Supplemental Methods

TRL Analysis. TRLs were analyzed by SDS-PAGE on 4-12% Bis-Tris gradient gels (NuPage, Invitrogen). Proteins were visualized by silver staining (Pierce) or after transfer to Immobilon-FL PVDF membrane (Millipore). Membranes were blocked with Odyssey Blocking Buffer (LI-COR Biosciences) for 1 hr and incubated overnight at 4°C with specific antibodies. Rabbit and mouse antibodies were incubated with secondary Odyssey IR dye antibodies (1:15,000 dilution) and visualized with Odyssey IR Imaging system (LI-COR Biosciences). Goat and guinea pig antibodies were incubated with HRP-conjugated secondary antibodies (Santa Cruz Biotechnology, 1:10,000 dilution) and visualized by SuperSignal chemiluminescent substrate for HRP detection (Pierce). Blots were analyzed by densitometry with ImageJ software (NIH) and the amount of each apolipoprotein was normalized to apoB. The values therefore are ratiometric and provided in arbitrary units. .

Western blot antibodies included: rabbit anti-mouse apoB (Abcam ab20737; 1:1,000 dilution), rabbit anti-mouse apoE (Meridian Life Sciences, K23100R, 1:1,000 dilution), rabbit anti-human (and mouse) hepatic lipase (Santa Cruz Biotechnology, sc-21007, 1:200 dilution), guinea pig anti-mouse LPL (antisera prepared from animals immunized with recombinant mouse LPL; 1:300 dilution; Gonzales and Esko, unpublished), rabbit anti-mouse apoAV (Santa Cruz Biotechnology, sc-66810, 1:200 dilution), mouse anti-human ApoE (Abcam, ab1906, 1:1,000 dilution), and mouse anti-human apoAV (Santa Cruz Biotechnology sc-32810, 1:200 dilution). Positive controls for Western blotting of LPL and HL consisted of clarified tissue homogenates prepared from heart and liver of a wildtype mouse, respectively. Freshly isolated tissue was homogenized in RIPA buffer and centrifuged at low-speed (2,000 x g). Protein concentration was measured by BCA assay.

Murine TRL binding to hepatocytes. Overnight fasted mice (n = 5 per preparation) were gavaged with 300 µl of corn oil (Sigma Aldrich) and 10 µCi of [11,12-^{3}H] retinol (Perkin-Elmer, 43.0 Ci/mmol). Three hours post-gavage, mice were sacrificed, blood was withdrawn by cardiac puncture, and postprandial [^{3}H]TRLs were isolated from plasma by ultracentrifugation. The samples were pooled from multiple tubes and the radiospecific activity of the particles was calculated by liquid scintillation counting and BCA protein assay.

In some experiments, Hep3B cells were incubated for 15 min at 37°C in serum free media in the absence or presence of 2 mU/ml each of recombinant heparin lyases I, II, and III. Treated and untreated cells were washed twice with serum-free growth medium, chilled on ice for 20 min and then incubated with 50 µg/ml [^{3}H]TRLs in serum-free medium at 4°C for 1 hr. Cells were rinsed with ice-cold PBS three times and solubilized by adding 0.1 M sodium hydroxide containing 0.1% sodium dodecyl sulfate for 45 min at room temperature. Bound TRLs was quantitated by liquid scintillation counting of the solubilized material, and the values were expressed as µg of TRL protein bound per mg of cell protein based on the radiospecific activity of the particles.

HSPG-TRL flotation assay. Hep3B cells were incubated for 24 hr in growth medium supplemented with 10% dialyzed fetal bovine serum (Gibco) containing 100 µCi/ml $Na[^{35}S]O_4$ (PerkinElmer Life Science). Cells were treated for 5 hr in serum-free medium with 250 nM phorbol myristic acid (Sigma-Aldrich) to induce shedding of [^{35}S]syndecan-1 ectodomains (1). Shed [^{35}S]ectodomains were isolated from conditioned medium and purified by anion-exchange chromatography as previously described (2). [^{35}S]ectodomains (~5,000 counts in 15 µL) were incubated with 0 - 1 µg hTRLs in 200 µl of a solution of iodixanol (OptiPrep Density Gradient Medium, Sigma-Aldrich) prepared in 150 mM NaCl (δ=1.019 g/ml) in the absence or presence of 1 µg of heparin (Celsus).

