Supporting Information

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SI Materials and Methods

Antibodies. Monoclonal anti-Na^+/K^+ ATPase, monoclonal anti-lactate dehydrogenase (LDH), monoclonal anti-flotillin 1, and monoclonal anti–β-galactosidase were from Epitomics; monoclonal anti–PEX-1, monoclonal anticalnexin, and monoclonal anti-early endosome antigen 1 (EEA1) were from BD Transduction; polyclonal anti-E1 was from Abcam; polyclonal anti-hexokinase I and horseradish peroxidase-conjugated, affinity-purified donkey anti-mouse and anti-rabbit IgGs were from Cell Signaling Technology. Polyclonal anti-GM130 was a generous gift from Joachim Seemann (University of Texas Southwestern Medical Center, Dallas, TX). Monoclonal anti-Niemann-Pick C2 (NPC2), designated IgG-13G4, was prepared by fusion of SP2-IL-6 mouse myeloma cells with splenic B-lymphocytes obtained from BALB/c mice immunized with nine injections of 50 μg of purified recombinant human GST-NPC2 (amino acids 20–151). Monoclonal IgG-13G4 (subclass 1) was purified from hybridoma culture supernatant by affinity chromatography on Protein G-Sepharose 4 Fast Flow columns.

Cell Viability Assay. Cell viability was assessed by release of LDH (1). On day 0, SV-589 cells were set up in medium A in a 12-well plate at 2.5×10^4 cells per well. On day 2, cells were switched to medium B. On day 3, cells were washed five times with ice-cold PBS at 4 °C and refed with 0.5 mL of ice-cold PBS containing varying amounts of the indicated protein. After incubation for 1 h at 4 °C, the PBS was removed from each well and centrifuged at $1,000 \times g$ for 5 min at 4 °C to remove any cells. Aliquots of the cell-free PBS solution (50 μL) were then assayed spectrophotometrically for LDH activity using 0.6 mM sodium pyruvate and 0.1 mM NADH as described by Lee et al. (2). LDH activity is expressed as percent NADH consumed per 50-μL of solution. Each value represents the mean of triplicate incubations.

Immunoblot Analysis. Samples were mixed with 5× SDS loading buffer, heated for 5 min at 95 °C, and then subjected to SDS/PAGE on an 8, 10, or 15% polyacrylamide gel. Proteins were transferred to Hybond-C extra nitrocellulose filters (GE Healthcare), blocked with BSA, lipoprotein-deficient serum, or nonfat dry milk, and then incubated with a 1:1,000 dilution of one of the following antibodies: anti-Na^+/K^+ ATPase, anti-flotillin 1, anti–β-galactosidase, anticalnexin, anti-GM130, anti-hexokinase I, anti–Pex-1, anti-EEA1, anti-E1, or anti-LDH. Incubation with anti-NPC2 was carried out at a concentration of 2.5 μg/mL. Bound antibodies were identified by incubation with anti-mouse IgG (1:5,000 dilution) or anti-rabbit IgG (1:5,000 dilution) and visualized by chemiluminescence (SuperSignal Substrate; Pierce). Filters were exposed to Premium X-ray Film (Phenix Research Products) at room temperature for 5–60 s.

1. Korzeniewski C, Callewaert DM (1983) An enzyme-release assay for natural cytotoxicity. *J Immunol Methods* 64(3):313–320.

