Supplementary Information

ApoC-III Modulates Clearance of Triglyceride-Rich Lipoproteins Through LDL Family Receptors

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SUPPLEMENTARY INFORMATION

SUPPLEMENTAL FIGURES

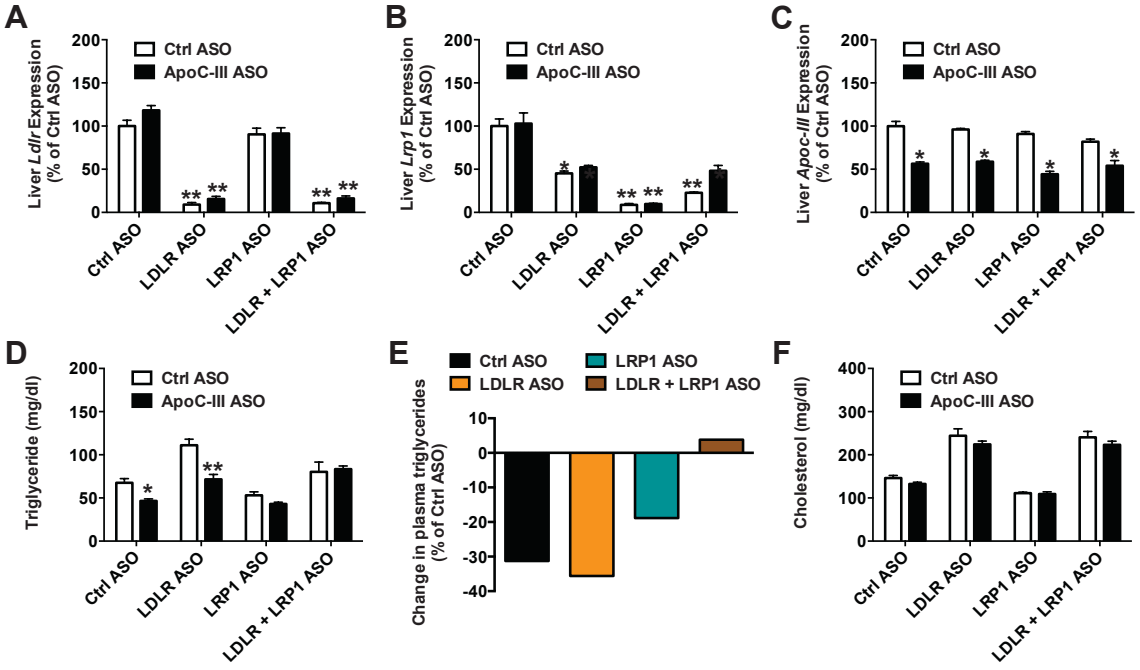

Figure S1. ApoC-III mediated TG reduction in wild-type mice treated with control ASO, Ldlr ASO, LRP1 ASO and a combination of LDLR and LRP1 ASOs. (A-C) Liver expression of (A) *Ldlr*, (B) *Lrp1* and (C) *ApoC3* RNA in wild-type mice treated with control ASO (45 mg/kg/week), Ldlr ASO (5 mg/kg/week), LRP1 ASO (5 mg/kg/week), a combination of LDLR (5 mg/kg/week) and LRP1 ASOs (5 mg/kg/week) mice, or a control ASO (45 mg/kg/week). A separate set of mice were treated simultaneously with apoC-III ASO (10 mg/kg/week). (D) Fasting plasma TG in wild-type mice treated for 4 weeks with the indicated ASOs. (E) Relative changes in plasma TG levels induced by apoC-III ASO in wild-type mice treated with control ASO, Ldlr ASO, LRP1 ASO and a combination of LDLR and LRP1 ASOs. (F) Fasting plasma cholesterol in wild-type mice treated for 4 weeks with control or apoC-III ASO. Values represent mean ± SEM (n = 4/group). *$p < 0.05$ and ** $p < 0.01$ compared to control ASO treated mice. ANOVA and Bonferroni post-hoc test.