For competition studies with monoclonal antibodies, hTRLs (1 µg) were mixed with the indicated concentration of purified antibody. The mixtures were incubated for 1 hr and centrifuged at 38,000 rpm (175,000 x g) for 3 hr at 18°C in a Beckman 42.2Ti rotor. The top 75 µl of each tube was removed and designated the top fraction. The remaining 125 µl was designated the bottom fraction. Both fractions were assayed for radioactivity by liquid scintillation counting, and the top fraction was expressed as a percentage of the total. In some experiments, [^{35}S]heparan sulfate was liberated from purified [^{35}S]HSPG ectodomains by beta-elimination (0.4 N sodium hydroxide for 24 hr at 4°C) and purified by anion-exchange chromatography.

Several mouse monoclonal antibodies against hTRL-associated proteins were used in competition experiments: unspecific mouse IgG (Jackson Immunoresearch, 015-000-003), anti-apoE (Abcam, ab1906), anti-apoAV (Santa Cruz Biotechnology, sc-32810), and anti-LPL (Abcam, ab21356). Ascites fluids with antibodies specific for apoB (MB2, MB3, MB11, MB19, MB43, and MB47) were a generous gift from Dr. Linda Curtiss (The Scripps Research Institute, La Jolla, CA) (3, 4). IgG was purified from ascites fluid on a 1 ml Protein G chromatography cartridge (Pierce) following directions provided by manufacturer. Inhibition curves were generated with each antibody up to a maximum of 1 µg/assay.

Immunoprecipitation and immunodepletion of TRLs. To separate apoB-48 bearing TRLs from apoB-100 bearing TRLs, purified apoB-100-specific mAb MB47 (10 µg) was incubated with 100 µl Protein G coated magnetic beads (Dynabeads, Invitrogen) for 15 min and washed twice with PBS using a magnet. Beads with captured IgG were resuspended in PBS with 10 µg of human TRLs and rotated gently overnight at 4°C. The beads were collected with a magnet, washed twice with PBS, and eluted with SDS-PAGE reducing buffer (Invitrogen). hTRLs, hTRLs cleared of apoB-100 containing

lipoproteins, and apoB-100 bearing lipoproteins bound to the beads were analyzed by SDS-PAGE and silver staining.

To deplete apoAV from hTRLs, 10 µg of anti-human apoAV antibody (Santa Cruz Biotechnology, sc-32810) was incubated with 100 µL Protein G coated magnetic beads (Dynabeads, Invitrogen) for 15 min and washed twice with PBS using a magnet. Beads with captured IgG (10 µg) were resuspended in PBS with 10 µg of hTRLs and rotated for 2 hr at room temperature. The beads were collected with a magnet and the supernatant was challenged with fresh beads two more times The final solution was collected and used for [^{35}S]HSPG binding experiments and Western blot analysis. Immunodepletion with nonspecific mouse IgG (Jackson Immunoresearch, 015-000-003) was performed in parallel as a control. Depletion of apoAV from mTRLs was accomplished under the same conditions with an anti-mouse apoAV antibody (Santa Cruz Biotechnology, sc-66810) using a nonspecific rabbit IgG as the control (Jackson Immunoresearch 011-000-003). The final solution was collected and used in hepatocyte binding assay and TRL clearance assays. Material bound to the beads was washed twice in PBS and eluted with SDS-PAGE reducing buffer (Invitrogen).

Reconstitution of apoE-deficient TRLs. Purified apoE-deficient [^{3}H]TRLs derived from *ApoE*$^{-/-}$ mice (50 µg) were incubated in the absence or presence of 50 µg recombinant human apoE3 (rhApoE3, R&D Systems) at 37°C for 1 hr in PBS (5). ApoE reconstituted [^{3}H]TRLs were reisolated by ultracentrifugation, and both the top (δ<1.006 g/ml) and bottom fractions (δ>1.006 g/ml) were collected. Incorporation of rhApoE3 was verified by SDS-PAGE with silver staining and Western blotting of top and bottom fractions using antibodies against human apoE (Abcam, ab1906, 1:1000), mouse apoAI (BioDesign K23500R; 1:200) and mouse apoAIV (Santa Cruz Biotechnology, sc-19036, 1:200). Reconstituted particles were used in binding studies described above.