2. Lee C-Y, Yuan JH, Goldberg E (1982) Lactate dehydrogenase isozymes from mouse. *Methods Enzymol* 89(Pt D):351–358.

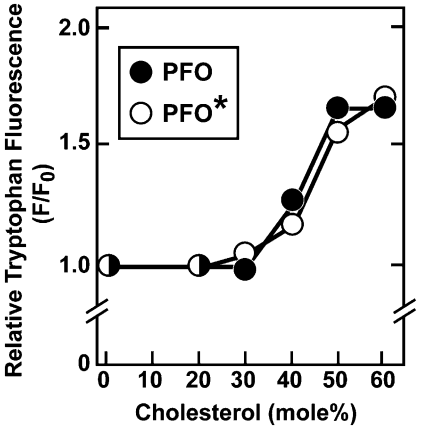

Fig. S1. Liposomes composed of dioleoylphosphatidylcholine (DOPC) and cholesterol were prepared as described previously (1). Perfringolysin O (PFO) and PFO* (a modified water-soluble PFO) were purified as described previously (1). Each reaction mixture, in 200 μL buffer A, contained 4 μM of purified PFO or PFO* and 800 μM liposomes (total lipid) composed of DOPC and the indicated amount of cholesterol. After incubation for 1 h at room temperature, tryptophan fluorescence from the samples was then measured (excitation wavelength, 290 nm; emission wavelength, 340 nm) using a Safire microplate reader (Tecan). F_0 is defined as the fluorescence from mixtures of protein and liposomes containing 0% cholesterol. Each value is the average of duplicate assays.

1. Sokolov A, Radhakrishnan A (2010) Accessibility of cholesterol in endoplasmic reticulum membranes and activation of SREBP-2 switch abruptly at a common cholesterol threshold. *J Biol Chem* 285(38):29480–29490.

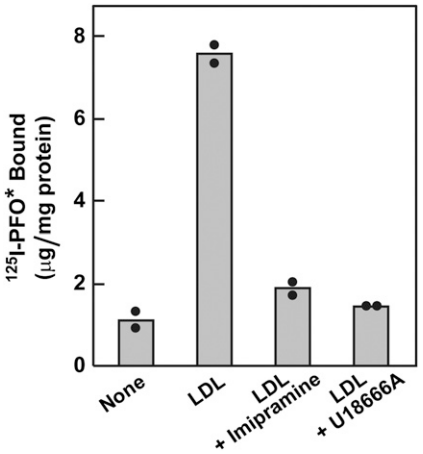

Fig. S2. Effect of imipramine and U18666A on LDL-derived cholesterol transport from lysosomes to plasma membrane. On day 0, SV-589 cells were set up in medium A at 1×10^5 cells per 60-mm dish. On day 2, cells were switched to lipoprotein-deficient medium C. On day 3, cells were refed with medium C containing 50 μM compactin and 50 μM sodium mevalonate and then incubated for 16 h at 37 °C. On day 4, cells received 2 mL of fresh medium E containing 50 μM compactin, 50 μM mevalonate, and 50 μg protein/mL of LDL in the presence of 1 μM U18666A or 50 μM imipramine, as indicated. After incubation for 5 h at 37 °C, the cells were washed five times as described in *Materials and Methods* and then incubated with 2 mL ice-cold buffer E containing 25 μg/mL ^{125}I-PFO* (6×10^3 cpm/μg). After 2 h at 4 °C, the total amount of cell surface binding of ^{125}I-PFO* was determined. Each bar denotes the average of duplicate incubations with individual values shown.

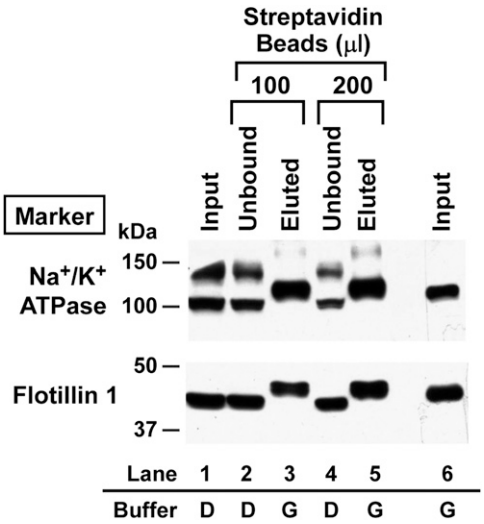

Fig. S3. Immunoblot analysis of the same purified plasma membranes used in Fig. 6*A*. Plasma membranes were purified with the indicated amount of streptavidin magnetic beads and then subjected to immunoblot analysis for the indicated marker in the presence of the indicated buffer. Note the difference in the migration of the two marker proteins when electrophoresed in buffer D (which contains no urea or thiourea) vs. buffer G (which contains 6 M urea and 2 M thiourea). Filters were exposed to film for 10 s lane 6 ("Input") contains the whole cell lysate incubated in buffer G before SDS/PAGE and immunoblotting. All samples were adjusted to the same final volume relative to the starting material.