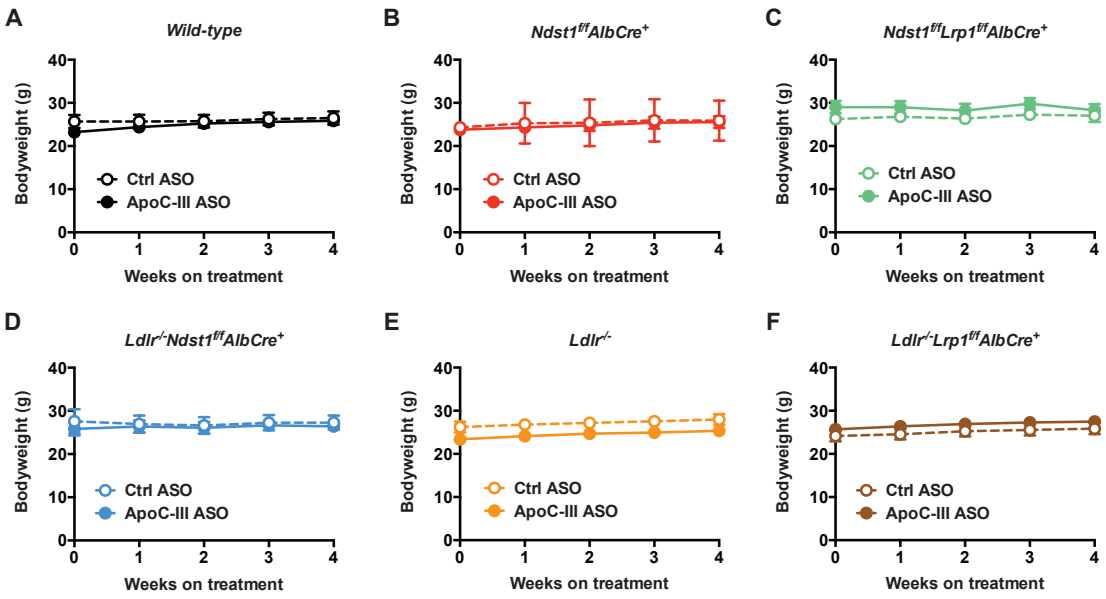

Figure S2. Impact of ApoC-III ASO on body weight of chow fed mutant mice. (A-F) Bodyweight changes of indicated mice over the 4-week apoC-III ASO treatment. Values represent mean ± SEM. ANOVA and Bonferroni post-hoc test.

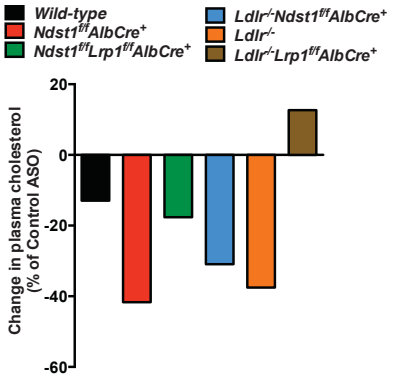

Figure S3. Impact of ApoC-III ASO on plasma cholesterol levels in high fat diet fed mutant mice. Relative changes in fasting plasma cholesterol levels induced by apoC-III ASO in mutant mice.

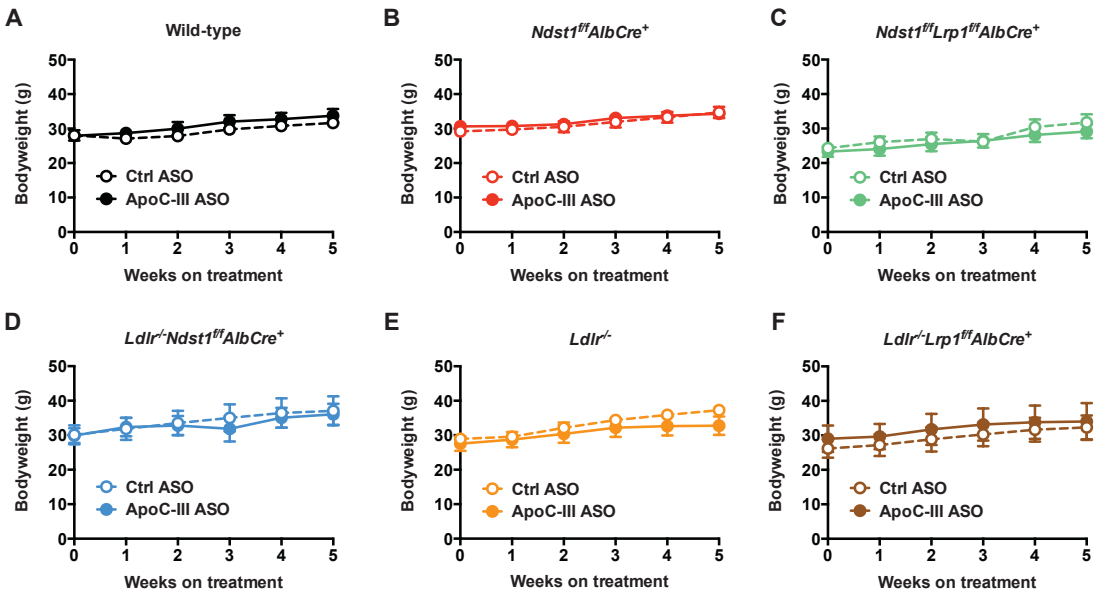

Figure S4. Impact of ApoC-III ASO on body weight of high fat diet fed mutant mice. (A-F) Bodyweight changes of indicated mice over the 5-week apoC-III ASO treatment. Values represent mean ± SEM. ANOVA and Bonferroni post-hoc test.