Clearance of [^{3}H]TRLs. Overnight fasted *Ndst1$^{f/f}$AlbCre$^-$* and *Ndst1$^{f/f}$AlbCre$^+$* mice (n = 3) were injected via the tail vein with purified [^{3}H]TRLs from wildtype or apoE-deficient mice. In some experiments purified [^{3}H]TRLs from *Ndst1$^{f/f}$AlbCre$^+$* and *apoE$^{-/-}$* mice were immunodepleted of apoAV as described above and injected into wildtype mice (n = 4 per group). Serial blood samples were taken by submandibular venous puncture at the indicated times. TRL clearance was measured by liquid scintillation counting of an aliquot of plasma and expressing the values at different time points relative to the number of counts in the circulation 1 min after injection.

Retinyl ester excursion. [11,12-^{3}H] retinol (25 µCi) (Perkin-Elmer, 43.0 Ci/mmol) in ethanol was mixed with 1 ml of corn oil (Sigma-Aldrich). Each mouse received 200 µl by oral gavage and blood was sampled at indicated times by submandibular puncture. Radioactivity in plasma was measured in duplicate by scintillation counting.

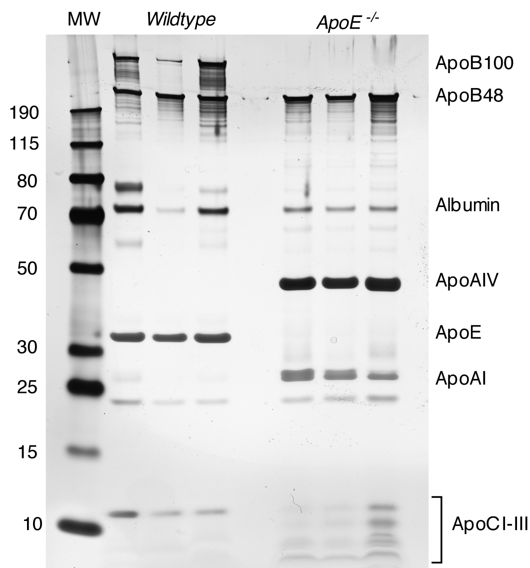
Plaque Analysis. Fasted 6-month old *Ndst1$^{f/f}$AlbCre$^-$;ApoE$^{-/-}$* (n = 8) and *Ndst1$^{f/f}$AlbCre$^+$;ApoE$^{-/-}$* (n = 12) mice were perfused with 10 ml of PBS following cardiac puncture. The heart and ascending aorta down to the iliac bifurcation were removed and incubated in formalin. The heart and adventitial tissue were removed, the aortas were incised longitudinally, pinned flat and stained for neutral lipids using Sudan IV. Images were acquired using a Sony DXC-960MD CCD color video camera mounted on 4x objective/Nikon 80i microscope. Lesion area of blinded samples was measured using ImageJ analysis software (NIH). The data are expressed as the Sudan IV positive lesion areas within the aortic arch.

