRNA or with *LDLR* siRNA for 72 h. The cultures were then transduced with EGFP-encoding VSV-G-LV (upper four panels) or EGFP-encoding LCMV-LV (lower four panels). Nuclei were counterstained with Hoechst 33258. EGFP expression (green) and nuclei (blue) were visualized by fluorescence microscopy. (*B*) Immunoblot of LDLR in WT fibroblasts 72 h after knockdown with the indicated siRNA. (*C*) Average ± SD of EGFP expression shown in *A*. data were normalized to nuclei numbers. ***$P < 0.0003$, $n = 4$. N.S., not significant ($P = 0.355$, $n = 3$).