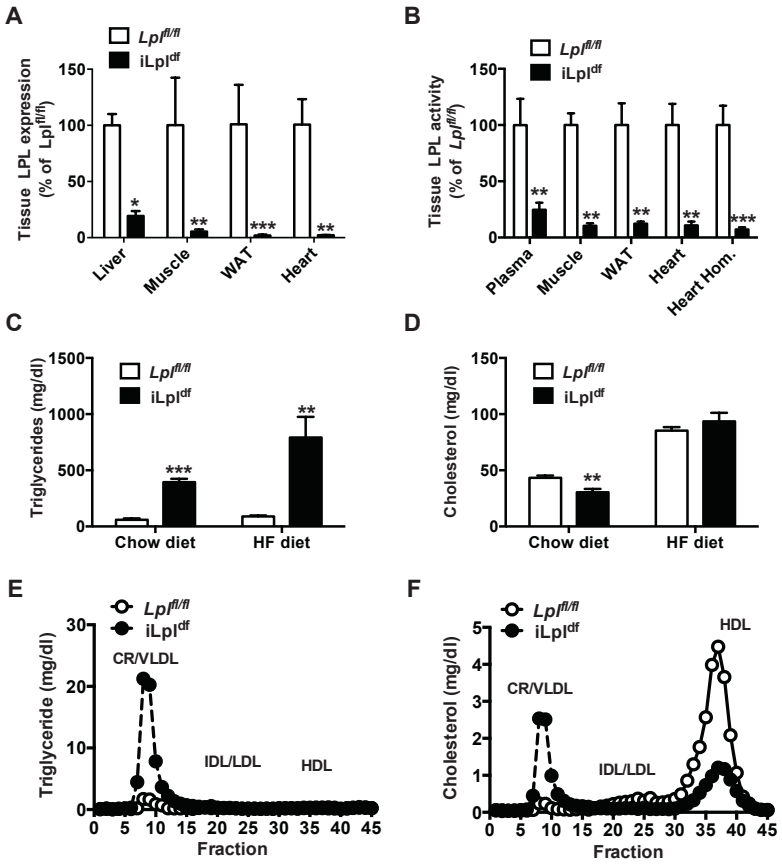

Figure S5. Characterization of the tamoxifen inducible LPL-deficient mice. (A) Liver, skeletal muscle, white adipose tissue (WAT) and heart expression of *Lpl* and *apoc-III* mRNA relative to *18s* in $Lpl^{fl/fl}$ mice treated with ($iLpl^{df}$) or without tamoxifen to induce inactivation of LPL expression 2 weeks after tamoxifen administration (n = 4-8/group). (B) Plasma, skeletal muscle, WAT and heart LPL activity levels 5 min after intravenous heparin (5 U/mouse) injection or a 30 min incubation in $Lpl^{fl/fl}$ mice and $Lpl^{fl/fl}$ mice treated with tamoxifen ($iLpl^{df}$) (n = 3/group). In addition similar LPL activity measurements were obtained in heart homogenates (heart hom.) in the absence of heparin. (C) Fasting plasma triglyceride and (D) cholesterol levels in $Lpl^{fl/fl}$ mice and $Lpl^{fl/fl}$ mice treated with tamoxifen ($iLpl^{df}$) on a chow diet or a high-fat diet. Plasma lipid levels were measured 2 weeks after the last tamoxifen administration. (E) FPLC profiles of lipoprotein TG in plasma from pooled fasted $Lpl^{fl/fl}$ mice and $Lpl^{fl/fl}$ mice treated with tamoxifen to induce

inactivation of LPL expression (iLpl^{df}) on a chow diet (n = 3 per group). (F) FPLC profiles of lipoprotein cholesterol in plasma from fasted *Lpl*$^{fl/fl}$ mice and *Lpl*$^{fl/fl}$ mice treated with tamoxifen (iLpl^{df}) on a chow diet. The elution positions of chylomicron remnant (CR)/very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL)/low-density lipoprotein (LDL), and high-density lipoprotein (HDL) are indicated. Values represent mean ± SEM. *p < 0.01, **p < 0.005 and ***p < 0.001 compared to *Lpl*$^{fl/fl}$ mice. ANOVA and Bonferroni post-hoc test.