References Cited

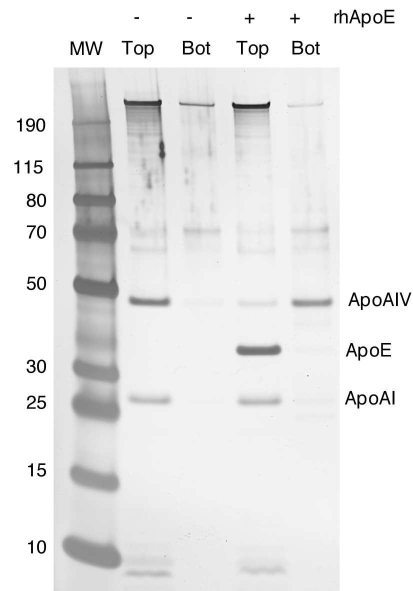
1. Deng, Y., Foley, E.M., Gonzales, J.C., Gordts, P.L., Li, Y., and Esko, J.D. 2012. Shedding of syndecan-1 from human hepatocytes alters very low density lipoprotein clearance. *Hepatology* 55:277-286.
2. Esko, J.D. 1993. Special considerations for proteoglycans and glycosaminoglycans and their purification. In *Current protocols in molecular biology*. F. Ausubel, R. Brent, B. Kingston, D. Moore, J. Seidman, J. Smith, K. Struhl, A. Varki, and J. Coligan, editors. New York: Greene Publishing and Wiley-Interscience. 17.12.11-17.12.19.
3. Curtiss, L.K., and Edgington, T.S. 1982. Immunochemical heterogeneity of human plasma apolipoprotein B. I. Apolipoprotein B binding of mouse hybridoma antibodies. *J Biol Chem* 257:15213-15221.
4. Young, S.G., Smith, R.S., Hogle, D.M., Curtiss, L.K., and Witztum, J.L. 1986. Two new monoclonal antibody-based enzyme-linked assays of apolipoprotein B. *Clin Chem* 32:1484-1490.
5. Ji, Z.S., Brecht, W.J., Miranda, R.D., Hussain, M.M., Innerarity, T.L., and Mahley, R.W. 1993. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J Biol Chem* 268:10160-10167.

Supplemental Figure 1. Analysis of apoE-deficient and reconstituted TRL particles. (A) Purified TRLs (1 µg) from wildtype and *ApoE*$^{-/-}$ mice were analyzed by SDS-PAGE and silver staining (n = 3 per genotype). The identification of the individual bands was determined by Western blotting with mAbs to apoE, apoB, and apoAIV, by molecular mass (apoC's), and by loss of the bands in the mutants. **(B)** *ApoE*$^{-/-}$ TRLs (lanes 1 and 2) were reconstituted with rhApoE3 (lanes 3 and 4) and purified by ultracentrifugation (δ < 1.006 g/ml). The top (lanes 1 and 3) and bottom (lanes 2 and 4) fractions of each flotation were analyzed by SDS-PAGE and silver staining or **(C)** by Western blotting for apoE, apoAIV, and apoAI.

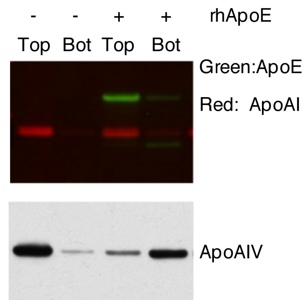
A



B

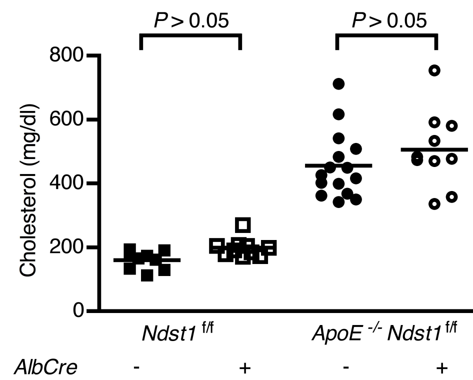


C



Supplemental Figure 2. Fasting plasma cholesterol from compound mutant mice.

Plasma cholesterol was measured in fasting plasma samples collected from *Ndst1*$^{f/f}$*AlbCre*$^{-}$ (n = 9, filled squares), *Ndst1*$^{f/f}$*AlbCre*$^{+}$ (n = 10, open squares), *Ndst1*$^{f/f}$*AlbCre*$^{-}$;*ApoE*$^{-/-}$ (n = 19, filled circles), and *Ndst1*$^{f/f}$*AlbCre*$^{+}$;*ApoE*$^{-/-}$ (n = 15, open circles) mice (8-weeks old).



Supplemental Figure 3. Analysis of apoB-restricted TRLs. Purified TRLs from *ApoB*$^{100/100}$ and *ApoB*$^{48/48}$ (1 μg, n = 3 per genotype) were analyzed by SDS-PAGE and silver staining. The identification of the individual bands was determined by molecular mass and by loss of the bands in the mutants.