Supplemental Data

Identification of Surface Residues on Niemann-Pick C2 (NPC2) Essential for Hydrophobic Handoff of Cholesterol to NPC1 in Lysosomes

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Figure Legend

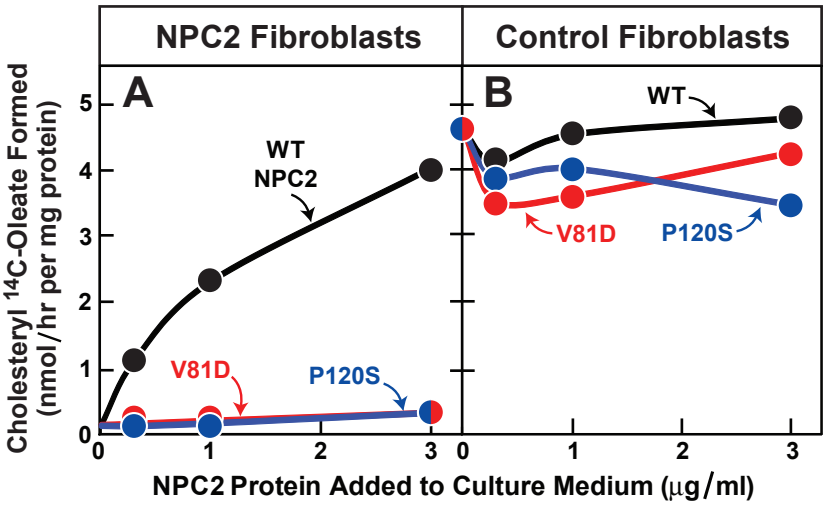
Figure S1. Ability of WT NPC2, but Not Mutant NPC2, to Rescue LDL-stimulated Cholesteryl Ester Formation in NPC2-deficient Fibroblasts

(A and B) Control and NPC2-deficient cells were set up for experiments as described in Experimental Procedures. On day 7, cells were switched to medium B containing 5% lipoprotein-deficient serum, 50 μM compactin, 50 μM sodium mevalonate, 60 μg protein/ml LDL, and the indicated concentration of either purified WT NPC2-FLAG protein or its mutant version (V81D or P120S). After incubation for 5 hr at 37°C, each cell monolayer was pulse-labeled for 1 hr with 0.2 mM sodium [^{14}C]oleate (4793 dpm/nmol), and the cellular content of cholesteryl [^{14}C]oleate and [^{14}C]triglycerides were determined. Each value is the average of duplicate incubations. Content of [^{14}C]triglycerides in NPC2-deficient fibroblasts treated with 60 μg/ml LDL and 3 μg/ml of WT, V81D, or P120S NPC2-FLAG proteins was 3.5, 4.0, and 3.8 nmol/hr per mg protein, respectively.

(C) Immunoblot analysis of cell extracts and culture media from WT and NPC2-deficient fibroblasts treated with purified NPC2 protein. Cell extracts were prepared as follows: cells from 1 60-mm dish were treated as above with the indicated WT or mutant NPC2 protein (3

μg/ml), washed twice with ice-cold PBS, and lysed in 300 μl of buffer B with 1% NP-40 and Protease Inhibitor Cocktail Set III (Calbiochem) for 30 min at 4°C on a rotator. The lysates were then centrifuged for 10 min at 15,000*g* at 4°C. The supernatant was collected and mixed with 40 μl of anti-FLAG M2-Agarose affinity beads and incubated overnight at 4°C on a rotator. The beads were washed three times with 1 ml ice-cold buffer B with 1% NP-40, and bead-bound protein was eluted by heating for 4 min at 95°C in 40 μl 75 mM Tris-HC1, 7.5% glycerol, 3% SDS 1.5% ß-mercaptoethanol, and 0.06% bromophenol blue. Aliquots from cells (representing 0.5 dish) and media (representing 0.012 dish) were then subjected to 13% SDS-PAGE, followed by immunoblot analysis with 1:3000 dilution of rabbit anti-human NPC2 antiserum. Filters were exposed to film at room temperature for 5-10 sec.

Figure S1



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Fibroblasts	Control				NPC2			
NPC2 Protein	None	WT	V81D	P120S	None	WT	V81D	P120S