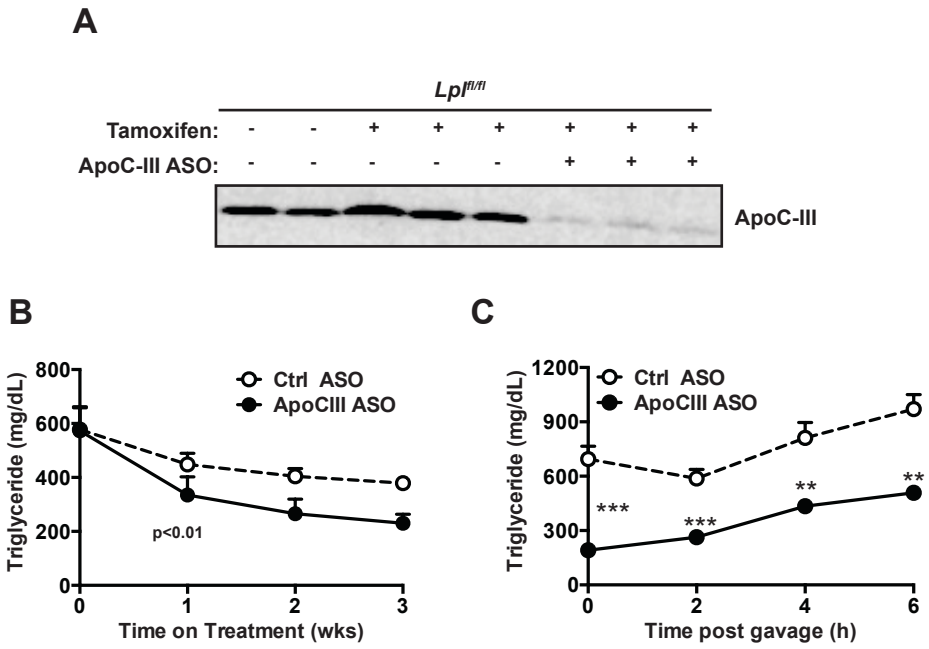

Figure S6. ApoC-III mediated TG reduction in LPL-deficient mice. (A) Western blot analysis of apoC-III on TRLs isolated from fasted tamoxifen-induced *Lpl*$^{fl/fl}$ mice treated with or without tamoxifen and/or apoC-III ASO. (B) Fasting plasma TG levels in tamoxifen-induced *Lpl*$^{fl/fl}$ mice treated for the indicated number of weeks with control or apoC-III ASO (n = 8/group). (C) Postprandial TG clearance in fasted tamoxifen-induced iLpl^{df} mice treated for 4 weeks with control or apoC-III ASO after giving 250 µL of corn oil by oral gavage. TG levels were measured at the indicated time points (n = 8/group). Values represent mean ± SEM. ** $p < 0.01$ and *** $p < 0.001$ compared to control ASO or tamoxifen-treated mice. ANOVA and Bonferroni post-hoc test.

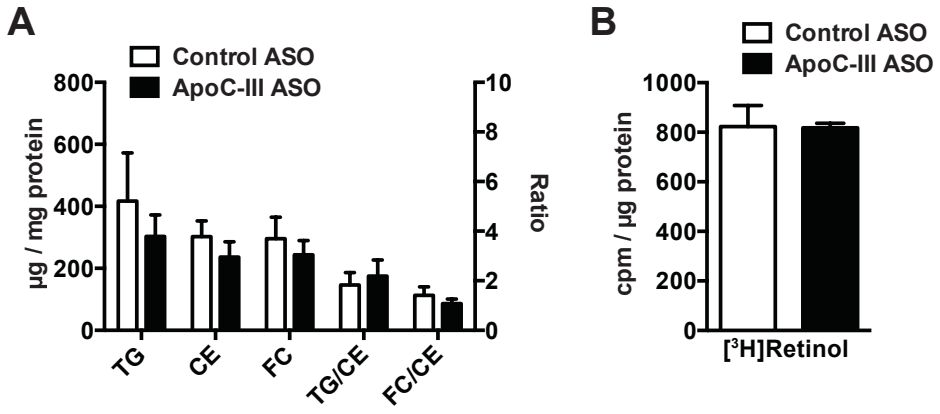

Fig. S7. [^{3}H]Retinol labeled TRL lipid Composition. (A) Analysis of triglyceride, cholesterol esters (CE) and free cholesterol (FC) levels per mg protein, ratio of free cholesterol to cholesterol esters, ratio of triglycerides to cholesterol esters and (B) retinol counts per µg protein of TRLs isolated 3 hours after corn oil gavage with 250 µl corn oil containing 5 *µCi* [^{3}H]Retinol] from *Ldlr*$^{-/-}$*Ndst1*$^{fl/fl}$*AlbCre*$^{+}$ mice on a chow diet treated for 4 weeks with control ASO or ApoC-III ASO. TRLs were prepared by ultracentrifugation (δ = 1.006 g/mL) (n = 3-6 mice per treatment group). T-test, ANOVA and Bonferroni post-hoc test.

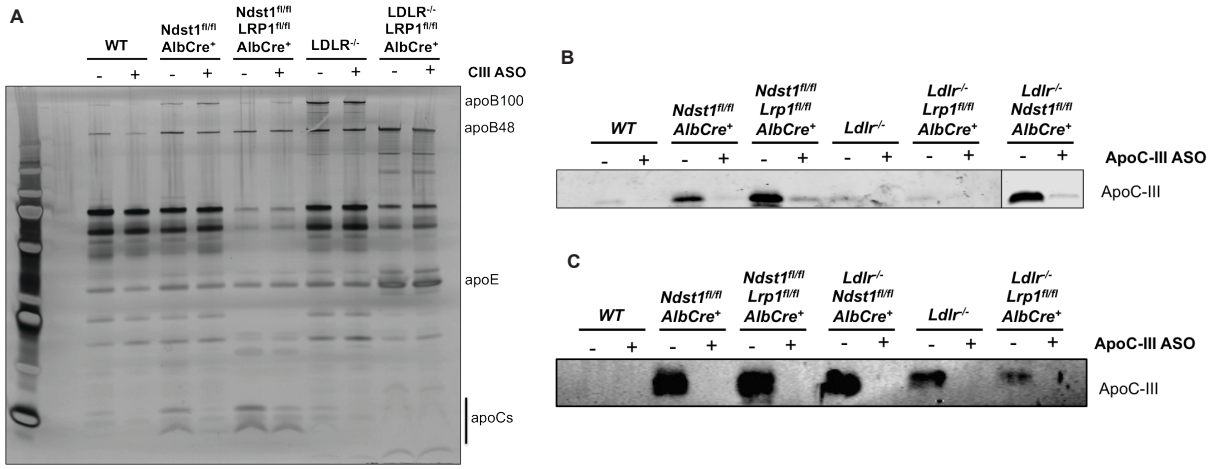

Fig. S8. TRL Apolipoprotein Composition. (A) SDS-PAGE analysis of plasma TRLs from indicated mice on a chow diet. TRLs were prepared by ultracentrifugation (δ = 1.006 g/mL) and pooled samples (n = 2-5 mice per pool) were separated on 4-12% SDS-PAGE gradient gels. The proteins were visualized using silver stain. The positions of apoB-100, apoB-48, apoE and apoCs are indicated on the right side. The lane on the left side contains protein MW markers. (B-C) Western blot analysis for apoC-III of pooled TRLs (5 μg) isolated from fasted mutant mice (n = 2-5/pool) on (B) a chow diet and (C) a high fat diet.

SUPPLEMENTAL TABLES

Table S1 Murine Antisense Oligonucleotide Sequences

Ionis number	Name	Target Gene	Sequence
ION 141923	Control ASO	N.A. (scramble)	5’-CCTTCCCTGAAGGTTCCTCC
ION 440726	apoC-III ASO	*apoC-III*	5'-CCAGCTTTATTAGGGACAGC-3'
ION 713852	LDLR ASO	*Ldlr*	5'-CTTTATCTTTAACCTC-3'
ION 793588	LRP1 ASO	*Lrp1*	5'-CCCAGTAGATGTTGCCTGCA-3'

Table S2 PCR Primers

Gene	Forward primer	Reverse primer
18s	5'-CCATCCAATCGGTAGTAGCG-3'	5'-GTAACCCGTTGAACCCCATT-3'
Apoc-III	5’-TGCAGGGCTACATGGAACAA-3’	5’-TCGGACTCCTGCACGCTACTT-3’
Ldlr	5'-GACCGCAGCGAGTACACCA-3'	5'-TCACCTCCGTGTCGAGAGC-3'
Lpl	5'-GCTGGTGGGAAATGATGTG-3'	5'-TGGACGTTGTCTAGGGGGTA-3'
Lrp1	5'-TGGTCTGATGTGCGGACTCA-3'	5'-AACAGATTTCGGGAGACCCA -3'
Tbp	5’-GAAGCTGCGGTACAATTCCAG-3’	5’-CCCCTTGTACCCTTCACCAAT-3’